E.A. klein Gebbinck

Synthesis of Model Compounds derived from Natural Clerodane Insect Antifeedants

Promotor: dr. Ae. de Groot, hoogleraar in de bio-organische chemie

Co-promotor: dr. B.J.M. Jansen, universitair docent organische chemie bij het laboratorium voor Organische Chemie

E.A. klein Gebbinck

Synthesis of Model Compounds derived from Natural Clerodane Insect Antifeedants

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Wageningen Universiteit,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 22 september 1999
des namiddags te vier uur in de Aula.

ISBN 90-5808-070-6

Stellingen

1. Daniewski's kwalificatie van verschillende marasmaan en lactaraan sesquiterpenen als 'very good antifeedants' is nogal overdreven.

   W.M. Daniewski, M. Gumulka, K. Ptaszynska, P. Skibicki, E. Bloszyk, B. Drozdz, S. Stromberg, T. Norin and M. Holub, *Eur. J. Entomol.*, **90**, 65-70 (1993); W.M. Daniewski, M. Gumulka, D. Przesmycka, K. Ptaszynska, E. Bloszyk and B. Drozdz, *Phytochemistry*, **38** (5), 1161-1168 (1995).

2. De konklusie van Enriz *et al* dat konformatieverschillen minder belangrijk zijn dan electronische effecten bij het verklaren van de verschillende antifeedant activiteiten van bacchotricuneatin A en salviarin is aan ernstige twijfel onderhevig.

   R.D. Enriz, H.A. Baldoni, E.A. Jauregui, M.E. Sosa, C.E. Tonn and O.S. Giordano, *J. Agric. Food Chem.*, **42**, 2958-2963 (1994).

3. Het door Appendino *et al* voorgestelde mechanisme voor de biosynthese van 3β-acetoxy-4β-hydroxypallenone en 3β-acetoxyplopanone in *Pallenis spinosa* (L.) Cass. is onjuist.

   G. Appendino, J. Jakupovic and S. Jakupovic, *Phytochemistry*, **46** (6), 1039-1043 (1997).

4. De verwachte stereokontrole in de bereiding van het decaline-deel van clerodin zal met de door Lallemand *et al* voorgestelde geconjugeerde additie op het enon (**2a**) waarschijnlijk niet bereikt worden.

   P.-H. Ducrot, A.-C. Hervier and J.-Y. Lallemand, *Synth. Comm.*, **26** (23), 4447-4457 (1996).

5. Bij de omlegging van 12b-(hydroxymethyl)-isoindoloisoquinoline onder invloed van sulfurylchloride is de rol van triethylamine minstens zo raadselachtig als die van pyridine.

   Y. Yoseki, S. Kusano, H. Sakata and T. Nagasaka, *Tetrahedron Lett.*, **40**, 2169-2172 (1999).

6. Het volledige gebrek aan standaardisatie bij het bepalen en rapporteren van insect antifeedant activiteiten staat een goed begrip van de onderliggende struktuur-activiteits relaties in de weg.

7. Het verdient aanbeveling om in leerboeken voor organische chemie niet te spreken van een aanval van een electrofiel deeltje op een electronenrijk molekuul, aangezien dit het gebruik van electronen-pijlen met een onjuiste richting in de hand kan werken.

   R.T. Morrison and R.N. Boyd, *Organic Chemistry*, 4th Ed., Boston, 1983, p. 601; J.F.J. Engbertsen en Ae. de Groot, *Inleiding in de bio-organische chemie*, 5e druk, Wageningen, 1992, p. 85 en 87.

8. De term 'rekeningrijden' is misleidend.

Stellingen behorende bij het proefschrift: '**Synthesis of Model Compounds derived from Natural Clerodane Insect Antifeedants**".

Wageningen, 22 september 1999 E.A. klein Gebbinck

Met dank aan Aede, Hans en Tommi.

Voorwoord

Het boekje vóór U vormt de neerslag van mijn promotieonderzoek naar verbindingen met insect antifeedant activiteit aan de voormalige Landbouwuniversiteit Wageningen. Naast de organische synthese is het vooral de wisselwerking met biologische aspecten geweest die dit onderwerp voor mij tot op de dag van vandaag zo boeiend heeft gemaakt. Achterom kijkend blijken de jaren dan ook voorbij gevlogen te zijn.

Op deze plaats wil ik een aantal mensen bedanken die aan de totstandkoming van dit proefschrift hebben bijgedragen. Allereerst Rinie Bouwman, die mij tijdens een groot deel van het onderzoek terzijde heeft gestaan. Dankzij haar creatieve en vasthoudende manier van werken konden synthetische problemen opgelost worden die anders wellicht waren blijven liggen. Haar inbreng is met name bij de hydroxy-furofuranen van doorslaggevende betekenis geweest. Daarnaast was zij een aangename labgenote, die mij altijd weer van de laatste nieuwtjes op de hoogte wist te brengen. Ook ben ik veel dank verschuldigd aan André Stork, die onverstoorbaar talloze onverwachte probleempjes uit de weg wist te ruimen en zo het werk aan de hydroxy-lactonen en de butenolides mede vorm heeft gegeven. Als lab- en kamergenoot was hij een prettige en betrouwbare kollega waarop altijd een beroep gedaan kon worden, behalve wanneer hij met spoed naar een mogelijke uitslaande brand op weg was. As an Erasmus exchange-student, Magalie Bourgois has also made her contibution to this Ph.D. Thesis, for which I am most grateful. Ben Jansen ben ik erkentelijk voor de inventieve oplossingen die hij wist aan te dragen om een syntheseroute vlot te trekken wanneer die dreigde vast te lopen en voor zijn bereidheid de handen uit de mouwen te steken wanneer meedenken alleen niet meer voldoende mocht zijn. Aede de Groot bood mij de mogelijkheid dit promotieonderzoek uit te voeren. Ik ben hem dankbaar voor de grote mate van vrijheid die hij heeft geboden, voor zijn niet aflatende belangstelling in het onderzoek, voor zijn aandrang om een punt te zetten en voor zijn geduld wanneer dit toch weer een komma werd.

Dit onderzoek was onderdeel van een gezamenlijk project van de vakgroepen Organische Chemie en Entomologie, met als doel de synthese en biologische evaluatie van modelverbindingen met insect antifeedant activiteit. De biotoetsen van de diverse stoffen zijn op bekwame wijze uitgevoerd door Lindy Messchendorp en Rieta Gols, onder begeleiding van Joop van Loon en Louis Schoonhoven. Ik ben hen zeer erkentelijk voor de prettige en open samenwerking en voor het enthousiasme waarmee zij hun bewondering voor de wereld der insecten met ons wilden delen. In dit verband wil ik ook de leden van de gebruikerscommissie van de Stichting voor Technische Wetenschappen,

P. Harrewijn, J. Henfling, A. Kerkenaar, C. Mombers en J. Moskal, danken voor hun interesse en betrokkenheid bij dit project.

Naast deze direkte medewerkers aan het onderzoek zijn er nog velen geweest die elk op eigen wijze aan dit proefschrift hebben bijgedragen. Waardevolle ondersteuning werd geleverd door Pim en Elbert (glc), Maarten, Cees, Hugo en Rien (massa en element-analyse), Bep (NMR), Pleun en Ronald (magazijn), Gert en Jurrie (glasblazerij), Hannie (hygiëne) en Elly, Ien en Ineke (secretariaat). Het onverwachte verlies van Bart Geurtsen (beheer) en Sies van der Kerk (onverwoestbaar humeur en kritische kanttekeningen op velerlei gebied) is nog steeds pijnlijk voelbaar. Marc en Adri waren altijd beschikbaar voor discussies over chemische en andere zaken en hebben daarmee op ideale wijze als klankbord gefungeerd. Ook Dennis, Wiet, Romano, Roel en Tommi hebben hieraan hun steentje bijgedragen. Dank ook aan de overige, niet met name genoemde mensen van de vakgroep Organische Chemie, voor de vriendschap en kollegiale samenwerking waardoor een sfeer ontstond waarin het goed vertoeven was.

Tot slot wil ik mijn ouders en Anja bedanken voor hun belangstelling, steun en begrip tijdens mijn studie en mijn promotieonderzoek. Zonder jullie was het nooit zo ver gekomen.

Edwin

Aan mijn Ouders
Aan Anja

Contents

Chapter 1

An Introduction to Insect Antifeedants

Abstract: Compounds with the ability to reduce or inhibit insect feeding have continued to attract attention for more than 60 years. In this period, thousands of plant species have been screened for the presence of antifeeding metabolites, several hundreds of compounds with antifeedant activity were reported and numerous investigations into their biological effects were carried out. Nevertheless, many questions regarding the chemical, biological and practical aspects of insect antifeedants are still open, and as a result commercial application of these compounds has until now been limited to a few examples. In this chapter an introduction into the field of insect antifeedants is presented and some aspects of their biological mode of action and practical application are discussed.

Table of Contents

1.1 - Some Historical Landmarks in Antifeedant Research

As a result of millions of years of attack by phytophagous insects, plants have developed an intricate defence system to protect themselves from insect predation. Apart from physical characteristics of the plant material, such as hairs or spikes or a waxy surface layer, this system often involves the use of secondary plant metabolites with various characteristics, such as insecticidal or insect growth-regulatory activity, insect repellency or compounds with the ability to prevent or reduce insect feeding, generally named 'insect antifeedants'.[1]

The use of parts or extracts of insect-resistant plants for the benefit of man has been recognized long ago. For instance, leaves and fruits of the tropical Neem tree (*Azadirachta indica*), well-known for its medicinal and insecticidal properties, have been in use in India and Sri Lanka for hundreds of years to protect books, clothes and stored foods from insect damage.[2] In Europe, the botanical insecticides rotenone and pyrethrum were introduced for agricultural use already around 1850.[3] Although unknown at the time, all these compounds or plant parts are now known to display antifeedant activity[4] against various insects, though this antifeedancy is not always their prime mode of action.

Scientific interest in the application of antifeedants in insect control can be traced back to the late 1920s, when Indian scientists used aqueous suspensions of ground Neem kernels to repel the desert locust (*Schistocerca gregaria*);[5] in 1937 Volkonsky discovered that leaves of the Neem tree contained chemicals that strongly inhibited feeding of this locust.[6] In the same period also a number of favorable laboratory and field tests were reported with various other synthetic (in)organic compounds or plant extracts with insect deterrent or repellent properties against pest insects.[7] However, due to the successes of the first neuroactive insecticides[8] in crop protection at that time, research into such 'Vergällungsstoffe' in the next decades remained limited to sporadic examples. It was not until the disadvantages of the early modern insecticides became apparent in the late 1950s and led to a search for new methods of insect control, that the unique properties of antifeedants were recognized by Jermy[9] and others[10] and renewed interest in their application in agriculture arose.

Initially, much of the attention was focussed on the application of various commercially available fungicides, based on copper or tin. Already in the 1930s some fungicides of the copper oxide type were known to discourage insects from feeding on treated foliage.[11a] Also, some organotin compounds had been in use since 1929 as mothproofing agents, due to their insecticidal and insect deterring properties.[10b] However, none of these early reports led to a practical application of an insect antifeedant in agriculture and the subject was largely forgotten until the antifeedancy of some triphenyltin-based fungicides was incidentally rediscovered by Ascher and coworkers in the early 1960s.[10a-d] Other reports of

antifeeding fungicides followed and eventually this research culminated in the first effective large-scale application of insect antifeedants, such as fentin (Ph_3SnOH) or copper(II)hydroxide, against the notorious Colorado potato beetle, *Leptinotarsa decemlineata*.[12]

At about the same time, researchers at the American Cyanamid Corporation developed the triazene AC24,055 (**10**) (Figure 1.3),[3,13] the first synthetic insect antifeedant for agricultural use. This compound deterred several chewing insects from feeding on leaves, but was ineffective against piercing-sucking or boring insects. Because of this limited spectrum of activity, development and registration of AC24,055 was halted in 1961, but taken up again in 1964, following the emergence of the resistant cotton bollworm, *Heliothis zea*, in the southern states of the USA.

Broad interest in antifeedants from natural sources arose in the early 1960s, following an observation by the German entomologist H. Schmutterer that, during a locust plague in the Sudan, Neem trees were the only green things left standing.[5] On closer investigation he noticed that, although the locusts settled on the trees in swarms, they always left without feeding. Already in 1962 succesful field trials were carried out with antifeeding Neem kernel suspensions in India.[5,14] Led by the belief that such natural pesticides could be ideal crop protection agents, especially in developing countries that often cannot afford to import modern synthetic pesticides, literally hundreds of studies have since then appeared in the literature, dealing with various aspects of Neem-based pesticides and their application.[15,16] Among the various antifeeding metabolites that have been isolated from this tree is the most powerful and versatile antifeedant known to date, the tetranortriterpene azadirachtin (**8**) (Figure 1.1). Although the isolation of this highly complex compound was already described in 1968,[17] its full structural elucidation was not completed until 17 years later.[18] Azadirachtin is a potent antifeedant against a broad range of pest insects, sometimes highly active at concentrations as low as 10^{-6} M. Furthermore, the compound displays toxic and growth-regulatory effects against many of these insects and also often interrupts insect reproduction. At the same time, azadirachtin appears to have no serious adverse effects on either benificial insect species or vertebrate organisms. Due to these properties, azadirachtin can be regarded as a near-perfect example of an ideal insect antifeedant. Although pure azadirachtin is not commercially available in the quantities required for practical application, a number of formulations based on Neem seed oil have been developed, with an azadirachtin content ranging from 0.09% to 30%.[19] After a trial period since 1985 on ornamentals and non-food crops only, some of these formulations are now registered for broad agricultural application in the USA.[19]

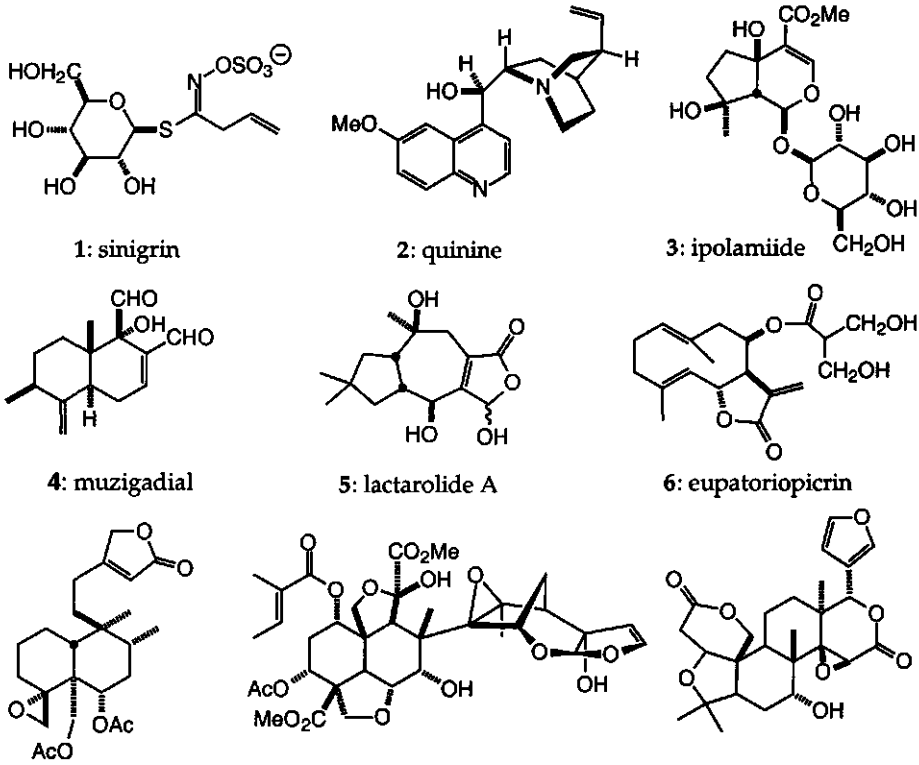
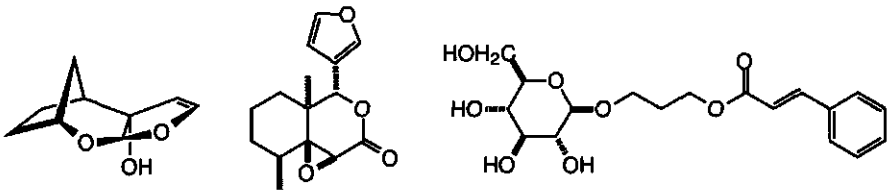


Figure 1.1: A small selection from the diverse array of natural products, isolated from various natural sources, for which insect antifeedant activity has been reported. [From ref. 20, 21, 22 (5) and 23 (9).]

Stimulated by the successes with the Neem tree, in a continuing effort since the early 1970s thousands of plant species[24] have been screened for the presence of antifeeding metabolites, yielding a bewildering array of hundreds of antifeeding compounds from the most diverse chemical classes, such as phenols, anthrachinones, carbohydrates, amino acids, alkaloids, coumarins, terpenes and many others.[20] Insect antifeedants have also been obtained from less obvious sources, such as algae or fungi. Although especially the class of terpenoid antifeedants[21] contains some promising representatives, in general these natural products were usually not sufficiently active against economically relevant pest species and/or only available from their sources in too limited quantities to warrant further development. As a result, few of them have been tested in the field and, apart from azadirachtin, none has, to my knowledge, ever reached the phase of registration.

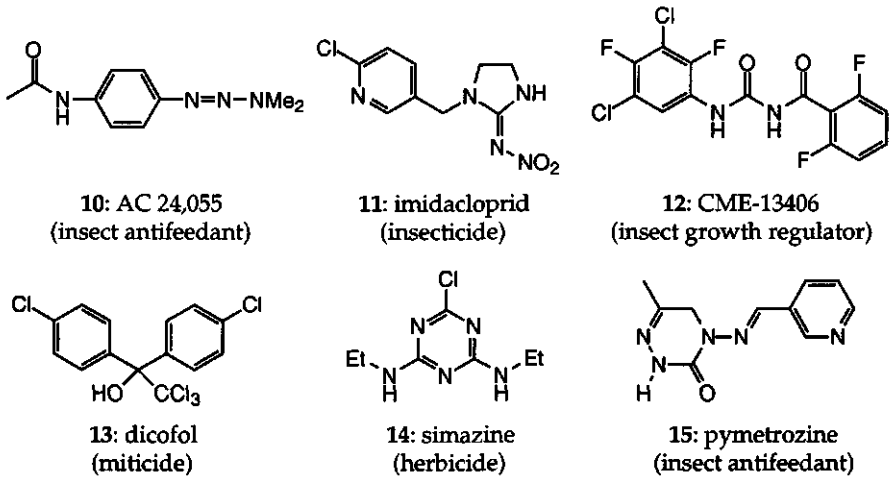
Antifeedants of synthetic origin have largely remained an area of purely academic interest. Apart from the isolation and structure elucidation of antifeedants from natural sources, the main contribution of organic chemistry to the study of antifeedants has been the synthesis of various derivatives of these natural compounds, in order to facilitate studies of the structure-activity relationships (SAR) within series of related antifeedants or to improve the stability of the natural products. Although a number of natural antifeedants, especially from the class of terpenes, have been prepared via total synthesis,[25] usually the employed synthetic routes are too long and complex for these approaches to be of much practical value.



16 17 18

Figure 1.2: Some synthetic antifeedants derived from natural products (**16,17**) or designed *de novo* (**18**). [From ref. 26-28.]

A more promising approach towards obtaining practical synthetic antifeedants would seem to be simplification of natural antifeedants while retaining the desirable biological properties (Figure 1.2). The viability of this principle has been demonstrated in cases where the simplified analogs (**16, 17**) of azadirachtin (**8**) and limonin (**9**), respectively, were shown to have retained (part of) their insect antifeedant activity in laboratory assays.[26,27] Alternatively, new antifeedants might be designed *de novo*, based on knowledge of the molecular interaction of allelochemicals with the insects' chemosensory system. For instance, Frazier and Lam synthesized the antifeeding carbohydrate-derivative (**18**) on the basis of a SAR of the interaction of glucose with the sugar-sensitive chemoreceptor of the tobacco budworm, *Heliothis virescens*.[28] Unfortunately, the agrochemical industry has shown only limited interest in the development of antifeedants for crop protection and, apart from some products which happened also to display some insect antifeedant activity (Figure 1.3), the only recent example of an industrial synthetic antifeedant is pymetrozine (**15**),[29] a potent antifeedant against sucking insects which was accidentally discovered and is presently under development worldwide for the control of aphids.



10: AC 24,055 (insect antifeedant)

11: imidacloprid (insecticide)

12: CME-13406 (insect growth regulator)

13: dicofol (miticide)

14: simazine (herbicide)

15: pymetrozine (insect antifeedant)

Figure 1.3: Some examples of synthetic agrochemicals which (also) display insect antifeedant activity (Their prime agricultural application is placed between brackets) [From ref. 13, 71, 77, 30 (**13,14**) and 29, respectively].

1.2 - Definition of Terms

Compounds that disrupt insect feeding are generally named insect antifeedants,[1] a term which has been defined by Munakata[31] as 'a chemical that inhibits feeding but does not kill the insect directly, the insect often remaining near the treated plant material and possibly dying through starvation'. The terms 'feeding deterrent', 'rejectant' and 'feeding or gustatory repellent' are often used synonymously for such substances.[32]

The multitude of terms used to describe this activity reflects the complexity of insect feeding behaviour, which generally includes scanning of the leaf surface with the sensory apparatus, followed by taking the first bite(s) to further evaluate the leaf contents ('nibbling'), and finally continuous feeding. Each of these separate phases can in principle be disturbed, leading to overall reduction or even inhibition of feeding. Primary antifeedants[15] are compounds that disrupt the phases of scanning or nibbling through interaction with the insect's chemoreceptors; a further discrimination between 'suppressants' (substances that suppress biting activity) and 'deterrents' (compounds that prevent continuous feeding) has been suggested.[32] Secondary antifeedants,[15] on the other hand, are compounds that have to be consumed in sufficient quantity before they reduce food intake due to some post-ingestive toxic effect.

The definition of antifeedancy is further complicated by the fact that it is often unknown which phase of feeding is interrupted or what the exact mode of action is. Also, some antifeedants have been found to act by a combination of both deterrency through chemoreception and toxic effects after ingestion. Furthermore, many insect species upon contact with antifeedants do not remain near the treated plant material but instead embark on a search for a more palatable food source, which may induce the use of the incorrect term 'repellency'.[33] Finally, death through starvation is only observed with the most potent antifeedants; less active compounds are usually only able to delay or reduce the phase of continuous feeding, especially when no untreated food is available to the insect.

In this thesis I will adopt the standard of most authors[34,35] to use the term 'insect antifeedant' or 'feeding inhibitor' to denote any substance that when contacted, prevents or interrupts feeding activity without directly killing the insect. It is important to stress that this term does *not* imply a specific mode of action. In my view, the terms 'feeding deterrent' or 'primary antifeedant' should be exclusively reserved for antifeedants which have been shown to act through interaction with the insect's chemoreceptors, since especially the term 'deterrent' is generally used in this fashion in discussions of host-plant acceptance behaviour by insects. On the other hand, compounds that after prolonged contact or ingestion are found to reduce or inhibit feeding, but do not necessarily induce a preference for untreated diet over treated diet, should be called 'secondary antifeedants', since their activity apparently is not based on direct chemoreception but is instead mediated through some post-ingestive toxic effect.

1.3 - Biological Techniques for the Detection of Antifeedancy

1.3.1 - Screening using Insect Feeding Bioassays

Insect feeding bioassays are the most direct method for the initial evaluation of the antifeedant activity of a test compound, which can be either a single chemical, a mixture of chemicals or even a whole (plant)extract. In such assays the test compound is applied onto, or mixed with, a suitable substrate that is palatable for the test insect. The amount of insect feeding on the treated substrate after a period of time, relative to the amount of feeding on untreated substrate, is taken as a measure of the antifeeding potency of the test compound.

The experimental setup of the assay can be widely varied in order to suit the specific needs of the test insect species.[32] Chewing insects can be presented with natural food (*e.g.* leaf discs or twig sections), dried food (*e.g.* wheat flour wafers) or artificial substrate (*e.g.* filter paper, glass fiber discs or styropor lamellae, made palatable with sucrose). Discs or plugs of agar or pressed cellulose can be

used for insects that need to tunnel into the substrate while they feed. Sucking insects can be tested on artificial medium, enclosed in parafilm sachets, while solutions of the test compound in water are employed for drinking insects. Other relevant parameters (such as light, temperature and relative humidity; developmental stage, physiological condition and age of the test insect; duration of the assay) need to be carefully controlled in order to yield reproducible results. Obviously, all these parameters must be mentioned in the experimental protocol. Also, since chemicals often only display antifeedant activity against some insect species, the species used in the assay must be described as accurately as possible, including the conditions under which it was reared or, for insects collected in the field, the geographic location where it was found.

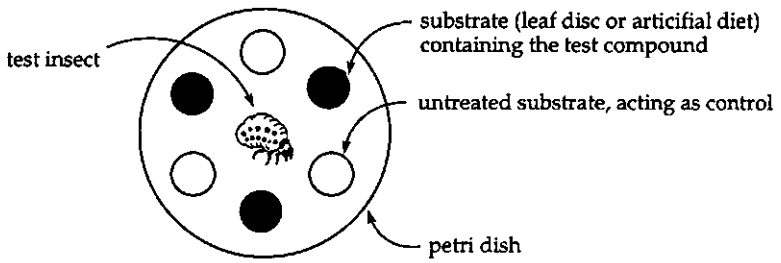


Figure 1.4: A schematic example of the experimental setup of a two-choice feeding bioassay. One or more insects are placed in an enclosed environment containing an equal amount of treated and untreated substrate. The insects are allowed to feed on the substrates for a period of time and are then removed. The antifeedant activity is determined by comparing the amounts eaten from both types of substrate (See also Figure 1.5).

Feeding assays have found widespread application in the screening of candidate chemicals, in monitoring the purification of crude plant extracts with antifeedant activity[36] and in the study of structure-activity relationships within series of structurally related antifeedants. Unfortunately, a lack of standardization has developed in the literature regarding virtually all experimental parameters and procedures. As a result the comparison of antifeedancy data from different research groups is often difficult, if not impossible, even in cases where the same insect species was used.[37]

Several applications of the basic principle are in use, of which the *two-choice* or *dual choice feeding assay* and the *no-choice feeding assay* are the most common. In the two-choice assay the preference of an insect for untreated substrate over treated substrate is recorded by allowing the insect to choose between treated and untreated substrate to feed upon. In the no-choice assay, the

insects are presented with either treated or untreated substrate, and the antifeedant activity is determined by comparing the amounts of feeding in both separate situations. Both test procedures have their advantages. The dual-choice assay is usually the more sensitive in detecting antifeedancy, since in this situation the insects can avoid compounds that are less palatable,[32] and the difference between treated and untreated substrate is thus largest. In a no-choice assay, insects often start feeding even on a less palatable substrate after some time; this behaviour is called (short-term) habituation and the compounds that are able to delay feeding for only a short period (usually some hours) are classified as 'relative antifeedants',[38] in contrast to 'absolute antifeedants' that inhibit feeding permanently. However, the no-choice situation is considered to be more representative for the actual situation that would occur upon application of an antifeedant in the field.[32]

1.3.2 - *Can Feeding Bioassays Help to Discriminate Between Primary and Secondary Antifeedancy?*

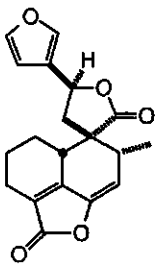
Feeding bioassays are 'end-point' assays, *i.e.* assays that record the cumulative result of insect behaviour over a period of time. Therefore, these assays cannot easily discriminate between different modes of action of a compound that all result in reduction or inhibition of insect feeding.

At first sight it might seem that a two-choice assay should be able to establish this difference, since antifeedants acting through feeding deterrency obviously should elicit a significant activity in this situation, contrary to secondary antifeedants that do not, by definition, induce a preference for untreated substrate over treated substrate and are therefore not *a priori* expected to show activity in a two-choice assay. Nevertheless, literature examples show that secondary antifeedants can display antifeedant activity in two-choice situations.[39,40] This can be rationalized by assuming that in such cases the insects fed normally on untreated substrate, but stopped feeding on either treated or untreated substrate for several hours after having consumed a small amount of treated substrate containing a high concentration of the toxin.[41]

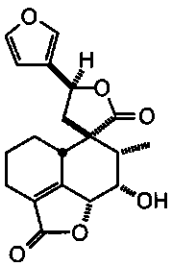
Obviously, no-choice assays are equally unable to establish a clear difference between primary and secondary antifeedants since, by definition, both should reduce or inhibit insect feeding in this situation.

When results of two-choice and no-choice assays are compared however, clues regarding the mode of action of the antifeedant can sometimes be obtained. For instance, when only treated substrate is available, the deterrent action of a primary antifeedant may not always be sufficiently strong to stop the insect from feeding for the entire duration of the assay; this leads to a lower antifeedant activity in the no-choice situation, relative to the corresponding two-choice assay

(see (**19**) in Figure 1.5). With secondary antifeedants however, this short-term habituation is less likely to occur since continued consumption of such a compound should make its toxic action more pronounced. In order to become tolerant to toxicants the insect must undergo physiological adaptations, for instance by increasing its metabolic activity through increased levels of detoxifying enzymes, which usually requires continuous exposure for a prolonged period (days).



19: teuscorolide
(feeding deterrent)



20: teucrin-A
(toxin)

Feeding assays with Colorado potato beetle:

	two-choice	no-choice	choice vs. no-choice
	AI ± SE [a]	AI ± SE [b]	SI ± SE [c]
19	25.2 ± 4.8	7.3 ± 5.3	4.3 ± 9.1
20	8.4 ± 3.6	51.0 ± 7.8	62.6 ± 2.3

Applied concentration: 300 ppm.

Figure 1.5: The effect of two natural 19-nor-clerodane diterpenes on the feeding behaviour of 4th-instar larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*), as determined in two-choice, no-choice, or choice vs. no-choice feeding bioassays (Selected examples; see ref. 39 for full details and discussion). (a) Average antifeedant index AI = [(C-T)/(C+T)] ×100% in which T is the amount consumed from the treated discs and C is the control disc consumption, both in a two-choice situation; SE is the standard error. (b) AI = [(C-T)/C]×100%, with C and T determined in a no-choice assay. (c) Average suppression index SI = [(C-T)/C]×100% in which T represents the total consumption from treated and control discs in a two-choice situation, while C is the consumption in a no-choice control assay containing only untreated discs.

Also, since secondary antifeedants after ingestion (temporarily) inhibit feeding of both treated and untreated substrate alike, the cumulative consumption of both treated and untreated substrate in a two-choice assay should be smaller than the amount eaten from the untreated substrate in the corresponding no-choice situation. Upon encountering primary antifeedants in a two-choice assay however, the insect can turn to untreated substrate to feed upon and the total amount of feeding in this case should therefore not be significantly different from the normal consumption, provided that the assay duration is sufficiently long for the insect to make up for the delays in its feeding behaviour caused by the unpalatable compound. A *choice vs. no-choice feeding assay*[42] (see

Figure 1.5) can be used in such cases to distinguish between these two modes of action of an antifeedant. Other experiments, in which the growth rate of an insect in relation to the food consumption rate is studied both with and without antifeedants in the insects' diet, can also be useful in such investigations.[39]

1.3.3 - *Direct Observation of Insect Feeding Behaviour*

The experimental setup of the no-choice feeding bioassay can also be used for direct observation of the effect of antifeedants upon the insects' feeding behaviour. In such *feeding behaviour observation* experiments the insect activity is classified (*e.g.* feeding, searching or resting) at regular intervals during a prolonged period of time. Also, the duration of the meals can be recorded. For sucking insects such as aphids, visual observations can be supplemented with electronic monitoring using the electrical penetration graph (EPG) technique,[43] in which the signals created when the insect inserts its stylet into the plant material are recorded. Comparison of the behaviour in the presence or absence of an antifeedant can help to distinguish between a pre-ingestive or post-ingestive mode of action of a compound (Figure 1.6). Also, direct observation may yield some insight into which particular phase of the feeding behaviour is affected.[44]

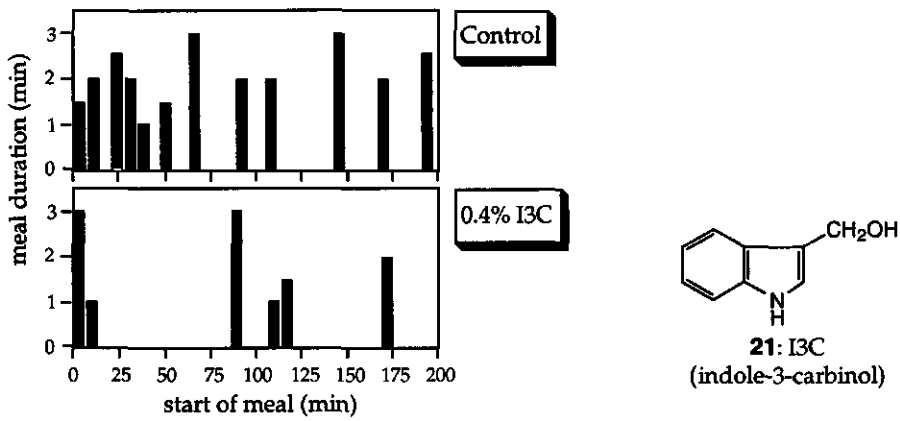


Figure 1.6: A representative example of a feeding behaviour observation experiment, demonstrating the effect of I3C on the temporal feeding behaviour of 6th-instar larvae of the fall armyworm, *Spodoptera frugiperda*. Statistical analysis of a number of such experiments (n=16) showed that the larvae, in the presence of I3C, had a lower number of meals and spent more time in long (>60 min) non-feeding pauses than the control caterpillars. However, the frequency distribution of the individual meal durations did not differ from control experiments, indicating that the reduction in total consumption was not due to feeding deterrency. [Adapted from ref. 87.]

1.4 - Antifeedancy through Sensory Chemoreception

1.4.1 - Chemoreception of Feeding Deterrents [32,45,46]

Insects can taste compounds through the interaction of these compounds with so-called chemoreceptors or sensilla, receptor organs that are specialized in the perception of non-volatile molecules. Taste receptors are found on the mouthparts of insects and sometimes also on the tarsi (lower legparts) and occur in the form of short pegs or hairs. These sensilla often contain three or four taste cells with long dendrites that are exposed to the environment through an opening at the tip of the sensillum. The taste cells can detect various chemicals, such as water, sugars, salts, amino acids or more complex plant metabolites, and subsequently produce nerve impulses. Contrary to the taste buds of vertebrates, the axons of insect chemoneurons are directly connected to the central nervous system (CNS), without any intervening synapses.[47] In the CNS, the various impulses are evaluated and a decision regarding the feeding behaviour is reached.

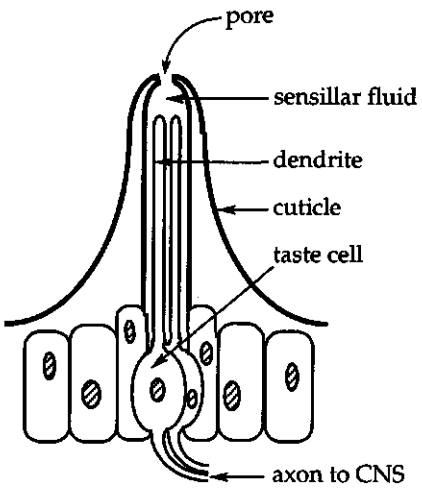


Figure 1.7: Schematic structure of an insect chemosensillum. Although only two taste cells are displayed here, often three or four neurons are found in a single taste hair. [Adapted from ref. 61.]

Electrophysiological techniques can be used to study the interaction of single compounds or mixtures with chemoreceptors at the cellular level. In such *electrophysiological bioassays* the electrical signals are recorded that are created when a single sensillum is brought into contact with a solution containing a test compound. Sometimes the impulses from individual neurons in a sensillum

can be distinguished on the basis of amplitude, shape and spike frequency of the generated action potentials, thus allowing the study of separate taste cells. Through such experiments can be identified which specific sensilla (or even which individual neurons) are sensitive to a specific test compound. Also, the nature of the effect of the compound on the chemoreceptor, *i.e.* stimulation or inhibition of the neuron(s), can be established.

Several types of taste cells responding to different kinds of stimuli have been identified in insects. Many insect species were found to have specialized cells primarily involved in the detection of specific feeding stimulants, appropriately named the 'sugar cell', 'water cell', 'amino acid cell', etc. Also, so-called 'broad spectrum cells' have been identified, which can detect a range of, for instance, secondary plant metabolites. Some insect species have one or more 'deterrent cells' that are specialized in detecting feeding deterrents.

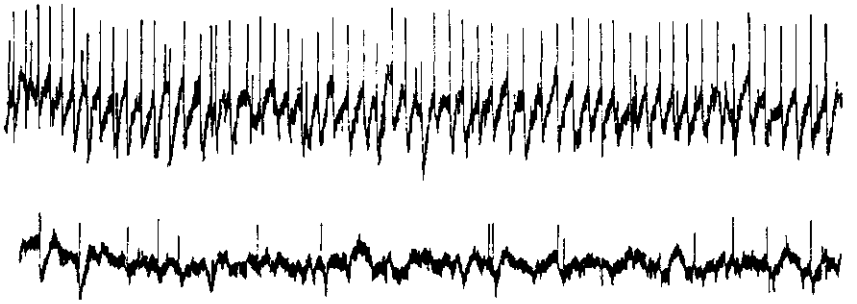


Figure 1.8: Example of an electrophysiological bioassay, showing the nerve action potentials of the deterrent cell in a maxillary taste hair of a larva of the large cabbage white butterfly (*Pieris brassicae*). Upper trace: Recorded response upon stimulation with an aqueous solution of azadirachtin (10^{-3} M) and NaCl (10^{-3} M); lower trace: control with NaCl only. [ref. 48.]

Unfortunately, the practice of insect chemoreception is more complicated than these simple names suggest. For instance, the 'sugar cell' of the *Boettcherisca* fleshfly is not only sensitive to carbohydrates, but also to amino acids and fatty acids.[49] Similarly, primary antifeedants do not always limit their action to specific 'deterrent cells', but often interact with the insects' sensory system via several cell-types and in various ways. According to a classification by Schoonhoven,[32] at least five modes of action of feeding deterrents on chemoreceptors can be distinguished:

(i) Stimulation of a deterrent receptor. In several insect species, mainly belonging to the order of Lepidoptera, specialized deterrent cells have been described, which are usually insensitive to feeding stimulants but are

stimulated upon contact with various classes of feeding deterrents. For instance, the large cabbage white butterfly (*Pieris brassicae*) was found to have deterrent neurons in both its medial and lateral sensilla, responding to phenolic acids and flavonoids (both) and to azadirachtin (medial only).[50]

(ii) Stimulation of broad spectrum receptors. In these cases a feeding deterrent was found to *stimulate* (see iii) the activity of one or more cells, that also responded to feeding stimulants, especially secondary plant compounds.

(iii) Inhibition of specific phagostimulant receptors, either momentarily or after a latency period. Several examples exist in which pure antifeedants or plant extracts with antifeedant activity were found to reduce or inhibit the activity of the 'sugar cell' in various insects. Similar effects have been described for other receptors, responding to inositol or salts. Often the inhibition is reversible and disappears after removal of the deterrent.

(iv) Stimulation of the activity of some cells while simultaneously inhibiting the activity of others, thereby changing an intricate and subtle 'sensory code' (see below). This is obviously a very complex process, consisting of a combination of the mechanisms described under (i)-(iii).

(v) Disruption of the activity of some specific cells, or even of all chemoreceptors, by evoking highly unnatural impulse patterns, often with high spike frequencies and emitted at intervals ('bursting') or even continuously. Conversely, substantial decreases in the total sensory activity have also been described.

Often a feeding deterrent acts via a combination of two or more of these neural reactions. Also, temporal actions, such as sensory adaptation (*i.e.* a decrease of the neural activity upon prolonged contact of a compound with the receptor) of one or more of the neurons involved, may further complicate the mode of action of a specific deterrent on the sensory system.

Through comparison of the results of electrophysiological assays with the feeding behaviour of the insect, as observed in feeding assays and feeding behaviour observational experiments, the relation between the insects' chemoreception of a food source and the resultant feeding behaviour can be investigated. A basic assumption in such studies is that there exists a direct relationship between the various impulses generated by the different neurons and the feeding behaviour, a 'neural' or 'sensory code'. Unfortunately, unravelling this code often turns out to be a difficult task. The number of sensilla involved can differ considerably between different insect groups, from only four to six sensilla in Lepidopterous larvae[51] to several thousand sensilla at the mouthparts of locusts.[52] Furthermore, the neural response is usually more or less compound-specific, thus different compounds with antifeedant activity may act on different neuron(s). Conversely, this means that different sensory codes

may still result in the same feeding behaviour. Also, the presence of mixtures of compounds at the chemoreceptor membrane can have effects not predictable from the individual responses to the pure compounds.[45a,46]

Due to this complexity, the present knowledge of sensory coding principles does not yet allow reliable predictions of the feeding behaviour on the basis of neural activity for most insect species. For a number of Lepidopteran insect species however, some promising correlations between neural activity and feeding behaviour have been obtained.[46] Several of these caterpillars require the combined input of only eight taste neurons for chemosensory host-plant acceptance. The feeding inhibition induced by antifeeding compounds was found to be strongly correlated with the firing rate of some deterrent neurons. For instance, the antifeedant effect of a Neem extract on *Pieris brassicae* larvae was mediated solely via the medial deterrent receptor; the inhibitory effect of some Neem compounds on the activity of the sugar and glucosinolate cells of *P. brassicae* did not play a significant role in it's feeding behaviour.[53] Similarly, the antifeedancy of drimane sesquiterpenes on *P. brassicae*[54] and of azadirachtin and related compounds on *Sp. littoralis* larvae[55] has also been correlated with deterrent cell activity.

1.4.2 - Molecular Mode of Action of Feeding Deterrents

The current understanding of the molecular mechanisms involved in the detection of molecules at the dendritic membrane of insect taste cells is still fragmentary. The basic premise is that, as in the signaltransduction mechanisms of neurons in the central nervous system of vertebrates and insects, the stimulus molecules upon contact with a receptor site on the polarizable mebrane elicit some reaction that changes the polarization of the membrane and thereby leads to the formation of an action potential. The interaction with the membrane is supposed to be more or less specific, depending on the molecular characteristics of both the stimulus and the receptor site, thus enabling molecular recognition of specific stimuli by the sensory system.

During electrophysiological studies on the 'sugar cell' of the blowfly, *Phormia regina*, four different receptor sites for the detection of sugars have been identified: a pyranose site, a furanose site, a D-galactose-sensitive site and a site reacting to 4-nitrophenyl-α-glucoside.[56] Similarly, three different receptor sites have been found on the sugar-neurons of *Boettcherisca* flies.[49] Furthermore, different receptor sites on deterrent cells have been reported for several caterpillar species.[46] Given the fact that many other insect taste cells are also sensitive to different types of stimuli with apparently unrelated molecular

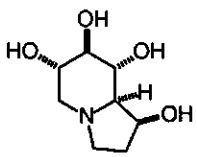
structures, it seems likely that the dendritic membrane of insect chemoreceptor neurons commonly contains multiple types of receptor sites.

Regarding the interaction of feeding deterrents with the dendritic membrane, several mechanisms can be envisaged. Stimulation of deterrent cells by structurally highly diverse antifeedant molecules might be mediated through a range of specific receptor proteins, that are specialized in recognizing classes of antifeedants with a high degree of molecular similarity. Alternatively, limited numbers of 'broad-spectrum' receptor proteins might be involved. Interaction of feeding deterrents with neurons that are also sensitive to feeding stimulants may result from the presence of receptor proteins, specifically tuned to recognize feeding deterrents, or might arise from interaction of the deterrent molecule with the receptor proteins involved in detection of the feeding stimulants, either through direct binding or via allosteric binding sites on such proteins. Also, it has been suggested that some feeding deterrents may act on the chemoreceptor membrane via rather non-specific association with the phospholipids that make up the membrane, thereby disturbing the normal membrane structure and changing ion conductivities.[57]

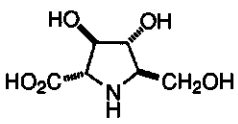
The present knowledge of molecular interactions at the chemosensory membranes does not allow us to discard any of these possibilities. However, as Schoonhoven *et al* pointed out,[45a] the fact that specific neurons are often stimulated by deterrents with highly diverse molecular structures while, at the same time, specific deterrent molecules can have different effects on different neurons, seems difficult to reconcile with the concept of one or a few types of deterrent receptor proteins. Thus, it seems likely that the molecular action of feeding deterrents is mediated through various biochemical transduction mechanisms, depending on the kind of antifeedant, the type of neuron and the species of insect involved.

A number of different models have been proposed to account for the molecular action of specific (kinds of) feeding deterrents. Several authors have postulated that membrane-bound α-glucosidases might constitute the receptor protein of the pyranose-site on the 'sugar cell' of the blowfly, *Phormia regina*.[58,59]

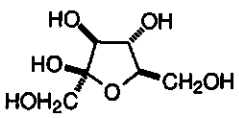
Figure 1.9



21: castanospermine



22: DMDP
(2,5-dihydroxymethyl-
3,4-dihydroxypyrrolidine)



23: β-D-fructofuranose

This receptor-site is thought to be connected to an ion-channel in the dendritic membrane, either directly[60a] or via a second messenger mechanism involving c-GMP acting on a protein kinase.[60b] Interestingly, polyhydroxyalkaloids as castanospermine (**21**) or DMDP (**22**) (Figure 1.9), which are known to inhibit α-glucosidases, were found to be potent feeding deterrents for a number of insect species. In experiments with larvae of the African armyworm, *Spodoptera littoralis*, these compounds were shown to stimulate neurons sensitive to deterrents but also to reduce the response of neurons sensitive to sugars, suggesting a possible relation between their deterrency and glucosidase-inhibitory modes of action.[61] Alternatively, since DMDP (but not castanospermine) was found to reduce the neural response to fructose (**23**) rather than to glucose, this compound might (also) act by blocking a putative furanose-site on the 'sugar cell' due to its structural resemblance to fructofuranose (**23**).

Another model of a potential taste-receptor protein is based on the action of glycine and GABA as recognized feeding stimulants for many insect species. While screening a series of direct antagonists of several major neuroreceptors in a two-choice feeding bioassay, Mullin *et al* noticed[62] that only antagonists associated with $GABA_A$- or glycine-sensitive neuroreceptors displayed potent antifeedant activity against western corn rootworm beetles, *Diabrotica virgifera virgifera* LeConte. Upon further investigation, also some known allosteric inhibitors of GABA binding were found to be antifeeding. In electrophysiological experiments, specific sensilla were found to be sensitive to GABA and glycine, to direct GABA-antagonists [strychnine (**26**)] and to allosteric GABA-antagonists [picrotoxinin (**27**)]. All of the antifeedants tested caused the firing of a single neuron, while the intensity of the neural response to antagonist stimulation was correlated with the observed order of antifeedant potency. Furthermore, saturable strychnine binding in the 10^{-8} M range was detected in biochemical preparations of *Diabrotica* mouthparts, indicating specific binding.

Based upon these observations, Mullin *et al* proposed the involvement of a transmembrane GABA/glycine-dependent chloride channel in the chemoreception of the amino acid-sensitive taste cell of the western corn rootworm (Figure 1.10a).[62] Upon interaction of amino acids with this neuroreceptor, the ion channel is opened and the resulting movement of chloride ions leads to taste cell stimulation and induces insect feeding. Competitive or non-competitive binding of GABA antagonists at, respectively, the amino acid binding site [*e.g.* bicuculline (**25**) and strychnine (**26**)] or at an allosteric site [*e.g.* picritoxinin (**27**) or some argophyllins (**28**)] would in this model result in an antifeedant effect. Furthermore, the presence of a second allosteric binding site is postulated in order to accomodate the binding of the phytosteroid cucurbitacin B (**29**), which is a potent feeding stimulator of the western corn

rootworm, probably through potentiation of GABA binding at the receptor. An attractive feature of this model is the ability to mediate the action of stimuli with a diverse range of molecular structures through a single receptor complex.

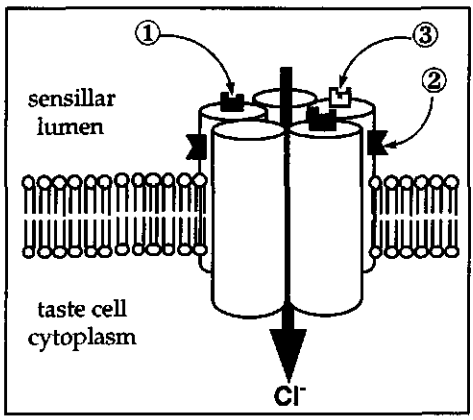


Figure 1.10a: Schematic representation of the GABA/glycine-dependent ionophore, proposed as a model of a taste cell neuro-receptor in the western corn rootworm by Mullin *et al.* The receptor consists of five subunits, forming an ion-channel that penetrates the dendritic membrane. GABA, glycine and direct antagonists bind to the amino acid site (1), the picritoxinin-site (2) binds allosteric antagonists and a steroidal site (3) binds the allosteric modulator of GABA-binding, cucurbitacin B. [Adapted from Ref. 62.]

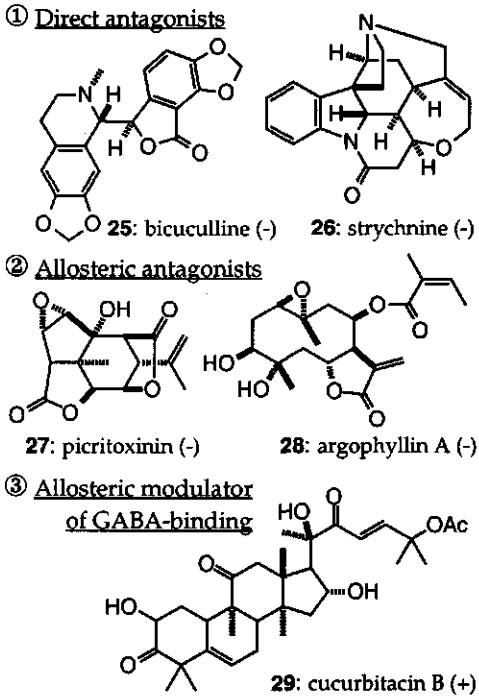


Figure 1.10b: Some compounds acting on the GABA/glycine-dependent ionophore. Compounds marked (-) are antifeeding; (+) denotes feeding stimulation.

In a series of studies[35] concerning the molecular mechanism of chemoreception of the American cochroach, *Periplanata americana*, Norris and coworkers found that the antifeeding potency of several 1,4-naphtoquinone-based antifeedants was related to their redox-potential, *i.e.* their ability to be reduced. Also, they isolated a sulfhydryl-containing lipoprotein from the chemosensillar dendritic membrane, the so-called redox-complex protein, which was shown *in vitro* to reversibly bind with naphtoquinones (*e.g.* **30**) and to subsequently reduce these electrochemically to the corresponding 1,4-dihydroxynaphtalene-derivatives (**31**). These observations have been incorporated into a model (Figure 1.11) of the energy-transduction mechanism between naphtoquinones and their chemoreceptor. In this model, an oxidizing naphtoquinone binds to the

receptor protein through a Michael-addition reaction with a sulfhydryl-group. Then, by being reduced, the oxidizing naphtoquinone withdraws hydrogen from the redox complex in the membrane and so increases the protonic potential across the dendritic membrane. If enough antifeedant molecules withdraw hydrogen from the redox chain complex (and thereby from the taste cell cytoplasm) in this fashion, the dendritic membrane becomes hyperpolarized so that a threshold action potential is less likely to develop and the rate of firing of the neuron is reduced. Thus, in this chemoreception mechanism the antifeedant 'message' is transformed from a molecular-oxidation state in the naphtoquinone to an increased protonic potential in the sensillar lumen and across the dendritic membrane, to a reduced inward flow of solutes (*e.g.* reduced transport of Na^+ through voltage-sensitive sodium channels), to a reduced rate of action potential generation and finally to fewer nerve-impulses per unit of time being sent to the CNS. The proposed model is supported by a wealth of biochemical evidence and quantitatively links several experimental parameters, such as the electrochemical effect of various naphtoquinones on the receptor complex, their relative potency

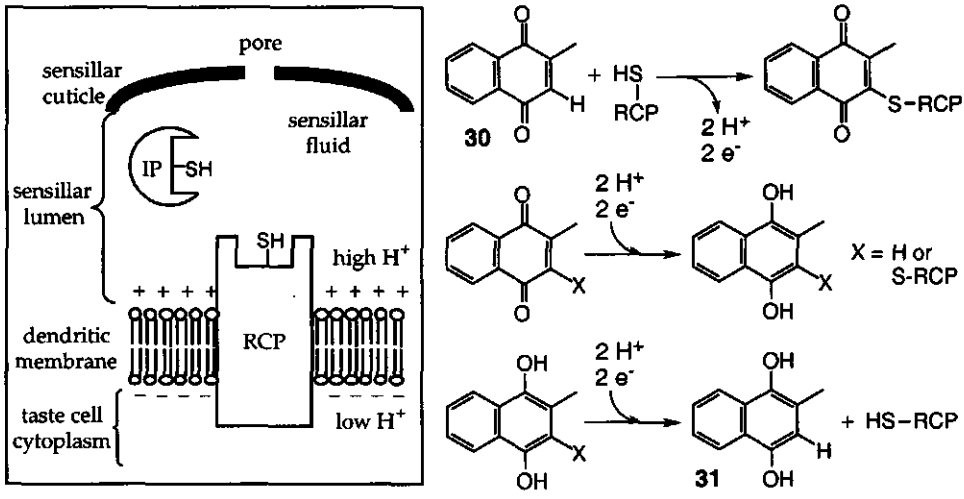


Figure 1.11: Schematic representation of the model of sensory perception of 1,4-naphtoquinone feeding deterrents by the American cockroach, *Periplanata americana*, as proposed by Norris [ref. 35]. RCP is the membrane-bound redox chain receptor protein; upon binding with this protein the naphtoquinone (**30**) is reduced and thereby withdraws hydrogens from the redox complex (nett reaction: $Q + 2H^+ + 2e^- \rightarrow QH_2$), leading to hyperpolarization of the dendritic membrane which inhibits the generation of action potentials. IP is a naphtoquinone-binding 'interfacin' protein, which is present in the sensillar fluid and is probably involved in naphtoquinone-transport and/or modulation of the sensory response to specific quinones.

in feeding bioassays and electrophysiological assays, and their binding affinities for the isolated redox complex protein.

Apart from having different redox potentials, the various naphtoquinone antifeedants may also differ in their ability to reach the sulfhydryl groups on the redox complex protein, depending on the specific structure of both the compound and of the local protein environment surrounding the reactive sulfhydryl group. Non-covalent interactions (*e.g.* steric hindering, hydrogen bonding, ionic bonding and hydrophilic vs. hydrophobic interactions) between the compound and the receptor protein thus can also help the receptor protein to recognize specific compounds as antifeedants.

Norris *et al* also isolated saline-soluble proteins from the chemosensillar pore contents of *P. americana*.[35] These proteins too were rich in sulfhydryl-groups and able to bind naphtoquinones. It is believed that such 'interfacin proteins' are involved in the transport of naphtoquinones and/or, through preferential binding of antifeedants with a specific structure, may also modulate the sensory response to these compounds and thus form yet another mechanism to discriminate between naphtoquinones with different structures.

1.4.3 - How do Antifeedants Interact With The Receptors?

Ideally, investigations on the nature of the interaction between antifeedants and their receptors should be based on studies correlating the effect of structural changes in the antifeedant molecule with changes in the binding affinity towards a taste receptor protein. Unfortunately, attempts to isolate such proteins from insects have been largely unsuccesful[61] and these data are thus not (yet) available.

Nevertheless, speculations about the possible nature of specific interactions between feeding inhibitors (or stimulants) and receptor proteins can be found in the literature, usually derived from studies relating the structure of antifeedants to the observed antifeedant activity or (better) to the activity in electrophysiological experiments. It is important to be aware of the imperfections of the biological data used for this purpose. Not only do many SAR-studies on antifeedant activity not address the possibility that structurally related compounds may act through different modes of action (*e.g.* primary vs. secondary antifeedancy), but even when only feeding deterrents acting on a single type of taste cell in one chemosensillum are considered, there always remains the possibility that several receptor types are involved. Furthermore, it is also conceivable that the antifeedant activity within such a series could differ due to differences in metabolic stability or the ability to penetrate membranes.

The capability to form covalent bonds has been suggested by several authors to be a possible factor relating the chemical structure of an antifeedant

molecule to its biological activity. For instance, during studies on insect antifeeding components in tropical plants, Kubo and Nakanishi noted that practically all antifeedants isolated (terpenes, alkaloids and other classes) contained electrophilic moieties.[34] Also, Norris has argued that upon comparison of the structures of antifeedants and related feeding stimulants, 'one is likely to find that those with antifeedant activity possess the more oxidized (or unsaturated) structures'.[35]

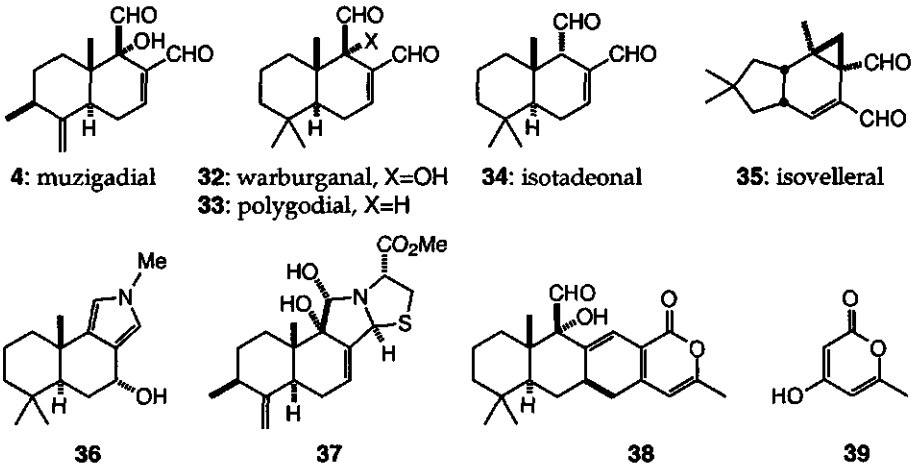


Figure 1.12: Natural antifeedants (**4**, **32-35**) containing a common α,β-unsaturated 1,4-dialdehyde moiety and some adducts (**36-38**) obtained upon reaction with nucleophilic reagents (methylamine, L-cysteine methyl ester and triacetic acid lactone (**39**), respectively). See text for discussion.

As discussed in the previous section, the activity of naphtoquinone antifeedants against the American cockroach has been partly ascribed to their ability to form Michael-adducts with sulfhydryl-groups on the receptor protein (see Figure 1.11). Another well-known example of feeding deterrents in which covalent interactions with the receptor have been proposed to be (partly) responsible for the biological activity are the natural drimane sesquiterpenes, such as muzigadial (**4**), polygodial (**32**) and warburganal (**33**).[21,63] These potent antifeedants are known to act on the chemoreceptor neurons of several insect species. Warburganal for instance was found to stimulate deterrent cells (*Pieris brassicae*[64]) and to block the excitability of sugar- and inositol-sensitive cells (*Spodoptera exempta*,[57a] *Manduca sexta*[57b]). SAR-studies with these compounds and related synthetic derivatives showed that both the C-9 aldehyde group and the enal-moiety are essential for antifeedancy, since their reduction greatly decreases the activity. Furthermore, apparently small structural modifications were found to have a large influence on the activity. For instance, epimerization

of polygodial at C-9 led to significant loss of activity, whereas introduction of an α-hydroxyl group in this position enhanced the antifeedant activity.

Ma reported that mixtures of warburganal with L-cysteine or dithiothreitol no longer caused the reduced excitability of the sugar- and inositol-neurons of *Sp. exempta* that was observed with warburganal alone and suggested that the enal moiety of warburganal might act as an -SH acceptor, reacting with sulfhydryl groups of the receptor protein in a Michael-type fashion.[57a] However, this model was not able to explain why epi-polygodial was a less potent antifeedant than polygodial, since both were found to react at equal rates with thiols under biomimetic conditions.[65]

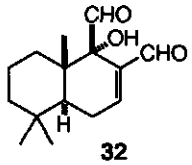
Therefore, Sodano and coworkers proposed that not a reaction with sulfhydryl groups but instead the binding to primary amine groups of a receptor protein constitutes the principal mode of action of these drimanes.[66] In model reactions polygodial reacted with methylamine to form the pyrrole-derivative (**36**), whereas isotadeonal (**34**) was unreactive, due to the larger distance between the aldehyde carbons. Furthermore, the reaction of muzigadial with L-cysteine methyl ester yielded derivative (**37**),[67] which seems to confirm that, when both primary amine groups and sulfhydryl-groups are present, these unsaturated drimane dialdehydes preferentially react with primary amines instead of thiols.

More recent studies, however, showed that Sodano's model still cannot satisfactorily explain all biological data. On comparison of the reactivity of the dialdehydes (**32**)-(**34**) towards the amino acids alanine and lysine, Jonassohn *et al* found that polygodial reacted about ten times faster than warburganal (see Figure 1.13), which in turn was more reactive than the two epimers, isotadeonal and C9-epi-warburganal (not shown).[68a-c] A similar order was observed in reactions with the nucleophilic triacetic acid lactone (**39**), leading to formation of pyranone adducts as (**38**). This order of reactivity cannot be reconciled with the observation that warburganal is an equipotent or even a more active antifeedant than polygodial, which indicates that interactions other than the simple covalent bonding to biological nucleophiles are (also) involved.

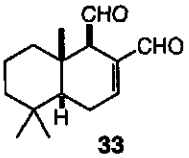
Interestingly, similar observations were made regarding the *in vitro* binding affinity of these dialdehydes towards some receptor types. Kubo and Ganjian noted that all the active drimane insect antifeedants taste hot and spicy to the human tongue wheras inactive derivatives are devoid of this pungent taste and suggested a correlation between these biological properties.[65] Other terpenes containing a similar unsaturated dialdehyde moiety, such as the marasmane-type sesquiterpene isovelleral (**35**), also exhibit both insect antifeedant activity[69] and a pungent taste. Stimulated by these findings, Sterner and coworkers determined the binding affinity of 17 drimane- and marasmane-type unsaturated dialdehydes for the vanilloid receptor, which is thought to be involved in the tasting of spicy

substances by the human tongue (see Figure 1.13).[68a,70] Upon comparison of the affinities of these 17 dialdehydes with their pungent taste, a good quantitative correlation was found (although not perfect; compare **32**,**35** with **33**).

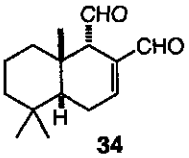
Furthermore, eight of these dialdehydes, including (**33**)-(**35**), also displayed specific *in vitro* affinity for the dopamine D1 receptor,[68a,d] whereas they were inactive against other CNS receptors tested. This binding affinity is directly related to the presence of an intact unsaturated dialdehyde moiety, as was shown



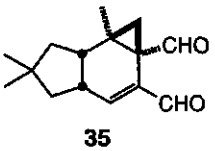
32



33



34



35

	32	**33**	**34**	**35**
Antifeedant activity				
Spodoptera exempta (AI ± SEM)[a]	62.3 ± 2.85	55.0 ± 6.81	10.9 ± 7.39	n.d.
Tribolium confusum, adults (TCA)[b]	n.d.	n.d.	n.d.	163.6
Reactivity towards nucleophiles				
L-cysteine ($T_{1/2}$, h)[c]	n.d.	0.28	n.d.	0.33
L-lysine ($T_{1/2}$, h)[d]	(35)	0.4 (2.6)	(580)	120
triacetic acid lactone (**39**) ($T_{1/2}$, h)[e]	1200	180	>2000	1100
Human pungency (nmol/tongue)[f]	2.0	0.43	21	2.2
Receptor affinity				
vanilloid receptor (IC_{50}, μM)[g]	6.8 ± 2.4	7.6 ± 0.9	43.2 ± 17.1	5.2 ± 0.9
dopamine D1 receptor (IC_{50}, μM)[h]	n.d.	0.46	5.3	0.31

Figure 1.13: Various properties of four insect antifeedants containing an unsaturated dialdehyde moiety. See text for discussion. (a) Antifeedant activity on larvae of the African armyworm, *Sp. exempta*, as measured in a two-choice feeding assay on artificial substrate (glass fibre disc/sucrose); mean antifeedant index AI = [(C-T)/(C+T)]×100 [ref. 71]. (b) Antifeedant activity on adult beetles of *Tribolium confusum*, as measured in a combined choice and no-choice test on wheat wafers; total coefficient of antifeedancy TCA = AI_{choice} + $AI_{no\text{-}choice}$ [ref. 72]. (c) Reactivity towards L-cysteine (2 mM) in buffer (pH 7.4) at 37°C [ref. 68a-c]. (d) Reactivity towards L-lysine (2 mM) in buffer (pH 7.4) at 37°C. Values in parenthesis with 0.4 mM lysine [ref. 68a-c]. (e) Reactivity towards triacetic acid lactone (**39**) in buffer (pH 7.4) at 37°C [ref. 68a-c]. (f) Minimum amount to cause pungent taste on the human tongue [ref. 68a]. (g) Affinity for specific [^{3}H]-resiniferatoxin binding sites in rat spinal cord preparations [ref. 68a]. (h) Inhibition of the specific binding of [^{3}H]-SCH 23390 to the dopamine D1 receptor [ref. 68a,d].

by the complete loss of dopamine binding affinity upon reduction of either the double bond or the unsaturated aldehyde carbonyl group of (**35**). Through molecular mechanics and semi-empirical computations the affinity was shown to be quantitatively correlated to a set of molecular parameters that describe the exact molecular structure of, and the electron density distribution within, the unsaturated dialdehyde moiety.

Unfortunately, the affinities for these receptor types, too, correlated poorly with the reactivity of the dialdehydes towards primary amines or (**39**). Even more striking is the observation that the affinities of polygodial and isovelleral for both receptor types are similar, despite the fact that marasmane-type dialdehydes are much more reactive towards sulfhydryl groups than towards amines (compare the reactivity of (**35**) towards cysteine and lysine) whereas drimane dialdehydes preferentially react with amines.

Based upon several lines of evidence, including those described above, Sterner and coworkers had to conclude[68a] that, although the bioactivities of drimane- and marasmane-type dialdehydes apparently are related to the presence of an unsaturated dialdehyde moiety in these molecules, the lack of correlation between the reactivity towards nucleophiles and various types of bioactivity (antifeedant activity, affinity for nerve cell receptors, pungency on the human tongue) indicates that these biological activities of drimane and marasmane antifeedants at least to some extent depend on interactions other than the reactivity towards biological nucleophiles.[73] In other words, even though a covalent bond with a sulfhydryl- or amine-group of the receptor may be part of the binding mechanism, other non-covalent interactions with the protein environment surrounding this nucleophilic group also influence the biological activity of these compounds and thus cannot be ignored.

Other types of compounds acting on receptors of the insects' chemosensory system may bind to the corresponding proteins through non-colvalent interactions only, without the need for covalent bonds to occur. For instance, the binding of sweet molecules, such as glucose, to the receptor sites is believed to be mediated through hydrogen bond formation.[45a] Frazier and Lam synthesized a series of glucose analogues in which the hydroxyl groups (potential hydrogen bond donors and/or acceptors) were individually replaced by fluorine (hydrogen bond acceptor capability only) or hydrogen.[28] These analogues were tested in electrophysiological assays to determine their ability to stimulate, relative to glucose, the sugar cells of two sensilla from larvae of the tobacco hornworm. In this way it was possible to map the hydrogen bonding requirements of the sugar receptors in the sensilla and to determine which hydroxyl groups of glucose were essential for recognition of the receptor sites. Interestingly, they subsequently replaced some non-critical hydroxyl groups by alkyl chains containing moieties

able to act as Michael acceptor. The rationale behind this approach was that the glucose-like moiety would guide the molecule towards the sugar receptor, enabling the Michael acceptor moiety to irreversibly block a functionally important thiol group, known to be present in the vicinity of the glucose receptor, thereby inhibiting this receptor. Indeed, some analogues (*e.g.* **18**, see Figure 1.2) were found to temporarily inhibit stimulation of the 'sugar-cell' by glucose in electrophysiological assays, and to reduce feeding of the larvae up to 77%, relative to untreated control, in feeding bioassays.

1.5 - Antifeedancy through Toxic Effects

1.5.1 - Reduction of Feeding due to Antibiosis

As was already mentioned previously, several recognized insect antifeedants are also known to invoke various toxic effects upon ingestion by insects. A well-documented case is the potent feeding deterrent azadirachtin, which in many insect species also disturbs normal insect growth and reproduction and has deleterious effects on the cells of various tissues, such as muscle, fat body and gut.[15] Conversely, a number of commercial insecticides and growth-regulators have been shown to display also antifeedant activity (see Figure 1.2).

It is clear that the food intake of an insect can be markedly reduced due to the occurrence of post-ingestive toxic effects. In studies of growth disruption of the locust *Locusta migratoria*, for instance, in which azadirachtin was applied in ways bypassing the mouthpart chemoreceptors (*e.g.* topically, via injection, or through cannulation via the mouth into the gut), the feeding of 5th instar larvae was found to be reduced up to 50% within 4 days of treatment.[15,74] Such type of feeding reduction through post-ingestive toxic effects has been labelled as 'secondary antifeedancy'.

Several examples have been reported in which sublethal concentrations of neuroactive insecticides were found to restrict insect feeding. With some insecticides, such as abamectin[75] or the pyrethroid cyfluthrin,[76] this feeding inhibition was accompanied by reduced mobility of the insects and the antifeedant action was therefore ascribed to the occurrence of a 'knockdown effect', characteristic of intoxication by such insecticides. Other neurotoxins were instead found to induce restless and increased probing behaviour of aphids (*e.g.* imidacloprid (**11**)[77,78]) or to cause tremulous movements of the insects' mouthparts (*e.g.* chlordimeform[79]), preventing normal biting behaviour. In all these cases the reduced food intake was not directly accompanied by increased mortality and sometimes the insects even recovered when placed on untreated food and resumed their normal feeding behaviour. Therefore, although the effect

of such compounds suggests that the mode of action in these cases resides in a toxic action on the CNS, the resulting insect feeding behaviour fits the definition of an insect antifeedant and thus justifies the term '(secondary) antifeedancy' to describe the effect observed with low concentrations of such compounds.

Another example of feeding inhibition due to a post-ingestive action is presented by the effect of pymetrozine (**15**) on the feeding behaviour of sucking insects, especially several aphid species.[29,80] This compound acts by disturbing the probing behaviour of aphids through blockage of stylet penetration (feeding initiation) and thereby results in inhibition of feeding. However, the aphids continue walking on the leaf surface and mortality (probably due to starvation) is slow. The rate of feeding inhibition varies with the method of contact: upon topical application or injection into the aphids the penetration behaviour is immediately disrupted. In feeding experiments on artificial diet or on whole plants (with pymetrozine either sprayed on the plant material or applied systemically via the roots) however, stylet penetration and sap uptake start normally and feeding is only interrupted after the required dose is ingested, which can take from 5 min to several hours, respectively. Also, the aphids do not display a preference for untreated plants in a choice situation. These observations show that the feeding inhibition is not due to a feeding deterrent action of pymetrozine, but is instead mediated through some post-ingestive effect. Although it could be shown that pymetrozine has a physiological effect on the nervous system of the locust[81] *Locusta migratoria*, none of the classical targets of neuroactive insecticides (*i.e.* the voltage-gated sodium channel, the nicotinic acetylcholine receptor, acetylcholine esterase or the GABA receptor) were affected by pymetrozine.[82] It thus seems likely that pymetrozine acts on the nervous system through a novel mode of action.[80a]

The occurrence of insect antifeedancy among toxic compounds is not limited to compounds with neurotoxic activity, as is demonstrated by reports of antifeedant activity being displayed by the insect growth hormone ecdysone[20] (syn.: ecdysterone), by inhibitors of chitin biosynthesis (CME-13406 (**12**)[83]) or mitochondrial respiration (rotenone[4a]) or by the bacterial endotoxin BT[84] (from *Bacillus thuringiensis*), which is known to act by perforating the insects' gut wall.[3] However, to my knowledge, in none of these cases has the mode of action of the observed feeding inhibition been determined and it is therefore unknown whether the inhibition results from pre- or post-ingestive effects.

It might be argued[32] that, since in the examples described above antifeedant action appears to result from the beginning of toxic effects that are normally associated with these compounds, it would be more logical to call these compounds (neuro)toxins instead of antifeedants. However, as was described in section 1.2, the term 'antifeedant' is historically defined on the basis of an observed reduction in food intake, without any need for statements about the

nature of the underlying mechanism(s). Differentiation between 'antifeedants' and 'toxins' would, in effect, mean the introduction of such a statement into the definition of antifeedancy, an unwelcome situation since for the majority of all compounds presently classified as insect antifeedants the mechanism of action is completely unknown. In my view, a division of the general class of 'antifeedants' into 'primary' and 'secondary' antifeedants, based on the mode of action of a particular antifeedant, should be preferred.

1.5.2 - Rapid Rejection of Toxic Foods

Due to the need for prior absorption and transport to target sites, toxic effects often only become evident after a relatively long period of time (seconds to minutes),[85] compared to the rapid occurrence of feeding deterrency (milliseconds).[32] For this reason, most investigations into the mode of action of insect antifeedants have focussed on mechanisms based on pre-ingestive chemosensory perception and have largely ignored the possibility of post-ingestive mechanisms being involved in rapid modulation of insect feeding behaviour. However, there is increasing evidence that toxic effects too can be capable of eliciting a rapid food rejection.

A striking demonstration of such rapid modulation was observed with larvae of the tobacco hornworm, *Manduca sexta*, which upon feeding on diets containing the alkaloid nicotine within 30 seconds abruptly stopped feeding.[86] In the same time period a significant proportion of the larvae exhibited twitching behaviour, characteristic of the toxic effect of nicotine on the CNS, although this did not result in mortality and apparently was of a transient nature since the larvae usually resumed feeding in a few seconds; it does show, however, that the ingested nicotine was absorbed within the same time as the rejection response. Although nicotine was found to elicit responses from two important chemosensilla, the recordings failed to plausibly link this sensory input to the rapid food rejection. Furthermore, when all mouthpart chemoreceptors were surgically removed from the larvae, the rejection response to nicotine remained unchanged, while a similar rejection response to caffeine (a known feeding deterrent to tobacco hornworm) was virtually eliminated. These experiments thus suggest that the rapid rejection response to nicotine is not mediated through pre-ingestive chemosensory perception, but instead through some post-ingestive feedback mechanism.

Glendinning and Slansky[87] have postulated two models of post-ingestive feeding regulatory mechanisms to account for these observations:

(i) In the 'passive response' model the insect is postulated to ingest a diet containing a toxicant until the onset of the toxic effect. Then, feeding is

halted until the toxic effect subsides and the insect resumes feeding on the diet. Thus, in this model the feeding behaviour is modulated in a dose-dependent manner via the pharmacological effect of the ingested toxicant.

(ii) Alternatively, the 'active response' model postulates the presence of *internal* chemoreceptors that detect the presence of secondary antifeedants after ingestion. Upon detection of a certain threshold concentration of the antifeedant, presumably lower than the concentration leading to intoxication, these receptors would be stimulated and thereby inhibit feeding until the concentration is reduced below the threshold level by metabolic and excretory activities and feeding can be safely resumed.

By preventing the insect from feeding on potentially lethal food sources, a post-ingestive feedback mechanism may allow an insect to become tolerant to specific toxicants upon prolonged exposure. When 6th instar larvae of the fall armyworm, *Spodoptera frugiperda*, were presented with a diet containing toxic but non-deterrent concentrations of indole-3-carbinol (I3C), the larvae were found to spend significantly less time feeding and to take more long (>60 min) non-feeding pauses between meals, relative to control caterpillars, leading to a drastic reduction in feeding during the 4-hour test-period (see Figure 1.6).[87] In contrast, the feeding behaviour of larvae that had been pre-exposed to I3C for two days did not differ significantly from control larvae. Since 2-day dietary exposure to I3C is known to induce increased detoxification enzyme activity in this species, including an increased rate of the oxidative metabolism of I3C, these experiments suggest that the putative mechanism of post-ingestive feedback might serve to protect the insect from consuming lethal amounts of I3C during the intermittent period that a metabolic defence against I3C-intoxication is acquired. A similar habituation of *Manduca sexta* to the secondary antifeedant nicotine is known to occur during 30-36 hours of continuous exposure.[86]

1.5.3 - Food Aversion Learning [88]

For some insect species, contact with food containing toxic compounds can influence the insects' feeding behaviour upon the next encounter with that food. After recovery of the symptoms of the toxic effect, the constitution of the unpalatable diet is apparently memorized and the insects subsequently avoid feeding on a similar food source. Such so-called 'food aversion learning' was for instance described[89a] for larvae of two polyphagous insect species (*Diacrisia virginica* and *Estigmene congrua*) that were fed *Petunia* ssp. leaves, known to be toxic to these insects. After recovery, it was found that the surviving larvae preferred significantly less to feed on *Petunia* leaves (compared to the

consumption of two other plant species) than did larvae that had not been previously exposed to *Petunia*.

More examples of food aversion learning have been reported, but such learning behaviour is not found with all insect species: when the experiments described above were repeated with tobacco hornworm (*Manduca sexta*) larvae on either *Petunia* ssp. or on plants treated with insecticide, no changes in the subsequent feeding behaviour were observed.[89b] Also, food aversion learning appears to occur more easily when the diet is less palatable, as was found for larvae of the American grasshopper (*Schistocerca americana*). After feeding on either *Spinacea oleracea* or *Brassica oleracea* plants, the larvae were injected in the abdomen with a 2% aqueous NHT solution. *Spinacea*-fed insects apparently associated the toxic effect of NHT with the nature of the plant material, because upon the next encounter with this plant the insects' meal size was found to be markedly reduced. With the more palatable *Brassica oleracea* plants, however, no food aversion learning took place after NHT injection.[90]

Apart from reacting upon adverse effects experienced during feeding, insects can also learn to avoid unpalatable food on the basis of sensory information only, by associating unpleasant taste stimuli at biting with other characteristics of the plant material. For instance, the food selection behaviour of *Locusta migratoria* on unpalatable food initially included palpation (scanning of the leaf surface with the sensory apparatus) and the taking of a test bite before the food was rejected. On subsequent occasions however, rejection was observed to follow immediately upon palpation, without biting.[91,92]

1.6 - Insect Antifeedants in Crop Protection

1.6.1 - Towards Practical Application of Antifeedants

Before practical application of a compound with insect antifeedant activity as a crop protection agent can be considered, several characteristics of the compound have to be examined. The most important characteristic is the action of the compound under realistic field conditions. As pointed out by Jermy,[30] the feeding bioassays used to initially identify compounds with antifeedant activity are not well suited to allow predictions of their practical applicability. The two-choice situation, often preferred in these assays because of its sensitivity, does not adequately represent the actual situation that occurs upon application of an antifeedant on a crop since especially crawling or mining insects are not able to cover large areas in search for food and find themselves in essentially a no-choice situation. Also, even when no-choice assays are employed, the assay duration is

often too short to reliably evaluate the possibility of habituation or to observe morphogenetic or toxic effects. Furthermore, because the feeding behaviour of insects can be expected to be determined by the total constitution of the plant material, rather than by the mere presence of a few stimulants and/or deterrents in the substrate, artificial diets cannot be reliably used in the evaluation of practical applicabiliy, nor can leaf discs because the physiology of such plant tissue may change during or after removal from the living plant and may also not adequately reflect possible changes in the plants' physiology caused by the presence of the antifeedant.

Therefore, the evaluation of antifeedants for potential practical use must start with long-term, no-choice experiments on whole plants. Because plants grown in a greenhouse can differ significantly in chemistry and tissue structure from field-grown plants, the source of the plants used in these experiments should be chosen in accordance with the anticipated application.[30] In the end, large scale field- or greenhouse-trials are still indispensible to evaluate the potential practical application of an antifeedant.

1 - Effective control of pest insects:
a. efficiently inhibits the feeding of the targeted pest insects and
b. preferably also displays long-term toxicity to the same insects.
c. active at very low concentrations.
d. no habituation or resistance should occur.
e. reasonably persistent when applied to a crop.

2 - No adverse effects on non-target organisms or crops
(*applies to both the antifeedant and it's metabolites*):
a. non-toxic to benificial insects, man , animals or other organisms.
b. not phytotoxic and no adverse effect on taste, smell or appearance of the product.

3 - Practical and economical in use:
a. compatible with other forms of (integrated) pest management.
b. preferably systemic action.
c. easily applicable.
d. constant supply and/or stable during storage.
e. low costs.
f. active against as many pest insects as possible.

Table 1.1: The characteristics of an ideal insect antifeedant (Adapted from ref. 21).

Apart from the antifeedant activity under field conditions, many other factors determine if a compound is suited for practical application. According to a compilation by van Beek and de Groot,[21] a practical antifeedant should meet as many as possible of the characteristics mentioned in Table 1.1. Most of these characteristics are self-evident, but a few merit some further discussion.

Long-term toxicity. As was mentioned before, upon prolonged contact with a feeding deterrent, especially in a no-choice situation, insects can become less sensitive to the feeding inhibitory activity of the antifeedant, a process called (short-term) habituation. As a result, the protecting effect of the compound decreases and in time feeding damage to the crop may rise to unacceptable levels. For a successful practical use, it is therefore desirable for an antifeedant to display also potent antibiotic properties against the target insects[21,30] (*e.g.* insecticidal or growth regulating activity): by ingesting small amounts of food the insects start accumulating the toxic compound which may eventually lead to increased mortality. Ideally, the antifeedant activity limits the amount of feeding damage to the crop during the period that is required for the slower antibiotic effects to become effective. Furthermore, a potent antibiotic activity would also introduce an insect population reducing component into the compound's spectrum of biological activity, which would reduce the risc that application of an antifeedant merely postpones the outbreak of an insect pest until the insects have become habituated or the compound has degraded. It is likely that the favourable experiences observed with azadirachtin in crop protection are at least partly due to the mixture of antifeeding and toxic effects it can display against insects.[30]

Resistance. In the previous sections, the possibility of short-term habituation (the development of tolerance for an antifeedant within one insect generation) has been noted with both primary and secondary antifeedants. Much less is known about the possibility of insect species aquiring heritable adaptation or resistance to the presence of antifeedants. Jermy has argued[30] that, since the host plant selection behaviour of oligophagous insects is mainly determined by the occurrence of feeding inhibitory compounds in non-host plants, the development of resistance to such substances would result in rapid changes in the host-plant range of these insects and because such changes are very rare events in nature, it appears unlikely that such adaptations could easily occur. Furthermore, Schoonhoven noted that, since many antifeedants may also have some toxic effect, such a behavioural adaptation must be accompanied by physiological adaptations, which further reduces the likelihood of such events.[32] Nevertheless, both authors recognize that changes in the food preference range of insects have sometimes been observed and the development of resistance to an antifeeding compound therefore cannot *a priori* be excluded completely.

Persistence. Because the successful application of antifeedants depends on an effective protection of the crop from feeding damage for an extended period of time, contrary to conventional insecticides that protect the crop by rapidly killing the pest insects, it is important that the antifeedant does not decompose too fast under field conditions. Unfortunately, the stability of especially the natural antifeedants tested so far appears to be rather limited, ranging from a few days (*e.g.* polygodial, ajugarin I[93]) to a few weeks (azadirachtin/Neem-extracts[94]). As a result, continued protection of the crop requires repeated application of these substances, which reduces their attractiveness. The stability of antifeedants can sometimes be improved through a proper formulation of the commercial product (for instance by including UV stabilizers) or by chemically modifying the antifeedant molecule.[94b] On the other hand, in view of the adverse environmental effects observed with the use of highly persistent insecticides in the past, neither the antifeedant nor its metabolites should be too stable either.

Systemic action. A compound that is taken up by a plant and is subsequently translocated to all plant parts is said to act systemically. Obviously, such a characteristic can be of great value for a practical antifeedant, since it would mean that newly formed plant parts, or surfaces not covered by the antifeedant, will also be protected. On the other hand, as with other types of insect control agents, accurately aimed and well-timed application techniques may (at least in principle) be able to counterbalance a lack of systemic activity. For applications in which the behaviour-modifying properties of an antifeedant are deliberately used to redirect the insects to specific plant parts, for instance because these parts are of less economical value or are treated with a conventional insecticide (see Section 1.6.2 for an example), systemic activity may even be a disadvantageous property.

1.6.2- Field Use of Antifeedants

At the moment, no antifeedant seems to meet all the conditions outlined in Table 1.1, although a few compounds (*e.g.* Neem-extracts/azadirachtin, pymetrozine) display enough of the desirable characteristics to have attracted commercial interest. As a result, the actual application of antifeedants in agriculture is still very limited. However, the principle of crop protection with antifeedants has been demonstrated to work under field conditions.

Fungicides based on Ph_3SnOH (fentin) or $Cu(OH)_2$ were shown to deter feeding and ovipositing behaviour of Colorado potato beetles (*Leptinotarsa decemlineata*) and to reduce the growth and survival of the larvae.[95a] In the field the copper-based fungicides reduced the larval densities on tomato plants by 44-100%,

relative to control plots, and thereby significantly increased the yield of tomatoes (control: ± 28 tons/ha; treated plots: ± 60 tons/ha). With potato and eggplants, only treated plants escaped total defoliation and survived long enough to be harvested.[95b] Similarly, fentin-treatment was found to suppress 2nd-generation larval densities well below levels necessary to cause yield reductions and thus allowed development of an effective pest management program without the need for any additional insecticide applications.[95c] Favourable results were also noted[11a-d] with fentin in field tests on sugar beet against larvae of the cotton leafworm (*Prodenia litura*). However, although these fungicides are approved agrochemicals, they are not totally devoid of adverse effects on non-target organisms and continuous use may lead to increased levels of copper or tin in the soil. Furthermore, trifen is known to be phytotoxic to some crops. It therefore remains doubtful whether widespread application of these fungicides for the purpose of insect control will be either attainable or desirable.

Many reports exist[15] of successful insect control in the field with Neem-based products or its major active constituent, azadirachtin, against various pests on economically important crops as cotton, corn and potato. Promising results have also been obtained in the protection of stored products, especially grains. Apart from its activity against insects, Neem-products were also found to display nematicidal, fungicidal and antiviral properties against some agricultural pest species. Although Neem-products are harmless to many benificial organisms, adverse effects are sometimes observed, for instance on some parasitoids of the sweetpotato whitefly (*Bemisia tabaci*), on a predator of the Colorado potato beetle or on bees.[15] However, in general Neem-products are more active against phytophagous pest insects than against benificial insects and it is believed that many of the problems mentioned can be overcome by properly timed and aimed application of the compound. Similarly, azadirachtin is not completely without effect on humans, but in view of the low amounts of the compound used in an agricultural application and its relatively rapid degradation under field conditions, it seems unlikely that these effects will be serious objections against the use of Neem-based products.

Apart from its potent antifeedant activity, azadirachtin also has various toxic and growth regulating effects on pest insects and it is likely that the excellent field results obtained with Neem-products can be partly ascribed to these properties.[30] However, good results have also been obtained with antifeeding natural products that seem to have no obvious toxic effects on insects. For instance, in laboratory assays the drimane dialdehyde polygodial (**33**) strongly deterred aphids (green peach aphid, *Myzus persicae*) from settling on Chinese cabbage leaves and was also found to reduce virus acquisition.[96] In subsequent field trials with winter barley, polygodial (at 25 g ha^{-1}) significantly reduced both the number of aphids (mainly bird-cherry aphid, *Rhopalosiphum padi*, and grain

aphid, *Sitobion avenae*) on the treated plots and the infestation of the plants with barley yellow dwarf virus (BYDV), a widespread and damaging pest on cereals, which is transmitted by these aphids.[97] As a result, the yield increased from 3.83 tons ha^{-1} (untreated plots) to 5.22 tons ha^{-1} (polygodial-treated plots), compared with 5.85 tons ha^{-1} for the standard cypermethrin treatment. Unfortunately, the polygodial treatment had to be repeated three times within one month to protect the plants (compared to only one treatment with cypermethrin) and the compound was therefore deemed insufficiently persistent for commercial use.

	leaf area (%) eaten on day					no. surviving the moult (%)
	1	2	3	4	total	
Laboratory assay						
Ajuga extract (at 5% wet leaf weight)	5	6	17	28	57	72
teflubenzuron	13	15	10	6	44	4
Ajuga extract + teflubenzuron	3	3	7	5	18	24
control	14	12	20	21	67	100
Field simulation test						
Ajuga extract (at 200% wet leaf weight)	6	7	5		18	23
teflubenzuron	19	12	5		36	0
Ajuga extract + teflubenzuron	3	4	3		10	0
control	21	34	31		86	100

Table 1.2: The effect of an antifeedant (extract of *Ajuga chamaepitys*) and an insect growth regulator (teflubenzuron), either singly or in combination, on the feeding and survival of larvae of the mustard beetle (*Phaedon cochleariae*) in leaf disc assays (laboratory assay) or on whole plants (field simulation test) of Chinese cabbage. For discussion see text. [Adapted from ref. 100.]

The limited stability of natural insect antifeedants under field conditions and the possibility of habituation by the pest insects are in general seriously hindering their acceptance as practical insect control agents. Another complicating factor is the lack of a population-reducing component in the biological activity of antifeedants lacking a toxic mode of action, due to which the outbreak of a pest may be only temporarily postponed until the antifeedant has degraded or the pest insects have habituated to it. Some authors[98] have expressed doubts whether insect control by semiochemicals[99] alone will be sufficiently robust to provide a sustainable crop protection. Therefore, several studies have evaluated the possibility of using antifeedants in conjunction with other insect control agents in an integrated strategy. For instance (Table 1.2), in laboratory assays extracts from *Ajuga* plants (*Ajuga chamaepitys* and *A. remota*), containing the natural

clerodane insect antifeedant ajugarin I (7) as the major active constituent, were shown to reduce the feeding of larvae of the mustard beetle (*Phaedon cochleariae*) on Chinese cabbage leaves; however the effect wore off within three days due to degradation and/or habituation, leading to severe leaf damage and a high number of surviving larvae after 4 days.[100] On the other hand, the chitin biosynthesis inhibitor teflubenzuron effectively controlled the insect pest by killing the larvae at the next moult, but during the preceding days the larvae still fed normally on the leaves, causing extensive damage. When the two treatments were combined, however, feeding remained low during the entire assay duration, probably due to the antifeeding effect of the *Ajuga* extract during the initial period being supported by the population-reducing action of teflubenzuron in the later stages of the experiment. As a result, the leaf damage was significantly lower for the combination strategy, compared to the treatments with either antifeedant of growth regulator alone. Similar results were obtained on isolated whole plants under field conditions (a field simulation experiment, see Table 1.2), although due to the higher concentration used in this experiment the effect of the antifeedant did not decrease during the three-day trial and also an increased mortality was observed, indicating that the *Ajuga* extract at higher concentrations may also display some toxic effect.[100]

In a related series of field experiments, Murray *et al* showed that the sequential treatment of potato plants with limonoid antifeedants, extracted from grapefruit seeds, followed by treatment with the *B. thuringiensis* endotoxin (a biological insecticide) led to significantly lower rates of colonization and ovipositing by adult Colorado potato beetles (*Leptinotarsa decemlineata*) than did either of the treatments alone.[101] The results of these and other experiments[102,103] thus seem to indicate that antifeedants can act as stress factors to increase the susceptibility of pest insects to insecticides and pathogens, even though their modes of action at first might seem to be contradictory (insecticides having to be ingested in order to act, whereas antifeedants act by reducing the amount of ingested food). However, there appears to be no general trend in the combined action of antifeedants and various insecticides, since antagonizing effects of antifeedants on insecticide potency have also been observed. Interestingly, Jermy[30] has pointed out that, since inhibition of feeding necessarily retards the development of an insect, antifeedants will also prolong their exposure to natural enemies and therefore might be ideal agents to combine with insect control schemes that mainly rely on biological control of insect pests.

A third approach to the application of antifeedants in insect control is to use the behaviour-modifying properties of these compounds in such a way as to drive the insects away from the plants (or plant parts) that are sensitive to insect attack towards less sensitive regions where they can be effectively controlled. A striking

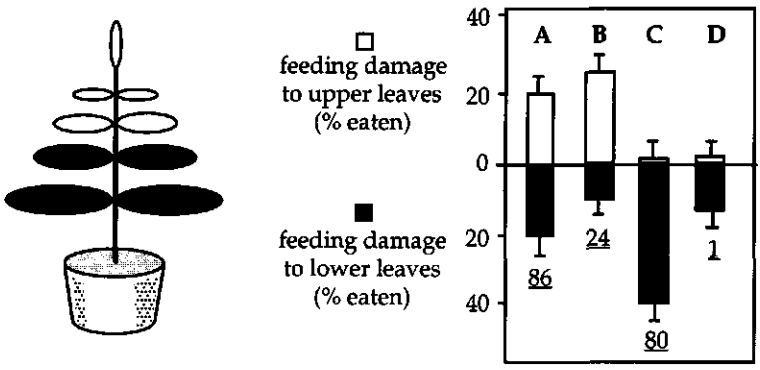


Figure 1.14: The effect of *Ajuga remota* extract applied to the upper leaves of whole mustard plants and/or teflubenzuron applied to the lower leaves on the feeding and survival of the mustard beetle (*Phaedon cochleariae*) in a field simulation experiment. The underlined numbers below the bars indicate the percentage of live adults recovered after 4 days. Treatment scheme: (**A**) control; (**B**) teflubenzuron applied to lower part; (**C**) *Ajuga* extract applied to upper part; (**D**) *Ajuga* extract and teflubenzuron applied to upper and lower parts, respectively. See text for discussion. [Adapted from ref. 100.]

demonstration of this approach was presented in a field simulation test with a combination of antifeeding *Ajuga remota* extracts and teflubenzuron to control mustard beetle larvae on mustard plants (Figure 1.14).[100] In this experiment the antifeedant and the growth-regulator were not applied together, but instead the upper leaves of the plant were treated with *Ajuga* extract, whereas teflubenzuron was applied on the lower leaves. As can be seen from the results, the sole application of teflubenzuron to the lower plant parts did not protect the upper parts from feeding damage, although clearly a reduction in the number of insects was observed. On the other hand, treatment of the upper plant parts with *Ajuga* extract alone induced a movement of the insects to the unprotected lower parts and thereby effectively prevented feeding damage to the upper leaves; however, the insect population was not significantly reduced by this treatment (compared to the control) and feeding damage to the upper leaves might still occur once the antifeedant becomes less effective through habituation and/or degradation. The most effective control was obtained when *Ajuga* extract and teflubenzuron were applied simultaneously to different plant parts, resulting in an almost complete protection of the upper leaves, combined with a high mortality of the larvae.

The strategy described above can also be applied on entire crops instead of on single plants; several examples regarding the field use of (different forms of) this principle have appeared in the literature under various names. For instance,

in the 'push-pull' strategy described by Pyke *et al*,[104] cotton pests of the genus *Heliothis* were concentrated in a small field area by the combined use of an attractive trap crop to lure the insects away and an antifeedant on the cotton plants to make this main crop unattractive to the insects. The insect population can subsequently be reduced through the application of an insecticide or a biological pathogen on the trap crop. In the similar 'stimulo-deterrent diversion strategy' (SDDS) proposed by Miller and Cowles,[105] onions are protected from the onion fly through the use of onion culls as an attractant in combination with a deterrent and a toxin protecting the main crop. A third example is the manipulation of the population density of the pea and bean weevil, *Sitona lineatus*, on a crop of field beans by the combined use of a Neem-extract as the deterrent component and the aggregation pheromone 4-methyl-3,5-heptanedione as the attractant, which together led to concentration of the pest insects in designated 'discard areas' and significantly reduced feeding damage to the crop.[106]

1.6.3 - Prospects of Antifeedants in Insect Control

It will be clear from the previous section that, in general, insect antifeedants have the potential to become a valuable addition to the existing arsenal of crop protection agents. Furthermore, considering the large number of natural compounds that were already found to have antifeedant activity and the huge reservoir of natural sources yet to be examined for antifeeding metabolites, it seems likely that many more interesting compounds will be discovered. Nevertheless, there still is a long way to go before the use of antifeedants in agriculture will be routine.

At present, a major limiting factor in antifeedants research and application is the limited availability, especially of natural insect antifeedants. Although many hundreds of natural compounds were shown to possess antifeedant activity in laboratory tests, only a handful of them have ever been evaluated for field use. In part this is due to the activity being deemed too low for practical application, but also the limited availability of many of these compounds from their sources has prevented a follow-up on the original observation. Even for compounds that have been used in field trials, the quantities available are usually not sufficiently abundant to allow widespread application at low cost. Also, the rather limited field stability of many of these compounds further increases the costs of their application.

Another difficulty in the development of natural products or extracts from natural sources for crop protection purposes is the registration process, which is mandatory for all compounds to be used in crop protection. In general, registration of a compound requires that extensive profiles on the toxicological effects of the compound on many organisms and on the environmental fate of

the compound are collected, both for the pure compound and for the mixture or formulation in which it is to be applied. If deemed safe, a registration can be granted, but only for a specific composition of the product and for a specific application. However, if the agent consists of a complex mixture, such as an antifeeding extract, all its constituents (or at least all the major ones) must be treated as separate compounds, which requires extensive research on many metabolites (a number of them most likely even being devoid of antifeedant activity), thereby making registration of an extract more expensive than of a single compound. Furthermore, the chemical content of a plant species often varies considerably with time, thereby creating new difficulties in maintaining constant extract composition. By intensifying the purification process of an extract, it is in principle possible to limit such problems, but this will also increase the cost of the product.

In view of these costs it seems likely that natural insect antifeedants will have difficulties to compete with the current pesticides and for the near future their use will probably be largely restricted to niche markets or as additives to conventional insect control agents. In the long term, however, when the current crop protection methods will be increasingly replaced by integrated pest management schemes with a heavy reliance on biological control methods, the need for selective and environmentally friendly pesticides will offer good opportunities for a more widespread application of antifeedants in insect control.

If the use of insect antifeedants is to become a reliable and accepted strategy in crop protection, then more known natural antifeedants must be tested in field trials against economically relevant insect species, in order to increase both the number of available practical antifeedants and the range of pest insects that can be effectively controlled through this strategy. Also, the problem of limited availability must be adressed, for instance via attempts to increase the production of antifeeding metabolites by their natural sources or through production of such compounds in tissue cultures. Furthermore, as was already suggested by Jermy,[30] by-products and waste materials from the food industry or from agriculture should be examined as a possible alternative source of natural antifeedants.

In my view, much more attention should be paid to the development of insect antifeedants of (semi)synthetic origin. Although most natural antifeedants are structurally far too complex to be synthesized on an economical basis, they can provide the inspiration for the synthesis of simplified analogues with structures based on those parts of the natural product that are known to be important for antifeedant activity. The viability of this approach has already been demonstrated in a number of cases where simplified analogues (such as **16** or **17**, see Chapter 3 for more examples) were shown to have retained (part of) their antifeedant activity in laboratory assays; however, to my knowledge none of these analogues has yet been evaluated in field tests. Other leads for the

development of such synthetic antifeedants might be derived from the observed antifeedancy of various commercial agrochemicals (Figure 1.2). Possibly, such compounds could eventually even be designed *de novo*, based on an understanding of the specific interactions of certain types of natural antifeedants with their receptors. Provided that the costs of such synthetic antifeedants are sufficiently low to compensate the potential partial loss of activity and/or specificity, relative to their natural templates, the application of synthetic antifeedants in insect control might be an attractive alternative to the use of insect antifeedants from natural sources.

1.7 - Notes and References

1. The term 'antifeedant' was first proposed by Ascher and Nissim to denote antifeeding compounds, *i.e.* 'compounds that prevent insects from feeding on plants without necessarily killing them'; K.S.R. Ascher and S. Nissim, *Proc. Mysore Symposium on Pesticides (Mysore, India, 21-23 December 1964)*, 221-227, 1965.
2. S. Adhikary, The Togo experience in moving from Neem research to its practical application for plant protection.; in: H. Schmutterer et al (Eds.), Natural pesticides from the Neem tree., Proceedings of the first international Neem conference, Rottach-Egern, 1980, p. 215-222.
3. R.J. Cremlyn, 'Agrochemicals. Preparation and mode of action.', Wiley, Chichester, 1991.
4. Antifeedant activity of (a) rotenone: G.A. Lane, D.R. Biggs, G.B. Russell, O.R.W. Sutherland, E.M. Williams, J.H. Maindonald and D.J. Donnell, *J. Chem. Ecol.*, **11**, 1713-1735 (1985); (b) pyrethrum: See ref. 40 and 41 in: L.M. Schoonhoven, *Stereoselective perception of antifeedants in insects.* In: E.J. Ariens, J.J.S. van Rensen and W. Welling (Eds.), *Stereoselectivity of pesticides - biological and chemical problems*, Chemicals in Agriculture Series, Vol. I, Elsevier, Amsterdam, 1988, p. 289-302.
5. National Research Council, *Neem: a tree for solving global problems.*, National Academy Press, Washington D.C., 1992.
6. M. Volkonsky, *Archs. Inst. Pasteur Algér.*, **15**, 427-432, 1937; as cited by L.M. Schoonhoven in ref. 32.
7. H.G. Guy, *J. Econ. Entomol.*, **29** (2), 467 (1936). See also references included in ref. 20.
8. The group of neuroactive insecticides or neurotoxins, sometimes also referred to as 'second generation' insecticides, consists of compounds that disrupt various vital biochemical signalling processes in the insects' nervous system. The discovery of these highly potent insecticides (chlorinated hydrocarbons (*e.g.* DDT, disc. 1939), organophospate and carbamate insecticides, synthetic pyrethrins) marks the beginning of the era of modern insect control. Most of the insecticides currently in use in agriculture still come from this group.

   The 'first generation' of insecticides consists of mainly botanical insecticides, such as nicotine, rotenone and natural pyrethrins, that were already in use prior to the 1940s. Insect growth regulators, such as juvenile hormone mimics or benzoylurea-based inhibitors of chitin synthesis, constitute the 'third generation' of insecticides. The latest development in the field of chemical insect control are the 'fourth generation insecticides', which modify the behaviour of insects by interacting with the insects'

sensory nervous system; sex pheromones and insect antifeedants are examples of such agents. See for instance: U. Eder and H.C. von Keyserlingk, *The challenge of finding new insecticides for a mature market.* In: H.C. von Keyserlingk *et al* (Eds.), *Approaches to new leads for insecticides.*, Springer-Verlag, Berlin, 1985, p. 1-8.

9. T. Jermy, *Entomol. Exp. Appl.*, **1**, 197-208 (1958).
10. W.H. Lange, *Chem. Week*, **89** (4), 46 (1961) and *Farm Chem.*, **125** (11), 22 (1962); D.P. Wright Jr., *Adv. Chem. Ser.*, **41**, 56 (1963).
11. (a) K.S.R. Ascher and S. Nissim, *Proc. Mysore Symposium on Pesticides (Mysore, India, 21-23 December 1964)*, 221-227, 1965; (b) K.R.S. Ascher and S. Nissim, *World Rev. Pest Control*, **3** (4), 188-211 (1964); (c) K.R.S. Ascher and G. Rones, *Int. Pest Control*, **6** (3), 6-8 (1964); (d) K.R.S. Ascher and S. Nissim, *Int. Pest Control*, **7** (4), (1965).
12. K.R.S. Ascher, *Phytoparasitica*, **7**, 117-137 (1979); A. Szentesi and T. Jermy, *Antifeedants of the Colorado potato beetle: an overview and outlook.* In: D.N. Ferro and R.H. Voss (Eds.), *Symposium on the Colorado potato beetle (Proceedings)*, Massachusetts Agricultural Experiment Station, Massachusetts University, Amherst, Res. Bull. 704, 1985, p. 17-27.
13. Based on Ascher and Nissim, Ref 11a. For more details, see ref. 10.
14. S. Pradhan and M.G. Jotwani, *Chem. Age India*, **19**, 756 (1968).
15. A.J. Mordue (Luntz) and A. Blackwell, *J. Insect Physiol.*, **39** (11), 903-924 (1993).
16. H. Schmutterer *et al* (Eds.), *Natural pesticides from the Neem tree (Azadirachta Indica A. Juss) and other tropical plants*, Proc. 1st Int. Neem Conf., GTZ, Eschborn, 1981; Proc. 2nd Int. Neem Conf., GTZ, Eschborn, 1984; Proc. 3rd Int. Neem Conf., GTZ, Eschborn, 1987.
17. J.H. Butterworth and E.D. Morgan, *J. Chem. Soc., Chem. Comm.*, 23-24 (1968).
18. (a) W. Kraus, M. Bokel, A. Klenk and H. Pöhnl, *Tetrahedron Lett.*, **26** (52), 6435-6438 (1985); (b) H.B. Broughton, S.V. Ley, A.M.Z. Slawin, D.J. Williams and E.D. Morgan, *J. Chem. Soc., Chem. Comm.*, 46-47 (1986); (c) C.J. Turner, M.S. Tempesta, R.B. Taylor, M.G. Zagorski, J.S. Termini, D.R. Schroeder and K. Nakanishi *Tetrahedron*, **43**, 2789-2803 (1987); (d) J.N. Bilton, H.B. Broughton, P.S. Jones, S.V. Ley, Z. Lidert, E.D. Morgan, H.S. Rzepa, R.N. Sheppard, A.M.Z. Slawin and D.J. Williams, *Tetrahedron*, **43**, 2805-2815 (1987); (e) W. Kraus, M. Bokel, A. Bruhn, R. Cramer, I. Klaiber, A. Klenk, G. Nagl, H. Pöhnl, H. Sadlo and B. Vogler, *Tetrahedron*, **43**, 2817-2830 (1987).
19. Database on pesticidal chemical ingredients and products, Department of Pesticide Regulation, California Environmental Protection Agency, http://www.cpdr.ca.gov; USEPA/OPP's Pesticide Products Database, http://www.cdpr.ca.gov/docs/epa/epamenu.htm.
20. R.F. Chapman, *Bull. Ent. Res.*, **64**, 339-363 (1974).
21. T.A. van Beek and Ae. de Groot, *Recl. Trav. Chim. Pays-Bas*, **105**, 513-527 (1986).
22. W.M. Daniewski, M. Gumulka, K. Ptaszynska, P. Skibicki, E. Bloszyk, B. Droszd, S. Stromberg, T. Norin and M. Holub, *Eur. J. Entomol.*, **90**, 65-70 (1993).
23. M.D. Bentley, M.S. Rajab, A.R. Alford, M.J. Mendel and A. Hassanali, *Entomol. Exp. Appl.*, **49**, 189-193 (1988).
24. For a compilation of almost 1500 plant species from 175 families, screened for the presence of feeding deterrents, see: M. Jacobsen, *Glossary of plant-derived insect deterrents*, CRC Press, Boca Raton, 1990.

25. Ae. de Groot and T.A. van Beek, *Recl. Trav. Chim. Pays-Bas*, **106**, 1-18 (1987).
26. S.V. Ley, D. Santafianos, W.M. Blaney and M.S.J. Simmonds, *Tetrahedron Lett.*, **28** (2), 221-224 (1987); W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson, S.C. Smith and A. Wood, *Pestic. Sci.*, **40**, 169-173 (1994).
27. M.D. Bentley, M.S. Rajab, M.J. Mendel and A.R. Alford, *J. Agric. Food Chem.*, **38** (6), 1400-1403 (1990).
28. J.L. Frazier and P.Y-S. Lam, *Three-dimensional mapping of insect taste receptor sites as an aid to novel antifeedant development*. In: L. Crombie (Ed.), *Recent advances in the chemistry of insect control II*, Royal Chemical Society, Cambridge, 1990. See also Section 1.4.3.
29. H. Kristinsson, *Pymetrozine - A new insecticide*. In: G.G. Briggs (Ed.), *Advances in the chemistry of insect control III*, Royal Chemical Society, Cambridge, 1994, p.85-102.
30. T. Jermy, *J. Chem. Ecol.*, **16** (11), 3151-3166 (1990).
31. K. Munakata, *Pure Appl. Chem.*, **42**, 57-66 (1975).
32. L.M. Schoonhoven, *Ent. Exp. Appl.*, **31**, 57-69 (1982).
33. V.G. Dethier, L.B. Browne and C.N. Smith, *J. Econ. Entomol.*, **53** (1), 134-136 (1960).
34. I. Kubo and K. Nakanishi, *Insect antifeedants and repellents from african plants*. In: P.A. Hedin (Ed.), *Host plant resistance to pests*, ACS Symp. Series, Vol. 62, American Chemical Society, Washington, 1977, p. 165-178.
35. D.M. Norris, *Anti-feeding compounds*. In: *Chemistry of plant protection*, Vol. 1, Springer Verlag, Berlin, 1986, p. 97-146.
36. Escoubas *et al* have developed an ingenious procedure to rapidly examine plant extracts for the presence of antifeeding components by combining a separation of the sample via thin layer chromatography with the biological assessment of the components through a feeding bioassay conducted on the same (agar-coated) silica gel TLC plate. See: P. Escoubas, L. Lajide and J. Mizutani, *Insecticidal and antifeedant activities of plant compounds*. In: P.A. Hedin, J.J. Menn and R.M. Hollingworth (Eds.), *Natural and engineered pest management agents*, ACS Symposium Series, ACS, Washington, 1994.
37. For a discussion of such problems, see Chapter 2.
38. S. Hosozawa, N. Kato and K. Munakata, *Agr. Biol. Chem.*, **38** (4), 823-826 (1974). In this report a number of plant compounds are described that initially showed high feeding inhibitory activity against 3rd instar larvae of *Sp. litura* F. in a two-choice situation. However, after about 2 hrs, when >90% of the control discs had been eaten, in some cases (with 'relative antifeedants') the larvae started consuming the treated discs while with other compounds (the 'absolute antifeedants') the larvae rather starved to death than to start eating the treated discs. In my view, the terminology regarding relative and absolute antifeedants thus applies to the no-choice situation, although at first sight the cited article seems to imply otherwise.
39. F. Ortega, B. Rodríguez and P. Castañera, *J. Chem. Ecol.*, **21** (9), 1375-1386 (1995).
40. See also: A.R. Alford, J.A. Cullen, R.H. Storch and M.D. Bentley, *J. Econ. Entomol.*, **80**, 575-578 (1987); M.J. Mendel, A.R. Alford and M.D. Bentley, *Entomol. Exp. Appl.*, **58**, 191-194 (1991).
41. The substrate must contain a rapidly acting toxin in a sufficiently high concentration, since otherwise the insect would be able to consume a large amount of the treated

substrate before experiencing any adverse effects and the two-choice antifeedant activity would consequently be low. For example: in two-choice assays with Colorado potato beetle larvae, the toxin teucrin-A (**20**) did not display antifeedant activity at lower concentrations (300 ppm, see Figure 1.5), while at high concentrations a significant activity was found (Antifeedant/suppression indices for (**20**) at 1000 ppm: two-choice AI=34.0±7.6%; no-choice AI=75.0±5.9%; SI=76.3±1.3%). [Data from ref. 39.]

42. K.F. Raffa and J.L. Frazier, *Entomol. Exp. Appl.*, **46**, 93-100 (1988).
43. W.F. Tjallingii, *Electrical recording of stylet penetration activities*. In: A.K. Minks and P. Harrewijn (Eds.), *Aphids, their biology, natural enemies and control.*, Vol. 2B, Elsevier, Amsterdam, 1988, p. 95-108.
44. F. Slansky and J.M. Scriber, *Food consumption and utilization*. In: G.A. Kerkut and C.I. Gilbert, *Comprehensive insect physiology, biochemistry and pharmacology.*, Vol. 4, Pergamon Press, Oxford, 1985, p. 87-163.
45. (a) L.M. Schoonhoven, W.M. Blaney and M.S.J. Simmonds, *Sensory coding of feeding in phytophagous insects*. In: E.A. Bernays (Ed.), *Insect-plant interactions*, Vol IV, CRC-Press, Boca Raton, 1992, p. 59-79; (b) L. Messchendorp, *Terpenoid antifeedants against insects: a behavioural and sensory study.*, Ph.D. Thesis, Wageningen Agricultural University, Wageningen, 1998, p. 4-9.
46. J.J.A. van Loon, *Entomol. Exp. Appl.*, **80** (1), 7-13 (1996).
47. C.A. Mullin, S. Chyb, H. Eichenseer, B. Hollister and J.L. Frazier, *J. Insect Physiol.*, **40** (11), 913-931 (1994).
48. L.M. Schoonhoven, *Entomol. Exp. Appl.*, **31**, 57-69 (1982).
49. I. Shimada, *J. Gen. Physiol.*, **71**, 19-36 (1978).
50. (a) W.-C. Ma, *Entomol. Exp. Appl.*, **12**, 584 (1969); (b) J.J.A. van Loon, *J. Comp. Physiol.*, A**166** (6), 889-900 (1990).
51. L.M. Schoonhoven, *What makes a caterpillar eat? The sensory code underlying feeding behavior*. In: R.F. Chapman *et al* (Eds.), *Perspectives in chemoreception and behavior*, Springer-Verlag, New York, 1987, p. 69.
52. R.F. Chapman, *Adv. Insect Physiol.*, **34**, 167 (1988).
53. L.-E. Luo, J.J.A. van Loon and L.M. Schoonhoven, *Physiol. Entomol.*, **20**, 134-140 (1995).
54. L. Messchendorp, J.J.A. van Loon and G.J.Z. Gols, *Entomol. Exp. Appl.*, **79**, 195-202 (1996).
55. M.S.J. Simmonds, W.M. Blaney, S.V. Ley, J.C. Anderson, R. Banteli, A.A. Denholm, P. Green, R.B. Grossman, C. Gutteridge, L. Jennens, S.C. Smith, P.L. Toogood and A. Wood, *Entomol. Exp. Appl.*, **77**, 69-80 (1995).
56. H. Wieczorek, I. Shimada and C. Hopperdietzel, *J. Comp. Physiol.*, A163, 413-419 (1988).
57. (a) W.-C. Ma, *Physiol. Entomol.*, **2**, 199-207 (1977); (b) J.L. Frazier, *The perception of plant allelochemicals that inhibit feeding*. In: L.B. Brattsten and S. Ahmad (Eds.), *Molecular aspects of insect-plant associations*, Plenum, New York, 1986, p. 1; (c) E.A. Bernays and R.F. Chapman, *Chemical deterrence of plants*. In: J.H. Law (Ed.), *Molecular entomology*, Alan R. Liss, New York, 1987, p. 107; V.G. Dethier, *Chem. Senses*, **12**, 251-263 (1987); (d) W.M. Blaney, M.S.J. Simmonds, S.V. Ley and P.S. Jones, *Entomol. Exp. Appl.*, **46**, 267-274 (1988).

58. K. Hansen, *Alpha-glucosidases as sugar receptor proteins in flies.* In: L. Jaenicke (Ed.), *25th Mosbacher Colloquim Ges. Biol. Chem.*, Springer Verlag, Heidelberg, 1974, p. 207-232; T. Amakawa, H. Kijima and H. Morita, *J. Insect Physiol.*, **21**, 1419-1425 (1975); J. Kijima, T. Amakawa, M. Nakashima and H. Morita, *J. Insect Physiol.*, **23**, 469-479 (1977); K. Hansen and H. Wieczorek, *Biochemical aspects of sugar reception in insects.* In: R.H. Cagan and M.R. Kare (Eds.), *Biochemistry of taste and olfaction*, Academic Press, New York, 1981, p. 139-162.

59. Alternatively, some α-glucosidases may instead be involved in the rapid elimination of sugars at the receptor site. See: M. Nakishima, K.-I. Enomoto, H. Kijima and H. Morita, *Insect Biochem.*, **12** (5), 579-585 (1982).

60. (a) H. Kijiama *et al*, *J. Gen Physiol.*, **91**, 29-47 (1988); (b) T Amakawa, M. Ozaki and K. Kawata, *J. Insect Physiol.*, **36** (4), 281-286 (1990).

61. M.S.J. Simmonds, W.M. Blaney and L.E. Fellows, *J. Chem. Ecol.*, **16** (11), 3167-3196 (1990).

62. C.A. Mullin, S. Chyb, H. Eichenseer, B. Hollister and J.L. Frazier, *J. Insect Physiol.*, **40** (11), 913-931 (1994).

63. B.J.M. Jansen and Ae. de Groot, *Nat. Prod. Reports*, 309-318 (1991).

64. L.M. Schoonhoven and Yan Fu-Shun, *J. Insect Physiol.*, **35**, 725-728 (1989).

65. I. Kubo and I. Ganjian, *Experientia*, **37**, 1063-1064 (1981).

66. (a) V. Caprioli, G. Cimino, R. Colle, M. Gavagnin, G. Sodano and A. Spinella, *J. Nat. Prod.*, **50**, 146-151 (1987); (b) M. D'Ischia, G. Prota and G. Sodano, *Tetrahedron Lett.*, **23** (32), 3295-3298 (1982); (c) G. Cimino, G. Sodano and A. Spinella, *Tetrahedron Lett.*, **43**, 5401-5410 (1987).

67. P.Y.S. Lam and J.L. Frazier, *Tetrahedron Lett.*, **28**, 5477-5480 (1987).

68. (a) M. Jonassohn, *Sesquiterpenoid unsaturated dialdehydes*, Ph.D. Thesis, Lund University, Sweden, 1996; (b) M. Jonassohn, O. Sterner and H. Anke, *Tetrahedron*, **52** (4), 1473-1478 (1996); (c) M. Jonassohn, R. Davidson, P. Kahnberg and O. Sterner, *Tetrahedron*, **53** (1), 237-244 (1997); (d) F. Bocchio, S. Kals-Hansen, K. Dekermenjian, O. Sterner and R. Witt, *Tetrahedron Lett.*, **33**(45), 6867-6870 (1992).

69. Unfortunately, to my knowledge (**35**) has not yet been tested for this property against the same insect species as the drimane antifeedants.

70. In analogy with the sugar- and inositol-sensitive neurons involved in the perception of warburganal, vanilloid receptors too contain thiol groups that are essential for binding activity. Also, sulfhydryl-reactive agents as N-ethyl maleimide are known to activate neurons sensitive to capsaicin, the hot-tasting constituent from hot peppers, which also interacts with the vanilloid receptor. [See ref. 68a, p. 61 and references cited therein.]

71. W.M. Blaney, M.S.J. Simmonds, S.V. Ley and R.B. Katz, *Physiol. Entomol.*, **12**, 281-291 (1987).

72. W.M. Daniewski, M. Gumulka, D. Przesmycka, K. Ptaszynska, E. Bloszyk and B. Drozdz, *Phytochemistry*, **38** (5), 1161-1168 (1995).

73. Other bioactivities of these compounds, such as antibiotic, antifungal and cytotoxic properties which may result from disruption of the cell membrane by reaction with membrane-associated proteins, probably depend more directly on the general reactivity of the dialdehyde functionality towards bionucleophiles. [ref. 68a.]

74. A.J. Mordue (Luntz) *et al, Physiol. Entomol.*, **10**, 431-437 (1985).

75. D.C. Deecher, J. Brezner and S.W. Tannenbaum, *J. Econ. Entomol.*, **83** (3), 710-714 (1990).

76. (a) L. Bernard and L. Lagadic, *Pesticide Biochem. and Physiol.*, **46**, 171-180 (1993); (b) L. Lagadic and L. Bernard, *Pesticide Science*, **38**, 323-328 (1993).

77. R. Nauen, *Pestic. Science*, **44**, 145-153 (1995); R. Nauen, H. Hungenberg, B. Tollo, K. Tietjen and A. Elbert, *Pestic. Sci.*, **53** (2), 133-140 (1998).

78. For antifeedant activity of imidacloprid against other insect species, see for instance: T.W. Drinkwater, *Crop Protection*, **13** (5), 341-345 (1994); T.W. Drinkwater and L.H. Groenewald, *Crop Protection*, **13** (6), 421-423 (1994). However, see also ref. 76b.

79. T. Shimizu, K. Matsuzawa and J.-I. Fukami, *Appl. Ent. Zool.*, **16**, 167-169 (1981).

80. (a) H. Kayser, L. Kaufmann, F. Schürmann and P. Harrewijn, *Brighton Crop Protection Conference - Pests and Diseases*, 737-742 (1994); (b) C.R. Flückiger, H. Kristinsson, R. Senn, A. Rindlisbacher, H. Buholzer and G. Voss, *Brighton Crop Protection Conference - Pests and Diseases*, **1**, 43-50 (1992); (c) H. Kristinsson, *Spec. Publ. - Royal Society of Chemistry*, **147**, 85-102 (1994); (d) D. Fuog, S.J. Fergusson and C. Flückiger, *Pymetrozine: a novel insecticide affecting aphids and whiteflies*. In: I. Ishaaya and D. Degheele (Eds.), *Insecticides with novel modes of action - Mechanisms and application*, Applied Agriculture Series, Springer-Verlag, berlin, 1998, p. 40-49..

81. Aphids are too small for this type of electrophysiological experiments.

82. Nor were chitin biosynthesis or mitochondrial respiration.

83. A.F. Tuttle and D.N. Ferro, *J. Econ. Entomol.*, **81** (2), 654-657 (1988).

84. N. Rajendran, E. Sivamani and K. Jayaraman *FEMS Microbiol. Lett.*, **122**, 103-108 (1994); N. Rajendran *et al, Synergistic action of plant defense chemicals and the endotoxin BT in relation to Hel. armigera and Spod. Litura*. In: T.N. Ananthakrishnan and A. Raman (Eds.), *Chemical ecology of the phytophagous insects.*, Oxford IBH Publ., New Delhi, 1993, p. 301-312.

85. J.L. Glendinning and F. Slansky Jr., *Physiol. Entomol.*, **19**, 173-186 (1994); J.P. Shapiro, *Arch. Biochem. Physiol.*, **11**, 65-78 (1989).

86. J.I. Glendinning, *J. Exp. Biol.*, **199**, 1523-1534 (1996).

87. J.I. Glendinning and F. Slansky Jr., *J. Comp. Physiol. A*, **176**, 337-345 (1995).

88. A. Szentesi and T. Jermy, *The role of experience in host plant choice by phytophagous insects*. In: E.A. Bernays (Ed.), *Insect-plant interactions*, Vol. II, CRC Press, Boca Raton, 1990, p. 39-74.

89. (a) V.G. Dethier, *Entomol.*, **5**, 321 (1980); (b) V.G. Dethier and M.T. Yost, *Physiol. Entomol.*, **4**, 125-130 (1979).

90. E.A. Bernays and J.C. Lee, *Entomol.*, **13**, 131 (1989).

91. W.M. Blaney and C. Winstanley, *Food selection behaviour in Locusta migratoria*. In: J.H. Visser and A.K. Minks (Eds.), *Proceedings of the 5th international symposium on insect-plant relationships (Wageningen, The Netherlands)*, PUDOC, Wageningen, 1982, p. 365. See also: W.M. Blaney, C. Winstanley and M.S.J. Simmonds, *Entomol. Exp. Appl.*, **38**, 35-40 (1985).

92. Similar behaviour has also been observed for larvae of the African armyworm (*Spodoptera exampta*). See: W.M. Blaney and M.S.J. Simmonds, *Experience: a modifier of neural and behavioral sensitivity*. In: V. Labeyrie, G. Fabres and D. Lachaise (Eds.), *Proceedings of the 6th symposium on insect-plant relationships (Pau, France)*, W. Junk Publishers, Dordrecht, 1987, p. 237.
93. Under (simulated) field conditions, the antifeedant activity of polygodial and ajugarin I was found to markedly decrease after a few days [ref. 97 and 100, respectively]. Apart from decomposition of the compounds, other processes (*e.g.* short-term habituation) may have been involved in reducing the effectiveness of these antifeedant treatments.
94. For photostability experiments with azadirachtin and related derivatives, see for instance: (a) J.B. Stokes and R.E. Redfern, *J. Environ. Sci. Health*, **A17** (1), 57-65 (1982); (b) M.A. Barnby, R.B. Yamasaki and J.A. Klocke, *J. Econ. Entomol.*, **82** (1), 58-63 (1989).
95. (a) J.D. Hare, P.A. Logan and R.J. Wright, *Environ. Entomol.*, **12**, 1470-1477 (1983); (b) J.D. Hare, *Environ. Entomol.*, **13**, 1010-1014 (1984); (c) J.D. Hare and R.E.B. More, *J. Econ. Entomol.*, **81**, 914-921 (1988).
96. J.A. Pickett, G.W. Dawson, D.C. Griffiths, A. Hassanali, L.A. Merritt, A. Mudd, M.C. Smith, L.J. Wadhams, C.M. Woodcock and Z.-N. Zhang, *Development of plant-derived antifeedants for crop protection*. In: R. Greenhalgh and T.R. Roberts (Eds.), *Pesticide science and biotechnology*, 1987, p. 125-128; R.W. Gibson, A.D. Rice, J.A. Pickett, M.C. Smith and R.M. Sawicki, *Ann. Appl. Biol.*, **100**, 55-59 (1982).
97. G.W. Dawson, D.C. Griffiths, A. Hassanali, J.A. Pickett, R.T. Plumb, B.J. Pye, L.E. Smart and C.M. Woodcock, *British Crop Protection Conference - Pests and Diseases*, 1986, p. 1001-1008; Y. Asakawa, G.W. Dawson, D.C. Griffiths, J.-Y. Lallemand, S.V. Ley, K. Mori, A. Mudd, M. Pezechk-Leclaire, J.A. Pickett, H. Watanabe, C.M. Woodcock and Z.-N. Zhang, *J. Chem. Ecol.*, **10**, 1845-1855 (1988); D.C. Griffiths, J.A. Pickett, L.E. Smart and C.M. Woodcock, *Pestic. Sci.*, **27**, 269-276 (1989).
98. See for instance ref. 106 and: J.A. Pickett, *Bull. Entomol. Res.*, **81**, 229-232 (1991).
99. Semiochemicals are compounds that modify the behaviour of insects by interacting with the insects' sensory nervous system.
100. D.C. Griffiths, S.P. Maniar, L.A. Merritt, A. Mudd, J.A. Pickett, B.J. Pye, L.E. Smart and L.J. Wadhams, *Crop Protection*, **10**, 145-151 (1991).
101. K.D. Murray, A.R. Alford, E. Groden, F.A. Drummond, R.H. Storch, M.D. Bentley and P.M. Sugathapala, *J. Econ. Entomol.*, **86** (6), 1793-1801 (1993).
102. H.S. Salama and A. Sharaby, *Berliner Insect Sci. Appl.*, **9**, 573-577 (1988).
103. B.S. Parmar and S. Dutta, *Neem Newsl.*, **3**, 3-5 (1986).
104. B. Pyke, M. Rice, B. Sabine and M. Zalucki, *Aust. Cotton Grower*, May-July 1987, 7-9 (1987).
105. J.R. Miller and R.S. Cowles, *J. Chem. Ecol.*, **16**, 3197-3212 (1990).
106. L.E. Smart, M.M. Blight, J.A. Pickett and B.J. Pye, *Crop Protection*, **13** (2), 127-135 (1994).

Chapter 2

Clerodane Diterpenes.

Introduction and Biological Activity, especially Insect Antifeedant Activity.

Abstract: During the last thirty years well over 750 secondary metabolites belonging to the class of clerodane diterpenes have been isolated from several plant species. Apart from the other types of biological activity displayed by some of its members, this class has particularly attracted attention for the more than 200 clerodane natural products and related (semi)synthetic derivatives showing various degrees of insect antifeedant activity. In this chapter, the structure, nomenclature and occurence of the clerodane diterpenoids are introduced. Furthermore a comprehensive and systematic compilation of all neo-clerodanes, structurally related natural products and (semi)synthetic derivatives which were reported tested for insect antifeedant activity to date is presented.

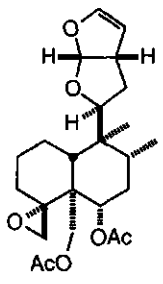
Table of Contents

This chapter will be published in a revised form: E.A. klein Gebbinck, B.J.M. Jansen and Ae. de Groot, *Insect antifeedant activities of clerodane diterpenes and related model compounds,* in preparation.

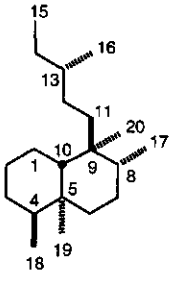
2.1 - Structure and Nomenclature

Terpenes are a major class of secondary plant metabolites, consisting of compounds with a carbon skeleton that is formally constructed by head-to-tail polymerisation of isoprene (isopentadiene) according to the 'isoprene rule',[1] sometimes followed by rearrangements to yield the final skeleton. Due to their build-up from five-carbon units, the class of terpenes can be further subdivided into hemiterpenes (terpenes containing a skeleton of only 5 carbon atoms, C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), etc.

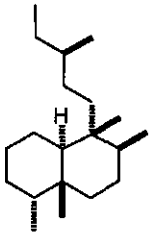
Within these classes, subgroups of terpenes can be distinguished that share a common carbon skeleton. One of these groups is centered on the natural insect antifeedant clerodin (**1**), a bitter principle isolated from the Indian bhat tree, *Clerodendron infortunatum*. Its name, carbon skeleton and absolute stereochemistry form the basis for the classification of a large group of related diterpenes that, according to the new terminology proposed by Rogers *et al*,[2] are now to be denoted as *neo*-clerodanes. The prefix '*neo*' originates from a reversal of the initially assigned clerodane stereochemistry and indicates an absolute configuration (**2a**) similar to clerodin at all asymmetric centers of the carbon skeleton; clerodane diterpenes with the opposite absolute stereochemistry (**2b**) are to be called *ent-neo*-clerodanes.



1: clerodin



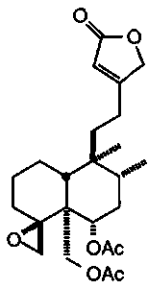
2a: *neo*-clerodane skeleton (altern.: *ent*-clerodane)



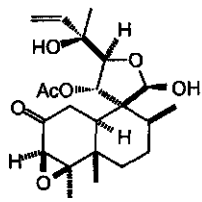
2b: *ent-neo*-clerodane (altern.: clerodane)

Piozzi[3] has advocated the use of an alternative nomenclature, in which clerodin and similar clerodane-type diterpenes (**2a**) are to be classified as *ent*-clerodanes, in order to stress the biogenetic relationship between such clerodanes and the *ent*-labdanes (see Section 2.2). A clear disadvantage of this system is that an inadvertant omission of the *ent*-prefix might easily go unnoticed, while in the Rogers-nomenclature the term 'clerodane' is (formally) meaningless. Nevertheless, both systems can be frequently encountered in the literature, although in more recent publications the nomenclature according to Rogers *et al* is usually preferred.

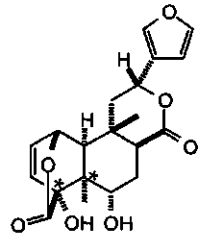
In correspondance with the IUPAC nomenclature for naming natural products,[4] the semi-systematic name of any clerodane-type diterpene is derived from the clerodane skeleton as the parent structure, with the prefix '*neo*' or '*ent-neo*' depending on the configuration of the majority of chiral centers of the carbon skeleton. Additional prefixes (α/β or R/S) are used to indicate a stereochemistry at any of these centers that deviates from the absolute configuration of the parent structure, and also to define the stereochemistry of non-hydrocarbon substituents attached to the clerodane skeleton. Some examples of this nomenclature applied to structurally diverse clerodane-type diterpenes are shown in Figure 2.1; it will be clear that, for practicality, usually the trivial names of these natural products are preferred.



3: ajugarin I; [6α,19-diacetoxy-4,18-epoxy-*neo*-clerod-13-en-15,16-olide]



4: heteroscyphone A; [(3β,11α,12αH,13R,20βOH)-11-acetoxy-3,4:12,20-diepoxy-2-oxo-*ent-neo*-clerod-14(15)-en-13,20-diol]



5: 2-dehydroarcangelisinol; [(1β,4αOH,5α,6α,12βH)-15,16-epoxy-4,6-dihydroxy-*ent-neo*-cleroda-2,13(16),14-triene-17,12:18,1-diolide]

Figure 2.1: Trivial and semi-systematic names of some clerodane diterpenes (see text for discussion). Configurationally inverted centers are indicated with an asterisk.

In the present thesis, the nomenclature according to Rogers *et al* will be used when the absolute configuration of a specific clerodane diterpene or group of clerodanes is to be indicated or implied. Terms as 'clerodane(-type) diterpene' or 'clerodanes' are considered to apply to any diterpene with the common carbon skeleton (either **2a** or **2b**) and will thus only be used when the absolute stereochemistry is unknown or irrelevant for the discussion.

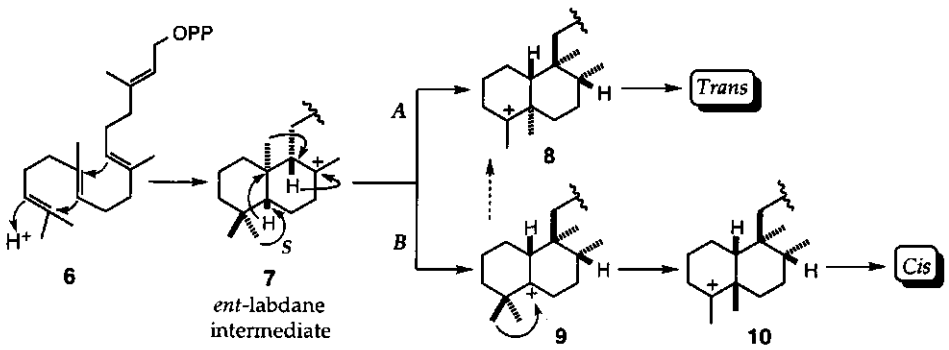
2.2 - Occurrence and Biosynthesis

Although the first reports of the isolation of clerodane diterpenes are dated as far back as the mid-19th century,[5] it was not until after the structure of clerodin (**1**) (isolation 1937, structure determination 1961, structure revision 1979) had been fully elucidated that such terpenes were identified as clerodanes. Since that time, the occurence of clerodane-type secondary metabolites was found to be a

widespread phenomenon among several hundreds of plant species from various families. Other sources of clerodanes include organisms from different taxonomic groups, such as fungi, bacteria and marine sponges. In a review by Merrit and Ley (1992),[6] well over 750 clerodane diterpenes were listed, grouped according to their taxonomic origin, and since then many more new clerodanes and sources have been reported.[7]

Particular types of clerodanes occur rather selectively within certain genera and species, suggesting that the distribution of these diterpenes in plants is under fairly rigid genetic control. For example, the occurrence of clerodane diterpenes with a furo[2,3b]furan-based sidechain at C-9, as in clerodin (**1**), appears to be limited to only five genera from the plant families Labiatae and Verbenaceae.[6] Due to such trends, the elucidation of the chemical terpene content can be of value for taxonomic purposes in order to establish relationships between different plant species or to distinguish between hybrids of differrent (sub)species that are morphologically indistinguishable.

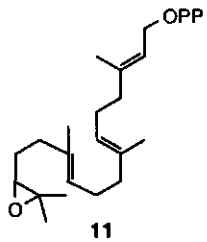
Biogenetically, the construction of the *neo*-clerodane carbon skeleton is believed to be an extension of the biosynthetic pathway towards labdane-type diterpenes, which in turn are formed by proton initiated cyclization of geranylgeranyl pyrophosphate (GGPP, **6**) through various parallel pathways. In order to yield trans-fused *neo*-clerodanes, the *ent*-labdane intermediate (**7**) can undergo a series of successive methyl and hydride shifts (Scheme 2.1, pathway *A*), which may possibly occur in a concerted fashion since all migrating groups are positioned anti with respect to the corresponding leaving groups. Cis-fused *neo*-clerodanes cannot be obtained in this manner, since the 5α,10β ring-fusion of the



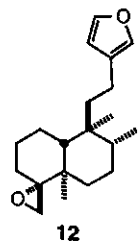
Scheme 2.1: Proposed biosynthetic pathway towards the trans- or cis-fused *neo*-clerodane carbon skeleton. The concerted pathway *A* includes a shift of the C-4α methyl group to C-5 (marked '*S*'); in pathway **B** this shift is not included in the initial sequence of steps towards (**9**), but may occur during the second phase, yielding a trans-fused skeleton (dotted arrow).

labdane precursor does not allow a concerted pathway towards a cis A/B ring junction. Therefore, a stepwise mechanism has been proposed (pathway *B*) in which the series of hydride and methyl shifts briefly pauses at the half-rearranged intermediate (**9**). Depending on which of the C-4 methyl groups subsequently migrates to C-5, either the trans-fused (Scheme 2.1, dotted arrow) or the cis-fused *neo*-clerodane skeleton can be obtained.

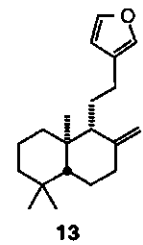
The exact nature of the oxidative steps involved in the construction of the various substitution patterns is still unknown. To my knowledge, no biosynthetic studies towards functionalization of clerodane diterpenes have been reported. In principle, the intermediates (**8**) and (**10**) may lose the positive charge through capture of an oxygen-containing nucleophile (*e.g.* water) or via expulsion of a proton under formation of a double bond (either endo- or exocyclic), which in turn creates opportunities for common enzyme systems to introduce oxidized functionalities at C-2, C-3, C-4 or C-18.[8] Alternatively, higher oxidized *neo*-clerodanes might originate from some oxidized form of GGPP; the epoxide (**11**), for example, has been postulated as a precursor in the biosynthesis of 3-keto-*neo*-clerodanes.[9] Similarly, it is not clear at which moment in the biosynthetic pathway the substitution pattern of the C-9 sidechain is formed. Functionalization could take place after formation of the clerodane ring system, for instance initiated by heterolytic cleavage of the C-OPP bond, but might also occur at an earlier stage, as was suggested for the biosynthesis of (**12**) from its proposed *ent*-furyllabdane precursor (**13**).[10]



11

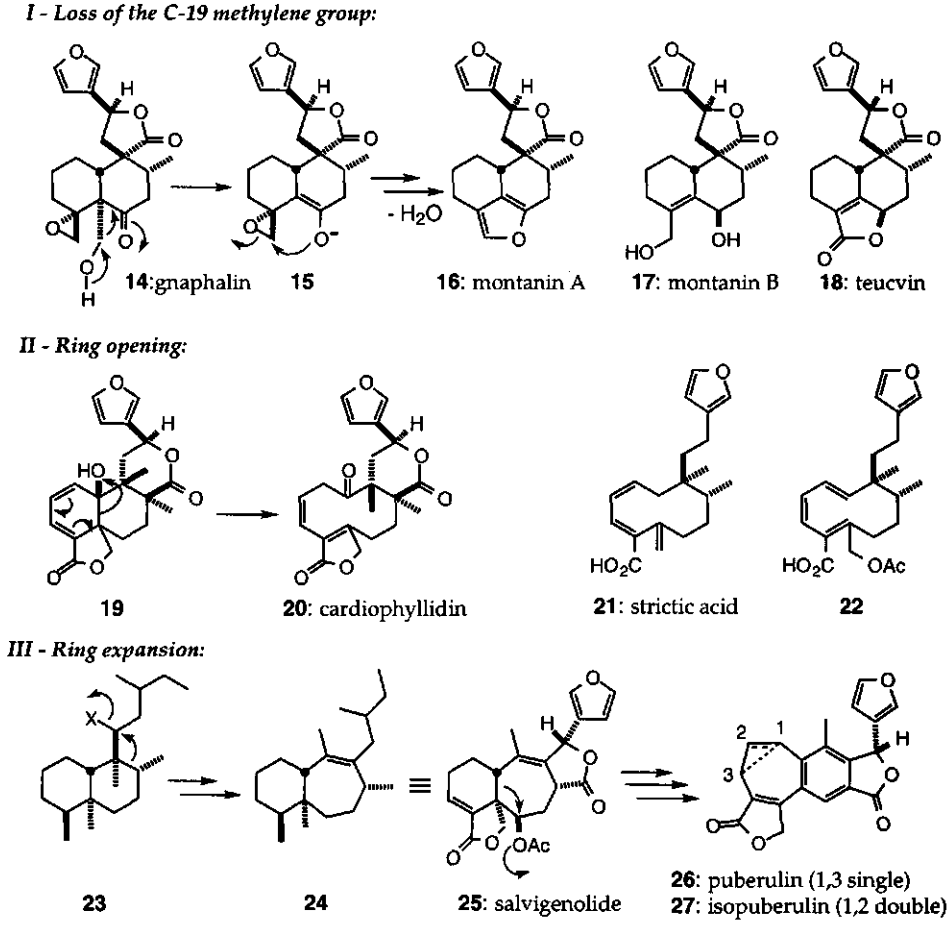


12



13

Apart from being biosynthetic end-products, some clerodane diterpenes may also act as the starting point for biotransformations towards other terpenes. For instance, for a series of *neo*-clerodane secondary metabolites isolated from *Teucrium* ssp., a tentative scheme for the biotransformation of relatively simple clerodanes into more complex ones has been proposed.[10] Some clerodanes may even undergo biotransformations involving skeletal rearrangement, as is suggested by the frequent co-isolation of both *neo*-clerodane diterpenes and structurally related diterpenes with a modified clerodane carbon skeleton from a single source. For example, in addition to numerous *neo*-clerodanes, plants of the



Scheme 2.2: Some examples of isolated natural products with a rearranged clerodane skeleton and their proposed biogenetic relationship to *neo*-clerodane precursors (see text for discussion).

genus *Teucrium* have yielded various 19-nor-*neo*-clerodane diterpenes, such as montanin A (**16**). Piozzi[3] has suggested that this compound might be biosynthetically related to a suitable 6-keto-*neo*-clerodane precursor as gnaphalin (**14**), which can eliminate formaldehyde in a retro-aldol fashion as an initial step towards (**16**) (Scheme 2.2-I). However, the isolation of montanin B (**17**) from the same source[11] appears to indicate that a similar pathway can be followed by precursors lacking a 6-keto substituent, but capable of generating a positive charge on C-4, for instance via acid-catalized opening of a 4,18 epoxide ring. It is conceivable that a range of 19-nor-*neo*-clerodanes found in *Teucrium* ssp.,[6] such

as teucvin (**18**) and related compounds, could result from further transformation of either (**16**) or (**17**).

Another skeletal rearrangement that is frequently encountered is the opening of the A/B ring system to a ten-membered ring (Scheme 2.2-II). González *et al* suggested that cardiophyllidin (**20**), isolated from *Salvia cardiophylla*, may have been biosynthetically derived from the hypothetical *neo*-clerodane precursor (**19**).[12] Indeed, several 10-hydroxy-*neo*-clerodanes structurally closely related to (**19**) have been found in other *Salvia* species. A similar mechanism has been proposed as the pathway for the biosynthesis of strictic acid (**21**).[13]

Clerodane diterpenes may also undergo ring expansion reactions to yield the [6.7] or [7.6] bicyclic ring system present in a number of natural products. The [6.7] system in salvigenolide (**25**), found in *Salvia fulgens*, is thought to have been formed by migration of a carbon-carbon bond from C-8,9 to C-8,11, initiated by departure of a leaving group X from C-11 (Scheme 2.2-III).[14] Interestingly, it has been suggested that this compound may undergo further skeletal rearrangements to the [3.6.6] tricyclic rearranged clerodane puberulin (**26**) and, subsequently, to the [7.6] system of isopuberulin (**27**), both isolated from *S. puberula*.[15]

2.3 - Biological Activity

Due to their bitter taste, clerodanes are often classified as 'bitter principles', a term generally used to denote extremely bitter tasting plant constituents, usually terpenoid or alkaloidal in nature, which often display a powerful physiological action.[16] At present, only a small fraction of all clerodanes isolated has actually been examined for any biological activity. However, of the clerodanes that were tested, only a few have been reported as apparently having no activity.[6]

It is a recognized principle of pharmacognosy that plant species used in folk medicine often provide valuable leads for the isolation of pharmacologically active plant metabolites. Interestingly, a number of plant species from which clerodanes have been isolated are considered to be of therapeutic value in the treatment of various illnesses.[6,17] Although this obviously is no rule, indeed in some cases the physiological activity of these plants has been attributed to the presence of specific clerodane secondary metabolites in the plant tissue. For example, the psychotropic effect of mexican mint (*Salvia divinorum*), employed in divinatory rites of the Mazatec indians, is ascribed to the presence of divinorin A (**28**, Figure 2.2), which was shown to have a sedative effect in a mouse bioassay.[18] Plaunols B-E (**29-32**), obtained from the Thai medicinal herb 'plan-noi' (*Croton Sublyratus*), displayed significant inhibitory activity against peptic ulcers

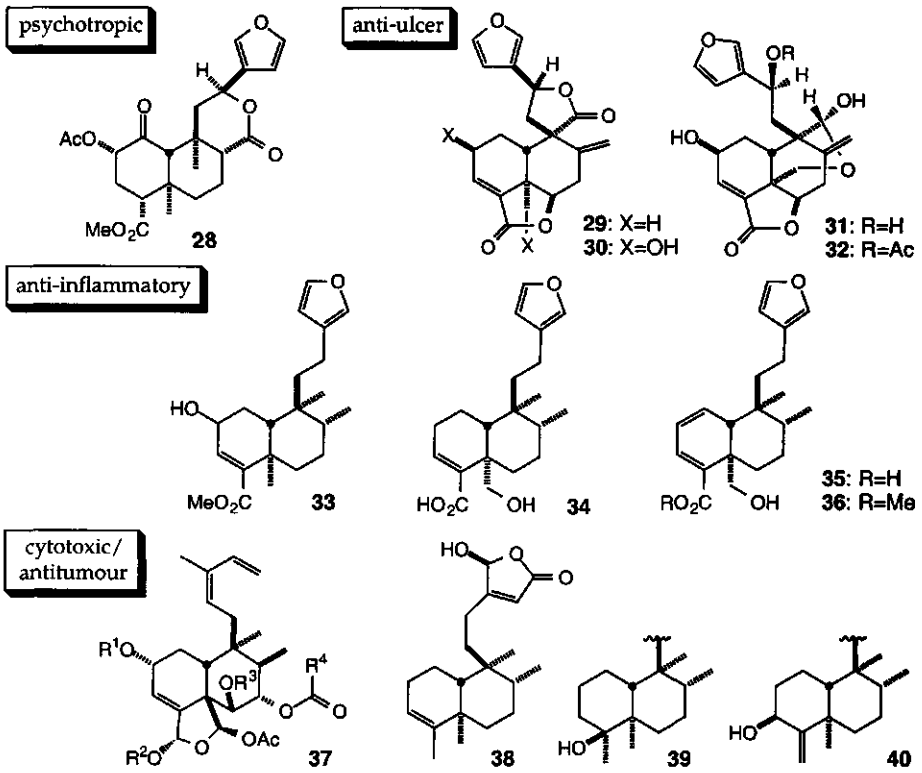


Figure 2.2: *Neo*-clerodane diterpenes with physiological activity on mammalian organisms or tissues (see text). (28) divinorin A, (29-32) plaunol B-E.

in rats.[19] Investigations of Australian medicinal plants yielded several clerodanes (**33-36**) with potential anti-inflammatory activity, due to their in-vitro inhibition of the cyclo-oxygenase and lipoxygenase pathways to prostaglandins and leukotrienes, respectively.[20] Furthermore, a number of clerodane diterpenes (**37-40**) with antitumour or cytotoxic activity have been isolated from various sources.[21,22]

A clerodane-type diterpene with physiological activity towards fish has been obtained from *Callicarpa maingayi*, a plant which is used on the Caroline and Phillipine islands to stun fish; its leaves were found to contain maingayic acid (**41**, Figure 2.3) which displays potent piscicidal activity.[23] Also, several clerodanes (**42-45**) with piscicidal activity towards killifish (*Oryzas latipes*) have been isolated from plants of the genus *Solidago*.[24]

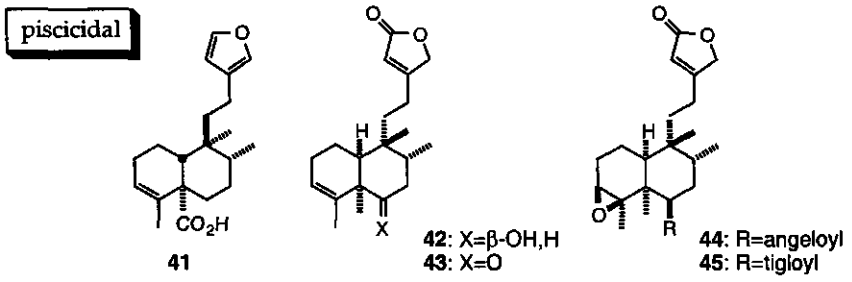


Figure 2.3: Natural clerodane-type diterpenes with physiological activity against fish. (**41**) maingayic acid, (**42**) solidagolactone IV, (**43**) solidagolactone V, (**44**) solidagolactone VII, (**45**) solidagolactone VIII.

Clerodanes displaying some sort of antimicrobial activity (Figure 2.4) have been obtained from highly different sources. Plant species from various genera contain (**46**),[25] which was reported to be as active towards Gram-positive bacteria as the antibiotic streptomycin.[26] The Chinese medicinal plant *Ajuga lupulina* yielded several diterpenes related to clerodin, such as lupulin A and B (**47**,**48**) and 14,15-dihydro-15-methoxy-clerodin (**49b**; *i.e.* **49a** with R=OMe).[27] Remarkably, the lupulins (**47**,**48**) readily displayed antibacterial activity against various species (*Escheria coli*, *Staphylococcus aurus* and *Pseudomonas aeruginosa* were used), but (**49b**) only showed activity after its hydrolysis to (**49a**). The fungus *Oidiodendron truncatum* produces the antibiotic fermenation product clerocidin (**50**), which acts on both Gram-positive and Gram-negative bacteria and also displays activity against P388 leukemia in mice.[28,29] Some structurally related antibiotic clerodanes were also found in this fungus (**51-53**) and in the plant *Premna schimperi* (**54**),[30] while bacteria of the genus *Kitasatosporis* yielded the antibiotic terpentecin (**55**), which also inhibits DNA synthesis.[31] Marine sponges of the genus *Agelas* contain several clerodane-type metabolites (**56-58**), inhibiting enzymatic reactions of Na,K-ATPase; these compounds were found both to disrupt the contractive responses of smooth muscles and to inhibit the growth of microorganisms.[32] Finally, an extract with antiviral and antitumour activity from the Columbian medicinal plant *Baccharis tricuneata* yielded several clerodane-type diterpenes;[33] however, to my knowledge, the observed bioactivity has not yet been firmly linked to the presence of these terpenes.

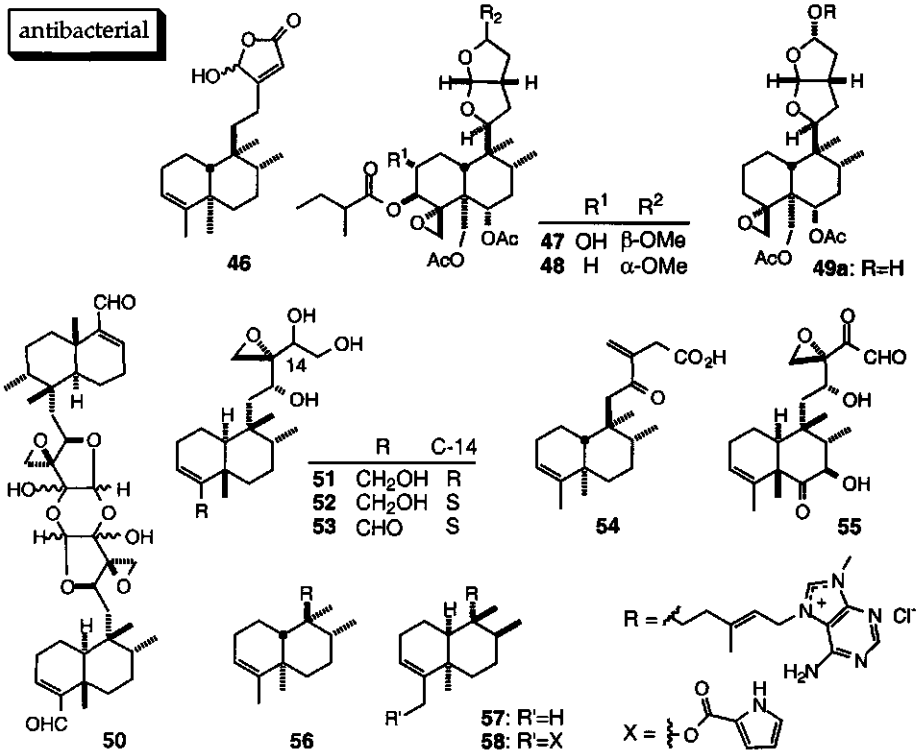


Figure 2.4: Clerodane-type diterpenes with antibacterial activity (see text for other activities). (**47**) lupulin A, (**48**) lupulin B, (**49a**) clerodin hemiacetal, (**50**) clerocidin (PR1350), (**51**) antibiotic PR1383, (**52**) hexahydroclerocidin (PR1389), (**53**) tetrahydroclerocidin (PR1421), (**55**) terpentecin.

As with most other terpenoids, the physiological role of clerodane secondary metabolites in the plant species from which they are isolated is obscure or unknown and for many a direct function in the plants' physiology may not even exist.[34] A possible exception is the diterpene gymnocolin (**59**, Figure 2.5), obtained from the liverwort *Gymnocolea inflata*.[35] This clerodane inhibits the root growth of cress seedlings; also it stimulates wheat seedling growth, though at the same time the coleoptiles of treated wheat plants remain shorter than the controls.[36] The possibility of a physiological activity of (**59**) on *G. inflata* itself, however, was not examined.

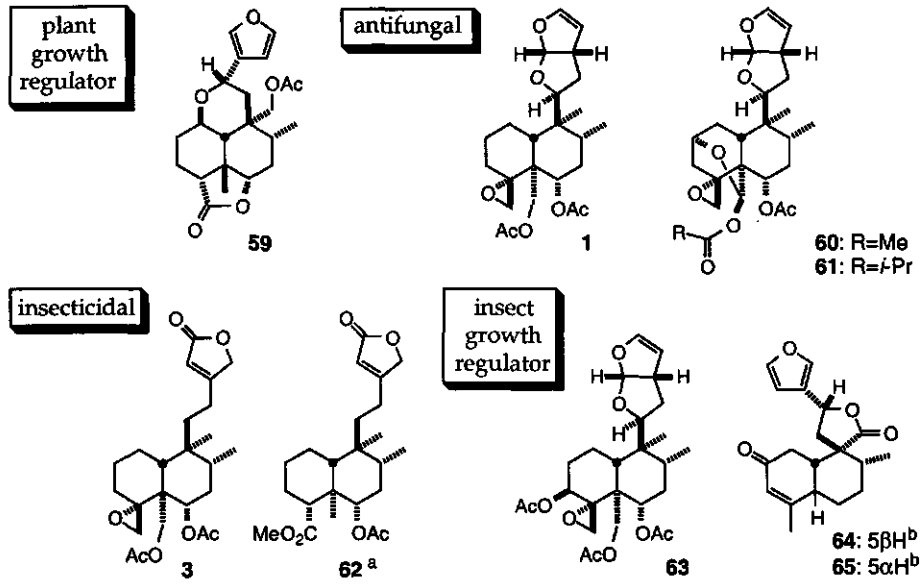


Figure 2.5: Clerodane diterpenes with physiological activity on plants, fungi or insects. (**59**) gymnocolin, (**1**) clerodin, (**60**) jodrellin A, (**61**) jodrellin B, (**3**) ajugarin I, (**62**) ajugarin IV, (**63**) 3-epi-caryoptin, (**64**) *cis*-dehydrocrotonin, (**65**) *trans*-dehydrocrotonin. *Notes:* (a) Displays also insect growth regulatory activity. (b) 19-Nor-clerodane.

The ability to synthesize clerodane metabolites without apparent plant physiological significance can nevertheless be of benefit to a plant species. There is ample evidence to suggest that such metabolites may act as a chemical defence mechanism to protect the plant from diseases or predation by phytophagous animals. For instance, clerodin (**1**) and some related jodrellins (**60**,**61**) were found to be inhibitors of the growth or spore germination of the plant pathogenic fungi *Fusarium oxysporum* and *Verticillium tricorpus*.[37] Furthermore, many clerodane diterpenes were reported to display biological activity against insects. Insecticidal activity has been found for the ajugarins I (**3**) and IV (**62**).[38] Ajugarin IV also displays insect growth regulating activity, as do 3-epi-caryoptin (**63**) and both *cis*- and *trans*-dehydrocrotonin (**64**) and (**65**).[39] However, the *neo*-clerodanes are best-known for their insect antifeeding properties, which is by far the most extensively studied bioactivity of these diterpenes. The insect antifeedant activity of clerodane diterpenes is discussed in the next section.

2.5.- Insect Antifeedant Activity

The class of *neo*-clerodane diterpenes has attracted considerable attention as a rich source of insect antifeedants. To date, well over 150 natural and semisynthetic clerodanes have been screened for this activity in laboratory assays, yielding several compounds with potent antifeedant activity[40] against various insect species. However, as with most other classes of natural antifeedants, to my knowledge no clerodanes have yet been tested under actual field conditions.

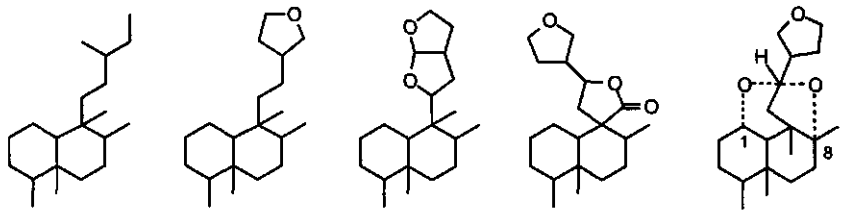
As was discussed in Chapter 1, natural insect antifeedants offer a number of properties that are desirable in future crop protection agents. For the use of antifeedants in agriculture to become common practice, a stable and cheap supply of compounds with high activity against economically relevant pest species is needed. In addition to the search for new antifeedants from natural sources, the preparation of more potent antifeedants through (semi)synthetic routes could be considered. Investigations into the structural elements required to evoke antifeedant activity in various insect species are essential for such a strategy to be successful.

In view of the extensive literature available on *neo*-clerodane antifeedants and the structural diversity of the compounds reported active, this class of diterpenes constitutes an attractive starting point for such investigations. Unfortunately, these antifeedancy data are at present only available in the form of a scattered series of reports in various journals; only a few papers reviewing parts of this literature have been published.[41] Furthermore, the data are severely plagued by a lack of standardization, with many researchers using different methods and definitions to determine and quantify essential parameters, such as the amount of compound applied and the degree of antifeedant activity found. These factors make the comparison of antifeedancy data from different sources difficult and thus hamper the search for meaningful structure-activity relationships (SAR's).

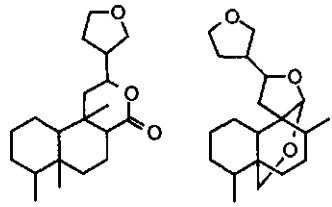
We therefore deemed it useful to establish a compilation of all test results on insect antifeedancy of *neo*-clerodanes reported to date. Also, we have attempted to increase the compatibility of these data by converting some of the results reported in the literature to the corresponding standardized parameters adopted in the compilation. The various aspects involved in the construction of this compilation are briefly discussed below.

Compounds. The compilation is resticted to diterpenes of natural or semisynthetic origin with either a clerodane carbon skeleton (**2**) or a structure arising from rearrangements of this skeleton. (Semi)synthetic fragments corresponding to substructures present in the these diterpenes were also included.

The compounds were arbitrarily sorted into groups reflecting the different types of sidechains attached to C-9 (Figure 2.6), ranging from simple acyclic chains (**66**), via sidechains containing one heterocycle (**67**) and various types containing two (separate or fused) heterocycles (**68-71**), to sidechains consisting of three such rings (**72**). Unless otherwise indicated, the diterpenes in the charts possess the *neo*-clerodane absolute stereochemistry.



66 (Chart 2.1) **67** (Chart 2.2) **68** (Chart 2.3) **69** (Chart 2.4) ‡ **70** (Chart 2.5)



71 (Chart 2.6) **72** (Chart 2.7)

Chart 2.8 - Diterpenes with a rearranged clerodane skeleton.

Chart 2.9 - Fragments with an intact decalin moiety.

Chart 2.10 - Fragments with an intact C-9 sidechain.

Figure 2.6: *Overview of the ordering scheme used in the compilation of antifeeding* ***neo*****-clerodane diterpenes and related compounds. The dashed bonds in (70) indicate an ether linkage to either C-1 or C-8. (‡) Including some 19-nor-*neo*-clerodanes with a similar sidechain.**

Insect species. The acceptance by a phytophagous insect of a plant as a suitable host depends on the capability of the insect's chemosensory system to detect plant tissue with favourable levels of (specific) feeding stimulants and feeding deterrents.[42a] Monophagous insect species (*i.e.* insects specialized to feed on a specific plant species) are often more sensitive to the presence of antifeedants than polyphagous insect species, that can accept a range of plant species as their host. Consequently, the antifeedant activity of a specific compound can differ significantly when tested on different insect species. Furthermore, several examples are known in which apparently small structural changes in an antifeedant molecule changed the activity against different insect species in

highly different manners.[42b] Nevertheless, conclusions on potential structure-activity relationships, based on comparison of the activity of related compounds on *different* insect species, have occasionally appeared in the literature.[41b,43] In my opinion, such conclusions should be treated with caution.

In the compilation, the antifeedant activities reported in the literature have been grouped according to the different test insect species used. The species themselves were sorted by insect order and are identified through their scientific (latin) name and, if possible, by a common name. When available, the developmental stage of a test insect was also indicated; other potential differences within a test species, for instance due to different geographic origin or conditions during culturing, have not been addressed due to lack of data.

Test substrate and protocol. Even when the same test insect species is used by various research groups, direct comparison of the antifeedancy data is sometimes complicated by other differences in the feeding bioassay. Apart from the incompatibility of bioassays with a different experimental design (*i.e.* two-choice- vs. no-choice assays, see Chapter 1), a major obstacle for such comparisons can be the use of different feeding substrates in the tests. Due to the sensitivity of insects to the chemical composition of their food, the activity of an antifeedant, presented on leaf discs from the insect's host-plant as the test substrate, may differ from the activity found when an artificial feeding substrate (*e.g.* glass-fibre, coated with sucrose for palatability) is used in the test. Such effects have been observed in practice[41a] and, as a consequence, caution should be exercised when comparing antifeedancy data from assays with different feeding substrates

The effects of other differences in the bioassay protocol are less easy to evaluate but cannot be discarded *a priori*. When comparing antifeedancy data from different sources, one should always be aware of possible effects due to differences other than the compounds tested; a useful comparison should therefore best be limited to data from highly similar assays.

In order to tackle such problems, in the Tables 2.1-2.9 the reported antifeedant activity was combined with information on the corresponding bioassay. This information is provided in the form of a code, *e.g.* A1a. The capital 'A' signifies that an artificial substrate was used (similarly, 'N' denotes natural substrates), while the numeral '1' codes for a specific artificial substrate and the letter 'a' distinguishes between different experimental protocols that all used the substrate A1. The code refers to a specific section of Table 2.10, in which details of the various bioassays are summarized.

Test compound concentration. Usually, the amount of test compound used is stated in the literature as the concentration of the applied test solution in ppm. It is important to realize that this practice does not always accurately reflect the

potency of the various compounds tested. For instance, if a large clerodane diterpene and a small fragment of this compound would be reported to be equally active at 100 ppm concentration (*i.e.* 100 mg/l), the diterpene actually is more potent because its mass is much larger and therefore less moles per volume were required to achieve this level of activity. Furthermore, between different test protocols the surface area of the employed test substrate may differ and thus influence the actual concentration of test compound that an insect encounters.

In my view, a better quantity to represent the amount of test compound would have been the compound density per unit surface area of the substrate, *i.e.* in mmol per cm^2. Although such a system was *de facto* used by Belles *et al* [44] and, occasionally, by Simmonds and coworkers,[45] it has unfortunately not been generally adopted. In correspondance with the large majority of antifeedancy data, the concentration in ppm was therefore chosen as standard parameter in the compilation; reported quantities differing from this standard were recalculated according to the procedures outlined in the appropriate sections of Table 2.10.

An additional word of caution should be mentioned regarding some of the literature test protocols in which the substrate is dipped into a test solution, instead of being treated with a specific volume. In such experiments it is usually unknown what the actual amount of compound present on the (dried) substrate is and such results should therefore be treated accordingly.

Antifeedant activity. A number of different systems are used in the literature to quantify insect antifeedant activity. In the compilation, the reported activities were as much as possible converted to a standard index of antifeedant activity. For activities determined in a two-choice situation we adopted the Antifeedant Index (AI) according to formula [1] (see below) as the standard quantity because this system covers a major part of the published data and can relatively easy be calculated from data reported according to different systems. A similar Antifeedant Index, defined by [2], was used for data obtained in no-choice feeding assays. Details on the procedures for conversion of reported quantities into these standard systems can be found in the corresponding assay protocols, specified in Table 2.10.

Two-choice assays:

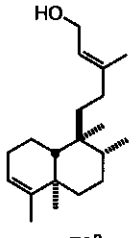
$$AI = \frac{(C - T)}{(C + T)} \times 100\% \quad [1]$$

No-choice assays:

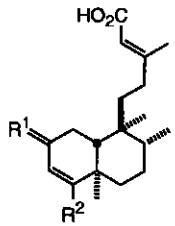
$$AI = \frac{(C - T)}{C} \times 100\% \quad [2]$$

AI is the Antifeedant Index in a two-choice [1] or no-choice [2] situation; C and T are the amounts eaten from control and treatment discs, respectively. A positive value indicates an antifeedant, while feeding stimulants have a negative AI value.

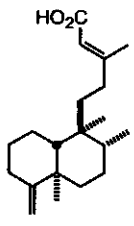
Chart 2.1: Natural and semisynthetic clerodane diterpenes with an acyclic C-9 sidechain.



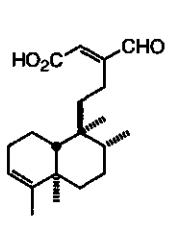
73^{n}



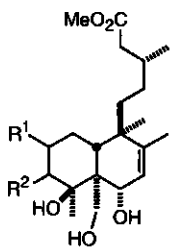
	R^1	R^2
74^{n}	H,H	Me
75^{d}	H,H	CHO
76^{d}	O	Me



77^{n}



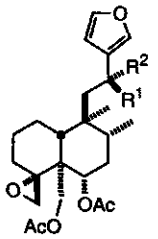
78^{n}



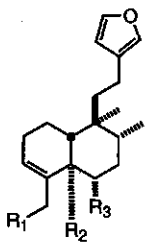
	R^1	R^2
79^{n}	α-OH	β-OH
80^{d}	α-OH	α-OH
81^{d}	β-OH	β-OH
82^{d}	β-OH	α-OH

Notes and compound names: (n) natural compound, (d) derived from a natural compound. (73) kolavenol.

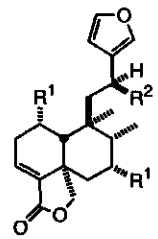
Chart 2.2.1: Natural and semisynthetic clerodane diterpenes with a 3-ethylfuran-based C-9 sidechain.



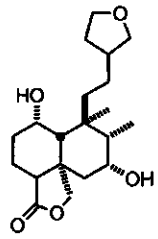
	R^1	R^2
83^{n}	OAc	H
84^{n}	OH	H
85^{d}	=O	



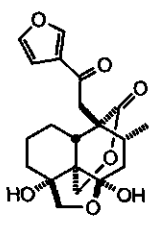
	R^1	R^2	R^3
86^{n}	H	CO_2H	OH
87^{d}	H	CO_2Me	OAc
88^{d}	OH	CH_2OH	H



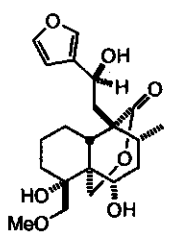
	R^1	R^2
89^{n}	H	H
90^{n}	OH	H
91^{n}	H	OAc



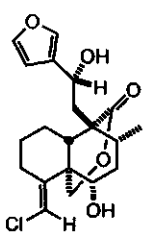
92^{d}



93^{d}



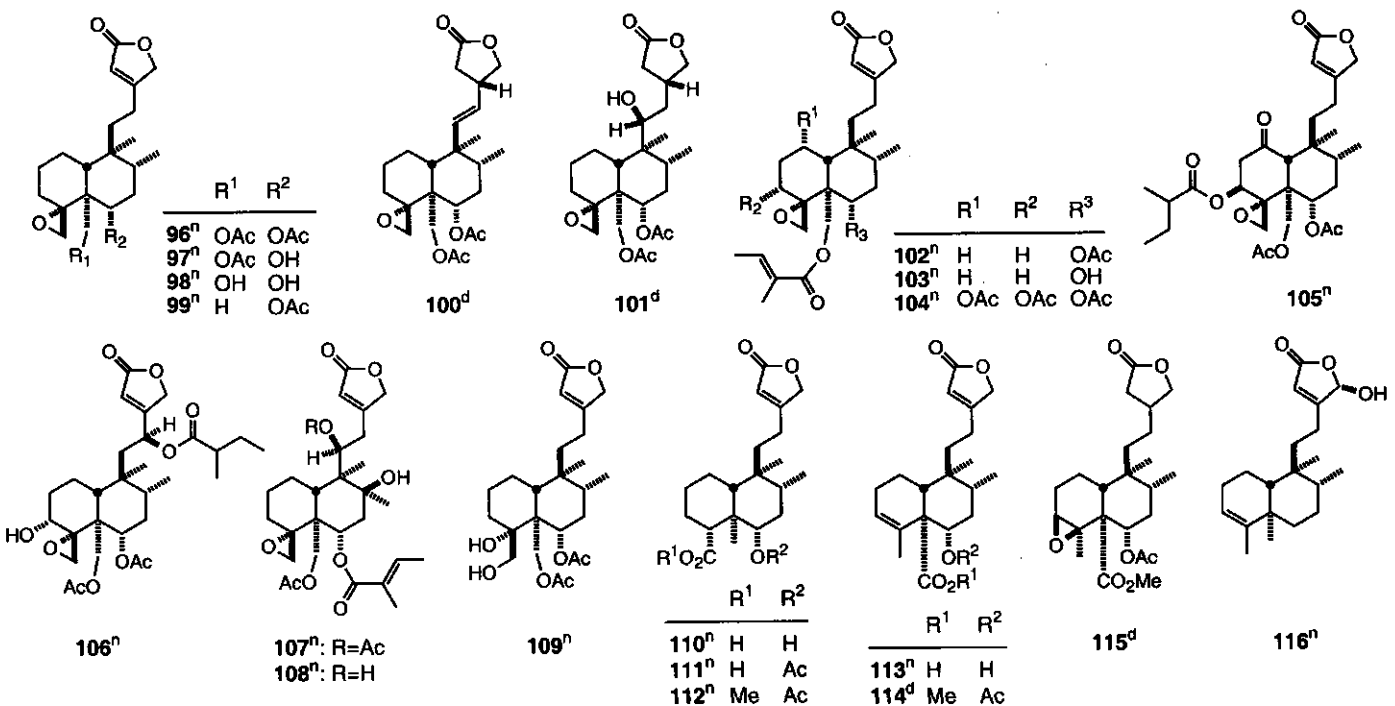
94^{d}



95^{d}

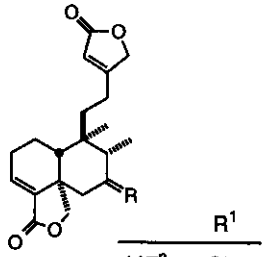
Notes and compound names: (n) natural compound, (d) derived from a natural compound. (84) 6,19-deacetylteumassilin; (86) kerlinic acid; (89) hawtriwa lactone; (90) bacrispine; (91) tanabalin; (92) hexahydrobacrispine; (93) 12-keto-teugnaphalodin.

Chart 2.2.2: Natural and semisynthetic clerodane diterpenes with a 3-ethylbutenolide-based C-9 sidechain.

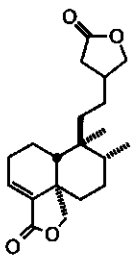


Notes and compound names: (n) natural compound, (d) compound derived from a natural product. (**96**) ajugarin I; (**97**) ajugarin II; (**98**) deacetylajugarin II; (**99**) ajugarin V; (**102**) ajugacumbin A; (**103**) ajugacumbin B; (**104**) ajugacumbin C; (**105**) ajugareptansone A; (**106**) 3α-hydroxy-ajugamarin F4; (**107**) scutalpin B; (**108**) scutalpin C; (**109**) ajugarin III; (**110**) ajugarin IV; (**113**) melisodoric acid.

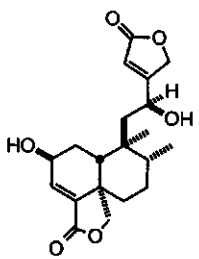
Chart 2.2.2 - Continued



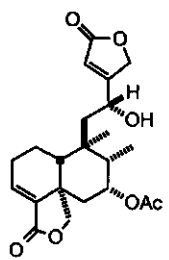
	R^1
117^n	α-OH,H
118	O



119^n



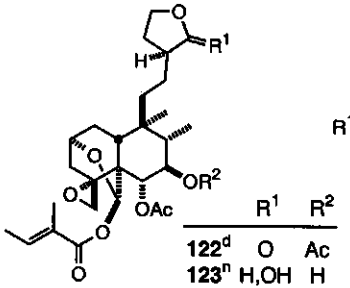
120^n



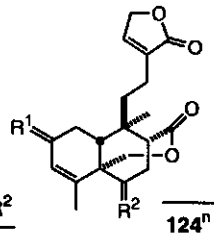
121^n

Notes and compound names: (n) natural compound, (d) compound derived from a natural product. (**120**) semiatrin; (**121**) kerlinolide.

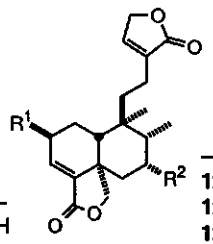
Chart 2.2.3: Natural and semisynthetic clerodane diterpenes with a 2-ethylbutenolide-based C-9 sidechain.



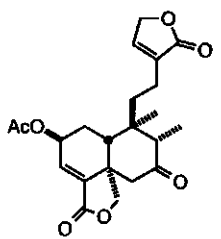
	R^1	R^2
122^d	O	Ac
123^n	H,OH	H



	R^1	R^2
124^n	H,H	α-OH,H
125^n	H,H	O
126^n	O	H,H



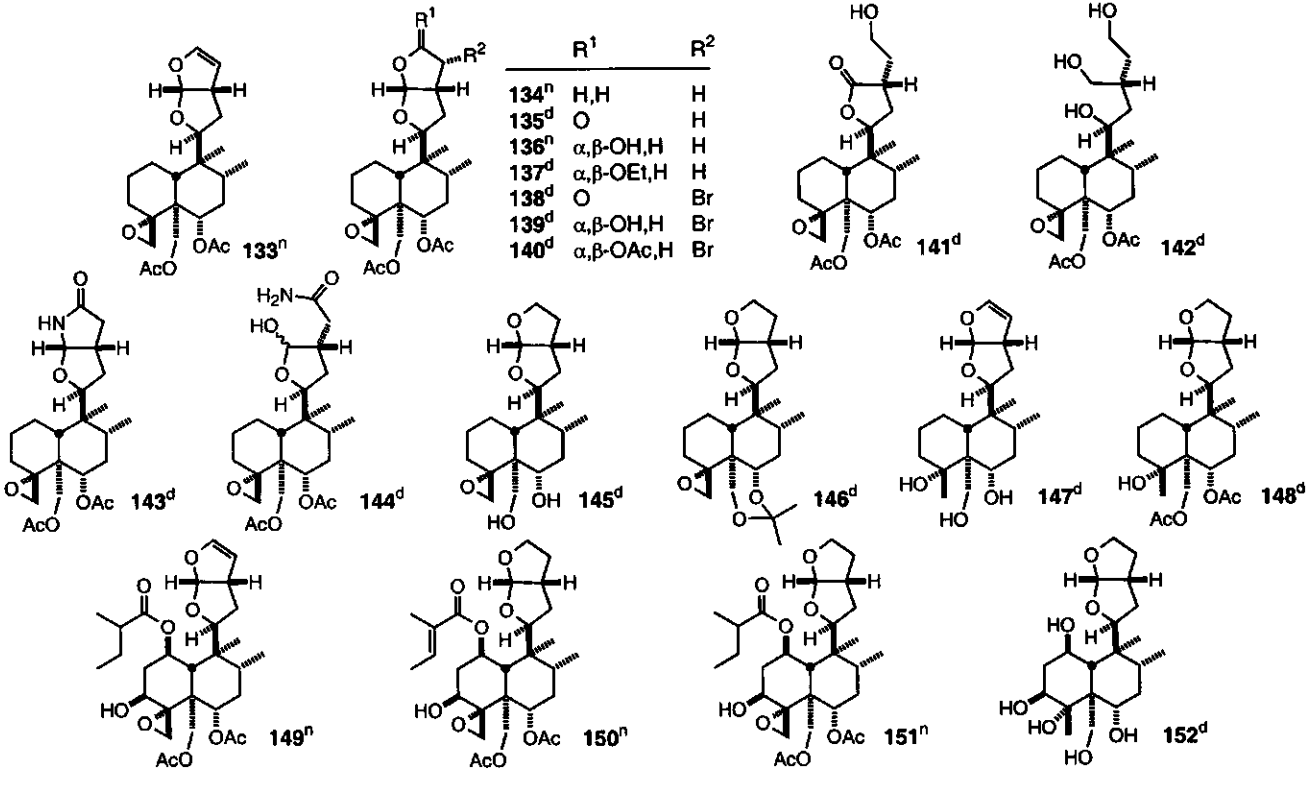
	R^1	R^2
127^n	H	OAc
128^d	OAc	H
129^n	OH	OH
130^n	OH	OAc
131^n	OAc	OH



132^d

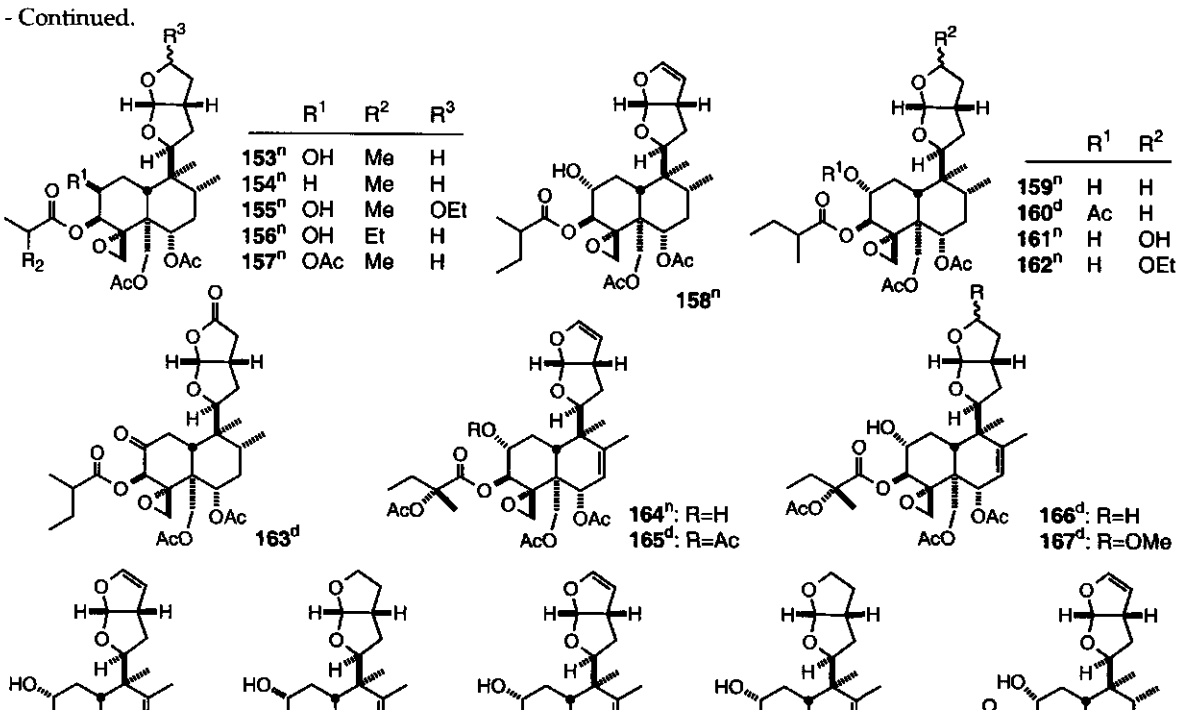
Notes and compound names: (n) natural compound, (d) compound derived from a natural product. (**123**) scutegalin B; (**124**) salvimadrensin; (**126**) salvimadrensinone.

Chart 2.3: Natural and semisynthetic clerodane diterpenes with a furo[2,3b]furan-based sidechain at C-9.



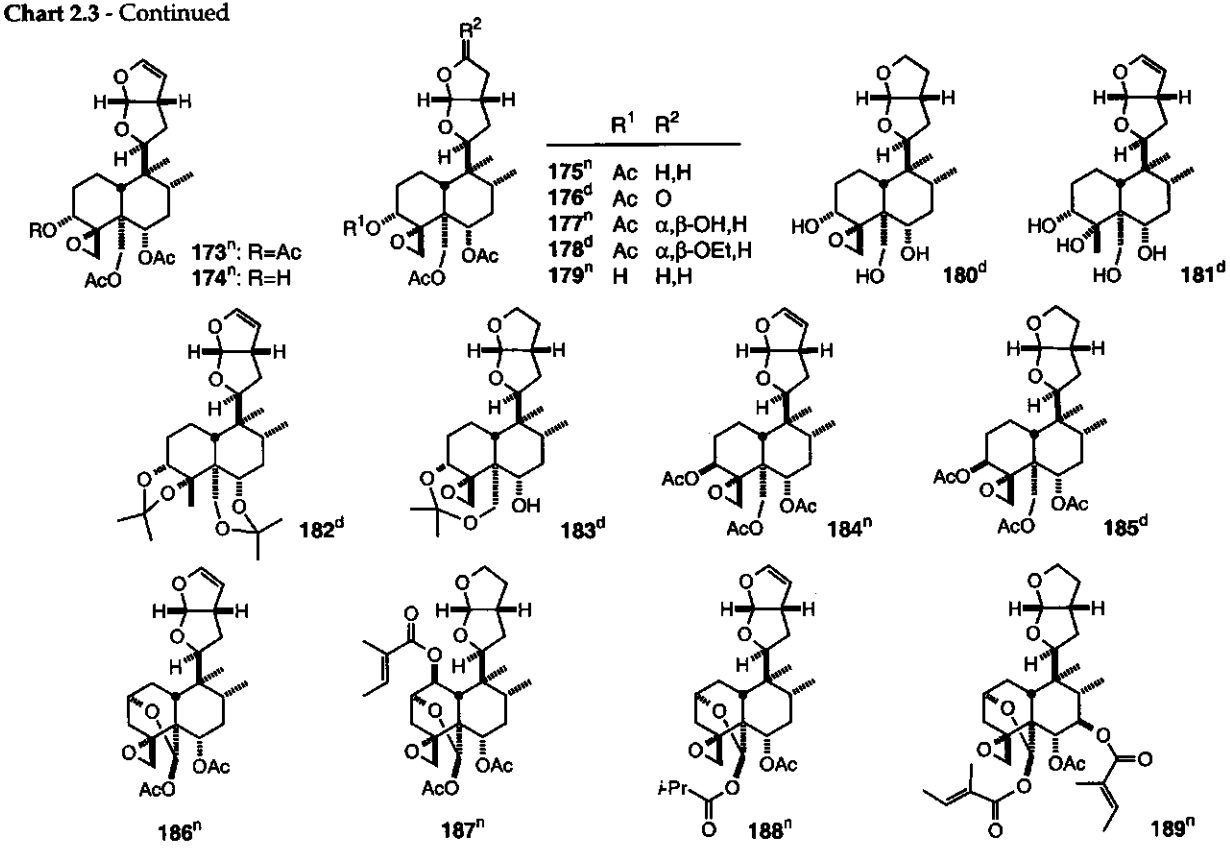
Notes and compound names: (n) natural product, (d) derivative from natural product. (**133**) clerodin; (**134**) dihydroclerodin; (**136**) clerodin hemiacetal; (**149**) 14,15-dehydroajugareptansin; (**150**) 3β-hydroxy-ajugavensin B; (**151**) ajugareptansin.

Chart 2.3 - Continued.



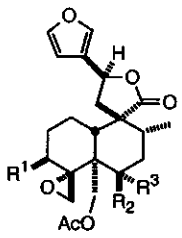
Notes and compound names: (n) natural product, (d) derivative from natural product. (**153**) ivain 1; (**154**) ivain 2; (**155**) ivain 3; (**156**) ivain 4; (**157**) 2-acetylivain 1; (**158**) ajugapitin; (**159**) 14,15-dihydro-ajugapitin; (**161**) 14-hydro-15-hydroxyajugapitin; (**162**) 15-ethoxy-14-hydroajugapitin; (**164**) clerodendrin A; (**172**) clerodendrin B.

Chart 2.3 - Continued

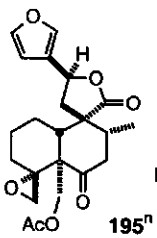


Notes and compound names: (n) natural product, (d) derived from natural product. (**173**) caryoptin; (**174**) caryoptinol; (**175**) dihydrocaryoptin; (**177**) caryoptin hemiacetal; (**179**) dihydrocaryoptinol; (**184**) 3-epicaryoptin; (**186**) jodrellin A; (**187**) 14,15-dihydrojodrellin T; (**188**) jodrellin B; (**189**) scutegalin A.

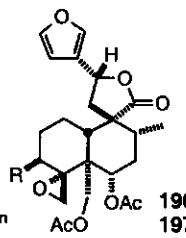
Chart 2.4: Natural and semisynthetic clerodane diterpenes, incorporating a C-9 sidechain based on an α-spiro-attached 4-(3-furyl)-γ-butyrolactone moiety, and some related natural products and derivatives.



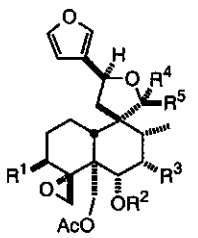
	R^1	R^2	R^3
190[n]	H	H	OH
191[n]	OAc	H	OH
192[n]	H	H	OAc
193[n]	H	OH	H
194[d]	H	OAc	H



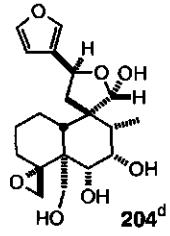
195[n]



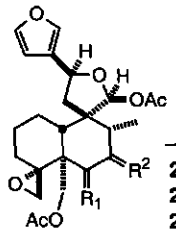
196[n]: R=H
197[n]: R=OAc



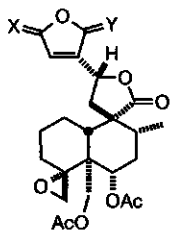
	R^1	R^2	R^3	R^4	R^5
198[d]	H	Ac	OH	OH	H
199[d]	H	Ac	OAc	OH	H
200[d]	H	H	OAc	OH	H
201[d]	H	Ac	OAc	OAc	H
202[n]	OAc	Ac	H	OAc	H
203[d]	H	H	OAc	H	OAc



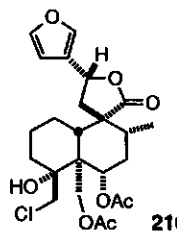
204[d]



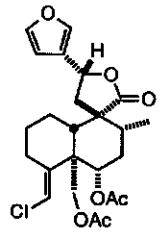
	R^1	R^2
205[n]	O	α-OH,H
206[d]	O	α-OAc,H
207[n]	α-OH,H	O



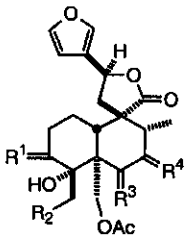
	X	Y
208a[d]	α-OAc,H	O
208b[d]	β-OAc,H	O
209a[d]	O	α,β-OH,H
209b[d]	α,β-OH,H	O



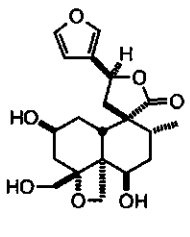
210[d]



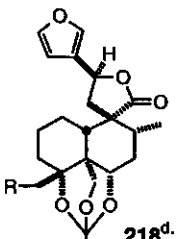
211[d]



	R^1	R^2	R^3	R^4
212[d]	H,H	Cl	α-OH,H	H,H
213[d]	H,H	Cl	O	H,H
214[n]	H,H	OAc	α-OH,H	O
215[n]	O	Cl	O	H,H
216[n]	O	Cl	α-OAc,H	H,H

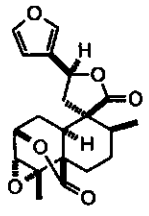


217[n]

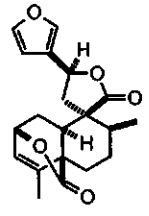


218[d]: R=OAc
219[d]: R=Cl

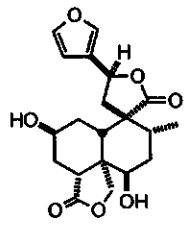
Chart 2.4 - Continued



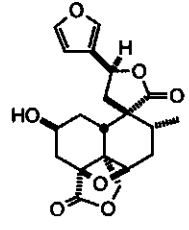
220^{n†}



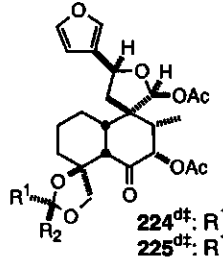
221^{n†}



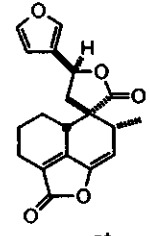
222^{n}



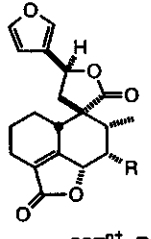
223^{n}



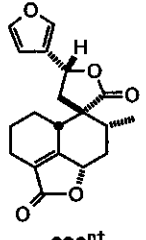
224^{d‡}: R^1=H, R^2=Me
225^{d‡}: R^1=Me, R^2=H



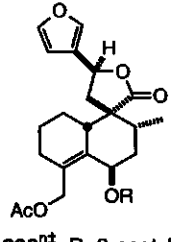
226^{n‡}



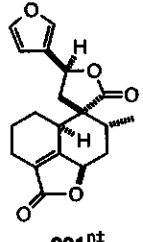
227^{n‡}: R=H
228^{n‡}: R=OH



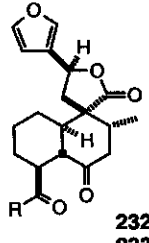
229^{n‡}



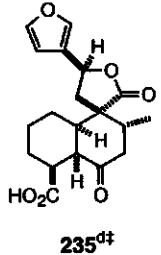
230^{n‡}: R=2-acetyl-glucose



231^{n‡}



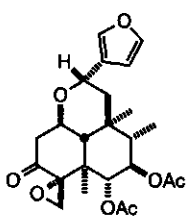
232^{d‡}: R=OH
233^{d‡}: R=OMe
234^{d‡}: $R=NH_2$



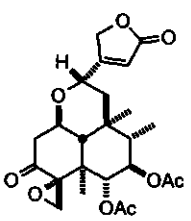
235^{d‡}

Notes and compound names: (n) natural product, (d) derived from natural product, (†) ent-*neo*-clerodane, (‡) 19-nor-clerodane.
(190) teucjaponin B; **(191)** teumicropodine; **(192)** 6-acetylteucjaponin B; **(193)** teucjaponin A; **(195)** 19-acetylgnaphalin; **(196)** montanin C; **(197)** teupyreinine; **(202)** teupyreinidin; **(205)** eriocephalin; **(206)** 7-O-acetyleriocephalin; **(207)** isoeriocephalin; **(214)** picropolinol; **(215)** tafricanin A; **(216)** tafricanin B; **(217)** teucroxide; **(220)** bartemidiolide; **(221)** deoxybartemidiolide; **(222)** dihydroteugin; **(223)** chamaedroxide; **(226)** teuscorolide; **(227)** teucvin; **(228)** teucrin A; **(229)** 12-epi-teucvin; **(230)** teuflavoside; **(231)** teucvidin.

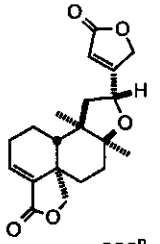
Chart 2.5: Natural clerodane diterpenes with a 3-ethylfuran- or 3-ethylbutenolide-based C-9 sidechain connected to the decalin fragment through a C-1,12 or C-8,12 ether-linkage.



236[n]



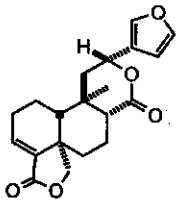
237[n]



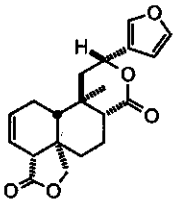
238[n]

Notes and compound names: (n) Natural compound. (**236**) cornutin A; (**237**) cornutin B; (**238**) kerlin.

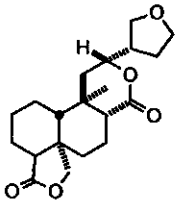
Chart 2.6: Natural and semisynthetic clerodane diterpenes, incorporating a C-9 sidechain based on a 5-(3-furyl)-δ-valerolactone with its C-α,β-bond fused with the C-9,10-bond of the decalin fragment.



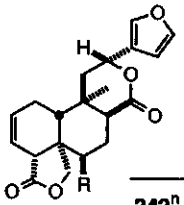
239[n]



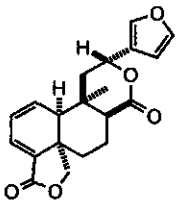
240[†]



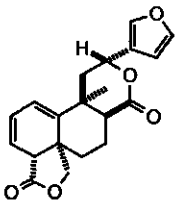
241[d]



	R
242[n]	H
243[n]	OH



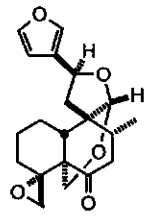
244[n]



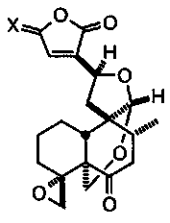
245[n]

Notes and compound names: (n) natural product, (d) derived from natural product, (†) Compound was described as salviarin , see structure compound (**242**), but structure presented in paper was C-9 epimer. (**239**) bacchotricuneatin A; (**242**) salviarin; (**243**) 6β-hydroxysalviarin; (**244**) linearolactone; (**245**) 1(10)-dehydrosalviarin.

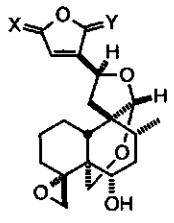
Chart 2.7: Natural and semisynthetic clerodane diterpenes, incorporating a C-9 sidechain based on an α-spiro-attached 4-(3-furyl)-γ-butyrolactone moiety, connected to C-19 through a C-19,20 ether-linkage



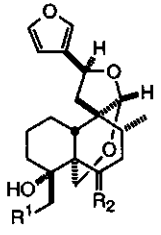
246[n]



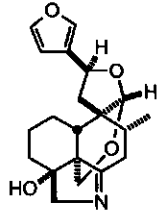
	X
247[d]	O
248a[d]	α-OAc,H
248b[d]	H,β-OAc



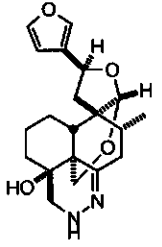
	X	Y
249[d]	H,H	O
250[d]	O	H,H



	R^1	R^2
251[d]	H	α-OH,H
252[d]	NMe_2	O



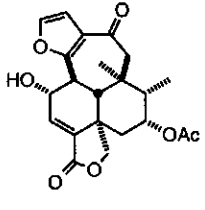
253[d]



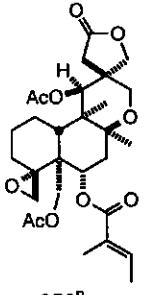
254[d]

Notes and compound names: (n) natural product, (d) derived from natural product. (**248**) teucrin P1.

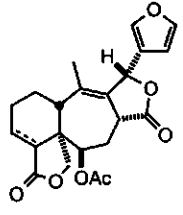
Chart 2.8: Natural diterpenes with a rearranged *neo*-clerodane skeleton.



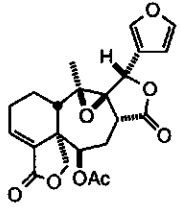
255[n]



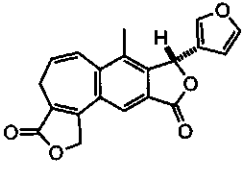
256[n]



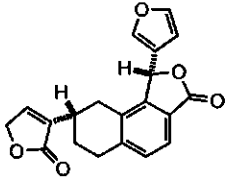
257[n]: 3,4 double bond
258[n]: 3,4 single bond, 4βH



259[n]



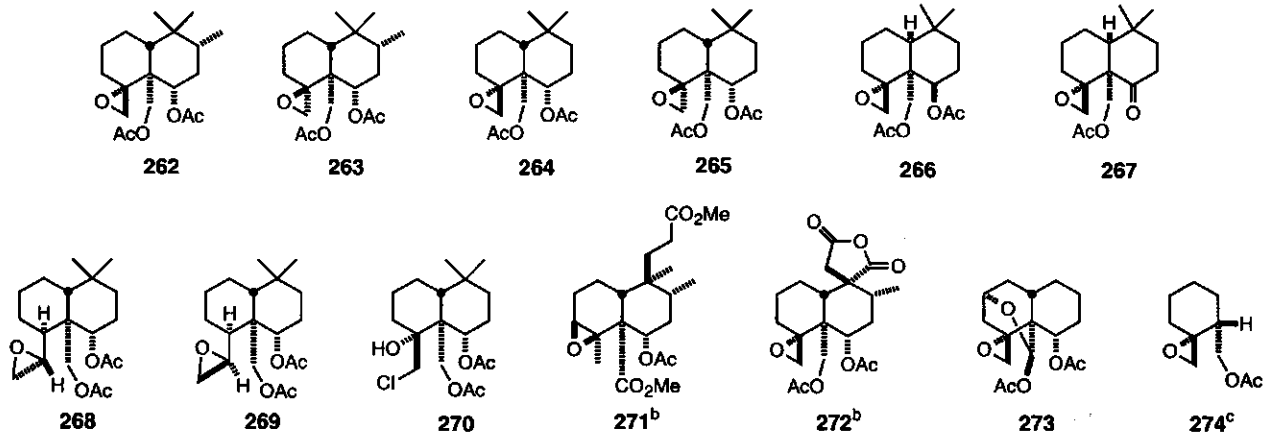
260[n]



261[n]

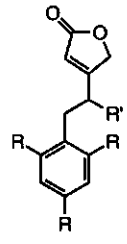
Notes and compound names: (n) natural compound. (**255**) salvisousolide; (**256**) scutalpin D; (**257**) salvigenolide; (**258**) dihydrosalvigenolide; (**259**) epoxysalvigenolide; (**260**) isosalvipuberuline; (**261**) tilifodiolide.

Chart 2.9: Synthetic and semisynthetic analogues of the decalin-fragment of clerodane diterpenes.[a]

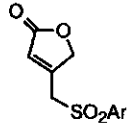


Notes: (a) All compounds are racemic and were obtained via total synthesis, unless indicated otherwise. (b) Compound was obtained by derivatization of a natural compound and is therefore presumed to be homochiral. (c) Epimeric mixture; α/β=7:3.

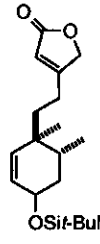
Chart 2.10: Synthetic and semisynthetic analogues of the C-9 sidechain-fragment of clerodane diterpenes.[a]



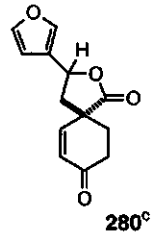
	R	R'
275	H	H
276	Me	H
277[c,d]	Me	SO_2Ar



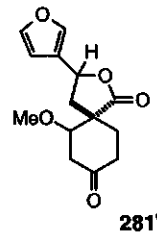
278[d]



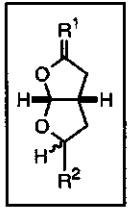
279[c]



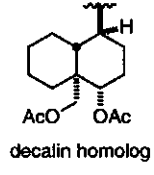
280[c]



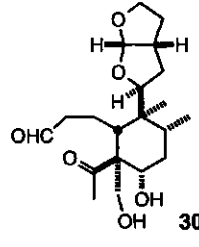
281[c]



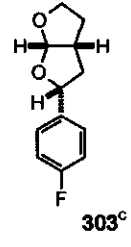
R^1	R^2 [(e)]				
	t-Bu	Ph	2-MePh	2,6-diMePh	'decalin homolog'
O	**282**	**286**	**290**	**294**	**298**
H,OH	**283**	**287**	**291**	**295**	**299**
H,OMe	**284**	**288**	**292**	**296**	**300**
H,OAc	**285**	**289**	**293**	**297**	**301**



decalin homolog



302[b]



303[c]

Notes: (a) All compounds are racemic and were obtained via total synthesis, unless indicated otherwise. (b) Compound was obtained by derivatization of a natural compound and is therefore presumed to be homochiral. (c) Synthesis not published; compound is probably racemic. (d) Ar not specified. (e) The compounds were obtained and tested as mixtures of mainly *cis*-isomer and variing amounts of the minor *trans*-isomer.

Table 2.1a: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and analogues against larvae of the Egyptian cotton leafworm (Lepidoptera: *Spodoptera littoralis*) as measured in two-choice feeding assays.[a]

	conc. (ppm)	AI (sem) [b] (%)	assay	ref.
79	*37* [c]	52.0 (6.8)	A1a	45
	3.7 [c]	10 (3.6)		
80	*37* [c]	-62 (16)	A1a	45
	3.7 [c]	-54 (1.3)		
81	*37* [c]	36 (9.7)	A1a	45
	3.7 [c]	1 (14)		
82	*37* [c]	33 (7.6)	A1a	45
	3.7 [c]	-12 (28)		
84	100	32.2 (22.16)	A1a,b	46,47
	10	21.7 (15.87)	A1a	46
86	100	-1 (12.8)	A1a	48
87	100	-8 (10.2)	A1a	48
93	100	9.6 (11.41)	A1a	46
	10	9.4 (10.62)		
94	100	-51.2 (7.88) *	A1a	57
95	100	-8.1 (0.47)	A1a	49
	10	-23.0 (9.87)		
96 [d]	300	*100* [e]	N2	50
	100	43.1 (7.31) *	A1a,b	46,47,49,51
	50	40 (11.3) *	A1a	51
	25	26 (14.8)		51
	10	34.5 (6.95) *		46
		36.8 (8.53) *		49
98	100	29.6 (8.96) *	A1a	46
	10	17.9 (9.65)		
100	100	24.8 (5.83)	A1b	47
101	100	48.8 (8.84)	A1b	47
105	*2500* [f]	*14.9 g*	N4	44
	1000 [f]	*-7.0 g*		
106	100	-32 (13.9)	A1a	52
107	100	26.9 (11.01)	A1a	53
108	100	96.8 (1.17)	A1a	53
114	100	-11 (9.8)	A1a	48
115	100	73 (5.2) *	A1a	48
117	100	32 (14.5) *	A1a	48
118	100	26 (6.8) *	A1a	48
120	100	57 (6.7) *	A1a	48
121	100	70 (10.3) *	A1a	48
122	100	21 (13.9)	A1a	54
123	100	-27 (12.0)	A1a	54
124	100	27 (10.2) *	A1a	48
125	100	20 (4.5)	A1a	48
126	100	3 (7.1)	A1a	48
127	100	29 (5.3) *	A1a	48
128	100	6 (2.5)	A1a	48
129	100	34 (4.6) *	A1a	48
130	100	54 (15.4) *	A1a	48
131	100	83 (8.3) *	A1a	48
132	100	-15 (11.1)	A1a	48
133	100	74.4 (8.44) *	A1a,b	47,51,55
	50	59 (6.2) *	A1a	51
	25	24 (9.8) *		51,55
	1	14 (19.6)		55
134	100	95.2 (16.64)	A1b	47
135	100	49.2 (9.64)	A1b	47
136	100	69.4 (13.31)	A1b	47
138	100	69.7 (18.31)	A1b	47
139	100	82.2 (12.13)	A1b	47
140	100	39.1 (11.61)	A1b	47
141	100	24.8 (7.76)	A1b	47
142	100	21.4 (4.84)	A1b	47
143	100	14.5 (6.06)	A1b	47
144	100	82.8 (13.73)	A1b	47
148	100	35.0 (13.61)	A1b	47
149	100	92 (5.5) *	A1a	52
	25	60 (15.9) *		
150	100	0.3 (30.1)	A1a	52
151	100	-41 (19.6)	A1a	52
	1000 [f]	*66.7 g*	N4	44
	100 [f]	*25.0 g*		
152	*2500* [f,h]	*19.8 g*	N4	44
153	*100* [f]	*60.0 g*	N4	44
	10 [f]	*14.9 g*		

Table 2.1a - Continued

	conc. (ppm)	AI (sem) (%)	assay	ref.
154	*100* [f]	*75.4 g*	N4	44
	10 [f]	*60.0 g*		
	1 [f]	*19.8 g*		
155	*100* [f]	*46.0 g*	N4	44
	10 [f]	*17.0 g*		
156	*100* [f]	*78.6 g*	N4	44
	10 [f]	*17.6 g*		
157	*100* [f]	*78.6 g*	N4	44
	10 [f]	*32.5 g*		
158	*100* [f]	*92.3 g*	N4	44
	10 [f]	*61.3 g*		
	1 [f]	*41.8 g*		
	0.1 [f]	*14.9 g*		
159	*100* [f]	*80.2 g*	N4	44
	10 [f]	*63.9 g*		
	1 [f]	*51.5 g*		
	0.1 [f]	*37.0 g*		
160	*100* [f]	*88.7 g*	N4	44
	10 [f]	*31.6 g*		
	1 [f]	*23.5 g*		
161	*100* [f]	*53.8 g*	N4	44
	10 [f]	*46.0 g*		
162	*1000* [f]	*80.2 g*	N4	44
	100 [f]	*43.9 g*		
163	*2500* [f]	*28.2 g*	N4	44
	1000 [f]	*35.0 g*		
186	100	92 (7.6) *	A1a	51,54-56
	50	100 (0.0) *		51
	25	53 (13.3) *		51,55
	10 [i]	53 (13.3) *		56
	1	43 (15.9) *		55,56
187	100	63 (7.8) *	A1a	51,54
	50	59 (4.2) *		51
	25	44 (4.5) *		
188	100	100 (0.0) *	A1a	51,54,55
	50	100 (0.0) *		51
	25	83 (10.3) *		51,55
	1	54 (14.4)		55
189	100	41 (18.6) *	A1a	54
190	100	48.9 (5.98) *	A1a	46,57
	10	43.0 (7.64) *		
193	100	12.9 (7.67)	A1a	46,57
	10	7.5 (6.98)		
194	100	10.4 (13.21)	A1a	46
	10	7.4 (10.41)		
195	100	23.7 (6.98)	A1a	46,57
		14.3 (9.24)	A1b	47
	10	21.9 (18.04)	A1a	46
196	100	6.5 (7.87)	A1a	46
		9.4 (8.54)		49
	10	7.5 (9.97)		46
		10.1 (8.31)		49
197	100	14.8 (6.98)	A1a	46
	10	12.6 (12.25)		
198	100	54.0 (9.72) *	A1a	58
199	100	-14.3 (12.18)	A1a	58
200	100	5.4 (9.29)	A1a	58
201	100	26.7 (11.23)	A1a	58
203	100	-21.1 (13.86)	A1a	58
204	100	30.4 (13.95) *	A1a	58
205	100	48.9 (5.98) *	A1a	46
		5.5 (8.95)		58
	10	40.9 (9.76) *		46
206	100	19.9 (12.87)	A1a	46
	10	18.5 (10.61)		
207	100	38.9 (16.98)	A1a	46
	10	33.1 (10.59)		
208a/b	100	23.7 (13.93)	A1a	49
(1:1)	10	12.5 (21.50)		
209a/b	100	-21.8 (10.37)	A1a	49
(j)	10	17.8 (12.49)		
210	100	-1.2 (5.02)	A1a	49
	10	-12.1 (9.15)		
211	100	-21.5 (9.67)	A1a	49
	10	-31.5 (12.87)		
212	100	8.8 (10.98)	A1a	57
213	100	21.0 (12.45) *	A1a	57
214	100	2.3 (13.47) *	A1a	57
217	100	14.8 (6.94)	A1a	46
	10	16.6 (7.42)		
218	100	-22.3 (9.04)	A1a	57
219	100	-30.6 (9.78) *	A1a	57
222	100	-9.9 (13.87)	A1a	46
	10	-18.6 (17.54)		

Table 2.1a - Continued

	conc. (ppm)	AI (sem) [b] (%)	assay	ref.
223	100	31.8 (11.87)	A1a	46
	10	25.6 (13.51)		
224	100	34.1 (13.71) *	A1a	58
225	100	-5.4 (12.00)	A1a	58
228	100	9.8 (11.87)	A1a	46
	10	8.7 (15.92)		
229	100	49.9 (3.87) *	A1a	46
	10	45.4 (9.61) *		
230	100	-9.8 (23.87)	A1a	46
	10	-2.6 (16.85)		
238	100	7 (21.2)	A1a	48
242	100	59 (5.2) *	A1a	48
243	100	66 (8.3) *	A1a	48
244	100	18 (8.9)	A1a	48
245	100	66 (8.4) *	A1a	48
246	100	-32.5 (7.51) *	A1a	49
	10	-17.7 (14.20)		
247	100	-11.7 (18.44)	A1a	49
	10	8.0 (11.63)		
248a/b (1:1)	100	6.8 (13.78)	A1a	49
	10	-29.5 (17.33)		
249	100	67.9 (10.29) *	A1a	49
	10	20.3 (7.24)		
250	100	-7.9 (11.93)	A1a	49
	10	5.5 (27.55)		
251	100	-22.3 (11.90)	A1a	49
	10	-34.3 (7.32)		
252	100	-45.0 (11.85)	A1a	49
	10	-36.1 (13.73)		
253	100	-42.7 (7.42) *	A1a	49
	10	6.8 (13.40)		
254	100	-43.2 (18.18)	A1a	49
	10	1.9 (12.32)		
255	100	13 (1.5)	A1a	48
256	100	-1.5 (11.29)	A1a	53
257	100	11 (2.4)	A1a	48
258	100	20 (13.3)	A1a	48
259	100	-7 (3.1)	A1a	48
260	100	39 (12.9) *	A1a	48
261	100	51 (10.4) *	A1a	48
264	100	1.8 (0.89)	A1b	47
266	100	-2.7 (7.93)	A1b	47
270	100	7.2 (8.24)	A1b	47
271	100	18 (4.8)	A1a	48
272	100	-16.9 (7.70)	A1a	49
	10	11.3 (4.30)		
273	1000	32 (8.8) *	A1a	56
	100	24 (4.7)		
275	*2500* [f]	*81.8* [g]	N4	44
	1000 [f]	*13.0* [g]		
276	*5000* [f]	*44.0* [g]	N4	44
	2500 [f]	*17.6* [g]		
277	*5000* [f]	*11.1* [g]	N4	44
	2500 [f]	*-14.5* [g]		
278	*2500* [f]	*62.6* [g]	N4	44
	1000 [f]	*16.3* [g]		
279	100	13.5 (5.33)	A1b	47
280	100	9.4 (9.33)	A1b	47
281	100	-5.3 (7.82)	A1b	47
303	10	20 (21.2)	A1a	59

Notes: (a) Values printed in italics are derived from the cited literature. (b) Mean two-choice Antifeedant Index, AI=[(C-T)/(C+T)]×100; sem = standard error of the mean. (c) Conc. originally given as 10^{-4} or 10^{-5} M. (d) Also insecticidal when injected into *Sp. litt.*; LD_{100}=0.001 ppm [ref. 38b]. (e) Minimum Inhibitory Concentration (MIC), which is the limiting concentration to cause 100% feeding inhibition. (f) Recalculated from the reported amount per leaf area in $\mu g/cm^2$. (g) Recalculated from the reported average FR_{50}; the corresponding standard deviation could not be converted and is therefore omitted. (h) Lower test concentrations omitted. (i) It is not clear whether the AI at 25 and 10 ppm is identical, or if a typing error has been made and '10 ppm' should read '25 ppm'. (j) Tested as mixture of regioisomers; ratio not known. (*) Significant difference between control and treatment discs (Wilcoxon's matched pairs test, $p<0.05$).

Table 2.1b: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and analogues against larvae of the Egyptian cotton leafworm (Lepidoptera: *Spodoptera littoralis*) as measured in no-choice feeding assays.[a]

	conc. (ppm)	AI (sem) [b] (%)	assay	ref.		conc. (ppm)	AI (sem) [b] (%)	assay	ref.		conc. (ppm)	AI (sem) [b] (%)	assay	ref.
86	100	*14 (14.8)*	A1d	48	**127**	100	*45 (19.1)*	A1d	48	**255**	100	*-7.8 (5.9)*	A1d	48
87	100	*0 (c)*	A1d	48	**128**	100	*6 (7.4)*	A1d	48	**257**	100	*5.9 (11.9)*	A1d	48
114	100	*6 (7.0)*	A1d	48	**129**	100	*49 (18.9)*	A1d	48	**258**	100	*9.8 (21.0)*	A1d	48
115	100	*71 (8.1)* *	A1d	48	**130**	100	*57 (20.0)*	A1d	48	**259**	100	*19.6 (10.3)*	A1d	48
117	100	*49 (19.1)*	A1d	48	**131**	100	*86 (6.2)* *	A1d	48	**260**	100	*15.7 (11.8)*	A1d	48
118	100	*33 (8.0)*	A1d	48	**132**	100	*0 (c)*	A1d	48	**261**	100	*68.8 (9.5)* *	A1d	48
120	100	*76 (6.3)* *	A1d	48	**238**	100	*43 (18.3)*	A1d	48	**262**	1000	*0-25* [d]	A3	17
121	100	*86 (8.2)* *	A1d	48	**242**	100	*65 (6.5)* *	A1d	48	**263**	1000	*0-25* [d]	A3	17
124	100	*41 (19.6)*	A1d	48	**243**	100	*86 (6.5)* *	A1d	48	**266**	e	f	g	60
125	100	*47 (18.9)*	A1d	48	**244**	100	*0 (c)*	A1d	48	**271**	100	*43 (23.6)*	A1d	48
126	100	*12 (16.4)*	A1d	48	**245**	100	*71 (5.7)* *	A1d	48					

Notes: (a) Values printed in italics are derived from the cited literature. (b) Mean no-choice Antifeedant Index, calculated from the reported average amounts consumed from the control (C) and treatment discs (T) according to: AI=[(C-T)/C]×100; standard error of the mean (sem) calculated from the corresponding sem's of C and T. (c) Standard error could not be calculated. (d) Originally reported as 'degree of antifeedant activity' with the same numerical value (see assay protocol A3). (e) Concentration not specified. (f) Reported as 'no significant activity'. (g) Assay on cotton (*Gosypium hirsatum*) leaves as feeding substrate; no-choice test not explicitly stated; no further details were given. (*) significant difference between control and treatment discs (Mann-Whitney U-test; $p<0.05$).

Table 2.1c: Reported AI_{50} values of natural clerodane diterpenes and related derivatives, estimated from two-choice or no-choice assays with larvae of the Egyptian cotton leafworm (Lepidoptera: *Spodoptera littoralis*) by means of probit analysis.

	AI_{50} [a] (ppm)	assay	ref.
	two-choice assay		
86	>1000	A1c	48
87	>1000	A1c	48
114	>1000	A1c	48
115	1	A1c	48
117	139	A1c	48
118	294	A1c	48
120	87	A1c	48
121	67	A1c	48
124	194	A1c	48
125	254	A1c	48
126	>1000	A1c	48
127	192	A1c	48
128	>1000	A1c	48
129	142	A1c	48
130	122	A1c	48
131	84	A1c	48
132	>1000	A1c	48
198	39	A1c	58
199	>1000	A1c	58
200	>1000	A1c	58
201	>1000	A1c	58
203	>1000	A1c	58
204	300	A1c	58
205	>1000	A1c	58
224	390	A1c	58
225	>1000	A1c	58
238	>1000	A1c	48
242	81	A1c	48
243	24	A1c	48
244	>1000	A1c	48
245	32	A1c	48
255	>1000	A1c	48
257	>1000	A1c	48
258	>1000	A1c	48
259	>1000	A1c	48
260	396	A1c	48
261	91	A1c	48
271	>1000	A1c	48
	no-choice assay[b]		
107	>1000	A1e	53
108	<1	A1e	53
122	>1000	A1e	54
123	870	A1e	54
189	0.4	A1e	54
256	930	A1e	53

Notes: (a) In the two-choice situation the AI_{50} is the concentration that gives an estimated Antifeedant Index of 50% [ref. 48,58], defined as AI=[(C-T)/(C+T)]×100. In the two-choice situation the AI_{50} is the estimated concentration required to decrease by 50% the amount eaten of a treated disc, relative to the control [ref. 53,54]; this corresponds to a no-choice Antifeedant Index value of AI=[(C-T)/C]×100=50%. (b) The denotation AI_{50} is preferred in this table over LD_{50}, as was used in the original literature [ref. 53,54], since the latter denotation is commonly used to refer to measurement of (insect)mortality instead of reduction in insect feeding.

Table 2.2: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and synthetic analogues against larvae of the fall armyworm (Lepidoptera: *Spodoptera frugiperda*) and the African armyworm (Lepidoptera: *Spodoptera exempta*) as measured in two-choice feeding assays.[a]

	conc. (ppm)	AI (sem) [b] (%)	assay	ref.
	Spodoptera frugiperda			
84	100	94.2 (1.55)	A1b	47
96	100	47.1 (7.31)	A1b	47
100	100	10.4 (6.74)	A1b	47
101	100	51.4 (5.57)	A1b	47
133	100	78.4 (3.80)	A1b	47
134	100	46.8 (9.13)	A1b	47
135	100	43.1 (7.63)	A1b	47
136	100	11.8 (14.14)	A1b	47
138	100	21.6 (15.85)	A1b	47
139	100	34.4 (13.34)	A1b	47
140	100	11.3 (4.27)	A1b	47
141	100	19.4 (5.75)	A1b	47
142	100	19.2 (6.95)	A1b	47
143	100	6.1 (8.44)	A1b	47
144	100	11.3 (9.49)	A1b	47
148	100	14.3 (3.81)	A1b	47
195	100	17.3 (7.14)	A1b	47
264	100	14.5 (5.91)	A1b	47
266	100	13.9 (9.34)	A1b	47
270	100	6.2 (7.62)	A1b	47
279	100	11.8 (6.84)	A1b	47
280	100	14.0 (6.41)	A1b	47
281	100	-3.3 (5.43)	A1b	47
303	10	18 (6.4)	A1a	59
	Spodoptera exempta			
84	100	38.0 (17.11)	A1b	47
96	100	*100* [c]	N2	38b,50,61
		73.1 (7.10)	A1b	47
97	100	*100* [c]	N3	38b,50,61
100	100	41.2 (4.97)	A1b	47
101	100	59.4 (3.49)	A1b	47
109	100	*100* [c]	N2	38b,61,62
133	100	75.8 (5.84)	A1b	47
134	100	94.4 (2.94)	A1b	47
135	100	51.4 (6.75)	A1b	47
136	100	84.2 (3.01)	A1b	47
138	100	64.8 (2.50)	A1b	47
139	100	63.6 (2.45)	A1b	47
140	100	55.2 (2.35)	A1b	47
141	100	36.4 (4.87)	A1b	47
142	100	34.2 (7.85)	A1b	47
143	100	26.6 (2.30)	A1b	47
144	100	75.0 (2.30)	A1b	47
148	100	29.3 (3.11)	A1b	47
195	100	44.4 (7.14)	A1b	47
264	100	19.5 (5.41)	A1b	47
266	100	14.9 (3.91)	A1b	47
270	100	26.7 (1.76)	A1b	47
279	100	34.2 (2.43)	A1b	47
280	100	9.1 (3.94)	A1b	47
281	100	13.3 (4.83)	A1b	47

Notes: (a) Values printed in italics were derived from the cited literature. (b) Mean two-choice Antifeedant Index, AI=[(C-T)/(C+T)]×100; sem = standard error of the mean. (c) Reported as Minimum Inhibitory Concentration (MIC), the limiting concentration to cause 100% inhibition of feeding.

Table 2.3: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and analogues against larvae of the tobacco cut leafworm (Lepidoptera: *Spodoptera litura*) as measured in two-choice feeding assays.[a]

	conc. (ppm)	AI[b] (%)	assay	ref.
133[c]	80[d]	*82-100*	N1	63
		100[e]		64,65
	50	*82-100*		63
		100[e]		65,66
	25	*54-82*		63
	12	*54-82*		
134[c]	50[d]	*82-100*	N1	63
		100[e]		65,66
	25	*54-82*		63
	12	*54-82*		
135	50[d]	*82-100*	N1	63
	25	*54-82*		
	12	*54-82*		
136[c]	50[d]	*82-100*	N1	63
		100[e]		65,66
	25	*54-82*		63
	12	*33-54*		
137	200[d]	*82-100*	N1	63
	100	*54-82*		
	80	*54-82*		
	50	*0-33*		
145	1000[f]	*0-33*	N1	63
146	1000[f]	*0-33*	N1	63
147	1000[f]	*0-33*	N1	63
164[c]	300	*100*[e]	N1	64,65
	200	*100*[e]		65,66
165	>1000	g	N1	64
166[h]	500	*100*[e]	N1	64
167[h]	15	*100*[e]	N1	64
168	>1000	g	N1	64
169	>1000	g	N1	64
170	>1000	g	N1	64
171	>1000	g	N1	64
172[h]	200	*100*[e]	N1	64,65
173[c]	200[d]	*82-100*	N1	63
		100[e]		65,66
	100	*0-33*		63
174[c]	200	*100*[e]	N1	65,66
175	80[d]	*82-100*	N1	63
		100[e]		65,66
	50	*54-82*		63
	25	*33-54*		
	12	*33-54*		
176[c]	500[d]	*82-100*	N1	63
	200	*54-82*		
	100	*0-33*		
177[c]	200[d]	*82-100*	N1	63
		100[e]		65,66
	100	*54-82*		63
	80	*54-82*		
	50	*54-82*		
	25	*33-54*		
	12	*33-54*		
178	1000	*54-82*	N1	63
	500	*33-54*		
	200	*0-33*		
	100	*0-33*		
179[c]	100	*100*[e]	N1	65,66
180	1000	*33-54*	N1	63
	500	*33-54*		
	200	*0-33*		
181	1000[f]	*0-33*	N1	63
182	1000[f]	*0-33*	N1	63
184	1000	*33-54*	N1	63
	500	*33-54*		
	200	*0-33*		
184[c]	200	*100*[e]	N1	65,66
185[c]	100	*100*[e]	N1	65,66
282	1000	*0-14.3*	N1	67,68

Table 2.3 - Continued

	conc. (ppm)	AI [b] (%)	assay	ref.
283	1000	*0-14.3*	N1	67,68
284	1000	*0-14.3*	N1	68
285	1000	*33.3-60.0*	N1	68
	500	*14.3-33.3*		
286	1000	*0-14.3*	N1	67,68
	500	*14.3-33.3*		
287	1000	*0-14.3*	N1	67,68
288	1000	*60.0-90.5*	N1	68
	500	*14.3-33.3*		
	250	*0-14.3*		
289	1000	*90.5-100*	N1	68
	500	*0-14.3*		
290	1000	*60.0-90.5*	N1	67,68
	500	*33.3-60.0*		
	250	*14.3-33.3*		
291	1000	*60.0-90.5*	N1	67,68
	500	*33.3-60.0*		
	250	*0-14.3*		
292	1000	*60.0-90.5*	N1	68
	500	*14.3-33.3*		
	250	*0-14.3*		
293	1000	*90.5-100*	N1	68
	500	*14.3-33.3*		
	250	*0-14.3*		
294	1000	*90.5-100*	N1	67,68
	500	*60.0-90.5*		
	250	*33.3-60.0*		
295	1000	*90.5-100*	N1	67,68
	500	*60.0-90.5*		
	250	*14.3-33.3*		
296	1000	*60.0-90.5*	N1	68
	500	*33.3-60.0*		
	250	*0-14.3*		
297	1000	*90.5-100*	N1	68
	500	*33.3-60.0*		
	250	*14.3-33.3*		
298	1000	*90.5-100*	N1	67,68
	500	*60.0-90.5*		
	250	*33.3-60.0*		
299	1000	*90.5-100*	N1	67,68
	500	*60.0-90.5*		
	250	*0-14.3*		
300	1000	*90.5-100*	N1	68
	500	*60.0-90.5*		
	250	*0-14.3*		
301	1000	*90.5-100*	N1	68
	500	*60.0-90.5*		
	250	*0-14.3*		
302	1000 [f]	*0-33.3*	N1	63

Notes: (a) Values printed in italics were derived from the cited literature. (b) Two-choice Antifeedant Index, AI=[(C-T)/(C+T)]×100; recalculated from the reported 'degree of antifeeding activity' (see assay protocol N1). (c) This compound has been classified by Munakata and coworkers as an 'absolute antifeedant' because, when applied at the concentration showing 100% antifeeding activity within 2 hrs, the larvae did not eat from the treatment discs even after an extended period of 24 hrs in a no-choice situation (the control discs in this two-choice assay were already eaten for over 90% after 2 hrs) and eventually starved to death [ref. 63,66]. (d) Higher test concentrations were omitted from the table. (e) Reported as the minimum concentration to cause 100% antifeeding activity (MIC). (f) Lower test concentrations were omitted from the table. (g) No 100% antifeeding activity found at 1000 ppm. (h) Compound acts as an 'absolute antifeedant' (see note c) at twice the concentration needed to cause 100% antifeeding activity within 2 hrs [ref. 64].

Table 2.4: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and analogues against larvae of the cotton bollworm (Lepidoptera: *Heliothis armigera*) or the tobacco budworm (Lepidoptera: *Heliothis virescens*) as measured in two-choice assays.

	conc. (ppm)	AI (sem) [a] (%)	assay	ref.
		Heliothis armigera		
84	100	63.2 (5.41) *	A1a,b	46,47
	10	16.9 (7.78)		
93	100	8.6 (10.41)	A1a	46
	10	2.4 (3.75)		
96	100	39.6 (9.65) *	A1a	46
	10	23.9 (9.86)		
98	100	23.9 (9.62)	A1a	46
	10	2.9 (5.65)		
101	100	50.2 (1.25)	A1b	47
134	100	37.4 (6.41)	A1b	47
139	100	24.5 (6.71)	A1b	47
190	100	29.8 (14.95)	A1a	46
	10	23.2 (6.58)		
193	100	3.9 (14.98)	A1a	46
	10	3.4 (14.67)		
194	100	4.6 (10.21)	A1a	46
	10	3.0 (18.41)		
195	100	11.7 (14.87)	A1a	46
		10.4 (4.17)	A1b	47
	10	19.5 (7.09)	A1a	46
196	100	4.9 (12.98)	A1a	46
	10	8.9 (15.98)		
197	100	8.8 (8.76)	A1a	46
	10	6.8 (3.71)		
205	100	23.9 (14.56)	A1a	46
	10	24.0 (7.22)		
206	100	16.9 (12.98)	A1a	46
	10	11.5 (5.68)		
207	100	24.8 (16.98)	A1a	46
	10	20.9 (7.98)		
217	100	9.8 (15.56)	A1a	46
	10	19.6 (6.46)		
222	100	-0.9 (11.87)	A1a	46
	10	-5.8 (5.0)		
223	100	18.9 (7.65)	A1a	46
	10	16.7 (13.65)		
228	100	11.9 (5.95)	A1a	46
	10	15.6 (6.52)		
229	100	29.6 (8.93)	A1a	46
	10	29.2 (8.55)		
230	100	2.8 (11.98)	A1a	46
	10	-2.9 (6.98)		
264	100	17.4 (4.72)	A1b	47
266	10	9.4 (4.11)	A1b	47
270	100	1.7 (7.41)	A1b	47
279	100	21.4 (5.41)	A1b	47
280	100	14.3 (5.95)	A1b	47
281	100	6.2 (7.21)	A1b	47
303	10	8 (11.3)	A1a	59
		Heliothis virescens		
84	100	91.8 (5.11)	A1b	47
101	100	48.6 (1.64)	A1b	47
195	100	12.7 (9.24)	A1b	47
264	100	14.7 (7.24)	A1b	47
266	100	9.1 (4.78)	A1b	47
	b	c	d	60
270	100	2.6 (1.67)	A1b	47
279	100	24.6 (1.34)	A1b	47
280	100	15.9 (5.44)	A1b	47
281	100	9.5 (7.94)	A1b	47
303	10	10 (3.4)	A1a	59

Notes: (a) Mean two-choice Antifeedant Index, AI=[(C-T)/(C+T)]×100; sem = standard error of the mean. (b) Test concentration was not specified. (c) Reported as 'no significant activity'. (d) Assay on cotton (*Gosypium hirsatum*) leaf; probably no-choice test, though not explicitly stated; no further details were given. (*) Significant difference between the amounts eaten from the control and treatment discs (Wilcoxon's matched pairs test, $p<0.05$).

Table 2.5: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and analogues against various other pest insect species of the order Lepidoptera.[a]

		conc. (ppm)	AI[b] (%)		assay	ref.
diamondback moth (*Plutella xylostella*) - larvae	**96**	*2500*[c]	*86.6* *** [d]	two-choice assay	N3	69
		250[c]	*85.6* *** [d]		N3	70
			68.5 * [d]		N3	69
		25[c]	*27.1* ** [d]		N3	70
			5.2 [d]		N3	69
		2.5[c]	*10.9* [d]		N3	70
	97	*250*[c]	*46.5* ** [d]		N3	70
		25[c]	*41.2* [d]			
large cabbage white butterfly (*Pieris brassicae*) - 5th instar larvae	**262**	100[e]	*95-100* [f]	no-choice assay	A3	17
		50	*75-95* [f]			
		25	*50-75* [f]			
	263	1000	*95-100* [f]		A3	17
		500	*75-95* [f]			
	274	1000	*0-25* [f]		A3	17
beet armyworm (*Spodoptera exigua*) - 5th instar larvae	**262**	1000	*0-25* [f]	no-choice assay	A3	17

Table 2.5 - Continued

		conc. (ppm)	AI [b] (%)		assay	ref.
European corn borer (*Ostrinia mubilalis*) - larvae	**164** and **172**	5000	*100* [g]	two-choice assay [h]	N1 [h]	65
Oriental tussock moth (*Euproctis subflava*) - larvae	**164** and **172**	1000	*100* [i]	two-choice assay [h]	N1 [h]	65
Acraea vesta[71] - larvae	**102**	50	*67-100* [j]	two-choice assay [k]	k	72
(Syn.: *Pareba vesta*)	**103**	200	*67-100* [j]	two-choice assay [k]	k	72
	104	200	*67-100* [j]	two-choice assay [k]	k	72
paddy armyworm (*Mythimna separata*)[71] - larvae	**102**	1000	*84.9* [l]	no-choice assay	N5	73
(Syn.: *Leucania separata*)	**231**	1000	*68.9* [l]	no-choice assay	N5	73
	232	1000	*100.0* [l]	no-choice assay	N5	73
	233	1000	*59.3* [l]	no-choice assay	N5	73
	234	1000	*27.3* [l]	no-choice assay	N5	73
	235	1000	*48.0* [l]	no-choice assay	N5	73
magpie moth (*Abraxas miranda*)[71] - larvae	**164**	5000	*100* [m]	two-choice assay [h]	N1 [h]	65
(Syn.: *Calospilos miranda*)	**172**	5000	*100* [m]	two-choice assay [h]	N1 [h]	65

Table 2.5 - Continued

		conc. (ppm)	AI [b] (%)		assay	ref.
pink bollworm (*Pectinophara gossypiella*)	**91**	100 µg/cm^2 [n]	≥ 82 [o]	two-choice assay [h]	N1 [h]	74
castor looper (*Achaea janata*)	**78**	p	q	p	p	75
	116	p	q	p	p	75

Notes: (a) Values printed in italics were derived from the cited literature. (b) Antifeedant Index, defined as AI=[(C-T)/(C+T)]×100 in the two-choice situation, or as AI=[(C-T)/C]×100 for no-choice assays. (c) Recalculated from the reported concentration in % (0.1% = *ca.* 250 ppm [ref. 69]). (d) Calculated from the reported amounts eaten from the control and treatment discs. (e) Higher concentrations were omitted from the table. (f) The value of this no-choice Antifeedant Index equals the originally reported 'degree of antifeeding activity' (see assay protocol N1). (g) Reported as '100% antifeeding activity'. (h) No details of this leaf disc assay were explicitly stated for this entry. (i) Originally reported as 'feeding was prevented'. (j) Reported as 'lowest effective concentration', which is assumed to be the lowest concentration to cause strong feeding inhibitory activity (*i.e.* >20% reduction in feeding, relative to control) according to ref. 76. (k) 'Host plant leaf disc method' with *Boehmeria nivea* leaves; assumed to be two-choice assay, however, no further details regarding this method were supplied, nor were any references given. (l) No-choice Antifeedant Index, defined as AI=[(C-T)/C]×100; value recalculated from the reported Antifeedant Index AI=[(C-T)/(C+T)]×100. (m) Reported as 'no inhibition of feeding below' specified concentration. (n) The concentration of the test solution could not be calculated from the reported data. (o) Originally reported as PC_{50}, which is the concentration at which less than 5% of the treatment discs are consumed in the time that over 50% of the control discs are eaten [ref. 41b]. (p) Not specified. (q) Reported as 'exhibited antifeedant activity'. (***) Significant difference between control and treatment discs, $p<0.001$. (**) $p<0.01$. (*) $p<0.05$.

Table 2.6: Reported insect antifeedant activities of natural clerodane diterpenes and related derivatives against larvae of the yellow mealworm (Coleoptera: *Tenebrio Molitor*), as measured in two-choice and no-choice feeding bioassays.[a]

	conc. (ppm)	two-choice AI [b] (%)	no-choice AI [c] (%)	assay	ref.
83	100	*84.0*	*54.12*	N6a,b	77,78,79
84	100	*8.0*	*-66.67*	N6a,b	77,79
85	100	*4.0*	*-37.87*	N6a,b	77,78,79
88	100	*18.0*	*5.07*	N6a,b	77,78
89	100	*88.0*	*55.22*	N6a,b	77,78,79
90	100	*84.0*	*54.52*	N6a,b	77,78,79
92	100	*26.0*	*6.39*	N6a,b	77,78,79
119	100	*18.0*	*-67.4*	N6a,b	77,78,79
192	100	*84.0*	*66.61*	N6a,b	77,79
220	100	*-18.0*	*-37.19*	N6a,b	77,79
221	100	*8.0*	*-68.24*	N6a,b	77,79
239	100	*86.0*	*70.77*	N6a,b	77,78,79
240	100	*6.0*	*10.2*	N6a,b	77,78
241	100	*32.0*	*2.37*	N6a,b	77,78,79

Notes: (a) Values printed in italics were derived from the cited literature. (b) Two-choice Antifeedant Index AI=[(C-T)/(C+T)] ×100; recalculated from the reported PFI value (see assay protocol N6b). (c) No-choice Antifeedant Index AI=[(C-T)/C]×100; recalculated from the original PFI value. The corresponding standard deviation was omitted (see assay protocol N6a).

Table 2.7: Reported insect antifeedant activities of natural clerodane and 19-nor-clerodane diterpenes against 4th instar larvae of the Colorado potato beetle (Coleoptera: *Leptinotarsa decemlineata*), as measured in two-choice, no-choice and choice vs. no-choice feeding assays.

	conc. (ppm)	two-choice AI (sem) [a] (%)	no-choice AI (sem) [b] (%)	two- vs. no-choice SI (sem) [c] (%)	assay	ref.
205	1000	23.8 (5.0) *	60.1 (3.2) ‡	18.7 (6.0)	N7a-c	80
	300	6.2 (6.9)	33.4 (6.3) ‡	6.9 (3.5)	N7a-c	80
	100	-7.8 (5.4)	-7.7 (11.1)	8.9 (5.9)	N7a-c	80
226	1000	32.9 (6.2) *	39.0 (5.2) ‡	20.9 (6.6)	N7a-c	80
	300	25.2 (4.8) *	7.3 (5.3)	4.3 (9.1)	N7a-c	80
	100	14.0 (8.4)	-10.1 (6.7)	12.3 (6.4)	N7a-c	80
227	1000	25.1 (5.8) *	77.8 (5.3) ‡	80.9 (2.8) ‡	N7a-c	80
	300	8.1 (4.6)	59.4 (2.6) ‡	52.9 (7.8) ‡	N7a-c	80
	100	5.5 (7.2)	45.6 (7.7) ‡	15.0 (8.8)	N7a-c	80
228	1000	34.0 (7.6) *	75.0 (5.9) ‡	76.3 (1.3) ‡	N7a-c	80
	300	8.4 (3.6)	51.0 (7.8) ‡	62.6 (2.3) ‡	N7a-c	80
	100	11.6 (4.6)	35.6 (9.3) ‡	18.3 (11.9)	N7a-c	80

Notes: (a) Two-choice assay: mean Antifeedant Index AI=[(C-T)/ (C+T)]×100; sem = standard error of the mean. (b) No-choice assay: mean Antifeedant Index AI=[(C-T)/C]×100. (c) Choice vs. no-choice assay: mean Suppression Index SI=[(C-T)/C]×100, in which C represents the amount eaten from the control discs in a no-choice assay, while T is the *total* amount consumed from *both control and treatment discs* in the corresponding two-choice assay. (*) Significant difference between control and treatment discs (Wilcoxon's signed rank test, $p \leq 0.05$). (‡) Significant difference between C and T (Dunnet two-tailed test, $p \leq 0.05$).

Table 2.8: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and analogues against other pest insect species of the order Coleoptera.[a]

		conc. (ppm)	AI[b] (%)		assay	ref.
mustard beetle (*Phaedon cochleariae*) - adults	**96**	*250*[c]	*100* ** [d]	two-choice assay	N3	70
		25[c]	*100* *** [d]			
		2.5[c]	*97.5* *** [d]			
		0.25[c]	*46.5* * [d]			
		0.025[c]	*17.9* * [d]			
	97	*25*[c]	*56.7* *** [d]	two-choice assay	N3	70
		2.5[c]	*7.7* * [d]			
		0.25[c]	*-5.0* [d]			
Western corn rootworm beetle (*Diabrotica virgifera vir.*) - adults	**245**[e]	ED_{50}=1.1 nmol/disc		f	f	81

Notes: (a) values printed in italics were derived from the cited literature. (b) Two-choice Antifeedant Index AI=[(C-T)/(C+T)] ×100. (c) Recalculated from the reported concentration in % (0.1% = *ca.* 250 ppm [ref. 69]). (d) Calculated from the reported amounts eaten from the control and treatment discs. (e) For comparison: azadirachtin displayed an ED_{50} of 0.39 nmol/disc in a similar bioassay [ref. 81]. (f) No details of the bioassay were specified. (***) Significant difference between treatment and control discs ($p<0.001$). (**) $p<0.01$. (*) $p<0.05$.

Table 2.9: Reported insect antifeedant activities of natural clerodane diterpenes, related derivatives and analogues against pest insect species from orders other than Lepidoptera or Coleoptera.[a]

		conc. (ppm)	AI[b] (%)		assay	ref.
Order: **Orthoptera**						
migratory locust (*Locusta migratoria*) - 5th instar larvae	**136**	100	*70*[c]	no-choice assay[d]	A2	82
	215	1000	*38*[c]	no-choice assay	A2	83
	216	100	*70*[c]	no-choice assay	A2	83
	264	100	*70*[c]	no-choice assay[d]	A2	82
	265	1000	e	no-choice assay[d]	A2	82
	266	1000	*72*[c]	no-choice assay[d]	A2	60
	267	1000	*<36*[c]	no-choice assay[d]	A2	60
	268	f	e	no-choice assay[d]	A2	82
	269	f	e	no-choice assay[d]	A2	82
desert locust (*Schistocerca gregaria*) - 5th instar larvae	**96**	*0.06*[g,h]	*100*[i]	two-choice assay	j	38b
vagrant grasshopper (*Schistocerca nitens nitens*) (Syn.: *S. vaga*[k])	**96**	*1000*[g]	*100*[i]	no-choice assay	l	38b
Order: **Homoptera**						
green peach aphid (*Myzus persicae*)	**96**	*2500*[m]	*12.5*[n]	two-choice assay	N3	81
	97	*250*[m]	*-6.7*[n]	two-choice assay	N3	81
Order: **Diptera**						
housefly (*Musca domestica*) - first instar larvae	**184**	4.5-8.6	o,p	no-choice assay	N8	39b
- third instar larvae		14.25-28.55	o,q			

Table 2.9 - Continued

		conc. (ppm)	AI (%)		assay	ref.
Order: **Hymenoptera**						
umbrella ant (*Atta cephalotes*)	**73** [r]	*5500* [s]	*47.4* ** [t]	two-choice assay	N9	84,85
leafcutting ant (*Acromyrmex octospinosus*)	**110**	0.50 mg/g [u]	*22.4* [t]	two-choice assay	N9	86
	111	0.50 mg/g [u]	*5,2* [t]	two-choice assay	N9	86
	236	0.33 mg/g [u]	*51.2* *** [t]	two-choice assay	N9	86
	237	0.33 mg/g [u]	*39.5* * [t]	two-choice assay	N9	86
Order: **Isoptera**						
subterranean termite (*Reticulitermes speratus*) - workers	**74**	*10000* [v]	*100* ‡ [w]	two-choice assay	N10	87
	75	*10000* [v]	*100* ‡ [w]	two-choice assay	N10	87
	76	*10000* [v]	*94.0* ‡ [w]	two-choice assay	N10	87
	77	*10000* [v]	*100* ‡ [w]	two-choice assay	N10	87
Insect species unspecified or unclear						
not specified	**99**	x	y	z	z	62
not specified	**112**	x	y	z	z	62
not specified	**113**	x	a a	x	x	88
not specified	**191**	bb	x	x	x	89
not specified	**202**	bb	x	x	x	89
Prodenia litura (Syn.:[71] *Spodoptera litura* or *Sp. littoralis*)	**193**	400	*67-100* [cc]	two-choice assay[dd]	ee	90

Table 2.9 - Continued

Notes: (a) Values printed in italics were derived from the cited literature. (b) Antifeedant Index, defined as AI=[(C-T)/(C+T)]×100 in the two-choice situation, or as AI=[(C-T)/C]×100 for no-choice assays. (c) The value of this no-choice Antifeedant Index equals the originally reported 'percentage of feeding inhibition' (see assay protocol A4). (d) No-choice assay not explicitly stated; see assay procedure A4 for discussion. (e) Activity reported as 'negligible' or 'not significant'. (f) Concentration not specified. (g) Recalculated from the reported concentration in µg/l. (h) Considering this low concentration, the qualification of this compound as a 'relatively strong' antifeedan [ref. 38b] against *Schist. gregaria* appears an understatement. (i) Originally reported as Minimum Inhibitory Concentration (MIC), which is the limiting concentration needed to cause 100% feeding inhibition. (j) Two-choice assay on glass fiber filters, impregnated with 0.1 M sucrose; no further details supplied. (k) The insect species was originally reported as *Schistocerca vaga*, which is synonymous to *S. nitens nitens* (other synonyms: *Gryllus nitens* or *Acridium vagum* [ref. 91]). (l) No-choice assay on leaves from corn seedlings; no further details supplied. (m) Recalculated from the reported concentration in % (0.1% = *ca.* 250 ppm [ref. 69]). (n) Calculated from the reported number of aphids settled on the control and treatment discs. (o) Decreased weight gain of the larvae during 144 hrs, relative to control larvae. (p) Dose-dependent delayed pupariation, decreased weight of puparia and increased mortality during the moult, all relative to control larvae. (q) Dose-dependent accelerated pupariation, decreased weight of puparia and increased mortality during the moult, all relative to control larvae. (r) Contrary to the statement in the review of Merrit and Ley [ref. 6], at the tested concentrations kolavenol (**75**) did *not* exhibit significant toxic activity towards either the ants themselves or towards the mutualistic ant fungus present in the fungal gardens of the *A. cephalotes* colony, which is used as the sole food source for the ant larvae [ref. 85]. (s) Recalculated from the reported concentration in mg/ml; 1.0 mg/ml corresponds to an approx. final amount of 20 µg of compound per flake. (t) Calculated from the reported number of control and treatment flakes taken. (u) Represents mg of compound per g of rye flakes. (v) Recalculated from the reported concentration in % (see assay protocol N10). (w) The originally reported antifeedant index AI=[T/(C-T)]×100 was converted into the standard two-choice Antifeedant Index as defined in note b; see assay protocol N10. (x) Not specified. (y) Reported as 'no antifeeding activity'. (z) Not specified; probably two-choice leaf disc assay. (aa) Melisodoric acid (**114**) was reported to 'show antifeedant activity'. (bb) Compound 'appeared to be devoid of such [antifeedant] activities' [ref. 85]. (cc) Originally reported as 'threshold concentration', which is the lowest concentration needed to cause strong feeding inhibitory activity (*i.e.* >20% reduction in feeding, relative to control) according to ref. 76. Recalculated according to the procedure described in assay protocol N1. (dd) Two-choice situation not explicitly stated. (ee) Leaf disc assay, similar to assay protocol N1. Cabbage leaf discs (15 mm diam.) were used and the compound was tested at three concentrations (10000, 2000 and 400 ppm) with three replicates per treatment. Feeding inhibitory activity was determined after 5 hrs. (***) Significant difference between control and treatment flakes ($p<0.001$). (**) $p<0.005$. (*) $p<0.01$. (‡) Significant difference between control and treatment discs (Mann-Whitney U-test, $p<0.05$).

Table 2.10: Assay protocols and recalculation procedures for the entries in Tables 2.1-2.9.

A - Assays using Artificial Substrates

A1a - The artificial substrate used consisted of glass-fibre discs (Whatman GF/A, 2.1 cm diam.), made palatable by the addition of 100 µl of an aqueous 50 mM solution of sucrose. The treatment discs were subsequently treated with 100 µl of a solution of the test compound in ethanol at a concentration specified in the tables. The discs were dried and weighed before being presented to the insects. In this two-choice feeding assay, insects (final instar larvae of *Sp. littoralis, Sp. frugiperda, Hel. virescens* or *Hel. armigera*), deprived of food for 4 hrs, were placed individually in Petri dishes (8.5 cm diam.), each with a control and treatment disc. Usually, 10-20 replicates[a] per concentration were used. The duration of the bioassay varied between treatments, since the assay was terminated after 50% of either disc was eaten or after 12, 18 or 24 hrs[a] if the insects had not eaten 50% of either disc. The discs were dried and then reweighed to determine the mass eaten of control (C) and treatment discs (T), from which the two-choice Antifeedant Index was calculated per replicate, according to $AI = [(C-T)/(C+T)]\times100$. The mean AI per test compound (and, if appropriate, per test concentration) and the corresponding standard error of the mean ($SEM = \sigma/\sqrt{n}$) were reported and are reproduced in the tables. The Wilcoxon matched pairs test was used to determine the statistical significance of the difference between control and treatment discs [ref. 48,58,59].

A1b - Protocol identical to A1a, except for the additional presence of 50 mM of sodium chloride in the employed sucrose solution. The sodium chloride was required as an electrolyte in the electrophysiological assays that were performed parallel to the feeding assays and its inclusion in the assay substrate allows both types of assay to be compared directly [ref. 47].

A1c - Protocol as in A1a. The compounds were tested at four concentrations (10, 100, 500 and 1 or 1000[a] ppm). Probit analysis was used to estimate the concentration required to obtain an antifeedant index of 50% (*i.e.* AI_{50}) [ref. 48,58].

A1d - In this no-choice feeding assay the test compounds are evaluated for their ability to suppress insect feeding for an extended period of time. The substrate and experimental protocol were similar to protocol A1a; however, in this assay the individual insects were presented with only a single disc from either the treatment or the control group (10 replicates per compound were used). Treatment and control assays were performed simultaneously and the bioassays were terminated when approx. 50% of the control discs had been consumed. The assay results were reported as the mean amount eaten of treatment (T) and control (C) discs; the statistical significance of the difference between C and T was evaluated via the Mann-Whitney U-test [ref. 48].

Table 2.10 - Continued

For inclusion in the tables the reported mean C and T were used to calculate the corresponding no-choice Antifeedant Index, according to AI = [(C-T)/C]×100, with the corresponding SEM calculated from the reported SEM's for C and T according to the standard rules for the carry-over of errors in calculated quantities.

A1e - Protocol as in A1d. The compounds were tested at six concentrations (0.01, 0.1, 1, 10, 100 and 1000 ppm) with 15 replicates per concentration. The assay was terminated after 16 hrs, the time needed for the insects to consume 50% of the control discs. Probit analysis was used to estimate the AI_{50},[b] which is the concentration required to reduce the amount eaten from the treated discs in 16 hrs by 50%, relative to the control discs [ref. 53,54].

A2 - This assay is poorly described in the cited literature [ref. 60,82,83]. The substrate consisted of glassfibre GF/A discs, made palatable with 5% glucose solution. Based on the similarity of the cited studies and the comparisons made by the authors between the obtained results, it seems likely that in all these studies a no-choice test was used, although this was only explicitly stated in ref. 83. The antifeedant activity was reported as the percentage of feeding inhibition, which is probably defined (analogously to A3 and N1) as:

$$\% \text{ feeding inhibition} = \left(1 - \frac{T}{C}\right) \times 100\% .$$

In the tables, this feeding inhibition was converted to the standard no-choice Antifeedant Index, AI = [(C-T)/C]×100. As in A3, the numerical value of this no-choice AI is identical to the originally reported feeding inhibition.

A3 - The substrate in this no-choice assay consisted of styropor lamellae (6 × 3 cm, density 0.015), which were dipped[b] into a 1:1 mixture of ethanol containing the test compound at the concentration indicated in the table (or solvent alone for the controls), and a 0.25 M solution of sucrose in water. After drying (30 min at 50°C) the lamellae were weighed and placed individually in the test arenas (10 × 7 × 3 cm), together with a moistened cotton wad in a plastic cover (to fulfil the drinking need of the larva). In each arena, one insect (fifth instar larvae of *Sp. littotalis*, *Sp. exigua* or *P. Brassicae*, starved for 3 hrs) was introduced and the amount of feeding was determined after 24 hrs by drying and reweighing the remaining substrate. The antifeeding activity was determined and reported in five classes, similar to protocol N1 [ref. 17].

In the tables, the originally reported antifeeding activity is included as the corresponding standard no-choice Antifeedant Index, according to AI = [(C-T)/C]×100.[c]

Table 2.10 - Continued

N - Assays using Natural Substrates

N1 - In this 'leaf disc method' the substrate consisted of sweet potato (*Ipomoea batatas*) leaf discs (20 mm diam). These discs were either dipped[b] momentarily into an acetone solution containing the test compound at the concentration indicated in the tables (treatment discs), or into acetone alone (control discs), and were air-dried. For a two-choice test, two treatment discs and two control discs were placed in a plastic cup (10 cm diam) and ten insects (third or fouth instar larvae of *Sp. litura*) were introduced. The assay was terminated when about 50% of the leaf area of the control discs was consumed (usually within 2 hrs). The consumed areas of control and treatment discs, respectively C and T, were measured and the feeding ratio FR = (T/C) was calculated. This ratio was used as an index of the degree of antifeeding activity of the test compound [ref. 65]:

Feeding Ratio	vs.	Antifeeding Activity
$FR = \left(\frac{T}{C}\right) \times 100\%$		100 - FR (%)
+ + + (0-10%)		+ + + (90-100%)

The degree of antifeeding activity of a test compound was usually reported as belonging to one of four (or five[a,d]) classes, *i.e.* as + + + (90-100%), + + (70-90%), + (50-70%) or (0-50%). An alternative quantity that has also been used is the concentration needed to obtain 100% antifeeding activity within 2 hrs (similar to MIC, see N2) [ref. 63-68].

For inclusion in the tables, the reported antifeeding activity was converted into the standard two-choice Antifeedant Index, AI = [(C-T)/(C+T)]×100. The equation:

$$T = \left(\frac{FR}{100}\right) \times C$$

allows T to be expressed as a multiple of C; subsequently the corresponding AI can be calculated. Test compounds for which the antifeeding activity was reported as a class are included in the tables with their corresponding AI-range (*e.g.* 70-90% antifeeding activity equals an AI ≈ 54-82%).

N2 - This leaf disc assay is similar to N1. Of the ten leaf discs (maize, *Zea mays*, for tests with *Sp. littoralis* (instar unknown); *Ricinis communis* with third instar larvae of *Sp. exempta*; [ref. 50]) used in this two-choice asssay, five were dipped[a] for 2 sec into a solution of the test compound in acetone and five were dipped in acetone only. The discs were placed in a Petri-dish in an alternate arrangement and ten insects were introduced. The assay duration varied from 2-24 hrs, depending on the potency of the test compound, during which the antifeeding activity was scored at regular intervals. The activity was reported as the Minimal Inhibitory Concentration, MIC, which is the minimal concentration required to completely inhibit the insects from feeding on the treatment discs [ref. 38b,50,61,62].

In the tables, these results were entered as AI = 100, with a note indicating the original measurement being a MIC.

Table 2.10 - Continued

N3 - The substrate for this two-choice assay consisted of a leaf of Chinese cabbage, placed in a Petri dish. A solution of the test-compound in ethanol at the concentration indicated in the table was painted[e] on to one half of the leaf (T), while the other half was treated with solvent only (C). An unspecified number of insects were introduced into the dish and after 2,[f] 24[g] or 48[h] hrs the antifeedant activity was evaluated. Per treatment 7[f]-10[h] replicates were used. The antifeedant activity was reported as either the average areas[g,h] of leaf consumed from, or the average number[f] of aphids settled on, respectively, C and T. The statistical significance of the difference between C and T was also determined [ref. 69,70].

For inclusion in the tables the reported average C and T were used to calculate the corresponding two-choice Antifeedant Index, according to AI = [(C-T)/(C+T)]×100.

N4 - The assays were performed in polythene Petri dishes (8.5 cm diam.) containing eight lettuce (*Lactuca sativa*) leaf discs (area 1 cm^2). For the two-choice test the leaf discs were alternately treated with either 10 µl of a solution of the test compound in acetone at the concentration indicated in the table[i] or with solvent alone. Five insects (fifth instar larvae of *Sp. littoralis*) were placed in each test arena and subsequently the consumed areas of the treated discs (CTD) and the control discs (CCD) were simultaneously measured every 30 min during the entire assay duration (4-5 hrs). Depending on the test compound, 2-10 replicates of each test were performed. The antifeedant activity was expressed as the mean Feeding Ratio, FR = [CTD/CCD], in combination with the SEM. For comparative purposes the authors recommended the use of FR_{50},[j] which is the Feeding Ratio when 50% of the total *control* area has been consumed. The required CTD_{50} and CCD_{50} values were obtained for each separate test by extrapolation of the nearest empirical values [ref. 44].

For inclusion in the tables, the reported FR_{50} value was recalculated to represent the standard two-choice Antifeedant Index, defined as AI = [(C-T)/(C+T)]×100. On the assumption that $CCD_{50} \equiv 50\%$, CTD_{50} can be calculated from FR_{50} according to:

$$FR_{50} = \frac{CTD_{50}}{CCD_{50}} \equiv \frac{CTD_{50}}{50} \leftrightarrow CTD_{50} = 50 \times FR_{50}.$$

Through substitution of CCD_{50} and CTD_{50} for C and T, the corresponding standard AI can be obtained. However, since the magnitude of the error associated with the assumption $CCD_{50} \equiv 50\%$ is unknown, it is *not* possible to calculate the SEM of this index (original SEM was ~0-36% of FR_{50} value).

N5 - No-choice assay at 1000 ppm concentration on fresh maize (*Zea mays*) leaf discs, three replicates per compound; no further details presented.[k] The activity was given as a (two-choice) Antifeedant Index AI = [(C-T)/(C+T)]×100 [ref. 73].

For inclusion in the tables the reported AI value was recalculated to represent the standard no-choice Antifeedant Index, defined as AI = [(C-T)/C]×100. With the use of the reported AI value, T can be expressed as a multiple of C. Substitution of this expression [1] into the definition of the

Table 2.10 - Continued

no-choice Antifeedant Index subsequently yields equation [2], from which the required AI value can be calculated.

$$AI = \left(\frac{C-T}{C+T}\right)\times 100 \leftrightarrow -T = \left[\left(\frac{AI}{100}-1\right)\Big/\left(\frac{AI}{100}+1\right)\right]\times C$$

$$= \text{const.} \times C \qquad [1]$$

$$AI = \left(\frac{C-T}{C}\right)\times 100 \xrightarrow{[1]} AI = (1 + \text{const.})\times 100 \qquad [2]$$

N6a - Carrot slices (2.5 cm diam., 0.5 cm thick) were coated either with 100 µl of an emulsion of the test compound in a mixture of water/methanol/acetone (90:5:5) and Triton CS-7 (0.1% v/v) at a concentration specified in the tables, or with 100 µl solvent blank alone. After drying the slices were weighed. For a no-choice assay, either six treatment slices or six control slices were presented to 20 insects in a plastic box; each test was performed in duplicate and repeated eight times. The slices were removed, reweighed and renewed every 24 hrs for the entire assay duration of 10 days. From the percentages consumed from the treated (T) and control slices (C) the antifeedant activity was calculated, which was expressed as the average Percentage of Feeding Inhibition PFI = [T/(C+T)]×100, and was reported together with the corresponding standard deviation [ref. 77-79].

For inclusion in the tables, the reported PFI value was recalculated to represent the standard no-choice Antifeedant Index, defined as AI = [(C-T)/C]×100. With the use of the reported PFI value, the required C can be obtained as a multiple of T through the following equation:

$$PFI = \left(\frac{T}{C+T}\right)\times 100 \leftrightarrow C = \left(\frac{100}{PFI}-1\right)\times T.$$

From this equation, all terms included in the definition of the Antifeedant Index can be expressed as multiples of T and the AI value can thus be calculated from the reported PFI value. However, the corresponding standard deviation *cannot* be calculated from the reported SD for the PFI, since it is not possible to express C or T in terms of PFI only, and was therefore omitted from the table. The standard deviation of the original PFI ranged from 5 to 16% of the reported value.

N6b - The protocol for this two-choice assay is largely identical to protocol N6a; however, in this case three treatment discs (T) and three control discs (C) were used per test arena and the assay duration was only 24 hrs. The antifeedant activity was expressed as the average Percentage of Feeding Inhibition PFI = [T/(C+T)]×100; no standard deviation reported [ref. 77].

For inclusion in the tables, the reported PFI value was recalculated to represent the standard two-choice Antifeedant Index AI = [(C-T)/(C+T)]×100, according to the procedure described under N6a.

Table 2.10 - Continued

N7a - The test arena consisted of Petri dishes (9 cm diam.), coated on their bottom half with 20 ml of 2.5% agar solution. Six potato (*Solanum tuberosum*) leaf discs (1.77 cm^2) were fitted in holes in the agar layer. For the choice assay the discs were alternately treated on the upper surface with either 10 µl of a solution of the test compound in acetone at the concentration indicated in the table or with solvent alone. After complete evaporation of the solvent, two insects (fourth instar larvae of *L. decemlineatae*), starved for 6 hrs, were placed in each test arena. Assays were conducted with 10 replicates per treatment and were terminated after consumption of 50% of the discs (control plus treated) or after 24 hrs. The remaining discs were oven-dried (60°C, two days) and weighed; comparison of their weights with the initial dry leaf disc weight (estimated from the mean weight of 100 oven-dried discs) lead to the mass eaten of control (C) and treatment discs (T), respectively. From these data the average two-choice Antifeedant Index AI = [(C-T)/ (C+T)]×100 and the corresponding standard deviation were calculated. Wilcoson's matched pairs test was used to determine the statistical significance of the difference between control and treatment discs [ref. 80].

N7b - The experimental set-up and protocol of the no-choice assay are similar to N6a; however, the test arena now only contained either treatment or control discs (six discs per arena). Treatment and control assays were performed simultaneously and the assays were terminated after 75% of the control discs was consumed. The average no-choice Antifeedant Index AI = [(C-T)/C]×100 and corresponding standard deviation were calculated and the significance of the difference between control and treatment discs was determined via the Dunnet two-tailed test [ref. 80].

N7c - For this choice vs. no-choice assay, a two-choice assay and a no-choice assay were simultaneously performed with the same test compound at equal concentrations, according to protocols N6a and N6b, respectively. The average Suppression Index was calculated from the equation SI = [(C-T)/C]×100, in which C represents the consumption from the control discs in the no-choice assay, while T represents the *total* consumption from *both control and treatment discs* in the choice assay. The significance of the difference between the consumption in both situations was determined via the Dunnet two-tailed test [ref. 80].

N8 - To asses housefly (*Musca domestica*) feeding and development in a no-choice situation, 6 g of flocked filter paper was stirred in a beaker while being sequentially treated with 8 ml of a solution containing the test compound in acetone (or solvent alone for the control) and, after air drying, with a solution of milk powder (2.7 g), corn starch (2.7 g) and dried yeast (0.6 g) in 26 ml of water. The treatment concentration was calculated, based on the total weight of the dry components of the diet. The medium was divided over three tubes (25 × 100 mm) and covered with muslin to obtain three replicates per treatment. Ten third instar larvae were used per replicate. The weight of the larvae, the number of larvae pupariating and the mortality rate were determined at regular intervals over a 6-day period [ref. 39b].

Table 2.10 - Continued

N9 - The substrate of this two-choice ant feeding deterrency bioassay consisted of pressed rye flakes, which were soaked in a solution containing the test compound at the indicated concentration (treatment flakes) or in solvent only (control flakes). For the assay 60 control and 60 treatment flakes were randomly arranged in a grid-like fashion. After 50% of the control flakes had been taken by the ants, the assay was terminated and the numbers of control and treatment flakes harvested by the ants were counted and reported. The significance of the difference between C and T was determined via a modified binomial test [ref. 85].

N10 - The test arena consisted of a Petri dish (55 mm diam.), coated on the bottom with a layer of agar (10 g/l) which was covered with sand. Filter paper discs (Whatman no.1, 2.0 cm diam.), placed on aluminum foil, were used as the feeding substrate. For this choice test, the Petri dish contained one disc treated with a 1% solution (25 µl) of the test compound (1% solution ≡ 80 µg compound/cm^2, which results from the use of a 10000 ppm solution [ref. 87]) and one disc treated with solvent; three replicates per treatment were used. Termites were allowed to feed on the discs for 14 days before the amount of feeding was measured, based on the remaining areas of the treatment and control discs, relative to the average initial disc area. The feeding inhibitory activity was reported as an Antifeedant Index, AI=[T/(C+T)]×100. The significance of the difference between C and T was evaluated via the Mann-Whitney U-test [ref. 87].

For inclusion in the tables, the reported AI value was converted to the standard two-choice Antifeedant Index, AI=[(C-T)/(C+T)]×100, based upon the expression of C as a multiple of T through the equation:

$$AI = \left(\frac{T}{C+T}\right)\times 100 \;\leftrightarrow\; C = \left(\frac{100}{AI} - 1\right)\times T\,.$$

Notes: (a) Depending on the study. (b) Due to this dipping procedure, the actual amount of test compound per lamellar area is unknown. (c) Contrary to protocol N1, however, in this case the values of the *no-choice* AI range are identical to the original activity class, as can be easily shown by calculations as in N1. (d) The five classes used were: + + + + (95-100%), + + + (75-95%), + + (50-75%), + (25-50%) and - (0-25%) [ref. 67,68]. (e) Since the total volume of the solution painted on the leaf was not reported, the actual amount of test compound per leaf area is unknown. (f) Assay with *Myzus persicae*. (g) With larvae of *Plutella xylostella*. (h) With adults of *Phaedon cochleariae*. (i) Recalculated from the given final amount of compound on the leaf in µg/cm^2, using the stated leaf area and the volume of the solution applied onto this area. The ppm-scale (0.01 µg/cm^2 dose equals 0.3 ppm) stated in the original article (and also cited in a previous review, ref. 41b) represents the amount of compound per dry leaf disc weight (average weight 33.65±0.07 mg; 0.01 µg of compound per leaf thus equals 3.0×10^{-5} wt% = 0.3 ppm). (j) FR_{75} was also reported, but is not included in these tables. (k) A note inserted in the no-choice assay protocol refers to the two-choice assay A1b in ref. 47.

2.5 - Notes and References

1. L. Ruzicka, A. Eschenmosher and H. Heusser, *Experientia*, **9**, 357 (1953).
2. D. Rogers, G.G. Ünal, D.J. Williams, S.V. Ley, G.A. Sim, B.S. Joshi and R. Ravindranath, *J. Chem. Soc. Chem. Comm.*, 97-99 (1979).
3. F. Piozzi, *Heterocycl.*, **15** (2), 1489-1503 (1981).
4. J. Rigaudy and S.P. Klesney, *IUPAC nomenclature of organic chemistry - Section F*, 4th Edition, Pergamon Press, Oxford, 1979.
5. The clerodane diterpene cascarillin, a bitter principle from the bark of cascarilla (*Croton eleuteria*), was already described in 1845, though its definite structure determination was not completed until 1962. See ref. 17, p. 1 and references cited therein.
6. A.T. Merritt and S.V. Ley, *Nat. Prod. Reports*, 243-287 (1992).
7. For instance, for a more recent review on new *neo*-clerodanes isolated from *Teucrium* ssp., see: F. Piozzi, *Heterocycl.*, **37** (1), 603-626 (1994).
8. Oxygenation pathways which have been considered are for instance: (a) allylic oxidation; (b) ene reaction; (c) epoxidation, followed by rearrangement. See: E. Fujita, Y. Nagao and M. Node, *Heterocycl.*, **5**, 793-838 (1976).
9. J.D. White and P.S. Manchand, *Bioorg. Chem.*, **11**, 337 (1978).
10. F. Piozzi, B. Rodriguez and G. Savona, *Heterocycl.*, **25**, 807-841 (1987).
11. Montanin A and B have been co-isolated from *T. montanum*; see: P. Malakov, G. Papanov and N. Mollov, *Tetrahedron Lett.*, 2025-2026 (1978).
12. A.G. González, J.R. Herrera, J.G. Luis, A.G. Ravelo, M.L. Rodriguez and E. Ferro, *Tetrahedron Lett.*, **29** (3), 363-366 (1988).
13. S. Pahup, C.S. Mahesh, C.J. Krishna and F. Bohlman, *Phytochem.*, **24**, 190 (1985); F. Bohlman, C. Zdero and S. Huneck, *Phytochem.*, **24**, 1027 (1985).
14. B. Esquivel, J. Cárdenas, A. Toscano, M. Soriano-García and L. Rodríguez-Hahn, *Tetrahedron*, **41** (16), 3213-3217 (1985).
15. L. Rodríguez-Hahn, B. Esquivel, A-A. Sánchez, J. Cárdenas, O.V. Tovar, M. Soriano-García and A. Toscano, *J. Org. Chem.*, **53**, 3933-3936 (1988).
16. W. Templeton, *An introduction to the chemistry of the terpenoids and steroids*, Butterworth&Co., London, 1969.
17. J.M. Luteyn, *Investigations into the total synthesis of insect antifeedant clerodanes*, Ph-D thesis, Wageningen Agricultural University, Wageningen, 1982
18. L.J. Valdes III, W.M. Butler, G.M. Hatfield, A.G. Paul and M. Koreeda, *J. Org. Chem.*, **49**, 4716-4720 (1984).
19. E. Kitazawa, A. Sato, S. Takahashi, H. Kuwano and A. Ogiso, *Chem. Pharm. Bull.*, **28** (1), 227-234 (1980).
20. *Biochemical assessment of anti-inflammatory compounds from medicinal plants.* In: S.J. Achmad, L. Makmur and F. Harjanto, *Progress in chemistry of medicinal plants in Asia*, Proceedings of the sixth Asian symposium on medicinal plants and species, January 24-28, 1989, Bandung, Indonesia, p. 515-524.
21. H. Itokawa, N. Totsuka, K. Takeya, K. Watanabe and E. Obata, *Chem. Pharm. Bull.*, **36** (4), 1585-1588 (1988).

22. X. Ma, I.-S. Lee, H.-B. Chai, K. Zaw, N.R. Farnsworth, D.D. Soejarto, G.A. Cordell, J.M. Pezzuto and A.D. Kinghorn, *Phytochem.*, **37** (6), 1659-1662 (1994)

23. C. Nishino, K. Kawaza and T. Mitsui, *Agr. Biol. Chem.*, **35** (12), 1921-1930 (1971).

24. T. Anthonsen and R. McCrindle, *Acta. Chim. Scand.*, **23**, 1068-1070 (1969); S. Manabe and C. Nishino, *Tetrahedron*, **42**, 3461-370 (1986).

25. H. Hagiwara, K. Inome and H. Uda, *J. Chem. Soc. Perkin Trans I*, 757-764 (1995) and references cited therein.

26. S. Habtemariam, A.I. Gray and P.G. Waterman, *Planta Med.*, **58**, 109 (1992).

27. H. Chen, R.X. Tan, Z.L. Liu and Y. Zhang, *J. Nat. Prod.*, **59**, 668-670 (1996).

28. N.R. Andersen and P.R. Rasmussen, *Tetrahedron Lett.*, **25** (4), 465-468 (1984) and *Ibid*, **25** (4), 469-472 (1984).

29. N.R. Andersen *et al*, *J. Antibiot.*, **36**, 753-760 (1983).

30. P.G. Waterman, *J. Nat. Prod.*, **53**, 13-22 (1990).

31. T. Tamamura *et al*, *J. Antibiot.*, **38**, 1664-1669 (1985), *Ibid*, **38**, 1819-1821 (1985) and *Ibid*, **41**, 648-654 (1988); K. Isshiki *et al*, *J. Antibiot.*, **39**, 1634-1635 (1986).

32. H. Wu, H. Nakamura, J. Kobayashi, M. Kobayashi, Y. Ohizumi and Y. Hirata, *Bull. Chem. Soc. Jpn.*, **59**, 2495-2504 (1986).

33. H. Wagner, R. Seitz, H. Lotter and W. Herz, *J. Org. Chem.*, **43** (17), 3339-3345 (1978).

34. The classical view on the biological role of terpenes is that most are waste products of metabolic routes to essential plant hormones and carotenoids. However, evidence from tracer studies shows that in plant tissues many of such terpenes are transferred into higher terpenes and that they are thus by no means inert, but appear to play a dynamic role in the plant's metabolism. It has been suggested that terpenes might be produced by plants especially during periods of dormancy in order to keep the enzyme systems in an active state (*e.g.* to keep respiratory coenzymes in a reduced form, etc.). See for instance: D.V. Banthorpe and B.V. Charlwood, *Biogenesis of terpenes.* In: A.A. Newman (Ed.), *Chemistry of terpenes and terpenoids*, Academic Press, London, 1972, p. 337-411, and references cited therein.

35. S. Huneck, Y. Asakawa, Z. Taira, A.F. Cameron, J.D. Connolly and D.S. Rycroft, *Tetrahedron Lett.*, **24**, 115-116 (1983).

36. S. Huneck and K. Schreiber, *Phytochem.*, **11**, 2429-2434 (1972).

37. M.D. Cole, P.D. Bridge, J.E. Dellar, L.E. Fellows, M.C. Cornish and J.C. Anderson, *Phytochem.*, **30** (4), 1125-1127 (1991).

38. (a) Insecticidal activities: ajugarin I (**3**) on Egyptian cotton leaf worm (*Spodoptera littoralis*): LD_{100} = 0.001 ppm [ref. 38b]; ajugarin IV (**64**) on silkworm (*Bombix mori*): LD_{95} = 500 ppm [ref. 38c]. (b) I. Kubo and K. Nakanishi, *Some terpenoid antifeedants from tropical plants.* In: H. Geissbühler (Ed.), *Advances in pesticide science: plenary lectures presented at the 4th international congress of pesticide chemistry, Zürich 1978*, Pergamon press, Oxford, 1979, p. 284-294. (c) I. Kubo, J.A. Klocke, I. Miura and Y. Fukuyama, *J. Chem. Soc. Chem. Comm.*, 618-619 (1982).

39. (a) Insect growth regulating activities: ajugarin IV (**64**) on pink bollworm (*Pectinophara gossypiella*): ED_{50} = 500 ppm [ref. 38c]; 3-epi-caryoptin (**65**) on housefly (*Musca domestica*) [ref. 39b]; *cis*- and *trans*-dehydrocrotonin (**66,67**) on tobacco budworm (*Heliothis virescens*) and pink bollworm (*P. gossypiella*): ED_{50} = 30 ppm [ref. 39c].

(b) J. Pereira and K.N. Gurudutt, *J. Chem. Ecol.*, **16** (7), 2297-2306 (1990). (c) I. Kubo, Y. Asaka and K. Shibata, *Phytochem.*, **30** (8), 2545-2546 (1991).

40. Obviously, terms as 'potent', 'strong' or 'good' antifeedant activity are highly subjective, especially since the degree of activity also depends on the test conditions under which this bioactivity was determined. With regard to the two-choice Antifeedant Index (AI) in laboratory assay conditions as used by Simmonds and co-workers (which was adopted as standard system to prepare the compilation of antifeedant activities presented in this chapter), a compound with an AI of 50% at 100 ppm test-concentration is considered to be a 'potent' antifeedant, while an AI of 95-100% is usually described as 'very potent' or 'strong' activity.

41. (a) K. Munakata, *Pure Appl. Chem.*, **42**, 57-66 (1975); (b) T.A. van Beek and Ae. de Groot, *Recl. Trav. Chim. Pays-Bas*, **105**, 513-527 (1986); (c) F. Camps and J. Coll, *Phytochemistry*, **32** (6), 1361-1370 (1993).

42. (a) For a discussion of the dependency of insect feeding behaviour on the presence of feeding stimulants or antifeedants, see: L.M. Schoonhoven, *Entomol. Exp. Appl.*, **31**, 57-69 (1982), and references cited therein.
(b) Several examples in which the antifeedant activity of a clerodane diterpene changes with the test insect species can be found in the compilation tables. This effect has also been observed with other classes of insect antifeedants, such as drimane-type sesquiterpenes or azadirachtin-related tetranor-triterpenoids. See for instance: W.M. Blaney, M.S.J. Simmonds, S.V. Ley and R.B. Katz, *Physiol. Entomol.*, **12**, 281-291 (1987); W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson and P.L. Toogood, *Entomol. Exp. Appl.*, **55**, 149-160 (1990).

43. J. Vader, *Studies towards the total synthesis of insect antifeedant clerodanes*, Ph-D thesis, Wageningen Agricultural University, Wageningen, 1989.

44. X. Belles, F. Camps, J. Coll and M.D. Piulachs, *J. Chem. Ecol.*, **11** (10), 1439-1445 (1985).

45. J.G. Urones, P. Basabe, A.M. Lithgow, I.S. Marcos, A. Jiménez, D. Díez, A. Gómez, A.J.P. White, D.J. Williams, M.S.J. Simmonds and W.M. Blaney, *Tetrahedron*, **51** (7), 2117-2128 (1995).

46. M.S.J. Simmonds, W.M. Blaney, S.V. Ley, G. Savona, M. Bruno and B. Rodríguez, *Phytochem.*, **28** (4), 1069-1071 (1989).

47. W.M. Blaney, M.S.J. Simmonds, S.V. Ley and P.S. Jones, *Entomol. Exp. Appl.*, **46**, 267-274 (1988).

48. M.S.J. Simmonds, W.M. Blaney, B. Esquivel and L. Rodriguez-Hahn, *Pestic. Sci.*, **47**, 17-23 (1996).

49. P.Y. Malakov, G.Y. Papanov, B. Rodríguez, M.C. de la Torre, M.S.J. Simmonds, W.M. Blaney and I.M. Boneva, *Phytochem.*, **37** (1), 147-157 (1994).

50. I. Kubo, Y.-W. Lee, V. Balogh-Nair, K. Nakanishi and A. Chapya, *J. Chem. Soc. Chem. Comm.*, 949-950 (1976).

51. M.D. Cole, J.C. Anderson, W.M. Blaney, L.E. Fellows, S.V. Ley, R.N. Sheppard and M.S.J. Simmonds, *Phytochem.*, **29** (6), 1793-1796 (1990).

52. P.D. Bremner, M.S.J. Simmonds, W.M. Blaney and N.C. Veitch, *Phytochem.*, **47** (7), 1227-1232 (1998).

53. D.M. Muñoz, M.C. de la Torre, B. Rodríguez, M.S.J. Simmonds and W.M. Blaney, *Phytochem.*, **44** (4), 593-597 (1997).

54. B. Rodríguez, M.C. de la Torre, B. Rodríguez, M. Bruno, F. Piozzi, G. Savona, M.S.J. Simmonds, W.M. Blaney and A. Perales, *Phytochemistry*, **33** (2), 309-315 (1993).

55. J.C. Anderson, W.M. Blaney, M.D. Cole, L.L. Fellows, S.V. Ley, R.N. Sheppard, and M.S.J. Simmonds, *Tetrahedron Lett.*, **30** (35), 4737-4740 (1989).

56. W.M. Blaney, A.C. Cuñat, S.V. Ley, F.J. Montgomery and M.S.J. Simmonds, *Tetrahedron Lett.*, **35** (27), 4861-4864 (1994).

57. B. Rodríguez, M.C. de la Torre, A. Perales, P.Y. Malakov, G.Y. Papanov, M.S.J. Simmonds and W.M. Blaney, *Tetrahedron*, **50** (18), 5451-5468 (1994).

58. M.C. de la Torre, G. Domínguez, B. Rodríguez, A. Perales, M.S.J. Simmonds and W.M. Blaney, *Tetrahedron*, **50** (47), 13553-13566 (1994).

59. W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson and P.L. Toogood, *Entomol. Exp. Appl.*, **55**, 149-160 (1990).

60. W.P. Jackson and S.V. Ley, *J. Chem. Soc. Perkin I*, 1516-1519 (1981).

61. I. Kubo and K. Nakanishi, *Insect Antifeedants and Repellents from African Plants*. In: P.A. Hedin (Ed.), *Host Plant Resistance to Pests*, ACS Symposium Series, Vol. 62, ACS, Washington, 1977, p. 165-178.

62. I. Kubo, Y. Fukuyama and A. Chapya, *Chem. Lett.*, 223-224 (1983).

63. S. Hosozawa, N. Kato and K. Munakata, *Agr. Biol. Chem.*, **38** (4), 823-826 (1974).

64. N.Kato, M. Takahashi, M. Shibayama and K. Munakata, *Agr. Biol. Chem.*, **36** (13), 2579-2582 (1972).

65. K. Munakata, *Insect Antifeedants of Spodoptera Litura in Plants*. In: P.A. Hedin (Ed.), *Host Plant Resistance to Pests*, ACS Symposium Series, Vol. 62, ACS, Washington, 1977, p.185-196.

66. S. Hosozawa, N. Kato, K. Munakata and Y.-L. Chen, *Agr. Biol. Chem.*, **38** (5), 1045-1048 (1974).

67. Y. Kojima and N. Kato, *Tetrahedron*, **37** (15), 2527-2538 (1981).

68. Y. Kojima and N. Kato, *Nippon Kagaku Kaishi*, (5), 712-720 (1981).

69. J.A. Pickett, G.W. Dawson, D.C. Griffiths, A. Hassanali, L.A. Merritt, A. Mudd, M.C. Smith, L.J. Wadhams, C.M. Woodcock and Z. Zhong-ning, *Development of plant-derived antifeedants for crop protection*. In: R. Greenhalgh and T.R. Roberts (Eds.), *Pesticide Science and Biotechnology*, Blackwell, Oxford, 1987, p. 125-128.

70. D.C. Griffiths, A. Hassanali, L.A. Merritt, A. Mudd, J.A. Pickett, S.J. Shah, L.E. Smart, L.J. wadhams and C.M. Woodcock, *Brighton Crop Protection Conference - Pests and Diseases - 1988*, 1041-1046 (1988).

71. (a) R. Couilloud, *Insects, Spiders, Mites & Ticks. Equivalences Between Scientific and Common English Names.*, CIRAD, Montpellier, 1991. (b) B.-C. Zhang, *Index of Economically Important Lepidoptera.*, CAB International, Wallingford UK, 1994.

72. Z. Min, S. Wang, Q. Zheng, B. Wu, M. Mizuno, T. Tanaka and M. Iinuma, *Chem. Pharm. Bull.*, **37** (9), 2505-2508 (1989).

73. C. Huan-Ming, M. Zhi-Da, M. Iinuma and T. Tanaka, *Heterocycles*, **43** (3), 611-617 (1996).

74. I. Kubo, V. Jamalamadaka, T. Kamikava, K. Takahashi, K. Tabata and T. Kusumi, *Chem. Lett.*, **6**, 441-442 (1996).

75. A.P. Phadnis, S.A. Patwardhan, N.N. Dhaneshwar, S.S. Tavale and T.N. Guro Row, *Phytochem.*, **27** (9), 2899-2901 (1988).

76. K. Wada and K. Munakata, *J. Agr. Food Chem.*, **16** (3), 471-474 (1968).

77. M.E. Sosa, C.E. Tonn and O.S. Giordano, *J. Nat. Prod.*, **57** (9), 1262-1265 (1994).

78. R.D. Enriz, H.A. Baldoni, E.A. Jauregui, M.E. Sosa, C.E. Tonn and O.S. Giordano, *J. Agric. Food Chem.*, **42**, 2958-2963 (1994).

79. J.M. Luco, M.E. Sosa, J.C. Cesco, C.E. Tonn and O.S. Giordano, *Pestic. Sci.*, **41**, 1-6 (1994).

80. F. Ortego, B. Rodríguez and P. Castañera, *J. Chem. Ecol.*, **21** (9), 1375-1386 (1995).

81. B. Esquivel, M. Zarate, A.D. Sanchez and L. Sanchez, *'Neo-Clerodane and Languidulane Diterpenoids from Salvia ramosa and S. karwinskii (Labiatae)'*. In: *10th Conference on Isoprenoids - Prague, 1995*, Book of Abstracts, p. 98-99.

82. S.V. Ley, D. Neuhaus, N.S. Simpkins and A.J. Whittle, *J. Chem. Soc. Perkin Trans. I*, 2157-2162 (1982).

83. J.R. Hanson, D.E.A. Rivett, S.V. Ley and D.J. Williams, *J. Chem. Soc. Perkin Trans. I*, 1005-1008 (1982).

84. T.D. Hubert and D.F. Wiemer, *Phytochem.*, **24** (6), 1197-1198 (1985).

85. J.J. Howard, J. Cazin Jr. and D.F. Wiemer, *J. Chem. Ecol.*, **14** (1), 59-69 (1988).

86. T.-B. Chen, D.L. Galinis and D.F. Wiemer, *J. Org. Chem.*, **57**, 862-866 (1992).

87. L. Lajide, P. Escoubas and J. Mizutani, *Phytochem.*, **40** (4), 1101-1104 (1995).

88. L. Rodríguez-Hahn, G. Martínez and J. Romo, *Rev. Latinoam. Quim.*, **4**, 93 (1973); as cited in: B. Esquivel *et al*, *Phytochem.*, **24** (8), 1769-1772 (1985) and ref. 41b.

89. S. Ladjel, K. Laamara, M.R.Y. Al-Hillo and M. Païs, *Phytochem.*, **37** (6), 1663-1666 (1994).

90. T. Miyase, H. Kawasaki, T. Noro, A. Ueno, S. Fukushima and T. Takemoto, *Chem. Pharm. Bull.*, **29** (12), 3561-3564 (1981).

91. V.M. Dirsh, *Genus Schistocerca (Acridomorpha, Insecta)*. In: E. Schimitschek, *Series Entomologica*, Vol. 10, Dr. W. Junk B.V. Publishers, The Hague, 1974, p. 92-97.

Chapter 3

Scope of the Thesis

Abstract: *In this chapter, our strategy in the search for bioactive model compounds derived from natural clerodane insect antifeedants is described. Based on some structure-activity relationships of the potent antifeedant azadirachtin, a structural modification is selected that may enhance the activity of such simplified model compounds. From these starting points, the target compounds of the present thesis are chosen and the scope of the thesis is discussed.*

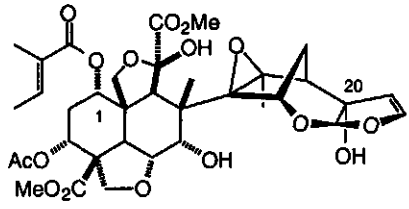
Table of Contents

3.1 - Approaches to Practical Insect Antifeedants

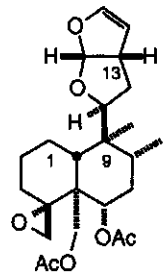
3.1.1 - Antifeedants of Synthetic Origin

As was discussed in Chapter 1, insect antifeedants from natural sources offer a number of properties that are highly desirable in future crop protection agents. The principle of insect control with antifeedants has been shown to work under field conditions and many of such compounds have been discovered. Nevertheless, only a few products, based on extracts from the Neem-tree that contain natural terpenes as azadirachtin (**1**), are actually in use today. One of the factors hampering the introduction of natural antifeedants in crop protection is the limited availability of many of these compounds, which prevents their widespread application at low cost. For the use of antifeedants in agriculture to become common practice, a stable and cheap supply of such compounds is therefore a first necessity.

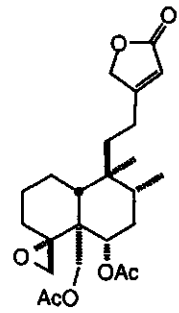
Figure 3.1



1: azadirachtin



2: clerodin



3: ajugarin I

Synthetic approaches might provide an alternative to the isolation of antifeedants from natural sources. So far, the emphasis has been largely on the total synthesis of natural insect antifeedants, but the synthetic routes involved are usually too long and complex to be of much practical value. A more promising approach would therefore seem to be to develop simplified analogues of such natural products that have retained as much of the desired biological properties as possible. Apart from being potential crop protection agents themselves, such simplified analogues may also serve as model compounds to investigate structure-activity relationships and thereby to provide a basis for the design of more potent synthetic insect antifeedants.

3.1.2 - *Simplified Analogues of Clerodane Diterpenes*

The class of clerodane diterpenes consists of well over 750 plant metabolites with a high degree of structural diversity. The members of this class display various types of biological activity and particularly clerodane diterpenes with insect antifeedant activity have attracted considerable attention (see Chapter 2).

The department of Organic Chemistry at the Wageningen Agricultural University has a long-standing interest in the chemistry of clerodane diterpenes, especially with respect to the total synthesis of complex natural products as clerodin (**2**) and ajugarin (**3**). During the course of these studies, a number of model compounds derived from fragments of such diterpenes were prepared and some of these were tested for insect antifeedant activity in collaboration with the department of Entomology (WAU).[1] However, in accordance with the results obtained by others with related model compounds,[2] none of these fragments displayed significant levels of antifeedant activity. Literature data on the activity of chemically modified clerodane antifeedants further showed that alterations in both halves of the antifeedant molecules can drastically influence their activity.[2]

It was concluded from these studies that both the decalin-fragment and the C-9 sidechain of these diterpenes are required to reach full potency.[1] However, model compounds that contain structural features from both halves soon become complicated themselves and were therefore considered to be unpractical as simplified bioactive analogues. Instead, we decided to concentrate upon one half of the molecule and to investigate the possibility to optimize the activity of such fragments.

In the search for modifications that could enhance the activity of clerodane fragments, we were struck by the structural resemblance between the furo[2,3b]furan sidechain of clerodin (**2**) and the furo[2,3b]pyran substructure present in azadirachtin (**1**). In view of this similarity, it was hypothesized that the introduction of structural features from the azadirachtin furo[2,3b]pyran substructure into fragments that resemble the clerodane C-9 sidechain fragments might increase the antifeedant activity of these model compounds. For the present thesis, the hydroxyl group present in the furo[2,3b]pyran substructure was selected as an interesting candidate for such a strategy. A background to motify this choice is presented in **Section 3.2**. The resulting target compounds and the scope of this thesis are described in **Section 3.3**.

3.2 - Some Structure-Activity Relationships of Azadirachtin

3.2.1 - Antifeedant Activity of Azadirachtin Fragments

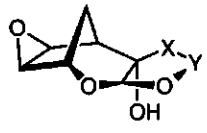
Several structure-activity studies have shown that functional groups throughout the azadirachtin molecule are important for the antifeedant activity, indicating that the activity resides in both halves of the molecule.[3] Nevertheless, both the decalin portion[4] (**16**, Chart 3.1) and the furo[2,3b]pyran fragment[5,6] (**4**) separately were found to display antifeedant activity (Table 3.1).[7,8,9] Remarkably, some further simplified model compounds were more active than fragments more closely mimicing the corresponding substructures present in azadirachtin [for instance, (**6**) vs. (**4**) and (**21**) vs (**16**)].

Because of the aforementioned structural resemblance between the corresponding substructures of azadirachtin and clerodin, the structure-activity relationships of the furo[2,3b]pyran fragment are of particular interest. The presence of an intact cyclic acetal moiety in the tricyclic ringsystem clearly is of importance for the antifeedant activity: ring opening of the C-ring (**10-13**) or complete removal of ring C (**14, 15**) or ring A (**9**) were found to result in considerable reduction in activity.

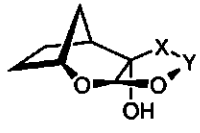
The absence of the C-10,11 epoxide moiety did not seriously affect the activity and even appeared to result in an activity increase at higher concentrations [(**4**) vs. (**6**), (**5**) vs. (**7**)]. Interestingly, the resulting model compounds (**6**) and (**7**) were as active as azadirachtin against *Spodoptera littoralis* larvae at 100 ppm, though at lower concentrations their activity decreased much faster than that of azadirachtin. For some other Lepidopteran insect species[10] however, (**6**) and (**7**) were considerably less active than azadirachtin (not shown). Similarly, reduction of the C-5,6 double bond [(**4**) vs. (**5**), (**6**) vs. (**7**)] resulted only in some loss of activity in case of bioassays with *Sp. littoralis*, but was detrimental for the activity on other Lepidopteran species[10] (not shown). The absolute stereochemistry of these simple model compounds seemed to be of little importance for the antifeedant activity [(**6a**) vs (**6b**)].

Unfortunately, no literature data are available on the importance of the C-7 hydroxyl group in these simple fragments. The only related C-7 dehydroxy model compound reported (**8**)[6] has an inappropriate relative stereochemistry for the crucial furo[2,3b]pyran ring system and is thus difficult to compare with (**7**). For indications on the possible role of such a hydroxyl group in insect antifeedant activity we therefore had to turn to structure-activity studies with the natural compound azadirachtin itself.

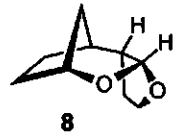
Chart 3.1: Simplified model compounds derived from azadirachtin, which have been tested for insect antifeedant activity.[a]



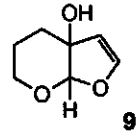
	X,Y
4[b]	CH=CH
5[b]	CH_2-CH_2



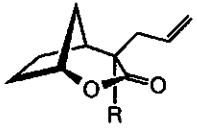
	X,Y
6a	CH=CH
6b[b]	CH=CH
7	CH_2-CH_2



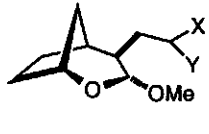
8



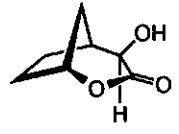
9



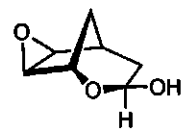
10: R=OH
11: R=H



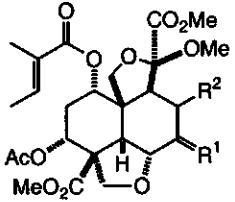
	X,Y
12	H,CH_2OH
13	CH_2



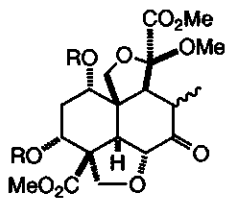
14



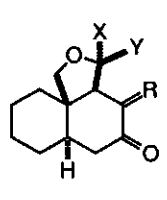
15



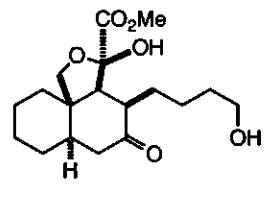
	R^1	R^2
16	α-OH,H	α-Me
17	O	α,β-Me
18	β-OH,H	β-Me



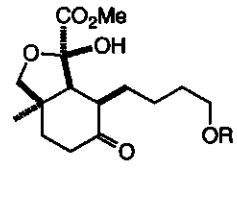
19: R=Ac
20: R=H



	R	X	Y
21	H,H	CO_2Me	OH
22	H,H	OH	CO_2Me
23	CH_2	CO_2Me	OH
24	β-Me,H	CO_2Me	OH



25



26: R=H
27: R=Bn

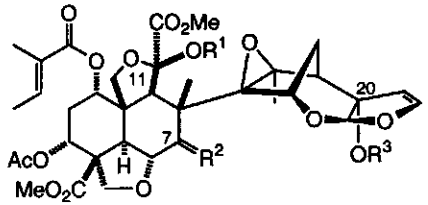
Notes: (a) All model compounds were tested as racemic mixtures, unless indicated otherwise. (b) Enantiomerically pure compound.

Table 3.1: Insect antifeedant activity of simplified model compounds, derived from azadirachtin, against larvae of the Egyptian cotton leaf worm (Lepidoptera: *Spodoptera littoralis*) in a two-choice feeding assay.[a]

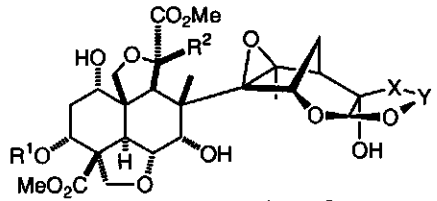
	conc.[b] (ppm)	conc.[b] (M)	AI (sem) [c,d] (%)	AI_{50}[e] (ppm)	AI_{50}[e] (M)	Ref.
4	*1.7*	1×10^{-5}	*20*	*> 1820*	$> 1\times10^{-2}$	4
	0.17	1×10^{-6}	*14*			4
5	*1.7*	1×10^{-5}	*21*	*>1840*	$> 1\times10^{-2}$	4
	0.17	1×10^{-6}	*11*			4
6a	100	6.0×10^{-4}	100.0 (0.00)			5
	10	6.0×10^{-5}	97.7 (8.89)			5
			98 (1.8) **			6
	1	6.0×10^{-6}	65.8 (2.27)			5
6b	*1.7*	1×10^{-5}	*64* #	*1.2*	7.0×10^{-6}	4
	0.17	1×10^{-6}	*20*			4
	0.017	1×10^{-7}	*9*			4
7	100	5.9×10^{-4}	100.0 (0.00)			5
	10	5.9×10^{-5}	69.1 (2.24)			5
			69 (2.2) *			6
	1.7	1×10^{-5}	*32* #	*714*	4.2×10^{-3}	4
	1	5.9×10^{-6}	54.8 (7.25)			5
	0.17	1×10^{-6}	*10*			4
8	10	6.5×10^{-5}	20 (12.2)			6
9 [f]	5000 [g]	35.2×10^{-3}	0 [h]			7
10	10		31 (8.7)			6
11	10		-4 (9.7)			6
12	10		41 (6.6)			6
13	10		10 (5.5)			6
14	10		16 (9.6)			6
15	10		41 (8.4)			6
16	*26.2*	5×10^{-5}	33 #	*> 5240*	$> 1\times10^{-2}$	4
	5.2	1×10^{-5}	29 #			4
	0.5	1×10^{-6}	22			4
	0.05	1×10^{-7}	11			4
17	*26.1*	5×10^{-5}	48 #	*42.8*	8.2×10^{-5}	4
	5.2	1×10^{-5}	49 #	*> 5240*		4
	0.5	1×10^{-6}	29 #			4
	0.05	1×10^{-7}	16		$> 1\times10^{-2}$	4
18	*26.2*	5×10^{-5}	31 #			4
	5.2	1×10^{-5}	30 #	*> 4980*		4
	0.5	1×10^{-6}	24			4
	0.05	1×10^{-7}	13		$> 1\times10^{-2}$	4
19	*24.9*	5×10^{-5}	7	*> 4140*		4
	0.5	1×10^{-6}	14			4
	0.05	1×10^{-7}	12		$> 1\times10^{-2}$	4
20	*20.7*	5×10^{-5}	30 #	9.4		4
	0.4	1×10^{-6}	22	820		4
	0.04	1×10^{-7}	16	> 1000	3.5×10^{-5}	4
21	100	3.7×10^{-4}	73 *	154	3.1×10^{-3}	8
22	100	3.7×10^{-4}	24	640	$> 3.6\times10^{-3}$	8
23	100	3.6×10^{-4}	39 *	95	5.5×10^{-4}	8
24	100	3.5×10^{-4}	44 *	93	1.9×10^{-3}	8
25	100	2.9×10^{-4}	-11		3.2×10^{-4}	8
26	100	3.3×10^{-4}	42 *		2.4×10^{-4}	9
27	100	2.6×10^{-4}	51 *			9

Notes: see Table 3.2.

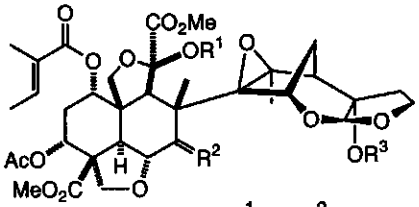
Chart 3.2: Azadirachtin and related compounds with modified hydroxy groups at C-7, C-11 and C-20.



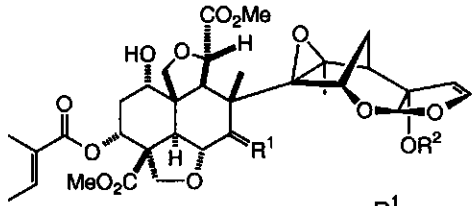
	R^1	R^2	R^3
1	H	α-OH,H	H
28	Me	α-OH,H	H
29	Me	α-OH,H	Me
30	H	O	H
31	Me	O	H



	R^1	R^2	X,Y
40	H	H	CH=CH
41	Ac	H	CH=CH
42	H	H	CH_2-CH_2
43	Ac	OH	CH_2-CH_2



	R^1	R^2	R^3
32	H	α-OH,H	H
33	Me	α-OH,H	H
34	H	O	H
35	Me	O	H
36	Ac	O	H
37	Me	β-OH,H	H
38	Me	α-OH,H	Me
39	Me	O	Me



	R^1	R^2
44	α-OH,H	H
45	H,H	Me

Table 3.2: Insect antifeedant activity of azadirachtin and related compounds against 6th instar larvae of the Egyptian cotton leafworm (Lepidoptera: *Spodoptera littoralis*) as measured in two-choice feeding assays.

	conc. (ppm)	conc. (M)	AI (sem) (%)	Ref
1	10	1.4×10^{-5}	100 (0.0)**	6,13,15
	7.2	1×10^{-5}	100	14a
	1	1.4×10^{-6}	99 (1.1)**	6,13,15
	0.7	1×10^{-6}	100	14a
	0.07	1×10^{-7}	96	14a
	0.007	1×10^{-8}	85	14a
	0.0007	1×10^{-9}	75	14a
28	7.3	1×10^{-5}	100	14a
	0.7	1×10^{-6}	96	14a
	0.07	1×10^{-7}	44	14a
29	7.5	1×10^{-5}	100	14a
	0.8	1×10^{-6}	78	14a
	0.08	1×10^{-7}	54	14a
30	10	1.4×10^{-5}	23 (12.3)	13
	7.2	1×10^{-5}	90	14a
	1	1.4×10^{-6}	21 (6.0)	13
	0.7	1×10^{-6}	73	14a
	0.07	1×10^{-7}	52	14a
31	7.3	1×10^{-5}	60	14a
	0.7	1×10^{-6}	54	14a
	0.07	1×10^{-7}	34	14a
32	7.2	1×10^{-5}	100	14a
	1	1.4×10^{-6}	100 (0.0) **	6,15
	0.7	1×10^{-6}	100	14a
	0.07	1×10^{-7}	100	14a
	0.007	1×10^{-8}	71	14a
	0.0007	1×10^{-9}	63	14a
33	10	10	100 (0.0) **	13
	7.4	7.4	30	14a
	1	1	100 (0.0) **	13
	0.7	0.7	26	14a
	0.07	0.07	15	14a
34	7.2	7.2	97	14a
	0.7	0.7	75	14a
	0.07	0.07	65	14a
35	10	1.4×10^{-5}	100 (0.0) **	13
	7.3	1×10^{-5}	97	14a
	1	1.4×10^{-6}	26 (14.0)	13
	0.7	1×10^{-6}	27	14a
	0.07	1×10^{-7}	17	14a
36	7.6	1×10^{-5}	86	14a
	0.8	1×10^{-6}	67	14a
	0.08	1×10^{-7}	9	14a
37	10	1.4×10^{-5}	46 (12.1) *	13
	7.4	1×10^{-5}	37	14a
	1	1.4×10^{-6}	35 (7.4) *	13
	0.7	1×10^{-6}	26	14a
	0.07	1×10^{-7}	8	14a
38	10	1.4×10^{-5}	95 (2.3) **	13
	7.2	1×10^{-5}	100	14a
	1	1.4×10^{-6}	66 (12.6) *	13
	0.7	1×10^{-6}	95	14a
	0.07	1×10^{-7}	73	14a
39	10	1.3×10^{-5}	100 (0.0) **	13
	7.5	1×10^{-5}	100	14a
	1	1.3×10^{-6}	29 (13.8) *	13
	0.8	1×10^{-6}	81	14a
	0.08	1×10^{-7}	51	14a
40	5.8	1×10^{-5}	100	14a
	1	1.7×10^{-6}	86 (1.9) **	6
	0.6	1×10^{-6}	86	14a
	0.06	1×10^{-7}	74	14a
41	6.2	1×10^{-5}	94	14a
	0.6	1×10^{-6}	89	14a
	0.06	1×10^{-7}	59	14a
42	1		63 (4.8) *	6
43	5.8	1×10^{-5}	81	14a
	1		75 (8.1) **	6
	0.6	1×10^{-6}	75	14a
	0.06	1×10^{-7}	34	14a
44	6.6	1×10^{-5}	100	14a
	1	1.5×10^{-6}	97 (3.9) **	6
	0.7	1×10^{-6}	97	14a
	0.07	1×10^{-7}	83	14a
45	6.6	1×10^{-5}	84	14a
	0.7	1×10^{-6}	63	14a
	0.07	1×10^{-7}	47	14a

Notes: see next page.

Notes Tables 3.1 and 3.2

(a) - Unless otherwise indicated, the two-choice feeding assays were performed according to the assay protocol routinely used by Blaney, Simmonds and coworkers (See for instance ref. 6). These assays employ an artificial substrate, consisting of glass-fibre discs, made palatable by the addition of 100 µl of an aqueous 50 mM solution of sucrose. Treatment discs were subsequently treated with 100 µl of a solution of the test compound in ethanol at a concentration specified in the tables. After termination of the assay, the discs were weighed to determine the mass eaten from the control (C) and treatment discs (T).

(b) - In view of the considerable differences in molecular weight of the test compounds, the concentrations of the test solutions used are expressed both in ppm and in M, to allow comparison of different experiments. Values printed in italics were calculated from the values given in the cited literature.

(c) - Mean two-choice Antifeedant Index, AI=[(C-T)/(C+T)]×100%; sem = standard error of the mean.

(d) - Some AI values (from ref. 4 and 14a) were derived from dose-response graphs depicted in the cited literature. In these graphs, the standard errors associated with the individual mean AIs were omitted for clarity; the sem values were reported to range from 4-15% (ref. 4) to 2-25% (ref 14a). The test solution concentration in these graphs ranged from 10^{-4} to 10^{-9} M. In the tables, only those data points from the graphs were included that give a good representation of the slope of the curve and/or that were needed for comparison with other test compounds.

(e) - AI_{50} is the concentration required to give a two-choice Antifeedant Index of 50%. This value was determined in the cited literature via probit analysis.

(f) - Assay protocol not specified in detail; see cited literature.

(g) - Lower test concentrations were omitted from the table.

(h) - Reported as 'no antifeedant activity'.

(**) - Significant difference between the control and treatment discs (Wilcoxon's matched pairs test, $p<0.01$).

(*) - Significant activity, $p<0.05$ (Wilcoxon's matched pairs test).

(#) - Significant activity, $p<0.05$ (Mann-Whitney U-test).

3.2.2 - *Hydroxyl Group Modification in Azadirachtin-like Compounds*

Several studies have indicated that the hydroxyl groups in azadirachtin and structurally related compounds are of importance for the biological activity. For instance, the growth inhibitory and lethal activity of azadirachtin against several insect species significantly decreased upon acetylation of OH-11 and OH-20.[11] Similar results were obtained upon alkylation, acetylation or silylation of the various hydroxyl groups, although the effects were less clear and varied between test compounds and insect species.[12]

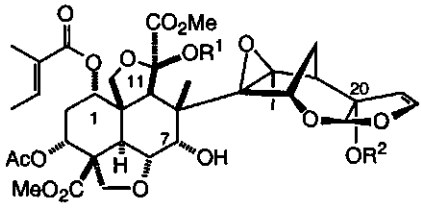
With regard to insect antifeedant activity, Blaney, Simmonds and coworkers have extensively studied the influence of hydroxyl group modification on the activity of azadirachtin-like compounds (Chart 3.2) against *Spodoptera littoralis* larvae (Table 3.2). Their initial investigations[13] seemed to indicate that especially the hydroxyl group at C-7 was important for activity, since both oxidation [see (**1**) vs. (**30**) and (**33**) vs. (**35**)] and epimerization [(**33**) vs. (**37**)] of this moiety resulted in decreased activity. In contrast, the antifeedant activity remained unchanged upon methylation of OH-11 [(**32**) vs. (**33**)]. Further methylation of (**33**) at OH-20 yielded the less potent derivative (**38**) and the activity of this compound could be even further decreased by C-7 oxidation [(**38**) vs. (**39**)]. However, in the presence of an existing C-7 keto group, methylation of OH-20 did not affect antifeedant activity [(**35**) vs. (**39**)]. Thus, the importance of the various hydroxyl groups of azadirachtin for the insect antifeedant activity appeared to follow the order: OH-7 > OH-20 > OH-11.

A later study from the same research groups showed results that differed from those discussed above.[14a,b] For instance, although oxidation of OH-7 still led to derivatives with decreased activity, the exact stereochemistry of this hydroxyl group no longer appeared to be of importance [(**33**) vs. (**37**)]. Furthermore, both methylation and acetylation of OH-11 now were found to reduce the activity [*e.g.* (**1**) vs. (**28**), (**32**) vs. (**33**)], even in the presence of a ketone at C-7 [(**30**) vs. (**31**), (**34**) vs. (**35**) and (**36**)]. Further methylation at OH-20 in these derivatives now either produced no significant change [(**28**) vs.(**29**)] or even resulted in an *increase* in antifeedant activity [(**33**) vs. (**38**), (**35**) vs. (**39**)]. In addition to the azadirachtins, some derivatives of azadirachtol (**40**) were examined.[6,14a,15] These compounds lack the hydroxyl group at C-11 and a comparison of the results for (**40-43**) shows that this change is accompanied by a reduction in activity at low concentrations. The simultaneous removal of OH-7 and methylation of C-20 [(**44**) vs. (**45**)] also decreased the antifeedant activity in this series.

Another study of the effects of hydroxyl group modification upon activity was reported by Morgan,[16] who found that upon silylation of either OH-11 (**46**) or

OH-20 (**47**) the feeding inhibitory effect against *Schistocerca gregaria* larvae at low concentrations was markedly reduced (Table 3.3). This result is of special interest since (**46**) is the only example in which OH-20 is modified while the hydroxyl groups at C-7 and C-11 have remained unchanged.

In conclusion, the results from several structure-activity studies seem to indicate that the hydroxyl groups at C-7 and C-11 are both of importance for the antifeedant activity of azadirachtin. The effect of OH-20 is less clear, since modification of this group was found to result both in decreased or increased activity, depending on the nature of the particular test compound. Also, in all cases but one, a modification of OH-20 was always studied in combination with changes at OH-7 and/or OH-11, which may have partially concealed the role of this hydroxyl group. Nevertheless, it seems that sufficient evidence is present to assume that the C-20 hydroxyl group, too, influences the biological activity of azadirachtin.



	R^1	R^3
1	H	H
46	Me_3Si	H
47	H	Me_3Si
48	Me_3Si	Me_3Si

Table 3.3: Feeding inhibitory effect[a] of some azadirachtin derivatives on 5th instar larvae of the desert locust (*Schistocerca gregaria*).

	Activity (%)[b] at concentration (ppm):				
	50	5	0.5	0.05	0.005
1	-	100	100	100	100
46	100	97	45	0	-
47	100	97	66	25	0
48	63	64	60	18	11

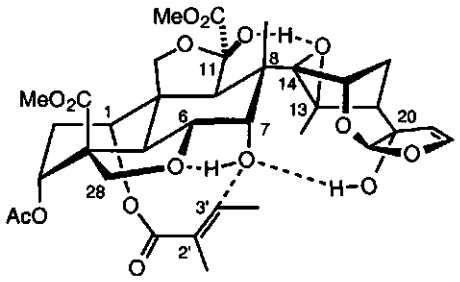
Notes: (a) Determined in a no-choice feeding assay on sucrose-impregnated filter paper. (b) 100% Activity represents total inhibition of feeding. [Adapted from ref. 16.]

3.2.3 - *Role of the Hydroxyl Groups in Azadirachtin Antifeedant Activity*

Although the discussion in the previous section showed that the hydroxyl groups at C-7, C-11 and C-20 in azadirachtin and its derivatives appear to play a role in the bioactivity of these compounds, the nature of this role was not touched upon. It has been suggested[17,18] that these groups are important because of their participation in an intramolecular network of hydrogen bonds that orients both halves of the molecule into some bioactive conformation. Alternatively, the hydroxyl groups could (also) be important for the recognition of azadirachtin by its hypothetical receptor site, via a direct interaction of these groups with the receptor site. The nature of the role of these groups is of importance for the question if these features (particularly OH-20) from

azadirachtin are relevant for the activity of the furo[2,3b]pyran fragment by itself, and therefore also determine whether introduction of such a hydroxyl group in the furo[2,3b]furan fragment of clerodin could increase the activity of such a model compound.

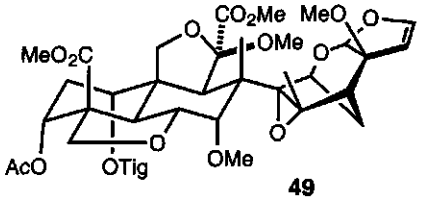
Figure 3.2: Schematic view of the intramolecular interactions stabilizing the preferred conformation of azadirachtin (**1**).[a]



Atoms[b]		Bond order[c] ($\times 10^{-2}$)
O_7	H_{20}	0.3
O_7	$C_{2'}$	0.0002
O_7	$C_{3'}$	0.004
O_7	$H_{3'}$	0.005
H_7	$O_{6,28}$	0.2
H_{11}	$O_{13,14}$	0.4

Notes:
(a) Dashed bonds indicate hydrogen bonding or charge- transfer complexation. (b) O_7 is the oxygen atom of OH-7, H_{20} is to the hydrogen atom of OH-20, etc. (c) Determined in the global minimum conformation, obtained by AM1 calculations. [Adapted from Ref. 17.]

The NMR spectra of azadirachtin display a clear dependency on the temperature, which is indicative of the presence of two or more distinct conformations.[19] In THF-d_8 or DMF-d_7, for instance, cooling of the solution to 180K produced doubling of every proton signal due to the presence of two conformers in a 2 : 1 ratio. Analysis has shown these effects to be located both in the orientation of the C-1 tiglate side-chain and in the rotation of the furo[2,3b]pyran fragment about the C-8/C-14 single bond (Figure 3.2). The rotational freedom of this fragment is restricted by an intramolecular network of hydrogen bonds between the two halves of the molecule, consisting of a strong interaction between OH-11 and the oxirane oxygen atom and a somewhat weaker bond between the hydrogen atom of OH-20 and the OH-7 oxygen atom. Ample evidence for these interactions has been found, both in the crystalline state and in solution. Disruption of these stabilizing hydrogen bonds disturbs the most preferred conformation and allows more open conformations to be populated, such as the conformer (**49**), obtained upon tri-methylation of azadirachtin.



49

This intramolecular hydrogen bond network could be involved in the biological activity of azadirachtin, for instance by orienting both halves of the molecule into some bioactive conformation.[17,18] In such a case, modification of the hydroxyl groups involved in this network could result in a disturbance of the bioactive conformation and should thereby lead to decreased activity of the derivatives. To examine this hypothesis, Baldoni *et al* [17] have analyzed the conformational behaviour of azadirachtin and some methyl ether derivatives via molecular mechanics and AM1 computations. Their calculations confirmed the presence of all intramolecular interactions deduced previously from experimental data and provided a quantitative description of the influence of rotation about the C-8/C-14 bond (Figure 3.3). The energy profile obtained for azadirachtin (**1**) showed three preferred conformations for the furo[2,3b]pyran rotation, separated by barriers which were sufficiently large to suggest the transition between these rotamers to be somewhat restricted. For the 11-O-monomethyl ether (**28**) a similar curve was obtained, with positions for the global and local minima similar to those of azadirachtin, though the energy differences between these conformations and the rotational barriers separating them were different. By contrast, the 11,20-O-dimethyl ether (**29**), was found to possess a global minimum that is clearly different from that of azadirachtin and also the energy changes associated with the transition from this global minimum to other minima (not shown) were enormous compared to those for (**1**) or (**28**).

From these and other[20] results it was concluded that the methylation of any single hydroxyl group would probably not critically disturb the structural conformation(s) involved in the bioactivity of azadirachtin. Double methylation

Figure 3.3: Potential energy curves (AM1 calculation) of azadirachtin (**1**, ○), azadirachtin-11-methyl ether (**28**, ■) and azadirachtin-11,20-dimethyl ether (**29**, ▲) upon rotation of the furo[2,3b]pyran fragment. [Adapted from ref. 17.]

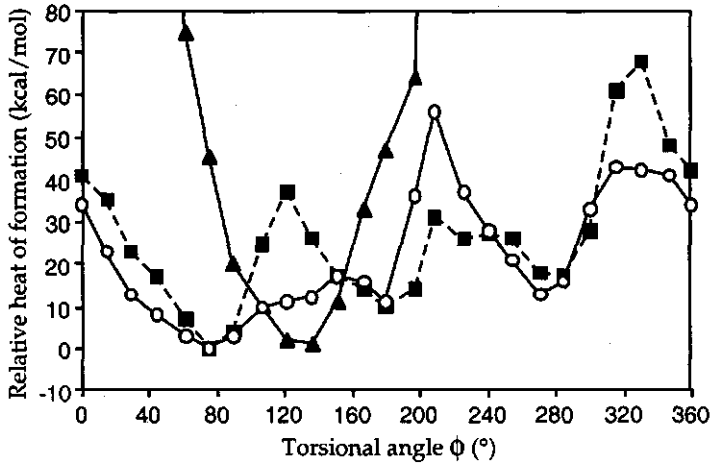
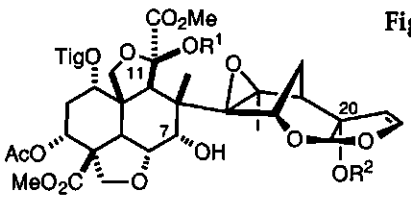
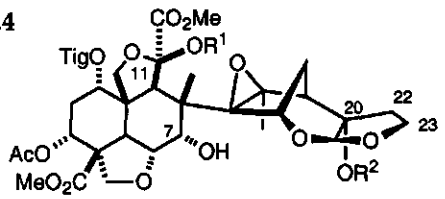


Figure 3.4





	R^1	R^2
1	H	H
28	Me	H
29	Me	Me

	R^1	R^2
46	Me_3Si	H
47	H	Me_3Si
50	H	Me

	R^1	R^2
32	H	H
33	Me	H
38	Me	Me

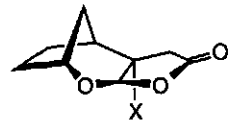
at C-11 and C-20 or trimethylation, however, introduced substantial changes in the conformational behaviour of the derivatives that could be responsible for reduced activity. These results are supported by some experimental data,[13] which indicated a significant decrease in the antifeedant activity of the trimethylated compound (**38**) in comparison to the corresponding derivatives (**32**) and (**33**) (see Table 3.2). However, later data on these derivatives, and on (**1**), (**28**) and (**29**) themselves, were in contrast with these findings since in this study the 11,20-dimethylated compounds (**29**) and (**38**) were equally or even more potent than the 11-O-monomethyl ethers (**28**) and (**33**).[14] Furthermore, the computational results on the 20-O-monomethyl ether (**50**) (which had an energy profile similar to (**28**)) are not supported by the biological data on the mono-TMS ethers (**46**) and (**47**) (see Table 3.3),[16] which displayed markedly reduced activity at lower concentrations, relative to azadirachtin.[21]

In my view, it is therefore still not proven beyond doubt that the role of the hydroxyl groups of azadirachtin in the bioactivity of this natural product lies mainly in their stabilizing a bioactive conformation through intramolecular hydrogen bonding. Alternatively, these hydroxyl groups could (also[22]) be important for the binding of azadirachtin to its hypothetical receptor site, via a direct interaction of these groups with the receptor site. The biological data reported on the effect of modifications to OH-7 [(**16**)-(**18**), table 3.1] or OH-11 [(**21**), (**22**)] could possibly be interpreted as support for the direct interaction of these groups with such a receptor site.

3.3 - Scope of the Thesis

This thesis is based on the hypothesis that the C-20 hydroxyl group of azadirachtin is of importance for the antifeedant activity of this natural compound and of the furo[2,3b]pyran model compounds (**6**) and (**7**) because of a direct interaction of this group with a receptor site. **Chapter 4** describes an attempt

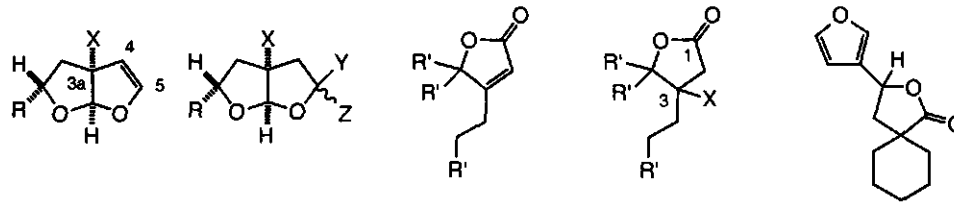
to synthesize some furo[2,3b]pyran model compounds (**51**) and (**52**), respectively with and without this hydroxyl moiety, which could be used in biological experiments to find supporting evidence for this hypothesis.



51: X=OH
52: X=H

The main goal of this thesis was the synthesis and biological evaluation of model compounds, derived from the C-9 sidechain fragments found in natural clerodane insect antifeedants. For this purpose, the furo[2,3b]furan ring system (**53a**, Figure 3.5), the 3-substituted butenolide (**55**) and the furanospirolactone (**57**) were chosen as target structures. In view of the structural resemblance of the hydroxy-furo[2,3b]pyran fragment of azadirachtin (**1**) to the furo[2,3b]furan substructure present in clerodin (**2**), the introduction of a similar hydroxyl group in these model compounds (*i.e.* X=OH in Figure 3.5) was selected as a modification that could potentially increase the antifeedant activity of such simple model compounds.

Figure 3.5



(**53a**): X=H
(**53b**): X=OH

(**54a**): X=H
(**54b**): X=OH

(**55**): R'=H or Me

(**56a**): X=H, R'=H or Me
(**56b**): X=OH, R'=H or Me

(**57**)

In **Chapter 5** the preparation of a series of furo[2,3b]furan-based analogues (**53a**, **54a**) with different substitution patterns at C-4,5 is presented. **Chapter 6** describes the synthesis of model compounds with a 3a-hydroxy-furo[2,3b]furan ring system (**53b**); a series of analogues (**54b**) incorporating this substructure with a C-4,5 substitution pattern as in Chapter 5 was prepared. **Chapter 7** is devoted to the synthesis of a series of butenolide-based model compounds and their 3-hydroxy derivatives (**55**, **56a,b**). In **Chapter 8** some approaches to the furano-spirolactone target structure (**57**) are discussed. Finally, in **Chapter 9** the results of antifeedancy bioassays with a number of model compounds from previous chapters against three different pest insect species are discussed.

3.4 - Notes and References

1. R.B.M. Geuskens, J.M. Luteijn and L.M. Schoonhoven, *Experientia*, **39**, 403 (1983).
2. For a comprehensive compilation of insect antifeeding clerodane diterpenes and structurally related compounds, see Chapter 2.
3. For reviews regarding the biological activity of azadirachtin, see: (a) A.J. Mordue (Luntz) and A. Blackwell, *J. Insect Physiol.*, **39** (11), 903-924 (1993); (b) T.A. van Beek and Ae. de Groot, *Recl. Trav. Chim. Pays-Bas*, **105**, 513-527 (1986); (c) S.V. Ley, A.A. Denholm and A. Wood, *Nat. Prod. Reports*, 109-157 (1993).
4. W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson, S.C. Smith and A. Wood, *Pestic. Sci.*, **40**, 169-173 (1994).
5. S.V. Ley, D. Santafianos, W.M. Blaney and M.S.J. Simmonds, *Tetrahedron Lett.*, **28** (2), 221-224 (1987).
6. W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson and P.L. Toogood, *Entomol. Exp. Appl.*, **55**, 149-160 (1990).
7. D. Pflieger and B. Muckensturm, *Tet. Lett.*, **28**, 1519-1522 (1987).
8. M.L. de la Puente, S.V. Ley, M.S.J. Simmonds and W.M. Blaney, *J. Chem. Soc., Perkin Trans. I*, 1523-1529 (1996).
9. M.L. de la Puente, R.B. Grossman, S.V. Ley, M.S.J. Simmonds and W.M. Blaney, *J. Chem. Soc., Perkin Trans. I*, 1517-1521 (1996).
10. Observed for *Spodoptera frugiperda, Heliothis virescens* and *H. armigera*. See ref. 6.
11. R.B. Yamasaki and J.A. Klocke, *J. Agric. Food Chem.*, **35** (4), 467-471 (1987).
12. M.S.J. Simmonds, W.M. Blaney, S.V. Ley, J.C. Anderson and P.L. Toogood, *Entomol. Exp. Appl.*, **55**, 169-181 (1990).
13. S.V. Ley, J.C. Anderson, W.M. Blaney, E.D. Morgan, R.N. Sheppard, M.S.J. Simmonds, A.M.Z. Slawin, S.C. Smith, D.J. Williams and A. Wood, *Tetrahedron*, **47** (44), 9231-9246 (1991).
14. (a) M.S.J. Simmonds, W.M. Blaney, S.V. Ley, J.C. Anderson, R. Bänteli, A.A. Denholm, P. Green, R.B. Grossman, C. Gutteridge, L. Jennens, S.C. Smith, P.L. Toogood and A. Wood, *Entomol. Exp. Appl.*, **77**, 69-80 (1995); (b) Remarkably, these differences were not mentioned or commented upon in this article.
15. S.V. Ley, J.C. Anderson, W.M. Blaney, P.S. Jones, Z. Lidert, E.D. Morgan, N.G. Robinson, D. Santafianos, M.S.J. Simmonds and P.L. Toogood, *Tetrahedron*, **45** (16), 5175-5192 (1989).
16. E.D. Morgan, *Strategy in the Isolation of Insect Control Substances from Plants*. In: H. Schmutterer, K.R.S. Acher and H. Rembold (Eds.), *Natural Pesticides from the Neem Tree*, Proceedings of the first International Neem Conference (Rottach-Egern, FRG, june 1980), GTZ Verlag, 1981, p. 43-52.
17. H.A. Baldoni, R.D. Enriz, E.A. Jáuregui and I.G. Csizmadia, *J. Molecular Structure (Theochem)*, **363**, 167-178 (1996).
18. S.V. Ley, K. Doherty, G. Massiot and J.-M. Nuzillard, *Tetrahedron*, **50** (42), 12267-12280 (1994).
19. (a) For a history of the structure elucidation of azadirachtin, see: D.A.H. Taylor, *Tetrahedron*, **43** (12), 2779-2787 (1987), and Ref. 3c. See further the following key-

papers: (b) W. Kraus, M. Bokel, A. Klenk and H. Pöhnl, *Tetrahedron Lett.*, **26** (52), 6435-6438 (1985); (c) H.B. Broughton, S.V. Ley, A.M.Z. Slawin, D.J. Williams and E.D. Morgan, *J. Chem. Soc., Chem. Comm.*, 46-47 (1986); (d) C.J. Turner, M.S. Tempesta, R.B. Taylor, M.G. Zagorski, J.S. Termini, D.R. Schroeder and K. Nakanishi *Tetrahedron*, **43**, 2789-2803 (1987); (e) J.N. Bilton, H.B. Broughton, P.S. Jones, S.V. Ley, Z. Lidert, E.D. Morgan, H.S. Rzepa, R.N. Sheppard, A.M.Z. Slawin and D.J. Williams, *Tetrahedron*, **43**, 2805-2815 (1987); (f) W. Kraus, M. Bokel, A. Bruhn, R. Cramer, I. Klaiber, A. Klenk, G. Nagl, H. Pöhnl, H. Sadlo and B. Vogler, *Tetrahedron*, **43**, 2817-2830 (1987).

20. A number of other methylated derivatives were examined (ref. 17). Azadirachtin-7-O-monomethyl ether and azadirachtin-20-O-monomethyl ether (**50**) displayed a conformational behaviour similar to (**28**), as did the azadirachtin-7,11-O-dimethyl and -7,20-O-dimethyl ethers. The azadirachtin-7,11,20-trimethyl ether (**49**) showed an energy profile as that for (**29**).
21. However, since a TMS group is much larger than a methyl group, it is possible that the energy profiles for (**46**) and (**47**) differ substantially from the potential energy curve of (**28**), thereby corresponding with the observed reduced antifeedant activitites.
22. These two hypothetical models of the role of the azadirachtin hydroxy groups in its bioactivity are not necessarily mutually exclusive. For instance, one could imagine the recognition of azadirachtin by a receptor protein to be enhanced by a correct 'bioactive' conformation of azadirachtin, which would allow the molecule to approach the active site on the protein whereupon the actual binding to the active site could occur via direct (*e.g.* hydrogen bonding) interactions between azadirachtin and amino acid residues of the protein.

Chapter 4

Synthesis of some Tricyclic Furo[2,3b]pyran Analogues of Azadirachtin, with and without the 7-Hydroxyl Group

Abstract: *In this chapter, some attempts are described towards the synthesis of tricyclic furo[2,3b]pyran model compounds derived from azadirachtin, both with and without a C-7 hydroxyl group. The 7-hydroxy-furo[2,3b]pyran model system was prepared according to literature procedures. The 7-dehydroxy-furo[2,3b]pyran model system could be prepared through a modified version of these procedures. Both isomers of this model system were formed, but only the isomer with inverted stereochemistry at C-3 and C-7 could be obtained in a pure form.*

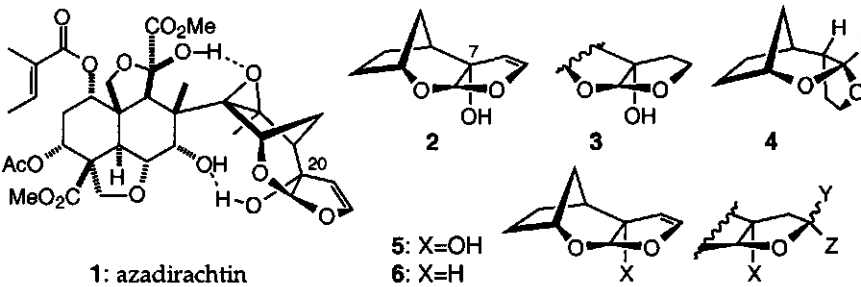
Table of Contents

4.1 - Introduction

One of the most powerful and versatile insect antifeedants known to date is the tetranortriterpene azadirachtin (**1**) from the Indian Neem tree (*Azadirachta indica*). This natural compound combines a potent antifeeding and growth disrupting activity against many different insect species with the absence of serious adverse effects on non-target organisms.[1] For this reason, azadirachtin is often considered as a rôle model of an environmentally friendly insecticide and it continues to attract attention, 30 years after its first isolation in 1968.[2]

Studies have demonstrated that various functional groups in both halves of the molecule contribute to the biological activity of azadirachtin.[1] However, both parts separately also displayed antifeedancy.[3] Especially analogues based on the tricyclic furo[2,3b]pyran fragment as (**2**) and (**3**) were as active against some insect species as azadirachtin itself at concentrations as low as 10 ppm.

Figure 4.1



1: azadirachtin

Despite many efforts, the present understanding of the structure-activity relationships controlling the antifeedant activity of azadirachtin is still limited. As was outlined in Chapter 3, some evidence[4] suggests that the presence of a free C-20 hydroxyl group is important to obtain full antifeedancy. It is unclear however, whether the importance of this group lies in its capability to participate in an intramolecular network of hydrogen bonds (indicated in Figure 4.1 by the dashed bonds), thereby positioning both halves of the molecule into a bioactive conformation,[5] or whether the presence of this group by itself is important for antifeedant activity, for instance through a direct interaction of this group with a receptor site.

Studies comparing the antifeedant activity of tricyclic furo[2,3b]pyran model compounds as (**5**) or (**6**), with and without a C-7 hydroxyl group, might contribute to a better understanding of the rôle of the corresponding C-20 hydroxyl group in azadirachtin. Since no decalin-fragment is present in these model compounds, their bioactivity cannot depend on the ability to form a network of intramolecular hydrogen bonds. An increased antifeedant activity of

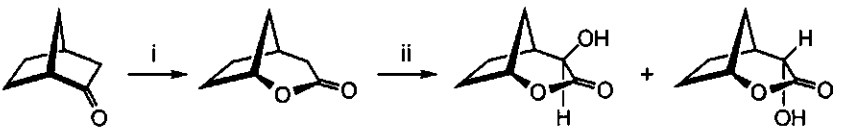
the hydroxyl-containing model compounds (**5**), relative to the *de*hydroxy analogues (**6**), could therefore be interpreted as support for the second hypothesis.

To our knowledge, no such C-7 dehydroxy model compounds have appeared in the literature, with the exception of compound (**4**),[6] which has the wrong stereochemistry to be of use in such studies and for which no synthetic details were reported. We therefore decided to undertake an attempt to prepare some 7-hydroxyfuro[2,3b]pyran model compounds (**5**) and the corresponding dehydroxy-derivatives (**6**).

4.2 - Synthesis of the 7-Hydroxyfuro[2,3b]pyran Model System

The 7-hydroxyfuropyran model system (**5**) was prepared according to the straightforward route developed by Anderson *et al*,[3a,7] with only minor modification of the described procedures. Baeyer-Villiger oxidation of norcamphor (**7**) with peracetic acid yielded the starting lactone (**8**)[8] in 75% yield (Scheme 4.1). The lithium enolate of (**8**) was α-hydroxylated in 54% yield with Vedejs' MoOPH complex (MoO_5•Py•HMPA),[9] affording an epimeric mixture of the hydroxylactones (**9a,b**) in a 14 : 1 ratio.

Scheme 4.1



7 **8** **9a** (*exo*) 14 : 1 **9b** (*endo*)

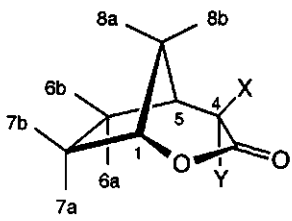
Reagents and conditions: (i) $MeCO_3H$, H_2SO_4-AcOH-water, 0°C, 1 hr (75%); (ii) 1) LDA, THF, -78°C, 1 hr; 2) MoOPH, r.t., 1 hr (54%).

In the literature[3a,7] the MoOPH oxidation was reported to take place exclusively from the sterically least hindered *exo*-face of lactone (**8**) to give isomer (**9a**) as the only product.[10] Although the ^{1}H NMR chemical shift values of our major isomer corresponded closely with those reported for (**9a**), we noted additional complexity in the coupling patterns of several protons. For instance, the H-4 signal was found to be a triplet instead of a singlet as reported for (**9a**).[7] Also, the signals previously assigned as H-1 and H-8' (identified as H-8b, see below) appeared as a multiplet and a double doublet, respectively, rather than as the singlet and doublet expected for (**9a**). In view of these differences we felt the need to confirm the *exo*-orientation of the hydroxyl group of our major isomer.

The protons H-1, H-4, H-5 and OH of the major isomer could be easily assigned from the ^{1}H NMR spectrum, but the remaining signals were more

complex and partially overlapped each other. With the help of a 2D ^{1}H-^{13}C correlation spectrum the positions of the protons H-6 - H-8 in the ^{1}H NMR spectrum could be established as the proton-pairs A, B and C, as depicted on the diagonal in Figure 4.2. Since the signal of the C-6 carbon atom in the ^{13}C NMR spectrum is well separated from both C-7 and C-8 by a downfield shift of the latter two (due to the inductive effect of the ring oxygen atom), the C1,2 pair could be identified as belonging to the H-6 protons. Also, the chemical shift values of the two H-8 protons were expected to differ significantly, due to the presence of H-8b in the local field generated by the carbonyl π-system, causing H-8b to shift downfield. Such a discriminating effect is lacking for the H-7 protons and it thus seemed reasonable to assign the B1,2 pair to the H-7 protons, while A1 and A2 are expected to be H-8b and H-8a respectively.

The relative stereochemistry of the major isomer could be deduced by analysis of the cross peaks found in the 2D ^{1}H-COSY spectrum (Figure 4.2). Apart from the usual geminal and vicinal couplings, some long range (*i.e.* ^{4}J) couplings were observed. This is not unusual for rigid molecules, especially when a W-like geometry exists for the σ-bonds connecting the coupled protons. The existence of



9a (*exo*): X=OH, Y=H
9b (*endo*): X=H, Y=OH

Assignment for *exo*-isomer (**9a**):

A1 = H-8b	B2 = H-7b
A2 = H-8a	C1 = H-6b
B1 = H-7a	C2 = H-6a

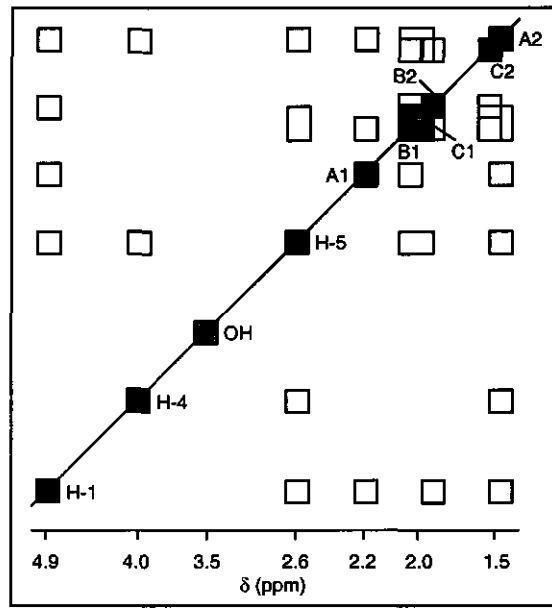


Figure 4.2: Schematic diagram of the 400 MHz symmetrical 2D ^{1}H-COSY spectrum of the major isomer (**9a**). Shaded or filled boxes represent the diagonal peaks corresponding to the 1D ^{1}H NMR spectrum, while the open boxes represent the observed cross peaks showing the coupling connectivity. For a discussion of the assignment of the signals A1-C2 and of the major isomer as the *exo*-isomer (**9a**), see text.

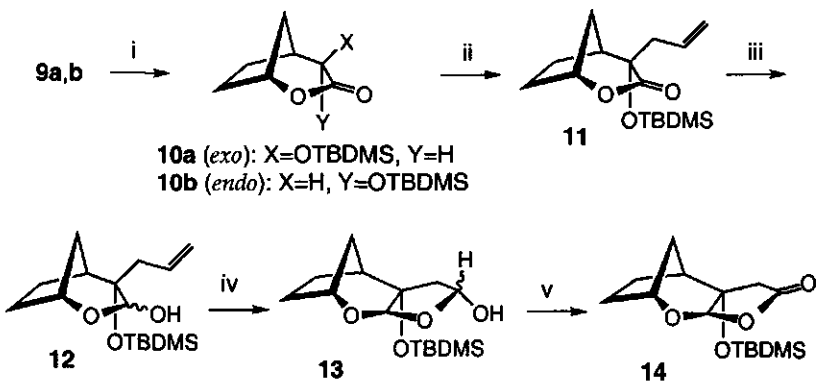
a long-range coupling between H-8b (A1) and one of the H-7 protons (B1) prompted the assignment of B1 as H-7a and of B2 as H-7b. Another long-range coupling between H-4 and A2 (H-8b) was considered to be indicative of an *endo*-orientation for H-4 and thus of the major isomer being the *exo*-isomer (**9a**). For the compound to be the *endo*-isomer (**9b**), this coupling would require A2 to be H-6b and thus A1 to be H-6a, which does not correspond with our previous assignment of the H-6 protons as proton pair C. Furthermore, A2 being H-6b would imply a long-range coupling between H-6b and H-1, which seems questionable since the geometry of the connecting bonds rather deviates from an ideal W-geometry.

The assignment of our major isomer as the *exo*-isomer (**9a**) was strengthened by some NOE experiments. The 1D NOE-difference spectrum obtained upon irradiation of H-4 showed one negative NOE for the hydroxyl proton and two positive NOE's: one for the H-5 proton and one for the overlapping signals A2 (H-8a) and C2. The latter NOE could be unequivocally assigned to the C2 signal with the help of a 2D NOESY spectrum. In the *exo*-isomer (**9a**) the distance between H-4 and H-8b obviously is too large (3.7Å [11]) to allow a NOE to occur and the observed effect can only be ascribed to H-6a, for which the distance to H-4 of 2.4Å is within NOE-range.[12] Signal C2 is thus identified as H-6a and C1 as H-6b, which is not in conflict with previous assignments. Contrary, for the *endo*-isomer (**9b**) a NOE of H-4 with H-8b would be expected (distance 2.6 Å), implying that C2 should be H-8b. This is in conflict with the previous identification of the A- and C-pairs as the H-8 and H-6 protons, respectively, and also contradicts our earlier expectation that H-8b should be shifted downfield, relative to the δ value for regular hydrocarbon protons. Furthermore, such an assignment would require unexpected long-range couplings, as of H-8b with both H-7 protons (B1,2) and of H-1 with both H-6 protons (A1,2).

In conclusion, the spectroscopic data best supported the assignment of our major product isomer as the *exo*-hydroxylactone (**9a**), which is in accordance with the preferred stereochemistry reported in the literature.

Next, the hydroxylactones (**9a,b**) were protected as the corresponding *tert*-butyldimethylsilyl ethers (**10a,b**), which were obtained in 93% yield as a 6.7 : 1 epimeric mixture[13] (Scheme 4.2). Allylation of (**10**) with LDA as the base afforded a crude reaction mixture containing 53% of starting material and 47% of both C-4 epimers of (**11**) in a 2 : 1 ratio (glc analysis). With KDA however, as described,[3a,7] only the major epimer was obtained in 74% isolated yield, together with 12.8% of

Scheme 4.2

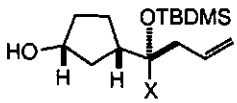


Reagents and conditions: (i) TBDMSCl, imidazole, DMF, 2 hrs (93%); (ii) 1) LDA, KO*t*-Bu, THF, -78°C, 30 min; 2) add (**10**), -78°C, 30 min; 3) $BrCH_2CH{=}CH_2$, -78°C to r.t., 8 hrs (71-74%); (iii) 1) DibalH (1.2 eq), toluene, -78°C, 1 hr; 2) $Na_2SO_4 \bullet 10H_2O$; (iv) 1) O_3, CH_2Cl_2, -78°C; 2) PPh_3 (1.3 eq), r.t., 24 hrs (39-43% over 2 steps); (v) TPAP (cat.), NMO (1.5 eq), 4Å mol. sieves, overnight (79-86%).

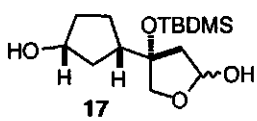
recovered starting material. The ^1H NMR chemical shift values of the product corresponded closely with those reported in the literature and the relative stereochemistry of (**11**) was consequently assigned as 4S.[14]

The allylated product (**11**) was reduced with a moderate excess[15] of DibalH at -78°C in toluene; no reaction was found to occur under similar conditions with ether as the solvent. The crude reduction product consisted of a mixture of the lactols (**12**) and the over-reduced diol (**15**), which were both present in about 40%. Furthermore, the presence of a small aldehyde signal (about 0.25H, *i.e.* 20% of the reduction product) in the ^1H NMR spectrum of the mixture indicated that (**12**) exists in equilibrium with its open hydroxy-aldehyde form (**16**), which explains the formation of the diol side-product in this step. A similar over-reduction has been reported for the reduction of both (**11**) and some 6-substituted derivatives of (**11**) under similar conditions, even when only 1.1 equiv. of DibalH were added slowly over a period of 6 hours.[7]

Ozonolysis of the crude mixture of reduction products and subsequent work-up with triphenylphosphine resulted in cleavage of the double bond to the corresponding aldehyde and cyclization to the furo[2,3b]pyran system in one step.



15: X=CH_2OH
16: X=CHO



17

The furopyranol (**13**) was obtained as a 3 : 1 mixture of C-5 epimers in a yield of 39-41% from (**11**). A more polar product fraction contained an inseparable mixture of triphenylphosphinoxide and a side product, which was identified as a 3 : 1 epimeric mixture of the hydroxycyclopentanyl-lactols (**17**) by comparison with a related compound described in the literature.[16]

The ^{1}H NMR data of the major isomer of (**13**) were sufficiently similar to those reported in the literature to assign the relative stereochemistry at C-7 as S, which is in agreement with the stereochemistry of the corresponding C-4 atom of the allylated lactone (**11**). For the minor isomer of (**13**), the ^{1}H and ^{13}C chemical shift values deviated only slightly from those of the major isomer, except for carbon atom C-11, which showed an upfield shift of almost 3 ppm from the value of the major isomer (all other carbon shifts were less than 1 ppm in magnitude). This caused some doubt whether the minor isomer might be epimeric at C-7, instead of at C-5. For this reason, the mixture of furopyranols (**13**) was oxidized with TPAP and NMO.[17] The corresponding furopyranone (**14**) was obtained in 79-86% as a single isomer, indicating that the minor isomer of (**13**) is indeed the C-5 epimer.

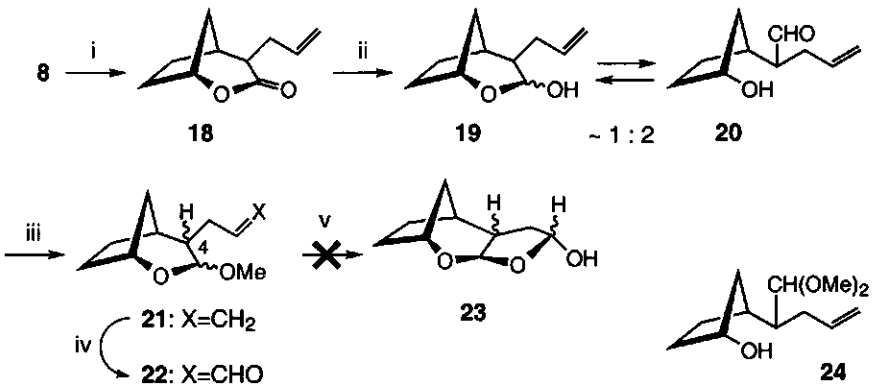
In conclusion, the results described above confirm that the desired 7-hydroxyfuropyran ring system can be conveniently prepared via the route developed by Anderson *et al*, albeit in a moderate overall yield (12% from norcamphor in 6 steps). The intermediate product (**13**) provides easy access to a range of differently C-5 functionalized model compounds (**5**) (*i.e.* Figure 4.1: Y=H and Z=H, OH, OMe, OAc or X,Y=O) via known methods.[1c,7] In view of the aim of the present study, however, the preparation of such a series of model compounds (**5**) has little value if the corresponding 7-dehydroxy analogues (**6**) are not available. We therefore first turned our attention to the synthesis of the 7-dehydroxyfuro[2,3b]pyran ring system.

4.3 - Synthesis of the 7-Dehydroxyfuro[2,3b]pyran Model System

4.3.1 - Preparation

As described in the previous section, the reactions of the enolate ions from (**8**) and (**10**) with several electrophilic reagents show a preference for the least hindered *exo*-face. We therefore anticipated that the synthesis of the 7-dehydroxyfuropyran model system (**6**) should be possible via the same approach as used for (**5**), simply by exclusion of the α-hydroxylation step of (**8**) from the sequence of reactions.

Scheme 4.3



Reagents and conditions: (i) 1) LDA, THF, -78°C, 30 min; 2) add (**8**), -78°C, 30 min; 3) $BrCH_2CH{=}CH_2$, -78°C to r.t., overnight (78-83%); (ii) 1) DibalH (1.2 eq), Et_2O or toluene, -78°C, 30 min - 1 hr; 2) $Na_2SO_4 \bullet 10H_2O$; (iii) MeOH, TsOH, 2 hrs; (iv) 1) O_3, CH_2Cl_2, -78°C; 2) PPh_3 (1.3 eq), r.t., 24 hrs; (v) 1N HCl, THF-water, overnight.

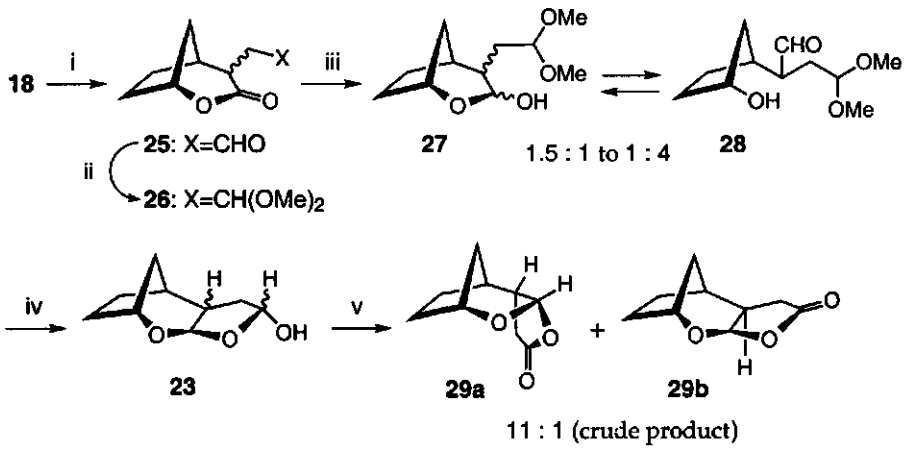
Allylation of (**8**) with LDA as the base yielded (**18**) in 83% yield (Scheme 4.3). Both tlc- and glc-analysis[18a] and ^{13}C-NMR spectroscopy[18b] indicated that the allylated product was obtained as a single isomer. Reduction of (**18**) with DibalH yielded a mixture of highly polar products. The presence of an aldehyde peak in the ^1H-NMR spectrum of the purified product indicated that it existed as a mixture[19] of the lactols (**19**) and its open hydroxy-aldehyde form (**20**) in a ratio of approx. 1 : 2. Contrary to the experiences in the previous section, *in situ* ozonolysis and acid-catalyzed cyclization of this mixture did not lead to the desired furopyranols (**23**) but instead yielded a complex mixture of highly polar products. We suspected that this might be due to sidereactions following the ozonolysis of (**20**) and therefore the reduction product was trapped by acetalization with methanol under acidic conditions. The methyl ethers (**21**) were obtained as a mixture of four isomers (no ring-opened derivative (**24**) was found), indicating that epimerization at C-4 had occurred. This is most likely due to the preference of the reduction product for its ring-opened form (**20**), in which epimerization can easily occur through the enol. The crude product (**21**) could be cleanly ozonolyzed to the corresponding aldehydes (**22**), but again the subsequent cyclization reaction failed to give the desired products (**23**).

In an attempt to circumvent these problems, the order of the oxidation and reduction steps in the reaction sequence was reversed (Scheme 4.4). However, epimerization at C-4 also occurred under the relatively mild reaction conditions during ozonolysis and subsequent work-up: the lactone-aldehyde (**25**) was obtained in 82% yield as a 1 : 1 epimeric mixture. The aldehyde was protected as

its dimethylacetal (**26**) in 73% yield by reaction with trimethylorthoformate under cerium-assisted conditions.[20]

Reduction of the lactone (**26**) repeatedly yielded mixtures of highly polar products, which were difficult to characterize. In analogy with previous results, the mixtures probably consisted of the lactol (**27**) and its ringopened hydroxy-aldehyde form (**28**), in a combined yield of 73-85%. Between different experiments, the aldehyde-content of the mixtures varied from 40-80%.

Scheme 4.4



Reagents and conditions: (i) 1) O_3, CH_2Cl_2, -78°C; 2) PPh_3 (1.3 eq), r.t., 24 hrs (82%); (ii) $CeCl_3 \bullet 7H_2O$ (1 eq), $(MeO)_3CH$ (10 eq), MeOH, r.t. (73%); (iii) 1) DibalH (1.2 eq), Et_2O, -78°C, 1 hr; 2) $Na_2SO_4 \bullet 10H_2O$ (73-85%); (iv) 1N HCl-THF (3:1), overnight (51%); (v) TPAP (cat), NMO, CH_2Cl_2, 4Å mol. sieves (45% after chromatography).

The acid-catalyzed cyclization of the (**27**)/(**28**) mixture afforded the furopyranols (**23**) as a mixture of highly polar diastereomers in 52% yield after chromatography. In order to facilitate separation of these isomers, the number of chiral centers in the molecule was reduced via ruthenium-catalyzed oxidation of the hemiacetal group of (**23**) to afford the corresponding furopyranone (**29**). The crude product was found to contain two isomers in a ratio of 11 : 1. After extensive chromatography two fractions were obtained, consisting of a 6 : 1 mixture of both isomers (least polar fraction, 27% yield) and of the pure major isomer (**29a**) (most polar fraction, 18% yield), respectively. Unfortunately, attempts to obtain the pure minor isomer (**29b**) failed.

4.3.2 - Determination of the Relative Stereochemistry

The relative stereochemistry of the major isomer (**29a**) was determined via NMR spectroscopy. Protons H-1 and H-3 were assigned directly from the ^{1}H NMR spectrum, while the positions of the remaining protons could be established as the signals A - E (as indicated in Figure 4.3) from the 2D ^{1}H-^{13}C correlation spectrum. The signals C1 - C3 belong to three protons connected to two different carbon atoms (*i.e.* a CH moiety and a CH_2 moiety); at this stage these signals could not be discriminated further because the corresponding peaks in the ^{13}C spectrum overlapped each other.

The ^{13}C NMR spectrum showed the presence of four CH_2 moieties in the molecule (associated with the signals A, C, D and E). In view of the expected downfield shift of the carbon atoms C-6, C-10 and C-11, relative to the most 'hydrocarbon-like' carbon atom C-9, signal E was assigned to both H-9 protons. Furthermore, the distinctive coupling patterns of the signals A1 and A2 in the ^{1}H NMR spectrum (as double doublet [J=17 and 9 Hz] and doublet [J=17 Hz], respectively) allowed their assignment as the H-6 proton-pair because this is the

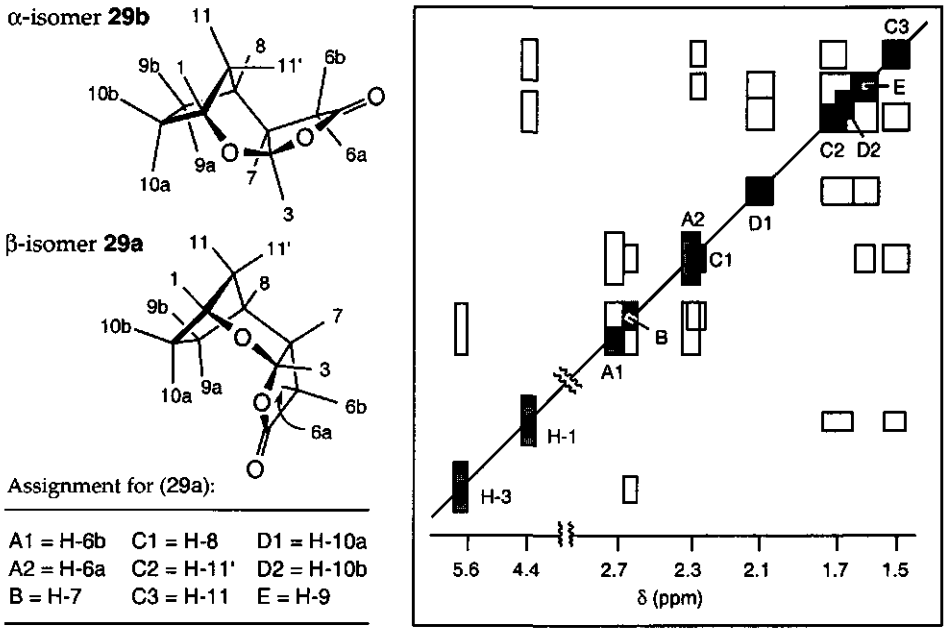


Figure 4.3: Schematic diagram of the 400 MHz non-symmetrical 2D ^{1}H-COSY spectrum of the major isomer (**29a**). Shaded or filled boxes represent the diagonal peaks corresponding to the 1D ^{1}H NMR spectrum, while the open boxes represent the observed cross peaks showing the coupling connectivity. For a discussion of the assignment of the signals A-E and of (**29a**) as the 7βH-isomer, see text.

only CH_2 moiety present with only one neighbouring proton (H-7); further discrimination of these protons into H-6a and H-6b was not yet possible because the (unknown) stereochemistry of C-7 determines which of the H-6 protons couples with H-7 to show a double doublet.

The remaining CH_2 protons (H-10, H-11) could be assigned via the 2D ^{1}H-COSY spectrum (Figure 4.3). All of these protons were expected to couple with proton H-1, with the exception of H-10a, which has a dihedral angle with H-1 of about 94° in both possible isomers (**29a** and **29b**)[21a] and for which the vicinal coupling constant should therefore approximate zero, according to the Karplus relationship.[22] The only remaining CH_2 signal for which no cross peak with H-1 could be found in the COSY spectrum was signal D1, allowing D1 to be assigned as H-10a and D2 as H-10b. For the H-9 proton-pair (E) a cross peak with signal C1 was observed, indicating that C1 belonged to the single H-8 proton. From this followed that the last CH_2 signals C2 and C3 had to be both H-11 protons. Finally, the assignment of the only remaining (CH-)signal B as proton H-7 was confirmed by the presence of a cross peak for B with H-3.

β-isomer (**29a**)		α-isomer (**29b**)		actual NOE diff. experiments[a]	
atom-pair	NOE exp.	atom-pair	NOE exp.	signal-pair	NOE found
H-3 - H-10a	no	H-3 - H-10a	yes	H-3 - D1 (H-10a)	no
H-6a - H-9a	yes	H-6a,b - H-9	no	A1/B - E	no
H-7 - H-9a	no	H-7 - H-9a	yes	(H-7/H-6^b) (H-9)	
H-6a,b - H-11'	no	H-6b - H-11'	yes	A1/B - C2/D2	yes
H-7 - H-11'	yes	H-7 - H-11'	no	(H-7/H-6^b) (H-11^b/H-10b)	

Table 4.1: Overview of the NOEs most relevant for the determination of the relative stereochemistry, both as the NOEs expected for the two possible isomers and as the NOEs actually found. For a discussion, see text. *Notes*: (a) Measured for the pure major isomer (**29a**). (b) Only one proton of the corresponding proton-pair; further assignment not yet known.

The relative stereochemistry of the major isomer (**29a**) was deduced from the results obtained from a number of 1D NOE difference experiments.[23] Table 4.1 lists a number of proton-pairs for which a NOE was expected in the possible isomers (**29a**) or (**29b**), based on the atom-atom distances[12] as determined by molecular mechanics calculations.[21b] The presence or absence of these NOEs in the actual NOE difference spectra was used to determine the relative stereochemistry. Upon irradiation of H-3 a NOE was observed for H-7 but not for H-10a (D1), as was expected for the 7αH-isomer (**29b**). Similarly, simultaneous

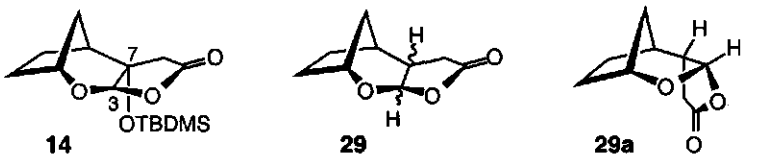
irradiation of the signals B and A1 (*i.e.* of H-7 and one of the H-6 protons) gave no NOE for the H-9 protons (E). This is again not in agreement with the expectation for (**29b**), since for this isomer a NOE with H-9 should have been present. For the 7βH-isomer (**29a**), however, this NOE may be absent, implying that signal A1 should be assigned to proton H-6b and A2 to H-6a. Furthermore, simultaneous irradiation of A1 and B produced a strong NOE for the overlapping signals D2 and C2 (*i.e.* H-10b and one of the H-11 protons). Since both H-10b and H-11 are out of NOE-range for H-6 or H-7 in both (**29a**) and (**29b**), this NOE could only be ascribed to dipolar coupling of H-11' with H-7 or H-6b, respectively, leading to the assignment of C2 as H-11' and C3 as H-11. This assignment was confirmed by the occurrence of a NOE of signal E (H-9) upon irradiation of H-11 (C3). In conclusion, the results of the NOE difference experiments best supported the assignment of the relative stereochemistry of the major isomer as the 7βH-isomer (**29a**).

4.4 - Concluding Remarks

The work described in this chapter was directed towards the synthesis of a series of tricyclic furo[2,3b]pyran model compounds derived from azadirachtin (**1**) (Figure 4.1). The aim was to prepare analogue pairs, each consisting of a model compound (**5**) with a C-7 hydroxyl group and its corresponding derivative (**6**) without this C-7 hydroxyl group. Comparison of the antifeedant activity within these pairs might provide insight into the rôle of the C-20 hydroxyl group of azadirachtin in its bioactivity.

The 7-hydroxy-furo[2,3b]pyran derivative (**14**) (Figure 4.4) with the same relative stereochemistry as in azadirachtin was prepared according to literature procedures. The 7-*de*hydroxy-furo[2,3b]pyran model system (**29**) could be prepared through a modified version of these procedures, but only isomer (**29a**) with an inverted stereochemistry at C-3 and C-7 (relative to the stereochemistry of the furo[2,3b]pyran fragment of azadirachtin) could be obtained in a pure form. Due to these and other experimental difficulties (such as the high polarity of the compounds and the constant necessity to work with mixtures of isomers), it was decided to stop research in this direction.

Figure 4.4



14 **29** **29a**

4.5 - Experimental Section

4.5.1 - General Experimental Conditions

All reagents were purchased from Aldrich, Janssen or Merck and were used without further purification, unless otherwise indicated. Dry reactions were performed under an a steady stream of dry nitrogen; flasks, syringes and other equipment used for these reactions were dried at 140°C and allowed to cool in an atmosphere of dry nitrogen. Dry tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use; dry CH_2Cl_2 was similarly distilled from CaH_2. Dry ether and toluene were obtained by storage of the distilled commercial solvents on sodium wire. Distilled pyridine was stored over KOH; other dry solvents were obtained by storage of the distilled material over molecular sieves.

Thin layer chromatography (tlc) was performed with Merck silicagel 60 F_{254} on plastic sheets. Flash column chromatography was performed using Merck silicagel (230-400 ASTM). Solvents used for column chromatography were always distilled prior to usage. Petrol refers to petroleum ether b.p. 40-60°C.

Gas chromatography (glc) was carried out on a Fisons GC 8000 or a Hewlett Packard 5890A gas chromatograph with a flame ionisation detector and a fused silica capillary column (DB-5+ or DB-17+), 30 m × 0.25 mm i.d., film thickness 0.25 μm. Peak areas were integrated electronically with a Fisons integrator DP700.

Melting points were determined on a C. Reichert, Vienna, apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 (90 MHz) or a Bruker AC 200E (200 MHz) or a Bruker Avance DPX 400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC 200E (50 MHz) or a Bruker Avance DPX 400 (100 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (δ 0.0). Infrared spectra were recorded in solution on a Jasco A-100 spectrometer. Mass spectral data and accurate mass measurements were determined on an AEI-MS-902 spectrometer equipped with a VG ZAB console and were obtained via electron impact (EI) ionisation, unless indicated otherwise. Elemental analyses were determined on a Carbo Elba elemental analyser 1106.

4.5.2 - Experimental Procedures

Compounds (8)-(13) were prepared according to cited literature procedures.

Synthesis of (1R*, 5S*)-2-oxabicyclo[3.2.1]octan-3-one (8).[8] A solution of 10 g (90.8 mmol) of nor-camphor in 40 ml of glacial acetic acid and 20 ml of conc. sulfuric acid was cooled on an ice bath while stirring mechanically in the dark. To this mixture was added dropwise over a period of 30 min 24 ml of a 32 wt% solution of peracetic acid in dilute acetic acid. The reaction mixture was stirred for a further 30 min at this temperature while the reaction was monitored by glc analysis. After complete disappearance of the starting material the reaction mixture was diluted with 200 ml of ethylacetate and poured into 200 ml of an ice-cold saturated solution of sodium bicarbonate in water. While stirring vigorously solid sodium bicarbonate was added until the pH of the mixture was 6.5-7. The organic layer was decanted and the water-salt slurry was extracted 5 times with 150 ml of ethyl acetate, decanting the organic layer after every extraction. The combined organic layers were dried with magnesium sulfate and filtered. Removal of the solvent under reduced pressure yielded 9.96 g of a clear, colourless oil with a pungent odour. Chromatography on 150 g of silicagel

with petrol-EtOAc (70-30) as the eluent yielded 8.6 g (68.2 mmol, 75.2%; lit.: 97% with purification by vacuum distillation instead of chromatography) of the lactone **(8)** as a clear, colourless and odourless oil, which formed white crystals upon standing at r.t.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.55-2.35 (m, 6H): H-6 - H-8; 2.40-2.57 (m, 2H): H-4 [δ 2.42 (dm, J≈20.1 Hz)] and H-5; 2.68 (dm, J=20.1 Hz, 1H): H-4'; 4.80 (br, 1H): H-1. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 29.2 (t); 31.8 (d): C-5; 32.4 (t); 35.8 (t); 40.6 (t); 81.0 (d): C-1; 170.8 (s): C-3.

Synthesis of (1R*, 4R*, 5S*)- and (1R*, 4S*, 5S*)-4-hydroxy-2-oxabicyclo[3.2.1] octan-3-one (9a) and (9b).[7] To a solution of 2.0 g (15.9 mmol) of the lactone (**8**) in 40 ml of anhydrous THF, stirred under nitrogen at -78°C, was added dropwise in approx. 5 min 8.8 ml (17.6 mmol) of a 2M-solution of lithium diisopropylamine in THF-heptane-ethylbenzene. The resulting yellow solution was stirred for 1 hr at -78°C before 8.3 g (19.5 mmol) of MoOPH was added in two portions. After 5 min the cooling bath was removed and the reaction mixture was slowly warmed to room temperature, during which time the MoOPH complex dissolved and the initial dark brown colour of the reaction mixture changed to greenish-yellow. After stirring vigorously for 1 hr, 50 ml of saturated aqueous sodium sulphite solution was added, followed by 50 ml of brine. Stirring was continued for another 15 min before the greenish-yellow reaction mixture was saturated with solid sodium chloride. The organic layer was separated and the remaining brine slurry was extracted with four 100 ml-portions of EtOAc. The combined organic layers were dried with $MgSO_4$, filtered through a pad of silicagel and concentrated under reduced pressure to give 2.0 g of a brown-yellow oil. Purification via chromatography on 50 g of silicagel with petrol-EtOAc (80-20 to 75-25) as the eluent afforded 1.2 g (8.4 mmol, 53%; lit.: 67%) of a pale yellow, waxy solid. NMR-analysis indicated the product to be a mixture of epimers (**9a**) : (**9b**) = 14.2 : 1; only one peak was observed upon glc-analysis.
Major (exo) isomer (**9a**): ^{1}H NMR ($CDCl_3$, 400 MHz, selected peaks): δ 1.53-1.66 (m, 2H): H-6a and H-8a; 1.84-2.06 (m, 3.0H): H-6b and H.7a,b; 2.25 (dd, J=13.53, 0.77 Hz, 1H): H-8b; 2.59-2.65 (m, 1H): H-5; 3.50 (br, 1H):OH; 4.01 (dd, J=1.68, 1.68 Hz, 1H): H-4; 4.89-4.92 (m, 1H): H-1. ^{13}C NMR ($CDCl_3$, 100 MHz, DEPT, selected peaks): δ 25.3 (t): C-6; 31.7 (t): C-7; 32.5 (t): C-8; 38.7 (d): C-5; 74.9 (d): C-4; 82.3 (d): C-1; 173.4 (s): C-3.
Minor (endo) isomer (**9b**): ^{1}H NMR ($CDCl_3$, 400 MHz, separated peaks only): δ 2.14 (br d, J=6.5 Hz, approx. 1H): H-8b; 2.74 (br q, J=5.5 Hz, 1H): H-5; 4.23 (dd, J=5.0, 2.0 Hz, 1H): H-4; 4.84-4.87 (m, 1H): H-1. ^{13}C NMR ($CDCl_3$, 100 MHz, DEPT, separated peaks only): δ 21.5 (t); 36.4 (t); 38.5 (d): C-5; 72.6 (d): C-4; 83.4 (d): C-1.

Synthesis of (1R*, 4R*, 5S*)- and (1R*, 4S*, 5S*)-4-(*tert*-butyldimethylsilyloxy)-2-oxabicyclo[3.2.1] octan-3-one (10a) and (10b).[7] The mixture of epimers (**9**) was treated overnight with *tert*-butyldimetylsilylchloride according to the described procedure. The crude product was purified via chromatography on silicagel with petrol-EtOAc (100-0 to 95-5) as the eluent to give a 6.7 : 1 mixture of epimers (**10**)[13a] in 93% yield (lit.: 94%) as a colourless oil, which crystallized overnight in the fridge into a white solid (m.p. 46-48°C; lit.: 48-49°C).
^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.10-0.19 (ms): $Si(CH_3)_2$; 0.90-0.93 (ms, 9H): $SiC(CH_3)_3$; 1.45-1.55 (m, 2H): H-6a and H-8a; 1.65-2.11 (m, 3H): H-6b and H.7a,b; 2.19-2.27 (m, 0.13H): H-8b minor isom.; 2.41 (dd, J=12.9, 1.0 Hz, 0.87H): H-8b major isom.; 2.44-2.49 (m, 0.87H): H-5 major isom.; 2.55 (dq, J=1.0, 5.7 Hz, 0.13 H): H-5 minor isom.; 3.95 (dd, J=2.7, 1.5 Hz, 0.87H): H-4 major isom.; 4.18 (dd, J=4.8, 1.8 Hz, 0.13H): H-4 minor isom.; 4.76-4.77 (m, 0.13H): H-1 minor isom.; 4.83-4.84 (m, 0.87H): H-1 major isom. ^{13}C NMR ($CDCl_3$, 100 MHz, DEPT, selected peaks): *Major isomer*: δ -4.9 (q) and -4.4 (q): $Si(CH_3)_2$; 18.5 (s): $Si\underline{C}(CH_3)_3$; 23.6 (t): C-6; 26.1 (q): $SiC(\underline{C}H_3)_3$; 31.4 (t): C-7; 32.1 (t): C-8; 40.5 (d):

C-5; 75.4 (d): C-4; 81.8 (d): C-1; 170.7 (s): C-3. *Minor isomer*: δ -5.2 (q) and -4.2 (q); 18.7 (s); 21.6 (t); 26.1 (q); 31.4 (t); 32.2 (t); 40.9 (d); 73.5 (d); 81.7 (d); 172.6 (s).

Synthesis of (1R*,4S*,5S*)- 4 -(*tert*-butyldimethylsilyloxy)- 4 -(prop-2-enyl)- 2 -oxabicyclo-[3.2.1]octan-3-one (11).[7] To a stirred mixture of 1.13 g (10.1 mmol) of potassium *tert*-butoxide and 40 ml of dry THF at -78°C was added dropwise 5.0 ml (10.0 mmol) of a 2M solution of LDA in THF/heptane/ethylbenzene. The resulting deep-red solution was stirred for 30 min before a solution of 1.80 g (7.0 mmol) of the lactones (**10a,b**) in 20 ml of THF was added dropwise in approx. 15 min. After stirring for an additional 30 min the pale-yellow solution was treated with 1.0 ml (11.6 mmol) of allylbromide. Stirring at -78°C was continued for 1 hr, while the progress of the reaction was monitored by glc-analysis. Subsequently the dry-ice was removed from the cooling bath and the reaction mixture was allowed to warm to room temperature. After an additional 6 hrs the reaction progressed no further and was quenched by the addition of 10 ml of saturated NH_4Cl-solution and 10 ml of water. The organic layer was separated and the water layer was extracted with three 50 ml-portions of EtOAc. The combined organic layers were washed twice with 15 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give a yellow oil (2.25 g). Chromatography on 60 g of silicagel with petrol-EtOAc (98-2 to 94-6) as the eluent afforded, in order of elution, 1.53 g (5.17 mmol; 74%) of (**11**) as a colourless oil and 229 mg (0.89 mmol; 12.8%) of starting material.
(**11**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.07 and 0.32 (2 s, 6H): $Si(CH_3)_2$; 0.89 (s, 9H): $SiC(CH_3)_3$; 1.60 (ddd, J=13.5, 5.3, 2.5 Hz, 1H): H-8a; 1.67-1.83 (m, 1H) and 1.83-2.08 (m, 2H) and 2.11-2.29 (m, 2H): H-6, H-7 and H-8b [δ 2.17 (d, J=13.2 Hz)]; 2.41-2.72 (m, 3H): H-5 [δ 2.52 (d, J=8.4 Hz)] and allyl H-1' [δ 2.49 (dd, J≈17.7, 8.5 Hz) and δ 2.66 (ddt, J=14.5, 5.6, 1.5 Hz)]; 4.76 (br m, 1H): H-1; 5.13 (dm, J=24.8 Hz, 1H): allyl H-3'(*E*); 5.15 (m, 1H): allyl H-3'(Z); 5.79-6.00 (m, 1H): allyl H-2'.

Reduction of the lactone (11). To a solution of 327 mg (1.1 mmol) of the lactone (**11**) in 20 ml of dry toluene, stirred at -78°C, was added dropwise 1.0 ml (1.5 mmol) of a 1.5M solution of diisobutyl aluminumhydride in toluene. After stirring for an additional hour at -78°C the reaction was quenched by addition of 2 g (6.2 mmol) of Glaubers' salt. The resulting slurry was diluted with 25 ml of EtOAc, warmed to room temperature and stirred for another 30 min. Then $MgSO_4$ was added and the mixture was filtered through a pad of hyflo. The residue was stirred up three times with 25 ml of EtOAc and filtered. The combined filtrates were concentrated under reduced pressure to yield 310 mg of a colourless oil. Tlc-analysis of the crude product mixture revealed two distinct spots, while the ^{1}H-NMR spectrum showed signals belonging to the desired lactols (**12**), their ringopened hydroxy-aldehyde form (**16**) and the over-reduced diol 2-(3'-hydroxy-cyclopentanyl)-2-TBDMSoxy-pent-4-enol (**15**)[7]. The product mixture was not purified, but was used as such in the next reaction.
^{1}H-NMR ($CDCl_3$, 90 MHz): δ 0.05 (br s, 6H): $Si(CH_3)_2$; 0.7 (br s, 9H): $SiC(CH_3)_3$; 0.9-2.6 (m, 13H); 3.4 (s, 1H): H-1 diol; 3.55 and 3.63 (2 s, 0.5H) OH lactols; 3.9-4.3 (m, 1H): H-1 lactols and H-3' diol; 4.55 and 4.63 (2 s, 0.5H): H-3 lactols; 4.8-5.1 (m, 2H): $CH_2CH=C\underline{H}_2$; 5.4-6.1 (m, 1H): $CH_2C\underline{H}=CH_2$; 9.5 (s, 0.25H): CHO ringopened hydroxy-aldehyde.

Synthesis of (1R*,3R*,5RS,7S*,8S*)- 7- (*tert*-butyldimethylsilyloxy)- 2, 4- dioxatricyclo-[6.2.1.0^{3,7}]undecan-5-ol (13).[7] A solution of 310 mg of the crude reduction product mixture (**12,15,16**) in 15 ml of CH_2Cl_2 was stirred at -78°C. An ozone/oxygen-mixture was bubbled through the solution until a deep blue colour appeared and tlc-analysis indicated that the starting materials had been completely transformed. The solution was purged with nitrogen to remove excess ozone and was then treated with 350 mg (1.3 mmol) of

triphenylphosphine. The reaction mixture was warmed to room temperature and stirred for 24 hrs. The solvent was removed under reduced pressure and the residual oil (771 mg) was purified via chromatography on 30 g of silicagel with petrol-EtOAc (90-10 to 60-40) as the eluent to yield, in order of elution, 129 mg (0.4 mmol; 39% over 2 steps) of a 3 : 1 epimeric mixture of the furopyranols (**13**) as a clear, colourless oil and 133 mg of a 3 : 1 mixture of the epimeric lactols **4-(3'-hydroxy-cyclopentanyl)-4-TBDMSoxy-2,3,4,5-tetrahydro-furan-2-ol** (**17**), contaminated with a trace of triphenylphosphinoxide, as an oil which solidified upon standing in the fridge.
(**13**): ^{1}H-NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.12 and 0.13 (2 s, 3.8H): $Si(CH_3)_2$ major epimer; 0.21 (2 s, 1.2H): $Si(CH_3)_2$ minor epimer; 0.88 (s, 6.8H): $SiC(CH_3)_3$ major epimer; 0.93 (s, 2.2H): $SiC(CH_3)_3$ minor epimer; 1.31-1.45 (m [incl. δ 1.39 (ddd, J=12.6, 5.2, 2.6 Hz)], 1H): H-11; 1.51-2.30 (m, 8H): H-6 and H-8 - H-11; 2.43-2.62 (m, 1H): H-6; 3.17 (d, J=10.7 Hz, 0.25H): OH minor epimer; 3.42 (br d, J≈6.6 Hz, 0.75H): OH major epimer; 4.31 (br, 0.25H): H-1 minor epimer; 4.40 (br, 0.75H): H-1 major epimer; 4.86 (s, 0.75H): H-3 major epimer; 5.02 (s, 0.25H): H-3 minor epimer; 5.59-5.80 (br m, 1H): H-5. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, selected peaks): *Major epimer*: δ -2.8 (q): $Si(CH_3)_2$; 18.1 (s): $SiC(CH_3)_3$; 24.5 (t): C-9; 25.7 (q): $SiC(CH_3)_3$; 28.3 (t): C-10; 35.8 (t): C-6; 42.2 (d): C-8; 43.2 (t): C-11; 76.3 (d): C-1; 83.9 (s): C-7; 102.4 (d) and 104.7 (d): C-3 and C-5. *Minor epimer* (separated peaks only): δ -1.8 (q); 24.9 (t); 25.8 (q); 28.7 (t); 35.5 (t); 40.5 (t); 41.8 (d); 75.7 (d); 101.8 (d); 104.5 (d).
(**17**): ^{1}H-NMR ($CDCl_3$, 90 MHz, selected peaks): δ 0.0-0.15 (2 s and br s, 6H): $Si(CH_3)_2$ both epimers; 0.8 (s, 9H): $SiC(CH_3)_3$; 1.0-2.5 (br m, 12H); 3.73 (d, J=6.5 Hz, 1.5H): H-5 major epimer; 3.86 (d, J=3.0 Hz, 0.5H): H-5 minor epimer; 4.05-4.3 (m, 1H); 5.33 (dd, J=6.0, 3.0 Hz, 0.25H): H-2 minor epimer; 5.56 (dd, J=6.0, 3.0 Hz, 0.75H): H-2 major epimer.

(1R*,3R*,7S*,8S*)- 7-(*Tert*-butyldimethylsilyloxy)- 2,4-dioxatricyclo[6.2.1.0^{3,7}]undecan-5-one (**14**). A solution of 118 mg (1.0 mmol) of 4-methylmorpholine-N-oxide (NMO) in 5 ml of dry CH_2Cl_2 containing 1 g of powdered 4Å molecular sieves was stirred for 10 min at room temperature before a solution of 202 mg (0.67 mmol) of the furopyranol (**13**) in 10 ml of CH_2Cl_2 was added. After stirring for another 5 min the reaction mixture was treated with 25 mg (0.07 mmol) of tetra-*n*-propylammonium perruthenate (TPAP). The resulting green solution was stirred for 2 hrs, during which time the initial green solution gradually darkened. After complete disappearance of the staring material, as judged by tlc-analysis, the mixture was diluted with 50 ml of CH_2Cl_2 and washed sequentially with 10 ml of saturated sodium sulphite solution, 10 ml of brine and 10 ml of satd. copper(II)sulphate solution. The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure to give 195 mg of a green-brown solid. The product was purified by chromatography on 25 g of silicagel with petrol-EtOAc (90-10) as the eluent, yielding 171 mg (0.57 mmol; 86%) of the furopyranone (**14**) as a white solid.
^{1}H-NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.14 (s, 6H): $Si(CH_3)_2$; 0.88 (s, 9H): $SiC(CH_3)_3$; 1.48 (ddd, J=12.8, 5.2, 2.6 Hz, 1H): H-11; 1.62-2.05 (m, 4H); 2.16-2.30 (m, 1H); 2.43 (d, J=17.9 Hz, 1H): H-6; 2.54 (t, J=5.4 Hz, 1H): H-8; 2.98 (d, J=17.8 Hz, 1H): H-6; 4.42 (br s, 1H): H-1; 5.21 (s, 2H): H-3. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, selected peaks): δ -2.7 and -2.6 (2 q): $Si(CH_3)_2$; 18.0 (s): $SiC(CH_3)_3$; 23.4 (t): C-9; 25.5 (q): $SiC(CH_3)_3$; 28.5 (t): C-10; 35.0 (t): C-6; 37.6 (t): C-11; 41.8 (d): C-8; 76.1 (d): C-1; 78.4 (s): C-7; 103.7 (d): C-3; 175.2 (s): C-5.

Synthesis of 4-(prop-2'-enyl)-2-oxabicyclo[3.2.1]octan-3-one (**18**). To a stirred solution of 1.9 ml (14.5 mmol) of diisopropylamine in 10 ml of THF under nitrogen at 0°C was added dropwise 9 ml (14.4 mmol) of a 1.6M solution of n-butyllithium in hexanes. After stirring for 20 min, the solution was cooled to -78°C. A solution of 1.5 g (11.9 mmol) of the lactone (**8**)

in 5 ml of THF was added dropwise in a period of 5 min, followed after 30 min by the dropwise addition of 1.34 ml (15.5 mmol) of neat allylbromide. The reaction mixture was stirred for 30 min at -78°C before the temperature was slowly raised to -10°C over a period of 3 hrs. After complete disappearance of the starting material, as judged by glc analysis, the reaction was quenched by addition of 10 ml of saturated ammoniumchloride solution and 10 ml of water. The mixture was warmed to room temperature and was extracted with 4x25 ml of ether. The combined organic layers were dried with magnesiumsulfate and concentrated under reduced pressure to yield 1.8 g of a yellow oil. The product was purified by chromatography on 40 g of silicagel with petrol-EtOAc (95-5) as the eluent, yielding 1.54 g (9.3 mmol; 78%) of the allyl lactone (**18**) as a clear, light-yellow oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.40-1.55 (dm,, J≈12 Hz, 1H): H-8a; 1.55-1.70 (dm, J=12 Hz, 1H): H-6a; 1.75-2.12 (m, 4H): H-6b, H-7 and H-8b; 2.12-2.32 (m, 1H): H-1'; 2.32-2.48 (br m, 2H): H-4 and H-5; 2.51-2.67 (dm, J≈18 Hz, 1H): H-1'; 4.76 (br, 1H): H-1; 5.01 (m, 1H): H-3'(*E*); 5.08 (m, 1H): H-3'(*Z*); 5.63-5.84 (m, 1H): H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 29.8 (t): C-6; 32.2 (t): C-8; 32.4 (t): C-7; 34.7 (d): C-5; 37.4 (t): C-1'; 50.1 (d): C-4; 80.9 (d): C-1; 117.5 (t): C-3'; 135.6 (d): C-2'; 173.5 (s): C-3.[24]

3-Methoxy- 4-(prop-2'-enyl)- 2-oxabicyclo[3.2.1]octane (21). A solution of 1.66 g (10.0 mmol) of the lactone (**18**) in 25 ml of dry ether was reduced with 12.5 ml (12.5 mmol) of a 1M solution of diisobutylaluminiumhydride in hexanes, according to the procedure described for the reduction of (**11**), to yield 1.60 g(9.5 mmol; 95%) of an oil consisting of the lactols (**19**) and the hydroxy-aldehydes (**20**) in approx. 3 : 7 ratio. The crude product mixture was used as such in the next reaction.
Crude product mixture (**19**)/(**20**): ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 0.7-2.9 (m, 11H); 3.42, 3.52, 3.60 and 3.66 (4 s, 0.3H): H-4 (**19**); 4.15-4.45 (br, 1H): H-1 (**19**) and H-3' (**20**); 4.53, 4.63 and 4.80 (3 s, 0.3H): H-3 (**19**); 4.93-5.14 (m, 2H): allyl H-3'(Z) (**19**)/H-5 (*Z*) (**20**) [δ 5.03 (d, J=10.5 Hz)] and allyl H-3'(*E*) (**19**)/H-5 (*E*) (**20**) [δ 5.05 (d, J=16.5 Hz)]; 5.5-6.05 (m, 1H): allyl H-2' (**19**)/H-4 (**20**); 9.65 (s, 0.7H): CHO (**20**).
The crude product mixture (**19**)/(**20**) was dissolved in methanol and a few drops of concentrated sulfuric acid were added. After stirring at room temperature for 60 hrs the starting materials had completely disappeared (tlc-analysis). Solid sodium bicarbonate was added and the mixture was filtered and concentrated under reduced pressure to give 1.60 g (8.8 mmol; 93%) of the methyl ethers (**21**), which were used as such in the next reaction.
^{1}H-NMR ($CDCl_3$, 90 MHz): δ 0.70-2.50 (br, 1H); 3.30 (s, 0.6H), 3.36 (s, 0.7H) and 3.44 (s, 1.7H): OCH_3; 4.15-4.55 (br m, 2H): H-1 and H-3; 4.80-5.20 (br dm, J≈12 Hz, 2H): allyl H-3'; 5.45-6.05 (br m, 1H) allyl H-2'.

3-Methoxy-2-oxabicyclo[3.2.1]octane-4-carbaldehyde (22). Ozonolysis of the crude methyl ethers (**21**), according to the procedure described for (**13**), gave a crude mixture of stereoisomers of the aldehyde (**22**) as an oil. The product mixture could be partially separated via careful chromatography on silicagel with petrol-EtOAc (90-10) as the eluent, yielding the most polar isomer of (**22**) in >90% purity (glc-analysis).
Crude product mixture (**22**): ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 1.00-1.35 (br, 2H); 1.35-2.50 (br, 8H); 2.50-2.70 (br, 1H); 3.25 (s, 1.9H) and 3.42 (s, 1.1H): OCH_3; 4.05-4.50 (br, 2H): H-1 and H-3; 9.55-9.65 (m, 1H): CHO. *Most polar isomer*: ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 1.05-1.90 (br, 6H); 1.90-2.60 (br, 4H); 3.30 (s, 3H): OCH_3; 4.10 (d, J≈6.5 Hz, 1H): H-3; 4.35 (br s, 1H): H-1; 9.55-9.65 (m, 1H): CHO.

Synthesis of 4-methylenecarbaldehyde-2-oxa-bicyclo[3.2.1]octan-3-one (25). A stirred solution of 1.4 g (8.4 mmol) of the allyl lactone (**18**) in 25 ml of CH_2Cl_2 was cooled under nitrogen atmosphere to -78°C on a dry-ice/acetone bath. An ozone/oxygen-mixture was

bubbled through the solution until a bright blue colour appeared and tlc-analysis indicated that the starting materials had been completely transformed. The solution was purged with nitrogen to remove excess ozone and then treated with 3.5 g (13.0 mmol) of triphenylphosphine. The reaction mixture was warmed to room temperature and stirred for 2 hrs. The solvent was removed under reduced pressure and the partly crystalline residue was taken up in 100 ml of EtOAc. To this solution was added 40 g of silicagel and the resulting slurry was stirred overnight. Filtration and evaporation of the solvent yielded 4.95 g of an oil, which partly crystallized. Chromatography on 100 g of silicagel with petrol-EtOAc (gradient elution, 85-15 to 65-35) as the eluent afforded 1.22 g (7.3 mmol, 86%) of a 1.1 : 1 mixture (glc-analysis) of the aldehydes (**25**) as an oil, which was sufficiently pure to be used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 90 MHz): δ 1.0-3.2 (m, 12H); 4.8 (br s,1H): H-1; 7.5-7.5 (m): traces of $P(O)Ph_3$; 9.7 (s, 1H): CHO.

Synthesis of 4-(2,2-dimethoxyethyl)-2-oxa-bicyclo[3.2.1]octan-3-one (26). To a stirred slurry of 2.4 g (6.4 mmol) of $CeCl_3 \bullet 7H_2O$ in 10 ml of methanol was added 7.0 ml (64 mmol) of trimethylorthoformate. After stirring for 5 min, a solution of 1.07 g (6.4 mmol) of the aldehydes (**25**) in 10 ml of methanol was added dropwise in approx. 10 min. The resulting clear solution was stirred at ambient temperature while the reaction was monitored via glc-analysis. After 4 hrs the starting material had disappeared and the reaction mixture was concentrated under reduced pressure. The residue was taken up in 50 ml of EtOAc and was washed with 30 ml of half-saturated sodiumbicarbonate solution. The organic layer was separated and the aqueous layer was extracted with 4x50 ml of EtOAc. The combined organic layers were washed with 20 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 1.16 g of a pale yellow oil. Purification by chromatography on 35 g of silicagel with petrol-EtOAc (70-30 to 45-55) as the eluent afforded 1.0 g (4.7 mmol; 73%) of the dimethylacetal (**26**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.41-2.13 (m, 9H); 2.35-2.45 (br m, 1H); 2.51 (br t, J=6.5 Hz, 1H): H-4; 3.29 and 3.34 (2 s, 6H): OCH_3; 4.57 (t, J=5.8 Hz, 1H): H-2'; 4.78 (br m, 1H): H-1.
^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 29.5 (t): C-6; 32.2 (t): C-7; 32.4 (t): C-8; 36.2 (t): C-1'; 36.7 (d): C-5; 46.7 (d): C-4; 52.4 (q) and 54.0 (q): OMe; 80.9 (d): C-1; 102.7 (d): C-2'; 173.7 (s): C-3.

Reduction of (26). To a stirred solution of 1.18 g (5.6 mmol) of the dimethylacetals (**26**) in 20 ml of anhydrous toluene, cooled to -78°C,. was added dropwise 4.0 ml (6.0 mmol) of a 1.5 M solution of diisobutyl aluminumhydride in toluene. After tlc-analysis indicated complete disappearance of the starting material (approx. 20 min), the mixture was diluted with 10 ml of ether and the reaction was quenched by addition of 4.3 g (13.4 mmol) of Glauber's salt and warmed to room temperature. After stirring for 45 min the turbid mixture was dried with $MgSO_4$ and filtered through a pad of hyflo. The solvent was removed under reduced pressure and the residue was twice redissolved in petrol and concentrated under reduced pressure to remove traces of toluene, finally affording 1.1 g of a clear, colourless oil. Chromatography on 35 g of silicagel with petrol-EtOAc (gradient elution, 80-20 to 50-50) as the eluent yielded 872 mg of a highly polar product mixture which was difficult to characterize. The mixture was used as such in the next reaction.

Between different experiments, the ^{1}H NMR spectrum of the crude mixture showed varying amounts of an aldehyde proton, indicating the presence of the ringopened hydroxy-aldehyde form (**28**); this compound could also be detected in the ^{13}C NMR spectrum. In analogy with previous results, the mixture probably consisted of the lactols (**27**) and the hydroxy-aldehyde (**28**), giving a combined yield of 73% of the crude reduction products.

General ^1H NMR characteristics of the product mixture ($CDCl_3$, 90 MHz): δ 1.0-2.5 (br m, 12-13H)[25a]; 3.2-3.5 (m [incl. δ 3.25 (br s)], 6H): OCH_3; 4.1-4.6 (br m): H-1 and H-2'; 9.55 (d, J=3 Hz, 0.4-0.8H):[25a] CHO of ring-opened aldehyde (**28**).
(**28**): ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, major peaks)[25b]: δ 27.9 (t): C-6; 32.0 (t): C-7; 34.9 (t): C-8; 37.9 (d): C-5; 39.7 (t): C-1'; 53.2 (d): C-4; 54.1 (q): OCH_3; 73.0 (d): C-1; 103.5 (d): C-2'; 204.4 (d): C-3.

Cyclization of the reduction products. A mixture of 212 mg (1.0 mmol) of the crude reduction products (**27**) and (**28**) in 4 ml of THF and 12 ml of an aqueous 1N hydrochloric acid solution was stirred vigorously overnight at room temperature. The reaction mixture was saturated with solid sodiumchloride and the resulting slurry was extracted with four 25 ml-portions of EtOAc. The combined extracts were washed with brine (10 ml), dried with $MgSO_4$ and concentrated under reduced pressure to yield 153 mg of a yellow, viscous oil. Chromatography on 10 g of silicagel with petrol-EtOAc (gradient elution, 80-20 to 0-100) yielded 87.1 mg (0.5 mmol; 51%) of the crude furopyranols (**23**) as a waxy solid. In view of the high polarity of the products, no further purification was attempted, and the crude product was used as such in the next reaction.
^1H NMR ($CDCl_3$, 90 MHz): δ 0.5-2.5 (br m, 12H); 3.2-3.9 (br m, 1H); 4.3 (br, 1H): H-1; 5.1-5.7 (br m, 2H): H-3 and H-5.

Synthesis of (1R*,3S*,7R*,8S*)- and (1R*,3R*,7S*,8S*)- 2,4-dioxatricyclo[6.2.1.$0^{3,7}$]-undecan-5-one (29a and 29b). A crude mixture of 600 mg (3.57 mmol) of the furopyranols (**23**) was oxidized with TPAP (65 mg, 0.18 mmol) and NMO (615 mg, 5.25 mmol) according to the procedure described for (**14**). Work-up as before yielded 339 mg of an oil containing an 11 : 1 mixture of two products and several major impurities. Purification via chromatography on 15 g of silicagel with petrol-EtOAc (80-20) as the eluent yielded, in order of elution, 161 mg (0.97 mmol, 27%) of a 6 : 1 mixture of both isomers of (**29**) as a solid and 107 mg (0.64 mmol, 18%) of the major isomer (**29a**) as white crystals.
Major (7βH-)isomer (**29a**): ^1H-NMR ($CDCl_3$, 400 MHz): δ 1.48 (ddd, J=10.9, 5.4, 3.0 Hz, 1H): H-11; 1.56-1.70 (m, 2H): H-9; 1.70-1.80 (m, 2H): H-10b and H-11'; 2.04-2.13 (m, 1H): H-10a; 2.29-2.34 (m, 1H): H-8; 2.35 (d, J=17.6 Hz, 1H): H-6a; 2.65 (ddd, J=9.0, 4.6, 4.6 Hz, 1H): H-7; 2.73 (dd, J=16.7, 8.8 Hz, 1H): H-6b; 4.40-4.45 (m, 1H): H-1; 5.67 (d, J=5.2 Hz, 1H): H-3. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 22.9 (t): C-9; 29.6 (t): C-10; 35.4 (t): C-6; 36.9 (d and t): C-8 and C-11; 38.8 (d): C-7; 76.4 (d): C-1; 101.9 (d): C-3; 174.2 (s): C-5.

4.6 - Notes and References

1. For reviews regarding the biological activity of azadirachtin, see: (a) A.J. Mordue (Luntz) and A. Blackwell, *J. Insect Physiol.*, **39** (11), 903-924 (1993); (b) T.A. van Beek and Ae. de Groot, *Recl. Trav. Chim. Pays-Bas*, **105**, 513-527 (1986); (c) S.V. Ley, A.A. Denholm and A. Wood, *Nat. Prod. Reports*, 109-157 (1993).
2. J.H. Butterworth and E.D. Morgan, *J. Chem. Soc., Chem. Comm.*, 23-24 (1968).
3. (a) S.V. Ley, D. Santafianos, W.M. Blaney and M.S.J. Simmonds, *Tetrahedron Lett.*, **28** (2), 221-224 (1987); (b) W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson and P.L. Toogood, *Entomol. Exp. Appl.*, **55**, 149-160 (1990); (c) W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson, S.C. Smith and A. Wood, *Pestic. Sci.*, **40**, 169-173 (1994).
4. See data from: (a) E.D. Morgan, *Strategy in the isolation of insect control substances from plants*. In: H. Schmutterer, K.R.S. Acher and H. Rembold (Eds.), *Natural pesticides from*

the Neem tree, Proceedings of the first International Neem Conference (Rottach-Egern, FRG, june 1980), GTZ Verlag, 1981, p. 43-52; (b) S.V. Ley, J.C. Anderson, W.M. Blaney, E.D. Morgan, R.N. Sheppard, M.S.J. Simmonds, A.M.Z. Slawin, S.C. Smith, D.J. Williams and A. Wood, *Tetrahedron*, **47** (44), 9231-9246 (1991); (c) M.S.J. Simmonds, W.M. Blaney, S.V. Ley, J.C. Anderson, R. Bänteli, A.A. Denholm, P. Green, R.B. Grossman, C. Gutteridge, L. Jennens, S.C. Smith, P.L. Toogood and A. Wood, *Entomol. Exp. Appl.*, 77, 69-80 (1995).

5. (a) S.V. Ley, K. Doherty, G. Massiot and J.-M. Nuzillard, *Tetrahedron*, **50** (42), 12267-12280 (1994); (b) H.A. Baldoni, R.D. Enriz, E.A. Jáuregui and I.G. Csizmadia, *J. Molecular Structure (Theochem)*, **363**, 167-178 (1996).
6. W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson and P.L. Toogood, *Entomol. Exp. Appl.*, **55**, 149-160 (1990).
7. J.C. Anderson, S.V. Ley, D. Santafianos and R.N. Sheppard, *Tetrahedron*, **47** (33), 6813-6850 (1991).
8. J. Meinwald and E. Frauenglass, *J. Am. Chem. Soc.*, **82**, 5235-5239 (1960).
9. E. Vedejs and J.E. Telschow, *J. Org. Chem.*, **41** (4), 740-741 (1976); E. Vedejs, *J. Org. Chem.*, **43** (2), 188-196 (1978).
10. However, for some 6-substituted derivatives of (**8**) this α-hydroxylation step was also reported to afford a mixture of both epimers [ref. 7].
11. Atom-atom distances in energy minimized molecular models, obtained via molecular mechanics calculations (conjugate gradient minimization method) with the Quanta software package using the CharmM force field. Relevant distances to H-4 (Å): *exo*-isomer (**9a**): H-5 2.6, H-6a 2.4, H-7a 3.8, H-8a 4.3, H-8b 3.7; *endo*-isomer (**9b**): H-5 2.4, H-8a 3.7, H-8b 2.6.
12. Usually a NOE can only be detected if the atom-atom distance is less than 3Å, and ideally it should be less than 2.5Å. See: H. Friebolin, *Basic One and Two-Dimensional NMR Spectroscopy*, VCH, Weinheim, 1991, p. 261.
13. (a) It is unclear whether the major isomer in our case was the *exo*-isomer (**10a**) or the *endo*-isomer (**10b**), since our NMR data are in conflict upon this issue with those reported in the literature [Relevant data (ref . 7) for *exo*-isomer (**10a**): H-4: δ 4.13 (dd, J=2.5, 1.5 Hz, 1H); H-1: δ 4.82 (br s, 1H)]; (b) The epimeric ratio apparently changed upon silylation of (**9**) to (**10**), but since in the next step an intermediate anion is formed at this position, the stereochemistry of (**10**) at C-4 is not of ultimate importance in the route towards the target compound (**5**).
14. For both (**9a,b**) and (**10a,b**) distinct differences can be observed in the δ values of the protons H-1, H-4, H-5 and H-8b for the two C-4 epimers (see data in the experimental section). Contrary, for (**11**) the δ values of H-1, H-5 and H-8a are identical to the literature values, while the δ value of H-8b is also well within the reported range (see Experimental Section and ref. 7).
15. Such an excess was found necessary for complete conversion of the starting material.
16. Compound (**41**) from ref. 7.
17. W.P. Griffith, S.V. Ley, G.P. Whitcombe and A.D. White, *J. Chem. Soc., Chem. Commun.*, 1625-1627 (1987).

18. (a) Only one product peak was detected using different chromatographic columns (DB-5 and DB-17+ columns were used) and temperature programs. (b) No double peaks suggesting the presence of two isomers were found.
19. In $CDCl_3$ under pH neutral conditions.
20. See: J.-L. Luche and A.L. Gemal, *J. Chem. Soc., Chem. Comm.*, 976-977 (1978); A.L. Gemal and J.-L. Luche, *J. Org. Chem.*, **44** (23), 4187-4189 (1979).
21. As determined in energy minimized molecular models, obtained via molecular mechanics calculations (conjugate gradient minimization method) with the Quanta software package using the CharmM force field. (a) Relevant dihedral angles (°) with H-1. β-isomer (**29a**): H-10a 95.2, H-10b 24.2, H-11 47.8, H-11' 74.3; α-isomer (**29b**): H-10a 93.3, H-10b 25.9, H-11 48.0, H-11' 73.0. (b) Relevant atom-atom distances (Å). β-isomer (**29a**): distance to H-3: H-1 4.0, H-6b 2.9, H-7 2.3, **H-10a 3.8**, H-11' 3.4; distance to H-7: H-6a 2.8, H-6b 2.4, H-8 2.4, **H-9a 3.8**, H-11 3.7, **H-11' 2.5**. α-isomer (**29b**): distance to H-3: H-1 3.8, H-6a/b 3.9, H-7 2.3, H-9a 2.9, **H-10a 2.4**, H-11' 4.0; distance to H-7: H-6a 2.3, H-6b 3.0, H-8 2.5, **H-9a 2.4**, H-10a 3.6, **H-11' 3.7**. Distances used in the determination of the relative stereochemistry are printed in boldface.
22. M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).
23. The following NOEs were observed for the pure major isomer (**29a**) through 1D NOE difference spectroscopy in $CDCl_3$ at 400 MHz: **H-3**: H-7; **A1/B**: H-3 (s), A2/C1 (s), D1 (negative NOE, w), C2/D2 (m); **C2/D2/E**: H-3 (w), H-1 (m), B (w), C1 (w), D1 (s), C3 (s); **C3**: H-1 (w), C1 (w), C2 (s), E (negative NOE, w). Irradiated signal(s) are printed in boldface. NOE strength is indicated relative to the other NOEs in the spectrum; w=weak, m=moderate, s=strong.
24. Assignments of these ^{1}H and ^{13}C NMR spectra were based on 400 MHz ^{1}H-^{13}C correlation spectroscopy and on analogy with the spectral assignments made for (**9a**).
25. (a) Amount varied for different experiments. (b) Derived from the ^{13}C NMR spectrum of a mixture containing 80% of (**28**).

Chapter 5

Synthesis of a Series of C-2 Modified Perhydrofuro[2,3b]furans

Abstract: *In this chapter, the preparation of a series of furo[2,3b]furan model systems related to the natural clerodane diterpene clerodin is described, using a known method for the construction of the furofuran ring system. The model compounds were equipped with different substitution patterns at positions C-2 and C-5, in order to facilitate studies of the effect of these modifications on the insect antifeedant activity of this type of system.*

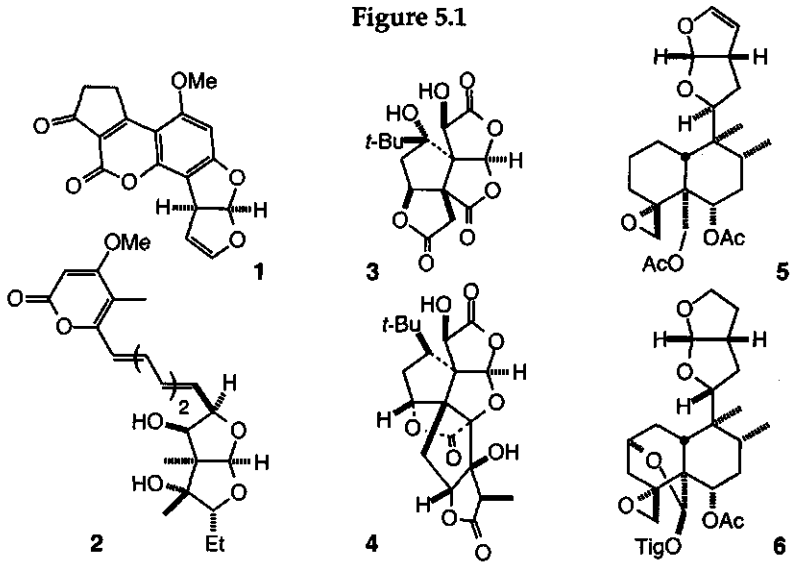
Table of Contents

This chapter will be published in a revised form, in combination with chapter 6 and part of chapter 9: E.A. klein Gebbinck, C.T. Bouwman, M. Bourgois, B.J.M. Jansen and Ae. de Groot, *Synthesis and insect-antifeedant activity of C-2 and C-5 substituted perhydrofurofurans and 3a-hydroxy-perhydrofurofurans (Part I), Tetrahedron*, accepted for publication.

5.1 - Introduction

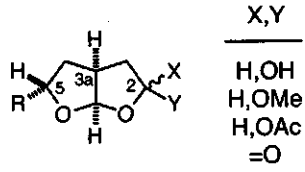
The furo[2,3b]furan ring system is a substructure that can frequently be encountered in natural products. Examples include fungal metabolites, such as the mycotoxins aflatoxin B_1 (**1**) and asteltoxin (**2**) from *Aspergillus* ssp., or secondary plant metabolites, such as bilobalide (**3**) and ginkgolide A (**4**) from the Ginkgo tree (Figure 5.1). This substructure is also found in a number of plant-derived clerodane diterpenes, such as clerodin (**5**)[1] and 11-episcutecyprin (**6**).[2]

Figure 5.1

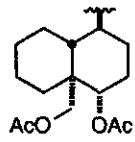


Clerodane diterpenes incorporating a furo[2,3b]furan subunit are well-known for their insect antifeedant activity against several pest insect species.[3] Several authors have identified the furo[2,3b]furan fragment of these diterpenes as one of the key structural features required for their biological activity.[4,5] In this respect it is interesting to note that insect antifeedant activity has also been reported for bilobalide and some ginkgolides.[6] It is therefore not surprising that the furo[2,3b]furan ring system has attracted attention as an interesting target structure in the search for simplified analogues of natural insect antifeedants. Model compounds (**7**) (Figure 5.2) based on this structure were found to display insect antifeedant activity in laboratory bioassays.[5] However, in comparison to their natural counterparts, these model compounds were markedly less potent and are therefore unsuitable candidates for application as crop protection agents.

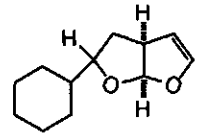
Figure 5.2



7: R= *t*-Bu, Ph, 2-MePh, 2,6-Me_2Ph and (**8**)



8



9

Remarkably, analogues with a cyclic enol ether at C-2/3, similar to the moiety present in some of the most potent natural clerodane antifeedants, were not included in this test series. In the natural antifeedants, reduction of this moiety was found to increase the activity of the resulting derivatives towards some insects, but to decrease the activity towards other species.[7,3] Also, it seems likely that for such simple model compounds lacking the decalin fragment, the activity will depend more critically upon the exact structure of the remaining fragment than it does in case of the complete natural counterparts.

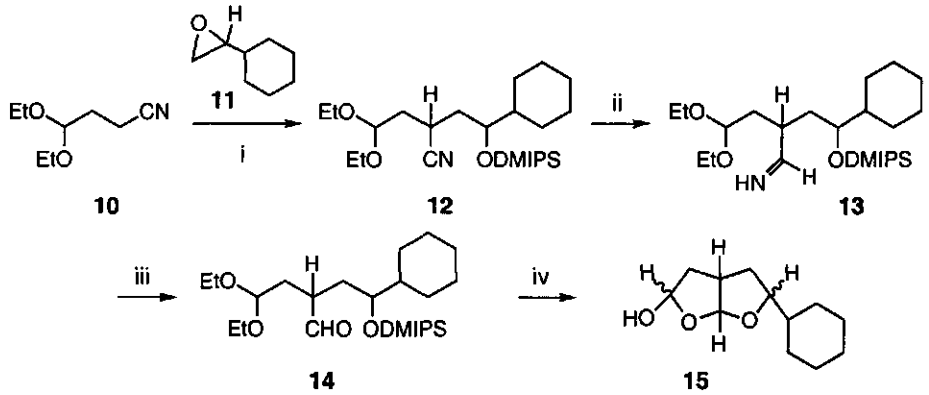
We therefore decided to prepare a series of furo[2,3b]furan model compounds with several different functional groups at C-2, including a cyclic enol ether as in (**9**), in order to study the effect of C-2 functionalization upon the antifeedant activity of such model compounds.

5.2 - Synthesis of a Series of C-2 Modified Perhydrofuro[2,3b]furans

5.2.1 - Preparation of the Perhydrofuro[2,3b]furan Ringsystem

The furo[2,3b]furan ringsystem was prepared via a route (Scheme 5.1) slightly modified from the methodology developed by Vader and coworkers.[8] Regioselective addition of the anion of the commercially available nitrile (**10**) onto cyclohexyl oxirane[9] (**11**), followed by trapping of the intermediate alkoxide with isopropyldimethylsilyl chloride, afforded the nitriles (**12**) in 85% yield. Reduction with diisobutylaluminium hydride and work-up with Glaubers' salt (sodium sulfate decahydrate) initially yielded an intermediate product (**13**) that displayed all the spectral characteristics expected for the aldehyde (**14**), except for the presence of an aldehyde functionality. Instead, the intermediate had an infrared absorbtion band at υ=1650 cm^{-1} and showed NMR signals at δ=4.4 and 7.5 ppm (^1H) and at δ=167 ppm (^{13}C), none of which are present in (**14**). These spectral data suggest the intermediate to be of imine character, which would be consistent with the mechanism of reduction of nitriles with DibalH.

Scheme 5.1



Reagents and conditions: (i) 1) LDA, THF, -78°C, 30 min; 2) add (**11**), 0°C to r.t., 20 hrs; c) DMIPSCl (85%); (ii) 1) DibalH, ether, -50°C, 1.5 hrs; 2) then Glaubers' salt; (iii) silicagel, 20 hrs (93%); (iv) 1N HCl, THF-water (98%).

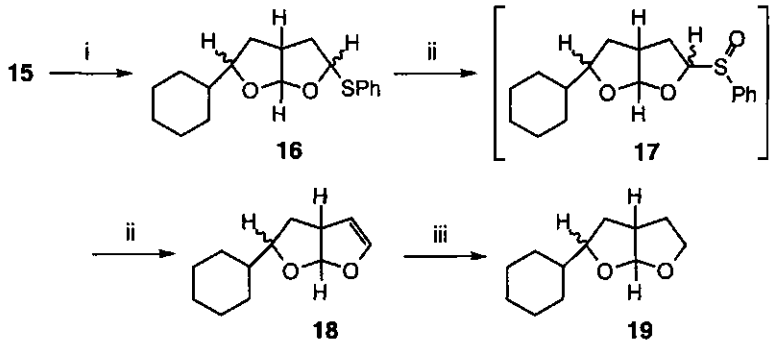
Apparently, the crystal water provided by the salt is able to displace diisobutylaluminium moiety from the iminic nitrogen atom but cannot effect its subsequent hydrolysis to the aldehyde. During purification attempts by chromatography, however, the intermediate was found to be partially transformed into the desired aldehyde (**14**). This observation was put to use in a simple method to obtain (**14**) by overnight treatment of the intermediate product with silicagel in EtOAc under common air, affording (**14**) in an overall yield of 93%. Finally, acid-catalyzed cyclization of (**13**) with 1N HCl in THF yielded the perhydrofuro[2,3b]furan-2-ols (**15**) in excellent yield as an inseparable mixture of all four diastereoisomers.

5.2.2 - Modification Reactions At C-2

The perhydrofuro[2,3b]furan-2-ols (**15**) were used as starting material in the synthesis of various C-2 functionalized furo[2,3b]furans. For the introduction of the enol ether functionality, we planned to use the phenylsulfoxide-based elimination strategy described by Anderson *et al.*[10] The perhydrofuro[2,3b]furan-2-ols (**15**) could be easily transformed into the corresponding 2-phenylsulfides (**16**) with thiophenol in the presence of an excess amount of borontrifluoride etherate as catalyst and as scavenger of water (Scheme 5.2). However, in the subsequent oxidation reaction with mCPBA, no sulfoxide (**17**) could be isolated and instead small amounts of (**15**) were found. From this we concluded that (**17**) apparently is rather unstable and quickly decomposes at temperatures above 0°C; a similar thermal instability has been reported for acyclic phenylthiomethyl ethers.[11] Therefore, the elimination procedure was modified so that the sulfoxide was

generated *in situ* and subsequently was rapidly eliminated to yield the desired tetrahydrofuro[2,3b]furans (**18**). In this procedure it proved to be important to quickly raise the temperature of the sulfoxide mixture to 130°C to facilitate a smooth elimination reaction; slow warming of the reaction mixture from 0°C to 130°C over a period of 10-20 min resulted in highly reduced yields of (**18**) due to the formation of several unidentified side-products. In this manner, the tetrahydrofuro[2,3b]furans (**18**) were obtained in 72% yield from (**16**) as an inseparable mixture of two diastereoisomers. Catalytic hydrogenation of (**18**) on palladium smoothly gave the corresponding perhydrofuro[2,3b]furans (**19**) in excellent yield.

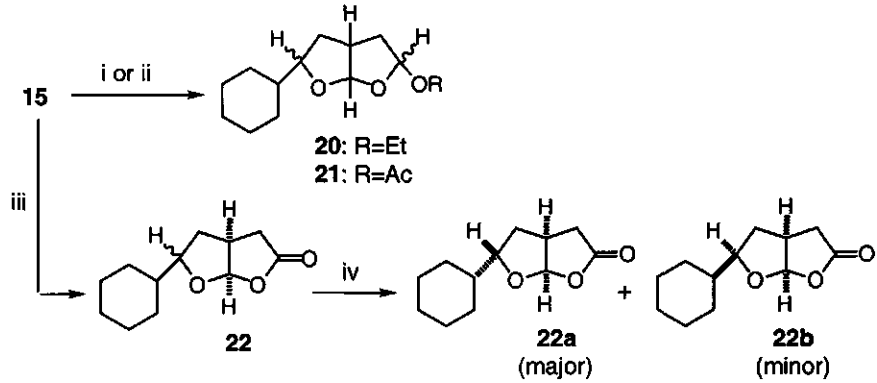
Scheme 5.2



Reagents and conditions: (i) PhSH, $BF_3 \bullet Et_2O$, ether, 0°C (93%); (ii) mCPBA, toluene, 0°C; then Et_3N, 110°C, 10 min (72%); (iii) H_2, Pd/C, MeOH (85%).

Conversion of the hemiacetal functionality of (**15**) into a mixed acetal as present in the methyl ether (**20**) was accomplished through acid-catalyzed acetalization with methanol (Scheme 5.3), yielding (**20**) in good yield as a mixture of stereoisomers. Acylation of (**15**) with acetyl chloride and pyridine gave the acetate (**21**) in 64% yield, also as a mixture of stereoisomers. In view of the complex nature of these mixtures, separation of the individual diastereoisomers was not attempted. Conversely, due to the removal of one asymmetric center, the oxidation of (**15**) with pyridinium dichromate afforded a mixture of only two diastereomers (**22a**) and (**22b**) which could be partially separated through careful chromatography. Based on the similarity of its ^1H NMR chemical shift values and coupling constants of H-3α, H3β, H-3a, H-5 and H-6a with those of corresponding perhydrofuro[2,3b]furan-2-ones reported in the literature[8], the relative stereochemistry of the less polar diastereoisomer (**22a**) was assigned as 3aS,5S,6aR. This is the same relative stereochemistry as found in the natural compound clerodin (**5**).

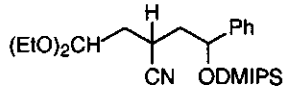
Scheme 5.3



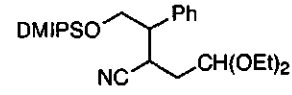
Reagents and conditions: (i) R=Et: EtOH (2 eq), TsOH, acetone (54 %); (ii) R=Ac: AcCl, pyr., CH_2Cl_2 (64%); (iii) PDC, CH_2Cl_2 (71%); (iv) repeated chromatography.

5.2.3 - Other Substituents at C-5

In addition to the furo[2,3b]furans described above, which contained different functional groups at C-2 and a *cyclo*-hexyl substituent at C-5, a number of model compounds with other C-5 substituents were synthesized in order to study the effect of the nature of this side chain on the antifeedant activity of this system. For this purpose, two straight-chain alkyl substituents (*n*-hexyl and *n*-decyl) and a phenyl substituent were selected. Both 2,3,3a,6a-tetrahydro-furo[2,3b]furans and perhydrofuro[2,3b]furan-2-ones were synthesized, to introduce some variation at C-2 into the series without the complication of a large number of stereoisomers.



23



24

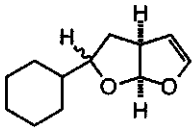
The preparation of these model compounds proceeded analogously to the methods described in the previous sections,[12,13] with the exception of the phenyl-substituted derivative. For this particular substituent, the addition of the lithium anion of (**11**) to the corresponding epoxide (*i.e.* styrenoxide) yielded an inseparable mixture of both regioisomers (**23**) and (**24**). This isomer mixture had to be converted to the stage of the final products (compounds (**26**) and (**30**), respectively) before the unwanted regioisomers could be removed via careful

chromatography. For the *n*-decyl- and the phenyl-substituted furo[2,3b]furan-2-ones, the pure 5S-stereoisomers (**28a**) and (**29a**) could be obtained via chromatography; the corresponding *n*-hexyl isomers however could not be separated.

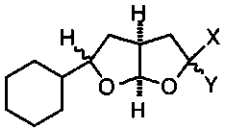
5.3 - Summary

In conclusion, we have prepared a series of furo[2,3b]furan-based analogues of the natural clerodane diterpene antifeedant clerodin (**5**), using a known method for the construction of the furofuran ring system. These simple model compounds (Figure 5.3) contain different substitution patterns at both C-2 and C-5, in order to facilitate studies of the effect of these modifications on the insect antifeedant activity of this type of system.

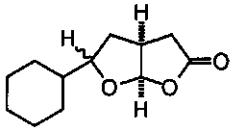
Figure 5.3



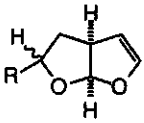
18



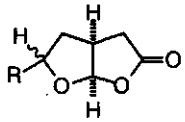
19: X,Y=H,H
15: X,Y=H,OH
20: X,Y=H,OEt
21: X,Y=H,OAc



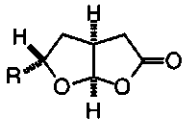
22
22a: 5S-epimer only
22b: 5R-epimer only



25: R=*n*-C_6H_{13}
26: R=Ph



27: R=*n*-C_6H_{13}
28: R=*n*-$C_{10}H_{21}$



28a: R=*n*-$C_{10}H_{21}$
29a: R=Ph

5.4 - Experimental Section

General experimental conditions were as described in the experimental section of Chapter 4.

Cyclohexyl-substituted Derivatives.

4-Cyclohexyl-2-(2',2'-diethoxyethyl)-4-(dimethyl-*iso*-propylsilyloxy)-butyronitrile (**12**). To an ice-cold, stirred solution of 3.7 ml (26.4 mmol) of diisopropylamine in 30 ml of anhydrous THF was added dropwise 16.4 ml (26.2 mmol) of a 1.6M solution of *n*-butyllithium in hexanes; stirring was continued for 30 min. After cooling to -78°C, 4.4 ml (26.2 mmol) of 3-cyanopropionaldehyde diethylacetal (**10**) in 5 ml of THF was added dropwise and the mixture was stirred for 30 min. Then, a solution of 3.06 g (24.3 mmol) of

cyclohexyl oxirane (**11**) in 10 ml of THF was added dropwise, the dry ice-acetone bath was removed and the stirred reaction mixture was allowed to warm overnight to room temperature. The resulting yellow mixture was cooled on an ice bath and 4.2 ml (26.7 mmol) of dimethyl-isopropylsilylchloride was added, after which the yellow colour disappeared; stirring was continued for 5 min at 0°C and then at room temperature for 2 hrs. The reaction mixture was poured into 100 ml of saturated aqueous sodium bicarbonate solution and 50 ml of water. The organic layer was separated and the aqueous layer was extracted with three 150 ml-portions of ether. The combined organic layers were washed twice with 100 ml of water and with 100 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield a yellow oil. Chromatography on 180 g of silicagel with petrol-EtOAc (99.5-0.5 to 97-3) as the eluent afforded 7.9 g (20.6 mmol; 85%) of a mixture of the nitriles (**12**) as a clear, pale yellow oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.05, 0.09 and 0.10 (3 s, 6H): $Si(CH_3)_2$; 0.8-1.25 (br m, 19H): $OCH_2C\underline{H}_3$ [δ 1.19 (2 t, J=7.0 Hz)], $SiCH(C\underline{H}_3)_2$ [δ 0.94 and 0.95 (2 d, J=5.9 Hz)], $SiC\underline{H}(CH_3)_2$ and *c*-hexyl H-3"-H-5"; 1.5-1.95 (m, 9H): H-3, H-1' and *c*-hexyl H-1", H-2", H-6"; 2.7-3.0 (m, 1H): H-2; 3.4-3.8 (m, 5H): H-4 and $OC\underline{H}_2CH_3$; 4.64-4.7 (m, 1H): H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ -3.7, -3.5 and -3.4 (3 q): $Si(CH_3)_2$; 15.1, 15.2 and 15.3: $Si\underline{C}H(CH_3)_2$ and $OCH_2\underline{C}H_3$; 17.0 (q): $SiCH(\underline{C}H_3)_2$; 23.6 and 25.0 (2 d): C-2; 26.3, 26.4, 26.6, 26.7, 27.4, 28.1, 28.3 and 28.8 (8 t): *c*-hexyl C-2"-C-6"; 35.9, 36.0, 36.4 and 36.9 (4 t): C-3 and C-1'; 43.0 and 44.5 (2 d): *c*-hexyl C-1"; 62.0, 62.2, 62.5 and 63.0 (4 t): $O\underline{C}H_2CH_3$; 73.6 and 74.0 (2 d): C-4; 100.8 and 101.0 (2 d): C-2'; 121.9 and 122.3 (2 s): C-1. MS: *m/e* (%) 341 (26), 340 (100), 338 (25), 294 (29), 266 (22), 220 (35), 169 (20), 124 (23), 103 (23), 75 (32). HRMS: calcd. (M-^{i}Pr): *m/e* 340.2308; found: *m/e* 340.2307.

4-Cyclohexyl- 2-(2',2'-diethoxyethyl)- 4-(dimethyl-*iso*-propylsilyloxy)- butyraldehyde (14). A stirred solution of 7.9 g (20.6 mmol) of a mixture of the nitriles (**12**) in 50 ml of dry ether, containing 0.2 ml of n-decane as internal standard, was cooled to -50°C (external temperature) on a dry-ice acetone bath. In approx. 20 min, 19 ml of a 1.5M solution of diisobutylaluminum hydride (28.5 mmol) in toluene[14] was added dropwise. Stirring at -40 to -50°C was continued for 1 hr while the progress of the reaction was monitored by glc-analysis (disappearance of the starting material with n-decane acting as control); after 45 min the reaction appeared to be complete. The reaction was quenched by addition of 20 g (62.1 mmol) of Glauber's salt and the mixture was warmed to room temperature. Stirring was continued for 1 hr, during which time the mixture turned into a slurry which had to be diluted with ether to facilitate stirring. $MgSO_4$ was added and the slurry was filtered through a pad of hyflo on a glassfilter. The solvent was evaporated under reduced pressure and the residual oil was twice taken up in petrol and re-concentrated to remove traces of toluene, yielding 7.9 g of the intermediate reduction product (**13**) as a colourless, viscous oil.
Intermediate product (**13**): The individual signals were assigned in accordance with the spectra of the final product (**14**), with signals not present in (**14**) printed in boldface. ^{1}H NMR ($CDCl_3$, 200 MHz): δ -0.01, 0.00, 0.01 and 0.02 (4 s, 6H): $Si(CH_3)_2$); 0.65-1.45 (br m, 22H): $SiCH(C\underline{H}_3)_2$ [δ 0.91 (d, J=6.2 Hz)], $SiC\underline{H}(CH_3)_2$, $OCH_2C\underline{H}_3$ [δ 1.14 (2 t, J=7.0 Hz)] and *c*-hexyl H-3"-H-5"; 1.45-1.95 (br m, 9H): H-3, H-1' and *c*-hexyl H-1", H-2", H-6"; 2.5 (br, 1H): H-2; 3.3-3.7 (br m, 5H): H-4 and $OC\underline{H}_2CH_3$; 4.37 (br, 0.3H); 4.45-4.7 (br m, 1H): H-2'; **7.5** (m, 0.6H); 9.5 (d, J=2.7 Hz, 0.1H): CHO. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ -3.2 and -3.5 (2 q): $Si(CH_3)_2$; 15.3 (d): $Si\underline{C}H(CH_3)_2$; 15.4 (q): $OCH_2\underline{C}H_3$; 17.2 (q): $SiCH(\underline{C}H_3)_2$; 26.6, 26.8, 26.9 and 28.5 (4 t): *c*-hexyl C-2"-C-6"; 34.0 and 36.5 (2 t): C-3 and C-1'; 37.4 (d): C-2; 43.8 (d): *c*-hexyl C-1"; 61.0, 61.5 and 62.0 (3 t): $O\underline{C}H_2CH_3$; 74.2 (d): C-4; 101.5 (d): C-2'; 166.7 (d). IR ($CHCl_3$): υ 1650 (m), 1740 (w) cm^{-1}.
The intermediate product (**13**) was dissolved in 150 ml of ethylacetate, 50 g of silicagel was added and the mixture was stirred overnight at room temperature. Filtration and

evaporation of the solvent under reduced pressure yielded 7.8 g of a less viscous, colourless oil. Purification via chromatography on 100 g of silicagel with petrol-EtOAc (99-1 to 97-3) as the eluent afforded 7.4 g (19.2 mmol; 93%) of a mixture of the aldehydes (**14**) as a clear, colourless oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ -0.03-0.10 (m s, 6H): $Si(CH_3)_2$; 0.65-1.45 (br m, 25H): $SiCH(C\underline{H}_3)_2$ [δ 0.91 (br d, J=6.6 Hz)], $SiC\underline{H}(CH_3)_2$, $OCH_2C\underline{H}_3$ [δ 1.10-1.25 (m t, J=7.0 Hz)] and *c*-hexyl H-3"-H-5"; 1.45-2.0 (br m, 9H): H-3, H-1' and *c*-hexyl H-1", H-2", H-6"; 2.40-2.65 (br m, 1H): H-2; 3.35-3.75 (m, 5H): H-4 and $OC\underline{H}_2CH_3$ [δ 3.35-3.52 (m q, J=7.0 Hz)]; 4.46 and 4.47 (2 t, J=5.7 Hz, 1H): H-2'; 9.55 (d, J=2.7 Hz, 1H): H-1. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ -3.6 (q): $Si(CH_3)_2$; 15.1 (d): $Si\underline{C}H(CH_3)_2$; 15.2 (q): $OCH_2\underline{C}H_3$; 17.0 and 17.1 (2 q): $SiCH(\underline{C}H_3)_2$; 26.4, 26.5, 26.6, 26.7, 27.7, 28.5, and 28.9 (7 t): c-hexyl C-2"-C-6"; 32.6, 33.5, 34.2 and 34.4 (4 t): C-3 and C-1'; 43.7 and 43.8 (2 d): *c*-hexyl C-1"; 44.5 and 45.3 (2 d): C-2; 61.7 and 62.1 (2 t): $O\underline{C}H_2CH_3$; 74.2 and 74.4 (2 d): C-4; 101.3 and 101.4 (2 d): C-2'; 204.2 and 204.3 (2 d): C-1. IR ($CHCl_3$): υ 1740 (s) cm^{-1}. MS: *m/e* (%) 298 (22), 297 (100), 257 (33), 251 (73), 213 (28), 185 (92), 177 (29), 157 (25), 103 (74), 75 (55), 73 (25). HRMS: calcd. (M - ^{i}Pr): *m/e* 343.2305; found: *m/e* 343.2305.

5-Cyclohexyl-perhydrofuro[2,3b]furan-2-ol (**15**). A mixture of 7.3 g (18.9 mmol) of the aldehydes (**14**) in 80 ml of THF and 80 ml of aqueous 1N HCl solution was stirred vigorously for 16 hrs at room temperature. Then, 100 ml of ether was added, the organic layer was separated and the aqueous layer was extracted with 50 ml of ether. The combined organic layers were washed sequentially with 50 ml of saturated aqueous sodium bicarbonate solution and 50 ml of brine. After drying with $MgSO_4$ and evaporation of the solvent under reduced pressure, 4.3 g of an oil was obtained. Chromatography on 100 g of silicagel with petrol-EtOAc (90-10 to 80-20) as the eluent yielded 3.9 g (18.5 mmol; 98%) of an inseparable mixture of all four stereoisomers of the furofuranol (**15**) as a colourless oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.8-2.3 (br m, 15H): H-3, H-4 and *c*-hexyl H-1'-H-6'; 2.70-2.97 (m, 0.7H) and 2.97-3.15 (m, 0.3H): H-3a; 3.56-3.80 (m, 0.7H), 3.89 (d, J=2.0 Hz, 0.2H), 3.98 (d, J=3.0 Hz, 0.3H) and 4.05-4.30 (m, 0.8H): H-5 and OH; 5.54 (ddd, J=5.2, 2.0, 0.8 Hz, 0.3H), 5.58-5.76 (m, 1.2H) and 5.83 (t, J=5.4 Hz, 0.5H): H-2 and H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 25.7, 25.8 (2), 25.9, 26.0 (2), 26.4, 28.7, 28.9, 30.0, 30.1 and 30.2 (12 t): *c*-hexyl C-2'-C-6'; 35.3, 36.0, 36.1 and 36.7 (4 t): C-3; 38.8 (2), 39.2 and 39.9 (4 t): C-4; 40.6, 41.5, 41.7, 42.2, 42.4, 43.0 and 43.2 (7 d): C-3a and *c*-hexyl C-1'; 82.5 (2), 85.4 and 86.1 (4 d): C-5; 98.5, 98.7, 98.9 and 100.8 (4 d): C-2; 107.7, 108.5, 110.4 and 110.5 (4 d): C-6a. MS: *m/e* (%) 194 (3), 168 (5), 148 (3), 129 (37), 112 (6), 111 (100), 96 (5), 95 (5), 83 (11), 82 (5), 81 (6), 69 (5), 67 (5), 55 (10), 43 (3), 41 (5). HRMS: calcd. (M-H_2O): *m/e* 194.1307; found: *m/e* 194.1304.

5-Cyclohexyl-2-phenylsulfide-perhydrofuro[2,3b]furan (**16**). To an ice-cold, stirred solution of 618 mg (2.9 mmol) of a mixture of the furofuranols (**15**) and 0.34 ml (3.3 mmol) of thiophenol in 25 ml of anhydrous ether, containing approx. 2 g of activated 4Å molecular sieves, was added dropwise via syringe 0.55 ml (4.5 mmol) of borontrifluoride etherate. After 30 min the reaction was quenched by the addition of 50 ml of an aqueous 4N sodiumhydroxide solution. The organic layer was separated and the waterlayer was extracted with 50 ml of ether. The combined organic layers were washed with 25 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 828 mg (2.7 mmol; 93%) of a mixture of all four diastereomers of the sulfide (**16**) as a clear, colourless oil. The crude product was sufficiently pure to be used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-1.50 (br m, 9H), 1.50-1.85 (br m, 6H) and 1.85-2.33 (m, 3H): H-3, H-4 and *c*-hexyl H-1'-H-6'; 2.50-2.68 (m, 0.3H) and 2.75-3.05 (m, 0.7H): H-3a; 3.56-3.82 (m, 0.8H) and 3.94-4.08 (m, 0.2H): H-5; 5.38-5.89 (m, 2H): H-2 and H-6a; 7.25-

7.35 (m, 3H) and 7.43-7.58 (m, 2H): Ph. MS: *m/e* (%) 197 (13), 195 (100), 177 (21), 151 (14), 133 (82), 109 (15), 91 (11), 81 (13), 67 (17), 55 (12). HRMS: calcd. (M^+): *m/e* 304.1497; found: *m/e* 304.1498.

2-Cyclohexyl-2,3,3a,6a-tetrahydrofuro[2,3b]furan (18). A solution of 740 mg (3.0 - 3.2 mmol) of mCPBA (70-75 wt% mCPBA, remainder mCBA and water) in 5 ml of anhydrous toluene was pre-dried in a dropping funnel containing activated 4Å molecular sieves. After 20 min, this solution was added dropwise in approx. 10 min to an ice-cold solution of 828 mg (2.7 mmol) of the sulfides (**16**) in 12 ml of toluene. Stirring at 0°C was continued until tlc-analysis indicated complete disappearance of the sulfides (approx. 10 min). The dropping funnel was replaced with a reflux condensor, 1.0 ml (7.2 mmol) of triethylamine was added and the flask containing the reaction mixture was placed in an oil bath, pre-heated at 130°C. The mixture was refluxed for 15 min, while the disappearance of the sulfoxide intermediate was monitored via tlc-analysis. Solvent and triethylamine were removed at the rotary evaporator under reduced pressure and the residual oil was purified by chromatography on 30 g of silicagel with petrol-EtOAc (99.5-0.5) as the eluent, affording 379 mg (1.95 mmol; 72%) of a 2 : 1 mixture of stereoisomers of the furofuran (**8**) as a colourless oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.70-2.10 (br m, 21 H): H-3 and *c*-hexyl H-1'-H-6'; 3.24-3.39 (m, 0.33H) and 3.40-3.52 (m, 0.66H): H-3a; 3.56-3.72 (m, 1H): H-2; 4.74 (t, J=2.6 Hz, 0.66H) and4.91 (t, J=2.6 Hz, 0.33H): H-4; 5.93 (d, J=6.7 Hz, 0.33 H) and 5.98 (d, J=6.2 Hz, 0.66H): H-6a; 6.19 (t, J=2.4 Hz, 0.33H) and 6.36 (t, J=2.5 Hz, 0.66H): H-5. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, selected peaks): Major isomer: δ 25.8, 26.4, 29.0, 29.6 and 30.3 (5 t): *c*-hexyl C-2'-C6'; 35.5 (t): C-3; 42.2 (d): *c*-hexyl C-1'; 46.4 (d): C-3a; 83.0 (d): C-2; 102.2 (d):C-4; 108.9 (d): C-6a; 146.0 (d): C-5. Minor isomer: δ 25.9, 26.4, 29.0, 29.6 and 30.3 (5 t): *c*-hexyl C-2'-C6'; 34.5 (t): C-3; 42.8 (d): *c*-hexyl C-1'; 46.2 (d): C-3a; 84.5 (d): C-2; 104.7 (d):C-4; 110.1 (d): C-6a; 143.9 (d): C-5. MS: *m/e* (%) 194 (100), 176 (27), 165 (58), 150 (15), 147 (52), 126 (16), 119 (15), 111 (52), 109 (50), 108 (18), 99 (14), 98 (17), 95 (31), 93 (16), 91 (22), 86 (42), 83 (44), 81 (60), 79 (25), 69 (26), 67 (78), 55 (48), 41 (36). HRMS: calcd. (M^+): *m/e* 194.1307; found: *m/e* 194.1310.

2-Cyclohexyl-perhydrofuro[2,3b]furan (19). A solution of 203 mg (1.0 mmol) of a mixture of the tetrahydrofurofurans (**18**) in 25 ml of methanol, containing 162 mg of 10% Pd/C, was hydrogenated in a Parr apparatus under hydrogen pressure (4 atm) at room temperature. After 20 min the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The crude product was purified via chromatography on silicagel with petrol-EtOAc (99.5-0.5 to 98-2) as the eluent, affording 167 mg (0.85 mmol; 85%) of a 1.5 : 1 mixture of isomers of the perhydrofurofurans (**19**) as a colourless oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-1.80 (br m, 15 H) and 1.80-2.16 (m, 3H): H-3, H-4 and *c*-hexyl H-1'-H-6'; 2.70-2.90 (m, 1H): H-3a; 3.47 (ddd, J=12.1, 7.3, 4.9 Hz, 0.4H): H-2 minor isomer; 3.68-3.90 (m, 2.6H): H-2 major isomer and H-5; 5.58 (d, J=5.4 Hz, 0.4H): H-6a minor isomer; 5.66 (d, J=5.1 Hz, 0.6H): H-6a major isomer. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, selected peaks): major isomer: δ 25.9, 26.4, 28.8 and 30.0 (4 t): *c*-hexyl C-2'-C6'; 32.7 (t): C-3; 36.5 (t): C-4; 42.4 (d): *c*-hexyl C-1'; 43.2 (d): C-3a; 68.1 (t): C-2; 84.1 (d): C-5; 108.8 (d): C-6a. minor isomer: δ 25.7, 25.8, 28.8 and 30.0 (4 t): c-hexyl C-2'-C6'; 32.5 (t): C-3; 34.5 (t): C-4; 42.7 and 42.9 (2 d): C-3a and *c*-hexyl C-1''; 65.8 (t): C-2; 83.7 (d): C-5; 108.7 (d): C-6a. MS: *m/e* (%) 152 (10), 113 (100), 69 (13). HRMS: calcd. ($M-C_6H_{11}$): *m/e* 113.0603; found: *m/e* 113.0602.

5-Cyclohexyl-2-ethoxy-perhydrofuro[2,3b]furan (20). A solution of 50 mg (0.24 mmol) of a mixture of the furofuranols (**15**), 25 mg (0.54 mmol) of ethanol and 5 mg (0.03 mmol) of

p-toluenesulfonic acid monohydrate in 10 ml of THF was stirred at room temperature for 2 days. Then, 5 ml of saturated aqueous sodium bicarbonate solution were added and the mixture was extracted with three 10 ml-portions of ether. The combined extracts were dried with $MgSO_4$ and concentrated under reduced pressure. According to tlc-analysis, the crude oil consisted of a mixture of the ethoxy-furo[2,3b]furans (**20**) as the major component, accompanied by a minor amount of the more polar starting material (**15**). Separation by chromatography on 10 g of silicagel with petrol-EtOAc (99-1) as the eluent yielded 32 mg (0.13 mmol; 54%) of the ethoxy-furofurans (**20**) as a mixture of all four diastereomers.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.76-1.45 (br m) and 1.45-2.10 (br m; total 21H): H-3, H-4, *c*-hexyl H-1'-H-6' and OCH_2CH_3; 2.67-2.85 (br, 0.7H) and 2.85-3.05 (br, 0.3H): H-3a; 3.25-3.55 (m, 0.4H), 3.55-3.87 (m, 0.5H) and 4.00 (ddd, J=10.1, 7.7, 5.8 Hz, 0.1H): H-5 and OCH_2CH_3; 5.02 (d, J=5.7 Hz, 0.3H) and 5.12 (d, J=5.3 Hz), 5.18 (dd, J=5.1, 1.4 Hz), 5.25 (t, J=4.7 Hz; total 0.7H): H-2; 5.63 (d, 5.2 Hz) and 5.67 (d, 5.8 Hz; total 0.2 H), 5.72 (d, 5.4 Hz, 0.4H) and 5.78 9d, 5.4 Hz, 0.4 H): H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): *Major isomers*: δ 15.1 (q): OCH_2CH_3; 25.8, 36.0, 26.4, 26.5, 28.9, 29.0, 30.1 and 30.2 (8 t): *c*-hexyl C-2'-C-6'; 36.5 and 36.6 (2 t): C-3; 38.2 and 39.6 (2 t): C-4; 40.5, 41.0, 42.4 and 42.8 (4 d): C-3a and *c*-hexyl C-1'; 62.6 and 62.9 (2 t): OCH_2CH_3; 82.3 (2 d): C-5; 103.4 and 103.8 (2 d): C-2; 108.5 and 110.2 (2 d): C-6a. *Minor isomers* (separated peaks only): δ 15.0 (q); 25.7, 26.0, 28.8, 29.8 (4 t); 35.1 and 35.7 (2 t); 41.6, 42.1, 43.0 and 43.4 (4 d); 62.7 and 63.9 (2 t); 86.2 (d); 104.3 and 105.2 (2 d); 108.1 and 108.5 (2 d). MS: *m/e* (%) 195 (12), 194 (12), 157 (63), 111 (100), 86 (16), 84 (25), 83 (10), 48 (23). HRMS: calcd. (M-OEt): *m/e* 195.1385; found: *m/e* 195.1383.

2-Acetyl-5-cyclohexyl-perhydrofuro[2,3b]furan (21). A solution of 100 mg (0.47 mmol) of a mixture of the furofuranols (**15**), 0.07 ml (0.98 mmol) of acetylchloride and 0.08 ml (0.99 mmol) of pyridine in 5 ml of dichloromethane was stirred at room temperature for 45 min. The reaction mixture was diluted with 50 ml of ether and 30 ml of water. The organic layer was separated and the aqueous layer was extracted with 50 ml of ether. The combined organic layers were washed with 30 ml of brine, dried with $MgSO_4$ and the solvents were evaporated under reduced pressure, yielding 94 mg of a pale yellow oil. Chromatography on 15 g of silicagel with petrol-EtOAc (90-10) as the eluent afforded 75 mg (0.30 mmol; 64%) of the acetates (**21**) as a mixture of isomers.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-2.35 (br m, 20H): H-3, H-4, $CH_3C(O)O$ [δ 1.99 and 2.01 (2 s)] and *c*-hexyl H-1'-H-6'; 2.78-3.10 (br m, 1H): H-3a; 3.60-3.85 (m, 1H): H-5; 5.73 (d, J=5.4 Hz, 0.4H) and 5.83 (d, J=5.0 Hz, 0.6H): H-6a; 6.34 (d, J=5.0 Hz) and 6.37 (d, J=8.5 Hz; 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 21.2 (q): $CH_3C(O)O$; 25.5, 25.7, 25.9, 26.0, and 26.3 (5 t): *c*-hexyl C-3'-C-5'; 28.8, 29.0, 39.9 and 30.1 (4 t): *c*-hexyl C-2', C-6'; 35.1 and 36.0 (2 t): C-3; 37.5 and 37.7 (2 t): C-4; 39.8 and 41.3, 42.1 and 43.4 (4 d): C-3a and *c*-hexyl C-1'; 82.4 and 87.1 (2 d): C-5; 98.5 and 98.7 (2 d): C-2; 109.6 and 109.9 (2 d): C-6a; 170.1 (2 s): $CH_3C(O)O$. MS: *m/e* (%) 195 (13), 194 (6), 171 (22), 133 (6), 112 (6), 111 (100), 81 (6), 67 (8), 54 (8), 43 (30), 41 (6). HRMS: calcd. (M-OAc): *m/e* 195.1385; found: *m/e* 195.1381.

Rac. (3aS,5S,6aR)- and (3aS,5R,6aR)-5-cyclohexyl-3a,4,5,6a-tetrahydrofuro[2,3b]-furan-2(3H)-one (22a) and (22b). A mixture of 2.01 g (9.5 mmol) of the furofuranols (**15**) and 4.3 g (11.4 mmol) of pyridinium dichromate in 40 ml of dichloromethane was stirred for 3 days at room temperature. The mixture was filtered through a pad of hyflo, dried with $MgSO_4$ and concentrated under reduced pressure. Purification via chromatography on 100 g of silicagel with petrol-EtOAc (95-5 to 90-10) as the eluent yielded, in order of elution, 290 mg (1.4 mmol; 15%) of the 5βH-isomer (**22a**) as a white solid (m.p. 90°C) and 1.12 g (5.3 mmol; 56%) of a mixture of C-5 epimers (**22a,b**) as an oil.

(**22a**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.8-2.0 (br m, 13H): H-4 and *c*-hexyl H-1'-H-6'; 2.37 (dd, J=18.6, 3.9 Hz, 1H): H-3β; 2.83 (dd, J=18.6, 10.6 Hz, 1H): H-3α; 3.1-3.2 (m, 1H): H-3a; 3.83 (ddd, J=9.6, 7.2, 6.2 Hz, 1H): H-5; 6.04 (d, J=5.5 Hz, 1H): H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.6, 25.8 and 26.2 (3 t): *c*-hexyl C-3'-C-5'; 28.7 and 29.7 (2 t): *c*-hexyl C-2', C-6'; 35.3 (t): C-4; 36.1 (t): C-3; 38.3 (d): C-3a; 42.0 (d): *c*-hexyl C-1'; 83.1 (d): C-5; 108.0 (d): C-6a; 175.5 (s): C-2. MS: *m/e* (%) 166 (10), 129 (12), 128 (12), 127 (100), 126 (24), 109 (23), 99 (13), 83 (12), 81 (17), 55 (15), 41 (12). HRMS: calcd. (M-C_6H_{11}): *m/e* 127.0395; found: *m/e* 127.0396. Anal: calcd. for $C_{12}H_{19}O_5$ ($C_{12}H_{18}O_3$): C, 68.54; H, 8.63; found: C, 68.29; H, 8.81.

n-Hexyl-substituted Derivatives.

2-(2',2'-Diethoxyethyl)-4-(dimethyl-*iso*-propylsilyloxy)-decanenitrile (**30**). Addition of the lithium anion of (**10**) to 4.2 ml (27.4 mmol) of 1,2-epoxyoctane according to the procedure described for (**12**) yielded, after purification via chromatography on silicagel with petrol-EtOAc (99-1) as the eluent, 7.9 g (20.6 mmol; 75%) of the nitriles (**30**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.0-0.10 (m [incl. δ 0.05, 0.08 and 0.09 (3 s)], 6H: $Si(CH_3)_2$; 0.70-1.00 (br m, 11H), 1.10-1.35 (m, 14H): H-6-H-10, , $SiCH(C\underline{H}_3)_2$ [δ 0.93 (d, J=6.0 Hz) and 0.94 (d, J=5.7 Hz)], $OCH_2C\underline{H}_3$ [δ 1.19-1.20 (3 t, J=7.1 Hz)] and $SiC\underline{H}(CH_3)_2$; 1.35-1.95 (m, 6H): H-3, H-5 and H-1'; 2.70-3.01 (m, 1H): H-2; 3.47-3.93 (m, 5H): H-4 and $OC\underline{H}_2CH_3$; 4.67 (2 t, J=5.3 Hz, 1H): H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ -3.9, -3.7, -3.5 and -3.4 (4 q): $Si(CH_3)_2$; 14.0 (q): C-10; 14.9 and 15.0 (2 d): $Si\underline{C}H(CH_3)_2$; 15.2 and 15.3 (2 q): $OCH_2\underline{C}H_3$; 17.0 (q): $SiCH(\underline{C}H_3)_2$; 23.6 and 24.8 (2 d): C-2; 22.5, 24.9, 29.3, 29.4, 31.8, 36.4, 36.9, 37.9, 39.4 and 39.7 (10 t): C-3, C-5-C-9 and C-1'; 62.1, 62.2, 62.5 and 62.9 (4 t): $O\underline{C}H_2CH_3$; 69.6 and 69.9 (2 d): C-4; 100.7 and 100.9 (2 d): C-2'; 121.9 and 122.2 (2 s): C-1. MS: *m/e* (%) 343 (19), 342 (100), 340 (39), 296 (35), 268 (18), 222 (76), 198 (14), 195 (9), 194 (14), 182 (11), 171 (15), 124 (10), 103 (29), 75 (28), 73 (11). HRMS: calcd. (M-^{i}Pr): *m/e* 342.2464; found: *m/e* 342.3012.

2-(2',2'-Diethoxyethyl)-4-(dimethyl-*iso*-propylsilyloxy)-decanal (**31**). Reduction of 5.3 g (13.8 mmol) of (**30**) with DibalH as described for (**14**), using toluene as the solvent, yielded, after purification by chromatography on 75 g of silicagel with petrol-EtOAc (95-5) as the eluent, 4.2 g (10.9 mmol; 79%) of the aldehydes (**31**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ -0.02 and 0.0 (2 s, 6H): $Si(CH_3)_2$; 0.64-0.95 (br m, 11H) and 1.05-1.32 (br m, 16H): H-6-H-10, $SiCH(C\underline{H}_3)_2$, $SiC\underline{H}(CH_3)_2$ and $OCH_2C\underline{H}_3$ [δ 1.13 (d t, J=7.0 Hz)]; 1.32-2.06 (m, 7H): H-3, H-5 and H-1'; 2.40-2.65 (br, 1H): H-2; 3.33-3.75 (m, 5H): H-4 and $OC\underline{H}_2CH_3$; 4.45 and 4.46 (2 t, J=5.7 Hz, 1H): H-2'; 9.53 (d, J=2.9 Hz) and 9.55 (d, J=2.5 Hz; total 1H): H-1. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ -3.7 and -3.5 (2 q): $Si(CH_3)_2$; 14.0 (q): C-10; 14.9 (d): $Si\underline{C}H(CH_3)_2$; 15.2 (q): $OCH_2\underline{C}H_3$; 17.0 (q): $SiCH(\underline{C}H_3)_2$; 22.6, 25.0, 25.1, 29.4, 31.8, 34.2, 34.3, 36.5, 36.9 and 37.6 (10 t): C-3, C-5-C-9 and C-1'; 44.4 and 45.3 (2 d): C-2; 61.8 and 62.1 (2 t): $O\underline{C}H_2CH_3$; 70.1 and 70.4 (2 d): C-4; 101.3 and 101.4 (2 d): C-2'; 204.1 and 204.3 (2 d): C-1. MS: *m/e* (%) 299 (78), 253 (71), 215 (27), 185 (100), 179 (30), 157 (28), 103 (42), 75 (44). HRMS: calcd. (M-^{i}Pr): *m/e* 345.2461; found: *m/e* 345.2463.

5-Hexyl-perhydrofuro[2,3b]furan-2-ol (**32**). Cyclization of 3.7 g (9.6 mmol) of (**31**) according to the procedure described for (**15**) yielded, after purification by chromatography on 70 g of silicagel with petrol-EtOAc (66-34) as the eluent, 1.16 g (5.4 mmol; 56%) of the furofuranols (**32**) as a mixture of all four stereoisomers.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-1.0 (br m, 3H): H-6', 1.05-1.50 (br, 10H) and 1.50-2.30 (br m, 6H): H-3, H-4 and H-1'-H-5'; 2.70-2.97 (br m, 0.7H) and 2.97-3.15 (m, 0.3H): H-3a;

3.40-4.12 (br m, 1.8H) and 2.97-3.15 (m, 0.3H): H-5 and OH; 5.15-5.90 (br m [incl. δ 5.82 (t, J=5.9 Hz)], 2H): H-2 and H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.0 (q): C-6'; 22.5, 23.8, 26.1, 27.9, 29.3, 31.7, 34.5, 35.3, 35.7, 36.2, 37.5, 38.2, 38.4, 38.7, 38.9, 39.3 and 39.8 (17 t): C-3, C-4 and C-1'-C-5'; 40.7, 41.6 (2) and 42.4 (4 d): C-3a; 78.1, 78.2, 81.0 and 81.6 (4 d): C-5; 98.5, 98.7, 99.0 and 100.9 (4 d): C-2; 108.0, 108.6 and 110.5 (3 d): C-6a.

5-Hexyl-2-phenylsulfide-perhydrofuro[2,3b]furan (33). Addition of thiophenol to 1.16 g (5.4 mmol) of (**32**) according to the procedure described for (**16**) yielded 1.3 g (4.3 mmol; 80%) of the sulfides (**33**) as a mixture of isomers. The crude product was used without further purification in the next reaction.
^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.55-0.9 (br t, J≈6.0 Hz, 3H): *n*-hexyl H-6'; 0.9-1.9 (br, 12H) and 1.9-2.35 (m, 2H): H-3, H-4 and *n*-hexyl H-1'-H-5'; 2.5-3.1 (br m, 1H): H-3a; 3.6-4.4 (br m, 1H): H-2; 5.2-5.8 (m, 2H): H-6a [δ 5.60 (d, J=4.5 Hz) and 5.70 (d, J=6.0 Hz)] and H-5; 6.9-7.3 (m, 3H): SPh H-3"-H-5"; 7.3-7.5 (m, 2H): SPh H-2", H-6". MS: *m/e* (%) 198 (13), 197 (100), 179 (11), 135 (21), 109 (13), 95 (12), 86 (23), 84 (37), 81 (11), 69 (13), 55 (14), 51 (11), 49 (34). HRMS: calcd. (M^+): *m/e* 306.1654; found: *m/e* 306.1651.

2-Hexyl-2,3,3a,6a-tetrahydrofuro[2,3b]furan (25). Sulphoxide elimination of 1.2 g (3.9 mmol) of (**33**) as described for (**18**) afforded the tetrahydrofurofurans (**25**) as a 1 : 1 mixture. Careful chromatography on 30 g of silicagel with petrol-EtOAc (99-1) as the eluent yielded 53 mg (0.27 mmol; 6.9%) of the least polar isomer of (**25**) and 528 mg (2.7 mmol; 69%) of a mixture of both isomers in a ratio of 1 : 1.25. Despite repeated chromatography, the most polar isomer could not be separated from the mixture in an analytically pure form.
^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.7-1.0 (br t, J≈4.5Hz, 3H): *n*-hexyl H-6'; 1.0-2.4 (br m, 12H): H-3 and *n*-hexyl H-1'-H-5'; 3.3-3.6 (m, 1H): H-3a; 3.8-4.3 (m, 1H): H-2; 4.80 (t, 3.0 Hz, 0.44H): H-4 least polar isomer; 5.0 (t, J=3.0 Hz, 0.55H): H-4 most polar isomer; 5.9-6.1 (m, 1H): H-6a most polar isomer [δ 6.00 (d, J=7.5 Hz)] and H-6a least polar isomer [δ 6.03 (d, J=6.0 Hz)]; 6.25 (t, 3.0 Hz, 0.55H): H-5 most polar isomer; 6.40 (t, 3.0 Hz, 0.44H): H-5 least polar isomer. MS: *m/e* (%) 196 (18), 167 (20), 145 (26), 127 (100), 111 (18), 98 (21), 97 (22), 95 (19), 84 (17), 83 (28), 81 (46), 73 (32), 72 (17), 70 (28), 69 (51), 66 (20), 57 (33), 55 (51), 43 (61), 41 (40). HRMS: calcd. (M^+): *m/e* 196.1463; found: *m/e* 196.1461.

5-Hexyl-3a,4,5,6a-tetrahydrofuro[2,3b]furan-2(3H)-one (27). Oxidation of 82 mg (0.38 mmol) of a mixture of the furofuranols (**32**) with PDC according to the procedure described for (**22**) gave, after chromatography on 10 g of silicagel with petrol-EtOAc (99-1) as the eluent, 50 mg (0.24 mmol; 63%) of an inseparable mixture of the furofuranones (**27**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.80-1.0 (br m, 3H) and 1.10-1.90 (br m, 14H): H-4 and *n*-hexyl. H-1'-H-6'; 2.35-2.53 (m, 1H) and 2.72-2.92 (m, 1H): H-3; 2.95-3.25 (br m, 1H): H-3a; 4.05-4.30 (br m, 1H): H-5; 5.94 (d, J=5.2 Hz, 0.25H): H-6a minor isom., 6.07 (d, J=5.6 Hz, 0.75H): H-6a major isom. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.0 (q): *n*-hexyl C-6'; 22.5, 29.0, 29.2, 31.6, 34.2, 35.2, 36.1, 36.6, 36.8 and 38.4 (10 t): C-3, C-4 and *n*-hexyl C-1'-C-5'; 38.5 (d): C-3a major isom.; 39.9 (d): C-3a minor isom.; 78.8 (d): C-5 major isom.; 83.3 (d): C-5 minor isom.; 108.1 (d): C-6a major isom.; 108.7 (d): C-6a minor isom.; 175.5 (s): C-2. MS: *m/e* (%) 127 (100), 109 (14), 99 (10), 98 (10), 97 (9), 83 (9), 70 (11), 55 (16), 43 (11), 41 (13). HRMS: calcd. (M^+): *m/e* 212.1412; found: *m/e* 212.1414.

n-Decyl-substituted Derivatives.

2-(2',2'-Diethoxyethyl)-4-TMSoxy-tetradecanenitrile (34). According to the procedure described for (**12**), the lithium anion of (**10**) was reacted at -78°C with 6.0 ml (27.5 mmol) of

1,2-epoxydodecane. After stirring for 2 hrs at 0°C the reaction was quenched by the addition of 4.0 ml (31.5 mmol) of chlorotrimethylsilane. Stirring was continued at room temperature for 1 hr before the reaction mixture was worked-up as described, yielding 11.0 g of a yellow oil as the crude product. Chromatography on 50 g of silicagel with petrol-EtOAc (98-2) as the eluent gave 9.5 g (22.9 mmol; 83%) of a mixture of the nitriles (**34**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.08, 0.09, 0.10, 0.11, 0.12 and 0.13 (6 s, 7H): $Si(CH_3)_3$; 0.84 (br 2 t, J≈6.0 Hz, 3H): H-14; 1.10-1.35 (br, 22H), 1.35-1.52 (br, 2H) and 1.52-1.90 (m, 3H): H-3, H-5-H-13, H-1' and $OCH_2C\underline{H}_3$ [δ 1.18 (3 t, J≈7.0 Hz)]; 2.65-2.97 (m, 1H): H-2; 3.40-4.92 (m, 5H): H-4 and $OC\underline{H}_2CH_3$; 4.66 (br t, J≈5.8 Hz, 1H): H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 0.3 (q): $Si(CH_3)_3$; 14.0 (q): C-14; 15.2 and 15.3 (2 q): $OCH_2\underline{C}H_3$; 23.8 and 24.7 (2 d): C-2; 22.6, 25.1, 29.3, 29.5, 31.8, 36.3, 36.7, 37.0, 37.9, 39.4 and 39.8 (11 t): C-3, C-5-C-13 and C-1'; 62.1 (2), 62.4 and 62.8 (4 t): $O\underline{C}H_2CH_3$; 69.7 and 70.0 (2 d): C-4; 100.7 and 100.8 (2 d): C-2'; 121.8 and 122.1 (2 s): C-1. MS: *m/e* (%) 400 (23), 398 (100), 369 (20), 352 (27), 278 (59), 243 (34), 227 (23), 226 (90), 182 (50), 110 (34), 103 (94), 75 (20), 73 (55). HRMS: calcd. (M-CH_3): *m/e* 398.3090; found: *m/e* 398.3090.

2-(2',2'-Diethoxyethyl)-4-TMSoxy-tetradecanal (35). *Reduction of 8.7 g (21.1 mmol) of* (**34**) with DibalH as described for (**14**), using toluene as the solvent, gave 9.1 g of an intermediate reduction product as an oil. Overnight treatment of this product in EtOAc solution with 25 g of silicagel as described yielded 7.6 g (18.2 mmol; 86%) of the crude aldehydes (**35**) as an oil, which was sufficiently pure to be used in the next reaction without further purification.
^{1}H NMR ($CDCl_3$, 200 MHz): δ -0.05-0.10 (m s, 9H): $Si(CH_3)_3$; 0.69-0.85 (br t, J≈6.3 Hz, 3H): H-14; 1.00-1.27 (br, 20H), 1.27-2.02 (br m, 7H): H-3, H-5-H-13, H-1' and $OCH_2C\underline{H}_3$ [δ 1.07 (t, J=7.1 Hz)]; 2.30-2.60 (br, 1H): H-2; 3.28-3.67 (br m, 5H): H-4 and $OC\underline{H}_2CH_3$; 4.37-4.45 (m; 1H): H-2', 9.47 (d, J=2.9 Hz) and 9.49 (d, J=2.2 Hz; total 1H): H-1. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 0.4 (q): $Si(CH_3)_3$; 14.1 (q): C-14; 15.3 (q): $OCH_2\underline{C}H_3$; 22.7, 25.2, 25.3, 29.3, 29.6, 29.7, 31.9, 34.0, 34.3, 36.8, 37.0 and 37.6 (12 t): C-3, C-5-C-13 and C-1'; 44.5 and 45.5 (2 d): C-2; 61.8 and 62.1 (2 t): $O\underline{C}H_2CH_3$; 70.0 and 70.6 (2 d): C-4; 101.3 and 101.4 (2 d): C-2'; 204.0 and 204.3 (2 s): C-1.

5-Decyl-perhydrofuro[2,3b]furan-2-ol (36). Cyclization of 6.9 g (16.6 mmol) of (**35**) according to the procedure described for (**15**) yielded, after purification by chromatography on 40 g of silicagel with petrol-EtOAc (95-5) as the eluent, 4.5 g (16.6 mmol; 100%) of the furofuranols (**36**) as a mixture of all four stereoisomers.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-0.95 (br t, J=5.7 Hz, 3.5H): *n*-decyl H-10'; 1.08-1.40 (br) and 1.40-2.27 (br m; total 24H): H-3, H-4 and *n*-decyl H-1'-H-9'; 2.71-2.95 (br m) and 2.95-3.13 (br m; total 1H): H-3a; 3.81-3.90 (br m) and 3.90-4.05 (br m; total 1.5H) and 4.12 (br d, J=4.5Hz, 0.3H) and 4.37-4.55 (br m, 0.3H): H-5 and OH; 5.50 (dd, J=5.4, 1.9 Hz), 5.57-5.72 (m [incl. δ 5.69 (t, J=5.5 Hz)]) and 5.81 (t, J=5.6 Hz; total 2H): H-2 and H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.1 (q): *n*-decyl C-10'; 22.6, 26.1, 26.2, 29.3, 29.5, 31.8, 34.5, 35.3, 35.7, 36.2, 37.5, 38.2, 38.4, 38.7, 38.8, 38.9, 39.3, 39.8 (18 t); 40.7, 41.6, 41.8 and 42.4 (4 d): C-3a; 78.1, 78.2, 80.9 and 81.6 (4 d): C-5; 98.5, 98.7, 98.9 and 100.8 (4 d): C-2; 107.9, 108.6, 110.5 and 110.6 (4 d): C-6a. MS: *m/e* (%) 129 (67), 112 (7), 111 (100), 100 (9), 83 (13), 82 (12), 69 (15), 58 (10), 55 (12), 43 (10). HRMS: calcd. (M-H_2O): *m/e* 252.2089; found: *m/e* 252.2091.

5-Decyl-3a,4,5,6a-tetrahydrofuro[2,3b]furan-2(3H)-one (28). Oxidation of 373 mg (1.38 mmol) of a mixture of the furofuranols (**36**) with PDC according to the procedure described for (**22**) gave 410 mg of a brown oil, which solidified in the freezer. Chromatography on 25 g of silicagel with petrol-EtOAc (96-4) as the eluent afforded, in order of elution, 123 mg (0.46 mmol; 33%) of the least polar isomer (**28a**) as a white solid

(mp 60-61°C) and 137 mg (0.51 mmol; 37%) of a 1 : 1.5 mixture of both isomers (**28**) as a clear, colourless oil.
Mixture of isomers (**28**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.81 (br t, J=5.9 Hz, 3H): *n*-decyl H-10'; 1.05-1.80 (m [incl. δ 1.19 (br s)], 17H); 2.30-2.48 (m, 2H); 2.68-2.88 (m, 1H); 2.91-3.20 (br m, 1H): H-3a; 3.98-4.25 (br m, 1H): H-5; 5.89 (d, J=5.2 Hz, 0.6H): H-6a most polar isomer; 6.02 (d, J=5.6 Hz, 0.4H): H-6a least polar isomer. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.1 (q): decyl C-10'; 22.7, 26.1, 29.3, 29.4, 29.6, 31.9, 34.3, 35.3 (8 t); 36.1, 36.6 and 36.9 (3t, most polar isom.); 38.5 (t); 38.6 (d): C-3a least polar isom.; 40.0 (d): C-3a most polar isom.; 78.9 (d): C-5 least polar isom.; 83.4 (d): C-5 most polar isom.; 108.1 (d): C-6a least polar isom.; 108.7 (d): C-6a most polar isom.; 174.3 (s): C-2 most polar isom.; 175.6 (s): C-2 least polar isom. MS: *m/e* (%) 129 (10), 127 (100), 111 (20), 109 (15), 99 (8), 98 (8), 97 (9), 83 (14), 81 (10), 70 (8), 69 (11), 57 (9), 55 (17), 43 (14), 41 (14). HRMS: calcd. (M^+): *m/e* 268.2038; found: *m/e* 268.2038. *Least polar (3aS*,5S*,6aR*)-isomer* (**28a**): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.87 (t, J=7.0 Hz, 3H): decyl H-10'; 1.20-1.35 (br), 1.35-1.45 (br m), 1.47-1.56 (br m) and (1.62-1.71 (br m; total 20H): decyl H-1'-H-9'; 1.73-1.82 (m, 2H): H-4; 2.41 (dd, J=18.8, 4.0 Hz, 1H): H-3β; 2.85 (dd, J=18.7, 10.6 Hz, 1H): H-3α; 3.15-3.19 (m, 1H): H-3a; 4.13 (ddd, J=12.1, 10.5, 5.3 Hz, 1H): H-5; 6.07 (d, J=5.6 Hz, 1H): H-6a. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.1 (q): decyl C-10'; 22.6, 25.9, 29.3, 29.5, 31.8 and 34.2 (6 t): decyl C-1'-C-9'; 35.2 (t): C-4; 38.4 (t): C-3; 38.5 (d): C-3a; 78.8 (d): C-5; 108.0 (d): C-6a; 175.4 (s): C-2. Anal: calcd. for $C_{16}H_{28}O_3$: C, 71.60; H, 10.52; found: C, 71.30; H, 10.68.

Phenyl-substituted Derivatives.

2 - (2',2'-Diethoxyethyl) - 4 - (dimethyl-*iso*-propylsilyloxy) - 4 - phenyl- butyronitrile (23). Addition of the lithium anion of (**10**) to 3.2 ml (27.4 mmol) of styrene oxide according to the procedure described for (**12**) yielded, after chromatography on silicagel with petrol-EtOAc (gradient elution, 99.5-0.5 to 25-1) as the eluent, 7.2 g of an oil, consisting of an inseparable mixture of 80% of (**23**) (15.3 mmol, 56% yield) and 20% of the regioisomeric **2-(2',2'-diethoxyethyl)-4-(dimethyl-*iso*-propylsilyloxy)-3-phenyl-butyronitrile (24)** (3.8 mmol, 14% yield), as determined by glc-analysis. The oil was used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz): δ -0.13 - 0.08 (ms, 8.4H): $SiCH_3$ (**23/24**); 0.55-1.02 (m) and 1.12-1.25 (m; total 18.4H): $OCH_2C\underline{H}_3$, $SiC\underline{H}(CH_3)_2$, and $SiCH(C\underline{H}_3)_2$ (**23/24**); 1.64-1.98 (m, 4H): H-3 and H-1' (**23**) and H-1' (**24**); 2.05-2.22 (m) and 2.52-2.70 (m; total 1H): H-2 (**23**); 2.75-3.25 (m, 0.8H): H-2 and H-3 (**24**); 3.40-4.05 (m, 6.4H): $OC\underline{H}_2CH_3$ (**23/24**) and H-4 (**24**); 4.61-4.89 (m, 2.4H): H-2' and H-4 (**23**) and H-2' (**24**); 7.15-7.35 (m, 7.5H): phenyl H-2"-H-6" (**23/24**). ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): [(**23**), selected peaks]: δ -4.7, -4.3, -4.2, -3.9 and -3.8 (5 q): $SiCH_3$; 14.3, 14.5 and 14.6 (3 d): $SiC\underline{H}(CH_3)_2$; 15.1 and 16.7 (2 q): $SiCH(\underline{C}H_3)_2$ and $O\underline{C}H_2CH_3$; 24.1 and 24.8 (2 d): C-2; 36.0 and 36.4 (2 t): C-1'; 42.5 and 43.5 (2 t): C-3; 62.0, 62.1, 62.5 and 62.6 (4 t): $OCH_2\underline{C}H_3$; 72.3 and 72.5 (2 d): C-4; 100.6 and 100.7 (2 d): C-2'; 121.6 and 121.7 (2 s): C-1; 125.6, 125.9, 127.5, 127.7, 128.2, 128.3, 128.4 and 128.6 (8 d): phenyl C-2"-C-6"; 143.3 and 144.0 (2 s): phenyl C-1". [(**24**), separated peaks only]: δ 28.9 and 29.6 (2 d): C-2; 34.1 and 34.6 (2 t): C-1'; 49.1 and 49.3 (2 d): C-3; 64.0 and 64.1 (2 t): C-4; 100.8 (d): C-2'; 120.5 (s): C-1; 137.5 (s): phenyl C-1". MS: *m/e* (%) 335 (23), 334 (100), 288 (63), 214 (46), 207 (19), 190 (45), 184 (29), 110 (46), 103 (19), 75 (21). HRMS: calcd. ($M-^iPr$): *m/e* 334.1838; found: *m/e* 334.1841.

2-(2',2'-Diethoxyethyl)-4-(dimethyl-*iso*-propylsilyloxy)-4-phenyl-butyraldehyde (37). The reduction of 7.2 g (19.1 mmol) of a 4 : 1 mixture of (**23**) and (**24**) with DibalH as described for (**14**), using toluene as the solvent, gave 7.2 g of an oil as the intermediate reduction product. The oil was dissolved in 100 ml of EtOAc and the solution was treated with

silicagel as described for 4 days, yielding 5.9 g of a pale yellow oil after filtration and evaporation of the solvent. Chromatography on 90 g of silicagel with petrol-EtOAc (95-5) as the eluent afforded 4.5 g (11.8 mmol; 62% combined yield) of a clear, colourless oil, consisting of an inseparable mixture of approx. 91% of the aldehydes (**37**) (both diastereomers in 1 : 1.5 ratio) and 9% of the regioisomeric **2-(2',2'-diethoxyethyl)-4-(dimethyl-*iso*-propylsilyloxy)-4-phenyl-butyraldehydes** (**38**), as determined by NMR spectroscopy. The aldehyde mixture was used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz, major peaks): δ -0.17 - 0.11 (ms, 6.8H): $SiCH_3$ (**37/38**); 0.63-1.06 (m) and 1.06-1.32 (m; total 14.8H): $OCH_2C\underline{H}_3$, $SiC\underline{H}(CH_3)_2$, and $SiCH(C\underline{H}_3)_2$ (**37/38**); 1.60-2.28 (m, 4H): H-3 and H-1' (**37**) and H-1' (**38**); 2.46-2.74 (br m, 1H): H-2 (**37**); 2.81-3.00 (m) and 3.06-3.20 (m; total 0.2H): H-2 and H-3 (**38**); 3.29-3.55 (m) and 3.55-3.80 (m; total 4.7H): $OC\underline{H}_2CH_3$ (**37/38**) and H-4 (**38**); 4.48-4.57 (m, 1H): H-2' (**37**); 4.37 (t, J=5.7 Hz, 0.1H): H-2' (**38**); 4.64-4.86 (m, 1H): H-4 (**37**); 7.15-7.40 (m, 5.9H): phenyl H-2"-H-6" (**37/38**); 9.5 (d, J=2.8 Hz, 0.4H) and 9.6 (d, J=2.8 Hz, 0.6H): H-1 (**37**); 9.7 (d, J=4.3 Hz, 0.1H): H-1 (**38**). ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, major peaks), (**37**) only: δ -4.1 and -3.8 (2 q): $SiCH_3$; 14.6 (2 d): $Si\underline{C}H(CH_3)_2$; 15.1, 15.2, 16.8 and 16.9 (4 q): $SiCH(\underline{C}H_3)_2$ and $OCH_2\underline{C}H_3$; 33.8 and 34.2 (2 t): C-1'; 40.5 and 40.7 (2 t): C-3; 44.6 and 45.6 (2 t): C-2; 62.0 (t) and 62.1 (2 t): $O\underline{C}H_2CH_3$; 72.5 and 73.3 (2 d): C-4; 101.3 and 101.4 (2 d): C-2'; 125.8, 126.0, 127.3, 127.4, 128.2 and 128.4 (6 d): phenyl C-2"-C-6"; 144.5 and 144.7 (2 s): phenyl C-1"; 203.6 and 203.8 (2 s): C-1. MS: *m/e* (%) 334 (27), 291 (64), 288 (20), 245 (100), 207 (66), 185 (46), 171 (22), 156 (20), 143 (19), 129 (24), 103 (58), 91 (23), 75 (48), 73 (25), 47 (20). HRMS: calcd. (M-^{i}Pr): *m/e* 337.1835; found: *m/e* 337.1835.

5-Phenyl-perhydrofuro[2,3b]furan-2-ol (**39**). Cyclization of 4.3 g (11.3 mmol) of the regioisomeric mixture of aldehydes (**37**) and (**38**) according to the procedure described for (**15**) yielded 3.0 g of a yellow oil. Removal of apolar fractions by rapid chromatography on 50 g of silicagel with petrol-EtOAc (66-33) as the eluent yielded 2.1 g (10.4 mmol; 92% combined yield) of a mixture of highly polar products, showing spectral characteristics of the furofuranols (**39**) and the regioisomeric **4-phenyl-perhydrofuro[2,3b]furan-2-ols** (**40**). The mixture was not further purified, but used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.7-1.0 (br m, 0.6H), 1.0-1.3 (m, 0.8H) and 1.4-2.6 (br m, 5H): incl. H-3 (**39/40**) and H-4 (**39**); 2.7-3.4 (br m, 1.5H): H-3a (**39/40**) and H-4 (**40**); 3.8-4.3 (br m, 1.5H): H-5 (**40**) and OH (**39/40**); 4.4-5.1 (br m, 1H): H-5 (**39**); 5.3-5.8 (br m, 1.5H): H-2 (**39/40**) and H-6a (**40**); 5.9 (d, J=5.4 Hz) and 6.0 (d, J=6.0 Hz; total 1H): H-6a (**39**).

5-Phenyl-2-phenylsulfide-perhydrofuro[2,3b]furan (**41**). Addition of thiophenol to 1.75 g (8.5 mmol) of the crude mixture of furofuranols (**39**) and (**40**) according to the procedure described for (**16**) yielded 2.30 g (7.7 mmol; 91% combined yield) of a yellow oil, mainly consisting of a mixture of all four isomers of (**41**). The crude product was used without further purification in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz, major peaks): δ 1.62-1.75 (m, 0.4H), 1.85-2.20 (m, 2.0H), 2.25-2.43 (m, 1.2H) and 2.51-2.80 (m, 0.4H): H-3 and H-4; 2.95-3.25 (m, 1H): H-3a; 5.02-5.15 (m, 1H): H-5; 5.33 (dd, J=10.4, 4.8 Hz, 0.1H), 5.53 (dd, J=8.8, 5.8 Hz, 0.3H) and 5.78 (t, J=6.1 Hz, 0.6H): H-2; 5.96 (d, J=5.3 Hz, 0.3H), 6.01-6.07 (m, 0.2H) and 6.12 (d, J=5.2 Hz, 0.6H): H-6a; 7.18-7.45 (m, 8H): SPh H-3'-H-5' and phenyl H-2"-H-6"; 7.48-7.65 (m, 2H): SPh H-2',H-6'. Minor peaks in the region δ 3.8-4.4 ppm indicate the presence of small amounts of the regioisomeric sulfides. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 38.1, 38.4 and 38.6 (3 t) and 39.8, 41.3 and 41.6 (3 t): C-3 and C-4; 42.4, 43.0 and 43.2 (3 d): C-3a; 79.8, 80.2 and 81.8 (3 d): C-5; 85.0, 86.2 and 86.7 (3 d): C-2; 109.9, 110.1, 110.9 and 112.2 (4 d): C-6a; 125.6-129.0 (16 d): SPh C-3'-C-5' and phenyl C-2"-C-6"; 131.2, 131.4, 131.9 and 132.2 (4 d): SPh C-2',C-6'; 134.6 and 134.7 (2 s): SPh C-1'; 140.5, 141.2, 141.9 and

142.6 (4 s): phenyl C-1". MS: *m/e* (%) 190 (12), 189 (100), 171 (15), 153 (15), 143 (17), 129 (33), 117 (22), 104 (12), 91 (31). HRMS: calcd. (M^+): *m/e* 298.1028; found: *m/e* 298.1026.

2-Phenyl-2,3,3a,6a-tetrahydrofuro[2,3b]furan (26). Sulphoxide elimination of 1.1 g (3.9 mmol) of the crude sulfide mixture (**41**) as described for (**18**) afforded, after repeated chromatography on 35 g of silicagel with petrol-EtOAc (gradient elution, 99.8-0.2 to 99-1) as the eluent, 342 mg of an oil, consisting for >85% (glc-analysis) of a mixture of both isomers of (**26**). The remainder consisted of several unidentified products, probably arising from the presence of regioisomers of (**41**) in the starting material. These byproducts were of similar polarity as the tetrahydrofurofurans (**26**) and were therefore impossible to remove. After extensive chromatography, only a small sample of the least polar, major (2S)-isomer (**26a**) could be obtained as a white solid (m.p. 32°C) of analytical purity.
Crude product mixture (assignments for (**26**) only): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.971.87-2.07 (m, 1.8H) and 2.21 (dd, J=12.4,4.7 Hz, 1.2H): incl. H-3; 2.50 (dd, J=7.5, 4.4 Hz) and 2.65 (dd, J=7.2, 4.7 Hz; total 0.4H); 2.90-3.22 (br, 0.3H); 3.22 (d, J=5.6 Hz, 0.2H); 3.46-3.61 (br m, 0.4H) and 3.66-3.75 (br m, 1H): incl. H-3a; 4.84 (t, J=2.6 Hz, 0.2H) and 4.92 (t, J=2.6 Hz, 0.9H): incl. H-4; 4.03 (d, J=7.5 Hz, 0.2H); 4.17 (dd, J=7.5, 6.2 Hz, 0.2H); 5.05 (dd, J=11.0, 4.8 Hz, 1.3H) and 5.19 (dd, J=7.5, 5.6 Hz, 0.3H): incl. H-5; 5.25-6.12 (m m, 0.7H); 6.15 (t, J=2.5 Hz, 0.2H); 6.19 (d, J=6.6 Hz, 0.2H) and 6.27 (d, J=6.1 Hz, 1H): incl. H-6a; 6.51 (t, J=2.4 Hz, 0.2H) and 6.54 (t, J=2.5 Hz, 0.8H): H-5. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): *Major peaks* [from major isomer of (**26**)]: δ 40.7 (t): C-3; 47.2 (d): C-3a; 79.8 (d): C-2; 102.1 (d): C-4; 109.2 (d): C-6a; 126.0, 127.7 and 128.4 (3 d): phenyl C-2'-6'; 140.1 (s): phenyl C-1'; 146.6 (d): C-5. *Minor peaks* [tentative assignment for minor isomer of (**26**) only]: δ 39.0 (t): C-3; 41.2 (t); 46.7 (d): C-3a; 50.8 and 55.3 (2 d); 72.9 (t); 79.0 (d); 80.4 (d): C-2; 103.1 (d); 104.0 (d): C-4; 109.7 (d); 110.6 (d): C-6a; 125.5, 126.6, 127.0, 128.0 and 128.8 (5 d); 142.5; 144.0; 144.4 (d); 146.4 (d): C-5. MS: *m/e* (%) 188 (30), 159 (40), 149 (11), 129 (14), 115 (12), 105 (22), 104 (100), 91 (37), 77 (14), 69 (12), 43 (24). HRMS: calcd. (M^+): *m/e* 188.0837; found: *m/e* 188.0836.

(2S*,3aS*,6aS*)-2-Phenyl-2,3,3a,6a-tetrahydrofuro[2,3b]furan (26a): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.97 (ddd, J=12.3, 11.2, 8.1 Hz, 1H): H-3α; 2.20 (dd, J=12.3, 4.8 Hz, 1H): H-3β; 3.65-3.75 (m, 1H): H-3a; 4.92 (t, J=2.6 Hz, 1H): H-4; 5.04 (dd, J=11.1, 4.8 Hz, 1H): H-2; 6.26 (d, J=6.1 Hz, 1H): H-6a; 6.54 (t, J=2.5 Hz, 1H): H-5; 7.20-7.42 (m, 5H): Ph. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 40.6 (t): C-3; 47.2 (d): C-3a; 79.8 (d): C-2; 102.1 (d): C-4; 109.1 (d): C-6a; 126.0, 127.7 and 128.4 (3 d): phenyl C-2'-6'; 140.0 (s): phenyl C-1'; 146.6 (d): C-5.

5-Phenyl-3a,4,5,6a-tetrahydrofuro[2,3b]furan-2(3H)-one (29). Oxidation of 340 mg (1.65 mmol) of a mixture of the furofuranols (**39**) and (**40**) with PDC according to the procedure described for (**22**) and chromatography of the crude product on 15 g of silicagel with petrol-EtOAc (80-20) as the eluent afforded, in order of elution, 90 mg (0.44 mmol; 27%) of the least polar isomer (**29a**) and 60 mg of a mixture of the most polar isomer of (**29**) and some inseperable side-products, probably arising from the regioisomeric furofuranols (**40**) present in the starting material.
(3aS*,5S*,6aR*)-isomer (**29a**)[8]: ^{1}H NMR ($CDCl_3$, 90 MHz): δ 2.11 (dd, J=7.5, 3.0 Hz, 2H): H-4; 2.55 (dd, J=18.0, 4.5 Hz, 1H): H-3β; 2.95 (dd, J=18.0, 9.0 Hz, 1H): H-3α; 3.16-3.60 (br m, 1H): H-3a; 5.18 (t, J=7.5 Hz, 1H): H-5; 6.30 (d, J=6.0 Hz, 1H): H-6a; 7.40 (br s, 5H): phenyl H-2"-H-6'.

5.5 - Notes and References

1. For recent reviews of natural clerodane diterpenes and their sources, see: A.T. Merrit and S.V. Ley, *Nat. Prod. Reports*, 243-287 (1992), and: F. Piozzi, *Heterocycl.*, **37** (1), 603-626 (1994).
2. P.Y. Malakov and G.Y. Papanov, *Phytochem.*, **46** (5), 955-958 (1997).
3. For an extensive survey of clerodane diterpenes with insect antifeedant activity, see Chapter 2.
4. R.B.M. Geuskens, J.M. Luteyn and L.M. Schoonhoven, *Experientia*, **39**, 403 (1983).
5. Y. Kojima and N. Kato, *Tetrahedron*, **37** (15), 2527-2538 (1981); Y. Kojima and N. Kato, *Nippon Kagaku Kaishi*, (5), 712-720 (1981).
6. See: T. Matsumoto *et al*, Agr. Biol. Chem., **51**, 249 (1987). Antifeedant activity against *Pieris rapae cruc.* on sweet potato leaf disc (500 µg dose) in a two-choice situation. Ginkgolide A: 98% feeding inhibition; bilobalide: 90% feeding inhibition.
7. W.M. Blaney, M.S.J. Simmonds, S.V. Ley and P.S. Jones, *Entomol. Exp. Appl.*, **46**, 267-274 (1988).
8. J. Vader, H. Sengers and Ae. de Groot, *Tetrahedron*, **45** (7), 2131-2142 (1989).
9. Prepared by mCPBA oxidation of vinylcyclohexane in 66% yield (bp. 72°C, 15 mmHg).
10. See: J.C. Anderson, S.V. Ley, D. Santafianos and R.N. Sheppard, *Tetrahedron*, **47** (33), 6813-6850 (1991) and references cited therein.
11. T. Mandai, K. Hara, T. Nakajima, M. Kawada and J. Otera, *Tetrahedron Lett.*, **24** (45), 4993-4996 (1983).
12. In the *n*-decyl series, the trimethylsilyl-group was used for hydroxyl group protection, instead of a dimethyl-*iso*-propylsilyl moiety as used in the other series.
13. For details of these preparations, see the experimental section: *n*-hexyl compounds (**30**)-(**33**), (**25**) and (**27**); *n*-decyl compounds (**34**)-(**36**), (**28**) and (**28a**); phenyl compounds (**23**), (**24**), (**37**)-(**41**), (**26**), (**26a**) and (**29a**).
14. Alternatively, an 1.0M solution of DibalH in hexanes has been used with similar results.

Chapter 6

Synthesis of a Series of 3a-Hydroxy-Perhydrofuro[2,3b]furans

Abstract: *In this chapter, a synthetic route to the 3a-hydroxy-perhydrofuro[2,3b]furan ring system is presented, which uses a stereoselective alkylation of cyanohydrin 1,3-acetonides as the key-step. This methodology is used for the preparation of a series of 3a-hydroxy-furo[2,3b]furan model compounds, derived from the C-9 sidechain of the natural clerodane-type insect antifeedant clerodin.*

Table of Contents

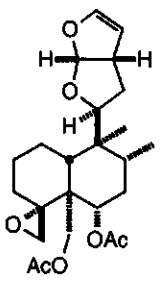
This chapter will be published in a revised form, in combination with chapter 5 and part of chapter 9: E.A. klein Gebbinck, C.T. Bouwman, M. Bourgois, B.J.M. Jansen and Ae. de Groot, *Synthesis and insect-antifeedant activity of C-2 and C-5 substituted perhydrofurofurans and 3a-hydroxy-perhydrofurofurans (Part I), Tetrahedron*, accepted for publication.

6.1 - Introduction

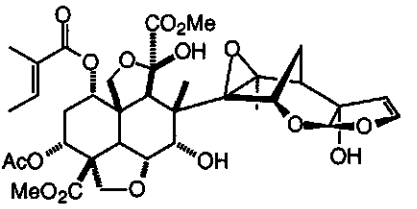
6.1.1 - Aim of the Study

Natural clerodane diterpenes incorporating a perhydrofuro[2,3b]furan subunit, such as clerodin (**1**), are well known for their antifeedant activity against several pest insect species.[1] These compounds constitute an interesting starting point in the search for structurally simple insect antifeedants. Studies have demonstrated that both the decalin-fragment[2] and the furo[2,3b]furan-fragment[3] of these diterpenes separately display antifeedant activity. Unfortunately, these simple models are generally much less potent than their parent compounds. Research into structural modifications that substantially increase this activity will be needed before application of such synthetic analogues as practical insect control agents can be considered.

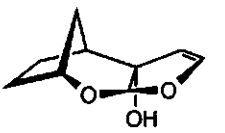
Figure 6.1



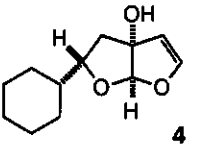
1: clerodin



2: azadirachtin



3



4

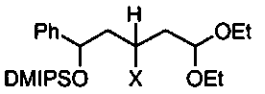
The tetranortriterpene azadirachtin (**2**) combines a potent antifeeding and growth disrupting activity against many different pest insect species with the absence of serious adverse effects on non-target organisms.[4] This natural compound is therefore often considered as a rôle model of an environmentally benign insecticide. Structure-activity studies have shown that for azadirachtin, too, the antifeedant activity resides in both halves of the molecule.[5] Nevertheless, simple analogues based on the furo[2,3b]pyran fragment, such as (**3**), were found to be as potent as azadirachtin itself at concentrations as low as 10 ppm.[6] Furthermore, some evidence suggests that the presence of a free C-20 hydroxyl group in (**2**) is required to obtain full antifeedant activity.[7,8]

In view of the structural resemblance of the hydroxy-furo[2,3b]pyran fragment of azadirachtin to the furo[2,3b]furan substructure present in clerodin, we envisioned that the introduction of a similar hydroxyl group into the latter moiety might increase the antifeedant activity of simple furo[2,3b]furans.[8,9] This

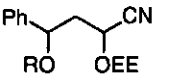
hypothesis could not be tested through literature data since the first clerodane diterpenes with this type of hydroxy-furo[2,3b]furan fragment have only very recently been isolated[10] and antifeedancy data for these compounds are not yet available. Also, to our knowledge no reports exist of synthetic compounds containing such a moiety, that have been examined for antifeedant activity.[11,12] Therefore, we decided to prepare a series of compounds, based on the 3a-*hydroxy*-tetrahydrofuro[2,3b]furan (**4**), in order to test this hypothesis through comparison of the antifeedant activity of these analogues with that of the corresponding 3a-*hydro*-furo[2,3b]furans described in the previous chapter.

6.1.2 - Synthetic Plan

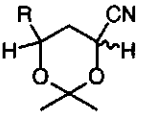
In our efforts towards the synthesis of 3a-hydroxy-perhydrofuro[2,3b]furans we first attempted to make use of the methodology described in the previous chapter by introduction of the hydroxyl group into a suitable intermediate obtained from this route. However, all attempts to functionalize the lithiated anions of either (**5**) or (**6**) through α-hydroxylation with MoOPh[13] or molecular oxygen[14] were unsuccesful and no trace of hydroxylated products was ever observed.



5: X=CN
6: X=CHO



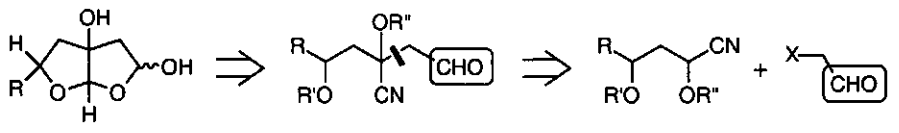
7a: R=TBDMS
7b: R=EE



8

We then embarked upon an alternative approach, involving an extension of the cyanohydrin-based[15] methodology developed in a parallel study for the synthesis of 3-hydroxy-2,3-dihydrofuran-based analogues (see Chapter 7). In order to gain access to the 3a-hydroxy-perhydrofurofuran ring system (Scheme 6.1), the cyanohydrins required for this modified approach needed to be equipped with an oxygen-containing functional group at C-4, as in (**7a**) or (**7b**). Unfortunately, neither of these cyanohydrins could be alkylated under the conditions previously

Scheme 6.1



9 **10** **11** **12**

Retrosynthetic scheme, outlining the synthetic strategy developed for the preparation of the 3a-hydroxy-perhydrofuro[2,3b]furan ringsystem (**9**). The boxed aldehyde group indicates a masked aldehyde functionality, such as an acetal or a double bond.

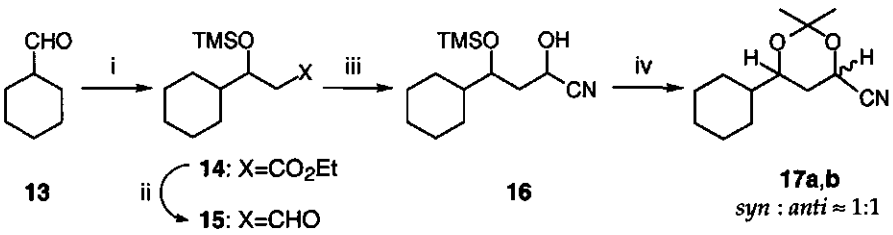
developed. A search of the literature revealed that examples of such an alkylation of γ-oxy-cyanohydrins are seldom. The only precedent we could find was a method developed by Rychnovsky and coworkers[16] for the stereoselective alkylation of the closely related cyanohydrin 1,3-acetonides (**8**). Therefore, we next turned our attention towards the utilization of such compounds in a synthesis of the 3a-hydroxy-perhydrofuro[2,3b]furan ring system.

6.2 - Synthesis of a Series of 3a-Hydroxy-perhydrofuro[2,3b]furans.

6.2.1 - Preparation of the Cyanohydrin 1,3-Acetonides.

The required cyanohydrin 1,3-acetonides (**17**) were prepared via a modified literature procedure (Scheme 6.2). Addition of the lithium enolate of ethylacetate[17] to cyclohexyl carbaldehyde (**13**) at low temperature yielded an intermediate alkoxide that was trapped as its silyl ether (**14**). Subsequent reduction of the ester afforded the aldehyde (**15**) in 66% yield from (**13**). The aldehyde was transformed into its cyanohydrin through the exchange of hydrocyanic acid from acetone cyanohydrin under basic conditions.[18] Protection of the resulting diastereomeric mixture of cyanohydrins (**16**) with acetone dimethylacetal subsequently yielded the cyanohydrin 1,3-acetonides (**17**) as a 1 : 1 mixture of *syn*- and *anti*-isomers[19] in 84% yield from (**15**). It is our experience that the use of this acetone cyanohydrin-based transformation is preferrable over the route described in the original literature,[16] since it circumvents the elaborate procedures associated with the use of trimethylsilyl cyanide as an (expensive) reagent for cyanohydrin formation and can easily be applied on a multi-gram scale.

Scheme 6.2

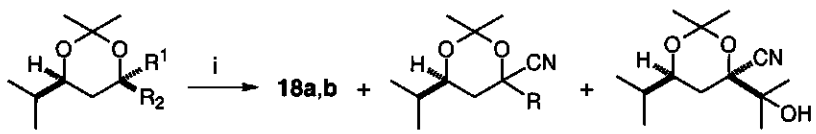


Reagents and conditions: (i) 1) $LiCH_2CO_2Et$, THF, -78°C; 2) TMSCl (71%); (ii) 1) DibalH, toluene, -78°C; 2) Glauber's salt (93%); (iii) Et_3N (cat.), $Me_2C(OH)CN$ (98%); (iv) $TsOH \bullet H_2O$ (cat.), $Me_2C(OMe)_2$.(86%).

6.3.2 - *Difficulties In the Alkylation of the Cyanohydrin 1,3-Acetonides*

Our first attempts to alkylate the cyanohydrin 1,3-acetonides (**17**) with bromoacetaldehyde dimethylacetal as the electrophile did not yield satisfactory results. We therefore decided to perform some experiments (Scheme 6.3) with the cyanohydrin 1,3-acetonides (**18**)[16a,b] to study the conditions required for alkylation of (**18**) and to compare our findings with those reported in the literature. The results of these experiments are summarized in Table 6.1.

Scheme 6.3



18a: R^1=CN, R^2=H (*anti*)[a] **19**[b] **20**
18b: R^1=H, R^2=CN (*syn*)[a]

Reagents and conditions: (i) 1) base, THF, -78°C; 2) excess electrophile RBr, -78°C to 0°C or r.t. (See Tables 6.1a,b for details). (a) Racemic. (b) Stereochemistry not determined.

To our surprise, we were unable to reproduce some of the results reported in the literature. Rychnovsky *et al* have reported the alkylation of (**18**) with 1-bromo pentane and 1.1 equiv. of LDA to give *anti*-(**19**) in a yield of 90%.[16a,b] In our hands (entry 1) this reaction performed poorly on several occasions, with results variing from 0-58% yield of (**19**). Attempts with the highly reactive allyl bromide also yielded only partial alkylation (entry 5). With the rather unreactive bromoacetaldehyde dimethylacetal we initially obtained yields of 10-26% of alkylated product, but lateron we were unable to reproduce these results (entry 8). The use of other strong bases[20] (entries 2-4,6,9) or the addition of HMPA as co-solvent[16d,f] (not shown) did not have benificial effects.

Usually, only limited quantities of starting material could be recovered from the product mixture, while the majority of this material had disappeared or had been transformed into several side-products. One of the major side products could be identified as the acetone adduct (**20**),[21] suggesting that the starting material (**18**) acts as a source for acetone, which subsequently adds to another molecule of (**18**). The formation of acetone could be imagined as a β-elimination type reaction via several pathways, as tentatively outlined in Scheme 6.4. Further reactions of the unstable intermediates (**21**) and (**22**) could possibly account for the observed loss of starting material.

Table 6.1: Summary of the results of a series of alkylation attempts with the model compound (**18**) using different combinations of bases and electrophiles (See text for discussion).

A Yields of alkylated product (**19**) under various reaction conditions.

entry	electrophile	base ‡	(**19**, %) [a]	n [b]
1	n-$C_5H_{11}Br$	LDA (1.2 eq)	0, 19, 58	3
2		LDEA (1.3 eq)	0, 40	2
3		LHMDS (1.2 eq)	12 [c], 17 [c]	2
4		KHMDS (1.2 eq)	14 [c]	1
5	$H_2C{=}CHCH_2Br$	LDEA (1.3 eq)	30, 35	2
6		LHMDS (1.5 eq)	25 [c,d]	1
7		LHMDS (4.0 eq)	75 - 82	4
8	$(MeO)_2CHCH_2Br$	LDA (1.3 eq)	0 - 26 [e]	5
9		LDEA (1.3 eq)	0	2
10		LHMDS (4.0 eq)	0	1

General reaction conditions: 1) base, THF, -78°C; 2) excess electrophile, -78°C to 0°C or r.t. (a) Isolated yields unless indicated otherwise; (b) Number of replicate experiments; (c) Determined by glc-analysis of a small sample with n-decane as internal standard; (d) Constant amount of (**19**) in the reaction mixture after 5 and 30 min at -78°C; (e) Accompanied by 4-19% of (**20**).

B Effect of different bases on the formation of the acetone adduct (**20**).

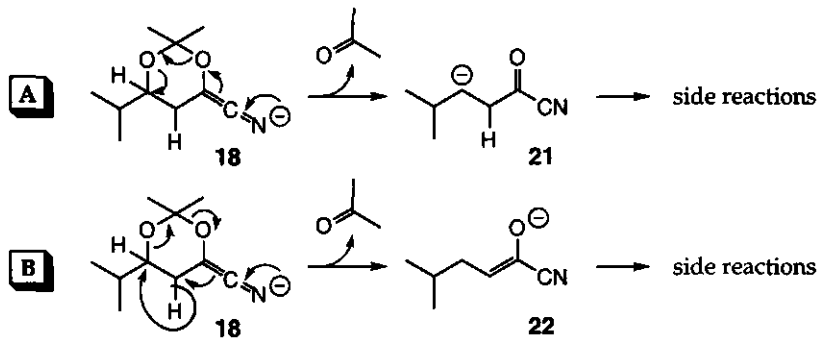
entry	without base [f]		base ‡	reaction mixture [f,g]		
	(**18a**, %)	(**18b**, %)		(**18a**, %)	(**18b**, %)	(**20**, %)
11	100	0	LDA (1.4 eq)	5	1.5	8
12	3	97	LDA (1.4 eq)	15	15	7
13	17	83	LDEA (1.3 eq)	8	11	9
14	100	0	LHMDS (1.3 eq)	42 [h]	35 [h]	<1 [h]
15	34	66	LHMDS (1.5 eq)	11	76	<1
16	3	97	LHMDS (4.0 eq)	11	78	3

Reaction conditions: 1) base, THF, -78°C, 30-40 min; 2) small sample quenched with water. (f) Composition determined via glc-analysis of an ether extract with n-decane as internal standard. (g) Refers to a single experiment per entry. (h) After 15 min with base at -78°C.

(‡) LDA = $LiN(i\text{-}Pr)_2$, LDEA = $LiNEt_2$, LHMDS = $LiN(SiMe_3)_2$, KHMDS = $KN(SiMe_3)_2$. LDA and LHMDS were both used as commercial solutions or were prepared *in situ* from n-BuLi and the corresponding amines; LDEA was prepared *in situ*; KHMDS was used as commercial solution in toluene.

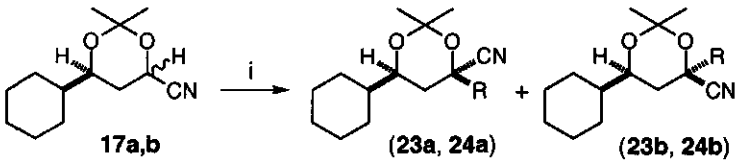
Although experiments with different bases initially did not lead to increased yields of alkylated product, on closer examination we observed that with LHMDS as base the loss of starting material was markedly reduced (entries 14-16). No significant amounts of acetone adduct (**20**) or other side-products were formed, so that in general the reaction mixtures appeared much cleaner, compared to attempts with LDA or LDEA (entries 11-13). Unfortunately, this increased stability of the anion-mixture was not translated directly into a higher yield of alkylated product. With 1.5 equiv. of LHMDS a mixture consisting of about 75% of starting material and 25% of allylated product (**19**) was formed (entry 6). Upon addition of an extra 2.5 equiv. LHMDS, however, the proportion of (**19**) slowly increased to 75%. Repeated experiments (entry 7) confirmed that the use of 3-4 equiv. of LHMDS was necessary to allow a reproducible alkylation of (**18**) with allyl bromide in about 3.5 hrs at -78 to -50°C, yielding (**19**, R=allyl) in 75-82% isolated yield.

Scheme 6.4



Although the reason for the initial difficulties with these alkylations was still poorly understood, we decided to apply our improved reaction conditions to the alkylation of the originally intended system (Scheme 6.5). Upon alkylation of a 1 : 1 mixture of the cyanohydrin 1,3-acetonides (**17a,b**) with bromoacetaldehyde dimethylacetal we obtained the alkylated product (**23**) in a yield of only 9% after chromatography, the remainder of the crude product being unchanged starting material. In preliminary experiments, similar products had been transformed into the desired 3a-hydroxy-perhydrofurofuran system,[22] but the low yield of the alkylation step was a serious limitation for further exploration of this reagent. However, in correspondance with the model experiments, the alkylation of (**17a,b**) with the more reactive allylbromide and 3-4 equivalents of LHMDS smoothly yielded the allylated products (**24a,b**) as a separable mixture of stereoisomers in a total yield of 86-91%.

Scheme 6.5



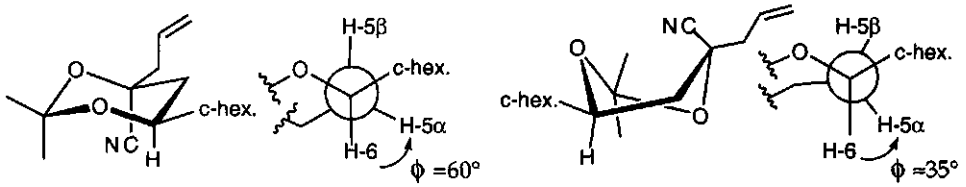
17a,b **(23a, 24a)** *anti*-isomer **(23b, 24b)** *syn*-isomer

R	isolated products (a+b, %)	*anti* : *syn*	recovered start. mat. (20, %)
23: $-CH_2CH(OMe)_2$	9	n.d.	80
24: $-CH_2CH=CH_2$	86-91	19 : 1 to 30 : 1	-

Reagents and conditions: (i) 1) 3-4 equiv. LHMDS, THF, -78°C, 45 min; 2) 3-5 equiv. RBr, -78°C to r.t., 18 hrs (**23**) or -78°C to -50°C, 3.5 hrs (**24**).

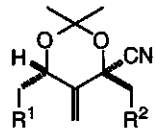
The configuration of the major isomer was established through analysis of the ^{13}C acetonide chemical shifts, according to a well-established method for discrimination between *syn*- and *anti*-1,3-diol acetonides.[23] The values of the acetonide methyl shifts of isomer (**24a**) (axial Me: δ 21.4 ppm, equatorial Me: δ 30.8 ppm) were indicative of the presence of a 1,3-diol acetonide moiety in a chair conformation with the largest substituents at C-4 and C-6 in an equatorial position.[24] This conformational assignment was supported by ^1H NMR data of (**24a**), which showed an axial-axial coupling of H-6 with H-5β (J=11.7 Hz), while H-6 was coupled to H-5α in an axial-equatorial fashion (J=2.1 Hz). Since the C-4 cyano-group is sterically much less demanding than the allyl substituent, it followed that in the above conformation the cyano group will occupy the axial position and the isomer (**24a**) thus had to possess an *anti* configuration (Figure 6.2). This stereochemical assignment is in correspondance with other C-4,6 substituted cyanohydrin 1,3-acetonides with *anti* configuration, which were reported to have identical ^{13}C NMR acetonide methyl shift values.[16] Also, the formation of an *anti*-adduct as the major product is in agreement with the favoured product configuration reported in the literature.[16]

Figure 6.2



anti-isomer **24a** *syn*-isomer **24b**

The analytical data of (**24b**) unambiguously showed that the minor product was a stereoisomer of (**24a**) and therefore this isomer was assigned a *syn* configuration. The expected twist conformation of this isomer (Figure 6.2) was confirmed by the magnitude of the ^1H coupling constants of H-6 with both H-5 protons (J=8.4 and 6.8 Hz, respectively). The twist conformation of (**24b**) is slightly distorted, probably due to the presence of the pseudo-axial cyano group at C-4, as became apparent from the pattern of ^{13}C acetonide shift values (δ 25.9, 28.3, 103.0 ppm) that partly deviated from the value ranges commonly observed for *anti*-1,3-diol acetonides with a perfect twist conformation.[24] Similar shift values have also been reported for a series of configurationally related cyanohydrin 1,3-acetonides of type (**25**), whose relative stereochemistry was independently established via NOE measurements.[25]



25: R^1 = Me or OTBDPS
R^2 = Ph or OBn

The formation of a mixture of *anti*- and *syn*-adducts (**24**) was an unexpected result, because generally this type of alkylation yields only products with the nitrile group in an axial position.[16,26] The only exception to this rule was reported for alkylation of the cyanohydrin 1,3-acetonides (**25**), for which the *syn*-product was found to be the preferred (or even the exclusive) isomer.[25a] No explanation was provided for this inversion of selectivity, which seems to be of an electronic nature since C-5 mono- or di-alkylated compounds are exclusively *trans*-alkylated.[16a,b] Under our modified reaction conditions, however, we found

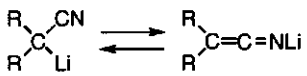
Table 6.2: Changes in the isomeric ratio[a] of both starting material and product during the alkylation of (**18**) with allylbromide (see text for discussion).

entry		reaction time (min)							
		0	30	60	90	105	180	210	overn.
1	**18a,b**	1 : 34 [b]	1 : 8	1 : 23		1 : 21			-
	19a,b	-	-	29 : 1		20 : 1			9 : 1 [c]
2	**18a,b**	1 : 8	1 : 7		1 : 26		1 : 11	-	
	19a,b	-	-		32 : 1		19 : 1	17 : 1 [c]	

Reaction conditions: 1) LHMDS (4.0 eq), THF, -78°C, 45-50 min; 2) allylBr (7.0 eq), -78°C to r.t., overnight (entry 1) or -78°C to -50°C, 3.5 hrs (entry 2). (a) Determined by glc-analysis of a small sample, quenched with satd. NH_4Cl-soln. and extracted with ether. (b) Isomeric ratio given as *anti* : *syn*. (c) 80-82% Yield of (**19a,b**, R=allyl) after work-up and chromatography.

a markedly reduced selectivity of alkylation, not only with (**24**), but also upon allylation of (**18**), which yielded (**19**, R=allyl) in a ratio of 9 : 1 to 17 : 1, depending on the temperature (Table 6.2). Analysis of samples taken during the timecourse of experiments with (**18**) showed that immediately after allylbromide addition the product ratio of (**19**) was about 30 : 1; this value gradually diminished as the allylation progressed. Remarkably, the isomer ratio of the products was always opposite to that of the starting material, regenerated upon quenching, for which the major isomer was *syn*.[27]

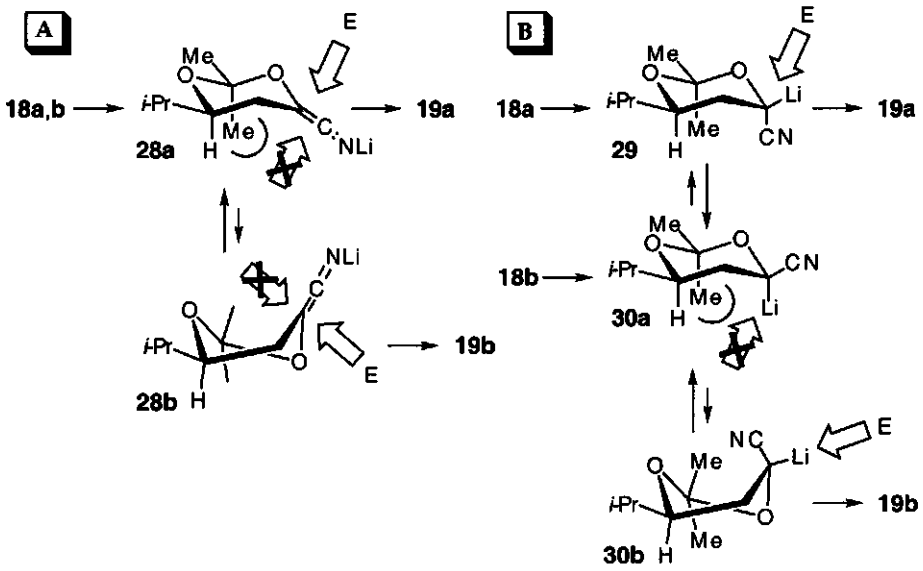
In order to understand these observations the structure of the lithiated anions of (**18a,b**) and the steric and conformational aspects governing their alkylation must be taken into account. The structure of nitrile-stabilized carbanions is usually represented by two tautomeric structures: an α-metallated nitrile with a regular sp3-hybridized α-carbon atom (**26**) and an enimine-like structure with the negative charge on the nitrogen atom and sp2-hybridization of the former α-carbon atom (**27**).[15b] However, the actual structure of a specific carbanion may divert from this uniform representation, for instance depending on the nature of the compound itself, on the particular base used or on the solvent (both the choice of solvent and base counterion may influence the aggregation state of the carbanion). Therefore, both tautomeric structures have to be considered in an attempt to rationalize the observed stereoselectivities.



26 **27**

The ^{13}C acetonide shift values of (**18a,b**) indicate that both isomers of the starting material have a chair conformation and in the 'enimine' model (**27**) both isomers will therefore yield the same structure (**28a**) upon deprotonation (Scheme 6.6A). The preferential formation of alkylated products with *anti*-configuration can easily be understood in this model: product formation occurs through β-face attack of the electrophile on the chair conformation (**28a**), affording the *anti*-adduct (**19a**). An α-face attack is blocked in this conformer, due to the presence of the axial methyl group at the 2-position, and the *syn*-adduct (**19b**) can therefore only be formed via the twist conformation (**28b**), which is energetically less favourable and thus less abundantly present in the reaction mixture. However, this model cannot explain the isomer ratio found for the regenerated starting material (**18**), since in view of the small size of a proton both faces of (**28**) should be equally accessible and an equimolar ratio of (**18a**) and (**18b**) would be expected.[28]

Scheme 6.6



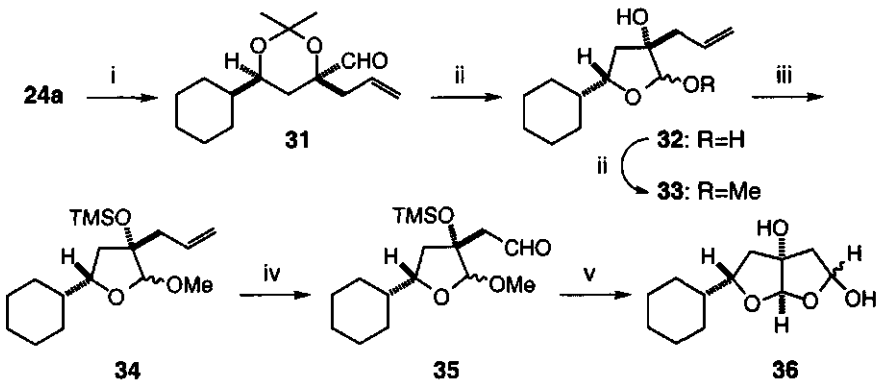
On the other hand, in the 'metallated nitrile' model (Scheme 6.6B) both faces of the carbanion are not identical towards proton addition and protonation of (**29**) therefore should yield only (**18a**). In effect, the isomer ratio of (**18**) found after quenching would be expected to reflect the actual ratio (**29**) : (**30**) present in the reaction mixture. As can be seen from Tables 6.1b (entries 14-16) and 6.2, this ratio is independent of the isomer ratio of the original starting material and instead appears to change into some equilibrium ratio with (**30**) as the major isomer.[29] However, as before, α-face alkylation of the thermodynamically preferred chair conformation (**30a**) of this isomer is blocked by the axial acetonide methyl group. The *syn*-adduct (**19b**) can only be formed from the less favourable twist-conformation (**30b**) and substantial amounts of (**19b**) are therefore only found after prolonged reaction time or at elevated temperatures. Due to the presence of the cyano group, isomerization of the lithium atom to the equatorial position apparently occurs more easily[30] and this gives room to rapid β-face alkylation and formation of the major adduct (**19a**).[31] In this way both the initially higher *anti*-selectivity and the initial increase of the isomer ratio of the starting material upon addition of allyl bromide can be rationalized.

Neither of these two models, however, provides much insight into the reason why our results deviate from those in the literature with respect to the amount of base needed and to the selectivity obtained. However, since our modified approach provided easy access to the required allylated cyanohydrin 1,3-acetonide (**24a**), this problem was of little practical significance and did not seriously interfere with our further synthetic plans.

6.2.3 - Preparation of the 3a-Hydroxy-Perhydrofuro[2,3b]furan Ring System

Two different strategies have been pursued to transform the *anti*-alkylated cyanohydrin 1,3-acetonide (**24a**) into a 3a-hydroxy-perhydrofuro[2,3b]furan ring system. In the first method (Scheme 6.7), the cyanohydrin was reduced with $LiAlH(OEt)_3$ to afford, after treatment of the iminic intermediate with silicagel,[32] the corresponding aldehyde (**31**) in 81% yield. This particular reducing reagent[33] was chosen because (**24a**) proved to be unreactive towards DibalH, as was also observed with other protected cyanohydrins (see Chapter 7). Furthermore, since the aluminate complex initially formed in the reduction is sufficiently stable at 0°C to prevent overreduction to the alcohol, an excess of the reducing agent could be used to ensure complete transformation of the starting material. This eliminated the need for a difficult chromatographic removal of traces of the nitrile (**24a**) from the product.

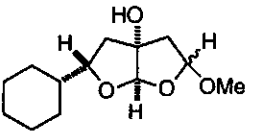
Scheme 6.7



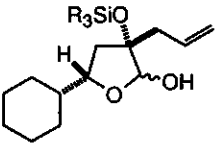
Reagents and conditions: (i) $LiAlH(OEt)_3$, ether, 0°C (81%); (ii) H^+, MeOH (**33**: 90%); (iii) TMSCl, imidazole, DMF (98%); (iv) 1) O_3, MeOH, -78°C; 2) PPh_3, r.t. (78%); (v) 4N HCl, THF, r.t., 4-10 days (69%).

Simultaneous acid-catalyzed deprotection and cyclization of (**31**) in methanol first gave rise to the formation of the diols (**32**) which, due to their highly polar nature, were difficult to characterize. Upon prolonged reaction, however, these intermediate products were smoothly transformed into a separable 1 : 1 mixture of C-2 epimers of the corresponding methyl ethers (**33**) in 90% yield from (**31**). Test experiments had indicated that the subsequent unmasking of the side-chain aldehyde group through ozonolysis of the allylic double bond in the presence of an unprotected hydroxyl group at C-3 suffered from low yields and limited reproducibility, due to the formation of several unidentified side-products. Therefore, the methyl ethers (**33**) were transformed

into their silylated analogues (**34**) prior to ozonolysis. The trimethylsilyl ether turned out to be the only suitable choice for this transformation, since larger silicon-based protective groups, such as the triethylsilyl group, could only be placed on the hydroxyl group of the 2αH-epimer of (**33**), the 2βH-epimer remaining unprotected. Subsequent ozonolysis of the double bond of (**34**) at -78°C in methanol, followed by oxidative work-up with triphenylphosphin, afforded the aldehydes (**35**) in 78% yield. Cyclization of these aldehydes in 4N hydrochloric acid and THF for 4 days yielded a mixture of the desired 3a-hydroxy-perhydrofuro[2,3b]furan-2-ols (**36**), together with their 2-methoxy derivatives (**37**) and some desilylated starting material. Separation and renewed treatment of the combined side-products with hydrochloric acid for another 10 days finally yielded (**36**) as a 1 : 1 mixture of C-2 epimers in a total yield of 69%.



37



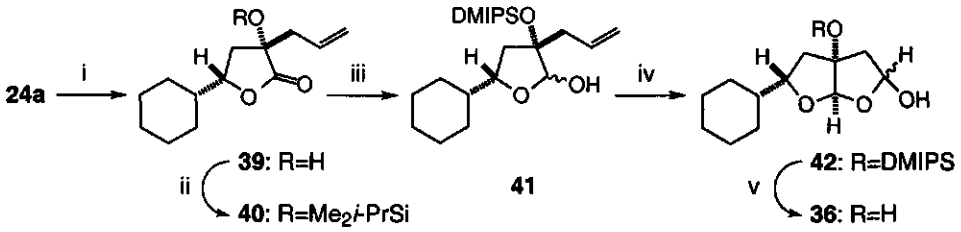
38

The tedious cyclization to the perhydrofurofuran ring system was a drawback in this approach and therefore another strategy was sought to avoid these problems. In their synthetic work towards furo[2,3b]pyran-based model compounds, Ley and coworkers routinely employed a different cyclization strategy in which the ozonolysis of an allylic double bond in the presence of an unprotected lactol functionality spontaneously effected the formation of the furo[2,3b]pyran skeleton.[34] To use this strategy in our system we required a lactol as (**38**) with the tertiary hydroxyl group protected as a silyl ether. This protection cannot be introduced into (**32**) itself due to the possibility of double silylation, nor at the stage of (**33**) since the subsequent hydrolysis of the methyl ether would probably experience problems similar to the cyclization of (**35**) and might also lead to loss of the protecting group. A different approach was therefore needed.

Preliminary experiments on the acid-catalyzed deprotection of the cyanohydrin 1,3-acetonides (**24**) had already indicated the potential of such systems to undergo an intramolecular Pinner reaction.[35] Upon optimization of the conditions it was found that (**24a**) could be converted in excellent yield into the corresponding 2-hydroxy-butyrolactone (**39**) by treatment with concentrated hydrochloric acid at elevated temperatures (Scheme 6.8). The hydroxyl group was protected in quantitative yield as its dimethyl-*iso*-propylsilyl ether; this protecting group was preferred over the trimethyl silyl group to minimize the risc of losing the protecting group during chromatographic purifications or of its accidental migration to the neighboring lactol hydroxyl group, to be created in the next step.

The lactone (**40**) was reduced with DibalH in toluene at -78°C to afford an 81% yield of the corresponding lactols (**41**). These were subsequently ozonolyzed according to the procedure described before, except that methanol was replaced by methylene chloride as the solvent to avoid the possible formation of any methoxy-acetals. Indeed, the intermediate lactol-aldehyde was found to cyclize smoothly under these conditions and gave the desired 3a-silyloxy-perhydrofuro[2,3b]furan-2-ols (**42**) in 82% yield. Finally, (**42**) was deprotected with an aqueous solution of hydrofluoric acid to afford in 86% yield a 1 : 1 mixture of C-2 epimeric perhydrofurofuran-2,3a-diols, which were identical in all respects to the diols (**36**) obtained previously.

Scheme 6.8



Reagents and conditions: (i) conc. HCl, MeOH, Δ (95%); (ii) DMIPSCl, imidazole, DMF (98%); (iii) DibalH, toluene, -78°C (81%); (iv) 1) O_3, CH_2Cl_2, -78°C; 2) PPh_3, r.t. (82%); (v) HF, MeCN-water (86%).

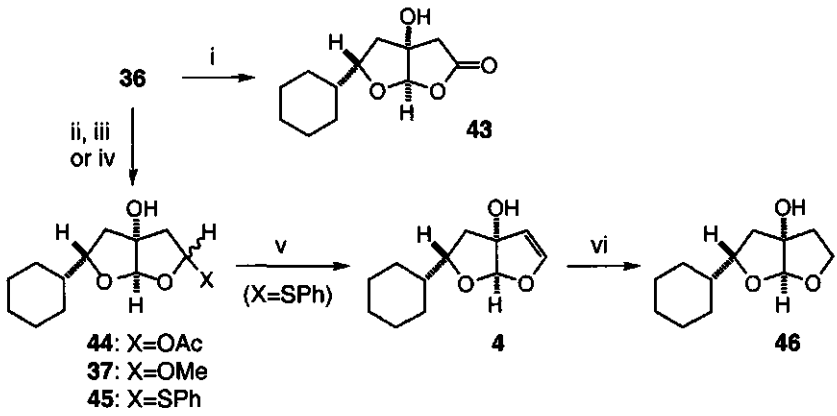
6.2.4 - Preparation of a Series of Test Compounds Through Derivatisation at C-2

With the synthesis of the 3a-*hydroxy*-perhydrofuro[2,3b]furan skeleton firmly established, we turned our attention to the preparation of a number of C-2 analogues for biological testing (Scheme 6.9). In order to allow evaluation of the influence of the 3a-hydroxyl group on the biological activity of these analogues, this series obviously had to contain the same C-2 functional groups as included in the 5-cyclohexyl-3a-*hydro*-perhydrofuro[2,3b]furan series described in the previous chapter.

Oxidation of the epimeric mixture of diols (**36**) with PDC gave a 75% yield of the 3a-hydroxy-perhydrofuro[2,3b]furanone (**43**) as a single product, thereby confirming that no partial epimerization at C-3a or C-5 had taken place during the transformation from (**24a**) to (**36**). Based on the stereochemistry established in the synthesis of (**24a**), the relative configuration of the 3a-hydroxy-perhydro-furo[2,3b]furan skeleton was assigned as (3aR*,5S*,6aS*), which is the same relative stereochemistry as found in the furo[2,3b]furan fragment present in the natural compound clerodin (**1**).

Acylation of a mixture of the diols (**36**) with acetylchloride and pyridine at 0°C yielded two inseparable acetates in 77% yield; the downfield shift of all H-2 protons in the ^1H spectrum of these epimers (**44**) indicated that under these conditions the hemiacetal functionality was acylated selectively, leaving the hydroxyl group at C-3a unaffected.

Scheme 6.9



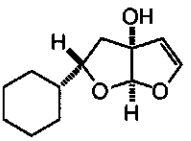
Reagents and conditions: (i) PDC (3.4 eq), CH_2Cl_2, 5 days (75%); (ii) AcCl (2 eq), pyridine (3 eq), CH_2Cl_2, 0°C, 1 hr (**44**, 77%); (iii) MeOH (2 eq), *p*-TsOH (cat.), THF, 48 hrs (**37**, 86%); (iv) PhSH (1.3 eq), $BF_3 \cdot Et_2O$ (cat.), 4Å mol. sieves, Et_2O, 0°C (**45**, 97%); (v) 1) mCPBA (1.1 eq), 4Å mol. sieves, toluene, 0°C, 10 min; 2) Et_3N (12 eq), Δ, 20 min (79%); (vi) H_2 (4 atm), Pd/C, EtOAc (73%).

The methyl ethers (**37**) could be obtained in 86% yield as a 1 : 1 mixture of C-2 epimers via acid-catalyzed acetalization of (**36**) with a moderate excess of methanol to avoid furo[2,3b]furan ring opening. A related reaction sequence was used for the preparation of the sulfides (**45**); contrary to the procedure used in the previous chapter, in this sequence only a catalytic amount of borontrifluoride etherate was employed, while the liberated water was absorbed by 4Å molecular sieves. The sulfides were obtained as a 4 : 1 mixture of epimers in 97% yield. Subsequent *in situ* oxidation of the mixture of sulfides (**45**) to the corresponding sulfoxides with mCPBA, followed immediately by its thermal elimination in the presence of triethylamine,[36] afforded the unsaturated tetrahydro-furo[2,3b]-furan-3a-ol (**4**) as a single isomer in 79% yield. Finally, the enol ether moiety was catalytically hydrogenated on palladium to give a 73% yield of the 3a-hydroxy-perhydrofuro[2,3b]furan (**46**).

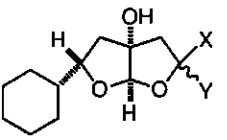
6.3 - Summary

In conclusion, we have developed a synthetic route to the 5-alkyl-3a-hydroxy-perhydrofuro[2,3b]furan ring system, using a modified version of the stereoselective alkylation of cyanohydrin 1,3-acetonides[16] as the key-step. Via this methodology, we have prepared a series of furo[2,3b]furan model compounds (Figure 6.3), derived from the natural insect antifeedant clerodin (**1**). The extra hydroxyl group on the C-3a bridgehead position of the ring system was introduced as a structural modification that may possibly increase the insect antifeedant potency of these model compounds.

Figure 6.3



(**4**)



(**46**): X,Y=H,H
(**36**): X,Y=H,OH
(**37**): X,Y=H,OMe
(**44**): X,Y=H,OAc
(**43**): X,Y=O

6.4 - Experimental Section

General experimental conditions were as described in the experimental section of Chapter 4.

Ethyl 3-cyclohexyl-3-(trimethylsilyloxy)-propanoate (14). To an ice-cold, stirred solution of 13 ml (61.6 mmol) of hexamethyl disilazane in 100 ml of anhydrous THF was added dropwise 39 ml (62.4 mmol) of a 1.6M solution of n-butyllithium in hexanes; stirring was continued at room temperature for 30 min. After cooling to -78°C, 5.1 ml (57.4 mmol) of anhydrous ethylacetate was added dropwise via syringe and the resulting solution was stirred for 45 min. Subsequently, the reaction mixture was treated dropwise with 6.0 ml (50 mmol) of cyclohexyl carbaldehyde, after 30 min followed by 9.0 ml (70.9 mmol) of chlorotrimethylsilane. Stirring was continued for 20 min at room temperature before the reaction mixture was poured into 50 ml of a saturated aqueous sodium bicarbonate solution and 150 ml of water. The organic layer was separated and the aqueous layer was extracted with three 150 ml-portions of petrol. The combined organic layers were washed with 100 ml of water and 100 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 16.1 g of a pale yellow oil. The crude product was distilled under reduced pressure in a bulb-to-bulb apparatus, yielding 9.67 g (35.6 mmol; 71%) of the ester (**14**) as a clear, colourless oil (b.p. 65°C at 0.7 mmHg), which was used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.09 (s, 9H): $Si(\underline{C}H_3)_3$; 0.8-1.45 (m, 9H): $OCH_2\underline{C}H_3$ [δ 1.26 (t, J=7.1 Hz)] and *c*-hexyl H-3'-H-5'; 1.55-1.85 (br m, 5H): *c*-hexyl H-1', H-2' and H-6'; 2.42 (d, J=7.3 Hz, 1H): H-2; 2.43 (d, J=5.3 Hz, 1H): H-2; 3.94 (ddd, J=7.3, 5.3, 5.0 Hz, 1H): H-3; 4.1 (q, J=7.1 Hz, 2H): $O\underline{C}H_2CH_3$. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 0.3 (q): $Si(CH_3)_3$; 14.2 (q): $OCH_2\underline{C}H_3$; 26.3 (3 t): *c*-hexyl C-3'-C-5'; 28.2 and 28.8 (2 t): *c*-hexyl C-2' and C-6'; 40.2 (t): C-2; 44.0 (d): *c*-hexyl C-1'; 60.2 (t): $O\underline{C}H_2CH_3$; 73.8 (d): C-3; 172.4 (s): C-1. MS: *m/e* (%) 257 (24), 243 (53), 229 (51), 197 (33), 188 (28), 145 (20), 110

(22), 103 (96), 95 (26), 83 (45), 75 (39), 73 (100), 71 (24), 67 (20), 57 (49), 55 (46), 41 (37). HRMS: calcd. (M-CH_3): *m/e* 257.1573; found: *m/e* 257.1574.

3-Cyclohexyl-3-(trimethylsilyloxy)-propanal (15). To a stirred solution of 8.5 g (31.2 mmol) of the ester (**14**) in 90 ml of anhydrous ether, cooled to -78°C, was added dropwise 21 ml (31.5 mmol) of a 1.5M solution of diisobutyl aluminumhydride in toluene. After stirring for 30 min the reaction was quenched by the addition of 10.05 g (31.2 mmol) of Glauber's salt. Stirring was continued at room temperature for 30 min before the reaction mixture was dried with $MgSO_4$ and filtered. The solvents were removed under reduced pressure to yield 6.6 g (28.2 mmol; 93%) of the aldehyde (**15**) as a pale yellow oil, which was used without further purification in the next reaction. A small sample (123 mg) was chromatographed on silicagel with petrol-EtOAc (98-2) as the eluent to yield, in order of elution, analytically pure (**15**) (74 mg) and the corresponding desilylated product (2-hydroxy-2-cyclohexylpropanal) which had been formed during chromatography.
(**15**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.0 (br s, 9H): $Si(CH_3)_3$; 0.6-1.4 (m, 6H): *c*-hexyl H-3'-H-5'; 1.4-1.8 (m, 5H): *c*-hexyl H-1', H-2' and H-6'; 2.2-2.55 (m, 2H): H-2; 3.9 (2 t, J=7.0 and 5.4 Hz, 1H): H-3; 9.7 (t, J=2.1 Hz, 1H): H-1. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 0.37 (q): $Si(CH_3)_3$; 26.2 (2) and 26.5 (3 t): *c*-hexyl C-3'-C-5'; 28.5 (2 t): *c*-hexyl C-2' and C-6'; 44.1 (d): *c*-hexyl C-1'; 48.4 (t): C-2; 72.3 (d): C-3; 202.7 (d): C-1.

4-Cyclohexyl-2-hydroxy-4-(trimethylsilyloxy)-butanenitrile (16). A mixture of 5.95 g (26.1 mmol) of the crude aldehyde (**15**), 3.5 ml (38.3 mmol) of acetone cyanohydrin and 0.2 ml (1.4 mmol) of triethylamine was stirred at room temperature for 45 min until tlc-analysis indicated complete transformation of the starting material. The reaction mixture was concentrated under reduced pressure to yield 6.49 g (25.5 mmol; 98%) of a 1 : 1 mixture of diastereomeric isomers of the cyanohydrin (**16**) as a yellow oil. The crude product was sufficiently pure to be used without further purification in the next reaction. A small sample (180 mg) was chromatographed on silicagel with petrol-EtOAc (10-1) as the eluent to afford, in order of elution, analytically pure (**16**) as a mixture of isomers (102 mg) and the corresponding desilylated diol, which had been formed during chromatography.
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.14 and 0.17 (2 s, 9H): $Si(CH_3)_3$; 0.75-1.35 (br m, 6H): *c*-hexyl H-3'-H-5'; 1.35-1.57 (br m, 1H): *c*-hexyl H-1'; 1.57-1.81 (br m, 5H): *c*-hexyl H-2' and H-6'; 1.81-2.10 (m, 2H): H-2; 3.67 (d, J=4.8 Hz, 0.5H): OH; 3.70-3.77 and 3.96-4.04 (2 m, 1H): H-3; 4.31 (d, J=7.6 Hz, 0.5H): OH; 4.54-4.68 (m, 1H): H-1. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 0.4 (q): $Si(CH_3)_3$; 26.3 (3), 26.9 and 27.3 (5 t): *c*-hexyl C-3'-C-5'; 29.0 (2), 35.7 and 37.6 (4 t): *c*-hexyl C-2' and C-6'; 43.7 and 43.9 (2 d): *c*-hexyl C-1'; 60.0 and 60.4 (2 d): C-1; 74.8 (2 d): C-3; 119.9 (2 s): CN.

(4R*, 6S*)-*Trans*- and (4S*,6S*)-*cis*-4-cyano-6-cyclohexyl-2,2-dimethyl-1,3-dioxane (17a,b). A mixture of 6.49 g (25.5 mmol) of the crude cyanohydrin (**16**), 5 ml (40.7 mmol) of 2,2-dimethoxypropane and 150 mg (0.8 mmol) of *p*-toluenesulfonic acid monohydrate in 25 ml of acetone was stirred at room temperature for 16 hrs until tlc-analysis indicated complete transformation of the starting material. The reaction mixture was diluted with 200 ml of ether and 80 ml of saturated aqueous sodium bicarbonate solution was added. The organic layer was separated and the aqueous layer was extracted with 100 ml of ether. The combined organic layers were washed with 100 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 5.32 g of a 1 : 1.1 mixture of both isomers as a yellow oil. Careful chromatography on 80 g of silicagel with petrol-EtOAc (98-2) as the eluent yielded, in order of elution, 630 mg (2.8 mmol; 11%) of the *anti*-isomer (**17a**) as a colourless oil, 3.74 g (16.8 mmol; 66%) of a mixture of isomers and 550 mg (2.5 mmol; 10%) of the *syn*-isomer (**17b**) as a white solid (m.p. 35.5-37°C).

Anti-isomer (**17a**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.8-1.45 (m, 9H): Me-1 [δ 1.35 (s, approx. 3H)] and *c*-hexyl H-3'-H-5'; 1.5-1.80 (m, 7H): Me-2 [δ 1.62 (s, approx. 3H)] and *c*-hexyl H-2', H-6'; 1.80-1.93 (m, 3H): H-5 and *c*-hexyl H-1' [approx. δ 1.9 (br d, J≈12.5 Hz)]; 3.81 (ddd, J=10.5, 6.9, 3.6 Hz, 1H): H-6; 4.83 (dd, J=5.8, 3.5 Hz, 1H): H-4. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 21.8 (q): Me-1; 25.7, 25.8, 26.4, 27.8 and 28.4 (5 t): *c*-hexyl C-2'-C-6'; 29.5 (q): Me-2; 31.0 (t): C-5; 42.1 (d): *c*-hexyl C-1'; 58.9 (d): C-4; 69.7 (d): C-6; 100.9 (s): C-2; 120.0 (s): CN. MS: *m/e* (%) 208 (78), 148 (86), 140 (48), 139 (100), 121 (78), 59 (48), 55 (19), 43 (55), 41 (26). HRMS: calcd. (M-CH_3): *m/e* 208.1338; found: *m/e* 208.1333. *Syn-isomer* (**17b**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-1.5 (m, 12H): Me [δ 1.4 (s, 6H)] and *c*-hexyl H-3'-H-5'; 1.5-1.95 (m, 7H): H-5 and *c*-hexyl H-1' [approx. δ 1.86 (br d, J≈10.1 Hz)], H-2', H-6'; 3.52 (dd, J=14.1, 6.7 Hz) and (ddd, J=10.0, 6.4, 4.0 Hz; total 1H): H-6 (two conformers); 4.71 (dd, J=10.5, 4.5 Hz) and (dd, J=8.2, 6.8 Hz; total 1H): H-4 (two conformers). ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 19.1 (q): Me-1; 25.7, 25.8 and 26.4 (3 t): *c*-hexyl C-3'-C-5'; 27.8 and 28.4 (2 t): *c*-hexyl C-2' and C-6'; 29.6 (q): Me-2; 31.9 (t): C-5; 42.3 (d): *c*-hexyl C-1'; 59.4 (d): C-4; 72.1 (d): C-6; 99.9 (s): C-2; 118.1 (s): CN. MS: *m/e* (%) 208 (50), 148 (37), 140 (100), 139 (40), 121 (20), 95 (13), 59 (42), 55 (12), 43 (36), 41 (12). HRMS: calcd. (M-CH_3): *m/e* 208.1338; found: *m/e* 208.1335. Anal: calcd. for $C_{13}H_{21}NO_2$: C, 69.92; H, 9.48; N,6.27; found: C, 70.05; H, 9.78; N, 6.21.

(4S*,6S*)- *Trans*- 4-cyano- 2,2-dimethyl- 4-[(1-hydroxy-1-methyl)-ethyl]- 6-*iso*-propyl- 1,3-dioxane (20). ^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.91 and 0.95 (2 d, J=7.5 Hz, 6H): $CH(C\underline{H}_3)_2$; δ 1.28 (s, 3H): Me-1; 1.38 and 1.40 (2 s, 6H): $C(OH)(C\underline{H}_3)_2$; 1.60-1.75 (m, 6H): H-5, Me-2 [δ 1.68 (s, 3H)] and $C\underline{H}(CH_3)_2$; 2.24 (br s, 1H): OH; 3.79 (ddd, J=9.5, 6.7, 4.4 Hz, 1H): H-6. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 17.7 and 18.0 (2 q): $CH(\underline{C}H_3)_2$; 21.4 (q): ax. Me; 22.7 and 24.3 (2 q): $C(OH)(\underline{C}H_3)_2$; 30.3 (t): C-5; 30.7 (q): eq. Me; 32.7 (d): $\underline{C}H(CH_3)_2$; 70.9 (d): C-6; 74.2 and 75.8 (2 s): C-4 and $\underline{C}(OH)(CH_3)_2$; 101.3 (s): C-2; 121.3 (s): CN. IR ($CHCl_3$): ν 3550 (br: OH); 2350 (w: CN) cm^{-1}. GC/MS: *m/e* (%) 226 (8: M-CH_3), 183 (5), 140 (6), 139 (8), 125 (9), 124 (10), 110 (20), 108 (10), 98 (23), 97 (6), 70 (40), 69 (42), 59 (100), 55 (22), 43 (71), 41 (34).

(4S*,6S*)-*Trans*- and (4R*,6S*)-*cis*-4-cyano-6-cyclohexyl-2,2-dimethyl-4-(prop-2-enyl)-1,3-dioxane (24a,b). A stirred solution of 6.1 ml (28.9 mmol) of hexamethyldisilazane in 50 ml of anhydrous THF was treated with 20 ml (32 mmol) of a 1.6M solution of *n*-butyllithium in hexanes. After 45 min the solution was cooled to -78°C and a solution of 2.15 g (9.6 mmol) of the acetonide (**17a,b**) as a mixture of *syn*- and *anti*-isomers in 10 ml of THF was added dropwise. Stirring was continued for 1 hr before 4.5 ml (52 mmol) of neat allylbromide was added. The temperature of the reaction mixture was slowly raised to about -50°C, while the progress of the reaction was monitored via glc-analysis. After 3.5 hrs the reaction was quenched by the addition of 200 ml of water. The organic layer was separated and the aqueous layer was extracted with three 100 ml-portions of petrol. The combined organic layers were washed with 80 ml of water and 80 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 3.31 g of a 30 : 1 mixture of the major *anti*-isomer (**24a**) and the minor *syn*-isomer (**24b**) as a yellow oil. The crude product was purified via chromatography on 60 g of silicagel with petrol-EtOAc (99.5-0.5 to 99-1) as the eluent, affording in order of elution 1.48 g (5.6 mmol; 58%) of the desired *anti*-isomer (**24a**) as an oil and 0.7 g (2.7 mmol; c.y. 28%) of a mixture of both isomers (**24a** : **24b** ≈ 9 : 1). Several repeated chromatographic purification steps of the isomer-mixture were required to finally obtain a small quantity of analytically pure minor isomer (**24b**).
Anti-isomer (**24a**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.8-1.4 (m, 9H): Me-1 [δ 1.36 (s, 3H)] and *c*-hexyl H-3"-H-5"; 1.44 (dd, J=13.5, 11.7 Hz, 1H): H-5β; 1.55-1.82 (m, 8H): H-5α [δ 1.78 (dd, J=13.0, 2.1 Hz, 1H)], Me-2 [δ 1.65 (s, 3H)] and *c*-hexyl H-2", H-6"; 1.90 (br d,

J=12.6 Hz, 1H): *c*-hexyl H-1"; 2.35-2.65 (m, 2H): allyl H-1'; 3.81 (ddd, J=11.6, 6.9, 2.0 Hz, 1H): H-6; 5.16-5.26 (m, 2H): allyl H-3'(*E*) [δ 5.22 (dm, J=16.6 Hz)], H-3'(*Z*) [δ 5.24 (dm, J=9.9 Hz)]; 5.77-5.91 (m, 1H): allyl H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 21.4 (q): ax. Me; 25.7, 25.9, 26.4, 27.9 and 28.5 (5 t): *c*-hexyl C-2"-C-6"; 30.8 (q): eq. Me; 36.1 (t): C-5; 42.1 (d): *c*-hexyl C-1"; 46.6 (t): allyl C-1'; 69.7 (s): C-4; 70.4 (d): C-6; 100.9 (s): C-2; 120.7 (t): allyl C-3'; 121.8 (s): CN; 130.0 (d): allyl C-2'. MS: *m/e* (%) 248 (100), 188 (83), 179 (75), 161 (13), 137 (11), 95 (69), 81 (11), 67 (15), 59 (41), 55 (18), 43 (34). HRMS: calcd. (M-CH_3): *m/e* 248.1651; found: *m/e* 248.1651. *Syn-isomer* (**24b**): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.80-1.81 (br m, 16H): Me-1 [δ 1.34 (s, 3H)], Me-2 [δ 1.57 (s, 3H)] and *c*-hexyl H-2"-H-6"; 1.85-2.09 (m, 3H): H-5a [δ 1.90 (dd, J=13.8, 6.8 Hz)], H-5b [δ 2.03 (dd, J=13.8, 8.4 Hz)] and *c*-hexyl H-1" [δ 1.88-2.08 (br)]; 2.39-2.62 (m, 2H): allyl H-1'; 3.54 (ddd, J=8.4, 7.6, 6.8 Hz, 1H): H-6; 5.16-5.26 (m, 2H): allyl H-3'(*E*) [δ 5.22 (dm, J=17.9 Hz)], H-3'(*Z*) [δ 5.24 (dm, J=10.0 Hz)]; 5.74-5.95 (m, 1H): allyl H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 25,9 (q): Me-1; 26.7 (2 t); 27.4 (t); 28.3 (q): Me-2; 28.8 (t); 29.5 (t); 38.4 (t): C-5; 43.3 (d): *c*-hexyl C-1"; 46.5 (t): allyl C-1'; 68.3 (s): C-4; 70.5 (d): C-6; 103.0 (s): C-2; 121.6 (t): allyl C-3'; 123.0 (s): CN; 131.3 (d): allyl C-2'. MS: *m/e* (%) 248 (94), 188 (96), 179 (59), 161 (16), 137 (15), 122 (37), 95 (89), 81 (19), 67 (24), 59 (100), 55 (18), 43 (48). HRMS: calcd. (M-CH_3): *m/e* 248.1651; found: *m/e* 248.1652.

(4S*,6S*)-*Trans*- 6-cyclohexyl- 2,2-dimethyl- 4-(prop-2-enyl)- 1,3-dioxane- 4-carbaldehyde (31). To a vigorously stirred solution of 13.5 mmol (2.6 eq) of lithium aluminumhydride in 50 ml of ether (prepared by diluting 13.5 ml of a commercial 1.0M etheral solution of $LiAlH_4$ with anhydrous ether) was added dropwise 1.6 ml (16.4 mmol; 3.2 eq) of anhydrous EtOAc while cooling on an ice-bath. After 15 min the resulting white slurry was treated dropwise with a solution of 1.37 g (5.2 mmol) of the *anti*-adduct (**24a**) in 15 ml of ether. Stirring at 0°C was continued for 1.5 hrs while the reaction was monitored via glc-analysis. The reaction was quenched by addition of 4.2 g (13 mmol) of Glauber's salt. The mixture was warmed to room temperature and stirred for 30 min, dried with $MgSO_4$, filtered and the solvent was evaporated. The residual oil was dissolved in 100 ml of EtOAc, 20 g of silicagel was added and the resulting slurry was stirred overnight. The slurry was filtered and the filtrate was concentrated under reduced pressure to give 1.34 g of a yellow oil. Chromatography on 25 g of silicagel with petrol-EtOAc (99-1) as the eluent afforded 1.12 g (4.2 mmol; 81%) of the aldehyde (**31**) as a colourless oil, which was used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.40-2.35 (br, 24H): H-5α [δ 1.90 (dd, J≈13, 3 Hz)], H-5β, Me-1 [δ 1.20 (s, approx. 3H)], Me-2 [δ 1.32 (s, approx. 3H)], *c*-hexyl H-1" [δ 2.10 (dm, J≈7 Hz)] and *c*-hexyl H-2" - H-6"; 3.20 (ddd, J≈12, 6, 3 Hz, 1H): H-6; 4.75-5.10 (m, 2H): allyl H-3'(*E*) [δ 4.90 (dm, J≈18 Hz)] and allyl H-3'(*Z*) [δ 4.97 (dm, J≈9 Hz)]; 5.30-5.75 (m, 1H): allyl H-2'; 9.50 (d, J≈2 Hz, 1H): CHO. IR ($CHCl_3$): ν 1730 cm^{-1}.

((2R*,3R*,5R*)- And (2S*,3R*,5R*)- 5-cyclohexyl- 3-hydroxy- 2-methoxy- 3-(prop-2-enyl)- tetrahydrofuran (33). A mixture of 250 mg (0.94 mmol) of the aldehyde (**31**) and 30 mg (0.15 mmol) of *p*-toluenesulfonic acid monohydrate in 10 ml of methanol was stirred overnight at room temperature, after which tlc-analysis indicated complete transformation of the starting material into a highly polar product. The solvent was removed under reduced pressure and the residue was dissolved in 75 ml of ether. Saturated aqueous sodium bicarbonate solution (20 ml) was added, the organic layer was separated and the aqueous layer was extracted with 25 ml of ether. The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure, yielding 206 mg (0.91 mmol) of the crude diol (**32**). Due to the highly polar nature of this diol, no attempt at purification was undertaken.

Crude product (**32**): ^{1}H NMR ($CDCl_3$, 90 MHz, selected peaks): δ 0.8-2.2 (br m, 17H); 2.4 (d, J=7.5 Hz, 2H): allyl H-1'; 2.55 (s, 1H): OH-3; 3.25 (d, J=6 Hz, 1H): OH-2; 3.5-3.8 (m, 1H): H-5; 4.9 (d, J=6 Hz, 1H): H-2; 5.1 (m, 1H): allyl H-3'(*E*); 5.25 (s, 1H): allyl H-3'(*Z*); 5.7-6.1 (m, 1H): allyl H-2'.
The crude diol (**32**) was dissolved in 15 ml of methanol and 25 mg (0.13 mmol) of *p*-toluenesulfonic acid monohydrate was added.[37] The mixture was stirred at room temperature while the progress of the reaction was monitored via tlc-analysis; after 2 days the reaction appeared complete. Work-up as described above yielded 204 mg (0.85 mmol; 90%) of a 1 : 1.4 mixture of C-2 epimers of (**33**) as a clear, colourless oil, which was sufficiently pure to be used without further purification in the next reaction. The isomers could be separated via chromatography on silicagel with petrol-EtOAc (95-5) as the eluent to give, in order of elution, the minor 2αH-isomer as a white solid (mp 84-85°C) and the major 2βH-isomer as an oil.
Minor 2αH-isomer: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.7-1.4 (br m, 6H): *c*-hexyl H-3"-H-5"; 1.4-1.85 (br m, 5H): *c*-hexyl H-1", H-2", H-6"; 1.98-2.07 (m, 2H): H-4; 2.3 (d, J=7.1 Hz, 2H): allyl H-1', 2.7 (s, 1H): OH; 3.4 (s, 3H): OCH_3; 3.56-3.68 (m, 1H): H-5; 4.4 (s, 1H): H-2; 5.08 (d, J=5.7 Hz, 1H): allyl H-3'(*E*); 5.15 (s, 1H): allyl H-3'(*Z*); 5.8-6.1 (m, 1H): allyl H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 25.8 (2 t) and 26.5 (t): *c*-hexyl C-3"-C-5"; 28.6 and 29.7 (2 t): *c*-hexyl C-2", C-6"; 39.7 and 41.0 (2 t): C-4; 44.9 (d): *c*-hexyl C-1"; 54.6 (q): OCH_3; 79.7 (s): C-3; 82.6 (d): C-5; 104.4 (d): C-2; 118.4 (t): allyl C-3'; 133.2 (d): allyl C-2'. MS: *m/e* (%) 180 (13), 167 (13), 139 (100), 121 (51), 97 (57), 95 (19), 85 (22), 83 (17), 81 (14), 67 (13), 55 (74), 41 (19). HRMS: calcd. (M-OCH_3): *m/e* 209.1542; found: *m/e* 209.1538. Anal: calcd. for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07; found: C, 70.03; H, 10.34. *Major 2βH-isomer*: ^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.8-1.35 (br m, 6H): *c*-hexyl H-3"-H-5"; 1.55-1.8 (br m, 5H): *c*-hexyl H-1", H-2", H-6"; 1.9 (br s, 1H): OH; 1.96-2.07 (m, 2H): H-4; 2.3-2.55 (m, 2H): allyl H-1"; 3.3 (s, 3H): OCH_3; 3.73-3.84 (m, 1H): H-5; 4.5 (s, 1H): H-2; 5.11 (s, 1H): allyl H-3'a; 5.18 (d, J=5.7 Hz, 1H): allyl H-3'b; 5.75-6.0 (m, 1H): allyl H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 25.8 (2 t) and 26.5 (t): *c*-hexyl C-3"-C-5"; 29.0 and 29.5 (2 t): *c*-hexyl C-2", C-6"; 39.6 and 39.9 (2 t): C-4; 43.0 (d): *c*-hexyl C-1", 54.3 (q): OCH_3; 81.4 (s): C-3; 81.6 (d): C-5; 108.7 (d): C-2; 118.9 (t): allyl C-3'; 134.0 (d): allyl C-2'. MS: *m/e* (%) 157 (33), 139 (100), 125 (16), 121 (47), 97 (57), 95 (18), 85 (37), 67 (18), 55 (77), 41 (26). HRMS: calcd. (M-OCH_3): *m/e* 209.1542; found: *m/e* 209.1539.

(2R*,3R*,5R*)- And (2S*,3R*,5R*)-5-cyclohexyl-2-methoxy-3-(trimethylsilyloxy)-3-(prop-2-enyl)-tetrahydrofuran (**34**). A solution containing 522 mg (2.2 mmol) of a 1 : 1 mixture of the epimeric methyl ethers (**33**), 0.7 ml (5.5 mmol) of trimethylsilyl chloride and 739 mg (11.0 mmol) of imidazole in 10 ml of anhydrous DMF was stirred at room temperature for 40 min. The reaction mixture was poured into 40 ml of half-saturated aqueous sodium bicarbonate solution and the resulting mixture was extracted with three 50 ml-portions of petrol. The combined organic layers were washed with 30 ml of water and 30 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure, to yield 667 mg (2.1 mmol; 98%) of a 1 : 1 mixture of C-2 epimers of (**34**) as a clear, colourless oil. The product was sufficiently pure to be used in the next reaction without further purification.
^{1}H NMR ($CDCl_3$, 200 MHz): δ -0.03 and 0.0 (2 s, 9H): $Si(CH_3)_3$; 1.7-0.6 (br m, 12H): *c*-hexyl H-1"-H-6"; 1.75-2.0 (br m, 2H): H-4; 2.14 (m, 1H): allyl H-1' epimer 1; 2.29 (m, 1H): allyl H-1' epimer 2; 3.1 (s, 3H): OCH_3; 3.2 and 3.57 (2 ddd, 1H): H-5; 4.21 and 4.53 (2 s, 1H): H-2; 4.9 (s) and 5.0 (m, total 2H): allyl H-3'; 5.5-5.9 (m, 1H): allyl H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.2 and 2.4 (2 q): $Si(CH_3)_3$; 43.0 and 45.2 (2 d): *c*-hexyl C-1"; 54.0 and 54.6 (2 q): OCH_3; 81.9 and 82.1 (2 d): C-5; 82.5 and 85.0 (2 s): C-3; 104.9 and 109.0 (2 d): C-2; 117.2 and 118.1 (2 t): allyl C-1'; 133.8 and 135.0 (2 d): allyl C-2'. MS: *m/e*

(%) 271 (14), 251 (9), 211 (13), 170 (16), 169 (100), 156 (10), 155 (21), 122 (37), 121 (19), 89 (10), 75 (11), 73 (58). HRMS: calcd. (M-OCH_3): *m/e* 281.1937; found: *m/e* 281.1940.

(2R*, 3S*, 5R*)- And (2S*, 3S*, 5R*)-5-cyclohexyl-2-methoxy-3-methylenecarbaldehyde-3-(trimethylsilyloxy)-tetrahydrofuran (35). A stirred solution of 637 mg (2.0 mmol) of a 1 : 1 epimeric mixture of the silylated methoxy acetals (**34**) in 30 ml of methanol and 10 ml of dichloromethane was cooled under nitrogen atmosphere on an dry-ice/acetone bath. An ozone/oxygen mixture was passed through the solution until a bright blue colour appeared and tlc-analysis indicated complete disappearance of the starting material. The solution was purged with nitrogen until the blue colour disappeared and then warmed to room temperature. Subsequently, 588 mg (2.2 mmol) of triphenylphosphin was added and the reaction mixture was stirred for 1 hr at ambient temperature. The solvent was removed under reduced pressure and the partly crystalline residue (1.26 gr) was purified by chromatography on 20 g of silicagel with petrol-EtOAc (99-1 to 95-5) as the eluent, yielding 498 mg (1.6 mmol; 78%) of a 1.2 : 1 mixture of C-2 epimers of the aldehyde (**35**) as a clear, colourless oil.
Least polar isomer: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.0 (s, 9H): $Si(CH_3)_3$; 0.65-1.7 (br m, 11H): *c*-hexyl H-1"-H-6"; 1.75 and 1.95 (2 dd, J=12.9, 7.3 Hz, 3H): H-4a and H-4b; 2.49 (dd, J=16.0, 2.7 Hz) and 2.62 (dd, J=16.0, 2.5 Hz; total 2H): $C\underline{H}_2CHO$; 3.2 (s, 3H): OCH_3; 3.6 (ddd, J=7.5, 7.3, 7.3 Hz, 1H): H-5; 4.7 (s, 1H): H-2; 9.6 (dd, J=2.7, 2.5 Hz, 1H): CHO. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.2 (q): $Si(CH_3)_3$; 25.7, 25.9 and 26.5 (3 t): *c*-hexyl C-3"-C-5"; 29.0 and 29.7 (2 t): *c*-hexyl C-2", C-6"; 42.2 (t): C-4; 42.8 (d): *c*-hexyl C-1"; 50.3 (t): $\underline{C}H_2CHO$; 54.7 (q): OCH_3; 81.7 (d): C-5; 83.3 (s): C-3; 108.9 (d): C-2; 201.7 (d): CHO. MS: *m/e* (%) 172 (14), 171 (100), 157 (9), 143 (10), 75 (7), 73 (15), 55 (25). HRMS: calcd. (M-OCH_3): *m/e* 283.1729; found: *m/e* 283.1726. *Most polar isomer*: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.0 (s, 9H): $Si(CH_3)_3$; 0.6-1.7 (br m, 11H): *c*-hexyl H-1"-H-6"; 1.8-2.0 (m, 3H): H-4a and H-4b; 2.36 (dd, J=15.0, 3.1 Hz) and 2.48 (ddd, J=15.0, 2.7, 1.4 Hz; total 2H): $C\underline{H}_2CHO$; 3.2 (s, 3H): OCH_3; 3.46 (ddd, J=8.9, 8.9, 6.8 Hz, 1H): H-5; 4.3 (s, 1H): H-2; 9.7 (dd, J=3.1, 2.7 Hz, 1H): CHO). ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.2 (q): $Si(CH_3)_3$; 25.7, 25.8 and 26.5 (3 t): *c*-hexyl C-3"-C-5"; 28.6 and 29.7 (2 t): *c*-hexyl C-2", C-6"; 39.2 (t): C-4; 45.0 (d): *c*-hexyl C-1"; 51.3 (t): $\underline{C}H_2CHO$; 54.0 (q): OCH_3; 81.3 (s): C-3; 82.0 (d): C-5; 104.6 (d): C-2; 201.9 (d): CHO. MS: *m/e* (%) 172 (13), 171 (100), 73 (13), 55 (19). HRMS: calcd. (M-CH_3): *m/e* 299.1679; found: *m/e* 299.1676.

(2R*,3aS*,5R*) - And (2S*,3aS*,5R*)-5-cyclohexyl-perhydrofuro[2,3b]furan-2,3a-diol (36). From 5-cyclohexyl-3-TMSoxy-2-methoxy-tetrahydrofuran-3-methylenecarbaldehyde (35): A mixture of 498 mg (1.6 mmol) of the aldehyde (**35**) in 15 ml of THF and 4 ml of an aqueous 4N solution of hydrochloric acid was stirred at room temperature for 4 days. The reaction mixture was poured into 75 ml of EtOAc and washed sequentially with water (10 ml), saturated aqueous sodiumbicarbonate solution (15 ml) and brine (25 ml). The combined washings were re-extracted with EtOAc (50 ml) and the combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure to yield a white solid (275 mg). Separation of the desired product from several side-products was achieved by chromatography on 15 g of silicagel with petrol-EtOAc (75-25 to 66-33) as the eluent to give, in order of elution, 99 mg of a mixture containing both desilylated starting material and the 2-methoxy-furofuran-3a-ol (**37**), and 167 mg (0.73 mmol, 46%) of an C-2 epimeric mixture of the furofuran-2,3a-diol (**36**) as a white solid. The mixture of side-products was taken up in 10 ml of THF and 5 ml of a 1N aqueous hydrochloric acid solution and was stirred for 10 days. Work-up as described before yielded another 82 mg (0.36 mmol, 23%) of (**36**).
From 5-cyclohexyl-3a-DMIPSoxy-perhydrofuro[2,3b]furan-2-ol (42): A stirred solution of 2.08 g (6.45 mmol) of a mixture of furofuranols (**42**) in 50 ml of acetonitrile at room

temperature was treated dropwise with a 40% solution of hydrogen fluoride in water (20 drops ≈ 1 ml) until tlc-analysis indicated complete transformation of the starting material. The reaction mixture was poured into 150 ml of water and extracted with four 100 ml-portions of ether. The combined extracts were washed with 80 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to afford 1.5 g of a white solid. The product was purified via chromatography on 40 g of silicagel with petrol-EtOAc (75-25 to 66-33) as the eluent to afford 1.26 g (5.5 mmol; 86%) of a mixture of C-2 epimers of the diol (**36**) as a white solid.
(**36**; 7 : 3 mixture[38]): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-2.01 (br m, 12H) and 2.01-2.50 (br m, 3H): H-3, H-4 and *c*-hexyl H-1'-H-6'; 2.60 (br, 0.3H) and 3.33 (br, 0.7H): OH; 3.77 (ddd, J=11.3, 7.2, 4.3 Hz, 0.7H): H-5; 3.96 (br, 0.3H) and 4.09 (br, 0.7H): OH; 4.20 (ddd, J=10.1, 8.6, 5.5 Hz, 0.3H): H-5; 5.45 (s, 0.3H) and 5.54 (s, 0.7H): H-6a; 5.65 (br, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.7, 25.8 and 26.3 (3 t): *c*-hexyl C-3'-C-5'; 28.5 and 29.6 (2 t): *c*-hexyl C-2', C-6'; 42.1 (t): C-3; 42.5 and 42.9 (2 d): *c*-hexyl C-1'; 44.9 and 47.1 (2 t): C-4; 83.7 and 84.2 (2 d): C-5; 86.9 and 87.8 (2 s): C-3a; 98.8 and 99.4 (2 d): C-2; 112.7 and 113.5 (2 d): C-6a. MS: *m/e* (%) 127 (23), 109 (10), 99 (29), 95 (10), 83 (10), 81 (17), 73 (10), 70 (12), 67 (9), 61 (11), 55 (17), 45 (17), 43 (100), 41 (11). HRMS: calcd. (M-H_2O): *m/e* 210.1256; found: *m/e* 210.1253.

(2R*,4R*)-2-Allyl-4-cyclohexyl-2-hydroxy-γ-butyrolactone (39). A stirred mixture of 2.5 g (9.6 mmol) of the cyanohydrin 1,3-acetonide (**24a**) and 3 ml of conc. hydrochloric acid in 30 ml of methanol was heated under reflux for 2 hrs. After cooling to room temperature the mixture was diluted with 50 ml of water and extracted with three 100 ml-portions of ether. The combined extracts were washed with 100 ml of a saturated aqueous sodiumbicarbonate solution, dried with $MgSO_4$ and concentrated under reduced pressure to give 2.2 g of a yellow solid. The crude product was purified via chromatography on 50 g of silicagel with petrol-EtOAc (95-5 to 85-15) as the eluent, yielding 2.04 g (9.1 mmol; 95%) of the hydroxy-lactone (**39**) as a white solid (m.p. 67-68°C).
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.80-1.90 (br m, 10H): *c*-hexyl H-2"-H-6"; 1.90-2.15 (m, 2H): H-3α [δ 2.01 (dd, J=22.9, 10.7 Hz)] and *c*-hexyl H-1"; 2.31-2.57 (m, 3H): H-3β and allyl H-1'; 2.91-3.23 (br, 1H): OH; 4.03 (ddd, J=9.0, 7.8, 6.8 Hz, 1H): H-4; 5.18 (dd, J=9.4, 0.6 Hz, 1H): allyl H-3'(*E*); 5.25 (d, J=0.7 Hz, 1H): allyl H-3'(Z); 5.72-5.90 (m, 1H): allyl H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.4, 25.6 and 26.2 (3 t): *c*-hexyl C-3"-C-5"; 27.5 and 28.9 (2 t): *c*-hexyl C-2", C-6"; 38.3 and 41.8 (2 t): C-3 and allyl C-1'; 42.8 (d): *c*-hexyl C-1"; 75.4 (s): C-2; 81.3 (d): C-4; 121.0 (t): allyl C-3'; 130.7 (d): allyl C-2'; 179.0 (s): C-1. MS: *m/e* (%) 224 (M^+, 2), 183 (11), 165 (16), 139 (76), 121 (59), 97 (55), 95 (31), 83 (19), 81 (15), 69 (17), 67 (17), 55 (100), 41 (49). HRMS: calcd. (M^+): *m/e* 224.1412; found: *m/e* 224.1415. Anal: calcd. for $C_{13}H_{20}O_3$: C, 69.61; H, 8.99; found: C, 69.64; H, 9.25.

(2R*,4R*) -2- Allyl -2- (dimethyl-*iso*-propylsilyloxy) - 4 - cyclohexyl- γ - butyrolactone (40). A mixture of 2.04 g (9.1 mmol) of the hydroxy-lactone (**39**), 2.0 ml (12.7 mmol) of dimethyl-*iso*-propylsilylchloride and 987 mg (14.5 mmol) of imidazole in 50 ml of anhydrous DMF was stirred overnight at room temperature. The reaction mixture was poured into 200 ml of saturated aqueous sodiumbicarbonate solution and the resulting mixture was extracted with three 100 ml-portions of petrol. The combined organic layers were washed with 100 ml of water, dried with $MgSO_4$ and concentrated under reduced pressure to yield 3.15 g of an oil. The crude product was purified via chromatography on 50 g of silicagel with petrol-EtOAc (98-2) as the eluent to give 2.88 g (8.9 mmol, 98%) of the silyloxy-lactone (**40**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.08 and 0.14 (2 s, 6H): $Si(CH_3)_2$; 0.60-1.82 (br m, 17H): $SiCH(C\underline{H}_3)_2$ [δ 0.92 (d, J=4.5 Hz)], $SiC\underline{H}(CH_3)_2$ and *c*-hexyl H-2"-H-6"; 1.85-2.03 (m, 2H): H-3α [δ 1.91 (dd, J=12.9, 9.8 Hz)] and *c*-hexyl H-1" [δ 1.95 (br d, J≈12.6 Hz)]; 2.31-2.54

(m, 3H): H-3β [δ 2.37 (dd, J=12.9, 6.0 Hz)] and allyl H-1'; 3.93 (ddd, J=9.7, 7.6, 6.0 Hz, 1H): H-4; 5.10 (dm, J=10.1 Hz, 1H): allyl H-3'(*E*); 5.18 (m, 1H): allyl H-3'(Z); 5.71-5.88 (m, 1H): allyl H-2'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ -2.4 and -2.2 (2 q): $Si(CH_3)_2$; 15.5 (d): $SiCH(CH_3)_2$; 16.8 (q): $SiCH(CH_3)_2$; 25.4, 25.6 and 26.2 (3 t): *c*-hexyl C-3"-C-5"; 27.5 and 28.8 (2 t): *c*-hexyl C-2", C-6"; 39.6 and 42.4 (2 t): C-3 and allyl C-1'; 42.8 (d): c-hexyl C-1"; 77.7 (s): C-2; 80.6 (d): C-4; 119.5 (t): allyl C-3'; 131.7 (d): allyl C-2'; 177.3 (s): C-1. MS: *m/e* (%) 281 (49), 266 (16), 263 (45), 253 (13), 189 (16), 169 (29), 161 (47), 127 (21), 101 (13), 95 (29), 75 (100), 73 (24), 59 (16). HRMS: calcd. (M-*i*-Pr): *m/e* 281.1573; found: *m/e* 281.1578.

(2R*,3R*,5R*)- And (2S*,3R*,5R*)-5-cyclohexyl-3-(dimethyl-*iso*-propylsilyloxy)-3-(prop-2-enyl)-tetrahydrofuran-2-ol (41). A stirred solution of 2.88 g (8.9 mmol) of the silyloxy-lactone (**40**) in 75 ml of anhydrous toluene was cooled on a dry-ice-acetone bath while 9.6 ml (9.6 mmol) of a 1.0M solution of diisobutyl aluminumhydride in hexanes was added dropwise in approx. 15 min. Stirring at -78°C was continued for 1.5 hrs before the reaction was quenched by the addition of 3.68 g (11.5 mmol) of Glauber's salt. The mixture was warmed to room temperature and stirred for another 30 min, during which time a homogenous white slurry formed. $MgSO_4$ was added and the mixture was filtered through a pad of hyflo. Evaporation of the solvents yielded 2.36 g (7.2 mmol, 81%) of a C-2 epimeric mixture of the lactols (**41**) as an oil, which was used in the next reaction without further purification.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.09, 0.10, 0.14 and 0.15 (4 s; total 6H): $Si(CH_3)_2$; 0.62-1.05 (br, 9H), 1.05-1.83 (br, 12H) and 1.90-2.08 (m, 2H): H-4, $SiCH(C\underline{H}_3)_2$ [δ 0.93 and 0.97 (2 d, J≈5.9 Hz)], $SiC\underline{H}(CH_3)_2$ and *c*-hexyl H-1'-H-6'; 2.25-2.50 (m, 2H): allyl H-1'; 2.95 (br d, J≈3.4 Hz, 0.3H): OH minor isomer; 3.49 (ddd, J=9.4, 8.0, 6.3 Hz, 0.6H): H-5 major isom.; 3.59 (d, J=5.7 Hz, 0.6H): OH major isom.; 3.82 (ddd, J=8.3, 8.2, 6.1 Hz, 0.3 H): H-5 minor isom.; 4.85 (d, J=5.8 Hz, 0.6H): H-2 major isom.; 5.04-5.14 (m, 2H): allyl H-3'(Z) [δ 5.07 (dm, J≈8.2 Hz)] and allyl H-3'(*E*) [δ 5.09 (dm, J≈17.8 Hz)]; 5.15-5.21 (m, 0.3H): H-2 minor isom.; 5.70-5.99 (m, 1H): allyl H-2'.

(2R*,3aS*,5R*) - And (2S*,3aS*,5R*) - 5-cyclohexyl - 3a - (dimethyl-*iso*-propylsilyloxy)-perhydrofuro[2,3b]furan-2-ol (42). A stirred solution of 2.36 g (7.2 mmol) of the lactols (**41**) in 75 ml of methylene chloride was cooled to -78°C. An oxygen-ozone mixture was passed through the solution until the colour turned blue and the starting material had disappeared, as judged by tlc-analysis. The solution was purged with nitrogen until the blue colour disappeared. Then, 1.93 g (7.4 mmol) of triphenylphosphin was added and the reaction mixture was warmed to room temperature. After tlc-analysis indicated complete transformation of the intermediate ozonide, stirring was continued for 4.5 hrs before the solvent was evaporated under reduced pressure to give 4.80 g of a colourless oil. The residue was purified via chromatography on 60 g of silicagel with petrol-EtOAc (99-1 to 90-10) as the eluent, yielding 1.95 g (5.9 mmol; 82%) of an inseparable 1 : 1 mixture of both C-2 epimers of the 3a-DMIPSoxy-furofuranol (**42**) as a white solid.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.08 (2 s) and 0.13 (s, total 6H): $Si(CH_3)_2$; 0.60-2.00 (br m, 21H) and 2.03-2.43 (m, 3H): H-3, H-4, $SiCH(C\underline{H}_3)_2$ [δ 0.91 and 0.93 (2 d, J=6.2 Hz)], $SiC\underline{H}(CH_3)_2$ and *c*-hexyl H-1'-H-6'; 3.51 (d, J=8.3 Hz, 0.5H): OH; 3.74 (ddd, J=10.8, 7.3, 4.7 Hz, 0.5H): H-5; 3.85 (br, 0.5H): OH; 4.17 (ddd, J=10.2, 7.2, 5.0 Hz, 0.5H): H-5; 5.43 and 5.52 (2 s, 1H): H-6a; 5.53-5.65 (m, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz): δ -2.1 (q): $Si(CH_3)_2$; 15.3 (d): $SiCH(CH_3)_2$; 16.7 (q): $SiCH(CH_3)_2$; 25.7 (2), 25.9 (2), 26.3 and 26.4 (6 t): *c*-hexyl C-3'-C-5'; 28.6, 29.5 and 29.6 (3 t): *c*-hexyl C-2', C-6'; 42.7 and 43.0 (2 d): *c*-hexyl C-1'; 43.3, 45.3, 46.3 and 48.0 (4 t): C-3 and C-4; 83.5 and 84.1 (2 d): C-5; 88.7 and 89.5 (2 s): C-3a; 98.9 and 99.8 (2 d): C-2; 112.6 and 114.1 (2 d): C-6a.

MS: *m/e* (%) 267 (12), 239 (31), 223 (11), 200 (16), 199 (100), 157 (18), 147 (29), 143 (12), 129 (20), 109 (11), 101 (16), 95 (14), 75 (32), 73 (18), 59 (14), 55 (17). HRMS: calcd. (M-*i*-Pr): *m/e* 285.1522; found: *m/e* 285.1518. Anal: calcd. for $C_{17}H_{32}O_4Si$: C, 62.17; H, 9.82; found: C, 62.10; H, 10.08.

(3aR*,5S*)-5-Cyclohexyl-3a-hydroxy-3a,4,5,6a-tetrahydrofuro[2,3b]furan-2(3H)-one (43). A mixture of 20 mg (0.09 mmol) of the furofuran-2,3a-diols (**36**) and 115 mg (0.31 mmol) of pyridinium dichromate in 10 ml of dichloromethane was stirred for 5 days at room temperature. The mixture was diluted with ether and filtered through a pad of hyflo. The solvents were removed under reduced pressure and the brownish residue was purified by chromatography on 10 g of silicagel with petrol-EtOAc (90-10 to 80-20) as the eluent, yielding 15 mg (0.07 mmol; 75%) of the 3a-hydroxy-furofuranone (**43**) as a white solid (m.p. 97-98.5°C).
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.80-1.35 (br m, 5H) and 1.40-1.80 (br m, 5H): *c*-hexyl H-2"-H-6"; 1.82-2.01 (br m, 2H): H-4β [δ 1.90 (dd, J=11.9, 11.9 Hz)] and *c*-hexyl H-1"; 2.22 (dd, J=12.7, 4.9 Hz, 1H): H-4α; 2.9 (s, 2H): H-3; 3.45 (br s, 1H): OH; 3.83 (ddd, J=11.1, 7.2, 4.9 Hz, 1H): H-5; 5.8 (s, 1H): H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.5, 25.7 and 26.2 (3 t): *c*-hexyl C-3"-C-5"; 28.5 and 29.4 (2 t): *c*-hexyl C-2" and C-6"; 42.1 (d): *c*-hexyl C-1"; 42.9 (t): C-3; 44.2 (t): C-4; 83.5 (s): C-3a; 84.5 (d): C-5; 112.4 (d): C-6a; 174.8 (s): C-2. Anal: calcd. for $C_{12}H_{19}O_5$ ($C_{12}H_{18}O_4$ + 0.5 mole H_2O): C, 61.26; H, 8.14; found: C, 61.61; H, 8.42.

(2S*,3aR*,5S*) - And (2R*,3aR*,5S*) - 2 - acetoxy - 5 - cyclohexyl - 3a - hydroxy - perhydrofuro[2,3b]furan (44). A stirred, ice-cold solution of 150 mg (0.66 mmol) of the furofuran-2,3a-diols (**36**) in 7.5 ml of dichloromethane was treated sequentially with 0.15 ml (1.9 mmol) of pyridine and 0.1 ml (1.4 mmol) of acetyl chloride. Stirring was continued for 1 hr at 0°C before the reaction mixture was poured into a mixture of 50 ml of ether and 25 ml of water. The organic layer was separated and the aqueous layer was extracted with 40 ml of ether. The combined organic layers were washed with 25 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 164 mg of a yellow solid. Purification of the crude product via chromatography on 20 g of silicagel with petrol-EtOAc (90-10 to 80-20) as the eluent afforded 137 mg (0.51 mmol; 77%) of an inseparable 1 : 4 mixture of both C-2 epimers of the acetate (**44**) as a white solid.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.78-1.33 (br m, 5H) and 1.33-2.00 (br m, 7H): H-4β [H-4β major isom.: δ 1.77 (dd, J=11.7, 11.7 Hz)], *c*-hexyl H-1" [δ 1.86 (br d, J=11.7 Hz)] and *c*-hexyl H-2"-H-6"; 2.03 (s, 0.6H): $CH_3C(O)$ minor isom.; 2.05 (s, 2.4H): $CH_3C(O)$ major isom.; 2.07-2.53 (several m, 3H): major isom.: H-4α [δ 2.12 (dd, J=12.2, 4.4 Hz)], H-3 [δ 2.24 (dd, J=14.8, 1.2 Hz)], H-3' [δ 2.38 (dd, J=14.7, 4.7 Hz)] and minor isom: H-3' [δ 2.55 (dd, J=14.7, 7.6 Hz)], H-4α, H-3; 2.74 (br, 1H): OH; 3.77 (ddd, J=11.5, 7.3, 4.3 Hz, 0.8H): H-5 major isom.; 4.06 (ddd, J=10.6, 7.5, 5.5 Hz, 0.2H): H-5 minor isom.; 5.47 (s, 0.2H): H-6a minor isom.; 5.53 (s, 0.8H): H-6a major isom.; 6.31-6.37 (2 m, 1H): H-2 minor isom. [δ 6.34 (dd, J=7.6, 1.2 Hz)] and H-2 major isom. [δ 6.35 (dd, J=4.9, 1.3 Hz)]. ^{13}C NMR ($CDCl_3$, 50 MHz, selected peaks): *Major isomer*: δ 21.4 (q): $\underline{C}H_3C(O)$; 25.6, 25.9 and 26.3 (3 t): *c*-hexyl C-3"-C-5"; 28.7 and 29.7 (2 t): *c*-hexyl C-2" and C-6"; 42.3 (d): *c*-hexyl C-1"; 43.8 and 44.5 (2 t): C-3 and C-4; 84.2 (d): C-5; 86.8 (s): C-3a; 98.9 (d): C-2; 114.0 (d): C-6a; 170.1 (s): $CH_3\underline{C}(O)$. *Minor isomer*: δ 21.4 (q); 25.6, 25.9, 26.3, 28.7 and 29.7 (5 t); 42.8 (d); 44.8 and 45.8 (2 t); 83.8 (d); 87.1 (s); 97.9 (d); 114.3 (d); 170.0 (s). MS: *m/e* (%) 211 (40), 187 (12), 183 (26), 181 (30), 165 (33), 164 (60), 142 (20), 137 (26), 127 (90), 121 (16), 110 (21), 109 (53), 99 (64), 95 (36), 86 (16), 83 (22), 81 (69), 71 (26), 67 (27), 55 (39), 43 (100). HRMS: calcd. (M-CH_3CO): *m/e* 227.1283; found: *m/e* 227.1286. Anal: calcd. for $C_{14}H_{22}O_5$: C, 62.20; H, 8.20; found: C, 62.42; H, 8.47.

(2R*,3aR*,5S*)- And (2S*,3aR*,5S*)- 5-cyclohexyl- 3a-hydroxy- 2-methoxy- perhydrofuro[2,3b]furan (37). A mixture of 150 mg (0.66 mmol) of the furofuran-2,3a-diols (**36**), 40 mg (1.25 mmol) of methanol and a few grains of *p*-TsOH in 50 ml of THF was stirred at room temperature for 48 hrs. The reaction mixture was poured into 20 ml of water and extracted with three 30 ml-portions of ether. The combined extracts were washed with 20 ml of saturated aqueous sodium bicarbonate solution, dried with $MgSO_4$ and concentrated under reduced pressure to give 154 mg of a yellow oil. The product was purified via chromatography on 10 g of silicagel with petrol-EtOAc (90-10) as the eluent, affording 137 mg (0.57 mmol; 86%) of the methyl ether (**37**) as a 1 : 1.7 mixture of C-2 epimers. Repeated chromatography yielded a small quantity of the pure minor (least polar) isomer as a white solid (m.p. 102°C).
Minor isomer: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.80-1.35 (br m, 5H) and 1.35-1.88 (br m, 6H): H-4β [δ 1.73 (dd, J=11.0, 11.0 Hz)] and *c*-hexyl H-2"-H-6"; 1.94 (br d, J=12.5 Hz, 1H): *c*-hexyl H-1"; 2.06 (dd, J=12.2, 4.4 Hz, 1H): H-4α; 2.13 (2 d, J_1=4.0 Hz and J_2=2.13 Hz, 2H): H-3; 2.78 (br s, 1H): OH; 3.36 (s, 3H): OCH_3; 3.78 (ddd, J=11.5, 7.5, 4.3 Hz, 1H): H-5; 5.12 (dd, J=3.5, 1.7 Hz, 1H): H-2; 5.40 (s, 1H): H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.7, 25.9 and 26.4 (3 t): *c*-hexyl C-3"-C-5"; 28.7 and 29.7 (2 t): *c*-hexyl C-2" and C-6"; 42.1 (t): C-3; 42.5 (d): *c*-hexyl C-1"; 44.5 (t): C-4; 54.9 (q): OCH_3; 84.2 (d): C-5; 86.8 (s): C-3a; 105.4 (d): C-2; 112.8 (d): C-6a. MS: *m/e* (%) 196 (41), 164 (34), 141 (65), 127 (41), 115 (31), 113 (100), 100 (50), 95 (32), 81 (79), 67 (36), 58 (74), 55 (49), 41 (33). HRMS: calcd. (M-OCH_3): *m/e* 211.1334; found: *m/e* 211.1333. Anal: calcd. for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15; found: C, 64.66; H, 9.43. *Major isomer*[39]: ^{1}H NMR ($CDCl_3$, 200 MHz, separated peaks only): δ 2.22 (s, 1H); 2.22-2.40 (m, 4H); 3.29 (s, 3H): OCH_3; 3.98 (ddd, J=10.5, 7.6, 5.5 Hz, 1H): H-5; 5.03 (dd, J=5.6, 1.3 Hz, 1H): H-2; 5.42 (2, 1H): H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz, separated peaks only): δ 42.8 (d): *c*-hexyl C-1"; 45.5 and 46.7 (2 t): C-3 and C-4; 54.7 (q): OCH_3; 83.4 (d): C-5; 87.5 (s): C-3a; 105.0 (d): C-2; 113.7 (d): C-6a.

(2R*,3aS*,5R*)- And (2S*,3aS*,5R*)- 5-Cyclohexyl- 3a-hydroxy- 2-phenylthio- perhydrofuro[2,3b]furan (45). To an ice-cold solution of 104 mg (0.45 mmol) of the diols (**36**) and 0.06 ml (0.6 mmol) of thiophenol in 25 ml of anhydrous ether, containing approx. 5 g of activated 4Å molecular sieves, was added via syringe 80 µl (0.65 µmol) of borontrifluoride etherate. After 30 min the reaction was quenched by the addition of 30 ml of an aqueous 2N sodiumhydroxide solution. The organic layer was separated and the waterlayer was extracted with two 50 ml-portions of ether. The combined organic layers were washed with 30 ml of water and 30 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 141 mg (0.44 mmol, 97%) of a 4 : 1 mixture of C-2 epimers of the sulfide (**45**) as a white solid, which was sufficiently pure to be used in the next reaction without further purification.The epimers could be separated by chromatography on silicagel with petrol-EtOAc (96-4 to 90-10) as the eluent to give, in order of elution, the major isomer (m.p. 91-92°C) and the minor isomer (m.p. 133-136°C), both as white solids.
Major isomer: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-1.30 (br m, 6H) and 1.30-1.96 (br m, 7H): H-4β [δ 1.77 (dd, J=11.8, 11.7 Hz)], *c*-hexyl H-1" [δ 1.89 (br d, J=12.4 Hz)] and *c*-hexyl H-2"- H-6"; 2.12 (dd, J=12.6, 4.9 Hz, 1H): H-4α; 2.24 (dd, J=14.3, 4.0 Hz, 1H): H-3; 2.48-2.62 (m, 2H): H-3 [δ 2.55 (dd, J=14.5, 6.8 Hz)] and OH [approx. δ 2.56 (br)]; 3.78 (ddd, J=11.1, 7.5, 4.7 Hz, 1H): H-5; 5.54 (s, 1H): H-6a; 5.67 (dd, J=6.8, 3.8 Hz, 1H): H-2; 7.15-7.30 (m, 3H): phenyl H-3'-H-5'; 7.38-7.50 (m, 2H): phenyl H-2' and H-6'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 25.6, 25.8 and 26.3 (3 t): *c*-hexyl C-3'-C-5'; 28.7 and 29.6 (2 t): *c*-hexyl C-2' and C-6'; 42.6 (d): *c*-hexyl C-1'; 43.7 and 45.7 (2 t): C-3 and C-4; 84.4 (d): C-5; 86.3 (d): C-2; 87.5 (s): C-3a; 113.2 (d): C-6a; 127.6, 129.0 and 131.8 (3 d): phenyl C-2-C-6; 133.7 (s): phenyl C-1. MS: *m/e* (%) 213 (13), 211 (100), 193 (17), 181 (14), 175 (17), 167 (13), 157 (12), 149 (36), 147 (12), 121 (24), 110 (12), 109 (11), 95 (21),

81 (16), 72 (11), 67 (21), 55 (16), 41 (13). HRMS: calcd. (M^+): *m/e* 320.1446; found: *m/e* 320.1445. Anal: calcd. for $C_{18}H_{24}O_3S$: C, 67.46; H, 7.55; found: C, 67.63; H, 7.77. *Minor isomer*: ^1H NMR ($CDCl_3$, 200 MHz): δ 0.80-2.28 (br m, 15H): H-4β [δ 1.72 (dd, J=11.6, 11.5 Hz)], H-3a and H-4 [δ 2.10-2.21 (m)], OH, *c*-hexyl H-1" [δ 1.92 (br d, J=12.7 Hz)] and *c*-hexyl H-2"-H-6"; 2.56 (dd, J=14.1, 6.8 Hz, 1H): H-3b; 5.40 (s, 1H): H-6a; 5.59 (ddd, J=14.3, 6.8, 6.8 Hz, 1H): H-2; 7.20-7.39 (m, 3H): phenyl H-3'-H-5'; 7.45-7.60 (m, 2H): phenyl H-2' and H-6'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 25.6, 25.8 and 26.4 (3 t): *c*-hexyl C-3"-C-5"; 28.5 and 29.5 (2 t): *c*-hexyl C-2" and C-6"; 42.6 (d): *c*-hexyl C-1'; 44.3 and 45.0 (2 t): C-3 and C-4; 84.0 (d): C-5; 85.3 (d): C-2; 87.8 (s): C-3a; 113.6 (d): C-6a; 127.5, 128.8 and 132.3 (3 d): phenyl C-2'-C-6'; 133.5 (s): phenyl C-1'. MS: *m/e* (%) 212 (12), 211 (100), 193 (18), 181 (48), 157(19), 149 (39), 121 (26), 110 (19), 109 (18), 99 (18), 95 (38), 83 (18), 81 (32), 71 (22), 69 (18), 67 (32), 55 (36), 43 (34), 41 (34). HRMS: calcd. (M^+): *m/e* 320.1446; found: *m/e* 320.1448.

(2S*,3aR*,6aR*)-2-Cyclohexyl-2,3,3a,6a-tetrahydrofuro[2,3b]furan-3a-ol (4). A solution of 40 mg (0.16-0.17 mmol) of mCPBA (70-75 wt% mCPBA, remainder mCBA and water) in 2 ml of anhydrous toluene was pre-dried in a dropping funnel containing activated 4Å molecular sieves. After 20 min, this solution was added dropwise in to an ice-cold solution of 48 mg (0.15 mmol) of the crude sulfide mixture (**45**) in 5 ml of toluene. Stirring at 0°C was continued until tlc-analysis indicated complete disappearance of the sulfide (approx. 10 min). The dropping funnel was replaced by a reflux condensor, 0.25 ml (1.8 mmol) of triethylamine was added and the flask containing the reaction mixture was placed in an oil bath, pre-heated at 130°C. The mixture was refluxed for approx. 20 min, while the disappearance of the sulfoxide intermediate was monitored via tlc-analysis. Then, both solvent and triethylamine were removed at the rotary evaporator under reduced pressure and the residual oil (92 mg) was purified by chromatography on 12 g of silicagel with petrol-EtOAc (95-5 to 90-10) as the eluent, affording 25 mg (0.12 mmol, 79%) of the tetrahydrofurofuran-3a-ol (**4**) as a white solid.
^1H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.75-1.38 (br m, 5H) and 1.38-1.80 (br m, 5H): *c*-hexyl H-2"-H-6"; 1.80-2.02 (br dd, 2H): H-3β [δ 1.91 (dd, J=11.7, 11.7 Hz)] and *c*-hexyl H-1"; 2.02-2.40 (br dd, 2H): H-3α [δ 2.17 (dd, J=12.0, 4.3 Hz)] and OH; 3.77 (ddd, J= 11.5, 7.4, 4.3 Hz, 1H): H-2; 5.01 (d, J=2.8 Hz, 1H): H-4; 5.65 (s, 1H): H-6a; 6.57 (d, J=2.8 Hz, 1H): H-5. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.7, 25.9 and 26.4 (3 t): *c*-hexyl C-3"-C-5"; 28.7 and 29.8 (2 t): *c*-hexyl C-2" and C-6"; 42.27 (d): *c*-hexyl C-1"; 42.3 (t): C-4; 85.6 (d): C-5; 90.5 (s): C-3a; 104.9 (d): C-3; 113.1 (d): C-6a; 150.4 (d): C-2.

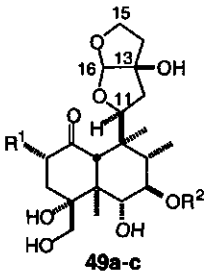
(2S*,3aS*,6aS*)-2-Cyclohexyl-perhydrofuro[2,3b]furan-3a-ol (46). A solution of 80 mg (0.38 mmol) of the tetrahydrofurofuran-3a-ol (**4**) in 30 ml of EtOAc, containing 47 mg of 10% Pd/C, was hydrogenated in a Parr apparatus under hydrogen pressure (4 atm) at room temperature. After 25 min the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The crude product was purified via chromatography on silicagel with petrol-EtOAc (92-8 to 85-15) as the eluent, affording 59 mg (0.28 mmol; 73%) of the perhydrofurofuran-3a-ol (**46**) as a white solid (m.p. 81-82.5°C).
^1H NMR ($CDCl_3$, 200 MHz): δ 0.75-1.78 (br m, 11H): *c*-hexyl H-2"-H-6"; 1.84 (dd, J=11.7, 11.7 Hz, 1H): H-3β; 1.95 (br d, J=11.7 Hz, 1H): *c*-hexyl H-1"; 2.13-2.27 (m, 3H): H-4 [δ 2.16 (dd, J=6.8, 6.8 Hz)] and H-3α [δ 2.22 (dd, J=12.5, 5.1 Hz)]; 2.4 (br s, 1H): OH; 3.85 (ddd, J=10.7, 7.5, 5.1 Hz, 1H): H-2; 3.9-4.1 (m, 2H): H-5; 5.3 (s, 1H): H-6a. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.7, 25.9 and 26.4 (3 t): *c*-hexyl C-3"-C-5"; 28.6 and 29.6 (2 t): *c*-hexyl C-2" and C-6"; 40.2 (t): C-3; 43.2 (d): *c*-hexyl C-1"; 44.2 (t): C-4; 68.2 (t): C-2; 84.7 (s): C-3a; 88.3 (d): C-5; 112.3 (d): C-6a. MS: *m/e* (%) 166 (11), 129 (100), 116 (10), 111

(21), 84 (10), 83 (88), 70 (19), 55 (36), 43 (40), 41 (14). HRMS: calcd. (M^+): *m/e* 212.1412; found: *m/e* 212.1409. Anal: calcd. for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50; found: C, 67.92; H, 9.69.

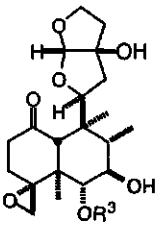
6.5 - Notes and References

1. For an extensive survey of insect antifeeding clerodane diterpenes and related compounds, see Chapter 2.
2. S.V. Ley, D. Neuhaus, N.S. Simpkins and A.J. Whittle, *J. Chem. Soc., Perkin Trans. I*, 2157-2162 (1982); R.B.M. Geuskens, J.M. Luteyn and L.M. Schoonhoven, *Experientia*, **39**, 403 (1983); W.M. Blaney, M.S.J. Simmonds, S.V. Ley and P.S. Jones, *Entomol. Exp. Appl.*, **46**, 267-274 (1988); W.M. Blaney, A.C. Cuñat, S.V. Ley, F.J. Montgomery and M.S.J. Simmonds, *Tetrahderon Lett.*, **35** (27), 4861-4864 (1994).
3. Y. Kojima and N. Kato, *Tetrahedron*, **37** (15), 2527-2538 (1981); Y. Kojima and N. Kato, *Nippon Kagaku Kaishi*, (5), 712-720 (1981).
4. For reviews regarding the biological activity of azadirachtin, see: A.J. Mordue (Luntz) and A. Blackwell, *J. Insect Physiol.*, **39** (11), 903-924 (1993); T.A. van Beek and Ae. de Groot, *Recl. Trav. Chim. Pays-Bas*, **105**, 513-527 (1986); S.V. Ley, A.A. Denholm and A. Wood, *Nat. Prod. Reports*, 109-157 (1993).
5. W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson, S.C. Smith and A. Wood, *Pestic. Sci.*, **40**, 169-173 (1994).
6. S.V. Ley, D. Santafianos, W.M. Blaney and M.S.J. Simmonds, *Tetrahderon Lett.*, **28** (2), 221-224 (1987).
7. (a) E.D. Morgan, *Strategy in the isolation of insect control substances from plants*. In: H. Schmutterer, K.R.S. Acher and H. Rembold (Eds.), *Natural pesticides from the Neem tree*, Proceedings of the first International Neem Conference (Rottach-Egern, FRG, june 1980), GTZ Verlag, 1981, p. 43-52; (b) S.V. Ley, J.C. Anderson, W.M. Blaney, E.D. Morgan, R.N. Sheppard, M.S.J. Simmonds, A.M.Z. Slawin, S.C. Smith, D.J. Williams and A. Wood, *Tetrahedron*, **47** (44), 9231-9246 (1991); (c) M.S.J. Simmonds, W.M. Blaney, S.V. Ley, J.C. Anderson, R. Bänteli, A.A. Denholm, P. Green, R.B. Grossman, C. Gutteridge, L. Jennens, S.C. Smith, P.L. Toogood and A. Wood, *Entomol. Exp. Appl.*, **77**, 69-80 (1995).
8. See Chapter 3 for a more elaborate discussion of this topic.
9. A similar model compound has also been proposed and synthesized by Lallemand and coworkers, ref. 11b. However, see also ref. 12.
10. (a) Recently, the first examples of *neo*-clerodane diterpenes containing a 3a-hydroxy-furo[2,3b]furan fragment were reported with the isolation of the scuterepenins A-C (Figure 6.4). To our knowledge, none of these compounds has yet been examined for insect antifeedant activity. See: H. Kizu, N. Sugita and T. Tomimori, *Chem. Pharm. Bull.*, **46** (6), 988-1000 (1998). (b) NMR data of the 3a-hydroxy-perhydrofuro[2,3b]furan fragment of (**49b**): ^{1}H NMR ($CDCl_3$, selected peaks): δ 2.1-2.2: H-12α, H-14α and H-14β; 2.21 (m): H-12β; 3.89 (ddd, J=9, 7.5, 7.5 Hz): H-15α; 4.02 (ddd, J=9, 7.5, 5 Hz): H-5β; 4.24 (dd, J=11, 6 Hz): H-11; 5.28 (s): H-16. ^{13}C NMR ($CDCl_3$, selected peaks): δ 40.3: C-14; 42.8: C-12; 68.3: C-15; 85.0: C-11; 87.7: C-13; 112.0: C-16.

Figure 6.4



49a-c

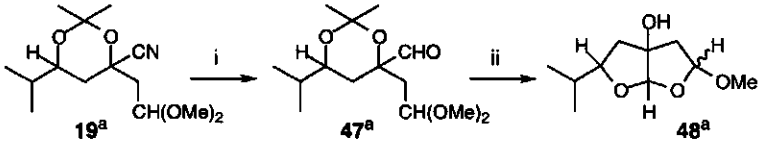


50a,b

49a: scuterepenin A_1; R^1=H, R^2=*trans*-cinnamoyl
49b: scuterepenin A_2; R^1=H, R^2=*c*-cin.
49c: scuterepenin B; R^1=OH, R^2=*t*-cin.
50a: scuterepenin C_1; R^3=*t*-cin.
50b: scuterepenin C_2; R^3=*c*-cin.

11. Only a few syntheses of 3a-hydroxy-furo[2,3b]furan-based compounds have been reported; see: (a) M. Jalali-Naini and J.Y. Lallemand, *Tetrahedron Lett.*, **27** (4), 497-500 (1986); (b) A.P. Brunetière, M. Leclaire, S. Bhatnagar and J.Y. Lallemand , *Tetrahedron Lett.*, **30** (3), 341-344 (1989); (c) E.R. Civitello and H. Rapoport, *J. Org. Chem.*, **59** (14), 3775-3782 (1994).
12. In 1989, Lallemand and coworkers announced that biological tests of such a compound (3a-hydroxy-3a,4,5,6a-tetrahydrofuro[2,3b]furan) were in progress (ref. 11b), but despite an extensive search of the available literature we were not able to find any report of these experiments.
13. E. Vedejs and J.E. Telschow, *J. Org. Chem.*, **41** (4), 740-741 (1976).
14. S. Selikson and D.S. Watt, *J. Org. Chem.*, **40** (2), 267-268 (1975).
15. For reviews on the use of protected cyanohydrins (α-hydroxy-nitriles) in synthesis, see: (a) J.D. Albright, *Tetrahedron*, **39** (20), 3207-3233 (1983); (b) (b) S. Arseniyadis, K.S. Kyler and D.S. Watt, *Addition and substitution reactions of nitrile-stabilized carbanions.* In: *Organic Reactions*, **31**, 1-367 (1984).
16. (a) S.D. Rychnovsky, S. Zeller, D.J. Skalitzky and G. Griesgraber, *J. Org. Chem.*, **55**, 5550-5551 (1990); (b) Supplementary material from ref. 16a; (c) S.D. Rychnovsky, G. Griesgraber, S. Zeller and D.J. Skalitzky, *J. Org. Chem.*, **56**, 5161-5169 (1991); (d) S.D. Rychnovsky and G. Griesgraber, *J. Org. Chem.*, **57** (5), 1559-1563 (1992); (e) S.D. Rychnovsky and R.C. Hoye, *J. Am. Chem. Soc.*, **116** (5), 1753-1765 (1994); (f) S.D. Rychnovsky, G. Griesgraber and J. Kim, *J. Am. Chem. Soc.*, **116** (6), 2621-2622 (1994).
17. M.W. Rathke, *J. Am. Chem. Soc.*, **92** (10), 3222-3223 (1970).
18. B. Cazes and S. Julia, *Tetrahedron*, **35**, 2655-2660 (1979).
19. The assignment of the relative stereochemistry of these compounds was based on comparison with spectral data of related cyanohydrin 1,3-acetonides from ref. 16.
20. All bases used in the original literature (ref. 16) were tried at least once.
21. Analytical data for this compound are presented in the experimental section.
22. The alkylated cyanohydrin 1,3-acetonides (**19**) with R=$CH_2CH(OMe)_2$) could be converted into the desired 3a-hydroxy-perhydrofurofuran systems (**48**) in moderate yield via the following two-step procedure:

Scheme 6.10



19[a] **47**[a] **48**[a]

Reagents and conditions: (i) 3 eq. $LiAlH(OEt)_3$, ether, 0°C; (ii) *p*-TsOH, MeOH, 48 hrs. (a) Stereochemistry not determined.

23. S.D. Rychnovsky and D.J. Skalitzky, *Tetrahedron Lett.*, **31** (7), 945-948 (1990); S.D. Rychnovsky, B. Rogers and G.J. Yang, *J. Org. Chem.*, **58** ,3511 (1993).

24. Reported (ref. 23) average ^{13}C chemical shift values (δ±s.d. in ppm): (a) *syn*-1,3-diol acetonides with a chair conformation: 19.62±0.39 and 30.02±0.11 (acetonide methyl shifts) and 98.50±0.56 (acetonide acetal shift); (b) *anti*-acetonides with a twist conformation: 24.70±0.34 (acetonide methyl shifts) and 100.43±0.11 (acetonide acetal shift). Note that for our cyanohydrin 1,3-acetonides the *nomenclature* (but not the actual configuration) is opposite to the 1,3-diol acetonides, due to the presence of the cyano group which has a higher priority according to the Cahn-Ingold-Prelog system.

25. (a) L.J. Brzezinski, D.D. Levy and J.W. Leahy, *Tetrahedron Lett.*, **35** (41), 7601-7604 (1994); (b) The average ^{13}C chemical shift values (δ±s.d. in ppm, n=4) for the cyanohydrin 1,3-acetonides of type (**25**) were : 26.18±0.33 and 27.05±0.40 (acetonide methyl shifts) and 101.93±0.13 (acetonide acetal shift).

26. The alkylation of (**18a,b**) with 1-bromopentane was reported to afford a *anti* : *syn* product ratio of 99.6 : 0.4 (ref. 16a), while in general selectivities of the order 200 : 1 are considered typical (see note 10 in ref. 25).

27. A similar effect was observed in the allylation of (**17**):

	reaction time (min)					
	50	120	180	210	240	crude product[d]
17a,b	1 : 51[a,b]	1 : 15	n.c.[c]	n.c.	n.c.	-
24a,b	37 : 1	28 : 1	25 : 1	20 : 1	20 : 1	19 : 1

Reaction conditions: 1) LHMDS (3.0 eq), THF, -78°C, 45 min; 2) allylbromide (5.4 eq), -78°C to -50°C, 3.5 hrs. (a) Determined by glc-analysis of a small sample, quenched with sat. NH_4Cl-soln. and extracted with ether. (b) Isomeric ratio reported as *anti* : *syn*. (c) Not calculable, due to too small glc peak areas of (**17**). (d) 86% Yield of (**24a**) after work-up and chromatography.

This example originates from an experiment that resulted in relatively poor selectivity. In several other attempts the initial (**24a,b**) ratio was as high as 48-50 : 1 after 90 min, resulting in a product ratio of 30-33 : 1 after work-up.

28. Even in case the size of the proton may not be entirely neglected or quenching with a naked proton is considered too much of an oversimplification, a (slight) excess of the *anti*-configuration (**18a**) would be expected instead of the observed preference for (**18b**).

29. It is not clear why isomer (**30**) with the axial lithium is preferred over (**29**) with lithium equatorial. This apparent behaviour is contrary to studies on both 2-lithio-tetrahydropyrans and 4-lithio-1,3-dioxanes, which indicated that in such compounds the isomer with lithium occupying an equatorial position is the thermodynamically favoured isomer. Although in (**29**) and (**30**) the cyano group probably is the main stabilizing element, the abovementioned effect could have been expected to lend additional stabilization to (**29**), thereby making it the major isomer in the equilibrium.
30. The lithio-pyrans and -dioxanes from ref. 29 are equilibrated at -20/-30°C.
31. Alternatively, (**30a**) might also be converted into (**28a**), from which β-face alkylation to (**19a**) could subsequently occur.
32. See Section 5.2.1.
33. M.N. Rerick, *The Chemistry of Mixed Hydrides.* In: R.L. Augustine (Ed.), *Reduction - Techniques and Application*, Marcel Dekker, New York, 1968, p. 71-74.
34. J.C. Anderson, S.V. Ley, D. Santafianos and R.N. Sheppard, *Tetrahedron*, **47**, 6813-6850 (1991), and references cited therein.
35. For reviews, see: J. March, *Advanced Organic Chemistry*, 3rd Ed., J. Wiley&Sons, New York, 1985, p. 792-793, and references cited therein.
36. T. Mandai, K. Hara, T. Nakajima, M. Kawada and J. Otera, *Tetrahedron Lett.*, **24** (45), 4993-4996 (1983). See also ref. 34.
37. Alternatively, a few drops of concentrated sulfuric acid have been used succesfully.
38. The epimeric ratio of different batches of (**36**) was found to vary from 5 : 3 to 7 : 3.
39. Determined from the ^{1}H and ^{13}C NMR spectra of a mixture of isomers (**37**); least polar (minor) isomer : most polar (major) isomer = 1 : 1.7.

Chapter 7

Syntheses towards Model Compounds based on 2,3-Dihydrofuran-3-ols and Butenolides

Abstract: *In this chapter, some attempts are described towards the synthesis of simple monocyclic compounds as bioactive analogues of natural clerodane antifeedants. The synthesis of a series of 3-alkyl-2,3-dihydrofuran-3-ol derivatives was unsuccessful, due to the instability of this moiety. Instead, a series of model compounds was prepared, based on 3-alkyl-3-hydroxy-γ-butyrolactones as the key target structure.*

Table of Contents

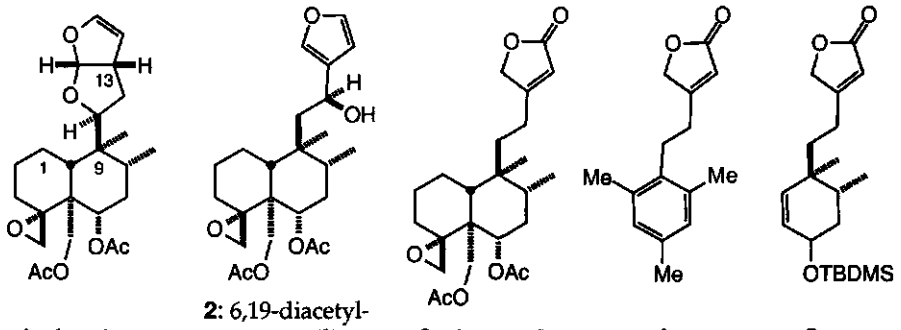
This chapter will be published in a revised form, in combination with part of chapter 9: E.A. klein Gebbinck, G.A. Stork, B.J.M. Jansen and Ae. de Groot, *Synthesis and insect-antifeedant activity of 2-substituted 2,3-dihydrofuran-3-ols and butenolides (Part II), Tetrahedron,* accepted for publication.

7.1 - Introduction

7.1.1 - Selection of the Target Structures

Several natural clerodane diterpenes display high insect antifeedant activity.[1] Especially clerodanes with a perhydrofuro[2,3b]furan subunit, such as clerodin (**1**), are among the most potent antifeedants known. This subunit has been recognized as an important feature for the biological activity of these natural compounds. For this reason, both we[2a,b] and others[3] have employed this structural element in the search for simplified bioactive analogues.

Figure 7.1



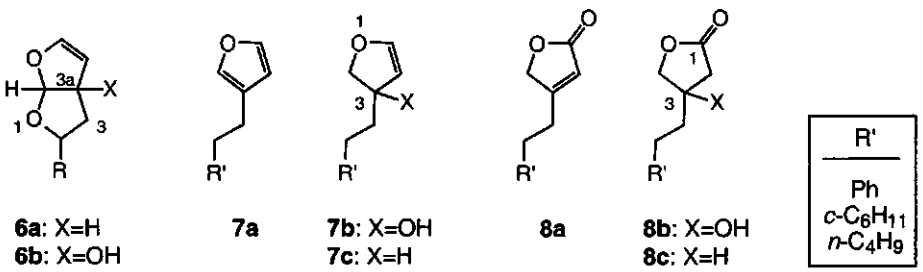
1: clerodin | **2**: 6,19-diacetyl-teumassilin | **3**: ajugarin I | **4** | **5**

However, perhydrofuro[2,3b]furans are not the only type of biologically active C-9 subunit encountered among clerodane antifeedants. A number of natural clerodanes with simple monocyclic C-9 sidechains, containing for instance a furan ring or a butenolide (*e.g.* (**2**) or (**3**), Figure 7.1), also exhibit significant antifeedant activity against various insect species.[4] Structural changes in the C-9 sidechains of these clerodanes are often associated with differences in activity. Also, both the decalin fragments and the C-9 sidechain separately display much weaker antifeedant activity than when combined in one molecule.[5] Such observations suggest that the antifeedancy of clerodane diterpenes as (**2**) or (**3**) partly resides in the C-9 subunit.

Due to their structural simplicity, monocyclic sidechains are attractive targets in the design of simplified bioactive analogues of natural antifeedants. Indeed, butenolide-based model compounds as (**4**)[6] and (**5**)[4a] were found to display moderate antifeedant activity against a number of insect species. However, the activity needs to be increased substantially before these model compounds can be considered for application as practical insect control agents.

In the previous chapter, the introduction of a hydroxyl group at position C-13 of the clerodane skeleton [*i.e.* at the C-3a atom of the perhydrofuro[2,3b]furan ring system (**6**)] was proposed as a strategy to potentially increase the antifeedant activity of the model compound (**6a**). Application of this reasoning (Figure 7.2) to the simple furan-based analogue (**7a**) would lead to compound (**7b**). This compound was considered an attractive target structure because of its structural similarity to the model compounds (**6b**), prepared before.[2b] The 2,3-dihydrofuran-3-ol moiety is known to be rather prone to aromatization[8-10] and the synthesis of (**7b**) was therefore expected to be potentially problematic. The existence of several precedents regarding the successful preparation of closely related compounds nevertheless seemed to merit some efforts towards the synthesis of (**7b**). In addition to (**7b**), the synthesis of derivatives (**7a**) and (**7c**) would be required to assess the effect on antifeedancy of the introduction of a C-3 hydroxyl group in such a ring system.

Figure 7.2



6a: X=H
6b: X=OH

7a

7b: X=OH
7c: X=H

8a

8b: X=OH
8c: X=H

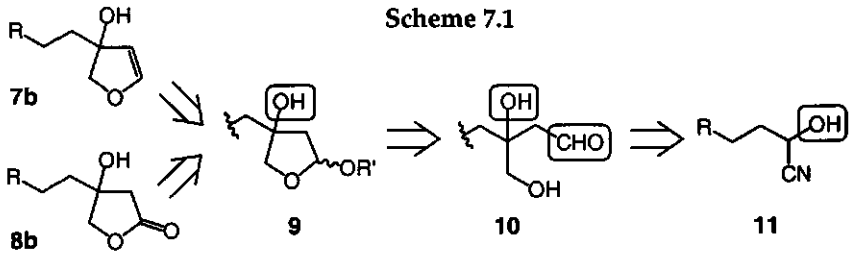
A second series of interesting target compounds (**8a-c**) was derived from the application of the same strategy to the butenolide-based model compound (**8a**). No serious problems were anticipated for the preparation of this series, since the key target structure (**8b**) was not expected to be unusually unstable.

Investigations on derivatives of (**4**) and (**6a**) have shown that the antifeedant activity of such model compounds can vary markedly with the nature of the substituent group R, even when only simple *tert*-butyl- or phenyl-groups are used.[3,6] We therefore decided to include a limited range of substituents R' (see Figure 2) in our series of test compounds (**7**) and (**8**), in order to examine their effect on antifeedancy.

7.1.2 - Synthetic Plan

For the preparation of the key target structures (**7b**) and (**8b**), we planned to use a route related to our previous synthesis of the 3a,4,5,6a-tetrahydrofuro[2,3b]furan-3a-ols (**6b**).[2b] In this approach (Scheme 7.1) the tertiary hydroxyl group is introduced via alkylation of a suitably protected cyanohydrin (**11**).[7] Subsequently, the cyano group is reduced to an alcohol, instead of to an aldehyde as was required for (**6b**). Cyclization of (**10**) then yields a central intermediate (**9**), from which both (**7b**) and (**8b**) should in principle be accessible.

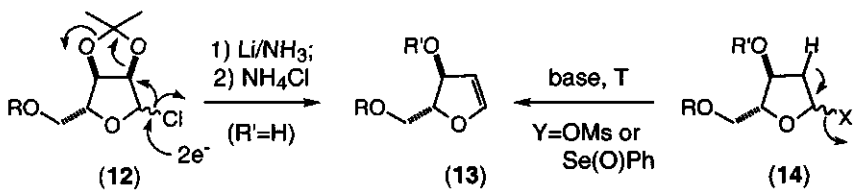
Scheme 7.1



Retrosynthetic scheme, outlining the planned synthetic approach in the preparation of the key target compounds (**7b**) and (**8b**). Boxed functional groups indicate the presence of an appropriate protecting group.

A number of syntheses of monocyclic 2,3-dihydrofuran-3-ol derivatives have appeared in the literature, mainly directed at the synthesis of furanoid glycals as (**13**). To our knowledge, no examples exist of compounds with this substructure, incorporating an alkyl substituent at the C-3 position. Two general approaches towards furanoid glycals can be distinguished (Scheme 7.2). Ireland and coworkers have developed a widely used method to prepare either pyranoid or furanoid glycals, based on the reductive elimination/fragmentation of a suitably activated precursor as (**12**).[8] Alternatively, various furanoid glycals have been obtained via elimination of a good C-5 leaving group from *e.g.* (**14**) under basic conditions, either at elevated temperature (X=OMs)[9] or at low temperature (X=Se(O)Ph).[10,11] The latter strategy was chosen for the planned synthesis of (**7b**) since it is most compatible with the route outlined above and because similar eliminations had also been used in our previous work (see Chapters 4-6).

Scheme 7.2

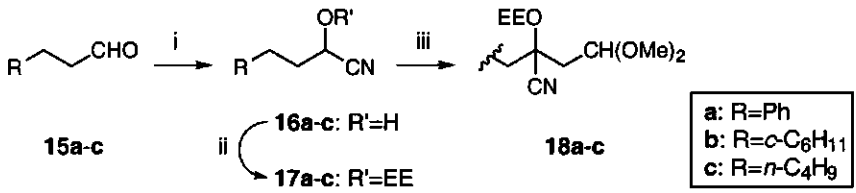


7.2 - Attempted Synthesis of 2,3-Dihydrofuran-3-ol-based Analogues

7.2.1 - Preparation of the Central Intermediate

The required cyanohydrins (**16a-c**) were prepared from the corresponding starting aldehydes (**15a-c**) through exchange of hydrocyanic acid from acetone cyanohydrin under basic conditions[12] (Scheme 7.3). Subsequently, the crude products were protected as the 2-ethoxyethyl (EE) ethers[13] (**17a,c**) by acid-catalyzed addition to ethylvinyl ether. In this way, (**17a**) and (**17c**) were easily obtained in 95-99% overall yield as 1 : 1 mixtures of diastereoisomers. The preparation of the cyclohexyl derivative (**17b**) was more difficult, due to the presence of several minor impurities in the crude product. After careful chromatographic purification, (**17b**) of sufficient purity (>92%, glc-analysis) was obtained in 84% yield from (**15b**).

Scheme 7.3



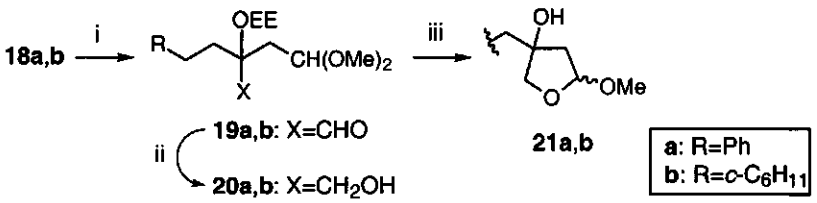
Reagents and conditions: (i) $Me_2C(OH)CN$ (1.0 eq), Et_3N (cat.) (95-99%); (ii) $EtOCH{=}CH_2$, TFA (cat.), 0°C (88-100%); (iii) 1) LDA (1.3 eq), THF-HMPA (1:1), -60°C, 30 min; 2) $BrCH_2CH(OMe)_2$, -60°C, 2 hrs (64-91%).

The protected cyanohydrins (**17a-c**) were alkylated with bromoacetaldehyde dimethylacetal, according to the general procedure developed by Stork *et al.*[14] The adducts (**18a**) and (**18c**) were obtained as 1 : 1 mixtures of diastereoisomers in good yields of 81% and 91%, respectively, while the cyclohexyl derivative (**18b**) was obtained in a somewhat lower yield of 64%.

The cyano group was reduced in two steps to the corresponding alcohol (Scheme 7.4). In our hands,[15] reduction of (**18a**) with DibalH failed repeatedly, even in refluxing ether. Similar problems had also been observed with other protected cyanohydrins during our synthesis of the perhydrofuro[2,3b]furan-3-ols (**6b**);[2b] these could be circumvented by the use of excess lithium triethoxyaluminiumhydride,[16] followed by hydrolysis of the resulting imine intermediates on silicagel. In this way, the aldehydes (**19a,b**) could be obtained in a reasonable yield of 64%. Subsequently, (**19a,b**) were reduced with lithium aluminiumhydride to afford the alcohols (**20a,b**) in 92-96% yield.

Finally, simultaneous deprotection and cyclization of (**20a,b**) under acidic conditions in methanol smoothly afforded (**21a,b**) in 82% yield as 1 : 2 mixtures of both diastereomeric isomers.

Scheme 7.4



Reagents and conditions: (i) 1) $LiAlH(OEt)_3$ (1.5-3.0 eq), Et_2O, 0°C, 2.5 hrs; 2) Glauber's salt, r.t., 1 hr; 3) silicagel, EtOAc, overnight (64%); (ii) $LiAlH_4$ (0.7-1.2 eq), Et_2O, 0°C, 15 min (92-96%); (iii) TsOH, MeOH (82%).

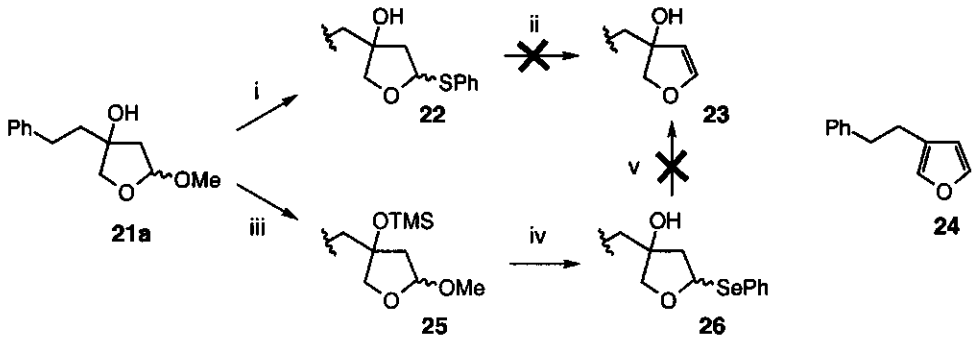
7.2.2 - Elimination Attempts towards the 3-Alkyl-2,3-dihydrofuran-3-ols

For the introduction of the cyclic enol ether functionality, we first tried the phenylsulfoxide elimination strategy, which had worked well on previous occasions.[2a,b] The phenylsulfide (**22**) could be prepared from (**21a**) in the usual way (Scheme 7.5), but attempts to convert this compound via its sulphoxide into the desired enol ether (**23**) failed. Usually a complex mixture of products was formed, containing 3-(2-phenylethyl)-furan (**24**) as the only identifiable component. This result corresponds with literature reports[8-10] on the behaviour of related furanoid glycals and suggested that (**23**) is an unstable compound with a tendency to aromatize under drastic conditions with expulsion of water.

We then turned our attention to the elimination of the corresponding phenylselenoxide derivatives. This type of elimination proceeds under much milder reaction conditions and has been succesfully applied in the synthesis of various 2,3-dihydrofuran-3-ol derivatives.[10,11] In addition, the hydroxyl group of (**21a**) was protected as a silyl ether in an attempt to lower the tendency of the ring system to aromatize during elimination.

Silylation of (**21a**) with TMSCl/hexamethyldisilazane (HMDSH) in pyridine gave the trimethylsilyl ether (**25**) in 74% yield; the sterically more demanding triethylsilyl group could not be introduced in (**21a**). Unfortunately, the TMS group was not stable under the conditions required for phenylselenide introduction: treatment of (**25**) with benzeneselenol and borontrifluoride etherate afforded the phenylseleno-alcohol (**26**) in 91% yield as a mixture of diastereomers[17] (2βH : 2αH = 2.6 : 1). In our hands, the oxidation and subsequent

Scheme 7.5



Reagents and conditions: (i) PhSH, $BF_3 \bullet Et_2O$, Et_2O, 0°C, 30 min (83%); (ii) mCPBA, toluene, 0°C; then Et_3N, 110°C, 10 min; (iii) TMSCl, HMDSH, pyridine (74%); (iv) PhSeH, $BF_3 \bullet Et_2O$, Et_2O, 0°C, 30 min (91%); (v) *t*-BuOOH (1.1 eq), $Ti(Oi\text{-}Pr)_4$ (1 eq), *i*-Pr_2NEt, CH_2Cl_2, 0°C, 45 min.

elimination of (**26**) with *t*-BuOOH in the presence of $Ti(Oi\text{-}Pr)_4$ [10,11] only led to rapid isomerization of (**26**) to its 2βH-isomer, which was recovered in approx. 70% yield. No traces of the 2,3-dihydrofuran-3-ol (**23**) or the corresponding furan (**24**) were found.

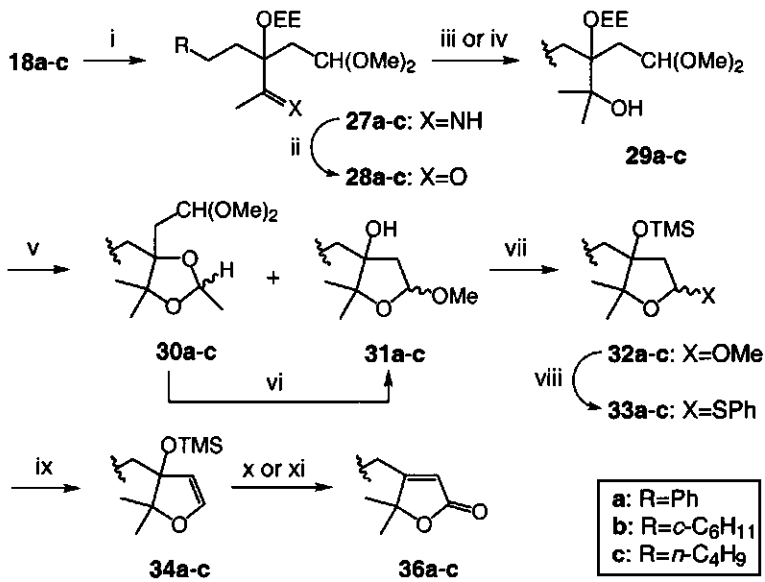
7.2.3 - 2,2-Dialkylated 3-Alkyl-2,3-dihydrofuran-3-ol Derivatives

In an attempt to obtain a more stable derivative of target structure (**7b**), it was decided to introduce two alkyl groups at the C-2 position of (**7b**), so that the possibility of aromatization to the corresponding furan would be blocked.

Addition of methyl lithium (Scheme 7.6) to the cyano group of (**18a-c**) initially yielded the imines (**27a-c**), which were sufficiently stable to withstand hydrolysis even in boiling water. These intermediates could be hydrolized without loss of the protecting group by heating for 24 hrs in a 10% NH_4Cl solution; the resulting ketones (**28a-c**) were obtained in 71-79% overall yield.

The introduction of a second methyl group via methyl lithium addition to (**28a-c**) was hampered by the formation of the lithium enolates of (**28**) as a major side reaction. However, after aqueous work-up, the alcohols (**29a-c**) could easily be separated from the ketones (**28a-c**) via chromatography, affording (**29a-c**) in 33-41% yield. By recycling the remaining starting material (41-46% recovery), a reasonable amount of the desired product could be obtained. Alternatively, the less basic $MeCeCl_2$ [18] could be used to minimize the enolisation of (**28**); in this way (**29a**) was obtained in one step from (**28a**) in 93-99% yield.

Scheme 7.6

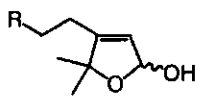


Reagents and conditions: (i) MeLi (1.6 eq), Et_2O, 0°C, 1.5 hrs; (ii) NH_4Cl, THF-water (1:1), 60-70°C, overnight (71-79% from **18**); (iii) MeLi, ether, 0°C (33-41% **29**, 41-46% **28**); (iv) $CeCl_3$;MeLi, THF, -78°C (93-99% **29a**); (v) TsOH (cat.), MeOH (56-60% **31**); (vi) TsOH (cat.), MeOH, 65°C, overnight; (vii) TMSCl, imidazole, DMF (89-100%); (viii) PhSH, $BF_3 \bullet Et_2O$ (1.5 eq), Et_2O, 4Å mol. sieves, 0°C, 30 min (98-100%); (ix) mCPBA, toluene, 0°C; then Et_3N, 110°C, 10 min; (x) (**36a,b**): 1) 40% HF, THF-water, 10 min; 2) Jones reagent, 0°C (58-61% from **33a,b**); (xi) (**36c**): 1) silicagel chromatography; 2) Jones reagent, 0°C (49% from **33c**).

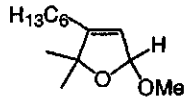
Cyclization of (**29a-c**) as before with *p*-toluenesulfonic acid in methanol unexpectedly yielded only 56-60% of the desired products (**31a-c**). In addition, an inseparable mixture of products was obtained, consisting of one diasteromer of (**31**) and both diastereomers of the cyclization product (**30a-c**). The formation of these unwanted side-products could not be suppressed by different reaction conditions: at elevated temperatures or after prolonged reaction time still about 25% of (**30**) remained present (glc-analysis). These compounds could only be converted completely into (**31a-c**) by repeated chromatographic separation of the fraction containing (**30a-c**), followed by renewed treatment with *p*-toluene-sulfonic acid in refluxing methanol.

The tertiary hydroxyl groups of (**31a-c**) were protected as their trimethylsilyl ethers in 89-100% yield. Subsequently, (**32a-c**) were transformed into the corresponding sulfides, yielding 98-100% of (**33a-c**) as 1 : 1 mixtures of isomers. *In situ* oxidation of (**33a-c**) to the sulphoxide, followed by thermal elimination as before, yielded crude products which could be identified as the desired enol ethers

(**34a-c**) from their ^{1}H NMR spectral data (Table 7.1). Upon purification of the crude products via chromatography on silicagel or florisil, the enol ethers (**34a,c**) were partly transformed into more polar products that lacked the TMS group. These rearrangement products were identified as the unsaturated lactols (**35a,c**) from their spectral data (Table 7.1) and from the rapid conversion of (**35c**) into the corresponding methyl ether (**37**) in MeOH/$CHCl_3$. Rearrangement of the enol ethers (**34**) did not occur during chromatography on neutral Al_2O_3 or reversed phase silicagel, but neither of these purification steps was sufficient to remove all impurities from the crude products. Finally, desilylation of the crude product (**34a**) with TBAF in THF and subsequent chromatography on silicagel yielded the same rearranged product (**35a**) as before.



35a: R=Ph
35c: R=*n*-C_4H_9



37

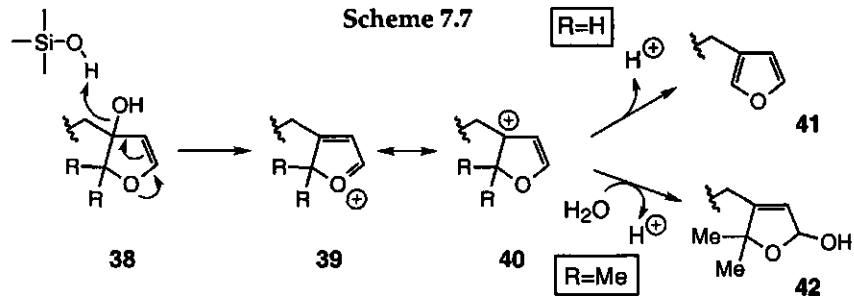
Table 7.1: Typical ^{1}H NMR spectral data† for the elimination- and rearrangement-products.

	H-4	H-5
34a,b	4.9 (d, J=3 Hz)	6.2 (d, J=3 Hz)
35a,c	5.3-5.5 (br s)	5.8-5.9 (br s, 0.3-0.4H) 6.0 (br s, 0.7-0.6H)
37	5.27 (2 d, J=1.2 Hz)	5.50 (2 d, J=1.3 Hz)

†) Measured in $CDCl_3$. Chemical shifts are reported in ppm, relative to TMS (δ=0).

The experiences with (**23**) and (**34a-c**) described above show that the 3-alkyl-2,3-dihydrofuran-3-ol ring system is rather unstable and that rearrangement reactions occur easily. This is probably due to the tertiary nature of the allylic hydroxyl group in (**38**), which can easily give a relatively stable cationic intermediate (**39**/**40**) after leaving (Scheme 7.7). Depending on the nature of the C-2 substituents in this intermediate, subsequent side-reactions can lead to aromatization to (**41**) or to capture of a nucleophile, *e.g.* water, leading to (**42**).

Scheme 7.7



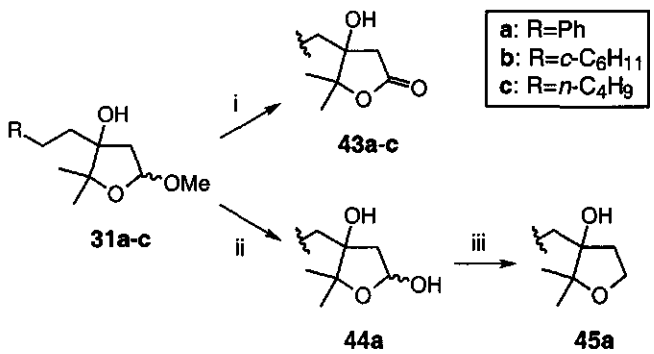
7.3 - Preparation of a Series of Butenolide-based Model Compounds

In view of the inherent instability of the key target structure (**7b**), we were forced to abandon further attempts into the synthesis of members of the planned test series (**7a-c**). Fortunately, several intermediates obtained during these attempts could be used in the preparation of some derivatives from the planned butenolide-based test series (**8a-c**).

In situ deprotection of the crude enol ethers (**34a,b**) with an aqueous 40% hydrogenfluoride solution (Scheme 7.6), followed by oxidation with Jones reagent, yielded the butenolides (**36a,b**) in an overall yield of 58-61% from the sulfides (**33a,b**). The butenolide (**36c**) was obtained by Jones oxidation of the silicagel rearrangement product (**35c**), affording (**36c**) in 49% overall yield from (**33c**). Attempts to reduce these compounds to the corresponding saturated lactones via catalytic hydrogenation on palladium failed, probably due to the C-2 methyl substituents blocking the approach to both faces of the butenolide ring.

Jones oxidation (Scheme 7.8) of the cyclization products (**31a-c**) afforded the 3-hydroxy-lactones (**43a-c**) in 85-100% yield. The diol (**44a**) could be obtained in 67% yield via acid-catalyzed partial hydrolysis of the cyclic methoxy-acetal (**31a**). Reduction of this diol with triethylsilane[19] gave the tetrahydrofuran-3-ol (**34a**) in 73% yield.

Scheme 7.8

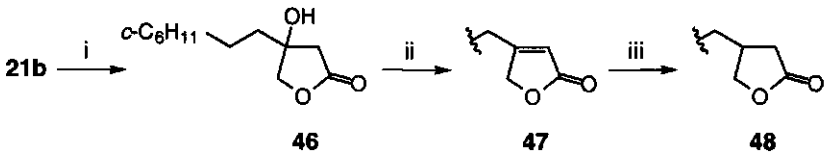


Reagents and conditions: (i) Jones reagent, acetone, 0°C, 10 min (85-100%); (ii) 4H HCl, THF, 30 min (67%); (iii) Et_3SiH (4 eq), $BF_3 \bullet Et_2O$ (2 eq), CH_2Cl_2, -78°C, 1 hr (73%).

The originally planned series of butenolide derivatives (**8a-c**) could be prepared from the central intermediate (**21b**). The 3-hydroxy-lactone (**46**) was obtained by Jones oxidation of (**21b**) in 79% yield (Scheme 7.9). In some test experiments, (**46**) was dehydrated under acidic conditions (conc. HCl, MeOH, reflux) to the butenolide (**47**). However, the crude product contained several major side products and over 72 hrs were required for complete transformation

of the starting material. In contrast, the elimination of (**46**) with $SOCl_2$ [20] was complete in 1 hr and yielded a mixture of (**47**) and its exocyclic double bond regioisomers; separation of these products by chromatography was difficult and pure (**47**) was obtained in only 39% yield. Finally, catalytic hydrogenation of (**47**) on palladium quantitatively yielded the corresponding lactone (**48**).

Scheme 7.9

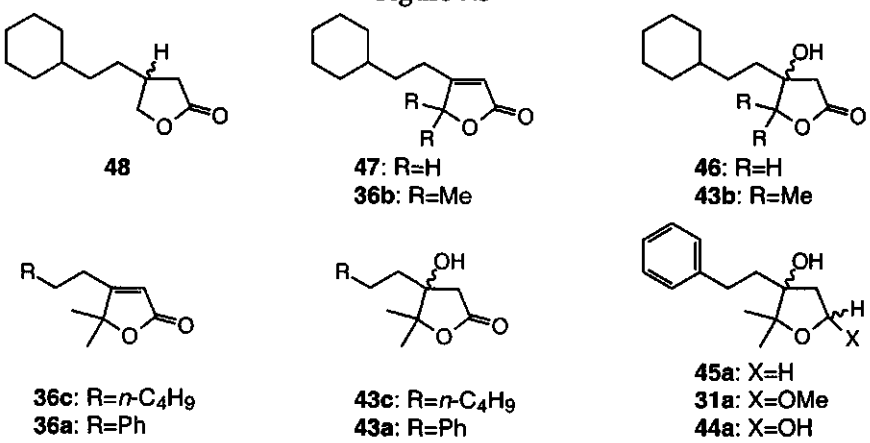


Reagents and conditions: (i) Jones reagent, acetone, 0°C (79%); (ii) $SOCl_2$ (5 eq), pyridine (20 eq), CH_2Cl_2, 0°C, 1 hr (39%); (iii) H_2, Pd-C, EtOAc, 6 hrs (100%).

7.4 - Summary

3-Alkyl-furans and 3-alkyl-butenolides were selected as attractive model compounds in the search for simplified bioactive analogues of natural clerodane insect antifeedants. The target structures were modified by introduction of a hydroxyl group at the C-3 position, in an attempt to increase the biological activity of these model compounds. The synthesis of a series of 3-alkyl-2,3-dihydrofuran-3-ol derivatives was unsuccessful, due to the instability of this moiety. Instead, a series of test compounds (Figure 7.3) was prepared, centered on 3-alkyl-3-hydroxy-γ-butyrolactones as the key target structure.

Figure 7.3



7.5 - Experimental Section

General experimental conditions were as described in the experimental section of Chapter 4.

2-(1-Ethoxyethoxy)-4-phenyl-butyronitril (17a). A mixture of 15 ml (0.11 mol) of hydrocinnamaldehyde (**15a**), 10 ml (0.11 mol; 1.0 eq) of acetone cyanohydrin and 0.2 ml (1.4 mmol) of triethylamine was stirred at r.t. for 2.5 hrs until tlc-analysis indicated complete disappearance of the starting material. The solvent was evaporated and the residue was dissolved in 50 ml of ether, washed with two 15 ml-portions of satd. sodium pyrosulphite soln. and two 15 ml-portions of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 17.5 g (0.11 mol; 99%) of the cyanohydrin (**16a**) as a yellow oil. The crude product was used without purification in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz, major peaks): δ 2.13-2.25 (m, 2H): H-3; 2.87 (t, J=7.7 Hz, 2H): H-4; 3.76 (br, 1H): OH; 4.44 (t, J=6.3 Hz, 1H): H-2; 7.21-7.41 (m, 5H): phenyl H-2' - H-6'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 30.6 (t); 36.5 (t); 60.3 (d): C-2; 120.1 (s): CN; 126.6 (d): phenyl C-4'; 128.6 (2 d): phenyl C-2',3',5',6'; 139.7 (s): phenyl C-1'.
A solution of 17.5 g (0.11 mol) of the crude cyanohydrin (**16a**) and 1.0 ml (13 mmol) of trifluoroacetic acid in 50 ml (0.52 mol; 4.75 eq) of ethylvinylether was stirred at 0°C for 20 hrs. The reaction mixture was diluted with 50 ml of ether, washed with three 20 ml-portions of satd. aqueous sodium bicarbonate soln. and two 20 ml-portions of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 25.7 g (0.11 mol; 100%) of a 1.3 : 1 mixture of diastereomeric isomers of the protected cyanohydrin (**17a**) as a pale-yellow oil. The crude product was sufficiently pure to be used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.21 and 1.25 (2 t, J=7.2 Hz; total 3H): $OCH_2C\underline{H}_3$; 1.39 and 1.42 (2 d, J=5.5 Hz; total 3H): $OCH(OEt)C\underline{H}_3$; 2.1-2.3 (2 t, J≈6.7 Hz, 2H): H-3; 2.87 (t, J=7.1 Hz, 2H): H-4; 3.44-3.76 (m, 2H): $OC\underline{H}_2CH_3$; 4.26 and 4.48 (2 t, J=6.6 Hz; total 1H): H-2; 4.84 and 4.95 (2 q, J=5.3 Hz; total 1H): $OC\underline{H}(OEt)CH_3$; 7.22-7.39 (m, 5H): phenyl H-2' - H-6'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.9 (2 q): $OCH_2\underline{C}H_3$; 19.5 (q): $OCH(OEt)\underline{C}H_3$; 30.8 (2 t); 35.1 and 35.5 (2 t); 61.3 (2 t): $O\underline{C}H_2CH_3$; 62.2 and 62.5 (2 d): C-2; 98.8 and 100.6 (2 d): $O\underline{C}H(OEt)Me$; 118.6 and 119.3 (2 s): CN; 126.431 (d): phenyl C-4'; 128.6 (3 d): phenyl C-2',3',5',6'; 139.8 (2 s): phenyl C-1'.

4-Cyclohexyl-2-(1-ethoxyethoxy)-butyronitril (17b). Hydrocyanation of 17 ml (0.11 mol) of 3-cyclohexylpropionaldehyde (**15b**), according to the procedure described for (**16a**), yielded 17.6 g of an oil containing the cyanohydrin (**16b**) and some major impurity. In view of the limited stability of unprotected cyanohydrins during chromatography, the oil was used in the next reaction without purification.
^{1}H-NMR (90 MHz, $CDCl_3$): δ 0.5-1.9 (br m, 15H): H-2, H-3 and *c*-hexyl H-1'-H-6'; 3.3-3.65 (br m, 1H): OH and impurity; 4.35 (t, J=6.0 Hz, 0.6H): H-2; 4.55 (t, J=4.5 Hz, 0.2H): impurity.
According to the procedure described for (**17a**), 17.6 g (105 mmol) of the crude cyanohydrin (**16b**) was reacted with ethylvinyl ether to give 25.3 g of a yellow oil. Partial purification of the crude product by chromatography on 135 g of silicagel with petrol-EtOAc (15-1) as the eluent yielded 22.0 g (92 mmol; 88%) of a 1 : 1 mixture of diastereomeric isomers of (**17b**) as an oil, which was used as such in the next reaction. A small sample was chromatographed to analytical purity.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.7-1.85 (br m, 21H); 3.35-3.70 (m, 2H): $OC\underline{H}_2CH_3$; 4.20 and 4.37 (2 t, J=6.6 Hz; total 1H): H-2; 4.76 and 4.85 (2 q, J=5.1 Hz; total 1H): $OC\underline{H}(OEt)CH_3$. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 15.0 (3 q): $OCH_2\underline{C}H_3$; 19.4 (q): $OCH(OEt)\underline{C}H_3$; 26.3 (3 t); 31.0 (t); 31.5 (t); 32.1 (t); 33.0 (t); 37.0 (d): *c*-hex C-1'; 60.8 and 61.3 (2 t): $O\underline{C}H_2CH_3$; 64.4 (d): C-2; 98.7 and 100.3 (2 d): $O\underline{C}H(OEt)Me$; 118.7 and 119.5

(2 s): CN. MS: *m/e* (%) 212 (3), 96 (2), 83 (3), 81 (3), 74 (4), 73 (100), 67 (3), 55 (5), 45 (16), 41 (2). HRMS: calcd. (M-HCN): 212.1776; found: 212.1773.

2-(1-Ethoxyethoxy)-octanenitril (17c). Hydrocyanation of 14.8 ml (0.11 mol) of heptanal (**15c**), according to the procedure described for (**16a**), yielded 14.8 g (0.10 mmol; 95%) of the cyanohydrin (**16c**) as an oil, which was used in the next reaction without purification.
^{1}H-NMR (90 MHz, $CDCl_3$): δ 0.7-1.05 (br m, 3H), 1.05-1.65 (br m, 8H) and 1.65-2.0 (br m, 2H): H-3 - H-8; 4.25 (br, 1H): OH; 4.45 (t, J=6.0 Hz, 1H): H-2.
According to the procedure described for (**17a**), 14.7 g (0.10 mol) of the crude cyanohydrin (**16c**) was protected as its ethoxyethyl ether, affording 21.9 g (0.10 mol; 100%) of a 1.1 : 1 mixture of diastereomeric isomers of the cyanohydrin (**17c**) as an oil, which was sufficiently pure to be used in the next reaction without purification.
^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.7-1.0 (br t, J=6 Hz, 3H): H-8; 1.0-2.2 (br m, 18H): H-3 - H-7, $OCH(OEt)C\underline{H}_3$ and $OCH_2C\underline{H}_3$; 3.2-3.8 (m, 2H): $OC\underline{H}_2CH_3$; 4.16 and 4.32 (2 t, J=6 Hz; total 1H): H-2; 4.73 and 4.80 (2 q, J=6 Hz; total 1H): $OC\underline{H}(OEt)CH_3$.

3-Cyano-3-(2-ethoxyethoxy)-5-phenyl-pentanal dimethylacetal (18a). A solution of 3.6 g (15.4 mmol) of the protected cyanohydrin (**17a**) in 10 ml of anhydrous THF and 20 ml of HMPA was added dropwise in 10 min to 10 ml of a 2M solution of lithium diisopropylamide in THF-hexanes while stirring under nitrogen atmosphere at -60°C. After 30 min the reaction mixture was treated dropwise with a solution of 3.4 g (20 mmol) of bromoacetaldehyde dimethylacetal in 10 ml of THF. Stirring at -60°C was continued for 2 hrs, while the progress of the reaction was monitored by glc-analysis. The reaction was quenched by addition of 100 ml of water and the resulting mixture was extracted with four 50 ml-portions of ether. The combined extracts were washed with two 50 ml-portions of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 6.0 g of a yellow oil. Purification via chromatography on 90 g of silicagel with petrol-EtOAc (10-1) afforded 4.0 g (12.5 mmol; 81 %) of (**18a**) as a 1 : 1 mixture of diastereomeric isomers.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.22 (2 q, J=6.3 Hz, 3H): $OCH_2C\underline{H}_3$; 1.37 and 1.39 (**2 d**, J=3.9 Hz; total 3H): $OCH(OEt)C\underline{H}_3$; 2.00-2.38 (m, 4H): H-2 and H-4; 2.70-2.95 (m, 2H): H-5; 3.35-3.40 (**m s**, 6H): OCH_3; 3.57 and 3.65 (**2 q**, J=6.4 Hz; total 3H): $OC\underline{H}_2CH_3$; 4.65 and 4.71 (**2 t**, J=5.5 Hz; total 1H): H-1; 5.05-5.21 (m, 1H): $OC\underline{H}(OEt)Me$; 7.15-7.40 (m, 5H): phenyl H-2' - H-6'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 15.1 (q): $OCH_2\underline{C}H_3$; 20.9 (q): $OCH(OEt)\underline{C}H_3$; 30.2 (t); 40.6 (2 t) and 41.2 (2 t): C-2; 53.1 (3 q): OCH_3; 60.8 (2 t): $O\underline{C}H_2CH_3$; 73.9 (s): C-3; 98.2 (2 d) and 100.7 (2 d): C-1 and $O\underline{C}H(OEt)Me$; 119.3 (2 s): CN; 126.2 (d): phenyl C-4'; 128.4 (2 d): phenyl C-2',3',5',6'; 140.5 (2 s): phenyl C-1'. MS: *m/e* (%) 295 (0.4), 289 (0.2), 282 (0.3), 281 (1.6), 200 (18), 199 (28), 168 (14), 167 (21), 91 (34), 89 (13), 75 (68), 73 (100), 59 (15). HRMS: calcd. (M-MeOH): 289.1678; found: 289.1684.

3-Cyano-5-cyclohexyl-3-(2-ethoxyethoxy)-pentanal dimethylacetal (18b). Alkylation of 5.0 g (20.9 mmol) of the partially purified cyanohydrin (**17b**) according to the procedure described for (**18a**) yielded, after purification by chromatography on silicagel with petrol-EtOAc (gradient elution, 99-1 to 97-3) as the eluent, 4.4 g (13.5 mmol; 64%) of a 1 : 1 mixture of diastereomeric isomers of (**18b**) as a colourless oil, which was used as such in subsequent reactions. A small sample was chromatographed to analytical purity.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.62-1.10 (br m); 1.10-1.30 (br m); 1.41-1.80 (br m); 1.91 (t, J=4.7 Hz, 1H): H-2; 1.97 (d, J=5.2 Hz, 1H): H-2'; 3.15-3.20 (m, 6H): OCH_3; 3.27-3.57 (m, 2H): $OC\underline{H}_2CH_3$; 4.46 and 4.53 (2 t, J=5.2 Hz; total 1H): H-1; 4.87 and 4.95 (2 q, J=4.9 Hz; total 1H): $OC\underline{H}(OEt)Me$. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.8 (2 q): $OCH_2\underline{C}H_3$; 20.6 and 20.9 (2 q): $OCH(OEt)\underline{C}H_3$; 26.1 (3 t); 30.9 (2 t); 32.9 (2 t); 36.0 and

36.6 (2 t); 37.3 (2 d): *c*-hexyl C-1'; 40.3 and 40.9 (2 t); 52.8 (4 q): OCH_3; 60.2 and 60.5 (2 t): $O\underline{C}H_2CH_3$; 74.1 (2 s): C-3; 97.6 (d): C-1; 100.4 (2 d): $O\underline{C}H(OEt)Me$; 119.2 (2 s): CN. MS: *m/e* (%) 312 (0.1), 254 (11), 222 (14), 220 (5), 206 (17), 75 (89), 73 (100), 59 (12), 55 (7), 45 (27). HRMS: calcd. (M-CH_3): 312.2175; found: 312.2176.

3-Cyano-3-(2-ethoxyethoxy)-nonanal dimethylacetal (18c). Alkylation of 3.3 g (15.4 mmol) of the crude cyanohydrin (**17c**) according to the procedure described for (**17a**) yielded, after purification via chromatography on silicagel with petrol-EtOAc (6-1) as the eluent, 4.2 g (14.0 mmol; 91%) of a 1 : 1 mixture of diastereomeric isomers of (**18c**) as a colourless oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.75-0.90 (br, 3H): C-9; 1.05-1.50 (br m, 14H); 1.70-1.95 (br m) and 2.01-2.12 (m; total 4H): H-2 and H-4; 3.29-3.36 (7 s, 7H): OMe; 3.40-3.75 (m, 2H): $OC\underline{H}_2CH_3$; 4.51, 4.59 and 4.65 (3 t, J≈5.4 Hz, 1H): H-1; 5.02 and 5.04 (2 q, J=5.1 Hz, 1H): $OC\underline{H}(OEt)Me$. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.0 and 15.1 (2 q): $OCH_2\underline{C}H_3$; 20.9 and 21.2 (2 q): $OCH(OEt)\underline{C}H_3$; 22.5 (t); 23.6 and 23.8 (2 t); 29.0 and 29.1 (2 t); 30.6 (t); 31.5 (t); 38.7 and 39.3 (2 t); 40.6 and 41.2 (2 t); 53.0, 53.1, 53.2 and 53.9 (4 q): OCH_3; 60.5 and 60.9 (2 t): $O\underline{C}H_2CH_3$; 74.3 (s): C-3; 97.9, 100.6, 100.8 and 103.0 (4 d): C-1 and $O\underline{C}H(OEt)Me$; 119.5 (s): CN. MS (CI): *m/e* (%): 302 (68), 270 (28), 258 (26), 212 (50), 198 (28), 194 (100), 180 (93), 171 (26). HRMS (CI): calcd. (M+H): 302.2321; found: 302.2328.

3-(2-Ethoxyethoxy)-1,1-dimethoxy-5-phenyl-pentane-3-carbaldehyde (19a). To an ice-cold solution of 6.7 mmol (1.67 eq) of lithium aluminiumhydride in 20 ml of anhydrous ether (prepared by dilution of a commercial 1.0M soln. of $LiAlH_4$ in ether) was added dropwise 1.0 ml (10.0 mmol) of anhydrous EtOAc in approx. 5 min while stirring vigorously. The resulting white slurry was stirred for another 30 min before a solution of 1.3 g (4.0 mmol) of the nitrile (**18a**) and 2 drops of *n*-decane in 10 ml of ether was added dropwise. Stirring at 0°C was continued, while the progress of the reaction was monitored via glc-analysis. After complete disappearance of the starting material (relative to *n*-decane as internal standard; approx. 1.5 hrs), the mixture was diluted with 50 ml of ether and the reaction was quenched by addition of 3.5 g (10.8 mmol) of Glaubers' salt. The resulting mixture was stirred at r.t. for 1 hr, dried with $MgSO_4$, filtered through a pad of hyflo and concentrated under reduced pressure to give 1.23 g of an oil. The residue was taken up in EtOAc (100 ml), 30 g of silicagel was added and the mixture was stirred overnight at r.t. The resulting slurry was filtered and concentrated to give 1.21 g of an oil. The crude product was purified by chromatography on 30 g of silicagel with petrol-EtOAc (95-5) as the eluent to yield 830 mg (2.6 mmol; 64%) of the aldehyde (**19a**) as a pale-yellow oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.14 and 1.17 (2 t, J=7.0 Hz; total 3H): $OCH_2C\underline{H}_3$; 1.39 and 1.40 (2 d, J=5.1 Hz; total 3H): $OCH(OEt)C\underline{H}_3$; 1.85-2.25 (m, 4H): H-2 and H-4; 2.45-2.85 (m, 2H): H-5; 3.34-3.56 (m, 8H): $OC\underline{H}_2CH_3$ and OCH_3 [δ 3.32 and 3.36 (2 s)]; 4.62 (dd, J=6.1, 4.7 Hz) and 4.68 (t, J=5.1 Hz; total 1H): H-1; 4.78 (q, J=5.1 Hz, 0.67 H) and 4.88 (q, J=5.1 Hz, 0.33 H): $OC\underline{H}(OEt)OMe$; 7.15-7.35 (m, 5H): phenyl H-2" - H-6"; 9.51 (s, 0.67H) and 9.57 (s, 0.33H): CHO. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.9 (2 q): $OCH_2\underline{C}H_3$; 20.4 (q): $OCH(OEt)\underline{C}H_3$; 28.6 and 29.1 (2 t); 32.0 (t); 35.2 and 35.4 (2 t); 38.6 (t); 52.6 (2 q) and 53.3 (2 q): OCH_3; 60.5 and 60.7 (2 t); 81.3 and 82.2 (2 s): C-3; 95.4 and 95.7 (2 d): $O\underline{C}H(OEt)Me$; 100.7 and 101.1 (2 d): C-1; 126.0, 128.2 and 128.4 (3 d): phenyl C-2" - C-6"; 141.5 (2 s): phenyl C-1"; 202.1 and 202.4 (2 s): CHO.

2-(2-Ethoxyethoxy)-2-(2,2-dimethoxyethyl)-4-phenyl-butanol (20a). To an ice-cold, stirred mixture of 60 mg (1.6 mmol) of lithium aluminiumhydride and 5 ml of anhydrous ether was added dropwise a solution of 784 mg (2.4 mmol) of the aldehyde (**19a**) in 10 ml of ether. After 15 min the reaction was quenched by addition of 1.0 g (3.1 mmol) of Glaubers' salt. The resulting slurry was stirred at r.t. for 30 min, dried with $MgSO_4$, filtered through a pad

of hyflo and concentrated under reduced pressure to give 813 mg of an oil. Purification by chromatography on 20 g of silicagel with petrol-EtOAc (gradient elution, 95-5 to 90-10) as the eluent afforded 726 mg (2.2 mmol; 92%) of the alcohols (**20a**) as a pale-yellow oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.22 (2 t, J=7.1 Hz, 3H): $OCH_2C\underline{H}_3$; 1.34 (d, J=5.3 Hz, 3H): $OCH(OEt)C\underline{H}_3$; 1.75-1.98 (m, 4H): H-3 and H-1'; 2.59-2.74 (m, 2H): H-4; 3.36 (3 s, 6H): OCH_3; 3.40-3.80 (m, 5H): H-1, OH and $OC\underline{H}_2CH_3$; 4.61 (t, J=4.8 Hz, 1H): H-2'; 4.99 (q, J=5.2 Hz, 1H): $OC\underline{H}(OEt)OMe$; 7.10-7.35 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 15.2 (q): $OCH_2\underline{C}H_3$; 20.4 (q): $OCH(OEt)\underline{C}H_3$; 29.6 (t); 34.5 and 35.6 (2 t); 37.4 and 37.8 (2 t); 52.7 (2 q): OCH_3; 59.0 (2 t): $O\underline{C}H_2CH_3$; 65.9 and 66.4 (2 t): C-1; 79.5 and 79.8 (2 s): C-2; 93.2 and 93.8 (2 d): $O\underline{C}H(OEt)Me$; 101.6 and 101.8 (2 d): C-2'; 125.7 (2 d) and 128.3 (d): phenyl C-2" - C-6"; 142.5 (s): phenyl C-1".

2-Methoxy-4-(2-phenylethyl)-tetrahydrofuran-4-ol (21a). A solution of 491 mg (1.5 mmol) of the alcohols (**20a**) and 29 mg (0.15 mmol) of *p*-toluenesulfonic acid monohydrate in 25 ml of anhydrous methanol was stirred at room temperature for 20 hrs. The purple reaction mixture was concentrated under reduced pressure and the residue was taken up in 50 ml of ether, washed with 10 ml of satd. aqueous sodium bicarbonate solution and 10 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 297 mg of an oil, consisting of both C-2 epimers of (**21a**) in 1 : 1.6 ratio (least polar isomer : most polar isomer). The crude product was sufficiently pure to be used as such in subsequent reactions; both isomers could be separated by careful chromatography on silicagel, using petrol-EtOAc (gradient elution, 98-2 to 90-10) as the eluent.
Least polar isomer: ^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.88-1.97 (m, 3H): H-3a [δ 1.92 (dd, J=13.3, 4.4 Hz)] and H-1'; 2.08 (d, J=13.4 Hz, 1H): H-3b; 2.74-2.82 (m, 2H): H-2'; 3.39 (s, 3H): OCH_3; 3.55 (s, 1H): OH; 3.82 (d, J=9.3 Hz, 1H) and 3.99 (d, J=9.3 Hz, 1H): H-5; 5.07 (d, J=4.5 Hz, 1H): H-2; 7.11-7.33 (m, 5H): Ph. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 31.0 (t) and 39.5 (t): C-1' and C-2'; 45.2 (t): C-3; 54.8 (q): OCH_3; 79.3 (s): C-4; 80.3 (t): C-5; 105.4 (d): C-2; 125.8, 128.3 and 128.4 (3 d): phenyl C-2" - C-6"; 142.1 (s): phenyl C-1". *Most polar isomer*: ^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.85-2.10 (m, 4H): H-3a, H-1' and OH; 2.28 (ddd, J=14.4, 5.8, 1.0 Hz, 1H): H-3b; 2.75 (d, J=10.5 Hz) and 2.78 (dd, J=10.4, 1.1 Hz; total 2H): H-2'; 3.37 (s, 3H): OCH_3; 3.74 (2 s, 2H): H-5; 5.18 (dd, J=5.8, 3.3 Hz, 1H): H-2; 7.14-7.33 (m, 5H): Ph. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 30.9 (t) and 39.4 (t): H-1' and H-2'; 47.3 (t): C-3; 55.3 (q): OCH_3; 76.7 (t): C-5; 81.0 (s): C-4; 105.3 (d): C-2; 126.0, 128.3 and 128.5 (3 d): phenyl C-2" - C-6"; 141.8 (s): phenyl C-1".

4-(2-Cyclohexylethyl)-2-methoxy-tetrahydrofuran-4-ol (21b). According to the procedure described for (**19a**), 1.0 g (3.0 mmol) of the nitriles (**18b**) was reduced with 3.0 equiv. of $LiAlH(OEt)_3$ in ether to give, after work-up and hydrolysis of the intermediate product on silicagel, 911 mg of a yellow oil. Chromatography on 20 g of silicagel with petrol-EtOAc (95-5) as the eluent yielded 634 mg (1.9 mmol; 64%) of a crude 1 : 1 diastereomeric mixture of the aldehydes (**19b**) as an oil, which was used as such in the next reaction.
^{1}H-NMR ($CDCl_3$, 90 MHz): δ 0.4-2.2 (br); 3.1-3.7 (br m, 8H): OCH_3 [δ 3.3 (s)] and $OC\underline{H}_2CH_3$; 4.4-4.9 (br m, 2H):$OCH_2C\underline{H}_3$; 9.4 (s, 0.5H) and 9.5 (s, 0.5H): CHO.
The aldehydes (**19b**) were reduced with $LiAlH_4$ according to the procedure described for (**20a**) to yield 572 mg (1.7 mmol; 96%) of the crude alcohols (**20b**) as a yellow oil. This crude product was cyclized as described for (**21a**) to give 371 mg of a yellow oil, consisting of both C-2 epimers of (**21b**) in 1 : 1.6 ratio. Careful chromatography on 10 g of silicagel with petrol-EtOAc (gradient elution, 98-2 to 90-10) afforded 111 mg (0.49 mmol; 29% yield from **20b**) of the least polar isomer of (**21b**) and 203 mg (0.89 mmol; 52%) of the most polar isomer.
Least polar isomer: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.70-0.95 (br m, 2H), 1.02-1.48 (br m, 7H) and 1.52-1.75 (br m, 7H): H-1', H-2' and *c*-hexyl H-1" - H-6"; 1.86 (dd, J=13.3, 4.6 Hz, 1H)

and 1.99 (d, J=13.4 Hz, 1H): H-3; 3.34 (s, 3H): OCH_3; 3.41 (s, 1H): OH; 3.77 (d, J=9.3 Hz, 1H) and 3.93 (d, J=9.2 Hz, 1H): H-5; 5.02 (d, J=4.4 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 26.4 (t); 26.7 (t); 32.2 (t); 33.3 (t); 34.9 (t); 38.1 (d): *c*-hexyl C-1"; 45.2 (t): C-3; 54.8 (q): OCH_3; 80.2 (s): C-4; 80.3 (t): C-5; 105.6 (d): C-2. *Most polar isomer*: ^1H NMR ($CDCl_3$, 200 MHz): δ 0.70-1.0 (br m, 2H), 1.02-1.40 (br m, 7H) and 1.51-1.77 (br m, 7H): H-1', H-2' and *c*-hexyl H-1" - H-6"; 1.83 (dd, J=14.3, 3.3 Hz, 1H): H-3a; 2.06 (br, 1H): OH; 2.20 (dd, J=14.2, 5.8 Hz, 1H): H-3b; 2.98 (s, 3H): OCH_3; 3.70 (s, 2H): H-5; 5.14 (dd, J=5.8, 3.3 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 26.3 (t); 26.6 (t); 32.0 (t); 33.3 (2 t); 34.9 (t); 38.0 (d): *c*-hexyl C-1"; 47.1 (t): C-3; 55.3 (q): OCH_3; 76.8 (t): C-5; 81.3 (s): C-4; 105.5 (d): C-2.

4-Hydroxy-4-(2-phenylethyl)-tetrahydrofuran-2-phenylselenide (26). Both diastereomers could be separated by chromatography on silicagel with petrol-EtOAc (gradient elution, 97-3 to 90-10) as the eluent. *Least polar isomer*, 2αH-(**26**): ^1H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.98 (d, J=16.6 Hz) and (t, J=2.9 Hz; total 2H): H-1'; 2.26 (ddd, J=14.2, 2.1, 0.7 Hz, 1H): H-3α; 2.50 (dd, J=14.2, 7.5 Hz, 1H): H-3β; 2.70 (s, 1H): OH; 2.79 (d, J=16.6 Hz) and (t, J=2.9 Hz; total 2H): H-2'; 3.81 (d, J=9.6 Hz, 1H) and 4.07 (d, J=9.6 Hz, 1H): H-5; 5.98 (dd, J=6.9, 2.1 Hz, 1H): H-2; 7.19-7.33 (m, 8H): SePh H-3" - H-5" and phenyl H-2"' - H-6"'; 7.62-7.66 (m, 2H): SePh H-2" and H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, selected peaks): δ 30.9 (t) and 40.4 (t): C-1' and C-2'; 47.2 (t): C-3; 79.7 (t): C-5; 80.0 (s): C-4; 83.3 (d): C-2; 126.1, 127.8, 128.4, 128.6, 129.2 and 134.2 (6 d): SePh C-2" - C-6" and phenyl C-2"' - C-6"'; 130.0 (s): SePh C-1"; 141.8 (s): phenyl C-1"'. *Most polar isomer*, 2βH-(**26**): ^1H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.90-2.08 (m, 4H): H-3a, H-1' and OH; 2.52 (ddd, J=14.3, 7.3, 1.5 Hz, 1H): H-3b; 2.71-2.79 (m, 2H): H-2'; 3.73 (dd, J=9.6, 1.6 Hz, 1H) and 3.86 (d, J=9.7 Hz, 1H): H-5; 6.04 (t, J=6.9 Hz, 1H): H-2; 7.17-7.29 (m, 8H): SePh H-3" - H-5" and phenyl H-2"' - H-6"'; 7.60-7.64 (m, 2H): SePh H-2" and H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, selected peaks): δ 31.0 (t) and 38.8 (t): C-1' and C-2'; 47.5 (t): C-3; 77.1 (t): C-5; 80.5 (s): C-4; 83.0 (d): C-2; 126.1, 127.6, 128.3, 128.5, 129.0 and 133.8 (6 d): SePh C-2" - C-6" and phenyl C-2"' - C-6"'; 130.1 (s): SePh C-1"; 141.5 (s): phenyl C-1"'.

3-(2-Ethoxyethoxy)-3-(2,2-dimethoxyethyl)-5-phenyl-pentan-2-one (28a). To an ice-cold, stirred solution of 2.15 g (6.7 mmol) of the nitrile (**18a**) in 50 ml of anhydrous ether was added dropwise via a syringe 6.0 ml (10.7 mmol) of a 1.6M solution of methyllithium in ether. Stirring was continued for 90 min, while the reaction was monitored via tlc-analysis. The reaction was quenched by addition of 25 ml of satd. aqueous ammoniumchloride solution. The organic layer was separated and the aqueous layer was extracted with three 25 ml-portions of ether. The combined organic layers were washed with 25 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 2.26 g (6.5 mmol) of the intermediate imine (**27a**).
^1H NMR ($CDCl_3$, 90 MHz): δ 1.0-1.3 (2 t, J=6.0 Hz, 3H): $OCH_2C\underline{H}_3$; 1.3-1.5 (d, J=6.0 Hz, 3H): $OCH(OEt)C\underline{H}_3$; 1.7-2.5 (br m, 9H): H-1, H-4, H-5 and H-1'; 3.1-3.7 (m, 8H): $OC\underline{H}_2CH_3$ and OCH_3 [δ 3.25-3.30 (3 s, 6H)]; 4.2 and 4.5 (2 t, J=4.5 Hz, 1H): H-2'; 4.7-5.1 (m, 1H): $OC\underline{H}(OEt)Me$; 6.9-7.4 (br m, 5H): phenyl H-2"-H-6"; 9.3-10.2 (br, 1H): -C(N$\underline{H}$)Me. IR ($CHCl_3$) : ν 1640 cm^{-1}.
A mixture of 2.26 g (6.5 mmol) of the crude imine (**27a**) in 50 ml of THF and 50 ml of a 10% aqueous ammoniumchloride solution was stirred at 60-70°C for 24 hrs until tlc-analysis indicated complete hydrolysis of the imine. The mixture was extracted with three 25 ml-portions of ether and the combined extracts were washed with 25 ml of brine, dried with $MgSO_4$ and concentrated to yield 2.25 g of a yellow oil. The crude product was purified via chromatography on 50 g of silicagel with petrol-EtOAc (5-1) as the eluent, affording 1.81 g (5.3 mmol; 79% from the nitrile) of the ketone (**28a**) as an oil.

^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.12 and 1.16 (2 t, J=6.5 Hz, 3H): $OCH_2C\underline{H}_3$; 1.38 and 1.39 (2 d, J=5.1 Hz, 3H): $OCH(OEt)C\underline{H}_3$; 1.8-2.6 (m, 9H): H-1 [δ 2.24 and 2.26 (2 s, 3H)], H-4, H-5 and H-1'; 3.25, 3.26, 3.28 and 3.30 (4 s, 6H): OCH_3; 3.42 and 3.46 (2 q, J=7.5 Hz, 2H): $OC\underline{H}_2CH_3$; 4.39 and 4.70 (2 t, J=5.6 Hz, 1H): H-2'; 4.76-4.90 (m, 1H): $OC\underline{H}(OEt)Me$; 7.10-7.29 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 15.0 (q): $OCH_2\underline{C}H_3$; 20.8 (q): $OCH(OEt)\underline{C}H_3$; 26.6 (q): C-1; 29.2 and 29.4 (2 t); 35.6, 37.2, 37.7 and 38.6 (4 t); 51.8, 52.6 and 53.1 (3 q): OCH_3; 60.8 and 61.0 (2 t): $O\underline{C}H_2CH_3$; 84.2 and 84.6 (2 s): C-3; 95.3 and 95.7 (2 d): $O\underline{C}H(OEt)Me$; 101.0 (d): C-2'; 125.8 and 126.0 (2 d) and 128.1, 128.2, 128.3 and 128.4 (4 d): phenyl C-2" - C-6"; 141.5 and 142.0 (2 s): phenyl C-1"; 211.1 (s): C-2. IR ($CHCl_3$) : ν 1710 cm^{-1}. MS (EI): *m/e* (%) 295 (2), 223 (7), 205 (15), 191 (18), 133 (12), 105 (9), 91 (20), 75 (21), 73 (100), 45 (40), 43 (9). MS (CI): *m/e* (%) 308 (10), 307 (100), 294 (6), 293 (31), 263 (7), 235 (9), 231 (8), 217 (4), 185 (5). HRMS (EI): calcd. ($M-CH_3CO$): 295.1909; found: 295.1909.

5-Cyclohexyl-3-(2-ethoxyethoxy)-3-(2,2-dimethoxyethyl)-pentan-2-one (28b). Addition of methyllithium to 1.52 g (4.6 mmol) of the nitrile (**18b**) and subsequent hydrolysis of the intermediate imine according to the procedure described for (**28a**) yielded, after purification via chromatography on silicagel with petrol-EtOAc (gradient elution, 7-1 to 5-1) as the eluent, 1.14 g (3.3 mmol; 71%) of the ketone (**28b**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz, major peaks): δ 0.65-1.30 (br m, 18H) and 1.50-1.70 (br, 9H); 1.96-2.06 (m, 1H) and 2.12 (d, J=7.2 Hz, 1H): H-1'; 2.11 and 2.15 (2 s, 3H): H-1; 3.17, 3.19, 3.20 and 3.24 (4 s, 6H): OCH_3; 3.30-3.41 (m, 3H): $OC\underline{H}_2CH_3$; 4.28 (t, J=5.5 Hz, 0.5H): C-2'; 4.58-4.75 (m, 1.5H): C-2' and $OC\underline{H}(OEt)Me$. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.8 and 14.9 (2 q): $OCH_2\underline{C}H_3$; 20.7 (q): $OCH(OEt)\underline{C}H_3$), 26.2 and 26.5 (2 t); 29.9, 30.0 and 30.2 (3 t); 32.7, 33.1 and 33.3 (3 t); 37.3 and 38.4 (2 t); 37.9 and 38.1 (2 d): *c*-hexyl C-1"; 51.8, 52.2, 52.8 and 53.4 (4 q): OCH_3; 60.8 and 61.0 (2 t): $O\underline{C}H_2CH_3$; 83.9 and 84.7 (2 s): C-3; 95.2 and 95.5 (2 d): C-2'; 101.0 (2 d): $O\underline{C}H(OEt)Me$; 211.1 and 211.4 (2 s): C-2. IR ($CHCl_3$): ν 1705 cm^{-1}. MS: *m/e* (%) 229 (14), 197 (20), 139 (18), 75 (17), 73 (100), 45 (20). HRMS: calcd. ($M-OCH_3$): 313.2379; found: 313.2387.

3-(2-Ethoxyethoxy)-3-(2,2-dimethoxyethyl)-nonan-2-one (28c). Addition of methyllithium to 3.9 g (13.0 mmol) of the nitrile (**18c**) and subsequent hydrolysis of the intermediate imine according to the procedure described for (**28a**) yielded, after purification via chromatography on silicagel with petrol-EtOAc (6-1) as the eluent, 3.15 g (9.9 mmol; 76%) of the ketone (**28c**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.7-0.9 (br, 3H): H-9; 0.9-1.35 (m, 15H) and 1.4-1.8 (m, 2H) and 1.8-2.2 (m, 5H): H-1 [δ 2.08 and 2.11 (2 s, 3H)], H-4-H-8, H-1' and $OCH_2C\underline{H}_3$; 3.13, 3.15, 3.17 and 3.20 (4 s, 6H): OCH_3; 3.33 and 3.36 (2 dq, J≈6.5 Hz, 2H): $OC\underline{H}_2CH_3$; 4.26 (t, J=5.0 Hz, 0.5H): C-2'; 4.55-4.72 (m, 1.5H): C-2' and $OC\underline{H}(OEt)Me$. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 13.9 (q): C-9; 14.8 and 14.9 (2 q): $OCH_2\underline{C}H_3$; 20.7 (q): $OCH(OEt)\underline{C}H_3$; 22.4, 22.5, 22.6 and 22.8 (4 t); 26.4 and 26.5 (2 q): C-1; 29.4 and 29.6 (2 t); 31.4 and 31.6 (2 t); 33.3 (t); 35.2 (t); 37.6 (t); 38.6 (t); 51.8, 52.2, 52.8 and 53.2 (4 q): OCH_3; 60.5 and 60.9 (2 t): $O\underline{C}H_2CH_3$; 84.2 and 84.8 (2 s): C-3; 95.1 and 95.5 (2 d): $O\underline{C}H(OEt)Me$; 101.0 (d): C-2'; 211.4 (s): C-2. IR ($CHCl_3$): ν 1710 cm^{-1}. MS: *m/e* (%) 287 (0.2), 203 (14), 197 (15), 185 (11), 171 (25), 113 (26), 75 (25), 73 (100), 45 (55), 43 (16). HRMS: calcd. ($M-OCH_3$): 287.2222; found: 287.222.

3-(2-Ethoxyethoxy)-3-(2,2-dimethoxyethyl)-2-methyl-5-phenyl-pentan-2-ol (29a).
A - Addition of MeLi. To an ice-cold, stirred solution of 4.49 g (13.3 mmol) of the ketone (**28a**) in 100 ml of anhydrous ether under dry nitrogen atmosphere was added dropwise via syringe 10 ml (16 mmol) of an 1.6M solution of MeLi in ether. After 90 min, 50 ml of brine

was added and the organic layer was separated. The aqueous layer was extracted with three 25 ml-portions of ether. The combined ether extracts were washed with 25 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 4.26 g of an oil. Careful chromatography on 100 g of silicagel with petrol-EtOAc (gradient elution, 10-1 to 3-1) as the eluent yielded, in order of elution, 2.09 g (6.2 mmol; 46%) of the starting material (**28a**), 0.58 g of a mixed fraction (**28a/29a**) and 1.55 g (4.4 mmol; 33%) of the desired alcohol (**29a**) as an oil.

B - Addition of $MeCeCl_2$. A solution of this cerium reagent was prepared *in situ* according to the procedures described by Imamoto and Greeves:[18] in a roundbottom flask, pulverized $CeCl_3 \bullet 7H_2O$ (5.9 g, 15.8 mmol) was heated at 100°C under high vacuum (0.01 mmHg) for 1 hr. The resulting pre-dried solid was finely ground in a mortar and then further dried at 140°C under high vacuum for 2 hrs to yield anhydrous $CeCl_3$ as a free-flowing white powder. After slow cooling to r.t. under dry argon atmosphere, the flask was placed on an ice-bath and 40 ml of ice-cold anhydrous THF was added via syringe; the resulting suspension was stirred overnight at r.t. under argon. Next, the milky suspension was stirred on an ultrasonic bath at r.t. for 1.5 hrs. The ultrasonic bath was removed and the dry $CeCl_3$-suspension was cooled to -78°C while stirring magnetically. Via syringe, 9.5 ml (15.2 mmol) of a 1.6M solution of MeLi in ether was added dropwise. After 1 hr the mixture was treated dropwise with a solution of 1.69 g (5.0 mmol) of the ketone (**28a**) in 35 ml of anhydrous THF. Stirring at -78°C was continued while the progress of the reaction was monitored by tlc-analysis. After complete conversion of the starting ketone (approx. 30 min) the reaction was quenched by addition of 15 ml of saturated NH_4Cl solution and the resulting mixture was extracted with ether (3×50 ml). The combined extracts were washed with two 25 ml-portions of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 1.76 g (4.9 mmol; 99%) of the alcohol (**29a**) as an oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.16-1.37 (m, 13H): C(OH)(CH_3)$_2$, OCH(OEt)CH_3 and OCH_2CH_3; 1.68-2.42 (m) and 2.55-2.87 (br m; total 7H): H-2, H-4 and H-5; 3.32-3.35 (3 s, 6H): OCH_3; 3.40-3.65 (m, 2H): OCH_2CH_3; 4.30 and 4.41 (2 s, 1H): OH; 4.65-4.73 (m, 1H): $CH(OMe)_2$; 5.00-5.12 (m, 1H): OCH(OEt)Me; 7.10-7.35 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 15.0 (q): OCH_2CH_3; 20.3 (q): OCH(OEt)CH_3; 25.5 and 25.6 (2 q): C(OH)(CH_3)$_2$; 30.8 (t); 34.5 and 35.2 (2 t); 37.7 and 38.0 (2 t); 51.5, 52.5, 52.8 and 53.7 (4 q): OCH_3; 58.6 and 58.8 (2 t): OCH_2CH_3; 75.3 (s): C-2; 84.5 (s): C-3; 94.0 and 94.4 (2 d): OCH(OEt)Me; 101.7 and 102.7 (2 d): C-2'; 125.7 (d): phenyl C-3"-C-5"; 128.3 (d): phenyl C-2" and C-6"; 143.2 (s): phenyl C-1". MS (EI): *m/e* (%) 295 (0.07), 233 (8), 191 (26), 133 (24), 105 (11), 91 (20), 75 (52), 73 (100), 59 (13), 45 (37), 43 (7). MS (CI): *m/e* (%): 307 (6), 293 (5), 291 (14), 278 (7), 277 (40), 261 (7), 247 (21), 234 (16), 233 (100), 219 (26), 201 (14). HRMS (EI): calcd. (M-C(CH_3)$_2$OH): 295.1909; found: 295.1903.

5-Cyclohexyl-3-(2-ethoxyethoxy)-3-(2,2-dimethoxyethyl)-2-methyl-pentan-2-ol (29b). The addition of methyllithium to 1.12 g (3.3 mmol) of the ketone (**28b**) was carried out according to procedure A, as described for (**29a**). Purification of the crude product (1.15 g) via chromatography on 40 g of silicagel with petrol-EtOAc (gradient elution, 10-1 to 3-1) as the eluent afforded, in order of elution: 514 mg (1.5 mmol; 45%) of the starting ketone (**28b**) and 493 mg (1.4 mmol; 41%) of the alcohol (**29b**) as an oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.72-1.0 (br, 3H); 1.0-1.4 (m, 21H); 1.53-1.82 (br, 9H); 1.85-2.35 (mm, 3H); 3.20-3.68 (m, 8H): OCH_2CH_3 and OCH_3 [δ 3.25-3.35 (ms, 6H)]; 4.30 and 4.35 (2 s, 1H): OH; 4.57 (2 t, J=4.7 Hz, 1H): $CH(OMe)_2$; 4.93 and 4.97 (dq, J=5.2 Hz, 1H): OCH(OEt)Me. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 15.1 (q): OCH_2CH_3; 20.3 and 20.4 (2 q): OCH(OEt)CH_3; 25.5 and 25.7 (2 q): C(OH)(CH_3)$_2$; 26.4 and 26.7 (2 t); 29.4 (t); 31.7 and 31.9 (2 t); 32.5 (t); 33.4 and 33.5 (2 t); 35.2 (t); 38.6 (t); 38.9 and 39.0 (2 d):

c-hexyl C-1"; 51.4, 52.4, 52.8 and 53.3 (4 q): OCH_3; 58.7 (t): $O\underline{C}H_2CH_3$; 75.3 (s): C-2; 84.8 (s): C-3; 94.1 and 94.4 (2 d): $O\underline{C}H(OEt)Me$; 101.7 and 102.6 (2 d): C-2'. IR ($CHCl_3$): ν 3350 cm^{-1} (br).

3-(2-Ethoxyethoxy)-3-(2,2-dimethoxyethyl)-2-methyl-nonan-2-ol (29c). The addition of methyllithium to 3.0 g (9.4 mmol) of the ketone (**28c**) was carried out according to procedure A, as described for (**29a**). Purification of the crude product (3.0 g) via chromatography on 100 g of silicagel with petrol-EtOAc (gradient elution, 10-1 to 3-1) as the eluent afforded, in order of elution: 1.24 g (3.9 mmol; 41%) of the starting ketone (**28c**), 310 mg of a mixed fraction of (**28c**) and (**29c**), and 1.29 g (3.9 mmol; 41%) of the alcohol (**29c**) as an oil.
^1H NMR ($CDCl_3$, 200 MHz): δ 0.75-0.90 (br t, J=6.6 Hz, 3H): H-9; 1.05-1.45 (br m, 22H); 1.45-2.30 (mm, 5H); 3.24-3.26 (3 s, 6H): OCH_3; 3.26-3.60 (m, 2H): $OC\underline{H}_2CH_3$; 4.27 (br, 1H): OH; 4.52-4.56 (m, 1H): $C\underline{H}(OMe)_2$; 4.90 and 4.95 (2 q, J=5.0 Hz, 1H): $OC\underline{H}(OEt)Me$. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.0 (q): C-9; 15.0 (q): $OCH_2\underline{C}H_3$; 20.2 and 20.3 (2 q): $OCH(OEt)\underline{C}H_3$; 22.6 (t); 24.3 and 24.4 (2 t); 25.3 and 25.6 (2 q): $C(OH)(\underline{C}H_3)_2$; 30.4 (t); 31.7 and 32.0 (2 t); 35.1 and 35.2 (2 t); 38.3 (t); 51.2, 52.2, 52.7 and 53.2 (4 q): OCH_3; 58.6 (t): $O\underline{C}H_2CH_3$; 75.2 (s): C-2; 84.5 and 84.7 (2 s): C-3; 94.0 and 94.3 (2 d): $O\underline{C}H(OEt)Me$; 101.6 and 102.5 (2 d): C-2'. MS: *m/e* (%) 213 (23), 171 (32), 113 (43), 75 (48), 73 (100), 45 (28). HRMS: calcd. ($M-H_2O-OCH(OEt)Me$): 227.2011; found: 227.2012. Anal: calcd. for $C_{18}H_{38}O_5$: C, 64.63; H, 11.45; found: C, 64.36; H, 11.57.

Cyclization of the alcohol (29a). A solution of 1.71 g (4.8 mmol) of the alcohol (**29a**) and 10 mg (0.06 mmol) of *p*-toluenesulfonic acid monohydrate in 25 ml of methanol was stirred overnight at r.t. The reaction mixture was poured into 10 ml of satd. aqueous sodium bicarbonate solution and extracted with three 25 ml-portions of ether. The combined extracts were washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 1.03 g of an oil. Purification via chromatography on 25 g of silicagel with petrol-EtOAc (6-1) as the eluent afforded 462 mg of an inseparable mixture, containing the least polar isomer of (**31a**) and both diastereomers of the unwanted cyclization product **4-(2,2-dimethoxyethyl)-4-(2-phenylethyl)-2,5,5-trimethyl-1,3-dioxolane (30a)** in a ratio of (**30a**) : (**31a**) ≈ 1 : 2. Further elution yielded 725 mg (2.9 mmol; 60%) of the most polar isomer of (**31a**).
Mixture of products (**30a**)/(**31a**): ^1H NMR (CDCl, 200 MHz): δ 1.05-1.40 (br m, 25H): $C(CH_3)_2$ (**31a**) [δ 1.13 (s, approx. 7H) and 1.34 (s)], $OCH(CH_3)O$ (**30a**) and $C(CH_3)_2$ (**30a**); 1.58-1.99 (br m, 6H) and 1.99-2.25 (br m, 8H): H-3 and H-1' (**31a**), H-1' and H-1" (**30a**); 2.40-2.85 (m, 4H) and 2.85-3.05 (m, 3H): H-2' (**31a**) and H-2" (**30a**); 3.32-3.39 (m s, 13H): OCH_3 (**31a**) and (**30a**); 3.56 (d, J=2.0 Hz, 2H); 4.55 (dd, J=6.2, 3.1 Hz, 0.5H) and 4.64 (t, J=4.8 Hz, 0.5H): H-2' (**30a**); 4.97 (d, J=4.1 Hz, 2H): H-2 (**31a**); 5.26 (q, J=4.9 Hz, 1H): H-2 (**30a**); 7.14-7.34 (br m, 15H): phenyl (**31a**) and (**30a**).^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): *Major peaks*; (**31a**): δ 23.4 (q) and 26.9 (q): $C(\underline{C}H_3)_2$; 31.0, 36.6 and 43.5 (3 t): C-3, C-1' and C-2'; 54.6 (q): OCH_3; 80.6 and 88.6 (2 s): C-4 and C-5; 103.0 (d): C-2; 125.8 (d) and 128.4 (2 d): phenyl C-2" - C-6"; 142.6 (s): phenyl C-1". *Minor peaks*; (**30a**): δ 21.5, 22.4, 22.6, 23.8 and 24.4 (5 q): $OCH(\underline{C}H_3)O$ and $C(\underline{C}H_3)_2$; 30.0 and 30.1 (2 t) and 34.4 (2 t): C-1" and C-2"; 36.2 (t): C-1'; 52.1, 53.7 and 53.8 (3 q): OCH_3; 82.7 (s); 83.8 and 83.9 (3 s): C-4 and C-5; 102.1 and 102.4 (2 d): C-2 and C-2'; 128.3 (d) and 142.4 (s): phenyl. MS: *m/e* (%) 307 (4), 201 (33), 192 (17), 160 (16), 159 (15), 133 (16), 105 (41), 91 (100), 87 (19), 85 (16), 75 (54), 43 (21), 31 (39). HRMS: calcd. (**30a**; M-H): 307.1909; found: 307.1910.
Most polar isomer (**31a**): ^1H NMR ($CDCl_3$, 200 MHz): δ 1.25 (s, 6H): $C(CH_3)_2$; 1.65-1.92 (m, 3H): H-1' and OH; 2.10 (dd, J=14.3, 4.9 Hz, 1H): H-3a; 2.40 (dd, J=14.3, 6.3 Hz, 1H): H-3b; 2.65 (ddd, J=12.9, 11.1, 6.5 Hz, 1H): H-2'a; 2.90 (ddd, J=13.7, 10.3, 6.6 Hz, 1H): H-2'b;

3.39 (s, 3H): OCH_3; 5.10 (dd, J=6.3, 4.9 Hz, 1H): H-2; 7.15-7.35 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 21.5 and 25.9 (2 q): $C(\underline{C}H_3)_2$; 30.9 (t); 37.1 (t); 45.9 (t); 55.5 (q): OCH_3; 83.3 (s); 86.1 (s); 103.3 (d); 125.9 (d): phenyl C-4"; 128.3 and 128.5 (2 d): phenyl C-2",3",5",6"; 142.2 (s): phenyl C-1". MS: *m/e* (%) 250 (0.3), 192 (77), 160 (50), 159 (23), 133 (20), 105 (35), 104 (13), 92 (15), 91 (72), 87 (100), 59 (15). HRMS: calcd. (M^+): 250.1569; found: 250.1566.

4-(2-Cyclohexylethyl)-5,5-dimethyl-2-methoxy-tetrahydrofuran-4-ol (31b). According to the procedure described for (**31a**), 460 mg (1.3 mmol) of the alcohol (**29b**) was cyclized to yield, after purification by chromatography on 10 g of silicagel with petrol-EtOAc (gradient elution, 7-1 to 3-1) as the eluent, in order of elution: 115 mg of an inseparable mixture of (**31b**) and the 1,3-dioxolane (**30b**), and 183 mg (0.71 mmol; 56%) of the 2-methoxy-tetrahydrofuran-4-ol (**31b**) as an oil.
(**31b**): ^1H NMR ($CDCl_3$, 200 MHz): δ 0.72-0.95 (m, 2H); 1.0-1.85 (m, 21H); 1.97 (dd, J=14.4, 4.9 Hz, 1H): H-3a; 2.28 (dd, J=14.4, 6.3 Hz, 1H): H-3b; 3.33 (s, 3H): OCH_3; 5.02 (dd, J=6.3, 4.9 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 21.5 (q), 26.0, 26,3 and 26.6 (3 q), 31.8 and 32.2 (2 t), 33.3 (t), 38.1 (d), 45.9 (t), 55.4 (q, OCH_3), 83.4 (s, C-4), 86.1 (s, C-5), 103.3 (d, C-2). MS: *m/e* (%) 207 (28), 139 (6), 123 (10), 102 (25), 95 (5), 87 (25), 69 (5), 55 (12), 43 (5). HRMS: calcd. (M-OCH_3): 255.1855; found: 255.1856.

5,5-Dimethyl-4-hexyl-2-methoxy-tetrahydrofuran-4-ol (31c). According to the procedure described for (**29a**), 1.1 g (3.3 mmol) of the alcohol (**29c**) was cyclized to yield, after purification by chromatography on 20 g of silicagel with petrol-EtOAc (gradient elution, 6-1 to 3-1) as the eluent, in order of elution: 295 mg of an inseparable mixture of (**31c**) and the 1,3-dioxolane (**30c**), and 431 mg (1.9 mmol; 57%) of the 2-methoxy-tetrahydrofuran-4-ol (**31c**) as an oil.
(**31c**): ^1H NMR ($CDCl_3$, 200 MHz): δ 0.72-0.95 (br t, J=6.0 Hz, 6H): H-6'; 1.1-1.55 (m, 16H): $C(C\underline{H}_3)_2$ and H-1'-H-5'; 1.84 (br s, 1H): OH; 1.96 (dd, J=14.3, 4.9 Hz, 1H): H-3a; 2.26 (dd, J=14.3, 6.3 Hz, 1H): H-3b; 3.31 (s, 1H): OCH_3; 5.00 (dd, J=6.3, 4.9 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.0 (q), 21.5 (q), 22.5 (t), 24.3 (t), 26.0 (q), 29.9 (t), 31.7 (t), 34.9 (t), 45.8 (t), 55.4 (q, OCH_3), 83.4 (s, C-4), 86.0 (s, C-5), 103.3 (d, C-2). MS: *m/e* (%) 215 (0.5), 199 (3), 172 (16), 115 (12), 113 (16), 102 (27), 87 (100), 85 (14), 70 (16), 59 (15), 55 (13), 43 (30), 41 (13). HRMS: calcd. (M-CH_3): 215.1647; found: 215.1644.

5,5-Dimethyl-2-methoxy-4-(2-phenylethyl)-4-(trimethylsilyloxy)-tetrahydrofuran (32a). To a stirred solution of 970 mg (3.9 mmol) of the tetrahydrofuran-4-ol (**31a**) in 15 ml of anhydrous DMF was added sequentially 811 mg (11.9 mmol) of imidazole and 0.6 ml (4.8 mmol) of chlorotrimethylsilane. After stirring overnight the reaction mixture was poured into 100 ml of water and extracted with three 50 ml-portions of petrol. The combined extracts were washed with 25 ml of water, dried with $MgSO_4$ and concentrated under reduced pressure to yield 1.23 g (3.8 mmol; 98%) of the silylated product (**32a**) as an oil, which was sufficiently pure to be used in the next reaction without further purification.
^1H NMR ($CDCl_3$, 200 MHz): δ 0.2 (s, 9H): $SiCH_3$; 1.29 (s, 6H): $C(CH_3)_2$; 1.77-1.97 (m, 2H): H-1'; 2.11 (dd, J=14.7, 4.9 Hz, 1H): H-3a; 2.51 (dd, J=14.7, 6.2 Hz, 1H): H-3b; 2.56-2.85 (m, 2H): H-2'; 3.40 (s, 3H): OCH_3; 5.08 (dd, J=6.2, 4.9 Hz, 1H): H-2; 7.15-7.37 (m, 5H): phenyl H-2' - H-6'. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.3 (q): $SiCH_3$; 23.1 and 26.1 (2 q): $C(\underline{C}H_3)_2$; 30.9 (t); 39.4 (t); 46.4 (t); 55.3 (q): OCH_3); 85.8 (s); 87.2 (s); 103.3 (d): C-2; 125.9 (d): phenyl C-4'; 128.3 and 128.5 (2 d) phenyl C-2", C-3", C-5" and C-6"; 142.5 (s): phenyl C-1". MS: *m/e* (%) 264 (15), 160 (13), 159 (100), 105 (11), 91 (25), 85 (9), 83 (12), 75 (10), 73 (22). HRMS: calcd. (M-CH_3): 307.1729; found: 307.1731.

4-(2-Cyclohexylethyl)-2-methoxy-5,5-dimethyl-4-(trimethylsilyloxy)-tetrahydrofuran (32b). Silylation of 239 mg (0.9 mmol) of the tetrahydrofuran-4-ol (**31b**), according to the procedure described for (**32a**), yielded 294 mg of an oil. Purification of the crude product by chromatography on 5 g of silicagel with petrol-EtOAc (25-1) as the eluent afforded 270 mg (0.8 mmol; 89%) of the silylether (**32b**) as a colourless oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.1 (s, 9H): $SiCH_3$; 0.70-0.98 (br, 2H); 0.98-1.30 (m, 12H); 1.45-1.75 (m, 7H); 1.96 (dd, J=14.7 and 5.0 Hz, 1H): H-3a; 2.30 (dd, J=14.6 and 6.2 Hz, 1H): H-3b; 3.32 (s, 3H): OCH_3; 4.97 (dd, J=6.2 and 5.0 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.1 (q): $SiCH_3$; 23.0 (q); 25.9 (q); 26.6 (2 t); 31.7 (t); 33.4 (2 t); 34.2 (t); 38.3 (d): *c*-hexyl C-1; 46.6 (t); 55.2 (q): OCH_3; 85.8 (s): C-5; 87.2 (s): C-4; 103.3 (d): C-2.

4-Hexyl-2-methoxy-5,5-dimethyl-4-(trimethylsilyloxy)-tetrahydrofuran (32c). Silylation of 315 mg (1.4 mmol) of the tetrahydrofuran-4-ol (**31c**) according to the procedure described for (**32a**) yielded 413 mg (1.4 mmol; 100%) of the 4-silyloxy-tetrahydrofuran (**32c**) as an oil, which was sufficiently pure to be used without further purification in the next reaction.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.0 (s, 9H, $SiCH_3$), 0.65-0.85 (br t, J=6.5 Hz, 3H): H-6'; 0.95-1.45 (br m, 18H); 1.88 (dd, J=14.7 and 5.9 Hz, 1H): H-3a; 2.28 (dd, J=14.7 and 5.9 Hz, 1H): H-3b; 3.24 (s, 3H): OCH_3; 4.90 (dd, J=5.8 and 5.9 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.2 (q): $SiCH_3$; 14.0 (q); 22.6 (t); 23.0 (q); 24.1 (t); 26.0 (q); 30.0 (t); 31.8 (t); 36.9 (t); 46.6 (t); 55.3 (q): OCH_3; 85.9 (s): C-5; 87.2 (s): C-4; 103.4 (d): C-2. HRMS: calcd. ($M-OCH_3$): 271.2093; found: 271.2090.

5,5-Dimethyl- 4-(2-phenylethyl)-2-phenylthio- 4-(trimethylsilyloxy)-tetrahydrofuran (33a). A stirred reaction mixture consisting of 800 mg (2.5 mmol) of (**32a**) in 25 ml of anhydrous ether and 25 beads of 4Å molecular sieve at 0°C was treated dropwise with 0.3 ml (2.9 mmol) of thiophenol, followed by 0.5 ml (4.1 mmol) of borontrifluoride etherate. Stirring at 0°C was continued for 30 min before the reaction was quenched by addition of 20 ml of 4M aqueous sodiumhydroxide solution. After stirring for 10 min at r.t. the organic layer was separated and the aqueous layer was extracted with three 25 ml-portions of ether. The combined organic layers were washed twice with brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 1.17 g of an oil. The crude product was purified via chromatography on 25 g of silicagel with petrol-EtOAc (95-1) as the eluent, affording 1.0 g (2.5 mmol; 100%) of a 1.3-1 mixture of C-2 epimers of the sulfide (**33a**) as a colourless oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.25 (2 s, 9H): $SiCH_3$; 1.25, 1.32 and 1.38 (3 s, 7H): $C(CH_3)_2$; 1.75-2.00 (m, 2H): H-1'; 2.24 (dd, J=14.2, 9.3 Hz, 0.57H) and 2.36 (dd, J=13.9 and 7.7 Hz, 0.43H): H-3a; 2.50-2.80 (m, 3H): H-3b and H-2'; 5.45 (t, J=7.4 Hz, 0.43H) and 5.65 (dd, J=9.3, 7.7 Hz, 0.57H): H-2; 7.15-7.40 (m, 9H): SPh H-3',4',5' and phenyl H-2" - H-6"; 7.48-7.58 (m, 2H): SPh H-2',6' . ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.3, 2.5 and 2.7 (3 q): $SiCH_3$; 23.2, 23.3, 25.3 and 26.2 (4 q): $C(\underline{C}H_3)_2$; 30.7 and 30.9 (2 t); 38.9 and 39.2 (2 t); 43.9 (t); 46.0 (t); 83.2 (s); 83.5 (d): C-2; 85.1 (s); 86.9 (s); 88.8 (s); 126.0 and 126.7 (2 d); 128.3, 128.6 and 128.9 (3 d); 130.7 and 130.8 (2 d); 136.0 and 136.4 (2 s): SPh C-1'; 142.2 (s): phenyl C-1". MS: *m/e* (%) 400 (0.1), 342 (1), 291 (33), 237 (22), 233 (31), 185 (20), 175 (45), 147 (90), 105 (29), 91 (100), 75 (15), 73 (49). HRMS: calcd. (M^+): 400.1892; found: 400.1893.

4-(2-Cyclohexylethyl)- 5,5-dimethyl- 4-(trimethylsilyloxy)- 2-phenylthio- tetrahydrofuran (33b). According to the procedure described for (**33a**), 270 mg (0.82 mmol) of (**32b**) was reacted with thiophenol to yield 325 mg (0.80 mmol; 98%) of a 1.2-1 mixture of C-2 epimers of the sulfide (**33b**) as an oil, which was sufficiently pure to be used in the next reaction without further purification.

^{1}H NMR ($CDCl_3$, 200 MHz, major peaks): δ 0.18 (d, 9H): $SiCH_3$; 0.77-1.05 (br, 2H); 1.05-1.40 (br [including δ 1.18 (s), δ 1.24 (s) and δ 1.31 (s)], 12H); 1.52-1.85 (br m, 7H); 2.17 (dd, J=14.1, 9.1 Hz) and 2.26 (dd, J=13.4, 7.5 Hz; total 1H): H-3a; 2.54 (dd, J=14.1, 7.0 Hz) and 2.57 (dd, J=13.4, 7.8 Hz; total 1H): H-3b; 5.39 (dd, J=7.6 and 7.6 Hz, 0.5H) and 5.60 (dd, J=9.0, 6.8 Hz, 0.5H): H-2; 7.15-7.35 (m, 3H): SPh H-3" - H-5"; 7.45-7.55 (m, 2H): SPh H-2",6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.4 (2 q): $SiCH_3$; 23.2 (2 q); 25.2 and 26.0 (2 q); 26.4 and 26.7 (2 t); 31.5 and 31.8 (2 t); 33.5 (t); 34.0 and 34.4 (2 t); 38.4 (2 d); 44.0 (t); 46.4 (t); 83.4 (2 d): C-2; 85.3 (s); 86.8 (s); 88.8 (s); 126.6 (d); 128.8 (d); 130.6 (d); 136.1 and 136.6 (2 s).

4-Hexyl-5,5-dimethyl-4-(trimethylsilyloxy)-2-phenylthio-tetrahydrofuran (33c). According to the procedure described for (**33a**), 290 mg (0.96 mmol) of (**32c**) was reacted with thiophenol to yield 363 mg (0.96 mmol; 100%) of a 1.5-1 mixture of C-2 epimers of the sulfide (**33c**) as an oil, which was sufficiently pure to be used in the next reaction without further purification.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.0 (d, 9H): $SiCH_3$; 0.62-0.85 (br m, 3H): hexyl H-6'; 0.9-1.5 (br m [including δ 1.0 (s), δ 1.05 (s) and δ 1.13 (br s)], 18H; 1.98 (dd, J=13.6, 10.0 Hz) and 2.09 (dd, J=14.4, 7.1 Hz; total 1H): H-3a; 2.34 and 2.42 (2 dd, J=10.9, 6.8 Hz; total 1H): H-3b; 5.22 (dd, J=7.4, 7.4 Hz, 0.58H) and 5.44 (dd, J=11.2, 6.5 Hz, 0.42H): H-2; 6.95-7.20 (m, 3H): SPh H-3"- H-5"; 7.25-7.40 (m, 2H): SPh H-2",6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 2.4 (2 q): $SiCH_3$; 14.1 (q); 22.7 (t); 23.2 (2 q); 24.0 (t); 24.3 (t); 25.2 (q); 26.1 (q); 30.0 (2 t); 31.8 (2 t); 36.6 (t); 37.1 (t); 44.0 (t); 46.3 (t); 83.5 (2 d): C-2; 85.3 (s); 86.9 (s); 88.8 (s); 126.6 (d); 128.8 (d); 130.6 (2 d); 136.1 and 136.7 (2 s). MS: *m/e* (%) 380 (0.3), 271 (42), 237 (29), 213 (54), 155 (25), 127 (100), 85 (27), 73 (62), 71 (32), 43 (23). HRMS: calcd. (M^+): 380.2205; found: 380.2206.

2,2-Dimethyl-3-(trimethylsilyloxy)-3-(2-phenylethyl)-2,3-dihydrofuran (34a). A solution of 780 mg (3.16-3.39 mmol) of mCPBA (70-75 wt% mCPBA, remainder mCBA and water) in 35 ml of anhydrous toluene was pre-dried in a dropping funnel containing activated 4Å molecular sieves. After 20 min, this solution was added dropwise to an ice-cold solution of 1.04 g (2.60 mmol) of the sulfides (**33a**) in 25 ml of toluene. Stirring at 0°C was continued until tlc-analysis indicated complete disappearance of the sulfide. The dropping funnel was replaced by a reflux condensor, 7.8 ml (56 mmol) of triethylamine was added and the flask containing the reaction mixture was placed in an oil bath, pre-heated at 120°C. The mixture was refluxed for approx. 10 min, while the disappearance of the sulfoxide intermediate was monitored via tlc-analysis. Then, both solvent and triethylamine were removed at the rotary evaporator under reduced pressure and the residual oil (1.0 g) was partially purified by chromatography on 75 g of aluminium oxide (activity grade III) with petrol as the eluent, affording 714 mg of an oil, consisting of the desired enol ether (**34a**) and a number of minor impurities (tlc-analysis).
^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.0 (s, 9H): $Si(CH_3)_3$; 1.0 (s, 3H) and 1.2 (s, 3H): $C(CH_3)_2$; 1.5-1.8 (m, 2H); 2.4-2.7 (m, 2H); 4.9 (d, J=3 Hz, 1H): H-4; 6.2 (d, J=3 Hz, 1H): H-5; 6.8-7.2 (m, 7H) and 7.2-7.4 (m, 1H): phenyl H-2" - H-6" from (**34a**) and impurities.

2,2-Dimethyl-3-(2-phenylethyl)-2,5-dihydrofuran-5-ol (35a). A solution of 600 mg (1.50 mmol) of the sulfides (**33a**) in 25 ml of toluene was sequentially oxidized and eliminated as described for (**34a**) to give 1.3 g of an oil. Repeated chromatography on silicagel (step I: 20 g of silicagel, petrol-EtOAc 20-1 (yielding 255 mg of partially purified product); step II: 6 g of silicagel, petrol-EtOAc 99-1, followed by 10-1) afforded 106 mg (0.49 mmol; 33%) of a 2 : 1 mixture of the rearranged enols (**35a**) as a viscous oil.

^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.25 (s, 3H) and 1.35 (s, 3H): $C(CH_3)_2$; 2.20-2.33 (m t, J=7.8 Hz, 2H); 2.72-3.00 (m, 2H); 5.45 (br s, 1H): H-4; 5.88 (br d, J=5.6 Hz, 0.34H): H-5 minor isomer; 6.00 (br s, 0.66H): H-5 major isomer; 7.11-7.34 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, major isomer only): δ 26.8 and 27.8 (2 q): $C(\underline{C}H_3)_2$; 28.0 (t); 33.5 (t); 88.6 (s): C-2; 103.7 (d): C-5; 118.4 (d): C-4; 126.0 (d): phenyl C-4"; 128.2 and 128.4 (2 d): C-2", C-3", C-5" and C-6"; 141.5 (s): C-1"; 153.0 (s): C-3.

4,4-Dimethyl-3-(2-phenylethyl)-but-2-en-4-olide (36a). To a stirred solution of 235 mg of the partially purified silyloxy-enolether (**34a**) in 10 ml of THF were added 9 drops of a 40% aqueous hydrogenfluoride solution. After tlc-analysis indicated complete disappearance of the starting material (approx. 15 min) the reaction mixture was cooled on an ice-bath and Jones reagent was added dropwise until an orange colour persisted. Stirring at 0°C was continued for 30 min before the reaction was quenched by the addition of ethanol. The reaction mixture was extracted with ether (3×15 ml), washed with 10 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 220 mg of an oil. The crude product was purified via chromatography on 5 g of silicagel with petrol-EtOAc (7-1) as the eluent to afford 107 mg (0.49 mmol) of the butenolide (**36a**) as an oil, in an overall yield of 58% from the sulfides (**33a**).
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.42 (s, 6H): $C(CH_3)_2$; 2.56 (dt, J=1.7, 7.7 Hz, and dt, J=1.7, 8.0 Hz; total 2H): H-1'; 2.94 (t, J=7.8 Hz, 2H): H-2'; 5.75 (t, J=1.7 Hz, 1H): H-2; 7.17-7.36 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 24.8 (q): $C(\underline{C}H_3)_2$; 28.7 (t); 32.9 (t); 87.3 (s): C-4; 114.4 (d): C-2; 126.7 (d): phenyl C-4"; 128.2 (d): C-3" and C-5"; 128.7 (d): C-2" and C-6"; 139.2 (s): C-1"; 172.4 (s) and 176.5 (s): C-1 and C-3. MS: *m/e* (%) 216 (5), 173 (5), 171 (28), 92 (7), 91 (100), 65 (5). HRMS: calcd. (M^+): 216.1150; found: 216.1150.

3-(2-Cyclohexylethyl)-4,4-dimethyl-but-2-en-4-olide (36b). According to the procedure described for (**34a**), 300 mg (0.74 mmol) of the sulfides (**33b**) were sequentially oxidized and eliminated to give 320 mg of an oil containing the silyloxy enolether (**34b**) and some unidentified impurities. The crude product was used as such in the next step.
^{1}H NMR ($CDCl_3$, 90 MHz, selected peaks): δ 0.0 (s, 9H): $Si(CH_3)_3$; 0.5-1.8 (br m, 21H): $C(CH_3)_2$, H-1', H-2' and *c*-hexyl H-1" - H-6"; 4.9 (d, J=3 Hz, 1H): H-4; 6.2 (d, J=3 Hz, 1H): H-5; 6.9-7.6 (m, 7.5H): phenyl H-2" - H-6" from (**34b**) and impurities.
A solution of 200 mg of the crude silyloxy enolether (**34b**) in 10 ml of THF was sequentially desilylated and oxidized as described for (**36a**), yielding 200 mg of the crude oxidation product. Purification by repeated chromatography on silicagel with petrol-EtOAc (gradient elution 99-1 to 96-4) as the eluent afforded 63 mg (0.28 mmol) of the butenolide (**36b**) in an overall yield of 61% from (**33b**).
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.78-1.05 (br m, 2H) and 1.05-1.82 (br m, 19H): $C(CH_3)_2$ [δ 1.42 (s, approx. 6H)], H-2' and *c*-hexyl H-1"-H-6"; 2.22 (dt, J=1.7, 7.9 Hz, 2H): H-1'; 5.65 (t, J=1.7 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 24.3 (t); 24.9 (q): $C(\underline{C}H_3)_2$; 26.1 (t); 26.4 (t); 33.1 (t); 34.3 (t); 37.3 (d): *c*-hexyl C-1"; 87.3 (s): C-4; 113.6 (d): C-2; 172.2 (s); 178.2 (s). MS: *m/e* (%) 222 (12), 207 (17), 179 (38), 163 (12), 162 (20), 161 (29), 135 (21), 127 (101), 111 (38), 96 (28), 65 (47), 81 (44), 67 (42), 55 (48), 43 (62), 41 (38). HRMS: calcd. (M^+): 222.1620; found: 222.1616.

3-Hexyl-4,4-dimethyl-but-2-en-4-olide (36c). According to the procedure described for (**34a**), 300 mg (0.79 mmol) of the sulfides (**33c**) were sequentially oxidized and eliminated to give 350 mg of an oil containing the silyloxy enolether (**34c**) and some unidentified impurities. Chromatography of the crude product on 10 g of silicagel with petrol-EtOAc

(initially 100-0 to remove some apolar side-products, then 0-100) as the eluent yielded 84 mg of an oil containing the rearranged unsaturated lactol (**35c**) as the main component.
(**35c**): ^{1}H NMR ($CDCl_3$, 90 MHz, selected peaks): δ 0.7-1.0 (br t, J≈6 Hz, 3H): hexyl H-6"; 1.0-1.75 (br m, 15H); 1.75-2.10 (br m, 2H); 5.30 (br s, 1H): H-4; 5.80 (br s, 0.33H) and 5.95 (br s, 0.66H): H-5.
For analytical purposes, a small sample of crude (**35c**) was transformed into the corresponding methyl ether (**37**) by treatment (approx. 10 min) with methanol in $CHCl_3$.
(**37**): ^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.74-0.90 (br t, J≈6.5 Hz, 4H); 1.10-1.39 (br [incl. δ 1.21 and 1.28 (2 s)], 15H); 1.39-1.60 (br m, 2H); 1.86-1.95 (br t, J≈7.1 Hz, 2H); 3.31 (s, 3H): OCH_3; 5.27 (d, J=1.3 Hz) and 5.28 (d, J=1.6 Hz; total 1H): H-4; 5.49 and 5.51 (2 d, J=1.2 Hz; total 1H): H-5. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, major peaks only): δ 14.1 (q): hexyl C-6'; 22.6 (t); 26.1 (t); 27.3 (t); 27.0 (q) and 27.8 (q) $C(\underline{C}H_3)_2$; 29.2 (t); 31.7 (t); 54.2 (q): OCH_3; 88.2 (s): C-2; 107.3 (d): C-5; 117.4 (d): C-4; 154.4 (s): C-3.
Next, the methyl ether (**37**) and the remainder of crude (**35c**) were combined and dissolved in 10 ml of acetone. The stirred solution was cooled on an ice-bath and Jones reagent was added dropwise until an orange colour persisted. The reaction was quenched by the addition of ethanol and the reaction mixture was extracted with three 15 ml-portions of ether. The combined extracts were washed with 10 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified via chromatography on 5 g of silicagel with petrol-EtOAc (5-1) as the eluent to afford 77 mg (0.39 mmol; 49% yield from **33c**) of the butenolide (**36c**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.79-0.95 (br m [incl. δ 0.86 (t, J=6.4 Hz)], 3H): H-6'; 1.18-1.45 (br m, 13H): $C(CH_3)_2$ [δ 1.41 (s, approx. 7H)] and H-3'-H-5'; 1.50-1.69 (br m, 2H): H-2'; 2.20 (dt, J=1.7, 7.6 Hz, 2H): H-1'; 5.65 (t, J=1.8 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.0 (q): C-6'; 22.5 (t); 24.8 (q): $C(\underline{C}H_3)_2$; 26.7 (t); 26.9 (t); 28.9 (t); 31.4 (t); 87.3 (s): C-4; 113.6 (d): C-2; 172.3 (s); 177.9 (s). IR ($CHCl_3$): ν 1740, 1630 cm^{-1}. MS: *m/e* (%) 181 (52), 154 (20), 153 (100), 111 (59), 109 (9), 96 (9), 81 (9), 69 (22), 67 (9), 54 (8), 43 (44), 41 (9). HRMS: calcd. (M^+): 196.1463; found: 196.1460.

3-Hydroxy-4,4-dimethyl-3-(2-phenylethyl)-butan-4-olide (43a). Jones reagent was added dropwise to a stirred solution of 100 mg (0.40 mmol) of the hydroxy methyl ether (**31a**) in 10 ml of acetone, cooled on an ice-bath, until an orange colour persisted and glc-analysis indicated complete conversion of the starting material (approx. 20 drops). Stirring at 0°C was continued for 30 min before the reaction was quenched by the addition of ethanol. The reaction mixture was extracted with ether (3×15 ml), washed with 10 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 100 mg of an oil. The crude product was purified via chromatography on 2.5 g of silicagel with petrol-EtOAc (5-1) as the eluent, affording 94 mg (0.40 mmol; 100%) of the hydroxylactone (**43a**) as an oil.
^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.34 (s, 3H) and 1.45 (s, 3H): $C(CH_3)_2$; 1.75-2.02 (m, 2H): H-1'; 2.45-2.98 (m, 5H): H-2a,b [δ 2.57 (d, J=18.0 Hz) and 2.77 (d, J=18.9 Hz)], H-2' and OH; 7.17-7.30 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 20.7 and 24.8 (2 q): $C(\underline{C}H_3)_2$; 30.5 (t); 36.8 (t); 41.9 (t): C-2; 79.8 (s); 90.3 (s); 126.3 (d): phenyl C-4"; 128.3 (d): phenyl C-3",5"; 128.6 (d): phenyl C-2",6"; 141.2 (s): C-1"; 174.8 (s): C-1. IR ($CHCl_3$): ν 3580, 3450 (br), 1760 cm^{-1}. Anal: calcd. for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74; found: C, 72.07; H, 7.94.

3-Hexyl-3-hydroxy-4,4-dimethyl-butan-4-olide (43c). According to the procedure described for (**43a**), 267 mg (1.2 mmol) of the hydroxy methyl ether (**31c**) was oxidized with Jones reagent to yield, after chromatography on 5 g of silicagel with petrol-EtOAc (5-1) as the eluent, 210 mg (0.98 mmol; 82%) of the hydroxylactone (**43c**) as an oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.77-0.90 (br m, 3H): H-6'; 1.15-1.28 (br m, 7H): H-2'-H-5'; 1.28 (s, approx. 3H) and 1.38 (s, 3H): $C(CH_3)_2$; 1.44-1.60 (br m, 2H): H-1'; 2.47 (d, J=17.5 Hz, 1H): H-2a; 2.64 (d, J=17.5 Hz, 1H): H-2b; 2.76 (br s, 1H): OH. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 14.0 (q): C-6'; 20.8 (q); 22.5 (t); 24.0 (t); 24.6 (q); 29.6 (t); 31.6 (t); 34.8 (t); 42.0 (t); 79.8 (s); 90.5 (s); 175.3 (s). MS: *m/e* (%) 128 (58), 116 (19), 86 (68), 84 (99), 71 (57), 59 (32), 58 (74); 49 (100), 43 (31). HRMS: calcd. (M-CH_3): 199.1334; found: 199.1337.

5,5-Dimethyl-4-(2-phenylethyl)-tetrahydrofuran-2,4-diol (44a). A mixture of 455 mg (1.82 mmol) of the hydroxy methyl ether (**31a**) in 30 ml of THF and 5 ml of 4M aqueous hydrochloric acid solution was stirred at r.t. for 30 min. The reaction mixture was poured into 100 ml of satd. aqueous sodiumbicarbonate solution and extracted with ether (4×25 ml). The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 455 mg of an oil. The crude product was purified via chromatography on 10 g of silicagel with petrol-EtOAc (4-1) as the eluent to yield, in order of elution, 50 mg (0.2 mmol; 11%) of starting material (**31a**) and 287 mg (1.22 mmol; 67%) of the diol (**44a**) as a 6 : 1 mixture of diastereomers.
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.12 and 1.38 (2 s, total 5.04H): $C(CH_3)_2$ major isom.; 1.25 and 1.32 (2 s, total 5.96H): $C(CH_3)_2$ minor isom.; 1.62-1.95 (m, 2H); 2.07-2.24 (m, 2H); 2.55-2.75 (m, 1H); 2.82-3.03 (m, 1H); 3.37 (br, 0.86H): OH major isom.; 3.89 (br, 0.14H): OH minor isom.; 4.25 (br, 0.77H): OH major isom.; 5.48 (d, J=4.0 Hz, 0.86H): H-2 major isom.; 5.65 (t, J=5.7 Hz, 0.14H): H-2 minor isom.; 7.15-7.37 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT, selected peaks): *Major isomer*: δ 23.3 and 26.8 (2 q): $C(\underline{C}H_3)_2$; 31.0 (t); 36.8 (t); 43.8 (t); 81.5 (s); 88.7 (s); 96.6 (d): C-2; 126.0 (d): phenyl C-4"; 128.4 and 128.5 (2 d): phenyl C-2",3",5",6"; 142.3 (s): phenyl C-1". *Minor isomer* (separated peaks only): 21.5 (q); 26.7 (q); 37.2 (t); 46.4 (t); 83.9 (s); 86.6 (s); 142.1 (s). IR ($CHCl_3$): ν 3575, 3480 (br), 3360 (br) cm^{-1}. MS: *m/e* (%) 218 (14), 178 (59), 160 (80), 134 (15), 133 (26), 105 (38), 104 (22), 92 (44), 91 (100), 85 (32), 73 (51), 59 (35). HRMS: calcd. (M-H_2O): 218.1307; found: 218.1303.

5,5-Dimethyl-4-(2-phenylethyl)-tetrahydrofuran-4-ol (45a). To a solution of 100 mg (0.42 mmol) of the diol (**44a**) in 5 ml of anhydrous CH_2Cl_2, stirred at -78°C, was added dropwise 0.1 ml (0.81 mmol) of borontrifluoride etherate, followed by 0.2 ml (1.25 mmol) of triethylsilane. Stirring was continued for 50 min until tlc analysis indicated complete conversion of the starting material. The reaction mixture was poured into 10 ml of satd. aqueous sodium bicarbonate solution and extracted with ether (15 ml). The extract was washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 100 mg of an oil. Purification of the crude product by chromatography on 2 g of silicagel with petrol-EtOAc (2-1) as the eluent yielded 67 mg (0.30 mmol; 73%) of (**45a**).
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 1.11 (s, 3H) and 1.26 (s, 3H): $C(CH_3)_2$; 1.68-1.90 (m, 2H); 2.05-2.25 (m, 2H); 2.69 (ddd, J=13.5, 10.6, 6.5 Hz, 1H): H-3a; 2.96 (ddd, J=13.7, 11.4, 6.0 Hz, 1H): H-3b; 3.81-3.99 (m, 2H): H-2; 7.15-7.35 (m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 221.1 and 23.9 (2 q): $C(\underline{C}H_3)_2$; 31.1 (t); 37.2 (t); 37.8 (t); 62.9 (t): C-2; 82.2 (s); 84.7 (s); 125.9 (d): phenyl C-4"; 128.3 and 128.4 (2 d): phenyl C-2",3",5",6"; 142.4 (s): phenyl C-1". MS: *m/e* (%) 163 (8), 162 (70), 144 (12), 133 (34), 105 (22), 92 (26), 91 (100), 59 (10). HRMS: calcd. (M-H_2O-CH_3): 187.1123; found: 187.1120.

3-(2-Cyclohexylethyl)-but-2-en-4-olide (47). Jones reagent was added dropwise to a stirred solution of 170 mg (0.75 mmol) of the hydroxy methyl ethers (**21b**) in 20 ml of acetone until an orange colour persisted and tlc-analysis indicated complete transformation of the starting material. Satd. aqueous sodiumbicarbonate soln. was added until a neutral pH was

obtained. The resulting mixture was extracted with ether (3×25 ml) and the combined extracts were washed with 10 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 155 mg of a yellow oil which solidified upon standing at room temperature Chromatography on 20 g of silicagel with petrol-EtOAc (95-5 to 90-10) as the eluent afforded 126 mg (0.59 mmol; 79%) of the hydroxy-lactone (**46**) as an oil which was sufficiently pure to be used as such in the next reaction.

3-(2-Cyclohexylethyl)-3-hydroxy-butan-4-olide (46): ^{1}H NMR ($CDCl_3$, 90 MHz): δ 0.60-1.45 (br m) and 1.45-2.10 (br m; total 16H): H-1', H-2' and *c*-hexyl H-1" - H-6"; 2.55 (br s, 2H): H-2; 2.70 (br s, 1H): H-3; 4.03-4.40 (m, 2H): H-4.
A solution of 35 mg (0.16 mmol) of the hydroxy-lactone (**46**), 0.06 ml (0.8 mmol) of thionylchloride and 0.13 ml (1.6 mmol) of pyridine in 5 ml of CH_2Cl_2 was stirred on an ice-bath until glc-analysis indicated complete disappearance of the starting material (approx. 60 min). The reaction mixture was concentrated under reduced pressure and the residue was dissolved in 20 ml of ether. The solution was washed with 5 ml of satd. aqueous sodiumbicarbonate soln. and 10 ml of brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield 28 mg of a brown oil. Careful chromatography on 10 g of silicagel with petrol-EtOAc (gradient elution, 98-2 to 96-4) afforded, in order of elution, 5 mg of a mixed fraction, consisting of the desired product (**47**) and two exocyclic double bond regioisomers in 1.6 - 1 ratio (glc-analysis), and 12 mg (0.06 mmol; 39%) of the analytically pure butenolide (**47**).
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.77-1.05 (br m, 2H), 1.05-1.37 (br m, 5H), 1.40-1.51 (m, 2H) and 1.55-1.85 (br m, 5H): H-2' [δ 1.44 (t, J=7.9 Hz) and 1.47 (t, J=7.4 Hz)] and *c*-hexyl H-1" - H-6"; 2.40 (t, J=7.9 Hz, 2H): H-1'; 4.72 (d, J=1.8 Hz, 2H): H-4; 5.81 (quintet, J=1.6 Hz, 1H): H-2. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 26.1 (t); 26.2 (t); 26.4 (t); 33.0 (t); 34.7 (t); 37.3 (d): *c*-hexyl C-1"; 73.1 (t): C-4; 115.2 (d): C-2; 171.0 (s) and 173.1 (s): C-1 and C-3.

3-(2-Cyclohexylethyl)-butan-4-olide (48). A solution of 10 mg (0.05 mmol) of the butenolide (**47**) in 20 ml of EtOAc, containing 15 mg of 10% Pd/C, was hydrogenated in a Parr apparatus under hydrogen pressure (4 atm) at room temperature. After 6 hrs, glc-analysis indicated complete transformation of the starting material into a single product. The reaction mixture was filtered and the solvent was evaporated under reduced pressure to yield 10 mg (0.05 mmol; 100%) of the butanolide (**48**).
^{1}H NMR ($CDCl_3$, 200 MHz, selected peaks): δ 0.67-1.00 (br m, 2H), 1.0-1.32 (br m, 7H), 1.32-1.50 (br m, 2H) and 1.50-1.85 (br m, 5H): H-1', H-2', *c*-hexyl H-1" [δ 1.62 (br d, J≈7.8 Hz)] and *c*-hexyl H-2" - H-6"; 2.10 (dd, J=16.2, 7.4 Hz, 1H): H-2a; 2.30-2.62 (m, 2H): H-2b [δ 2.54 (dd, J=22.6, 7.8 Hz)] and H-3 [δ 2.45 (ddd, J≈21.8, 15.0, 7.4 Hz); 3.85 (dd, J=9.0, 7.0 Hz, 1H): H-4a; 4.35 (dd, J=9.0, 7.3 Hz, 1H): H-4b. ^{13}C NMR ($CDCl_3$, 50 MHz, DEPT): δ 26.3 (t); 26.6 (t); 30.5 (t); 33.3 (t); 34.6 (t): C-2; 35.1 (t); 36.0 (d): C-3; 37.6 (d): *c*-hexyl C-1"; 73.5 (t): C-4; 177.3 (s): C-1.

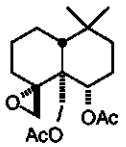
7.6 - Notes and References

1. For a compilation of all reported insect antifeeding clerodane diterpenes and related compounds, see Chapter 2.
2. (a) See Chapter 5. (b) See Chapter 6.
3. Y. Kojima and N. Kato, *Tetrahedron*, **37** (15), 2527-2538 (1981); Y. Kojima and N. Kato, *Nippon Kagaku Kaishi*, (5), 712-720 (1981).

4. See for instance: (a) W.M. Blaney, M.S.J. Simmonds, S.V. Ley and P.S. Jones, *Entomol. Exp. Appl.*, **46**, 267-274 (1988). (b) M.S.J. Simmonds, W.M. Blaney, S.V. Ley, G. Savona, M. Bruno and B. Rodríguez, *Phytochem.*, **28** (4), 1069-1071 (1989). For a complete account of all antifeedancy reports on (**2**) or (**3**), see ref. 1.
5. It has been suggested that the antifeedant activity of clerodin (**1**) and other clerodane antifeedants with a perhydrofuro[2,3b]furan subunit is a synergetic effect, related to the presence of (elements from) both the decalin fragment and the furofuran fragment in one molecule. (See: R.B.M. Geuskens, J.M. Luteyn and L.M. Schoonhoven, *Experientia*, **39**, 403 (1983).) In analogy, the data collected in Table 2 support the proposal of a similar hypothesis regarding the antifeedant activity of (**2**) and (**3**).

Table 7.2: Two-choice insect antifeedant activity of *neo*-clerodane diterpenes (**1-3**) and some related model compounds (**5**,**49**) against larvae of the fall armyworm (*Spodoptera frugiperda*) and the African armyworm (*Sp. exempta*).

	(1)	(2)	(3)	(5)	(49)
Sp. frugiperda	78 (4)[a,b]	94 (2)	47 (7)	12 (7)	15 (6)
Sp. exempta	76 (6)	38 (17)	73 (7)	34 (2)	20 (5)



49

Notes: (a) Measured at 100 ppm concentration in a two-choice test on artificial substrate (Glass-fibre/sucrose); (b) Activity reported as AI (sem), with AI = mean Antifeedant Activity = [(C-T)/(C+T)]×100% and sem = standard error of the mean. [Adapted from Ref. 4a,b]

6. X. Belles, F. Camps, J. Coll and M.D. Piulachs, *J. Chem. Ecol.*, **11** (10), 1439-1445 (1985).
7. For reviews on the use of protected cyanohydrins in synthesis, see: (a) J.D. Albright, *Tetrahedron*, **39** (20), 3207-3233 (1983); (b) S.Arseniyadis, K.S. Kyler and S.D. Watt, *Organic Reactions*, **31**, 1-367 (1984).
8. R.E. Ireland, S. Thaisrivongs, N. Vanier and C.S. Wilcox, *J. Org. Chem.*, **45** (1), 48-61 (1980).
9. J.A. Walker, II, J.J. Chen, D.S. Wise and L.B. Townsend, *J. Org. Chem.*, **61** (6), 2219-2221 (1996).
10. M. Kassou and S. Castillón, *Tetrahedron Lett.*, **35** (30), 5513-5516 (1994).
11. The selenoxide-mediated elimination strategy was also used in a synthesis of 2-heptyl-2,3-dihydrofuran. See: A.P Brunetière and J.Y. Lallemand, *Tetrahedron Lett.*, **29** (18), 2179-2182 (1988).
12. B. Cazes and S. Julia, *Tetrahedron*, **35**, 2655-2660 (1979).
13. 2-Ethoxyethyl or trimethylsilyl groups are most commonly used for protecting cyanohydrins. For TMS-protected *aliphatic* cyanohydrins however, alkylation reactions usually fail (ref. 7b), probably due to intramolecular migration of the silyl protecting group to the α-carbon atom of the intermediate cyanohydrin anion. See: E.L.M. van Rozendaal, S.J.T. Kuster, E.T. Rump and H.W. Scheeren, *Synth. Comm.*, **24** (3), 367-374 (1994).

14. G. Stork and L. Maldonado, *J. Am. Chem. Soc.*, **93** (20), 5286-5287 (1971) and **96** (16), 5272-5274 (1974).

15. In the literature, reduction of TMS- or TBDMS-protected cyanohydrins with DibalH to the corresponding aldehydes has been reported. See for instance: P.R. Ortiz de Montellano and W.A. Vinson, *J. Am. Chem. Soc.*, **101** (8), 2222-2224 (1979); M. Hayashi, T. Yoshiga, K. Nakatani, K. Ono and N. Oguni, *Tetrahedron*, **50** (9), 2821-2830 (1994).

16. See: M.N. Rerick, *The chemistry of mixed hydrides*. In: R.L. Augustine (Ed.), *Reduction - Techniques and application*, Marcel Dekker, New York, 1968, p. 71 and p. 73-74.

17. Analytical data for these isomers are included in the experimental section.

18. T. Imamoto, N. Takiyama, K. Nakamura, T. Hatajima and Y. Kamiya, *J. Am. Chem. Soc.*, **111** (12), 4392-4398 (1989); N. Greeves and L. Lyford, *Tetrahedron Lett.*, **33** (33), 4759-4760 (1992).

19. See: E. Calzada, C.A. Clarke, C. Roussin-Bouchard and R.H. Wightman, *J. Chem. Soc. Perkin Trans. I*, 517-518 (1995) and references cited therein.

20. G.A. Danilova, V.I. Mel'nikova and K.K. Pivnitsky, *Tetrahedron Lett.*, **27** (22), 2489-2490 (1986).

Chapter 8

Synthesis of a Model Compound with a Furano-Spirolactone Structure

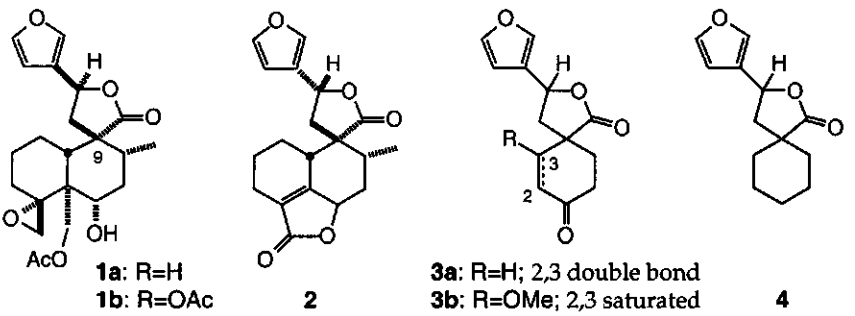
Abstract: *In this chapter, two different approaches are described towards the synthesis of a model compound (**4**) with a furano-spirolactone moiety, derived from the C-9 subunit of the natural neo-clerodane insect antifeedant teucjaponin B.*

Table of Contents

8.1 - Introduction

One of the C-9 sidechains most frequently encountered in natural *neo*-clerodane diterpenes[1] is a 4-(3-furyl)-butyrolactone moiety, which is connected to the decalin fragment in a spiro-fashion at the α-position of the lactone ring (see Scheme 8.1). Several of these C-9 furano-spirolactone diterpenes, such as teucjaponin B (**1a**)[2a,b] and its 6-acetate (**1b**)[3] or the 19-nor-clerodane 12-epi-teucvin (**2**),[2a] display significant antifeedant activity against various insect species.[4]

Scheme 8.1



1a: R=H
1b: R=OAc

2

3a: R=H; 2,3 double bond
3b: R=OMe; 2,3 saturated

4

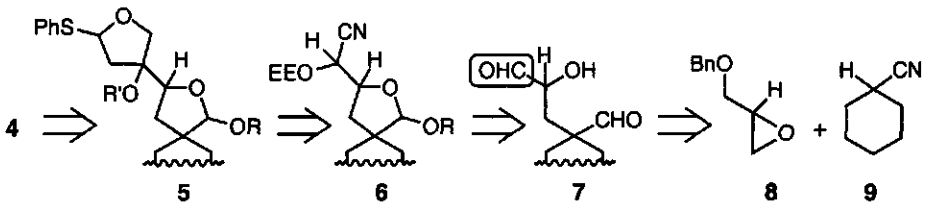
The data regarding the structure-activity relationships of these clerodanes are still fragmentary. Changes in the C-9 subunit of these compounds are often found to markedly affect the antifeedant activity.[4] Also, while (**1b**) is a potent antifeedant for *Spodoptera littoralis* larvae, the removal of either the furan ring[5] or the complete C-9 moiety[6a] results in compounds that are virtually inactive towards this insect, as are model compounds (**3a,b**)[6a] lacking the decalin fragment. These observations suggest that the antifeedancy of such diterpenes depends on structural features from both halves of the molecule.[7]

In continuation of the strategy adopted in previous chapters, our efforts were directed at the synthesis of model compounds derived from the C-9 subunit of these diterpenes. Since few other syntheses of such furano-spirolactones appear to have been published,[8] we set out to find a simple and flexible approach towards model compounds as (**4**). We were especially interested in the possibility to use the failed attempts towards 3-hydroxy-2,3-dihydrofurans described in the previous chapter as a means to construct the required furan ring.

8.2 - Attempted Synthesis of a Furano-Spirolactone via a Cyanohydrin-based Approach

Our first approach towards the synthesis of the furano-spirolactone target compound (**4**) was derived from the methodology employed in previous chapters (Scheme 8.2). From the experiences gained in Chapter 7, we anticipated that the application of the usual phenylsulfoxide-based elimination strategy to the sulfide (**5**) should in principle lead to formation of the 3-furyl ring in the target structure (**4**). Furthermore, the required 3-alkoxy-tetrahydrofuran moiety of (**5**) might be prepared from the protected cyanohydrin (**6**) through the methods used previously. Finally, the usual retrosynthetic ringopening of the central spirolactol ring of (**6**) to the γ-hydroxyaldehyde (**7**) showed this moiety to be accessible via addition of the nitrile (**9**) to an epoxide incorporating a masked carbaldehyde group, such as the benzyloxymethyl-substituted epoxide (**8**).

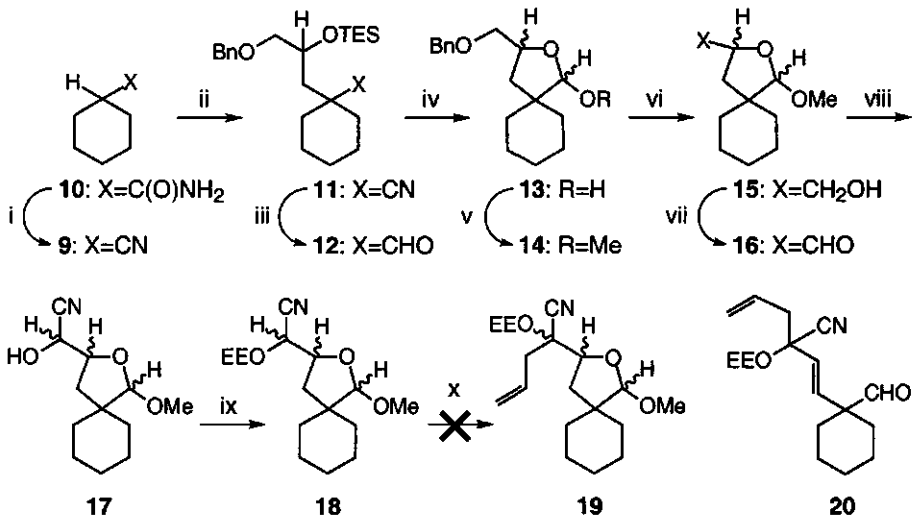
Scheme 8.2



Retrosynthetic scheme, outlining the planned approach to the furano-spirolactone target compound (**4**). Boxed functional groups are present in a masked form.

Cyclohexylcarbonitrile (**9**) was prepared in 80% yield by dehydration[9] of the carbamide (**10**) with thionylchloride (Scheme 8.3). Addition of the lithium-anion of (**9**) to benzylglycidyl ether (**8**)[10] at 0°C in THF, followed by trapping of the intermediate alkoxide with triethylsilyl chloride, afforded the silyloxy-nitrile (**11**) in 70% yield. The reduction of (**11**) with diisobutylaluminium hydride yielded a mixture of the aldehyde (**12**) and its iminic reduction intermediate;[11] treatment of this mixture with silicagel in EtOAc led to complete conversion of the intermediate, affording the silyloxy-aldehyde (**12**) in 96% yield. The construction of the central spirolactol ring system was completed by the simultaneous desilylation and cyclization of (**12**) with aqueous hydrofluoric acid to yield a 3 : 1 mixture of diastereomers of the lactol (**13**). Finally, the lactol moiety was protected by acid-catalyzed acetalization in methanol, which gave the corresponding methoxy acetals (**14**) in 87% yield from (**12**).

Scheme 8.3

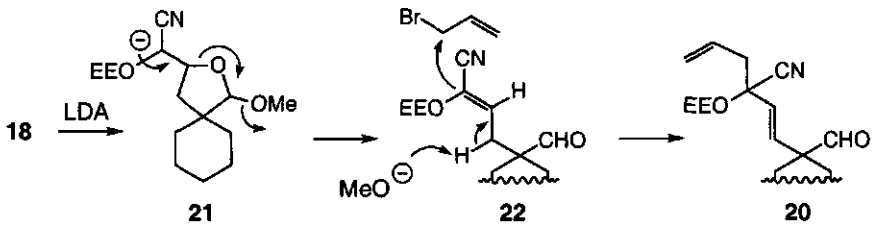


Reagents and conditions: (i) $SOCl_2$, Δ (80%); (ii) 1) LDA, THF, -78°C, 30 min; 2) add (**9**), 0°C; 3) TESCl (70%); (iii) 1) DibalH, Et_2O, -40°C; 2) Glaubers' salt; 3) silicagel, 16 hrs; (96%); (iv) HF, THF-water; (v) H_2SO_4 (cat.), MeOH (87% from **12**); (vi) H_2, Pd-C, EtOAc (100%); (vii) 1) oxalylchloride, DMSO, CH_2Cl_2, -55°C; 2) Et_3N; (viii) $Me_2C(OH)CN$, Et_3N; (ix) EVE, TFA, 0°C (57% from **15**); (x) 1) LDA, THF-HMPA (4:1), -60°C, 30 min; 2) $H_2C{=}CHCH_2Br$, 2 hrs.

The next stage in the synthetic route involved the transformation of the masked carbaldehyde group from (**14**) into a protected cyanohydrin. Hydrogenolysis of the benzyl ether on Pd/C quantitatively gave the alcohols (**15**). After a number of unsuccesful attempts with different oxidizing reagents,[12] (**15**) could finally be oxidized to the corresponding aldehydes in a practical manner by means of a Swern oxidation.[13] The resulting crude aldehydes (**16**) were treated with acetone cyanohydrin and triethylamine to yield a complex mixture of the cyanohydrins (**17**). Subsequently, crude (**17**) was protected as its 1-ethoxyethyl (EE) ether by acid-catalyzed addition to ethylvinyl ether. After purification, the protected cyanohydrins (**18**) were obtained in an overall yield of 57% from (**15**).

Unexpectedly, attempts to allylate (**18**) with allylbromide according to the procedure used in the previous chapter did not yield the desired adduct (**19**), but instead gave a mixture of three products, which featured an aldehyde group while the methoxy group had disappeared (^1H NMR). After repeated chromatographic steps, one of these products could finally be separated from the mixture and was identified as the rearrangement product (**20**). The formation of

Scheme 8.4



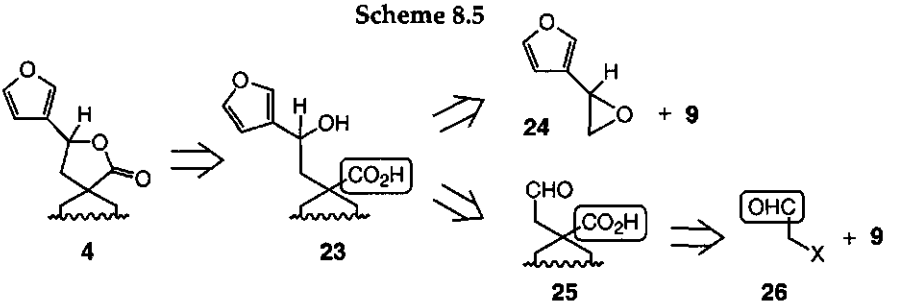
this compound can be rationalized (Scheme 8.4) through the occurence of a β-elimination reaction in the cyanohydrin-anion (**21**), which leads to opening of the cyclic acetal with expulsion of methoxide anion as leaving group. The intermediate aldehyde (**22**) may subsequently be deprotonated at the γ-position of the α,β-unsaturated nitrile moiety, followed by migration of the double bond and addition of allylbromide to the resulting negative charge on the α-carbon atom to yield the rearranged product (**20**).

8.3 - Alternative Approaches towards the Furano-Spirolactone Moiety

In our efforts towards the synthesis of the furano-spirolactone (**4**) we next embarked upon an alternative tactic, in which the early introduction of an intact furyl-moiety precedes the formation of the central lactone ring. Two different approaches were examined (Scheme 8.5).

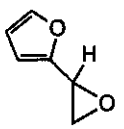
A very short route towards (**4**) consists of the addition of the nitrile (**9**) to a furano-epoxide as (**24**). Several related γ-hydroxynitriles are known to easily undergo cyclization to the corresponding (spiro)lactones under acidic[14] or basic conditions[15] and therefore this route could in principle lead to the desired target structure (**4**) in just two steps. However, the regiochemistry of the nitrile-addition to the epoxide (**24**) was considered as potentially problematic. Although addition

Scheme 8.5

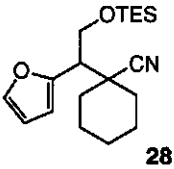


Retrosynthetic scheme, outlining some alternative approaches to the furano-spirolactone target (**4**). Boxed functional groups are present in a masked form.

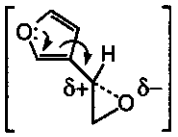
of a nucleophile to an epoxide under basic conditions is usually directed primarily by steric factors, arylsubstituted epoxides may experience reduced regioselectivity during ring opening due to the capability of the aryl π-system to stabilize a developing positive charge at C-1. This effect may be reinforced by the presence of a heteroatom in the aromatic ring: contrary to the general behaviour of arylsubstituted epoxides, furyl-2-oxirane (**27**) undergoes alcoholysis *without* any catalyst present and with ringopening exclusively taking place next to the aromatic ring.[16] We therefore first examined the feasibility of such a route with the epoxide (**27**).[17a] Indeed, addition of (**9**) to this epoxide yielded the adduct (**28**) with the unwanted regiochemistry as the only product.[16b] Since we expected a 3-furyl-substituent to be equally capable of stabilizing a positive charge at C-1 (*e.g.* as depicted in **24a**), the anticipated route appeared to be unsuitable for preparation of the desired moiety.



27



28



24a

In an alternative approach (Scheme 8.5), the 3-furyl ring is introduced into (**23**) as an organometal reagent by addition to a masked acid-aldehyde (**25**), for instance a cyano-aldehyde as (**30**) (Scheme 8.6). The rather volatile nitrile (**29**) was obtained in a moderate yield of 66% after distillation by allylation of the lithium-anion of (**9**) with allylbromide. Ozonolysis of the double bond of (**29**), followed by reductive work-up with triphenylphosphin, initially yielded a mixture of products. Apart from the expected aliphatic protons, the ^{1}H NMR spectrum of this mixture displayed signals characteristic of both aldehyde protons (δ=9.9 ppm) and acetal protons (δ=4.8-5.0 ppm) in a 1 : 1 ratio. The latter signals are indicative for the presence of polymeric forms of (**30**) in the mixture. Overnight treatment of the product mixture with silicagel effected the decomposition of these acetals and gave the desired cyano-aldehyde (**30**) in 73% overall yield.

Addition of 3-lithiofuran[18] to (**30**), followed by silylation of the intermediate alkoxide with triethylsilylchloride, gave the desired product (**31b**) in a rather low yield (Table 8.1, entry 1), while no starting material could be recovered. As a working hypothesis, we assumed that (**30**) was (also) converted into its lithium enolate by the lithiofuran acting as a base, upon which further aldol reactions might account for the loss of starting material. Indeed, with the less basic 3-furyl-dichlorocerate[19] (entry 2) we obtained 66% of recovered starting material in addition to the furyl adduct (**31a**), indicating a suppression of the side

Scheme 8.6

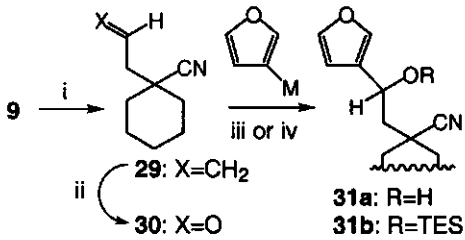
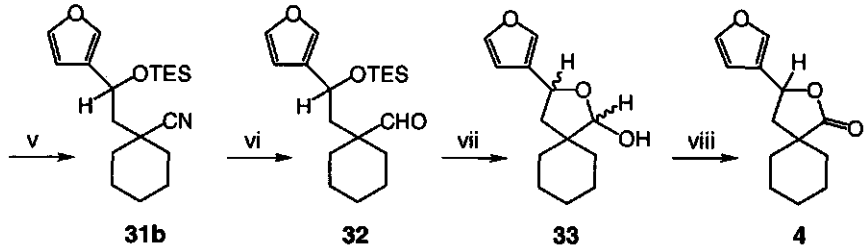


Table 8.1

entry	M	equiv.	**30** (%)	**31a/b** (%)
1	Li	1.2	0	39 (**b**)
2	$CeCl_2$	1.2	66	26 (**a**)
3	$CeCl_2$	3.0	0	67 (**a**)



31b **32** **33** **4**

Reagents and conditions: (i) 1) LDA, THF, -78°C, 15 min; 2) $H_2C{=}CHCH_2Br$, 1.5 hrs (66%); (ii) 1) O_3, CH_2Cl_2, -25°C; 2) PPh_3, r.t.; 3) silicagel, EtOAc, 16 hrs (73%); (iii) For M=Li: 1) 3-Br-furan, BuLi, THF, -78°C, 5 min; 2) add (**30**), -78°C, 60 min; 3) TESCl; (iv) For M=$CeCl_2$: 1) 3-Br-furan, BuLi; 2) $CeCl_3$, -78°C, 1 hr; 3) add (**30**), -78°C, 75 min; (v) TESCl, imidazole, DMAP (cat.), DMF (84%); (vi) 1) DibalH, Et_2O, -50°C; 2) Glaubers' salt; 3) silicagel, EtOAc, 24 hrs (90%); (vii) HF, THF-H_2O (67%); (viii) PDC (1.5 eq), CH_2Cl_2 (48%).

reactions. By increasing the amount of furanocerium reagent to 3 equivalents the yield of the alcohol (**31a**) could be increased to 67% (entry 3). Unfortunately, *in situ* quenching of the reaction with triethylsilylchloride did not lead to silylation of (**31a**), probably due to the increased stability of the intermediate cerium-oxygen complex compared to lithium. The alcohol (**31a**) was silylated in 84% yield to give the silyloxy-nitrile (**31b**), which was subsequently reduced with DibalH, affording the aldehyde (**32**) in 90% yield. Simultaneous desilylation and cyclization of (**32**) with hydrofluoric acid as before gave the lactols (**33**) in 67% yield. Finally, oxidation of (**33**) with an excess of pyridinium dichromate afforded the desired furano-spirolactone (**4**) in a moderate yield of 48% after chromatography.

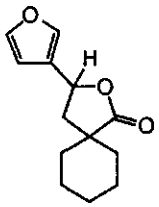
8.3 - Summary

This chapter describes some approaches towards the synthesis of the furano-spirolactone model compound (**4**) (Figure 8.2). Initially, it was planned to use the facile aromatization of a 3-hydroxy-2,3-dihydrofuran ring system, that occured as an unwanted side reaction in the previous chapter, as a means to construct the required furan ring. This approach had to be abandoned however, because the

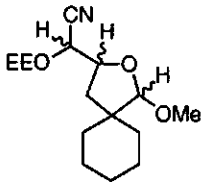
cyanohydrin intermediate (**18**) was unexpectedly prone to β-elimination and could not be allylated as intended.

In an alternative approach, the furan ring was introduced via a regioselective addition of 3-furyl-dichlorocerate to the aldehyde group of (**30**). Next, the central lactone ring was constructed according to the methods used in previous chapters, to give the furano-spirolactone (**4**) in 16% overall yield from (**30**).

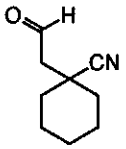
Figure 8.2



4



18



30

8.4 - Experimental Section

General experimental conditions were as described in the experimental section of Chapter 4.

Cyclohexylcarbonitrile (**9**). A mixture of 6.3 g (49.5 mmol) of cyclohexylcarbamide (**10**) and 7.6 ml (104 mmol) of thionylchloride was stirred at reflux-temperature for 1 hr. After cooling to room temperature, an 1M aqueous solution of sodium hydroxide was added and the mixture was extracted with three 50-ml portions of ether. The combined extracts were dried with $MgSO_4$ and the solvent was removed by distillation at atmospheric pressure. The residue was purified by bulb-to-bulb distillation under reduced pressure, affording 4.3 g (39.4 mmol, 80%) of the nitrile (**9**) as an oil (b.p. 100-110°C at 35-40 $mmHg^{20}$).
^1H NMR ($CDCl_3$, 200MHz): δ 1.23-1.50 (br m) and 1.50-1.90 (br m; total 12H): H-2 - H-6; 2.52-2.64 (m, 1H): H-1. ^{13}C NMR ($CDCl_3$, 50MHz, DEPT): δ 24.0, 25.1, 29.4 and 29.6 (4 t): C-2 - C-6; 27.9 (d): C-1; 122.6 (s): CN.

1-[1-(Benzyloxy)-2-(triethylsilyloxy)-prop-3-yl]-cyclohexylcarbonitrile (**11**). To a stirred solution of 1.65 g (15.1 mmol) of cyclohexylcarbonitrile (**9**) in 20 ml of anhydrous THF, cooled to -78°C, was added dropwise 9.1 ml (18.2 mmol) of a 2M solution of lithium-diisopropylamide in THF. After 30 min, a solution of 2.73 g (16.6 mmol) of racemic benzylglycidylether[9] (**8**) in 10 ml of THF was added dropwise at -78°C. Stirring was continued at 0°C while the progress of the reaction was monitored by glc-analysis. After 90 min, 3.0 ml (17.9 mmol) of triethylsilylchloride was added and the reaction mixture was stirred for 1 hr at room temperature. The reaction mixture was poured into 40 ml of water and 10 ml of saturated aqueous sodiumbicarbonate solution. The organic layer was separated and the aqueous layer was extracted with four 25 ml-portions of ether. The combined organic layers were washed twice with 25 ml of water, dried with $MgSO_4$ and concentrated under reduced pressure to yield 6.91 g of a yellow oil. The crude product was

purified by chromatography on 130 g of silicagel with petrol-EtOAc (98-2 to 97-3) as the eluent, affording 4.10 g (10.6 mmol, 70%) of the nitrile (**11**) as a clear, pale yellow oil.
^{1}H NMR ($CDCl_3$, 200MHz): δ 0.61 and 0.63 (2 q, J=7.9 Hz, 6H): $SiCH_2CH_3$; 0.90-0.99 (m t, J≈7.5 Hz, 9H): $SiCH_2CH_3$; 1.03-1.35 (br m) and 1.50-2.20 (br m; total 12H): H-3'a and H-3'b [δ 1.91 and 2.12 (2 br d, J≈13 Hz)] and *c*-hexyl H-2 - H-6; 3.37 (dd, J=9.5, 5.5 Hz) and 3.44 (dd, J=9.5, 5.0 Hz; total 2H): H-1'a and H-1'b; 4.13-4.20 (br m, 1H): H-2'; 4.52 (s, 2H): OCH_2Ph; 7.25-7.40 (br m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50MHz, DEPT): δ 5.2 (t): $SiCH_2CH_3$; 6.9 (q): $SiCH_2CH_3$; 22.8, 22.9, 25.2 and 36.4 (4 t): *c*-hexyl C-2 - C-6; 37.4 (s): *c*-hexyl C-1; 44.7 (t): C-3'; 68.9 (d): C-2'; 73.2 (t) and 74.7 (t): C-1' and OCH_2Ph; 123.5 (s): CN; 127.6, 127.8 and 128.3 (3 d): phenyl C-2" - C-6"; 138.0 (s): phenyl C-1".

1-[1-(Benzyloxy)-2-(triethylsilyloxy)-prop-3-yl]-cyclohexylcarbaldehyde (12). A stirred solution of 4.10 g (10.6 mmol) of the nitrile (**11**) in 50 ml of anhydrous ether was cooled to -50°C (external temperature) on a dry-ice acetone bath. In approx. 10 min, 14 ml of a 1M solution of diisobutylaluminum hydride (14.0 mmol) in hexanes was added dropwise. Stirring at -40 to -50°C was continued for 2 hrs while the progress of the reaction was monitored by glc analysis; after 75 min only a trace of starting material remained. The reaction was quenched by addition of 7.0 g (21.7 mmol) of Glauber's salt and the resulting mixture was stirred for 1 hr at room temperature. $MgSO_4$ was added and after stirring for 5 min the slurry was filtered through a pad of hyflo on a glassfilter. The solvent was removed under reduced pressure to afford 4.09 g of an oil, containing a mixture of the desired aldehyde (**12**) and its imine reduction intermediate.
^{1}H NMR ($CDCl_3$, 90MHz): δ 0.30-2.10 (br, 26H): H-3', *c*-hexyl H-2 - H-6, $SiCH_2CH_3$ [δ 0.55 (br t, J≈7.5 Hz, approx. 5H)] and $SiCH_2CH_3$ [δ 0.70-1.05 (br m, approx. 9H)]; 3.10-3.50 (br m, 2H): H-1'; 3.70-4.05 (br m, 1H): H-2'; 4.35-4.55 (m incl. s [δ 4.40], 2H): OCH_2Ph; 7.10-7.40 (br, 5H): phenyl H-2" - H-6"; 9.37 (s, 0.3H): CHO. IR ($CHCl_3$): υ **1640** (C=NH), 1720 (CH=O) cm^{-1}.
The residual oil was dissolved in 130 ml of ethylacetate, 40 g of silicagel was added and the mixture was stirred for 4 hrs. Filtration and evaporation of the solvent under reduced pressure yielded 3.98 g (10.2 mmol, 96%) of the aldehyde (**12**) as a clear, faint yellow oil, which was used as such in the next step.
^{1}H NMR ($CDCl_3$, 90MHz): δ 0.40-2.15 (br, 26H): H-3' [δ 1.80 (d, J≈6 Hz)], *c*-hexyl H-2 - H-6, $SiCH_2CH_3$ [δ 0.40-0.80 (br m, approx. 5H)] and $SiCH_2CH_3$ [δ 0.80-1.15 (br m, approx. 8H)]; 3.15-3.65 (m, 2H): H-1'; 3.80-4.10 (m, 1H): H-2'; 4.45-4.65 (m incl. s [δ 4.57], 2H): OCH_2Ph; 7.20-7.55 (br, 5H): phenyl H-2" - H-6"; 9.45 (s, 1H): CHO. IR ($CHCl_3$): υ 1720 cm^{-1}.

3-(Benzyloxymethyl)-1-methoxy-2-oxaspiro[4.5]decane (14). Over a period of 22 hrs, a solution of 3.98 g (10.2 mmol) of the aldehyde (**12**) in 30 ml of THF and 10 ml of water was sequentially treated with 0.5 ml-portions of a 40% aqueous hydrofluoric acid solution (a total of 3.0 ml was added) until a constant pH value of 2 was obtained and tlc-analysis indicated complete conversion of the starting material into a more polar product. Then, 50 ml of ether and 25 ml of water were added, the organic layer was separated and the aqueous layer was extracted with three 25 ml-portions of ether. The combined organic layers were dried with $MgSO_4$ and the solvent was evaporated under reduced pressure to afford 3.0 g of a mixture of the lactols (**13**) as a partly crystalline, yellow oil. The crude product was sufficiently pure to be used in the next reaction without purification.
^{1}H NMR ($CDCl_3$, 90MHz, selected peaks): δ 1.15-2.10 (br, 15H); 3.15-3.85 (br m, 4H): H-4 and H-1'; 4.20-4.55 (m, 1H): H-3; 4.60 (s, 2H): OCH_2Ph; 4.95 (s, 0.75H) and 5.10 (s, 0.25H): H-1; 7.20-7.50 (br, 5H): phenyl H-2" - H-6". IR ($CHCl_3$): υ 3600, 3410 (br) cm^{-1}.

A mixture of 2.97 g (approx. 10.7 mmol) of the crude lactols (**13**) and 60 µl (1.1 mmol) of conc. sulfuric acid in 25 ml of methanol was stirred at room temperature for 1 hr. Solid sodium bicarbonate was added until no more gas evolved and the solvent was removed under reduced pressure. The residue was dissolved in a mixture of 50 ml of water and 50 ml of ether, the organic layer was separated and the aqueous layer was extracted with three 25 ml-portions of ether. The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography on 80 g of silicagel with petrol-EtOAc (98.5-1.5 to 97-3) as the eluent to afford 2.54 g (8.7 mmol; 87% overall yield from (**12**)) of a 1 : 1 mixture of the acetals (**14**) as an oil.
^{1}H NMR ($CDCl_3$, 200MHz): δ 1.20-1.65 (br, 13H); 1.77-1.92 (m, 1H); 3.29 (s, 1.6H) and 3.36 (s, 1.4H): OCH_3; 3.36-3.57 (m, 2H): H-1'; 4.25-4.40 (m, 1H): H-3; 4.50-4.67 (m, 3H): H-1 and OCH_2Ph; 7.20-7.40 (br m, 5H): phenyl H-2" - H-6". ^{13}C NMR ($CDCl_3$, 50MHz, DEPT): *Major isomer*: δ 22.7, 23.7, 26.1, 31.7, 33.6 and 37.7 (6 t): C-4 and C-6 - C-10; 46.6 (s): C-5; 54.8 (q): OCH_3; 73.2 and 73.3 (2 t): C-1' and OCH_2Ph; 77.2 (d): C-3; 109.3 (d): C-1; 127.5, 127.7 and 128.3 (3 d): phenyl C-2" - C-6"; 138.4 (s): phenyl C-1". *Minor isomer*: (separated peaks only): δ 22.9, 23.9, 25.9, 31.8, 36.1 and 37.9 (6 t); 47.4 (s); 54.5 (q); 75.2 (t); 75.7 (d); 110.0 (d); 138.3 (s).

1-Methoxy-2-oxaspiro[4.5]decane-3-carbaldehyde (16). A solution of 2.54 g (8.7 mmol) of the acetals (**14**) in 50 ml of EtOAc, containing 25 mg of 10% Pd/C was hydrogenated in a Parr-apparatus under hydrogen pressure (4 atm) for 1.5 hrs until tlc-analysis indicated complete conversion of the starting material. The reaction mixture was filtered and concentrated under reduced pressure to yield 1.72 g (8.7 mmol, 100%) of the alcohols (**15**) as an oil, which was used without purification in the subsequent step.

To a stirred solution of 1.0 ml (11.5 mmol) of oxalyl chloride in 25 ml of dry CH_2Cl_2, cooled to -55°C on an acetone/dry ice bath, was added dropwise 2.0 ml (28.2 mmol) of dry dimethylsulfoxide. After 5 min a solution of 1.72 g (8.6 mmol) of the crude alcohols (**15**) in 10 ml of CH_2Cl_2 was added and stirring at -55°C was continued until tlc-analysis indicated complete conversion of the starting material (approx. 1.5 hrs). Then, 6.0 ml (43.0 mmol) of triethylamine was added, upon which a thick slurry was formed. After addition of 50 ml of CH_2Cl_2 to facilitate stirring, the reaction mixture was warmed to room temperature and stirred for 1 hr. Water (50 ml) was added, the organic layer was separated and the aqueous layer was extracted with three 50 ml-portions of CH_2Cl_2. The combined organic layers were washed sequentially with 50 ml of a 1% aqueous hydrochloride solution and twice with 30 ml of water, dried with $MgSO_4$ and concentrated under reduced pressure, affording 2.04 g of the crude aldehydes (**16**) as a turbid yellow oil, which was used as such in the next reaction.
^{1}H NMR ($CDCl_3$, 90MHz, selected peaks): δ 0.60-1.65 (br, 13.5H) and 1.65-2.30 (br m, 3H): H-4 and H-6 - H-10; 3.25-3.47 (br, 3H): OCH_3; 3.95-4.60 (br m, 1.6H): incl. H-3; 4.65 (s, 0.3H) and 4.67 (s, 0.7H): H-1; 9.58 (d, J≈3 Hz, 0.7H) and 9.64 (s, 0.3H): CHO.

3-[Cyano-(1-ethoxyethoxy)-methyl]-1-methoxy-2-oxaspiro[4.5]decane (18). A mixture of 2.04 g of the crude aldehydes (**16**), 0.8 ml (8.8 mmol) of acetone cyanohydrin and 0.1 ml (0.7 mmol) of triethylamine was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to afford 2.03 g of a yellow oil. The product was separated by chromatography on 40 g of silicagel with petrol-EtOAc (97-3 to 90-10) as the eluent, yielding 1.63 g of the crude cyanohydrins (**17**) as a faintly yellow oil, which was used as such without purification in the next step.
^{1}H NMR ($CDCl_3$, 90MHz, selected peaks): δ 0.65-1.70 (br, 11.5H) and 1.70-2.30 (m, 2.5H): H-4, H-6 - H-10; 3.34-3.44 (3 s, 3H): OCH_3; 4.10-4.70 (m, 3H): H-1, H-3 and H-1'.

A mixture of 1.63 g (7.2 mmol) of (**17**), 15.0 ml (0.16 mol) of ethylvinyl ether and 0.1 ml (1.3 mmol) of trifluoroacetic acid was stirred at room temperature for 10 hrs. The reaction mixture was poured into 10 ml of a saturated aqueous sodium bicarbonate solution, the organic layer was separated and the aqueous layer was extracted with two 25 ml-portions of ether. The combined organic layers were washed twice with 10 ml of water and dried with $MgSO_4$. The solvents were removed under reduced pressure and the residual oil (2.09 gr) was purified by chromatography on 40 g of silicagel with petrol-EtOAc (97-3) as the eluent to yield 1.47 g (4.9 mmol; overall yield 57% from (**15**)) of a complex mixture of stereoisomers of the protected cyanohydrin (**18**) as a colourless oil.
^{1}H NMR ($CDCl_3$, 200MHz): δ 1.10-1.27 (br, 4H): $OCH_2C\underline{H}_3$ [δ 1.21 (2 t, J≈6.5 Hz, 3H)]; 1.27-1.79 (br, 15H) and 1.85-2.10 (br m, 1H): H-4, H-6 - H-10 and $OCH(OEt)C\underline{H}_3$; 3.33-3.37 (6 s, 3H): OCH_3; 3.40-3.82 (br m, 2H): $OC\underline{H}_2CH_3$; 4.10-4.36 (br m, 1.6H) and 4.41-4.48 (m, 0.4H): H-3 and H-1'; 4.55-4.61 (6 s, 1H): H-1; 4.81-4.96 (m, 1H): $OC\underline{H}(OEt)CH_3$. ^{13}C NMR ($CDCl_3$, 50MHz, DEPT): δ 14.8 and 15.0 (2 q): $OCH_2\underline{C}H_3$; 19.5 (q): $OCH(OEt)\underline{C}H_3$; 22.6, 22.9, 23.5, 25.8, 25.9, 31.4, 31.6, 33.2, 33.7, 35.7, 37.1 and 38.9 (12 t): C-6 - C-10; 46.9 and 47.5 (2 s): C-5; 55.0 (br q): OCH_3; 61.1 and 61.3 (2 t): $O\underline{C}H_2CH_3$; 65.4, 66.0, 66.9, 67.7, 68.5 and 68.7 (6 d): C-1'; 76.0, 76.4, 77.0, 77.2, 77.6 and 78.3 (6 d): C-3; 99.0 (2 d) and 100.9 (2 d): $O\underline{C}H(OEt)CH_3$; 109.6 (d), 109.9 (d) and 110.3 (2 d): C-1; 117.0 and 117.9 (2 s): CN.

Allylation of (18). To a stirred solution of 500 mg (1.7 mmol) of the protected cyanohydrins (**18**) in 20 ml of anhydrous THF and 5 ml of HMPA, cooled to -60°C, was added dropwise 1.0 ml of a 2M solution of lithium diisopropylamide in THF-hexanes. After 30 min the yellow reaction mixture was treated dropwise with 0.2 ml (2.3 mmol) of allylbromide. Stirring at -60°C was continued for 2 hrs before the reaction was quenched by addition of 20 ml of water. The mixture was extracted with ether (4×25 ml). The combined extracts were washed with two 25 ml-portions of brine, dried with $MgSO_4$ and concentrated under reduced pressure to give 483 mg of a yellow oil. The product fraction was isolated by chromatography on 15 gr of silicagel with petrol-EtOAc (97-3) as the eluent, to afford 161 mg of an oil, consisting of a mixture of 3 products in a ratio of 1.3 : 1 : 1.2 (glc-analysis). Separation of the products was difficult and after repeated chromatography on silicagel with petrol-EtOAc (99-1) only a small amount (15 mg) of pure (**20**) could be obtained.
Product mixture: ^{1}H NMR ($CDCl_3$, 200MHz, selected peaks): δ 1.06-1.67 (br, 46H), incl. [δ 1.06-1.22 (m m)] and [δ 1.32 and 1.33 (2 d, J=5.3 Hz)]; 1.76-2.00 (br, 6H); 2.28-2.71 (m, 6H), incl. [δ 2.34 (d, J=8.4 Hz, 3H)] and [δ 2.47 and 2.64 (2 ddm, J≈13.7, 6.2 Hz, 3H)]; 3.21-3.80 (m, 6H); 4.80 and 4.81 (2 q, J=5.3 Hz, 1.5H); 5.06 (q, J=5.3 Hz, 1.5H); 5.14 (dm, J=18.8 Hz) and 5.17 (dm, J≈8.9 Hz; total 3H); 5.28 (d, J=16.3 Hz, 1H); 5.43 (d, J=16.3 Hz, 1H); 5.57-5.83 (m, 3H); 5.88 (d, J=16.4 Hz, 1H); 9.28 (s, 1H); 9.41 (s, 1H). ^{13}C NMR ($CDCl_3$, 50MHz, DEPT): δ 14.8 and 15.1 (2 t); 19.6, 20.7 and 21.1 (3 t); 22.1, 25.3, 30.6, 30.9 (2), 31.0 and 33.9 (7 t); 44.9 and 45.3 (2 t); 50.1 (s); 52.2 and 52.6 (2 s); 60.8, 61.0 and 62.4 (3 t); 76.3, 76.4, 77.1, 77.2 and 77.7 (5 s); 97.6 and 98.1 (2 d); 101.3 (d); 113.5 (q); 117.6 and 118.0 (2 q); 120.9 (t); 124.3 (d); 128.5 (q); 129.6, 129.8, 129.9 and 130.4 (4 d); 135.0 and 136.5 (2 d); 201.3 and 201.4 (2 d); 205.2 (d). IR ($CHCl_3$): υ 2200 (C≡N),1720 (CH=O), 1640 (C=C) cm^{-1}.
(*E*) -1- [3-cyano- 3-(1-ethoxyethoxy)- hexa-1,5-dien-1-yl]- cyclohexylcarbaldehyde (20): ^{1}H NMR ($CDCl_3$, 200MHz, *selected peaks*): δ 1.10-1.75 (br, 16H) and 1.81-2.03 (br, 2H): H-2 - H-6, $OCH_2C\underline{H}_3$ [δ 1.21 (t, J≈6.2 Hz)] and $OCH(OEt)C\underline{H}_3$ [1.28 (d, J=5.2 Hz)]; 2.52 (ddm, J=12.8, 7.8 Hz, 1H) and 2.70 (ddm, J=15.9, 6.0 Hz, 1H): H-4'; 3.45-3.82 (m, 2H): $OC\underline{H}_2CH_3$; 4.80-4.98 (m [incl. δ 4.85 (q, J=5.2 Hz)], 1H): $OC\underline{H}(OEt)CH_3$; 5.12-5.27 (m, 2H): H-6' (*Z*) [δ 5.19 (dm, J=8.5 Hz)] and H-6'(*E*) [δ 5.22 (dm, J=18.0 Hz)]; 5.32 (d, J=16.2 Hz, 1H): H-2'; 5.61-5.88 (m, 1H): H-4'; 5.93 (d, J=16.2 Hz, 1H): H-1'; 9.33

(s, approx. 1H): CHO. ^{13}C NMR ($CDCl_3$, 50MHz, DEPT, major peaks only): δ 14.9 (q): $OCH_2\underline{C}H_3$; 21.2 (q): $OCH(OEt)\underline{C}H_3$; 22.1, 22.2, 25.3, 31.0, 31.1 (5 t): C-2 - C-6; 45.4 (t): C-4'; 52.6 (s): C-1; 61.0 (t): $O\underline{C}H_2CH_3$; 77.2 (s): C-3'; 97.6 (d): $O\underline{C}H(OEt)CH_3$; 118.1 (s): CN; 121.0 (t): C-6'; 129.6 and 129.9 (2 d): C-1' and C-2'; 136.5 (d): C-5'; 201.4 (s): CHO.

1-(Prop-2-enyl)-cyclohexylcarbonitrile (29). To an ice-cold solution of 3 ml (22.9 mmol) of diisopropylamine in 25 ml of dry THF was added dropwise 14 ml (22.4 mmol) of a 16.M solution of *n*-butyllithium in hexanes; stirring was continued for 30 min. The mixture was cooled to -78°C and a solution of 2.0 g (18.3 mmol) of the nitrile (**9**) in 10 ml of THF was added dropwise, after 30 min followed by 3.7 ml (42.8 mmol) of neat allylbromide. Stirring at -78°C was continued while the progress of the reaction was monitored by tlc-analysis. After 90 min, 20 ml of water was added and the mixture was warmed to room temperature. The organic layer was separated and the water layer was extracted with two 25 ml-portions of ether. the combined organic layers were dried with $MgSO_4$ and concentrated at a rotary evaporator under reduced pressure (approx. 60 mbar) for 15 min. The resulting oil was distilled under reduced pressure in a short-path distillation apparatus to yield 503 mg of a fraction (bp 32-35°C at 11 mmHg) consisting of a mixture of (**29**) and (**9**) (respectively 84% and 11%; glc-analysis), and 1.8 g (12.1 mmol; 66%) of the nitrile (**29**) (bp 90-92°C at 11 mmHg) of >97% purity (remainder (**9**); glc-analysis).
^1H NMR ($CDCl_3$, 90MHz): δ 0.95-2.15 (br m, 10H): H-2 - H-6; 2.30 (d, J=7.5 Hz, 2H): allyl H-1'; 5.15 (d, J=16.0 Hz) and 5.20 (d, J=9.0 Hz; total 2H): allyl H-3'; 5.65-6.23 (m, 1H): allyl H-2'.

2-(1-Cyano-cyclohexyl)-acetaldehyde (30). An ozone/oxygen mixture was passed through a stirred solution of 1.8 g (12.1 mmol) of (**29**) in 20 ml of CH_2Cl_2, cooled to -18°C on an ice-salt bath, until a bright blue colour appeared and tlc-analysis indicated complete disappearance of the starting material. The solution was purged with nitrogen until the blue colour disappeared. Then 3.5 g (13.4 mmol) of triphenylphosphin was added and the mixture was stirred at room temperature for 4 hrs. Silicagel (20 gr) was added and stirring was continued overnight. The resulting slurry was filtered and the solvent was removed at a rotary evaporator under reduced pressure (approx. 60 mbar) to afford 5.8 g of a partly crystalline residue, from which the product was obtained by chromatography on 100 g of silicagel with petrol-ether (9-1 to 6-4) as the eluent, yielding 1.34 g (8.8 mmol; 73%) of the cyano-aldehyde (**30**) as a clear, colourless oil.
^1H NMR ($CDCl_3$, 90MHz): δ 0.75-2.50 (br m, 12H): *c*-hexyl H-2' - H-6'; 2.60 (d, J=3.0 Hz, 2H): $C\underline{H}_2CHO$; 9.85 (m, 1H): CHO.

2-(1-Cyano-cyclohexyl)-1-(3-furyl)-ethanol (31a). The 3-furyl-$CeCl_2$ reagent was prepared *in situ* according to the procedures described by Imamoto[21] and Greeves:[22] 2.3 g (6.2 mmol) of finely ground $CeCl_3 \bullet 7H_2O$ was heated at 140°C under high vacuum for 2 hrs to yield a free-flowing white powder. After slow cooling to room temperature under dry nitrogen atmosphere, the flask was placed on an ice-bath and 20 ml of ice-cold anhydrous THF was added via syringe under vigorous magnetic stirring. Next, the resulting milky suspension was stirred on an ultrasonic bath at room temperature for 1.5 hrs. The ultrasonic bath was removed and the dry $CeCl_3$-suspension was cooled to -78°C while stirring magnetically. In a separate reaction flask, 3.8 ml (6.1 mmol) of a 1.6M solution of *n*-butyllithium in hexanes was added to a solution of 0.54 ml (6.0 mmol) of 3-bromofuran in 10 ml of dry THF at -78°C. After stirring for 5 min, the resulting black solution was added dropwise *via canula* in approx. 5 min to the stirred $CeCl_3$-suspension. Stirring at -78°C was continued for 1 hr before a solution of 304 mg (2.0 mmol) of the cyano-aldehyde (**30**) in 10 ml of dry THF was added dropwise in approx. 10 min. The resulting clear reaction mixture was stirred for

75 min at -78°C while the progress of the reaction was monitored by glc-analysis. After complete disappearance of the starting material (**30**), 10 ml of saturated aqueous ammonium chloride solution and 5 ml of water were added and the mixture was warmed to room temperature. The reaction mixture was extracted with two 25 ml-portions of EtOAc[23] and the combined extracts were washed twice with 10 ml of water, dried with $MgSO_4$ and concentrated under reduced pressure to yield 392 mg of a brown oil. Purification of the crude product by chromatography on 20 g of silicagel with petrol-EtOAc (97-3 to 94-6) as the eluent afforded 295 mg (1.3 mmol; 67%) of (**31a**) as an oil.
^{1}H NMR ($CDCl_3$, 90MHz): δ 0.87-2.43 (br m, 13H): H-2 and *c*-hexyl H-2'-H-6'; 2.47-2.72 (br, 1H): OH; 5.00 (dd, J=9, 3 Hz, 1H): H-1; 6.47 (s, 1H): furyl H-4"; 7.47 (s, 2H): furyl H-2" and H-5". ^{1}H NMR ($CDCl_3$, 200MHz, separated peaks only): δ 5.03 (dd, J=9.1, 3.4 Hz, 1H); 6.39-6.40 (m, 1H); 7.35-7.40 (m, 2H). ^{13}C NMR ($CDCl_3$, 50MHz, DEPT): δ 22.9, 23.0, 25.2, 35.9 and 36.6 (5 t): *c*-hexyl C-2'-C-6'; 37.7 (s): *c*-hexyl C-1'; 47.8 (t): C-2; 64.1 (d): C-1; 108.3 (d): furyl C-4"; 123.6 (s): CN; 129.3 (s): furyl C-3"; 138.9 and 143.6 (2 d): furyl C-2" and C-5". IR ($CHCl_3$): ν 3580 (br) and 3450 (sh): OH; 2210: C≡N

1-[2-(Triethylsilyloxy)-2-(3-furyl)-ethyl]-cyclohexylcarbonitrile (31b). A mixture of 278 mg (1.3 mmol) of the cyano-alcohol (**31a**), 0.26 ml (1.5 mmol) of triethylsilylchloride, 260 mg (3.8 mmol) of imidazole and a catalytic amount of 4-dimethylaminopyridine in 5 ml of anhydrous DMF was stirred overnight at room temperature. The reaction mixture was poured into 25 ml of water and 5 ml of saturated aqueous sodiumbicarbonate solution and the resulting mixture was extracted with four 25 ml-portions of petrol. The combined extracts were washed twice with 10 ml of water, dried with $MgSO_4$ and concentrated under reduced pressure to afford 463 mg of an oil. The crude product was purified by chromatography on 15 g of silicagel with petrol-EtOAc (99-1) as the eluent to yield 356 mg (1.1 mmol; 84%) of the silyloxy-nitrile (**31a**) as a clear, colourless oil.
^{1}H NMR ($CDCl_3$, 90MHz): δ 0.3-0.7 (m, 6H): $SiC\underline{H}_2CH_3$; 0.7-2.35 (br m, 24H): *c*-hexyl H-2 - H-6, ethyl H-1' and $SiCH_2C\underline{H}_3$; 5.00 (dd, J=8, 5 Hz, 1H): ethyl H-2'; 6.40 (s, 1H): furyl H-4"; 7.35 (s, 2H): furyl H-2" and H-5".

1-[2-(Triethylsilyloxy)-2-(3-furyl)-ethyl]-cyclohexylcarbaldehyde (32). According to the procedure described for (**12**), 356 mg (1.1 mmol) of the nitrile (**31b**) was reduced with diisobutylaluminium hydride. After treatment of the intermediate reduction product mixture with silicagel in EtOAc for 24 hrs, 324 mg (1.0 mmol; 90%) of the crude aldehyde (**32**) was obtained as an oil, which was used without purification in the next reaction.
^{1}H NMR ($CDCl_3$, 90MHz): δ 0.3-0.7 (m, 6H): $SiC\underline{H}_2CH_3$; 0.7-1.05 (m, 9H): $SiCH_2C\underline{H}_3$; 1.05-2.30 (m, 15H): ethyl H-1'a [δ 1.69 (dd, J=14.4, 3.6 Hz)], ethyl H-1'b [δ 2.13 (dd, J=15.0, 9.0 Hz)] and *c*-hexyl H-2 - H-6; 4.76 (dd, J=10.2, 3.3 Hz, 1H): ethyl H-2'; 6.40 (s, 1H): furyl H-4"; 7.29 and 7.34 (2 s, total 2H): furyl H-2" and H-5"; 9.45 (s, 1H): CHO.

3-(3-Furyl)-2-oxaspiro[4.5]decan-1-ol (33). Cyclization of 324 mg (1.0 mmol) of the crude aldehyde (**32**) with hydrofluoric acid according to the procedure described for (**14**) gave 223 mg of a yellow oil. The crude product was was purified by chromatography on 10 g of silicagel with petrol-EtOAc (95-5) as the eluent to yield 143 mg (0.64 mmol; 67%) of the lactols (**33**) as an oil.
^{1}H NMR ($CDCl_3$, 90MHz): δ 0.70-1.85 (br): H-6 - H-10; 1.85-2.35 (br m, 2H): H-4; 3.0-3.25 (br, 1H): OH; 4.95-5.40 (br m, 2H): H-1 and H-3; 6.15-6.55 (m, 1H): furyl H-4"; 7.35 (s, 2H): furyl H-2' and H-5'.

3-(3-Furyl)-2-oxaspiro[4.5]decan-1-one (4). A mixture of 137 mg (0.62 mmol) of the lactols (**33**) and 350 mg (0.93 mmol) of pyridinium dichromate in 10 ml of dichloromethane was

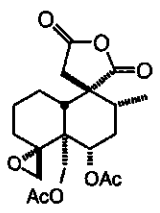
stirred at room temperature for 2 days until tlc-analysis indicated complete disappearance of the starting material. The reaction mixture was poured into 100 ml of ether and filtered through a pad of hyflo on a glass filter. The filtrate was concentrated under reduced pressure to yield a brown oil, which was reconcentrated from toluene (1×) and petrol (2×) to remove traces of pyridine. Chromatography on 10 g of silicagel with petrol-EtOAc (98-2) as the eluent gave 64.9 mg (0.30 mmol; 48%) of the furano-spirolactone (**4**) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 90MHz): δ 0.65-2.10 (br m, 13H): H-4a [δ 2.15 (dd, J=12.0, 10.5 Hz)] and H-6 - H-10; 2.60 (dd, J=13.0, 6.5 Hz, 1H): H-4b; 5.39 (dd, J=10.2, 6.3 Hz, 1H): H-3; 6.40 (s, 1H): furyl H-4"; 7.40 and 7.45 (2 s, total 2H): furyl H-2" and H-5".

8.5 - Notes and References

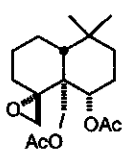
1. For reviews on natural clerodane diterpenes, see: A.T. Merrit and S.V. Ley, *Nat. Prod. Reports*, 243-287 (1992) and F. Piozzi, *Heterocycl.*, **37** (1), 603-626 (1994).
2. (a) M.S.J. Simmonds, W.M. Blaney, S.V. Ley, G. Savona, M. Bruno and B. Rodríguez, *Phytochem.*, **28** (4), 1069-1071 (1989). (b) B. Rodríguez, M.C. de la Torre, A. Perales, P.Y. Malakov, G.Y. Papanov, M.S.J. Simmonds and W.M. Blaney, *Tetrahedron*, **50** (18), 5451-5468 (1994).
3. M.E. Sosa, C.E. Tonn and O.S. Giordano, *J. Nat. Prod.*, **57** (9), 1262-1265 (1994) and J.M. Luco, M.E. Sosa, J.C. Cesco, C.E. Tonn and O.S. Giordano, *Pestic. Sci.*, **41**, 1-6 (1994).
4. For examples of other C-9 furano-spirolactone clerodanes and/or other insect species, see the extensive survey of clerodane diterpenes and related compounds that were reported tested for insect antifeedant activity in Chapter 2.
5. P.Y. Malakov, G.Y. Papanov, B. Rodríguez, M.C. de la Torre, M.S.J. Simmonds, W.M. Blaney and I.M. Boneva, *Phytochem.*, **37** (1), 147-157 (1994).
6. W.M. Blaney, M.S.J. Simmonds, S.V. Ley and P.S. Jones, *Entomol. Exp. Appl.*, **46**, 267-274 (1988).
7. (a) It has been suggested that the antifeedant activity of clerodane antifeedants with a perhydrofuro[2,3b]furan subunit is a synergetic effect, related to the presence of (elements from) both the decalin fragment and the furofuran fragment in one molecule [ref. 7b]. In analogy, the data collected in Table 2 suggest a similar effect in the activity of (**1a**). However, the absence of a 6-acetyl group in (**1a**) complicates the comparison with (**34**,**35**); unfortunately, (**1b**) has not been tested on *Sp. littoralis*.

Table 8.2: Two-choice insect antifeedant activity of the *neo*-clerodane diterpene teucjaponin B (**1a**) and some related model compounds against larvae of the Egyptian cotton leafworm (*Spodoptera littoralis*).

	AI (sem)[a,b]	ref.
(**1a**)	48.9 (5.98)	2a,b
(**34**)	-16.9 (7.70)	5
(**35**)	1.8 (0.89)	6
(**3a**)	9.4 (9.33)	6
(**3b**)	-5.3 (7.82)	6



34



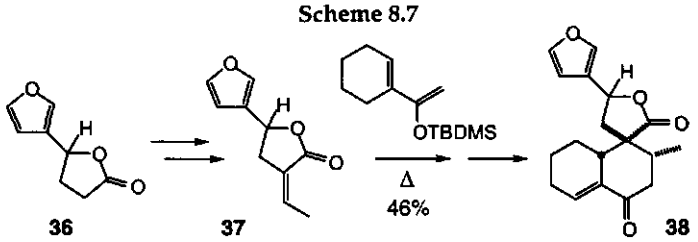
35

Notes: (a) Mean Antifeedant Index, AI = [(C-T)/(C+T)]×100%; sem = standard error of the mean; (b) Measured at 100 ppm concentration in a two-choice test on artificial substrate (Glass-fibre/sucrose);

(b) R.B.M. Geuskens, J.M. Luteyn and L.M. Schoonhoven, *Experientia*, **39**, 403 (1983).

8. (a) Actually, we are not aware of *any* publication concerning full synthetic details on the preparation of such model compounds. The only example of such a synthesis known to us is a preliminary account of the synthetic intermediate (**38**) in a planned route towards some natural clerodanes by S.V. Ley and coworkers (Scheme 8.7). However, to our knowledge no details of this work have been published. See: S.V. Ley, *Synthesis of insect antifeedants*. In: R. Greenhalgh and T.R. Roberts (Eds.), *Pesticide science and biotechnology (Proceedings of the sixth international congress on pesticide chemistry, Ottawa, Canada, 10-15 August, 1986)*, Blackwell Scientific Publications, Oxford, 1987, p. 25-34.

Scheme 8.7



(b) To our knowledge, no synthetic details of the model compounds (**3a,b**) have been published either. However, since Ley and coworkers were also involved in this work [ref. 6], it seems likely that these analogues were prepared via a similar Diels-Alder-based strategy as in Scheme 8.7. (c) For a related approach using a Diels-Alder reaction with allenic lactones for the construction of the spiro-attachment to the decalin ring system, see: M.E. Jung, C.N. Zimmerman, G.T. Lowen and S.I. Khan, *Tetrahedron Lett.*, **34** (28), 4453-4456 (1993).

9. See: B.S. Furniss, A.J. Hannaford, P.W.G. Smith and A.R. Tatchell, *Vogel's textbook of practical organic chemistry*, 5th Ed., Longman, Harlow, 1989, p. 716-717.

10. Benzylglycidylether (**8**) was easily prepared by BF_3-catalyzed addition of benzyl alcohol to racemic epichlorohydrin, followed by an intramolecular Williamson reaction with sodiumhydroxide. See: S. Takano, Y. Sekiguchi, M. Setoh, T. Yoshimitsu, *Heterocycles*, **31** (9), 1715-1719 (1990).

11. Similar imine intermediates were previously observed upon reduction of other nitriles; see Chapters 5-7.

12. Other standard oxidation methods were found to be unsuitable for transformation since they afforded complex product mixtures (PDC, NDC and TPAP/NMO) or because no reaction took place (TEMPO, MnO_2).

13. K. Omura and D. Swern, *Tetrahedron*, **34**, 1651-1660 (1978).

14. See chapter 6.

15. S. Takano, S. Yamada, H. Numata and K. Ogasawara, *J. Chem. Soc., Chem. Comm.*, 760-761 (1983).

16. (a) B. Alcaide, C. Biurrun, J. Plumet and E. Borredon, *Tetrahedron*, **44**, 9719-9724 (1992); (b) B. Alcaide, C. Biurrun and J. Plumet, *Tetrahedron*, **50** (18), 5555-5560 (1994).

17. (a) The epoxide (**27**) was easily prepared from furfural, according to the procedure described in : M.E. Borredon, M. Delmas and A Gaset, *Tetrahedron*, **43**, 3945-3954 (1987). (b) Characteristic ^1H NMR signals for **1-[2-(triethylsilyloxy)-1-(2-furyl)-ethyl]-cyclohexylcarbonitril (28)** in the crude product: ^1H NMR ($CDCl_3$, 90 MHz, separated

peaks only): δ 2.90 (t, J=6.0 Hz, 1H) : H-1'; 4.01 and 4.05 (2 d, J=6.0 Hz; total 2H): H-2'; 6.15-6.40 (br m, 2H): furyl H-3" and H-4"; 7.30 (s, 1H): furyl H-5". No other peaks were present in the 4-6 ppm area.

18. Y. Fukuyama, Y. Kawashima, T. Miwa and T. Tokoroyama, *Synthesis*, 443-444 (1974).
19. See: L.A. Paquette and R.E. Maleczka Jr., *J. Org. Chem.*, **57** (26), 7118-7122 (1992) and references cited therein.
20. A more laborious vacuum distillation of the crude product in a standard distillation apparatus fitted with a vigreux column similarly gave pure (**9**) with a boiling point of 65-68°C at 12 mmHg [Lit.: b.p. 62-67°C at 12 mmHg; O.H. Oldenziel, D. van Leusen and A.M. van Leusen, *J. Org. Chem.*, **42** (19), 3114-3118 (1977)], which was identical in all respects to the product obtained by bulb-to-bulb distillation.
21. T. Imamoto, N. Takiyama, K. Nakamura, T. Hatajima and Y. Kamiya, *J. Am. Chem. Soc.*, **111** (12), 4392-4398 (1989); N. Greeves and L. Lyford, *Tetrahedron Lett.*, **33** (33), 4759-4760 (1992).
22. N. Greeves and L. Lyford, *Tetrahedron Lett.*, **33** (33), 4759-4760 (1992).
23. The use of ether as a solvent in the extraction step was found to result in a difficult separation of the organic and aqueous layers.

Chapter 9

Insect Antifeedant Activity of some Model Compounds derived from Clerodane Diterpenes

Abstract: *In this chapter, the results of antifeedancy bioassays with a number of model compounds from the Chapters 5-7 are discussed. Larvae of the large cabbage white butterfly (Pieris brassicae) and the Colorado potato beetle (Leptinotarsa decemlineata) and nymphs of the peach-potato aphid (Myzus persicae) were used in these tests.*

Table of Contents

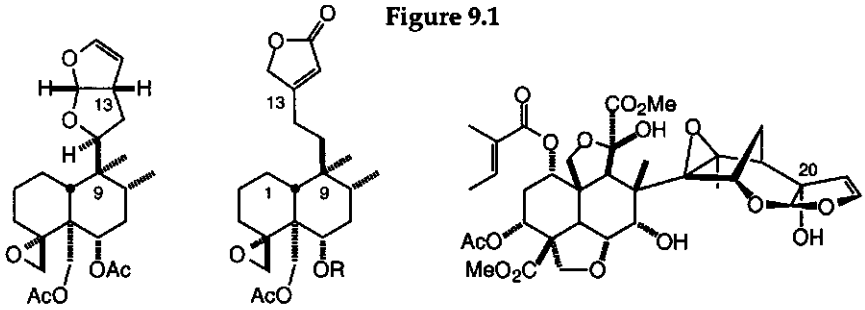
Parts of this chapter will be published in a revised form, in combination with chapters 5 and 6 (E.A. klein Gebbinck, C.T. Bouwman, M. Bourgois, B.J.M. Jansen and Ae. de Groot, *Synthesis and insect-antifeedant activity of C-2 and C-5 substituted perhydrofurofurans and 3a-hydroxy-perhydrofurofurans (Part I), Tetrahedron,* accepted for publication) and in combination with chapter 7 (E.A. klein Gebbinck, G.A. Stork, B.J.M. Jansen and Ae. de Groot, *Synthesis and insect-antifeedant activity of 2-substituted 2,3-dihydrofuran-3-ols and butenolides (Part II), Tetrahedron,* accepted for publication).

9.1 - Introduction

Several members of the class of clerodane diterpenes are known to display antifeedant activity against various insect species, especially from the order Lepidoptera. A number of synthetic model compounds, derived from (fragments of) these natural products, were reported to have retained some of this biological activity.[1] Such simplified analogues are of interest as potential starting points in the development of new crop protection agents.

In the preceding chapters the preparation of series of test compounds was described. These series consisted of simplified analogues derived from the C-9 sidechain fragments of clerodin (**1**) and of the ajugarins (**2a,b**). Furthermore, some modified model compounds were prepared, in which a tertiary hydroxyl group was introduced at the carbon atom corresponding with the C-13 position in the clerodane skeleton. The reason for this modification was the structural similarity between the furofuran substructure of clerodin and the furopyran fragment present in the potent antifeedant azadirachtin (**3**). The C-20 hydroxyl group in the latter fragment is believed to be important for the antifeedant activity of azadirachtin.[2] Also, this furopyran fragment separately already is a potent antifeedant for some insects species.[3]

Figure 9.1



1: clerodin

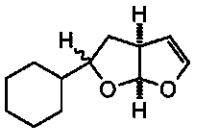
2a: R=Ac; ajugarin I
2b: R=H; ajugarin II

3: azadirachtin

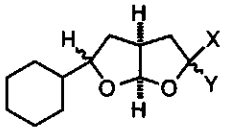
A number of these model compounds (Figure 9.2) have been screened* for insect antifeedant activity against species from different insect orders; larvae of *Pieris brassicae* (order: Lepidoptera) and *Leptinotarsa decemlineata* (Coleoptera) and nymphs of *Myzus persicae* (Homoptera) were used in these tests. In this chapter the results of these experiments are discussed.

*) These biological assays were performed by L. Messchendorp and G.J.Z. Gols at the department of Entomology, Wageningen Agricultural University, as part of a doctoral dissertation[4] (L.M.) under the supervision of J.J.A. Van Loon and L.M. Schoonhoven. We are grateful for their kind permission to reproduce their results in this chapter.

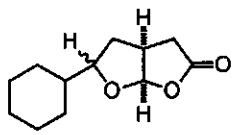
Figure 9.2[a]



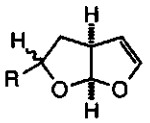
4



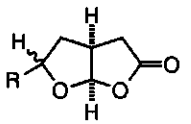
5: X,Y=H,H
6: X,Y=H,OH
7: X,Y=H,OEt
8: X,Y=H,OAc



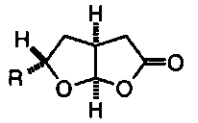
9
10: 5S-epimer only
11: 5R-epimer only



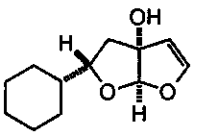
12: R=*n*-C_6H_{13}
13: R=Ph



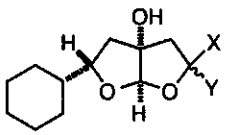
14: R=*n*-C_6H_{13}
15: R=*n*-$C_{10}H_{21}$



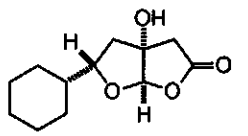
16: R=*n*-$C_{10}H_{21}$
17: R=Ph
18: R=*t*-C_4H_9



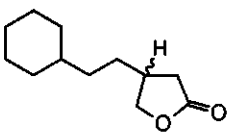
19



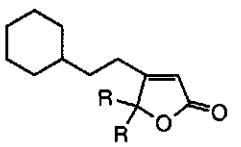
20: X,Y=H,H
21: X,Y=H,OH
22: X,Y=H,OMe
23: X,Y=H,OAc



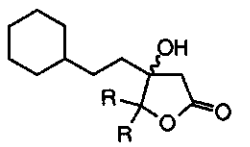
24



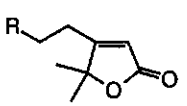
25



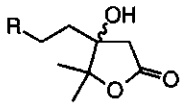
26: R=H
28: R=Me



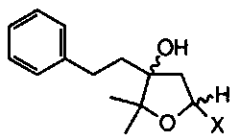
27: R=H
29: R=Me



30: R=*n*-C_4H_9
32: R=Ph



31: R=*n*-C_4H_9
33: R=Ph



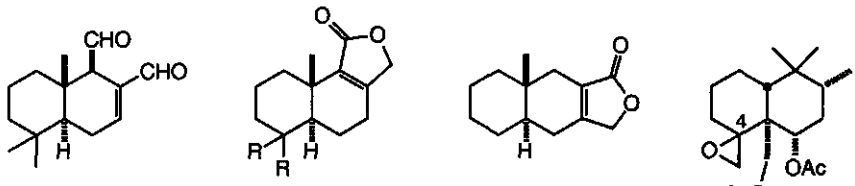
34: X=H
35: X=OMe
36: X=OH

Notes a) All test compounds are racemates.

9.2 - Large Cabbage White Butterfly (*Pieris Brassicae*)

Vegetable crops as cauliflower, broccoli, red or white cabbage and Brussels sprouts (all varieties of *Brassica oleracea*) are damaged by a wide range of insect pests.[5] Among these pests are a number of Lepidopteran insect species, of which the larvae can cause considerable feeding damage that reduces both yield and quality of the crop. One of these, the large cabbage white butterfly (*Pieris brassicae*), has been widely employed in studies related to insect-plant relationships, including investigations into the effects of several well-known insect antifeedants. For instance, drimane sesquiterpenes as polygodial (**37**) or isodrimenin (**38**) and various analogues (**39**, **40**) derived from these natural compounds have been shown to disrupt the feeding behaviour of *Pieris brassicae* larvae.[4,6] Antifeedant activity has also been reported for azadirachtin (**3**).[7]

Figure 9.3



37: polygodial

38: R=Me; isodrimenin
39: R=H

40

41a: 4α-O
41b: 4β-O

So far, the class of *neo*-clerodane insect antifeedants has received little attention in studies with *Pieris brassicae*. To our knowledge, no clerodane diterpene has ever been examined for insect antifeedant activity against this species. However, in no-choice bioassays on artificial substrate, some simplified analogues (**41a,b**) derived from the decalin fragment of such diterpenes showed promising levels of activity.[8] It was therefore of interest to examine the model compounds (**4-36**) for possible insect antifeedant activity against *Pieris brassicae*. These bioassays[9] were conducted with 5th instar larvae, using cabbage leaf discs as the substrate. All test compounds were applied in 5 mM concentration. The results of the experiments are reproduced in the Tables 9.1-9.3.

9.2.1 - Furofuran Model Compounds: No-Choice Bioassay

In screening compounds for potential antifeedant activity a no-choice bioassay is often considered to be more appropriate than a two-choice assay, because the no-choice situation is most representative of the application of an antifeedant in the field.[10] For this reason, the furofuran model compounds (**4-9**), (**12**) and (**13**) were

first tested in a no-choice situation. The bioassay was conducted in two consecutive periods of 1.5 hrs each, in order to determine if the larvae might possibly be able to adapt to the presence of less palatable compounds in their diet (short-term habituation). Conversely, a significant increase in the feeding inhibitory activity of a test compound during the second period, relative to its activity in the first period, could be indicative of the occurrence of some post-ingestive effect.

Table 9.1: Insect antifeedant activities of the model compounds (**4-9, 12** and **13**) against 5th instar larvae of the large cabbage white butterfly (Lepidoptera: *Pieris brassicae*) in a no-choice bioassay on cabbage leaf discs. [a]

	AI (sem) [b,c] (%)			AI (sem) [b,c] (%)	
	0-1.5 hrs [d]	1.5-3 hrs [d]		0-1.5 hrs [d]	1.5-3 hrs [d]
4	-9.3 (5.2)	-12.3 (4.3)	**9**	22.4 (5.0) **	13.9 (4.3) **
5	6.7 (6.8)	5.4 (6.9)	**12**	20.3 (5.3) **	10.6 (4.6) *
6	18.6 (7.6) *	15.9 (7.0) *	**13**	-1.7 (6.2)	-2.6 (3.0)
7	18.3 (8.5)	9.5 (5.8)			
8	16.7 (5.4) *	3.0 (3.8)	**40**	47.0 (3.4) ***	66.1 (2.3) ***

Notes: (a) Adapted from ref. 4. (b) Mean Antifeedant Index, AI = [(C-T)/C]×100; sem=standard error of the mean. (c) Originally reported as the 'Inhibition Percentage' (I.P.) of identical value. (d) The no-choice assay was divided over two consecutive periods, each 90 min long. Leaf discs were renewed between periods. Column '0-1.5 hrs' contains the results obtained in the first period. (***) Statistically significant difference between control and treatment discs (Mann-Whitney U test); $p<0.001$. (**) $p<0.01$. (*) $p<0.05$.

Although some of the compounds did produce a statistically significant reduction in the amount of feeding (Table 9.1), only low levels of activity were detected. Compounds (**5**) and (**13**) were inactive under these test conditions, while (**4**) even somewhat stimulated the food intake of the larvae. For the compounds showing an antifeeding effect, the initial activity usually decreased in the second period, suggesting the occurrence of short-term habituation. Such a rapid habituation has previously been observed for *Pieris brassicae* larvae in similar experiments with drimane-type antifeedants.[4,11] None of the compounds showed signs of post-ingestive toxicity.

The levels of antifeedant activity of the furofuran model compounds in the present assay were markedly lower than those found previously for the decalin fragments (**41a,b**) on an artificial substrate (glucose-coated styropor lamellae).[8] This does not necessarily imply that these furofurans are less active than the decalin fragments, since the antifeedant activity of a compound is often greater when tested on an artificial substrate than when a leaf disc is used.[12]

Furthermore, the decalin fragments were applied onto the artificial substrate by dipping the substrate into a test solution of the reported concentration; due to this procedure the actual amount of test compound present *on the substrate* is not known and may well have been higher than for the furofurans.[13]

9.2.2 - Furofuran Model Compounds: Two-Choice Bioassay

In view of the low levels of activity in the no-choice tests, the antifeedancy assays of the furofuran model compounds (**4-25**) with *Pieris brassicae* larvae were continued in a two-choice situation (Table 9.2). Such choice assays are often more sensitive because the insects can easily avoid food containing less palatable substances, which makes the difference between the diet treated with antifeedants and the non-treated diet more pronounced.[14] Indeed, compounds (**5**) and (**13**) (inactive in the no-choice test) now displayed antifeeding activity. On the other hand, compound (**4**) (stimulated feeding in the no-choice test) had become virtually inactive in the two-choice situation. The other compounds tested in both situations in general did not show increased levels of activity in the two-choice experiment; in some cases (**6, 9, 12**) the activity had even somewhat decreased. In the literature, differences between the activity in no-choice vs. two-choice assays have sometimes been used to discuss possible modes of action of the test compounds involved (*e.g.* sensory feeding deterrency vs. toxicity), but in our case neither the observed differences nor the actual levels of activities themselves were deemed sufficiently secure to allow such speculations.

Apart from the usual variance associated with the mean Antifeedant Index (AI), the values obtained in this two-choice assay were demonstrated to contain an additional uncertainty. The testing of the series (**4-24**) was conducted over a period of several days. Unexpectedly, for experiments that were repeated on different days, the results were found to vary in magnitude. It is not known whether this variation was due to temporal changes in the insect population or if other (external) factors were involved. Reference compound (**40**) was tested on every day and the AI varied from 23±10% to 62±10% (nine replicated measurements); model compounds (**9, 10, 13** and **24**) were also tested repeatedly (N=2-3) and for these measurements the results varied no more than 11%.[15] For the repeated tests, this day-to-day variation was accounted for in Table 9.2 by taking the mean of the different results as the final value. However, the other compounds included in this table (and in Table 9.3) were only tested on a single day and it seems likely that these values too are subject to an extra uncertainty of similar magnitude.

In this two-choice assay, 8 of the 21 test compounds displayed statistically significant antifeedant activity and the most active compound (**19**) was more potent than the reference compound (**40**). In view of the applied concentration,

however, none of the compounds can be considered as a particularly strong antifeedant for *Pieris brassicae* larvae.

Table 9.2: Insect antifeedant activities of the furofuran model compounds (**4-24**) against 5th instar larvae of the large cabbage white butterfly (Lepidoptera: *Pieris brassicae*) in a two-choice bioassay on cabbage leaf discs. [a]

	AI (sem) [b] (%)		AI (sem) [b] (%)		AI (sem) [b] (%)
4	-2.5 (7)	**12**	11 (9)	**20**	24 (9) *
5	26 (7) **	**13**	*11 (14)* [c]	**21**	16 (6) **
6	10 (9)	**14**	-11 (8)	**22**	18 (10)
7	20 (9)	**15**	41 (11) **	**23**	9 (7)
8	16 (9)	**16**	10 (6)	**24**	*9 (4)* [e]
9	*10 (6)* [c]	**17**	18 (9) *		
10	*9 (3)* [c,d]	**18**	28 (11) *		
11	24 (8) *	**19**	54 (12) **	**40**	*33 (4)* ** [f]

Notes (a) Adapted from Ref 4. Values printed in italics were calculated from the reported data. (b) Mean Antifeedant Index AI=[(C-T)/(C+T)]×100; sem=standard error of the mean. (c) Average AI value, calculated from the results reported for the same experiment on two different days. Average sem calculated according to standard procedures regarding multiplication of errors in repeated measurements. (d) The results of these two experiments with (**10**) differed substantially: in one experiment compound (**10**) displayed statistically significant antifeedancy (AI=19±7*), whereas it was almost inactive in the other (AI=8±7). (e) Average value from three experiments. See note c. (f) Average value from four experiments (AI range: 23±10 to 39±9). See note c. (**) Statistically significant difference between control and treatment areas (Wilcoxon's matched pairs test); $p<0.01$. (*) $p<0.05$.

9.2.3 - Some tentative Structure-Activity Relationships

Due to the rather high variance among individual mean AIs, relative to their magnitudes, it is difficult to discriminate between the activities of the different test compounds. Furthermore, since both the individual measurements and the differences between them often are devoid of a satisfactory degree of statistical significance, it is inappropriate to regard these differences with a high level of confidence. On the other hand, a lack of statistical significance for an observation does not necessarily imply that the observation is wrong and therefore some tentative, qualitative comparisons may still provide an indication for the underlying structure-activity relationships.

With regard to the nature of the furofuran ring system it seems that the presence of an oxygen-containing functional group at C-2 is less favourable, since

such derivatives appear to be somewhat less active than the corresponding dihydro-compounds. This trend is observed for both the regular furofurans [compare (**5**) with (**6-8**)] and the modified 3a-hydroxy-furofurans [(**20**) vs. (**21-23**)]. The presence of a cyclic enol ether in the ring system was found to significantly increase the activity in case of the 3a-hydroxy compound (**19**), but had a deleterious effect on the activity of (**4**). The validity of these trends could not be supported with literature data since no antifeedancy assays of *neo*-clerodane diterpenes against *Pieris brassicae* larvae have been reported. A survey of the results obtained with other Lepidopteran species[1] shows that no unequivocal picture appears to exist: hydrogenation of the cyclic enol ether moiety in the furofuran side chain of different clerodane insect antifeedants may either increase or decrease the potency of the molecule, depending on both the structure of the compound and the insect species used in the test. Similarly, several examples can be found of antifeedants with C-2 oxygenated furofuran sidechains being either more or less potent than the corresponding dihydro-derivatives.

Within this test series the choice of alkyl substituent at C-5 appeared not to be of great importance; a slightly higher activity could be observed for either large (*n*-decyl, *e.g.* **15**) or bulky (*t*-butyl, **18**) substituents. In combination with a cyclic enol ether in the ring system, a *c*-hexyl group at C-5 (**4**) was less active than the corresponding phenyl-compound (**13**), but this effect was not observed in the furofuran-2-one ring system (**10** vs. **17**). In view of the limited stability of the enol ether moiety in this system,[16] it is conceivable that the inactivity of (**4**) in these tests was caused by (partial) degradation of this compound in the test solution. The stereochemistry at C-5 appeared to be less important (**10**[17] vs. **11**), although some effect cannot be excluded in view of observed differences between some pure compounds and their epimeric mixtures (*e.g.* **16** vs. **15**).

With the exception of the ring system with the cyclic enol ether moiety (*i.e.* compound **19**), all test compounds containing a 3a-hydroxyl group (**20-24**) were found to be about equally active as their counterparts (**5-9**) without such a hydroxyl group. This test series would therefore seem to support the conclusion that the presence of a 3a-hydroxyl group in the furofuran ring system has no effect on the antifeedant activity, at least not for *Pieris brassicae*.

9.2.4- Antifeedancy Assay of the Lactone Model Compounds

The lactone model compounds (**25-36**) were tested in a two-choice assay as before,[9] though with cabbage leaf discs of a different commercial variety.[18] Half of the compounds displayed statistically significant antifeedant activity (Table 9.3), with the most active compound (**33**) reaching levels almost equal to the reference compound (**40**) included in these tests. In view of the applied concentration,

however, none of these compounds can be considered as a potent antifeedant for *Pieris brassicae* larvae.

It has been suggested[19] that the biological activity of various types of antifeedants might be related to the presence of electrophilic moieties in the molecules. Compound (**26**) contains an electrophilic butenolide system, which is also present in several natural insect antifeedants such as the ajugarins (**1**). Modification of this butenolide through hydrogenation (**25**) or via formal addition of water onto the double bond (**27**) indeed resulted in a complete loss of the antifeedant activity.

Table 9.3: Insect antifeedant activities of the lactone model compounds (**25-36**) against 5th instar larvae of the large cabbage white butterfly (Lepidoptera: *Pieris brassicae*) in a two-choice bioassay on cabbage leaf discs (5 mM concentration).[a]

	AI (sem) [b] (%)		AI (sem) [b] (%)		AI (sem) [b] (%)
25	-9.2 (12)	**30**	9 (9)	**35**	23 (8) **
26	35 (8) **	**31**	11 (9)	**36**	5 (8)
27	-4 (10)	**32**	33 (10) **		
28	26 (6) **	**33**	36 (7) **		
29	15 (8)	**34**	28 (10) **	**40**	*50 (4)* ** [c]

Notes (a) Adapted from ref. 4. Values printed in italics were calculated from the reported data. (b) Mean Antifeedant Index AI=[(C-T)/(C+T)]×100; sem = standard error of the mean. (c) Average value from four experiments (AI range: 42±7 to 62±10). See also note c, Table 9.2. (**) Statistically significant difference between control and treatment areas (Wilcoxon's matched pairs test); $p<0.01$. (*) $p<0.05$.

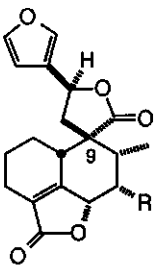
Introduction of two methyl groups at carbon atom C-4 gave rise to a series of C-4 dialkylated model compounds (**28-36**). This substitution led to some loss of activity in case of the butenolide system (**28** vs. **26**), but substantially increased the antifeedant activity of the related 3-hydroxy lactone (**29**, compared to **27**). Remarkably, for such C-4 dialkylated compounds the presence of the C-2,3 unsaturation in general appears to be of lesser importance, since the 3-hydroxy lactones (**29, 31, 33**) were roughly equally active as the corresponding butenolides (**28, 30, 32**). However, further degradation of the electrophilic moiety by replacing the lactone (**33**) with a cyclic ether (**34**) or an acetal (**35**) did result in partial loss of activity, and the cyclic half-acetal (**36**) was found to be inactive. The nature of the C-3 alkyl sidechain also had an impact on the activity of the model compounds, generally favouring a phenethyl substituent (**32, 33**) over its cyclohexyl-

equivalent (**28, 29**), which in turn was sometimes preferred over a straight-chain alkyl substituent (**28** vs. **30**, however see also **29** vs. **31**).

9.3 - Colorado Potato Beetle (*Leptinotarsa decemlineata*)

The Colorado potato beetle (Coleoptera: *Leptinotarsa decemlineata*) is a serious pest on potato which occurs throughout North America and has also been introduced into Europe, including The Netherlands. Both larvae and adults cause feeding damage to foliage and stems, resulting in reduced yields or even death of the plants. In North America, populations of the beetle have become resistant to most conventional insecticides and new approaches are therefore urgently needed.[20]

Figure 9.4



42a: R=H; teucvin
42b: R=OH; teucrin A

Insect antifeedant activity against *L. decemlineata* larvae has been reported for some 19-nor-clerodanes with a C-9 furano-lactone substructure; the natural compounds teucvin (**42a**) and teucrin A (**42b**) were found to be potent antifeedants, especially in a no-choice situation, suggesting a post-ingestive mode of action.[21] However, to our knowledge no clerodane diterpenes with other types of C-9 sidechain have ever been examined for antifeedant activity. For this reason, the furofuran model compounds (**4-9**), (**12**) and (**13**) were tested against 4th instar *L. decemlineata* larvae in a no-choice assay on potato (*Solanum tuberosum*) leaf discs.[22] All test compounds were applied in 5 mM concentration.

The test results (Table 9.4) indicated that these simple furofuran model compounds had little overall effect on the feeding of the larvae. Most compounds were either inactive during the entire assay period (**7, 9** and **12**) or initially showed some feeding stimulatory activity that gradually diminished to inactivity during the second period (**5, 6** and **8**). The only compounds with residual activity in the second period were the weak feeding stimulant (**4**) and the phenyl-substituted furofuran (**13**), which was the only compound found to display statistically significant (but weak) antifeeding activity.

In view of the low activity of these compounds, no further screening experiments were performed.

Table 9.4: Insect antifeedant activities of the model compounds (**4-9, 12** and **13**) against 4th instar larvae of the Colorado potato beetle (Coleoptera: *Leptinotarsa decemlineata*) in a no-choice bioassay on potato leaf discs.[a]

	AI (sem) [b,c] (%)			AI (sem) [b,c] (%)	
	0-1.5 hrs [c]	1.5-3 hrs [c]		0-1.5 hrs [c]	1.5-3 hrs [c]
4	-7.1 (8.1)	-17.4 (6.0)	**9**	-6.3 (9.3)	-4.8 (5.7)
5	-20.2 (11.7)	0.8 (6.3)	**12**	-1.7 (8.0)	1.0 (5.9)
6	-13.5 (6.1)	3.8 (4.7)	**13**	8.7 (8.5)	15.9 (5.5) **
7	-4.8 (9.2)	3.0 (9.1)			
8	-21.3 (5.2)	6.3 (4.9)	**38**	28.2 (6.1) **	29.7 (5.0) **

Notes: (a) Adapted from ref. 4. (b) Mean Antifeedant Index, AI = [(C-T)/C]×100; sem=standard error of the mean. (c) For further details regarding this no-choice assay, see notes for Table 9.1. (**) Statistically significant difference between control and treatment discs (Mann-Whitney U test); $p<0.01$.

9.4 - Green Peach Aphid (*Myzus persicae*)

Contrary to the insect species in the previous sections, aphids are piercing-sucking insects: they feed directly on the plant saps in the phloem by inserting their stylet (mouthparts shaped like a hollow needle) into the plants' vascular bundles. Aphids are among the most important insect pests on plants. In addition to the direct injury caused by tapping the plants' nutrients, aphids inflict damage by excreting honeydew, a sugery liquid on which moulds can grow. Even more destructive is their ability to transmit plant viruses; especially the green peach aphid (Homoptera: *Myzus persicae*) is notorious in this respect because it can transmit several different virusses. This aphid species can be a pest on various crops in the Netherlands, including economically important crops as wheat, potato or sugar beet.[20]

Because aphids feed directly on plant saps, feeding cannot easily be quantified as usual by measuring the amount of diet that is consumed. Instead, antifeedancy bioassays with aphids are based on the numbers of insects that have settled on the respective control or treatment surfaces. In the literature such assays on Chinese cabbage leaf discs have been used to determine the antifeedant activity of the *neo*-clerodanes ajugarin I (**2a**) and II (**2b**) against *M. persicae*.[23]

The experiments discussed in this section employed an assay,[24] in which an artificial diet was enclosed between two parafilm layers; the test solutions were painted on the outside of the bottom surface of this parafilm sachet (two-choice situation). In this way, the furofuran model compounds **(4-10)**, **(12-14)**, **(16-18)** and **(24)** were tested against nymphs of *Myzus persicae*. All test compounds were applied in 1000 ppm concentration; due to the different molecular weights, the molar concentration thus slightly varied between the test compounds.

Table 9.4: Insect antifeedant activities of several furofuran model compounds against nymphs of the green peach aphid (Homoptera: *Myzus persicae*) in a two-choice bioassay on parafilm sachets with artificial diet.[a]

	conc.[b] (mM)	AI (sem)[c,d] (%)		conc.[b] (mM)	AI (sem)[c,d] (%)
4	*5.2*	29 (19)	**13**	*5.1*	*-5 (12)*[e]
5	*5.1*	*1 (12)*[e]	**14**	*4.7*	*0 (9)*[f]
6	*4.7*	31 (20)	**16**	*3.7*	-19 (18)
7	*4.2*	-10 (17)	**17**	*4.9*	-41 (16) *
8	*3.9*	10 (19)	**18**	*5.4*	5 (21)
9	*4.7*	20 (19)	**24**	*4.4*	-18 (18)
10	*4.7*	29 (14)			
12	*5.1*	*4 (12)*[e]	**37**	*4.2*	*56 (6)*[g]

Notes (a) Adapted from ref. 4. Values printed in italics were calculated from the reported data. (b) Test solutions 1000 ppm concentration. (c) Mean Antifeedant Index AI=[(C-T)/(C+T)]×100; sem=standard error of the mean. C and T are numbers of aphids settled on control and treatment areas, respectively. (d) Originally reported as 'Deterrency Index' (D.I.) with similar numerical value. (e) Average value from two experiments. See also note c, Table 9.2. (f) Average value from three experiments. (g) Average value from five experiments. (*) Statistically significant difference between control and treatment areas (Wilcoxon's matched pairs test); $p<0.05$.

In general, the ability to deter the aphids from settling on the treated side of the diet was found to be rather weak or absent for the test compounds included in this series; some compounds even appeared to constitute an attractant for the aphids. The assay results suffered from a high variance of the individual mean AIs, relative to their magnitudes, and the results from some repeated measurements also varied considerably. As a result, only a single compound **(17)** elicited an (attractive) effect on the aphids with a reasonable level of statistical significance.

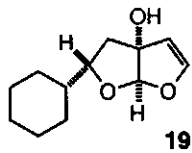
In view of the unsatisfactory results obtained with this assay, no further compounds were tested against *Myzus persicae*.

9.5 - Summary

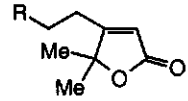
A number of model compounds (Figure 9.2), derived from natural clerodane diterpenes and prepared as described in the Chapters 5-7, were tested for insect antifeedant activity against species from different insect orders.

Against larvae of the large cabbage white butterfly (*Pieris brassicae*) several of the test compound displayed statistically significant antifeedant activity, though none of the compounds was found to be highly active. For the test series of furo[2,3b]furan analogues (**4-24**) the presence of a C-3a hydroxyl group did not generally increase the activity of the test compounds, although (**19**, Figure 9.5) was the most potent compound within this series. Similarly, in the butyrolactone series the butenolides (**28,30,32**) were about equally active as the corresponding 3-hydroxy compounds (**29,31,33**). These results would therefore seem to support the conclusion that the presence of such a hydroxyl group in these model compounds has no effect on the antifeedant activity, at least not for *Pieris brassicae* larvae.

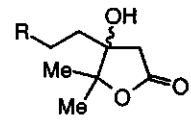
Figure 9.5



19



(28,30,32)†



(29,31,33)†

Note: (†) R=*c*-C_6H_{11}, *n*-C_4H_9 or Ph, respectively.

A limited range of model compounds was examined for antifeedant activity in bioassays with larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) or nymphs of the green peach aphid (*Myzus persicae*). None of the compounds tested showed statistically significant antifeedant activity.

9.6 - Notes and References

1. For an overview of the antifeedant activity of natural and (semi)synthetic clerodane diterpenes and related compounds, see Chapter 2.
2. Chapter 3 provides a discussion of the background leading to this hypothesis.
3. See: W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson, S.C. Smith and A. Wood, *Pestic. Sci.*, **40**, 169-173 (1994).; S.V. Ley, D. Santafianos, W.M. Blaney and M.S.J. Simmonds, *Tet. Lett.*, **28** (2), 221-224 (1987); W.M. Blaney, M.S.J. Simmonds, S.V. Ley, J.C. Anderson and P.L. Toogood, *Entomol. Exp. Appl.*, **55**, 149-160 (1990). Some of these data are also reproduced in Chapter 3.

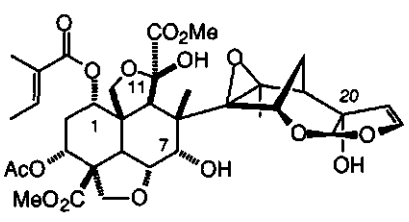
4. L. Messchendorp, *Terpenoid antifeedants against insects: a behavioural and sensory study.*, Ph.-D. thesis, Wageningen Agricultural University, Wageningen, 1998.
5. A. Blaakmeer, *Infochemicals in a tritrophic system. - Interactions between Brassica, Pieris and Cotesia.*, Ph.-D. thesis, Wageningen Agricultural University, Wageningen, 1994, p. 6-10.
6. L. Messchendorp, G.J.Z. Gols and J.J.A. van Loon, *Entomol. Exp. Appl.*, **79**, 195-202 (1996).
7. (a) S. Arpaia and J.J.A. van Loon, *Entomol. Exp. Appl.*, **66**, 39-45 (1993); (b) L.-E. Luo, J.J.A. van Loon and L.M. Schoonhoven, *Physiol. Entomol.*, **20**, 134-140 (1995).
8. R.B.M. Geuskens, J.M. Luteijn and L.M. Schoonhoven, *Experientia*, **39**, 403 (1983).
9. (a) In the *P. brassicae* bioassays the substrate consisted of discs (area 3.80 cm^2/disc) of cabbage leaves (*Brassica oleracea* var. *gemmifera* cv. Titurel). The discs were painted on the upper surface with 10 µl of a solution of the test compound at the indicated concentration dissolved in distilled water containing 2% of ethanol and 2% of Tween-80 (treatment discs) or with 10 µl of distilled water containing 2% of ethanol and 2% of Tween-80 (control discs) and were air-dried for 30 min. The *P. brassicae* larvae used were reared on cabbage plants and were 24-72 hrs into its fifth instar stadium.
   For a two-choice assay, three treatment discs and three control discs were placed in a glass Petri dish and one larva was introduced. After feeding for 3 hrs, the remaining leaf disc areas were measured and the amount of feeding from treatment (T) and control discs (C) was determined by comparison with the mean area of three reference discs.
   In the no-choice assays, the larvae were individually presented with a single leaf disc (either treatment or control), which was replaced by a fresh disc after 1.5 hrs of feeding. The amount of feeding was determined via the remaining leaf disc areas as before and was compensated for differences in feeding rate due to differences in larval weight.
   From these data the appropriate Antifeedant Indices (two-choice or no-choice, see tables for definitions) were calculated.
   (b) For full experimental details, see ref. 4 and 6.
10. L.M. Schoonhoven, *Ent. Exp. Appl.*, **31**, 57-69 (1982).
11. L. Messchendorp, J.J.A. van Loon and G.J.Z. Gols, *Behavioural observations of Pieris brassicae larvae indicate multiple mechanisms of action of analogous drimane antifeedants, Entomol. Exp. Appl.*, Accepted for publication (1998).
12. For an example of this effect with drimane-type antifeedants on several Lepidopteran insects, see: W.M. Blaney, M.S.J. Simmonds, S.V. Ley and R.B. Katz, *Physiol. Entomol.*, **12**, 281-291 (1987).
13. Some drimane sesquiterpenes and related analogs have been tested under identical conditions as the furofuran model compounds (ref. 4 and 11) and a number of these were significantly more potent than the furofurans. One of the most active compounds was also included in the present bioassay as reference compound (**40**); the antifeedant indices from both experiments with (**40**) were found to be nearly identical, indicating that both sets of results can be compared quantatively.
14. An example of such a situation with *Pieris brassicae* larvae was observed in leaf disc assays of drimane sesquiterpenes and related analogues (ref. 4 and 11) , in which most test compounds were more active in the two-choice test than in the no-choice test.
15. Not 11% of the magnitude of the mean AI, but (mean AI ± sem) ± 11%.

16. For instance, exposure of a solution of (**12**) in CH_2Cl_2 to direct sunlight for 1 week resulted in the transformation of (**12**) into a complex mixture of products, with a half-life of approximately 24 hrs. Contrary, a solution of (**12**) subjected to the same conditions but shielded from sunlight remained unaffected (E.A. klein Gebbinck, unpublished results).
17. See note d from Table 9.2.
18. These assays were conducted with leaf discs of *Brassica oleracea* var. *gemmifera* cv. Cyrus.
19. (a) I. Kubo and K. Nakanishi, *Some terpenoid insect antifeedants from tropical plants.* In: H. Geissbühler (Ed.), *Advances in pesticide science: plenary lectures presented at the 4th international congress of pesticide chemistry, Zürich, 1978*, Part 2, Pergamon Press, Oxford, 1979, p. 284-294. (b) D.M. Norris, *Anti-Feeding Compounds.* In: *Chemistry of plant protection*, Vol. I, Springer Verlag, Berlin, 1986, p. 97-146.
20. (a) L.P. Pedigo, *Entomology and pest management*, Macmillan Publishing Company, New York, 1991. (b) J.A. de Jong, *Algemene gewasbescherming*, Educaboek, Culemborg, 1991.
21. F. Ortega, B. Rodríguez and P. Castañera, *J. Chem. Ecol.*, **21** (9), 1375-1386 (1995).
22. (a) The *L. decemlineata* assays were performed according to the no-choice protocol described before (ref. 9) with potato leaf discs (area 2.27 cm^2/disc), painted on the upper surface with 10 µl of either treatment or control solution. The larvae were reared on potato leaves and were in their fourth instar stadium.
(b) For full experimental details, see ref. 4 and G.J.Z. Gols, J.J.A. van Loon and L. Messchendorp, *Entomol. Exp. Appl.*, **79**, 69-76 (1996).
23. J.A. Pickett, G.W. Dawson, D.C. Griffiths. A. Hassanali, L.A. Merritt, A. Mudd, M.C. Smith, L.J. Wadhams, C.M. Woodcock and Z. Zhong-ning, *Development of plant-derived antifeedants for crop protection.* In: R. Greenhalgh and T.R. Roberts (Eds.), *Pesticide science and biotechnology*, 1987, p. 125-128; D.C. Griffiths, A. Hassanali, L.A. Merritt, A. Mudd, J.A. Pickett, S.J. Shah, L.E. Smart, L.J. Wadhams and C.M. Woodcock, *Brighton crop protection conference - Pests and diseases*, 1988, p. 1041-1046.
24. (a) For this *M. persicae* bioassay a double layer set-up was used: one half of the lower surface of a parafilm layer, stretched over a plastic ring (2.7 cm diameter), was painted with 10 µl of a solution of the test compound in ethanol at the indicated concentration, while the other half was painted with ethanol only (two-choice situation). After drying, a parafilm sachet containing two compartments filled with artificial diet was stretched over the upper surface of the first parafilm layer. 20-25 Nymphs of *Myzus persicae* (5-6 days old, reared on oil seed rape plants (*Brassica napus*)) were placed on the painted lower side of this test set-up (diet sachet on top). After 24 hrs, the number of nymphs present on the treatment (T) and control (C) halves of the diet was counted. From these data, an antifeedant index was determined (see table for definition).
(b) For full experimental details, See ref. 4 and L. Messchendorp, G.J.Z. Gols, J.J.A. van Loon, *Behavioural effects and sensory detection of drimane deterrents in Myzus persicae and Aphis gossypii nymphs, J. Chem. Ecol.*, **24** (9), 1433-1446 (1998).

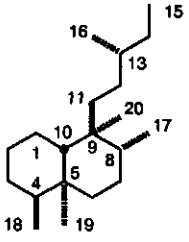
Summary

■ Insect antifeedants are compounds with the ability to reduce or inhibit insect feeding without directly killing the insect. In general, such compounds offer a number of properties that are highly desirable in environmentally friendly crop protection agents. Often, insect antifeedants still display considerable biological activity at low concentrations. Furthermore, the effect is often directed specifically against a narrow group of pest-insect species, leaving benificial insects and other species unharmed. For these reasons, insect antifeedants have the potential to become a valuable addition to the existing arsenal of crop protection agents. However, although the principle of insect control using antifeedants has been shown to work under field conditions, many questions regarding the chemical, biological and practical aspects of insect antifeedants are still open. As a result, commercial application of these compounds has until now been limited to a few examples, mainly consisting of extracts of the Neem tree containing the highly potent tetranortriterpene azadirachtin (**1**). In **Chapter 1** an introduction into the field of insect antifeedants is provided and some aspects of their biological mode of action and practical application are discussed.

Figure 10.1



1: azadirachtin

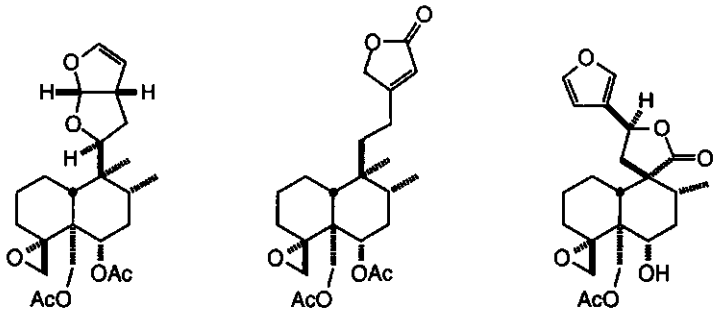


2: *neo*-clerodane skeleton

■ Broad interest in insect antifeedants from natural origin has arisen in the early 1960s and since then, thousands of plant species have been screened for the presence of antifeeding metabolites, yielding hundreds of different antifeedants from diverse chemical classes. Especially the class of *neo*-clerodane diterpenes (**2**) has attracted considerable attention as a source of natural insect antifeedants. The members of this class, consisting of well over 750 metabolites from (mostly) plant sources with a high degree of structural diversity, display various types of biological activity. In **Chapter 2**, the structure, occurrence and general biological activity of the clerodane diterpenes are introduced. Furthermore, a comprehensive compilation is presented of the more than 200 natural clerodane diterpenes and related (semi)synthetic derivatives that have been tested for insect antifeedant activity.

■ One of the factors hampering the introduction of insect antifeedants in crop protection is the limited availability of many of these compounds, which prevents their widespread application at low cost. In the efforts to solve this availability problem, synthetic approaches might provide an alternative to the isolation of antifeedants from natural sources. The synthetic routes involved in the total synthesis of natural insect antifeedants are usually too long and complex to be of much practical value. A more promising approach would therefore be to develop simplified analogues of such natural products, which have retained as much of the desired biological properties as possible. Apart from being potential crop protection agents themselves, such simplified analogues may also serve as model compounds to investigate structure-activity relationships and thus to provide a basis for the design of more potent synthetic insect antifeedants.

Figure 10.2

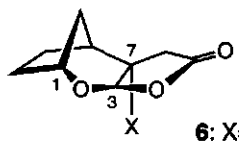


3: clerodin **4**: ajugarin I **5**: teucjaponin B

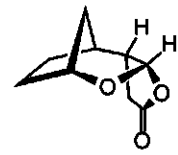
In the present thesis, *neo*-clerodane diterpenes as clerodin (**3**), ajugarin I (**4**) and teucjaponin B (**5**) were chosen as starting points in a search for such potentially active model compounds. From the literature it is known that both halves of these molecules (*i.e.* the decalin-fragment and the C-9 sidechain) are involved in the bioactivity of these diterpenes. Both parts separately also display antifeedant activity, but these fragments are much less potent than their parent compounds and are therefore unsuitable candidates for practical application. Model compounds that consist of structural features from both halves soon become complicated themselves and were therefore considered to be an unpractical approach towards simplified bioactive analogues. Instead, we decided to concentrate upon one half of the molecule and to investigate the possibility to optimize the activity of such fragments.

In the search for modifications that could enhance the activity of clerodane fragments, we were struck by the structural resemblance between the furo[2,3b]furan sidechain of clerodin (3) and the furo[2,3b]pyran substructure present in azadirachtin (1). In view of this similarity, it was hypothesized that the introduction of structural features from azadirachtins furo[2,3b]pyran substructure into clerodane C-9 sidechain fragments might increase the antifeedant activity of these model compounds. Especially the hydroxyl group present in the furo[2,3b]pyran substructure was seen as an interesting candidate for such a strategy. As is discussed in **Chapter 3**, the hydroxyl groups at C-7, C-11 and C-20 of azadirachtin appear to be important for its bioactivity, possibly because of a direct interaction of these groups with a natural receptor site.

Figure 10.3



6: X=OH
7a: X=H

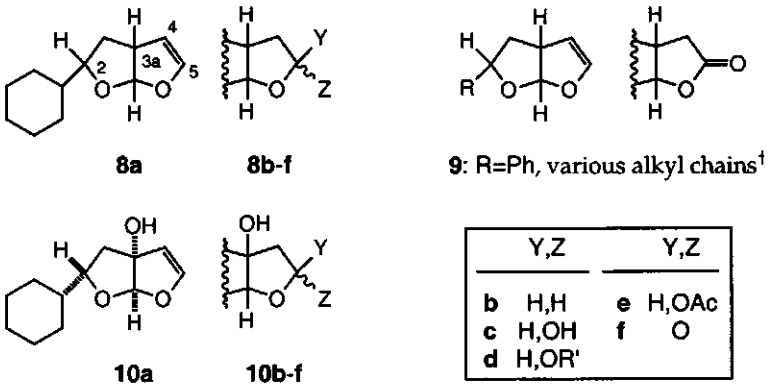


7b

■ In **Chapter 4**, an attempt is described to prepare some tricyclic furo[2,3b]pyran model compounds (**6**) and (**7a**), respectively with and without a hydroxyl group at C-7 (Figure 10.3). The objective of these efforts was to gain some insight into the role of this group in the bioactivity of the furo[2,3b]pyran fragment via comparison of the antifeedant activity within this pair of model compounds. The 7-hydroxy-furo[2,3b]pyran model system (**6**) with the same relative stereochemistry as in azadirachtin was prepared according to literature procedures. The 7-*de*hydroxy-furo[2,3b]pyran model system (**7**) could be prepared through a modified version of these procedures, but only isomer (**7b**) with inverted stereochemistry at C-3 and C-7 could be obtained in a pure form. Due to its incorrect stereochemistry, this compound was not suited for the intended goal and therefore these efforts were not continued.

■ Some of the most potent clerodane insect antifeedants (*e.g.* clerodin (**3**)) contain a furo[2,3b]furan-based C-9 sidechain and therefore this fragment has frequently been used in clerodane model compounds. As proposed above, the introduction of a hydroxyl group at position C-3a of this fragment might increase the antifeedant activity of such model compounds. To examine this hypothesis, furo[2,3b]furan model compounds with and without a hydroxyl group at C-3a were prepared (see Figure 10.4).

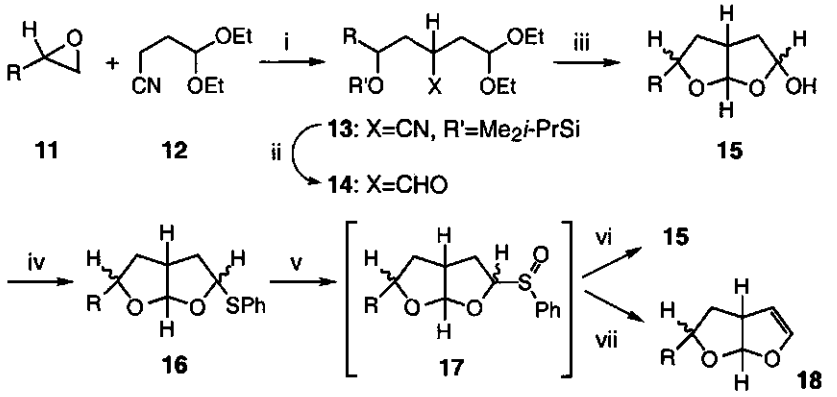
Figure 10.4



Note: (†) See Chapter 5 for details on the exact compounds synthesised.

Chapter 5 describes the synthesis of a series of 3a-*hydro*-furo[2,3b]furan-based analogues (**8-9**), using a modified version of a literature method for the construction of the furo[2,3b]furan ring system (Scheme 10.1). These model compounds were equipped with various functional groups at C-4,5 (**9a-f**) and with different C-2 alkyl- and aryl-substituents (**10**), to facilitate studies of the effect of these modifications on the insect antifeedant activity of this type of system. The enol ethers (**18**) were obtained in a one-pot reaction from the sulfides (**16**) via a modified oxidation-elimination sequence, involving rapid elimination of the unstable sulphoxides (**17**).

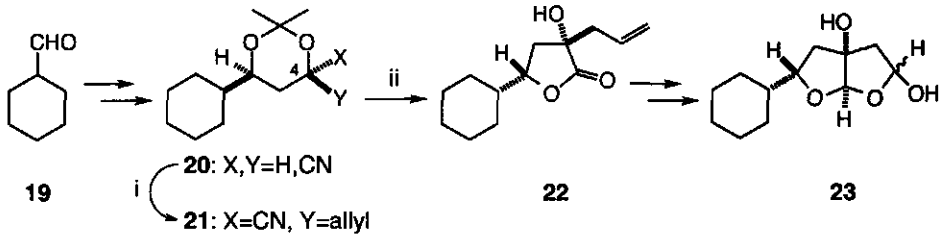
Scheme 10.1



Key-reagents and -conditions: (i) 1) LDA; 2) DMIPSCl; (ii) DibalH; (iii) H^+; (iv) PhSH, $BF_3 \bullet Et_2O$; (v) mCPBA, 0°C; (vi) warm to r.t.; (vii) add Et_3N, then *rapid* heating to 130°C .

Chapter 6 describes the synthesis of the corresponding series of 3a-*hydroxy*-furo[2,3b]furan model compounds (**10a-f**). For the preparation of these model compounds a novel synthetic route was developed, using a stereoselective allylation of the cyanohydrin 1,3-acetonides (**20**) as the key step (Scheme 10.2). An excess of the strong base LHMDS was found to be necessary to obtain a good yield of (**21**) and its C-4 epimer, in a best ratio of 30 : 1. Deprotection of (**21**) and subsequent Pinner condensation yielded the hydroxy-lactone (**22**), which could be smoothly converted into the desired 3a-hydroxy-furo[2,3b]furan ring system. The required C-4,5 functional groups were introduced into this system in essentially the same manner as before.

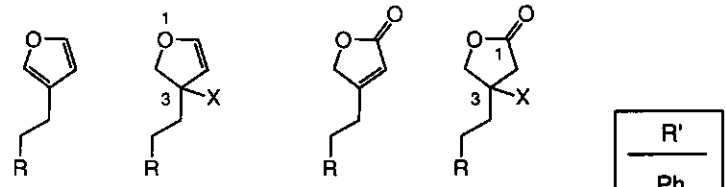
Scheme 10.2



Key-reagents and -conditions: (i) 1) 3-4 eq LHMDS, 2) allylBr; (ii) conc. HCl, Δ.

■ A number of natural antifeeding clerodanes incorporate monocyclic C-9 sidechains, for instance with a furan ring or a butenolide (*e.g.* as in ajugarin (**4**)). Due to their structural simplicity, such monocyclic sidechains were considered attractive targets in the design of simplified bioactive analogues. In line with the strategy adopted in previous chapters, we were interested in model systems as (**24**) and (**25**), with and without a hydroxyl group at the position corresponding with carbon atom C-13 in the clerodane skeleton (Figure 10.5). The cyclic enol ether (**24b**) was of particular interest because of its close similarity to the furo[2,3b]furan model compounds prepared before.

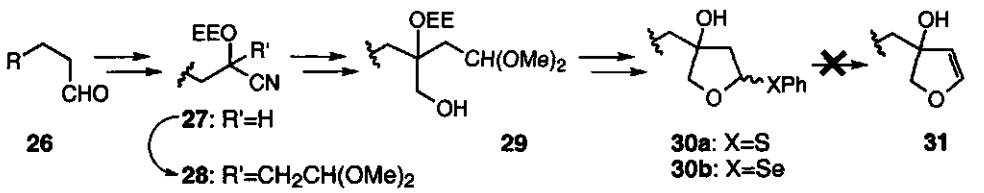
Figure 10.5



24a **24b**: X=OH **24c**: X=H **25a** **25b**: X=OH **25c**: X=H

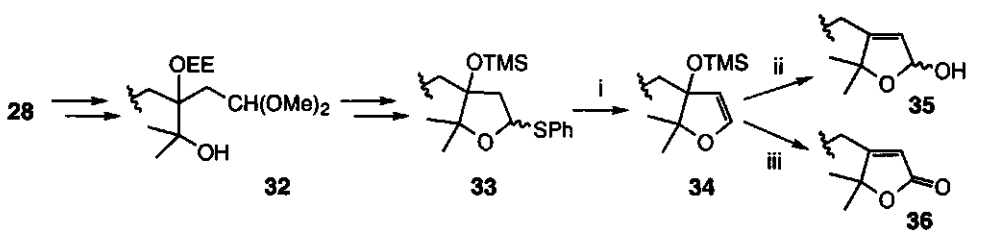
In **Chapter 7** some attempts at the preparation of such compounds are described. Our synthetic approach was centered on the alkylation of a protected cyanohydrin (**27**), followed by transformation of the cyano group to an alcohol and cyclization to afford the required 3-hydroxy-tetrahydrofuran ring system (Scheme 10.3). Attempts at the introduction of the enol ether moiety using an oxidation-elimination method as before failed for both the sulfides (**30a**) or the selenides (**30b**), yielding only the corresponding furans.

Scheme 10.3



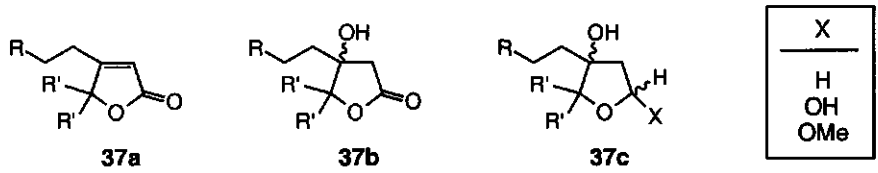
When this aromatization was blocked by the introduction of two methyl substituents at position C-2 of the ring system, the oxidation-elimination sequence produced the expected crude enol-ethers (**34**) (Scheme 10.4). Upon chromatographic purification of these compounds however, again a rearrangement reaction occurred that gave the unsaturated alcohols (**35**) as the main product. Due to this inherent instability of the cyclic enol ether moiety in these ring systems, further attempts towards the synthesis of furan-based model compounds as (**24**) were not undertaken and instead the attention was focussed on the related butyrolactone model compounds. Such compounds (**36**) could be easily obtained from the crude enol ethers (**34**) by a one-pot procedure. A series of model compounds (**37**) was prepared (Figure 10.6).

Scheme 10.4



Key-reagents: (i) 1) mCPBA, 0°C; 2) Et_3N, 130°C; (ii) silicagel chromatography; (iii) 1) HF; 2) CrO_3, H_2SO_4.

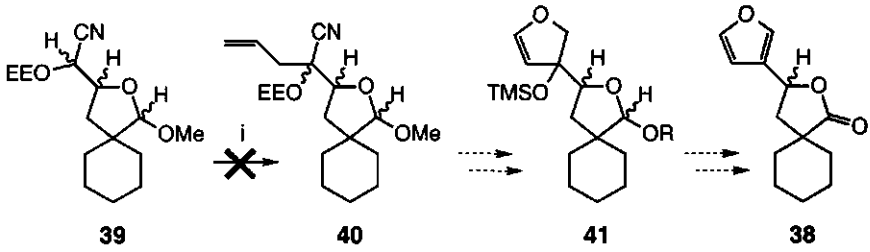
Figure 10.6



R=Ph, *c*-C_6H_{11}, *n*-C_4H_9; R'=H, Me. See Chapter 7 for the exact compounds synthesized

■ One of the C-9 sidechains most frequently encountered in natural clerodane diterpenes is the 4-furano-spirolactone moiety, for instance as present in teucjaponin B (**5**) (Figure 10.2). A number of these diterpenes have been shown to display insect antifeedant activity and therefore model compounds as (**38**) (see Scheme 10.5) are of interest in the search for simplified bioactive analogues. To our knowledge however, no synthetic routes towards this moiety have been published. **Chapter 8** describes some approaches towards the synthesis of this model compound. In our initial attempt (Scheme 10.5) it was planned to use a rearrangement reaction of the type found in Chapter 7 as the key-step, to convert the 3-hydroxy-2,3-dihydrofuran moiety of (**41**) into a furan group.

Scheme 10.5

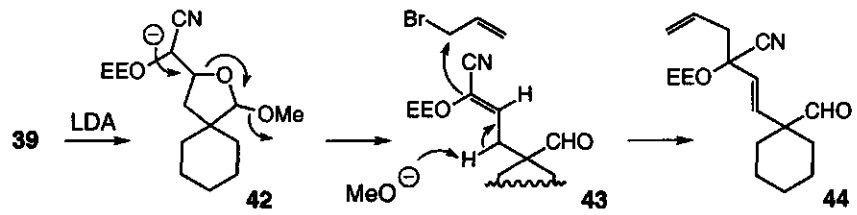


39 **40** **41** **38**

Key-reagents: (i) 1) LDA; 2) $BrCH_2CH{=}CH_2$.

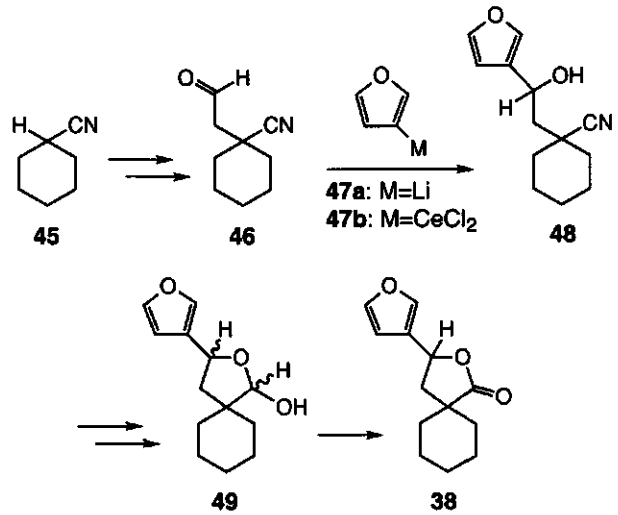
This route required the alkylation of the complex cyanohydrin derivative (**39**), but instead of the desired compound (**40**) a number of different products were obtained. One of these was identified as the rearranged product (**44**), probably resulting from ring opening and subsequent loss of methanol from the anion of (**39**) (Scheme 10.6). The formation of this product showed (**39**) to be prone to β-elimination and therefore this approach was not continued

Scheme 10.6



In an alternative approach (Scheme 10.7) the addition of a 3-metallated furyl reagent onto the cyano-aldehyde (**46**) was used to construct the required skeleton (**48**). In order to suppress enolization of the aldehyde as a major side-reaction, in this reaction the cerium reagent (**47b**) was found to be preferrable over the lithiated equivalent (**47a**). Subsequent reduction of the cyano group and cyclization of the resulting hydroxy-aldehyde smoothly yielded the furano-spirolactol (**49**), which was oxidized to the desired model compound (**38**).

Scheme 10.7

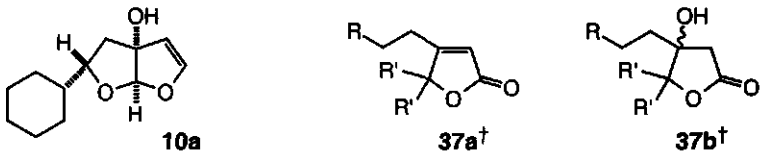


■ In **Chapter 9** the results of insect antifeedancy bioassays against various insect species with a number of model compounds from Chapters 5-7 are presented.

Against larvae of the large cabbage white butterfly (*Pieris brassicae*) several of the test compound displayed statistically significant antifeedant activity, though none of the compounds was found to be highly active. For the test series

of furo[2,3b]furan analogues (**8-10**) the presence of a C-3a hydroxyl group did not generally increase the activity of the test compounds, although (**10a**) was the most potent compound within this series. Similarly, in the butyrolactone series the 3-hydroxy compounds (**37b**) were about equally active as the corresponding butenolides (**37a**).

Figure 10.7



10a **37a**† **37b**†

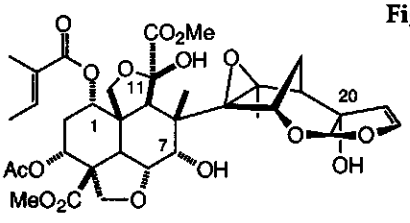
Note: (†) R=Ph, *c*-C_6H_{11}, *n*-C_4H_9; R'=H, Me.

For larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) and for nymphs of the green peach-potato aphid (*Myzus persicae*), none of the compounds tested showed statistically significant antifeedant activity.

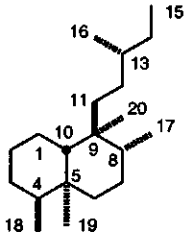
Samenvatting

■ Insect antifeedants zijn stoffen die de vraat van insekten verminderen of voorkomen zonder het insekt direct te doden. In het algemeen hebben dergelijke stoffen enkele eigenschappen die zeer gewenst zijn in milieuvriendelijke gewasbeschermingsmiddelen. Zo vertonen insect antifeedants vaak nog aanzienlijke activiteit bij lage concentraties. Verder is de activiteit vaak specifiek gericht tegen een beperkte groep van plaaginsekten. Om deze redenen hebben stoffen met insektenvraatremmende werking de potentie om een waardevolle aanvulling te worden op het bestaande arsenaal van gewasbeschermingsmiddelen. Hoewel het principe van gewasbescherming met antifeedants zich reeds in veld-situaties bewezen heeft, zijn veel vragen omtrent de chemische, biologische en praktische aspecten van het gebruik van insektenvraatremmende stoffen nog onbeantwoord. Als gevolg hiervan is de commerciële toepassing van dergelijke middelen tot nu toe beperkt gebleven tot enkele voorbeelden, voornamelijk extracten van de Neem boom met daarin het zeer aktieve tetranortriterpeen azadirachtine (**1**). In **Hoofdstuk 1** wordt een inleiding op het gebied van de insect antifeedants gegeven en worden enkele aspecten van het biologische werkingsmechanisme en van de praktische toepassing besproken.

Figuur 11.1



1: azadirachtine

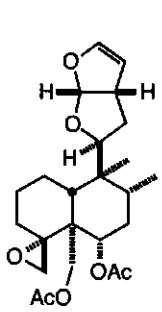


2: *neo*-clerodaan skelet

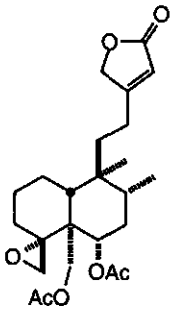
■ In het begin van de zestiger jaren is er een brede belangstelling voor insect antifeedants van natuurlijke oorsprong ontstaan. Sindsdien zijn duizenden plantensoorten onderzocht op de aanwezigheid van metabolieten met insektenvraatremmende werking, hetgeen honderden verschillende antifeedants uit diverse chemische klassen heeft opgeleverd. Vooral de klasse der *neo*-clerodaan diterpenen (**2**) heeft veel belangstelling getrokken als een bron van natuurlijke insect antifeedants. De ruim 750 leden van deze klasse van (grotendeels planten-)metabolieten hebben een grote diversiteit in struktuur en vertonen verscheidene soorten van biologische aktiviteit. **Hoofdstuk 2** geeft een inleiding in de struktuur, het voorkomen en de algemene biologische aktiviteit van clerodaan diterpenen. Daarnaast bevat dit hoofdstuk een overzicht van de ruim 200 natuurlijke clerodaan diterpenen en hun (semi)synthetische derivaten die op insektenvraatremmende werking zijn onderzocht.

■ Eén van de oorzaken van de moeizame invoering van het gebruik van insect antifeedants als gewasbeschermingsmiddelen is de beperkte beschikbaarheid van veel van deze stoffen, hetgeen hun toepassing op grote schaal en tegen lage kosten verhindert. Bij de inspanningen om dit probleem op te lossen kunnen synthetische benaderingen wellicht een alternatief bieden voor de isolatie van antifeedants uit natuurlijke bronnen. De syntheseroutes die gebruikt worden voor de totaalsynthese van natuurlijke insektenvraatremmers zijn meestal te lang en te gecompliceerd om hierbij van veel praktisch nut te zijn. De ontwikkeling van vereenvoudigde analoga van dergelijke natuurprodukten, met behoud van zoveel mogelijk van de gewenste biologische eigenschappen, lijkt daarom een veelbelovender aanpak. Dergelijke vereenvoudigde analoga kunnen wellicht direkt als gewasbeschermingsmiddel gebruikt worden en kunnen bovendien dienen als model verbindingen in studies naar struktuur-werkingsrelaties en daarmee een basis vormen voor het ontwerp van meer aktieve synthetische insect antifeedants.

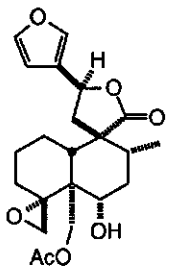
Figuur 11.2



3: clerodin



4: ajugarin I

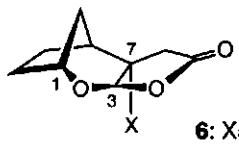


5: teucjaponin B

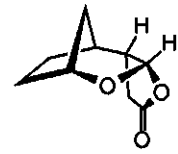
In dit proefschrift zijn de *neo*-clerodaan diterpenen clerodin (**3**), ajugarin I (**4**) en teucjaponin B (**5**) gekozen als beginpunten in een zoektocht naar mogelijk aktieve modelverbindingen. Uit de literatuur is bekend dat beide helften van deze molekulen (m.a.w. het decaline-deel en de C-9 zijketen) betrokken zijn bij de biologische aktiviteit van deze diterpenen. Afzonderlijk vertonen beide delen ook insektenvraatremmende werking, maar dergelijke fragmenten zijn aanzienlijk minder aktief dan de originele diterpenen en zijn daarom minder geschikt voor een praktische toepassing. Modelverbindingen die bestaan uit elementen van beide delen van deze diterpenen worden al snel komplex en een dergelijke aanpak werd daarom niet erg praktisch geacht. In plaats daarvan werd besloten om uit te gaan van één helft van het molekuul en te onderzoeken of de biologische aktiviteit van dergelijke fragmenten verbeterd kon worden.

In de zoektocht naar modificaties die de aktiviteit van clerodaan-fragmenten zouden kunnen versterken werden wij getroffen door de overeenkomsten in de struktuur van de furo[2,3b]furan zijketen van clerodin (**3**) en van de furo[2,3b]pyran deelstruktuur in azadirachtine (**1**). Vanwege deze overeenkomst werd aangenomen dat de introductie van elementen uit de furo[2,3b]pyran deelstruktuur van azadirachtine in de clerodaan C-9 zijketen fragmenten wellicht de aktiviteit van deze modelverbindingen zou kunnen vergroten. Met name de hydroxy-groep uit de furo[2,3b]pyran deelstruktuur werd beschouwd als een interessante kandidaat in een dergelijke strategie. Zoals wordt besproken in **Hoofdstuk 3**, lijken de hydroxy-groepen op C-7, C-11 and C-20 in azadirachtine belangrijk te zijn voor de biologische aktiviteit van deze verbinding, wellicht als gevolg van een direkte interaktie tussen deze groepen en een natuurlijke receptor.

Figuur 11.3



6: X=OH
7a: X=H

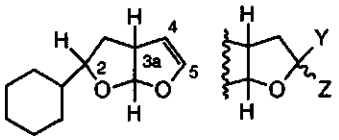


7b

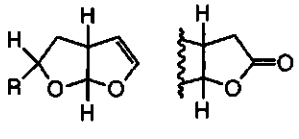
■ In **Hoofdstuk 4** wordt een poging beschreven om de tricyclische furo[2,3b]pyran modelverbindingen (**6**) en (**7a**), respectievelijk met en zonder een C-7 hydroxy-groep, te synthetiseren (Figuur 11.3). Het doel van deze pogingen was het verkrijgen van enig inzicht in de rol van deze hydroxy-groep in de biologische aktiviteit van het furo[2,3b]pyran fragment, door de vraatremmende werking van dit paar van modelverbindingen te vergelijken. Het 7-hydroxy-furo[2,3b]pyran model systeem (**6**) met dezelfde relatieve stereochemie als azadirachtine werd bereid volgens een literatuur procedure. Het *7-de*hydroxy-furo[2,3b]pyran model systeem (**7**) kon bereid worden via een gewijzigde versie van deze procedure, maar alleen de isomeer (**7b**) met een geïnverteerde stereochemie op C-3 en C-7 kon in zuivere vorm verkregen worden. Deze verbinding was vanwege de onjuiste stereochemie niet geschikt voor het gestelde doel en daarom werden deze pogingen niet voortgezet.

■ Omdat enkele van de meest aktieve clerodaan insect antifeedants, zoals clerodin (**3**), een furo[2,3b]furan C-9 zijketen hebben wordt dit fragment regelmatig gebruikt in modelverbindingen. De introductie van een hydroxy-groep op positie C-3a van dit fragment zou de insektenvraatremmende werking van dergelijke model-verbingingen wellicht kunnen verbeteren. Om deze hypothese te onderzoeken werden furo[2,3b]furan modelverbindingen met en zonder een hydroxy-groep op C-3a gesynthetiseerd (zie Figuur 11.4).

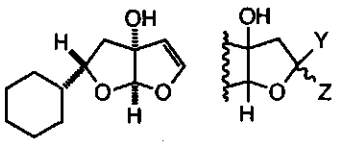
Figuur 11.4



8a **8b-f**



9: R=Ph, various alkyl chains†



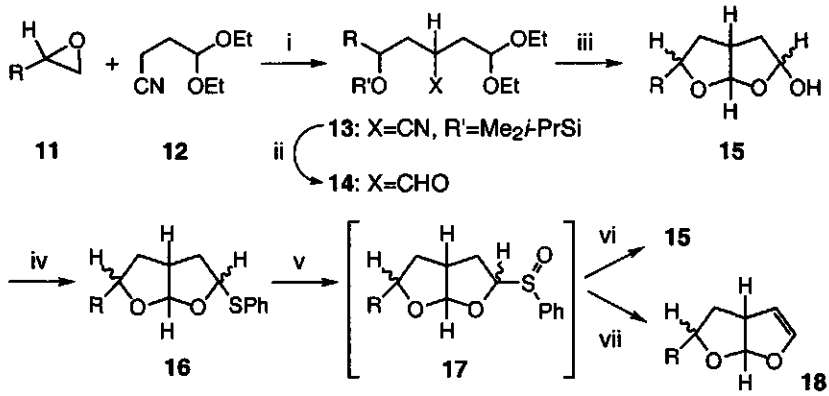
10a **10b-f**

Y,Z		Y,Z	
b	H,H	**e**	H,OAc
c	H,OH	**f**	O
d	H,OR'		

Note: (†) See Chapter 5 for details on the exact compounds synthesised.

Hoofdstuk 5 beschrijft de synthese van een serie 3a-*hydro*-furo[2,3b]furan analoga (**8-9**) volgens een gewijzigde literatuur methode voor de konstruktie van het furo[2,3b]furan ring systeem (Schema 11.1). Deze modelverbindingen werden voorzien van verschillende functionele groepen op C-4,5 (**9a-f**) en van verschillende C-2 alkyl- en aryl-substituenten (**10**), om het effect van deze modificaties op de insektenvraatremmende werking van dit type systemen te kunnen bestuderen. De enol ethers (**18**) werden in een één-pots reaktie verkregen uit de sulfides (**16**) via een gewijzigde oxidatie-eliminatie methode, gebaseerd op een snelle eliminatie van het onstabiele sulfoxide (**17**).

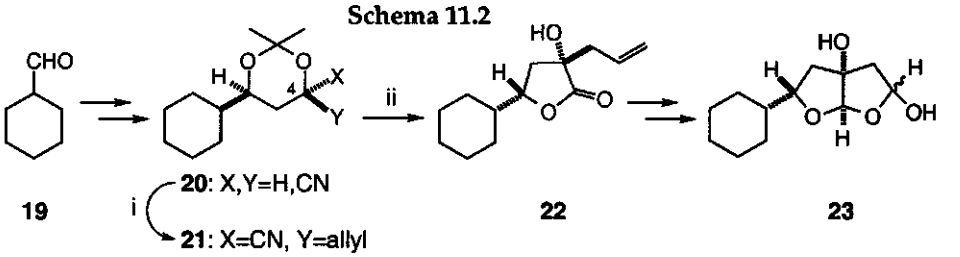
Schema 11.1



Sleutel-reagentia en -reaktieomstandigheden: (i) 1) LDA; 2) DMIPSCl; (ii) DibalH; (iii) H^+; (iv) PhSH, $BF_3 \bullet Et_2O$; (v) mCPBA, 0°C; (vi) opwarmen tot kamertemp.; (vii) Et_3N toevoegen, vervolgens *snel* verwarmen tot 130°C .

Hoofdstuk 6 beschrijft de synthese van de bijbehorende serie 3a-*hydroxy*-furo[2,3b]furan modelverbindingen (**10a-f**). Voor de bereiding van deze modelverbindingen werd een nieuwe syntheseroute ontwikkeld, met de stereoselectieve allylering van de cyanohydrin 1,3-acetonides (**20**) als de belangrijkste stap (Schema 11.2). Het bleek noodzakelijk om een overmaat van de sterke base LHMDS te gebruiken om een goede opbrengst te verkrijgen van verbinding (**21**) en diens C-4 epimeer, in een beste verhouding van 30 : 1. Ontscherming van (**21**), gevolgd door Pinner kondensatie, leverde het hydroxylacton (**22**) dat gemakkelijk in het gewenste 3a-hydroxy-furo[2,3b]furan ringsysteem kon worden omgezet. De benodigde functionele groepen op C-4,5 werden in dit systeem ingevoerd op vrijwel identieke wijze als voorheen.

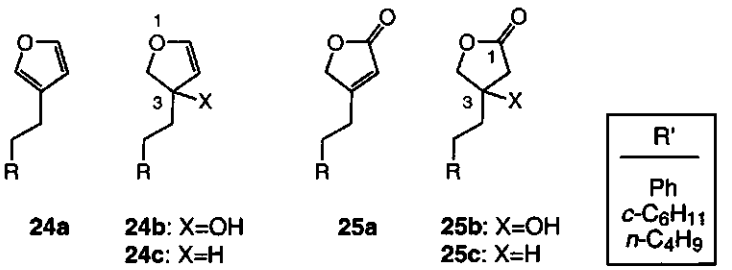
Schema 11.2



Sleutelreagentia en -reaktieomstandigheden: (i) 1) 3-4 eq LHMDS, 2) allylBr; (ii) conc. HCl, Δ.

■ Een aantal natuurlijke clerodaan antifeedants bevat een monocyclische C-9 zijketen, bijvoorbeeld met een furan ring of met een butenolide (zoals bijvoorbeeld in ajugarin I (**4**)). Vanwege hun eenvoudige struktuur werden zulke monocyclische zijketens beschouwd als aantrekkelijke doelen in het ontwerp van vereenvoudigde analoga met biologische aktiviteit. Overeenkomstig de strategie van de vorige hoofdstukken, waren wij met name geïnteresseerd in modelverbindingen als (**24**) en (**25**), met en zonder een hydroxygroep op de positie die overeenkomt met koolstofatoom C-13 in het clerodaan skelet (Figuur 11.5). Met name de cyclische enol ether (**24b**) was interessant vanwege de gelijkenis met de furo[2,3b]furan modelverbindingen van voorheen.

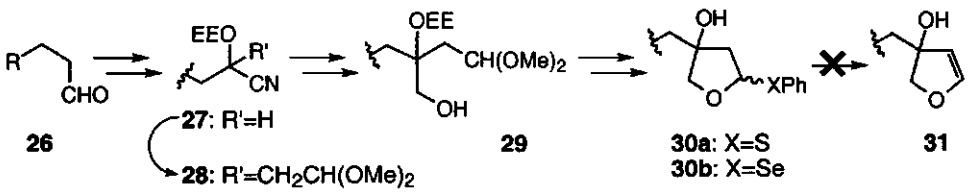
Figuur 11.5



24a **24b**: X=OH **24c**: X=H **25a** **25b**: X=OH **25c**: X=H

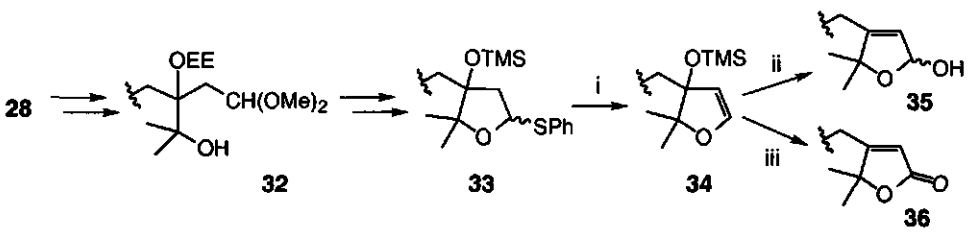
In **Hoofdstuk 7** worden enkele pogingen tot de synthese van dergelijke verbindingen beschreven. Onze synthetische benadering was gebaseerd op de alkylering van het beschermde cyanohydrin (**27**), gevolgd door omzetting van de cyano groep in een alkohol en cyclisatie tot het gewenste 3-hydroxy-tetrahydrofuran ring systeem (Schema 11.3). Pogingen om een enol ether eenheid in te voeren volgens de voorheen gebruikte oxidatie-eliminatie methodiek faalden voor zowel de sulfides (**30a**) als voor de selenides (**30b**), en leverden alleen de overeenkomstige furanen.

Schema 11.3



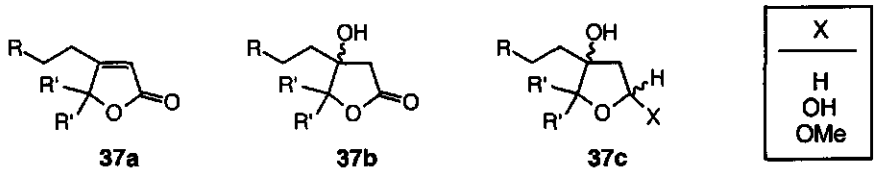
Wanneer de mogelijkheid tot aromatisatie werd geblokkeerd door invoering van twee methyl substituenten op positie C-2 van het ring systeem, leverde de oxidation-elimination procedure de verwachtte enol-ethers (**34**) als ruw product (Schema 11.4). Zuivering van deze producten via kolom-chromatografie leidde echter opnieuw tot een omleggingsreaktie, waarbij het onverzadigde alkohol (**35**) werd gevormd als belangrijkste product. Vanwege deze inherente instabiliteit van de enol ether eenheid in dit ringsysteem werden geen verdere pogingen tot bereiding van modelsystemen als (**24**) ondernomen. In plaats daarvan werd de aandacht gericht op de verwante butyrolacton model-verbindingen, zoals (**36**). Deze konden eenvoudig via een één-pots reaktie gesynthetiseerd worden vanuit de ruwe enol ethers (**34**). Op deze wijze werd een serie modelverbindingen (**37**) verkregen (Figuur 11.6).

Schema 11.4



Sleutelreagentia en -reaktieomstandigheden: (i) 1) mCPBA, 0°C; 2) Et_3N, 130°C; (ii) kolom-chromatografie op silica; (iii) 1) HF; 2) CrO_3, H_2SO_4.

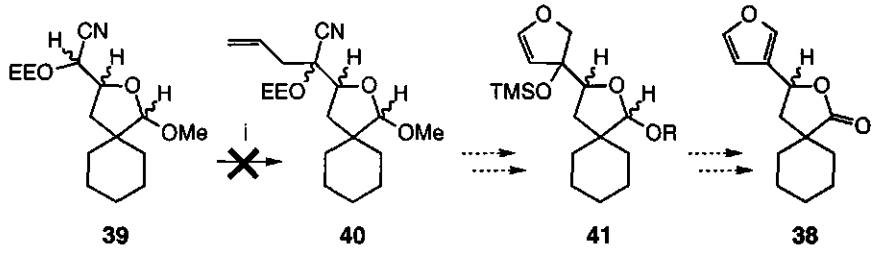
Figuur 11.6



R=Ph, *c*-C_6H_{11}, *n*-C_4H_9; R'=H, Me. Zie Hoofdstuk 7 voor de precieze modelverbindingen

■ Eén van de meest voorkomende C-9 zijketens in natuurlijke clerodaan diterpenen is de 4-furano-spirolacton eenheid, zoals bijvoorbeeld aanwezig in teucjaponin B (**5**) (Figuur 11.2). Omdat een aantal van deze diterpenen een insektenvraatremmende werking vertoont, zijn modelverbindingen als (**38**) (zie Schema 11.5) van interesse voor de zoektocht naar vereenvoudigde analoga met biologische aktiviteit. Voorzover bekend zijn er nog geen syntheseroutes naar deze eenheid gepubliceerd. **Hoofdstuk 8** beschrijft enkele onderzochte syntheseroutes naar deze modelverbinding. In de eerste poging (Schema 11.5) werd geprobeerd om een omleggingsreaktie zoals gevonden in Hoofdstuk 7 toe te passen voor de omzetting van de 3-hydroxy-2,3-dihydrofuran groep van (**41**) in een furan ring.

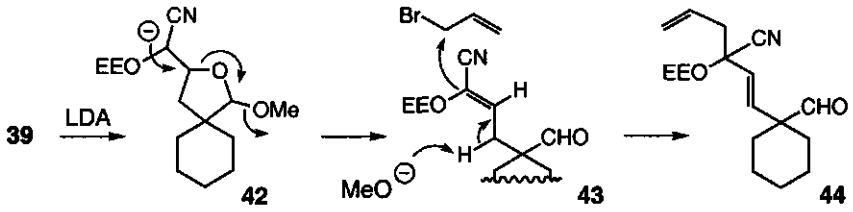
Schema 11.5



Sleutelreagentia: (i) 1) LDA, HMPA-THF; 2) $BrCH_2CH{=}CH_2$.

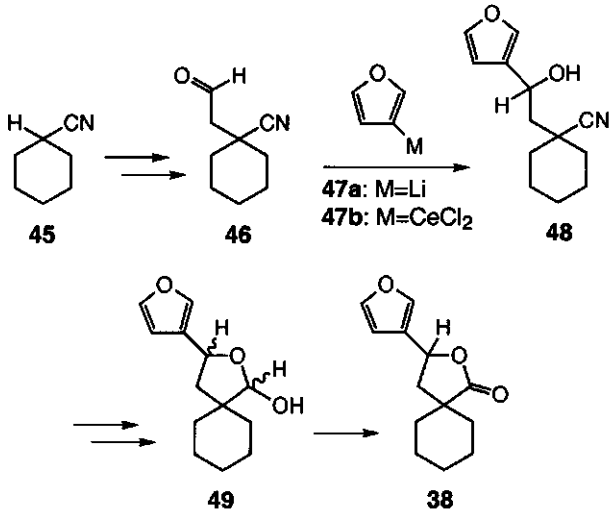
Voor deze route moest het komplexe cyanohydrin (**39**) geallylleerd worden, maar in plaats van de gewenste verbinding (**40**) werd een aantal andere producten verkregen. Eén van deze producten kon geïdentificeerd worden als het omleggingsproduct (**44**), dat vermoedelijk gevormd werd via ringopening en verlies van methanol in het anion van (**39**) (Schema 11.6). De vorming van een dergelijk product geeft aan dat (**39**) gevoelig is voor β-eliminatie en daarom werd deze benadering niet voortgezet.

Schema 11.6



Een alternatieve route (Schema 11.7) was gebaseerd op de additie van een gemetalleerd 3-furyl reagens op het cyano-aldehyde (**46**), waarmee het vereiste skelet (**48**) verkregen kon worden. In deze reaktie bleek het gebruik van het cerium-reagens (**47b**) voordelen to bieden boven het lithium equivalent (**47a**), omdat daarmee de enolisatie van het aldehyde als belangrijke nevenreaktie onderdrukt kon worden. Reductie van de cyano groep en cyclisatie van het resulterende hydroxy-aldehyde leverde vervolgens het furano-spirolactol (**49**), dat tenslotte geoxideerd werd tot de gewenste modelverbinding (**38**).

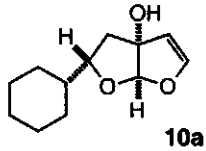
Schema 11.7



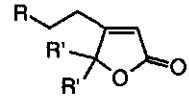
■ In **Hoofdstuk 9** worden de resultaten van een aantal biotoetsen beschreven, waarin een aantal modelverbindingen uit de hoofdstukken 5-7 zijn getest op hun insektenvraatremmende werking tegen verschillende insektensoorten.

Met larven van het grote koolwitje (*Pieris brassicae*) werd voor verscheidene modelverbindingen een statistisch significante remming van de insektenvraat gevonden, maar geen enkele van de getestte verbindingen vertoonde een hoge aktiviteit. De aanwezigheid van een 3a-hydroxy-groep in de serie furo[2,3b]furan analoga (**8-10**) leidde niet tot een algemene vergroting van de antifeedant aktiviteit, hoewel (**10a**) wel de meest aktieve modelverbinding in deze serie bleek te zijn. In de serie butyrolacton analoga bleek de vraatremmende werking van de butenolides (**37a**) en van de verwante 3-hydroxy verbindingen (**37b**) van een vergelijkbaar nivo te zijn.

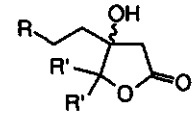
Figuur 11.7



10a



37a



37b

R=Ph, *c*-C_6H_{11}, *n*-C_4H_9; R'=H, Me.

Met larven van de Coloradokever (*Leptinotarsa decemlineata*) en met nymfen van de groene perzikbladluis (*Myzus persicae*) werd voor geen van de getestte modelverbindingen een statistisch significante insektenvraatremming gevonden.

Curriculum Vitae

Edwinus Antonius klein Gebbinck werd geboren op 23 juli 1966 te Arnhem. Na het doorlopen van het HAVO behaalde hij in 1985 het VWO-diploma aan het Thomas à Kempis College te Arnhem. In datzelfde jaar begon hij zijn doctoraal studie aan de Katholieke Universiteit Nijmegen. De afstudeerfase omvatte een uitgebreide hoofdvakstage Organische Chemie onder leiding van prof. dr. B. Zwanenburg, gevolgd door een bijvakstage Biochemie onder leiding van prof. dr. J. de Pont. Het doctoraalexamen werd afgelegd op 28 oktober 1991.

Vanaf 1 november 1991 tot 1 januari 1996 was hij als onderzoeker in opleiding (OIO) werkzaam bij de vakgroep Organische Chemie van de Landbouwuniversiteit Wageningen. Het tijdens deze periode uitgevoerde onderzoek, verricht onder leiding van dr. B.J.M. Jansen en prof. dr. Ae. de Groot, staat beschreven in dit proefschrift.