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This thesis has been corrected to my satisfaction

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ADVANCEMENTS IN NATURAL PRODUCTS CHEMISTRY

Chemical Constituents of

Aplysilla pallida
Aplysilla rosea
Aplysilla var. sulphurea
Orthoscutilla maculata

And

Approaches to the synthesis of aplysulphurin

A thesis submitted in partial fulfilment of the requirements for admission to the degree of

DOCTOR OF PHILOSOPHY

by

STEPHEN IMRE TOTH

The University of Sydney

March, 1990

PREFACE

The work presented in this thesis was carried out under the supervision of Professor W.C. Taylor in the Department of Organic Chemistry, University of Sydney during the period March 1985 to March 1990.

All the results recorded in this thesis are those of the author unless otherwise stated. Some of the results have been described elsewhere:

Novel diterpenes from the marine sponge *Aplysilla pallida*. RACI Organic Chemistry Division, 11th National Conference, Townsville, July 1989.

and

Chemistry International Conference, 3rd Asian Chemical Congress, Division of Medicinal and Agricultural Chemistry, Brisbane, August 1989.

ABSTRACT

This thesis is divided into two main parts, both of which are in the field of natural products chemistry.

Part 1: The Chemical Constituents of *Aplysilla pallida, Aplysilla rosea, Aplysilla var. sulphurea* and *Orthoscutilla maculata*

Part 1 deals with the collection, extraction, isolation and structural elucidation of a number of novel diterpenes from the marine sponges *Aplysilla pallida* and *Aplysilla rosea*. Another marine sponge *Aplysilla var. sulphurea* and a bryozoan *Orthoscutilla maculata* were also investigated but revealed no new novel metabolites. In all twenty nine metabolites were isolated, fifteen having new structures. All collections, extractions, isolations and structural determinations were conducted by the author.

The sponge *Aplysilla pallida* was found to contain four novel diterpenes aplypallidenone, aplypallidoxone, aplypallidione and aplypallidioxone. Their structures were determined by extensive spectroscopic methods. The structure of aplypallidenone and aplypallidoxone were further confirmed by X-ray crystallography.

From the sponge *Aplysilla rosea*, previously investigated by Karuso in our department, twenty-two diterpene metabolites were isolated, eleven of which were new compounds (AROSST-1 to -11).

The sponge *Aplysilla var. sulphurea*, so named because of its morphological and colour similarities but chemically difference with

Aplysilla sulphurea yielded only two previously isolated diterpenes 15, 16-diacetoxyspongin and 16-oxospongian.

The bryozoan *Orthoscutilla maculata* was also investigated and found to contain only one previously isolated alkaloid 1-ethyl-β-carboline.

Part 2: Approaches to the synthesis of Aplysulphurin

Aplysulphurin was the first diterpene of its type to be isolated. Due to its novel structure and one hitherto not investigated, steps towards the total synthesis were therefore undertaken.

ACKNOWLEDGEMENTS

I wish to offer my sincere thanks to Professor W.C. Taylor for giving me the opportunity to undertake this project and for his help, encouragement and friendly discussions throughout the course of this work.

I would also like to thank the technical, professional and academic staff of the Department of Organic Chemistry especially Dr J. Nemorin, Dr B. Rowe, Miss S. Boyd and Mr N. Bampos for ^{1}H n.m.r. spectra, Dr I. Brown, Mr I. Smyth and Mr A. Schenk for mass spectra, Mr R. Appio for i.r. and u.v. spectra and Dr K. Picker for all things h.p.l.c.

I extend my thanks to Dr P. Karuso and Dr A. Poiner for introducing me to marine natural products chemistry and for their assistance in the initial collections of sponge material. A special thanks is also due to Mr C. Kasparian for his willingness and invaluable assistance in collecting the sponge, even under unpleasant conditions. I would also like to extend a special thanks to Dr M. Garson of the Department of Chemistry University of Wollongong for her assistance in collecting the sponges and helpful discussions.

I am also deeply indebted to Miss R. Walton for the typing of much of this thesis, and to Mr P. Hatton and Mr R. Dancer for their friendship, assistance in putting together this thesis and many helpful discussions. Thanks is also due to Mrs D. Hugh and Mr T. Romero of the Electron Microscope Unit, University of Sydney for their expertise, technical assistance and helpful discussion in the use of the scanning electron microscope.

I would also like to offer my thanks to the other research students within the Department of Organic Chemistry and School of Chemistry for making my stay at the University of Sydney an enjoyable one.

Finally I offer my deepest love and thanks to my parents, my sister

and my young brother for their neverending love, support, understanding and help throughout the course of this study.

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PART 1

The Chemical Constituents of

Aplysilla pallida

Aplysilla rosea

Aplysilla var. sulphurea

and

Orthoscutilla maculata.

1.1 Introduction

The study of natural products chemistry prior to 1970 mainly involved terrestrial organisms, for example plants, animals and fungi. The marine environment however, remained largely neglected. This was primarily due to two areas of difficulty. Firstly, the logistics involved in the collection of material and secondly, securing enough material so that when extracted a sufficient amount of each pure compound could be obtained for structural determination.

However, the last two decades have seen an explosive growth of interest and literature in the field of marine natural products chemistry. This has mainly been in response to the many technological advances, particularly in the instruments used for purification and structural elucidation, for example high performance liquid chromatography (h.p.l.c.) and high field nuclear magnetic resonance spectroscopy (n.m.r.). Moreover, the increasing popularity of SCUBA (Self Contained Underwater Breathing Apparatus) diving has also made the ocean's resources more accessible.

Marine organisms have been detected in the fossil record as far back as the precambrian era (ca 850 my)[1]. In the course of this long evolutionary history a vast diversity of organisms have evolved, specializing into certain modes of life and habitats. In doing so the aquatic organisms have had to develop unique chemical and/or mechanical mechanisms for survival.

Therefore, through the course of time many metabolic, physiological and ecological alternatives have been developed. The rich array of unusual chemical compounds, which are often indicators of novel biosynthetic pathways, are the traces that remain of these diverse

evolutionary experiments. The discovery of interesting new compounds, many of which possess structural types not found from terrestrial sources, had given the incentive for further research into the chemistry of the marine environment.

Many of these novel compounds have also been found to possess potent biological activity, for example as antiviral, antibacterial and antitumor agents. In the future these may give rise to new and useful pharmaceuticals.

Today, the search for new products from marine sources, especially those with new structural types and possible pharmaceutical use is being carried out in many institutes around the world. One group of aquatic animals which has been given a lot attention, because of the variety of novel and active compounds isolated from them, are the sponges.

Sponges (phylum Porifera) are primitive filter feeding, benthic, metazoans. They grow on the substrate from the seashore through to the abysses of the deep ocean trenches, and some, although to a far lesser degree, in fresh water streams and lakes. To date there are some 5000 species and accounts for much of the epifaunal biomass.

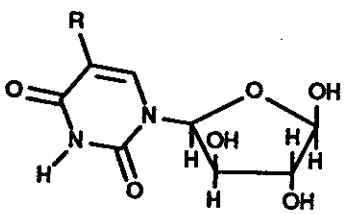
The sponge is a very simple organism which is one reason for its evolutionary success. As the phylum name Porifera suggests, the sponge is made up of a myriad of pores: an elaborate network of channels and chambers.[2]

The sponge body is made up of three main parts. A one cell thick external epithelium layer (pinacoderm) isolating the sponge from the external environment and an internal flagellated epithelium (choanoderm) isolating the sponge from the internal environment. Between these two thin layers is a third region, the mesohyl, which can vary in composition and extent but always includes some reproductive cells and some skeletal

material. The skeletal material may be either calcareous siliceous or made of spongin fibres.[2]

Sponges feed on microscopic particles of organic matter which they absorb from large volumes of sea water. This is achieved by pumping water at low pressure through their elaborate network of channels and chambers. Both the pumping of water and collecting of food is instrumented by the flagellated (choanocyte) cells which line the internal surface of the chambers[2] (Plate 1-2, 15-16; Fig 1).

Motivation in the search for new and interesting compounds from sponges was stimulated, perhaps more than any other discovery, by the isolation of two unusual nucleosides (1) and (2) from the Jamaican sponge *Tethya crypta* in 1950 by Bergmann and Feeney[3].



(1) R = H

(2) R = Me

These two compounds were found to have antitumor and antiviral activity and indeed served as model compounds for the synthesis of other compounds with similar powerful biological activity[4]. Hence the search for other potentially useful drugs from the marine environment, especially sponges, has progressed with great enthusiasm.

Another optimistic challenge which has presented itself is to turn the biochemical diversity of compounds isolated from sponges to advantage in taxonomy.

Historically, classification of sponges has been primarily based on

the characteristics of the spicule skeleton. However this has proved to be inadequate in many cases, particularly in the classification of one important group, the Keratosa[7] or Horny[8] sponges (class Demospongiae).

Chemotaxonomy utilises the presence or absence and biochemical relationship of particular compounds and, together with the ultrastructural, histological and reproductive characteristics, could offer a more convenient method, or at least supplement existing methods of sponge classification.

Early work into the possible use of chemotaxonomy on sponges was conducted by Bergmann in which he investigated the structural variations and distribution of sterols in a large variety of species from Demospongiae.[5,6]

The amino acids, alkaloid and terpene content of sponges has also been looked at and hold promise that they may also be useful in taxonomy.[2]

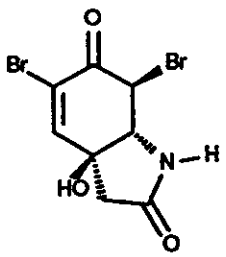
As mentioned above, the usual method of classification of sponges by their spicules is not possible in the so called group known as the 'horny' sponges.[8].

Horny sponges are devoid of mineral spicules and have a skeleton comprised only of spongin fibres. It is within this group that chemotaxonomy has proven to be of key importance.

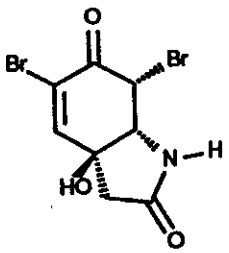
Recently Bergquist has reclassified the 'horny' sponges groups into three separate orders:[9,10] the Verongidae, Dictyoceratida and Dendroceratida. In this reclassification biochemical aspects were taken into consideration together with the arrangement of the spongin fibre network.

Sponges within the order Verongidae have a spongin fibre skeleton which supports a heavily collagenous matrix. Chemically they are typified by secondary metabolites derived from tyrosine. Such examples are those

isolated from sponges of the genus *Aplysina*, (3), (4)[11] and (5), (6)[12].

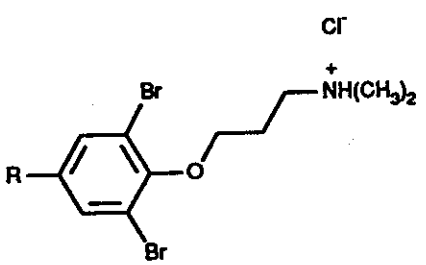


(3)

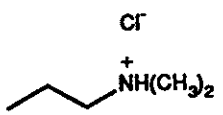


(4)

A. cavernicola



(5) R =



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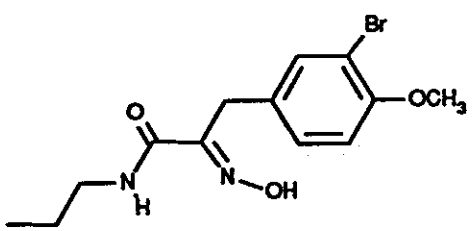


Aplysina sp.

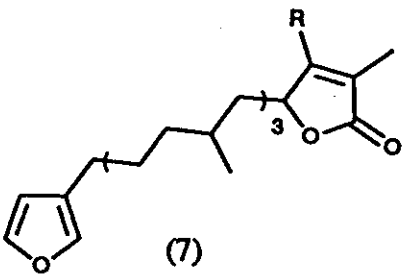
The orders Dictyoceratida and Dendroceratida on the other hand are characterised chemically by a rich terpenoid content.[2]

The major differentiating aspects between these two orders is the complexity of the spongin fibres and the type of terpenoids found in the sponges so far analysed.

The spongin skeletons of dictyoceratid sponges are always constructed upon an

anastomosing pattern involving primary and secondary fibres (fig.2)[2].

The chemistry of Dictyoceratid terpenes is characterised by being predominantly of sesterterpene (C25) origin. These include such structural varieties as furanosesterterpenes (7), tetracyclic sesterterpenes (8) and truncated C21 and C22 sesterterpenes (9).



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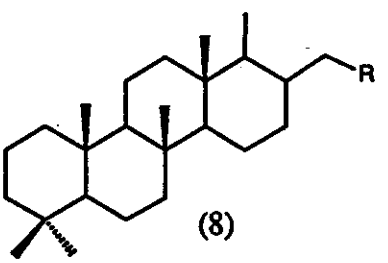
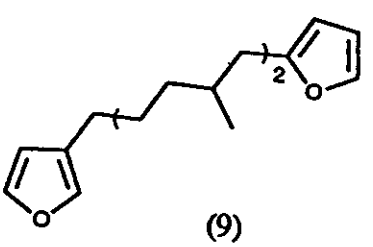


(8)



(9)

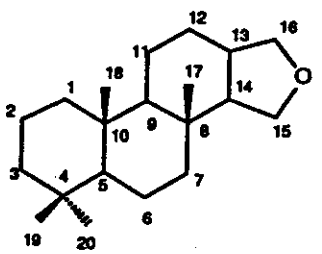
In addition to sesterterpenes, cyclic sesquiterpenes, polyprenyl quinols and diterpenes have also been isolated from Dictyoceratida.[2]

The third order within the 'horny' sponges, Dendroceratida, is both the smallest and also the least studied of the three orders. It contains three families: Aplysillidae (5 genera), Dictyodendrillidae (2 genera) and Halisoridae (1 genus).

In contrast to the Dictyoceratida, the spongin skeleton of members of the Dendroceratida is composed of spongin fibres arranged in a dendritic pattern. In small specimens of the Aplysilla genus they may be simple

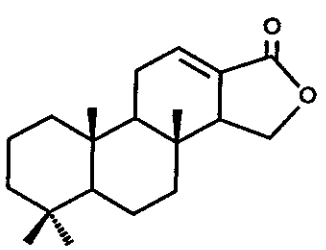
upright fibres (Plates 11-13; fig, 3).[2,8]

The sponges under investigation here belong to the family Aplysillidae (genus *Aplysilla*). This group of sponges has been largely neglected by chemists probably due to their difficulty in collecting. However a number of species have been investigated, the metabolites of which may be formally derived from the hypothetical tetracyclic precursor spongian (10).[13]



(10)

The first report of metabolites containing the spongian skeleton was from *Spongia officinalis* from which isoagatholactone (11) was isolated.[14]



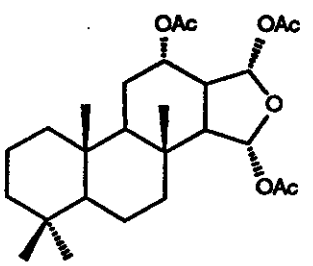
(11)

Since this first report other *Spongia* species, examined from a variety of locations, have also given metabolites containing the spongian or spongian derived backbone.[15,16,17] Oxidation in these metabolites has been directed into the A ring and the C11, C12 and C13 positions of the C ring.

The majority of metabolites containing the spongian skeleton or spongian derived skeleton to date are from sponges of the order Dendroceratida. Within these metabolites, oxidation has been mainly directed towards C6 and C7 of the B ring and at C17.

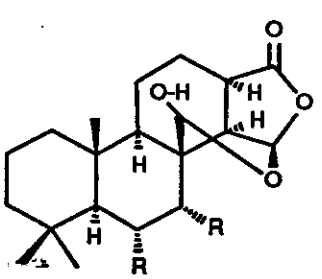
It is believed that the oxidation pattern within these sponges may give some lead into a chemotaxonomic representation of sponges between the two orders as well as their evolutionary development.[17a]

An initial extraction of a sponge identified as *Aplysilla rosea* (family Aplysillidae) from New Zealand waters revealed only one metabolite, aplysillin (12).[18]



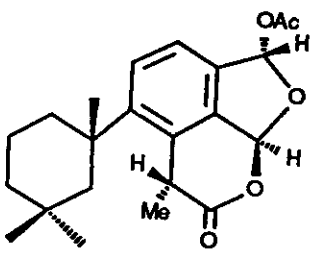
(12)

In contrast to the New Zealand sample, extraction of *Aplysilla rosea* collected from Sydney waters (N.S.W.) provided a series of lactone acetals, the aplyroseols, with general structure (13)[19].



(13)

In some species of aplysillids, the hypothetical precursor spongian (10) has undergone deep seated biogenetic rearrangements including cleavage of the B and C rings. Examples of this are aplysulphurin (14)[20] and aplytandiene-1 (15)[21] isolated from *Aplysilla sulphurea* and *Aplysilla tango* respectively.



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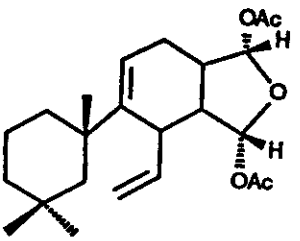

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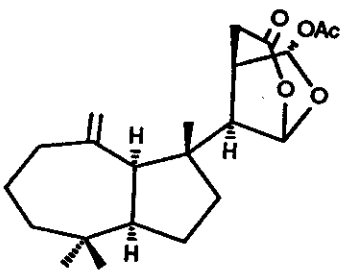


(16)

Further rearrangements of the rings may also occur as in the case of aplyviolene (16)[22] isolated from *Chelonaplysilla violacea* (family Aplysillidae). A number of other similar compounds were also isolated from this sponge collected from New Zealand waters[21].

A list of terpenoids which have been isolated from sponges bearing a spongian or spongian derived backbone is presented in Section 1.2.5.

In light of our group's initial interest in the metabolites of the Aplysillidae for chemotaxonomy, as well as the probable chemical defence role and hence biological activity of their secondary metabolites, two further members of this family, *Aplysilla pallida* and *Aplysilla var. sulphurea* have been extracted.

Also, an initial large scale collection of *Aplysilla rosea* by the author as part of an honours project prompted a further investigation for other unisolated minor metabolites.

The second major taxonomic group of colonial animals which has been gaining respect and interest from chemists are the Bryozoans (Ordovician - Recent), which are also sedentary colonial filter feeding invertebrates like sponges. There are some 4000 living species known today, of which the majority are marine.

The Bryozoan is made up of individual zooids encased in a cylindrical skeleton which are linked together forming a colony. The skeleton in the majority of cases is made of calcium carbonate, however in a few examples it is membranous.

The individual zooids resemble small cnidarian polyps (e.g. coral polyps) in that they deploy a ring of tentacles out of an orifice in their skeleton when feeding. But the bryozoan is a higher order of animal, having a freely suspended gut with both mouth and anus, and a rudimentary nervous system. The tentacles, when danger strikes, retract back into the skeleton and a flap (operculum) covers the orifice protecting the zooid.

The feeding habit of the bryozoan zooids is similar to that of sponges. The tentacles direct water towards the mouth and the organic matter is filtered out.

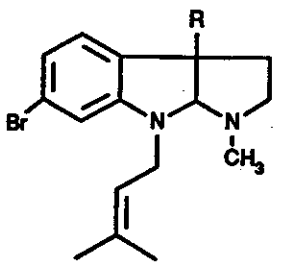
Today three classes of Bryozoa are recognised: the Phylactolaemata, Stenolaemata and the Gymnolaemata.

The Phylactolaemata contains 12 genera all of which are fresh water varieties. The Stenoloemata are marine bryozoans divided into five orders of which only one, the Cyclostomata, exists today. The final class, the Gymnolaemata, contains over 3000 species and is the most important class existing today.[23,24]

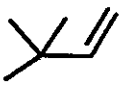
The chemistry of this extensive invertebrate phylum has largely been neglected, which is in part due to difficulties in securing enough material for serious investigations. This lack of progress into this large animal group is clearly shown by the few accounts of chemical

literature presented in a review by Christopherson in 1985.[25]

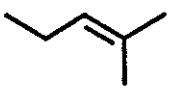
However bryozoans lately have emerged as an interesting source of biologically active compounds. Most of the compounds isolated to date have been alkaloids such as those from *Flustra foliacea* (family Frustridae) (17), (18) or from *Chartella papyracea* (family Frustridae) having general structure (19).[25]

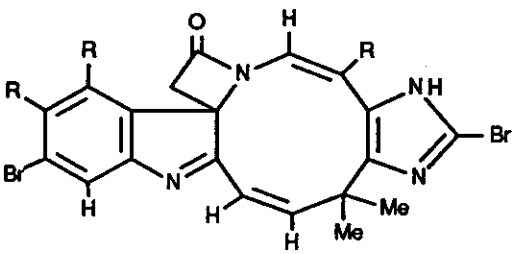


(17) R =



(18) R =





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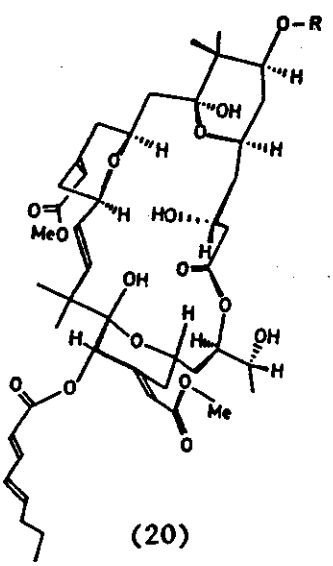
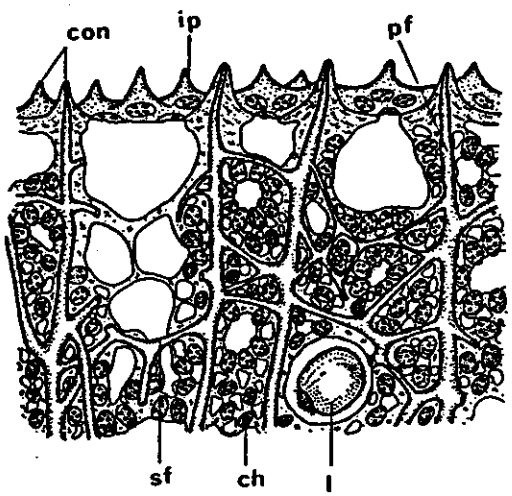


(20)

Recently the bryozoan *Bugula neritina* also has attracted considerable interest due to the isolation of the potent antineoplastic metabolite bryostatin-1 (20).[25]

It was in light of this lack of progress and the potential of finding interesting new metabolites that work was commenced on the bryozoan *Orthoscutilla maculata* (family Catenicellidae) collected from Jervis Bay (N.S.W. coast).

Fig 1. : A choanocyte cell showing the long flagellum (F), apical colar of cytoplasmic tenticles (T) and rounded cell body (B). (Source: Bergquist[2])



con - conules
f - fibres
l - larva
ip - inhalent pores
xc - exhalent canals (osculum)
ch - choanocytes

Fig 2. : Diagrammatic section at right angles to the surface of a dictyoceratid sponge. Note the primary (pf) and secondary (sf) fibres which make up the spongian network.

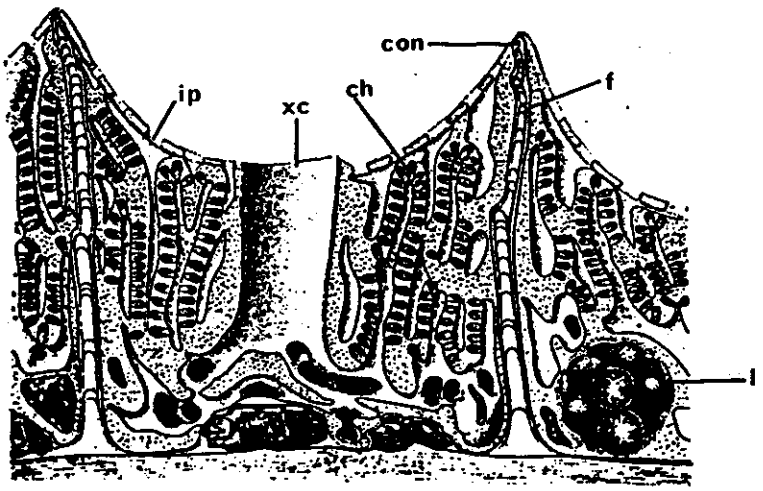
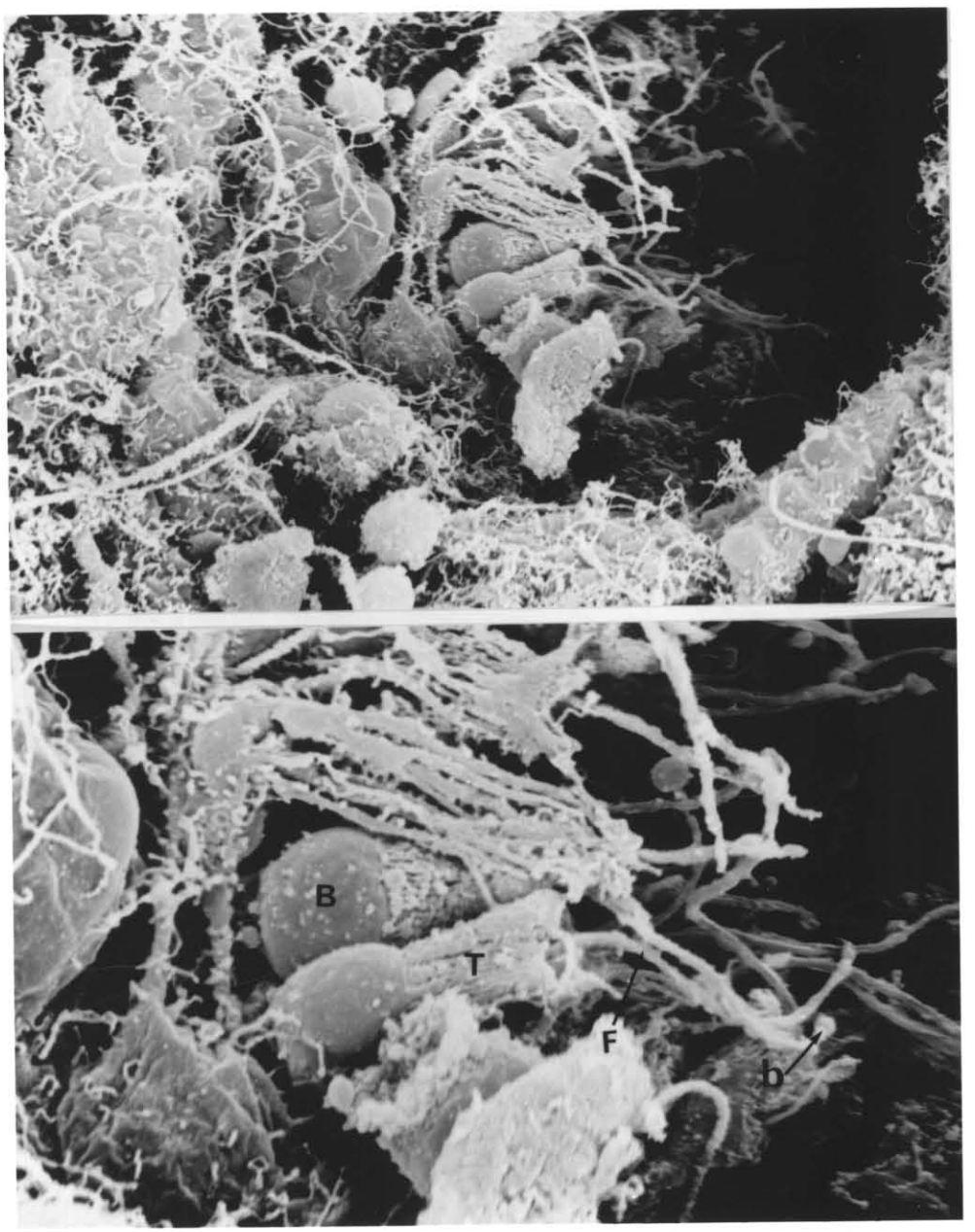


Fig 3. : Diagrammatic section at right angles to the surface of an aplysillid sponge. Note in these sponges the spongian fibres (f) are simple upright fibres and display no anastomosing (hence a Dendroceratid).

Plate 1. : Scanning electron micrograph of a chamber within the sponge *Aplysilla tango*, showing choanocyte cells which execute both the pumping of water through the sponge elaborate network of pores and chambers, as well as the feeding by filtering out the organic matter from the sea water. (x1500)

Plate 2. : Close up of the choanocyte cells showing the long flagellum (F)with an apparent bulbous tip (B), apical collar of cytoplasmic tenticles (T) and rounded cell body (B). (x3600)


B
T
F
b

1.2 DISCUSSION

The marine sponge *Aplysilla pallida* was collected from two locations; Bare Is. Sydney and Bass (Pig) Is. Wollongong. The samples were immediately transported back to the laboratory, freeze dried and then extracted with chloroform/ethyl acetate (1:1). Four new diterpenes were isolated and charaterised by extensive spectroscopic methods: aplypallidenone (21), aplypallidoxone (22), aplypallidione (23) and aplypallidioxone (24). The structures of aplypallidenone and aplypallidoxone were further confirmed by X-ray crystallography (Appendix 1). Due to the difficulty in finding and collecting material, several collections were made at various times of the year. Therefore the two most concentrated diterpenes, aplypallidenone (21) and aplypallidoxone (22), were quantified in the various samples to ascertain if any geographical or seasonal variation in terpene concentrations was occuring.

To the best of the author's knowledge, the *A. pallida* metabolites (21)-(24) are the first products isolated having the diterpene carbon skeleton (25), which is being tentatively called pallidane. These metabolites may be formally derived from spongian (10) in which the C15 carbon is oxidatively removed (see Section 1.2.1F). Hence a similar numbering system (as are the ring letters) to that used in spongian (10), and shown in (25), has been adopted in this text.

Aplypallidenone (21) on treatment with methanol afforded a mixture of (21) and the Michael adduct, as the enol (26). Completion of the reaction was never achieved and the product could not be purified by chromatography (flash silica): only a 95% conversion to the Michael adduct was achieved even after a week in AR grade methanol (^{1}H n.m.r.), and the adduct then slowly reverted to aplypallidenone after removal of the methanol (t.l.c.). The enol (26) on treatment with acetic anhydride/pyridine (1:1)

afforded aplypallidenone and the trapped enol acetate (27) as a minor product (Scheme 1). By returning the reaction mixture to methanol for 24 hours, removing the solvent before adding acetic anhydride/pyridine for 24 hours and repeating this cycle several times, the yield of enol acetate was increased. The enol acetate could then be purified by column chromatography (flash silica).

As part of an Honours project to obtain and purify the pentacyclic diterpene aplyroseol-1 (30) in sufficient quantity for 2D ^{13}C - ^{1}H n.m.r. studies, a large scale collection of the sponge *Aplysilla rosea* was made. Although originally extracted by Karuso, a large scale collection by the author provided an opportunity to examine the sponge for further minor metabolites. The previously isolated metabolites, ambliofuran (28), hexahydroambliofuran (29), aplyroseol-1 (30), -3 (31), -4 (32), -5 (33), -6 (34) and lactones (35) and (36), together with dendrillol-1 (37) and -2 (38) from the New Zealand sponge *Dendrilla rosea* were found.[19,26] Eleven new compounds AROSST-1 (39) -11 (49) were also isolated, six of which have skeletons based on the *ent*-isocopalane (50)[27] system [compounds (39)-(44)], two on the pallidane (25) system [compounds (45)-(46)] and three on the spongian (10) system [compounds (47)-(49)].

The structures of compounds (30)-(36) were identified by direct comparison of the ^{1}H n.m.r. spectra with those of the authentic samples. The structures of compounds (37)-(38) were identified by comparison with the spectral data presented in the literature.[26] The structures of all new compounds, (39)-(49), were assigned by exhaustive spectroscopic analysis.

The absolute stereochemistry of aplyroseol-1 (30) was determined by X-ray crystal structure determination of its p-bromobenzoate derivative (51), prepared by the treatment of (30) with p-bromobenzoyl

chloride/N,N-dimethylaminopyridine (DMAP)in dichloromethane (Scheme 2). It was also reasonable to infer that all the aplyroseol metabolites have the same absolute stereochemistry.

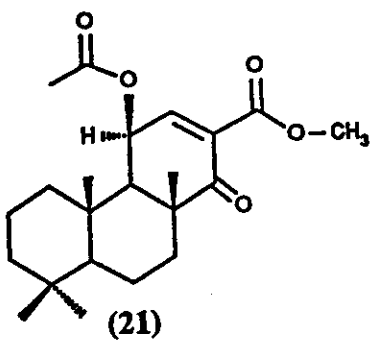
Treatment of AROSST-3 (41) with acetic anhydride/pyridine gave a compound identical to AROSST-4 (42). This therefore allowed the assignment of the regioisomers AROSST-4 (42) and AROSST-5 (43) (Scheme 3). Treatment of the lactone (35) with lithium aluminium hydride afforded the triol (52) which, when treated with dimethyl sulphoxide at 140° or refluxed with triphenyl phosphine in carbon tetrachloride, yielded the cyclic ether (53) (Scheme 4). Although not completed, reduction of the C7 alcohol would then yield the hypothetical precursor spongian (10).

The sponge *Aplysilla var. sulphurea* was collected from Bare Is, Sydney and immediately transported to the laboratory, freeze dried and then extracted with chloroform/ethyl acetate (1:1). Only one patch has been found of this sponge, which the author observed to have a very rapid regeneration. A subsequent second collection of this sponge showed no seasonal variations in the diterpene metabolites. Only two previously reported diterpenes, 15,16-diacetoxyspongian (54) and 16-oxospongian (55), were isolated in both samples collected.[17,21]

The catenicellid bryozoan *Orthoscutilla maculata* (family Catenicellidae) was collected from a location known as 'Torpedo Tubes' in the waters of Jervis Bay. The material was transported back to Sydney, freeze dried and then extracted with ammoniated methanol. The resulting extract was then partitioned between ethyl acetate and hydrochloric acid solution (3 M). The ethyl acetate extract yielded only fatty acids and triglycerides which were not analysed. The hydrochloric acid solution upon basification (pH 14) with ammonia solution and extraction with

chloroform yielded only one alkaloid, 1-ethyl-β-carboline (56), previously isolated from another bryozoan, *Castaticella hastata* (family Catenicellidae),[28] and from the roots of a tropical African tree *Hannoa klaineana*.[29]

In Section 1.2.5. a list of sponges and their metabolites containing the spongian or spongian-derived backbone is presented.



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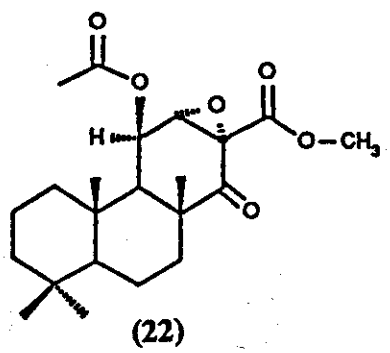

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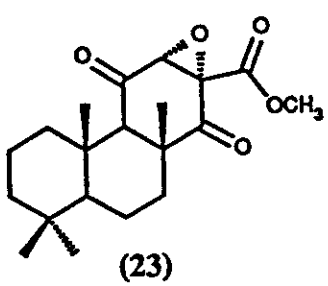

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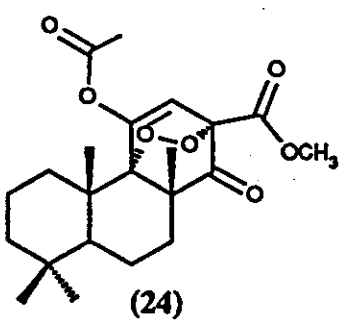

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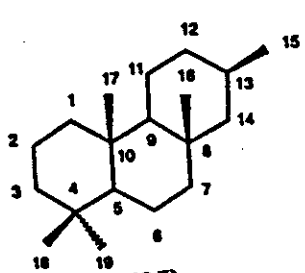
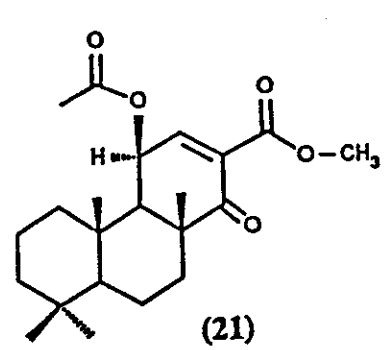


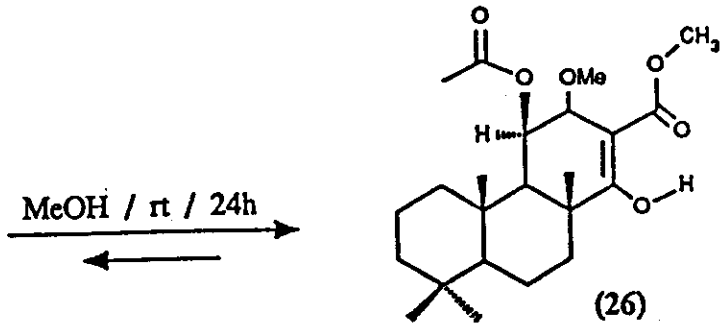
(25)

Palli-dane



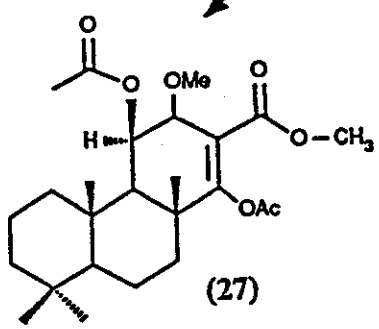
(21)

Aplypallidenone



(26)

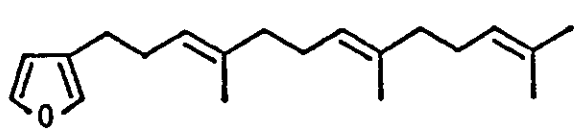
Ac_2O / py / rt / 24 h.



(27)

+ Aplypallidenone

Scheme 1



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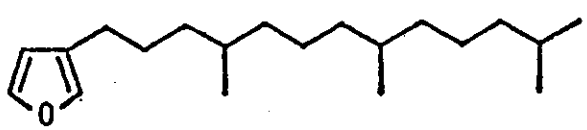

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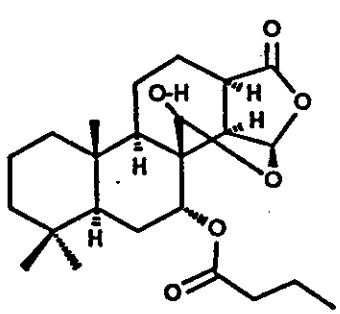

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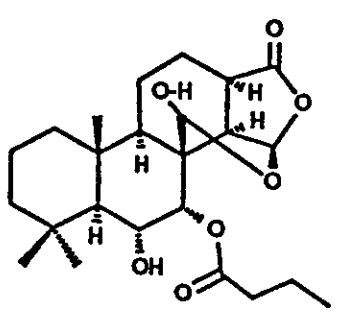

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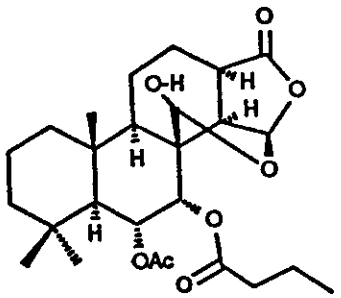

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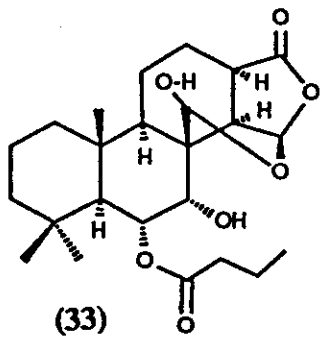

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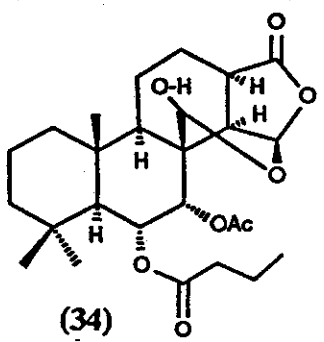

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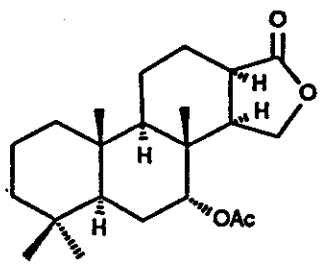

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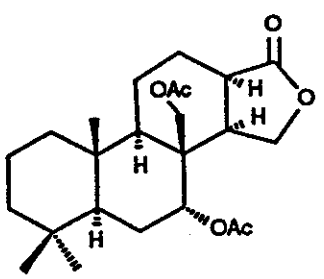

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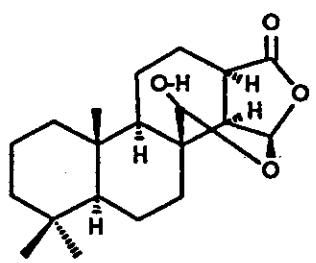

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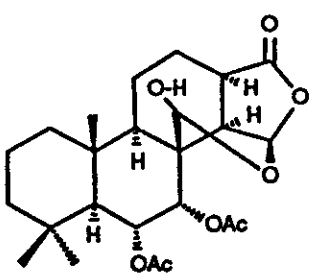

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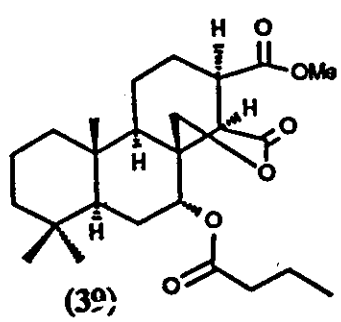

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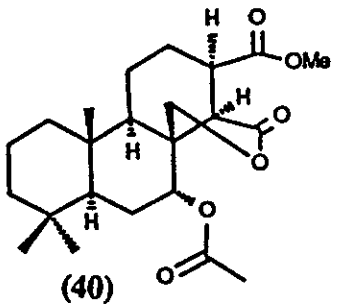

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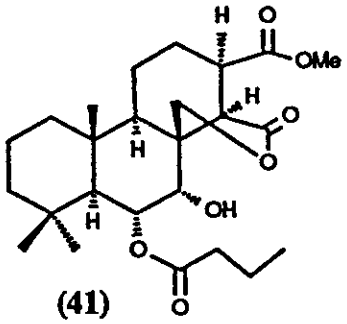

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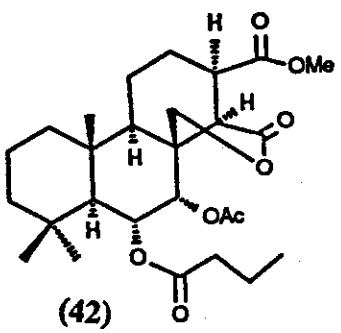

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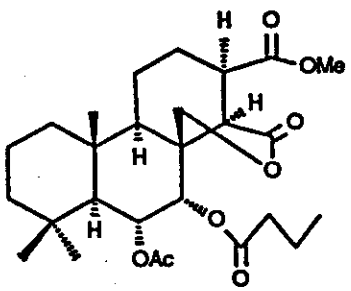

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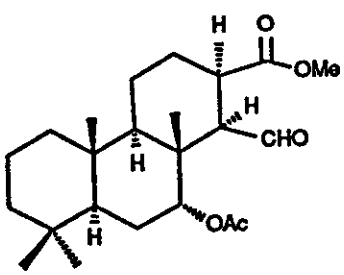

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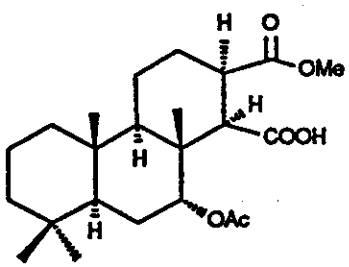

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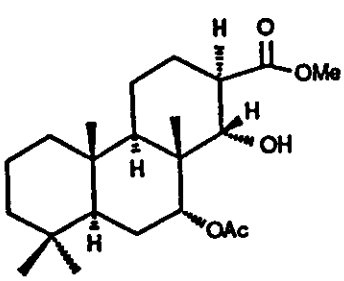

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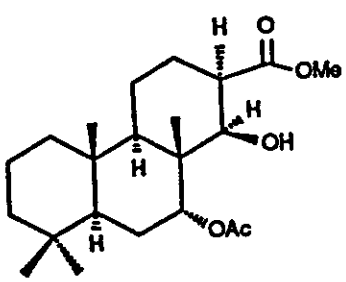

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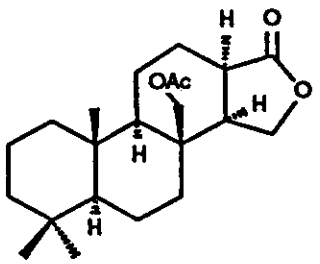

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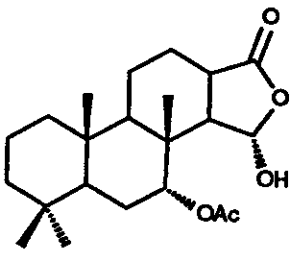

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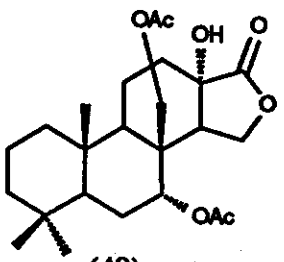

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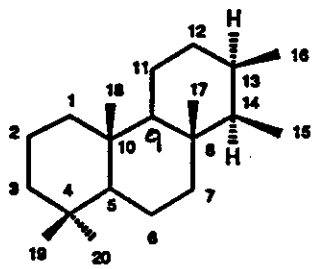
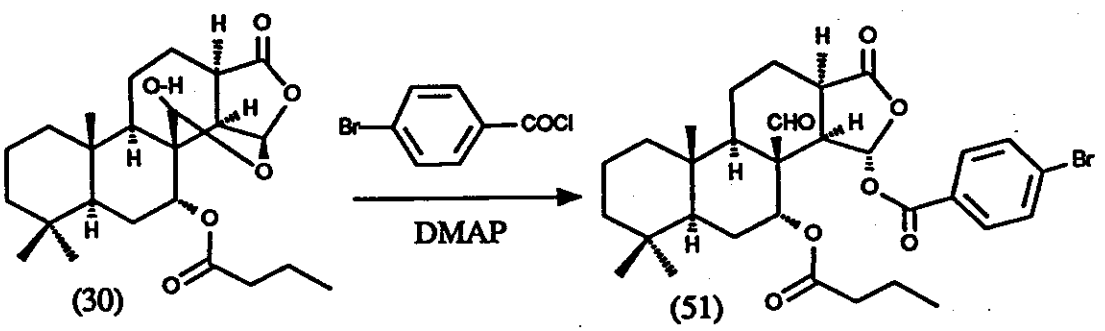


(50)

ent-Isocopalane



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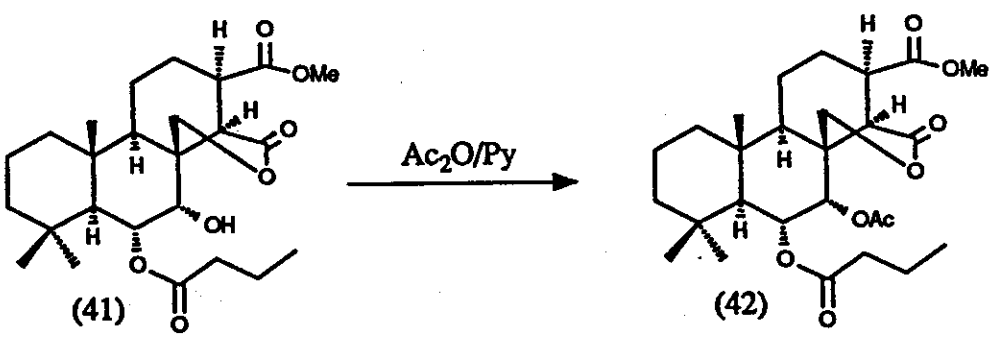

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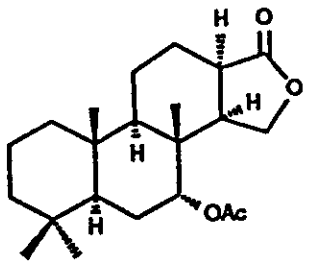
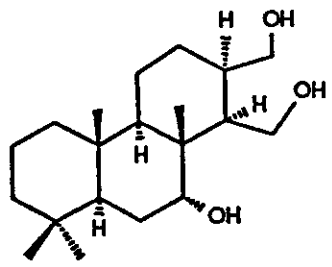


(35)

$LiAlH_4$

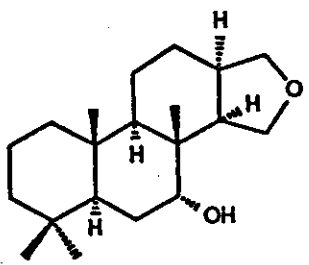
Et_2O



(52)

DMSO/140°
or $PPh_3/CCl_4/CH_3CN$

reflux



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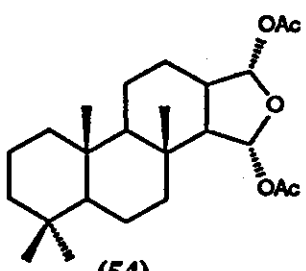
Scheme 4



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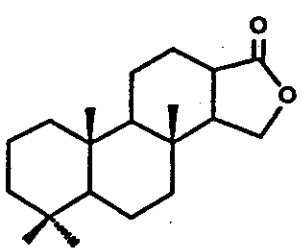

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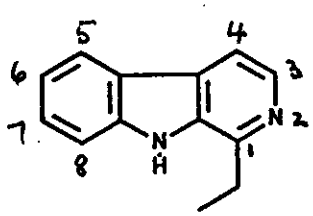


(56)

1.2.1 *Aplysilla pallida*

Aplysilla pallida (Plates 3-4, 11-14) is a rare, creamy white, encrusting sponge of the order Dendroceratida, which on extraction yielded four new crystalline diterpenes. Six collections of *A. pallida* were made: four from Bare Is., Sydney (May 1985, Jan 1988, Sept 1988 and Jan 1989) and two from Bass (Pig) Is., Wollongong (Aug 1988 and Dec 1988).

A. pallida from the Bare Is., Sydney location was collected at a depth of 10-15 m and was only ever found on the ceiling of small crevices and caves, away from direct sunlight. These crevices tended to be narrow and hence, particularly during the winter season (water temp. 14°), made collection of material very tedious. However *A. pallida* from the Bass Is., Wollongong location was collected at a depth of 23-27 m. At this depth the sponge, when found, was out in the open which made collecting much easier.

The sponge material, once transported back to the laboratory, was freeze dried and extracted with chloroform/ethyl acetate (1:1). The crude extract was then purified by gravity column chromatography (flash silica) and then further purified by crystallisation.

The two major diterpene metabolites, aplypallidenone (21) and aplypallidoxone (22), were first isolated and the concentrations within the sponge quantified (Table 1). They were also tested for biological activity (antileukemic (P388), antiviral and antibacterial) the results of which are presented in Section 1.2.1F.

From the combination of the remaining fractions, purification on flash silica followed by crystallisation yielded the further two metabolites, aplypallidione (23) and aplypallidioxone (24).

A more polar sterol fraction (^{1}H n.m.r. spectrum, t.l.c.) was also present but was not investigated.

Plate 3. : A underwater photograph of *Aplysilla pallida*.

Plate 4. : A preserved sample (ethanol/water, 7:3) of *A. pallida*. Note the extremely flat and delicate habit of the sponge which made it difficult to collect in quantity.

Table 1 Diterpene Concentrations from *A. pallida*

Locality[1]	Month (y)	C[2] (g)	21 mg(%)	22 mg(%)	21 + 22 (%)
A	May(1985)	6.2	105(1.7)	71(1.1)	2.8
A	Jan(1988)	21.6[3]	398.5(1.7)	605.5(2.8)	4.5
B	Aug(1988)	13.9	245(1.8)	175.5(1.3)	3.1
A	Sept(1988)	5.0	14(0.3)	108(2.2)	2.5
B	Dec (1988)	9.8	58(0.6)	136(1.4)	2.0
A	Jan (1989)	8.7	157(1.8)	113(1.3)	3.1

[1] A Collected at Bare Is. Sydney

B Collected at Bass (Pig) Is. Wollongong

[2] C mass of dry weight sponge; collections were of single colonies

[3] Collection incorporated more than one sponge colony collected over a two week period.

(A) Aplypallidenone (21)

Aplypallidenone (21) is a white, crystalline, neutral, optically active, tricyclic diterpene. It was shown to have a molecular formula of $C_{22}H_{32}O_5$ by high resolution mass spectrometry. By means of extensive spectroscopic studies the structure (21), with stereochemistry, was assigned to this compound.

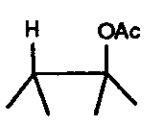
The electron impact mass spectrum (e.i.m.s.) showed a weak molecular ion peak at *m/z* 376 (5%), which was supported by the chemical ionization mass spectrum (c.i.m.s.) which gave an $[M + H]^+$ ion at *m/z* 377 as the base

peak. High resolution mass matching of the molecular ion (*m/z* 376) in the e.i.m.s. substantiated the molecular formula as $C_{22}H_{32}O_5$. In addition, the fully decoupled ^{13}C nuclear magnetic resonance (^{13}C n.m.r.) spectrum showed 22 signals; the fully coupled ^{13}C n.m.r. spectrum together with the integration of the proton nuclear magnetic resonance (^{1}H n.m.r.) spectrum (Fig 4) indicated the presence of 32 protons. Therefore, from the molecular formula, seven units of unsaturation are present.

An intense broad absorption peak in the infrared (i.r.) spectrum at 1740 cm^{-1} and three quaternary carbon signals in the ^{13}C n.m.r. spectrum at δ 165.3, 169.8 and 200.6 indicated the presence of three carbonyl functions: two esters and a ketone respectively.

A singlet in the ^{1}H n.m.r. spectrum at δ 2.13 (integrating to three protons) together with a sharp quartet at δ 21.3 ($^{1}J_{C-H}$ 130 Hz) in the ^{13}C n.m.r. spectrum and a strong absorption peak at 1246 cm^{-1} in the i.r. spectrum established one of the ester functions as an acetate. Both the e.i.m.s. and c.i.m.s. showed the full loss of a molecule of acetic acid, fragmentation occurring in two stages: initial loss of ketene (42 daltons) giving peaks at *m/z* 334 in the e.i.m.s. and *m/z* 335 in the c.i.m.s., and subsequent loss of water (18 daltons) gave the *m/z* 316 and 317 peaks in the e.i.m.s. and c.i.m.s. respectively.

As the acetoxy group fragments with the full loss of acetic acid there must be a β-proton available for β-elimination. This indicates partial structure (A).



(A)

The ^1H n.m.r. spectrum contained a three proton singlet at δ 3.81 and, together with a sharp quartet in the ^{13}C n.m.r. spectrum at δ 52.4 ($^1J_{C-H}$ 147.6 Hz), indicated the presence of a methoxy group. From the chemical shift in the ^1H n.m.r. spectrum the methoxy group must be part of a methyl ester group.

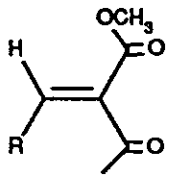
The ultraviolet (u.v.) spectrum (λ_{max} cyclohexane: 211 nm, ϵ 9640; 336 nm, ϵ 60) and a doublet in the ^1H n.m.r. spectrum at δ 7.24 (J 5.4 Hz) was good evidence for an α,β-unsaturated carbonyl system. Further analysis of the ^{13}C n.m.r. spectrum showed two signals in the olefinic region: a singlet at δ 132 and a doublet of doublets at δ 144 (J 166 Hz, 5.4 Hz). The chemical shifts of the carbonyl carbons for both the methyl ester (δ 165.3) and ketone (200.6) were further upfield than those for α,β-saturated systems (cf. δ 174.4 for methyl n-octanoate and 208.8 for cyclohexanone).

These upfield carbonyl resonances together with the characteristically large coupling of $^1J_{C-H}$ 166 Hz (δ 144) for the olefinic carbon and an olefinic absorption peak (1686 cm^{-1}) in the i.r. spectrum, provided further evidence for an α,β-unsaturated carbonyl system.

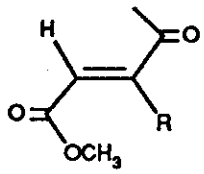
Additionally, the chemical shift of both carbonyl carbons indicated that both the methyl ester and the ketone are in conjugation with the olefinic bond in the α,β-unsaturated system.

Since the olefinic carbons appear as a doublet and a singlet, and as there is only one olefinic proton in the ^1H n.m.r. spectrum (δ 7.24), the α,β-unsaturated carbonyl system must be trisubstituted with the proton residing β to the carbonyl.

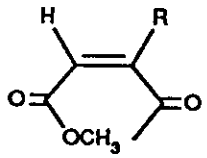
Part structures (B)-(G) fulfill all the above criteria accounting for all the possibilities.



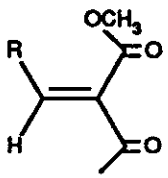
(B)



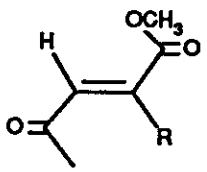
(D)



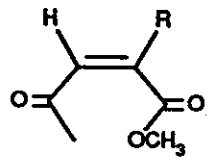
(F)



(C)



(E)



(G)

As already mentioned, the β-proton at δ 7.24 in the ^{1}H n.m.r. spectrum has a coupling of J 5.4 Hz. This magnitude indicated that the protons coupling must be vicinal rather than allylic. Hence part strutures (D)-(G) were eliminated.

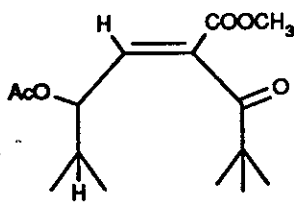
Furthermore, on closer analysis of the ^{13}C n.m.r. spectrum, the ketone carbonyl signal (δ 200.6) was found to be broader than the methyl ester carbonyl signal (δ 165.3). This situation is best explained by part structure (B) : the broadening being due to a *trans* - $^{3}J_{C-H}$ coupling of the ketone carbonyl carbon to the β-proton.

Spin-spin decoupling experiments in the ^{1}H n.m.r. spectrum of the proton at δ 7.24 (J 5.4 Hz) showed it was coupled to a proton at δ 5.83 (J 5.4, 4.2 Hz). This proton could be associated with a doublet in the ^{13}C n.m.r. spectrum at δ 64.8 (J 149.8 Hz). These δ values as well as the magnitude of the $^{1}J_{C-H}$ coupling ($^{1}J_{C-H}$ 149.8 Hz) meant that the proton must be attached to a carbon bearing an oxygen. As the only oxygen source which has not been assigned is the acetate, this clearly indicates that the proton at δ 5.83 in the ^{1}H n.m.r. spectrum is both α to the acetate and allylic to the α,β-unsaturated carbonyl system.

Further decoupling experiments in the ^1H n.m.r. spectrum showed the α-acetoxy proton to be coupled to an aliphatic proton resonating as a doublet at δ 1.69 (*J* 4.2 Hz). This proton, since there were no further couplings, is therefore situated next to two quaternary carbons.

Also, as there are no further downfield protons in the ^1H n.m.r. spectrum (> δ 2.00), the ketone carbonyl must also reside next to two quarternary carbons.

From the data presented above partial structure (B) could now be extended to (H).



(H)

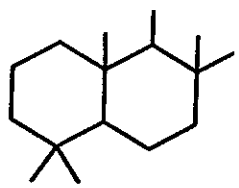
Partial structure (H) consequently accounts for all the oxygens as well as four of the seven units of unsaturation. As the ^{13}C n.m.r., i.r. and ^1H n.m.r. spectra indicated no further unsaturation the parent hydrocarbon is tricyclic.

Of particular diagnostic value was the mass spectrum (scheme 5). Two strong peaks, *m/z* 184 (45%) and 192 (16%) were found by high resolution mass matching to be $C_8H_8O_5$ and $C_{14}H_{24}$ respectively. Together they represent the full complement of the molecular formula $C_{22}H_{32}O_5$.

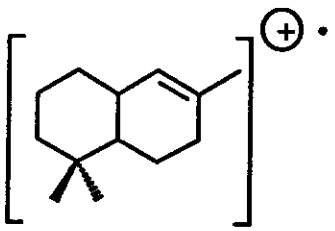
The fragment *m/z* 184 which contains all the oxygens corresponds to that of partial structure (H). This then further fragmented with the loss of ketene (42 daltons) to give a peak at *m/z* 142.

The remaining moiety of the molecular formula, once partial structure (H) was established, was $C_{14}H_{24}$ (*m/z* 192).

Analysis of the remaining features of the ^1H n.m.r. spectrum in both $CDCl_3$ (Fig 4) and C_6D_6 (Fig 5) together with the ^{13}C n.m.r. spectrum leads to the elucidation of the $C_{14}H_{24}$ fragment as partial structure (I).



(I)



(J)
m/z 177

The ^1H n.m.r. spectrum ($CDCl_3$) showed four methyl groups attached to quaternary carbons: two singlets at δ 0.85 and 0.87 and two fine doublets at δ 1.21 (J 0.85 Hz) and 1.42 (J 0.7 Hz). This was also substantiated by the ^{13}C n.m.r. spectrum which contained three singlets (δ 33.2, 38.6 and 45.9) and four quartets (δ 17.4, 21.6, 21.7 and 33.5).

The remaining signals in the ^1H n.m.r. spectrum (δ 0.75 - 2.00, 12 x H) were part of an aliphatic system. From the ^{13}C n.m.r. spectrum they constituted five methylene (δ 18.0, 18.4, 35.4, 39.2 and 41.5) and two methine (δ 53.2 and 56.6) resonances.

One of the methine groups (δ_H 1.69) has already been assigned as the aliphatic proton β to the acetoxy group in (H); this therefore leaves eleven unassigned protons (five methylenes and one methine).

Four of the eleven aliphatic resonances in the ^1H n.m.r. spectrum were isolated systems (δ 0.79, 1.00, 1.13 and 1.97). The A ring in partial structure (I) was suggested by two of these resonances, δ 1.00 (ddd J 13, 13, 4 Hz) and 1.13 (ddd, J 13, 13, 4 Hz) which were, from their multiplicity and magnitude of the coupling, reminiscent of H1α and 3α protons in the aplyroseol series of compounds (30)-(34): a J 13 Hz geminal coupling, a J 13 Hz *trans*-diaxial coupling and a J 4 Hz axial-equatorial coupling.

The B ring of partial structure (I) was suggested upon analysis of the ^1H n.m.r. spectrum obtained in benzene (C_6D_6).

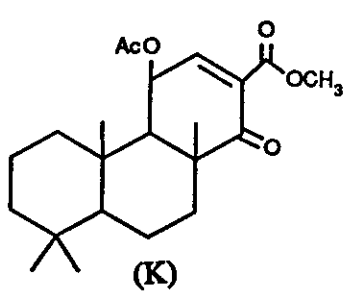
In the ^1H n.m.r. spectrum obtained in chloroform ($CDCl_3$) the resonance at δ 0.79 was clearly a doublet of doublets (J 11.4, 2.2 Hz). The absence of three or more couplings indicated it as a methine attached to a methylene. Unfortunately however the resonances pertaining to the protons of this methylene were obscured by other aliphatic resonances.

When the ^1H n.m.r. spectrum was obtained in benzene (C_6H_6, Fig 2), further dispersion of the aliphatic resonances was obtained.

Decoupling of the methine at δ 0.37 (δ 0.79 in $CDCl_3$) demonstrated it clearly to be coupled to a slightly second order methylene proton at δ 1.51 having four couplings : one geminal, two *trans*-diaxial and an axial-equatorial. This in turn was shown, by decoupling experiments, to be coupled to a proton resonating as a multiplet at δ 1.96 by a small ($\simeq J$ 4 Hz) coupling. This multiplet also has one large coupling indicating it was geminally coupled and part of a methylene group.

Therefore, from the n.m.r. data presented above, together with the observation of a further fragmentation ion in the mass spectrum at *m/z* 177 ($C_{13}H_{21}$) attributable to ion (J), established the $C_{14}H_{24}$ moiety as partial structure (I).

To account for the data presented above the full structure of aplypallidenone without stereochemistry was settled as (K). The numbering system shown (as are the ring letters) being based upon that of pallidane (25).

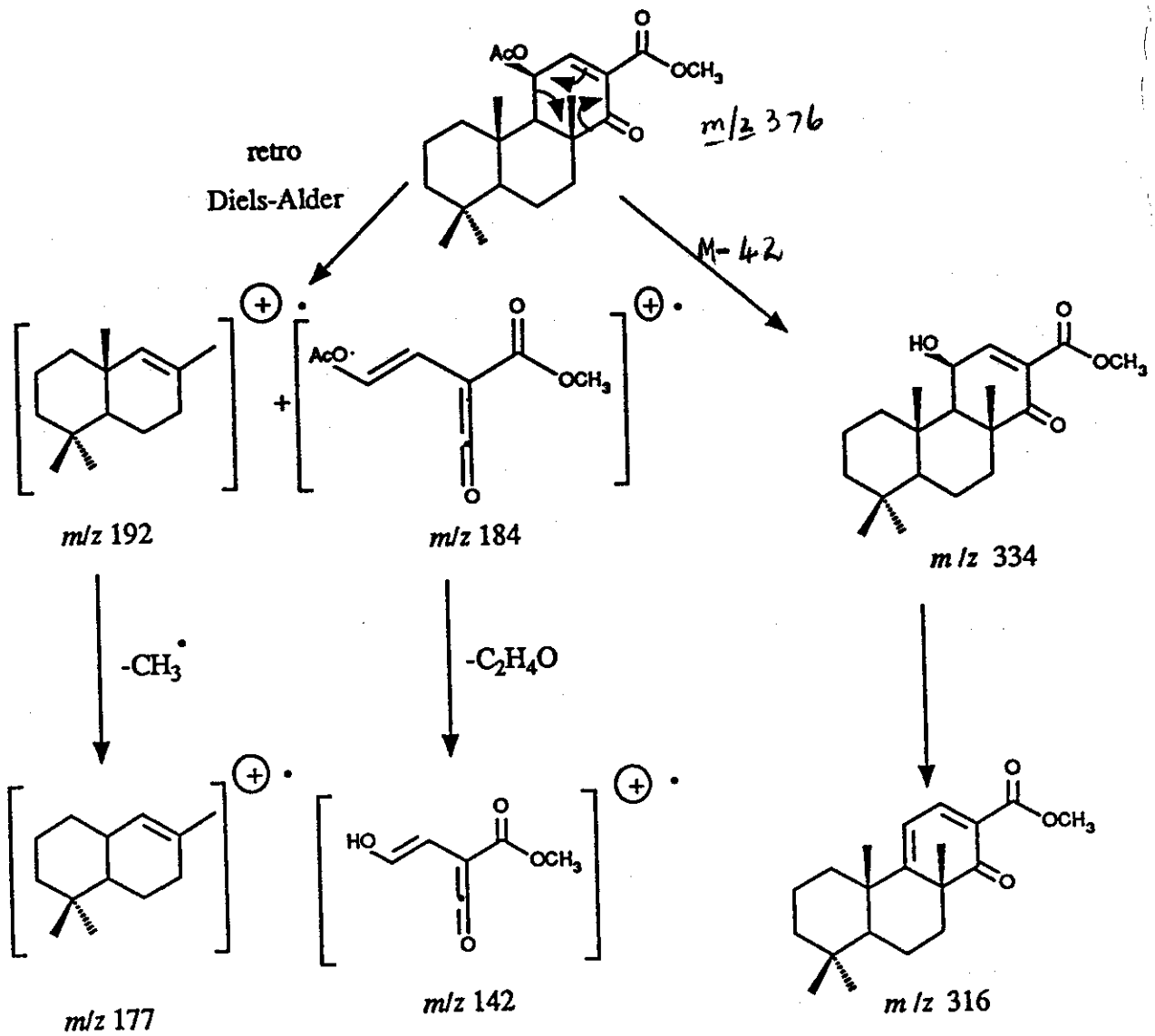


(K)

Fragmentation Pattern of Aplypallidenone (21).

Fragments Determined by High Resolution

Mass Matching (e.i.m.s.)



Scheme 5

The e.i.m.s., giving peaks *m/z* 192 and 186 may now be envisaged as the result of a reverse Diels-Alder fragmentation as shown in scheme 5.

(1) 2D-COSY Spectrum

A proton-proton two dimensional correlated spectrum (2D-COSY) of aplypallidenone (Fig 6) was recorded, allowing the assignment of all protons to be established through the coupling connectivities between protons.

The 2D-COSY spectrum confirmed the connectivities already established by decoupling experiments in the 1D spectrum. In addition the 2D-COSY spectrum together with n.O.e. experiments (Table 2) enabled the connectivities and chemical shifts of all the protons in the A and B rings, as well as some small long range connectivities to be determined.

The 2D-COSY spectrum was obtained by the method of Bax and Freeman[30] with a $90^{\circ}-t_1-45^{\circ}$ pulse sequence (COSY-45).

In the COSY-45 experiment the final 45° pulse has the effect of orientating cross peaks of couplings of negative sign ($^2J_{H-H}$ and $^4J_{H-H}$) parallel to the 45° axis (negative slope) while couplings of positive sign $^3J_{H-H}$ are orthogonal to the 45° axis (positive slope). Hence, by inspection of the 2D-COSY spectrum for the slope of the cross peak it is often possible to determine the relative sign of the coupling constant (negative for geminal, positive for vicinal coupling).

An expansion of the aliphatic proton envelope is shown in Fig 6. Whereas in the 1D ^1H n.m.r. spectrum the vicinal methylene protons, as well as the geminal protons, for both H1α and 3α were obscured by other aliphatic protons, in the 2D-COSY spectrum it was clearly visible that both H1α and 3α were mutually coupled to two vicinal protons (δ 1.48, H2α; 1.62, H2β).

Other connectivities which were observed for H1α and 3α were two geminal protons (δ 1.41 and 1.65). Using n.O.e. experiments, the assignments of 1α, 1β, 3α, and 3β were possible: irradiation of H11 gave n.O.e.'s to both H1α (δ 1.00) and 1β (δ 1.65) while irradiation of 19Me gave n.O.e.'s to both H3α (δ 1.13) and 3β (δ 1.41).

Also evident from the 2D-COSY spectrum was H5α (δ 0.79) possessing two vicinal couplings to H6β (δ 1.51) and 6α (δ 1.71) which were coupled to each other.

In addition H7β (δ 1.97) also showed connectivities to H6α and 6β as well as to H7α (δ 1.58). The close proximity of the two axially disposed protons, H7α and H6β, result in producing the second order pattern in H7β invoked by virtual coupling.

Finally, two long range connectivities are also discernible. The 'W' coupling connectivity between H9 and the two methyl groups 16Me (δ 1.42, *J* 0.7 Hz) and 17Me (δ 1.21, *J* 0.85 Hz) are clearly visible. This implies the two methyl groups 16 and 17 are positioned *trans* with respect to H9 across the C8-C9 and C10-C9 bond respectively. Therefore by convention H9 is in the α-face while the methyl groups are in the β-face.

(II) Relative Stereochemistry

Aplypallidenone contains five chiral carbons. The relative stereochemistry of these sites was determined by nuclear Overhauser enhancement (n.O.e.) experiments (Table 2). The resultant enhancements were analysed with the help of molecular models.

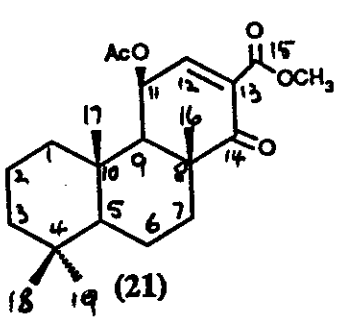
As already stated, the small long range 'W' coupling between H9α and the two methyl groups, 16Me and 17Me, implies H9 and the two methyl groups are on opposite faces of the molecule: H9 in the α-face (H9α) and the two methyl groups in the β-face.

Furthermore n.O.e. irradiation at the 16Me enhanced the 17Me as well as H7β (δ 1.97). Further n.O.e. irradiation of the 17Me showed the reciprocal enhancement in 16Me and as well showed the chemical shift of the axial 18Me to be δ 0.85.

Upon irradiation of H12, a n.O.e. enhancement was observed in H11; when H11 was irradiated it gave the reciprocal enhancement in H12 as well as enhancing H9α, H1α and H1β. Enhancements observed in the later three protons indicated H11 was also α disposed (H11α). Finally irradiation of H5α confirmed, with enhancement at H1α, H3α and H9α, that they are all close in space and therefore α disposed.

Therefore the final structure of aplypallidenone with relative stereochemistry was settled as (21). The absolute stereochemistry is probably that depicted in structure (21) on the basis of biogenetic arguments (Section 1.2.4).

To resolve any doubts of the structure and relative sterochemistry, a single crystal X-ray analysis of aplypallidenone was carried out. The analysis validated the structure of (21), proposed by spectroscopic arguments (Appendix 1).



(21)

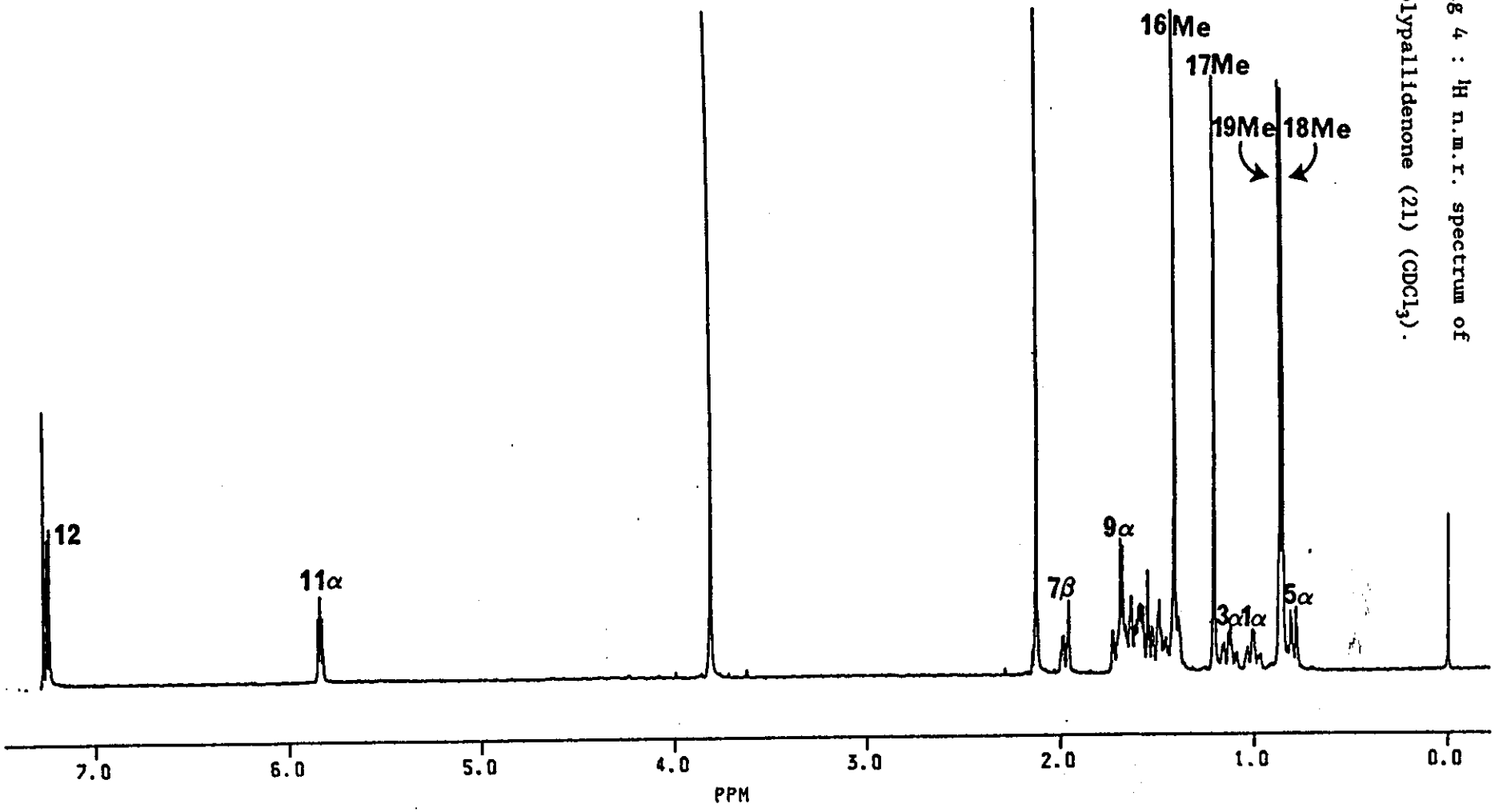


Fig 4 : ^1H n.m.r. spectrum of Aplypallidenone (21) ($CDCl_3$).

Fig 5 : ^1H n.m.r. spectrum of Aplypallidenone (21) (C_6D_6). Expansion of upfield portion.

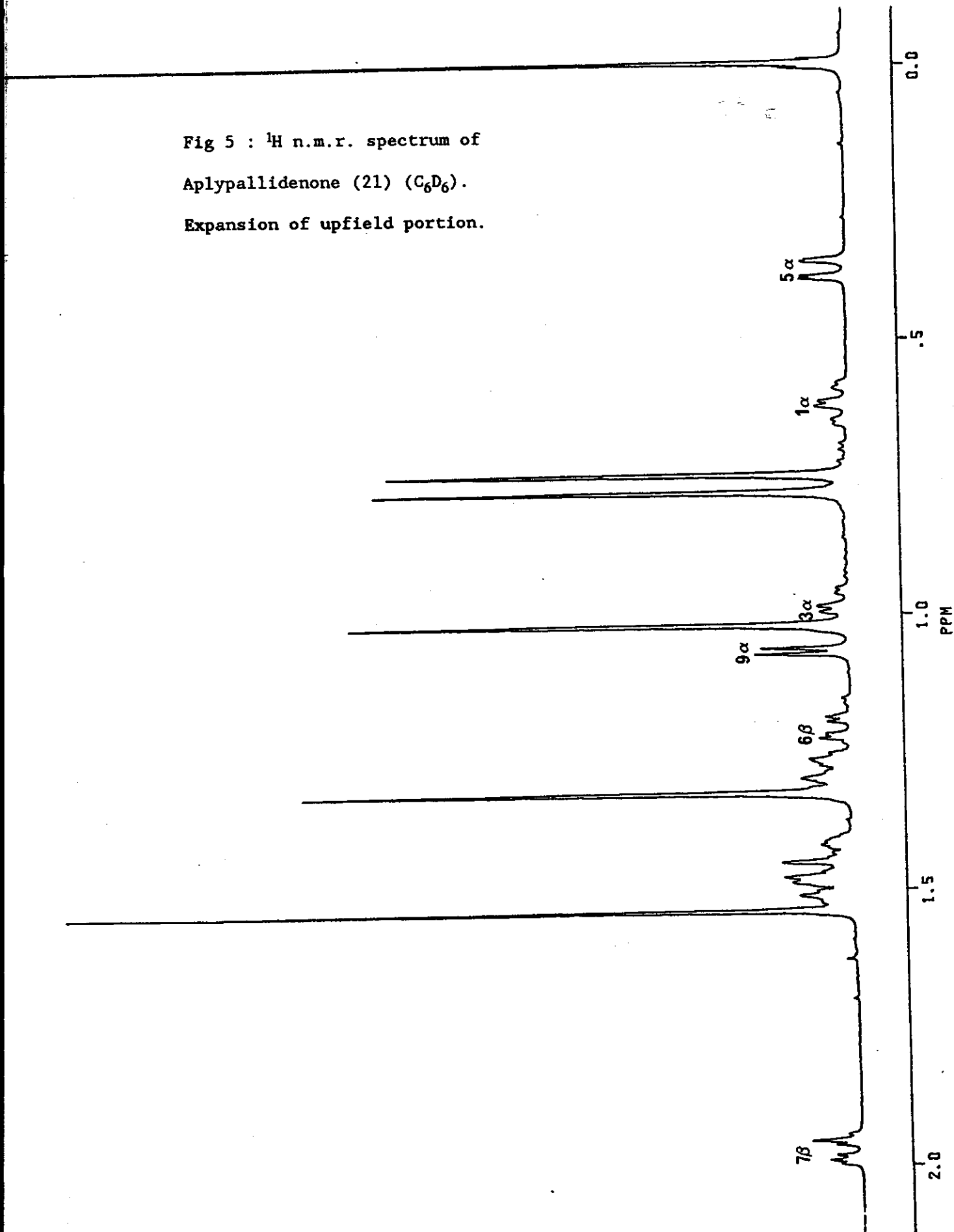


Fig 6 : 2D-COSY spectrum of

Aplypallidenone (21) ($CDCl_3$).

Expansion of upfield portion.

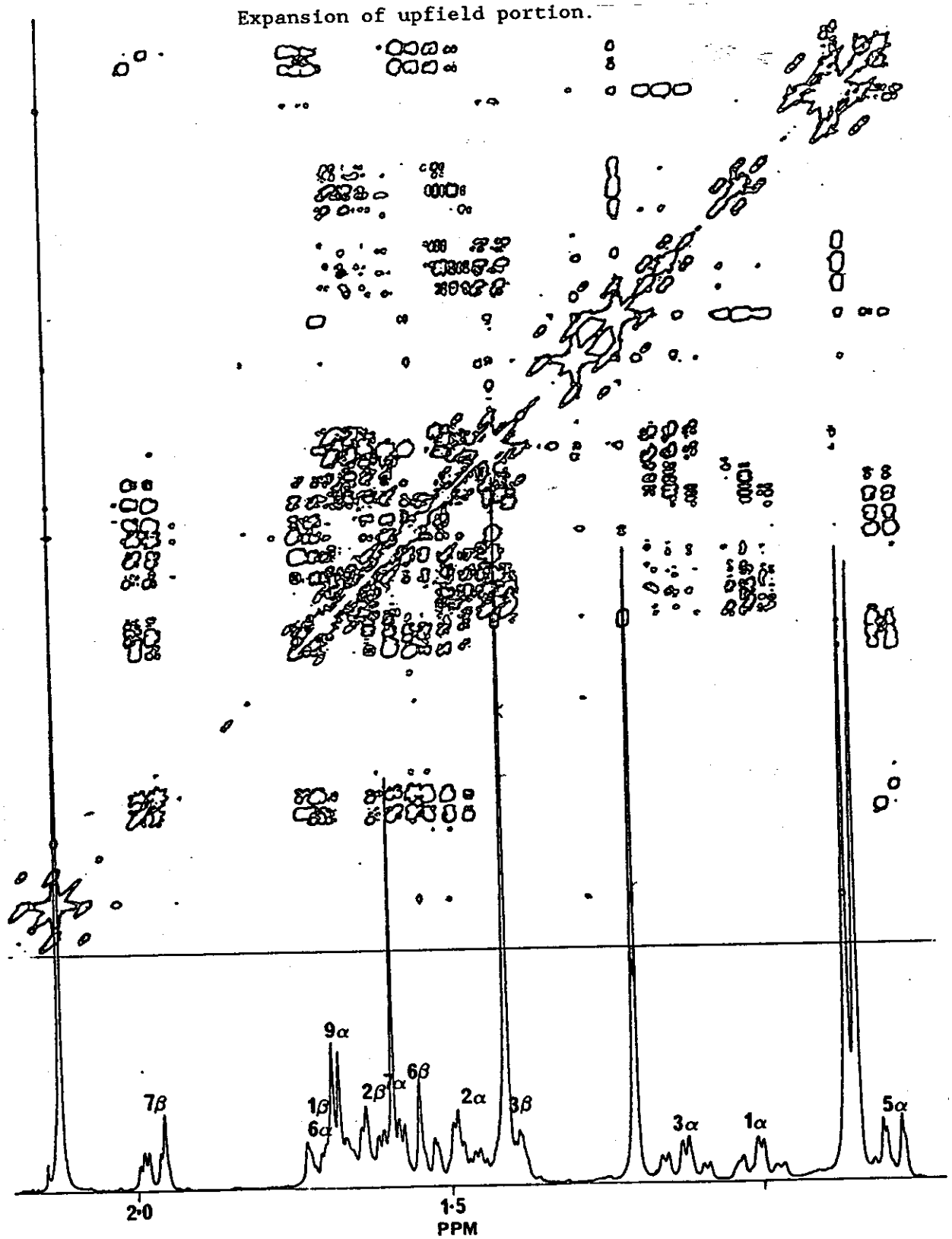


Table 2. ^{1}H–^{1}H nuclear Overhauser enhancements in aplypallidenone (21)

^{1}H irradiated	Signals enhanced (%)
16Me	H7β (1.1), 17Me(3.1).
17Me	16Me(2.0), 18Me(2.6).
18Me	17Me(3.0).
19Me	H3α(1.0), H3β(1.0), H6α(3.0).
H5α	H1α(2.9), H3α(5.1), H9α(2.7)
H11α	H12(6.8), H9α+H1β(6.9)*, H1α(2.7).
H12	H11α(6.8).

* These protons are superimposed so the percentage n.O.e. is collective.

(B) Aplypallidoxone

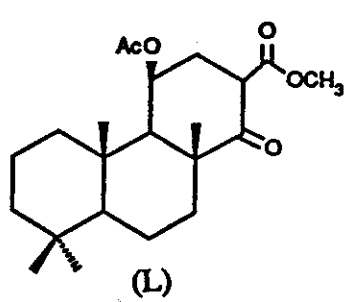
The second compound isolated was aplypallidoxone (22) which was a white crystalline, optically active, neutral solid. The e.i.m.s. showed a weak molecular ion peak at *m/z* 392 (<1%). This was supported by the c.i.m.s. which gave an [M + H] peak, *m/z* 393, as the base peak. In addition the fully decoupled ^{13}C n.m.r. spectrum showed 23 signals; the ^{13}C-DEPT together with the integration of the protons in the ^{1}H n.m.r. spectrum indicated the presence of 32 protons. Therefore from the

molecular formula seven units of unsaturation are again present.

The ^1H n.m.r. spectrum of both aplypallidenone and aplypallidoxone (Fig 7) had many similar features. For example, characteristic signals pertaining to the A, B and C rings of aplypallidenone were also present in aplypallidoxone : H5α (δ 0.77), H1α (δ 0.98), H3α (δ 1.12), H7β (δ 1.99) and H11α (δ 5.89). The ^1H n.m.r. spectrum also showed four methyl groups attached to quaternary carbons: two singlets at δ 0.83 (18Me) and 0.85 (19Me), and two fine doublets at δ 1.14 (17Me, J 0.9 Hz) and δ 1.44 (16Me, J 0.75 Hz).

The i.r. spectrum showed two carbonyl absorption bands, at 1750 and 1713 cm^{-1}, while in the ^{13}C n.m.r. spectrum three carbonyl signals were present at δ 166.2, 169.7 and 201.9; two esters and a ketone respectively. In the ^1H n.m.r. spectrum, two three proton singlets at δ 2.12 and 3.82 confirmed the presence of an acetate and a methyl ester.

The above spectral evidence therefore suggested that aplypallidoxone was very similar to aplypallidenone (21). By analogy with (21), aplypallidoxone had partial structure (L), the relative stereochemistry being confirmed by n.O.e. experiments (table 3).



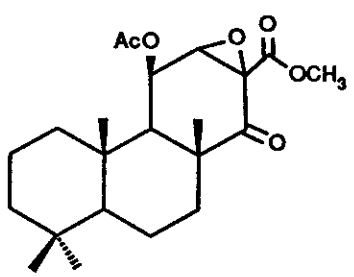
(L)

The u.v. spectrum showed only end absorption and the i.r. spectrum was absent of an olefinic absorption band. This suggested that the α,β-unsaturated carbonyl system present in (21) was no longer present in

aplypallidoxone. This was also reflected in the ^{13}C n.m.r. and ^{1}H n.m.r. spectra in which the olefinic carbons (C12 and C13) and the β-proton (H12) of (21) were absent.

Instead two new carbons were found : a methine carbon at δ 47.1 (C12) and a quaternary carbon at δ 55.8 (C13). The ^{1}H n.m.r. spectrum also showed a new signal at δ 3.55 (H12) as a fine doublet (J 2.5 Hz) which was coupled to the proton geminal to the acetoxy group (H11α). From its chemical shift, H12 is consistent with a proton also geminal to an oxygen.

Since the molecular formula ($C_{22}H_{32}O_6$) contained only one extra oxygen to that of aplypallidenone ($C_{22}H_{32}O_5$) it was deduced that an oxirane ring must be present at C12 and C13. This is further supported by the chemical shifts of C12 (δ 47.1) and C13 (δ 55.8). Thus aplypallidoxone, without stereochemistry at C12 and C13, was settled as (M).

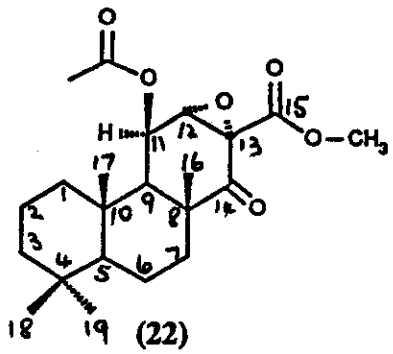


(M)

Due to the small coupling of J 2.5 Hz between H12 and equatorial H11α it was not possible to distinguish, by coupling constants whether H12 was α or β face disposed. A n.O.e. experiment with irradiation of H12 only enhanced H11α, and hence, was also inconclusive.

A single crystal X-ray determination of aplypallidoxone (Appendix 1), however clearly showed the epoxide oxygen as α-disposed and

unequivocally confirmed the structure and relative stereochemistry as shown in (22).



(22)

Table 3. ^{1}H–^{1}H nuclear Overhauser enhancements in aplypallidoxone (22).

^{1}H irradiated	Signal enhanced (%)
16Me	17Me(2.8), H7β(1.7).
17Me	16Me(3.1), 18Me(2.3).
18Me	17Me(3.0).
19Me	H3α(1.6), H3β(1.1), H6α(2.7).
H5α	H9α(10.7).
H11α	H1α(4.0), H1β(8.4), H9α(4.7), H12β(9.7).
H12β	H11α(5.5).

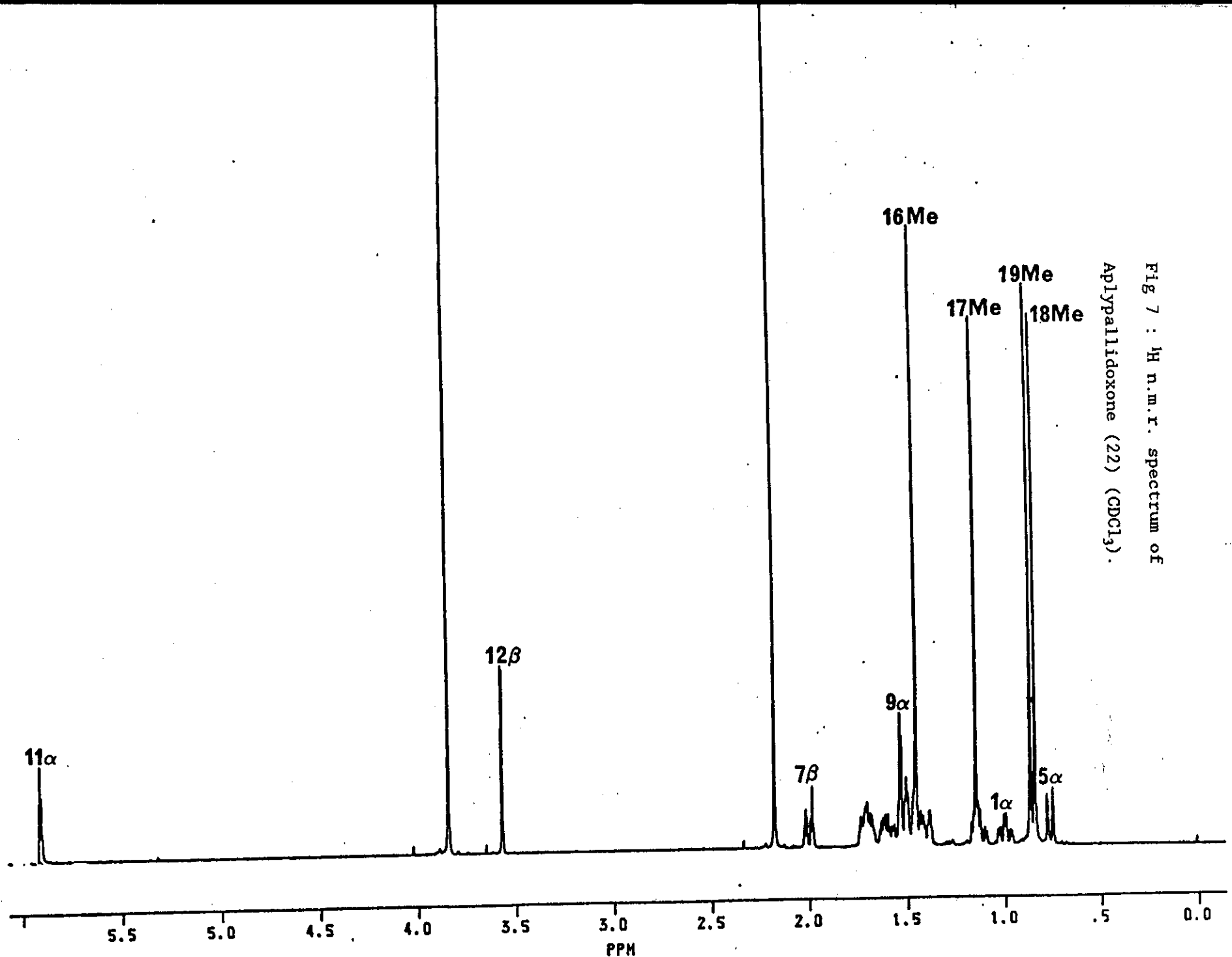


Fig 7 : ^1H n.m.r. spectrum of Aplypallidoxone (22) ($CDCl_3$).

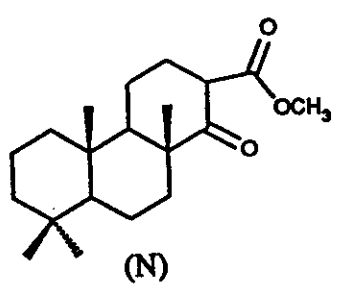
(C) Aplypallidione

Aplypallidione (23), a very minor metabolite, was isolated as a white crystalline neutral compound. High resolution mass matching of the molecular ion peak *m/z* 348, indicated a molecular formula $C_{20}H_{28}O_5$. This was further substantiated by the ^{13}C n.m.r. and ^{1}H n.m.r. spectra which showed 20 carbons and 28 protons respectively.

The ^{1}H n.m.r. spectrum of aplypallidione (Fig 8) showed many similarities with those of aplypallidenone (21) and aplypallidoxone (22) including the characteristic signals associated with the A and B rings [H5α (δ 0.71), H1α (0.78), H3α (δ 1.13) and H7β (δ 1.96) the chemical shift of which indicated the presence of the C14 ketone] and the methyl ester group (a three proton singlet at δ 3.84).

However the signal associated with the acetate group in (21) and (22) was absent in aplypallidione.

The ^{1}H n.m.r. spectrum also showed signals for four quaternary methyl groups, two singlets at δ 0.83 and 0.86 and two fine doublets at δ 1.23 (*J* 0.8 Hz) and 1.26 (*J* 0.9 Hz). Hence partial structure (N) was indicated.



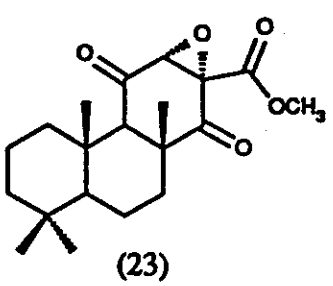
(N)

The i.r. spectrum showed two carbonyl absorption bands at 1758 and 1720 cm^{-1} which, together with three carbonyl signals in the ^{13}C n.m.r. spectrum at δ 164.3, 199.4 and 202.3, indicated the presence of three carbonyl functions: an ester (methyl ester) and two ketones respectively.

Two of these carbonyls have already been established as the methyl ester and C14 ketone in partial structure (N). The absence of the acetate and the presence of a third new ketone carbonyl suggested the acetate was replaced by a ketone function. This was further substantiated by two singlets in the ^{1}H n.m.r. spectrum, one at δ 2.70 assigned to H9α and consistent with a methine α to a carbonyl. The other at δ 3.59 was assigned to H12 and was consistent with a proton α to both an oxygen and carbonyl function. Neither H9α or H12 showed further coupling to any other protons which confirmed the ketone on C11.

The downfield chemical shift of H1β (δ 2.09) and 17 Me (δ 1.23) added further support to the C11 ketone, the downfield shift being due to the anisotropic deshielding by the C11 ketone group.

Hence four of the five oxygens were assigned to the methyl ester and the two ketones. The remaining oxygen, already shown to be attached to C12, was therefore assigned to an oxirane ring reminiscent of aplypallidoxone (22). The relative stereochemistry of the oxirane oxygen into the α-face is based on that of aplypallidoxone which was shown to be α-disposed by X-ray crystallography. Hence the final structure of aplypallidione was settled as (23).



(23)

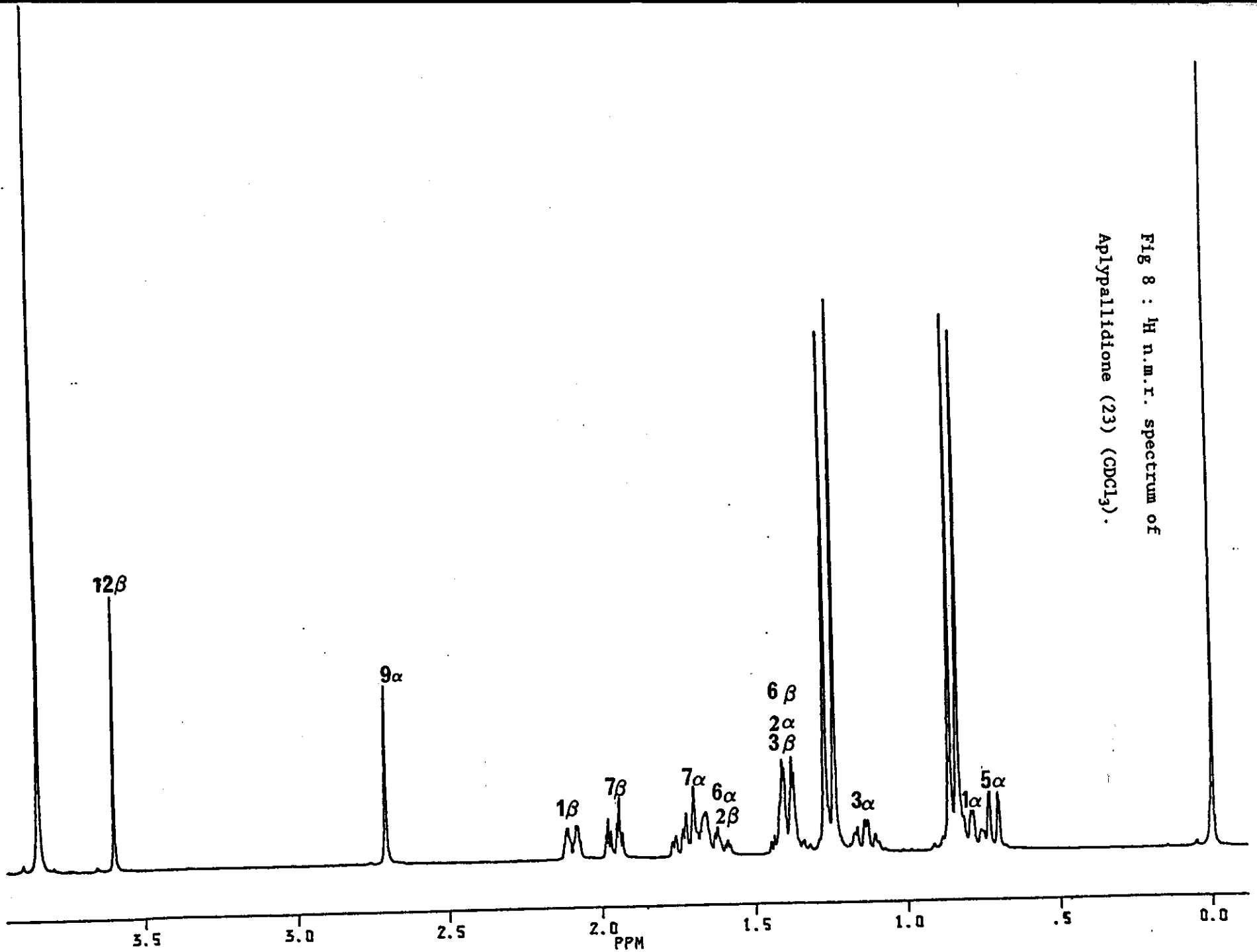


Fig 8 : ^1H n.m.r. spectrum of Aplypallidione (23) ($CDCl_3$).

(D) Aplypallidioxone

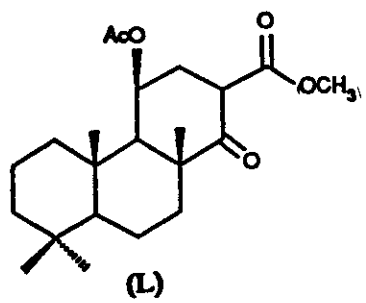
Aplypallidioxone (24), the second minor metabolite, was isolated as a white crystalline neutral compound. The e.i.m.s. gave only a very weak molecular ion peak at *m/z* 406 (<1%). The first major fragment ions were at *m/z* 364, 346 and 336 which correspond to the loss, from the molecular ion, of ketene (42 daltons), acetic acid (60 daltons) and the initial loss of ketene followed by carbon monoxide (42 + 28 daltons) respectively. The molecular formula, $C_{22}H_{30}O_7$, was confirmed by high resolution mass matching of the *m/z* 364 ($C_{20}H_{28}O_6$), 346 ($C_{20}H_{26}O_5$) and 336 ($C_{19}H_{28}O_5$) peaks. In addition the fully decoupled ^{13}C n.m.r. spectrum showed 22 signals (six methyls, five methylenes, two methines and nine quaternary signals, by DEPT) and the ^1H n.m.r. spectrum (Fig. 9) indicated the presence of 30 protons. From the molecular formula eight units of unsaturation were indicated.

The i.r. spectrum showed two carbonyl absorptions at 1744 (broad) and 1693 cm^{-1}, together with three signals in the carbonyl region of the ^{13}C n.m.r. spectrum at δ 163.9, 166.2 and 201.6 indicated the presence of two esters and a ketone respectively. The u.v. spectrum was featureless above 200 nm. In the ^1H n.m.r. spectrum two, three- proton singlets at δ 2.25 and 3.88 substantiated the ester functions as an acetate (δ_c 166.2) and methyl ester (δ_c 163.9) respectively.

^1H n.m.r. spectrum also showed four quarternary methyl groups; two singlets at δ 0.86 (18Me) and δ 0.89 (19Me), and two fine doublets at δ 1.28 (17Me; *J* 0.8 Hz) and δ 1.37 (16Me; *J* 0.8 Hz) and hence by analogy with the previously isolated metabolites, (21)-(23), aplypallidioxone contained partial structure (L)

The absence of a signal associated with a proton α to an acetate [δ 5.83 in (21) and 5.89 in (22)] and with the presence of two olefinic

signals in the ^{13}C n.m.r. spectrum, a quaternary carbon at δ 156.2 (C11) and a methine carbon at δ 110.4 (C12), which could be associated with a sharp singlet in the ^{1}H n.m.r. spectrum at δ 6.64 (H12), indicated the acetate was part of an enol acetate system, the olefinic group being situated between C11 and C12.



(L)

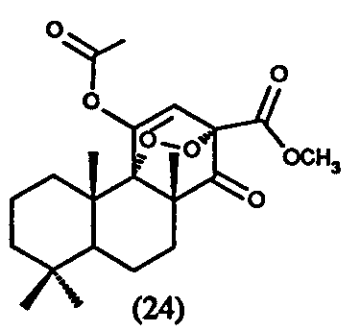
Partial structure (L) together with the olefinic functions accounted for five of the seven oxygens and seven of the eight units of unsaturation. Since only one methine remained to be assigned, and as there were no allylic one-proton singlets in the ^{1}H n.m.r. spectrum indicating H9α, the remaining two oxygens were therefore present as a peroxide linkage between C9 and C13: the remaining methine being assigned to C5. The peroxide function also accounted for the final unit of unsaturation.

The 2D-COSY spectrum (Fig 10) combined with n.O.e. experiments (Table 4) allowed the chemical shifts of all protons to be determined as well as confirming the proposed structure and relative stereochemistry.

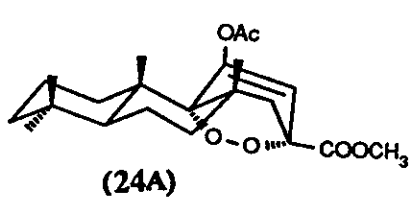
In the 1D ^{1}H n.m.r. spectrum (Fig 9), three of the eleven aliphatic ring protons (A and B ring protons) resonated as isolated systems. Two of these resonances at δ 1.22 (ddd, J 13.4, 13.4, 4.2 Hz) and δ 1.36 (dddd, J 13.4, 3.4, 3.4, 1.6 Hz) were assigned to H3α and H3β respectively (based on their multiplicities and magnitude of couplings). The assignments were

further confirmed by the 2D-COSY spectrum, which showed these two to be geminally coupled, and from n.O.e. experiments in which an enhancement was observed in H3α upon irradiation of 19Me (δ 0.89). The 2D COSY spectrum showed clear couplings to H2α (δ 1.47), $H_{2\beta}$ (δ 1.67) as well as a long range 'W' coupling from H3β to H1β (δ 1.72). Further examination of couplings from H1β, H2β and H2α then revealed H1α at δ 1.83.

Also showing n.O.e. enhancements upon irradiation of 19Me were two further resonances, a broad doublet at δ 1.70 (J 12.6 Hz) and a doublet of doublets at δ 1.78 (J 12.8, 2.6 Hz). These were shown, by the 2D-COSY spectrum, to be vicinally coupled and hence were assigned to H6α (δ 1.70) and H5α (δ 1.78). The 2D-COSY spectrum also showed clear couplings to H6β (δ 1.52) and H7β (δ 1.81) both of which showed further mutual coupling to the remaining isolated system at δ 1.95 (H7α). This therefore also confirmed the oxidation occuring on the C9 carbon. Additionally, the chemical shifts of the three axially disposed protons, H1α (δ 1.83), H5α (δ 1.78) and H7α (δ 1.95) confirmed the peroxide function was also α-disposed. From the molecular model of aplypallidioxone (24) the C ring, due to the α-peroxide between C9 and C13 would be predicted to exist in a boat conformation as shown in structure (24A).



(24)



(24A)

Fig 9 : ^1H n.m.r. spectrum of Aplypallidioxone (24) ($CDCl_3$).

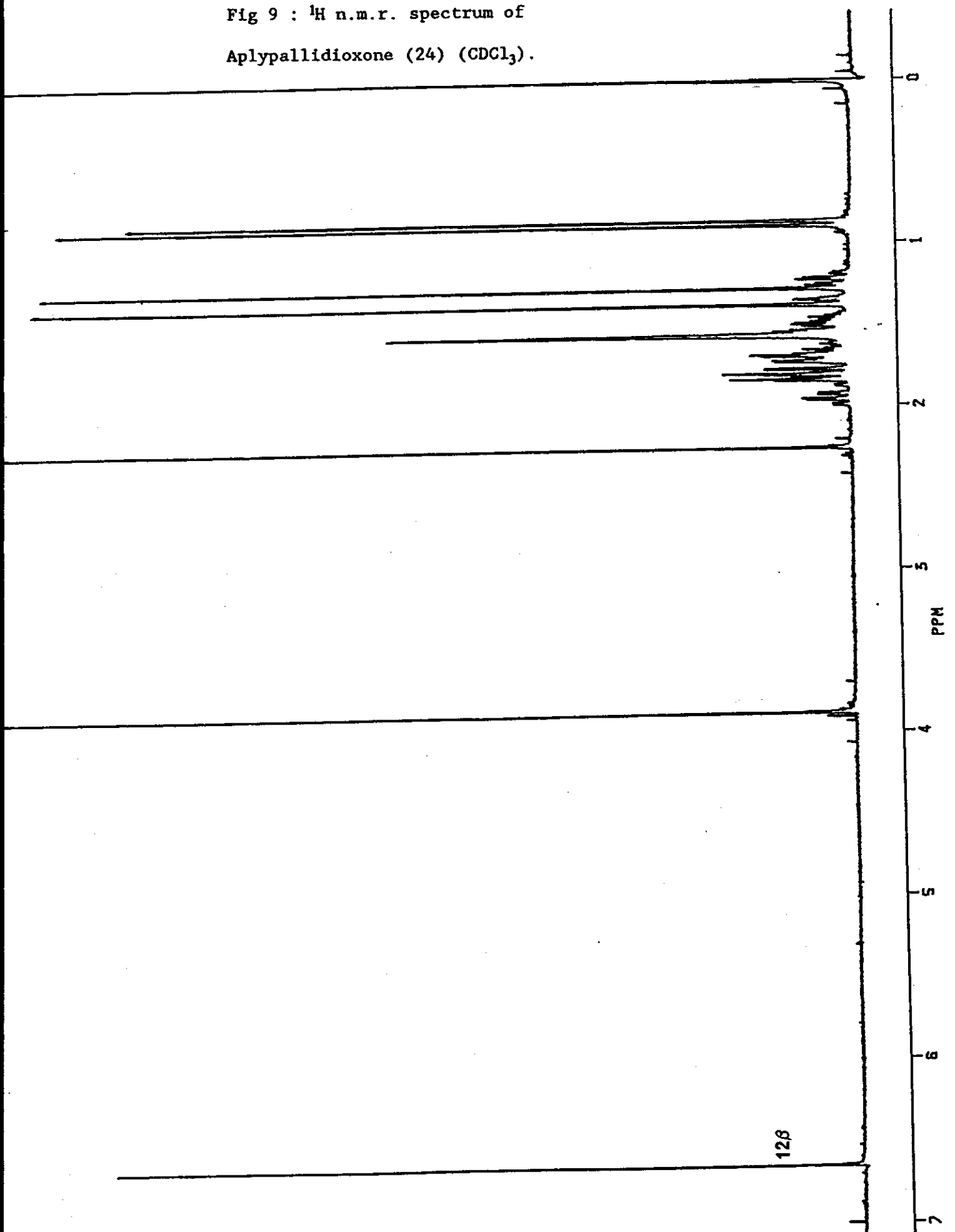


Fig 10 : 2D-COSY spectrum of

Aplypallidioxone (24) ($CDCl_3$).

Expansion of upfield portion.

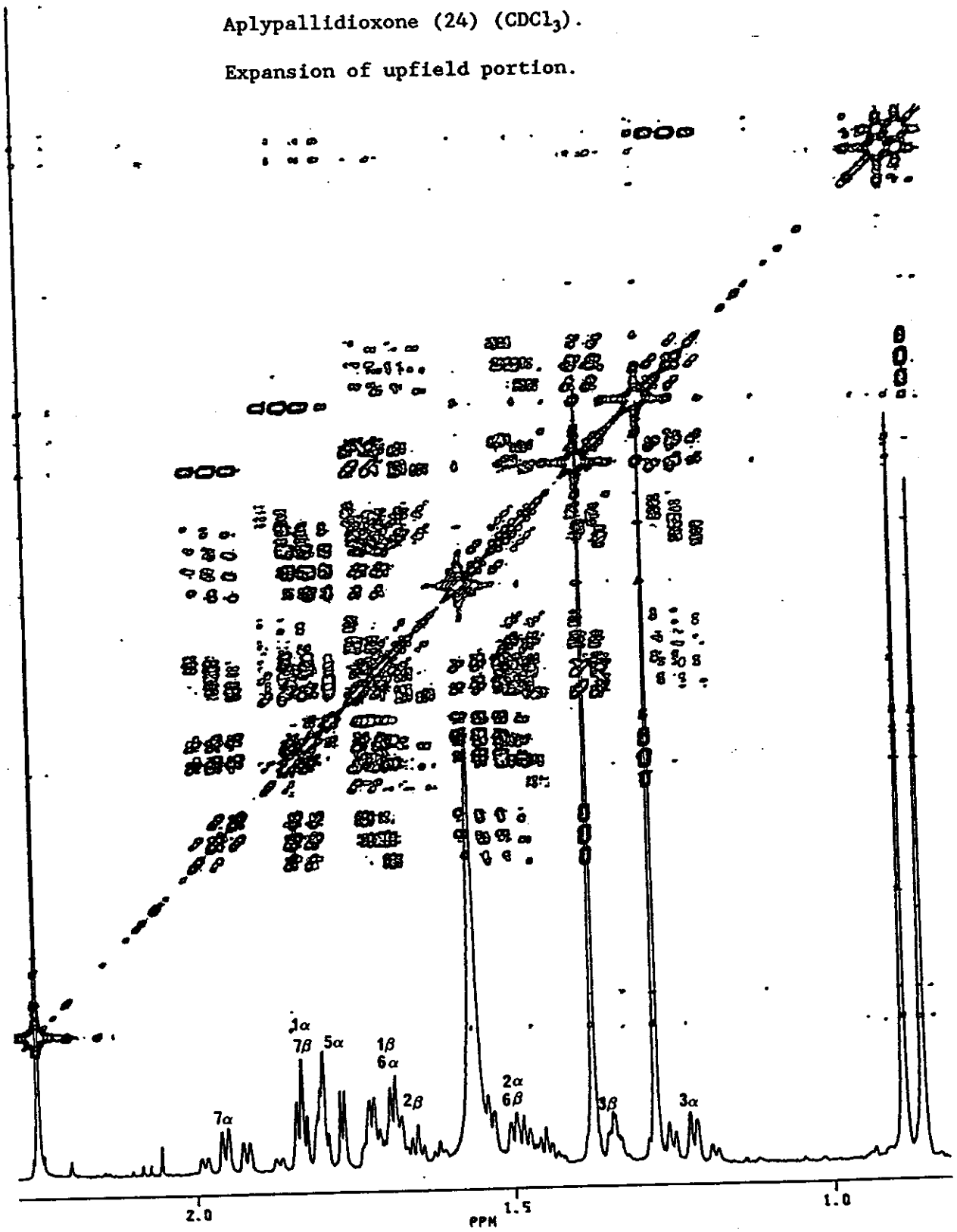
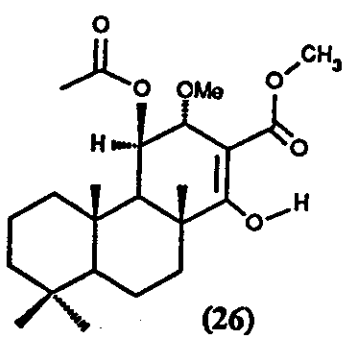


Table 4 ^1H-^1H nuclear Overhauser enhancements in aplypallidioxone (24)

^1H irradiated	Signals enhanced (%)
18Me (δ 0.86)	17Me(2.0), H3β(0.6)
19Me (δ 0.89)	H3α(1.4), H6α(2.1), H5α(1.8)

(E) Chemical Reactions: When the u.v. spectrum of aplypallidenone (21) was recorded in ethanol on two separate occasions, it was noticed that the two absorptions obtained at λ_{max} 217.6 and 248.8 nm were at different intensities hence indicating a chemical reaction was occuring. To investigate this reactivity aplypallidenone (21) was stirred with methanol for 24 h which, upon removal of the solvent, yielded two products, a minor product identified as the starting material aplypallidenone (t.l.c. and ^1H n.m.r. spectrum), and a major product identified as the enol (26) by ^1H n.m.r. spectroscopy. Attempts to purify the product by chromatography using flash silica gel however failed, with the starting material aplypallidenone being recovered as the only major compound. The enol when left standing, slowly reverted to aplypallidenone hence showing the reversibility of the reaction.



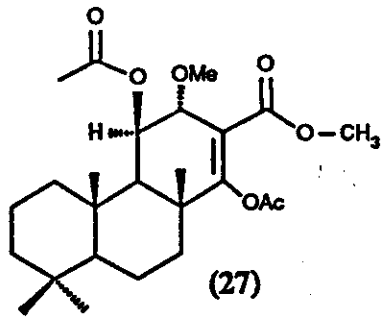
(26)

In the ^1H n.m.r. spectrum the enol proton resonated as a sharp singlet at δ 12.8 due to intramolecular hydrogen bonding with the C15 carbonyl. H12, originally resonating at δ 7.24 in aplypallidenone (21), appeared in (26) as a fine doublet (J 2.4 Hz) at δ 3.82 indicating it to be α to the methoxide function observed as a three proton singlet at δ 3.49. Otherwise the remainder of the ^1H n.m.r. spectrum was similar to that of aplypallidenone with four methyl signals [δ 0.83 (18Me), 0.85 (19Me), 1.03 (17Me) and 1.51 (16Me) the assignments confirmed by n.O.e. experiments [Table (5)], an acetate signal at δ 2.05 and a doublet of doublets (J 2.4, 2.2 Hz) at δ 5.51 (H11α).

Decoupling experiments in the ^1H n.m.r. spectrum confirmed the proton resonating at δ 3.82 (J 2.4 Hz) as H12, and revealed H9α resonating at δ 1.63 (J 2.2 Hz) by irradiation of H11α at δ 5.51.

The stereochemistry depicted at C12, with the methoxide in the α-face, was based on chemical arguments in which methanol attacks in a Michael fashion from the less sterically hindered α-face augmented by a very small (0.4%) n.O.e. measurement in H9α on irradiation of the methoxide groups.

To stabilize the enol and prevent it from reverting to aplypallidenone, which therefore would also allow it to be purified by chromatography, the enol-acetate (27) was prepared by stirring the enol (26) in acetic anhydride/pyridine (1:1) for 24 h. This afforded the enol-acetate in low yield with the major product being in fact aplypallidenone. However by returning the mixture to methanol for 24 h, removing the solvent and again stirring in acetic anhydride/pyridine for 24 h and repeating this cycle several times the yield of the enol-acetate (27) was increased. It was then purified by column chromatography using flash silica gel.



(27)

(27)

The enol acetate (27) was isolated as a colourless, sticky gum. The highest peaks in the e.i.m.s. at *m/z* 349 and *m/z* 317 were confirmed by high resolution mass matching to correspond to molecular formulas $C_{21}H_{33}O_4$ [$M^+ - C_2H_2O - COOCH_3$] and $C_{20}H_{29}O_3$ [$M^+ - 2 \times C_2H_2O - COOCH_3$] respectively. The i.r. spectrum showed two carbonyl absorption bands at 1768 and 1730 cm^{-1} and an olefinic absorption band at 1642 cm^{-1} which together with five quaternary signals in the ^{13}C n.m.r. spectrum at δ 113.6, 164.5, 165.7, 168.0 and 170.2 substantiated the presence of three esters and the olefinic group of an enol-ester function. In the ^1H n.m.r. spectrum four downfield three-proton singlets at δ 2.07, 2.22, 3.50 and 3.72 confirmed the ester functions as two acetate groups (δ 2.07 and 2.22), a methyl ester (δ 3.72) and the remaining methyl signal substantiated the presence of the C12 methoxyl group.

Also evident in the ^1H n.m.r. spectrum were four aliphatic methyl groups (δ 0.83, 0.84, 1.03 and 1.38) and H11α resonating as a doublet of doublets at δ 5.54 (J 2.0, 1.8 Hz) which was found by decoupling experiments in the ^1H n.m.r. spectrum to be mutually coupled to H12β at δ 4.03 (J 2.0 Hz) and H9α at 1.63 (J 1.8 Hz).

Fig 11 : ^1H n.m.r. spectrum of Enol acetate (26) ($CDCl_3$).

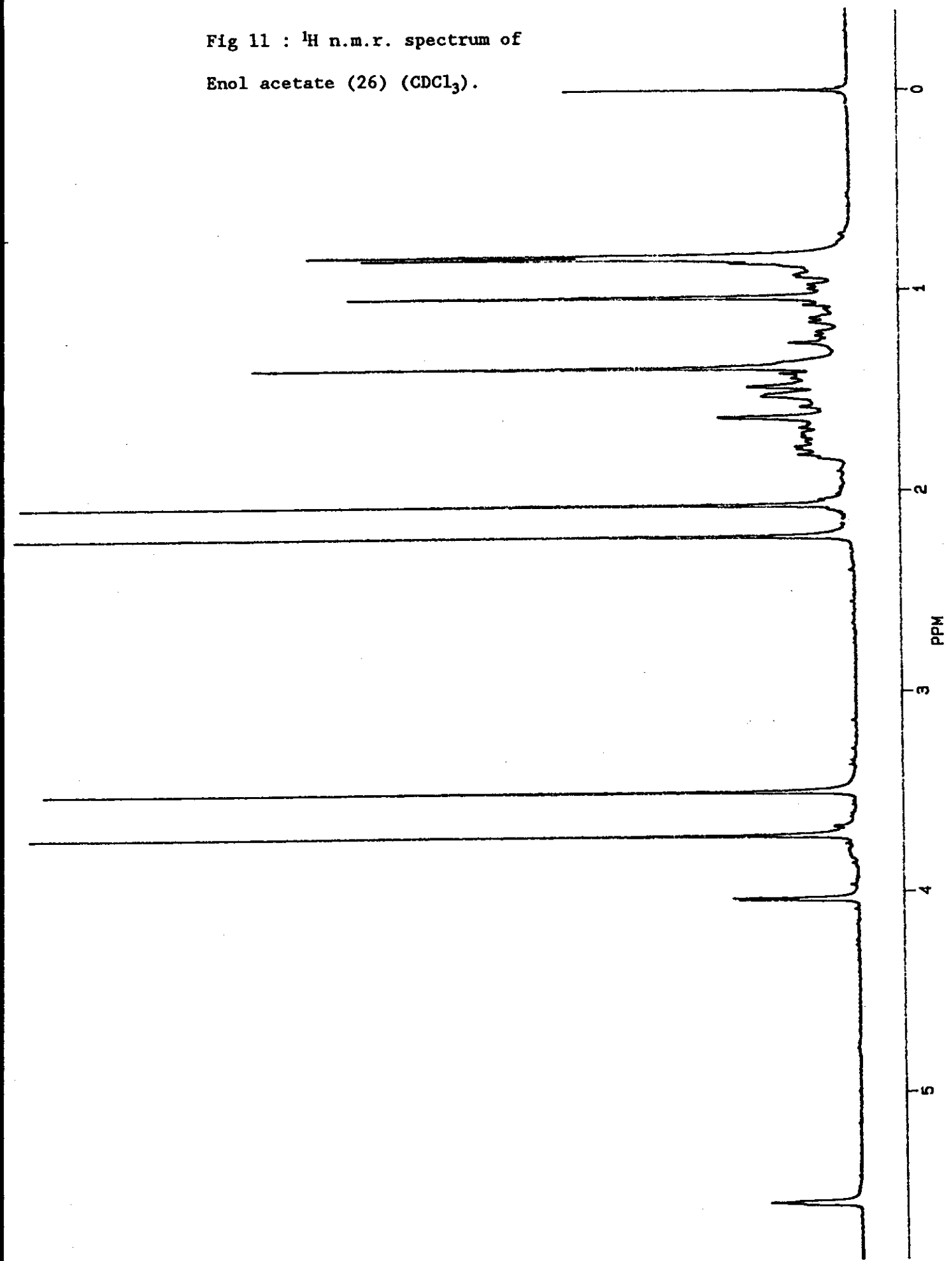


Table 5. $^{1}H-^{1}H$ nuclear Overhauser enhancements in (26)

^{1}H irradiated	Signal enhanced (%)
16Me	17Me(3.1)
17Me	18Me(3.2), 16Me(3.6)
18Me	17Me(3.3), H3β(1.8)
19Me	H3α(1.8), H3β(1.4)
OCH_3	H9α(0.4), H11α(2.1), H12β(4.1)
H11α	H9α(4.8), H1β(9.4), OCH_3(6.0), H12β(7.8)
H12β	OCH_3(7.5), H11α(4.6).

(F) Biological Activity: The antibacterial antileukemic and antiviral activity of aplypallidenone (21) and aplypallidoxone (22).

The hypothesis as to the role of the secondary metabolites in sponges, and for that matter many marine organisms, is that they are present as a chemical weapon which defends the organism from potential predators.

From previous work completed in our department on the relationship between sponges of the Aplysillidae family and the nudibranchs which feed on them, Karuso[31] found that *Chromodoris tasmaniensis* which feeds on the sponge *Aplysilla rosea* concentrated certain metabolites from the sponge by up to ten times while Poiner[21] found that *C. thompsoni* concentrated the two major diterpene metabolites in *Chelanaplysilla violacea* by up to three times.

To test the antifeedant properties of the two most concentrated

metabolites isolated from *A. rosea* [aplyroseal-1 (30) and -6 (34)] and from *C. violacea* [aplyviolene (100) and aplyviolacene (101)],[22] Poiner conducted some fish feeding experiments in which fish pellets were impregnated with the metabolites and then fed to the fish.[21]

The results of all four metabolites tested showed positive antifeedant properties at both sponge and nudibranch concentrations. The aplyroseal compounds also showed toxic effects at both concentrations with some fish dying within three hours of eating the impregnated pellets. Therefore these results lend support to the hypothesis mentioned above.

A potential use of natural defence chemicals, because of their inherent biological activity, are as pharmaceuticals. In light of the role of the diterpenoids in the Aplysillidae sponges as a chemical defence system and hence their potential as pharmaceuticals the two most concentrated diterpenoids from *A. pallida*, aplypallidenone (21) and aplypallidoxone (22), were tested for antibacterial, antiviral cytotoxic and antileucemic activity and the results shown in Table (6). The antiviral, cytotoxic, and antileucemic tests for aplypallidenone however must be viewed with some caution.

In the initial testing of the metabolites, using the paper disc method, the compounds were dissolved and applied to the paper discs with methanol. It was subsequently found that aplypallidenone reacts with methanol (see Section 1.2.1E) to give the Michael adduct (26), as the enol, and hence depending on how long aplypallidenone was left in methanol would determine the ratio of enol (26) to aplypallidenone (21).

A further point to be noted however, is that the reaction is relatively slow and reversible and under the conditions of the test the enol (26) may revert back to aplypallidenone giving a correct result for the natural product (21). This point gained favour when in a subsequent antibacterial test of aplypallidenone, the compound was applied to the

paper discs dissolved in dichloromethane and this was compared with aplypallidenone which had been initially left in methanol for 48 h. The results, shown in Table (7), showed there was only a slight reduction in the activity of the enol sample compared with that of aplypallidenone. This could indicate one of two situations, either the enol is just as active as the natural product or is reverting back to the natural product under the conditions of the test. Hence the enol acetate (27), prepared by acetylation of the enol with acetic anhydride/pyridine would be an interesting compound to test for activity.

Table 6.: Biological Activity of aplypallidenone (21) and aplypallidoxone (22).

Compound	Antiviral	Cytotoxic (ng/ml)	P388 (ng/ml)	Antibacterial[a] (mm)[b]			
				Sa	Bs	Ec	Ps
aplypallidenone	X	10	90	19	23	21	X
aplypallidoxone	X	X	X	X	X	X	X

a paper disk method aplypallidenone 750 μg/disc
aplypallidoxone 1000 μg/disc

b zone of inhibition in mm.

Sa *Staphylacaccus aureus* gram (+)

Bs *Bacillus subtillis* gram (+)

Ec *Escherichia coli* gram (-)

Ps *Pseudomonae auginosa* gram (-)

X Denotes no activity observed

Table 7.: Biological Activity of aplypallidenone (21) and enol (26).

Compound	Solvent[a]	Conc.[b]	Sa	Bs
aplypallidenone	MeOH	750	19	22
aplypallidoxone	CH_2Cl_2	750	16	22
enol (26)	CH_2Cl_2	750	13	19

[a] Solvent used to apply compound to disc

[b] µg/disc

As can be seen in Table 6 the metabolite aplypallidoxone (22) is completely void of any activity in the tests carried out. This, together with the high reactivity of aplypallidenone towards alcohols, giving the 1,4-Michael adduct, suggests the mode of action within this defense system is as a Michael acceptor.

Michael acceptors as chemical defence weapons are not uncommon in nature having been found for example in fungi, higher plants and, more interestingly, in the defence secretion of certain species of termites.

In a study by Quennedey *et al.*[32] on the defence secretion from termites of the genus *Schedorhinotermes*, which contain as the active compounds long chain C12, C14 and C16-1-alken-3-ones, it was found that a topical application or exposure of its vapour to ants led to toxic effects in the ants. Prestwich[33] therefore speculated, since no wound was necessary to bring on the toxic effect, that the mechanism of action is one in which the long aliphatic side chain of the enone facilitates penetration through the lipophilic ant cuticle, and then a subsequent Michael type addition of a biological sulfhydryl group to the vinyl ketone could result

in the observed toxicity. Preswich also found that the soldier and worker termites were unaffected by their own poisons due to a detoxification in which the α,β-unsaturated ketone is reduced to the ketone.[33]

Therefore, by analogy with the termite defence secretion the aliphatic A and B rings of aplypallidenone could assist in entering the predator's circulatory system, possibly via the lipophilic system. Then a Michael type addition to the C ring, possibly by sulfhydryl groups as speculated by Prestwich in the ants, could lead to a toxic effect in the predator. It also remains possible that aplypallidoxone (22), due to its complete inactivity to the tests conducted may be a method of detoxification in the sponge, and furthermore, may be present in the sponge as a method of storage of a patent cytotoxic compound which is then converted to active aplypallidenone when necessary. A further possibility of a symbiotic organism in the sponge producing the active compounds which are then detoxified by the sponge was discounted since the sponges in the Aplysillidae family are not known to carry symbionts.[31]

1.2.2 *Aplysilla rosea*

Aplysilla rosea Barrois (Plate 5), a pink-red encrusting sponge of the Dendroceratida order of sponges was collected and the secondary metabolites extracted. All collections of sponge material for a large scale extraction were conducted off the northern headlands of Bondi Beach between the months of Apr - Jun, 1984 (water temp 14 - 20°).

Large scale extraction of the freeze dried sponge followed by exhaustive gravity column and high performance liquid chromatography (h.p.l.c.) yielded 22 diterpenes (Table 8): nine diterpenes previously isolated from *A. rosea* (28) - (36),[19] two isolated from the New Zealand sponge, *Dendrilla rosea* (37) - (38),[26] and eleven new diterpenes (39)-(49).

The known diterpenes (28) - (36), originally isolated in our department, were identified by direct comparison of the ^{1}H n.m.r. and mass spectra with those of the authentic samples. The structures of dendrillol-1(37) and -2(38) were established by comparison of spectral data recorded in the literature.[26] All new diterpenes (39) - (49) were identified by extensive spectroscopic investigations.

The absolute stereochemistry of the p-bromobenzoate derivative (51) of aplyroseol-1 (30) was determined by X-ray crystallography (Appendix 1), from which it was inferred that all the aplyroseol metabolites have the same absolute stereochemistry.

The following notes apply to Table 8:

[1] % diterpene concentration of dry weight sponge material.

A % concentration from *A. rosea* isolated by the auther.

B % concentration from *A. rosea* isolated by Karuso.[19]

C % concentration from *D. rosea* isolated by Karuso.[26]

Plate 5. : An underwater photograph of *Aplysilla rosea*. The nudibranchs feeding on the sponge are *Chromodoris tasmaniensis*.

Table 8. Diterpene concentrations from *A. rosea*.

Compound	Structure	% dry weight[1]		
		A	B	C
Ambliofuran	28	0.23	0.22	—
Hexa-H-furan	29	0.0017	0.01	—
Aplyroseol-1	30	0.14	0.11	0.026
Aplyroseol-2	74	—	0.003	0.003
Aplyroseol-3	31	0.019	0.08	0.009
Aplyroseol-4	32	0.060	0.08	—
Aplyroseol-5	33	0.12	0.09	0.018
Aplyroseol-6	34	0.069	0.11	0.005
Dendrillol-1	37	0.00093	—	0.048
Dendrillol-2	38	0.0052	—	0.002
Dendrillol-3	76	—	—	0.002
Dendrillol-4	77	—	—	0.004
Lactone A	35	0.022	0.001	0.001
Lactone B	36	0.056	0.030	—
AROSST-1	39	0.0045	—	—
AROSST-2	40	0.0017	—	—
AROSST-3	41	0.0083	—	—
AROSST-4	42	0.0036	—	—
AROSST-5	43	0.0093	—	—
AROSST-6A	44A	0.0033	—	—
AROSST-7	45	0.0021	—	—
AROSST-8	46	0.0018	—	—
AROSST-9	47	0.00019	—	—
AROSST-10	48	0.00080	—	—
AROSST-11	49	0.00076	—	—

(A) AROSST-1 (39)

AROSST-1(39) was isolated as a colourless, neutral gum. High resolution mass matching of the molecular ion, *m/z* 434, established a molecular formula $C_{25}H_{38}O_6$. This was further substantiated by the ^{13}C n.m.r. and ^1H n.m.r. spectra which showed signals for twenty five carbons and thirty eight protons respectively. From the molecular formula there are seven units of unsaturation.

In the i.r. spectrum two strong carbonyl absorptions at 1774 and 1735 cm^{-1} together with three carbonyl signals in the ^{13}C n.m.r. spectrum at δ 172.4, 172.8 and 176.0 indicated the presence of three ester carbonyls.

A three proton triplet at δ 1.00 (*J* 7.5 Hz) coupled to a two proton multiplet at δ 1.71 which in turn was coupled to another two proton multiplet at δ 2.36 in the ^1H n.m.r. spectrum (Fig 12) substantiated the presence of a butyrate ester. The e.i.m.s. also showed the prominent loss of butyric acid (88 daltons) from the molecular ion giving *m/z* 346. This was confirmed by high resolution mass matching which gave a molecular formula $C_{21}H_{30}O_4$ $[M^+ - CH_3CH_2CH_2COOH]$.

The methyl group of a methyl ester was evident from a three proton singlet at δ 3.64 in the ^1H n.m.r. spectrum and from the occurrence of a fragment peak at *m/z* 314 in the mass spectrum corresponding to the loss of CH_3OH $[M^+ - CH_3CH_2CH_2COOH - CH_3OH]$.

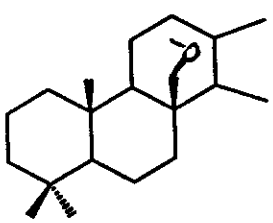
The final carbonyl was assigned to a γ-lactone. This was suggested by both the carbonyl absorption at 1774 cm^{-1} and the mass spectrum which showed a strong fragment peak *m/z* 288 corresponding to the $[M^+ - CH_3CH_2CH_2COOH - CO - CH_2O]$ ion.

The methyl ester, butyrate and γ-lactone accounted for all six oxygens and three units of unsaturation. As no further unsaturation was indicated

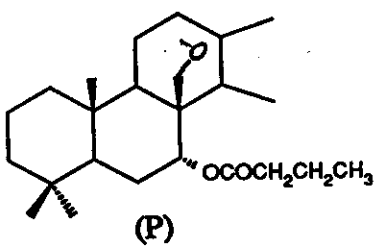
(^{1}H n.m.r., ^{13}C n.m.r. and U.V. spectra) the parent hydrocarbon was tetracyclic.

The ^{1}H n.m.r. spectrum also showed three quaternary methyl signals (δ 0.77, 0.78 and 0.80), and two doublet of doublet of doublets at 0.94 (J 13, 13, 3.8 Hz) and 1.17 (J 13.8, 13.8, 4.5 Hz), reminiscent of H1α and H3α respectively in previous aplyroseols.

This indicates a diterpene structure in which, as in aplyroseols (30)-(34), the fourth methyl group had been oxidised. Hence partial structure (O) was suggested.



(O)

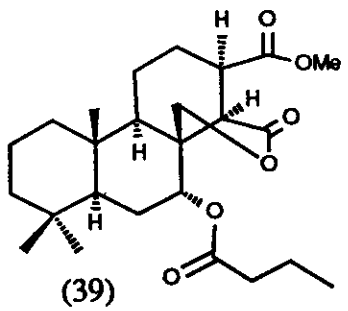


(P)

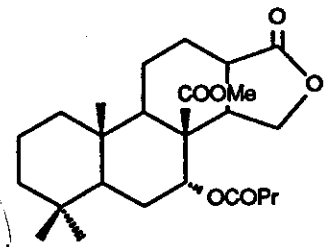
The methine proton α to the butyroxy group (δ_H 4.86, δ_C 74.0) was clearly visible in the ^{1}H n.m.r. spectrum as a fine doublet of doublets (J 2.8 2.6 Hz). This is consistent with a proton both equatorial and β to a methylene. Decoupling experiments in the ^{1}H n.m.r. spectrum showed the proton α to the butyroxy group (δ 4.86) to be coupled, as expected, to two methylene protons. These in turn were coupled to a single proton resonating at δ 1.30, evident as a doublet of doublets: one axial-axial (J 13.2 Hz) coupling and one axial equatorial (J 2.4 Hz) coupling. The data presented above are reminiscent of the β ring in aplyroseol-1(30) and hence partial structure (P) may now be envisaged.

From the evidence presented above thus far three structures (39), (57)

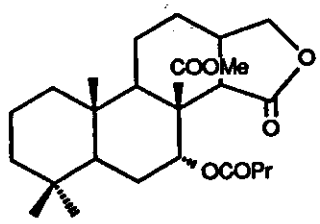
and (58) were suggested.



(39)



(57)



(58)

Two resonances in the ^1H n.m.r. spectrum at δ 2.84 and 2.96, the chemical shifts of which are consistent with methines α to carbonyls, suggested the structure of AROSST-1 was (39). This was further substantiated by two doublets in the ^1H n.m.r. spectrum at δ 4.02 (J 10.0 Hz) and 4.36 (J 10.0 Hz) which were coupled together. These resonances could be associated with a methylene carbon signal in the ^{13}C n.m.r. spectrum at δ 74.0, and are consistent with a pair of geminal protons attached to a carbon (C17) bearing one oxygen. As there were no futher couplings to either protons this confirms the structure (39) assigned to AROSST1.

Further analysis of the two α-carbonyl methines resonances, H13 (δ 2.84) and H14 (2.96), revealed that the C ring in AROSST-1 must reside in a boat conformation rather than the usual chair conformation. This was evident from decoupling experiments.

Decoupling experiments in the ^1H n.m.r. spectrum showed the proton H13, evident as a doublet of doublet of doublets (J 11.4, 6.7, 4.1 Hz), was coupled to three protons; H14 (J 6.7 Hz) and both H12β (J 11.4 Hz) and H12α (J 4.1 Hz). The large couplings exhibited by H13 indicated it must be in a pseudo-axial orientation: giving an axial-axial coupling (J 11.4Hz) to H12β and an axial-equatorial coupling (J 4.1Hz) to H12α.

In addition, H14, which has already been shown to be coupled to H13,

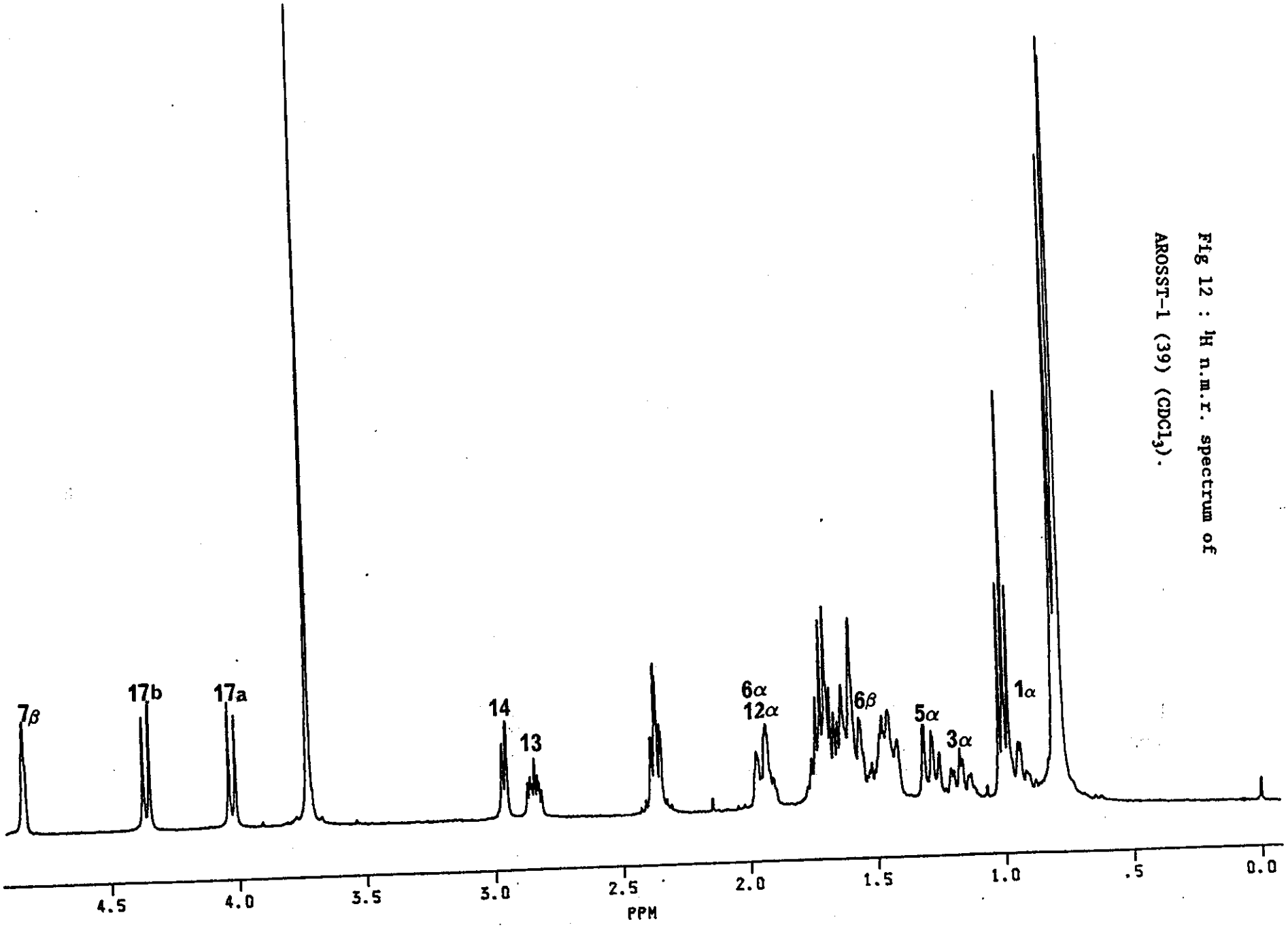


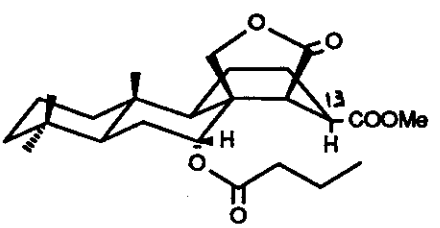
Fig 12 : ^1H n.m.r. spectrum of AROSST-1 (39) ($CDCl_3$).

also contained a small (*J* 1.5 Hz) long range coupling with H12α. This implies that these two protons, H14 and H12α, and the carbons between them adopt a 'W' conformation in one plane. Hence H14 must be equatorially disposed.

The above observation of large couplings in H13 and a 'W' coupling in H14 can only be accounted for if the C ring adopts a boat conformation.

Molecular model studies of AROSST-1 supported this proposal and provided a rationale for the boat conformation. The C ring, in the chair conformation, imposes a β-axial orientation on the C13 methyl ester group and also imposes a strained twist on the γ-lactone. However in the boat conformation the bulky methyl ester group becomes equatorial and the twist in the γ-lactone is relieved. These two effects therefore overcome the usual preference for the chair conformation of a cyclohexane ring system.

Thus the final structure of AROSST-1 was settled as (39), the conformation of which is shown in structure (39A).



(39A)

(B) AROSST-2 (40)

AROSST-2(40) was isolated as a colourless neutral gum. High resolution mass matching of the molecular ion, *m/z* 406, established a molecular formula $C_{25}H_{34}O_6$. This was further substantiated by the ^{13}C n.m.r. and ^{1}H n.m.r. spectra which showed signals for twenty three carbons

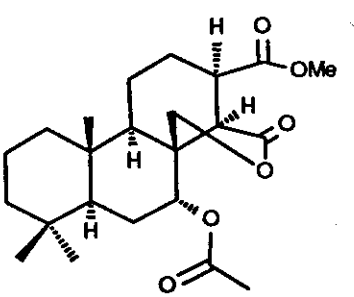
and thirty four protons respectively.

The ^{13}C n.m.r., ^{1}H n.m.r. and i.r. spectrum of AROSST12 were virtually identical to those of AROSST1.

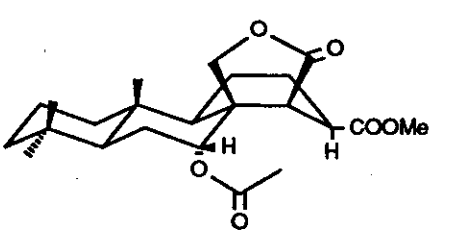
In the ^{1}H n.m.r. spectrum (Fig 13) all the charateristic signals of AROSST1 were present, with one notable difference: it lacked the signals associated with a butyrate ester. Instead these were replaced by a three proton singlet at δ 2.13 assigned to an acetate. The e.i.m.s. also showed a peak at *m/z* 346 due to the loss of a molecule of acetic acid (60 daltons) from the parent ion. This therefore suggested AROSST12 had structure (40).

Closer analysis of the protons H13 (δ 2.85) and H14 (δ 2.95) again showed H13 with large couplings (*J* 11.0, 6.8, 4.2 Hz) and H14 possessing a long range coupling (*J* 6.8, 1.6 Hz). Decoupling experiments, as in AROSST1, showed H13 coupled to H14, 12α and 12β. As well, H14 was shown to be coupled to H12α by a small (*J* 1.6 Hz) long range 'W' coupling.

Therefore, as in AROSST-1, the C ring of AROSST-2 resides in a boat conformation, relieving the stress on the γ-lactone and placing the methyl ester group in an equatorial orientation. N.O.e. experiments (Table 9) further supported this conformation with a n.O.e. being observed from H14 to H7β and *vice versa*.



(40)



(40A)

N.O.e. experiments also allowed the chemical shifts of the two geminal

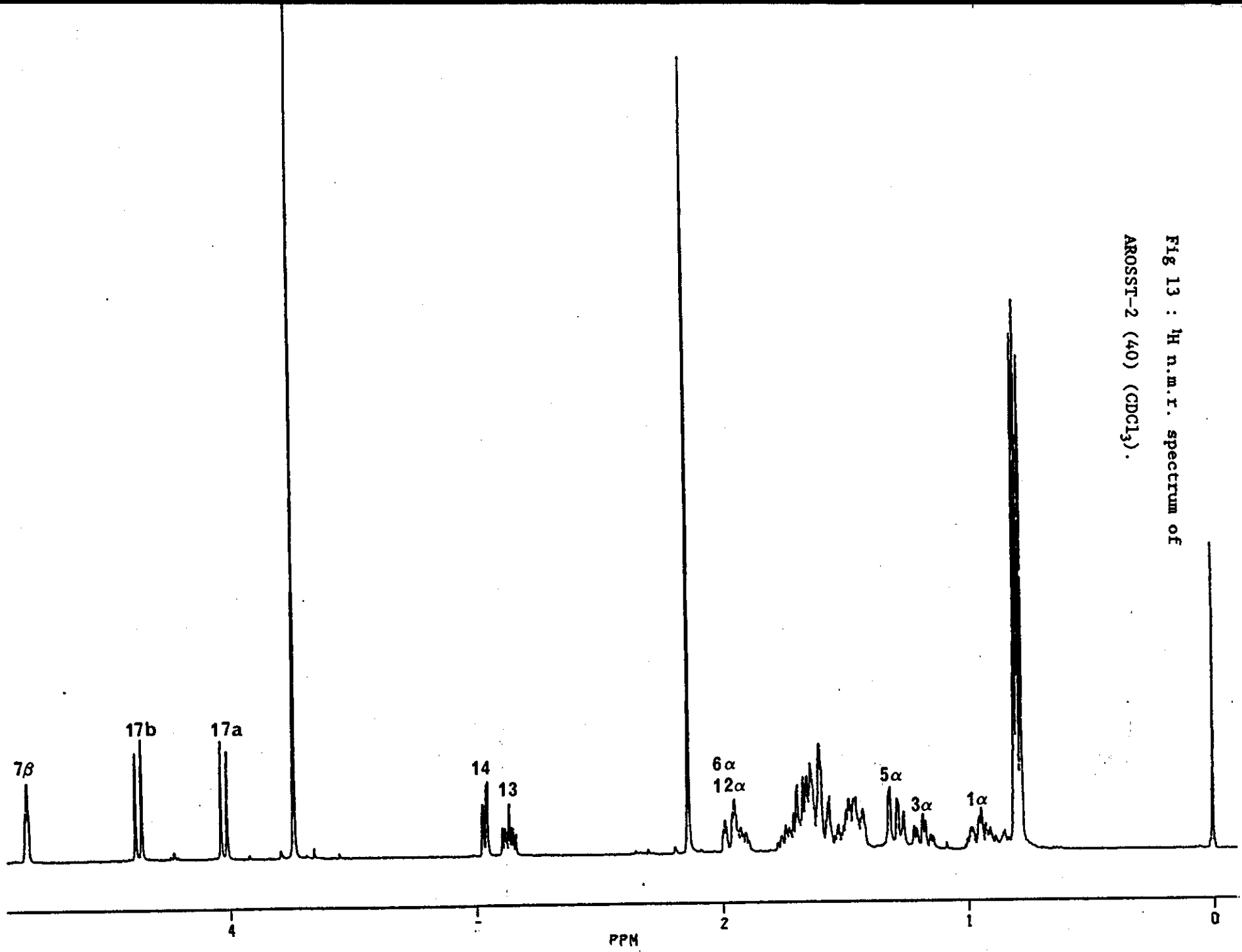


Fig 13 : ^1H n.m.r. spectrum of AROSST-2 (40) ($CDCl_3$).

protons, H17a (pointing away from the ring) and H17b (pointing into the ring) to be assigned; irradiation of H7β gave a n.O.e. to H17α (δ 4.01) and by inference H17β (δ4.36) was assigned.

In conclusion the final structure of AROSST12 was therefore settled as (40) the conformation of which is shown in structure (40A).

Table 9. ^{1}H-^{1}H nuclear Overhauser enhancements in AROSST-2 (40).

^{1}H irradiated	Signals enhanced (%)
H7β	H17a(3.7), H14(5.7).
H14	H7β(4.3).

(C) AROSST-3 (41)

AROSST-3 (41) was isolated as a white, crystalline, neutral solid. Mass spectroscopy established a molecular formula of $C_{25}H_{38}O_7$. The most prominent peak in the mass spectrum was *m/z* 362 corresponding to the molecular ion minus a molecule of butyric acid (88 daltons). The molecular formula was substantiated by both the fully decoupled ^{13}C n.m.r. spectrum and integration of the ^{1}H n.m.r. spectrum in which twenty five carbons and thirty eight protons respectively were shown. From the molecular formula there are seven units of unsaturation.

The i.r. spectrum showed two strong carbonyl absorptions at 1772 and 1734 cm^{-1}. Together with three carbonyl signals in the ^{13}C n.m.r. spectrum

(δ 172.6, 173.2, 176.6), indicated the presence of three ester functions.

In the ^1H n.m.r. spectrum (Fig. 14) a three proton triplet (*J* 7.4 Hz) at δ 0.97 coupled to a two proton multiplet at δ 1.68, which was further coupled to another two proton multiplet at δ 2.34, confirmed the presence of a butyrate ester already implied by the mass spectrum (*m/z* 362 [M^+ - $CH_3CH_2CH_2COOH$]).

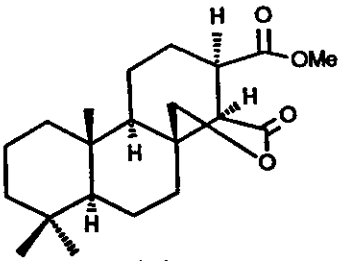
The methoxyl group of a methyl ester was also indicated by a three proton singlet at δ 3.73, and in the mass spectrum with a peak at *m/z* 330 corresponding to the loss of CH_3OH [M^+ - $CH_3CH_2CH_2COOH$ - CH_3OH].

A γ-lactone was also again suggested by the carbonyl absorption in the i.r. spectrum (1772 cm^{-1}) and the chemical shift of the lactone carbonyl carbon (δ 176.6).

A broad absorption in the i.r. spectrum (3531 cm^{-1}) and a broad singlet in the ^1H n.m.r. spectrum at δ 2.55, which was temperature dependent, indicated the presence of an alcohol group.

The methyl and butyrate esters, γ-lactone and alcohol accounted for all seven oxygens together with three units of unsaturation. Therefore the parent hydrocarbon was again tetracycylic.

Two protons at δ 2.86 (ddd, *J* 11.4, 7.0, 3.6 Hz) and 3.54 (dd, *J* 7.0, 1.5 Hz) which by decoupling experiments were shown to be coupled together (*J* 7.0 Hz) and also mutually coupled to H12α were reminiscent of H13 and H14 respectively in AROSST-1(39) and -2(40) indicating the C ring of AROSST-3 also existed in a boat conformation. N.O.e. experiments (Table 10) further substantiated this with a n.O.e. enhancement being observed in H7β upon irradiation of H14 and *vice versa*. This therefore, according to the evidence presented, indicated that partial structures (Q), (R) and (S) are present with the C ring in partial structure (Q) being in a boat conformation.



(Q)

$-OCOCH_2CH_2CH_3$

(R)

$-OH$

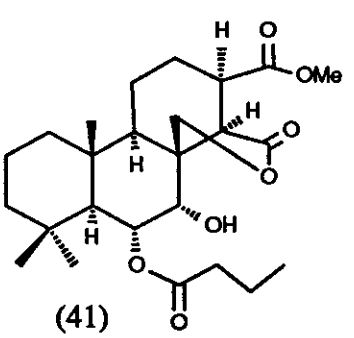
(S)

The regiochemistry of the alcohol and butyroxy groups in AROSST-3 was elucidated from the chemical shifts and magnitude of the coupling of the protons α to these groups.

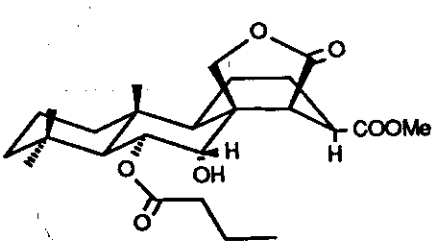
Decoupling experiments in the ^1H n.m.r. spectrum of a fine doublet at δ 3.54 (J 2.4Hz) found it coupled to a resonance at δ 5.35 evident as a doublet of doublets (J 12.2, 2.4 Hz). This in turn was coupled to an aliphatic doublet (J 12.2 Hz) at δ 1.96.

The chemical shifts and magnitude of the coupling are consistent with an equatorial proton α to an alcohol (δ 3.54, H7β) coupled to an axial proton α to a butyroxy group (δ 5.35, H6β) which is further connected to an axial aliphatic proton recognised as H5α.

The final structure of AROSST-3 was therefore settled as (41) the conformation of which is shown in structure (41A).



(41)



(41A)

Fig 14 : ^1H N.m.r. spectrum of AROSST-3 (41) ($CDCl_3$).

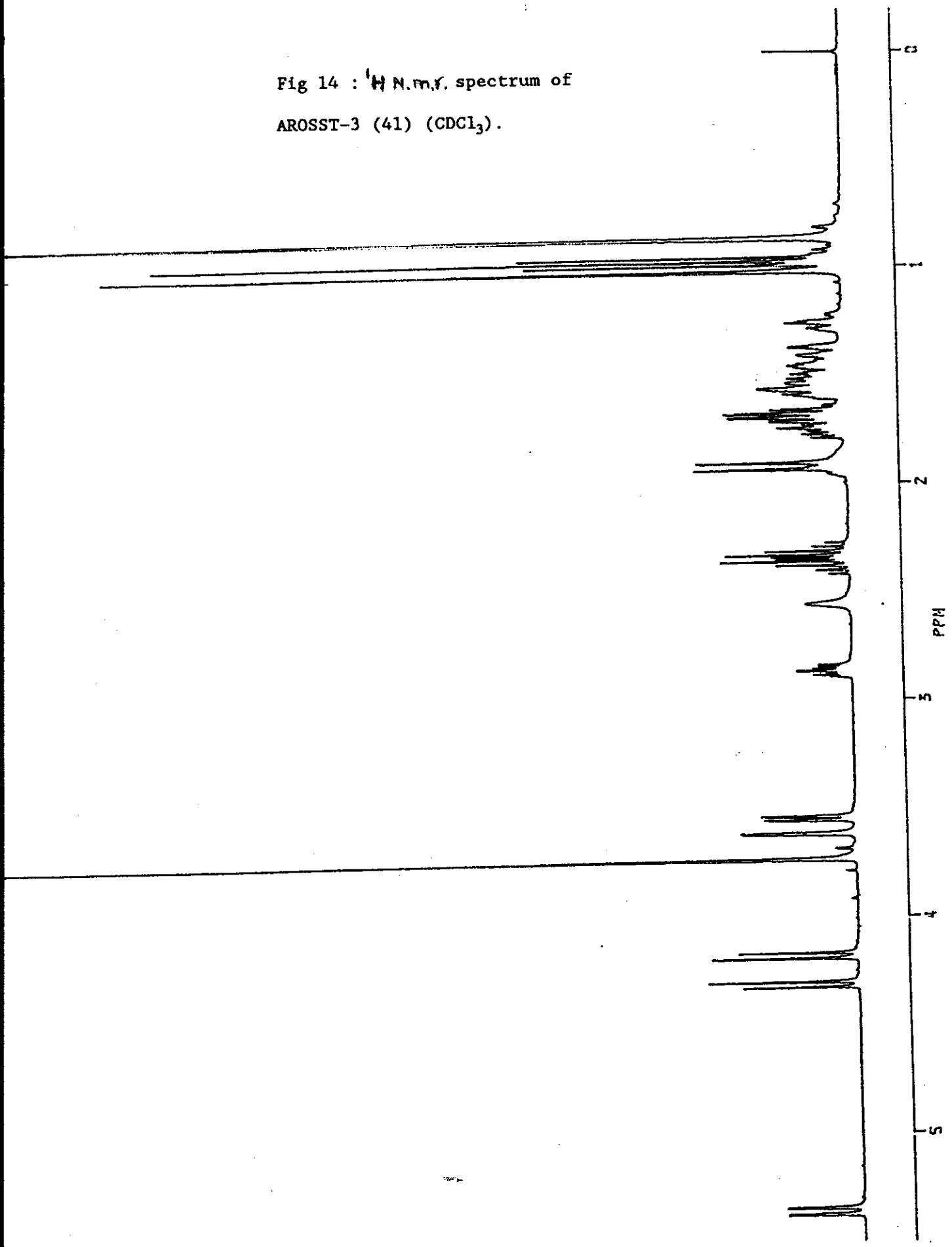


Table 10. ^{1}H-^{1}H nuclear Overhauser enhancements in AROSST-3 (41).

^{1}H irradiated	Signals enhanced (%)
H7β	H6β(4.9), H17a(3.6), H14(3.0), OH(5.1).
H14	H7β(4.4)

(D) AROSST-4 (42) and AROSST-5 (43)

Two very similar compounds, AROSST-4 (42) and AROSST-5 (43), were isolated by h.p.l.c. as colourless crystals. High resolution mass matching of the molecular ion peaks of both compounds (*m/z* 492) indicated a molecular formula of $C_{27}H_{40}O_8$. The mass spectrum of both compounds gave peaks at *m/z* 461 and 362 corresponding to the loss of methoxide (methyl ester) and butyric acid (butyrate ester) respectively.

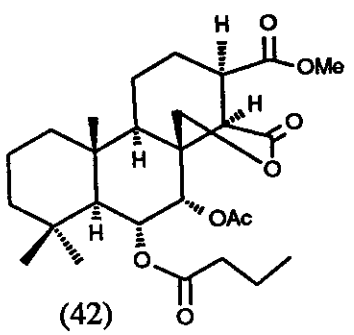
The ^{13}C n.m.r. and i.r. spectra were virtually identical for both compounds. In ^{13}C n.m.r. spectra, four carbonyl resonances at δ 170.0, 172.6, 172.9 and 175.3 in AROSST-4 and at 170.1, 172.5, 172.6 and 175.3 in AROSST-5 were due to the acetate, butyrate, methyl ester and γ-lactone. The i.r. spectrum confirmed the presence of a strained γ-lactone with strong carbonyl absorbtion bands at 1777 cm^{-1} in AROSST-4 and 1776 cm^{-1} in AROSST-5.

The ^{1}H n.m.r. spectrum for both compounds (Figs 15 and 16) were also very similar and substantiated the presence of the acetate, butyrate and methyl ester in both compounds. The major differences between the two

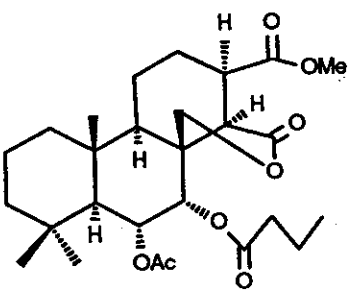
spectra were the chemical shifts of the acetate and butyrate signals, together with the protons α to these groups, and the chemical shifts of H13 and H14.

In AROSST-4 the acetate resonates at δ 2.17 (AROSST-5:δ 1.99) and the butyrate methyl group at δ 0.94 (AROSST-5: δ 1.03), β-methylene δ 1.64 (AROSST-5: δ 1.75) and α-methylene δ 2.20 (AROSST-5: 2.42). The protons α to these groups, H6β resonates at δ 5.28 (AROSST-5: δ 5.26) and H7β at δ 5.14 (AROSST-5: δ 5.17). The protons H13 and H14 resonated as a two proton multiplet centred at δ2.77 in AROSST-4 while in AROSST-5 they occurred at δ 2.76 and 2.82 respectively.

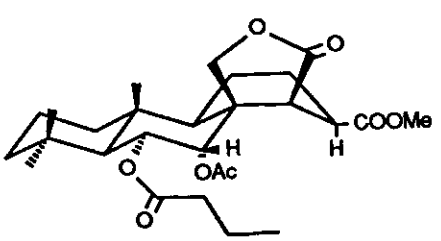
This led to the conclusion that AROSST-4 and AROSST-5 were regioisomers and given structures (42) and (43) interchangeably. Again, because both H13 and H14 were shown, by a 2D-COSY spectrum (Fig 16 and 17: AROSST-5) and decoupling experiments in the 1D ^{1}H n.m.r. spectrum (AROSST-4), to be mutually coupled to H12α, the C ring for both compounds resides in a boat conformation as in AROSST-1 (39), -2 (40) and -3 (41).



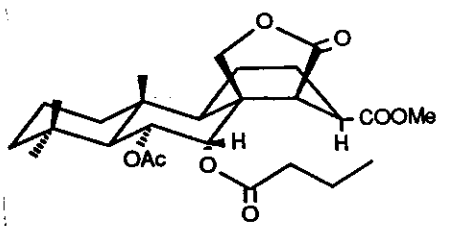
(42)



(43)



(42A)



(43A)

Fig 15 : ^1H n.m.r. spectrum of AROSST-4 (42) ($CDCl_3$).

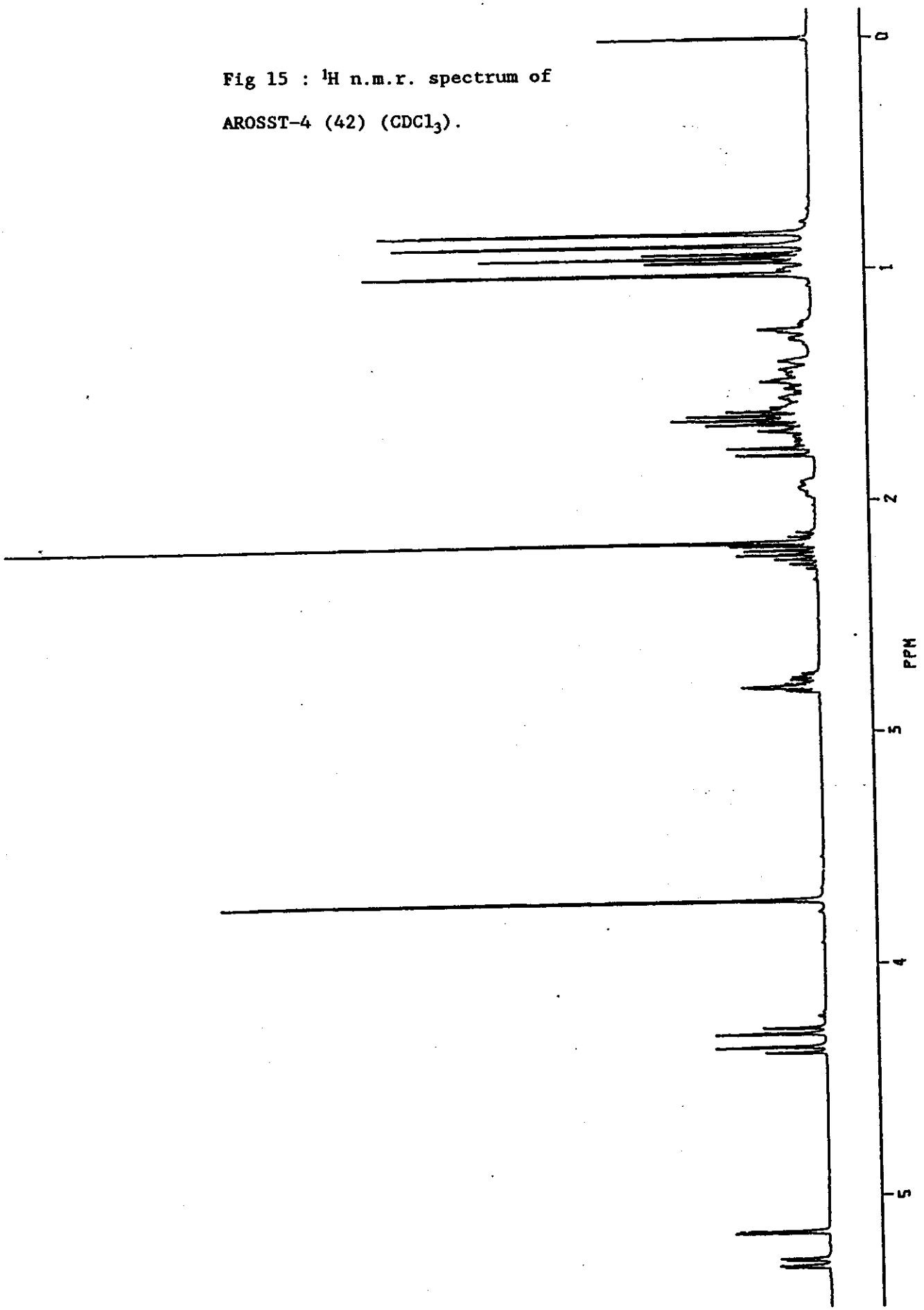


Fig 16 : 2D-COSY spectrum of AROSST-5 (43) ($CDCl_3$).

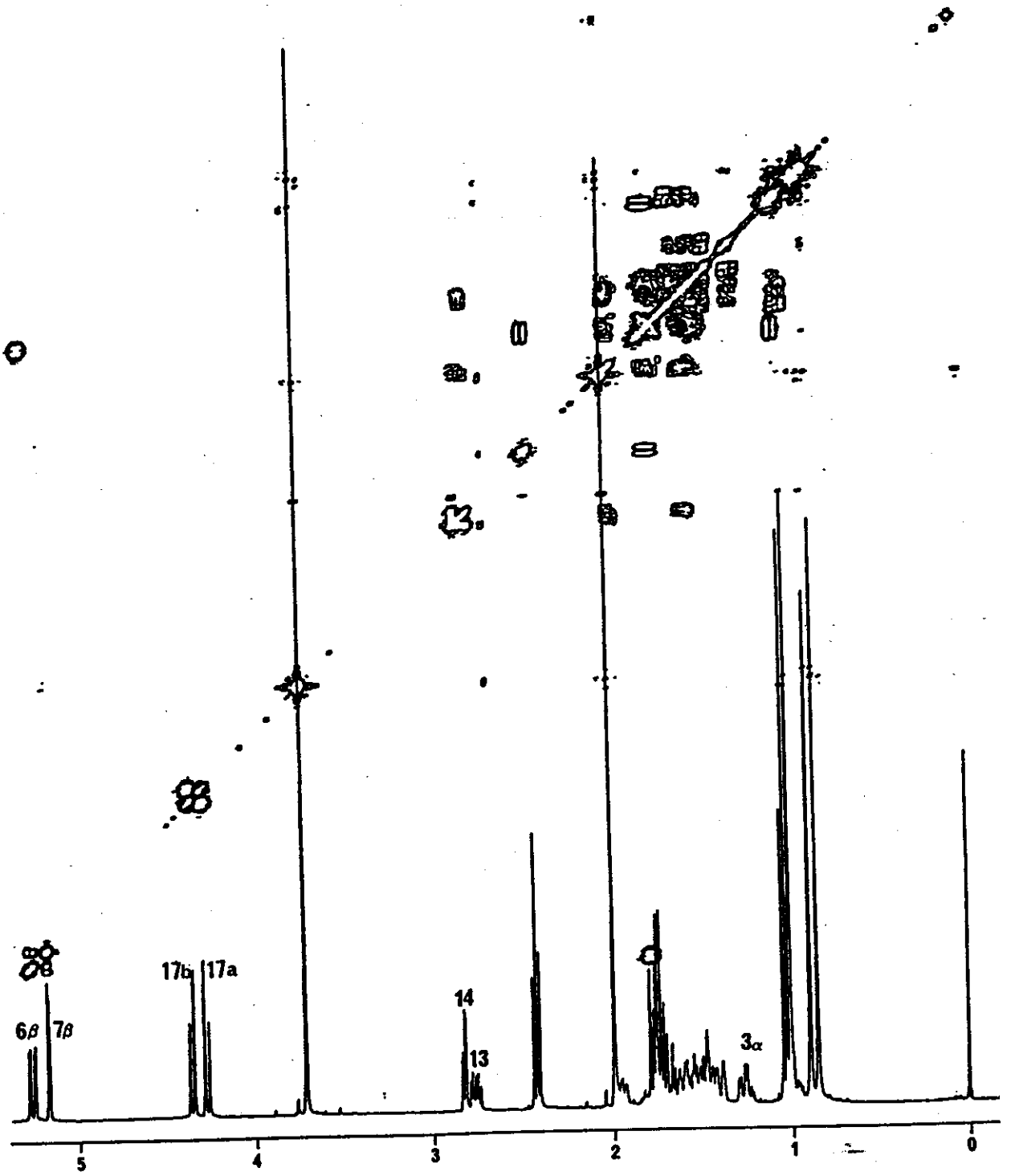
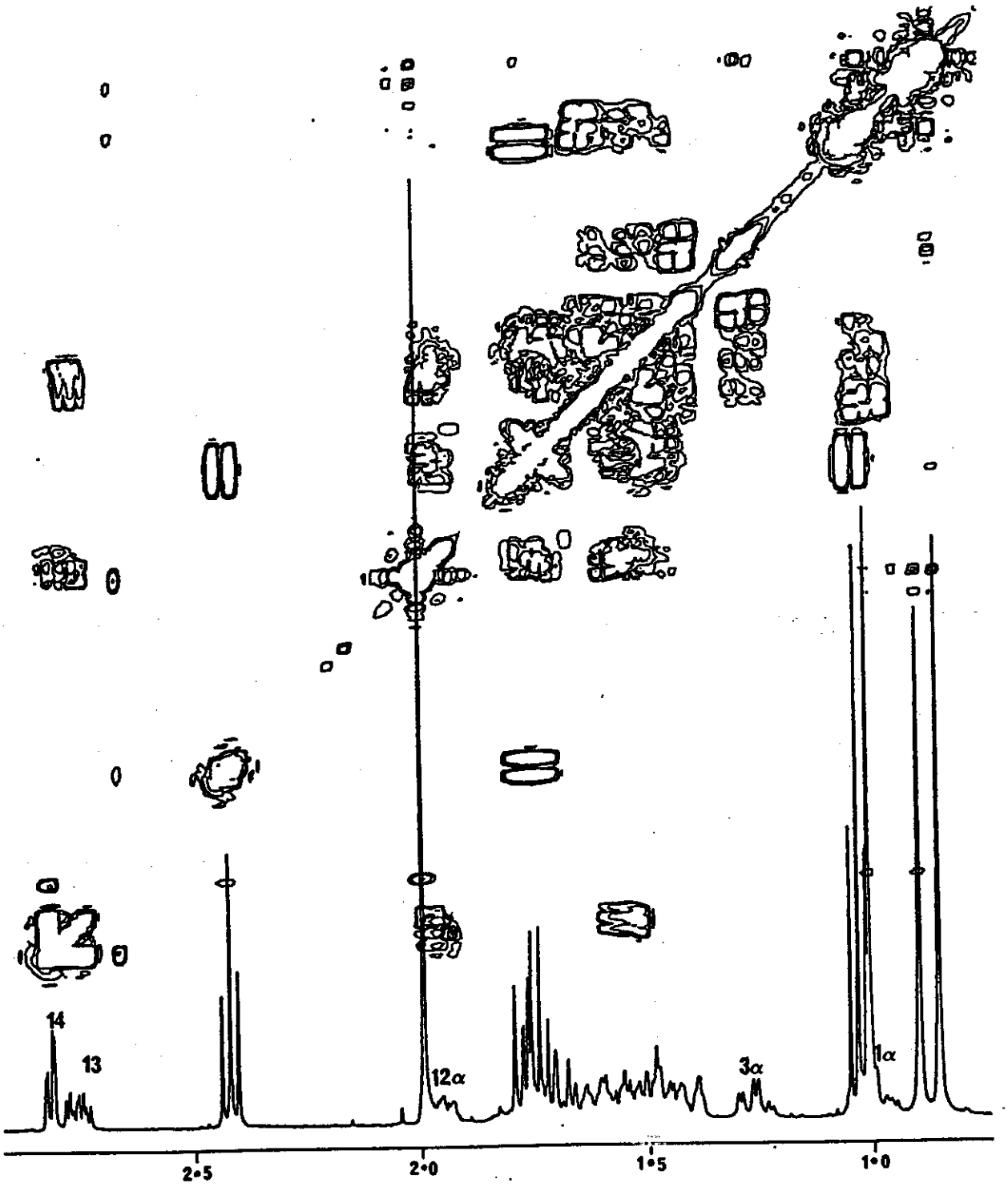


Fig 17 : 2D-COSY spectrum of

AROSST-5 (43) ($CDCl_3$).

Expansion of upfield portion.



Unfortunately, it was impossible to distinguish which of the two, AROSST-4 or AROSST-5, was the acetate of AROSST-3.

Curiously the α-CH_2, being close to a chiral centre and hence slightly non-equivalent, usually is present as a second order multiplet. However, in AROSST-5 the α methylene protons are equivalent resulting in an interpretable first order pattern.

The problem of which regioisomer was which, was resolved by the acetylation of AROSST-3. This proved that AROSST-4 was in fact the acetate of AROSST-3 and hence had structure (42) and by inference AROSST-5 had the structure (43) the conformations of which are shown in structures (42A) and (43A) respectively

(E) AROSST-6 (44)

AROSST-6 (44) was isolated as a colourless glass. Unfortunately the sample autooxidised giving the acid product AROSST-6A (44A) before full spectral measurements could be obtained. However, the oxidation product also proved beneficial in assigning the regiochemistry in AROSST-6.

AROSST-6A was isolated as a neutral polar white powder with a molecular formula $C_{23}H_{36}O_6$. The e.i.m.s. showed the parent ion, *m/z* 408, only weakly (<1%). High resolution mass matching of two fragment ions, *m/z* 366 [$C_{23}H_{36}O_6$ - C_2H_2O] and *m/z* 348 [$C_{23}H_{36}O_6$ - CH_3COOH], together with the ^{13}C n.m.r. spectrum, which showed twenty three carbons, served as proof of the molecular formula. From the molecular formula there are six units of unsaturation.

The i.r. spectrum (1732 cm^{-1}) together with the ^{13}C n.m.r. spectrum (δ 170.0, 174.2 and 177.2) indicated the presence of three carbonyl functions.

An additional broad i.r. absorption (3500-2500 cm^{-1}) and a broad

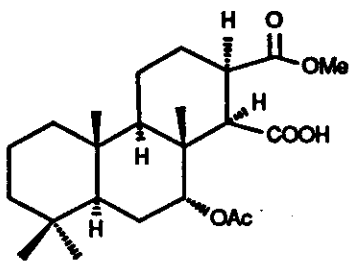
signal in the ^1H n.m.r. spectrum (60MHz) at δ 7.50, which was temperature dependent, implied one of the carbonyls was a carboxylic acid. In addition, two-three proton singlets at δ 2.07 and 3.64 in the ^1H n.m.r. spectrum established the other two carbonyl function as an acetate and a methyl ester respectively. The acetate was further supported by the mass spectrum [*m/z* 320, $M^+ - CH_3COOH$] and an additional i.r. absorption at 1252 cm^{-1}.

From the ^1H n.m.r. spectrum (Fig. 18), four quaternary methyl singlets at δ 0.75 (20Me), 0.77 (19Me), 0.83 (18Me) 1.08 (17Me) [the assignments based on n.O.e. results (Table 11)] indicated a diterpene structure in which, unlike the previous aplyroseols, the 17Me group remained unoxidised.

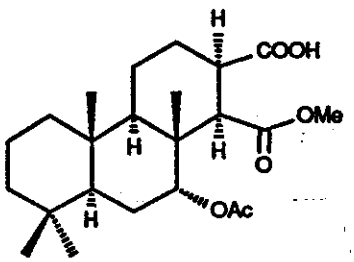
The proton α to the acetoxy groups (δ 5.29) was clearly visible as a fine doublet of doublets, reminiscent of equatorial H7β adjacent to a methylene as in AROSST-1 (39) and -2 (40). From decoupling experiments the two methylene protons, H6α (δ 1.84) and H6β (δ 1.68) were located. Further decoupling of H6α then located H5α in a three proton multiplet centred at δ 1.17.

Other features in the downfield region of the ^1H n.m.r spectrum were signals at δ 3.22 (ddd, *J* 5.3, 5.3, 1.8 Hz), a doublet at δ 2.90 (*J* 5.3 Hz) and a multiplet at δ 2.37 (dddd, *J* 13.2, 5.3, 3.0, 1.8 Hz). Decoupling experiments in the ^1H n.m.r. spectrum established the proton resonating at δ 2.90 to be coupled to the proton resonating at δ 3.22: this in turn was coupled to two methylene protons, one of which resonated at δ 2.37, the other within a four proton multiplet centred at δ 1.42.

The chemical shifts and multiplicities of the two protons resonating at δ 2.90 and 3.22 are consistent for protons H14 and H13 respectively, which are adjacent to carbonyl functions. The evidence presented therefore suggested the structures of AROSST-6A was one of two isomer (44A) or (44B).



(44A)



(44B)

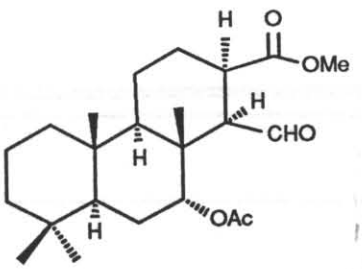
The *cis* assignment as depicted in the two isomers (44A) and (44B), was substantiated by the magnitude of the coupling (*J* 5.3 Hz) and n.O.e. experiments: a n.O.e. was recorded from H13 to H14 and *vice versa* (Table 11). This was also reasonable on biogenetic grounds.

The decision as to which isomer was correct was made by referring to the ^{1}H n.m.r. spectrum of AROSST-6.

In the ^{1}H n.m.r. spectrum of AROSST-6, a singlet at δ 9.77 indicating the presence of an aldehyde function: which was oxidised to a carboxylic acid.

By comparing the two ^{1}H n.m.r spectra, it was found that the protons assigned H13 and H14 in AROSST-6 were at δ 3.16 and 2.63 respectively; in AROSST-6A they were at δ 3.22 and 2.90 respectively.

Therefore, since the proton H14 was found to be more downfield on oxidation of the aldehyde to the carboxylic acid it unequivocally proves the structure of AROSST-6A as (44A) and hence the natural product isolated was (44).



(44)

Table 11. ^{1}H-^{1}H nuclear Overhauser enhancements in AROSST-6A (44A).

^{1}H irradiated	Signals enhanced (%)
H7β	17Me(4.6).
H13	H14(7), H12β(2.8).
H14	H13(8).
17Me	18Me(2.6), H7β(2.3), H6β+H11β(3.1).*
18Me	17Me(2.3).
19Me	H3β(0.9), H6β(2.4), H6α(0.7).
20Me	H5α(2.4), H3β(0.8) H6α(2.5).

* These protons are superimposed so the percentage is collective

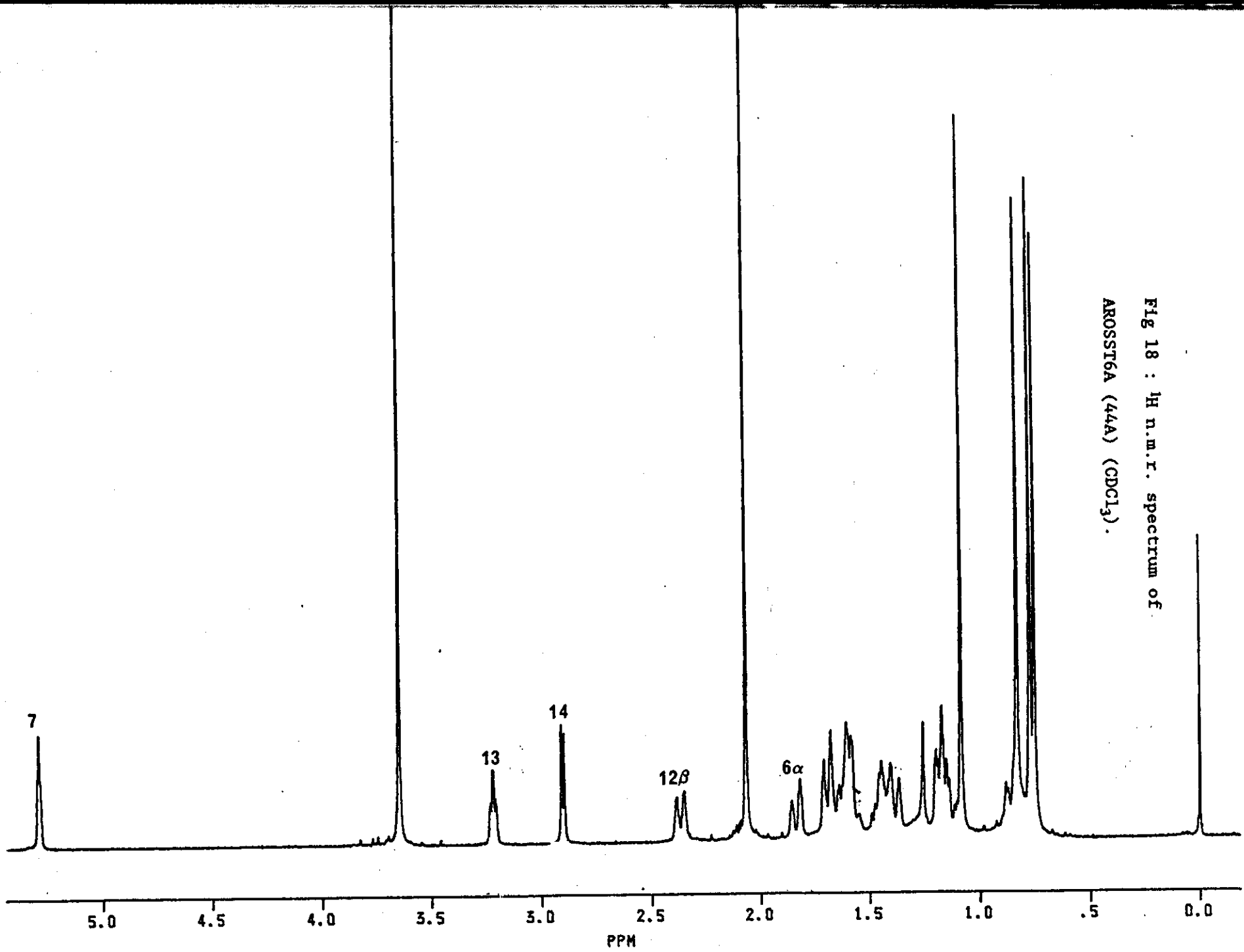


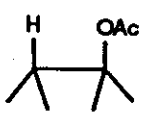
Fig 18 : ^1H n.m.r. spectrum of AROSST6A (44A) ($CDCl_3$).

(F) ARROST-7 (45)

AROSST-7 was isolated as a colourless gum with molecular formula $C_{22}H_{36}O_5$. The highest peak in the e.i.m.s. was at *m/z* 379 (<1%) due to the loss of a proton from the molecular ion. The molecular formula was determined from the high resolution mass matching of the *m/z* 320 [M^+ - CH_3COOH] and *m/z* 302 [M^+ - CH_3COOH - H_2O] peaks. The molecular formula was further supported by the ^{13}C n.m.r. (DEPT and NORD) in which 22 carbons were indicated with 35 protons attached: integration of the ^1H n.m.r. spectrum indicated thirty six protons hence one proton was attached to an oxygen. From the molecular formula five units of unsaturation were present.

A strong carbonyl absorption in the i.r. spectrum at 1732 cm^{-1} together with two carbonyl signals in the ^{13}C n.m.r. at δ 169.9 and 174.1, indicated the presence of two ester functions.

An absorption at 1231 cm^{-1} and a three proton singlet at δ 2.13 in the ^1H n.m.r. spectrum (Fig. 19) substantiated one of the ester functions as an acetate. The e.i.m.s. also showed prominent loss of acetic acid (60 daltons) giving the peak at *m/z* 320. Since the acetoxy group fragments with the full loss of acetic acid, there must be a β-proton available for β-elimination; hence partial structure (A) occurs.



(A)

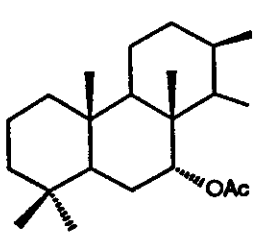
Another three proton singlet at δ 3.67 in the ^1H n.m.r. spectrum and a methyl signal in the ^{13}C n.m.r. (DEPT) at δ 51.3 disclosed the other carbonyl function as a methyl ester.

The presence of an alcohol function, already suggested by the ^{13}C n.m.r. (only thirty five protons attached to carbons) was confirmed by the i.r. spectrum (ν_{OH} 3420 cm^{-1}) and the ^{1}H n.m.r. spectrum which showed a broad singlet at δ 3.91 which exchanged with D_2O. The e.i.m.s. also showed a prominent peak at *m/z* 302 due to the initial loss of acetic acid and subsequent loss of water (18 daltons) from the molecular ion.

The ^{13}C n.m.r. spectrum, showing two signals at δ_C 77.8 and 80.4, also confirmed the presence of two carbons bearing oxygens (acetate and alcohol).

The alcohol, acetate and methyl ester accounted for all the five oxygens as well as two units of unsaturation. Since the acetate and the methyl group of the methyl ester account for three of the 22 carbons the basic hydrocarbon skeleton must contain only 19 carbons. As there are three units of unsaturation unaccounted for, the parent hydrocarbon must be tricyclic.

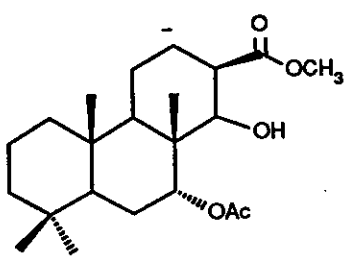
The tricyclic diterpene structure (T) of a degraded spongian system was therefore proposed. This was indicated by the presence in the ^{1}H n.m.r. spectrum of four methyl groups (δ_H 0.76, 0.78, 0.82 and 0.84) and a doublet of doublets at δ 4.86 (*J* 2.8, 2.8 Hz) reminiscent of H7β in AROSST-1 (39) and -2 (40), and by analogy with previously isolated spongian diterpenes. Therefore partial structure (T) was envisaged.



(T)

The presence of a proton α to the alcohol was clearly evident in the ^{1}H n.m.r. spectrum as a doublet at δ 4.12 (J 2.8 Hz). This proton was coupled to a proton resonating as a fine doublet of doublet of doublets (coupling could not be resolved) at δ 2.63: this signal in turn showed further coupling from two protons resonating as a broad doublet at δ 2.10 (J 14 Hz) and an overlapping two proton multiplet at δ 1.86.

As the proton α to the alcohol contained only one coupling, it was evident that the alcohol was β to a quaternary carbon. The chemical shift of the proton at δ 2.63 indicated that it must be α to the methyl ester group; therefore the methyl ester group is β to the alcohol function. Therefore the structure of AROSST-7, without stereochemistry at C14 was extended to (U).



(U)

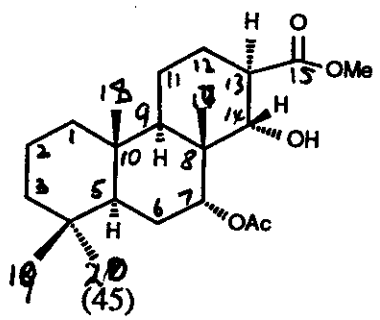
A 2D-COSY spectrum (Fig. 19 and 20) was obtained which showed all the connectivities already determined by decoupling experiments in the 1D spectrum. In addition the 2D-COSY spectrum together with n.O.e. experiments enabled the chemical shifts of all protons to be assigned. N.O.e. experiments (Table 12) also substantiated the relative stereochemistry within partial structure (45) and established the relative stereochemistry at C14.

The equatorial and β-face assignment of H7β was confirmed by a n.O.e. enhancement from 16Me. A n.O.e. enhancement from 16Me to H14 and *vice*

versa also showed that H14 is β-face disposed. This assignment was reinforced by mutual n.O.e. enhancements between of H14 and H7β which could only occur if H14 was equatorial: β face disposed.

Furthermore, upon irradiation, 17Me gave a n.O.e. to H6β and H11β; 19Me gave an n.O.e. to H5α H3α and H6α; H5α and H1α both gave a n.O.e. to H9α. These n.O.e. experiments in conjunction with the 2D-COSY allowed the chemical shifts of the protons on C1, C3, C6, C9 and C11 to be assigned.

The only other chiral centre yet to be assigned is C13. The relative stereochemistry of this site is probably as depicted in (45) based on biogenetic arguments[13] and by analogy with other aplyroseol compounds. Therefore the final structure of AROSST-7 was settled as (45).



(45)

Table 12. ^{1}H-^{1}H nuclear Overhauser enhancements in AROSST-7 (45).

^{1}H irradiated	Signals enhanced (%)
H1α	H9α(6.1).
H5α	H9α(6.8).
H7β	H14(4.5), 17Me(3.8).
H14	H7β(4.1), 17Me(3.9).
17Me	H6β(2.4), H11β(2.9).
	H14(2.9), H7β(2.8).
20Me	H5α+H3α(3.5), H6α(2.3).

Fig 19 : 2D-COSY spectrum of

AROSST-7 (45) ($CDCl_3/D_2O$).

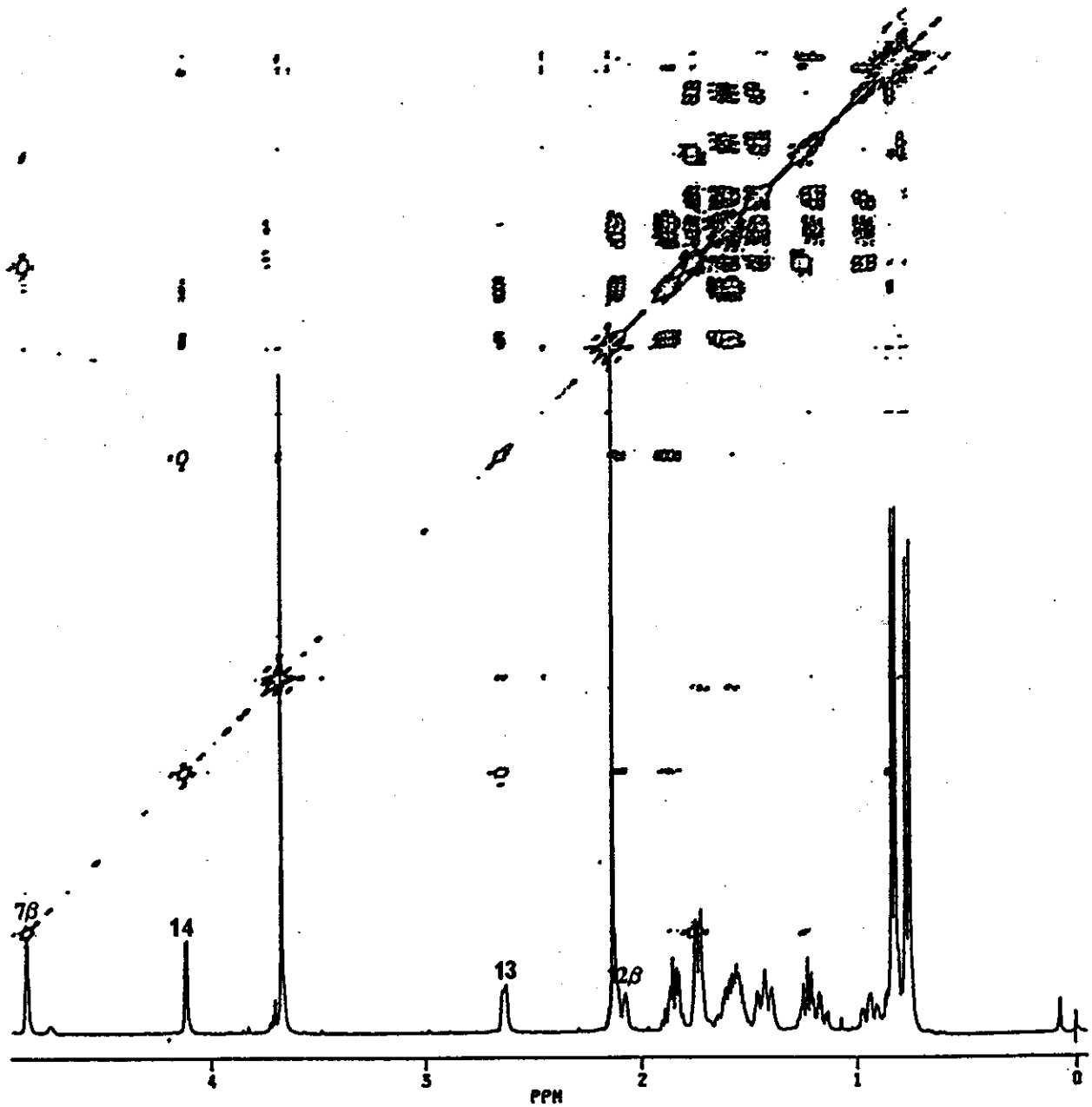
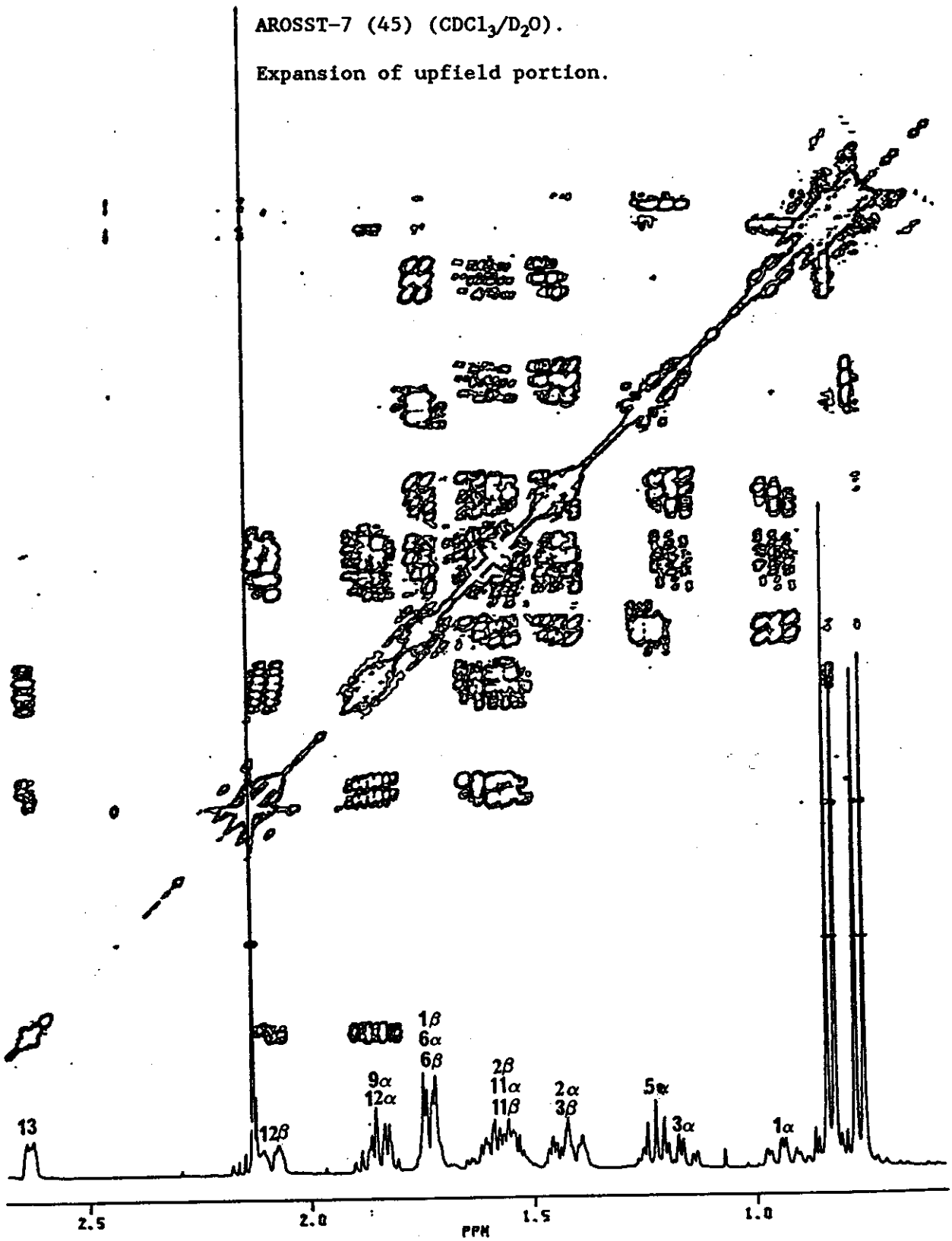


Fig 20 : 2D-COSY spectrum of AROSST-7 (45) ($CDCl_3/D_2O$). Expansion of upfield portion.



(G) AROSST-8 (46)

AROSST-8 (46) was isolated as a colourless gum which crystallised on standing and had a molecular formula $C_{22}H_{36}O_5$. The highest peak in the e.i.m.s. was at *m/z* 348 (<1%) due to the loss of methanol from the molecular ion. The molecular formula $C_{22}H_{36}O_5$ was confirmed by high resolution mass matching of the *m/z* 320 [M^+ - CH_3COOH] and *m/z* 302 [M^+ - CH_3COOH - H_2O] peaks. The ^{13}C n.m.r. spectrum (noise decoupled and DEPT sequence) showed the presence of 22 carbons with 35 protons attached. The ^1H n.m.r. spectrum indicated 36 protons and hence one proton was attached to an oxygen.

In the ^1H n.m.r. spectrum (Fig 21 and 22), characteristic signals pertaining to the A and B rings and the methyl ester of AROSST-7 (45) were present in AROSST-8. This includes an acetate singlet at δ 2.10, a fine doublet of doublets at δ 4.96 recognized as the proton H7β (α to an acetate function) and a three proton singlet at δ 3.74 due to the methyl ester.

Four quaternary methyl singlets (δ 0.77, 0.78, 0.79 and 0.83) together with a multiplet reminiscent of H1α (δ 0.88) further confirmed the presence in AROSST-8 of the A and B rings of (45). The methyl ester was indicated by a three proton singlet at δ 3.74.

The alcohol function already suggested by the ^{13}C n.m.r. spectrum (only 35 protons connected to carbons) was confirmed by the ^1H n.m.r. spectrum which showed a sharp doublet at δ 4.10 which exchanged with D_2O. Both the D_2O exchanged spectrum, as well as decoupling experiments in the ^1H n.m.r. spectrum, revealed the hydroxyl proton to be coupled to a proton resonating at δ 3.82 (H14) which was further shown, by decoupling experiments and the 2D COSY spectrum (Fig 21 and 22), to be coupled to a proton resonating at δ 2.82 (H13). This proton in turn was further

Fig 21 : 2D-COSY spectrum of AROSST-8 (46) ($CDCl_3$).

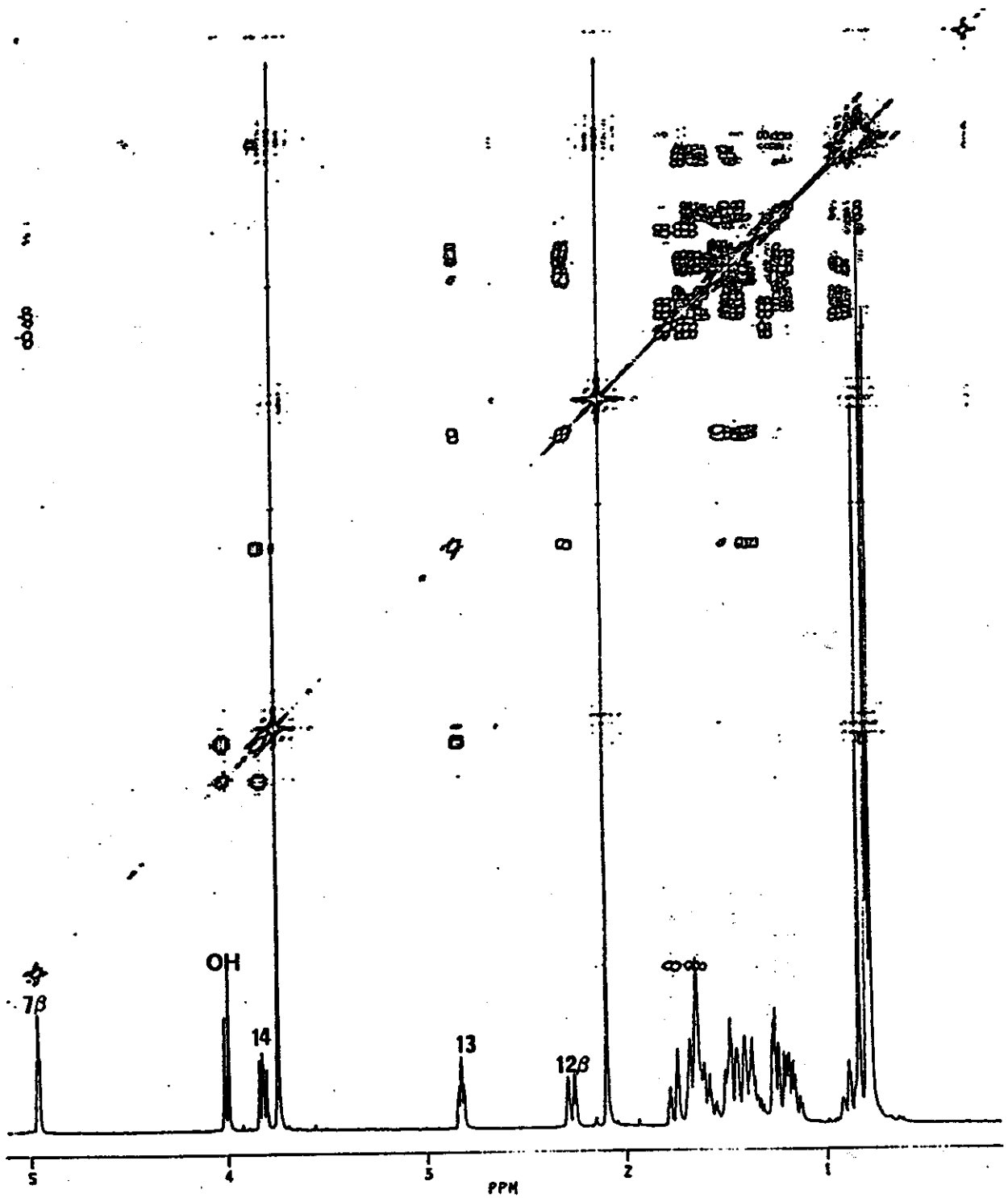
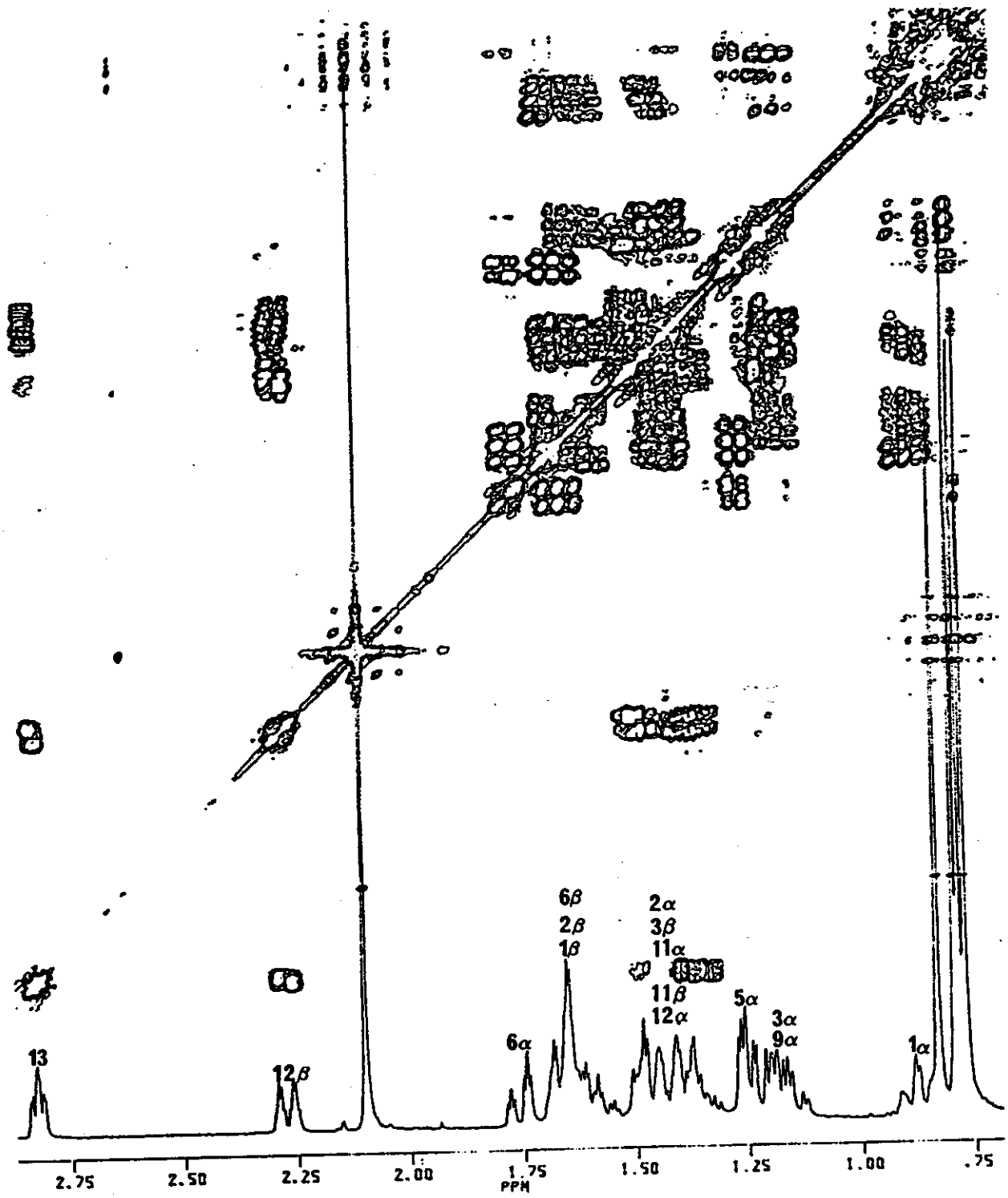


Fig 22 : 2D-COSY spectrum of

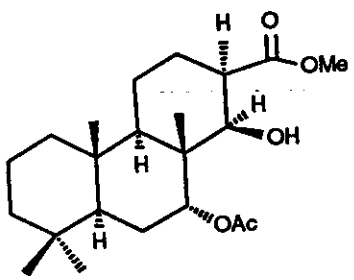
AROSST-8 (46) ($CDCl_3$).

Expansion of upfield portion.



coupled to two protons, one resonating at δ 2.27 (H12β) and the other resonating within a five proton multiplet centred at δ 1.43 (H12α).

Thus AROSST-8 was found to be an isomer of AROSST-7 (45) and assigned the structure (46). The relative stereochemistry of (46) was based on that of AROSST-7 with H14 assigned to the α-face by inference from coupling constant between H13 and 14 (*J* 6.3 Hz). All other spectral data was consistent with the proposed structure.



(46)

(H) AROSST-9 (47).

A very minor constituent isolated from the sponge was AROSST-9 (47). The molecular formula $C_{22}H_{34}O_4$ was determined by high resolution mass matching of the molecular ion peak, *m/z* 362 in the e.i.m.s. There are six units of unsaturation in the structure.

The presence of an acetate was indicated by the ^1H n.m.r. spectrum (Fig 23 and 24) which showed a three proton singlet at δ 2.10. This was further substantiated by the mass spectrum which showed two fragmentation ions at *m/z* 320 ($C_{20}H_{32}O_3$) and 302 ($C_{20}H_{30}O_2$), the molecular formulae being established by high resolution mass matching and corresponded to the loss of ketene and acetic acid respectively. The presence of a major peak due to the loss of ketene (*m/z* 302) suggested the acetoxy group had no β-hydrogen available to undergo β-elimination.

The presence of only three quaternary methyl groups was indicated by the ^{1}H n.m.r. spectrum which showed three methyl singlets at δ 0.81, 0.86 and 1.02. The absence of a fourth quaternary methyl signal and the presence of an AB system centred at δ 4.49 indicated the C17 methyl groups was oxidised and contained the acetate function.

The lactone D ring of (47), with the carbonyl at C16 (as opposed to C15), was indicated by a pair of doublet of doublets in the ^{1}H n.m.r. spectrum at δ 3.99 (J 11.6, 7.1 Hz) (H15α) and 4.34 (J 11.6, 4.3 Hz) (H15β) which are consistent with a methylene α to an oxygen function and reminisent of the D rings in the lactones (35) and (36). These protons were shown by decoupling experiments in the 1D ^{1}H n.m.r. spectrum and from the 2D–COSY spectrum (Fig. 23 and 24), to be geminally coupled together and mutually coupled to a proton resonating at δ 1.74 (H14). This proton in turn displayed further coupling to a proton resonating as a fine second order multiplet at δ 2.84 (H13), the chemical shift of which was consistent with a proton α to a carbonyl group. This proton in turn exhibited further coupling with two protons, resonating as a second order multiplet at δ 2.14 (H12β) and within an overlapping three proton multiplet at δ 1.73 (H12α). The second order pattern observed for H13 and H12β being imposed by virtual coupling due to ~~the~~ the close proximity of H12α and H11α both situated at δ 1.73.

The 2D-COSY spectrum also revealed two long range 'W' couplings and together with n.O.e. experiments enabled the relative sterochemistry of (47) to be determined.

The small 'W' coupling between H17a (δ 4.09) and H9α (δ 1.14) of J 1.5 Hz, and between H17b (δ 4.89) and H14 (δ 1.74) of J 1.8 Hz consequently showed H17a and 17b to be β face disposed while H14 and 9 are α face disposed. The *cis* configuration between H13 and H14 was based on the fine second order multiplet observed for H13(δ 2.84), which hence only contains

Fig 23 : 2D-COSY spectrum of AROSST-9(47) ($CDCl_3$).

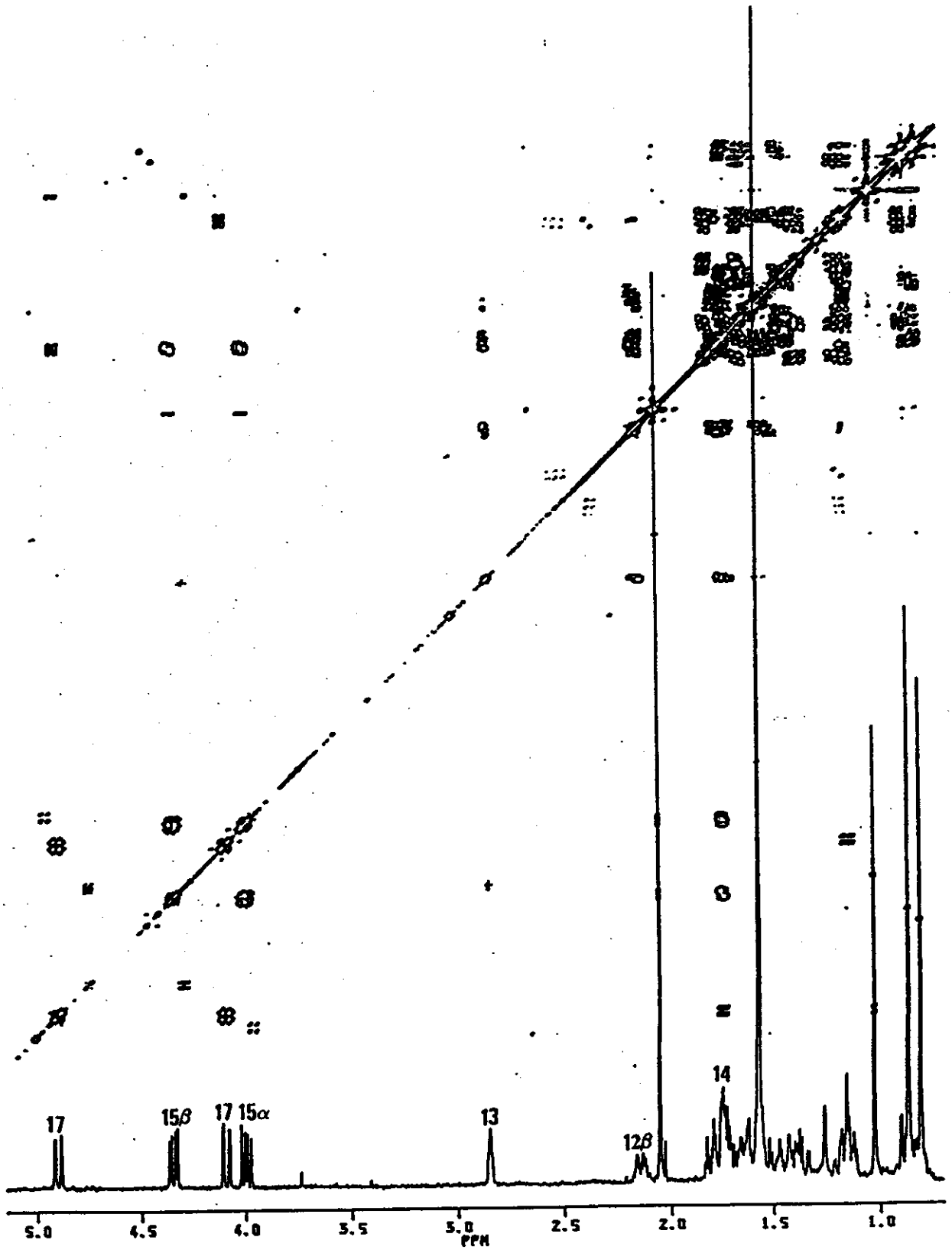
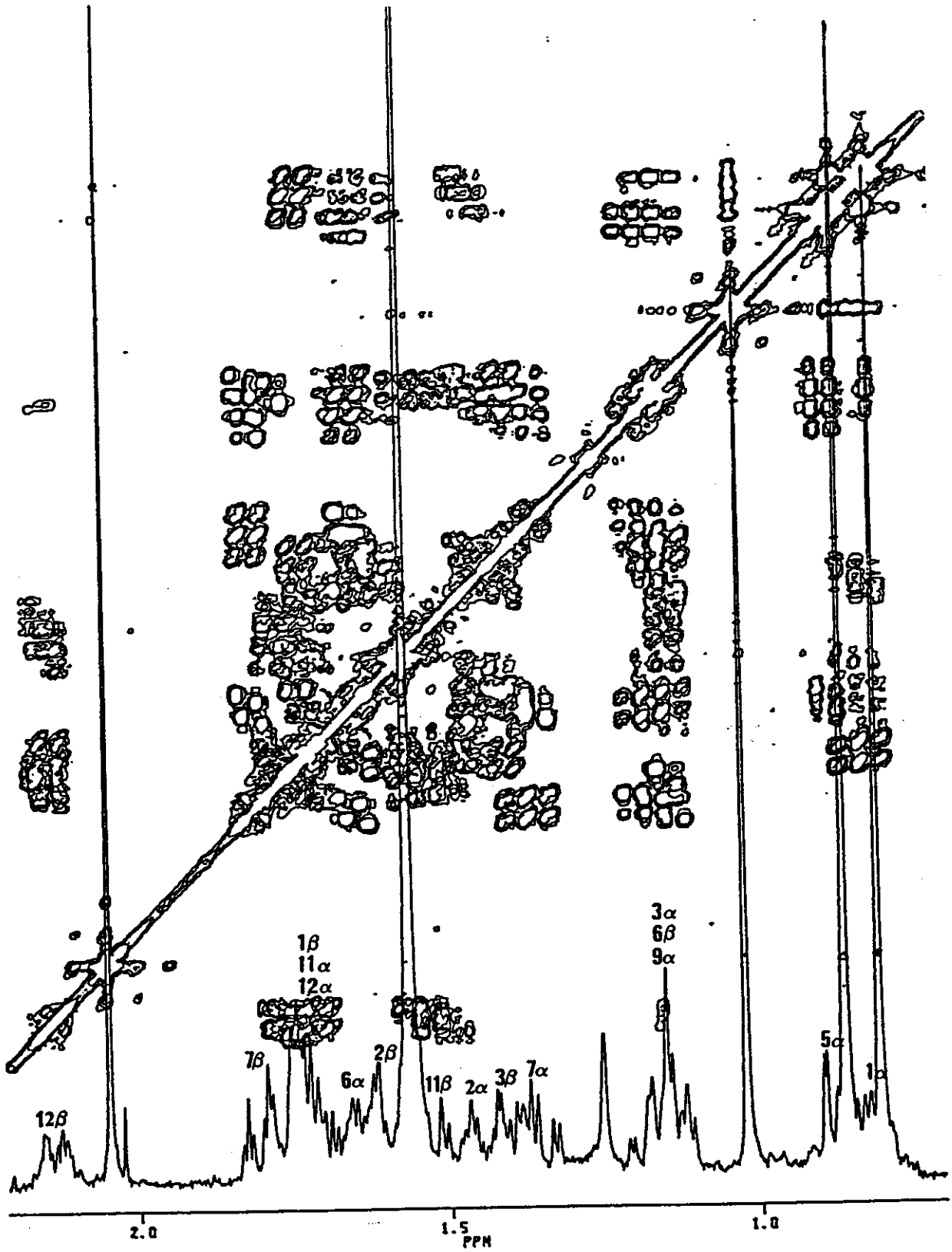


Fig 24 : 2D-COSY spectrum of

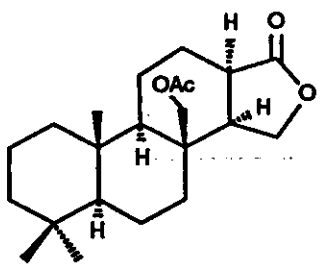
AROSST-9 (47) ($CDCl_3$).

Expansion of upfield portion.



small couplings (the reverse *trans* configuration would have given two large *trans*-diaxial couplings).

A n.O.e. (Table 13) observed between 18Me and H17β (δ 4.89) and 19Me confirmed that these groups were disposed on the β-face. The configuration at C5 was based on biogenetic considerations. Therefore the final structure of AROSST-9 was settled as (47).



(47)

Table 13. ^{1}H-^{1}H nuclear Overhauser enhancements in AROSST-9 (47).

^{1}H irradiated	Signals enhanced (%)
H17b	18Me(7).
18Me	19Me(2), H17b(4).

(I) AROSST-10

AROSST-10 (48) was isolated as a colourless gum with a molecular formula $C_{22}H_{34}O_5$. The highest peak in the e.i.m.s. at *m/z* 360 was due to the loss of a molecule of water. The first two major fragments were at *m/z* 318, due to the loss of a molecule of acetic acid from the molecular ion, and at *m/z* 300 due to the subsequent loss of a molecule of water from

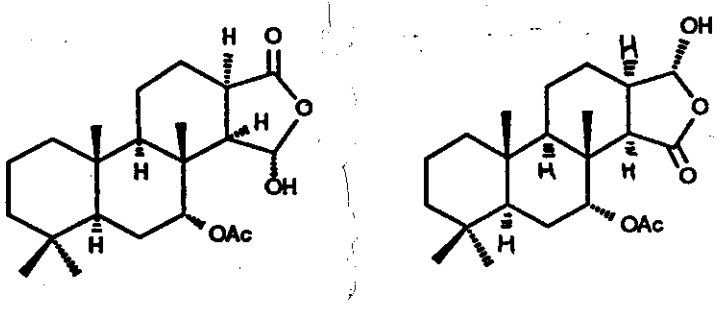
the *m/z* 318 parent ion. The molecular formula was determined from the high resolution mass matching of the *m/z* 318 ($C_{20}H_{30}O_3$) and *m/z* 300 ($C_{20}H_{28}O_2$) and *m/z* 300 ($C_{20}H_{28}O_2$) peaks, and from the ^{13}C n.m.r. and ^1H n.m.r. spectra which showed 22 carbons (noise decoupled spectra) and 34 protons respectively. From the molecular formula there are six units of unsaturation present.

The acetonyl, γ-lactone and hydroxyl groups gave rise to absorptions in the i.r. spectrum at 1726, 1775 and 3363 cm^{-1} respectively. The acetonyl and γ-lactone were further substantiated by two carbonyl signals in the ^{13}C n.m.r. spectrum at δ 170.8 and 178.6 respectively. In the ^1H n.m.r. spectrum (Fig. 25) the acetoxyl methyl resonated as a three proton singlet at δ 2.13. A broad singlet at δ 3.77 which was also temperature dependent further substantiated the hydroxyl group.

The acetate, γ-lactone and hydroxyl group accounted for all five oxygens and two units of unsaturation. As no further unsaturation was indicated (^{13}C n.m.r and i.r. spectra) the remaining C_{20} hydrocarbon, containing four units of unsaturation, must be tetracyclic. The ^1H n.m.r. spectrum also showed four quaternary methyl signals and by analogy with the structures of other aplyroseal metabolites particularly lactone (35) and AROSST-9 (47), AROSST-10 also contains the spongian skeleton (10).

The ^1H n.m.r. spectrum also contained two other signals, apart from the hydroxyl signal (δ 3.77), in the downfield region. The first, a fine doublet of doublets at δ 5.05 (*J* 2.9, 2.9 Hz), which together with a signal in the ^{13}C n.m.r. spectrum at δ 74.1, was reminiscent of H7β in the structures of such aplyroseals as (30), (35) and (36). Hence the acetonyl group was assigned to C7. The remaining downfield signal, resonating as a broad singlet ($W_{h/z}$ 5 Hz) at δ 5.56, together with a signal in the ^{13}C n.m.r. spectrum at δ 96.6, indicated the presence of a hemiacetal, the hemiacetal alcohol being placed in the α-face due to a zero coupling and

hence orthogonal geometry of the hemiacetal proton to the adjacent proton. Structures (48) and (48A) were therefore suggested.



(48) (48A)

The assignment of structure (48) to AROSST-10 was based on the chemical shift of H13, H14 and H12β, found as isolated systems in the midfield region of the ^{1}H n.m.r. spectrum. N.O.e. experiments (Table 14) further substantiated the proposed structure (48), as well as the relative stereochemistry of the hemiacetal.

H13 resonated as a doublet of doublets at δ 2.95 (J 8.2, 8.2, 0.8 Hz), the chemical shift of which indicated it to be α to a carbonyl. H14 on the other hand resonated as a doublet at δ 2.46 (J 8.2 Hz). A further proton resonating at δ 2.30 as a broad doublet of doublets (J 14.4, 4.8 Hz), and found by decoupling experiments in the ^{1}H n.m.r. spectrum to be coupled to H13 (J 0.8 Hz), was assigned to equatorial H12β, the downfield shift indicating it lying in the deshielding zone of the carbonyl.

The above evidence therefore indicated the γ-lactone carbonyl to be at C16 and the hemiacetal to be at C15. N.O.e. experiments also further confirmed the hemiacetal to C15 as well as indicating H15 is β-disposed when n.O.e. enhancements were observed in H15β (and *vice versa*) on irradiation of H7β or 17Me.

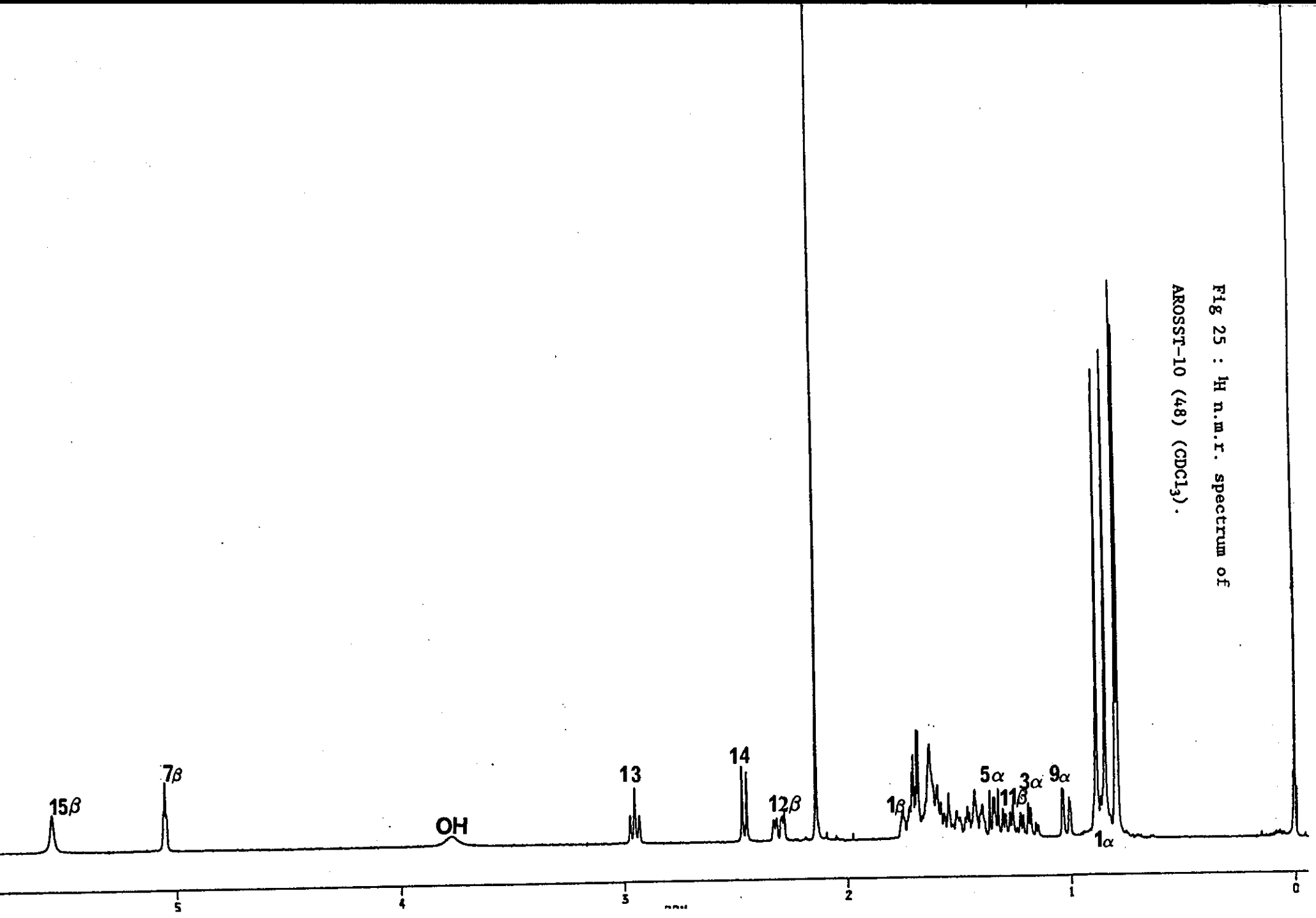


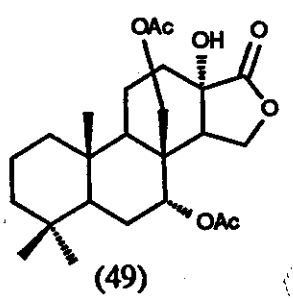
Fig 25 : ^1H n.m.r. spectrum of AROSST-10 (48) ($CDCl_3$).

Table 14. ^{1}H-^{1}H nuclear Overhauser enhancements in AROSST10 (48)

^{1}H irradiated	Signals enhanced (%)
18Me	19Me(5.3)
17Me	H7β(2), H15β(2)
H7β	17Me(3), H15β(7.1)
H15β	17Me(5.3), H7β(6.8)

(J) AROSST-11

AROSST-11 (49) the third of the three new minor metabolites containing the spongian (10) skeleton, was isolated as a colourless gum by h.p.l.c.



(49)

The ^{13}C n.m.r. spectrum (fully decoupled) showed signals for 24 carbons: the ^{1}H n.m.r. spectrum indicated 36 protons (integration). the e.i.m.s., although failing to give a molecular ion peak did however, by high resolution mass matching of the prominent peaks at *m/z* 376 ($C_{22}H_{32}O_5$ [M^+ – CH_3COOH]), *m/z* 334 ($C_{20}H_{30}O_4$ [M^+ – CH_3COOH – C_2H_2O]), and *m/z* 316 ($C_{20}H_{28}O_3$[M^+ – 2 x CH_3COOH]) established a molecular formula of $C_{24}H_{36}O_7$.

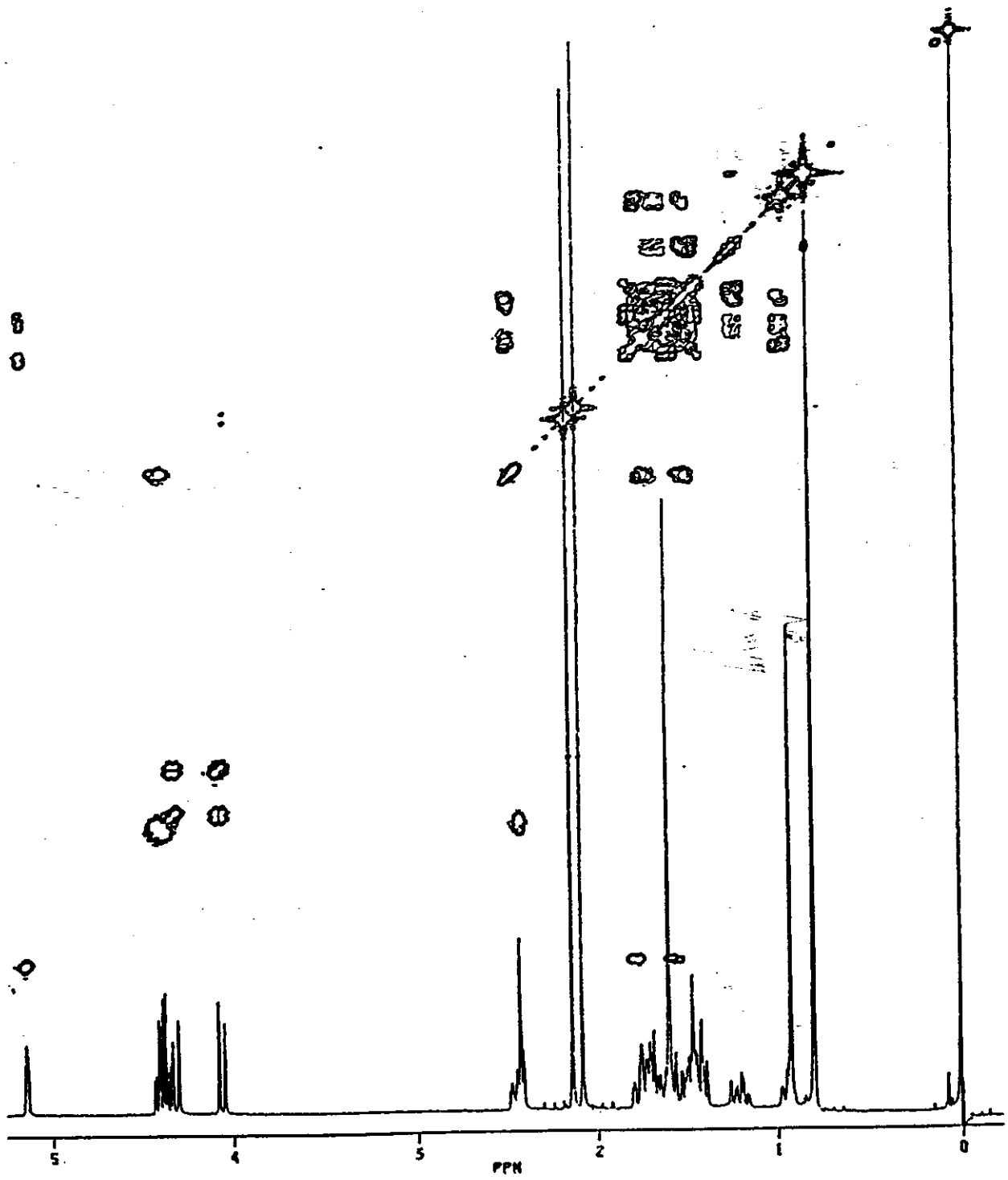
The i.r. spectrum contained two ester carbonyl absorptions at 1735 and 1774 cm^{-1} together with an OH absorption at 3345 cm^{-1}. The presence of two acetates was confirmed by two methyl singlets in the ^{1}H n.m.r. spectrum at δ 2.08 and 2.14 together with two ester carbonyl signals in the ^{13}C n.m.r. spectrum at δ 170.1 and 170.2. The presence of a γ-lactone, as suggested by the i.r. spectrum (1774 cm^{-1}), was confirmed by a further ester carbonyl signal in the ^{13}C n.m.r. spectrum at δ 177.6. Similarly the alcohol group, already suggested by the broad absorption band in the i.r. spectrum (3345 cm^{-1}), was further confirmed by the ^{13}C n.m.r. spectrum (only 35 protons attached to carbons, by DEPT) and the ^{1}H n.m.r. spectrum which showed a temperature dependant broad singlet at δ 2.40.

A comparison of the ^{1}H n.m.r. spectra of both AROSST-11 (Fig.26) and that of lactone (36) showed it to contain many similar features including, in the upfield region, a six proton singlet (two methyl groups) at δ 0.79 and a three proton multiplet at δ 0.92 together with the two acetate singlets (δ 2.08 and 2.14) already mentioned above. The absence of a fourth methyl group indicated the C17 methyl group was oxidised. In the downfield region, two AB quartets centred at δ 4.19 and 4.39, and a fine doublet of doublets at δ 5.14 were reminiscent of $(H17)_2$ $(H15)_2$ and H7β respectively in (36).

The major differences between the two ^{1}H n.m.r. spectra ~~was~~ were, in AROSST-11 the absence of a signal pertaining to H13 [δ 2.65 in (36)] and the inclusion of an OH signal (δ 2.40). The absence of both further unsaturation (i.r., ^{13}C n.m.r. and ^{1}H n.m.r. spectra), and of a downfield signal in the ^{1}H n.m.r. spectrum, indicating a proton α to an alcohol, together with the presence of a quaternary carbon in the ^{13}C n.m.r. spectrum at δ 73.8 suggested a tertiary alcohol was present (ie. quaternary carbon) and hence assigned to C13.

Decoupling experiments in the ^{1}H n.m.r. spectrum also showed that the

Fig 26 : 2D-COSY spectrum of AROSST-11 (49) ($CDCl_3$).



two further couplings in the AB system centred at δ 4.39 [δ 4.36 (*J* 5.7 Hz) and δ 4.41 (*J* 2.2 Hz)] were coupled to a doublet of doublets at δ 2.42 (*J* 5.7, 2.2 Hz) and assigned to H14. The absence of a further coupling in H14 from H13 further substantiated the oxidation of C13 to the alcohol.

The relative stereochemistry of the C13-alcohol as α-disposed was based on biogenetic grounds; all other ring oxidations in the aplyroseol series of compounds have been directed to the α-face.

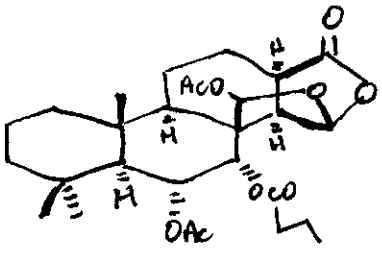
The *p*-Bromobenzoate of Aplyroseol-1 (51).

Features of the ^{1}H n.m.r. spectrum of (51) included a sharp singlet at δ 9.99 due to an aldehyde arising from the opening of the cyclic hemiacetal system in aplyroseol-1 and an AA'BB' system centred at δ 7.70 arising from the *p* - bromobenzoyl group. Signals due to the butyrate function were also present at δ 2.73 (α - CH_2), 1.69 (β - CH_2) and 0.98 (Me).

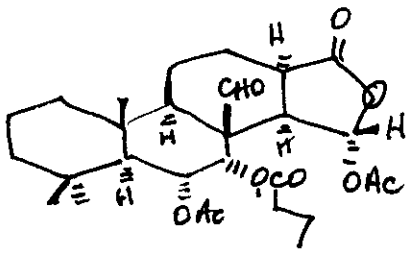
An interesting feature in the ^{1}H n.m.r. is the presence of a singlet at δ 6.37 (H15) and a doublet at δ 2.88 (H14) which was coupled to a multiplet at δ 3.12 (H13). Therefore since the vicinal coupling constant between H14 and H15 is zero, which can only arise if H14 and H15 are in an axial-equatorial orientation with a dihedral angle of approximately 90°, H15 is in an equatorial orientation and H14 in an axial orientation ($J_{13,14}$ 8.2 Hz).

The n.O.e enhancement of H15 upon irradiation of H7β (Table 15) also supports this assignment indicating the benzoyl group is axial and an inversion of configuration at C15 has occured.

Faulkner *et al.*[40] also reported that in the acetylation of aplyroseol-6 (34) a 1:1 mixture of the two isomers (34A) and (34B) were



(34A)



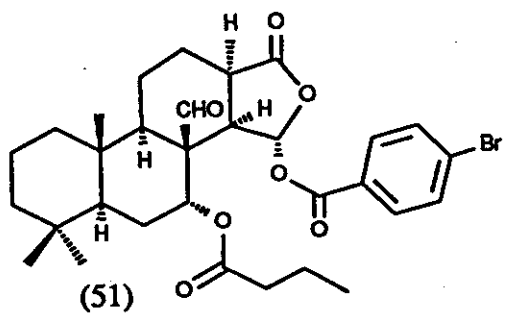
(34B)

obtained, (34B) also having an inversion of configuration at C15.

Although both the e.i.m.s and c.i.m.s. failed to show the molecular ion peak, high resolution mass matching of the highest peaks in the e.i.m.s. at *m/z* 516 and 514 [$M^+ - CH_3(CH_2)_2COOH$] did however confirm that a bromine was present in the *p*-substituted benzoyl group.

The structure of (51) including the inversion of configuration at C15 and the absolute stereochemistry was finally confirmed by X-ray diffraction analysis (Appendix 1).

The benzoylation therefore proceeds via first a base catalysed opening of the hemiacetal followed by an opening and subsequent closing of the lactol to give the less sterically hindered alkoxy isomer which can then be approached and trapped by the bulky *p*-bromobenzoyl group.



(51)

Table 15.: ^1H-^1H nuclear Overhauser enhancements in (51).

^1H irradiated	Signals enhanced (%)
H7β	H15(15).
H14	H13(7.7), H15(5.5).
H13	H14(10).

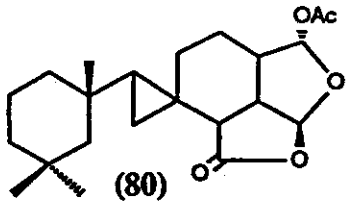
Chemical Reactions. The lactone (35), first isolated from *A. rosea* by Karuso[19], was re-extracted by the author from *A. rosea* and isolated in a higher concentration than that originally reported (see Table 6). Due to the quantity of sample collected (149 mg) and because of its close relationship to the hypothetical precursor spongian (10), a synthetic scheme to (10) was proposed (Scheme 4).

The lactone (35) on treatment with lithium aluminium hydride in ether afforded the triol (52). In the i.r. spectrum, the absence of any carbonyl absorption bands and the presence of a strong broad OH absorption band at 3323 cm^{-1} indicated the complete reduction of (35) to the triol (52). The presence of the alcohols was further substantiated by the e.i.m.s. which although only showing the molecular ion peak *m/z* 324 weakly (< 1 %), did show fragment ions at *m/z* 306, 288 and 273. High resolution mass matching of the *m/z* 288 and 273 peaks confirmed them as resulting from the loss of two units of water (*m/z* 288) followed by the loss of a methyl group (*m/z* 273) from the molecular ion (*m/z* 324). In the ^1H n.m.r. spectrum the four methylene protons α to the C15 and C16 alcohols, $(H15)_2$ and $(H16)_2$ resonated as a three proton multiplet from δ 3.40 to 3.53 together with a one proton doublet of doublets at δ 3.88 (*J* 11.2, 7.0 Hz). H7β resonated as a fine doublet of doublets at δ 3.62 (*J* 2.8, 2.8 Hz) consistent with a cyclohexane ring equatorial proton α to an alcohol.

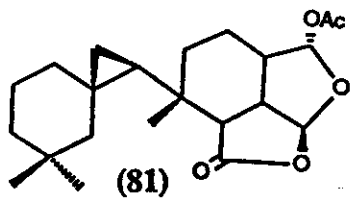
The second step in the scheme was a cyclization of the C15, C16 alcohols to the cyclic ether (53). Two procedures for cyclizing 1,4-diols were investigated. The first method, reported by Gillis and Beck[34], involved heating the triol (52) in dimethyl sulphoxide at 140° for 24 h

while the second method, reported by Erickson and Fry[35] involved refluxing (52) in a solution of triphenylphosphine, carbon tetrachloride and acetonitrile for 24 h. Both afford the cyclic ether (53) in reasonable yields.

The cyclic ether (53) also only gave a very weak molecular ion peak in the e.i.m.s. at *m/z* 306 (1%) which corresponded to a molecular formula $C_{20}H_{34}O_2$. The molecular formula was further substantiated by the high resolution mass matching of the first two fragment ions *m/z* 304 [$C_{20}H_{32}O_2(M^+ - H_2)$] and *m/z* 302 [$C_{20}H_{30}O_2(M^+ - 2 \times H_2)$] together with the ^{13}C n.m.r. spectrum which showed signals for 20 carbons. The i.r. spectrum showed signals for 20 carbons. The i.r. spectrum showed an OH absorption band for the C7 alcohol at 3435 cm^{-1} and was further supported by the ^{13}C n.m.r. spectrum which showed 33 protons attached to 20 carbons (by DEPT), and the mass spectrum which showed the prominent loss of water (18 daltons) from the molecular ion (*m/z* 306) to give *m/z* 288. The α-hydroxy proton H7β resonated at δ 3.62 as a fine doublet of doublets (*J* 3.2, 2.4 Hz), consistent with an equatorial proton α to an alcohol. The ^1H n.m.r. spectrum also clearly showed the two protons, H13 and H14, as a multiplet at δ 2.47 and a doublet of doublets at δ 2.67 (*J* 6.2, 5.4 Hz) respectively. Subsequent decoupling experiments in the ^1H n.m.r. spectrum involving H13 and H14 enabled the methylene protons, H15α, H15β, H16α and H16β, α to the other oxygen to be assigned.



(80)



(81)

1.2.3 *Aplysilla var. sulphurea*

Aplysilla var. sulphurea is a yellow encrusting sponge of the order Dendroceratida. The sponge in general appearance and texture is not unlike that of *Aplysilla sulphurea*, the major difference being the slightly greater thickness attained by *A. var. sulphurea*.

An initial extraction of *A. sulphurea* by Karuso[20] yielded aplysulphurin (14) and a second metabolite aplysulphuride [(80) or (81)], the structure of which could not be confidently assigned.

However on all subsequent collections no aplysulphuride was obtained and it was believed the initial collection was contaminated by a morphologically similar sponge.[31,36] Consequently a search for this sponge was commenced.

Only one patch of *A. var. sulphurea* was ever found. It was located on the top surface of a small underwater cave at Bare Is. Sydney. All other collections of structurally similar yellow encrusting sponges from outside this cave proved to be *A. sulphurea*: they contained only aplysulphurin (t.l.c.).

Fortunately the regeneration of *A. var. sulphurea* was rapid and two collections were made, one in Jan 1989 (summer) and another in May 1989 (winter).

The winter collection (water temp. 16°) yielded only one metabolite identified as 15,16-diacetoxyspongian (54) while in the summer collection (water temp. 20°), (54) and another metabolite 16-oxospongian (55) were isolated.

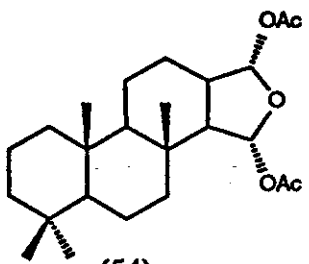
From the crude extract no resonances pertaining to the cyclopropyl ring of aplysulphuride were indicated in the ^{1}H n.m.r. spectrum.

Both (54) and (55) have been previously reported in the literature, (54) isolated from *Spongia officinalis* (order Dictyoceratida)[16] and (55)

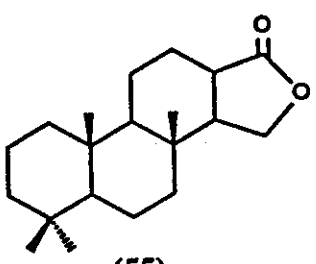
synthetically prepared by the hydrogenation of another metabolite from *S. officinalis*.[17] Poiner also isolated (54) and (55) from the sponge *Chelonaplysilla violacea* (order Dendroceratida).[21]

The structures of the two metabolites isolated from *A. var. sulphurea* (54) and (55) were established by the direct comparison of their ^1H n.m.r. spectra [Fig 27 (54); Fig 28 (55)] and mass spectra with those of the authentic samples isolated by Poiner.

Although the desired sponge containing aplysulphuride was not found another sponge morphologically similar to *A. sulphurea* had been found and extracted.



(54)



(55)

Table 16.: Diterpenes from *A. var. sulphurea*

Collection (g)	(54) mg (%)	(55) mg (%)
Jan 1989 (5.3)	129(2.4)	23(0.4)
May 1989 (1.98)	28.7(1.45)	–

Fig 27 : ^1H n.m.r. spectrum of

15,16-Diacetoxyspongian (54) ($CDCl_3$).

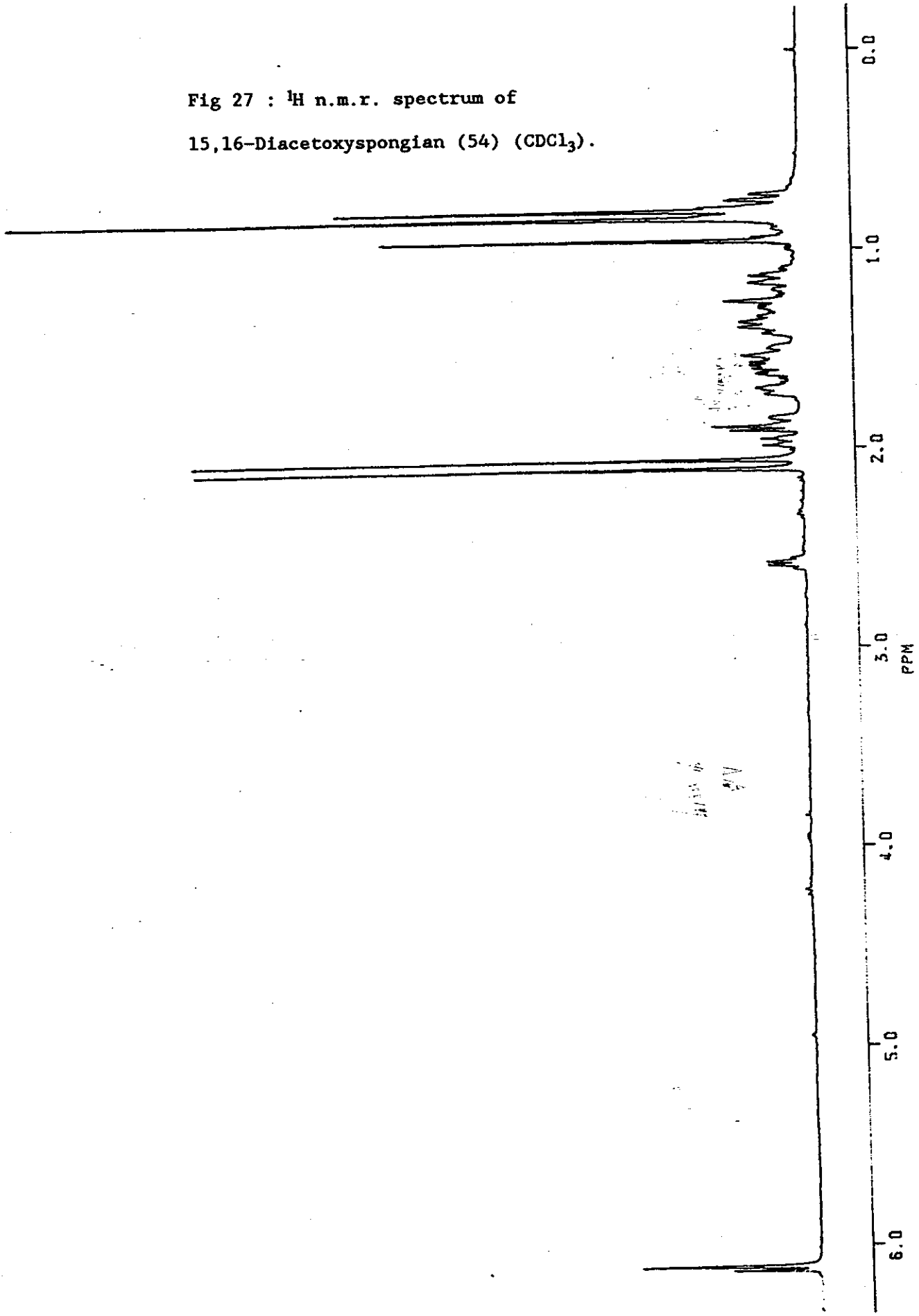
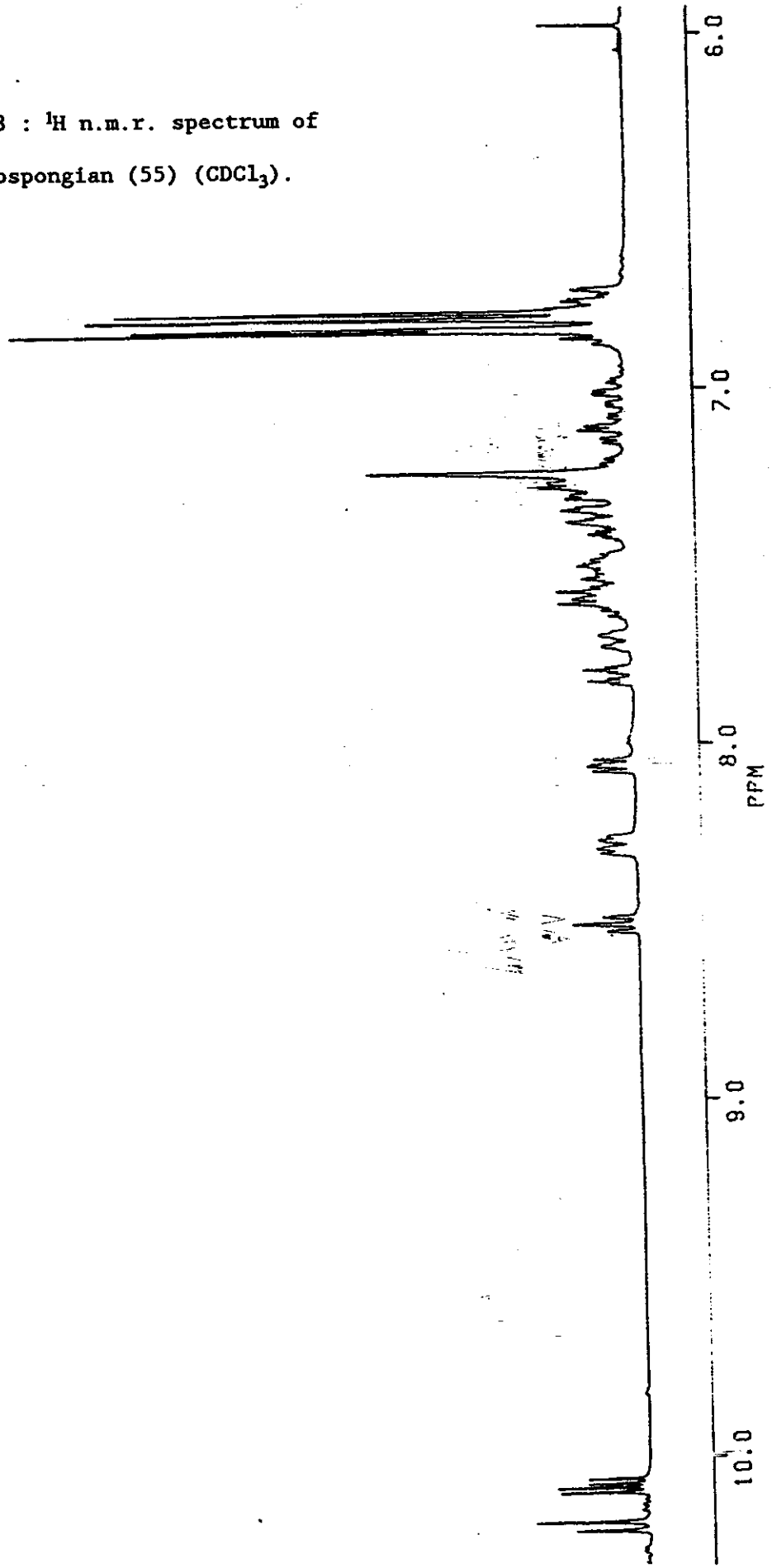


Fig 28 : ^1H n.m.r. spectrum of 16-Oxospongian (55) ($CDCl_3$).



1.2.3 Biogenesis

To arrive at the absolute stereochemistry shown for the *A. var. sulphurea*, *A. rosea* and *A. pallida* diterpenes, it was necessary first to consider the biogenesis of the system.

It is reasonable to assume that the compounds are all related to the hypothetical tetracyclic diterpene, spongian (10). Although it is peculiar to compounds of marine origin the spongian skeleton is a regular terpenoid system, closely related to many terrestrially-derived diterpenoids. Indeed the stereospecific cyclization, proposed by Fenical, of all *trans*-geranylgeranyl pyrophosphate to give (10), involves only well known chemistry.[13]

Isoagathalactone (11), isolated from *Spongia officinalis*, was the first such diterpene containing the spongian backbone. The absolute stereochemistry was determined by chemical correlation with grindelic acid of known stereochemistry.[14] The same sponge also yielded three further compounds, (60)-(62), which may be considered precursors of isoagathalactone.[16] Another sponge, *Spongia sp.* closely related to *S. officinalis* and collected from the Great Barrier Reef afforded variously oxidised tetracyclic furanoditerpenes, (70)-(73), which can be thought of as derived from (10). Their absolute stereochemistry was determined by CD and ORD studies.[15]

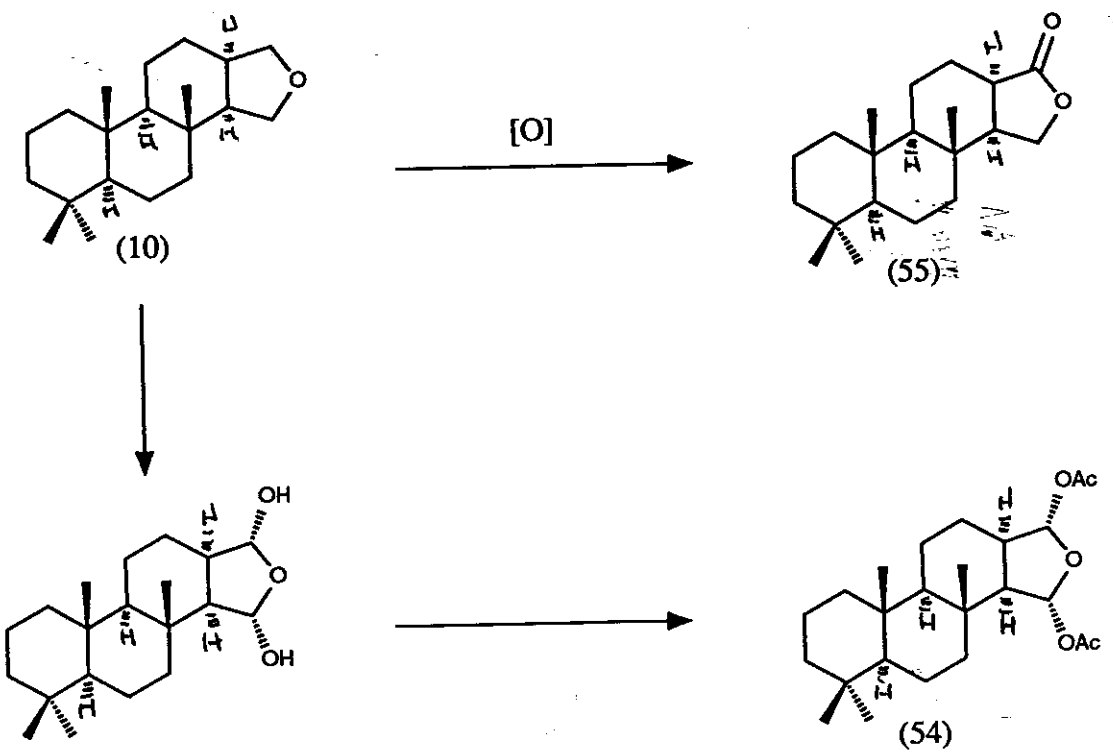
As described in Section 1.2.2K of this thesis, part of the further study into the constituents of *A. rosea* involved the determination of the absolute sterochemistry of aplyroseol-1 (30). This was achieved by a single crystal X-ray determination of the p-bromobenzoate derivative (51) of aplyroseol-1.

From the results, the absolute stereochemistry is the same as that proposed for spongian (10), and it may also be inferred that all the

aplyroseols have the same stereochemistry.

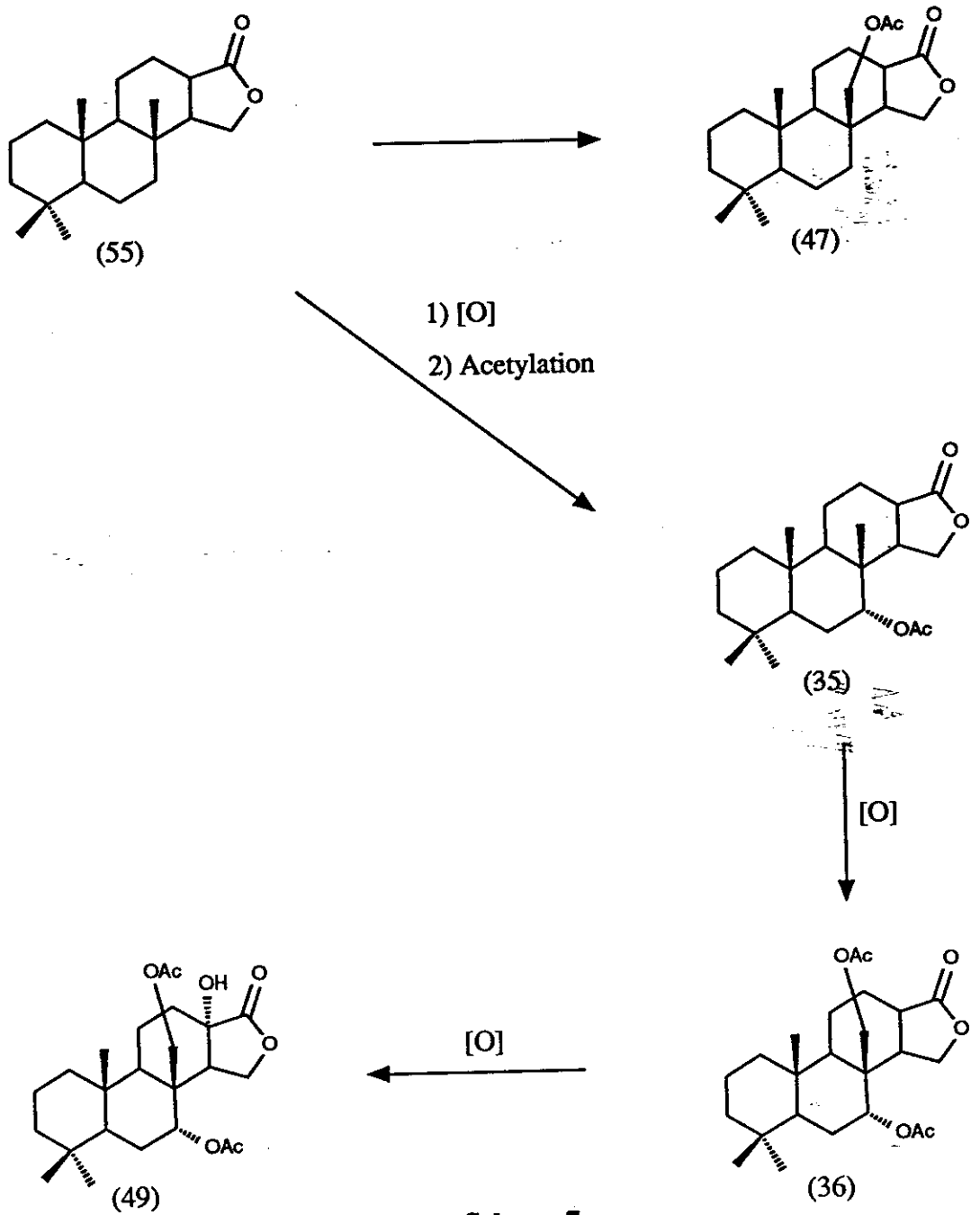
As the spongian derived metabolites isolated from *S. officinalis*, *Spongia sp.* and *A. rosea* all have the same absolute sterochemistry, it is reasonable to postulate that all the spongian derivatives originated from the one configuation of (10).

It is a reasonable assumption that the two diterpenes isolated from *A. var. sulphurea*, (54) and (55), have the same absolute stereochemistry as proposed for (11), isolated from *S. officinalis*. The biogenesis of these compounds may be assumed, since they both contain the full spongian skeleton, to be simply the result of oxidation of spongian. Oxidation at C16 yields (55) while oxidation followed by acetylation of both C16 and C15 would afford (54) (Scheme 6).



Scheme 6

Schemes (7)-(11) suggest possible biogenetic pathways for the *A. rosea* metabolites. Starting with (55) which has been isolated from both *A. var. sulphurea* and *Chelonaplysilla violacea*, oxidation and acetylation of C17 would result in AROSST-9 (47) (Scheme 7).



Scheme 7

Alternately (Scheme 7), oxidation and acetylation at C7 results in the lactone (35). Further acetylation of C17 would then give lactone (36) which then upon oxidation of C13 gives AROSST-11 (49).

From the lactone (35) (Scheme 8), oxidation of C15 would give the alcohol AROSST-10 (48). Methylation of the lactone carbonyl of (48), followed by ring opening of (I) could give the methyl ester-aldehyde AROSST-6 (44). This aldehyde was isolated as the natural product (identified by ^{1}H n.m.r.) however it was found to oxidise rapidly to the acid (44A) which was then fully characterised.

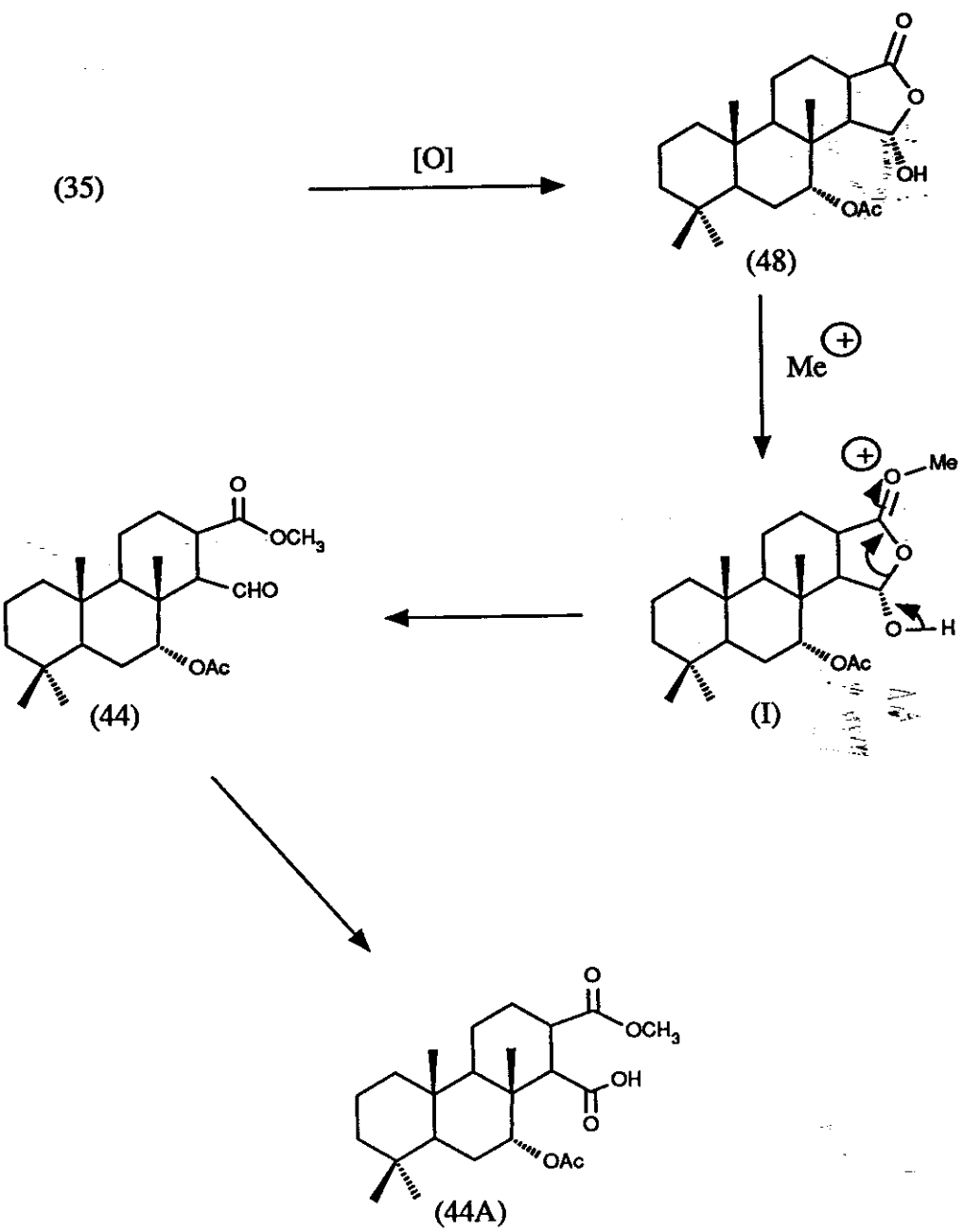
The biogenesis of the aplyroseols (30)-(34) (Scheme 9) may also be envisaged as starting from (55). With oxidations and esterification occurring at C7 and/or C6 could give (II). This upon further oxidation at C15 to the alcohol (III) [which has been shown can occur by the isolated product AROSST-10 (48)] and then C17 to the aldehyde could then give the intermediate (IV).

From all the spongian derived metabolites thus far isolated which have been oxidised at C15, the alcohol has resided in an α-orientation, for example (15), (48) and (54). Therefore it would not be unreasonable to suggest that the alcohol in intermediate (IV) would also reside in an α-orientation. Hence first an isomerization at C15 giving the β-alcohol intermediate (V) followed by acetal formation would then give the aplyroseols (30)-(34).

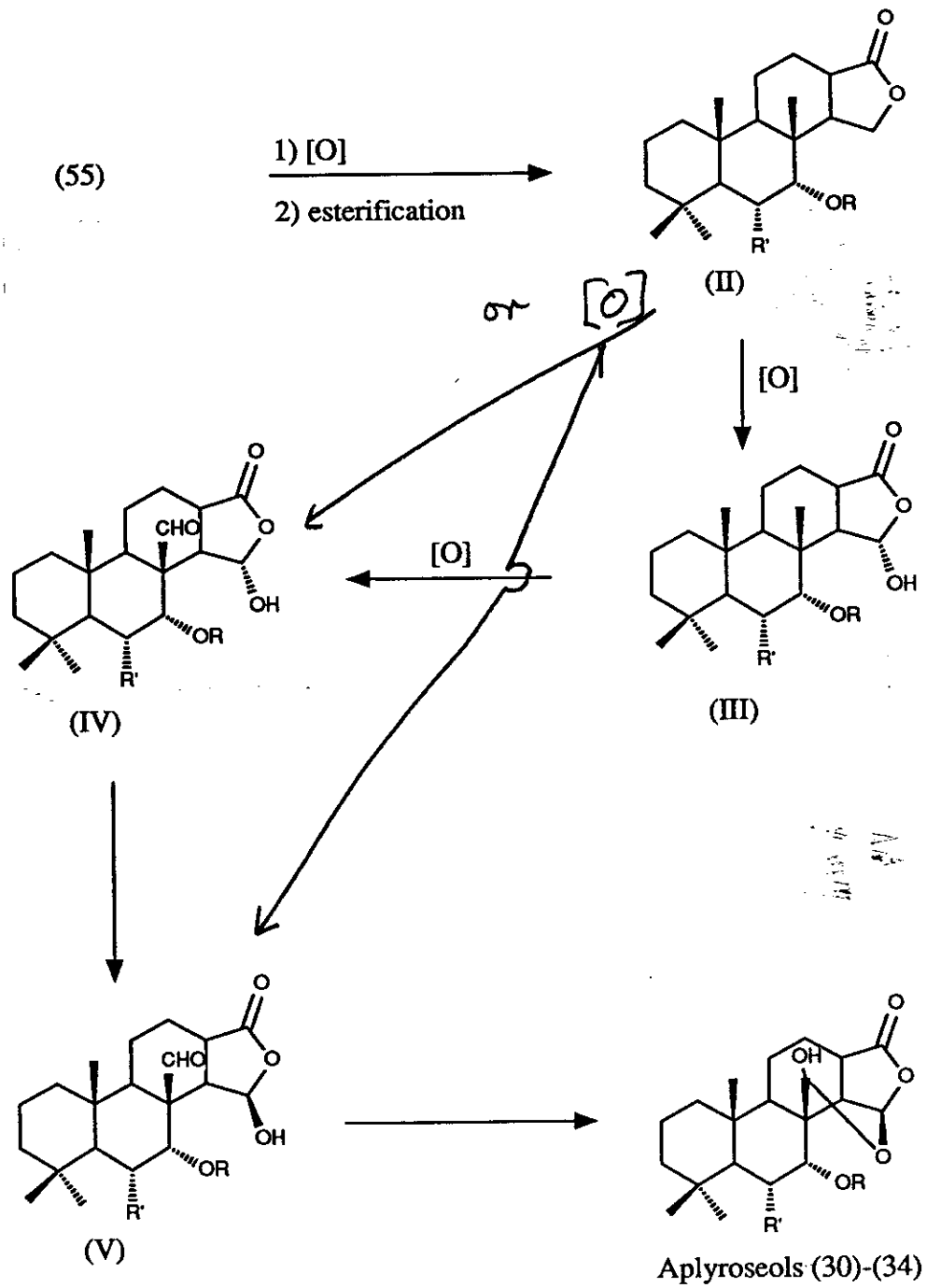
Alternatively, (Scheme 10) methylation of the lactone carbonyl in (IV) followed by ring opening could then give the dialdehyde (VI), which via a Cannizzaro-type reaction (oxidation/reduction) could be converted to intermediate (VII). Lactonization of this intermediate could then give AROSST-1(39) to -5(43).

The biogenesis of AROSST-7 (45) and AROSST8 (46) (Scheme 11) may be regarded as an extension from AROSST-6 (44). The ease of aerial oxidation

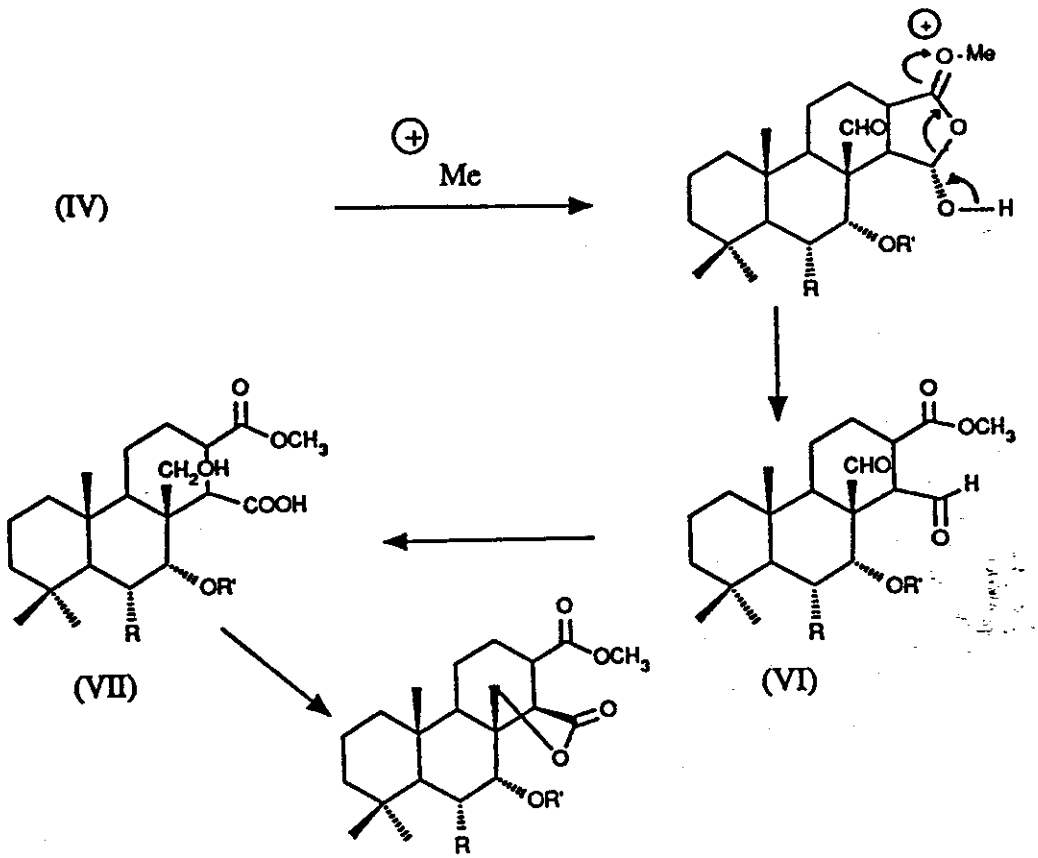
encounted in the case of AROSST-6 to the acid (44A) may indicate that in the natural system this oxidation could also proceed with ease. Further oxidation to the hydroperoxide (VIII) which upon decarbonylation to the ketone (IX) followed by α or β face reduction could give AROSST-8 (46) and AROSST-7 (45) respectively.



Scheme 8

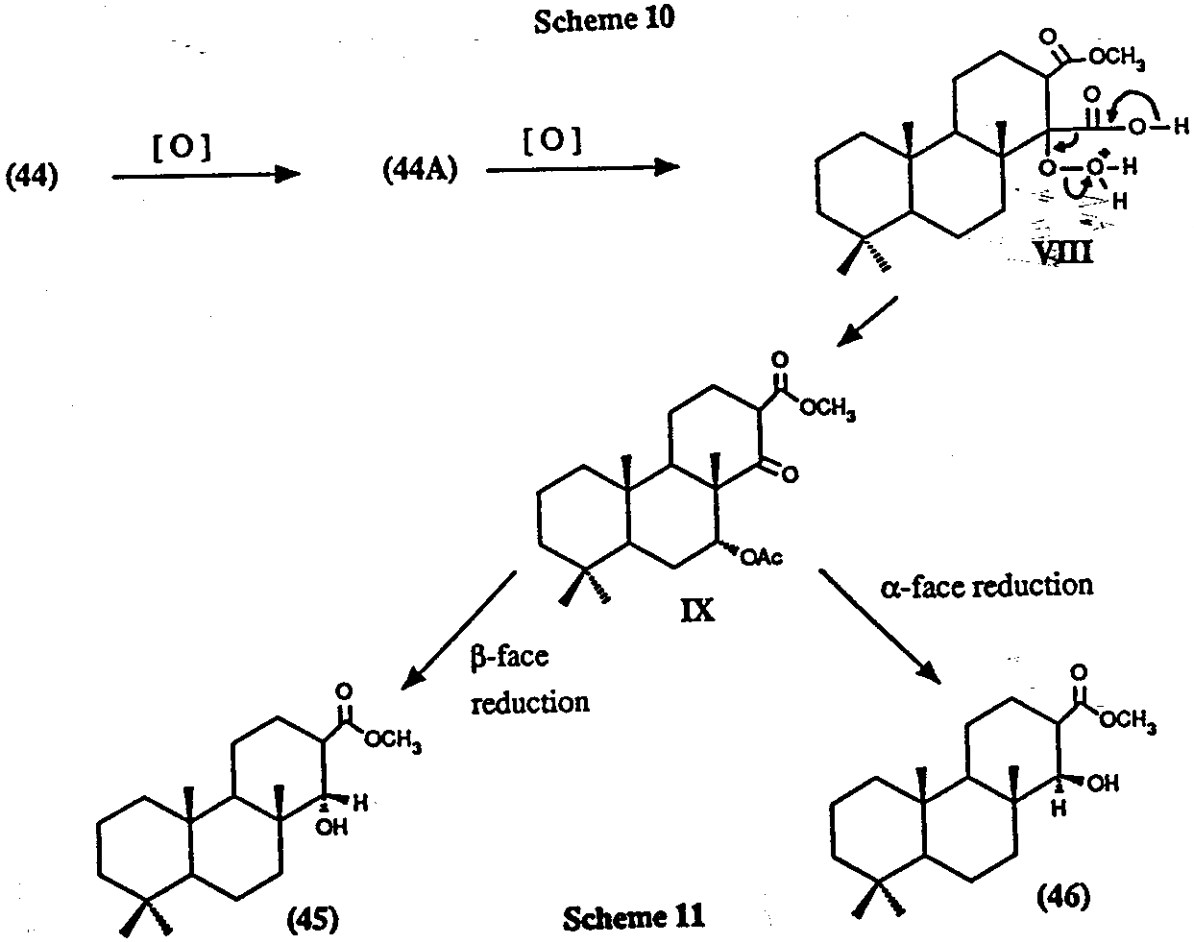


Scheme 9



AROSST-1 (39) to -5 (43)

Scheme 10

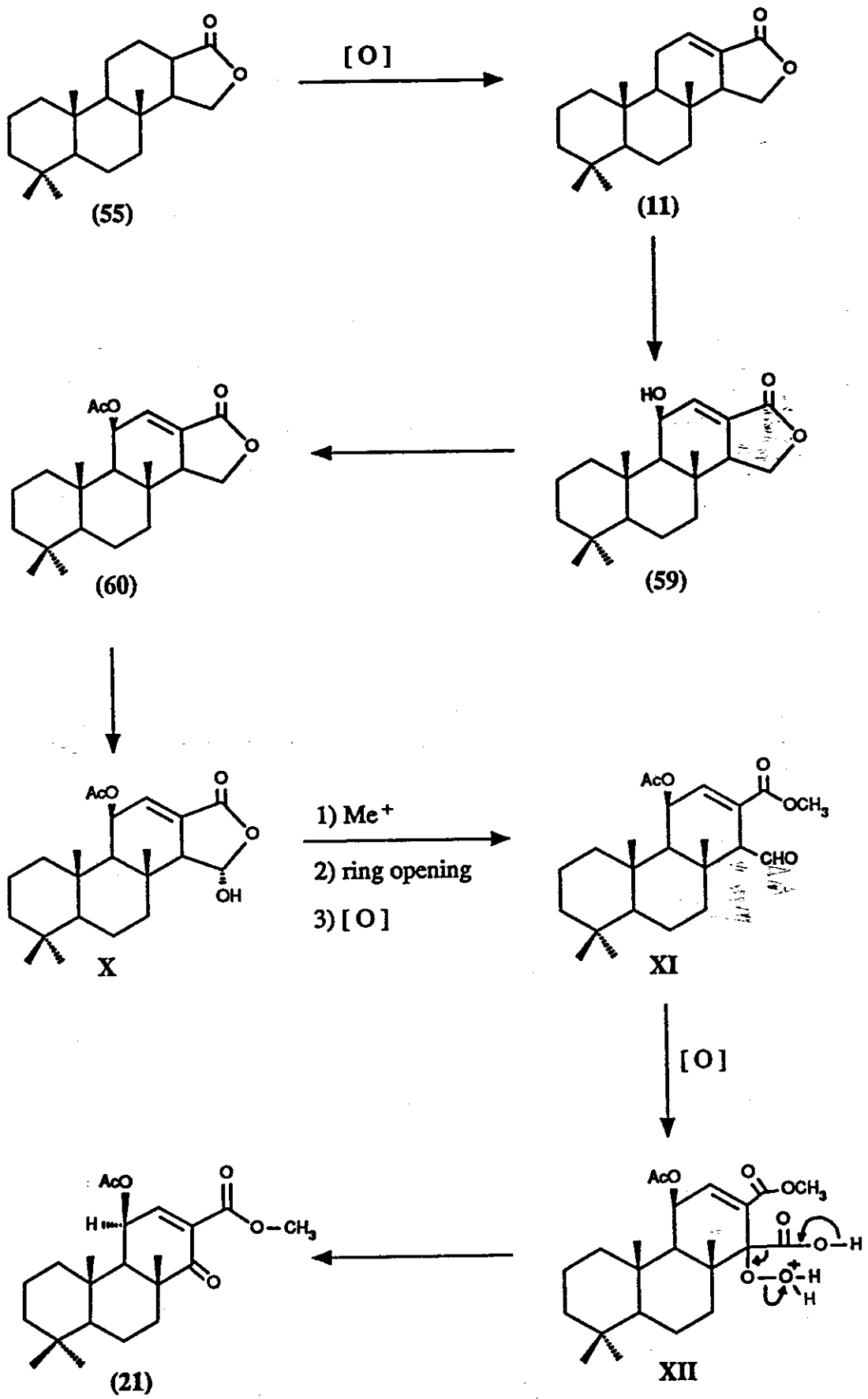


Scheme 11

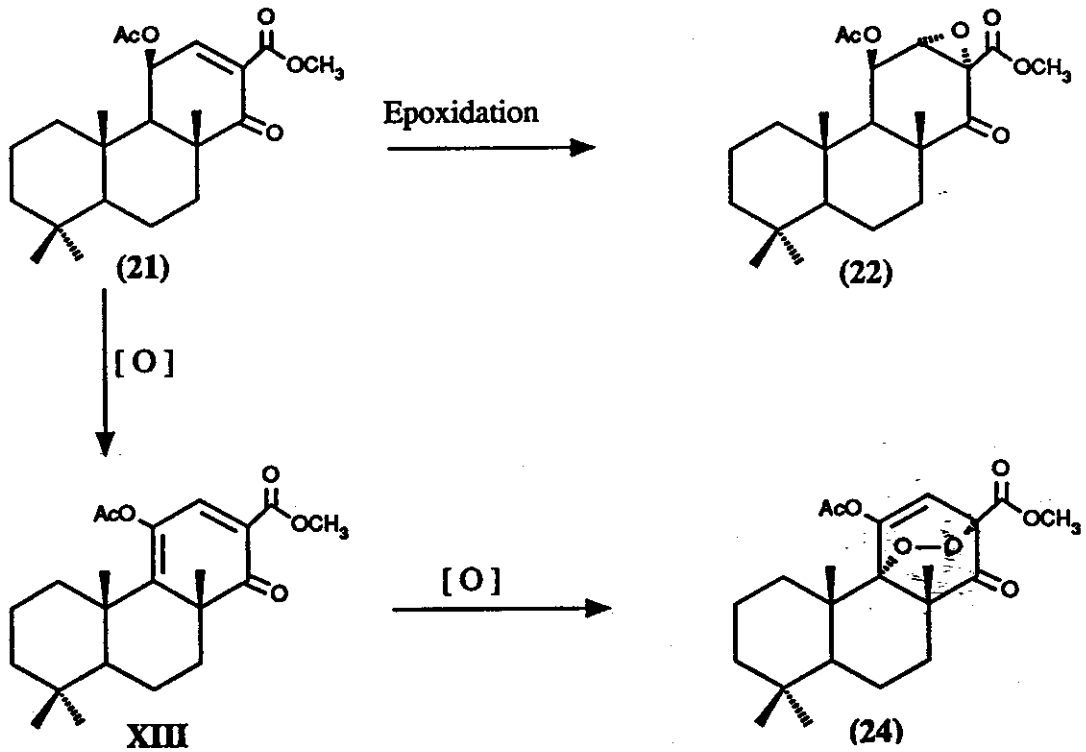
The biogenesis of the *A. pallida* metabolites (Schemes 12-14) may also follow a similar pathway as that proposed for the *A. rosea* metabolites. However, oxidation occurs at different locations.

Again starting from the lactone (55) oxidation to the α-β unsaturated lactone gives isogathotholactone (11). Further C11 allylic oxidation followed by acetylation would give (59) and (60) respectively. The compounds (59)-(60) have also been previously isolated from *Spongia officinalis*. Oxidation at C15 to the β-alcohol (X) proceeded by methylation of the lactone carbonyl, ring opening and oxidation could then give the intermediate (XI) similar to AROSST-6A (44A). Further oxidation of the C14 acid to the hydroperoxide (XII) followed by decarbonylation could give aplypallidenone (21) (Scheme 12). Epoxidation of (21) (Scheme 13) then would give aplypallidoxone (22). Alternatively, oxidation of aplypallidenone (21) to the diene (XIII), could then further undergo oxidation to form the aplypallidioxone (24).

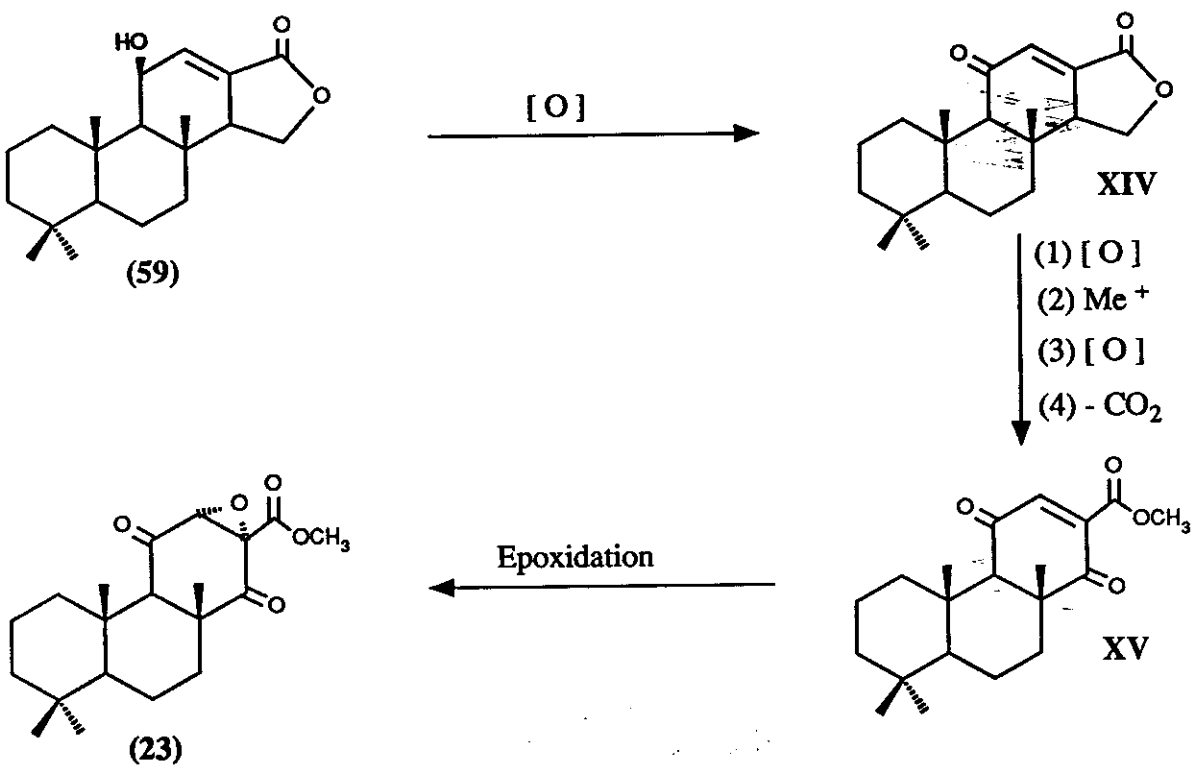
A further oxidation of (59) (Scheme 14) to the ketone (XIV), followed by an oxidation, methylation and ring opening, then further oxidation to the peroxide and finally decarbonylation could then give the diketone (XV). Epoxidation of this diketone would then result in aplypallidione (23).


[O]
(55)
(11)
HO
(59)
AcO
(60)
AcO
OH
X
1) Me+
2) ring opening
3) [O]
AcO
OCH3
CHO
XI
[O]
AcO
OCH3
XII
AcO
H
O—CH3
(21)

Scheme 12



Scheme 13



Scheme 14

1.2.5 Sponges Containing Spongian Derived Metabolites

One of the aims of a chemical study into the constituents of marine sponges is its possible application to taxonomy. However for this to become applicable a large enough number of samples must be examined and correlated with the existing taxonomic base.

One important group in which chemotaxonomy could show great benefit is within the so called 'Horny' sponges (Keratosa): the Verongids, Dictyoceratids and Dendroceratids. This group of sponges have no spicules, the traditional method by which sponges are identified. Hence, the use of the metabolites present in these sponges in conjunction with other ultrastructural, histological and reproductive characteristics may offer a reliable method for identification within this group, and reinforce the identifications in other groups of sponges.

Already, the Verongids, although morphologically similar to the other two orders, are found to be chemically distinct. They are, to date, all characterised by possessing brominated tyrosine derivatives and a high steroid content. On the other hand sponges belonging to the orders Dictyoceratida and Dendroceratida are characterised by a high terpene content.[2]

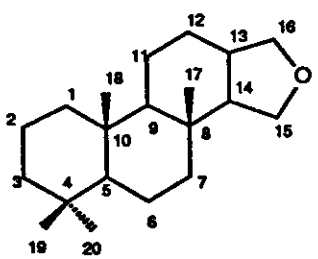
An extension to the use of chemotaxonomy is its possible ability to show convergent evolution and affinities within different groups of sponges. For example three genera within the order Dictyoceratida (Smenospongia, Fasciospongia and Therectandia) have been found to possess brominated amino acid derivatives. These genera are now viewed as intermediates between the Verongida and the Dictyoceratida. The histology, pigmentation and fibre structure within the above genera, although not identical with those of Verongida, do show some similarities.[2]

However it is only now, with the recent increase in the number of

Dendroceratids investigated, that a possible affinity of this group with the Dictyoceratids is emerging.

The major biological difference between the two orders is the arrangement of their spongin fibres. In Dictyoceratids they are constructed upon a complex anastomosing pattern of primary and secondary fibres (Fig 2). In Dendroceratids, the pattern is less complex with spongin fibres arranged in a dendridically ramified pattern (Fig 3).

Set out below are the sponges and their terpenoid metabolites which may be derived from the hypothetical precursor spongian (10).



(10)

In the arrangement an attempt has been made to group together these sponges and their metabolites in such a way as to show a possible affinity between the sponge genera and between the two orders Dictyoceratida and Dendroceratida.

Although the first isolation of a metabolite with the spongian backbone was from *Spongia officinalis*,[14] a Dictyoceratid, the majority have been from the order Dendroceratida.

Bergquist (1980)[9] has listed three families containing nine genera in the order Dendroceratida. They are as follows:-

Order - Dendroceratida (Phylum; Porifera; Class, Demospongiae; subclass ceractinomorpha)

Family - Aplysillidae

Genus - *Aplysilla*, *Pleraplysilla*, *Chelonaplysilla*, *Darwinella*, *Dendrilla*, *Hexadella*.

Family - Dictyodendrillidae

Genus - *Dictyodendrilla*, *Igernella*

Family - Halisarcidae

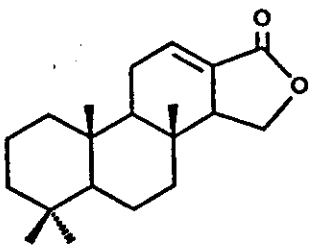
Genus - *Halisarca*

Spongia Officinalis (Dictyoceratida):[14,16,17]

The first reported metabolite containing the spongian backbone was isogatholactone (11), isolated from *S. officinalis* collected in the Bay of Naples, Italy.[14] A further report on the metabolites from this sponge described the diterpenes (54) and (60)-(62).

The extraction of *S. officinalis* collected from the Canary Is. also yielded (11) together with aplysillin (12) and the new but similar diterpenes (63) - (66).[17]

From still another specimen, also identified as *S. officinalis*, the new diterpenes (67)-(69) were isolated.[37] In this case however the sponge species may have been misidentified due to the atypical nature of these metabolites to those of the other *S. officinalis* metabolites.



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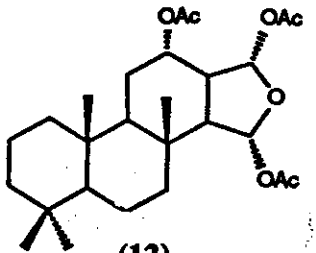
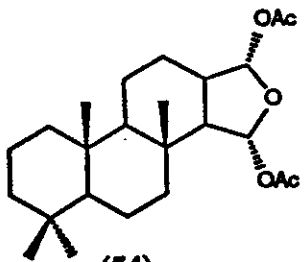


(12)



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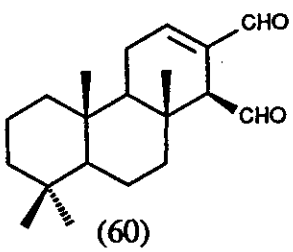

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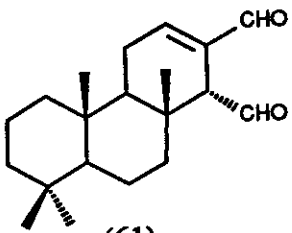

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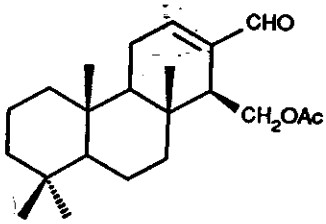
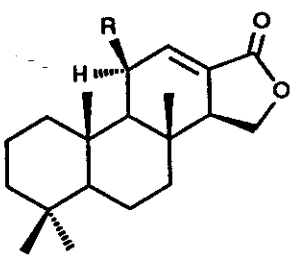
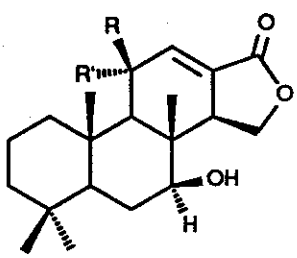


(62)



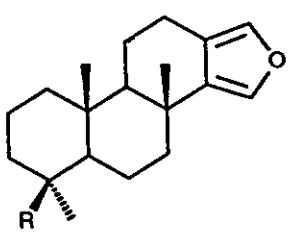
(63) R=OH

(64) R=OAc



(65) R=H R'=OH

(66) R=OH R'=H



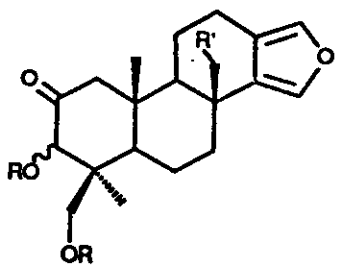
(67) R=Me

(68) R=CHO

(69) R=COOH

Spongia sp:[15]

Eight new tetracyclic furan diterpenes (70)-(73) were isolated from a closely related sponge to *S. officinalis*, described as *Spongia sp.*, and collected from the Great Barrier Reef, Australia, eight new tetracyclic furan diterpenes were isolated.



3α and 3β

(70) R=R'=H

(71) R=Ac R'=H

(72) R=H R'=Ac

(73) R=R'=Ac

Other *Spongia* species which have been investigated to date have yielded sesterterpene metabolites containing structures such as (8) including one collection of *S. officinalis*.[38] Yet other species of *Spongia* have yielded furanoterpenes of the type (7) and (9) including *S. officinalis*.[39]

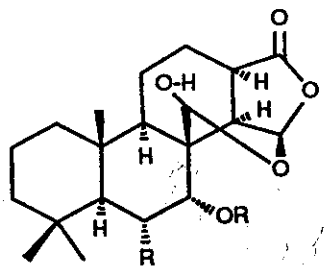
Aplysilla var. sulphurea:

This sponge which is very similar in appearance to *A. sulphurea* was extracted and the diterpenes isolated (54) and (55) discussed in Section 1.2.3.

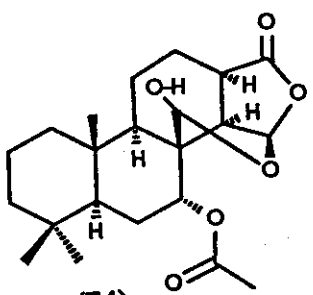
Aplysilla rosea:

The first report of the extraction of *A. rosea* described the isolation of the triacetate aplysillin (12), from a New Zealand specimen.[18] More recently, some sponge samples of *A. rosea* from N.S.W. waters have yielded a series of alcohol diterpenes named the aplyroseols (30) - (34) and (74) together with the lactones (35)-(36).[19] Current results, described in Section 1.2.2, from a large scale collection of *A. rosea*, also collected from N.S.W. waters, again was found to contain (30)-(36) together with the further diterpenes (37)-(49). In another report, a sponge described as *Aplysilla sp.* was found to contain two of the aplyroseols, (30) and (31),

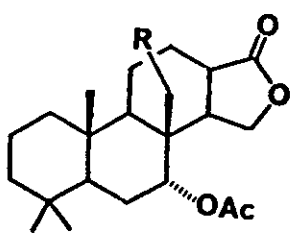
together with another diterpene (75). From the constituents and the fact that the authors describe this sponge, from Port Phillip Bay, as pink and encrusting, it is probably *A. rosea*.[40]



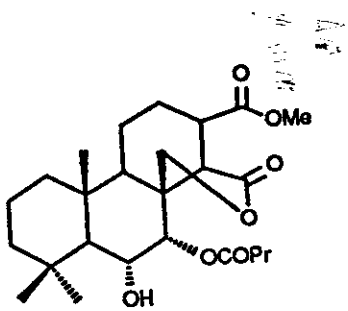
	R^1	R^2
(30)	COC_3H_7	H
(31)	COC_3H_7	OH
(32)	COC_3H_7	OAc
(33)	H	$OCOC_3H_7$
(34)	Ac	$OCOC_3H_7$



(74)



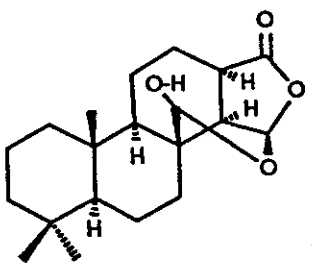
(35) R= H
(36) R= OAc



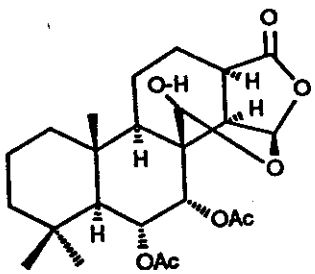
(75)

Dendrilla rosea:[26]

The aplyroseols (30), (31), (33), (34) and (74) together with the dendrillols (37)-(38) and (76)-(77) were isolated from a New Zealand collection of *D. rosea*.



(37)



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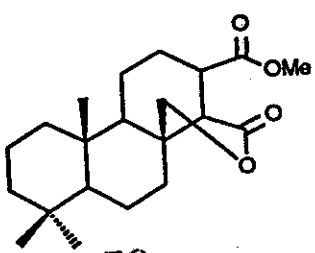

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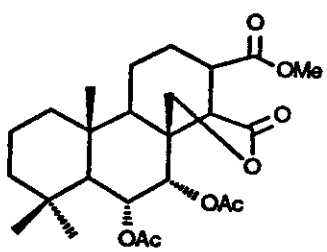
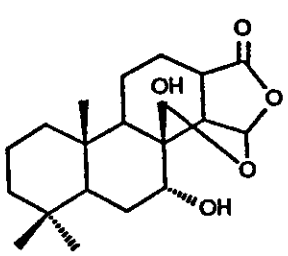


(77)

Igemella notabilis (Family Dictyodendridae):[41]

The two aplyroseols (30) and (74) together with the diol (78) were extracted from the Caribbean sponge *I. notabilis*.



(78)

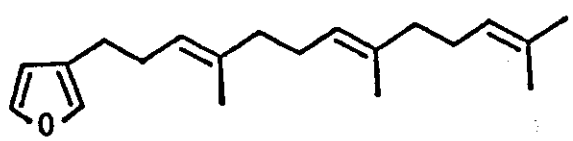
Aplysilla pallida (Family Aplysillidae):

The diterpenes from *A. pallida* (21) - (24) are described in Section 1.2.1

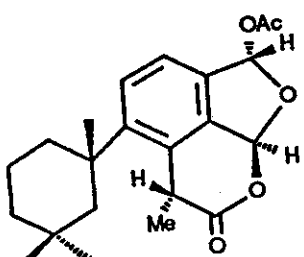
Again the three six-membered rings of the spongian system are present and hence may be derived from spongian.

Darwinella sp.:[26]

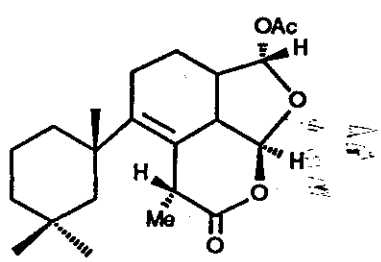
This encrusting sponge collected from New Zealand waters was reported as being formerly referred to as *Aplysilla rosea*. It was found to contain the furanoditerpene (28) together with aplysulphurin (14) and as the major constituent tetrahydroaplysulphurin-1 (79). No trace of aplysillin (12), as was reported from the first extraction of *A.rosea*, was detected in the sample.



(28)



(14)



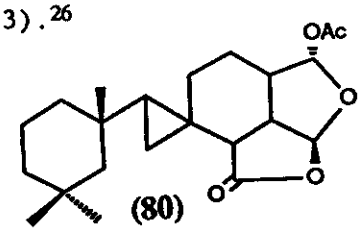
(79)

Darwinella oxeata:[26]

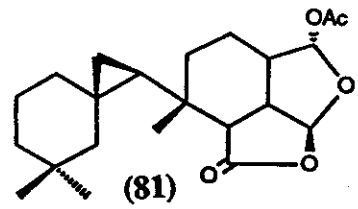
Specimens of this sponge have been extracted from two countries Australia (N.S.W. south coast) and New Zealand (Leigh, Poor Knights and Dunedin). In the Australian samples, which were referred to as *Aplysilla sulphurea*, two compounds were isolated: aplysulphurin (14) and aplysulphuride (80) or (81) (the exact structure was not established).[20,31] However aplysulphuride was only obtained in the first collection of *A. sulphurea*; all subsequent collections were free of aplysulphuride. Hence this metabolite remains a mystery and has been suggested that it may be

from a morphologically similar sponge to that of *A. sulphurea*.[31,36]

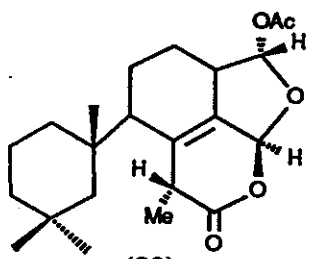
From the New Zealand specimens, referred to as *D. oxeata*, four metabolites were obtained; aplysulphurin (14), tetrahydroaplysuphurin-1 (79) and two other minor new metabolites tetrahydroaplysulphurin-2 (82) and -3 (83).[26]



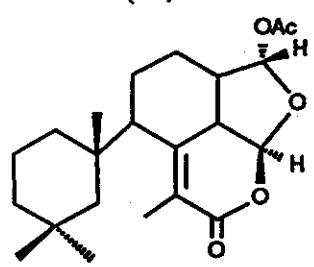
(80)



(81)



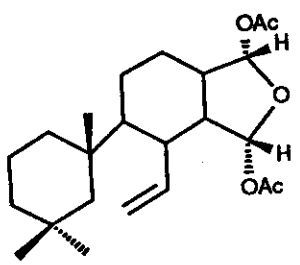
(82)



(83)

Dendrilla membranosa:[42,43]

From the Antarctic specimens of *D. membranosa* the two metabolites (84) and (85), related to those of *Darwinella oxeata*, were isolated.[42] Later work on this sponge also yielded the yellow pigment (86)[43].



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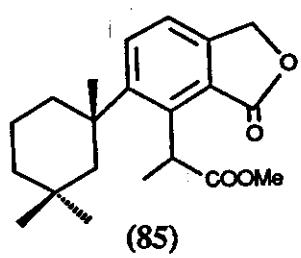
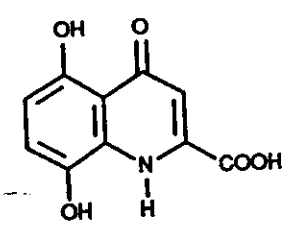


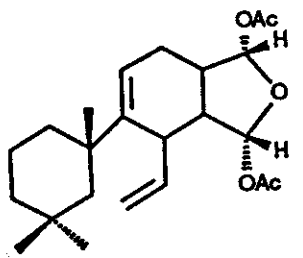
(85)



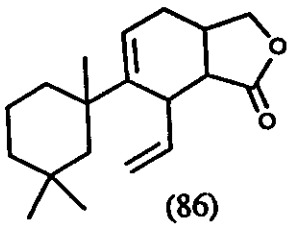
(86)

Aplysilla tango:[21]

Aplysilla tango, a new species collected from Jervis Bay, N.S.W. was found to contain (15) along with two related compounds (86) and (87) together with two further compounds (88) and (89).



(15)



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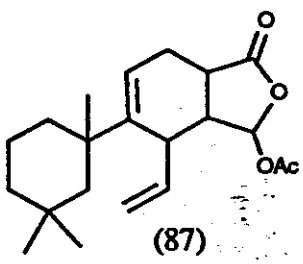
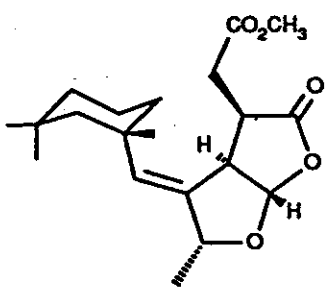
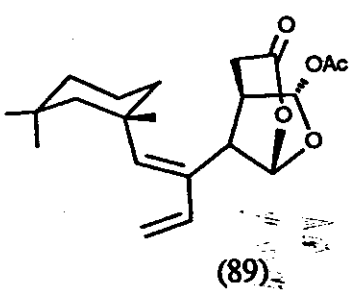


(87)



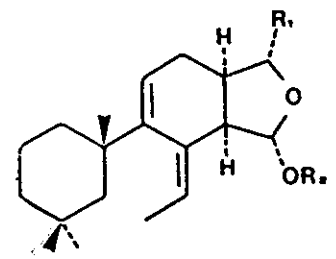
(88)



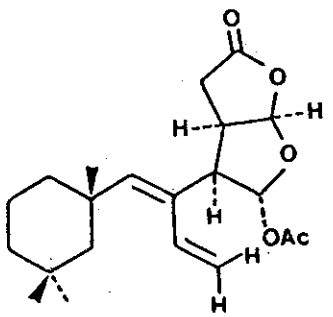
(89)

Dictyodendrilla sp.:

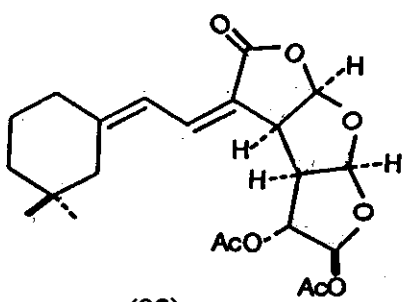
Bergquist established this genus in 1980[9] and stated that species previously assigned to the genus *Spongionella* (Order Dictyoceratida) and which have a reticular rather than dendridic skeleton should be referred to the genus *Dictyodendrilla* (Order Dendroceratida). Five diterpenes were isolated from the sponge referred to as *Spongionella gracilis*, (15)[44] which has also been isolated from *A. tango*, along with four others (90)-(92)[46] and (93)[45].



90 $R_1 = H$ $R_2 = Ac$
91 $R_1 = H$ $R_2 = H$



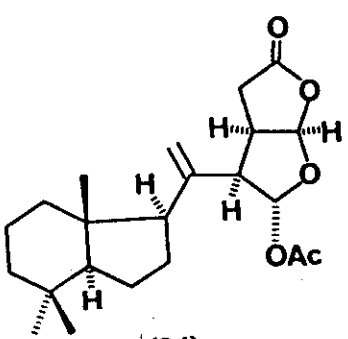
(92)



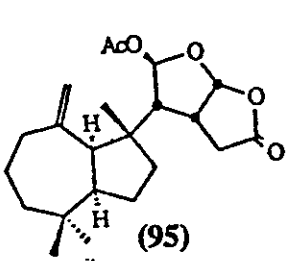
(93)

Dendrilla sp.:[47,48,49]

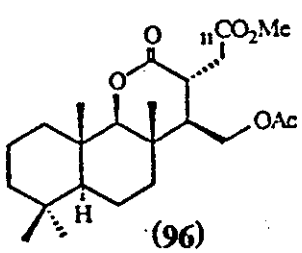
A sponge described as a *Dendrilla sp.* collected from Palau, West Caroline Is. was extracted and the compounds norrisolide (94), dendrillolides (95)-(99) were isolated.



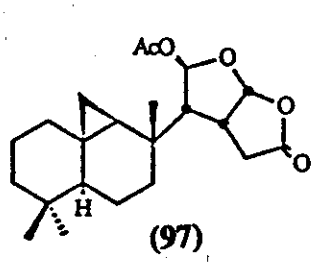
(94)



(95)



(96)



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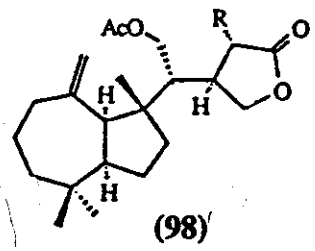
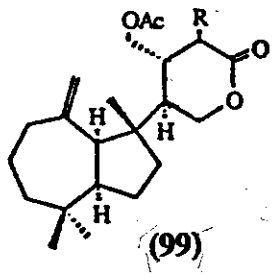


(98)



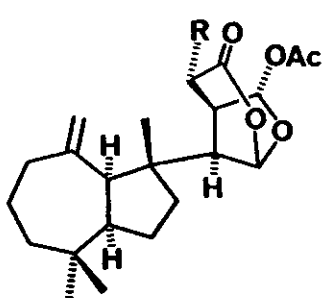
(99)

Chelonaplysilla violacea:[21,22]

The sponge *Chelonaplysilla violacea* was examined from three locations; Sydney, (N.S.W.), Jervis Bay (N.S.W. South Coast) and from Leigh New Zealand.

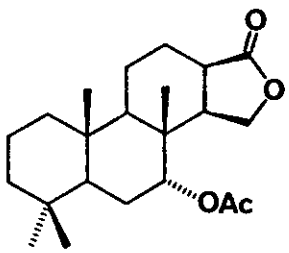
From both the Sydney and Jervis Bay specimens four compounds, (54)-(55) and (100)-(101) were isolated. Both (54) and (55) have been previously reported, (54)[16] being isolated from *S. officinalis* and (55) synthetically prepared by hydrogenation of (63) also from *S. officinalis*.[17] Compound (54)and (55) were also recently found in *Aplysilla var. sulphurea* (Section 1.2.3).

From the New Zealand specimen thirteen diterpene metabolites, (35), (55), (94), (95), (99), (100) and (102)-(108) were isolated.



(100) R = H

(101) OAc



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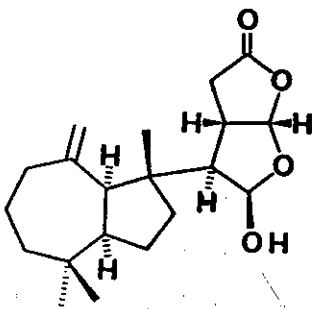

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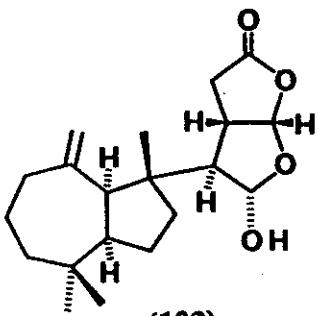

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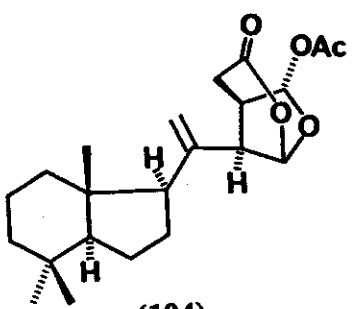

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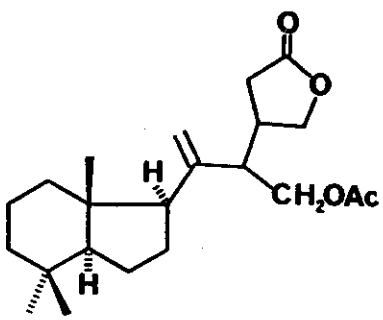

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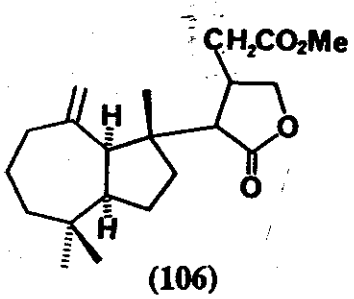

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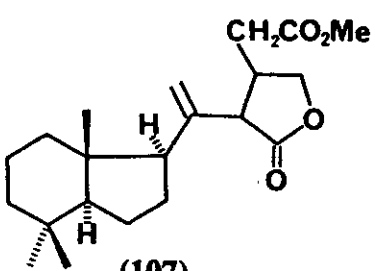

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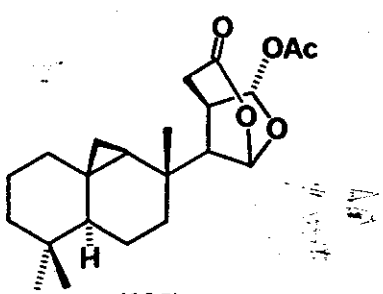
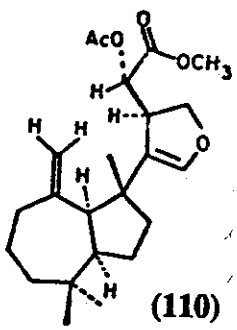


(108)

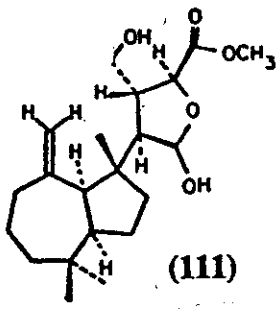
Dysidea sp. (Order Dictyoceratida):[50]

Two species of *Dysidea*, both collected in the Gulf of Suez and described as *Dysidea sp.*1 and *Dysidea sp.*2, were extracted, both yielding compounds structurally similar to those of *Dendrilla sp.* (order Dendroceratida) and *Chelonaplysilla violacia* (order Dendroceratida).

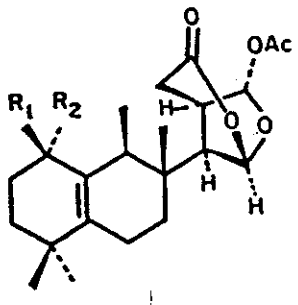
The extracts of *Dysidea sp.*1 afforded the diterpene (101) together with seven other new diterpenes (109)-(115). The extract of *Dysidea sp.*2 on the other hand also afforded (109) together with the further new diterpenes (116)-(118).



(110)



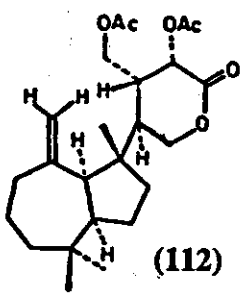
(111)



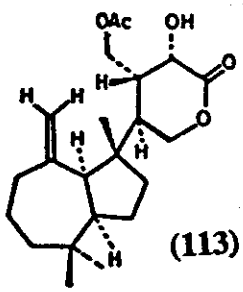
(109) $R_1 = R_2 = H$

(115) $R_1 = H, R_2 = OH$

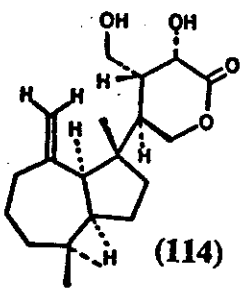
(116) $R_1 = OH, R_2 = H$



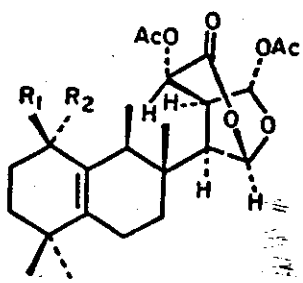
(112)



(113)



(114)



(117) $R_1 = R_2 = H$

(118) $R_1 = OH, R_2 = H$

For any sort of chemotaxonomic work to be applicable it is important to have a good taxonomic representation of species and the taxonomy of the species must be correct. For example two sponges which were originally referred to in earlier papers as belonging to the *Aplysilla* genus, *A. sulphurea* and *A. rosea* were then placed in the genus *Darwinella* (Order Dendroceratida). It also remains possible that *A. tango* is also a member

of *Darwinella*. These sponges remain a point of confusion since the genus *Darwinella* contains spongin spicules[8] while the aplysillids are devoid of any such spicules. In this review the genus *Darwinella* was retained, since the chemical work and taxonomic identification of both *D. oxeata* and *D. rosea* were conducted in the same laboratory and by the same group.

The isolation of both identical and similar metabolites from the two sponges identified as *Aplysilla rosea* and *Dendrilla rosea*, leaves in question whether these two sponges are one and the same but growing in different forms depending on habitat available.

However even with these shortcomings at the generic level, the distinction at the ordinal level seems less confused. Chemically two points of affinity between the orders Dictyoceratida and Dendroceratida can be envisaged: between some species of the genera *Spongia* and *Aplysilla* or *Dendrilla*, and between species of the genera *Dysidea* and those of *Chelonaplysilla* and *Dendrilla*.

Biologically, Bergquist[2] has found that within the Dictyoceratids the family Dysideidae most resemble the Dendroceratids; the Dysideidae having retained a simple histology and eurypylous choanocyte chambers but developing the anastomosing fibre skeleton. It is this anastomosing skeleton which has allowed the evolution of bodies of great coherence and size in the Family Spongiidae. Hence both the biological and chemical evidence supports an affinity between the two orders Dictyoceratida and Dendroceratida.

The similarity of the metabolites between the *Spongia* species and those of the *Dendrilla* and *Aplysilla* species may be a case of convergent evolution of the chemical pathways to produce similar compounds.

It has also been anticipated that the oxidation pattern and structural types of the spongian metabolites may be used in taxonomy: *Spongia* species having oxidation in the A and C rings while those of the Dendroceratids

contained oxidation in the B ring, never in the A and C rings. In some species of Dendroceratids rearrangement of the B ring is also possible.[21,31]

However with the discovery of the diterpenes metabolites from *Dysidea sp.*1 and 2 together with those from *Aplysilla pallida*, show that the chemotaxonomic situation is not as clear-cut as first anticipated.

The metabolites of the *Dysidea* species are oxidised and rearranged in the B ring while those of *A.pallida*, which is definitely a Dendroceratid (see Section 1.3.1) are oxidised at C11, C12 and C13; in the C ring. These two situations were described as being atypical for their respective orders. Hence this shows the importance of correlating both biological and chemical data as well as having a large enough representation of sponge species before any chemotaxonomic application can be confidently made.

1.2.6 *Orthoscuticella maculata*

Orthoscuticella maculata Busk (family: Vittaticellidae) (Plates 6-10) is a member of the Catenicelliform Bryozoa (Phylum: Bryozoa, Class: Gymnolaemata, Order: Cheilostomata, Suborder; Ascophoran).

A single collection was made from 'Torpedo Tubes' on the northern side of Jervis Bay. A sample of the bryozoan was identified by Dr R. Wass of the Department of Geology and Geophysics, University of Sydney.

Description[23,24,52,53,54].

Vittaticellidae Bryozoa are characterised by a cattenicellid growth habit. These growth habits are defined by individual calcified zoids which are connected together by chitinous nodes to form an erect, flexible (jointed) colony (Plate 6). In the fossil record members of the catencelliform bryozoans are represented by many hundreds of unconnected zooecia and are very rarely found as colonies. Because of this, early taxonomic studies are littered with species names based on a single specimen without any consideration for intercolonical variations or correlations between ovicelled and non ovicelled zooecia

The zooecia may be either single zooecia (Plates 7-8), or two zooecia (a geminate pair) in a mother-daughter relationship (Plates 9-10). These zooecia, both single and geminate pair may or may not be ovicelled.

The catenicelliform bryozoan colonies are able to withstand considerable current action because of their flexible structures. They have been recorded throughout the world in current-dominated environments and form the major part of the biota in these environments. For example, they have been recorded in areas with currents varying from 50 cm/sec to 3 m/sec and to depths of more than 200 m.

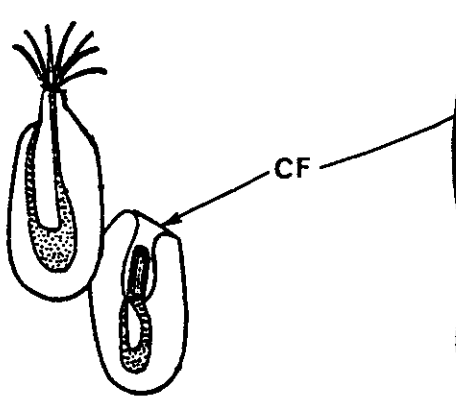


Fig 29

Fig 30

Feeding in catenicelliform bryozoa is achieved by extending a ring of tentacles from the aperture (Fig. 29). These tentacles, in a similar fashion to the choanocyte cells in sponges, direct water towards the mouth which then filters out organic material. The extrusion of these tentacles in Ascophoran bryozoa is achieved by expanding the ascus which is controlled by muscles. The aperture is covered by a cuticle flap (CF) (Fig 30) hinged at the base of the aperture. This flap opens when feeding and is closed for protection.

Identification of the catenicelliform bryozoans is based on the branching pattern exhibited in the colonies, the shape and symmetry of the zoids and the pattern presented on the zooecia, e.g. the number of infracostal windows in the frontal wall, the shape of the zoids and their symmetry effect and the branching pattern of a colony, particularly in the catenicellids. There is a regular pattern of formation, position and number of zoids in the family Vittaticellidae.

The scanning electron microscopy prints (Plates 7–10) are those of *Orthoscuticella maculata*, showing two single zooecia (Plate 7-8) and two geminate pairs (Plate 9-10).

The singlets (Plate 7) are about 0.6 mm long and 0.3 mm wide. It has a semicircular orifice (O) with a proximal lip normal to the growth direction. The orifice is as long as it is wide (0.15 x 0.15 mmm), the

width being similar to the distance apart of the two most distal windows (W) in the frontal wall.

The frontal wall is dominated by seven infracostal windows (W). These windows are uncalcified areas which are covered by a thickened cuticle. The number of windows is fairly constant within species and is used in part for identification.

The proximolateral chambers (P) are long and narrow, extending nearly the complete length of the frontal wall. These chambers are partitions of body cavity separated from the perigastric coelom by an internal calcareous wall. This calcareous wall is perforated by ten or so communication pores (CP). Externally the chambers are covered by an uncalcified cuticle (Fig 30).

There also three distolateral chambers: the inferior (I), superior (S) and median distolateral chambers (M). The first two are similar in composition to the proximolateral chambers and communicate with the median distolateral chamber, which in turn is connected to the perigastric coelom by communication pores.

With these external cuticle covers the living zoids appears as a rather featureless undecorated animal (Fig. 30) and only when chemically cleaned do the true patterns arise.

At the distal end of the zoid is the median distal chamber (MD) which is connected by a joint to the next distal zoid.

In the doublets (Plate 9-10) the daughter zoid (D) is always situated above the mother zoid (MO). The daughter zoid is not connected to its mother by a chitinous joint but is broadly adherent to it. The daughter zoid has in general five windows; the mother has seven as in the singlets.

In both singlets and doublets a line fusion (IF) is seen where the components of the two bilateral components meet.

Plate 6. : *Orthoscutilla maculata*.

Plate 7. : *Orthoscutilla maculata*. Frontal view of a singlet zoid. (x160)

O, orifice
W, window
P, proximolateral chambers
CP, communication pores
LF, line fusion

Plate 8. : *Orthoscutilla maculata*. Basal view of a singlet zoid. (x240)

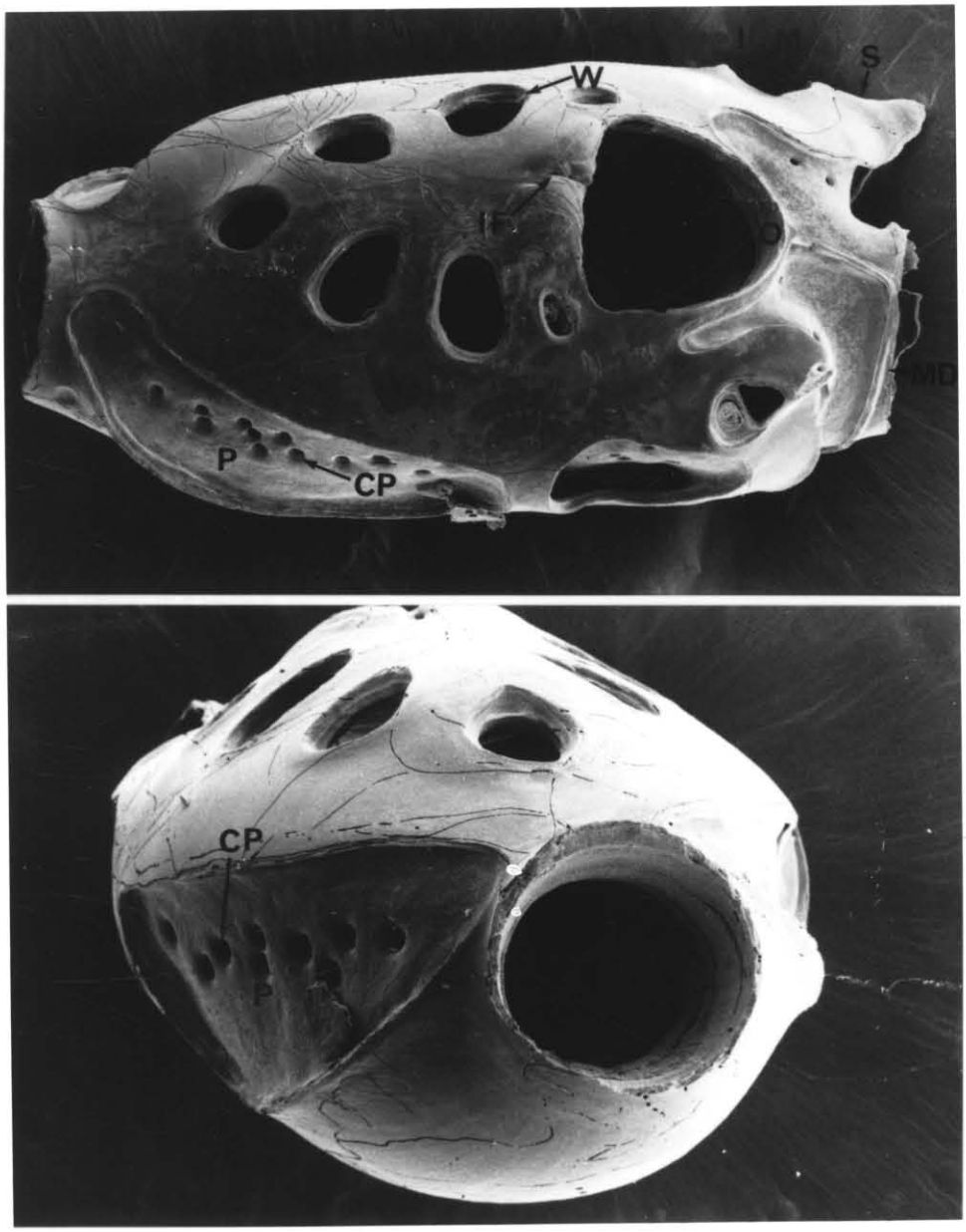
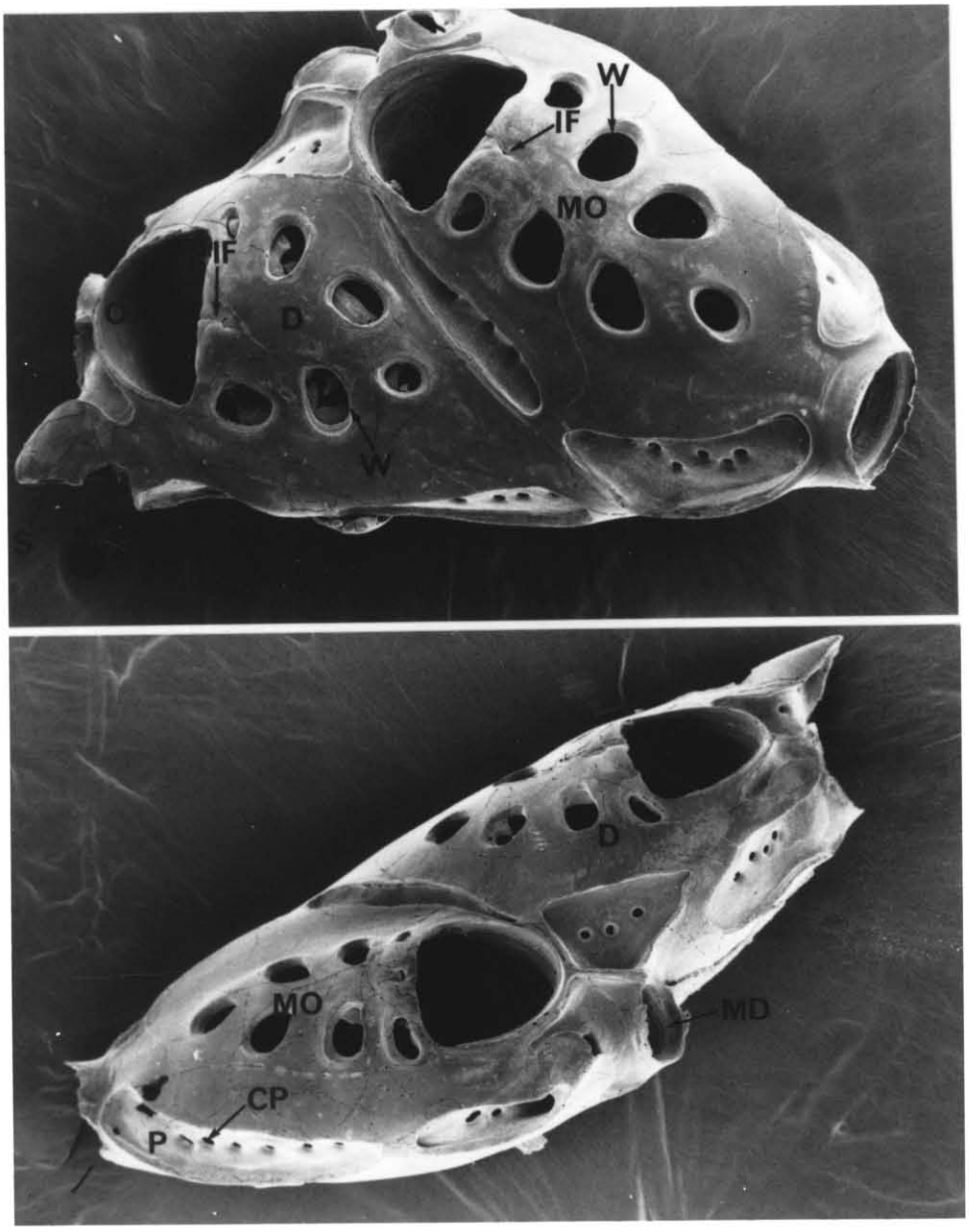

W
S
MD
P
CP
CP
P

Plate 9. : *Orthoscutilla maculata*. Frontal view of a doublet [Mother (MO)-daughter (D)] zoid. (x130)

Plate 10. : *Orthoscutilla maculata*. Frontal view of a doublet [mother (MO)-daughter (D)] zoid. (x120)


W
IF
MO
IF
D
W
D
MO
MD
CP
P

Collection and Extraction

The bryozoan *Orthoscuticella maculata* was collected at 'Torpedo Tubes', Jervis Bay. The bryozoan was found as fine brown orange feather like growths which arose from the substrata to a height of around 10 cm. The growth occurred on the exposed vertical rock face within a thin channel at a depth of 25 m. The material was removed from the rock by hand and gathered in a stocking. The bryozoan was then placed in a plastic bag and transported back to Sydney. The wet bryozoan (about 50 g) was then freeze dried for 48 hours reducing the weight to 33 g. The major portion of this mass is however due to the calcium carbonate shell.

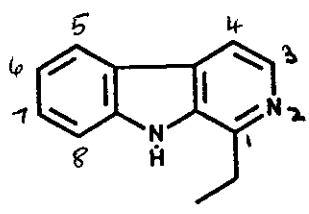
The dried bryozoan was then ground to a powder and extracted by shaking the material in ammoniated methanol (5 x 250 ml) for 24 hours. The extracts were filtered and the solvent removed under vacuum. Ethyl acetate (50 ml) was then added to the dark brown gum and then extracted with 10 % hydrochloric acid solution (5 x 20 ml).

The ethyl acetate extract was then washed with brine, dried over sodium sulphate and the solvent removed under vacuum, yielding a dark brown gum (250 mg). The gum was then chromatographed under gravity on flash silica with dichloromethane and then increasing portions of ethyl acetate/dichloromethane. ^{1}H n.m.r. spectral analysis of the fractions revealed only long chain lipids, triglycerides and a cholesterol type sterol fraction. Therefore no further work was performed on these fractions.

Basification of the acid extracts obtained (ammonia solution, 10M) followed by extraction with chloroform (5 x 50 ml) and removal of the solvent (under vacuum) yielded a dark brown gum (20 mg). Mayer's reagent test of this extract showed positive, indicating the presence of alkaloids. The gum was then subjected to flash silica chromatography

eluting with chloroform/methanol (100/0 to 90/10) to afford an alkaloid fraction. Progress of the column was followed by t.l.c., observed under U.V. light and developed with Dragendoff reagent. Further purification was achieved by h.p.l.c., first using $CHCl_3$/MeOH/NEt_3 (97.7/2/0.3) and then finally with $CHCl_3$/MeOH (95/5). This then afforded pure 1-ethyl-β-carboline (2 mg, 6 x 10^{-3} % of dry bryozoan).

Comparison of the ^1H n.m.r. and mass spectra with those reported in literature[29] confirmed the identity of this compound. There have only been two previous reports in the literature of the metabolite 1-ethyl-β-carboline (56). The first report was from the roots of the tropical African tree *Hannoa klaineana*.[29] It was isolated together with four other similar β-carbolines. The only other report was from another catenicellid bryozoan *Costaticella hastata*.[28] However, decoupling experiments in the ^1H n.m.r. spectrum indicated that minor reassignment of the ^1H n.m.r. spectrum was in order (refer to experimental Section 1.3.4).



(56)

1.3 EXPERIMENTAL

Except where otherwise stated the following generalizations apply.

Melting points are uncorrected and were determined on a Reichert melting point apparatus. Optical rotations were determined for chloroform solutions on a Perkin-Elmer 241 polarimeter. Analyses were performed by the Australian Microanalytical Service, Melbourne. Ultraviolet spectra were measured for ethanol solutions on a Perkin-Elmer 402 spectrometer and infrared spectra for chloroform solutions on a Bio-Rad FTS 20/80 spectrometer by Mr. R. Appio. Electron impact mass spectra (low and high resolution) were obtained with an A.E.I. MS902 spectrometer equipped with a DS30 data system by Dr I. Brown, Mr M. Smyth or Mr A. Schenk. Chemical ionization (methane) mass spectra were obtained with an A.E.I. MS30 spectrometer. Gas-liquid chromatography was performed on a Hewlett-Packard 402 instrument with a 1.46 x 0.002 m column packed with 3% OV-17 on Gas Chrom Q.

^1H nuclear magnetic resonance spectra were measured at 400 MHz for deuterochloroform solutions on a Bruker WM-400 or a Varian XL-400 instrument by Dr B. Rowe, Dr J. Nemorin, Miss S. Boyd or Mr N. Bampos. ^1H n.m.r. spectra measured at 200 MHz on a Bruker HX-200 instrument by the author. For routine high resolution studies 64 x 2^{10} data points were used for a spectral window of 3000 Hz, giving digital resolution of 0.09 Hz, and Lorentz to Gaussian multiplication was applied to the acquired f.i.d.. ^1H spin-lattice relaxation times were obtained by the inversion-recovery pulse sequence, the null-point method being used; partially-relaxed spectra were obtained in a similar manner. Chemical shifts and coupling constants were derived from first-order considerations

and were confirmed by double resonance experiments wherever possible. Nuclear Overhauser enhancement spectra were obtained at 400 MHz in the difference mode by the general method of Hall and Sanders,[51] 16 transients (preceeded by two dummy scans) being collected after preirradiation at a selected frequency for 2-5s ($\geq 5xT_1$). Up to 10 frequencies and one control were run in sequence, and the cycle repeated 15-20 times, which gave total accumulations of 200-400 transients per irradiation. The f.i.d. runs were carried out with 16×2^{10} data points over 4000 Hz; a line broadening of 1Hz was applied to each f.i.d. before processing (subtraction of the control f.i.d. and Fourier transformation of the difference f.i.d.). The irradiation power ('45L') was less than optimum for saturation and the enhancements observed are less than theoretically possible. Results are therefore only qualitatively comparable; enhancements down to 0.1% were routinely detectable. 2D shift-correlated (COSY) spectra were obtained by the general method of Bax and Freeman[30] with a $90^{\circ}-t_1-45^{\circ}$ pulse sequence using appropriate phase cycling to ensure quadrature detection in the F_1 dimension. Thirty two or 48 transients (preceded by 2 dummy scans) were collected after delays of 1 sec; 2×2^{10} data points were collected in the F_2 dimension over 2500 Hz, and 512 increments collected in the F_1 dimension (zero filled to 1×2^{10}), giving a $2^{10} \times 2^{10}$ matrix for absolute value calculation. A shift sine-bell window was usually applied in both dimensions.

^{13}C n.m.r. spectra were measured in the noise-decoupled mode with Jeol FX-60Q (15 MHz), Varian XL-400 (100 MHz) or Bruker WM-400 (100.62 MHz) instruments, by Dr B. Rowe, Dr J. Nemorin, Miss S. Boyd or Mr N. Bampos. Coupled spectra with gated decoupling for n.O.e. and with selective low-power ^1H decoupling were obtained by the procedures previously described. All n.O.e. and 2D n.m.r. experiments were measured by Prof.

W.C. Taylor and transformed by the author.

The single crystal X-ray structures of aplypallidenone, aplypallidoxone and the *p*-bromobenzoate of aplyroseol-1 were measured by Dr T. Hambley with an Enraf-Nonius CAD4-F diffractometer with MoKα radiation.

Merck silica gel (40-63 μm) was used for column chromatography. Higher performance liquid chromatography was carried out with a Waters 6000A solvent delivery system; for preparative separations either a normal phase or reverse phase Whatman 50 by 0.94 cm/Partisil 10μm M9 column was used with r.i. detection. For thin-layer chromatography Merck $60F_{254}$ silica gel precoated plastic sheets were used; elution was with ethyl acetate/dichloromethane (3/7) and vizualization was effected by spraying with 2% vanillin in concentrated sulphuric acid followed by heating at 100° for 1 min.

All solvents were distilled. Light petroleum had b.p. 60-65°.

All sponge and bryozoan samples were collected by the author on SCUBA. Underwater photographs were taken with a Nikonos-V camera equipped with a 35 mm lens and a 2:1 macrotube with an Aquasea-100 flash on Ektichrome-200 film by either Dr P. Karuso or Dr A. Poiner. All sponge scanning electron micrograph sample preparations, measuring and photography were performed by the author on a JSM-35C scanning electron microscope. Sponge samples were prefixed in ethanol/water (7:3) for 4 months, then critical point dried (CO_2), the dried samples mounted on aluminium discs with carbon dag and coated with a 25 nm gold film.

Bryozoan samples for scanning electron microscopy were prepared by Mr D. Salt, Department of Geology and Geophysics, University of Sydney.

Samples were cleaned chemically overnight in 10% potassium hypochlorite solution, washed overnight in distilled water and air dried. Micrographs were obtained either by Mr D. Salt or the author.

1.3.1 *Aplysilla Pallida*

(A) Description

Aplysilla pallida (Plates 3-4, 11-14) is a rare, white to creamy, thin encrusting sponge very similar in appearance to *A. sulphurea* (Plate 1) and *A. tango*.[21] The crusts are 1 - 2 mm thick, very soft and collapsible in texture. The inhalent pores are exceedingly small, while the oscula are 0.5 - 1 mm wide, flat with the surface and numerous.

The white creamy colour of the living sponge showed no change on death. The spirit specimens (Ethanol/water 10:30) also retained their natural colour. The sponge surface is covered with conules (Plate 15) 1 mm high and 4 mm apart. The conuli are the result of flexible spongin fibres (Plates 12-15) which rise from the basal spongin mat through the mesohyl to the surface where it supports the epidermis in a tent-like fashion (Fig 3). These spongin fibres are smooth, upright and either simple (Plate 12) or dendrically ramified fibres (Plate 13-14). The height of both simple and ramified fibres are around 2.5 mm. The width of the simple fibres is around 0.2 mm, while those of ramified fibres is 0.2 mm at the base which taper to 0.05 mm at the distal ends of their final ramifications, which terminate in the conuli.

Since these fibres obviously display no astomosing confirms the sponge is a Dendroceratid, not a Dictyoceratid. Comparison of the appearances of *A. tango* and *A. sulphurea* with those of *A. pallida* indicated it as an

Plate 11. : Scanning electron micrograph of a simple spongin fibre from the sponge *Aplysilla pallida* which arise from the the basal spongian mat, pass through the mesohyl to the surface where they support the epidermis in a tent like fashion producing the conules (C) in the sponge surface (Fig 3). (x32)

Plate 12. : A ramified spongin fibre which also arises from the basal spongian mat giving rise to a pair of conules (C) on the sponge surface such as those in Plate 15. (x32)

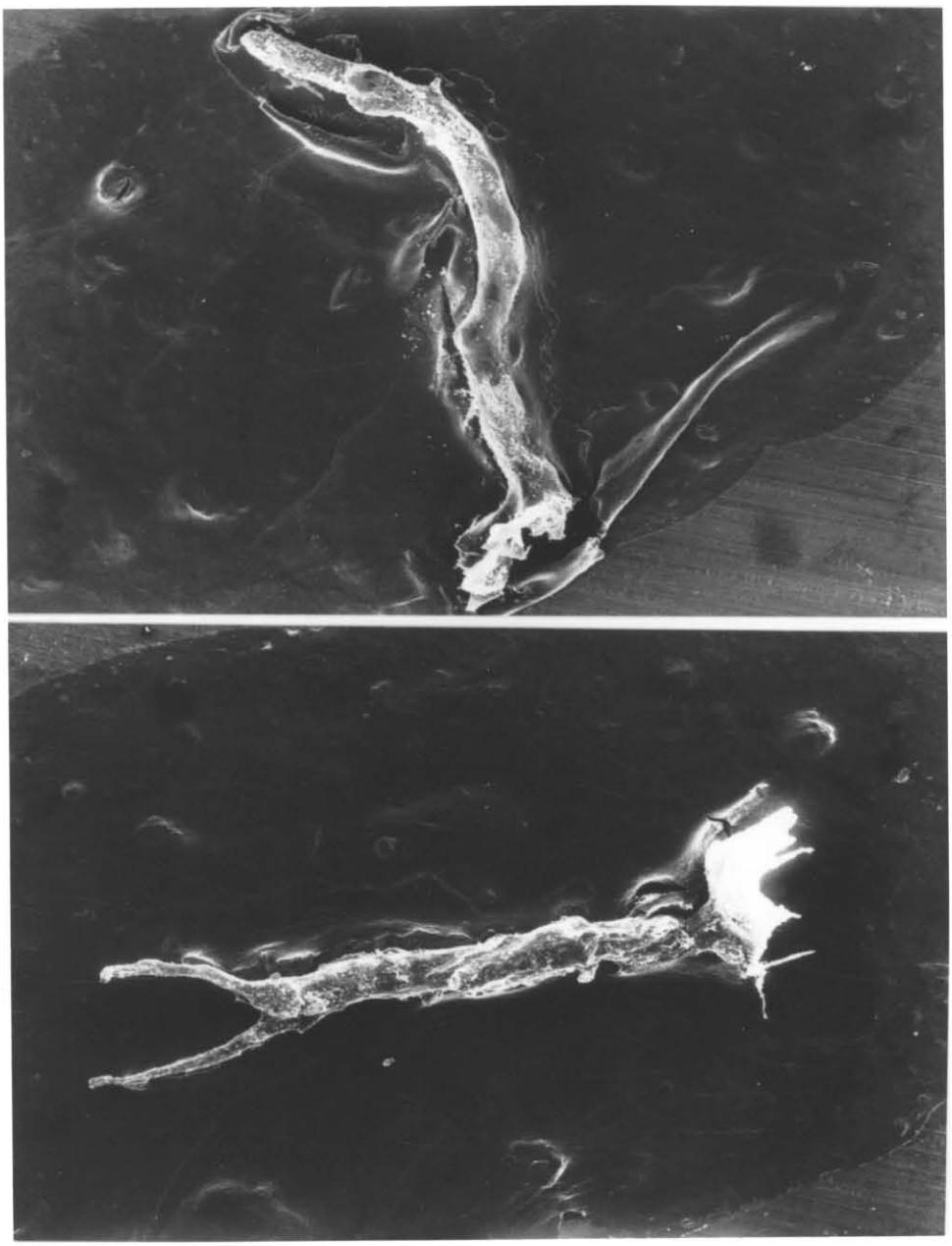
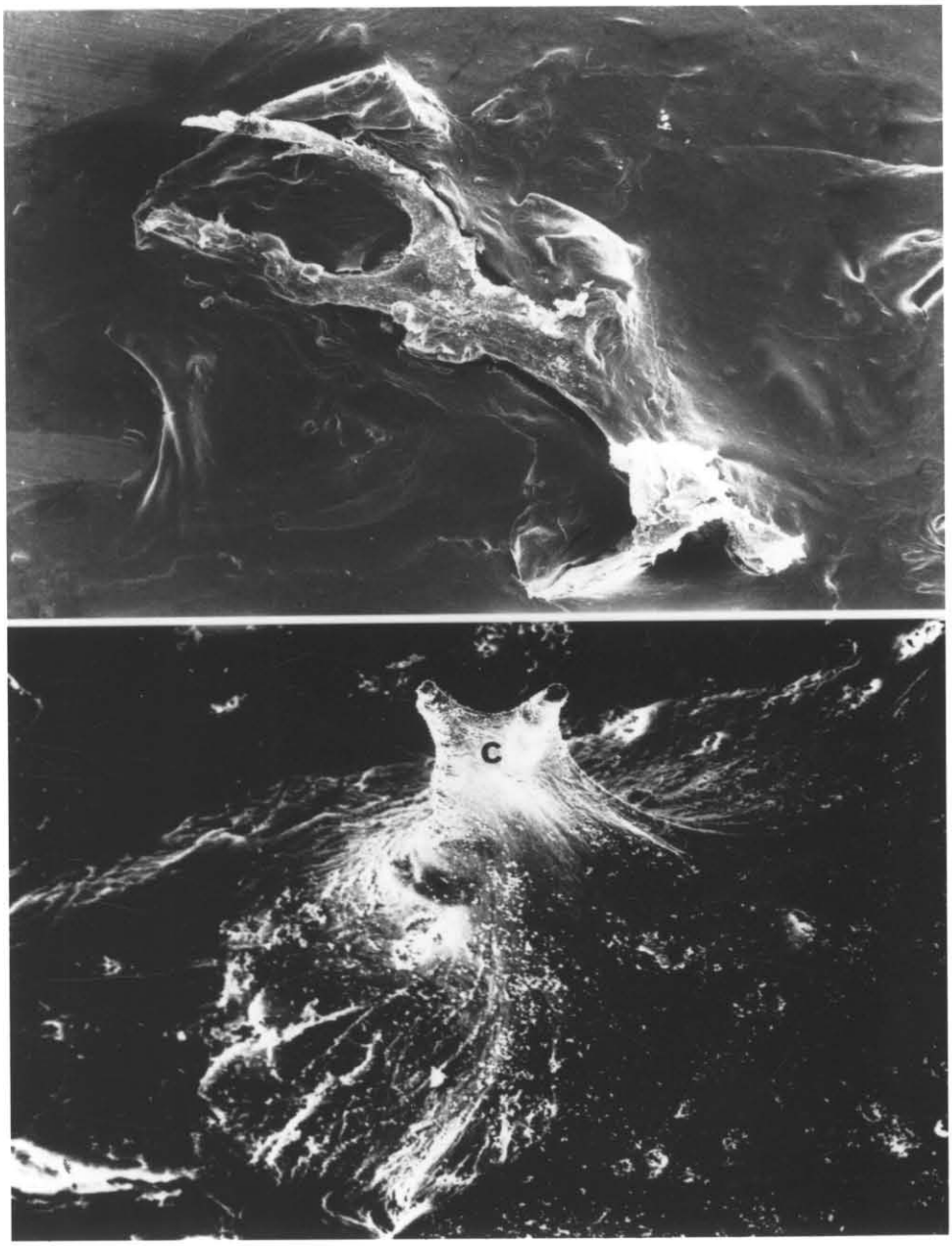


Plate 13. : Another ramified spongin fibre from from *A. pallida*. (x32)

Note that since the fibres display no anastomosing confirms the sponge is within the the order Dendroceratida and not a member of the order Dictyoceratida. By analogy with Fig 3 also confirms the sponge as an aplysillid type.

Plate 14. : A pair of conules (C) arising from a ramified spongin fibre such as those in Plates 13 and 14.


C

Aplysillid.

(B) Collection and Extraction

Aplysilla pallida was collected from two locations Bare Is, Sydney and Bass (Pig) Is, Wollongong. Specimens from the Bare Is. Sydney location were collected at a depth of 10 - 15 m and only ever found on the ceilings of small crevices and caves; away from direct sunlight. All other white creamy and grey sponges found exposed proved (by t.l.c.) not to be *A. pallida*.

Exposed crusts of *A.pallida* were however found to grow within a depth range 23 - 27 m at Bass Is Wollongong, The only other exposed crust found was at 20 m from 'The Cave', Jervis Bay.

This could possibly indicate that *A. pallida* is to a certain extent photophobic and hence either shades itself or grows at greater depths. No ecological work was however performed to prove this point.

There were six collections made of *A. pallida* on SCUBA; four from Bare Is. Syndey (May 1985, Jan 1988, Sept 1988 and Jan 1989) and two from Bass Is, Wollongong (Aug 1988 and Dec 1988).

Due to the delicate texture and scarcity of the sponge only small quantities of material could be found and collected. All collections made, except for Jan 1988 (21.6 g collected over 6 dives), were of single colonies which were extracted separately.

The collection of the Bare Is. specimens were particularly difficult. Since they were situated on the ceilings of narrow caves it was necessary to remove the SCUBA tank and BCD (Buoyancy Control Device). Then, holding them by hand or placing them in the cave, one could then squeeze in and, without exhaling, scrape the sponge off the substrate with a paint scraper. The scrapings were then, while exhaling, passed to the buddy

diver who gathered them in a stocking. This process often proved uncomfortable and labourious particularly in the cold winter waters or particularly when one cave was also occupied by an irate moray eel.

The removal of the Bass Is. samples were far simpler, as they were exposed on the rock surface. This was however, offset due to the scarcity of the sponge and the greater depth, which restricted bottom time available for collecting.

The sponge material, once collected, was then immediately transported back to the laboratory, frozen in liquid nitrogen, freeze dried and then extracted.

In all, six collections of *A. pallida* were made over four years yielding a total 62.5g dry weight sponge (approx. 110 g wet sponge). Typically, the dried sponge (6.2 g, Aug 1988 collection) was placed into a flask with chloroform/ethyl acetate (1:1, 100ml) stoppered and shaken for 24 hours. The extract was then filtered (Buchner funnel) and the solvent removed. This process was carried out five times and then followed by a methanol extraction. Analysis of the methanol extract (normal and reverse phase t.l.c.) showed only base line material and hence was not further investigated.

Evaporation of the chloroform/ethyl acetate extract afforded a yellow-brown gum. T.l.c. of the gum developed with dichloromethane showed two major compounds as a yellow (aplypallidoxone, R_f 0.25) and pink (aplypallidenone, R_f 0.15) spots when visualized with 2% vanillin concentrated sulphuric acid solution. These two products were found to vary in concentration among specimens (Table 1). The gum was then chromatographed on a flash silica column under gravity, eluting with dichloromethane and very slowly increasing the polarity with incremented amounts of ethyl acetate. This afforded crystalline, almost pure diterpenes aplypallidoxone [(22, 71 mg] and aplypallidenone [(22), 105 mg]

which were then further purified by recrystallization. Crystals for the X-ray crystal structure analysis (Appendix 1) of aplypallidenone were prepared by slow evaporation in an *n*-hexane solution. Those prepared for aplypallidoxone were crystallised by the slow diffusion mixing of dichloromethane/light petroleum (30 - 40°) at -15°.

T.l.c. analysis and subsequent combination of the like fractions from all the specimens, followed by chromatography on flash silica as above, afforded the further two diterpenes aplypallidione [(23), 5 mg] and aplypallidioxone [(24), 9 mg]. These were also further purified by crystallization from the slow diffusion mixing of dichloroform/light petroleum (30 - 40°) at 4°.

1.3.2 *Aplysilla rosea*

(A) Description

Aplysilla rosea (Plate 6) is a rose to cherry red encrusting sponge, 2 - 5 mm thick; however in some cases sections of the sponge were raised to form thick fleshy labose nodules.

The skeleton is composed of a basal spongin mat from which arise dendridically ramifying upright isolated fibres which pass through the mesohyl and support the epidermis in a tent like fashion (Fig 3) accounting for the connulose nature of the sponge.

The inhalent pores are very small and arranged in roughly polygonal groups over the entire surface. The exhalant canals (oscula) are 2 - 5 mm wide and about 5 cm apart, and situated on the summit of tubular volcano-like processes.

The red colour of the living sponge is imperfectly converted to dark

brown on death. The sponge, once dry, is stable but on contact with water turns brown. This process is accelerated by base but it is reversed by acid, e.g. an orange colour is formed on treatment with acetic acid. This is typical behaviour for the guanidine pigments present in Aplysillid sponges.[2]

The sponge has a large geographical distribution having been found in the Mediterranean Sea, Atlantic and Pacific oceans. It also has a large vertical distribution being found from a depth of 5 m at Port Jackson, N.S.W. to 640 m in Scotland.[8]

(B) Collection and Extraction

The method of collection was similar to that used for *Aplysilla pallida* and was carried out between the months Apr - Jun, 1984 (water temp 14 - 16°). Again, due to the time of collecting, this often proved to be uncomfortable in the cold, winter Sydney waters. This was, however, offset by the ease of locating and removing the red coloured sponge which was often found on exposed rock surfaces.

The sponge material, once collected, was immediately transported back to the laboratory, frozen in liquid nitrogen and freeze dried. The dried sponge was then stored at -15° in an airtight, light proof container until extracted.

Altogether 675 g of dry weight sponge was collected in 1984 over a three month intensive diving period. The dry sponge was ground to a powder and extracted in a Soxholet extractor with light petroleum (60 - 70°) for 1.5 weeks, changing solvent every 48 hours. This was then followed by a dichloromethane and methanol extraction, each of 1 week duration and again changing the solvent every 48 hours. The light petroleum and dichloromethane extracts were then combined, the solvent

removed and the resulting dark green gum (45.6 g) chromatographed on flash silica gel. Elution with light petroleum yielded the two furanoditerpenes ambliofuran [(28), 1600 mg] and hexahydroambliofuran [(29), 11.3 mg], which were further purified by distillation (kugelrohr 200°/0.05 mm Hg) and preparative gas-liquid phase chromatography (3% OV-17 on Gas Chrom Q column at 158°) respectively. Further elutions with ethyl acetate/light petroleum (0/100 to 100/0) yielded fractions containing aplyroseol-1 (30). These were combined and further chromatographed on flash silica under gravity eluting with dichloromethane and very slowly increasing the polarity with ethyl acetate. Finally, recrystallization yielded pure (30), (464 mg). Further purification of the combined fractions which contained the lactone (35) by chromatography on flash silica gel and eluting with ethyl acetate/dichloromethane (0/100 to 20/80), followed by h.p.l.c. with ethyl acetate/light petroleum and/or isopropanol/light petroleum yielded (35) (130 mg), together with the new diterpenes AROSST-1 [(39), 31 mg], AROSST-7 [(45); 14.2 mg] and AROSST-6 (44). AROSST-6 however autooxidised to the acid AROSST-6A [(44A), 22.3 mg] before complete spectral measurements were obtained.

Although the methanol extracts (120 g) consisted mostly of inorganic sea salts, t.l.c. did indicate the presence of terpenes. Partitioning between ethyl acetate/water, the t.l.c. of the ethyl acetate extract showed the presence of polar terpenes also present in the light petroleum extract. The extract was therefore chromatographed on flash silica under gravity eluting with ethyl acetate/light petroleum (0/100 to 100/0). The fractions were analysed by t.l.c. and fractions alike with those from the light petroleum extract were combined. The resulting fractions were then further fractionated on flash silica gel under gravity eluting with ethyl acetate/dichloromethane and then subjected to h.p.l.c. eluting with ethyl acetate/light petroleum and/or isopropanol/light petroleum. This gave the

Plate 15. : Scanning electron micrograph of a chamber within the sponge *Aplysilla var. sulphurea* showing choanocytes feeding cells. (x2000)

Plate 16. : Close up of choanocyte cells showing the long flagellum (F), apical colar of cytoplasmic tenticles (T) and rounded cell body (B). (x4800)


F
T
B

known diterpenes aplyroseol-1 [(30); 490 mg], -3 [(31); 130 mg], -4 [(32); 406 mg], -5 [(33); 843 mg), -6 [(34); 466 mg), lactones-1 [(35), 19 mg], -2 [(36); 380 mg] and dendrillol -1 [(37); 6.3 mg] and -2 [(38); 35 mg] together with the new diterpenes AROSST-8 [(46); 12.1 mg], AROSST-3 [(41); 55.5 mg], AROSST-5 [(43); 62.8 mg)], AROSST-4 [(42); 24.6 mg], AROSST-2 [(40); 11.5 mg], AROSST-9 [(47); 1.3 mg] AROSST-10 [(48); 5.4 mg] and AROSST-11 [(49); 5.1 mg]. The compounds isolated together with their concentrations in dry weight sponge are summarised in Table 8 which also shows the results previously obtained by Karuso from *A. rosea* in 1984[19] and a New Zealand sponge *Dendrilla rosea* in 1986.[26]

1.3.3 *Aplysilla var. sulphurea*

(A) Description

Aplysilla var. sulphurea (Plates 15-16) is a bright yellow encrusting sponge very similar to *Aplysilla sulphurea* except that it attains a greater thickness of 5 - 10 mm. Only one crust had ever been found. It was located at a depth of 15 m on the ceiling of a narrow cave at Bare Is, Sydney. All other morphologically similar yellow sponges collected proved to contain aplysulphurin (14) and hence were *A. sulphurea*.

The crust was very soft and collapsible in texture. The sponge surface is covered with connuli about 5 mm apart and 2 mm high which result from the dendrically ramified spongin fibre skeleton. These fibres rise from the basal spongin mat, pass through the mesohyl and extend to the surface.

As in *A. sulphurea* the inhalent pores are very small and arranged in roughly polygonal groups over the entire surface. The exhalent canals

(oscular) are 2 - 5 mm wide and about 5 mm apart.

The yellow colour of the sponge, as in *A. sulphurea*, is also gradually converted to blue on death but at a far reduced rate: within an hour of collection most of the yellow of *A. sulphurea* is blue, in *A. var. sulphurea* only a small portion has changed. When both sponges were added to preserving solution (Ethanol/water 70:30) the colour conversion to blue/purple was almost instantaneous in both sponges. In the dried sponge the yellow colour was stable.

(B) Collection and Extraction

Two collections were made, one in Jan 1989 (5.3 g) and another in May 1989 (1.98 g). The sponge was noticed to have a quite rapid and successful regeneration.

The method of collection from the narrow cave was exactly the same as that used to collect the Bare Is. samples of *A. pallida* (section 2.3.1). The sponge material collected was immediately transported back to the laboratory, frozen in liquid nitrogen and freeze dried.

The dried sponge was then extracted with chloroform/ethyl acetate (1:1, 100 ml) by stirring in a stoppered flask. The extract was then filtered (Buchner funnel) and the solvent removed. This process was carried out three times resulting in a yellow-brown gum. The gum was then applied to a flash silica column and eluted under gravity with light petroleum/ethyl acetate (100/0 to 50/50) yielding the two diterpenes (54) and (55) and a sterol fraction which was not analysed.

1.3.4 Characterisation

Aplypallidenone (21)

Methyl ($5R^*$, $8S^*$, $9S^*$, $10R^*$, $11S^*$)-11-acetoxy-14-oxopallid-12-en-15-oate (21)

Aplypallidenone was crystallised as colourless needles from cyclohexane m.p. 99 - 101°. $[\alpha]_D$ +135°, $[\alpha]_{578}$ +141°, $[\alpha]_{546}$ +158°, $[\alpha]_{436}$ +206° (c 2.41). (Found: 376.2269. $C_{22}H_{32}O_5$ requires 376.2249). λ_{max} (cyclohexane) 211, 336nm(ϵ 9640, 60). ν_{max} 2953, 1740, 1686, 1437, 1390, 1370, 1295, 1271, 1246, 1035, 1022, 993 cm^{-1}. ^1H n.m.r. δ 0.79, dd, $J_{5\alpha,6\beta}$ 11.4 Hz, $J_{5\alpha,6\alpha}$ 2.2 Hz, H5α; 0.85, s, $W_{h/2}$ 1.3 Hz, 18Me; 0.87, s, $W_{h/2}$ 0.9 Hz, 19Me; 1.00, dt, J_{gem} 12.9 Hz, $J_{1\alpha,2\beta}$ 12.9 Hz, $J_{1\alpha,2\alpha}$ 4.0 Hz, H1α; 1.13, dt, J_{gem} 12.9 Hz, $J_{3\alpha,2\beta}$ 12.6 Hz, $J_{3\alpha,2\alpha}$ 4 Hz, H3α; 1.21, d, J 0.85 Hz, 17Me; 1.41, bd, J_{gem} 12.9 Hz, H3β; 1.42, d, J 0.70 Hz, 16Me; 1.48, H2α; 1.51, H6β; 1.58, H7α; 1.62, H2β; 1.65, bd, J_{gem} 12.9 Hz, H1β; 1.69, d, $J_{9\alpha,\ 11\alpha}$ 4.2 Hz, H9α; 1.71, H6α; 1.97, m, H7β; 2.13, s, $OCOCH_3$; 3.81, s, $COOCH_3$; 5.83, dd, $J_{11\alpha,12}$ 5.4 Hz, $J_{11\alpha,9\alpha}$ 4.2 Hz, H11α; 7.24, d, $J_{12,11\alpha}$ 5.4 Hz, H12. ^1H n.m.r. (C_6D_6) δ 0.37, H5α; 0.62, H1α; 0.75, s, 18Me; 0.78, s, 19Me; 0.99, H3α; 1.02, s, 17Me; 1.07, d, $J_{9\alpha,11\alpha}$ 4.2 Hz, H9α; 1.21, m, H6β; 1.25 - 1.35, m, H2α, H3β; 1.32, s, 16Me; 1.39-1.56, m, H1β, H7α, H2β, H6α; 1.54, s, $OCOCH_3$; 1.98, H7β; 3.44, s, $COOCH_3$; 5.64, dd, $J_{11\alpha,12}$ 5.4 Hz, $J_{11\alpha,9\alpha}$ 4.2 Hz, H11α; 7.14, d, $J_{12,11\alpha}$ 5.4 Hz, H12. ^{13}C n.m.r. δ 17.4, q, $^1J_{C,H}$ 125.7 Hz, Me; 18.0, t, $^1J_{C,H}$ 126.0 Hz, CH_2; 18.4, t, $^1J_{C,H}$ 125.6 Hz, CH_2; 21.3, q, $^1J_{C,H}$ 130 Hz, $OCOCH_3$; 21.6, q, CH_3; 21.7, q, CH_3; 33.2, s, C4 or C10; 33.5, q, $^1J_{C,H}$ 124.4 Hz, CH_3; 35.4, t, $^1J_{C,H}$ 131.5 Hz, CH_2; 38.6, s, C4 or C10; 39.2, t, $^1J_{C,H}$ 124.6 Hz, CH_2; 41.5, t, $^1J_{C,H}$ 123.5 Hz, CH_2; 45.9, s, C8; 52.4, q, $^1J_{C,H}$

147.6 Hz, $COOCH_3$; 53.2, d, $^1J_{C,H}$ 117.7 Hz, C5 or C9; 56.6, d, $^1J_{C,H}$ 119.3 Hz, C5 or C9; 64.8, d, $^1J_{C,H}$ 149.8 Hz, C11; 132.0, s, C13; 144.0, d, $^1J_{C,H}$ 166.2 Hz, C12; 165.3, s, $COOCH_3$; 169.8, s, $OCOCH_3$; 200.6, s, C14. Mass spectrum (e.i.) *m/z* 376(5%), 345(4), 334(6), 316(5), 302(6), 284(4), 269(4), 239(2), 192(16), 184(45), 142(100), 123(33), 109(23), 95(36), 81(34), 69(51), 55(37). Mass spectrum (c.i.) *m/z* 377(100%), 345(6), 335(15), 317(25), 285(18), 193(9), 184(18), 177(16), 142(52), 123(16), 109(9), 95(13), 81(10), 69(15), 55(10).(Found: 345.2054. $C_{21}H_{29}O_4$ requires 345.2066; found: 334.2141. $C_{20}H_{30}O_4$ requires 334.2144; found: 192.1878. $C_{14}H_{24}$ requires 192.1878; found: 184.0377. $C_8H_8O_5$ requires 184.0378; found: 177.1622. $C_{13}H_{21}$ requires 177.1643; found: 142.0260. $C_6H_6O_4$ requires 142.0266).

Aplypallidoxone (22)

Methyl (5*R**, 8*S**, 9*S**, 10*R**, 11*S**, 12*R**, 13*R**)-11-acetoxy-12,13-epoxy-14-oxopallidan-15-oate (22)

Aplypallidoxone was crystallised from cyclohexane as fine colourless needles m.p. 143 - 144°. Crystals for X-ray crystal structure determination were grown by slow diffusion mixing of isopentane/ether at 4° giving colourless prisms m.p. 143 - 144°. $[\alpha]_D$ -32°, $[\alpha]_{578}$ -34°, $[\alpha]_{546}$ -41°, $[\alpha]_{436}$ -100° (c 2.55). (Found: 392.2215. $C_{22}H_{32}O_6$ requires 392.2198). λ_{max} 299nm(ϵ 43). ν_{max} 2948, 1750, 1713, 1460, 1441, 1390, 1370, 1296, 1245, 1038, 990 cm^{-1}. ^1H n.m.r. δ 0.77, dd, $J_{5\alpha,6\beta}$ 11.8 Hz, $J_{5\alpha,6\alpha}$ 2.0 Hz, H5α; 0.83, s, $W_{h/2}$ 1.2 Hz, 18Me; 0.85, s, $W_{h/2}$ 0.8 Hz, 19Me; 0.98, dt, J_{gem} 12.8 Hz, $J_{1\alpha,2\beta}$ 12.8 Hz, $J_{1\alpha,2\alpha}$ 4.0 Hz, H1α; 1.12, dt, J_{gem} 13.6 Hz, $J_{3\alpha,2\beta}$ 13.6 Hz, $J_{3\alpha,2\alpha}$ 2.2 Hz, H3α; 1.14, d, *J* 0.9 Hz, 17Me; 1.39,

bd, J_{gem} 13.6 Hz, H3β; 1.40 - 1.63, m, $(H2)_2$, H6β, H7α; 1.44, d, J 0.75Hz, 16Me; 1.52, d, $J_{9\alpha,11\alpha}$ 3.2 Hz, H9α; 1.69, bd, J_{gem} 13 Hz, H6α; 1.71, bd, J_{gem} 12.8 Hz, H1β; 1.99, m, H7β; 2.12, s, $OCOCH_3$; 3.55, d, $J_{12\beta,11\alpha}$ 2.5 Hz, H12β; 3.82, s, $COOCH_3$; 5.89, dd, $J_{11\alpha,9\alpha}$ 3.2 Hz, $J_{11\alpha,12\beta}$ 2.5 Hz, H11α. ^{13}C n.m.r. δ 17.7, CH_3; 18.1, CH_2; 18.2, CH_2; 21.0, CH_3; 21.3, CH_3; 21.5, CH_3; 33.2, C4 or C10; 33.4, CH_3; 35.7, CH_2; 38.5, C4 or C10; 39.6, CH_2; 41,4, CH_2; 46.5, C8; 47.1, CH; 52.9, $COOCH_3$; 55.8, C13; 56.5, CH; 57.8, CH; 65.5, CH; 166.2, C15; 169.7, $OCOCH_3$; 201.9, C14. Mass spectrum (e.i.) *m/z* 392(1%), 377(1), 333(1), 322(1), 304(2), 289(4), 255(2), 245(2), 217(2), 182(10), 138(10), 123(27), 109(17), 95(24), 81(27), 69(39), 55(28), 43(100). Mass spectrum (c.i.) *m/z* 393(100%), 377(12), 364(8), 333(55), 322(5), 315(95), 305(23), 283(15), 273(14), 255(10), 245(6), 229(27), 182(10), 163(9), 123(13), 109(6), 95(7), 43(5). (Found: 377.1981. $C_{21}H_{29}O_6$ requires 377.1964; found: 333.2068. $C_{20}H_{29}O_4$ requires 333.2065; found: 322.2109. $C_{19}H_{30}O_4$ requires 322.2144).

Aplypallidione (23)

Methyl (5*R**, 8*S**, 9*S**, 10*R**, 12*S**, 13*R**)-12,13-epoxy-11,14-dioxopallidan-15-oate (23).

The diketone aplypallidione (23) (0.007 g, 0.01%) was isolated as colourless needles from the slow diffusion mixing of light petroleum (30-40°) and dichloromethane at 4° m.p. . (Found: 348.1937. $C_{20}H_{28}O_5$ requires 348.1937). λ_{max} 294 nm(ϵ 58). ν_{max} 2962, 2930, 1758, 1720, 1592, 1490, 1451, 1296, 1176, 1082, 966 cm^{-1}. ^1H n.m.r. δ 0.71, dd, $J_{5\alpha,6\beta}$ 12.2 Hz, $J_{5\alpha,6\alpha}$ 2.0 Hz, H5α; 0.78, dt, J_{gem} 12.8 Hz, $J_{1\alpha,2\beta}$ 12.8 Hz, $J_{1\alpha,2\alpha}$ 3.6 Hz, H1α; 0.83, s, $W_{h/2}$ 1.9 Hz, 18Me; 0.86, s, $W_{h/2}$ 1.7 Hz, 19Me; 1.13,

dt, J_{gem} 13.6 Hz, $J_{3\alpha,2\beta}$ 13.6 Hz, $J_{3\alpha,2\alpha}$ 4.2 Hz, H3α; 1.23, d, J 0.8 Hz, 17Me; 1.26, d, J 0.9 Hz, 16Me; 1.39, m, H2α, H3β, H6β; 1.55 - 1.78, m, H2β, H6α, H7α; 1.96, m, H7β; 2.09, dddd, J_{gem} 12.8 Hz, $J_{1\beta,2\beta}$ 3.3 Hz, $J_{1\beta,2\alpha}$ 3.3 Hz, $J_{1\beta,3\beta}$ 1.6 Hz, H1β; 2.70, s, H9α; 3.59, s, H12; 3.84, s, $COOCH_3$. ^{13}C n.m.r. δ 16.3; 17.5; 17.9; 20.3; 21.6; 33.1; 33.3; 34.6; 37.2; 39.1; 41.6; 52.2; 53.4; 54.9; 56.4; 59.1; 61.2; 164.3; 199.4; 202.3. Mass spectrum *m/z* 348(13%), 333(15), 315 (2), 305(2), 289(9), 261(9), 198(11), 123(86), 109(31), 95(51), 69 (100), 55(50). (Found: 333.1714. $C_{19}H_{25}O_5$ requires 333.1702; found: 305.1750. $C_{18}H_{25}O_4$ requires 305.1753; found: 289.1798. $C_{18}H_{25}O_3$ requires 298.1803).

Aplypallidioxone (24)

Methyl (5R*, 8S*, 9R*, 10R*, 13R*)-11-acetoxy-9,13-epidioxy-14-oxopallidan-11-en-15-oate (24).

The peroxide aplypallidioxone (24) (0.004 g, 0.006%) was isolated as colourless needles from the slow diffusion mixing of light petroleum (30–40°) and dichloromethane at 4° m.p. 170–171°. (Found: 364.1852 $C_{20}H_{28}O_6$ [$C_{22}H_{30}O_7-C_2H_2O$] requires 364.1886). λ_{max} no peaks. ν_{max} 2958, 2930, 1744, 1693, 1459, 1263, 1179, 1106, 1012 cm^{-1}. ^1H n.m.r. δ 0.86, s, $W_{h/2}$ 1.8 Hz, 18Me; 0.89, s, $W_{h/2}$ 1.5 Hz, 19Me; 1.22, dt, J_{gem} 13.4 Hz, $J_{3\alpha,2\beta}$ 13.4 Hz, $J_{3\alpha,2\alpha}$ 4.2 Hz, H3α; 1.28, d, $J_{17Me,1\alpha}$ 0.8 Hz, 17Me; 1.36, dddd, J_{gem} 13.4 Hz, $J_{3\beta,2\beta}$ 3.4 Hz, $J_{3\beta,2\alpha}$ 3.4 Hz, $J_{3\beta,1\beta}$ 1.6 Hz, H3β; 1.37, d, $J_{16Me,7\alpha}$ 0.8 Hz, 16Me; 1.47, H2α; 1.52, H6β; 1.67, H2β; 1.70, bd, J_{gem} 12.6 Hz, H6α; 1.72, H1β; 1.78, dd, $J_{5\alpha,6\beta}$ 12.8 Hz, $J_{5\alpha,6\alpha}$ 2.6 Hz, H5α; 1.81, ddd, J_{gem} 13.4 Hz, $J_{7\beta,6\beta}$ 3.8 Hz, $J_{7\beta,6\alpha}$ 3.0 Hz, H7β; 1.83, m, H1α; 1.95, dt, J_{gem} 13.4 Hz, $J_{7\alpha,6\beta}$ 13.4 Hz, $J_{7\alpha,6\alpha}$ 4.2 Hz, H7α; 2.25, s, $OCOCH_3$; 3.88, s, $COOCH_3$; 6.64,

s, H12. ^{13}C n.m.r. δ 17.3, CH_2; 17.9, CH_2; 18.6, CH_3; 21.7, CH_3; 22.0, CH_3; 23.6, CH_3; 32.0, CH_2; 32.1, CH_2; 33.4, CH_3; 33.6, C; 41.2, CH_2; 41.9, C; 47.2, CH, C5; 47.7, C; 53.3, OCH_3; 83.4, C9; 89.5, C13; 110.4, CH, C12; 156.2, C11; 163.9, C15; 166.2, $OCOCH_3$; 201.6, C14. Mass spectrum *m/z* 406(<1%), 391(<1), 378(<1), 364(3), 346(1), 336(3), 318(23), 305(12), 244(16), 229(11), 185 (20), 137(54), 123(39), 109(39), 95(44), 81(45), 69(100). (Found: 346.1778. $C_{20}H_{26}O_5$ requires 346.1780; found: 336.1935. $C_{19}H_{28}O_5$ requires 336.1936).

1,4 Michael Adduct (26)

Aplypallidenone (21) (0.050 g) was dissolved in methanol (10 ml) and stirred at room temperature for 24h. Evaporation of the solvent yielded (26) as a slightly yellow gum. ^1H n.m.r. δ 0.83, s, 18Me; 0.85, s, 19Me; 0.86, m, H5α; 0.96, dt, J_{gem} 12.5 Hz, $J_{1\alpha,2\beta}$ 12.5 Hz, $J_{1\alpha,2\alpha}$ 4.2 Hz, H1α; 1.03, s, 17Me; 1.15, dt, J_{gem} 13.5 Hz, $J_{3\alpha,2\beta}$ 13,5 Hz, $J_{3\alpha,2\alpha}$ 4.2 Hz, H3α; 1.38, bd, J_{gem} 13.5 Hz, H3β; 1.42 - 1.68, m, $(H2)_2$, $(H6)_2$, H7α; 1.51, s, 16Me; 1.63, d, $J_{9\alpha,11\alpha}$ 2.2 Hz, H9α; 1.71, bd, J_{gem} 12.5 Hz, H1β; 2.05, s, $OCOCH_3$; 2.06, m, H7β; 3.49, s, OCH_3; 3.79, s, $COOCH_3$; 3.82, d, $J_{12\beta,11\alpha}$ 2.4 Hz, H12β; 5.51, dd, $J_{11\alpha,12\beta}$ 2.4 Hz, $J_{11\alpha,9\alpha}$ 2.2 Hz, H11α; 12.80, s, OH.

Enol Acetate (27)

The Michael adduct (26) was then stirred with a solution of acetic anhydride/pyridine (1:1, 10 ml) at room temperature for 24h. The solvent was evaporated and the residue taken up in methanol (10 ml), stirred for 24h and the solvent evaporated. Repeating this cycle five times followed

by chromatography on flash silica gel eluting with dichloromethane/ethyl acetate (100/0 - 90/10) gave two products. The first to be eluted was identified as starting aplypallidenone (21) (t.l.c. and ^{1}H n.m.r.). The second to be eluted was the enol acetate (27) as a colourless gum. (Found: 349.2357. $C_{21}H_{33}O_4$ [$C_{25}H_{38}O_7$-$COOCH_3$-C_2H_2O] requires 349.2378; found: 317.2124. $C_{20}H_{29}O_3$ [$C_{25}H_{38}O_7$-$COOCH_3$-C_2H_2O-CH_3OH] requires 317.2116). λ_{max} 217 nm (ϵ 8867). ν_{max} 2951, 2935, 1768, 1730, 1642, 1437, 1370, 1241, 1115, 1079, 1033 cm^{-1}. ^{1}H n.m.r. (200MHz) δ 0.80 - 2.10, m, $(H1)_2$, $(H2)_2$, $(H3)_2$, H5α, $(H6)_2$, $(H7)_2$; 0.83, s, Me; 0.84, s, Me; 1.03, s, Me; 1.38, s, Me; 1.63, d, $J_{9\alpha,11\alpha}$ 1.8 Hz, H9α; 2.07, s, $OCOCH_3$; 2.22, s, $OCOCH_3$; 3.50, s, OCH_3; 3.72, s, $COOCH_3$; 4.03, d, $J_{12\beta,11\alpha}$ 2.0 Hz, H12β; 5.54, dd, $J_{11\alpha,12\beta}$ 2.0 Hz, $J_{11\alpha,9\alpha}$ 1.8 Hz, H11α. ^{13}C n.m.r. (50MHz) δ 17.8, CH_3; 18.3, 2xCH_2; 21.0, CH_3; 21.1, CH_3; 21.2, CH_3; 21.6, CH_3; 33.2, CH_3 and C; 37.4, C; 37.8, CH_2; 39.1, CH_2; 40.6, C; 41.6, CH_2; 50.9, CH_3; 51.7, CH_3; 56.6, CH, C5 or C9; 58.2, CH, C5 or C9; 67.1, CH, C12; 76.8, CH, C11; 113.6, C13; 164.5, C14 or C15; 165.7, C14 or C15; 168.0, $OCOCH_3$; 170.2, $OCOCH_3$. Mass spectrum *m/z* 349 (40%), 317 (30), 198 (10), 165 (22), 143 (13), 123 (11), 109 (10), 95 (16), 81 (13), 69 (24), 43 (100).

Ambliofuran (28)

Ambliofuran (1.6 g, 0.23%) was isolated as a colourless oil b.p. (Kugelrohr) 200°/0.05 mmHg (lit.[19] 190°/0.05mmHg). ^{1}H n.m.r. δ 1.59, bs, 3xCH_3; 2.00, m, 4xH; 2.07, m, 4xH; 2.24, td, *J* 7.7, 7.2 Hz, $(H2')_2$; 2.45, bt, *J* 7.7 Hz, $(H1')_2$; 5.10, m, H7', H11'; 5.17, tdd, *J* 7.2, 3.0, 2.5 Hz, H3'; 6.27, m, H4; 7.20, m, H2; 7.33, m, H5.

Hexahydroambliofuran (29)

Hexahydroambliofuran (11.3 mg, 0.0017%) was separated as a colourless oil by g.l.c. ^{1}H n.m.r. δ 0.86, d, J 7 Hz, $(H13')_3$; 0.87, d, J 7 Hz, $3xCH_3$; 1.0–1.3, m, 16xH; 1.47, m, 3xH; 2.37, t, J 7.5 Hz, $(H1')_2$; 6.25, bs, H4; 7.20, bs, H2, 7.33, t, J 1.6 Hz, H5.

Aplyroseol-1 (30)

Aplyroseol-1 (0.954 g, 0.14%) was isolated as clear colourless needles from light petroleum/dichloromethane m.p. 196–198° (lit.[19] 190–192°). ^{1}H n.m.r. δ 0.78, s, Me; 0.82, s, Me; 0.93, m, H1α; 0.94, s, Me; 0.98, t, J 7.5 Hz, $OCOCH_2CH_2CH_3$; 1.18, td, J 13, 5 Hz, H3α; 1.33, dd, J 13.2, 2.5 Hz, H5α; 1.37–1.76, m, H1β, $(H2)_2$, H3β, H6β, H9α, H11α, H12α; 1.69, m, $OCOCH_2CH_2CH_3$; 1.84, ddd, J 14.8, 3.5, 2.5 Hz, H6α; 1.96, qd, J 13.1, 4.2, H11β; 2.28, bd, J 13.5 Hz, H12β; 2.37, m, $OCOCH_2CH_2CH_3$; 2.78, ddd, J 11.4, 7.9, 1.2 Hz, H13; 2.91, dd, J 11.4, 6.1 Hz, H14; 3.77, bs, OH; 4.75, dd, J 3.5, 2.5 Hz, H7; 5.53, d, J 6.1 Hz, H15; 6.05, d, J 1.8 Hz, H17. Mass spectrum *m/z* 402(<1%), 332(2), 314(4), 286(75), 258 (48), 243(9), 230(10), 229(15), 163(15), 162(17), 137(40), 124(35), 109 (57), 81(32), 79(18), 71(50), 69(45), 60(30), 55(44), 43(100).

Aplyroseol-3 (31)

Aplyroseol-3 (0.130 g, 0.019%) was isolated as clear colourless fibres from light petroleum/ dicloromethane m.p. 210–213° (lit.[19] 214–215°). ^{1}H n.m.r. δ 0.95, m, H1α; 0.98, s, Me; 1.01, t, J 7.4 Hz,

$OCOCH_2CH_2CH_3$; 1.02, s, Me; 1.10, s, Me; 1.24, dt, *J* 13.5, 4.2 Hz, H3α; 1.35–1.80, m, H1β, $(H2)_2$, H3β, H5α, H9α, H11α, H12α; 1.74, m, $OCOCH_2CH_2CH_3$; 1.98, dq, *J* 13.2, 4.2 Hz, H11β; 2.37, bd, *J* 14.2 Hz, H12β; 2.46, m, $OCOCH_2CH_2CH_3$; 2.75, m, H13, H14; 3.18, bs, OH; 4.18, ddd, *J* 11.6, 6.3, 2.7 Hz, H6β; 4.92, d, *J* 2.7 Hz, H7β; 5.56, d, *J* 1.8 Hz, H17; 6.06, m, H15. Mass spectrum *m/z* 436(<1%), 418(<1), 403(1), 400(1), 385(2), 348(1), 302(30), 245 (24), 71(58), 43(100)

Aplyroseol-4 (32)

Aplyroseol-4 (0.406 g, 0.069%) was isolated by h.p.l.c. as a colourless glass m.p. 97–99° (lit.[19] 125–127°). ^1H n.m.r. δ 0.87, s, Me; 0.95, m, H1α; 0.97, s, Me; 1.03, t, *J* 7.4 Hz, $OCOCH_2CH_2CH_3$; 1.05, s, Me; 1.25, dt, *J* 13.2, 4.2Hz, H3α; 1.35–1.80, m, H1β, $(H2)_2$, H3β, H9α, H11α, H12α; 1.74, m, $OCOCH_2CH_2CH_3$; 1.76, d, *J* 12.4 Hz, H5α; 1.98, s, $OCOCH_3$; 2.00, dq, *J* 13.2, 4.2 Hz, H11β; 2.38, m, H12β; 2.42, m, $OCOCH_2CH_2CH_3$; 2.72, m, H13, H14; 3.47, d, *J* 2.1 Hz, OH; 4.98, d, *J* 2.5 Hz, H7β; 5.36, dd, *J* 12.4, 2.5 Hz, H6β; 5.56, d, *J* 2.1 Hz, H17; 6.03, m, H15. Mass spectrum *m/z* 463(<1%), 372(<1), 344(4), 330(2), 320(1), 302(5), 286(5), 274(14), 256 (17), 105(15), 71(23), 55(12), 43(100).

Aplyroseol-5 (33)

Aplyroseol-5 (0.843 g, 0.12%) was isolated as clear colourless fibres from light petroleum/dichloromethane m.p. 212–215° (lit.[19] 214–217°). ^1H n.m.r. δ 0.87, s, Me; 0.96, t, *J* 7.5 Hz, $OCOCH_2CH_2CH_3$; 0.97, m, H1α; 0.99, s, Me; 1.03, s, Me; 1.25, td, *J* 13.6, 4.2 Hz, H3α; 1.44–1.75, m,

H1β, $(H2)_2$, H3β, H9α, H11α, H12α; 1.66, m, $OCOCH_2CH_2CH_3$; 1.83, d, *J* 12.1 Hz, H5α; 1.97, dq, *J* 13.2, 4.0 Hz, H11β, 2.20, d, *J* 2.7 Hz, OH; 2.32, m, $OCOCH_2CH_2CH_3$; 2.36, m, H12β; 2.80, m, H13; 3.26, bs, OH; 3.44, dd, *J* 2.7, 2.4 Hz, H7β; 3.58, dd, *J* 11.6, 6.3 Hz, H14; 5.39, dd, *J* 12.1, 2.4 Hz, H6β; 5.59, d, *J* 3.4 Hz, H17; 6.07, d, *J* 6.3 Hz, H15. Mass spectrum *m/z* 389(<1%), 348(2), 340(14), 330(6), 302(36), 179(34), 123(34), 109(42), 71(72), 43(100).

Aplyroseol-6 (34)

Aplyroseol-6 (0.466 g, 0.069%) was isolated by h.p.l.c. as a colourless glass m.p. 114–118° (lit.[19] 133–135°). ^1H n.m.r. δ 0.87, s, Me; 0.93, t, *J* 7.4 Hz, $OCOCH_2CH_2CH_3$; 0.98, s, Me; 0.98, m, H1α; 1.05, s, Me; 1.25, dt, *J* 13.4, 4.0 Hz, H3α; 1.35–1.80, m, H1β, $(H2)_2$, H3β, H9α, H11α, H12α; 1.61, m, $OCOCH_2CH_2CH_3$; 1.76, d, *J* 12.2 Hz, H5α; 2.02, m, H11β; 2.18, s, $OCOCH_3$; 2.20, m, $OCOCH_2CH_2CH_3$; 2.37, bd, *J* 14.6 Hz, H12β; 2.73, m, H13, H14; 3.75, d, *J* 2.4 Hz, OH; 4.96, d, *J* 2.5 Hz, H7β; 5.37, dd, *J* 12.2, 2.5Hz, H6β; 5.66, d, *J* 2.4 Hz, H17; 6.02, m, H15. Mass spectrum *m/z* 463(<1%), 372(13), 344(2), 302(19), 286(8), 274(19), 256 (15), 71(42), 43(100).

Lactone (35)

The lactone (35) (0.149 g, 0.022%) was isolated as fine colourless fibres from light petroleum/dichloromethane m.p. 179–180° (lit.[21] 179–180°). $[\alpha]_D$ −40.9°, $[\alpha]_{578}$ −42.8°, $[\alpha]_{546}$ −48.6°, $[\alpha]_{436}$ −82.2° (c 1.99). ^1H n.m.r. δ 0.79, s, 2xMe; 0.85, s, Me; 0.86, m, H1α; 0.93, s,

Me; 1.05, dd, H5α; 1.17, dt, H3α; 1.24–1.78, m, H1β, $(H2)_2$, H3β, $(H6)_2$, H9α, $(H11)_2$, H12α; 2.09, s, $OCOCH_3$; 2.30, m, H12β; 2.50, dd, *J* 7.6, 5.1 Hz, H14; 2.58, m, H13; .6, 5.1 Hz, H14; 3.98, dd, *J* 10.5, 5.1 Hz, H15α; 4.23, d, *J* 10.5 Hz, H15β; 4.93, m, H7β. Mass spectrum *m/z* 302(24%), 287(19), 259(4), 217(11), 149(8), 137(28), 123(24), 109(21), 95(26), 81(33), 43(100).

Lactone (36)

The lactone (36) (0.380 g, 0.056%) was isolated by h.p.l.c. as a colourless gum. ^1H n.m.r. δ 0.79, s, 2xMe; 0.87, m, H1α; 0.92, s, Me; 1.18, m, H3α, H5α; 1.30–1.80, m, H1β, $(H2)_2$, H3β, $(H6)_2$, H9α, $(H11)_2$, H12α; 2.09, s, $OCOCH_3$; 2.12, s, $OCOCH_3$; 2.36, m, H12β; 2.62, m, H13, H14; 3.95, dd, *J* 10.2, 5.6 Hz, H15α; 4.06, d, *J* 13.2 Hz, H17a; 4.42, dd, *J* 13.2, 1.3Hz, H17b; 4.59, d, *J* 10.2 Hz, H15β; 5.30, m, H7β. Mass spectrum *m/z* 360(2%), 318(8), 300(31), 288(15), 287(24), 285(9), 123(24), 109(32), 43(100).

(5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*)-7,15,16-trihydroxy-*ent*-isocopalane (52)

A mixture of the lactone (35) (21 mg, 0.058 mmol) and lithium aluminium hydride (excess) in dry ether (20 ml) was heated under reflux for 6 hours. The mixture was then acidified with hydrochloric acid (3 M) and extracted with ethyl acetate (6 x 20 ml). The combined ethyl acetate extracts were then washed with saturated sodium bicarbonate solution (2 x 20 ml), brine (2 x 20 ml), and the solvent evaporated. Benzene (10 ml) was added and evaporation of the solvent yielded a colourless powder (17

mg, 90%). (Found: 288.2430. $C_{20}H_{32}O$ [$C_{20}H_{36}O_3$–2xH_2O] requires 288.2453; found: 273.2218. $C_{19}H_{29}O$ [$C_{20}H_{36}O_3$–2xH_2O–CH_3] requires 273.2218). ν_{max} 3323br, 2953, 2926, 2856, 1462, 1368, 1021 cm^{-1}.

^1H n.m.r. (CD_3OD/D_6–DMSO) δ 0.78, s, Me; 0.82, s, Me; 0.84, s, Me; 0.85, s, Me; 0.88, m, H1α; 1.15–1.51, m, 9xH; 1.58–1.75, m, 3xH; 1.77, dt, *J* 14.4, 2.8 Hz, H6β; 2.01, m, H13, H14, H12β; 3.40–3.53, m, 3xH; 3.62, dd, *J* 2.8, 2.8 Hz, H7β; 3.88, dd, *J* 11.2, 7.0Hz, 1xH. Mass spectrum *m/z* 324(<1%), 306(14), 288(10), 273(10), 261(7), 245(5), 233(5), 220(5), 207(17), 177(18), 149(23), 123(51), 109(70), 95(65), 81(72), 69(91), 55(100).

(5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*)-7-hydroxyspongian (53)

Method 1

Using the method of Gillis and Beck[34] the triol (52) (17 mg) in dimethylsulfoxide (2 ml) was heated at 140 - 145° for 14 h. Water (50 ml) was then added and the product extracted into ether (3 x 20 ml). The combined ether extracts were washed with water (2 x 20 ml), brine (2 x 20 ml), dried over anhydrous sodium sulfate and the solvent evaporated. Chromatography on flash silica gel eluting with ethyl acetate/light petroleum (1:5) gave 7-hydroxyspongian (53) as a colourless glass (12 mg, 75%). (Found: 304.2392. $C_{20}H_{32}O_2$ [$C_{20}H_{34}O_2$–H_2] requires 304.2402). ν_{max} 3435br, 2954, 2928, 1464, 1390, 1230, 1058, 885 cm^{-1}. ^1H n.m.r. (200MHz) δ 0.72–1.83, m, 13xH; 0.82, s, Me; 0.86, s, Me; 0.87, s, Me; 0.97, s, Me; 1.86, ddd, *J* 14.4, 13.2, 2.4 Hz, H6β; 2.47, m, H13; 2.64, dd, $J_{14,13}$ 6.2 Hz, $J_{14,15\alpha}$ 5.4 Hz, H14; 3.49, dd, J_{gem} 11.4 Hz, $J_{16\alpha,13}$ 7.6 Hz, H16α; 3.62, dd, $J_{7\beta,6\beta}$ 2.4 Hz, $J_{7\beta,6\alpha}$ 3.2 Hz,

H7β; 3.72, dd, J_{gem} 9.0 Hz, $J_{15\alpha,14}$ 5.4 Hz, H15α; 3.81, d, J_{gem} 11.4 Hz, H16β; 3.90, d, J_{gem} 9.0 Hz, H15β. ^{13}C n.m.r. (50MHz) δ 16.0, CH_3; 16.6, CH_2; 17.2, CH_3; 18.5, CH_2; 21.8, CH_3; 23.8, CH_2; 26.1, CH_2; 32.6, C; 33.2, CH_3; 37.5, CH; 37.6, C; 39.5, C; 39.7, CH_2; 42.0, CH_2; 43.7, CH; 47.0, CH; 51.8, CH; 69.9, CH_2, C15 or C16; 71.6, CH_2, C15 or C16; 72.7, CH, C7. Mass spectrum *m/z* 306(1%), 304(2), 302(4), 288(18), 273(11), 203(10), 150(100), 137(40), 123(48), 109(46), 95(43), 81(49), 69(55), 55(57), 41(63). (Found: 302.2252. $C_{20}H_{30}O_2$ [$C_{20}H_{34}O_2$-2xH_2] requires 302.2245; found: 288.2435. $C_{20}H_{32}O$ requires 288.2453; found: 273.2225. $C_{19}H_{29}O$ requires 273.2218).

Method 2

Using the method of Barry and Evans[35] a solution of the triol (52) (11 mg, 0.034 mmol), carbon tetrachloride (2 ml), and acetonitrile (1 ml) was heated under reflux for 24 h. The solution was then cooled and the solvent evaporated. The residue was chromatographed on flash silica gel eluting with ethyl acetate/light petroleum (1:9) to give (53) in 70% yield

Benzoylation of Aplyroseol-1 (30)

A solution aplyroseol-1 (30) (40 mg, 0.095 mmol), *p*-bromobenzoyl chloride (160 mg, 0.71 mmol) and *N*,*N*-dimethylaminopyridine (120 mg, 0.95 mmol) in dichloromethane (15ml) was stirred at room temperature (25°) for 72h. The solvent was removed under reduced pressure to give a clear glass. Purification by h.p.l.c. (ethyl acetate / dichloromethane,1:19) gave one major product as a clear glass (45 mg, 79%) which was crystallised by the slow diffusion mixing of dichloromethane and light

petroleum (30-40°) at room temperature for 96h to give the *p*-bromobenzoate, (5R, 7S, 8R, 9S, 10R, 13S, 14S, 15R)-7,15-dihydroxyspongian-16,17-dione-7-butyrate-15-*p*-bromobenzoate (2) as colourless needles m.p. 179-182°. (Found:C, 61.56; H, 6.83. $C_{31}H_{39}O_7Br$ requires C, 61.70; H, 6.47%). λ_{max} 247.6nm (ϵ 15389). ν_{max} 2933, 2908, 2873, 1800, 1739, 1591, 1264, 1258, 1089, 1012, 972, 958, 942, 935 cm^{-1}. ^1H n.m.r. δ 0.73, s, 19Me; 0.75, s, 20Me; 0.78, s, 18Me; 0.88, t, *J* 7.6 Hz, $OCOCH_2CH_2CH_3$; 0.93, m, H1α; 1.16, m, H3α; 1.26, dd, $J_{5\alpha,6B}$ 13 Hz, $J_{5\alpha,6\alpha}$ 2.3 Hz, H5α; 1.38-2.00, m, $(H2)_2$, H3β, $H6_B$, H9, $(H11)_2$, H12α; 1.60, m, $OCOCH_2CH_2CH_3$; 1.74, m, H1β; 1.19, m, H6α; 2.27, m, $OCOCH_2CH_2CH_3$; 2.55, m, H12β; 2.89, dd, $J_{14,13}$ 8.2 Hz, $J_{14,15}$ 0 Hz, H14; 3.12, br dd, *J* 6.8 Hz, *J* 8.2 Hz, H13; 5.70, m, H7; 6.37, d, $J_{14,15}$ 0 Hz, H15; 7.57 and 7.81, AA'BB' system, Ar-H; 9.99, s, CHO. Mass spectrum (e.i.) *m/z* 516(<1%), 514(<1), 487(1), 485(1), 403(3), 332(8), 314(31), 286(63), 285(78), 257(29), 229(10), 185(51), 183(53), 123(29), 71(69), 43(100). (Found: 514.1351. $C_{27}H_{31}O_5Br$ [$C_{31}H_{39}O_7Br - CH_3(CH_2)_2COOH$] requires 514.1355; found: 485.1342. $C_{26}H_{30}O_4Br$ requires 485.1328; found: 403.2488, $C_{24}H_{35}O_5$ requires 403.2484; found: 314.1862, $C_{20}H_{26}O_3$ requires 314.1882; found: 286.1937, $C_{19}H_{26}O_2$ requires 286.1933; found: 257.1879, $C_{18}H_{25}O$ requires 257.1905). Mass spectrum (c.i. methane) *m/z* 517(<1%), 515(<1), 403(48), 315(37), 287(24), 203(13), 201(15), 41(100).

Dendrillol-1 (37)

Dendrillol-1 was isolated by h.p.l.c. as colourless needles (6.3 mg, 0.00093%). (lit.[26] 229-231°). ^1H n.m.r. δ 0.83, s, Me; 0.85, s, Me; 0.87, m, H1α; 0.92, s, Me; 0.95, dd, J12.5, 2.7 Hz, H5α; 1.12, m, H7α, H3α; 1.20-1.76, m, 9xH; 1.86, m, 1xH; 1.96, H11β; 2.39, m, H12β; 2.58, dd, *J*

11.6, 6.1 Hz, 2.71, m, H13, OH; 5.50, d, *J* 1.8 Hz, H17; 6.09, d, *J* 6.1 Hz, H15. Mass spectrum *m/z* 304(<1%), 288(2), 281(1), 275(4), 264(1), 256(1), 246(4), 237(2), 232(1), 205(3), 176(5), 163(8), 149(6), 123(7), 109(9), 95(8), 58(14), 43(100).

Dendrillol-2 (38)

Dendrillol-2[26] (35 mg, 0.0052%) was isolated by h.p.l.c. as a colourless glass m.p. 120-124°. ^{1}H n.m.r. δ 0.88, s, Me; 0.98, s, Me; 0.99, m, H1α; 1.05, s, Me; 1.26, m, H3α; 1.08-1.35, m, 9xH; 1.99, s, $OCOCH_3$; 2.02, m, 1xH; 2.20, s, $OCOCH_3$; 2.38, m, 1xH; 2.74, m, H13, H14; 3.73, bs, OH; 4.98, d, *J* 2.7 Hz, H7β; 5.36, dd, *J* 12.2, 2.7, H6β; 5.65, bs, H17; 6.03, m, H15. Mass spectrum *m/z* 450(<1%), 346(5), 345(20), 302(26), 286(14), 274(30), 256(35), 43(100).

AROSST-1 (39)

Methyl (5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*)-7-butyroxy-15,17-epoxy-15-oxo-*ent*-isocopalan-16-oate (39)

AROSST-1 (31 mg, 0.0045%) was isolated by h.p.l.c. as a colourless gum. (Found: 434.2670. $C_{25}H_{38}O_6$ requires 434.2668). λ_{max} no peaks. ν_{max} 2956, 2933, 1774, 1735, 1463, 1438, 1384, 1318, 1230, 1174, 1041 cm^{-1}. ^{1}H n.m.r. δ 0.77, s, Me; 0.78, s, Me; 0.80, s, Me; 0.94, dt, J_{gem} 13 Hz, $J_{1\alpha,2\beta}$ 13 Hz, $J_{1\alpha,2\alpha}$ 3.8 Hz, H1α; 1.00, t, J 7.5Hz, $OCOCH_2CH_2CH_3$; 1.17, dt, J_{gem} 13.8 Hz, $J_{3\alpha,2\beta}$ 13.8 Hz, $J_{3\alpha,2\alpha}$ 4.5 Hz, H3α; 1.30, dd, $J_{5\alpha,6\beta}$ 13.2 Hz, $J_{5\alpha,6\alpha}$ 2.4 Hz, H5α; 1.40-1.78, m, H1β, $(H2)_2$, H3β, H6β, H9α, $(H11)_2$, H12β; 1.71, m,

$OCOCH_2CH_2CH_3$; 1.95, m, H6α, H12α; 2.36, m, $OCOCH_2CH_2CH_3$; 2.84, ddd, $J_{13,12\beta}$ 11.4 Hz, $J_{13,14}$ 6.7 Hz, $J_{13,12\alpha}$ 4.1 Hz, H13; 2.96, dd, $J_{14,13}$ 6.7 Hz, $J_{14,12\alpha}$ 1.5 Hz, H14; 3.71, s, $COOCH_3$; 4.02, d, J_{gem} 10.0 Hz, H17a; 4.36, d, J_{gem} 10.0 Hz, H17b; 4.84, dd, $J_{7\beta,6\alpha}$ 2.8 Hz, $J_{7\beta,6\beta}$ 2.6 Hz, H7β. ^{13}C n.m.r. δ 13.7, CH_3; 14.2, CH_3; 16.3, CH_2; 18.2, CH_2; 18.6, CH_2; 21.2, CH_2; 21.3, CH_3; 23.3, CH_2; 32.5, C; 33.0, CH_3; 36.6, CH_2; 37.7, C; 38.4, CH_2; 38.6, CH; 41.7, CH_2; 44.8, CH; 45.6, CH; 46.0, C; 48.4, CH; 51.8, OCH_3; 71.4, CH_2, C17; 74.0, CH, C7; 172.4; 172.8; 176.1, C15. Mass spectrum *m/z* 434(5%), 419(1), 403(3), 363(12), 346(29), 331(17), 314(8), 288(22), 123(14), 105(9), 91(12), 71(27), 43(100). (Found: 403.2507. $C_{24}H_{35}O_5$ requires 403.2484; found: 346.2163. $C_{21}H_{30}O_4$ requires 346.2114).

AROSST-2 (40)

Methyl (5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*)-7-acetoxy-15,17-epoxy-15-oxo-*ent*-isocopalan-16-oate (40).

AROSST-2 (11.5 mg, 0.0017%) was isolated by h.p.l.c. as a colourless gum. (Found: 406.2363. $C_{23}H_{34}O_6$ requires 406.2355). λ_{max} no peaks. ν_{max} 3022, 2956, 2931, 1775, 1738, 1375, 1242, 1231, 1176, 1040, 797 cm^{-1}. ^1H n.m.r. δ 0.77, s, $W_{h/2}$ 2.2 Hz, Me; 0.79, s, $W_{h/2}$ 2.2 Hz, Me; 0.80, s, $W_{h/2}$ 2.2 Hz, Me; 0.94, m, H1α; 1.17, dt, J 13.2, 4.2 Hz, H3α; 1.29, dd, J 13.2, 2.8 Hz, H5α; 1.40-1.78, m, H1β, $(H2)_2$, H3β, H6β, H9α, $(H11)_2$, H12β; 1.90, m, H12α; 1.96, td, J_{gem} 15.4 Hz, $J_{6\alpha,5\alpha}$ 2.8 Hz, $J_{6\alpha,7\beta}$ 2.8 Hz, H6α; 2.13, s, $OCOCH_3$; 2.85, ddd, $J_{13,12\beta}$ 11.0 Hz, $J_{13,14}$ 6.8 Hz, $J_{13,12\alpha}$ 4.2 Hz, H13; 2.95, dd, $J_{14,13}$ 6.8 Hz, $J_{14,12\alpha}$ 1.6 Hz, H14; 3.73, s, $COOCH_3$; 4.01, d, J_{gem} 10.1 Hz, H17a; 4.36, d , J_{gem} 10.1 Hz, H17b; 4.81, dd, $J_{7\beta,6\beta}$ 3.0 Hz, $J_{7\beta,6\alpha}$ 2.8 Hz, H7β. ^{13}C n.m.r. δ 14.2; 16.4; 18.2; 21.2; 21.3; 21.4; 23.1; 32.5; 33.1;

37.7; 36.2; 36.7; 41.6; 44.7; 45.5; 45.9; 46.3; 52.1; 71.5; 74.2; 170.0; 173.0; 176.3. Mass spectrum *m/z* 406(18%), 391(5), 375(12), 363(22), 346(72), 331(34), 314(25), 300(13), 288(48), 271(14), 123(49), 109(37), 91(47), 69(73), 43(100). (Found: 391.2106. $C_{22}H_{31}O_6$ requires 391.2120; found: 375.2177. $C_{22}H_{31}O_5$ requires 375.2171; found: 346.2125. $C_{21}H_{30}O_4$ requires 346.2144).

AROSST-3 (41)

Methyl (5*S*, 6*R*, 7*S*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*)-6-butyroxy-15,17-epoxy-7-hydroxy-15-oxo-*ent*-isocopalan-16-oate (41).

AROSST-3 (55.9 mg, 0.0083%) was isolated by h.p.l.c. as a clear colourless glass. (Found: 450.2623. $C_{25}H_{38}O_7$ requires 450.2617). λ_{max} no peaks. ν_{max} 3531br, 2955, 2934, 1772, 1734, 1437, 1385, 1231, 1178, 1097, 1041 cm^{-1}. ^1H n.m.r. δ 0.86, s, Me; 0.87, s, Me; 0.97, t, *J* 7.4 Hz, $OCOCH_2CH_2CH_3$; 0.98, m, H1α; 1.02, s, Me; 1.25, dt, *J* 13.2, 4.0 Hz, H3α; 1.37, bd, *J* 13.2 Hz, H3β; 1.37-1.85, m, H1β, $(H2)_2$, H9α, $(H11)_2$, H12β; 1.68, m, $OCOCH_2CH_2CH_3$; 1.92, d, $J_{5\alpha,6\beta}$ 12.2 Hz, H5α; 1.92, m, H12α; 2.34, m, $OCOCH_2CH_2CH_3$; 2.55, bs, OH; 2.86, ddd, $J_{13,12\beta}$ 11.4 Hz, $J_{13,14}$ 7.0 Hz, $J_{13,12\alpha}$ 3.6 Hz, H13; 3.54, dd, $J_{14,13}$ 7.0 Hz, $J_{14,12\alpha}$ 1.5 Hz, H14; 3.61, d, $J_{7\beta,6\beta}$ 2.4 Hz, H7β; 3.73, s, $COOCH_3$; 4.17, d, J_{gem} 10.1 Hz, H17a; 4.31, d, J_{gem} 10.1 Hz, H17b; 5.35, dd, $J_{6\beta,5\alpha}$ 12.2 Hz, $J_{6\beta,7\beta}$ 2.4 Hz, H6β. ^{13}C n.m.r. δ 13.6; 15.2; 16.7; 18.0; 18.2; 21.2; 22.1; 32.5; 36.0; 36.7; 38.3; 38.8; 39.2; 42.1; 43.1; 44.7; 47.2; 49.4; 51.9; 71.6; 72.0; 73.3; 172.6, 173.2; 176.6. Mass spectrum *m/z* 450(1%), 435(1), 419(2), 362(56), 347(6), 330(24), 315(6), 302(5), 280(6), 272(6), 123(10), 109(11), 71(40), 43(100). (Found: 435.2396. $C_{24}H_{35}O_7$ requires 435.2382; found: 419.2468. $C_{24}H_{35}O_6$ requires

419.2433).

AROSST-4 (42)

Methyl (5*S*, 6*R*, 7*S*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*,)-7-acetoxy-6-butyroxy-15,17-epoxy-15-oxo-*ent*-isocopalan-16-oate (42).

AROSST-4 (24.5 mg, 0.0036%) was isolated by h.p.l.c. as a colourless glass. (Found: 492.2700. $C_{27}H_{40}O_8$ requires 492.2723). λ_{max} no peaks. ν_{max} 2956, 2935, 1777, 1744, 1464, 1438, 1377, 1370, 1235, 1178 cm^{-1}. ^1H n.m.r. δ 0.83, s, $W_{h/2}$ 2.1 Hz, Me; 0.88, s, $W_{h/2}$ 2.3 Hz, Me; 0.94, t, J 7.5 Hz, $OCOCH_2CH_2CH_3$; 0.98, m, H1α; 1.00, s, $W_{h/2}$ 1.8 Hz, Me; 1.25, dt, J 13.6, 4.0 Hz, H3α; 1.41, bd, J 13.6 Hz, H3β; 1.43-1.80, m, H1β, $(H2)_2$, H5α, H9α, $(H11)_2$, H12β; 1.64, m, $OCOCH_2CH_2CH_3$; 1.97, m, H12α; 2.17, s, $OCOCH_3$; 2.20, m, $OCOCH_2CH_2CH_3$; 2.77, m, H13, H14; 3.71, s, $COOCH_3$; 4.27, d, J_{gem} 10.4 Hz, H17a; 4.35, d, J_{gem} 10.4 Hz, H17b; 5.14, d, $J_{7\beta,6\beta}$ 2.3 Hz, H7β; 5.28, dd, $J_{6\beta,5\alpha}$ 12.2 Hz, $J_{6\beta,7\beta}$ 2.3 Hz, H6β. ^{13}C n.m.r. δ 13.8, CH_3; 15.5, CH_3; 16.5, CH_2; 17.7, CH_2; 17.9, CH_2; 20.6, CH_2; 20.8, CH_3; 21.8, CH_3; 32.6, C; 36.1, CH_3; 36.4, CH_2; 38.4, CH_2; 38.5, CH; 39.2, C; 43.1, CH_2; 43.7, CH; 45.5, CH; 46.4, C; 50.6, CH; 51.1, OCH_3; 70.0, CH, C6 or C7; 71.1, CH_2, C17; 74.1, CH, C6 or C7; 170.0; 170.6; 172.9; 175.3, C15. Mass spectrum *m/z* 492(7%), 477(2), 461(4), 362(51), 335(8), 334(10), 330(18), 316(12), 149(12), 123(10), 109(15), 81(17), 71(70), 43(100). (Found: 477.2498. $C_{26}H_{37}O_8$ requires 477.2488; found: 461.2549. $C_{26}H_{37}O_7$ requires 461.2549).

AROSST-5 (43)

Methyl (5*S*, 6*R*, 7*S*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*,)-6-acetoxy-7-butyroxy-15,17-epoxy-15-oxo-*ent*-isocopalan-16-oate (43).

AROSST-5 (62.5 mg, 0.0093%) was isolated by h.p.l.c. as colourless needles. (Found: 492.2707. $C_{27}H_{40}O_8$ requires 492.2723). λ_{max} no peaks. ν_{max} 2955, 2935, 1776, 1740, 1242, 1178, 1054 cm^{-1}. ^1H n.m.r. δ 0.81, s, $W_{h/2}$ 2.2 Hz, Me; 0.86, s, $W_{h/2}$ 2.4 Hz, Me; 1.00, m, H1α; 1.01, s, Me; 1.03, t, *J* 7.4 Hz, $OCOCH_2CH_2CH_3$; 1.26, dt, *J* 13.5, 4.4 Hz, H3α; 1.41, bd, *J* 13.5 Hz, H3β; 1.43-1.80, m, H1β, $(H2)_2$, H9α, $(H11)_2$, H12β; 1.75, dt, *J* 7.4, 7.4 Hz, $OCOCH_2CH_2CH_3$; 1.78, d, $J_{5\alpha,6\beta}$ 12.3 Hz, H5α; 1.96, m, H12α; 1.99, s, $OCOCH_3$; 2.42, t, *J* 7.4 Hz, $OCOCH_2CH_2CH_3$; 2.76, ddd, $J_{13,12\beta}$ 12.0Hz, $J_{13,14}$ 6.4 Hz, $J_{13,12\alpha}$ 4.0 Hz, H13; 2.82, dd, $J_{14,13}$ 6.4 Hz, $J_{14,12\alpha}$ 1.6 Hz, H14; 3.71, s, $COOCH_3$; 4.27, d, J_{gem} 10.2 Hz, H17a; 4.36, d, J_{gem} 10.2 Hz, H17b; 5.17, d, $J_{7\beta,6\beta}$ 2.4 Hz, H7β; 5.26, dd, $J_{6\beta,5\alpha}$ 12.3 Hz, $J_{6\beta,7\beta}$ 2.4 Hz, H6β. ^{13}C n.m.r. δ 13.7; 15.4; 16.4; 17.9; 18.7; 20.4; 21.3; 21.8; 32.6; 36.1; 36.4; 38.3; 38.4; 39.1; 43.0; 43.8; 45.5; 46.2; 50.8; 52.0; 70.1, C6 or C7; 71.1, C17; 73.6, C6 or C7; 170.1; 172.5; 172.6; 175.3. Mass spectrum *m/z* 492(4%), 461(2), 433(10), 362(34), 345(3), 344(4), 331(7), 316(6), 285(4), 71(40), 55(18), 43(100). (Found: 461.2534. $C_{26}H_{37}O_7$ requires 461.2539).

AROSST-6 (44) and AROSST-6A (44A)

AROSST-6 was isolated by h.p.l.c. as a colourless glass however it oxidised to AROSST-6A before full spectroscopic measurements were obtained.

Methyl (5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*,)-7-acetoxy-*ent*-isocopalan-

15-al-16-oate (44)

^{1}H n.m.r. δ 0.78, s, Me; 0.80, s, Me; 0.85, s, Me; 1.07, s, Me; 1.09-1.79, m, 13xH; 2.03, s, $OCOCH_3$; 2.44, bd, *J* 10 Hz, 1xH; 2.63, d, J 5 Hz, H14; 3.16, m, H13; 3.68, s, $COOCH_3$; 5.32, bs, H7β; 9.77, s, CHO.

15H, 16Methyl (5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*)-7-acetoxy-*ent*-isocopalan-15,16-dioate (44A).

AROSST-6A (44A) was isolated as a white powder. (Found: 348.2324. $C_{21}H_{32}O_4$ [$C_{23}H_{36}O_6$-CH_3COOH] requires 348.2300). λ_{max} no peaks. ν_{max} 3500-2500br, 2955, 2929, 1732, 1464, 1443, 1389, 1366, 1254, 1181, 1639 cm^{-1}. ^{1}H n.m.r. δ 0.75, s, 20Me; 0.77, s, 19Me; 0.83, s, 18Me; 0.87, m, H1α; 1.08, s, 17Me; 1.17, m, H3α, H5α, H9α; 1.42, m, H2α, H3β, H11α, H12α; 1.60, m, H2β, H11β; 1.68, m, H1β, H6β; 1.84, ddd, J_{gem} 15.0 Hz, $J_{6\alpha,5\alpha}$ 3.6 Hz, $J_{6\alpha,7\beta}$ 2.3 Hz, H6α; 2.06, s, $OCOCH_3$; 2.37, dddd, *J* 13.2, 5.3, 3.0, 1.8 Hz, H12β; 2.90, d, $J_{14,13}$ 5.3 Hz, H14; 3.22, ddd, $J_{13,14}$ 5.3 Hz, $J_{13,12\alpha}$ 5.2 Hz, $J_{13,12\beta}$ 1.8 Hz, H13; 3.64, s, $COOCH_3$; 5.29, dd, $J_{7\beta,6\beta}$ 3.4 Hz $J_{7\beta,6\alpha}$ 2.3 Hz, H7β. ^{13}C n.m.r. δ 14.9; 15.6; 17.5; 18.5; 21.0; 21.2; 22.2; 27.3; 32.6; 32.9; 37.5; 39.7; 40.3; 40.8; 41.9; 48.1; 50.0; 51.4; 54.7; 75.3; 170.0; 174.2; 177.2. Mass spectrum *m/z* 408(<1%), 366(13), 348(25), 333(10), 316(6), 301(8), 289(8), 242(19), 207(16), 189(22), 119(14), 93(12), 81(14), 43(100). (Found: 366.2389. $C_{21}H_{34}O_5$ requires 366.2406; found: 333.2102. $C_{20}H_{29}O_4$ requires 333.2066; found: 316.2060. $C_{20}H_{28}O_3$ requires 316.2038).

AROSST-7 (45)

Methyl (5*S*, 7*R*, 8*S*, 9*R*, 10*S*, 13*R*, 14*S*)-7-acetoxy-14-hydroxypallidan-15-oate (45).

AROSST-7 (14.2 mg, 0.0021%) was isolated by h.p.l.c. as a colourless gum. (Found: 320.2338. $C_{20}H_{32}O_3$ [$C_{22}H_{36}O_5$–CH_3OOH] requires 320.2351). λ_{max} no peaks. ν_{max} 3558br, 2954, 2929, 1733, 1439, 1365, 1264, 1231, 1189, 1024 cm^{-1}. ^1H n.m.r. δ 0.76, s, $W_{h/2}$ 1.8 Hz, 20Me; 0.78, s, $W_{h/2}$ 2.0 Hz, 19Me; 0.82, d, $J_{18Me,9\alpha}$ 0.8 Hz, 18Me; 0.84, s, $W_{h/2}$ 1.6 Hz, 17Me; 0.94, dt, *J* 13, 3.7 Hz, H1α; 1.17, dt, *J* 13, 4.2 Hz, H3α; 1.23, H5α; 1.41, H3β; 1.43, H2α; 1.51, H11α; 1.57, H2β; 1.60, H11β; 1.73, H1β, H6α, H6β; 1.85, H9α; 1.86, H12α; 2.10, bd, *J* 14 Hz, H12β; 2.13, s, $OCOCH_3$; 2.63, m, H13; 3.67, s, $COOCH_3$; 3.91, bs, OH; 4.12, d, $J_{14,13}$ 2.8 Hz, H14; 4.86, dd, $J_{7\beta,6\beta}$ 2.8 Hz, $J_{7\beta,6\alpha}$ 2.8 Hz, H7β. ^{13}C n.m.r. δ 15.5, CH_3; 17.9, CH_2; 18.4, CH_2; 18.9, CH_3; 21.3, CH_2, CH_3; 21.7, CH_3; 23.6, CH_2; 32.7, C; 32.9, CH_3; 37.0, C; 39.7, CH_2; 42.0, C, CH_2; 45.1, CH_2; 45.6, CH; 48.2, CH; 51.3, OCH_3; 77.8, CH, C7 or C14; 80.4, CH, C7 or C14; 168.9; 174.1. Mass spectrum *m/z* 379(<1%), 320(8), 302(20), 289(4), 287(4), 259(3), 218(9), 191(14), 178(9), 167(10), 149(9), 124(20), 109(33), 94(100). (Found: 302.2264. $C_{20}H_{30}O_2$ requires 302.2245; found: 287.2013. $C_{19}H_{27}O_2$ requires 287.2011).

AROSST-8 (46)

Methyl (5*S*, 7*R*, 8*S*, 9*R*, 10*S*, 13*R*, 14*R*)-7-acetoxy-14-hydroxypallidan-15-oate (46).

AROSST-8 (12.1 mg, 0.0018%) was isolated by h.p.l.c. as a colourless gum

which crystallised on standing. (Found: 320.2350. $C_{20}H_{32}O_3$ [$C_{22}H_{36}O_5$–CH_3COOH] requires 320.2351). λ_{max} no peaks. ν_{max} 3486br, 2956, 2930, 1723, 1464, 1389, 1257, 1200, 1161 cm^{-1}. ^1H n.m.r. δ 0.77, s, $W_{h/2}$ 1.8 Hz, Me; 0.78, s, $W_{h/2}$ 2.2 Hz, Me; 0.79, s, $W_{h/2}$ 1.6 Hz, Me; 0.83, s, $W_{h/2}$ 2.2 Hz, Me; 0.88, m, H1α; 1.18, m, H3α, H9α; 1.25, dd, $J_{5\alpha,6\beta}$ 13.0 Hz, $J_{5\alpha,6\alpha}$ 2.6 Hz, H5α; 1.43, m, H2α, H3β, H11α, H11β, H12α; 1.63, m, H1β, H2β, H6β; 1.76, dt, J 15, 3, 3 Hz, H6α; 2.10, s, $OCOCH_3$; 2.27, m, H12β; 2.82, ddd, J 6.3, 6.0, 2.1 Hz, H13; 3.74, s, $COOCH_3$; 3.82, dd, $J_{14,OH}$ 8.3 Hz, $J_{14,13}$ 6.3 Hz, H14; 4.10, d, J 8.3 Hz, OH; 4.96, dd, J 3.0, 3.0 Hz, H7β. ^{13}C n.m.r. δ 13.5, CH_3; 15.6, CH_3; 17.1, CH_2; 18.5, CH_2; 21.2, CH_3; 21.3, CH_3; 22.6, CH_2; 25.7, CH_2; 32.7, C; 33.0, CH_3; 37.2, C; 39.7, CH_2; 41.9, CH_2; 42.4, CH; 43.6, C; 48.3, CH; 52.0, OCH_3; 52.4, CH; 73.5, CH, C7; 73.5, CH, C14; 170.2; 175.4. Mass spectrum *m/z* 348(<1%), 320(29), 302(37), 287(7), 259(3), 243(4), 227(5), 218(17), 191(30), 178(17), 124(51), 109(72); 94(100), 81(36), 69(48), 55(57). (Found: 302.2266. $C_{20}H_{30}O_2$ requires 302.2246).

AROSST-9 (47)

(5*S*, 8*S*, 9*R*, 10*S*, 13*R*, 14*R*)-17-acetoxy-16-oxospongian (47).

AROSST-9 (1.3 mg, 0.00019%) was isolated by h.p.l.c. and evaporation of the solvent gave a clear crystalline product. (Found: 362.2457. $C_{22}H_{34}O_4$ requires 362.2457). ^1H n.m.r. δ 0.81, s, $W_{h/2}$ 1.8 Hz, 19Me; 0.83, m, H1α; 0.86, s, $W_{h/2}$ 1.8 Hz, 20Me; 0.89, m, H5α; 1.02, s, $W_{h/2}$ 2.1 Hz, 18Me; 1.14, H9α; 1.15, m, H3α, H6β; 1.37, dt, J 13.5, 4.0 Hz, H7α; 1.51, m, H2α, H3β, H11β; 1.64, m, H2β, H6α; 1.73, m, H1β, H11α, H12α; 1.74, H14; 1.81, td, J 13.5, 3.2 Hz, H7β; 2.05, s, $OCOCH_3$; 2.14, m, H12β; 2.84, m, H13; 3.99, dd, J_{gem} 11.6 Hz, $J_{15\alpha,14}$ 7.1 Hz, H15α; 4.09, dd, J_{gem} 12.5 Hz, $J_{17a,9\alpha}$ 1.5 Hz,

H17a; 4.34, dd, J_{gem} 11.6 Hz, $J_{15\beta,14}$ 4.3 Hz, H15β; 4.89, dd, J_{gem} 12.5 Hz , $J_{17b,14}$ 1.8 Hz, H17b. Mass spectrum *m/z* 362(16%), 320(10), 302(62), 287(23), 274(10), 259(6), 246(11), 231(12), 217(14), 205(21), 145(26), 137(28), 123(55), 91(54), 81(87), 69(100). (Found: 320.2330. $C_{20}H_{32}O_3$ requires 320.2351; found: 302.2254. $C_{20}H_{30}O_2$ requires 302.2245).

AROSST-10 (48)

(5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*R*, 15*S*)-7-acetoxy-15-hydroxy-16-oxospongian (48).

AROSST-10 was isolated by h.p.l.c. as a colourless gum. (Found: 318.2190. $C_{20}H_{30}O_3$ [$C_{22}H_{34}O_5$–CH_3COOH] requires 318.2195). λ_{max} no peaks. ν_{max} 3363br, 2959, 2930, 1775, 1726, 1465, 1444, 1376, 1254, 1089, 1036, 936 cm^{-1}. ^1H n.m.r. δ 0.79, s, $W_{h/2}$ 1.8 Hz, 19Me; 0.80, s, $W_{h/2}$ 1.6 Hz, 20Me; 0.84, d, *J* 0.9 Hz, 18Me; 0.86, m, H1α; 0.88, s, $W_{h/2}$ 1.6 Hz, 17Me; 1.02, dd, $J_{9\alpha,11\beta}$ 12.4 Hz, $J_{9\alpha,11\beta}$ 2.4 Hz, H9α; 1.18, dt, *J* 13.5, 4.4 Hz, H3α; 1.28, dq, J_{gem} 12.6 Hz, $J_{11\beta,9\alpha}$ 12.6 Hz, $J_{11\beta,12\alpha}$ 12.6 Hz, $J_{11\beta,12\beta}$ 4.8 Hz, H11β; 1.34, m, H5α; 1.38–1.80, m, $(H2)_2$, H3β, $(H6)_2$, H11α, H12α; 1.73, dddd, J_{gem} 12.7 Hz, $J_{1\beta,2\beta}$ 3.3 Hz, $J_{1\beta,2\alpha}$ 3.3 Hz, $J_{1\beta,3\beta}$ 1.5Hz, H1β; 2.13, s, $OCOCH_3$; 2.30, bdd, J_{gem} 14.4 Hz, $J_{12\beta,11\beta}$ 4.8 Hz, H12β; 2.46, d, $J_{14,13}$ 8.2 Hz, H14; 2.95, dt, $J_{13,14}$ 8.2 Hz, $J_{13,12\alpha}$ 8.2 Hz, $J_{13,12\beta}$ 0.8 Hz, H13; 3.77, bs, OH; 5.05, dd, $J_{7\beta,6\beta}$ 2.9 Hz, $J_{7\beta,6\alpha}$ 2.9 Hz, H7β; 5.56, bs, $W_{h/2}$ 5.0 Hz, H15. ^{13}C n.m.r. δ 16.0; 16.3; 17.4; 18.4; 21.2; 21.3; 23.2; 32.7; 32.9; 34.2; 37.3; 36.2; 39.8; 41.8; 44.4; 47.6; 47.7; 51.4; 74.1, C7; 96.6, C15; 170.8, $OCOCH_3$; 178.6, C16. Mass spectrum *m/z* 360(3%), 318(76), 304(30), 300(16), 285(13), 244(76), 229(43), 159(16), 137(100), 123(57), 109(43), 95(49), 81(67), 69(76), 55(73), 43(85). (Found: 300.2102. $C_{20}H_{28}O_2$ requires

300.2089).

AROSST-11 (49)

(5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 13*S*, 14*R*)-7,17-diacetoxy-13-hydroxy-16-oxospongian (49).

AROSST-11 (5.1 mg, 0.00076%) was isolated by h.p.l.c. as a colourless gum. (Found: 376.2227. $C_{22}H_{32}O_5$ [$C_{24}H_{36}O_7$–CH_3COOH] requires 376.2249). λ_{max} no peaks. ν_{max} 3345br, 2958, 2929, 1774, 1735, 1472, 1375, 1233, 1143, 1039 cm^{-1}. ^1H n.m.r. δ 0.79, s, 2xMe; 0.92, s, $W_{h/2}$ 2.0 Hz, 18Me; 0.94, dt, *J* 12.6, 3.9 Hz, H1α; 1.19, dt, *J* 13.4, 4.0 Hz, H3α; 1.41, dd, $J_{5\alpha,6\beta}$ 13.0 Hz, $J_{5\alpha,6\alpha}$ 2.1 Hz, H5α; 1.43, H3β; 1.48, m, H2α, H11β; 1.53, ddd, J_{gem} 14.8 Hz, $J_{6\beta,5\alpha}$ 13.0 Hz, $J_{6\beta,7\beta}$ 2.1 Hz, H6β; 1.62, m, H9α, H2β; 1.74, m, H1β, H11α, H12α; 1.78, ddd, J_{gem} 14.8 Hz, $J_{6\alpha,7\beta}$ 3.7 Hz, $J_{6\alpha,5\alpha}$ 2.1 Hz, H6α; 2.08, s, $OCOCH_3$; 2.14, s, $OCOCH_3$; 2.40, s, OH; 2.42, dd, $J_{14,15\alpha}$ 5.7 Hz, $J_{14,15\beta}$ 2.2 Hz, H14; 2.46, m, H12β; 4.06, d, J_{gem} 13.2 Hz, H17a; 4.31, d, J_{gem} 13.2 Hz, H17b; 4.36, dd, J_{gem} 9.7 Hz, $J_{15\alpha,14}$ 5.7 Hz, H15α; 4.41, dd, J_{gem} 9.7 Hz, $J_{15\beta,14}$ 2.2 Hz, H15β; 5.14, dd, $J_{7\beta,6\alpha}$ 3.7 Hz, $J_{7\beta,6\beta}$ 2.1 Hz, H7β. ^{13}C n.m.r. δ 16.1, CH_3; 17.2, CH_2; 18.4, CH_2; 20.9, CH_3; 21.2, CH_3; 21.3, CH_3; 30.6, CH_2; 30.8, CH_2; 32.6, C; 32.9, CH_3; 37.4, C; 39.9, CH_2; 41.6, CH_2; 41.7, C; 47.4, CH; 47.7, CH; 50.5, CH; 63.6, CH_2, C15 or C17; 67.2, CH_2, C15 or C17; 70.1, CH, C7; 73.8, C, C13; 170.1, $OCOCH_3$; 170.2, $OCOCH_3$; 177.6, C16. Mass spectrum *m/z* 376(2%), 334(4), 316(34), 303(20), 301(13), 298(6), 259(45), 187(20), 145(22), 123(38), 109(59), 95(33), 81(40), 69(72), 55(63), 43(100). (Found: 334.2170. $C_{20}H_{30}O_4$ requires 334.2144; found: 316.1994. $C_{20}H_{28}O_3$ requires 316.2038).

Acetylation of AROSST-3 (41).

A solution of AROSST-3 (10 mg) in acetic anhydride/pyridine (1 : 1, 2 ml) was stirred at room temperatue for 24 h. T.l.c. indicated the presence of one product. The solvent was evaporated and the residue taken up in dichloromethane and filtered through flash silica gel. Evaporation of the solvent yielded a colouless glass, the ^{1}H n.m.r. spectrum of which was identical to that of AROSST-4 (42).

(5*R**, 8*R**, 9*R**, 10*R**, 13*R**, 14*R**, 15*R**, 16*S**)-15,16-diacetoxyspongian (54)

15,16-diacetoxyspongian (54) was isolated by column chromatography on flash silica gel as a colourless crystalline product. ^{1}H n.m.r. δ 0.73, dd, *J* 12, 2.3 Hz, 1xH; 0.82, s, Me; 0.84, s, Me; 0.85, s, Me; 0.95, s, Me; 0.80-1.90, m, 15xH; 1.90, d, *J* 7.7Hz, H14; 1.96, m, 1xH; 2.06, s, $OCOCH_3$; 2.10, s, $OCOCH_3$; 2.58, dddd, *J* 7.7, 7.6, 7.3, 1.7 Hz, H13; 6.09, s, H15; 6.11, d, *J* 7.6Hz, H16. Mass spectrum *m/z* 286(48%), 272(16), 147(37), 137(67), 135(22), 109(11), 95(20), 91(19), 55(28), 43(100).

(5*R**, 8*S**, 9*S**, 10*R**, 13*S**, 14*S**)-16-oxospongian (55)

16-oxospongian (55) was isolated by column chromatography on flash silica gel as a colourless crystalline product. ^{1}H n.m.r. δ 0.78, m, H9α; 0.80, m, H1α; 0.80, s, Me; 0.82, s, Me; 0.85, s, Me; 0.86, s, Me; 1.00-1.90, m, 15xH; 2.09, dd, *J* 8.0, 5.4 Hz, H14; 2.30, bdd, *J* 14, 5 Hz, H12β; 2.53, ddd, *J* 8.0, 8.0, 0.6 Hz, H13; 4.10, dd, *J* 9.8, 5.4 Hz, H15α; 4.21, d, *J*

9.8 Hz, H15β. Mass spectrum *m/z* 304(9%), 289(17), 191(100), 180(10), 124(17), 109(33), 95(42), 69(60).

1-Ethyl-β-carboline (56)

1-Ethyl-β-carboline (2 mg, 0.006%) was isolated by h.p.l.c. as a slightly yellow crystalline product. ^{1}H n.m.r. δ 1.49, t, *J* 7.6 Hz, CH_3CH_2Ar; 3.19, q, *J* 7.6 Hz, CH_3CH_2Ar; 7.30, m, H6; 7.56, m, H7, H8; 7.85, *J* 5.4 Hz, H4; 8.13, d, *J* 8 Hz, H5; 8.40, d, *J* 5.4 Hz, H3; 8.44, bs, N-H. Mass spectrum *m/z* 196(81), 195(100), 168(29), 154(6), 140(8). In ref. 29, δ 7.56 is assigned to H6,H7 and δ 7.30 to H8.

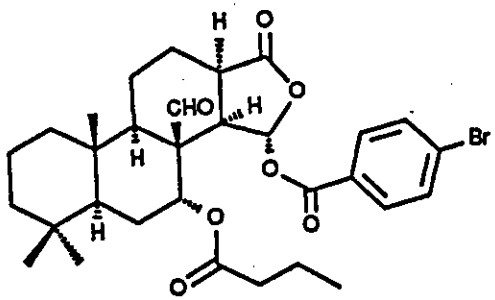
APPENDIX 1

The X-ray Crystal structure of:-

p-Bromobenzoate of Aplyroseol-1 (51)

Aplypallidenone (21)

Aplypallidoxone (22).



(51)

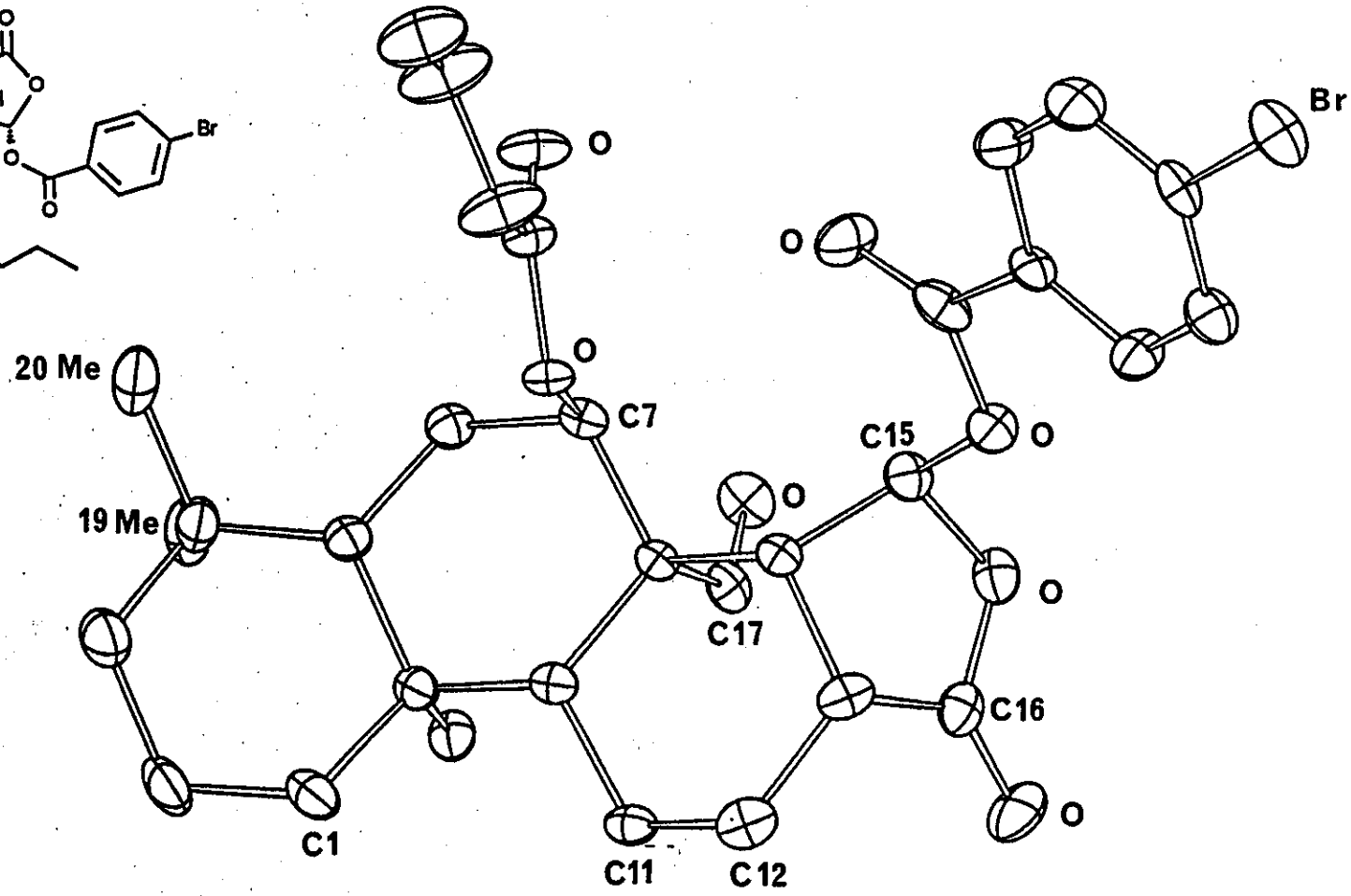


Table 1. Positional parameters for (51).

	10^4x	10^4y	10^4z
Br(1)	-2345(1)	-2499(1)	-469(1)
C(1)	184(11)	-2086(4)	53(8)
C(2)	1360(13)	-2324(4)	1070(8)
C(3)	3179(13)	-2025(4)	1445(8)
C(4)	3782(11)	-1491(4)	824(7)
C(5)	2589(12)	-1258(4)	-200(8)
C(6)	763(12)	-1549(4)	-596(8)
C(7)	5846(13)	-1179(4)	1344(10)
O(1)	6832(9)	-1338(3)	2271(6)
O(2)	6292(7)	-691(3)	575(5)
C(8)	8141(12)	-340(4)	1026(7)
O(3)	8643(8)	0(3)	-21(5)
C(9)	7734(10)	188(3)	1939(7)
C(10)	6825(11)	725(4)	1066(8)
C(11)	7928(11)	639(5)	-24(8)
O(4)	8241(10)	1029(3)	-788(5)
C(12)	6771(11)	1428(4)	1579(8)
C(13)	8662(11)	1612(4)	2420(7)
C(14)	9294(10)	1085(3)	3411(6)
C(15)	9724(9)	397(3)	2821(6)
C(16)	11410(10)	440(4)	2030(7)
O(5)	12717(8)	34(3)	2017(5)
C(17)	10274(9)	-145(3)	3793(6)
O(6)	8472(7)	-301(2)	4323(4)
C(18)	8439(13)	-908(4)	4861(8)

C(19)	6424(15)	−1017(5)	5305(11)
C(20)	6343(17)	−1567(6)	6160(12)
C(21)	4431(17)	−1721(6)	6585(11)
O(7)	9787(9)	−1293(3)	4951(6)
C(22)	11884(11)	89(4)	4796(7)
C(23)	11353(14)	732(4)	5402(7)
C(24)	10984(12)	1313(4)	4452(7)
C(25)	12918(12)	1498(4)	3883(7)
C(26)	10260(14)	1923(4)	5100(8)
C(27)	11636(17)	2099(4)	6271(8)
C(28)	11915(18)	1520(4)	7176(9)
C(29)	12689(15)	889(4)	6620(7)
C(30)	12504(19)	316(5)	7547(8)
C(31)	14966(15)	955(5)	6518(9)

Table 2. Bond Lengths (Å) for (51)

C(1)-Br(1)	1.895(7)	C(2)-C(1)	1.36(1)
C(6)-C(1)	1.37(1)	C(3)-C(2)	1.36(1)
C(4)-C(3)	1.36(1)	C(5)-C(4)	1.37(1)
C(7)-C(4)	1.55(1)	C(6)-C(5)	1.36(1)
O(1)-C(7)	1.17(1)	O(2)-C(7)	1.35(1)
C(8)-O(2)	1.45(1)	O(3)-C(8)	1.42(1)
C(9)-C(8)	1.51(1)	C(11)-O(3)	1.36(1)
C(10)-C(9)	1.51(1)	C(15)-C(9)	1.58(1)
C(11)-C(10)	1.50(1)	C(12)-C(10)	1.52(1)
O(4)-C(11)	1.19(1)	C(13)-C(12)	1.50(1)
C(14)-C(13)	1.53(1)	C(15)-C(14)	1.56(1)
C(24)-C(14)	1.56(1)	C(16)-C(15)	1.52(1)
C(17)-C(15)	1.53(1)	O(5)-C(16)	1.19(1)
O(6)-C(17)	1.44(1)	C(22)-C(17)	1.50(1)
C(18)-O(6)	1.35(1)	C(19)-C(18)	1.51(2)
O(7)-C(18)	1.18(2)	C(20)-C(19)	1.45(2)
C(21)-C(20)	1.45(2)	C(23)-C(22)	1.51(2)
C(24)-C(23)	1.56(2)	C(29)-C(23)	1.53(1)
C(25)-C(24)	1.55(1)	C(26)-C(24)	1.53(1)
C(27)-C(26)	1.51(1)	C(28)-C(27)	1.52(1)
C(29)-C(28)	1.52(1)	C(30)-C(29)	1.55(1)
C(31)-C(29)	1.54(2)		

Table 3. Bond angles (°) for (5)

C(2)-C(1)-Br(1)	118.9(6)	C(6)-C(1)-Br(1)	119.2(6)
C(6)-C(1)-C(2)	122.0(7)	C(3)-C(2)-C(1)	118.8(8)
C(4)-C(3)-C(2)	120.5(8)	C(5)-C(4)-C(3)	120.2(7)
C(7)-C(4)-C(3)	116.3(8)	C(7)-C(4)-C(5)	123.4(7)
C(6)-C(5)-C(4)	120.4(8)	C(5)-C(6)-C(1)	118.2(8)
O(1)-C(7)-C(4)	124.8(8)	O(2)-C(7)-C(4)	108.9(8)
O(2)-C(7)-O(1)	126.2(7)	C(8)-O(2)-C(7)	113.0(6)
O(3)-C(8)-O(2)	104.1(6)	C(9)-C(8)-O(2)	110.2(6)
C(9)-C(8)-O(3)	106.6(6)	C(11)-O(3)-C(8)	109.3(6)
C(10)-C(9)-C(8)	99.9(6)	C(15)-C(9)-C(8)	112.2(5)
C(15)-C(9)-C(10)	115.3(6)	C(11)-C(10)-C(9)	103.1(6)
C(12)-C(10)-C(9)	116.9(7)	C(12)-C(10)-C(11)	116.2(7)
C(10)-C(11)-O(3)	108.4(7)	O(4)-C(11)-O(3)	121.5(8)
O(4)-C(11)-C(10)	130.0(9)	C(13)-C(12)-C(10)	113.3(6)
C(14)-C(13)-C(12)	113.0(6)	C(15)-C(14)-C(13)	111.2(5)
C(24)-C(14)-C(13)	114.7(6)	C(24)-C(14)-C(15)	114.0(5)
C(14)-C(15)-C(9)	107.4(5)	C(16)-C(15)-C(9)	106.8(5)
C(16)-C(15)-C(14)	112.4(5)	C(17)-C(15)-C(9)	109.6(5)
C(17)-C(15)-C(14)	112.1(5)	C(17)-C(15)-C(16)	108.3(6)
O(5)-C(16)-C(15)	124.7(7)	O(6)-C(17)-C(15)	107.7(5)
C(22)-C(17)-C(15)	111.4(6)	C(22)-C(17)-O(6)	108.8(6)
C(18)-O(6)-C(17)	115.9(6)	C(19)-C(18)-O(6)	109.9(7)
O(7)-C(18)-O(6)	124.5(8)	O(7)-C(18)-C(19)	125.6(8)
C(20)-C(19)-C(18)	115.7(8)	C(21)-C(20)-C(19)	118.9(9)
C(23)-C(22)-C(17)	113.6(6)	C(24)-C(23)-C(22)	111.4(6)
C(29)-C(23)-C(22)	114.5(7)	C(29)-C(23)-C(24)	116.7(6)
C(23)-C(24)-C(14)	107.6(6)	C(25)-C(24)-C(14)	109.3(6)

C(25)-C(24)-C(23)	112.6(6)	C(26)-C(24)-C(14)	109.5(6)
C(26)-C(24)-C(23)	108.4(6)	C(26)-C(24)-C(25)	109.3(6)
C(27)-C(26)-C(24)	112.9(7)	C(28)-C(27)-C(26)	112.4(8)
C(29)-C(28)-C(27)	112.8(7)	C(28)-C(29)-C(23)	109.4(8)
C(30)-C(29)-C(23)	109.3(7)	C(30)-C(29)-C(28)	106.8(7)
C(31)-C(29)-C(23)	114.3(7)	C(31)-C(29)-C(28)	110.5(8)
C(31)-C(29)-C(30)	106.3(9)		

Table S2. Thermal parameters ($x10^3$) for (51)

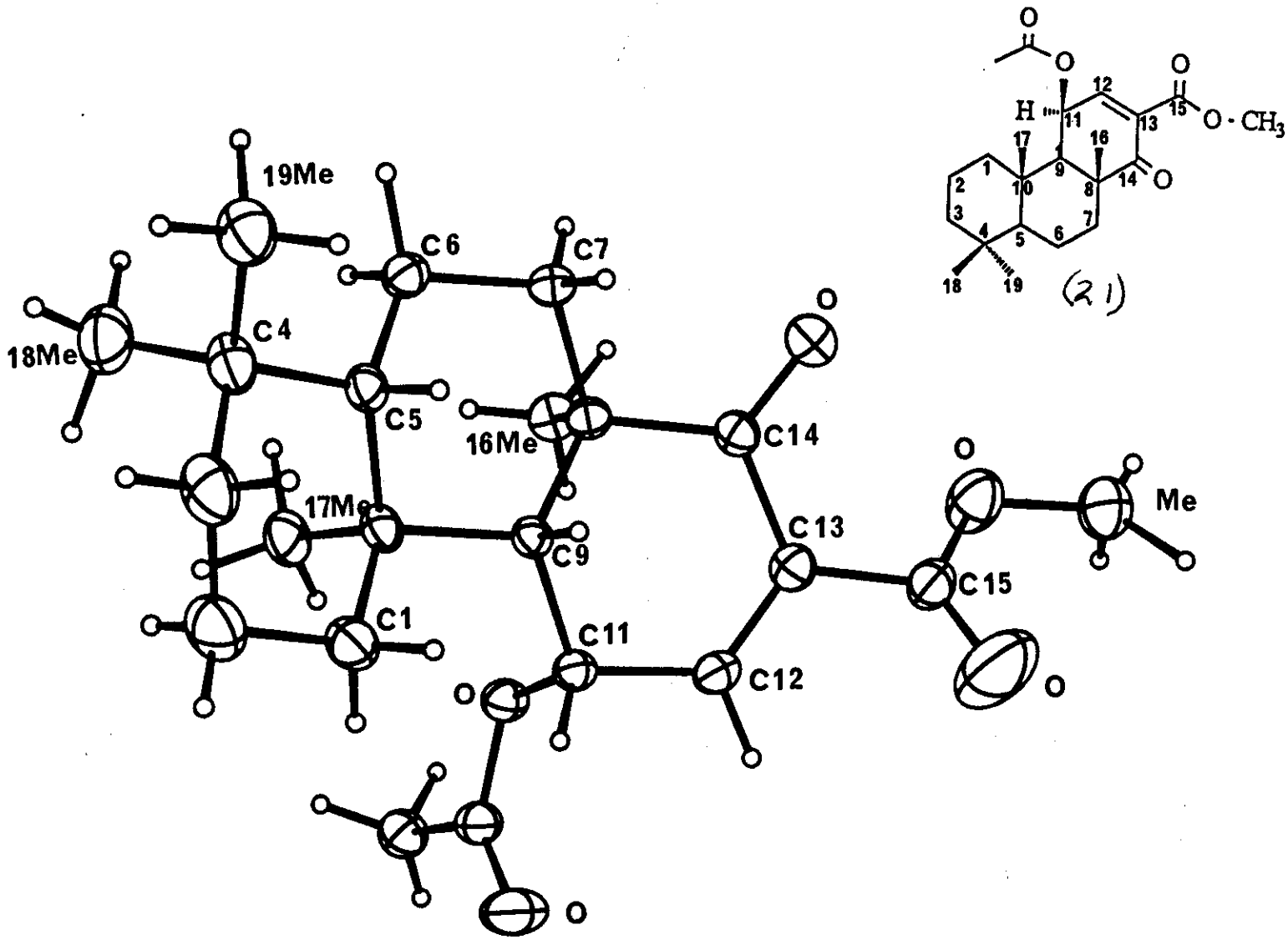
	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Br(1)	44(1)	81(1)	95(1)	-32(1)	17(1)	-19(1)
C(1)	31(1)	54(1)	62(1)	-26(1)	10(1)	-4(1)
C(2)	62(1)	61(1)	81(1)	3(1)	11(1)	-21(1)
C(3)	61(1)	62(1)	73(1)	11(1)	-10(1)	-9(1)
C(4)	41(1)	39(1)	53(1)	-6(1)	12(1)	-17(1)
C(5)	52(1)	61(1)	58(1)	12(1)	2(1)	-16(1)
C(6)	53(1)	58(1)	71(1)	-10(1)	5(1)	-17(1)
C(7)	41(1)	43(1)	88(1)	-15(1)	9(1)	-13(1)
O(1)	58(1)	71(1)	80(1)	22(1)	-28(1)	-10(1)
O(2)	44(1)	53(1)	57(1)	2(1)	0(1)	-23(1)
C(8)	39(1)	49(1)	56(1)	-1(1)	-3(1)	-10(1)
O(3)	51(1)	64(1)	47(1)	-6(1)	14(1)	-13(1)
C(9)	31(1)	38(1)	46(1)	0(1)	10(1)	-5(1)
C(10)	30(1)	57(1)	64(1)	17(1)	5(1)	0(1)
C(11)	40(1)	66(1)	46(1)	0(1)	-2(1)	-12(1)
O(4)	76(1)	92(1)	56(1)	24(1)	14(1)	-22(1)
C(12)	38(1)	65(1)	71(1)	13(1)	18(1)	7(1)
C(13)	48(1)	37(1)	56(1)	6(1)	21(1)	5(1)
C(14)	35(1)	35(1)	49(1)	2(1)	20(1)	3(1)
C(15)	24(1)	33(1)	43(1)	-1(1)	8(1)	-7(1)
C(16)	32(1)	49(1)	53(1)	-10(1)	1(1)	-4(1)
O(5)	37(1)	64(1)	73(1)	-7(1)	19(1)	6(1)
C(17)	26(1)	34(1)	54(1)	0(1)	16(1)	2(1)
O(6)	39(1)	31(1)	54(1)	12(1)	11(1)	2(1)
C(18)	41(1)	42(1)	58(1)	13(1)	0(1)	0(1)

C(19)	54(1)	97(1)	129(1)	65(1)	32(1)	8(1)
C(20)	75(1)	119(1)	142(1)	76(1)	46(1)	15(1)
C(21)	85(1)	110(1)	138(1)	56(1)	40(1)	3(1)
O(7)	51(1)	47(1)	110(1)	27(1)	6(1)	10(1)
C(22)	42(1)	46(1)	51(1)	3(1)	7(1)	-4(1)
C(23)	49(1)	44(1)	48(1)	2(1)	15(1)	-8(1)
C(24)	53(1)	38(1)	41(1)	-1(1)	21(1)	-2(1)
C(25)	50(1)	55(1)	45(1)	0(1)	13(1)	-13(1)
C(26)	76(1)	42(1)	67(1)	-11(1)	29(1)	-8(1)
C(27)	124(1)	55(1)	64(1)	-26(1)	38(1)	-19(1)
C(28)	116(1)	66(1)	63(1)	-9(1)	28(1)	-13(1)
C(29)	90(1)	68(1)	39(1)	-1(1)	5(1)	-16(1)
C(30)	127(1)	101(1)	47(1)	-9(1)	0(1)	-12(1)
C(31)	91(1)	99(1)	58(1)	-13(1)	-17(1)	-13(1)

Table S3. Hydrogen atom positional ($\times 10^3$) and thermal ($\times 10^2$) parameters for (61)

	x	y	z	U_{11}
H(2)	91(1)	-269(1)	152(1)	5(1)
H(3)	405(1)	-219(1)	216(1)	5(1)
H(4)	506(1)	-126(1)	111(1)	5(1)
H(5)	304(1)	-87(1)	-64(1)	5(1)
H(8)	918(1)	-63(1)	141(1)	6(1)
H(9)	686(1)	6(1)	253(1)	5(1)
H(10)	537(1)	67(1)	86(1)	6(1)
H(12A)	563(1)	146(1)	204(1)	7(1)
H(12B)	659(1)	174(1)	90(1)	8(1)
H(13A)	975(1)	167(1)	193(1)	4(1)
H(13B)	843(1)	203(1)	282(1)	3(1)
H(14)	814(1)	102(1)	384(1)	3(1)
H(16)	1143(1)	82(1)	150(1)	4(1)
H(17)	1079(1)	-52(1)	341(1)	4(1)
H(19A)	542(1)	-109(1)	459(1)	24(1)
H(19B)	608(1)	-60(1)	571(1)	24(1)
H(20A)	731(2)	-146(1)	688(1)	22(1)
H(20B)	676(2)	-196(1)	576(1)	22(1)
H(21A)	461(2)	-209(1)	715(1)	30(1)
H(21B)	343(2)	-182(1)	589(1)	30(1)
H(21C)	398(2)	-133(1)	701(1)	30(1)
H(22A)	1210(1)	-25(1)	542(1)	7(1)
H(22B)	1313(1)	16(1)	445(1)	4(1)
H(23)	1005(1)	67(1)	568(1)	6(1)
H(25A)	1338(1)	111(1)	347(1)	8(1)

H(25B)	1397(1)	164(1)	453(1)	8(1)
H(25C)	1261(1)	186(1)	329(1)	8(1)
H(26A)	892(1)	183(1)	530(1)	7(1)
H(26B)	1020(1)	230(1)	454(1)	8(1)
H(27A)	1295(2)	223(1)	606(1)	10(1)
H(27B)	1105(2)	247(1)	666(1)	9(1)
H(28A)	1288(2)	165(1)	788(1)	11(1)
H(28B)	1062(2)	142(1)	744(1)	3(1)
H(30A)	1298(2)	-9(1)	723(1)	7(1)
H(30B)	1110(2)	26(1)	766(1)	7(1)
H(30C)	1331(2)	42(1)	833(1)	7(1)
H(31A)	1516(2)	131(1)	595(1)	11(1)
H(31B)	1546(2)	54(1)	622(1)	11(1)
H(31C)	1571(2)	106(1)	732(1)	11(1)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C6
C7
C4
18Me
C5
16Me
C14
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17Me
C9
C13
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Me
C15
C1
C11
C12
O
O
O

O
O
O
12
H
11
13
15
O · CH3
17
16
1
9
2
10
8
14
O
3
4
5
6
7
18
19

(21)

Table 1. Positional parameters (x10) for Aplypallidenone C22 H30 O5 (21)

O(1)	8775(3)	1418(2)	4120(1)
O(2)	6063(3)	851(3)	3943(2)
O(3)	6437(6)	5699(6)	4677(2)
O(4)	8808(4)	5804(3)	5114(1)
O(5)	11237(4)	5680(2)	4257(1)
C(1)	8530(4)	2565(3)	3822(1)
C(2)	7850(4)	3509(3)	4203(1)
C(3)	8740(4)	4474(3)	4381(1)
C(4)	10568(4)	4667(3)	4204(1)
C(5)	11522(4)	3579(3)	3947(1)
C(6)	10205(3)	2990(2)	3548(1)
C(7)	10941(4)	2099(3)	3107(1)
C(8)	9506(6)	1868(4)	2697(2)
C(9)	10141(9)	1311(6)	2170(2)
C(10)	11501(7)	2112(5)	1913(1)
C(11)	13065(6)	2342(3)	2271(1)
C(12)	12402(4)	2851(2)	2820(1)
C(13)	13809(4)	3201(3)	3224(1)
C(14)	13086(4)	4091(3)	3639(1)
C(15)	7442(4)	625(3)	4136(1)
C(16)	7920(6)	-537(3)	4413(2)
C(17)	7873(5)	5387(4)	4736(1)
C(18)	8095(10)	6698(7)	5480(2)
C(19)	12119(5)	2746(3)	4416(1)
C(20)	11606(6)	857(3)	3338(2)
C(21)	14201(8)	1187(5)	2324(3)
C(22)	14155(8)	3325(4)	1995(2)

Table 2. Bond lengths (Å) for Aplypallidenone C22 H30 O5 (21)

C(1)-O(1)	1.459(3)	C(15)-O(1)	1.342(4)
C(15)-O(2)	1.195(4)	C(17)-O(3)	1.169(5)
C(17)-O(4)	1.270(4)	C(18)-O(4)	1.439(5)
C(4)-O(5)	1.220(4)	C(2)-C(1)	1.491(4)
C(6)-C(1)	1.534(4)	C(3)-C(2)	1.327(4)
C(4)-C(3)	1.494(4)	C(17)-C(3)	1.486(4)
C(5)-C(4)	1.531(4)	C(6)-C(5)	1.559(4)
C(14)-C(5)	1.535(4)	C(19)-C(5)	1.546(4)
C(7)-C(6)	1.569(4)	C(8)-C(7)	1.528(5)
C(12)-C(7)	1.565(4)	C(20)-C(7)	1.550(4)
C(9)-C(8)	1.525(6)	C(10)-C(9)	1.506(7)
C(11)-C(10)	1.522(6)	C(12)-C(11)	1.557(4)
C(21)-C(11)	1.535(6)	C(22)-C(11)	1.523(5)
C(13)-C(12)	1.530(4)	C(14)-C(13)	1.518(4)
C(16)-C(15)	1.483(5)		

Table 3. Bond angles (°) for Aplypallidenone C22 H30 O5 (21)

C(15)-O(1)-C(1)	117.5(2)	C(18)-O(4)-C(17)	119.4(4)
C(2)-C(1)-O(1)	107.9(2)	C(6)-C(1)-O(1)	111.9(2)
C(6)-C(1)-C(2)	112.0(2)	C(3)-C(2)-C(1)	124.7(3)
C(4)-C(3)-C(2)	120.2(3)	C(17)-C(3)-C(2)	119.3(3)
C(17)-C(3)-C(4)	120.5(3)	C(3)-C(4)-O(5)	119.7(3)
C(5)-C(4)-O(5)	122.2(3)	C(5)-C(4)-C(3)	118.1(2)
C(6)-C(5)-C(4)	105.5(2)	C(14)-C(5)-C(4)	108.1(2)
C(14)-C(5)-C(6)	110.1(2)	C(19)-C(5)-C(4)	106.1(2)
C(19)-C(5)-C(6)	115.9(2)	C(19)-C(5)-C(14)	110.7(2)
C(5)-C(6)-C(1)	113.0(2)	C(7)-C(6)-C(1)	115.6(2)
C(7)-C(6)-C(5)	117.4(2)	C(8)-C(7)-C(6)	107.7(3)
C(12)-C(7)-C(6)	105.1(2)	C(12)-C(7)-C(8)	107.7(2)
C(20)-C(7)-C(6)	113.3(2)	C(20)-C(7)-C(8)	110.2(3)
C(20)-C(7)-C(12)	112.5(3)	C(9)-C(8)-C(7)	113.9(4)
C(10)-C(9)-C(8)	111.2(4)	C(11)-C(10)-C(9)	113.6(4)
C(12)-C(11)-C(10)	108.1(3)	C(21)-C(11)-C(10)	111.8(4)
C(21)-C(11)-C(12)	113.7(3)	C(22)-C(11)-C(10)	106.7(3)
C(22)-C(11)-C(12)	109.3(3)	C(22)-C(11)-C(21)	107.0(4)
C(11)-C(12)-C(7)	116.9(2)	C(13)-C(12)-C(7)	110.0(2)
C(13)-C(12)-C(11)	115.5(3)	C(14)-C(13)-C(12)	110.1(2)
C(13)-C(14)-C(5)	113.6(2)	O(2)-C(15)-O(1)	122.7(3)
C(16)-C(15)-O(1)	111.6(3)	C(16)-C(15)-O(2)	125.7(3)
O(4)-C(17)-O(3)	122.0(4)	C(3)-C(17)-O(3)	123.1(4)
C(3)-C(17)-O(4)	114.9(3)		

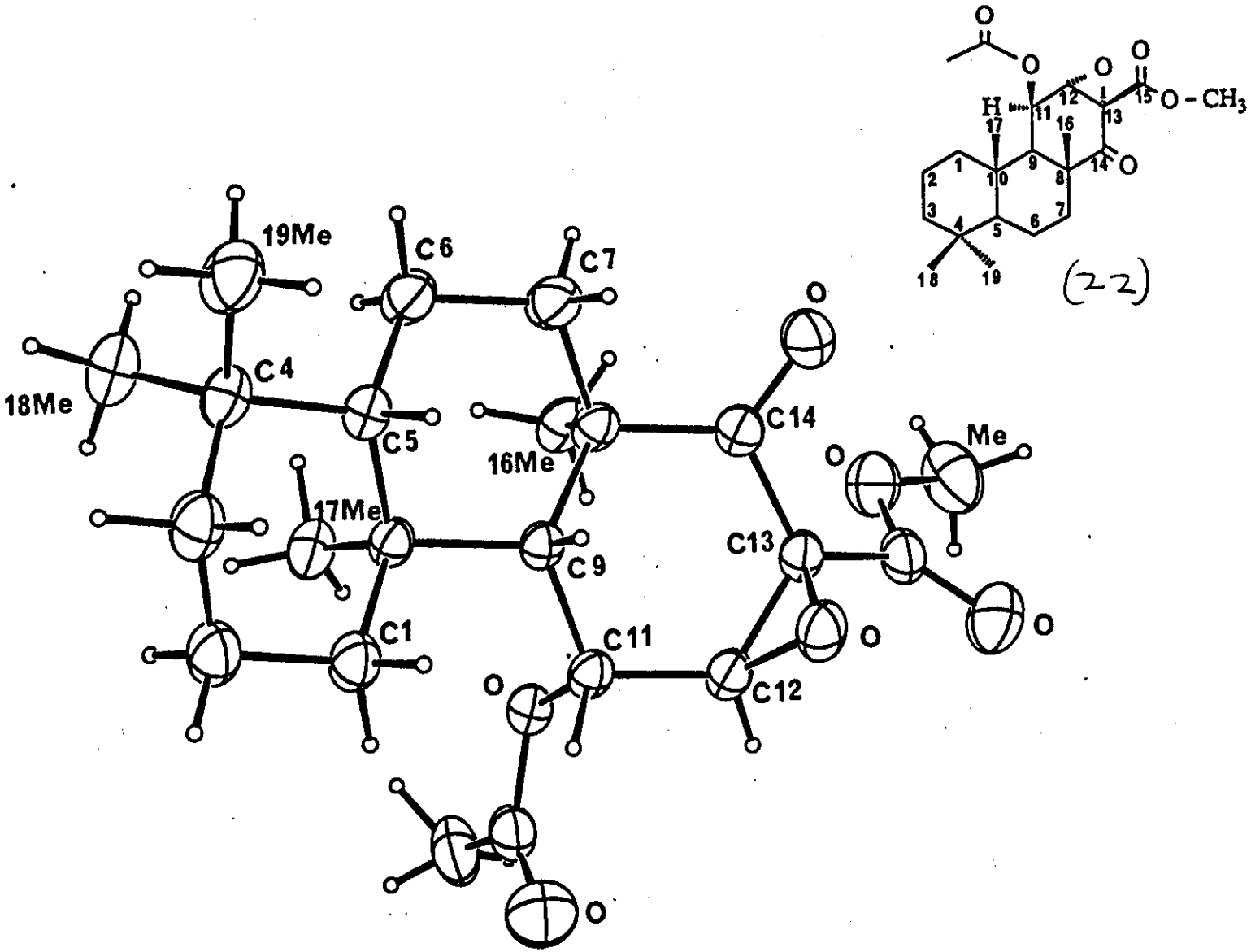
Table S2. Thermal parameters ($x10^3$) for Aplypallidenone C22 H30 O5 (21)

O(1)	48(1)	50(1)	61(1)	12(1)	-3(1)	-6(1)
O(2)	57(1)	83(1)	150(1)	41(1)	-26(1)	-24(1)
O(3)	95(1)	291(1)	209(1)	-168(1)	-45(1)	84(1)
O(4)	83(1)	124(1)	81(1)	-45(1)	-13(1)	38(1)
O(5)	76(1)	64(1)	93(1)	-26(1)	18(1)	-18(1)
C(1)	40(1)	45(1)	51(1)	6(1)	-3(1)	-1(1)
C(2)	41(1)	58(1)	61(1)	7(1)	3(1)	3(1)
C(3)	54(1)	56(1)	50(1)	0(1)	1(1)	3(1)
C(4)	51(1)	51(1)	43(1)	-3(1)	-4(1)	-2(1)
C(5)	42(1)	49(1)	45(1)	4(1)	-4(1)	-1(1)
C(6)	42(1)	36(1)	44(1)	2(1)	-2(1)	-1(1)
C(7)	60(1)	35(1)	53(1)	-1(1)	5(1)	-3(1)
C(8)	74(1)	65(1)	66(1)	-17(1)	3(1)	-21(1)
C(9)	111(1)	98(1)	81(1)	-45(1)	12(1)	-32(1)
C(10)	118(1)	86(1)	55(1)	-26(1)	15(1)	-15(1)
C(11)	91(1)	52(1)	63(1)	-8(1)	22(1)	-2(1)
C(12)	54(1)	37(1)	52(1)	-1(1)	8(1)	2(1)
C(13)	45(1)	52(1)	60(1)	7(1)	4(1)	4(1)
C(14)	41(1)	53(1)	52(1)	4(1)	-6(1)	-6(1)
C(15)	48(1)	56(1)	62(1)	2(1)	2(1)	-8(1)
C(16)	67(1)	56(1)	78(1)	12(1)	8(1)	-7(1)
C(17)	56(1)	72(1)	68(1)	-12(1)	2(1)	13(1)
C(18)	98(1)	99(1)	89(1)	-44(1)	17(1)	9(1)
C(19)	57(1)	65(1)	51(1)	13(1)	-8(1)	1(1)
C(20)	85(1)	38(1)	87(1)	7(1)	22(1)	4(1)
C(21)	113(1)	69(1)	116(1)	-13(1)	50(1)	21(1)
C(22)	109(1)	79(1)	70(1)	-3(1)	33(1)	-11(1)

Table 5a. Hydrogen atom positional ($x10^3$) and thermal' ($x10^2$) parameters for Aplypallidenone C22 H30 O5 (21)

	x	y	z	U_{11}
H(1)	765(4)	243(3)	357(1)	4(1)
H(2)	665(6)	340(3)	430(1)	7(1)
H(6)	983(4)	369(3)	333(1)	4(1)
H(8A)	891(5)	268(4)	264(1)	6(1)
H(8B)	868(6)	140(4)	285(2)	8(1)
H(9A)	918(6)	119(5)	195(2)	9(1)
H(9B)	1059(9)	67(6)	221(3)	12(1)
H(10A)	1188(8)	180(5)	160(2)	12(1)
H(10B)	1100(8)	306(6)	176(2)	12(1)
H(12)	1183(4)	363(3)	272(1)	4(1)
H(13A)	1437(4)	245(3)	340(1)	5(1)
H(13B)	1495(5)	364(3)	304(1)	6(1)
H(14A)	1264(4)	482(3)	344(1)	4(1)
H(14B)	1391(5)	421(3)	387(1)	6(1)
H(16A)	866(6)	-34(4)	477(2)	9(1)
H(16B)	707(8)	-79(5)	456(2)	11(1)
H(16C)	872(9)	-106(6)	425(2)	14(1)
H(18A)	688(8)	737(5)	529(2)	11(1)
H(18B)	849(9)	710(6)	552(2)	10(1)
H(18C)	764(9)	617(6)	580(3)	14(1)
H(19A)	1270(5)	206(4)	430(1)	7(1)
H(19B)	1128(5)	236(3)	462(1)	7(1)
H(19C)	1278(5)	329(4)	467(1)	7(1)
H(20A)	1083(8)	64(5)	355(2)	12(1)
H(20B)	1289(8)	89(5)	349(2)	12(1)
H(20C)	1155(6)	27(4)	303(2)	9(1)

H(21A)	1535(9)	122(6)	265(3)	14(1)
H(21B)	1347(8)	35(6)	243(2)	13(1)
H(21C)	1476(9)	100(6)	205(3)	13(1)
H(22A)	1440(5)	302(3)	163(2)	7(1)
H(22B)	1541(7)	340(4)	217(2)	10(1)
H(22C)	1346(6)	408(4)	202(1)	8(1)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C13
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C12
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O
O
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H
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(22)

Table 1. Positional parameters ($x10^4$) for Aplypallidoxone (22)

	10^4 x	10^4 y	10^4 z
O(1)	1249(1)	487(1)	7455(1)
O(2)	885(1)	-2422(6)	7812(2)
O(3)	1220(1)	-1350(5)	4971(2)
O(4)	196(1)	494(6)	3722(2)
O(5)	538(1)	3487(5)	4345(2)
O(6)	1774(1)	2541(5)	4387(2)
C(1)	1519(1)	-557(5)	6803(2)
C(2)	1064(1)	-549(5)	5802(2)
C(3)	1114(1)	758(6)	4995(2)
C(4)	1668(1)	1942(5)	5124(2)
C(5)	2057(1)	2308(5)	6182(2)
C(6)	2106(1)	349(5)	6806(2)
C(7)	2590(1)	367(5)	7854(2)
C(8)	2632(1)	-1751(6)	8290(2)
C(9)	3183(2)	-2092(7)	9170(3)
C(10)	3725(1)	-1649(7)	8880(3)
C(11)	3755(1)	469(6)	8481(2)
C(12)	3175(1)	857(6)	7621(2)
C(13)	3136(1)	2908(6)	7130(3)
C(14)	2665(1)	2913(7)	6125(3)
C(15)	935(1)	-652(7)	7914(2)
C(16)	676(2)	592(10)	8542(3)
C(17)	564(1)	1526(7)	4267(2)
C(18)	23(2)	4535(12)	3736(4)
C(19)	1749(2)	4072(6)	6533(3)
C(20)	2455(2)	1838(7)	8601(3)
C(21)	3887(2)	1998(8)	9327(3)

C(22)	4262(2)	499(10)	8030(3)

Table 2. Bond lengths (Å) for Aplypallidoxone (22)

C(1)-O(1)	1.446(3)	C(15)-O(1)	1.359(4)
C(15)-O(2)	1.192(5)	C(2)-O(3)	1.430(3)
C(3)-O(3)	1.432(4)	C(17)-O(4)	1.198(4)
C(17)-O(5)	1.316(4)	C(18)-O(5)	1.453(4)
C(4)-O(6)	1.204(3)	C(2)-C(1)	1.500(4)
C(6)-C(1)	1.530(3)	C(3)-C(2)	1.462(4)
C(4)-C(3)	1.509(4)	C(17)-C(3)	1.497(4)
C(5)-C(4)	1.520(4)	C(6)-C(5)	1.559(4)
C(14)-C(5)	1.534(4)	C(19)-C(5)	1.545(4)
C(7)-C(6)	1.578(3)	C(8)-C(7)	1.533(4)
C(12)-C(7)	1.562(4)	C(20)-C(7)	1.539(4)
C(9)-C(8)	1.532(4)	C(10)-C(9)	1.500(5)
C(11)-C(10)	1.530(5)	C(12)-C(11)	1.567(4)
C(21)-C(11)	1.525(5)	C(22)-C(11)	1.525(5)
C(13)-C(12)	1.523(5)	C(14)-C(13)	1.522(4)
C(16)-C(15)	1.475(6)		

Table 3. Bond angles (°) for Aplypallidoxone (22)

C(15)-O(1)-C(1)	116.3(2)	C(3)-O(3)-C(2)	61.5(2)
C(18)-O(5)-C(17)	118.8(4)	C(2)-C(1)-O(1)	104.8(2)
C(6)-C(1)-O(1)	113.0(2)	C(6)-C(1)-C(2)	114.3(2)
C(1)-C(2)-O(3)	118.1(2)	C(3)-C(2)-O(3)	59.3(2)
C(3)-C(2)-C(1)	121.7(2)	C(2)-C(3)-O(3)	59.2(2)
C(4)-C(3)-O(3)	111.2(2)	C(4)-C(3)-C(2)	119.3(2)
C(17)-C(3)-O(3)	116.5(2)	C(17)-C(3)-C(2)	118.5(2)
C(17)-C(3)-C(4)	117.8(2)	C(3)-C(4)-O(6)	118.1(2)
C(5)-C(4)-O(6)	124.2(2)	C(5)-C(4)-C(3)	117.6(2)
C(6)-C(5)-C(4)	109.6(2)	C(14)-C(5)-C(4)	107.8(2)
C(14)-C(5)-C(6)	109.6(2)	C(19)-C(5)-C(4)	102.8(2)
C(19)-C(5)-C(6)	115.1(2)	C(19)-C(5)-C(14)	111.4(3)
C(5)-C(6)-C(1)	114.5(2)	C(7)-C(6)-C(1)	115.2(2)
C(7)-C(6)-C(5)	115.4(2)	C(8)-C(7)-C(6)	107.9(2)
C(12)-C(7)-C(6)	105.2(2)	C(12)-C(7)-C(8)	108.7(2)
C(20)-C(7)-C(6)	113.2(2)	C(20)-C(7)-C(8)	108.5(3)
C(20)-C(7)-C(12)	113.0(2)	C(9)-C(8)-C(7)	113.4(3)
C(10)-C(9)-C(8)	111.1(3)	C(11)-C(10)-C(9)	114.8(3)
C(12)-C(11)-C(10)	107.9(2)	C(21)-C(11)-C(10)	110.8(3)
C(21)-C(11)-C(12)	114.9(3)	C(22)-C(11)-C(10)	107.1(3)
C(22)-C(11)-C(12)	108.0(3)	C(22)-C(11)-C(21)	107.9(3)
C(11)-C(12)-C(7)	116.6(2)	C(13)-C(12)-C(7)	110.1(2)
C(13)-C(12)-C(11)	114.7(2)	C(14)-C(13)-C(12)	110.5(3)
C(13)-C(14)-C(5)	113.8(3)	O(2)-C(15)-O(1)	123.2(3)
C(16)-C(15)-O(1)	110.9(4)	C(16)-C(15)-O(2)	125.8(4)
O(5)-C(17)-O(4)	125.5(3)	C(3)-C(17)-O(4)	124.7(4)
C(3)-C(17)-O(5)	109.7(3)		

Table S2. Thermal parameters ($x10^3$) for Aplypallidoxone (22)

O(1)	46(1)	50(1)	44(1)	-2(1)	19(1)	-3(1)
O(2)	110(1)	78(1)	103(1)	10(1)	55(1)	-23(1)
O(3)	67(1)	48(1)	51(1)	-11(1)	17(1)	-1(1)
O(4)	73(1)	108(1)	76(1)	12(1)	-17(1)	-24(1)
O(5)	59(1)	75(1)	66(1)	9(1)	12(1)	25(1)
O(6)	61(1)	74(1)	53(1)	17(1)	21(1)	2(1)
C(1)	44(1)	33(1)	45(1)	-3(1)	13(1)	-2(1)
C(2)	44(1)	43(1)	46(1)	-5(1)	13(1)	-4(1)
C(3)	46(1)	46(1)	41(1)	-3(1)	11(1)	0(1)
C(4)	46(1)	39(1)	47(1)	6(1)	16(1)	8(1)
C(5)	42(1)	36(1)	49(1)	0(1)	14(1)	0(1)
C(6)	41(1)	32(1)	39(1)	-4(1)	15(1)	2(1)
C(7)	43(1)	38(1)	42(1)	-5(1)	11(1)	-3(1)
C(8)	52(1)	53(1)	53(1)	7(1)	5(1)	0(1)
C(9)	69(1)	56(1)	57(1)	10(1)	2(1)	0(1)
C(10)	55(1)	66(1)	62(1)	2(1)	1(1)	13(1)
C(11)	41(1)	65(1)	54(1)	-10(1)	6(1)	0(1)
C(12)	41(1)	48(1)	45(1)	-8(1)	12(1)	-1(1)
C(13)	47(1)	58(1)	66(1)	1(1)	13(1)	-12(1)
C(14)	49(1)	61(1)	66(1)	19(1)	15(1)	-10(1)
C(15)	53(1)	80(1)	46(1)	8(1)	16(1)	-8(1)
C(16)	68(1)	133(1)	46(1)	2(1)	24(1)	7(1)
C(17)	49(1)	80(1)	41(1)	7(1)	13(1)	-3(1)
C(18)	60(1)	141(1)	75(1)	38(1)	26(1)	46(1)
C(19)	59(1)	31(1)	67(1)	-6(1)	10(1)	2(1)
C(20)	48(1)	69(1)	51(1)	-19(1)	13(1)	-1(1)
C(21)	60(1)	84(1)	65(1)	-22(1)	-4(1)	-4(1)

C(22)	46(1)	105(1)	81(1)	-6(1)	15(1)	4(1)

Table 5i. Hydrogen atom positional ($x10^3$) and thermal' ($x10^2$) parameters for Aplypallidoxone (22)

	x	y	z	U_{11}
H(1)	157(1)	-197(5)	704(2)	4(1)
H(2)	70(1)	-89(5)	583(2)	5(1)
H(6)	225(1)	-55(4)	644(2)	3(1)
H(8A)	265(1)	-270(5)	773(2)	5(1)
H(8B)	226(1)	-209(6)	844(2)	6(1)
H(9A)	318(2)	-339(6)	941(3)	7(1)
H(9B)	315(1)	-119(6)	972(2)	6(1)
H(10A)	375(1)	-254(6)	836(3)	7(1)
H(10B)	410(2)	-186(6)	946(3)	7(1)
H(12)	319(1)	-11(4)	710(2)	3(1)
H(13A)	350(1)	317(5)	695(2)	6(1)
H(13B)	306(1)	386(6)	758(2)	5(1)
H(14A)	265(1)	420(6)	585(3)	6(1)
H(14B)	279(1)	176(7)	565(3)	8(1)
H(16A)	76(2)	38(11)	914(5)	13(1)
H(16B)	95(4)	169(19)	886(7)	24(1)
H(16C)	33(2)	102(9)	822(4)	12(1)
H(18A)	14(4)	572(19)	388(7)	26(1)
H(18B)	-27(2)	411(8)	392(3)	11(1)
H(18C)	-1(2)	433(7)	311(4)	9(1)
H(19A)	137(1)	370(6)	657(2)	7(1)
H(19B)	199(1)	453(6)	715(2)	6(1)
H(19C)	169(1)	514(7)	604(3)	7(1)
H(20A)	208(2)	205(6)	856(2)	6(1)
H(20B)	267(2)	165(7)	921(3)	9(1)
H(20C)	261(2)	325(8)	853(3)	10(1)

H(21A)	363(2)	192(7)	979(3)	10(1)
H(21B)	429(2)	173(7)	975(3)	9(1)
H(21C)	386(2)	340(9)	910(4)	11(1)
H(22A)	419(2)	-44(7)	744(3)	9(1)
H(22B)	462(2)	7(7)	850(3)	9(1)
H(22C)	435(2)	183(8)	786(3)	9(1)

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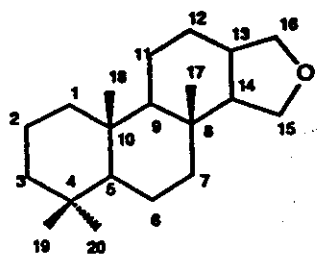
PART 2

Approaches to the synthesis of Aplysulphurin

2.1 Introduction

The chemical constituents of marine organisms, in contrast to terrestrial organisms, have only relatively recently been examined, much of the interest being sustained by the novel compounds isolated from them. Many of these novel compounds contain structures not yet found from terrestrial sources and possess interesting biological activity which may in the future prove to be potential pharmaceuticals.

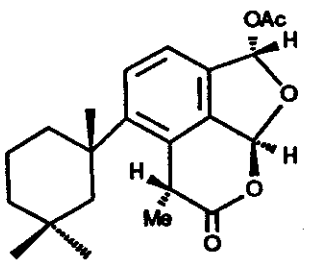
Sponges in particular have shown themselves to be a profitable source of novel and biologically active metabolites. One such group of sponges, the order Dendroceratida, have proven to be a rich source of interesting and novel diterpenoids which contain the spongian (1) or spongian derived backbone.



(1)

Karuso in 1984 isolated two new and novel diterpene metabolites, aplysulphurin (2)[1,2] and aplysulphuride (3 or 4)[2] from the sponge *Aplysilla sulphurea* (Order Dendroceratida, family Aplysillidae)

The structures were identified by extensive spectroscopic methods; aplysulphurin (2) being further established by X-ray crystallography.



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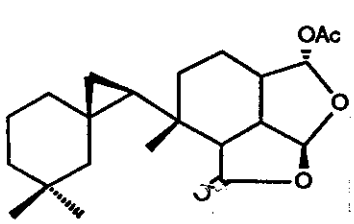

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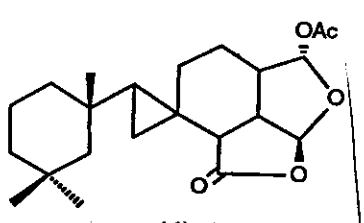
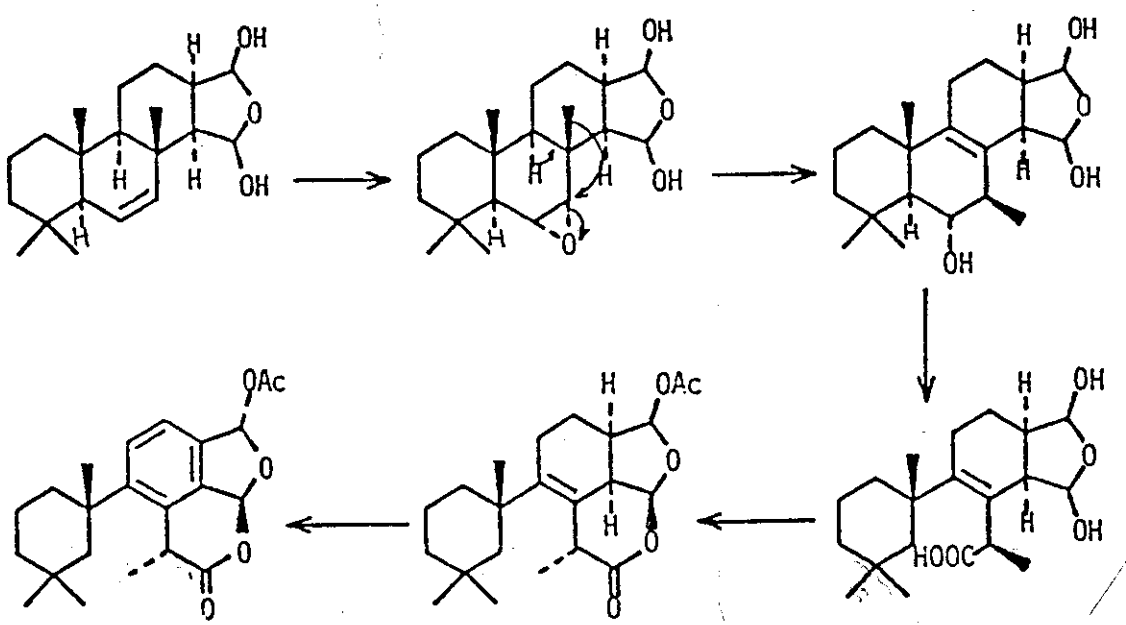


(4)

Aplysilla sulphurea Schulze (Plate 17) is a yellow encrusting sponge which grows to a thickness of 3-10 mm. It is widely distributed throughout the world and has been recorded with a vertical distribution from 2 to 640 m.[3] The samples extracted by Karuso were collected from 'The Docks' Jervis Bay by SCUBA at a depth range of 10-30 m.

In this thesis work was undertaken directed towards a study into possible schemes for the total synthesis of aplysulphurin.

Aplysulphurin (2) is a neutral, optically active, tetracyclic, aromatic lactone diterpene. The biogenesis of (2) was first discussed by Karuso (Scheme A)[2] and is thought to commence with an oxidation at C6 and C7 in the B ring of spongian (1) followed by migration of 17Me to C7. The ensuing cleavage of the C5-C6 bond (radical fragmentation or oxidative cleavage) and supply of a new hydrogen to C5 could eventually generate a carboxyl group capable of lactonising with the C15 hydroxyl. This may then be followed by acetylation of the C16 hydroxyl group and aromatization of the C ring. This sequence was preferred over other possible mechanisms since it gave the required stereochemistry and is biologically feasible.



Scheme A

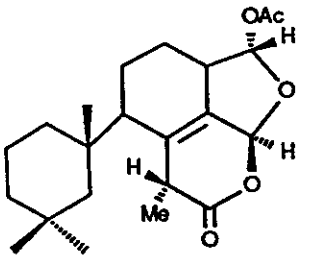
Plate 17. : An underwater photograph of *Aplysilla sulphurea* (yellow sponge), *Aplysilla tango* (orange sponge in bottom left hand corner) and *Aplysilla rosea* (red sponge in top left hand corner).

Aplysuphurin (2) was the first diterpene of its type to be isolated. Since then other similar metabolites have been isolated such as (5), (6) and (7) from *Darwinella sulphurea*,[4] (8) from *Dendrilla membranosa*[5] and (9) and (10) from the dorid nudibranch *Chromadoris macfarlandi*.[6]

The secondary metabolites of sponges have also drawn a lot interest both ecologically and pharmaceutically. Ecologically because the secondary metabolites may be present in the sponge as a chemical defence system. Therefore, because of their possible ecological value, which inherently requires them to have biological activity, they may also be of interest as potentially useful pharmaceuticals.

Aplysilla sulphurea is one such sponge in which its secondary metabolites may act as a chemical defence system. The sponge is, as mentioned above, a thin, yellow encrusting sponge, however it has no physical means of defending itself. Hence it is likely to possess a chemical defence system. Aplysulphurin (2) was the major metabolite isolated together with the minor metabolite (3 or 4). Because of this emphasis on one diterpene metabolite, whose function may be as a chemical defence system, its possible biological activity may prove to possess interesting pharmacological properties.

Because of aplysulphurin's interesting structure and possible pharmacological properties, and a diterpene structure hitherto unsynthesised, it presented a challenge for synthesis. Indeed a total synthesis of (2) is necessary before medicinal potential can be realised. *Aplysilla sulphurea*, because of its thin encrusting structure, is difficult to collect in large enough quantities for serious pharmacological testing and, if useful, possible economic potential.



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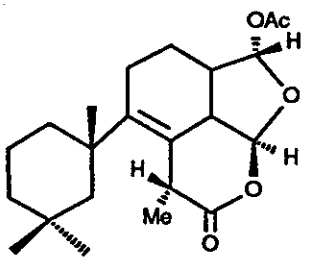

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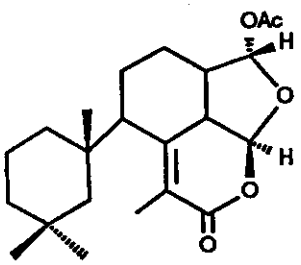

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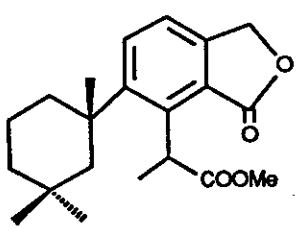

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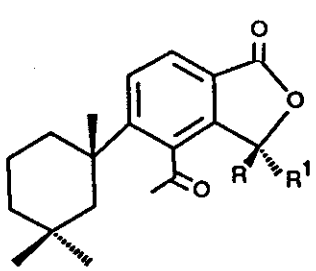
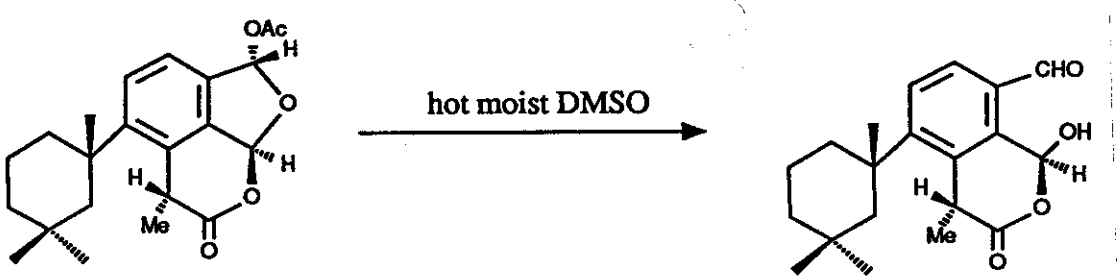


(9) R = OAc R^1 = H

(10) R = H R^1 = OAc

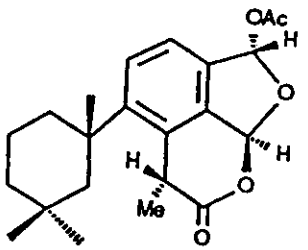
2.2 Discussion

When Karuso treated aplysulphurin (2) with hot moist dimethyl sulphoxide (DMSO) the product isolated was an unstable crystalline product identified as the lactal aldehyde (11).[1] This reaction provided an insight into a possible synthetic route for the preparation of aplysulphurin which could involve initial formation of the carbon-carbon bond linking the A and B ring followed by the formation of the C and D rings.

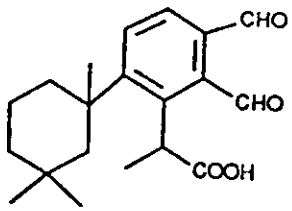


(2) (11)

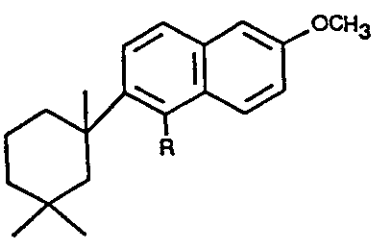
In the retrosynthetic analysis of aplysulphurin (Scheme 1) the last stage of the synthesis could involve a closure of the acid-phthalaldehyde (12), forming the C and D rings. It was anticipated that (12) may be obtained from a 6-cyclohexyl-2-methoxy-naphthyl derivative (13) appropriately substituted in the 5 position from which the side chain may be built on. Ozonolysis of the aromatic ring in (13) could then afford (12). The 6-cyclohexyl-2-methoxynaphthyl derivative (13) in turn, was anticipated, could be obtained from the condensation of 4,4-dimethylcyclohexane-1,3-dione (15) with 2-bromo-6-methoxy-1-naphthoic acid (16) via a Hurtley reaction with subsequent reduction of the ketones in (14).



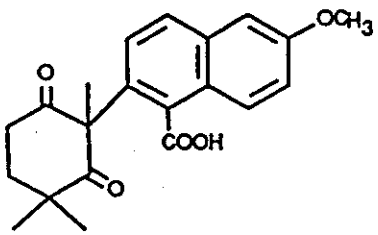
(2)



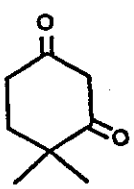
(12)



(13)



(14)



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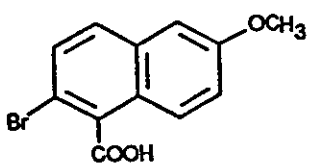
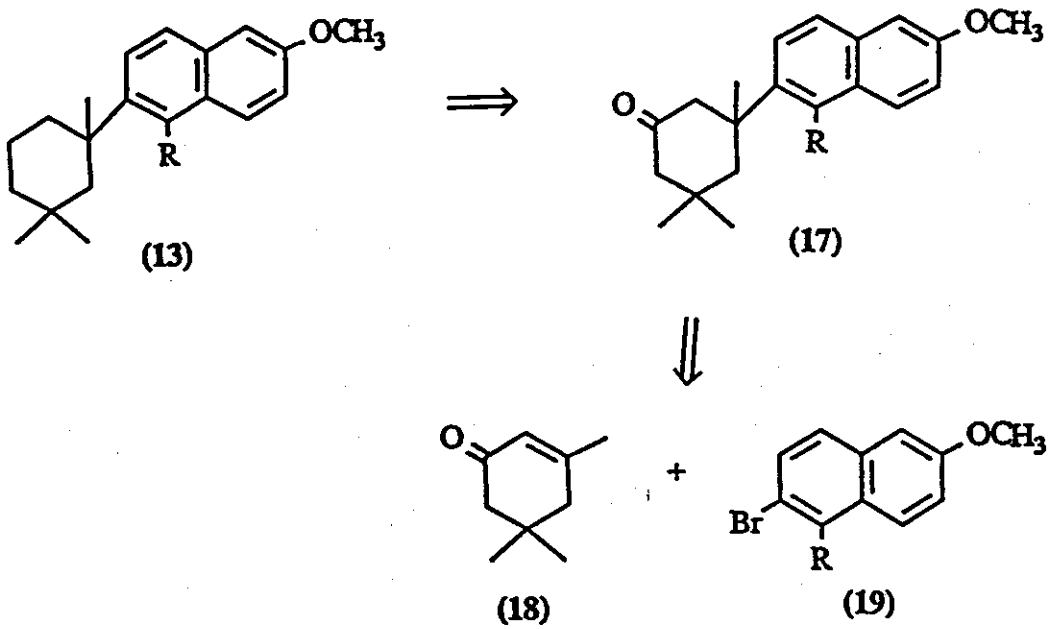


(15) (16)

Scheme 1

An alternative approach to the preparation of (13) is shown in Scheme (2) where the addition of an appropriately 5-substituted-

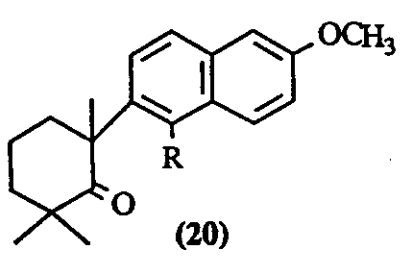
6-bromo-2-methoxynaphthalene (19) is added to isophorone (18) via a 1,4-Michael addition. The subsequent reduction of the ketone (17) would then yield (13).



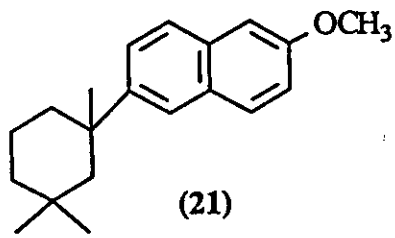
Scheme 2

In light of the large amount of work which had been completed in our department on the Hurtley reaction[7] a synthetic scheme to aplysulphurin involving this reaction was first considered (Scheme 1).

From an initial study by Karuso into a possible synthetic route to aplysulphurin, the model compound (20) was prepared. However, subsequent attempts to reduce the carbonyl function failed.[8]



Since this carbonyl function would also be present in our synthetic strategy [See Scheme 1 (14)] it became evident that an initial study into possible methods of reducing the carbonyls in (14) was in order. Hence a synthetic route, outlined in scheme 3, towards the model compound (21) was first investigated.

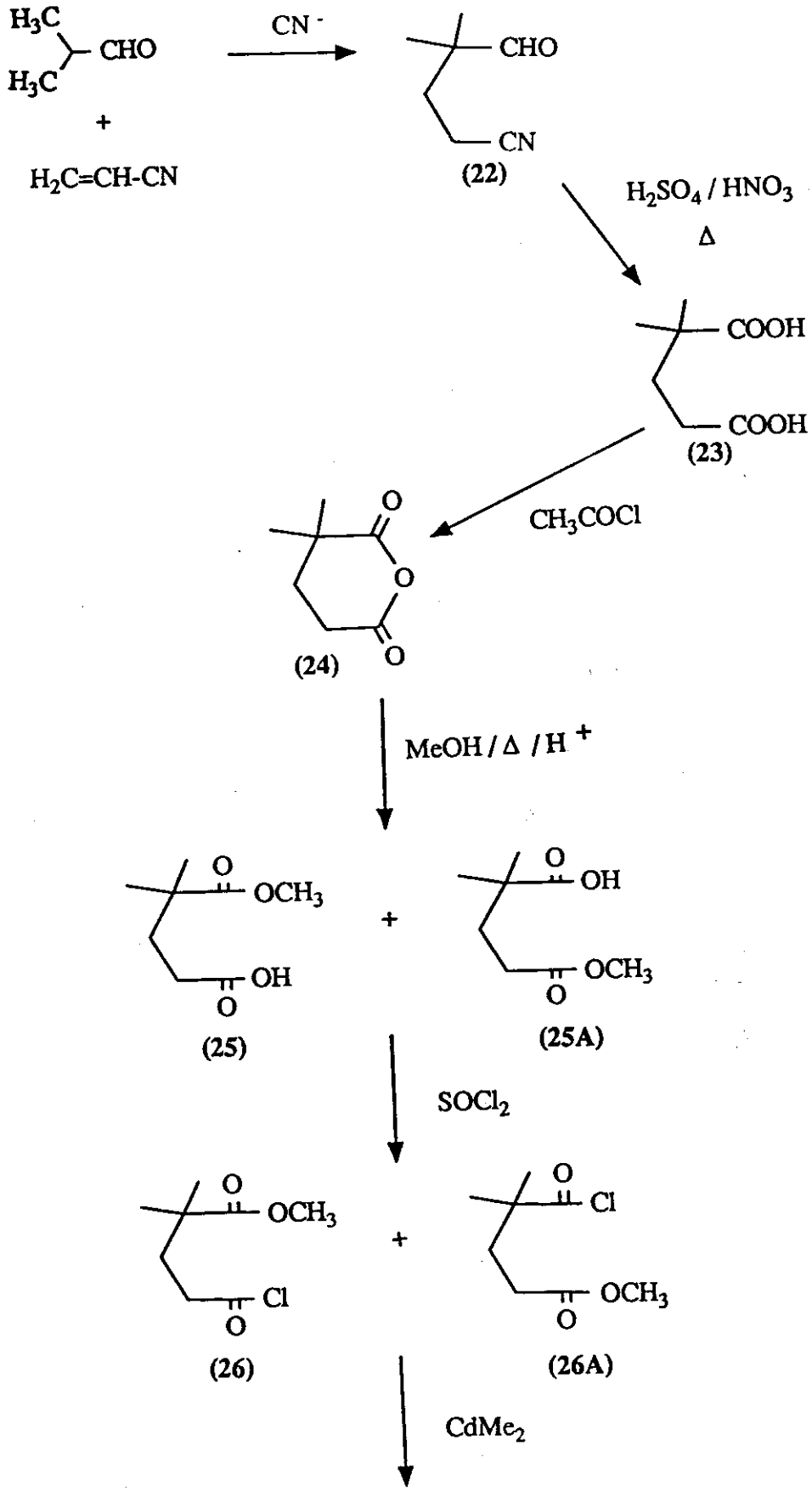


(21)

The preparation of 4,4-dimethylcyclohexane-1,3-dione (15) was first investigated by Champagne *et al.* in 1964[9] and has been adopted in our preparation. The arylation of the β-diketone (15) with 6-methoxy-2-naphthyllead triacetate (33) followed the method of Pinhey *et al.*[10]

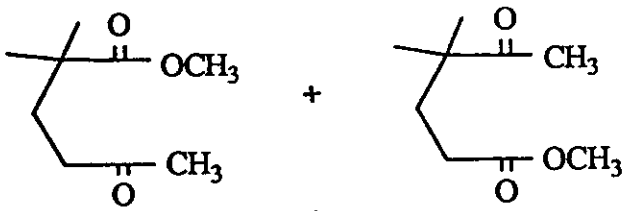
The first step in Scheme 3 was a condensation of isobutyraldehyde with acrylonitrile, using cyanide as the base, to give the corresponding cyanobutyraldehyde (22) in 79% yield. This was then oxidised with a solution of nitric acid and sulphuric acid (1:1) to the glutaric acid (23) (75%) which was then cyclised to the anhydride (24) (63%) with acetyl chloride.

Treatment of (24) with methanol provided the monomethyl ester (25) in 76% yield which upon further treatment with thionyl chloride gave the corresponding acyl chloride (26) in 96% yield. The acyl chloride (26) was then reacted with dimethyl cadmium (prepared by the addition of cadmium chloride to the Grignard of methyl iodide) yielding the ketone (27) in 79%


H3C
CHO
H3C
+
H2C=CH-CN
CN -
CHO
CN
(22)
H2SO4 / HNO3
Δ
COOH
COOH
(23)
CH3COCl
O
O
O
(24)
MeOH / Δ / H +
O
OCH3
OH
O
(25)
+
O
OH
OCH3
O
(25A)
SOCl2
O
OCH3
Cl
O
(26)
+
O
Cl
OCH3
O
(26A)
CdMe2

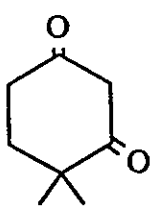
Scheme 3

Scheme 3 continued



(27) (27A)

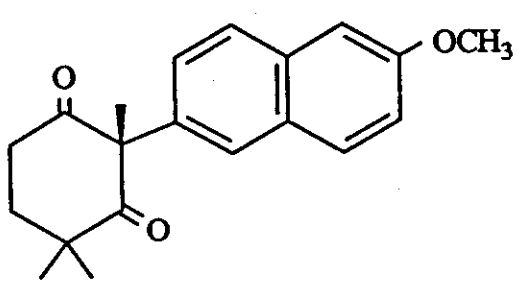
MeOH / MeO^-



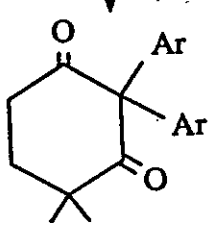
(15)

(1) (33)

(2) MeI

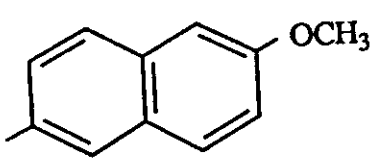


(28)



(29)

Ar =



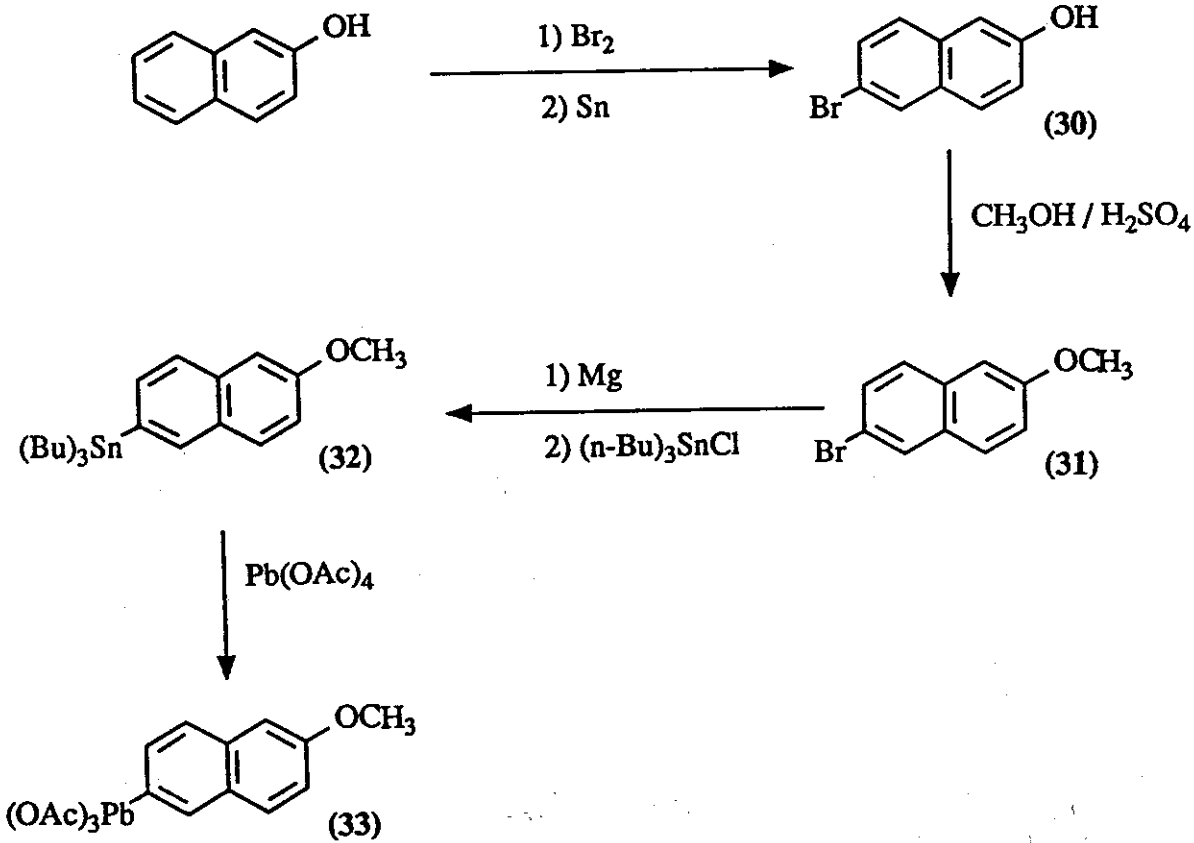
yield. All three compounds (25), (26) and (27) were found by ^1H n.m.r. to be of two isomers as shown in Scheme 3. No attempt was made to separate the two isomeric forms since in the next cyclization step both isomers would give the same product.

The cyclization of (27) to the β-diketone, 4,4-dimethylcyclohexane-1,3-dione (15) was accomplished via a Claisen condensation using sodium methoxide as the base, the product being obtained in 71% yield.

With the synthesis of the β-diketone (15) now achieved, the next step was to attempt a monoarylation in the α-position. The method used was a reaction explored by Pinhey and Rowe,[10] in which aryl lead triacetates were condensed with β-diketones. Although in their attempts at condensing dimedone with the two aryl lead triacetates, *p*-methoxyphenyllead triacetate and *p*-tolyllead triacetate, had only given the corresponding diarylated products, the naphthyl series had not been explored. It was also anticipated that with the increase in steric hindrance provided by the two methyl groups in (15), it may lead to the monoarylated product.

The desired naphthyllead triacetate (33), was therefore prepared using the method of Kozyrod[11] (Scheme 4) starting from β-naphthol.

Bromination of β-naphthol using the method of Koelch[12] gave 6-bromo-2-naphthol (30). Methylation of (30) was effected with methanol and sulphuric acid giving 6-bromo-2-methoxynaphthalene (31)[13] in 71% yield. The Grignard of (31) was then prepared by the method of Kidwell *et al.*[13] and treated with tributyltin chloride according to the general method of Eaborn *et al*[14] which gave the stannane, 6-methoxy-2-naphthyl-tributyl stannane (32) in 70% yield. Transmetalation to the lead triacetate (33) was then effected in 60% yield using dried lead tetraacetate according to the method of Kozyrod.[11]

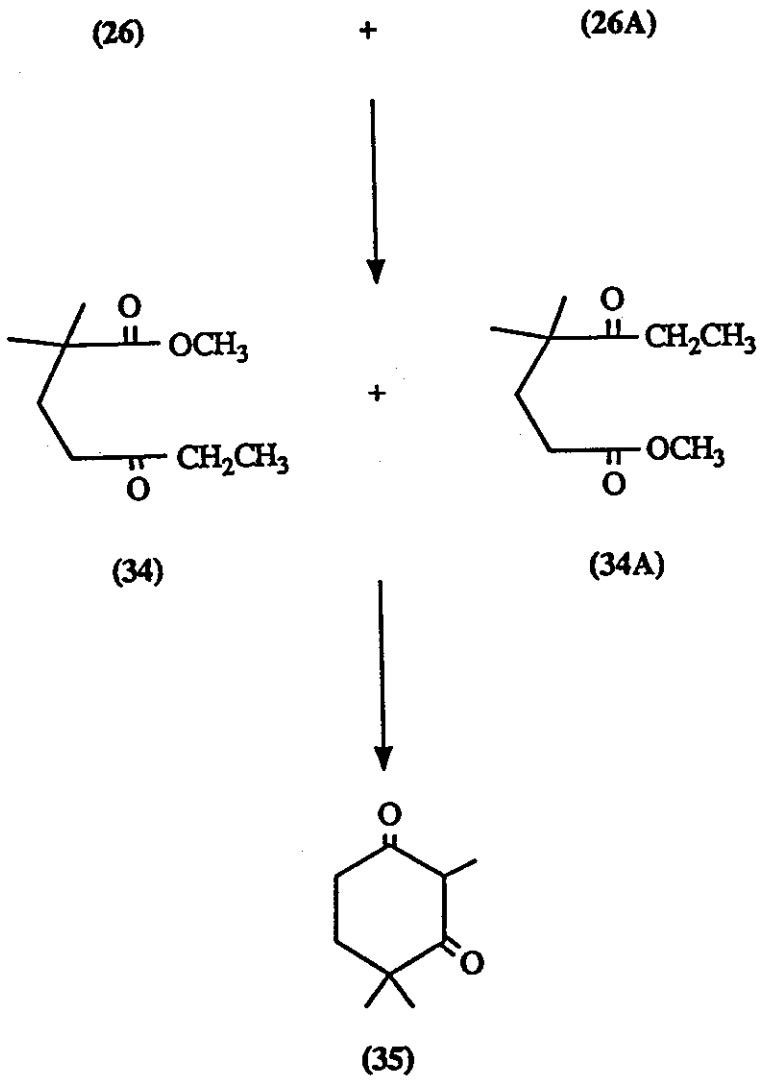


Scheme 4

The naphthyllead triacetate (33) (1 equiv) was then condensed with the β-diketone (15) (1 equiv) according to the method of Pinhey and Rowe[10] (Scheme 3). T.l.c. of the reaction mixture indicated the starting β-diketone was still present even after 24 h.

The product obtained upon work up and crystallization was found to have an empirical formula of $C_{15}H_{14}O_2$ and gave a molecular ion peak of *m/z* 452; hence a molecular formula of $C_{30}H_{28}O_4$. This information coupled with the ^1H n.m.r. spectrum which showed signals pertaining to methoxy and aromatic protons integrating to 6 and 12 protons respectively therefore confirmed the product obtained was in fact the diarylated product 2,2-bis(6-methoxy-2-naphthyl)-4,4-dimethylcyclohexane-1,3-dione (29).

Since the diarylated product was obtained from (15), Scheme 5 in which the α-methyl-β-diketone 2,4,4-trimethylcyclohexane-1,3-dione (35) is prepared, was then proposed, the α-methyl groups therefore preventing diarylation from occurring together with introducing the 7'Me groups of aplysulphurin.



Scheme 5

The preparation of (35) was achieved by treating the acyl chloride (26), prepared as in Scheme 3 with diethylcadmium (prepared by the

addition of cadmium bromide to the Grignard of ethyl bromide) giving the ketone (34) in 80% yield. This was then cyclised by the same method as that in Scheme 3 giving the product (35) in 70% yield. However (35) was found to be a highly unstable crystalline solid which decomposed rapidly to give a pungent yellow oil. All attempts to arylate (35) with the naphthyllead triacetate (33) met with failure giving numerous products which were not analysed.

Therefore the two schemes 3 and 5 were deemed not to be synthetically useful for the preparation of the model compound (21).

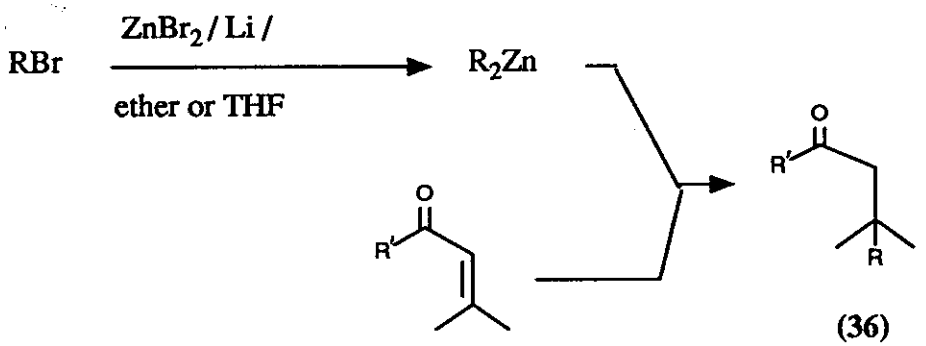
The second approach which may be employed in the preparation of aplysulphurin outlined in the retrosynthetic analysis Scheme 2 was therefore investigated. The approach utilises a reaction explored by Luche *et al.*[15] (outlined in Scheme 6) in which diorganozinc reagents are prepared ultrasonically and then reacted with α,β unsaturated carbonyl compounds to give the 1,4 Michael addition product. In the preparation of anhydrous zinc bromide, lithium foil and an alkyl or aryl bromide in dry ether and under nitrogen are sonicated at 0°.

Sonication is ceased when all the lithium foil has been consumed leaving the diorganozinc reagent as a black precipitate. To this zinc reagent a solution of an α,β unsaturated carbonyl compound together with a catalytic amount of anhydrous nickel acetylacetonate [$Ni(acac)_2$] in ether is added and stirred overnight at room temperature. On workup the 1,4-Michael addition product (36) is obtained.

The initial reaction is believed to be the formation of the organolithium reagent, the formation being assisted by the cavitation energy induced by the ultrasonic vibrator. Two of these organolithiums then undergo a transmetallation with the zinc bromide present to form the diorganozinc reagent.

The major disadvantage of this method is however that only one of the organo groups (alkyl or aryl) is used up. Hence half of the organobromo compound is wasted which would be a major concern if it were expensive.

To investigate the potential of this method and subsequent reactions which may be employed in the preparation of aplysulphurin, again the preparation of the model compound (21) was first investigated.

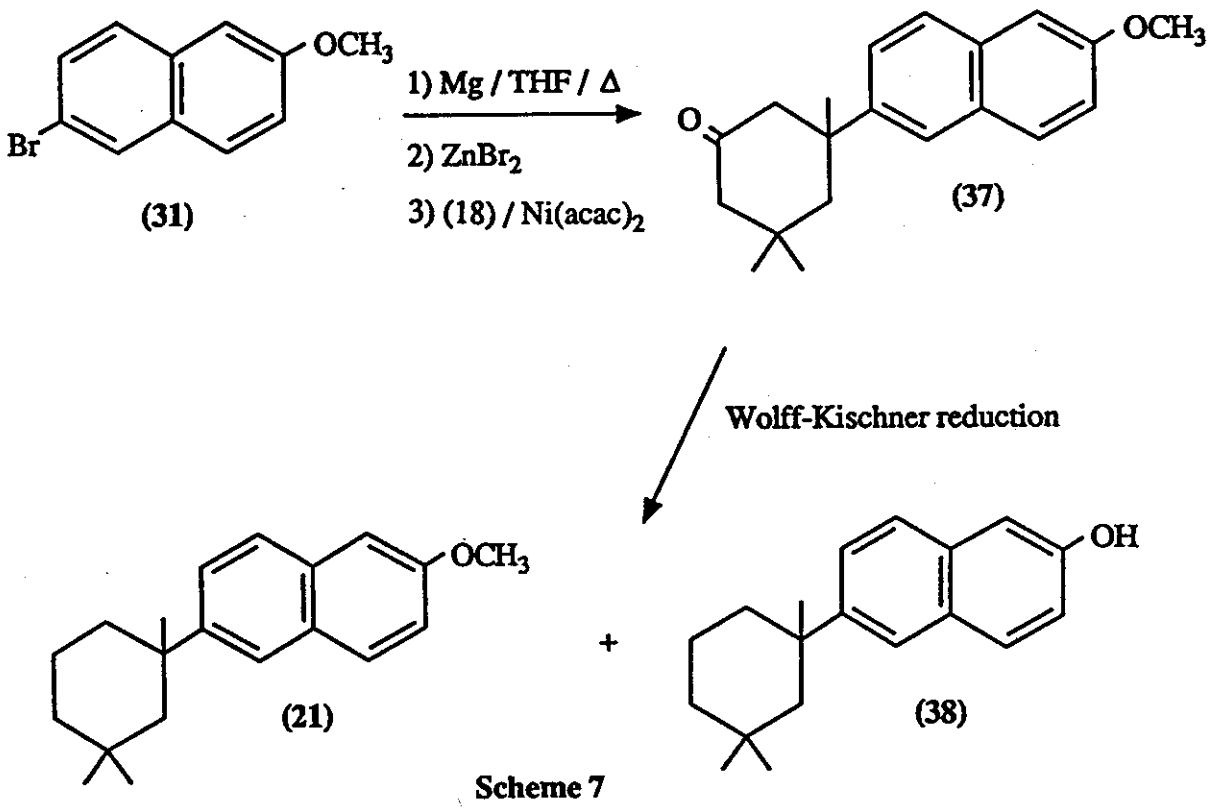


Scheme 6

However when the above method was applied to 6-bromo-2-methoxy-naphthalene (31) and isophorone (18) added, only the two starting materials were recovered. Heating the mixture during sonification to 40° in tetrahydrofuran (THF) also gave no reaction. In a later paper by Luche *et al.*[16] it was reported that in a number of cases for which it was difficult to obtain good results in ether, improved reactions were achieved when sonification was conducted in toluene/THF, the cavitation energy being transferred more efficiently. Applying this method to (31) also met with failure; only starting materials were recovered. Therefore it became obvious that the initial lithiation was not occuring.

To ascertain whether the lack of reactivity was due to the 2-methoxy groups or the extra aromatic ring system, reactions with both 1- and 2-bromonaphthalene under varying conditions and in both ether and

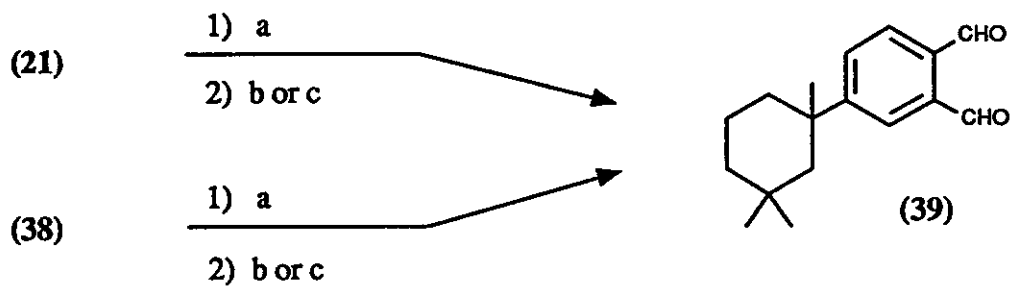
THF/toluene solvents were run. All attempts were however unsuccessful with again only the starting material being recovered. Hence the extra aromatic ring prevents the formation of the lithium reagents.



Scheme 7

It was however possible to form the diaryl zinc reagent by first preparing the Grignard reagent of 6-bromo-2-methoxynaphthalene using the method of Kidwell *et al*,[13] then cooling the reaction mixture to 0° and adding anhydrous zinc bromide (Scheme 7). Isophorone (18) with a catalytic amount of $Ni(acac)_2$ in ether was then added. Upon work up and Kugelrohr distillation the pure 3-(2-methoxy-6-naphthyl)-3,5,5-trimethylcyclohexanone (37) was obtained in 81% yield with respect to isophorone. The ketone was then reduced by the usual Wolff-Kishner

reduction method to yield the model compound 1-(2-methoxy-6-naphthyl) -1,3,3-trimethylcyclohexane (21) in 40% yield together with 1-(2-hydroxy-6-naphthyl)-1,3,3-trimethylcyclohexane (38) in 46% yield. The fact that (38) was obtained may be rationalised by ipso substitution of the methoxy group with hydroxide. The production of the naphthol (38) however was not deleterious, since this compound could also be used in the following ozonolysis reaction to form the *o*-phthalaldehyde (39).



a. O_3 / MeOH / DCM / -78° b. DMS c. PPh_3

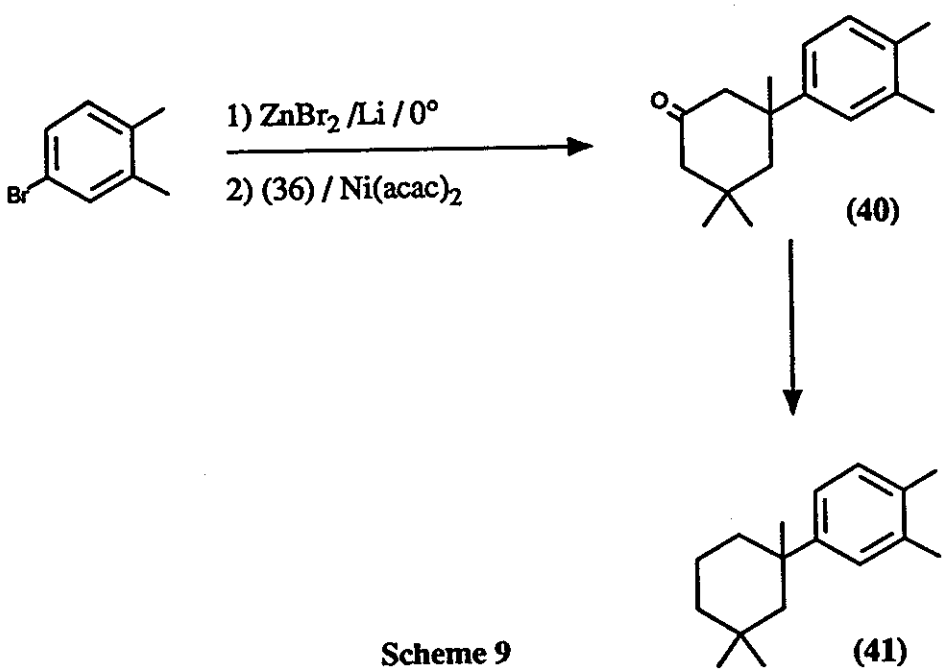
Scheme 8

The ozonolysis of both (21) and (38) (Scheme 8) were carried out in a solution of dichloromethane/methanol (2:1) at -78°. Using the methods of Pappas *et al*,[17,18] (Scheme 8) the reduction of the ozonide with dimethylsulphide[17] yielded from (21) and (38) the *o*-phthalaldehyde (39) in 58 and 60% yields respectively. On the other hand, reduction with triphenylphosphine[18] yielded (39) in 67 and 66% from (21) and (38) respectively.

An alternative approach to the preparation of the *o*-phthalaldehyde

(39) involved the preparation of 4-(1,3,3-trimethylcyclohexyl)-*o*-xylene (41) and is outlined in Schemes (9-10).

This avenue was pursued since there existed a report by Raphalen[19] for the preparation of 2-bromo-5,6-dimethylbenzoic acid which could be used as a precursor for the B, C and D rings of aplysulphurin.



Scheme 9

The preparation of (41) (Scheme 9) involved using the method Luche *et al.*[15] in which the organozinc reagent was prepared by sonication of a mixture of 4-bromo-*o*-xylene, lithium foil and zinc bromide in ether at 0°. To the resulting zinc reagent was added a solution of isophorone in ether with a catalytic amount of anhydrous $Ni(acac)_2$. Upon work up this yielded the ketone (40) in an 85% yield. Reduction of the ketone (40) by the usual Wolff-Kishner method afforded (41) in 96% yield.

Two avenues for effecting the oxidation of the two benzylic methyl groups to the *o*-phthalaldehyde (39) were investigated.

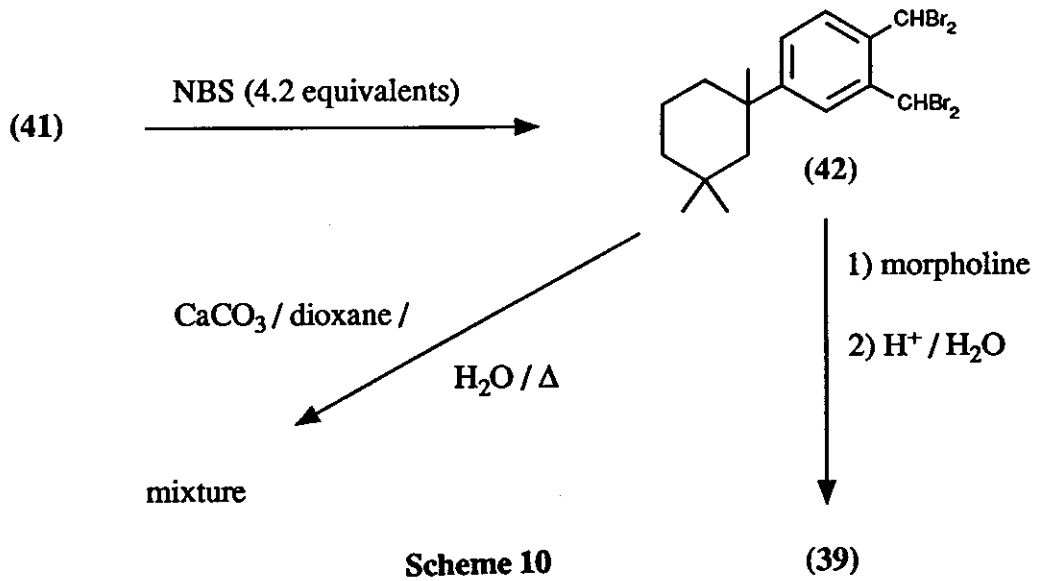
Both involved initial bromination of the benzylic methyl groups to either the tetrabromo product (42)(Scheme 10) or to the dibromo product (43) (Scheme 11) which is then hydrolysed to the diol (44) (Scheme 11).

In Scheme 10 bromination of (41) with 4.1 equivalents of N-bromosuccinimide (NBS) yielded the tetrabromo-*o*-xylyl product (42) in 80% yield. Hydrolysis of this product by refluxing with calcium carbonate in dioxane/water (1:1) yielded a mixture containing a great number of products which were not further analysed.

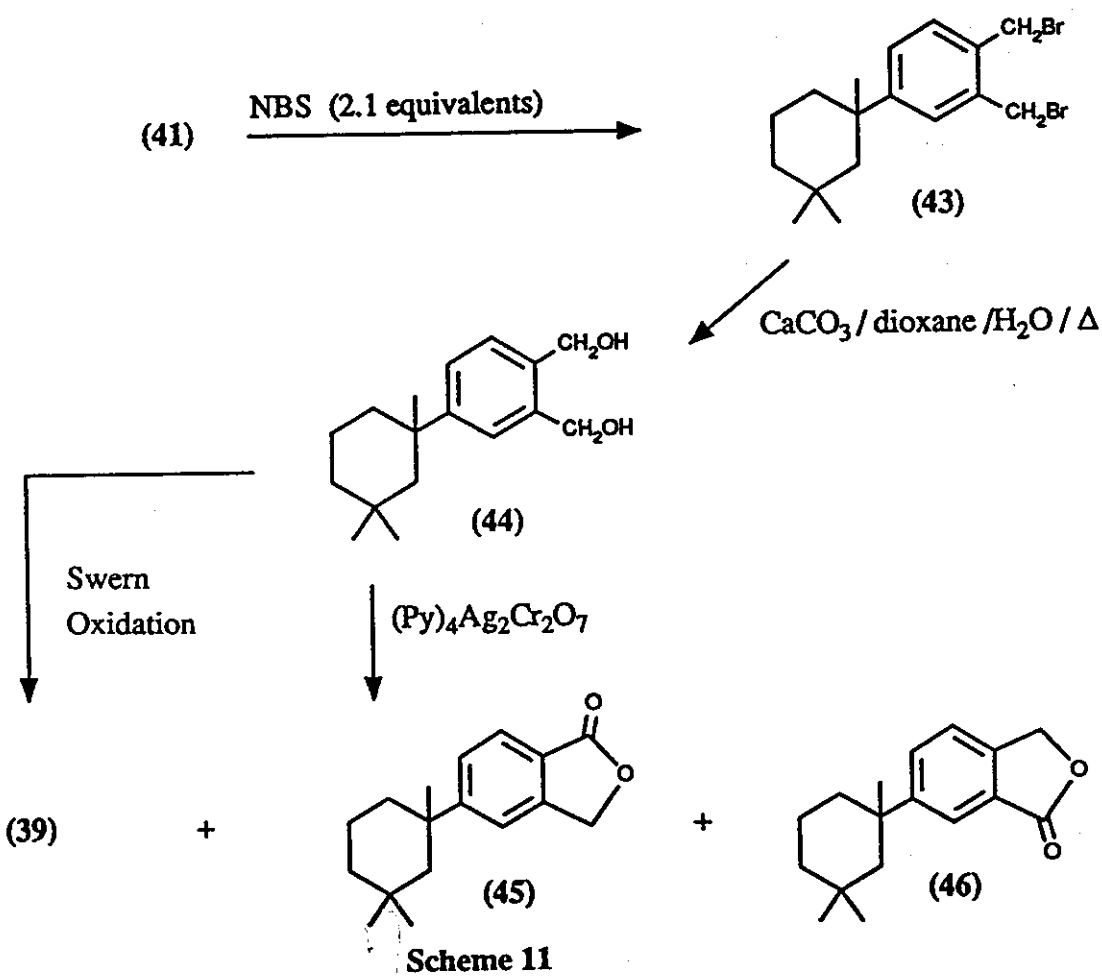
Another method of preparing *o*-phthalaldehydes from tetrabromoxylenes was reported by Kerfanto and Soyer[20] in which the tetrabromoxylenes are first converted to the tetramorpholine product by stirring with morpholine at 50°. the excess morpholine is then removed by distillation and the residue hydrolysed with 10M hydrochloric acid to yield the phthalaldehydes. When this method was applied to (42) a 58% yield of the *o*-phthalaldehyde (39) was obtained.

Alternatively, bromination of (41) with 2.1 equivalents of N-bromosuccinimide afforded the dibromo-*o*-xylene (43) which was then immediately hydrolysed by refluxing with calcium carbonate in dioxane/water (1:1) for 24 h to give the dialcohol (44) in 76% yield.

Two methods for effecting the oxidation of the dialcohol (44) to the phthalaldehyde (39) were tested. The first was oxidation with tetrakis(pyridine) silver dichromate a new reagent developed by Firouzabadi *et al.*[21] This gave both (39) and the two phthalides isomers (45) and (46) in a ratio of 13:10:6 respectively (^{1}H n.m.r.). However oxidation using the method Swern *et al.*,[22] in which alcohols are treated with dimethylsulphoxide and oxalyl chloride in dichloromethane at 0° and followed by triethylamine gave (39) in a 71% yield.



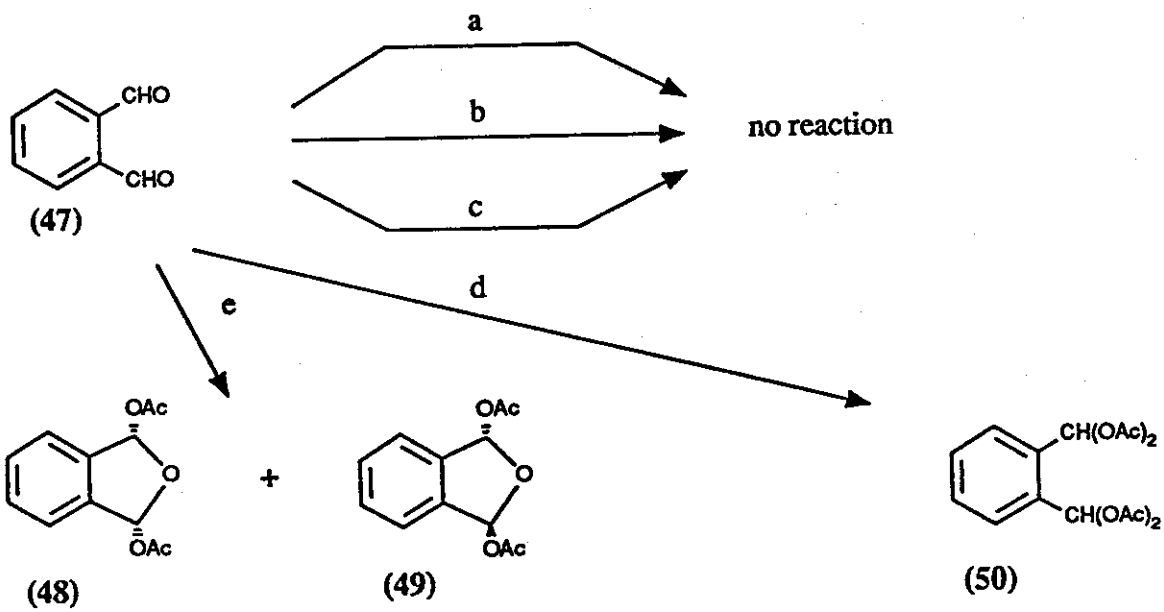
Scheme 10



Scheme 11

Although the method of Firouzabadi *et al.*[21] gave (39) in only relatively low yield (42%), with the major products being the phthalides (45) and (46), it was however an extremely convenient method and the products were easily purified by column chromatography.

The final step was then to cyclise the dialdehyde to form the model diacetoxy phthalan compound (51). A search throughout the literature revealed that these systems have to date not been explored.



a. Ac_2O / H_20 (5 drops) b. Ac_2O / HCl (3 M, 5 drops)

c. Ac_2O / HCl (6 M, 5 drops) d. Ac_2O / HCl (10 M, 5 drops)

e. Ac_2O (1 equivalent) / $BF_3.Et_2O$ (1 equivalent) / Et_2O

Scheme 12

As a test reaction for the cyclization, *o*-phthalaldehyde (47) was prepared by the method of Pappas *et al*;[18] ozonlysis of β-naphthol followed by reduction with triphenylphosphine and of hyrolysis with 6M hydrochloric acid.

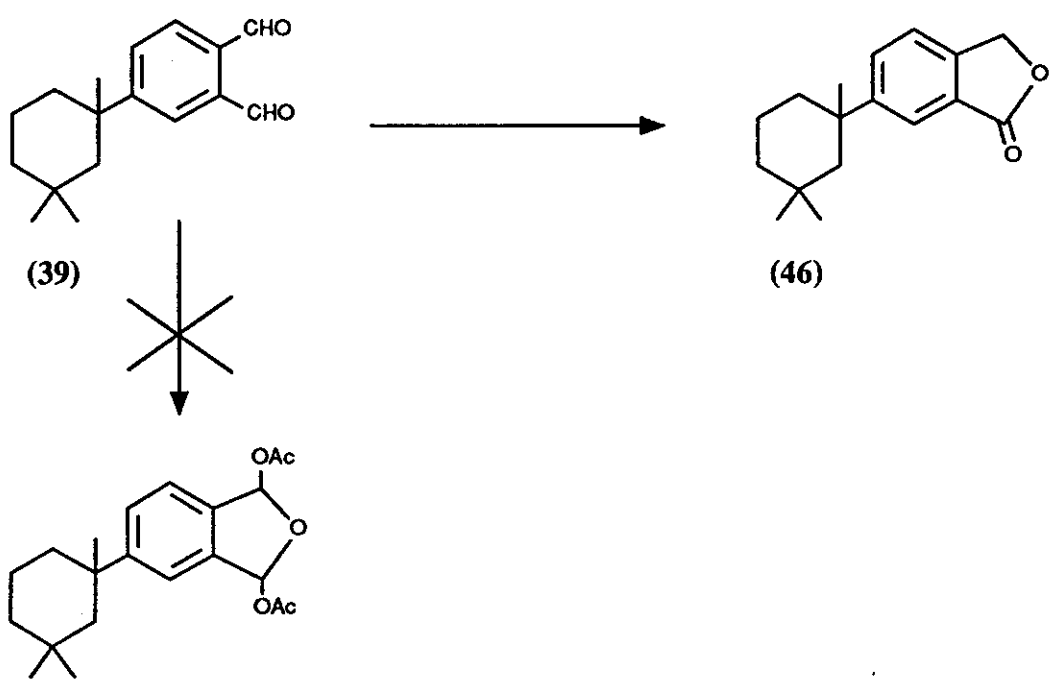
The first reaction attempted for the cyclization used acetic anhydride as solvent to which a few drops of water were added; however only starting

material was recovered. Similarly catalytic amounts of 3M or 6M hydrochloric acid also gave no reaction.

However the use of 10M hydrochloric acid as catalyst gave a white crystalline compound which was identified as *o*-phthalaldehyde tetraacetate (50).

Eventually it was found that boron trifluoride etherate with 1 equivalents of acetic anhydride in ether effected the desired reaction giving 1,3-diacetoxyphthalan as both the *cis* (48) and *trans* (49) isomers in a ratio of 5:9 and overall yield of 95%. The ^1H n.m.r. (200 MHz) spectrum showed two signals at δ 2.12 and 2.14 integrating to six protons (2 x $OCOCH_3$), two signals at δ 7.26 and 7.46 integrating to two protons (α-acetoxyl protons) and a signal at δ 7.5 integrating to four protons (aromatic protons).

Application of this method to (39) however, curiously yielded only the phthalide (46)(Scheme 13).

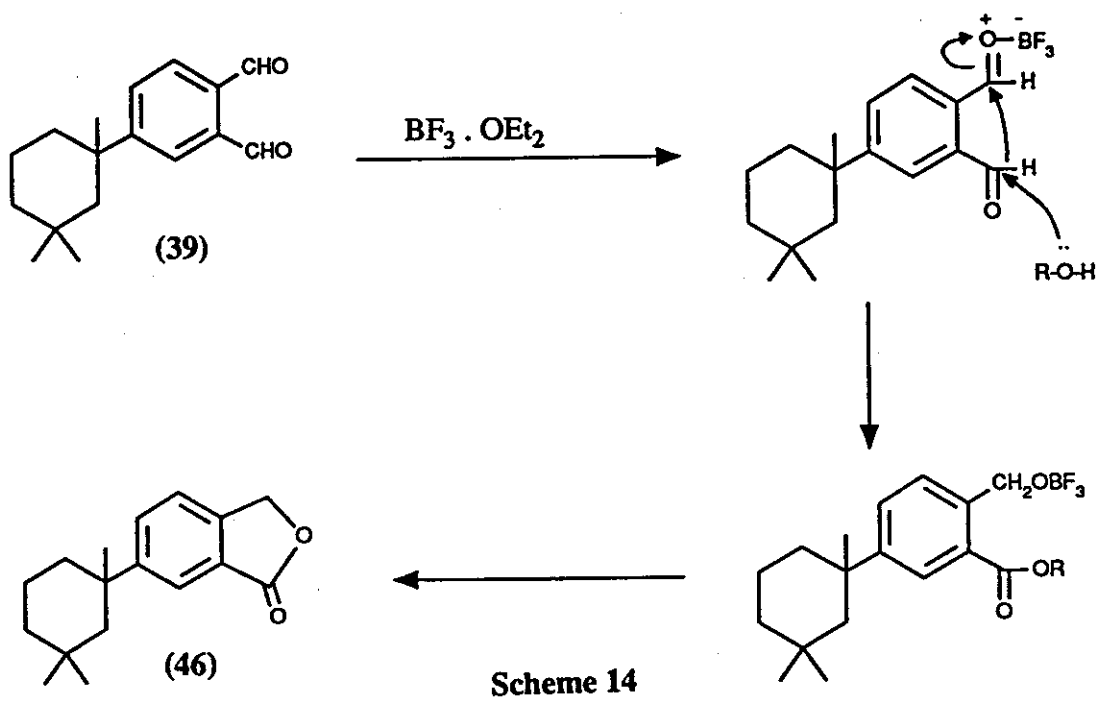


(39) (46) (51)

Scheme 13

This was evident from the ^1H n.m.r. spectrum which showed all the characteristic signals of the cyclohexane ring and aromatic protons but showed a broadened two proton singlet at δ 5.3 indicating one of two isomers (45) or (46).

Distinguishing between these two isomers was achieved by inspection of the aromatic signals and a comparison of the ^1H n.m.r. spectra (90 MHz)of the product with that of the phthalide mixture (45) and (46) obtained from the oxidation of the diol (44) by the method of Firouzabadi. A broadened singlet at δ 7.9, indicating an aromatic proton, possessing only *meta* coupling, was next to the phthalide carbonyl; hence establishing the structure (46).



Scheme 14

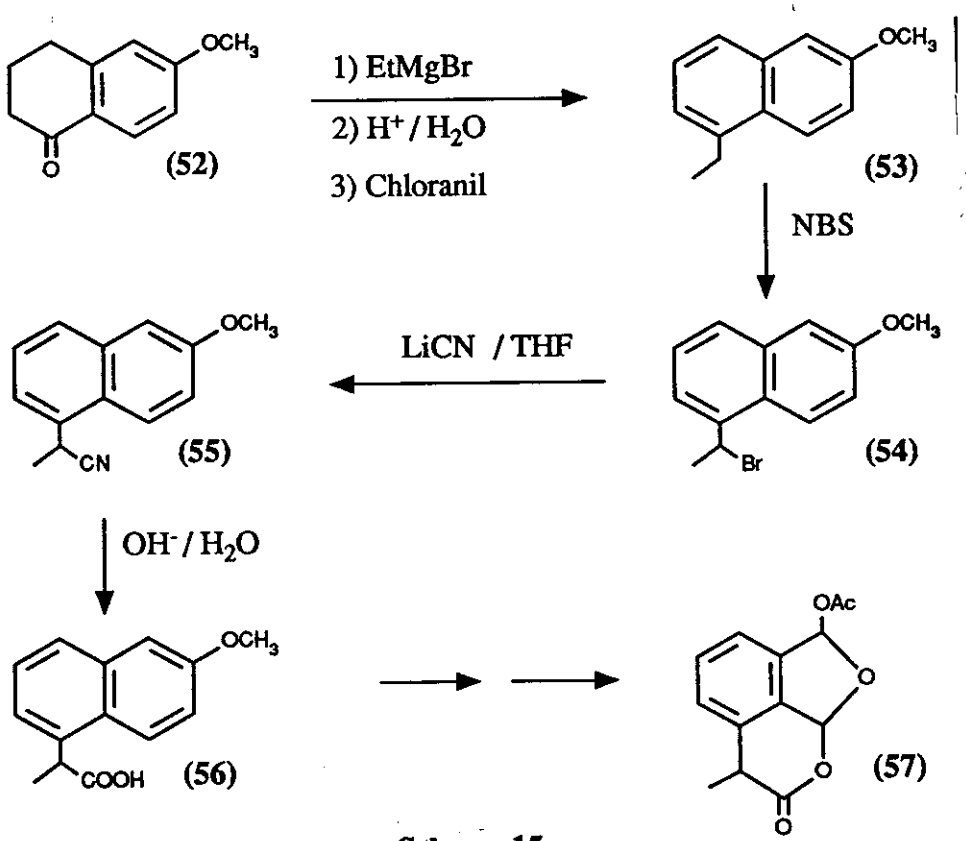
The mechanism for this may be interpreted as depicted in Scheme (14). Initial attack by boron trifluoride on the electronically favoured

aldehyde group *para* to the cyclohexane ring followed by attack of acetic acid or water then hydride transfer would give either the mixed anhydride (which could hydrolyze to the acid on work up) or the acid which could then lactonise to the phthalide (46).

Although not producing the desired diacetoxy phthalan (51) this cyclization is potentially useful for the preparation of other natural products including Membranolide (8), isolated from an Antarctic Dendroceratid sponge identified as *Dendrilla membranosa*.[5]

In the light of the formation of the phthalide (46) in the cyclization of the *o*-phthalaldehyde (39) [Scheme 13] it was decided to also investigate the preparation of (57); aplysulphurin minus the A ring.

Starting from 6-methoxy-α-tetralone (52) and treating it with the Grignard of ethyl bromide followed by dehydration (with 3M hydrochloric acid) and aromatization with chloranil yielded 2-methoxy-5-ethylnaphthalene (53) in 55% yield. Bromination of (53) with 1.1 equivalents of N-bromosuccinimide yielded α-bromo-5-ethyl-2-methoxy-naphthalene (54). Cyanation of the α-bromonaphthalene was effected using the method of Kurihara *et al*[23] in which organohalides and tosylates are refluxed with lithium cyanide (prepared by the method of Livinghouse[24]) in THF. This method when applied to (54) gave the nitrile (55) in 94% yield. Hydrolysis of the nitrile by refluxing in an ethanolic solution of 10M potassium hydroxide followed by work up with hydrochloric acid (3M) gave the acid 2-(2-methoxy-5-naphthyl)-propionic acid (56) in 90% yield. Ozonolysis of (56) followed by reduction of the ozonide, cyclization and acetylation could then yield (57).



Scheme 15

There still remained the problem of finding a method to connect a suitable 5-substituted-2-methoxy naphthyl derivative to isophorone. This might be achieved by initial *o*-lithiation of a suitably 5-substituted methoxynaphthalene i.e. lithiation into the 6 position, followed by organozinc or organocuprate formation and 1,4 Michael addition to isophorone.

A good 5-substituent could be a carboxylic acid function since derivatives can be prepared which have good co-ordinating and directing ability. One such derivative considered was the oxazoline since these functions are known to have strong *ortho*-directing ability in the lithiation of aromatic rings.[25] However a recent paper by Meyers *et al*[26]

reported that 1- and 2- naphthyl oxazolines when treated with organolithium reagents afforded 1,4 addition products rather than *ortho*-lithiation, this being due to the decrease in aromaticity in the naphthalene rings. In the light of these Michael type addition products with oxazolines, a further possibility may be as an N-substituted amide which should, with the removal of the amide proton, prevent any addition products forming both to the amide and aromatic ring. Then a second equivalent of an organolithium reagent could give the *ortho*-lithiated product. As yet these possibilities have not been fully tested and hold potential for further work.

Although the synthesis of aplysulphurin has not been completed some interesting chemistry has been observed in systems that have not otherwise been investigated.

In the arylation of the β-diketone (15) only the diarylated product (29) was obtained. The α-methyl-β-diketone (35) therefore prepared was found to be unstable (probably oxidising readily to a peroxide with molecular oxygen) and hence deemed not synthetically useful for our purposes.

A number of methods of preparing the *o*-phthalaldehyde (39) were investigated including ozonolysis of the naphthyls (21) and (38), and oxidation of the benzylic methyl groups of (41).

Investigations into the preparation of the diacetoxy phthalan from *o*-phthalaldehyde (47) were successful, however applying the method to (39) gave only the phthalide (46).

Preparations towards the synthesis of (57), aplysulphurin minus the A ring, from 6-methoxy-α-tetralone also presented some interesting preparative methods including the use of lithium cyanide to prepare the nitrile (55) conveniently and in high yield.

2.3 Experimental

Microanalyses were performed by the Australian Mineral Development Laboratories, Melbourne and at the University of Sydney facility. ^{1}H n.m.r. spectra were recorded on Varian EM 390, XL 100, and Bruker HX 200 and WM 400 spectrometers, with samples dissolved in deuterochloroform containing tetramethylsilane as internal reference. Unless otherwise stated, ^{1}H n.m.r. spectra were recorded on the Brucker HX 200. Infrared spectra were recorded on a Perkin Elmer 221 or a Bio-Rad FTS spectrometer. Samples were done as chloroform solutions, except where otherwise indicated. Ultraviolet spectra were recorded on a Hitachi 150-20 spectrometer as ethanol solutions.

Electron impact mass spectra were recorded on an AEI MS9 spectrometer at 70 eV which was connected to a DS 390 data handling system when high resolution spectra were required.

Thin-layer chromatography (t.l.c.) was carried out on silica gel (Merck HF_{254} or $PF_{254+360}$). Merck silica gel 60 (230-400 mesh ASTM) was used for flash chromatography. Merck silica gel (60 PF_{254} gipshaltig) was used for preparative centrifugal chromatography (Chromatotron model 7924 Harrison Research U.S.A.).

Melting points were recorded on a Reichert melting point stage and are uncorrected.

Tetrahydrofuran was predried over sodium and then stored over sodium and sodium hydride in the presence of benzophenone under nitrogen. Prior to use, the tetrahydrofuran was distilled into the reaction vessel under

nitrogen. Light petroleum refers to the fraction of b.p. 60-65°.

2,2-Dimethyl-4-cyanobutanal (22)

A solution of isobutyraldehyde (500 g) and acrylonitrile (500 g) with hydroquinone (1.0 g) under a nitrogen atmosphere was heated to reflux whereupon a solution of potassium cyanide in acrylonitrile (25% w/v, 50 ml) was added slowly, keeping the reaction under control. When all the potassium cyanide solution had been added, the contents were further heated, keeping the temperature between 80-90° for 1.5 h. The solution was then cooled, acidified with hydrochloric acid (10 M), the crude mixture filtered and the product extracted into ether.

The ether extracts were then dried over magnesium sulphate, the solvent removed, toluene (250 ml) added and then fractionally distilled to yield the product as a clear oil (685 g, 79%), b.p. 102-106°/2 mm Hg (lit.[9] 106-108°/water pump pressure). ^{1}H n.m.r. (100 MHz) δ 1.00, s, 6H, 2 x CH_3; 1.74, t, *J* 8 Hz, 2H, $C\underline{H}_2C(CH_3)_2$; 2.22, t, *J* 8 Hz, 2H, $CH_2C\underline{H}_2CN$; 9.30, s, C<u>H</u>O.

2,2-Dimethylglutaric acid (23)

2,2-Dimethyl-4-cyanobutanal (5.0 g), nitric acid (70%, 170 ml) and sulphuric acid (98%, 26 ml) were heated until brown fumes were evolved. The solution was then cooled to 40° and the remaining quantity of the cyanoaldehyde (22) (345 g) was added slowly, maintaining the temperature between 35-40°. The solution was then left overnight at room temperature then heated for 2 h at 70° and again left overnight, the crude product crystallising out. The solid product was filtered off and washed with hydrochloric acid (10 M, 200 ml). A second crop was also collected by the evaporation of the mother liquor. The combined crops were then dried yielding a white powder (338 g, 75%), m.p. 84-85° (lit.[9] m.p.84-85°). ^{1}H

n.m.r. (100 MHz) δ 1.20, s, 6H, 2 x CH_3; 1.86, t, *J* 7.5 Hz, 2H, $C\underline{H}_2C(CH_3)_2$; 2.36, t, *J* 7.5 Hz, 2H, $CH_2C\underline{H}_2CO_2H$; 10.31, bs, COO<u>H</u>.

2,2-Dimethylglutaric anhydride (24)

2,2-Dimethylglutaric acid (100 g) and acetyl chloride (70 ml) were stirred overnight at room temperature. The excess acetyl chloride was then removed by distillation and the crude product distilled to yield a clear glass on solidification (55 g, 63%) (bp 90-98°/0.04 mm Hg) m.p.36-38° (lit.[9] 38-40°). ^{1}H n.m.r. (90 MHz) δ 1.35, s, 6H, 2 x CH_3; 1.84, *J* 6.0 Hz, 2H, $C\underline{H}_2C(CH_3)_2$; 2.78, t, *J* 6 Hz, 2H, $C\underline{H}_2COO$.

Monomethyl 4,4- and 2,2-dimethylglutarate (25) and (25A)

2,2-Dimethylglutaric anhydride (20 g) and methanol (8.5 ml) were refluxed for 1.25 h. The solution was then cooled to room temperature and the product extracted into sodium hydrogencarbonate solution (10%, 3 x 50 ml). The combined carbonate extracts were then acidified with hydrochloric acid (3 M) to pH 3.4 and the product extracted into ether. The combined ether extracts were then dried with anhydrous magnesium sulphate, the solvent removed and the crude product distilled to yield a clear oil (18.8 g, 76.7%) b.p.90-100 °/0.07 mm Hg (lit.[9] 120°/1.6 mm Hg). ^{1}H n.m.r. (100 MHz) (both isomers) δ 1.22, s, 12H, 4 x CH_3; 1.2-1.7, m, 8H, 4 x CH_2; 3.67, s, 6H, 2 x OCH_3; 10.8, bs, OH.

4-Methyl-4-methoxycarbonylpentanoyl chloride (26) and (26A)

The methyl ester (25) and (25A) (70 g) was stirred with thionyl chloride (100 ml) for 26 h. The excess thionyl chloride was removed by distillation and the product distilled to yield a clear oil (74 g, 96%) b.p.65-70°/1.0 mm Hg (lit.[9] 70°/ 1.2 mm Hg). ^{1}H n.m.r. (100 MHz) δ 1.21 and 1.32, s, 12H, $C(C\underline{H}_3)_2COCl$ and $C(C\underline{H}_3)_2COOCH_3$; 1.87, m, 4H, $C\underline{H}_2C(CH_3)_2$; 2.15,

t, *J* 7.5 Hz, 2H, $C\underline{H}_2CO$; 2.62, t, *J* 7.5 Hz, 2H, $C\underline{H}_2CO$; 3.69, s, 6H, 2 x OCH_3.

Methyl 4,4- and 2,2-dimethyl-5-oxohexanoate (27) and (27A)

To activated magnesium (1.41 g, 0.058 mol) in dry ether (10 ml) under nitrogen and stirred with a mechanical stirrer, methyl iodide (4.0 ml, 0.064 mol) in ether (10 ml) was added slowly, maintaining a steady reflux. After all the methyl iodide had been added, the mixture was stirred for a further 0.5 h, then cooled in an ice bath. Then to the vigorously stirred Grignard solution, cadmium chloride (5.68 g, 0.031 mol), previously dried at 100° for three days, was added slowly. After the addition was complete the mixture was further stirred at room temperature for 1 h. The ether was then removed by distillation and the residue taken up in dry benzene (20 ml) and further distilled until 10 ml of benzene was removed. Benzene (20 ml) was further added and the reaction mixture cooled to 0°. The acyl chloride mixture (26) and (26A) (10 g, 0.052 mol) in benzene (20 ml) was then added slowly to the vigorously stirred cadmium reagent. After the addition of the acyl chloride was complete the mixture was heated at reflux for 1 h and then cooled to 0° whereupon sulphuric acid (3 M, 50 ml) was added and further stirred for 1 h. The product was then extracted into ether, the ether/benzene extract dried over anhydrous magnesium sulphate, the solvent removed and the crude product distilled to yield a slightly yellow oil (6.61 g, 74%) b.p.70-78°/1.6 mm Hg (lit.[9] 72°/2 mm Hg). ^{1}H n.m.r. (100 MHz) (both isomers) δ 1.12 and 1.15, s, 12H, $C(C\underline{H}_3)_2CO$ and $C(C\underline{H}_3)_2CO_2CH_3$; 1.68-1.98, m, 4H, 2 x $C\underline{H}_2C(CH_3)_2$; 2.05-2.38, mm, 10H, $COC\underline{H}_2$, $CH_3O_2CC\underline{H}_2$, 2 x $COC\underline{H}_3$; 3.64, s, 6H, 2 x OCH_3.

Methyl 4,4- and 2,2-dimethyl-5-oxoheptanoate (34) and (34A)

To activated magnesium (1.41 g; 0.058 mol) in dry ether (10 ml) under nitrogen, and stirred with a mechanical stirrer, ethyl bromide (7.85 ml, 0.06 mol) in dry ether (10 ml) was added slowly maintaining a steady reflux. After all the ethyl bromide had been added, the mixture was stirred for a further 0.5 h, then cooled in an ice bath. Then to the vigorously stirred Grignard solution, cadmium bromide (8.44 g, 0.031 mol) previously dried at 100° for three days was added slowly. After the addition was complete the mixture was further stirred at room temperature for 1 h. The ether was then removed by distillation and the residue taken up in dry benzene (20 ml) and further distilled until 10 ml of benzene was removed. Benzene (20 ml) was further added and the reaction mixture cooled to 0°. The acyl chloride mixture (26) and (26A) (10 g, 0.052 mol) in benzene (20 ml) was then added slowly to the vigorously stirred cadmium reagent. After the addition of the acyl chloride was complete the mixture was heated at reflux for 1 h and then cooled to 0° whereupon sulphuric acid (3 M, 50 ml) was added and further stirred for 1 h. The product was then extracted into ether, the ether/benzene extract dried over anhydrous magnesium sulphate, the solvent removed and the crude product distilled to yield a clear oil (7.43 g, 77%) b.p.95°/1.0 mm Hg. (Found: C, 64.3; H 9.9. $C_{10}H_{18}O_3$ requires C, 64.5; H, 9.7%) λ_{max}: 278.4 (ϵ 0.18), 202.4 (ϵ 0.72) nm. ν_{max}(liquid film): 2975, 1740, 1710, 1200, 1140 cm^{-1}. ^1H n.m.r. (100 MHz) δ 0.97 and 0.99, t, *J* 7 Hz and 7 Hz, 2 x CH_3, 6H, $C(CH_3)_2COCH_2\underline{C}H_3$ and $CH_2COCH_2\underline{C}H_3$; 1.14 and 1.18, s, 12H, $C(\underline{C}H_3)_2CO$ and $C(\underline{C}H_3)_2CO_2CH_3$; 1.65–1.90, m, 4H, $\underline{C}H_2C(CH_3)_2$; 2.05–2.30, m, 4H, $COCH_2\underline{C}H_2$ and $CH_3O_2CCH_2\underline{C}H_2$; 2.48 and 2.52, q, *J* 7 Hz and 7 Hz, 4H, $C(CH_3)_2COCH_2CH_3$ and $CH_2COCH_2CH_3$; 3.65 and 3.66, s, 6H, 2 x OCH_3. Mass spectrum *m/z* 157(4%), 155(4), 129(26), 115(17), 97(61), 69(100).

4,4-Dimethylcyclohexane-1,3-dione (15)

To powdered sodium (0.67 g) in dry ether (20 ml), methanol (2 ml) was added and then stirred at room temperature for 24 h. The methyl ketone (27) and (27A) (5 g) was then added and stirring continued for a further 2 h. The resulting mixture was then acidified with hydrochloric acid (3 M), extracted with ether, the ether extract dried over anhydrous magnesium sulphate and the solvent removed. Light petroleum (2 ml) was then added to the resulting oil, vigorously shaken and then allowed to stand, allowing the product to crystallise. The product was then filtered off and recrystallized from ethyl acetate/ light petroleum to yield white needle crystals (2.29 g, 71%) m.p. 104-105° (lit.[9] 105°). ^{1}H n.m.r. (100 MHz) δ 1.15 and 1.22, s, 6H, $C(CH_3)_2$; 2.00-1.70, m, $\underline{CH_2}C(CH_3)_2$; 2.75-2.35, m, $OCC\underline{H_2}$; 3.46, s, 5.38, s, 6.86, s, 2H.

2,4,4-Trimethylcyclohexane-1,3-dione (35).

To powdered sodium (0.62 g) in ether (20 ml), methanol (2 ml) was added and stirred at room temperature for 24 h. The ethyl ketone (34) (5 g) was then added and stirring continued for a further 2 h. The resulting mixture was then acidified with hydrochloric acid (3 M), extracted with ether, the ether extracts dried over magnesium sulphate and the solvent removed. Light petroleum (2 ml) was then added to the resulting oil, vigorously shaken and then allowed to stand, allowing the product to crystallise. The product was then filtered off and recrystallised from ethyl acetate/light petroleum to yield an unstable clear crystalline solid (2.90 g, 70%) m.p. 115-116° with prior softening at 104-108°. (Found: 154.1006. $C_9H_{14}O_2$ requires 154.0994). λ_{max} 261 nm (ϵ 91). ν_{max} (nujol mull) 3700-2300 br, 1580, 1460, 1380, 1355, 1095 cm^{-1}. ^{1}H n.m.r. (100MHz) δ 1.88-1.10, m, 3 x Me, 1 x CH_2; 2.55, t, *J* 6 Hz, $COC\underline{H_2}CH_2$; 3.72 and 6.08, q and bs respectively, 1H. Mass spectrum: *m/z* 154 (55%), 126 (17), 111 (25), 98 (100), 83 (26), 70 (80), 56 (81).

2,2-Bis(6-methoxy-2-naphthyl)-4,4-dimethylcyclohexane-1,3-dione (29)

A solution of 4,4-dimethylcyclohexane-1,3-dione (52.7 mg, 0.376 mmol), 6-methoxy-2-naphthyllead triacetate (204 mg, 0.377 mmol) and pyridine (0.03 mg, 0.376 mmol) in chloroform (10 ml) were stirred for 24 h at 40° (the reaction mixture being monitored by t.l.c). After 24 h the solution was diluted with chloroform (10 ml), sulphuric acid (3 M, 15 ml) was added and the solution filtered. The residue was washed with chloroform (2 x 5 ml), the combined chloroform extracts dried with anhydrous magnesium sulphate and the solvent removed. Ether (2 ml) was then added and the precipitate filtered. The precipitate was then dissolved in chloroform, filtered through flash silica gel, the solvent removed and the resulting solid recrystallised from ether/dichloromethane to yield fine clear diamond crystals (63.8 mg, 75%) m.p. 216-218°. (Found: C, 79.7; H 6.3. $C_{30}H_{28}O_4$ requires: C 79.6; H, 6.2). λ_{max} (EtOH) 330.0, 226.0 nm ϵ 691.5, 16503. ν_{max} 2960, 1725, 1695, 1635, 1605, 1480, 1390, 1265, 1030, 850 cm^{-1}. ^1H n.m.r. (100 MHz) δ 1.27, s, $C(C\underline{H}_3)_2$; 1.73, t, J 7 Hz, $C\underline{H}_2C(CH_3)_2$; 2.86, t, J 7 Hz, $COC\underline{H}_2$; 3.89, s, 2 x $OC\underline{H}_3$; 6.90-7.90, m, 12H, ArH. Mass spectrum *m/z* 452(100%), 424(35), 398(2), 369(3), 340(5), 328(7), 327(33), 326(10), 252(8), 239(13), 211(19), 163(37).

6-Bromo-2-methoxynaphthalene (31)

6-Bromo-2-naphthol was prepared from β-naphthol using the method of Koelsch.[12] Methylation of 6-bromo-2-napthol (50 g) was carried out using the method of Kidwell *et al*..[13] to give 6-bromo-2-methoxynaphthalene which was purified by distillation, b.p.114-120°/0.25 mm Hg to yield a hard wax (38 g, 71%) m.p.102-108° (lit.[13] 101.5-103°). The hard wax was then recrystallised from acetone/water to yield a white powder (37 g, 69%) m.p.107-108°.

6-Methoxy-2-naphthyltributylstannane (32)

6-Methoxy-2-naphthylmagnesium bromide was prepared using the method of Kidwell *et al.*[13] and treated with chlorotributylstannane according to the general method of Eaborn *et al.*[14] to give 6-methoxy-2-naphthyltributylstannane (74%) as a colourless oil b.p.(Kugelrohr) 220-230°/0.15 mm [lit.[11] (Kugelrohr) 230°/0.1 mm Hg]

6-Methoxy-2-naphthylead triacetate (33)

Dried lead tetraacetate (1.0 g, 2.3 mmol) was stirred with 6-methoxy-2-naphthyltributylstannane (1.0 g, 2.2 mmol) and mercury (II) acetate (36 mg, 0.11 mmol) in chloroform (20 ml) at 40° for 2 h. The reaction mixture was cooled, filtered through celite, the solvent removed and the residual oil allowed to solidify. Light petroleum (10 ml) was added, and the precipitate was filtered off and washed with light petroleum (3 x 6 ml). Crystallization from chloroform/light petroleum then gave 6-methoxy-2-naphthylead triacetate (1.04 g, 85%) as yellow needles m.p.146-148° (lit.[11] 146-149°) ^{1}H n.m.r. δ 2.14, s, 9H, 3 x $OCOCH_3$; 3.93, s, 3H, OCH_3; 7.00-8.10, m, 6H, ArH.

4-Bromo-*o*-xylene

4-Bromo-*o*-xylene was prepared from *o*-xylene (140 g) according to the method of Wisansky and Anabacher[27] to yield a clear oil (156.5 g, 64%) b.p.210-216° (lit.[27] 92-94°/14-15 mm Hg)

3-(4'-*o*-Xylyl)-3,5,5-trimethylcyclohexanone (40)

4-Bromo-*o*-xylene (13.69 g, 74 mmol), lithium foil (1.03 g, 148 mmol) and anhydrous zinc bromide (8.37 g, 37 mmol) in dry ether (20 ml) under nitrogen were sonicated at 0° until all the lithium foil was consumed

leaving a black precipitate. Sonification was discontinued and to the black suspension a solution of isophorone (5.11 g, 37 mmol) and anhydrous $Ni(acac)_2$ (0.1 g) in dry ether (20 ml) was added and then stirred at room temperature (20°) for 24 h. The solution was then poured onto saturated ammonium chloride solution and the product extracted into ether (3 x 100 ml). The combined ether extracts were washed with water (100 ml), brine (2 x 50 ml) and dried over anhydrous sodium sulphate. Evaporation of the solvent and chromatography of the residual oil on flash silica gel with ethyl acetate/light petroleum (1:9) gave, after recrystallization from light petroleum, 3-(4'-*o*-xylyl)-3,5,5-trimethylcyclohexanone as clear colourless crystals (5.9 g, 67%) m.p.86-87°. (Found: C, 83.83; H, 10.06 $C_{17}H_{24}O$ requires C, 83.61; H, 9.84 %) λ_{max}: 275.6, 268 nm (ϵ 431, 474). ν_{max}: 3000, 1700, 1450, 1290, 1215, 815 cm^{-1}. ^1H n.m.r. δ 0.44, s, 3H, 5α-CH_3; 1.04, s, 3H, 5β-CH_3; 1.34, s, 3H, 3β-CH_3; 1.88, d, $J_{4\beta,4\alpha}$ 14.2 Hz, H4β; 2.03-2.30, m, 3H, H4α, $(H6)_2$; 2.35, d, $J_{2\beta,2\alpha}$ 13.7 Hz, H2β; 3.03, bd, $J_{2\alpha,2\beta}$ 13.7 Hz, H2α; 7.02-7.14, m, 3H, ArH. Mass spectrum (e.i.) *m/z* 244(86%), 229(70), 187(68), 173(46), 146(100), 131(20), 99(24), 69(26), 55(33).

1,3,3,-Trimethyl-1-(3',4'-dimethyl-1'-phenyl)cyclohexane (41)

A solution of 3,5,5-trimethyl-3-(4-*o*-xylyl)-cyclohexanone (40) (2.0 g, 8.2 mmol) and hydrazine hydrate (40 ml) in digol (80 ml) was refluxed for 1 h. The solution was cooled, potassium hydroxide (1 g) added, distilled until the solution temperature was 190° and then further refluxed for 24 h. The cooled solution was then poured onto water (250 ml) and the product extracted into ether (3 x 100 ml). The combined ether extracts were washed with water (2 x 100 ml), brine (2 x 100 ml), dried over anhydrous sodium sulphate and the solvent removed. The residual oil was chromatographed on flash silica gel eluting with dichloromethane, then

distilled to give 1,3,3-trimethyl-1-(3',4'-dimethyl-1-phenyl)cyclohexane as a clear colourless oil (1.8 g, 95%) b.p.(Kugelrohr) 120°/0.2 mm Hg. (Found: 230.2032. $C_{17}H_{26}$ requires 230.2034). λ_{max}: 388, 275, 268, 215 nm (ϵ 33, 309, 376, 9512). ν_{max} 2949, 2924, 1507, 1465, 1456, 1386, 1364, 967, 818 cm^{-1}. ^1H n.m.r. δ 0.43, s, 3H, 3α-Me; 0.91, s, 3H, 3β-Me; 1.13, s, 3H, 1β-Me; 1.16-1.72, m, 5H, $(H4)_2$, $(H5)_2$, H6β; 1.37, d, $J_{2\beta,2\alpha}$ 14.2 Hz, H2β; 1.96, bd, $J_{2\alpha,2\beta}$ 14.2 Hz, H2α; 2.20, m, H6α; 2.22, s, 3H, $ArCH_3$; 2.25, s, 3H, $ArCH_3$; 6.99-7.15, m, 3H, ArH. Mass spectrum (e.i.) *m/z* 230(88%), 215(100), 201(9), 187(12), 173(13), 159(79), 146(24), 133(90), 119(49), 109(9), 69(12).

1,3,3-Trimethyl-1-[3',4'-bis(hydroxymethyl)-1'-phenyl)-cyclohexane (44)

1,3,3-Trimethyl-1-(3',4'-dimethyl-1'-phenyl)-cyclohexane (41) (0.5 g, 2.2 mmol) was dissolved in carbon tetrachloride (20 ml). *N*-bromosuccinimide (0.85 g, 4.8 mmol) and benzoyl peroxide (0.1 g) were added to the solution and the reaction mixture was illuminated and refluxed with a 500 W light globe until all the solid suspension floated on the surface. The reaction mixture was then cooled to 0° and filtered through celite. The residue was further washed with carbon tetrachloride (2 x 10 ml) and the solvent removed. To the residual oil was added dioxane (30 ml), water (30 ml) and calcium carbonate (7.5 g) and the reaction mixture was refluxed for 24 h with stirring. The mixture was then cooled and acidified with hydrochloric acid (10 M). Water (100 ml) was added and the product extracted into ether (3 x 50 ml). The combined ether extracts were washed with water (2 x 50 ml), brine (2 x 50 ml), dried over anhydrous sodium sulphate and the solvent removed. The crude product was purified by chromatography on flash silica gel eluting with ethyl acetate and then recrystallization from light petroleum gave 1,3,3-trimethyl-1-[3',4'-bis(hydroxymethyl)-1'-phenyl]-cyclohexane as fine

needle crystals (432 mg, 76%) m.p.88–89°. (Found: C, 77.59, H, 9.97 $C_{17}H_{26}O_2$ requires C, 77.86, H, 9.92%). λ_{max} 264.0, 216.4 nm (ϵ 395, 10461). ν_{max} 3402 br, 2954, 2928, 1613, 1465, 1455, 1231, 1010 cm^{-1}. ^1H n.m.r. δ 0.38, s, 3H, 3α– Me; 0.91, s, 3H, 3β–Me; 1.34, s, 3H, 1β–Me; 1.18–1.77, m, 5H, $(H4)_2$, $(H5)_2$, H6β; 1.40, d, $J_{2\beta,2\alpha}$ 13.7 Hz, H2α; 2.24, bd, $J_{6\alpha,6\beta}$ 14.2 Hz, H6α; 3.05, bs, 2H, 2 x OH; 4.70, s, 2H, $ArC\underline{H}_2OH$; 4.72, s, 2H, $ArC\underline{H}_2OH$; 7.27–7.35, m, 3H, ArH. Mass spectrum (e.i.) *m/z* 262(74), 260(65), 247(39), 244(43), 229(24), 199(74), 165(52), 147(39), 143(71), 131(44), 105(32), 91(33), 69(56), 41(70).

1,3,3-Trimethyl-1-[3',4'-bis(dibromomethyl)-1'-phenyl]-cyclohexane (42)

1,3,3,-Trimethyl-1-(3',4'-dimethyl-1-phenyl)-cyclohexane (41) (0.5g, 2.2 mmol) was dissolved in carbon tetrachloride (20 ml). *N*-Bromosuccinimide (1.63 g, 9.2 mmol) and benzoyl peroxide (0.1 g) were added and the reaction mixture illuminated and refluxed with a 500 W light globe until all the solid suspension was floating on the surface. The reaction mixture was then cooled to 0° and filtered through celite. The residue was further washed with carbon tetrachloride (2 x 10 ml) and the solvent removed. The residual oil was then chromatographed on flash silica gel eluting with light petroleum and the resulting gum distilled at b.p.(Kugelrohr) 220°/0.3 mm Hg. On standing the gum solidified to yield a soft white wax (1.02 g, 86%) m.p.111–113°. (Found: C, 37.21; H, 4.01. $C_{17}H_{22}Br_4$ requires C, 37.39; H, 4.03%). λ_{max} 218 nm (ϵ 24567). ν_{max} 2955, 2930, 2867, 1606, 1466, 1243, 1137, 968, 835, 649 cm^{-1}. ^1H n.m.r. δ 0.39, s, 3H, 3α–Me; 0.92, s, 3H, 3β–Me; 1.16, s, 3H, 1β–Me; 1.18–1.70, m, 5H, $(H4)_2$, $(H5)_2$, H6β; 1.43, d, $J_{2\beta,2\alpha}$ 13.9 Hz, H2β; 1.99, bd, $J_{2\alpha,2\beta}$ 13.9 Hz, H2α; 2.24, bd, $J_{6\alpha,6\beta}$ 14.4 Hz, H6α; 7.06, bs, $\underline{W}_{h/z}$ 5.5 Hz, 4'-$ArC\underline{H}Br_2$; 7.19, bs, $\underline{W}_{h/z}$ 5.5 Hz, 3'-$ArC\underline{H}Br_2$; 7.35, m, H6'; 7.51, m, H5'; 7.70, bs, $\underline{W}_{h/z}$ 7.8 Hz, H2'. Mass spectrum (e.i.) *m/z* 550 / 548 / 546 / 544 / 542

(all < 1%), 535 / 533 / 531 / 529 / 527 (all< 1%), 469 / 467 / 465 / 463 (all < 32%), 389 / 387 / 385 (all < 16%), 373 / 371 / 369 (all < 29%) 307 / 305(100%), 201(8), 125(20).

Attempted 1,4-Michael Addition of 6-Bromo-2-methoxynaphthalene via Luche *et al*[15,16] method.

By using the same method as described for the preparation of (40), in which the zinc reagent of 4-bromo-*o*-xylene (prepared by sonicating at 0° a mixture of anhydrous zinc bromide, lithium and 4-bromo-*o*-xylene in ether) was reacted with isophorone, it was attempted to prepare the zinc reagent of 6-bromo-2-methoxynaphthalene then react this also with isophorone. However after 12 h of sonification, addition of isophorone and work up only starting materials were retrieved. Using the same procedure except heating to 40° in tetrahydrofuran or in tetrahydrofuran/toluene (5:1) also gave no reaction. Attempts to prepare the zinc reagent of 1 and 2-bromonaphthalene and add this to isophorone in a 1,4-Michael addition also failed with only starting materials being retrieved.

3,5,5-Trimethyl-3-(6'-methoxy-2'-naphthyl)-cyclohexanone (37)

To 6-methoxy-2-naphthylmagnesium bromide, prepared using the method of Kidwell *et al*.[13], [from 6-bromo-2-methoxynaphthalene (7.09 g, 30 mmol), magnesium (1.09 g, 95 mmol) and anhydrous tetrahydrofuran (50 ml)] under nitrogen and cooled to 0°, was added anhydrous zinc bromide (3.37 g, 15 mmol) (prepared by sublimation of hydrated zinc bromide at 300°/0.1 mm Hg) and stirred for 0.5 h. A solution of isophorone (18) (2.06 g, 15 mmol) and $Ni(acac)_2$ (0.1 g) in tetrahydrofuran (20 ml) was added and then the reaction mixture allowed to warm to room temperature and stirred for a further 24 h. The reaction mixture was then poured onto saturated ammonium chloride solution and the product extracted into ether (3 x 50

ml). The combined ether extracts were washed with water (100 ml), brine (2 x 50 ml) and dried over anhydrous sodium sulphate. Evaporation of the solvent and chromatography of the residual oil on flash silica gel eluting with ethyl acetate/light petroleum (1:5) followed by distillation gave 3,5,5-trimethyl-3-(6'-methoxy-2'-naphthyl)-cyclohexane as a slightly yellow gum (3.70 g, 88%) b.p.(Kugelrohr) 250°/0.05 mm Hg. (Found: C, 81.03; H, 8.18; $C_{20}H_{24}O_2$ requires C, 81.01; H, 8.10%). λ_{max} 333.2, 318.8, 271.2, 261.6, 231.2 (ϵ 1853, 1472, 5451, 5833, 14855). ν_{max} 2950, 1705, 1625, 1600, 1475, 1450, 1380, 1270, 1020, 840 cm^{-1}. ^1H n.m.r. (400 MHz) δ 0.36, s, 3H, 5α-Me; 1.04, s, 3H, 5β-Me; 1.41, s, 3H, 3β-Me; 1.95, d, $J_{4\beta,4\alpha}$ 14.2 Hz, H4β; 2.11, ddd, $J_{6\alpha,6\beta}$ 13.8 Hz, $J_{6\alpha,2\alpha}$ 1.8 Hz, $J_{6\alpha,4\alpha}$ 1.8 Hz, H6α; 2.24, d, $J_{6\beta,6\alpha}$ 13.8 Hz, H6β; 2.36, ddd, $J_{4\alpha,4\beta}$ 14.2 Hz, $J_{4\alpha,2\alpha}$ 1.8 Hz, $J_{4\alpha,6\alpha}$ 1.8 Hz, H4α; 2.46, d, $J_{2\beta,\ 2\alpha}$ 14.2 Hz, H2β; 3.18, ddd, $J_{2\alpha,2\beta}$ 14.2 Hz, $J_{2\alpha,4\alpha}$ 1.8 Hz, $J_{2\alpha,6\alpha}$ 1.8 Hz, H2α; 3.89, s, 3H, ArOC$\underline{H}_3$; 7.06-7.76, m, 6H, ArH. Mass spectrum *m/z* 296(79%), 281(49), 239(33), 225(100), 198(61), 183(28),167(25), 155(27), 105(19), 91(33), 83(37), 69(28), 55(37).

Wolff-Kishner Reduction of (37)

A solution of 3,5,5-trimethyl-3-(6'-methoxy-2'-naphthyl)-cyclohexanone (37) (2.96 g, 10 mmol) and hydrazine hydrate (20 ml) in digol (100 ml) was refluxed for 1 h. The solution was cooled, potassium hydroxide (1.0 g) added, distilled until the solution temperature was 190° and then further refluxed for 5 h. The cooled solution was then poured onto water and the product extracted into ether (3 x 100 ml). The combined ether extracts were washed with water (2 x 100 ml), brine (2 x 100 ml), dried over anhydrous sodium sulphate and the solvent removed. The residual oil was chromatographed on flash silica gel eluting with ethyl acetate/light petroleum (1:4). the first compound to be eluted 1,3,3-trimethyl-1-(6'-methoxy-2'-naphthyl)-cyclohexane (21) was then

distilled yielding a slightly yellow gum (1.13 g, 40%) b.p.(Kugelrohr) 210-230°/0.5 mm Hg. (Found: C, 84.36; H, 9.48. $C_{20}H_{26}O$ requires C, 85.1; H, 9.12%). λ_{max} 333.2, 318.8, 271.2, 262.0, 231.6 nm. (ϵ 2098, 1748, 5360, 5593, 82794). ν_{max} 2925, 1600, 1455, 1260, 1010, 820 cm^{-1}. ^1H n.m.r. (400 MHz) δ 0.35, s, 3H, 3α-Me; 0.91, s, 3H, 3β-Me; 1.21, s, 3H, 1β-Me; 1.21-1.83, m, 5H, $(H4)_2$, $(H5)_2$. H6β; 1.44, d, $J_{2\beta,2\alpha}$ 13.6 Hz, H2α; 2.11, bd, $J_{2\alpha,2\beta}$ 13.6 Hz, H2α; 2.38, m, H6α; 3.89, s, 3H, $ArOCH_3$; 7.08-7.73, m, 6H, ArH. Mass spectrum *m/z* 282(100), 267(34), 253(11), 239(5), 211(30), 198(16), 185(34), 171(41), 128(9), 109(14), 69(33).

The second compound to be eluted gave, after recrystallisation from light petroleum 1,3,3-trimethyl-1-(6'-hydroxy-2-naphthyl)-cyclohexane (38) as a slightly pink powder (1.23 g, 46%) m.p.90-95° then 122-123°. (Found: C, 85.08; H, 9.12; $C_{19}H_{24}O$ requires C, 85.07; H, 8.96%0. λ_{max} 334.8, 322.4, 272.4, 262.8, 254.0, 229.2 nm (ϵ 2072, 1808, 5154, 5116, 4062, 97054). ν_{max} 3311 br, 2953, 2927, 1637, 1607, 1465, 1389, 1176, 867 cm^{-1}. ^1H n.m.r. δ 0.38, s, 3H, 3α-CH_3; 0.93, s, 3H, 3β-CH_3; 1.14, -1.89, m, 5H, $(H4)_2$, $(H5)_2$, H6β; 1.20, s, 3H, 1β-CH_3; 1.45, d, $J_{2\beta,2\alpha}$, 13.9 Hz, H2β; 2.11, bd, $J_{2\alpha,2\beta}$ 13.9 Hz, H2α; 2.39, bd, $J_{6\alpha,6\beta}$ 13.2 Hz, H6α; 5.01, bs, ArOH; 7.00-7.76, m, 6H, ArH. Mass spectrum *m/z* 268(85), 253(49), 225(6), 211(12), 197(55), 171(62), 157(70), 144(7), 128(7), 109(17), 69(46).

Preparation of 4-(1',3',3'-Trimethyl-1'-cyclohexyl)-*o*-phthalaldehyde (39)

Method 1. Hydrolysis of 1,3,3-Trimethyl-1-[3',4'-bis(dibromomethyl)-1-phenyl]cyclohexane (42)

A mixture of (42) (1.0 g, 1.8 mmol) and morpholine (10 ml) were heated at 50° for 3 h. The excess morpholine was then removed by distillation under reduced pressure and the residue taken up in hydrochloric acid (10 M, 20 ml) and shaken for 0.5 h. Water (100 ml) was then added and the

product extracted with ether (3 x 50 ml). The combined ether extracts were washed with water (50 ml), saturated sodium bicarbonate solution (2 x 50 ml), brine (2 x 50 ml) and then dried over anhydrous sodium sulphate. Evaporation of the solvent and distillation yielded 4-(1',3',3'-trimethyl-1'-cyclohexyl)-*o*-phthalaldehyde as a clear gum (0.31 g, 86%) b.p.(Kugelrohr) 150°/1 mm Hg. (Found: 258.1615. $C_{17}H_{22}O_2$ requires 258.1620). λ_{max} 266.8 nm (ϵ 3840). ν_{max} 2955, 2930, 1778, 1698, 1596, 1466, 1275, 1198 cm^{-1}. ^1H n.m.r. δ 0.34, s, 3H, 3'α-Me; 0.93, s, 3H, 3'β-Me; 1.10-1.77, m, 5H, $(H4')_2$, $(H5')_2$, H6'β; 1.18, s, 3H, 1'β-Me; 1.49, d, $J_{2'\beta,2'\alpha}$ 14.2 Hz, H2'β; 2.08, bd, $J_{2'\alpha,2'\beta}$ 14.2 Hz, H2'α; 2.31, m, H6'α; 7.78, dd, *J* 8.1; 2.1 Hz, H5; 7.91, d, *J* 8.1 Hz, H6; 8.01, d, *J* 2.1 Hz, H3. Mass spectrum *m/z* 258(100, 243(10), 215(8), 201(2), 189(9), 175(20), 161(20), 146(49), 133(71), 105(49), 91(49), 69(73), 55(44).

Method 2 Oxidation of 1,3,3-Trimethyl-1-[3',4'-bis(hydroxymethyl)-1'-phenyl]cyclohexane (44)

A) Oxidation with tetrakis(pyridine) silver dichromate ($Py_4Ag_2Cr_2O_7$)

Using the general method of Firouzabadi *et al.*,[21] the dialcohol (44) (200 mg, 0.76 mmol) was dissolved in benzene, the oxidising agent (4.56 g, 6.1 mmol) was added and the reaction mixture refluxed for 6 h. The reaction mixture was cooled, filtered through celite and the residue washed with benzene (2 x 10 ml). Evaporation of the solvent and chromatography of the residue on flash silica gel eluting with ethyl acetate/light petroleum (1:9) yielded two products. The first to be eluted was the *o*-phthalaldehyde (39) (86 mg, 44%) as a yellow gum. The second product eluted were the two phthalide isomers (45) and (46) which were inseparable by flash silica chromatography and were determined to be

in a 2:1 ratio respectively from the ^{1}H n.m.r. spectrum.

5-(1',3',3'-Trimethyl-1'-cyclohexyl)-phthalide (45). ^{1}H n.m.r. δ 0.35, s, 3H, 3'α-Me; 0.93, s, 3H, 3'β-Me; 1.18, s, 3H, 1'β-Me; 1.12-1.74, m, 6H; 2.05, m, H2'α; 2.30, m, H6'α; 5.30, s, 2H, $(H3)_2$; 7.49, bs, H4; 7.58, bd, *J* 8 Hz, H6; 7.84, d, *J* 8 Hz, H7.

6-(1',3',3'-Trimethyl-1'-cyclohexyl)-phthalide (46). ^{1}H n.m.r. δ 0.34, s, 3H, 3'α-Me; 0.92, s, 3H, 3'β-Me; 1.16, s, 3H, 1'β-Me; 1.12-1.74, m, 6H; 2.05, m, H2'α; 2.30, m, H6'α; 5.30, s, 2H, $(H3)_2$; 7.42, m, H4; 7.73, dd, *J* 8.2, 2.0 Hz, H5; 7.94, d, *J* 2.0 Hz, H7.

Isomers (45) and (46). (Found: 258.1620. $C_{17}H_{22}O_2$ requires 258.1620). λ_{max} 282, 274, 237, 203 nm (ϵ 1806, 1750, 10320, 33218). ν_{max} 2955, 2930, 1757, 1619, 1466, 1456, 1354, 1050, 1009 cm^{-1}. Mass spectrum *m/z* 258(5%), 243(34), 211(24), 196(28), 175(20), 161(37), 153(6), 145(11), 115(9), 69(12), 41(15).

(B) via Swern Oxidation[22]

To a solution of oxalyl chloride (540 mg, 0.36 ml, 4.2 mmol) in dichloromethane (25 ml) cooled to -30°, was added a solution of dimethylsulphoxide (660 mg, 0.6 ml, 8.4 mmol) in dichloromethane (5 ml) over 2 min. This was followed by a solution of the diol (44) (250 mg, 0.95 mmol) over a 5 min period and then stirred for 0.5 h. Triethylamine (1.94 g, 2.68 ml, 19 mmol) was added and the reaction mixture allowed to warm to room temperature then further stirred for 0.5 h whereupon water (25 ml) was added. The product was then extracted into dichloromethane (3 x 25 ml) and the combined dichloromethane extracts washed with hydrochloric acid (3 M, 2 x 50 ml), sodium bicarbonate solution (5%, 2 x 50 ml) and water (2 x 50 ml). The solvent was evaporated and the residue taken up in benzene which again was evaporated. The residual oil was then

chromatographed on flash silica gel eluting with ethyl acetate/light petroleum (1:9) giving the o-phthalaldehyde (39) (210 mg, 85%).

Method 3. Ozonolysis of naphthyls (21) and (38)

A) Reduction of Ozonide with Dimethyl Sulphide

Using the general method of Pappas *et al.*[17] a solution of the naphthol (38) (200 mg, 0.75 mmol) in methanol (25 ml) and dichloromethane (50 ml) was cooled to -60°. Ozonized oxygen was passed through the solution until a permanent blue colour in the solution remained. The solution was then flushed with nitrogen and dimethyl sulphide (0.17 ml, 2.25 mmol) added. The solution was then stirred at -10° for 1 h, then at 0° for 1 h and finally at room temperature for 1 h. The solvent was then evaporated and the residue partitioned between ether and water. The aqueous extract was further extracted with ether (2 x 20 ml) and the combined ether extracts washed with brine (2 x 20 ml) and dried over sodium sulphate. Evaporation of the solvent followed by chromatography on flash silica gel eluting with ethyl acetate/light petroleum (1:9) afforded the o-phthalaldehyde (39) (110 mg, 58%). Using the above method on the methoxynaphthyl (21) afforded the o-phthalaldehyde in 60% yield.

B) Reduction of Ozonide with Triphenylphosphine

Using the general procedure of Pappas *et al.*.[18] a solution of the naphthol (38) (400 mg, 1.50 mmol) in dichloromethane (50 ml) and methanol (25 ml) was cooled to -60° and treated with ozonised oxygen until the solution turned blue. The solution was then flushed with nitrogen, triphenylphosphine (910 mg, 3.50 mmol) added over 10 min and then allowed to slowly warm to room temperature. The solvent was evaporated and ether (10 ml) added to the residue. The triphenylphosphine oxide was filtered

off and washed with an additional 10 ml of ether. Upon evaporation of the ether the residue was heated with hydrochloric acid (3 M, 15 ml) for 0.5 h then cooled, water (35 ml) added and the product extracted into ether (3 x 50 ml). The combined ether extracts were washed with sodium bicarbonate solution (10%, 2 x 50 ml), dried over sodium sulphate and the solvent evaporated. The residue was then chromatographed on flash silica gel eluting with ethyl acetate/ light petroleum (1:9) affording the o-phthalaldehyde (39) (258 mg, 67%). Using the above method on the methoxynaphthyl (21) afforded the o-phthalaldehyde in 66% yield.

Cis and *trans*-1,3-Diacetoxyphthalan (48) and (49).

(a) o-Phthalaldehyde (47) was prepared from β-naphthol (5 g) by the method of Pappas *et al.*.[18] and purified by distillation giving a crystalline solid m.p.51-52° (lit.[18] 54-55°).

(b) Initial attempts to cyclise and acetylate o-phthalaldehyde to 1,3-diacetoxyphthalan by dissolving o-phthalaldehyde (0.5 g) in acetic anhydride (15 ml) and adding either water (5 drops), hydrochloric acid (3 M, 5 drops) or hydrochloric acid (6 M, 5 drops) met with failure with only starting o-phthalaldehyde being retrieved. When hydrochloric acid (10 M, 5 drops) was added and stirred for 24 h followed by the addition of water (50 ml) and extraction into ether in the usual way gave only the α,α,α',α'-tetraacetoxy-o-xylene was recovered quantitatively. However a solution of o-phthalaldehyde when treated with acetic anhydride and boron trifluoride etherate afforded the desired 1,3-diacetoxyphthalan in high yield.

To a solution of o-phthalaldehyde (0.5 g, 3.73 mmol) in dry ether (20 ml) and contained in a round bottom flask and stoppered by a superseal, was added sequentially acetic anhydride (0.77 g, 7.46 mmol) then boron

trifluoride etherate (0.72 ml) and stirred for 48 h. The resulting mixture was then filtered through celite and the residue washed with ether (35 ml). The filtrate was then washed with water (3 x 50 ml), brine (2 x 50 ml) and dried over anhydous sodium sulphate. Evaporation of the solvent and chromatography of the residue on flash silica gel eluting with ethyl acetate/dichloromethane (1:19) gave the *cis* (48) and *trans* (49) isomers of 1,3-diacetoxyphthalan in a 5:9 ratio (0.57 g, 65%). (Found: 177.0566. $C_{10}H_9O_3$ [M^+ - $OCOCH_3$] requires 177.0552; found: 135.0448. $C_8H_7O_2$ [M^+ - $OCOCH_3$ - C_2H_2O] requires 135.0446). ν_{max} 1751, 1736, 1368, 1231, 960 cm^{-1}. ^1H n.m.r. (*cis* isomer) δ 2.14, s, 6H, 2 x $OCOCH_3$; 7.26, s, 2H, H1 and H3; 7.49-7.52, m, 4H, ArH. ^1H n.m.r. (*trans* isomer) δ 2.12, s, 6H, 2 x $OCOCH_3$; 7.45-7.51, m, 6H, ArH, H1 and H3. Mass spectrum *m/z* 177(58%), 135 (62), 105(13), 77(6), 43(100).

Attempted Acetylation of the *o*-Phthalaldehyde (39)

Using the method described above except using the o-phthalaldehyde (39) (200 mg, 0.78 mmol) yielded after workup and chromatography only one isolated product identified by the ^1H n.m.r. spectrum to be 6-(1',3',3'-trimethyl-cyclohexyl)-phthalide (46). ^1H n.m.r. (90 MHz); δ 0.3, Me; 0.9, Me 1.2, Me; 5.3, s, $(H3)_2$; 7.4, m, H4; 7.7, m, H5; 7.9, bs, H7.

5-Ethyl-2-methoxynaphthalene (53)

To a solution of ethyl magnesium bromide [prepared from ethyl bromide (18.6 g, 171 mmol) and magnesium (4.86 g, 100 mmol) in ether (50 ml)] was added a solution of 6-methoxy-α-tetralone (10 g, 57 mmol) and stirred for 24 h. Hydrochloric acid (3 M, 100 ml) was then added carefully and the

mixture stirred for a further 3 h. The product was then extracted into ether (3 x 100 ml), washed with saturated sodium bicarbonate solution (2 x 100 ml), brine (2 x 100 ml) and the solvent removed. The residual oil was then taken up in toluene (250 ml), chloranil (14.0 g, 57 mmol) added and then refluxed for 24 h. The solvent was then removed and the residual oil chromatographed on flash silica gel eluting with light petroleum and finally distillation (Kugelrohr) 120°/0.5 mm Hg afforded 5-ethyl-2-methoxynaphthalene as a white solid (7.10 g, 67%). ^{1}H n.m.r. δ 1.31, t, 3H, *J* 7.4 Hz, $ArCH_2\underline{CH_3}$; 3.01, q, 2H, *J* 7.4 Hz, $Ar\underline{CH_2}CH_3$; 3.85, s, 3H, $ArOCH_3$; 7.03-7.95, m, 6H, ArH.

α-Cyano-5-ethyl-2-methoxynaphthalene (55)

To a solution of 5-ethyl-2-methoxynaphthalene (200 mg, 1.1 mmol) in carbon tetrachloride (15 ml) was added *N*-bromosuccinimide (210 mg, 1.2 mmol) and benzoyl peroxide (0.1 g). The reaction mixture was then illuminated and refluxed with a 500 W light globe until all the solid suspension floated on the surface. The reaction mixture was then cooled to 0° and filtered through celite, the residue being further washed with carbon tetrachloride (2 x 10 ml) and the solvent removed. The residual oil was then taken up in tetrahydrofuran (20 ml) and lithium cyanide [prepared by the method of Livinghouse[24]] (110 mg, 3.3 mmol) was added and the solution refluxed for 6 h. Water (50 ml) was added and the product extracted into ether (3 x 50 ml), the combined ether extracts washed with brine (2 x 50 ml), dried over anhydrous sodium sulphate and the solvent evaporated. Chromatography of the residual oil on flash silica gel eluting with light petroleum/dichloromethane (3:7) followed by distillation yielded α-cyano-5-ethyl-2-methoxynaphthalene as an orange gum (200 mg, 91%) b.p.(Kugelrohr) 210°/1 mm Hg. (Found: C, 79.60; H, 6.19; N, 6.57. $C_{14}H_{13}NO$ requires C, 79.62; H, 6.16; N, 6.64 %). λ_{max} 329.6, 315.6,

285.2, 274.8, 265.6, 230.0 nm (ϵ 2236, 1758, 4266, 5368, 4530, 66817). ν_{max} 3110, 2940, 2245, 1628, 1604, 1516, 1437, 1375, 1260, 1231, 1175, 1038, 850 cm^{-1}. ^1H n.m.r. δ 1.63, d, 3H, *J* 7.5 Hz, $CH_3CH(CN)Ar$; 3.81, s, 3H, OCH_3; 4.41, q, 2H, *J* 7.5 Hz, $CH_3CH(CN)Ar$; 7.05–7.75, m, 6H, ArH. Mass spectrum *m/z* 211(88%), 196(100), 182(35), 153(73), 141(40), 126(39), 115(38), 98(18), 63(29).

2-(6'-Methoxy-1'-naphthyl)-propionic Acid (56)

An ethanolic solution of the nitrile (55)(0.5 g, 2.4 mmol) and potassium hydroxide solution (10 M, 20 ml) was heated to reflux for 5 h then acidified with hydrochloric acid (3 M) to pH 1 and extracted with ethyl acetate (3 x 50 ml). The combined ethyl acetate extracts were then extracted with saturated sodium bicarbonate solution (3 x 20 ml) which was then acidified with hydrochloric acid (3 M) and then reextracted with ethyl acetate (3 x 20 ml). The combined ethyl acetate extracts were then washed with brine (3 x 20 ml) and dried over anhydrous sodium sulphate. The solvent was removed to give 2-(6'-methoxy-1-naphthyl)-propionic acid (0.46 g, 83%). ν_{max} 3500–2500 br, 1709, 1627, 1515, 1436, 1255, 1231, 1039, 850 cm^{-1}. ^1H n.m.r. δ 1.64, d, 3H, *J* 7.4 Hz, $CH_3CH(COOH)Ar$; 3.91, s, 3H, OCH_3; 4.47, q, *J* 7.4 Hz, $CH_3CH(Ar)COOH$; 7.12–7.71, m, 5H, ArH; 7.98, d, *J* 14 Hz, ArH.

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