SYNTHESIS OF KAURENOIDS FROM GIBBERELLINS

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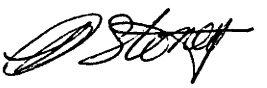
Declaration

The work described herein is the authors own work, unless otherwise stated, and was carried out in the Research School of Chemistry, the Australian National University, under the supervision of Professor L. N. Mander. None of the material has been submitted in any part or form for a degree at this or any other university.



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Abstract

The objectives of this thesis have been focussed in two main areas. The first involved the isotopic labelling of gibberellins (GA's) at the C(17) position to enable metabolic tracking, isotope dilution assays and confirmation of tentative structural assignments. A number of GA's were labelled: these included the tricarboxylic acids GA_{13} and GA_{25}, which were labelled with deuterium at the C(17) position by cleaving the 16,17-methylene by oxidation to the norketone, and incorporating a deuterated methylene unit via the Lombardo-Oshima reaction; GA_{20} was also labelled at the C(17) position, but with a ^{14}C-radio isotope via Wittig methylenation. This compound was then utilised as a model for the major objective which involved the 17-^{14}C-radio isotope labelling of GA_{95}, a recently discovered gibberellin isolated from *Prunus cerasus* (flowering cherry). This necessitated many functional group manipulations to give the correct skeletal requirements. It became imperative to perform the skeletal changes in a particular sequence, in order to preserve the very labile Δ^1-ene, which is highly prone to undergo rearrangement to the isolactone in an acidic environment.

The second area of research dealt with the expansion of the B-ring in gibberellins to give access *inter alia* to the *Rabdosia* family of kaurenes. Kaurenes are a complex group of tetracyclic diterpenoids which have shown interesting therapeutic potential, such as antibacterial and antitumour activity. Their complexity makes them an attractive target for synthesis due to the dense array of functionality and multiple stereogenic centres.

A study was therefore undertaken to explore the prospect of utilising gibberellins as a source of semi-synthetic kaurenes, drawing on the experience already gained in the manipulation of gibberellins. This was achieved by the Lewis acid-catalysed rearrangement of a 6β-hydroxy-gibberellin-7α-carboxaldehyde derived (**a**) from GA_3, resulting in acyloin rearrangement (ring expansion) to give the 6-membered B-ring of an *ent*-20-norkaurene derivative, and (**b**) from GA_{15} to afford an advanced intermediate for the preparation of the *Rabdosia* family of kaurenoids.

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CHAPTER 1

INTRODUCTION TO GIBBERELLINS AND KAURENOIDS

1.1 The Discovery of Gibberellins

1.1.1 History

Gibberellins were originally discovered in 1926[1] in Japan, when a pathologist, Kurosawa was investigating the 'bakanae' disease of rice seedling plants. The disease, which was reported in the early part of the 19th Century[2], caused infected plants to grow abnormally tall and spindly. Many of the mature plants died before reaching the stage of fruiting, resulting in seriously reduced crop yields (up to 40%) throughout Japan and the Asian area. This disease was shown to be caused by an infection from the fungus *Fusarium moniliforme* . In 1912, Sawada demonstrated that a substance produced by *Gibberella fujikuroi*, the 'perfect', or sexual stage of the fungus *Fusarium moniliforme* caused the infection[3]. When this fungus was cultured on a synthetic medium, the filtrate, when applied to healthy plants, caused the same effects of bakanae disease as those observed in infected plants[1]. Some years later, in 1938, the Japanese agricultural chemists, Yabuta and Sumiki[4] succeeded in isolating from the culture filtrate a crystalline fraction with the same biological activity as that of the fungus. Work was disrupted during World War II, but in 1954, an ICI group[5] reported the isolation of an active principle from the fermentation of *G. fujikuroi* which proved to have plant growth-promoting activity. They named this substance gibberellic acid, which was later given the trivial name of GA_3 (Figure 1). In 1958, MacMillan and Suter[6] reported the isolation of a gibberellin (GA_1) from immature seeds of scarlet runner bean *Phaseolus multifloris*, and it is now established that Gibberellins control many aspects of plant growth and development. They have been shown to be widely distributed throughout the plant Kingdom, with over 120 naturally occurring gibberellins now known.

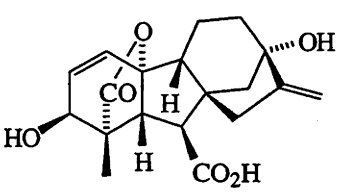


Figure 1. Structure of GA_3.

1.1.2 Structure and Nomenclature

Gibberellins are a group of highly functionalised tetracyclic diterpenoids arranged in the form of four fused carbocyclic rings which are designated as A, B, C and D (Figure 2). The nomenclature of the gibberellins throughout the experimental section is based on the *ent*-gibberellane carbon skeleton (a)[7] (Figure 2), which is related to ent-kaurene (b) and the steroids.

Figure 2. (a) Numbering system used for Gibberellins based on *ent*-gibberellane, (b) numbering system used for kaurenes.

Once the structure of a new gibberellin is established, it is allocated a number in the series, GA_1 to GA_n[8].

Gibberellins are divided into two subgroups. The 19-carbon atom gibberellins form the majority of gibberellins (approximately two thirds) and possess a 19,10-γ-lactone bridge, of which GA_9 may be regarded as the parent compound (Figure 3). Differences within the group are mainly accounted for by the location and number of hydroxy groups, and additional double bonds. Those gibberellins possessing the full 20-carbon skeleton of the diterpenoids are *ent*-gibberellanes, of which GA_{12} may be regarded as the parent compound (Figure 3). The carbon at position 20 exists in a number of oxidation levels, ranging from methyl through to carboxyl. Further variations arise through the addition of one or more hydroxyl groups to various positions on the gibberellin skeleton.

Figure 3.

1.1.3 The Value of Gibberellin Research

For socio-economic reasons, it has become desirable to control some aspects of plant growth and development. This has been achieved to some extent, by the bulk use of fertilisers, pesticides and herbicides. However, environmental concerns now discourage the use of such chemicals. Since GA's are recognised as one of the most important classes of plant bioregulators, playing a central role in plant growth and development, their use may have many potential commercial applications[9-12]. With the availability of reasonable quantities of some gibberellins from the fermentation of the fungus *Gibberella fujikuroi*, plant hormone research has escalated, bringing forth an understanding of the intricate action of these bioregulators. However, as yet commercial applications have been limited, mainly due to cost. Those applications that have been commercially successful include inducement of flowering in ornamental plants; reduced time required for malting in the brewing of beer; increased berry size of seedless grapes; increased storage life of fruit; and control of russet, a scablike skin disorder in apples[13]. Other useful applications have been to change the sex expression of plants, increase vegetative growth, and induce sprouting. Gibberellins can induce flowering in "long day" plants (i.e. flower buds are induced by a photoperiod of a minimum duration)[14,15], and also in plants that require a low temperature (vernalisation) for flower induction, when these conditions are not operating. Seeds usually have a dormant period either due to a hard seed coat which must first be removed, or the seed embryo is not fully developed at the time of shedding. There may also be the presence of germination inhibitors. This dormant period for some species may be broken by exposure to red light, dark, or low temperature. Gibberellins can replace the red light/dark requirement or the low temperature requirement (vernalisation) and terminate dormancy[16]. In the case of fruiting plants, once pollination and fertilisation have occurred the ovary develops into a fruit containing seeds. However, some plants can develop a seedless fruit from an ovary that has not been fertilised (parthenocarpy). Gibberellins, auxin, and cytokinin can cause parthenocarpy to occur, and have been used commercially on tomatoes, cucumbers, peachs, apples, pears and grapes[17].

Some commercial success has also been obtained by suppressing the production of gibberellins by GA antagonists or inhibitors, to enhance cultivated crop yields. With increased knowledge of these compounds comes greater understanding of their biosynthesis, bioactivity and metabolism. This leads naturally to the design of synthetic gibberellins which would possess particular properties, or the production of inhibitors of GA biosynthesis. Therefore, it would seem that we are possibly at the dawn of their large scale use throughout agriculture.

1.2 *Structure / Activity Relationships*

Although shoot elongation in intact plants, caused by cell elongation and cell division, is the most prominent physiological effect of Gibberellins, GA's play a role in almost every aspect of plant growth and development. The relationship between structure and biological activity is extremely important, and many inroads into this area have been made.

It has been observed that the most biologically active gibberellins possess a free 7-carboxyl group, a 19,10-γ-lactone bridge and a 3β-hydroxy function with or without a 1,2-double bond[18-20]. Examples of GA's exhibiting these features are GA_1, GA_3, GA_4, GA_7, GA_{30}, and GA_{32}#. In general, the C_{20} gibberellins show lower bioactivity than their C_{19} counterparts[21], activity probably being due to metabolic conversion into C_{19}-GA's. GA's such as GA_8, GA_{29}, GA_{34} and GA_{30}, all of which possess a 2β-hydroxy group have very low bioactivity, while GA's possessing additional carboxyl groups at C-4 and C-10 (tricarboxylic acids) similarly show extremely low biological activity. In fact, the GA's of this class (ie. GA_{13}, GA_{17}, GA_{25}, GA_{28}, GA_{39}), are thought to be artefacts or end products of biosynthesis. Also, the presence or absence of a 13-hydroxy has an effect on bioactivity and has been found to be species dependent.

1.3 *Biosynthetic Pathways of Gibberellins*

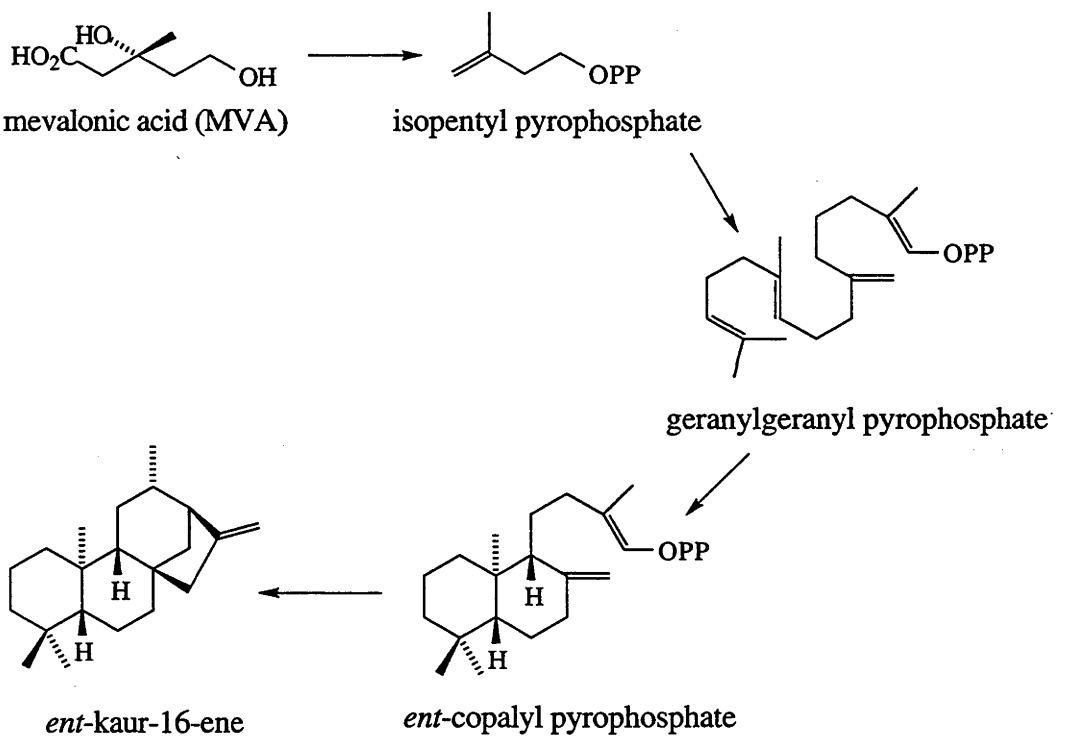
1.3.1 Fungal GA's

The biosynthesis of GA's in the fungus, *Gibberella fujikuroi* was first studied by Birch *et al*[22], in the late 1950's. Feeding 2-^{14}C-acetic acid and 2-^{14}C-mevalonic acid (MVA) to the culture media, revealed that both labelled carbons were incorporated into GA_3. It has since been established that the GA biosynthesis in the fungus *G. fujikuroi* consists of three stages: (A) the conversion of mevalonic acid into *ent*-kaur-16-ene; (B) the conversion of *ent*-kaur-16-ene into GA_{12}-7-aldehyde; and (C) the conversion of GA_{12}-7-aldehyde into C_{20} and C_{19} GA's with hydroxylation at various carbon atoms[23-26].

See Appendix I for GA structures.

1.3.1.1 Stage A: Mevalonic Acid to ent-*kaur-16-ene*

Schecter and West[27], during their studies, identified the intermediates between MVA and *ent*-kaur-16-ene by preparing a soluble enzyme system from a cell-free extract of mycelia from *G. fujikuroi*. By incubating with 2-^{14}C-mevalonate (MVA), adenosine triphosphate (ATP) and $MgCl_2$, they effected the conversion of 2-^{14}C-MVA into *ent*-kaur-16-ene. Copalol and geranylgeraniol were also isolated, but are not intermediates, as their resuspension into a *G. fujikuroi* culture did not yield GA's. Both *trans*-geranylgeranyl pyrophosphate and *ent*-copalyl pyrophosphate, (Scheme 1) were converted successfully by this enzyme system into *ent*-kaur-16-ene. Many studies on higher plants have also shown the above view to be correct[28]. There has been evidence for the intermediacy of mevalonic acid-5-phosphate, mevalonic acid-5-pyrophosphate and isopentyl pyrophosphate (Oster and West, 1968[29]).



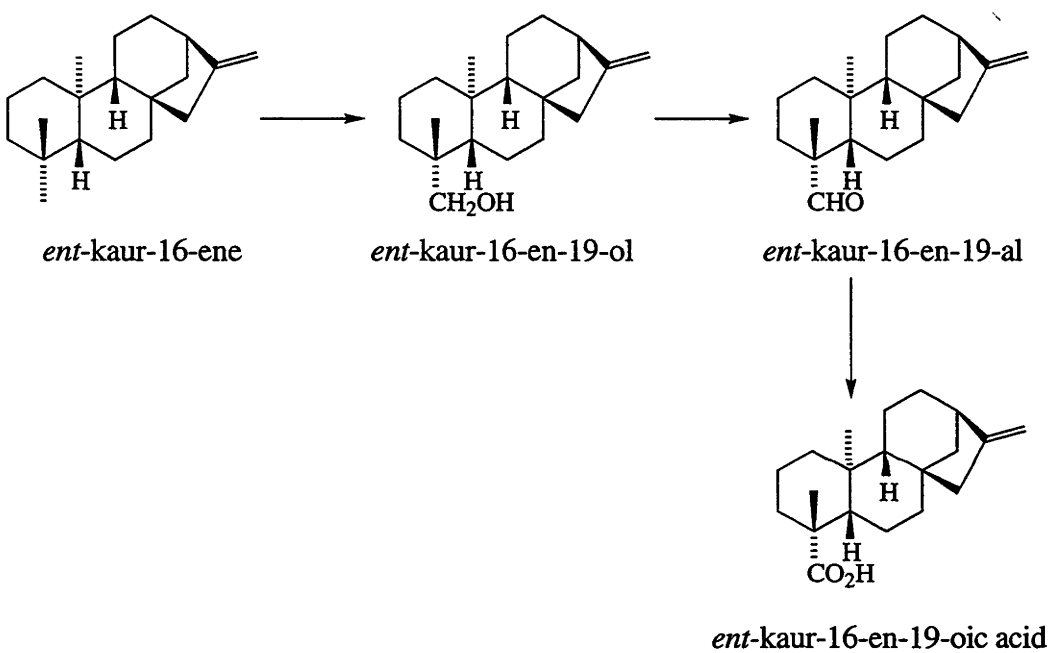
Scheme 1. Conversion of MVA into ent-kaur-16-ene.

1.3.1.2 Stage B: ent-*Kaur-16-ene to* GA_{12}*-7-aldehyde*

There are two processes at this stage, the first being the sequential oxidation of C(19) from *ent*-kaur-16-ene to *ent*-kaur-16-en-19-oic acid, and the introduction of a 7β-hydroxyl into the *ent*-kaurene skeleton. The second stage is the contraction of the

six-membered B ring of *ent*-kaurene to a five membered ring to form the gibberellin skeleton.

The conversion of *ent*-kaur-16-ene[25], *ent*-kaur-16-en-19-ol[28,30,31], *ent*-kaur-16-en-19-al[32], *ent*-kaur-16-en-19-oic acid[33] and *ent*-7α-hydroxykaur-16-en-19-oic acid[34,35] into GA_3 (Scheme 2), was shown by feeding these ^{14}C-labelled substrates to the culture media of *G. fujikuroi*. The products were identified by combinations of radio-thin layer chromatography (RTLC), gas chromatography-radio chromatography (GC-RC), and isotope dilution.

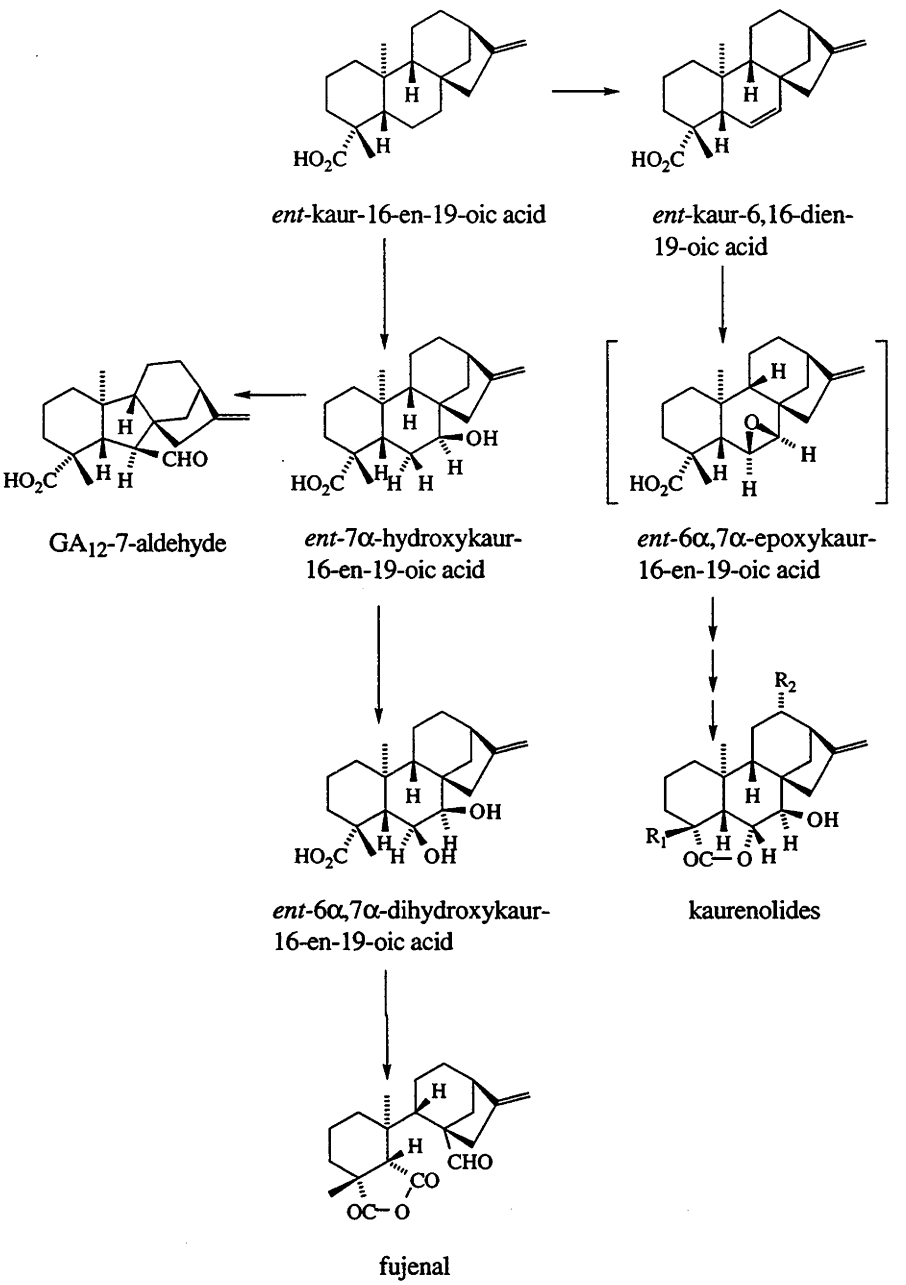


ent-kaur-16-ene *ent*-kaur-16-en-19-ol *ent*-kaur-16-en-19-al

ent-kaur-16-en-19-oic acid

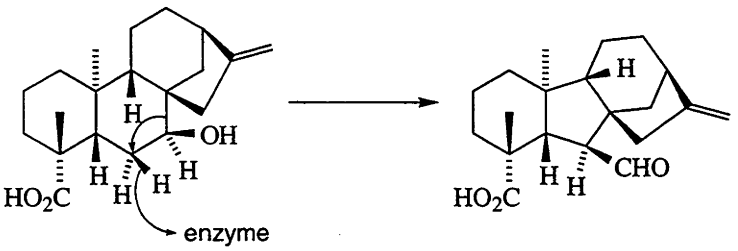
Scheme 2. Biosynthesis of *ent*-kaur-16-en-19-oic acid from *ent*-kaur-16-ene.

A branching in the pathway occurs during the oxidation of the B ring. This branching determines whether GA's or other metabolites, such as kaurenolides and fujenal are biosynthesised. It was shown that *ent*-kaur-16-en-19-oic acid can be converted to *ent*-7α-hydroxykaur-16-en-19-oic acid or *ent*-kaur-6,16-dien-19-oic acid (Scheme 3)[36]. It is presumed that the latter is converted to the *ent*-6α,7α-epoxide intermediate, and further modified to the kaurenolides. *ent*-7α-Hydroxykaur-16-en-19-oic acid is converted into GA_{12}-aldehyde, and also *ent*-6α,7α-dihydroxykaur-16-en-19-oic acid, by microsomes from *G. fujikuroi*[37]. The dihydroxy acid is not a precursor to GA's but is converted to fujenal.



Scheme 3.

The ring contraction step has received considerable attention[38-40], using microsomal enzyme preparations from higher plant systems and cultures of *G. fujikuroi* with isotopically labelled substrates. The results show that the formation of GA_{12}-aldehyde occurs with the loss of the *ent*-6α and 7α-hydrogens, with retention of the *ent*-6β-hydrogen. The ring contraction involves the abstraction of the *ent*-6α-H followed by the migration of C(8) to C(6) with the extrusion of C(7), affording the GA_{12}-7-aldehyde (Scheme 4).

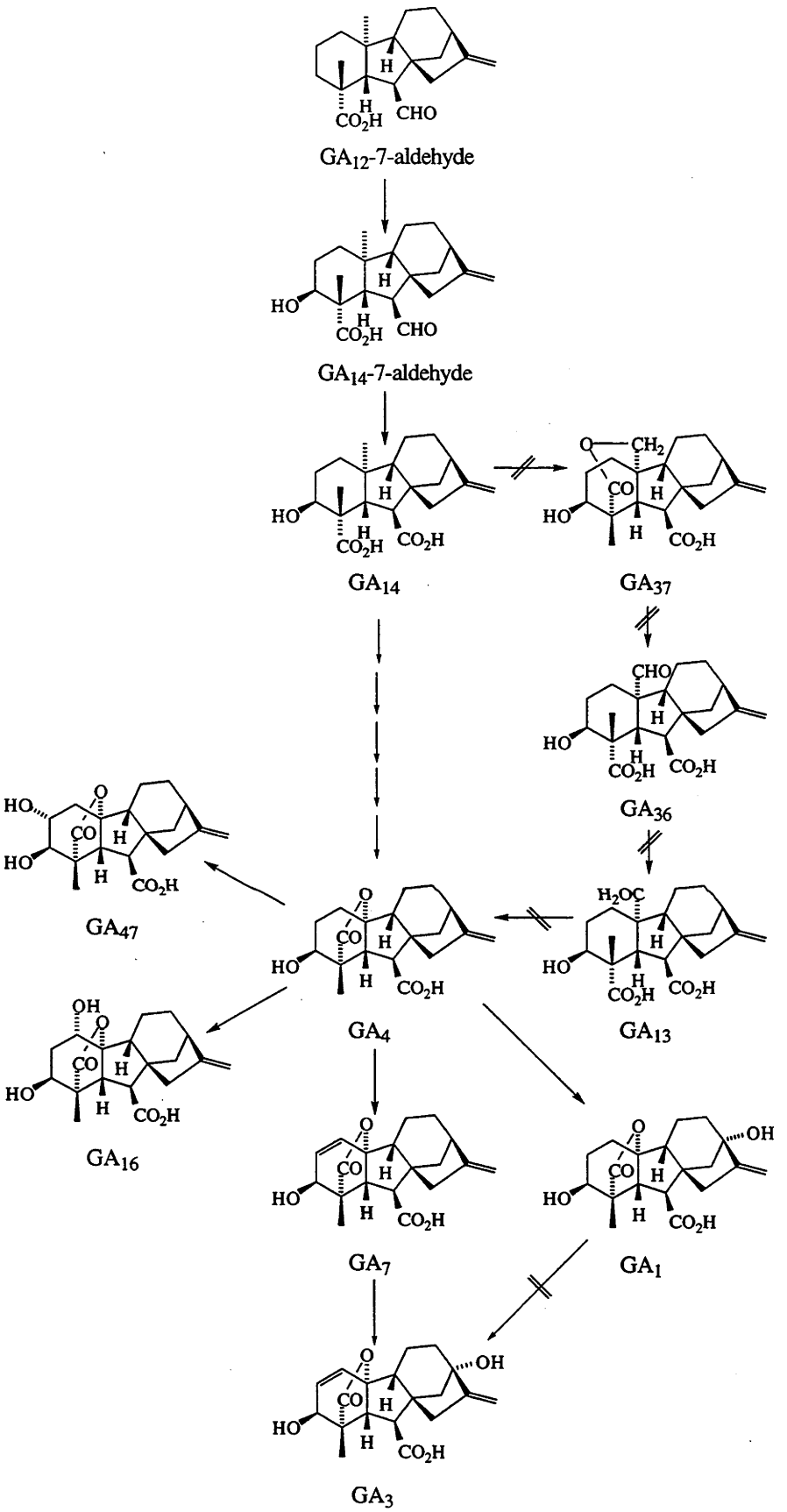


Scheme 4. Formation of GA_{12}-aldehyde through B-ring contraction.

1.3.1.3 Stage C: GA_{12}-7-aldehyde to C_{20}- and C_{19}-GA's

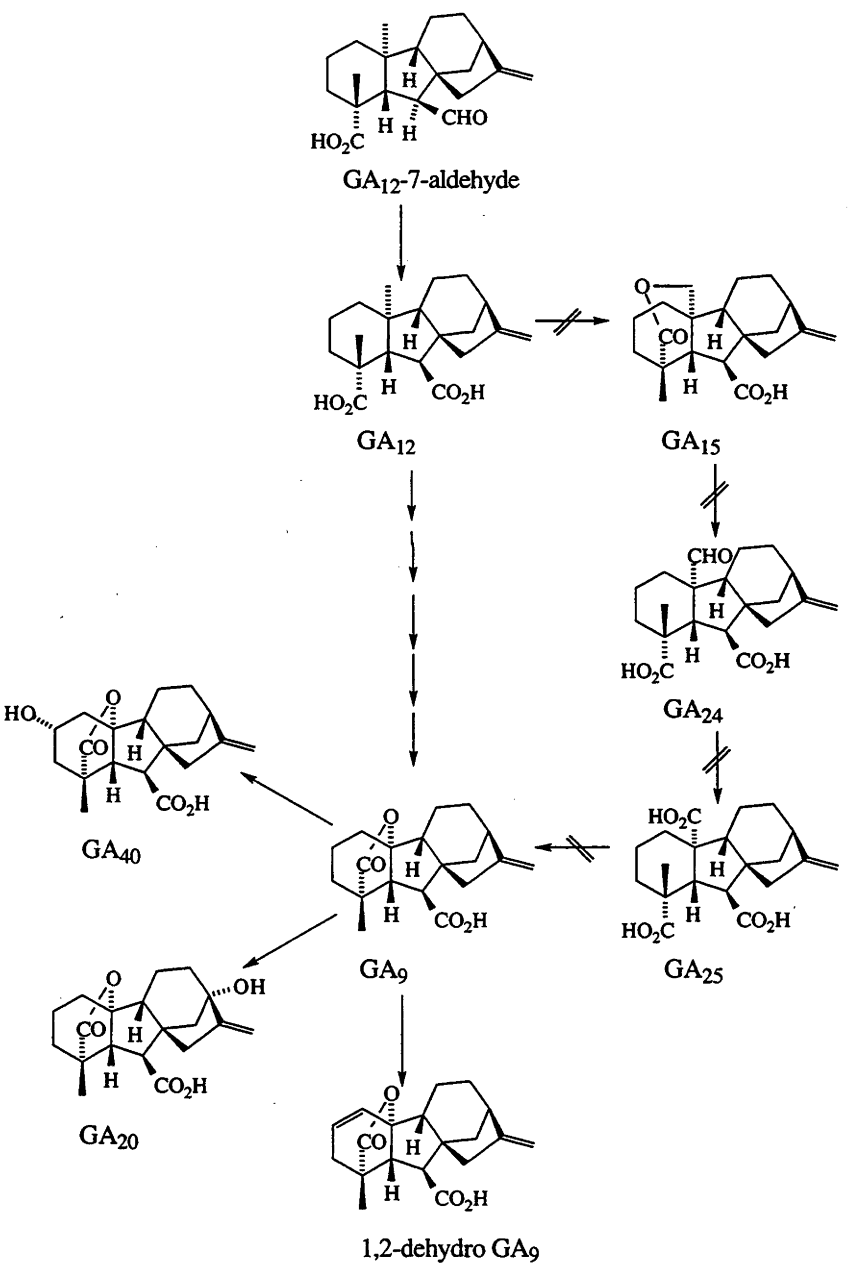
This stage also involves two processes: the loss of C(20) during the formation of C_{19}-GA's from C_{20}-GA's; and the introduction of hydroxyl(s). The detailed mechanism for the loss of the C(20) is unknown, but the variation in substituent at C(10) (ie, methyl through to carboxyl) show a stepwise oxidation with the eventual release of CO_2. However, experiments to elucidate the fine detail still remain inconclusive[41,42].

Through the use of different wild type strains of *G. fujikuroi* and from single gene mutants blocked for GA biosynthesis, a number of routes have been established for the pathway from GA_{12}-7-aldehyde. The most comprehensive of these comes from feeding studies with the mutant strain, B1-41a[43], which arrests endogenous GA biosynthesis by 97.5% at the *ent*-kaur-16-en-19-al to *ent*-kaur-16-en-19-oic acid stage. The major pathway in the fungus involves the early 3β-hydroxylation of GA_{12}-7-aldehyde to GA_{14}, via the aldehyde, (Scheme 5). C(20) is then lost to give GA_4, although the steps involved have not yet been identified. GA_{37}, GA_{36} and GA_{13} have been isolated as possible intermediates[41], but none are formed from GA_{14}, nor metabolised to GA_4 in cultures of the mutant B1-41a. GA_4 can then undergo 1,2-dehydrogenation to GA_7, 1α-hydroxylation to GA_{16}[41,44], or 2α-hydroxylation to GA_{47}[45]. This is in direct contrast with the 1β and 2β-hydroxylations found in higher plants. GA_3 is then obtained from the 13-hydroxylation of GA_7. It was also shown during the study that GA_1 was not converted to GA_3[44].



Scheme 5. Early 3β-hydroxylation pathway in *G. fujikuroi* using mutant B1-41a.

The early 3β-hydroxylation pathway is accompanied by a minor non-early 3β-hydroxylation pathway (Scheme 6). GA_{12}-7-aldehyde is converted to GA_9 via GA_{12}[41] in a similar fashion to that by which GA_{14} is converted to GA_4. The 1,2-dehydrogenation of GA_9 gives Δ^1-GA_9, parallel to the metabolism of GA_4 to GA_7 in cultures of the mutant, B1-41a[46], but late 3β-hydroxylation of GA_9 to GA_4 does not occur. GA_9 does, however, undergo 2α-hydroxylation to give GA_{40}, or 13-hydroxylation to give GA_{20}.



Scheme 6. Non-early 3β,13-hydroxylation pathway in *G. fujikuroi* using mutant B1-41a.

The biologically inactive tricarboxylic acids have been found to be artefacts of GA biosynthesis in the fungus *G. fujikuroi*, and as such, accumulate as waste products or are catabolised[47,48].

1.3.2 Higher Plants

1.3.2.1 Stage A and Stage B

The biosynthetic pathway from MVA to GA_{12}-7-aldehyde is the same for both *G. fujikuroi* and higher plants. Information on the intermediates in the pathway was obtained from higher plant sources such as the endosperm-nucellus of immature seeds, cell free preparations of young seeds, young fruit, young seedlings and shoot tips from different species including *Marah macrocarpus*[28], *Cucurbita maxima*[49], *Zea mays*[50], *Hordeum vulgare*[51], *Pisum sativum*[52], *Phaseolus coccineus*[53], and many more.

However, the pathway from GA_{12}-aldehyde depends upon the organism in question, and so GA_{12}-aldehyde is more or less at a branch point from which many systems have been studied and different pathways observed. A brief outline of the major pathways follows.

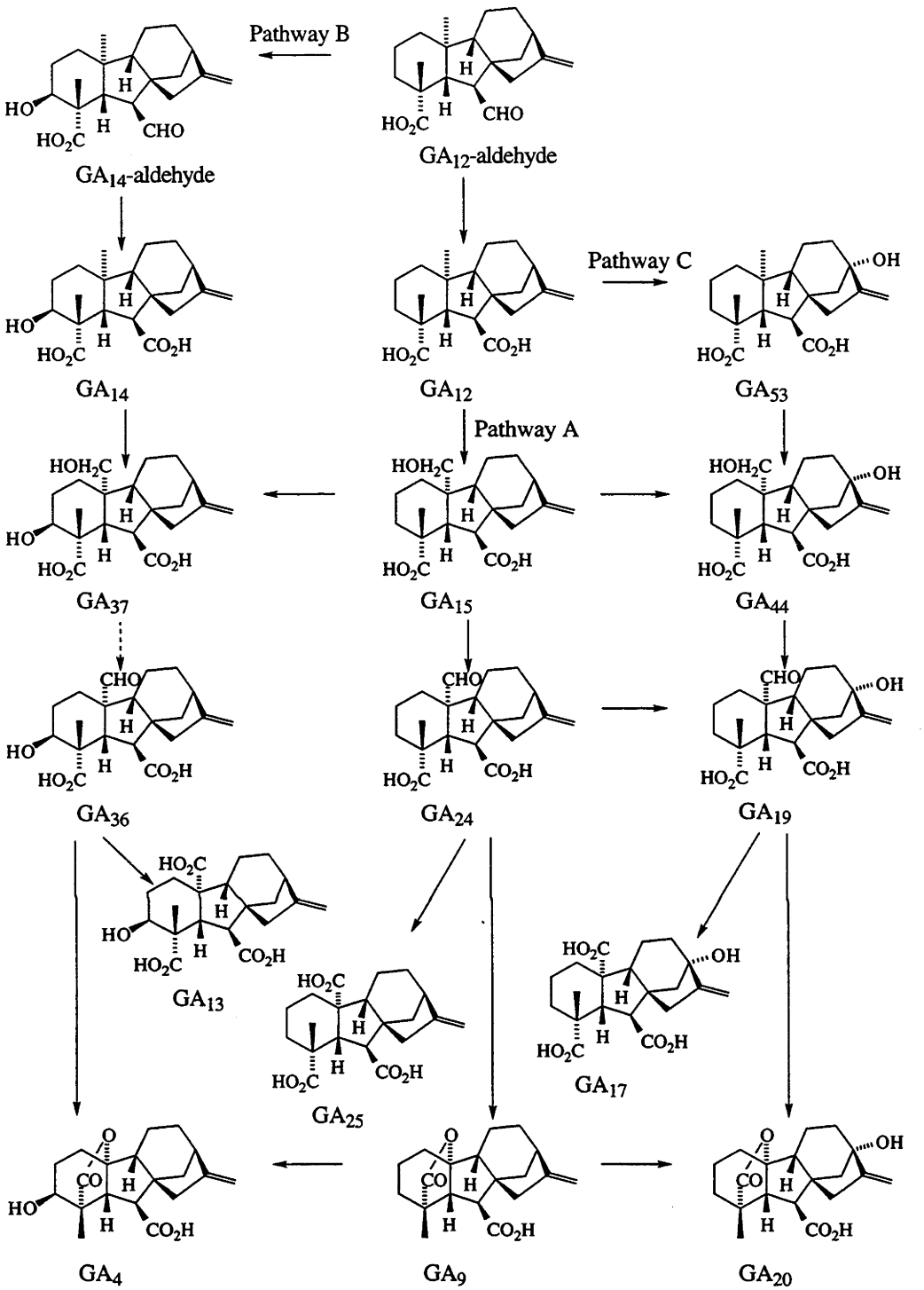
1.3.2.2 Stage C

There is a common sequence for the conversion of C_{20} GA's to C_{19} GA's. This involves the progressive oxidation of C(20) to the 20-hydroxy derivative (isolated as the δ-lactone), followed by oxidation to the 20-aldehyde, and finally oxidation to the γ-lactone, with loss of C(20) as CO_2. The progressive oxidation of C(20) may occur before or after 3β-hydroxylation and 13-hydroxylation of GA_{12}-aldehyde and GA_{12}, giving rise to three main pathways to C_{19} GA's: (i) non early 3β,13-hydroxylation; (ii) early 13-hydroxylation and (iii) early 3β-hydroxylation. These pathways were identified by making use of *in vitro* and *in vivo* studies of higher plants. *In vitro* studies were carried out using extracts of seeds containing relatively high concentrations of GA's. Isolating and studying the enzymes from seed homogenates also made it possible to establish specific steps in the pathways. Much of the information obtained from *in vivo* metabolism studies has been gathered using the shoots of *Z. mays*[54]. It has been observed that most of the endogenous GA's are formed via the early 13-hydroxylation pathway indicated by the *in vitro* studies.

The non-early 3β,13-hydroxylation pathway from GA_{12}-aldehyde to GA_9 may be seen in Scheme 7, (Pathway A). Oxidation of GA_{12} occurs at C(20) to give the 20-alcohol, isolated as the δ-lactone, GA_{15}. GA_{15} is further oxidised at C(20) as the opened lactone during *in vitro* studies[55,56] to form the aldehyde, GA_{24}, or as the δ-lactone during *in vivo* studies[57]. GA_{24} is then converted to GA_9 with the loss of C(20) as CO_2. 3β-Hydroxylation of GA_9 to GA_4, and 13-hydroxylation of GA_9 to GA_{20} have been shown to occur *in vivo* only.

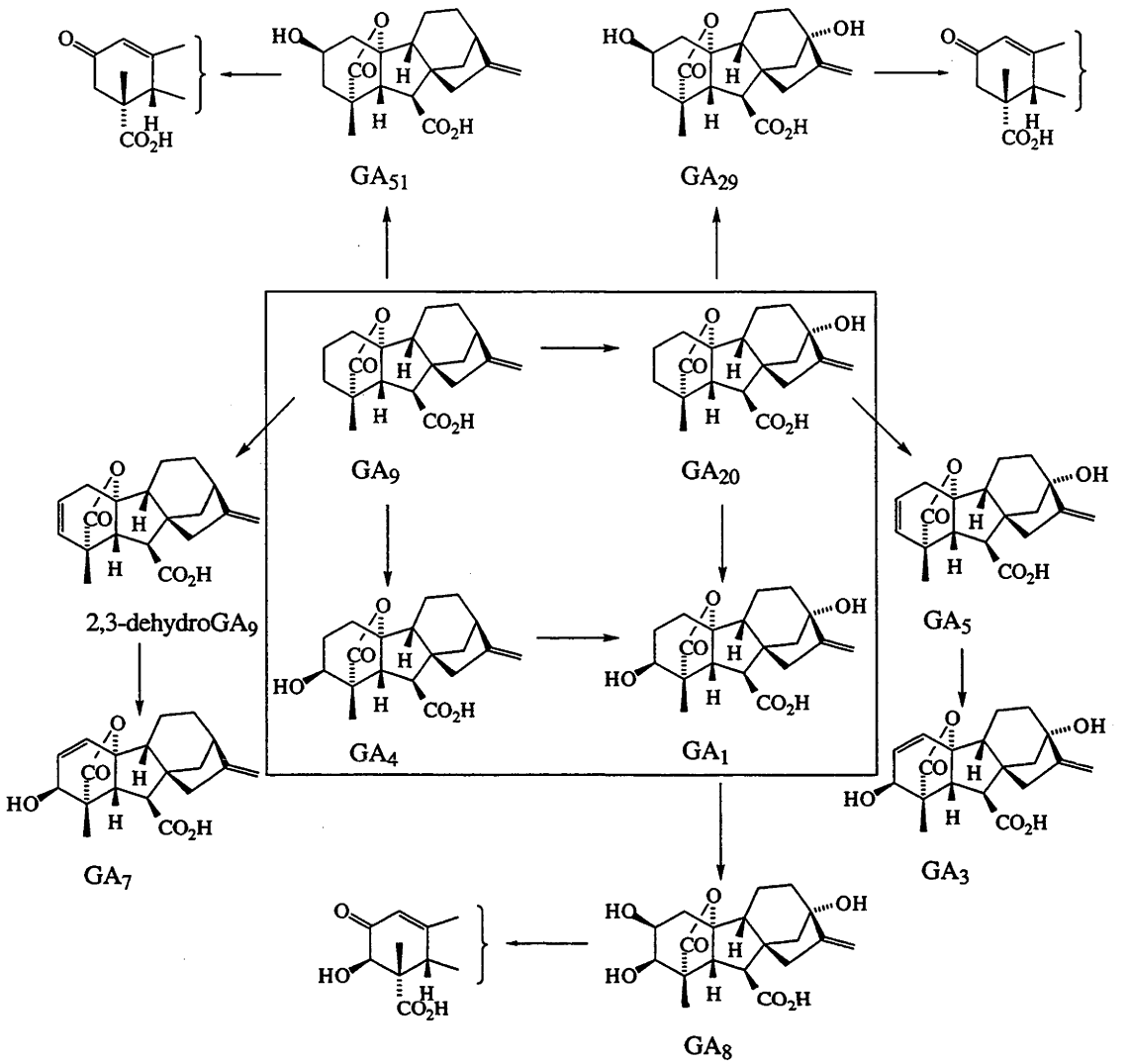
The early 3β-hydroxylation pathway from GA_{12}-aldehyde to GA_4 was established using *C. maxima*[58-60] for studying all but one of the *in vitro* steps (Scheme 7, Pathway B). 3β-Hydroxylation occurs before C(20) oxidation to form GA_{14}, via the aldehyde. This is then followed by the progressive oxidation of C(20), until it is lost as CO_2 to give GA_4. It has not been concluded how GA_{36} is formed from GA_{37}, but that it may involve the opened lactone is a possibility[59]. 13-Hydroxylation of GA_4 occurs to give GA_1, only during *in vivo* studies (Scheme 8).

For the early 13-hydroxylation pathway to GA_{20} it has been observed that GA_{53} is not formed from GA_{53}-aldehyde, but from GA_{12}-aldehyde via GA_{12} (Scheme 7, Pathway C). The system used to establish the steps from GA_{12}-aldehyde to GA_{19} was the germinating grain of *H. vulgare in vitro*[57]. The conversion of GA_{19} to GA_{20} is only assumed due to the metabolism of GA_{44} to GA_{20} as well as GA_{19}. Again, GA_{19} is formed from the opened lactone of GA_{44} *in vitro*, and from the δ-lactone *in vivo*. GA_{20} can be further metabolised to give GA_1, and may also undergo 2,3-dehydrogenation to give GA_5[58,59]. GA_9 gives 2,3-dehydro-GA_9[61] with some *in vitro* and *in vivo* systems (Scheme 8) but has not yet been isolated as a naturally occurring GA. GA_5 is then converted to GA_3 by the unique *ene*-hydroxylation rearrangement reaction[62,63].



Scheme 7. Biosynthetic Steps established from GA_{12}-aldehyde to C_{19}-GA's for Higher Plants.

The C(20)-aldehydes are the immediate precursors of the C_{19}-GA's but can also be oxidised to the inactive C_{20}-GA tricarboxylic acids such as GA_{25} from GA_{24}[59], GA_{13} from GA_{36}[59], and GA_{17} from GA_{19}[64] (Scheme 7).



Scheme 8. Further Metabolism of C_{19}-GA's.

After further hydroxylation of the gibberellin skeleton (recorded at the 1β, 2α, 3β, 12α and 18 positions) and the introduction of olefinic bonds, there are two sequences that lead to gibberellin inactivity. The first is 2β-hydroxylation which has been observed in both *in vitro* and *in vivo* systems[62]. This is generally the last step in the biosynthetic sequence and may lead to degradation via catabolism to give GA_{29}, GA_{51}, and GA_8 and their 2-oxo-catabolites (Scheme 8). There are also other catabolites, such as the 16β,17-dihydrodiols which are probably the products of non-specific oxidation of the 16,17-double bond[65,66].

Conjugation, as glucosyl esters or glucosyl ethers[67], is the second method of removing GA activity. It is thought that these GA conjugates may be storage forms of GA's, and that enzymatic hydrolysis should release the active GA moiety when it is required[67]. However, this hypothesis has not yet been proven.

1.3.3 Conclusion

By knowing the various pathways of Gibberellin biosynthesis, and the enzymes involved in transformations, a better understanding of plant growth regulation is obtained. Since certain GA's trigger different plant growth responses, the relationship between GA structure and their bioactivity is an area of research interest, *per se*, while underpinning the development of commercial applications.

1.4 Synthesis

1.4.1 Identification and Characterisation

In order to study the biosynthesis, structure, and the role gibberellins play in plant growth and development, many techniques of analysis have been adopted. If enough material is available then full structural analysis is carried out, including nuclear magnetic resonance (NMR) spectroscopy and single crystal X-ray diffraction. Unfortunately, gibberellins are usually only available in very small quantities (micrograms to nanograms), often as a complex mixture of a number of GA's. The isolation of pure material is very laborious and the quantities of material obtained are frequently extremely low. It therefore, becomes less feasible to get full spectroscopic data. However, it is possible to glean information as to the molecular structure of a gibberellin based on its chromatographic behaviour and its mass spectrometric (MS) fragmentation pattern by comparison with the fragmentation patterns of fully characterised GA's. The proposed gibberellin structure can then be synthesised from the more readily available fungal GA's. In some circumstances the molecule may be isotopically labelled in order to study its metabolic pathway.

1.4.2 Synthesis of Gibberellins using Fungal Cultures

Fungal cultures can produce a wide range of gibberellins in reasonable quantities. The rarer GA's can be obtained by making use of the fact that the enzymes of the fungus *G. fujikuroi* are not substrate specific, making it possible to produce many non-fungal GA's. This process can be enhanced by using mutant strains, such as B1-41a[43], in which normal GA biosynthesis is blocked at an early stage.

1.4.3 Total Synthesis of Gibberellins

The total synthesis of Gibberellins has proven to be a challenging research area and has excited a great deal of competitive creativity[68,69]. A number of research groups have focussed their attention on the total synthesis of these types of compounds and so many different approaches have been ventured. One of the greatest difficulties associated with total synthesis lies in establishing the correct stereochemistry at the ring junctions, that is, a *trans* ring junction between the A and B rings, while the CD-ring junction is *cis,* and the relationship between H-9 and the C-10 substituent is *anti* (Fig 2a). There are also other asymmetric centres to consider, and the additional problem of introducing functionality into the rings. Many approaches have not proceeded to a successful conclusion, and those that have, are lengthy (requiring more than 20 steps), and are extremely inefficient (producing only a few milligrams of the target compound).

1.4.4 Partial Synthesis of Gibberellins

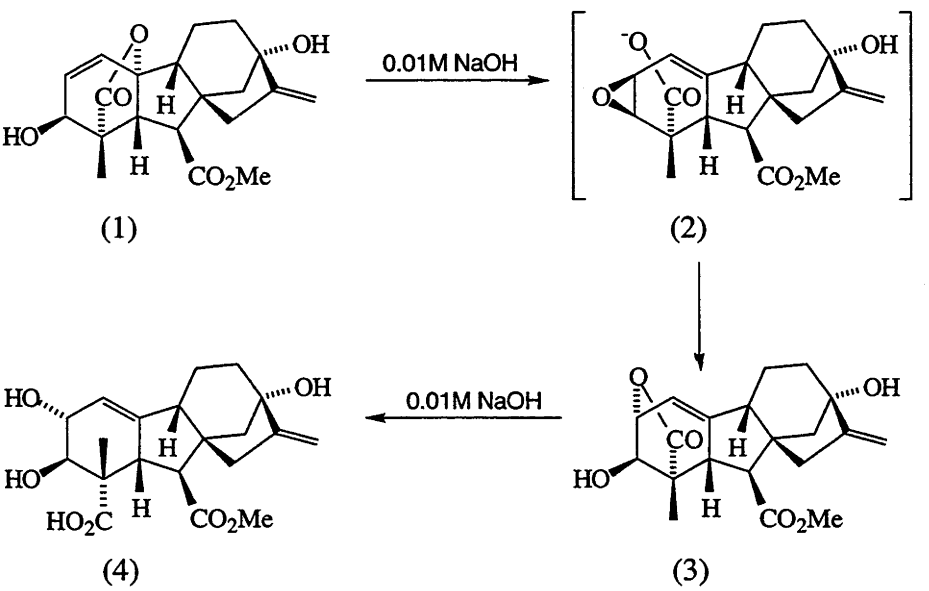
As mentioned previously (section 1.1.3) some gibberellins, namely GA_3, GA_4 and GA_7, are available in reasonable quantities by fermentation, and may therefore be utilised as starting materials. Partial synthesis from such compounds has been the most successful way of confirming tentative new structures. Over 120 naturally occurring gibberellins have been found to exist and most of these are accessible through interconversion of the more available GA's. Almost every carbon atom in the gibberellin skeleton has been hydroxylated, and double bonds have been placed at various positions.

These highly functionalised compounds are sensitive to a wide range of reaction conditions and can readily undergo rearrangement; manipulation of these types of compounds is rarely straight forward. Although a considerable amount of synthetic methodology and experience concerning gibberellins has built up over the past few decades, gibberellins never fail to surprise the organic chemist. A subtle structural change can affect the reactivity of functional groups, even at a remote distance.

1.4.4.1 General Procedures for Gibberellin Manipulation

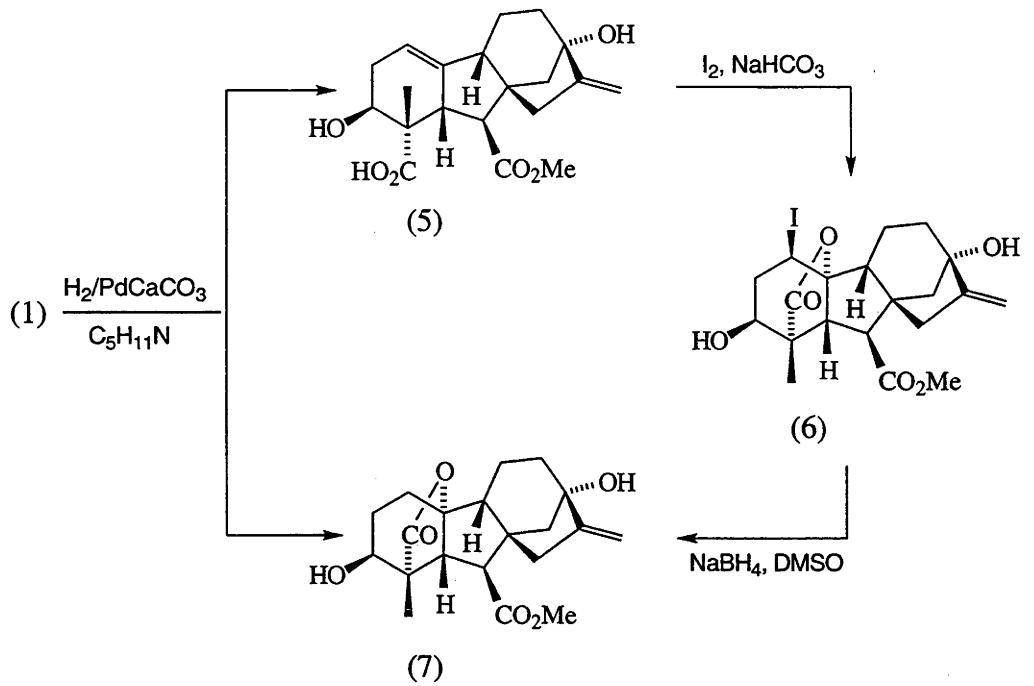
There are a number of standard procedures that exist for the chemical manipulation of GA's. Protection and deprotection are widely used throughout GA chemistry to facilitate other chemical manipulations. The use of protecting groups, particularly the conversion of the 7-carboxylic acid to the 7-methyl ester, also makes handling these compounds considerably easier, (ie. improved solubility, reduced

polarity). However, removal of the ester protecting groups can prove troublesome. For example, the hydrolysis of a C_{19} gibberellin, proceeds extremely slowly since the 10,19-lactone is preferentially hydrolysed, retarding hydrolysis of the 7-methyl ester function to an even greater extent than that due to steric hindrance. The process becomes even more complicated for a Δ^1-ene-3β-ol derivative, such as in GA_3. For this type of compound (**1**), the lactone is converted into the isolactone (**3**) through the 2β,3β-epoxy 19-carboxylate intermediate (**2**), (Scheme 9). In this case, it becomes necessary to protect the 3-hydroxyl first. Under normal circumstances (without the Δ^1-ene-3β-ol), the lactone can be re established by heating at reflux in acetic acid. An alternative hydrolysis procedure which is usually successful, is O-alkyl cleavage using iodide ion[70] or lithium propanethiolate in HMPA[71,72].



Scheme 9. Lactone Rearrangement of GA_3.

Another problem associated with GA chemistry is discrimination between the two double bonds in each of the GA_3 and GA_7 molecules, and considerable effort has been spent on this area, the main problem being the selective reduction of the Δ^1-ene. This can be accomplished to a certain extent by hydrogenation in the presence of an amine base[73] (Scheme 10), but the major product is the diene acid (**5**). The GA_1-type skeleton can, however be obtained through iodolactonisation to form **6**, followed by removal of the halogen, however.



Scheme 10. Hydrogenolysis of the allylic lactone.

Alternatively, the 17-methylene group may be epoxidised before hydrogenation, and then re-established by deoxygenation[74,75]. Another common procedure is to cleave the 17-methylene by ozonolysis or osmium tetraoxide, allowing the formation of the 16-norketone. The 17-methylene can be reintroduced at the appropriate time by utilising the Wittig reaction with methylene triphenylphosphorane[76,77]. In some cases it may be advantageous to use the Lombardo/Oshima[78] procedure for methylenation using dibromomethane/titanium chloride/zinc metal. Both of these reactions provide an ideal opportunity for introducing an isotopic label into a molecule.

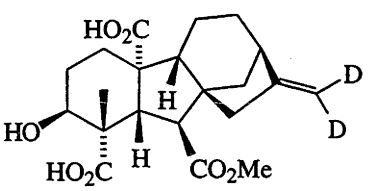
Another quite common procedure used in the partial synthesis of GA's is removal of the 3-hydroxyl and/or 13-hydroxyl. This is usually done by the conversion of the hydroxyl to either a halide[79], thioester[80], thioamide[81], mesylate[82] or methyloxalyl ester[83], followed by reduction using *n*-tributyltin hydride under standard free radical reaction conditions. The latter ester function has proven particularly useful for removing the 13-hydroxyl[83].

As mentioned above, most of the carbons in the gibberellin skeleton have been hydroxylated. Many procedures and chemical manipulations have been employed to this end, the details of which, have been highlighted in a review on gibberellin research[13].

1.5 Aims and Goals of This Project

1.5.1 Isotopic Labelling of Gibberellins

One of the objectives of this work was to partially synthesise 17,17-dideutero-GA_{13} (**8**), and 17,17-dideutero-GA_{25} (**9**) for the purpose of checking their concentration in plants by isotope dilution analysis using GC-mass spectrometry. Deuterated GA_{13} was required in order to compare catabolism between C_{19}- and C_{20}-GA's, whereas deuterated GA_{25} was to be used in the quantification of GA_{25} in transgenic plants, in which a pumpkin gene overexpresses GA 20-oxidase, causing the production of tricarboxylic acids. Through the synthetic scheme used, a naturally occurring GA was also partially synthesised. The deuterium labels were introduced by utilising the Lombardo/Oshima procedure[78].



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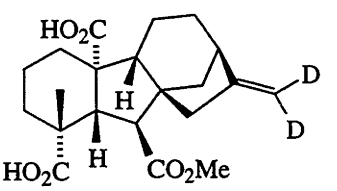


(9)

Figure 4. Deuterated GA_{13} and GA_{25}.

A further objective was to produce ^{14}C labelled GA_{20} which was required by a research group in Minnesota, to help in tracking the metabolic pathway involved in the conversion of GA_{19} to GA_{20}. Other planned studies include observing the effect on GA_{20} metabolism by varying the conditions operating on certain plant systems, such as photoperiod and auxin levels. This synthesis also served as a model for ^{14}C labelling the fairly recently discovered GA_{95}, a significantly more difficult task. It was hoped that a study using labelled GA_{95} (Figure 5) may shed some light on the formation of gibberellins possessing a 3β-hydroxyl and Δ^1-ene, such as GA_3, and to uncover the mechanism involved in C(3) oxygenation of GAs possessing ring-A alkene bonds.

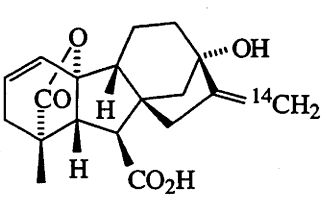
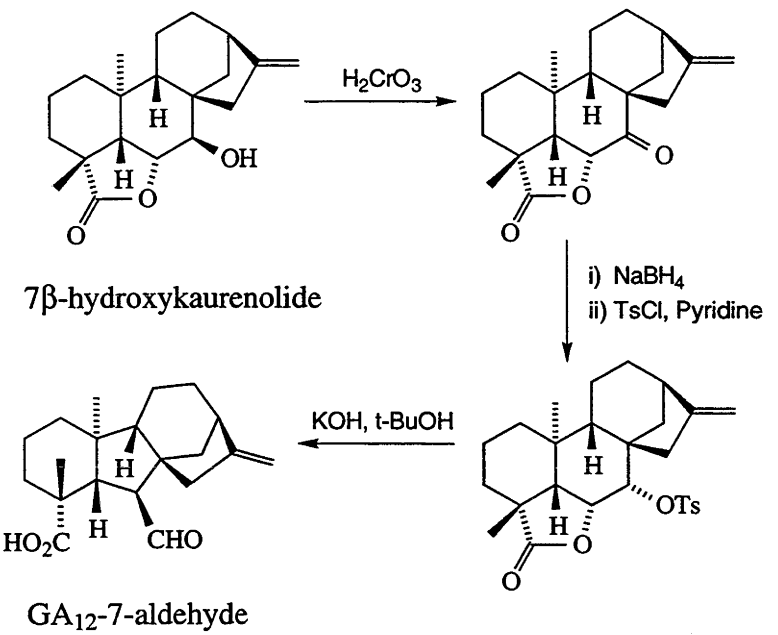


Figure 5. ^{14}C-labelled GA_{95}.

1.5.2 Conversion of Gibberellins into Kaurenoids

Access to the kaurenoids, (the biosynthetic precursors of GAs) has been facilitated by the availability of several kaurenolides produced during the commercial fermentation of *G. fujikuroi*. Moreover, these kaurenolides have served as useful substrates for the preparation of a variety of C_{20}-GAs (Scheme 11).



Scheme 11. Synthesis of GA_{12}-aldehyde from 7β-hydroxykaurenolide.

Current strains of the fungus, however, produce only trace amounts of these useful compounds. It was therefore, of interest to determine whether it would be possible to convert gibberellins back into kaurenoids as a means of gaining access to these important intermediates. In addition, there was also the prospect of gaining better access to biologically active kaurenoids obtained from plant sources in very small amounts. Some notable kaurenoids which have shown therapeutic potential[84] and form an attractive goal for synthesis are enmein[85], oridonin[86] and shikodonin[87] (Figure 6).

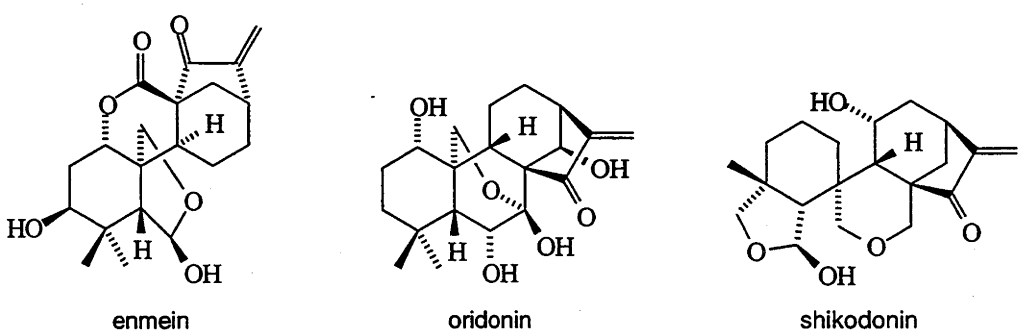


Figure 6. Interesting kaurenoids from the Rabdosia family which have antibacterial and antineoplastic properties.

CHAPTER 2

ISOTOPIC LABELLING OF GIBBERELLINS

2.1 Labelling Studies

Isotopically labelled gibberellins have been used extensively to study the biosynthetic pathways involved in gibberellin transport, metabolism and the mode of action of gibberellins, as well as locate where these processes are taking place[88-92]. These studies are performed by applying a suitably labelled substrate to an intact plant, fungal culture or cell-free preparation. Analysis of the products is usually performed by GC-mass spectrometry. However, labelling studies are often not as straightforward as one would wish, for several reasons. First, it must be made certain that the correct substrate is applied in the correct concentration, such that it is similar to that of the native compound. Secondly, GA biosynthesis can be activated or deactivated during the life cycle of the plant, thus necessitating the incorporation of the labelled substrate at the correct stage of the plant's development. Thirdly, biosynthetic pathways may differ from species to species of a given plant, and also from organ to organ of the same species. Therefore, anomalous results can easily be obtained if great care is not exercised in the choice of plant or the organ of the plant used. Fourthly, many enzymes are non-specific, which may result in the formation of products from the metabolism of the labelled substrate which correspond to endogenous GA's, but which are artefacts of the "feeding" process. It is therefore, necessary to ensure that the metabolites are also normally endogenous to the system studied.

The type of isotope used and the position into which it is incorporated in the gibberellin largely depends on the purpose of the study. The choice, however, is somewhat limited by the availability of methods of incorporating the isotopic label into the required substrate. Stable isotopes, such as deuterium, ^{13}C and ^{18}O have the advantage of being less troublesome to manage, and also a high incorporation can be achieved, making identification by MS easier. However, detection is limited to MS or NMR. Tritium, although an unstable isotope, has been found to be very useful in labelling studies. Tritiated compounds may be prepared with a very high specific activity, so for low metabolic conversions where the product is at low concentration, it makes detection easier. ^{14}C has a specific activity of up to 1000 times less than tritium, but is more easily detected, making it perhaps more widely accepted where specific activity is not an issue. It is generally used both as a radiotracer and as a heavy isotope for MS detection.

There are a number of points to consider when choosing the site for an isotopic label; will the isotope be retained by the substrate throughout the GA metabolism, or is the site likely to be lost during an enzymatic reaction? There is also the possibility of rearrangement occurring at the labelled site, making identification of the labelled product quite difficult. It has been found that the C(17) position usually remains intact throughout GA metabolism, so this site is eminently suitable for isotopic labelling. In this way the label serves not only as a radiotracer, but allows quantification studies to be done. Conversely, if the mechanism of a certain enzymatic reaction is being studied, it would be best to place a heavy isotope of carbon or oxygen at the reaction site, and investigate the fate of the label after the reaction (eg, fate of C(20) during the conversion of C_{20} GA's to C_{19} GA's)[93].

2.1.1 Methods of Incorporation of Isotopic labels into Gibberellins

Biochemical methods of incorporating isotopic labels have involved feeding labelled acetate and/or labelled mevalonate to the fungus *Gibberella fujikuroi.* Product analysis was initially executed by degradative studies and later by chromatographic techniques[94]. Chemical methods of incorporating a label generally involve the introduction of the label at the latest possible stage of a synthetic scheme so as to contain costs or, in the case of ^{3}H and ^{14}C, to minimise handling of radioactive materials..

Since the labelling studies involved in this work are only concerned with labelling at the C(17) position, the following discussion will only mention procedures of incorporating a label at this site.

The D-ring is the site most used for the introduction of heavy isotopic labels, namely ^{13}C and ^{14}C, although deuterium and tritium may also be incorporated. The 17-methylene group can be cleaved oxidatively as previously mentioned (1.4.4.1) and re-established with an isotopically labelled Wittig salt. In this way deuterium, tritium, ^{13}C or ^{14}C may be introduced. Some caution is required, however, as the basic conditions of the Wittig olefination can cause scrambling of the deuterium or tritium label between the C(15) and C(17) positions and may also lead to epimerisation in the case of a 3β-hydroxyl, unless it is protected. If a 13-hydroxyl is present, protection may be required to prevent acyloin rearrangement (C/D-ring rearrangement)[95]. However, a bulky protecting group may result in extended reaction times, and so acetates or TMS ethers are usually employed as C(13)-protecting groups. For deuterium labelling of the terminal olefin, an effective alternative is the Lombardo/Oshima reaction[78] which can give

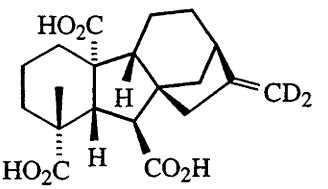
greater than 95% incorporation of two deuterium atoms. No scrambling of the label between C(15) and C(17) occurs, and the 3β-hydroxyl is not epimerised. However the yields are not always good, especially if a 13-hydroxyl is present.

Once the label has been incorporated, the only step usually remaining is the deprotection of functional groups, and so the handling of the labelled substrate is kept to a minimum and the radioactivity is well contained.

CHAPTER 3

17,17-DEUTERIUM LABELLING OF GA_{13} AND GA_{25}

3.1 Protection of GA_{13} and Removal of the 3β-Hydroxyl



(9)

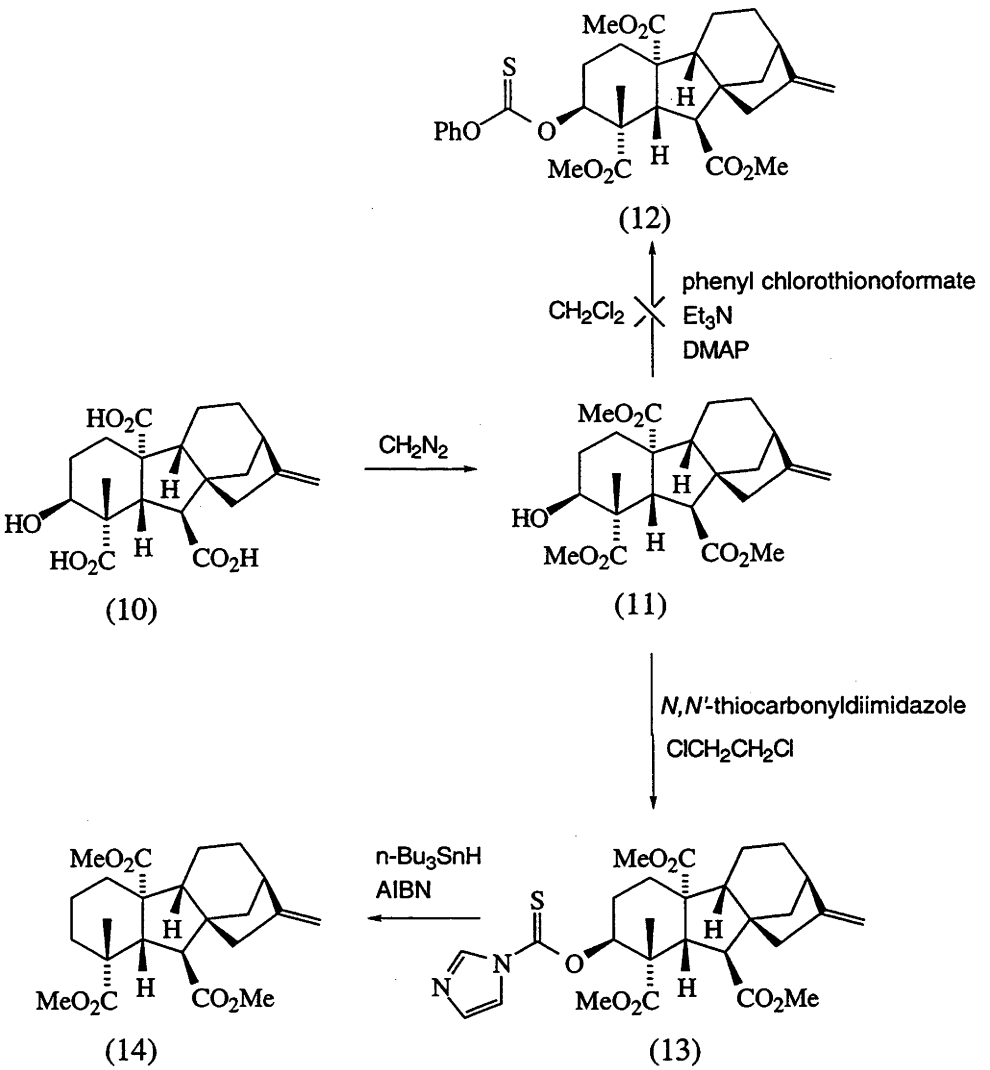
Figure 7. Deuterated GA_{25}

A research group at Long Ashton Research Station at the University of Bristol required deuterated GA_{25} (**9**) for the purpose of monitoring its concentration in plants by isotope dilution analysis using GC-mass spectrometry. In order to synthesise GA_{25}, the closely related GA_{13} (**10**) was envisaged as an appropriate precursor since it can be obtained from the fermentation of *Gibberella fujikuroi.* Moreover, the only structural change required for the conversion of GA_{13} to GA_{25} is the removal of the 3β-hydroxy group.

The C(17) position in GA_{25} was chosen as the labelling site as it would not be lost due to metabolism. It is also quite convenient to label C(17) since this terminal methylene group can be cleaved and reinstated using a variety of methods, namely the Wittig reaction or the Lombardo procedure[78], both of which lend themselves to isotope incorporation.

The first step in the partial synthesis was to protect the three carboxy groups in GA_{13}. The substrate was therefore, protected as the trimethyl ester **11** (Scheme 12). Methylation was achieved using diazomethane, and the methyl esters appeared as three singlets between 3.5 and 3.8 ppm in the ^1H nuclear magnetic resonance (nmr) spectrum. The next step was removal of the 3β-hydroxyl to give the GA_{25} derivative. A well investigated procedure for deoxygenation of gibberellins involves the conversion of the hydroxyl to a phenoxythiocarbonyl ester[96] followed by deoxygenation by means of stannane reduction[97]. However, attempts to produce the phenoxythiocarbonyl derivative (**12**) using phenyl chlorothionoformate failed, and the 3β-hydroxy derivative was re-isolated and purified. A second attempt to form a suitable carbonyl derivative was made using *N,N'*-thiocarbonyl-diimidazole[98,99]. This proved to be successful, producing the thioester **13** in high yield (92%, after purification), as a white crystalline solid. The ^1H nmr spectrum showed that the 3α-H had shifted downfield from 4.0 ppm to 5.9 ppm.

Free radical based deoxygenation using tri-*n*-butyltin hydride in benzene at reflux with azoisobutyronitrile (AIBN) as a radical initiator[97], afforded the 7,19,20-trimethyl ester GA_{25}, (**14**) in 77% yield. The success of the *N,N'*-thiocarbonyldiimidazole over phenyl chlorothionoformate in producing a thioester, may be due to the imidazolide being a better leaving group than chloride.

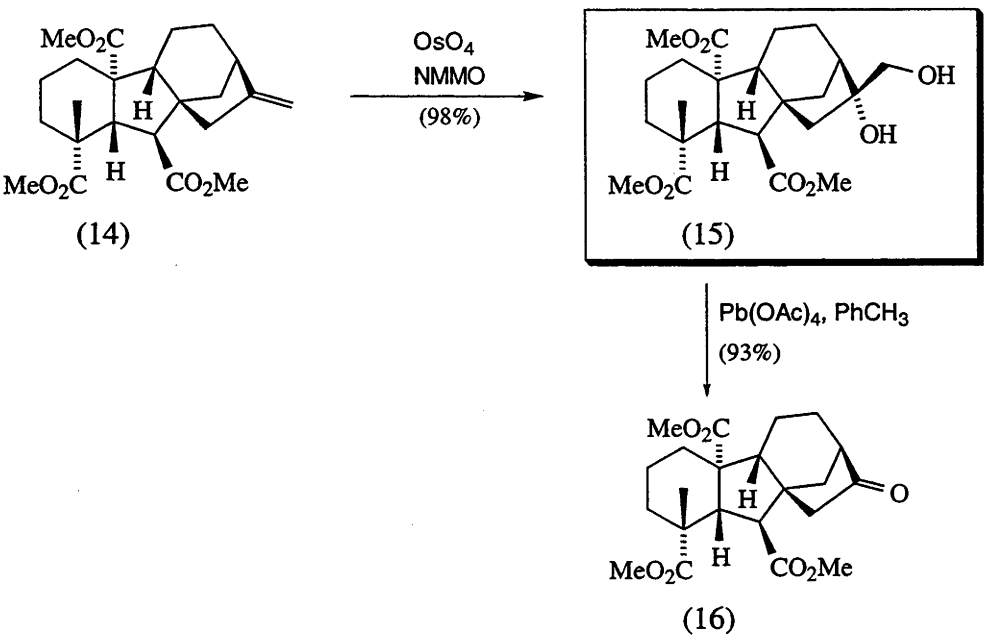


Scheme 12.

3.2 Formation of GA_{25}-16,17-Diol and the 16-Norketone

It was envisaged that the deuterium label would be incorporated using a Lombardo reaction. This necessitated the conversion of the 16-methylene group to the 17-nor-16-one function.

This was accomplished by dihydroxylation to form the 16,17-diol trimethyl ester of GA_{25} (**15**) first, using a catalytic amount of osmium tetroxide with a co-oxidant. The procedure followed, used 4-methylmorpholine-*N*-oxide to re-oxidise the osmate ester according to the procedure of VanRheenen et al.[100]. The 16,17-diol of the GA_{25}-trimethyl ester was of some interest after isolation and structure confirmation. It was used to confirm a tentative structure for a naturally occurring gibberellin recently isolated from plant extracts by Dr Patrick Blake of Horticultural Research International.



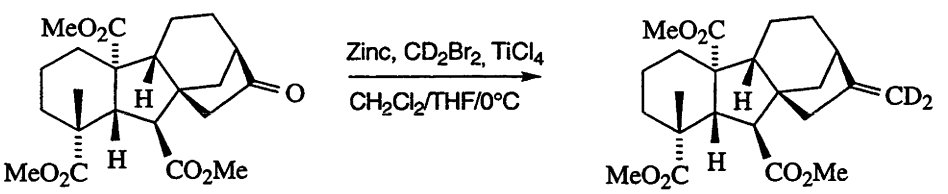
Scheme 13.

Subsequent treatment of the diol with lead tetraacetate oxidatively cleaved the C(17)-hydroxyl to give the GA_{25}-trimethyl ester-16-norketone (**16**). This two-step oxidation procedure proved to be highly efficient and superior to the more direct method using osmium tetroxide-periodate[101].

3.3 Deuterium Labelling

As mentioned earlier, the Lombardo/Oshima methylenation reaction has distinct advantages over the Wittig methylenation when deuterium labelling is involved. Specific deuterium incorporation can be obtained using this procedure, whereas scrambling of the label between C(15) and C(17) can occur during Wittig methylenation. The active species (thought to be similar to the Tebbe reagent[102], $Cp_2TiCH_2AlCl(CH_3)_2$) is preformed by the addition of $TiCl_4$ to a suspension of activated zinc dust in THF at -40°C with CD_2Br_2

(Scheme 14). A thick grey slurry is formed which can be stored at 4°C, but which gives best results after approximately 36-48 hours. The reagent is added portion-wise to the substrate and the reaction is monitored by thin layer chromatography (tlc). GA_{25}-trimethyl ester was labelled successfully using this procedure (**17**). Although the 17,17-deuterium atoms do not show up in the ^{1}H nmr spectrum, the C(17) should be seen in the ^{13}C nmr spectrum as a doublet of triplets due to the spin-spin coupling between the deuteriums and the carbon atom. However, the intensity was much diminished and in the ^{13}C nmr spectrum obtained, the two triplets were hidden in the base line. C(16), on the other hand, was observed at 156.3 ppm as expected for a quaternary methylene, as opposed to 220.9 ppm for the 17-nor-16-one. The mass spectrum also showed a molecular ion peak for 406, corresponding to the expected mass of the labelled GA_{25}-methyl ester.



(16) (17)

Scheme 14.

3.4 Deprotection

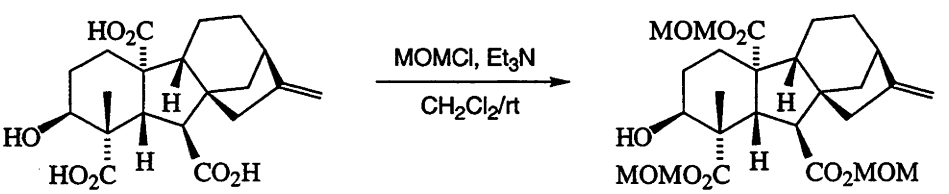
The last remaining step was the deprotection of the three methyl esters. A common procedure used for the hydrolysis of methyl esters from similar substrates, is to heat at reflux in aqueous 2M sodium hydroxide and methanol. This procedure, however, was not effective in removing the protecting groups, since after one week the major constituent was starting material with a mixture of mono and dimethyl esters. The procedure was repeated in diglyme to boost the temperature, but this also gave a mixture consisting mainly of starting material. Treatment of ester **17** with potassium tert-butoxide in DMSO at room temperature, and also at reflux also proved ineffective at removal of the protecting groups. In an attempt to transesterify the trimethyl ester to a compound which might be more easily deprotected, the ester **17** was dissolved in acetonitrile, and treated with sodium iodide and chlorotrimethyl silane, but no reaction took place. The difficulty encountered for the demethylation can possibly be explained by the fact that C(19) and C(20) lie in a sterically hindered environment accentuated by the concave shape of the α-face of the gibberellin skeleton. The Johnson-Bartlett procedure[103] using lithium

propanethiolate in HMPA was contemplated, but in view of the difficulties expected in isolation of the very polar tricarboxylic acid, more labile protecting groups were employed instead.

3.5 *Protection / Deprotection of GA_{13} as the Tris-Methoxymethyl Ester*

Since the methyl ester protecting groups proved to be so difficult to remove, a new approach seemed necessary. Methoxymethyl (MOM) esters have proven to be quite useful throughout gibberellin partial synthesis. Their formation is fairly simple and their removal has been readily achieved using a variety of procedures.

GA_{13}, was dissolved in dichloromethane and treated with triethylamine and chloromethylmethyl ether for 10 minutes to afford the tris-MOM ester **18** (Scheme 15). A trial deprotection was investigated as the next course of action before continuing any further with the partial synthesis. Thus, the tris-MOM ester **18** was treated with a known amount of hydrochloric acid generated from trimethylsilyl chloride and methanol. This resulted in the partial deprotection of GA_{13} tris-MOM ester, but total deprotection was not achieved. The soft Lewis acid, magnesium bromide, in ether at room temperature[104] has been shown to cleave MOM esters, but this proved to be ineffective towards the compound under investigation, which showed no change when subjected to this treatment. However, 1M zinc chloride in methanol at reflux overnight was successful in fully deprotecting the ester to give GA_{13} (**10**). Having established that MOM protecting groups can be satisfactorily removed, the synthesis of the deuterated GA_{25} was continued.

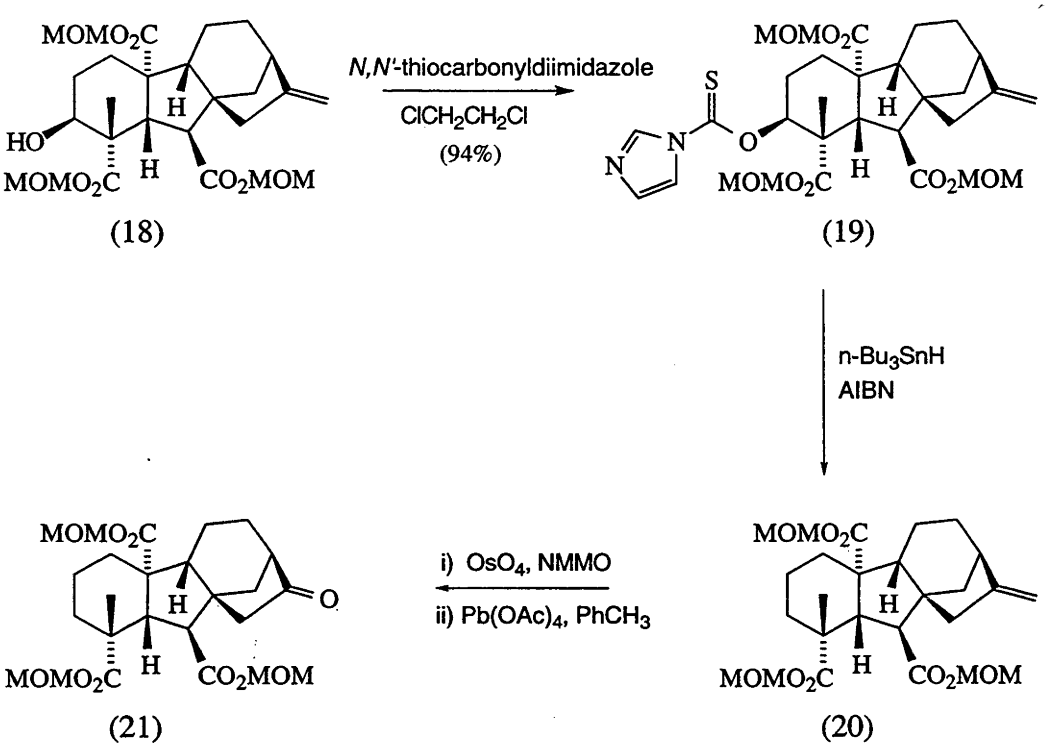


(17) (18)

Scheme 15.

The GA_{13} tris-methoxymethyl ester was deoxygenated at C(3) as for **11** by treatment with *N,N'*-thiocarbonyldiimidazole to form the 3β-imidazolide **19** in a 94% yield, followed by deoxygenation with *n*-tributyltin hydride to give the GA_{25} tris-MOM

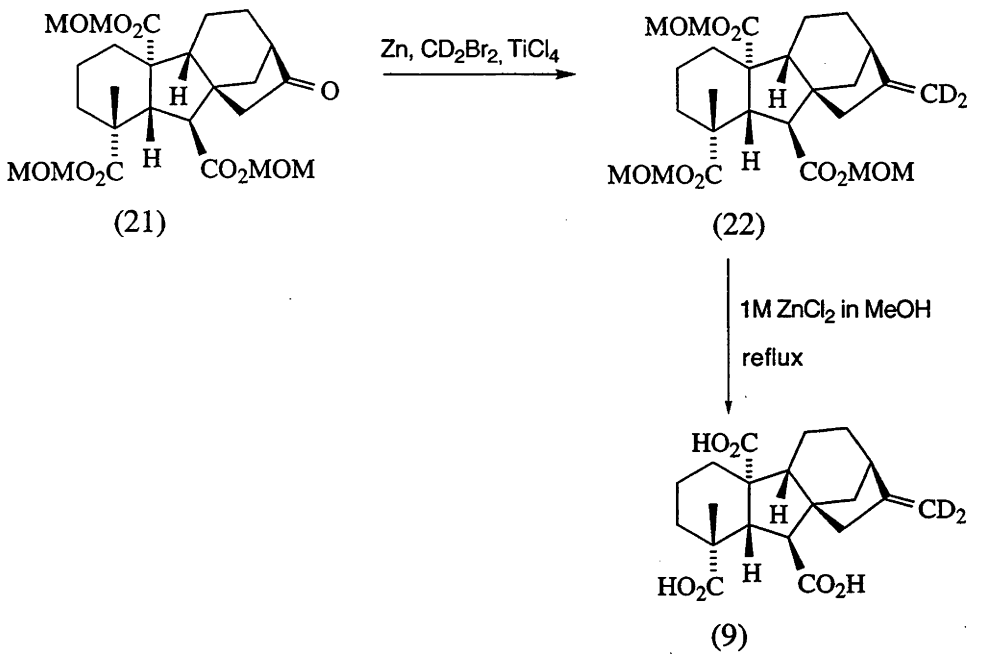
ester (**20**), then the 16-norketone (**21**) was formed as before by treatment with osmium tetroxide-NMMO followed by lead tetraacetate.



Scheme 16.

3.6 Deuteration / Deprotection of GA_{25}-tri-MOM Ester

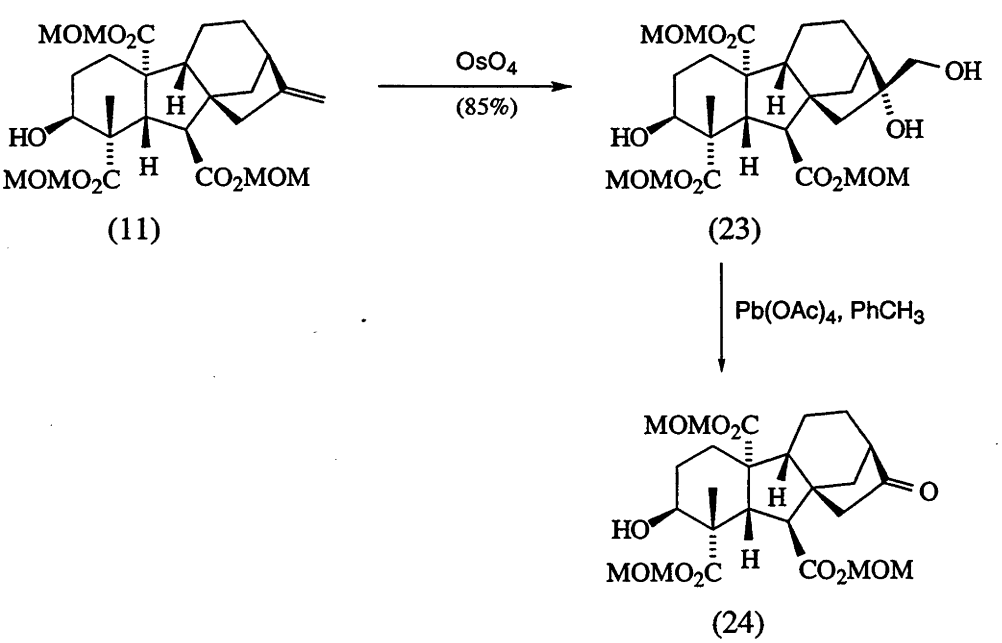
The norketone **21** was treated with the Lombardo/Oshima reagent dropwise until no starting material remained as indicated by tlc analysis. Following the work-up procedure, 17,17-d_2-GA_{25} tris-MOM ester (**22**) was purified by flash column chromatography to give a colourless oil in 62% yield, (Scheme 17). The deprotection step was carried out as for the trial deprotection with 1M zinc chloride in methanol at reflux overnight. A small amount of starting material, mono and diesters remained and were separated by flash column chromatography from the deuterated GA_{25} parent acid. The 17,17-dideutero-GA_{25} (**8**) was characterised and gave satisfactory spectroscopic data.



Scheme 17.

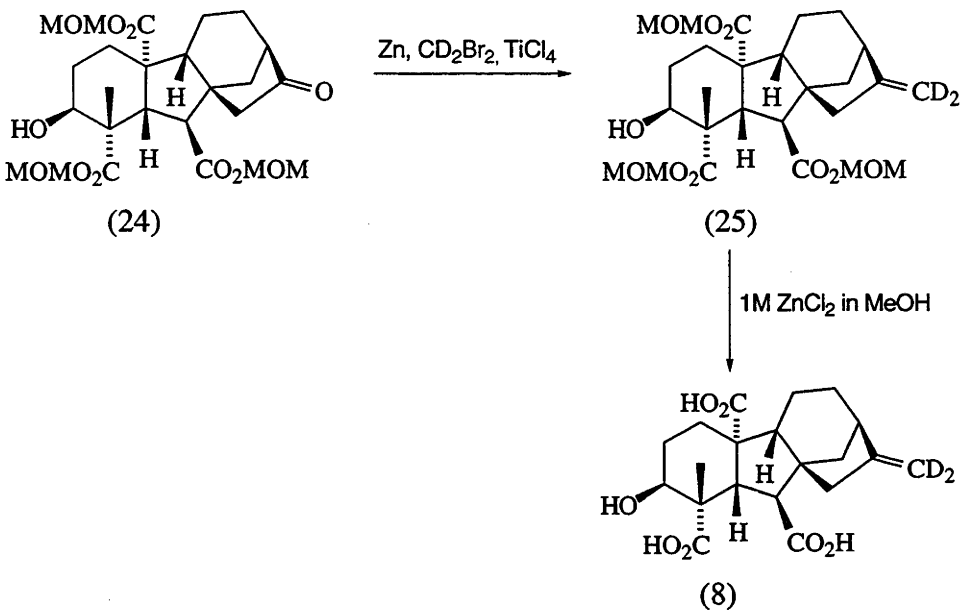
3.7 Deuterated GA_{13}

GA_{13} was also required as an isotopically labelled substrate for the purpose of quantifying C_{20}-GA catabolism relative to C_{19}-GA catabolism for fast growing plants such as sugar cane, which appear to be metabolised via the early 3β-hydroxylation pathway. To this end, GA_{13}-tri MOM ester (**11**) was oxidised to the 17-nor-16-one (**24**) via the 16,17-diol (**23**), (Scheme 18).



Scheme 18.

The norketone **24** was subsequently methylenated using the Lombardo/Oshima reagent, incorporating two deuterium labels at C(17) in 57% yield, (**25**). Deprotection afforded 17,17-dideutero-GA_{13} (**9**) which was characterised spectroscopically, (Scheme 19).



Scheme 19.

3.8 Quantification of GA_{25} in Studies on Transgenic Plants

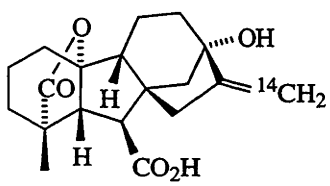
The deuterated GA_{25} substrate(**9**) is currently being used in the quantification of GA_{25} in plants, which have been transformed with a pumpkin gene that over-expresses GA 20-oxidase. This enzyme produces biologically inactive tricarboxylic acids rather than C_{19}-GA's, leading to accumulation of GA_{25} and reduction in growth.

CHAPTER 4

^{14}C-LABELLING OF GA_{20}

4.1 Introduction

GA_{20} is a product of the early 13-hydroxylation pathway during the metabolism of gibberellins in higher plants. A ^{14}C radiolabelled derivative of GA_{20} (**26**) was required by a number of research groups for the purpose of tracking this metabolic pathway and studying how certain enzymes and environmental factors influence the levels of GA_{20} production in different plant species. The ^{14}C-GA_{20} would also be used as an internal control. As the label was required to be preserved in the substrate throughout the metabolic processes in order to follow the pathway involved, the C(17) position was chosen as the site for the ^{14}C-label.



(26)

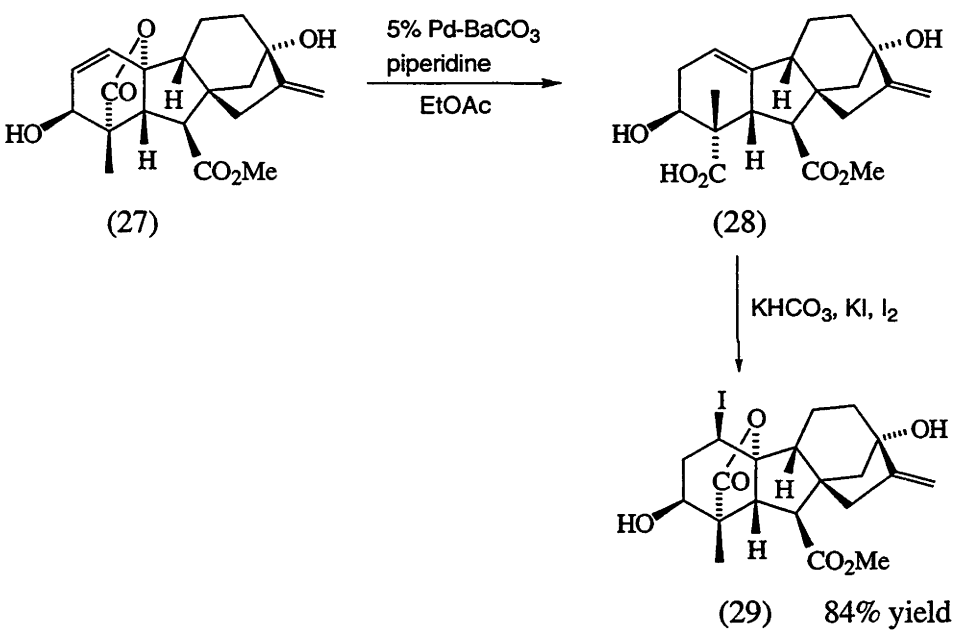
Figure 8.

Initial investigations were centred upon the incorporation of the ^{14}C-label utilising a Wittig methylenation using labelled methyl triphenylphosphonium iodide. In order that manipulations of radiolabelled material were kept to a minimum, the Wittig methylenation step was proposed as one of the last synthetic operations, followed by deprotection of the 7-carboxylic acid and the 13-hydroxy group. The removal of protecting groups on the 7-carboxylic acid of gibberellins can sometimes prove to be problematical. Thus, protection and deprotection of a suitable substrate was investigated before looking at the desired synthesis of GA_{20}. GA_9 was chosen for this model investigation and was protected as the 7-MOM ester. Although deprotection of this molecule was forthcoming, the MOM ester did not withstand the reaction conditions of the Wittig methylenation. Protection of the 7-acid as its methyl ester was next investigated. The 7-methyl ester was completely stable during the methylenation step, but deprotection with sodium hydroxide in methanol opened the A-ring lactone as well as removing the methyl ester. The lactone could be closed again by refluxing in acetic acid, and this "two step" procedure gave good yields of the free acid. Hence, this latter approach was adopted.

4.2 *Partial Synthesis of GA_{20} from GA_3*

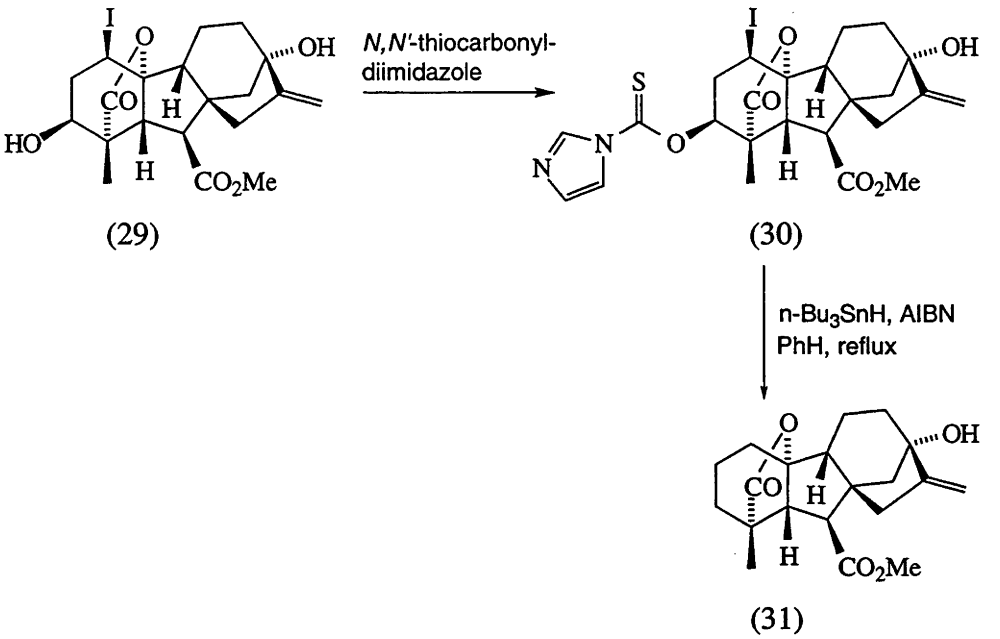
The partial synthesis of GA_{20} from GA_3, involved the hydrogenolysis of the 1,2-double bond in the A-ring, and deoxygenation of the 3β-hydroxyl.

GA_3 was protected as the 7-methyl ester **27** and then reduced with hydrogen using palladium on barium carbonate in the presence of piperidine to form the hydrogenolysis product **28**.[73] Iodolactonisation was carried out on **28** by treatment with potassium hydrogen carbonate, potassium iodide and iodine in tetrahydrofuran (THF) for 30 minutes. The iodolactone (**29**) was isolated as a white crystalline solid in 84% yield. A ^1H nmr spectrum showed H(1) to be a doublet at approximately 4.4 ppm, indicative of a proton geminal to an iodo substituent.



Scheme 20.

The next step involved the removal of the 1-iodide and 3β-hydroxyl, (Scheme 21). This was achieved by first converting the 3β-hydroxyl into a thiocarbamate group by treatment with *N,N'*-thiocarbonyldiimidazole.[81] This gave thiocarbamate **30**, as a white crystalline solid (87% yield) which was dissolved in benzene and heated at reflux, followed by the addition of tri-*n*-butyltin hydride and AIBN as initiator. This procedure successfully reduced both the iodide and thiocarbamate functions to give the desired GA_{20} methyl ester (**31**) in 70% yield.



Scheme 21.

4.3 *Protection of the 13-Hydroxyl*

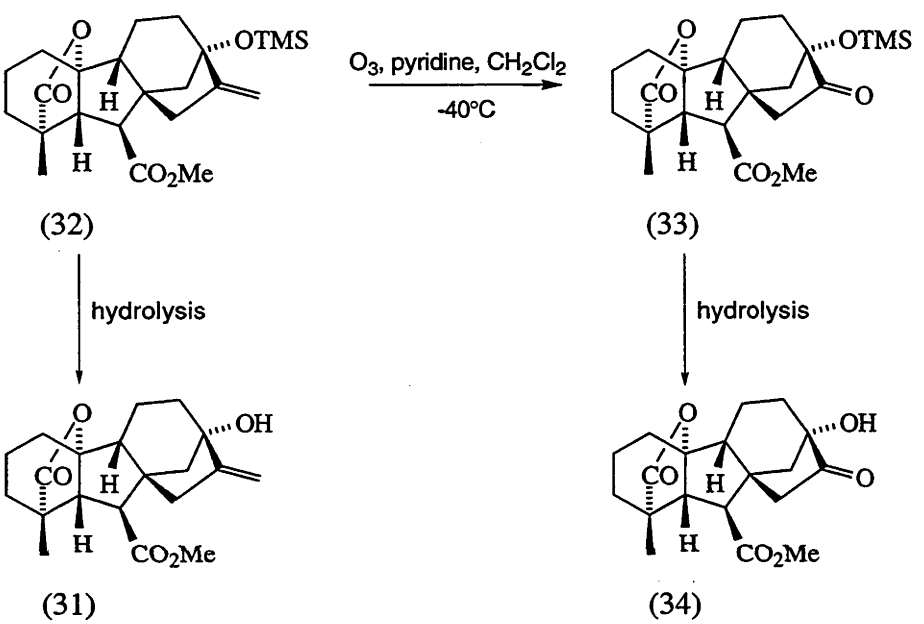
The 13-hydroxyl in **31** was protected using trimethylsilyl chloride (TMSCl) and diisopropylethylamine in dichloromethane to give ether **32**, (Scheme 22). Unfortunately, the TMS ether is extremely acid sensitive and had not only been known to hydrolyse during the work up procedure, but also upon storage. For this reason, flash column chromatography was performed using silica gel which had been buffered with triethylamine in the eluting solvent.

Scheme 22.

4.4 *Formation of the 16-Norketone*

Ozonolysis was chosen as the method for cleaving the 16,17-methylene group. Thus, olefin **32** was dissolved in dichloromethane/pyridine (4:1) and the temperature lowered to -78°C. Ozone was bubbled through the solution until the olefin could no

longer be detected by tlc, which was 4 minutes. It was found that the addition of excess ozone resulted in the decomposition of the substrate. Four equivalents of dimethylsulphide were added dropwise and the reaction mixture was allowed to warm to room temperature. After flash column chromatography, again using a buffered eluting solvent, only 35% yield of the desired 16-norketone **33** was obtained as a white foam. Some losses occurred due to the fact that the trimethylsilyl ether hydrolysed during ozonolysis and during chromatography. The GA_{20}-7-methyl ester (**31**) was recovered (20%) and a small amount of GA_{20}-7-methyl ester-16-norketone (**34**) was also recovered. No other products were isolated or identified (Scheme 23).

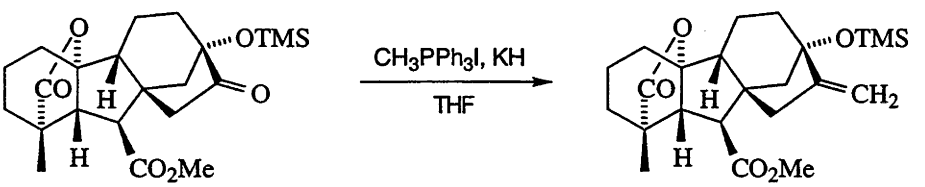


Scheme 23.

4.5 Wittig Methylenation Followed by Deprotection

The Wittig methylenation involved the use of ^{14}C-labelled methyl triphenylphosphonium iodide. Trial reactions were performed with unlabelled phosphorane to ascertain the optimal reaction conditions required. Salt-free conditions were required to prevent side reactions taking place. Because of the expense and the nature of the isotopically labelled phosphorane, any excess had to be kept to an absolute minimum, and approximately three equivalents were determined to be sufficient. Only a very small quantity of the labelled GA_{20} substrate was required, (approximately 1-2 mg), and so the Wittig methylenation was conducted on a small quantity of the protected norketone. The model reaction using the unlabelled ylide was performed on the same scale as that required for radiolabelling. Ylides are moisture and air sensitive, and because of the small scale of the reaction, all moisture had to be excluded and great care

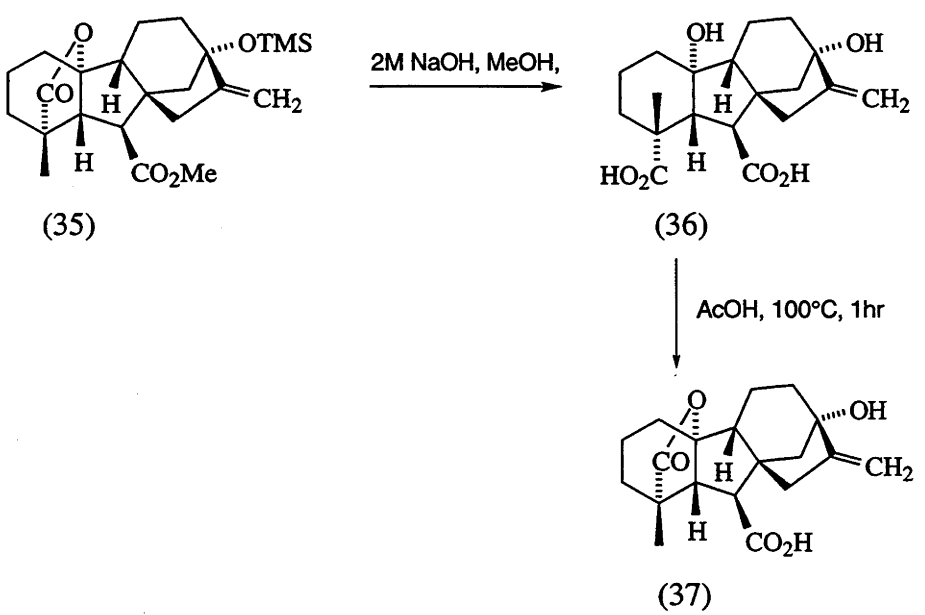
was required to maintain anaerobic conditions. The bright yellow ylide was pre-formed in a separate flask by the addition of 35 equivalents of sodium hydride to the phosphonium salt in THF. The mixture was heated to 30°C to ensure that all of the phosphorane had been formed. The stirrer was then switched off and any particulate matter was allowed to settle, so that the yellow solution could be cannulated into a flask containing the dry norketone (**33**). As soon as tlc analysis indicated that no starting material remained, the solvent was removed. The ^1H nmr spectrum of the crude product showed the protons of the olefin **35** resonating at 4.9 and 5.2 ppm (Scheme 24).



(33) (35)

Scheme 24.

The deprotection was carried out by the addition of a 6:1 mixture of 2M sodium hydroxide and methanol respectively to the crude olefin **35**. The mixture was heated to 110°C overnight, cooled, and brought to pH 3-4 by 2M hydrochloric acid. Of course, the 19,10-lactone was hydrolysed under these conditions to give the 19-carboxylic acid and the 10-hydroxyl, but the methyl ester and the TMS ether functions were also successfully removed.



(35) (36) (37)

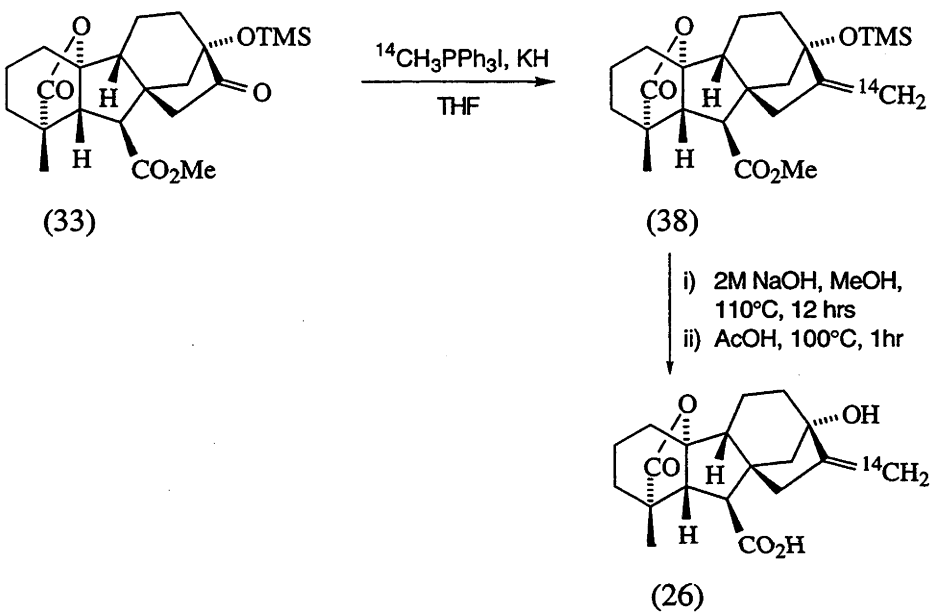
Scheme 25.

Once the solvent had been removed from the reaction vessel, acetic acid was added to the residue which was then heated with stirring to 100°C for 1 hour. After filtering and column chromatography, GA_{20} was obtained as the free acid **37**, (Scheme 25).

4.6 Incorporation of the Radio-Isotope

The above Wittig procedure was repeated using the ^{14}C-methyl triphenylphosphonium iodide. All glassware and reagents were thoroughly dried and placed under an argon atmosphere. The 7-methyl ester 13-TMS ether 16-norketone (**33**) was azeotroped with dry, distilled benzene to remove any moisture that may have been present. The labelled phosphonium salt was also azeotroped with dry, distilled benzene and heated overnight at 60°C under high vacuum. It was found to carry a high static charge and difficulty was encountered in weighing out the material. THF was added to the Wittig salt followed by potassium hydride, and the mixture was stirred as for the trial reaction (Section 4.5). After settlement of particulate matter the solution was cannulated into the flask containing the norketone **33** and a very faint yellow colour persisted. Unfortunately, the labelled phosphorane contained a yellow dye, possibly as a visual aid during handling. This interfered by masking the bright yellow colour of the ylide being formed, and made it difficult to tell whether the ylide had been formed at all. Unfortunately, no product was observed by tlc analysis. Considering that the trial reaction with unlabelled phosphorane proceeded well, the only conclusion that could be made from this lack of reaction, was that the labelled methyl triphenylphosphonium iodide was contaminated. However, no steps to prove this could be taken at the time, as nmr, mass spectroscopy or elemental analysis were impossible to perform due to the radioactivity of the substance. The phosphorane was later found to contain a large amount of water as an impurity. Although an attempt was made to dry the phosphonium salt by azeotroping with dry benzene, it was not soluble, and so any water trapped within the crystals had not been removed. Despite having added 35 equivalents of sodium hydride to form the ylide, this most likely only partially dried the phosphorane. In fact, no ylide (or very little) had been formed at all. It appeared that about half the mass of the weighed labelled phosphorane was actually taken up by water and coloured dye. The problem was eventually overcome by using an extremely large excess of sodium hydride (>1000 equivalents) to dry the phosphorane and to also form the ylide. Although the phosphorane still contained the yellow dye, the ylide once formed could be distinguished by a brighter yellow colour. The ylide was then cannulated through a needle, tightly packed with glass wool to act as a filter into a solution of the norketone **33**. This proved to be highly effective, allowing no sodium hydride to pass through. Tlc analysis showed

that the labelled olefin **38** had indeed been formed. However, it was thought necessary to chromatograph the crude olefin (even though this would involve more handling), to remove the yellow dye and the triphenylphosphine oxide. This resulted in a 74% yield of the radiolabelled olefin being isolated. The deprotection step was then carried out. The deprotected radiolabelled GA_{20} (**26**) was purified by reverse phase high pressure liquid chromatography (HPLC) to give 2.5 mg (45% yield) of the ^{14}C-labelled GA_{20} over the three steps (labelling and deprotection). This afforded 263 μCi of GA_{20} with a specific activity of 56 μCi/μmole.



Scheme 26.

4.7 Biological Studies Using ^{14}C-GA_{20}

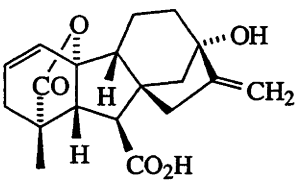
A research group in Minnesota require the ^{14}C-GA_{20} to help track the metabolic pathway involved in the conversion of GA_{19} to GA_{20}. Other uses of this radiolabelled substrate include: observing the effect of seeds and auxin on GA_{20} metabolism in pea pericarp; comparing GA_{20} metabolism in light-independent photomorphogenetic (lip1) mutant with that of the wildtype; use as an internal standard and marker in plant extracts and during GA purification, as well an for injections into eucalyptus trees and hybrid aspen to study GA metabolism and transport *in vivo*; and observing the effects of photoperiod on GA metabolism in hybrid aspen.

CHAPTER 5

^{14}C-LABELLING OF GA_{95}

5.1 *Introduction*

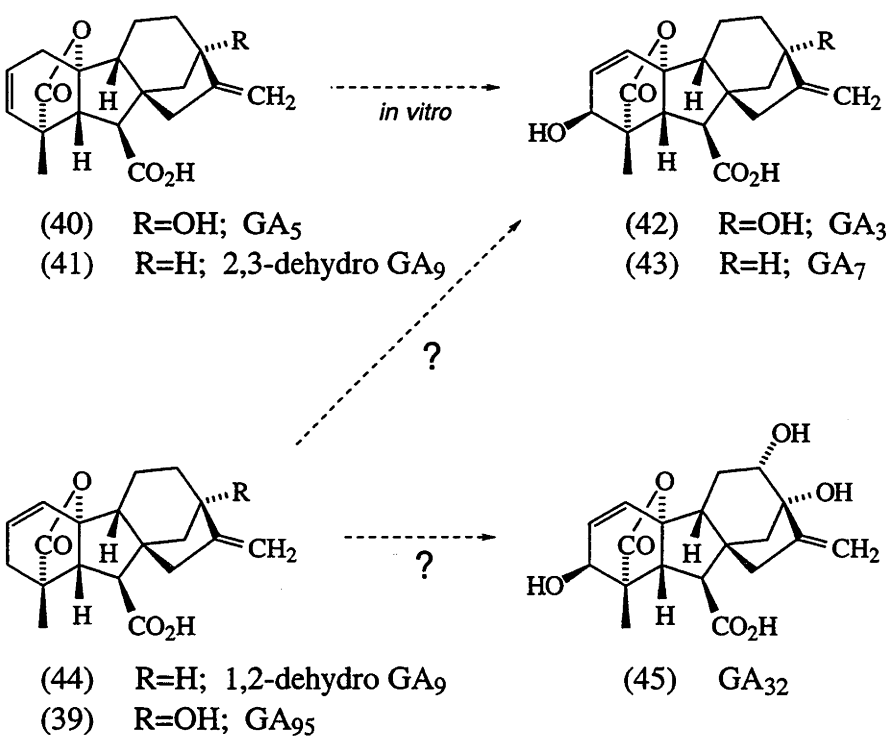
GA_{95} (**39**) is a recently discovered gibberellin, isolated from *Prunus cerasus* (sour cherry). Its structure determination was confirmed by partial synthesis, and the identification of endogenous material was made possible by the preparation of the 17,17-deuterated derivative[105]. There was speculation that this unique structural type might be involved in one or more biosynthetic pathways. It was expected that metabolic studies with labelled GA_{95} would give insight into A-ring hydroxylation and some understanding of the oxidising enzymes involved in the formation of GA_3 (**42**), GA_7 (**43**), *etc*[106].



(39)

Figure 9.

The combination of a 3β-hydroxyl and a Δ^1-double bond that is present in GA_3, (**42**) has been shown to be formed biosynthetically in maize from a Δ^2-double bond. Although GA_5 (**40**) possesses a Δ^2-double bond, it appears not to be the precursor of GA_3, or GA_{32} (**45**) (the most potent GA plant bioregulator) in *Prunus persica*[107,108].



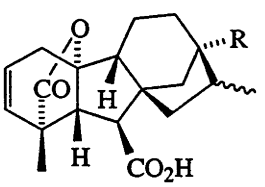
(40) R=OH; GA_5
(41) R=H; 2,3-dehydro GA_9
(42) R=OH; GA_3
(43) R=H; GA_7
(44) R=H; 1,2-dehydro GA_9
(39) R=OH; GA_{95}
(45) GA_{32}

Scheme 27.

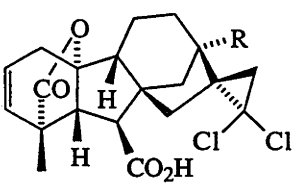
Similarly, GA_7, **43** has also been shown to be formed *in vitro* from the 2,3-dehydro GA_9, **41** in *Marah* and *Malus*[109]. This GA_9 derivative has not been isolated as a natural gibberellin, however, perhaps because it does not accumulate due to its slow formation and fast metabolism. Alternatively, its Δ^1-isomer **44** (GA_{120}) may be the precursor of GA_7. In fact, **44**, has very recently been found in *P. persica* by Koshioka[110]. It therefore follows that the analogue of **44**, namely GA_{95} (**39**), may be the precursor to GA_3, and perhaps GA_{32}, which has been isolated along with GA_{95} in several *Prunus* species[105].

The objective of this study was to prepare ^{14}C-labelled GA_{95}, which would then be used in feeding studies, so that its metabolism in *Marah macrocarpus* and various *Prunus* species may be followed.

It was hoped that through these feeding studies, an understanding of the mechanism involved in 3β-hydroxylation would be gained. Another objective was to study the effect of some semi-synthetic gibberellin growth retardants, such as dihydro-GA_5 (**46**), and dichloromethano-GA_5 (**47**) on 3β-hydroxylation. It has been proposed that these growth retardants act as competitive inhibitors of the enzyme responsible for effecting 3β-hydroxylation[111].



(46)



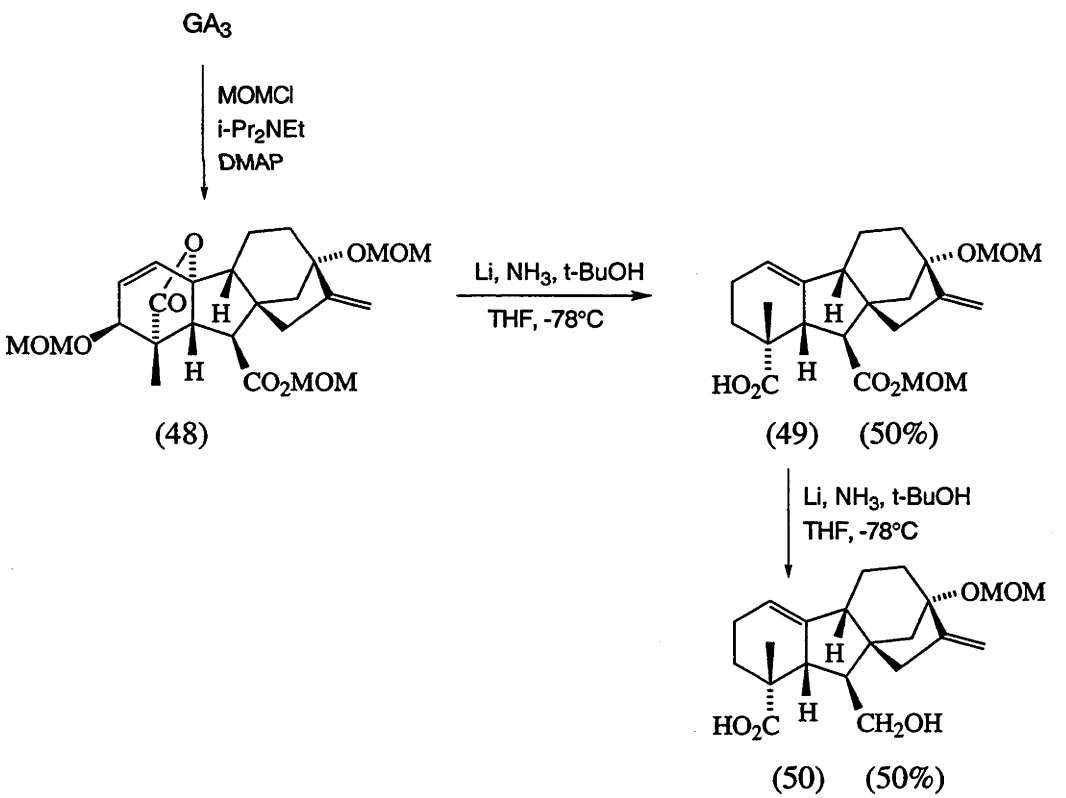
(47)

Figure 10.

The proposed route for preparing the radiolabelled GA_{95} was based on an earlier synthesis[105], but changed slightly in order to minimise handling of the gibberellin after incorporation of the ^{14}C-isotope at the C(17) position. The readily available GA_3 was used as starting material, which required removal of the 3β-hydroxy group as one of the major transformations. The allylic lactone in the A-ring of GA_{95} was found to be especially labile and easily rearranged to the isolactone. Thus, it was necessary and convenient to mask the Δ^1-double bond through part of the synthesis. For the same reason, the 7-carboxylic acid was protected as the methoxymethyl ester in the hope that its eventual removal would not disturb the allylic lactone.

5.2 *Reductive Elimination in the A-Ring*

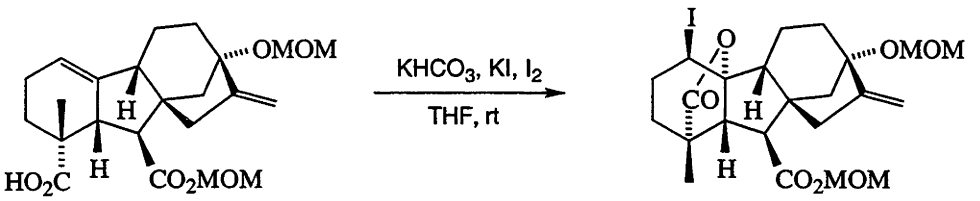
GA_3 was protected as the 3β,13-bismethoxymethyl ether 7-methoxymethyl ester, **48** by treatment with methoxymethyl chloride and diisopropylethylamine in dichloromethane in the presence of DMAP. The protected GA_3 derivative (**48**) was then subjected to reduction by dissolving lithium in liquid ammonia[112]. For the methyl ester, it is usual for these types of dissolving metal reductions to turn a persistent blue colour for a few seconds upon the addition of a slight excess of lithium, which indicates that complete reduction of the A-ring has occurred but that the ester group is still intact. However, in the system being studied, once the A-ring functionality had been reduced, the 7-MOM ester began to be reduced also. Hence, it was very difficult to establish the correct end-point for the reduction of the 3β-MOM ether. It was, therefore, necessary to follow the reaction by tlc analysis. The best yield was obtained when tlc indicated that no starting material remained. A 1:1 mixture of the desired acid **49** with carbinol **50** was then obtained, which could be separated by flash column chromatography.



Scheme 28.

5.3 *Iodolactonisation*

The reduction product was then transformed into the iodolactone **51**, by dissolving the acid **49** in THF and treating with potassium hydrogen carbonate, potassium iodide and iodine. The reaction proceeded smoothly to afford the 1-iodo lactone as a white solid in 88% yield. Evidence for the formation of the desired product could be seen in the ^{1}H nmr spectrum with the H(1) resonance at approximately 4.5 ppm, and in the ^{13}C nmr spectrum, the resonance from C(10) at 93.9 ppm.

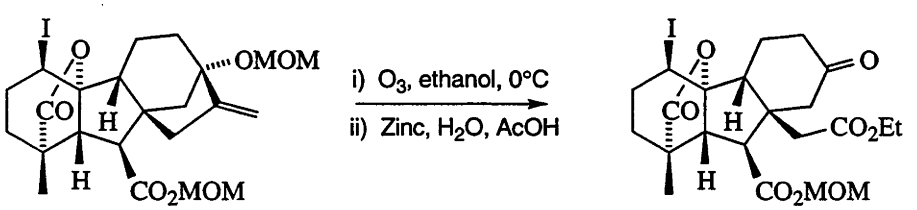


(49) (51) (88%)

Scheme 29.

5.4 *Ozonolysis*

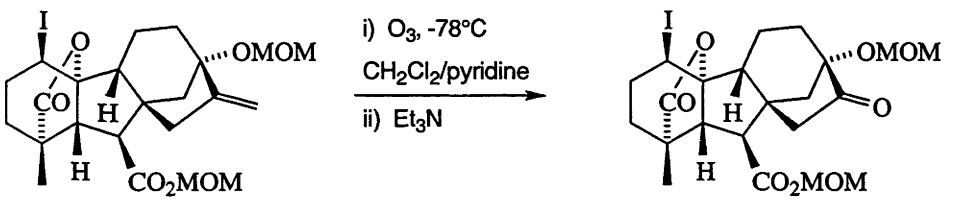
Because of the poor yield obtained during ozonolysis in section 4.4 using dimethyl sulfide as the reducing agent, alternative procedures were investigated for the GA_{95} substrate. At first ozonolysis was carried out in ethanol using zinc and acetic acid to decompose the ozonide. However, the crude ^{1}H nmr indicated that the major product was the 13-ketone-16-ethyl ester, (**52**), due to oxidative cleavage of the C(13)-C(16) bond.



(51) (52)

Scheme 30.

The procedure was repeated using a mixture of dichloromethane/methanol as the solvent, and triethylamine to decompose the intermediate peroxy methyl acetal. Unfortunately, cleavage of C(13)-C(16) bond occurred again, the crude ^{1}H nmr indicating that the 13-ketone-16-methyl ester was the major product. In order to circumvent this problem, a solvent mixture of dichloromethane/pyridine (4:1) was used with triethylamine as the reducing agent which alleviated the problem. However, to avoid decomposition products the reaction was not driven to completion; yields for ozonolysis were then reasonable, although still not very high: 56% for the desired norketone **53** and 16% of recovered starting material.



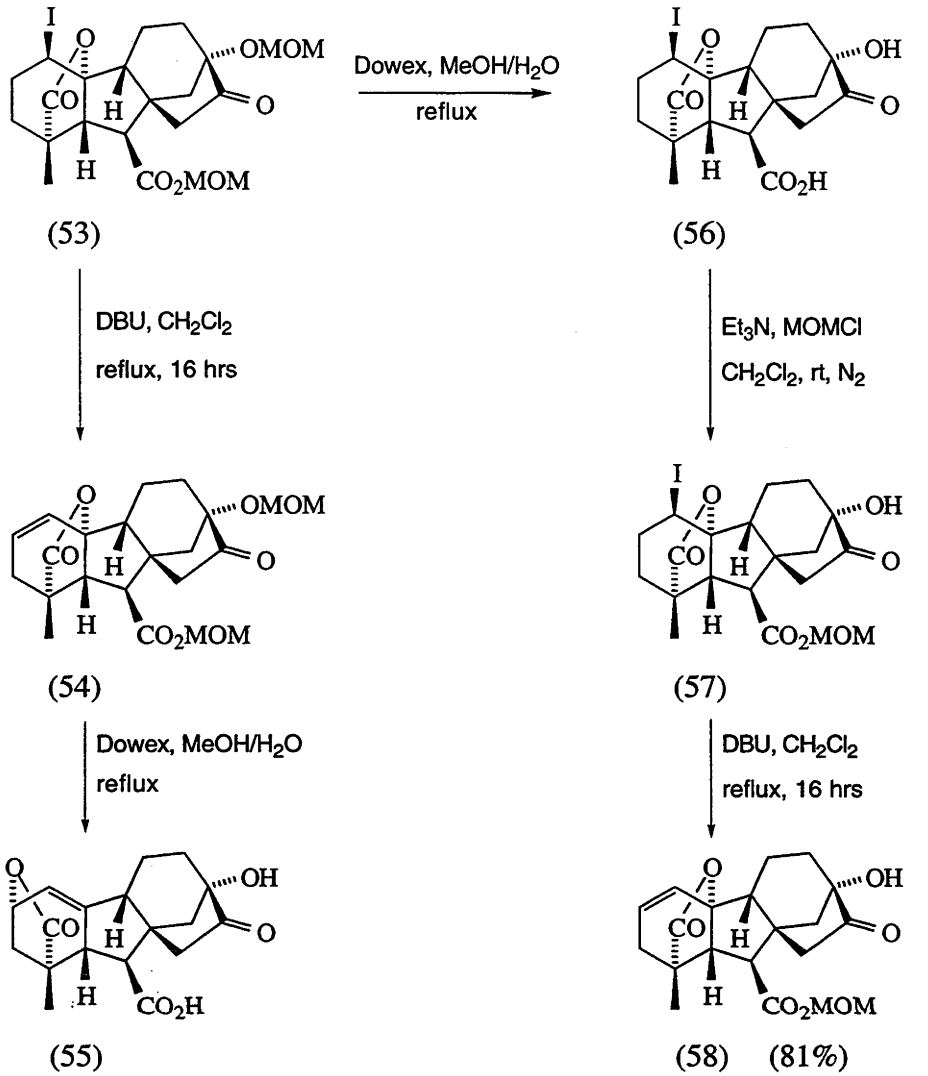
(51) (53)

Scheme 31.

5.5 Removal of 13-MOM Ether

Because of the extreme sensitivity of the allylic lactone structure in GA_{95}, it was vital to remove the 13-MOM ether prior to introducing the Δ^1-double bond, as treatment of the didehydro derivative **54** with Dowex resin[113], necessary for 13-MOM removal, rearranged the A-ring to afford the isolactone **55**, as experienced by Nakayama *et al.* when isolating GA_{95}[106].

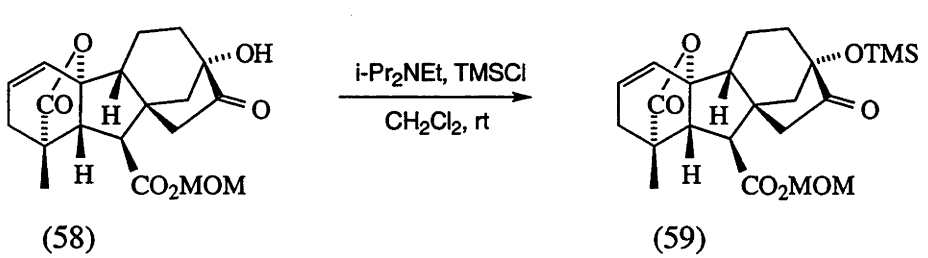
The norketone **53** was treated with Dowex resin in methanol/water (5:1) which removed both the 7-MOM ester and 13-MOM ether after heating at reflux overnight. To reinstate the 7-MOM ester selectively, the deprotected compound **56** was again treated with MOMCl, but triethylamine was used as the base instead of diisopropylethylamine and DMAP. Elimination of HI from **57** was achieved by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane. Once the Δ^1-double bond had been reinstated, the excess of DBU was removed by carefully washing with cold 1M HCl. GA_{95}-7-methoxymethyl ester-16-norketone (**58**) was isolated as a white crystalline solid (81% yield). H(2) was observed in the ^{1}H nmr spectrum as a doublet split into a triplet at approximately 5.9 ppm, and H(1) as a broad doublet at approximately 6.2 ppm.



(53) (56) (54) (57) (55) (58) (81%)

Scheme 32.

The TMS ether was successfully incorporated by treating the norketone with TMSCl in the presence of DMAP. An excess of diisopropylethylamine was also used to remove free HCl formed during the reaction, thus reducing the possibility of rearranging the A-ring. Purification was carried out by flash column chromatography using a buffered solvent system, thereby affording the TMS ether (**59**) as a colourless oil (73% yield).

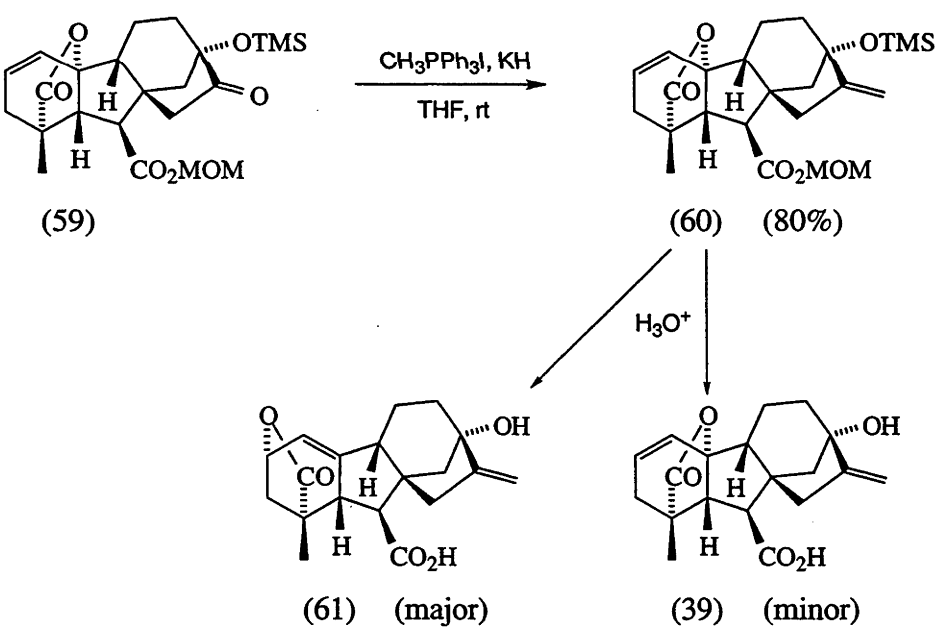


(58) (59)

Scheme 33.

5.6 *Methylenation and Deprotection*

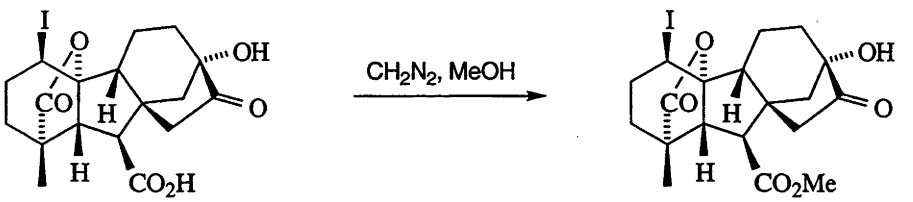
The Wittig methylenation of the 7-MOM ester-13-TMS ether was carried out first with the non-labelled methyltriphenylphosphonium iodide in exactly the same way as GA_{20} was methylenated. This resulted in an 80% yield of GA_{95}-7-MOM ester-13-TMS ether (**60**), with the 17,17 protons resonating at 4.9 and 5.2 ppm in the ^1H nmr spectrum. However, all attempts to deprotect the ester by acid catalysed hydrolysis resulted in the formation of a high percentage of the GA_{95}-isolactone **61**, as evidenced by resonances at 4.87 ppm for H(2) occurring as a triplet and 5.95 ppm of H(1) occurring as a doublet in the ^1H nmr spectrum . Dimethylboron bromide was also used at -78°C in dichloromethane for a very short period of time (approximately 5 mins.), but this resulted in a mixture of GA_{95} and the isolactone, and did not succeed in removing the 13-MOM ether. Because purification techniques (ie. HPLC) resulted in the formation of more isolactone formation, this route was therefore, considered inappropriate and it was decided that the 7-carboxylic acid should be protected as the methyl ester prior to Wittig olefination and then removed by the lithium thiolate procedure[103].



Scheme 34.

5.7 Protection as the 7-Methyl Ester-13-TMS Ether

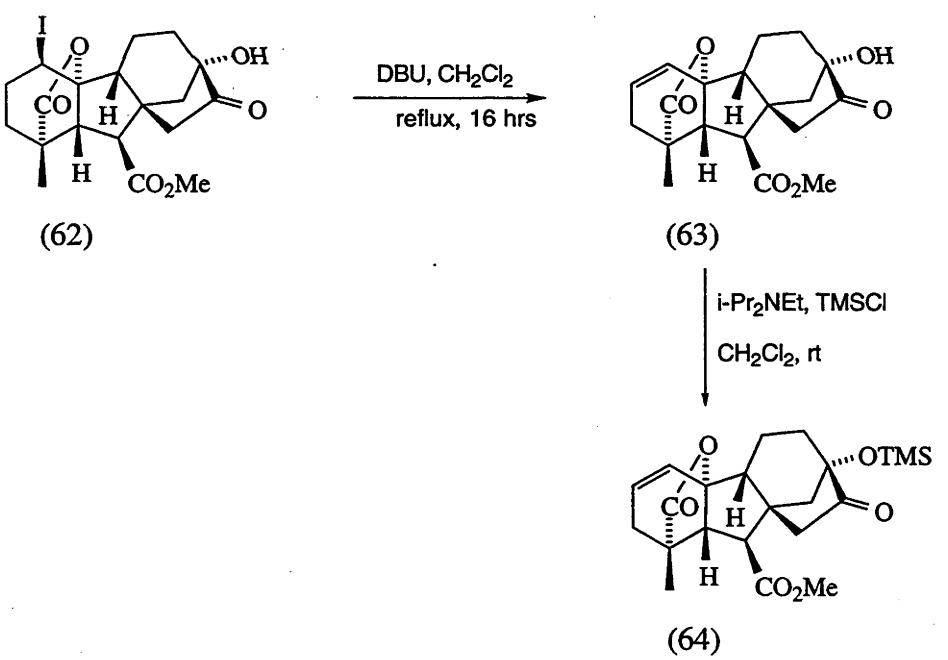
After deprotection of **53** using Dowex resin, the free acid **56** was protected as the 7-methyl ester **62** using diazomethane in methanol.



(56) (62)

Scheme 35.

The 1-iodide was eliminated as HI by treating **62** with DBU in dichloromethane to afford the 1,2-double bond in the GA_{95} derivative **63**. The 13-TMS ether was incorporated as before (section 5.5) with the same precautions to prevent isolactone formation.



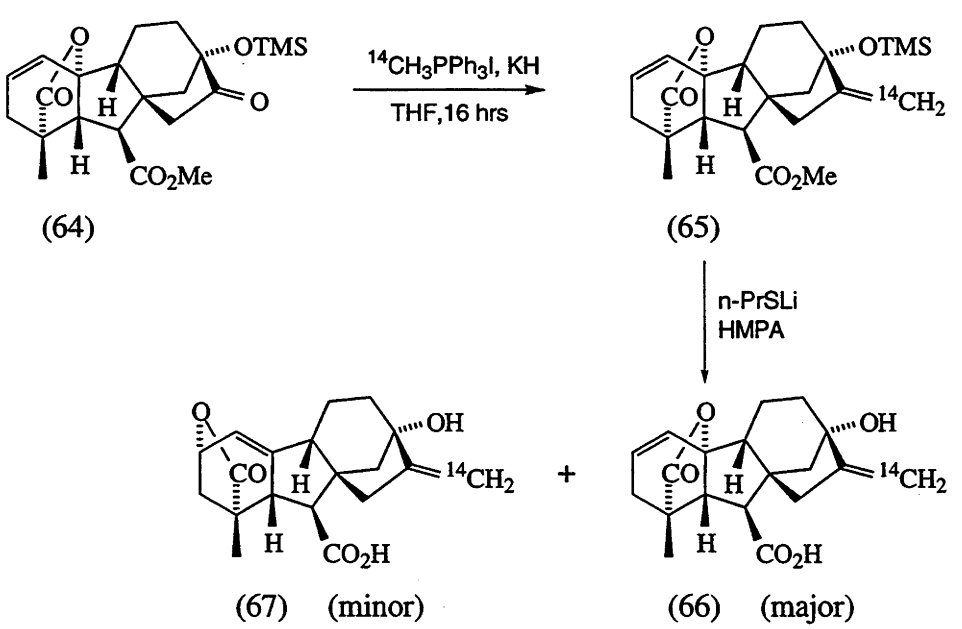
(62) (63) (64)

Scheme 36.

5.8 *Methylenation-Incorporating ^{14}C Isotope label; and Deprotection*

The radiolabelling procedure was carried out with all necessary precautions taken into consideration. This step was a replica of that described in section 4.6. Anhydrous conditions were maintained throughout the procedure, which was successful in producing 4.9 mg (74%) of the 17-^{14}C-radiolabelled GA_{95} derivative **65** after flash column chromatography.

The methyl ester was carefully treated with lithium thiolate by taking a solution of ester **65** in hexamethylphosphoramide (HMPA) and adding lithium thiopropoxide according to the standard procedure used in the Mander research group[103]. The ^{14}C-labelled GA_{95} substrate was purified by reverse phase HPLC. Any isolactone (**67**) which had formed throughout the reaction procedure or during HPLC was successfully removed at this time. The solvents used throughout HPLC were removed by freeze drying the labelled compound, as previously[105], since removal under reduced pressure resulted in isolactone formation due to acetic acid being present in the solvent system. This procedure afforded 180 μCi of the pure labelled GA_{95} (**66**) with a specific activity of 56 μCi/μmole. Throughout the synthesis, reaction conditions and yields were not optimised due to the urgency of the required labelled GA_{95}.



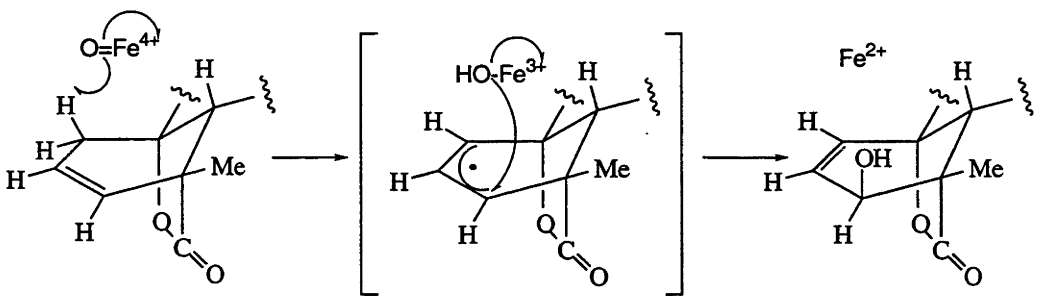
(64) (65) (67) (minor) (66) (major)

Scheme 37.

5.9 *Results of Feeding Study*

The ^{14}C-labelled GA_{95}, prepared above was incubated in tris buffer with a cell free enzyme preparation from *Marah macrocarpus*. GCMS analysis showed that only one product was formed, namely GA_3[114].

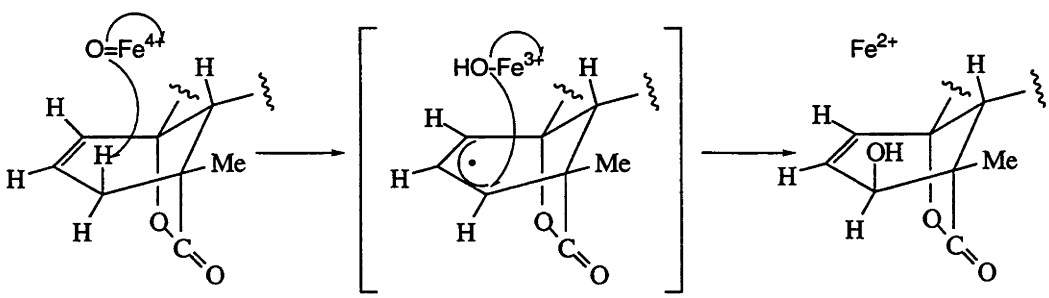
When observing the formation of GA_7, (**43**) from **41** (section 5.1, Scheme 27), it has been shown that the enzyme abstracts the 1β-hydrogen[109]. There are two possible pathways for this observation: (i) suprafacial oxygenation at C(3) concerted with hydrogen removal; or (ii) initial abstraction of hydrogen to form an allylic free radical, delocalised over carbons 1,2 and 3 with quenching by an oxygen ligand at C(3), as seen in Scheme 38.



(41) 2,3-dehydro GA_5 (43) GA_7

Scheme 38.

Formation of GA_3 (**42**) from GA_{95} (**39**) is consistent with the formation of an allylic free radical with initial abstraction of the C(3) hydrogen shown in Scheme 39.



(39) GA_{95} (42) GA_3

Scheme 39.

The radiolabelled GA_{95} will be used again in collaboration with Teikyo University Utsunomiya, Japan, to ascertain if GA_{95} is the biosynthetic precursor of GA_3 and/or GA_{32} in *Prunus cerasus* and other *Prunus* species.

CHAPTER 6

CONVERSION OF GA'S INTO KAURENOIDS BY EXPANSION OF THE B-RING

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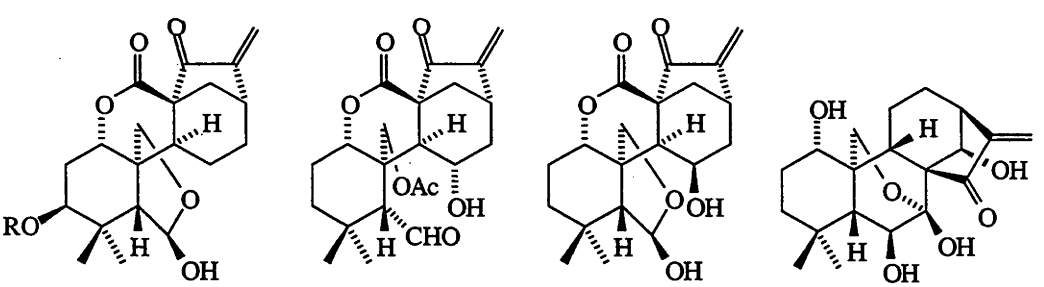
BACKGROUND

6.1 *Introduction*

Kaurenoids are a complex group of tetracyclic diterpenoids that have proven their utility in traditional Chinese medicine for the treatment of a range of conditions (especially those from the Rabdosia genus), from gastrointestinal disorders to cancer therapy[84], and anti-HIV activity[115,116]. Their isolation from plant species is restricted to very small quantities, sparking investigations into the chemical synthesis of these compounds. Thus, the preparation of semi-synthetic kaurenoids from gibberellins was an attractive possibility with the experience already gained in transformations of GA_3 to reinforce this approach.

6.2 *The Kaurenoids: Activity and Isolation*

Many kaurenoids were available in the past as by products of GA production from the fermentation of *G. fujikuroi*. However, in recent years with the use of current strains, only trace amounts of kaurene derivatives are being produced. In 1958, enmein (**68**) one of the major diterpenoid constituents of the *Rabdosia* genus was isolated, prompting an investigation into its structure[85].

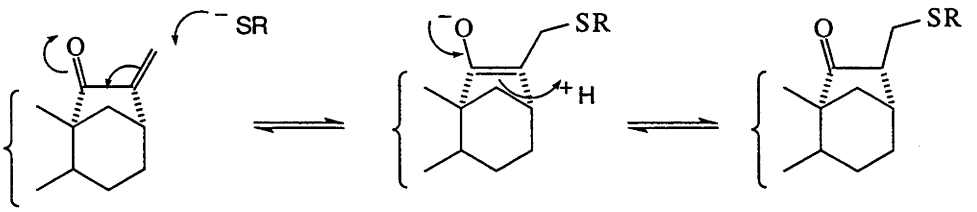


(68) R = H
(72) R = Ac

(69) (70) (71)

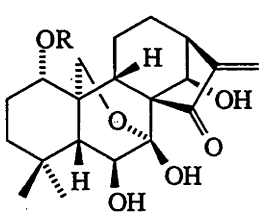
Figure 11.

Kubota and co-workers[117] reported high antibacterial activity for enmein (**68**), isodonal (**69**), nodosin (**70**), oridonin (**71**), and enmein-3-acetate (**72**) against Gram-positive bacteria. It was concluded that antibacterial activity depended upon the presence of the α-methylene-cyclopentanone moiety due to a Michael-type addition by a sulfhydryl containing enzyme (Scheme 40).

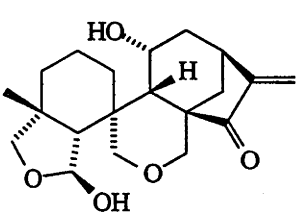


Scheme 40. Michael-type addition of a sulfhydryl enzyme to the α-methylene-cyclopentanone moiety.

In vivo antitumour activity against Ehrlich ascites carcinoma in mice was also examined and it was assumed that the α-methylenecyclopentanone moiety also played a role in antitumour activity by deactivating S-H enzymes (or S-H coenzymes)[116,119]. Oridonin (**71**) and lasiokaurin (**73**) were shown to possess even greater activity, which was attributed to H-bonding between the C(6)-OH and C(15)-carbonyl groups; the hydroxy groups at C(7) and C(14) were assumed to play a role as binding sites to specific enzymes in the tumour cells. Spirolactone-type diterpenoids have also been studied and shikodonin (**74**) has shown antitumour activity[87].



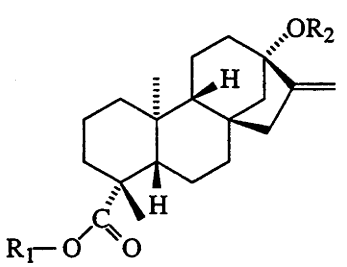
(71) R = H
(73) R = Ac



(74)

Figure 12.

Some kaurenes have also been shown to be efficient inhibitors of gibberellin biosynthesis in *G. fujikuroi*, and so may provide a basis for the design of novel plant growth regulators[120]. The potential of stevioside (**75**) and related rubosides as non-nutritive sweeteners has been investigated for a number of years[121] with patents being registered for their isolation and applications.



(75)

R_1 = β-D-glucosyl
R_2 = β-D-sophorosyl

Figure 13.

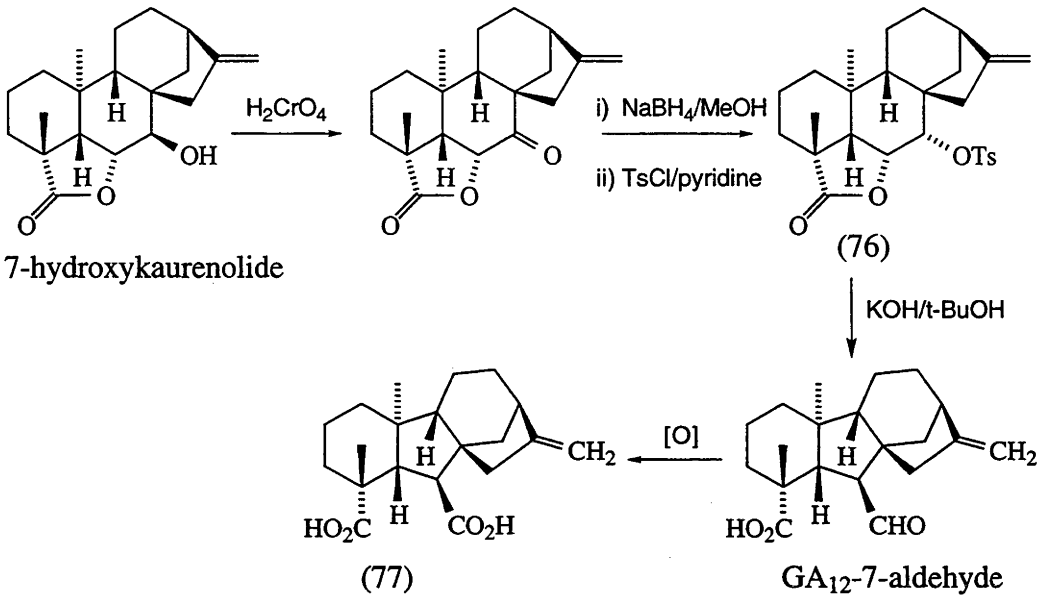
The above brief outline illustrates the therapeutic potential and possible industrial uses for kaurenoids and provides an impetus to produce these compounds synthetically. As a consequence, our attention has been focussed upon the conversion of the more accessible gibberellins into kaurene derivatives. Moreover, by extending the availability of kaurenes, access to the complete set of intermediates in the biosynthetic sequence in plants would then be provided, from kaurene through to GA_3.

6.3 Biological and Chemical Transformations of Kaurenes into Gibberellins

In the past, when kaurenes were produced as byproducts from the fermentation of *G. fujikuroi*, they were utilised in the formation of rare gibberellins. Transformations of kaurenes to GA's were possible by either (a) biological means or (b) chemical synthesis.

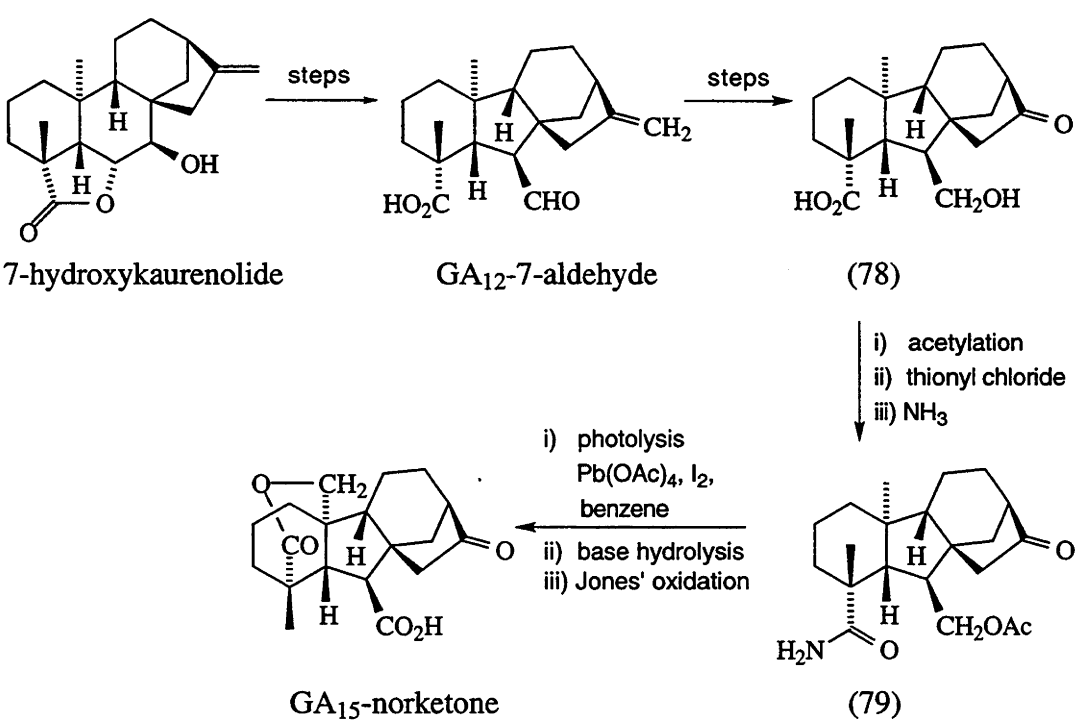
(a) By feeding isotopically labelled kaurene derivatives to the fungus, *G. fujikuroi*, it has been possible to trace the biosynthetic pathway of the labelled gibberellins produced[122]. These studies were made possible through the use of plant growth retardants which blocked normal GA biosynthesis at the stage between mevalonate and *ent*-kaur-16-ene. Hence, any gibberellins isolated should only have been biosynthesised from the labelled kaurene derivative.

(b) The first partial synthesis of a naturally occurring gibberellin was that of GA_{12} from 7β-hydroxykaurenolide, which was made available by isolation from the fermentation of *G. fujikuroi*[123]. The 7β-hydroxykaurenolide was converted in three steps to the 7α-toluene-*p*-sulphonate **76** which then underwent a pinacol-like B-ring contraction using potassium hydroxide in boiling *t*-butyl alcohol. This resulted in the formation of GA_{12}-7-aldehyde which was oxidised to acid **77** with Jones' reagent (Scheme 41).



Scheme 41.

For the ring contraction step to occur it proved essential to have the 7-tosylate group aligned antiperiplanar with the migrating C(6)-C(7) bond. It was therefore, necessary to invert the stereochemistry at C(7) by oxidation followed by borohydride reduction. Incorporating a ^{14}C-label into the C(17) position of the gibberellin after ring contraction, has allowed for the partial synthesis of labelled GA_{12}[123]. This five step sequence still remains the best procedure to obtain GA_{12} and its labelled derivative. GA_{15}-norketone has also been partially synthesised from 7-hydroxykaurenolide[124], through the transanular oxidation of the acetate-amide (**79**), Scheme 42.



Scheme 42.

6.4 *Proposed Synthesis of Kaurenoids from Gibberellins*

There are many challenges to be met in the synthesis of kaurenes. First, the need to address the densely arrayed functionality found in the biologically active members of this family of molecules. Secondly, the kaurenoids provide a challenging goal from a stereochemical standpoint with their multiple stereogenic centres. These factors make total synthesis extremely difficult and so it has been achieved only with long multi-step sequences and often poor overall yields. Moreover, in the main, the targets have been the simpler derivatives.

A study was therefore, proposed which would explore the prospect of utilising gibberellins as a source of semi-synthetic kaurenes. Such a conversion would take advantage of the extensive knowledge and wealth of experience already gained in the transformations of GA's, exploiting their highly functionalised nature to facilitate the preparation of the more complex kaurenoids.

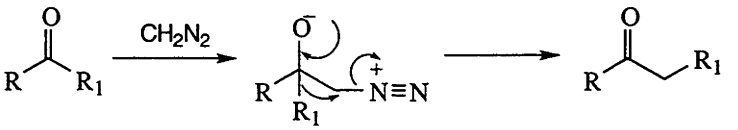
To convert a GA into a kaurenoid requires a one carbon expansion of the B-ring. Initial investigations were centred upon a GA_3-type skeleton due to its ready availability. The main objective was to extend the methodology to a C_{20} gibberellin, with a view to synthesising the more complex compounds which characteristically show greater therapeutic potential. Comprehensive reviews are available on the vast literature surrounding ring expansion methods that have been developed[125,126,127]. Therefore, only a selection of some of the more salient methods that may be of use in the context of the synthetic aims of this investigation are presented.

6.5 *Ring Expansion Reactions*

There are a number of procedures in which ring expansion can be effected. Often the main driving force for ring rearrangements is the release of energy from ring-strain.

6.5.1 *Diazoalkane: Carbon Insertion*

Diazomethane may act as a carbon nucleophile and functions by the insertion of a methylene unit followed by a migration step similar to that of a pinacol rearrangement as illustrated in Scheme 43.



Scheme 43.

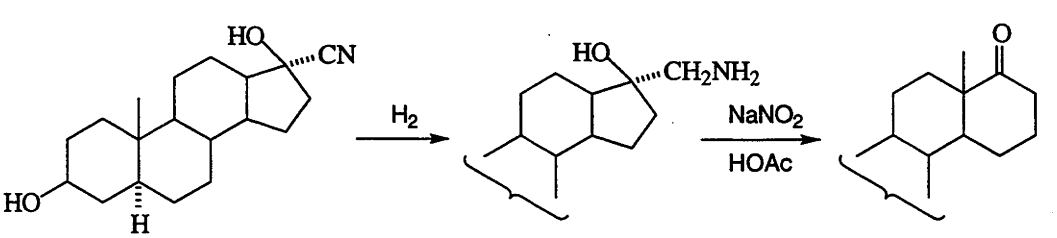
The main disadvantages of using diazoalkanes for ring expansion are that their use on unsymmetrical ketones can give rise to two products, with the result that control of regioselectivity can become a problem. The products can also continue to react with the diazomethane. Sometimes an epoxide can be the main product (Scheme 44).

Scheme 44.

The initial addition of diazoalkane to the carbonyl carbon can be catalysed by a variety of Lewis acids. For bridged bicyclones (at least), the regiochemistry of the carbon bond migration is largely dependent on conformational, rather than electronic factors[127].

6.5.2 Tiffeneau-Demjanov Carbon Insertion

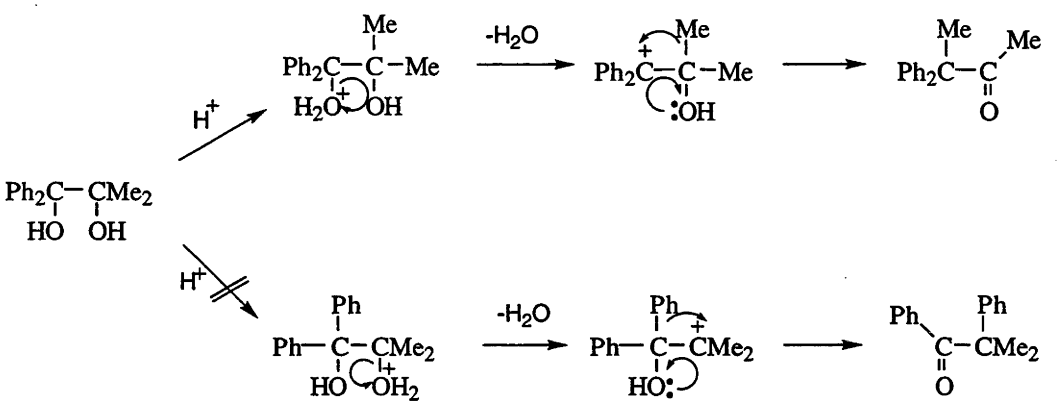
The Tiffeneau-Demjanov reaction is similar to the diazomethane based carbon insertion, in that a single carbon atom is inserted into a ring, and has often been successful where reactions with diazomethane have proven to be problematical. First of all, the ketone must be converted to an amino alcohol via the cyanohydrin. Ring expansion is brought about by treating this amino alcohol with nitrous acid. This procedure has provided one route to D-homosteroids[128].



Scheme 45.

6.5.3 *Pinacol Rearrangement*

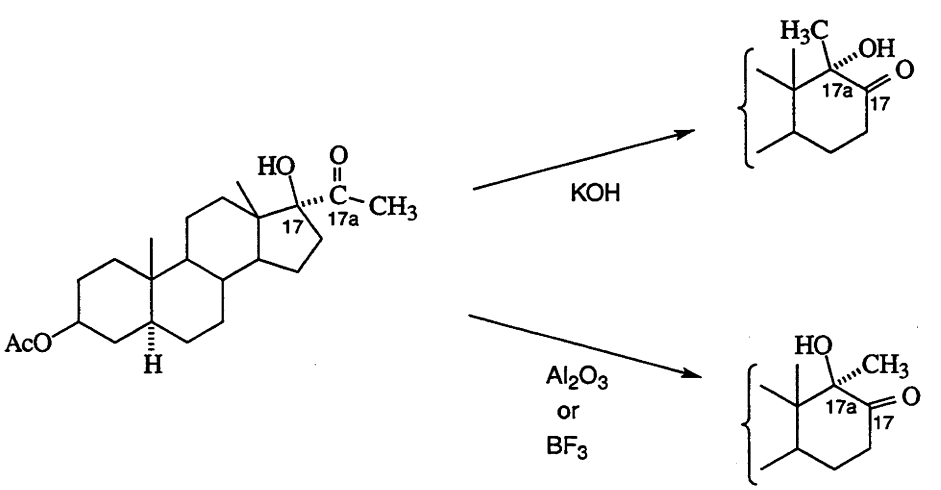
The Pinacol rearrangement is the migration of a functional group to a cationic carbon atom by acid catalysed rearrangement of 1,2-diols. For migration to take place the starting material must be able to adopt a conformation in which the leaving group is *antiperiplanar* to the group which is migrating. The migratory aptitude is of the order Ph > Me_3C > Me_2CH_2 > OMe > H. However, it must be noted that the hydroxyl which leaves is the one which can give the more stable carbonium ion, and this takes precedence over migratory aptitude.



Scheme 46.

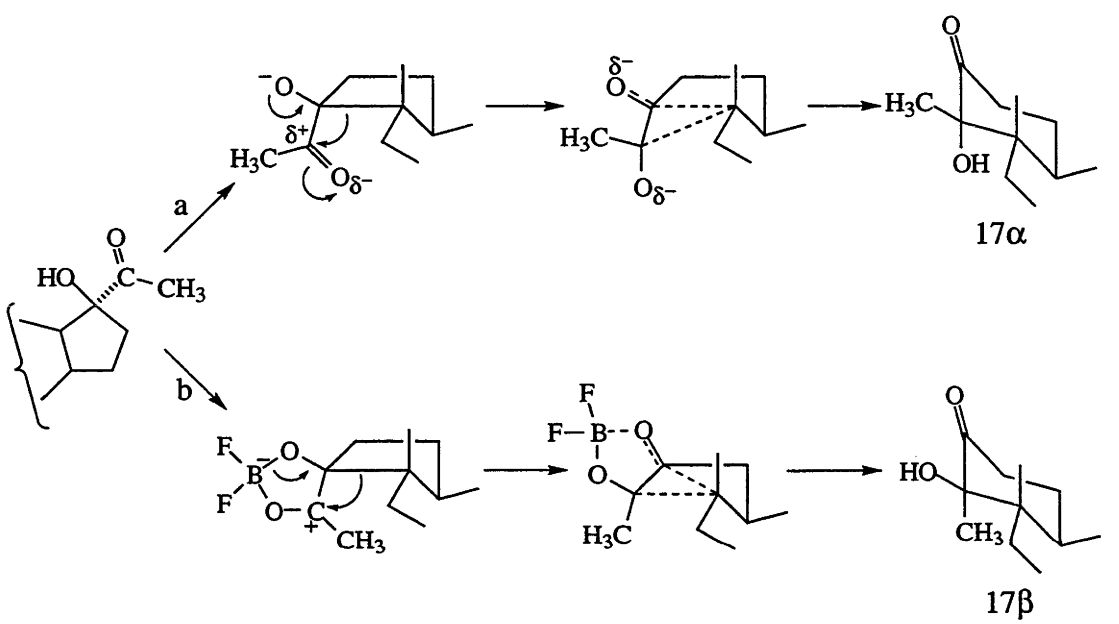
Thus, in the above example it is the methyl group that is found to migrate and not phenyl as would otherwise be expected.[129]

There are a great many examples of pinacol-like rearrangements in the literature[130-137]. The ring expansion of the D-ring in steroids has been facilitated by a number of methods of pinacol-like (acyloin) rearrangements promoted by Lewis acids or alkaline catalysis. One rearrangement which generated a lot of interest was the rearrangement of the 17α-hydroxy-20-keto steroids into ketols[130].



Scheme 47.

The geometry about carbon 17a was determined and found to be dependent on the reagent used to catalyse the rearrangement, by manipulating the orientation of the carbonyl group in the transition state. The reasoning behind these reagent-controlled rearrangements can be explained in the following way. Base-catalysed homoannulation (a) involves the removal of the 17β-hydroxy proton and produces a *trans alignment* due to the repulsion by the resulting negative charge on the oxygen, forcing the polarised carbonyl oxygen to assume a remote position(a, Scheme 48). Conversely, a Lewis acid such as borontrifluoride etherate coordinates with the two oxygen functions forcing them into a *cis* relationship with respect to each other (b, Scheme 48).



Scheme 48.

6.6 *Strategy for the B-Ring Expansion of GA3*

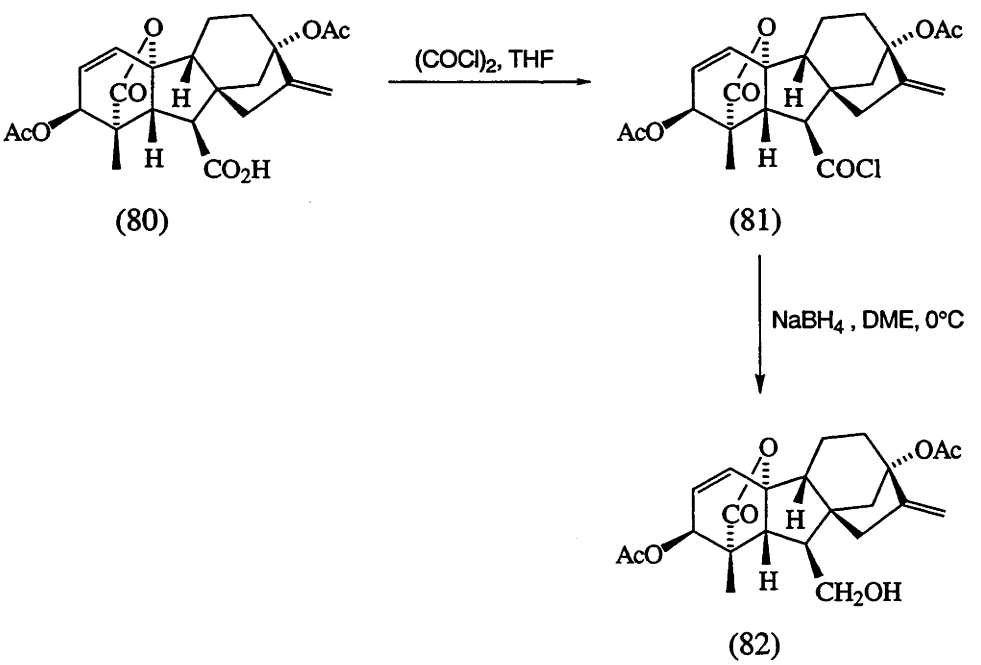
Given the literature precedent for ring expansion using pinacol rearrangements, it was expected that this protocol could be employed for the ring expansion of the gibberellins. To this end, initial investigations focussed on the pinacol-like rearrangement of a 6,7-diol with the primary alcohol activated as a mesylate to promote the rearrangement. Initially, GA_3 was chosen as a suitable model compound with as much functionality protected or removed in order to reduce the possibility of any side reactions.

CHAPTER 7

RING EXPANSION OF A GA_3-TYPE DERIVATIVE

7.1 *Functional Group Protection and Formation of the 7-Hydroxyl*

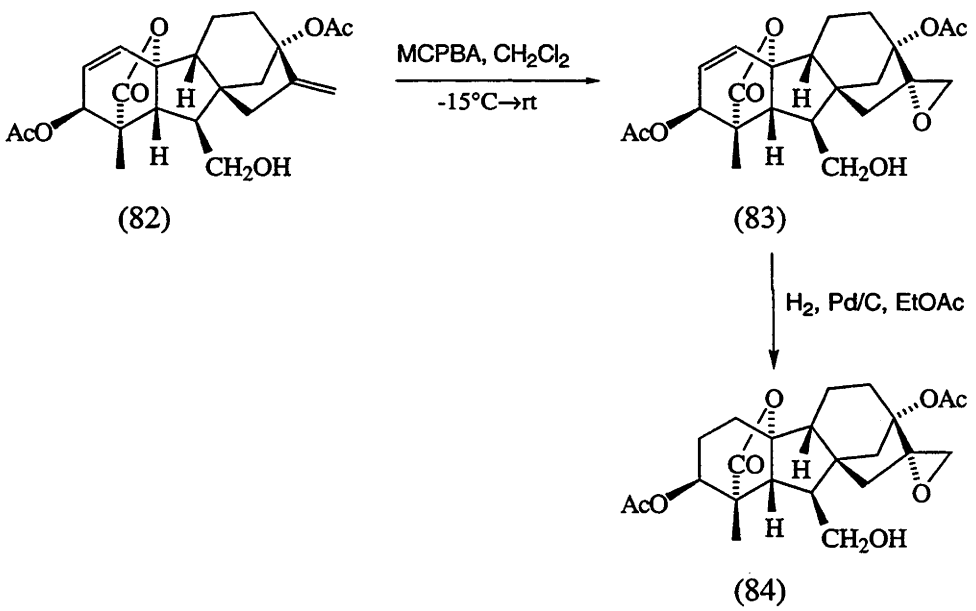
The 3,13-hydroxy groups of GA_3 were first protected as acetates using acetic anhydride, DMAP and diisopropylethylamine in dichloromethane. The next step in the synthesis was to form a carbinol at the C(7) position. It was envisaged that the simplest way to do this was by first forming the 7-acyl chloride[138], (in comparison to the formation of the anhydride[139]), and then to reduce this product to the 7-hydroxy derivative. To this end, the acyl chloride **81** was formed by the addition of the diacetate **80** in THF containing pyridine, via cannula, to a solution of oxalyl chloride in THF at -40°C. The acid chloride was filtered through celite and no other purification was carried out due to its sensitivity. C(7) was associated with a resonance at 173.9 ppm in the ^{13}C nmr spectrum, corresponding to an acid chloride. Reduction of the acid chloride was carried out by dissolving **81** in dry dimethoxyethane to which sodium borohydride was then added at 0°C, with some effervescence being observed. After 4 hrs the acid chloride had been reduced to the alcohol **82**, which was isolated as a white crystalline solid. The two protons attached to C(7) gave a multiplet between 3.67 and 3.85 in the ^1H nmr spectrum.



Scheme 49.

7.2 7-Hydroxyl Elimination to the 6-Ene

The next step in the sequence was the formation of the Δ^6-ene. Differentiation between the C(6) and C(16) enes would be required, and so it was found necessary to mask the Δ^{16}-ene prior to formation of the former function. This was effected by forming an epoxide at the C(16), C(17) position which could be reduced back at a later stage in the synthesis. Epoxidation was effected using *m*-chloroperbenzoic acid in dichloromethane, giving rise to a white crystalline solid in 77% yield after work-up. The respective C(17) protons were observed as doublets at 2.76 and 3.07 ppm, respectively, in the ^{1}H nmr spectrum. Since the Δ^1-ene could also prove problematic, and since this double bond was not required at a later stage, it was hydrogenated using a palladium catalyst under a hydrogen atmosphere to give the GA_1-type skeleton (**84**). The ^{1}H nmr spectrum showed the "disappearance" of protons H(1) and H(2) from 6.38 and 5.82 ppm respectively.

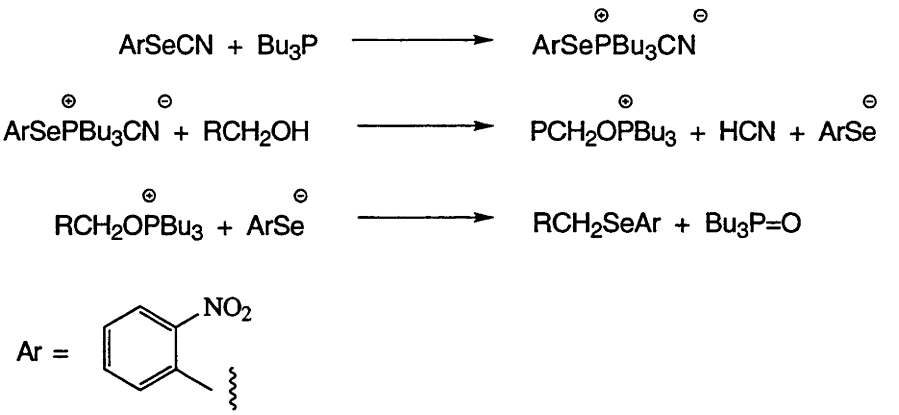


(82) (83) (84)

Scheme 50.

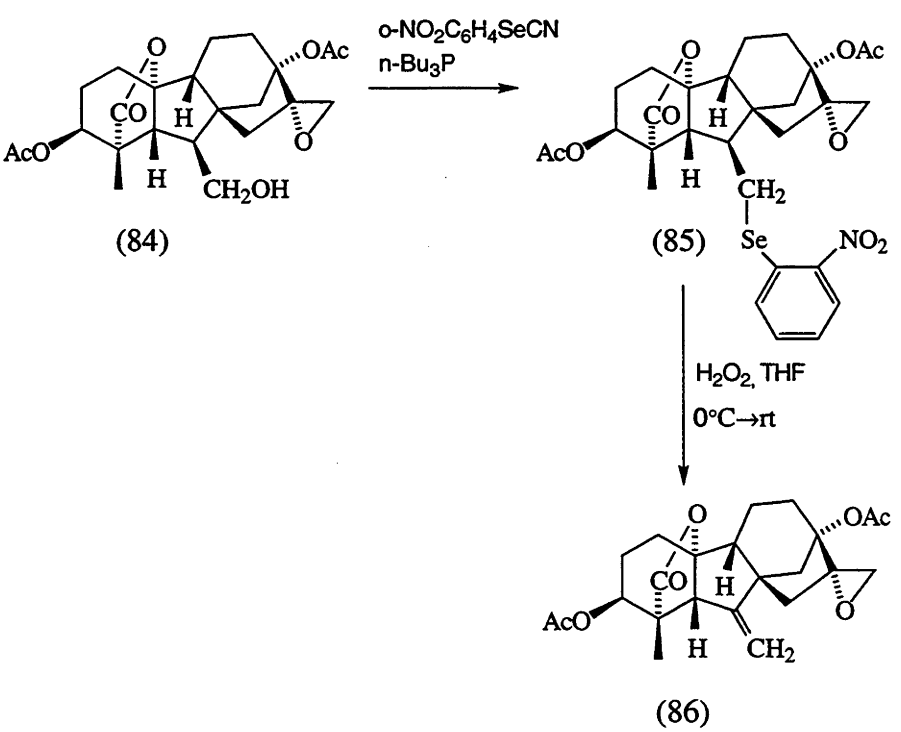
Formation of the Δ^6-ene was first attempted by forming the 7-mesylate, followed by treatment with DBU in toluene. However, this led to decomposition, and the starting material could not be recovered.

Elimination of the hydroxy group to form the exocyclic alkene was eventually carried out according to the procedure of Grieco *et al*[140]. This involves the direct one-step conversion of a primary alcohol to an alkyl aryl selenide using *o*-nitrophenyl selenocyanate[141] in THF at room temperature in the presence of tri-*n*-butylphosphine. The proposed mode of reaction is as follows:



The GA-*o*-nitrophenyl selenide (**85**) was formed in 89% yield as a yellow powder, the aromatic protons showing up very distinctly in the ^1H nmr spectrum between 7.36 and 8.28 ppm.

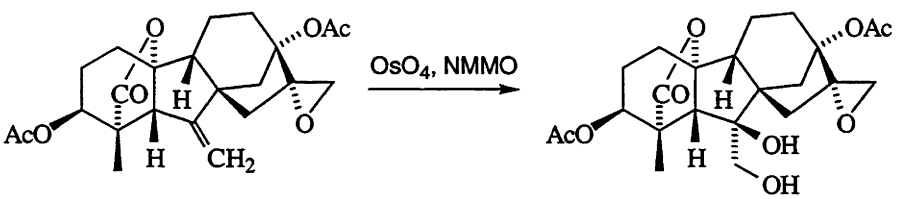
Elimination[142] of the derived primary alkyl *o*-nitrophenyl selenoxide was effected by stirring the selenide in a mixture of THF and 30% hydrogen peroxide at 0°C for 1.5 hrs, and warming to room temperature over 10.5 hrs, by which time no starting material was detected by tlc. The alkene (**86**) was isolated as a white solid in 90% yield. The protons of C(7) were located at 5.17 and 5.31 ppm in the ^1H nmr spectrum and C(7) itself was observed at 113.0 ppm in the ^{13}C nmr spectrum with C(6) at 151.5 ppm.



Scheme 51.

7.3 *Formation of the 6,7-Diol*

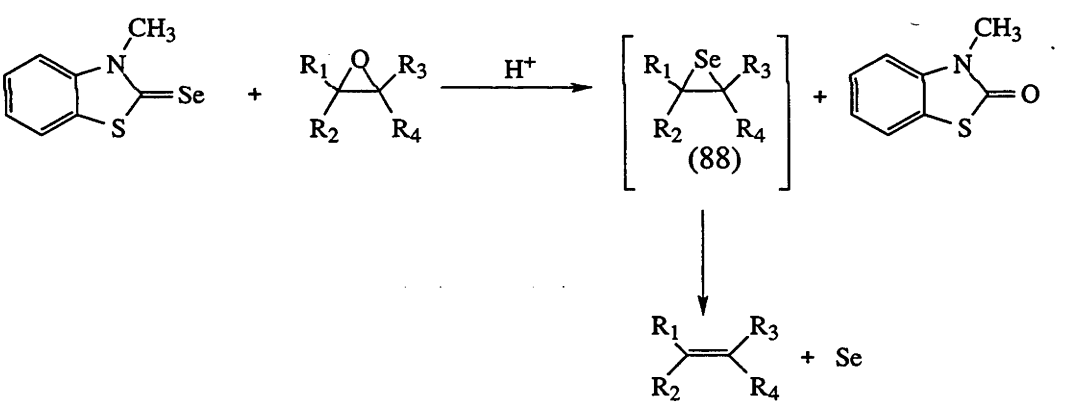
Dihydroxylation was performed on the 6-exocyclic methylene with OsO_4 using *N*-methylmorpholine *N*-oxide as a co-oxidant[100]. The mixture was stirred in acetone/water at 30°C for 9 days, after which time, a small amount of starting material still remained. However, the reaction mixture was worked up, the two compounds separated by flash chromatography, and the starting olefin recycled.



(86) (87)

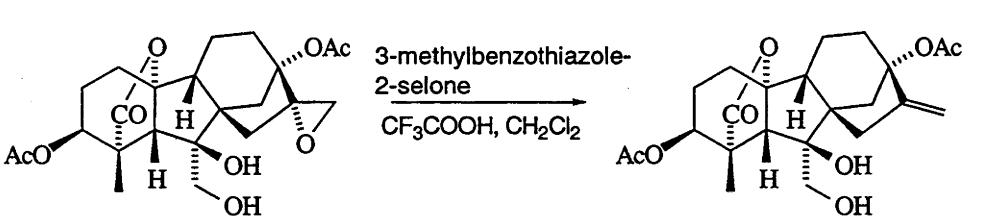
Scheme 52.

The 16-exocyclic methylene was then reinstated by deoxygenation of the epoxide using 3-methylbenzothiazole-3-selone in the presence of trifluoroacetic acid. First of all, an episelenide is formed (**88**) in the presence of the acid, then extrusion of selenium from **88** gives the alkene:



Scheme 53.

Unfortunately this procedure resulted in only a modest yield of the olefin, (48%), and no other compounds were recovered.



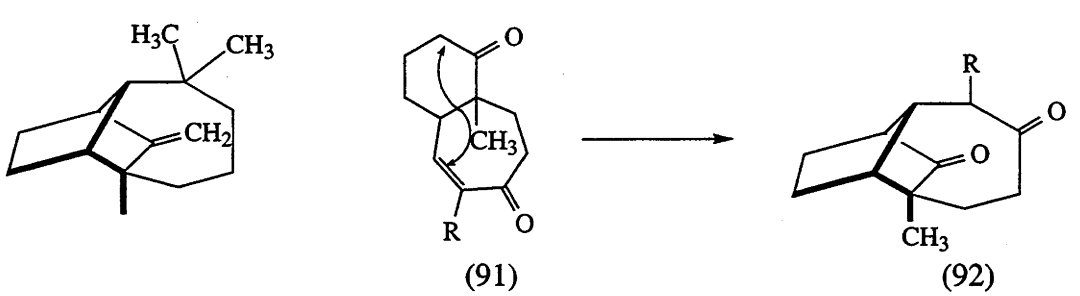
(87) (89)

Scheme 54.

In order to determine the stereochemistry of this diol, NOE experiments[143,144] were performed. Carbon and proton assignments were made through COSY and HETCOR spectra[143,144]. Prior to a NOESY experiment, a GNOE experiment was conducted (one dimensional gradient NOE experiment). In this experiment, a one dimensional spectrum is obtained showing only the resonances to which there is an NOE. The resulting spectrum has a large negative peak for the peak of interest at the centre, and small positive peaks indicating the resonances to which there is an NOE. When observing the H(7) protons a number of peaks were observed including 6-OH, H(15)α, H(5), H(15)β, H(14)α, H(1) and the largest of these H(14)β. An approximate 50% enhancement of H(14)β over the other peaks indicated that the 7-methylene was on the α-face of the molecule. To expand on this result, a NOESY experiment was also conducted. This clearly showed a cross peak correlating to an NOE between the 7-methylene and the H(14)β indicating that these protons are in close proximity to one another, but no cross peaks were observed for H(5) or the H(15) protons. This indicated that the OsO_4 would have approached the gibberellin molecule from the β-face, which is consistent with the reported protonation of a GA-6-enolate to afford a 6-epi-GA derivative[139].

7.4 Mesylation and Ring Expansion

Corey et al[131] used a variation of the pinacol rearrangement in their total synthesis of longifolene (**90**). The synthetic plan was to begin with a simple homodecalin precursor (**91**) which would cyclise to (**92**) by means of an intramolecular Michael addition.

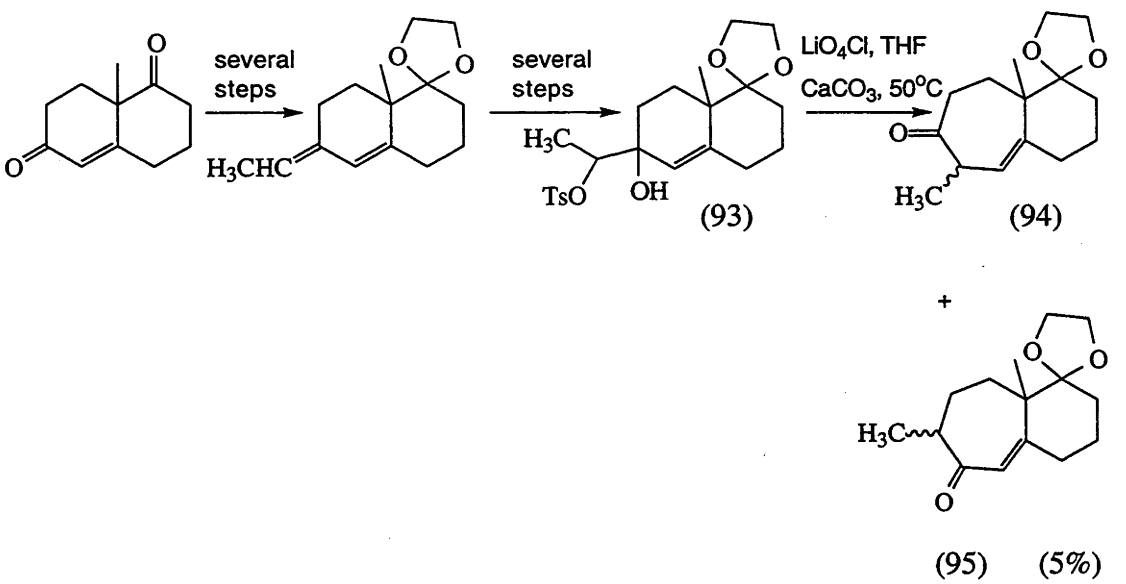


(91) (92)

Scheme 55.

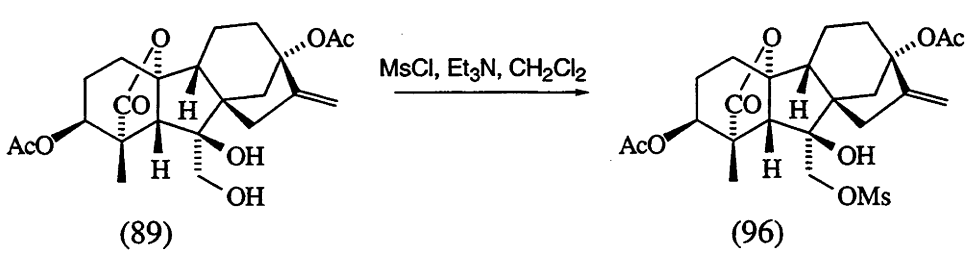
The authors decided to form the secondary mono-*p*-toluenesulfonate derivative (**93**), (Scheme 56), in order to form a better leaving group and in effect, activate the site for pinacol rearrangement. Because of the sensitivity of the ketal functionality and the

allylic *tert*-hydroxyl which could undergo acid catalysed elimination, the ring-expansion was to be carried out under essentially neutral conditions. The solvent used was THF, and lithium perchlorate was added until saturation occurred to facilitate ionisation of the *p*-toluenesulfonyloxy function. Calcium carbonate was also added to buffer the solution. The reaction mixture was heated at 50°C for 60 hrs, after which time a mixture of stereoisomers having different orientations of the methyl α to the carbonyl group were obtained in 48% overall yield (**94**). The alternative ring expansion product (**95**) was obtained in 5% yield. The authors had anticipated that the ring expansion would favour **94** over the alternative **95** because of the π electron participation of the migrating group, as opposed to the less favourable alkyl rearrangement for **95**.



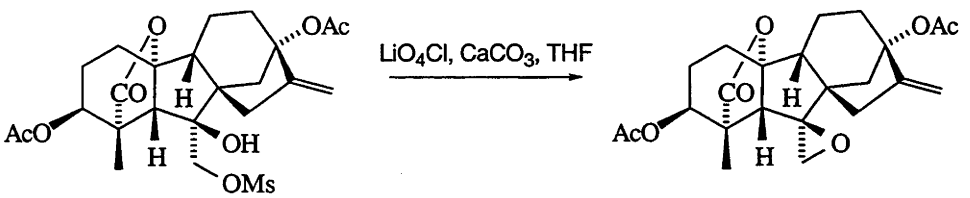
Scheme 56.

Prompted by this success, the 7-carbinol in **89** was to be derivatised so as to establish a good leaving group. An attempt to form the tosylate failed, and so the mesylate was produced by dissolving the diol in dichloromethane and adding triethylamine and methanesulfonyl chloride at -10°C[145]. The mesylate protons resonated as a large singlet at 3.15 ppm in the ^{1}H nmr spectrum.



Scheme 57.

Ring expansion of the mesylate was attempted via Corey's[131] ring enlargement procedure. The mesylate in THF was added to a suspension of lithium perchlorate and calcium carbonate in THF, followed by heating at 60°C for 5 days. However, after work-up and inspection of NMR spectra, in addition to starting material, only the 6,7-epoxide (**97**) could be detected.



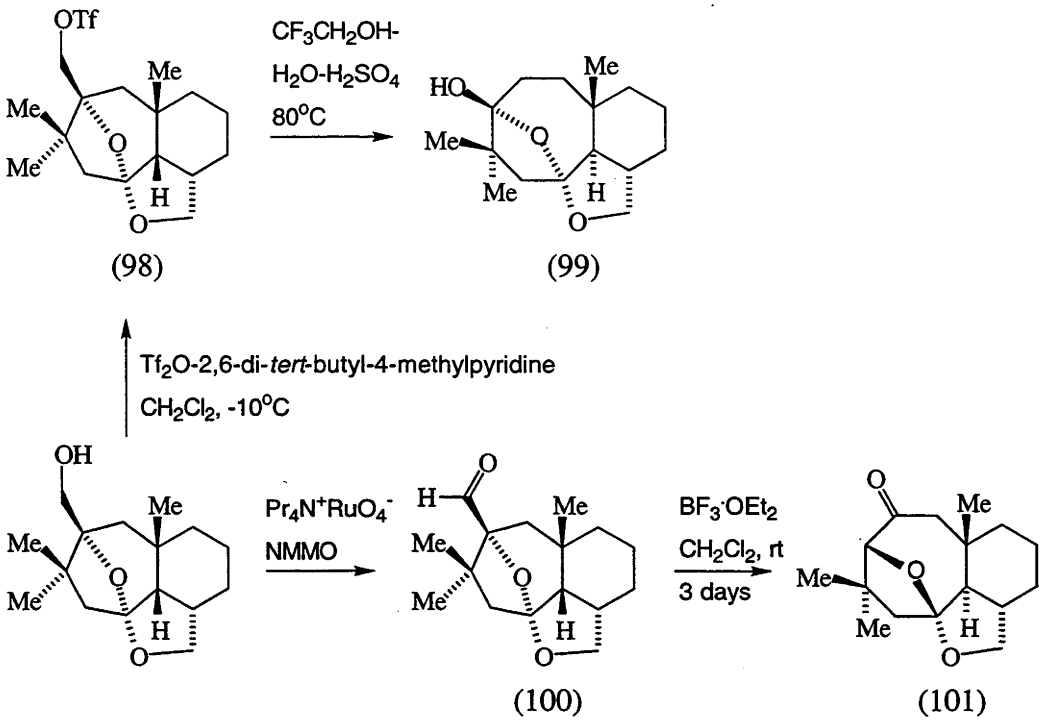
Scheme 58

The C(7) protons of the β-epoxide and a small amount of its epimer were observed in the ^1H nmr as two doublets at 2.73 and 3.16 ppm respectively.

The preparation of the analogous triflate was also attempted, but proved to be too unstable, eliminating to form the epoxide. Following Nagai's[146] procedure for ring enlarging an epoxide with boron trifluoride etherate, the 6,7-epoxide (**97**) was dissolved in benzene and treated with 3 equivalents of boron trifluoride etherate at room temperature. This resulted only in the formation of the aldehyde, however.

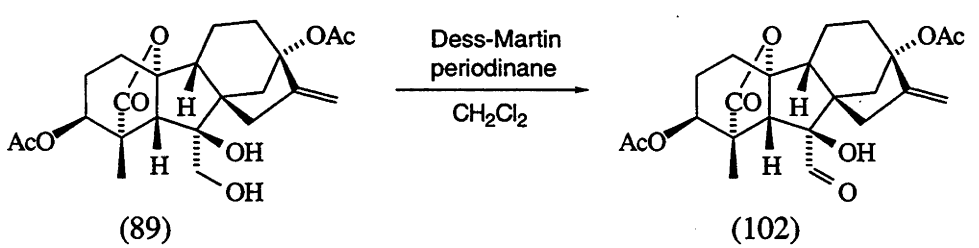
7.5 Acyloin Rearrangement of the Hydroxy Aldehyde

With the above ring expansion procedures showing no promise, attention was now focussed on the acyloin rearrangement that had been used for ring expansion of steroids[130] and which was also undertaken by Magnus and co-workers[132] for the formation of the eight membered B-ring of Taxol (Scheme 59). They had treated triflate **98** with CF_3CH_2OH-H_2O which had successfully resulted in conversion into the spiro-hemiketal **99**, but they had also formed aldehyde **100**, which underwent ring expansion followed by an acyloin-like rearrangement when treated with $BF_3{\cdot}OEt_2$ to give ketone **101**.



Scheme 59.

It was hoped that the strain in the GA skeleton would be of a sufficient magnitude as to favour rearrangement. In preparation for an acyloin rearrangement, the diol **89** was oxidised to the hydroxy aldehyde **102** using the Dess-Martin procedure. This proved successful as assessed by the observation of the proton on the 7α-aldehyde as a singlet at 9.67 ppm in the ^{1}H nmr spectrum of the product.

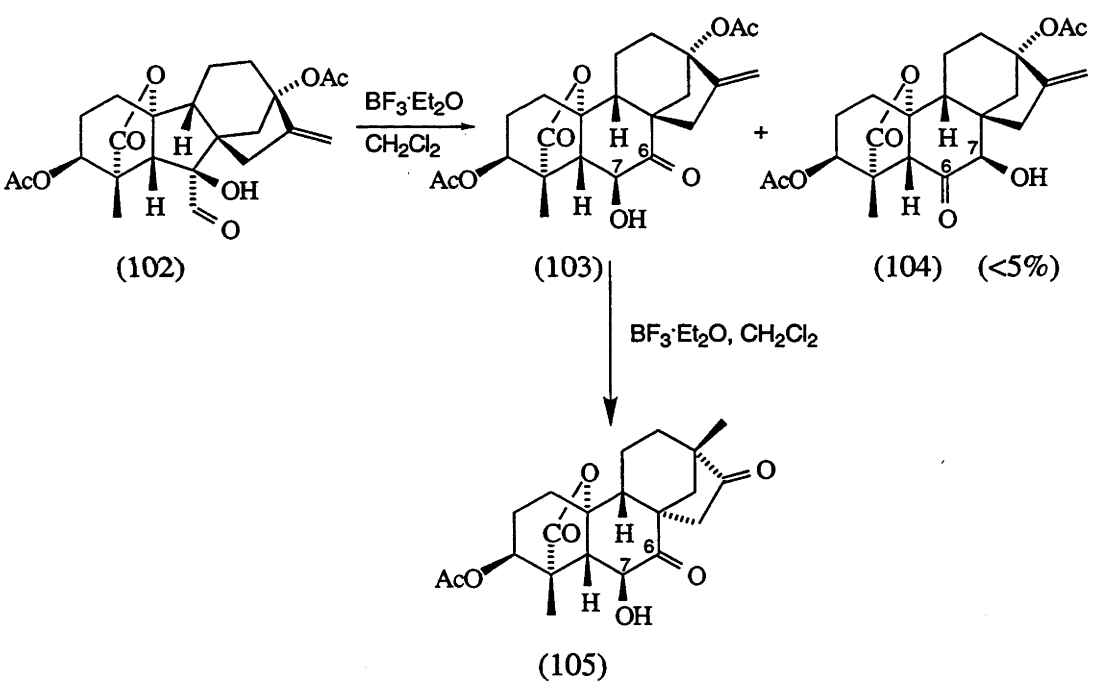


Scheme 60.

The hydroxy aldehyde (**102**)was then dissolved in dry dichloromethane and boron trifluoride etherate was added at room temperature. The mixture was stirred overnight and tlc indicated three new products. At least two of the products proved to be ring expanded compounds, one of which appeared to be a ring expanded compound that had also undergone C/D-ring rearrangement (**105**), (Scheme 61).

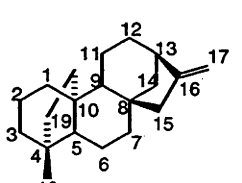
The structures of these products were determined by ^1H nmr spectra. For the main product, it was rationalised that the C(5)-C(6) bond was the migrating bond, giving (**103**), since H(5) was shown to be a doublet, and H(6)§ a doublet of doublets in the expected chemical shift range, with a vicinal coupling constant of 10 Hz in the ^1H nmr spectrum[147]. The structure of (**105**) was determined by the obvious lack of the C(17) methylene protons, loss of the 13-acetate, and the appearance of a second methyl peak at 1.35 ppm. The C(5)-C(6) bond was again the migrating bond as the chemical shift and coupling pattern for H(5) and H(6) was similar to that of (**103**). The third product, could not be identified immediately, since it could not be isolated and was only observed by tlc.

It is not surprising that the beyerane analogue (**105**) is obtained, as it would be expected that the labile D-ring would undergo rearrangement when exposed to Lewis acids. The prolonged exposure to boron trifluoride etherate engenders D-ring rearrangement and so it was hoped that a shorter reaction time would prevent this process from occurring.



Scheme 61.

§ The carbon and proton numbering system of the ring expanded products is based on the kaurene numbering system.



The reaction was repeated exactly as before except that the reaction time was reduced to 30 mins. No starting material remained after this time, and no C/D-ring rearrangement was observed. When the reaction was repeated on a larger scale, the third product mentioned above, was isolated and identified as **104**, resulting from the alternative migration of the C(8)-C(6) bond.

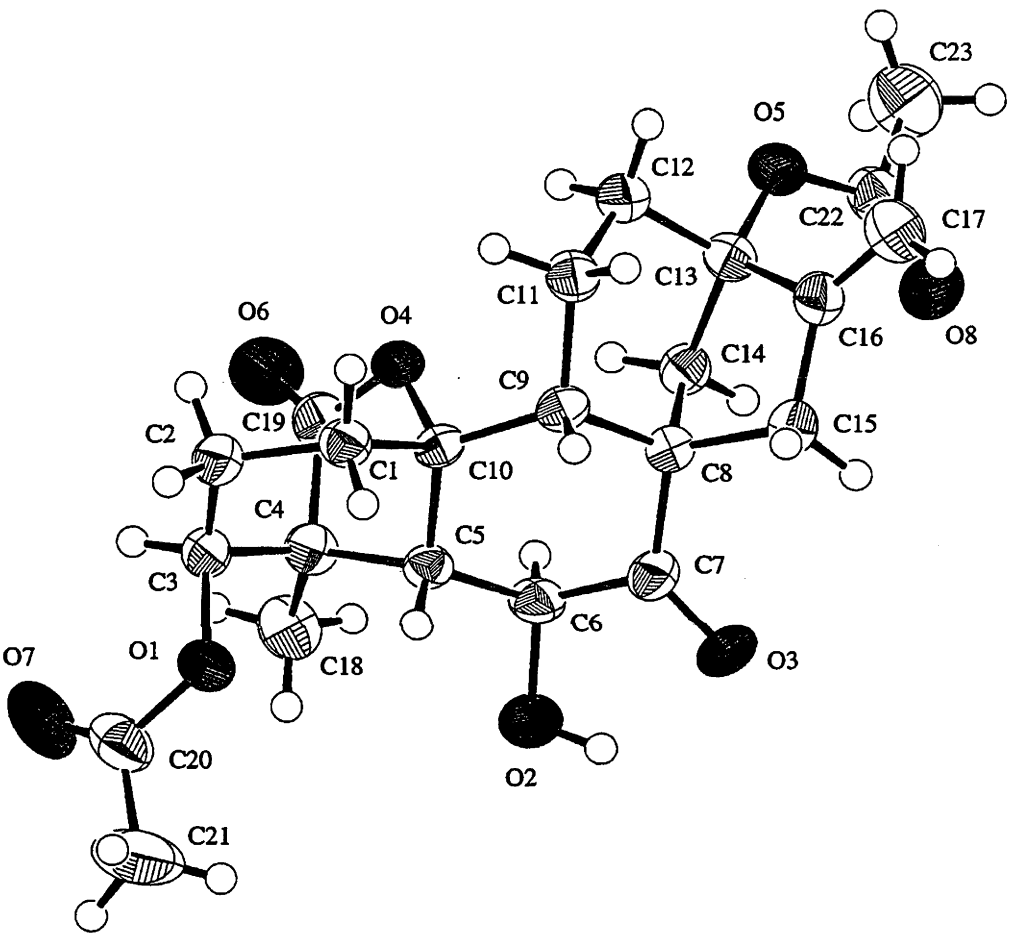
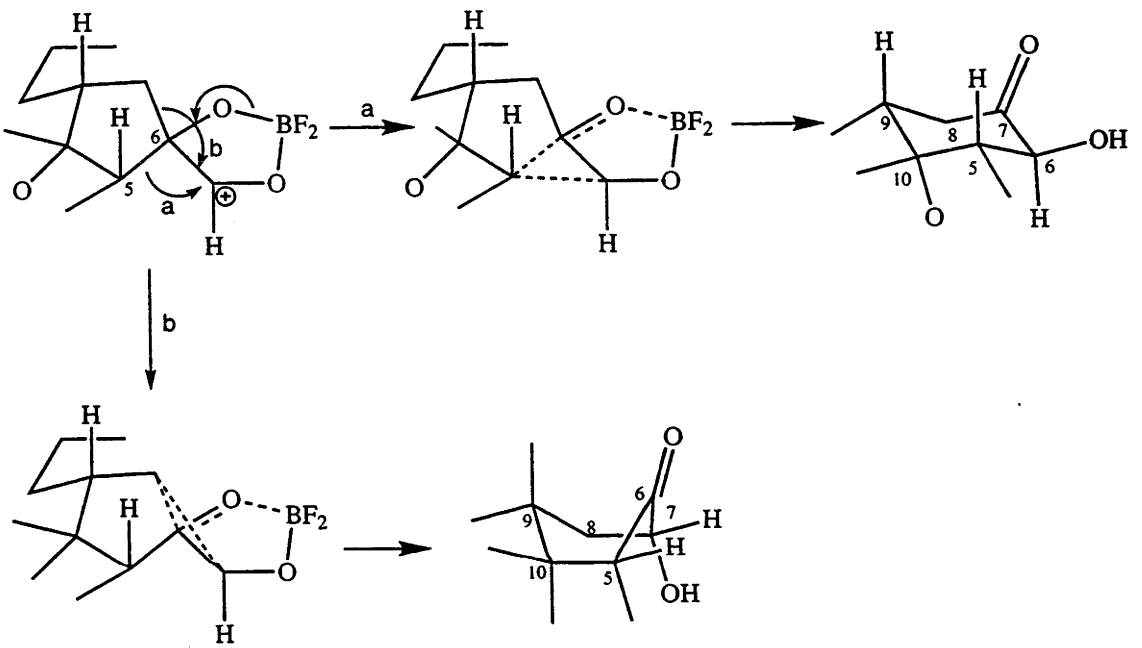


Figure 15.

The assignment of **103** was confirmed by a single crystal X-ray structure£ which clearly showed a C(7) carbonyl and a 6β-hydroxyl (Figure 15). It was also instrumental in showing a change of conformation in the C-ring. In gibberellins, the C-ring is normally in a quasi boat conformation[148]. But following the ring expansion, the C-ring had adopted a chair conformation.

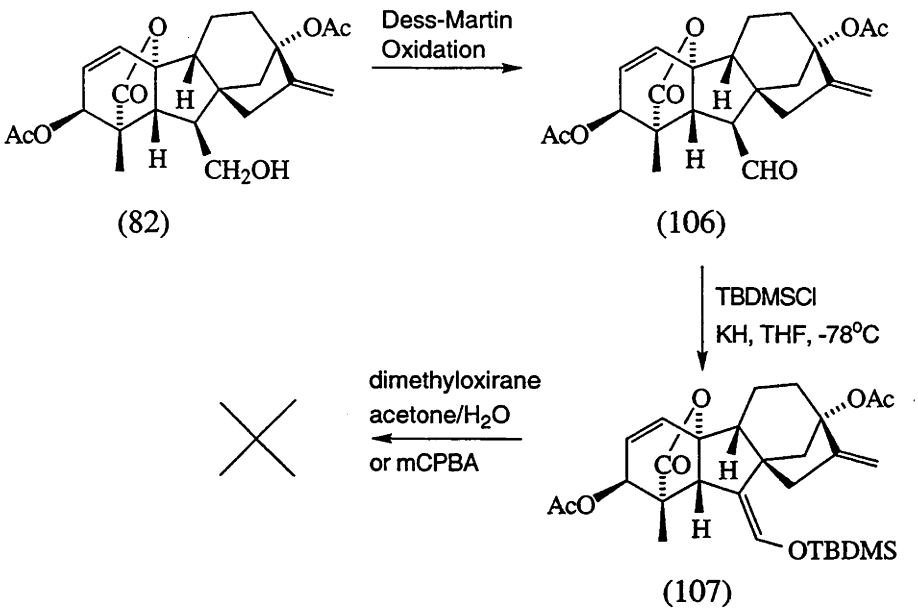
£ See Appendix II for X-ray Structure Report.

The energy released by this conformational change would contribute to additional strain relief in the gibberellin skeleton. The X-ray structure clearly shows **103** to possess a chair conformation for the B-ring. If **103** is assumed to be the kinetic product of the ring expansion, then its formation may be regarded as the more favourable due to the chair-like transition state for the C(5)-C(6) bond migration (a). Whereas, migration of the C(8)-C(6) bond (b) would lead to a boat conformation for the B-ring, a less favourable pathway in terms of strain which is exhibited by the less than 5% yield for this process (Scheme 62).



Scheme 62.

The steps in this synthetic sequence are generally high yielding, apart from the deoxygenation of epoxide **87**. However, this step could be avoided by either protecting the 16-methylene with a different function or by preparing the hydroxy aldehyde (**102**) by an alternative route. It would indeed, improve the synthesis if the hydroxy aldehyde could be prepared directly from aldehyde (**106**). **106** was formed by oxidising the alcohol **82** with Dess-Martin reagent in dichloromethane. The *t*-butyldimethylsilyl enol ether **107** was then prepared by dissolving the dry aldehyde **106** and *t*-butyldimethylsilyl chloride in dry THF at -78°C. Potassium hydride was added to the mixture, which was allowed to warm to room temp with stirring. An attempt to oxidise the enol ether with dimethyldioxirane to the hydroxy aldehyde failed however, and aldehyde **106** was recovered. Oxidation of the enol ether with *m*-chloroperbenzoic acid was attempted, but was also unsuccessful.

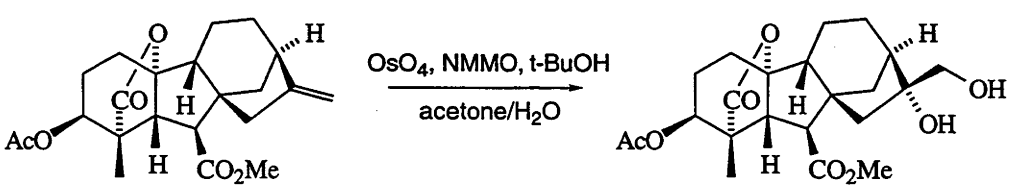


(82) (106) (107)

Scheme 63.

7.6 D-Ring Expansion

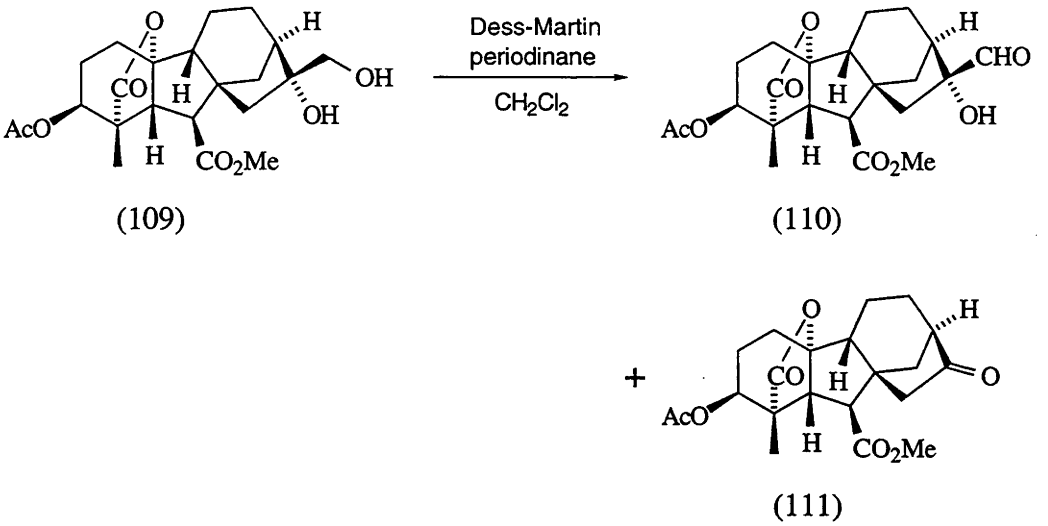
To further probe these ring expansions, an attempt was made to expand the 5-membered D-ring of a gibberellin. GA_4-methyl ester, 3β-acetate was readily available and so was chosen as a suitable substrate for investigation. A three step synthesis was all that was required in order to obtain a suitable intermediate, as the 16-methylene group needed only to be dihydroxylated, oxidised to the hydroxy aldehyde, and then treated with boron trifluoride etherate. Using standard reaction conditions, the 16-ene (**108**) was oxidised to the diol (**109**) in a quantitative yield using OsO_4 with NMMO as co-oxidant.



(108) (109)

Scheme 64.

The diol was then oxidised using the Dess-Martin procedure, which afforded the hydroxy aldehyde (**110**) in 30% yield and also the 16-norketone (**111**) due to over oxidation (22% yield).

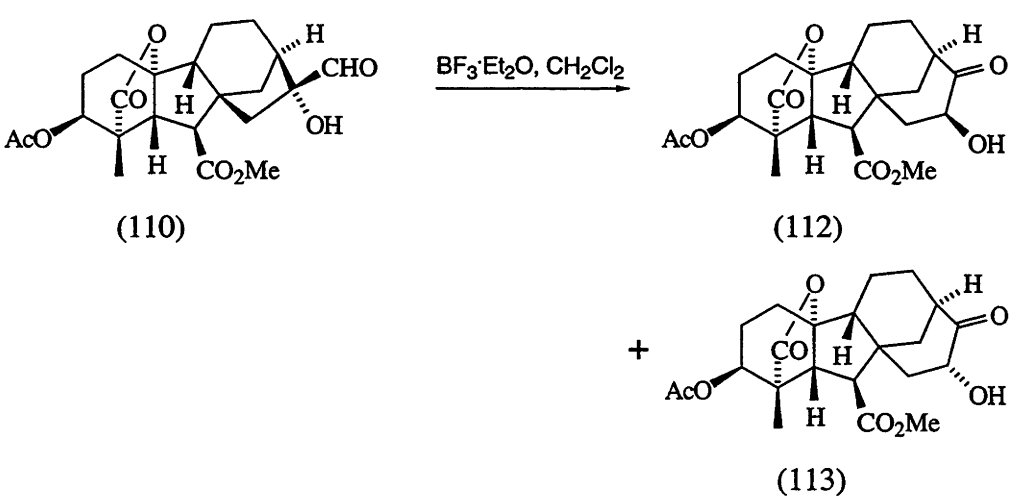


(109) (110)

(111)

Scheme 65.

The hydroxy aldehyde was taken up in dry dichloromethane and treated with 1.3 equivalents of boron trifluoride etherate which, after 30 mins, furnished a 1:1 mixture of ketols, **112** and **113** in a quantitative yield. The two epimers could not be completely separated by chromatography and only tentative ^1H nmr assignments could be made. In both cases, it was proposed that the C(15)-C(16) bond migrates to C(17) forming a 6-membered ring supposedly in a chair conformation. Approximately 50% of the material has the C(17)-OH on the α-face of the molecule with the remainder on the β-face.



(110) (112)

(113)

Scheme 66.

7.7 Continuing Studies

Having successfully ring expanded the B-ring to a 6-membered ring, a further study designed to explore the accessibility of the C_{20} kaurenoid lactones was considered, making it possible to synthesise some of the more complex kaurenoids from a C_{20}-gibberellin.

CHAPTER 8

RING EXPANSION IN GA_{15}

8.1 *Introduction to C_{20}-Kaurenoids*

Following on from the success of the B-ring expansion model study in Chapter 7, our attention was now focussed on the preparation of a C_{20} kaurene derivative, with the intention of producing complex kaurenoids that show interesting therapeutic potential[84]. Of particular interest are the *seco*-B-ring diterpenoids of the *Rabdosia* family (see Chapter 6.2) which have shown antibacterial and antitumor activity such as enmein[85], nodosin[149], oridonin[86], and shikodonin[87].

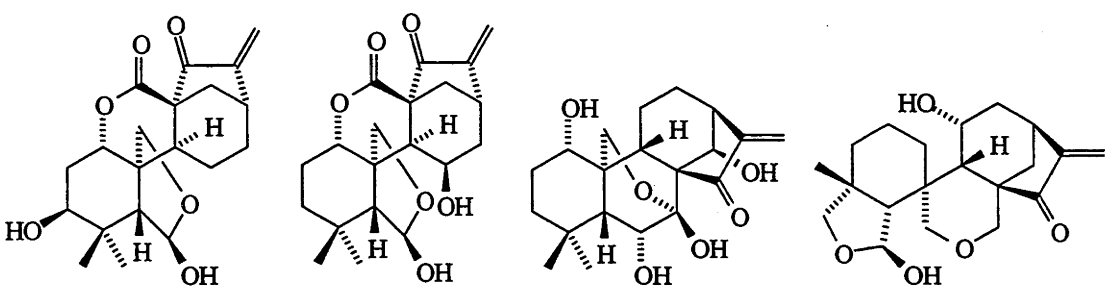
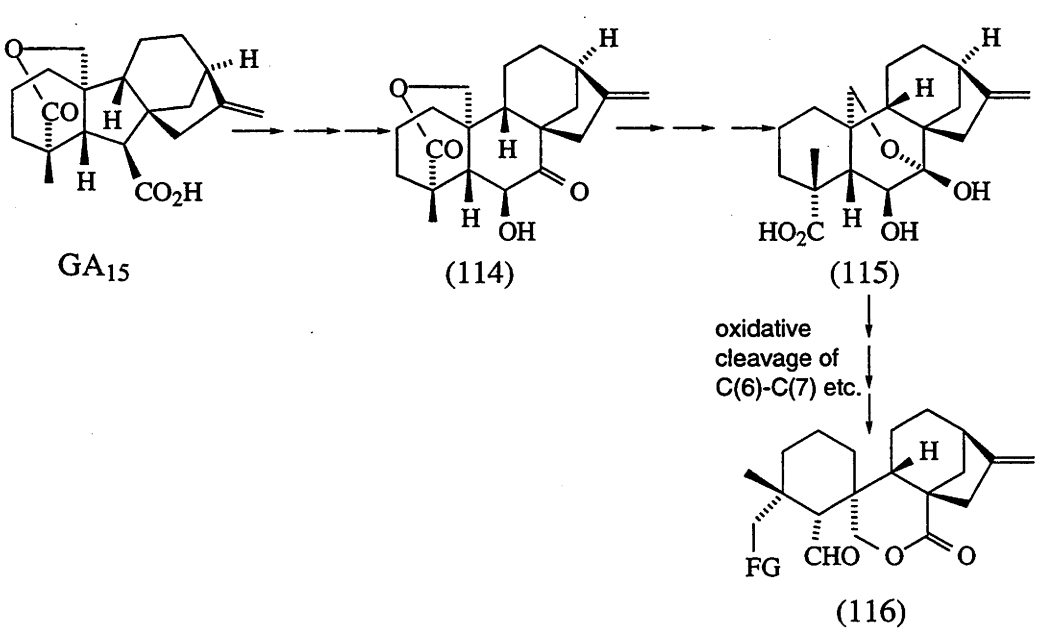
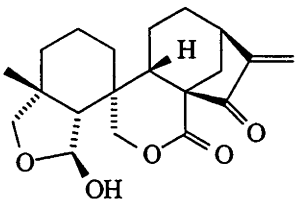


Figure 16.

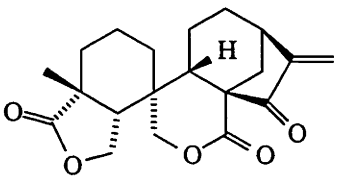
By starting with a C_{20}-gibberellin, most of the skeletal requirements for the above complex derivatives are already in place. It was hoped that ketol **114** could be prepared from the gibberellin, GA_{15} (Scheme 67), using the methodology established in Chapter 7. Ketol **114** could then be transformed into **116**, via the hemiacetal **115**, by following the strategy used in the synthesis of 15-desoxyeffusin[150], a shikodonin-type spirosecokaurene. This synthetic strategy would then give access to kaurenoids macrocalyxoformin (**117**)[151] and longirabdolactone (**118**)[152], thus establishing a potential pathway to the more complex derivatives above (Fig. 17).



Scheme 67.



(117)



(118)

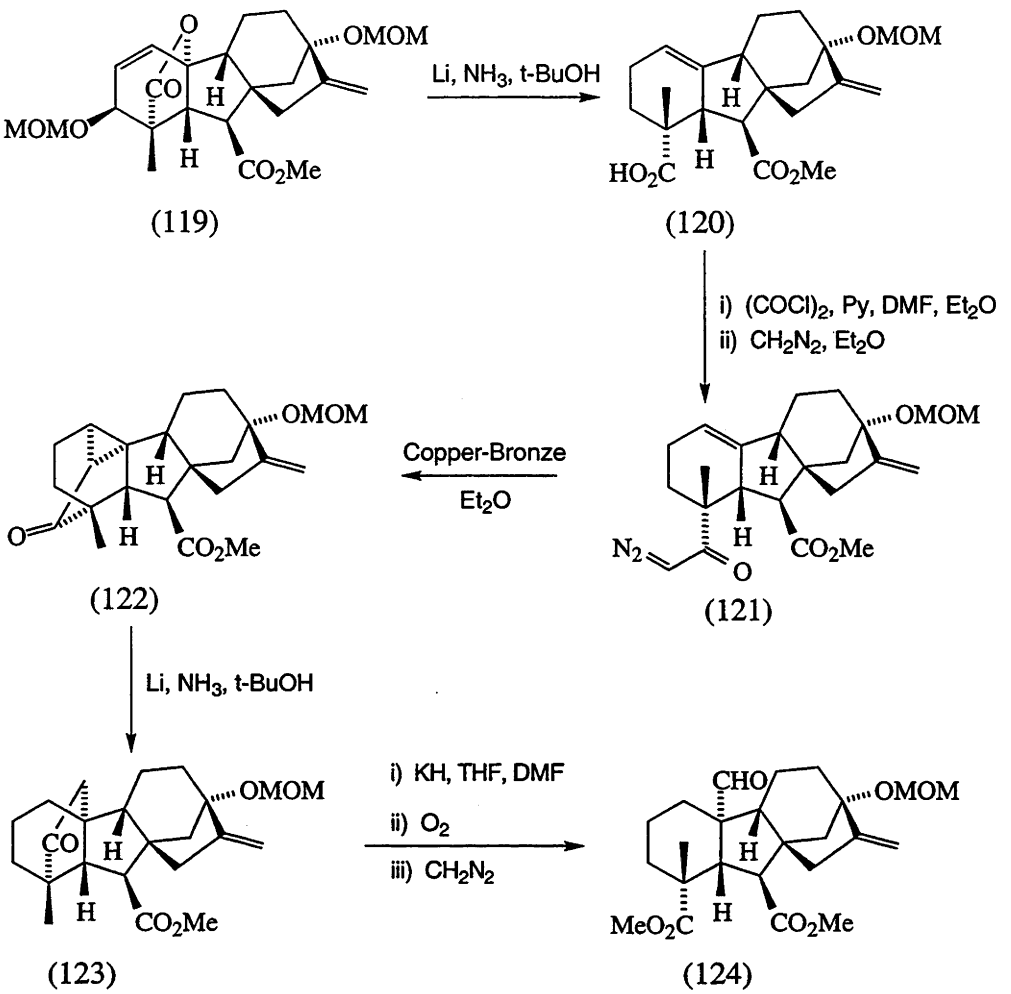
Figure 17.

It was unclear if a C_{20}-gibberellin would ring expand under the conditions used in Chapter 7, as the C(20)-lactone is orientated differently to the model compound studied and the driving force necessary for ring expansion may be different. However, the ease with which ring expansion was achieved on the model compound gave us confidence that a B-ring expansion in a C_{20} substrate was feasible, and so a synthetic route was mapped out.

It was proposed that the synthesis would begin with GA_3, which would be converted to GA_{19}, through a well established pathway for preparing C_{20}-gibberellins. After removal of the 13-hydroxy group, a similar route would be followed to that in Chapter 7.

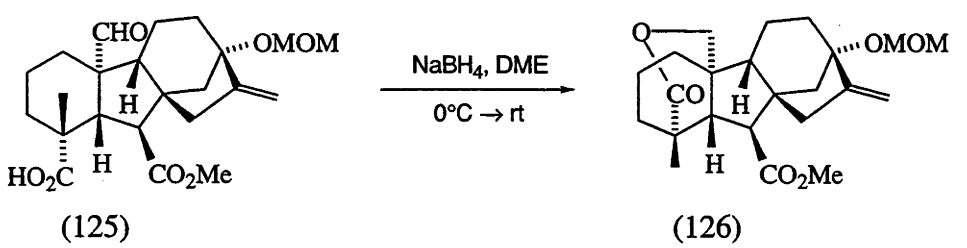
8.2 Synthesis of GA_{19}

The stereoselective synthesis of the 13-hydroxy-C_{20} gibberellin (GA_{19}) derivative **124** from readily available GA_3 methyl ester di-MOM ether **119** was accomplished by Dawe *et al*[112] in 1985. This synthesis involves an intramolecular cyclopropanation of diazoketone **121** to give the cyclopropylketone **122**. The C(20) carbon is introduced in its correct position by a regioselective lithium/ammonia reduction of the cyclopropyl ketone to produce the cyclopentanone **123**, (Scheme 68).



Scheme 68.

Reduction of the GA_{19} derivative (**125**)* to GA_{44} was accomplished by dissolving this compound in dimethoxyethane at O°C and adding sodium borohydride. After 4 hours the mixture was worked up in the usual way giving 88% of the lactone (**126**). In the ^1H nmr spectrum the 20-pro-*S* proton occurred at 4.09 ppm as a doublet of doublets with a geminal coupling of 12.3 Hz and a ^4J coupling of 2.3 Hz to H(1). The 20-pro-*R* proton occurred at 4.40 ppm as a simple doublet.

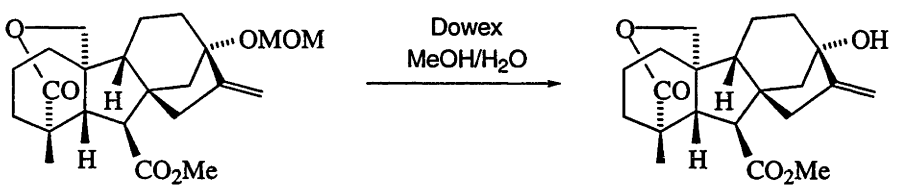


Scheme 69

* The GA_{19}-7-methyl ester-13-MOM ether was a greatly appreciated donation from Mr Bruce Twitchin.

8.3 *Removal of the 13-Hydroxyl*

The 13-hydroxyl is not present in the seco-kaurenes of interest, and so the decision was made to remove it early on in the synthesis. First, the methoxymethyl ether was removed by heating the substrate in methanol/water for 3 hours with Dowex (H^+) resin. The mixture was then filtered to remove the resin and worked up in the usual way to afford methyl ester **127** in good yield.

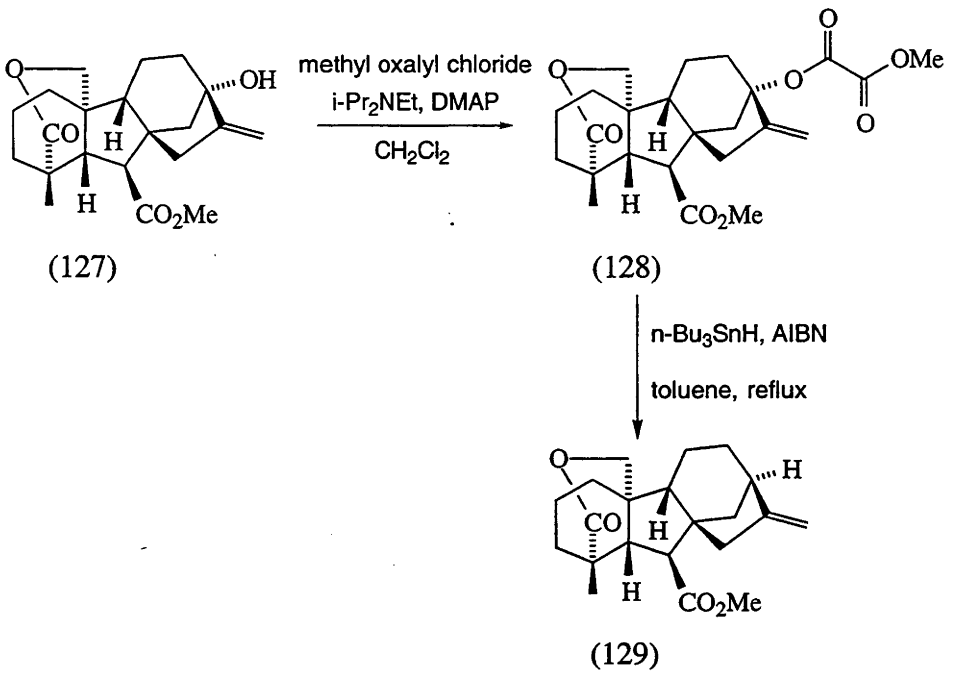


(126) (127)

Scheme 70.

Two different procedures were tried in order to deoxygenate the 13-hydroxyl. At first the 13-hydroxyl was converted to the 13-iodide by heating the substrate in toluene with triphenyl phosphine, imidazole and iodine at reflux. After 1.5 hours, no starting material remained and a dark blue spot at a higher R_f value was visualised by tlc after spraying with vanillin. The iodide was isolated in 59% yield after chromatography and was subjected to radical reduction conditions to give the GA_{15} derivative in 72% yield. However, due to the low yield obtained for the iodide a different procedure was studied.

The free alcohol **127** was taken up in dichloromethane with diisopropylethylamine and DMAP. Methyloxalyl chloride was added, and after stirring overnight at room temperature, no starting material remained. After work up and chromatography, this procedure yielded approximately 90% of the methyloxalyl product **128**. The methoxyl of the methyloxalyl group gave rise to a singlet at 3.87 ppm in the ^1H nmr spectrum. This compound then underwent radical deoxygenation with tributyltin hydride in toluene at reflux with the initiator AIBN for 3 hours[153]. This produced the GA_{15} derivative in 88% yield (**129**) with some recovered starting material (approx. 10%).

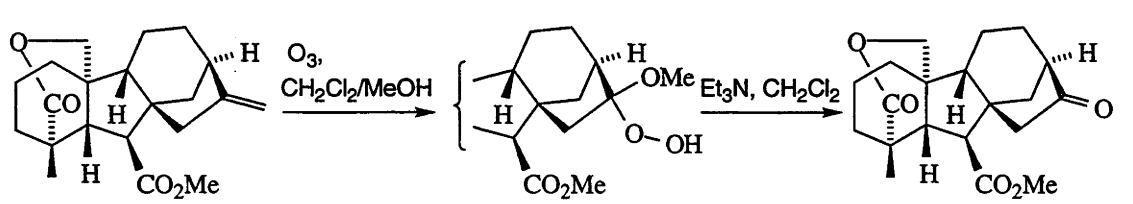


(127) (128) (129)

Scheme 71

8.4 Reduction of the 7-Carboxylic Acid

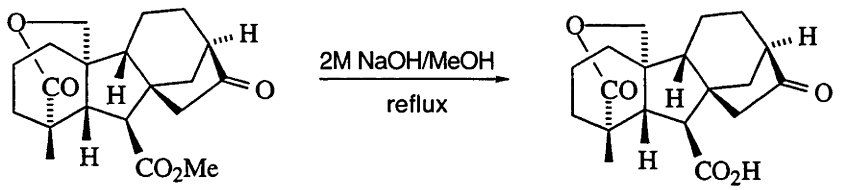
To prevent the formation of a 16,17-diol during dihydroxylation of the Δ^6-ene, the 16,17- exocyclic double bond required some form of protection. To avoid the problem encountered during the model study (Chapter 7.3) of obtaining a low yield on removal of the epoxide, the 16-methylene was instead oxidised to the 16-norketone by ozonolysis (with the intention that the methylene group could be restored in high yield by means of a Wittig reaction). This was initiated by dissolving the olefin **129** in dichloromethane/ methanol, lowering the temperature to -78°C and bubbling ozone through the solution. The resulting methoxyhydroperoxide **130** was treated with dimethyl sulfide with the expectation of obtaining the norketone **131**, but was recovered unchanged. The hydroperoxy acetal eventually did decompose to the 16-norketone, however, by removing all solvent, dissolving the residue in dichloromethane only, and stirring the mixture with triethylamine for 20 hours.



(129) (130) (131)

Scheme 72.

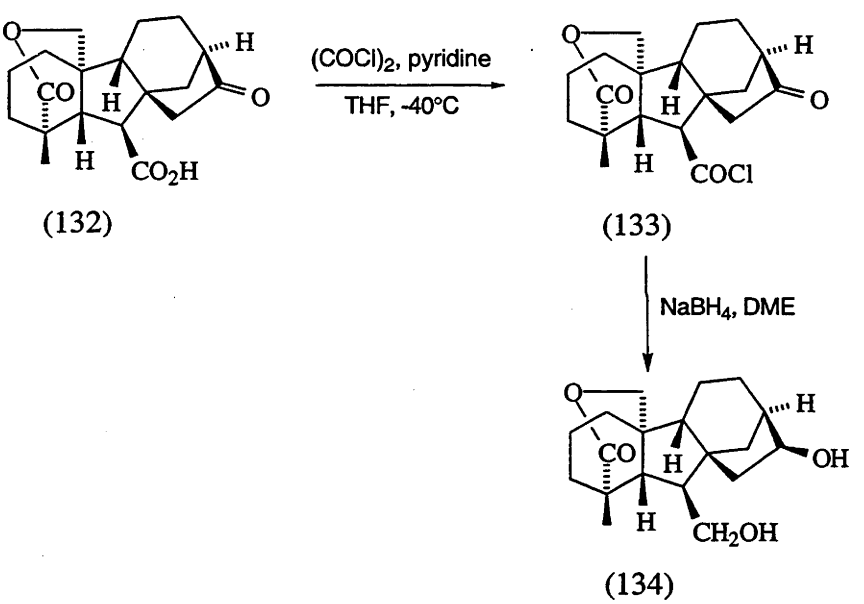
The 7-methyl ester was hydrolysed using methanol/sodium hydroxide at reflux, and then acidifying to pH 3-4 to form the 7-carboxylic acid **132** in 94% yield.



(131) (132)

Scheme 73.

The acid chloride was synthesised and reduced in the same way as for the C_{19}-model compound (Chapter 7.1) to give the 7-carbinol **134** in 82% overall yield. During reduction of the acid chloride, the 16-norketone was also reduced to the 16β-hydroxyl, as indicated by the doublet of triplets at 4.2 ppm in the ^{1}H nmr spectrum.



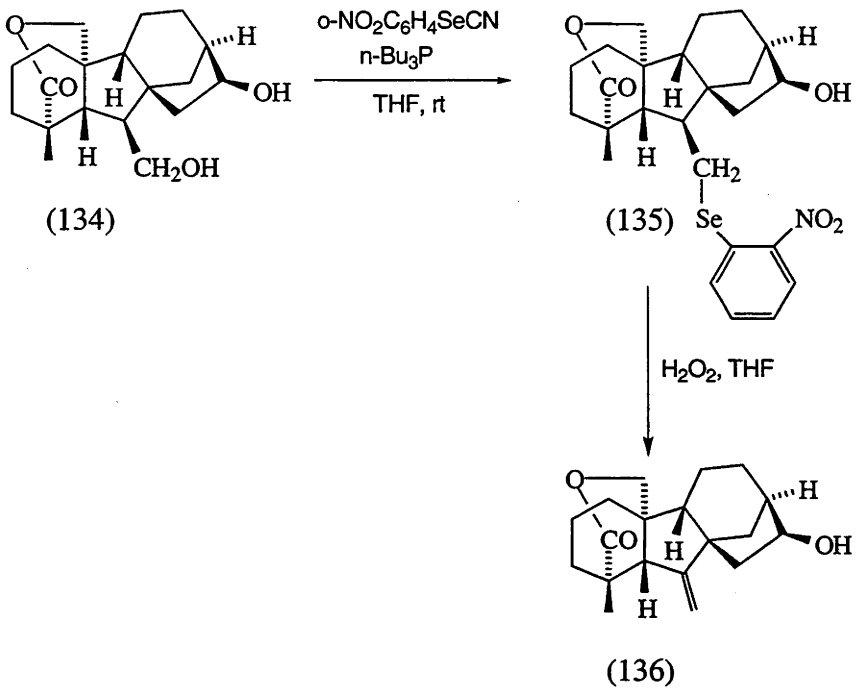
(132) (133) (134)

Scheme 74.

8.5 Formation of the 6-Ene by Elimination

Elimination via the Grieco procedure[140] successfully produced the Δ^6-ene. The alcohol was dissolved in THF and *o*-nitrophenyl seleno-cyanate was added, followed by *n*-tributylphosphine. After one hour of stirring at room temperature the 7-selenyl ester **135** was formed selectively, being a primary alcohol and less sterically hindered than the 16β-hydroxy group. The selenyl ester **135** was isolated as dark orange crystals in 90% yield. The C(7) protons occurred at 2.83 and 3.50 ppm as multiplets in the ^{1}H nmr

spectrum. Subsequent oxidative elimination with 30% hydrogen peroxide at 0°C in THF afforded the 6-methylene derivative **136** in 78% yield.

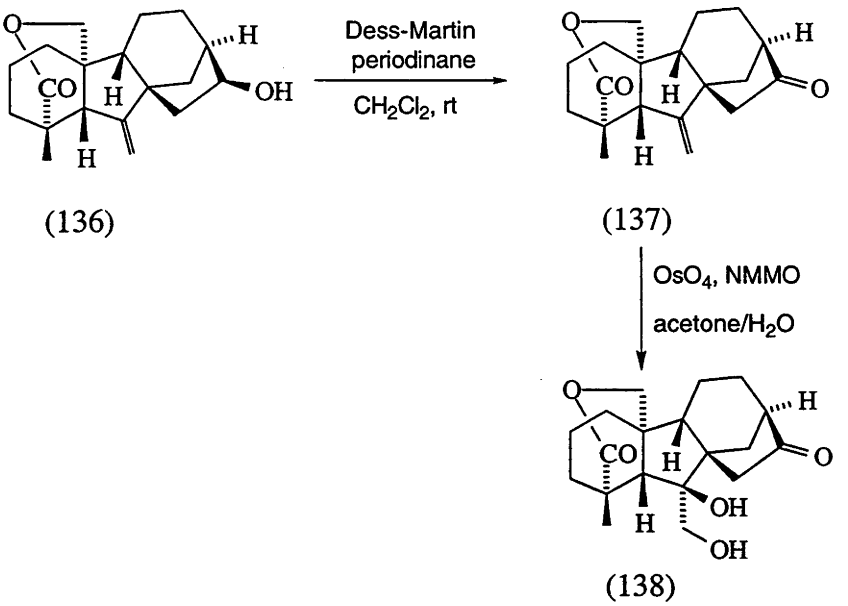


(134) (135) (136)

Scheme 75

8.6 Formation of the 6,7-Diol

To form the 6,7-diol, the 6-methylene **136** was dissolved in acetone/water (5/1), and osmium tetroxide was added with NMMO. Tlc analysis showed product formation with a very low R_f value. However, the 6,7-diol could not be successfully purified by flash column chromatography due to its polarity. Alkene **136** was therefore, oxidised to the 16-ketone prior to dihydroxylation with osmium tetroxide. Treatment of **136** with the Dess-Martin reagent in dichloromethane furnished the 16-ketone **137**, which was then dihydroxylated with osmium tetroxide and NMMO as previously described, over four weeks to give the 6,7-diol **138** in 76% yield. The C(7) protons were seen as doublets at 3.76 and 3.85 ppm with the 6-hydroxy group (which was H-bonded) at 3.65 ppm in the ^1H nmr spectrum.

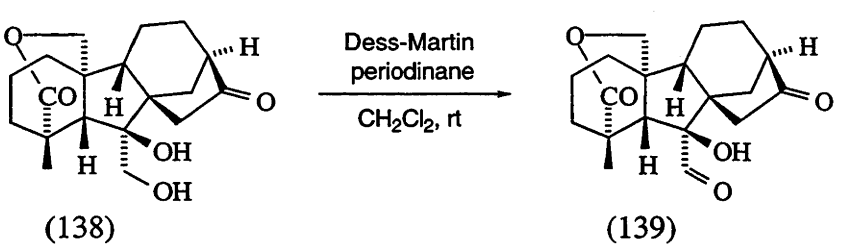


(136) (137) (138)

Scheme 76.

8.7 *Oxidation to the Hydroxy Aldehyde and Rearrangement to the Ketol*

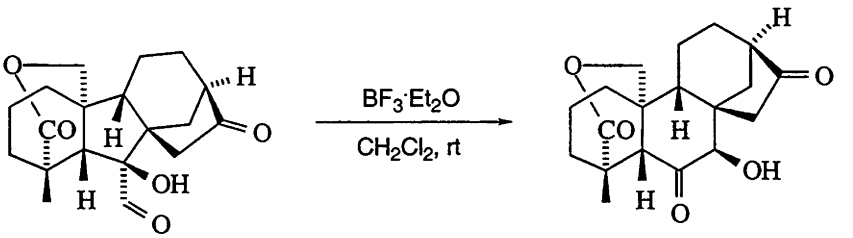
The 6,7-diol **138** was then oxidised to the hydroxy aldehyde **139**, again using the Dess-Martin procedure in 89% yield.



(138) (139)

Scheme 77.

The ring expansion was carried out in dichloromethane at room temperature with the addition of 1.3 equivalents of boron trifluoride etherate. After 30 minutes of stirring, a new product was formed as seen by tlc analysis. Although the ring expansion had proceeded smoothly, the expected 6-hydroxy-7-one had not been formed. Instead, the isomeric 7-hydroxy-6-one **140** was the only product isolated in 100% yield. This was apparent by examination of the ^1H nmr spectrum which showed H(5) as a singlet at 2.26 ppm, H(7α) as a broad singlet at 3.86, and 7β-OH (hydrogen bonded) as a singlet at 3.49 ppm.



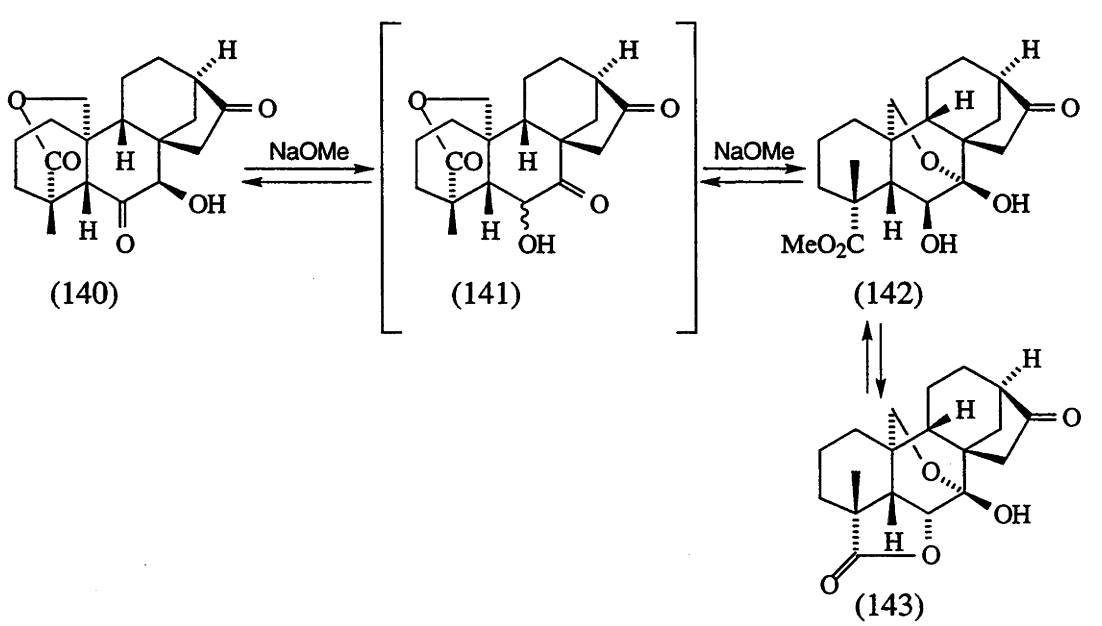
(139) (140)

Scheme 78.

The difference in outcome for the C_{20} rearrangement may be attributed to different steric factors caused by the repulsion between C(20) and C(11). This interaction appears to be relieved more effectively and rapidly by migration of the C(8)-C(6) bond even though this leads to a boat conformation for the B-ring. For the C_{19}-series (Chapter 7), this steric demand is reduced because of the smaller 10α-oxygen substituent, which is also angled away from C(11) due to the 19,10-γ-lactone bridge [ie. in the 5-membered ring, the 10α-oxygen is "pulled" towards C(4)]. Thus, for the C_{19}-series a chair-like transition state [C(5)-C(6) bond migration] takes precedence over steric repulsion between the 10α-oxygen and C(11).

8.8 Rearrangement to the Hemiacetal

Although ketol **140** can not undergo direct rearrangement to the hemiacetal (**142**), base catalysed isomerisation of the ketol might afford the desired ketol **141**. This could then undergo methanolysis and rearrangement to afford the hemiacetal (**142**).



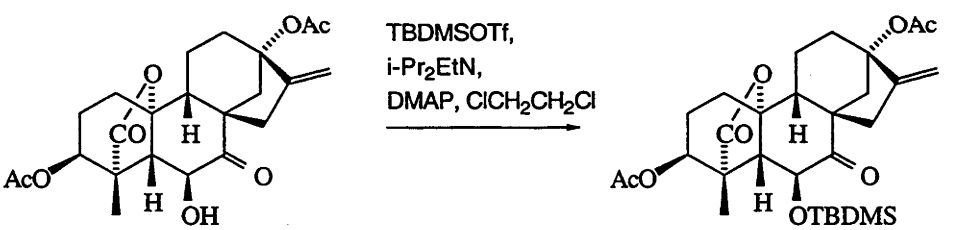
(140) (141) (142) (143)

Scheme 79.

Methoxide attack directly on **140** to afford a 20,6-acetal would also be possible, but the product appears from molecular models to be more highly trained than **142**. Unfortunately, time did not allow further investigations. However, the rearrangement has since been achieved by Dr George Adamson[154]. The ketol **140** was treated with sodium methoxide in methanol for 20 hrs, followed by diazomethane, thereby affording a 2:1 mixture of acetals **142** and **143**.

8.9 Incorporation of the 15-Hydroxyl

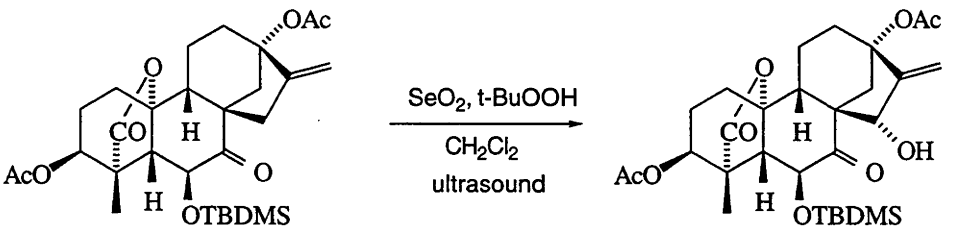
Preliminary investigations were undertaken to ascertain the feasibility of introducing a carbonyl group at the C(15) position which is present in many of the more complex kaurenoids. This had previously been attempted in the synthesis of effusin[150]. The 15-desoxyeffusin derivative had been subjected to allylic oxidation using selenium dioxide and *tert*-butylhydroperoxide[155]. However, this failed to furnish the 15-hydroxy derivative. Thus, incorporation of a 15-hydroxy group at an earlier stage was examined by taking the ring expanded product of Chapter 7.5 (**103**) and treating it with *t*-butyl-dimethylsilyl triflate (TBDMSOTf), Scheme 80.



(103) (144)

Scheme 80.

Having protected the secondary alcohol, the ring expanded product underwent allylic oxidation with selenium dioxide and *t*-butylhydroperoxide under sonication to afford the 15α-hydroxy derivative (**145**), (Scheme 81). The 15β-proton appeared as a broad singlet at 4.40 ppm in the ^{1}H nmr spectrum.



(144) (145)

Scheme 81.

This last step has proven that a 15-hydroxy group and thence a 15-oxo function could be incorporated after the ring expansion step.

8.10 Conclusion

The synthetic sequences illustrated in this chapter demonstrate a viable route to obtaining kaurenoids from gibberellins and offers many opportunities for preparing some of the more complex and interesting examples of this family of compounds. Synthetic studies are continuing in this area, in the hope of manipulating acetals **142** and **143** to form macrocalyxoformin (**117**) and longirabdolactone (**118**), as well as endeavouring to shorten the number of steps required in the existing transformations.

Having paved the way to synthesising complex kaurenoids from gibberellins, there now exists great potential for expanding on this research area, such as assigning tentative new structures, derivatising particular compounds in order to produce heightened biological activity, and isotopic labelling for the purpose of metabolic tracking. The highly functionalised nature of gibberellins, and the wide range of synthetic methodology already existing for further functionalising these complex compounds, allows for numerous possibilities. For example, the optional retention of functionality at C(3) and/or C(13), plus procedures for introducing functionality into the GA molecule at C(1)[156-158], C(11)[159,160], C(12)[161,162], C(14)[163,164] and C(18)[165] in combination with those described above could give access to the complete range of known kaurenoids.

CHAPTER 9

EXPERIMENTAL

9.1 General Experimental

9.1.1 Instrumentation

Melting points were recorded on a Reichert hot-stage apparatus and are uncorrected. All infrared spectra (ν_{max}) were recorded on a Perkin-Elmer 683 Infrared Spectrophotometer and referenced to the characteristic peak at 1601 cm^{-1} for polystyrene. ^{1}H and ^{13}C nmr spectra were recorded on a Varian Gemini 300 MHz instrument. Chemical shifts are reported as values in parts per million (δ ppm). For proton spectra recorded in chloroform, the residual peak of $CHCl_3$ was used as the internal reference (7.26 ppm), while the central peak of $CDCl_3$ (77.0 ppm) was used as the reference for carbon spectra. Multiplicities are abbreviated: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. Distortionless enhancement by polarisation transfer (DEPT) and the attached proton test (APT) were used in the assignment of carbon spectra[143,144]. Two dimensional NMR experiments were used to assign the molecular framework[143,144]. Low resolution EI mass spectra (70eV) were recorded on a VG Micromass 7070F double focussing mass spectrometer. High resolution mass spectra (exact mass) were also determined on the 7070F instrument by peak matching. Microanalyses were carried out by the Australian National University Analytical Services Unit, Canberra. C,H,N analyses were measured on a Carlo Erba EA 1106 CHN-O instrument.

9.1.2 Chromatography

Analytical thin layer chromatography (tlc) was conducted on Merck glass backed tlc plates coated with 0.2 mm thick silica gel 60 GF_{254}, or Merck aluminium backed tlc sheets with silica gel 60 F_{254}. The developed plates were visualised under shortwave ultraviolet light, exposure to iodine vapour, or stained with 13% (*w/v*) vanillin in concentrated sulfuric acid at 180°C. All flash column chromatography was carried out using the flash technique as reported by Still[166] using Merck Kieselgel 60 and distilled analytical reagent (AR) grade solvents as indicated.

9.1.3 Reaction Conditions

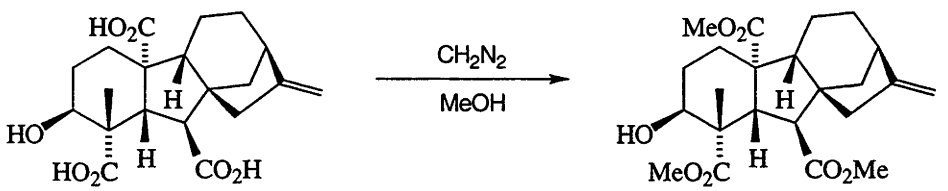
All reactions requiring dry solvent were performed in flame dried flasks, under a dry nitrogen atmosphere. Syringes and needles were pre-dried in an oven (110°C) and cooled in an anhydrous atmosphere prior to use. Stirring was by internal magnetic follower. After filtration of solutions from drying reagents, the bulk of the solvent was removed on a Büchi rotatory evaporator. The last traces of solvent were removed under high vacuum.

9.1.4 Reagents and Solvents

Many reagents were commercially available (Aldrich, Merck) and were used as supplied. Where necessary, solvents and reagents used in reactions were purified according to well established procedures[167]. Tetrahydrofuran (THF), diethyl ether, benzene, and dimethoxyethane (DME) were purified by distillation from sodium benzophenone ketyl under nitrogen. Dichloromethane was distilled from calcium hydride under nitrogen. Triethylamine and diisopropylethylamine were stored under nitrogen over activated molecular sieves. Ethanol-free ethereal diazomethane was prepared from Diazald® (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) purchased from Aldrich.

9.2 Experimental for Chapter 3

Trimethyl *ent*-3α-Hydroxygibberell-16-ene-7,19,20-trioate (11)



(10) (11)

To a stirred solution of GA_{13} (1.00 g, 2.64 mmol) in MeOH (10 ml) was added dropwise, an ethereal solution of diazomethane until the yellow colour persisted. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:2) as eluting solvent to give GA_{13}-7,19,20-trimethyl ester (911 mg, 82%) as a white foam.

Rf 0.46 (ethyl acetate:hexane, 1:2). **mp** 157-159°C (Lit.[30] 117-119°C). **IR** (KBr disc) 1703, 1725, 3460 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92-1.09 (1H, m), 1.23 (3H, s, H18), 1.23-2.32 (12H, m), 2.58 (1H, d, J = 12.6 Hz, H5), 2.58 (1H, m, H13), 3.59 (3H, s, $-CO_2CH_3$), 3.65 (3H, s, $-CO_2CH_3$), 3.75 (3H, s, $-CO_2CH_3$), 3.88 (1H, d, J = 12.8 Hz, H6), 3.97 (1H, br s, H3), 4.79 (1H, br s, H17), 4.88 (1H, br s, H'17).

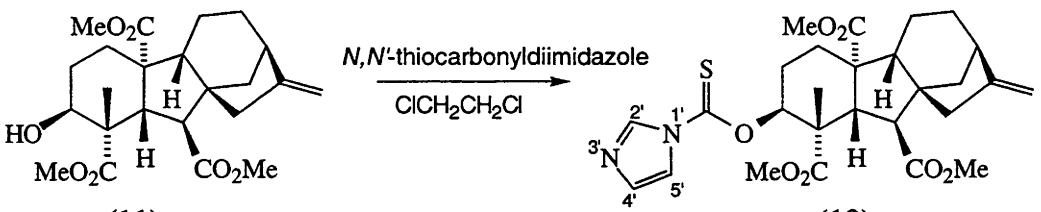
^{13}C NMR (75 MHz, $CDCl_3$): δ 18.6 (C11), 22.6 (C18), 28.9 (C1), 30.0 (C2), 31.5 (C12), 36.1 (C14), 39.3 (C13), 46.0 (C15), 49.2 (C4), 49.9 (C5), 50.0 (C8), 50.6 (C6), 50.9, 51.3, 51.4 (3x-$CO_2\underline{C}H_3$), 56.1 (C9), 56.9 (C10), 70.3 (C3), 105.9 (C17), 156.7 (C16), 174.6, 175.2, 175.3 (C7, C19, C20).

MS (EI) *m/z* 420 (M^+, 7%), 388 (45), 360 (17), 328 (100), 310 (41), 300 (60), 282 (60), 268 (55), 241 (28), 223 (43).

HRMS (EI) *m/z*: calc'd for M^+ $C_{23}H_{32}O_7$: 420.2148; found: 420.2154.

Microanalysis $C_{23}H_{32}O_7$ requires: C 65.70, H 7.67; found: C 65.45, H 7.72.

Trimethyl *ent*-3α-Imidazol-1'-ylthiocarbonyloxygibberell-16-ene-7,19, 20-trioate (13)



(11) (13)

To a solution of the starting alcohol (20 mg, 0.048 mmol) in dry 1,2-dichloroethane (3 ml), under an atmosphere of N_2 was added *N,N*-thiocarbonyldiimidazole (44.1 mg, 0.196 mmol), and the mixture stirred at reflux for 16 h. The solvent was removed under reduced pressure, and the residue suspended in water (3 ml). The product was extracted with ethyl acetate (2x10 ml), washed with water (5 ml), and saturated brine (5 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give an orange oil. Purification was carried out by flash chromatography on silica gel using ethyl acetate:hexane (1:4) as eluting solvent, to give the product (23.5 mg, 92%) as a white powder.

Rf 0.32 (ethyl acetate:hexane, 1:4). **mp** 50-52°C. **IR** (CH_2Cl_2) 1262, 1269, 1386, 1727 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17-2.60 (14H, m), 1.20 (3H, s, H18), 2.61 (1H, d, J = 12.5 Hz, H5), 3.62 (3H, s, $-CO_2CH_3$), 3.71 (6H, s, 2 x $-CO_2CH_3$ overlapped), 3.88 (1H, d, J = 12.5 Hz, H6), 4.84 (1H, br s, H17), 4.90 (1H, br s, H'17), 5.87 (1H, br s, H3), 7.08 (1H, br s, H'5), 7.66 (1H, br s, H'4), 8.40 (1H, br s, H'2).

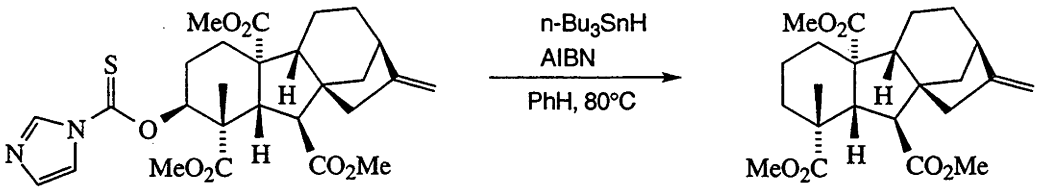
^{13}C NMR (75 MHz, $CDCl_3$): δ 18.5 (C11), 22.6 (C18), 25.8 (C1), 31.3 (C2), 31.4 (C12), 36.3 (C14), 39.3 (C13), 46.1 (C15), 49.2 (C4), 50.2 (C8, $-CO_2\underline{C}H_3$ overlapped), 50.3 (C5), 51.2, 51.5 (2x$-CO_2\underline{C}H_3$), 52.0 (C6), 56.3 (C9), 56.8 (C10), 86.3 (C3), 106.3 (C17), 117.4 (C4'), 131.0 (C5'), 136.9 (C2'), 156.0 (C16), 173.6, 174.0, 174.9 (C7, C19, C20), 182.7 (-OC(S)N).

MS (EI) *m/z* 530 (M^+, 30%), 403 (51), 371 (50), 343 (60), 311 (100), 283 (100), 251 (48), 223 (70), 181 (34), 129 (52).

HRMS (EI) *m/z*: calc'd for M^+ $C_{27}H_{34}N_2O_7S$: 530.2087; found: 530.2088.

Microanalysis $C_{27}H_{34}N_2O_7S$ requires: C 61.11, H 6.46, N 5.28; found: C 61.00, H 6.53, N 4.96.

Trimethyl *ent*-Gibberell-16-ene-7,19,20-trioate (14)



(13) (14)

The above imidazolide (631 mg, 1.19 mmol) was taken up in dry benzene (70 ml) and heated at reflux under N_2 for 10 minutes. Tributyltin hydride (640 µl, 2.38 mmol) was added *via* syringe followed by AIBN (40 mg, 10% wt). The resulting mixture was heated at reflux for a further 30 mins, after which the solvent was removed under reduced pressure. The product was purified by flash chromatography on silica gel using ethyl acetate:hexane (1:10), to give a yellow oil. Residual tin by-products were removed by taking the residue up in diethyl ether (30 ml) and washing with ammonia (3x15 ml), and chromatographed again to give GA_{25} trimethyl ester (369 mg, 77%) as a white solid.

Rf 0.28 (ethyl acetate:hexane, 1:10). **mp** 77-79°C. **IR** (KBr disc) 1729 cm^{-1}.

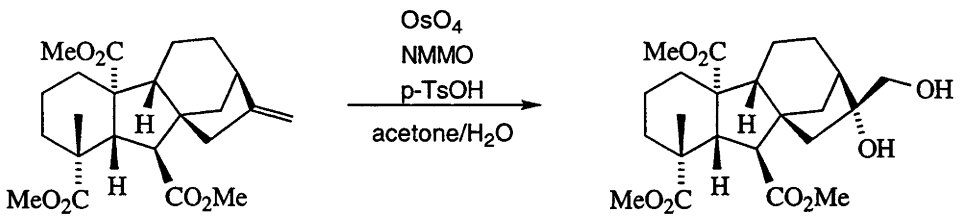
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00–2.58 (16H, m), 1.13 (3H, s, H18), 2.13 (1H, d, J = 12.6 Hz, H5), 3.59, 3.85, 3.71 (3x3H, s, $-CO_2CH_3$), 3.84 (1H, d, J = 12.6 Hz, H6), 4.81 (1H, br s, H17), 4.89 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ18.5 (C11), 21.5 (C2), 28.4 (C18), 31.5 (C12), 36.4 (C14), 36.5 (C1), 37.7 (C3), 39.5 (C13), 44.9 (C4), 46.0 (C15), 50.0 (C8), 50.8 (C5, $-CO_2\underline{C}H_3$ overlapped), 51.2, 51.3 (2x-$CO_2\underline{C}H_3$), 56.3 (C6), 56.6 (C9), 57.0 (C10), 105.8 (C17), 156.5 (C6), 174.5, 175.6, 176.0 (C7, C19, C20).

MS (EI) *m/z* 404 (M^+, 32%), 374 (71), 372 (74), 342 (35), 328 (26), 312 (93), 284 (100), 255 (26), 225 (73), 183 (38).

HRMS (EI) *m/z*: calc'd for M^+ $C_{23}H_{32}O_6$: 404.2199; found: 404.2190.

Trimethyl *ent*-16β,17-Dihydroxygibberell-16-ene-7,19,20-trioate (15)



(14) (15)

To the above olefin (107 mg, 0.265 mmol) in acetone/water (8/1 ml), was added ten small crystals of *p*-toluenesulfonic acid, and *N*-methyl morpholine-*N*-oxide (37 mg, 0.317 mmol). One crystal of osmium tetroxide was dissolved in t-butyl alcohol (1 ml) and added to the above mixture, which was stirred at room temperature for 3 h. The reaction mixture was then diluted with water (5 ml) and extracted with ethyl acetate (2x10 ml). The organic extracts were combined and washed with water (5 ml), and saturated brine (5 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give the crude product as a brown oil (114 mg, 98%). Purification was carried out by flash chromatography on silica gel using ethyl acetate:hexane (4:1), giving a white crystalline solid which was recrystallised from ether/heptane to give pure 16α,17-dihydroxy-GA_{25} trimethyl ester, (79% Yield).

Rf 0.18 (ethyl acetate:hexane, 2:1). **mp** 47°C. **IR** (KBr disc) 1143, 1169, 1198, 1230, 1729, 2930, 3437 cm^{-1}.

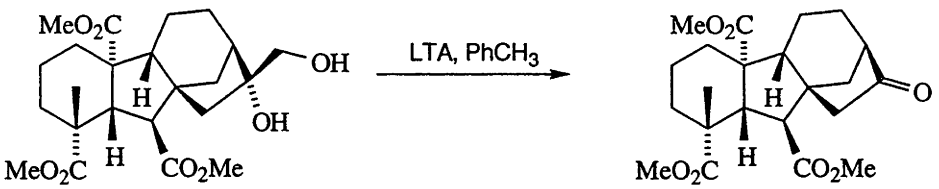
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88-2.04 (15H, m), 1.11 (3H, s, H18), 2.07 (1H, d, *J* = 12.6 Hz, H5), 2.43 (1H, m, H15α), 3.58-3.76 (2H, m, H17), 3.60, 3.65, 3.74 (3x3H, s, $-CO_2CH_3$), 3.86 (1H, d, *J* = 12.6 Hz, H6).

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.7 (C11), 21.4 (C2), 21.9 (C12), 28.3 (C18), 34.6 (C14), 36.3 (C1), 327.6 (C3), 43.1 (C13), 44.8 (C4), 50.4 (C8), 50.9 ($-CO_2\underline{C}H_3$), 51.3 ($-CO_2\underline{C}H_3$), 51.5 (C15), 51.5 ($-CO_2\underline{C}H_3$), 51.7 (C6), 56.5 (C5), 57.0 (C10), 58.1 (C9), 66.8 (C17), 82.2 (C16), 174.3, 175.8, 176.0 (C7, C19, C20).

MS (EI) *m/z* 406 (M-32$^+$, 14%), 375 (25), 347 (18), 315 (27), 287 (40), 245 (17), 227 (18), 185 (24), 129 (28), 105 (42).

Microanalysis $C_{23}H_{34}O_8$ requires: C 63.00, H 7.81; found: C 62.85, H 8.02.

Trimethyl *ent*-16-Oxo-17-norgibberellane-7,19,20-trioate (16)



(15) (16)

To the starting diol **15** (136 mg, 0.310 mmol) in dry toluene (15 ml) was added lead tetraacetate (417 mg, 0.929 mmol). The mixture was stirred under nitrogen at room temp for 2.5 h, after which, tlc indicated that no starting material remained. The mixture was diluted with water (8 ml) and extracted with ethyl acetate (2x10 ml). The combined organic fractions were washed with saturated sodium bicarbonate (2x10 ml), and saturated brine (15 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The crude product was purified by flash chromatography on silica gel using ethyl acetate:hexane, (1:3) as eluting solvent to give the pure norketone **16** as a white solid (117 mg, 93%).

Rf 0.32 (ethyl acetate:hexane, 1:3). **mp** 141°C. **IR** (KBr disc) 1197, 1169, 1721, 1739, 2952 cm^{-1}.

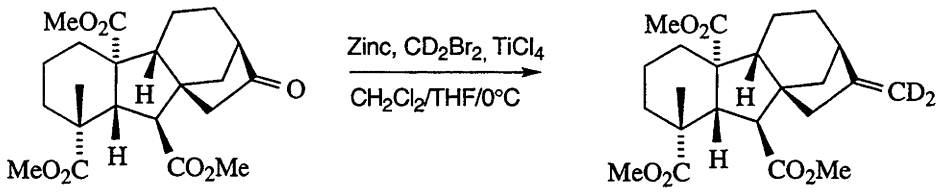
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86-2.19 (14H, m), 1.13 (3H, s, H18), 2.12 (1H, d, J = 12.8 Hz, H5), 2.36 (1H, m, H13), 2.48 (1H, m, H15α), 3.62, 3.65, 3.72 (3x3H, s, -CO_2CH_3), 3.96 (1H, d, J = 12.7 Hz, H6).

^{13}C NMR (75 MHz, $CDCl_3$): δ 19.4 (C11), 21.4 (C2), 24.9 (C12), 28.2 (C18), 33.2 (C14), 36.1 (C1), 37.5 (C3), 44.9 (C13), 45.0 (C4), 48.5 (C8), 51.1, 51.4, 51.6 (3x-$CO_2\underline{C}H_3$), 52.0 (C5), 52.3 (C15), 56.5 (C6), 56.6 (C9), 57.3 (C10), 174.3, 174.0, 174.8 (C7, C19, C20), 220.9 (C16).

MS (EI) *m/z* 406 (M^+, 6%), 374 (74), 314 (49), 286 (35), 207 (48), 105 (50), 91 (84), 79 (53), 59 (100), 55 (91).

Microanalysis $C_{22}H_{30}O_7$ requires: C 65.01, H 7.44; found: C 64.76, H 7.76.

Trimethyl *ent*-(17,17-D_2)-Gibberell-16-ene-7,19,20-trioate (17)



(16) (17)

The Lombardo reagent[78] (approximately 4.5 ml in suspension) was added by a Pasteur pipette to a solution of the 17-norketone **16** (90 mg, 0.22 mmol) in dry dichloromethane (5 ml), with stirring under a nitrogen atmosphere. Tlc analysis indicated that the reaction was complete after 1 min. The reaction mixture was quenched with saturated sodium bicarbonate (5 ml) and extracted with ethyl acetate (2x10 ml). The combined organic phases were washed with 2M hydrochloric acid (2x5 ml), water (10 ml), and saturated brine (10 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure to give a clear oil. Chromatography on silica gel using ethyl acetate:hexane (1:7) afforded the desired dideuterated compound **17** (62 mg, 70%) as a colourless oil.

Rf 0.34 (ethyl acetate:hexane, 1:7). **IR** (film) 1144, 1168, 1197, 1230, 1730, 2948 cm^{-1}.

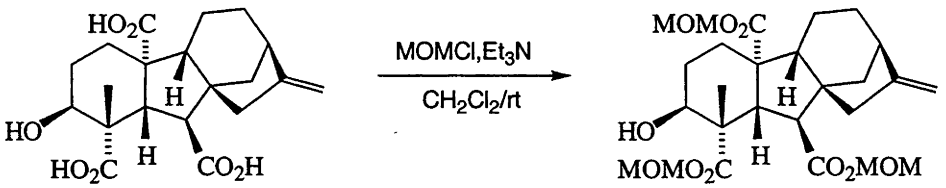
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99–2.16 (14H, m), 1.12 (3H, s, H18), 2.09 (1H, d, J = 12.3 Hz, H5), 2.45 (1H, m, H15α), 2.55 (1H, m, H13), 3.59, 3.64, 3.70 (3x3H, s, -CO_2CH_3), 3.83 (1H, d, J = 12.6 Hz, H6).

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.5 (C11), 21.4 (C2), 28.4 (C18), 31.5 (C12), 36.3 (C14), 36.5 (C1), 37.6 (C3), 39.4 (C13), 44.9 (C4), 45.9 (C15), 49.9 (C8), 50.8 (C5), 50.9 (-$CO_2\underline{C}H_3$), 51.2 (-$CO_2\underline{C}H_3$), 51.3 (-$CO_2\underline{C}H_3$), 56.2 (C6), 56.6 (C9), 56.9 (C10), 156.3 (C16), 174.5, 175.5, 176.0 (C7, C19, C20).

MS (EI) *m/z* 406 (M^+, 13%), 374 (20), 314 (66), 286 (100), 227 (72), 143 (14), 129 (16).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{30}D_2O_6$: 406.2355, found 406.2336.

Tri(methoxymethyl) *ent*-3α-Hydroxygibberell-16-ene-7,19,20-trioate (18)



(10) (18)

To the free acid **10** (50 mg, 0.132 mmol) in dry dichloromethane (5 ml) was added triethylamine (92 µl, 0.66 mmol), followed by chloromethyl methyl ether (104 µl, 1.32 mmol) and the mixture stirred at room temperature under an atmosphere of nitrogen for 10 min. Saturated sodium bicarbonate (1 ml) was added and the mixture was diluted with water (5 ml), and extracted with ethyl acetate (2x5 ml). The combined organic phases were washed with water (5 ml), 2M hydrochloric acid (5 ml), water (5 ml), and saturated brine (5 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure to give a clear oil. Purification was carried out by flash chromatography on silica gel using ethyl acetate:hexane (1:2), to give **18** as a clear oil (51 mg, 79%). The oil was crystallised using ether:heptane to give a white solid.

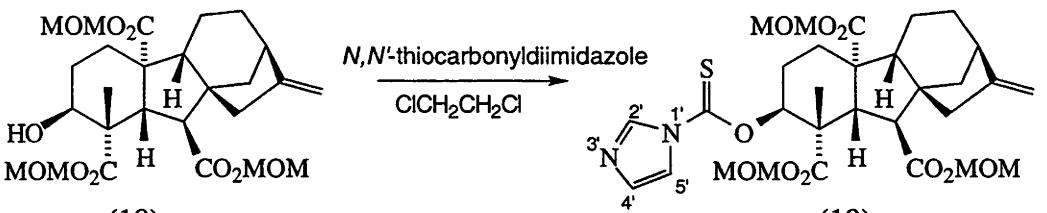
Rf 0.27 (ethyl acetate:hexane, 1:2). **mp** 99-101°C. **IR** (KBr disc) 923, 941, 1081, 1136, 1157, 1711, 1743, 3513 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10-2.37 (13H, m), 1.33 (3H, s, H18), 2.57 (1H, m, H13), 2.61 (1H, d, *J* = 12.7 Hz, H5), 3.48, 3.51, 3.53 (3x3H, s, -$OCH_2OC\underline{H}_3$), 3.98 (1H, d, *J* = 12.6 Hz, H6), 3.40 (1H, s, H3), 4.82 (1H, br s, H17), 4.90 (1H, br s, H'17), 5.02, 5.41 (2x1H, ABd, *J* = 6.0 Hz, -$OC\underline{H}_2OCH_3$), 5.10, 5.33 (2x1H, ABd, *J* = 6.0 Hz, -$OC\underline{H}_2OCH_3$), 5.23, 5.25 (2x1H, ABd, *J* = 4.9 Hz, -$OC\underline{H}_2OCH_3$), .

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.5 (C11), 22.8 (C18), 28.9 (C1), 29.8 (C2), 31.4 (C12), 36.2 (C14), 39.3 (C13), 45.8 (C15), 49.4 (C4), 49.9 (C5), 50.0 (C8), 50.5(C6), 56.3 (C9), 56.9 (C10), 57.6, 57.6, 58.0 (3x-$OCH_2O\underline{C}H_3$), 70.6 (C3), 90.2, 90.9, 91.6 (3x-$O\underline{C}H_2OCH_3$), 106.1 (C17), 156.3 (C16), 174.1, 174.3, 174.4 (C7, C19, C20).

MS (EI) *m/z* 510 (M^+, 9%), 448 (69), 420 (86), 416 (86), 404 (50), 359 (50), 326 (74), 313 (76), 285 (62), 269 (100), 241 (51), 225 (45).
Microanalysis $C_{26}H_{38}O_{10}$ requires: C 61.16, H 7.50; found: C 61.38, H 7.83

Tri(methoxymethyl) *ent*-3α-Imidazol-1'-ylthiocarbonyloxygibberell-16-ene-7,19,20-trioate (19)



(18) (19)

To a solution of the starting alcohol (792 mg, 1.55 mmol) in dry 1,2-dichloroethane (80 ml) under an atmosphere of N_2, was added *N,N'*-thiocarbonyldiimidazole (1.66 g, 9.31 mmol), and the mixture stirred at reflux for 16 h. The solvent was removed under reduced pressure, and the residue was suspended in water (20 ml). The product was extracted with ethyl acetate (2x30 ml), and the combined organic phases were washed with water (30 ml), and saturated brine (30 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give an orange oil. Purification was carried out by flash chromatography on silica gel using ethyl acetate:hexane (1:2) as eluting solvent, to give **19** (906 mg, 94%) as a clear oil.

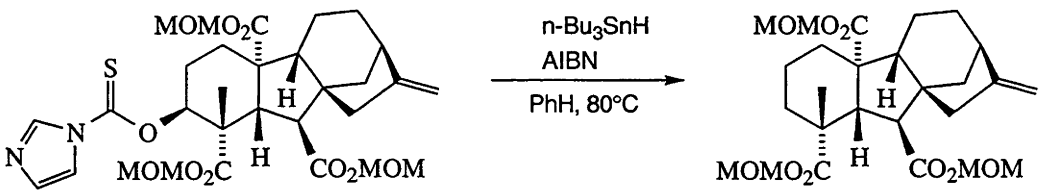
Rf 0.35 (ethyl acetate:hexane, 1:2). **IR** ($CDCl_3$) 1092, 1138, 1164, 1230, 1282, 1384, 1728, 2944 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18-2.51 (13H, m), 1.29 (3H, s, H18), 2.60 (1H, m, H13), 2.64 (1H, d, J = 12.6 Hz, H5), 3.49 (6H, s, 2x-$OCH_2OC\underline{H}_3$ overlapped), 3.53 (3H, s, -$OCH_2OC\underline{H}_3$), 3.98 (1H, d, J = 12.6 Hz, H6), 4.85 (1H, br s, H17), 4.92 (1H, br s, H'17), 5.06, 5.44 (2x1H, ABd, J = 5.8 Hz, -$OC\underline{H}_2OCH_3$), 5.13, 5.27 (2x1H, ABd, J = 6.0 Hz, -$OC\underline{H}_2OCH_3$), 5.25, 5.30 (2x1H, ABd, J = 6.1 Hz, -$OC\underline{H}_2OCH_3$), 5.89 (1H, s, H3), 7.09 (1H br s, H5'), 7.66 (1H br s, H4'), 8.44 (1H, br s, H2').

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.4 (C11), 22.7 (C18), 285.8 (C1), 31.1 (C2), 31.3 (C12), 36.3 (C14), 39.3 (C13), 45.9 (C15), 49.4 (C8), 50.2 (C5), 51.9 (C6), 56.5 (C9), 56.8 (C10), 57.6, 57.8, 58.1 (3x-$OCH_2O\underline{C}H_3$), 77.1 (C4), 83.3 (C3), 90.5, 91.3, 92.2 (3x-$O\underline{C}H_2OCH_3$), 106.5 (C17), 117.4 (C4'), 131.1 (C5'), 137.0 (C2'), 155.7 (C16), 172.8, 173.5, 174.1 (C7, C19, C20), 182.7 (-OC(S)N).

MS (EI) *m/z* 620 (M^+, 48%), 559 (35), 493 (6), 431 (31), 386 (57), 342 (82), 269 (92), 223 (56), 157 (100).
HRMS (EI) *m/z* calc'd for M^+ $C_{30}H_{40}N_2O_{10}S$: 620.2404, found 620.2407.

Tri(methoxymethyl) *ent*-Gibberell-16-ene-7,19,20-trioate (20)



(19) (20)

The above imidazolide (881 mg, 1.42 mmol) was taken up in dry benzene (90 ml) and heated to reflux under nitrogen for 10 minutes. Tributyltin hydride (765 µl, 2.84 mmol) was added *via* syringe followed by AIBN (47 mg, 10% wt). The resulting mixture was heated at reflux for a further 1 h, after which the solvent was removed under reduced pressure. The product was purified by flash chromatography on silica gel using ethyl acetate:hexane (1:5), to give a clear oil. Residual tin by-products were removed by taking the residue up in ethyl acetate (60 ml) and washing with ammonia (3x25 ml), and chromatographed again to give GA_{25} tri(methoxymethyl) ester (507 mg, 72%) as a white solid.

Rf 0.30 (ethyl acetate:hexane, 1:5). **mp** 89-91°C. **IR** (KBr disc) 1163, 1205, 1729, 1745, 2939 cm^{-1}.

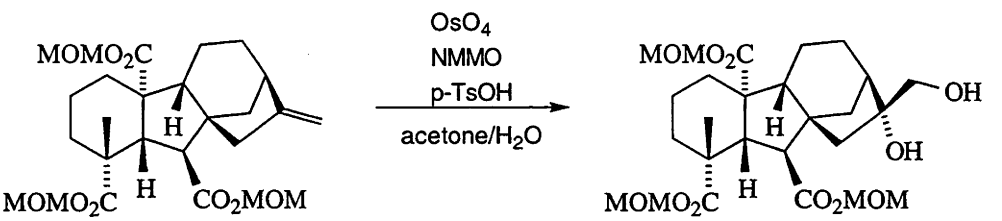
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03-2.20 (14H, m), 1.22 (3H, s, H18), 2.16 (1H, d, *J* = 12.5 Hz, H5), 2.50 (1H, m, H15α), 2.57 (1H, m, H13), 3.48, 3.50, 3.51 (3x3H, s, $-OCH_2OC\underline{H}_3$), 3.95 (1H, d, *J* = 12.5 Hz, H6), 4.81 (1H, br s, H17), 4.89 (1H, br s, H'17), 5.05, 5.37 (2x1H, ABd, *J* = 6.1 Hz, $-OC\underline{H}_2OCH_3$), 5.10, 5.32 (2x1H, ABd, *J* = 6.1 Hz, $-OC\underline{H}_2OCH_3$), 5.22, 5.25 (2x1H, ABd, *J* = 6.0 Hz, $-OC\underline{H}_2OCH_3$), .

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.3 (C11), 21.4 (C2), 28.4 (C18), 31.4 (C12), 36.1 (C14), 36.5 (C1), 37.4 (C3), 39.4 (C13), 45.1 (C4), 45.7 (C15), 49.9 (C8), 50.7 (C5), 56.4 (C6), 56.4 (C9), 56.9 (C10), 57.4, 57.5, 57.9 (3x-$OCH_2O\underline{C}H_3$), 90.0, 90.8, 91.4 (3x-$O\underline{C}H_2OCH_3$), 106.0 (C17), 156.1 (C16), 173.9, 174.5, 175.0 (C7, C19, C20).

MS (EI) *m/z* 494 (M^+, 21%), 465 (20), 432 (79), 417 (28), 404 (32), 315 (57), 269 (68), 225 (79), 91 (100).

HRMS (EI) *m/z* calc'd for M^+ $C_{26}H_{38}O_9$: 494.2516, found 494.2514.

Tri(methoxymethyl) *ent*-16β,17-Dihydroxygibberellane-7,19,20-trioate



(20)

To the above olefin (49 mg, 0.099 mmol) in acetone/water (10/1 ml), was added 10 small crystals of *p*-toluenesulfonic acid and *N*-methyl morpholine-*N*-oxide (46 mg, 0.398 mmol). One crystal of osmium tetroxide was dissolved in *t*-butyl alcohol (1 ml) and added to the above mixture, which was stirred at room temperature for 3 hours. The reaction mixture was then diluted with water (5 ml) and extracted with ethyl acetate (2x15 ml). The organic extracts were combined and washed with water (12 ml), and saturated brine (15 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give the crude product as a brown oil (51 mg, 98%). Purification was carried out by flash chromatography on silica gel using ethyl acetate:hexane (4:1), giving the expected diol as a white crystalline solid (76% yield).

Rf 0.28 (ethyl acetate:hexane, 4:1). **mp** 72-74°C. **IR** (KBr disc) 1739, 2932, 3428 cm^{-1}.

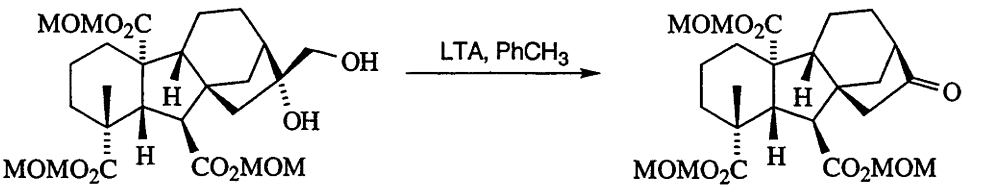
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01-2.13 (15H, m), 1.20 (3H, s, H18), 2.10 (1H, d, *J* = 12.6 Hz, H5), 2.50 (1H, m, H15α), 3.48 (3H, s, $-OCH_2O\underline{C}H_3$), 3.52 (3H, s, $-OCH_2O\underline{C}H_3$), 3.54 (3H, s, $-OCH_2O\underline{C}H_3$), 3.57-3.56 (2H, m, H7), 3.97 (1H, d, *J* = 12.7 Hz, H6), 5.05, 5.36 (2x1H, ABd, *J* = 6.0 Hz, $-O\underline{C}H_2OCH_3$), 5.10, 5.34 (2x1H, ABd, *J* = 6.0 Hz, $-O\underline{C}H_2OCH_3$), 5.26, 5.28 (2x1H, ABd, *J* = 3.3 Hz, $-O\underline{C}H_2OCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.6 (C11), 21.4 (C2), 21.8 (C12), 28.4 (C18), 34.5 (C14), 36.0 (C1), 37.4 (C3), 42.8 (C13), 45.0 (C4), 50.4 (C8), 51.1 (C15), 51.6 (C5), 56.3 (C6), 57.0 (C10), 57.5, 57.9, 58.5 (3x-$OCH_2O\underline{C}H_3$), 58.5 (C9), 66.6 (C17), 82.4 (C16), 90.0, 90.8, 91.5 (3x-$O\underline{C}H_2OCH_3$), 173.7, 174.9, 175.0 (C7, C19, C20).

MS (EI) *m/z* 528 (M^+, 6%), 497 (17), 466 (44), 435 (10), 405 (61), 315 (55), 287 (86), 269 (43), 241 (67), 185 (30).

HRMS (EI) *m/z* calc'd for M^+ $C_{26}H_{40}O_{11}$: 528.2571, found 528.2590.
Microanalysis $C_{26}H_{40}O_{11}$ requires: C 59.08, H 7.63; found: C 59.22, H 7.92.

Tri(methoxymethyl) *ent*-16-Oxo-17-norgibberellane-7,19,20-trioate (21)



(21)

To the above diol (326 mg, 0.619 mmol) in dry toluene (70 ml) was added lead tetraacetate (824 mg, 1.86 mmol). The mixture was stirred under nitrogen at room temp for 5 min, after which, tlc indicated that no starting material remained. The mixture was diluted with water (50 ml) and extracted with ethyl acetate (2x50 ml). The combined organic fractions were washed with saturated sodium bicarbonate (2x50 ml), and saturated brine (50 ml), then dried (sodium sulfate) and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel using ethyl acetate:hexane, (1:2) as eluting solvent to give the pure norketone **21** as a clear oil (305 mg, 99%).

Rf 0.30 (ethyl acetate:hexane, 1:2). **IR** (film) 920, 1088, 1144, 1157, 1741 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04-2.22 (14H, m), 1.22 (3H, s, H18), 2.16 (1H, d, *J* = 12.8 Hz, H5), 2.37 (1H, m, H13), 2.52 (1H, m, H15α), 3.49, 3.51, 3.52 (3x3H, s, $-OCH_2OC\underline{H}_3$), 4.06 (1H, d, *J* = 12.8 Hz, H6), 5.05, 5.38 (2x1H, ABd, *J* = 6.0 Hz, $-OC\underline{H}_2OCH_3$), 5.08, 5.31 (2x1H, ABd, *J* = 6.0 Hz, $-OC\underline{H}_2OCH_3$), 5.23, 5.34 (2x1H, ABd, *J* = 6.1 Hz, $-OC\underline{H}_2OCH_3$), .

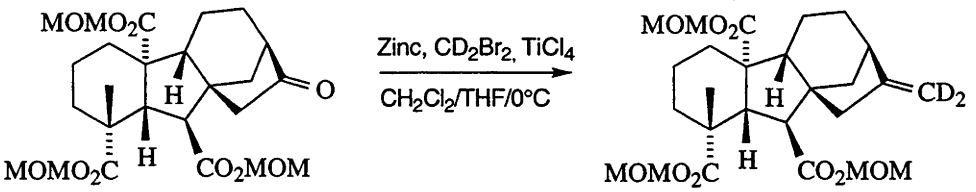
^{13}C NMR (75 MHz, $CDCl_3$): δ 19.2 (C11), 21.4 (C2), 24.9 (C12), 28.2 (C18), 33.3 (C14), 35.9 (C1), 37.3 (C3), 44.8 (C13), 45.2 (C4), 48.4 (C8), 51.8 (C5), 52.0 (C15), 56.4 (C6), 56.6 (C9), 57.2 (C10), 57.6, 57.7, 57.9 (3x-$OCH_2O\underline{C}H_3$), 90.4, 91.0, 91.6 (3x-$O\underline{C}H_2OCH_3$), 173.7, 174.0, 174.8 (C7, C19, C20) 220.5 (C16).

MS (EI) *m/z* 496 (M^+, 23%), 435 (37), 434 (83), 390 (30), 344 (30), 328 (37), 273 (43), 227 (33), 149 (45).

HRMS (EI) *m/z* calc'd for M^+ $C_{25}H_{36}O_{10}$: 496.2308, found 496.2309.

Microanalysis $C_{25}H_{36}O_{10}$ requires: C 60.47, H 7.31; found: C 60.31, H 7.04.

Tri(methoxymethyl) *ent*-(17,17-D_2)-gibberell-16-ene-7,19,20-trioate (22)



(21) (22)

The Lombardo reagent[78] (approximately 5.7 ml in suspension) was added by a Pasteur pipette to a solution of the 17-norketone **21** (265 mg, 0.534 mmol) in dry dichloromethane (25 ml), with stirring under a nitrogen atmosphere. Tlc analysis indicated that the reaction was complete. The reaction mixture was quenched with saturated sodium bicarbonate (10 ml) and extracted with ethyl acetate (2x20 ml). The combined organic phases were washed with 2M hydrochloric acid (2x15 ml), water (15 ml), and saturated brine (15 ml), then dried (sodium sulfate) and the solvent was removed under reduced pressure to give a clear oil. Chromatography on silica gel using ethyl acetate:hexane (1:5) afforded the desired dideuterated compound **22** (165 mg, 62%) as a colourless oil.

Rf 0.31 (ethyl acetate:hexane, 1:5). **IR** ($CDCl_3$) 1723, 2932 cm^{-1}.

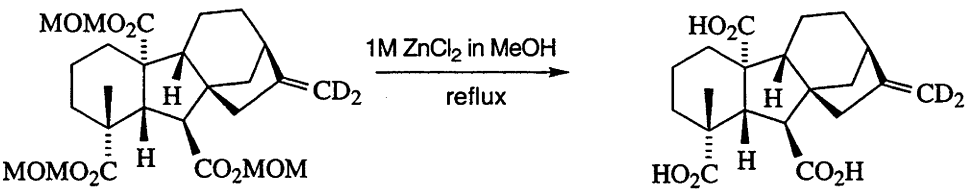
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03-2.20 (14H, m), 1.22 (3H, s, H18), 2.16 (1H, d, J = 12.5 Hz, H5), 2.52 (1H, m, H15α), 2.57 (1H, m, H13), 3.48, 3.50, 3.51 (3x3H, s, -$OCH_2OC\underline{H}_3$), 3.95 (1H, d, J = 12.6 Hz, H6), 5.05, 5.37 (2x1H, ABd, J = 5.9 Hz, -$OC\underline{H}_2OCH_3$), 5.11, 5.25 (2x1H, ABd, J = 6.0 Hz, -$OC\underline{H}_2OCH_3$), 5.22, 5.33 (2x1H, ABd, J = 6.0 Hz, -$OC\underline{H}_2OCH_3$), .

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.4 (C11), 21.5 (C2), 28.5 (C18), 31.5 (C12), 36.2 (C14), 36.6 (C1), 37.5 (C3), 39.4 (C13), 45.2 (C4), 45.7 (C15), 50.0 (C8), 50.8 (C5), 56.5 (C6), 56.5 (C9), 57.8 (C10), 57.5, 57.6, 58.0 (3x-$OCH_2O\underline{C}H_3$), 90.1, 90.9, 91.5 (3x-$O\underline{C}H_2OCH_3$), 156.0 (C16), 174.0, 174.6, 175.1 (C7, C19, C20).

MS (EI) *m/z* 493 (M^+, 4%), 434 (47), 406 (82), 390 (40), 361 (41), 344 (44), 317 (100), 299 (41), 271 (93), 227 (91).

HRMS (EI) *m/z* calc'd for M^+ $C_{26}H_{36}D_2O_9$: 496.2641, found 496.2646.

ent-(17,17-D_2)-Gibberell-16-ene-7,19,20-trioic Acid (9)



(22) (9)

The 17-dideutero GA_{25} tri(methoxymethyl) ester (20 mg, 0.040 mmol) was taken up in 1M zinc chloride in methanol (5 ml) and the mixture was heated at reflux overnight. The mixture was diluted with ethyl acetate (20 ml) and washed with sodium dihydrogen phosphate buffer (pH4) (2x10 ml), and saturated brine (10 ml). The aqueous washing's were extracted with ethyl acetate (15 ml) and the organic phase was washed with saturated brine (10 ml). The combined organic phases were dried (sodium sulfate), and the solvent was removed under reduced pressure. The residue was chromatographed on silica using ethyl acetate:hexane:methanol:acetic acid, (66:33:1:1) to give the deuterated product **9** as a white solid (10.9 mg, 74%), plus a small amount of non-deprotected gibberellin.

Rf 0.37 (ethyl acetate:hexane:methanol:acetic acid, 66:33:1:1).

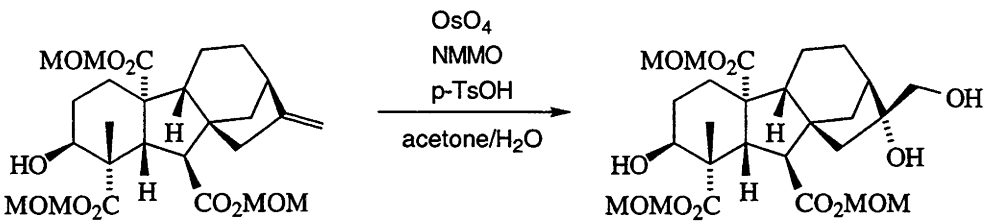
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00-2.19 (14H, m), 1.23 (3H, s, H18), 1.74 (1H, s, H3), 2.41 (1H, apparent d, J = 12.2 Hz, H15α), 2.55 (1H, apparent t, H13), 3.94 (1H, d, J = 12.5 Hz, H6).

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.6 (C11), 21.3 (C2), 28.2 (C18), 31.5 (C12), 35.5 (C3), 36.3 (C15), 37.5 (C1), 30.4 (C13), 45.3 (C4), 46.3 (C14), 49.8 (C8), 51.0 (C6), 56.0 (C5), 56.6 (C9), 57.0 (C10), 157.0 (C16), 178.0 (C7), 179.4 (C19), 181.6 (C20).

MS (EI) *m/z* 364 (M^+, 3%), 346 (33), 318 (6), 300 (73), 272 (100), 257 (9), 229 (56), 203 (22), 159 (27).

HRMS (EI) *m/z* calc'd for M^+ $C_{20}H_{24}D_2O_6$: 364.1855, found 364.1860.

Tri(methoxymethyl) *ent*-3α,16β,17-Trihydroxygibberellane-7,19,20-trioate (23)



(11) (23)

To the above olefin (560 mg, 1.10 mmol) in acetone/water (48/6 ml), was added 10 small crystals of *p*-toluenesulfonic acid and *N*-methyl morpholine-*N*-oxide (154 mg, 1.32 mmol). One crystal of osmium tetroxide was dissolved in *t*-butyl alcohol (1 ml) and added to the above mixture, which was stirred at room temperature for 8 hours. The reaction mixture was then diluted with water (20 ml) and extracted with ethyl acetate (2x40 ml). The organic extracts were combined and washed with water (20 ml), and saturated brine (20 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give the crude product as a white solid (510 mg, 85%). Purification was carried out by flash chromatography on silica gel using ethyl acetate:hexane (4:1), giving a white crystalline solid (**23**), (73% yield).

Rf 0.09 (ethyl acetate:hexane, 2:1). **IR** (film) 1042, 1733, 2928, 3385 cm^{-1}.

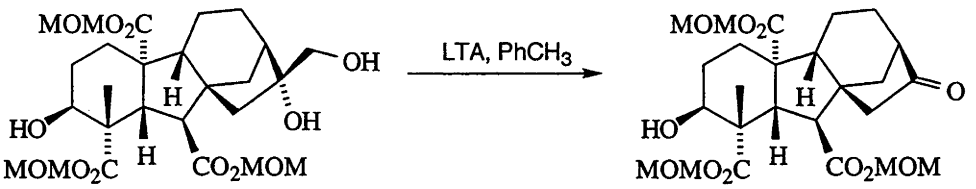
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (3H, s, H18), 1.48-2.32 (15H, m), 2.52 (1H, d, *J* = 13.1 Hz, H5), 3.47, 3.51, 3.53 (3x3H, s, $-OCH_2O\underline{CH_3}$), 3.64 (2H, m, H7), 3.95 (1H, s, H3), 3.99 (1H, d, *J* = 13.0 Hz, H6), 5.01, 5.25 (2x1H, ABd, *J* = 6.0 Hz, $-O\underline{CH_2}OCH_3$), 5.08, 5.33 (2x1H, ABd, *J* = 6.0 Hz, $-O\underline{CH_2}OCH_3$), 5.24, 5.38 (2x1H, ABd, *J* = 5.9 Hz, $-O\underline{CH_2}OCH_3$), .

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8 (C11), 21.6 (C12), 22.9 (C18), 29.8 (C1), 29.7 (C2), 34.1 (C14), 42.8 (C13), 49.4 (C8), 49.6 (C5), 50.5 (C4), 51.2 (C6), 51.5 (C15), 57.0 (C9), 57.0, 57.6, 58.0 (3x-$OCH_2O\underline{C}H_3$), 58.4 (C10), 66.9 (C17), 70.6 (C3), 82.2 (C16), 90.1, 90.9, 91.6 (3x-$O\underline{C}H_2OCH_3$), 173.8, 174.3, 174.7 (C7, C19, C20).

MS (EI) *m/z* 544 (M^+, 2%), 513 (6), 482 (33), 451 (96), 420 (72), 375 (71), 358 (66), 329 (100), 301 (86), 285 (96), 227 (43), 183 (41).

HRMS (EI) *m/z* calc'd for M^+ $C_{26}H_{40}O_{12}$: 544.2520, found 544.2514

Tri(methoxymethyl) *ent*-3α-Hydroxy-16-oxo-17-norgibberellane-7,19, 20-trioate (24)



(23) (24)

To the starting diol **23** (459 mg, 0.843 mmol) in dry toluene (120 ml) was added lead tetraacetate (1.12 g, 2.53 mmol). The mixture was stirred under nitrogen at room temperature for 5 minutes, after which, tlc indicated that no starting material remained. The mixture was diluted with water (80 ml) and extracted with ethyl acetate (2x100 ml). The combined organic fractions were washed with saturated sodium bicarbonate (2x100 ml), and saturated brine (100 ml), then dried (sodium sulfate) and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel using ethyl acetate:hexane (1:1), as eluting solvent to give the pure norketone (**24**) as a clear oil (296 mg, 68%).

Rf 0.32 (ethyl acetate:hexane, 1:1). **IR** (film) 1161, 1717, 1733, 1744, 2965, 3511 cm^{-1}.

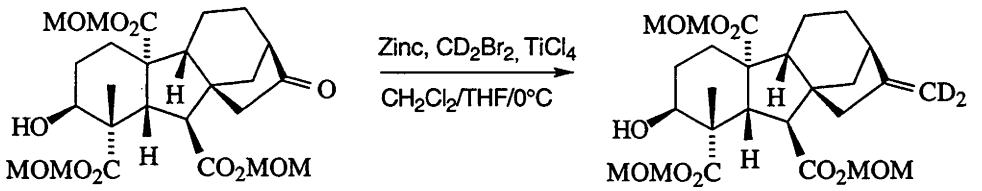
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (3H, s, H18), 1.43-2.38 (13H, m), 2.60 (1H, d, *J* = 13.0 Hz, H5), 3.47, 3.49, 3.51 (3x3H, s, $-OCH_2O\underline{C}H_3$), 3.95 (1H, s, H3), 4.05 (1H, d, *J* = 13.0 Hz, H6), 5.00, 5.28 (2x1H, ABd, *J* = 6.0 Hz, $-OC\underline{H}_2OCH_3$), 5.05, 5.32 (2x1H, ABd, *J* = 6.0 Hz, $-OC\underline{H}_2OCH_3$), 5.21, 5.39 (2x1H, ABd, *J* = 6.0 Hz, $-OC\underline{H}_2OCH_3$), .

^{13}C NMR (75 MHz, $CDCl_3$): δ 19.2 (C11), 22.7 (C18), 24.8 (C12), 29.0 (C1), 29.5 (C2), 33.0 (C14), 44.7 (C13), 48.4 (C8), 49.4 (C4), 49.6 (C5), 51.4 (C6), 52.0 (C15), 56.4 (C9), 57.1 (C10), 57.6, 57.7, 58.0 (3x-$OCH_2O\underline{C}H_3$), 70.4 (C3), 90.4, 91.0, 91.7 (3x-$O\underline{C}H_2OCH_3$), 173.7, 173.8, 174.1 (C7, C19, C20), 220.6 (C16).

MS (EI) *m/z* 512 (M^+, 21%), 451 (18), 418 (100), 405 (25), 374 (33), 344 (36), 315 (48), 271 (55), 227 (21).

HRMS (EI) *m/z* calc'd for M^+ $C_{25}H_{34}O_{11}$: 512.2258, found 512.2249.

Tri(methoxymethyl) *ent*-3α-Hydroxy-(17,17-D_2)-gibberell-16-ene-7,19, 20-trioate (25)



(24) (25)

The Lombardo reagent[78] (approximately 2 ml of suspension) was added by a Pasteur pipette to a solution of the 17-norketone **24** (27 mg, 0.052 mmol) in dry dichloromethane (2.5 ml), with stirring under a nitrogen atmosphere. Tlc analysis indicated that the reaction was complete after one min. The reaction mixture was quenched with saturated sodium bicarbonate (2 ml) and extracted with ethyl acetate (2x5 ml). The combined organic phases were washed with 2M hydrochloric acid (2x5 ml), water (5 ml), and saturated brine (5 ml), then dried (sodium sulfate) and the solvent was removed under reduced pressure to give a clear oil, (26.5 mg). Chromatography on silica gel using ethyl acetate:hexane (1:2) afforded the desired dideuterated compound **25** (15 mg, 57%) as a colourless oil and some starting material was recovered (3.9 mg, 15%).

Rf 0.24 (ethyl acetate:hexane, 1:2). **IR** (film) 1718, 1728, 1739, 2936 cm^{-1}.

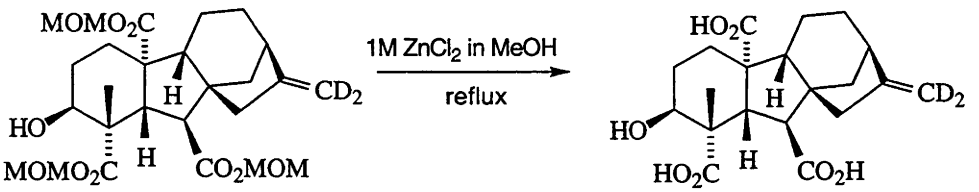
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10-2.58 (14H, m), 1.32 (3H, s, H18), 2.61 (1H, d, *J* = 12.8 Hz, H5), 3.48, 3.51, 3.52 (3x3H, s, -$OCH_2OC\underline{H}_3$), 3.97 (1H, d, *J* = 12.8 Hz, H6), 3.99 (1H, s, H3), 5.02, 5.24 (2x1H, ABd, *J* = 6.0 Hz, -$OC\underline{H}_2OCH_3$), 5.10, 5.33 (2x1H, ABd, *J* = 6.0 Hz, -$OC\underline{H}_2OCH_3$), 5.22, 5.41 (2x1H, ABd, *J* = 5.9 Hz, -$OC\underline{H}_2OCH_3$), .

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.5 (C11), 22.8 (C18), 28.9 (C1), 29.8 (C2), 31.4 (C12), 36.2 (C14), 39.2 (C13), 45.7 (C15), 49.4 (C4), 49.9 (C5), 50.0 (C8), 50.6 (C6), 56.3 (C9), 56.9 (C10), 57.5, 57.6, 58.0 (3x-$OCH_2O\underline{C}H_3$), 70.6 (C3), 90.2, 90.9, 91.6 (3x-$O\underline{C}H_2OCH_3$), 156.1 (C16), 174.1, 174.2, 174.4 (C7, C19, C20).

MS (EI) *m/z* 512 (M^+, 2%), 466 (3), 450 (19), 422 (58), 388 (15), 359 (28), 333 (28), 315 (52), 287 (38), 271 (70).

HRMS (EI) *m/z* calc'd for M^+-62 $C_{24}H_{30}D_2O_8$: 450.2223, found 450.2228.

ent-3α-Hydroxy-(17,17-d_2)gibberell-16-ene-7,19,20-trioc Acid (8)



(25) (8)

The 17-dideutero GA_{13} tri(methoxymethyl) ester (15 mg, 0.029 mmol) was taken up in 1M zinc chloride in methanol (10 ml) and the mixture was heated at reflux for 16 h. The mixture was diluted with ethyl acetate (20 ml) and washed with sodium dihydrogen phosphate buffer (pH4) (2x10 ml), and saturated brine (10 ml). The aqueous washing's were extracted with ethyl acetate (15 ml) and the organic phase was washed with saturated brine (10 ml). The combined organic phases were dried (sodium sulfate) and the solvent was removed under reduced pressure to give the crude product as a white solid (5 mg, 45%).

Rf 0.13 (ethyl acetate:hexane:methanol:acetic acid, 66:33:1:1).

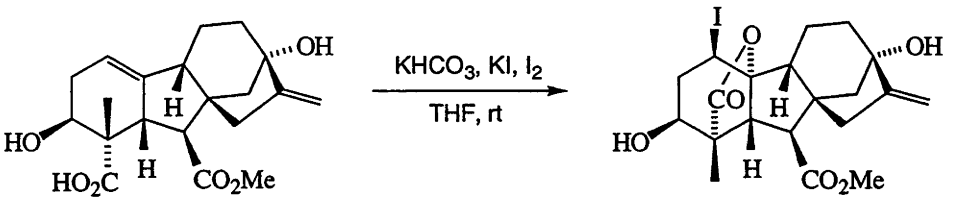
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00-2.40 (13H, m), 1.21 (3H, s, H18), 2.54 (1H, apparent d, J = 12.6 Hz, H5), 2.54 (1H, m, H13), 3.88 (1H, d, J = 12.6 Hz, H6), 3.91 (1H, s, H3).

MS (EI) *m/z* 380 (M^+, 1%), 362 (11), 316 (100), 288 (66), 270 (95), 243 (24), 227 (38).

HRMS (EI) *m/z* calc'd for M^+-18 $C_{20}H_{22}D_2O_6$: 362.1698, found 362.1698.

9.3 Experimental for Chapter 4

ent-1α-Iodo-3α,10β,13-trihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (29)[168]



(28) (29)

To the diene acid **28** (202 mg, 0.558 mmol) in THF (6 ml), was added potassium hydrogen carbonate (559 mg, 5.58 mmol) in water (5 ml), potassium iodide (463 mg, 2.79 mmol), and iodine (1.14 g, 4.46 mmol) in THF (1 ml). The dark brown mixture was stirred at room temp for 30 min. Saturated sodium thiosulfate (13 ml) was added to the mixture which became colourless upon addition. The product was extracted with diethyl ether (2x15 ml) and the combined organic phases were washed with saturated brine (20 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The iodolactone **29** was recrystallised from dichloromethane:heptane to give white crystals (230 mg, 84%).

Rf 0.30 (ethyl acetate:hexane, 1:1). **mp** 139-141°C. **IR** ($CHCl_3$) 1215, 1428, 1733, 1779, 3028, 3619 cm^{-1}.

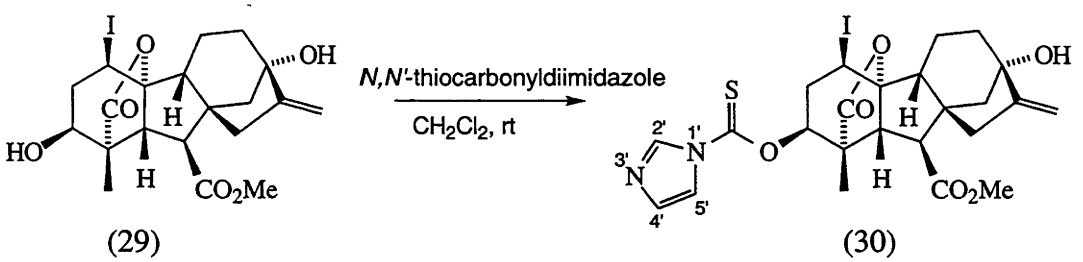
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17 (3H, s, H18), 1.50-2.73 (11H, m), 2.66 (1H, d, J = 9.8 Hz, H6), 3.72 (3H, s, 7-CO_2CH_3), 3.82 (1H, d, J = 9.9 Hz, H5), 3.93 (1H, s, H3), 4.39 (1H, d, J = 5.9 Hz, H1), 4.97 (1H, br s, H17), 5.24 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4 (C18), 16.9 (C11), 20.2 (C1), 37.8, 37.9 (C2, C12), 42.2 (C15), 45.2 (C14), 48.7 (C6), 49.2 (C8), 50.6, 50.9 (C5, C9), 52.1 (7-$CO_2\underline{C}H_3$), 54.7 (C4), 70.6 (C3), 78.1 (C13), 94.9 (C10), 107.7 (C17), 156.3 (C16), 172.2 (C7), 177.1 (C19).

MS (EI) *m/z* 488 (M^+, 11%), 456 (3), 429 (13), 361 (5), 343 (7), 329 (7), 301 (7), 283 (7), 255 (11), 239 (10), 135 (25).

HRMS (EI) *m/z* calc'd for M^+ $C_{20}H_{25}O_6I$: 488.0696, found 488.0698.

ent-10β,13-Dihydroxy-3α-imidazol-1'-ylthiocarbonyloxy-1α-iodo-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (30)



(29) (30)

To a solution of the starting alcohol (196 mg, 0.402 mmol) in dry dichloromethane (10 ml), under an atmosphere of nitrogen was added *N,N'*-thiocarbonyldiimidazole (143 mg, 0.803 mmol), and imidazole (27 mg, 0.40 mmol), and the mixture was stirred at room temperature for 7 hours. The solvent was removed under reduced pressure, and the residue was suspended in water (20 ml). The product was extracted with ethyl acetate (2x30 ml), washed with water (20 ml), and saturated brine (20 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure to give an orange oil. Purification was carried out by flash chromatography on silica gel using ethyl acetate:hexane (1:2) as eluting solvent, to give the product (208 mg, 87%) as a white powder, which was recrystallised from ether:hexane.

Rf 0.31 (ethyl acetate:hexane, 1:2). **mp** 104°C. **IR** (KBr disc) 1093, 1734, 1788, 3393 cm^{-1}.

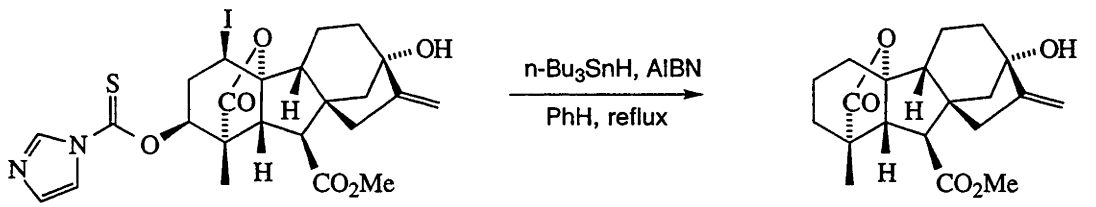
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (3H, s, H18), 1.52-2.91 (11H, m), 2.71 (1H, d, *J* = 10.4 Hz, H6), 3.75 (3H, s, 7-CO_2CH_3), 4.01 (1H, d, *J* = 10.3 Hz, H5), 4.47 (1H, d, *J* = 5.0 Hz, H1), 5.01 (1H, br s, H17), 5.30 (1H, br s, H'17), 5.69 (1H, apparent d, *J* = 2.3 Hz, H3), 7.09 (1H, br s, H'5), 7.79 (1H, br s, H'4), 8.51 (1H, br s, H'2).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (C18), 16.8 (C11), 19.5 (C1), 37.7 (C2), 37.9 (C12), 42.0 (C15), 44.5 (C14), 49.1 (C8), 49.8, 50.3, 50.8 (C5, C6, C9), 52.2 (7-$CO_2\underline{C}H_3$), 53.2 (C4), 77.6 (C13), 79.2 (C3), 94.4 (C10), 107.8 (C17), 117.8 (C'4), 130.8 (C'5), 137.1 (C2'), 156.1 (C16), 171.6 (C7), 174.9 (C19), 182.0 (-OC(S)N).

MS (EI) *m/z* 598 (M^+, 32%), 239 (33), 221 (10), 129 (36), 105 (19), 69 (100).
HRMS (EI) *m/z*: calc'd for M^+ $C_{24}H_{27}N_2O_6SI$: 598.0635; found: 598.0634.

Microanalysis $C_{24}H_{27}N_2O_6SI$ requires: C 48.17, H 4.55, N 4.68, I 21.21; found: C 48.36, H 4.69, N 4.42, I 21.33.

ent-10β,13-Dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (31)



(30) (31)

The above imidazolide (160 mg, 0.268 mmol) was taken up in dry benzene (15 ml) and heated at reflux under nitrogen for 10 minutes. *n*-Tributyltin hydride (144 µl, 0.536 mmol) was added *via* syringe followed by AIBN (9 mg, 10% wt). The resulting mixture was heated to reflux for a further 10 mins, after which the solvent was removed under reduced pressure. The residue was taken up in diethyl ether (10 ml) and DBU (160 µl, 1.07 mmol) was added. The mixture was stirred while a 0.1M solution of I_2 in diethyl ether was added until the brown colour persisted. The solution was then passed through a plug of silica gel using diethyl ether, and the solvent was removed under reduced pressure. The product was purified by flash chromatography on silica gel using ethyl acetate:hexane (1:1), to give a white crystalline solid. Residual tin by-products were removed by taking the residue up in ethyl acetate (10 ml) and washing with ammonia (3x5 ml). The residue was again chromatographed to give the 3-deoxy lactone (**31**) (65 mg, 70%) as a white solid, which was recrystallised from ethyl acetate:hexane.

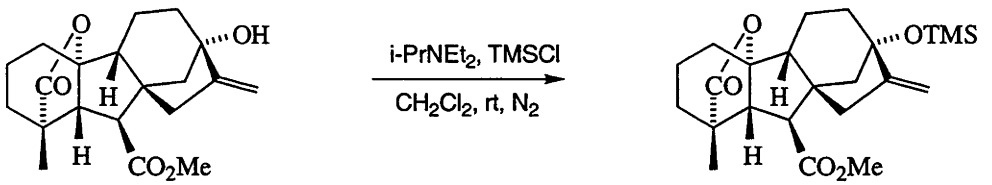
Rf 0.17 (ethyl acetate:hexane, 1:2). **mp** 144-148°C (lit.[169] 187°C). **IR** (KBr disc) 991, 1712, 1775, 2932, 3508 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (3H, s, H18), 1.33-2.07 (15H, m), 2.49 (1H, d, *J* = 10.0 Hz, H5), 2.62 (1H, d, *J* = 10.0 Hz, H6), 3.65 (3H, s, 7-CO_2CH_3), 4.87 (1H, br s, H17), 5.18 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8 (C18), 16.9 (C11), 19.0 (C2), 30.2 (C1), 34.0 (C3), 38.1 (C12), 42.7 (C15), 44.8 (C14), 48.7, 49.2 (C4, C8), 51.1 (C6), 51.8 (7-$CO_2\underline{C}H_3$), 52.7 (C9), 58.2 (C5), 77.7 (C13), 92.6 (C10), 106.9 (C17), 156.5 (C16), 172.8 (C7), 179.0 (C19).

MS (EI) *m/z* 346 (M^+, 12%), 314 (22), 303 (14), 286 (12), 199 (11), 159 (15), 135 (26), 129 (16), 115 (16), 105 (29).
HRMS (EI) *m/z* calc'd for M^+ $C_{20}H_{26}O_5$: 346.1780, found 346.1779.

ent-10β-Hydroxy-13-trimethylsilyloxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (32)



(31) (32)

To the above methyl ester **31** (127 mg, 0.368 mmol) in dry dichloromethane (10 ml) was added diisopropylethylamine (320 µl, 1.84 mmol), trimethylsilyl chloride (233 µl, 1.84 mmol) and DMAP (catalytic), and the mixture stirred under nitrogen at room temp for 1 h. The mixture was diluted with ethyl acetate (20 ml), washed with 2 M hydrochloric acid (2x10 ml), water (15 ml), and saturated brine (15 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure. The crude mixture was chromatographed on silica gel using ethyl acetate:hexane (1:5), with a trace amount of triethylamine to buffer the silica gel, to give the pure silylated product **32** as a colourless oil (94 mg, 61%).

Rf 0.37 (ethyl acetate:hexane, 1:4). **IR** (film) 840, 1143, 1736, 1776, 2951 cm^{-1}.

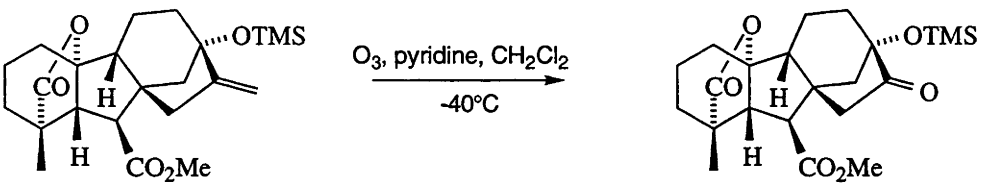
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.1 (9H, s, 13-OSi$(CH_3)_3$), 1.07 (3H, s, H18), 1.37-2.10 (15H, m), 2.53 (1H, d, J = 9.9 Hz, H5), 2.67 (1H, d, J = 9.9 Hz, H6), 3.71 (3H, s, 7-CO_2CH_3), 4.89 (1H, br s, H17), 5.17 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 2.1 (13-OSi$(CH_3)_3$), 17.0 (C18), 17.2 (C11), 19.2 (C2), 30.3 (C1), 34.3 (C3), 40.2 (C12), 42.6 (C15), 44.6 (C14), 48.8, 49.7 (C4, C8), 51.5 (C6), 51.8 (7-$CO_2\underline{C}H_3$), 52.8 (C9), 58.4 (C5), 79.7 (C13), 92.8 (C10), 107.2 (C17), 156.5 (C16), 173.0 (C7), 179.1 (C19).

MS (EI) *m/z* 418 (M^+, 100%), 403 (19), 375 (58), 359 (18), 301 (16), 207 (28), 73 (32).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{34}O_5Si$: 418.2176, found 418.2162.

***ent*-10β-Hydroxy-13-trimethylsilyloxy-16-oxo-17,20-dinorgibberellane-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (33)**



(32) (33)

Through a solution of the olefin **32** (80 mg, 0.191 mmol) in dichloromethane/pyridine (8/2 ml) at -78°C, was bubbled ozone until no starting material was observed by tlc (approximately 4 minutes). Dimethylsulfide (56 µl, 0.766 mmol) was added dropwise to the stirred solution which was then warmed to room temp over a period of 1 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:3), with a trace amount of triethylamine to buffer the silica gel, affording the norketone **33** as a white foam (28 mg, 35%). The free 13-hydroxy olefin (34) was also recovered (13 mg, 20%) as well as a very small amount of free 13-hydroxy norketone.

Rf 0.26 (ethyl acetate:hexane, 1:3). **IR** (KBr disc) 919, 1114, 1162, 1173, 1738, 1759, 1784, 2953 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.14 (9H, s, 13-$OSi(CH_3)_3$), 1.11 (3H, s, H18), 1.42-2.18 (14H, m), 2.41 (1H, dd, H15α), 2.59 (1H, d, J = 10.2 Hz, H5), 2.73 (1H, d, J = 10.2 Hz, H6), 3.74 (3H, s, 7-CO_2CH_3).

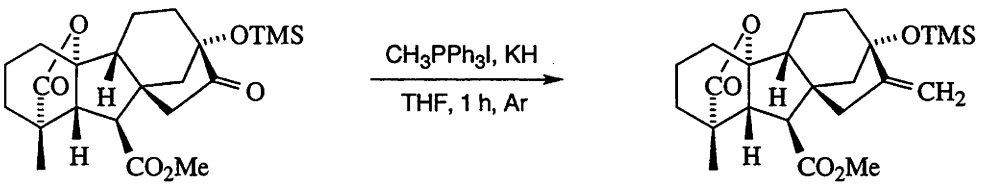
^{13}C NMR (75 MHz, $CDCl_3$): δ 1.9 (13-$OSi(CH_3)_3$), 17.0 (C18), 17.1 (C11), 19.1 (C2), 30.2 (C1), 34.1 (C3), 34.2, 42.4, 48.0 (C12, C14, C15), 46.4 (C8), 49.1 (C4), 52.2 (7-$CO_2\underline{C}H_3$), 52.8, 53.4 (C6, C9), 58.2 (C5), 80.5 (C13), 92.4 (C10), 172.3 (C7), 178.9 (C19), 216.4 (C16).

MS (EI) *m/z* 420 (M^+, 12%), 392 (56), 364 (100), 305 (21), 158 (13), 143 (29), 130 (18), 73 (61).

HRMS (EI) *m/z* calc'd for M^+ $C_{22}H_{32}O_6Si$: 420.1968, found 420.1956.

Microanalysis $C_{22}H_{32}O_6Si$ requires: C 62.83, H 7.67; found: C 62.60, H 7.93.

ent-10β-Hydroxy-13-trimethylsilyloxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (35)



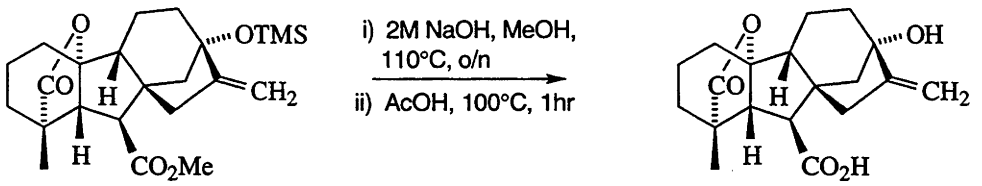
(33) (35)

Methyltriphenylphosphonium iodide (20 mg, 0.050 mmol) was placed in a flask which was then subjected to high vacuum, and heated to 60°C with rapid stirring for 16 h. The flask was then filled with Argon and dry THF (3 ml) was added with clean potassium hydride (10 mg, 0.250 mmol), and the mixture stirred rapidly under nitrogen with heating (30°C) for 30 min. The stirrer was turned off and the yellow solution was cannulated into a dry flask containing the starting norketone 33 (3.0 mg, 0.007 mmol). The mixture was stirred at room temp for 1 h. Tlc indicated that the reaction had almost gone to completion. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:5), as eluting solvent. The solvent was removed by blowing a stream of nitrogen over the flask containing the product (**35**) (2.3 mg, 79%).

Rf 0.37 (ethyl acetate:hexane, 1:4).

See **32** for relative data.

***ent*-10β,13-Dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone (37)**



(35) (37)

The alkene **35** was transferred to a 1 ml reacti-vial with a stirrer. Methanol (150 µl) and 2M sodium hydroxide (150 µl) were added to the vial, which was then capped and placed in an oil bath at 110°C overnight with stirring. The mixture was transferred to a separating funnel and a small amount of methyl red indicator was added. 1M hydrochloric acid (approx. 350 µl) was added to bring the mixture to a pH of 3. The solvent was removed under a stream of nitrogen. Acetic acid (1 ml) was added to the reacti-vial which was then capped and placed into an oil bath at 100°C for 1 hour. The acetic acid was removed under a stream of nitrogen and the residue was passed through a plug of silica gel using ethyl acetate:hexane (1:2). The solvent was changed to ethyl acetate, but the free acid **37** did not elute from the silica gel column until straight methanol was used. The free acid was collected and the solvent removed under a stream of nitrogen to give a white solid (0.8 mg, 44%).

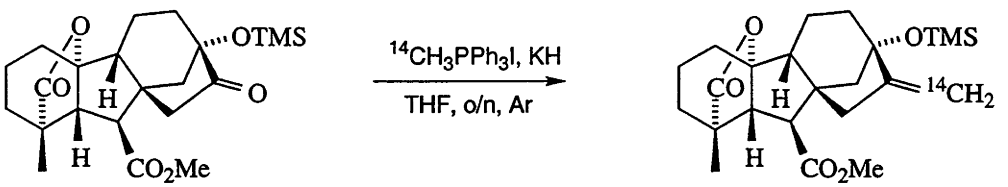
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12 (3H, s H18), 1.38-2.16 (14H, m), 2.35 (1H, dt, J_1 = 16 Hz, J_2 = 3 Hz, H15α), 2.51 (1H, d, J = 10 Hz, H5), 2.66 (1H, d, J = 10 Hz, H6), 4.94 (1H, br s, H17), 5.24 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.3 (C11 and C18 overlapped), 20.3 (C2), 31.9 (C1), 35.7 (C3), 39.8 (C12), 44.1 (C15), 46.0 (C14), 50.3, 50.6 (C4, C8), 52.4 (C6), 54.5 (C9), 59.6 (C5), 79.8 (C13), 94.3 (C10), 108.0 (C17), 157.4 (C16), 177.2 (C7), 181.0 (C19).

MS (EI) *m/z* 332 (M^+, 35%), 314 (100), 289 (72), 286 (31), 268 (17), 244 (22), 199 (12), 163 (17), 135 (23).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{24}O_5$: 332.1624, found 332.1629.

***ent*-[17-^{14}C]-10β-Hydroxy-13-trimethylsilyloxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (38)**

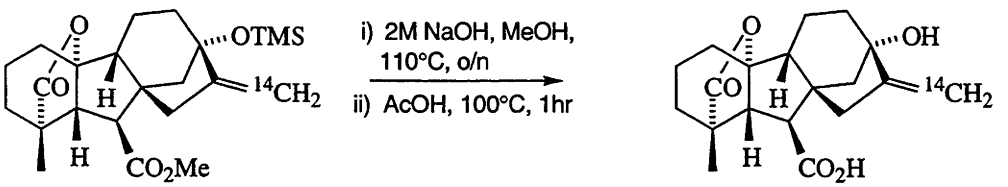


(33) (38)

To a flask containing [^{14}C]methyltriphenylphosphonium iodide (20 mg, 0.0492 mmol) in dry THF (3 ml) was added a large spatula load of clean potassium hydride, and the mixture stirred rapidly under nitrogen with heating (30°C) for 45 min. The stirrer was turned off and the yellow solution was cannulated through a needle, plugged with glass wool to act as a filter, into a dry flask containing the starting norketone **33** (7.4 mg, 0.0176 mmol). The mixture was stirred at room temp overnight. Tlc indicated that the reaction had almost gone to completion. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:5), as eluting solvent. The solvent was removed by blowing a stream of nitrogen over the flask containing the product (**38**) (5.5 mg, 74%).

Rf 0.37 (ethyl acetate:hexane, 1:4).

ent-[17-^{14}C]-10β,13-Dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone (26)

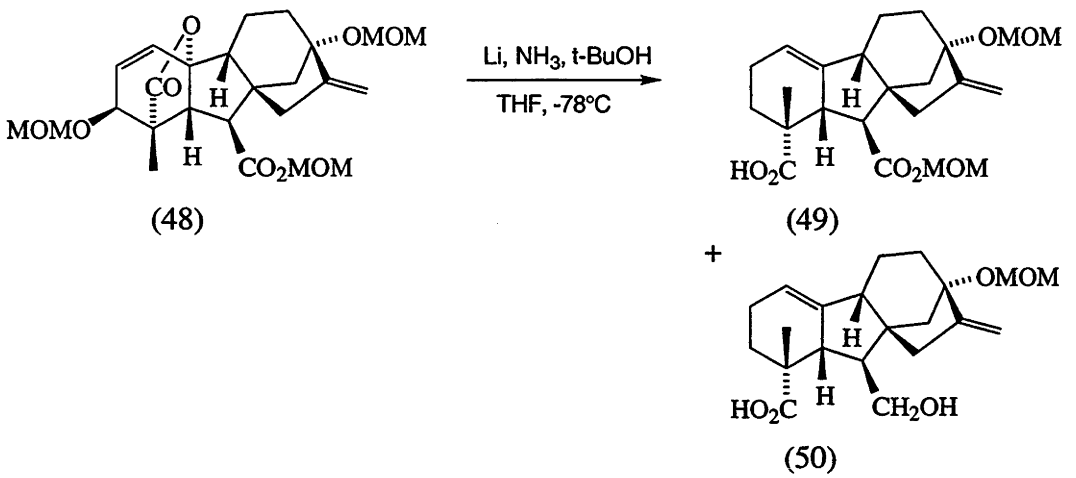


(38) (26)

The labelled alkene **38** was transferred to a 1 ml reacti-vial with a stirrer. Methanol (150 µl) and 2M sodium hydroxide (150 µl) were added to the vial, which was then capped and placed in an oil bath at 110°C over night with stirring. The mixture was transferred to a separating funnel and a small amount of methyl red indicator was added. 1M hydrochloric acid (approx. 350 µl) was added to bring the mixture to a pH of 3. The mixture was then extracted with 10% butan-2-ol in ethyl acetate (4x1.5 ml) and the combined organic phases were placed into a clean separating funnel and washed with 50% brine (2 ml), and saturated brine (2 ml), then dried (sodium sulfate) and filtered through a Pasteur pipette containing a plug of cotton wool, into a 3 ml reacti-vial. The solvent was removed under reduced pressure. Acetic acid (1 ml) was added to the reacti-vial which was then capped and placed into an oil bath at 100°C for 1 hour. The acetic acid was removed under a stream of nitrogen and the residue was passed through a plug of silica gel using ethyl acetate:hexane (1:2). The solvent was changed to ethyl acetate, but the free acid **26** did not elute from the silica gel column until straight methanol was used. The free acid was collected and the solvent removed under a stream of nitrogen to give a white solid (4.1 mg). The product was further purified using reverse phase HPLC, (2.5 mg, 45% over 3 steps). Radiochemical yield of 266 µCi.

9.4 Experimental for Chapter 5

ent-13-Methoxymethoxy-20-norgibberell-1(10),16-diene-7,19-dioic Acid 7-Methoxymethyl Ester (49)



(48) (49) (50)

The protected GA_3 derivative **48** (8.00 g, 16.7 mmol) was dissolved in dry THF (200 ml) containing *t*-butyl alcohol (11.8 ml, 0.125 mol). After cooling to -78°C, liquid ammonia (approximately 800 ml) was distilled into the flask from a sodium amide solution. Lithium metal (approximately 990 mg, 0.143 mol) was added in small pieces with vigorous stirring until tlc showed no remaining starting material. The reaction was then quenched with saturated ammonium chloride (200 ml) and the ammonia was removed under a gentle stream of nitrogen. The white, solid residue was dissolved in phosphoric acid (10%, 100 ml), and ethyl acetate (300 ml), the layers were separated and the aqueous layer further extracted with ethyl acetate (2x100 ml). The combined organic phases were washed with saturated brine (3x100 ml) to pH4, then dried (sodium sulfate) and the solvent removed under reduced pressure to give a mixture of acids **49** and **50**, as a yellow oil. The mixture was separated by flash chromatography on silica gel using ethyl acetate:hexane (1:2), to give both the acid **49** (4.07 g, 58%) and the carbinol **50** (3.58 g, 42%) as colourless oils.

Rf 0.24 (ethyl acetate:hexane, 1:2). **IR** (film) 739, 1266, 1733, 2929 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (3H, s, H18), 1.46-2.53 (12H, m), 2.61 (1H, d, *J* = 15.0 Hz, H15α), 2.85 (1H, br s, H6), 3.15 (1H, d, *J* = 6.0 Hz, H5), 3.36, 3.50 (2x3H, s, -$OCH_2OC\underline{H}_3$), 4.59, 4.79 (2x1H, ABd, J = 7.1 Hz, 13-$OC\underline{H}_2OCH_3$), 5.03 (2H, br s, H17), 5.26 (2x1H, ABd, 7-$OC\underline{H}_2OCH_3$), 5.35 (1H, br s, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.3 (C11), 23.1 (C2), 26.4 (C18), 34.5 (C3), 36.7 (C12), 39.2 (C15), 43.2 (C8), 45.0 (C14), 46.3 (C9), 49.9 (C4), 50.0 (C6), 51.0 (C5), 55.0 (13-$OCH_2O\underline{C}H_3$), 57.7 (7-$OCH_2O\underline{C}H_3$), 84.3 (C13), 90.4 (7-$O\underline{C}H_2OCH_3$), 91.5 (13-$O\underline{C}H_2OCH_3$), 106.8 (C17), 113.7 (C1), 140.5 (C10), 151.1 (C16), 175.6 (C7), 180.9 (C19).

MS (EI) *m/z* 420 (M^+, 8%), 390 (18), 375 (24), 358 (68), 344 (34), 330 (94), 314 (88), 296 (64), 258 (72), 268 (100), 255 (58), 223 (82).

HRMS (EI) *m/z*: calc'd for M^+ $C_{23}H_{32}O_7$: 420.2148; found: 420.2149.

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ent-13-Methoxymethoxy-7-hydroxy-20-norgibberell-1(10),16-diene-19-oic Acid (50)

Rf 0.51 (ethyl acetate:hexane:acetic acid:methanol, 100:100:1:1). **IR** (film) 739, 1266, 1699, 2935 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82-2.58 (14H, m), 1.29 (3H, s, H18), 2.80 (1H, d, *J* = 15.9 Hz, H15α), 3.37 (3H, s, 13-$OCH_2OC\underline{H}_3$), 3.63 (2H, m, H7), 4.58, 4.78 (2x1H, ABd, J = 7.0 Hz, 13-$OC\underline{H}_2OCH_3$), 5.01 (2H, br s, H17), 5.34 (1H, br s, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 18.5 (C11), 23.3 (C2), 26.9 (C18), 34.2 (C3), 36.6 (C12), 38.3 (C15), 42.9 (C8), 44.5 (C14), 46.2 (C9), 46.8 (C6), 48.6 (C4), 50.1 (C5), 54.9 (13-$OCH_2O\underline{C}H_3$), 65.5 (C7), 84.3 (C13), 91.2 (13-$O\underline{C}H_2OCH_3$), 106.0 (C17), 113.8 (C1), 140.2 (C10), 152.1 (C16), 180.6 (C19).

MS (EI) *m/z* 362 (M^+, 15%), 344 (69), 330 (31), 316 (38), 299 (100), 282 (100), 267 (70), 237 (81), 213 (74).

HRMS (EI) *m/z*: calc'd for M^+ $C_{21}H_{30}O_5$: 362.2093; found: 362.2081.

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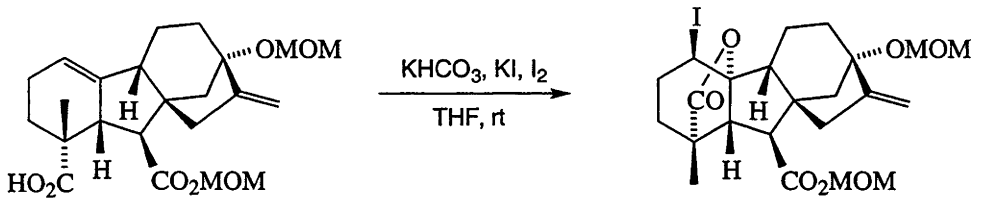
ent-10β Hydroxy-1α-iodo-13-methoxymethoxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone 7-Methoxymethyl Ester (51)



(49) (51)

To the diene acid **49** (192 mg, 0.457 mmol) in THF (6 ml), was added potassium hydrogen carbonate (460 mg, 4.57 mmol) in water (4 ml), potassium iodide (380 mg, 2.29 mmol), and iodine (930 mg, 3.66 mmol) dissolved in THF (1 ml). The dark brown mixture was stirred at room temp for 30 min. Saturated sodium thiosulfate (10 ml) was added to the mixture which became colourless upon addition. The product was extracted with diethyl ether (2x15 ml), the combined organic phases were washed with saturated brine (20 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The iodo lactone **51** was chromatographed on silica gel using ethyl acetate:hexane (1:4), to give a white solid (184 mg, 88%), plus some starting material (35 mg).

Rf 0.24 (ethyl acetate:hexane, 1:4). **mp** 91-92°C. **IR** (KBr disc) 1035, 1083, 1151, 1165, 1743, 1781, 2954 cm^{-1}.

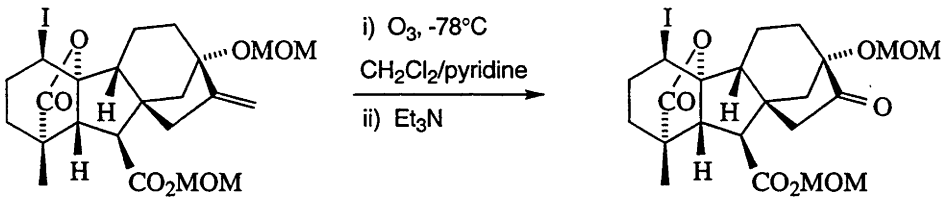
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (3H, s, H18), 1.50-2.46 (13H, m), 2.66 (1H, d, *J* = 10.0 Hz, H6), 3.33, 3.46 (2x3H, s, -$OCH_2OC\underline{H}_3$), 3.43 (1H, d, *J* = 10.1 Hz, H5), 4.53 (1H, br s, H1), 4.51, 4.73 (2x1H, ABd, J = 7.2 Hz, 13-$OC\underline{H}_2OCH_3$), 5.04 (1H, br s, H17), 5.14 (1H, br s, H'17), 5.17, 5.35 (2x1H, ABd, J = 6.0 Hz, 7-$OC\underline{H}_2OCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6 (C11), 16.8 (C18), 27.3 (C1), 30.7, 31.6 (C2, C3), 37.6 (C12), 40.9 (C15), 42.6 (C14), 48.7 (C8), 49.4 (C4), 50.7, 51.2, 54.8 (C5, C6, C9), 55.1 (13-$OCH_2O\underline{C}H_3$), 57.7 (7-$OCH_2O\underline{C}H_3$), 83.1 (C13), 90.6 (7-$O\underline{C}H_2OCH_3$), 91.7 (13-$O\underline{C}H_2OCH_3$), 93.9 (C10), 108.5 (C17), 152.6 (C16), 171.4 (C7), 178.1 (C19).

MS (EI) *m/z* 546 (M^+, 78%), 531 (57), 514 (28), 501 (90), 485 (50), 471 (56), 357 (68), 343 (78), 329 (96), 313 (87), 297 (79), 258 (86), 269 (100), 255 (72), 223 (86).
HRMS (EI) *m/z*: calc'd for M^+ $C_{23}H_{31}O_7I$: 546.1115; found: 546.1109.

Microanalysis $C_{23}H_{31}O_7I$ requires: C 50.56, H 5.72; found: C 50.69, H 5.59.

***ent*-10β-Hydroxy-1α-iodo-13-methoxymethoxy-16-oxo-17,20-dinorgibberellane-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (53)**



(51) (53)

Ozone was bubbled through a solution of the olefin **51** (200 mg, 0.366 mmol) in dichloromethane/pyridine (12/3 ml) at -78°C until no starting material was observed by tlc (approximately 7 min). Triethylamine (204 µl, 1.47 mmol) was added drop wise to the stirred solution which was then warmed to room temperature over a period of 1 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:4), to afford the norketone **53** as a clear oil (112 mg, 56%). Some starting material was also recovered (33 mg, 16%).

Rf 0.16 (ethyl acetate:hexane, 1:4).

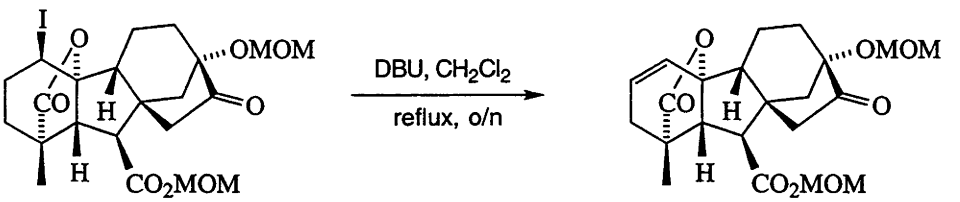
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (3H, s, H18), 1.54-2.48 (13H, m), 2.69 (1H, d, *J* = 10.2 Hz, H6), 3.28, 3.44 (2x3H, s, -$OCH_2OC\underline{H}_3$), 3.44 (1H, d, *J* = 10.2 Hz, H5), 4.50 (1H, br s, H1), 4.56, 4.73 (2x1H, ABd, *J* = 7.5 Hz, 13-$OC\underline{H}_2OCH_3$), 5.15, 5.37 (2x1H, ABd, *J* = 6.0 Hz, 7-$OC\underline{H}_2OCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.5 (C11), 16.8 (C18), 26.6 (C1), 30.5, 31.3 (C2, C3), 31.4, 47.8 (C14, C15), 37.6 (C12), 46.0 (C8), 49.6 (C4), 51.3, 52.4, 54.6 (C5, C6, C9), 55.7 (13-$OCH_2O\underline{C}H_3$), 57.9 (7-$OCH_2O\underline{C}H_3$), 82.5 (C13), 90.7 (7-$O\underline{C}H_2OCH_3$), 92.5 (13-$O\underline{C}H_2OCH_3$), 93.5 (C10), 170.7 (C7), 177.7 (C19), 215.2 (C16).

MS (EI) *m/z* 548 (M^+, 12%), 520 (34), 505 (28), 458 (20), 375 (36), 331 (100), 303 (57), 285 (61), 271 (72), 259 (66), 213 (69).

HRMS (EI) *m/z*: calc'd for M^+ $C_{22}H_{29}O_8I$: 548.0907; found: 548.0904.

***ent*-10β-Hydroxy-13-methoxymethoxy-16-oxo-17,20-dinorgibberell-1-ene-7,19-dioic Acid 19,10-Lactone 7-Methoxymethyl Ester (54)**



(53) (54)

A solution of the iodide (444 mg, 0.910 mmol) in dry dichloromethane (20 ml) was treated with DBU (967 µl, 6.48 mmol) and heated at reflux overnight. The mixture was diluted with ethyl acetate (30 ml), washed with 1M hydrochloric acid (2x20 ml), water (4x20 ml), and saturated brine (20 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane, (1:2) to give the alkene **54** as a clear oil (215 mg, 66%).

Rf 0.26 (ethyl acetate:hexane, 1:2).

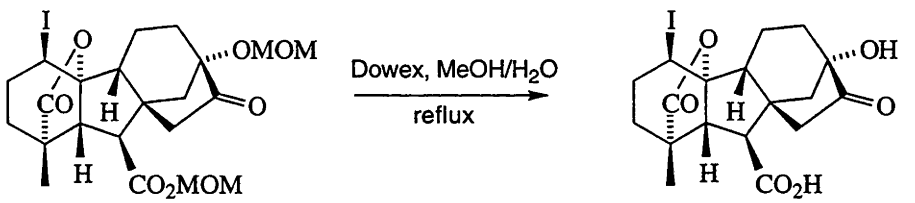
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (3H, s, H18), 1.73-2.39 (11H, m), 2.82 (1H, d, *J* = 10.6 Hz, H6), 2.98 (1H, d, *J* = 10.6 Hz, H5), 3.32, 3.46 (2x3H, s, $-OCH_2OC\underline{H}_3$), 4.60, 4.80 (2x1H, ABd, *J* = 7.5 Hz, $13-OC\underline{H}_2OCH_3$), 5.17, 5.40 (2x1H, ABd, *J* = 6.0 Hz, $7-OC\underline{H}_2OCH_3$), 5.90 (1H, br d, *J* = 9.3 Hz, H2), 6.16 (1H, br d, *J* = 9.3 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8 (C11), 17.5 (C18), 31.7 (C3), 37.6, 37.7, 48.3 (C12, C14, C15), 47.5 (C8), 47.9 (C4), 51.6, 52.2, 56.2 (C5, C6, C9), 55.8 ($13-OCH_2O\underline{C}H_3$), 57.9 ($7-OCH_2O\underline{C}H_3$), 82.5 (C13), 89.5 (C10), 90.9 ($7-O\underline{C}H_2OCH_3$), 92.6 ($13-O\underline{C}H_2OCH_3$), 129.0 (C2), 131.3 (C1), 171.2 (C7), 178.9 (C19), 215.3 (C16).

MS (EI) *m/z* 420 (M^+, 3%), 389 (16), 375 (10), 359 (27), 314 (36), 301 (31), 286 (44), 270 (47), 257 (100), 242 (51), 225 (48), 211 (60), 197 (61), 169 (60).

HRMS (EI) *m/z*: calc'd for M^+ $C_{22}H_{28}O_8$: 420.1784; found: 420.1781.

ent-10β-Hydroxy-1α-iodo-13-hydroxy-16-oxo-17,20-dinorgibberellane-7,19-dioic Acid 19, 10-Lactone (56)



(53) (56)

A solution of the iodo lactone **53** (90 mg, 0.164 mmol) in methanol/water (15/3 ml) containing Dowex 50W resin (90 mg) was heated at reflux overnight. After filtration through celite, the solvent was removed under reduced pressure. The residue was taken up in ethyl acetate (30 ml), washed with 20% potassium dihydrogen phosphate (20 ml), and saturated brine (20 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure to give a mixture of the acid **56**, (45 mg, 55%) with the starting material and some 13-methoxymethoxy ether.

Rf 0.24 (ethyl acetate:hexane:methanol:acetic acid, 66:33:1:1). **mp** 105-107°C. **IR** (KBr disc) 1098, 1753, 1785, 2934, 3434 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14 (3H, s, H18), 1.16-2.47 (13H, m), 2.69 (1H, d, J = 10.0 Hz, H6), 3.38 (1H, d, J = 10.2 Hz, H5), 4.50 (1H, d, J = 4.3 Hz, H1).

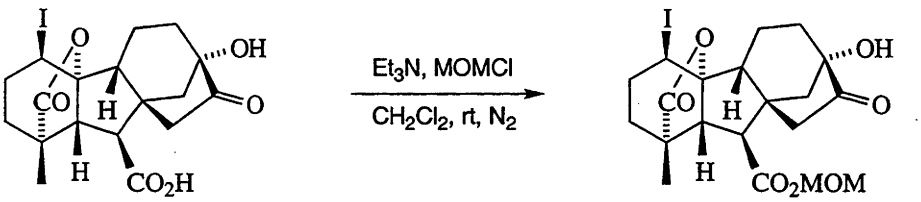
^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6 (C11), 16.8 (C18), 26.8 (C1), 30.4, 31.3 (C2, C3), 32.2, 47.3 (C14, C15), 40.4 (C12), 45.8 (C8), 49.6 (C4), 51.3, 52.1, 54.4 (C5, C6, C9), 78.7 (C13), 93.6 (C10), 174.0 (C7), 178.1 (C19), 218.6 (C16).

MS (EI) *m/z* 460 (M^+, 10%), 414 (4), 333 (3), 315 (11), 287 (58), 259 (100), 241 (53), 213 (56).

HRMS (EI) *m/z*: calc'd for M^+ $C_{18}H_{21}O_6I$: 460.0383; found: 460.0393.

Microanalysis $C_{18}H_{21}O_6I$ requires: C 46.97, H 4.60, I 27.57; found: C 46.96, H 4.76, I 27.47.

***ent*-10β,13-Dihydroxy-1α-iodo-16-oxo-17,20-dinorgibberellane-7,19-dioic Acid 19,10-Lactone 7-Methoxymethyl Ester (57)**



(56) (57)

A solution of the acid **56** (100 mg, 0.217 mmol) in dry dichloromethane (4 ml), with triethylamine (152 µl, 1.087 mmol) and chloromethyl methyl ether (86 µl, 1.087 mmol), was stirred at room temp under nitrogen for 15 min. The mixture was then quenched with saturated sodium bicarbonate (3 ml) and extracted with ethyl acetate (10 ml). The organic phase was washed with 2 M hydrochloric acid (5 ml), water (5 ml), and saturated brine (5 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure to give the ester **57** as a foam (108 ml, 99%).

Rf 0.36 (ethyl acetate:hexane, 1:1). **mp** 54°C. **IR** (KBr disc) 1089, 1145, 1752, 1785, 2935, 3452 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15 (3H, s, H18), 1.56-2.52 (13H, m), 2.72 (1H, d, *J* = 10.1 Hz, H6), 3.46 (3H, s, 7-$OCH_2OC\underline{H}_3$), 3.48 (1H, d, *J* = 10.2 Hz, H5), 4.53 (1H, d, *J* = 5.0 Hz, H1), 5.25, 5.31 (2x1H, ABd, *J* = 6.0 Hz, 7-$OC\underline{H}_2OCH_3$).

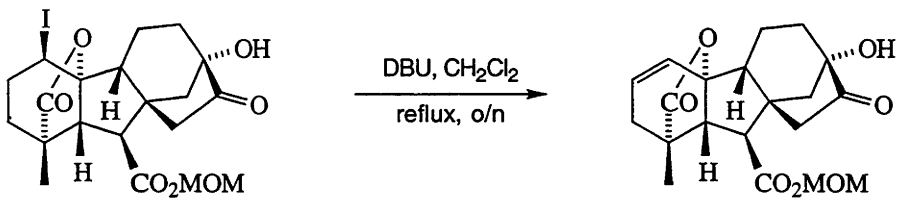
^{13}C NMR (75 MHz, $CDCl_3$): δ 16.7 (C11), 16.8 (C18), 26.8 (C1), 30.5, 31.4 (C2, C3), 32.6, 47.1 (C14, C15), 40.6 (C12), 46.1 (C8), 49.6 (C4), 51.8, 52.4, 54.6 (C5, C6, C9), 58.0 (7-$OCH_2O\underline{C}H_3$), 78.5 (C13), 91.0 (7-$O\underline{C}H_2OCH_3$), 93.5 (C10), 170.8 (C7), 177.7 (C19), 217.4 (C16).

MS (EI) *m/z* 504 (M^+, 1%), 476 (1), 414 (4), 315 (10), 287 (59), 259 (100), 241 (56), 213 (63), 157 (25).

HRMS (EI) *m/z*: calc'd for M^+ $C_{20}H_{25}O_7I$: 504.0645; found: 504.0657.

Microanalysis $C_{20}H_{25}O_7I$ requires: C 47.63, H 5.00; found: C 48.04, H 4.77.

ent-10β,13-Dihydroxy-16-oxo-17,20-dinorgibberell-1-ene-7,19-dioic Acid 19,10-Lactone 7-Methoxymethyl Ester (58)



(57) (58)

A solution of the iodide (117 mg, 0.233 mmol) in dry dichloromethane (10 ml) was treated with DBU (278 µl, 1.86 mmol) and heated at reflux overnight. The mixture was diluted with ethyl acetate (15 ml), washed with 1M hydrochloric acid (2x12 ml), water (4x12 ml), and saturated brine (12 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane (1:1), to give the alkene **58** as a white solid (71 mg, 81%), which was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.20 (ethyl acetate:hexane, 1:1). **mp** 145-146°C. **IR** (film) 1089, 1144, 1733, 1750, 1778, 2967, 3400 cm^{-1}.

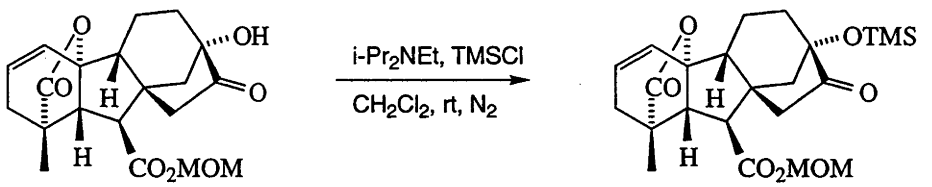
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (3H, s, H18), 1.72-2.52 (11H, m), 2.83 (1H, d, J = 10.7 Hz, H6), 3.00 (1H, d, J = 10.7 Hz, H5), 3.46 (3H, s, 7-OCH_2OCH_3), 5.25, 5.31 (2x1H, ABd, J = 6.0 Hz, 7-OCH_2OCH_3), 5.91 (1H, br d, J = 9.3 Hz, H2), 6.16 (1H, br d, J = 9.3 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.9 (C11), 17.5 (C18), 32.8, 47.5 (C14, C15), 37.6 (C3), 40.6 (C12), 47.5, 47.9 (C4, C8), 52.0, 52.2, 56.2 (C5, C6, C9), 58.0 (7-OCH_2OCH_3), 78.5 (C13), 89.4 (C10), 90.9 (7-OCH_2OCH_3), 129.0 (C2), 131.3 (C1), 171.2 (C7), 178.9 (C19), 217.5 (C16).

MS (EI) *m/z* 376 (M^+, 7%), 358 (4), 344 (8), 332 (20), 315 (46), 287 (63), 270 (41), 257 (88), 242 (67), 213 (100), 197 (56), 169 (45), 155 (48), 143 (54).

HRMS (EI) *m/z*: calc'd for M^+ $C_{20}H_{24}O_7$: 376.1522; found: 376.1522.

ent-10β-Hydroxy-13-trimethylsilyloxy-16-oxo-17,20-dinorgibberell-1-ene-7,19-dioic Acid 19,10-Lactone 7-Methoxymethyl Ester (59)



(58) (59)

To the above methoxymethyl ester (37 mg, 0.098 mmol) in dry dichloromethane (3 ml) was added diisopropylethylamine (86 µl, 0.492 mmol), trimethylsilyl chloride (38 µl, 0.295 mmol) and DMAP (cat) and the mixture stirred under nitrogen at room temperature for 1 h. The mixture was diluted with ethyl acetate (10 ml), washed with 2M hydrochloric acid (2x7 ml), water (8 ml), and saturated brine (8 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The crude mixture was chromatographed on silica gel using ethyl acetate:hexane (1:3), with a trace amount of triethylamine to buffer the silica gel, to give the pure silylated product as a colourless oil (32 mg, 73%), which was crystallised using ether:hexane.

Rf 0.58 (ethyl acetate:hexane, 1:1). **mp** 89-91°C. **IR** (KBr disc) 1137, 1739, 1761, 1775, 2958 cm^{-1}.
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.13 (9H, s, 13-OSi(CH_3)$_3$), 1.23 (3H, s, H18), 1.68-2.48 (11H, m), 2.78 (1H, d, J = 10.7 Hz, H6), 2.97 (1H, d, J = 10.7 Hz, H5), 3.46 (3H, s, 7-$OCH_2OC\underline{H}_3$), 5.25, 5.31 (2x1H, ABd, J = 6.0 Hz, 7-$OC\underline{H}_2OCH_3$), 5.89 (1H, br d, J = 9.3 Hz, H2), 6.15 (1H, br d, J = 9.3 Hz, H1).

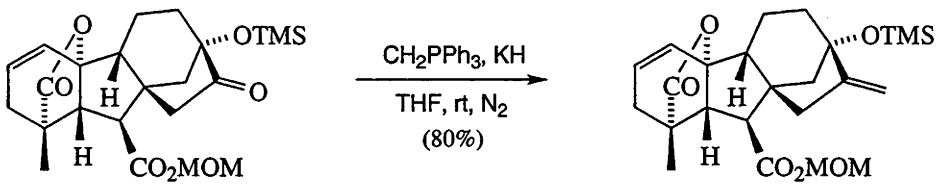
^{13}C NMR (75 MHz, $CDCl_3$): δ 1.91 (13-OSi(CH_3)$_3$, 17.0 (C11), 17.5 (C18), 34.0, 47.9 (C14, C15), 37.7 (C3), 42.0 (C12), 47.3, 47.9 (C4, C8), 51.6, 52.4, 56.1 (C5, C6, C9), 58.0 (7-$OCH_2O\underline{C}H_3$), 80.4 (C13), 89.5 (C10), 91.0 (7-$O\underline{C}H_2OCH_3$), 129.0 (C2), 131.2 (C1), 171.2 (C7), 179.0 (C19), 216.0 (C16).

MS (EI) *m/z* 448 (M^+, 3%), 434 (27), 420 (81), 392 (78), 375 (84), 304 (74), 285 (28), 241 (30), 157 (47), 143 (58).

HRMS (EI) *m/z*: calc'd for M^+ $C_{23}H_{32}O_7Si$: 448.1917; found: 448.1930.

Microanalysis $C_{23}H_{32}O_7Si$ requires: C 61.58, H 7.19; found: C 61.23, H 7.55.

***ent*-10β-Hydroxy-13-trimethylsilyloxy-20-norgibberell-1,16-diene-7,19-dioic Acid 19,10-lactone 7-Methoxymethyl Ester (60)**



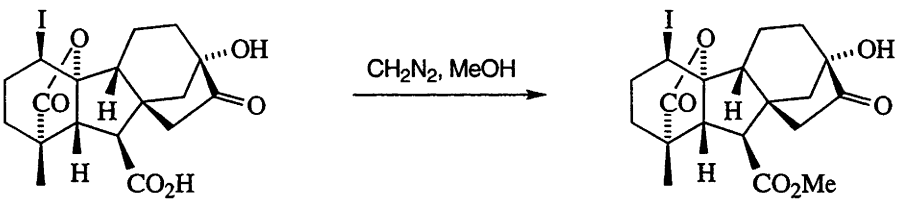
(59) (60)

To a flask containing methyltriphenylphosphonium iodide (35 mg, 0.087 mmol) in dry THF (3 ml) was added a large spatula load of clean potassium hydride, and the mixture was stirred rapidly under nitrogen with heating (30°C) for 45 minutes. The stirrer was turned off and the yellow solution was cannulated through a needle, plugged with glass wool to act as a filter, into a dry flask containing the starting norketone **59** (13 mg, 0.029 mmol). The mixture was stirred at room temperature overnight. Tlc indicated that the reaction had gone to completion. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:5), as eluting solvent. The solvent was removed under reduced pressure to give the product (10.3 mg, 80%).

Rf 0.22 (ethyl acetate:hexane, 1:5).

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.18 (9H, s, 13-OSi$(CH_3)_3$), 1.22 (3H, s, H18), 1.58-2.38 (11H, m), 2.77 (1H, d, J = 10.6 Hz, H6), 2.96 (1H, d, J = 10.6 Hz, H5), 3.48 (3H, s, 7-$OCH_2OC\underline{H}_3$), 4.91 (1H, br s, H17), 5.22 (1H, br s, H'17), 5.27, 5.30 (2x1H, ABd, J = 6.0 Hz, 7-$OC\underline{H}_2OCH_3$), 5.87 (1H, br d, J = 9.3 Hz, H2), 6.16 (1H, br d, J = 9.3 Hz, H1).

***ent*-10β,13-Dihydroxy-1α-iodo-13-hydroxy-16-oxo-17,20-dinorgibberellane-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (62)**



(56) (62)

To the acid **56** (132 mg, 0.396 mmol) in methanol (5 ml), was added dropwise, an ethereal solution of diazomethane until the yellow colour persisted. The solvent was removed under reduced pressure to give the methyl ester **62** as a white foam (136 mg, quantitative), which was recrystallised from ether:heptane (1:10).

Rf 0.51 (ethyl acetate:hexane, 1:1). **mp** 54-55°C. **IR** (KBr disc) 1278, 1735, 1753, 1789, 2953 cm^{-1}.

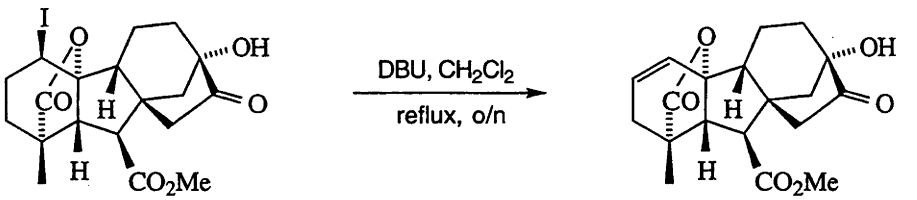
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (3H, s, H18), 1.57-2.53 (13H, m), 2.70 (1H, d, *J* = 10.2 Hz, H6), 3.73 (1H, d, *J* = 10.5 Hz, H5), 3.75 (3H, s, 7-CO_2CH_3), 4.52 (1H, d, *J* = 5.0 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.7 (C18), 16.8 (C11), 26.8 (C1), 30.6. 31.5 (C2, C3), 32.8, 47.3 (C14, C15), 40.6 (C12), 46.2 (C8), 49.7 (C4), 51.7, 52.4, 52.5 (C5, C6, C9), 54.7 (7-$CO_2\underline{C}H_3$), 78.7 (C13), 93.5 (C10), 171.6 (C7), 177.8 (C19), 217.6 (C16).

MS (EI) *m/z* 474 (M^+, 15%), 446 (9), 431 (11), 347 (12), 301 (90), 287 (100), 273 (76), 259 (72), 241 (93), 213 (91).

HRMS (EI) *m/z*: calc'd for M^+ $C_{19}H_{23}O_6I$: 474.0539; found: 474.0539.

ent-10β,13-Dihydroxy-16-oxo-17,20-dinorgibberell-1-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (63)



(62) (63)

A solution of the iodide (150 mg, 0.316 mmol) in dry dichloromethane (10 ml), was treated with DBU (378 µl, 2.53 mmol) and heated at reflux overnight. The mixture was diluted with ethyl acetate (15 ml), washed with cold 1M hydrochloric acid (2x12 ml), water (4x12 ml), and saturated brine (12 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane, (1:1) to give a mixture of the alkene **63** as a white solid (39 mg, 36%), and the iodide (71 mg, 47%). **63** was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.26 (ethyl acetate:hexane, 1:1). **mp** 118-119°C. **IR** (solution cell, $CHCl_3$) 1734, 1752, 1772 cm^{-1}.

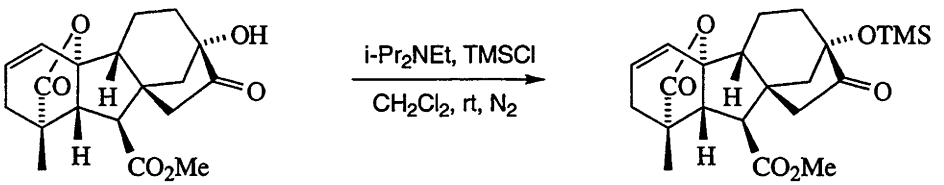
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (3H, s, H18), 1.72-2.48 (11H, m), 2.80 (1H, d, J = 10.9 Hz, H6), 2.98 (1H, d, J = 10.9 Hz, H5), 3.74 (3H, s, 7-CO_2CH_3), 5.90 (1H, br d, J = 9.3 Hz, H2), 6.15 (1H, br d, J = 9.2 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.9 (C18), 17.5 (C11), 32.9, 47.7 (C14, C15), 37.7 (C3), 40.5 (C12), 47.8, 47.9 (C4, C8), 51.9, 52.3, 56.4 (C5, C6, C9), 54.6 (7-$CO_2\underline{C}H_3$), 78.6 (C13), 89.5 (C10), 129.0 (C2), 131.3 (C1), 172.0 (C7), 178.9 (C19), 217.5 (C16).

MS (EI) *m/z* 346 (M^+, 7%), 328 (23), 314 (31), 302 (52), 287 (18), 271 (100), 259 (44), 242 (50), 213 (83), 197 (93), 157 (74).

HRMS (EI) *m/z*: calc'd for M^+ $C_{19}H_{22}O_6$: 346.1416; found: 346.1422.

ent-10β-Hydroxy-16-oxo-13-trimethylsilyloxy-17,20-dinorgibberell-1-ene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (64)



(63) (64)

To the above methyl ester (32 mg, 0.093 mmol) in dry dichloromethane (3 ml) was added diisopropylethylamine (81 µl, 0.462 mmol), trimethylsilyl chloride (35 µl, 0.277 mmol) and DMAP (catalytic), and the mixture stirred under nitrogen at room temp for 1 h. The mixture was diluted with ethyl acetate (10 ml), washed with 2M hydrochloric acid (2x7 ml), water (8 ml), and saturated brine (8 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The crude mixture was chromatographed on silica gel using ethyl acetate:hexane (1:3), with a trace amount of triethylamine to buffer the silica gel, to give the pure silylated product **64** as a colourless oil (32 mg, 73%) which was crystallised using ether:hexane.

Rf 0.27 (ethyl acetate:hexane, 1:3). **mp** 107-108°C. **IR** ($CHCl_3$) 1737, 1756, 1774, 2951 cm^{-1}.

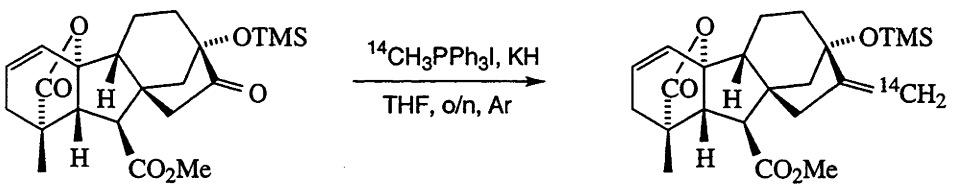
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.15 (9H, s, 13-$OSi(CH_3)_3$, 1.22 (3H, s, H18), 1.58-2.45 (11H, m), 2.77 (1H, d, J = 10.7 Hz, H6), 2.97 (1H, d, J = 10.6 Hz, H5), 3.75 (3H, s, 7-CO_2CH_3), 5.89 (1H, br d, J = 9.3 Hz, H2), 6.15 (1H, br d, J = 9.3 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 1.9 (13-$OSi(CH_3)_3$, 17.0 (C18), 17.5 (C11), 34.0, 48.1 (C14, C15), 37.8 (C3), 42.0 (C12), 47.3, 47.9 (C4, C8), 51.4, 52.2, 56.2 (C5, C6, C9), 52.4 (7-$CO_2\underline{C}H_3$), 80.4 (C13), 89.6 (C10), 129.1 (C2), 131.2 (C1), 172.0 (C7), 179.0 (C19), 216.2 (C16).

MS (EI) *m/z* 418 (M^+, 4%), 390 (85), 362 (100), 345 (10), 330 (20), 285 (16), 255 (12), 216 (18), 195 (28).

HRMS (EI) *m/z*: calc'd for M^+ $C_{22}H_{30}O_6Si$: 418.1812; found: 418.1809.

***ent*-[17-^{14}C]-10β-Hydroxy-13-trimethylsilyloxy-20-norgibberell-1,16-diene-7,19-dioic Acid 19,10-Lactone 7-Methyl Ester (65)**



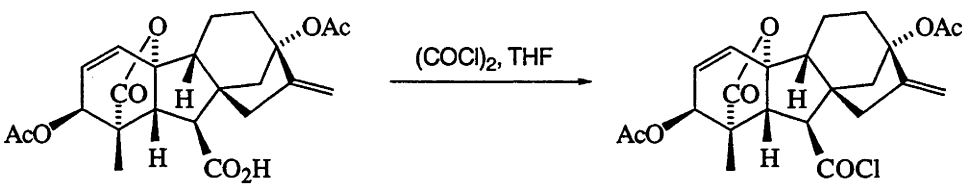
(64) (65)

To a flask containing [^{14}C]methyltriphenylphosphonium iodide (24 mg, 0.059 mmol) in dry THF (3 ml) was added a large spatula load of clean potassium hydride, and the mixture was stirred rapidly under nitrogen with heating (30°C) for 45 min. The stirrer was turned off and the yellow solution was cannulated through a needle, plugged with glass wool to act as a filter, into a dry flask containing the starting norketone **64** (8 mg, 0.019 mmol). The mixture was stirred at room temp overnight. Tlc indicated that the reaction had almost gone to completion. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:5), as eluting solvent. The solvent was removed by blowing a stream of nitrogen over the flask containing the product **65** (4.9 mg, 74%). Radiochemical yield 180 μCi.

Rf 0.37 (ethyl acetate:hexane, 1:4).

9.5 Experimental for Chapter 7

ent-7-Chloro-3α,13-diacetoxy-7-oxo-20-norgibberell-1,16-dien-19-oic Acid 19,10-Lactone (81)



(80) (81)

A solution of the acid **80** (100 mg, 0.232 mmol) in dry THF (2 ml) containing pyridine (282 µl, 3.48 mmol) was added *via* cannula to oxalyl chloride (203 µl, 2.32 mmol) in dry THF (5 ml) at -40°C under nitrogen. The solution was allowed to warm to room temp, and stirred for 4 h. The reaction mixture was filtered through a sintered funnel and washed into a dry flask with dry benzene. Residual oxalyl chloride was removed by azeotroping with benzene (4x10 ml), and the residue was filtered through a plug of celite into a dry flask. The solvent was removed under reduced pressure to give the acid chloride (**81**) (~100%) as a yellow oil.

Rf 0.81 (ethyl acetate:hexane, 1:1).

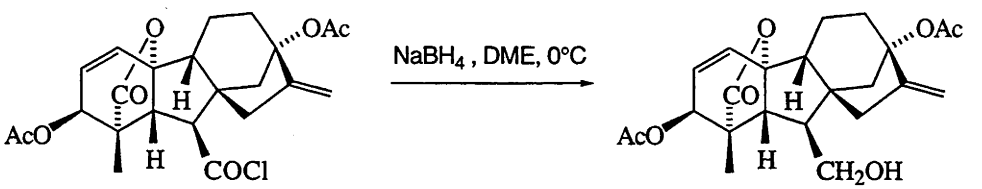
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (3H, s, H18), 1.67-2.65 (9H, m), 2.05, 2.13 (2x3H, s, -OCOC$\underline{H}_3$), 3.25 (1H, d, J = 10.8 Hz, H6), 3.32 (1H, d, J = 10.8 Hz, H5), 5.06 (1H, br s, H17), 5.24 (1H, br s, H'17), 5.35 (1H, d, J = 3.6 Hz, H3), 5.89 (1H, dd, $J_{2,1}$ = 9.2 Hz, $J_{2,3}$ = 3.7 Hz, H2), 6.36 (1H, d, J = 9.2 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3 (C18), 17.3 (C11), 21.3, 22.5 (2x-OCO$\underline{C}H_3$), 36.4 (C12), 40.2 (C14), 42.4 (C15), 51.9 (C9), 52.0 (C6), 52.6 (C8), 54.9 (C4), 62.3 (C5), 70.4 (C3), 84.1 (C13), 89.9 (C10), 109.7 (C17), 141.8 (C1), 146.6 (C2), 153.0 (C16), 170.4, 170.5 (2x-O$\underline{C}OCH_3$), 173.9 (C7), 176.8 (C19).

MS (EI) *m/z* 448 (M^+, 24%), 406 (50), 352 (24), 301 (19), 281 (53), 255 (33), 238 (71), 221 (100), 196 (65), 155 (64).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{25}O_7{}^{35}Cl$: 448.1289; found: 448.1287.

***ent*-3α,13-Diacetoxy-7-hydroxy-20-norgibberell-1,16-dien-19-oic Acid 19,10-Lactone (82)**



(81) (82)

To the starting acid chloride (**81**) (225 mg, 0.502 mmol) in dry, distilled dimethoxyethane (10 ml) at 0°C under nitrogen, was added sodium borohydride (25 mg, 0.661 mmol). The mixture was stirred at 0°C and allowed to warm slowly to room temp over 4 h. Acetic acid (2 ml) was added and the mixture was stirred for a further 20 min, then diluted with ethyl acetate (20 ml). The mixture was washed with saturated sodium bicarbonate (4x10 ml), and saturated brine (1x10 ml), then dried over sodium sulfate. The solvent was removed under reduced pressure to give the crude alcohol. Purification was carried out by silica gel chromatography using ethyl acetate:hexane (1:1), as eluting solvent to give pure alcohol (**82**) as a white crystalline compound (187 mg, 90%), which was recrystallised from ethyl acetate:hexane (1:10).

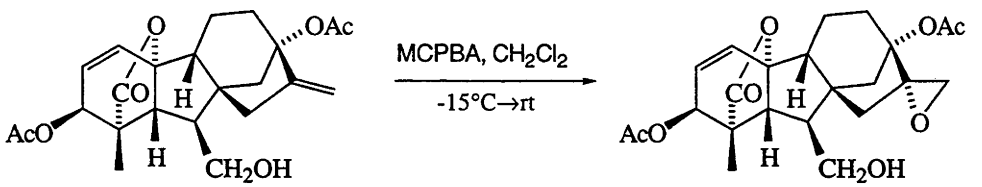
Rf 0.28 (ethyl acetate:hexane:methanol:acetic acid, 100:100:1:1). **mp** 62°C. **IR** (KBr disc) 1734, 1776, 2934, 3482 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (3H, s, H18), 1.65-2.36 (9H, m), 2.00, 2.09 (2x3H, s, -$OCOCH_3$), 2.66 (1H, d, J = 10.6 Hz, H5), 2.83 (1H, dt, J_1 = 18 Hz, H15α), 3.69-3.85 (2H, m, H7), 4.98 (1H, br s, H17), 5.11 (1H, br s, H'17), 5.30 (1H, d, J = 3.5 Hz, H3), 5.81 (1H, dd, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H2), 6.37 (1H, d, J = 9.5 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9 (C18), 16.7 (C11), 20.7, 22.0 (2x-OCO$\underline{C}H_3$), 36.3 (C12), 41.1 (C14), 46.3 (C15), 50.2 (C9), 50.8 (C5, C6 overlapped), 51.8 (C8), 52.2 (C4), 61.9 (C7), 70.9 (C3), 84.2 (C13), 90.4 (C10), 106.9 (C17), 128.6 (C1), 134.7 (C2), 153.7 (C16), 169.9 (-O$\underline{C}OCH_3$, -O$\underline{C}OCH_3$ overlapped), 177.4 (C19).

MS (EI) *m/z* 416 (M^+, 10%), 374 (46), 281 (56), 239 (57), 223 (58), 221 (100), 209 (75), 195 (50), 155 (63).
HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{28}O_7$: 416.1835; found: 416.1837.
Microanalysis $C_{23}H_{28}O_7$ requires: C 66.33, H 6.78; found: C 66.27, H 7.01.

ent-3α,13-Diacetoxy-16β,17-epoxy-7-hydroxy-20-norgibberell-1-en-19-oic Acid 19,10 Lactone (83)



(82) (83)

To the starting alcohol **82** (343 mg, 0.822 mmol) in dichloromethane (4 ml) at -15°C (ice/salt bath), was added 50% *m*-chloroperbenzoic acid (312 mg, 1.81 mmol), (pre-dried over sodium sulfate) in dichloromethane (6 ml). The mixture was stirred at -15°C and allowed to warm to room temp overnight. Tlc showed no remaining starting material, and the mixture was diluted with ethyl acetate (20 ml), washed with saturated sodium bicarbonate (2x10 ml), and saturated brine (1x10 ml), then dried (sodium sulfate). The solvent was removed under reduced pressure to give the crude epoxide **83**, which was purified by silica gel chromatography using ethyl acetate:hexane (1:1), as eluting solvent to give the pure epoxide as a white crystalline solid (275 mg, 77%), which was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.26 (ethyl acetate:hexane, 3:1). **mp** 91.5-93.5°C. **IR** ($CHCl_3$) 1250, 1728, 1755, 2880, 2940, 2970, 3040, 3500 cm^{-1}.

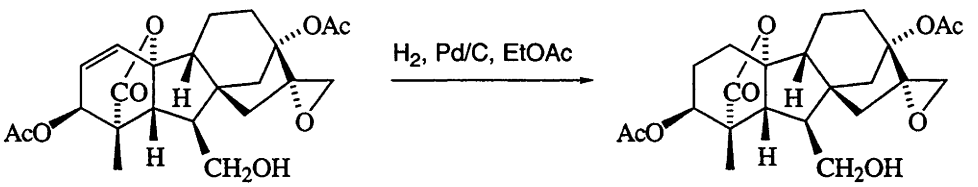
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (3H, s, H18), 1.72-2.40 (10H, m), 1.97, 2.08 (2x3H, s, $-OCOCH_3$), 2.66 (1H, d, J = 10.7 Hz, H5), 2.76 (1H, d, J = 5.3 Hz, H17), 3.07 (1H, d, J = 5.3 Hz, H'17), 3.62-3.84 (2H, m, H7), 5.29 (1H, d, J = 3.7 Hz, H3), 5.82 (1H, dd, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.7 Hz, H2), 6.38 (1H, d, J = 9.3 Hz, H1).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6 (C18), 16.9 (C11), 20.5, 21.4 (2x-OCO<u>C</u>H$_3$), 32.6 (C12), 40.6 (C14), 41.1 (C15), 46.8 (C9), 88.7 (C8), 50.1 (C17), 51.3 (C6), 51.4 (C5), 52.1 (C4), 61.4 (C7), 66.6 (C16), 70.6 (C3), 80.0 (C13), 90.2 (C10), 128.5 (C1), 134.3 (C2), 169.8, 169.9 (2x-O<u>C</u>OCH$_3$), 177.4 (C19).

MS (EI) *m/z* 432 (M^+, 26%), 390 (37), 297 (50), 237 (37), 209 (58), 195 (35), 169 (36), 155 (61).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{28}O_8$: 432.1784; found: 432.1780.

ent-3α,13-Diacetoxy-7-16β,17-epoxy-hydroxy-20-norgibberellan-19-oic Acid 19,10 Lactone (84)



(83) (84)

To a solution of the above epoxide (230 mg, 0.531 mmol) in ethyl acetate (15 ml), was added 10% palladium on charcoal (27.6 mg, 12% wt). The resulting mixture was stirred under a hydrogen atmosphere at room temp for 3 h. The mixture was then filtered through a plug of celite and the solvent removed under reduced pressure to give the saturated epoxide (**84**) as a white solid (231 mg, 100%), which was recrystallised from ether:hexane.

Rf 0.26 (ethyl acetate:hexane, 3:1). **mp** 164.5-166.5°C. **IR** ($CHCl_3$) 1733, 1774, 2933, 3620 cm^{-1}.

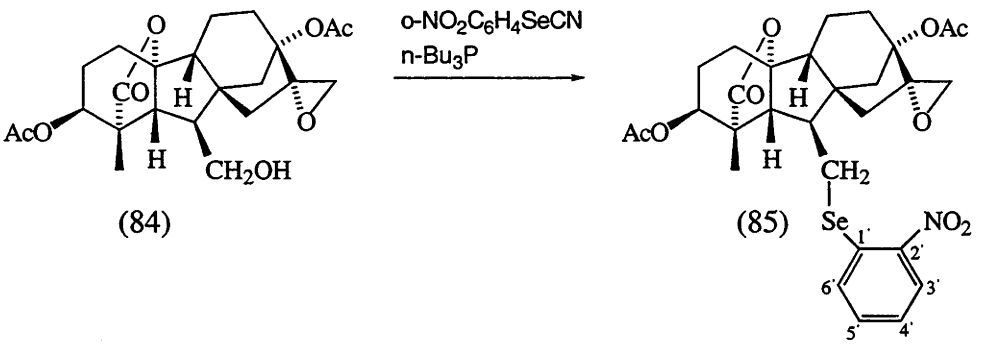
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.19 (3H, s, H18), 1.50-2.17 (12H, m), 1.99, 2.11 (2x3H, s, -$OCOCH_3$), 2.36 (1H, d, J = 13.7 Hz, H5), 2.39 (1H, d, J = 10.9 Hz, H15), 2.56 (1H, apparent dd, J_1 = 13.2 Hz, J_2 = 6.6 Hz, H6), 2.78 (1H, d, J = 5.3 Hz, H17), 3.10 (1H, d, J = 5.3 Hz, H'17), 3.60-3.83 (2H, m, H7), 4.95 (1H, apparent t, J = 2.7 Hz, H3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.7 (C18), 17.2 (C11), 21.0, 21.5 (2x-OCO$\underline{C}H_3$), 25.2 (C2), 27.2 (C1), 32.7 (C12), 40.9 (C14), 41.7 (C15), 47.2 (C9), 48.0 (C8), 50.2 (C5, C17 overlapped), 53.2 (C6, C4 overlapped), 61.7 (C7), 66.2 (C16), 71.9 (C3), 80.3 (C13), 93.1 (C10), 169.9, 170.0 (2x-O$\underline{C}OCH_3$), 177.0 (C19).

MS (EI) *m/z* 434 (M^+, 7%), 392 (100), 374 (37), 335 (32), 257 (68), 239 (33), 211 (60), 157 (51).

Microanalysis $C_{23}H_{30}O_8$ requires: C 63.58, H 6.96; found: C 63.28, H 7.25.

***ent*-3α,13-Diacetoxy-16β,17-epoxy-7-(2'-nitrobenzeneselenenyl)-20-norgibberellan 19-oic Acid 19,10-Lactone (85)**



To the alcohol **84** (230 mg, 0.528 mmol) in THF (10 ml), was added *o*-nitrophenyl selenocyanate (264 mg, 1.16 mmol), followed by the drop wise addition of *n*-tributylphosphine (290 µl, 1.16 mmol) under an atmosphere of nitrogen at room temperature. The resulting mixture was stirred for 1 hour, after which, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel (ethyl acetate:hexane, 1:1) to give the *o*-nitrophenyl selenide **85** (290 mg, 89%) as a yellow powder, which was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.28 (ethyl acetate:hexane, 1:1). **mp** 103-105°C. **IR** (KBr disc) 1239, 1740, 1773 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (3H, s, H18), 1.53-2.25 (12H, m), 2.00, 2.10 (2x3H, s, $-OCOCH_3$), 2.31 (1H, d, J = 13.4 Hz, H15α), 2.40 (1H, d, J = 11.1 Hz, H14β), 2.67 (1H, d, J = 9.2 Hz, H5), 2.78 (1H, d, J = 5.4 Hz, H17), 2.90 (1H, dd, J_{gem} = 10.8 Hz, J_{vic} = 4.8 Hz, H7), 3.05 (1H, dd, J_{gem} = 10.8 Hz, J_{vic} = 7.5 Hz, H'7), 3.13 (1H, d, J = 5.4 Hz, H'17), 4.98 (1H, apparent t, H3), 7.36 (1H, apparent t, J = 7.0 Hz, H'4), 7.43 (1H, d, J = 8.2 Hz, H'6), 7.55 (1H, apparent t, J = 7.0 Hz, H'5), 8.28 (1H, d, J = 8.3 Hz, H'3).

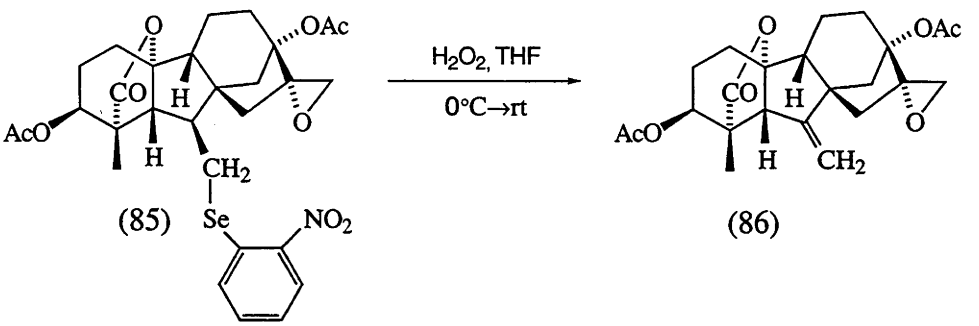
^{13}C NMR (75 MHz, $CDCl_3$): δ 14.8 (C18), 17.4 (C11), 20.9, 21.5 (2x-OCO$\underline{C}H_3$), 25.1 (C2), 27.5 (C1), 27.7 (C7), 32.7 (C12), 41.2 (C14), 42.2 (C15), 43.3 (C9), 49.2 (C8), 50.4 (C17), 52.6 (C5), 53.2 (C4), 55.3 (C6), 65.4 (C16), 71.8 (C3), 80.4 (C13), 92.4 (C10), 125.7 (C'3), 126.3 (C'4), 128.7 (C'6), 132.3 (C'1), 133.7 (C'5), 146.5 (C'2), 169.8 ($-O\underline{C}OCH_3$, $-O\underline{C}OCH_3$ overlapped), 176.4 (C19).

MS (EI) *m/z* 619 (M^+, 70%), 589 (15), 376 (26), 357 (27), 315 (37), 297 (58), 211 (62), 186 (100), 155 (56).

HRMS (EI) *m/z* calc'd for M^+ $C_{29}H_{33}O_9NSe$: 619.1321; found: 619.1311.

Microanalysis $C_{29}H_{33}O_9NSe$ requires: C 56.31, H 5.38; found: C 56.38, H 5.66.

ent-3α,13-Diacetoxy-16β,17-epoxy-20-norgibberell-6-en-19-oic Acid 19,10-Lactone (86)



A solution of the selenide (**85**) (53 mg, 0.086 mmol) in a mixture of THF (2 ml) and 30% hydrogen peroxide (97 µl, 0.860 mmol) was stirred at 0°C for 1.5 h and then allowed to warm to room temp over 10.5 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:2), to give the olefin as a clear oil (33 mg, 90%). The olefin was recrystallised from dichloromethane:hexane to give a white crystalline solid.

Rf 0.33 (ethyl acetate:hexane, 1:1). **mp** 160-163°C. **IR** (KBr disc) 1221, 1739, 1753, 1768, 2936 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31(3H, s, H18), 1.53-2.20 (12H, m), 1.97, 2.10 (2x3H, s, -$OCOCH_3$), 2.51 (1H, dd, J_1 = 2.4 Hz, J_2 = 11.3 Hz, H15α), 2.80 (1H, d, J = 5.2 Hz, H17), 3.06 (1H, d, J = 5.1 Hz, H'17), 3.20 (1H, apparent t, J = 2.7 Hz, H5), 4.98 (1H, dd, J_1 = 2.6 Hz, J_2 = 2.9 Hz, H3), 5.17 (1H, d, J = 2.7 Hz, H7), 5.31 (1H, d, J = 3.0 Hz, H'7).

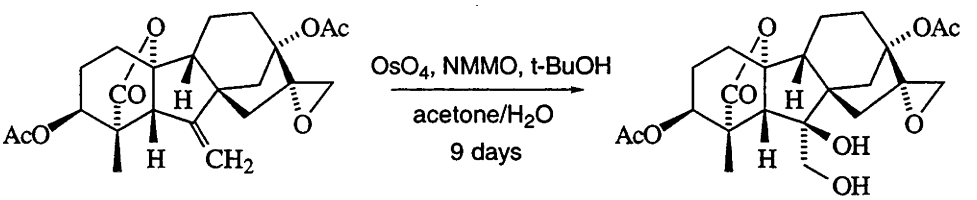
^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3 (C18), 17.1 (C11), 21.0, 21.6 (2x-OCO$\underline{C}H_3$), 25.2 (C2), 26.6 (C1), 32.3 (C12), 42.1 (C14), 46.8 (C15), 49.4 (C17), 50.4 (C8), 51.5 (C9), 53.0 (C4), 53.4 (C5), 65.4 (C16), 72.9 (C3), 81.0 (C13), 92.2 (C10), 113.0 (C7), 151.5 (C6), 169.7, 169.8 (2x-O$\underline{C}OCH_3$), 176.3 (C19).

MS (EI) *m/z* 416 (M^+, 100%), 374 (51), 356 (42), 312 (76), 270 (37), 252 (46), 211 (61), 149 (49).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{28}O_7$: 416.1835; found: 416.1825.

Microanalysis $C_{23}H_{28}O_7$ requires: C 66.33, H 6.78; found: C 65.99, H 7.10.

***ent*-3α,13-Diacetoxy-6α,7-dihydroxy-16β,17-epoxy-20-norgibberellan-19-oic Acid 19,10-Lactone (87)**



(86) (87)

To a solution of the olefin (**86**) (65 mg, 0.153 mmol) in acetone/water (5/1 ml) was added *N*-methylmorpholine-*N*-oxide (89 mg, 0.763 mmol), and osmium tetroxide (catalytic) dissolved in *t*-butyl alcohol (100 µl), and the mixture stirred at 30°C for 9 days. Sodium bisulfite (10 mg) was added to the mixture, which was then diluted with water (4 ml) and stirred for another 30 min. The mixture was extracted with ethyl acetate (2x10 ml), and the combined organic phases were washed with water (12 ml), and saturated brine (12 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane (2:1), to give the diol **87**, (55 mg, 66%) as a white solid, plus some starting olefin (11.3 mg, 17%). The diol was recrystallised from ether:hexane.

Rf 0.10 (ethyl acetate:hexane, 2:1). **mp** 169-171°C. **IR** (KBr disc) 1233, 1262, 1719, 1740, 1776, 2955, 3422, 3513 cm^{-1}.

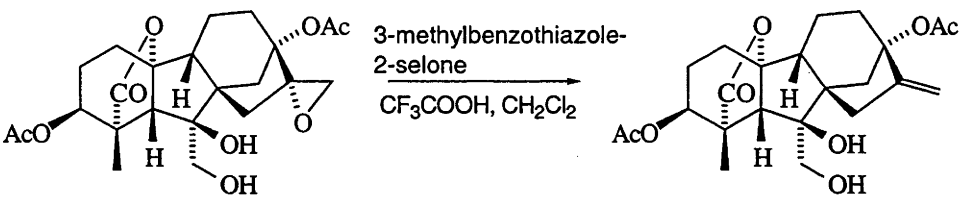
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31(3H, s, H18), 1.57-2.17 (11H, m), 1.97, 2.12 (2x3H, s, -$OCOCH_3$), 2.55 (1H, d, *J* = 13.5 Hz, H15α), 2.61 (1H, d, *J* = 10.8 Hz, H14), 2.80 (1H, d, *J* = 5.5 Hz, H17), 2.90 (1H, s, H5), 3.06 (1H, d, *J* = 5.5 Hz, H'17), 3.38 (1H, br s, 6-OH), 3.74 (2H, d, *J* = 2.8 Hz, H7), 4.91 (1H, br s, H3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.5 (C18), 17.3 (C11), 21.1, 21.8 (2x-OCO$\underline{C}H_3$), 25.2 (C2), 27.3 (C1), 32.8 (C12), 38.2 (C14), 41.2 (C15), 47.0 (C9), 50.3 (C17), 52.0 (C8), 54.5 (C4), 61.8 (C5), 62.5 (C16), 64.7 (C7), 75.0 (C3), 82.6 (C13), 82.7 (C10), 92.9 (C6), 170.0, 170.3 (2x-O$\underline{C}OCH_3$), 177.1 (C19).

MS (EI) *m/z* 450 (M^+, 11%), 419 (82), 408 (100), 390 (28), 377 (31), 359 (44), 331 (33), 315 (74), 289 (50), 271 (68), 255 (65), 227 (66), 197 (51), 171 (44).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{30}O_9$: 450.1890; found: 450.1894.
Microanalysis $C_{23}H_{30}O_9$ requires: C 61.32, H 6.71; found: C 61.12, H 6.96.

ent-3α,13-Diacetoxy-6α,7-dihydroxy-20-norgibberell-16-en-19-oic Acid 19,10-Lactone (89)



(87) (89)

A solution of the diol (**87**) (176 mg, 0.391 mmol) in dichloromethane (8 ml), with 3-methylbenzothiazole-2-selone (98 mg, 0.430 mmol) and trifluoroacetic acid (33 µl, 0.430 mmol), was stirred under nitrogen at room temp. The solution turned a dark yellow colour. After 6 h the now brown solution, was diluted with ethyl acetate (15 ml), washed with water (2x10 ml), and saturated brine (10 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane, (1:1 changing to 2:1), to give the alkene (**89**) as a clear oil (81 mg, 48%).

Rf 0.11 (ethyl acetate:hexane, 1:1). **IR** ($CDCl_3$) 1044, 1241, 1372, 1454, 1733, 1766, 2944, 3535, 3622 cm^{-1}.

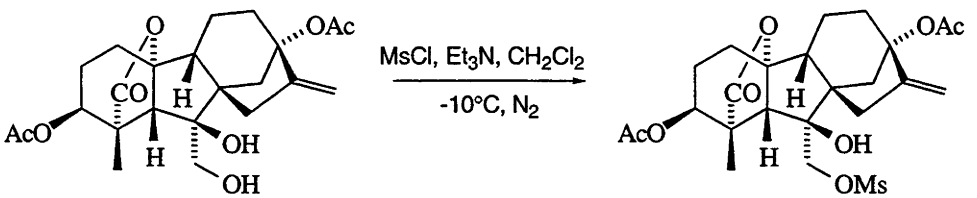
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (3H, s, H18), 1.54-1.70 (2x1H, m, H1, H12), 1.85-1.90 (4x1H, m, H2, H11), 1.97-2.10 (3x1H, m, H1, H9, H14), 2.00, 2.11 (2x3H, s, -$OCOCH_3$), 2.13-2.20 (1H, m, H12), 2.25 (1H, d, J = 17.3 Hz, H15β), 2.53 (1H, d, J = 12.4 Hz, H14), 2.86 (1H, s, H5), 2.90-2.96 (1H, m, H15), 3.67 (2H, br s, H7), 4.90 (1H, dd, J_1 = 2.9 Hz, J_2 = 2.6 Hz, H3), 4.94 (1H, br s, H17), 5.00 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.5 (C18), 17.64 (C11), 21.1, 22.1 (2x-$OCO\underline{C}H_3$), 25.3 (C2), 27.4 (C1), 36.7 (C12), 37.2 (C14), 41.2 (C15), 47.1 (C9), 52.0 (C8), 55.8 (C4), 61.6 (C5), 64.8 (C7), 75.1 (C3), 82.7 (C13), 86.2 (C6), 93.1 (C10), 105.7 (C17), 149.7 (C16), 170.0, 170.1 (2x-$O\underline{C}OCH_3$), 177.2 (C19).

MS (EI) *m/z* 434 (M^+, 58%), 392 (84), 356 (38), 344 (100), 314 (36), 299 (72), 257 (58), 239 (91), 209 (59), 197 (72).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{30}O_8$: 434.1941; found: 434.1944.

***ent*-3α,13-Diacetoxy-6α-hydroxy-7-methanesulfonyloxy-20-norgibberell-16-ene-19-oic Acid 19, 10-Lactone (96)**



(89) (96)

To the starting diol (**89**) (61 mg, 0.142 mmol) in dry dichloromethane (10 ml) at -10°C, was added triethylamine (197 µl, 1.42 mmol), and methanesulfonyl chloride (44 µl, 0.566 mmol), and the resulting mixture was stirred under nitrogen for 3 h. Ice was added to the mixture which was then extracted with ethyl acetate (2x15 ml). The combined organic phases were washed with water (12 ml), and saturated brine (12 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane (1:1), to give a white solid (54 mg, 74%), which was recrystallised from ethyl acetate:hexane (1:10).

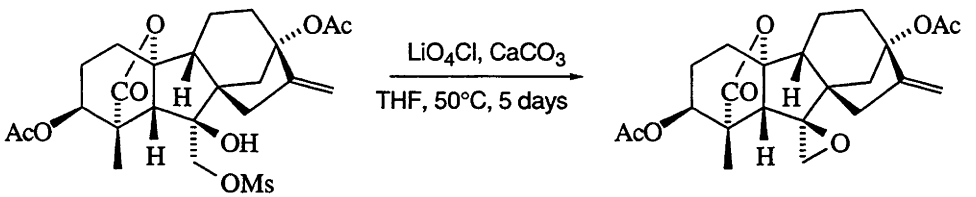
Rf 0.18 (ethyl acetate:hexane, 1:1). **mp** 169-171°C. **IR** (KBr disc) 1233, 1262, 1719, 1740, 1776, 2955, 3422, 3513 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (3H, s, H18), 1.58-2.16 (10H, m), 2.00, 2.14 (2x3H, s, $-OCOCH_3$), 2.30 (1H, br d, J = 15.1 Hz, H15β), 2.67 (1H, dd, J_1 = 10.0 Hz, J_2 = 2.2 Hz, H14), 2.91 (1H, br d, J = 15.5 Hz, H15α), 2.95 (1H, s, H5), 3.15 (3H, s, $7\text{-}OSO_2CH_3$), 4.10 (1H, d, J = 10.1 Hz, H7), 4.44 (1H, d, J = 10.1 Hz, H'7), 4.94 (1H, dd, J_1 = 3.0 Hz, J_2 = 2.7 Hz, H3), 4.97 (1H, br s, H17), 5.01 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.9 (C18), 17.5 (C11), 21.1 ($-OCO\underline{C}H_3$), 22.0 ($-OCO\underline{C}H_3$), 25.2 (C2), 27.2 (C1), 36.7 (C12), 37.3 (C14), 37.7 ($7\text{-}OSO_2CH_3$), 40.9 (C15), 47.0 (C9), 51.8 (C8), 56.4 (C4), 62.3 (C5), 72.0 (C7), 75.1 (C3), 81.8 (C13), 85.5 (C6), 93.0 (C10), 106.0 (C17), 148.5 (C16), 169.5 ($-O\underline{C}OCH_3$), 170.0 ($-O\underline{C}OCH_3$), 176.3 (C19).

MS (EI) *m/z* 512 (M^+, 16%), 470 (61), 417 (25), 374 (25), 356 (45), 343 (73), 279 (31), 239 (41), 149 (100).
HRMS (EI) *m/z* calc'd for M^+ $C_{24}H_{32}O_{10}S$: 512.1716; found: 512.1717.
Microanalysis $C_{24}H_{32}O_{10}S$ requires: C 56.24, H 6.29; found: C 56.08, H 6.72.

ent-3α,13-Diacetoxy-6α,7-epoxy-20-norgibberell-16-en-19-oic Acid 19,10-Lactone (97)



(96) (97)

The mesylate (**96**) (54 mg, 0.105 mmol) was dissolved in dry THF (2 ml), and added to a stirred suspension of lithium perchlorate (112 mg, 1.05 mmol) and calcium carbonate (105 mg, 1.05 mmol), (oven-dried) in THF (5 ml). The mixture was stirred under an atmosphere of nitrogen at 60°C for 5 days. The mixture was diluted with ethyl acetate (15 ml), and washed with water (5x10 ml), saturated brine (10 ml), dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane, (1:3) to give a high R_f spot by tlc, which corresponded to the epoxide (**97**) (1 mg, 2.3%) and a lower R_f spot, the starting mesylate (35.5 mg, 66%).

Rf 0.64 (ethyl acetate:hexane, 1:1). **mp** 76-78°C. **IR** (KBr disc) 1237, 1741, 1781, 2933 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18-2.42 (13H, m), 1.19 (3H, s, H18), 2.00, 2.09 (2x3H, s, -$OCOCH_3$), 2.73 (1H, d, *J* = 3.8 Hz, H7), 2.93 (1H, s, H5), 3.16 (1H, d, *J* = 3.7 Hz, H'7), 4.92 (1H, br s, H17), 5.02 (1H, br s, H3), 5.05 (1H, br s, H'17).

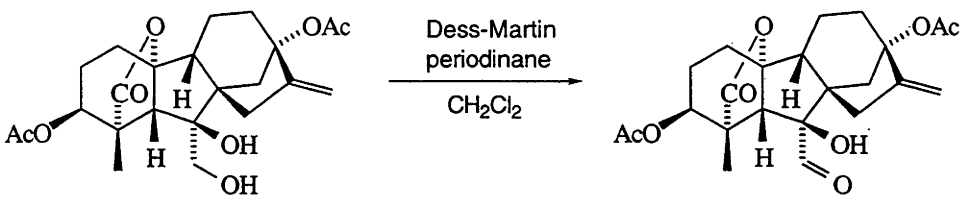
^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9 (C18), 17.3 (C11), 21.0, 21.9 (2x-OCO$\underline{C}H_3$), 25.2 (C2), 27.2 (C1), 36.8 (C12), 38.9 (C14), 39.9 (C15), 47.4 (C7), 49.2 (C9), 48.5 (C4), 51.9 (C8), 53.6 (C5), 65.5 (C6), 73.4 (C3), 84.8 (C13), 91.4 (C10), 106.9 (C17), 150.3 (C16), 169.9, 169.8 (2x-O$\underline{C}OCH_3$), 176.4 (C19).

MS (EI) *m/z* 416 (M^+, 59%), 374 (100), 356 (78), 314 (35), 238 (48), 252 (72), 239 (57), 223 (66), 197 (51), 135 (55).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{28}O_7$: 512.1716; found: 512.1717.

Microanalysis $C_{23}H_{28}O_7$ requires: C 66.33, H 6.78; found: C 66.30, H 6.69.

***ent*-3α,13-Diacetoxy-6α-hydroxy-7-oxo-20-norgibberell-16-en-19-oic Acid 19,10-Lactone (102)**



(89) (102)

A solution of the diol (**89**), (56 mg, 0.129) in dichloromethane (5 ml) containing Dess-Martin reagent (71 mg, 0.168 mmol), was stirred at room temp overnight under an atmosphere of nitrogen. The solution was diluted with ethyl acetate (10 ml) and sodium thiosulfate (1 g) dissolved in saturated sodium bicarbonate (5 ml) was added to the mixture, which was stirred for a further 30 min. The organic phase was separated and washed with saturated sodium bicarbonate (8 ml), water (8 ml), and saturated brine (10 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane, (1:2) to give the aldehyde **102** as a white crystalline solid (51 mg, 91%), which was recrystallised from ether:hexane (1:10).

Rf 0.30 (ethyl acetate:hexane, 1:2). **mp** 145-147°C. **IR** (KBr disc) 1704, 1739, 1774, 2959 cm^{-1}.

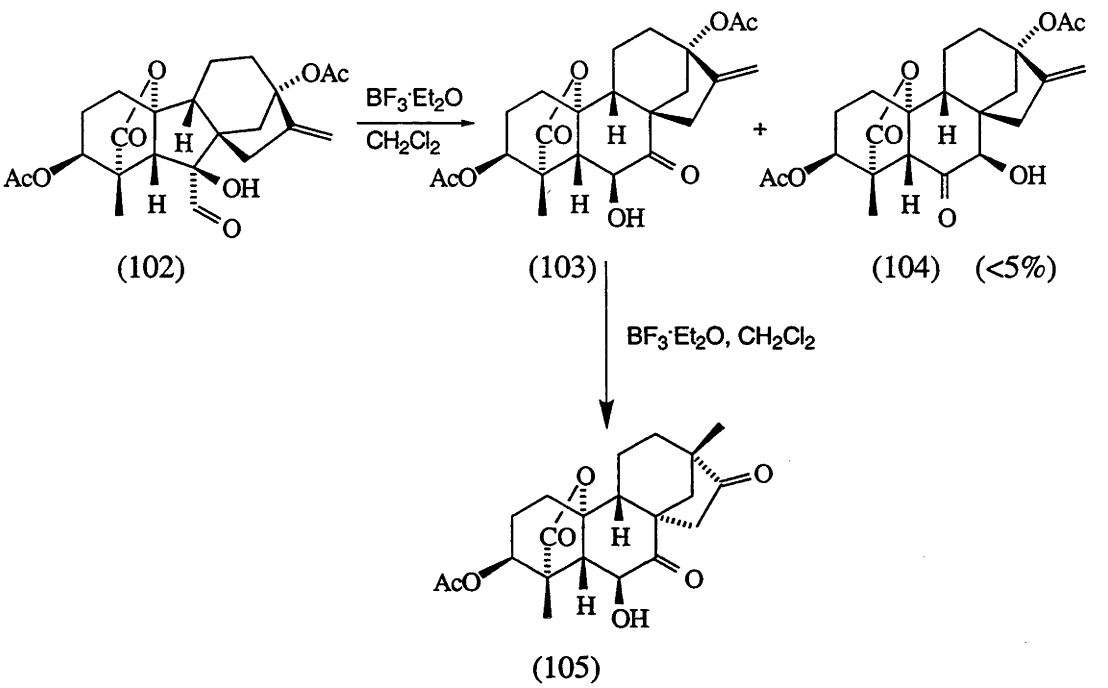
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (3H, s, H18), 1.61-2.38 (12H, m), 2.00, 2.12 (2x3H, s, $-OCOCH_3$), 2.63 (1H, m, H15α), 3.05 (1H, s, H5), 4.12 (1H, br s, 6-OH), 4.94 (1H, br s, H17), 5.03 (1H, br s, H3), 5.14 (1H, br s, H'17), 9.67 (1H, s, 7-CHO).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4 (C18), 17.1 (C11), 20.9, 21.7 (2x-OCO$\underline{C}H_3$), 24.9 (C2), 27.9 (C1), 35.1 (C14), 36.1 (C12), 42.3 (C15), 50.7 (C4), 51.5 (C9), 54.4 (C8), 61.3 (C5), 72.2 (C3), 84.8 (C13), 86.6 (C6), 91.4 (C10), 107.6 (C17), 150.5 (C16), 169.6, 169.8 (2x-O$\underline{C}OCH_3$), 176.9 (C19), 200.6 (C7).

MS (EI) *m/z* 432 (M^+, 6%), 390 (10), 360 (26), 272 (7), 255 (10), 231 (25), 172 (100), 149 (53).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{28}O_8$: 432.1784; found: 432.1776.
Microanalysis $C_{23}H_{28}O_8$ requires: C 63.88, H 6.53; found: C 63.59, H 6.64.

***ent*-3α,13-Diacetoxy-6α-hydroxy-7-oxo-20-norkaur-16-en-19-oic Acid 19,10-Lactone (103)**



To the hydroxy aldehyde (**102**), (5 mg, 0.0116 mmol) in dry dichloromethane (1 ml), was added boron trifluoride etherate (1.9 µl, 0.0154 mmol), and the mixture was stirred at room temp for 30 min under an atmosphere of nitrogen. The mixture was diluted with ethyl acetate (5 ml) and washed with water (2 ml), and saturated brine (2 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give the ketol **103** as a white solid (4.4 mg, 88%), with the 6,7-isomer **104** (<5%). Treatment of **102** (10 mg, 0.023 mmol) as above, but increasing the reaction time to 16 h, yielded the norbeyerane derivative **105** (3.9 mg, 43%), **103** (4.8 mmol, 48%), and **104** (<5%). Ketol **103** was recrystallised from dichloromethane:hexane (1:10)

Rf 0.20 (ethyl acetate:hexane, 1:2). **mp** 205-206.5°C. **IR** (KBr disc) 1209, 1246, 1717, 1740, 1770, 3503 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (3H, s, H18), 1.34-1.52 (1H, m, H1), 1.62-1.68 (1H, m, H12), 1.78 (1H, d, J = 8.5 Hz, H9), 1.89-2.22 (6H, m), 2.03, 2.10 (2x3H, s, -$OCOCH_3$), 2.44 (1H, m, H12β), 2.48 (1H, m, H14), 2.56 (1H, dd, J_1 = 2 Hz, J_2 = 10.0 Hz, H14), 2.61 (1H, d, J = 10.0 Hz, H5), 2.30 (1H, dt, J_1 = 3.0 Hz, J_2 = 17.0 Hz, H15α), 3.62 (1H, d, J = 3.5 Hz, 6-OH), 4.19 (1H, dd, J_1 = 3.5 Hz, J_2 = 10.0 Hz, H6), 4.95 (1H, br s, H17), 4.98 (1H, br s, H3), 5.00 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 15.8 (C18), 21.1 (C11), 21.9 (2x-OCO<u>C</u>H$_3$), 25.1 (C2), 29.8 (C1), 36.2 (C12), 37.3 (C15), 38.7 (C14), 46.4 (C9), 52.3 (C8), 53.4 (C4), 57.1 (C5), 69.9 (C6), 71.4 (C3), 84.7 (C13), 85.3 (C10), 105.1 (C17), 147.5 (C16), 169.2 (-O<u>C</u>OCH$_3$), 169.9 (-O<u>C</u>OCH$_3$), 176.5 (C19), 209.3 (C7).

MS (EI) *m/z* 432 (M^+, 33%), 390 (63), 372 (45), 355 (33), 330 (25), 312 (29), 284 (30), 268 (24), 239 (24), 167 (29), 149 (83).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{28}O_8$: 432.1784; found: 432.1786.

Microanalysis $C_{23}H_{28}O_8$ requires: C 63.88, H 6.53; found: C 63.58, H 6.39.

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ent-3α,13-Diacetoxy-7α-hydroxy-6-oxo-20-norkaur-16-ene-19-oic Acid 19,10-Lactone (104)

Rf 0.10 (ethyl acetate:hexane, 1:2).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (3H, s, H18), 1.50-2.43 (12H, m), 1.95 (3H, s, -OCOC<u>H</u>$_3$), 2.06 (3H, s, -OCOC<u>H</u>$_3$), 2.16 (1H, s, H5), 2.43 (1H, m, H12β), 3.59 (1H, s, 7-OH), 3.86 (1H, s, H7), 4.79 (1H, br s, H3), 4.81 (1H, br s, H17), 4.89 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2 (C18), 20.9, 21.9 (2x-OCO<u>C</u>H$_3$), 24.9 (C11), 29.5 (C2), 30.2 (C1), 37.2 (C12), 37.6 (C15), 38.8 (C14), 42.3 (C9), 49.2, 49.4 (C4, C8), 53.8 (C5), 73.1 (C3), 82.2, 85.9 (C10, C13), 90.2 (C7), 104.2 (C17), 149.5 (C16), 169.6 (-O<u>C</u>OCH$_3$), 170.0 (-O<u>C</u>OCH$_3$), 176.2 (C19), 208.8 (C6).

MS (EI) *m/z* 432 (M^+, 50%), 414 (6), 390 (100), 372 (76), 344 (16), 330 (43), 312 (48), 284 (56), 239 (42), 211 (35), 178 (39), 149 (93).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{28}O_8$: 432.1784; found: 432.1780.

***ent*-3α-Acetoxy-7,16-dioxo-6α-hydroxy-20-norbeyeran-19-oic Acid 19,10-Lactone (105)**

Rf 0.11 (ethyl acetate:hexane, 1:2). **mp** 91-92°C. **IR** (film) 1210, 1238, 1739, 1778, 2933, 3467 cm^{-1}.

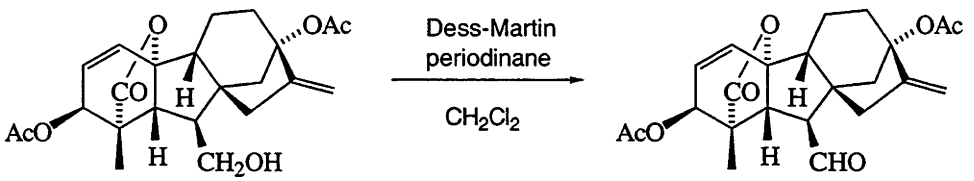
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (3H, s, H18), 1.35 (3H, s, H17), 1.20-2.21 (11H, m), 2.11 (3H, s, 3-$OCOCH_3$), 2.30 (1H, dd, J_1 = 2.8 Hz, J_2 = 12.7 Hz, H14), 2.67 (1H, d, J = 10.0 Hz, H5), 3.03 (1H, dd, J_1 = 3.6 Hz, J_2 = 18.0 Hz, H15), 3.66 (1H, d, J = 3.7 Hz, 6-OH), 4.06 (1H, dd, J_1 = 3.6 Hz, J_2 = 10.0 Hz, H6), 4.98 (1H, br s, H3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.0 (C18), 19.5 (C17), 20.4 (C11), 21.0 (3-$OCO\underline{C}H_3$), 24.9 (C2), 29.6 (C1), 35.6, 43.4, 44.0 (C12, C14, C15), 48.2, 49.0 (C8, C16), 48.2 (C9), 53.8 (C4), 57.1 (C5), 70.4 (C6), 71.4 (C3), 83.9 (C10), 169.9 (3-$O\underline{C}OCH_3$), 176.0 (C19), 210.4 (C7), 215.4 (C13).

MS (EI) *m/z* 390 (M^+, 100%), 372 (21), 348 (18), 330 (49), 284 (43), 256 (47), 199 (29).

HRMS (EI) *m/z* calc'd for M^+ $C_{21}H_{26}O_7$: 390.1679; found: 390.1687.

***ent*-3α,13-diacetoxy-7-oxo-20-norgibberell-1,16-dien-19-oic Acid 19,10-Lactone (106)**



(82) (106)

To the starting alcohol (**82**) (44 mg, 0.105 mmol), in dry dichloromethane (3 ml) was added the Dess-Martin reagent (54 mg, 0.127 mmol) at room temp, and the mixture was stirred for 4 hrs under an atmosphere of nitrogen. The solution was diluted with ethyl acetate (10 ml) and sodium thiosulfate (1 g) dissolved in saturated sodium bicarbonate (5 ml) was added to the mixture, which was stirred for a further 30 min. The organic phase was separated and washed with saturated sodium bicarbonate (8 ml), water (8 ml), and saturated brine (10 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane, (1:2) to give the aldehyde **106** as a white crystalline solid (40 mg, 91%), which was recrystallised from ether:hexane (1:10).

Rf 0.33 (ethyl acetate:hexane, 1:2). **mp** 158-159°C. **IR** (film) 1239, 1259, 1727, 1743, 1775 cm^{-1}.

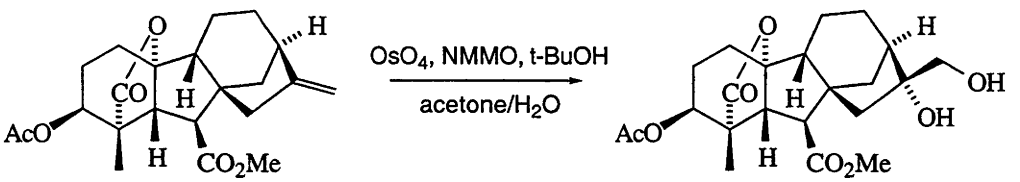
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (3H, s, H18), 1.17-2.40 (8H, m), 2.01, 2.12 (2x3H, s, 3-$OCOCH_3$, 13-$OCOCH_3$), 2.53 (1H, m, H15α), 2.81(1H, dd, $J_{5,6}$ = 11.0 Hz, $J_{6,7}$ = 2.0 Hz, H6), 3.29 (1H, d, J = 11.0 Hz, H5), 5.00 (1H, br s, H17), 5.15 (1H, br s, H'17), 5.32 (1H, d, J = 4.0 Hz, H3), 5.87 (1H, dd, $J_{2,3}$ = 4Hz, $J_{2,1}$ = 9.0 Hz, H2), 6.36 (1H, d, J = 9.0 Hz, H1), 9.84 (1H, d, J = 2.0 Hz, H7).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.7 (C18), 16.4 (C11), 20.7, 21.9 (3-$OCO\underline{C}H_3$, 13-$OCO\underline{C}H_3$), 36.2 (C12), 40.4, 41.6 (C14, C15), 50.6, 50.9, 51.1 (C5, C6, C9), 51.9 (C8), 55.9 (C4), 70.0 (C3), 83.3 (C13), 89.7 (C10), 108.3 (C17), 129.1 (C1), 133.9 (C2), 152.6 (C16), 169.7, 169.8 (3-$O\underline{C}OCH_3$, 13-$O\underline{C}OCH_3$), 176.7 (C19), 199.6 (C7).

MS (EI) *m/z* 414 (M^+, 11%), 372 (32), 354 (12), 312 (12), 281 (48), 267 (24), 250 (28), 239 (47), 221 (100), 192 (35), 132 (37).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{26}O_7$: 414.1679; found: 414.1680.

ent-3α-Acetoxy-10β,16β,17-trihydroxy-20-norgibberellan-19-oic Acid 19,10-Lactone Methyl Ester (109)



(108) (109)

To a solution of the olefin (**108**) (100 mg, 0.257 mmol) in acetone/water (8/1 ml), was added *N*-methyl morpholine-*N*-oxide (151 mg, 1.29 mmol), and osmium tetroxide (catalytic) dissolved in *t*-butyl alcohol (100 µl), and the mixture was stirred at room temperature over night. Sodium bisulfite (15 mg) was added to the mixture which was diluted with water (6 ml) and stirred for a further 30 min. The mixture was extracted with ethyl acetate (2x15 ml), and the combined organic phases were washed with water (20 ml), and saturated brine (20 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane (2:1), to give the diol (**109**), (108 mg, quant) as a white solid, which was recrystallised from ether:hexane.

Rf 0.10 (ethyl acetate:hexane, 2:1). **mp** 188-189°C. **IR** (KBr disc) 1053, 1232, 1734, 1742, 2957, 3438, 2955, 3527 cm^{-1}.

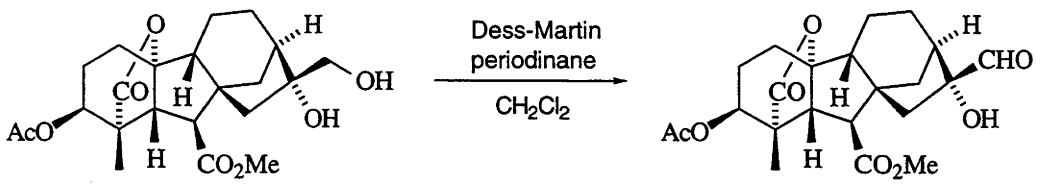
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (3H, s, H18), 1.36-2.15 (14H, m), 2.13 (3H, s, 3-$OCOCH_3$), 2.71 (1H, d, J = 10.6 Hz, H5), 3.00 (1H, d, J = 10.7 Hz, H6), 3.57-3.79 (2H, m, H17), 3.74 (3H, s, 7-CO_2CH_3), 4.95 (1H, t, J_1 = 3.4 Hz, J_2 = 2.1 Hz, H3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (C18), 15.9 (C11), 20.9 (3-$OCO\underline{C}H_3$), 21.3, 35.2 (C12, C14), 25.2 (C2), 27.2 (C1), 42.9 (C13), 49.7 (C15), 51.6 (C6), 51.7 (C8), 51.9 (7-$CO_2\underline{C}H_3$), 52.2 (C5), 52.9 (C4), 54.8 (C9), 66.7 (C17), 71.2 (C3), 82.8 (C16), 93.5 (C10), 170.0 (3-$O\underline{C}OCH_3$), 172.9 (C7), 176.8 (C19).

MS (EI) *m/z* 422 (M^+, 1%), 404 (3), 391 (100), 362 (17), 331 (42), 300 (97), 287 (82), 258 (18), 244 (63), 227 (51), 199 (28), 183 (38).

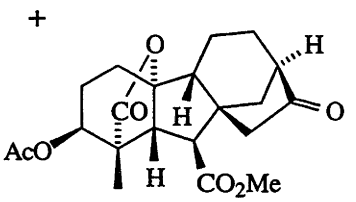
HRMS (EI) *m/z* calc'd for M^+ $C_{22}H_{30}O_8$: 422.1941; found: 422.1935.

***ent*-3α-Acetoxy-10β,16β-dihydroxy-17-oxo-20-norgibberellan-19-oic acid 19,10-Lactone Methyl Ester (110)**



(109) (110)

+



(111)

A solution of the diol (**109**), (108 mg, 0..256) in dichloromethane (8 ml) containing Dess-Martin reagent (142 mg, 0.335 mmol), was stirred at room temp overnight under an atmosphere of nitrogen. The solution was diluted with ethyl acetate (20 ml), and sodium thiosulfate (2 g) dissolved in saturated sodium bicarbonate (10 ml) was added to the mixture, which was stirred for a further 30 min. The organic phase was separated and washed with saturated sodium bicarbonate (20 ml), water (20 ml), and saturated brine (20 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane, (1:3, 1:2, 1:1) to give the aldehyde (**110**) as a white crystalline solid (32.9 mg, 30%), and the 16-norketone (21 mg, 22 %).

Rf 0.33 (ethyl acetate:hexane, 1:1). **mp** 180-183°C. **IR** (KBr disc) 1226, 1739, 1755, 2952, 3468 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (3H, s, H18), 1.37-2.33 (14H, m), 2.15 (3H, s, 3-$OCOCH_3$), 2.77 (1H, d, J = 10.5 Hz, H5), 3.08 (1H, br s, 16-OH), 3.16 (1H, d, J = 10.5 Hz, H6), 3.75 (3H, s, 7-CO_2CH_3), 4.98 (1H, br s, H3), 9.80 (1H, s, H17).

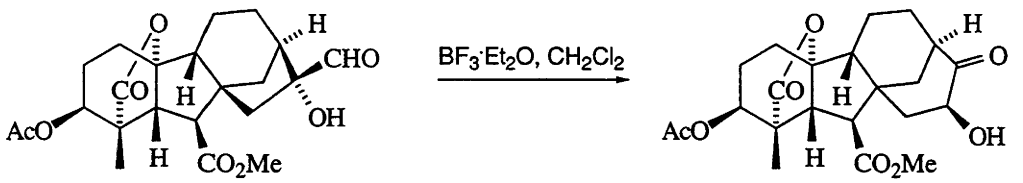
^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3 (C18), 15.9 (C11), 20.9 (C12), 21.0 (3-$OCO\underline{C}H_3$), 25.2 (C2), 27.2 (C1), 36.2 (C14), 46.0 (C13), 47.9 (C15), 51.8 (C6), 52.1 (C5), 52.1 (7-$CO_2\underline{C}H_3$), 52.8, (C4, C8), 54.4 (C9), 71.3 (C3), 86.2 (C16), 93.4 (C10), 170.1 (3-$O\underline{C}OCH_3$), 172.9 (C7), 176.7 (C19), 203.3 (C17).

MS (EI) *m/z* 420 (M^+, 24%), 391 (93), 360 (32), 331 (44), 316 (72), 287 (100), 256 (43), 244 (90), 199 (45), 183 (66).

HRMS (EI) *m/z* calc'd for M^+ $C_{22}H_{28}O_8$: 420.1784; found: 420.1788.

Microanalysis $C_{22}H_{28}O_8$ requires: C 62.85, H 6.71; found: C 62.47, H 6.84.

ent-3α-Acetoxy-10β,16α-dihydroxy-16a-oxo-_D_-homo-17,20-dinorgibberellan-19-oic acid 19,10-Lactone Methyl Ester (112)



(110) (112)

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(113)

To the hydroxy aldehyde (**110**), (10 mg, 0.0238 mmol) in dry dichloromethane (1 ml), was added boron trifluoride etherate (3.9 µl, 0.0316 mmol), and the mixture was stirred at room temp for 30 min under an atmosphere of nitrogen. The mixture was diluted with ethyl acetate (5 ml) and washed with water (2x2 ml), and saturated brine (2 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give a 1:1 mixture of ketols, **112** and **113** (10.5 mg crude), which would not separate cleanly by chromatography.

Rf 0.23 (ethyl acetate:hexane, 1:1).

(112) ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (3H, s, H18), 1.60-2.11 (13H, m), 2.12 (3H, s, 3-$OCOCH_3$), 2.42 (1H, d, J = 10.1 Hz, H5), 2.56 (1H, m, H15), 3.10 (1H, d, J = 10.0 Hz, H6), 3.72 (3H, s, 7-CO_2CH_3), 4.10 (1H, dd, J_1 = 9.7 Hz, J_2 = 2.7 Hz, H17), 4.96 (1H, br d, J = 1.7 Hz, H3).

ent-3α-Acetoxy-10β,16β-dihydroxy-16a-oxo-_D_-homo-17,20-dinorgibberellan-19-oic acid 19,10-Lactone Methyl Ester (113)

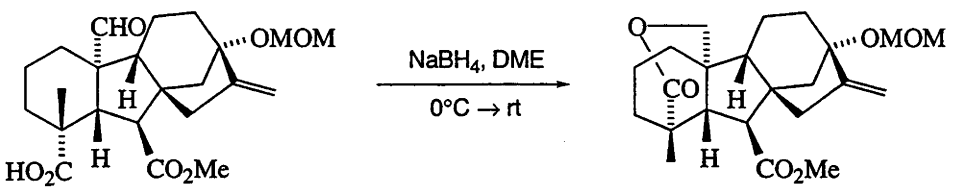
(113) ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (3H, s, H18), 1.38-2.35 (13H, m), 2.16 (3H, s, 3-$OCOCH_3$), 2.52 (1H, d, J = 11.4 Hz, H5), 2.92 (1H, m, H15), 3.20 (1H, d, J = 11.2 Hz, H6), 3.70 (3H, s, 7-CO_2CH_3), 4.47 (1H, J_1 = 11.3 Hz, J_2 = 3.8 Hz, H17), 4.99 (1H, br s, H3).

MS (EI) *m/z* 420 (M^+, 8%), 418 (10), 402 (29), 360 (24), 332 (26), 314 (32), 288 (61), 270 (34), 244 (100), 228 (40), 183 (41).

HRMS (EI) *m/z* calc'd for M^+ $C_{22}H_{28}O_8$: 420.1784; found: 420.1787.

9.6 Experimental for Chapter 8

ent-20-Hydroxy-13-methoxymethoxy-gibberell-16-ene-7,19-dioic Acid 19,20-Lactone Methyl Ester (126)



(125) (126)

To the starting aldehyde (**125**) (250 mg, 0.595 mmol) in dry, distilled dimethoxy ethane (25 ml) at 0°C under nitrogen, was added sodium borohydride (68 mg, 1.78 mmol). The mixture was stirred at 0°C and allowed to warm slowly to room temp over 4 h. Acetic acid (5 ml) was added and the mixture was stirred for a further 20 min, then diluted with ethyl acetate (35 ml). The mixture was washed with saturated sodium bicarbonate (4x30 ml), and saturated brine (1x30 ml), then dried (sodium sulfate). The solvent was removed under reduced pressure to give the crude lactone. Purification was carried out by silica gel chromatography using ethyl acetate:hexane (1:3), as eluting solvent to give pure lactone **126**, as a white crystalline compound (211 mg, 88%), which was recrystallised from ethyl acetate:hexane.

Rf 0.39 (ethyl acetate:hexane, 1:2). **mp** 153-154°C. **IR** (KBr disc) 1039, 1149, 1731 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12 (3H, s, H18), 1.19-2.25 (16H, m), 2.77 (1H, d, J = 12.6 Hz, H6), 3.37 (3H, s, 13-OCH_2OCH_3), 3.70 (3H, s, 7-CO_2CH_3), 4.09 (1H, dd, J_{gem} = 12.3 Hz, ^4J = 2.3 Hz, 20-pro-S-H), 4.40 (1H, d, J_{gem} = 12.3 Hz, 20-pro-R-H), 4.54, 4.73 (2x1H, ABd, J = 7.1 Hz, 13-$OC\underline{H}_2OCH_3$), 4.97 (1H, d, J = 3.0 Hz, H17), 5.10 (1H, d, J = 3.0 Hz, H'17).

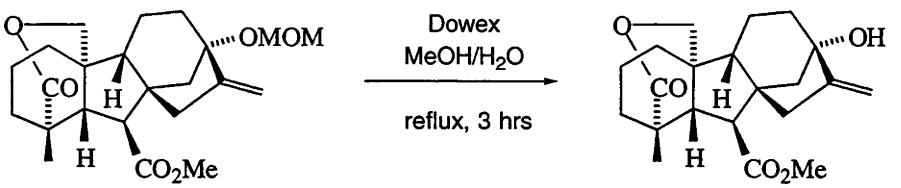
^{13}C NMR (75 MHz, $CDCl_3$): δ 16.3 (C11), 20.4 (C2), 22.9 (C18), 37.7 (C1), 38.0 (C3), 39.5 (C14), 40.3 (C12), 41.2 (C10), 42.3 (C4), 45.1 (C15), 47.1 (C8), 51.6 (C6), 51.7 (7-$CO_2\underline{C}H_3$), 52.8 (C5), 55.0 (C9), 55.1 (13-$OCH_2O\underline{C}H_3$), 74.0 (C20), 93.4 (C13), 91.7 (13-$O\underline{C}H_2OCH_3$), 107.2 (C17), 153.1 (C16), 173.0, 174.9 (C7, C19).

MS (EI) *m/z* 404 (M^+, 100%), 389 (80), 373 (34), 359 (22), 344 (54), 331 (80), 313 (34), 299 (62), 285 (80), 271 (72), 255 (42), 243 (44), 225 (50), 211 (72), 179 (76).

HRMS (EI) *m/z* calc'd for M^+ $C_{23}H_{32}O_6$: 404.2199; found: 404.2211.

Microanalysis $C_{23}H_{32}O_6$ requires: C 68.29, H 7.97; found: C 68.31, H 8.25.

ent-13,20-Dihydroxy-gibberell-16-ene-7,19-dioic Acid 19,20-Lactone Methyl Ester (127)



(126) (127)

To MOM ether **126** (73 mg, 0.180 mmol) in methanol (30 ml) and water (6 ml) was added Dowex 50W resin (75 mg) and the mixture was stirred at reflux for 3 h. The mixture was filtered to remove the resin and the solvent was removed under reduced pressure. The residue was taken up in ethyl acetate (15 ml) and washed with saturated brine (10 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure to give the free alcohol (**127**), which was purified by silica gel chromatography using ethyl acetate:hexane (1:1) as the eluting solvent to give pure **127** (48 mg, 74%).

Data previously reported[168].

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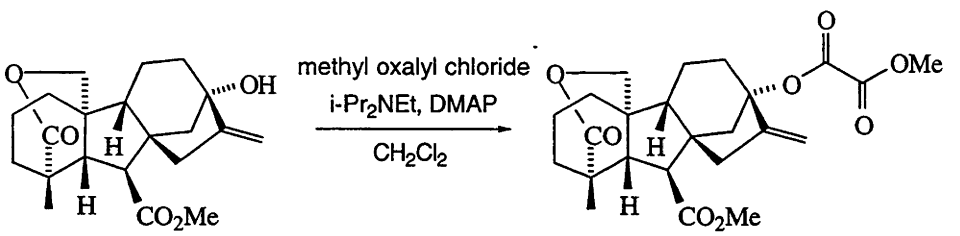
ent-20-Hydroxy-13-methyloxalyloxy-gibberell-16-ene-7,19-dioic Acid 19,20-Lactone Methyl Ester (128)



(127) (128)

To the alcohol (**127**), (154 mg, 0.427 mmol) in dry dichloromethane (15 ml), was added diisopropylethylamine (447 µl, 2.56 mmol), dimethylaminopyridine (catalytic), and methyloxalyl chloride (236 µl, 2.56 mmol). The mixture was stirred at room temp under an atmosphere of nitrogen overnight. The mixture was diluted with ethyl acetate (30 ml), and washed with saturated sodium bicarbonate (25 ml) and saturated brine (25 ml). The aqueous washings were then extracted with ethyl acetate (15 ml), the combined organic phases were dried (sodium sulfate), and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane (1:2), to give **128** as a white crystalline solid, (171 mg, 90%), which was recrystallised from dichloromethane:hexane.

Rf 0.27 (ethyl acetate:hexane, 1:2). **mp** 195-197°C. **IR** (KBr disc) 1162, 1206, 1724, 1747, 2938 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12 (3H, s, H18), 1.22-2.47 (16H, m), 2.78 (1H, d, J = 12.6 Hz, H6), 3.69 (3H, s, 7-CO_2CH_3), 3.87 (3H, s, 13-$O(CO)_2OCH_3$), 4.10 (1H, d, J_{gem} = 12.5 Hz, ^4J = 2.4 Hz, 20-pro-S-H), 4.39 (1H, d, J_{gem} = 12.2 Hz, 20-pro-R-H), 5.02 (1H, br s, H17), 5.23 (1H, br s, H'17).

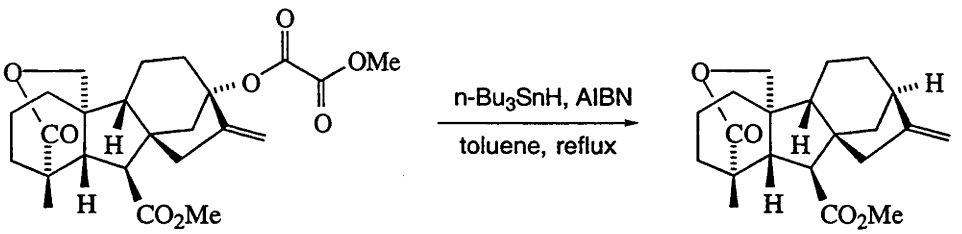
^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6 (C11), 20.5 (C2), 23.1 (C18), 35.8 (C1), 38.2 (C3), 39.4 (C14), 39.6 (C12), 41.4 (C10), 42.5 (C4), 43.8 (C15), 48.3 (C8), 51.4 (C6), 51.9 (7-CO_2CH_3), 52.8 (C5), 53.4 (13-$O(CO)_2OCH_3$), 54.9 (C9), 73.9 (C20), 87.4 (C13), 108.6 (C17), 151.5 (C16), 156.2, 158.1 (2x-$O(\underline{C}O)_2OCH_3$), 172.8, (C7), 174.8 (C19).

MS (EI) *m/z* 446 (M^+, 100%), 415 (17), 400 (10), 386 (36), 341 (50), 310 (31), 282 (74), 237 (81).

HRMS (EI) *m/z* calc'd for M^+ $C_{24}H_{30}O_8$: 446.1941; found: 446.1944.

Microanalysis $C_{24}H_{30}O_8$ requires: C 64.56, H 6.77; found: C 64.67, H 7.05.

ent-20-Hydroxy-gibberell-16-ene-7,19-dioic Acid 19,20-Lactone Methyl Ester (129)



(128) (129)

To the oxalate (**128**), (170 mg, 0.381 mmol), at reflux in dry, degassed toluene (20 ml), was added tri-*n*-butyltin hydride (154 µl, 0.571 mmol) followed by AIBN (catalytic), and the mixture stirred at reflux under nitrogen for 3 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica using hexane at first, and then ethyl acetate:hexane, (1:4) to give the 13-deoxy product as a white solid (115 mg, 88%), and some recovered starting oxalate (21 mg, 12%). **129** was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.74 (ethyl acetate:hexane, 1:1). **mp** 198°C (lit.[171] 198-200°C). **IR** (KBr disc) 1727, 2935 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97-2.12 (15H, m), 1.12 (3H, s, H18), 2.15 (1H, d, J = 12.9 Hz, H5), 2.62 (1H, m, H13), 2.75 (1H, d, J = 12.6 Hz, H6), 3.67 (3H, s, 7-CO_2CH_3), 4.07 (1H, d, J_{gem} = 12.2 Hz, ^4J = 2.4 Hz, 20-pro-S-H), 4.39 (1H, d, J_{gem} = 12.2 Hz, 20-pro-R-H), 4.79 (1H, br s, H17), 4.92 (1H, br s, H'17).

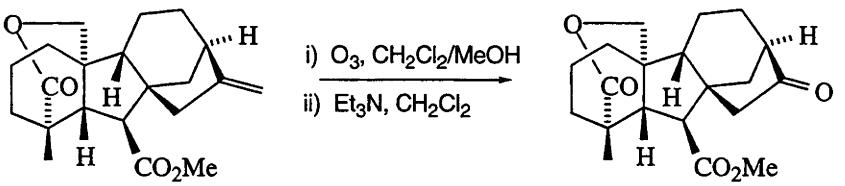
^{13}C NMR (75 MHz, $CDCl_3$): δ 15.8 (C11), 20.6 (C2), 23.2 (C18), 31.3 (C12), 36.3 (C1), 38.2 (C3), 39.2 (C13), 39.8 (C14), 41.7 (C10), 42.5 (C4), 46.0 (C15), 49.6 (C8), 51.7 (7-$CO_2\underline{C}H_3$), 51.9 (C6), 52.4 (C5), 55.6 (C9), 74.3 (C20), 106.5 (C17), 156.6 (C16), 173.5, (C7), 175.2 (C19).

MS (EI) *m/z* 344 (M^+, 4%), 312 (28), 298 (20), 284 (71), 239 (100), 195 (29), 155 (15), 129 (15).

HRMS (EI) *m/z* calc'd for M^+ $C_{21}H_{28}O_4$: 344.1988; found: 344.1986.

Microanalysis $C_{21}H_{28}O_4$ requires: C 73.23, H 8.19; found: C 72.91, H 8.50.

ent-20-Hydroxy-16-oxo-17-norgibberellane-7,19-dioic Acid 19,20-Lactone Methyl Ester (131)



(129) (131)

Ozone was bubbled through a solution of the alkene (**129**), (70 mg, 0.203 mmol) in dichloromethane/methanol (9/3 ml) at -78°C for 40 sec. Tlc indicated that no starting material remained and so the solvent was removed by bubbling nitrogen through the solution. The residue was taken up in dichloromethane (12 ml), and triethylamine (113 ml, 0.813) was added. The mixture was stirred for 20 h at room temp. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:2, 1:1), to give the norketone (**131**) as a white solid (55 mg, 78%), which was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.35 (ethyl acetate:hexane, 1:1). **mp** 175-176°C (lit.[171] 238-240°C). **IR** (KBr disc) 1727, 2933 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12 (3H, s, H18), 1.20-2.20 (15H, m), 2.14 (1H, d, J = 12.7 Hz, H5), 2.42 (1H, m, H13), 2.79 (1H, d, J = 12.8 Hz, H6), 3.69 (3H, s, 7-CO_2CH_3), 4.12 (1H, d, J_{gem} = 11.9 Hz, ^4J = 2.1 Hz, 20-pro-S-H), 4.43 (1H, d, J_{gem} = 12.2 Hz, 20-pro-R-H).

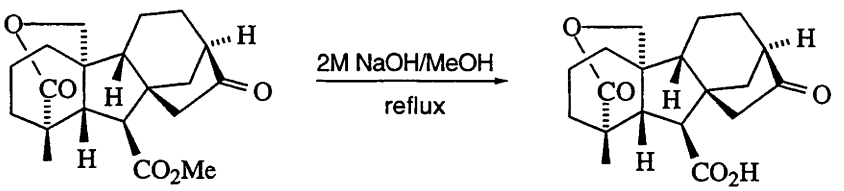
^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6 (C11), 20.4 (C2), 23.0 (C18), 24.3 (C12), 34.0 (C1), 38.1 (C3), 39.6 (C14), 41.8 (C10), 42.4 (C4), 44.6 (C13), 48.2 (C8), 51.9 (C15, 7-$CO_2\underline{C}H_3$ overlapped), 52.5 (C5), 52.8 (C6), 55.9 (C9), 73.8 (C20), 172.9, (C7), 174.7 (C19), 219.6 (C16).

MS (EI) *m/z* 346 (M^+, 100%), 318 (78), 300 (13), 286 (58), 273 (37), 258 (41), 241 (52), 227 (33), 213 (43), 201 (52), 187 (54), 145 (49).

HRMS (EI) *m/z* calc'd for M^+ $C_{20}H_{26}O_5$: 346.1780; found: 346.1787.

Microanalysis $C_{20}H_{26}O_5$ requires: C 69.34, H 7.56; found: C 68.90, H 7.50.

ent-20-Hydroxy-16-oxo-17-norgibberellane-7,19-dioic Acid 19,20-Lactone (132)



(131) (132)

A solution of the methyl ester (**131**), (102 mg, 0.294 mmol) in methanol (450 µl) was treated with 2M sodium hydroxide (5.6 ml) and heated at reflux for 7 h. The solvent was removed under reduced pressure and the residue was taken up in methanol (80 ml). The mixture was then acidified with Dowex 50 W H^+ resin to pH 3-4, with cooling. The resin was filtered off and the solvent was removed under reduced pressure to give acid **132** as a white solid, (92 mg, 94 %), which was recrystallised from ethanol-ether-heptane.

Rf 0.13 (ethyl acetate:hexane:methanol:acetic acid, 100:100:1:1). **mp** 264°C. **IR** (KBr disc) 1158, 1704, 1739 cm^{-1}.

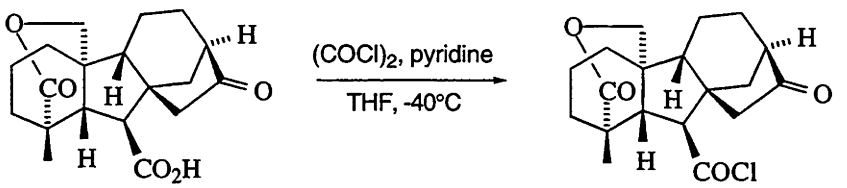
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (3H, s, H18), 1.21-2.27 (15H, m), 2.12 (1H, d, J = 12.8 Hz, H5), 2.48 (1H, m, H13), 2.83 (1H, d, J = 12.8 Hz, H6), 4.15 (1H, d, J_{gem} = 12.2 Hz, ^4J = 2.1 Hz, 20-pro-S-H), 4.45 (1H, d, J_{gem} = 12.2 Hz, 20-pro-R-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6 (C11), 20.4 (C2), 23.0 (C18), 24.3 (C12), 34.0 (C1), 38.1 (C3), 39.7 (C14), 41.9 (C10), 42.5 (C4), 44.7 (C13), 48.2 (C8), 51.9 (C15), 52.4 (C5), 52.6 (C6), 55.9 (C9), 73.9 (C20), 175.1 (C19), 176.5 (C7), 220.4 (C16).

MS (EI) *m/z* 332 (M^+, 100%), 314 (6), 304 (43), 286 (87), 273 (26), 258 (43), 245 (38), 229 (17), 215 (18), 201 (35), 187 (25), 159 (22), 145 (29).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{24}O_5$: 332.1624; found: 332.1628.

ent-7-Chloro-20-hydroxy-7,16-dioxo-17-norgibberellane-7,19-dioic Acid 19,20-Lactone (133)



(132) (133)

The starting acid **132** (100 mg, 0.301 mmol) was dissolved in dry THF (2 ml) and pyridine (365 µl, 4.51 mmol) was added. To a two-necked flask containing oxalyl chloride (262 µl, 3.01 mmol) in dry THF (5 ml) at -40°C under nitrogen, was added *via* cannular the above mixture. The solution was allowed to warm to room temperature, and after 5 h no starting material remained. The reaction mixture was filtered through a sintered funnel and washed into a dry flask with dry benzene. Residual oxalyl chloride was removed by azeotroping with benzene (4x10 ml) and the residue was filtered through a plug of celite into a dry flask. The solvent was removed under reduced pressure to give the acid chloride (**133**) (~100%) as a yellow oil.

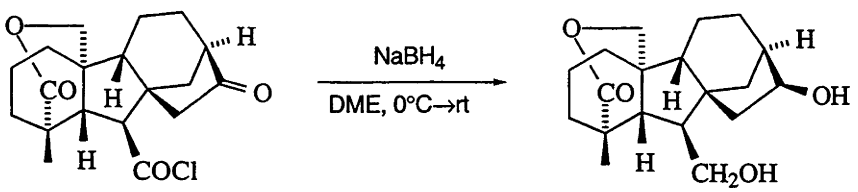
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (3H, s, H18), 1.28-2.31 (15H, m), 2.04 (1H, d, J = 12.1 Hz, H5), 2.51 (1H, m, H13), 3.32 (1H, d, J = 12.1 Hz, H6), 4.16 (1H, d, J_{gem} = 11.8 Hz, ^4J = 2.1 Hz, 20-pro-S-H), 4.42 (1H, d, J_{gem} = 12.1 Hz, 20-pro-R-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.7 (C11), 20.3 (C2), 24.0 (C18), 24.3 (C12), 33.9 (C1), 38.0 (C3), 39.5 (C14), 41.6 (C10), 42.5 (C4), 44.6 (C13), 48.9 (C8), 51.1 (C15), 53.6 (C5), 56.4 (C9), 64.4 (C6), 73.4 (C20), 174.0 (C7), 174.2 (C19), 217.9 (C16).

MS (EI) *m/z* 350 (M^+, 100%), 332 (3), 315 (69), 286 (79), 273 (48), 259 (53), 243 (53), 229 (47), 213 (23), 199 (38), 187 (40), 159 (27).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{23}O_4Cl$: 350.1285; found: 350.1287.

ent-7,16α,20-Trihydroxy-17-norgibberellan-19-oic Acid 19,20-Lactone (134)



(133) (134)

To the above acid chloride (**133**) (0.301 mmol) in dry, distilled dimethoxyethane (6 ml) at 0°C under nitrogen, was added sodium borohydride (57 mg, 1.51 mmol). The mixture was stirred at 0°C and allowed to warm slowly to room temp overnight. Acetic acid (20 drops) was added and the mixture was stirred for a further 20 minutes, then diluted with ethyl acetate (15 ml). The mixture was washed with saturated sodium bicarbonate (4x8 ml), and saturated brine (10 ml), then dried over sodium sulfate. The solvent was removed under reduced pressure to give the crude alcohol. Purification was carried out by silica gel chromatography using ethyl acetate:hexane, 1:1 as eluting solvent to give pure alcohol **134**, as a white crystalline compound (80 mg, 82%), which was recrystallised from ethyl acetate:hexane (1:10).

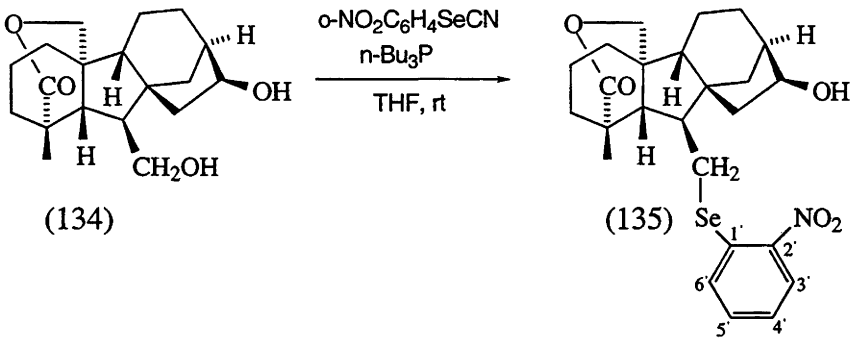
Rf 0.21 (ethyl acetate:hexane:methanol:acetic acid, 66:33:1:1). **mp** 249°C. **IR** (KBr disc) 1159, 1724, 1738, 2937, 3412, 3521 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88-1.81 (15H, m), 1.26 (3H, s, H18), 1.94 (1H, d, J = 11.6 Hz, H5), 2.18 (1H, m, H13), 2.37 (1H, dd, J_1 = 11.6 Hz, J_2 = 12.3 Hz, H6), 3.16 (1H, br s, 7-OH), 3.69 (1H, m, H7), 3.84 (1H, m, H'7), 3.98 (1H, d, J_{gem} = 12.0 Hz, ^4J = 2.2 Hz, 20-pro-S-H), 4.17 (1H, td, $J_{15\text{-}16}$ = 6.5 Hz, 6.8 Hz, $J_{13\text{-}16}$ = 4.0 Hz, H16), 4.40 (1H, d, J_{gem} = 12.0 Hz, 20-pro-R-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9 (C11), 17.4 (2), 20.4 (C12), 23.8 (C18), 35.7 (C1), 36.6 (C13), 38.4 (C3), 40.9 (C14), 41.4 (C10), 42.6 (C4), 44.5 (C15), 48.7 (C8), 49.1 (C5), 50.9 (C6), 58.9 (C9), 61.0 (C7), 72.0 (C16), 74.9 (C20), 177.1 (C19).

MS (EI) *m/z* 320 (M^+, 4%), 32 (16), 284 (100), 272 (8), 257 (19), 244 (36), 225 (37), 213 (37), 199 (24), 185 (16), 143 (22).
HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{28}O_4$: 320.1988; found: 320.1986.
Microanalysis $C_{19}H_{28}O_4$ requires: C 71.22, H 8.81; found: C 70.84, H 8.66.

***ent*-16α,20-Dihydroxy-7-(2'-nitrobenzeneselenenyl)-17-norgibberellan-19-oic Acid 19,20-Lactone (135)**



(134) (135)

To the diol (**134**) (150 mg, 0.462 mmol) in THF (20 ml), was added *o*-nitrophenyl seleno-cyanate (1.05 g, 4.62 mmol), followed by the dropwise addition of *n*-tributylphosphine (1.15 ml, 4.62 mmol) under an atmosphere of nitrogen at room temp. The resulting mixture was stirred for 1 h, after which, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel (ethyl acetate:hexane:methanol:acetic acid, 100:100:1:1) to give the *o*-nitrophenyl selenide (**135**) (210 mg, 90%) as dark orange crystals.

Rf 0.43 (ethyl acetate:hexane:methanol:acetic acid, 66:33:1:1). **mp** 197-199°C. **IR** (KBr disc) 1019, 1037, 1090, 1717, 2872, 2914, 2928, 2961 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87-2.03 (15H, m), 1.42 (3H, s, H18), 2.23 (2x1H, m, H5, H6 overlapped), 2.47 (1H, dd, J_{gem} = 13.5 Hz, J_{vic} = 7.7 Hz, H15β), 2.83 (1H, m, H7), 3.50 (1H, m, H'7), 4.02 (1H, d, J_{gem} = 12.1 Hz, 20-pro-S-H), 4.30 (1H, m, H16), 4.42 (1H, d, J_{gem} = 12.1 Hz, 20-pro-R-H), 7.31 (1H, dd, J_1 = 7.0 Hz, J_2 = 8.3 Hz, H'4), 7.42 (1H, d, J = 7.0 Hz, H'6), 7.54 (1H, dd, J_1 = 6.8 Hz, J_2 = 8.3 Hz, H'5), 8.26 (1H, d, J = 6.9 Hz, H'3).

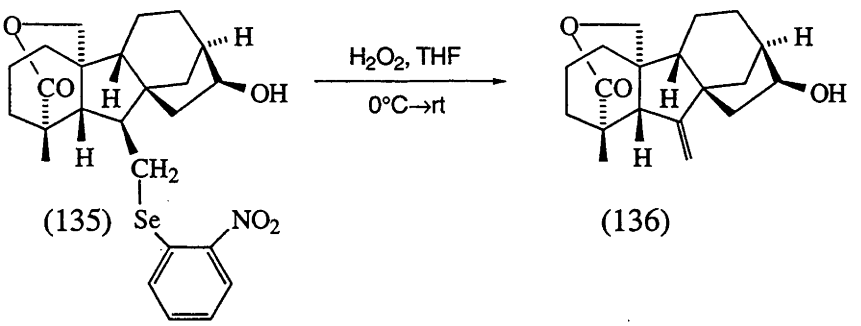
^{13}C NMR (75 MHz, $CDCl_3$): δ 15.1 (C11), 17.3 (2), 20.4 (C12), 25.3 (C18), 28.8 (C7), 36.8 (C1), 37.1 (C13), 38.8 (C3), 41.5 (C14), 41.9 (C10), 43.0 (C4), 44.4 (C15), 46.3 (C5), 49.5 (C8), 56.4 (C9), 59.5 (C6), 72.0 (C16), 74.4 (C20), 125.5 (C'4), 126.3 (C'3), 128.9 (C'6), 133.1 (C'1), 133.8 (C'5), 146.7 (C'2), 175.6 (C19).

MS (EI) *m/z* 505 (M^+, ^{80}Se, 91%), 503 (M^+, ^{78}Se, 54%), 488 (43), 486 (22), 429 (8), 382 (2), 303 (13), 285 (28), 257 (51), 227 (37).

HRMS (EI) *m/z* calc'd for M^+ $C_{25}H_{31}O_5{}^{80}SeN$: 505.1367; found: 505.1386.

Microanalysis $C_{25}H_{31}O_5SeN$ requires: C 59.52, H 6.19, N 2.78; found: C 59.32, H 6.11, N 2.70.

ent-16α,20-Dihydroxy-17-norgibberell-6-en-19-oic Acid 19,20-Lactone (136)



A solution of the selenide (**135**) (210 mg, 0.417 mmol) in THF (40 ml) containing 30% hydrogen peroxide (473 µl, 4.17 mmol), was stirred at 0°C for 1.5 h, and then allowed to warm to room temp over 10.5 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using ethyl acetate:hexane (1:2), to give the olefin as a clear oil (98 mg, 78%). The olefin was recrystallised from dichloromethane/hexane to give a white crystalline solid.

Rf 0.31 (ethyl acetate:hexane, 1:1). **mp** 161°C. **IR** (KBr disc) 1158, 1166, 1702, 2920, 2939, 3480 cm^{-1}.

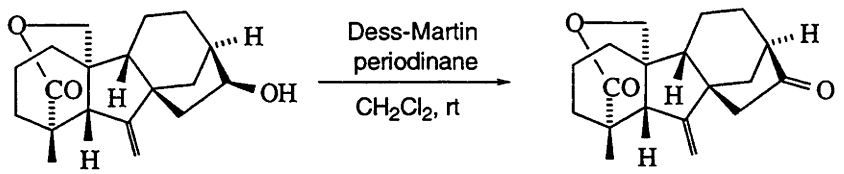
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (1H, m, H1), 1.21-2.04 (14H, m), 1.41 (3H, s, H18), 2.18 (1H, s, H5), 2.37 (1H, m, H13), 3.92 (1H, d, J_{gem} = 11.7 Hz, 20-pro-S-H), 4.19 (1H, d, J_{gem} = 11.5 Hz, 20-pro-R-H), 4.35 (1H, m, H16), 4.91 (1H, d, J = 2.7 Hz, H7), 5.03 (1H, d, J = 2.7 Hz, H'7).

^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2 (C11), 17.8 (2), 20.3 (C12), 23.0 (C18), 33.5 (C1), 37.2 (C3), 37.4 (C13), 40.5 (C14), 53.3 (C15), 41.5, 41.8 (C4, C10), 49.3 (C8), 55.2 (C9), 57.0 (C5), 72.3 (C16), 74.6 (C20), 104.4 (7), 156.0 (C6), 175.9 (C19).

MS (EI) *m/z* 302 (M^+, 55%), 284 (36), 260 (16), 244 (100), 226 (30), 212 (23), 199 (34), 186 (35), 152 (34).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{26}O_3$: 302.1882; found: 302.1883.

***ent*-20-Hydroxy-16-oxo-17-norgibberell-6-en-19-oic Acid 19,20-Lactone (137)**



(136) (137)

A solution of the alcohol (**136**), (68 mg, 0.225) in dichloromethane (10 ml) with Dess-Martin reagent (143 mg, 0.338 mmol), was stirred for 3 h under an atmosphere of nitrogen. The solution was diluted with ethyl acetate (20 ml), and sodium thiosulfate (1 g) dissolved in saturated sodium bicarbonate (5 ml) was added to the mixture, which was stirred for another 30 min. The organic phase was separated and washed with saturated sodium bicarbonate (15 ml), water (15 ml), and saturated brine (15 ml), then dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane (1:1), to give the norketone (**137**) as a white crystalline solid (60 mg, 89%), which was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.27 (ethyl acetate:hexane, 1:1). **mp** at 165°C reformed very fine needles which then melted at 192-194°C. **IR** (KBr disc) 1148, 1725, 1737, 2939 cm^{-1}.

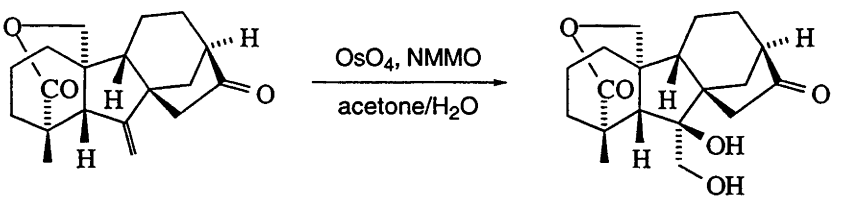
^{1}H NMR (300 MHz, $CDCl_3$): δ1.07-2.30 (13H, m), 1.44 (3H, s, H18), 2.10 (1H, d, J = 17.5 Hz, H15), 2.20 (1H, s, H5), 2.27 (1H, dd, J_1 = 17.5 Hz, J_2 = 3.4 Hz, H'15), 2.48 (1H, dd, J = 10.5 Hz, H13), 3.98 (1H, d, J_{gem} = 11.5 Hz, 20-pro-S-H), 4.25 (1H, d, J_{gem} = 11.6 Hz, 20-pro-R-H), 5.05 (1H, s, H7), 5.15 (1H, s, H'7).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6 (C11), 20.2 (C2), 22.9 (C18), 24.9 (C12), 31.9 (C1), 37.0 (C3), 40.4 (C14), 41.4, 41.9 (C10, C4), 44.7 (C13), 47.6 (C8), 54.6, 54.9 (C9, C5), 56.1 (C15), 74.3 (C20), 105.8 (7), 154.0 (C6), 175.2 (C19), 220.7 (C16).

MS (EI) *m/z* 300 (M^+, 100%), 282 (6), 272 (21), 258 (28), 242 (84), 227 (25), 213 (22), 199 (43), 185 (27), 151 (55).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{24}O_3$: 300.1725; found: 300.1725.

***ent*-6α,7,20-Trihydroxy-16-oxo-17-norgibberellan-19-oic Acid 19,20-Lactone (138)**



(137) (138)

To a solution of the olefin (**137**) (60 mg, 0.200 mmol) in acetone/water (5/1 ml), was added *N*-methyl morpholine-*N*-oxide (117 mg, 1.00 mmol), and osmium tetroxide (catalytic) dissolved in *t*-butylalcohol (100 µl), and the mixture was stirred at 40°C for 4 weeks. Sodium bisulfite (10 mg) was added to the mixture, which was diluted with water (4 ml) and stirred for another 30 min. The mixture was extracted with ethyl acetate (2x10 ml), then the combined organic fractions were washed with water (12 ml), and saturated brine (12 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure. The residue was chromatographed on silica using ethyl acetate:hexane (2:1), to give the diol (**138**), (51 mg, 76%) as a white solid, plus some starting olefin (5 mg, 8%). **138** was recrystallised from dichloromethane:hexane (1:5).

Rf 0.36 (ethyl acetate:hexane, 2:1). **mp** 197-198°C. **IR** (KBr disc) 1144, 1708, 1727, 1743, 2948 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ1.22-2.51 (14H, m), 1.39 (3H, s, H18), 1.92 (1H, s, H5), 2.45 (1H, m, H13), 2.48 (1H, d, J = 16.8 Hz, H15), 3.65 (2H, br s, 6-OH, 7-OH), 3.76, 3.85 (2x1H, ABd, J = 10.1 Hz, H7, H'7), 4.11 (1H, d, J_{gem} = 11.9 Hz, 20-pro-S-H), 4.43 (1H, d, J_{gem} = 12.5 Hz, 20-pro-R-H).

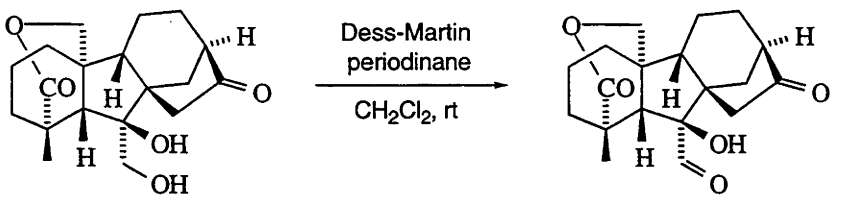
^{13}C NMR (75 MHz, $CDCl_3$): δ 16.7 (C11), 20.3 (C2), 22.5 (C18), 24.7 (C12), 29.7 (C1), 38.1 (C3), 40.9, 41.46 (C4, C10), 41.0 (C14), 45.1 (C13), 52.5 (C8), 53.1 (C15), 56.0 (C9), 58.6 (C5), 63.7 (C7), 75.6 (C20), 80.9 (C6), 176.9 (C19), 222.4 (C16).

MS (EI) *m/z* 334 (M^+, 1%), 316 (1), 303 (100), 286 (8), 257 (47), 239 (24).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{26}O_5$: 334.1780; found: 334.1781.

Microanalysis $C_{19}H_{26}O_5$ requires: C 68.24, H 7.84; found: C 67.83, H 8.03.

***ent*-6α,20-Dihydroxy-7,16-dioxo-17-norgibberellan-19-oic Acid 19,20-Lactone (139)**



(138) (139)

A solution of the diol (**138**), (40 mg, 0.120) in dichloromethane (8 ml) with Dess-Martin reagent (76 mg, 0.180 mmol), was stirred overnight under an atmosphere of nitrogen. The solution was diluted with ethyl acetate (15 ml), and sodium thiosulfate (1.0 g) dissolved in saturated sodium bicarbonate (5 ml) was added to the mixture, which was stirred for a further 30 min. The organic phase was separated and washed with saturated sodium bicarbonate (8 ml), water (8 ml), and saturated brine (8 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure. The residue was chromatographed on silica using ethyl acetate:hexane (1:1), to give the hydroxy aldehyde (**139**) as a white crystalline solid (60 mg, 89%).

Rf 0.36 (ethyl acetate:hexane, 1:2). **IR** (film) 1038, 1155, 1705, 1727, 3403 cm^{-1}.

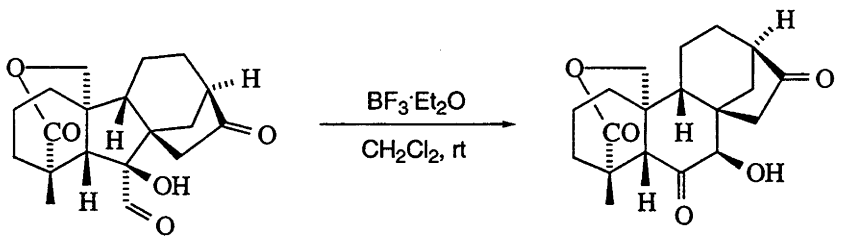
^{1}H NMR (300 MHz, $CDCl_3$): δ1.15 (3H, s, H18), 1.34-2.57 (14H, m), 2.07 (1H, s, H5), 2.46 (1H, m, H13), 2.53 (1H, d, J = 18.1 Hz, H15), 4.20 (1H, br s, 6-OH), 4.33 (1H, d, J_{gem} = 12.9 Hz, 20-pro-S-H), 4.66 (1H, d, J_{gem} = 12.9 Hz, 20-pro-R-H), 9.83 (1H, s, H7).

^{13}C NMR (75 MHz, $CDCl_3$): δ 16.7 (C11), 20.4 (C2), 21.7 (C18), 24.0 (C12), 29.6 (C1), 39.1, 40.8 (C4, C10), 40.4 (C3), 41.2 (C14), 44.7 (C13), 51.3 (C15), 53.0 (C8), 55.5 (C9), 60.4 (C5), 74.9 (C20), 87.2 (C6), 174.1 (C19), 201.9 (C7), 219.1 (C16).

MS (EI) *m/z* 332 (M^+, 19%), 314 (17), 303 (100), 286 (10), 275 (40), 257 (65), 245 (48), 229 (22).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{24}O_5$: 332.1624; found: 332.1639.

ent-7α,20-Dihydroxy-6,16-dioxo-17-norkauran-19-oic Acid 19,20-Lactone (140)



(139) (140)

To the hydroxy aldehyde (**139**), (5 mg, 0.0151 mmol) in dry dichloromethane (1 ml), was added boron trifluoride etherate (2.5 µl, 0.0200 mmol), and the mixture was stirred at room temp for 30 min under an atmosphere of nitrogen. The mixture was diluted with ethyl acetate (5 ml) and washed with water (2 ml), and saturated brine (2 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure to give the ketol (**140**) as a white solid (5 mg, 100%), which was recrystallised from ethyl acetate:hexane (1:10).

Rf 0.23 (ethyl acetate:hexane, 1:2). **mp** 186-187°C.

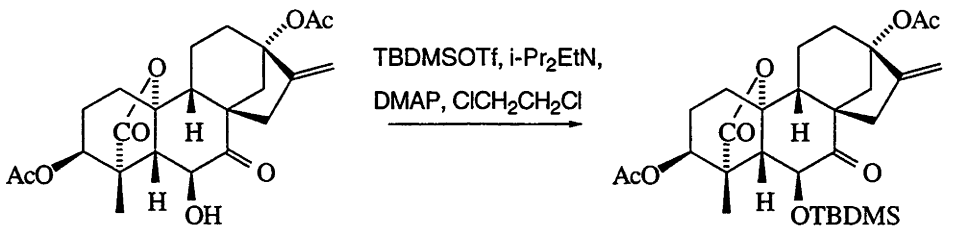
^{1}H NMR (300 MHz, $CDCl_3$): δ1.27 (3H, s, H18), 1.37-2.54 (15H, m), 2.26 (1H, s, H5), 3.49 (1H, s, 7-OH), 3.86 (1H, br s, H7), 4.14 (1H, d, J_{gem} = 12.1 Hz, 20-pro-S-H), 4.83 (1H, d, J_{gem} = 12.1 Hz, 20-pro-R-H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 17.6 (C11), 20.4 (C2), 22.4 (C18), 28.4 (C12), 29.6 (C1), 35.1 (C3), 39.0 (C14), 40.0 (C10), 43.6 (C13), 46.1 (C9), 45.0 (C4), 51.4 (C15), 51.6 (C8), 55.3 (C5), 73.4 (C20), 82.5 (C7), 174.7 (C19), 210.5 (C6), 218.5 (C16).

MS (EI) *m/z* 332 (M^+, 36%), 314 (16), 304 (25), 286 (22), 275 (100), 258 (29), 243 (24), 231 (22), 149 (56).

HRMS (EI) *m/z* calc'd for M^+ $C_{19}H_{24}O_5$: 332.1624; found: 332.1636.

ent-3α,13-Diacetoxy-10β-hydroxy-7-oxo-6-_t_-butyldimethylsilyloxy-20-norkaur-16-en-19-oic Acid 19,10-Lactone (144)



(103) (144)

To the ketol (**103**), (7.7 mg, 0.0178 mmol) in 1,2-dichloroethane (2 ml) was added diisopropylethylamine (186 µl, 1.07 mmol), *t* -butyldimethylsilyl triflate (164 µl, 0.712 mmol) and 4-dimethylamino pyridine (1 mg, 0.009 mmol), and the mixture stirred at room temp for 24 h under an atmosphere of nitrogen. The mixture was diluted with ethyl acetate (10 ml), washed with saturated sodium bicarbonate (2x5 ml), and saturated brine (6 ml), then dried (sodium sulfate), and the solvent removed under reduced pressure. The residue was chromatographed twice on silica gel using ethyl acetate:hexane (1:3), to give the TBDMS ether (**144**) as a clear oil (6 mg, 63%) plus some starting ketol.

Rf 0.61 (ethyl acetate:hexane, 1:2).

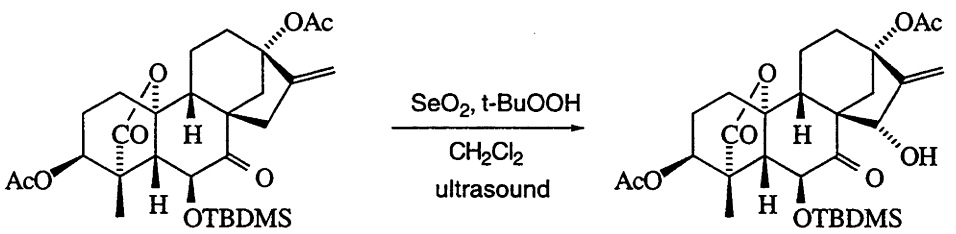
^{1}H NMR (300 MHz, $CDCl_3$): δ -0.02, 0.07 (2x3H, s, 6-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.89 (9H, s, 6-OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$), 1.29 (3H, s, H18), 1.43-2.62 (12H, m), 2.05, 2.08 (2x3H, s, -OCOC$\underline{H}_3$), 2.78 (1H, d, *J* = 10.0 Hz, H5), 3.15 (1H, m, H15α), 4.43 (1H, d, *J* = 10.0 Hz, H6), 4.95, 4.97, 5.01 (3H, br s, H3, H17, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ -4.3, -3.4 (2x6-OSi($\underline{C}H_3$)$_2$C(CH_3)$_3$), 16.8 (C18), 18.3 (6-OSi(CH_3)$_2\underline{C}$(CH_3)$_3$), 20.4 (C11), 21.2, 21.9 (2x-OCO$\underline{C}H_3$), 25.1 (C2), 25.9 (6-OSi(CH_3)$_2$C($\underline{C}H_3$)$_3$), 30.4 (C1), 36.1 (C12), 37.8, 38.1 (C14, C15), 47.0 (C9), 53.0, 53.3 (C4, C8), 57.4 (C5), 71.9 (C3), 76.9 (C6), 85.5, 85.6 (C10, C13), 104.9 (C17), 147.8 (C16), 169.3 (-O$\underline{C}OCH_3$), 169.9 (-O$\underline{C}OCH_3$), 176.7 (C19), 208.3 (C7).

MS (EI) *m/z* 531 (M^+-15, 3%), 503 (54), 489 (100), 429 (53), 385 (17), 325 (48), 149 (26).

HRMS (EI) *m/z* calc'd for M^+(-15) $C_{28}H_{39}O_8Si$: 531.2414; found: 531.2418

ent-3α,13-Diacetoxy-10β,15β-dihydroxy-7-oxo-6-_t_-butyldimethylsilyloxy-20-norkaur-16-en-19-oic Acid 19,10-Lactone (145)



(144) (145)

To the silyl ether (**144**), (6 mg, 0.0112 mmol) in dry dichloromethane (500 μl), was added selenium dioxide (3.7 mg, 0.0337 mmol), followed by *t*-butyl hydroperoxide (1 drop) and the mixture sonicated for 3 h. The mixture was diluted with ethyl acetate (5 ml) and washed with 1M hydrochloric acid (2 ml) and water (2 ml). The aqueous washings were extracted with ethyl acetate (5 ml), and the combined organic fractions were washed with saturated sodium bicarbonate (5 ml), saturated brine (5 ml), dried (sodium sulfate) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:hexane (1:2), to give the 15α-hydroxy kaurene (**145**) as a clear oil (3.6 mg, 58%).

Rf 0.24 (ethyl acetate:hexane, 1:2). **IR** (film) 1211, 1239, 1739, 1783, 2933, 3111 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ -0.00, 0.03 (2x3H, s, 6-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.93 (9H, s, 6-OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$), 1.27 (3H, s, H18), 1.42-2.90 (11H, m), 2.06, 2.08 (2x3H, s, -OCOC$\underline{H}_3$), 2.82 (1H, d, J = 9.5 Hz, H5), 4.40 (1H, br s, H15β), 4.43 (1H, d, J = 9.5 Hz, H6), 4.97 (1H, br s, H3), 5.33 (1H, br s, H17), 5.47 (1H, br s, H'17).

^{13}C NMR (75 MHz, $CDCl_3$): δ -4.3, -3.7 (2x6-OSi($\underline{C}H_3$)$_2$C(CH_3)$_3$), 16.5 (C18), 18.1 (6-OSi(CH_3)$_2\underline{C}$(CH_3)$_3$), 20.9 (C11), 21.4, 21.8 (2x-OCO$\underline{C}H_3$), 25.1 (C2), 25.7 (6-OSi(CH_3)$_2$C($\underline{C}H_3$)$_3$), 30.3 (C1), 34.6 (C14), 34.8 (C12), 46.4 (C9), 53.1 (C4), 57.4 (C5), 58.6 (C8), 71.7 (C3), 76.4 (C15), 77.1 (C6), 84.2 (C13), 85.4 (C10), 111.1 (C17), 150.9 (C16), 169.5, 169.8 (2x-O$\underline{C}$OCH_3), 176.1 (C19), 212.8 (C7).

MS (EI) *m/z* 505 (M^+-57, 19%), 461 (6), 445 (100), 385 (15), 357 (6), 341 (19), 285 (12).

HRMS (EI) *m/z* calc'd for M^+(-57) $C_{25}H_{33}O_9Si$: 505.1894; found: 505.1885.

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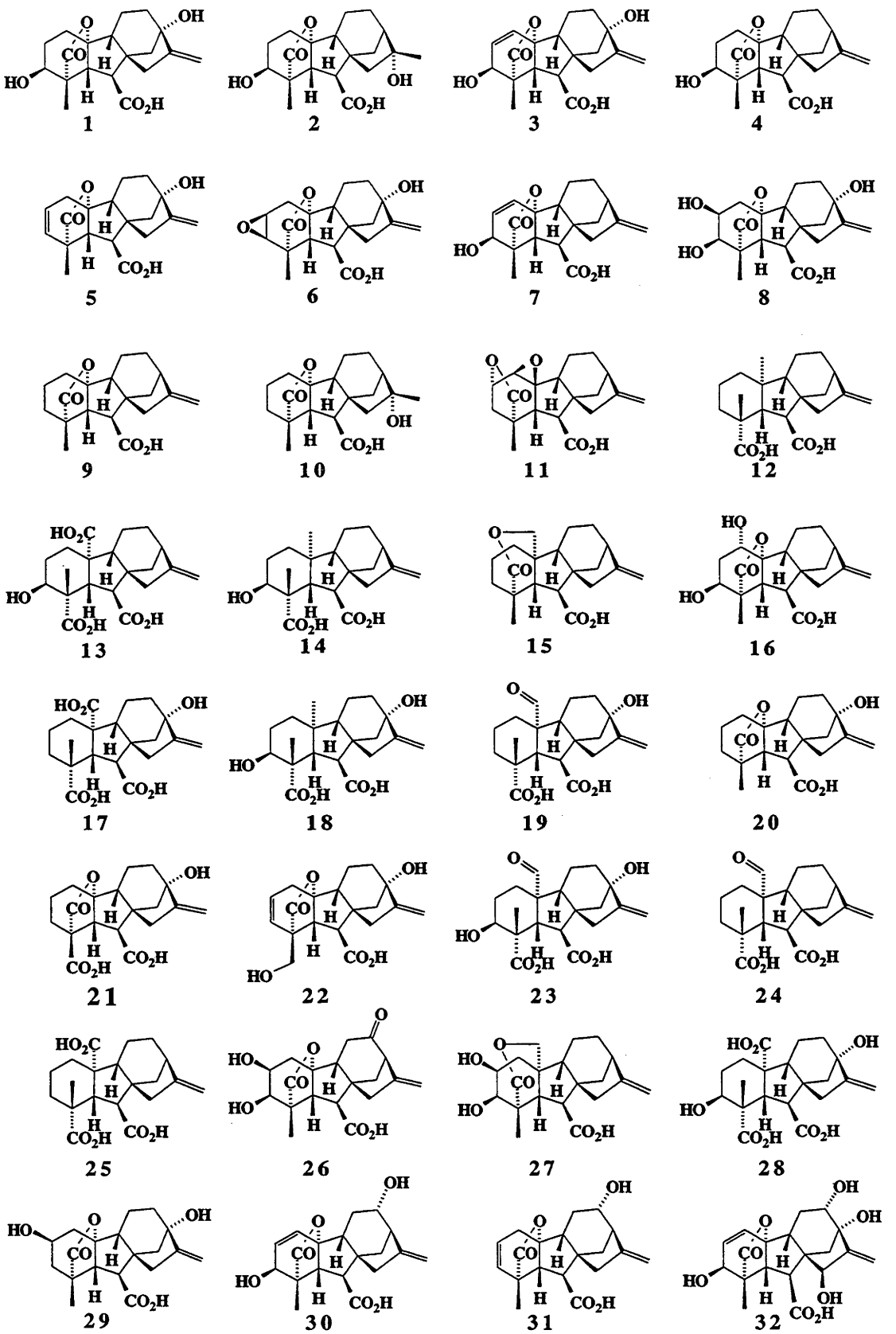
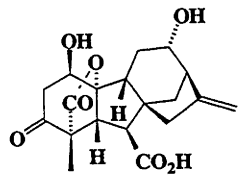
Appendix I

Complete set of gibberellin structures in order of discovery.





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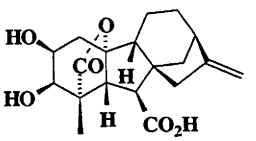

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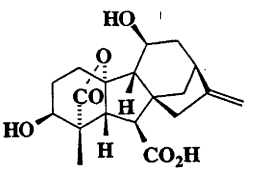

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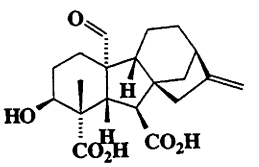

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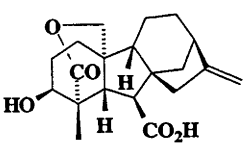

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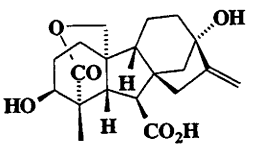

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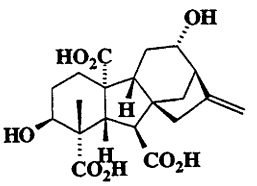

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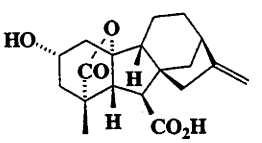

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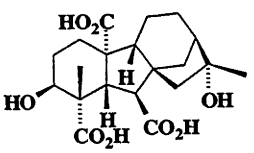

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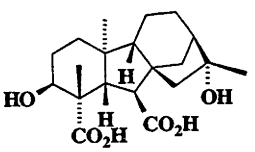

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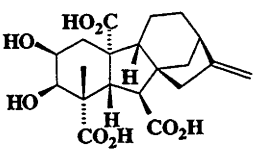

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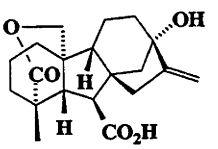

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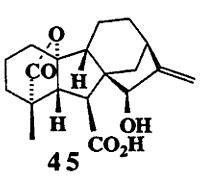

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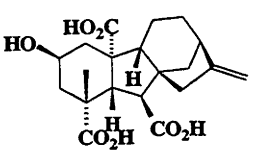

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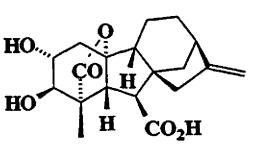

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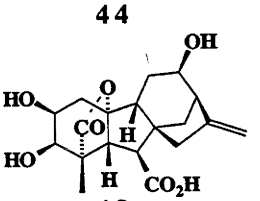

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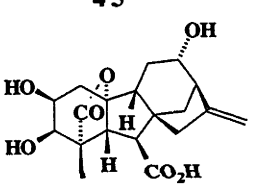

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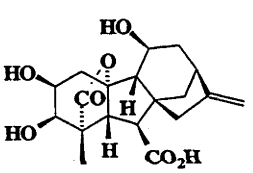

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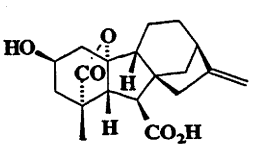

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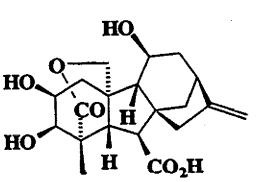

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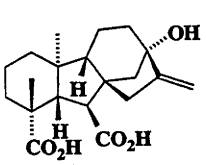

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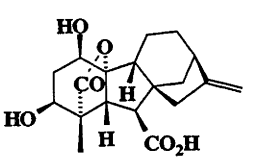

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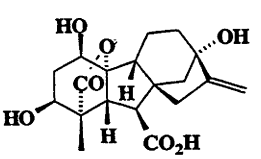

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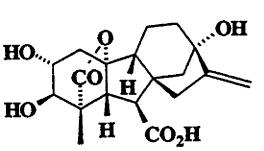

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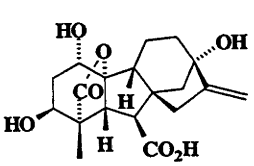

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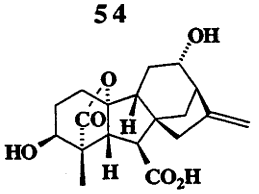

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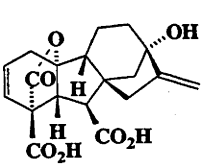

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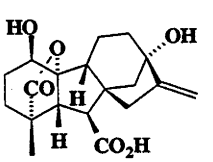

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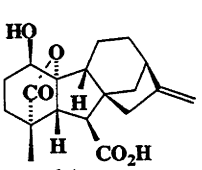

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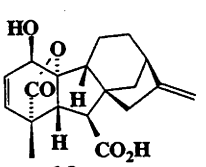

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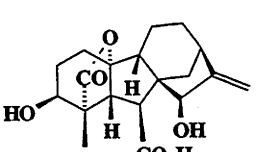

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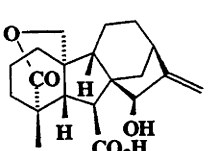

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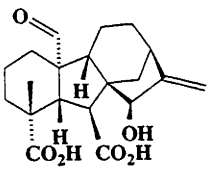
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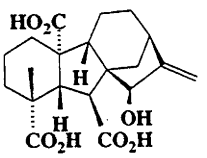
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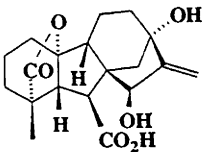
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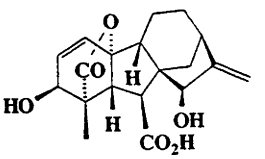
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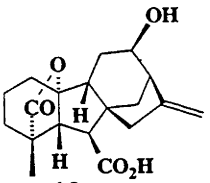
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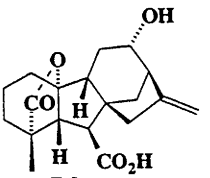
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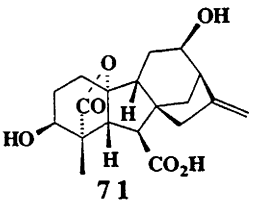
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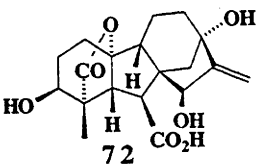
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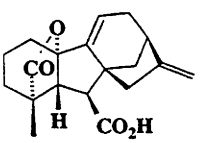
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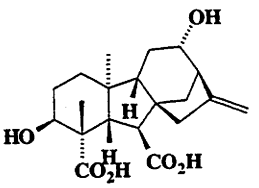
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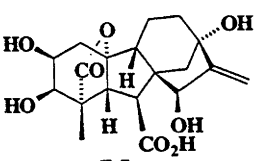
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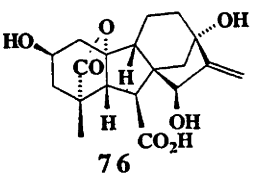
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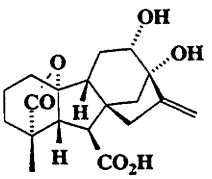
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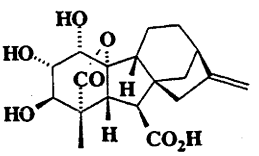
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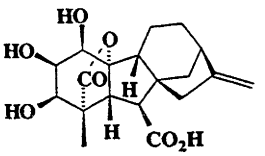
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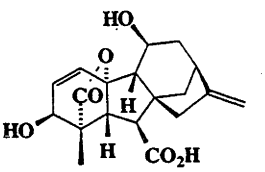
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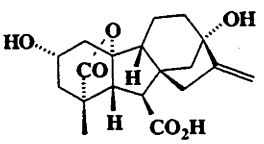
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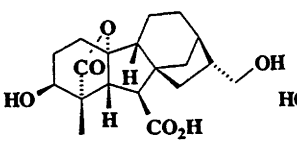
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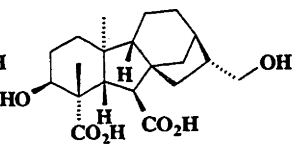
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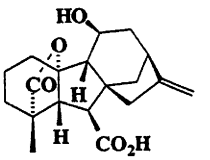
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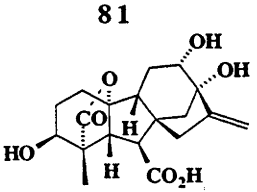
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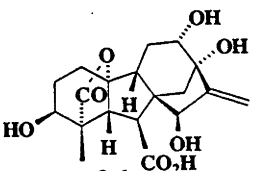
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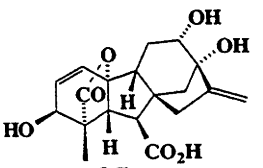
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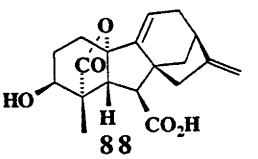
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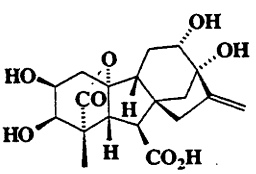
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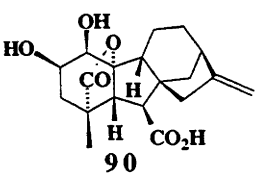
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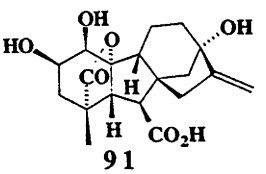
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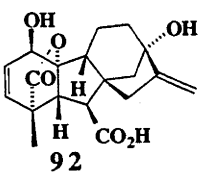
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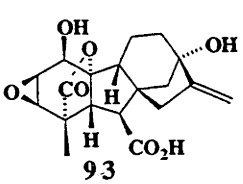
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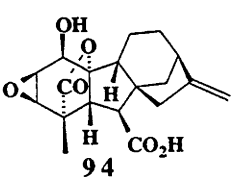
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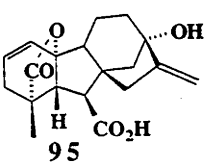
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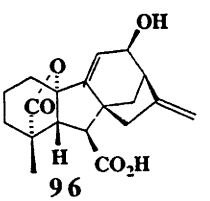
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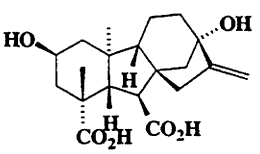

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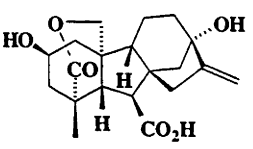

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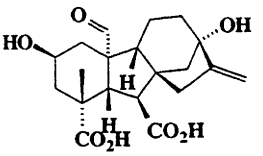

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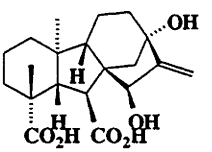

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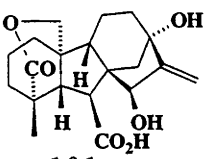

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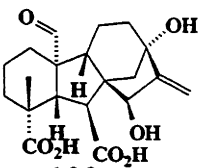

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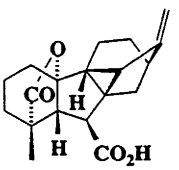

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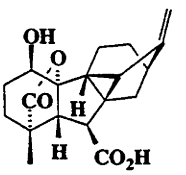

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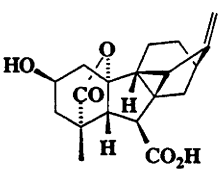

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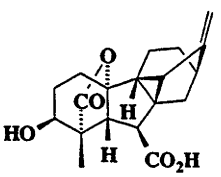

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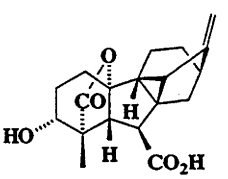

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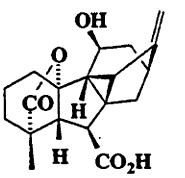

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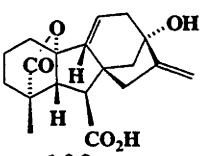

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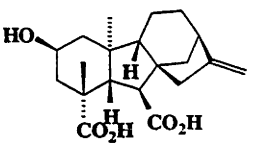

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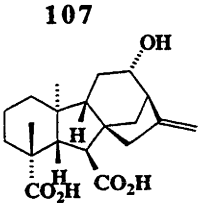

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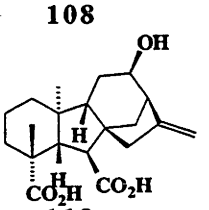

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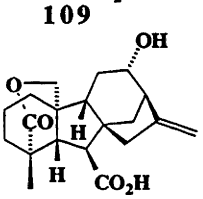

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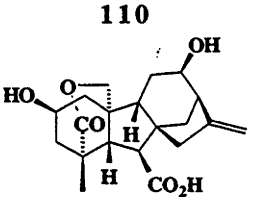

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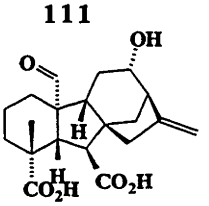

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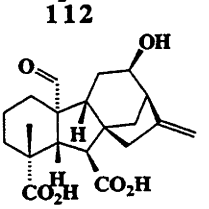

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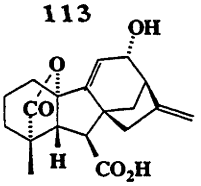

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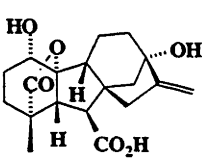

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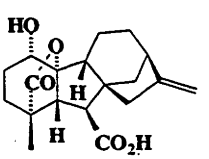

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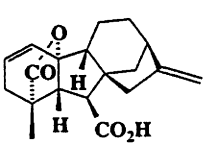

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Appendix II

X-ray Structure Data for *ent*-3α,13-Diacetoxy-6α-hydroxy-7-oxo-20-norkaur-16-en-19-oic Acid 19,10-Lactone (103)

Experimental

Data Collection

A fragment having approximate dimensions of 0.17 × 0.19 × 0.29 mm was cleaved from a colourless needle of $C_{23}H_{28}O_8$ and was mounted on a quartz fibre. All measurements were made on a Rigaku AFC6R diffractometer with graphite monochromated Cu-Kα radiation and a 12kW rotating anode generator.

Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 23 carefully centered reflections in the range $100.72 < 2\theta < 109.71°$ corresponded to a primitive orthorhombic cell with dimensions:

a = 6.552(1) Å
b = 13.705(2) Å
c = 23.789(1) Å
V = 2136.1(5) Å^3

For Z = 4 and F.W. = 432.47, the calculated density is 1.35 g/cm^3. The systematic absences of:

h00: h ≠ 2n
0k0: k ≠ 2n
00l: l ≠ 2n

uniquely determine the space group to be:

$P2_12_12_1$ (#19)

The data were collected at a temperature of 23 ± 1°C using the ω-2θ scan technique to a maximum 2θ value of 120.1°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.29° with a take-off angle of 6.0°. Scans of (1.00 + 0.30 tan θ)° were made at a speed of 8.0°/min (in omega). The weak reflections ($I < 10.0\sigma(I)$) were rescanned (maximum of 4 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 0.5 mm, the crystal to detector distance was 400 mm, and the detector aperture was 7.0 × 7.0 mm (horizontal × vertical).

Data Reduction

A total of 1886 reflections was collected. The intensities of three representative reflection were measured after every 150 reflections. No decay correction was required.

The linear absorption coefficient, μ, for Cu-Kα radiation is 8.1 cm^{-1}. An analytical absorption correction was applied which resulted in transmission factors ranging from 0.83 to 0.89. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction was applied (coefficient = 9.5×10^{-6}).

Structure Solution and Refinement

The structure was solved by direct methods[1] and the remaining atoms were located in subsequent difference electron-density maps. The non-hydrogen atoms were refined with anisotropic displacement factors. Most hydrogen atoms were observed in a difference map but were included at calculated positions (those of the acetate groups oriented to best fit the difference map peaks) and not refined though they were periodically recalculated. The exception was the hydrogen atom on O(2) which was refined positionally in the least-squares, starting with the coordinates from the difference map. The final cycle of full-matrix least-squares refinement[2] was based on 1777 observed reflections ($I > 3.0\sigma(I)$) and 284 variable parameters and converged (largest parameter shift was 0.07 times its esd) with unweighted and weighted agreement factors of:

$$R = \Sigma \, ||F_o| - |F_c|| \, / \, \Sigma \, |F_o| = 0.030$$

$$R_w = [(\Sigma \, w \, (|F_o| - |F_c|)^2 \, / \, \Sigma \, w \, F_o^2)]^{1/2} = 0.042$$

The standard deviation of an observation of unit weight[3] was 2.67. The weighting scheme was based on counting statistics and included a factor ($p = 0.020$) to downweight the intense reflections. Plots of $\Sigma \, w \, (|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, $\sin\theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.12 and -0.15 $e^-/Å^3$, respectively.

Neutral atom scattering factors were taken from Cromer and Waber[4]. Anomalous dispersion effects were included in F_{calc}[5]; the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley[6]. The values for the mass attenuation coefficients are those of Creagh and Hubbel[7]. All calculations were performed using the teXsan[8] crystallographic software package of Molecular Structure Corporation.

EXPERIMENTAL DETAILS

A. Crystal Data

Empirical Formula	$C_{23}H_{28}O_8$
Formula Weight	432.47
Crystal Color, Habit	colourless, needle
Crystal Dimensions	0.17 × 0.19 × 0.29 mm
Crystal System	orthorhombic
Lattice Type	Primitive
No. of Reflections Used for Unit Cell Determination (2θ range)	23 (100.7 - 109.7°)
Omega Scan Peak Width at Half-height	0.29°
Lattice Parameters	a = 6.552(1)Å b = 13.705(2) Å c = 23.789(1) Å V = 2136.1(5) Å^3
Space Group	$P2_12_12_1$ (#19)
Z value	4
D_{calc}	1.345 g/cm^3
F_{000}	920.00
μ(Cu-Kα)	8.05 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC6R
Radiation	CuKα (λ = 1.54178 Å) graphite monochromated
Take-off Angle	6.0°
Detector Aperture	7.0 mm horizontal 7.0 mm vertical
Crystal to Detector Distance	400 mm
Temperature	23.0°C

Scan Type	ω-2θ
Scan Rate	8.0°/min (in ω) (up to 4 scans)
Scan Width	(1.00 + 0.30 tan θ)°
$2\theta_{max}$	120.1°
No. of Reflections Measured	Total: 1886
Corrections	Lorentz-polarization Absorption (trans. factors: 0.8349 - 0.8879) Secondary Extinction (coefficient: 9.5×10^{-6})

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SIR92)
Refinement	Full-matrix least-squares
Function Minimized	Σ w ($\|F_o\|$ - $\|F_c\|$)^2
Least Squares Weights	$1/\sigma^2(F_o) = 4F_o^2/\sigma^2(F_o^2)$
p-factor	0.02
Anomalous Dispersion	All non-hydrogen atoms
No. Observations (I > 3.0σ(I))	1777
No. Variables	284
Reflection/Parameter Ratio	6.26
Residuals: R; R_w	0.030 ; 0.042
Goodness of Fit Indicator	2.67
Max Shift/Error in Final Cycle	0.07
Maximum peak in Final Diff. Map	0.12 $e^-/Å^3$
Minimum peak in Final Diff. Map	-0.15 $e^-/Å^3$

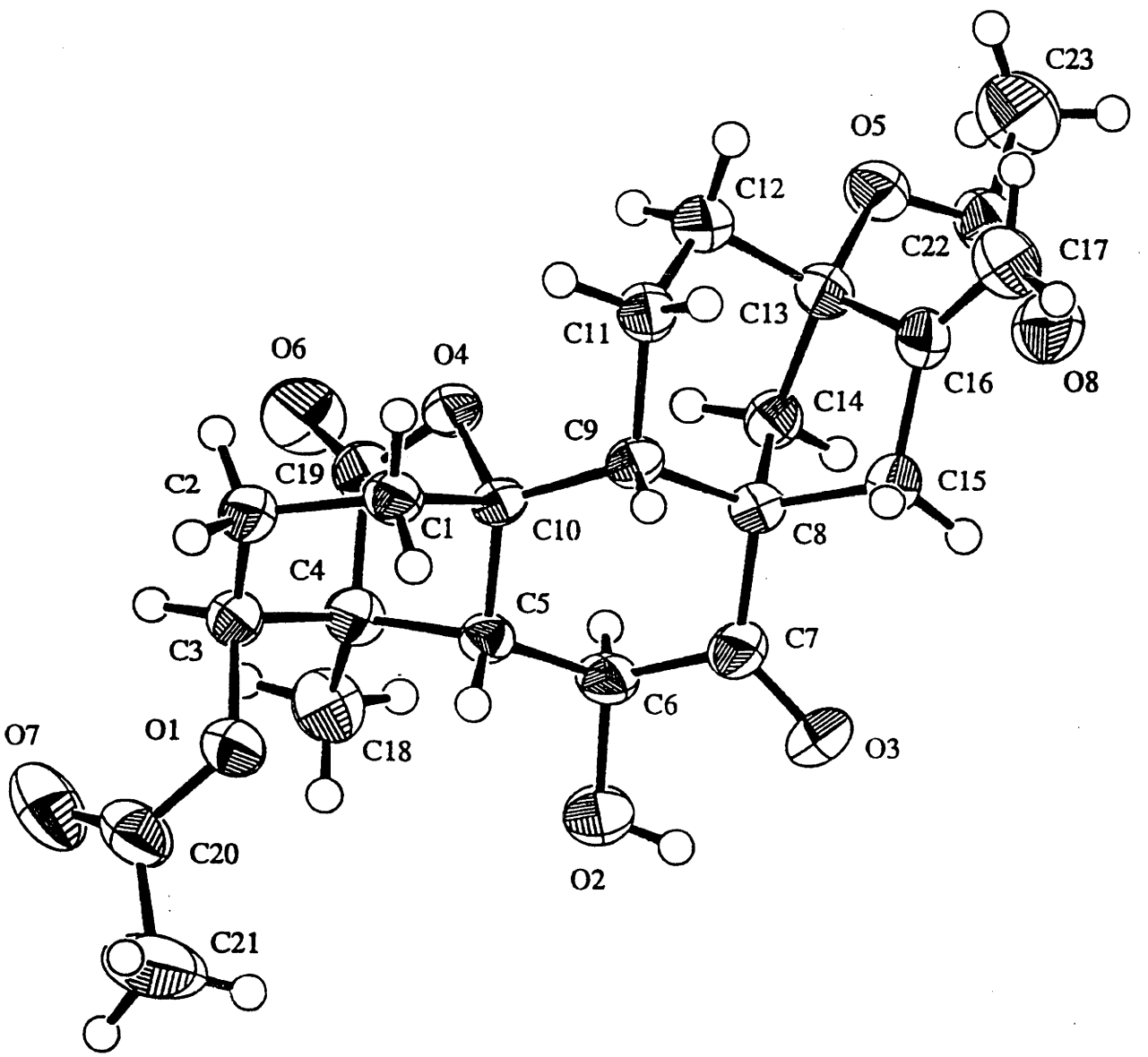

Figure 18. Thermal ellipsoid diagram of $C_{23}H_{28}O_8$ with labelling of selected atoms. Ellipsoids show 50% probability levels except for hydrogen atoms which are drawn as spheres of arbitrary radius.

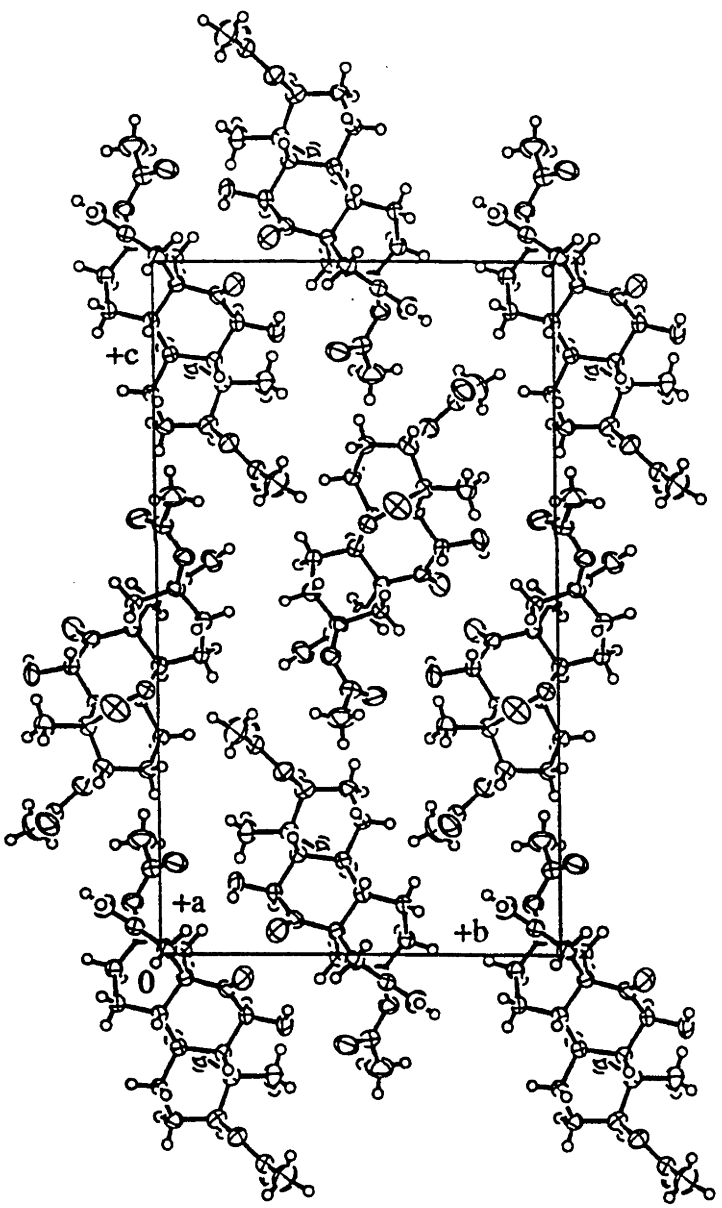

Figure 19. Unit cell packing diagram for $C_{23}H_{28}O_8$, projected down the *a* axis. Ellipsoids show 50% probability levels except for hydrogen atoms which are drawn as spheres of arbitrary radius.

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{23}H_{28}O_8$

atom	x	y	z	B_{eq}
O(1)	0.7081(3)	0.1875(1)	0.73891(7)	3.47(4)
O(2)	0.7954(4)	0.3093(1)	0.89920(9)	4.55(5)
O(3)	1.0373(4)	0.2063(2)	0.96637(9)	5.07(6)
O(4)	0.4674(3)	0.0307(1)	0.87809(7)	2.76(4)
O(5)	0.5144(3)	-0.0681(1)	1.07250(7)	3.49(4)
O(6)	0.1823(3)	0.1059(2)	0.85134(10)	5.27(6)
O(7)	0.4950(4)	0.2738(2)	0.68458(10)	5.50(7)
O(8)	0.6709(4)	0.0385(2)	1.13015(8)	5.09(6)
C(1)	0.7335(4)	-0.0064(2)	0.80952(10)	2.78(5)
C(2)	0.5865(5)	0.0270(2)	0.76346(10)	3.14(6)
C(3)	0.5398(4)	0.1356(2)	0.7648(1)	3.02(6)
C(4)	0.5130(4)	0.1748(2)	0.8254(1)	2.77(5)
C(5)	0.7105(4)	0.1535(2)	0.85785(10)	2.33(5)
C(6)	0.7342(5)	0.2124(2)	0.9123(1)	2.99(6)
C(7)	0.8874(5)	0.1633(2)	0.9507(1)	3.07(6)
C(8)	0.8354(4)	0.0600(2)	0.9674(1)	2.58(5)
C(9)	0.8107(4)	-0.0039(2)	0.91379(10)	2.44(5)
C(10)	0.6893(4)	0.0423(2)	0.86654(10)	2.24(5)
C(11)	0.7407(4)	-0.1086(2)	0.9292(1)	2.96(5)
C(12)	0.5899(5)	-0.1151(2)	0.9787(1)	3.13(6)
C(13)	0.6542(4)	-0.0473(2)	1.0262(1)	2.79(6)
C(14)	0.6431(4)	0.0570(2)	1.00445(10)	2.78(6)
C(15)	0.9937(5)	0.0150(2)	1.0077(1)	3.23(6)
C(16)	0.8776(5)	-0.0636(2)	1.0388(1)	2.97(6)
C(17)	0.9546(5)	-0.1378(2)	1.0670(1)	4.37(7)
C(18)	0.4323(5)	0.2789(2)	0.8258(1)	4.00(7)
C(19)	0.3648(4)	0.1038(2)	0.8525(1)	3.26(6)
C(20)	0.6622(6)	0.2567(2)	0.7006(1)	4.09(7)
C(21)	0.8538(8)	0.3058(3)	0.6808(2)	7.1(1)
C(22)	0.5365(5)	-0.0193(2)	1.1210(1)	3.82(7)
C(23)	0.3713(6)	-0.0437(3)	1.1618(1)	5.70(9)
H(1)	0.7216	-0.0751	0.8138	3.3338
H(2)	0.8688	0.0095	0.7984	3.3338
H(3)	0.4619	-0.0077	0.7677	3.7597
H(4)	0.6453	0.0118	0.7280	3.7597
H(5)	0.4186	0.1478	0.7440	3.6218
H(6)	0.8241	0.1660	0.8341	2.7893
H(7)	0.6060	0.2147	0.9309	3.5891
H(8)	0.9449	-0.0113	0.8993	2.9284
H(9)	0.6770	-0.1363	0.8971	3.5430
H(10)	0.8585	-0.1456	0.9386	3.5430
H(11)	0.4577	-0.0971	0.9661	3.7466
H(12)	0.5869	-0.1803	0.9923	3.7466
H(13)	0.5227	0.0680	0.9831	3.3272
H(14)	0.6502	0.1032	1.0342	3.3272
H(15)	1.1044	-0.0124	0.9873	3.8711
H(16)	1.0440	0.0627	1.0331	3.8711
H(17)	1.0983	-0.1461	1.0692	5.2340
H(18)	0.8663	-0.1831	1.0850	5.2340
H(19)	0.4069	0.2986	0.8634	4.7932
H(20)	0.5306	0.3211	0.8094	4.7932

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{23}H_{28}O_8$ (cont...)

atom	x	y	z	B_{eq}
H(21)	0.3091	0.2819	0.8048	4.7932
H(22)	0.9253	0.2634	0.6561	8.5007
H(23)	0.8197	0.3642	0.6615	8.5007
H(24)	0.9375	0.3208	0.7122	8.5007
H(25)	0.4226	-0.0386	1.1990	6.8276
H(26)	0.3255	-0.1085	1.1553	6.8276
H(27)	0.2608	0.0003	1.1570	6.8276
H(28)	0.905(6)	0.317(3)	0.920(1)	5.5000

$B_{eq} = 8/3\ \pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}(aa^*bb^*)\cos\gamma + 2U_{13}(aa^*cc^*)\cos\beta + 2U_{23}(bb^*cc^*)\cos\alpha)$

Table 2. Anisotropic Displacement Parameters for $C_{23}H_{28}O_8$

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	0.043(1)	0.046(1)	0.0419(10)	-0.0059(10)	0.0002(9)	0.0094(8)
O(2)	0.085(2)	0.0284(9)	0.059(1)	-0.012(1)	-0.015(1)	0.0014(9)
O(3)	0.073(2)	0.053(1)	0.066(1)	-0.032(1)	-0.028(1)	0.009(1)
O(4)	0.0279(9)	0.0391(9)	0.0380(9)	-0.0058(9)	0.0007(8)	0.0043(8)
O(5)	0.049(1)	0.047(1)	0.0358(10)	-0.0095(10)	0.0056(10)	0.0022(8)
O(6)	0.022(1)	0.092(2)	0.086(2)	-0.001(1)	0.002(1)	0.025(1)
O(7)	0.088(2)	0.062(1)	0.058(1)	0.022(2)	-0.007(1)	0.019(1)
O(8)	0.080(2)	0.070(1)	0.044(1)	-0.023(2)	0.003(1)	-0.008(1)
C(1)	0.041(1)	0.032(1)	0.033(1)	-0.003(1)	0.002(1)	-0.001(1)
C(2)	0.046(2)	0.040(1)	0.033(1)	-0.008(1)	-0.002(1)	-0.003(1)
C(3)	0.030(1)	0.046(1)	0.039(1)	-0.006(1)	-0.005(1)	0.008(1)
C(4)	0.027(1)	0.037(1)	0.041(1)	0.004(1)	0.000(1)	0.004(1)
C(5)	0.025(1)	0.031(1)	0.032(1)	-0.002(1)	0.002(1)	0.002(1)
C(6)	0.052(2)	0.025(1)	0.037(1)	-0.004(1)	0.001(1)	-0.001(1)
C(7)	0.046(2)	0.036(1)	0.035(1)	-0.008(1)	-0.002(1)	-0.007(1)
C(8)	0.033(1)	0.032(1)	0.033(1)	-0.004(1)	-0.001(1)	0.000(1)
C(9)	0.026(1)	0.030(1)	0.036(1)	-0.001(1)	0.003(1)	-0.003(1)
C(10)	0.023(1)	0.029(1)	0.034(1)	-0.003(1)	0.001(1)	-0.001(1)
C(11)	0.046(2)	0.029(1)	0.037(1)	0.001(1)	-0.004(1)	-0.001(1)
C(12)	0.046(2)	0.035(1)	0.038(1)	-0.006(1)	-0.002(1)	0.001(1)
C(13)	0.036(2)	0.039(1)	0.031(1)	-0.003(1)	0.003(1)	0.003(1)
C(14)	0.040(2)	0.033(1)	0.033(1)	0.000(1)	0.000(1)	-0.004(1)
C(15)	0.040(2)	0.044(2)	0.040(1)	-0.003(1)	-0.009(1)	0.002(1)
C(16)	0.043(2)	0.038(1)	0.032(1)	-0.001(1)	-0.004(1)	-0.001(1)
C(17)	0.059(2)	0.051(2)	0.055(2)	0.003(2)	-0.009(2)	0.011(1)
C(18)	0.047(2)	0.046(2)	0.059(2)	0.015(2)	-0.004(2)	0.004(1)
C(19)	0.029(2)	0.053(2)	0.042(1)	0.001(1)	0.001(1)	0.004(1)
C(20)	0.081(3)	0.035(1)	0.039(1)	0.002(2)	0.004(2)	0.003(1)
C(21)	0.118(4)	0.066(2)	0.085(3)	-0.030(3)	0.023(3)	0.019(2)
C(22)	0.062(2)	0.049(2)	0.035(1)	-0.002(2)	0.003(1)	0.002(1)
C(23)	0.078(3)	0.091(3)	0.048(2)	-0.011(2)	0.022(2)	-0.002(2)

The general temperature factor expression:
$\exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$

Table 3. Interatomic Distances (Å) Involving Non-Hydrogen Atoms for $C_{23}H_{28}O_8$

atom	atom	distance	atom	atom	distance
O(1)	C(3)	1.450(3)	O(1)	C(20)	1.349(3)
O(2)	C(6)	1.422(3)	O(3)	C(7)	1.205(3)
O(4)	C(10)	1.488(3)	O(4)	C(19)	1.351(3)
O(5)	C(13)	1.461(3)	O(5)	C(22)	1.341(3)
O(6)	C(19)	1.196(3)	O(7)	C(20)	1.183(4)
O(8)	C(22)	1.205(4)	C(1)	C(2)	1.529(4)
C(1)	C(10)	1.539(3)	C(2)	C(3)	1.520(4)
C(3)	C(4)	1.549(4)	C(4)	C(5)	1.534(3)
C(4)	C(18)	1.521(4)	C(4)	C(19)	1.518(4)
C(5)	C(6)	1.535(3)	C(5)	C(10)	1.544(3)
C(6)	C(7)	1.514(4)	C(7)	C(8)	1.509(4)
C(8)	C(9)	1.555(3)	C(8)	C(14)	1.538(4)
C(8)	C(15)	1.542(4)	C(9)	C(10)	1.516(3)
C(9)	C(11)	1.549(3)	C(11)	C(12)	1.540(4)
C(12)	C(13)	1.522(4)	C(13)	C(14)	1.522(4)
C(13)	C(16)	1.511(4)	C(15)	C(16)	1.511(4)
C(16)	C(17)	1.319(4)	C(20)	C(21)	1.500(6)
C(22)	C(23)	1.491(4)			

Table 4. Interatomic Distances (Å) Involving Hydrogen Atoms for $C_{23}H_{28}O_8$

atom	atom	distance	atom	atom	distance
O(2)	H(28)	0.88(4)	C(1)	H(1)	0.95
C(1)	H(2)	0.95	C(2)	H(3)	0.95
C(2)	H(4)	0.95	C(3)	H(5)	0.95
C(5)	H(6)	0.95	C(6)	H(7)	0.95
C(9)	H(8)	0.95	C(11)	H(9)	0.95
C(11)	H(10)	0.95	C(12)	H(11)	0.95
C(12)	H(12)	0.95	C(14)	H(13)	0.95
C(14)	H(14)	0.95	C(15)	H(15)	0.95
C(15)	H(16)	0.95	C(17)	H(17)	0.95
C(17)	H(18)	0.95	C(18)	H(19)	0.95
C(18)	H(20)	0.95	C(18)	H(21)	0.95
C(21)	H(22)	0.95	C(21)	H(23)	0.95
C(21)	H(24)	0.95	C(23)	H(25)	0.95
C(23)	H(26)	0.95	C(23)	H(27)	0.95

Table 5. Interatomic Angles (°) Involving Non-Hydrogen Atoms for $C_{23}H_{28}O_8$

atom	atom	atom	angle	atom	atom	atom	angle
C(3)	O(1)	C(20)	117.5(2)	C(10)	O(4)	C(19)	108.9(2)
C(13)	O(5)	C(22)	118.9(2)	C(2)	C(1)	C(10	112.5(2)
C(1)	C(2)	C(3)	113.9(2)	O(1)	C(3)	C(2)	108.6(2)
O(1)	C(3)	C(4)	108.2(2)	C(2)	C(3)	C(4)	112.5(2)
C(3)	C(4)	C(5)	107.8(2)	C(3)	C(4)	C(18)	111.7(2)
C(3)	C(4)	C(19)	104.2(2)	C(5)	C(4)	C(18)	117.9(2)
C(5)	C(4)	C(19)	101.8(2)	C(18)	C(4)	C(19)	112.1(2)
C(4)	C(5)	C(6)	114.2(2)	C(4)	C(5)	C(10)	100.3(2)
C(6)	C(5)	C(10)	114.5(2)	O(2)	C(6)	C(5)	109.5(2)
O(2)	C(6)	C(7)	111.1(2)	C(5)	C(6)	C(7)	110.0(2)
O(3)	C(7)	C(6)	120.6(2)	O(3)	C(7)	C(8)	124.2(3)
C(6)	C(7)	C(8)	115.2(2)	C(7)	C(8)	C(9)	109.7(2)
C(7)	C(8)	C(14)	111.2(2)	C(7)	C(8)	C(15)	112.8(2)
C(9)	C(8)	C(14)	111.7(2)	C(9)	C(8)	C(15)	110.7(2)
C(14)	C(8)	C(15)	100.6(2)	C(8)	C(9)	C(10)	115.3(2)
C(8)	C(9)	C(11)	111.0(2)	C(10)	C(9)	C(11)	114.1(2)
O(4)	C(10)	C(1)	107.5(2)	O(4)	C(10)	C(5)	102.6(2)
O(4)	C(10)	C(9)	109.3(2)	C(1)	C(10)	C(5)	107.1(2)
C(1)	C(10)	C(9)	111.9(2)	C(5)	C(10)	C(9)	117.7(2)
C(9)	C(11)	C(12)	115.1(2)	C(11)	C(12)	C(13)	110.8(2)
O(5)	C(13)	C(12)	105.5(2)	O(5)	C(13)	C(14)	114.2(2)
O(5)	C(13)	C(16)	115.4(2)	C(12)	C(13)	C(14)	107.9(2)
C(12)	C(13)	C(16)	109.0(2)	C(14)	C(13)	C(16)	104.6(2)
C(8)	C(14)	C(13)	100.4(2)	C(8)	C(15)	C(16)	104.5(2)
C(13)	C(16)	C(15)	106.6(2)	C(13)	C(16)	C(17)	125.8(3)
C(15)	C(16)	C(17)	127.3(3)	O(4)	C(19)	O(6)	121.7(3)
O(4)	C(19)	C(4)	110.4(2)	O(6)	C(19)	C(4)	127.9(3)
O(1)	C(20)	O(7)	124.3(3)	O(1)	C(20)	C(21)	109.9(3)
O(7)	C(20)	C(21)	125.8(3)	O(5)	C(22)	O(8)	124.2(3)
O(5)	C(22)	C(23)	111.7(3)	O(8)	C(22)	C(23)	124.1(3)

Table 6. Interatomic Angles (°) Involving Hydrogen Atoms for $C_{23}H_{28}O_8$

atom	atom	atom	angle	atom	atom	atom	angle
C(6)	O(2)	H(28)	102(2)	C(2)	C(1)	H(1)	108.7
C(2)	C(1)	H(2)	108.7	C(10)	C(1)	H(1)	108.7
C(10)	C(1)	H(2)	108.7	H(1)	C(1)	H(2)	109.5
C(1)	C(2)	H(3)	108.4	C(1)	C(2)	H(4)	108.4
C(3)	C(2)	H(3)	108.4	C(3)	C(2)	H(4)	108.4
H(3)	C(2)	H(4)	109.5	O(1)	C(3)	H(5)	109.2
C(2)	C(3)	H(5)	109.2	C(4)	C(3)	H(5)	109.2
C(4)	C(5)	H(6)	109.1	C(6)	C(5)	H(6)	109.1
C(10)	C(5)	H(6)	109.1	O(2)	C(6)	H(7)	108.7
C(5)	C(6)	H(7)	108.7	C(7)	C(6)	H(7)	108.7
C(8)	C(9)	H(8)	105.1	C(10)	C(9)	H(8)	105.1
C(11)	C(9)	H(8)	105.1	C(9)	C(11)	H(9)	108.0
C(9)	C(11)	H(10)	108.0	C(12)	C(11)	H(9)	108.0
C(12)	C(11)	H(10)	108.1	H(9)	C(11)	H(10)	109.5
C(11)	C(12)	H(11)	109.2	C(11)	C(12)	H(12)	109.1
C(13)	C(12)	H(11)	109.2	C(13)	C(12)	H(12)	109.1
H(11)	C(12)	H(12)	109.5	C(8)	C(14)	H(13)	111.7
C(8)	C(14)	H(14)	111.7	C(13)	C(14)	H(13)	111.7
C(13)	C(14)	H(14)	111.7	H(13)	C(14)	H(14)	109.5
C(8)	C(15)	H(15)	110.7	C(8)	C(15)	H(16)	110.7
C(16)	C(15)	H(15)	110.7	C(16)	C(15)	H(16)	110.7
H(15)	C(15)	H(16)	109.5	C(16)	C(17)	H(17)	120.0
C(16)	C(17)	H(18)	120.0	H(17)	C(17)	H(18)	120.0
C(4)	C(18)	H(19)	109.4	C(4)	C(18)	H(20)	109.5
C(4)	C(18)	H(21)	109.5	H(19)	C(18)	H(20)	109.5
H(19)	C(18)	H(21)	109.5	H(20)	C(18)	H(21)	109.5
C(20)	C(21)	H(22)	109.5	C(20)	C(21)	H(23)	109.4
C(20)	C(21)	H(24)	109.4	H(22)	C(21)	H(23)	109.5
H(22)	C(21)	H(24)	109.5	H(23)	C(21)	H(24)	109.5
C(22)	C(23)	H(25)	109.5	C(22)	C(23)	H(26)	109.5
C(22)	C(23)	H(27)	109.5	H(25)	C(23)	H(26)	109.5
H(25)	C(23)	H(27)	109.5	H(26)	C(23)	H(27)	109.5

Table 7. Torsion Angles (°) Involving Non-Hydrogen Atoms for $C_{23}H_{28}O_8$

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
O(1)	C(3)	C(2)	C(1)	79.6(3)	O(1)	C(3)	C(4)	C(5)	-61.6(2)
O(1)	C(3)	C(4)	C(18)	69.5(3)	O(1)	C(3)	C(4)	C(19)	169.2(2)
O(2)	C(6)	C(5)	C(4)	-77.4(3)	O(2)	C(6)	C(5)	C(10)	167.7(2)
O(2)	C(6)	C(7)	O(3)	0.4(4)	O(2)	C(6)	C(7)	C(8)	179.7(2)
O(3)	C(7)	C(6)	C(5)	121.9(3)	O(3)	C(7)	C(8)	C(9)	-123.1(3)
O(3)	C(7)	C(8)	C(14)	112.9(3)	O(3)	C(7)	C(8)	C(15)	0.8(4)
O(4)	C(10)	C(1)	C(2)	50.0(3)	O(4)	C(10)	C(5)	C(4)	-38.7(2)
O(4)	C(10)	C(5)	C(6)	84.1(3)	O(4)	C(10)	C(9)	C(8)	-81.2(3)
O(4)	C(10)	C(9)	C(11)	48.9(3)	O(4)	C(19)	C(4)	C(3)	91.3(2)
O(4)	C(19)	C(4)	C(5)	-20.7(3)	O(4)	C(19)	C(4)	C(18)	147.7(2)
O(5)	C(13)	C(12)	C(11)	-173.8(2)	O(5)	C(13)	C(14)	C(8)	167.8(2)
O(5)	C(13)	C(16)	C(15)	-144.1(2)	O(5)	C(13)	C(16)	C(17)	41.8(4)
O(6)	C(19)	O(4)	C(10)	173.4(3)	O(6)	C(19)	C(4)	C(3)	-86.3(4)
O(6)	C(19)	C(4)	C(5)	161.7(3)	O(6)	C(19)	C(4)	C(18)	34.7(4)
O(7)	C(20)	O(1)	C(3)	-4.4(4)	O(8)	C(22)	O(5)	C(13)	3.3(4)
C(1)	C(2)	C(3)	C(4)	-40.1(3)	C(1)	C(10)	O(4)	C(19)	85.0(2)
C(1)	C(10)	C(5)	C(4)	74.3(2)	C(1)	C(10)	C(5)	C(6)	-162.9(2)
C(1)	C(10)	C(9)	C(8)	159.9(2)	C(1)	C(10)	C(9)	C(11)	-70.0(3)
C(2)	C(1)	C(10)	C(5)	-59.6(3)	C(2)	C(1)	C(10)	C(9)	170.0(2)
C(2)	C(3)	O(1)	C(20)	132.8(2)	C(2)	C(3)	C(4)	C(5)	58.3(3)
C(2)	C(3)	C(4)	C(18)	-170.6(2)	C(2)	C(3)	C(4)	C(19)	-49.3(3)
C(3)	O(1)	C(20)	C(21)	177.0(2)	C(3)	C(2)	C(1)	C(10)	41.1(3)
C(3)	C(4)	C(5)	C(6)	163.3(2)	C(3)	C(4)	C(5)	C(10)	-73.7(2)
C(4)	C(3)	O(1)	C(20)	-104.8(2)	C(4)	C(5)	C(6)	C(7)	160.2(2)
C(4)	C(5)	C(10)	C(9)	-158.6(2)	C(4)	C(19)	O(4)	C(10)	-4.4(3)
C(5)	C(6)	C(7)	C(8)	-58.9(3)	C(5)	C(10)	O(4)	C(19)	27.7(2)
C(5)	C(10)	C(9)	C(8)	35.2(3)	C(5)	C(10)	C(9)	C(11)	165.3(2)
C(6)	C(5)	C(4)	C(18)	35.7(3)	C(6)	C(5)	C(4)	C(19)	-87.4(3)
C(6)	C(5)	C(10)	C(9)	-35.9(3)	C(6)	C(7)	C(8)	C(9)	57.7(3)
C(6)	C(7)	C(8)	C(14)	-66.3(3)	C(6)	C(7)	C(8)	C(15)	-178.4(2)
C(7)	C(6)	C(5)	C(10)	45.2(3)	C(7)	C(8)	C(9)	C(10)	-44.0(3)
C(7)	C(8)	C(9)	C(11)	-175.6(2)	C(7)	C(8)	C(14)	C(13)	-167.2(2)
C(7)	C(8)	C(15)	C(16)	155.7(2)	C(8)	C(9)	C(11)	C(12)	36.5(3)
C(8)	C(14)	C(13)	C(12)	-75.2(3)	C(8)	C(14)	C(13)	C(16)	40.7(2)
C(8)	C(15)	C(16)	C(13)	-12.4(3)	C(8)	C(15)	C(16)	C(17)	161.5(3)
C(9)	C(8)	C(14)	C(13)	70.0(2)	C(9)	C(8)	C(15)	C(16)	-81.0(2)
C(9)	C(10)	O(4)	C(19)	153.4(2)	C(9)	C(11)	C(12)	C(13)	-42.7(3)
C(10)	C(5)	C(4)	C(18)	158.7(2)	C(10)	C(5)	C(4)	C(19)	35.5(2)
C(10)	C(9)	C(8)	C(14)	79.7(2)	C(10)	C(9)	C(8)	C(15)	-169.1(2)
C(10)	C(9)	C(11)	C(12)	-95.7(3)	C(11)	C(9)	C(8)	C(14)	-51.9(3)
C(11)	C(9)	C(8)	C(15)	59.3(3)	C(11)	C(12)	C(13)	C(14)	63.8(3)
C(11)	C(12)	C(13)	C(16)	-49.3(3)	C(12)	C(13)	O(5)	C(22)	179.2(2)
C(12)	C(13)	C(16)	C(15)	97.5(2)	C(12)	C(13)	C(16)	C(17)	-76.5(3)
C(13)	O(5)	C(22)	C(23)	175.4(3)	C(13)	C(14)	C(8)	C(15)	-47.5(2)
C(14)	C(8)	C(15)	C(16)	37.2(2)	C(14)	C(13)	O(5)	C(22)	-62.5(3)
C(14)	C(13)	C(16)	C(15)	-17.7(3)	C(14)	C(13)	C(16)	C(17)	168.2(3)
C(16)	C(13)	O(5)	C(22)	58.8(3)					

Table 8. Non-bonded Contacts out to 3.60 Å for $C_{23}H_{28}O_8$

atom	atom	distance	ADC	atom	atom	distance	ADC
O(2)	O(8)	3.300(4)	55703	O(2)	C(22)	3.318(4)	55703
O(2)	C(23)	3.560(4)	55703	O(3)	O(6)	3.207(4)	65501
O(3)	C(6)	3.351(3)	55703	O(3)	C(14)	3.388(3)	55703
O(3)	C(7)	3.514(4)	55703	O(6)	C(7)	3.152(4)	45501
O(6)	C(5)	3.164(3)	45501	O(6)	C(9)	3.225(3)	45501
O(6)	C(10)	3.365(3)	45501	O(6)	C(1)	3.465(4)	45501
O(6)	C(6)	3.585(4)	45501	O(7)	C(1)	3.367(4)	65604
O(7)	C(17)	3.377(4)	65402	O(7)	C(11)	3.509(3)	65604
O(8)	C(18)	3.209(4)	55703				

Potential Hydrogen Bond

Donor---H	Acceptor	ADC	D...A	D---H	H...A	D-H...A
O(2)---H(28)	O(3)	55501	2.657(3)	0.88(4)	2.07(4)	124(3)

The ADC (atom designator code) specifies the position of an atom in a crystal. The 5-digit number shown in the table is a composite of three one-digit numbers and one two-digit number: TA (first digit) + TB (second digit) + TC (third digit) + SN (last two digits). TA, TB and TC are the crystal lattice translation digits along cell edges a, b and c. A translation digit of 5 indicates the origin unit cell. If TA = 4, this indicates a translation of one unit cell length along the a-axis in the negative direction. Each translation digit can range in value from 1 to 9 and thus ± 4 lattice translations from the origin (TA=5, TB=5, TC=5) can be represented.

The SN, or symmetry operator number, refers to the number of the symmetry operator used to generate the coordinates of the target atom. A list of symmetry operators relevant to this structure are given below.

For a given intermolecular contact, the first atom (origin atom) is located in the origin unit cell and its position can be generated using the identity operator (SN=1). Thus, the ADC for an origin atom is always 55501. The position of the second atom (target atom) can be generated using the ADC and the coordinates of the atom in the parameter table. For example, an ADC of 47502 refers to the target atom moved through symmetry operator two, then translated -1 cell translations along the a axis, +2 cell translations along the b axis, and 0 cell translations along the c axis.

An ADC of 1 indicates an intermolecular contact between two fragments (eg. cation and anion) that reside in the same asymmetric unit.

Symmetry Operators:

(1)	X,	Y,	Z	(2)	1/2-X,	-Y,	1/2+Z
(3)	1/2+X,	1/2-Y,	-Z	(4)	-X,	1/2+Y,	1/2-Z

Table 9. Selected Least Squares Planes for $C_{23}H_{28}O_8$

Plane number 1	
Atoms defining plane	Distance
O(1)	0.001(2)
O(7)	0.003(2)
C(20)	-0.007(3)
C(21)	0.005(4)
Additional Atoms	Distance
C(3)	-0.075

Plane number 2	
Atoms defining plane	Distance
O(5)	-0.001(2)
O(8)	-0.002(3)
C(22)	0.007(3)
C(23)	-0.004(4)
Additional Atoms	Distance
C(13)	-0.096

Plane number 3	
Atoms defining plane	Distance
O(4)	-0.001(2)
O(6)	-0.006(3)
C(4)	-0.003(3)
C(19)	0.012(3)
Additional Atoms	Distance
C(3)	-1.507
C(5)	0.505
C(10)	-0.135
C(18)	0.761

Plane number 4	
Atoms defining plane	Distance
O(3)	-0.002(3)
C(6)	-0.001(3)
C(7)	0.004(3)
C(8)	-0.001(3)
Additional Atoms	Distance
O(2)	-0.004
C(5)	1.227
C(9)	1.230
C(14)	-1.320
C(15)	-0.035

Plane number 5	
Atoms defining plane	Distance
C(13)	0.013(3)
C(15)	0.010(3)
C(16)	-0.031(3)
C(17)	0.019(3)
Additional Atoms	Distance
O(5)	-0.780
C(8)	0.397
C(12)	1.446
C(14)	-0.372

Plane number 6

Atoms defining plane	Distance
C(13)	-0.106(3)
C(14)	0.051(2)
C(15)	-0.065(3)
C(16)	0.108(3)
Additional Atoms	**Distance**
C(8)	-0.688

Plane number 7

Atoms defining plane	Distance
C(8)	0.0
C(14)	0.0
C(15)	0.0
Additional Atoms	**Distance**
C(13)	1.104
C(16)	0.885

Summary

plane	mean deviation	χ^2
1	0.0040	8.5
2	0.0034	6.8
3	0.0056	22.2
4	0.0021	3.5
5	0.0182	195.9
6	0.0824	4285.2
7	0.0000	0.0

Dihedral angles between planes (°)

plane	1	2	3	4	5	6
2	73.04					
3	167.67	94.67				
4	30.67	75.84	148.20			
5	8.86	81.53	175.84	29.10		
6	161.49	88.80	6.46	148.17	170.27	
7	31.56	64.99	141.80	61.23	37.54	136.10

Table 10. Ring Puckering Analysis for $C_{23}H_{28}O_8$[9,10]

Ring 1 5-membered O(4) → C(10) → C(5) → C(4) → C(19) →
q_2 = 0.397(2)Å φ_2 = 246.7(4)°
[envelope]

Ring 2 5-membered C(8) → C(14) → C(13) → C(16) → C(15) →
q_2 = 0.474(3)Å φ_2 = 21.0(4)°
[half-chair]

Ring 3 6-membered C(1) → C(2) → C(3) → C(4) → C(5) → C(10) →
q_2 = 0.273(3)Å φ_2 = 62.2(6)°
q_3 = 0.397(2)Å
Q = 0.666(3)Å θ = 155.9(3)° φ = 62.2(6)°
[chair tending towards envelope]

Ring 4 6-membered C(5) → C(6) → C(7) → C(8) → C(9) → C(10) →
q_2 = 0.156(3)Å φ_2 = 297.1(10)°
q_3 = -0.461(3)Å
Q = 0.486(3)Å θ = 161.3(4)° φ = 297.1(10)°
[chair]

Ring 5 6-membered C(8) → C(9) → C(11) → C(12) → C(13) → C(14) →
q_2 = 0.265(3)Å φ_2 = 284.8(6)°
q_3 = 0.590(3)Å
Q = 0.647(3)Å θ = 24.2(3)° φ = 284.8(6)°
[chair tending towards half-chair]

References and Footnotes

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2. Least-Squares in teXsan:

   Function minimized: $\Sigma w(|F_o|-|F_c|)^2$ where

   $w = 4F_o^2/\sigma^2(F_o^2) = [\sigma^2(F_o) + (pF_o/2)^2]^{-1}$

   and $F_o^2 = S(C-RB)/Lp$

   $\sigma^2(F_o^2) = [S^2(C+R^2B) + (pF_o^2)^2]/Lp^2$

   S = Scan rate

   C = Total integrated peak count

   R = Ratio of scan time to background counting time

   B = Total background count

   Lp = Lorentz-polarization factor

   p = p-factor = 0.020

3. Standard deviation of an observation of unit weight:

   $[\Sigma w(|F_o|-|F_c|)^2/(N_o-N_v)]^{1/2}$

   where: N_o = number of observations

   N_v = number of variables

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