UNIVERSITY OF SOUTHAMPTON

THE SYNTHESES OF TWO INSECT DEFENCE SECRETIONS

A Thesis Submitted for the Degree of
Doctor of Philosophy

by

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To Mum and Dad

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ABSTRACT

FACULTY OF SCIENCE

CHEMISTRY

Doctor of Philosophy

THE SYNTHESES OF TWO INSECT DEFENCE SECRETIONS

By Malcolm John Crook

A synthesis of 4,11-epoxy-cis-eudesmane, the major component of the defensive secretion of the termite Amitermes evuncifer, has been investigated. Beginning with (-)-carvone, hydrogenation followed by a Robinson ring annelation reaction yielded epi-α-cyperone. Epoxidation, reduction to an intermediate diol, and then oxidation gave epi-carrisone. Hydrogenation under controlled conditions, separation of the cis-hydrogenated product followed by a Bamford-Stevens reaction yielded epi-γ-eudesmol which on reaction via a mercury (II) bridged intermediate gave intramolecular ether formation and afforded 4,11-epoxy-cis-eudesmane.

The synthesis of a novel dialdehyde, cavidial found in the defensive secretion of Ancistrotermes cavithorax has also been studied. The major route involved formation of the analogous diol from the Diels-Alder reduction of 2,6,6-trimethyl-1-vinyl cyclohexene and dimethyl acetylenedicarboxylate followed by hydrogenation of the trisubstituted double bond and reaction of the two ester groups to yield 3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-naphthalene-1,2-dimethanol. Spectral analysis showed this product to have a cis fused ring junction. A range of conditions has been utilised to achieve oxidation to the required dial, but none have been totally successful. The major product formed, in most cases, was epi-confertifolin. Reaction of the diol using differential protection followed by a series of oxidation/deprotection steps has also been investigated. Progress to a suitable intermediate has been made, but the final reaction failed to yield cavidial.

The chemical composition of the cuticular waxes of both male and female warble flies Hypoderma bovis and H. lineatum have been investigated. The major constituents were found to be straight and monomethyl branched alkanes; however, spectral analysis is presented that suggests the presence of two unusual trimethyl branched alkanes.

ACKNOWLEDGEMENTS

This work was carried out under the supervision of Professor Ray Baker and I would like to record my gratitude for his friendship, continued interest and helpful advice during the period of these studies. I would also like to thank Dr. D.A. Evans (now of Fisons Ltd) for his friendship and help both at primary degree level and during my first year of research.

I am indebted to many other members of the Chemistry Department, and particularly the Insect Group, who have assisted me over the last three years and made my stay at Southampton enjoyable. I would particularly like to mention Dr. Ted Parton, who completed a proportion of the project on warble flies, Dr. Colin Broomfield, Dr. Bill Speed, Richard Herbert and Dave Selwood. My special thanks to Dr. Philip Parsons for his many ideas and hospitality in New York.

I would like to express my sincere gratitude to my parents for making the whole thing possible, for their enthusiasm and support over the last six years and finally to thank my fiancée, Linda, for her friendship and tolerance.

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C O N T E N T S

CHAPTER 1

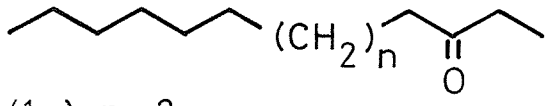
Introduction

The research of chemists and biologists has established the existence and importance of chemical cues in many aspects of insect behaviour. Pheromones are chemicals secreted by a member of an insect species that elicits a definite behavioural response in other members of the same species, and generally these chemicals have been found to regulate most stages of the development of insects such as mating, egg-laying and foraging[1-4]. The study of insect pheromones has attracted great attention because of their scientific interest and the possibilities for their use in the control of pest insects[5].

The defence mechanisms of some insects may also involve the use of chemicals. These studies involved the synthesis of two compounds secreted by different species of termites when defending themselves. Other aspects of termite biology are beyond the scope of this report, but may be found in other literature[6-8].

1.1 Defence in Termites

The predator-prey relationships existing between termites, and their major predators, ants, are well known[9]. Most species of termites have a soldier caste which defends the nest by use of well developed mandibles capable of cutting and biting[8]. Some species have developed a primitive method of defence which allows a toxic or repellent chemical to be applied to the foe[10,11]. An example of this is the termite *Mastotermes darwinienis*. This termite produces p-benzoquinone which combines with amino-acids in the saliva to produce a dark latex that entangles the victim[12]. Other Termitidae have developed a frontal gland for defence. This gland is situated in the middle of the head above the mandibles, and when required the secretion flows from a small pore - the fontanelle. The use of both mechanical and chemical means of defence is common to many species of termites, and the chemicals secreted have been found to be of many structural types. The soldier caste of *Macrotermes subhyalinus* secretes a mixture of n-alkanes, branched alkanes and alkenes that have been shown to immobilise ant predators[13]. *Schedorhinotermes lamanianus* also secretes straight chain compounds but these are multifunctional[14]. Three series of ketones (1a,b,c), αβ-unsaturated ketones (2a,b,c) and dienones (3a,b,c)



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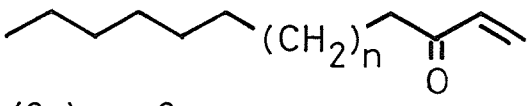

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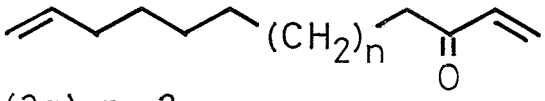

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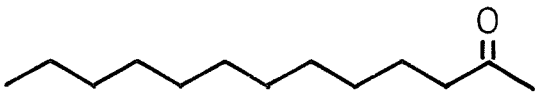

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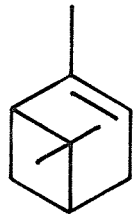

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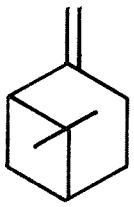

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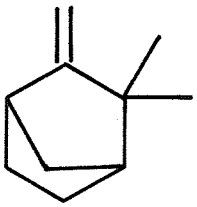

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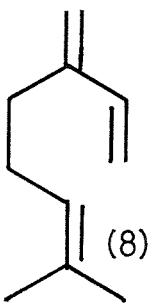

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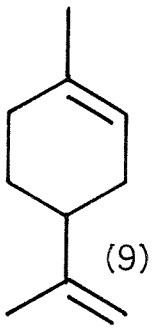

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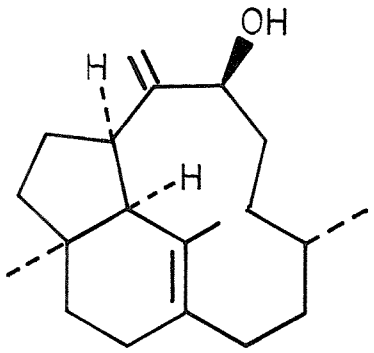

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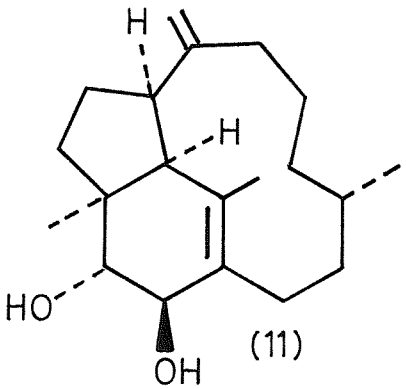

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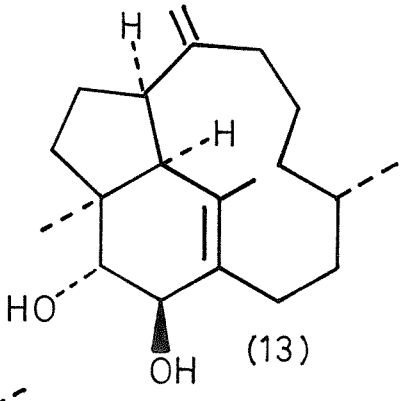

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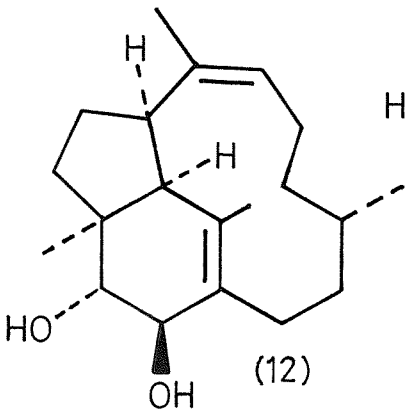

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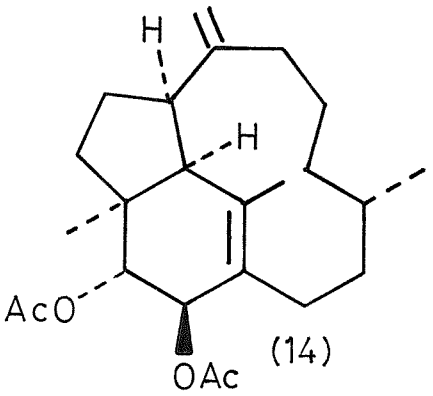
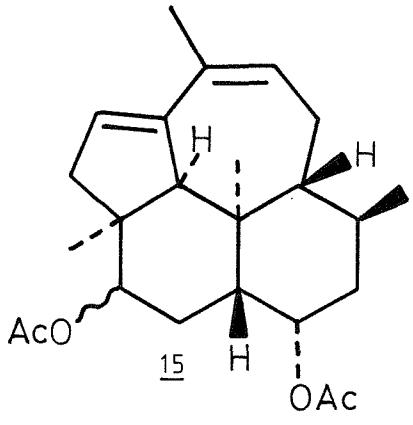


(14)

have been observed in the secretion of the major soldiers. The minor soldiers have ten components, the nine mentioned above and tridecan-2-one (4). One unusual example is 1-nitro-*trans*-pentadecene secreted by *Prorhinotermes simplex;* this is a rare example of a naturally occurring nitro-compound[15]. The soldier caste of the termite *Trinervitermes gratiosus* contains in addition to five monoterpenes [α-pinene (5), β-pinene (6), camphene (7), myrcene (8), and limonene (9)][16], an interesting series of novel diterpenes with a "trinervitene" structure[17]. *T. gratiosus* has both a major and minor caste of soldier termite and each produces a different defence secretion. The major soldiers secrete: α-pinene (5), camphene (7), myrcene (8), limonene (9), and diterpenes (10), (11), (12) and (13); the minor soldiers secrete α-pinene (5), β-pinene (6), camphene (7), limonene (9) and diterpenes (10), (11), (13) and (14). One other method of defence, used by a few species of termites is a spray ejected at attackers by nozzle-like heads of the soldiers. Studies of these secretions have also shown the presence of diterpene structures. An example is the secretion isolated from the species *Nasutitermes kempae* which produces two "kempene" type diterpenoids (15) and (16)[18].



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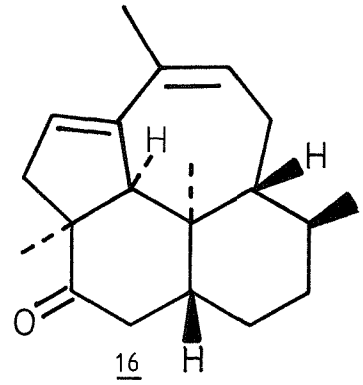

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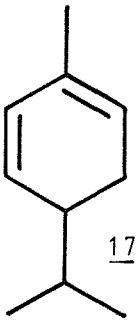
It has been reported that some ants produce secretions from their poison glands and these are repellent to termites. *Myrmicana eumenoids* produces a mixture of monoterpenes repellent to some species of termites of the Macroterminae family including some species of *Ancistrotermes*[19]. Myrcene (8), α-phellandrene (17) and β-phellandrene (18), have all been

found to be repellent to *Reticulitermes lucifigus santonensis*[7,20]. It is hoped that a fuller understanding of predator-prey relationships may provide clues to future control programmes. With this in mind, the soldiers of two species of termites have been analysed to determine the composition of their defence secretions.



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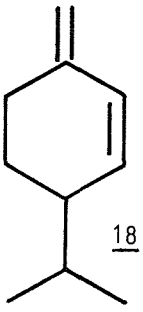

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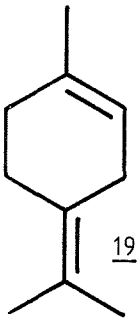

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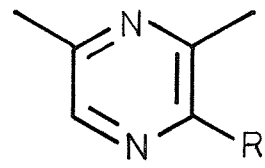
1.2 Defence in *Amitermes*

The termites of *Amitermes* species have developed a chemical method of defence as well as the usual methods of physical defence, such as cutting and biting. Previous work by Moore *et al*[12] has shown the defence secretion of *Amitermes* spp. to contain hydrocarbons. Extracts from *A. herbertensis*, *A. laurensis* and *A. vitiosus* were found to have α-pinene (5), β-pinene (6) and terpinolene (19) as major constituents. The secretion from *A. unidentatus* contained mainly straight chain ketones[21] and *A. ionnbergianus* seemed to contain no components at all[22].

1.3 Defence in *Amitermes evuncifer*

Amitermes evuncifer Silvestri (Isoptera, Termitidae, Amitermitinae) is common to many parts of Africa. The soldiers of this species have a well developed frontal gland and defence behaviour has been observed when this species is disturbed[21]. Some species of ants only attack one species of termite, but the major predator of *A. evuncifer* is the ant species *Odontomachus troglodytes*[19] which will attack any termite colony. This opportunist predator has been found to produce a series of related 2,6-dimethyl-3-alkylpyrazines (20). These are thought to play a part in alarm and defence behaviour.[23]

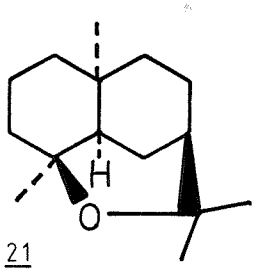


20 R = Et, n-C_4H_9,

n-C_5H_{11}, n-C_6H_{13}.

The mixture of components secreted by *A. evuncifer* has been extracted from specimens obtained from Nigeria. Analysis by gas-chromatography showed the volatile part of the extract to be mainly one compound (>90%), plus a number of minor components; the components were identified by spectroscopy. The major component was found to be a novel sesquiterpene ether, 4,11-epoxy-*cis*-eudesmane (21)[24]; the quantity being about 400 µl/soldier. The minor components were found to be 10-*epi*-eudesma-3,11-diene [(22), 0.8 µg/soldier], *epi*-caparrapi oxide [(23), 1.2 µg/soldier], caparrapi oxide [(24), 0.4 µg/soldier] and *cis*-β-ocimene [(8), 0.8 µg/soldier][22]. Caparrapi oxide (24) and *epi*-caparrapi oxide (23) were both identified by coelution with synthetic samples and detailed comparison of spectral data[25]. *Cis*-β-ocimene (8) was identified (with special attention being paid to the stereochemistry of the double bonds) by comparison of its spectral data, with data of an authentic sample[26,27]. 4,11-Epoxy-*cis*-eudesmane (21) has been synthesised by an enantiomer specific route to establish the absolute configuration[21].

In recent studies of another species, *A. messinae*, an East African termite, the presence of (21) has been suggested, cooccurring with limonene (9).



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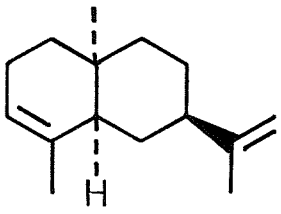

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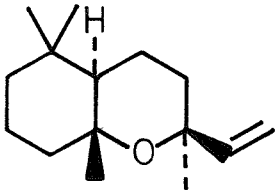

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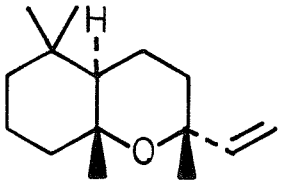

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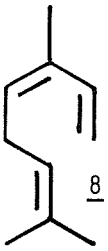

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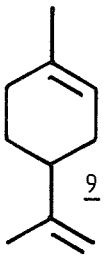

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1.4 Preliminary Studies of Toxicity

Studies on the toxicity of the defence secretion of *A. evuncifer* have been performed to examine the activity of the crude extract[21]. The mixture was tested on several species of ants, and was found to be very repellent to the opportunist predator *Odontomachus troglydytes*, but not so active against other species.

Further biological testing on the major component of the defence secretion would require larger quantities of material and so synthetic routes to 4,11-epoxy-*cis*-eudesmane (21) were explored.

1.5 Previous Synthetic Studies on Eudesmanes

4,11-Epoxy-*cis*-eudesmane (21) is a novel member of the eudesmane sesquiterpenes. Other naturally occurring members of the eudesmane group may be found in a catalogue by Devon and Scott[28]. The general structure (Figure 1) is common to all eudesmanes, and many syntheses of members of this group have been published (Figure 2)[29].

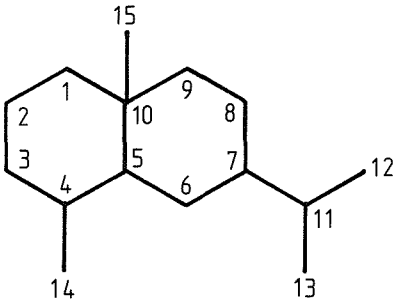


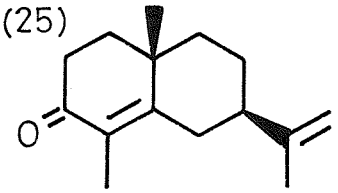
Figure 1.

For completeness, two other groups are also included in Figure 2. These are the *nor*-eudesmanoids, biosynthetically degraded eudesmanes [e.g. *nor*-ketoagaro furan (41), chamaecynone (42), and 4-hydroxy-*iso*-chamaecynone (43)], and an example of a rearranged, non-isoprenoid eudesmane [occidol (44)].

1.6 Synthesis of the Eudesmane Sesquiterpenes

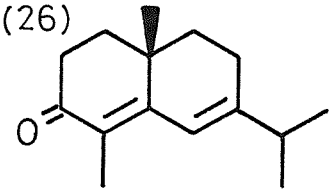
The first synthetic problem for the routes to these structures is the construction of the 2-methyldecalin system common to all eudesmanes. There are two major methods of preparing a bicyclic system, a Robinson ring annelation reaction and a Diels-Alder electrocyclic reaction. The former method is the one used in the majority of the published syntheses. The three carbon side-chain (eventually at C-7) may be present in the starting methyl cyclohexanone, but in some syntheses it has been introduced at a later stage. Stereochemical control is not a major problem with compounds of this class. Many eudesmanes have a *trans*-decalin nucleus, with the three carbon function in an equatorial position, this allows thermodynamic control to be exerted. Those eudesmanes having a *trans*-relationship between the side chain and the angular methyl group have been synthesised via 7-*epi*-cyperone (55), the kinetically formed isomer when dihydrocarvone (53) is condensed with ethyl vinyl ketone (54), or an equivalent molecule such as a 1,3-haloketone or a Mannich base methiodide (Scheme 1)[30,31].

(25)



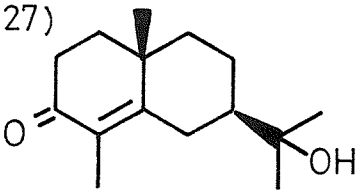
α-CYPERONE

(26)



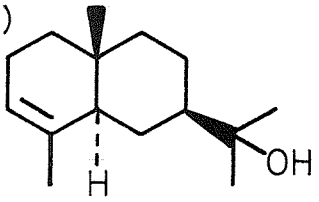
β-CYPERONE

(27)



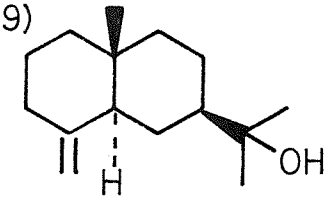
CARRISONE

(28)



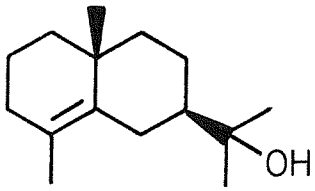
α-EUDESMOL

(29)



β-EUDESMOL

(30)



δ-EUDESMOL

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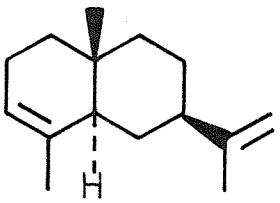
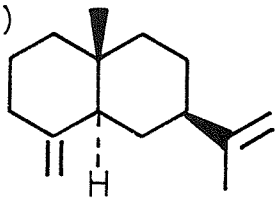


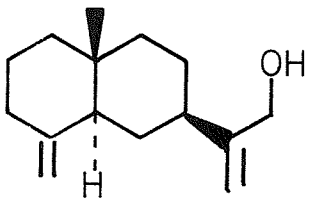
α-SELINENE

(32)



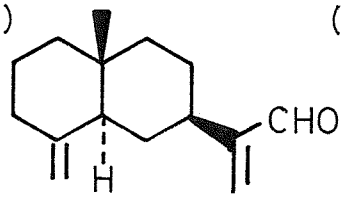
β-SELINENE

(33)



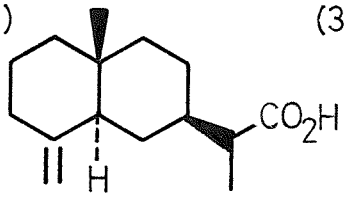
COSTOL

(34)



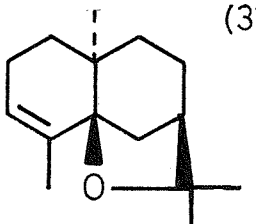
COSTAL

(35)



COSTIC ACID

(36)



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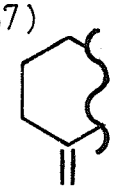
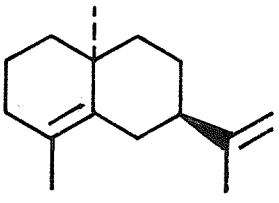


α-AGAROFURAN

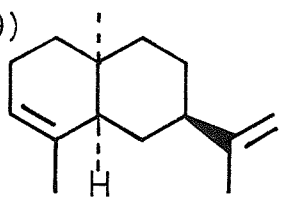
β-AGAROFURAN

(38)



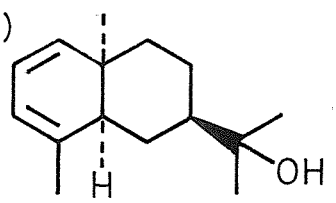
7β,10α-SELINA-4,11-DIENE

(39)



5β,7β,10α-SELINA-3,11-DIENE

(40)



OCCIDENTALOL

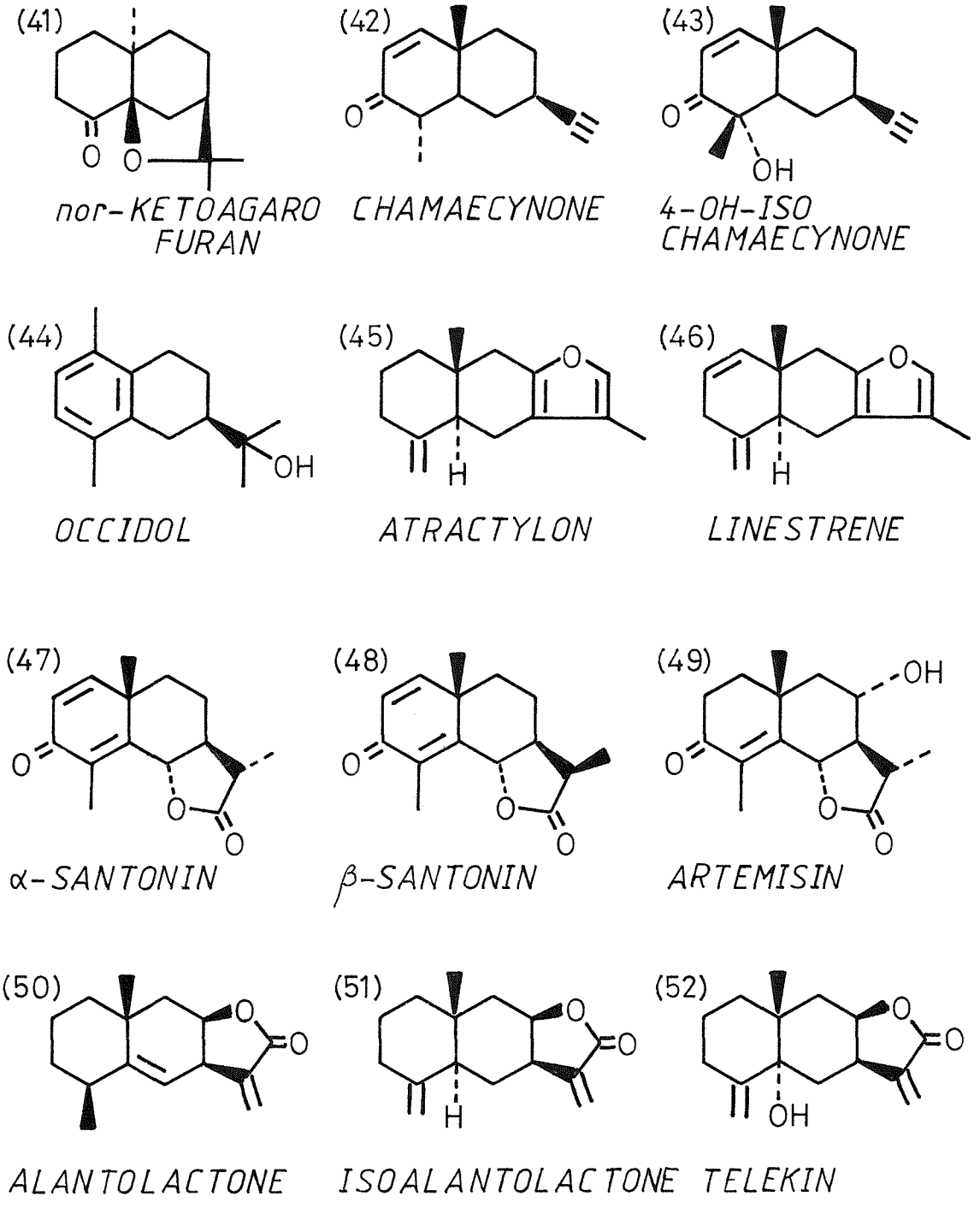
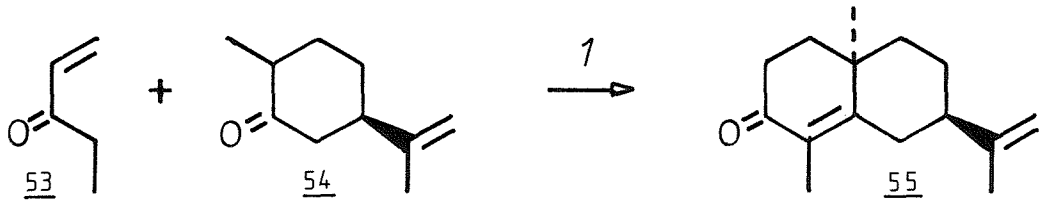


Figure 2: NATURALLY OCCURING EUDESMANES.

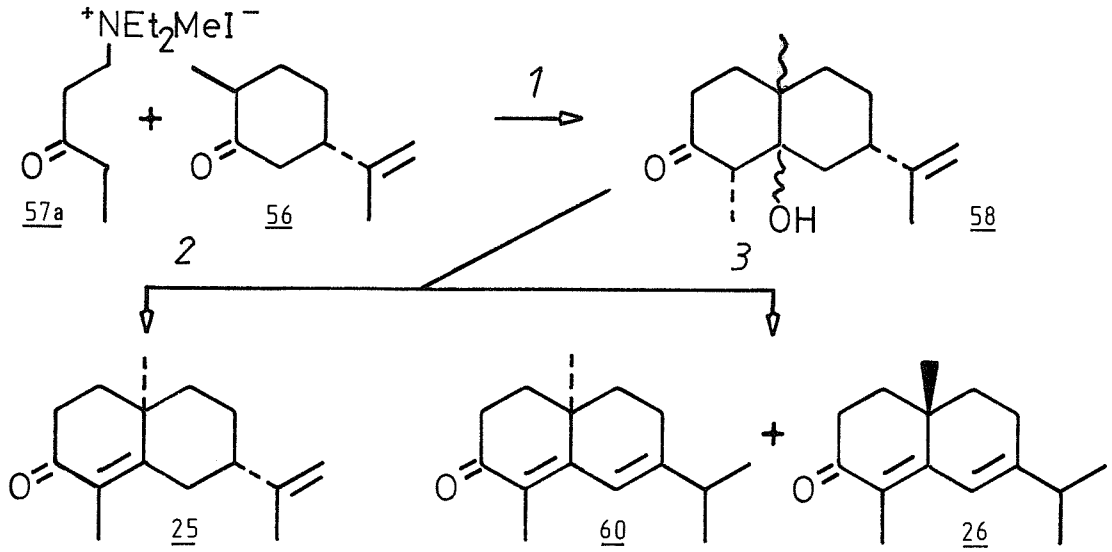


Scheme 1: 1⊢ base

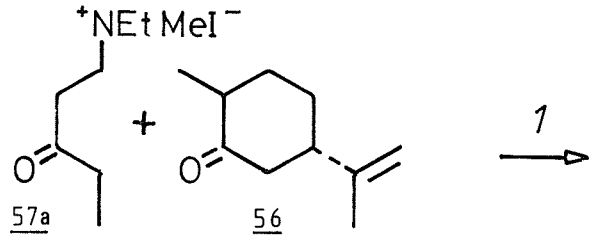
1.6.1 Synthesis of α- and β-Cyperones (25 and 26)

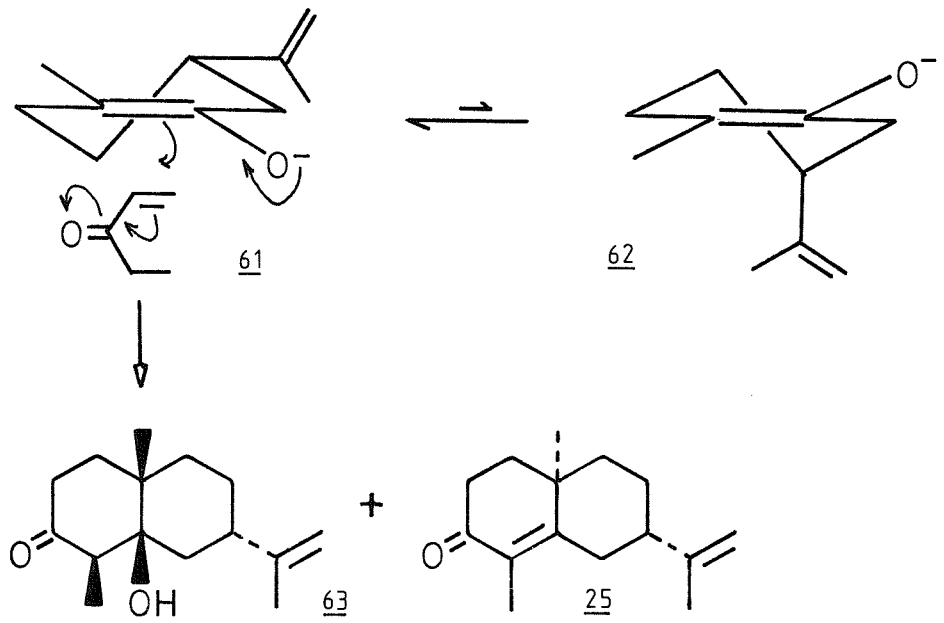
The earliest recorded work on these structures is reported by Adamson, McQuillan, Robinson and Simonsen, in the reported synthesis of α- and β-cyperone [(25) and (26), Scheme 2][32]. Condensation of (-)-dihydrocarvone (56) with 1-diethylaminopentan-3-one methiodide (57a) resulted in the formation of ketol (58) not the uncyclised ketone suggested by the authors. The ketol (58) was then dehydrated with sodium ethoxide in benzene to give α-cyperone (25). However, subsequent investigation of this reaction has shown the product to be a mixture of α-cyperone (25) and its C-10 epimer, *epi*-α-cyperone (55), with the latter predominating. Dehydration of the ketol (58) with 50% sulphuric acid, gave two products (60) and (26) in a 80:20 ratio. In further investigations of this reaction[33], dihydrocarvone (56) was condensed with methiodide (57a) to give a mixture of ketol (63) and α-cyperone [(25), Scheme 3]. The formation of isomer (63), as the major product of this reaction, is the result of axial alkylation of the more stable equatorial isopropenyl enolate anion of ketone (61). This result is not unusual for a Michael addition reaction on dialkylated cyclohexanones. The α-cyperone was purified via its oxime to yield pure material in 3% yield.

β-Cyperone (26) has been synthesised as a racemate from carvenone (64); no yield was reported (Scheme 4)[34].

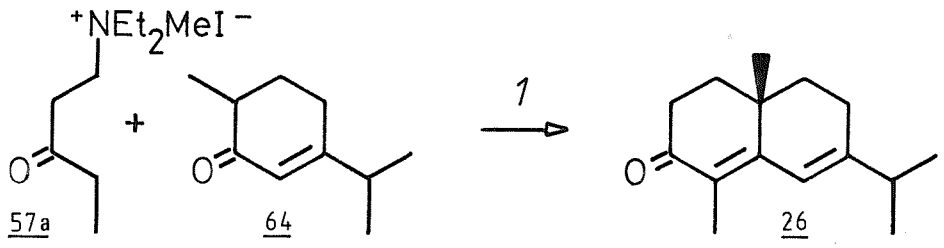


Scheme 2: 1⊢ $NaNH_2$ 2⊢ EtO^- 3⊢ H_2SO_4

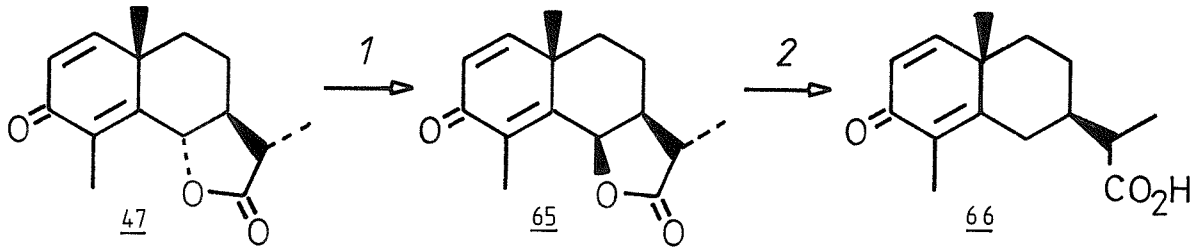


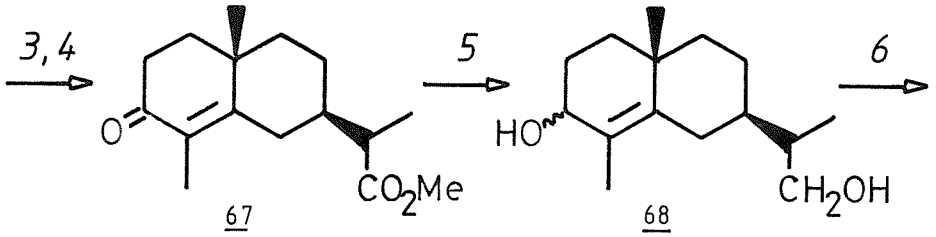


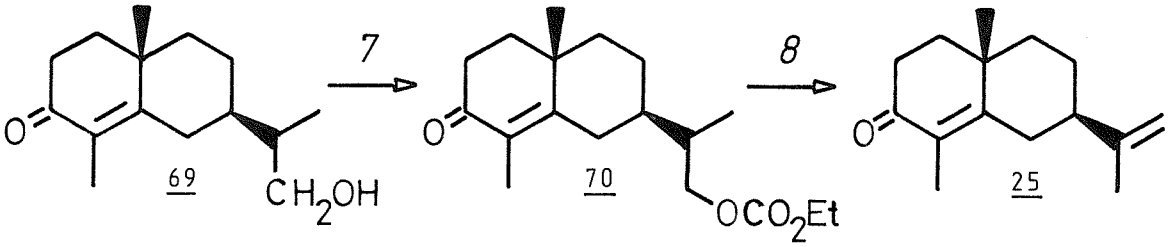
Scheme 3: 1⊢ $NaNH_2 / Et_2O$



Scheme 4: 1 ▻ KOH







Scheme 5: 1 ▻ HCl / DMF 2 ▻ Zn / AcOH 3 ▻ CH_2N_2
4 ▻ H_2 / $(Ph_3P)_3RhCl$ 5 ▻ $LiAlH_4$ 6 ▻ DCDQ
7 ▻ $ClCO_2Et$ / pyr 8 ▻ 400° C

From the examples shown, it can be seen that the yield of annelation reactions is low, and attempts were made to prepare α-cyperone (25) by a relay synthesis from a readily available sesquiterpene precursor. α-Santonin (47) is one such starting material used by Piers (Scheme 5). α-Santonin is epimerised at C-6 to give (-)-6-*epi*-santonin (65). Reduction of the lactone by zinc in acetic acid followed by methylation of the resulting acid and reduction of the disubstituted olefin with Wilkinson's catalyst gave the enone-ester (67). Lithium aluminium hydride reduction gave a mixture of diastereome ric diols (68), which were oxidised by a radical mechanism to give enone-alcohols (69). Esterification to give the carbonate ester (70), followed by the pyrolysis gave α-cyperone (25) and enone-alcohol (69) in 61% and 32% yield respectively.

1.6.2 Synthesis of Carrisone (27)

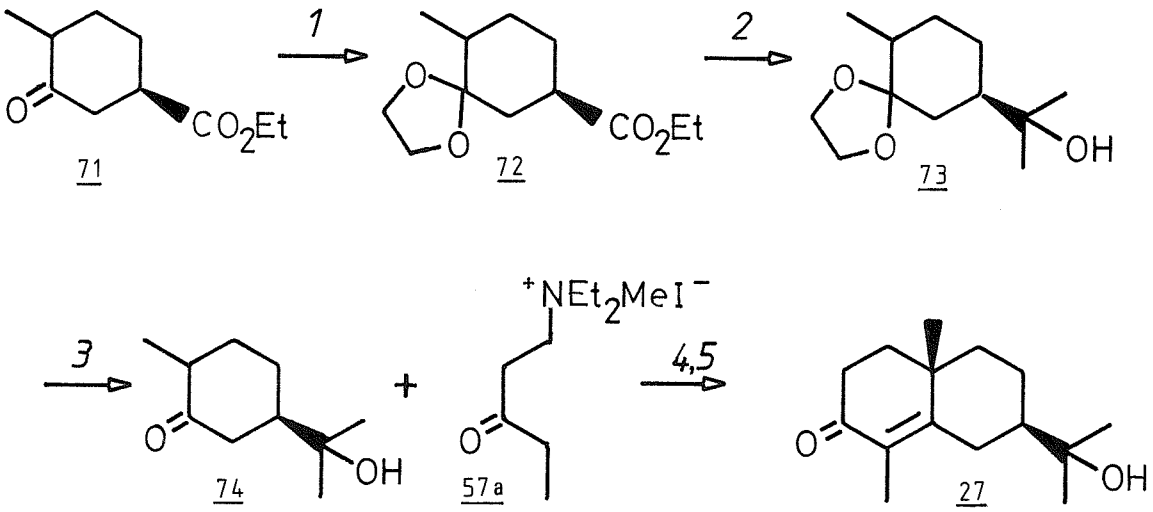
The first synthesis of carrisone (27) was reported by Mukherji *et al* (Scheme 6)[36]. Ketoalcohol (74) was prepared as shown, and then was annelated with the methiodide (57a). The required product (27) was isolated as its 2,4-dinitrophenylhydrazone derivative in an unspecified yield. As before, the C-10 epimer is probably the major product from the annelation reaction.

Carrisone (27) was also prepared by a relay synthesis from α-cyperone (25) by Pinder (Scheme 7)[37,38]. The previously synthesised α-cyperone (25), epoxidation of the *exo*-methylene group and then reduction of both the ketone and epoxide, with lithium aluminium hydride gave diol (76). Oxidation with manganese dioxide gave (+)-carrisone (27) in high overall yield (not specified).

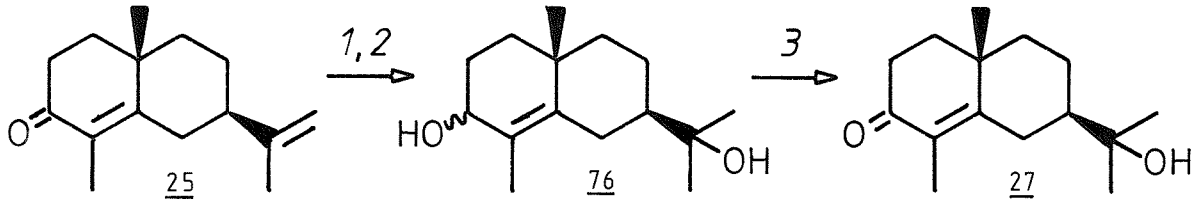
1.6.3 Synthesis of α-,β- and γ-Eudesmols (28, 29 and 30)

A relay synthesis of γ-eudesmol (30) was reported by Pinder in 1963 (Scheme 8)[38]. Their previously prepared (+)-carrisone (27) was converted to the dithioketal (77) and then to (-)-γ-eudesmol (30) by Raney nickel reduction.

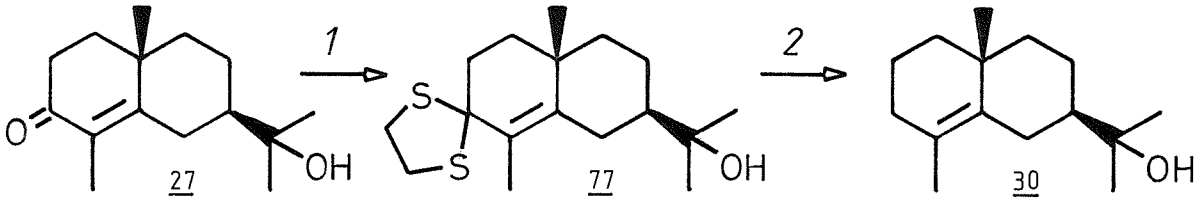
Marshall's synthesis of β-eudesmol (29) from octalone (78) was the first reported method where the side chain at C-7 was introduced during the synthetic route (Scheme 9)[39,40]. Octalone (78) was protected as the ethylene ketal (79), hydration of the double bond followed by oxidation



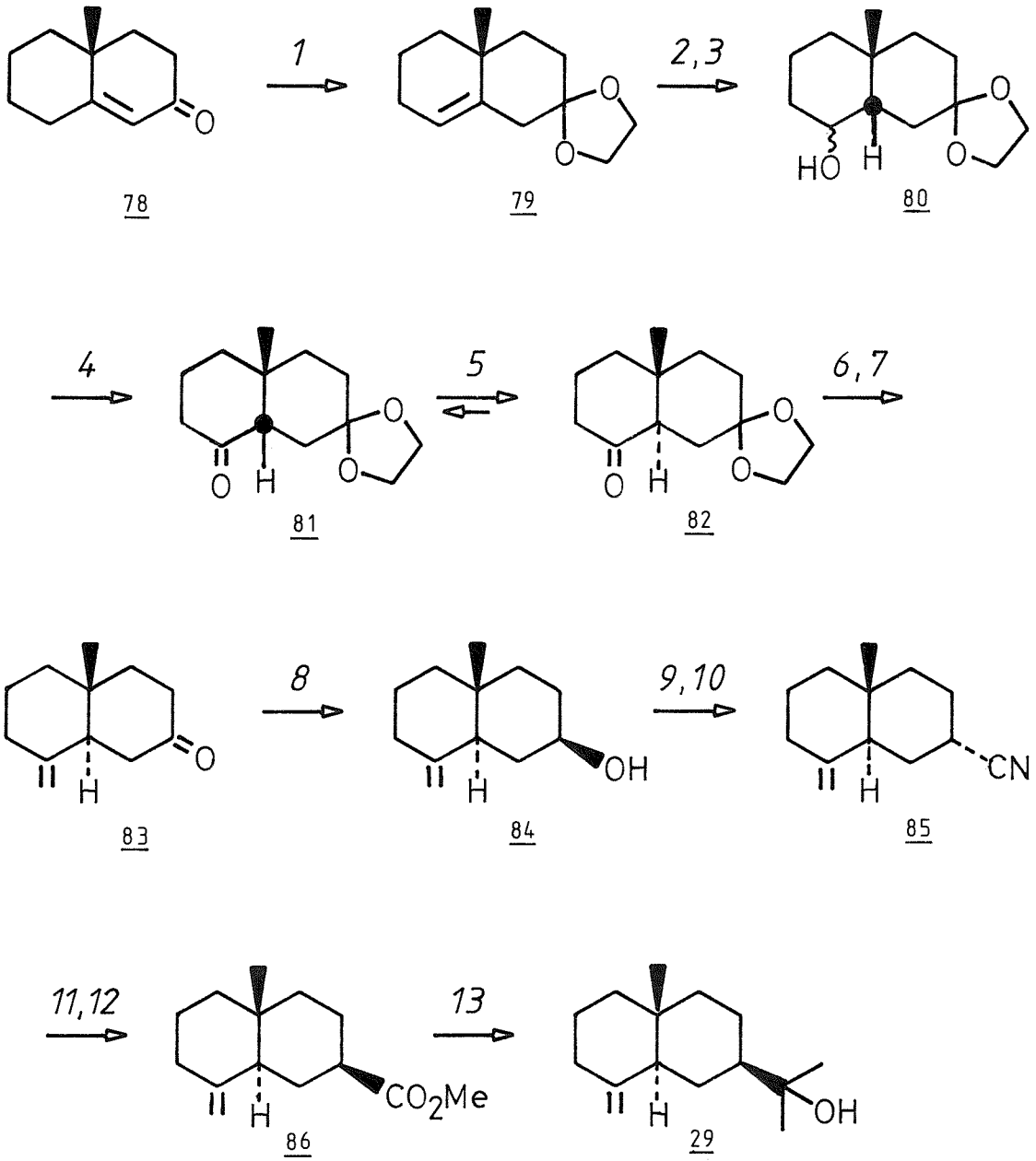
Scheme 6: 1⊢ $(CH_2OH)_2$ / H^+ 2⊢ MeMgI 3⊢ H_3O^+ 4⊢ $NaNH_2$ 5⊢ separate



Scheme 7: 1⊢ MCPBA 2⊢ $LiAlH_4$ 3⊢ MnO_2



Scheme 8: 1⊢ $(CH_2SH)_2$ / BF_3 2⊢ Raney Ni [H_2]



78 79 80 81 82 83 84 85 86 29

Scheme 9: 1▻ $(CH_2OH)_2/H^+$ 2▻ B_2H_6 3▻ H_2O_2 4▻ $H_2Cr_2O_7$ 5▻ pTsOH [3:1 trans:cis] 6▻ $Ph_3P{=}CH_2$ 7▻ H_3O^+ 8▻ $LiAlH_4$ 9▻ pTsOH 10▻ NaCN 11▻ $(CH_2OH)_2$ / 11▻ OH^- / 160°C 12▻ CH_2N_2 13▻ MeLi

afforded ketone (81). Equilibration to the more stable *trans*- ring junction was now possible. A Wittig reaction gave the required *exo*-methylene group at C-4. Manipulation of the protected ketone (83), via an alcohol (84), a tosylate and a cyanide (85), followed by hydrolysis and methylation gave the ester-olefin (86). The reaction of methyl lithium on this olefin yielded β-eudesmol (29).

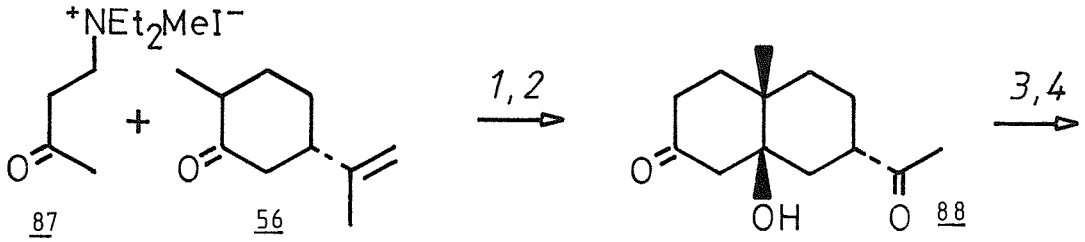
Pinder's synthesis of β-eudesmol (29), used the more usual annelation reaction (Scheme 10)[41]. (-)-Dihydrocarvone (56) was condensed with 1-diethylaminobutan-3-one methiodide (87), which gave the more favourable intermediate ketal; ozonolysis then gave the ketoalcohol (88). This has a *trans* relationship between the side chain and the angular methyl group. Equilibration allowed the side chain to take the preferred equatorial position, and lead to dehydration. Protection of the more reactive αβ-unsaturated ketone gave intermediate (89). A Grignard reaction gave the isopropanol side chain, and then the dithioketal was removed with Raney nickel to yield *nor*-γ-eudesmol (91). Hydration of the double bond gave a diastereomeric mixture of diols (92), which were oxidised and equilibrated with acidic alumina to the more stable *trans* ring junction. The final stage was a Wittig reaction yielding β-eudesmol (29).

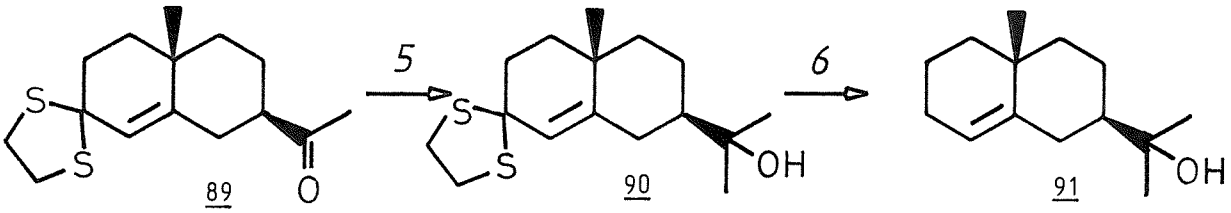
Later, work on β- and γ-eudesmol syntheses were modifications of previous routes already mentioned. Heathcock's[42] and Vig's[43] syntheses of γ-eudesmol are two examples. γ-Eudesmol has also been prepared by Marshall.[44]

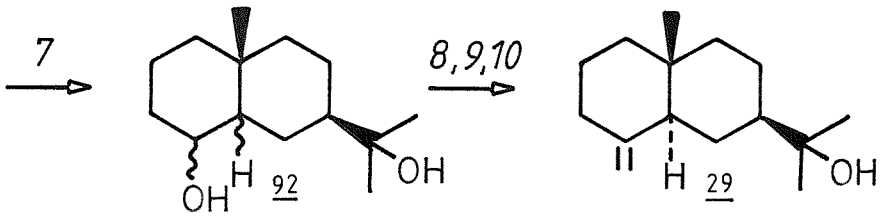
There is only one reported synthesis of the other double bond isomer, α-eudesmol [(28), Scheme 11][45]. It involves another relay synthesis from carrisone (27). Birch reduction of carrisone to the *trans* ring junction ketoalcohol (93), followed by a Bamford-Stevens reaction yielded α-eudesmol (28).

1.6.4 Synthesis of α- and β-Agarofurans (36 and 37)

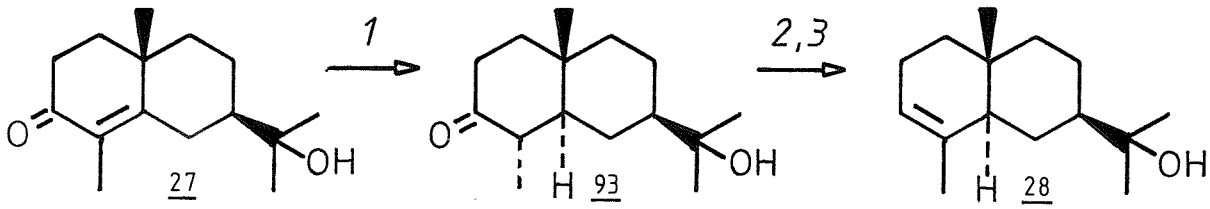
In the synthesis of the eudesmane structures so far, a *trans* ring junction and a *cis* relationship between the C-7 side chain and the angular methyl group have been common features. The agarofurans have a *trans* relationship between the side chain and the angular methyl group, so these compounds are ideally made by the annelation reaction. Consideration



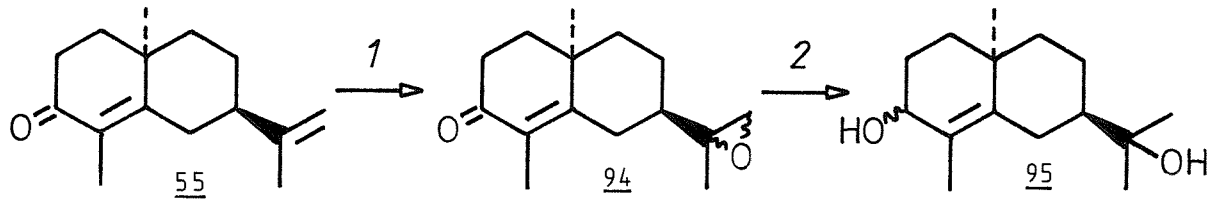


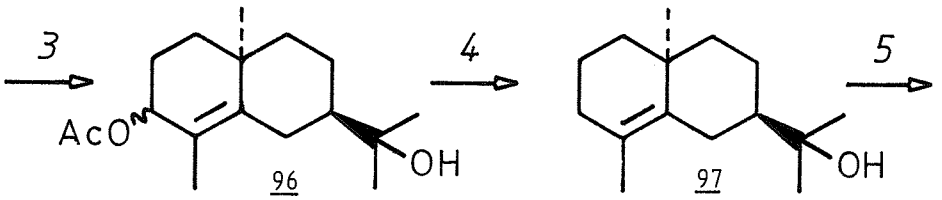


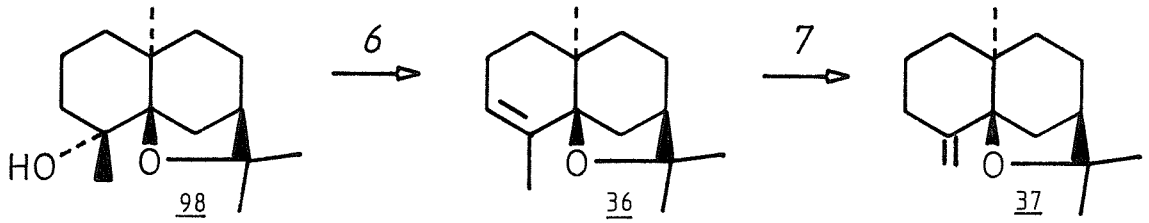
Scheme 10: 1⊳ $DMSO^-$ 2⊳ O_3 3⊳ HCl / EtOH 4⊳ $(CH_2SH)_2$ / BF_3 5⊳ MeMgI 6⊳ Raney Ni 7⊳ B_2H_6, H_2O_2 8⊳ $H_2Cr_2O_7$ 9⊳ Al_2O_3 10⊳ $Ph_3P{=}CH_2$



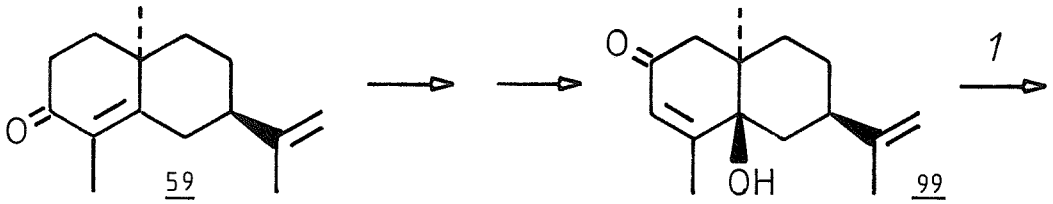
Scheme 11: 1⊳ Li / NH_3 2⊳ $pTsNHNH_2$ / HCl 3⊳ CH_2ONa | CH_2OH

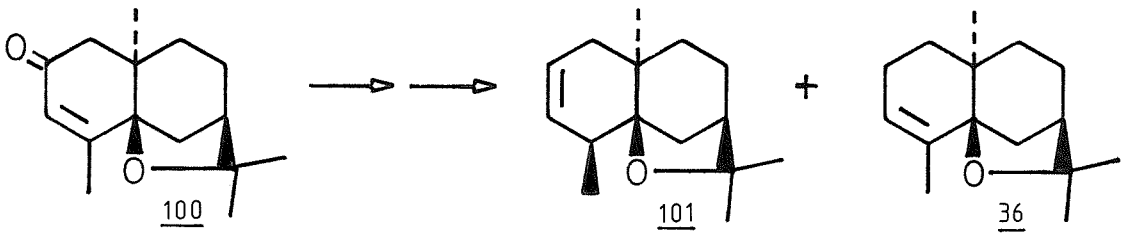






Scheme 12: 1⊳ MCPBA 2⊳ $LiAlH_4$ 3⊳ Ac_2O 4⊳ Li/NH_3 5⊳ MCPBA 6⊳ $SOCl_2/pyr$ 7⊳ $h\nu$ / iPrOH

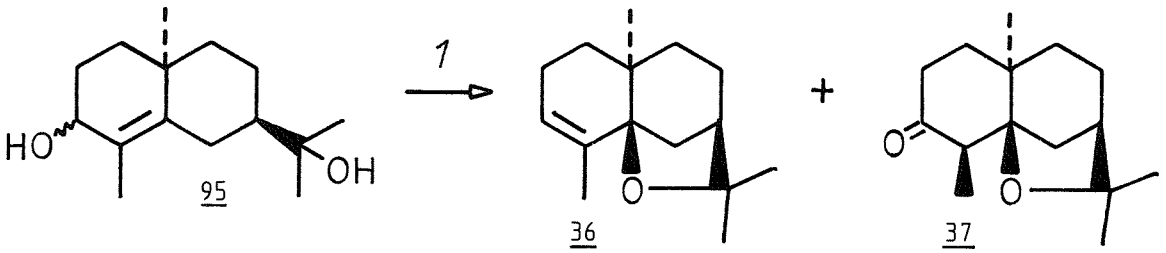




Scheme 13: 1⊳ Al_2O_3

of Scheme 11 shows 7-*epi*-α-cyperone (55) to be a good starting material. Marshall and Pike reported a synthesis that afforded both agarofurans from the same route (Scheme 12)[46]. Epoxidation of the *exo*-methylene group of *epi*-α-cyperone (55) followed by lithium aluminium hydride reduction gave diol (95). Acetylation of the secondary alcohol followed by Birch reduction, cleaved the acetate to yield 10-*epi*-γ-eudesmol (97). Epoxidation lead to cyclisation on work up (98), and dehydration with thionyl chloride in pyridine yielded α-agarofuran (36). This was then photochemically isomerised to β-agarofuran (37). A lower yield, longer synthesis was published by Barrett and Buchi[47], the key step of which is shown in Scheme 13. The cyclisation was carried out with acidic alumina. It was noted that this reaction may be of significance when applied to other ether formations.

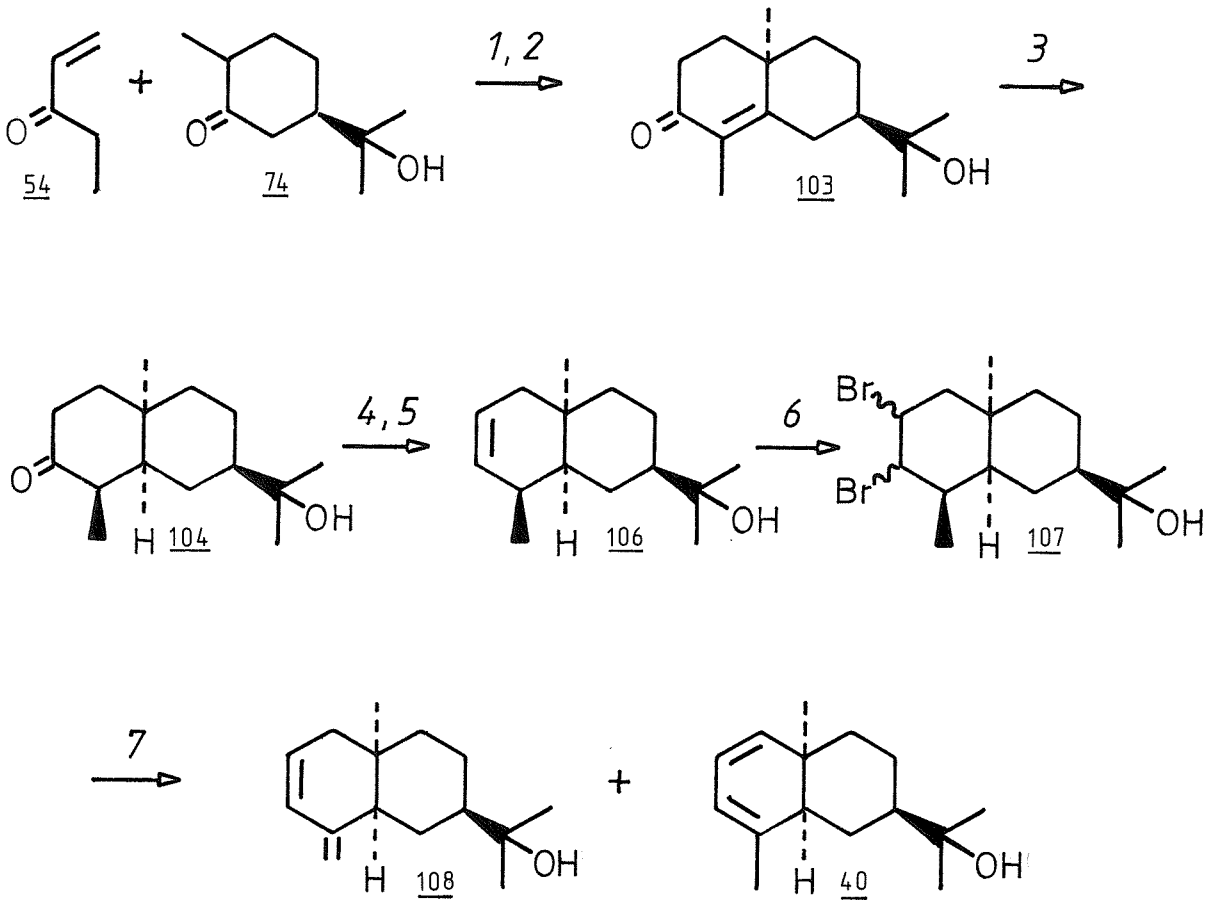
One final synthesis of α-agarofuran showed another interesting ring closure (Scheme 14)[48]. The readily available ketol (95) was reacted with p-toluenesulphonic acid to yield to cyclised products, α-agarofuran [(36) in 80% yield] and a by product [(102) in 20% yield].



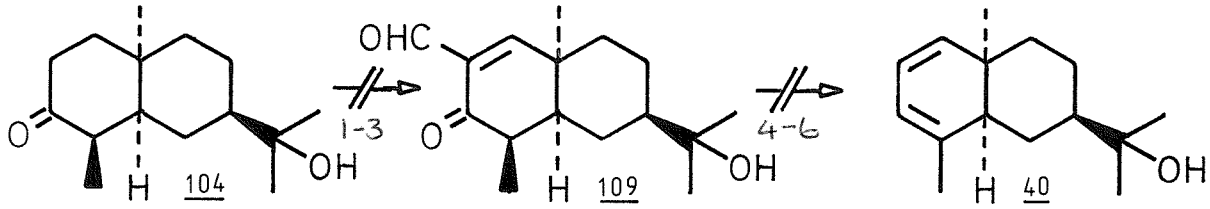
Scheme 14: 1⊢ TsOH

1.6.5 Synthesis of Occidentalol (40)

Occidentalol (40), found occurring naturally in the eastern white cedar *Thuja occidentalis*[49], has been synthesised by a number of different methods. The first synthesis was reported by Heathcock (Scheme 15)[50]. (+)-Dihydrocarvone was oxymercurated to give dihydrocarvone hydrate (74). Condensation with ethyl vinyl ketone (54) yielded a ketol which was then dehydrated with methanolic hydrochloric acid to give *epi*-carrisone (103).



Scheme 15: 1⊳ $NaNH_2$ 2⊳ HCl / MeOH 3⊳ H_2 / Pd on C
4⊳ $pTsNHNH_2$ 5⊳ MeLi 6⊳ Br_2 7⊳  / heat



Scheme 16: 1⊳ HCO_2Et / NaH 2⊳ Br_2 3⊳ LiCl / Li_2CO_3
4⊳ $(Ph_3P)_3RhCl$ 5⊳ $LiAlH_4$ 6⊳ pTsOH

Hydrogenation over palladium on charcoal in acetic acid gave a 3:1 mixture of *cis:trans* ring junctioned dihydro-*epi*-carrisones, which were separated to yield pure *cis*-dihydro-*epi*-carrisone (104). Reaction using Shapiro's modification of the Bamford-Stevens reaction gave olefin (106). Bromination to the dibromide (107) followed by dehydrobromination gave a mixture of occidentalol (40) and an isomer (108).

Subsequent work (Scheme 16)[51] has converted *cis*-dihydro-*epi*-carrisone (104) to the αβ-unsaturated aldehyde (109) by formylation, bromination and dehydrobromination. Occidentalol (40) was then obtained by heterogeneous hydrogenation, reduction with lithium aluminium hydride and elimination of the corresponding tosylate. Another synthesis via a Diels-Alder reaction is presented in the next section.

1.6.6 The Eudesmane Skeleton via a Diels-Alder Reaction

Recent work has shown that the Diels-Alder reaction can be used to give the eudesmane skeleton. A general example is shown in Figure 3.

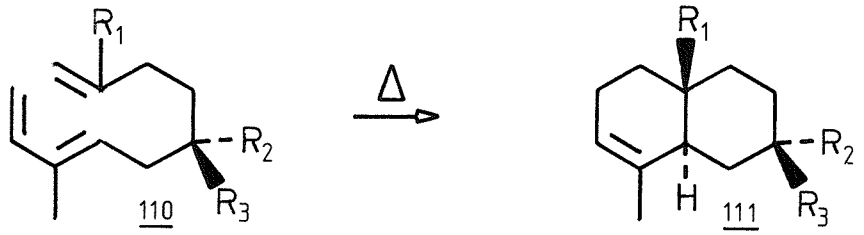
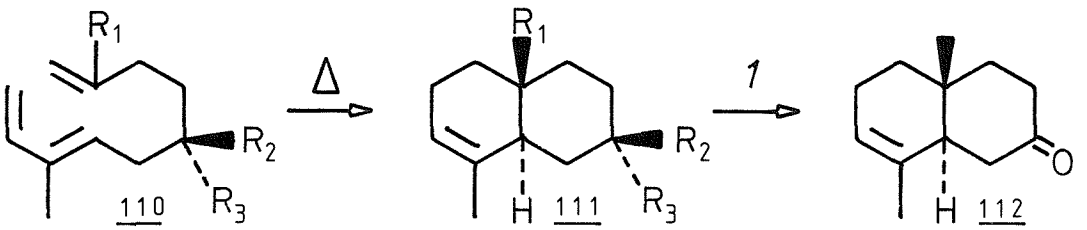


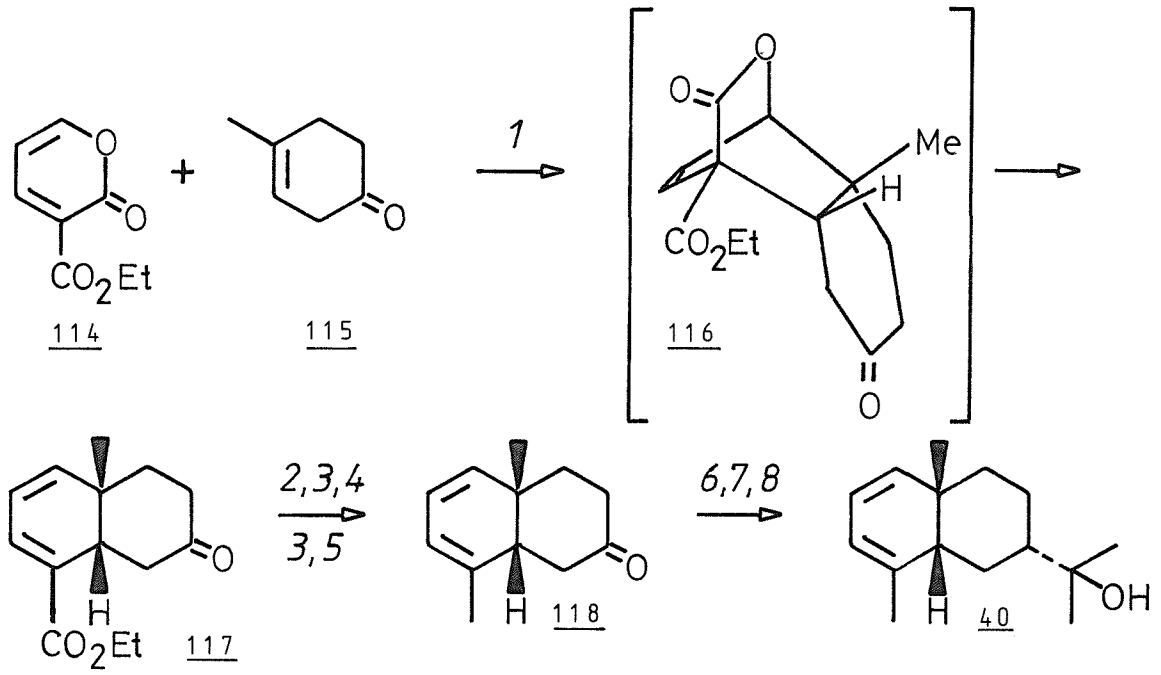
Figure 3.

Ideally, to obtain a eudesmane skeleton, R_1 = methyl, R_2 = isopropyl and R_3 = hydrogen. Wilson has used this reaction to synthesise selina-3,7(11)-diene [(113), Scheme 17][52]. The preparation of the intermediate trienes (110 a-e) are described in preceding papers[53,54]. The trienes were heated at 160-200°C in toluene and the products were isolated with 100% *trans* ring junction configuration, in varying yield depending on the substituents (R_1, R_2 and R_3). The selinene (113) was prepared by heating (110e) at 200° in toluene. Two ethers were produced and these were hydrolysed on work up to afford a mixture of alcohols (111e). Oxidation yielded keto-olefin (112) which was subsequently converted to selina-4,7(11)-diene



	R_1	R_2	R_3	Temp.	R_1	R_2	R_3	%
a	H	H	H	160°C	H	H	H	95%
b	H	H	OH	160°C	H	H	OH	24%
					H	OH	H	33%
c	Me	H	H	190°C	Me	H	H	30%
d	Me	H	OH	190°C	decomposed			—
e	Me	H	OTMS	200°C	Me	H	OTMS	34%
					Me	OTMS	H	54%

Scheme 17: *1⊳ oxidise 2⊳ Wittig*



Scheme 18: *1⊳ heat 2⊳ $(CH_2OH)_2$ / H^+ 3⊳ $LiAlH_4$ 4⊳ pyr. SO_3 5⊳ H^+ 6⊳ diethyl-1-(methylthio)-ethyl phosphonate 7⊳ $HgCl_2$ 8⊳ MeLi*

(113) in high yield. A review of electrocyclic reactions that yield decalin systems has been published by Oppolzer[55].

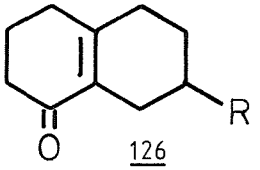
Another example of using a Diels-Alder reaction to obtain the eudesmane skeleton has been used in the synthesis of occidentalol [(40), Scheme 18][56]. The key step being the formation of the decalin system by a Diels-Alder reaction followed by a retro Diels-Alder reaction.

1.6.7. Other Ring Closures Used in the Synthesis of Eudesmanes

Elemol (119) has been used as a starting material in the synthesis of cryptomeridial (122)[57]. The diene system of elemol (119) was reacted in a mercury (II) catalysed ring closure to give a decalin system (Scheme 19), which on borohydride work up yielded cryptomeridial (122) in good yield.

Occidentalol (40) has been synthesised by a long route using an acid catalysed ring closure. The interesting reaction of this synthesis is shown in Scheme 20[58].

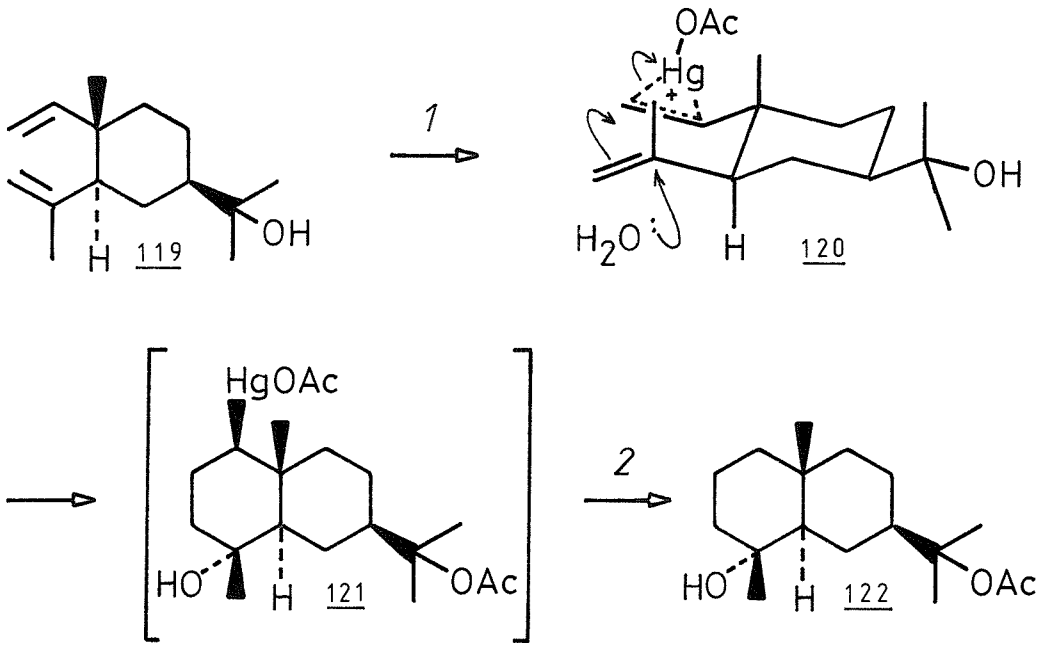
Finally, several authors[59-62] have published routes to eudesmanes using a lithium cuprate reaction on a system similar to that shown below:



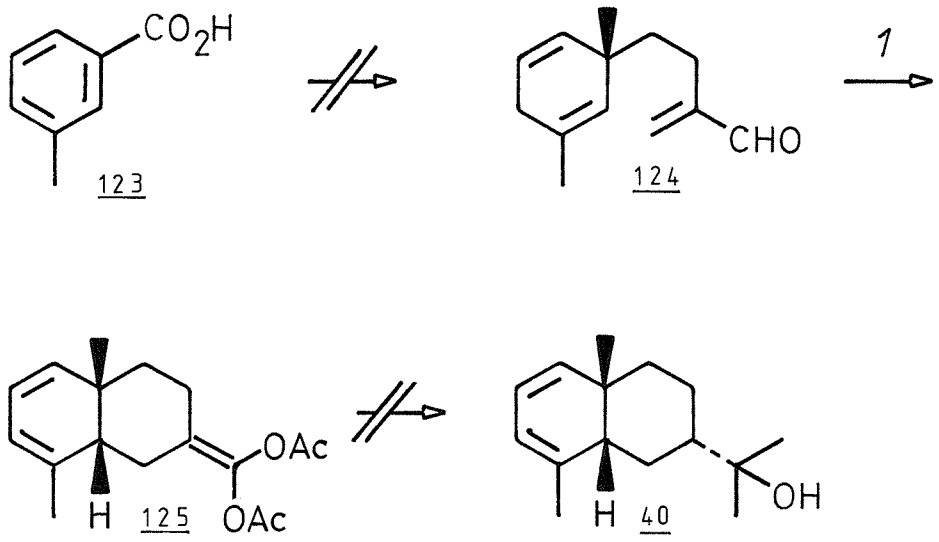
126

The advantage of this type of synthetic intermediate is the ease of their preparation. However, a major disadvantage is the lack of specificity of the addition. It is unlikely that optically pure molecules could be prepared in this way.

Further syntheses of eudesmane sesquiterpenes may be found in books by ApSimon[29] and Nakanishi[63].



Scheme 19: 1⊢ $Hg(OAc)_2$ /aq.THF 2⊢ $NaBH_4$



Scheme 20: 1⊢ Ac_2O / $HClO_4$

1.6.8. Synthesis of 4,11-Epoxy-*cis*-Eudesmane (21)

Optically active 4,11-epoxy-*cis*-eudesmane (21) has been previously synthesised in small quantities. This was due to problems associated with the separation of (21) from a byproduct also formed in the ultimate stage of the reaction sequence. 4,11-Epoxy-*cis*-eudesmane (21) has been found to be repellent to some species of ants and larger quantities of material would allow further, more vigorous testing for repellency and toxicity. Other synthetic schemes aimed at the preparation of larger quantities of (21) were considered and these are discussed in Chapter 2.

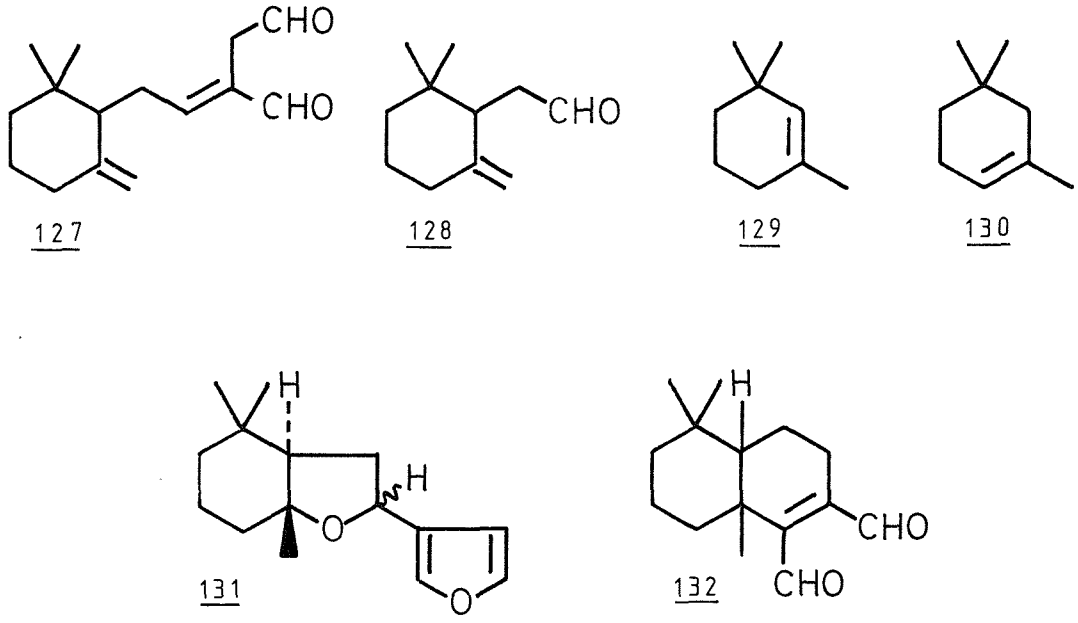
1.7 Defence in *Ancistrotermes cavithorax*

Ancistrotermes spp. have a well developed form of chemical defence as well as a physical defence capability. The secretion is contained in a gland that extends from the mouthparts to the abdomen and is secreted when the termites are disturbed.

Ancistrotermes cavithorax Sjostedt (Isoptera, Termitidae, Macrotermitinae) is common to parts of West Africa. It is interesting that predation of this species is less than expected for Macrotermitinae species and it has been suggested the reason is the chemical method of defence employed by these termites[64]. The nests of *A. cavithorax* are defended by two castes of soldiers, the *major* and the *minor* soldiers, and samples of each have been collected at Mokwa (in Nigeria). The defence secretions of both castes have been analysed by Briner[65] and subsequently published[66].

The *minor* soldiers were found to secrete a dialdehyde ($\sim$ 2 μg per soldier), and this compound constituted about 90% of the volatile components of the extract. This dialdehyde was named ancistrodial (127) and identified as the structure in Figure 5. The geometry of the double bond has been verified as (E) by total synthesis of both the (E) and (Z) isomers from (±)-γ-cyclohomocitral (128), followed by comparison with the natural product[65,66]. The absolute configuration at the chiral centre is unknown at present.

The *major* soldiers, however, were found to secrete a mixture of compounds. The major components were a furan, named ancistrofuran [(131), 50 μg per soldier] and a dialdehyde [(132), 30 μg per soldier]. The minor



127 128 129 130

131 132

Figure 5.

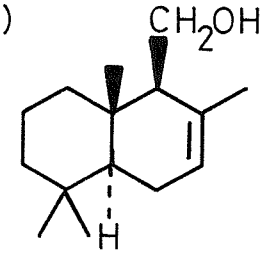
components found were toluene, and a mixture of α- and β-cyclogeraniolenes, (129) and (130) respectively.

The structure of the cyclogeraniolenes was established by comparison of spectral details with prepared samples. The structure of ancistrofuran (131) has been proved by total synthesis[65], but the absolute configuration has not been assigned. The dialdehyde structure herein named cavidial (132) has not been synthesised.

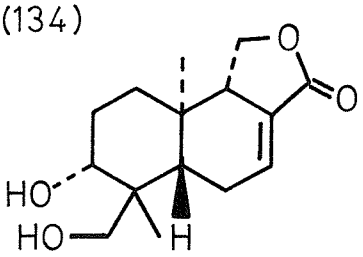
Preparation of cavidial (132) was undertaken to check the assigned structure and so synthetic routes to the drimane sesquiterpenes were explored.

1.8. Previous Synthetic Studies on Drimanes

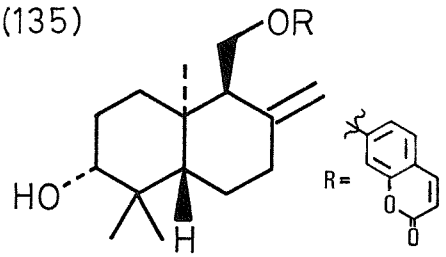
If the assigned structure of cavidial (132) is correct, it is a novel member of the drimane sesquiterpenes. The general structure (Figure 6) is common to all drimanes and nearly all the known members of this group have been synthesised (Figure 7). The natural sources of all the drimanes are listed in Table 1.

(133) 

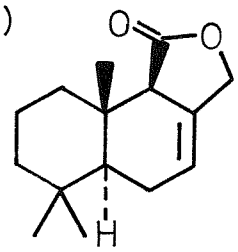
DRIMENOL

(134) 

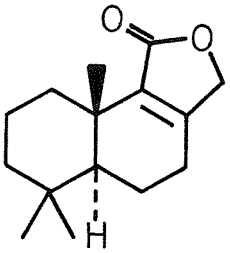
IRESIN

(135) 

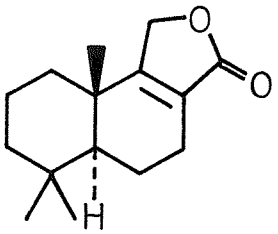
FARNESIFEROL

(136) 

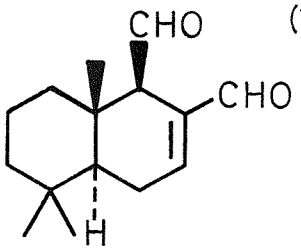
DRIMENIN

(137) 

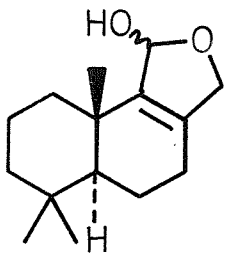
ISODRIMENIN

(138) 

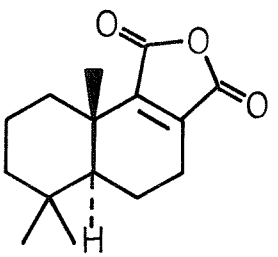
CONFERTIFOLIN

(139) 

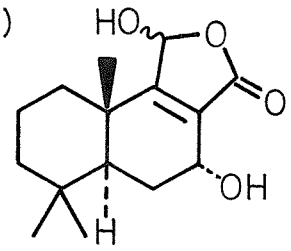
POLYGODIAL

(140) 

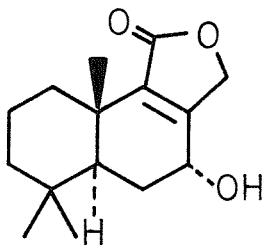
VALDIVOLIDE

(141) 

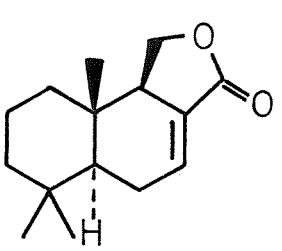
WINTERIN

(142) 

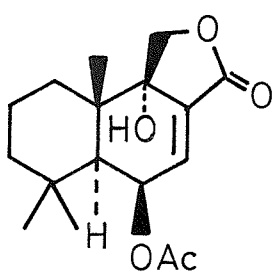
FUGEIN

(143) 

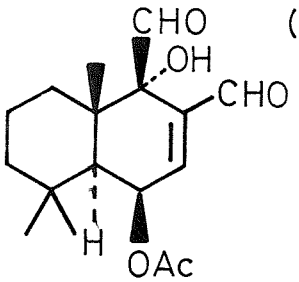
FUTRONOLIDE

(144) 

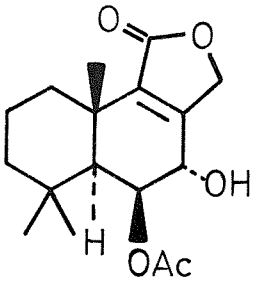
CINNAMOLIDE

(145) 

CINNAMOSOLIDE

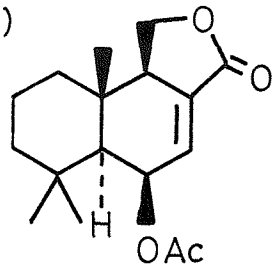
(146) 

CINNAMODIAL

(147) 

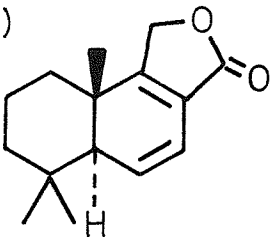
UGANDENSOLIDE

(148)



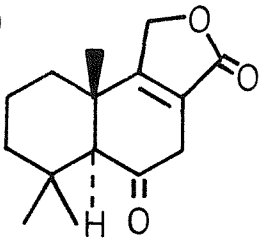
BEMAVRIVOLIDE

(149)



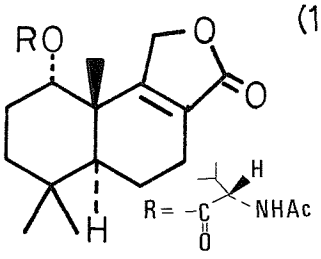
BEMADIENOLIDE

(150)



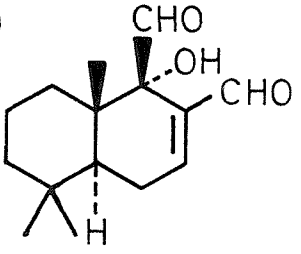
FRAGROLIDE

(151)



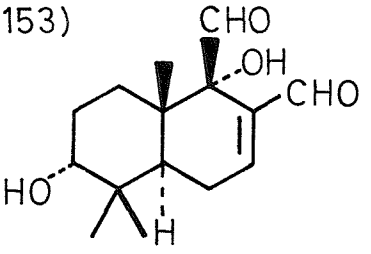
PURPURIDE

(152)



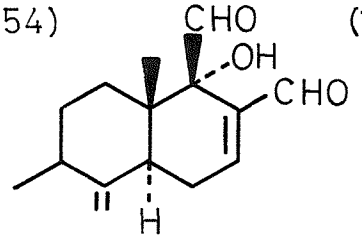
WARBURGANAL

(153)



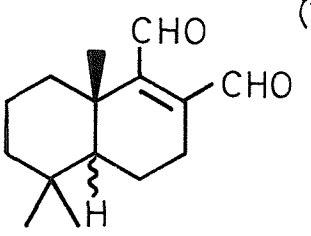
3β-OH-WARBURGANAL

(154)



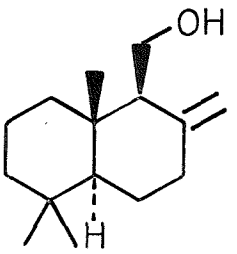
MUZIGADIAL

(155)



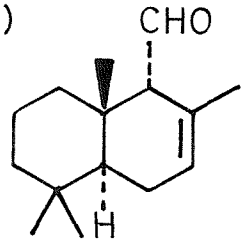
CAVIDIAL

(156)



ALBICANOL

(157)



ISOTADEONAL

Figure 7: NATURALLY OCCURING DRIMANES.

TABLE 1

No	Name	From	References Occurrence	References Synthesis
133	Drimenol	*Drimys winteri* *Bazzaria trilobata*	67, 68, 69	86, 87, 88 100
134	Iresin	*Drimys winteri*	70, 71, 72	-
135	Farnisiferol	*Drimys winteri*	73, 74	90
136	Drimenin	*Drimys winteri*	67, 75	91, 92, 103, 105
137	Isodrimenin	*Drimys winteri*	67, 75	91, 92 100, 102
138	Confertifolin	*Drimys winteri* *Drimys confertifolia*	67, 75	100, 102
139[a]	Polygodial[a]	*Polygonium hydropiper* *Drimys lanceolata*	72, 76	97, 106
140	Valdivolide	*Drimys winteri*	67, 72	102
141	Winterin	*Drimys winteri*	67	101, 102 103
142	Fugein	*Drimys winteri*	67	
143	Futronolide	*Drimys winteri*	67, 77	77
144	Cinnamolide	*Cinnamosma fragrans*	78, 79	95, 97
145	Cinnamosolide	*Cinnamosma fragrans*	78, 79	-

TABLE 1 (Continued)

No	Name	From	References Occurrence	References Synthesis
146	Cinnamodial[b]	*Cinnamosma fragrams* *Warburgia ugandensis* *Diplophyllum albicans*	78, 79, 80	-
147	Ugandensolide	*Warburgia ugandensis*	79	-
148	Bemaviolide	*Cinnamosma fragrans*	81	-
149	Bemadienolide	*Cinnamosma fragrans*	81	82, 102
150	Fragrolide	*Cinnamosma fragrans* *Warburgia ugandensis*	79, 81	102
151	Purpuride	*Penicillium purporogenum*	82, 83	-
152	Warburganal	*Warburgia ugandensis*	80	98, 106 112, 113
153	3β-OH-Warburganal	*Warburgia ugandensis* *Warburgia stuhlmanii*	84	-
154	Muzigadial	*Warburgia ugandensis*	84	-
155	Cavidial	*Ancistrotermes cavithorax*	65	-
156	Albicanol	*Diphophyllum albicans*	80	-
157	Isotadeonal	*Polygonium hydropiper*	72	92, 93

[a]Also known as Tadeonal

[b]Also known as Ugandesidial

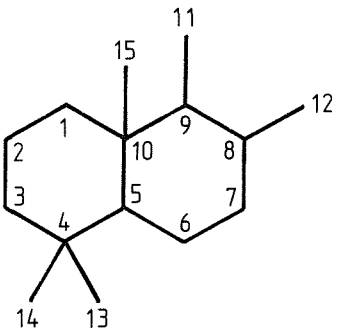


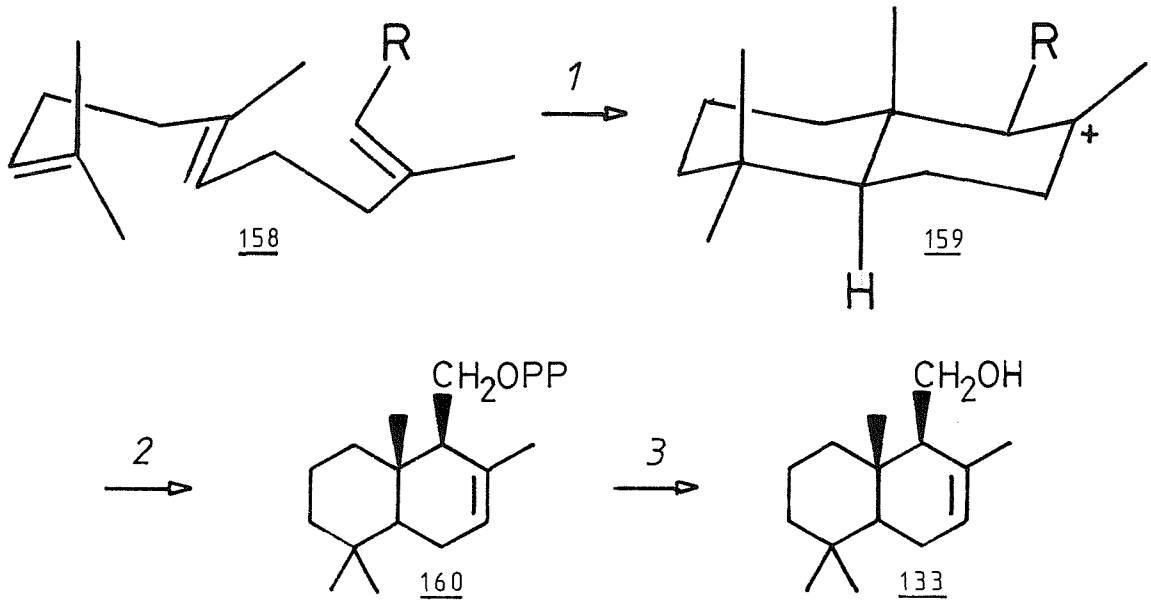
Figure 6.

The drimane sesquiterpenes have a bicyclofarnesol skeleton with a substitution pattern similar to many di- and tri-terpenes. The biosynthesis of bicyclofarnesols has been studied thoroughly from farnesol pyrophosphate [(158), Scheme 21]; the biogenesis of farnesol pyrophosphate is discussed in Chapter 2. Cyclisation of farnesol pyrophosphate under acid catalysed conditions gave a bicyclic carbonium ion (159); elimination to olefin (160) followed by hydrolysis led to drimenol (133), the first member of the naturally occurring drimanes[68]. The drimane series all have *trans* ring junctions and this is implied by the suggested mechanism, but it is interesting that irisin (134) and farnesiferol (135) have the opposite *trans* configuration at the ring junction.

Drimane sesquiterpenes have been synthesised in a number of ways, but the original preparations imitated the biosynthetic pathway.

1.8.1. Synthesis of Drimanes via Biosynthetic Type Cyclisations

The original work was completed by Caliezi and Schinz in 1949[85]. Cyclisation of farnesic acid (161) with formic acid - sulphuric acid gave a mixture of two acids (Scheme 22). One acid was isolated as a crystalline solid in 10% yield, and was subsequently found to be bicyclofarnesic acid (162)[86]. The other product was also isolated, but was later shown to be a mixture. Reduction of bicyclofarnesic acid (162) with lithium aluminium hydride gave a racemic alcohol, later shown to be (±)-drimenol (133)[86,87]. One of the components in the by product mixture was found to be the epimeric acid (163), the reduction of which gave the epimeric alcohol, (±)-epidrimenol (164). Later work by Stork and

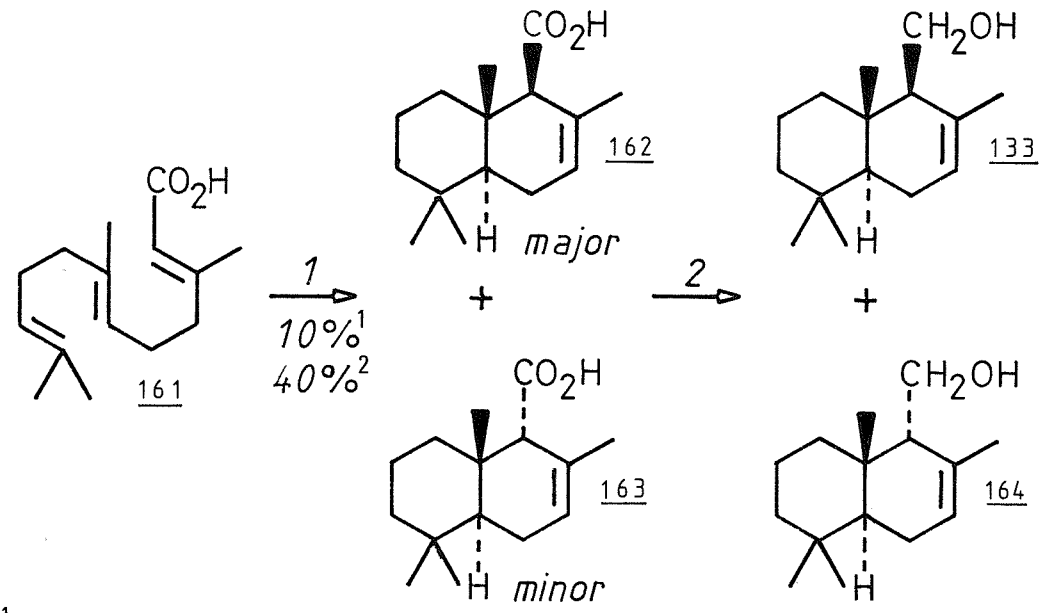


Scheme 21: *1⊢ acid catalysed cyclisation 2⊢ elimination 3⊢ hydrolysis PP = pyrophosphate*

Burgstahler[87] used a similar cyclisation reaction using boron trifluoride etherate (Scheme 23). This reaction gave a higher yield (40%) of the bicyclic acid (162), again reduction yielded (±)-drimenol (133).

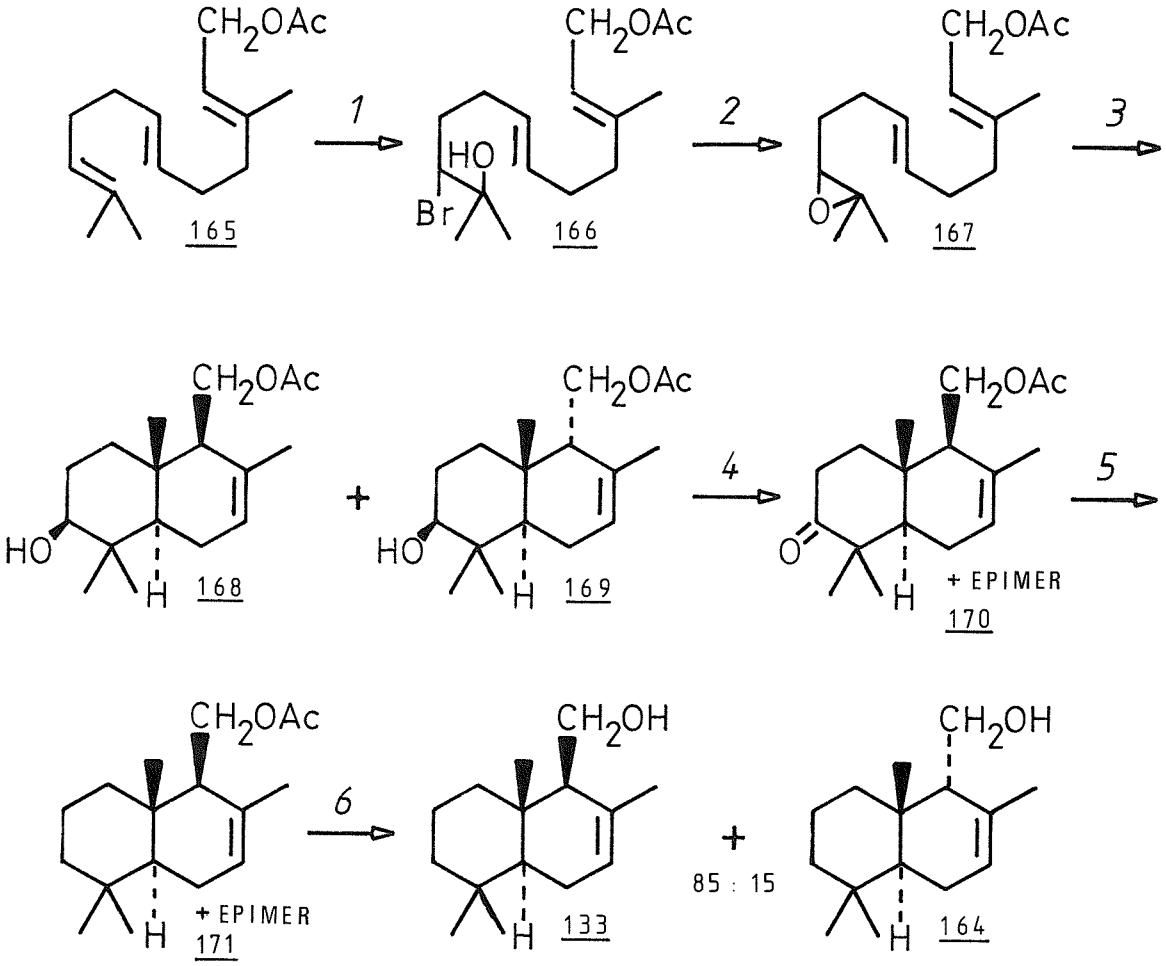
Van Tamelen *et al* were examining the cyclisations of polyenes (Scheme 24)[88]. Farnesyl acetate (165) was brominated selectively at the tertiary centre to give the bromohydrin (166). This was eliminated with base to give epoxide (167), cyclisation with boron trifluoride gave a mixture of two acetate alcohols, (168) and (169) in an 85:15 mixture, in "modest" yield. These were converted to (±)-drimenol (133) and (±)-epidrimenol (164). Cyclisation of methyl farnesate (172) was achieved with N-bromo-succinimide (NBS)[89] but the cyclic bromides (173) and (174) were only obtained in "minute" yield. Reduction gave a mixture of (±)-drimenol (133) and epimer [(164), Scheme 25].

Figure 7 shows two structures which have a reverse ring geometry, irisin (134) and farnesiferol (135). (±)-Farnesiferol (135) was synthesised by van Tamelen *et al* by a similar route to the one used to prepare drimenol [(133), Scheme 26][90]. Condensation of *cis,trans-*



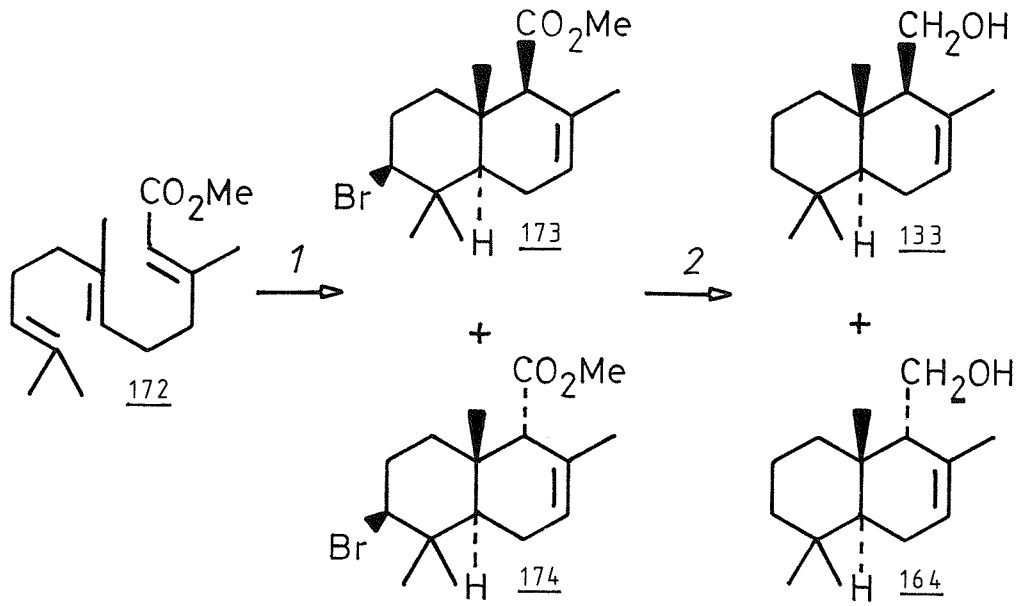
[1] **Scheme 22:** 1⊳ HCO_2H / H_2SO_4, 30°C 2⊳ $LiAlH_4$

[2] **Scheme 23:** 1⊳ $BF_3.Et_2O$ 2⊳ (i) CH_2N_2 (ii) $LiAlH_4$

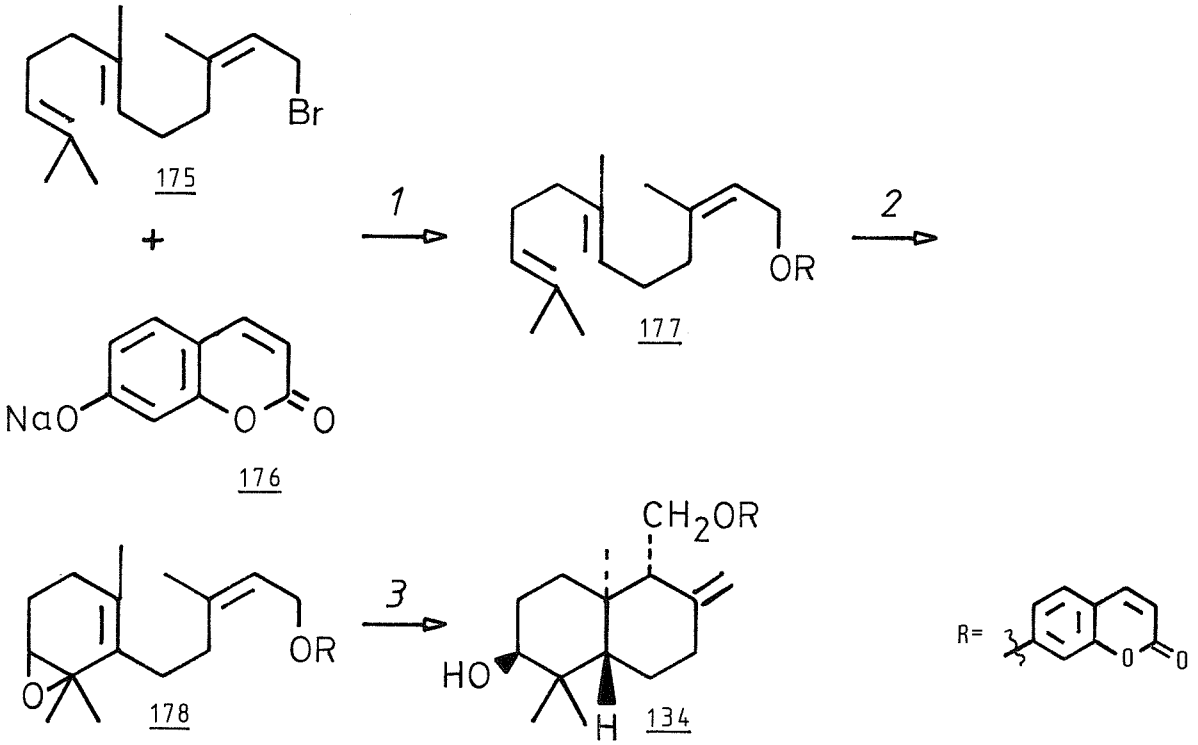


Scheme 24: 1⊳ NBS/H_2O 2⊳ OH^- 3⊳ $BF_3.Et_2O$/PhH

4⊳ oxidise 5⊳ (i) $(CH_2SH)_2$ / $BF_3.Et_2O$ (ii) Ni 6⊳ $LiAlH_4$



Scheme 25: 1⊳ *NBS / aq. THF* 2⊳ $LiAlH_4$



Scheme 26: 1⊳ *DMF* 2⊳ *i* *NBS / H_2O*
ii K_2CO_3 */ MeOH* 3⊳ $BF_3.Et_2O$ */ PhH*

farnesyl bromide (175) with the sodium salt of umbelliferone (176) yielded *cis,trans*-umbelliprenin (177). Treatment of the epoxide (178), prepared via the bromohydrin, with boron trifluoride etherate afforded a mixture containing 2% (±)-farnesiferol (134).

Another example of a biosynthetic cyclisation was carried out by Kitahara *et al* (Scheme 27)[91,92]. The bicyclic system was prepared by consecutive cyclisations. The first step had been completed previously by Stork and Burgstahler[88], Kitahara completed the second cyclisation using stannic chloride to give bicyclofarnesic acid (162) in an increased yield of 55% (Scheme 28). After esterification to (180), photooxidation followed by decomposition of the intermediate hydroperoxides with potassium iodide, afforded mixture of three esters, (181), (182) and (183). Separation of the required ester (183) followed by acid hydrolysis, allowed rapid lactonisation to the required products, a mixture of (±)-drimenin (136) and (±)-isodrimenin (137). These two compounds had been previously found in the hexane extract of the bark of a South American tree, *Drimys winteri*[75], co-occuring with drimenol (133).

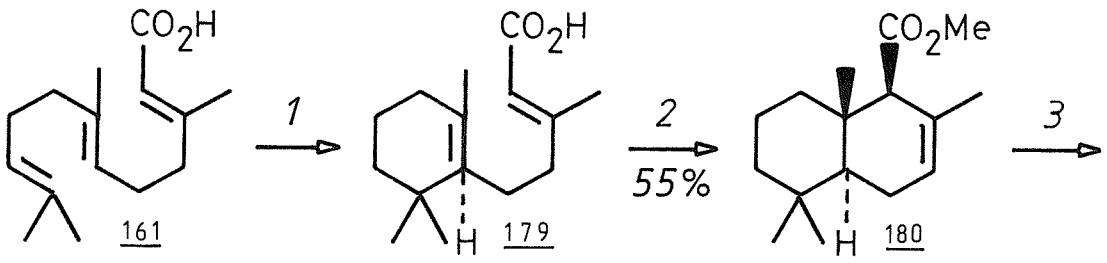
Studies on the preparation of iresin have been reported[93,94], but a total synthesis has not been completed.

Drimenol (133), *epi*-drimenol (164), drimenin (136) and isodrimenin (137) were then used as precursors to other naturally occurring drimanes.

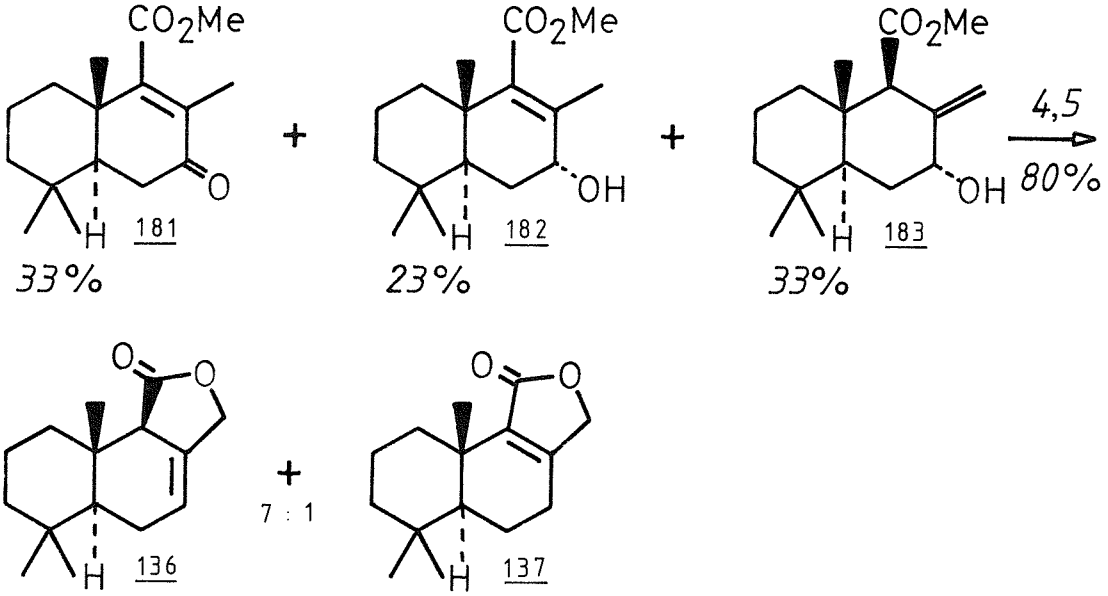
1.8.2. Relay Syntheses of Some Other Drimanes

Cinnamolide (144), a lactone isolated by some Italian workers[78], was synthesised using a relay synthesis by Kitahara *et al* (Scheme 29)[95,96]. Drimenin (136) was reduced with lithium aluminium hydride to intermediate diol (184), oxidation with manganese dioxide then yielded cinnamolide (144). This was later converted to bemadienolide (149)[82].

Polygodial (139), isolated from an extract of the tree *Polygonium hydropiper*[76], was prepared from cinnamolide [(144), Scheme 30][97]. The lactone ring was hydrolysed and the resulting acid methylated to give the alcohol-ester (185). Oxidation to an aldehyde followed by protection yielded the dimethyl acetal (186). The ester was reduced to an alcohol, oxidation to an aldehyde gave the monoprotected dialdehyde (187), which on deprotection gave the dialdehyde, polygodial (139).

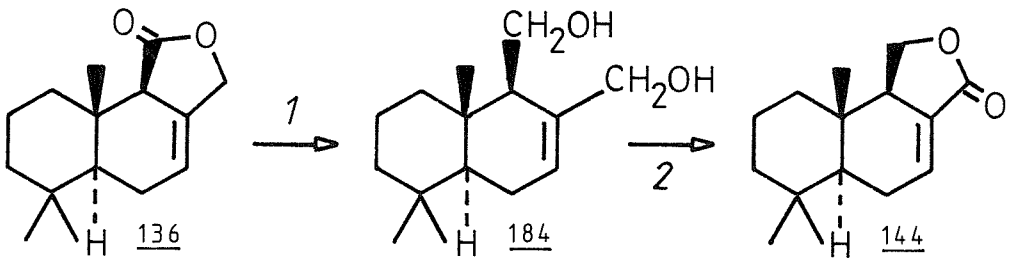


Scheme 27.

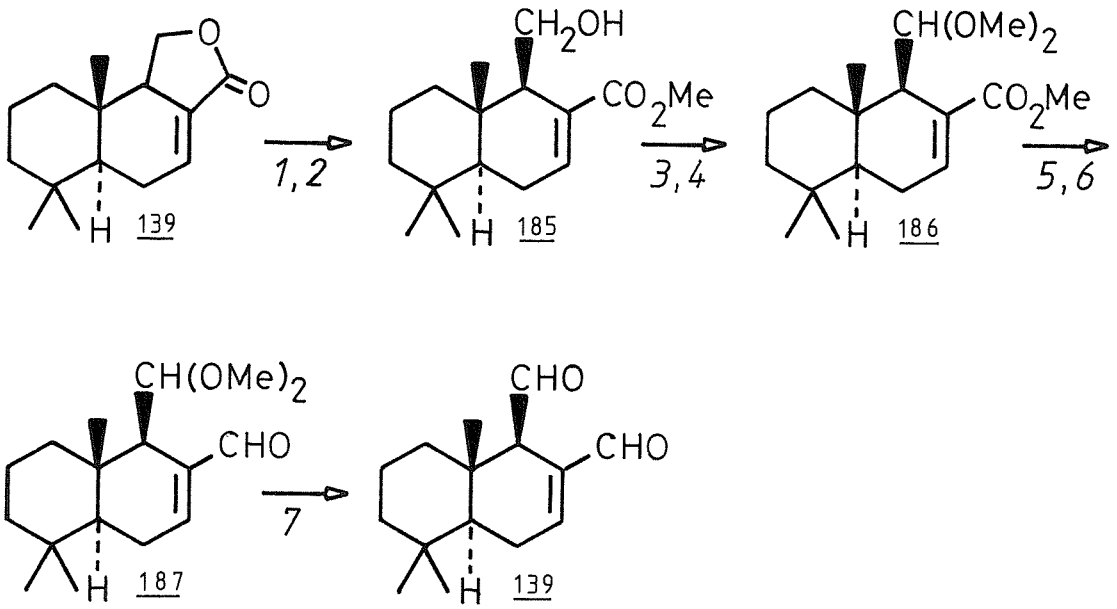


Scheme 28: 1⊳ $BF_3.Et_2O$ 2⊳ (i) $SnCl_4$ (ii) esterify

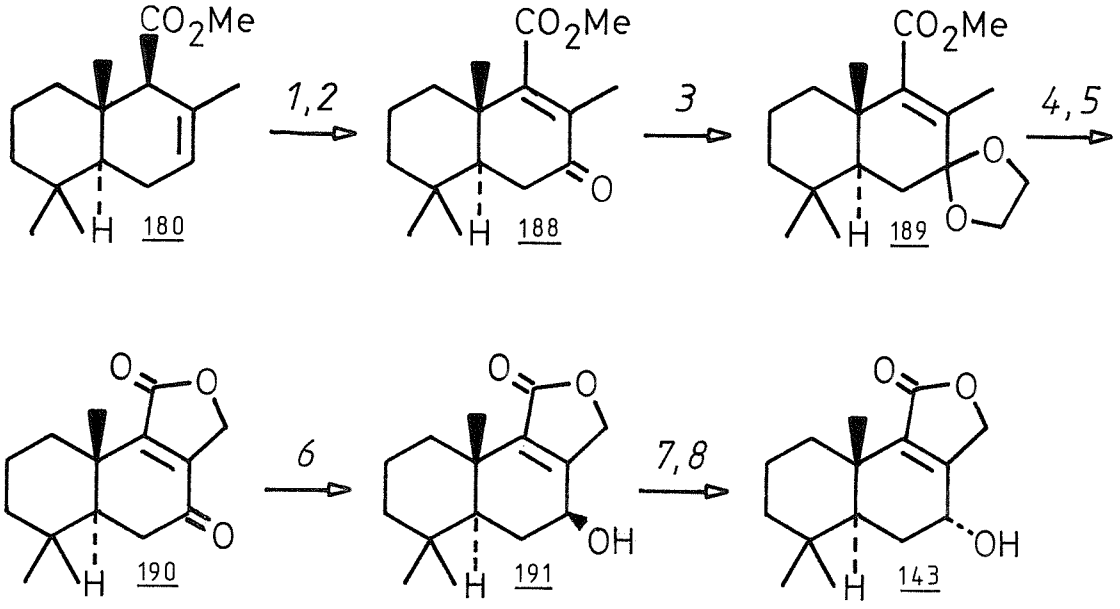
3 ⊳ O_2/hv/sensitizer 4⊳ KI 5⊳ separate 6⊳ H_2SO_4/H_2O



Scheme 29: 1⊳ $LiAlH_4$ 2⊳ MnO_2



Scheme 30: 1⊢ KOH/MeOH 2⊢ CH_2N_2 3⊢ CrO_3.2pyr 4⊢ $CH(OMe)_3$ 5⊢ $LiAlH_4$ 6⊢ MnO_2 7⊢ $(CO_2H)_2$ /aq.acetone



Scheme 31: 1⊢ hν / O_2/sensitizer 2⊢ CrO_3. 2pyr 3⊢ $(CH_2OH)_2$ / H^+ 4⊢ NBS 5⊢ H_2O 6⊢ $NaBH_4$ 7⊢ PBr_3 8⊢ H_2O

Another example, also reported by Kitahara, was the synthesis of futronolide [(143), Scheme 31][77]. Methyl bicyclofarnesate (180) previously prepared in the synthesis of drimenol (Scheme 28), was used as the starting material. Photooxidation of (180) followed by quenching yielded an alcohol which was oxidised to the αβ-unsaturated ketone (188), and protected as ketal (189). Allylic oxidation of the methyl group, via a bromide, and hydrolysis afforded lactone (190). Borohydride reduction gave an alcohol (191) which was epimerised to the required stereochemistry (via a bromide) to futronolide (143).

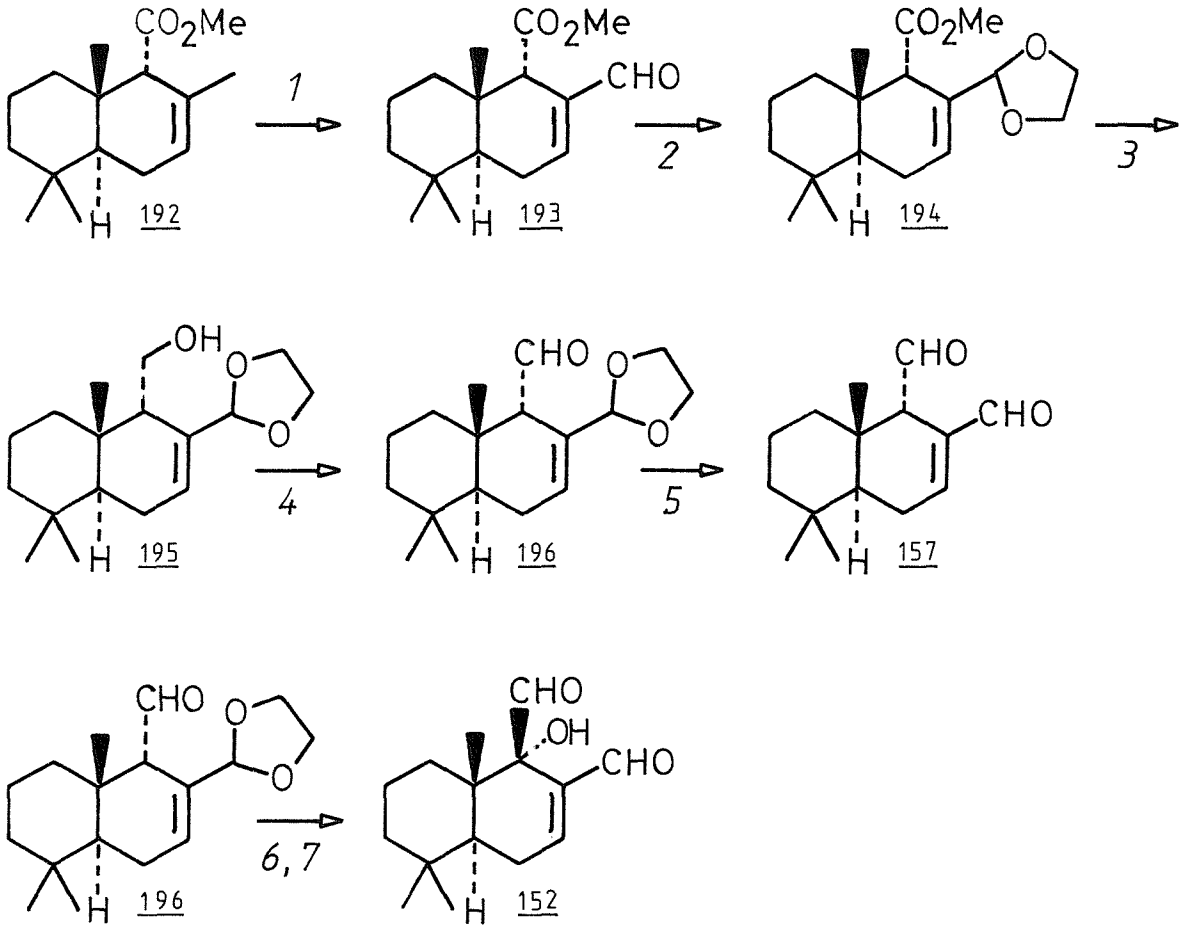
The most recently published biosynthetic type route was reported by Ohsuka and Matsukawa[98]. They synthesised (±)-warburganal (157) and isotadeonal (152) from methyl-(±)-9-epidrimate [(192), Scheme 32]. Allylic oxidation of the starting material (192) with selenium dioxide gave aldehyde ester (193). Protection as the acetal (194) followed by lithium aluminium hydride reduction and Collins oxidation gave the mono protected aldehyde (196). Acid hydrolysis of the acetal yielded the dialdehyde, (±)-isotadeonal (157). (±)-Warburganal (152) was obtained by the oxidation procedure of Vedejs *et al*[99] on the monoprotected acetal (196).

1.8.3. Drimane Structures by Degradative Procedures

As stated earlier, drimane sesquiterpenes have structures which are similar to some diterpenes. These diterpenes have been degraded, sometimes by lengthy procedures, to give naturally occurring drimanes.

One such diterpene, available in large quantities, was dehydroabietane (197). It can be degraded to drimic acid [(99), Scheme 33], and this can be used to prepare drimane sesquiterpenes.

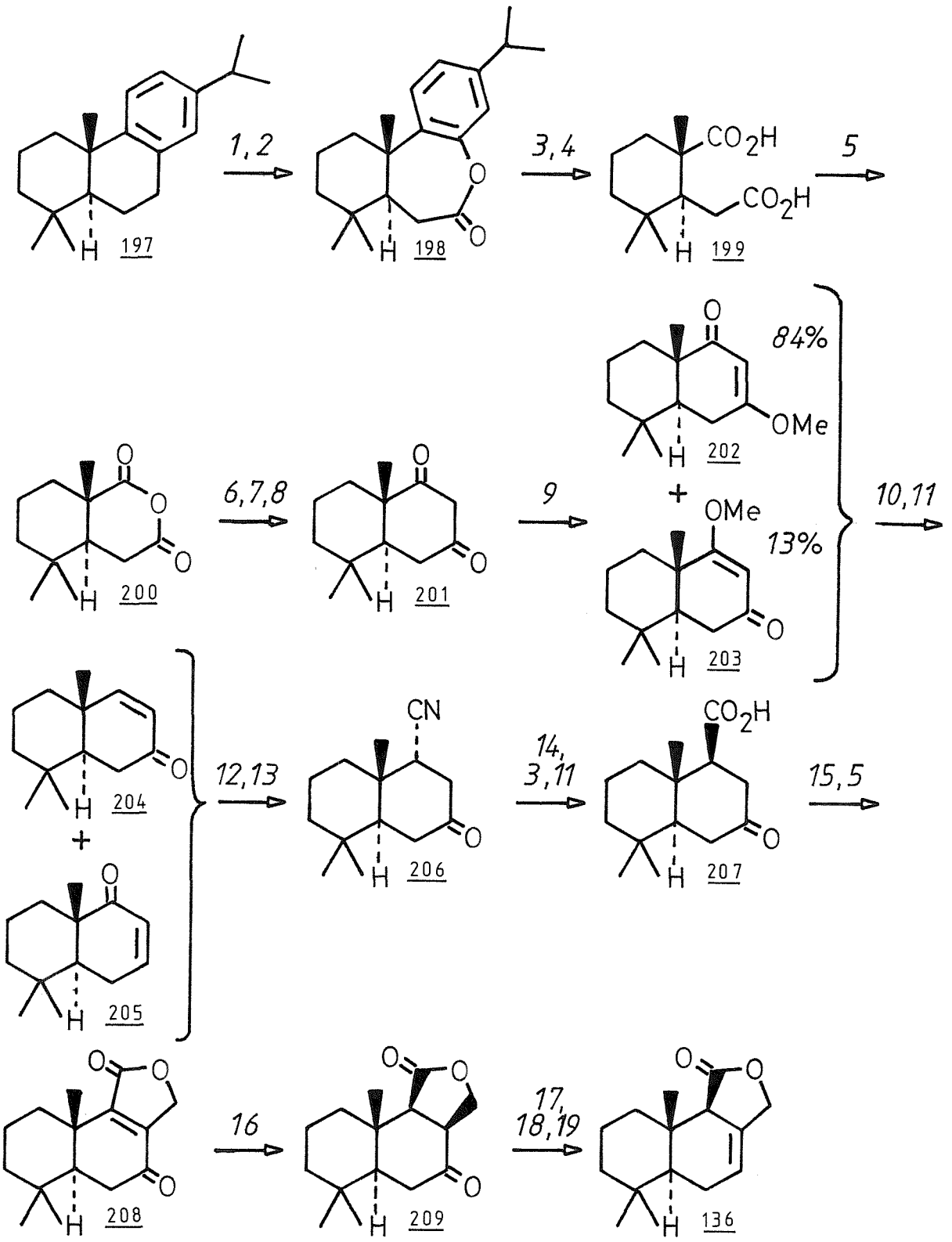
The idea of degradative synthesis was first reported by Wenkert in the synthesis of drimenin [(136), Scheme 33][100]. Benzylic oxidation of dehydroabietane (197) yielded a ketone, which on oxidation under Baeyer-Villiger conditions afforded lactone (198). Hydrolysis of the lactone, followed by ozonolysis yielded a diacid, drimic acid (199). Drimic anhydride (200), was obtained by dehydration, and this was converted to diketone (201) by alkylation, methylation of the acid, and base catalysed cyclisation. Treatment with acidic methanol gave two enol ethers, (202)



Scheme 32: *1⊢ SeO_2 2⊢ $(CH_2OH)_2$ /H^+ 3⊢ $LiAlH_4$ 4⊢ Collins reagent 5⊢ H^+ 6⊢ Li-hexamethyldisilazamide/HMPA 7⊢ oxodiperoxymolybdenum(pyridine)(HMPA) [MoOPH]*

and (203) in 84% and 13% yields respectively. These were converted to enones (204) and (205). Separation of the major component (204) was then followed by a Michael addition of cyanide to give nitrile (206). Protection, epimerization and hydrolysis of the acid and ketal afforded keto-acid (207). Treatment with ethyl formate and base yielded a hydroxy-methylene derivative which lactonised to (208). Hydrogenation, reduction of the ketone, followed by tosylation and elimination yielded drimenin (136).

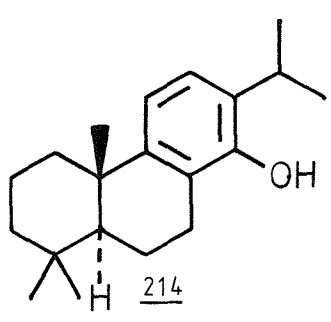
As before, this product was used in relay syntheses; isodrimerin (137), drimenol (133) and confertifolin (138) were prepared by this route (Scheme 34). This was the first reported synthesis of confertifolin (138), isolated from an extract of a tree, *Drimys confertifolia*[75].



Scheme 33: 1⊢ CrO_3 2⊢ TFA 3⊢ KOH 4⊢ O_3 5⊢ Ac_2O 6⊢ Me_2Cd 7⊢ CH_2N_2 8⊢ tBuOK 9⊢ $MeOH/H^+$ 10⊢ $LiAlH_4$ 11⊢ H_3O^+ 12⊢ separate 13⊢ KCN 14⊢ $(CH_2OH)_2/H^+$ 15⊢ $HCO_2Et/tBuOH$ 16⊢ H_2 / Pd on C 17⊢ $NaBH_4$ 18⊢ TsCl 19⊢ DMSO

One further example of degradative synthesis was reported by Pelletier and Ohtsaka (Scheme 35)[101]. They took podocarpone derivative (212) and converted it via phenol derivative (213) to winterin (141).

Recently Japanese workers have prepared a whole range of drimanes from degradative syntheses. The important feature of the route used, was the optical active products achieved by the use of chiral starting materials[102]. Various dehydroabietic acids were used, some of which are shown below:



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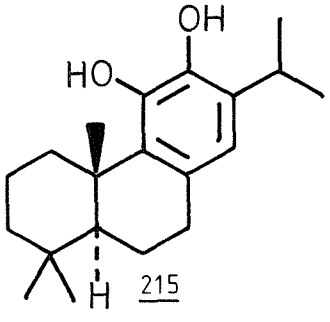

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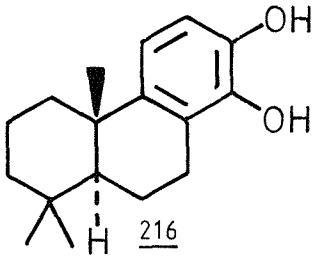

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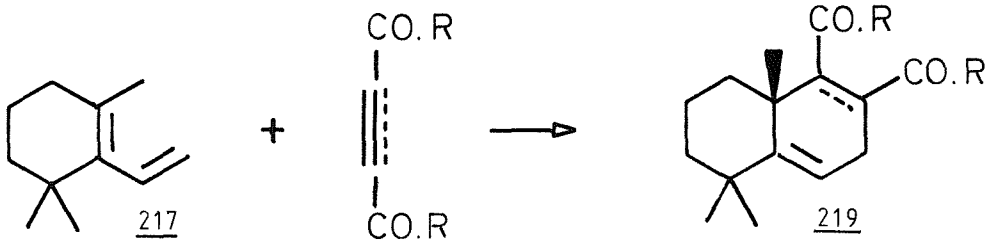
The optically active products were produced after a short degradative procedure. (+)-Confertifolin (138) was obtained from (214) and (216), (+)-valdivolide (140), (+)-winterin (141), (+)-fragrolide (150) and (+)-bemadienolide (149) from (214). Dehydroabietic acid derivative (215) was used as a precursor to (+)-isodrimenin (138).

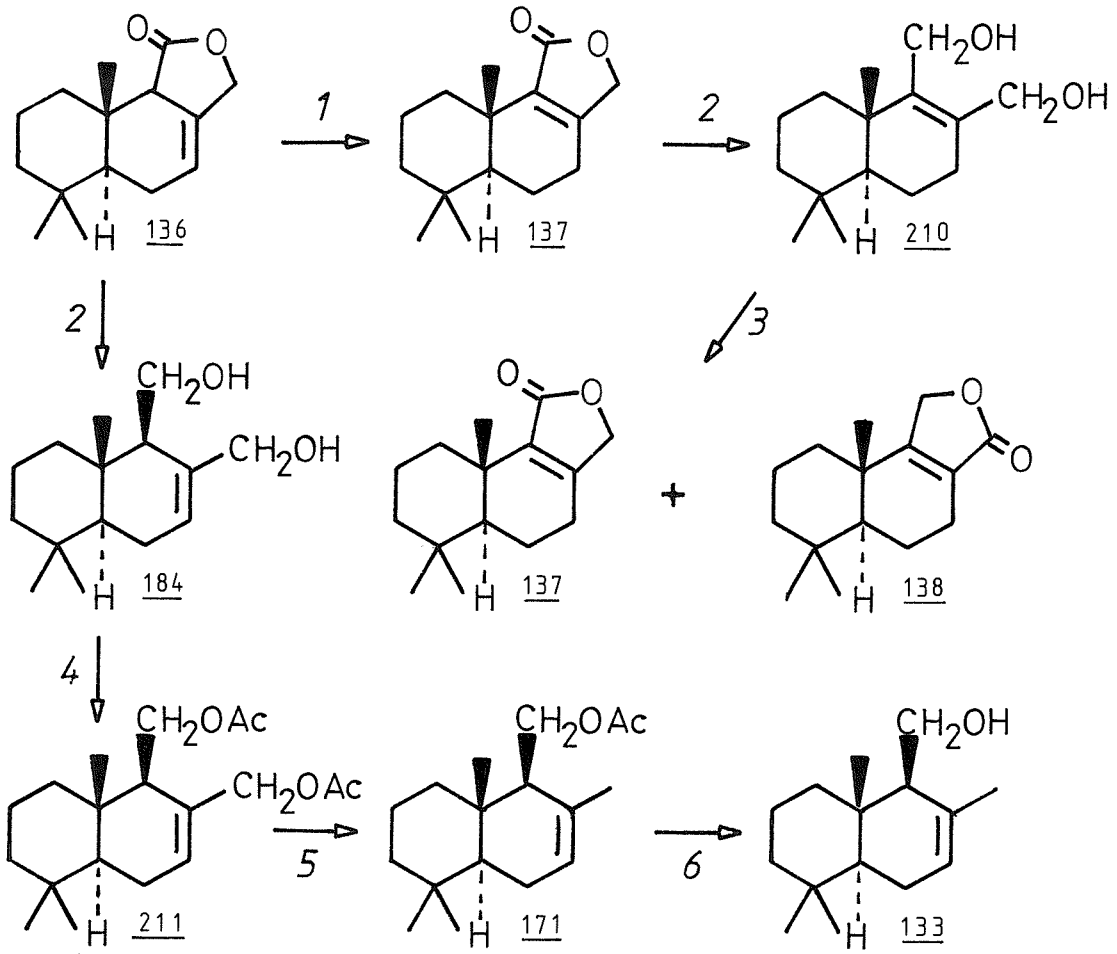
1.8.4. Drimane Structures by Cycloaddition Reactions

The final method of constructing the drimane skeleton is by a cycloaddition reaction. It would be possible to produce a drimane structure using the Diels-Alder reaction below, (Figure 8):



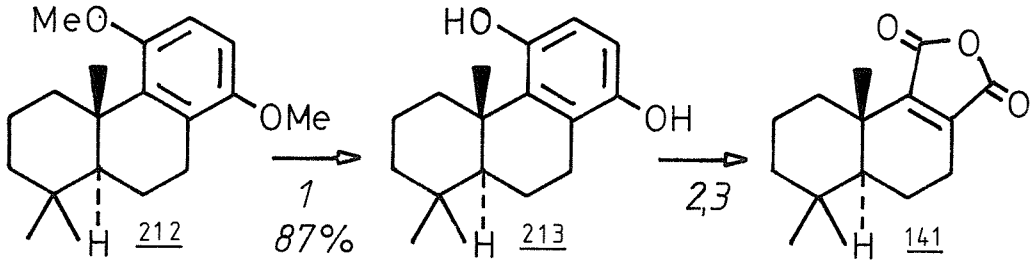
217 218 219

Figure 8.



Scheme 34: 1⊢ base 2⊢ $LiAlH_4$ 3⊢ MnO_2

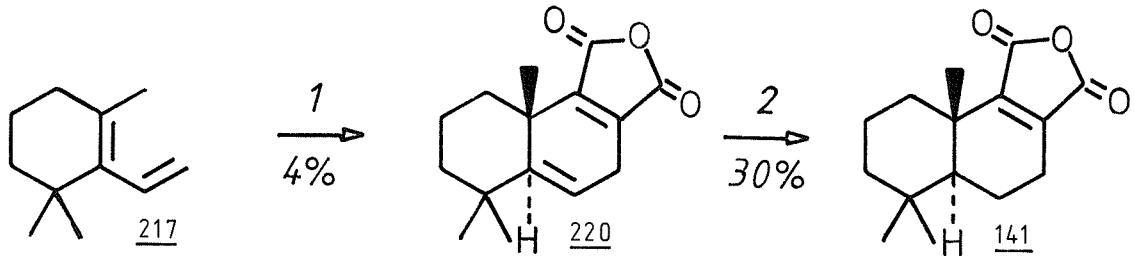
4⊢ Ac_2O 5⊢ Li / NH_3 6⊢ NaOH / EtOH



Scheme 35: 1⊢ BBr_3 2⊢ O_3 3⊢ oxidise

By using either a disubstituted olefin or an acetylene (218) with diene (217); a structure such as (219) could be prepared as either an olefin or a diene.

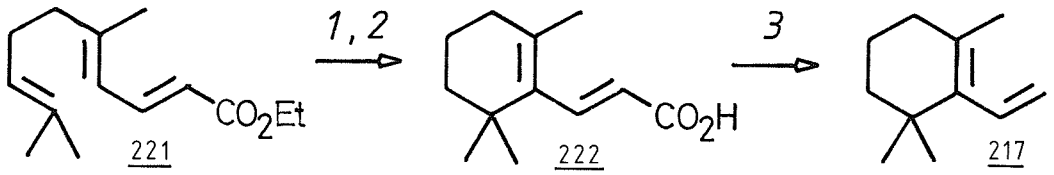
This reaction was first used by Brieger in his synthesis of (±)-winterin [(141), Scheme 36][103].



Scheme 36: 1⊢ $HO_2C-\equiv-CO_2H$ /Δ 2⊢ H_2 /Pd on C

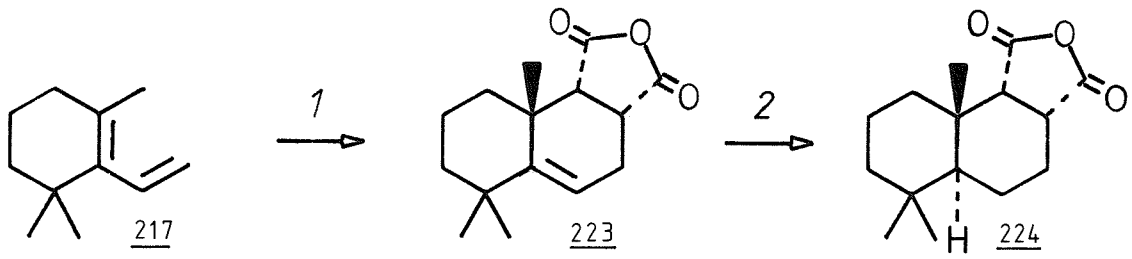
The Diels-Alder reaction between diene (217) and acetylene dicarboxylic acid afforded (±)-dehydrowinterin (220) in 4% yield. Hydrogenation over a palladium on charcoal catalyst, gave (±)-winterin (141) in 30% yield after crystallisation.

The diene was prepared by the method of Royal[104] from ethyl citrylidine acetate [(221), Scheme 37].



Scheme 37: 1⊢ H_2SO_4/H_3PO_4 2⊢ NaOH 3⊢ Δ

Dihydrowinterin (224) has also been prepared in a similar way by Compos *et al* (Scheme 38)[105]. The same diene (217) was heated with maleic

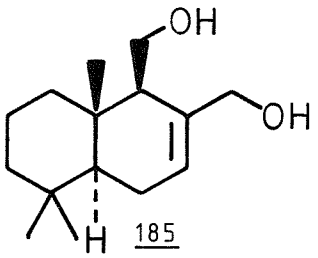


217 223 224

Scheme 38: *1⊢ maleic anhydride/Δ 2⊢ H_2/cat.*

anhydride to give cyclic product (223), which on hydrogenation gave dihydrowinterin (224). It is interesting that both hydrogenations have been reported to give *trans* ring junctions.

A Diels Alder method of preparing diol (185) has been developed by Nakanishi[106].



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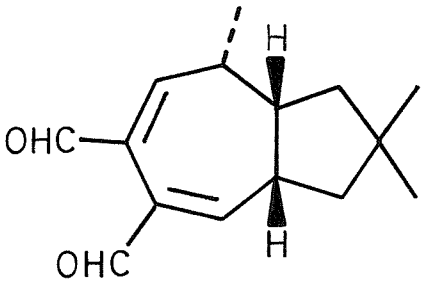
(This will be discussed fully in Chapter 3). This intermediate may be converted to drimenol (133)[75,100], cinnamolide (144)[95], bemadienolide (149)[82] drimenin (136)[106] and polygodial (139)[106].

1.9 Naturally Occurring Dialdehydes

The proposed structure of cavidial (123) is very interesting because only a few dialdehydes are found occurring naturally. Apart from the drimane sesquiterpene dialdehydes shown in Figure 6, very few other dials are known.

Magnusson isolated two sesquiterpene dialdehydes from *Lactanus* spp[107,108] with structures (225) and (226). Helminthosporal (227), was isolated from *Bipolaris serokiniano*[109].



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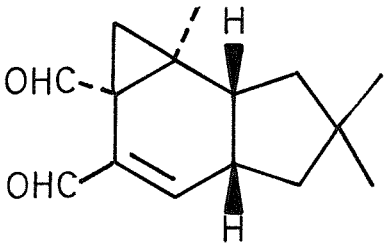

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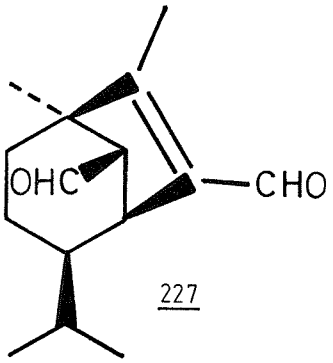

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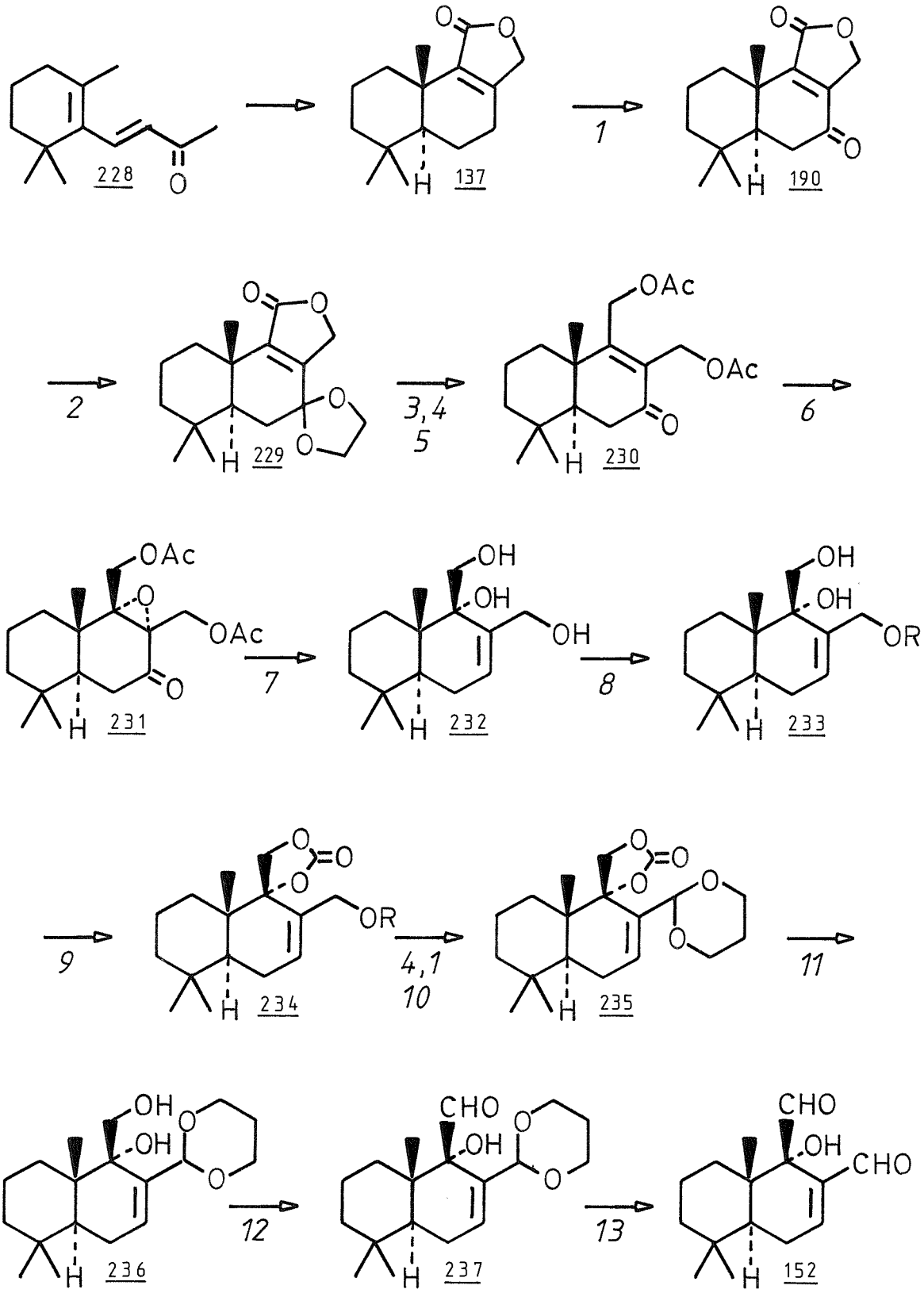
Of these, only Helinthosporal (227) has been synthesised[110,111]. Most of the known drimane sesquiterpene dialdehydes have been prepared; some have already been mentioned, but there are other syntheses that involved the use of different routes to those already discussed.

The first was published by Oishi *et al*[112]. (±)-Isodrimenin (137) was synthesised on a large scale from β-ionone [(228), the route was not published]. The subsequent synthetic steps are shown in Scheme 39. Oxidation of (±)-isodrimenin (137) with chromium trioxide in acetic acid yielded ketone (190), which was protected as a ketal (229). Lithium aluminium hydride reduction followed by ketal hydrolysis and acetylation gave diacetate (230). Epoxidation with hydrogen peroxide gave the



Scheme 39: 1▻ CrO_3 2▻ $(CH_2OH)_2 / H^+$ 3▻ $LiAlH_4$ 4▻ H^+
5▻ Ac_2O 6▻ $H_2O_2 / NaOH$ 7▻ NH_2NH_2 8▻ $tBuMe_2SiCl = RCl$
9▻ NN'-carbonyl diimide 10▻ $HO(CH_2)_3OH / H^+$ 11▻ NaOH
12▻ Moffatt 13▻ acetone / H^+

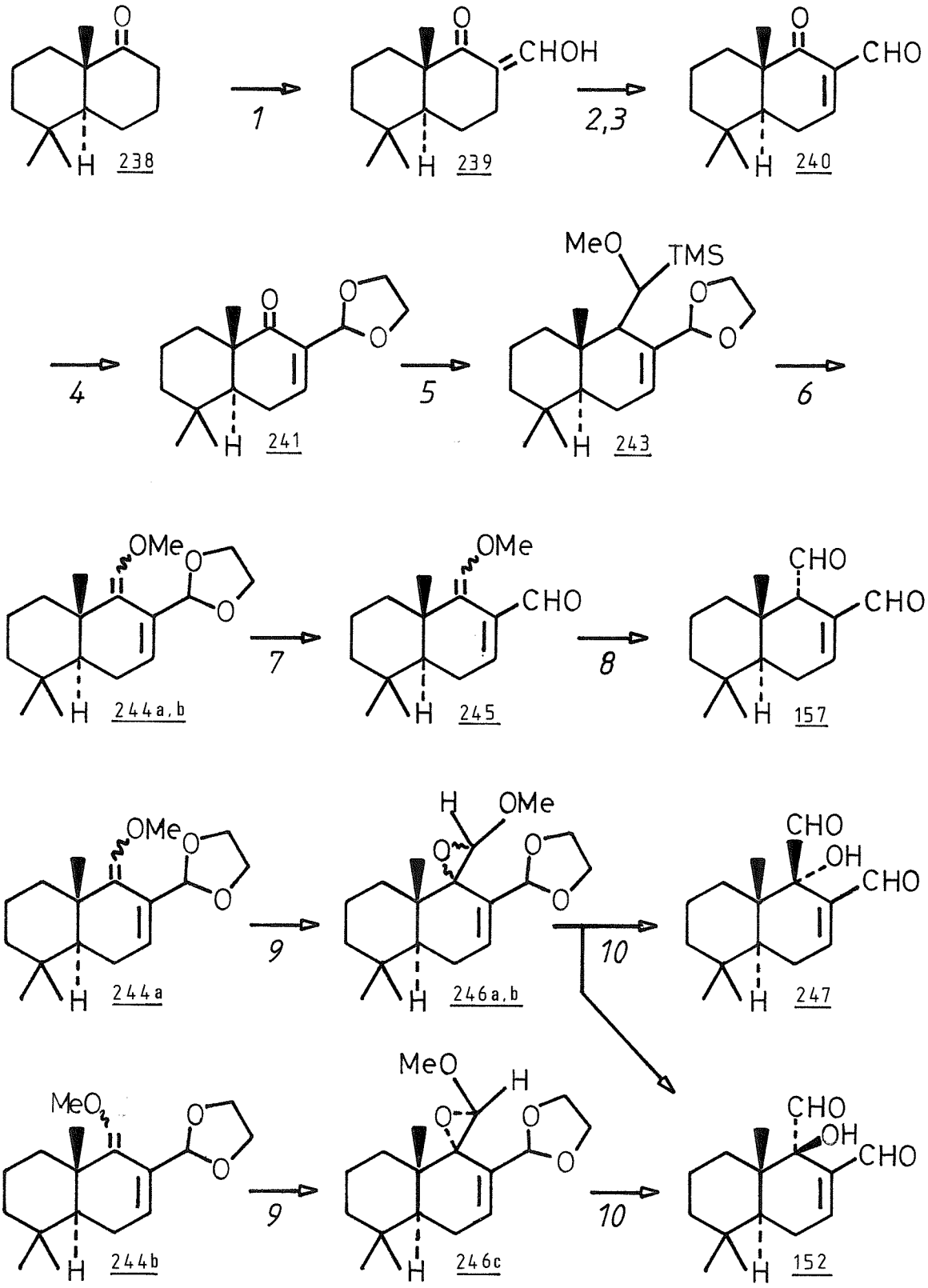
α-epoxide (231), due to steric hinderance of the top face, and triol (232) was formed by treatment with hydrazine hydrate. Preferential protection of the less hindered primary alcohol was achieved with t-butyldimethylsilyl chloride, and the remaining alcohols were converted to a carbonate ester (234). Hydrolysis of the silyl group followed by oxidation and protection yielded acetal (235). Finally, hydrolysis of the ester to (236), Moffatt oxidation of the primary alcohol and hydrolysis of the acetal gave warburganal (152) in 20% overall yield from (±)-isodrimenin (137).

This impressive synthesis has been recently followed by a novel route published by Kende and Blacklock (Scheme 40)[113]. This route afforded both warburganal (152) and isotadeonal (157). 5,5,9-Trimethyl-*trans*-1-decalone (238)[114] was formylated and dehydrogenated under mild conditions to give ketoaldehyde (240), which was subsequently protected as the acetal (241). Normal Wittig type reagents were found to be unreactive with (241), but the method of Magnus and Roy[115] using lithium methoxy-(trimethylsilyl)-methylide (242) proceeded smoothly to give a mixture of ethers (243). Elimination of trimethylsilanol with potassium hydride afforded a 3:1 mixture of enolethers (244, a,b). Hydrolysis under mild conditions yielded monoaldehydes (245), or under more vigorous conditions isotadeonal (157).

Epoxidation of enol ether (244 a), with buffered m-chloroperbenzoic acid (MCPBA) gave a 4:1 mixture of epoxides (246 a,b), the mild hydrolysis of which afforded a 4:1 mixture of *epi*-warburganal (247) and warburganal (152). Epoxidation of enol ether (244 b) gave one epoxide (246 c) which was hydrolysed to give warburganal (152).

1.10. Synthesis of Cavidial (150)

A dialdehyde has been isolated from the defence secretion of *Ancistrotermes cavithorax* and a structure has been assigned (150). Similar isomeric dialdehydes have been synthesised by other workers, but these have only been published recently. Synthetic routes to cavidial were explored, all of which used the Diels-Alder reaction to construct the bicyclic system (Chapter 3). A total synthesis of the structure postulated would prove the structure of cavidial to be (150).



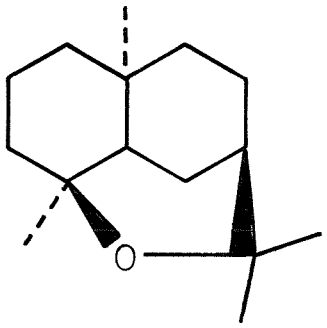
Scheme 40: 1⊳ NaH / HCO_2Et 2⊳ DDQ 3⊳ silica 4⊳ $(CH_2OH)_2 / H^+$ 5⊳ [242], see text 6⊳ KH 7⊳ aq. HCl 8⊳ aq. H_2SO_4 / acetone 9⊳ MCPBA 10⊳ H_3O^+

CHAPTER 2

Synthesis of 4,11-epoxy-*cis*-eudesmane

2.1 Synthesis of 4,11-epoxy-*cis*-eudesmane (21)

In the previous chapter, the extraction of soldiers from the termite species *Amitermes evuncifer* was described and the major component (greater than 90% of the volatile constituents) was isolated. From spectral analysis and microchemical reactions, it was suggested that this compound was 4,11-epoxy-*cis*-eudesmane (21)[24]. The absolute configuration of this molecule was confirmed by synthesis, and is as shown below[116]:



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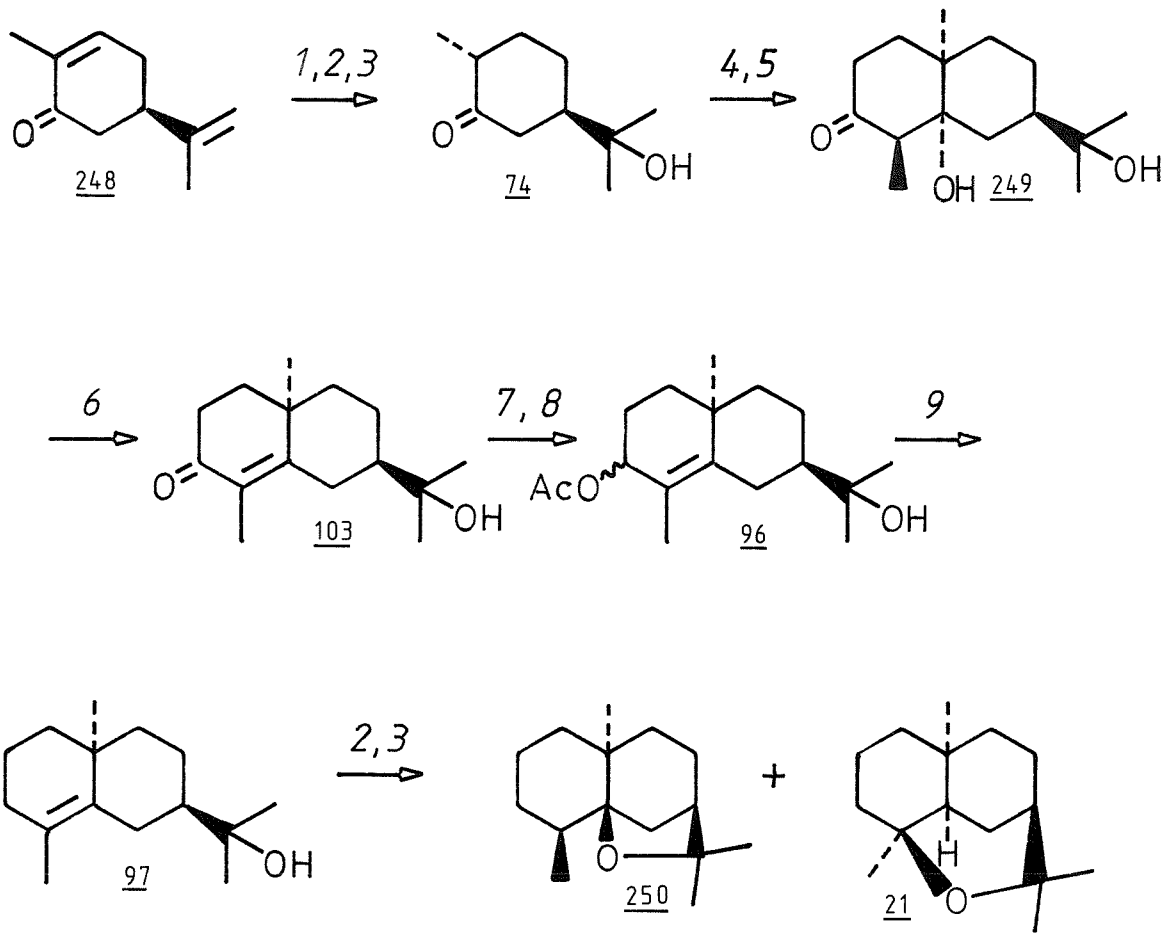
A summary of this route is presented in Scheme 41. *Epi*-γ-eudesmol (97) was synthesised from (-)-carvone (248) by a modification of the method used by Marshall and Pike (Scheme 12)[46]. Oxymercuration of (97) with mercuric acetate in aqueous tetrahydrofuran gave an *in situ* cyclisation, the products (21) and (250) being isolated after reduction with alkaline sodium borohydride. 4,11-Epoxy-*cis*-eudesmane (21) and the bi-product, *iso*-dihydroagarofuran (250) were isolated in 80% yield as a 3:1 mixture (Scheme 41).

The synthesis has advantages; the sequence has relatively few steps and the final product has optical activity, but the major disadvantage was the difficulty in separating the final products. On a small scale, isolation of the products for spectral analysis was achieved by preparative gas chromatography. On a large scale separation (to obtain quantities of the defence secretion for field trials and toxicity testing), preparative g.c. was not viable. 4,11-Epoxy-*cis*-eudesmane (21) and dihydroagarofuran (250) were found to codistil and coelute by high performance liquid chromatography, so it was necessary to consider other synthetic schemes.



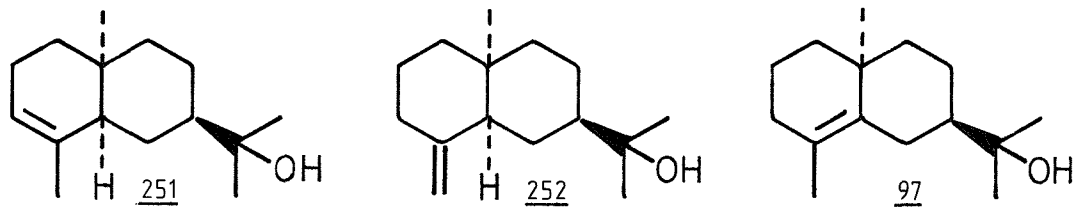
248 74 249 103 96 97 250 21

Scheme 41: 1⊢ $Zn / NaOH / EtOH$ 2⊢ $Hg(OAc)_2$ / aq.THF

3⊢ $NaOH / NaBH_4$ 4⊢ NaH 5⊢ $ClCH_2CH_2CO.CH_2CH_3$

6⊢ $EtOH / HCl$ 7⊢ $LiAlH_4$ 8⊢ Ac_2O /pyr 9⊢ Li / NH_3

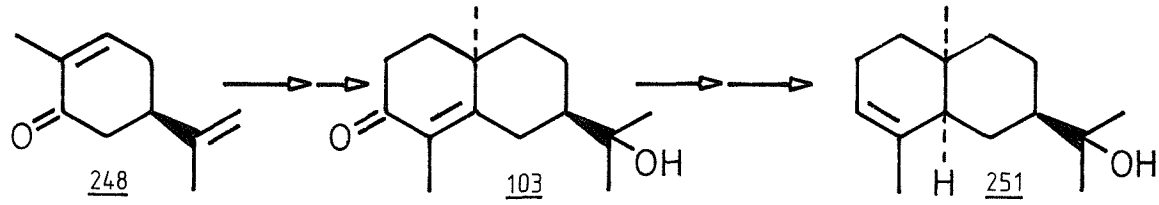
In the published route, the cyclic ether was formed by using a mercury (II) catalysed intramolecular ring closure of the tertiary alcohol on tetrasubstituted olefin (97); this reaction will be discussed in more detail in a later section. It can be seen that the tertiary alcohol may also take part in a ring closure reaction with two other olefins, *epi*-α-eudesmol (251) and *epi*-β-eudesmol (252).



251 252 97

Epi-β-eudesmol (252) could be prepared by a similar route to that used for β-eudesmol [(29), Scheme 9], but the step required to introduce the *exo*-methylene group (for example a Wittig reaction) would probably lead to equilibrium of the *cis*-ketoalcohol to the more thermodynamically stable *trans*-ketoalcohol as previously observed[39-41].

Epi-α-eudesmol (251) was prepared from carvone [(248), Scheme 42].



248 103 251

Scheme 42:

The synthesis of *epi*-carrisone (103) from carvone (248) was achieved by one of many routes, hydrogenation followed by separation afforded *cis*-dihydro-*epi*-carrisone (104), an intermediate similar to that used in the synthesis of α-eudesmol [(28), Scheme 11][45]. The Bamford-Stevens reaction

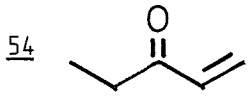
on (104) should yield *epi*-α-eudesmol (251), which could then by cyclised using a mercury (II) catalyst to give 4,11-epoxy-*cis*-eudesmane (21).

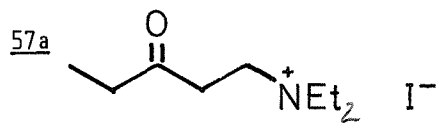
2.2 Annelation Reactions

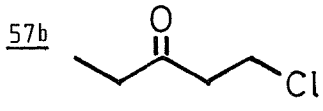
2.2.1 The Robinson Annelation Reaction

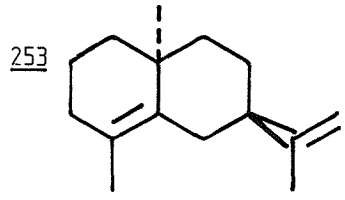
The Robinson annelation reaction is one of the most synthetically useful applications of the Michael reaction[117a]. Generally, the reaction involves the formation of a new six-membered ring by the intramolecular aldol condensation of an initial Michael adduct, and then subsequent dehydration to an αβ-unsaturated ketone. Two general procedures have been adopted. The first involves the formation of the uncyclised adduct from methyl vinyl ketone (or equivalent) and a relatively acidic methylene compound (.e.g an enolate anion) under mild conditions. This is followed by treatment with pyrrolidine and acetic acid to afford the intermediate ketol, dehydration with acid then yields the required product. The second procedure involves the slow addition of methyl vinyl ketone (or equivalent) to a cold ethereal solution of the active methylene compound to form the aldol product which may then be dehydrated with acid or base.

The commonest method employed in decalin syntheses is the second procedure, and this was used in this work. The carbon chain fragment to be added, nominally a vinyl ketone, must be a Michael acceptor; for the intermediate required in this scheme, a five carbon fragment must be added. Three such molecules are readily available: ethyl vinyl ketone (54), 1-diethylaminopentan-3-one (57a) and 1-chloropentan-3-one (57b).







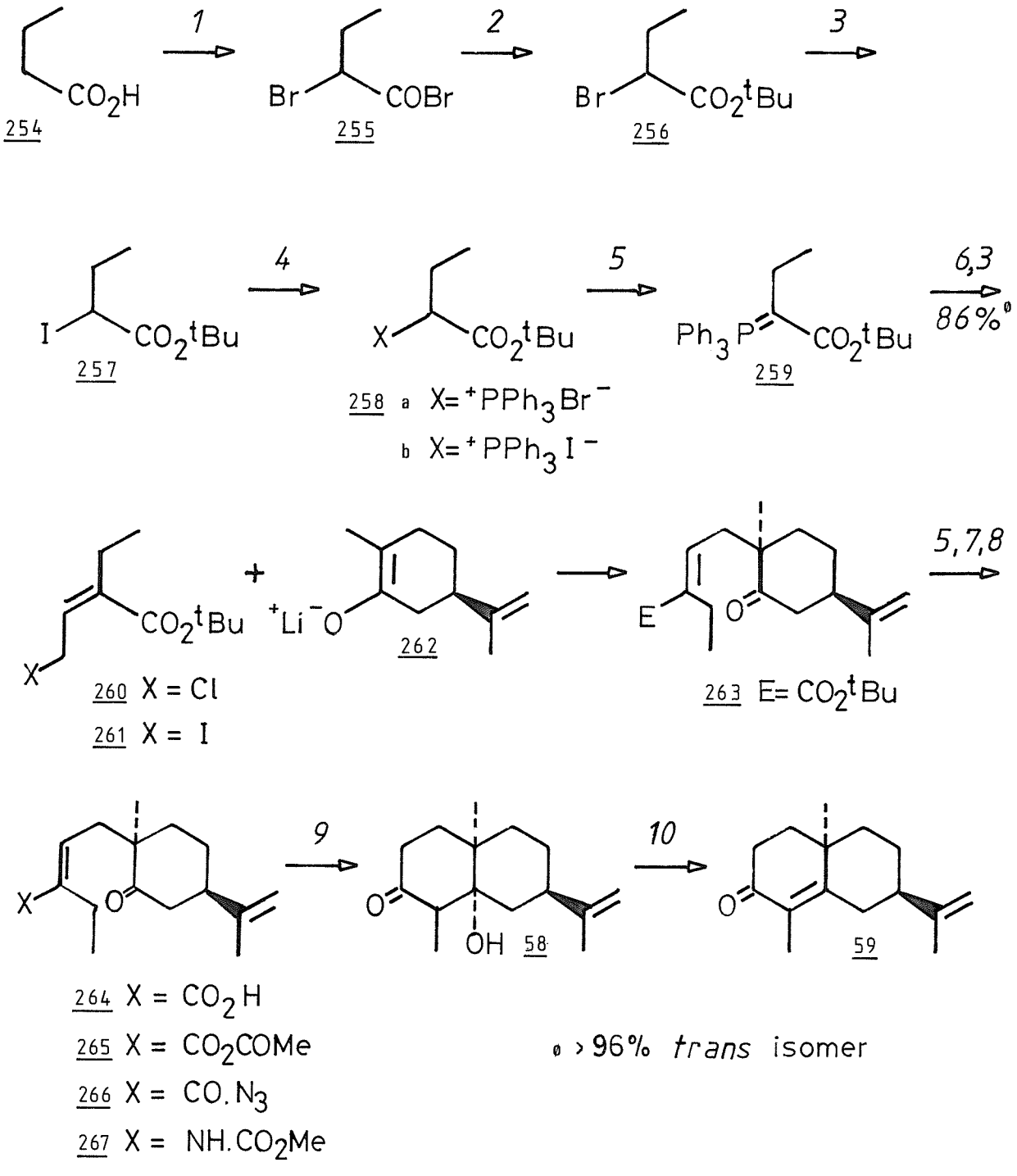


All the earlier work on eudesmane chemistry involved the use of (54) or (57a), and it was not until 1970 in a synthesis of *epi*-γ-selinene (253) by Klein and Rojahn[118], that 1-chloropentan-3-one (57b) was reported to be used in an annelation reaction. We have shown 1-chloropentan-3-one (57b) to be the best 'Michael acceptor' available,[a] ethyl vinyl ketone (54) was found to polymerise easily and 1-diethylaminopentan-3-one (57a) was not as easy to prepare. (1-Chloropentan-3-one was synthesised by a Friedel-Crafts reaction of propionyl chloride with ethylene and aluminium chloride[119]. Propionyl chloride was prepared by heating propionic acid with benzoyl chloride[120].)

2.2.2 Annelations via Tiglate Esters

The main problem associated with the Robinson ring annelation reaction is the poor yield obtained, typically less than 50%. A novel route devised by Stotter has more synthetic steps than the standard method, but the published yields (better than 70%) justify its inclusion in a study of annelation reactions. The synthesis of a decalin system involves the use of tiglate esters (Scheme 43). To obtain the tiglate ester[121], butyric acid [butanoic acid , (254)] is brominated with red phosphorus and bromine[122] to give the α-bromide acid bromide (255). Esterification with t-butanol (2-methylpropan-2-ol) in pyridine gave the corresponding α-bromoester (256)[123], which was then converted to the α-iodoester (257) with sodium iodide in acetone[124]. Reaction of the α-iodoester (257) with triphenyl phosphine yielded the phosphonium iodide (258b), from which the phosphorane (259) can be prepared as a white crystalline solid, after treatment with sodium hydroxide[125]. A Wittig reaction between chloroacetaldehyde, obtained from chloroacetaldehyde dimethyl acetal, and the phosphorane (259) yielded the chlorotiglate ester (260). Stotter found that the condensation of the tiglate ester with an enolate anion proceeded in better yield if the iodotiglate ester (261) were used[126], so the chlorotiglate ester (260) was converted to the corresponding iodo-compound (261) with sodium iodide in acetone. The enol acetate of a methyl cyclohexanone was then prepared[127,128] and reaction with methyl lithium gave the enolate anion[129]. To obtain epi-α-cyperone (59) as a final product, the lithium enolate anion of dihydrocarvone (262) would be required, and condensation with the iodotiglate ester (261) led to

[a] EVK (54) was formed *in situ* from (57b)



Scheme 43: *1⊢ P/Br$_2$ 2⊢ tBuOH/pyr 3⊢ NaI/acetone 4⊢ PPh$_3$ 5⊢ NaOH 6⊢ ClCH$_2$CHO 7⊢ ClCO$_2$Me 8⊢ NaN$_3$ 9⊢ KOH 10⊢ dehydrate*

intermediate (263) by nucleophilic displacement of iodide. A series of reactions was then used to transform the ester to a ketone group on the ring via a modified Curtius reaction[130]. The ester (263) was saponified to acid (264) and then from this mixed anhydride (265) was prepared. Transformation to acyl azide (266) was followed by rearrangement to the N-methylvinyl carbamate (267), and treatment with potassium hydroxide gave ketoalcohol (58). Dehydration afforded *epi*-α-cyperone (59).

When this reaction sequence was attempted, good yields were obtained for the first three steps (73%, 82% and 95% respectively), but the preparation of phosphonium salt (258b) and phosphorane (259) were found to proceed in lower yields than reported. (Yields of 33% and 31% were observed for these reactions compared to the reported yields of 54% and 85%). The reaction to the corresponding phosphonium bromide (258a) also gave a poor yield of 30%.

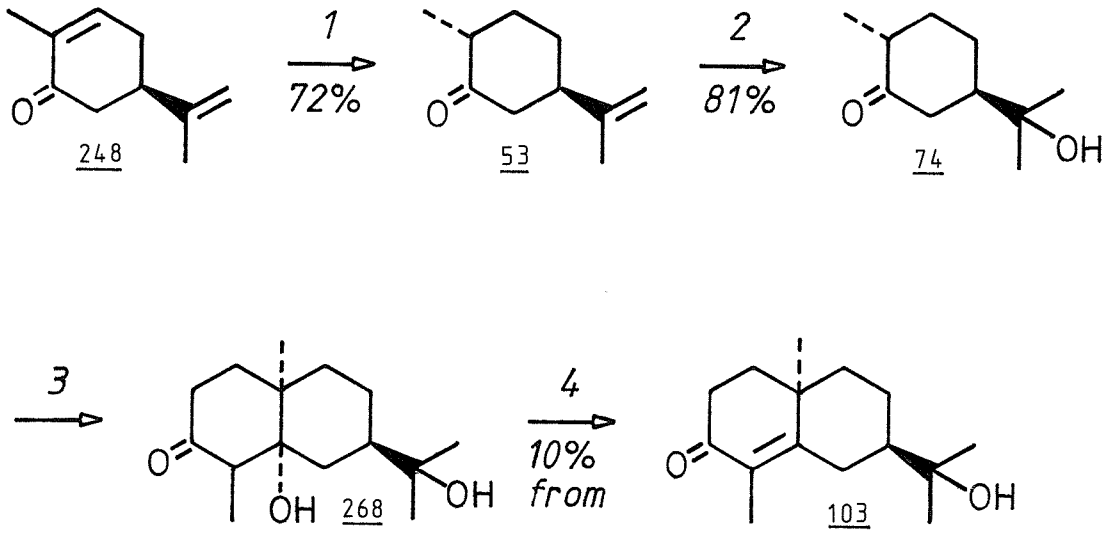
These yields were not high enough to merit further investigation because the overall yield of the reaction sequence would have been lower than for the conventional annelation reaction.

2.3 Synthesis of *epi*-carrisone (103)

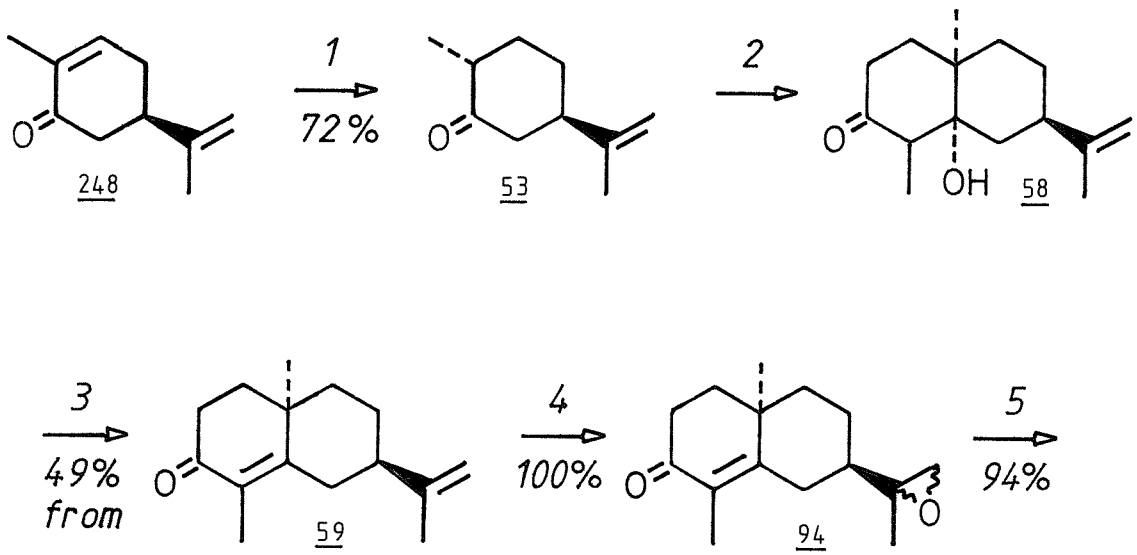
2.3.1 via Dihydrocarvone Hydrate (74)

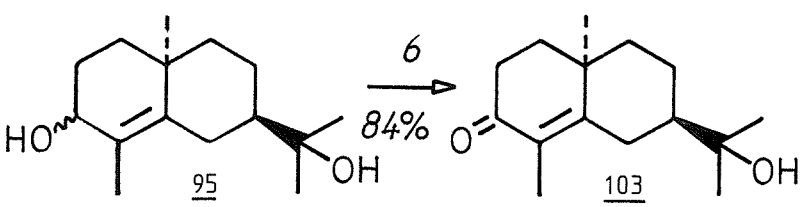
The method used to prepare *epi*-carrisone [(103), Scheme 44] was a modification of Marshall and Pike's synthesis of α-agarofuran (36), and Heathcock's synthesis of occidentalol (40)[50].

(-)-Carvone (248) was reduced to (+)-dihydrocarvone (53) with activated zinc dust and ethanolic caustic soda[131]. Activation of the zinc dust, achieved by washing with 5% hydrochloric acid, water and ethanol[132], was found to increase the yield of the reduction by 10% to 72%. (Care must be taken with activated zinc dust as it is pyrophoric.) Dihydrocarvone (53) was then hydrated by the Markownikov addition of water. The oxymercuration-demercuration procedure of Brown and Geoghegan[133] was used to give dihydrocarvone hydrate (74) in 81% yield. Annelation with 1-chloropentan-3-one has been reported to proceed to the ketol (268) in a yield of 40%[21]. This reaction was repeated many times varying the conditions of temperature, the rate of addition of the ketone and concentration of reactants, but a maximum yield of only 10% was recorded.



Scheme44: 1⊢ $Zn/NaOH/aq.EtOH$ 2⊢ $Hg(OAc)_2/aq.THF$
3⊢ $NaH/ClCH_2CH_2CO.CH_2CH_3$ 4⊢ $HCl/EtOH$





1⊢ $Zn/NaOH/EtOH$
2⊢ annelation
3⊢ $HCl/EtOH$
4⊢ MCPBA
5⊢ $LiAH_4$
6⊢ P.D.C. / DMF

Scheme 45.

With this low yield reaction so early in the sequence, an alternative procedure was required for the synthesis of *epi*-carrisone (103).

2.3.2 via *epi*-α-cyperone (59)

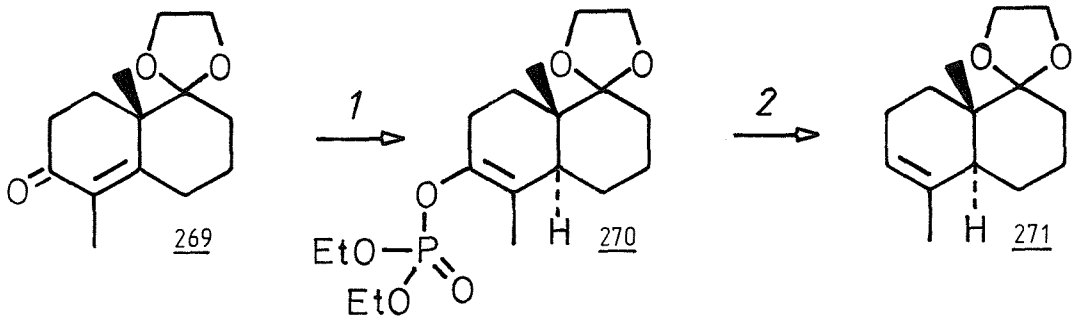
Carrisone (27) has been previously prepared from α-cyperone (25) by Pinder (Scheme 7)[37,38]; if *epi*-α-cyperone (46) could be prepared, this would allow a higher overall yield synthesis of *epi*-carrisone [(103), Scheme 45].

Reaction of (+)-dihydrocarvone (53) with 1-chloropentan-3-one yielded the required ketol (58) in 34% yield after distillation. Dehydration with ethanolic hydrochloric acid followed by distillation gave (-)-*epi*-α-cyperone (59) in 84% yield, $[\alpha]_D = -121^0$; an overall yield of 28% from dihydrocarvone (53). It was found that an increase in yield for one reaction was obtained when the intermediate was not purified, yields of 45% were not uncommon. Epoxidation with m-chloroperbenzoic acid (MCPBA) gave epoxide (94) in 98% yield, which without purification was reduced with lithium aluminium hydride to afford a mixture of diastereoisomeric diols (95) in 94% yield. Oxidation with pyridinium dichromate in dimethylformamide (pdc in DMF)[134] afforded (-)-*epi*-carrisone (103) in 84% yield, $[\alpha]_D = -160^0$. The diols were also oxidised using the following reagents: manganese dioxide in chloroform[135], chromium trioxide in hexamethylphosphoramide (HMPA)[136,137], chromium trioxide in pyridine[138], and pyridinium chlorochromate (pcc) in methylene chloride[139]. All gave satisfactory yields between 70-90%, but for large scale preparations pdc in DMF was chosen because only 1¼ molar equivalents of oxidising agent were required.

The overall yield for the preparation of *epi*-carrisone (103) from carvone (248) was 19%. This compares favourably with the previously reported overall yield of 15% obtained by synthesis via dihydrocarvone hydrate (74)[21].

2.4 Synthesis of *epi*-α-eudesmol (251) From *epi* -carrisone (103)

It was necessary to synthesise a trisubstituted olefin from an αβ-unsaturated system. Only one method, published by Ireland in 1969 (Scheme 46)[140] was available.

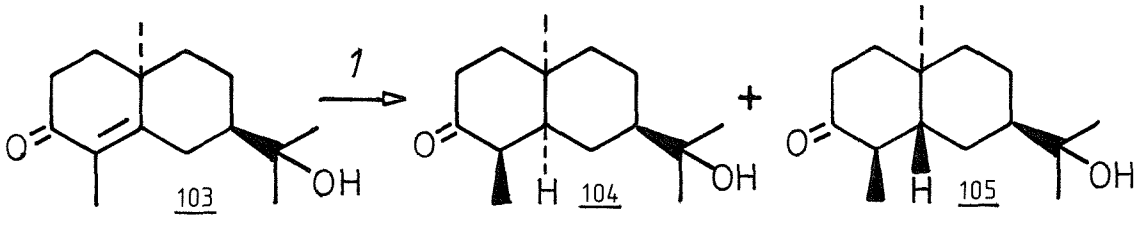


Scheme 46: 1⊢ *i* Li/NH_3 *ii* $(EtO)_2POCl$
2⊢ $Li / EtNH_2 / tBuOH$

Unfortunately, the ring junction produced after the Birch reduction of the αβ-unsaturated ketone was *trans*[50], the *cis* ring junction was required and so another method had to be sought.

2.5.1Synthesis of Dihydro-*epi*-carrisone (104, 105)

Hydrogenation of the αβ-unsaturated system of (103) was considered. Ideally, the only isomer produced on hydrogenation would be the *cis* isomer (104), with little or no contamination by the *trans* isomer [(105), Scheme 47].



Scheme 47: 1⊢ H_2 / catalyst

Selective hydrogenations of this type have been studied extensively in steroid chemistry[117b], high percentage convertions to mainly the *cis* isomer have been obtained, but the solvent used has been shown to have a marked effect on the ratio of *cis:trans* products. The hydrogenation of

4-cholesten-3-one (272) over palladium on calcium carbonate in different solventshas been studied (Scheme 48)[141], and the highest *cis:trans* ratio was obtained in acetonitrile. *Epi*-carrisone (103) was hydrogenated under similar conditions, but no reaction was observed. It was assumed that steric restraints were affecting the reaction. Other catalysts such as palladium on charcoal and Adams' catalyst were tried in acetic acid, methanol and acetonitrile (Table 2).

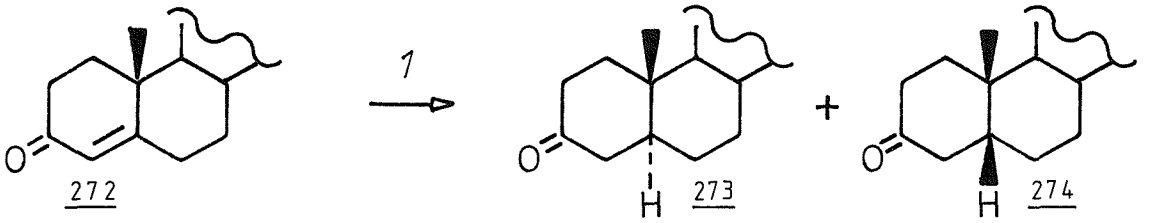
TABLE 2

Catalyst	Solvent	Product Ratio		Yield
		cis	*trans*	
$Pd/CaCO_3$	CH_3CN	-	-	0%
$Pd/CaCO_3$	MeOH	-	-	0%
Pd/C 5%	MeOH	3	1	80%
Pd/C 10%	MeOH	3	1	77%
Pd/C 10%	CH_3CN	3	1	63%[a]
Pd/C 10%	ACOH	3	1	60%[b]
PtO_2	MeOH	2	1	82%

[a]Reaction not complete after overnight stirring

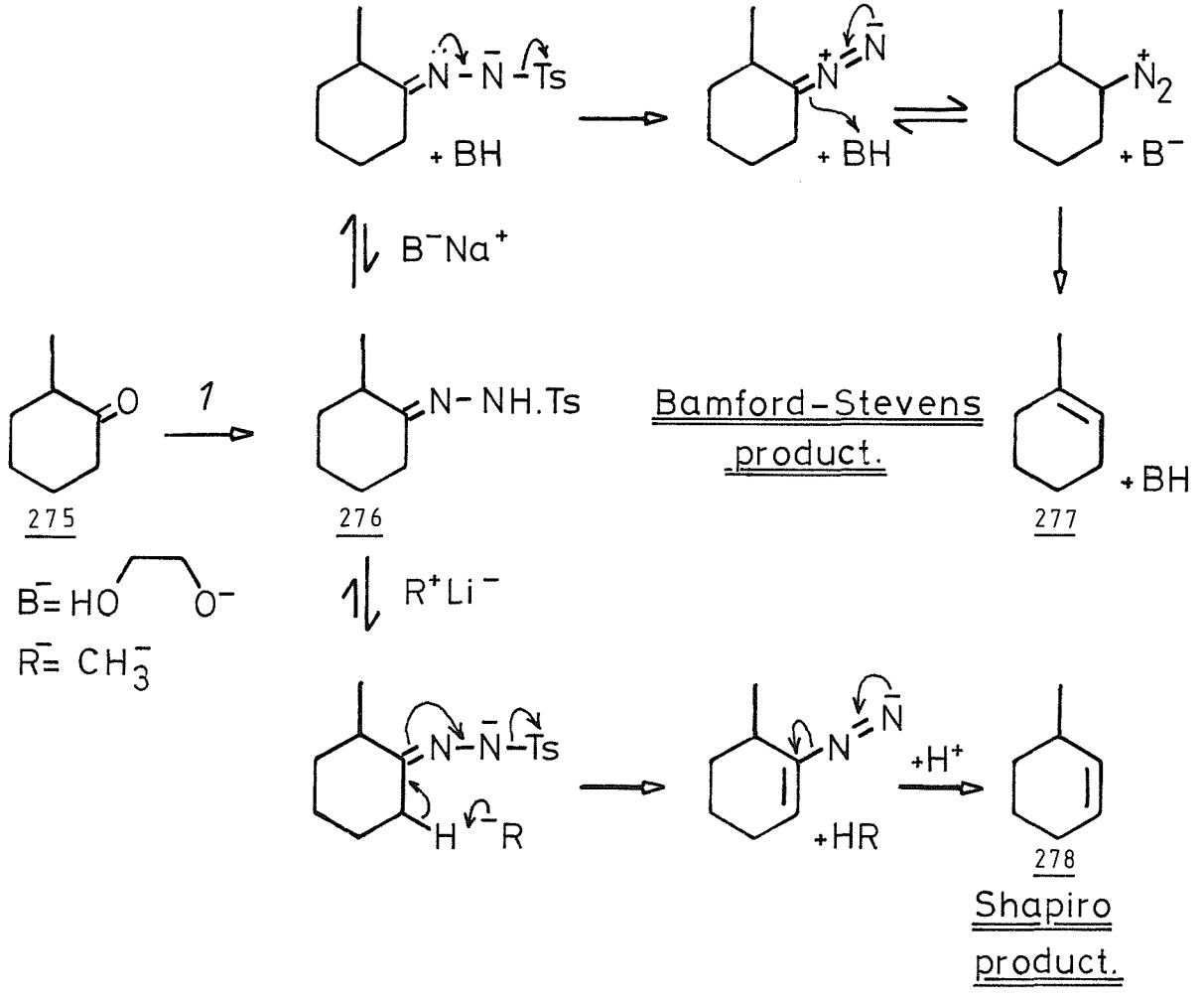
[b]Product mixture showed elimination products

With these results, 10% palladium on charcoal was chosen as the most suitable catalyst, and methanol was used as the solvent. This method was similar to that used by Amano and Heathcock[50]. The *trans* isomer was identified by Amano and Heathcock as the same product as obtained from the Birch reduction of *epi*-carrisone (103). This was found to be the minor product from the hydrogenation reaction. The other isomer therefore had a *cis* ring junction, and this postulate was supported by the spectral data. The previous yield of *cis*-dihydro-*epi*-carrisone (104) was 32%[50].



solvent	trans	cis
CH_3CN	5%	95%
benzene	23%	77%
THF	39%	61%
$(CH_3)_2CHOH$	50%	50%

Scheme 48: 1⊢ H_2 (1 atm.) $Pd/CaCO_3$



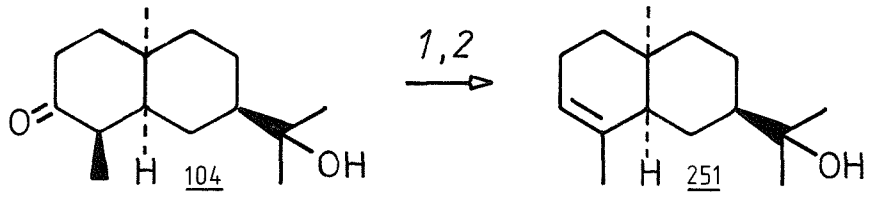
Scheme 49: 1⊢ $pTsNH.NH_2$ / HCl

As the Table shows,a 3:1 ratio was obtained, and separation of the hydrogenation product mixture was achieved by using flash column chromatography (Experimental Section, Chapter 4), to give *cis*-dihydro-*epi*-carrisone (91) in 30-35% yield (greater than 95% pure). High performance liquid chromatography afforded 100% purity material for spectroscopic analysis.

2.5.2Synthesis of *epi*-α-eudesmol (251) from *cis*-dihydro-*epi*-carrisone (104)

The reaction of a ketone to give an olefin has been previously described by Bamford and Stevens[142]. The ketone was reacted with p-toluene sulphonyl hydrazine (also known as tosyl hydrazine) to give the corresponding hydrazone, and then this was eliminated with a base to yield the more substituted olefin. The Shapiro-Heath modification[143], involving the elimination of the hydrazone with an alkyl lithium reagent, afforded the least substituted olefin. These reactions have been reviewed by Shapiro[144]. An example is 2-methylcyclohexanone [(275), Scheme 49]; treatment of the hydrazone (276) under Bamford-Stevens conditions (with the monosodium salt of ethylene glycol at 160°C) yields 1-methylcyclohexene (277), treatment of the hydrazone under Shapiro-Heath conditions (methyl lithium in THF) leads to the formation of 3-methylcyclohexene (278). The mechanisms of these reactions have been suggested[144-146] and are presented in Scheme 49.

The synthesis of *epi*-α-eudesmol (251) from *cis*-dihydro-*epi*-carrisone (104) requires the formation of more substituted olefin (Scheme 50), so the Bamford-Stevens reaction conditions were relevant.



Scheme 50: 1⊢ $TsNH.NH_2$ / HCl 2⊢ $NaOCH_2CH_2OH$ / Δ

The conditions previously used in the synthesis of α-eudesmol (29)[45], were repeated (see Scheme 11). Ketone (104) was refluxed with 1:1 molar equivalents of p-toluenesulphonylhydrazine in methanol with an acid catalyst, the hydrazone was isolated and then heated on an oil bath with sodium in ethylene glycol at 180°C for 2 hours. The required product, *epi*-α-eudesmol (251) was isolated in 19% yield after purification $[\alpha]_D$ = +65°. None of the other double bond isomer was visible by NMR. In the synthesis of α-eudesmol (29), the reported yield for the Bamford-Stevens reaction was 55%[45], so a series of experiments was conducted aimed at increasing the yield of this reaction, the results are summarised below (Table 3).

TABLE 3

Temperature	Time	Yield[a] of (251)
180°C	1 hr	10%[b]
180°C	2 hr	19%
160°C	1 hr	30%[b]
160°C	2 hr	40%
150°C	2 hr	51%
120°C	2 hr	10%
100°C	2 hr	0%

[a]After purification

[b]Reaction not complete by gc

The results show that 180°C is not the ideal temperature for this substrate, and that by lowering the temperature an increased yield may be obtained. All further reaction mixtures were pyrolysed at 140°C-150°C. Each of the above reactions was completed on about ½ gram of substrate; it was found in later reactions that increasing the scale to 2 grams of ketone (104) led to a decrease in the isolated yield of product to 30%.

2.6 Ring Closure of Olefin Alcohols to Cyclic Ethers

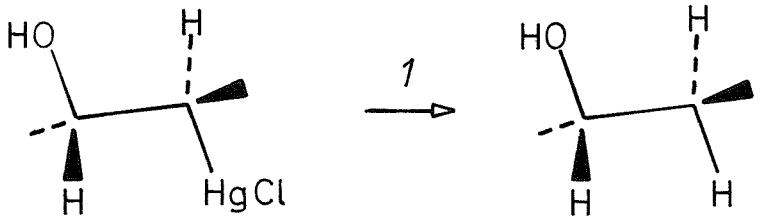
Ring closure of olefin-alcohol (251) to 4,11-epoxy-*cis*-eudesmane (21) was achieved by the intramolecular nucleophilic attack of the tertiary alcohol on the olefin. For this reaction to occur, the electrophilic character of the double bond must be altered, and this has been accomplished in many ways by previous workers.

2.6.1 Cyclisation via Mercury Bridged Intermediates

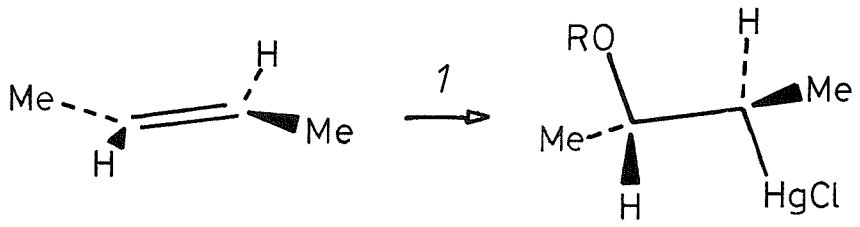
The complexation of mercury (II) ions with olefins to give charged mercury bridged intermediates has been known since 1892[147], later work has shown the properties of these bridged intermediates[148,149].

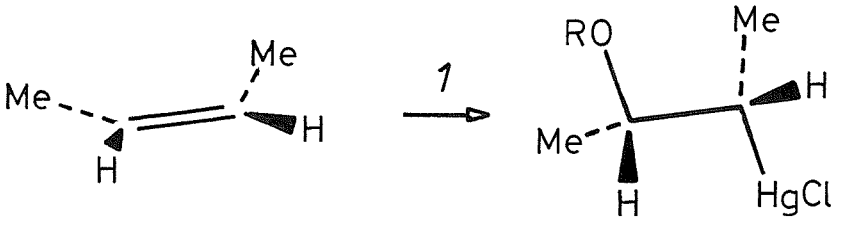
The addition of mercury to a double bond to give a π-complex is an electrophilic addition reaction. Subsequent attack by nucleophiles leads to σ-bonded mercury compounds which may be isolated. A range of nucleophiles may be used and these are summarised in Scheme 51. The product is usually formed by *trans* addition of the nucleophile and mercury substituent; *cis* and *trans* isomers of alkenes give different diastereoisomeric products. A few examples of *cis* addition are known, an example of which is norbornene [(279), Scheme 51], but this only occurs when steric hinderance or strained systems are present. More detailed descriptions of reactions and mechanisms of these species may be found in reviews by Chatt[150] and Zefirov[151].

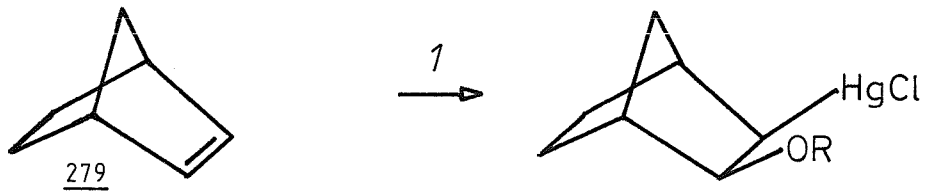
The reaction of water with these mercury bridged intermediates has been found to be a useful reaction as it allows Markownikov addition with retention of configuration. The intermediate σ-bonded mercury complex was reduced with sodium borohydride (Scheme 52)[133].



Scheme 52: 1⊢ $NaBH_4$

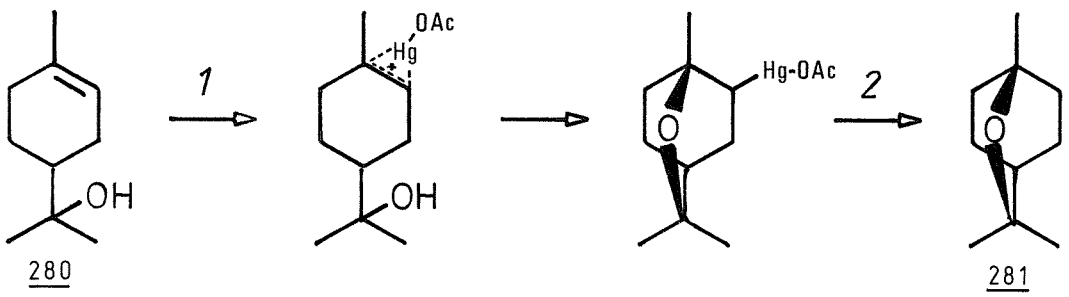






Scheme 51: *1⊳ $Hg(OAc)_2$ / NaCl / ROH*

R = H, alkyl, acyl



Scheme 53: *1⊳ $Hg(OAc)_2$ / aq. THF 2⊳ $NaBH_4$*

If an alcohol replaced water as the solvent, an ether resulted[152].

When this reaction was tried with an internal oxygen nucleophile present, it was possible to get intramolecular attack to give a cyclic ether[153,154]. The synthesis of the major constituent of eucalyptus oil (281) from terpineol [(280), Scheme 53] was one reported example[155]. In some molecules, the alcohol reacted at either end of the mercury complex to give two possible ether products. Work by Bordwell and Douglas[156] showed that by alteration of the reaction conditions, either kinetic or thermodynamic control could be exerted. Cycloocta-1,5-diene (282) was used in these control reactions (Scheme 54). Thermodynamic control led to the formation of the six membered ring ether (283), kinetic control yielded the five-membered ring ether (284).

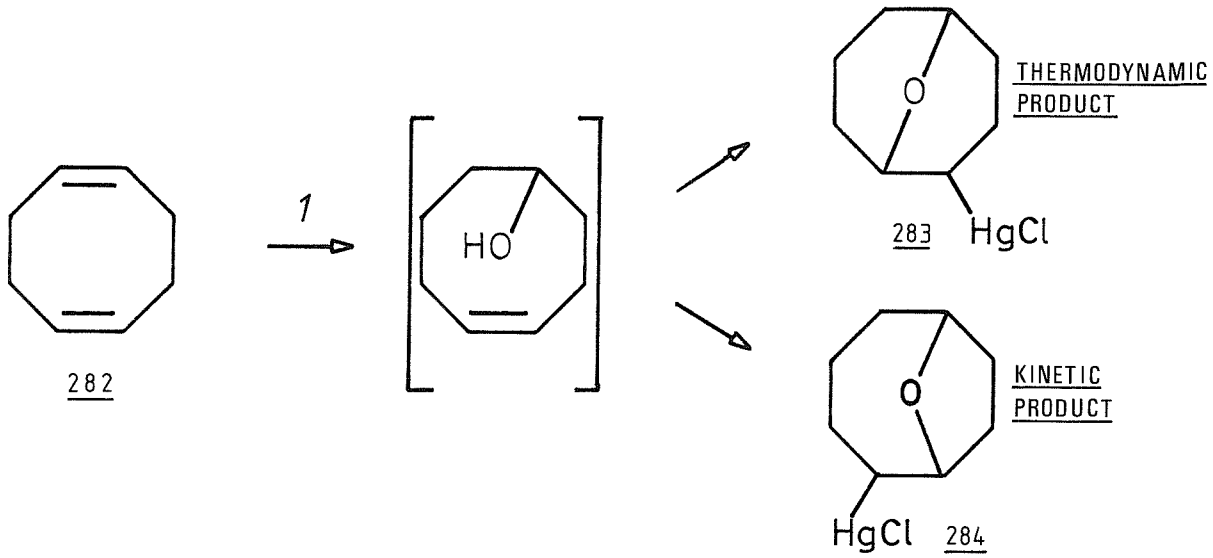
2.6.2 Cyclisation via Selenium Bridged Intermediates

Recent work by Lysenko *et al.*[157,158] has suggested the use of phenylselenyl chloride for ring closures of the type being discussed. Using a model compound, 4-cyclohepten-1-methanol (285), cyclisation to both a saturated (286) and unsaturated (287) product was achieved via a selenium stabilised intermediate (Scheme 55)[157]. The reaction was then used to synthesise the muscarines a group of naturally occurring compounds[158].

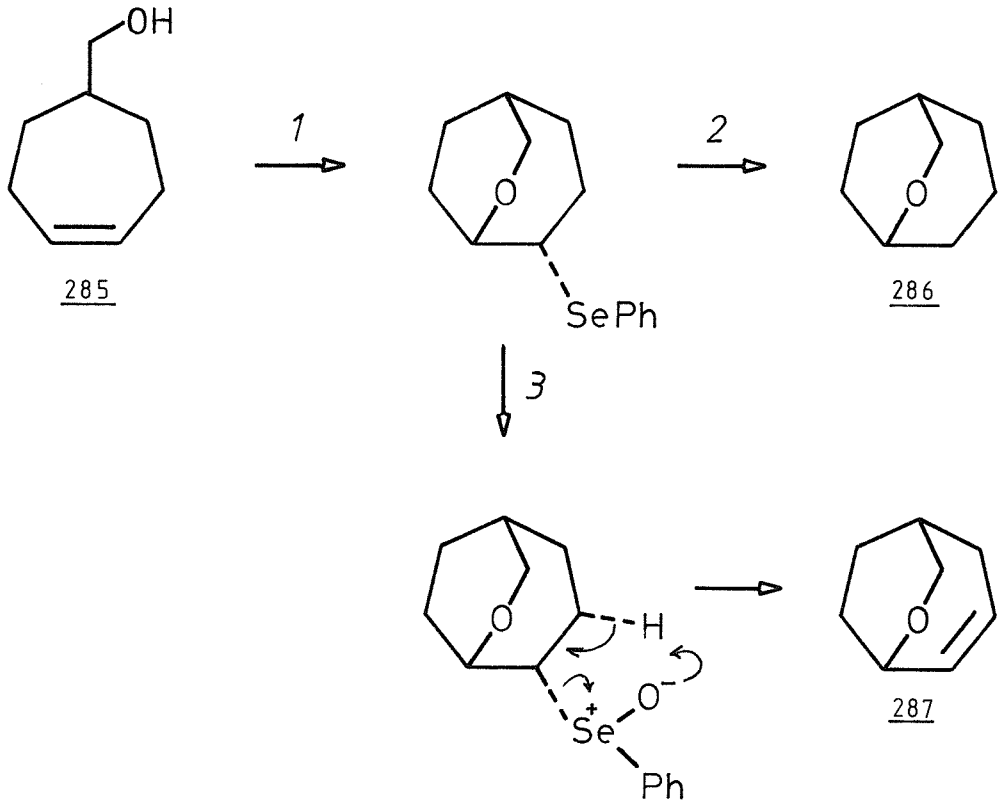
2.6.3 Other Cyclisation Reactions

Palladium stabilised intermediates have been used to prepare cyclic ether systems (Scheme 56)[159]. Palladium (II) acetate was used catalytically in this reaction; copper (II) acetate and oxygen were added to the reaction mixture to reoxidise the palladium (0), formed during the reaction mechanism, to palladium (II). Reaction rate was very dependent on substituents R_1, R_2 and R_3, and by comparing *epi*-α-eudesmol (251) with known examples, that do not cyclise when reacted with palladium (II) acetate because of steric crowding, it seemed unlikely that (251) would react to yield 4,11-epoxy-*cis*-eudesmane (21).

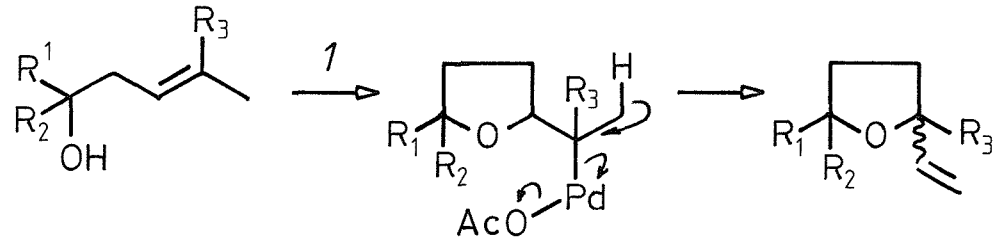
N-bromosuccinimide[160], N-iodosuccinimide[161] and iodine[162], have all been used to activate olefins towards nucleophilic attack. The halide in the intermediate acts very similarly to the mercury (II) ion previously discussed; ring closure then gives the corresponding halide. Reduction



Scheme 54: 1⊢ $Hg(OAc)_2$ /NaCl



Scheme 55: 1⊢ PhSeCl 2⊢ Raney Ni 3⊢ H_2O_2



Scheme 56: 1⊢ $Pd(OAc)_2$ / $Cu(OAc)_2$ / O_2 / aq.MeOH

of the halide to a hydride is usually facile, but the ring closure of *epi*-α-eudesmol (251) would yield a neo-pentyl halide, and previous studies[65] have shown such halides resistant to hydrogenolysis.

2.7 The Ring Closure of *epi*-α-eudesmol (251) to 4,11-Epoxy-*cis*-eudesmane (21)

2.7.1 With Mercuric Acetate

The reaction of *epi*-α-eudesmol (251) with mercuric acetate in 50% aqueous tetrahydrofuran was carefully studied to obtain a maximum yield of 4,11-epoxy-*cis*-eudesmane (21); the parameters of temperature, molar excess of mercuric acetate and reaction time were varied. The results are summarised in Table 4.

TABLE 4

Reaction number	Mole eq of $Hg(OAc)_2$	Solvent	Time	Temp	Yield[a]
1	1	aq THF	12 hrs	20°C	nil
2	2	aq THF	12 hrs	20°C	nil
3	4	aq THF	12 hrs	20°C	4%
4	8	aq THF	12 hrs	20°C	10%
5	8	aq THF	36 hrs	20°C	10%
6	8	THF	12 hrs	20°C	nil
7	8	aq THF	12 hrs	60°C	8%

[a]By gc assay

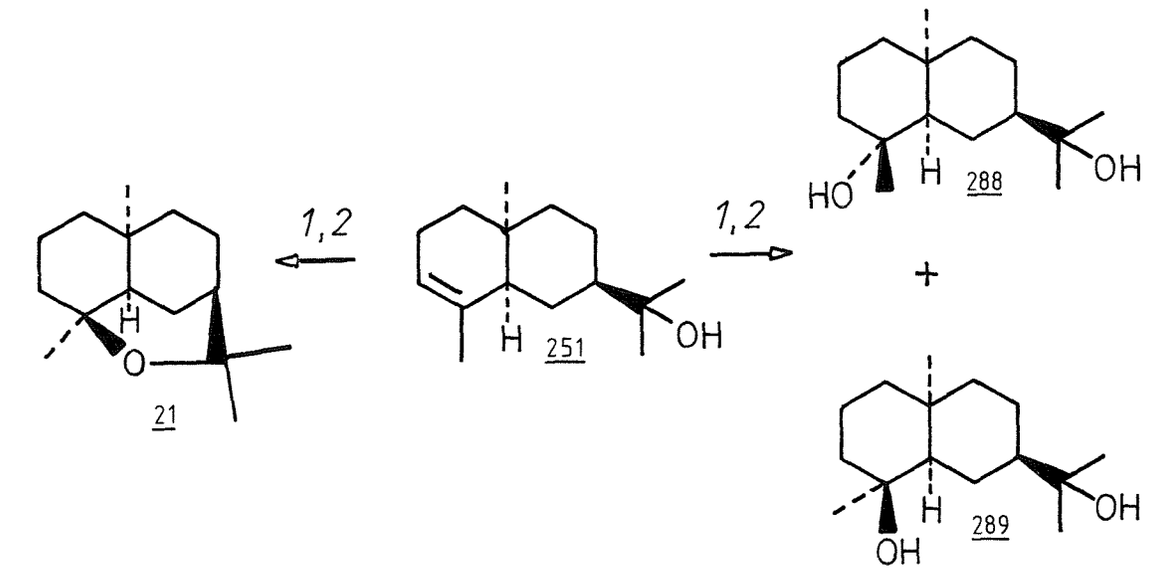
With one or two molar equivalents of mercuric acetate, stirring at room temperature overnight in aqueous THF, no reaction products were detected (tlc and glc). Four equivalents of mercuric acetate were found to give 8% conversion of starting material to one component (gc assay); with an eight molar excess, 20% conversion to one component was observed. Greater than an eight molar excess of mercuric acetate did not affect the percentage conversion. Retention time and coinjection studies of the product and a sample of naturally occurring 4,11-epoxy-*cis*-eudesmane (21) showed them to be the same compound.

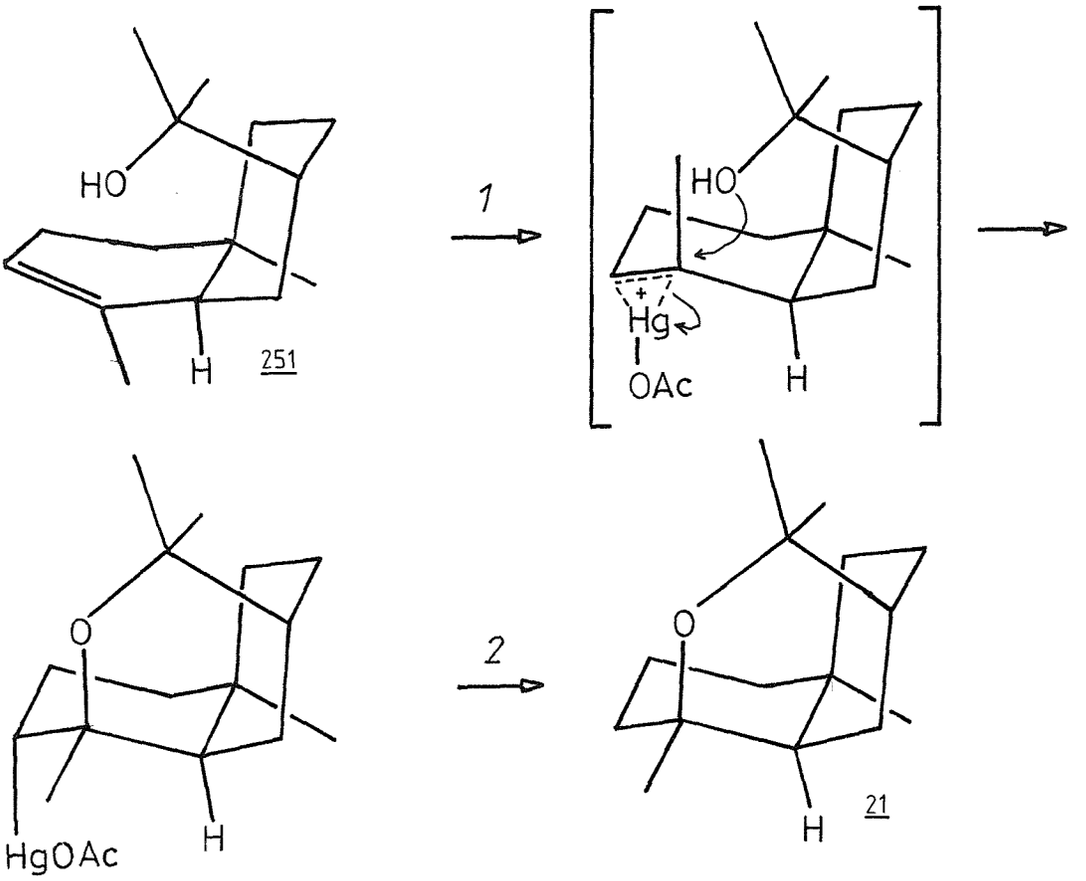
During subsequent reactions, the following observations were made. The initial result using an eight molar excess of mercuric acetate was repeatable; increasing the reaction time did not affect the percentage conversion or the ratio of products by more than 10%; neat THF gave no reaction products, 25% aqueous THF gave low conversion, only products from hydration of the double bond [(288) and (289), Scheme 57] were observed with water as the solvent; the use of a different batch of mercuric acetate did not affect the reaction product or the percentage conversion; and temperature increases only decreased the product yield.

In previous studies by Brown *et al.*,[92] sodium chloride was added to the reaction mixture to afford the intermediate σ-mercury chloride, rather than the acetate. This was repeated, with both a catalytic and molar amount of sodium chloride, but product yield was unchanged.

The yield of 4,11-epoxy-*cis*-eudesmane (21) from reaction 4 (Table 4) was found to be 10%, and 52% of unreacted starting material was recovered. The reaction of *epi*-α-eudesmol (251) with mercuric acetate in water yielded a mixture of hydrated products [(288) and (289), Scheme 57], the remaining 39% of products from this reaction arose from hydration of the double bond.

The results from early reactions were complicated by the appearance of another component after work up, this was later identified as 2,6-(ditert-butyl)-4-methylphenol, an antioxidant added to commercial grade THF. It was found that the presence of antioxidant only reduced the yield of 4,11-epoxy-*cis*-eudesmane to 9%, but more starting material was recovered from the reaction mixture because the yields of the hydration products was reduced.





Scheme 57: 1⊳ $Hg(OAc)_2$ /aq. THF 2⊳ $NaBH_4$

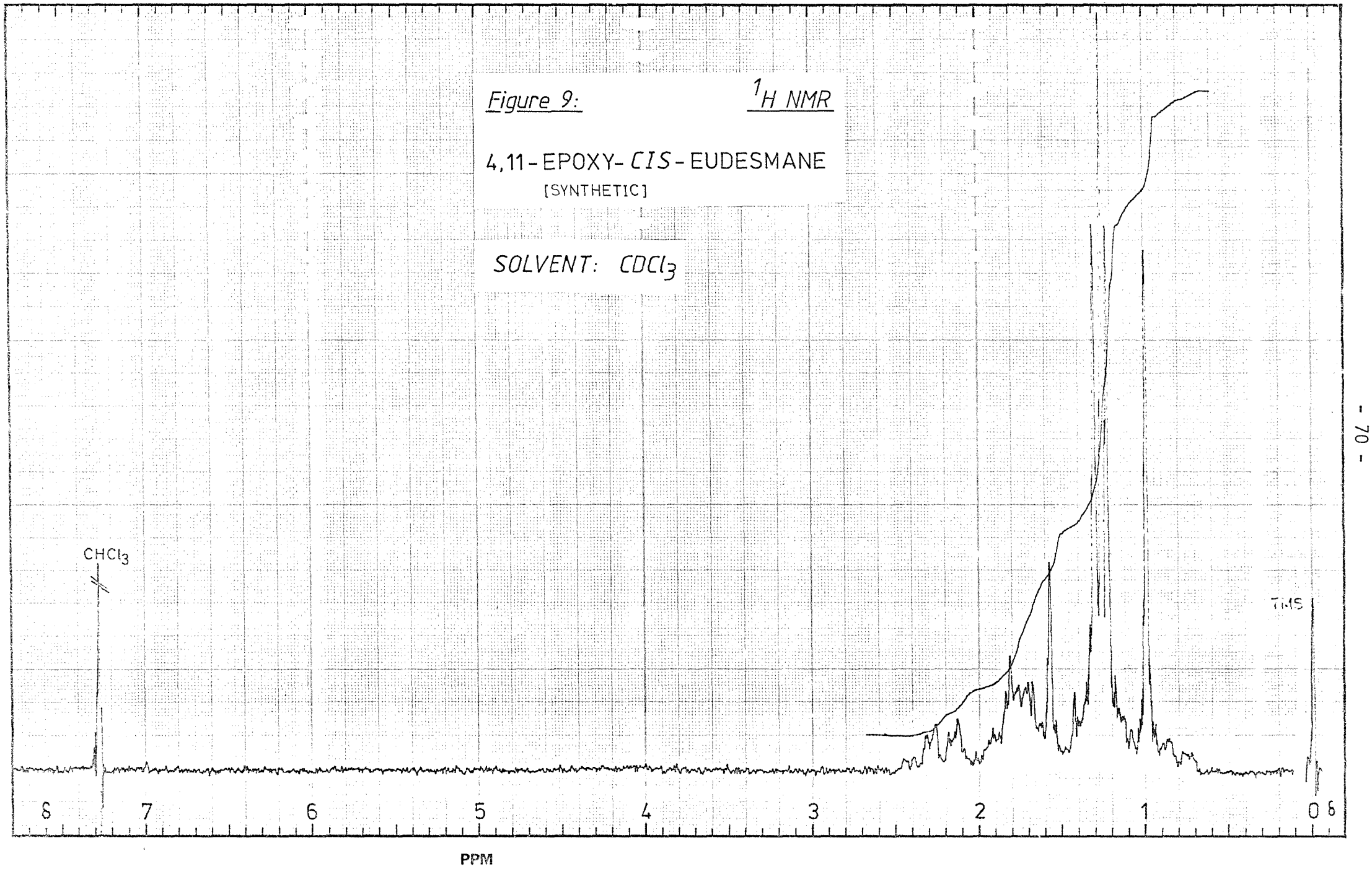


Figure 9:
^1H NMR
4,11-EPOXY-*CIS*-EUDESMANE
[SYNTHETIC]
SOLVENT: $CDCl_3$
$CHCl_3$
TMS
8
7
6
5
4
3
2
1
0 δ
PPM

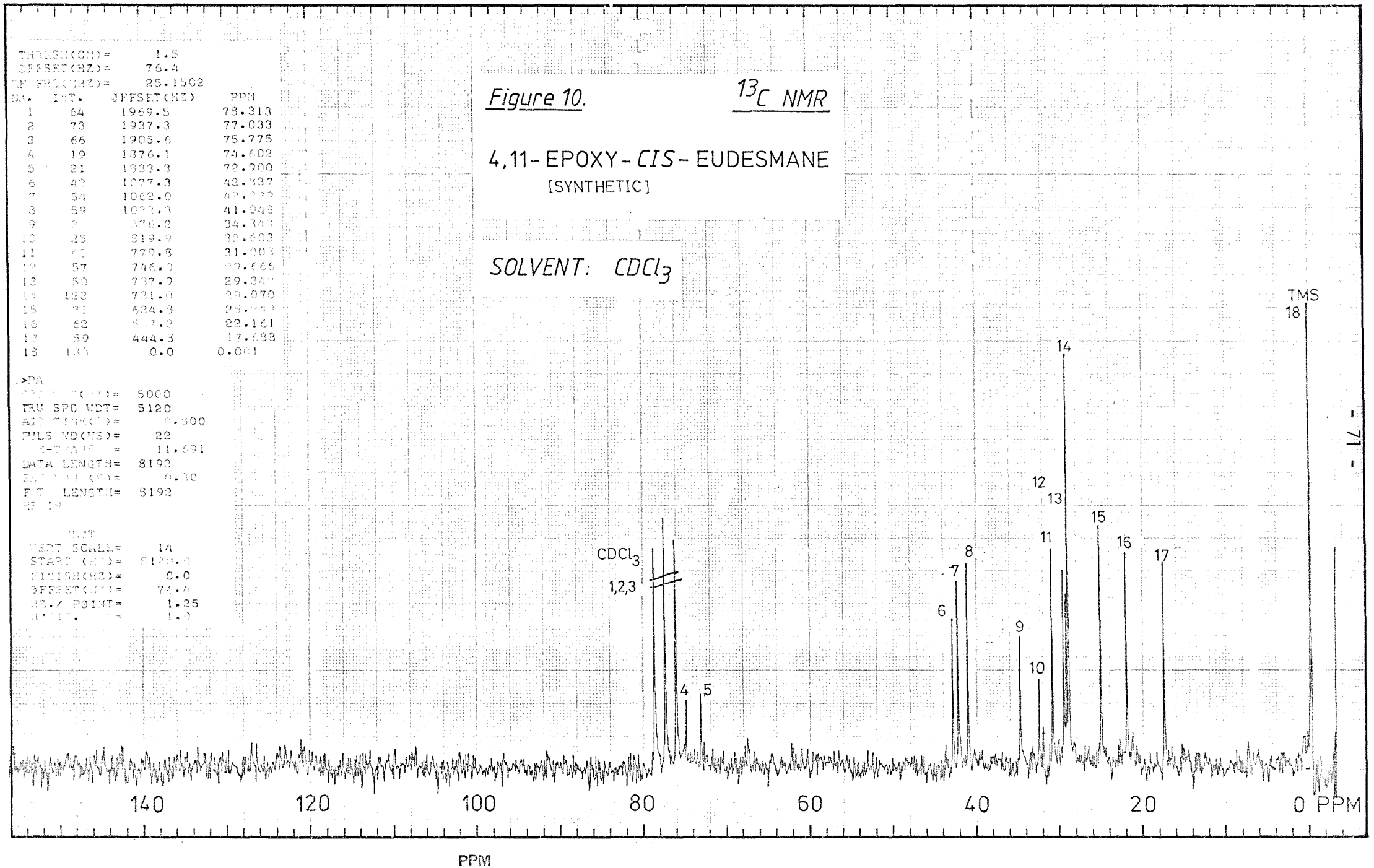


Figure 10.
13C NMR
4,11-EPOXY-CIS-EUDESMANE
[SYNTHETIC]
SOLVENT: CDCl3
TMSS.(GN)= 1.5
OFFSET(HZ)= 76.4
OF FRQ(MHZ)= 25.1502
NO. INT. OFFSET(HZ) PPM
1 64 1969.5 78.313
2 73 1937.3 77.033
3 66 1905.6 75.775
4 19 1876.1 74.602
5 21 1833.3 72.900
6 42 1077.3 42.837
7 54 1062.0 42.228
8 59 1022.3 41.043
9 26 876.2 34.843
10 25 819.9 32.603
11 63 779.3 31.003
12 57 746.0 29.666
13 50 737.9 29.347
14 122 731.6 29.070
15 71 634.8 25.243
16 62 557.3 22.161
17 59 444.3 17.668
18 133 0.0 0.001
>PA
SW(HZ)= 5000
TRU SPC WDT= 5120
ADJ TIME(SEC)= 0.300
PULS WD(US)= 22
D-TIME = 11.691
DATA LENGTH= 8192
D1(SEC)= 0.30
FT LENGTH= 8192
WF EM
PLT
VERT SCALE= 14
START (HZ)= 5120.0
FINISH(HZ)= 0.0
OFFSET(HZ)= 76.4
HZ/ POINT= 1.25
INTG. = 1.0
CDCl3
1,2,3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
TMS
18
140
120
100
80
60
40
20
0 PPM
PPM

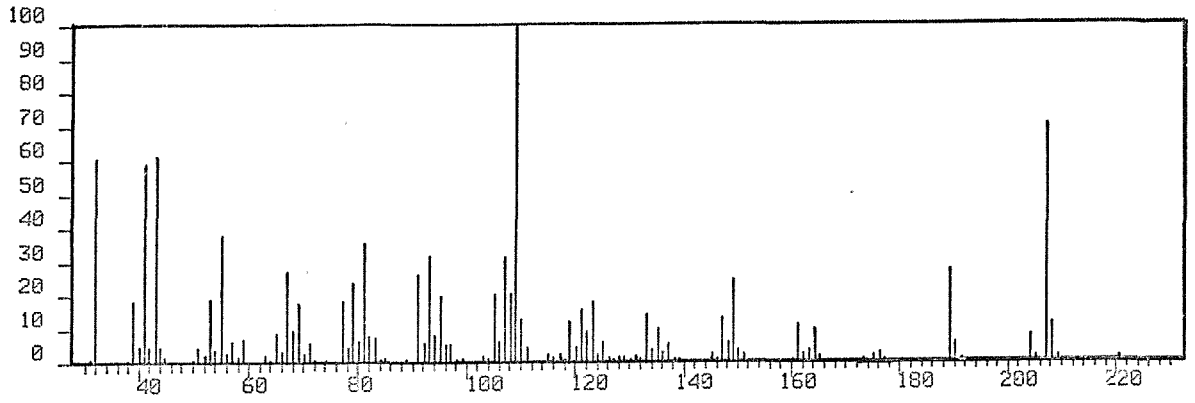


Figure 11: Mass Spectrum of 4,11-epoxy-*cis*-eudesmane.

The required product was separated from starting material by flash chromatography (see Experimental, Chapter 4), and analysis confirmed the reaction product to be 4,11-epoxy-*cis*-eudesmane (21), all spectral data identical to the natural product[21]. The sample was optically active, $[\alpha]_D = -20^o$.

^{1}H NMR (Figure 9, $CDCl_3$, 100 MHz, δ): 1.00(3H,s,CH_3-C); 1.23, 1.26, 1.31(3H,s,CH_3-C-O).

^{13}C NMR (Figure 10, $CDCl_3$, ppm): 17.7, 22.1, 25.2, 29.0, 29.0, 29.3, 29.6, 31.0, 32.6, 34.8, 41.0, 42.2, 42.8, 72.9, 74.6.

MS (m/e): 222(M^+, 0.2%), 208(11%), 207(70%), 189(27%), 149(24%), 123(18%), 109(100%), 107(30%), 93(31%), 91(26%), 81(35%), 79(24%), 67(27%), 55(37%), 43(61%).

IR (thin film, cm^{-1}): 3000-2840, 1460, 1380, 1370, 1360, 1240, 1130, 1090, 1020, 970, 810.

The accepted mechanism of this reaction was the complex formation between the olefin and mercury (II) on the least hindered α-face of *epi*-α-eudesmol (251). Nucleophilic attack by the oxygen at the most

electrophilic centre led the cyclic ether and the σ-mercury complex. Borohydride reduction then cleaved the mercury complex to yield 4,11-epoxy-*cis*-eudesmane [(21), Scheme 57].

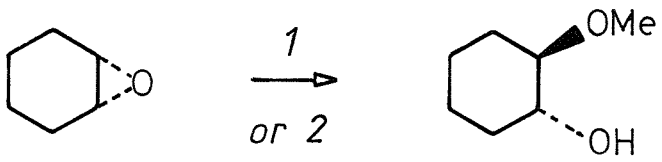
2.7.2 With Phenyl Selenyl Chloride

Epi-α-eudesmol (251) was reacted under the conditions used by Lysenko[157,158]. The alcohol was added to 1.1 molar equivalents of phenyl selenyl chloride in 50% petroleum ether (40-60°C) in methylene chloride solvent at -78°C. The mixture was stirred under N_2 for ½ hour, and then at room temperature for ½ hour; assay of the product mixture showed no remaining starting material. Both oxidative cleavage with H_2O_2 and reductive cleavage with Raney nickel yielded a complex mixture of products which did not contain 4,11-epoxy-*cis*-eudesmane (21).

2.8 Other Cyclisation Methods

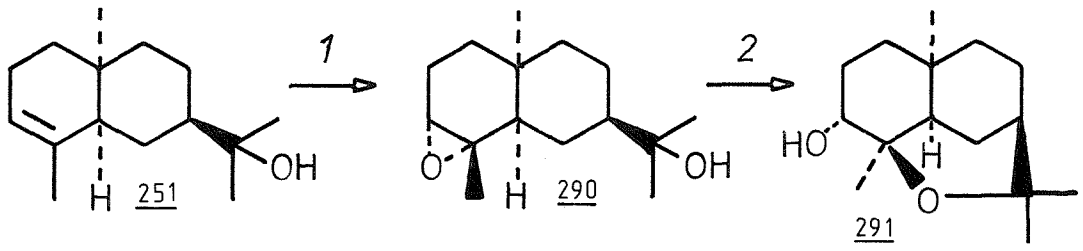
2.8.1 Ring Closure of 3,4-Epoxy-*epi*-α-Eudesmol (290)

In previous studies by other workers, nucleophiles have been used to attack epoxide rings[163-165]. If an alcohol, for example methanol, and an epoxide are mixed with an acid catalyst, a diol monoether is the product (Scheme 58); catalysts that have been used include sulphuric acid[163] and acidic alumina[164].

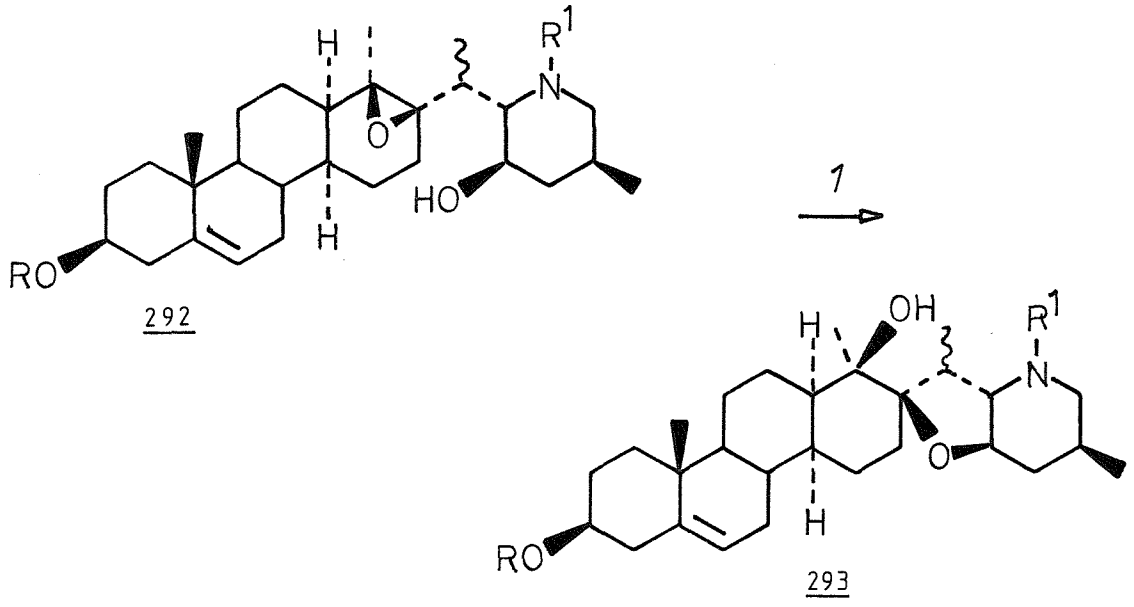


Scheme 58: 1⊢ H_2SO_4 / CH_3OH 2⊢ CH_3OH / acidic alumina

For the preparation of 4,11-epoxy-*cis*-eudesmane [(21), Scheme 59], *epi*-α-eudesmol (251) could be epoxidised (the α-epoxide would be formed due to steric hinderance) and then nucleophilic attack of the tertiary alcohol on the epoxide (290), under acid or base catalysed conditions,



Scheme 59: 1= MCPBA 2= acid catalyst



Scheme 60: 1= KOH / DMSO

should give ether-alcohol (291). Hydrogenolysis of the alcohol would then yield 4,11-epoxy-*cis*-eudesmane (21). There is a precedent in the literature for this reaction sequence (Scheme 60)[165].

When a model of structure (292) was made, the alcohol group was positioned under the plane of the epoxide, allowing reaction (via the alkoxide) to the 5-membered ring ether (293). When a model of 3,4-epoxy-*epi*-α-eudesmol (290) was made, a similar positioning was found, but steric restraints did not allow the alcohol and the epoxide to be as close as in the previous example.

Epi-α-eudesmol (251) was converted to epoxide (290) with MCPBA in methylene chloride in 99% yield. The epoxide (290) was treated with potassium hydroxide in dimethylsulphoxide (DMSO) overnight at room temperature, but no reaction was observed. Stronger bases, such as potassium hydride in THF and potassium in toluene under reflux were tried, in both cases the alkoxide was seen to form but only starting material was obtained on work up. Following these unsuccessful reactions using bases, acid catalysis was attempted.

The epoxide (290), dilute sulphuric acid and THF were stirred overnight at room temperature. Reaction products were seen, but these resulted from dehydration of the starting material, glacial acetic acid gave a similar product mixture.

The epoxide (290), acidic alumina and THF were stirred overnight at room temperature. Filtration of the alumina followed by analysis by gc showed a reaction product (not derived from dehydration) and unreacted starting material. The reaction was repeated under reflux overnight. Work up, followed by gc assay of the product mixture, showed 100% reaction of the starting material to two products, in a 4:1 ratio. The major product was believed to be the required product (291) and the other product was identified as dehydrated starting material. Separation followed by spectral analysis showed the product was not alcohol-ether (291). The spectral data are listed below:

^{1}H NMR: (100 MHz,$CDCl_3$,δ): 0.84(3H,s); 1.20(6H,s); 4.26(1H,t,J=2½Hz); 4.88(2H,dxd,J=3Hz,9Hz)

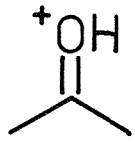
IR (thin film, cm^{-1}): 3360, 3000-2800, 1635, 1450, 1350, 905.

MS (MS30, m/e): 238(0.3%), 220(5%), 205(6%), 162(92%), 147(72%), 133(45%), 119(41%), 107(41%), 106(36%), 105(45%), 93(38%), 91(39%), 59(100%), 43(38%).

M^+ measured 238.2133 $C_{15}H_{26}O_2^+$, calculated 238.1933.

The spectral data of the product indicated protons in the olefinic region of the NMR spectrum, this is not consistent with structure (291).

The ir spectrum shows an alcohol and an olefin. The mass spectrum shows the presence of m/e 59 (as base peak) due to the ion below:



$C_3H_7O^+$ m/e 59

This ion is characteristic of an isopropanol side chain, and is common to all structures of this type. The ion (M^+-18) is also seen (usually indicative of an alcohol in the molecule), but this is not normally seen in compounds with an isopropanol side chain. It is, therefore, likely that another alcohol is present in the molecule.

Three signals observed in the NMR spectrum were assigned to methyl groups, two coincident at 1.20δ and one at 0.84δ; the former are those on the side chain adjacent to a carbon attached to an oxygen, the latter is the bridgehead methyl groups; this shows the loss of one methyl group from the starting material. Other signals of interest were observed at 4.26 and 4.88. At 4.26δ, a triplet that integrates as 1 proton. This signal is in the position for a proton on a carbon also attached to an oxygen atom (Tables value 3.9δ), but it can be seen that the actual signal is downfield; this suggests that the carbon to which the proton is attached, is also bonded to an olefinic system (Tables value 4.13δ). The triplet splitting may be attributed to an adjacent methylene group. The other signals found at 4.88δ, are two olefinic protons. The coupling constant of 3Hz suggests germinal coupling and therefore an *exo*-methylene group. The coupling constant of 9Hz is very large for olefinic coupling and suggests non equivalence of the two protons. Therefore the postulated structure for this product (294) is shown in Figure 12, also included is the proposed mechanism for its formation.

This seems to be the first example of such a reaction under acidic conditions, although similar reactions have been obtained with diphenyl diselenide[166,167] and bases such as the lithium dialkylamides[168-170], lithium alkyls[171,172] and potassium t-butoxide[173].

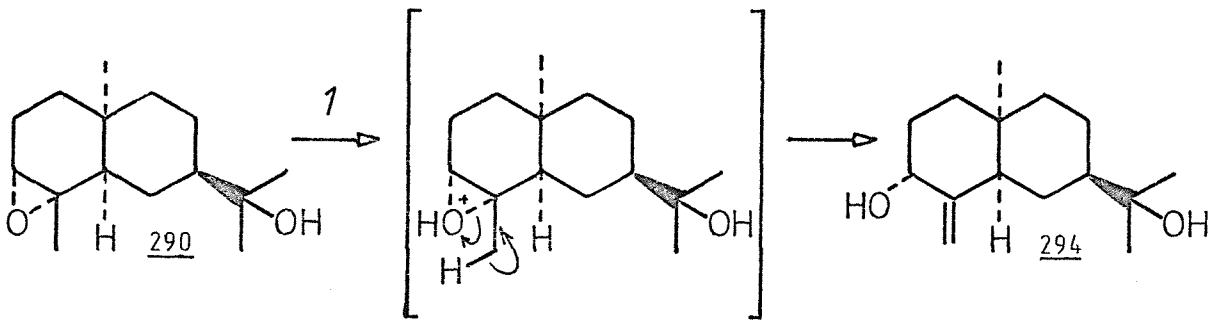


Figure 12: 1⊢ acidic alumina

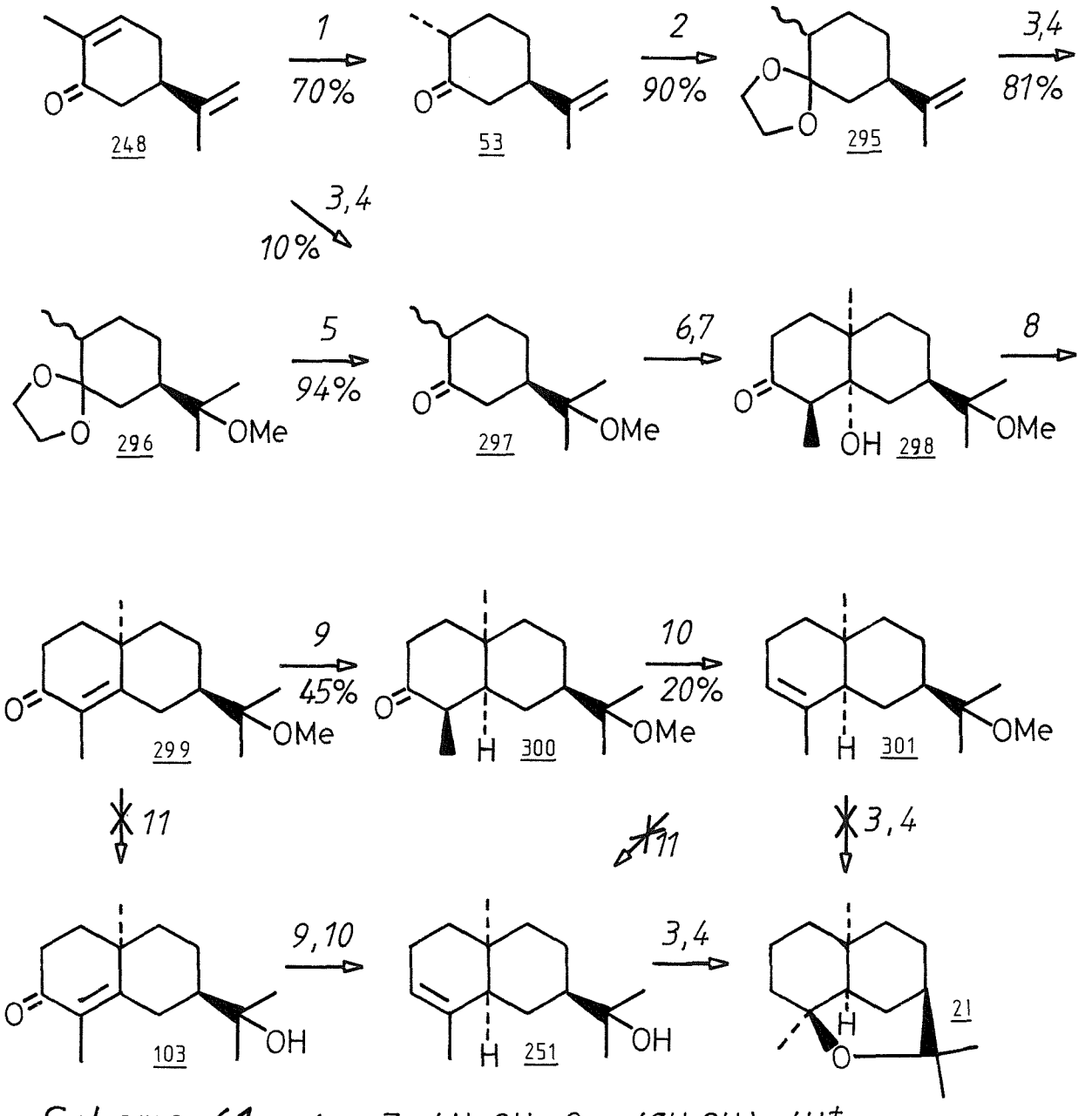
With this compound resulting from the reaction, it was not possible to prepare 4,11-epoxy-*cis*-eudesmane (21) by this route, if a method of hydrogenolysis of the alcohol functionality could be found (294) could be a precursor to *epi*-β-eudesmol (252). This could then be converted to 4,11-epoxy-*cis*-eudesmane (21) by a mercury catalysed cyclisation.

2.8.2 Ring Closure of *Epi*-α-Eudesmol Methyl Ether (301)

The final planned synthetic route to 4,11-epoxy-*cis*-eudesmane (21) involved the mercury catalysed cyclisation of *epi*-α-eudesmol methyl ether (301). The advantage of this route was the alcohol was protected throughout the reaction sequence and the final intermediate (301) could possibly be cyclised to the required product [(21), Scheme 61].

2.8.2.1 Synthesis of *Epi*-α-Eudesmol Methyl Ether (301)

(+)-Dihydrocarvone (53) was methoxymercurated by stirring with mercuric acetate in methanol in the dark overnight. The product was obtained by sodium borohydride work up. The reaction product contained many components including the required ether (297), but the reaction was of little use since a mixture of products were obtained. It was postulated that the borohydride work up was affecting the ketonic functionality so ketone (53) was protected as the ketal (295) in over 90% yield by refluxing in benzene with ethylene glycol and p-toluene sulphonic acid. Methoxymercuration of the ketal yielded 81% of the protected methyl ether (296) as a clear oil. Dihydrocarvone hydrate methyl ether (297)



Scheme 61: 1⊢ $Zn / NaOH$ 2⊢ $(CH_2OH)_2 / H^+$

3⊢ $Hg(OAc)_2$ / aq. THF 4⊢ $NaBH_4$ 5⊢ acetone / H^+

6⊢ NaH 7⊢ $ClCH_2CH_2CO.CH_2CH_3$ 8⊢ HCl / EtOH

9⊢ H_2 / Pd on C 10⊢ Bamford-Stevens 11⊢ demethylation SEE TEXT

was obtained in 94% yield by acid catalysed transketalisation in acetone. An overall yield of 69% from dihydrocarvone (53) was obtained. The optical activity of the molecule was lost temporarily due to epimerisation of the α-methyl to the ketone during protection. Annelation of the methyl ether (297) under the conditions previously described, yielded the intermediate ketol methyl ether (298), subsequent dehydration with methanolic hydrochloric acid led to dehydration products, not the required methyl ether (299).

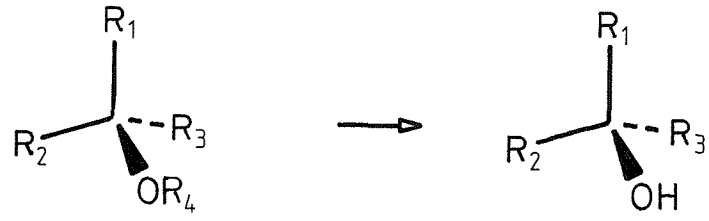
A milder method of dehydration was sought, so the intermediate ketol (298) was refluxed with florisil in benzene[174]. After reaction overnight only starting material was observed. The ketol (298) was refluxed overnight with basic alumina in benzene, only one product resulted with 100% conversion (gc assay) in 53% yield. This product had spectral data that was in accordance with *epi*-α-cyperone methyl ether (299), the optical activity had returned, $[\alpha]_D = -55^o$. Hydrogenation of (299) over palladium on charcoal gave a 3:1 *cis:trans* ratio of hydrogenated ethers. It was assumed that the *cis* (300) would predominate as found in previous cases. The two ring junction isomers were separated by flash column chromatography (see Experimental, Chapter 4), to afford pure *cis*-dihydro-*epi*-carrisone methyl ether (300), in 45% yield from (299). Compound (300) was then refluxed with p-toluenesulphonic acid and hydrochloric acid in methanol to prepare the corresponding hydrazone. Thermal elimination with sodium in ethylene glycol at 150°C under nitrogen for 1½ hours yielded one major product in 20% yield after purification. Spectral analysis identified the product as *epi*-α-eudesmol methyl ether (301), $[\alpha]_D = +20^o$.

2.8.2.2 Ring Closure of *Epi*-α-Eudesmol Methyl Ether (301)

Ring closure of this intermediate was undertaken with mercuric acetate (8 molar excess) in aqueous THF, the conditions discussed in an earlier section. Borohydride work up showed mainly starting material and a small amount of hydrated product but no 4,11-epoxy-*cis*-eudesmane (21). The reaction conditions were changed but still none of the required product was obtained.

The ring closure of the ether oxygen on the mercury complexed double bond is far less likely than with a more electronegatively charged species such as an alcohol. Ethers are known to possess electronegative character, an example of which is demonstrated by the boron trifluoride etherate complex which is easily formed from bubbling boron trifluoride through ether. It could be that the mercury bridged intermediate is not electrophilic enough to attract a poor nucleophile such as an ether.

Deprotection of either of the intermediate ethers [(299) and (301)] would lead to the corresponding alcohols [(103) and (251) respectively] and thus allow reaction to 4,11-epoxy-*cis*-eudesmane (21) as carried out before (See Scheme 61). Many methods exist to form an alcohol from an ether (Scheme 62) but primary (R_1=R_2=H) and secondary (R_1=H) ethers have



Scheme 62.

been found to be far easier to deprotect to the corresponding alcohols[175-179] than tertiary ethers (R_1, R_2, R_3≠H). There are no reported examples of the deprotection of tertiary ethers to alcohols, although reactions to give olefins or halides are known[180,181]. A few of the standard methods of deprotection were tried; reaction of both (299) and (301) with boron tribromide in methylene chloride[175-177] yielded complex mixtures of dehydrated and rearranged products, trimethylsilyl chloride and sodium iodide in acetonitrile[178] and chloroform[179] gave similar mixtures.

Unfortunately, this scheme of reactions could not be used to prepare 4,11-epoxy-*cis*-eudesmane because it was not possible to get intermediate (301) to undergo cyclisation or deprotect (299) and (301) to the corresponding alcohols.

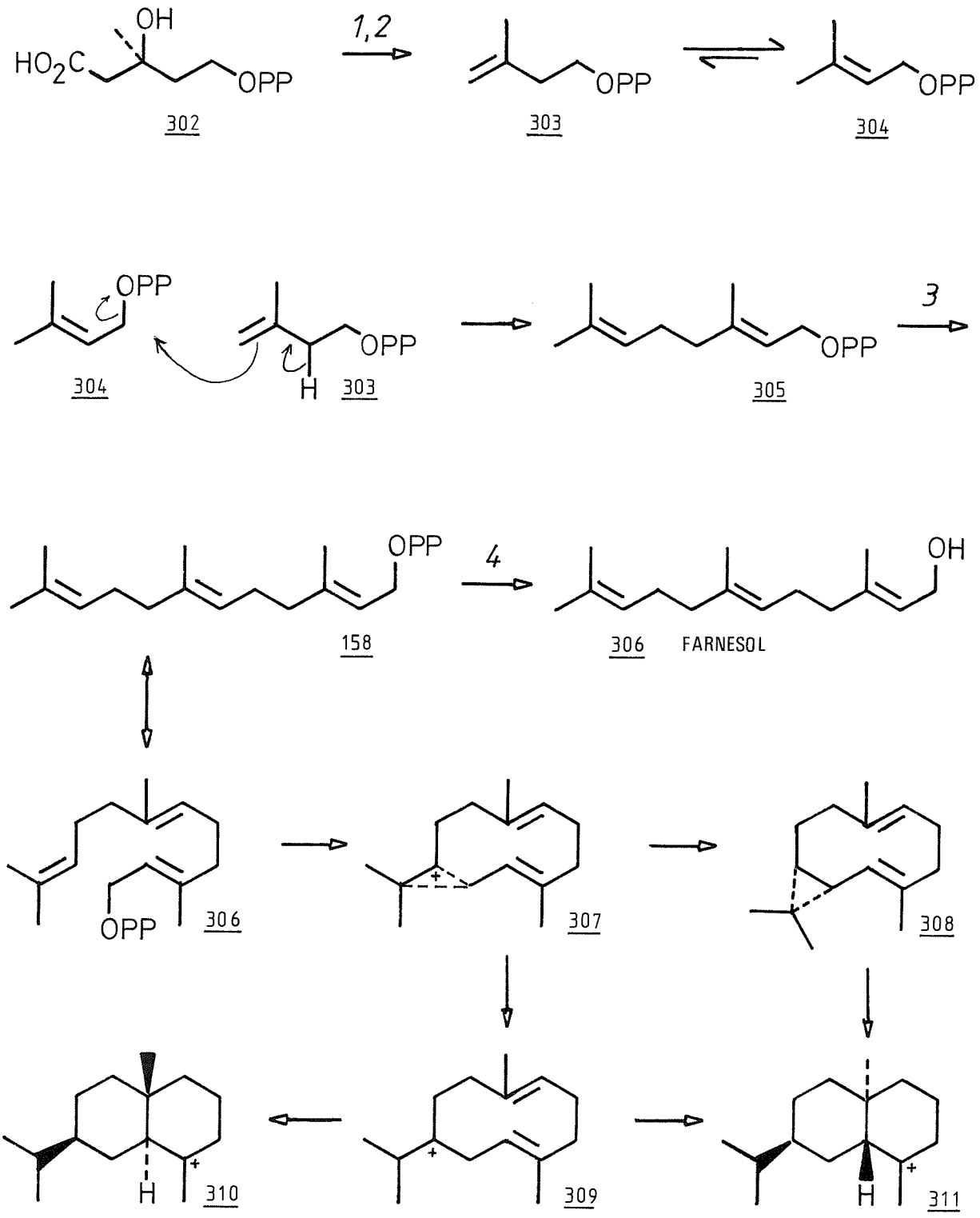
The failure of this route prompted all attention to be paid to the large scale synthesis of 4,11-epoxy-*cis*-eudesmane using the mercuration demercuration of *epi*-α-eudesmol (251) as the final step.

2.9 Large Scale Synthesis of 4,11-Epoxy-*cis*-Eudesmane

The reaction sequence used has been shown in Schemes 45, 47, 51 and 57. The scaling up of reactions generally resulted in a decrease in yields. The synthesis of *epi*-carrisone (103) was found to proceed in an overall yield of 13% from carvone (248) compared to the smaller scale yield of 19%. Yields for the annelation and dehydration step decreased to 25%, epoxidation was as efficient at 95%, lithium aluminium hydride reduction proceeded in 91% yield and oxidation with pyridinium dichromate yielded *epi*-carrisone (103) in 69%. Hydrogenation and separation gave *cis*-dihydro-*epi*-carrisone (104) in a yield of 35%. The Bamford-Stevens reaction afforded 30% of *epi*-α-eudesmol (251), and cyclisation with mercuric acetate yielded a mixture of starting material (251) and 4,11-epoxy-*cis*-eudesmane (21) in a ratio of 6:1 (gc assay). Separation by flash chromatography yielded two fractions, *epi*-α-eudesmol (251) and 4,11-epoxy-*cis*-eudesmane (21). The *epi*-α-eudesmol (251) recovered was cyclised with mercuric acetate under the same conditions to yield a similar mixture of products. *Epi*-α-eudesmol (251) was again separated and cyclised, this procedure was repeated twice more. The fractions containing 4,11-epoxy-*cis*-eudesmane (21) were collected and (21) was purified further on another flash column; 0.25 grams of material were obtained (14% yield).

2.10 Biosynthesis of Eudesmane Sesquiterpenes

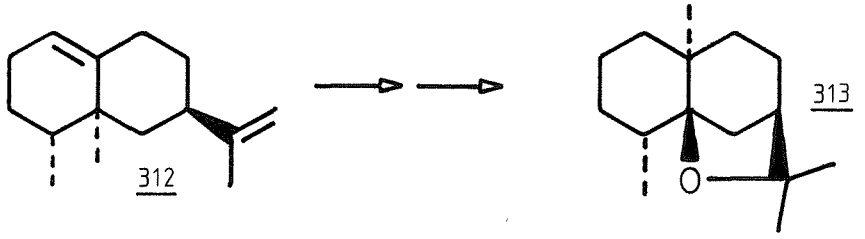
Many papers have been published suggesting biosynthetic pathways to the eudesmane skeleton. Labelling experiments have proved the precursor to all the alicyclic, monocyclic and bicyclic sesquiterpenes to be farnesyl pyrophosphate (158), which is derived from acetyl coenzyme A; via mevalonic acid [(302), Scheme 63][182-185].



Scheme 63: 1⊢ decarboxylate 2⊢ dehydrate 3⊢ repeat 4⊢ hydrolysis

Farnesyl pyrophosphate (158), when in conformation (306), may ring close under acid catalysed conditions to either cyclopropane system (308) or germacrane carbonium ion (309), via intermediate (307). Intermediate (309) is the precursor to both the eudesmane series (310) and the selinane series (311) of sesquiterpenes. These pathways have been reviewed by Rucker[186] and Cordell[187].

At present, labelling studies have not been conducted on 4,11-epoxy-*cis*-eudesmane (21), but a dihydroagarofuran (313) has been found to be a metabolite of valencene (312) in *Enterobacteria* spp. (Scheme 64)[188].



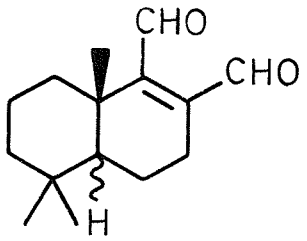
Scheme 64.

2.11 Conclusions

4,11- Epoxy-*cis*-eudesmane (21), a naturally toxic compound, has been previously isolated. A number of synthetic routes to this structure have been explored, in view of a large scale synthesis of (21). The successful route allowed 0.25 gm of the required component to be prepared with an overall yield of 0.2% from l-carvone (248). Other routes included the use of different internal ether cyclisations and ring annelation reactions, but these failed to yield 4,11-epoxy-*cis*-eudesmane (21). The available material will now be forwarded for toxicity testing.

3.1. Introduction

In Chapter 1, the extraction of soldiers from the termite species *Ancistrotermes cavithorax* was described. The *major* soldiers were found to contain a novel dialdehyde, and from the spectral data structure (155) has been suggested. An unambiguous synthesis was required to confirm this assignment.



155

Schemes to cavidial (155) were considered.

3.2. Routes to Cavidial (155)

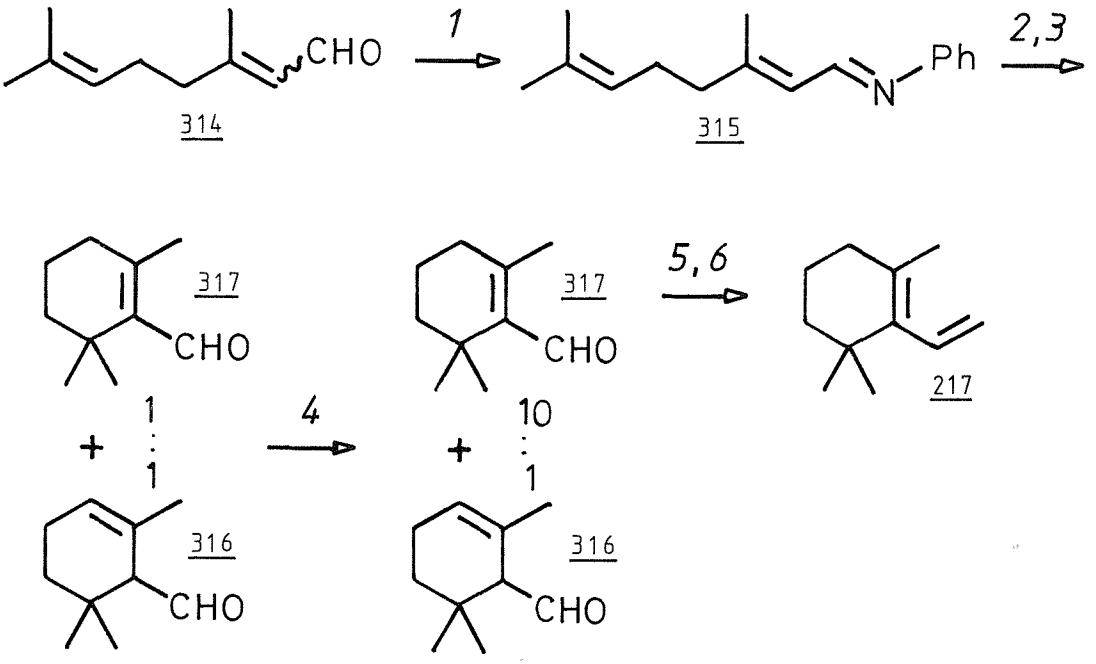
The proposed structure of cavidial (155) was very similar to that of winterin (141), previously synthesised by Brieger (Scheme 36)[103]. That route allowed the bicyclic skeleton to be constructed by a Diels-Alder reaction in poor yield. The reaction of the diene (217) with acetylene dicarboxylic acid gave anhydride (220) in 4% yield. It was hoped that by changing the reaction conditions, an adduct could be obtained in high yield which could then be used as an intermediate in the synthesis of cavidial (155).

3.2.1. Synthesis of 1-vinyl-2,6,6-trimethylcyclohexene (217)

The envisaged Diels-Alder reactions all required diene (217) which was prepared in a two stage synthesis from the readily available citral (314, Scheme 65)[189]. Citral (314), a mixture of geranial and neral, was converted to an imine (315) by the addition of 1 equivalent of aniline in ether. The citral anil (315) in ether was then decanted from the water that had been produced and added dropwise to 95% sulphuric acid at -20°C under nitrogen. Ether

CHAPTER 3

Synthetic Studies of Cavidial



Scheme 65: 1⊢ aniline / Et_2O 2⊢ H_2SO_4 3⊢ steam distil 4⊢ KOH / MeOH 5⊢ distil 6⊢ $Ph_3P{=}CH_2$

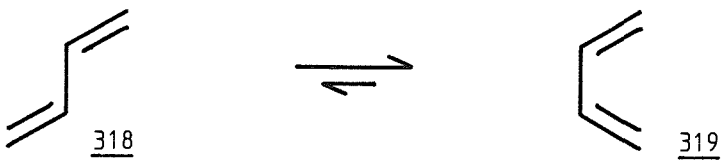
extraction of the brown oily product mixture yielded two major products in a 1:1 ratio; these were shown to be α-cyclocitral (316) and β-cyclocitral (317) respectively. This mixture was then treated with two molar equivalents of potassium hydroxide in methanol to alter the product ratio to 1:10 α(316):β-cyclocitral (317). Distillation afforded pure β-cyclocitral (317) in 60% yield, bpt 56-58°C @ 25 mmHg; and α-cyclocitral (316) in 6% yield, bpt 48-52°C @ 2.5 mmHg. The yield of this reaction was found to decrease when the scale of the reaction was increased due to an increase in polymer formation.

β-Cyclocitral [(317), >99% pure] was then reacted with methylene triphenylphosphorane (prepared from triphenylphosphine and potassium t-butoxide in refluxing THF under a nitrogen atmosphere) at 0°C under nitrogen. Work up and distillation yielded diene (217) in 60% yield, bpt 54-56°C @ 2 mmHg.

3.2.2. Diels Alder Reactions of Diene (217) with Selected Dienophiles

Previously reported reactions have shown 1-vinyl-2,6,6-trimethyl-cyclohexene (217) to react with dienophiles. Brieger showed diene (217) to react with acetylene dicarboxylic acid to give anhydride [(220), Scheme 36] in 4% yield[103]. Campos *et al* reacted diene (217) with maleic anhydride and obtained anhydride [(223), Scheme 38] in 21% yield[105].

The low yields of these reactions may be predicted if the Diels Alder reactions of dienes are considered. Open chain dienes exist in the conformational equilibrium below; but only the cisoid conformer (319) will undergo



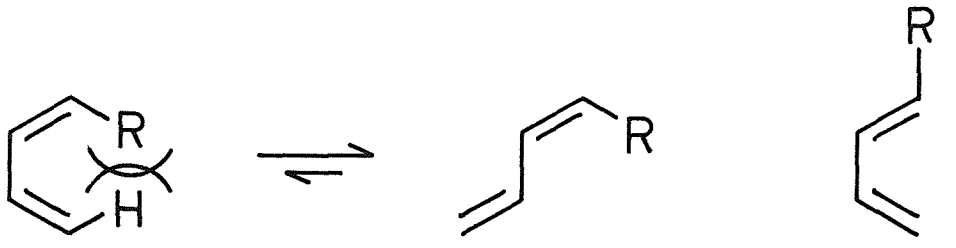
318 319

Scheme 66.

Diels-Alder reactions. It is then possible to show that if a molecule is held in the S-*trans* conformation (318), reaction with a dienophile will be impossible; conversely cycloaddition will be facilitated by a molecule held in the cisoid conformer (317) by substituents at positions 2 and 3.

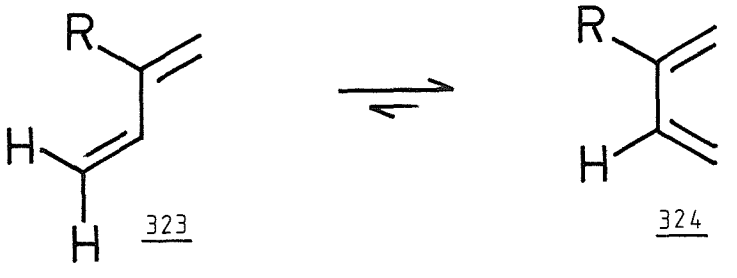
Cis-1-substituted butadienes are much less reactive than the corresponding *trans*-dienes. An example is the reaction of Z-2,4-pentadiene (320) with maleic anhydride which provides only a 4% yield of the cycloadduct[192], whereas the E compound (322) affords a quantitative yield of adduct. The low yield obtained must be due to the equilibration of (320) to the transoid conformer (321) which is unable to react in cycloaddition reactions.

2-Substituted butadienes (324) also exist in the dynamic equilibrium shown in Scheme 67; but if the substituent is bulky, rotation about the



320 321 322

single bond [to the transoid isomer (323)] is hindered and therefore the ability to react in cycloaddition reactions will be enhanced.[193]

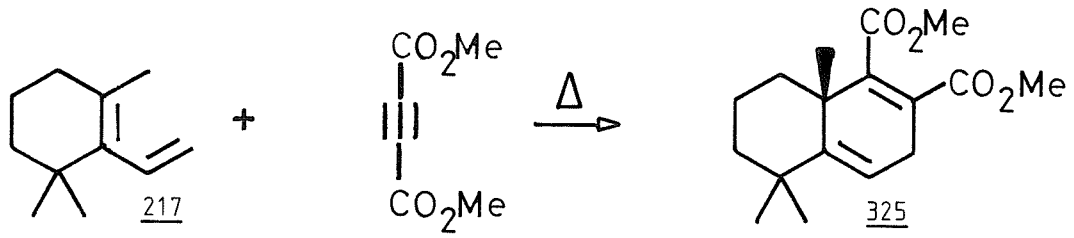


323 324

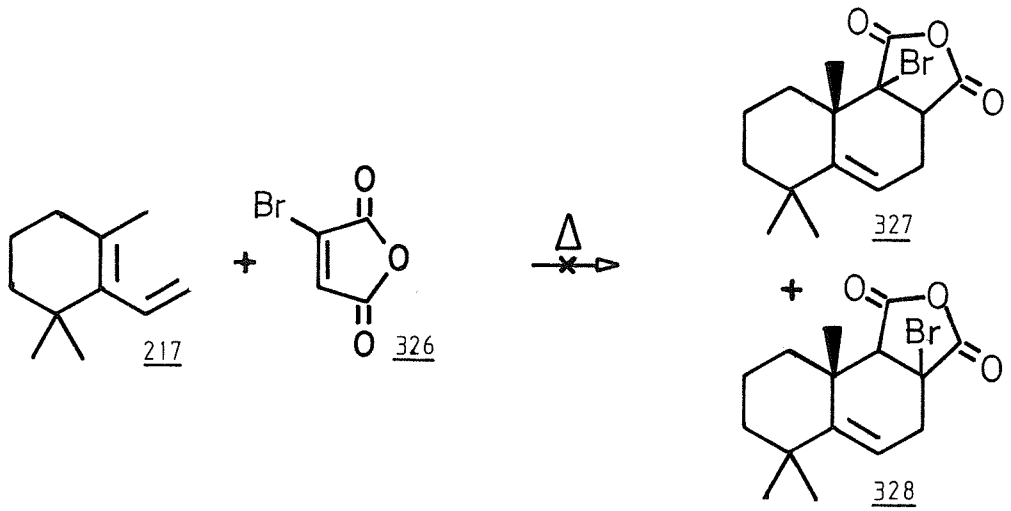
Scheme 67.

Diene (217) is highly substituted and both factors that effect the conformation are operating. The molecule contains a methyl group in the 2-position preventing it from obtaining a s-*cis* coplanar conformation. However, the 6,6-dimethyls will not allow the diene to adopt a coplanar s-*trans* conformation. This suggests that diene (217) is held in a S-*cis* conformation but cannot achieve a coplanar orientation. The predicted outcome of these factors would be a reluctance of diene (217) to react with dienophiles.

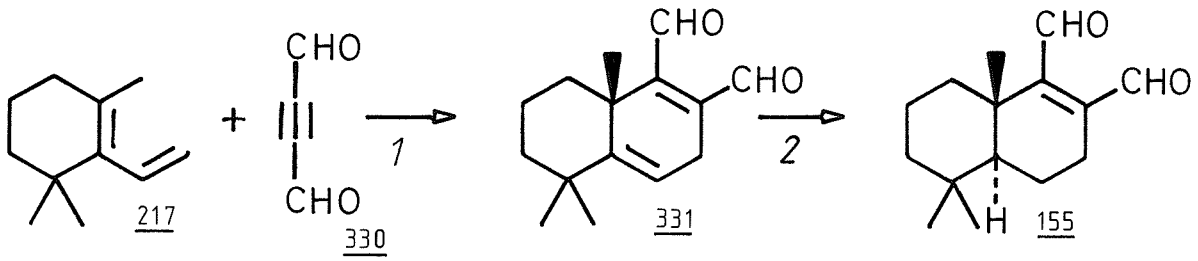
In this work, the reaction reported by Brieger was repeated, but it was found to be difficult to isolate the anhydride product. Dimethyl acetylene dicarboxylate (DMAD) was used instead of the diacid and the yields of the cycloaddition improved significantly (Scheme 68).



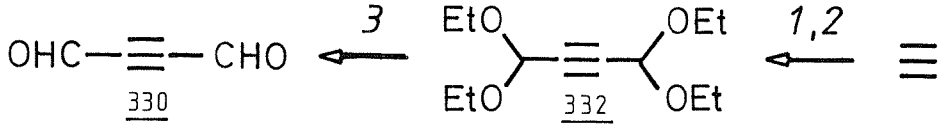
Scheme 68.



Scheme 69.

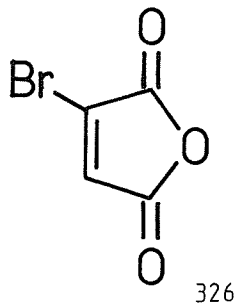


Scheme 70: 1⊢ 0°C / CH_2Cl_2 2⊢ H_2 / catalyst



Scheme 71: 1⊢ EtMgBr / Et_2O 2⊢ $HC(OEt)_3$ 3⊢ HCOOH

Bromomaleic anhydride (326) has been reacted with diene systems such as (329) in Diels-Alder reactions[124]. When bromomaleic anhydride was reacted with diene [(217) Scheme 69] under a variety of conditions, only unreacted starting material and decomposition products were observed.



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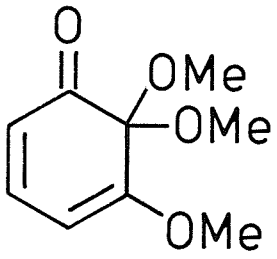

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330

Recently the preparation and reactions of acetylene dicarbaldehyde (330) have been discussed[195,196]. This dialdehyde has been reported to react with activated dienes in Diels-Alder reactions; a similar adduct with diene (217) would yield an intermediate (331).that on hydrogenation would afford cavidial [(155), Scheme 71]. The dialdehyde (330) was prepared from acetylene via the tetraethoxy derivative [(332), Scheme 71][194] and reacted with diene (217) under the reported conditions. Work up procedures showed only the presence of the starting diene (217) and polymer. Changing the reaction conditions did not afford the required product.

Many different Diels-Alder reactions of diene (217) were tried but poor or no yields were obtained - supporting the conclusions previously suggested. By the use of very forcing conditions, dimethylacetylene dicarboxylate reacts with diene (217) in fairly high yield. A series of reactions were completed to optimise the yields of this reaction.

3.2.3. Diels Alder Reaction of Diene (217) with DMAD to give Dimethyl 3,5,6,7,8,8a-Hexahydro-5,5,8a-Trimethyl-1,2-Naphthalenedicarboxylate (325)

One equivalent of diene (217) and 1.2 equivalents of DMAD were heated together at a preset temperature overnight in either a sealed tube or in a flask under nitrogen. As specified below, the volatile components

(unreacted starting materials) were removed by distillation to leave a yellow oil which was chromatographed by flash column chromatography (see Experimental, Chapter 4). The results are recorded (Table 4).

TABLE 4

Temperature °C	Vessel	Yields %	
		Product (325)[a]	Byproduct[a]
80	tube[b]	0%	0%
100	tube[b]	40%	3%
120	tube[b]	87%	3%
140	tube[b]	63%	8%
160	tube[b]	50%	30%
200	tube[b]	10%	80%
120	tube[b]	60%	5%
120	flask[d]	80%	10%

[a]After purification by column chromatography

[b]Sealed under vacuum

[c]Sealed with air atmosphere

[d]Under a nitrogen atmosphere

Two products were usually formed, their ratio being controlled by the reaction conditions. The required product (325) was separated from the byproduct by column chromatography and then the unknown was analysed spectroscopically, the data is listed below:

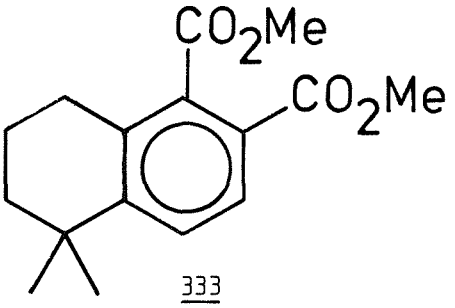
^{1}H NMR(60MHz,$CDCl_3$,δ): 1.25(6H,s,2xCH_3); 1.5-1.9(4H,m,2xCH_2); 2.4-2.8 (2H,m,benzyl CH_2); 3.85,3.92(3H,s,OCH_3); 7.48, 7.85(2H,ABq,J=9Hz,aromatic H).

IR(Film, cm^{-1}): 3100-2850, 1740, 1725(C=O), 1600(aromatic CH), 1440, 1300-1250(CO), 1200, 1160, 1100.

MS(MS30,m/e): 276(M^+,0.6%), 245(43%), 244(100%), 229(76%), 201(29%), 186(33%), 158(26%), 143(28%), 141(28%), 129(39%), 128(55%), 127(23%), 115(57%), 91(16%).

UV(pentane, max): 241(ε = 9400), 281(1500), 289(1450).

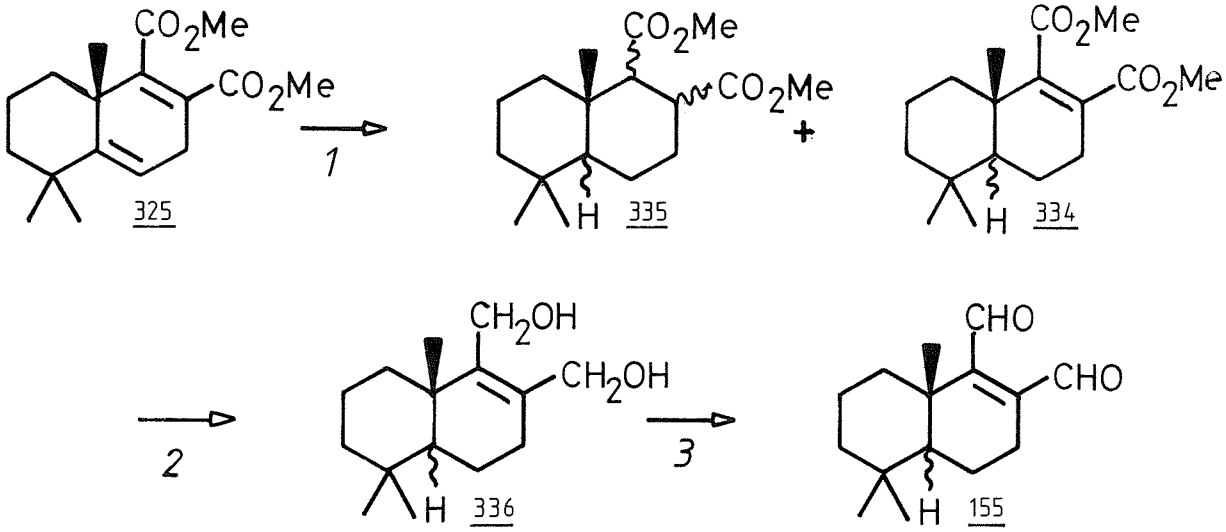
From this data the byproduct, obtained as a clear oil, was identified as 5,6,7,8-tetrahydro-5,5-dimethyl-1,2-naphthalenedicarboxylate (333). This compound has been previously reported as the major product from the pyrolysis of diester (325) at 200°C in triglyme[198]. It was proposed that it was formed by the thermal elimination of the bridgehead methyl group.



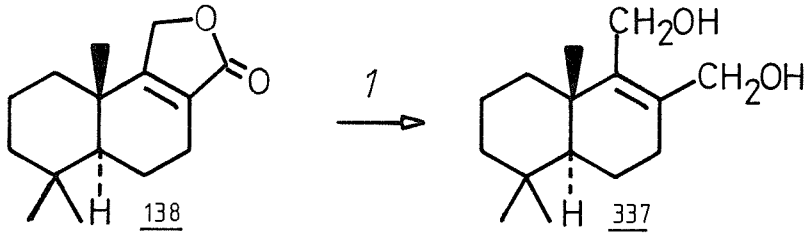
333

A series of reactions has been carried out to optimise the conditions in the preparation of diester (325) from diene I(217), Scheme 68]. As predicted, forcing conditions were required to give a high yield product.

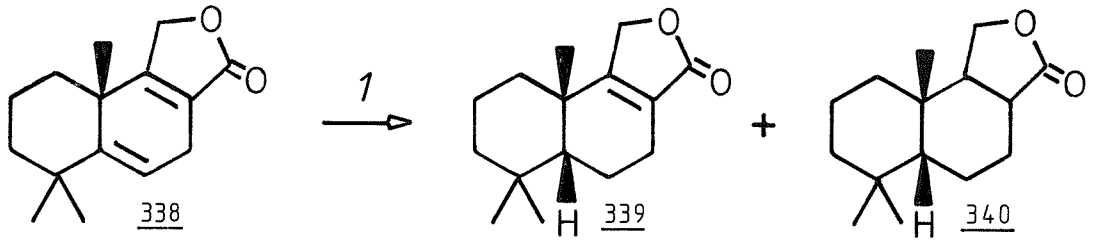
At low temperatures (< 100°C) little or no reaction was observed and only unreacted starting materials were recovered. At much higher temperatures (> 150°C), another unwanted product was formed with diester (325) which identified as aromatic system (333). At 120°C, in a flask under nitrogen, a good yield of the required diester was produced but byproduct was also formed. The best conditions were found to be in a sealed tube (under vacuum) overnight in a thermostatically controlled oil bath at 120°C.



Scheme 72: 1⊳ H_2 / catalyst 2⊳ $LiAlH_4$ 3⊳ oxidation



Scheme 73: 1⊳ $LiAlH_4$



Scheme 74: 1⊳ H_2 / Pd on C

Column chromatography of the crude product yielded diester (325) as colourless crystals (mpt 50-51^{o}C) in 87% yield.

3.3. Synthesis of Cavidial (155) from Diester (325)

Diester (325) has now been available in high yield from a readily available starting material, a route to dialdehyde (155) from 325) was now considered (Scheme 72).

Selective hydrogenation of the non-conjugated double bond should be possible under standard conditions to give diester (334). Partial reduction or total reduction to diol (336) followed by oxidation should yield dialdehyde (155).

3.3.1. Synthesis of Dimethyl 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethyl-1,2-Naphthalenedicarboxylate (334) by Hydrogenation of Diester (325)

The hydrogenation of diester (325) was attempted under the standard conditions of stirring overnight under an atmosphere of hydrogenation over a palladium on charcoal catalyst in methanol. On work up only starting material was observed. More forcing conditions of platinum dioxide (Adam's catalyst) in methanol and palladium on charcoal catalyst in glacial acetic acid also failed to give any products. However, the hydrogenation of diester (325) in glacial acetic acid over platinum dioxide gave a complex mixture of products after stirring for 2 days. Analysis of the product mixture showed one major product and one significant product. The mixture was separated by either silver ion column chromatography[199] or by distillation using a spinning-band microstill. Spectral analysis of the major product (Obtained in > 95% purity by both methods of separation) showed a monounsaturated diester of structure (334). The other product (> 80% pure) was found to be the dihydrogenated product (335). The other byproducts were assumed to be rearranged products formed by the use of very forcing hydrogenation conditions. The required diester (334) was isolated as a clear oil (bpt 105-108^{o}C @ 0.15 mm Hg) and yields in excess of 30% were not uncommon. The stereochemistry of the ring junction of (334) could not be determined at this stage.

3.3.2. Partial Reduction of Dimethyl 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethyl-1,2-Naphthalenedicarboxylate (334) to Cavidial (155).

A few methods for the reduction of esters to aldehydes have been reported. All involve the controlled addition of one molar equivalent of hydride to the ester to give the dialdehyde, without further reduction by a second molar equivalent of hydride to give the alcohol. There has not been any reported examples of the reduction of diesters to dialdehydes. The reported reagents include the inverse addition of lithium aluminium hydride at -70°C[200], the addition of di-isobutylaluminium hydride (DIBAL) at -70°C[201,202], and stirring with sodium aluminium hydride at -60°C[203].

All three methods of reduction were tried, all gave a mixture of products, but none contained the required dialdehyde. This result was not surprising for a molecule that contains two ester functionalities both of which can be reduced to two different oxidation levels.

3.3.3. Total Reduction of Diester (334) to 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethyl-1,2-Naphthalenedimethanol (336)

Diester (334) was reduced with lithium aluminium hydride in ether under reflux for 2 hours. The product (336), a white powder mpt 124-126°C, was isolated in 98% yield. It was now possible to assign the ring junction of the diol (336) and therefore diester (334). Tanis has previously reduced naturally occurring confertifolin [(138), known to have a *trans* ring junction] with lithium aluminium hydride to the corresponding diol [(337), Scheme 73][204]. The NMR spectrum of diol (337) showed a multiplet of signals at 4.10 and 4.18δ. These were assigned to the methylene groups adjacent to the alcohols. The NMR spectrum of the diol, obtained from the reduction of diester (334), showed a doublet of signals at 4.09 and 4.26δ; these were also assigned as the methylene protons adjacent to the alcohols. The NMR samples had been run in the same solvent (Figure 13) therefore, the spectral changes must be due to a structural difference. As previously indicated, the diol (337) obtained from the reduction of confertifolin (138) has a *trans* ring junction. This implied that diol (336), and therefore diester (334), was of *cis* ring junction stereochemistry.

The hydrogenation of diester (334) to give a *cis* ring junction was surprising as both anhydrides (220) and (223) have been hydrogenated and

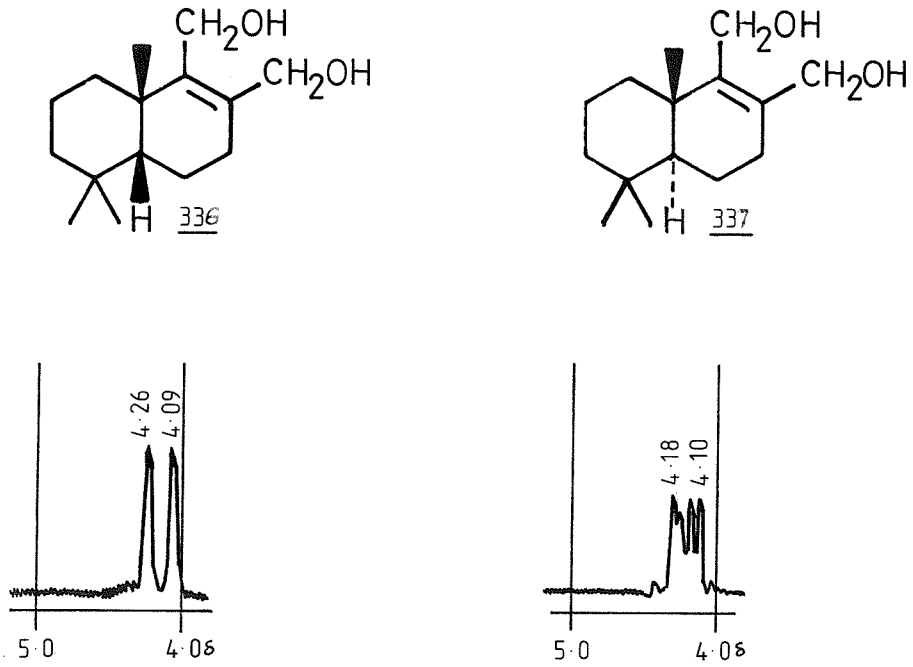


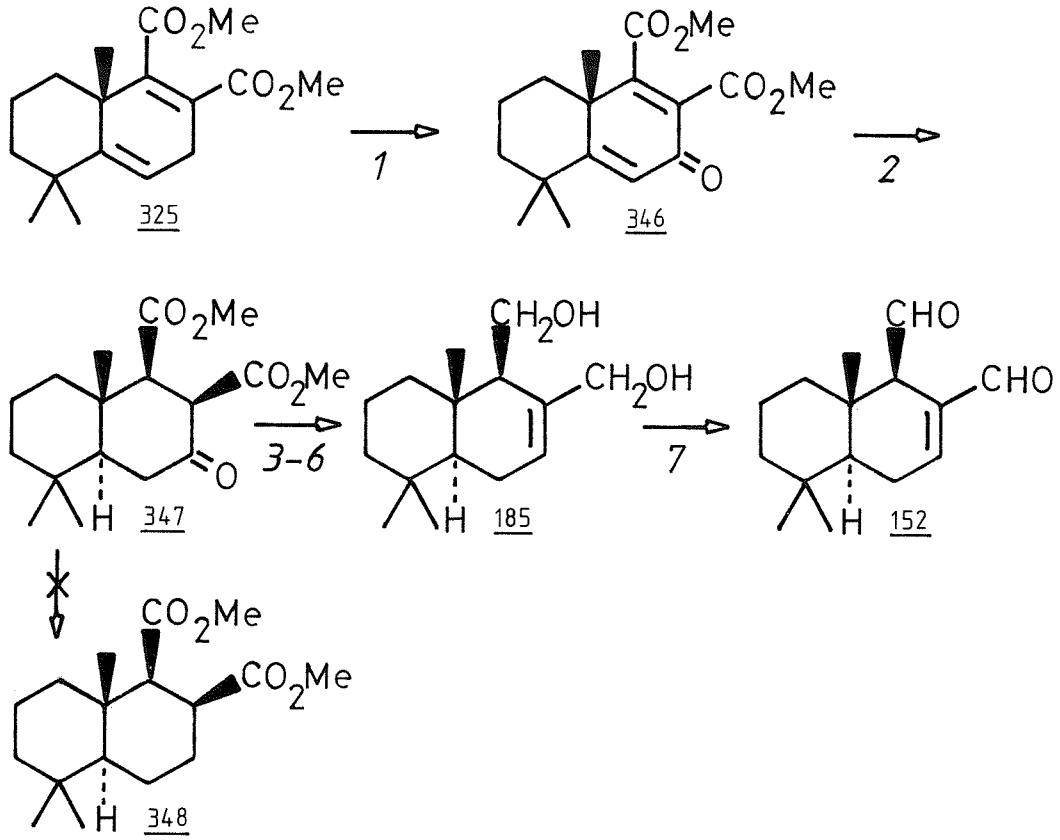
Figure 13: *THE N.M.R. SPECTRA (4.0-5.0δ) OF DIOLS (336)&(337).*

a *trans* ring junction obtained[103,105]. It has been reported that when lactone (338) is hydrogenated, a mixture of two hydrogenated lactones (339) and (340) are produced, both having a *cis* ring junction (Scheme 74)[204].

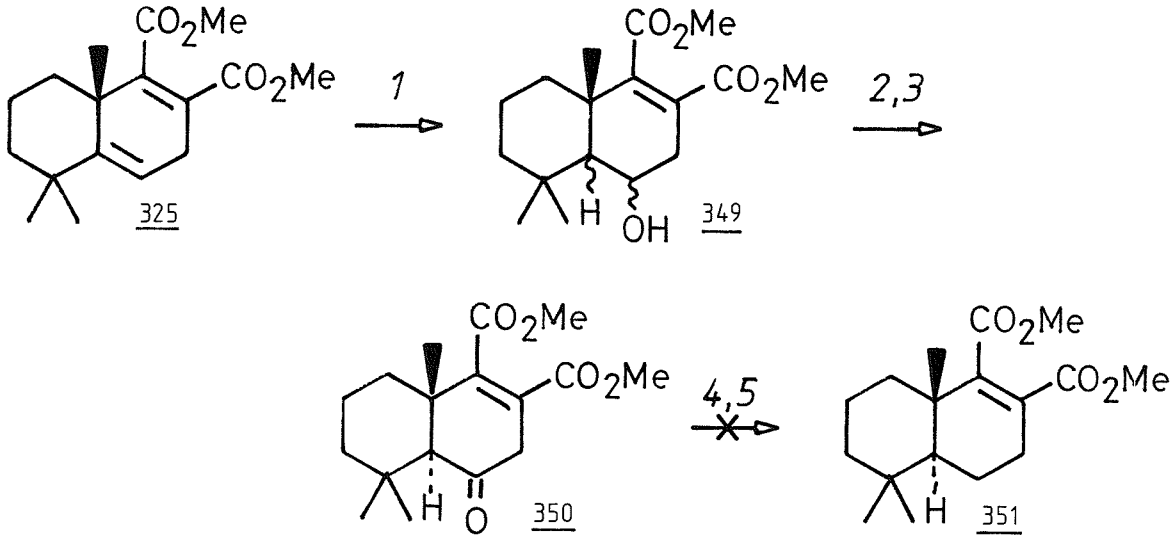
Trans ring junctions have been obtained when similar systems containing a benzene ring fused to the decalin skeleton have been hydrogenated. Compounds (341)-(344) all give single hydrogenated products that have a *trans* ring junction only.

Birch reduction of α,β-unsaturated systems contained in a decalin system (e.g. 345) usually give a *trans* reduction product[117c], but compound (346) derived from diester (325) will not react under Birch reduction conditions[204].

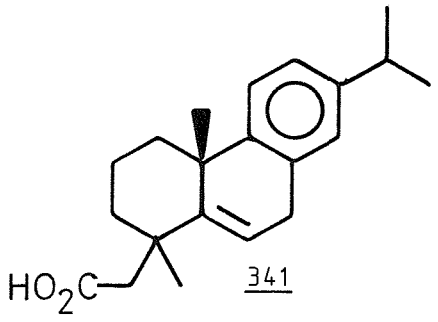
However, (346) has been hydrogenated under normal conditions in the synthesis of warburganal [(152), Scheme 75][106], to yield a product containing a *trans* ring junction. Unfortunately, the authors showed the



Scheme 75: 1▻ $CrO_3.2pyr.$ 2▻ H_2 / Pd on C 3▻ $NaBH_4$ / MeOH 4▻ MsCl / $EtNH_2$ 5▻ DBU 6▻ $LiAlH_4$ 7▻ several steps



Scheme 76: 1▻ B_2H_6 2▻ [O] 3▻ H^+ 4▻ HS⌒SH 5▻ Ni^R



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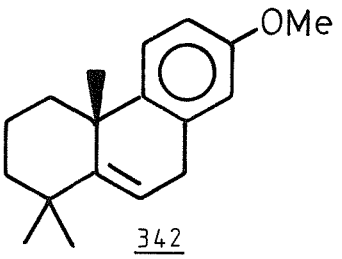

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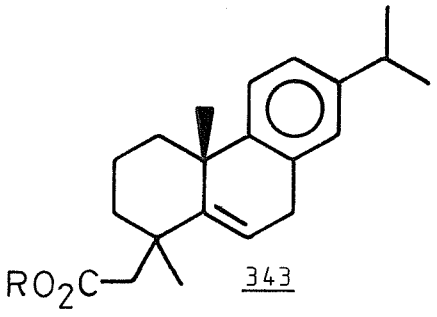

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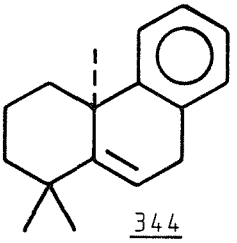

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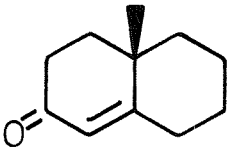

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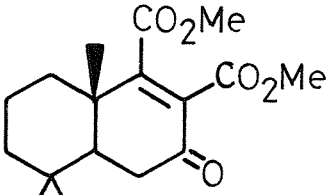

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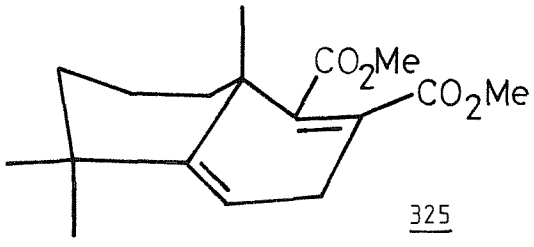
hydrogenolysis of the keto-group to be impossible. A similar problem was also observed when a ketone was introduced into compound (325) by hydroboration of the trisubstituted double bond to alcohol (349) followed by oxidation and equilibration to the *trans*-ketone [(350), Scheme 76][204].

Many conditions were tried by the authors but hydrogenolysis of the ketone was found not to be possible.

The hydrogenations discussed were clearly affected by the configuration of the molecule concerned and the differences in the direction of reaction may be explained by consideration of molecular models.

The olefin would be absorbed on the catalyst surface in a way that enables the maximum overlap of π-orbitals between olefin and catalyst. For diester (325), the molecular conformation adopted would decide the stereochemistry of the hydrogenation product. Models suggested the most favourable conformation was that shown below:

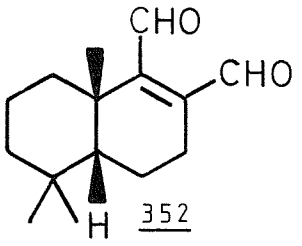


325

In most cases, the chair conformation is favoured by cyclohexane systems, but for (325) this required twisting the ester groups out of the double bond plane. This steric compression made the boat conformation more favourable, and therefore the β-face more accessible to hydrogenation. Hydrogenation from the β-face leads to a product with a *cis*-ring junction. A similar conformation was seen for lactone (338).

When anhydride (220) or a benzenoid molecule (e.g. 341) was considered a different conformation was observed when the molecular model was constructed. Ring A was flatter due to the planar substituent, presenting the α-face, the least hindered side of the molecule, to the catalyst surface. The result of this would be a product with a *trans*-ring junction.

With the data available, it seemed that the required diol (337) could not be prepared by this method. It was not known whether cavidial (155) had a *cis* or *trans* ring junction so the synthesis was continued using the *cis*-diol (336) to prepare *cis*-cavidial (352).

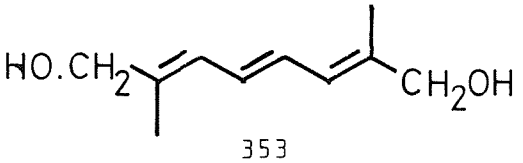


352

3.3.4. Oxidation of Diol (336) to Cavidial (352) with Manganese Dioxide and Chromium Reagents

Many reagents have been used to prepare dialdehydes from diols, but none of the reported examples have the same substitution pattern as diol (336).

Manganese dioxide has been used to oxidise diol (353) in the synthesis of multiunsaturated systems[205]; chromium trioxide/pyridine complex has been used to oxidise cyclobutadiene-1,2-dimethanol (354), the intermediates being stabilised as an iron carbonyl complex[206]; the diol obtained from the reduction of phthalic anhydride with lithium aluminium hydride has been oxidised with selenium dioxide to yield benzene-1,2-dicarbaldehyde[207,208].



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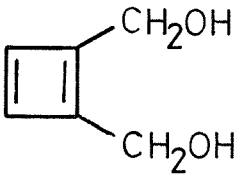

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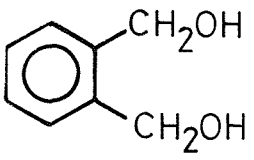


355

Diol (336), obtained from diester (325) as previously described, was oxidised with an excess of activated manganese dioxide in chloroform[135]. Analysis of the product mixture showed one major product, but this did not have any signals in the NMR spectrum characteristic of an aldehyde. The spectral details are listed below:

^{1}H NMR(60MHz,$CDCl_3$,): 0.65, 0.98, 1.19(3H,s,CH_3).

MS(MS30,m/e): 234(M^+,80%), 219(100%), 164(30%), 152(41%), 151 (72%), 150(39%), 91(45%), 41(51%).

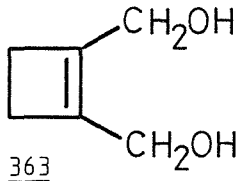
IR(solution,CCl_4,cm^{-1}): 3100-2840, 1762(C=O), 1450, 1375.

This data suggests that a lactone ring has been formed. (The infrared spectrum shows of band at 1762 cm^{-1} which is in the unsaturated γ-lactone range). The product was assigned structure (357) and was probably formed by the mechanism shown in Scheme 77. A similar reaction has been used before in the synthesis of confertifolin (138)[209].

Oxidation using the common chromium reagents were tried using the recommended excess of reagent. Chromium trioxide/pyridine complex[138], pyridinium dichromate[134] and pyridinium chlorochromate[139] all gave lactone and the isomeric lactone (358) in varying yields. When only one molar equivalent of pdc was used, in an attempt to prepare the intermediate lactol (356), only starting material and lactone (357) were isolated. This suggests that the initial lactol formation is a rate determining step. The oxidation[210] involving oxalyl chloride, DMSO and diol (336), followed by work up with triethylamine; yielded a complex mixture of products that did not contain any of the required dialdehyde product.

The formation of lactones from the oxidation of diols is not unusual, and has been noted by many workers (Scheme 78)[211,212]. In a recent study by Sondheimer[213], the oxidation of cyclic diols has been studied rigorously. It was found that the products from the oxidation of cyclic diols (363-365) obtained by the DIBAL reduction of the corresponding diesters, varied with ring size. When (363) and (364) were oxidised, the corresponding dialdehydes were formed in > 95% yield; however, the oxidation product from

diol (365) was lactone (366), a product analogous to that obtained from the oxidation of diol (336) mentioned earlier.



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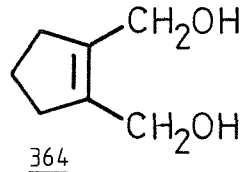

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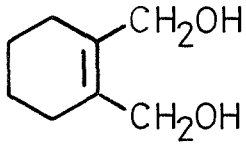

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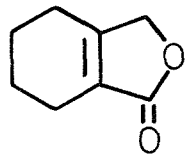

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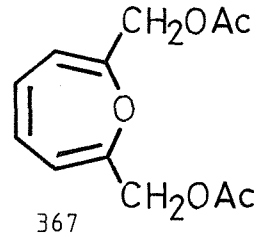

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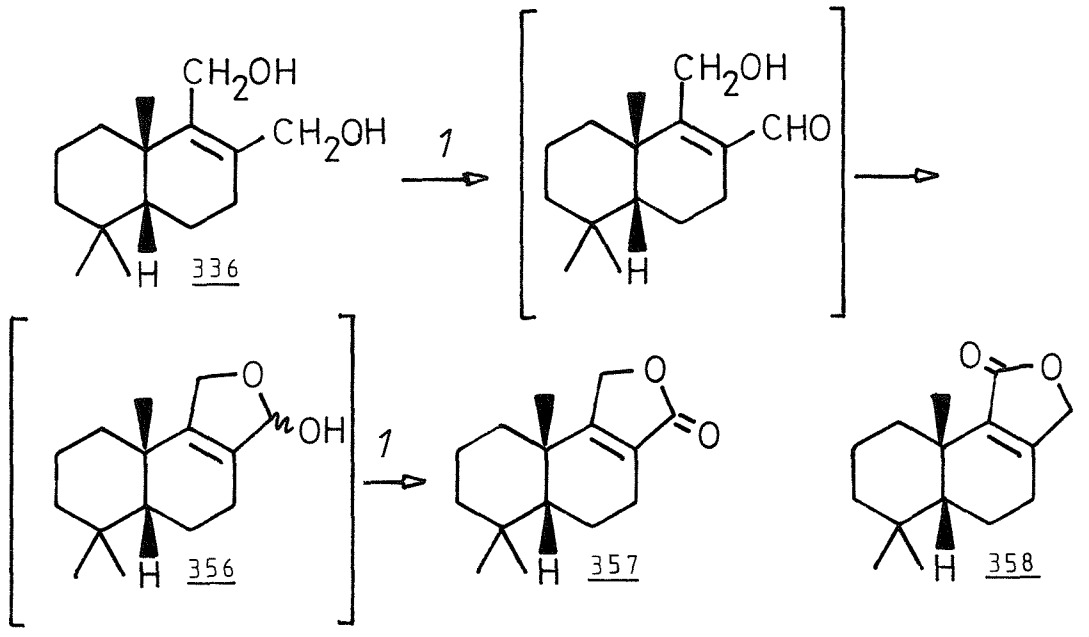
With the failure of the oxidation methods listed, others were tried.

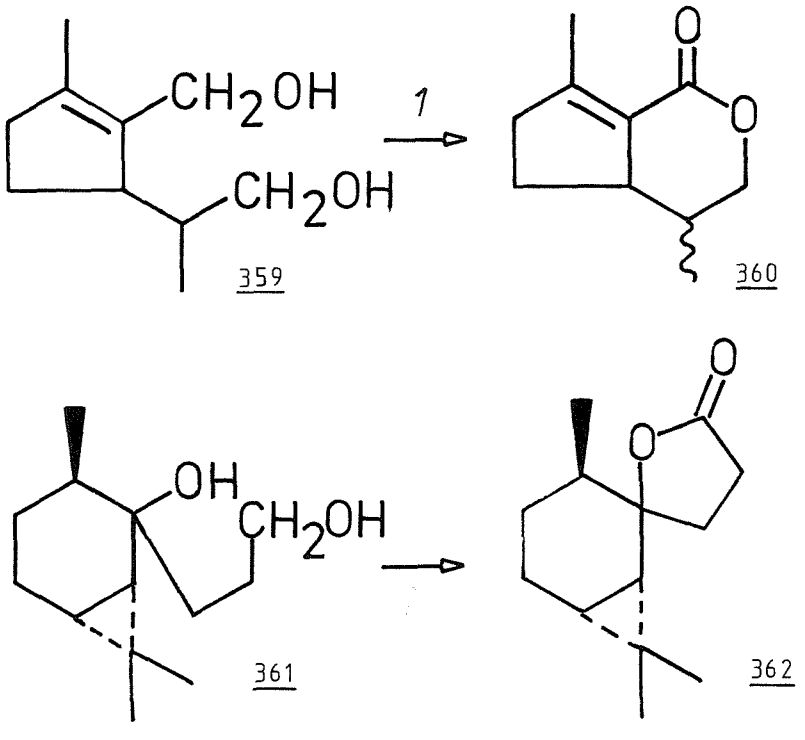
3.3.5. Other Oxidations of Diol (336)

Diol (336) was oxidised with selenium dioxide by a similar method to that reported for benzene-1,2-dimethanol (356)[207,208]. Powdered selenium dioxide and the diol were heated in an open flask to 130-140°C (oil bath) until water was no longer condensed at the top of the tube. The product was washed with water and then heated with the open flame of a bunsen burner for 1 minute. Benzene-1,2-dimethanol (356) was found to give the corresponding dialdehyde; diol (336) only yielded high molecular weight products on treatment with selenium dioxide.

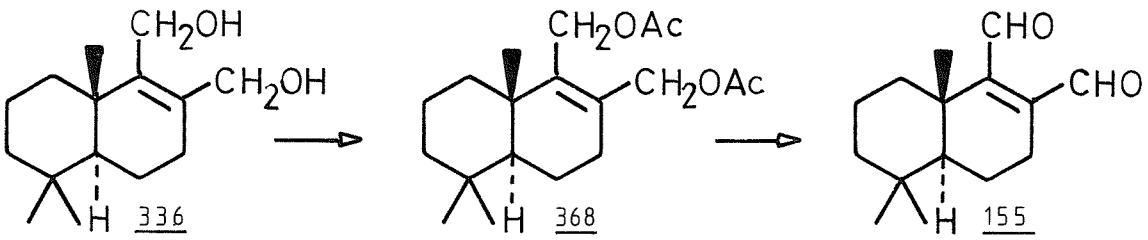
One further method of oxidation tried involved the allylic oxidation of the diacetate of diol (336) with manganese dioxide. Previously used in the oxidation of diacetate (367)[214], the conditions were repeated on diacetate [(368), Scheme 79].



Scheme 77: 1⊳ MnO_2



Scheme 78: 1⊳ MnO_2 2⊳ $CrO_3.2pyr.$



Scheme 79: 1⊢ Ac_2O /pyr. 2⊢ MnO_2/acetone

Diol (336) was stirred overnight with 4 equivalents of acetic anhydride in pyridine. Work up yielded diacetate (368) as a clear oil in 95% yield. Oxidation of diacetate (368) with manganese dioxide in acetone overnight did not give any reaction product; extended reaction time at higher temperature also failed to yield any reaction product.

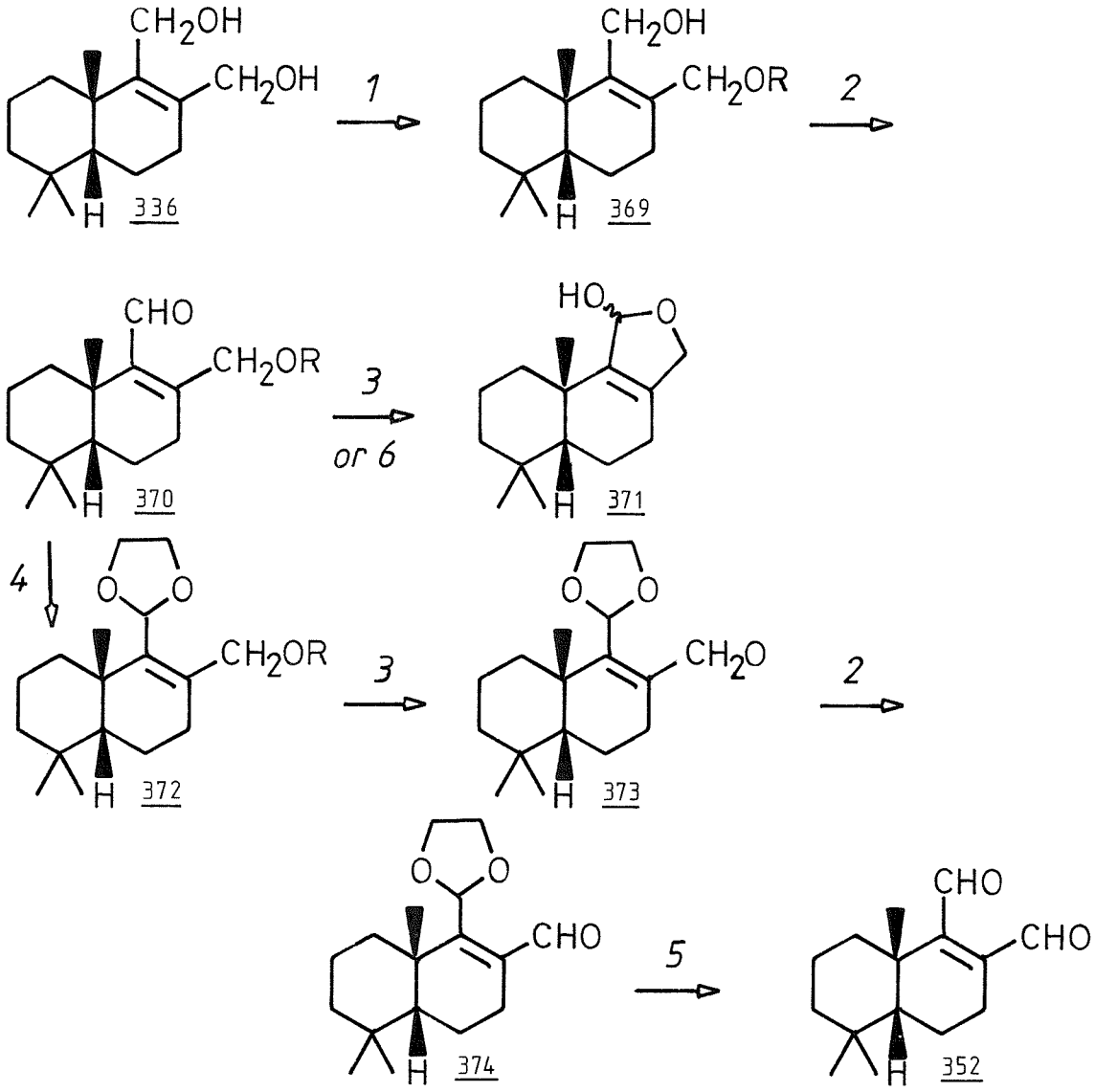
With the failure of the direct oxidation methods, other synthetic schemes had to be considered. The notable point of the oxidation of diol (336) was the preferential oxidation of one alcohol in the presence of another. It seemed likely that this preference would be extended to formation of a monoprotected diol.

A longer route which utilised this difference in reactivity was considered. The mono-protected diol would then take part in further reactions where intramolecular reactions could not occur.

3.4. The Synthesis of Cavidial (352) via a Monoprotected Diol Intermediate

The proposed route to cavidial is shown in Scheme 80. Monoprotection of diol (336) would give intermediate (369) by preferential reaction at the least hindered alcohol. Oxidation followed by protection of the aldehyde would yield a diprotected aldehyde-alcohol intermediate (372).

Preferential deprotection of the alcohol followed by oxidation would give acetal-aldehyde (374) which could possibly be deprotected to



Scheme 80: 1⊢ *monoprotection* 2⊢ *p.d.c. / DMF* 3⊢ $Bu_4N^+F^-$ / *THF* 4⊢ $(CH_2OH)_2$ 5⊢ *aq. acetone* 6⊢ $(EtO)_3CH$ / H^+

give a dialdehyde (352). Different protecting groups for the protection of diol (336) were considered.

3.4.1. Monoprotection of Diol (336)

Several protection groups were considered, the criteria for choice being ease of addition and removal and stability to oxidation and mildly acidic conditions. From the large number of possibilities, the following were investigated: acetate, tetrahydropyranyl (THP) ether, methoxyethoxymethyl

(MEM) ether and t-butyldimethylsilyl (TBDMS) ether. The conditions for addition and removal are shown in Table 5.

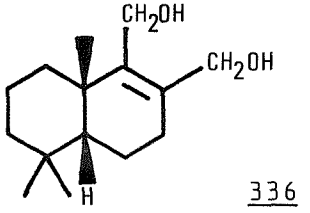
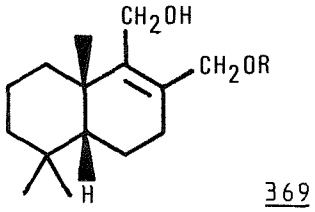
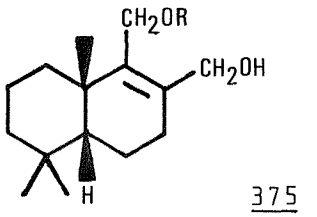
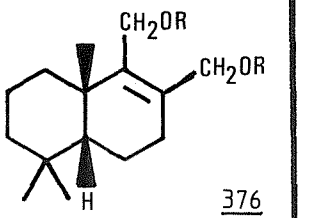
TABLE 5

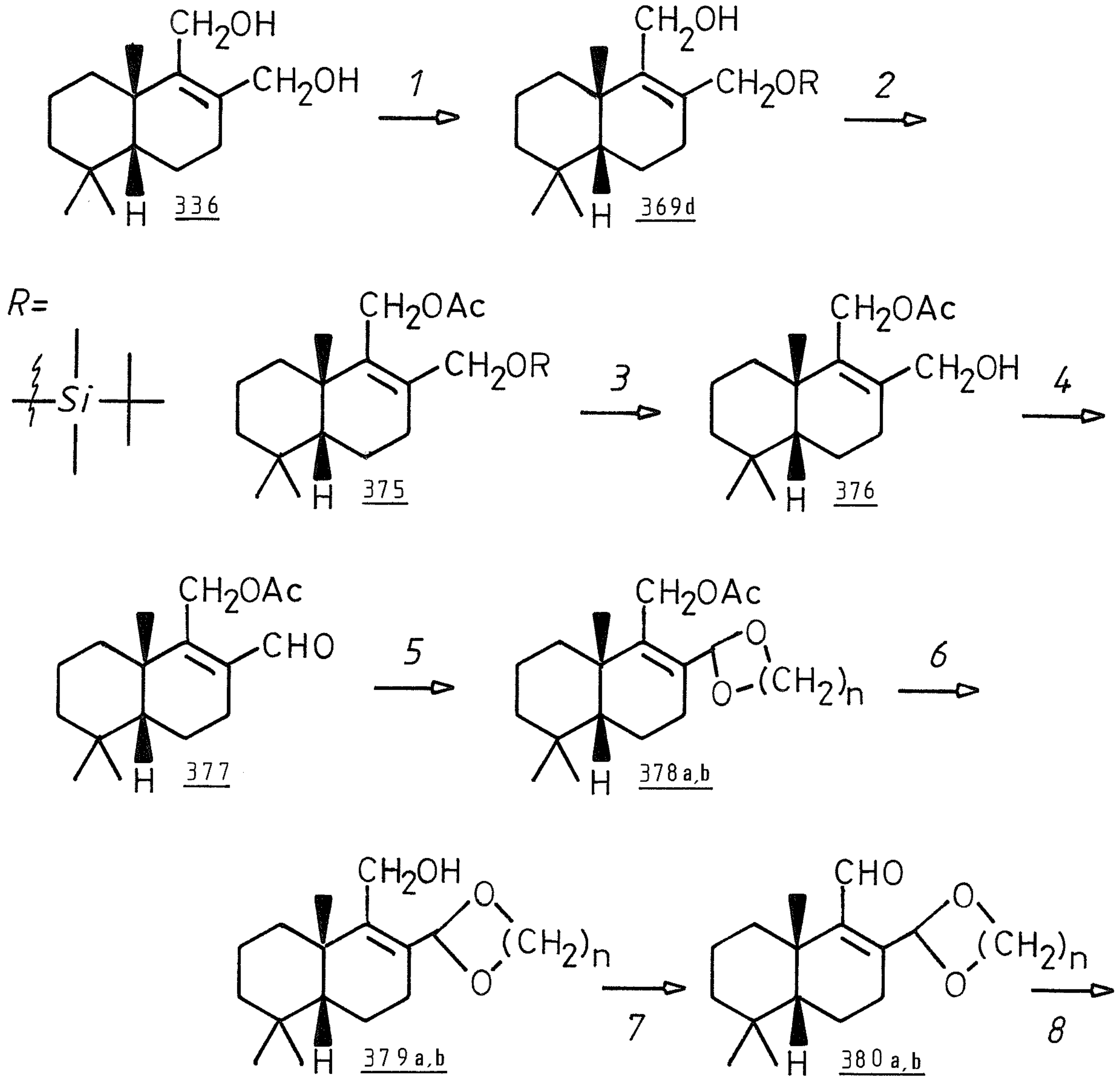
Group	Addition	Removal	Ref.
Acetate	Ac_2O, pyr	K_2CO_3, MeOH KOH, MeOH $LiAlH_4$	215
THP	DHP, TsOH	H_2O, HOAC	215,216
MEM	mem chloride/ DIPA	$ZnBr_2$ or $TiCl_4$	217
$tBuMe_2Si$	$tBuMe_2SiCl$ imidazole DMF	AcOH, aq THF $Bu_4N^+F^-$	218

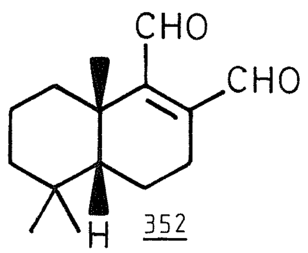
Each protecting group was reacted with diol [(336), Scheme 81], to find the reagent which produced the highest specificity of reaction (Table 6).

From the results shown in Table 6, t-butyldimethylsilyl chloride gave the best ratio of (369):(375) and the best overall yield of (369) so this group was used in all further schemes. Silyl alcohol (369) was removed from the other compounds in the reaction mixture [(336), (375):(376)] by flash column chromatography (see Experimental, Chapter 4) or by HPLC.

TABLE 6

Prot. Group	PERCENTAGE COMPOSITION OF PRODUCT MIXTURE			
	 336	 369	 375	 376
1. 2 eq Ac_2O 2. 5 eq pyridine stir o/night, rt a. R = $CH_3.C=O$	-	17%	17%	38%
1. 2 eq MEMCl 2. 5 eq DIPA, CH_2Cl_2 stir o/night, rt b. R = $CH_3OCH_2CH_2OCH_2$-	22%	30%	18%	12%
1. 2 eq DHP cat. TSOH, Et_2O c. R = 	4%	60%	12%	10%
1. 1 eq tBDMSCl 5 eq imidazole DMF, 0^oC d R= 	8%	80%	8%	4%





1 ⇾ tBuMe$_2$SiCl / imidazole / DMF

2 ⇾ Ac_2O / pyr.

3 ⇾ $Bu_4N^+F^-$/THF

4 ⇾ p.d.c. / DMF

5 ⇾ $HO(CH_2)_nOH$

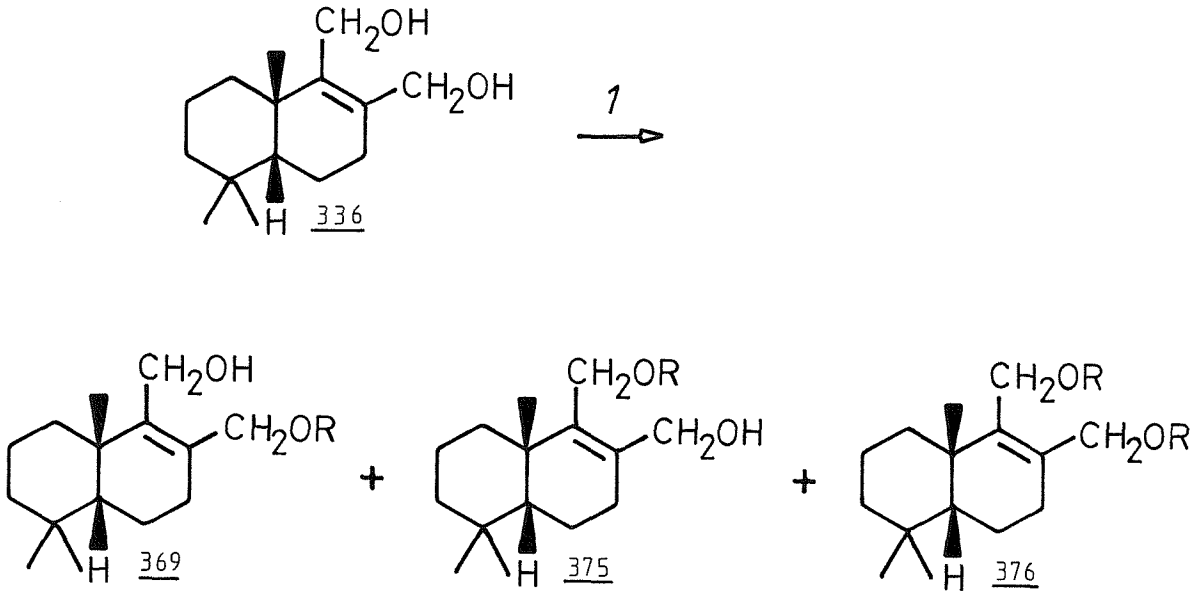
6 ⇾ K_2CO_3 / MeOH

7 ⇾ [O]

8 ⇾ acetone / H^+

a: n=2 b: n=3

Scheme 82.



Scheme 81: *1⊢ protection conditions (tables 5 & 6)*

3.4.2. Oxidation of 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethylnaphthalene-1-Methanol-2-t-Butyldimethylsilyloxymethyl (369d)

As in Scheme 80, the monoprotected diol (369d) was oxidised with pdc in DMF at $0^{o}C$ for 2 hours. The product (370), isolated as a colourless oil, exhibited a singlet at 10.16δ in the ^{1}H NMR spectrum attributed to the aldehyde proton and a band at 1685 cm^{-1} in the IR spectrum also associated with the aldehyde group.

Deprotection of the silyl-aldehyde (370) with tetrabutylammonium fluoride in THF at room temperature did not yield an aldehyde alcohol, but another product later found to be lactol (371).

3.4.3. Protection of 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethylnaphthalene-1-Carboxldehyde-2-t-Butyldimethylsilyloxymethyl (370) as an Acetal

Silyl-aldehyde (370) was refluxed with ethylene glycol in benzene overnight with p-toluenesulphonic acid catalyst[219]. On work up, only starting material was isolated. Even after extended reaction time (3 days), none of the required product had been formed. It was assumed that the ethylene

glycol group was too bulky and that steric hinderance was blocking reaction at the aldehyde group. When silyl-aldehyde (370) was subjected to the conditions of triethyl orthoformate in methanol with p-toluene sulphonic data of this product showed a loss of the silyl group (IR loss of 1255 cm^{-1} and 1075 cm^{-1} bands) and the loss of the aldehyde signal in the NMR. Further interpretation of the data suggested the structure to be (371), a cyclic acetal formed by the deprotection of the alcohol group followed by ring closure on the protected aldehyde.

This had not been anticipated so another route was considered.

3.5. The Synthesis of Cavidial (352) via a Differently Protected Diol

The problem with the previous route was the failure of the protection of the aldehyde due to steric hinderance and the instability of the silyl protecting group to certain reaction conditions. The next route proposed (Scheme 82) involved the manipulation of protecting groups so that the alcohol at C-2 could be oxidised and protected.

3.5.1. Synthesis of 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethylnaphthalene-1-Acetoxymethyl-2-t-Butyldimethylsilyloxymethyl (375)

The monoprotected diol (369d) was prepared as before. Acetylation of this intermediate was achieved by stirring overnight with a ten fold excess of acetic anhydride in pyridine. The product, silyl acetate (375), was obtained as a clear oil in 75% yield and was characterised by the retention of the silyl absorption bands in the IR (1260 and 1090 cm^{-1}) and the appearance of the acetate absorption at 1740 cm^{-1}. A signal at 1.99 τ (the CH_3 of the acetate group) in the NMR spectrum was also observed.

3.5.2. The Deprotection of (375) to 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethylnaphthalene-1-Acetoxymethyl-2-Methanol (376)

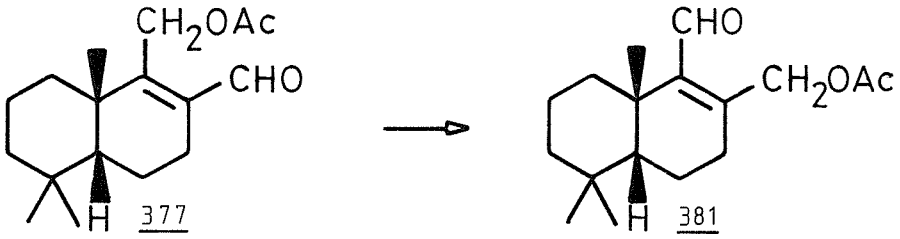
Removal of the silyl protecting group could be undertaken in a number of ways. Acetic acid in aqueous THF[220] failed to yield the required acetate-alcohol (376). Boron trifluoride etherate complex in THF gave a poor yield of (376), but tetrabutylammonium fluoride (1M in THF) removed the silyl protecting group smoothly to yield (376) in quantitative yield.

The characteristic bands of the silyl group were lost from the IR spectrum and were replaced by a strong absorption at 3440 cm^{-1} consistent with an alcohol product.

3.5.3. The Oxidation of (376) to 3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-Trimethylnaphthalene-1-Acetoxymethyl-2-carbaldehyde (377)

Oxidation of acetate-alcohol (376) was achieved using pdc in DMF at $0^{o}C$ for 2 hours. The product, acetate-aldehyde (377), was obtained as a colourless oil in 77% yield. The IR spectrum exhibited bands at 1730 and 1660 cm^{-1} assigned to the acetate and aldehyde groups respectively.

It was observed that acetate-aldehyde (377) was unstable overnight. The new product was another acetate-aldehyde (381) and it seemed likely that this was a rearrangement product of [(377), Scheme 83].

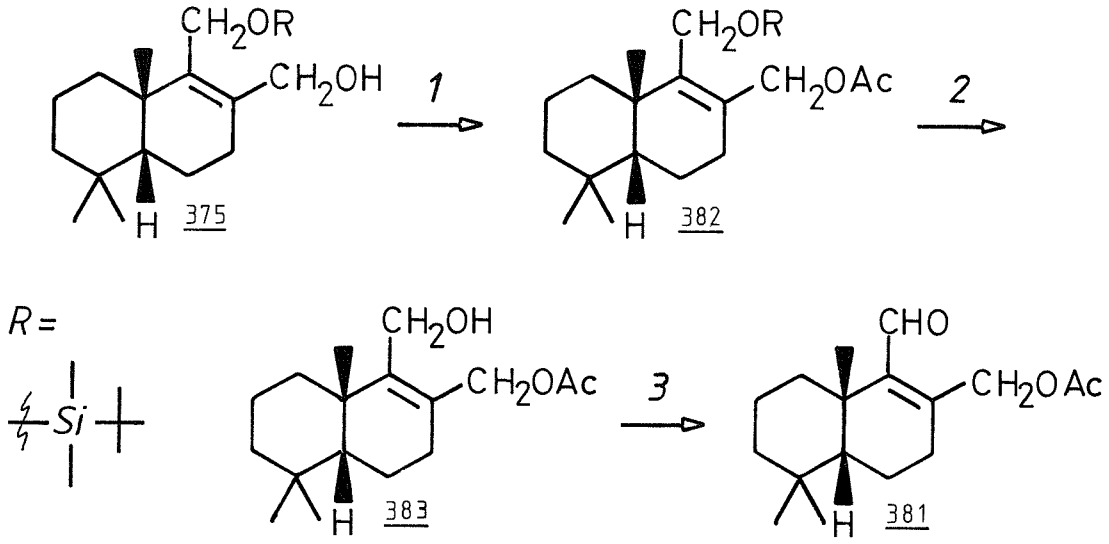


Scheme 83: 1⊢ *r.t. / overnight*

The rearrangement could be retarted by storage in the fridge, but when possible, the compound was used soon after preparation.

The structure of the rearranged product was confirmed by synthesis (Scheme 84). Silyl alcohol (375d), isolated from the monoprotection of diol [(336), Scheme 81] by HPLC, was protected as the acetate (382), desilylated to acetate-alcohol (383) and oxidised to acetate-aldehyde (381). Comparison of the spectral data showed this acetate-aldehyde and the rearrangement product to be the same compound.

It was previously found that silyl-aldehyde (370) could not be protected as an ethylene ketal (372) due to steric hinderance. Acetate-aldehyde (381) was subjected to similar protection conditions, but again no reaction was observed.



Scheme 84: 1⊳ Ac_2O / pyr. 2⊳ $Bu_4N^+F^-$/THF
3⊳ p.d.c. / DMF

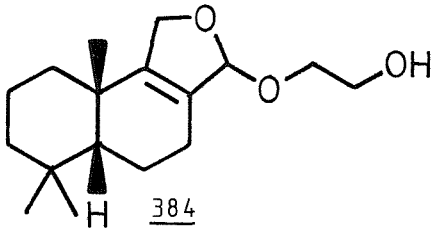
3.5.4. The Protection of (377) as an Ethylene Acetal (378a)

Acetate-aldehyde (377) was refluxed overnight with ethylene glycol in benzene with p-toluene sulphonic acid catalyst in a Dean and Stark apparatus (Scheme 82, n = 2). Work up yielded acetate-acetal (378a) in 89% as a clear oil. The product was characterised by the loss of the aldehyde absorption in the IR spectrum and the aldehyde signal in the NMR spectrum, and the appearance of a singlet at 3.85 δ due to the methylene protons of the acetal group.

3.5.5. Saponification of the Acetate-Acetal (378a) to Alcohol-Acetal (379a)

Many methods are available to deprotect acetate protecting groups. The most commonly used are sodium hydroxide or potassium hydroxide in ethanol[221], lithium aluminium hydride in ether[222], and potassium carbonate in methanol[223]. The latter was found to give alcohol-acetal (379a) as a colourless oil in 80% yield. The spectral data showed a loss of the characteristic band of acetate and appearance of a strong band at 3550 cm^{-1} indicating an alcohol had been formed in the IR spectrum.

The newly formed alcohol was found to be unstable, within 1 hour of formation, > 50% had rearranged to another compound. The addition of p-toluene sulphonic acid in methanol led to total rearrangement within 1 hour. The rearranged product was postulated as (384). A similar reaction



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has been recently reported by Oishi *et al*[224].

The formation of acetal (384) showed that a change of protecting group was required. A six membered ring acetal was chosen to replace the five membered ring acetal used in Scheme 82.

3.5.6. The Protection of (377) as Propylene Acetal (378b)

Acetate-aldehyde [(377), prepared as before, Scheme 82] was refluxed overnight with propane-1,3-diol and p-toluenesulphonic acind in benzene with azeotropic removal of water. Acetate acetal [(378b), Scheme 82, n=3], was obtained as a clear oil in 89% yield. The IR spectrum of the compound showed the loss of the aldehyde peak, the NMR showed the complex pattern of signals associated with a six-membered ring acetal.

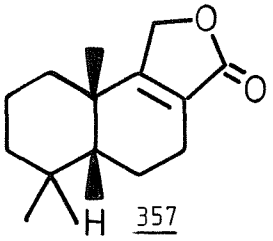
3.5.7. Saponification of Acetate-Acetal (378b) to Alcohol-Acetal (379b)

Acetate-acetal (378b) was stirred overnight with potassium carbonate in methanol at room temperature. The product, alcohol-acetal (379b) was isolated as a clear oil in 72% yield after purification by flash column chromatography. This acetal was stable enough to store in the fridge without rearrangement to any byproducts.

3.5.8. Oxidation of Alcohol-Acetal (379b)

Many oxidations were tried to convert (379b) to aldehyde-alcohol (380b) but none were successful. Chromium trioxide/pyridine complex, pdc in DMF, pcc in methylene chloride, MnO_2 in chloroform, Moffatt oxidation (oxalyl chloride, DMSO, methylene chloride and triethylamine at -78°C), the Oppenhauer oxidation[225] and the milder oxidation conditions of pcc on alumina[226] were all tried but none yielded the required product. In most cases a complex mixture of products were obtained. In each case the product mixture was taken and stirred overnight with 3% concentrated hydrochloric acid in acetone in an attempt to detect dialdehyde products but none were seen.

One interesting reaction was the mixture obtained from the Moffatt oxidation of alcohol acetal (379b). The attempted deprotection with dilute acid yielded a 4:1 mixture of two products which were identified as the same products as found in the oxidation of diol (336) namely (357) and (358).



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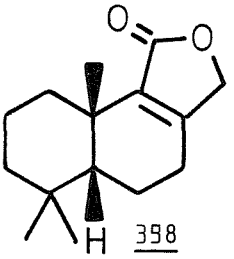

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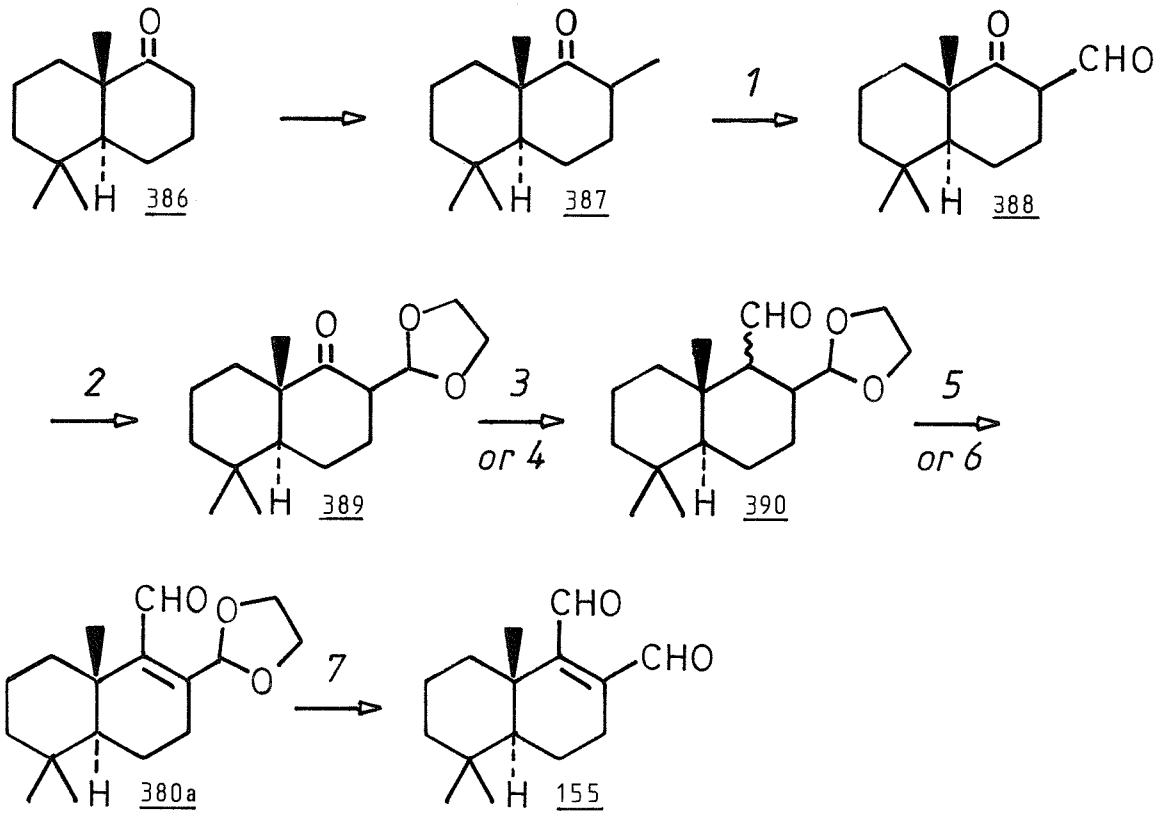
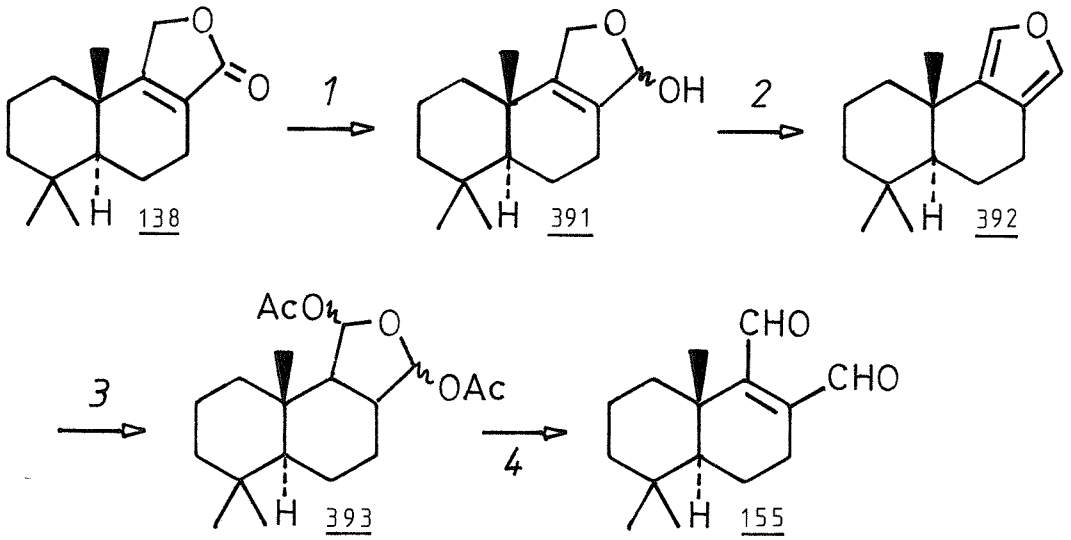
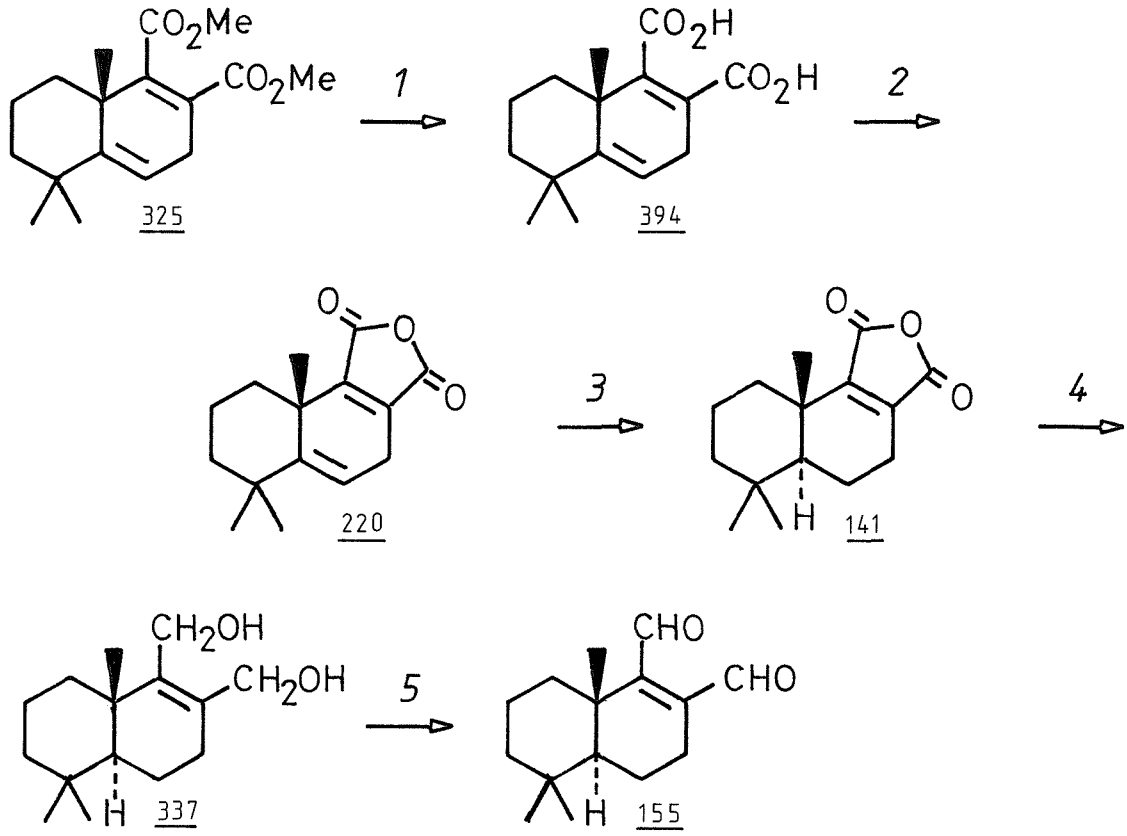
The mechanism for this reaction was not verified, but it was likely that the protecting group of the aldehyde was being removed under the Moffatt reaction conditions. The resulting aldehyde-alcohol would cyclise to an intermediate lactol which under the reaction conditions would oxidise to the lactones shown above (cf. Scheme 77).



Scheme 85: 1⊳ i CrO_2Cl_2 / / CCl_4 ii H_2O[227] 2⊳ $(CH_2OH)_2$ / H^+ 3⊳ i $Me_3S^+O\ I^-$ / NaH / DMSO ii $BF_3.Et_2O$[228] 4⊳ CH_3NO_2 / piperidine[229] 5⊳ PhSeCl / THF[230] 6⊳ i Br_2 / $CHCl_3$ ii $PhNEt_2$[231,2] 7⊳ aq. acetone / H^+



Scheme 86: 1⊳ DIBAL[233] 2⊳ DIBAL[234,5] 3⊳ $Pb(OAc)_4$[236] 4⊳ H^+



Scheme 87: *1⊢ KOH / MeOH 2⊢ Δ / Ac_2O*
3⊢ H_2 / Pd on C 4⊢ $LiAlH_4$ 5⊢ see text

3.6. Other Schemes to Cavidial (352)

Some routes to cavidial (352) have been tried, and the basic skeleton has been constructed. Difficulties associated with protecting groups for diol (336) have been overcome but the protecting group used for the penultimate has caused several problems. The solution to the problem may be a change in protecting group e.g. the use of (385)[227a,b], or perhaps a

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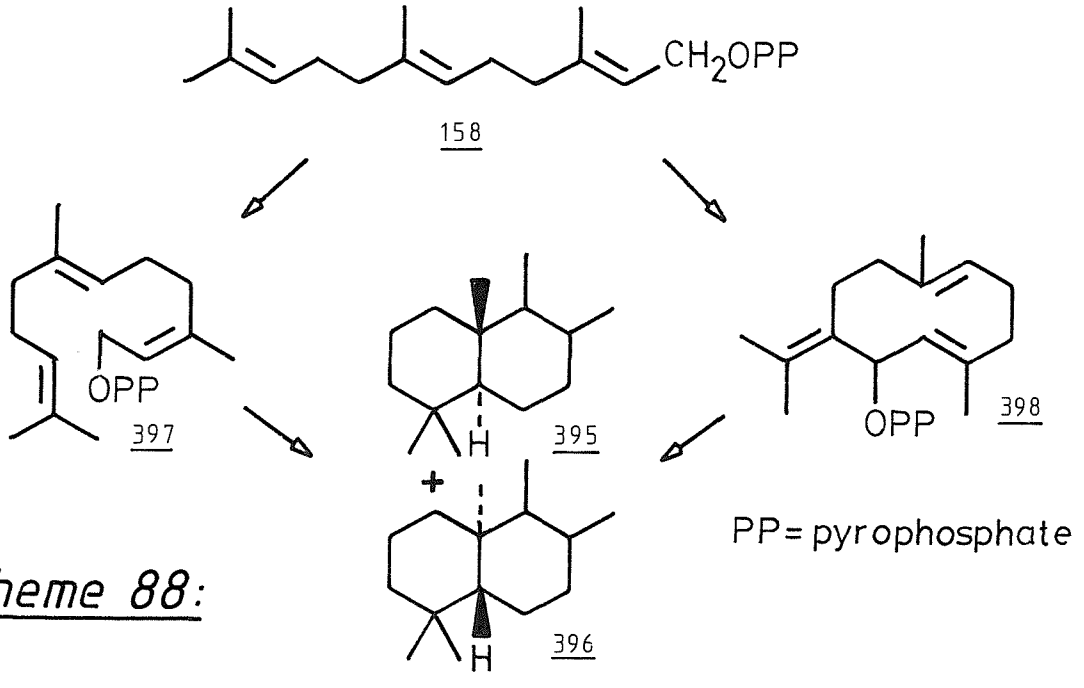
completely different scheme involving a different approach to the dialdehyde functionality (e.g. Schemes 85, 86).

The *trans* isomer of cavidial could possibly be prepared from the corresponding anhydride [(220), Scheme 87] via the diacid (394) derived from diester (325). Reduction with hydrogen with a palladium catalyst would give winterin (141) which could then be reduced to diol (337) with lithium aluminium hydride. The diol could then be taken through reaction scheme 82. It is possible that the *trans* ring junctioned intermediates would not react to give the anomalous products observed with the *cis*-isomers.

3.7. Biosynthesis of Drimane Sesquiterpenes

The drimanes (395) and iresanes (396) constitute the small group of bicyclofarnesol sesquiterpenes possessing enantiomeric skeletons. Their biogenesis is assumed to involve direct *trans*-antiparallel cyclisation of farnesyl pyrophosphate (158) in conformations (397) or (398) to give both skeletons (Scheme 88). The biosynthesis of farnesyl pyrophosphate (158) was explained in Chapter 2 (Scheme 63). Many of the drimane group of sesquiterpenes have been synthesised utilising a biogenetic type cyclisation (see earlier sections) but few paths have been studied by radiolabelling techniques. However, routes to higher terpenes have been attempted and have suggested the proposed biosynthetic pathway to be correct[237,238].



Scheme 88:

3.8. Conclusions

A dialdehyde compound, named cavidial (155) has been isolated from the termite soldiers of *Ancistrotermes cavithorax* and synthetic routes to this proposed structure have been explored. The first route, via diol (336) followed by oxidation, yielded a lactone rather than the required dialdehyde. Comparison of the spectral data with the diol of known *trans*-ring junction configuration has shown the diol synthesised in this work to have a *cis*-ring junction. Further work has shown the *cis*-dialdehyde difficult to prepare from the *cis*-diol via a monoprotected intermediate. An ideal protecting group to give a monoprotected diol has been found and high yields have been obtained but the latter stages of this synthetic route have furnished unstable intermediates. Changing the aldehyde protecting group afforded an alcohol-acetal (379b) with greater stability, but all attempts to oxidise this compound failed.

Further work is necessary to find a new route, possibly using already prepared intermediates, to cavidial so that proof of structure may be achieved.

CHAPTER 4

Experimental

4.1. General Techniques

THF was dried by distillation under nitrogen from benzophenone and sodium wire. Benzene was dried by distillation from sodium, pyridine was dried by distillation from potassium hydroxide, DMF was dried by distillation at reduced pressure from calcium hydride. Pet. ether refers to the 40^{o}-60^{o}C bp fraction of petroleum benzin. Triethylamine and diethyl ether were dried by distillation from sodium wire, ethyl acetate was used as received.

Melting points were determined on a Reichert Koffler melting point apparatus, and are uncorrected.

Infrared spectra (IR) were recorded on a Perkin-Elmer 157G grating IR spectrometer using the 1603 and 1025 cm^{-1} lines of polystyrene as calibrants. Ultraviolet spectra (uv) were recorded on a Pye Unicam SP 800 UV spectrometer with spectroscopic ethanol as the solvent.

Proton magnetic resonance spectra were recorded on a Perkin Elmer R12 (60 MHz) spectrometer or on a Varian XL-100 (100 MHz) spectrometer, carbon-13 spectra were also obtained on the latter. Chemical shifts are reported in parts per million on the δ scale relative to a tetramethylsilane internal standard or Si-Me for silicon containing compounds. In NMR descriptions s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and J = coupling constants in Hertz. Mass spectra (ms) were recorded on either an AEI MS-12 spectrometer equipped with a direct insertion probe and an all glass heated inlet system (AGHIS) or an AEI (Kratos) MS30 equipped with a direct insertion probe and a Data General DS50 data handling system.

Thin layer chromatographic analyses (TLC) were performed on precoated Merck Kieselgel 60F (254), Macherey-Nagel MN Sil G-25 UV 254 or MN ALOX-25 UV 254 plates.

TLC spots were made visible with UV light, dipping in an iodine vapour tank or by spraying with a vanillin in sulphuric acid mixture.

Open column chromatography was performed utilizing Merck 100-200 mesh silica. Flash chromatography was performed according to the procedure of Still *et al*[243] using Macherey-Nagel MN Kiesel gel 60 (230-400 mesh) eluted with the solvents described.

A Perkin Elmer M-131 T microstill was used for distillations requiring the separation of components with boiling point differences of less than 10°C.

4.2. Experimental Details

(+)-5β-(2-propyl)-2α-methylcyclohexanone (dihydrocarvone, 53)

Zinc dust (260 g, 4 mol) was stirred in 2% hydrochloric acid (400 ml) for 1 minute. Filtration, followed by washing with water (2l) and ethanol (2l), gave activated zinc. Sodium hydroxide (136 g, 3.4 mol) was dissolved in ethanol (1300 ml) and water (700 ml) and l-carvone (248, 100 g, 0.67 mol) was added. The zinc dust was added portionwise over 2 hours to the mixture under reflux. After the addition, refluxing was continued for 16 hrs. The zinc was filtered off and washed with aq. ethanol, the ethanol was then removed from the filtrate to yield two layers. Steam distillation followed by ether extraction of the distillate, drying and evaporation of the ether yielded dihydrocarvone (53, 73.3 g, 72%).

^{1}H NMR(60MHz, $CDCl_3$):	1.03(3H,d,6Hz,CH_3); 1.75(3H,s,CH_3,C=C); 4.76(2H, broad s,C=CH_2).
MS(MS12,m/e):	152(M^+,17%), 95(62%), 82(42%), 81(43%), 68(63%) 67(100%), 55(36%), 41(78%), 39(52%).
IR(film,cm^{-1}):	3080, 2970, 2940, 2860, 1710(C=O), 1642(C=C), 1450, 1380, 1240, 890.
$[\alpha]_D = +13^o$	(lit[240] $[\alpha]_D = +19^o$)

(+)-5β-(2-hydroxy-2-propyl)-2α-methylcyclohexanone (dihydrocarvone hydrate, 74)

Dihydrocarvone (53, 27 g, 0.178 mol) was added to a well stirred suspension of mercuric acetate (56.7 g, 0.178 mol) in tetrahydrofuran (200 ml)

and water (200 ml). Decolouration occurs after 15 seconds. After 5 minutes, sodium hydroxide (3M aq. solution, 200 ml) and sodium borohydride (0.3M solution in 3M NaOH, 200 ml) were added successively; mercury was deposited. The mixture was filtered using celite (filtering aid) and the residue was washed with ethyl acetate. Removal of the THF followed by separation of the aqueous layer, drying and evaporation of the organic layer afforded dihydrocarvone hydrate (74, 17 g, 81%) as a semi crystalline solid.

^{1}H NMR(60MHz, $CDCl_3$): 1.02(3H,d,J=6Hz,CH_3); 1.19, 1.22(3H,s,CH_3C-O)

MS(MS12,m/e): 170(M^+,0.5%), 152(M^+-18,6%), 81(35%), 68(26%), 67(27%), 59(100%), 55(25%), 43(39%), 41(47%), 39(22%).

IR(film, cm^{-1}): 3420(broad,OH), 2980, 2940, 2770, 1705(C=O), 1450, 1380, 1220, 1155, 1120, 1050, 1020, 915.

$[\alpha]_D = +27^o$ (lit $[\alpha]_D^{240} = +30^o$)

(+)-5α,11-dihydroxy-4α(H)-10-*epi*-eudesman-3-one (268)

Dihydrocarvone hydrate (74, 1 g, 5.88 mmol) was added to THF (10 ml) and the mixture was refluxed with sodamide (0.26 g, 90%, 6.67 mmol) overnight. The solution was cooled to 0°C and 1-chloropentan-3-one (0.73 gm, 6.05 mmol) was added dropwise over 10 minutes. The mixture was stirred for 1 hour. Aqueous ammonium chloride was added, the organic layer separated, washed with water and dried. Removal of the solvent followed by distil lation of the low molecular weight material yielded a yellow oil that solidified on standing. Recrystallisation from ether-petroleum ether (40°-60°C) yielded white crystals of (268, 0.15 g, 10%), mpt 192-195°C. (Lit[241] mpt 195-196°C [from petroleum ether, CH_2Cl_2]).

^{1}H NMR(60MHz,d^6-acetone): 0.98(3H,d,7Hz,CH_3); 1.09, 1.11, 1.24(3H,s,CH_3).

MS(MS12,m/e): 254(M^+,0.5%), 236(22%), 221(15%), 170(19%), 152(100%), 81(20%), 68(22%), 67(23%), 59(75%), 43(39%), 41(31%).

IR(film, cm^{-1}): 3500-3400(broad,OH), 1705(C=O).

$[\alpha]_D = +56^o$

(-)-11-hydroxy-10-*epi*-eudesm-4-en-3-one (10-*epi*-carrisone, 103)

The diol (268, 0.1 g, 0.39 mmol) was stirred overnight in conc. hydrochloric acid (2 ml) and ethanol (10 ml). Neutralisation ether extraction followed by drying and evaporation of the ether yielded a white solid (103, 74 mg, 81%), which on recrystalisation from ether/petroleum ether (40^o-60^oC) gave white crystals mpt $54\frac{1}{2}$-56^oC (lit[242] 56^oC).

^1H NMR(60MHz,CCl_4): 1.18, 1.20, 1.24(3H,s,CH_3); 1.8(3H,broad s, CH_3-C=C).

MS(MS12,m/e): 236(M^+,10%), 221(21%), 218(M^+-18,65%), 172(29%), 59(100%), 43(50%), 41(32%), 39(25%).

IR(film, cm^{-1}): 3400(broad,OH), 1650(C=O), 1620(C=C).

UV(EtOH, nm max): 253(ε = 15,500)

$[\alpha]_D = -150^o$

(-)-5α-hydroxy-4α(H)-10-*epi*-eudesm-11-en-3-one (58)

Dihydrocarvone (53, 20.1g, 0.132 mol) was stirred with sodamide (5.15 g, 0.132 mol) in THF (120 ml) overnight. The solution was cooled to 0^oC and 1-chloropentan-3-one (16.7 g, 0.139 mol) in THF (40 ml) was added dropwise of $\frac{1}{2}$ hour. The mixture was stirred at room temperature for 3 hours. Aqueous ammonium chloride was added, the organic layer separated, washed, dried and the solvent evaporated to yield a yellow oil. Chromatography showed the oil to be a mixture of a new product and starting material. Distillation at 0.4 mmHg yielded two fractions i) 80-86^oC shown to be starting material (12.8 g, 64%), and ii) 140-160^oC, the required product (58, 10.8 g, 34%) as a immobile yellow oil. Crystallisation from ether/petroleum ether (40-60^oC) yielded white crystals, mpt 96-98^oC.

^{1}H NMR(60MHz,$CDCl_3$): 1.01(3H,d,J=7.3Hz,CH_3); 1.27(3H,s,CH_3); 1.71 (3H,s,CH_3C=C); 1.92(2H,broad s, CH_2C-O); 2.41 (2H,m,CH_2-C=O), 4.80(2H, broad s, C=CH_2).

MS(MS12,m/e): 236(M^+,17%), 218(M^+-18,6%), 152(93%), 109(100%), 81(15%), 67(16%), 55(24%), 43(21%), 41(38%).

IR(solution $CHCl_3$,cm^{-1}): 3620, 3520(OH), 2760-2960, 1710(C=O), 1645(C=C), 1455, 1380.

$[\alpha]_D = -13^o$

(-)-10-*epi*-eudesma-4,11-dien-3-one (10-*epi*-cyperone, 59)

Keto-alcohol (58, 10.7 gm, 45 mmol) was stirred in ethanol (175 ml) and conc. hydrochloric acid (15 ml) for three days. Neutralisation and extraction followed by drying and evaporation of the solvent yielded a yellow oil (59, 9.9 g, 84%). Distillation afforded a clear liquid bpt 170-172^{o}C @ 2mmHg.

^{1}H NMR(100MHz,$CDCl_3$): 1.24(3H,s,CH_3); 1.73, 1.83(3H,s,CH_3,C=C); 2.3-2.4(4H,m,CH_2-C=O,CH_2-C=C); 4.62, 4.80(1H,s,=CH).

MS(MS30,m/e) 218(M^+,71%), 175(72%), 161(76%), 147(91%), 133 (71%), 132(74%), 107(71%), 93(74%), 91(82%), 41 (100%).

IR(film, cm^{-1}): 3480(broad,OH), 2860-3000, 1710(C=O), 1660(C=C), 1605(C=C), 1450, 1380, 1320, 1200, 1100, 1020, 890.

UV(EtOH, nm max): 252(ε = 5379), 298(819).

$[\alpha]_D = -121^o$

11,12-epoxy-10-*epi*-eudesm-4-en-3-one (94)

Epi-α-cyperone (59, 23 g, 0.106 mol) was dissolved in chloroform (240 ml) and MCPBA (25.8g, 0.127 mmol) was added. The mixture was stirred at

room temperature overnight. The reaction product was washed with freshly prepared saturated sodium metabisulphite, sodium bicarbonate solution and brine. The organic layer was separated, dried, and the solvent evaporated to afford a clear oil (94, 24.6 gm, 98%). Distillation of a small portion led to decomposition.

^{1}H NMR(100MHz,$CDCl_3$):	1.23(3H,s,CH_3); 1.32(3H,s,CH_3C-O); 1.76(3H,s, CH_3,C=C); 2.48, 2.60(2H,dxd,J=5,10Hz,epoxide CH_2).
MS(MS30,m/e):	234(M^+,1.4%), 177(100%), 176(96%), 161(37%), 134(37%), 134(34%), 133(16%), 119(17%), 105 (15%), 91(15%).
IR(film,cm^{-1}):	3040-2850, 1660(C=O), 1610(C=C), 1455, 1380, 1360, 1320, 1040, 750.

3,11-dihydroxy-10-*epi*-eudesm-4-ene (95)

Epoxide (94, 16 g, 68.4 mmol) was dissolved in ether (100 ml) and added dropwise to a cooled suspension of lithium aluminium hydride (5.2 g, 0.136 mol) in ether (600 ml). The mixture was stirred at room temperature for 6 hours. Water (5.2 ml), sodium hydroxide (15% aqueous solution, 5.2 ml) and then water (15.6 ml) were added dropwise, and stirred for 15 minutes to produce a granular precipitate. Filtration through celite, followed by washing the aluminates with ether (100 ml), and removal of the ether yielded a pale yellow oil (95, 15.4 g, 94%). This was used without further purification.

^{1}H NMR(60MHz,$CDCl_3$):	0.82, 1.13, 1.17(3H,s,CH_3), 1.67(3H,s,CH_3,C=C).
MS(MS30,m/e):	220(M^+-18,100%), 205(25%), 202(20%), 162(29%), 147(39%), 123(28%), 109(25%), 105(24%), 96(25%), 82(70%), 81(34%), 59(38%).
IR(film,cm^{-1}):	3400(broad,OH), 3040-2840, 1645(C=C), 1460, 1380, 1020.

(-)-11-hydroxy-10-*epi*-eudesm-4-en-3-one (10-*epi*-carrisone, 103)

The diol (95, 16.3 g, 68.5 mmol) was added in DMF (50 ml) to pyridinium dichromate (32.2 g, 85.6 mmol) in DMF (150 ml) at 0°C. The mixture was stirred at 0°C for 4 hours and then diluted with water (2 l). Ether extraction, followed by drying and removal of solvent yielded the required product as a pale yellow oil (103, 13.6 g, 84%). Column chromatography on a small portion yielded a white crystalline solid, mpt 55-56°C. All spectral data was identical to that previously reported $[\alpha]_D = -160^o$.

(-)-11-hydroxy-4α(H)-10-*epi*-eudesman-3-one (*cis*-dihydro-*epi*-carrisone, 104)

Epi-carrisone (103, 15.6 g, 66.1 mmol) was dissolved in methanol (300 ml) and hydrogenated overnight over palladium on charcoal (100 mg). Hydrogen (1.42 l, 96% of the expected amount) was absorbed. Filtration of the catalyst followed by removal of the solvent yielded a 3:1 mixture of *cis* and *trans* dihydro-*epi*-carrisones (104 and 105, 11.3 g, 72%). Separation by flash column chromatography (4 x 3 g substrate, 100 g SiO_2, 25% pet. ether in ether solvent) yielded *cis*-dihydro-*epi*-carrisone (104, 6.1 g, 39%, 100% pure), *trans*-dihydro-*epi*-carrisone (105, 2.18 g, 14%, 100% pure) and a mixture of 80% *cis* and 20% *trans* (2.98 g, 19%). *Cis*-dihydro-*epi*-carrisone (104) was a white crystalline solid, mpt, 87-88°C (lit[241] 88.5-89.5°C).

^{1}H NMR (100MHz,$CDCl_3$): 0.98(3H,d,J=7Hz,CH_3); 1.16(6H,s,2xCH_3), 1.32 (3H,s,CH_3), 2.3(2H,m,CH_2-C=O), 2.98(1H,m,CH).

MS(MS30,m/e): 238(M^+,0.2%), 220(M^+-18,1.2%), 180(38%), 110(15%), 95(15%), 85(13%), 84(100%), 81(11%), 59(30%), 55(12%).

IR(film,cm^{-1}): 3420(broad,OH), 3000-2840, 1710(C=O), 1390, 1160, 1140.

$[\alpha]_D = -19^o$

Trans-dihydro-*epi*-carrisone (105) was a clear oil.

^{1}H NMR(100MHz,$CDCl_3$):	1.02(3H,d,J=6Hz,MHz); 1.18(3H,s,CH_3); 1.24(6H, s,3xCH_3); 3.48(1H,q,J=6Hz,CH); 4.03(1H,quin, J=6Hz,CH).
MS(MS30,m/e):	238(M^+?0.6%), 220(M^+-18,7%), 180(79%), 125(23%), 123(29%), 109(22%), 108(30%), 96(21%), 95(30%), 84(100%), 81(21%), 59(93%), 55(23%).
IR(film,cm^{-1}):	3400, 3000-2840, 1705, 1380, 1160, 1125.

(+)-11-hydroxy-10-*epi*-eudesm-3-ene (*epi*-α-eudesmol, dihydrooccidentalol, 251)

Cis-dihydro-*epi*-carrisone (104, 1 gm, 4.2 mmol) was refluxed for 1 hour with p-toluenesulphonyl hydrazine (1.84 g, 5.7 mmol) and hydrochloric acid (0.5 ml) in ethanol (100 ml). The ethanol was removed by distillation and then the residue was taken up in ethylene glycol (270 ml) containing dissolved sodium (1.67 g, 72.6 mmol).

This was heated at 140°C under nitrogen, until the evolution of nitrogen ceased (about 2 hours). The product was poured into water (1 l) and extracted thoroughly with ether. The ether extract was washed with brine and dried. Removal of the solvent gave a yellow oil. The yellow oil was purified by flash column chromatography (10 g SiO_2, 50% ether in pet. ether) to yield the required product as a clear oil (251, 0.26 g, 28%). Further chromatography on a small sample yielded white crystals mpt 85-86°C (lit[243] 86-87.5°C).

^{1}H NMR(100MHz,$CDCl_3$):	0.86(3H,s,CH_3); 1.17(6H,s,2xCH_3); 1.68(3H,m, CH_3.C=C); 5.26(1H,m,C=CH).
^{13}C NMR(25.2MHz,$CDCl_3$, ppm):	137.1, 119.1, 72.9, 49.4, 47.5, 40.9, 31.4, 30.4, 29.6, 27.1, 26.8, 26.1, 23.2, 22.8, 22.7.
MS(MS30,m/e):	222(M^+,2.1%), 207(M^+-15,1.6%), 204(M^+-18,29%), 161(16%), 149(39%), 109(61%), 107(18%), 93(16%), 59(100%), 43(17).

IR(film,cm^{-1}): 3480(broad,OH), 3000-2840, 1635, 1440, 1370, 1120.

$[\alpha]_D = +65^o$

(-)-4,11-epoxy-10-*epi*-eudesmane (21)

Epi-α-eudesmol (25l, 1.94, 8.74 mmol) was dissolved in THF (10 ml) and added dropwise to a stirred suspension of mercuric acetate (22 g, 68.9 mmol) in THF (125 ml) and water (125 ml). After stirring for 24 hours, sodium hydroxide (3M aqueous, 60 ml) and sodium borohydride (0.5M in 3M NaOH) were added. The mercury that was deposited was filtered and washed with ethyl acetate. The organic washings were combined, washed with brine and then dried. Removal of the solvent gave a mixture of the required product (21) and starting material. Separation by flash column chromatography (100 gm SiO_2, 10% ether in pet ether) yielded the product as a colourless liquid (21, 0.17 g, 9%) and the starting material (25l, 0.97g, 55%) which was used again.

^1H NMR(100MHs,$CDCl_3$): 1.00, 1.23, 1.26, 1.31(3H,s,CH_3).

^{13}C NMR(25.2MHz,$CDCl_3$, ppm): 74.6, 72.9, 42.8, 42.2, 41.0, 34.8, 32.6, 31.0, 29.7, 29.3, 29.1 (x2), 25.2, 22.2, 17.7.

MS(MS30,m/e): 222(M^+,0.2%), 207(70%), 189(27%), 149(24%), 109(24%), 109(100%), 107(30%), 93(31%), 91 (26%), 81(35%), 79(24%), 67(27%), 55(37%), 43(61%), 41(58%).

IR(film,cm^{-1}): 3020-2840, 1480, 1460, 1450, 1380, 1375, 1360, 1265, 1245, 1220, 1175, 1130, 1090, 1040, 1020, 970, 810, 780.

$[\alpha]_D = +17^o$ (lit[240]: $+19^o$)

3,4-epoxy-11-hydroxy-10-*epi*-eudesmane (3,4-epoxy-*epi*-α-eudesmol, 29O)

Epi-α-eudesmol (25l, 0.11 g, 0.5 mmol) was stirred with MCPBA (0.107 g, 0.62 mmol) in chloroform (5 ml) for 30 mins. After work up by washing

with sodium metabisulphate solution and sodium bicarbonate solution, the organic extract was washed with brine. After drying, removal of the solvent yielded a clear oil (290, 0.117, 99%).

^{1}H NMR(60MHz,CCl_4): 0.84(3H,s,CH_3); 1.14(6H,s,2xCH_3); 1.24(3H,s, epoxide, CH_3); 2.85(1H,m,epoxide CH).

MS(MS30,m/e): 238(M^+,0.3%), 165(22%), 125(66%), 107(28%), 95(47%), 84(37%), 81(27%), 59(71%), 43(100%), 41(31%).

IR(film,cm^{-1}): 3460(broad,OH), 3000-2840, 1460, 1380, 895, 730.

3-11-dihydroxy-3β(H)-10-*epi*-eudesm-14-ene (294)

The epoxide (290, 70 mg, 0.29 mmol) was refluxed overnight with alumina (100 mg) in THF (5 ml). Filtration followed by removal of the solvent yielded a mixture of components which were separated by flash column chromatography (10 g SiO_2, 50% ether in pet. ether) to yield the product (294, 35 mg, 50%).

^{1}H NMR(100MHz,$CDCl_3$,δ): 0.84(3H,s); 1.20(6H,s); 4.26(1H,t,J=2½Hz); 4.88(2H,dxd,J=3Hz,9Hz).

IR(thin film,cm^{-1}): 3360, 3000-2800, 1635, 1450, 1350, 905.

MS(MS30,m/e): 238(0.3%), 220(5%), 205(6%), 162(92%), 147(72%), 133(45%), 119(41%), 107(41%), 106(36%), 105(45%), 93(38%), 81(39%), 59(100%), 43(38%).

M^+ measured 238.2133 $C_{15}H_{26}O_2^+$, calculated 238.1933.

5β-(2-propenyl)-2-methylcyclohexanone ethylene ketal (295)

Dihydrocarvone (53, 13 g, 85.3 mmol) ethylene glycol (7.5 ml, 135 mmol) & p-toluenesulphonic acid (20 mg) were refluxed in benzene (100 ml) overnight in a Dean and Stark apparatus. The product mixture was diluted with water, washed with sodium bicarbonate solution and brine and then

dried. Removal of the solvent yielded a clear oil (295, 16.7 g, 99%). Bpt 112-4°C @ 17 mmHg.

^{1}H NMR(60MHz,CCl_4): 0.84(3H,d,J=5.3Hz,CH_3); 1.68(3H,s,CH_3,C=C); 3.87(4H,s,O-CH_2-CH_2-O); 4.64(2H,broad s,C=CH_2).

MS(MS30,m/e): 181(M^+-15,1.7%), 155(52%), 139(33%), 103(54%), 95(29%), 81(30%), 69(34%), 67(34%), 59(63%), 55(53%), 43(100%), 41(74%).

IR(film,cm^{-1}): 2980, 2940, 2880, 1645(C=C), 1455, 1265, 1095, 1020, 890, 805.

5β-(2-methoxy-2-propyl)-2-methylcyclohexanone ethylene ketal (296)

The protected ketone (295, 15 g, 76.5 mmol) in methanol (50 ml) was added to a stirred suspension of mercuric acetate (24.5 gm, 76.8 mmol) in methanol (150 ml). Sodium hydroxide solution (3M, 77 ml) and sodium borohydride (0.5M in 3M NaOH, 77 ml) were added and mercury was deposited immediately. Filtration through celite followed by washing of the filter cake with ethyl acetate yielded an extract which was washed with brine and then dried. Removal of the solvent yielded the required product as a colourless oil (296, 14.1 g, 81%). Bpt: 94-96°C @ 0.4 mmHg.

^{1}H NMR(60MHz,CCl_4): 0.80(3H,d,J=6Hz,CH_3); 1.02, 1.04(3H,s,CH_3); 3.11(3H,s,O-CH_3); 3.91(4H,s,O-CH_2-CH_2-O).

MS(MS30,m/e): 228(M^+,0.8%), 155(100%), 113(26%), 99(14%), 73(80%), 55(12%), 43(13%), 41(10%).

5β-(2-methoxy-2-propyl)-2-methylcyclohexanone (297)

The methoxyketal (296, 11g, 48.2 mmol) was refluxed overnight in 10% aqueous acetone (200 ml) with p-toluenesulphonic acid (3 gm). The product mixture was washed with sodium bicarbonate solution and brine, and then dried over magnesium sulphate. Removal of the solvent yielded the required product as a clear oil (297, 8.9 g, 94%). This compound was used without further purification.

^{1}H NMR(60MHz,CCl_4):	0.96(3H,d,J=6.7Hz,CH_3); 1.12(6H,s,2xCH_3); 3.16 (3H,s,O-CH_3).
MS(MS30,m/e):	184(M^+,0.3%), 81(24%), 73(100%), 69(38%), 57, (27%), 55(29%), 43(30%).
IR(film,cm^{-1}):	2980, 2940, 2880, 1710(C=O), 1460, 1385, 1370, 1255, 1255, 1190, 1150, 1140, 1080.

5-hydroxy-11-methoxy-10-*epi*-eudesm-4-en-3-one (298)

The ketone (297, 8 g, 43.5 mmol) was stirred with sodamide (1.7 g, 43.6 mmol) in THF (100 ml) under reflux overnight. The mixture was cooled to 0°C and 1-chloropentan-3-one (5.25, 43.5 mmol) in THF (10 ml) was added dropwise at this temperature. The reaction mixture was then warmed to room temperature and stirred for 1 hour. Saturated ammonium chloride solution was added, the organic layer separated, washed with brine and dried. Removal of the solvent showed the reaction product to be a mixture of the required methoxyketol and starting material. The starting material was distilled away to leave methoxyketol (298, 5.6 g, 48%).

^{1}H NMR(60MHz,d^5-pyridine):	1.00(6H,s,2xCH_3); 1.08(3H,s,CH_3); 1.38(3H, s,CH_3); 2.07(3H,s,O-CH_3).
MS(MS30,m/e):	268(M^+,0.1%), 250(M^+-18,10%), 218(22%), 178(21 %), 73(100%), 43(11%).
IR(film,cm^{-1}):	3500(broad,OH), 3000-2840, 1705(C=O), 1460, 1385, 1370, 1080, 995.

(-)-11-methoxy-10-*epi*-eudesm-4-en-3-one (*epi*-carrisone methyl ether, 299)

The methoxyketol (298, 5.6 g, 20.9 mmol) was stirred with alumina (20 gm) overnight at room temperature. Filtration followed by removal of solvent yielded *epi*-carrisone methyl ether (299) as a pale yellow oil. Purification by flash column chromatography (100 g, pet. ether) yielded (299) as a clear oil (5.22 g, 80%), 39% overall yield from (298).

^{1}H NMR(60 MHz,CCl_4): 1.04, 1.11, 1.20(3H,s,CH_3); 1.70(3H,s,CH_3,C=C); 3.12(3H,s,O-CH_3).

MS(MS30,m/e): 219(M^+-31,3.3%), 218(M^+-32,19%), 203(4%), 178 (11%), 163(4%), 74(4%), 73(100%), 55(4%), 43 (4%), 41(6%).

IR(solution $CHCl_3$,cm^{-1}): 3020-2820, 1650(C=O), 1615(C=C), 1460, 1385, 1370, 1360, 1070.

$[\alpha]_D = -55^o$

(-)-11-methoxy-4α(H)-10-*epi*-eudesman-3-one (300)

α-*Epi*-carrisone methyl ether (299, 2g, 8 mmol) was stirred overnight in methanol (25 ml) over a palladium on charcoal catalyst (10%, 50 mgs) under a hydrogen atmosphere.

One molar equivalent (195 ml) of hydrogen was taken up. Filtration of the catalyst through celite, followed by evaporation of the solvent yielded a 3:1 mixture (GC analysis) of *cis* and *trans* hydrogenated products. Flash column chromatography (100g SiO_2, 100% petroleum ether) of the mixture yielded pure *cis* product (300, 0.9 gm, 45%) as a clear oil.

^{1}H NMR(60MHz,$CDCl_3$): 0.91(3H,d,J=7Hz,CH_3); 1.08, 1.10, 1.29(3H,s,CH_3); 2.63(2H,broad s,CH_2CO), 3.17(3H,s,O-CH_3).

IR($CHCl_3$ solution, cm^{-1}): 3040-2840, 1695, 1460, 1385, 1370, 1270, 1255.

(+)-11-methoxy-10-*epi*-eudesm-3-ene (*epi*-α-eudesmol methyl ether, 301)

The *cis*-hydrogenated product (300, 1.4 gr, 5.6 mmol) was refluxed with p-toluene sulphonyl hydrazine (1.6 g, 8.6 mmol) and a few drops of hydrochloric acid in methanol (100 ml) for 1 hour. The methanol was removed by evaporation and the yellow residue was taken up in ethylene glycol (30 ml). Sodium, (1.8 g) dissolved in ethylene glycol, (170 ml) was added and the mixture was heated at 200^o under nigrogen until no further evolution of gas was observed (1½ hours). The reaction products were allowed to cool and then were extracted thoroughly with ether (3x150 ml).

The volume was reduced (150 ml) and the extract was washed with water and brine. Drying followed by the removal of the solvent yielded a yellow oil. Purification by flash column chromatography (100 g SiO_2, petroleum ether) yielded the required product as a clear oil (301, 0.24 g, 20%).

^1H NMR(60MHz,CCl_4):	0.87(3H,s,CH_3); 1.04(6H,s,CH_3); 1.27(3H,s, C=C-CH_3); 3.1(3H,s,OCH_3); 5.2(1H,broad,s, C=CH).
MS(MS30,m/e):	221(M^+-15, 0.4%), 109(1.7%), 107(1.9%), 74(5.1 %), 73(100%), 72(2.1%), 55(2.4%), 45(2.0%), 41(1.7%).
IR(film,cm^{-1}):	2970, 2940, 1650, 1470, 1460, 1380, 1370, 1265, 1085, 1040, 805, 780.

2,6,6-trimethyl-cyclohexene-1-carbaldehyde[β-cyclocitral, (317)]

Citral (314, 65 ml, 0.42 mol) was added to aniline (34 ml, 0.43 mol) in ether (60 ml). After 1 hour the etheral solution of citral anil (315) was decanted and added dropwise to 95% sulphuric acid at -20°C over a period of 1 hour. The mixture was stirred for a further hour at -15°C. The thick oil was poured onto ice (1.5 kg) and extracted thoroughly with ether. Separation followed by drying and removal of the ether yielded a yellow liquid which on analysis was found to be two major components in a 1:1 ratio. Distillation of these components afforded α-cyclocitral (316, 13.4 g, 32%) and β-cyclocitral (317, 11.8 g, 28%). Treatment of the 1:1 mixture before distillation, with sodium hydroxide (7 gm) in methanol (130 ml) at 0°C yielded a 10:1 ratio of β-cyclocitral (317):α-cyclocitral (316). Distillation yielded pure β-cyclocitral (317, 25.2 gm, 60%) and α-cyclocitral (316, 2.5 gm, 6%). α-Cyclocitral (316): Bpt 48-52°C @ 2.5 mmHg.

^1H NMR(60MHz,CCl_4):	0.92(3H,s,CH_3), 0.99(3H,s,CH_3); 1.59(3H,d,J=2Hz, C=C-CH_3);, 5.7(1H, broad s,C=CH); 9.43(1H,d, J=5.5Hz,C=C-CHO).

IR(film,cm^{-1}): 1720, 1640

β-Cyclocitral (317): Bpt 56-58°C @ 2.5 mmHg.

^1H NMR(60MHz,CCl_4): 1.18(6H,s,2xCH_3); 2.09(3H,s,C=C-CH_3), 10.1(1H, s,CHO).

MS(MS12,m/e): 152(M^+,6.1%), 137(100%), 123(73%), 119(81%), 109(71%), 81(74%), 67(70%), 41(80%).

IR(film,cm^{-1}): 1686, 1630.

1-vinyl-2,6,6-trimethylcyclohexene (217)

Triphenylphosphine methiodide (29.2 g, 72.3 mmol) was stirred with reflux under nitrogen with potassium t-butoxide (8.1 g, 71.9 mmol) in THF (200 ml) for 2 hours. The orange solution of the phosphorane was cooled to 0°C and β-cyclocitral (317, 10 g, 63.8 mmol) was added dropwise over ½ hour. The mixture was stirred for 1 hour at 0°C and 1 hour at room temperature and then saturated aqueous ammonium chloride (100 ml) was added. The organic layer was separated, the aqueous layer washed with ether and the extracts were dried. Removal of solvent followed by distillation (54-56°C @ 2 mmHg) afforded diene (217) as a clear liquid in 58% yield.

^1H NMR(60MHz,CCl_4): 0.99(6H,s,2xCH_3); 1.1-2.0(6H,m,CH_2's), 1.67(3H, Broad s,C=C-CH_3); 4.92(1H,dxd,J=2,18Hz,C=CH_2); 5.25(1H,dxd,J=2,12Hz,C=CH_2); 6.22(1H,dxd,J=12,18Hz, C=CH).

MS(MS12,m/e): 150(M^+,28%), 135(100%), 119(46%), 107(36%), 93 (39%), 91(30%), 79(39%), 41(38%).

IR(film,cm^{-1}): 2970, 2940, 2880, 1620, 1440, 1275, 1045, 915, 890.

Dimethyl-3,5,6,7,8,8a-hexahydro-5,5,8a-trimethyl-1,2-naphthalene dicarboxylate (325)

Dimethylacetylene dicarboxylate (DMAD, 2.3 ml, 18.3 mmol) was heated with diene (217, 2.5 gm, 16.7 mmol) in a sealed tube under vacuum at 120°C overnight. Flash column chromatography (100 g SiO_2, 1:1 ether, petroleum ether) gave diester (325) in 87% yield. Further purification followed by crystallisation from ether, petroleum ether yielded colourless crystals, mpt 50-51°C.

^{1}H NMR(100MHz, $CDCl_3$):	1.14(3H,s,CH_3); 1.20(6H,s,2xCH_3); 1.45(3H,s,CH_3); 1.2-2.0(6H,m,CH_2's); 2.98(1H,dxd,J=3,22.3Hz,3-H); 2.88(1H,dxd,J=6,22.3Hz,3-H); 3.71, 3.79(3H,s,CO_2CH_3); 5.69(1H,dxd,J=3.6Hz,4-H).
MS(MS12,m/e):	292(M^+,1.3%), 260(24%), 245(100%), 233(37%), 213(75%), 163(33%), 119(30%), 110(38%), 59(24%).
IR(Film,cm^{-1}):	3040-2880, 1760, 1670, 1640, 1480, 1295, 1090, 1060.
UV(hexane, λ_{max}):	233 nm (ε 3200).

Dimethyl-3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-1,2-naphthalene dicarboxylate (334)

Diester (325, 15 g, 51.1 mmol) was stirred in acetic acid (100 ml) over Adams catalyst (platinum dioxide) under a hydrogen atmosphere for 48 hours. The catalyst was removed by filtration through celite and the filtrate was neutralised by the careful addition of solid sodium carbonate. Ether extraction followed by drying and removal of the solvent yielded a thick yellow oil. Analysis showed a mixture of four major components (gc assay), from which the two major components were obtained by distillation on a spinning-band microstill. Spectral analysis showed the major component to be the required monohydrogenated product (334, 6 gm, 40%, bpt 105-110°C / 1.5 mmHg) and the other major product to be the tetrahydroderivative dimethyl-1,2,3,4,4a,5,6,7,8,8a-decahydro-5,5,8a-trimethyl-[4aβ,8aβ]-1,2-naphthalene dicarboxylate (335, 2.25 g, 15%, bpt 114-118°C @ 1.5 mmHg).

Dihydroproduct (334):

^{1}H NMR(100MHs,$CDCl_3$):	0.96, 0.99(3H,s,CH_3); 1.27(3H,s,CH_3); 1.0-2.2 (9H); 2.2-2.8(2H,m,3-H's); 3.73,3.82(3H,s, CO_2CH_3).
MS(MS12,m/e):	294(M^+,6%), 247(33%), 235(29%), 234(100%), 105(32%), 91(29%), 44(53%), 41(41%), 40(73%).
IR(film,cm^{-1}):	2980, 2940, 2880, 1740, 1720, 1640, 1480, 1460, 1440, 1390, 1370, 1305, 1260, 1220, 1150, 1065, 1035.
UV(hexane,λ_{max}):	235nm(2710).

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1,2-dimethanol (336)

Hydrogenated diester (334, 6 g, 20.4 mmol) was added dropwise to a suspension of lithium aluminium hydride (1.55 g, 40.8 mmol) in ether (200 ml), and the mixture was refluxed for 1 hour. The dropwise addition of water (1.6 ml), sodium hydroxide solution (15%, 1.6 ml) and then water (4.8 ml) followed by stirring for ½ hour afforded a granular precipitate of aluminates which was removed by filtration through celite. Removal of the solvent yielded diol (336, 9.78 g, 98%) as a white crystalline solid, mpt 124-126^{o}C.

^{1}H NMR(100MHz,$CDCl_3$):	0.94, 1.01, 1.12(3H,s,CH_3); 1.1-2.1(11H); 2.29 (2H,m,3-H's); 4.13, 4.29(2H,s,CH_2-O).
^{13}C NMR(25.2MHz,$CDCl_3$):	142.5(s), 137.7(s), 64.1(t), 58.5(t), 49.9(d), 41.9(t), 37.9(s), 36.9(t), 34.6(s), 32.7(q), 31.9(q), 28.6(t), 25.4(q), 20.2(t), 19.0(t).
MS(MS12,m/e):	220(M^+-H_2O,7%), 109(13%), 107(18%), 91(15%), 69(19%), 55(17%), 44(76%), 41(26%), 40(100%).
IR($CDCl_3$,cm^{-1}):	3610, 3420, 2920, 2860, 1640, 1475, 1460, 1395, 1365, 1205, 985, 720.

1,3,4,5,5a,6,7,8,8a,9,9a-decahydro-6,6,9a-trimethyl-1-oxo-[5aα,9aβ,9bβ]-naphtho-[1,2c]-furan (5-*epi*-confertifolin, 357).

Diol (336, 48 mg, 0.204 mmol) was oxidised with chromium trioxide (0.121 gm, 1.21 mol) and pyridine (0.20 ml, 2.42 mmol) in methylene chloride (5 ml) for 15 minutes. The product mixture was passed down a florisil column with methylene chloride eluant. Removal of the solvents by evaporation followed by analysis showed the presence of one major product. Spectral analysis showed this clear oil to be lactone (357, 16 mg, 37%).

^{1}H NMR(60MHz,CCl_4):	0.65, 0.98, 1.19(3H,s,CH_3), 2.4(2H,m,C=C-CH_2); 4.1(2H,broad s,CH_2-CO).
MS(MS30,m/e):	234(M^+,79%), 219(100%), 164(30%), 152(41%), 151(72%), 150(39%), 91(45%), 41(51%).
IR(film,cm^{-1}):	3040, 2980-2840, 176, 1680, 1450, 1390, 1375, 1365, 1080, 1080-1000, 890, 690.

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1-methanol-2-t-butyldimethylsilyloxymethyl (369d)

Diol (336, 400 mg, 1.66 mmol) tbutyldimethylsilyl chloride (254 mg, 1.68 mmol) and imidazole (308 mg, 4.53 mmol) in DMF (13 ml) at 0°C. The mixture was stirred at 0°C for 15 minutes and 15 minutes at room temperature and then diluted with water (200 ml). After ether extraction and washing with brine, the product solution was dried and the solvent evaporated. Flash column chromatography (100 gm, SiO_2, petroleum ether) yielded silyl-alcohol (369 d, 586 mg, 80%) as a clear oil. From the product mixture, diol (336, 31 mg, 8%) and disilyl diol (376d, 31 mg, 4%) were also isolated.

Monosilyl product (369d):

^{1}H NMR(60MHz,$CDCl_3$,internal Si-CH_3 standard):	0.00(6H,s,CH_3-Si); 0.84(9H,s,tBu); 0.80, 0.91, 1.02(3H,s,CH_3); 1.0-2.5(8H); 4.1(4H,m,CH_2-O).
MS(MS30,m/e):	334(M^+-H_2O, 3.5%), 203(76%), 119(37%), 107(37%) 105(62%), 95(37%), 75(100%), 73(42%).

IR(film,cm^{-1}): 3400, 2980, 2950, 1675, 1465, 1390, 1260, 1050, 835, 770.

Disilyl product (376d):

^1H NMR(60MHz,$CDCl_3$,internal Si-CH_3 standard): 0.00(12H,s,Si-CH_3); 0.83(3H,s,CH_3); 0.85(18H, s,tBu); 1.06(6H,s,CH_3); 1.1-2.5(11H); 4.20(4H, m,CH_2-O).

MS(MS30,m/e): 466(M^+, 0.7%), 335(22%), 334(76%), 252(21%), 251(100%), 147(46%), 138(49%), 140(94%), 69 (18%).

IR(film,cm^{-1}): 2980, 2970, 2950, 2930, 1675, 1475, 1465, 1390, 1365, 1260, 1090, 1050, 1010, 835, 770.

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1-acetoxy-methyl-2-tbutyldimethylsilyloxymethyl (375)

Silyl alcohol (369d, 701 mg, 1.99 mmol) was stirred overnight with acetic anhydride (2 ml, 21 mol) in pyridine (10 ml). The product mixture was extracted with ether, washed with sodium bicarbonate and brine and then dried. Removal of the solvent yielded a clear oil (375, 560 mg, 71%).

^1H NMR(60MHz,$CDCl_3$,internal Si-CH_3 standard): 0.00(6H,s,Si-CH_3); 0.82(3H,s,CH_3); 0.87(9H,s, tBu); 0.94, 1.04(3H,s,CH_3); 1.99(3H,s,$OCOCH_3$); 1.1-2.5(11H); 4.07(2H,s,Si-O-CH_2); 4.66 (2H,d,J=12Hz,AcO-CH_2).

MS(MS30,m/e): 334(M^+-60,16%), 251(38%), 203(59%), 147(21%), 119(31%), 117(100%), 75(54%), 73(40%).

IR(film,cm^{-1}): 2980, 2970, 2935, 1740, 1655, 1475, 1465, 1460, 1390, 1380, 1370, 1260, 1235, 1090, 1050, 1020, 840, 775.

3,4,4a,5,6,7,8,8a-Octahydro-5,5,8a-trimethyl-[5aβ,8aβ]-naphthalene-1-acetoxymethyl-2-methanol (376)

Silyl acetate (375, 390 mg, 0.990 mmol) in THF (5 ml) was stirred with tetrabutyl ammonium fluoride (1M in THF, 1.48 ml, 1.48 mmol) for ½ hour. Flash column chromatography (10 gm, SiO_2, 50% petroleum ether in ether) yielded pure product as a clear oil (376, 0.274 g, 99%)½

^{1}H NMR(100MHz,$CDCl_3$):	0.89, 0.98, 1.08(3H,s,CH_3); 2.06(3H,s,$OCOCH_3$); 1.1-2.5(12H), 4.12(2H,d,J=12Hz,2-CH_2); 4.83 (2H,d,J=12.5Hz,1-CH_2).
MS(MS30, recorded as TMS ether, m/e):	292(M^+-60), 24%), 210(20%), 209(100%), 119(39%), 117(18%), 103(17%), 75(23%), 73(75%).
IR(film,cm^{-1}):	3440, 3000-2860, 1725, 1650, 1470, 1440, 1370, 1360, 1230, 1190, 1170, 1120, 1090, 1020, 970, 950.

3,4,4,a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1-acetoxymethyl-2-carbaldehyde (377)

Acetate-alcohol (376, 109mδ, 0.39 mmol) in DMF (1 ml) was added dropwise to pdc (525 mg, 0.49 mmol) in DMF (2 ml) at 0°C. After stirring at this temperature for 2 hours, the product was poured into water (50 ml), extracted with ether, washed with brine and dried. Removal of the solvent followed by flash column chromatography (100g silica, 20% ether in petroleum ether), yielded a clear oil (377, 82 mg, 77%).

^{1}H NMR(100MHz,CCl_4):	0.86, 1.02, 1.18(3H,s,CH_3); 2.08(CH_3,s,0.$COCH_3$) 1.1-1.6(8H); 1.6-2.5(3H); 5.12(2H,d, J=12.5Hz,1-CH_2); 10.15(1H,s,CHO).
MS(MS30,m/e):	278(M^+, 0.4%), 236(31.1%), 218(28%), 205(24%), 203(23%), 135(100%), 69(22%), 43(40%), 41(24%).
IR(film,cm^{-1}):	3010, 2980-2960, 2860, 1730, 1660, 1620, 1470, 1445, 1370, 1360, 1220, 1040, 960, 750.

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1-acetoxymethyl-2-carbaldehyde ethylene acetal (378a)

Acetate-aldehyde (377, 47 mg, 0.17 mmol) ethylene glycol (0.1 ml, 1.8 mmol) and a catalytic amount of p-toluenesulphonic acid, were refluxed overnight with benzene (30 ml) in a Dean-Stark apparatus. The product mixture was washed with sodium bicarbonate solution, dried and the solvent removed to give the product as a clear oil (378a, 48mg, 89%).

^{1}H NMR(100MHz,CCl_3): 0.91, 0.97, 1.10(3H,s,CH_3); 2.01(13H,s,O.$COCH_3$); 1.1-1.6(8H); 1.6-2.5(3H): 3.85(4H,m,O-CH_2CH_2-O); 4.68(2H,d,J=13Hz,1-CH_2); 5.50(1H,s, O-CH-O).

MS(MS30,m/e): 322(M^+,0.2%); 263(15%), 262(28%), 247(22%), 179(83%), 73(100%), 55(13%), 45(17%), 43(31%).

IR(film, cm^{-1}): 3920, 3860, 1730, 1470, 1450, 1375, 1360, 1260, 1140, 1105, 1070, 1020, 990, 975, 915.

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1-t-butyldimethyl silyloxymethyl-2-methanol (375d)

Diol (336, 600 mg, 2.52 mmol) was silylated with t-butyldimethylsilyl chloride (417 mg, 2.77 mmol) and imidazole (714 mg, 12.6 mmol) in DMF (10 ml) at room temperature for 30 minutes. After diluting with water (100 ml) the products were extracted with ether. The extract was dried and the solvent removed to leave a clear oil. Analysis by gas chromatography showed a mixture of the two possible silyl-alcohols (369d) and (375d). Analysis by HPLC showed a 1:1 mixture of components (eluant heptane) which when separated on a preparative scale, afforded the two silyl-alcohols. Silyl alcohol (375d, 283 mg) was isolated in 32% yield.

^{1}H NMR(60MHz,$CDCl_3$,internal Si-CH_3 standard): 0.00(6H,s,CH_3-Si), 0.86(9H,s,tBu); 0.80, 0.93, 1.05(3H,s,CH_3); 4.1(4H,m,CH_2-O).

MS(MS30,m/e): 334(M^+-18,4%), 203(85%), 119(25%), 107(30%), 105(55%), 95(32%), 75(100%), 73(41%).

IR(film,m/e): 3400, 2980-2860, 1675, 1465, 1255, 1045, 840, 775.

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8β]-naphthalene-1-methanol -2-acetoxymethyl (383)

Monosilylalcohol (375d, 280 mg, 0.80 mmol) was acetylated with acetic anhydride (0.07 ml, 7.95 mmol) in pyridine (2 ml) over night. The product mixture was diluted with water, extracted with ether and dried. Removal of the solvent yielded (382) as a pale yellow oil (378 mg, 76%). The silyl protecting group was removed by stirring with tetrabutylammonium fluoride (0.5 ml, 1M in THF) for 30 minutes at room temperature. Dilution with water, extraction with ether and washing with brine was followed by drying. Removal of the solvent yielded (383) as a pale yellow oil (143 mg, 85%).

^{1}H NMR(100MHz,$CDCl_3$): 0.85, 0.97, 1.10(3H,s,CH_3), 2.04(3H,s,O.CO.CH_3); 1.1-2.6(12H); 4.26(2H,s,CH_2OH); 4.69 (2H,d,J=12Hz,CH_2OAc).

MS(MS30, recorded as the TMS ether, m/e): 292(M^+-60,29%), 277(17%), 210(18%), 209(100%), 147(12%), 117(10%), 75(17%), 73(63%).

IR(film,cm^{-1}): 3440, 2960-2840, 1700, 1645, 1470, 1440, 1370, 1230, 1020, 975, 955.

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-napthalene-1-carbaldehyde-2-acetoxymethyl (381)

Acetate alcohol (383, 136 mg, 0.486 mmol) was added to pdc (230 mg, 0.607 mmol) in DMF (2 ml) at 0°C and stirred for three hours. After dilution with water (100 ml), the product was extracted with ether, dried and the solvent removed to yield a yellow oil. The product mixture was purified by flash column chromatography (10g silica petroleum ether) to afford a clear oil (381, 82 mg, 61%).

^{1}H NMR(60MHz,CCl_4):	0.86, 0.97, 1.23(3H,s,CH_3); 1.98(3H,s,O.CO.CH_3); 1.1-2.6(11H); 4.76(2H,s,CH_2OAc); 10.11(1H,s, CHO).
MS(MS30,m/e):	278(M^+,0.4%), 232(29%), 217(100%), 149(30%), 69 (38%), 44(31%), 43(47%), 41(28%).
IR(film,cm^{-1}):	2960-2940, 2875, 1750, 1680, 1460, 1360, 1380, 1370, 1240, 1030, 975, 790, 760.

3,4,4a,5,6,7,8,8a-octahydro-5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1-acetoxymethyl-2-carboxaldehyde propylene acetal (378b)

Acetate-aldehyde (377, 0.780 g, 2.81 mmol) propane-1,3-diol (2.1 g, 28.1 mmol) and a catalytic amount of p-toluenesulphonic acid were refluxed with benzene (100 ml) overnight in a Dean and Stark apparatus. The product mixture was washed firstly with sodium bicarbonate solution, then brine and finally dried. The solvent was removed to yield a clear oil (378b, 0.93g, 98%).

^{1}H NMR(60MHz,CCl_4):	0.89, 0.94, 1.06(3H,s,CH_3); 1.15-2.60(13H), 1.96(3H,S,O.CO.CH_3); 3.9(4H,m,O-CH_2); 4.53 (1H,d,J=12Hz,CH_2-OAc); 5.25(1H,s,O-CH-O).
MS(MS30,m/e):	336(M^+,5.2%), 277(55%), 276(53%), 261(40%), 203(24%), 193(64%), 87(100%), 43(34%).
IR(film,cm^{-1}):	3000-2870, 1740, 1675, 1480, 1460, 1455, 1430, 1390, 1380, 1370, 1240, 1150, 1110, 1025, 100, 980, 960, 925, 860, 790.

3,4,4a,5,6,7,8,8a-octahydro5,5,8a-trimethyl-[4aβ,8aβ]-naphthalene-1-methanol-2-carbaldehyde propylene ketal (379b)

Acetate-acetal (378b, 400 mg, 1.19 mmol) was stirred overnight with potassium carbonate (200 mg) in methanol (10ml). Filtration of the resulting pale yellow solution, to remove the potassium carbonate, followed by evaporation of the solvent yielded a pale yellow oil (379b, 0.35 g, 81%).

^{1}H NMR(60MHz,CCl_4):	0.89, 0.97, 1.08(3H,s,CH_3); 1.1-2.5(14H), 4.0 (6H,m,O-CH_2); 5.19(1H,s,O-CH-O).
MS(MS30, recorded as TMS ether, m/e):	366(M^+,16%), 276(36%), 261(27%), 193(100%), 87(76%), 75(20%), 73(49%), 41(22%).
IR(film,cm^{-1}):	3550, 2990-2890, 2860, 1650, 1475, 1760, 1755, 1395, 1370, 1150, 1110, 1080, 1045, 1030, 985.

CHAPTER 5

Chemical Composition of the Cuticular Waxes of *H. bovis* and *H. lineatum*

5.1.1. Introduction

Two-winged flies, or true flies of the order Diptera, make up a very large order. About 70,000 species are known, of which about 5,200 are known in Britain. The order is made up of species of different shapes and sizes, the major feature being two wings (hence the name of the order, Greek di = two). There are the stout bodied house-flies and blow-flies, the hover-flies and horse-flies, the slender crane-flies and mosquitos and all the small flies usually referred to as gnats and midges.

Flies have adapted to feed on a wide range of foodstuffs, from decaying matter to blood, and the order makes a great difference to the ecology of the world. Some flies are agricultural pests (e.g. Carrot-flies); others scavange decaying matter (e.g. dung-flies); the flower feeders also act as pollenators; but there are species that cause health and economic problems to man. The blood sucking flies, such as mosquitos and the tsetse fly, carry dangerous diseases that include malaria and yellow fever. The common house-fly is not a blood sucker, but in feeding on filthy and sweet things, it can contaminate human food and spread diseases. Veterinary pests include the green blow-flies (Lucilia spp) - the maggots of which eat the flesh of sheep - and the warble fly - whose larvae burrow through the hide of cattle.

The fly family Oestridae contains a number of large fluffy flies whose larvae tend to be internal parasites of animals. Included in this family are warble flies and the sheep nostril fly (*Oestrus ovis*). The flies of interest in these studies are from the *Hypoderma* species known as warble flies in Britain, but as the "cattle grub", the "bot fly" or the "bomb fly" in other countries.

There are six species of *Hypoderma: H. actaeon* (affects red deer); *H. aeratum* and *H. crossii* (affects goats); *H. diana* (affects red deer and goats); *H. bovis* and *H. lineatum* (affects cattle)[244]. Of these, only *H. bovis, H. lineatum* and *H. diana* are found in Britain, the climate of which suits these three species best. Climatic conditions have been found to be important to the survival of *Hypoderma* species[245]. For example, *H. lineatum* may be found in warmer subtropic areas, whereas *H. bovis* prefers the more temperate regions of the Northern Hemisphere[246].

Warble flies have been found to be parasites in cattle mainly, but infections have been found in horses[247,248], sheep[244], goats[244] and deer[244,249]. Infection in man is rare, but cases have been reported in rural and isolated areas. The warble fly migrates into the central nervous system[250,251], or into the conjunctival sac[252].

Severe economic damage is caused each year by warble fly infestation. The financial loss to cattle farmers in 1975 was estimated at £13 million. These losses are made up of lost weight on the cow, because contaminated meat must be discarded (£7 million); cowhide damage preventing holed hides from being sold (£3 million); and lost milk production (£3 million)[253]. These losses have been the subject of many total eradication schemes in both the British Isles and abroad[250,254], and although large research programmes on the biology of warble flies have been undertaken, very little work has been done on the chemical aspects of the species.

5.1.2. Life Cycles of *H. bovis* and *H. lineatum*

The life cycles of the *Hypoderma* species have been studied since 1895, when Ruser attempted to unravel the complex behaviour of grubs whilst under the hide of the cow[245]. There was confusion in the literature until 1926, when Laake discovered the main differences in the life cycles between the two species. Many detailed reports of the life cycles have been published[244,255] on the effect of larval development on cattle tissue[256] so only a summary will be presented.

The cycle starts in May by the mating of adult flies on the cow. Oviposition sites may be found on the belly and upper leg[245,257]. Egg laying takes place on the same day as, or the day following copulation, depending on fine weather. Up to 400 eggs are laid per fly, but the mortality rate is about 70%[258]. This amount of eggs takes several days to lay, because each egg is laid on a separate hair (*H. lineatum* may lay up to 15 eggs/hair[244]). Hatching occurs 5-7 days after laying and the grub tunnels by way of the hair follicle, under the skin[257]. At this stage the grub (the first instar larva) is 1½-3 mm in length. It migrates under the skin through the subcutaneous tissue at a rate of 50 mm per day towards the shoulder of the cow to rest in the epidural fat around the spine, growing in size along its journey (*H. lineatum* comes to rest in the

oesophagus[259]). The larvae then remain here throughout the winter period, only to begin further migration into the back of the cow in the Spring. Once in position, the larva opens an emergence hole through the hide, moults twice (second instar and third instar larvae) whilst feeding on the subcontaneous and necrotic material of the host. This presence of a foreign body in the hide of the cow causes a large swelling and an open sore called a warble (from which the fly gets its common name). The larva has a short fat spiny case with a corrugated exterior. It remains in the hide until late April (early April for *H. lineatum*[246]). When it emerges, through the previously opened hole, it drops to the ground and forms a tough pupal case 15-20 mm in length. This case has a suture from where the fly is able to emerge 3-5 weeks later, after pupation. The fly emerges as a large-headed, hairy, bee-like insect of length 12-15mm[246]. Its life span of around 6 days is short, but during this time the adults do not feed at all. The source of food is the fat accumulated whilst in the host[257,259]. Generally, the female flies are larger than the male flies and *H. bovis* specimens tend to be larger than *H. lineatum* specimens. Otherwise the external appearance of the two species is very similar.

The attraction and mating responses of warble flies are still not totally understood. *H. bovis* females have been reported to search for males which aggregate around cattle. *H. lineatum* males have been observed to aggregate along streams flowing through pasture land[260]. The flight of adult warble flies seems to be restricted to areas of masked sunlight around midday. Recognition between different species in Britain is not necessary because of their staggered emergence times and short life span *H. bovis* and *H. lineatum* flies never actually meet; but in some countries the two species fly at the same time, so that some form of recognition must be used. No reports have appeared yet, suggesting either visual or chemical cues are involved. It is possible that some form of chemical stimulus is involved. Other members of the Diptera family have been found to use chemical forms of recognition, but proof could be difficult, as no bioassay is available.

5.1.3. Chemical Communication in Other Species of Diptera

Much effort has been directed at the compounds that control behavioural patterns of flies. Many are common pests, and an understanding of the communication of these insects may lead to some form of control.

For example, the females of the common housefly, *Musca domestica*, have been found to contain (Z)-9-tricosene[261], and this is an attractant to the male[262]. Synthetic pheromone has been found to elicit mating behaviour when males are close to the sample[268]. (Z)-9-Tricosene has been used to increase the effectiveness of traps. 0.12% (Z)-9-Tricosene and 5% demetilan (an insecticide) are used in the trap together[264].

The courtship behaviour of the lesser housefly, *Fannia canniculars*, has been shown to be mediated by (Z)-9-pentacosene[265]. This compound has been found to attract males[266].

Later work on two species of stable flies, *Fannia femorlis*[267], and *Fannia pusio*[268], has found a straight chain alkene (Z)-11-hentricontene as the mating stimulant. A third species of stable fly, *Stomoxyes calcitrans* has several branched alkanes and alkenes that are active, of which 15-methyltritriacontane and 15,19-dimethyltritriacontane are more active[269]. Further studies has shown mixtures of (Z)-9-hentricontene, (Z)-9-tritriacontene, 13-methyl-1-hentricontene to be even more attractive[270].

The tsetse fly, *Glossina morsitans morsitans*, has three ultrashort range active components (contact pheromones): 15,19-dimethylheptatricontane and 15,19,23-trimethylheptatriacontane[271].

The mosquito, *Culiseta inornata* has also been reported to have a non-volatile contact pheromone, but at present this has not been identified[272].

These rather involatile compounds are usually described as control pheromones rather than attractants. The term "attractant" is more often associated with the volatile components used by Lepidoptera.

The above compounds all resemble one another in general structure. They also resemble the type of components found in the cuticular waxes of insects. The usual method of identification of the contact pheromones is to compare the gas-chromatograms of male and female extracts, and then to find and identify the differences. Synthesis of these components yields enough sample to test for activity with live flies.

CHEMICAL NAME [Merck index no.][2]	TRADE NAMES	DOSE[1]	TIME [days][3]	REF.
COUMAPHOS 2543	ASUNTOL CO-RAL	b	14[4]	276 277
CRUFOMATE 2598	RUELENE	a,c	15-27	277
FENCHLORPHOS 8022	ETROLENE	b	15	277
FENTHION 3919	BAYTEX	d	4	278 279 280
TRICHLOPHON 9303	DYVON NEGAVON	a b	14 14	277 281

FOOTNOTES:

[1] a 6% aq.liquid
b 2% aq.liquid
c 8·3% emulsion
d 30mg/kg

[2] for structure, formula, etc.

[3] average time to kill a larva

[4] varies

TABLE 7: COMMERCIAL INSECTICIDES AVAILABLE FOR THE TREATMENT OF WARBLE FLY INFESTATIONS.

5.1.4. Insecticides and Control Methods

Although research has been carried out for many decades, no totally effective method of control has been found. In 1920, derris - the active component of which is rotenone - was found to kill larvae. Derris is still used as a cheap, effective method of control[273]. After the last war, an act of Parliament - "The Warble Fly (Dressing of Cattle) Order, (1948)" - was passed, which required the dressing of all infected cattle with derris between March and June. This act was repealed in 1964, due to difficulties with its enforcement . The recent situation has been reviewed and "The Warble Fly (England and Wales) Order, (1978)" has been passed, outlining procedures for treatment, and movement of warble fly infected cattle[274]. This is the first stage in a five year plan to eradicate warble fly infections from Britain.

The research into finding a good insecticide has been difficult. Many new techniques have had to be developed to combat the problems of using cattle as experimental animals (their size is the major problem)[259]. Experiments have been conducted on implanting larvae into rabbits, dogs, and other small animals, and these are reviewed by Beesley[275]. Many problems are encountered by using small experimental animals, the main one being that the dose required to kill the warble fly larvae, kills the host animal as well.

There are some reasonably successful insecticides used at present (Table 7). Dressing may be carried out during two periods of the year: during October, when it is necessary to dress every cow to prevent infection; or during March, when it is only necessary to treat affected cows. Both times of treatment have advantages, but the latter is more common, due to the lower cost of treating the affected cow rather than the whole herd; treatment of the whole herd usually means no infection[277].

One important factor to be considered is the residues that may be found in the parts of the cow that are for human consumpton (i.e. meat and milk). The levels observed are quoted in ppm and depend on the insecticide used and the time that the measurement is made after treatment. A 4% solution of trichlorophon leads to an immediate 0.4 ppm of the compound in the milk, but after six hours this is reduced to 0.1 ppm. Ruelene, however, is more toxic and it is suggested by the manufacturers that treated cows should not be slaughtered until four weeks after treatment[273]. A further factor for consideration is the affect of these chlorinated compounds on cows. Loss of weight, paralysis of the hind-quarter, and shock have been detected. One beneficial side effect is the loss of any cattle lice infection[273,282].

Greater than 99% control has been reported in some countries[289], but total control by any insecticide has not been reported. Less common treatments used throughout the world include scrubbing with paradichlorobenzene (France), burning the hairs from the hindquarters with mustard oil (Northern India), and a treatment with a mixture of iodine, arsenious acid, kerosine, nicotine and lime in carbontetrachloride injected into the cysts (Asia)[244].

New treatments that have been suggested for control include injection of insect juvenile hormone to effect the larval stages within the body of the cow[284] and sex attractant baited traps in the "field"[285].

5.2. Collection Procedures

There are many methods to obtain samples of warble flies. Removal by squeezing from the open sores on the back of the cow was tried and over twenty specimens were obtained (third instar larvae), but it was found that these samples died before emergence.

Other methods involve plastic containers attached to the back of the cow, over the emergence hole, to catch the larvae as it wriggles out[259], or covering the cow in plastic sheeting[286,287]. The problem with plastic containers is that the cows, irritated by the boxes, remove them by rubbing against trees. The most successful method makes use of an experimental herd, whereby the cattle are stood over a grating. On emergence, the larvae are allowed to fall to the floor and they may then be collected from under the grating. This is how the fly specimens used were obtained. We studied Canadian warble flies (*H. bovis* and *H. lineatum*) and British warble flies (*H. bovis*). (We are grateful to Professor Weiutraub of Lethbridge, Canada, and ICI Ltd. for supplying these samples). The larvae were placed on moist cotton wool at laboratory temperature and allowed to pupate and emerge naturally.

We obtained as live flies seven of the species *H. bovis* (2 males and 5 females), 5 of *H. lineatum* (4 males and 1 female) from Canada and 2 of *H. bovis* (1 male and 1 female) from Britain. After emergence, the flies were separated according to sex and species. It was found that the flies survived 1-6 days, but they did not fly very much, possibly due to the low ambient temperature. Dead flies were placed in vials (that had been washed in chromic acid, aqueous sodium bicarbonate and distilled water), covered in purified pentane, and stored in the fridge. Cuticle extracts were either analysed by gas chromatography or removed from the vial, placed in another vial and the volume reduced by blowing at the surface with a stream of dry nitrogen. This concentrated solution could then be analysed by a gas chromatography - mass spectrometry system.

Second instar larvae were also obtained from the hides of slaughtered cattle from an abbatoir in Southern England. These were cleaned and stored on cotton wool in the fridge.

5.3. Analytical Techniques

5.3.1. Gas Chromatography (GC)

GC was carried out on a Pye 104 GC machine with a programmable oven. Glass columns (3 m x 2 mm internal diameter glass tubing) packed with 2% or 5% OV101 on Diatomite CLQ 100-120 mesh were used. Nitrogen carrier gas was used (rate: 25 ml/min).

5.3.2. Capillary Gas Chromatography

Capillary GC was carried out on a Varian 3700 series GC using a glass capillary column (50m x 0.3 mm) filled with SE30 column packing. A split injector was used (ratio 50:1) with helium carrier gas (rate: 2-3ml/min).

5.3.3. Gas Chromatography - Mass Spectrometry (GC - MS)

Preliminary surveys on cuticular composition were carried out on an AEI MS12 spectrometer, interfaced by a Watson-Biemann porous glass separator to a Pye 104 oven. The later, more comprehensive work, was done using an AEI MS30 spectrometer, interfaced by a Reihage jet separator to a Pye 204 oven. The spectral information was recorded and stored on a DS50 data system with interactive facility. Columns used were similar to the above, but the MS30 uses helium as a carrier gas.

5.3.4. GC Microscale Chemical Reactions

(i) the removal of an alcohol from the GC trace was carried out by microscale silylation. N,O-bis(trimethylsilyl)acetamide (BSA, $\frac{1}{2}$ µl) was added to the concentrated cuticular extract (1 µl) and left for five minutes. The resulting mixture (1 µl) was injected on to the column as before. Alcohols were found to increase in retention time, due to the formation of the trimethylsilyl ether derivative. 100% transformation was observed on standard alcohols.

(ii) Alkenes were detected by the addition of bromine water (2%), or bromine in carbon tetrachloride (2%)[288]. The latter was found to be easier to use because immiscibility problems were eliminated. The bromine solution (3 µl) was added to the concentrated extract (1 µl) and left to react for five minutes. The product mixture (1 µl) was then injected on to the Gc column. Peaks previously assigned to olefins were then seen to be removed. (The resulting dibromo olefin rarely appeared on the GC trace). Standard solutions of alkenes were seen to be removed totally on addition of bromine in carbon tetrachloride.

5.4. Results

5.4.1. GC analysis of Extracts

The extract of the cuticle of each fly was obtained as stated in Section 4.2. All extracts were analysed, so some idea of the reproducibility of results could be ascertained. It was hoped that the traces of British and Canadian warble flies (*H. bovis*) could be compared as well as the differences between the two difference species (*H. bovis* and *H. lineatum*). The main interest though, was the difference between the males and females of each species. Sample traces of the different sexes and species are shown in Figures 14-19.

5.4.2. Interpretation of GC Traces

Figures 14-19 show many clear similarities between the species and sexes, although many distinct differences are also visible. The extract from two flies of the same sex and species were found to give identical GC traces. Comparing British (Figure 14, 15) and Canadian (Figures 16, 17) samples of *H. bovis* the traces appear almost identical, except for slight concentration differences in the more volatile region. The differences between the two sexes are more pronounced, the males have a trio of peaks in the volatile region not present in the female extracts. However, in contrast with the males of *H. bovis*, the males of *H. lineatum* (Figure 18) have many more peaks in the volatile region. The females of *H. lineatum* (Figure 19) have no major components in the same region.

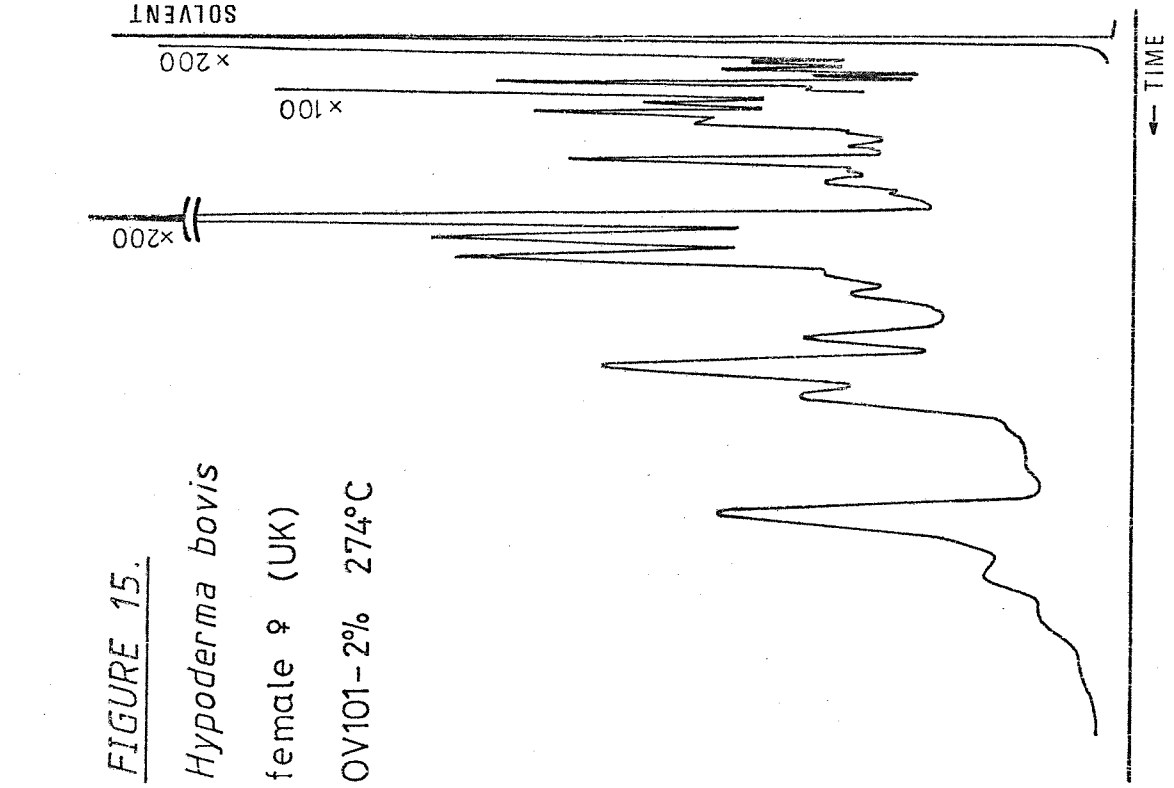


FIGURE 15.

Hypoderma bovis

female ♀ (UK)

OV101-2% 274°C

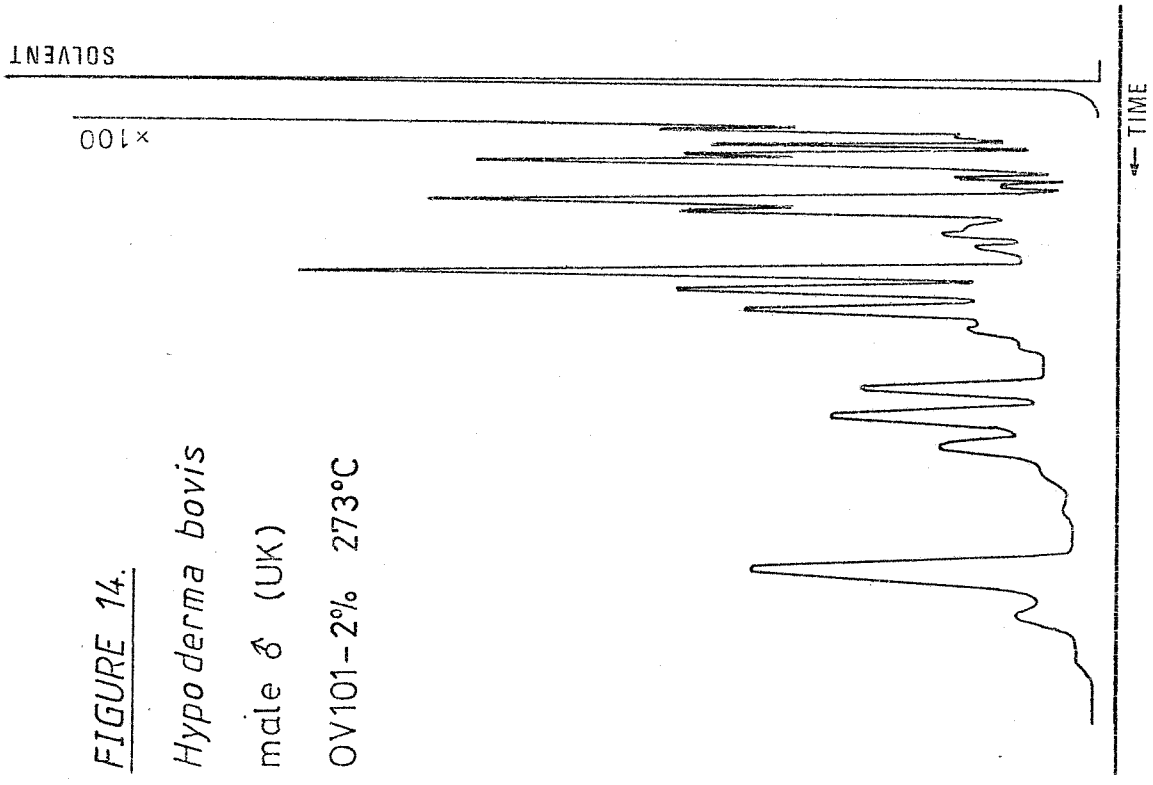


FIGURE 14.

Hypoderma bovis

male ♂ (UK)

OV101-2% 273°C

TYPICAL G.C.'s OF EXTRACTS FROM *H. bovis*

ORIGIN: UK

5.4.3. Microscale Chemical Reactions

Each specimen was tested in the way explained in Section 3.1 and was found that only one peak in each trace was affected by both microscale chemical reactions (Figures 16, 17, 18, 19 peak marked Ø), suggesting that it is an unsaturated alcohol. All the other peaks remained unaffected by these reactions, so this was taken to suggest that the other components were saturated hydrocarbons (probably a mixture of normal and branched alkanes).

5.4.4. Gas Chromatography - Mass Spectrometry (GC-MS)

Unfortunately, larger quantities of material are required for analysis by GC-MS than by GC. This meant that with the samples available, this technique could not be applied to *H. lineatum* males and the British samples of *H. bovis*.

The results are shown in Tables 8-11. GC-MS showed the main components of the chromatograms to be normal alkanes: tricosane, pentacosane, heptacosane and nonacosane in males of *H. bovis*; tricosane, heptacosane and nonacosane in the females. Only heptacosane and nonacosane were found in female extracts of *H. lineatum*. Heptacosane was the major component in each sample. The remaining components were assumed to be branched alkanes; analysis of the peaks showed them to contain multiple components. These are listed as branched alkanes, mainly 7,9,11 and 13-monomethyl branching was found (see 5.4.6. - the mass spectra of alkanes).

It was possible to suggest structures for some of the components of the male extract of *H. lineatum*. By considering the retention time of the peak on the chromatogram and comparing it with those of known compounds of the other extracts, structures could be proposed. It was disappointing that some of the more volatile components of the male, and not present in the female, could not be identified. Any pheromone that existed would be found in this region of the chromatogram.

Estimated amounts of each component present in each extract are quoted for general comparison between the different sexes and species.

TYPICAL G.C.'s OF EXTRACTS FROM

H.lineatum

ORIGIN: CANADA

FIGURE 18.

Hypoderma lineatum

male ♂ (Canada)

OV101-5% 282°C

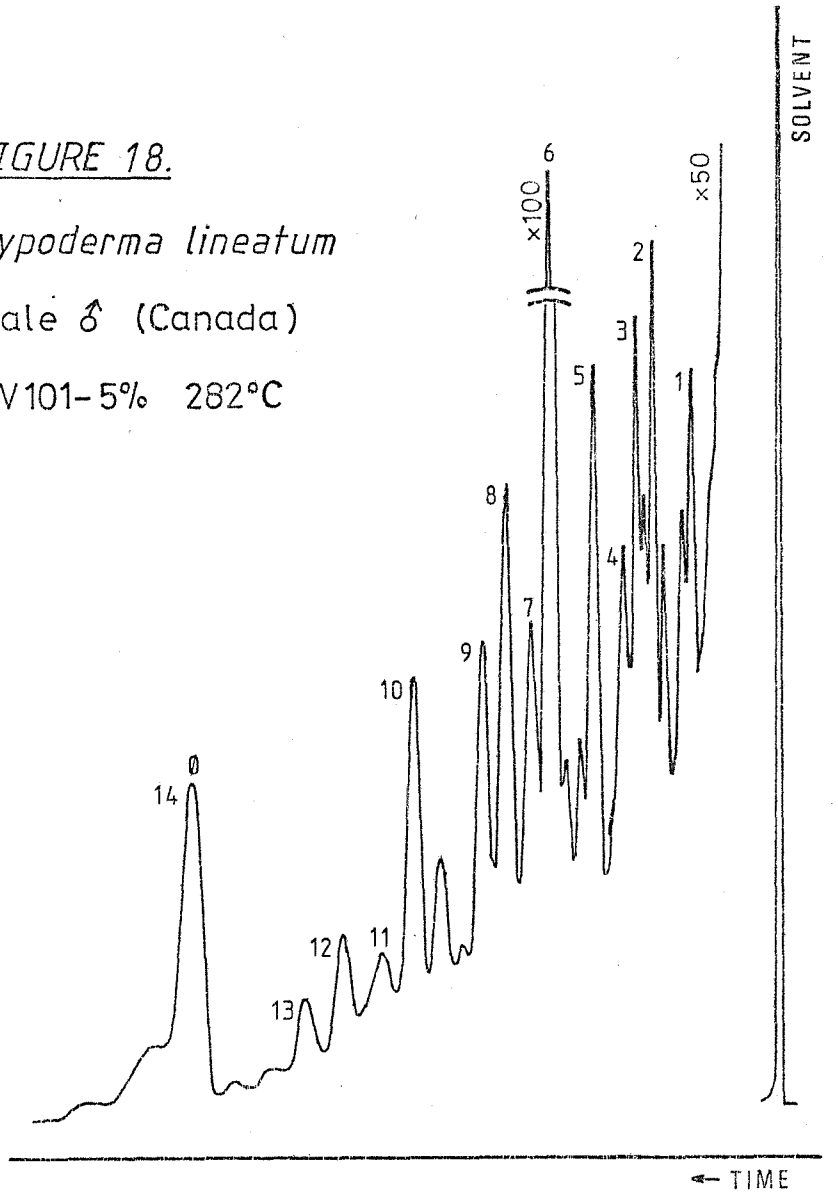
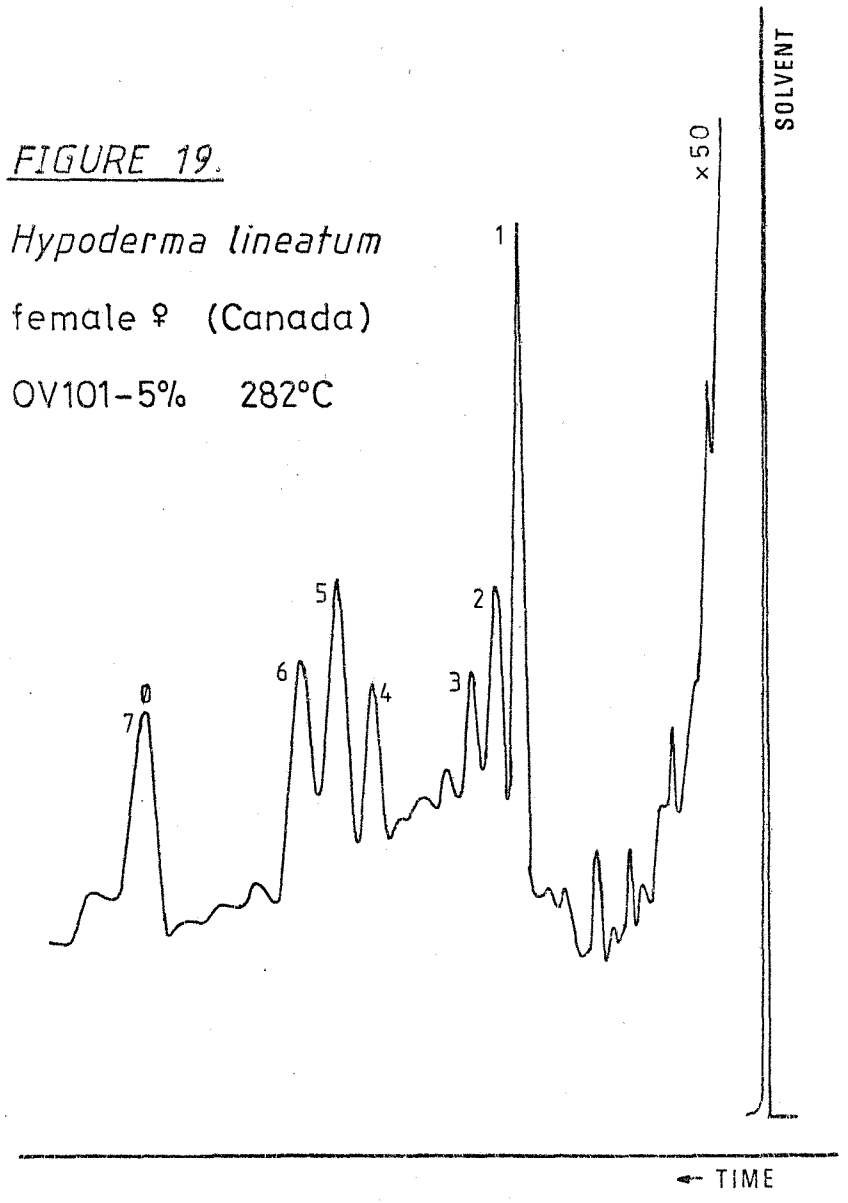


FIGURE 19.

Hypoderma lineatum

female ♀ (Canada)

OV101-5% 282°C



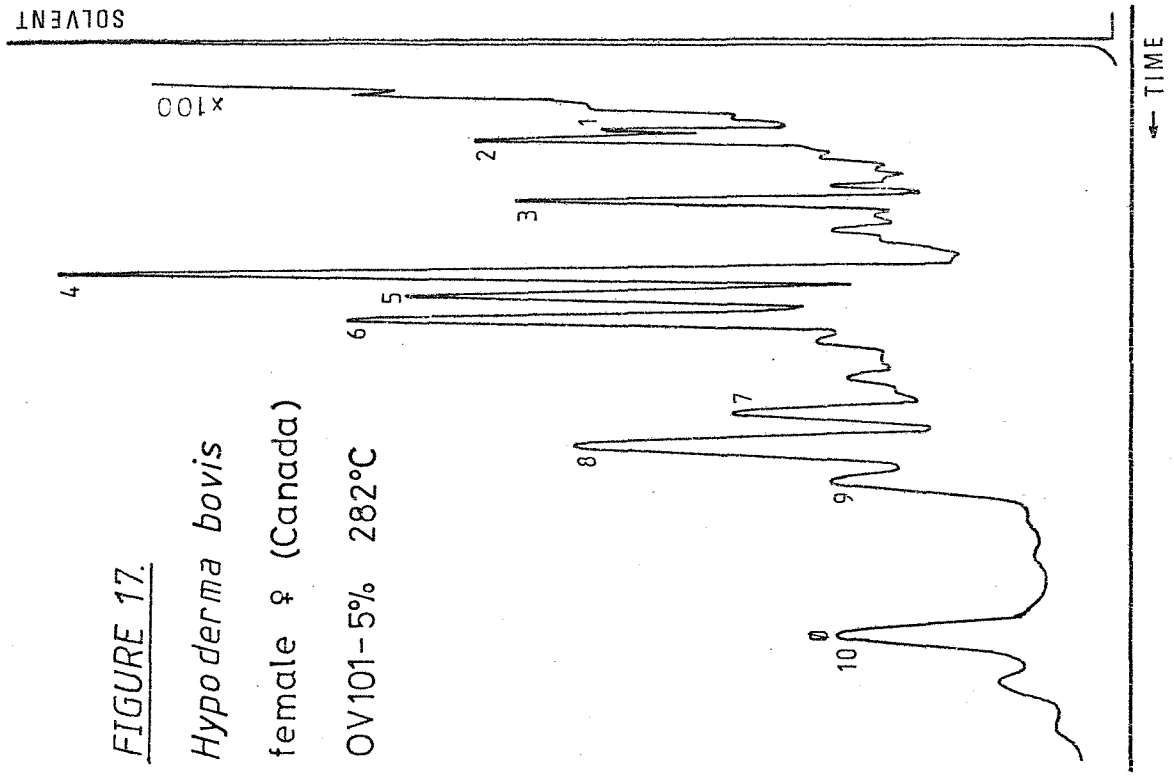


FIGURE 17.
Hypoderma bovis
female ♀ (Canada)
OV101-5% 282°C

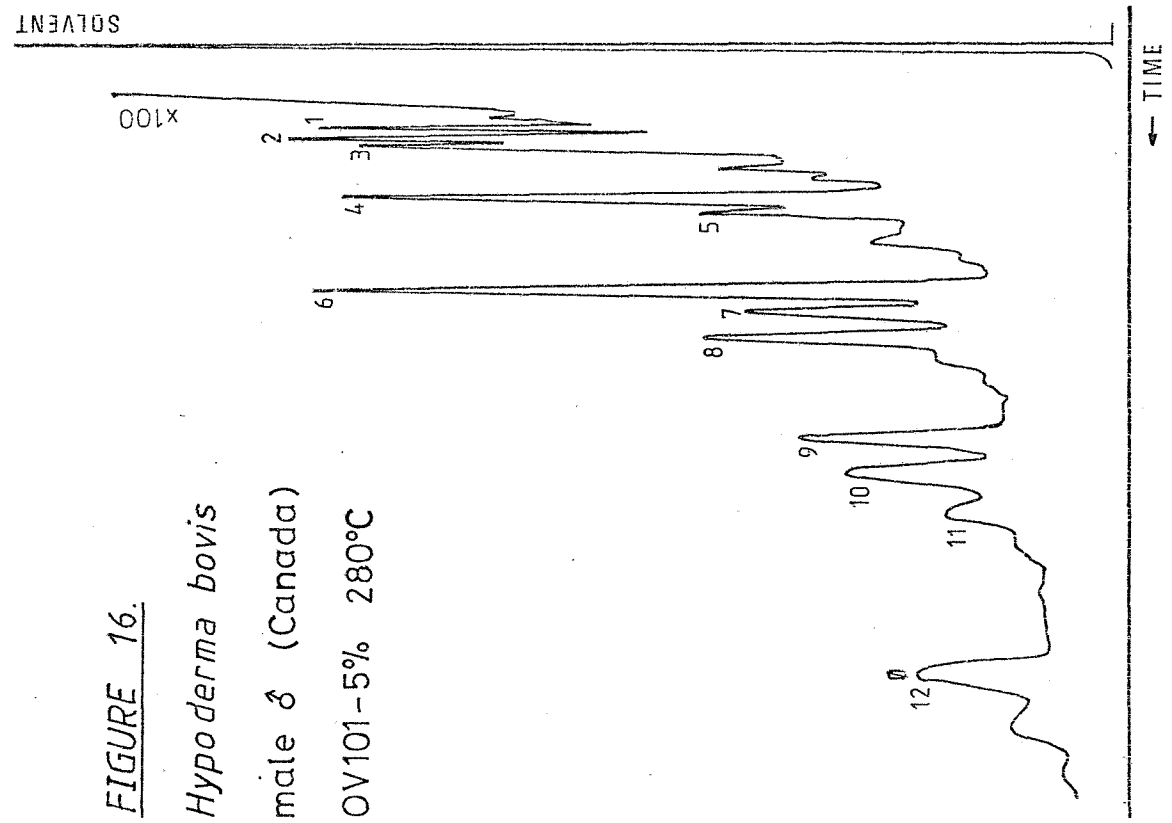


FIGURE 16.
Hypoderma bovis
male ♂ (Canada)
OV101-5% 280°C

TYPICAL G.C.'s OF EXTRACTS FROM
H. bovis
ORIGIN: CANADA

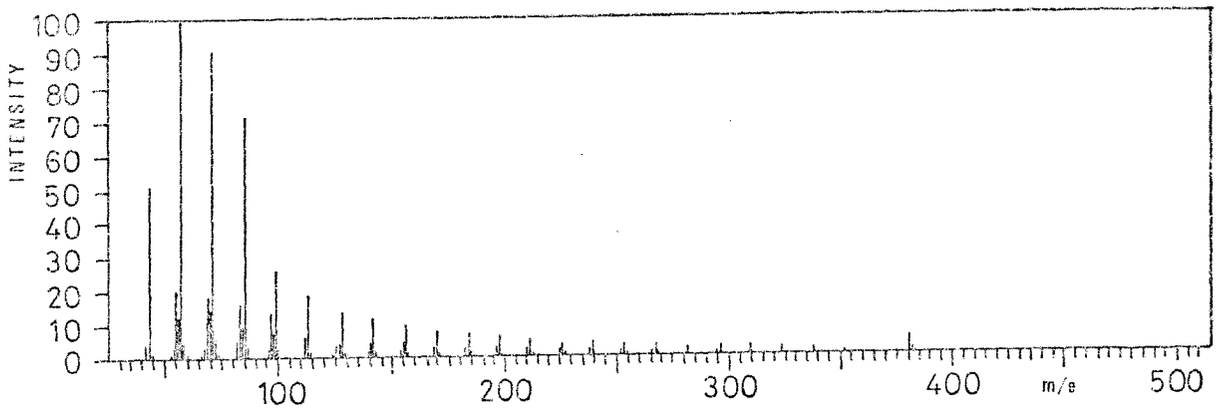


FIGURE 20: MASS SPECTRUM OF n-HEPTACOSANE.

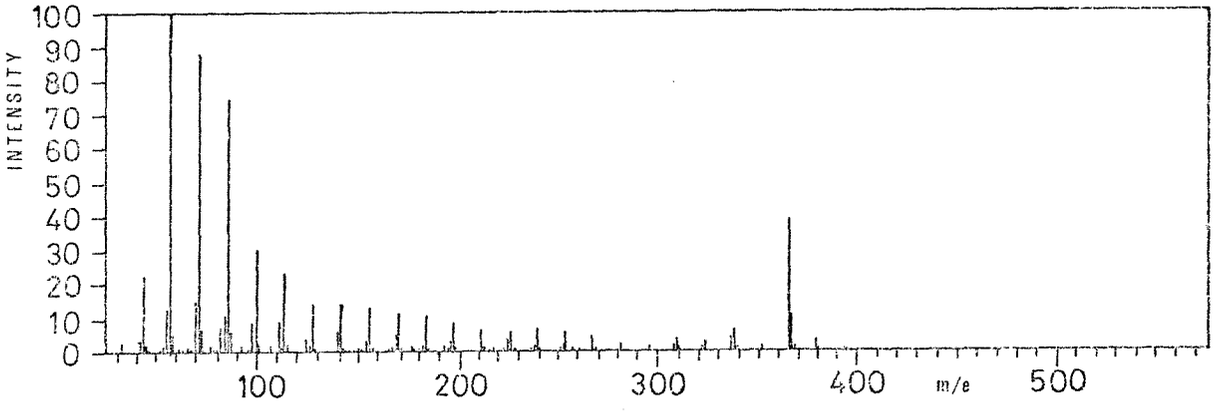
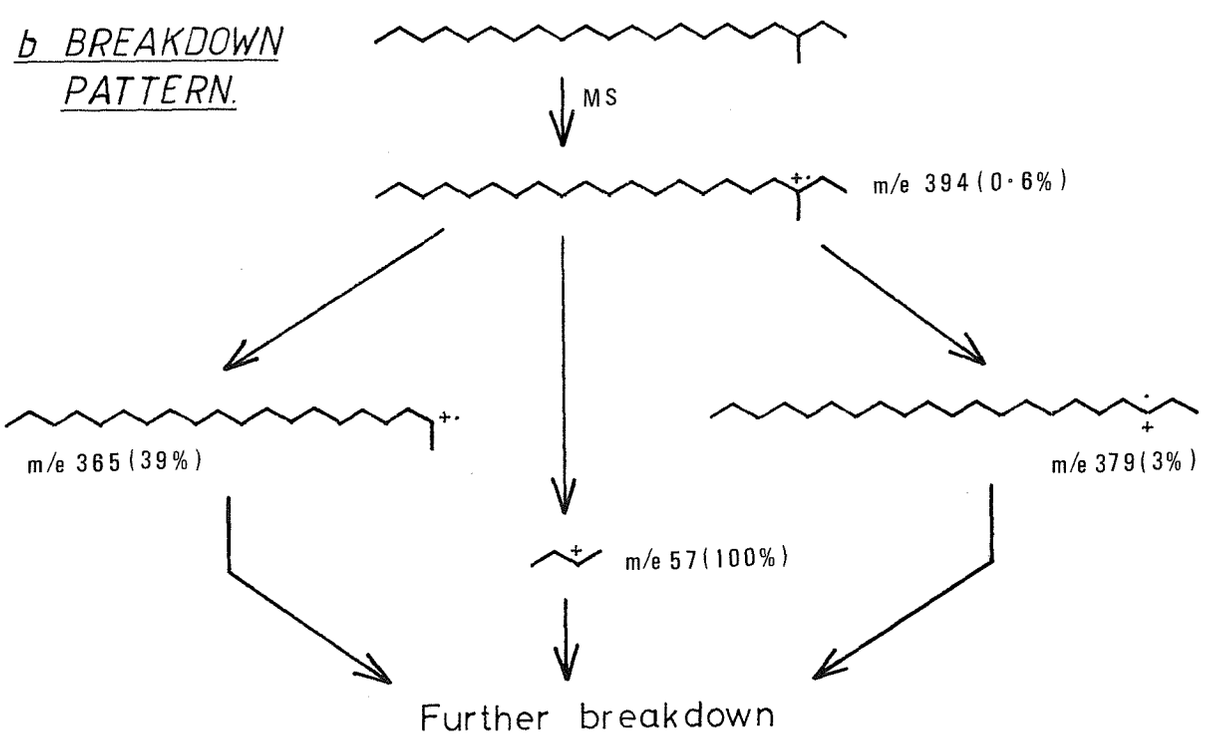


FIGURE 21: a MASS SPECTRUM OF 3-METHYL HEPTACOSANE

b BREAKDOWN PATTERN.



PEAK No.[1]	MWt	FORMULA	STRUCTURE	PEAK SIZE µg/insect[2]
1	338	$C_{24}H_{50}$	(399)[3]	5
2	324	$C_{23}H_{48}$	n-tricosane	4
3	352	$C_{25}H_{52}$	(400)[3]	8
4	352	$C_{25}H_{52}$	n-pentacosane	9
5	366	$C_{26}H_{54}$	[4]	2
6	380	$C_{27}H_{56}$	n-heptacosane	14
7	394	$C_{28}H_{58}$	[5]	5
8	394	$C_{28}H_{58}$	3-methyl heptacosane	6
9	408	$C_{29}H_{60}$	n-nonacosane	7
10	422	$C_{30}H_{62}$	[6]	4
11	422	$C_{30}H_{62}$	3-methyl nonacosane	2
12	386	$C_{27}H_{46}O$	cholesterol	8

FOOTNOTES:

1 see Figure 16.
2 for a typical insect
3 see text
4 mixture of 7-methyl, 9-methyl & 11-methylpentacosanes
5 mainly 11-methylheptacosane
6 mixture of 9-methyl,11-methyl & 13-methylnonacosanes

TABLE 8: GC-MS DATA FOR H.bovis (CANADA). ♂

5.4.5. The GC Characteristics of Hydrocarbons

Generally, on non-polar phases (such as OV101), it is observed that the normal alkane of a series is eluted after the branched alkanes of the same molecular weight. The n-alkane series is eluted in order of increasing molecular weight. The change in retention time between a normal alkane and branched alkane is dependent on the number of branches and

PEAK No.[1]	MWt	FORMULA	STRUCTURE	PEAK SIZE µg/insect[2]
1	324	$C_{23}H_{48}$	n-tricosane	1
2	338	$C_{24}H_{50}$	5-methyl tricosane	2
3	366	$C_{26}H_{54}$	[3]	3
4	380	$C_{27}H_{56}$	n-heptacosane	24
5	394	$C_{28}H_{58}$	[4]	8
6	394	$C_{28}H_{58}$	3-methyl heptacosane	10
7	408	$C_{29}H_{60}$	n-nonacosane	10
8	422	$C_{30}H_{62}$	[5]	10
9[6]	422	$C_{30}H_{62}$	3-methyl nonacosane	10
10	386	$C_{27}H_{46}O$	cholesterol	15

FOOTNOTES:

1 see Figure 17
2 for a typical insect
3 mixture of methylpentacosanes
4 mixture of methylheptacosanes
5 mainly 7-methylnonacosane

TABLE 9: GC - MS data for H.bovis (CANADA). ♀

where they occur in the molecule. This is the basis of Kovat's Indeces for relative retention times[290]. As has already been suggested, some of the peaks in the chromatogram are made up of many different isomers. It was found that by using a capillary column, these multiple peaks could be resolved into a series of peaks, but at the time of this work integrated capillary GC-MS was not available.

PEAK No.[1]	MWt	FORMULA	STRUCTURE	PEAK SIZE ug/insect[2]
1	[3]			1
2	[3]			1
3	[3]			1
4	[3]			1/2
5	[3]			2
6[4]	380	$C_{27}H_{56}$	n-heptacosane	9
7[4]	394	$C_{28}H_{58}$	[5]	1
8[4]	394	$C_{28}H_{58}$	3-methyl heptacosane	2
9	[3]			1
10[4]	408	$C_{29}H_{60}$	n-nonacosane	3
11[4]	422	$C_{30}H_{62}$	[6]	1/2
12[4]	422	$C_{30}H_{62}$	3-methyl nonacosane	1/2
13	[3]			1/2
14[4]	386	$C_{27}H_{46}O$	cholesterol	2

FOOTNOTES:

1 see Figure 18
2 for a typical insect
3 spectrum not recorded
4 spectrum not recorded, suggested structure.
5 mixture of methylheptacosanes
6 mixture of methylnonacosanes

TABLE 10: GC-MS DATA FOR H. lineatum (CANADA) ♂

PEAK No.[1]	MWt	FORMULA	STRUCTURE	PEAK SIZE ug/insect[2]
1	380	$C_{27}H_{56}$	n-heptacosane	12
2	394	$C_{28}H_{58}$	[3]	4
3	394	$C_{28}H_{58}$	3-methyl heptacosane	2
4	408	$C_{29}H_{60}$	n-nonacosane	3
5	422	$C_{30}H_{62}$	[4]	5
6	422	$C_{30}H_{62}$	3-methyl nonacosane	4
7	386	$C_{27}H_{46}O$	cholesterol	10

FOOTNOTES:

1 see Figure 19
2 for a typical insect
3 mixture of methylheptacosanes
4 mixture of methylnonacosanes

TABLE 11: GC-MS DATA FOR H.lineatum (CANADA) ♀

5.4.6. The Mass Spectra of Alkanes

The spectra recorded on the straight chain normal alkanes are easily identified. A molecular ion is usually observed. (M^+-15), loss of a methyl radical, is not very favourable and therefore rarely observed. The series (M^+-29) loss of an ethyl radical, (M^+-43) loss of a propyl radical, (M^+-57) loss of a butyl radical, and so on, is observed in increasing intensity until a maximum is seen at m/e 57 ($C_4H_9^{+\cdot}$) - the most stable cation radical. This pattern may be seen in Figure 20, the mass spectrum of heptacosane.

The mass spectra of some of the other peaks is confusing, due to the number of different components under the envelope of the peak, but knowing the type of compound is a branched alkane and probably only branched as a methyl group in one place, structures may be postulated. Such assumptions have been made in previous work on the tsetse fly[271]. The molecular ion is rarely seen in the spectrum of a branched compound, so molecular weight is

assigned by the relative position of the peak on the chromatogram with respect to the normal alkanes.

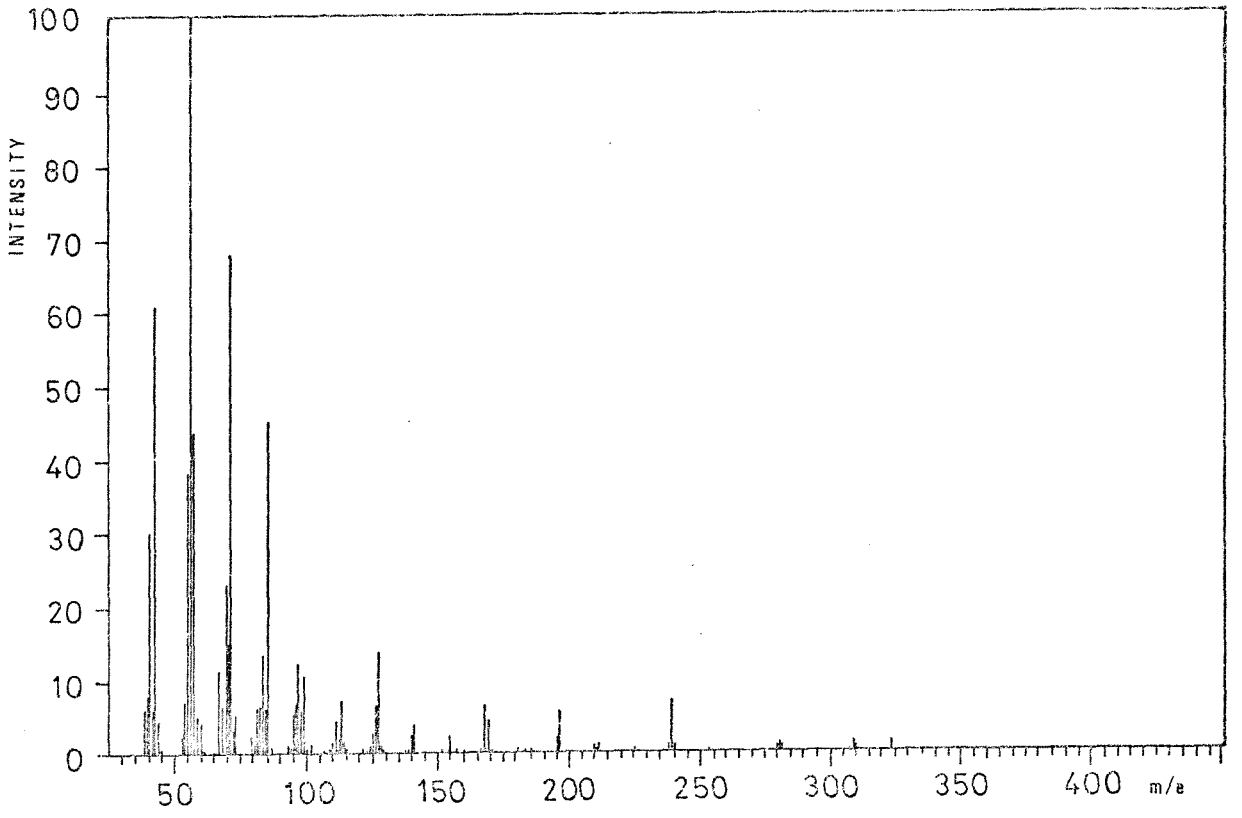
The isomers observed, all seemed to branch in odd positions (3-methyl, 5-methyl, 7-methyl, 9-methyl, 11-methyl and 13 methyl were all present). Other compounds, with other branching positions, may be present in smaller quantities, but not in sufficient amount to be observed by mass spectrometry. This phenomenon may be due to the way these compounds are biosynthesised (see Section 5.6).

Fragmentation in the mass spectrum is the key to the identification of branching patterns. 3-Methyl heptacosane seems to occur in one peak, not contaminated by any other branched compounds (the mass spectrum may be found in Figure 21a). Assignment of some of the peaks of the mass spectral breakdown pattern may be found in Figure 21b.

Important factors to note are that the charge prefers to be on the more stable secondary centre, and the electron on the primary site of the longest chain. Loss of a methyl group is not seen due to the unfavourability of the process. Loss of ethyl, more favourable is seen. As previously suggested for long-chain alkane mass spectra, m/e 57 is the base peak. Finally, there is a peak at m/e 364 (11%). This is indicative of a branching point. It shows the formation of the olefin charged complex, seen more often in branched alkanes than in normal straight-chain alkanes.

In our search for a pheromone we turned our attention away from compounds in both male and female samples (i.e. compounds with 26 carbon atoms or more), to components found in the more volatile part of the chromatogram. In Figures 16 and 17, it is possible to see two major components in the extract from *H. bovis* females. These were identified as tricosane and 5-methyltricosane. The extract from the males, however, has four major peaks: tricosane, pentacosane, and two other components (399) and (400). The mass spectra are not as simple as those of normal straight chained alkanes or monomethyl branched alkanes. We therefore considered the possibility of multibranching.

This would not be the first example of multibranched alkanes as a constituent of an extract from a fly. 15,19,23-Trimethylheptatriacontane has been found to be a contact pheromone in the tsetse fly[271]. Compound (399), the mass spectrum of which may be found in Figure 22, was found to exhibit ions at 309 and 323. It would be unusual to see a molecular ion,



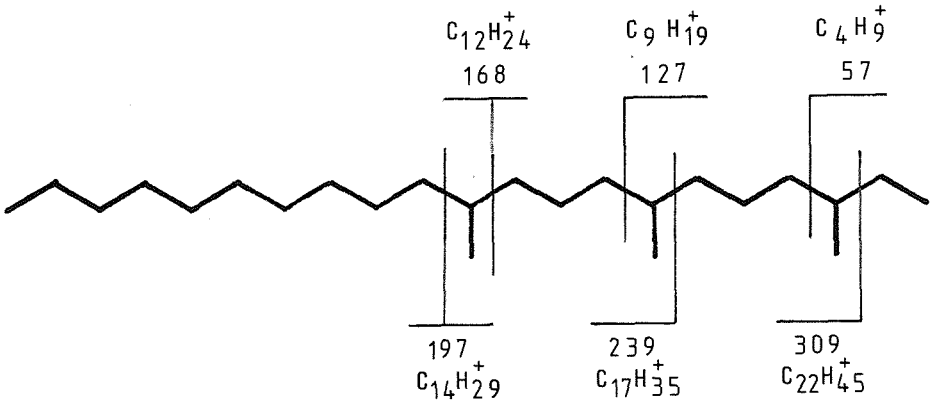


FIGURE 22: 3,7,11-TRIMETHYLHENEICOSANE - mass spectrum & major ions.

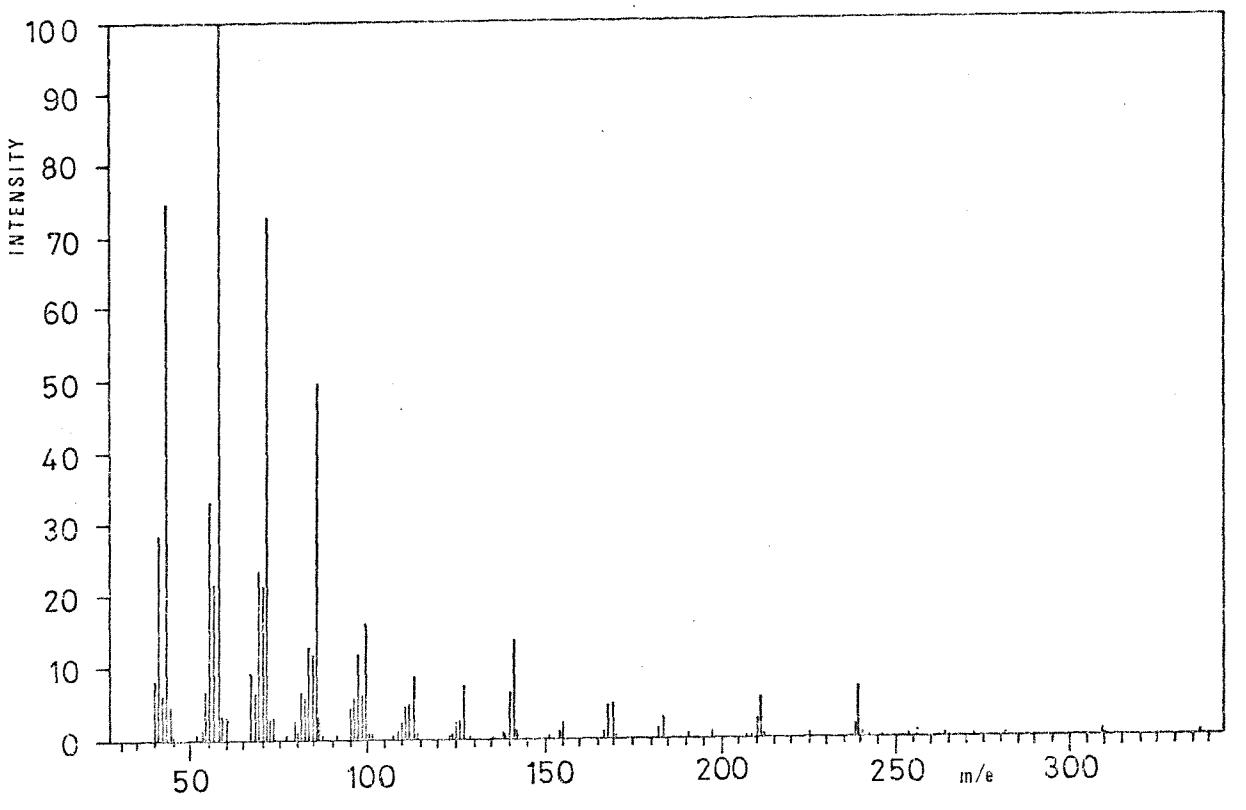
so these peaks could be assigned as (M^+-29) and (M^+-15) respectively, showing a molecular weight of 338. This is consistent with what we would expect a component of such a molecular weight to be eluted on a GC trace. Major fragments are seen to contain 9,12,14 and 17 carbon atoms (masses 127, 168, 197, 239).

Previous work on the tobacco hornworm, *Manduca sexta*[290,291], found that in the mass spectra of branched alkanes, when comparing consecutive ions of the form C_nH_{2n} and C_nH_{2n+1}; the former predominates if n is greater than 8 and less than eighteen, unless the fragment contained another branch, in which case C_nH_{2n+1} predominates. Looking at Figure 22, the even mass at 168($C_{12}H_{24}$, n=12) is more abundant than the odd mass at 169 ($C_{12}H_{25}$, n=12). The odd mass at 127(C_9H_{19}, n=9) is more abudant than the even ion at 126 (C_9H_{18}, n=9). This suggests that the latter contains the branch. No ion is seen at 295(M^+-43, loss of a propyl radical), suggesting a branch at carbon 3. From this it is possible to prepose the structure of (399) as 3, 7,11-trimethylheneicosane.

The mass spectrum of (400) is shown in Figure 23. The spectrum is similar to that of component (399). Peaks are seen at 336 and 323 and are assigned (M^+-15) and (M^+-27), so it is likely that the molecular weight is 352. No ion is seen at 295 (i.e. M^+-57, loss of a butyl radical) suggesting a branch at carbon 4. Predominant peaks in the spectrum contain 10, 12, 15 and 17 carbon atoms (fragment masses 141, 168, 211 and 239 respectively). Again, comparing the even and odd mass ions of these fragments, 169(4.9%) is larger than 168(4.8%), but 168 is the largest of all the even mass ions. The inference is that the twelve carbon fragment contains a branch. This suggests the structure of (400) to be 4,8,12-trimethyldocasane, a higher homologue of (399).

5.4.7. Kovat's Indices

The hypothesis of branching on long-chain hydrocarbons can be tested by considering retention times and their relation to chain length. The retention index values (RI) are called Kovat's Indices[290]. Experimental values were calculated by using the formula:



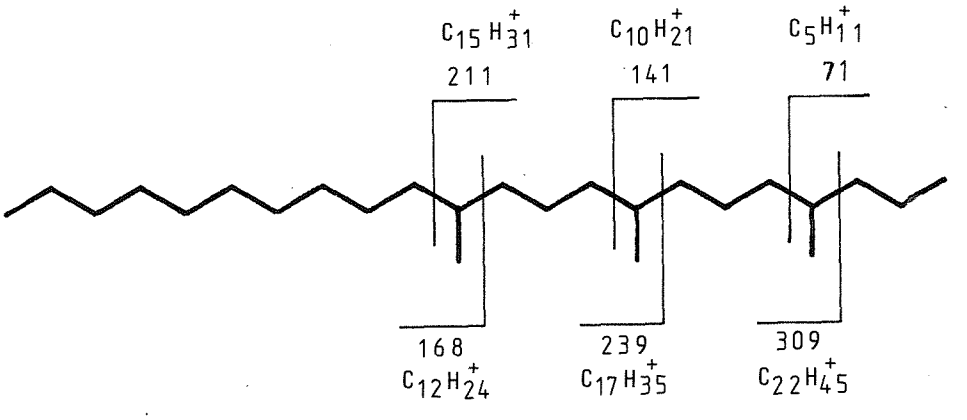


FIGURE 23: 4,8,12-TRIMETHYLDOCOSANE - mass spectrum & major ions.

$$RI = 100n + 200\left[\frac{\ln(X) - \ln(A)}{\ln(B) - \ln(A)}\right]$$

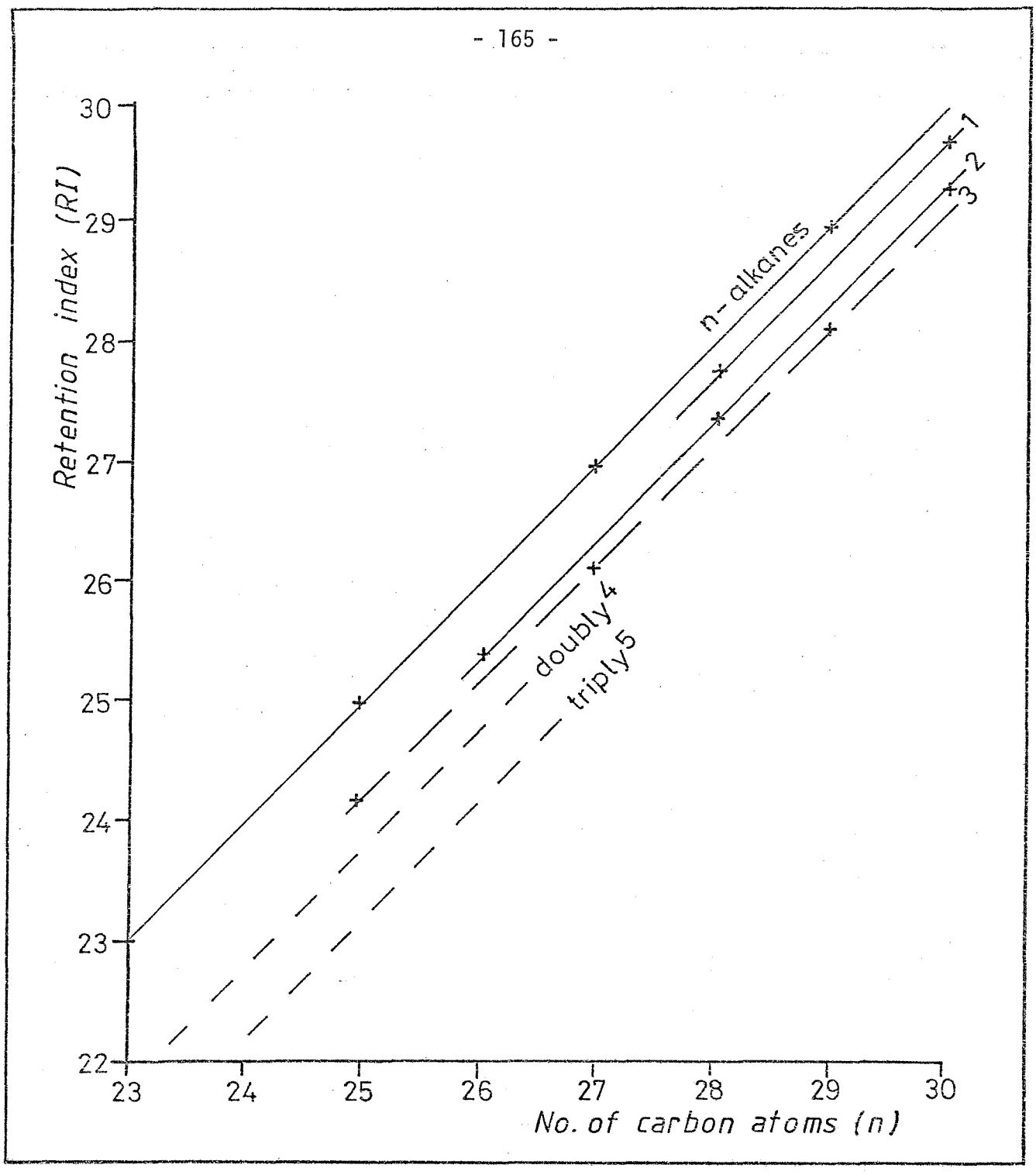
where n = carbon number
X = retention time of unknown from A
A = of HC (same chain length)
B = of HC (A's chain length + 2 carbon atoms)
HC = normal hydrocarbon

When these experimental values are plotted against chain length (Figures 24, 25), it can be seen that a straight line is obtained by joining the points representing the n-alkanes. Similarly, a line joining points representing alkanes of a similar branching pattern (e.g. 3-methyl branched) is also a straight line. Figure 11 shows the plot of RI against chain length for *H. bovis*, and Figure 12 shows the plot for *H. lineatum*. It is important to note that all "similar" hydrocarbons are joined by lines that are parallel.

Previous studies of this type - for example studies of eggs of the tobacco hornworm[291] have shown that branching in a particular place in a molecule leads to a consistent decrement from the index of a normal straight chain hydrocarbon of the same number of carbon atoms. On OV101 (5%) they found a decrement of 70 units per branch, whereas in another study by Mold *et al*[292], 62 units per branch was found using an SE60 (6%) column.

Following this approach, two broken lines have been drawn to correspond to molecules with two and three branches. It has been estimated from the mixture of 7,11,13-mono-methyl branched compounds that a methyl in the middle of the chain gives a decrement of 60 units. Therefore, 2 branches and three branches give a decrement of 120 and 180 units respectively. It can be seen from the positions of (399) and (400) on the graph that they have more than two branches, but not three branches in the middle of the molecule. This supports the structures assigned to (399) and (400).

From the retention indices already calculated for other components, a table of approximate decrements for particular methyl branches may be



FOOTNOTES:

1 *3-methyl alkanes*

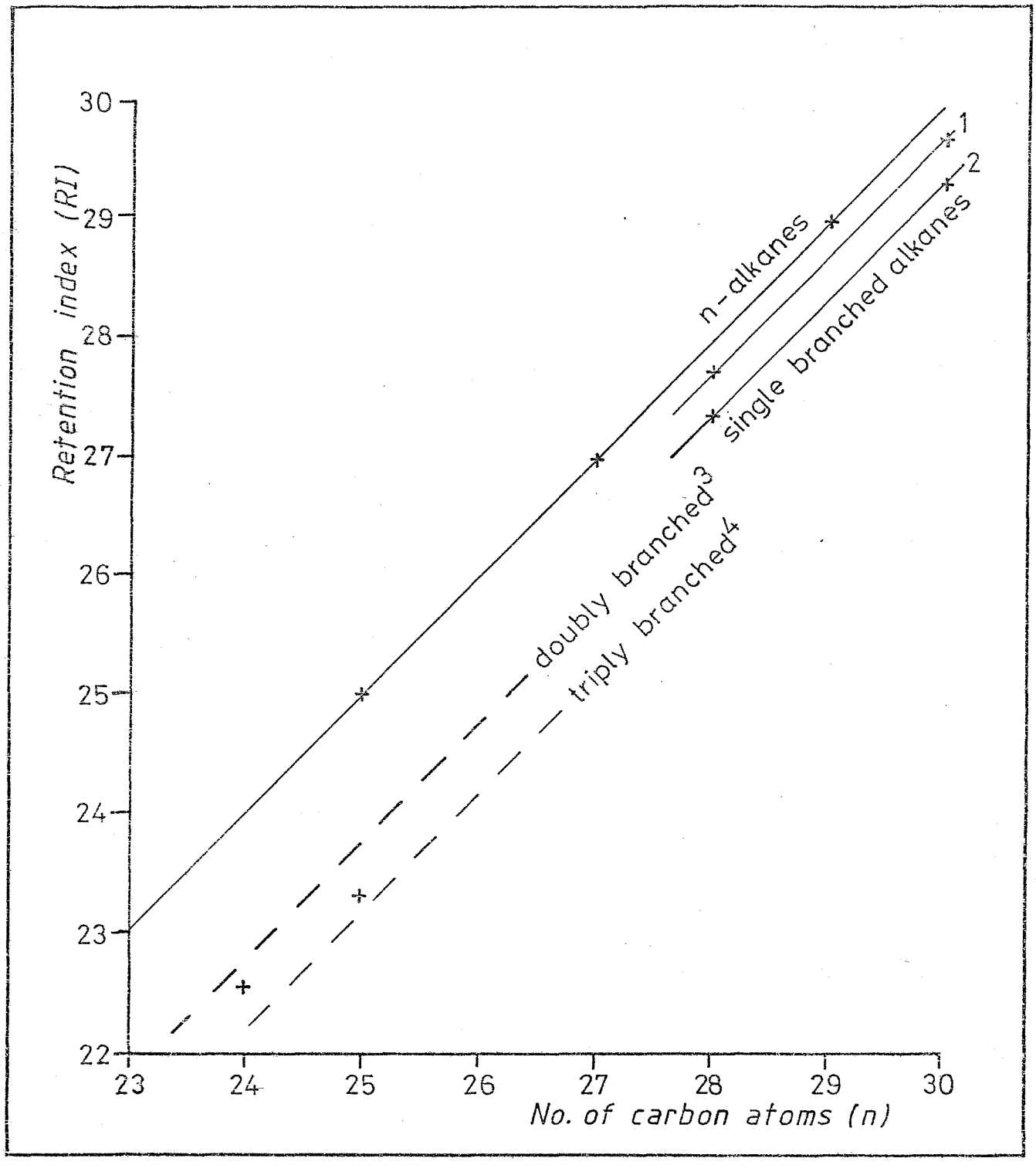
2 *7-, 9-, 11- or 13-methyl alkanes*

3 *series of unidentied alkanes (H. lineatum males)*

4 *calculated from (100x n) - (2xI)* } *where n=as above*

5 *calculated from (100x n) - (3xI)* } *I=60, see text*

FIGURE 25:



FOOTNOTES:

1 *3-methyl alkanes*

2 *7-, 9-, 11- or 13-methyl alkanes*

3 *calculated from (100 x n) - (2 x I)* } *where n = as above*

4 *calculated from (100 x n) - (3 x I)* } *I = 60, see text*

FIGURE 24.

MEASURED DECREMENT	BRANCHING POSITION	ESTIMATED DECREMENT
28	3-methyl	
	4-methyl	42
54	5-methyl	
60	7-methyl	
	8-methyl	61
62	11-methyl	
	12-methyl	64
66	13-methyl	

TABLE 12: KOVAT'S INDEX CORRECTION FACTORS

proposed. From this, the unknown indices for other branching positions may be estimated. These are shown in Table 12.

From these figures it is now possible to calculate a theoretical value for the retention indeces for the two unknown compounds (399) and (400). For 3,7,11-trimethylheneicosane (399), the theoretical retention index would be:

$$(24 \times 100) - (28 + 60 + 62) = 2250 \text{ units}$$

The experimental value was 2248 units. Similarly, for 4,8,12-trimethyldocosane (400), the theoretical value would be:

$$(25 \times 100) - (42 + 61 + 64) = 2333 \text{ units}$$

The experimental value was 2332 units. Good agreement is obtained by this method between calculated and theoretical values for compounds (399) and (400). It is likely, by using the same column conditions, that structures which are as yet unknown, may be postulated using the graph of chain length against retention index.

Figure 25 shows chain length against retention index for *H. lineatum*. It is of similar construction to Figure 24, the points assigned to n-alkanes (from the mass spectra of females) have been joined by a straight line, as

PEAK No.[1]	PROPOSED STRUCTURE
1	n-tricosane
2	[2]
3	n-pentacosane
4	methylpentacosane[4]
5	[3]
6	n-heptacosane
7	methylheptacosane[4]
8	3-methylheptacosane
9	[3]
10	n-nonacosane
11	methylnonacosane[4]
12	3-methylnonacosane
13	[3]

FOOTNOTES:

1 see Figure 18

2 no structure can be proposed

3 see text

4 branching point unknown

TABLE 13: PROPOSED STRUCTURES FOR COMPONENTS FOUND IN THE EXTRACT FROM *H. lineatum* ♂

have those assigned to mono-methyl branched alkanes. The two lines for double and triple branching patterns have also been added. It is possible to suggest structures for some of the peaks as yet not assigned. In Table 13, suggested structures are shown for the peaks of Figure 18.

Most of the compounds in the male extract of *H. lineatum* seem to be those found in the other samples of warble flies analysed. In addition, a series of three compounds, as yet unidentified can be seen (see footnote 3 of Figure 25). It would be interesting to identify this series of compounds, but this would require more material for analysis. The diagram also allows us to conclude that *H. lineatum* do not seem to have any triply branched alkanes as part of the cuticle lipids.

5.4.8. Cholesterol in Warble Fly Extracts

The final major peak seen in each trace has been identified as cholesterol. It was found that this component of the trace was affected by both microscale bromination and silylation. The mass spectrum showed a parent ion at m/e 386. An authentic sample of cholesterol coinjected with the peak on the chromatogram, as did the trimethylsilyl ether. (Coinjection studies were carried out by taking ½ µl of natural extract mixture and ½ µl of a similar concentration solution of cholesterol in pentane and injecting the two components on the column at the same time. If the cholesterol was the component in the extract, the peak assigned to cholesterol would get larger, relative to the rest of the components. If it were not cholesterol, an extra peak would be visible.) A mass spectrum of recrystallised cholesterol gave good agreement with the spectrum obtained from the fly extracts. Large quantities of cholesterol were found in all of the flies, but it was difficult to calculate the precise amount in each fly, because cholesterol is not very soluble in pentane (the solvent used in extraction).

It was interesting to find cholesterol on the cuticle of the warble fly because it has not been reported to be present in any extract obtained from other flies. At this point, it was decided to find the source of cholesterol.

As previously stated, warble flies do not feed during their lifetime as flies, so the source of cholesterol must be the host. Therefore, cholesterol should be found in the larva. A second instar larva *(H. bovis)* was available, and this was ground in a mortar and pestle with purified methylene chloride (5 ml). The resulting extract was decanted and filtered. The GC of the solution showed no peaks at all, no volatile organics or cholesterol seemed to be present. The lack of volatile organics was not surprising, the

larva is protected by the host, so no protective cuticular wax is needed. Cholesterol had been expected. It therefore followed that the cholesterol was present in another form, possibly as a fatty acid ester - too involatile for GC analysis.

Two larvae were digested by refluxing in 10% aqueous sodium hydroxide for several hours. The mixture was extracted with methylene chloride, and the extract washed with aqueous ammonium hydroxide and dried with anhydrous magnesium sulphate. Concentration followed by GC analysis, showed the presence of cholesterol (confirmed by coinjection studies and mass spectrometry). About 70 µg/larva was found to be present. Small amounts of hydrocarbons were also observed.

5.5. Discussion

We have found the study of warble flies difficult because of four major reasons:

(i) the larva can only live and develop within the host. Removal leads to death.

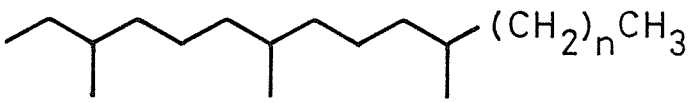
(ii) the adult flies live for one week during one month of the year

(iii) No bioassay has been devised for testing of extracts and likely attractive volatile components

(iv) the supply of flies to study has been poor, and structure elucidation of constituents, in such low concentrations, is difficult.

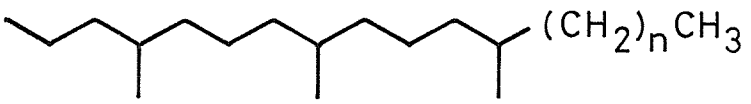
The flies must have a method of finding a mate, either visual or chemical. It is known that tsetse flies aggregate near the host, but observations show this not to be so for warble flies[260,293]. Unusually, it seems that females actively hunt for males, who lie in wait. The males will chase any likely object, even pebbles, thrown past them[260]. Therefore, it is interesting that lipids (399) and (400) are found in the extracts of the males of *H. bovis*. These could be contact pheromones that lead to recognition of mates (as in the case of the tsetse fly[271]). Lack of material prevented any such compounds being found in *H. lineatum*, should any be present.

Work on a South American ant, *Atta columbica*, showed the presence of triply-branched alkanes at the 3,7,11 and 4,8,12 positions[294]. Triply-branched alkanes are also found in *Atta sexdens* of the form (401) and (402).



401

n = 19, 21 or 23



402

A series similar to (401) is also found in *Atta cephalotes isthmicola*. Retention index decrements for (401) and (402) were calculated to be 160 and 180 units respectively, both on OV101 (1%). These do not agree with our values of 152 and 168 units for (399) and (400) respectively, but this could be due to different column materials and column conditions. Mass spectral data in these papers is only presented schematically, but the breakdown patterns observed do give good agreement with those obtained for (399) and (400).

The other hydrocarbons seen in the extract (the n-alkanes and mono-branched alkanes in the range C_{23}-C_{30}) are frequently found in cuticular lipids, whereas the triply-branched alkanes are less common. Biosynthesis of n-alkanes could occur by repeated joining and reduction of acetic acid units followed by decarboxylation of the acid group. The branches could be caused by the incorporation of propionic acid units, but this has not been proven.

The presence of cholesterol is interesting, as it has not been found in the cuticular waxes of other species of Diptera. Flies are not able to biosynthesise steroid structures, so they require an intake of readily cyclised substrate. This is especially important as the insect moulting hormone, ecdysone, is steroidal. It has been found that there is a dietary requirement of either cholesterol (from a host animal) or stigmasterol or ergosterol (from a host plant). The lack of steroids in the diet of a fly leads to restricted development and sterility in adult flies[296,297,298].

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