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ERRATA

Page	Line	
40	20	should read: at least three cardenolides ...
42	35	" " : *aureus*.[120]
48	31	" " : This methodology ...
66		(125) → (127) conditions (ii) should read: Pd-$SrCO_3$/57%
69	17	should read: with a strong absorption ...
69	18	" " : Absorption peaks ...
76	3	" " : cyclohexane (96) and the ...
80	20	" " : less congested β-face ...
80	32	" " : at the transition state ...
83	18	" " : hydride attack on (150) should ...
84	2	" " : dihedral angles, ...
97	3	" " : carbocyclic skeleton were overcome ...
100	4	" " : The absorption bands ...

UNIVERSITY OF SOUTHAMPTON

PROSTAGLANDINS IN INSECTS
AND
MODEL STUDIES ON THE PANICULIDES

A thesis submitted for the degree of
Doctor of Philosophy

by

Colin Leslie Gibson

Department of Chemistry

March 1983

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With Love
To My Parents and Sandra

"The best-laid scheme o' mice and men
Gang aft a'gley."

(Robert Burns)

ACKNOWLEDGEMENTS

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CONTENTS

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

CHEMISTRY

Doctor of Philosophy

PROSTAGLANDINS IN INSECTS AND MODEL STUDIES ON THE PANICULIDES

By Colin Leslie Gibson

Extracts of the guts of Acheta domesticus and the haemolymph of Actias perneyi larvae have been analysed for the presence of prostaglandins. Two prostaglandin-like compounds were detected in each extract as the methyl ester, methoxime and trimethylsilyl ether derivatives by gas chromatography. Limited structural information was gained by gas chromatography-mass investigation of these components. The mass spectral data of these derivatives suggested that the components had several trimethylsilyl ether groups, one of which was thought to be primary and another allylic. Derivatisation work in conjunction with gas chromatography on extracts of the haemolymph from Actias perneyi larvae indicated that there was no carbonyl or carboxylic acid group present in these compounds.

Model studies directed towards the design of a total synthesis of (1aβ, 2aβ,6α,6aβ)-(±)-2,2a,6,6a-tetrahydro-6-hydroxy-1a-(hydroxymethyl)-5-(4-methyl-3-pentenyl)oxireno[f]benzofuran-4(1aH)-one (paniculide B) and (1aβ, 2aβ,6aβ)-(±)-1a,2,2a,6a-tetrahydro-1a-(hydroxymethyl)-5-(4-methyl-3-pentenyl)oxireno[f]benzofuran-4,6-dione (paniculide C) are described. The basic carbocyclic skeleton for the model system was constructed by reaction of dilithioacetate with trans-2,3-epoxycyclohexan-1-ol which yielded (3aβ,4β,7aβ)-(±)-hexahydro-4-hydroxy-2(3H)-benzofuranone after lactonisation. Two approaches involving the alkylation of 1,3-cyclohexandione with ethyl bromoacetate were found to be unsuitable for the construction of the required skeleton. Further modifications of (3aβ,4β,7aβ)-(±)-hexahydro-4-hydroxy-2(3H)-benzofuranone were carried out to introduce an α-hydroxy epoxide moiety onto the cyclohexyl ring. An investigation into the introduction of a latent $\Delta^{\alpha,\beta}$-butenolide olefin via α-phenylthio and α-methylthio ethers was carried out. On the basis of these model studies two synthetic strategies to paniculides B and C are proposed.

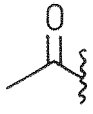
The total synthesis of (±)-(6E,10E)-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraen-3-ol (geranyllinalool) beginning from (6E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol), involving a Carroll rearrangement is described. The synthetic material and the natural (R)-(-)-(6E,10E)-geranyllinalool isolated from the defence secretion of Reticulitermes lucifugus soldiers were found to have identical ^{1}H NMR, infrared and mass spectra. Biological testing on the synthetic material indicated that it had insect antifeedant activity and significant insect toxicity.

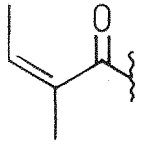
ABBREVIATIONS

Solvents and Reagents

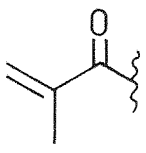
*m*CPBA = *meta*-Chloroperoxybenzoic acid
DBN = 1,5-Diazabicyclo[4.3.0]non-5-ene
DCC = N,N'-Dicyclohexylcarbodiimide
DMAP = N,N-Dimethyl-4-aminopyridine
DME = 1,2-Dimethoxyethane
DMF = N,N-Dimethylformamide
DMSO = Dimethylsulphoxide
HMPA = Hexamethylphosphoramide
LDA = Lithium diisopropylamide
LiTMP = Lithium 2,2,6,6-tetramethylpiperidine
PCC = Pyridinium chlorochromate
py = Pyridine
THF = Tetrahydrofuran
TMEDA = N,N,N',N'-Tetramethylethylenediamine
*p*TsOH = *para*-Toluenesulphonic acid

Derivatives

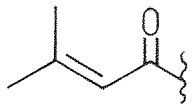
Ac = 

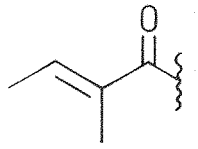
Ang = 

*t*BDMS = *tert*-Butyldimethylsilyl

Meth = 

MOM = Methoxymethyl
Ms = Methanesulphonate

Sen = 

Tig = 

TES = Triethylsilyl
TMS = Trimethylsilyl

Standard abbreviations for prostaglandin derivatives have been used in this thesis:-

PG-*t*BDMS = Prostaglandin *tert*-butyldimethylsilyl ether
PG-Et = Prostaglandin ethyl ester
PG-ME = Prostaglandin methyl ester
PG-MO = Prostaglandin methoxime
PG-TMS = Prostaglandin trimethylsilyl ether

General

GC = Gas chromatography
GC-MS = Gas chromatography - mass spectrometry
HPLC = High performance liquid chromatography
RIA = Radioimmunoassay

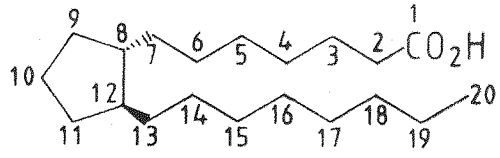
CHAPTER ONE

Prostaglandins in Insects

1.1 INTRODUCTION

1.1.1 History

The prostaglandins (PG) are a group of naturally occurring, biologically active, monocyclic, C_{20}, oxygenated fatty acids based on the hypothetical prostanoic acid skeleton (1).



Prostanoic Acid (1)

The discovery of prostaglandins and their potent and manifold biological properties has stimulated a huge amount of research from scientists of various disciplines. The importance of prostaglandins is equalled only by the earlier discovery of steroids and penicillins.

From the historical point of view, Kurzrok and Lieb in 1930 demonstrated that human seminal plasma could induce smooth muscle stimulation when applied to the human uterus.[1] Shortly thereafter, von Euler[2] and Goldblatt[3] demonstrated independently the presence of a vasodepressor agent and a stimulating factor of muscles in extracts of human seminal plasma and sheep vesicular glands. Von Euler showed that the biological activity was due to a lipid soluble material with acidic properties and coined the name, prostaglandin, erroneously believing that the substance was produced in the prostate gland.[4] Eliasson subsequently found that most prostaglandins in human ejaculate are secreted by the seminal vesicles rather than the prostate gland.[5]

In 1957 Bergström and Sjövall first isolated pure crystalline substances from sheep seminal vesicles which they called prostaglandin E (PGE) and prostaglandin F (PGF) and elucidated their structures.[6] A short time later, Bergström, Sjövall and Samuelsson were able to differentiate and then isolate 13 different substances.[7,8,9]

1.1.2 Structure and Nomenclature

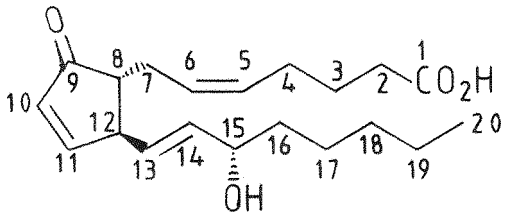
The naturally occurring prostaglandins isolated to date fall into nine basic categories according to the functionality on the cyclopentane

ring (Figure 1.1). These nine groups are referred to by the letters A-I which were derived in the following way: the first successful purification of prostaglandins in Sweden was accomplished by partition of the crude mixture between ether and phosphate buffer.[6] Prostaglandin E was obtained from the ether phase (*e*ther) while prostaglandin F was isolated from the aqueous phase (*f*osfat). Treatment of prostaglandin E with *a*cid gave prostaglandin A while *b*ase treatment gave prostaglandin B. As other prostaglandins were discovered they were given the appropriate letters to fill the gaps and to extend the sequence.

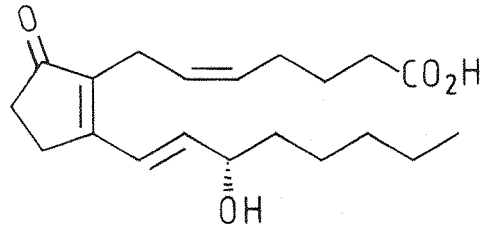
The E and F series prostaglandins contain a hydroxy function at C-11, but whereas the E series possess a ketone moiety at C-9, the F series have a second hydroxy group at this position. The A-C prostaglandins can be regarded as dehydration products of the E series, having the C-9 ketone group and a double bond in the cyclopentane ring. The A prostaglandins have a double bond at the 10 position while in the B series it is positioned between C-8 and C-12 and in the C series it is at the 11 position. The D series are isomeric with the E series having a keto group at C-11 and an α-hydroxy at C-9. The G and H prostaglandins are *endo*peroxides which have an α-*endo*peroxide bridge between C-9 and C-11. The I series are bicyclic with a cyclic enol ether moiety between C-6 and C-9. All prostaglandins have a (13E) double bond and an α-hydroxy group at C-15 except for the G series which have a C-15 hydroperoxide moiety.

The nine groups of prostaglandins are further subdivided into three groups according to the degree of unsaturation occurring in the sidechains, denoted by the numerical subscripts 1,2 or 3. Thus PGE_1 (6) has only the (13E) double bond while PGE_2 (7) has a second (5Z) double bond and PGE_3 (8) has a third (17Z) double bond. An additional subscript in the case of the F series defines the stereochemistry of the C-9 hydroxy group.

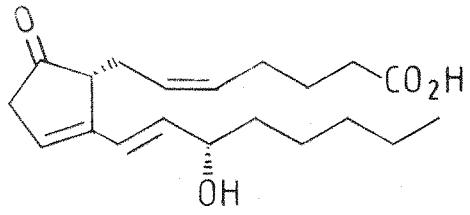
The thromboxanes which are closely related to prostaglandins have been isolated recently.[10] These compounds have an oxacyclohexane ring in place of the cyclopentane ring and they induce aggregation of blood platelets and cause thrombus formation. Two classes of thromboxane have been isolated, TXA_2 (14) and TXB_2 (15).



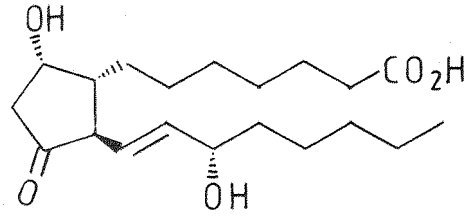
PGA_2 (2)



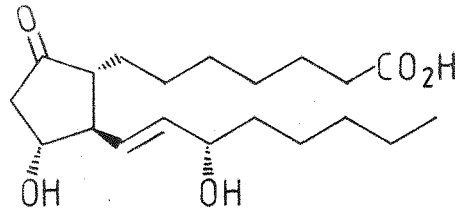
PGB_2 (3)



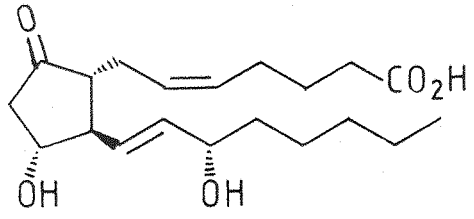
PGC_2 (4)



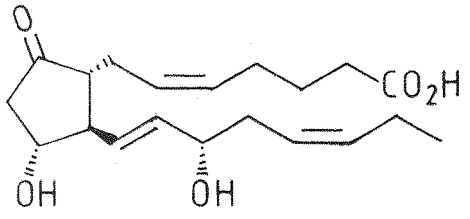
PGD_1 (5)



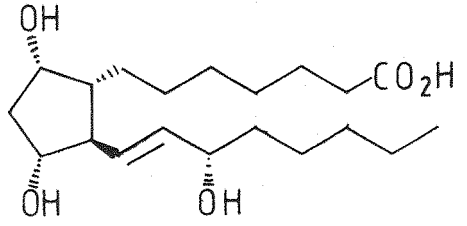
PGE_1 (6)



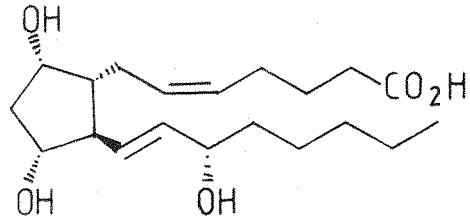
PGE_2 (7)



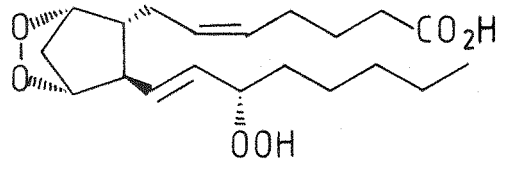
PGE_3 (8)



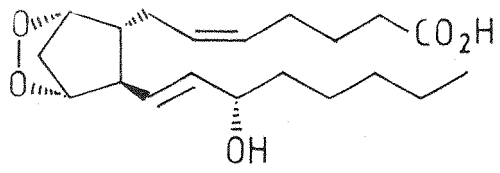
$PGF_{1\alpha}$ (9)



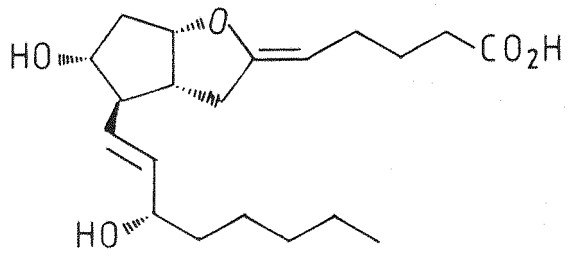
$PGF_{2\alpha}$ (10)



PGG_2 (11)

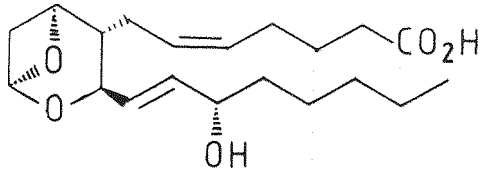


PGH_2 (12)

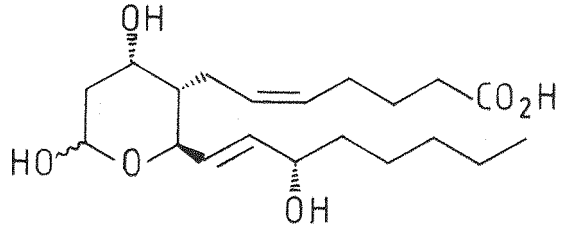


PGI_2 (13)

Figure 1.1: Prostaglandin Structure and Nomenclature



TXA_2 (14)



TXB_2 (15)

1.1.3 Occurrence and Biological Properties

The prostaglandins are ubiquitous in mammals;[11] the richest sources (ca. 300μg/g) of prostaglandins are the seminal fluids of man and sheep. Lower concentrations (ca. 1μg/g) are detectable in tissue and body fluids from *inter alia* the uterus, ovaries, eye, brain, thymus, bronchials, pancreas, lungs and kidney. They have diverse rôles in mammalian physiology and are considered to be modulators of intracellular metabolism[12,13] as well as being involved in platelet aggregation.[14] They have rôles in renal,[15] haemodynamic,[12] cardiovascular[16] and respiratory activities.[17]

Prostaglandins are closely associated with reproduction especially in the human where they are implicated at every level from conception to birth. In the female, prostaglandins may be involved in menstruation, parturition, steroidgenesis, luteolysis, ovulation, ovum transport and sperm transport.[18] In the male, prostaglandins may be involved in spermatogenesis, steroidgenesis and emptying of the male accessory glands.[19]

To-date there has been relatively little information concerning the distribution of prostaglandins in the lower animals. Prostaglandins have been found in the urinary bladder of the frog and prostaglandin synthetase has been found in mussels and lobsters.[11] Large quantities of 15-epi-PGA_2 and it's acetate, methyl ester have been found in the gorgonian coral *Plexaura homomalla* (Esper).[20,21] These reports were followed shortly by the equally surprising and much more useful finding that the prostaglandins (15S)-PGA_2 (2) and (15S)-PGE_2 (7), having the natural (mammalian) configuration at C_{15}, were found in equally large

amounts in varieties of *P. homomalla* collected in the Caribbean area.[22] This coral has also been found to contain 5-*trans*-PGA_2.[23] Corey *et al.*, have detected prostaglandin synthetase activity in the microsomal fraction from homogenates of *P. homomalla*.[24] Recently the soft coral *Lobophyton depressum* was shown to contain 18-acetoxy-(15S)-$PGF_{2\alpha}$-11-acetate, (15S)-$PGF_{2\alpha}$-11-acetate and their methyl esters.[25]

In the plant kingdom, PGA_1 has been found in the yellow onion[26] while the red alga *Gracilaria lichenoides* was shown to contain both PGE_2 (7) and $PGF_{2\alpha}$ (10).[27]

1.1.4 Prostaglandins in Insects

The first report of prostaglandin biosynthesis in insects was made by Destephano *et al.* in 1974, who reported the *in vitro* synthesis of PGE_2 (7) from arachidonic acid by the reproductive tract of the male house cricket *Acheta domesticus*.[28] The report that large quantities of endogenous PGE_1 (6) occur in the spermatophore of *A. domesticus* has now been withdrawn.[29] In a later study, Destephano *et al.* showed that prostaglandin synthetase was present in the testes, seminal vesicles and spermatophore of male *A. domesticus*.[29] The enzyme was not detected in the bursa copulatrix, spermathecae and oviducts from virgin females, while substantial activity was measured in the same tissue from mated females. A PGE_2 (7) like material was detected by radioimmunoassay (RIA) in *A. domesticus* mated female reproductive tissue at a level of 589 pg per insect, but not in virgin females. Substantial levels of PGE_2 (7) like material was also found in the testes (458 pg/cricket) whereas levels in the remainder of the reproductive tract were lower (84 pg/cricket). In *in vivo* studies, injected PGE_1 (6), PGE_2 (7), and to a smaller degree $PGE_{2\alpha}$ (10) stimulated oviposition in virgin females in a dose dependent fashion.

The authors tentatively concluded from these results that oviposition in *A. domesticus* is stimulated by post-copulatory prostaglandin biosynthesis in the female reproductive tract. It was thought that *A. domesticus* females acquired prostaglandin synthetase from the male, where it originated in the testes, so the enzyme could be considered as a 'primer pheromone'. In addition, prostaglandins were implicated in the mechanism of sperm transport.[28,29]

Whole body extracts of virgin and mated female crickets *Teleogryllus commodus* were shown to contain PGE_2 (7) by HPLC separation of the *p*-bromophenacyl ester. The amounts of PGE_2 (7) in the mated female (140 ng/female) were almost twice that of virgin females (80 ng/female).[30] The presence of PGE_2 (7) in the spermathecae of *T. commodus* mated females (500 pg/spermathaca) and its virtual absence from those of virgin females and spermatophores has also been shown by HPLC.[31] Substantial prostaglandin biosynthetic activity was present in the spermatophore and spermathecae of mated female *T. commodus*, while the precursor of PGE_2 (7), arachidonic acid was detected in the spermathecae of virgin females (2.2% of total fatty acid content).[31] In *in vivo* studies only small quantities of PGE_2 (7) injected into the oviduct of virgin females were necessary to stimulate mating induced egg release.[31,32]

A possible rôle for prostaglandins in egg laying in *T. commodus* was postulated on the basis of these results, which was similar to the hypothesis of Destephano *et al.*[28,29,31]

The presence of a fat soluble material in the reproductive organs of the male silkmoth *Bombyx mori*, which stimulates smooth muscle contraction, has been shown to be due to prostaglandins. The active material was separated into two types of prostaglandins, PGE (4.192 µg/g wet wt.) and PGF (2.53 µg/g wet wt.) which could be further resolved into PGE_1 (6), PGE_2 (7), $PGF_{1\alpha}$ (9) and $PGF_{2\alpha}$ (10) respectively. This analysis was based on TLC behaviour, enzymic assay, UV absorption and fluorescence emission spectroscopy.[33] In further work Yamaja Setty *et al.* showed that PGF was present in the reproductive organs of mated female *B. mori* (14 µg/animal), but was absent in virgin silkmoths using an enzymic assay. Prostaglandins E were also detected in the reproductive organs in virgin (15.6 µg/animal) and mated females (29.8 µg/animal), but the level of PGE was increased on mating. Prostaglandin PGE_2 (7) and to a lesser extent PGE_1 (6) and $PGF_{2\alpha}$ (10) when injected into virgin female silkmoths, stimulated oviposition.[34]

In contrast to the case for both *A. domesticus* and *T. Commodus* these observations led to the postulation that in the silkmoth *B. mori*, PGE_2 (7) and $PGF_{2\alpha}$ (10) are transferred during copulation, rather than prostaglandin synthetase. These transferred prostaglandins may then stimulate post-mating oviposition.[33,34]

Low levels of prostaglandin like compounds (PGE_2 (7), $PGF_{2\alpha}$ (10)) were detected by RIA in reproductive tissue of virgin and mated females of the cabbage looper *Trichoplusia ni* (Hübner). A threefold increase in PGE_2 equivalents was found in mated female reproductive tissue (71 pg/insect) with respect to virgin female tissue (24.3pg/insect). The mean level of $PGF_{2\alpha}$ equivalents for virgin females was 15.8 pg/insect while mated females contained 31.7 pg/insect, this represents a two fold increase over the virgin female. The male reproductive tract of *T. ni* was also found to contain PGE_2 (7) by RIA (27.6 pg/insect). The elevated levels of PGE_2 (7) and $PGF_{2\alpha}$ (10) in mated *T. ni* females were explained by the receipt of prostaglandins or prostaglandin synthetase from the male *via* the semen.[35]

The discovery of endogeneous PGE_2 (7) in the saliva, salivary glands and larvae of the cattle tick *Boophilis microplus* was achieved by TLC characteristics and bioassay on a muscle preparation.[36] In addition the saliva of the engorged tick *B. microplus* is known to contain two pharmacologically active components. One of these was thought to be a prostaglandin (or mixture) from its Sephadex LH20 elution profile and its deactivation by 15-hydroxy prostaglandin dehydrogenase.[37] It is suggested that prostaglandins could have a rôle in the feeding mechanism of this tick where they may be important in the initiation and maintenance of host lesions.[36]

Shemesh *et al.* incubated salivary glands, ovaries, testes and eggs from the tick *Hyalomma anatolicum excavatum* and found moderate levels of PGE_2 (7) and PGF by RIA. Endogenous prostaglandins in non-cultured organs were undetectable (<0.02 ng/gonad) by RIA. However, measurable amounts of endogenous PGE_2 (7) and PGF were found in these organs in both sexes following six days of feeding. High levels of PGF and PGE_2 (7) were also found in tick eggs (19.6 and 13.2 ng/g tissue, respectively). As in the case of *B. microplus* PGE_2 (7) was implicated in the feeding mechanism of *H. anatolicum excavatum*.[38]

1.1.5 Microscale Analysis of Prostaglandins

Because of the very low quantities of prostaglandins present in most locations, only those methods of determination which can detect nanogram amounts are of practical importance. Only a limited number of methods meet this requirement: (a) biological assays, (b) RIA, (c) enzymic

assay, (d) HPLC, (e) gas chromatography combined with mass spectrometry (GC-MS). The biological methods are very sensitive but they lack specificity and accuracy due to the interference from substances other than prostaglandins. HPLC is less sensitive than the biological methods although it is quicker and easier to operate. The detection methods used for HPLC (liquid scintillation counting, ultraviolet absorption, fluorescence and refractive index) are, however, not specific for the prostaglandins, and this has led to its infrequent usage. GC-MS, however, is increasingly proving to be one of the most sensitive and selective physical methods of prostaglandin analysis.

The structural elucidation of prostaglandins and their metabolites has been dominated by the use of GC-MS. In particular the sensitivity of the technique (nanogram level) and the objectivity of the information derived, coupled with the ability to work without rigorous sample purification has led to its routine use.

1.1.6 Prostaglandin Derivatives for Gas Chromatography

The prostaglandins are difficult to chromatograph because the presence of several polar groups renders prostaglandins susceptible to thermal decomposition and irreversible adsorption on active sites of the column. The analysis of prostaglandins by GC has one major disadvantage in that it is essential to protect all the functional groups to improve chromatography.

The carboxylic acid group of prostaglandins are generally protected as the ester of which the methyl ester is the most common, primarily because of the relative ease with which diazomethane can be generated. Alternative esters including the ethyl and trimethylsilyl[39,40] and pentafluorobenzyl[41] have been used. The use of the trimethylsilyl derivatization of prostaglandins has the advantage that the hydroxy and carboxylic acid groups can be protected in a single step.

The prostaglandins of the E series possess a thermally unstable β-ketol moiety, which even when the 11α-hydroxy group is protected will result in elimination unless the 9-keto function is protected. The ketonic functions in prostaglandins are protected by conversion to the O-alkyl oxime. The most common O-alkyl oxime is the O-methyl oxime[42] and O-ethyl oxime[43] followed by the O-benzyl oxime.[44] The ketone groups of the A and B series prostaglandins are thermally stable

and thus do not have to be protected for GC. The β-ketol moiety of the E series is unstable in base giving the corresponding B series prostaglandin, thus the E series can be chromatographed without oximation by conversion to the B series with methanolic potassium hydroxide.[45,46]

The hydroxy groups of the prostaglandins are most commonly protected as the trimethylsilyl ether (TMS)[42,47] or as the acetate[42,47] as both are readily prepared and are relatively stable. An increasingly popular derivative for the protection of the hydroxy group is the *t*-butyldimethylsilyl ether (*t*BDMS) which is 10^4 times more stable towards hydrolysis than the trimethylsilyl ether and has better mass spectral characteristics. The mass spectral base peak of the *t*-butyldimethylsilyl ethers are generally as a result of the loss of the *t*-butyl radical (M-57) and can be regarded as a pseudo form of chemical ionization.[48,49,50]

1.1.7 Summary

Endogenous prostaglandins and prostaglandin synthetase have been observed in a variety of insect sources, where they are mainly associated with reproductive organs and to a lesser extent the saliva and salivary glands of ticks. Prostaglandins have been shown to induce oviposition when administered to virgin females.

The prostaglandins have been implicated in insect reproduction where they may be of significant importance. In this context it is envisaged that prostaglandin synthetase is transferred to the female, which results in prostaglandin biosynthesis and subsequent oviposition. In the case of the silkmoth *Bombyx mori* PGE_2 (7) and $PGF_{2\alpha}$ (10) are transferred during copulation and these prostaglandins then stimulate oviposition. In short, prostaglandin synthetase and prostaglandins are regarded as primer pheromones in insect reproduction.

The prostaglandins may play an important rôle in the feeding mechanism of ticks, as they are thought responsible for the dilution of superficial bovine skin capillaries and increased skin permeability.

The identification of endogenous prostaglandins in insect tissue has to date relied on TLC and HPLC characteristics, RIA, enzymic assay

and bioassay. These methods, however, are not specific and only give tentative identifications of prostaglandins. There has, therefore, been no unequivocal chemical identification of endogenous prostaglandins or similar compounds from insect tissue.

The purpose of this study was to establish the structures of prostaglandin like compounds from insect tissues and body fluids. It was envisaged that GC-MS would provide the most objective structural information at the low levels involved. In order to determine the structures of insect prostanoid material an investigation of the guts and testes of male *Acheta domesticus* and of the haemolymph from *Actias perneyi* larvae was proposed.

1.2 Mass Spectral Characteristics of Prostaglandin Derivatives

1.2.1 Literature Mass Spectra

The most suitable derivatives for GC-MS analysis appeared to be the methyl ester, methoxime, trimethylsilyl ether (PG-ME-MO-TMS) or the methyl ester, trimethylsilyl ether (PG-ME-TMS) for ketone and non-ketone containing prostaglandins respectively. The corresponding *t*-butyldimethylsilyl ethers (PG-ME-MO-*t*BDMS and PG-ME-*t*BDMS) could also be of use, especially if used in conjunction with the trimethylsilyl ether.

The analysis of prostaglandin mass spectra was aided by comparison with the published spectra for the trimethylsilyl ethers of these derivatives for PGA_1,[51] PGB_1,[51] PGD_1,[42] PGD_2,[52] PGE_1,[42,53,54] PGE_2,[42,53,54] 6-*oxo*-$PGF_{1\alpha}$,[52,55] $PGF_{1\alpha}$,[53,54,56] and $PGF_{2\alpha}$.[53,54,56] In addition, the spectral details of the *t*-butyldimethylsilyl ethers of these derivatives for $PGF_{1\alpha}$,[48] $PGF_{2\alpha}$[48] and 6-*oxo*-$PGF_{1\alpha}$[50,57] were also consulted. Comparisons were also made with the published spectra of the trimethylsilyl ethers of these derivatives for other deoxy and hydroxy prostaglandins.[58]

1.2.2 Derivatisation and Fragmentation Patterns of Standard Prostaglandin Derivatives

An investigation into the derivatisation method of Green[42] and Maclouf *et al.*[53] and of the spectral fragmentation patterns of standard prostaglandins was carried out in order that expertise in handling these sensitive compounds could be acquired. In addition, subtle changes from

the observed and reported ion intensities would be invaluable in prostaglandin structural analysis.

The major fragmentations of the ME-MO-TMS and ME-TMS derivatives for PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ are given in Tables 1.1-1.4 respectively.

Protection of the E series as the ME-MO-TMS derivative gave two peaks by GC. The first eluting peak was assigned the 'minor' and the second as the 'major' (ratio 1:3). These two components were assumed to be the syn and anti isomers of the methoxime group.[42]

The base peak in all the mass spectra (Tables 1.1-1.4) is the m/z 73 ion which is due to the trimethylsilyl cation (Me_3Si^+) and is indicative of the trimethylsilyl ether group. A prominent ion at m/z 75 is observed which is again due to the trimethylsilyl ether functionalities and corresponds to the $[Me_2Si = OH]^+$ fragment. In general the high mass ions are relatively weak and the lower masses are intense, in contrast to the literature spectra for these derivatives.

The ions at m/z 199 and m/z 173 are common to all spectra and are a result of loss of the C13-C20 side chain to give the m/z 199 ion (Fragment A) followed by expulsion of acetylene to give Fragment B (see Figure 1.2).[39,59]

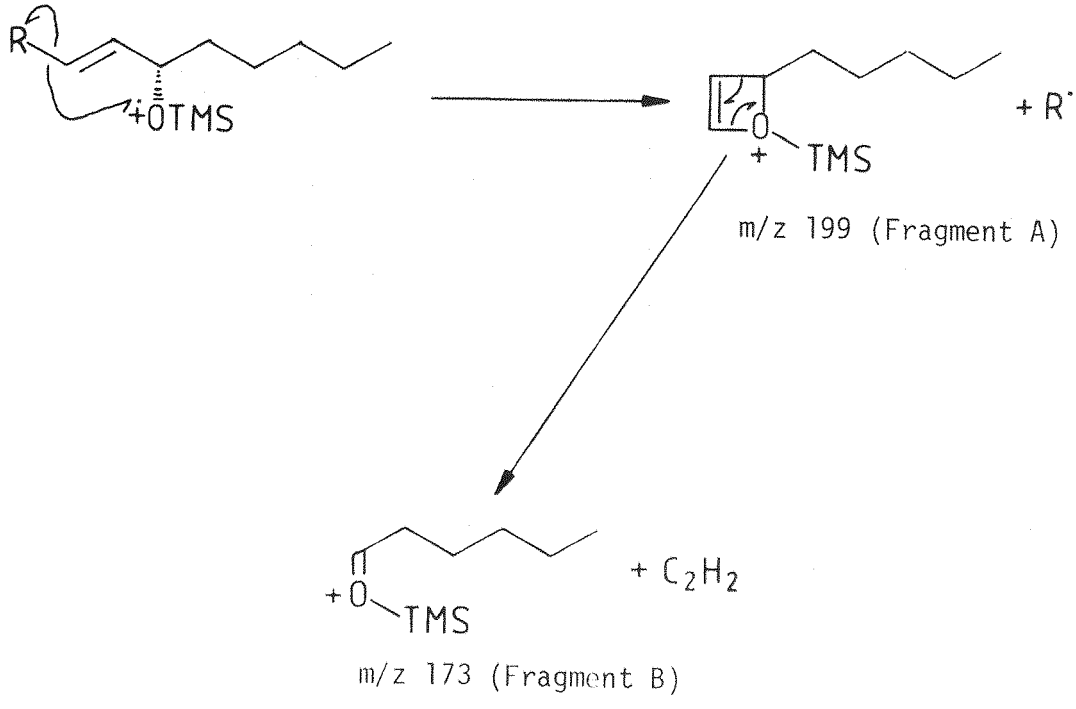


Figure 1.2: Genesis of m/z 199 and m/z 173 ions

m/z	Intensity (%)		Assignment[42]
	Minor	Major	
541	0.1	-	M
526	0.8	0.4	M-15
510	3.3	0.3	M-31
470	14.8	-	M-71
420	6.0	-	M-(90+31)
380	12.4	-	M-(90+71)
368	-	6.0	M-173
308	4.0	-	M-(90+143)
297	-	26.0	M-(199+45)
225	-	10.8	M-(173+143)
199	23.3	4.6	A
173	8.6	-	B
129	44.0	5.7	C
75	51.0	33.0	$Me_2Si{=}\overset{+}{O}H$
73	100	100	Me_3Si^+

Table 1.1 The Mass Spectral Fragmentation Patterns of the Minor and Major Isomers of PGE_1-ME-MO-TMS.

m/z	Intensity (%)		Assignment[42]
	Minor	Major	
539	-	0.25	M
524	-	1.25	M-15
508	2.1	3.0	M-31
468	4.4	0.2	M-71
449	-	1.8	M-90
438	1.2	0.4	M-101
418	3.0	1.2	M-(90+31)
378	4.0	0.4	M-(90+71)
366	1.8	3.0	M-173
365	3.0	-	M-174
348	2.0	1.3	M-(101+90)
308	2.9	1.1	M-(141+90)
295	3.9	22.0	M-(199+45)
225	5.3	25.4	M-(173+141)
199	4.0	2.0	A
173	7.4	6.0	B
129	10.0	6.0	C
89	86.0	8.0	-
75	42.0	35.0	$Me_2Si{=}\overset{+}{O}H$
73	100	100	Me_3Si^+

Table 1.2 The Mass Spectral Fragmentation Patterns of the Minor and Major Isomers of PGE_2-ME-MO-TMS.

m/z	Intensity	Assignment[39,54,59]
571	0.3	M-15
515	1.6	M-71
496	1.7	M-90
425	4.7	M-(90+71)
237	2.2	$C_{14}H_{25}OSi^+$ D
217	6.0	A
199	2.0	
191	15.5	M-(2x90+71+143+1) B
173	4.0	
147	10.0	$Me_3SiOSiMe_2^+$ C
129	10.3	
75	13.0	$Me_2Si{=}\overset{+}{O}H$
73	100	Me_3Si^+

Table 1.3 Major Fragmentations of $PGF_{1\alpha}$-ME-TMS

m/z	Intensity (%)	Assignment[39,54,59]
513	0.3	M-71
511	2.1	M-73 (TMS)
494	0.6	M-90
423	13.0	M-(90+71)
404	1.4	M-(2x90)
333	1.5	M-(2x90+71)
314	1.4	M-(3x90)
307	15.0	-
237	13.0	$C_{14}H_{25}OSi^+$ D
217	12.0	A
199	6.0	
191	39.0	M-(2x90+71+141+1) B
173	16.0	
147	18.o	$Me_3SiOSiMe_2^+$ C
129	15.0	
75	33.0	$Me_2Si{=}\overset{+}{O}H$
73	100	Me_3Si^+

Table 1.4 Major Fragmentations of $PGF_{2\alpha}$-ME-TMS.

The high intensity of the m/z 129 ion observed in all the spectra is indicative of the allylic trimethylsilyl ether function in prostaglandins and is assigned Fragment C (Figure 1.3).[55,60]

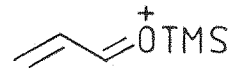


Figure 1.3: Structure of Fragment C (m/z 129)

The expulsion of trimethylsilanol (m/z 90) is common to all these spectra. The loss of a methyl radical (m/z 15) is also observed which is a common elimination of trimethylsilyl ethers. Elimination of $C_5H_{11}^{\cdot}$ (m/z 71) is another prominent fragmentation which occurs from allylic cleavage of the C15-C16 bond to give a doubly stabilized cation (see Figures 1.4 and 1.5).

Several ions in the mass specta of the 2-series prostaglandins show the loss of m/z 141 which results from allylic cleavage of the C7-C8 bond. In addition the expuslion of m/z 101 occurs by cleavage of the C4-C5 bond α to the double bond (Figure 1.4).[42] However, a number of ions in the spectra of the 1-series prostaglandins (PGE_1,$PGF_{1\alpha}$) show the loss of m/z 143 from cleavage of the C7-C8 bond (Figure 1.5).[42]

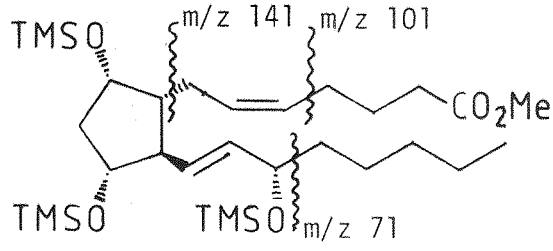


Figure 1.4: Common Fragmentations of the 2-Series Prostaglandins

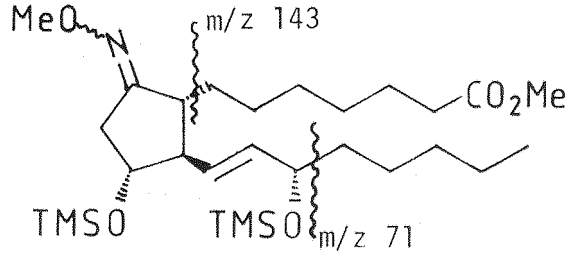
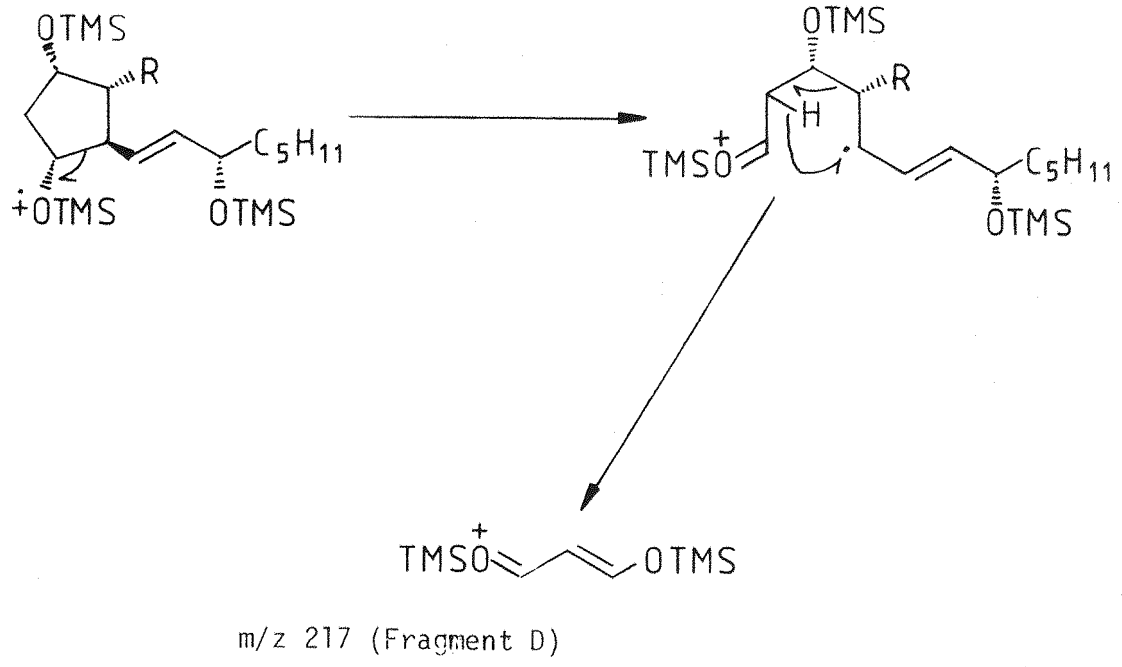


Figure 1.5: Common Fragmentations of the 1-Series Prostaglandins

The mass spectra of the F series prostaglandins show several ions which lose trimethylsilanol (m/z 90) and the molecular ion of $PGF_{2\alpha}$-ME-TMS gives three such successive eliminations. The ion at m/z 147 ($[Me_3SiOSiMe_2]^+$) is common in polyhydroxy TMS compounds containing two or more trimethylsilyl ether groups on adjacent carbon atoms or brought near each other through expuslion of the central part of the molecule.[61] In addition the spectra of the F-series show a prominent ion at m/z 217 (Fragment D) which is derived from fragmentation of the cyclopentane ring (see Figure 1.6).[59]



m/z 217 (Fragment D)

Figure 1.6: Genesis of the m/z 217 ion of the F-series Prostaglandins

The loss of a methoxy radical (m/z 31) from several ions in the mass spectra of the E-series prostaglandins is observed.[42] In addition, a number of ions eliminate the methoxime group (m/z 45).[42]

1.3 Analysis of *Acheta domesticus* Tissue

1.3.1 Gas Chromatographic Studies

Extracts of the guts and testes from 120 male *A. domesticus* were made by a modification of the method of Jouvenaz *et al.*[45] Derivatization of these extracts was carried out as per the method of Green[42] and Maclouf *et al*[53] to give the ME-MO-TMS derivative.

Examination of the derivatized extract of the testes by gas chromatography did not yield any prostaglandin like materials. However, analysis of the derivatized gut extract showed two components eluting in the prostaglandin GC region. The first eluting peak (major) was of greater intensity than thesecond (minor) in contrast to the E-series prostaglandins. Gas chromatography and co-injection studies indicated that these compounds were different to a number of authentic prostaglandin derivatives. The level of these two components together was estimated at 5ng per insect.

1.3.2 GC-MS of *A. domesticus* Extract

The derivatized extract of 120 *A. domesticus* guts was analysed by GC-MS of a 10µl aliquot (Table 1.5 and Figures 1.7 and 1.8).

The mass spectrum of the major component (Figure 1.7) shows a base peak at m/z 103 due to the $[Me_3SiOCH_2]^+$ fragment, indicative of a primary trimethylsilyl ether function.[58,60] The high intensity of the m/z 129 ion (62%) may result from an allylic trimethylsilyl ether (cf standard prostaglandins). However, the absence of the m/z 199, 173 ions would indicate that the C13-C20 alkyl chain of prostaglandins is absent. The loss of m/z 71 which is a prominent prostaglandin elimination is observed in the sequence:- m/z 408 → m/z 337. The presence of the m/z 147 ion (19%) which is assigned to the $[Me_3SiOSiMe_2]^+$ fragment, indicates that we have two or more trimethylsilyl ether groups close to each other (cf F series). In contrast to the prostaglandins the ion m/z 73 is not the base peak and the m/z 75 ion is absent from the spectrum. The highest mass observed is m/z 483 indicating that either nitrogen is present or, more likely, that the molecular ion is not seen.

The minor component (Figure 1.8) shows similar mass spectral characteristics to the major component but has a base peak of m/z 129. There is an absence of the m/z 199, 173 ions again indicating that the C13-C20 side chain of prostaglandins is not present. This component shows the loss of two trimethylsilanol molecules in the sequence:-

$$m/z\ 485 \rightarrow m/z\ 395 \rightarrow m/z\ 305$$

which would indicate the presence of two or more trimethylsilyl ether groups. This is further supported by the high intensity of the m/z 147 ion (45%). Interestingly, we see the loss of m/z 101 in the sequence

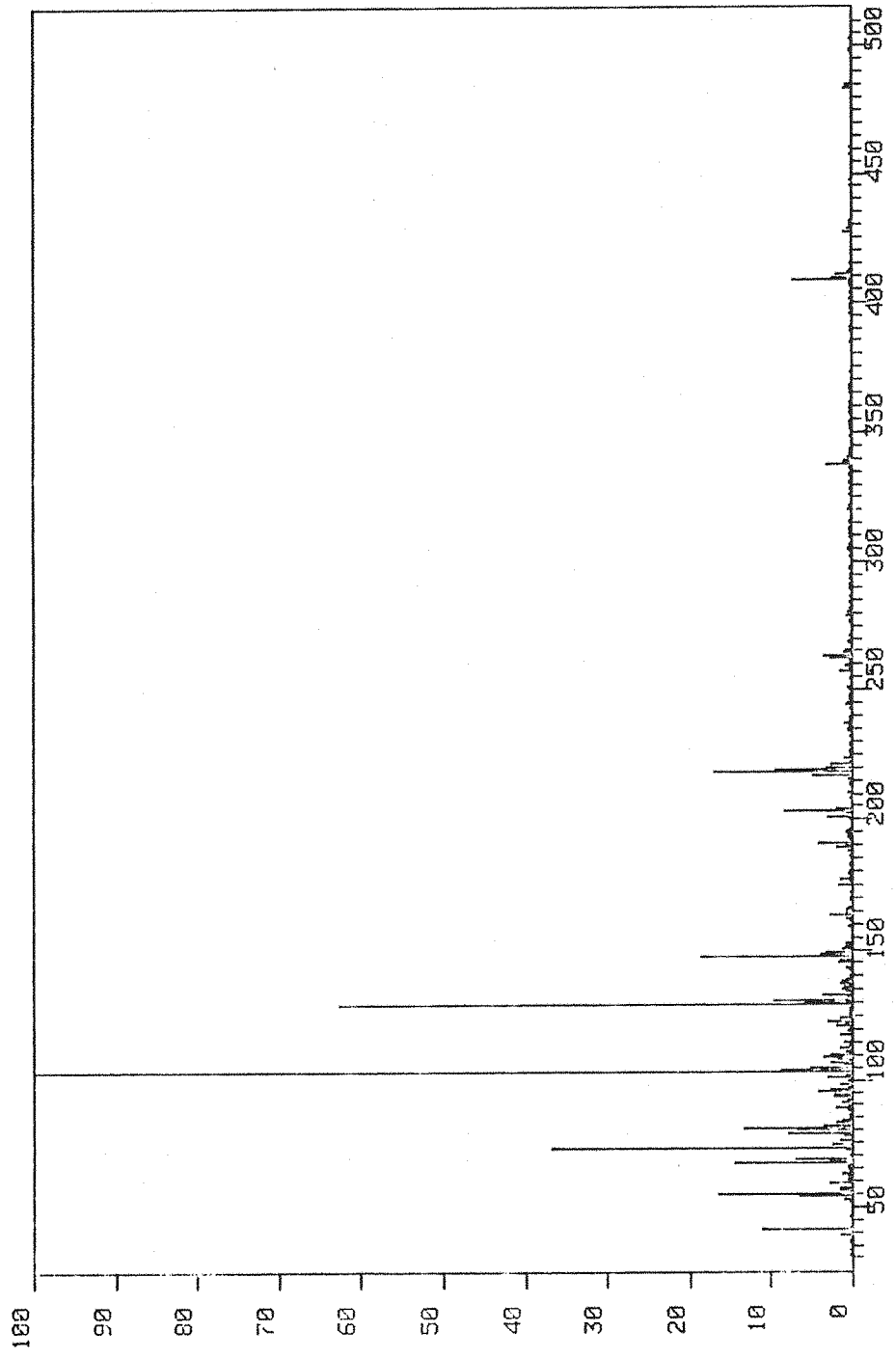
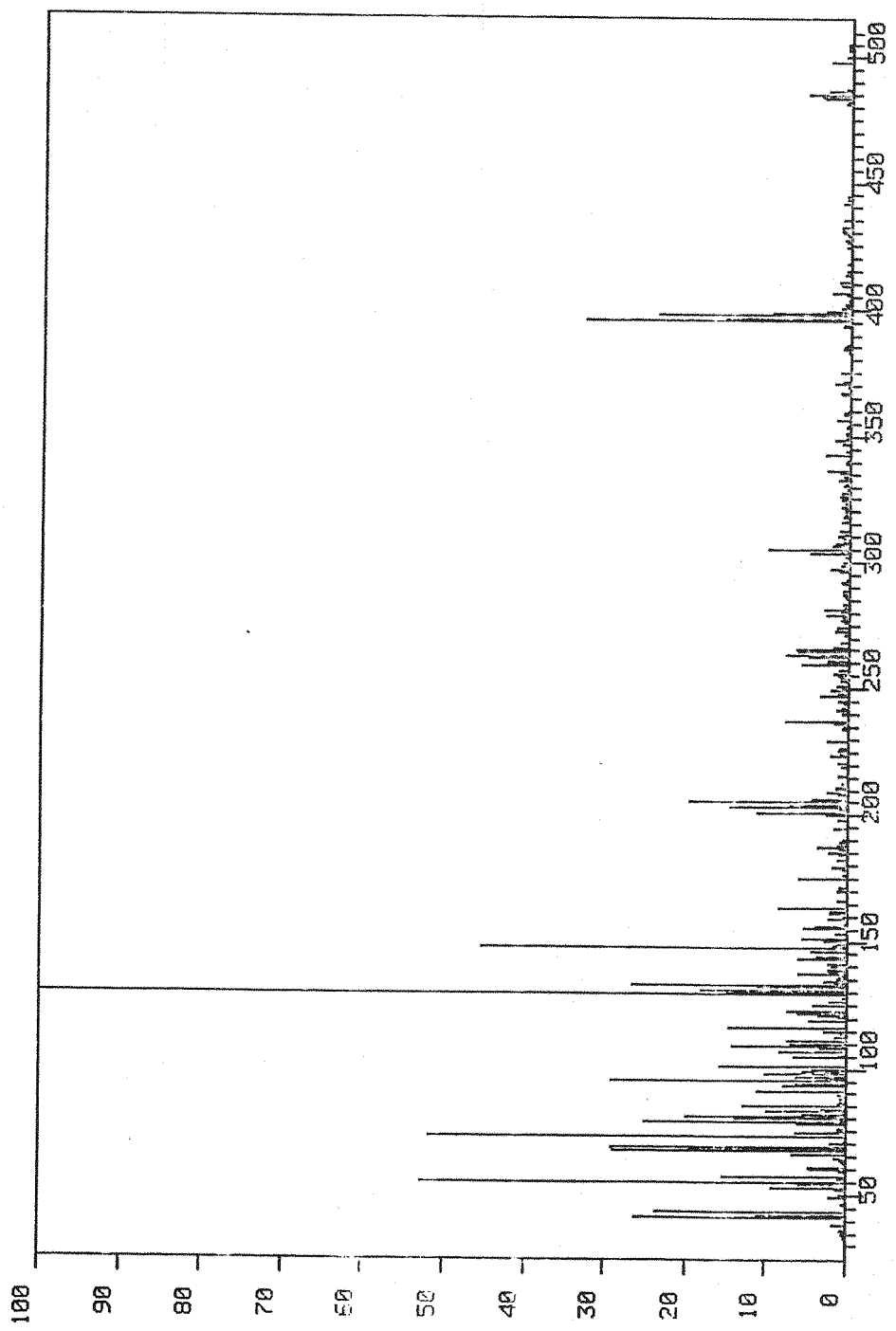


Figure 1.7: Mass Spectrum of ME-MO-TMS Derivative of the Major Component from an Extract of 120 *Acheta domesticus* Guts

Figure 1.8: Mass Spectrum of ME-MO-TMS Derivative of the Minor Component from an Extract of 120 *Acheta domesticus* Guts



m/z	Intensity (%)	
	Major	Minor
498	-	2.4
485	-	5.3
484	-	3.6
483	0.2	3.0
409	2.0	-
408	7.0	-
398	-	9.6
397	-	23.9
396	-	15.5
337	2.8	-
263	3.3	7.7
221	2.5	-
220	3.0	-
219	9.0	-
218	16.9	-
205	-	19.6
203	8.2	14.3
201	-	11.3
191	4.0	-
147	18.6	45.3
133	3.5	26.5
131	9.6	18.1
129	62.0	100
123	-	7.4
116	-	14.6
109	3.3	14.2
105	5.0	-
103	100	-
101	3.0	15.8
98	-	10.3
95	4.0	29.0
91	-	11.0
85	-	12.9
83	-	9.7
81	13.0	20.0
80	6.5	-
79	8.0	25.0
73	37	51.9
69	69	29.0
68	-	19.5
67	74.4	28.9
55	16.6	52.0
41	11.0	26.0

Table 1.5 GC-MS of ME-MO-TMS Derivatives for the Major and Minor Components from *Acheta domesticus*

m/z	Intensity (%)	
	Major (Peak a)	Major (Peak b)
498	-	0.6
496	0.2	3.5
481	2.0	13.3
425	1.6	0.8
411	-	3.5
410	1.5	3.1
408	3.4	4.9
407	3.3	-
406	9.2	13.6
397	-	9.9
395	-	11.6
393	-	40.9
337	2.6	4.1
335	3.7	3.4
281	-	19.3
221	3.3	5.3
219	11.8	-
218	22.4	-
203	8.9	8.6
191	9.0	-
147	30.0	66.0
133	6.0	-
131	9.7	22.0
129	65.4	100
121	5.1	7.6
117	5.8	16.3
108	9.7	18.0
103	100	60.0
101	5.3	11.8
95	13.6	29.0
93	8.3	16.2
91	5.7	13.8
81	9.0	21.0
79	17.2	31.5
75	18.9	16.0
73	55.0	98.8
67	14.1	29
55	9.0	14.6

Table 1.6 GC-MS of ME-MO-TMS Derivatives for the Major and Minor Components from *Actias pernyi*

m/z 498 → m/z 397 indicative of the 2 series prostaglandins. The m/z 73 ion is again not the base peak and the m/z 75 ion is not observed. The highest mass detected is at m/z 498 and is too low for a prostaglandin molecular ion, but it shows the loss of m/z 15 with an ion at m/z 483 (30%).

The general mass spectral characteristics of these two components are not those expected for a prostaglandin or prostaglandin analogue.

1.4 Analysis of *Actias perneyi* Haemolymph

1.4.1 Gas Chromatographic Studies

Extracts of the haemolymph from 50 *Actias perneyi* larvae were made and derivatized as above. Gas chromatography again showed two components in the prostaglandin region (Figure 1.9). Co-injection studies again indicated that the observed components were different to a number of authentic prostaglandin derivatives. The combined levels of these components in the haemolymph was estimated at 20ng per larvae.

A gas chromatographic study of various derivatives of the haemolymph was carried out to deduce the functional groups present. Omission of either the esterification or oximation step gave the MO-TMS and ME-TMS derivatives respectively. Analysis by GC of both these derivatives showed two components with similar retention times to the ME-MO-TMS derivative. However, failure to include the trimethylsilylation step (ME-MO) gave no peaks by GC. The use of diazoethane to give the Et-MO-TMS derivative was also carried out and this gave two peaks by GC with identical retention times to the ME-MO-TMS derivative.

1.4.2 GC-MS of ME-MO-TMS and Et-MO-TMS Derivatives

The above extracts were analysed by GC-MS of the ME-MO-TMS and Et-MO-TMS derivatives. The spectral details were identical and, therefore, only those for the ME-MO-TMS case are given (Table 1.6, Figures 1.10,1.11).

The mass spectrum of the major component (a) shows a base peak at m/z 103 and again this is indicative of a primary trimethylsilyl ether group (Figure 1.10). The high intensity of the m/z 129 ion (65%) indicates the possibility of an allylic trimethylsilyl ether. However, there is again an absence of ions at m/z 199 and 173 and therefore this

Figure 1.9: Gas Chromatogram of the ME-MO-TMS Derivatised Extract of Haemolymph from 50 *Actias permeyi* Larvae

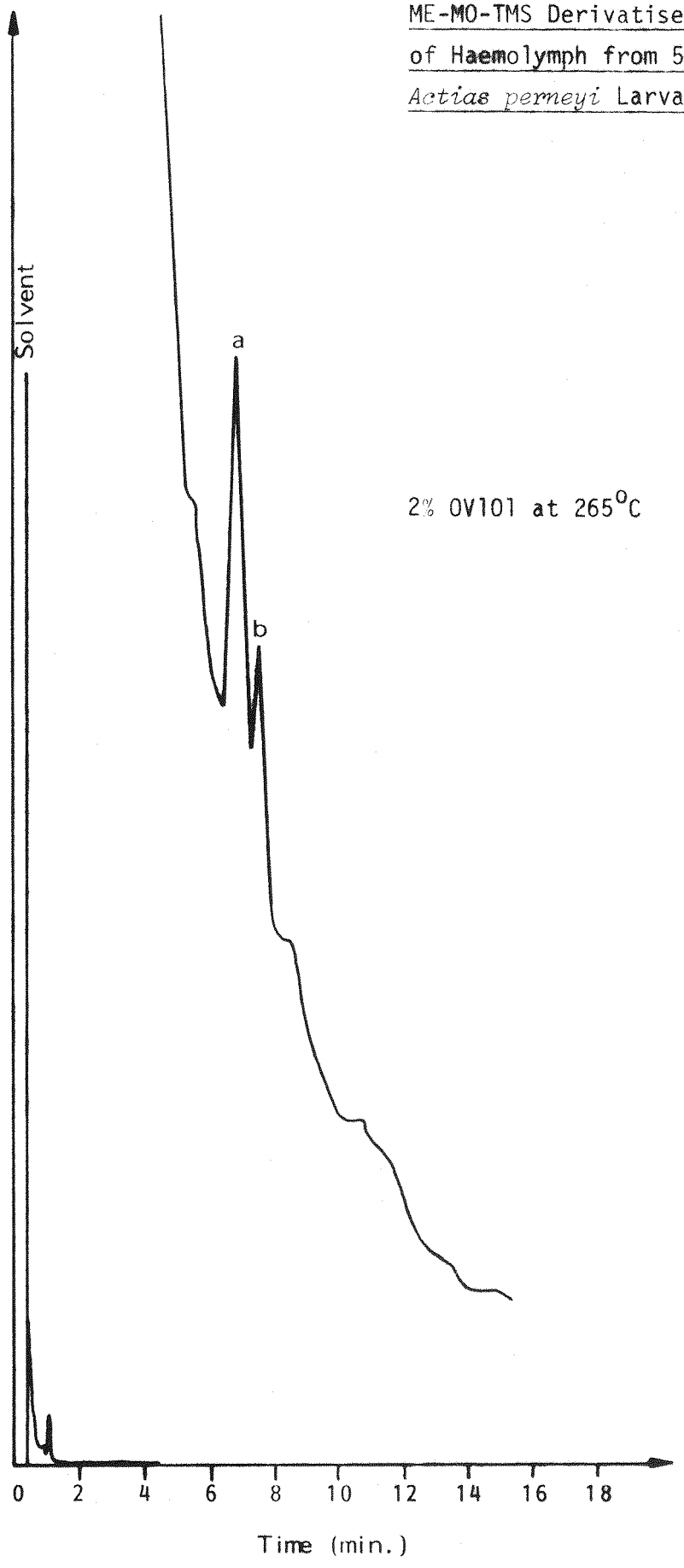


Figure 1.10: Mass Spectrum of ME-MO-TMS Derivative of the Major Component (a) from an extract of Haemolymph from 50 *Actias permeyi* Larvae

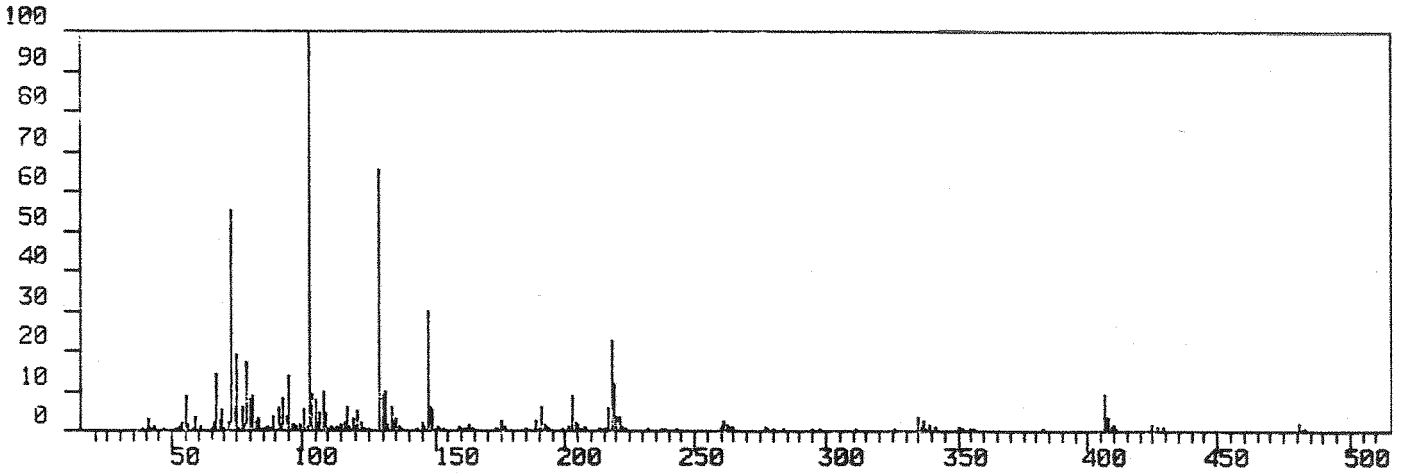
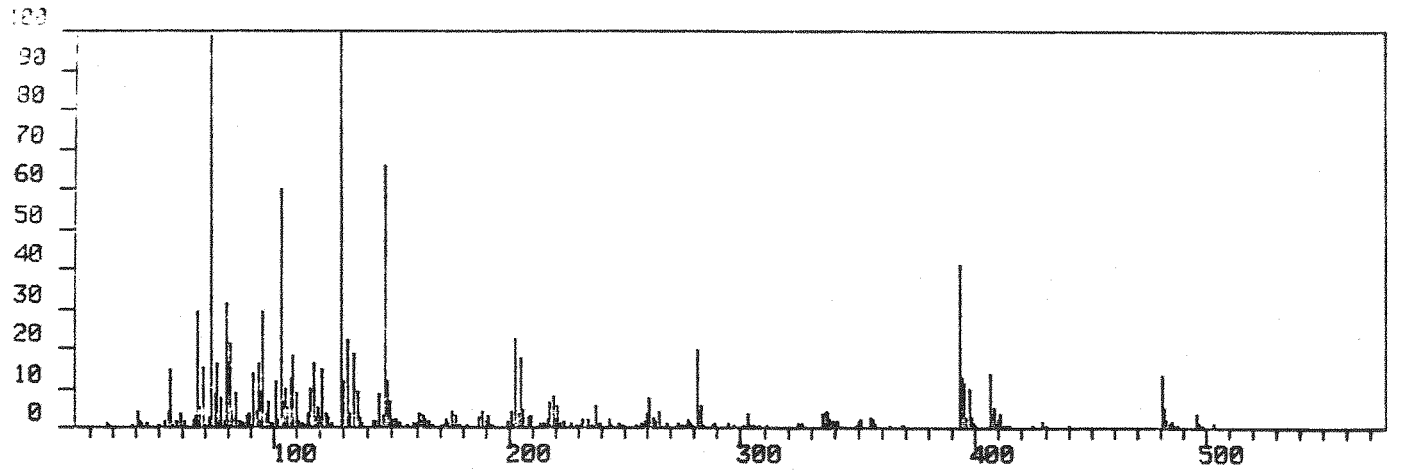


Figure 1.11: Mass Spectrum of ME-MO-TMS Derivative of the Minor Component (b) from an extract of Haemolymph from 50 *Actias permeyi* Larvae



component lacks the C13-C20 side chain of standard prostaglandins. The presence of several trimethylsilyl ether groups is indicated by the ion at m/z 147 (30%). The heaviest ion observed at m/z 496 (0.2%) is not necessarily the molecular ion. This ion, however, shows the fragmentations; M-15 (m/z 481 (2%)), M-71 (m/z 425 (1.6%)), M-90 (m/z 406 (9.2%)) and M-90-71 (m/z 335 (3.7%)).

The minor component (b) shows a base peak at m/z 129 with intense ions at m/z 147 (66%) and m/z 103 (60%) (Figure 1.11). The heaviest mass observed is at m/z 498 (0.8%). However, the ion at m/z 496 (3.5%) shows the fragmentations: M-15 (m/z 481 (13%)), M-71 (m/z 425 (0.8%)), M-90 (m/z 406 (13.6%)), M-103 (m/z 393 (41%)) and M-90-71 (m/z 335 (3.7%)). The loss of m/z 101 (cf 2 series prostaglandins) is observed in the sequence m/z 496 → m/z 395.

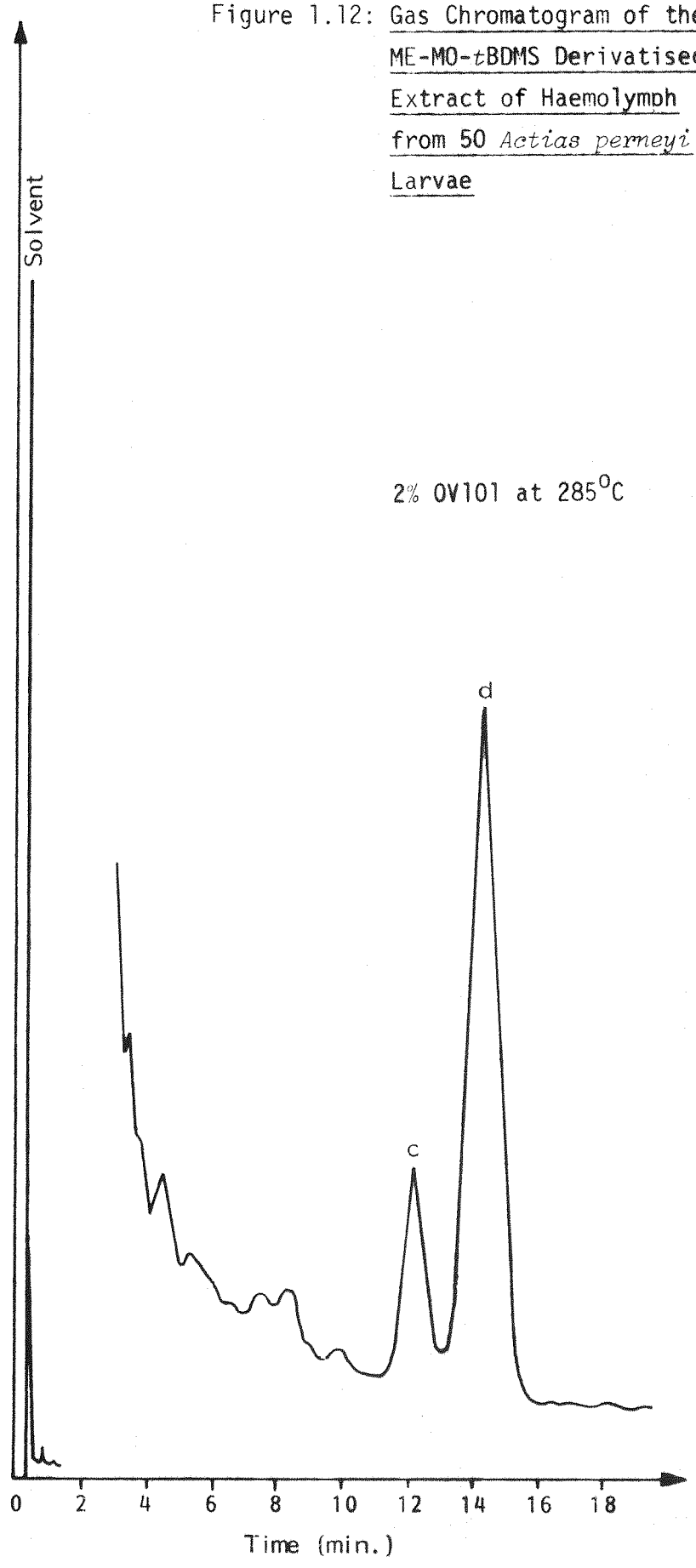
The general mass spectral characteristics of these two components are similar to each other and to the corresponding *Acheta domesticus* components.

1.4.3 GC and GC-MS Studies on ME-MO-*t*BDMS Derivative

Protection of the hydroxy groups as the *t*-butyldimethylsilyl ethers was carried out on a haemolymph extract of 50 *Actias perneyi* larvae to give the ME-MO-*t*BDMS derivative. The derivatized extract again showed two components in the prostaglandin region by GC (see Figure 1.12). In contrast to the corresponding trimethylsilyl ethers, the minor component (c) had a shorter retention time than the major component (d). Co-injection studies indicated that these components were different to a number of derivatized prostaglandin standards.

This derivatized extract was examined by GC-MS which showed that the two components had similar mass spectral characteristics (Table 1.7, Figures 1.13, 1.14). The highest mass observed is m/z 614 (cf the trimethylsilyl ethers of m/z 496) which shows the fragmentation M-15 (m/z 599). There is no evidence for the loss of m/z 57 which is the reported major fragmentation for *t*-butyldimethylsilyl ethers.[48] The loss of m/z (57+132) and m/z (57+2x132) would also be expected for these derivatives but these fragmentations are not observed. There exists, however, the possibility that the molecular ion is not observed and hence these fragmentations go undetected. The base peak in both these

Figure 1.12: Gas Chromatogram of the ME-MO-*t*BDMS Derivatised Extract of Haemolymph from 50 *Actias perneyi* Larvae



m/z	Intensity (%)	
	Minor (Peak c)	Major (Peak d)
614	0.12	1.3
599	0.5	-
573	0.9	10
572	1.0	18
571	2.3	38
384	2	8
383	7	28
327	-	5
313	4	2
303	1.7	7
171	-	12
147	20	20
133	-	12
115	13	15
101	27	-
89	22	22
88	40	-
83	13	-
75	37	35
73	100	100
69	17	-
67	13	-
59	10	14
57	28	11
56	-	20
55	29	-
43	33	-
41	-	50

Table 1.7 GC-MS of ME-MO-*t*BDMS Derivatives for Major and Minor Components from *Actias perneyi*.

Figure 1.13: Mass Spectrum of ME-MO-*t*BDMS Derivative of the Minor Component (c) from an extract of Haemolymph from 50 *Actias permeyi* Larvae

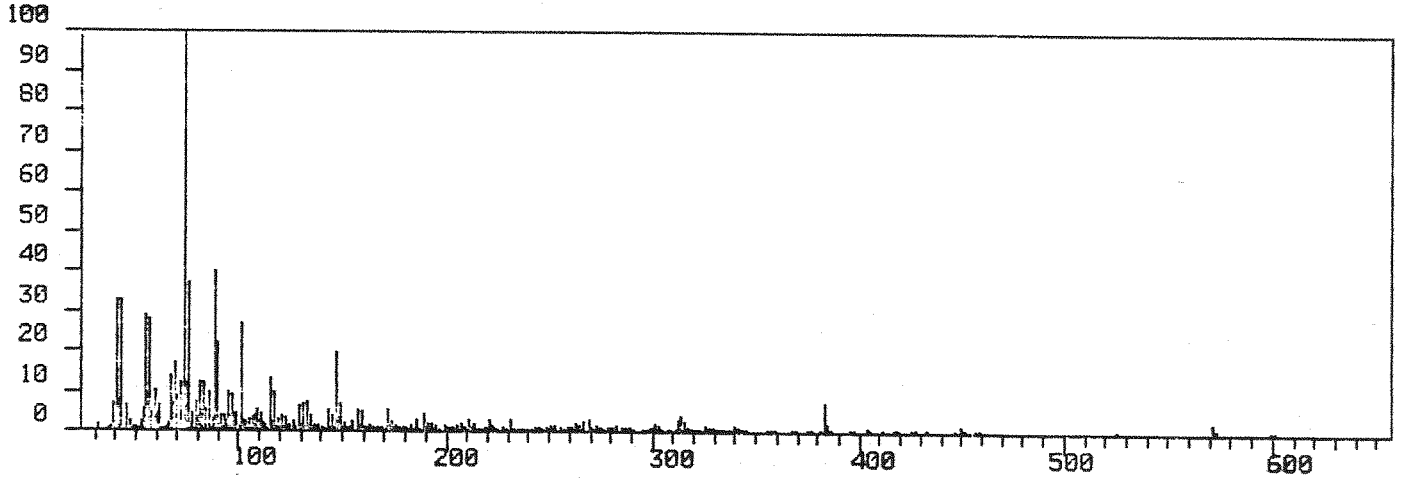
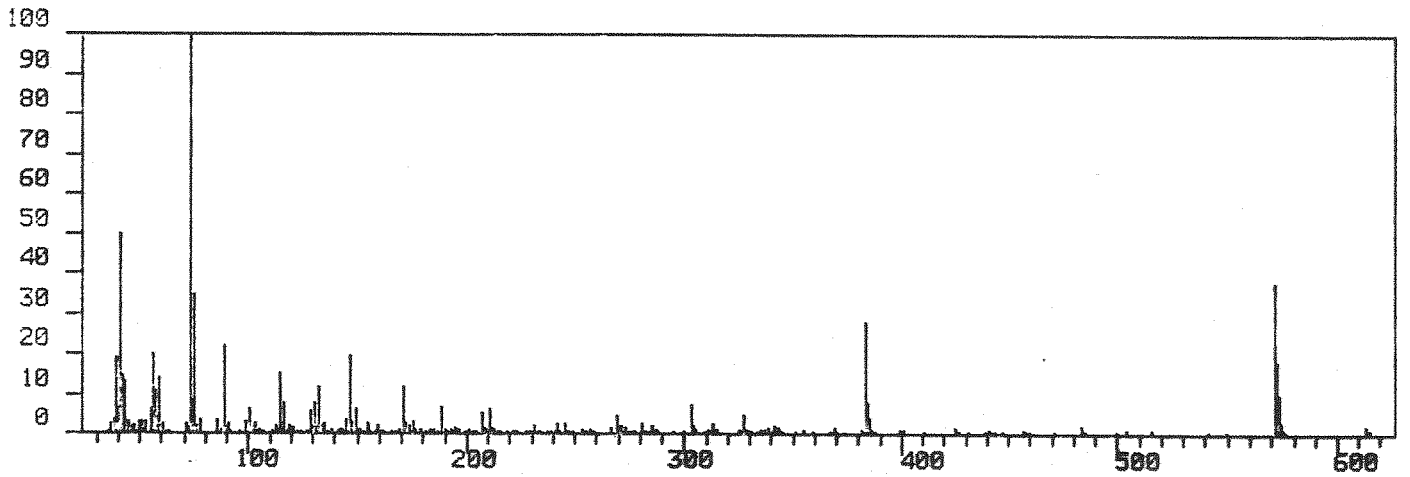


Figure 1.14: Mass Spectrum of ME-MO-*t*BDMS Derivative of the Major Component (d) from an extract of Haemolymph from 50 *Actias permeyi* Larvae



spectra is surprisingly m/z 73 and not the expected m/z 115 for the *t*-butyldimethylsilyl cation. In the trimethylsilyl derivatives a prominent ion at m/z 103 was observed. However, there is no corresponding ion at m/z 145 which might be expected for the *t*-butyldimethylsilyl derivative.

1.5 Discussion

The derivatisation work on extracts of *Actias perneyi* haemolymph indicated that there was no ketone or free carboxylic acid groups present in these compounds. The absence of a carboxyl group was also indicated by the identical mass spectral characteristics for both the ME-MO-TMS and Et-MO-TMS derivatives. However, the possibility that an ester moiety already exists in these compounds prior to derivatization cannot be discounted. There was strong evidence for the presence of hydroxy groups, since omission of the silylation step gave no peaks by GC.

The mass spectral data for all these components shows that they are non-prostanoid since they do not give the expected fragmentation patterns. There is, in addition, no evidence for the presence of a C13-C20 prostaglandin alkyl chain due to the absence of the ions at m/z 199 and 173. In all cases the highest observed mass is too low for a prostanoid, although it seems likely that the molecular ion is not detected. This was the case in the mass spectra of the standard prostaglandin derivatives where the molecular ions were weak (<0.25%) or not observed. As a result of the absence of the molecular ion no indication of the molecular formula by high resolution mass spectra was possible.

The fragmentation patterns of these compounds indicate the presence of several trimethylsilyl ether groups as shown by the loss of m/z 90 (trimethylsilanol) and by the presence of the m/z 147 ion. The high intensity of the m/z 103 ion strongly suggests that one of the trimethylsilyl ether groups is primary, while the ion at m/z 129 indicates that another may be allylic.

It is clear that a full structure is unlikely to be deduced from mass spectral data alone, and, therefore, an NMR study of these components would be invaluable. However, NMR has the distinct disadvantage over GC-MS in that relatively larger amounts of materials are required (ca. 1.5μg). An NMR study, therefore, requires the availa-

bility of large quantities of these compounds. A large scale extraction programme was undertaken to provide sufficient quantities of these components for an NMR study. However, due to problems with insect supplies, only 1,456 larvae have been extracted to-date (3,000 required).

Once the large scale extraction of haemolymph is completed a method of purification will be required to provide pure samples of the two components. HPLC seems to be uniquely suited to the problems involved from the viewpoint of high resolution, good sample recovery and short purification times. In addition, it is not necessary to derivatise prostaglandins due to the recent availability of variable wavelength detectors which are relatively sensitive (ng).[62,63] As a result of these factors, HPLC is becoming increasingly popular for the separation and identification of prostaglandins and related arachidonic acid metabolites.[30,31,64,65]

Completion of the large scale extraction and subsequent HPLC purification is required to provide large amounts of material for NMR analysis. Chemical ionization mass spectroscopy would also provide invaluable information on the molecular ions of the purified components. In addition, microscale reactions may provide useful structural evidence. Catalytic hydrogenation, ozonolysis and methoxymercuration-demercuration would provide information on the presence, number and position of double bonds.[47,66] Reduction with lithium aluminium hydride would also be useful as this would indicate the presence and number of ester groups.

1.6 Conclusion

Derivatised extracts of the guts from male *Acheta domesticus* and of the haemolymph from *Actias perneyi* larvae were found to contain two components with gas chromatographic properties similar to those of authentic prostaglandin derivatives.

Gas chromatography-mass spectroscopic analysis showed that these components had different spectral characteristics to standard prostaglandin derivatives. There was no evidence for the presence of a molecular ion and hence no postulation of the molecular formula could be made. However, the spectral data of the derivatives indicated that these components appear to have several trimethylsilyl ether groups,

one of which may be primary and another allylic. The absence of a ketone functionality and carboxyl group was shown by derivatisation and GC-MS studies.

Limited structural information was obtained by GC-MS of these derivatives, and it was concluded that larger quantities of these components would be needed for structural elucidation by NMR, in conjunction with chemical ionization mass spectrometry.

1.7 Experimental

1.7.1 Materials

The standard prostaglandins were kindly supplied by May and Baker Ltd., Dagenham, Essex.

All glassware was cleaned in chromic acid, then soaked in aqueous sodium bicarbonate, washed with distilled water and dried. Solvents were redistilled before use in all glass apparatus and their purity checked by GC.

Pentane (99%) was obtained from BDH Chemicals Ltd. and methanol (Gold Label) from Aldrich Chemical Company Ltd. The peroxides in diethyl ether (Koch-Light) were removed by washing twice with aqueous sodium metabisulphite (5%) and then twice with distilled water, followed by distillation from anhydrous calcium chloride, under nitrogen. Chloroform (Koch-Light) was passed down a column of basic alumina (Grade 1) and then distilled under nitrogen. Pyridine (BDH) was distilled from and stored over potassium hydroxide. Dimethylformamide (BDH) was stirred over calcium hydride and barium oxide and then fractionated with the first and last 20% being discarded.

Methoxyamine hydrochloride (Pierce Chemical Co.), bis(trimethylsilyl)acetamide (Aldrich Chemical Co.), imidazole (L. Light and Co. Ltd.) and *t*-butyldimethylchlorosilane (Aldrich Chemical Co.) were all used without further purification.

1.7.2 Gas Chromatography

Gas chromatography was conducted using a Pye 104 chromatograph fitted with a 10' x $\frac{1}{8}$" glass column containing 2% OV101 on diatomite CLQ (100-120 mesh). The carrier gas used was nitrogen at 25ml min^{-1} and the column temperature was maintained at 265°C or 285°C (for TMS and *t*BDMS derivatives respectively). The chromatograph was equipped with a flame ionization detector with a hydrogen and air pressure of 0.85kg cm^{-2} and 0.5kg cm^{-2} respectively.

1.7.3 Gas Chromatography-Mass Spectrometry

GC-MS were recorded on a Kratos MS-30 spectrometer connected to a Pye 204 chromatograph via an all glass jet separator at 350°C (electron energy 70eV, trap current 50μA, source temperature 250°C). The mass spectrometer data was analysed using a Nova-3 computer (Data General) equipped with a DS 50S data system (Kratos). The GC conditions were as described above except that the carrier gas was helium.

1.7.4 Tissue and Haemolymph Preparation

The insect tissue from *Acheta domesticus* was collected in a similar manner to that of Destephano *et al.*[28,29] Adult *A. domesticus* (Xenopus Ltd.) were sexed on arrival and were provided with water and bran pellets. The males were then dissected under iced Ringer solution (NaCl (208.6mmol l^{-1}), KCl (3.1mmol l^{-1}), $NaHCO_3$ (2mmol l^{-1}), $CaCl_2$ (5.4mmol l^{-1}) at pH7.0) and the guts (minus the crop and accessory glands) and testes were quickly removed and stored separately in methanol under nitrogen at -20°C.

A culture of *Actias permeyi* was reared from the first and second instar to the final larval instar and then sacrificed. The haemolymph was removed into an equal volume of ethanol and was stored under nitrogen at -20°C.

1.7.5 Extraction of Insect Tissue and Larval Haemolymph

Endogenous prostaglandins from insect tissue and haemolymph were extracted by a modification of the method described by Jouvenaz *et al.*[45]

To the insect tissue or body fluid in alcohol was added aqueous citric acid (1ml, 1M, pH5.5). The resulting sample was homogenized by maceration and ultrasonification. The precipitate was collected by centrifugation and then washed twice with an equal volume of ether and methanol (1:1). The combined extracts were concentrated to an aqueous acid solution (ca. 1ml) and then extracted with six equal volumes of pentane to remove inactive lipids. The resulting aqueous residue was extracted with three equal volumes of ether and chloroform (4:1). The combined ether-chloroform extracts were then blown to dryness by a rapid nitrogen stream.

1.7.6 Derivatisation Method

The derivatisation of prostaglandins to give the methyl ester, methoxime, trimethylsilyl ether derivative (PG-ME-MO-TMS) was based on the methods of Green[42] and Maclouf *et al.*[53] To the dry extract from above or the standard prostaglandin (100µg) was added an excess of diazomethane in ether prepared according to Fales *et al.*[67] Excess reagent and solvent was removed rapidly after two minutes with a stream of nitrogen. The resulting sample was treated with 50µl of a solution of methoxyamine hydrochloride (25mg) in pyridine (0.5ml) and left overnight at room temperature. The pyridine was removed by a stream of nitrogen and the residue was treated with bis(trimethylsilyl)acetamide (50µl) at room temperature for one hour. Excess reagent was removed by a nitrogen stream and the residue was taken up into pentane before injection into the gas chromatograph.

The use of the *t*-butyldimethylsilyl ether protection was based on the methods reported for the F series.[48,49] To the dry methyl ester of the prostaglandin (500µg) or extract prepared as above was added a solution of methoxyamine hydrochloride in pyridine (250µl, 25mg/500µl). The mixture was left overnight at room temperature then the pyridine was removed by a stream of nitrogen. To the residue was added a solution of imidazole in dimethylformamide (500µl, 2.5M) followed by a solution of *t*-butyldimethylchlorosilane in dimethylformamide (500µl, 1M). This mixture was left at room temperature overnight in a desiccator whereupon water (2ml) was added and the mixture was extracted three times with pentane (2ml). The resulting organic layer was concentrated and then injected into the gas chromatograph for analysis.

An investigation into the *t*-butyldimethylsilisation of prostaglandins was carried out in some pilot work. These studies showed that the use of pyridine as the solvent in the silylation gave low yields of derivatised products ($PGF_{2\alpha}$-ME-*t*BDMS (65%)). In contrast the use of dimethylformamide as solvent gave excellent yields of these derivatives (PGE_2 ME-MO-*t*BDMS (84%), $PGF_{1\alpha}$ ME-*t*BDMS (94%)).

In the derivatisation of the F series the oximation step was omitted to give the PG-ME-TMS or PG-ME-*t*BDMS derivatives.

1.7.7 Control

A blank extraction and derivatisation (in the absence of insect tissue or body fluid) was carried out to ensure that artifacts were not present. Analysis of the derivatised blank extract showed no interfering peaks by GC. The possibility of syringe artifacts was discounted by injection of a solvent blank prior to the GC analysis of insect extracts.

The efficiency of the extraction procedure was determined by the extraction and derivatisation of $PGF_{2\alpha}$ (10) in the absence of a biological matrix. The results showed that 90% ± 5% recovery of $PGF_{2\alpha}$-ME-TMS could be achieved with less than 0.5% in the pentane washes.

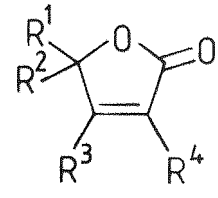
CHAPTER TWO

Model Studies on the Paniculides

2.1 INTRODUCTION

2.1.1 The $\Delta^{\alpha,\beta}$-Butenolides (2(5H)-Furanones)

Compounds that contain the $\Delta^{\alpha,\beta}$-butenolide moiety (2(5H)-furanones) (16) occur widely in nature[68,69,70] and possess a wide range of biological activities.[71,72] Such compounds appear throughout the plant kingdom from the simple metabolites of lichens, mold and fungi[73] to the more complex terpenoids of the families Compositae and Labiatae[70] through to the steroidal glycosides of the familes Ranunculaceae, Liliaceae, Scrophulariaceae and Apocyanaceae.[74] In the animal world, $\Delta^{\alpha,\beta}$-butenolides have been observed in such diverse species as sponges,[75-78] coral,[79-81] butterflies[82-84] and insects.[85] In the latter two families they appear to play a significant rôle as chemical defence weapons. The $\Delta^{\alpha,\beta}$-butenolides are found to have plant growth inhibitory properties,[71,86,87] insect antifeedant and insecticidal activity.[88] They also have antifungal[89,90] and widespread antibacterial activity.[91,92] Tremendous interest has also been shown in the cardiac glycosides (cardenolides) which have the remarkable ability to reduce the frequency, but increase the amplitude of the heart beat.[74] An increasing number of $\Delta^{\alpha,\beta}$-butenolides exhibit cytotoxic and/or tumour inhibitory properties towards cancers,[93,95] although in some cases they are carcinogenic.[96,97]



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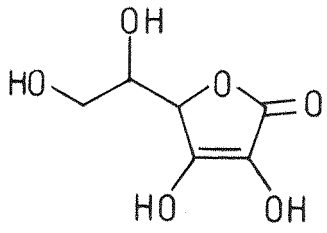
2.1.2 Biologically Active $\Delta^{\alpha,\beta}$-Butenolides

2.1.2.1 Structurally Simple $\Delta^{\alpha,\beta}$-Butenolides

The simple $\Delta^{\alpha,\beta}$-butenolides in general possess both antibacterial[71] and carcinogenic activity.[96,97] Vitamin C (17) is undoubtedly the most physiologically active of the simple $\Delta^{\alpha,\beta}$-butenolides and it has been established that the activity is due to the unsaturated lactone system.[98] The antibiotic and vesicant agent present in extracts of buttercups and many species of *Ranunculus*, *Anemone* and *Clematis* has been shown to be



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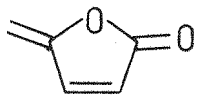

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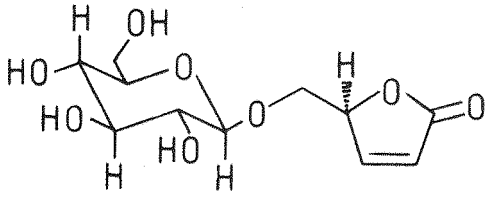

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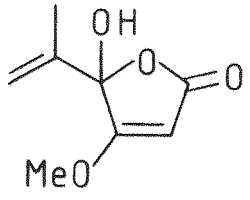

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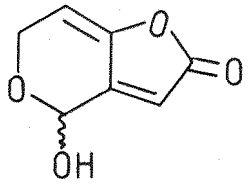

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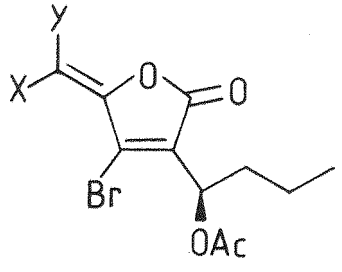


(22a) X=H, Y=Br
(22b) X=Br, Y=H
(22c) X=I, Y=H
(22d) X=H, Y=I
(22e) X=H, Y=Cl
(22f) X=Br, Y=Br

the lactone protoanemonin (18).[99] This compound exists in the buttercup as the glycoside, ranunculin (19) from which protoanemonin (18) and glucose are obtained either by crushing the plant or by alkaline hydrolysis.[100] Penicillic acid (20) is another structurally simple $\Delta^{\alpha,\beta}$-butenolide with both antibacterial and carcinogenic activity.[101] It is found in many *Penicillium* species but is less active than penicillin to Gram-positive bacteria while it exhibits greater activity against Gram-negative bacteria. A number of species of *Penicillium* and *Aspergillus* have yielded the antibiotic and fungistatic patulin (21), which has been isolated under the additional names claviformin, clavacin, clavatin or expansin.[71] More recently a series of six closely related antimicrobial C_9 halobutenolides (22a-f) have been found in the red seaweed *Delisea fimbriata*.[102]

2.1.2.2 Sesquiterpene $\Delta^{\alpha,\beta}$-Butenolides

Significant biological activity in certain highly oxygenated sesquiterpenes has been uncovered. The structural diversity of these materials is renowned, but Jacobi *et al.* have considered the subtle features which are held in common by these sesquiterpenes.[103] Namely,

(i) They either contain a furan ring or a functionality in principle derivable from a furan.

(ii) The more biologically active compounds contain an oxygen function adjacent to the furan or lactone ring juncture.

(iii) Most of the stereochemically interesting features are contained about the periphery of a single ring.

These points would suggest that $\Delta^{\alpha,\beta}$-butenolides may possess biological activity since this functionality is derivable from a furan.

An increasing number of sesquiterpene $\Delta^{\alpha,\beta}$-butenolides have been found in a variety of sources,[69,70] however, the small amounts of material isolated has, in the majority of cases, prohibited their biological testing. A comprehensive review of those sesquiterpene $\Delta^{\alpha,\beta}$-butenolides without any biological activity is beyond the scope of this work due to the large numbers involved.

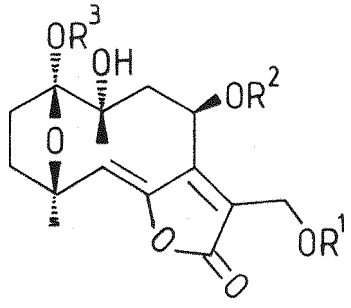
The sesquiterpene $\Delta^{\alpha,\beta}$-butenolides which have biological activity and satisfy the above empirical conditions include piptocarphins A-F (23a-f) which were isolated from cytotoxic extracts of *Piptocarpha chontalensis*.[104] These structurally related germacranolides all exhibited cytotoxic activity against the 9kB human nasopharynx carcinoma cells. Piptocarphins A (23a) and C (23c) also showed weak activity in the P388 lymphoid leukemia system.

An investigation of the roots and aerial parts of *Chloranthus japonicus* which are used as antifungal and antiinflammatory agents in Chinese medicine has led to the isolation of six moderately cytotoxic sesquiterpenes, chloranthalactones A-E (24a-e) and atractylenolide III (25).[105] In addition, chloranthalactone A (24a) has been shown to have remarkable antifungal activity[106] and atractylenolide III (25) has been reported to show antiinflammatory effects.[107]

Methanolic extracts of *Elephantopus scaber* have been shown to exhibit antitumour activity. In addition to lupeol and stigmasterol these extracts were found to contain 11,13-dihydrodeoxyelephantopin (26), a new sesquiterpene lactone, which was postulated to be responsible for the antitumour activity of the extract.[108] Elephantin (27a) and elephantopin (27b) two cytotoxic sesquiterpenes structurally related to (26) have been found in dried extracts of the plant *Elephantopus elatus*.[109] These sesquiterpenes also showed significant tumour inhibitory activity against the Walker 256 intramuscular carcinoma in rats. It should be noted, however, that elephantin (27a) and elephantopin (27b) contain an α-methylene-γ-butyrolactone moiety which has been shown to be responsible for the cytotoxic activity in a number of sesquiterpenes.[110]

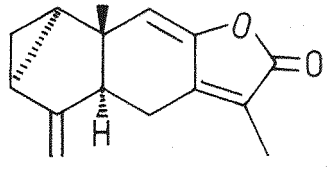
2.1.2.3 Diterpene $\Delta^{\alpha,\beta}$-Butenolides

A large number of naturally occurring diterpene $\Delta^{\alpha,\beta}$-butenolides have been isolated,[69,70] but only those which show significant biological activity will be discussed. Three structurally related diterpene triepoxides, triptolide (28a), tripdiolide (28b) and triptonide (28c) were isolated from cytotoxic extracts of *Tripterygium wilfordii*. All these compounds showed cytotoxic activity against KB cell cultures. In addition, triptolide (28a) and tripdiolide (28b) showed significant antileukemic activity against the L1210 and P388 leukemias in mice.[111] Another epoxy diterpene, jolkinolide B (29) was isolated from *Euphorbia jolkini* and

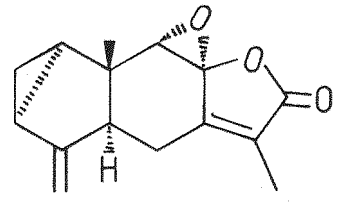


(23a-f)

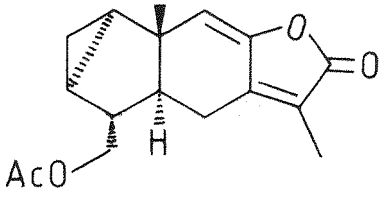
	R^1	R^2	R^3
(23a)	Ac	Meth	H
(23b)	Ac	Tig	H
(23c)	H	Meth	H
(23d)	Ac	H	H
(23e)	Ac	Meth	Et
(23f)	Et	Meth	H



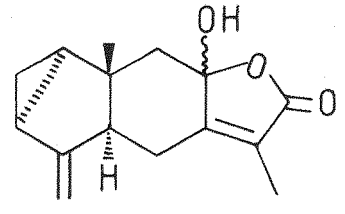
(24a)



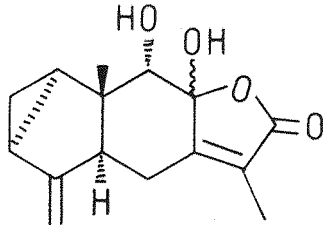
(24b)



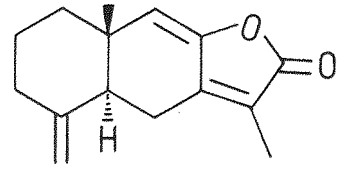
(24c)



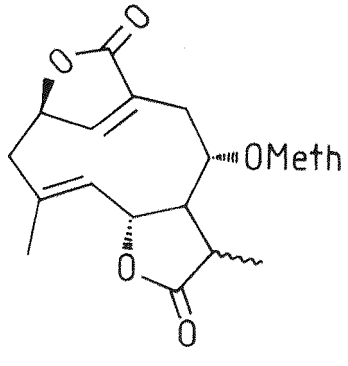
(24d)



(24e)



(25)



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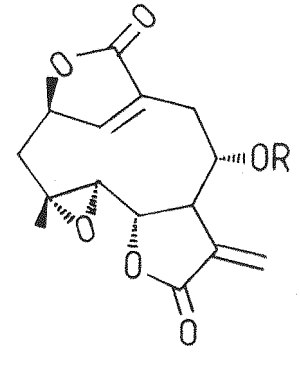
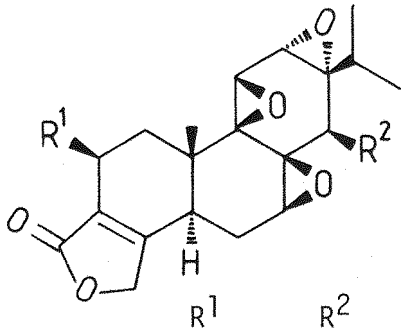
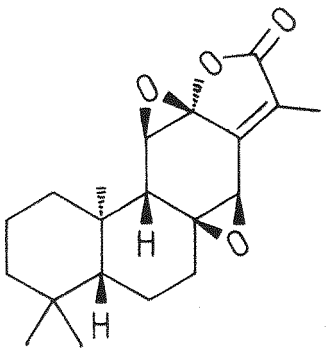


(27a) R = Sen

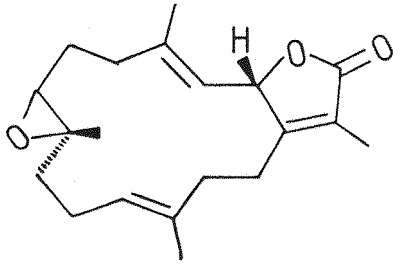
(27b) R = Meth



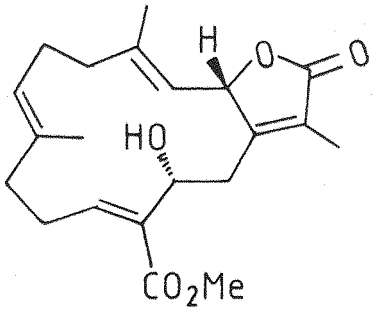
	R^1	R^2
(28a)	H	OH
(28b)	OH	OH
(28c)	H	=O



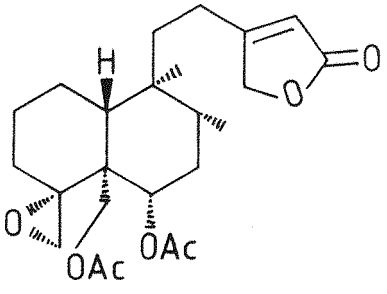
(29)



(30)



(31)



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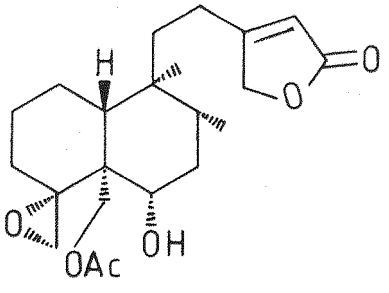

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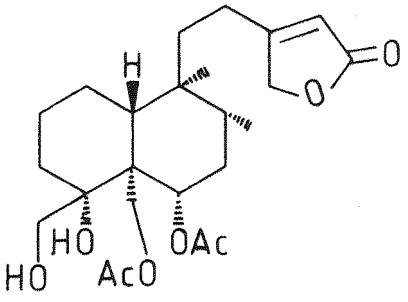
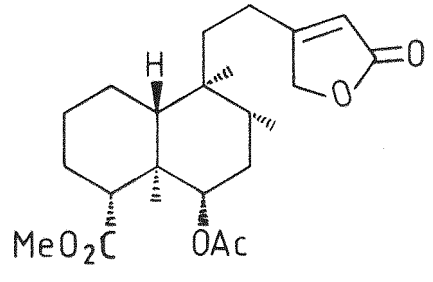


(34)



(35)

this showed growth inhibition of cultured Hela cells.[112]

The soft bodied coral *Sarcophytum glaucum* has been found to contain the cembranolide, sarcophine (30) which is believed to be one of the repellants, protecting the coral against predators.[80] Recently a new cembranolide (31) was isolated from *Sarcophytum* species. This compound was found to be reponsible for the central nervous system effects demonstrated by the crude extract which stimulated transient convulsant responses in mice.[79]

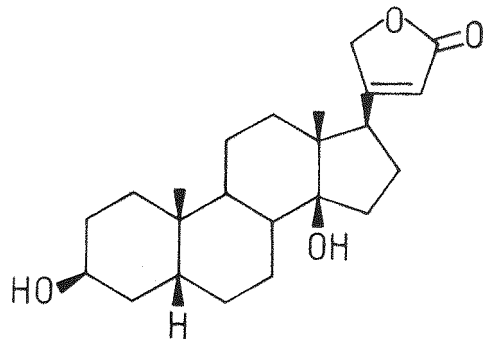
The observation that leaves of *Ajuga remota* are not attacked by African armyworms *Spodoptera exigua* has led to the isolation of three moderately strong insect antifeedants, ajugarin I (32), II (33) and III (34).[113] A fourth neo-clerodane diterpene ajugarin IV (35) has also been isolated recently from *Ajuga remota*. In contrast to ajugarins I-III (32-34), ajugarin IV (35) exhibited insecticidal activity against the silkworm *Bombyx mori* and growth inhibitory activity against pink bollworm *Pectinophora gossypiella*.[114]

2.1.2.4 Cardenolides (Cardiac Glycosides)

With the exception of the squill and toad venoms and a few other substances of alkaloid nature, the cardiac drugs are all non-nitrogeneous glycosides (cardenolides).[71,74] Hydrolytic or enzymic cleavage of the sugar residue yields the genins or aglycones, which are steroid derivatives. The cardenolides of medical importance are obtained chiefly from the foxgloves (*Digitalis*), but many plants of the families Ranunculaceae, Liliceae, Scropulariacea and Apocyanaceae also contain these compounds. The crude extracts of these plants are mixtures of many closely related cardenolides in which one or more aglycone may be associated with several different sugars.

The cardenolides, typified by the aglycone digitoxigenin (36) are steroids carrying a $\Delta^{\alpha,\beta}$-butenolide ring at the 17 position, nearly always with the β-orientation. This unsaturated lactone is considered essential for activity since reduction or ring opening reduces activity. There is a wide variation in the oxidation pattern around the steroid nucleus, but the tertiary hydroxy group at C14 is also essential for activity. Apart from D-glucose and L-rhamnose, the sugars attached to the steroid nucleus are rather uncommon and are either deoxy or dideoxy

sugars.



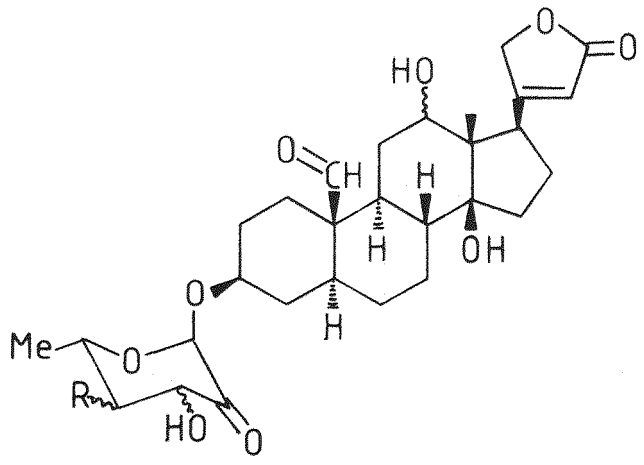
(36)

The physiological activity of the cardenolides, resides chiefly with the aglycone, although the sugar moiety has a definite significance. The sugars are thought to control water solubility, cell penetration and the persistance of the cardiac action. Only the glycosides are important therapeutically as they reduce the frequency but increase the amplitude of the heart beat. The aglycones, however, are medically valueless as they are convulsive poisons.

Naturalists have observed for more than a century that the insect eating vertebrates, particularly birds, avoid eating butterflies of the family Danaidae. During their larval stages these butterflies sequester cardenolides from their food plants, milkweeds of the family Asclepiadaceae.[82-84] The cardenolides like most other drugs produce side effects and in addition to their cardiac activity they have the ability to activate the nerve centre in the brain that controls vomiting. As a consequence, danaine butterflies are poisonous to bird predators which rapidly learn to reject these species.

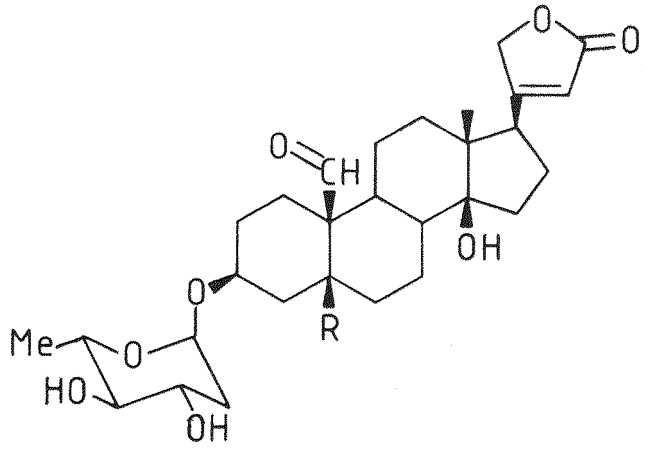
In a study of the monarch butterly *Danaus plexippus* which had been reared on the milkweed *Asclepias curassavica* it was found that both the host and butterfly contained at least three carednolides. These were shown to be calactin (37a), calotropin (37b) and calotoxin (37c).[82,83] Analogous cardiac poisons were also detected in *Danaus chrysippus* and the grasshopper *Paekilocerus bufonius*.[84]

In addition to the cardiac and emetic activities, a number of



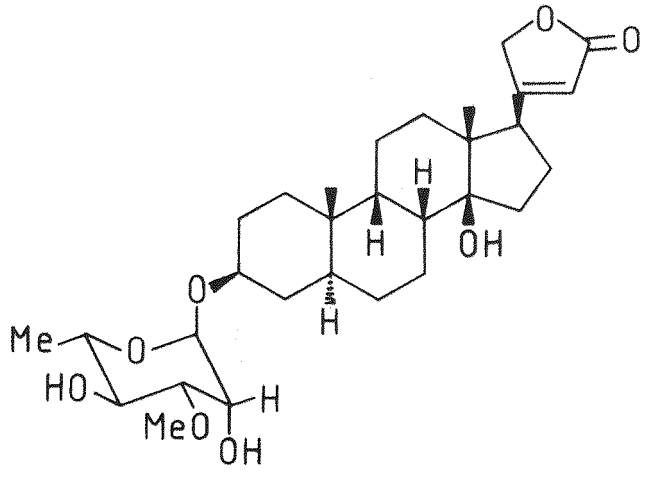
(37a) R = OH

(37b,c) R = H



(38a) R = H

(38b) R = OH



(39)

cardenolides have been found to have cytotoxic activities. Calotropin (37b) is one such compound which has been found to be the cytotoxic principle in extracts of *Asclepias curassavica*.[115] This plant is widely used in folk medicine for treating cancer and warts. Two similar cardenolides apocannoside (38a) and cymarin (38b) were isolated from *Apocynum cannabinum*. These two compounds were held responsible for the cytotoxicity of the plant extract which was sufficiently high to warrant the testing of these compounds in *in vivo* tumour systems.[116] More recently the new cardenolide 3'-O-methylevonoside (39) isolated from extracts of *Thevetia ahouia* has been shown to have strong cytotoxic activity against human epidermoid carcinoma of the nasopharynx.[117]

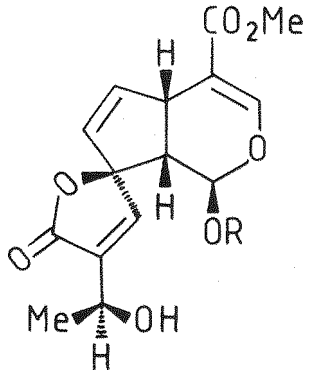
2.1.2.5 Other Biologically Active $\Delta^{\alpha,\beta}$-Butenolides

Plumieride (40a) has been isolated from extracts of *Aspergillus niger* and *Plumeria obtusifolia*.[86,87] This compound along with its hydrolysis product, plumieridine (40b), show plant growth inhibitory properties in the dwarf rice bioassay. It was concluded that the structural feature responsible for the observed inhibitory activity was the unsaturated lactone moiety.[86]

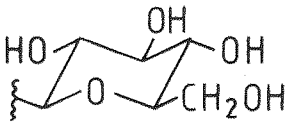
In a study of over 150 synthetic steroids for cytotoxic activity, the 25 most active compounds were all found to contain a $\Delta^{\alpha,\beta}$-butenolide ring fused to the 16,17-positions. Two of these lactones (41) and (42) were found to have very significant cytotoxic activity. In addition they showed antimicrobial activity and marginal *in vivo* antitumour activity against S-180 and T-4 lymphoma implanted in mice with a low animal toxicity.[118]

A group of five structurally related, angular azaphilones, the deflectins (43a-e), have been isolated from the mycelia of *Aspergillus deflectus*. Besides the inhibitory effects on the growth of bacteria and fungi, these compounds showed lytic activity towards bacteria and erythrocytes and cytotoxic activity towards cells of the Ehrlich carcinoma in mice.[119]

The antimicrobial sesterterpene palauolide (44) has been isolated from metholic extracts of a *Palauan* sponge. Palauolide (44) was shown to be responsible for the antimicrobial activity of the crude extract, the pure compound inhibited growth of *Bacillus subtilis* and *Staphyloccus aureus*.[190]

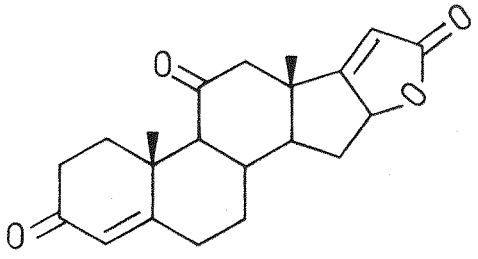


(40a) R=

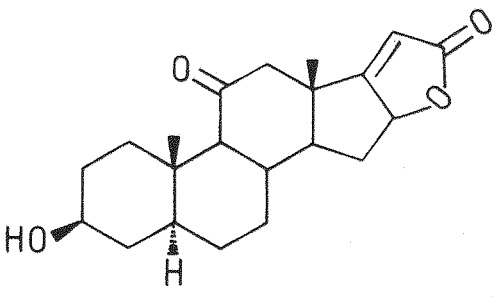


(40b) R=H

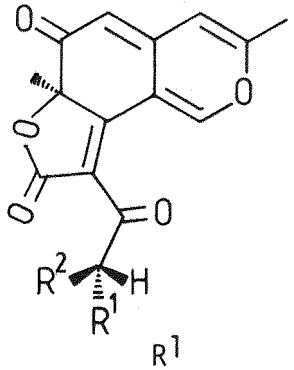
(40a,b)



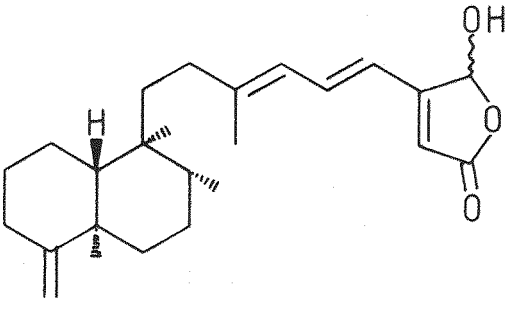
(41)



(42)



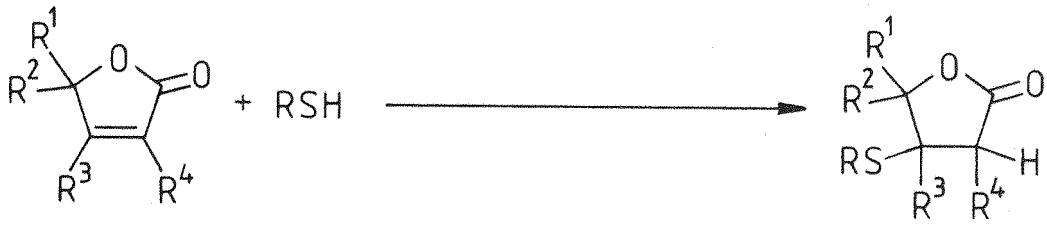
	R^1	R^2
(43a)	nC_6H_{13}	H
(43b)	nC_8H_{17}	H
(43c)	$nC_{10}H_{21}$	H
(43d)	nC_8H_{17}	Me
(43e)	$nC_{10}H_{21}$	Me



(44)

2.1.3 Mechanism of Antibiotic and Carcinogenic Activities

The antibiotic activity of $\Delta^{\alpha,\beta}$-butenolides has been explained by their ability to react rapidly with cysteine and also with certain enzymes containing thiol groups which are important for the normal function of micro-organisms.[98,121] In this context, it is envisaged that the thiol groups undergo Michael addition (1,4-conjugate addition) to the α,β-unsaturated system thus destroying an essential metabolite or interfering with an enzyme system (Scheme 2.1).[91,122]



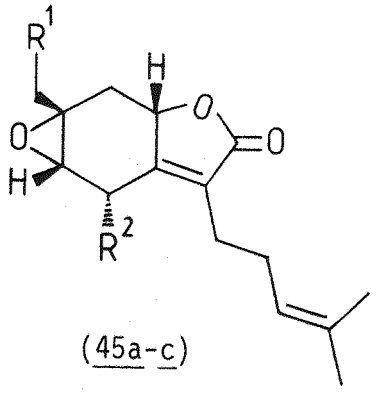
(16)

Scheme 2.1

It also seems possible that a similar reaction with a cell component or metabolite may also be concerned with the carcinogenic action of the $\Delta^{\alpha,\beta}$-butenolides.[96a,97]

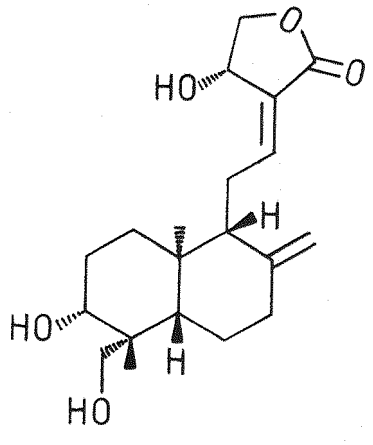
2.1.4. The Paniculides

Three highly oxygenated sesquiterpene lactones, paniculide A (45a), B (45b) and C (45c) were isolated in 1968 by Overton *et al.* from callus cultures derived from hypocotyl and stem tissues of *Andrographis paniculata* Nees (Acanthaceae).[123,124] Andrographolide (46), a major constituent of the whole plant could not be detected in this culture system. The structures of the paniculides (45a-c) were initially determined by elemental composition in conjunction with their spectral properties, most notably high field NMR data. More recently the absolute configuration of paniculide B (45b) has been determined by an X-ray crystal structure determination of the bis-*p*-bromobenzoate.[125]



(45a-c)

	R^1	R^2	Yield (mg/l)
(45a)	H	OH	3-5
(45b)	OH	OH	20-25
(45c)	OH	=O	8-12



(46)

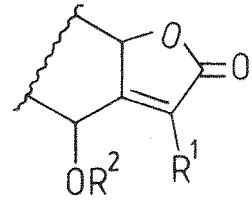
Insufficient quantities of the paniculides (45a-c) were available for biological testing. They contain, however, all three structural requirements proposed by Jacobi *et al.*[103] for biological activity in sesquiterpenes (see Section 2.1.2.2). The presence of the $\Delta^{\alpha,\beta}$-butenolide function in these compounds would suggest some biological action from the aforegoing discussion on the biological activity of this functionality.

The possible biological activity of these compounds, in addition to their novel structure, makes the paniculides (45a-c) an interesting and challenging synthetic proposition.

2.1.5 Approaches to the Carbocyclic Skeleton of the Paniculides

2.1.5.1 Synthesis of $\Delta^{\alpha,\beta}$-Butenolides

The wide range of biological activities of the $\Delta^{\alpha,\beta}$-butenolides has stimulated considerable research into the synthesis of these compounds.[98,126] However, few synthetic efforts to-date have been devoted to the construction of $\Delta^{\alpha,\beta}$-butenolides with an oxygenated centre adjacent to the ring juncture as shown in partial structure (47).[103,127-130]



(47)

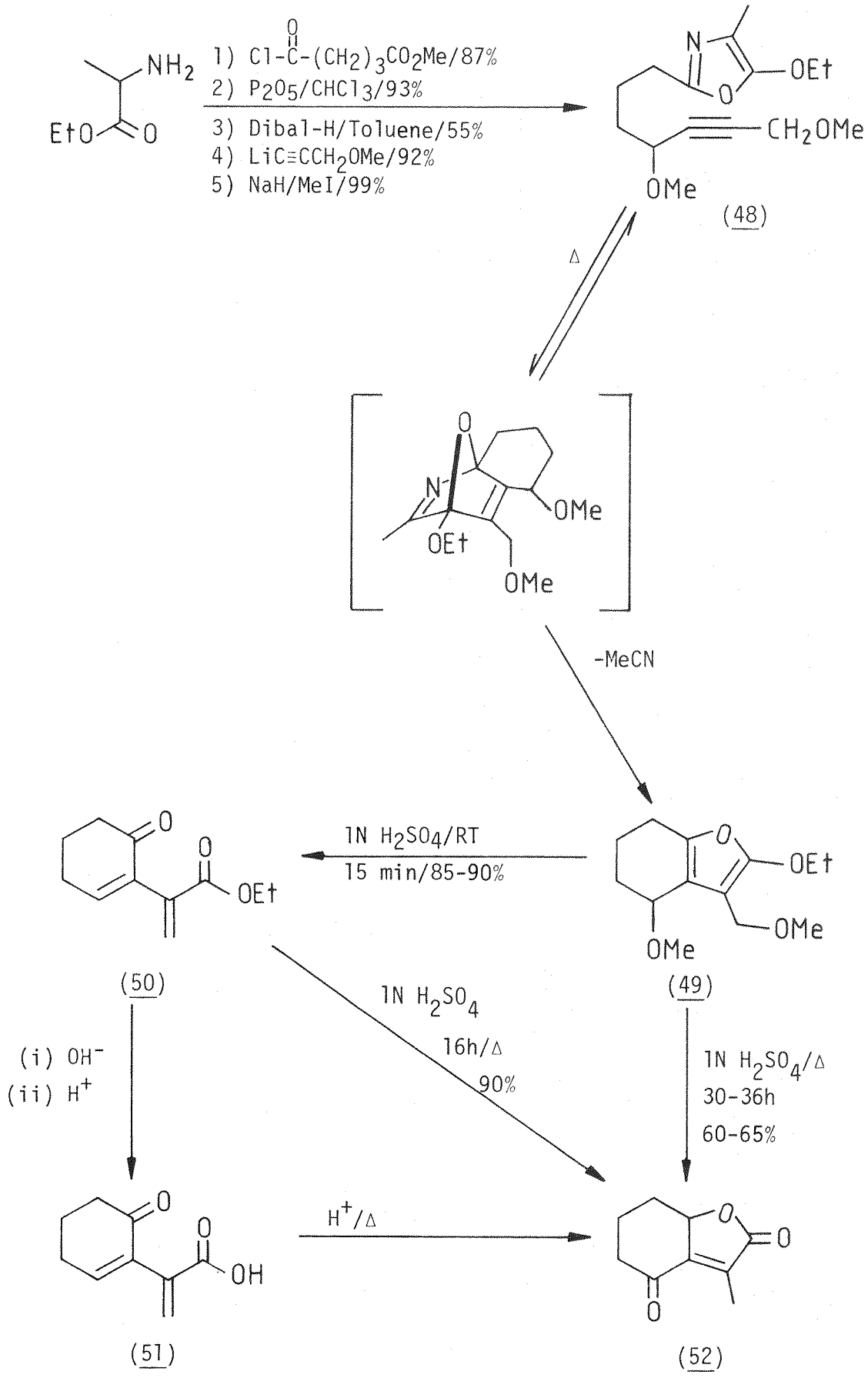
a) Bis-Heteroannulation

At the time of starting this work, there existed only one report directed towards the basic carbocyclic skeleton; this was the work of Jacobi *et al.*[103] This method involved the preparation of an acetylenic oxazole (48) which, on heating in ethylbenzene, underwent thermal rearrangement to give ethoxy furan (49). On treatment with mild aqueous acid (1N H_2SO_4) the ethoxy furan (49) gave the methylene ester (50). Heating the methylene ester (50) or the corresponding acid (51) in aqueous acid at reflux resulted in formation of the $\Delta^{\alpha,\beta}$-butenolide (52). Under more vigorous conditions, ethoxy furan (49) could be transformed directly to $\Delta^{\alpha,\beta}$-butenolide (52) (Scheme 2.2).

This approach was considered to be too lengthy and inapplicable with labile substituents to be applied to the synthesis of the paniculides (45a-c) particularly paniculide B (45b) and C (45c).

b) Oxidation of a Furan

A second approach to the basic carbocyclic skeleton which involved


1) Cl-C(=O)-(CH2)3CO2Me/87%
2) P2O5/CHCl3/93%
3) Dibal-H/Toluene/55%
4) LiC≡CCH2OMe/92%
5) NaH/MeI/99%
(48)
Δ
-MeCN
1N H2SO4/RT
15 min/85-90%
(49)
(50)
1N H2SO4
16h/Δ
90%
(i) OH⁻
(ii) H⁺
1N H2SO4/Δ
30-36h
60-65%
H⁺/Δ
(51)
(52)

Scheme 2.2

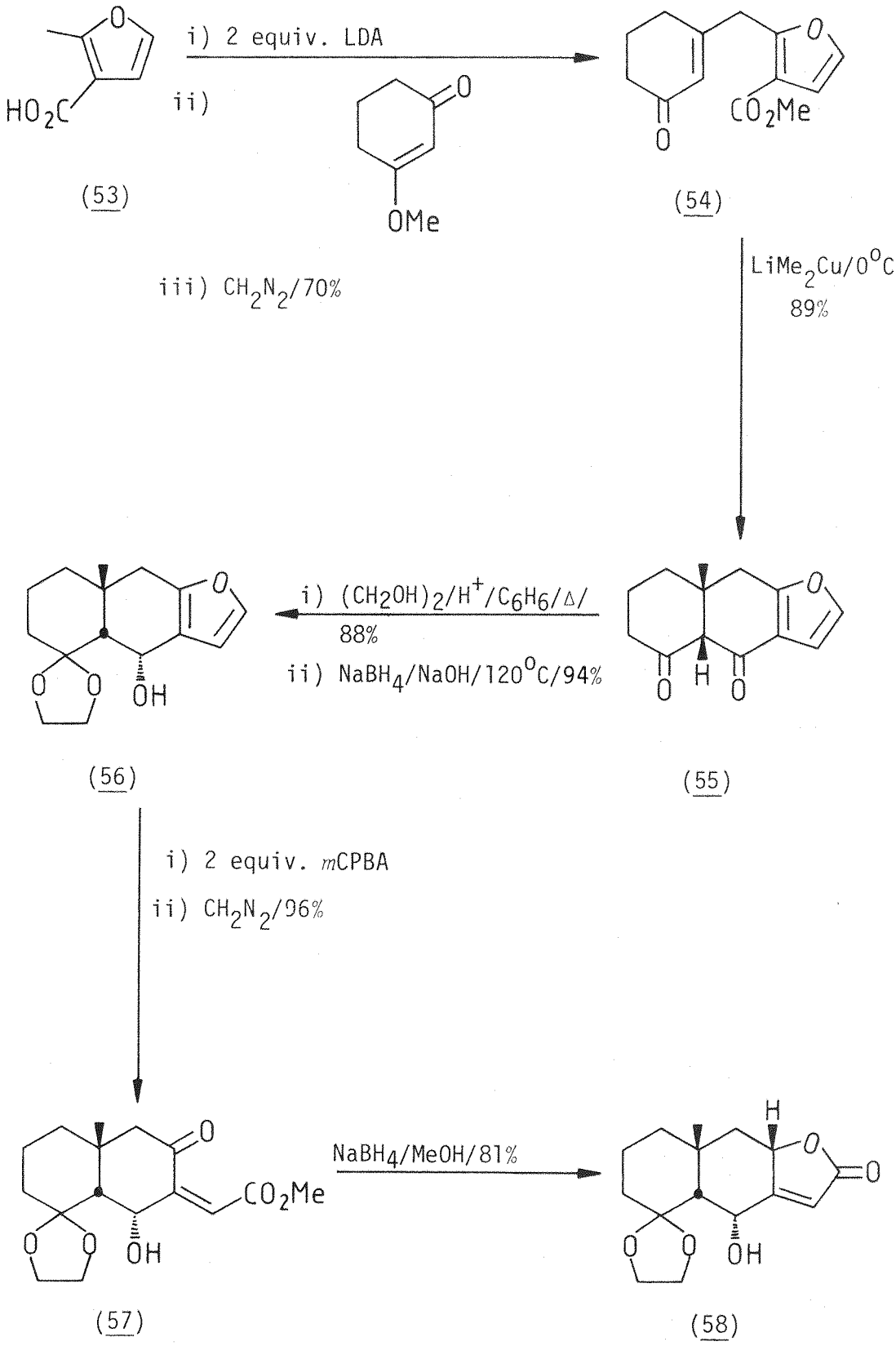
the alkylation of 2-methyl-3-furanoic acid (53) and oxidation of a furan was published during the course of this work. This process was achieved by reaction of the dianion of (53) with 3-methoxy-2-cyclohexen-1-one and treatment with diazomethane to give the methyl ester (54). Conjugate 1,4-addition of lithium dimethyl cuprate with methyl ester (54) and subsequent cyclisation yielded dione (55). Mono acetalisation of the dione (55) and stereospecific sodium borohydride reduction afforded the hydroxy furan (56). Oxidation of the alcohol (56) with two equivalents of *meta*-chloroperoxybenzoic acid and methylation resulted in the formation of unsaturated ester (57). Stereospecific sodium borohydride reduction gave the $\Delta^{\alpha,\beta}$-butenolide (58) (Scheme 2.3).[127]

This methodology is not readily applicable to the synthesis of the required skeleton for the paniculides (45a-c).

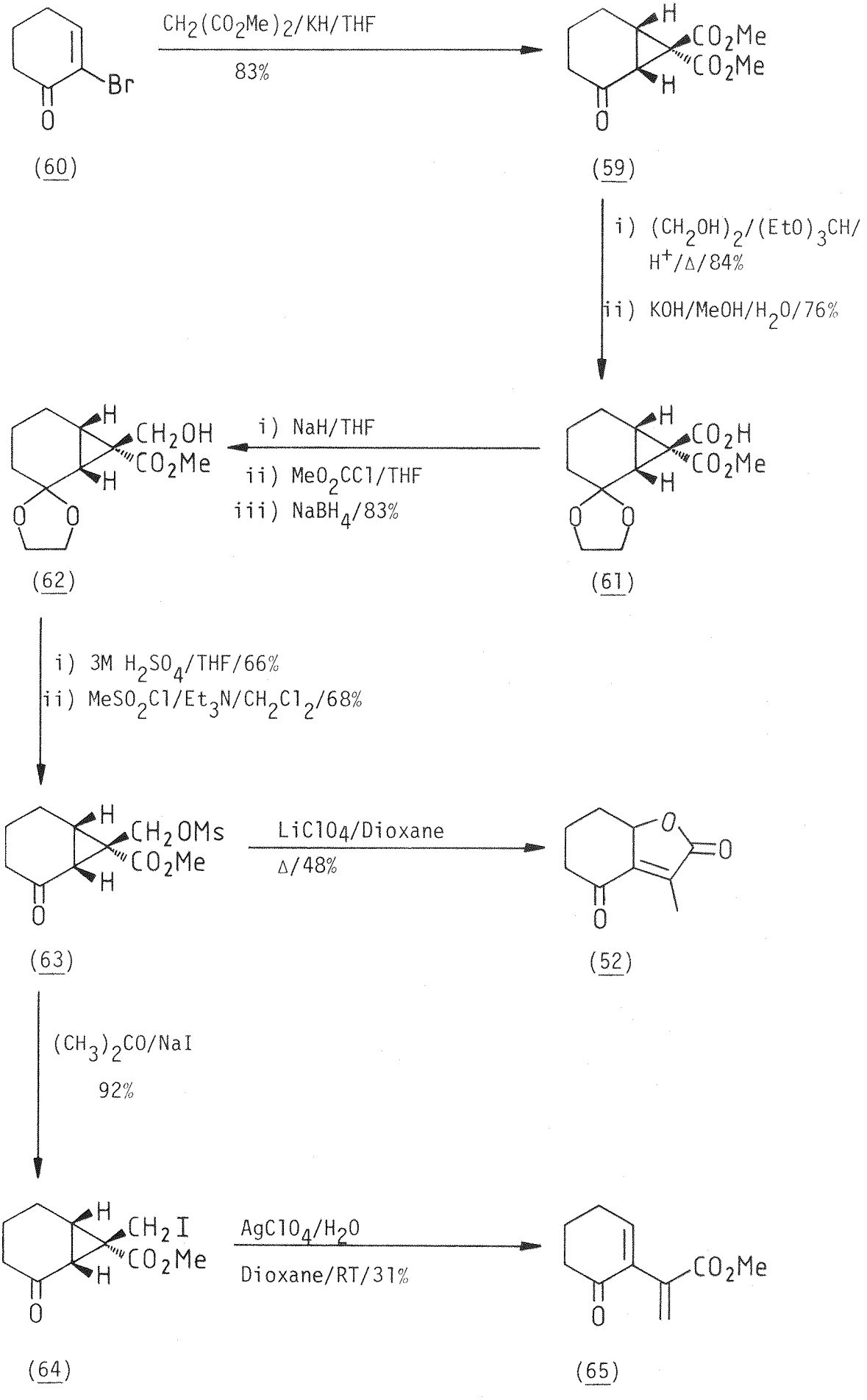
c) Rearrangement of Oxygen-Functionalised Cyclopropylcarbinyl Compounds

More recently solvolytic rearrangements of functionalised cyclopropylcarbinyl derivatives have been used for the synthesis of allylically oxygenated $\Delta^{\alpha,\beta}$-butenolides.[128] The key intermediate for the preparation of the cyclopropyl compounds was the keto diester (59) which was prepared by the reaction of 2-bromocyclohexen-1-one (60) with dimethylmalonate in the presence of potassium hydride. Acetalisation of the keto diester (59) and hydrolysis with methanolic potassium hydroxide gave exclusively the *exo* acid (61). Treatment of *exo* acid (61) with sodium hydride and then methyl chloroformate and subsequent sodium borohydride reduction yielded the *exo*-hydroxy methyl ester (62). Deprotection of the acetal group in (62) by acid hydrolysis and reaction with methanesulphonyl chloride afforded the mesylate (63). Treatment of the mesylate (63) with sodium iodide in acetone yielded the iodide (64). When mesylate (63) was heated under reflux with lithium perchlorate in dioxane $\Delta^{\alpha,\beta}$-butenolide (52) was formed in 48% yield. However, rearrangement of the iodide (64) in aqueous dioxane with silver perchlorate at room temperature afforded the diene ester (65) in 31% yield (Scheme 2.4).

This methology is very lengthy and the yields of the solvolysis step are only moderate. It was therefore decided that this approach was not suitable for a synthesis of the paniculides (45a-c).


i) 2 equiv. LDA
ii)
OMe
iii) CH_2N_2/70%
$LiMe_2Cu/0^oC$
89%
i) $(CH_2OH)_2/H^+/C_6H_6/\Delta/$
88%
ii) $NaBH_4$/NaOH/120°C/94%
i) 2 equiv. *m*CPBA
ii) CH_2N_2/96%
$NaBH_4$/MeOH/81%
(53)
(54)
(55)
(56)
(57)
(58)

Scheme 2.3


CH2(CO2Me)2/KH/THF
83%
(60)
(59)
i) (CH2OH)2/(EtO)3CH/
H+/Δ/84%
ii) KOH/MeOH/H2O/76%
i) NaH/THF
ii) MeO2CCl/THF
iii) NaBH4/83%
(62)
(61)
i) 3M H2SO4/THF/66%
ii) MeSO2Cl/Et3N/CH2Cl2/68%
LiClO4/Dioxane
Δ/48%
(63)
(52)
(CH3)2CO/NaI
92%
AgClO4/H2O
Dioxane/RT/31%
(64)
(65)

Scheme 2.4

d) Vinylfuranone Annulation

During the preparation of this thesis, a fourth route to the $\Delta^{\alpha,\beta}$-butenolide skeleton was published which utilised the reaction between 2,5-dihydro-3-phenylthio-4-vinylfuran-2-one (66) and ketones as shown in Scheme 2.5.[129] The ketone (67) in basic conditions undergoes exclusive 1,6-conjugate addition followed by an aldol type cyclisation to afford (68). Dehydration with thionyl chloride yields (69) which was then oxidised to the sulphoxide (70). Allylic sulphoxide-sulphonate rearrangement (Ac_2O,pyridine) gave the acetoxy-perhydrobenzofuranone (71) as a mixture of epimers with respect to the acetate group.

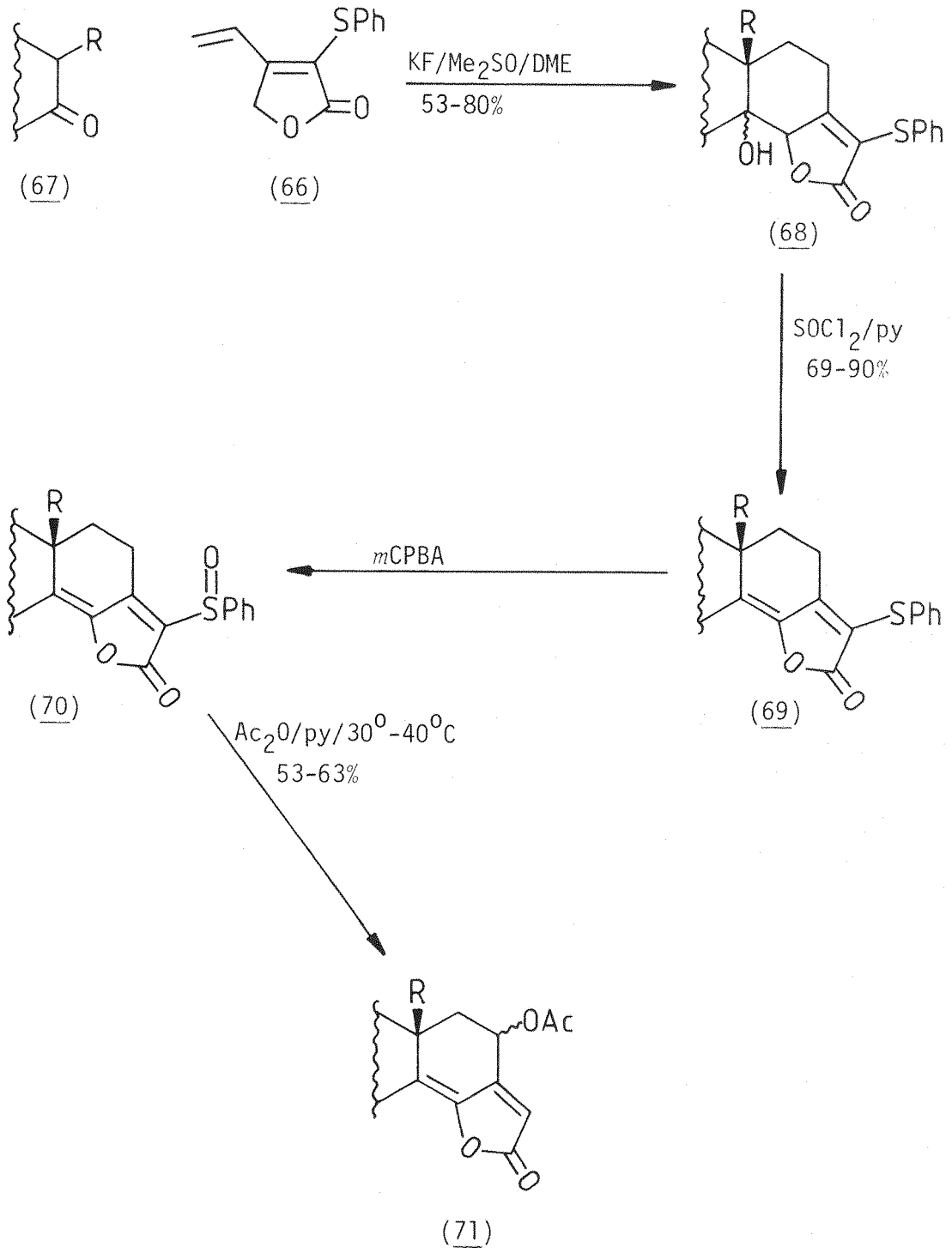
Although this method has been used recently in the synthesis of paniculide A (45a) (see Section 2.1.6.1)[130] it appears somewhat inefficient. The $\Delta^{\alpha,\beta}$-butenolide double bond is introduced along with a second olefin in the cyclohexyl ring. Specific catalytic reduction of the second olefin is not possible and hence the $\Delta^{\alpha,\beta}$-butenolide double bond has to be removed and then regenerated. In addition catalytic reduction of these enol esters generally results in substantial hydrogenolysis.

2.1.5.2 $\Delta^{\alpha,\beta}$-Butenolides from γ-Butyrolactones

An alternative approach to the basic $\Delta^{\alpha,\beta}$-butenolide skeleton involves the modification of a suitable γ-butyrolactone. This can be achieved by introduction of a leaving group α to the lactone carbonyl and then elimination. The elimination can proceed to give either the required $\Delta^{\alpha,\beta}$-butenolide or an α-methylene lactone depending on the relative steric requirements for the elimination (syn or anti).

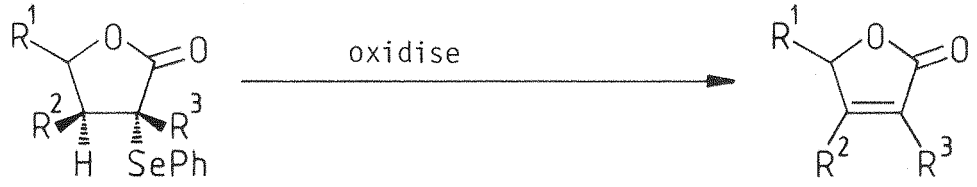
a) Alkylphenylselenoxides

The facile syn elimination of selenoxides has been used extensively for the synthesis of $\Delta^{\alpha,\beta}$-butenolides and α-methylene-γ-butyrolactones from the corresponding α-phenylselenyl lactones.[131-134] These methods, are, however, dependent upon the proper stereochemical relationship between the α-phenylselenyl substituent and the adjacent methine. It has been observed that selenoxides derived from (72) in which there


R
O
(67)
SPh
O
O
(66)
KF/Me2SO/DME
53-80%
R
OH
O
SPh
(68)
SOCl2/py
69-90%
R
SPh
O
(69)
mCPBA
R
O
SPh
O
(70)
Ac2O/py/30°-40°C
53-63%
R
OAc
O
(71)

Scheme 2.5

exists the possibility for two syn modes of elimination, results in >90% yield of the endocyclic olefin (73) despite the statistical preference for exocyclic olefin formation (Scheme 2.6).[132] The high propensity for endocyclic olefin formation thus provides a useful $\Delta^{\alpha,\beta}$-butenolide synthesis.

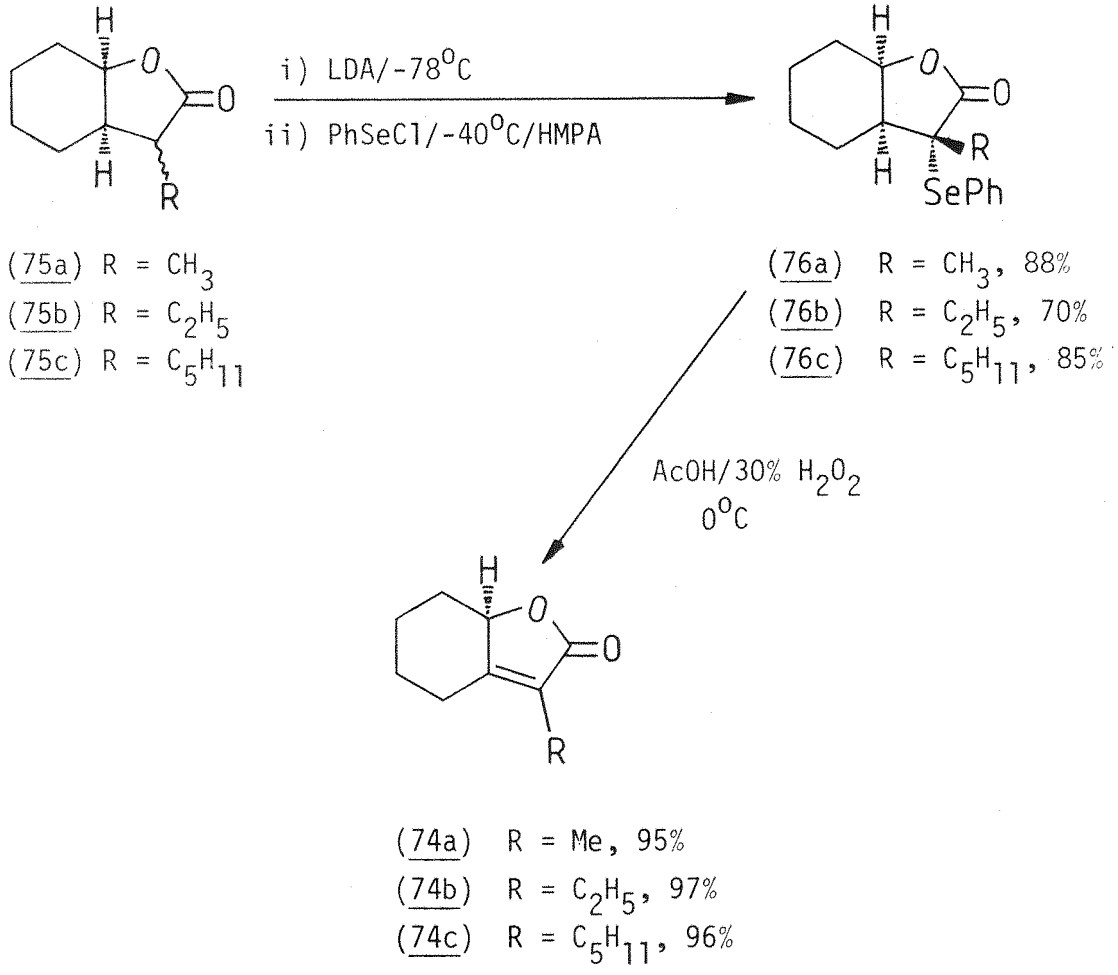


(72) (73)

Scheme 2.6

This approach has been utilised in the synthesis of a series of fused $\Delta^{\alpha,\beta}$-butenolides (74a-c) from the alkylated *cis*-γ-butyrolactones (75a-c).[132] Thus the anion α to the lactone carbonyls in (75a-c) were prepared by the action of lithium diisopropylamide (LDA) in the presence of hexamethylphosphoramide (HMPA). This anion was quenched with phenylselenyl chloride to afford the *exo* selenides (76a-c) as the exclusive products. This stereospecific introduction of the phenylselenyl group establishes the required syn relationship between the substituent and the adjacent methine proton; thus oxidation and syn elimination of the selenoxide gave the $\Delta^{\alpha,\beta}$-butenolides (74a-c) (Scheme 2.7).

If, however, the order of alkylation and phenylselenylation is reversed then the relative geometry about the carbon atom α to the lactone carbonyl is inverted. Reaction of the enolate derived from the *cis*-fused lactone (77) with diphenyl diselenide gave the α-phenylselenyl compound (78). Methylation of (78) using the conditions described above, yielded the lactone (79) free from the isomeric lactone (75a). This establishes an anti-relationship between the α-phenylselenyl substituent and the adjacent methine. Consequently, oxidation to the selenoxide and syn elimination gave the α-methylene-γ-butyrolactone (80) exclusively (Scheme 2.8).[131]

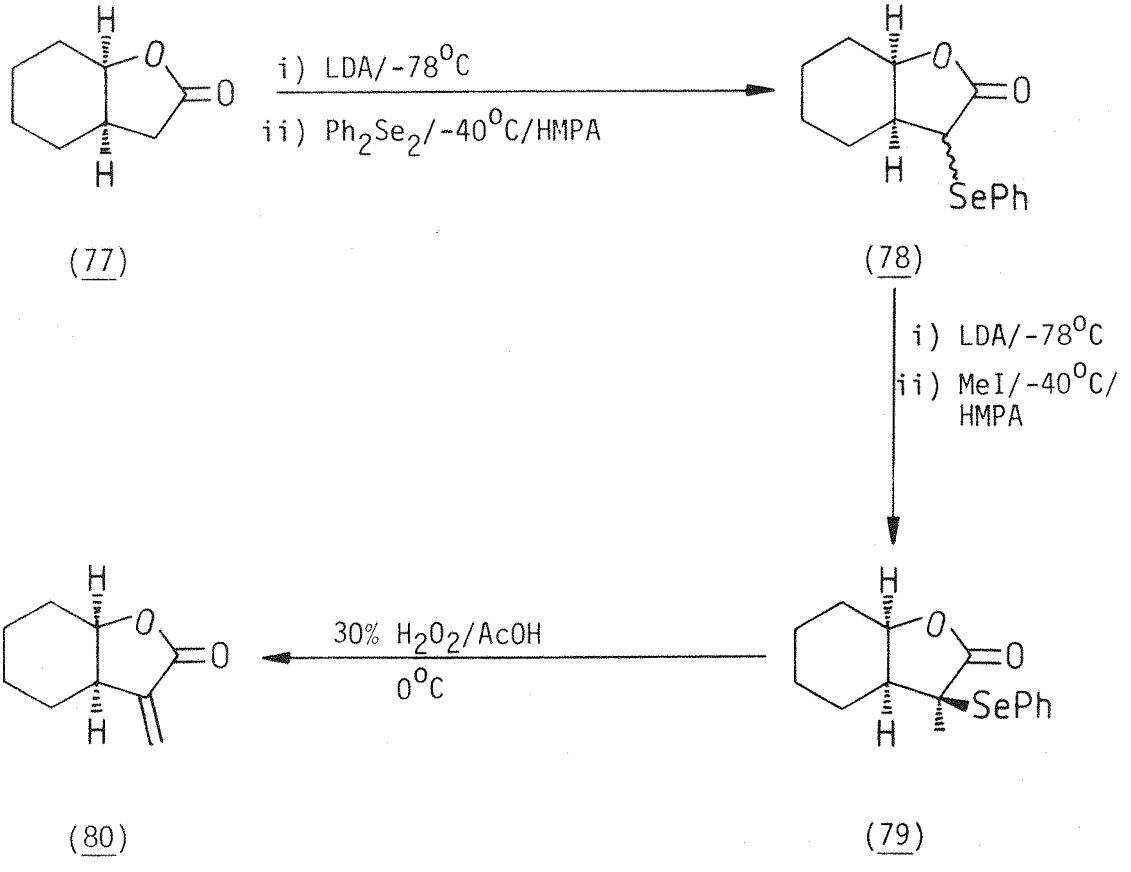


(75a) R = CH_3
(75b) R = C_2H_5
(75c) R = C_5H_{11}

(76a) R = CH_3, 88%
(76b) R = C_2H_5, 70%
(76c) R = C_5H_{11}, 85%

(74a) R = Me, 95%
(74b) R = C_2H_5, 97%
(74c) R = C_5H_{11}, 96%

Scheme 2.7



(77)

(78)

(79)

(80)

Scheme 2.8

b) Alkylphenylsulphoxides

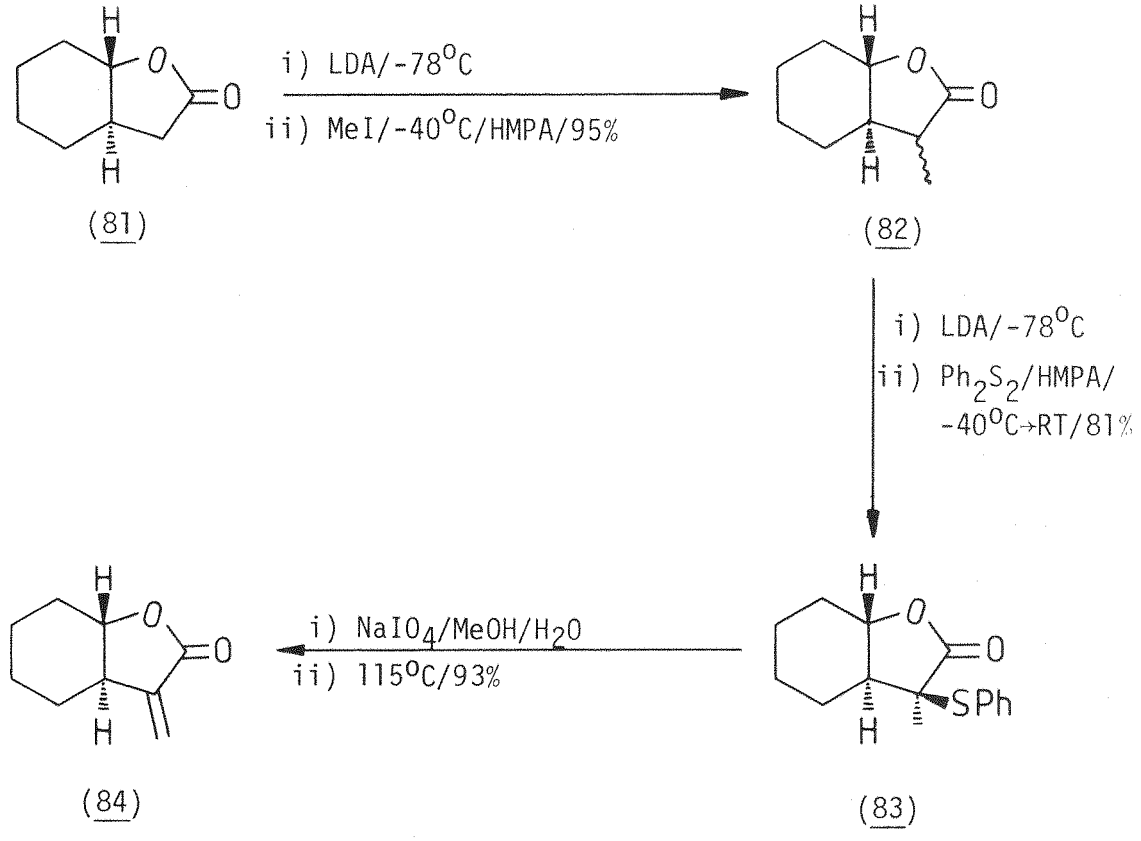
The stereoelectronic factors governing the sulphoxide eliminations are analogous to the selenoxide case. The syn elimination of sulphoxides has been used for the synthesis of $\Delta^{\alpha,\beta}$-butenolides and α-methylene-γ-butyrolactones.[135-139] This process is, however, less facile, requiring higher temperatures (ca. 115°C) than the corresponding selenoxides which eliminate at or below room temperature.

The success of this method is again dependent upon the proper stereochemical relationship between the α-phenylsulphenyl substituent and the adjacent methine proton. This is exemplified by a consideration of the *trans* fused-γ-butyrolactone (81). In this case, alkylation with methyl iodide gave the methylated lactone (82). Stereospecific introduction of the α-phenylsulphenyl group by α-anion formation and treatment with diphenyl disulphide afforded the sulphenated lactone (83). In lactone (83) the anti relationship between the α-phenylsulphenyl group and the adjacent methine precludes endocyclic olefin formation. Hence, oxidation of (83) with sodium metaperiodate and subsequent thermolysis yielded the α-methylene lactone (84) (Scheme 2.9).[135]

The isomeric α-phenylsulphenyl lactone (85) could be prepared from γ-butyrolactone (81) by α-phenylsulphenylation and subsequent stereospecific introduction of a methyl group. It should be noted that the α-phenylsulphenyl group is now syn to the adjacent methine, consequently oxidation and thermolysis gave exclusively the $\Delta^{\alpha,\beta}$-butenolide (86) (Scheme 2.10).[136,137]

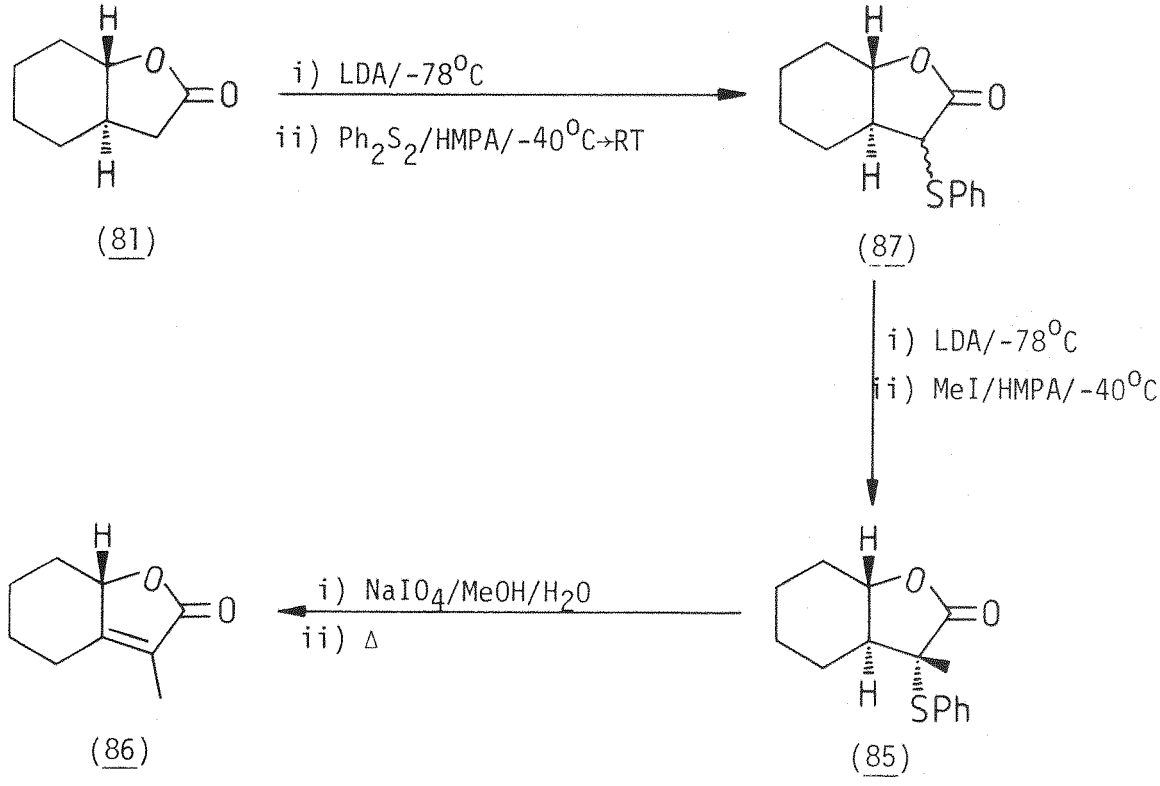
c) Dehydrobromination

In contrast to selenoxides and sulphoxides the elimination of hydrogen bromide requires an antiperiplanar transition state and consequently the stereochemical considerations are the reverse of these cases. Thus, treatment of the *cis* lactone (75a) with excess triphenylmethyllithium in the presence of TMEDA gave the corresponding enolate, which upon quenching with 1,2-dibromoethane yielded the α-bromolactone (88). Dehydrobromination of (88) with excess diazabicyclononane (DBN) in toluene at reflux resulted in the formation of the α-methylene-γ-butyrolactone (80) as the sole product (Scheme 2.11). This is a result of the *cis* relationship between the bromine and the adjacent methine



(81) (82) (83) (84)

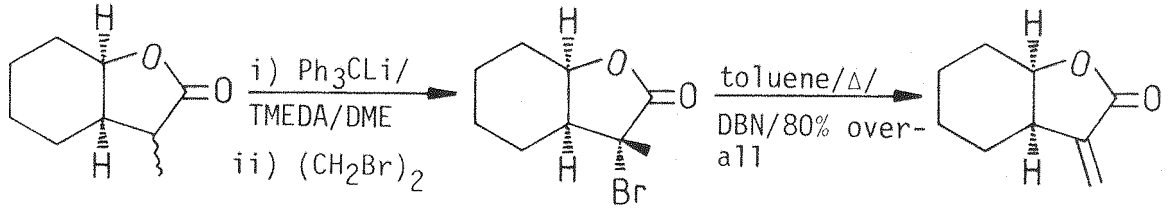
Scheme 2.9



(81) (87) (85) (86)

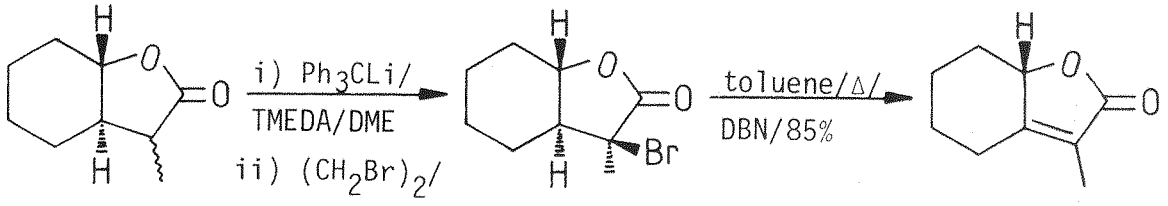
Scheme 2.10

proton in (88), consequently *trans* elimination can only give the exocyclic olefin (80). In contrast the α-bromo-*trans*-lactone (89) formed from the *trans*-lactone (82) gave exclusively the $\Delta^{\alpha,\beta}$-butenolide (74a) under a variety of elimination conditions (Scheme 2.12).[140]



(75a) (88) (80)

Scheme 2.11

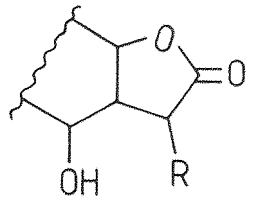


(82) (89) (74a)

Scheme 2.12

2.1.5.3 Synthesis of γ-Butyrolactones

The above methodology for the conversion of a γ-butyrolactone to a $\Delta^{\alpha,\beta}$-butenolide requires the synthesis of the basic γ-butyrolactone moiety with an oxygenated centre adjacent to the ring juncture as shown in partial structure (90). Although this ring system has been the objective of synthetic projects in a number of laboratories,[141-149] the number of basically different approaches is limited.



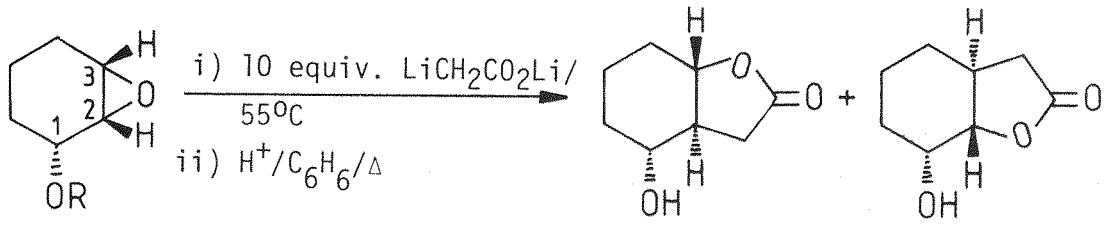
(90)

a) Opening α-Oxy Epoxides with Dilithioacetate

One route to the γ-butyrolactone (90) involves the opening of a vicinal, oxygen substituted epoxide with dilithioacetate. This elegant approach has been studied by Danishefsky *et al.* using α-hydroxy and α-trimethylsiloxyl epoxides which exert important directing effects.[141] Thus, the reaction of the *cis*-hydroxy epoxide (91) with 10 equivalents of dilithioacetate followed by acid workup and lactonisation gave a 66% combined yield of lactones (92) and (93) in a 3:1 ratio (Scheme 2.13, Table 2.1). These lactones (92) and (93) result from SN_2 attack at C2 and C3 respectively. However, when the same reaction was carried out on the corresponding silyl ether (94), the hydroxy lactones (92) and (93) were obtained in a ratio of 1:3.2 respectively (Scheme 2.13, Table 2.1).

Similar reactions were conducted on the *trans* hydroxy epoxide (95) and its silyl ether (96). Compound (95) afforded a 61% yield of 3.2:1 ratio of lactones (97) and (98), whereas (96) gave a 50% yield now in a 1:4.5 ratio (Scheme 2.14, Table 2.1).

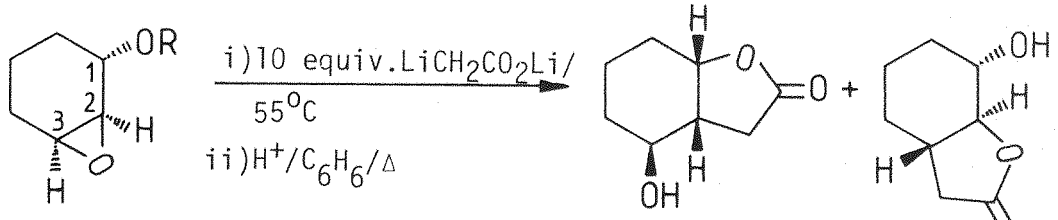
This approach has been utilised by Danishefsky *et al.* In the synthesis of the α-methylene-γ-butyrolactones vernolepin (99) and vernomenin (100).[142] More recently, Schlessinger *et al.* have synthesised eriolanin (101) by this methodology.[143]



(91) R = H
(94) R = TMS

(92) (93)

Scheme 2.13



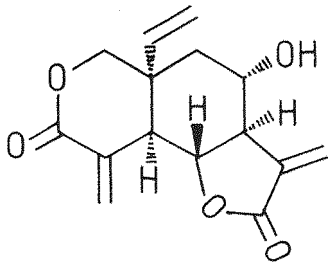
(95) R = H
(96) R = TMS

(97) (98)

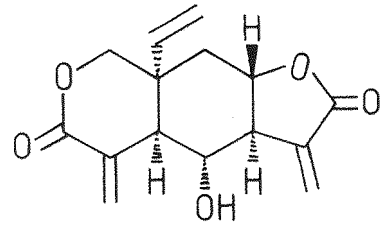
Scheme 2.14

Epoxide	Product Ratio	Yield
(91)	(92):(93) = 3:1	66%
(94)	(92):(93) = 1:3.2	-
(95)	(97):(98) = 3.2:1	61%
(96)	(97):(98) = 1:4.5	50%

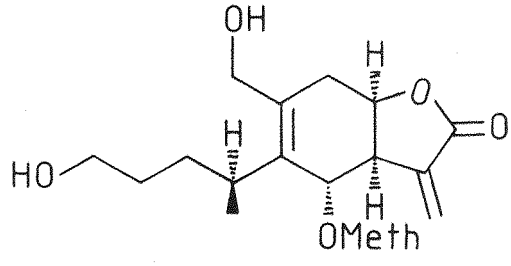
Table 2.1: Dilithioacetate Epoxide Opening of (91), (94), (95) and (96).



(99)



(100)

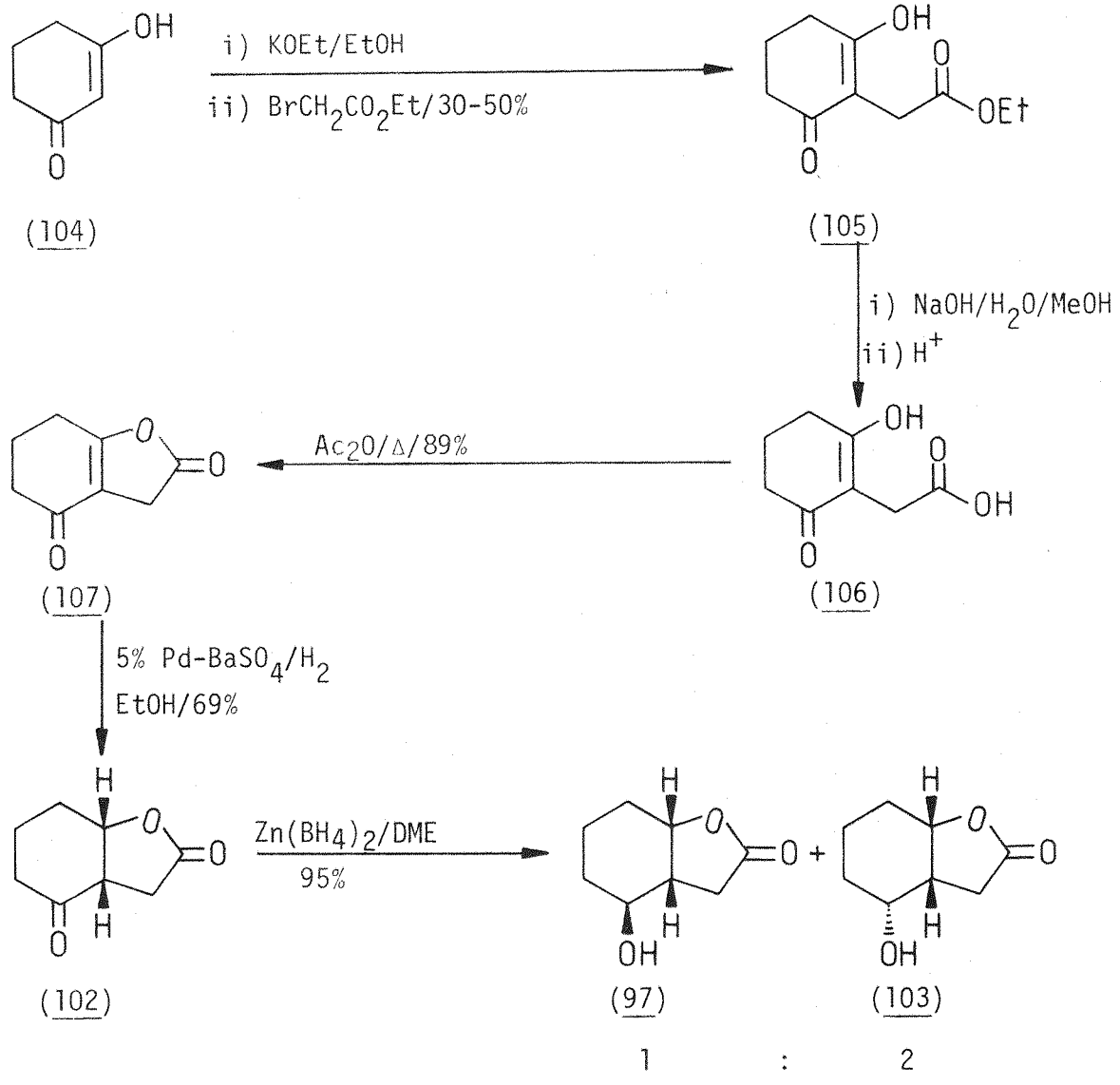


(101)

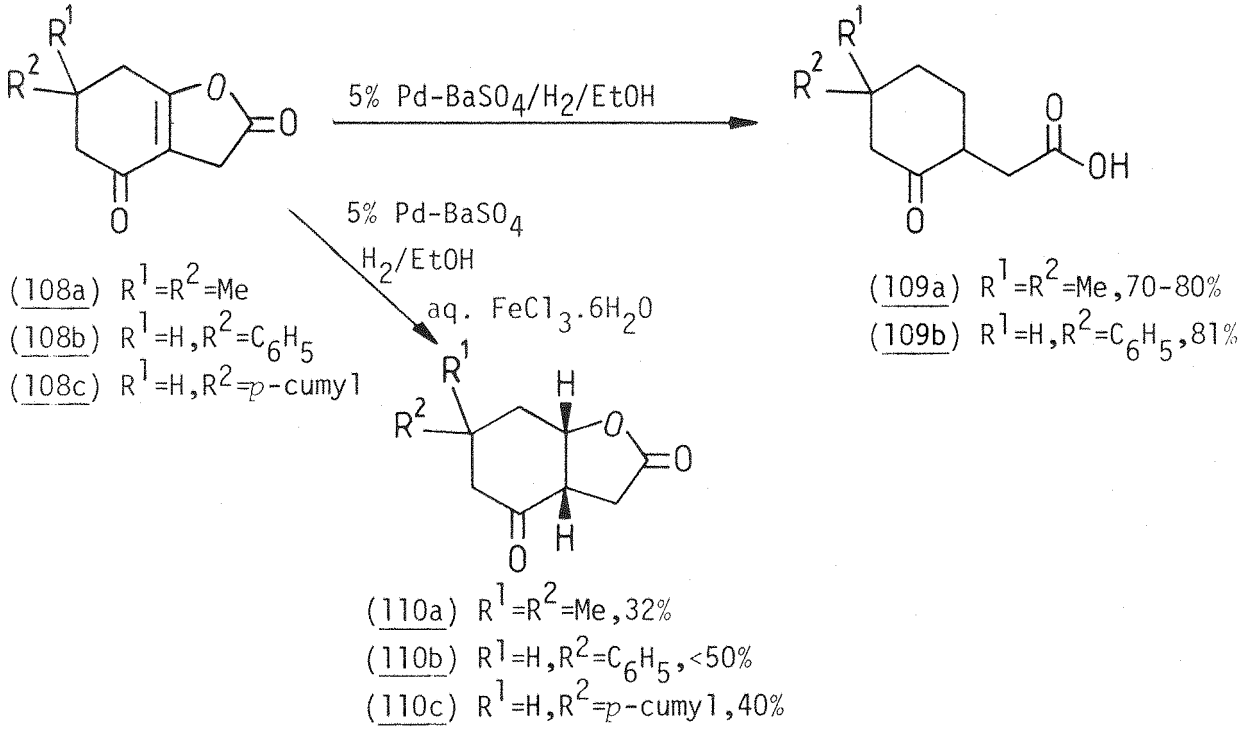
b) Hydrogenation of an Enol Lactone

This method has been used for the synthesis of the keto lactone (102) and a mixture of the hydroxy lactones (97) and (103).[144] This involved the alkylation of 1,3-cyclohexanedione (104) with ethyl bromoacetate which afforded the alkylated dione (105).[145,146] Saponification of (105) with sodium hydroxide gave the acid (106) which was cyclised to the enol lactone (107) with acetic anhydride.[147] Catalytic reduction of (107) with palladium on barium sulphate gave the keto lactone (102) and subsequent reduction with zinc borohydride afforded a mixture of the hydroxy lactones (97) and (103) in a 1:2 ratio respectively (Scheme 2.15).

It should be noted, however, that in a study of a series of related enol lactones (108a-c) Rosenmund *et al.* observed high yields of hydrogenolysis under similar conditions to give keto acids (109a,b) and none of the keto lactones (110a,b). However, low yields of the keto lactones (110a-c) were observed using this catalyst system poisoned with iron III chloride (Scheme 2.16).[146] This type of approach, although direct may suffer problems of hydrogenolysis in the reduction step.



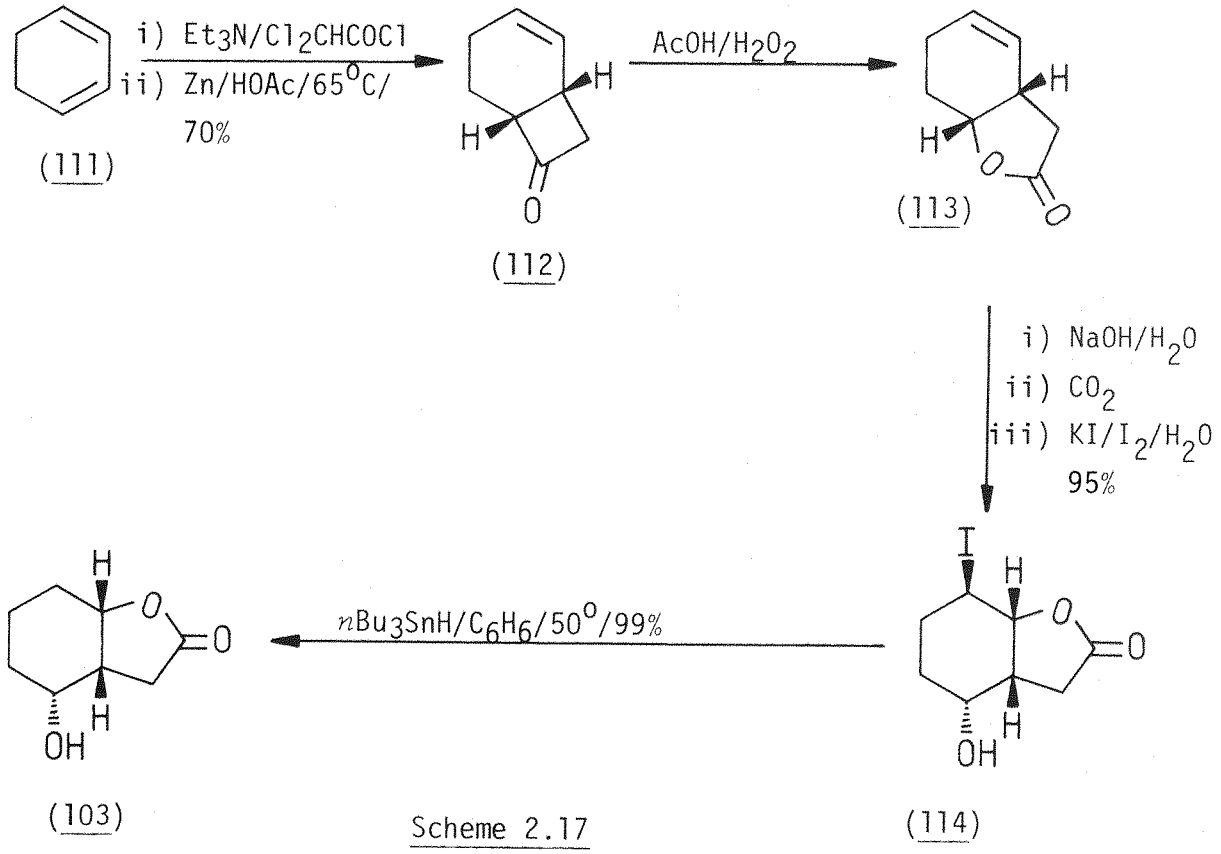
Scheme 2.15



Scheme 2.16

c) Baeyer-Villiger Oxidation of a Cyclobutanone Derivative

A third route to the γ-butyrolactone skeleton (90) involved mono-addition of dichloroketene to 1,3-cyclohexadiene (111), subsequent dechlorination yielded the cyclobutanone (112). Baeyer-Villiger oxidation resulted in the formation of the olefinic γ-butyrolactone (113) which when subjected to saponification and iodolactonisation, gave the hydroxy lactone (114). De-iodination of (114) using tributyl tin hydride in benzene at 50°C afforded the hydroxy lactone (103) (Scheme 2.17).[148]



(111) (112) (113) (114) (103)

Scheme 2.17

Although this methodology has been used by Grieco *et al.* in the synthesis of eriolanin (101),[149] this approach is less direct than those above. In addition a suitably substituted 1,3-diene required for a synthesis of the paniculides (45a-c) would not be readily available.

2.1.6 Synthesis of Paniculide A (45a)

2.1.6.1 Photoaddition of 1,1-Diethoxyethylene to an Enone

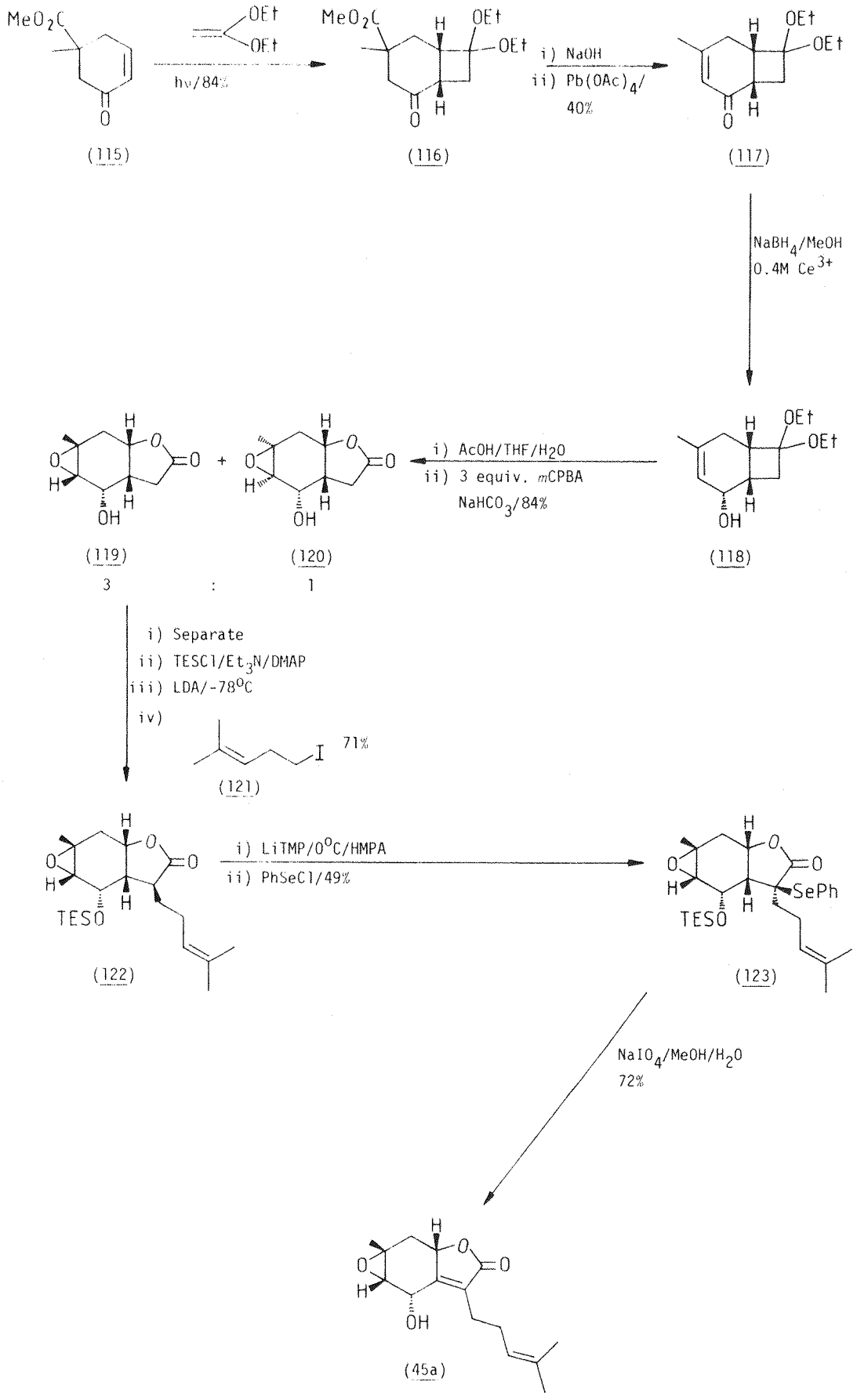
It should be noted that during the course of this work Smith *et al.* published the total synthesis of (±)-paniculide A (45a) which is outlined in Scheme 2.18.[150]

The synthesis started from enone (115) which underwent a [2+2] cycloaddition with 1,1-diethoxyethylene and gave the bicyclic ketone (116). Subsequent saponification followed by oxidative-decarboxylation with lead tetraacetate afforded the bicyclic enone (117). Stereospecific reduction of (117) with sodium borohydride in the presence of cerium(III) gave the allylic alcohol (118). Subsequent deacetalisation and oxidation with *meta*-chloroperoxybenzoic acid yielded a 3:1 mixture of epoxy lactones (119) and (120) which were separated by chromatography. Protection of (119) as the triethylsilyl ether (TES) and subsequent alkylation with 1-iodo-4-methylpent-3-ene (121) afforded the alkylated lactone (122). Treatment of (122) with lithium tetramethylpiperidine (LiTMP) followed by phenylselenyl chloride afforded the *exo* selenide (123) exclusively. Subsequent oxidative-elimination with concomitant removal of the TES group gave (±)-paniculide A (45a).

This stereocontrolled synthesis was therefore achieved in 10 steps with a 4.2% overall yield from enone (115). However, this synthesis contains a low yield step in the oxidative-decarboxylation of (116) to (117) (40% yield). Although the deprotection and epoxidation of (118) proceeds in good yield it gives a mixture of (119) and (120) in a 3:1 ratio. These factors along with the envisaged difficulty in application to the synthesis of paniculide B (45b) and C (45c) severely limit this synthetic approach.

2.1.6.2 Vinylfuranone Annelation Method

During the preparation of this thesis a second total synthesis of paniculide A (45a) was reported (Scheme 2.19).[130] This was based on a novel vinylfuranone annelation method already described in Section 2.1.5.1.


MeO2C
OEt
OEt
hν/84%
(115)
(116)
i) NaOH
ii) Pb(OAc)4/
40%
(117)
NaBH4/MeOH
0.4M Ce3+
i) AcOH/THF/H2O
ii) 3 equiv. mCPBA
NaHCO3/84%
(118)
(119)
3
:
(120)
1
i) Separate
ii) TESCl/Et3N/DMAP
iii) LDA/-78°C
iv)
I
71%
(121)
TESO
(122)
i) LiTMP/0°C/HMPA
ii) PhSeCl/49%
SePh
(123)
NaIO4/MeOH/H2O
72%
OH
(45a)

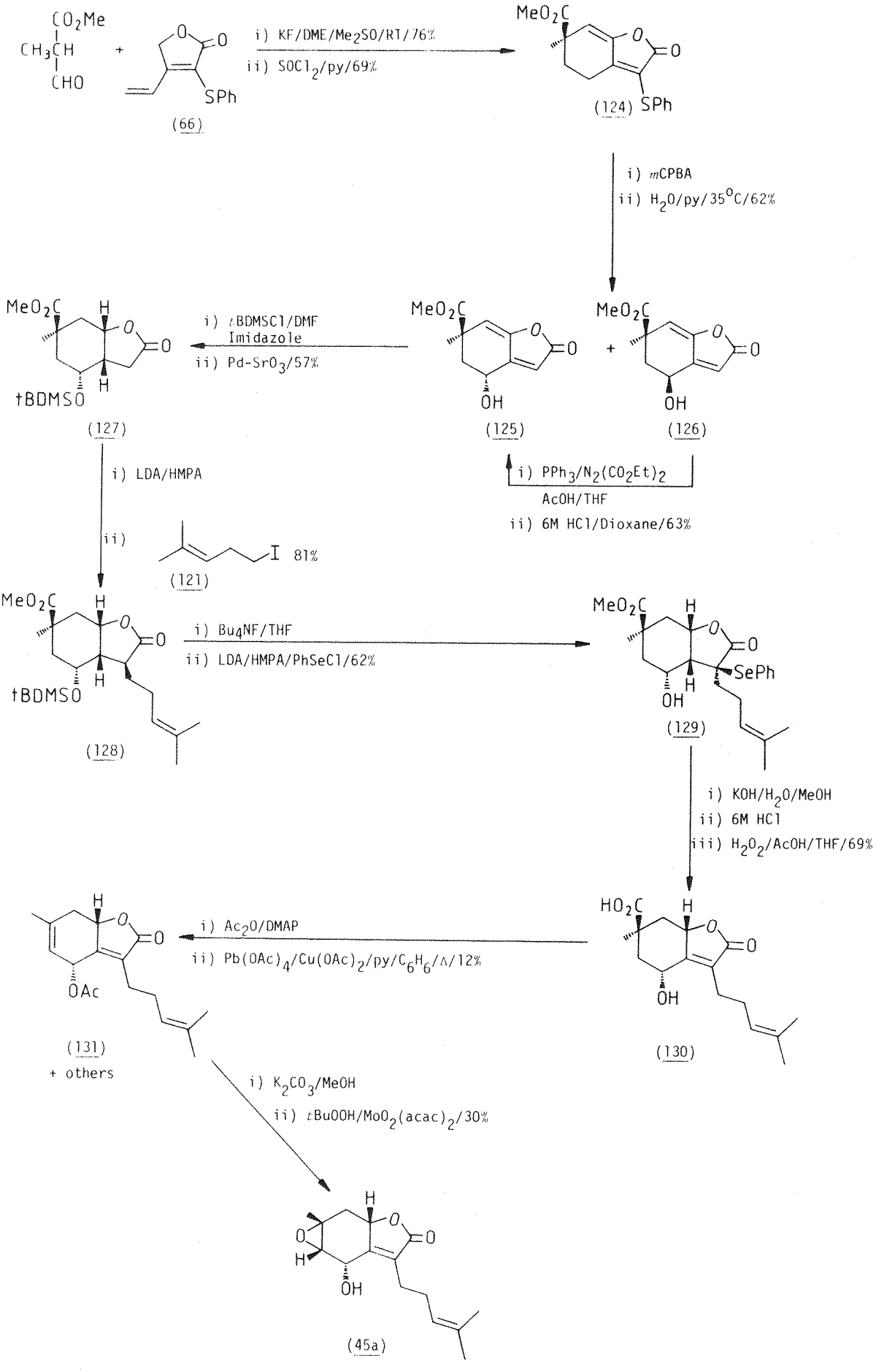
Scheme 2.18

Annelation of methyl-α-formylpropionate with 2,5-dihydro-3-phenylthio-4-vinylfuran-2-one (66) and then dehydration gave the annelated product (124). Peracid oxidation of (124) and then treatment with pyridine and water yielded a separable mixture of hydroxy lactones (125) and (126) in a 56:44 ratio. Hydroxy lactone (126), however, could be recycled to (125) by the Mitsunobu inversion followed by hydrolysis. The hydroxy lactone (125) was then protected as the *tert*-butyldimethylsilyl ether (*t*BDMS) and then hydrogenation gave the lactone (127). Alkylation of (127) with 1-iodo-4-methylpent-3-ene (121) yielded (128), which after desilylation and selenylation gave exclusively the *exo* selenide (129). Saponification of (129) and then oxidative elimination yielded the $\Delta^{\alpha,\beta}$-butenolide (130). Subsequent acetylation and oxidative-decarboxylation with lead tetraacetate gave a mixture of olefins, from which olefin (131) was isolated. Hydrolysis of the acetate group followed by metal-mediated epoxidation afforded (±)-paniculide A (45a).

This synthesis was achieved in 15 steps with a 0.2% overall yield from (66) and compares less favourably with the synthesis of Smith *et al.*[150] The low yield for the oxidative-decarboxylation step (12%) and the fact that the $\Delta^{\alpha,\beta}$-butenolide olefin in (125) is removed and then re-introduced at a later stage make this synthesis inefficient. In addition the sulphoxide-sulphenate rearrangement ((124) → (125) + (126)) produced a mixture of alcohols (125) and (126) in a 56:44 ratio which have to be separated. These limitations render this approach inapplicable to the synthesis of paniculides B (45b) and C (45c).

2.1.7 Proposed Study

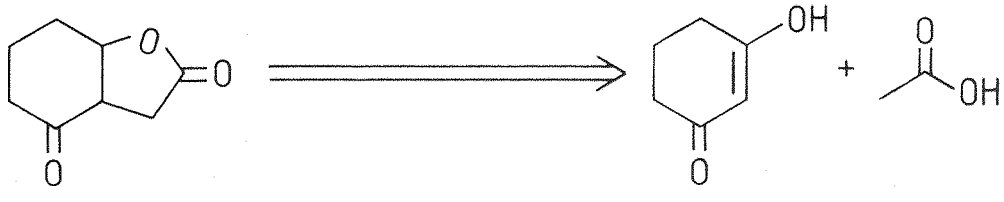
Our interests were directed towards the paniculides (45a-c) because of their possible biological activity and their novel structures. Paniculides B (45b) and C (45c) appeared more interesting targets since these are structurally more complex than paniculide A (45a) and are obtained in larger quantities from the tissue cultures of *Andrographis paniculata*. Paniculide C (45c) has been prepared from paniculide B (45b) *via* mild oxidation of the secondary allylic alcohol.[123,125] Therefore, a total synthesis of paniculide B (45b) would also constitute a total synthesis of paniculide C (45c).


CO2Me
CH3CH
CHO
SPh
(66)
i) KF/DME/Me2SO/RT/76%
ii) SOCl2/py/69%
MeO2C
(124) SPh
i) mCPBA
ii) H2O/py/35°C/62%
MeO2C
tBDMSO
(127)
i) tBDMSCl/DMF
Imidazole
ii) Pd-SrO3/57%
MeO2C
OH
(125)
MeO2C
OH
(126)
i) PPh3/N2(CO2Et)2
AcOH/THF
ii) 6M HCl/Dioxane/63%
i) LDA/HMPA
ii)
I 81%
(121)
MeO2C
tBDMSO
(128)
i) Bu4NF/THF
ii) LDA/HMPA/PhSeCl/62%
MeO2C
OH
SePh
(129)
i) KOH/H2O/MeOH
ii) 6M HCl
iii) H2O2/AcOH/THF/69%
HO2C
OH
(130)
i) Ac2O/DMAP
ii) Pb(OAc)4/Cu(OAc)2/py/C6H6/Δ/12%
OAc
(131)
+ others
i) K2CO3/MeOH
ii) tBuOOH/MoO2(acac)2/30%
OH
(45a)

Scheme 2.19

A retrosynthetic analysis of paniculide B (45b) (Scheme 2.20) indicates that the epoxide moiety could be generated from the corresponding allylic alcohol (132) using a Sharpless directed epoxidation.[151] The allylic alcohol (132) could, in turn, be generated from the ketone (133) using the Reich[152]-Sharpless[153] phenylselenation-oxidative elimination protocol followed by reduction. The $\Delta^{\alpha,\beta}$-butenolide olefin could be introduced in a similar fashion (see Section 2.1.5.2) to give retrosynthetically the lactone (134). Alkylation α to the lactone carbonyl is possible, so we are left with the keto lactone (135) as the sub-goal. Further retrosynthetic analysis of (135) indicated that it could be derived from the 1,3-dione (136) and an acetic acid residue.

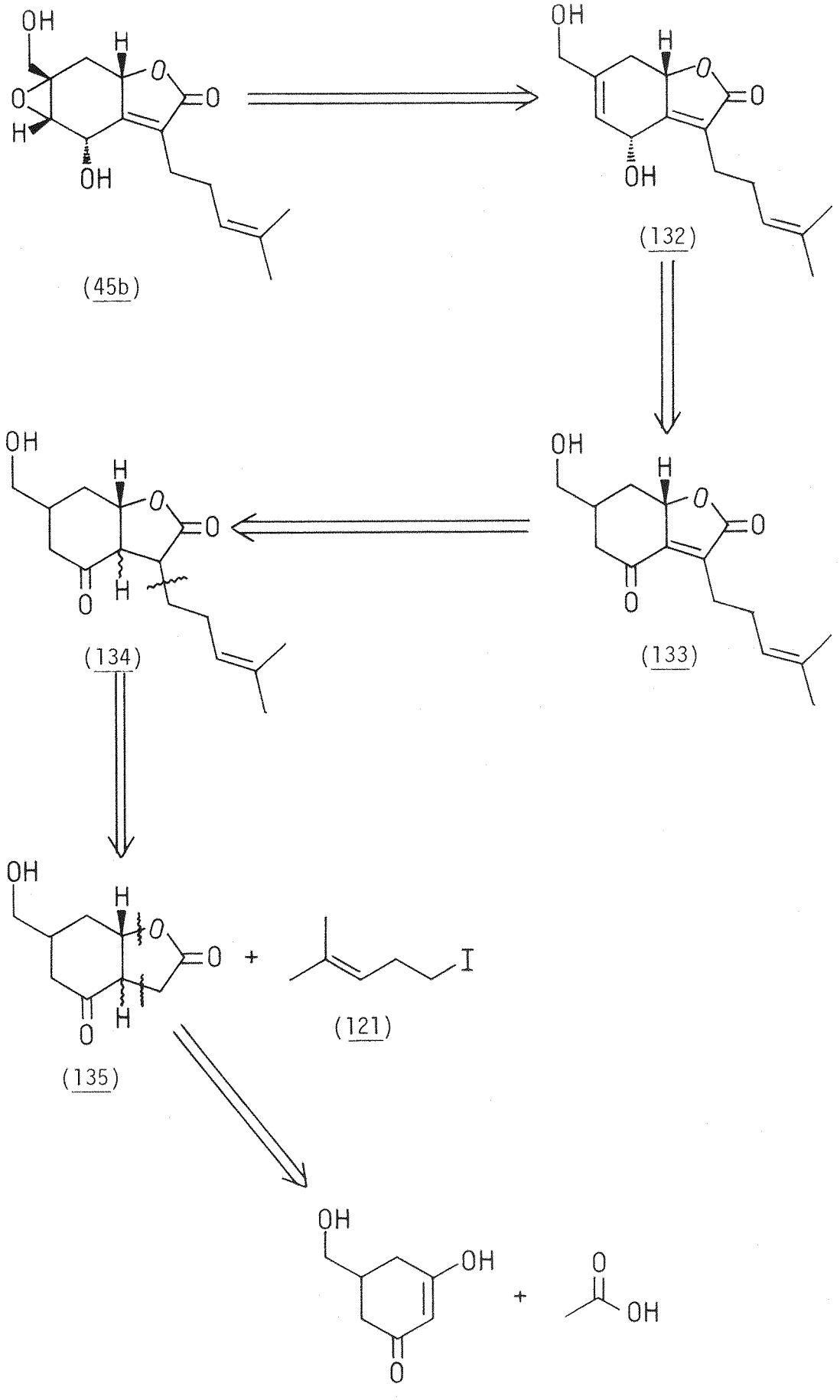
It was thought wise to study any synthetic strategies on the paniculides (45a-c) with a model system. It was envisaged that these strategies would utilise the stereospecific introduction of selenium or sulphur to generate the $\Delta^{\alpha,\beta}$-butenolide olefin and later reduction of a ketone to an alcohol. In order to ease the problems of assigning stereochemistry it was decided to carry out synthetic studies towards the paniculides (45a-c) on a simple system. The system that was chosen lacked the hydroxy methylene group of paniculides B (45b) and C (45c) so we have the sub-goal (102) which is derivable from 1,3-cyclohexane-dione (104) (Scheme 2.21).



(102) (104)

Scheme 2.21

It was therefore decided to investigate synthetic strategies to paniculide B (45b) and C (45c) with a model system. The aim was then to utilise these methods in the total synthesis of these two natural products.



Scheme 2.20

2.2 Results and Discussion

2.2.1 Attempts to Prepare Keto Lactone (102)

2.2.1.1 Sodium Borohydride Reduction of an Enol Ether

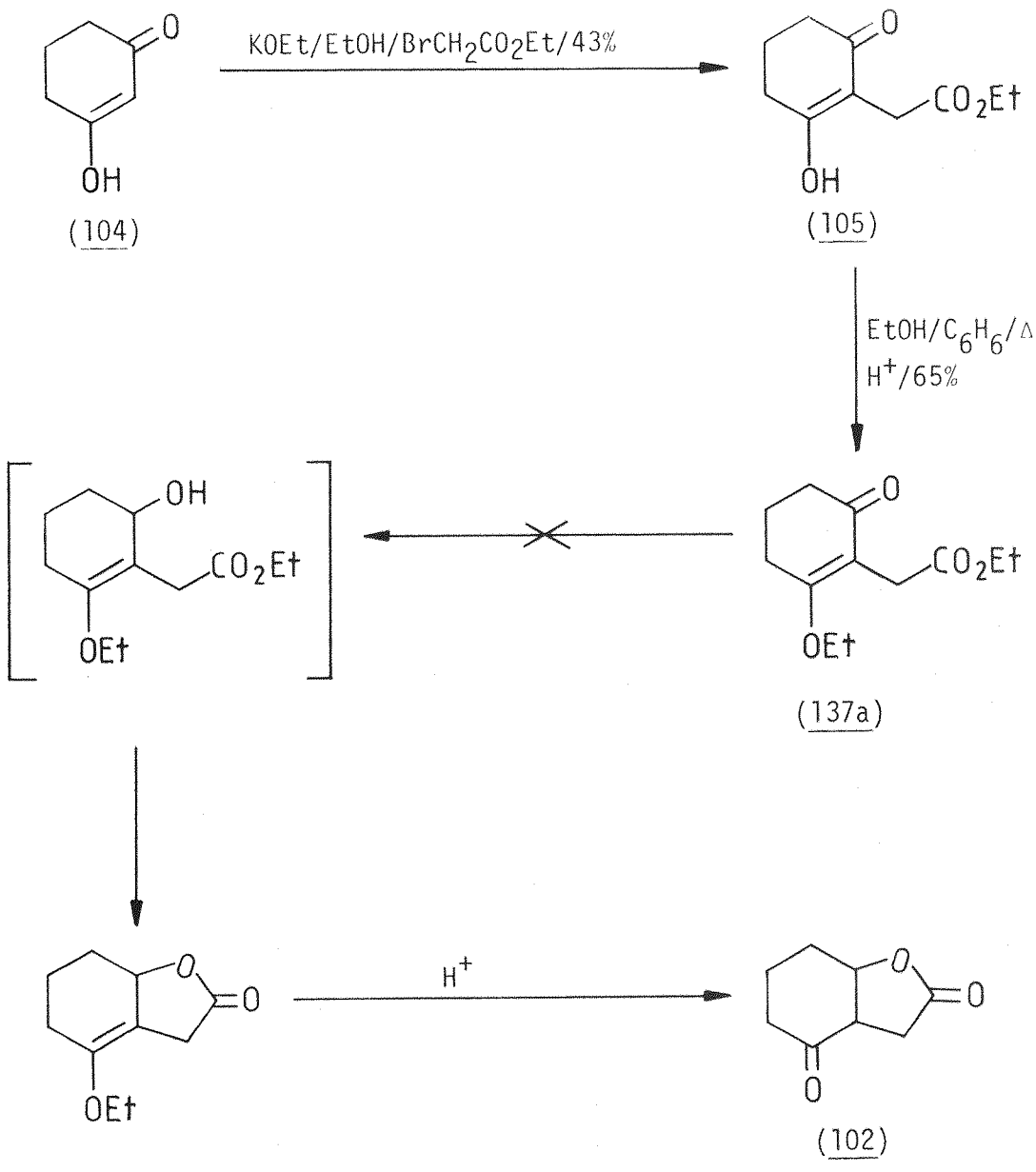
Mono enol ether (137a) could be prepared from 1,3-cyclohexanedione (104) by alkylation with ethyl bromoacetate, followed by mono protection of the 1,3-diketo moiety. It was envisaged that the enol ether (137a) should undergo sodium borohydride reduction to give the keto lactone (102) on hydrolysis (Scheme 2.22). Unfortunately, the predominant product from these reductions was lactone (138a) resulting from 1,4- followed by 1,2-addition of hydride (Scheme 2.23). In addition, an unidentified product (139a) was also isolated under a variety of conditions (Table 2.2). Under similar conditions the isobutoxy enol ether (137b), which was chosen in an attempt to reduce 1,4-addition, gave predominantly the isobutoxy lactone (138b) again resulting from 1,4-addition of hydride (Scheme 2.23, Table 2.2).

The ^{1}H NMR (60MHz, CCl_4) of the unknown (139a) indicated the presence of an ethyl ester or enol ether with a triplet at δ1.25 (J=8Hz) and a quartet at δ4.4 (J=8Hz). Very little other information could be obtained from the ^{1}H NMR. The thin film IR of (139a) indicated the presence of a hydroxy group with a strong absorbtion between 3700-3100cm^{-1}. Absorbtion peaks at 1780cm^{-1} and 1730cm^{-1} gave evidence for the presence of a lactone and ketone moiety respectively. The mass spectrum of (139a) showed no evidence of a molecular ion and no useful fragmentation patterns could be deduced. The base peak of this mass spectrum was observed at m/z 41 with other pominent ions at m/z 81(70%), 68(40%), 67(69%), 57(56%), 55(85%), 43(55%) and 39(57%) (see Section 2.5.4.3).

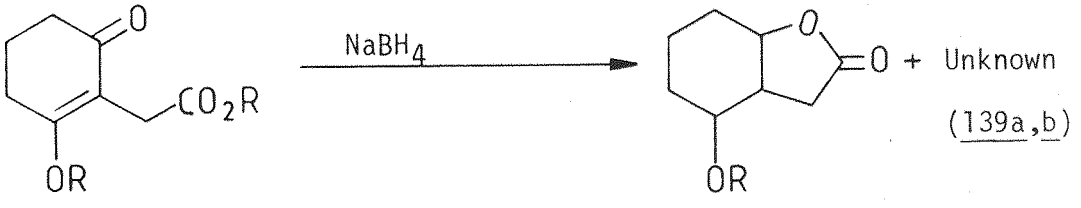
The problems with 1,4-addition of hydride coupled with the lower isolated yielded of products led to the abandoning of this approach.

2.2.1.2 Catalytic Reduction of an Enol Lactone

A second approach to the keto lactone (102) was attempted which involved saponification of diketo ester (105) to yield the diketo acid (106). The acid (106) was then cyclised to enol lactone (107) in 73% yield by stirring in dry ethyl acetate with dicyclohexylcarbodiimide (DCC). This method was found to be superior to the literature



Scheme 2.22



(137a) R = Et
(137b) R = iBu

(138a) R = Et
(138b) R = iBu

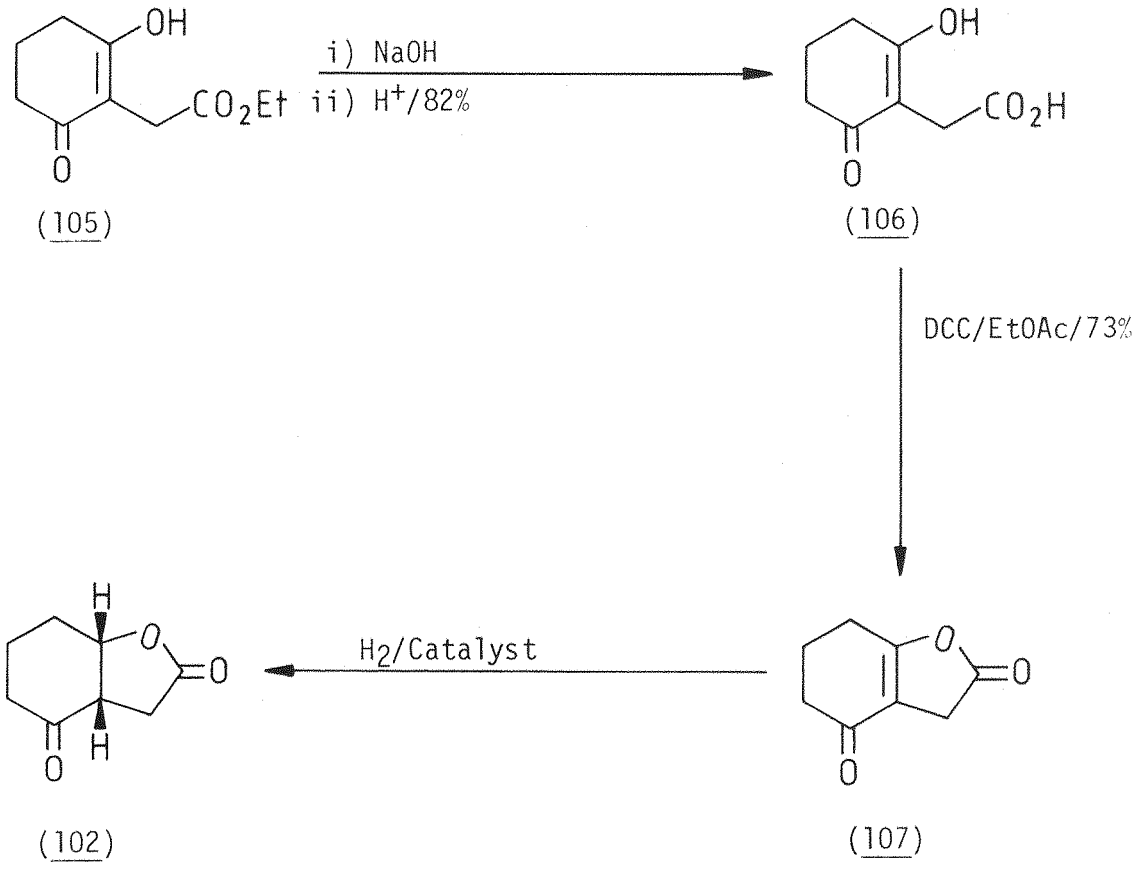
Scheme 2.23

Conditions	Yield[a] (137a) or (137b)	Yield[a] (138a) or (138b)	Yield[a] (139a) or (139b)
(137a)/$NaBH_4$/MeOH 0^oC/2h.	47%	49% (24%)	4%
(137a)/$NaBH_4$/EtOH 20^oC/12h.	28%	45% (15%)	26%
(137a)/$NaBH_4$/EtOH 40^oC/4h.	-	63%	37%
(137a)/$NaBH_4$/MeOH 0^oC-20^oC/12h. 0.1N NaOH	-	86%	14%
(137b)/$NaBH_4$/MeOH 0^oC-20^oC/12h. 0.1N NaOH	-	82% (12%)	18%

[a] Yields were determined by gas chromatography (5% OV101 200^oC) except those in parentheses which are isolated yields.

Table 2.2: Results of Sodium Borohydride Reduction of (137a) and (137b)

method[144,147] of heating (106) in acetic anhydride (40% yield). It was hoped that enol lactone (107) could be catalytically reduced to keto lactone (102) (Scheme 2.24, see Section 2.1.5.3 (b)).



(105) (106) (107) (102)

Scheme 2.24

The catalytic reduction of (107) has been reported using palladium on barium sulphate.[144] However, several analogues of (107) were found to undergo extensive hydrogenolysis with this catalyst.[147] These analogues could be reduced in low yields with palladium on barium sulphate provided that it was poisoned with iron III chloride[147] (see Section 2.1.5.3 (b)). In view of these hydrogenolysis problems it was envisaged that catalytic reduction of (107) with rhodium catalysts would give high yields of the keto lactone (102). The rhodium catalysts are known to give minimum amounts of hydrogenolysis in other systems.[154] The reduction of (107) was therefore investigated using a variety of reaction conditions and catalysts (Table 2.3).

Reduction No.	Catalyst	Pressure (atm.)	Solvent
1	5% $Pd/BaSO_4$	1	EtOH
2	5% $Pd/BaSO_4$	1	EtOH ($FeCl_3$ $6H_2O$)
3	5% $Pd/BaSO_4$	1	EtOAc ($FeCl_3$ $6H_2O$)
4	5% Rh/C	4	EtOH
5	5% Rh/C	4	EtOAc
6	5% Rh/Al_2O_3	4	EtOAc
7	$RhCl(PPh_3)_3$	1	EtOH

Table 2.3: Conditions used for Catalytic Reduction of (107)

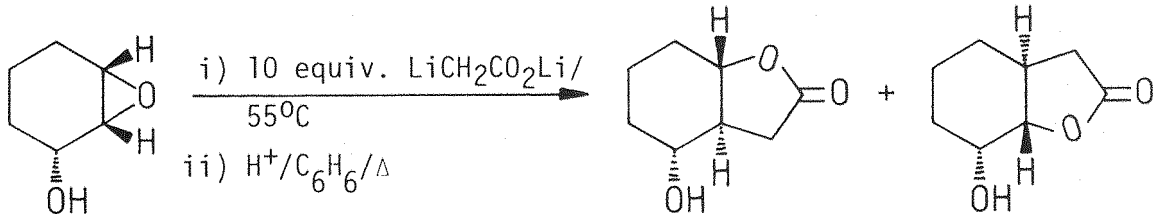
In reductions 1, 2 and 3 the fate of the starting material (107) was monitored by GC (5% OV101, 150^{o}-$220^{o}C$ at $4^{o}C\ min^{-1}$). This showed the appearance of a new peak, but this along with the starting material (107) diminished very rapidly. GC-MS of the reaction mixture showed that the new peak was the required keto lactone (102). In reductions 4,5 and 6 analysis of the reaction mixture by GC showed the presence of very little volatile material. NMR and IR analysis showed no evidence for the formation of keto lactone (102)but indicated that hydrogenolysis had occurred. Analysis of the products from reduction 7 showed that the enol lactone (107) had opened to give the starting diketo ester (105).

In view of the difficulties with hydrogenolysis in these reductions, it was decided to pursue a different approach to the required keto lactone (102).

2.2.2 Synthesis of Keto Lactone (102) *via* Epoxide Opening with Dilithioacetate

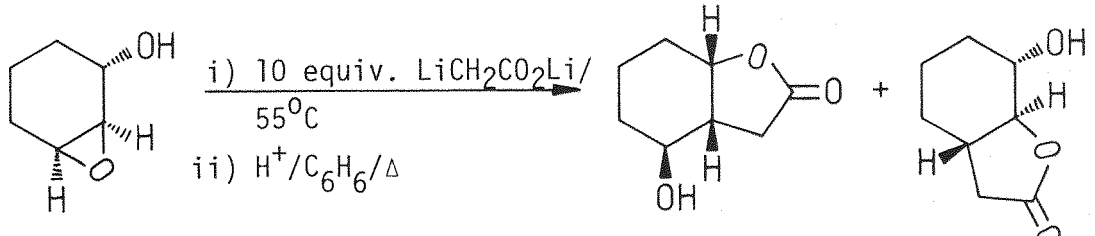
It was hoped that generation of the keto lactone (102) could be achieved *via* the method of Danishefsky *et al.*[141] which involved the opening of α-hydroxy epoxides with dilithioacetate (Schemes 2.13 and 2.14, see Section 2.1.5.3 (a)). The *cis* fused lactone (97) appeared

more attractive than the *trans* fused lactone (92) since it was anticipated that stereochemical control could be better effected in the former due to the 'butterfly' shape of this molecule.



(91) (92) (93)

Scheme 2.13

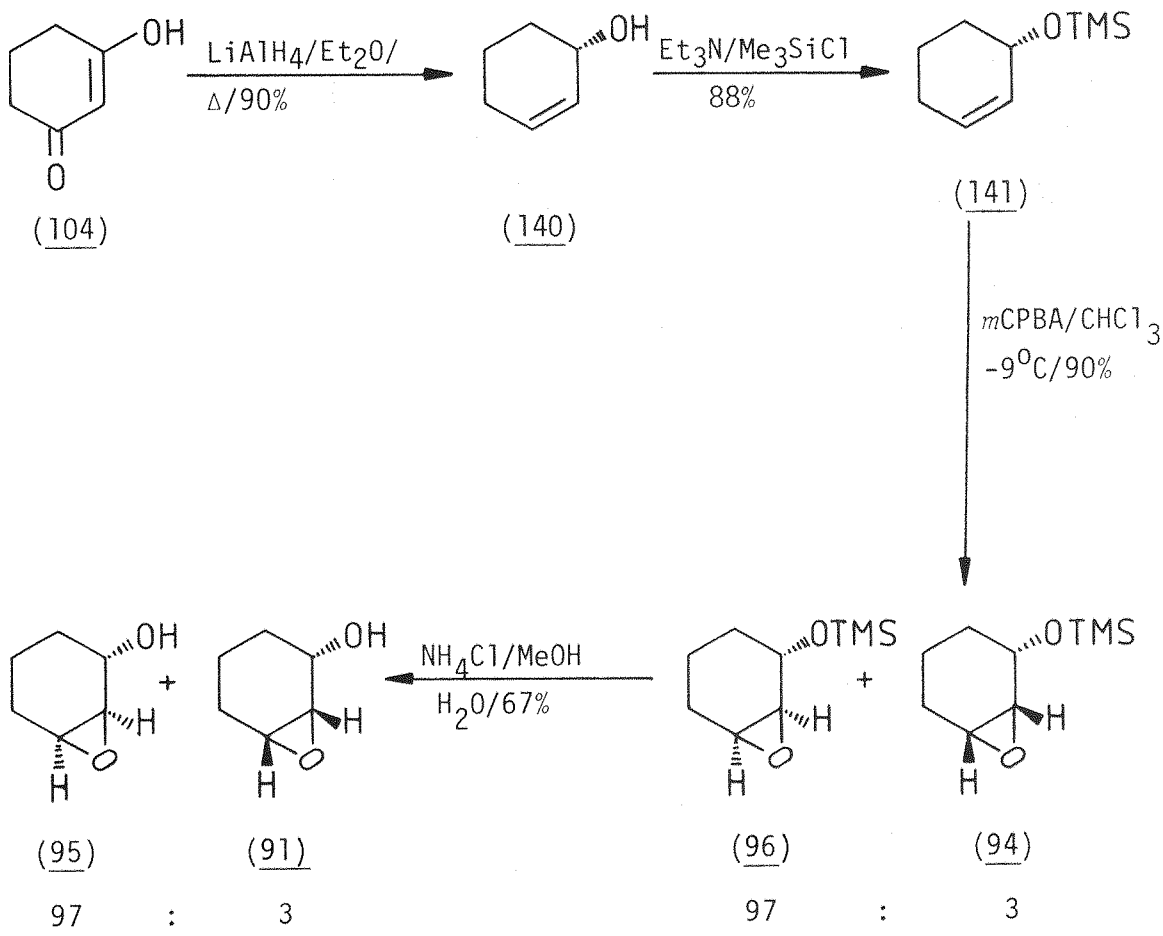


(95) (97) (98)

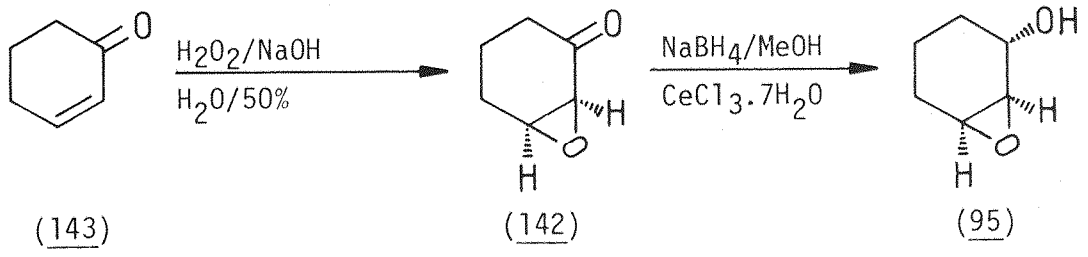
Scheme 2.14

2.2.2.1 Synthesis of *trans*-2,3-Epoxycyclohexan-1-ol (95)

The above methodology requires the synthesis of *trans*-2,3-epoxycyclohexan-1-ol (95) which could be obtained from 2-cyclohexen-1-ol (140) by a modification of the method of Chavdarian and Heathcock.[155] Thus, lithium aluminium hydride reduction of 1,3-cyclohexanedione (104) gave 2-cyclohexen-1-ol (140) in 90% yield. Subsequent protection of the hydroxyl group in (140) as the trimethylsilyl ether proceeded in 88% yield



Scheme 2.25



Scheme 2.26

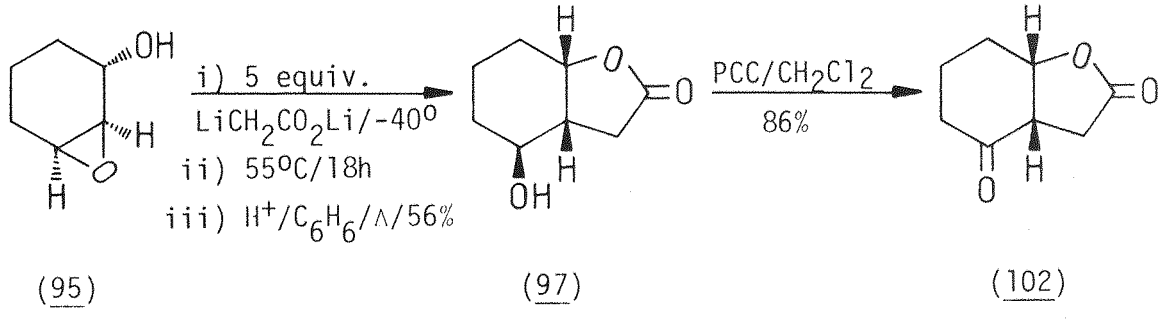
For clarity only one enantiomer is drawn in Schemes 2.25-2.35.

to give silyl ether (141). Epoxidation of (141) with *meta*-chloroperoxybenzoic acid at -9°C gave a 90% yield of *trans*-(2,3-epoxy)trimethylsiloxycyclohexane (90) and the *cis* isomer (94) in the ratio of 97:3 respectively. Deprotection gave the required *trans*-2,3-epoxycyclohexan-1-ol (95) contaminated with the *cis* isomer (91) in 67% combined yield (ratio 97:3 respectively) (Scheme 2.25).

A second synthesis of (95) was carried out which utilised the stereospecific reduction of 2,3-epoxycyclohexan-1-one (142) with sodium borohydride in the presence of cerium III chloride.[156] Thus, epoxidation of 2-cyclohexen-1-one (143) with alkaline hydrogen peroxide gave 2,3-epoxycyclohexan-1-one (143) in 50% yield.[157] Sodium borohydride reduction of (143) in methanolic cerium III chloride (0.4mol/l) afforded an 88% yield of (95) in greater than 97% GC purity (5% FFAP, 148°-200°C at 5°C min^{-1}) (Scheme 2.26).

2.2.2.2 Dilithioacetate Epoxide Opening of *trans*-2,3-Epoxycyclohexan-1-ol (95)

With *trans*-2,3-epoxycyclohexan-1-ol (95) in hand, an investigation of its reaction with dilithioacetate was carried out. Thus, treatment of (95) with five equivalents of dilithioacetate at -40°C followed by heating to 55°C for 18h gave after work up and lactonisation the hydroxy lactone (97) in 56% yield. In contrast to the work of Danishefsky *et al.*[141] (see Section 2.1.5.3 (a)), (97) was the sole product with no evidence for the formation of the regio isomer (98). The hydroxy lactone (97) was readily oxidised with pyridinium chlorochromate (PCC) in methylene chloride and gave the required keto lactone (102) in 86% yield (Scheme 2.27).



(95) (97) (102)

Scheme 2.27

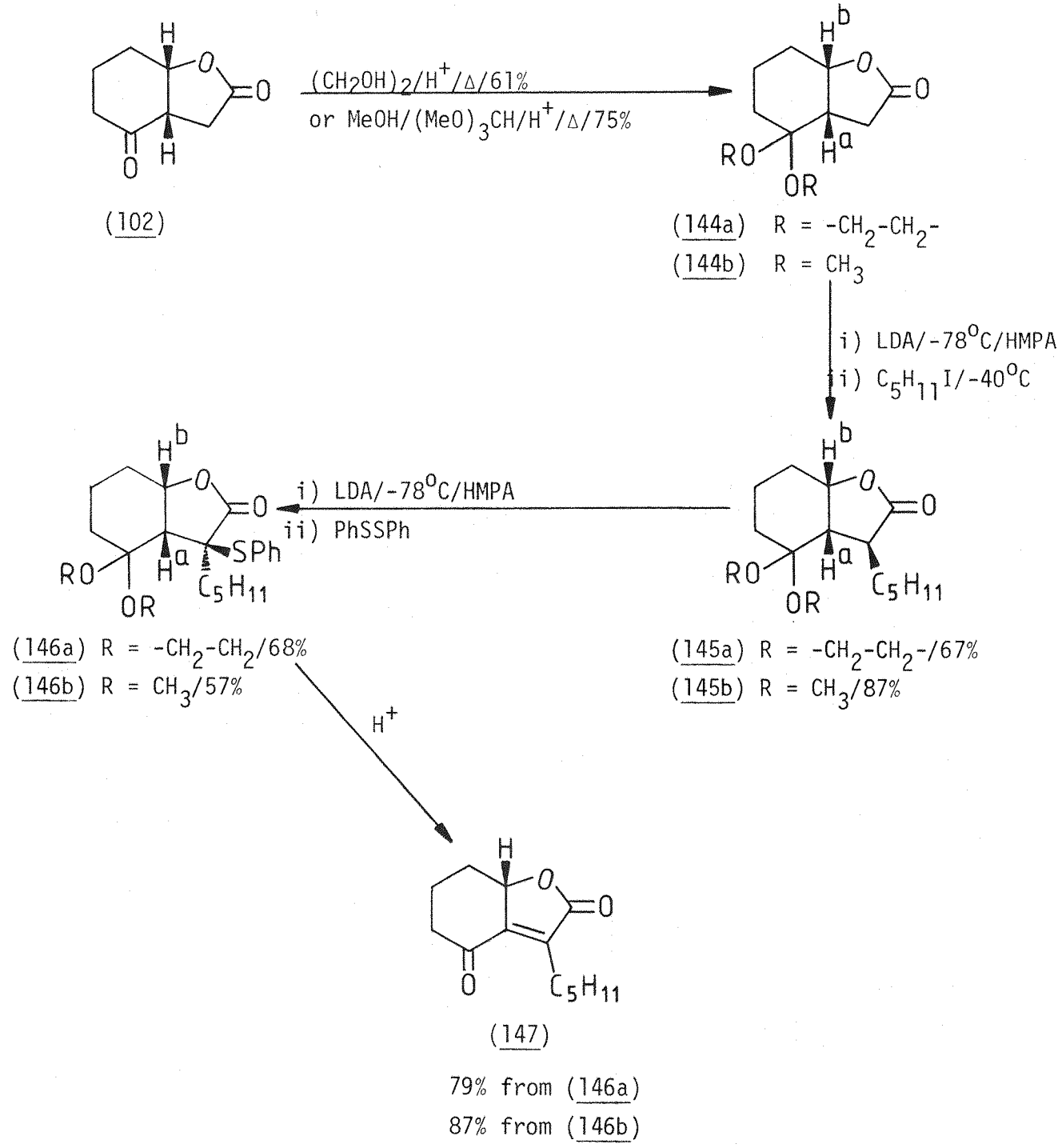
2.2.3 Modifcation of Carbocyclic Skeleton

With an efficient route to the keto lactone (102), studies on the synthetic strategies towards the paniculide (45a-c) could be carried out. These require the modification of the basic carbocyclic skeleton of (102) by introduction of an alkyl group on the lactone ring. In addition, a suitable leaving group must be introduced stereospecifically α to the lactone carbonyl to act as a latent $\Delta^{\alpha,\beta}$-butenolide olefin. The cyclohexyl ring requires the introduction of an allylic alcohol residue which on stereospecific epoxidation should give the *cis*-α-hydroxy epoxide moiety as required for the paniculide skeleton.

2.2.3.1 Protection of the Ketone Moiety in (102) as the Ethylene Acetal

With the above factors in mind, protection of the ketone group in (102) with ethylene glycol under standard Dean & Stark conditions gave the ethylene acetal (144a). Alkylation α to the lactone carbonyl in (144a) could be readily achieved by anion formation with LDA in the presence of HMPA followed by the addition of 1-iodopentane which afforded alkylated acetal (145a). Treatment of (145a) with LDA at -78°C followed by diphenyl disulphide at -20°C yielded the sulphenylated lactone (146a) (Scheme 2.28). The sulphenylated lactone (146a) was found to be homogeneous by TLC, GC (5% OV101, 182°-300° at 5°C min^{-1}) and 100MHz ^1H NMR and it was presumed that phenylsulphenylation had occurred from the β-face to give the *exo* sulphide (146a). Evidence for this specific β-face introduction of the phenylsulphenyl group came from an examination of the 100MHz ^1H NMR resonances of the ring junction protons, H^a and H^b in (145a) and (146a). On introduction of the phenylsulphenyl group the $H^a$and H^b protons shifted from δ2.37 and δ4.71 in (145a) to δ2.55 and δ5.45 respectively in (146a). If we have the *exo* sulphide (146a), then the ring junction protons H^a and H^b lie on the β-face along with the aromatic ring. These protons, therefore, lie in the ring current of the phenyl group and this causes a downfield shift. If, however, phenylsulphenylation had occurred from the α-face then the phenyl ring would not have such a large influence on these two protons.

Considerable difficulties were encountered in the removal of the acetal protecting group in (146a). Several conditions were employed in attempts to remove the acetal group:-



Scheme 2.28

Compound	H^a	H^b
(144a)	δ2.4-2.72	δ4.67
(145a)	δ2.37	δ4.71
(146a)	δ2.55	δ5.45
(144b)	δ2.8-3.08	δ4.62
(145b)	δ2.57-2.71	δ4.44-4.64
(146b)	δ2.48	δ5.06

Table 2.4: ^{1}H NMR Resonances of H^a,H^b in (144a,b)-(146a,b)

HCl (2N)/THF/18h.

HCl (2N)/THF/reflux/18h.

HCl (6N)/THF/18h.

Acetone/*p*TsOH/reflux/18h.

10% Oxalic acid/THF/reflux/18h.

All these reaction conditions failed to remove the acetal protecting group and starting material was recovered. However, treatment of (146a) with a 1:1 mixture of THF and concentrated hydrochloric acid yielded the keto lactone (147) which resulted from elimination of the phenylsulphenyl moiety in addition to removal of the acetal protecting group (Scheme 2.28).

It was thought that the strong aqueous acid conditions required for the removal of the ethylene acetal protecting group was responsible for the elimination of thiophenol. It was, therefore, anticipated that a ketone protecting group was required, which could be removed under milder conditions.

2.2.3.2 Protection of the Ketone Moiety in (102) as the Dimethyl Acetal

The dimethyl acetals are more readily hydrolysed by dilute acids than the corresponding ethylene acetals, hence it was decided to use the dimethyl acetal as the ketone protecting group. Thus, reaction of the keto lactone (102) with trimethylorthoformate in methanol at reflux afforded the dimethyl acetal (144b). As before the acetal (144b) could be alkylated by reaction with LDA in the presence of HMPA followed by treatment with 1-iodopentane which gave the alkylated lactone (145b). Exclusive β-face introduction of the phenylsulphenyl moiety was achieved by reaction of (145b) with LDA in the presence of HMPA followed by treatment with diphenyl disulphide which gave the homogeneous *exo* sulphide (146b). The evidence for this specific β-face introduction of the phenylsulphenyl group again came from an 100MHz ^{1}H NMR analysis of the ring junction protons H^a and H^b in (145b) and (146b) (Table 2.4). Acid hydrolysis of (146b) with 2N aqueous hydrochloric acid in THF yielded the keto lactone (147) again resulting from deprotection of the acetal and elimination of thiophenol (Scheme 2.28).

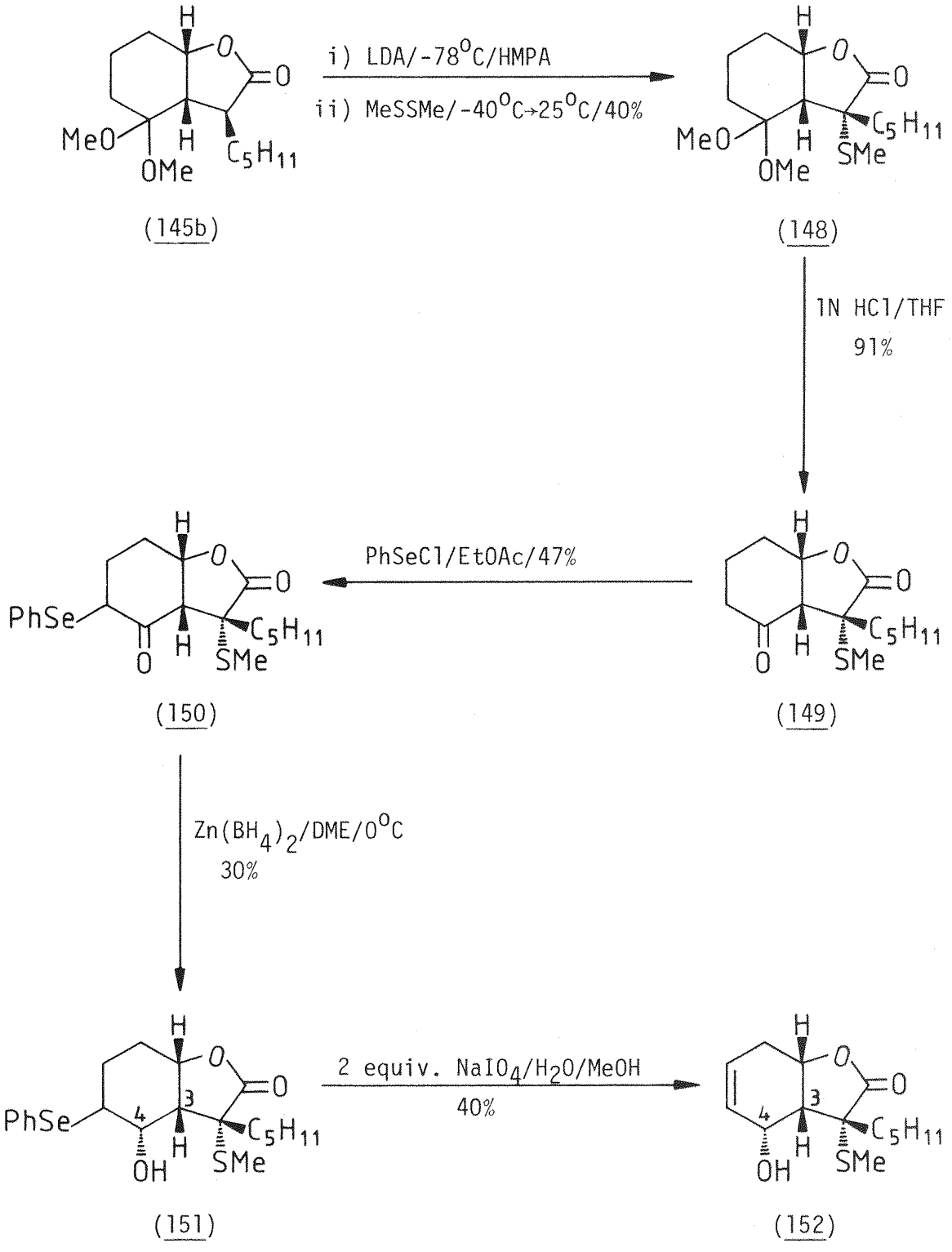
2.2.3.3 Use of an α-Methylthio Ether Moiety as a Latent Olefin

a) Chemical Transformations and X-ray Study

In view of the above problems with elimination of thiophenol on unmasking the protected ketone group in (146a) and (146b) it was decided to use a more stable α-alkylthio ether. Lactone enolates have been quenched with dimethyl disulphide to give the corresponding α-methylthio ethers.[136] These derivatives appeared attractive as it was anticipated that these would be more stable to acidic conditions.

The alkylated lactone (145b) was treated with LDA in the presence of HMPA to form the lactone enolate, subsequent quenching with dimethyl disulphide afforded the α-methylthio ether (148) (Scheme 2.29). In this case, however, it was not possible to carry out an ^{1}H NMR study of the bridgehead protons to assign the stereochemistry of methylsulphenylation. Consequently, the molecular structure of (148) had to be determined by a single crystal X-ray diffraction study. A single crystal of (148) obtained by recrystallisation from pentane was found to contain two crystallographically independent molecules (A and B) which had nearly identical geometry. Molecule A is shown in the ORTEP drawing (Figure 2.1) which shows that the α-methylthio group had been introduced exclusively from the α-face, giving the stereochemistry as shown. This result contrasts with the corresponding α-phenylthio ether (146b) where sulphenylation proceeded from the sterically less conjested β-face. In the reaction of the enolate derived from (145b) with diphenyl disulphide, the sulphenating agent can only approach from the β-face due to the steric bulk of this group, hence the *exo* sulphide (146b) predominates. In the case of the reaction between the enolate derived from (145b) and dimethyl disulphide, the sulphenating agent is sufficiently small enough to approach from either the α or β-face. The limiting factor, therefore, is the relative steric compression at the transition state. In the case of attack from the least hindered β-face the change from sp^2 to sp^3 hybridisation of the carbon atom α to the lactone carbonyl, causes steric compression between the relatively bulkyl pentyl chain and the cyclohexyl ring. In contrast, attack from the α-face results in a lower steric compression of the pentyl chain at the transtion state, hence the *endo* sulphide (148) predominates.

In view of the long delay experienced in obtaining the results of the X-ray structural determination of (148) further synthetic transformations on (148) were carried out as outlined in Scheme 2.29. Thus,



(145b) (148) (149) (150) (151) (152)

Scheme 2.29

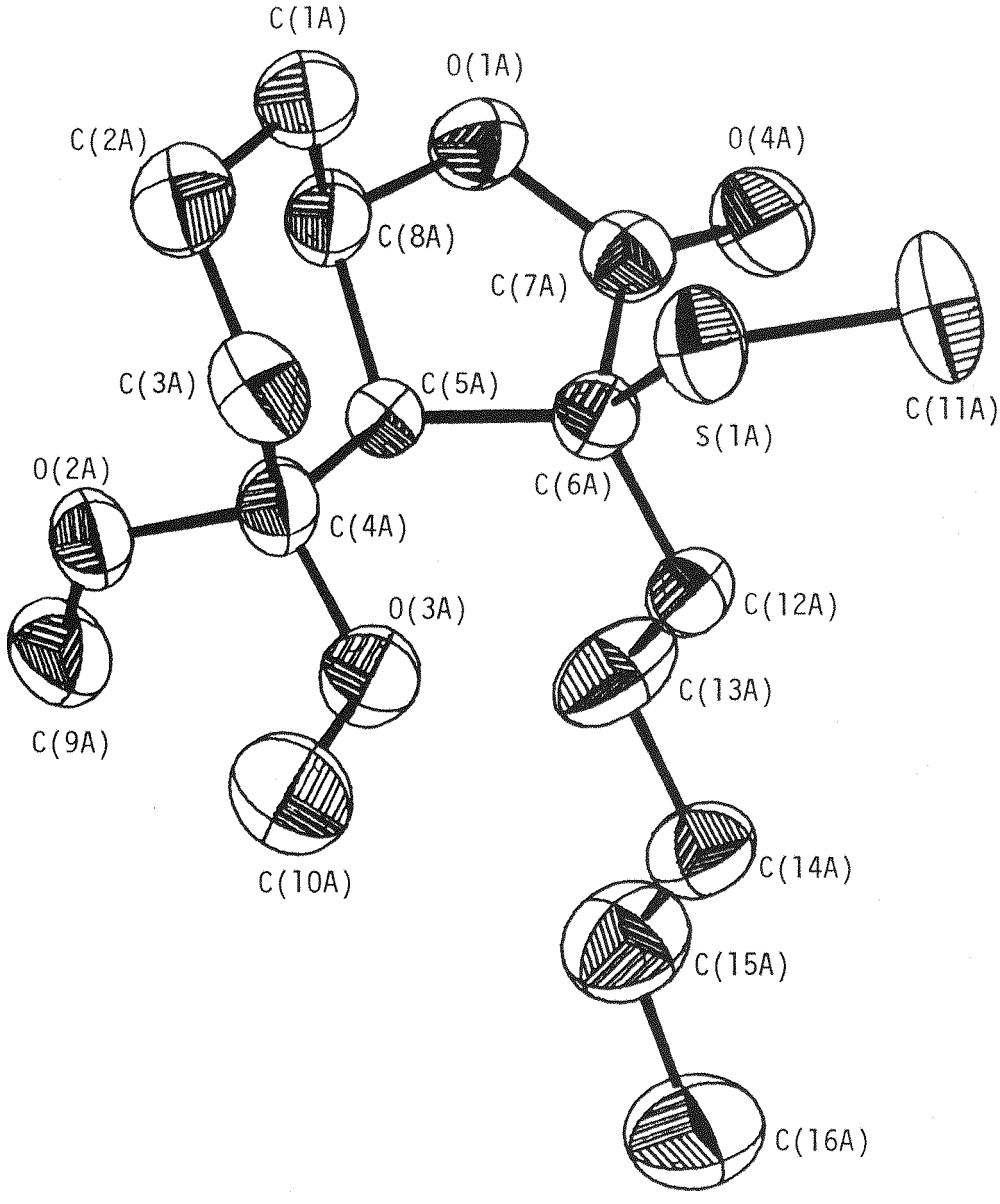


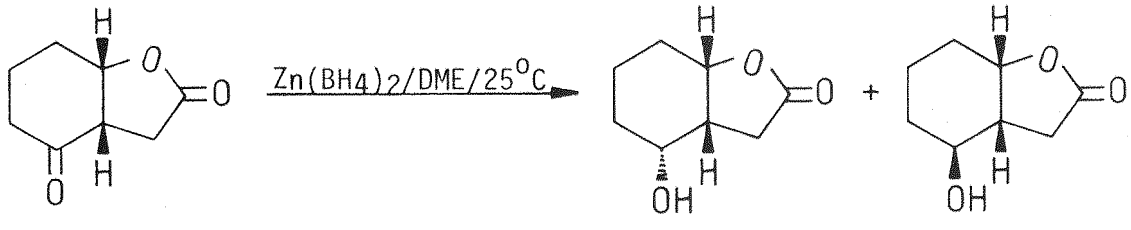
Figure 2.1: ORTEP Drawing of Molecule A of (148)
Showing 50% Probability Thermal Ellipsoids

deprotection of the acetal (148) was carried out under mild acid hydrolysis (1N HCl/THF) which afforded the keto lactone (149) without elimination of the α-methylthio ether moiety. Regiospecific introduction of the phenylselenyl residue was achieved by stirring (149) in ethyl acetate in the presence of phenylselenyl chloride which gave the selenide (150). Stereospecific reduction of (150) with zinc borohydride in dimethoxyethane (DME) yielded the seleno alcohol (151) which, on oxidative elimination of phenylselenic acid afforded the allylic alcohol (152).

It was at this stage that the structural determination of (148) was completed which indicated the *trans* relationship between the α-methylthio moiety and the adjacent methine (Figure 2.1). Consequently oxidative elimination of this group would result in the formation of an exocyclic olefin (see Section 2.1.5.2). In view of the 'incorrect' stereochemistry about the carbon α to the lactone carbonyl, it was decided to pursue a different approach to the modification of the carbocyclic skeleton.

b) Evidence for Stereochemistry of Hydroxy Group in (151) and (152)

It would be expected that hydride attack of (150) should occur from the least sterically conjested β-face to give (151) with the stereochemistry shown (Scheme 2.29). It has been reported that when (102) is treated with zinc borohydride predominant β-facial addition of hydride is observed to give a 1:2 mixture of (97) and (103) resulting from α and β-facial attack of borohydride respectively (Scheme 2.30).[144]

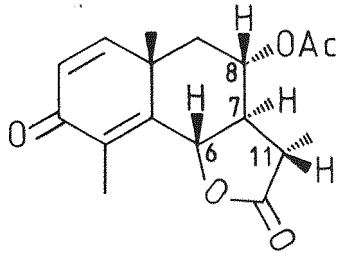


(102) (103) (97)

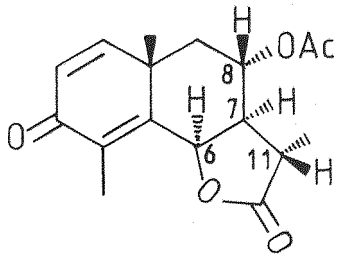
2 : 1

Scheme 2.30

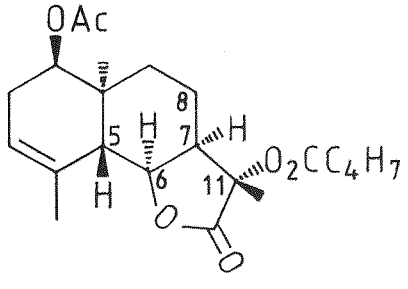
Because of the dependence of vicinal coupling constants on dihydral angles, spin-spin coupling data can be used to obtain stereochemical information. Artemisin acetate (153), which has the all *trans* relationship of the hydrogens as shown, has been found to have the following coupling constants; $J_{6,7}$ = 11.6Hz; $J_{8,9ax}$ = $J_{7,8}$ = 10.9Hz; $J_{7,11}$ = 11.5Hz.[158] On the other hand, in 6-epiartemisin acetate (154), $J_{6,7}$ = 5.7Hz; $J_{8,9ax}$ = $J_{8,7}$ = 10.5Hz.[158] Thus, in (153) where H-6 and H-7 are *trans* we have a coupling constant of ∿11Hz, while in (154) these protons are *cis* so we observe a coupling constant of ∿6Hz. This factor is further exemplified in eudesmanolide (155) where $J_{5,6}$ = 10.4Hz; $J_{6,7}$ = 7.3Hz; $J_{7,8}$ = 8.6Hz; $J_{7,8}$ = 6.0Hz.[159] The observation that protons with a *cis* relationship have coupling constants between 1 and 7Hz while those of a *trans* relationship lie between 8 and 13Hz have been used extensively to deduce the stereochemistry in these sytems.[160-164] Application of these factors to (151) and (152) where $J_{3,4}$ = 5Hz indicates that H-3 and H-4 are *cis* and consequently that the hydroxy group lies on the α-face, resulting from β-face addition of borohydride.



(153)



(154)



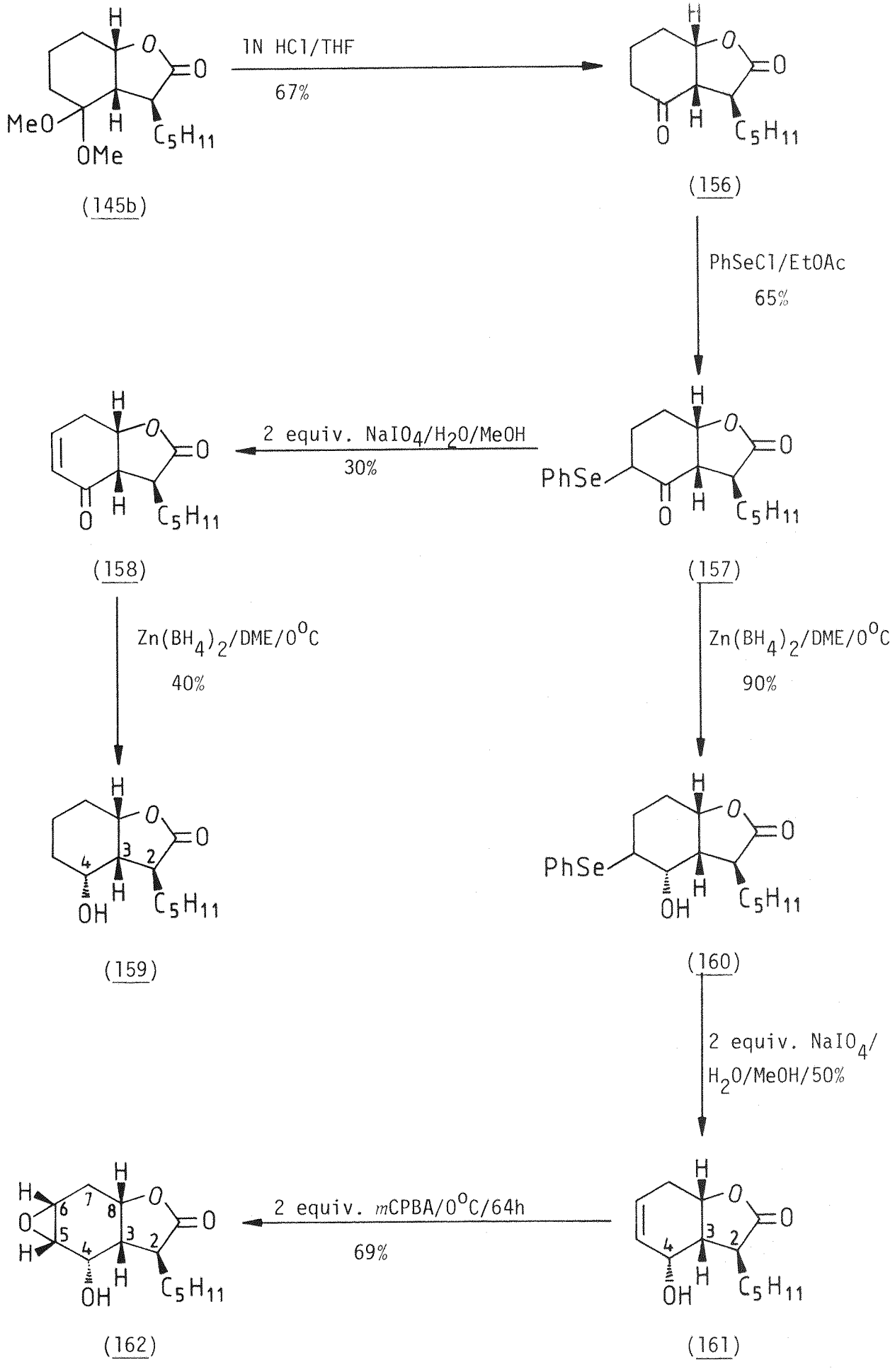
(155)

2.2.3.4 Synthetic Transformations of the Cyclohexyl Ring in (145b)

As a result of the difficulties with the α-phenylthio ethers (146a,b) and the α-methylthio ether (148) it was decided to first carry out the synthetic transformations on the cyclohexyl ring in (145b) and then introduce the latent $\Delta^{\alpha,\beta}$-butenolide olefin *via* the α-phenylsulphenyl or phenylseleno ether. The introduction of a phenylsulphenyl or phenylseleno residue at this later stage should proceed stereospecifically from the β-face. An additional advantage is that this approach would overcome the problem of carrying a labile group through several synthetic transformations. These modifications of the cyclohexyl ring in (145b) require the conversion of the acetal moiety to an allylic alcohol and then stereospecific epoxidation to introduce the *cis*-α-hydroxy epoxide moiety of the paniculides (45a-c).

a) Synthetic Studies on (145b)

The synthetic transformations carried out on the cyclohexyl ring of (145b) are shown in Scheme 2.31. Deprotection of the acetal group in (145b) with dilute acid (1N HCl/THF) afforded the keto lactone (156). Treatment of the keto lactone (156) with phenylselenyl chloride in ethyl acetate afforded (157) resulting from regiospecific introduction of the phenylselenyl moiety. Oxidation and syn elimination of the phenylselenyl residue yielded the enone (158), which on subsequent reduction with zinc borohydride in DME gave exclusively alcohol (159). In contrast to literature precedence,[165-168] the saturated alcohol (159) resulted from 1,4- followed by 1,2-addition of hydride to the enone system in (158). An analysis of the 100MHz ^{1}H NMR spectrum of (159) showed that $J_{3,4}$ = 6Hz, which indicated that H-3 and H-4 were *cis* to each other and hence we have the stereochemistry as shown. Thus, hydride addition had occurred exclusively from the β-face as expected. To overcome this problem the selenide (157) was reduced with zinc borohydride in DME which gave the alcohol (160). Subsequent oxidation and in situ elimination of phenylselenic acid afforded the allylic alcohol (161). The 100MHz ^{1}H NMR spectrum of (161) indicated that $J_{3,4}$ = 4Hz and, therefore, that the C-3 and C-4 protons have a *cis*-disposition, thus substantiating the stereochemistry of the C-4 hydroxy group as shown. Oxidation of (161) with *meta*-chloroperoxybenzoic acid at 0°C for 64h afforded epoxide (162) as the sole product.


1N HCl/THF
67%
(145b)
(156)
PhSeCl/EtOAc
65%
2 equiv. NaIO4/H2O/MeOH
30%
(158)
(157)
Zn(BH4)2/DME/0°C
40%
Zn(BH4)2/DME/0°C
90%
(159)
(160)
2 equiv. NaIO4/
H2O/MeOH/50%
2 equiv. mCPBA/0°C/64h
69%
(162)
(161)

Scheme 2.31

b) 100MHz ^{1}H NMR Spectrum of Epoxide (162)

The 100MHz ^{1}H NMR of epoxide (162) is shown in Table 2.5 and Figure 2.2. The one proton multiplet at δ4.7 and triplet at δ3.98 could be unambiguously assigned to H-8 and H-4 respectively on the grounds of their chemical shifts.[141,144,148] In a similar manner the multiplet at δ3.4 was assigned to the two epoxide methine protons H-5 and H-6.[169] The triplet at δ0.89 corresponded to the C-13 methyl group, while the broad envelope between δ1.1-1.8 resulted from the methylene protons on C-9 → C-12. Dilution of the sample resulted in an upfield shift of the δ2.95 doublet, thus this resonance corresponded to the hydroxyl proton which must, therefore, be coupled to the adjacent H-4 methine (J=7Hz).

The remaining assignments were made with the assistance of decoupling experiments. Thus, the multiplets at δ2.68 and δ2.31 were perturbed on irradiation at δ4.74 (H-8) while the signal at δ2.08 collapsed to a double doublet (J=16, >2Hz). This indicated that the resonances at δ2.68, 2.31 and 2.08 were coupled to H-8 and, therefore, must result from the methylene protons at C-7 and the H-3 methine. Irradiation at δ3.4 (H-5,6) collapsed the multiplet at δ2.68 to a double doublet (J=16,8Hz) which was superimposed on a multiplet. The signal at δ2.08 also collapsed to a double doublet (J=16,6Hz) on decoupling H-5,6.

Thus, decoupling the epoxide protons H-5,6 showed that they were coupled to protons with signals at δ2.08 and δ2.68, which were attributable to the two protons on C-7. It was concluded from this result that H-3 resonates at δ2.31. The fact that the signal at δ2.08 (J =16,6,<2Hz) collapsed to a double doublet (J =16,<2Hz) on irradiation at δ4.74 (H-8) indicated that it was coupled to H-8 with a coupling constant of 6Hz. It followed that the δ2.68 resonance which collapsed to a double doublet (J=16,8Hz) on decoupling H-5,6 must couple to H-8 with a coupling constant of 8Hz. It was clear from the aforegoing discussion on coupling constants between vicinal protons (see Section 2.2.3.3. (b)) ($J_{H,H\,cis}$ = 1-7Hz, $J_{H,H\,trans}$ = 8-13Hz), that the multiplet at δ2.68 must result from the C-7 proton *trans* to H-8 i.e. H-7α ($J_{7\alpha,8}$ = 8Hz) while the signal at δ2.08 resulted from the C-7 proton *cis* to H-8 i.e. H-7β ($J_{7\beta,8}$ = 6Hz).

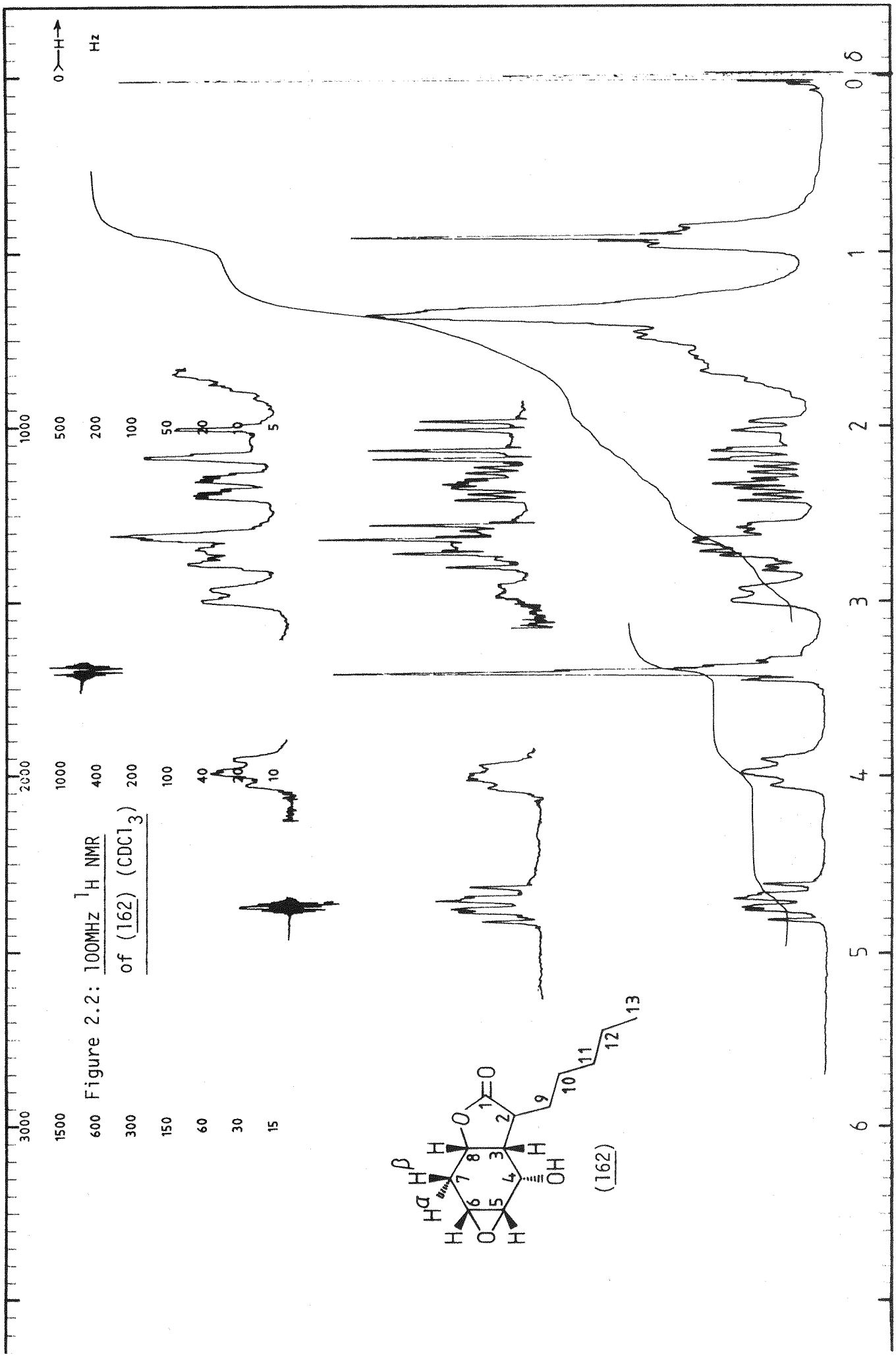


Figure 2.2: 100MHz ^{1}H NMR of (162) ($CDCl_3$)

Chemical Shift (δ)	Signal Pattern	Integration	Assignment
4.70	m	1H	H-8
3.98	t; J=7Hz	1H	H-4
3.4	m	2H	H-5,6
2.95	d; J=7Hz	1H	OH
2.68	m	2H	H-2, H-7α
2.31	m	1H	H-3
2.08	ddd; $J_{7\beta,7\alpha}$=16Hz; $J_{7\beta,8}$=6Hz; $J_{7\beta,6}$<2Hz	1H	H-7β
1.8-1.1	b, envelope	8H	C-9 → C-12 methylenes
0.89	t; $J_{12,13}$ = 6Hz	3H	C-13 Me

Table 2.5: 100MHz ^{1}H NMR Spectrum of (162)

Decoupling the H-4 proton (δ3.98) caused the doublet at δ2.95 (OH) to collapse to a singlet and the multiplet at δ2.31 (H-3) to be perturbed as expected. In addition irradiation at δ2.95 (OH) resulted in the collapse of the triplet at δ3.98 (H-4) to a doublet (J=7Hz); protons H-3 and H-4, therefore, were coupled to each other with a coupling constant of 7Hz. Irradiation at δ2.68 (H-7α) resulted in the signal at δ2.31 (H-3) to collapse to a triplet (J=7Hz). This result could be explained if H-2 and H-7α resonances coincide, as was anticipated for the expected shift of H-2 (∿ δ2.2-2.5). It, therefore, followed from this result that $J_{3,4} = J_{3,8} = 7Hz$. Decoupling H-7α (δ2.68) resulted in the collapse of the δ2.08 resonance to a double doublet (J=6, <2Hz) which indicated that $J_{7\alpha,7\beta} = 16Hz$ as anticipated.

c) Comments on the Stereochemistry of Epoxide (162)

In a study of a series of *cis*-α-hydroxy epoxides it was observed in the case of cyclohexyl and cyclopentyl rings that the ^1H NMR resonances of the epoxide methines were coincident.[169] In contrast in the corresponding *trans* case the ^1H NMR resonances of the epoxide methines were separated by approximately 0.2ppm.[169] In the 100MHz ^1H NMR of epoxide (162) (Figure 2.2, Table 2.5) the resonances of the epoxide methines H-5,6 occur as a sharp multiplet (δ3.4, J<2Hz). The signals due to these two protons are coincident and, therefore, the epoxide and hydroxyl groups must be *cis* to each other.

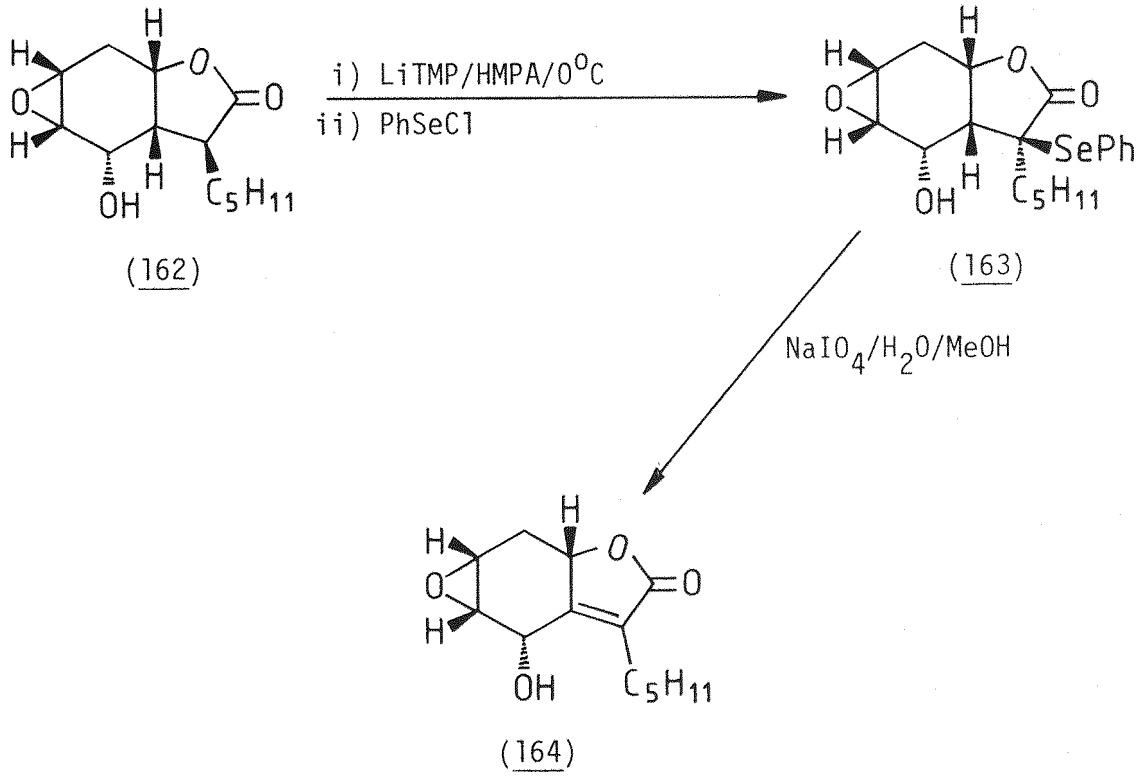
The 100MHz ^1H NMR of allylic alcohol (161) showed that $J_{3,4} = 4Hz$ from which it was deduced that the hydroxyl group lay on the α-face. If this is the case then the hydroxy moiety in (162) must have the same relative stereochemistry. Further evidence for this comes from the above decoupling experiments where it was observed that $J_{3,4} = 7Hz$, indicating that H-3 and H-4 are *cis* and, therefore, that we have the stereochemistry as shown.

It is hoped to determine the stereochemistry of (162) unequivocally with a single crystal X-ray structural determination. However, this is work which is in hand and, as yet, has not been completed.

2.3 Future Work

2.3.1 Model Work

In the model study all that remains to be done is the introduction of the $\Delta^{\alpha,\beta}$-butenolide olefin. It is envisaged that this would be achieved by stereospecific introduction of an α-phenylselenyl group in (162) *via* α-anion formation with lithium tetramethylpiperidine (LiTMP) and subsequent quenching with phenylselenyl chloride to give *exo* selenide (163). Oxidative syn elimination of phenylselenic acid from selenide (163) should give the $\Delta^{\alpha,\beta}$-butenolide (164) (Scheme 2.32) thus completing these studies. These transformations have, however, essentially been carried out in the two reported syntheses of paniculide A (45a) (see Sections 2.1.6.1 and 2.1.6.2).[130,156] Consequently, it is not strictly necessary to carry out these transformations since it can be regarded that the model work is formally complete.



(162) (163) (164)

Scheme 2.32

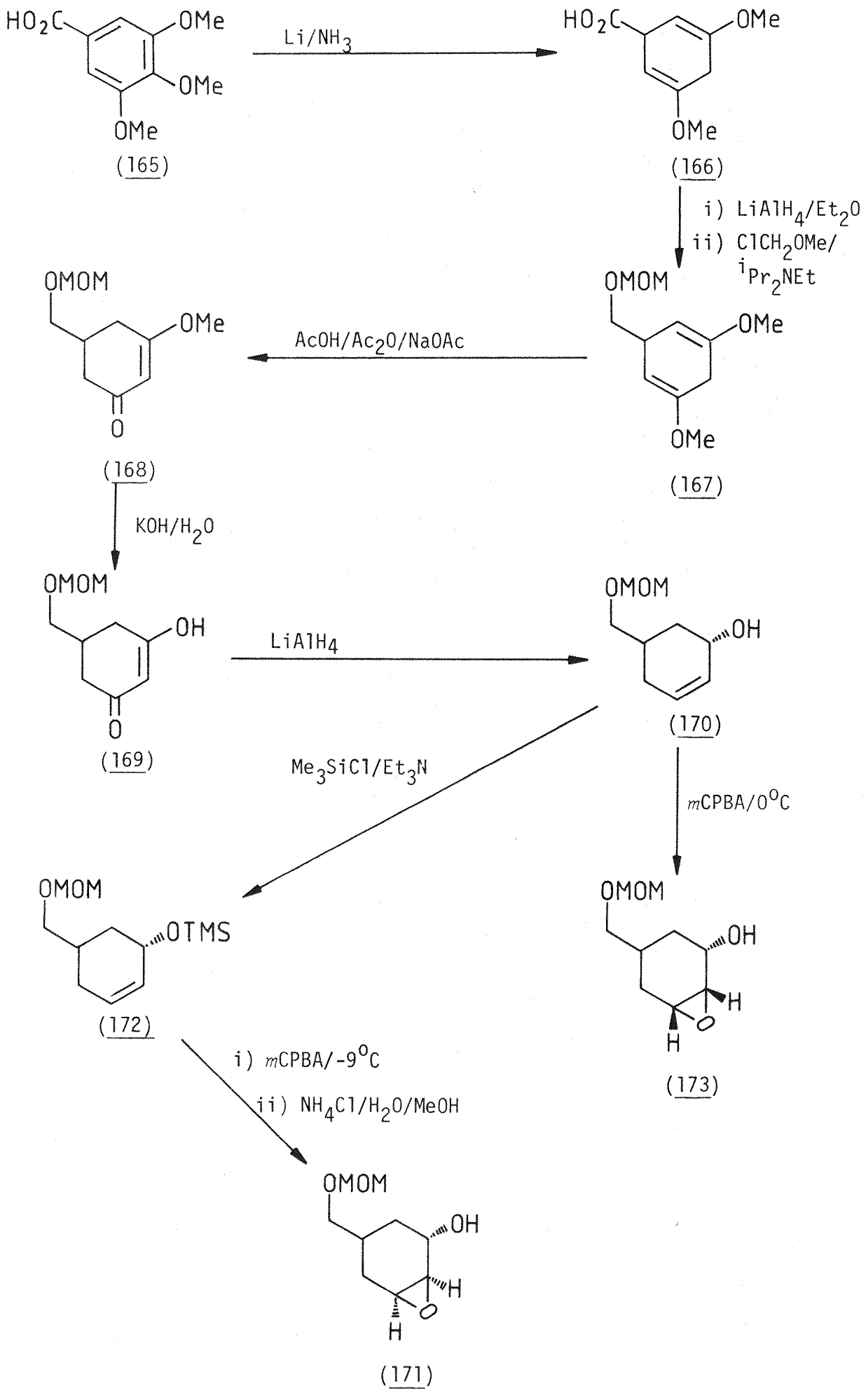
2.3.2 Proposed Synthesis of Paniculide B (45b) and C (45c)

It is hoped that the synthetic methods described in this thesis will be utilised in the total synthesis of paniculide B (45b) and C (45c).

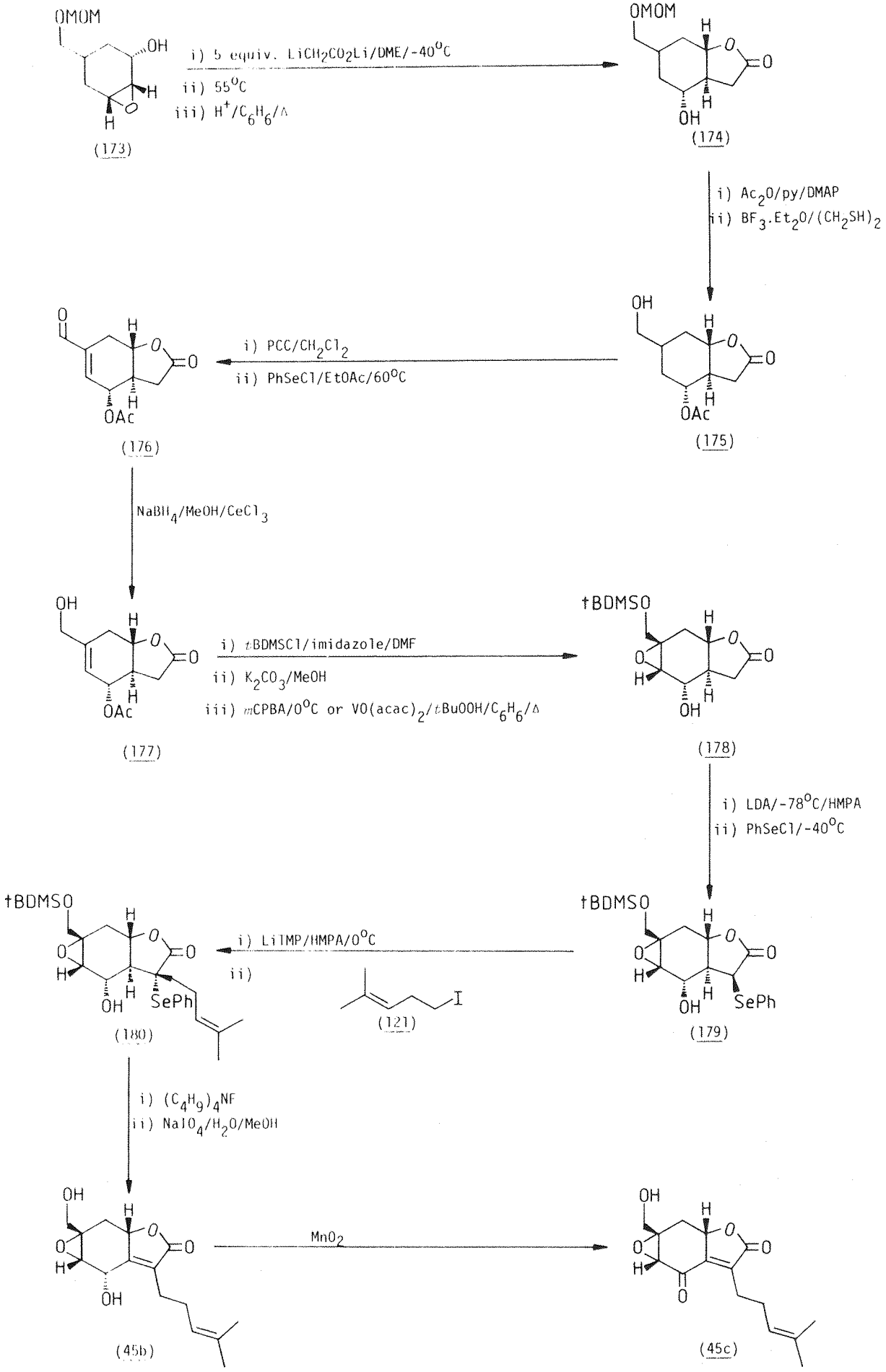
The proposed synthesis will be initiated by a Birch reduction of 3,4,5-trimethoxybenzoic acid (165) which should give 3,5-dimethoxy-1,4-dihydro benzoic acid (166).[170,171] Reduction of the acid residue in (166)[170] and protection of the resulting hydroxy methyl group with chloromethyl methyl ether would be expected to give the ether (167).[143] Solvolysis of the methoxy methyl ether (167) with acetic acid in the presence of acetic anhydride and sodium acetate should afford the vinylogous ester (168) which, on alkaline hydrolysis, would be expected to yield the 1,3-dione (169).[170] As in the model work, reduction of 1,3-dione (169) with lithium aluminium hydride should give the allylic alcohol (170). At this point there are two options open, either *trans*-α-hydroxy epoxide (171) can be prepared in a similar fashion to the model system (see Section 2.2.2.1) by protection as the trimethylsilyl ether (172) followed by epoxidation with *meta*-chloroperoxybenzoic acid and subsequent hydrolysis. Alternatively, the *cis*-α-hydroxy epoxide (173) can be prepared by epoxidation[169] of (170) with *meta*-chloroperoxybenzoic acid at 0°C (Scheme 2.33).

2.3.2.1. Use of *cis*-α-Hydroxy Epoxide (173)

The epoxides (171) and (173) can be used for two slightly different approaches to (±)-paniculides B (45b) and C (45c), the case for the *cis*-α-hydroxy epoxide (173) is outlined in Scheme 2.34. Thus, reaction of (173) with excess dilithioacetate and subsequent lactonisation would be predicted to give the *trans* fused lactone (174). Acetylation of the alcohol residue in (174) followed by removal of the methoxy methyl ether moiety using boron trifluoride etherate and ethanedithiol[143] should yield the alcohol (175). Oxidation of the primary alcohol residue in (175) with PCC and subsequent treatment with phenylselenyl chloride in ethyl acetate should give the unsaturated aldehyde (176).[143] Sodium borohydride reduction of (176) in the presence of cerium III chloride[172] would be anticipated to give allylic alcohol (177). Protection of the primary alcohol residue in (177) as the *tert*-butyldimethylsilyl ether and deprotection of the acetate moiety followed by stereospecific epoxidation using *meta*-chloroperoxybenzoic acid[169] or metal mediated epoxidation[151] should give the epoxide (178). Treatment of (178) with LDA in the presence of HMPA should afford the selenide (179). Alkylation of (179) by α-anion formation with LiTMP and quenching with 1-iodo-4-methyl-pent-3-ene (121) would be anticipated to give the alkylated lactone (180)


HO2C
OMe
OMe
OMe
(165)
Li/NH3
HO2C
OMe
OMe
(166)
i) LiAlH4/Et2O
ii) ClCH2OMe/
iPr2NEt
OMOM
OMe
OMe
(167)
AcOH/Ac2O/NaOAc
OMOM
OMe
O
(168)
KOH/H2O
OMOM
OH
O
(169)
LiAlH4
OMOM
OH
(170)
Me3SiCl/Et3N
mCPBA/0°C
OMOM
OTMS
(172)
OMOM
OH
H
O
H
(173)
i) mCPBA/-9°C
ii) NH4Cl/H2O/MeOH
OMOM
OH
H
O
H
(171)

Scheme 2.33


OMOM
OH
(173)
i) 5 equiv. $LiCH_2CO_2Li$/DME/-40°C
ii) 55°C
iii) $H^+/C_6H_6/\Delta$
OMOM
(174)
i) Ac_2O/py/DMAP
ii) $BF_3.Et_2O/(CH_2SH)_2$
OH
OAc
(175)
i) PCC/CH_2Cl_2
ii) PhSeCl/EtOAc/60°C
(176)
$NaBH_4$/MeOH/$CeCl_3$
(177)
i) tBDMSCl/imidazole/DMF
ii) K_2CO_3/MeOH
iii) mCPBA/0°C or $VO(acac)_2/t$BuOOH/C_6H_6/Δ
tBDMSO
(178)
i) LDA/-78°C/HMPA
ii) PhSeCl/-40°C
tBDMSO
SePh
(179)
i) LiTMP/HMPA/0°C
ii)
I
(121)
(180)
i) $(C_4H_9)_4NF$
ii) $NaIO_4/H_2O$/MeOH
(45b)
MnO_2
(45c)

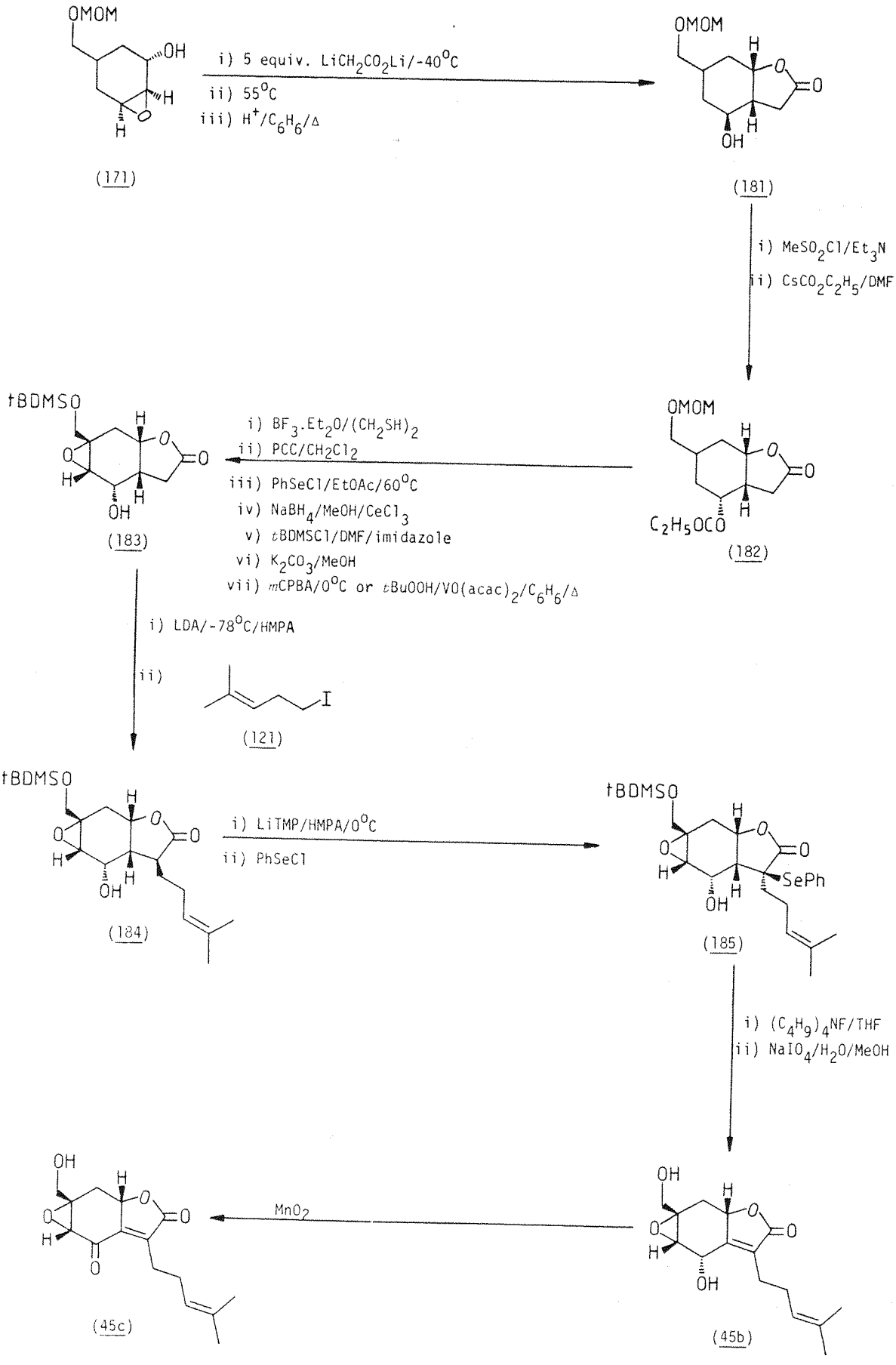
Scheme 2.34

stereospecifically. Desilylation of (180) with tetrabutylammonium fluoride and subsequent oxidative syn elimination of phenylselenic acid would be expected to afford (±)-paniculide B (45b), which could be converted to (±)-paniculide C (45c) by mangenese dioxide oxidation.[123,125]

2.3.2.2 Use of *trans*-α-Hydroxy Epoxide (171)

An alternative strategy to (±)-paniculide B (45b) and C (45c) which involves the use of *trans*-α-hydroxy epoxide (171) is outlined in Scheme 2.35. Thus, reaction of epoxide (171) with excess dilithioacetate and subsequent lactonisation should yield the *cis* fused lactone (181). Unfortunately, the secondary alcohol function in (181) has the incorrect stereochemistry necessary for a synthesis of (±)-paniculide B (45b) and C (45c) and, therefore, requires inversion. The inversion can be carried out by treatment of (181) with methanesulphonyl chloride in triethylamine and subsequent reaction with cesium propionate in DMF[173] which should afford the propionate (182) with the correct relative stereochemistry. The subsequent transformations of propionate (182) to epoxide (183) are identical to the above case for the conversion of *trans* fused lactone (174) to epoxide (178) (Scheme 2.34). Alkylation of epoxide (183) with 1-iodo-4-methylpent-3-ene (121) *via* α-anion formation with LDA in the presence of HMPA would be predicted to yield the alkylated lactone (184). Phenylselenylation of (184) by α-anion formation with LiTMP in the presence of HMPA at $0^{o}C$ followed by quenching with phenylselenyl chloride would be anticipated to afford, stereospecifically, the selenide (185). As for the *trans* fused lactone (180) desilylation of (185) and oxidative syn elimination of the phenylselenyl residue would be expected to yield (±)-paniculide B (45b) which, on oxidation with manganese dioxide should give (±)-paniculide C (45c).[123,125]

This approach is slightly longer than the route involving the *trans* fused lactones (174)→(180) (Scheme 2.34) and requires two extra steps in the preparation of the α-hydroxy epoxide (171) (Scheme 2.33). In addition, the alcohol (181) requires a two step inversion to propionate (182) to generate the correct relative stereochemistry for the paniculide skeleton (Scheme 2.35). However, this route has the advantage that the stereochemical considerations of alkylation and phenylselenylation are better defined due to the 'butterfly' shape of the *cis* fused lactone (183). It is envisaged that both these routes will be attempted in future synthetic studies on paniculide B (45b) and C (45c).


OMOM
OH
H
O
(171)
i) 5 equiv. $LiCH_2CO_2Li$/-40°C
ii) 55°C
iii) $H^+/C_6H_6/\Delta$
OMOM
H
O
O
OH
H
(181)
i) $MeSO_2Cl/Et_3N$
ii) $CsCO_2C_2H_5$/DMF
tBDMSO
OMOM
C_2H_5OCO
(182)
i) $BF_3.Et_2O/(CH_2SH)_2$
ii) PCC/CH_2Cl_2
iii) PhSeCl/EtOAc/60°C
iv) $NaBH_4$/MeOH/$CeCl_3$
v) tBDMSCl/DMF/imidazole
vi) K_2CO_3/MeOH
vii) mCPBA/0°C or tBuOOH/$VO(acac)_2/C_6H_6/\Delta$
(183)
i) LDA/-78°C/HMPA
ii)
I
(121)
tBDMSO
(184)
i) LiTMP/HMPA/0°C
ii) PhSeCl
tBDMSO
SePh
(185)
i) $(C_4H_9)_4NF$/THF
ii) $NaIO_4/H_2O$/MeOH
OH
(45b)
MnO_2
OH
(45c)

Scheme 2.35

2.4 Conclusion

Model studies directed towards the design of a total synthesis for paniculides B (45b) and C (45c) have been carried out. Initial problems in obtaining the required carbocyclic skeleton was overcome by reaction of *trans*-2,3-epoxycyclohexan-1-ol (95) with excess dilithioacetate which gave regiospecifically and in good yield (3aβ,4β,7aβ)-(±)-hexahydro-4-hydroxy-2(3H)-benzofuranone (97) after lactonisation. Further modifications of the basic carbocyclic skeleton in (97) were carried out, including the introduction of a *cis*-α-hydroxy epoxide moiety into the cyclohexyl ring. However, several problems were encountered in attempts to introduce a latent $\Delta^{\alpha,\beta}$-butenolide olefin. Elimination of thiophenol was a major problem when the α-phenylthio ether was used as a masked olefin. On changing to the α-methylthio ether, sulphenylation occurred specifically from the sterically conjested α-face to generate the incorrect stereochemistry about the carbon α to the lactone carbonyl. This specific α-facial introduction of the methylthio residue generated an anti relationship between the thio ether and the adjacent methine, consequently oxidation and syn elimination of methylsulphenic acid would have given an α-methylene-γ-butyrolactone and not the required $\Delta^{\alpha,\beta}$-butenolide. It was, therefore, concluded that the introduction of a latent $\Delta^{\alpha,\beta}$-butenolide olefin should be left to a later stage where problems with elimination would not arise. The use of a phenylselenyl or phenylsulphenyl residue at this stage would also overcome the problem of α-facial attack.

Two syntheses of paniculide B (45b) were proposed on the basis of this model work, both of which start from the readily available 3,4,5-trimethoxybenzoic acid (165). These two syntheses of paniculide B (45b) would also constitute a formal total synthesis of paniculide C (45c) since mild oxidation of the former yields the latter.

2.5 Experimental

2.5.1 Purification of Reagents and Solvents

Dry reagents and solvents were prepared as outlined in Table 2.6. In addition, acetic acid was purified by heating under reflux with 2-5% potassium permanganate followed by distillation. Traces of water were removed by treatment with triacetyl borate. This was prepared by heating boric acid and acetic anhydride (1 part to 5 respectively) at

Table 2.6: Methods Used for Drying Reagents and Solvents

Reagent/Solvent	Drying Agent
Benzene[a]	Na
Diisopropylamine[b,c]	CaH_2
Dimethoxyethane[d]	$CaH_2/LiAlH_4$
Dimethyl disulphide[c]	CaH_2
Diphenyl disulphide[e]	P_2O_5
Ethanol	Mg/I_2
Ether[a]	Na
Ethyl acetate	P_2O_5
Ethyl bromoacetate[f]	-
Ethylene glycol	1% Na
Hexamethylphosphoramide[g]	CaH_2
1-Iodopentane[g]	CaH_2
Methanol	Mg/I_2
Methylene chloride[h]	Al_2O_3
Tetrahydrofuran[b]	Na/benzophenone
Toluene[a]	Na
Triethylamine[b,c]	CaH_2

a) Solvent treated with sodium wire and left to stand for 24h.

b) Pre-dried over potassium hydroxide.

c) Distilled from calcium hydride at atmospheric pressure, under nitrogen.

d) Stirred over calcium hydride for 16h, then heated under reflux with added lithium aluminium hydride.

e) Dried over phosphorus pentoxide at 0.1mm Hg.

f) Distilled at reduced pressure, 58^{o}-60^{o}C at 15mm Hg.

g) Distilled from calcium hydride at reduced pressure.

h) Passed through a column of basic alumina, Grade 1.

60°C; cooling and filtration afforded white crystals of triacetyl borate. The acetic acid was heated with 2-3 fold excess of borate estimated for reaction with the water present; distillation under nitrogen gave anhydrous acetic acid.[142b]

All solvents used for purification by chromatography or recrystallisation were distilled prior to use.

'Ether' in all cases refers to diethyl ether and petroleum ether corresponds to the fraction boiling between 40°-60°C.

2.5.2 General Procedures

Removal of solvents was carried out by evaporation at reduced pressure (ca. 15mm Hg) using a rotary evaporator.

'Flash' column chromatography was performed according to the procedure of Still *et al.*[174] using Macherey-Nagal Kieselgel 60 (230-400 mesh) with the solvents described. Analytical TLC was carried out on pre-coated silica gel plates (Merck Kieselgel 60F (254)) and were visualised by UV fluorescence or spraying with an aqueous solution of potassium permanganate, methanolic 2,4-dinitrophenylhydrazine or vanillin in methanolic sulphuric acid.

2.5.3 Instrumentation

Gas chromatography (GC) was carried out on a 3.2mm x 10' column of 5% FFAP on diatomite AAW/DMCS (100-120 mesh) between 80°-240°C or a similar column of 5% OV101 on diatomite CLQ (100-120 mesh) between 70°-300°C. All other conditions were as described previously.

Gas chromatography - mass spectrometry (GC-MS) was carried out essentially as described previously except the GC-MS interface was maintained at 250°C with a source temperature of 100°C using the above GC columns. Probe mass spectra were also recorded on the same spectrometer under the same conditions as above. Chemical ionization mass spectra (CI-MS) were obtained on the same spectrometer in the CI mode with ammonia as the reagent gas.

Infra-red spectra (IR) were recorded using a Perkin-Elmer 157G grating spectrophotometer as thin films on sodium chloride plates or as 10% solutions in chloroform or carbon tetrachloride in sodium chloride solution cells (cell thickness = 0.1mm). The absorbtion bands are given in wavenumbers (cm^{-1}) relative to a polystyrene standard and are described with the following abbreviations; s = strong, m = medium, w = weak, b = broad.

^1H NMR spectra were obtained at 60MHz using a Perkin-Elmer R-12A spectrometer or a Hitachi-Perkin-Elmer R-24B high resolution spectrometer, 100MHz ^1H NMR were recorded using a Varian Associates XL-100-12 (deuterium lock) spectrometer. Tetramethylsilane was used an an external standard for organosilicon compounds and as an internal standard in all other cases. Peak positions are quoted on the δ scale relative to tetramethylsilane (zero) using the following abbreviations: s = singlet, d = doublet, t = triplet, dd = double doublet, q = quartet, dt = doublet of triplets, ddd = double doublet of doublets, m = multiplet, b = broad. Coupling constants, J, are expressed in hertz.

Melting points were determined using an electrothermal electrically heated block or a Reichert Koffler hot stage melting point apparatus and are uncorrected.

Elemental analyses were carried out at the micro-analytical laboratory, University College, London.

2.5.4 Experimental Procedures

2.5.4.1 2,6-Dioxocyclohexylacetic acid, ethyl ester (105)

2,6-Dioxocyclohexylacetic acid, ethyl ester (105) was prepared from 1,3-cyclohexanedione (104) by the method of Stetter *et al.*[145] Recrystallisation from water gave white needles (43%), m.p. 84.5° - 86°C (Lit.[145] m.p. 85°C).

2.5.4.2 2-Ethoxy-6-oxocyclohex-1-enylacetic acid, ethyl ester (137a)

A solution of 2,6-dioxocyclohexylacetic acid, ethyl ester (105) (4.1g, 20.7mmol) and *p*-toluenesulphonic acid (0.24g) in dry benzene (90ml) and dry ethanol (50ml) was heated under reflux with the azeotropic

removal of water (azeotrope removed at a rate of 10ml/h) until the temperature of the vapour reached 78°C (ca. 8h). The remaining solvent was removed by evaporation and the residue dissolved in benzene (75ml). The resulting solution was washed with 10% aqueous sodium hydroxide solution (35ml) and then with water until the washings were neutral (x5,35ml) and dried ($MgSO_4$). Removal of the solvent gave an orange oil (3.1g) which was purified by Kugelrohr distillation (139°-142°C at 0.075mm Hg, Lit.[175] b.p. 80°-82°C at 0.02,, Hg) to give (137a) as a light yellow oil (3.04g, 13.45mmol, 65%).

2.5.4.3 Sodium borohydride reduction of (137a)

2-Ethoxy-6-oxocyclohex-1-enylacetic acid, ethyl ester (137a) was subjected to a variety of sodium borohydride reduction conditions as outlined in Table 2.2 (see Section 2.2.1.1), two typical procedures are presented here.

(a) Procedure 1

To a stirred solution of 2-ethoxy-6-oxocyclohex-1-enylacetic acid, ethyl ester (137a) (1g, 4.42mmol) in methanol (22ml) was added slowly sodium borohydride (168mg, 4.39mmol) at 0°C. This solution was stirred at 0°C for 2h whereupon the solvent was evaporated and the residue taken up in water (20ml) which was extracted with ether (x3, 20ml). The organic extracts were dried ($MgSO_4$) and then concentrated to give a clear oil. Purification by flash column chromatography (ether/petroleum ether 9:1)† gave hexahydro-4-ethoxy-2(3H)-benzofuranone (138a) (191.2mg, 1.04mmol, 24%) as a colourless oil. High resolution gas chromatography mass spectrometry (5% FFAP, 245°C) gave $C_{10}H_{16}O_3$ for m/z 184.1045 (calc. m/z = 184.1099).

^{1}H NMR (60MHz, CCl_4): 1.15(t,J=8Hz,3H,CH_3), 1.4-1.95(b envelope, 6H,3xCH_2), 2.25-2.4(m,1H,$\underline{CH}CH_2CO_2$-), 2.55-2.9(m,2H,$\underline{CH_2}CO_2$-), 3.2-3.7(m,3H, $CH_3\underline{CH_2}O$ +$\underline{CH}OEt$), 4.5 (q,J=5Hz,1H,$\underline{CH}O_2CCH_2$-).

IR (thin film): 1780(s,C=O lactone).

GC-MS (5% FFAP,245°C,m/z): 184(M^+,13%), 138(M-EtOH,41%), 85(100%), 67 (37%), 57(99%), 55(40%), 44(35%), 43(30%), 41 (50%).

† Under these conditions (137a) remained on the baseline and was not recovered.

(b) Procedure 2

To a stirred solution of 2-ethoxy-6-oxocyclohex-1-enylacetic acid, ethyl ester (137a) (0.5g, 2.2mmol) in ethanol (10ml) at 0°C was added slowly sodium borohydride (85.6mg, 2.2mmol). This solution was stirred at 0°C for 1h then at room temperature for 16h. Workup and purification, as above, gave (138a) (60.6mg, 0.33mmol, 15%) and an unknown (139a) (77.6mg) as colourless oils.

Spectral Details of (139a)

^{1}H NMR (60MHz, CCl_4): 1.25(t,J=8Hz,CH_3), 1.1-2.1(b envelope), 2.25-2.65(m), 4.2(q,J=8Hz,CH_2CH_3).

IR (thin film): 3700-3100(bs,OH), 1780(s,C=O lactone), 1730(s,C=O).

GC-MS(5%FFAP,245°C,m/z): 81(70%), 68(40%), 67(69%), 57(56%), 55(85%), 43(55%), 41(100%), 39(57%).

2.5.4.4 2-Isobutoxy-6-oxocyclohex-1-enylacetic acid, isobutyl ester (137b)

A solution of 2,6-dioxocyclohexylacetic acid, ethyl ester (105) (8.8g, 4.44mmol) and *p*-toluenesulphonic acid (0.12g) in benzene (80ml) and isobutyl alcohol (13.2g, 17.8mmol) was heated under reflux for 22h with the azeotropic removal of water. Evaporation of the solvent afforded an orange oil which was dissolved in benzene (100ml), washed with 10% aqueous sodium hydroxide solution (100ml) and then with water until the washings were neutral (x3, 100ml). The resulting organic extract was dried ($MgSO_4$) and evaporation of the solvent yielded an orange oil (11.39g) which was distilled (156-162°C at 0.02mm Hg) to give (137b) (10.62g, 37.7mmol, 85%) as a colourless oil. High resolution mass spectrometry gave $C_{16}H_{26}O_4$ for m/z 282.1924 (calc. m/z = 282.1831).

^{1}H NMR (60MHz, CCl_4): 0.9(d,J=7Hz,6H,2xCHCH_3), 0.95(d,J=7Hz,6H, 2xCHCH_3), 1.8-2.3(b envelope,6H,2xCHCH_3 + 2xCH_2), 2.6(t,J=6Hz,2H,CH_2COC=C), 3.15(s, 2H,CH_2CO_2iBu), 3.75(d,J=7Hz,2H,OCH_2CH$(CH_3)_2$), 3.8(d,J=7Hz,2H,OCH_2CH$(CH_3)_2$).

IR (thin film): 1745(s,C=O ester), 1650(s,C=O enone), 1615(s,C=C).

MS (probe,m/z): 282(M^+,9%), 226(M-56,4%), 153(34%), 152(56%), 126(42%), 125(56%), 98(12%), 57(100%), 55(17%), 41(52%)

2.5.4.5 Sodium Borohydride Reduction of (137b)

To a stirred solution of sodium borohydride (124mg, 3.28mmol) in methanol (12.5ml) and 0.1M aqueous hydroxide solution (0.5ml) at 0^oC was added dropwise 2-isobutoxy-6-oxocyclohex-1-enylacetic acid, isobutyl ester (137b) (826mg, 2.93mmol). The resulting solution was allowed to warm slowly to room temperature and then stirred overnight. This solution was then cooled to 0^oC and a further portion of sodium borohydride (120mg, 3.17mmol) was added slowly and then stirred at room temperature for 16h. The solvent was evaporated and the residue was taken up in water (10ml) and then extracted with chloroform (x3, 10ml). The combined organic extracts were concentrated to ca. 5ml to which was added ice (5g) and then concentrated sulphuric acid (1ml). The resulting mixture was stirred for 1h and then extracted with chloroform (x3, 10ml) and the combined extracts were dried over magnesium sulphate. Evaporation of the solvent gave a yellow oil (370mg) which was purified by flash column chromatography (ether/petroleum ether, 1:1) to give hexahydro-4-isobutoxy-2(3H)-benzofuranone (138b) (76mg, 0.36mmol, 12%) as a colourless oil

^{1}H NMR (60MHz, $CDCl_3$): 0.9(d,J=7Hz,6H,2xCHC$\underline{H}_3$), 1.3-2.0(b envelope, 7H,3xCH_2 + $CH_2C\underline{H}(CH_3)_2$), 2.35-2.75(m,3H, $C\underline{H}_2CO_2$ + $C\underline{H}CH_2CO_2$), 3.1 and 3.25(2xd, J=7Hz,2H,O$C\underline{H}_2$CH(Me)$_2$), 3.4-3.65(m,1H,$C\underline{H}$OiBu), 4.55(q,J=5Hz,1H,$C\underline{H}O_2CCH_2$).

IR (thin film): 1780(s,C=O lactone).

MS (probe, m/z): 212(M^+,5%), 155(M-57,11%), 138(M-iBuOH, 95%), 111(29%), 94(32%), 79(30%), 67(37%), 57(100%), 55(36%), 41(81%).

2.5.4.6 2,6-Dioxocyclohexylacetic acid (106)

2,6-Dioxocyclohexylacetic acid (106) was prepared from the ethyl ester (105) by the method of Rosenmund *et al.*[146] Recrystallisation from ethyl acetate - petroleum ether gave white micro crystals (82%) m.p. 160°-162°C (Lit. m.p. 162°-163°C,[146] 156-157°C[147]).

2.5.4.7 3,5,6,7-Tetrahydro-2,4-benzofurandione (107)

To a stirred solution of 2,6-dioxocyclohexylacetic acid (106) (0.51g, 2.99mmol) in dry ethyl acetate (40ml) under a nitrogen atmosphere was added dicyclohexylcarbodiimide (0.704g, 3.42mmol) in dry ethyl acetate (10ml). The resulting solution was stirred under nitrogen for 4h, during which time a white precipitate of dicyclohexylurea formed. The precipitate was removed by filtration and the solvent was reduced to 5ml and refiltered. Evaporation of the solvent gave a crude solid which was purified by flash column chromatography (ethyl acetate/petroleum ether, 9:1) to afford (107) (0.33g, 2.18mmol, 73%) as a white solid. Recrystallisation from cyclohexane gave an analytical sample, m.p. 64°-66°C (Lit.[147] m.p. 65°-66°C), b.p. 156°-160°C at 0.8mm Hg (Kugelrohr distillation).

2.5.4.8 Catalytic Reduction of (107)

3,5,6,7-Tetrahydro-2,4-benzofurandione (107) was subjected to a variety of hydrogenation conditions as outlined in Table 2.3 (see Section 2.2.1.2). The typical experimental procedures for reduction at atmosphere and medium pressure are given here.

a) Reduction of (107) at Atmospheric Pressure ($Pd\text{-}BaSO_4/FeCl_3 \cdot 6H_2O$/EtOH)

A solution of 3,5,6,7-tetrahydro-2,4-benzofurandione (107) (192.6mg, 1.27mmol) in a 0.5mM ethanolic solution of iron(III)chloride hexahydrate (1ml) was stirred under a hydrogen atmosphere in the presence of palladium on barium sulphate (5%, 100mg) for 1h. The catalyst was then removed by filtration and evaporation of the solvent gave an orange oil (166.6mg). The reaction was monitored by GC (5% OV101, 150°-220°C at 4°C min^{-1}) which showed the appearance of a product peak (177°C) but this along with (107)

($171^{o}C$) rapidly disappeared. Gas chromatography - mass spectrometry indicated that the product peak was *cis*-hexahydro-2,4-benzofurandione (102).

Crude ^{1}H NMR (60MHz,DMSO-d_6): 1.1-2.1 (b envelope), 2.1-2.75(m), 2.95-3.35(m), 4.95(q,J=6Hz).

Crude IR (thin film): 3720-2500(bs,CO_2H?), 1715(bm,C=O acid?), 1675(bm).

GC-MS (5% OV101, 150^{o}-$220^{o}C$ at $4^{o}C\ min^{-1}$, m/z): 154(M^{+},10%), 126(M-CO,32%), 98(58%), 84(31%), 70(33%), 55(100%), 54(48%), 42(79%), 41(33%), 39(36%).

b) Reduction of (107) at Medium Pressure (Rh-Al_2O_3/EtOAc/60 p.s.i.)

A solution of 3,5,6,7-tetrahydro-2,4-benzofurandione (107) (200mg, 1.3mmol) in dry ethyl acetate (5ml) was hydrogenated in the presence of 5% rhodium-on-alumina (27mg) for 24h at 60 p.s.i. in a Parr apparatus. After removal of the catalyst by filtration and evaporation of the solvent, a viscous yellow oil remained (195mg). GC analysis (5% OV101, 150^{o}-$300^{o}C$ at $4^{o}C\ min^{-1}$) of this oil showed the presence of very little volatile material ((102) and (107) would be detected.)

Crude ^{1}H NMR (60MHz,DMSO-d_6): 0.7-1.75(b envelope), 1.8-2.45(m), 2.75-2.95(m), 3.3-3.5(m), 4.1(q,J=6Hz), 4.65(q,J=6Hz).

Crude IR (thin film): 3700-2700(bs.CO_2H?), 1720(m,C=O acid?), 1670(bm).

2.5.4.9 2-Cyclohexen-1-ol (140)

To a stirred suspension of lithium aluminium hydride (14.8g, 0.39 mol) in dry ether (500ml) at $0^{o}C$ was added slowly 1,3-cyclohexanedione (104) (32.3g, 0.28mol) over a 1h period. The resulting suspension was stirred at $0^{o}C$ for 1h until the evolution of hydrogen had ceased, then it was heated under reflux for 3h. At the conclusion of this period, the suspension was cooled ($0^{o}C$) and then poured onto iced water (200ml) then

20% aqueous potassium sodium tartrate solution (300ml) was added and the ether layer separated. The aqueous layer was extracted with ether (x3, 250ml) and the combined ether extracts were washed with brine (x2, 100ml) and dried ($MgSO_4$). Evaporation of the solvent gave a yellow oil (25.9g). Distillation afforded (140) (24.5g, 0.25mol, 90%) as a colourless oil, b.p. 82^o-84^oC at 32mm Hg (Lit.[176] b.p. 120^o at 45mm Hg).

A large scale reduction of 1,3-cyclohexanedione (104) (66g, 0.57mol) with lithium aluminium hydride (27.7g, 0.73mol) gave an 88% yield of (140) (49.5g, 0.5mol).

2.5.4.10 3-Trimethylsilyloxycyclohexene (141)

3-Trimethylsilyloxycyclohexene (141) was prepared from 2-cyclohexen-1-ol (140) by the method of Chavdarian and Heathcock[155] to give (141) as a colourless oil in 90% yield, b.p. 65^o-66^oC at 17mm Hg (Lit.[155] b.p. 64^o-66^oC at 15mm Hg).

2.5.4.11 *trans*-(2,3-Epoxy)trimethylsilyloxycyclohexene (96)

To a solution of 3-trimethylsilyloxycyclohexene (141) (35.97g, 0.212mol) in chloroform (300ml), cooled to -9^oC was added 80% *meta*-chloroperoxybenzoic acid (50.21g, 1.1 equiv.) over a 1h period. The resulting suspension was stirred at -9^oC for 1h then at room temperature for 4h. After filtration to remove the major portion of *meta*-chlorobenzoic acid, the chloroform solution was washed sequentially with saturated aqueous sodium sulphite (x2, 500ml), 5% aqueous sodium bicarbonate (x3, 500ml), water (x3, 200ml) and brine (200ml) and dried over sodium sulphate. Evaporation of the solvent and then distillation of the residue (b.p. 81^o-83^oC at 10mm Hg, Lit.[155] b.p. 92^o-95^oC at 15mm Hg) gave (96) (35.68g, 0.191mol, 90%) as a colourless oil. Analysis of this oil by GC (5% FFAP, 96^o-200^oC at 5^oC min^{-1}) indicated that it was 97% pure, the sole contaminant being *cis*-(2,3-epoxy)trimethylsilyloxycyclohexene (94).

2.5.4.12 *trans*-2,3-Epoxycyclohexan-1-ol (95)

A solution of *trans*-(2,3-epoxy)trimethylsilyloxycyclohexane (96) (23.3g, 0.125mol) in methanol (300ml) and saturated aqueous ammonium chloride (200ml) was stirred at room temperature for 2h, whereupon it was diluted with water (200ml). After evaporation of the methanol and salting out with ammonium sulphate the aqueous residue was extracted with chloroform (x3, 150ml). The chloroform extracts were combined, washed with brine (100ml) and dried over magnesium sulphate, evaporation of the solvent gave a yellow oil (13.2g). Distillation gave (95) (9.51g, 83.4mmol, 67%) as a colourless oil, b.p. 58°C-61°C at 0.1mm Hg (lit.[155] b.p. 65°-66°C at 0.3mm Hg). GC analysis (5% FFAP, 150°-200°C at 5°C min^{-1}) again showed (95) to be 97% pure, the *cis*-2,3-epoxycyclohexan-1-ol (91) was the sole contaminant.

2.5.4.13 2,3-Epoxycyclohexan-1-one (142)

A solution of 2-cyclohexen-1-one (143) (3.85g, 40.1mmol) in methanol (40ml) and aqueous hydrogen peroxide (30%, 11.5ml) was cooled to 15°C and then 6N aqueous sodium hydroxide (3.3ml) was added dropwise with stirring over a period of 1h. During the addition the temperature of the reaction mixture was maintained between 15°-20°C. The resulting solution was stirred at room temperature for 2h, then poured into water (50ml). The aqueous solution was saturated with sodium chloride and extracted with chloroform (x3, 100ml). The combined chloroform extracts were washed with water and dried over sodium sulphate, evaporation of the solvent gave a light yellow oil of 2,3-epoxycyclohexan-1-one hydrate (3.1g, 23.85mmol, 59%).[177] The hydrate was dissolved in benzene (200ml) and this solution was heated under reflux for 2½h with the azeotropic removal of water. Evaporation of the solvent afforded a yellow oil (2.56g) which was subjected to Kugelrohr distillation (b.p. 82°-85°C at 11mm Hg, Lit.[157] b.p. 75°-78°C at 10mm Hg) to yield (142) (2.25g, 20mmol, 50%) as a colourless oil.

2.5.4.14 *trans*-2,3-Epoxycyclohexan-1-ol (95)

A second preparation of *trans*-2,3-epoxycyclohexan-1-ol (95) was carried out using the method of Rucker *et al.*[156] Thus sodium borohydride (384mg, 10.1mmol) was added in portions at room temperature to a stirred

solution of 2,3-epoxycyclohexan-1-one (142). (1.13g, 10.06mmol) in methanolic cerium(III)chloride heptahydrate (25ml, 0.4mol l^{-1}). Stirring was continued for 20 min after hydrogen evolution had ceased and the solution poured into brine (50ml) and extracted with chloroform (x4, 25ml). The solvent was evaporated after drying over magnesium sulphate to give a light yellow oil (1.32g) which was subjected to Kugelrohr distillation (86^{o}-92^{o} at 0.15mm Hg, Lit.[155] b.p. 65-66^{o} at 0.3mm Hg) to give (95) as a colourless oil (1.01g, 8.87mmol, 88%). Analysis of this oil by GC (5% FFAP, 148^{o}-200^{o}C at 5^{o}C min^{-1}) indicated that it was greater than 97% pure.

2.5.4.15 (3aβ,4β,7aβ)-(±)-Hexahydro-4-hydroxy-2(3H)-benzofuranone (97)

To a solution of dry diisopropylamine (14ml, 100mmol) in dry dimethoxyethane (200ml) under nitrogen at -50^{o}C was added slowly *n*-butyllithium in hexane (1.6M, 64ml, 100mmol). This solution was stirred at -50^{o}C for 30 min, whereupon a solution of dry acetic acid (2.85ml, 3g, 50mmol) in dry dimethoxyethane (25ml) was added to give a white suspension. The suspension was heated to 52^{o}C with stirring and was maintained at that temperature for 1¾h.

To the above suspension at -38^{o}C was added over 30 min a solution of *trans*-2,3-epoxycyclohexan-1-ol (95) (1.14g, 10mmol) in dry dimethoxyethane (25ml). The resulting mixture was stirred at -38^{o}C for 30 min then allowed to slowly warm to room temperature and then stirred at 55^{o}C for 18h. At the conclusion of this period the reaction mixture was cooled to -10^{o}C and water (50ml) was added and the organic solvents were evaporated. The resulting aqueous residue and reaction flask were washed with water (25ml) and ether (50ml). The aqueous layers were acidified with concentrated hydrochloric acid to pH 2 at -5^{o}C, saturated with sodium chloride and then extracted with chloroform (x3, 100ml). The combined organic extracts were dried over sodium sulphate and the solvent was evaporated to give a yellow oil (2.1g). This oil was dissolved in toluene (250ml) which was then evaporated (azeotropic removal of acetic acid) and the residue was dried at 0.1mm Hg for 1½h to give a yellow oil (1.34g). The resulting oil was suspended in benzene (100ml) and the mixture stirred at reflux beneath a phase-separating head (charged with activated 4Å molecular sieves) for 4½h in the presence of *p*-toluenesulphonic acid (17mg). Evaporation of the solvent and purification by

flash column chromatography with ethyl acetate as the eluant gave (97) (876.8mg, 5.62mmol, 56%) as white microcrystals, m.p. 73.5°-75.5°C (Lit.[141] m.p. 68°-70°C). High resolution mass spectrometry gave $C_8H_{12}O_3$ for m/z 156.0935 (calc. m/z = 156.0786).

^{1}H NMR (100MHz,$CDCl_3$): 1.25-2.0(b envelope,6H,3xCH_2), 2.04-2.33 (m,1H,C<u>H</u>CH_2CO_2-), 2.69(d,J=4Hz,2H, $CHCH_2CO_2$-), 3.3-3.6(m,2H,C<u>H</u>OH + O<u>H</u>), 4.69(m,1H,C<u>H</u>O_2CCH_2-).

On dilution of the sample the multiplet between δ3.3-3.6 became a triplet of doublets (J_d=4Hz, J_t=9Hz, 1H,C<u>H</u>OH) and a broad resonance appeared at δ2.4-2.7 (1H,O<u>H</u>).

IR ($CHCl_3$): 3610(w, free OH), 3600-3200(w, H bonded OH), 1775(s,C=O lactone).

MS (Probe, m/z): 156(M^+,1%), 138(M-H_2O,39%), 112(66%), 70(81%), 67(45%), 60(47%), 57(100%), 55(68%), 43(43%).

2.5.4.16 *cis*-Hexahydro-2,4-benzofurandione (<u>102</u>)[144]

To a rapidly stirred suspension of pyridinium chlorochromate[178] (3.25g, 15.09mmol, 1.5 equiv.) in dry methylene chloride (20ml) was added in one portion a solution of (3aβ,4β,7aβ)-(±)-hexahydro-4-hydroxy-2(3H)-benzofuranone (<u>97</u>) (1.53g, 9.84mmol) in methylene chloride (20ml). This mixture was stirred at room temperature for 15h, whereupon the supernatant was decanted from the black gum. The insoluble residue was washed thoroughly with ethyl acetate (x3, 40ml) and the combined organic solutions were passed through a short plug of silica. Evaporation of the solvent gave a brown oil which was purified by flash column chromatography with ethyl acetate as the eluant to give (<u>102</u>)[144] (1.3g, 8.44mmol, 86%) as an oil which, on standing, solidified to a white solid, m.p. 69°-71°C. High resolution mass spectrometry gave $C_8H_{10}O_3$ for m/z 154.0730 (calc. m/z = 154.0630).

^{1}H NMR (100MHz, $CDCl_3$): 1.8-2.32(m,4H,2xCH_2), 2.36-2.49(m,3H, $C\underline{H}_2COC\underline{H}CH_2CO_2$), 3.0-3.28(m,2H,$C\underline{H}_2CO_2$), 4.9-5.1(m,1H,$C\underline{H}O_2CCH_2$).

IR ($CHCl_3$): 1785(s,C=O lactone), 1720(s,C=O ketone).

MS (Probe, m/z): 154(M^+,30%), 126(M-CO,41%), 98(83%), 84($C_4H_4O_2$,38%), 70(36%), 55(100%), 54(39%), 42(59%).

2.5.4.17 *cis*-Hexahydrospiro[benzofuran-4(2H),2'-[1,3]dioxolan]-2-one (144a)

A stirred solution of *cis*-hexahydro-2,4-benzofurandione (102) (683.4mg, 4.43mmole) and *p*-toluenesulphonic acid (42mg) in dry benzene (100ml) and dry ethylene glycol (0.5ml, 0.55g, 8.86mmol) was heated at reflux for 2½h under a Dean and Stark separator charged with activated molecular sieves (5Å). Evaporation of the solvent gave an oil and purification by flash column chromatography (ethyl acetate/petroleum ether, 4.7:1) gave (144a) (537.5mg, 2.7mmole, 61%) as a white solid. Recrystallisation from ethyl acetate/cyclohexane gave white needles, m.p. 81°-83°C (Lit.[144] m.p. 86°-87°C). Elemental analysis gave C=60.63, H=7.26; calc. for $C_{10}H_{14}O_4$ C=60.59, H=7.12 and high resolution mass spectrometry gave $C_{10}H_{14}O_4$ for m/z 198.0983 (calc. m/z = 198.0892).

^{1}H NMR (100MHz, $CDCl_3$): 1.24-2.04(b envelope,6H,3xCH_2), 2.4-2.72 (m,3H,$C\underline{H}C\underline{H}_2CO_2$), 3.99(s,4H($C\underline{H}_2O)_2$), 4.6-4.76 (m,1H,$C\underline{H}O_2CCH_2$-).

IR ($CHCl_3$): 1775(s,C=O lactone).

GC-MS (5% OV101,200°C,m/z): 198(M^+,4%), 170(M-CO,2%), 113(6%), 100(20%), 99($C_5H_7O_2$,100%), 86(32%), 55(28%), 42(9%), 41(7%), 40(10%).

3.5.4.18 *cis*-Hexahydro-4,4-dimethoxy-2(3H)-benzofuranone (144b)

A stirred solution of *cis*-hexahydro-2,4-benzofurandione (102) (1.3g, 8.44mmol) in dry methanol (5ml) containing a catalytic quantity of *p*-toluenesulphonic acid (11mg) was heated to reflux under a nitrogen atmosphere. To this solution was added trimethyl orthoformate (1.34g, 12.67mmol, 1.5 equiv.) and the resulting mixture was heated under reflux for 15 min, whereupon the solvent was evaporated to afford a yellow oil. Purification by flash column chromatography (ethyl acetate/petroleum ether, 1:1) yielded (144b) (1.263g, 6.31mmol, 75%) as a white crystalline solid. Recrystallisation from cyclohexane/ethyl acetate gave an analytical sample of (144b), m.p. 53°-55°C. High resolution mass spectrometry gave $C_{10}H_{14}O_4$ for m/z 200.1005 (calc. m/z = 200.10048).

^{1}H NMR (100MHz,$CDCl_3$): 1.26-2.2(b envelope,6H,3xCH_2), 2.47 (ABXdd,J=8,10Hz,2H,$\underline{CH}_2CO_2$-), 2.8-3.08 (m,1H,$\underline{CH}CH_2CO_2$-), 3.18(s,3H,OCH_3), 3.21 (s,3H,OCH_3), 4.52-4.72(m,1H,$\underline{CH}O_2CCH_2$).

Irradiation at δ4.7 perturbed both the δ2.8-3.08 multiplet and the δ1.26-2.2 envelope at ∿ δ1.5 and 2.1.

IR ($CHCl_3$): 1770(s,C=O lactone).

GC-MS (5% OV101,190°C,m/z): 200(M^+,3%), 169(M-OCH_3,22%), 141(M-OCH_3-CO,11%), 102(13%), 101($C_5H_9O_2$,100%), 97(16%), 88(13%), 81(89%), 55(10%).

2.5.4.19 (3β,3aβ,7aβ)-(±)-Hexahydro-3-pentylspiro[benzofuran-4(2H),2'-[1,3]dioxolan]-2-one

To a stirred solution of lithium diisopropylamide (3.25mmol, 1.2 equiv.) (prepared from dry diisopropylamine (0.46ml, 3.25mmol) and *n*-butyllithium in hexane (1.57M, 2ml) under nitrogen at -78°C) in anhydrous tetrahydrofuran (3ml) was added dropwise a solution of *cis*-hexahydrospiro[benzofuran-4(2H),2'-[1,3]dioxolan]-2-one (144a) (537.5mg, 2.71mmol) in tetrahydrofuran (5ml) over a 2h period. To the resulting solution was added hexamethylphosphoramide (0.57ml, 3.25mmol) and this mixture was stirred at -78°C for 30 min, whereupon a solution of 1-iodo-pentane (0.43ml, 644mg, 3.25mmol) in tetrahydrofuran (5ml) was added

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dropwise. The reaction mixture was stirred at -78°C for 30 min then at -40°C for 3h and quenched by the addition of saturated aqueous ammonium chloride (10ml). The organic solvents were evaporated and the resulting aqueous solution was extracted with chloroform (x3, 20ml), drying the combined extracts over sodium sulphate, filtration through silica and evaporation of the solvent afforded a yellow oil (1.33g). Purification of the resulting oil by flash column chromatography (ethyl acetate/petroleum ether, 1:2.3) gave (145a) (484.7mg, 1.81mmol, 67%) as a colourless oil. Analysis of this oil by GC (5% OV101, 243°C) indicated that it was a single component with a purity of 98.6%. High resolution gas chromatography - mass spectrometry gave $C_{15}H_{24}O_4$ for m/z 268.1691 (calc. m/z = 268.1674).

^{1}H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.2-1.85(b envelope, 14H,7xCH_2), 2.37(t,J=5Hz,1H,C<u>H</u>CH(C_5H_{11})CO_2-), 2.52(m,1H,C<u>H</u>CO_2-), 3.97(s,4H,(CH_2O)$_2$), 4.62-4.78(m,1H,C<u>H</u>O_2CCH-).

Irradiation at δ4.7 caused the triplet at δ2.37 to collapse to a doublet (J=5Hz). In addition, part of the δ1.2-1.85 envelope was perturbed at δ1.8. The multiplet at δ4.62-4.78 collapsed to a doublet (J=5Hz) on irradiation at δ1.8 and was perturbed on decoupling at δ2.35.

IR ($CHCl_3$): 1765(s,C=O lactone).

GC-MS (5% OV101,243°C,m/z): 268(M^+,12%), 211(30%), 198(10%), 197(M-C_5H_{11},40%), 100(11%), 99($C_5H_7O_2$,100%), 86(12%), 55(9%).

2.5.4.20 <u>(3β,3aβ,7aβ)-(±)-Hexahydro-4,4-dimethoxy-3-pentyl-2(3H)-benzofuranone (145b)</u>

To a stirred solution of lithium diisopropylamide (4.58mmol, 1.2 equiv.) (prepared from dry diisopropylamine (0.64ml, 4.58mmol) and *n*-butyllithium in hexane (1.56M, 2.9ml) under nitrogen at -78°C) in anhydrous tetrahydrofuran (5ml) was added over a 3h period a solution of *cis*-hexahydro-4,4-dimethoxy-2(3H)-benzofuranone (144b) (764.5mg, 3.8mmol) in tetrahydrofuran (10ml). On completion of the addition the mixture was

stirred at -78°C for 1½h then hexamethylphosphoramide (0.8ml, 4.58mmol) was added and the solution stirred for a further 30 min. To the resulting orange solution was added 1-iodopentane (2ml, 15.3mmol, 4 equiv.) in tetrahydrofuran (5ml) and the reaction mixture was stirred at -78°C for 30 min then at -40°C for 16h. At the conclusion of this period the reaction was quenched by the addition of saturated aqueous ammonium chloride (10ml) and the organic solvents were evaporated. The resulting aqueous solution was extracted with ethyl acetate (x3, 20ml), drying the combined extracts over sodium sulphate, filtration through silica and evaporation of the solvent afforded a yellow oil. Purification by flash column chromatography (ethyl acetate/petroleum ether, 1:3.4) gave (145b) (896mg, 3.32mmol, 87%) as a colourless oil. Analysis of (145b) by GC (5% OV101 180°-300°C at 5°C min^{-1}) indicated that it was a single component with evidence for on column thermal decomposition. Elemental analysis gave C = 66.41, H = 9.38; calc. for $C_{15}H_{26}O_4$, C = 66.64, H = 9.69, while high resolution mass spectrometry gave $C_{15}H_{26}O_4$ for m/z 270.1849 (calc. m/z = 270.1831).

^{1}H NMR (100MHz,$CDCl_3$): 0.88(t,J=6Hz,3H,CH_3), 1.14-2.09(b envelope, 14H,7xCH_2), 2.58-2.71(m,2H,$\underline{CH}\underline{CH}(C_5H_{11})CO_2$), 3.18(s,3H,$OCH_3$), 3.21(s,3H,$OCH_3$), 4.44-4.64(m,1H,$\underline{CH}O_2CCH$).

Irradiation at δ4.53 perturbed the δ1.14-2.09 envelope at δ1.7 and the δ2.58-2.71 multiplet. Decoupling at δ1.7 perturbed the multiplets at δ4.44-4.64 and δ2.58-2.71.

IR ($CHCl_3$): 1765(s,C=O lactone).

MS (Probe, m/z): 270(M^+,9%), 239(M-OCH_3,11%), 213(M-C_4H_9, 17%), 199(M-C_5H_{11},24%), 102(15%), 101 ($C_5H_9O_2$,100%), 88(17%), 55(21%), 43(14%), 41(21%).

2.5.4.21 (3β,3aβ,7aβ)-(±)-Hexahydro-3-pentyl-3(phenylthio)spiro[benzofuran-4(2H),2'-[1,3]dioxolan]-2-one (146a)

To a stirred solution of lithium diisopropylamide (2.12mmol, 1.2 equiv.) (prepared from dry diisopropylamine (0.3ml, 2.12mmol) and *n*-butyl-

lithium in hexane (1.57M, 1.36ml) under nitrogen at -78°C) in dry tetrahydrofuran (3ml) was added dropwise over a 2h period a solution of (3β,3aβ,7aβ)-(±)-hexahydro-3-pentylspiro[benzofuran-4(2H),2'-[1,3]dioxolan]-2-one (145a) (474.7mg, 1.77mmol) in tetrahydrofuran (5ml). After addition was complete the reaction was stirred at -78°C for 30 min then hexamethylphosphoramide (0.37ml, 2.12mmol) was added and the mixture stirred for a further 30 min. To the resulting orange solution was added quickly a solution of diphenyl disulphide (468.4mg, 2.14mmol) in tetrahydrofuran (3ml) to give an immediate deep red colouration. The red solution was stirred at -78°C for 30 min, -20°C for 30 min and then warmed to room temperature. The reaction was quenched by the addition of saturated aqueous ammonium chloride (20ml). The organic solvents were evaporated and the resulting aqueous solution was extracted with ethyl acetate (x3, 50ml). Evaporation of the solvent after drying (Na_2SO_4) gave a yellow oil which was subjected to flash column chromatography (ethyl acetate/petroleum ether, 1:2.125) to afford (146a) (455.7mg, 1.2mmol, 68%) as a cream coloured solid, m.p. 116°-118.5°C. Attempted recrystallisations with a variety of solvents led to extensive decomposition. Analysis by GC (5% OV101, 182°-298°C at 5°C min^{-1}) indicated that (146a) was a single component with evidence for on column thermal decomposition. High resolution mass spectrometry gave $C_{21}H_{28}O_4S$ for m/z 376.1923 (calc. m/z = 376.1708).

^1H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.1-2.0(b envelope, 14H,7xCH_2), 2.55(d,J=5Hz,1H,CHC(SPh)(C_5H_{11})CO_2-), 3.84-4.05(m,4H,($CH_2O)_2$), 4.38-4.50(m,1H,CHO_2C), 7.24-7.32(m,3H, *o*+*p*Ar-H), 7.37-7.44(m,2H,*m*Ar-H).

IR ($CHCl_3$): 1770(s,C=O lactone), 695(w,Ar-H).

MS (Probe,m/z): 376(M^+,3%), 367(18%), 267(M-PhS,100%), 179(7%), 109(7%), 99($C_5H_7O_2$,43%), 86(10%), 55(18%), 43(7%), 41(9%).

2.5.4.22 (3β,3aβ,7aβ)-(±)-Hexahydro-4,4-dimethoxy-3-pentyl-3-(phenylthio)-2(3H)-benzofuranone (146b)

(3β,3aβ,7aβ)-(±)-Hexahydro-4,4-dimethoxy-3-pentyl-3-(phenylthio)-2(3H)-benzofuranone (146b) was prepared from (3β,3aβ,7aβ)-(±)-hexahydro-4,4-

dimethoxy-3-pentyl-2(3H)-benzofuranone (145b) in 57% yield using the conditions described above. Workup and isolation as above gave an oil (0.31g) which was purified by flash column chromatography (ether/petroleum ether, 1:1) to give a colourless oil which on trituration with petroleum ether/ethyl acetate (1:3) produced (146b) (77.5mg, 0.205mmol, 57%) as white micro crystals, m.p. 126°-127.5°C. Analysis by GC (5% OV101, 184°-300°C at 5°C min^{-1}) indicated that (146b) was a single component. Elemental analysis gave C = 66.11, H = 7.93; calc. for $C_{21}H_{30}O_4S$, C = 66.64, H = 7.99, while high resolution mass spectrometry gave $C_{21}H_{30}O_4S$ for m/z 378.1738 (calc. m/z = 378.1865).

^1H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.09-2.24(b envelope,14H,7xCH_2), 2.48(d,J=5Hz,1H, $CHC(SPh)(C_5H_{11})CO_2$-), 3.19(s,3H,OCH_3), 3.22(s,3H,OCH_3), 5.06-5.23(m,1H,CHO_2C-), 7.24-7.39(m,3H,*o*+*p*ArH), 7.42-7.58(m,2H, *m*Ar-H).

Irradiation at δ5.15 caused the doublet at δ2.48 to collapse to a singlet.

IR ($CHCl_3$): 1770(s,C=O lactone), 695(w,Ar-H).

MS (Probe, m/z): 378(M^+,7%), 346(M-OCH_3,5%), 269(M-PhS, 100%), 237(M-PhS-OCH_3,31%), 111(70%), 110(27%), 109(50%), 101($C_5H_9O_2$,32%), 55(30%), 41(18%).

2.5.4.23 (±)-5,6,7,7a-Tetrahydro-3-pentyl-2,4-benzofurandione (147)

(3β,3aβ,7aβ)-(±)-Hexahydro-3-pentyl-3-(phenylthio)spiro[benzofuran-4(2H),2'-[1,3]dioxolan]-2-one (146a) (54.5mg, 0.14mmol) was dissolved in 1ml of a mixture of tetrahydrofuran and concentrated hydrochloric acid (5:3). The resulting solution was stirred at room temperature for 24h, whereupon it was diluted with water (5ml). The mixture was extracted with ethyl acetate (x3, 10ml) and the combined organic layers were dried over sodium sulphate. Evaporation of the solvent afforded a yellow oil (55mg) which on purification by flash column chromatography (ethyl acetate/petroleum ether, 1:2.03) yielded

(147) (24.6mg, 0.11mmol, 79%) as an unstable yellow oil. High resolution mass spectrometry gave $C_{13}H_{18}O_3$ for m/z 222.1516 (calc. m/z = 222.1256).

^1H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.2-2.84(b envelope, 14H,7xCH_2), 4.88-5.06(m,1H,CHO_2C-).

IR ($CHCl_3$): 1765(s,C=O lactone), 1705(m,C=O ketone), 1655(w,C=C).

MS (Probe, m/z): 222(M^+,24%), 204(72%), 175(100%), 166(35%), 161(53%), 149(53%), 147(34%), 55(60%), 41(61%), 39(48%).

In addition (147) was prepared from (3β,3aβ,7aβ)-(±)-hexahydro-4,4-dimethoxy-3-pentyl-3-(phenylthio)-2(3H)-benzofuranone (146b). Thus, to a solution of (146b) (11.8mg, 0.03mmol) in redistilled tetrahydrofuran (0.5ml) was added 2N aqueous hydrochloric acid (0.5ml). The resulting solution was stirred at room temperature for 30 min and then poured into water (5ml). This aqueous mixture was extracted with ethyl acetate (x3, 5ml) and the combined organic extracts were dried over magnesium sulphate. Evaporation of the solvent gave a yellow oil (11.5mg) which was purified by flash column chromatography (ethyl acetate/petroleum ether, 1:2.03) to afford (147) (6mg, 0.027mmol, 87%) as an unstable colourless oil.

2.5.4.24 (3α,3aβ,7aβ)-(±)-Hexahydro-4,4-dimethoxy-3-(methylthio)-3-pentyl-2(3H)-benzofuranone (148)

To a stirred solution of lithium diisopropylamide (5.8mmol) (prepared from dry diisopropylamine (0.8ml, 5.8mmol) and *n*-butyllithium in hexane (1.57M, 3.7ml) under nitrogen at -78°C) in tetrahydrofuran (6ml) was added (3β,3aβ,7aβ)-(±)-hexahydro-4,4-dimethoxy-3-pentyl-2(3H)-benzofuranone (145b) (1.3g, 4.83mmol) in tetrahydrofuran (10ml) over a 2h period. The resulting solution was stirred at -78°C for 1h then for a further 30min after the addition of hexamethylphosphoramide (1ml, 1.04g, 5.8mmol). To this orange solution was added dry dimethyl disulphide (683mg, 7.25mmol, 1.5 equiv.) in tetrahydrofuran (10ml). The mixture was stirred at -78°C for 30 min, -20°C for 30 min and then at room temperature for 17h, then quenched by the addition of saturated aqueous ammonium chloride (10ml). Evaporation of the organic solvents and extraction with

ethyl acetate (x3, 25ml), filtration through silica and drying over sodium sulphate followed by removal of the solvent afforded a yellow oil (1.6g). Purification by flash column chromatography (ether/petroleum ether, 1:4 then 1:1.5) yielded (148) (644.6mg, 2.03mmol, 42%) as an oil which solidified on standing. In addition, starting material (145b) (585.2mg, 2.17mmol, 45%) was recovered (ether/petroleum ether, 1:1.5). Recrystallisation of (148) from pentane gave colourless cubic crystals m.p. 90°-92.5°C, elemental analysis of which gave C = 60.77, H = 8.98. Calc. for $C_{16}H_{28}O_4S$ C = 60.73, H = 8.92. High resolution mass spectrometry gave $C_{16}H_{28}O_4S$ for m/z 316.1727 (calc. m/z = 316.1708).

^{1}H NMR (100MHz, $CDCl_3$): 0.88(t,J=6Hz,3H,CH_3), 1.18-1.74(b envelope,8H,4xCH_2), 1.8-2.31(m,7H, 3xCH_2 + C$\underline{H}$C(SMe)(C_5H_{11})CO_2-), 2.1 (s,3H,SCH_3), 3.2(s,3H,OCH_3), 3.24(s,3H, OCH_3), 4.44(dt,J_d = 10Hz,J_t = 8Hz,1H, C$\underline{H}O_2$C-).

Irradiation at δ4.45 caused the δ1.8-2.31 multiplet to be perturbed (C$\underline{H}$C(SMe)(C_5H_{11})CO_2 + C$\underline{H}_2CHO_2C$ + 2xCH_2).

IR ($CHCl_3$): 1775(s,C=O lactone).

MS (Probe, m/z): 316(M^+,8%), 285(M-OCH_3,7%), 284(M-CH_3OH, 11%), 269(M-SCH_3,13%), 237(M-CH_3OH-SCH_3, 58%), 176(18%), 175(13%), 111(32%), 110(30%), 109(100%), 101 ($C_5H_9O_2$,14%), 55(19%).

2.5.4.25 Single Crystal X-Ray Structural Determination of (148)

A single crystal of (3α,3aβ,7aβ)-(±)-hexahydro-4,4-dimethoxy-3-(methylthio)-3-pentyl-2(3H)-benzofuranone (148) obtained by recrystallisation from pentane was found to be triclinic with: a = 15.964(2)Å, b = 13.261(2)Å, c = 9.019(1)Å, α = 110.821(8)°, β = 89.566(8)°, γ = 103.478(9)°, U = 1729.55Å^3, D_c = 1.215g^{-1} cm^3, D_o = 1.26(2)g^{-1} cm^{-3}, Z = 4, F(ooo) = 688, μ(Mo-Kα) = 1.59cm^{-1}. Distribution of the 'E's' indicated the centrosymmetric space group $P\bar{1}$ and was confirmed by subsequent analysis.

The data was collected on a 'CAD-4' diffractometer at Queen Mary College, London, using graphite monochromated Mo-Kα radiation (λ = 0.71ϕ7Å) at room temperature. The cell dimensions were determined from 25 accurately centred reflections. Three check reflections showed that deterioration had occurred and a linear correction - 'FADE' was used to overcome this problem. After suitable averaging 4233 unique reflections were obtained (θ_{min} 1.5° to θ_{max} 22°). No absorption correction was used (μ = 1.59cm^{-1} MoKα) and treating these reflections where 6(F)>2F as "less-thans" gave 3048 reflections that were used in the subsequent structure determination and refinement. The most probable solution from MULTAN (combined F.O.M. 2.8032) yielded all the non-hydrogen atoms. Subsequent least squares isotropic refinement reduced R to 0.134. Introducing an empirical weighting scheme ($w = 1/6^2F + 0.0003F_o^2$) and anistropic refinement of all non-hydrogen atoms reduced R to 0.1014. A difference synthesis revealed the majority of the hydrogen atoms and these were introduced into the model in geometrically calculated positions (d(C-H) ≈ 1.08Å). A common isotropic temperature factor was used for the tertiary and secondary hydrogen atoms but a different isotopic temperature factor was used for methyl hydrogen atoms since these atoms were not subjected to the same geometric constraints. Final least squares refinement gave R = 0.0656, R_w = 0.057 and a difference electron density map showed no significant features in the range -0.51Å < electron density < 0.49Å. The number of parameters was 405 and the ratio of reflections to parameters was 7.53.

2.5.4.26 (3α,3aβ,7aβ)-(±)-Hexahydro-3-(methylthio)-3-pentyl-2,4-benzofurandione (149)

A solution of (3α,3aβ,7aβ)-(±)-hexahydro-4,4-dimethoxy-3-(methylthio)-3-pentyl-2(3H)-benzofuranone (148) (482.4mg, 1.53mmol) in redistilled tetrahydrofuran (30ml) and 1N aqueous hydrochloric acid (10ml) was stirred for 30 min, whereupon brine (35ml) was added. Extraction with ethyl acetate (x3, 50ml), drying over sodium sulphate and evaporation of the solvent afforded a yellow oil (441mg). Purification by flash column chromatography (ether/petroleum ether, 1:1.08) yielded (149) (375.4mg, 1.39mmol, 91%) as a colourless oil. Elemental analysis gave C = 62.0, H = 7.96; calc. for $C_{14}H_{22}O_3S$ C = 62.19, H = 8.2, while high resolution mass spectrometry gave $C_{14}H_{22}O_3S$ for m/z 270.120 (calc. m/z = 270.129).

^{1}H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.15-1.8(b envelope, 8H,4xCH_2), 1.85-2.12(m,2H,$\underline{CH_2}CH_2C$=O), 2.2(s,3H,SCH_3), 2.2-2.4(m,4H,$CO_2CH\underline{CH_2}CH_2\underline{CH_2}C$=O), 3.52(d,J=8Hz,1H,$\underline{CH}C(SMe)(C_5H_{11})CO_2$-), 4.72(q,J=8Hz,1H,$\underline{CH}O_2C$).

The quartet at δ4.72 was perturbed on irradiation at δ3.52, decoupling at δ2.3 caused this resonance to collapse to a doublet (J=8Hz). The doublet at δ3.52 collapsed to a singlet on irradiation at δ4.72, in addition the multiplet at δ2.2-2.4 was perturbed.

IR (CCl_4): 1780(s,C=O lactone), 1720(s,C=O ketone).

MS (Probe, m/z): 270(M^+,67%), 224(62%), 223(M-SCH_3,52%), 196(42%), 155(86%), 139(100%), 55(94%), 43(45%), 41(55%).

2.5.4.27 (3α,3aβ,7aβ)-(±)-Hexahydro-3-(methylthio)-3-pentyl-5-(phenylseleno)-2,4-benzofurandione (150)

To a stirred solution of (3α,3aβ,7aβ)-(±)-hexahydro-3-(methylthio)-3-pentyl-2,4-benzofurandione (149) (275mg, 1.02mmol) in dry ethyl acetate (9ml) under a nitrogen atmosphere was added phenylselenyl chloride (393.5mg, 2.05mmol). The resulting red solution was stirred until it had turned pale yellow (18h). At this point, water (10ml) was added and the organic layer drawn off. The resulting aqueous layer was extracted with ethyl acetate (x2, 25ml). The combined organic extracts were dried over sodium sulphate and removal of the solvent afforded an orange oil (600mg). Flash column chromatography (ether/petroleum ether, 1:1.63) afforded (150) (203.7mg, 0.48mmol, 47%) as a yellow oil. High resolution mass spectrometry gave $C_{20}H_{26}O_3SSe$ for m/z 426.055 (calc. m/z = 426.0768).

^{1}H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.06-1.52(b envelope, 7H,3x$\underline{CH_2}$+$\underline{CH}$), 1.64-1.8(m,2H,CH_2), 1.8-2.08(m,2H,CH_2), 2.2(s,3H,SCH_3), 2.22-2.42(m,1H,CH), 3.67(d,J=8Hz,1H,$\underline{CH}C(SMe)(C_5H_{11})CO_2$-), 4.23(dd,J=6,11Hz,1H, $\underline{CH}SePh$), 4.72(q,J=8Hz,1H,$\underline{CH}O_2C$), 7.22-7.4 (m,3H,*o*+*p*Ar-$\underline{H}$), 7.52-7.66 (m,2H,*m*Ar-$\underline{H}$).

The doublet at δ3.67 collapsed to a singlet on irradiation at δ4.72. Decoupling at δ4.24 resulted in perturbation of the multiplets at δ1.88-2.2 and δ2.22-2.4.

IR ($CHCl_3$): 1770(s,C=O lactone), 1715(m,C=O ketone), 690(w,Ar-H).

MS (Probe,m/z): 426(M^+,23%), 241(37%), 157(PhSe,32%), 87(29%), 77(46%), 67(29%), 55(100%), 43(49%), 41(86%), 39(44%).

The molecular ion m/z 426.055 (^{80}Se) showed a selenium isotope pattern with ions at m/z 428 (^{82}Se,5%), 424 (^{78}Se,10%), 422 (^{76}Se,4%).

3.5.4.28 (3α,3aβ,4α,7aβ)-(±)-Hexahydro-4-hydroxy-3-(methylthio)-3-pentyl-5-(phenylseleno)-2(3H)benzofuranone (151)

To a stirred solution of (3α,3aβ,7aβ)-(±)-hexahydro-3-(methylthio)-3-pentyl-5-(phenylseleno)-2,4-benzofurandione (150) (193.7mg, 0.45mmol) in anhydrous dimethoxyethane (5ml) at 0°C under nitrogen was added a freshly prepared 0.5M zinc borohydride[165] solution in dimethoxyethane (0.5ml, 0.25mmol). The resulting solution was stirred at 0°C for 2h then at room temperature for 22h. At the conclusion of this period, saturated aqueous sodium hydrogen tartrate solution was added dropwise until the evolution of hydrogen ceased (ca. 1ml), followed by water (4ml) and the aqueous mixture was extracted with chloroform (x3, 10ml). The combined organic extracts were dried over sodium sulphate and evaporation of the solvent yielded a yellow oil (208.3mg). The oil was purified by flash column chromatography (ether/petroleum ether, 1:1) to afford (151) as an oil which on trituration with ether gave a white solid (60mg, 0.14mmol, 31%). Recrystallisation from ether gave white needles m.p. 132°-135°C, elemental analysis of which gave C = 55.84, H = 6.64; calc. for $C_{20}H_{28}O_3SSe$, C = 56.2, H = 6.6.

^1H NMR (100MHz, $CDCl_3$): 0.9(t,J=6Hz,3H,CH_3), 1.08-1.48(b envelope, 6H,3xCH_2), 1.54-1.94(b envelope, 5H, 2xCH_2+OH), 2.01(s,3H,SCH_3), 2.04-2.37 (m,3H,CH_2CHO_2C+CHSePh), 3.83-4.0(dd, J=5,4Hz,1H,CHC(SMe)(C_5H_{11})CO_2-),

4.12-4.26(m,1H,CHOH), 5.06(t,J=4Hz,1H, CHO_2C-), 7.24-7.37(m,3H,*o*+*p*Ar-H), 7.5-7.65(m,2H,*m*Ar-H).

On dilution of the sample, part of the δ1.54-1.94 broad envelope centred at δ1.87 was shifted upfield.

The multiplet at δ2.04-2.37 and the double doublet at δ3.83-4.0 were perturbed on irradiation at δ5.06. Decoupling the multiplet at δ4.19 caused the multiplet at δ2.04-2.37 to be perturbed. Irradiation at δ3.9 or δ2.28 caused the triplet at δ5.06 to collapse to a doublet (J=4Hz). The multiplet at δ4.12-4.26 was perturbed on irradiation at δ2.28.

IR (CCl_4): 3660-3200(m,OH), 1765(s,C=O lactone), 1580(w,aromatic C=C), 680(m,Ar-H).

MS (Probe, m/z): 428(M^+,10%), 271(M-PhSe,100%), 81(30%), 79(30%), 78(34%), 77(46%), 57(28%), 55(55%), 43(41%), 41(52%).

The molecular ion m/z 428 (^{80}Se) showed a selenium isotope pattern with ions at m/z 430 (^{82}Se,3%), 426 (^{78}Se,5%), 424 (^{76}Se,2%).

2.5.4.29 (3α,3aβ,4α,7aβ)-(±)-3a,4,7,7a-Tetrahydro-4-hydroxy-3-(methylthio)-3-pentyl-2(3H)-benzofuranone (152)

To a stirred solution of (3α,3aβ,4α,7aβ)-(±)-hexahydro-4-hydroxy-3-(methylthio)-3-pentyl-5-(phenylseleno)-2(3H)-benzofuranone (151) (55mg, 0.129mmol) in redistilled methanol (3ml) and tetrahydrofuran (1ml) at 0°C was added a solution of sodium periodate (59.5mg, 0.278mmol) in the minimum volume of water . When addition was complete, the reaction was stirred at 0°C for 2h and then at room temperature for 2h. The reaction mixture was filtered and the precipitate was washed with methanol (x3, 10ml). The combined filtrate and washings were concentrated to yield a yellow oil which was dissolved in ether and dried over sodium sulphate. Evaporation of the solvent yielded a yellow oil (72.1mg) which was purified by flash column chromatography (ether/petroleum ether, 3:1) to afford (152) (13.9mg, 0.05mmol, 40%) as a white crystalline solid.

Recrystallisation from pentane gave colourless needles, m.p. 97.5°C-101°C, elemental analysis of which gave C = 61.84, H = 8.07; calc. for $C_{14}H_{22}O_3S$ C = 62.19, H = 8.2. High resolution mass spectrometry gave $C_{14}H_{22}O_3S$ for m/z 270.1264 (calc. m/z = 270.12896).

^{1}H NMR (100MHz, $CDCl_3$): 0.93(t,J=6Hz,3H,CH_3), 1.2-1.52(b envelope, 5H,2xCH_2+CH), 1.58-1.88(b envelope,4H, CH_2+CH+OH), 1.99-2.2(m,1H,CHC(SCH_3)(C_5H_{11}) CO_2-), 2.12(s,3H,SCH_3), 2.25-2.46(m,2H, CH_2CHO_2C), 4.06-4.32(b,1H,CHOH), 5.08-5.24(m,1H,CHO_2C-), 5.9-6.32(m,2H,CH=CH).

Dilution of the sample caused part of the broad envelope centred at δ1.76 to shift upfield. Irradiation at δ5.16 resulted in a simplification of the multiplets at δ2.25-2.46 and δ1.99-2.22. The multiplet at δ1.99-2.22 was perturbed on irradiation at δ4.19. Decoupling the resonance at δ2.38 resulted in a simplification of the multiplets at δ5.08-5.24 and δ5.9-6.32.

IR (CCl_4): 3570(w,Free OH), 3600-3250(w,OH), 1760(s,C=O lactone).

MS (Probe, m/z): 270(M^+,0.34%), 222(M-MeSH,2%), 85(65%), 83(100%), 55(16%), 48(19%), 47(37%), 43(16%), 41(27%), 35(12%).

2.5.4.30 (3β,3aβ,7aβ)-(±)-Hexahydro-3-pentyl-2,4-benzofurandione (156)

Aqueous hydrochloric acid (1M, 20ml) was added to a solution of (3β,3aβ,7aβ)-(±)-hexahydro-4,4-dimethoxy-3-pentyl-2(3H)-benzofuranone (145b) (667.5mg, 2.47mmol) in redistilled tetrahydrofuran (30ml) and this homogeneous mixture was stirred for 2h. At the conclusion of this period, brine (35ml) was added and the resulting aqueous mixture was extracted with ethyl acetate (x3, 50ml) and the combined extracts were dried over sodium sulphate. Evaporation of the solvent yielded a yellow oil (564mg) which was purified by flash column chromatography (ether/petroleum ether, 2.3:1) to afford (156) (370.3mg, 1.65mmol, 67%) as a white fluffy solid. Recrystallisation from pentane yielded white needles, m.p. 38.5°-39°C, elemental analysis gave C = 69.46, H = 8.90;

calc. for $C_{13}H_{20}O_3$ C = 69.61, H = 8.90. High resolution mass spectrometry gave $C_{13}H_{20}O_3$ for m/z 224.1403 (calc. m/z = 224.1414).

^{1}H NMR (60MHz, $CDCl_3$): 0.9(t,J=6Hz,3H,CH_3), 1.1-1.9(b envelope, 8H,4xCH_2), 1.95-2.25(m,4H,$\underline{CH_2}\underline{CH_2}CHO_2C$), 2.45(t,J=6Hz,2H,$\underline{CH_2}COCH$), 2.8(dd,J=6,5Hz, 1H,$\underline{CH}CH(C_5H_{11})CO_2$-), 3.0-3.3(m,1H,$\underline{CH}$ $(C_5H_{11})CO_2$-), 4.95(q,J=6Hz,1H,$\underline{CH}O_2CCH$ (C_5H_{11})).

IR (CCl_4): 1785(s,C=O lactone), 1720(s,C=O ketone).

MS (Probe, m/z): 224(M^+,1.7%), 196(M-CO,1.7%), 180(M-CO_2, 9%), 154(100%), 126(72%), 97(46%), 81(26%), 67(40%), 55(67%), 42(36%), 41(62%).

2.5.4.31 (3β,3aβ,7aβ)-(±)-Hexahydro-3-pentyl-5-(phenylseleno)-2,4-benzofurandione (157)

To a stirred solution of (3β,3aβ,7aβ)-(±)-hexahydro-3-pentyl-2,4-benzofurandione (156) (358mg, 1.6mmol) in dry ethyl acetate under a nitrogen atmosphere was added phenylselenyl chloride (373mg, 1.95mmol, 1.2 equiv.). The resulting red solution was stirred until it turned pale yellow (2h), whereupon water (10ml) was added and the organic layer was drawn off. The aqueous layer was then extracted with ethyl acetate (x3, 25ml) and the combined organic layers were dried over sodium sulphate. Evaporation of the solvent gave an orange oil which was purified by flash column chromatography (ether/petroleum ether, 1:1.2) to afford (157) (390.2mg, 1.03mmol, 64%) as a light yellow oil.

^{1}H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.12-1.82(b envelope, 9H,4x$\underline{CH_2}$+$\underline{H}CHCH_2CHO_2C$), 1.94-2.44(m,3H, $HC\underline{H}C\underline{H_2}CHO_2C$),3.08-3.36(m,2H,$\underline{CH}C\underline{H}(C_5H_{11})$ CO_2-), 3.9(t,J=4Hz,1H,PhSe$\underline{CH}$CO-), 4.86-5.06(m,$\underline{CH}O_2C$-), 7.16-7.36(m,3H,*o*+*p*Ar-$\underline{H}$), 7.39-7.66(m,2H,*m*Ar-$\underline{H}$).

The multiplets at δ1.94-2.44 and δ3.08-3.36 were perturbed on irradiation at δ4.96. Decoupling the resonances at δ2.3 or δ2.1 resulted

in the collapse of the multiplet at δ4.86-5.06 to a doublet (J=6Hz). The triplet at δ3.9 collapsed to a doublet (J=4Hz) on irradiation at δ2.1. Irradiation at δ1.7 caused the multiplet at δ3.08-3.36 to be perturbed.

IR (CCl_4): 1785(s,C=O lactone), 1710(s,C=O ketone), 690(m,Ar-H).

MS (Probe, m/z): 380(M^+,70%), 378($C_{19}H_{24}O_3{}^{78}Se$,43%), 157(PhSe,49%), 78(43%), 77(43%), 69(51%), 55(100%), 41(56%).

The molecular ion m/z 380 showed a selenium isotope pattern with ions at m/z 382 (^{82}Se,16%), 378 (^{78}Se,43%), 377 (^{77}Se,14%), 376 (^{76}Se, 16%).

3.5.4.32 (3β,3aβ,7aβ)-(±)-3,3a,7,7a-Tetrahydro-3-pentyl-2,4-benzofurandione (158)

A solution of sodium periodate (126.3mg, 0.59mmol) in the minimum volume of water was added to a stirred solution of (3β,3aβ,7aβ)-(±)-hexahydro-3-pentyl-5-(phenylseleno)-2,4-benzofurandione (157) (105.2mg, 0.28mmol) in redistilled methanol (5ml) and tetrahydrofuran (1ml) at 0°C. The reaction was stirred at 0°C for 30 min, then at room temperature for 1h. The reaction mixture was filtered and the precipitate washed with methanol (x3, 10ml). The combined filtrate and washings were concentrated to give a yellow oil which was dissolved in ether and dried over sodium sulphate. Evaporation of the solvent and purification by flash column chromatography (ether/petroleum ether 4.5:1) afforded (158) (18.6mg, 0.084mmol, 30%) as a light yellow oil. High resolution mass spectrometry gave $C_{13}H_{18}O_3$ for m/z 222.112 (calc. m/z = 222.1256).

^1H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.1-1.9(b envelope, 8H,4xCH_2), 2.74-3.0(m,4H,$C\underline{H}_2CHO_2CC\underline{H}(C_5H_{11})C\underline{H}$-), 5.04(q,J=6Hz,1H,$C\underline{H}O_2CCH$-), 6.17 ($dt,J_d$=10Hz,$J_t$=2Hz,1H,HC=C$\underline{H}$C=O), 6.88 ($dt,J_d$=10Hz,$J_t$=4Hz,1H,C$\underline{H}$=CHC=O).

The multiplet at δ2.74-3.0 was perturbed on irradiation at δ5.04. Irradiation at δ2.82 resulted in the collapse of the quartet at δ5.04 to a singlet while the two doublet of triplets at δ6.17 and δ6.88 gave two doublets (J=10Hz). Decoupling the doublet of triplets at δ6.88 caused the signal at δ6.17 to collapse to a triplet (J=2Hz) and perturbed the δ2.74-3.0 multiplet. Irradiation at δ6.17 resulted in the collapse of the doublet of triplets at δ6.88 to a triplet (J=4Hz) while the multiplet at δ2.74-3.0 was perturbed.

IR (CCl_4): 1785(s,C=O lactone), 1680(s,C=O enone).

MS (Probe, m/z): 222(M^+,1%), 152(M-C_5H_{10},43%), 95(27%), 94(20%), 84(19%), 69(34%), 68(100%), 55(21%), 41(21%).

2.5.4.33 (3β,3aβ,4α,7aβ)-(±)-Hexahydro-4-hydroxy-3-pentyl-2(3H)-benzofuranone (159)

To a stirred solution of (3β,3aβ,7aβ)-(±)-3,3a,7,7a-tetrahydro-3-pentyl-2,4-benzofurandione (158) (17.3mg, 0.078mmol) in anhydrous dimethoxyethane (2ml) at 0°C under nitrogen was added a freshly prepared 0.5M zinc borohydride[165] solution in dimethoxyethane (100μl, 0.05mmol). This solution was stirred at 0°C for 2h then at room temperature for 2h. Saturated aqueous sodium hydrogen tartrate was then added dropwise until the evolution of hydrogen ceased, then water (1ml) was added and the resulting aqueous mixture was extracted with chloroform (x3, 10ml). The combined organic extracts were dried over sodium sulphate and evaporation of the solvent gave a light yellow oil. Purification by flash column chromatography with ether as the eluant afforded (159) (7.5mg, 0.033mmol, 43%) as a colourless oil. High resolution mass spectrometry gave $C_{13}H_{22}O_3$ for m/z 226.1523 (calc. m/z = 226.1569).

^1H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.18-2.08(b envelope, 15H,7xCH_2+OH), 2.39(q,J=6Hz,1H,CHCH(C_5H_{11})CO_2-), 2.71(q,J=6Hz,1H,CH(C_5H_{11})CO_2-), 3.95-4.1(m,1H,CHOH), 4.55(q,J=6Hz, 1H,CHO_2C-).

IR (CCl_4): 3660-3320(w,OH), 1785(s,C=O lactone).

MS (Probe, m/z): 226(M^+,3%), 208(M-H_2O,3%), 156(M-C_5H_{10}, 100%), 138(M-C_5H_{10}-H_2O,67%), 111(30%), 99(22%), 97(30%), 81(20%), 67(24%), 55(37%).

2.5.4.34 (3β,3aβ,4α,7aβ)-(±)- Hexahydro-4- hydroxy-3-pentyl-5-(phenylseleno)-2(3H)-benzofuranone (160)

To a stirred solution of (3β,3aβ,7aβ)-(±)-hexahydro-3-pentyl-5-(phenylseleno)-2,4-benzofurandione (157) (390mg, 1.03mmol) in anhydrous dimethoxyethane (10ml) at 0°C under nitrogen was added dropwise a freshly prepared 0.5M zinc borohydride[165] solution in dimethoxyethane (1.15ml, 0.575mmol). The reaction mixture was stirred at 0°C for 2h then at room temperature for 30 min. The reaction was cooled to 0°C and saturated aqueous sodium hydrogen tartrate was added until hydrogen evolution ceased, then water (10ml) was added. Extraction with chloroform (x3, 25ml) and drying (Na_2SO_4) afforded after evaporation of the solvent a yellow oil (394.6mg). Purification by flash column chromatography (ether/petroleum ether, 1.5:1) yielded (160) (352.5mg, 0.93mmol, 90%) as a colourless oil. High resolution mass spectrometry gave $C_{19}H_{26}O_3Se$ for m/z 382.1017 (calc. m/z = 382.1047).

^1H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.15-2.0(b envelope,11H,5xCH_2+OH), 2.05-2.89(m, 5H,$CH_2CHO_2CCH(C_5H_{11})CH$+PhSeCH), 3.54-3.8(m,1H,CHOH), 4.56-4.74(m,1H,CHO_2C), 7.2-7.4(m,3H,*o*+*p*Ar-H), 7.50-7.68(m,2H, *m*Ar-H).

IR (CCl_4): 3600-3300(w,OH), 1785(s,C=O lactone), 680(s,Ar-H).

MS (Probe, m/z): 382(M^+,81%), 225(M-PhSe,14%), 207(M-PhSe-H_2O,81%), 179(M-PhSe-H_2O-CO,56%), 157.95 (PhSe,100%), 95(81%), 81(55%), 78(52%), 67(52%).

The molecular ion at m/z 382 showed a selenium isotope pattern with ions at m/z 384 (^{82}Se,16%), 380 (^{78}Se,38%), 379 (^{77}Se,15%), 378 (^{76}Se, 15%).

2.5.4.35 (3β,3aβ,4α,7aβ)-(±)-3a,4,7,7a-Tetrahydro-4-hydroxy-3-pentyl-2(3H)-benzofuranone (161)

To a stirred solution of (3β,3aβ,4α,7aβ)-(±)-hexahydro-4-hydroxy-3-pentyl-5-(phenylseleno)-2(3H)-benzofuranone (160) (329.5mg, 0.87mmol) in redistilled methanol (15ml) and tetrahydrofuran (2ml) at 0°C was added a solution of sodium periodate (416.9mg, 1.95mmol, 2 equiv.) in the minimum volume of water. The reaction was stirred at 0°C for 30 min and then at room temperature for 24h. The reaction mixture was filtered and the precipitate washed with methanol (x3, 15ml). The combined filtrate and washings were concentrated to afford a yellow oil which was dissolved in ethyl acetate and dried over sodium sulphate. Evaporation of the solvent gave a dark yellow oil (383.3mg) which was subjected to flash column chromatography (ether/petroleum ether,4:1) to give (161) (97.1mg, 0.43mmol, 50%) as a colourless oil. High resolution mass spectrometry gave $C_{13}H_{20}O_3$ for m/z 224.1325 (calc. m/z = 224.1413).

^{1}H NMR (100MHz, $CDCl_3$): 0.9(t,J=6Hz,3H,CH_3), 1.1-1.9(b envelope, 8H,4xCH_2), 2.15-2.86(m,5H,$CH_2CHO_2CCH(C_5H_{11})CH$+OH), 4.0-4.16(m,1H,CHOH), 4.72-4.92(m,1H,CHO_2CCH-), 5.73-6.04(m,2H,CH=CH-).

Irradiation at δ5.99 or δ5.79 resulted in the collapse of the multiplet at δ4.0-4.16 to a doublet (J=4Hz) while the resonance at δ2.15-2.86 was perturbed. The signal at δ2.15-2.86 was also perturbed on irradiation at δ4.82. Decoupling at δ2.7 resulted in the collapse of the multiplet at δ4.72-4.92 to a triplet (J=6Hz) while the resonance at δ2.15-2.86 was also perturbed. These two multiplets were also simplified on irradiation at δ2.34.

IR (CCl_4): 3620-3200(m,OH), 1770(s,C=O lactone), 730(w,HC=CH).

MS (Probe, m/z): 224(M^+,0.9%), 206(M-H_2O,4%), 154(M-C_5H_{10}, 38%), 95(41%), 71(23%), 70(100%), 69(20%), 67(27%), 55(38%), 41(37%).

2.5.4.36 (1aβ,2aβ,5β,5aβ,6α,6aβ)-(±)-Hexahydro-6-hydroxy-5-pentyl-oxireno[f]benzofuran-4(1aH)-one (162)

To a stirred solution of (3β,3aβ,4α,7aβ)-(±)-3a,4,7,7a-tetrahydro-4-hydroxy-3-pentyl-2(3H)-benzofuranone (161) (79.9mg, 0.35mmol) in dry methylene chloride (5ml) at 0°C was added slowly 80% *meta*-chloroperoxybenzoic acid (173mg, 0.8mmol, 2.2 equiv.). The homogeneous solution was stirred at 0°C for 64h, washed with saturated aqueous sodium sulphite (5ml), 5% aqueous sodium bicarbonate (5ml) and then brine (5ml) and dried (Na_2SO_4). Evaporation of the solvent gave an oil (107.3mg) which was purified by flash column chromatography (ethyl acetate/ether, 1:5.6) to give (162) as an oil which, on trituration with petroleum ether afforded a white solid (58mg, 0.24mmol, 69%). Recrystallisation from pentane yielded white needles, m.p. 43.5°-45.5°C, elemental analysis gave C = 64.79, H = 8.32; calc. for $C_{13}H_{20}O_4$, C = 64.98, H = 8.39. High resolution chemical ionization mass spectrometry gave $C_{13}H_{24}O_4N$ (MNH_4^+) for m/z 258.1612 (calc. 258.1705).

^1H NMR (100MHz, $CDCl_3$): 0.89(t,J=6Hz,3H,CH_3), 1.1-1.8(b envelope, 8H,4xCH_2), 2.08(ddd,J=16,6,<2Hz,1H, HCHCHO$_2$C), 2.2-2.40(m,1H,CHCH(C_5H_{11})CO_2-), 2.54-2.80(m,2H,CH(C_5H_{11})CO_2+HCH CHO$_2$C), 2.95(d,J=7Hz,1H,OH), 3.32-3.44 (m,2H,CH—O—CH), 3.98(t,J=7Hz,1H, CHOH), 4.58-4.68(m,1H,CHO$_2$CCH).

IR (CCl_4): 3620-3200(m,OH), 1765(s,C=O lactone).

MS (Probe, m/z): 170(42%), 125(23%), 98(46%), 97(30%), 83(100%), 82(23%), 79(19%), 55(21%).

CI-MS (NH_3, m/z): 259(^{13}C satellite MNH_4^+,18%), 258(MNH_4^+, 100%), 241(MNH_4-H_2O,46%), 223(11%), 142(10%), 83(12%), 55(13%), 41(12%).

CHAPTER THREE

Synthesis of (6E,10E)-Geranyllinalool, a Component of the Defence Secretion from Soldiers of *Reticulitermes lucifugus*

3.1 Introduction

3.1.1 The Termite Society

Termites are insects which belong to the order Isoptera and number over 2000 species. They are structurally related to cockroaches (Blattonidae) but differ greatly in being social insects living in colonies. All colonies are polymorphic, having three main castes (reproductives, workers and soldiers) with separate functions.

3.1.2 Termite Defence

The termites are a constantly available food source for various predators such as insectivorous mammals, birds, ants and other anthropods. Consequently, the termites have evolved a soldier caste whose task is to defend the colony.

The soldier caste of termites utilise both physical and chemical forms of defence which can be used separately or in conjunction. Physical defence involves the use of mandibles which have evolved into powerful weapons. Soldiers using this form of defence are large and their mandibles are designed for cutting, piercing and biting. The shape and precise action of the mandibles varies among the genera. A second form of physical defence is employed by some termites; soldiers with large heads plug the entrances to the nest and present a formidable barrier to invading ants.

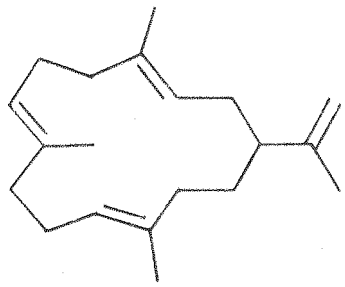
Many species of termite also use a chemical secretion in the defence mechanism. With species such as *Amitermes* and *Macrotermes* the opponent is firmly grasped and a secretion is allowed to flow into the wound. The secretion can also be applied onto the opponent using a labrial brush which involves physical contact but the mandibles do not cut the cuticle, e.g. *Rhinotermes*. The most highly evolved form of defence is used by the nasute termites who totally avoid any contact with the enemy. In these termites the head capsule has developed into an elongated rostrum called the nasus through which a gluey viscous secretion is ejected. The *Ruptitermes* also have a chemical defence which does not require physical contact. In these cases the termites burst themselves and spread large volumes of a sticky fluid onto the foe.

3.1.3 Composition of Soldier Secretions

A great variety of different compounds have been identified as components of the defence secretion of termites. These include quinones, monoterpenes, sesquiterpenes, long chain ketones and nitro compounds. A complete review of these materials is beyond the scope of this work. However, such information has been described in detail elsewhere.[179]

A few diterpene hydrocarbons have been found in the frontal gland secretion of termites: Cembrene A (186), previously isolated as a trail pheromone in the Australian termite *Nasutitermes exitiosus*[180] and (3Z)-Cembrene A (187) are two of the four diterpene hydrocarbons produced by *Cubitermes umbratus*.[181,182] A third component cubitene (188) has a novel diterpene skeleton with an irregular isoprenoid structure,[183] while the fourth component biflora-4,10(19),15-triene (189) has an unusual bicyclic skeleton.[184]

A large number of oxygenated diterpenes have been found in termite secretions of the genera *Trinervitermes* and *Nasutitermes*. The soldiers of *Trinervitermes gratiosus* produce a secretion which contains α-pinene (190), β-pinene (191), camphene (192), limonene (193) and terpinolene (194). Dissolved in this mixture were the oxygenated diterpenes (195), (196), (197), (198) and (199). Three other diterpenes were present in small amounts but were not identified.[185] A large number of similar tricyclic oxygenated diterpenes ('trinervitenes') have now been identified in soldier secretions of the families *Trinervitermes* and *Nasutitermes*.[179,186-190] A closely related series of tetracyclic oxygenated diterpenes ('kempenes') have also been isolated from termite soldiers.[191,192] Two such 'kempenes' (200) and (201) have been identified in the soldiers of *Nasutitermes kempae*.[193] The diterpene 3α-hydroxy-15(16)-rippertene (202), isolated from *Grallatotermes africanus* soldiers,[194] is a tetracyclic compound related to the 'kempenes' but which has undergone a 1,2-methyl shift. A bicyclic oxygenated diterpene (203) has been isolated from the soldier secretion of *Nasutitermes princeps* which was identified by an X-ray diffraction study.[195]



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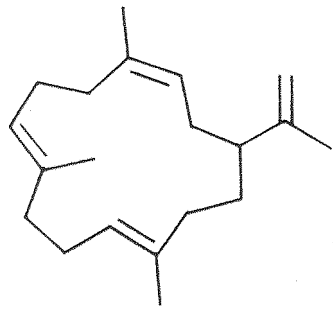

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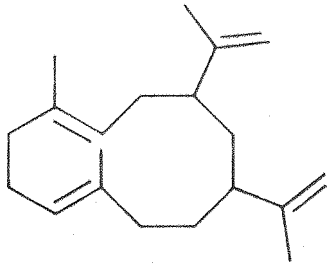

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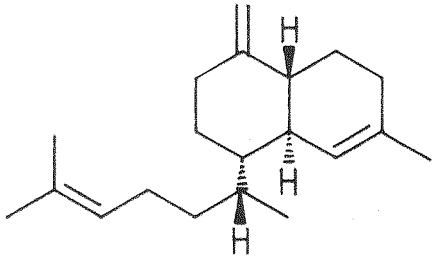

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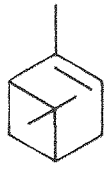

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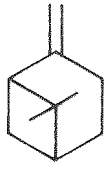

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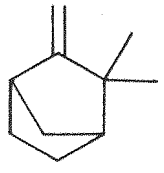

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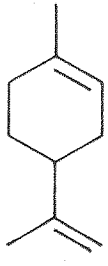

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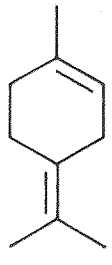

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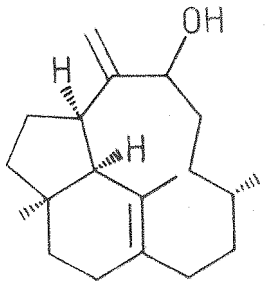

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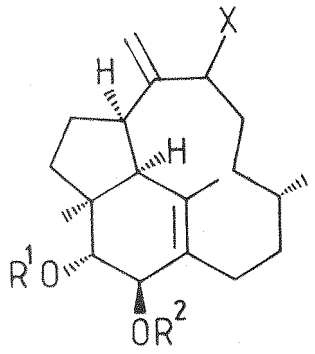
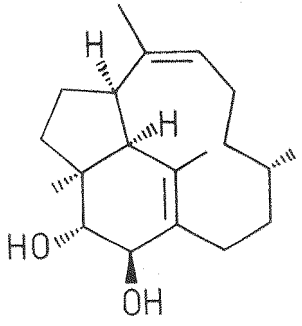


(196) $R^1 = R^2 = X = H$
(197) $R^1 = R^2 = H$, X = OAc
(198) $R^1 = R^2 = Ac$, X = OH



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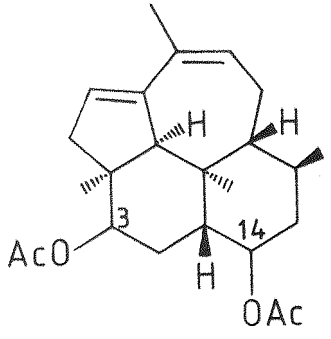
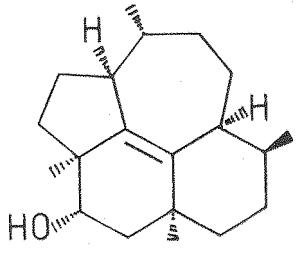
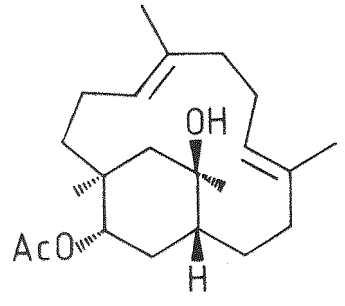


(200) 3α-OAc, 14β-OAc
(201) 3β-OAc, 14α-OAc



(202)

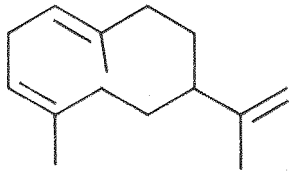


(203)

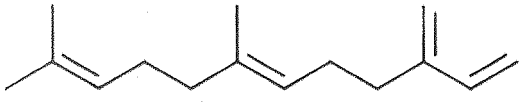
3.1.4 Defence in *Reticulitermes lucifugus*

The soldiers of *Reticulitermes* have mandibles of the biting type,[196] they also have a frontal gland in the head capsule. Thus, both physical and chemical defence mechanisms are possible. When soldiers of *R. lucifugus* are provoked, they lunge in the direction of the 'intruder', closing their mandibles violently. If the mandibles close around a thin object, the labium moves backwards and forwards. A few seconds after the initial provocation, secretion from the frontal gland runs down onto the labium and mandibles.[197]

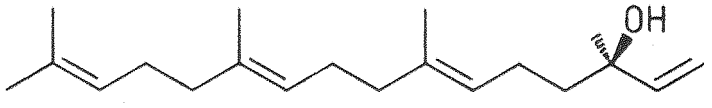
A recent study of the frontal gland secretion and whole extracts of *R. lucifugus* soldiers showed that the major component was a diterpene alcohol together with two sesquiterpene hydrocarbons.[197,198] The sesquiterpene hydrocarbons were found to be germacrene A (204) (10µg/insect) and β-farnesene (205) (1µg/insect) by preparative GC and GC-MS. The diterpene alcohol (40µg/insect) was isolated by reversed phase HPLC and chracterisation by GC-MS, 100MHz ^{1}H NMR and optical rotation indicated that it was the isoprenoid alcohol(R)-(-)-(6E,10E)-geranyllinalool (206).



(204)

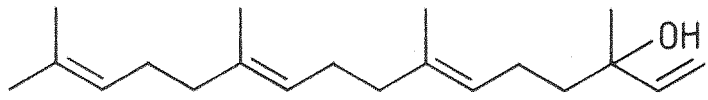


(205)



(206)

It is possible the (R)-(-)-(6E,10E)-geranyllinalool (206) may have some biological activity, since it is the major component of the defence secretion of *R. lucifugus*. Insufficient quantities of the alcohol (206) were available from the termite (ca. 7mg) to test for possible activity. Biological testing on (206) would, therefore, require larger quantities which would only be available by synthesis. Thus, a total synthesis of (±)-(6E,10E)-geranyllinalool (207) was contemplated.

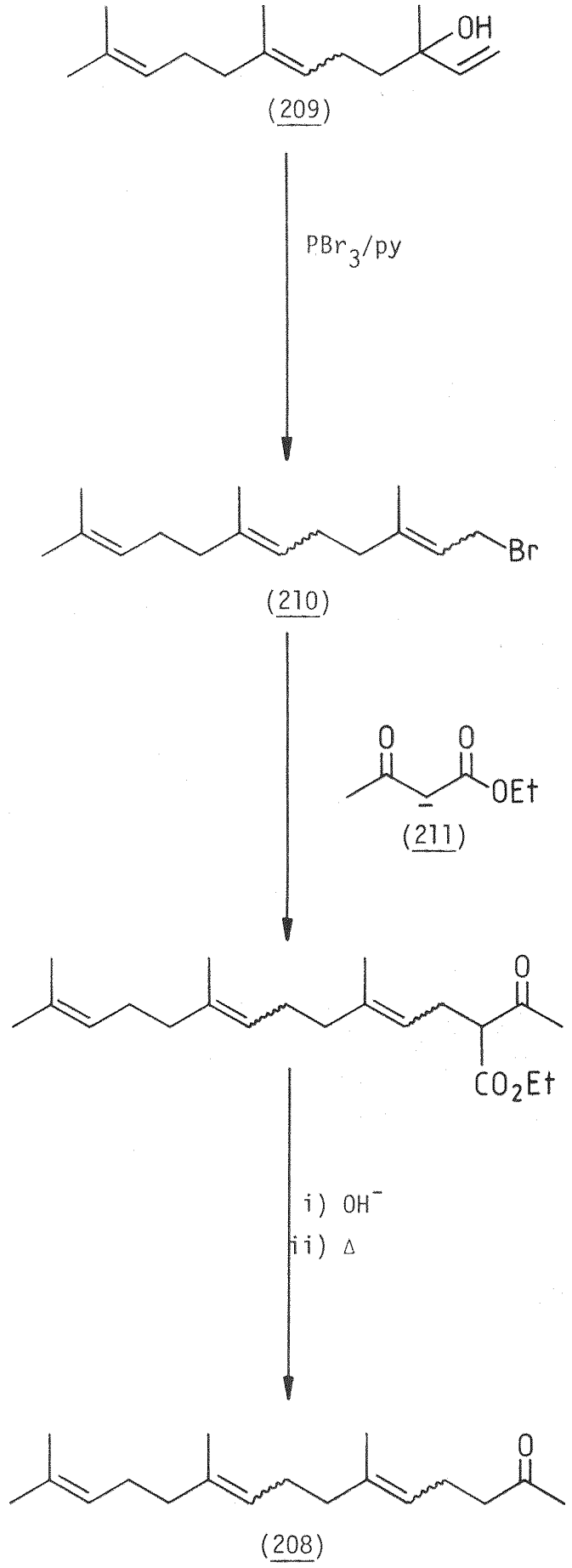


(207)

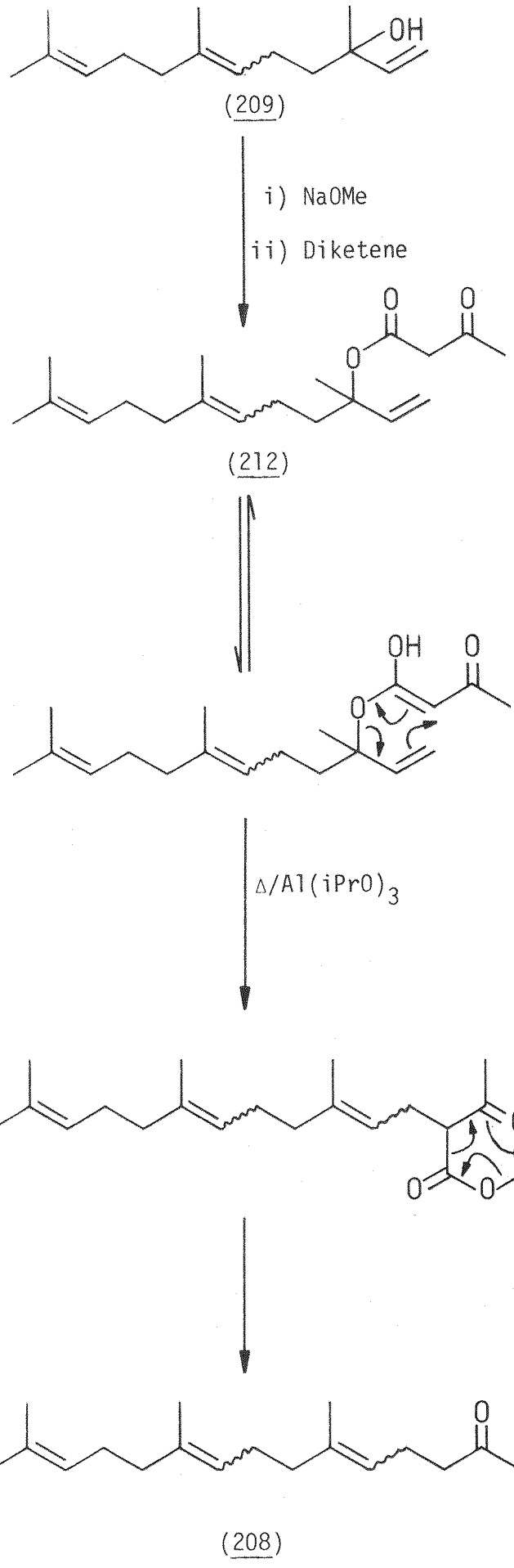
3.1.5 Previous Synthetic Approaches to Geranyllinalool (207)

The synthesis of a mixture of (6E,10E),(6E,10Z),(6Z,10Z) and (6Z,10E)-geranyllinalool (207) was first reported by Ruzicka *et al.*[199] in 1939 and a number of workers have reported the synthesis of this mixture subsequently.[200-204] In all cases the synthesis proceeds *via* a mixture of (5E,9E),(5E,9Z), (5Z,9Z) and (5Z,9E)-farnesylacetone (208) which is generally obtained from a mixture of (6E) and (6Z)-nerolidol (209).

There are three basic approaches to the transformation of (6E) and (6Z)-nerolidol (209) to a mixture of (5E,9E), (5E,9Z), (5Z,9Z) and (5Z,9E)-farnesylacetone (208). The first method is that of Ruzicka *et al.*[199] in which bromination of nerolidol (209) with phosphorus tribromide gave farnesyl bromide (210). Treatment of the bromide (210) with the anion of ethyl acetoacetate (211) followed by saponification and decarboxylation gave farnesylacetone (208) (Scheme 3.1).[200,201]
The second approach involved the *trans* esterification of nerolidol (209) with diketene or ethyl acetoacetate which gave nerolidyl acetoacetate (212). Subsequent heating in the presence of aluminium isopropoxide afforded farnesylacetone (208) *via* a Carroll rearrangement (Scheme 3.2).[201-203]



Scheme 3.1



Scheme 3.2

The third method involved the use of a Claisen rearrangement by an acid catalysed *trans* etherification of isopropenyl methyl ether with nerolidol (209) followed by a [3,3] sigmatropic rearrangement which gave farnesylacetone (208) (Scheme 3.3).[205]

The farnesylacetone (208) was then converted to geranyllinalool (207) either by reaction with sodium acetylide which gave geranyldehydrolinalool (213) followed by catalytic hydrogenation[199-203] or by the one step reaction with vinyl magnesium bromide[204] (Scheme 3.4).

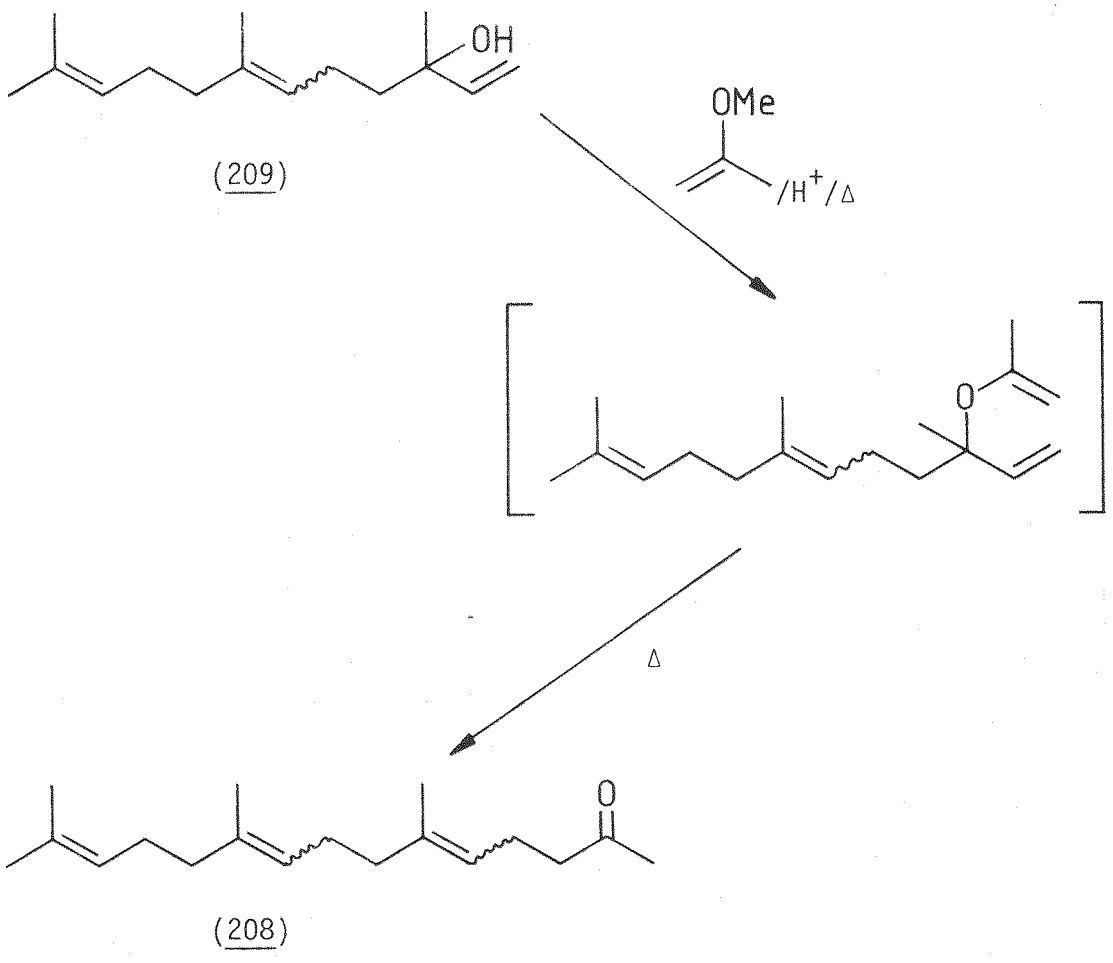
3.1.6 Double Bond Geometry

The use of (6E)-nerolidol (209) in the above reaction schemes would ensure the (9E) stereochemistry in farnesylacetone (208) and hence the (10E) stereochemistry in geranyllinalool (207). However, the trisubstituted double bond at the 5 position in farnesylacetone (208) is generated non-specifically in the above reaction schemes. A synthesis of (6E,10E)-geranyllinalool (207) from (6E)-nerolidol (209) will, therefore, require the separation of the (5E,9E) and (5Z,9E) isomers of farnesylacetone (208). Such a separation has been reported by chromatography on alumina of the semicarbazone of (5E,9E) and (5Z,9E)-farnesylacetone,[206] but it was hoped that separation of (5E,9E) and (5Z,9E)-farnesylacetone (208) could be achieved using silver nitrate impregnated silica gel column chromatography.[207-210]

3.2 Results and Discussion

3.2.1 Model Work

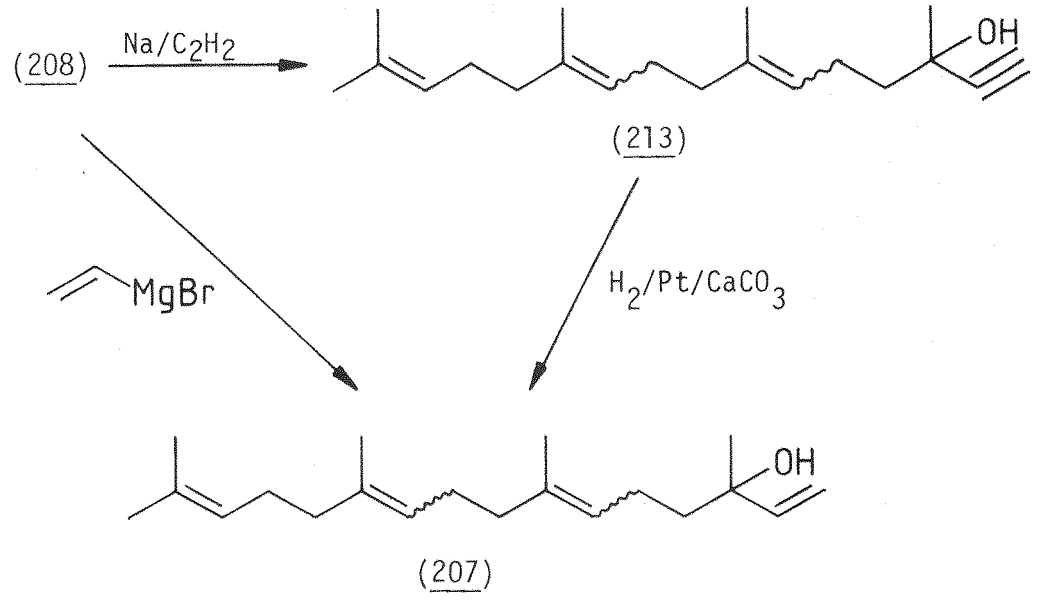
To find the optimum route to farnesylacetone (208) an investigation of the synthesis of (5E) and (5Z)-geranylacetone (214) from linalool (215) was carried out using the above approaches (Scheme 3.5).



(209)

(208)

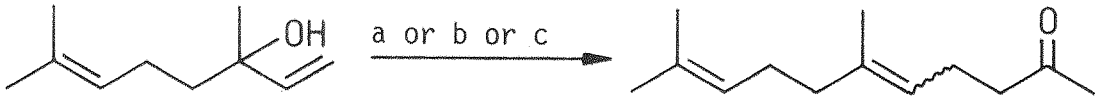
Scheme 3.3



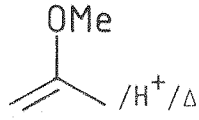
(213)

(207)

Scheme 3.4



(215) (214)

a) 

b) (i) PBr_3/py (ii) $CH_3\bar{C}OCHCO_2Et$ (211) (iii) NaOH/Δ

c) (i) Diketene/NaOMe (ii) Δ/$Al(iPrO)_3$

Scheme 3.5

Analysis of the three possible routes to farnesylacetone (208) from nerolidol (209) (see Section 2.1.5) showed that the route utilising the Claisen rearrangement method would involve a single step (Scheme 3.3). The yields of these rearrangements were generally greater than 80%[205] and thus this route appeared to be the most attractive. However, attempted Claisen rearrangement reactions with linalool (215) gave mainly rearrangement of the hydroxyl group and elimination products (Table 3.1) (Scheme 3.5 conditions (a))

Alcohol	Reagent	Catalyst	Conditions
(215)	2,2-Dimethoxypropane	H_3PO_4	Sealed tube/120°C/15h
(215)	2,2-Dimethoxypropane	H_3PO_4	Sealed tube/150°C/17h
(215)	Acetone	*p*TsOH	Toluene/Δ/12h
(215)	2,2-Dimethoxypropane	*p*TsOH	Xylene/100°C/15h
(215)	Isopropenyl methyl ether	*o*-nitrobenzoic acid	Xylene/Δ/15h

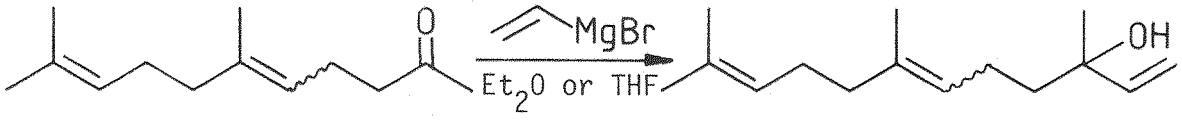
Table 3.1: Conditions of Claisen Rearrangement Reactions with Linalool (215)

The method of Ruzicka *et al.*[199] was investigated with linalool (215) (Scheme 3.5, conditions (b)) which also gave low yields of geranylacetone (214) (ca. 17%). The literature yields for this method (25%,[199] 48%,[200]) are at best moderate, therefore, it was concluded that

this was an inherently low yield route and consequently was not suitable.

In contrast to the above two routes, test reactions on linalool (215) using the Carroll rearrangement method (Scheme 3.5, conditions (c)) gave good yields of geranylacetone (214) (66%). This route was, therefore, chosen for the synthesis of farnesylacetone (208).

Initial experiments on the conversion of geranylacetone (214) to nerolidol (209) with vinyl magnesium bromide gave low yields (36%) with ether as the solvent (Scheme 3.6). However, under identical conditions with THF as the solvent, excellent yields of nerolidol (209) (71%) were obtained. This observation is generally found for alkenyl magnesium halides and is thought to be due to the greater availability of the lone pair of electrons in THF. Thus, THF stabilises the alkenyl magnesium halide by complex formation and, therefore, prevents disproportionation.[211]

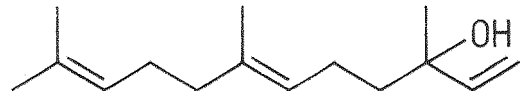


(214) (209)

Scheme 3.6

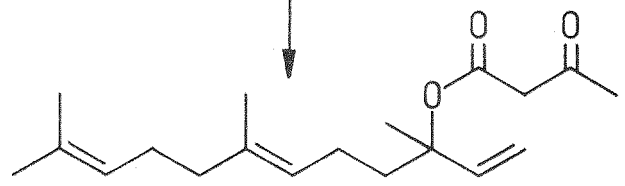
3.2.2 Synthesis of (±)-(6E,10E)-Geranyllinalool (207)

The synthesis of (±)-(6E,10E)-geranyllinalool (207) from (6E)-nerolidol (209) is shown in Scheme 3.7. Thus, treatment of (6E)-nerolidol (209) with diketene in the presence of sodium methoxide gave (6E)-nerolidyl acetoacetate (212) in 93% yield. Subsequent heating of (212) to 140°-170°C in the presence of aluminium isopropoxide afforded a mixture of (5E,9E) and (5E,9Z)-farnesylacetone (208) in the ratio of 3:2 respectively. The (5E,9E) and (5Z,9E) isomers of farnesylacetone (208) were separated by two chromatographs on silver nitrate impreg-



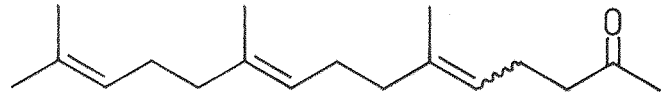
(209)

NaOMe/Diketene/Toluene
93%



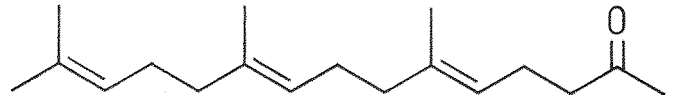
(212)

$\Delta/Al(iPrO)_3$
59%



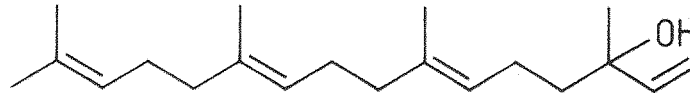
(208)
(5E,9E):(5Z,9E) = 3:2

Separate/$AgNO_3$ (30%) - Silica



(5E,9E)-(208) >98%

/THF/66%



(6E,10E)-(207)

Scheme 3.7

nated silica (30% loading)[207,208] with pentane/ether (3:1) as eluant. This separation afforded (5E,9E)-farnesylacetone (208) in greater than 98% purity by GC while the (5Z,9E) isomer was obtained in 93% purity. Reaction of (5E,9E)-farnesylacetone with vinyl magnesium bromide in THF yielded (±)-(6E,10E)-geranyllinalool (207) in 66% yield.

3.2.3 Assignment of All *trans* Stereochemistry

3.2.3.1 ^{1}H NMR

The 100MHz ^{1}H NMR of synthetic (±)-(6E,10E)-geranyllinalool (207) is given in Table 3.2 and Figure 3.1 and was assigned in accordance with the literature case for(R)-(-)-(6E,10E)-geranyllinalool (206).[212]

Shift (δ)	Signal Pattern	Integration	Assignment[212]
5.9	dd, J=18,10Hz	1H	H-2
5.18	dd, J=18,2Hz	)	H^a-1
5.1	b signal	) 5H	H-6,10,14
5.04	dd, J=10,2Hz	)	H^b-1
2.02	b envelope	12H	C-4,5,8,9,12,13 CH_2's
1.66	s	3H	C-16 CH_2
1.6	s	9H	C-17,18,19 CH_2's
1.28	s	3H	C-20 CH_2

Table 3.2: 100MHz ^{1}H NMR of (±)-(6E,10E)-Geranyllinalool (207)

The ^{1}H NMR of synthetic (±)-(6E,10E)-geranyllinalool (207) (Figure 3.1, Table 3.2) shows two singlets at δ1.66 and δ1.6 in a 1:3 ratio, which are due to the methyl groups on the trisubstituted olefins.[213,215] The methyl groups in trisubstituted olefins of the E configuration (216) have been found to resonate at δ1.56 or δ1.65, while the methyl groups of the corresponding Z isomer (217) gave rise to a signal at δ1.7.[214,215] The expected ratio for the δ1.66 and δ1.6 singlets from the above discussion should be 1:3 for the (6E,10E) isomer, while a ratio of 1:1 would be anticipated for the (6Z,10E) isomer. The observed ratio of these two signals in the ^{1}H NMR of (207) is 1:3 and hence the all '*trans*' (6E,10E) geometry can be assigned to (207).

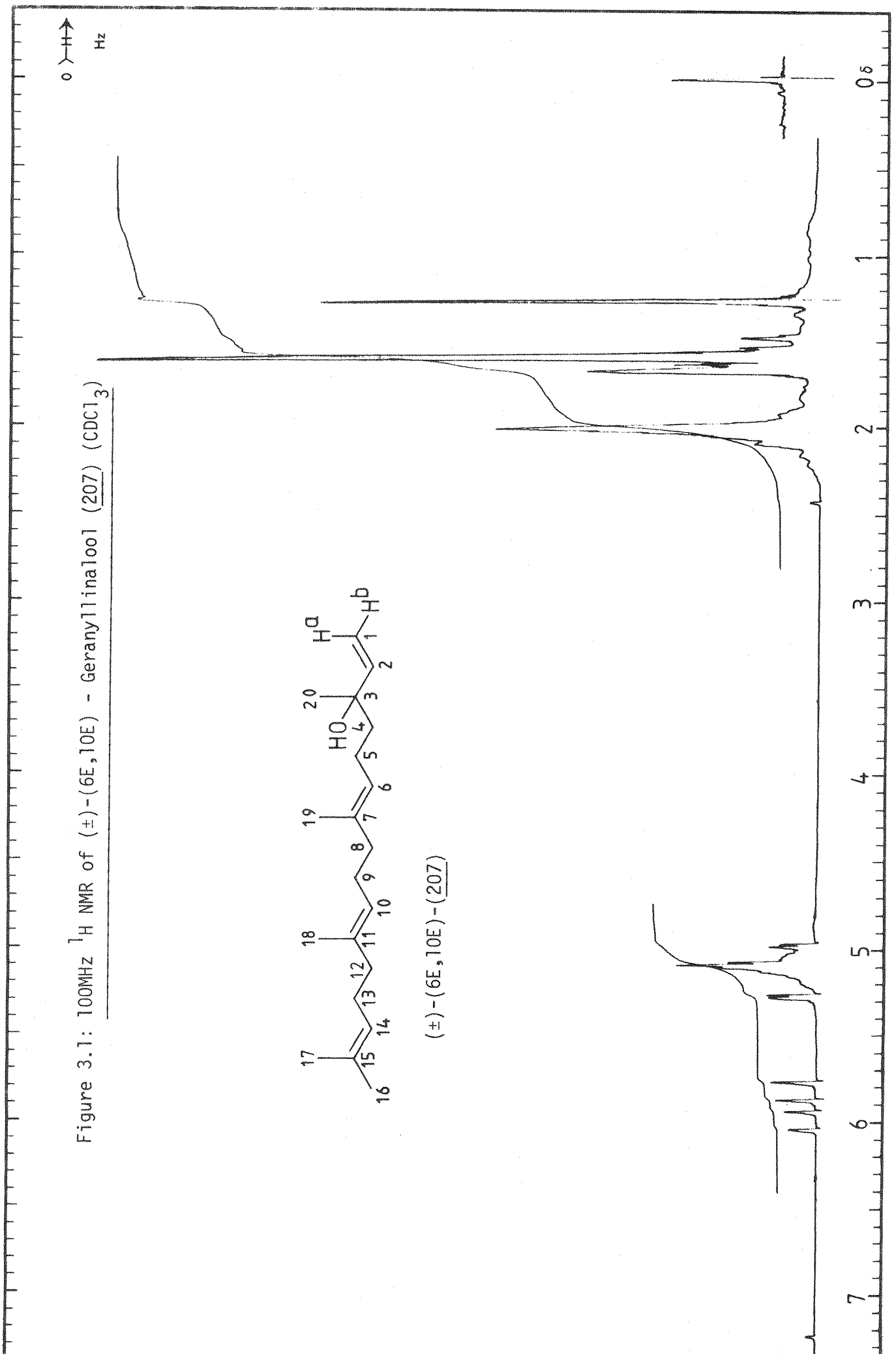
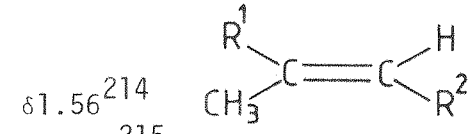
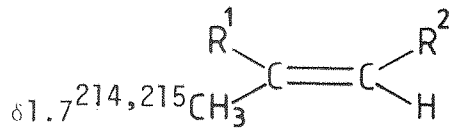


Figure 3.1: 100MHz ^1H NMR of (±)-(6E,10E) - Geranyllinalool (207) ($CDCl_3$)



(216)



(217)

3.2.3.2 ^{13}C NMR

Further evidence for the (6E,10E) geometry of (207) was obtained by analysis of the ^{13}C NMR of (207) (Figure 3.2, Table 3.3) which was assigned by comparison with the published spectra of related terpenoids.[216] It has been observed that the average difference between *cis* and *trans* α carbons in a trisubstituted double bond is about 8 ppm for methyl and methylene carbons.[217] As for the ^{1}H NMR case, the methyl groups in a trisubstituted double bond of the E configuration resonate upfield of the corresponding Z configuration methyl group in the ^{13}C NMR (Δ ppm = 8).[217] In the ^{13}C NMR of (207) (Figure 3.2, Table 3.3) there are three methyl groups between 16.02 and 17.66 ppm but only one methyl group at 25.71 ppm. The expected ratio for the (6E,10E) isomer from the above discussion should be 3:1 respectively, while for (6Z,10E) isomer a ratio of 1:1 would be anticipated. The observed ratio for the trisubstituted olefin methyl groups in the areas ∿ 16 ppm and 26 ppm is 3:1 respectively and hence (207) has the 'all *trans*' geometry.

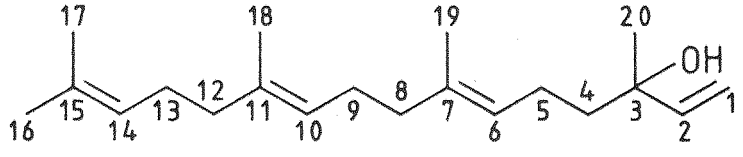
3.2.4 Comparison of the Natural and Synthetic Geranyllinalool Spectra

The 100MHz ^{1}H NMR of (R)-(-)-(6E,10E)-geranyllinalool (206) from *Reticulitermes lucifugus* soldiers[197,198] is given in Figure 3.3.† Comparison of the ^{1}H NMR spectra of the synthetic (Figure 3.1) and

† courtesy of A.H. Parton

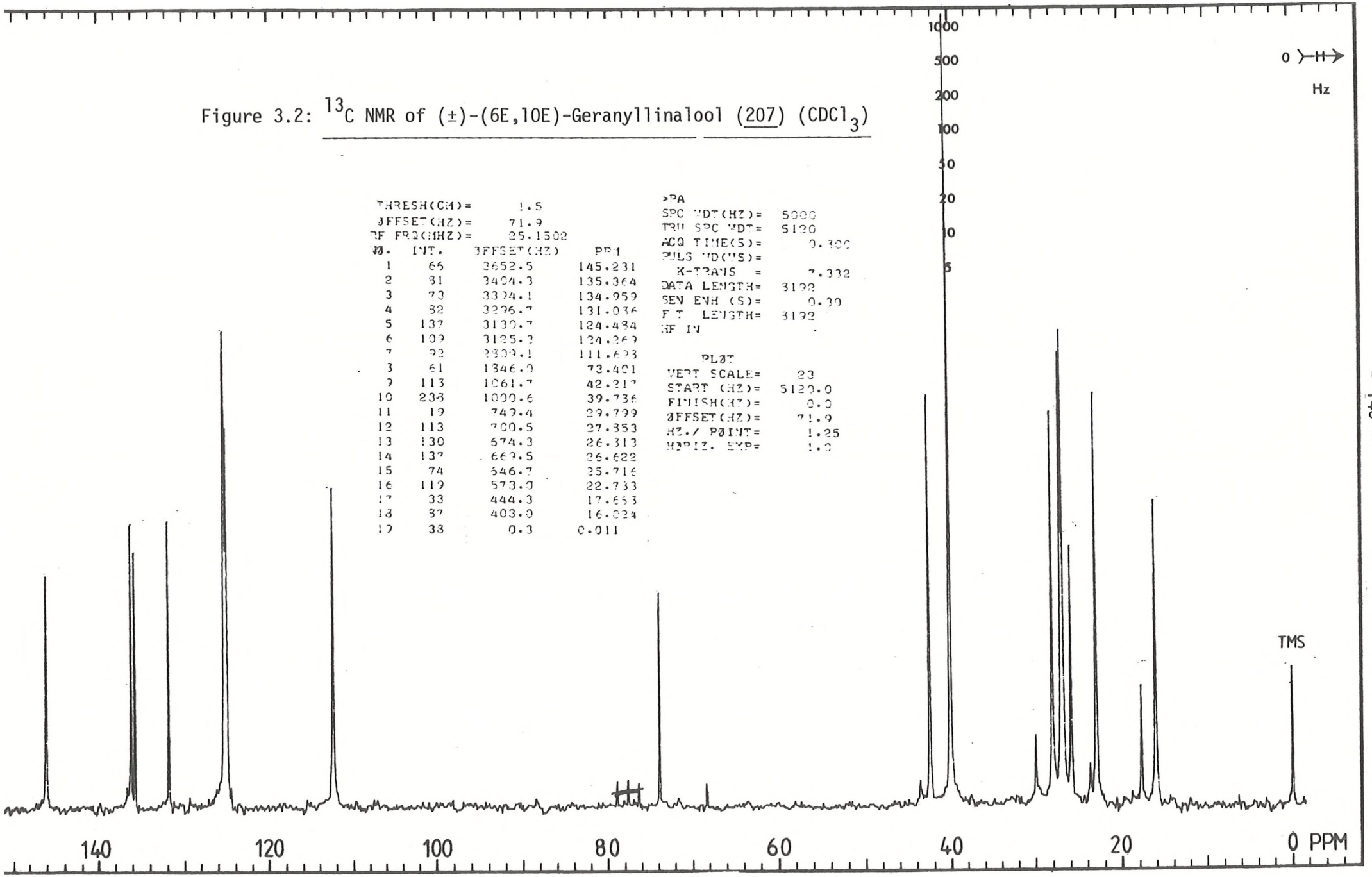
Shift (ppm)	Off Resonance Signal Pattern	Integration	Assignment[216]
145.22	d	66	C-2
135.35	s	81	)
134.95	s	73	) C-11,7)
131.07	s	82	C-15
124.47	d	137	)
124.26	d	109	) C-14,10,6)
111.68	t	92	C-1
73.40	s	61	C-3
42.20	t	118	C-4
39.78	t	238	C-12,8
27.84	-	113	C-20
26.80	-	130	C-13
26.61	-	137	C-9
25.71	-	74	C-16
22.77	t	119	C-5
17.66	q	33	C-17
16.02	q	87	C-18,19

Table 3.3: ^{13}C NMR of (±)-(6E,10E)-Geranyllinalool (207)



(±)-(6E,10E)-(207)

Figure 3.2: ^{13}C NMR of (±)-(6E,10E)-Geranyllinalool (207) ($CDCl_3$)



natural geranyllinalool (Figure 3.3) showed that they were identical in every respect. The ten most intense ions in the GC-MS of the natural[197] and synthetic geranyllinalool were the same with the same base peak (m/z 69) (see Section 3.4.4.9). The infra-red spectra of the natural product (CCl_4 solution)[197] showed the same absorptions as the synthetic material (thin film) except the hydroxy absorption was very sharp in the natural product (see Section 3.4.4.9).

3.2.5. Biological Activity

Preliminary trials of biological activity using synthetic (±)-(6E,10E)-geranyllinalool (207) indicated that it was toxic to *Camponotus vagus* and *Crematogaster scutellaris* on topical application. In feeding tests, quantities equivalent to those found in 10 soldiers were repellent to these species.[198]

Biological trials were also carried out on the leaf cutting ant, *Atta cephalotes* by A.H. Parton using the 'cut and apply' technique.[218] After treatment, the ants were kept in perspex petri dishes with moist filter paper and their state of health was determined after 24h. Four states of healt were recognised:-

A - Normally active; upright with good balance. Score 0

B - Slow moving; awkward, clumsy or jerky movements; uneven balance. Score 1

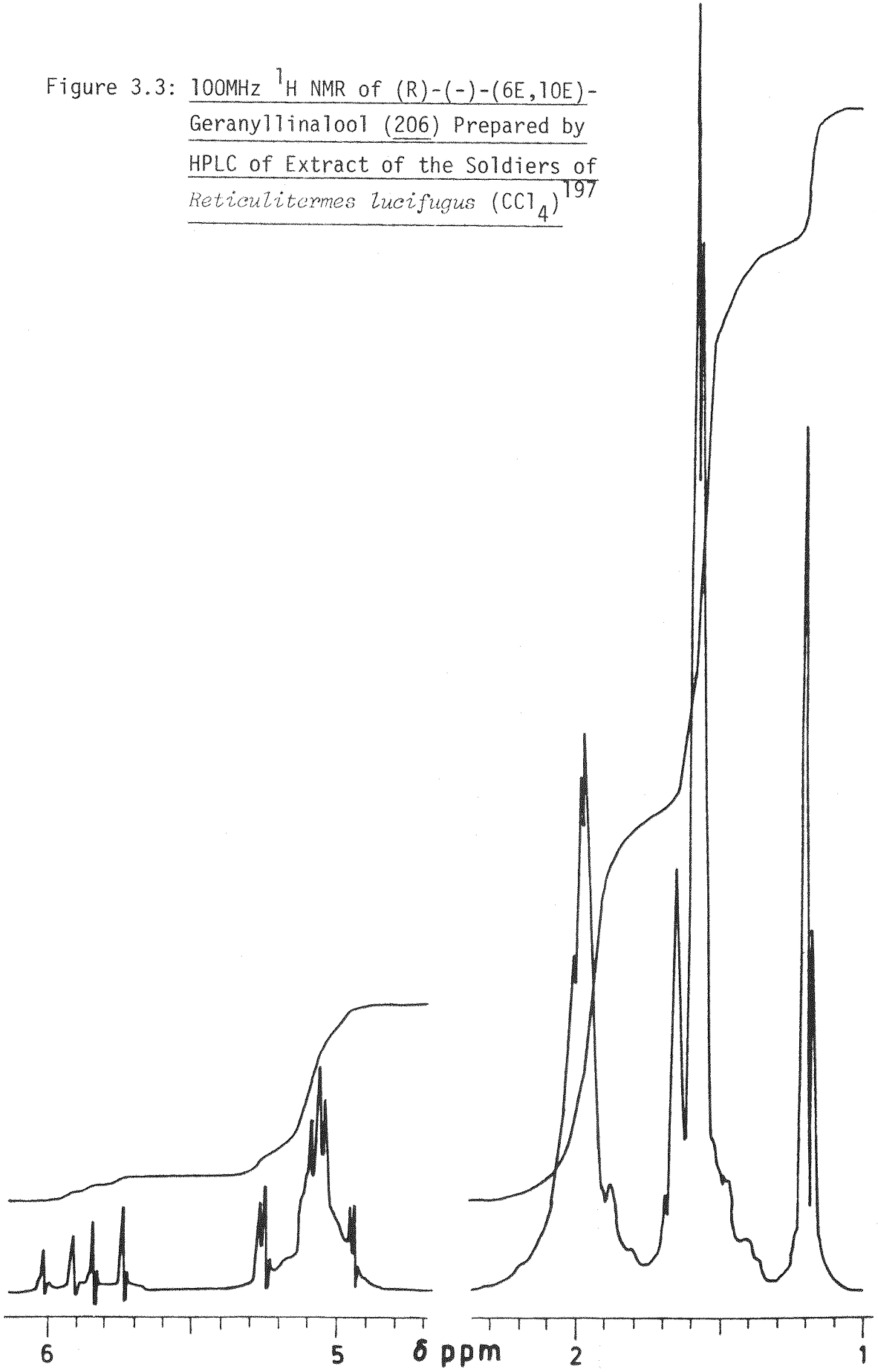
C - Debilitated; nearly dead. Score 2

D - Dead. Score 3

The results for a blank (cut but no topical application); ethanol control (cut + 1µl ethanol); 2µg, 5µg, 10µg and 50µg of (±)-(6E,10E)-geranyllinalool (207) each in 1µl of ethanol are given in Table 3.4.[218]

Analysis of these results gave an LD_{50} value somewhere between 10-5µg of (207). This value is lower than the quantities of (R)-(-)-(6E,10E)-geranyllinalool (206), available in the soldiers of *Reticulitermes lucifugus* (ca. 40µg/soldier).[197,198] This factor would, therefore, indicate that (206) is a toxic component of the defence secretion of *R. lucifugus* soldiers.

Figure 3.3: 100MHz ^{1}H NMR of (R)-(-)-(6E,10E)-Geranyllinalool (206) Prepared by HPLC of Extract of the Soldiers of *Reticulitermes lucifugus* (CCl_4)[197]



Treatment	Sample No.	Number				Percentage				Average Score 0.0 - 3.0
		A	B	C	D	A	B	C	D	
Blank	15	14	0	0	1	94	0	0	6	0.2
Ethanol	31	20	2	2	7	65	6	6	23	0.9
2μg (207)	24	17	6	0	1	71	25	0	4	0.4
5μg (207)	24	5	10	5	4	21	42	21	17	1.5
10μg (207)	32	7	3	2	20	22	9	6	63	2.1
50μg (207)	7	0	0	0	7	0	0	0	100	3.0

Table 3.4: Results of Biological Trials of (±)-(6E,10E)-Geranyllinalool (207) on the Leaf Cutting Ant, *Atta cephalotes*[218]

3.3 Conclusion

The synthesis of (±)-(6E,10E)-geranyllinalool (207) was achieved from (6E)-nerolidol (209) *via* a Carroll rearrangement which gave a mixture of (5E,9E) and (5Z,9E)-farnesylacetone (208) in a 3:2 ratio respectively. The (5E,9E)-farnesylacetone (208) was obtained in greater than 98% purity by silver nitrate impregnated silica gel column chromatography of the mixture. This, on treatment with vinyl magnesium bromide, afforded the required (±)-(6E,10E)-geranyllinalool (207).

Comparison of the 100MHz ^{1}H NMR, infra-red and mass spectra of (±)-(6E,10E)-geranyllinalool (207) with the (R)-(-)-(6E,10E)-geranyllinalool (206), found in the soldiers of *Reticulitermes lucifugus*, showed the spectral details to be identical.

Biological testing on (±)-(6E,10E)-geranyllinalool (207) indicated that it was toxic to *Atta cephalotes*, *Camponotus vagus* and *Crematogaster scutellaris*. The LD_{50} was found to be between 5-10µg per ant in the case of *Atta cephalotes*, which was significantly lower than the quantities found in *Reticulitermes lucifugus* soldiers (40µg/soldier). In feeding tests, quantities equivalent to 10 soldiers were repellent to *Camponotus vagus* and *Crematogaster scutellaris*.

3.4 Experimental

3.4.1 Purification of Reagents and Solvents

The purity of 3,7-dimethyl-1,6-octadien-3-ol (linalool) (215) was checked by GC (5% OV101, 162°C) before use and was used without further purification. (±)-(6E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (*trans* nerolidol) (209), a gift from the Takasago Chemical Company Ltd., Japan, was found to be greater than 98% the (6E) isomer by GC (5% FFAP, 200°C) and was used as supplied. Phosphorus tribromide (BDH), ethyl acetoacetate (BDH), diketene (Aldrich Chemical Co.) and vinyl bromide (Aldrich Chemical Co.) were all distilled before use. Aluminium isopropoxide (Hopkins and Williams) was sublimed (135°C at 17mm Hg) immediately before use. All other solvents and reagents were purified as described previously (see Section 2.5.1).

3.4.2 General Procedures

These were as described earlier (see Section 2.5.2).

3.4.3 Instrumentation

The gas chromatographic analysis of the (5E,9E) and (5Z,9E) isomers of 6,10,14-trimethyl-5,9,13-pentadecatrien-2-one (farnesylacetone) (208) was carried out on a 3.2mm x 20' column of 5% FFAP on diatomite AAW/DMCS (100-120 mesh) maintained at 240°C. All other conditions and columns were as described previously (see Section 2.5.3).

Noise decoupled ^{13}C NMR spectra were recorded at 25.15MHz on a Varian Associates XL-100-12 spectrometer with deuterochloroform as the solvent. Peak positions are reported using the δ scale with the internal standard, tetramethylsilane as zero. All other instrumentation was as described in Section 2.5.3.

3.4.4 Experimental Procedures

3.4.4.1 6,10-Dimethyl-5,9-undecadien-2-one (Geranylacetone) (214)

6,10-Dimethyl-5,9-undecadien-2-one (geranylacetone) (214) was prepared from 3,7-dimethyl-1,6-octadien-3-ol (linalool) (215) *via* 1-bromo-3,7-dimethyl-2,6-octadiene by the method of Weichet *et al.*[203] Purification by flash column chromatography (ether/petroleum ether, 1:2.3) afforded a mixture of (5E) and (5Z)-(214) (2.13g, 10.98mmol, 17%) in a 7:3 ratio respectively (assayed by GC, 5% FFAP, 150°C) (b.p. 76°-78°C at 0.25mm Hg, Lit. b.p. 66°-69°C at 0.03mm Hg,[205] 126°-130° at 12mm Hg[203]).

3.4.4.2 3,7-Dimethyl-1,6-octadien-3-acetoacetate (linalyl acetoacetate)

This was prepared from 3,7-dimethyl-1,6-octadien-3-ol (linalool) (215) by the method of Kimel and Cope.[219] Distillation afforded 3,7-dimethyl-1,6-octadien-3-acetoacetate (10.1g, 42.4mmol, 65%) as a colourless oil (b.p. 78°-80°C at 0.04mmHg, Lit.[219] b.p. 71°-74°C at 0.006mm Hg).

3.4.4.3 6,10-Dimethyl-5,9-undecadien-2-one (Geranylacetone) (214)

6,10-Dimethyl-5,9-undecadien-2-one (geranylacetone) (214) was prepared from 3,7-dimethyl-1,6-octadien-3-acetoacetate (linalyl acetoacetate) by the method of Kimel and Cope.[219] Distillation (b.p. 76°-78°C at 0.25mm Hg, Lit. b.p. 66°-69°C at 0.03mm Hg,[205] 126°-130°C at 12mm Hg[203]) of the residue yielded (214) (5.4g, 27.8mmol, 66%) as a mixture of(5E)and (5Z)isomers in the ratio of 1.7:1 respectively (by GC, 5% FFAP, 150°C).

3.4.4.4 3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (Nerolidol) (209)

To a stirred suspension of magnesium turnings (133mg, 5.54mmol) in tetrahydrofuran (25ml) under a nitrogen atmosphere was added two drops of 1,2-dibromoethane. To this mixture was added freshly distilled vinyl bromide (0.62g, 5.75mmol) and the reaction was stirred for 30 min to generate a light brown solution. To this cooled solution (0°C) was added a solution of 6,10-dimethyl-5,9-undecadien-2-one (geranylacetone) (214) (1.0g, 5.17mmol) in tetrahydrofuran over a 1h period. The reaction was stirred at 0°C for 4h, whereupon iced water (10ml) was added, followed by 2N HCl (10ml). The organic layer was separated and the aqueous residue was extracted with ether (x3, 25ml). The combined organic extracts were concentrated to give a brown oil which was dissolved in ether (50ml) and washed with water (50ml). Drying (Na_2SO_4) and evaporation of the solvent gave a light brown oil which was purified by Kugelrohr distillation (b.p. 98°-101°C at 0.08mm Hg, Lit. b.p. 94°C at 0.18mm Hg,[201] 108°-112°C at 2mm Hg[203]) to give (209) (0.82g, 3.68mmol, 71%) as a colourless oil. Analysis by GC (5% FFAP, 200°C) showed that (209) was a mixture of(6E)and (6Z)isomers in the ratio of 1.7:1 respectively.

Under identical conditions with ether as the solvent a 36% yield of (209) was obtained.

3.4.4.5 (6E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-acetoacetate (nerolidyl acetoacetate) (212)

The synthesis of 3,7,11-trimethyl-1,6,10-dodecatrien-3-acetoacetate (212) was carried out by a modification of the method of Kimel *et al.*[219,220] Thus, to a solution of (6E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol ((6E)-nerolidol) (209) (15g, 67.7mmol) and sodium methoxide (74mg) in

anhydrous toluene under nitrogen was added dropwise diketene (6.24g, 74.3mmol) over a 2h period. The resulting solution was stirred for 16h then worked up by washing sequentially with 2N sulphuric acid (100ml), saturated aqueous sodium bicarbonate solution (100ml) and then with water (100ml) until the washings were neutral. The resulting toluene extract was dried over magnesium sulphate and evaporation of the solvent afforded (212) (19.22g, 62.8mmol, 93%) as a yellow oil. This residue was subjected to an attempted distillation but at 0.03mm Hg this did not distil below 130°C.†

Crude NMR (60MHz, $CDCl_3$): 1.55(s,3H,$CH_3COCOCH_2$-), 1.6(s,6H,2xCH_3C=CH), 1.66(s,3H,CH_3C=CH-), 1.95(t,J=6Hz,8H,4xCH_2), 2.25(s,3H,$CH_3COCH_2CO_2$-), 3.3(s,2H,$CH_3COCH_2CO_2$-), 4.95-5.3(m,4H,2xCH=C+C=CH_2), 6.0(dd, J=10,18Hz,1H,CH=CH_2).

Crude IR (thin film): 1745(s,C=O ester), 1720(s,C=O ketone), 1650 (m,C=C), 995(m,RCH=CH_2), 930(m,RCH=CH_2), 830(w,R_2C=CHR).

Crude MS (Probe, m/z): 107(36%), 93(78%), 81(31%), 80(24%), 69(100%), 55(29%), 43(51%), 41(38%).

3.4.4.6 (5E,9E) and (5Z,9E)-6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one (Farnesylacetone) (208)

The Carroll rearrangement of (6E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-acetoacetate (212) was carried out by a modification of the method of Kimel *et al.*[219,220] Thus, a mixture of (212) (19.1g, 62.4mmol) and freshly sublimed aluminium isopropoxide (220mg) were stirred vigorously under a nitrogen atmosphere and heated to 140°-170°C until the evolution of carbon dioxide had ceased (3h).* Distillation of the residue (b.p. 126°-129°C at 0.225mm Hg, Lit.[205] b.p. 109°-111°C at 0.07mm Hg) afforded (208) (9.57g, 36.5mmol, 59%) as a colourless oil. Analysis of this oil by GC (3.2mm x 20' column of 5% FFAP, 240°C) indicated that it was a mixture of the (5E,9E) and (5Z,9E) isomers in the ratio of 3:2 respectively.

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† These acetoacetates undergo the Carroll rearrangement at 140°C.[219,220]

* Evolution of CO_2 was followed by bubbling the nitrogen from the reaction vessel through lime water.

3.4.4.7 Silver Nitrate Column Chromatography

The separation of the (5E,9E) and (5Z,9E) isomers of 6,10,14-trimethyl-5,9,13-pentadecatrien-2-one (208) was achieved by two chromatographs on a column of 30% silver nitrate impregnated silica gel (100g). Aliquots of the mixture (2g) were eluted with pentane and diethyl ether (3:1) and collected in 25ml fractions . This gave (5E,9E)-(208) which was found to be greater than 98% the all *trans* isomer, in addition the (5Z,9E) isomer of (208) was obtained in 93% purity((5E,9E) and (5Z,9E) isomers assayed on 3.2mm x 20' column of 5% FFAP at 240°C).

The 30% silver nitrate impregnated silica gel was prepared by drying a mixture of silica gel (Merk, Kieselgel 60 (70-230 mesh)) (100g) and 10% aqueous silver nitrate (300ml) on a rotary evaporator (4h) followed by heating to 95°C for 1h.[207,208]

(5E,9E) Isomer

^{1}H NMR (100MHz, $CDCl_3$): The 100MHz ^{1}H NMR of (5E,9E)-(208) is given in Table 3.5.

^{13}C NMR (25.15MHz, $CDCl_3$): The ^{13}C NMR of (5E,9E)-(208) is given in Table 3.6.

GC-MS (5% FFAP, 240°C): 262(M^+,0.1%), 219(M-CH_3CO,0.14%), 93(6%), 81(16%), 69(59%), 68(6%), 67(7%), 43(100%), 41(38%), 39(6%).

IR (thin film): 1740(s,C=O ketone), 1670(w,C=C), 1360 (s, -$COCH_3$), 840(m,R_2C=CHR).

(5Z,9E) Isomer

^{1}H NMR (100MHz, $CDCl_3$): The 100MHz ^{1}H NMR of (5Z,9E)-(208) is given in Table 3.7.

^{13}C NMR (25.15MHz, $CDCl_3$): The ^{13}C NMR of (5Z,9E)-(208) is given in Table 3.8.

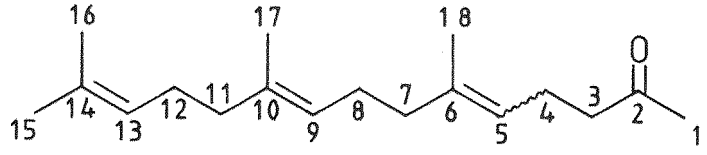
GC-MS (5% FFAP, 240°C): 262(M^+,0.2%), 219(M-CH_3CO,0.16%), 81(35%), 69(88%), 67(17%), 55(19%), 53(18%), 43(100%), 41(86%), 39(19%).

Shift (δ)	Signal Pattern	Integration	Assignment
5.08	t, J = 6Hz	3H	H-5,9,13
2.4	t, J = 8Hz	2H	C-3 CH_2
2.3	t, J = 8Hz	2H	C-4 CH_2
2.15	s	3H	C-1 CH_3
2.0	t, J = 6Hz	8H	C-7,8,11,12 CH_2's
1.66	s	3H	C-15 CH_3
1.6	s	9H	C-16,17,18 CH_3's

Table 3.5: ^{1}H NMR Data for (5E,9E)-(208)

Shift (δ)	Signal Pattern	Integration	Assignment
5.07	t, J = 6Hz	3H	H-5,9,13
2.38	t, J = 8Hz	2H	C-3 CH_2
2.29	t, J = 8Hz	2H	C-4 CH_2
2.14	s	3H	C-1 CH_3
2.04	t, J = 6Hz	8H	C-7,8,11,12 CH_2's
1.66	s	6H	C-15,18 CH_3's
1.6	s	6H	C-16,17 CH_3's

Table 3.7: ^{1}H NMR Data for (5Z,9E)-(208)



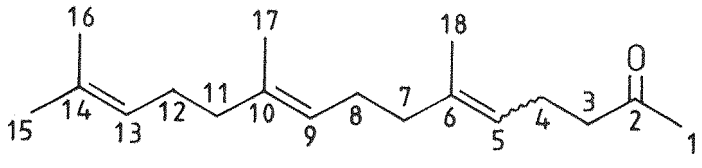
(208)

Shift (ppm)	Off Resonance	Assignment[216]
208.60	s	C-2
136.27	s	C-6,10
134.99	s	
131.07	s	C-14
124.53	d	C-9,13
124.21	d	
122.81	d	C-5
43.73	t	C-3
39.79	t	C-7,11
29.81	-	C-1
26.84	t	C-12
26.60	t	C-8
25.70	-	C-15
22.52	t	C-4
17.66	q	C-16
15.97	q	C-17,18

Table 3.6: ^{13}C NMR Data for (5E,9E)-(208)

Shift (ppm)	Off Resonance	Assignment[216]
208.60	s	C-2
136.51	s	C-6,10
135.28	s	
131.17	s	C-14
124.49	d	C-13,9
124.11	d	
123.50	d	C-5
44.00	t	C-3
39.84	t	C-11
31.93	t	C-7
29.81	-	C-1
26.79	t	C-12
26.52	t	C-8
25.70	-	C-15
23.39	-	C-18
23.36	t	C-4
17.67	q	C-16
15.98	q	C-17

Table 3.8: ^{13}C NMR Data for (5Z,9E)-(208)



(208)

IR (thin film): 1740(s,C=O ketone), 1675(w,C=C), 1360 (s,$COCH_3$), 840(M,R_2C=CHR).

3.4.4.8 (±)-(6E,10E)-3,7,11,15-Tetramethyl-1,6,10,14-hexadecatetraen-3-ol (Geranyllinalool) (207)

To a stirred suspension of magnesium turnings (200mg, 83.3mmol) in tetrahydrofuran (25ml) under nitrogen was added two drops of 1,2-dibromoethane. Vinyl bromide (930mg, 86.9mmoles) was distilled directly into the reaction vessel (acetone/liq. N_2 condenser) giving rise to an exothermic reaction. This mixture was stirred at room temperature for 30 min to generate a homogeneous solution. To the resulting light brown solution at 0^oC was added dropwise a solution of (5E,9E)-6,10,14-trimethyl-5,9,13-pentadecatrien-2-one (208) (1.91g, 73mmol) in tetrahydrofuran (25ml). The reaction was stirred at 0^oC for 4h whereupon iced water (10ml) followed by 2N HCl (10ml) was added. The organic layer was separated and the aqueous residue was extracted with ether (x3, 50ml). The solvent from the combined organic extracts was evaporated and the residue was dissolved in ether (100ml) and washed with water (100ml). The resulting organic extract was dried over sodium sulphate and evaporation of the solvent afforded a yellow oil. Purification by flash column chromatography followed by Kugelrohr distillation yielded (207) (1.39g, 47.9mmol, 66%) as a colourless viscous oil, b.p. 122^o-126^oC at 0.02mm Hg (Lit. b.p. 130^o-132^oC at 0.2mm Hg,[206] 144^o-146^o at 0.32mm Hg[203]).

^1H NMR (100MHz, $CDCl_3$): The 100MHz ^1H NMR of (207) is given in Table 3.2 and Figure 3.1 (see Section 3.2.3.1).

^{13}C NMR (25.15MHz, $CDCl_3$): The ^{13}C NMR of (207) is given in Table 3.3 and Figure 3.2 (see Section 3.2.3.2).

IR (thin film): 3650-3200 (s,OH), 1640(w,C=C), 995(m,RCH=CH_2), 920(m,RCH=CH_2), 840(w,R_2C=CHR).

GC-MS (5% FFAP, 240^oC, m/z): 290(M^+,0.7%), 272(M-H_2O,1%), 135(10%), 107(14%), 95(14%), 93(29%), 81(38%), 71(16%), 69(100%), 67(15%), 55(17%), 43(15%), 41(34%).

3.4.4.9 Spectral Details of Natural R-(-)-(6E,10E)-3,7,11,15-Tetramethyl-1,6,10,14-hexadecatetraen-3-ol (206)[218]

^{1}H NMR (100MHz, CCl_4): 1.27(s,3H,CH_3COH), 1.6(s,9H,3xCH_3C=C-), 1.66(s,3H,CH_3C=C-), 2.0(b envelope,12H, 6xCH_2), 5.05(dd,J=10,2Hz,1H,C=C$\overset{H}{\underset{H}{\diagup\diagdown}}$), 5.11(b signal,3H,3xC=CH), 5.22(dd, J=18,2Hz,1H,C=C$\overset{H}{\underset{H}{\diagup\diagdown}}$), 5.93(dd,J=18,10Hz, 1H,CH=CH_2).

IR (CCl_4): 3620(s, free OH), 3550(w,H bonded OH), 1645(w,C=C), 995(w,RCH=CH_2), 925(m,RCH=CH_2).

GC-MS (m/z): 290(M^+,1.2%), 272(M-H_2O,3%), 135(15%), 107(19%), 95(16%), 93(34%), 81(41%), 71(13%), 69(100%), 67(12%), 55(13%), 41(13%).

REFERENCES

1. R. Kurzrok and C.C. Lieb, *Proc. Soc. Exp. Biol. Med.*, 1930, 26, 268.

2. U.S. von Euler, *J. Physiol.*, 1935, 84, 21.

3. M.W. Goldblatt, *J. Physiol.*, 1935, 84, 208.

4. U.S. von Euler, *J. Physiol.*, 1936, 88, 213.

5. R. Eliasson, *Acta Physiol. Scand.*, 1957, 39, 141.

6. S. Bergström and J. Sjövall, *Acta Chem. Scand.*, 1957, 11, 1086.

7. S. Bergström, F. Dressler, R. Ryhage, B. Samuelsson and J. Sjövall, *Arkiv. Kemi.*, 1962, 19, 563.

8. S. Bergström, R. Ryhage, B. Samuelsson and J. Sjövall, *Acta Chem. Scand.*, 1962, 16, 501.

9. S. Bergström and B. Samuelsson, *J. Biol. Chem.*, 1962, 237, 3005.

10. (a) M. Hamberg, B. Samuelsson and J. Svensson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, 72, 2994; (b) G.B. Kolata, *Science*, 1975, 190, 770; (c) E.F. Ellis, O. Olez, L. Roberts, N.A. Payne, B. Sweetman, A.S. Nies and J.A. Oates, *Science*, 1976, 193, 1135.

11. E.J. Christ and D.A. van Dorp, *Biochim. Biophys. Acta*, 1972, 270, 537.

12. P.W. Ramwell and J.E. Shaw, "Prostaglandins", *Ann. N.Y. Acad. Sci.*, 1971, 180.

13. T.O. Oesterling, W. Morozowich and T.J. Roseman, *J. Pharm. Sci.*, 1972, 61, 1861.

14. J.B. Smith and D.E. Macfarlane in 'The Prostaglandins', ed., P.W. Ramwell, Plenum Press, New York, 1974, Vol. 2, p. 293.

15. U.S. von Euler and R. Eliasson, 'Prostaglandins', Academic Press, New York, 1967.

16. J. Nakano in 'Prostaglandins: Pharmacology and Therapeutic Advances', ed. M.F. Cuthbert, Heinman, London, 1973.

17. P.W. Ramwell, 'The Prostaglandins', Plenum Press, New York, 1973, Vol. 1.

18. M.P. Embrey, 'The Prostaglandins in Human Reproduction', Churchill Livingston, New York, 1976.

19. P.B. Curtis-Prior, 'Prostaglandins: An Introduction to their Biochemistry, Physiology and Pharmacology', North Holland Publishing Co., New York, 1976, p. 159.

20. A.J. Weinheimer and R.L. Spragins, *Tetrahedron Lett.*, 1969, 5183.

21. R.J. Light and B. Samuelsson, *Eur. J. Biochem.*, 1972, 28, 232.

22. W.P. Schneider, R.D. Hamilton and L.E. Rhuland, *J. Amer. Chem. Soc.*, 1972, 94, 2122.

23. G.L. Bundy, E.G. Daniels, F.H. Lincoln and J.E. Pike, *J. Amer. Chem. Soc.*, 1972, 94, 2124.

24. E.J. Corey, W.N. Washburn and J.C. Chen, *J. Amer. Chem. Soc.*, 1973, 95, 2054.

25. S. Carmely and Y. Kashman, *Tetrahedron Lett.*, 1980, 875.

26. K.A. Attrep, J.M. Mariani Jr., and M. Attrep Jr., *Lipids*, 1973, 8, 484.

27. R.P. Gregson, J.E. Marwood and R.J. Quin, *Tetrahedron Lett.*, 1979, 4505.

28. U.E. Brady, D.B. Destephano and R.E. Lovins, *Prostaglandins*, 1974, 6, 71.

29. U.E. Brady and D.B. Destephano, *J. Insect Physiol.*, 1977, 23, 905.

30. I. Ganjian, W. Loher and I. Kubo, *J. Chromatogr.*, 1981, 216, 380.

31. I. Ganjian, I. Kubo, W. Loher, D. Stanley-Samuelson and S.S. Tobe, *Proc. Natl. Acad. Sci. U.S.A.*, 1981, 78, 7835.

32. W. Loher, *Ent. Exp. & Appl.*, 1979, 25, 107.

33. B.N. Yamaja Setty and T.R. Ramaiah, *Insect Biochem.*, 1979, 9, 613.

34. B.N. Yamaja Setty and T.R. Ramaiah, *Indian J. Exper. Biol.*, 1980, 18, 539.

35. (a) D.V. Hagan, Ph.D. Thesis, University of Georgia, 1979;
(b) D.V. Hagan and U.E. Brady, *J. Insect Physiol.*, 1982, 29, 761.

36. R.J. Hart, G.A. Higgs, C. Potter, J.R. Vane and R.G. Wilson, *Bull. Ent. Res.*, 1976, 66, 665.

37. K.C. Binnington, R.G. Dickinson, J.E. O'Hagan, M.H. Hegarty and M. Schotz, *Aust. J. Exp. Biol. Med. Sci.*, 1976, 54, 475.

38. F. Meleguir, A. Hadani, M. Shemesh, A. Shklar and L.S. Share, *Bull. Ent. Res.*, 1979, 69, 381.

39. D.M. Desiderio, *GC-MS News*, 1979, 7, 32.

40. M.G. Horning and F. Vane, *Anal. Lett.*, 1969, 2, 357.

41. S.R. Shaw and A.J.F. Wickramsinge, *Prostaglandins*, 1974, 4, 903.

42. K. Green, *Chem. Phys. Lipids*, 1969, 3, 2541.

43. D.M. Desiderio and B.S. Middleditch, *Prostaglandins*, 1972, 2, 195.

44. U. Axen, *Anal. Chem.*, 1973, 45, 479.

45. D.A. van Dorp, G.H. Jouvenaz and D.H. Nugteren, *Prostaglandins*, 1973, 3, 175.

46. J.C. Frölich, J. Throck-Watson and B.J. Sweetman, *Prostaglandins*, 1973, 3, 75.

47. P.F. Crain, D.M. Desiderio and J.A. McCloskey, *Methods in Enzymology*, 1975, 35, 359.

48. T.A. Baillie and A.R. Brash, *Biomed. Mass Spec.*, 1978, 5, 346.

49. K. Bricknell and M.E. Conolly, *Biochem. Med.*, 1980, 23, 119.

50. A.C. Bazan and D.R. Knapp, *J. Chromatogr.*, 1982, 236, 201.

51. B.J. Sweetman and J. Throck-Watson, *Org. Mass Spectrom.*, 1974, 9, 39.

52. J. Roselló, E. Gelpi, M. Rigaud, J. Durand and J.C. Breton, *Biomed. Mass Spectrom.*, 1981, 8, 149.

53. P. Chebroux, J. Durand, J. Maclouf and M. Rigaud, *Prostaglandins*, 1976, 11 , 1000.

54. E.W. Horton, M. Los and C.J. Thompson, *Life Sciences*, 1970, 9, 983.

55. A.F. Cockerill, D.N.B. Mullen, D.J. Osbourne J.R. Boot and W. Dawson, *Biomed. Mass Spectrom.*, 1977, 4, 358.

56. K. Hirano and M. Sugiura, *J. Chromatogr.*, 1974, 90, 169.

57. P.W. Kelly, I. Cooper, S.K. Smith and M.H. Abel in 'Prostaglandins, Prostacyclin and Thromboxane Measurement', eds. J.M. Boeynaems and A.G. Herman, Martinus Nijhoff, The Hague, 1980, p.142.

58. J.G.A.M. Raaijmakers, *J. Chromatogr.*, 1977, 138, 373.

59. D.M. Desiderio and B.S. Middleditch, *Adv. Mass Spectrom.*, 1974, 6, 173.

60. H. Budzikiewicz, C. Djerassi and D.H. Williams, 'Mass Spectrometry of Organic Compounds', Holden-Day, San Francisco, 1967, p. 475.

61. C.F. Poole in 'Handbook of Derivatives for Chromatography', eds. K. Blau, G.S. King, Heyden & Son Ltd., London, 1977, pp. 183-200.

62. A. Terragno, R. Rydzik and N.A. Terragno, *Prostaglandins*, 1981, 21, 101.

63. S. Inayama, H. Hori, T. Shibata, Y. Ozawa, K. Yamagami, M. Imazu and H. Hayashida, *J. Chromatogr.*, 1980, 194, 85.

64. A.R. Whorton, K. Carr, M. Smigel, L. Walker, K. Ellis and J.A. Oates, *J. Chromatogr.*, 1979, 163, 64.

65. I. Alam, K. Ohuchi and L. Levine, *Anal. Biochem.*, 1979, 93, 339.

66. P. Abley, F.J. McQuillen, D.E. Minnikin, K. Kusamran, K. Maskens and N. Polgar, *J. Chem. Soc., Chem. Commun.*, 1970, 348.

67. J.F. Babashak, H.M. Fales and T.M. Jaouni, *Anal. Chem.*, 1973, 45, 2302.

68. F.M. Dean, 'Naturally Occurring Oxygen Ring Compounds', Butterworths, London, 1963, pp. 53-81.

69. T.K. Devon and A.I. Scott, 'Handbook of Naturally Occurring Compounds', Academic Press, New York, 1972, Vol. II.

70. (a)'Terpenoids and Steroids', ed. K.H. Overton (Specialist Periodical Reports), The Chemical Society, London, 1971-1976, Vol. 1-6;
(b) 'Terpenoids and Steroids', ed. J.R. Hanson (Specialist Periodical Reports), The Chemical Society, London, 1977-1979, 1981, 1982, Vol. 7-11.

71. L.J. Haynes, *Quart. Rev.*, 1948, 2, 46.

72. G. Pattenden, *Fortschr. Chem. Org. Naturst.*, 1978, 35, 133.

73. L.J. Haynes and J.R. Plimmer, *Quart. Rev.*, 1960, 14, 292.

74. P.G. Marshall in 'Rodd's Chemistry of Carbon Compounds', ed. S. Coffey, Elsevier, New York, 1970, Vol. IID, pp. 360-421.

75. G. Cimino, S. DeStephano, A. Guerriero and L. Minale, *Tetrahedron Lett.*, 1975, 1417.

76. G. Cimino in 'Marine Natural Products Chemistry', eds. D.J. Faulkner and W.H. Fenical, Plenum Press, New York, 1977, pp. 61-86.

77. D.J. Faulkner, *Tetrahedron Lett.*, 1973, 3821.

78. I. Rothberg and P. Shubiak, *Tetrahedron Lett.*, 1975, 769.

79. R. Kazlauska, J.A. Baird-Lambert, P.T. Murphy and J.R. Wells, *Aust. J. Chem.*, 1982, 35, 61.

80. J. Bernstein, U. Shmeuli, E. Zadock, Y. Kashman and I. Nëeman, *Tetrahedron*, 1974, 30, 2815.

81. F.J. Schmitz and E.D. Lorance, *J. Org. Chem.*, 1971, 36, 719.

82. L.P. Brower, *Scientific American*, 1969, 220, 22.

83. L.P. Brower and S.C. Glazier, *Science*, 1975, 188, 19.

84. D.M. Kirpotin and K.L. Gladilin, *Priroda (Moscow)*, 1969, 108; *Chem. Abs.*, 1970, 72, 64115k.

85. T. Reichstein, *Cron. Chim.*, 1967, 15, 3; *Chem. Abs.*, 1970, 72, 77821n.

86. W. Schliemann and G. Adam, *Phytochemistry*, 1982, 21, 1438.

87. G. Adam, N.H. Khoi, C. Bergner and N.T. Lien, *Phytochemistry*, 1979, 18, 1399.

88. J. Meinwald, G.D. Prestwich, K. Nakanishi and I. Kubo, *Science*, 1978, 199, 1167.

89. K. Sakurai, H. Matsumoto, J. Adachi, *Yakugaku Zasshi*, 1968, 88, 919; *Chem. Abs.*, 1968, 67, 94792j.

90. Jap. P. 73 28 646/1973; *Chem. Abs.*, 1974, 80, P.117147w.

91. A. Dal Pozzo, A. Dansi and E. Meneghini, *Boll. Chim. Farm.*, 1974, 113.

92. C.J. Cavallito in 'Medicinal Chemistry', ed. S. M. Suter, Wiley, New York, 1951, pp. 221-235.

93. S.M. Kupchan, Y. Aynehchi, J.M. Cassady, A.T. McPhail, G.A. Sim, H.K. Schnoes and A.L. Burlingame, *J. Amer. Chem. Soc.*, 1966, 88, 3674.

94. M. Ito, T. Iwata and C. Tsukida, *Heterocycles*, 1982, 19, 1385.

95. S.D. Joland, J.J. Hoffmann, K.H. Schram and J.J. Cole, *J. Org. Chem.*, 1982, 47, 3151.

96. (a) F. Dickens and H.E.H. Jones, *Br. J. Cancer*, 1961, 15, 85; (b) F. Dickens and H.E.H. Jones, *ibid.*, 1963, 17, 100.

97. F. Dickens, *Br. Med. Bull.*, 1964, 20, 96.

98. A.A. Avetisyan and M.T. Dangyan, *Russ. Chem. Rev.*, 1977, 46, 643.

99. H. Baer, M. Holden and B.C. Seegal, *J. Biol. Chem.*, 1946, 162, 65.

100. M.H. Benn and L.J. Yelland, *Can. J. Chem.*, 1968, 46, 729.

101. (a) R.A. Raphael, *J. Chem. Soc.*, 1947, 805; (b) R.A. Raphael, *Nature*, 1947, 160, 261.

102. J.J. Sims, J.A. Pettus Jr., and R.M. Wing, in 'Marine Natural Products Chemistry', eds. D.J. Faulkner and W.H. Fenical, Plenum Press, New York, 1977, pp. 205-209.

103. P.A. Jacobi and T. Craig, *J. Amer. Chem. Soc.*, 1978, 100, 7748.

104. P.L. Cowall, J.M. Cassady, C.-J. Chang and J.F. Kozlowski, *J. Org. Chem.*, 1981, 46, 1108.

105. M. Uchida, Y. Koike, G. Kusano and Y. Kondo, *Chem. Pharm. Bull.*, 1980, 28, 92.

106. J. Kawabata, S. Tahara, J. Mitzutani, A. Furusaki, H. Hashida and T. Matsumoto, *Agric. Biol. Chem.*, 1979, 43, 885.

107. K. Endo, T. Taguchi, F. Taguchi, H. Hikino, J. Yamahara and H. Fujimura, *Chem. Pharm. Bull.*, 1979, 27, 2954.

108. L.B. DeSilva, W.H.M.W. Herath, R.C. Jennings, M. Mahendran and G.E. Wannigama, *Phytochemistry*, 1982, 21, 1173.

109. S.M. Kupchan, Y. Aynehchi, J.M. Cassady, H.K. Schnoes and A.L. Burlingame, *J. Org. Chem.*, 1969, 34, 3867.

110. S.M. Kupchan, M.A. Eakin and A.M. Thomas, *J. Med. Chem.*, 1971, 14, 1147.

111. S.M. Kupchan, W.A. Court, R.G. Dailey Jr., C.J. Gilmore and R.F. Bryan, *J. Amer. Chem. Soc.*, 1972, 94, 7194.

112. D. Uemura, C. Katayama and Y. Hirata, *Tetrahedron Lett.*, 1977, 283.

113. I. Kubo, Y.-W. Lee, V. Balogh-Nair, K. Nakanishi and A. Chapya, *J. Chem. Soc., Chem. Commun.*, 1976, 949.

114. I. Kubo, J.A. Klocke, I. Miura and Y. Fukuyama, *J. Chem. Soc., Chem. Commun.*, 1982, 618.

115. S.M. Kupchan, J.R. Knox, J.E. Kelsey and J.A. Saenz-Renauld, *Science*, 1964, 146, 1685.

116. S.M. Kupchan, R.J. Hemingway and R.W. Doskotch, *J. Med. Chem.*, 1964, 7, 803.

117. S.D. Joland, J.J. Hoffmann, J.R. Cole, M.S. Tempesta and R.B. Bates, *J. Org. Chem.*, 1981, 46, 1946.

118. J.E. Pike, J.E. Grady, J.S. Evans and C.G. Smith, *J. Med. Chem.*, 1964, 7, 348.

119. H. Anke, T. Kemmer and G. Höfle, *J. Antibiot., (Tokyo)*, 1981, 34, 923.

120. B. Sullivan and D.J. Faulkner, *Tetrahedron Lett.*, 1982, 23, 907.

121. C.J. Cavillito and T.H. Haskell, *J. Amer. Chem. Soc.*, 1945, 67, 1991.

122. W.B. Geiger and J.E. Conn, *J. Amer. Chem. Soc.*, 1945, 67, 112.

123. A.J. Allison, D.N. Butcher, J.D. Connolly and K.H. Overton, *J. Chem. Soc., Chem. Commun.*, 1968, 1493.

124. D.N. Butcher and J.D. Connolly, *J. Exp. Bot.*, 1971, 22, 314.

125. P. Anastasis, I. Freer, C. Gilmore, H. Mackie, K. Overton and S. Swanson, *J. Chem. Soc., Chem. Commun.*, 1982, 268.

126. Y.S. Rao, *Chem. Rev.*, 1976, 76, 625.

127. M. Tada, *Chem. Lett.*, 1982, 441.

128. P.F. Hudrlik, D.T.-W. Chou and M.A. Stephenson, *J. Org. Chem.*, 1982, 47, 2987.

129. F. Kido, Y. Noda and A. Yoshikoshi, *J. Amer. Chem. Soc.*, 1982, 104, 5509.

130. F. Kido, Y. Noda and A. Yoshikoshi, *J. Chem. Soc., Chem. Commun.*, 1982, 1209.

131. P.A. Grieco and M. Miyashita, *J. Org. Chem.*, 1974, 39, 120.

132. P.A. Grieco, C.S. Pogonowski and S. Burke, *J. Org. Chem.*, 1975, 40, 542.

133. P.A. Grieco and M. Nishizawa, *J. Chem. Soc., Chem. Commun.*, 1976, 582.

134. M. Watanabe and A. Yoshikoshi, *Chem. Lett.*, 1980, 1315.

135. P.A. Grieco and J.J. Reap, *Tetrahedron Lett.*, 1974, 1097.

136. B.M. Trost, T.N. Salzmann and K. Hiroi, *J. Amer. Chem. Soc.*, 1976, 98, 4887.

137. R.B. Gammill, C.A. Wilson and T.A. Bryson, *Synth. Commun.*, 1975, 5, 245.

138. K. Iwai, M. Kawai, H. Kosugi and H. Uda, *Chem. Lett.*, 1974, 385.

139. J.L. Herman, M.H. Berger and R.H. Schlessinger, *J. Amer. Chem. Soc.*, 1973, 95, 7922.

140. (a) A.E. Greene, J.-C. Muller and G. Ourisson, *Tetrahedron Lett.*, 1972, 2489; (b) A.E. Greene, J.-C. Muller and G. Ourisson, *ibid.*, 1972, 3375.

141. S. Danishefsky, M.-Y. Tsai and T. Kitahara, *J. Org. Chem.*, 1977, 42, 394.

142. (a) S. Danishefsky, T. Kitahara, P.F. Schuda and S.J. Etheredge, *J. Amer. Chem. Soc.*, 1976, 98, 3028; (b) S. Danishefsky, T. Kitahara, P.F. Schuda and S.J. Etheredge, *ibid.*, 1977, 99, 6066.

143. (a) M.R. Roberts and R.H. Schlessinger, *J. Amer. Chem. Soc.*, 1981, 103, 724; (b) R.H. Schlessinger in 'Organic Synthesis: Today and Tomorrow', eds. B.M. Trost and C.R. Hutchinson, Pergamon Press, New York, 1981,p. 251.

144. T.A. Eggelte, J.J.J. de Boer, H. de Koning and H.O. Huisman, *Synth. Commun.*, 1978, 8, 353.

145. H. Stetter and W. Dierichs, *Chem. Ber.*, 1952, 85, 61.

146. H.W. Rosenmund, H. Herzberg and H. Schütt, *Chem. Ber.*, 1954, 87, 1258.

147. B.M. Goldschmidt, B.L. van Duuren and C. Mercado, *J. Chem. Soc., (C)*, 1966, 2100.

148. P.A. Grieco, N. Marinovic and M. Miyashita, *J. Org. Chem.*, 1975, 40, 1670.

149. (a) P.A. Grieco, T. Oguri, S. Gilman and G.T. DeTitta, *J. Amer. Chem. Soc.*, 1978, 100, 1616; (b) P.A. Grieco, T. Ogura and S. Gilman, *ibid.*, 1980, 102, 5886.

150. A.B. Smith III and R.E. Richmond, *J. Org. Chem.*, 1981, 46, 4816.

151. (a) K.B. Sharpless and T.R. Verhoeven, *Aldrichimica Acta*, 1979, 12, 63; (b) K.B. Sharpless and R.C. Michaelson, *J. Amer. Chem. Soc.*, 1973, 95, 6136; (c) T. Katsuki and K.B. Sharpless, *J. Amer. Chem. Soc.*, 1980, 102, 5976.

152. H.J. Reich, J.M. Renga and I.L. Reich, *J. Amer. Chem. Soc.*, 1975, 97, 5434.

153. K.B. Sharpless, R.F. Lauer and A.Y. Teranishi, *J. Amer. Chem. Soc.*, 1973, 95, 6137.

154. (a) J.C. Sircar and A.I. Meyers, *J. Org. Chem.*, 1965, 30, 3206;
(b) P.A. Bartlett and K.K. Jernstedt, *Tetrahedron Lett.*, 1980, 1607;
(c) W.F. Berkowitz and S.S. Choudy, *Tetrahedron Lett.*, 1981, 22, 1075.

155. C.G. Chavdarian and C.H. Heathcock, *Synth. Commun.*, 1976, 6, 277.

156. G. Rücker, H. Horster and W. Gajewski, *Synth. Commun.*, 1980, 10, 623.

157. H.O. House and R.L. Wasson, *J. Amer. Chem. Soc.*, 1957, 79, 1488.

158. L.M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry', Pergamon Press, New York, Vol. 2, 2nd Edition, 1969, p. 295.

159. H. Holub, M. Buděšínský, Z. Smitalová, D. Šaman and U. Rychlewska, *Tetrahedron Lett.*, 1982, 23, 4583.

160. J.T. Pinhey and S. Sternhell, *Aust. J. Chem.*, 1965, 18, 543.

161. R.B. Bates, V. Prochazka and Z. Cekan, *Tetrahedron Lett.*, 1963, 877.

162. P.A. Grieco, N. Nishizawa, T. Oguri, S.D. Burke and N. Marinovic, *J. Amer. Chem. Soc.*, 1977, 99, 5773.

163. W. Herz, *J. Org. Chem.*, 1982, 47, 3991.

164. M. Ando, A. Akahane, H. Yamaoka and K. Takase, *J. Org. Chem.*, 1982, 47, 3909.

165. P. Crabbé, G.A. Garcia and C. Rius, *J. Chem. Soc., Perkin Trans. 1*, 1973, 810.

166. A. Rüttimann, A. Wick and A. Eschenmoser, *Helv. Chim. Acta*, 1975, 58, 1451.

167. E.J. Corey, N.H. Anderson, R.M. Carlson, J. Paust, E. Vedejs, I. Vlattas and R.E.K. Winter, *J. Amer. Chem. Soc.*, 1968, 90, 3245.

168. P. Crabbé, A. Guzmán and M. Vera, *Tetrahedron Lett.*, 1973, 3021.

169. T. Itoh, K. Jitsukawa, K. Kaneda and S. Teranishi, *J. Amer. Chem. Soc.*, 1979, 101, 159.

170. O.L. Chapman and P. Fitton, *J. Amer. Chem. Soc.*, 1963, 85, 41.

171. M.E. Kuehne and B.F. Lambert, *J. Amer. Chem. Soc.*, 1959, 81, 4278.

172. J.L. Luche, *J. Amer. Chem. Soc.*, 1978, 100, 2226.

173. R.D. Rieke, P.T.-J. Li, T.P. Barns and S.T. Uhm, *J. Org. Chem.*, 1981, 46, 4323.

174. W.C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, 43, 2923.

175. S.V. Sunthankar, C.S. Menon and D.V. Telang, *Indian J. Chem.*, 1971, 9, 786.

176. A.S. Dreiding and J.A. Hartman, *J. Amer. Chem. Soc.*, 1953, 75, 939.

177. P.A. Wender, J.M. Erhardt and L.J. Letende, *J. Amer. Chem. Soc.*, 1981, 103, 2114.

178. E.J. Corey and J.W. Suggs, *Tetrahedron Lett.*, 1975, 2647.

179. R. Baker and S. Walmsley, *Tetrahedron*, 1982, 38, 1899.

180. A.J. Birch, W.V. Brown, J.E.T. Corrie and B.P. Moore, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2653.

181. G.D. Prestwich, *J. Chem. Ecol.*, 1979, 5, 459.

182. D.F. Wiemer, J. Meinwald, G.D. Prestwich and I. Miura, *J. Org. Chem.*, 1979, 44, 3950.

183. G.D. Prestwich, D.F. Wiemer, J. Meinwald and J. Clardy, *J. Amer. Chem. Soc.*, 1978, 100, 2560.

184. D.F. Wiemer, J. Meinwald, G.D. Prestwich, B.A. Solheim and J. Clardy, *J. Org. Chem.*, 1980, 45, 191.

185. G.D. Prestwich, *Insect. Biochem.*, 1977, 7, 91.

186. G.D. Prestwich, S.P. Tanis, E.G. Pilkiewicz, I. Miura and K. Nakanishi, *J. Amer. Chem. Soc.*, 1976, 98, 6062.

187. (a) J. Vrkoč, M. Buděšínský and P. Sedmera, *Collect Czech. Chem. Commun.*, 1978, 43, 1125; (b) J. Vrkoč, M. Buděšínký and P. Sedmera, *ibid*, 1978, 43, 2478.

188. A. Dupont, J.C. Braekman, D. Daloze, J.M. Pasteels and B. Tursch, *Bull. Soc. Chim. Belg.*, 1981, 90, 485.

189. J. Vrkoč, M. Buděšínský, J. Krecek and I. Hrdý, 'Proc. 8th Int. Congr., I.U.S.S.I.', Wageningen, 1977, 320.

190. G.D. Prestwich, S.G. Spanton, S.H. Goh and Y.P. Tho, *Tetrahedron Lett.*, 1981, 22, 1563.

191. G.D. Prestwich, S.H. Goh and Y.P. Tho, *Experientia*, 1981, 37, 11.

192. G.D. Prestwich, J.W. Lauher and M.S. Collins, *Tetrahedron Lett.*, 1979, 3827.

193. G.D. Prestwich, B.A. Solheim, J. Clardy, F.G. Pilkiewicz, I. Miura, S.P. Tanis and K. Nakanishi, *J. Amer. Chem. Soc.*, 1977, 99, 8082.

194. G.D. Prestwich, S.G. Spanton, J.W. Lauher and J. Vrkoč, *J. Amer. Chem. Soc.*, 1980, 102, 6825.

195. J.C. Braekman, D. Daloze, A. Dupont, J. Pasteels, B. Tursch, J.C. Declerq, G. Germain, and M. van Meerssche, *Tetrahedron Lett.*, 1980, 2761.

196. J. Deligne, *Forma. Functo.*, 1971, 4, 176.

197. A.H. Parton, Ph.D. Thesis, University of Southampton, 1981.

198. R. Baker, A.H. Parton and P.E. Howse, *Experientia*, 1982, 38, 297.

199. L. Ruzicka and G. Firmenich, *Helv. Chim. Acta*, 1939, 22, 392.

200. E. Demole and E. Lederer, *Bull. Soc. Chim. France*, 1958, 1128.

201. I.N. Nazarov, B.P. Gussev and V.I. Gunar, *Zh. Obshch. Khim.*, 1958, 28, 1444.

202. G.A. Vorob'eva, I.K. Sarycheva and N.A. Preobrazhenskiĭ, *Zh. Obshch. Chim.*, 1959, 29, 2314.

203. J. Weichet, L. Blāha and V. Kvita, *Collect. Czech., Chem. Commun.*, 1960, 25, 1914.

204. P.O. Vig, J.C. Kapur, J. Singh and B. Vig, *Indian J. Chem.*, 1969, 7, 574.

205. G. Saucy and R. Marbet, *Helv. Chim. Acta*, 1967, 50, 2091.

206. E.I. Kozlov, M.Ts. Yanotovskii and G.I. Samokhvalov, *Zh. Obshch. Khim.*, 1964, 34, 2748.

207. J.C. Stowell, *J. Org. Chem.*, 1970, 35, 244.

208. L.J. Morris, *Chem. Ind.*, 1962, 1238.

209. W.L. Roelofs. A. Comeau, A.S.Hill and G. Milicevic, *Science*, 1971, 174, 297.

210. W.L. Roelofs, R.J. Bartwell, A.S. Hill, R.T. Carde and L.H. Waters, *J. Econ. Entomol.*, 1972, 65, 1276.

211. H. Normant, *Adv. Org. Chem.*, 1960, 2, 1.

212. B. Kimland and T. Norin, *Acta Chem. Scand.*, 1967, 21, 825.

213. L.M. McDonough, D.A. George, B.A. Butt, J.M. Ruth and K.R. Hill, *Science*, 1972, 177, 177.

214. F.J. Ritter, I.E.M. Brüggemann-Rotgans, P.E.J. Verwiel, C.J. Persoons and E. Talman, *Tetrahedron Lett.*, 1977, 2617.

215. G.W.K. Cavill and P.J. Williams, *Aust. J. Chem.*, 1969, 22, 1737.

216. E. Breitmaier and W. Voelter, '^{13}C NMR Spectroscopy: Methods and Applications', Verlag Chemie, Weinheim, 1974.

217. J.B. Stothers and N.K. Wilson, *Top. Stereochem.*, 1967, 8, 1.

218. A.H. Parton, personal communication.

219. W. Kimel and A.C. Cope, *J. Amer. Chem. Soc.*, 1943, 65, 1992.

220. W. Kimel, N.W. Sax, S. Kaiser, C.G. Eichmann, G.O. Chase and A. Ofner, *J. Org. Chem.*, 1958, 23, 153.