CYCLOHEXENE OXIDE ANTIBIOTICS AND RELATED METABOLITES

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by

Rujee Kyokajee Duke

Research School of Chemistry
Canberra, A.C.T.

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DECLARATION

The work described in this thesis is the candidate's own unless stated otherwise and has not been submitted in support of an application for any other degree. It was carried out at The Australian National University (1975 to 1978) under the supervision of Professor R. W. Rickards.

Rujee K. Duke

Rujee Kyokajee Duke

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SUMMARY

The work described in this thesis is primarily concerned with the absolute configuration and synthesis of eupenoxide, a cyclohexene oxide antibiotic.

Eupenoxide was isolated from an unidentified species of *Eupenicillium* as a fungistatic substance active against the phytopathogenic fungus, *Phytophthora cinnamomi*. The structure and relative stereochemistry of eupenoxide had been previously established by Quinn and Rickards as (1*R**,4*S**,5*R**,6*S**)-2-[(*E*)-hept-1′-enyl]-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol. In this thesis, chiroptical studies of eupenoxide derivatives show that eupenoxide has the (1*R*,4*S*,5*R*,6*S*) absolute configuration.

Synthesis of the carbocyclic nucleus of eupenoxide was achieved by cycloaddition of the novel compounds (*E*,*E*)-1,4-bis(*t*-butyldimethylsilyloxy)buta-1,3-diene and 4-acetoxybut-2-ynal. Because of the lability of the synthetic intermediates, the protecting groups in both the diene and dienophile were found to be crucial for the success of the synthesis. The epoxide group was introduced by the oxidation of the disubstituted double bond in the cyclohexa-1,4-diene intermediate and a Wittig reaction enabled the attachment of the unsaturated side-chain. The acyclic double bond was photochemically isomerised to give the required *E* stereochemistry.

In the removal of the silyl and the acetoxyl protecting groups to generate (±)-eupenoxide, it was found that the eupenoxide had become unstable as a result of the acid treatment. The conditions required for the isolation of (±)-eupenoxide in stable form were investigated.

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CHAPTER 1

INTRODUCTION TO CYCLOHEXENE OXIDE ANTIBIOTICS

1.1 General

The term "antibiotic" was introduced into the literature by Waksman[1] in 1942, although it had been realised for many years that certain microorganisms were able to exert antagonistic effects upon other microorganisms. In many cases, the antagonist was shown to produce an active, isolable substance, the antibiotic, which was responsible for the biological activity. The discovery of the antibiotic penicillin by Fleming[2] in 1929 marked the beginning of a new era in medicine and since that time hundreds of antibiotics have been isolated and tested for specific biological activity. Only a small number of these compounds, however, have found clinical application.

Already in 1942, Wilkins and Harris[3] observed that *Aspergillus terreus* produced an antibiotic substance, but it was not until 1949 that it was isolated by Abraham and Florey[4]. The antibiotic substance, terreic acid (1), was finally characterised by Sheehan *et al.*[5] in 1958 as the first member of the cyclohexene oxide antibiotic series. Since then other members of the cyclohexene oxide group of antibiotics and related compounds (1)-(16) [Table 1] and other closely related compounds (17)-(22) [Table 2] have been isolated from microorganisms,[6-16,26-28] higher plants[17-24] and a mollusc[25].

The compounds of primary concern contain a cyclohexene oxide nucleus in which the carbocyclic ring is highly oxygenated and often contains a double bond. Carbon substituents are common amongst all members of this class and may be grouped as follows. The one-carbon substitutents are methyl as in (1), (2) and (3) and hydroxymethyl as in (4) and (5): Also

Table 1

Naturally Occurring Cyclohexene Oxide Antibiotics and Related Compounds

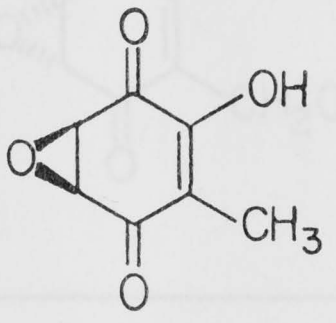
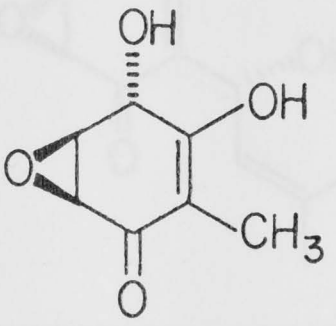
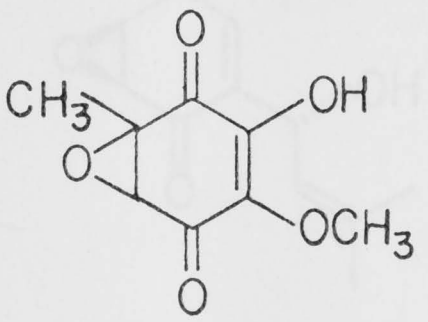
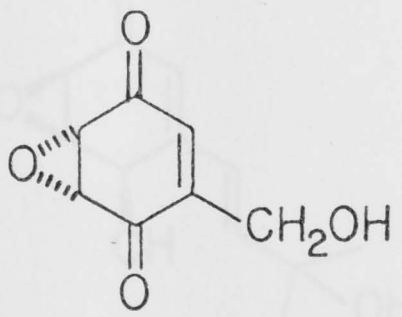
Name	Structure	Source
Terreic acid (1)		*Aspergillus terreus*[5] *A. flavipes*[6]
Terremutin (2)		*A. terreus*[7] *A. cirvinus*[8]
Fumigatin oxide (3)		*A. fumigatus*[9]
Phyllostine (4)		*Phyllosticta* species[10,11]

Table 1 (continued)

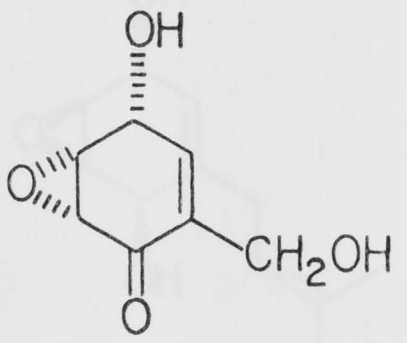
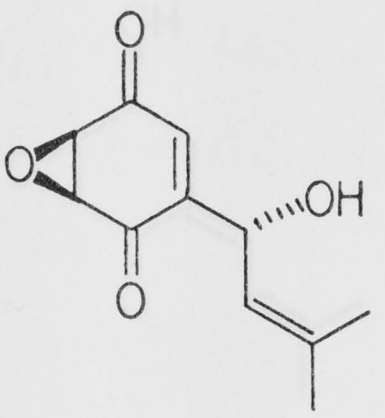
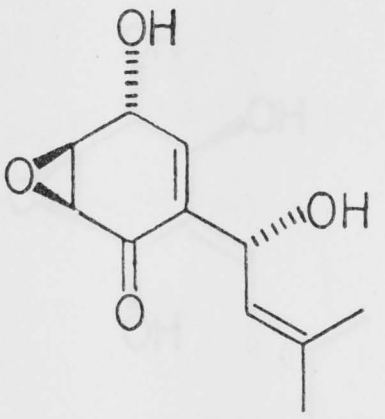
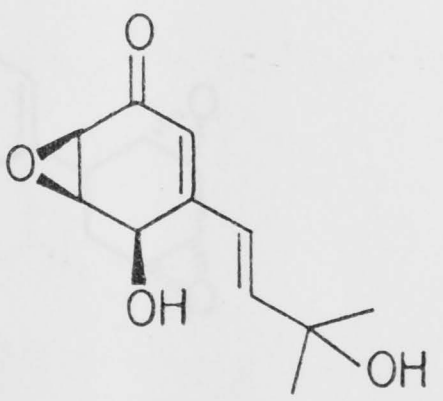
Name	Structure	Source
Epoxydon (Phyllosinol) (5)		*Phoma terrestris*[12] *Phyllosticta* species[13]
Panepoxydione (6)		*Panus chonchatus*[14] *Panus rudis*[14]
Panepoxydon (7)		*Panus chonchatus*[14] *Panus rudis*[14]
Isopanepoxydon (8)		*Panus chonchatus*[14]

Table 1 (continued)

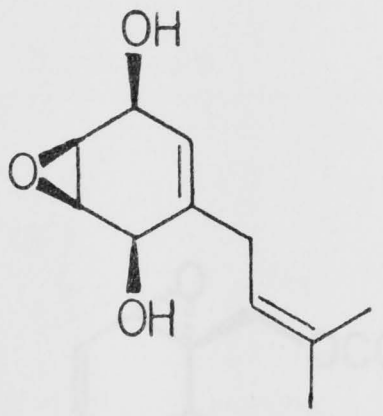
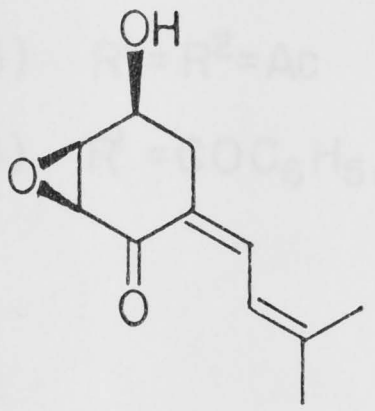
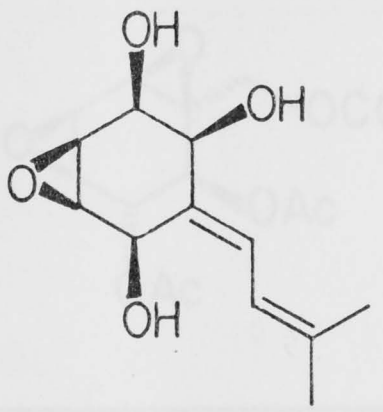
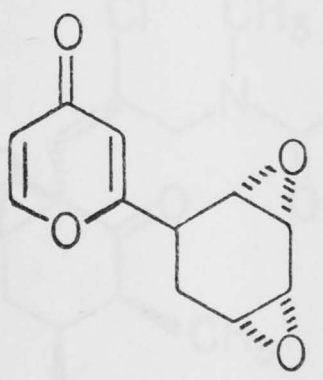
Name	Structure	Source
7-Desoxypanepoxydol (9)		*Panus rudis*[14]
Neopanepoxydon (10)		*Panus rudis*[14]
Neopanepoxydol (11)		*Panus chonchatus*[14]
LL-Z1220 (12)		Undetermined fungal species[15,16]

Table 1 (continued)

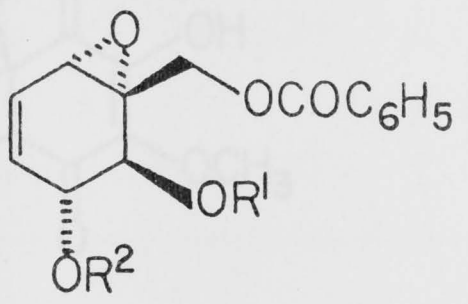
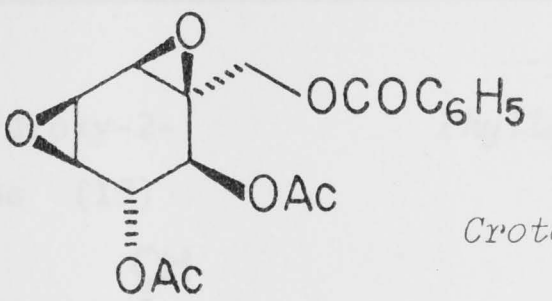
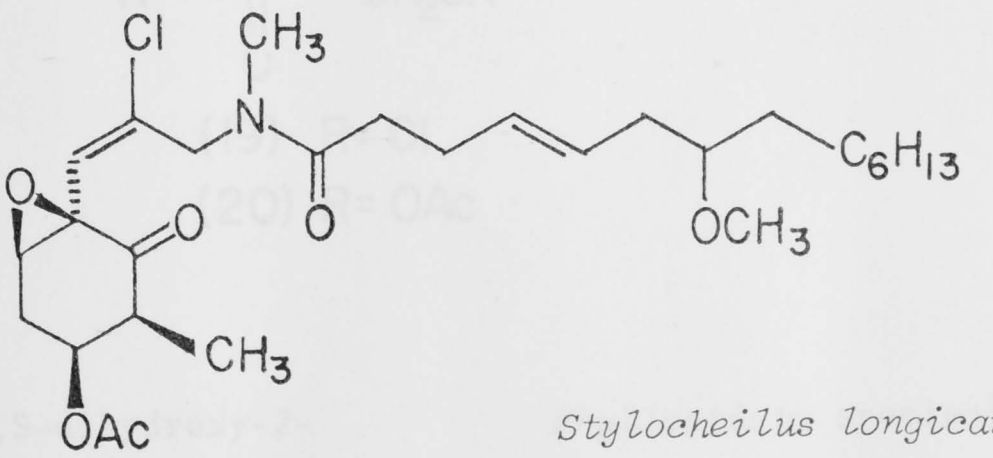
Name	Structure	Source
Senepoxide (13)	 (13) $R^1 = R^2 = Ac$ (14) $R^1 = COC_6H_5, R^2 = H$	*Uvaria catacarpa*[17]
Pipoxide (14)		*Piper hookeri*[18]
Crotepoxide (15)		*Croton macrostachys*[19-21] *Piper futokadzura*[22] *Piper hookeri*[23] *Piper brachystachyum*[24]
Stylocheilamide (16)		*Stylocheilus longicauda*[25] (mollusca)

Table 2

Compounds Related to Naturally Occurring Cyclohexene Oxide Derivatives

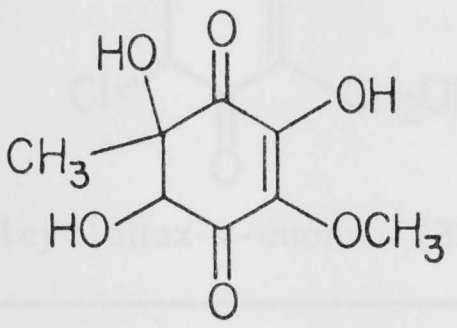
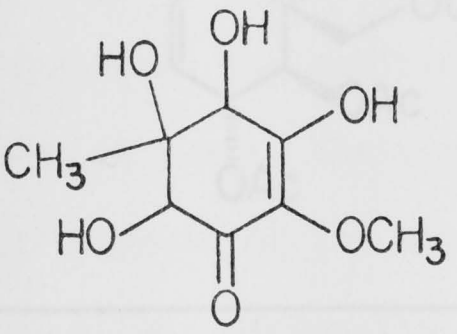
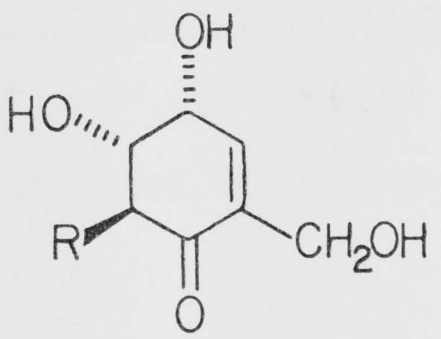
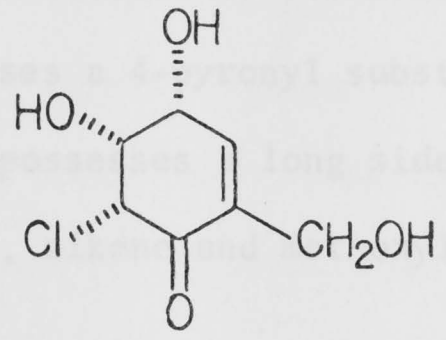
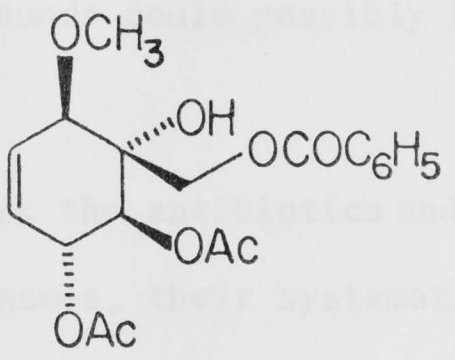
Name	Structure	Source
Spinulosin-hydrate (17)		*A. fumigatus*[26]
Spinulosin quinol-hydrate (18)		*A. fumigatus*[26]
(4*R*,5*R*,6*S*)-6-Chloro-4,5-dihydroxy-2-hydroxymethylcyclohex-2-enone (19)	 (19) R = Cl	*Phyllosticta* species[27,28]
(4*R*,5*R*,6*S*)-6-Acetoxy-4,5-dihydroxy-2-hydroxymethylcyclohex-2-enone (20)	(20) R = OAc	*Phyllosticta* species[27,28]

Table 2 (continued)

Name	Structure	Source
(4*R*,5*R*,6*R*)-6-Chloro-4,5-dihydroxy-2-hydroxymethylcyclohex-2-enone (21)		*Phyllosticta* species[27,28]
Selenol (22)		*Uvaria catacarpa*[17]

related to hydroxymethyl is the benzoyloxymethyl substituent which has been found to occur in all of the compounds from plants, that is in (13), (14) and (15). Compounds in the panepoxydon series carry isopentenyl side-chains as in (9), (10) and (11), which can also be hydroxylated as in (6), (7) and (8). More unusual substituents were observed with LL-Z1220 (12) which possesses a 4-pyronyl substituent and with a compound from a mollusc (16) which possesses a long side-chain containing chloro-vinyl, disubstituted amide, alkene and methoxyl functionalities.

As well as the cyclohexene oxide antibiotics and related compounds discussed, there are also other structurally similar coexisting compounds (17)-(22)[17,26-28]. These compounds could possibly be derived from the ring opening of the epoxide.

For convenience in this work the antibiotics and the related compounds will be referred to by trivial names, their systematic names being used only when required. Many aspects of the chemistry, synthesis, biosynthesis and biological activity of highly oxygenated cyclohexane derivatives, including cyclohexene oxides, have been reviewed recently by Ichihara[29], also Ichihara and Sakamura[30].

1.2 Determination of Structure and Stereochemistry

After considering the general techniques used in the determination of the structure, and relative and absolute stereochemistry of the cyclo-hexene oxide antibiotics, specific examples will be discussed.

Structure

Chemical transformations, formation of derivatives, functional group analyses and spectroscopic studies were the basis for structural assign-ments in this area. Acetylation, reductive acetylation, aromatisation and reduction were the key reactions carried out with the cyclohexene oxide

antibiotics; these results, together with the elemental analyses, enabled the determination of the carbon framework and distribution of oxygen atoms in the antibiotics.

Functional group analysis tests, namely ferric chloride, periodate, thiosulphate, 2,4-dinitrophenylhydrazine, Kuhn-Roth oxidation, Fehling and Tollens' reagents have been used to assist in the structural determination of the antibiotics, but in later years these types of analyses have been largely superseded by the use of physical data.

Electronic and infrared spectra indicated the chromophoric properties of the antibiotic and, in some cases, mass spectral evidence enabled the position of epoxide linkages in the carbocyclic skeleton to be determined.[17,18]

Much structural information concerning cyclohexene oxide antibiotics was obtained from extensive studies by proton magnetic resonance spectroscopy (^{1}H n.m.r.). The environment of the protons could be assessed from their chemical shifts and analysis of coupling constants aided by double resonance experiments (spin-spin decoupling) enabled relationships amongst the protons to be established.[31] However, in recent years ^{13}C nuclear magnetic resonance spectroscopy (^{13}C n.m.r.) has played a major role in determining the carbocyclic skeleton and its substitution pattern.[15] Carbon attached to oxygen can be easily recognised by its chemical shift which also allows the hybridisation state of the carbon to be assigned. The proton-coupled ^{13}C n.m.r. spectrum enables the determination of the number of protons attached to each carbon.[32] This information allows a reduction in the number of chemical transformations required, and in some cases such transformations are no longer necessary.

Relative Stereochemistry

For the assignment of the stereochemistry mainly spectroscopic studies were used. In general, relative stereochemistry was established by extensive

^{1}H n.m.r. studies of the antibiotic and/or derivatives. Although ^{1}H n.m.r. spectroscopy is a very good method for stereochemical studies in general, sometimes incorrect stereochemical assignments can be made using this technique[7,33] because the cyclohexane or cyclohexene ring adopts an abnormal conformation due to the strain caused by the accommodation of the epoxide ring.

Absolute Stereochemistry

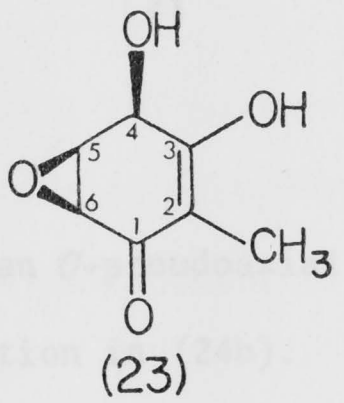
Various physical techniques have been used for absolute stereochemical assignment. For antibiotics which already possessed suitable chromophores or could conveniently be transformed into compounds with suitable chromophoric properties (*e.g.* a conjugated or non-conjugated carbonyl function) from which the Cotton effects could be observed, the absolute stereochemistry was obtained from studies of the chiroptical properties, namely optical rotatory dispersion (ORD) and circular dichroism (CD).[34] Horeau's method[35] for determining the chirality of a secondary alcohol has been used. However, the most frequently used technique was X-ray crystallography. Finally, in some members, the absolute stereochemistry followed simply from chemical conversion into closely related members whose absolute stereochemistry had already been determined.

Terreic acid (1) *and Terremutin* (2)

The structure of terreic acid (1) was indicated by chemical transformations, ultraviolet and infrared and also ^{1}H n.m.r. studies. Terreic acid, when treated with acetic anhydride in the presence of boron trifluoride, gave 2,3,4,5,6-pentaacetoxytoluene, which was identical with the product formed by reductive acetylation of 2,5,6-trihydroxy-3-methyl-1,4-benzoquinone. The action of dilute base upon the antibiotic produced a mixture of quinones, from which 2,3,5,6-tetraacetoxytoluene was obtained

after reductive acetylation. A comparison of the ^{1}H n.m.r. spectra of (1) and 2,3-epoxy-1,4-naphthoquinone indicated the presence of protons attached to the epoxide group in the antibiotic. On the basis of the foregoing, the structure of terreic acid (1) was determined to be 2-hydroxy-3-methyl-5,6-epoxycyclohex-2-en-1,4-dione.

Initially, structure (23) was proposed[7] for terremutin (2). The relative stereochemistry was deduced by analysis of the ^{1}H n.m.r. spectrum in which the coupling constants between H-4 and H-5, and H-4 and H-6 are approximately 1 Hz. A $^3J_{4,5}$ value of 1 Hz is consistent with the coupling constant calculated by the Karplus equation[36] for vicinal protons as in structure (23), in which the epoxide ring and the hydroxyl group at C-4 are *cis*.

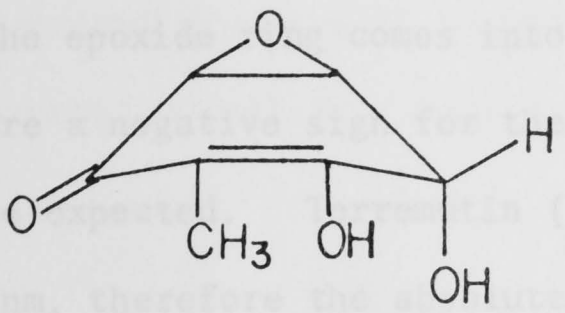


(23)

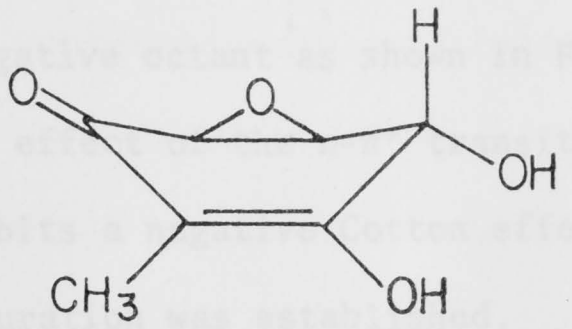
Later, Read *et al.*[33] synthesised the two stereoisomers (2) and (23) and clarified the stereochemistry for terremutin. Long-range W coupling between H-4 and H-6 is not observed in the isomer (23), nor in epoxydon (5) and other related compounds which have the epoxide ring *cis* to the hydroxyl group. Analysis of the ^{1}H n.m.r. spectra of terremutin and its derivatives gave the revised structure (2) for terremutin.

Long-range W couplings have been used to elucidate the relative stereochemistry of intermediates in syntheses of epoxydon (5) and its isomer[37] and also of senepoxide (13)[38] and crotepoxide (15)[39].

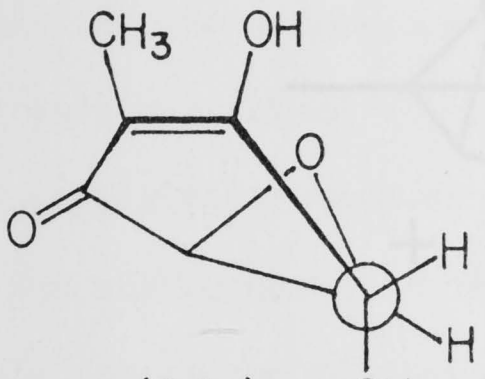
Dreiding models suggest two possible conformers for terremutin, (24a) and (24b) which are depicted in (24c) and (24d) respectively when viewed along the C-4 to C-5 bond.



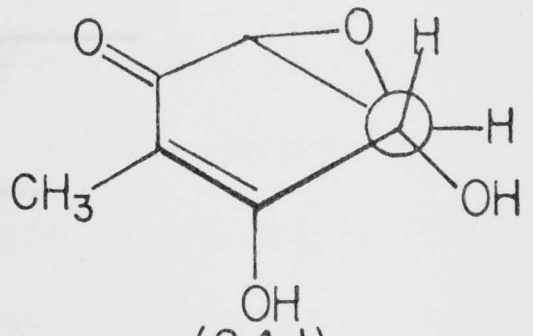
(24a)



(24b)



(24c)



(24d)

The epoxide ring assumes an *O*-pseudoaxial conformation in (24a) and an *O*-pseudoequatorial conformation in (24b). In (24c) the substituents at C-4 and C-5 take a staggered conformation with regard to each other so that there is less interaction between the substituents. Also (24a) provides more favourable resonance possibilities between the delocalised electrons in the oxirane ring and the π-electrons of the carbonyl group,[12] therefore conformer (24a) is considered more stable. Read *et al.*[40] synthesised several epoxyketone derivatives and found that all epoxyketones synthesised have the *O*-pseudoaxial conformation.

The absolute stereochemistry of terremutin (2) was deduced from its CD properties by application of the "reverse" Octant Rule for α,β-epoxyketones.[41] The presence of a double bond α to the ketone in the carbocyclic ring does not alter the rule because the Cotton effect derived from an α,β-epoxyketone is stronger than that from an enone.[12]

In the application of the "reverse" Octant Rule to the conformer (24a) the epoxide ring comes into the negative octant as shown in Fig.1, therefore a negative sign for the Cotton effect of the n-π* transition could be expected. Terremutin (2) exhibits a negative Cotton effect at 318 nm, therefore the absolute configuration was established.

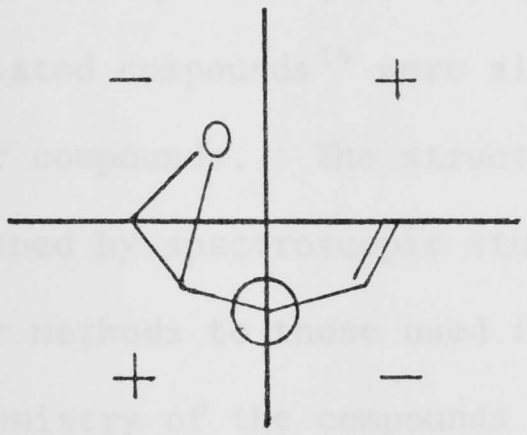


Fig. 1

Dehydrogenation of terremutin (2) gave terreic acid (1); therefore the absolute chiralities of the epoxide groups in terremutin and terreic acid were identical. Hence the absolute stereochemistry of terreic acid (1) was established.

Fumigatin oxide (3)

A study of the relationship between asthma and fungi[9] led to the isolation of fumigatin oxide (3). The notable similarities between the physical and chemical properties of (3) and terreic acid (1) suggested a hydroxy-1,4-benzoquinone nucleus. Spectroscopic studies of fumigatin oxide and its derivatives led to the determination of the structure (3).

Epoxydon (5)

Epoxydon was isolated from a culture filtrate of *Phoma S1019* in the course of screening for antitumour compounds by Closse *et al.*[12] The structure (5) was assigned to this compound by spectroscopic studies of the antibiotic and its derivatives. The *cis* relationship between the epoxide and hydroxyl groups was established from analysis of the ^1H

n.m.r. spectrum of a derivative of epoxydon. Absolute stereochemistry was determined from the CD properties of epoxydon by the application of the "reverse" Octant Rule in a similar fashion to the case of terremutin (2).

Panepoxydon (7) *and Related Compounds* (6), (8)-(11)

Panepoxydon and related compounds[14] were also isolated as a result of screening for antitumour compounds. The structure and stereochemistry of panepoxydon were determined by spectroscopic studies of (7) and its derivatives using similar methods to those used for epoxydon (5). The structures and stereochemistry of the compounds (6) and (8)-(11) were determined by comparison of their spectroscopic data with (7).

Phyllostine (4)

Phyllostine was isolated as a metabolite of *Phyllosticta* sp. together with phyllosinol.[10,11] The structure of phyllosinol was found to be identical with that of epoxydon (5) by comparison of the ^{1}H n.m.r. and CD spectra. Structural elucidation of phyllostine[11] was carried out using spectroscopic methods, and selective oxidation of epoxydon at C-4 gave a compound which was identical to phyllostine, therefore establishing the absolute stereochemistry of (4).

Senepoxide (13)

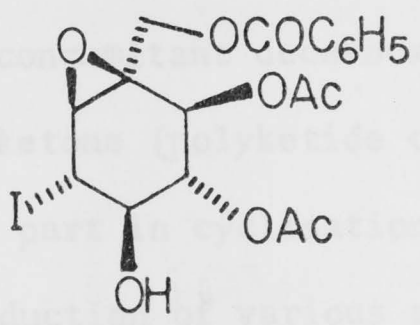
Uvaria catacarpa is distributed in the highlands of Madagascar and the fruit has been used as a traditional drug in France. From the fruit senepoxide (13) was isolated[17] and its structure determined by derivatisation and spectroscopic studies. Application of Horeau's rule to several derivatives of (13) gave a tentative absolute stereochemistry for (13)[17] which was confirmed by CD studies[17] and also X-ray studies.[42]

Pipoxide (14)

Plants belonging to the genus *Piper* are known to possess considerable medicinal properties,[18,22] and investigation of *Piper hookeri*[18] led to the isolation of pipoxide. The structure (14) was determined from spectroscopic data of pipoxide and its derivatives.

Crotepoxide (15)

Crotepoxide, which has a similar structure to that of senepoxide (13) and pipoxide (14), was first isolated by Kupchan *et al.*[19-21] as an anti-tumour substance from the fruit of *Croton macrostachys* collected in Ethiopia. It was identical with futoxide isolated from the leaves and stems of *Piper futokadzura* in Japan by Takahashi[22]. In India, crotepoxide has also been isolated from the stems of *Piper hookeri*[23] and the leaves of *Piper brachystachyum*[24]. The structure was deduced by spectroscopic studies of the compound and its derivatives. X-ray structural analysis of the iodohydrin (25)[21] established the absolute configuration for crotepoxide (15).



(25)

LL-Z1220 (12) *and Stylocheilamide* (16)

The structure of LL-Z1220 has been determined by spectroscopic studies on the antibiotic and derivatives[15,16]. Chemical and spectral properties of (12) indicated that the benzene dioxide has the *cis* configuration,[16] but the absolute stereochemistry has not been reported.

A mollusc metabolite, stylocheilamide (16) was referred to in a review by Scheuer[25], but its chemistry was not discussed.

1.3 Biosynthesis

Cyclohexene oxide antibiotics and related compounds have been isolated from many sources including plants, fungi and a mollusc.

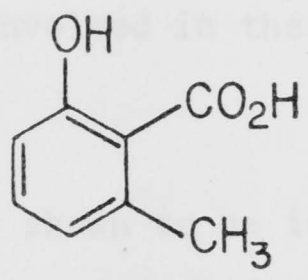
Biosynthetic studies of the compounds from the plant sources (13)-(15) and (22) have not been reported in the literature. However, a theory regarding their biosynthetic origins in the 'shikimate' pathway has been proposed[43] but this theory still needs to be substantiated by feeding experiments.

Some compounds from fungal sources, terreic acid (1),[44,45] fumigatin oxide (3)[26,46] and epoxydon (5)[47] have been shown to be derived from polyketide intermediates.

The polyketides are a structurally diverse group of naturally occurring compounds produced by the acylpolymalonate biosynthetic route. The route involves condensation of an acetyl or another acyl coenzyme A unit with malonyl coenzyme A units, with concomitant decarboxylation, giving rise to a β-polyketone. The β-polyketone (polyketide chain) possesses activated methylene groups which can take part in cyclisations and substitutions. The latter process allows the introduction of various groups into the polyketide, notably methyl from methionine, isoprenoid units from their pyrophosphates and halogen from a halonium ion source. Besides cyclisation and substitution, oxidation, reduction and dehydration can also occur in the polyketide chain, therefore a wide range of natural products can be derived from the polyketide precursors.[48,49]

Cyclisation by internal aldol type condensation of a tetraketide intermediate in which the C-5 carbonyl has been reduced is known to give

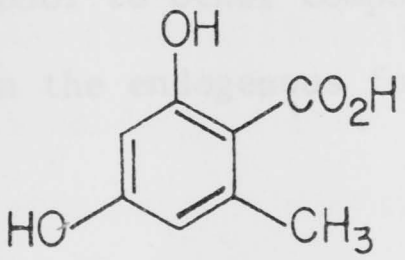
rise to 6-methylsalicylic acid (26).[48,49a] Decarboxylation followed by oxidation of 6-methylsalicylic acid has been demonstrated, by feeding



(26)

experiments, to be the biosynthetic route to terreic acid (1)[44,45] and epoxydon (5)[47].

Although the 'acetate' origin has been well established for fumigatin oxide (3)[26,46], the identity of the possible aromatic intermediate which links the tetraketide precursor with (3) has not been established by feeding experiments. Orsellinic acid (27) was assumed to be the aromatic precursor of fumigatin oxide[46] on the basis of their coexistence and from literature reports on the biosynthesis of toluquinones in *Aspergillus fumigatus* from orsellinic acid.[46] Also on the basis of literature reports,



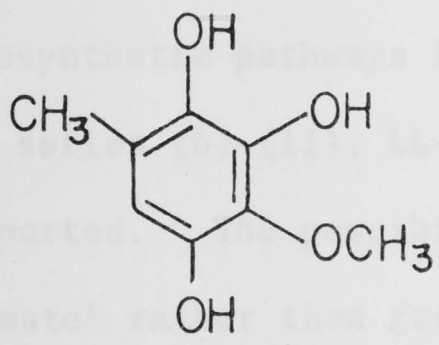
(27)

the methyl in the methoxyl group of (3) was assumed to be derived from methionine.[46]

Turner[49b] pointed out that it was unlikely that orsellinic acid (27) was a precursor of fumigatin oxide (3) for the following reasons: 6-methyl-

salicylic acid (26), not orsellinic acid (27), was incorporated into terreic acid (1), and molecular oxygen was the precursor of the epoxide.[44,45] This is in accord with Patterson's suggestion[50] that 6-methylsalicylic acid, rather than orsellinic acid, was involved in the formation of toluquinones which lack the 3-hydroxyl group.

Fumigatin oxide (3) has been shown to be incorporated into fumigatin quinol (28),[26,46] fumigatin (29)[46] and spinolusin quinol-hydrate (18)[26]. This indicated that fumigatin quinol was derived from fumigatin oxide and fumigatin and spinulosin quinol-hydrate were derived from fumigatin quinol.[26,46]



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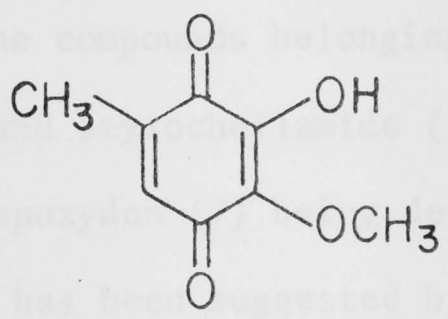
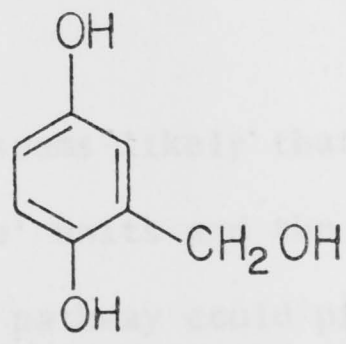


(29)

Gentisyl alcohol (30) was postulated to be a very near precursor of epoxydon (5), and ^{14}C-labelled gentisyl alcohol was found to be incorporated (25%) into epoxydon[47] offering support for this hypothesis. Conversion of epoxydon and/or gentisyl alcohol to other compounds could be the cause of low incorporation rather than the endogenous formation of epoxydon as suggested by Nabeta *et al.*[47]



(30)

The compounds related to epoxydon, (19) and (21), have been shown to be derived non-enzymatically from epoxydon (5)[47]. The coexistence of spinulosin-hydrate (17) with spinulosin quinol-hydrate (18) and fumigatin oxide (3), and also the coexistence of phyllostine (4) with epoxydon (5) implies their common biosynthetic origin. The structural similarities between terreic acid (1) and terremutin (2), together with a close relationship between *Aspergillus terreus* and its mutant, from which (1) and (2) were respectively isolated, implies the 'acetate' origin for terremutin.

Other Compounds

The biosynthetic pathways leading to the compounds belonging to the panepoxydon series (6)-(11), LL-Z1220 (12) and stylocheilamide (16) have not been reported. The possibility of panepoxydon (7) being derived from 'shikimate' rather than from 'acetate' has been suggested by Turner.[49c] However, panepoxydon (7) and related metabolites (6), (8)-(11) could also be postulated to originate from a carbocyclic triketide skeleton where the isoprenoid side-chain could be derived from substitution of the triketide intermediate with isopentenyl pyrophosphate.

The condensation with concomitant decarboxylation between an acyl coenzyme A unit, arising from a C_9-acid of 'shikimate-acetate' origin, and a malonyl coenzyme A unit could give rise to a 'shikimate-acetate' intermediate which could on cyclisation, dehydration and oxidation yield LL-Z1220 (12).

For stylocheilamide (16), it seems likely that the acyl part of the side-chain originates from 'acetate' units and the C-1 units originate from methionine. The 'shikimate' pathway could provide a C_6-C_3 precursor for the carbocyclic nucleus and the three-carbon unit linking the carbocyclic nucleus with the rest of the chain. Alternatively, the

carbocyclic nucleus could be derived from a polyketide precursor; however, the biosynthetic origin of the three-carbon unit in this pathway is not clear.

Chlorinated marine natural products are believed to be formed by electrophilic substitution in a process involving chloronium ions, or equivalents, derived from chloride ions present in sea water.[51]

1.4 Biological Activity

The cyclohexene oxide group of antibiotics displays a diverse spectrum of biological activity, including activity against bacteria, fungi, tumours and plants.

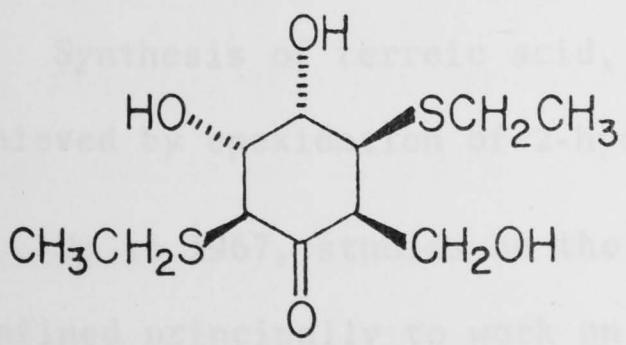
Terreic acid (1) showed *in vitro* activity against gram-positive and gram-negative bacteria and fungi, although *in vivo* tests were not promising.[52] The unnatural enantiomer (+)-terreic acid showed similar activity to that of the natural enantiomer (1) against *Staphylococcus aureus*, and the racemate was slightly more active than either enantiomer alone.[53] While (+) and (-) synthetic terreic acid showed activities of 97% and 90% respectively relative to natural (-)-terreic acid (1), the racemic terreic acid showed an activity of 125%.[53] Cycloserine is the other reported case of the unnatural enantiomer of an antibiotic possessing biological activity.[54] Both natural and unnatural enantiomers were found to be active against *Escherichia coli B* and the racemate was even more active than either enantiomer alone.[54]

LL-Z1220 (12) showed activity against both gram-positive and gram-negative bacteria and fungi.[15,16] However, epoxydon (5) appeared from the organisms tested to be more effective against gram-negative bacteria and phycomycetes.[55]

Besides antibiotic activity, many cyclohexene oxide antibiotics and related compounds show antitumour activity. For instance, terreic acid (1) completely inhibited mitosis of HeLa cells at 6.25 μg/ml[56], while epoxydon (5) at 1 μg/ml[12], panepoxydon at 1 μg/ml[14] and panepoxydione at 0.16 μg/ml[14] were shown to inhibit cell growth *in vitro* in mice mast tumour cells P-815 up to 50%. Crotepoxide (15) has been found to exhibit significant activity against Lewis lung carcinoma and Walker intramuscular carcinoma in mice.[19-21]

Epoxydon (5) has been shown to exert phytopathogenic effects on various plants, for example, red and white clover, alfalfa, soybean, potato, grape, oats and millet.[55] These effects were measured by leaflet testing at 100-500 μg/ml, and application of 10 μg/ml of epoxydon to the stem of red clover was found to cause scorching of the stem which later spread to the leaves.[55] Phyllostine (4), a close relative of epoxydon (5), also showed phytotoxic activity.[10,11] Other than the activities mentioned, epoxydon at low concentrations promoted root elongation and inhibited coleoptile growth.[55] Both promoting and inhibiting effects were reduced by the addition of cysteine or glutathione.[55]

Epoxydon (5) has two possible sites for reaction with mercapto groups, an α,β-unsaturated ketone and an epoxide. The mode of action of epoxydon was illustrated by reactions with ethanethiol and 2-aminoethanethiol resulting in the products (31) and (32) respectively.[57]



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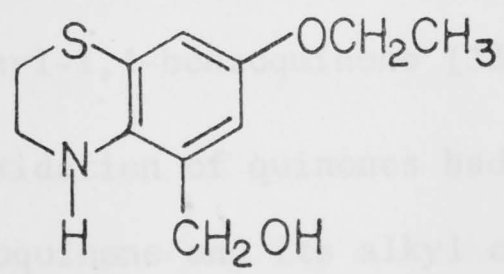


(32)

The results suggested that the activity in (5) was associated with both the epoxide and the α,β-unsaturated ketone functions. Work on anti-microbial activity of 1,4-benzoquinone and its derivatives, and 1,4-benzoquinone oxide derivatives,[58] indicated that compounds containing both α,β-unsaturated ketone and epoxide groups show additivity in activity.

1.5 Synthesis

The synthesis of some cyclohexene oxide antibiotics and related compounds has been reviewed by Ichihara[29], and Ichihara and Sakamura[30].

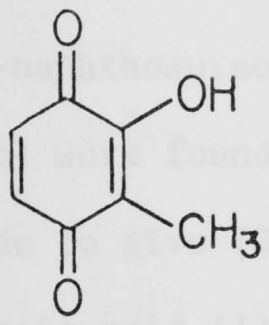
Most members of this group of cyclohexene oxide antibiotics and related compounds are of low molecular weight, therefore the problem of building a complex carbon skeleton is seldom encountered. However, frequent difficulties arise because the cyclohexene or cyclohexane ring is polyoxygenated and many of the synthetic intermediates required are unstable, often undergoing facile aromatisation. Because their stereochemistry is usually quite complex, efficient stereoselective syntheses are desirable. Many stereoselective reactions utilised for the syntheses of cyclohexene oxide metabolites exploit neighbouring group and steric effects.

Some specific examples which lead to the synthesis of cyclohexene oxide metabolites will be discussed.

Terreic acid (1) *and Terremutin* (2)

Synthesis of terreic acid, a 1,4-benzoquinone oxide derivative, was achieved by epoxidation of 2-hydroxy-3-methyl-1,4-benzoquinone (33).[53,59]

Up to 1967, studies on the direct epoxidation of quinones had been confined principally to work on 1,4-naphthoquinone and its alkyl or alkoxy derivatives. The reaction was thought to be initiated by nucleophilic attack by hydroperoxide anion on the quinone double bond followed



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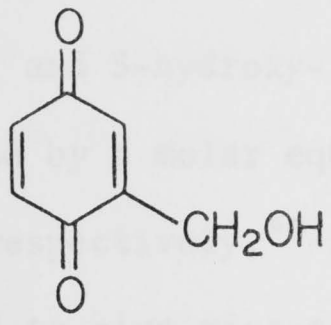
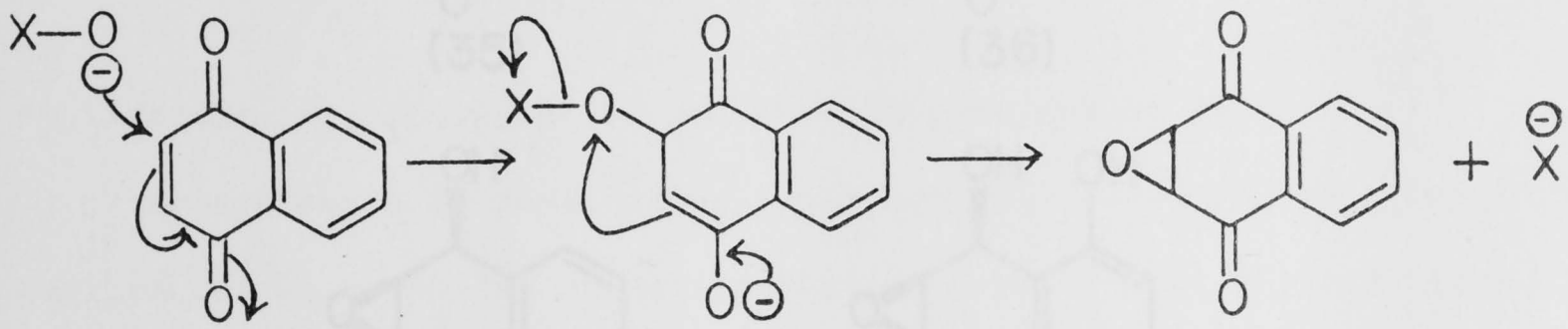


(34)

by intramolecular nucleophilic substitution to produce the epoxide, (Scheme 1).[59]



Scheme I

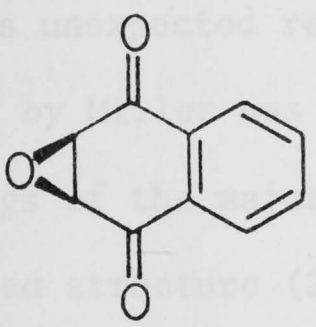
In the epoxidation of 1,4-benzoquinone and its derivatives the epoxides formed were found to be easily decomposed under the alkaline conditions.[59] This method of epoxidation is therefore limited to alkali-stable compounds.

In 1967, Moore[60] synthesised 1,4-benzoquinone oxide derivatives by the oxidation of alkyl derivatives of 1,4-benzoquinones with *t*-butyl-hydroperoxide and triton B. In this case, the alkyl substituent was considered to stabilise the epoxide. However, all attempts[61] to oxidise 2-hydroxymethyl-1,4-benzoquinone (34) to the corresponding epoxides failed.

A good method for direct epoxidation of quinones was introduced by Read *et al.*[59] In this method, quinones were oxidised at pH 9 with

sodium perborate. Oxidation of 2-hydroxy-3-methyl-1,4-benzoquinone (33) by this method gave terreic acid (1) in 16% yield.

Because 1,4-naphthoquinone oxide (35) and 5-hydroxy-1,4-naphthoquinone oxide (36) were found to be reduced by a molar equivalent of sodium borohydride to give (37) and (38) respectively,[40] the similar reduction of terreic acid (1) was expected to give rise to structure (23).[33] In the borohydride reduction of terreic acid (1) (5*S*,6*R*)-(2-hydroxy-



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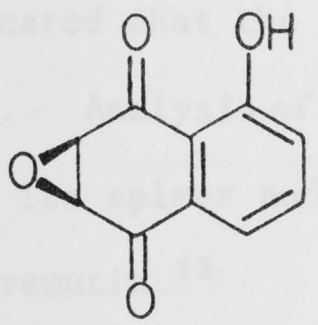

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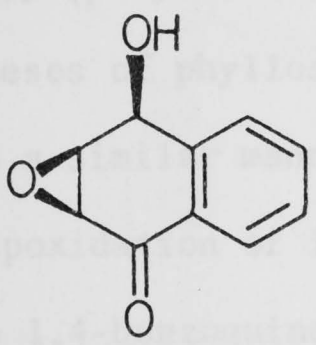

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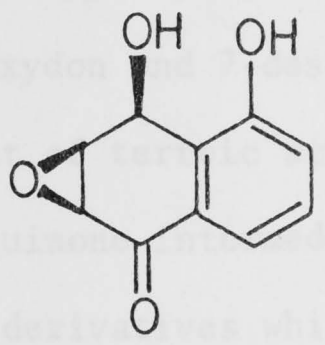
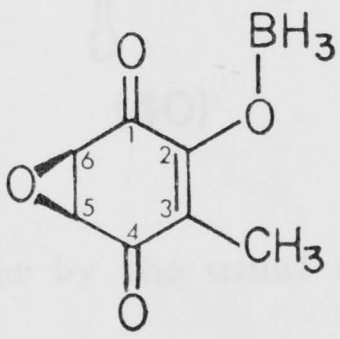


(38)

3-methyl-5,6-epoxycyclohex-2-en-1,4-dione) competing attack at the C-4 carbonyl was not anticipated because the acidic enolic hydroxyl group was expected to be the first functional group attacked to give (39) as an intermediate. In (39), the reducing centre of the borohydride



(39)

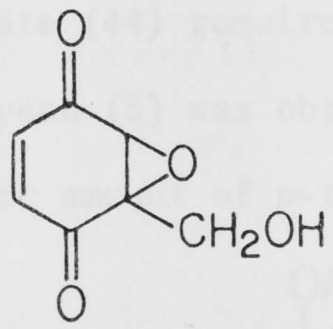
and the C-1 carbonyl group were juxtaposed and the electrophilic character of the C-4 carbon would be low. Sterically, attack by the reagent from the opposite side to the epoxide group was also expected because of the *O*-pseudoaxial conformation of the epoxide. Therefore terreic acid (1) was expected to give rise to (23) as the major product.[33]

However, when terreic acid (1) was reduced, with two molar equivalents of sodium borohydride, terremutin (2) was not a major product but a minor product. This unexpected result indicated that the structure (23) proposed for terremutin by Miller was incorrect. Analysis of vicinal and long-range proton couplings of the major product, its epimer and their derivatives gave the revised structure (2) for terremutin.[33]

Phyllostine (4), *Epoxydon* (5) *and 7-Desoxypanepoxydol* (9)

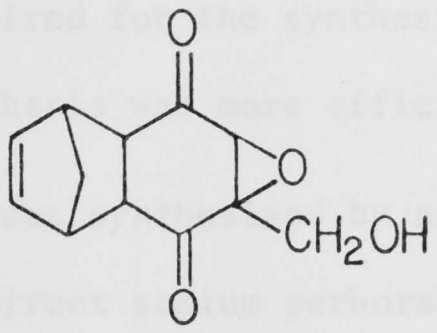
The syntheses of phyllostine, epoxydon and 7-desoxypanepoxydol were carried out in a similar manner to that of terreic acid (1) and terremutin (2). Epoxidation of 1,4-benzoquinone intermediates by sodium perborate gave 1,4-benzoquinone oxide derivatives which, in some cases, were reduced to obtain the required natural product.[61-64]

Direct epoxidation of 2-hydroxymethyl-1,4-benzoquinone (34) gave phyllostine (4) and its position isomer (40). As it was not possible



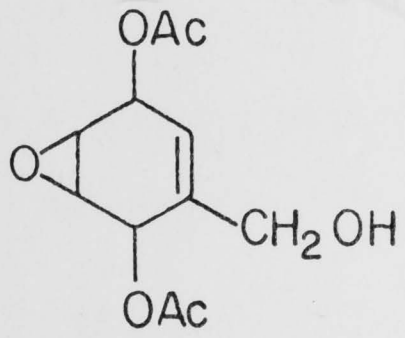
(40)

to separate the position isomer by the usual chromatographic methods, compound (40) was converted into the cyclopentadiene adduct (41) and phyllostine was then isolated.[61]



(41)

In the synthesis of epoxydon (5), the neighbouring group effect was utilised in the selective hydrolysis of the acetoxyl function of the intermediate (42).[62] In this case the hydroxymethyl caused the



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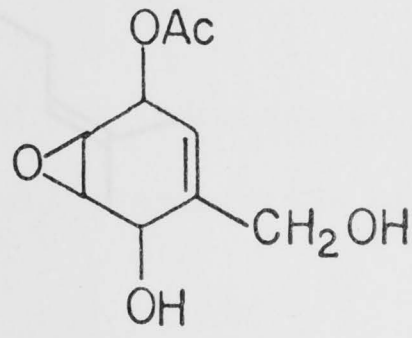
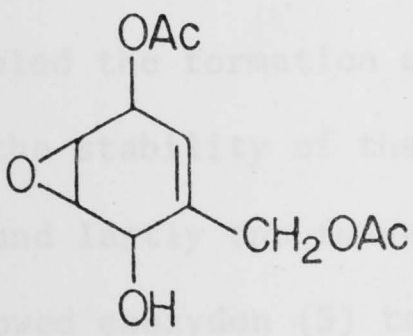


(43)

neighbouring acetoxyl group to be selectively hydrolysed giving rise to (43). Protection of the primary hydroxyl group in (43) followed by oxidation and deprotection gave epoxydon (5).[62]

A slightly different synthetic route to epoxydon employed an acyl rearrangement.[63] The intermediate (44) required for the oxidation step leading to the synthesis of epoxydon (5) was obtained directly by heating a solution of (45) and a catalytic amount of *p*-toluenesulphonic acid.



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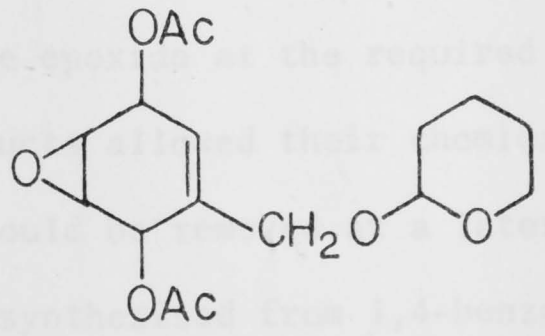
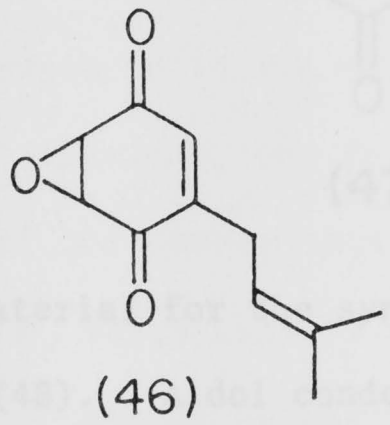


(45)

Since the extra steps involving protection and deprotection of the primary hydroxyl group were not required for the synthesis of epoxydon by the acyl rearrangement, this synthesis was more efficient.

7-Desoxypanepoxydol (9) was synthesised by a similar method to that used for epoxydon.[64] Direct sodium perborate epoxidation of 2-isopentenyl-1,4-benzoquinone yielded a mixture of (46) and its position isomer. Zinc borohydride reduction of (46) gave 7-desoxypanepoxydol in low yield (<3%).



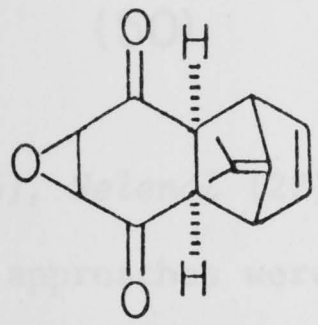
(46)

Alder *et al.*[65] synthesised 1,4-benzoquinone oxide by Diels-Alder reaction of 1,4-benzoquinone with cyclopentadiene followed by epoxidation of the adduct and thermolysis to yield the required epoxide. Because of the drastic conditions (420 °C) used during the retro Diels-Alder reaction, some substituted 1,4-benzoquinone oxides prepared by this method underwent decomposition.[66]

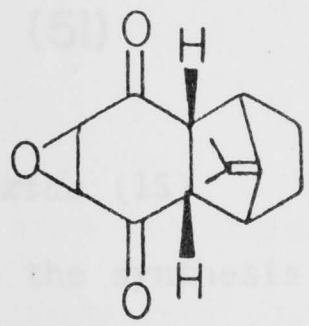
Replacing the cyclopentadiene moiety with dimethylfulvene enabled the thermolysis to be carried out in an organic solvent at a considerably lower temperature (<200 °C).[66] This method had the following advantages: firstly, it enabled the formation of the epoxide at the required double bond, secondly the stability of the adducts allowed their chemical transformation and lastly the fulvene could be removed at a later stage. This method allowed epoxydon (5) to be synthesised from 1,4-benzoquinone with an overall yield of 10%, which was a far better yield than that

obtained from the previous multistep non-stereoselective synthesis[62,63] (1% from gentisyl alcohol).

Cycloaddition of dimethylfulvene and 1,4-benzoquinone gave a 1:1 mixture of *exo* and *endo* adducts which, without isolation, were epoxidised to yield the corresponding epoxides (47a) and (47b).[66] One of the epoxides

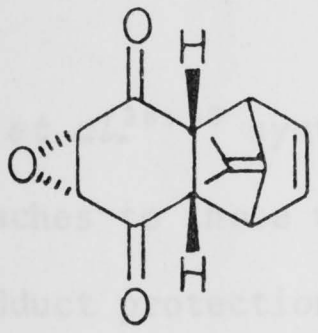


(47a)

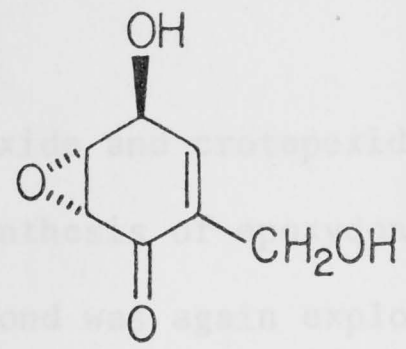


(47b)

could be used as a starting material for the syntheses of phyllostine (4), epoxydon (5) and epiepoxydon (48). Aldol condensation of one of the *endo*

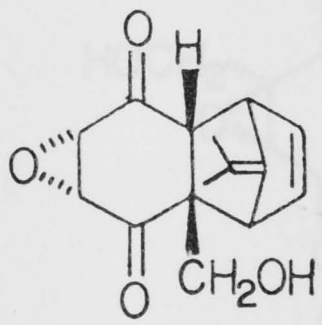


(47c)



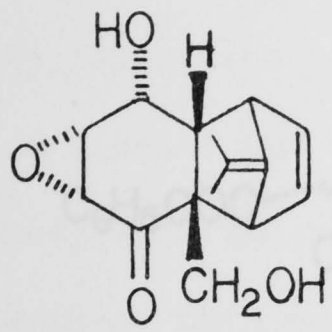
(48)

isomers (47c) with formaldehyde gave (49) which on retro Diels-Alder reaction gave phyllostine (4).[37] Reduction of (47c) yielded a mixture

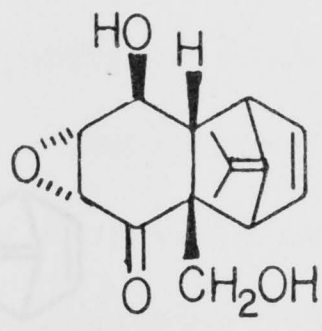


(49)

of alcohols which on aldol condensation with formaldehyde gave (50) and (51). Retro Diels-Alder reactions of (50) and (51) gave epoxydon (5) and epiepoxydon (48) respectively.[37]



(50)

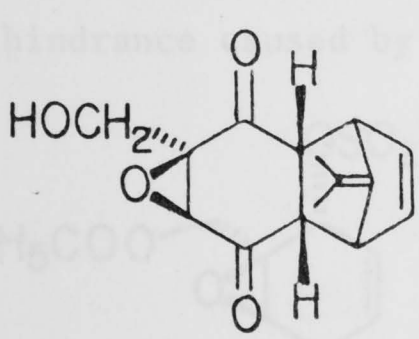


(51)

Senepoxide (13), *Selenol* (22) *and Crotepoxide* (15)

Two main approaches were employed in the synthesis of senepoxide and crotepoxide. In the first approach, the formation of epoxide groups was achieved by the oxidation of the double bond or by internal SN_2 displacement. The second approach, which will be subsequently discussed, involves the formation of epoxide groups by the rearrangement of the epidioxides.

Ichihara *et al.*[38,39] synthesised senepoxide and crotepoxide using similar approaches to those used for the synthesis of epoxydon (5). Diels-Alder adduct protection of a double bond was again exploited and a neighbouring group effect was utilised in the reduction of a carbonyl function in (52) to the corresponding alcohol (53). The regio- and stereoselectivity observed in the borohydride reduction of (52) was



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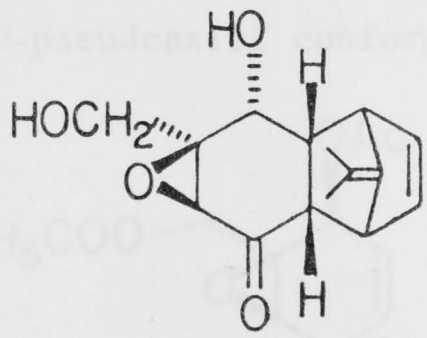
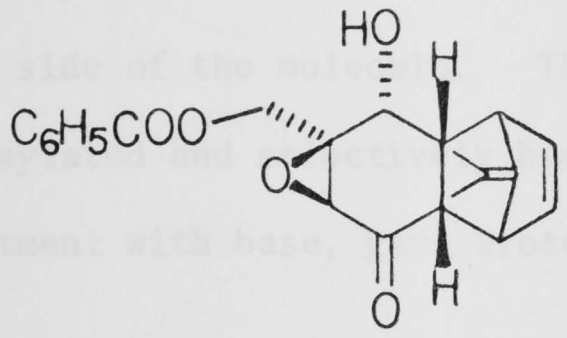


(53)

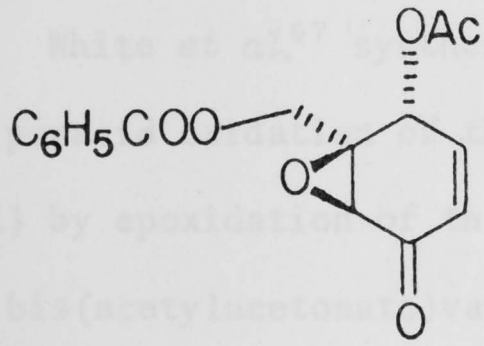
suggested[39] to arise from the coordination of the borohydride with the hydroxymethyl group, and the steric hindrance caused by the fulvene part of the molecule, preventing the hydride attack from underneath.

Compound (54) was a common intermediate for the synthesis of senepoxide (13) and crotepoxide (15).[38,39] In the synthesis of



(54)

senepoxide (13), compound (54) was acetylated and thermolysed to yield (55). Epoxidation of the thermolysis product (55) gave the diepoxide (56) which was regioselectively reduced with hydrazine then acetylated to give senepoxide (13).[38]



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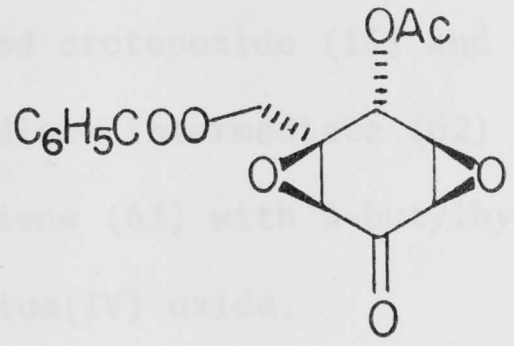
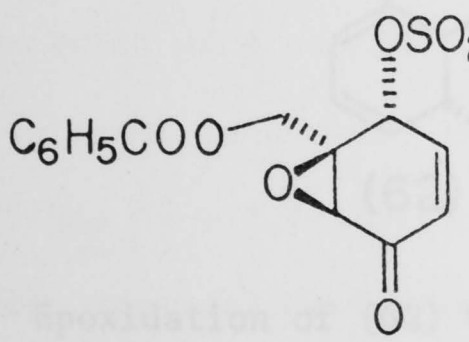


(56)

In the synthesis of crotepoxide (15), compound (54) was mesylated and thermolysed to yield (57). Zinc borohydride reduction of (57) gave the hydroxyl exclusively *cis* to the epoxide group, possibly due to the steric hindrance caused by an *O*-pseudoaxial conformation of the epoxide



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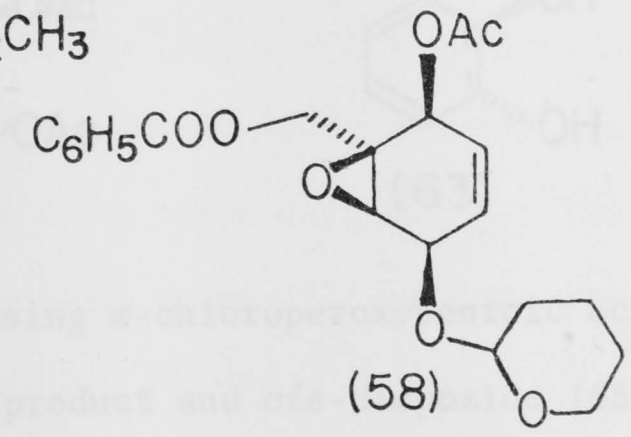
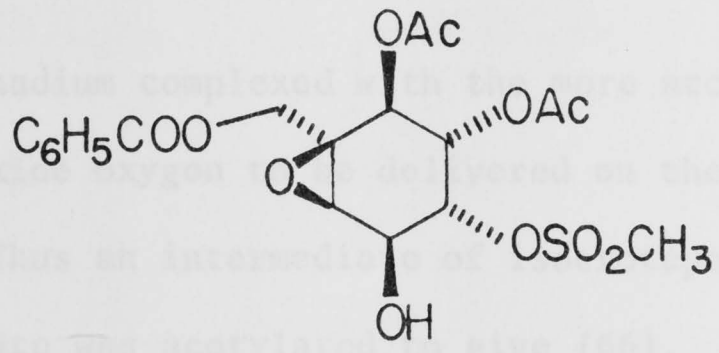


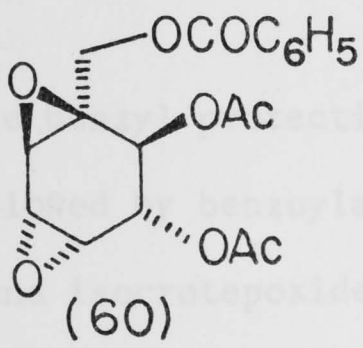
(58)

ring.[39] SN_2 displacement of the mesylate by acetate followed by the formation of a tetrahydropyranyl ether gave intermediate (58). Hydroxylation of (58), by osmium tetroxide, occurred stereoselectively from the least hindered side of the molecule. The hydroxylated product was acetylated, mesylated and selectively hydrolysed to yield compound (59) which, on treatment with base, gave crotepoxide (15).[39]



(59)

White *et al.*[67] synthesised crotepoxide (15) and epicrotepoxide (60) by peracid oxidation of the diene intermediate (62) and isocrotepoxide (61) by epoxidation of the diene (63) with *t*-butylhydroperoxide catalysed by bis(acetylacetonato)vanadium(IV) oxide.



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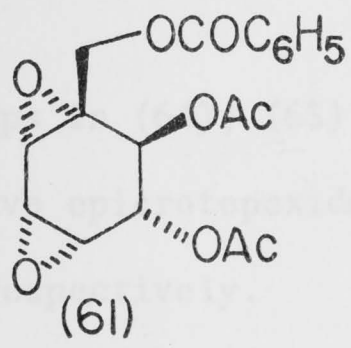

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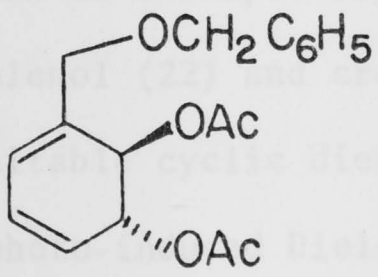
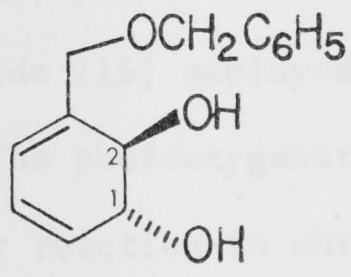
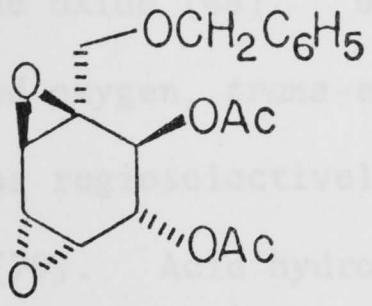


(62)



(63)

Epoxidation of (62) using *m*-chloroperoxybenzoic acid yielded *trans*-diepoxide (64) as a major product and *cis*-diepoxide (65) as a minor product. Since the latter is required for the synthesis of crotepoxide, epoxidation by this method is not suitable. In the epoxidation of the



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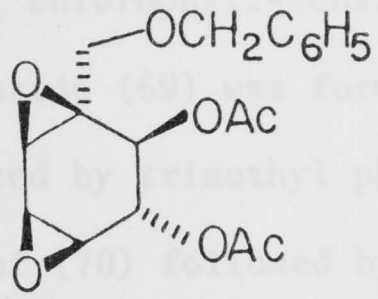
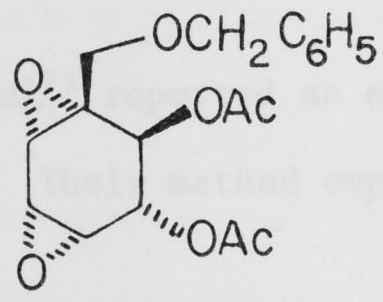


(65)

diene (63), the vanadium complexed with the more accessible C-1 hydroxyl and caused the epoxide oxygen to be delivered on the same side as this hydroxyl group. Thus an intermediate of isocrotepoxide (61) was obtained and this intermediate was acetylated to give (66).



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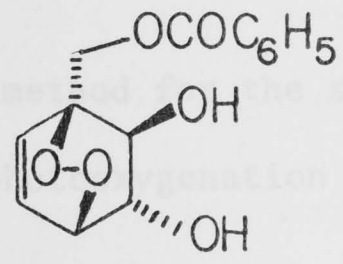


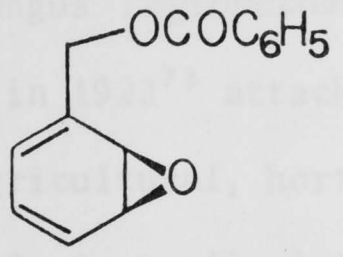
(67)

Removal of the benzyl protecting groups in (64), (65) and (66) by hydrogenolysis followed by benzoylation gave epicrotepoxide (60), crotepoxide (15) and isocrotepoxide (61) respectively.

Another approach to the synthesis of the plant metabolites, senepoxide (13), selenol (22) and crotepoxide (15) employed photooxygenation of a suitable cyclic diene. The photooxygenation reaction is analogous to a photo-induced Diels-Alder reaction in which the peroxide is formed by a 1,4-addition of singlet oxygen.[68] The peroxides can undergo rearrangement under both thermal[69] and photochemical[70] conditions to afford *cis*-1,3-diepoxides.

White *et al.*[67] photooxygenated the diol (63) to yield the peroxide (67) which on acetylation, followed by thermal rearrangement, yielded an intermediate (65) in the synthesis of crotepoxide (15).

Ganem *et al.*[71] synthesised senepoxide (13) and selenol (22) by photooxygenation of arene oxide (68). By using chlorophyll-sensitised photochemically generated oxygen, *trans*-endoperoxide (69) was formed exclusively. The endoperoxide was regioselectively reduced by trimethyl phosphite to yield a diepoxide (70). Acid hydrolysis of (70) followed by acetylation gave senepoxide (13) which on methanolysis yielded selenol (22).



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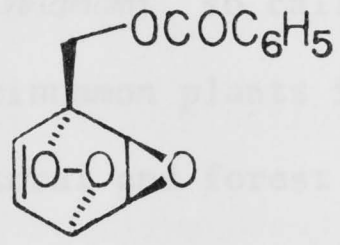

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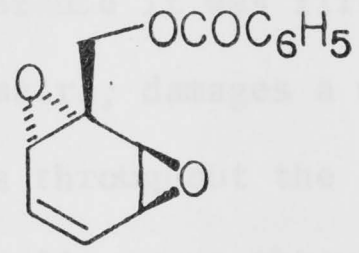
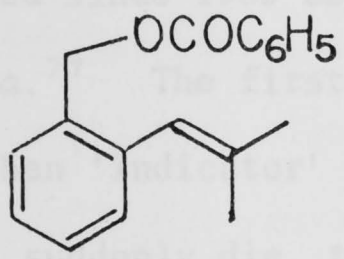


(70)

Matsumoto *et al.*[72] reported an elegant method for the synthesis of crotepoxide (15). Their method employed photooxygenation of a styrene



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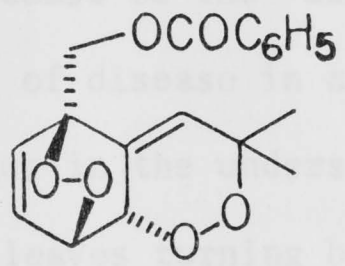
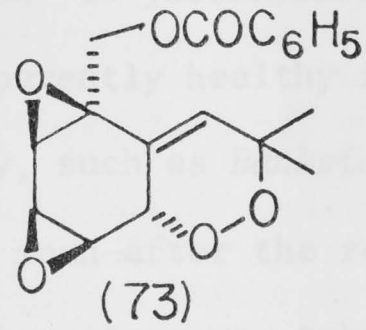


(72)



(73)

derivative (71) which gave a mixture of the peroxide (72) and its isomer. Thermal isomerisation of (72) yielded a *cis*-diepoxide (73) which on subsequent chemical transformations gave crotepoxide (15).

CHAPTER 2

INTRODUCTION TO EUPENOXIDE

2.1 *Phytophthora cinnamomi*

The fungus *Phytophthora cinnamomi*, so called because it was first identified in 1922[73] attacking cinnamon plants in Sumatra, damages a wide range of agricultural, horticultural and forest crops throughout the world.[74] In Australia interest in the fungus originally arose through its attack on crops such as pineapples, avocados and peaches, and on flowering plants such as rhododendrons.[75] It is also responsible for severe damage to chestnut and oak forests in the U.S.A. and Europe.[75,76]

In Western Australia, this microscopic soil-borne fungus has been recognised since 1965 as the cause of the 'die back' of jarrah, *Eucalyptus marginata*.[77] The first sign of disease in an apparently healthy forest occurs when 'indicator' species in the understorey, such as *Banksia grandis*, suddenly die, their leaves turning brown soon after the root tips are damaged by the fungus.[78] The jarrah trees themselves may take years to succumb, perhaps after several attempts at regeneration by producing small clumps of regrowth along the main branches and trunk.[79]

At first, the root rot disease in Australia was thought to be confined to jarrah but now it is also believed to be responsible for serious problems in certain native forests of Tasmania,[75b,80-82] eastern and southern Victoria, southern New South Wales,[75b,80-82] and to a lesser extent in other areas.[75b,80-82]

In Australia many attempts have been made to combat the disease. Several control measures have been introduced, including drainage of the wet land and trenching to contain the diseased area, and also replanting

of several resistant species into the 'die back' area.[82] A number of hygiene measures[75,80,82] have been implemented to restrict the spread of the fungus, but these measures apparently do not stop the disease from spreading slowly.

Meanwhile, research into many aspects of controlling the disease has been in progress. One area of research has looked into the possibility of using the mycorrhizal activities of basidiomycete fungi to control *P. cinnamomi* in forest situations through antibiosis.[83]

Mycorrhiza can be described as the wide-spread association between fungal hyphae and the roots of higher plants. In many instances both plant and fungus derive benefit from the association; in others, the fungus is clearly the parasite but the injury to the host is negligible. If the balance is disturbed, however, the fungus may cause severe root disease.[84]

Controlling disease by adding to soil alien microorganisms which produce antibiotics alone was not successful.[85] However, there were indications[86] that the soil fungi which produce antibiotics might be used more effectively in protecting a host against pathogenic infection if they were mycorrhizal on the host, or at least active in the root rhizosphere.

Eighty species of forty-five basidiomycete genera, collected from eucalyptus forests near Canberra (Australian Capital Territory), on attempted laboratory culture gave isolates from 33 species in 21 genera. The *in vitro* antagonism of these isolates to *P. cinnamomi* was studied, and 16 genera were found to exhibit antagonism to *P. cinnamomi* in agar culture. The effect of these basidiomycetes on *P. cinnamomi* was found to be fungistatic rather than fungicidal. Some of the basidiomycetes which exhibited strong antagonism were postulated to produce antibiotics.[83]

Two of the strongly inhibitory basidiomycetes were selected to investigate the possibility of these fungi secreting antibiotics. Both basidiomycetes indeed produced antibiotics ; 3,5-dichloro-4-methoxybenzoic acid was isolated from *Naematoloma fasciculare*[87] and from another basidiomycete, a species of *Eupenicillium*, eupenoxide was isolated.[88]

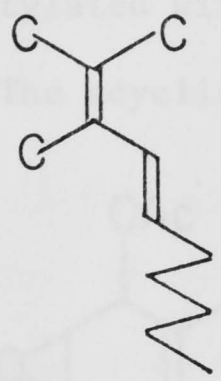
2.2 Isolation of Eupenoxide

The contribution in the area of cyclohexene oxide antibiotics from our research group started with the finding of eupenoxide. Quinn and Rickards[88] isolated eupenoxide and determined its structure, relative stereochemistry and biogenetic origin.

Eupenoxide was isolated from the culture filtrate of an unidentified species of *Eupenicillium*. Purification was effected by preparative thin layer chromatography (t.l.c.) on silica gel and gel-permeation chromatography.

2.3 Structure Determination

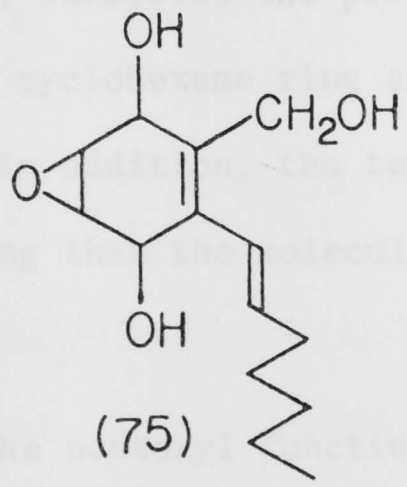
The structure of eupenoxide was determined from the spectroscopic data of eupenoxide and its triacetate. The ^{13}C n.m.r. spectrum of eupenoxide indicated the presence of four olefinic carbons, two of which were shown to be disubstituted and the others tetrasubstituted by off-resonance proton decoupling. The ultraviolet absorption at 240 nm (ε 20000) indicated that the double bonds were conjugated.[89a] The *E* stereochemistry of the olefinic protons was evident from the size of the coupling constant ($^{3}J_{AB}$ 16 Hz).[31a] Analysis of the methylene signals in the ^{13}C n.m.r. and ^{1}H n.m.r. spectra enabled assignment for the rest of the side-chain, hence establishing the partial structure (74) for eupenoxide.



(74)

The ^{1}H n.m.r. showed three exchangeable protons indicating the presence of three hydroxyl groups, and this was confirmed by the ability of eupenoxide to form a triacetate (76). Upon acetylation, a proton α to a secondary alcohol has been shown to shift to lower field than the protons α to a primary alcohol.[31b] Upon this basis, the formation of eupenoxide triacetate enabled the presence of a primary and two secondary alcohols to be established. The chemical shifts belonging to the four protons adjacent to the hydroxyl groups also suggested that they were all allylic.[31]

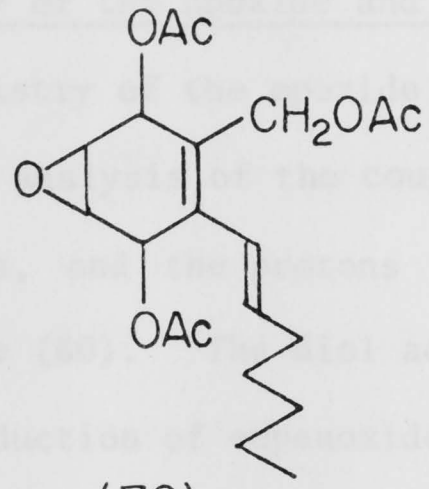
Eupenoxide contained four degrees of unsaturation, two of which were the double bonds, and because of the absence of a carbonyl function, the other two degrees of unsaturation were deduced to arise from the presence of two rings in the molecule. Based upon the foregoing evidence, the structure of eupenoxide was determined to be (75).



(75)

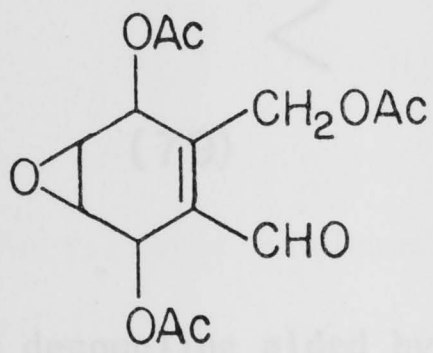
2.4 The Relative Stereochemistry of the Secondary Hydroxyl Groups

Eupenoxide (75) when acetylated with acetic anhydride and pyridine yielded a triacetate (76). The acyclic double bond was then selectively

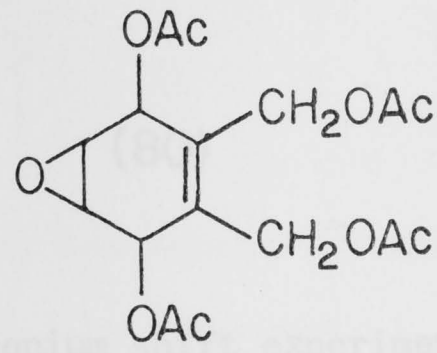


(76)

ozonised to yield an aldehyde (77) and hexanal, the latter confirming the presence of a C_5-straight chain. Sodium borohydride reduction of (77) followed by acetylation gave a tetraacetate (78) from which the relative stereochemistry of the secondary hydroxyl groups was determined.



(77)



(78)

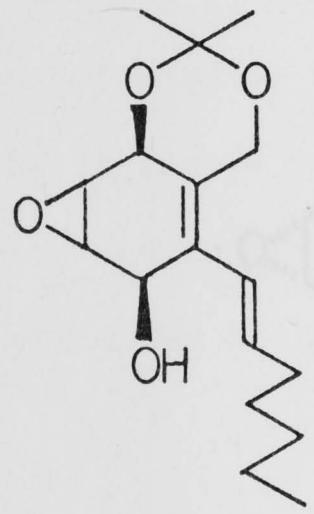
The ^{1}H n.m.r. spectrum of (78) indicated the presence of a plane of symmetry perpendicular to the cyclohexene ring and passing through the epoxide and olefin groups. In addition, the tetraacetate (78) exhibited no optical activity, confirming that the molecule possessed a plane of symmetry.

A *trans* arrangement of the acetoxyl functions could not give rise to this symmetry. Therefore the relative stereochemistry of the secondary

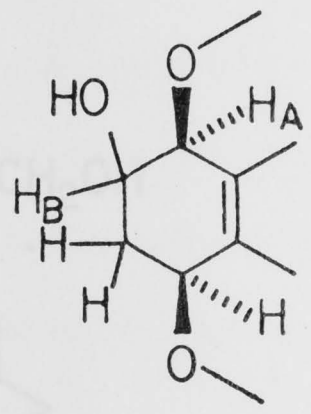
acetoxyl groups in (78) was deduced to be *cis*, thus establishing the *cis* stereochemistry between the hydroxyl groups in eupenoxide.

2.5 Relative Stereochemistry of the Epoxide and the Secondary Hydroxyl Groups

The relative stereochemistry of the epoxide and hydroxyl groups in eupenoxide was determined by analysis of the coupling constants between the carbocyclic methylene protons, and the protons on the vicinal oxygens in the diol acetonide (80). The diol acetonide was prepared by lithium aluminium hydride reduction of eupenoxide acetonide (79), itself obtained from acid-catalysed condensation of eupenoxide and 2,2-dimethoxypropane.

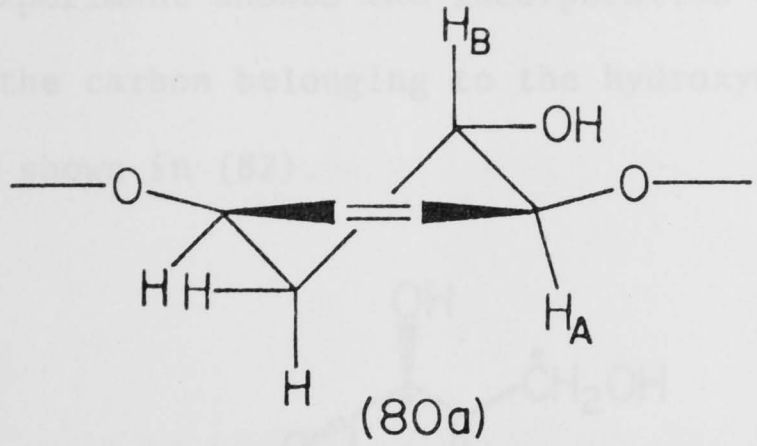


(79)



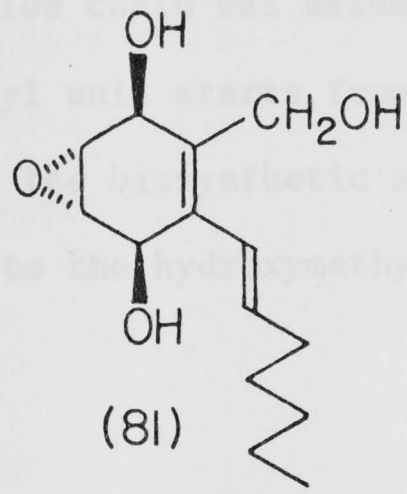
(80)

Homonuclear decoupling aided by europium shift experiments carried out upon the diol acetonide (80) enabled the assignment and measurement of all the ring-proton coupling constants in (80). The axial configuration of the proton (H_B) adjacent to the newly formed hydroxyl group was indicated by a large coupling (13 Hz) with a proton in the methylene group, thus establishing the equatorial configuration of the newly-formed hydroxyl group. This, together with other ring-proton coupling constants, enabled the cyclohexene skew chair of structure (80a) to be derived for the diol acetonide (80). The proton (H_A) exhibiting only one coupling was the methine proton vicinal to the hydroxyl group derived from the epoxide group.



(80a)

The 8 Hz coupling exhibited by this proton is consistent with H_A and H_B being approximately coplanar. This established the *trans* relationship between the two vicinal oxygens, therefore establishing structure (81) without regard to absolute stereochemistry.



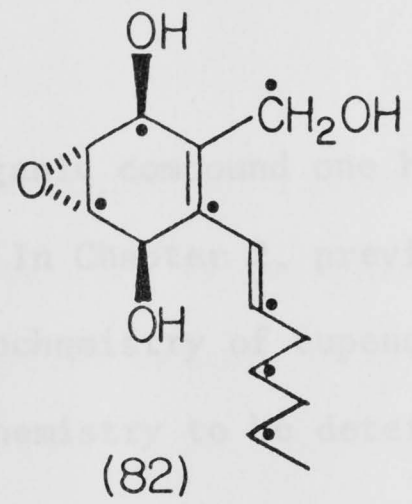
(81)

Therefore the systematic nomenclature for eupenoxide can be represented as (1*R**,4*S**,5*R**,6*S**)-2-[(*E*)-hept-1′-enyl]-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol. In the synthesis of eupenoxide (81), for convenience, the bicyclic intermediates were numbered in the same manner as eupenoxide (81). Also for simplicity, systematic names were not used in the discussion of eupenoxide derivatives.

2.6 Biosynthesis of Eupenoxide

The biosynthesis of eupenoxide was shown to be similar to other members of this class of antibiotics. Comparison of ^{13}C n.m.r. spectra of natural eupenoxide and [1-^{13}C]acetate enriched eupenoxide obtained

from a feeding experiment showed the incorporation of seven acetate units, and that the carbon belonging to the hydroxymethyl group had been enriched as shown in (82).



(82)

Therefore the heptaketide chain was assembled in the usual fashion. Chain initiation by an acetyl unit starts from the methyl group in the heptenyl side-chain, and in the biosynthetic sequence the terminal carboxyl group was reduced to the hydroxymethyl group.

2.7 Present Work

The present work by the author is concerned with various aspects of the stereochemistry and chemistry of eupenoxide (81). In Chapter 3 the work leading to the determination of the absolute configuration will be described, and the synthesis of eupenoxide will be discussed in Chapters 4, 5 and 6.

CHAPTER 3

THE ABSOLUTE STEREOCHEMISTRY OF EUPENOXIDE

3.1 Introduction

To describe fully an organic compound one has to define both its structure and chirality.[90a] In Chapter 2, previous work leading to the structure and relative stereochemistry of eupenoxide (81) was described, leaving the absolute stereochemistry to be determined.

Various methods for the determination of absolute stereochemistry are available, such as chemical degradation to compounds of known absolute stereochemistry, Horeau's direct method[35], X-ray analysis and chiroptical methods[34,90-92]. For eupenoxide (81), one convenient method is the measurement of chiroptical properties.

3.2 Chiroptical Theory

Optically active compounds are capable of rotating the plane of polarisation of light, by unequal absorption (circular dichroism, CD) and by unequal velocity of transmission (optical rotatory dispersion, ORD), of the left and right components of circularly polarised light.[34,90-92]

The combination of unequal absorption (CD) and unequal velocity of transmission (ORD) of left and right circularly polarised light in the region in which optically active absorption bands are observed is a phenomenon called the "Cotton effect".[92] The Cotton effect associated with an optically active absorption band of a given compound manifests itself by a circular dichroism curve and an anomalous optical rotatory dispersion curve.[92]

Optical activity is associated with electronic transitions occurring in a chiral environment of a chromophore. Optically active chromophores

have been classified by Moscowitz[93] into two major groups:

(i) inherently symmetric but dissymmetrically perturbed chromophores (inherently achiral but chirally perturbed chromophores);

(ii) inherently dissymmetric chromophores (inherently chiral chromophores).

Any optical rotation associated with electronic transitions of chromophores belonging to group (i) arises from a chiral perturbation due to the environment. A classical example of this type is the carbonyl chromophore in saturated ketones, which only becomes optically active when at least one of the two groups bound to the carbonyl is chiral. Other examples are given by unstrained olefins, benzene derivatives, ethers, alcohols, carboxylic acids, esters and thiocyanates.

The perturbation of the chromophore by a chiral environment is not required in a chromophore which belongs to group (ii), as the chromophore itself is already chiral, being non-planar. However, a chiral disposition of the substituents, for instance an asymmetric carbon atom, is necessary in some cases to stabilise one chiral form over the other enantiomeric form. Non-planar α,β-unsaturated ketones, non-planar dienes, polyenes, aromatic hydrocarbons and dithio- and trithiocarbonates are examples of chromophores in group (ii).

A third class of optically active chromophores is formed by homo-conjugated systems[94] such as β,γ-unsaturated ketones and non-conjugated dienes, where the molecule contains at least two inherently achiral chromophores [group (i)] which are separated by two or more than one single but interacting bonds and are chirally disposed to each other. The properties of these systems resemble those of inherently chiral chromophores [group (ii)].

Another classification has been made by Snatzke[95] who has suggested

that the molecule may be divided into 'spheres', starting with the chromophore and then going to the more remote atoms. The first sphere is the chromophore itself and the 'chiral first sphere' coincides with Moscowitz's inherently dissymmetric chromophore. The second sphere is the ring in which the chromophore is included or attached, in the case of cyclic compounds, and the third sphere comprises rings or groups attached to the second sphere, and this pattern is repeated. In the higher spheres the effects on the chromophore become insignificant.[34a,95]

If several centres of asymmetry are present, the one nearest to the chromophore primarily determines the sign and magnitude of the Cotton effect, the more remote ones having only minor influence.[34a,95]

The absolute configuration of a compound of unknown chirality can be determined by examination of the experimental Cotton effects in the light of available octant, quadrant and sector rules, or by correlation, *i.e.*, comparison of the observed optical properties with those of a model compound of known stereochemistry.[90b]

3.3 Chiroptical Properties of Eupenoxide

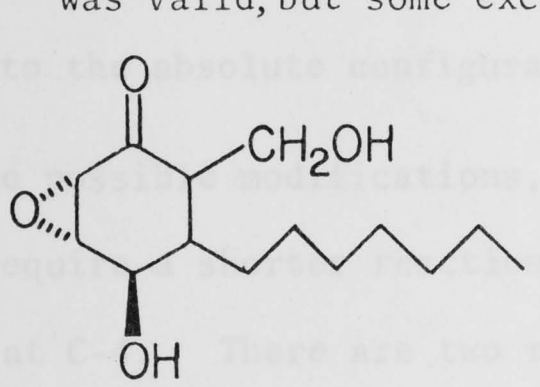
The major contribution to the chiroptical properties of eupenoxide (81) arises from the interacting orbitals in the diene chromophore, and the sign of the Cotton effect will be determined by the helicity, if any, of the diene chromophore and the asymmetric perturbations of the double bonds in the diene chromophore by the secondary hydroxyl group. As the epoxide group is further away from the diene chromophore, it will have only a minor influence on the sign and magnitude of the Cotton effect.

The absolute stereochemistry of eupenoxide could not be determined by use of the diene chromophore itself because, for compounds such as eupenoxide which possess an endocyclic double bond conjugated to an acyclic bond there is

no rule relating the sign of the Cotton effect to the absolute stereochemistry. In addition, as there were no suitable model dienes of known absolute stereochemistry, it was necessary to derivatise eupenoxide (81) so that the absolute stereochemistry could be deduced from the ORD or CD data by correlation with the model compounds, or directly by the existing empirical rules.

Hydrogenation of both double bonds in eupenoxide (81) followed by selective oxidation of a secondary hydroxyl group would give the dehydrotetrahydroeupenoxides (83) or (84) containing an epoxyketone chromophore. The absolute configuration of "such epoxide groups in general" could be deduced from the epoxyketone rule.[34b,41] However, hydrogenation would generate two more asymmetric centres, one of which would be α to the ketone.

In most of the cases studied, the epoxyketone rule for n-π* transitions[34b,41] was valid, but some exceptions[96,97] were found if the chiralities



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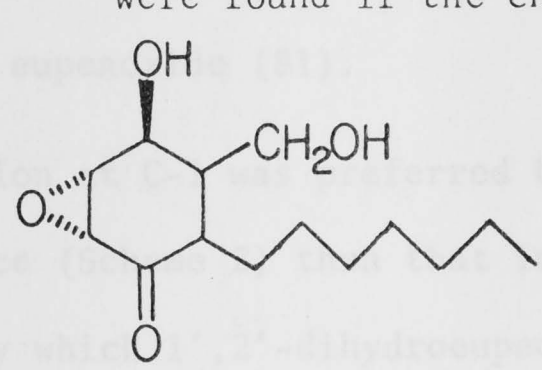


(84)

of the other substituents on the six-membered ring were much larger and their Cotton effects at this transition were of opposite sign to that of the epoxyketones.

The π-π* Cotton effect of the epoxyketone chromophore[96] could probably enable the chirality of the epoxide group to be determined, as the sign of the Cotton effect at this transition is not affected by the contributions from the remainder of the molecule.[96] However, more data is needed in order to establish the generality and limitations of this rule[96]. Therefore complete hydrogenation of the double bonds in eupenoxide (81) was not considered to be a good method for the determination of the absolute

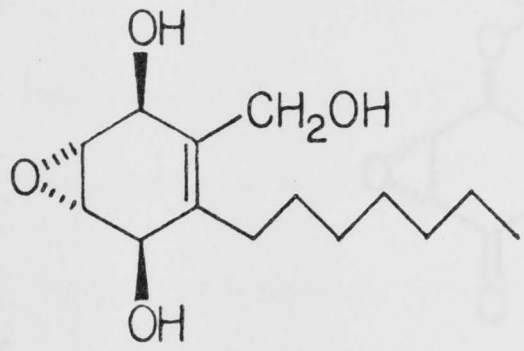
configuration of eupenoxide. Furthermore, hydrogenation of (81) from either the α- or β-face and through 1,2- and 1,4-addition is likely to lead to a mixture of isomers; hence other alternatives for the modification of eupenoxide (81) were considered.

3.4 Modification of Eupenoxide for Chiroptical Studies (i)

Hydrogenation of the heptenyl side-chain and oxidation of the secondary hydroxyl group at C-1 or C-4 of eupenoxide (81) would lead to a compound possessing epoxide ring and double bond substituents bound to a carbonyl group. There are rules from which the chirality of the epoxide ring can be evaluated for compounds having these chromophoric properties. In addition, there are compounds possessing similar chromophores, namely epoxydon (5)[12] and panepoxydon (7)[14], and comparison of the chiroptical properties of the modified eupenoxide with those of (5) and (7) would then lead to the absolute configuration of eupenoxide (81).

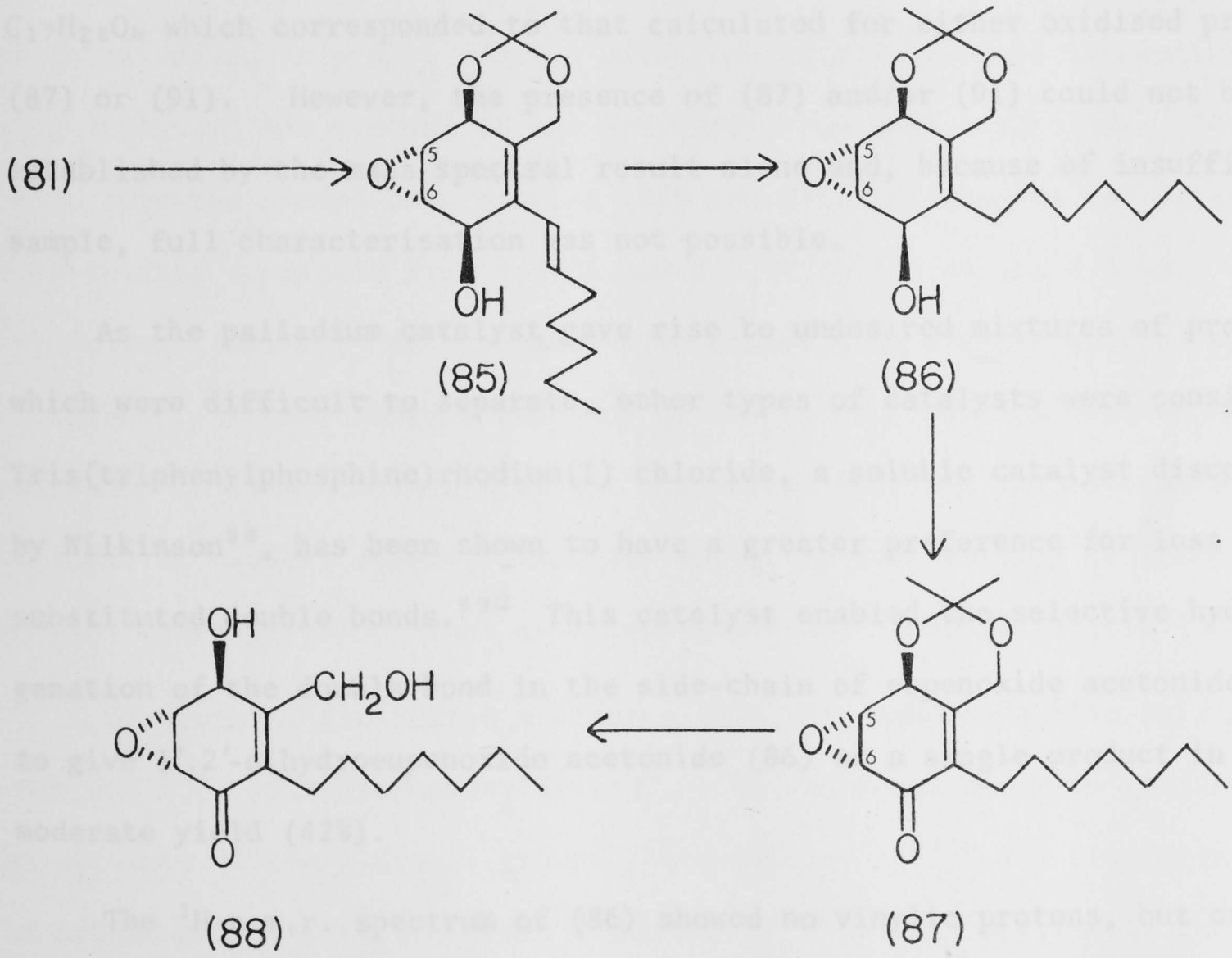
Of the possible modifications, oxidation at C-1 was preferred because it would require a shorter reaction sequence (Scheme 2) than that involving oxidation at C-4. There are two routes by which 1',2'-dihydroeupenoxide acetonide (86) can be prepared. The side-chain of eupenoxide (81) can be hydrogenated followed by the formation of the acetonide or, alternatively, the acetonide could be formed first.

Hydrogenation of the side-chain of eupenoxide (81) using 10% palladium on calcium carbonate gave a low yield of 1',2'-dihydroeupenoxide (89). Extensive decomposition occurred during purification on the silver nitrate impregnated thin layer phase which was necessary to separate the 1',2'-hydrogenated product from the starting material and therefore the initial formation of the acetonide was considered instead.

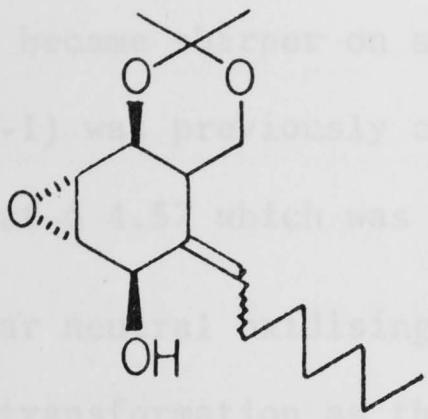


(89)

Using the same catalyst, eupenoxide acetonide (85) was hydrogenated yielding a mixture of the required 1 ,2 -addition product (86) and a 1 ,4 -addition product (90). The ^{1}H n.m.r. spectrum of the crude reaction product showed an additional multiplet at δ 5.8-5.4 indicating the presence of this latter species (90). This multiplet could belong to H-1′ of (90), as the olefinic protons in the starting material resonated at lower field (δ 6.2-6.02) and the 1 ,2 -addition product did not possess any olefinic



Scheme 2



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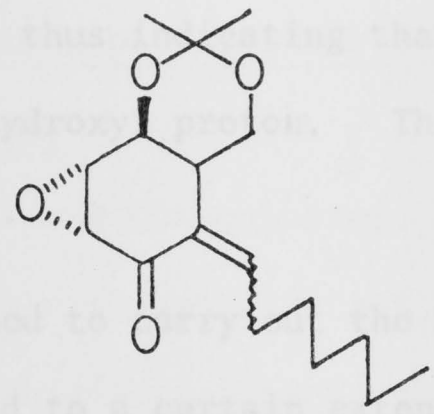


(91)

protons. The mixture could not be separated by the chromatographic methods attempted so, without further purification, it was oxidised with manganese dioxide and purified by preparative t.l.c. to give a fraction (5%) which gave a positive colour test with 2,4-dinitrophenylhydrazine. The mass spectrum of this fraction indicated a molecular formula of $C_{17}H_{28}O_4$ which corresponded to that calculated for either oxidised product (87) or (91). However, the presence of (87) and/or (91) could not be established by the mass spectral result alone and, because of insufficient sample, full characterisation was not possible.

As the palladium catalyst gave rise to undesired mixtures of products which were difficult to separate, other types of catalysts were considered. Tris(triphenylphosphine)rhodium(I) chloride, a soluble catalyst discovered by Wilkinson[98], has been shown to have a greater preference for less substituted double bonds.[99a] This catalyst enabled the selective hydrogenation of the double bond in the side-chain of eupenoxide acetonide (85) to give 1',2'-dihydroeupenoxide acetonide (86) as a single product in moderate yield (42%).

The ^1H n.m.r. spectrum of (86) showed no vinylic protons, but otherwise the spectrum was very similar to that of the starting material with additional saturated methylene intensity. The D_2O exchanged ^1H n.m.r. spectrum allowed assignments of H-1 and H-4. The signal at δ 4.26 was

broad but became sharper on addition of D_2O, thus indicating that this proton (H-1) was previously coupled to the hydroxyl proton. This left a signal at δ 4.57 which was assigned to H-4.

A near neutral oxidising agent was needed to carry out the next chemical transformation as the acetonide, and to a certain extent the epoxide, is labile to acid. Manganese dioxide or buffered pyridinium chlorochromate[100] were possibilities but the latter was chosen because the reaction in general is more rapid,[100] and does not require a large excess of oxidant.[99b,100]

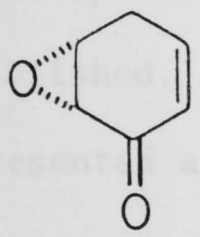
1′,2′-Dihydroeupenoxide acetonide (86) was oxidised with buffered pyridinium chlorochromate to yield the ketone acetonide (87). The ultraviolet spectrum λ_{max} 312, 249 nm (ε 50, 7850) and infrared absorption at 1675 cm^{-1} [101a] confirmed the presence of an α,β-unsaturated ketone group.[89b,101a] The infrared and ^1H n.m.r. spectra were free of any hydroxyl absorption, which indicated the removal of the hydroxyl group upon oxidation. The ^1H n.m.r. spectrum showed both protons (H-5 and H-6) attached to the epoxide group shifted downfield from δ 3.23 and δ 3.38 to δ 3.49 and δ 3.69 respectively, which is in agreement with deshielding effects exerted by the C-1 carbonyl. Long-range W coupling of 0.8 Hz was observed between H-4 and H-6, which is in accordance with the long-range coupling values observed in compounds of closely related structure where the epoxide ring is *trans* to the adjacent hydroxyl group, such as epiepoxydon (48)[37] and terremutin (2)[7,23].

Hydrolysis of the ketone acetonide (87) with aqueous acetic acid gave 1-dehydro-1′,2′-dihydroeupenoxide (88). The infrared spectrum confirmed the presence of the hydroxyl groups (3660-3100 cm^{-1})[101b] and retention of the α,β-unsaturated ketone group (1680 cm^{-1})[101a]. All protons in the ^1H n.m.r. spectrum were in agreement with the structure (88).

3.5 The Chiralities of 1-Dehydro-1′,2′-dihydroeupenoxide acetonide (87) and 1-Dehydro-1′,2′-dihydroeupenoxide (88)

The compounds (87) and (88) possess the same chromophores, an epoxide ring and a double bond on opposite sides of a carbonyl function, in similar chiral environments. Contributions to the chiroptical properties of (87) and (88) arise mainly from the interacting orbitals in the epoxide, ketone and double bond chromophores and the sign of the Cotton effect will be determined by the chiral environment of these chromophores. The influence of chirality at C-4 will be minor relative to the dominant chirality of the epoxide ring which behaves as a part of the chromophore through delocalisation.[41]

The chromophores in (87) and (88) can be envisaged as being comprised of two partial chromophores, an epoxyketone and an enone chromophore. For both partial chromophores, there are rules which link the chirality of the cross-conjugated epoxyketone chromophore with a positive or negative sign of the n-π* Cotton effect.[12,34b] When a molecule possesses a cross-conjugated chromophore of this type it can assume two possible conformations, which are depicted as (92a) and (92b) for the particular case of a compound possessing an epoxide ring with the chirality illustrated in (92). In the first conformation (92a) only the epoxide ring lies below the plane bisecting the C=O bond, and in the second conformation (92b) both the epoxide ring and the double



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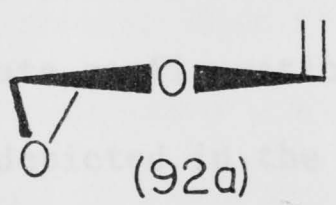
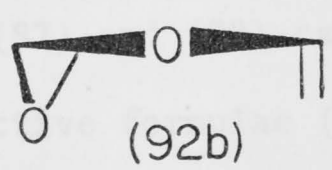


(92a)

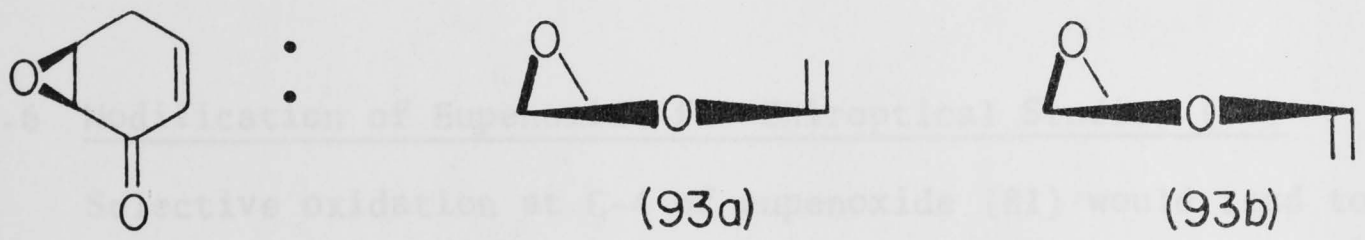


(92b)

bond lie below this plane.[34b] In both conformations (92a) and (92b), the contribution to the sign of the n-π* Cotton effect from the epoxyketone partial chromophore is positive. The enone partial chromophore contributes

positively to the sign of the n-π* Cotton effect in conformation (92a) and negatively in conformation (92b).[34c,102] However, the dominant chromophore is the epoxyketone; therefore the overall sign is controlled by this chromophore and the sign of the resulting Cotton effect will be the same, *i.e.* positive, in both conformations (92a) and (92b).[12,34b]

In the enantiomeric epoxide (93), the two conformations can be depicted as (93a) and (93b). The contribution to the sign of the n-π*



(93) : (93a) (93b)

Cotton effect from the epoxyketone partial chromophore is negative in both conformations (93a) and (93b). The enone partial chromophore again contributes positively and negatively to the sign of the n-π* Cotton effect in (93a) and (93b) respectively.[34c,102] The same argument as above holds and the sign of the resulting n-π* Cotton effect in both conformations (93a) and (93b) will be negative.

In the area of the n-π* absorption both (87) and (88) gave positive Cotton effects in the CD; therefore, from the above rules, the epoxide group in each compound is below the plane bisecting the carbonyl group as depicted in (92). Since the oxygen at C-4 in eupenoxide (81) has been shown to be *trans* to the epoxide group,[88] the chiralities at C-4 in (87) and (88) were also established. Hence, the absolute configuration of (87) and (88) can be represented as (4*S*,5*R*,6*R*), as depicted in the respective formulae (87) and (88).

The molar coefficient of dichroic absorption (Δε) values for both (87) and (88) are relatively small (344 nm, Δε +0.13 and 336 nm, Δε +0.19 respectively) compared to epoxydon (5) (341 nm, Δε +4.70)[12] and panepoxydon (7) (341 nm, Δε -1.81).[14] This could be explained in terms of epoxydon and

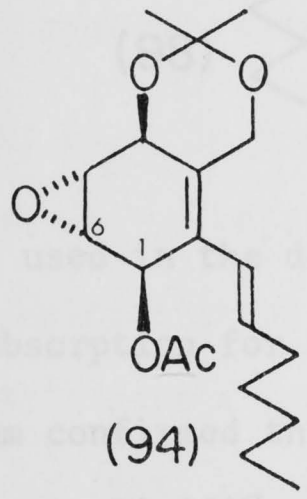
panepoxydon not being perfect models. These compounds not only lack substituents at C-3 but the chiralities at C-4 are also opposite to those of compounds (87) and (88). In addition, the chirality of the side-chain in panepoxydon would also exert some effect on the magnitude of the Cotton effect. Even though the absolute configuration of eupenoxide (81) could be established from these results alone, more evidence was desired to support these results because of the low $\Delta\varepsilon$ values. The next section therefore describes the modification and absolute stereochemistry of another modified eupenoxide.

3.6 Modification of Eupenoxide for Chiroptical Studies (ii)

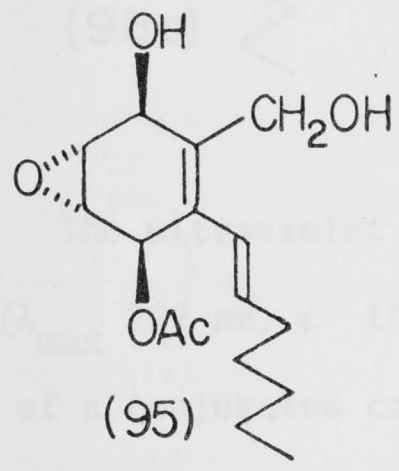
Selective oxidation at C-4 of eupenoxide (81) would lead to a compound possessing an epoxydienone chromophore, which could also be used for the determination of the absolute stereochemistry of eupenoxide (81). Furthermore, it has been observed that compounds possessing chromophores of this type give rise to a considerably larger n-π^* Cotton effect than the corresponding epoxyenones.[14,103]

Selective oxidation of eupenoxide (81) at C-4 required protection of the other secondary and primary hydroxyl groups. Protection of the secondary hydroxyl group was achieved by acetylation of eupenoxide acetonide (85) with acetic anhydride/pyridine to give eupenoxide acetonide acetate (94). The presence of an acetoxyl group in the product was indicated by the absorption at 1740 cm^{-1} in the infrared spectrum.[101c] For simplicity in this discussion, numbering as depicted in (94), rather than the systematic numbering, will be used. The ^1H n.m.r. spectrum confirmed the structure of the product, showing a signal at δ 2.09 corresponding to the acetoxyl group, a downfield shift of H-1 (δ4.59 → 5.83) and an upfield shift of H-6 (δ 3.45 → 3.29) upon acetylation. The downfield shift of 1.24 ppm of H-1 was in agreement with the shift of a proton adjacent to a secondary hydroxyl group,[31b] indicating the acetylation of this hydroxyl group. Acetylation of the C-1 hydroxyl caused an upfield shift of H-6 and enabled the

assignment of the protons attached to the epoxide ring (H-5 and H-6) in the starting material (85). Since the signal at δ3.45 of (85) showed a 0.12 ppm upfield shift upon acetylation of compound (85), this signal corresponded to H-6 and therefore the other signal at δ 3.26 could be assigned to H-5. This shift was caused by the shielding effect of the carbonyl of the acetoxyl group.

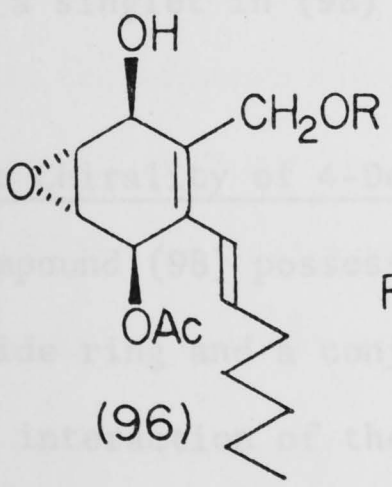


(94)



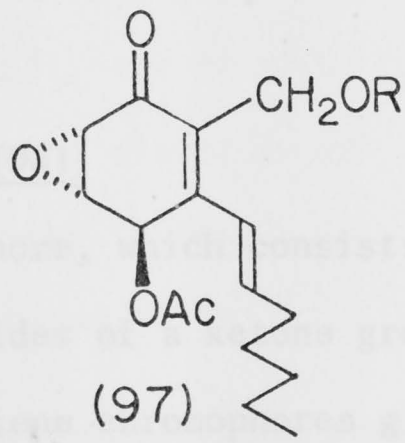
(95)

Deketalisation of (94) with aqueous acetic acid gave eupenoxide-1-acetate (95). The presence of hydroxyl groups was indicated by infrared absorption at 3650-3100 cm^{-1} [101b] and was confirmed by two exchangeable protons in the ^1H n.m.r. spectrum. Other signals in the ^1H n.m.r. spectrum could be assigned to structure (95). Selective protection of the hydroxymethyl group in the diol (95) was then achieved by the formation of a trityl ether (96). The *p',p'*-dimethoxy derivative was chosen rather than trityl itself because it is more easily removed.[104a]



(96)

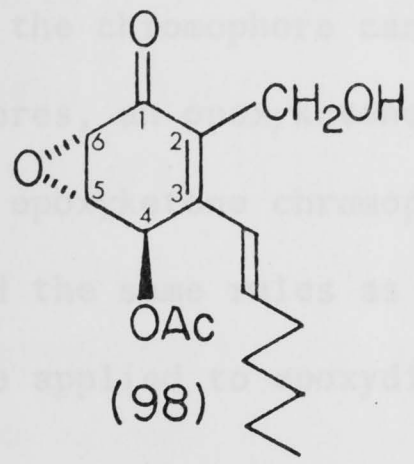
R = *p',p'*-Dimethoxytrityl



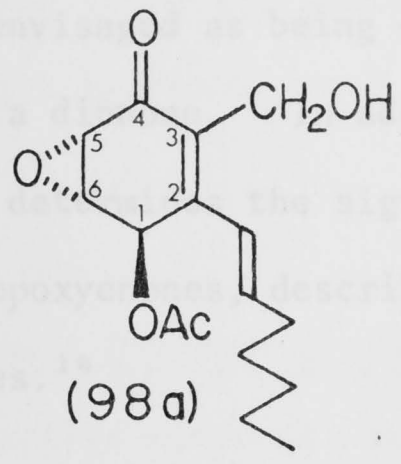
(97)

In view of its instability, the *p',p'*-dimethoxytrityl ether (96) was, without purification, oxidised with manganese dioxide to give (97) which

on hydrolysis with aqueous acetic acid gave (98). For convenience, the numbering system as depicted in (98a), rather than the systematic numbering



(98)



(98a)

in (98), will be used in the discussion. The ultraviolet spectrum showed characteristic absorption for a dienone (λ_{max} 292 nm, ε 16000).[89b] The infrared spectrum confirmed the presence of a conjugated carbonyl group by an absorption at 1668 cm^{-1},[101a] and indicated the presence of hydroxyl (3620-3240 cm^{-1})[101b] and acetoxyl (1745 cm^{-1}) functions. In the ^1H n.m.r. spectrum of (98a), the hydroxyl group was confirmed by the presence of an exchangeable proton and the acetoxyl group by a methyl singlet at δ 2.11. The long-range W coupling of 0.8 Hz between H-1 and H-5 indicating the *trans* relationship between the epoxide ring and the substituent at C-1, was again observed. The downfield shifts of H-5 (0.33 ppm) and of H-6 (0.11 ppm) indicated that the oxidation had occurred at C-4. This was confirmed by the collapse of the AB quartet observed for the hydroxymethyl signals in (95) to a singlet in (98) due to the destruction of the chirality at C-4.

3.7 The Chirality of 4-Dehydroeupenoxide-1-acetate (98)

Compound (98) possesses an epoxydienone chromophore, which consists of an epoxide ring and a conjugated diene on opposite sides of a ketone group. Orbital interaction of the epoxide, ketone and the diene chromophores gives rise to the chiroptical properties of (98). The sign of the Cotton effect will be determined by the chiral environment of these chromophores. The influence of the chirality at C-4 will be minor relative to the dominant

chirality of the epoxide which becomes a part of the chromophore through delocalisation.[41]

Here again, the chromophore can be envisaged as being composed of two partial chromophores, an epoxyketone and a dienone. In addition, the chirality of the epoxyketone chromophore determines the sign of the n-π* Cotton effect and the same rules as for epoxyenones, described in (92) and (93), can also be applied to epoxydienones.[14]

Compound (98) gave, in the area of the n-π* absorption, a negative Cotton effect (344 nm, Δε -0.71) and from the rules described, it can be deduced that the epoxide group in (98) has the configuration depicted in (93). Isopanepoxydon (8)[14] gave a positive Cotton effect for the n-π* transition, opposite to that of (98), suggesting the opposite chiralities for the epoxide groups in the two compounds. This result is in agreement with that deduced from the formulae (92) and (93). As the secondary hydroxyl groups in eupenoxide (81) are *trans* to the epoxide ring,[88] the carbon carrying the acetoxyl function in (98) has the *R* configuration, compound (98) can then be described as (4*R*,5*S*,6*S*)-4-acetoxy-3-[(*E*)-hept-1′-enyl]-2-hydroxymethyl-5,6-epoxycyclohex-2-en-1-one.

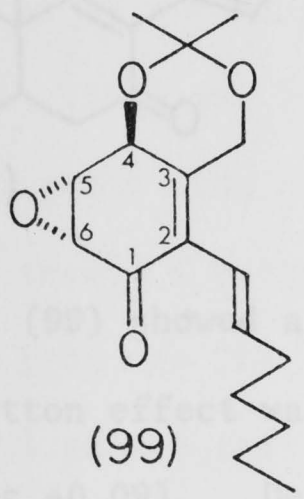
The results from the studies of compounds (87), (88) and (98) all led to the conclusion that the epoxide group in eupenoxide (81) had the configuration as depicted in the eupenoxide formula. Previous work has shown that the secondary hydroxyl groups in eupenoxide (81) are of opposite chiralities[88] (§ 2.4). On the basis of the foregoing, the structure of eupenoxide can be designated as (1*R*,4*S*,5*R*,6*S*)-2-[(*E*)-hept-1′-enyl]-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol, as depicted in (81).

Although the Cotton effect of (98) was approximately 3-4 times those of (87) and (88), it was still small compared to isopanepoxydon (8) which has a Δε value of +5.04.[14] However, isopanepoxydon (8) could not be

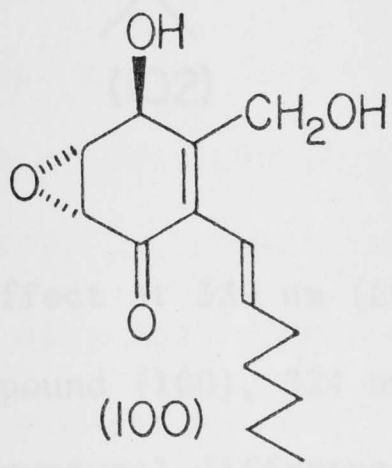
considered as a perfect model for correlation of optical properties with compound (98) because it lacked the substituent at C-2 and the chirality at C-4 is opposite to that of (98). In addition, the substituents at C-4 in both compounds are different, being a hydroxyl group in (8) and an acetoxyl group in (98).

3.8 1-Dehydroeupenoxide acetonide (99) and 1-Dehydroeupenoxide (100)

The reactivity of many biologically active compounds such as epoxydon (5) and phyllostine (4), are ascribable at least partly to an α,β-unsaturated ketone.[55,58] α,β-Unsaturated ketones are able to react with various nucleophiles; for example, the mercapto groups of proteins and amino acids, by 1,4-addition processes. For this reason, compounds (99) and (100) were prepared for biological studies. However, the lability of these compounds did not permit biological testing to be carried out.



(99)

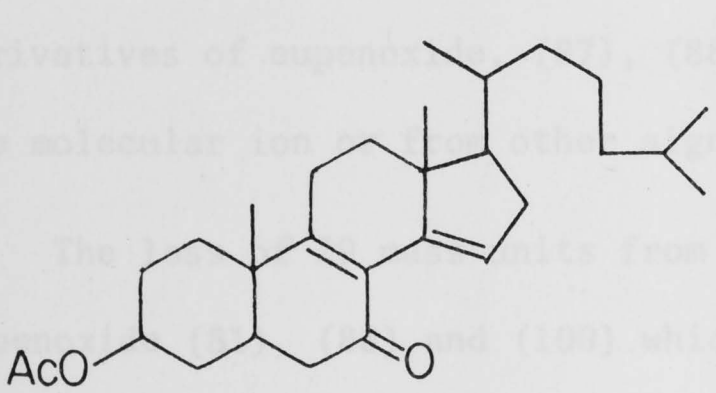


(100)

Oxidation of compound (85) by manganese dioxide gave compound (99). The presence of the α,β-unsaturated ketone function in compound (99) was indicated by the infrared absorption at 1680 cm^{-1}.[101a] The ^1H n.m.r. spectrum showed a downfield shift of the protons attached to the epoxide ring, H-5 (δ 3.26 → 3.50) and H-6 (δ 3.45 → 3.70) indicative of oxidation at C-1. Other signals in the ^1H n.m.r. spectrum could be assigned to structure (99).

Acid hydrolysis of the acetonide (99) by aqueous acetic acid gave the diol (100). The presence of hydroxyl and α,β-unsaturated ketone groups was indicated by the infrared absorptions at 3600-3100 cm^{-1} [101b] and 1680 cm^{-1} [101a] respectively. Two exchangeable protons in the ^1H n.m.r. spectrum of (100) confirmed the presence of the hydroxyl groups. Other signals in the ^1H n.m.r. spectrum of this compound could be assigned to the structure (100).

The ultraviolet absorptions λ_{max} 273, 216.5 nm (ε 4260, 12200) for compound (99) and λ_{max} 266 sh, 213 nm (ε 4160, 14000) for compound (100) are consistent with the similar chromophores in the compounds (101) and (102). Compounds (101) and (102) showed ultraviolet absorptions, λ_{max} 298, 224 nm (ε 5060, 15600) and λ_{max} 284 nm (ε 6310) respectively.[89c]



(101)

(102)

Compound (99) showed a negative Cotton effect at 330 nm (Δε -0.15) but a positive Cotton effect was observed for compound (100), 324 nm (Δε +0.12) and 342 nm (Δε +0.09). Despite the small structural differences between compounds (99) and (100), these compounds showed opposite Cotton effects indicating that the use of cross-conjugated systems of this type for the prediction of absolute stereochemistry is hazardous.

3.9 Some Mass Spectral Characteristics of Eupenoxide and its Derivatives

Eupenoxide (81) and derivatives (85)-(89), (94), (95), (98)-(100), showed considerably complex EI mass spectra. However, high resolution mass measurement and the presence of some metastable ions in the mass

spectra enabled some generalisations to be made for the mass spectral characteristics of these compounds.

The loss of water[105a,106a] from the molecular ion was observed in eupenoxide (81) and the non-acetonide derivatives carrying hydroxyl groups (88), (89), (95), (98) and (100). The acetonide derivatives carrying a hydroxyl group (85) and (86) showed a hydroxyl loss[105a,106a] from the $M^+-CH_3COCH_3$ ion. Losses of acetic acid[105b] and/or ketene[106b] from the molecular ions or major ions were observed in compounds carrying acetoxyl functions (94), (95) and (98).

α-Fission, giving rise to an M^+-CH_3 ion[105c] and the loss of acetone from the molecular ion, was always evident in the mass spectra of the acetonide derivatives (85)-(87), (94) and (99). For the more oxidised derivatives of eupenoxide, (87), (88), (98)-(100), the loss of CO[105d] from the molecular ion or from other significant ions was always evident.

The loss of 29 mass units from the molecular ion was observed in eupenoxide (81), (88) and (100) which could have arisen from the fission of an ethyl fragment from the alkyl side-chain or from the loss of CHO. Compound (98) showed a metastable ion at *m/z* 179.6 which indicated that *m/z* 205 arose from *m/z* 234. High resolution mass measurement of ions at *m/z* 234 and 205 showed that the loss of 29 mass units was due to the loss of CHO. Formyl loss from compounds possessing an epoxide group had been observed in other systems.[105e] By analogy, the loss of 29 mass units from the molecular ion of (81), (88) and (100) could also arise from alkyl rearrangement of the epoxide group.

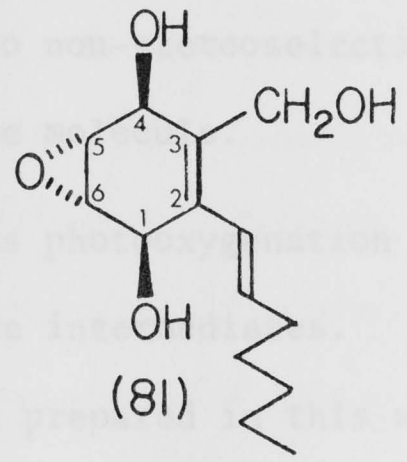
Lastly, eupenoxide (81) and some derivatives, (87) and (100), each showed a loss of oxygen from the molecular ion which is common amongst compounds carrying an epoxide group.[106c]

CHAPTER 4

SYNTHESIS OF EUPENOXIDE — CYCLOADDITION REACTIONS

4.1 Introduction

Although microbial production is probably the most convenient source of eupenoxide (81), a total synthesis, in addition to being a challenge in itself, would provide structural confirmation and would also enable the preparation of stereoisomers and analogues. Moreover, a successful synthesis of eupenoxide could possibly be extended to the synthesis of other cyclohexene oxide antibiotics.



(81)

Eupenoxide is a highly oxygenated compound with complex stereochemistry. The stereochemical requirements are that the epoxide ring be *trans* to the hydroxyl groups and an *E* double bond be present in the side-chain. The most difficult aspect of a synthetic approach to eupenoxide would be the stereoselective introduction of the oxygen functionalities. Since the intermediates in the synthesis of a polyoxygenated molecule such as eupenoxide could undergo facile isomerisation and aromatisation, the later steps in the synthesis would need to be carried out under mild conditions.

Reported methods for the syntheses of cyclohexene oxide metabolites to date have been described in § 1.5. The key reactions carried out were the direct epoxidation of 1,4-benzoquinone intermediates[37-39,61-64] or the photooxygenation of cyclic diene derivatives.[67,71,72]

In the first process, the formation of a hydroxyl group *trans* to the epoxide ring was achieved[62] by utilising the bulkiness of the dimethylfulvene protecting group. The addition of hydride from the least hindered side of the benzoquinone oxide ring resulted in the hydroxyl group being formed *trans* to the epoxide ring. In principle, this steric hindrance caused by the fulvene moiety could be used for the stereoselective formation of hydroxyl groups *trans* to an epoxide ring in the synthesis of eupenoxide (81). However, this method could not be applied to the synthesis of eupenoxide (81) because, after the reduction of the carbonyl groups, the side-chain could not be introduced by alkylation or aldol condensation. Moreover, the introduction of the side-chains prior to the reduction of the carbonyl group could lead to non-stereoselective reduction due to steric hindrance from both sides of the molecule.

The second process involves photooxygenation of cyclic diene derivatives to give endoperoxide intermediates. Senepoxide (13)[67] and crotepoxide (15)[63,68] have been prepared in this way. An endoperoxide intermediate could provide the required *cis* stereochemistry for the hydroxyl groups in eupenoxide (81) through the reductive opening of the peroxide linkage[68]. This route, although attractive, has its disadvantages because the synthesis of the required diene would need a rather long synthetic sequence, some stages of which could be difficult to achieve.

4.2 Synthetic Plan for Eupenoxide

A synthesis which could provide full stereochemical control of all asymmetric centres in eupenoxide (81) and would allow facile variation of the side-chain in a later reaction sequence was desirable. In addition, a synthetic route which could easily be extended to the syntheses of other cyclohexene oxide antibiotics and related compounds would be an advantage.

A Diels-Alder reaction, as well as providing the six-membered ring nucleus by a (4+2) cycloaddition[107-110,111a], would also allow full stereochemical control of the substituents of C-1 and C-4 in eupenoxide by the *cis* principle of addition[107-110,111a]. These substituents would provide stereochemical control for the subsequent epoxidation reaction[99d,114b,115b].

The double bond which is to be oxidised later to an epoxide could be generated by cycloaddition of a 1,4-dioxygenated-1,3-diene with a dienophile. Using an acetylenic dienophile, the tetrasubstituted double bond required in eupenoxide (81) could be introduced directly by the cycloaddition reaction. A hydroxymethyl and a functional group which would allow the extension of the unsaturated side-chain were required as substituents on the acetylene. An aldehyde was selected as the functional group for various reasons. Firstly, it would make the acetylene a stronger dienophile,[107,110,111a] thus permitting the cycloaddition reaction to be carried out under conditions in which the product was stable. Secondly, it would allow the extension of the side-chain. Thirdly, it would reduce the reactivity of the resulting tetrasubstituted double bond towards peroxy acid oxidants,[114a,115a] thereby providing the regioselectivity for the epoxidation reaction.

The development of the alkyl side-chain in a later reaction sequence would be advantageous for the following reasons. If the side-chain were to be introduced in the cycloaddition reaction itself, (*E*)-dec-4-en-2-ynal would be required as a dienophile for the synthesis of eupenoxide (81), and each analogue would require a separate synthesis starting with the preparation of a dienophile. Analogues could not then be produced readily from a common intermediate. In addition, it has been observed that sp^2 hybridised carbons γ,δ to a carbonyl compete with α,β-unsaturated sp hybridised carbons in cycloaddition processes,[116] and thus the cycloaddition of a substituted (*E*)-but-4-en-2-ynal may not give rise to the required intermediate for the synthesis

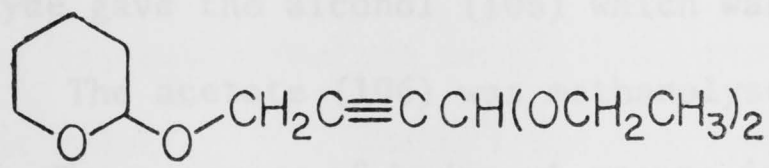
of eupenoxide (81) or its analogues. The problem could possibly be overcome by masking the sp^2 carbons as sp carbons, that is by using dienophiles possessing an α,β,γ,δ-diynal system. However, this method suffers from the disadvantage of difficult alteration of the side-chain, and requires a lengthy synthetic sequence. The resulting triple bond in the side-chain would require hydrogenation to give a double bond, followed by isomerisation to give the *E* stereochemistry.

4.3 Preparation of the Dienophiles

It was necessary to prepare two dienophiles because the dienophile prepared initially did not give the required cyclohexa-1,4-diene intermediate from the cycloaddition reaction.

An acetylenic aldehyde possessing a suitably protected hydroxymethyl group was required in the synthesis of eupenoxide (81). Some methods for the preparation of α,β-acetylenic aldehydes have been reported in the literature.[111b,117,118a,119] Most of these methods involve the hydrolysis of acetylenic acetals,[111b,117,118a] although an acetylenic immonium salt has also been hydrolysed to yield an acetylenic aldehyde.[118b] It is also possible to obtain acetylenic aldehydes by oxidation of acetylenic alcohols.[117,119]

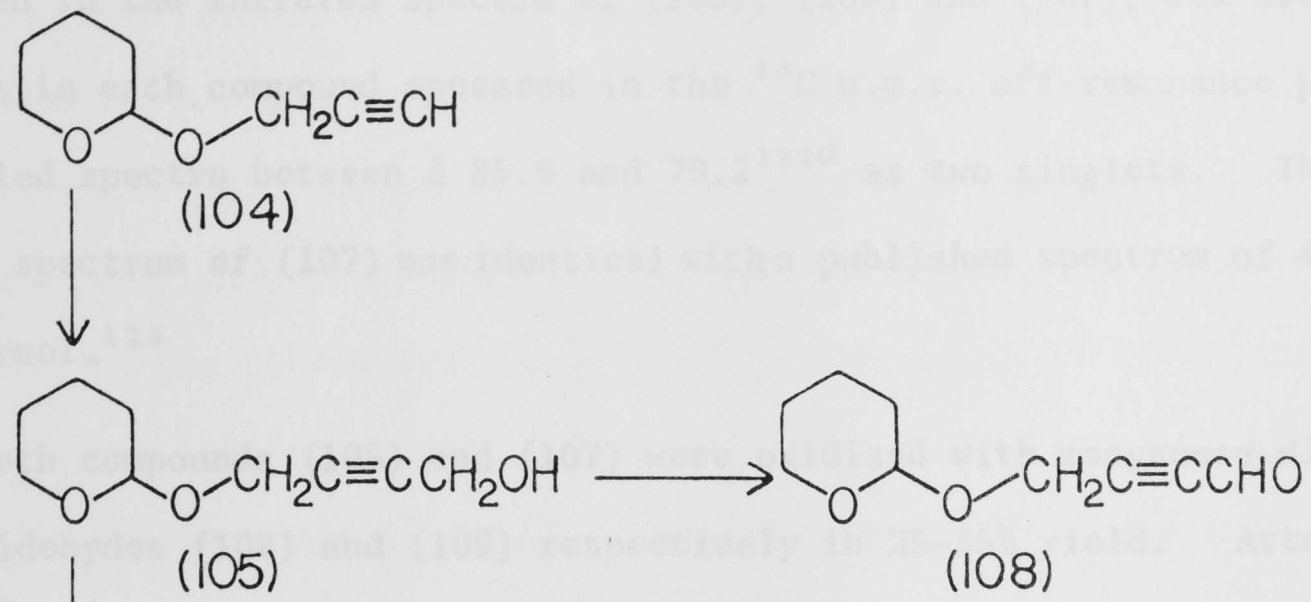
Initially, preparation of the required acetylenic aldehyde (108) was attempted by the hydrolysis of the diethyl acetal (103) which was prepared

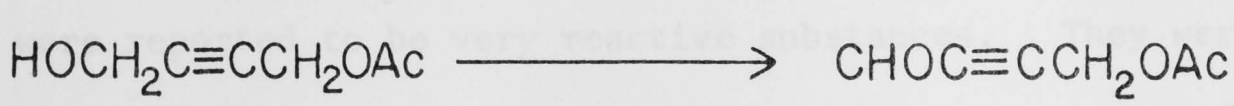


(103)

from propargyl alcohol by a known procedure.[120] Hydrolysis of (103) with 50% aqueous acetic acid yielded a mixture of products. This showed that the hydrolysis of the diethyl acetal did not proceed selectively.

The complication involving the hydrolysis of the diethyl acetal (103) was avoided by preparing the acetylenic aldehydes (108) and (109) by oxidation





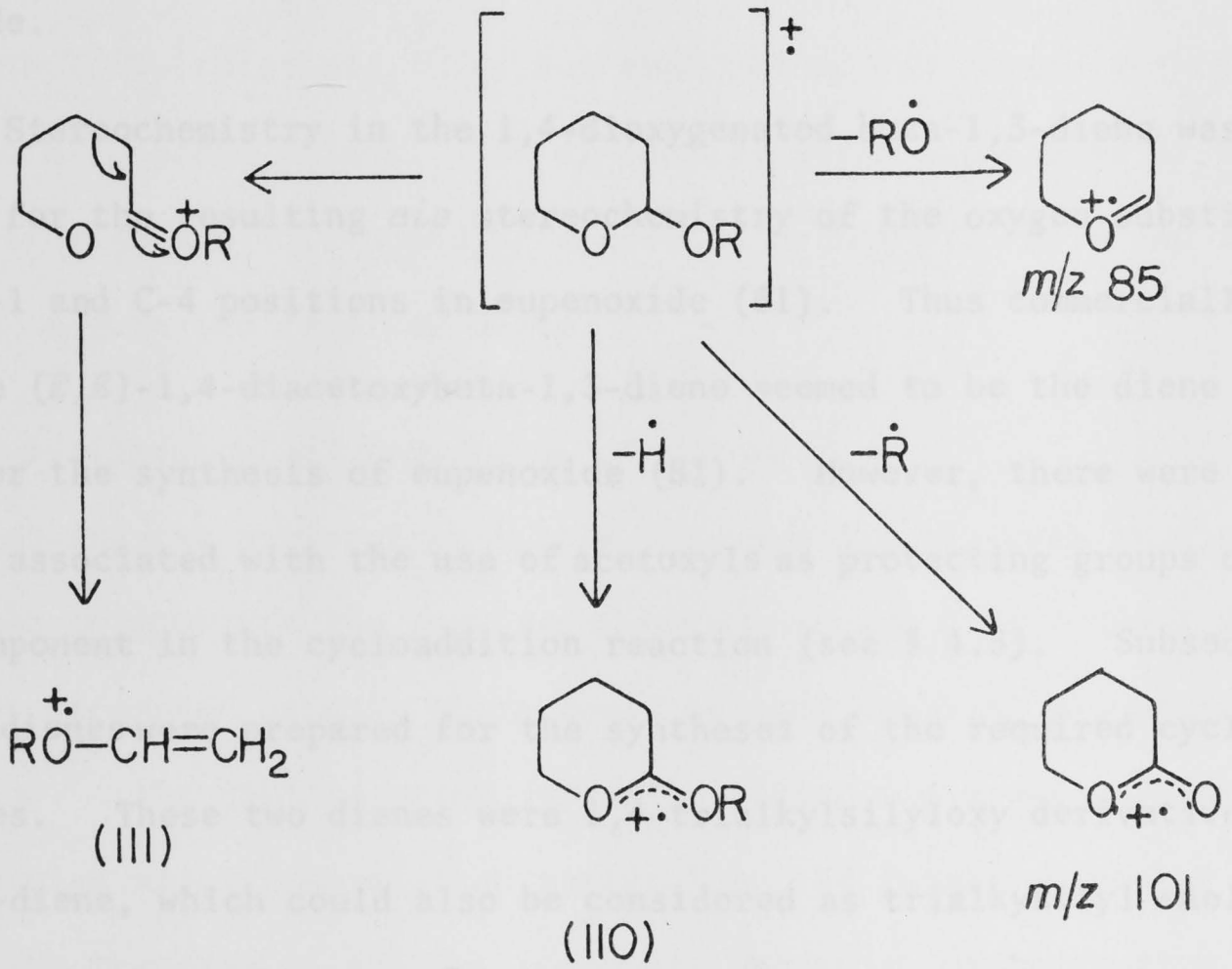
of the appropriate acetylenic alcohols. Starting from propargyl alcohol, the hydroxy group was protected by an addition reaction with 2,3-dihydropyran to give the tetrahydropyran-2-yl derivative (104).[120] Condensation of (104) with formaldehyde gave the alcohol (105) which was acetylated to give the acetate (106). The acetate (106) was methanolysed to yield the hydroxy acetate (107). The presence of hydroxyl groups in (105) and (107) was indicated by the infrared absorptions at 3700-3030 cm^{-1} [101b] and the exchangeable protons in the ^1H n.m.r. spectra. The infrared spectra of (106) and (107) also indicated the presence of acetoxyl groups,[101c] which was confirmed by the resonance at δ 2.06 in the ^1H n.m.r. spectra. Although typical triple bond absorption, *i.e.* bands between 2260-2190 cm^{-1}, was not

observed in the infrared spectra of (105), (106) and (107), the acetylenic carbons in each compound appeared in the ^{13}C n.m.r. off-resonance proton decoupled spectra between δ 85.6 and 79.2[122a] as two singlets. The ^{1}H n.m.r. spectrum of (107) was identical with a published spectrum of 4-acetoxy-but-2-ynol.[123]

Both compounds (105) and (107) were oxidised with manganese dioxide to give aldehydes (108) and (109) respectively in 25-35% yield. Attempted oxidation of (107) with pyridinium chlorochromate or nickel peroxide led to extensive decomposition and very little aldehyde was detected. The instability of the acetylenic aldehydes (108) and (109) could be one of the reasons for the low yields obtained from the oxidation of the alcohols (105) and (107). Both aldehydes (108) and (109) were colourless liquids which turned slightly yellowish-brown on contact with air and were, in general, used immediately after purification for the cycloaddition reactions. 1-Hydroxyalk--2-yn-4-ones were reported to be very reactive substances. They were found to be sensitive to light, heat and air, and remained unchanged only for a short period of time when stored in the dark at temperatures below -20°.[124]

Both compounds (108) and (109) exhibited two absorptions in the infrared region due to the triple bond: 2240(w), 2200(s) for (108) and 2270(w), 2200(s) cm^{-1} for (109), in agreement with those reported for acetylenic compounds.[101d] In many cases dialkyl alkynes show a weak band followed by a stronger band at lower frequency for the triple bond stretching frequencies, due to a Fermi-resonance effect.[101d] The ultraviolet spectra of the compounds (108) and (109) showed absorptions for both n-π* (around 330 nm) and π-π* (around 215 and 265 nm) transitions which were in accordance with the reported absorptions of α,β-acetylenic aldehydes and ketones.[125] In each compound (108) and (109), the aldehyde function was clearly detected in the ^{1}H n.m.r. spectrum as a low field singlet in the vicinity of δ 9.2, and in the ^{13}C n.m.r. spectrum as a low field proton coupled doublet in the vicinity of δ 176.[122b]

Compounds (105), (106) and (108) exhibited very similar mass spectral fragmentation patterns, characteristic of compounds carrying the tetrahydropyranyl ether function. Molecular ions were not observed in (105), (106) or (108), but (105) and (108) showed the loss of the anomeric hydrogen, belonging to the tetrahydropyranyl ether, to form ion (110)[105f] [*m/z* 169 for (105) and *m/z* 167 for (108)]. Compound (106) showed the loss of the anomeric hydrogen from M^+-COCH_3. The ions *m/z* 85[105f] and *m/z* 101[105f], typical of the tetrahydropyranyl ethers, were observed for all three



compounds. The ions *m/z* 112 and 110 corresponding to the type (111)[105f] were observed in compounds (105) and (108) respectively.

The mass spectra of compounds (107) and (109) showed molecular ions at *m/z* 128 and 126 respectively, as well as base peaks at *m/z* 43 which indicated the presence of the acetoxyl group.[105b,106b] Ion *m/z* 97 in the mass spectra of (107) and (109), corresponding to M^+-CH_2OH and M^+-CHO respectively,

indicated the presence of hydroxymethyl and formyl groups in the respective compounds.

4.4 Preparation of The Dienes

Introduction

In our preliminary investigation of the approach to the synthesis of eupenoxide, eupenoxide triacetate was found to be smoothly deacetylated by methanolic ammonia to give eupenoxide (81). Therefore acetate was considered to be a protecting group of potential use in the synthesis of eupenoxide.

E,E Stereochemistry in the 1,4-dioxygenated buta-1,3-diene was required for the resulting *cis* stereochemistry of the oxygen substituents at the C-1 and C-4 positions in eupenoxide (81). Thus commercially available (*E,E*)-1,4-diacetoxybuta-1,3-diene seemed to be the diene of choice for the synthesis of eupenoxide (81). However, there were some problems associated with the use of acetoxyls as protecting groups on the diene component in the cycloaddition reaction (see § 4.5). Subsequently two more dienes were prepared for the syntheses of the required cyclohexa-1,4-dienes. These two dienes were 1,4-trialkylsilyloxy derivatives of buta-1,3-diene, which could also be considered as trialkysilyl enol ethers.

Reported Preparations of Trialkylsilyl Enol Ethers

Several methods have been used in the preparation of trialkylsilyl enol ethers.[126,127-136] In general, trialkylsilyl enol ethers are prepared from ketones or aldehydes, either directly or through the enolates.[127-136] The direct methods employ the treatment of carbonyl compounds with a mixture of trimethylsilyl chloride and triethylamine in the presence of zinc chloride[131] or in the presence of dimethylformamide.[132] Some oxygenated buta-1,3-dienes have been prepared by this method. These include

(1*E*)-1-methoxy-3-trimethylsilyloxybuta-1,3-diene[133] and 2-trimethylsilyloxybuta-1,3-diene[134,135] which were prepared directly from the corresponding ketones (*E*)-4-methoxybut-3-en-2-one and but-3-en-2-one respectively. Treatment of enolate anions with a silylating agent, such as trimethylsilyl chloride or *t*-butyldimethylsilyl chloride, has been widely used in the preparation of silyl enol ethers.[127-130] Two derivatives of buta-1,3-diene, 2-trimethylsilyloxybuta-1,3-diene and (*E*,*E*)-1,4-bis(trimethylsilylthio)-buta-1,3-diene have been prepared in this way. But-3-en-2-one was deprotonated with lithium diisopropylamide and silylated with trimethylsilyl chloride to give 2-trimethylsilyloxybuta-1,3-diene.[135] The preparation of (*E*,*E*)-1,4-bis(trimethylsilylthio)buta-1,3-diene employed a less common method for generation of enolate anions. The di-enolate anion was formed by nucleophilic attack on the corresponding diacetate by methyllithium and then silylated to give the required diene.[136]

(E,E)-1,4-bis(Trialkylsilyloxy)buta-1,3-dienes

Concerning the preparation of the required dienes, even though it may be possible to generate the required di-enolate anion from butan-1,4-dial, it was not attempted because there could be some problems associated with this method. The enolate anion initially formed could undergo inter- and/or intramolecular aldol condensation faster than the formation of the second anion. The required di-enolate anion was therefore prepared from (*E*,*E*)-1,4-diacetoxybuta-1,3-diene (112). Generation of the di-enolate anion from (112) with methyllithium followed by silylation with either trimethylsilyl chloride or *t*-butyldimethylsilyl chloride furnished the required product (113) or (114).

(112)

(113)

(114)

The diene (113) decomposed immediately on contact with moisture, thus making the preparation and manipulation of this compound cumbersome.

The olefinic protons in all three dienes (112), (113) and (114), exhibited AA'XX' spin systems in their ^{1}H n.m.r. spectra. The AA'XX' spin system arises from four magnetically non-equivalent nuclei of which there are two sets of symmetrically equivalent nuclei. Also the chemical shift difference between these two sets of symmetrically equivalent nuclei is large compared with the coupling constants.[121b] The AA'XX' spin system indicated that each double bond had the same stereochemistry. *Z,E* Stereochemistry could not give rise to the two sets of symmetrically equivalent protons required for the AA'XX' spin system. The double bonds were confirmed to have the same stereochemistry by ^{13}C n.m.r. spectroscopy. The ^{13}C n.m.r. spectra of (112) and (113) each showed two resonances for the olefinic carbons, indicating the presence of two sets of symmetrically equivalent carbons.

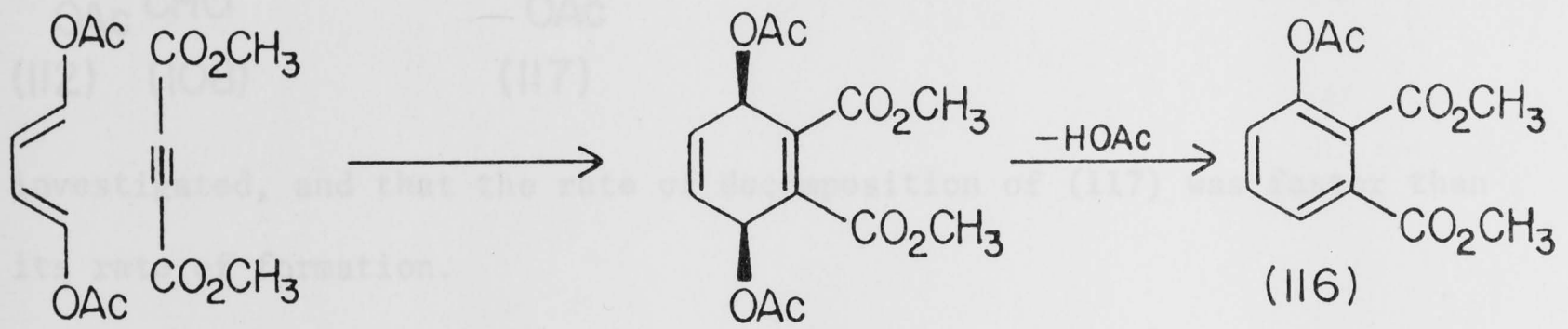
The mass spectra of both (113) and (114) showed very little fragmentation upon electron impact. Each spectrum showed two strong ions, a molecular ion and an ion at *m/z* 73, in addition to a weaker ion at *m/z* 75. Djerassi *et al*[105i] showed that the ions at *m/z* 75 and 73 in the mass spectra of trimethylsilyl ethers corresponded to ions $\overset{+}{H}O{=}Si(CH_3)_2$ and $\overset{+}{S}i(CH_3)_3$ respectively. From the mass spectral studies of several compounds containing *t*-butyldimethylsilyl ethers, Phillipou[137] showed that the trimethylsilyl cation in the mass spectra of compounds possessing *t*-butyldimethylsilyl ether functions was formed by methyl migration upon electron impact.

4.5 Cycloaddition Reactions

(E,E)-1,4-Diacetoxybuta-1,3-diene

Diels-Alder cycloadditions of (*E,E*)-1,4-diacetoxybuta-1,3-diene (112) have been widely investigated using electrophilic olefins and, to a much

lesser extent, electrophilic acetylenes.[138-141] Only three acetylenic dienophiles, propiolic acid[141], ethyl propiolate[141] and dimethyl acetylenedicarboxylate[139-141] have been studied in connection with (112). Of the three acetylenic dienophiles, only dimethyl acetylenedicarboxylate reacted cleanly with (112) to give a cyclohexa-1,4-diene (115) which under more severe conditions eliminated acetic acid to yield an aromatic compound (116).[140,141] This result clearly demonstrated that dimethyl acetylenedicarboxylate, which has two electron-withdrawing substituents on the acetylenic carbons, is more electrophilic in character in comparison to propiolic acid and ethyl propiolate. It has been shown that, in general,



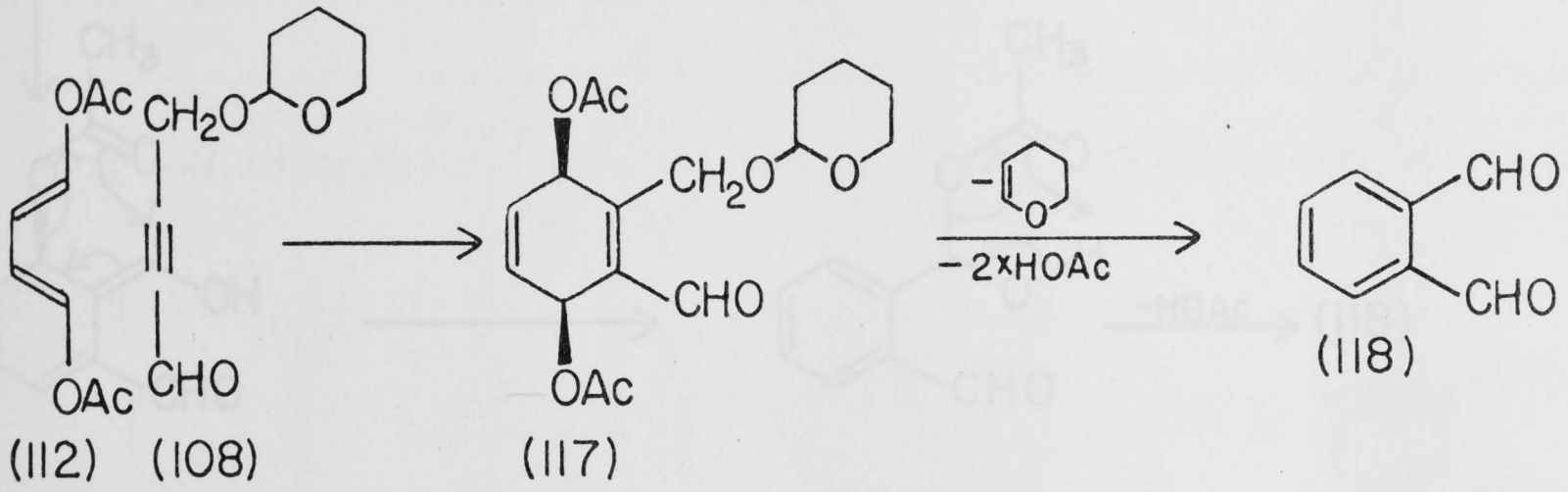
(112) (115) (116)

the reactivity of the dienophile increases with the electron-withdrawing ability of the substituents.[107,110,111a]

Dudkowski and Becker[112] measured the rates of cycloaddition of phenylacetylenes of the general formula C_6H_5-C≡C-R with tetracyclone and the following relative rates were observed: CH_3, 1; CH_2OH, 2; CO_2CH_3, 6.7; CHO, 8.9. In view of the higher reactivity conferred by the aldehyde substituent, the reactivity of the dienophile (108) would not be expected to differ greatly from that of dimethyl acetylenedicarboxylate.

The reaction of (*E*,*E*)-1,4-diacetoxybuta-1,3-diene (112) and the dienophile (108) in refluxing xylene under an atmosphere of nitrogen was attempted. A large percentage of starting materials still remained after 4 days and a low yield of a product was isolated. The product was shown to be identical to *o*-phthalaldehyde by t.l.c. examination and ultraviolet

and mass spectra. The ^1H n.m.r. spectrum of the product showed signals identical to those of *o*-phthalaldehyde in the aromatic and aldehydic proton regions, but some low intensity signals were also observed in the high field region indicating the presence of a small amount of impurity. The required cyclohexa-1,4-diene (117) was not detectable. The result indicated that the cycloaddition had occurred rather slowly under the conditions

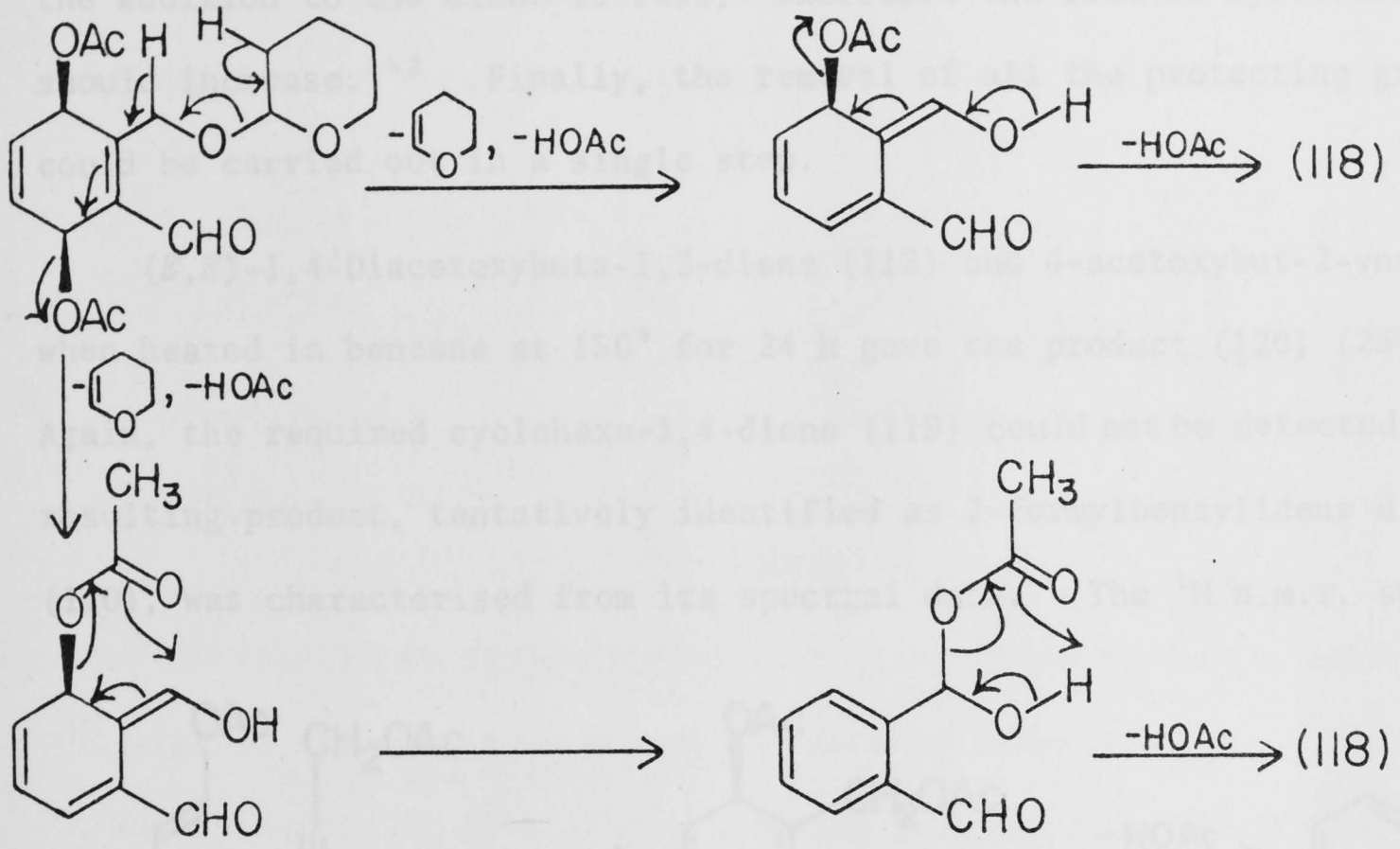


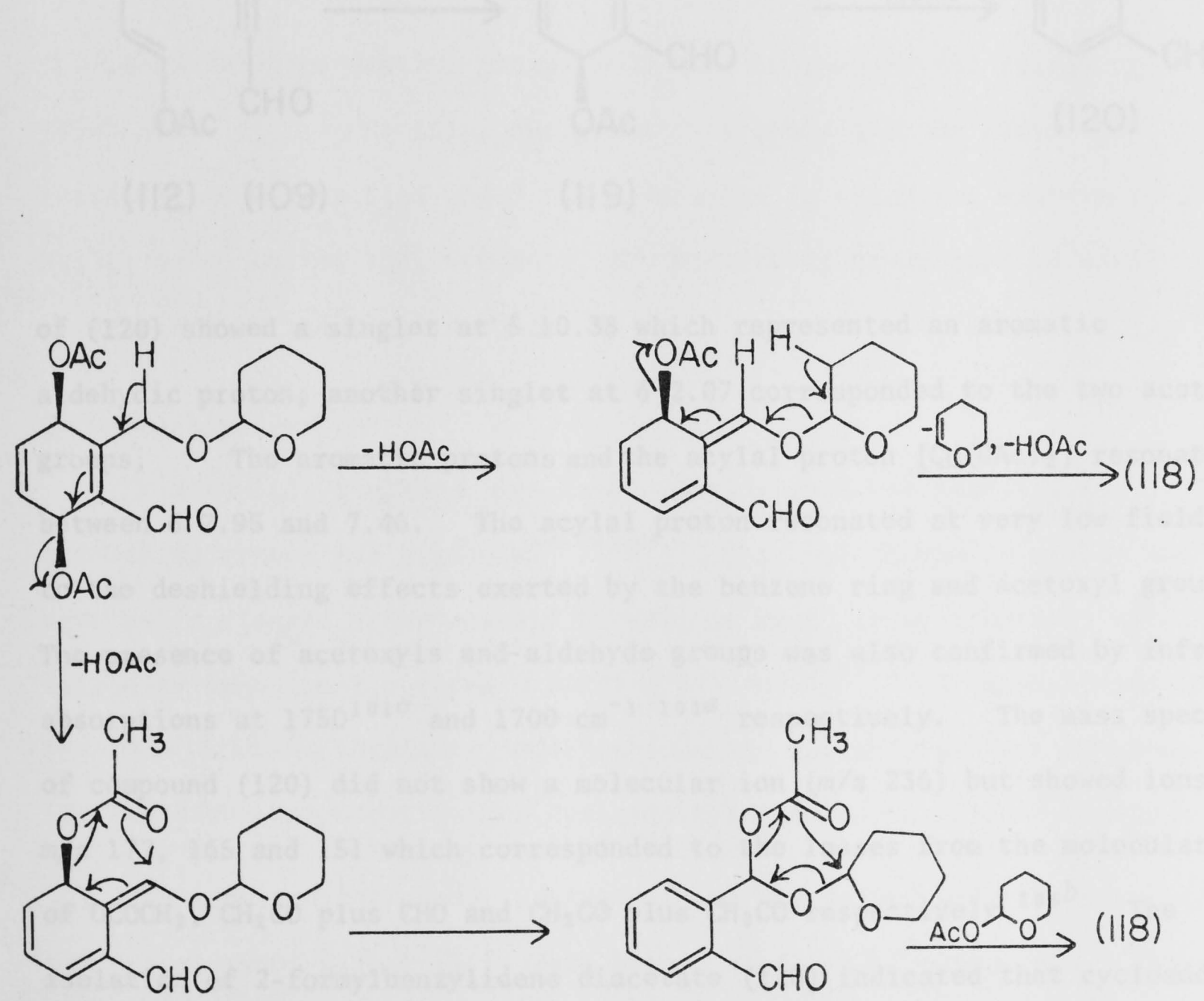
investigated, and that the rate of decomposition of (117) was faster than its rate of formation.

The elimination of dihydropyran and two equivalents of acetic acid from (117) is required for the formation of (118). It cannot be predicted from the result whether it was the dihydropyran or the acetic acid which eliminated first. Two of the possible elimination processes are shown in Scheme 3.

If the dihydropyran eliminated first, alteration of the protecting group in the dienophile to a functional group of greater resistance to elimination would be necessary. However, if acetic acid eliminated first, a diene with poorer leaving groups than the acetoxyl group would have to be used.

Initially a modification was carried out on the dienophile whereby the tetrahydropyranyl ether group was replaced with an acetoxyl group for the following reasons. Firstly, the acetate may be less likely to undergo thermal elimination than the tetrahydropyranyl ether. Secondly, since the acetoxyl is an electron-withdrawing group, it should increase the reactivity of the dienophile.[112,113] Thirdly, the acetoxyl group is considerably smaller than the tetrahydropyranyl ether group, consequently the steric requirement in

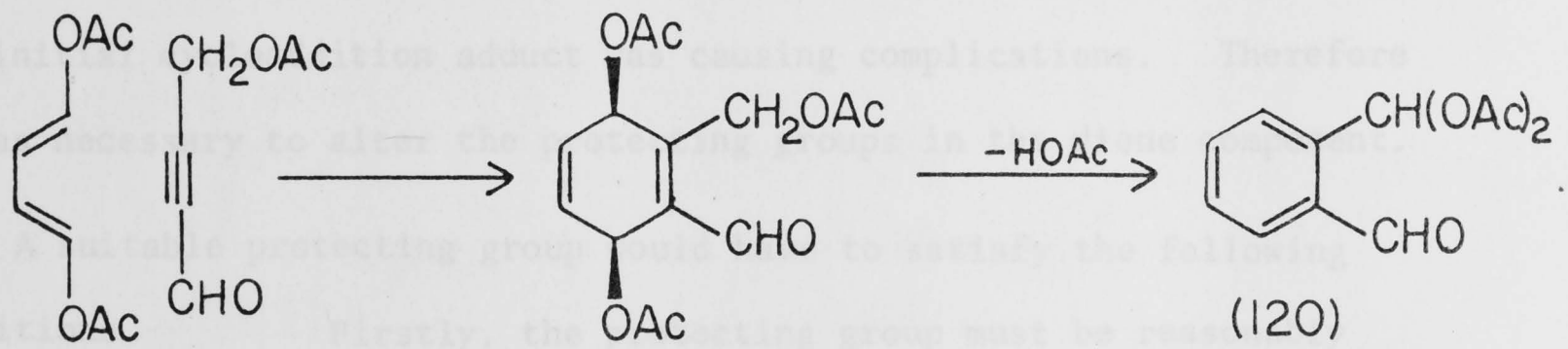

OAc H
H
O
O
CHO
OAc
-HOAc
(118)
CH3
OH


OAc H
H
O
O
CHO
OAc
-HOAc
(118)
CH3
OH
AcO

Scheme 3

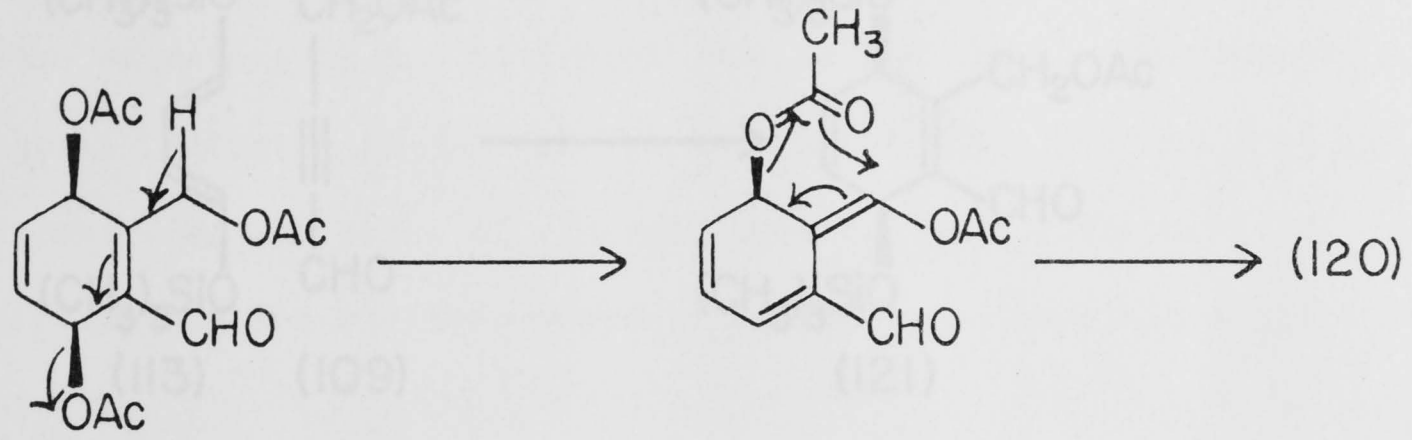
the addition to the diene is less; therefore the rate of cycloaddition should increase.[142] Finally, the removal of all the protecting groups could be carried out in a single step.

(*E*,*E*)-1,4-Diacetoxybuta-1,3-diene (112) and 4-acetoxybut-2-ynal (109) when heated in benzene at 150° for 24 h gave the product (120) (25%). Again, the required cyclohexa-1,4-diene (119) could not be detected. The resulting product, tentatively identified as 2-formylbenzylidene diacetate (120), was characterised from its spectral data. The ^{1}H n.m.r. spectrum



of (120) showed a singlet at δ 10.38 which represented an aromatic aldehydic proton; another singlet at δ 2.07 corresponded to the two acetoxyl groups. The aromatic protons and the acylal proton [$CH(OAc)_2$] resonated between δ 7.95 and 7.46. The acylal proton resonated at very low field due to the deshielding effects exerted by the benzene ring and acetoxyl groups. The presence of acetoxyls and aldehyde groups was also confirmed by infrared absorptions at 1750[101c] and 1700 cm^{-1} [101e] respectively. The mass spectrum of compound (120) did not show a molecular ion (*m/z* 236) but showed ions at *m/z* 177, 165 and 151 which corresponded to the losses from the molecular ion of $OCOCH_3$, CH_2CO plus CHO and CH_2CO plus CH_3CO respectively.[106b] The isolation of 2-formylbenzylidene diacetate (120) indicated that cycloaddition had led to the formation of the required product (119), which had then decomposed to form (120). Again, the rate of decomposition of (119) must be faster than its rate of formation.

Elimination of acetic acid from (119), followed by allylic rearrangement as shown in Scheme 4, could lead to the formation of (120).



Scheme 4

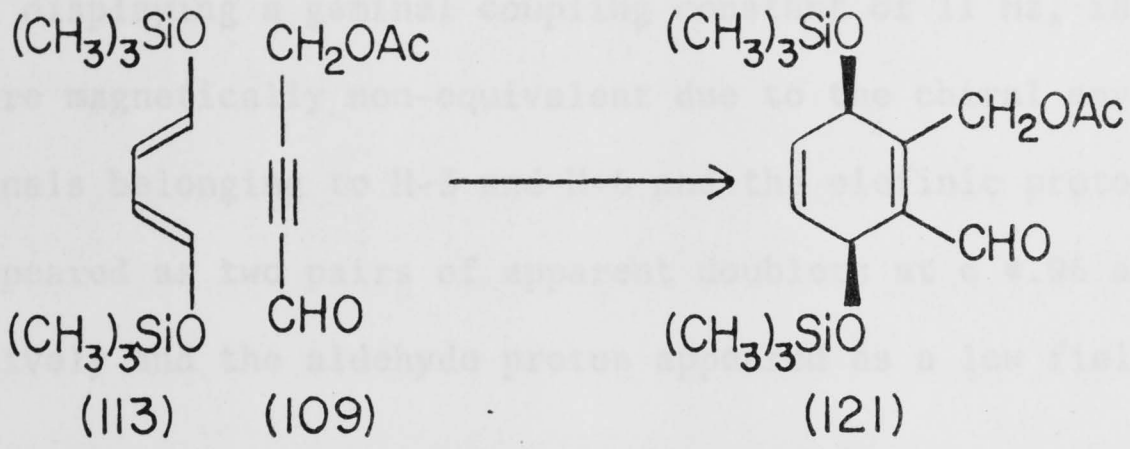
The results, so far, indicated that elimination of acetic acid from the initial cycloaddition adduct was causing complications. Therefore it was necessary to alter the protecting groups in the diene component.

A suitable protecting group would have to satisfy the following conditions. Firstly, the protecting group must be reasonably resistant to elimination under the conditions in which the experiment was to be conducted and, secondly, the protecting group must be easily removable from the final product.

Trimethylsilyl ethers

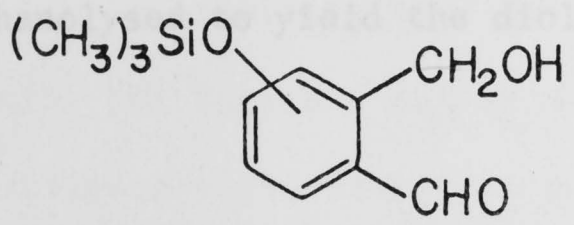
Trimethylsilyl ethers have found considerable use recently as protecting groups for alcohols.[104b,127] They are thermally stable and are easily cleaved by hydrolysis in acid or base, or by treatment with tetraalkylammonium fluorides in aprotic solvents.[104b,143] Trimethylsilyl ethers thus fulfilled the necessary conditions and are suitable protecting groups for the diene component in the cycloaddition and deprotection reactions.

The required cyclohexa-1,4-diene (121) was formed when the diene (113) and 4-acetoxybut-2-ynal (109) were heated at 110-120° overnight. The cycloaddition product could be partially purified by vacuum distillation at 110-120°, but attempted further purification by preparative g.l.c. led to extensive decomposition. Analysis by g.l.c.-mass spectrometry indicated

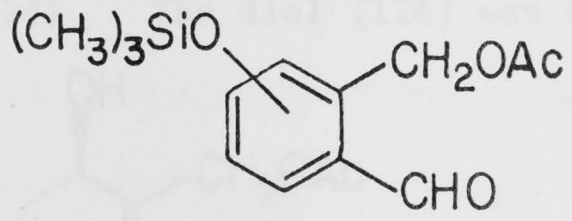


(113) (109) (121)

that the decomposition products were *o*-phthalaldehyde (118), and the aldehydes (122) and (123). A low percentage of (121) was also observed.



(122)



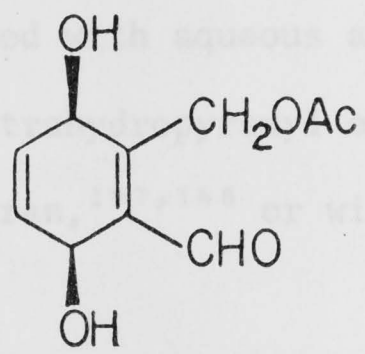
(123)

Direct comparison of the mass spectra of (118) and *o*-phthalaldehyde verified their identity. The mass spectrum of (122) showed ions at *m/z* 194, 177 and 134 that corresponded to M^+-CH_2O, $M^+-(H_2O$ plus CHO) and $M^+-(CH_3)_3SiOH$ respectively. In the mass spectrum of (123), the ions at *m/z* 266, 177 and 134 corresponded to the molecular ion, $M^+-(CH_3COOH$[106k] plus CHO), and $M^+-(CH_2CO$ plus $(CH_3)_3SiOH)$ respectively. An alternative source of the ion at *m/z* 134 from (122) and (123) could be *o*-phthalaldehyde formed by a thermal elimination process.

The cyclohexa-1,4-diene (121) was characterised by its mass spectral and ^1H n.m.r. properties. The mass spectrum of (121) was very informative; besides a molecular ion at *m/z* 356, it also showed ions at 341, 296 and 230 which correspond to M^+-CH_3,[105i] M^+-CH_3COOH,[105b] and a diene from a retro Diels-Alder fragmentation[105l] respectively. The ^1H n.m.r. spectrum indicated that the distilled material contained a high percentage of (121). Other than the methyl signals of the trimethylsilyl

and acetoxyl groups, the allylic methylene protons appeared as an AB quartet displaying a geminal coupling constant of 11 Hz, indicating that they were magnetically non-equivalent due to the chiral environment. The signals belonging to H-3 and H-6 and the olefinic protons (H-4 and H-5) appeared as two pairs of apparent doublets at δ 4.96 and 5.79 respectively and the aldehyde proton appeared as a low field singlet at δ 10.02.

Purification of (121) by preparative t.l.c. was also attempted; however, it was found that the trimethylsilyl ether protecting groups were methanolysed to yield the diol (124). The diol (124) was characterised



(124)

from its ^{1}H n.m.r. spectrum. Apart from the lack of methyl signals of the protecting group, the ^{1}H n.m.r. spectrum of (124) was virtually identical with that of (121), therefore establishing the structure of (124).

Although the trimethylsilyl ether protecting group fitted the requirement of being resistant to thermal elimination, it was, however, unstable in the protic conditions which were needed for the purification and subsequent chemical transformations. A more suitable protecting group was therefore required.

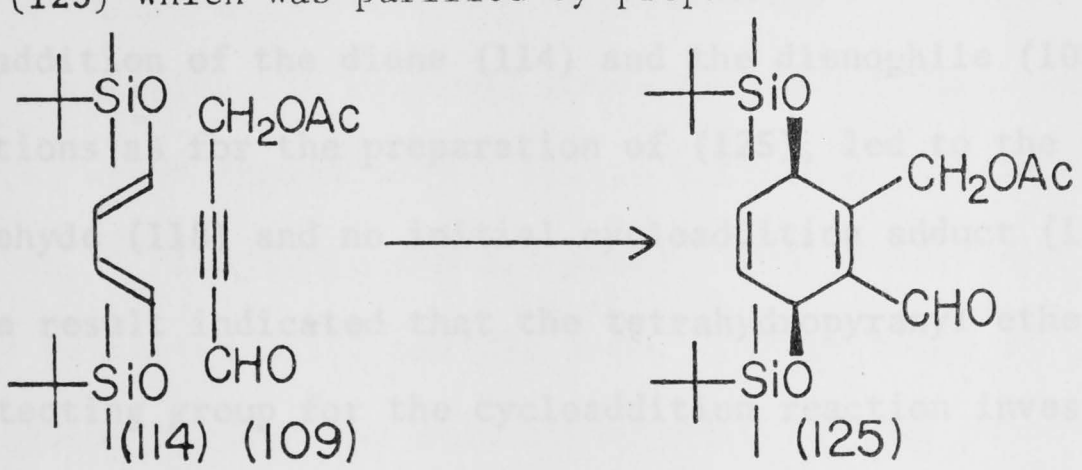
t-*Butyldimethylsilyl ethers*

The *t*-butyldimethylsilyl ether seemed to be the protecting group of choice for the synthesis of eupenoxide (81) for the following reasons. The hydrolysis of silyl ethers has been shown to be markedly retarded by bulky alkyl groups on silicon. For example, the hydrolysis of triethyl-

silyl ethers and *t*-butyldimethylsilyl ethers are slower than that of trimethylsilyl ethers by the factors of about 10^2 and 10^4 respectively in acid or base.[128,144,145]

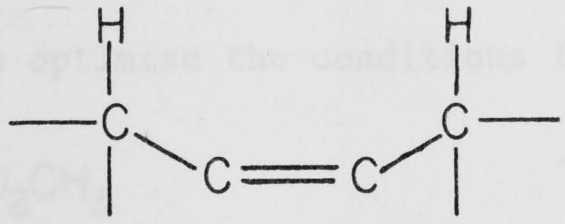
Stork and Hudrlik[128] first showed the utility of the *t*-butyldimethylsilyl group for protecting enols as *t*-butyldimethylsilyl enol ethers. These enol ethers have been found to be much more resistant to hydrolysis than trimethylsilyl enol ethers.[127,128] Also, Corey and co-workers[146,147] and Ogilvie and co-workers[148] have shown that *t*-butyldimethylsilyl ethers are useful protecting groups for alcohols. Because of their greater hydrolytic stability they are easier to work with than the trimethylsilyl ethers, yet they are easily cleaved with aqueous acetic acid[147,148] (at approximately the same rate as tetrahydropyranyl ethers), with tetrabutylammonium fluoride in tetrahydrofuran,[147,148] or with ferric chloride in acetic anhydride.[149]

Cycloaddition of (*E*,*E*)-1,4-bis(*t*-butyldimethylsilyloxy)buta-1,3-diene (114) with 4-acetoxybut-2-ynal (109) at 110-120° yielded the crude cyclohexa-1,4-diene (125) which was purified by preparative t.l.c.



The diene (125) showed several characteristic fragmentations in the mass spectrum. Other than the molecular ion at *m/z* 440, ions at *m/z* 383 and 381 were observed and these corresponded to the losses of $C(CH_3)_3$ and $OCOCH_3$[106b] from the molecular ion. The loss of *t*-butyl from the molecular ion is characteristic of compounds carrying the *t*-butyldimethylsilyl ether function.[150-152] The cyclohexa-1,4-diene nucleus was confirmed by a diene component (*m/z* 314) formed by a retro Diels-Alder process.[105l]

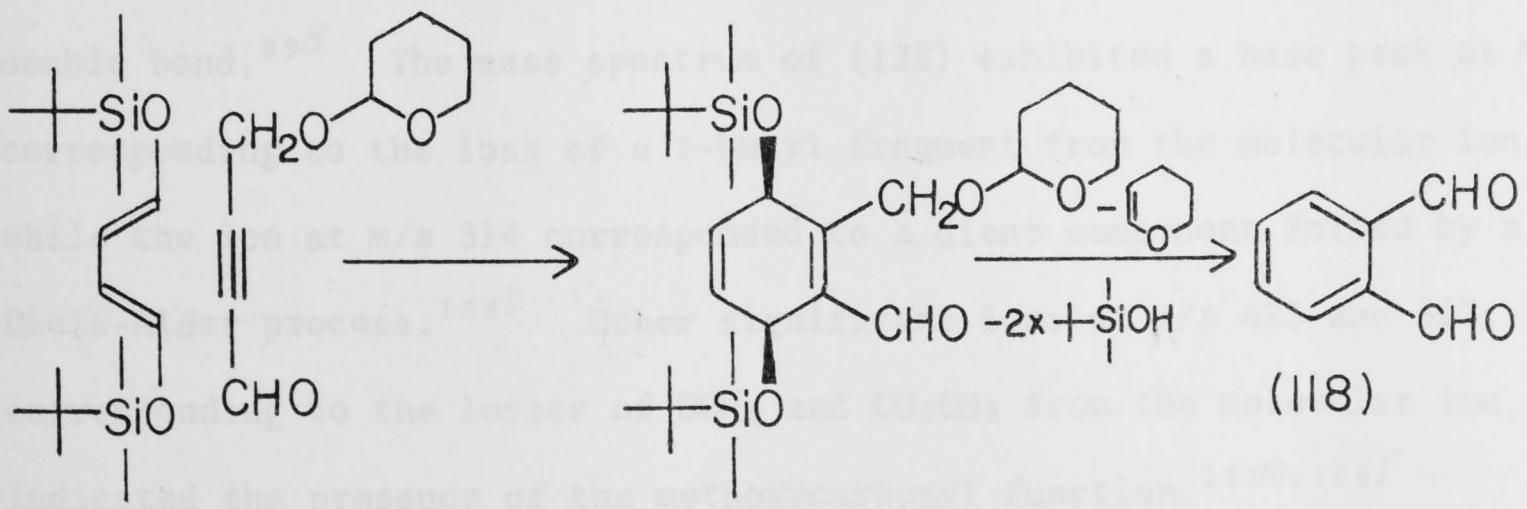
The ultraviolet spectrum of (125) showed a high extinction coefficient for the n-π* transition (332.5 nm, ε 490) which could be a result of the interaction of the α,β-unsaturated aldehyde with the homoconjugated double bond.[89 d] As was the case with compound (121), the allylic methylene protons in the ^{1}H n.m.r. spectrum of (125) appeared as an AB quartet exhibiting geminal coupling of 11 Hz due to their chiral environment.[31d,121c] The coupling constant $^3J_{4,5}$ 10.2 Hz was consistent with vicinal coupling between the olefinic protons. The notably large homoallylic long-range coupling between H-3 and H-6 of 5.3 Hz was comparable to that observed (1-5 Hz) in compounds having a *cis* arrangement between the protons as in (126).[121d]



(126)

This observation indicated that no isomerisation had occurred during or after the cycloaddition reaction and confirmed the *cis* stereochemistry of the substituents at C-3 and C-6.

The cycloaddition of the diene (114) and the dienophile (108), using the same conditions as for the preparation of (125), led to the formation of *o*-phthalaldehyde (118) and no initial cycloaddition adduct (127) was observed. The result indicated that the tetrahydropyranyl ether was not a suitable protecting group for the cycloaddition reaction investigated.

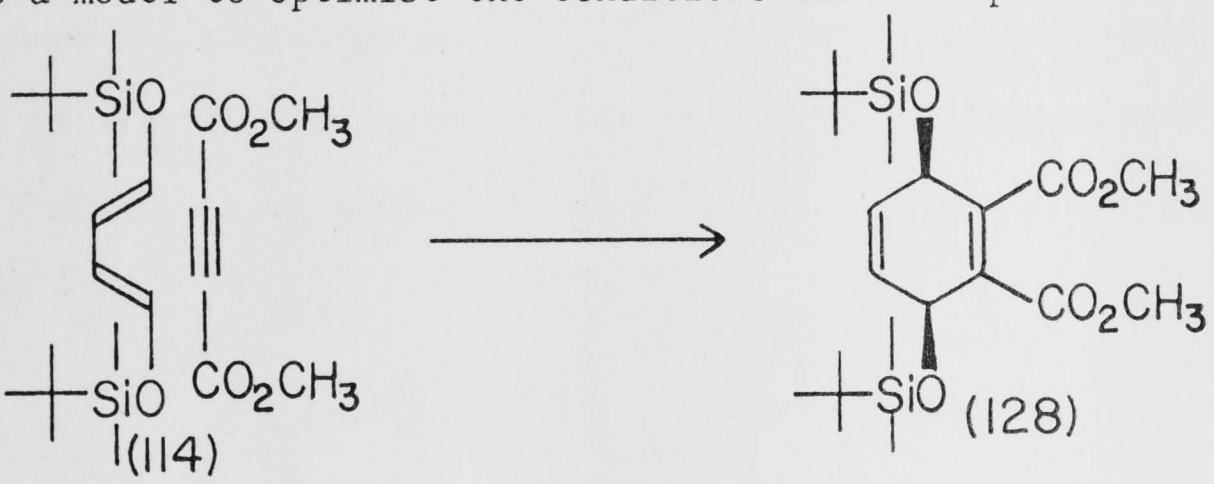


(114) (108) (127) (118)

It is not clear why the tetrahydropyranyl ether protecting group in both of the initially formed cyclohexa-1,4-dienes (117) and (127) would undergo a facile elimination reaction producing 2,3-dihydropyran and the corresponding aromatic intermediates. However, in the case of attempted preparation of (117) the possibility of an initial elimination of acetic acid cannot be excluded.

Model Compound

Because of difficulties encountered in the attempted epoxidation of the cyclohexa-1,4-diene intermediate (125) (see § 5.2) the more readily available, similarly substituted, cyclohexa-1,4-diene (128) was prepared for use as a model to optimise the conditions for the epoxidation reaction.



The diene (114) readily underwent cycloaddition with dimethyl acetylenedicarboxylate yielding the required model compound (128). Unlike dimethyl maleate, which showed only a single ultraviolet absorption maximum at 209 nm,[153] the cyclohexa-1,4-diene (128) exhibited a shoulder at 273.5 nm and maxima at 282.5 and 306.5 nm. These could be due to orbital interactions between the main chromophore and the homoconjugated double bond.[89d] The mass spectrum of (128) exhibited a base peak at M^+-57 corresponding to the loss of a *t*-butyl fragment from the molecular ion,[150-152] while the ion at *m/z* 314 corresponded to a diene component formed by a retro Diels-Alder process.[105l] Other significant ions at *m/z* 425 and 397, corresponding to the losses of OCH_3 and CO_2CH_3 from the molecular ion, indicated the presence of the methoxycarbonyl function.[105m,106f]

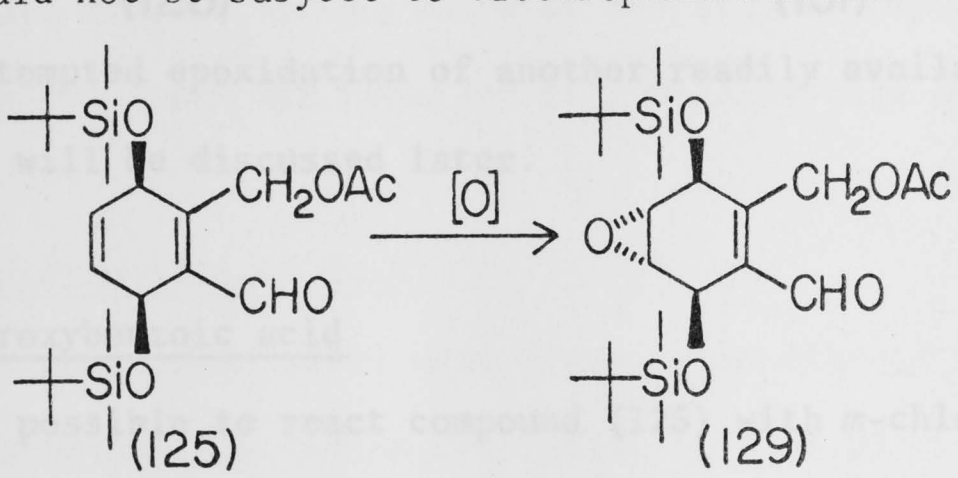
The ^{1}H n.m.r. spectra of (125) and (128) showed that the two methyl groups attached to each silicon had different chemical shifts. The intrinsic difference between the methyl groups could arise from the effects of a nearby asymmetric centre[31d,122c] formed by the cycloaddition reaction.

CHAPTER 5

SYNTHESIS OF EUPENOXIDE — EPOXIDATION REACTIONS

5.1 Introduction

In the synthesis of eupenoxide, a selective epoxidation at the disubstituted double bond of the cycloaddition product (125) was required to yield the intermediate (129). Selectivity of the epoxidation reaction could be achieved by an epoxidising agent such as a peroxy acid. Epoxidation with peroxy acids is generally considered as an electrophilic addition since it is facilitated by electron donation to the double bond and electron withdrawal from the peroxy group.[99d,114a,115a] Because the tetrasubstituted double bond in (125) was conjugated with an electron-withdrawing aldehyde function, it would not be subject to electrophilic attack. In addition, it

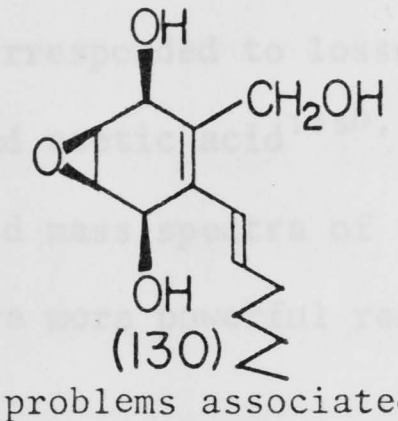


(125) (129)

has been observed that the stereochemistry of an epoxide formed on a disubstituted double bond is influenced by the allylic substituents.[114b] Therefore, in the synthesis of eupenoxide (81), the *t*-butyldimethylsilyl ethers would not only protect the hydroxyl groups but would also provide the steric hindrance necessary for the formation of an epoxide ring *trans* to the hydroxyl groups.[114b,115b,154]

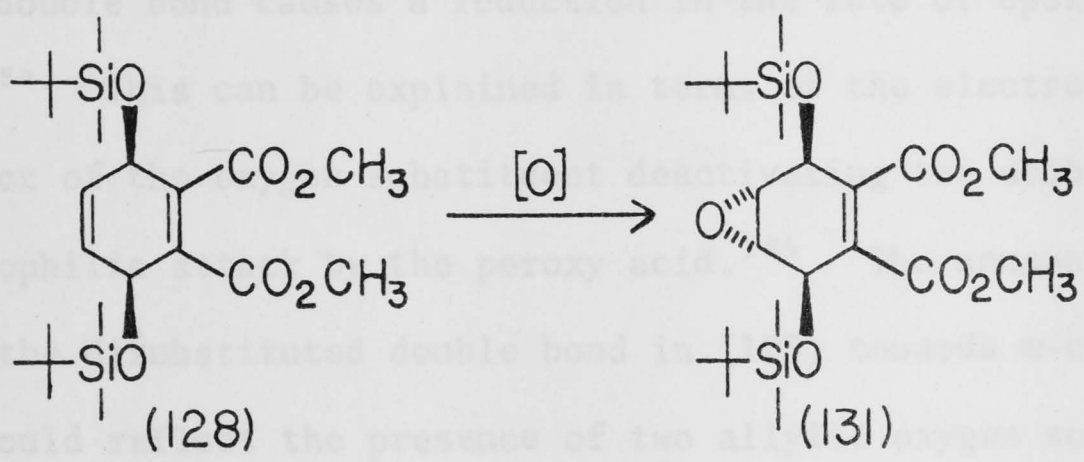
It would also be possible, after the removal of the protecting groups from (125), to form the epoxide ring *cis* to the resulting hydroxyl groups.[99d,114b,115b,154] In this case the reagent would coordinate or hydrogen-bond with the allylic hydroxyl groups, thus producing the epoxide ring on the same side.[99d,114b,115b,154]

Therefore compound (125) could also serve as an intermediate for the synthesis of the isomeric eupenoxide (130).



(130)

Because there were a few problems associated with the epoxidation of (125), the more readily prepared model compound (128) was used to establish the reaction conditions, the product in this case being (131). The



(128) (131)

unsuccessful attempted epoxidation of another readily available cyclohexa-1,4-diene (115) will be discussed later.

5.2 *m*-Chloroperoxybenzoic acid

It was not possible to react compound (125) with *m*-chloroperoxybenzoic acid under normal conditions. Forcing conditions were introduced by Kishi *et al*[155] whereby an olefin, *m*-chloroperoxybenzoic acid and a catalytic amount of a free radical scavenger were heated under reflux in 1,2-dichloroethane. On using these conditions with (125) for three hours, a low yield of product (4%) was obtained and most of the starting material (56%) was recovered.

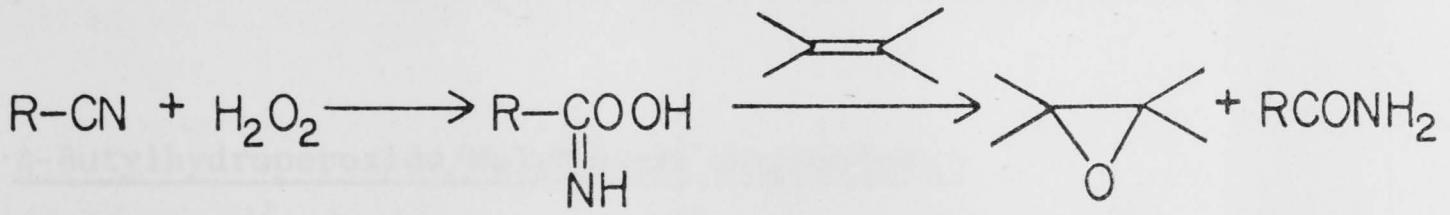
The product was tentatively identified as the required epoxide (129) by analysis of the ^{1}H n.m.r. spectrum of the reaction mixture. Apart from other signals, a low intensity signal situated very close to the aldehyde proton of the starting material and a broad signal of low intensity at δ 3.3-3.2 were observed, suggesting the presence of the aldehyde and the

protons attached to the epoxide group of (129). The assigned structure (129) was supported by the mass spectrum of the partially purified product. The ions at *m/z* 399 and 339 corresponded to losses of *t*-butyl[150-152] from the molecular ion (*m/z* 456) and acetic acid[105b,106b] from *m/z* 399 respectively. Comparison of the ^{1}H n.m.r. and mass spectra of this product with those obtained from later work, where more powerful reagents were used, confirmed this identification.

It has been shown that introduction of an oxygen substituent in the vicinity of a double bond causes a reduction in the rate of epoxidation by peroxy acids.[154] This can be explained in terms of the electron-withdrawing inductive effect of the oxygen substituent deactivating the double bond towards electrophilic attack by the peroxy acid.[154] The unusually low reactivity of the disubstituted double bond in (125) towards *m*-chloroperoxybenzoic acid could reflect the presence of two allylic oxygen substituents. Thus a more reactive agent was required to epoxidise this double bond.

5.3 Phenylperoxycarboximidic acid

Payne[156] found that epoxides were formed when alkenes were treated with hydrogen peroxide in a medium consisting of a nitrile buffered at pH 8, preferably with sodium hydrogen carbonate[157] or phosphate[158]. This reaction probably involves the formation of non-isolable peroxycarboximidic acids (132) which act as epoxidising agents, presumably through a mechanism of the same type as that proposed for the peroxy acid oxidations.[115c]

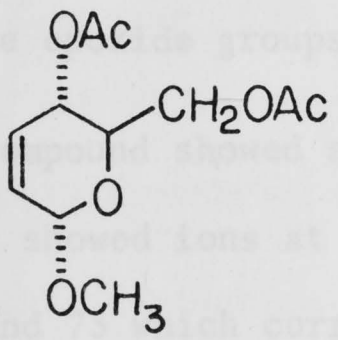


(132)

The rate determining step in the reaction was reported to be the addition of the hydroperoxide anion to the nitrile, and consequently differently substituted olefins, such as hex-1-ene and 2-methylbut-2-ene,

were epoxidised at the same rate.[114c] This is in contrast to the rates of reaction of ethylenic compounds with peroxy acids, which are highly dependent upon the degree of substitution at the double bond.[114a]

An example in the literature[159] showed that a double bond carrying three allylic oxygens could be epoxidised using this method. Methyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-*D*-*erythro*-hex-2-enopyranoside (133) could be epoxidised in good yield with benzonitrile/hydrogen peroxide yielding epoxy derivatives (134) and (135) in the ratio of 2:3. However, when compound (125) was reacted with benzonitrile/hydrogen peroxide, numerous



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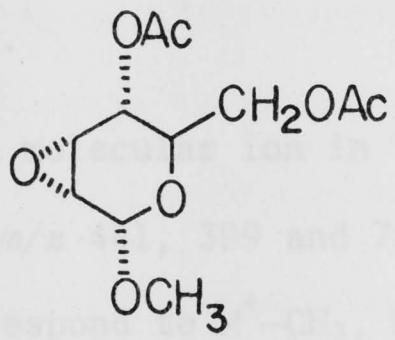

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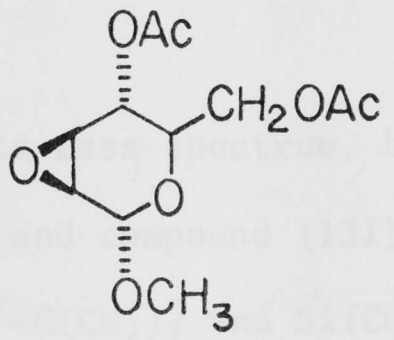


(135)

products were formed as judged by t.l.c. examination, and the ^{1}H n.m.r. spectrum of the reaction mixture did not show any of the required epoxide (129).

No epoxides were obtained on reaction of the model compound (128) and compound (115) with benzonitrile/hydrogen peroxide. In the reaction of (128), low recovery of the starting material (30%) indicated its decomposition and/or decomposition of the product. The isolation of an aromatic product (116) from the reaction of (115) indicated that, in this case, the decomposition was at least partly due to the starting material.

5.4 *t*-Butylhydroperoxide/Molybdenum hexacarbonyl

Reaction of olefins with hydroperoxides, catalysed by molybdenum hexacarbonyl and bis(acetylacetonato)vanadium(IV) oxide has been shown to give high yields of epoxides.[160,161] In general, the molybdenum catalysed reaction was found to give a higher yield of epoxide than the reaction catalysed by vanadium with the exception of allylic alcohols, which complex

readily with vanadium.[161] The fact that molybdenum hexacarbonyl was able to catalyse the epoxidation of 1,4-dichlorobut-2-ene[161] was encouraging as the disubstituted double bond carries two chlorines in allylic positions, and therefore the reactivity of this double bond should be comparable with that of the disubstituted double bonds in (125) and (128).

The model compound (128) and the intermediate (125) were epoxidised by *t*-butylhydroperoxide catalysed by molybdenum hexacarbonyl. In the ^1H n.m.r. spectra of the products (129) and (131) the presence of epoxides was clearly evident from the resonances at δ3.28-3.26 due to the protons attached to the epoxide groups.

Neither compound showed a molecular ion in its mass spectrum, but compound (129) showed ions at *m/z* 441, 399 and 73 and compound (131) at *m/z* 457, 415 and 73 which correspond to M^+-CH_3, $M^+-C(CH_3)_3$ and $\overset{+}{Si}(CH_3)_3$, characteristic of *t*-butyldimethylsilyl derivatives.[150-152]

The ultraviolet spectrum of (129) showed a normal absorption for an α,β-unsaturated aldehyde (229.5 nm, ε 8100)[89b], confirming that epoxidation had not occurred at the tetrasubstituted double bond. This observation was further confirmed by the α,β-unsaturated aldehyde absorption in the infrared spectrum at 1690 cm^{-1}.[101e] The infrared absorption at 1730 cm^{-1} indicated the presence of the methoxycarbonyl group in compound (131). This was confirmed by the signal at δ 3.8 in the ^1H n.m.r. spectrum. Signals in the vicinity of δ 0.9 and δ 0.1-0.2 due to the methyls of the silyl protecting groups were observed in both (129) and (131).

The respective vicinal couplings between the protons attached to the epoxide group and the allylic methine protons enabled the expected *trans* relationship between the epoxide and the *t*-butyldimethylsilyloxy groups to be established in compounds (129) and (131). For simplicity, only compound (129) will be referred to in this discussion as the same argument establishes the stereochemistry in compound (131).

Dreiding models indicated dihedral angles between H-5 and H-4 or H-6 and H-1 of approximately 50° or 120° for *trans* stereochemistry and approximately 0° or 70° for *cis* stereochemistry. Application of the Karplus equations[36] gave $^3J_{4,5} \equiv {}^3J_{1,6}$ 3.2 or 2.1 Hz for *trans* and 8.2 or 0.8 Hz for *cis* stereochemistry, which were comparable to the coupling constants of 2.1 or 1.3 Hz for *trans* and 5.1 or 0.6 Hz for *cis* stereochemistry calculated using the Tori equation[162]. A coupling constant, $^3J_{4,5} \equiv {}^3J_{1,6}$ of 2.3 Hz was observed in compound (129), thus a *trans* relationship between the epoxide and the *t*-butyldimethylsilyloxy groups in (129) was established. Compound (131) showed a corresponding coupling constant, $^3J_{3,4} \equiv {}^3J_{5,6}$, of 1.5 Hz, so by analogy the *trans* stereochemistry between the epoxide and *t*-butyldimethylsilyloxy groups was also established.

The epoxidation reaction with *t*-butylhydroperoxide and molybdenum hexacarbonyl, however, was difficult to control and the yield of epoxide obtained by this method was never more than 20%, often considerably less. Therefore another epoxidising agent was investigated.

5.5 *p*-Nitroperoxybenzoic acid

The powerful electron-withdrawing nitro group, on substitution into an aromatic peroxy acid, substantially enhances the acid's electrophilic character.[163,164] The nitro group is more activating than the chloro group, and it has been found that *p*-nitroperoxybenzoic acid is 5-20 times more reactive than peroxybenzoic acid[163-165], whereas *m*-chloroperoxybenzoic acid was found to be only slightly more reactive.[163,166] This greatly enhanced reactivity, coupled with outstanding stability, often makes *p*-nitroperoxybenzoic acid the reagent of choice for olefins which epoxidise slowly.[164,167] Furthermore, the low solubility of the reaction product, *p*-nitrobenzoic acid, effectively removes it from the reaction medium, thus minimising possible oxirane-ring opening.

Of the three methods which achieved epoxidation, *p*-nitroperoxybenzoic acid was found to give the best yield of required product from the model compound (128) or from the intermediate (125), and therefore this method was used. The relatively low yields (30%) of these reactions may be due to the instability of the starting material under the reaction conditions, resulting in the formation of aromatic by-products (see § 5.7). Comparison of the ^{1}H n.m.r. spectra of these aromatic products and those of the mixtures obtained from epoxidation by *t*-butylhydroperoxide and molybdenum hexacarbonyl indicated the formation of aromatic products common to both reactions.

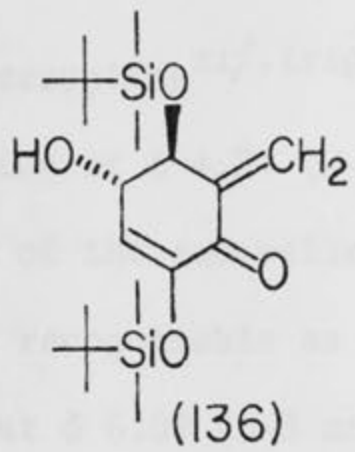
Forcing conditions[155] for the epoxidation of the diacetoxy compound (115) by *p*-nitroperoxybenzoic acid led only to decomposition of the starting material. After two days, the ^{1}H n.m.r. spectrum of the reaction mixture did not show any of the expected resonances for protons attached to epoxide groups (δ 3.5-3.1), and starting material (45%) was recovered. An explanation for this result could be as follows: the greater electron-withdrawing inductive effect exerted by the acetoxyl groups, compared with the corresponding trialkylsilyloxy groups, caused the disubstituted double bond in (115) to be less reactive than that in (128). In agreement, a double bond possessing an allylic acetate has been observed to epoxidise more slowly than that possessing an allylic ether.[168,169]

5.6 *o*-Nitroperoxybenzoic acid

Because Silbert and Konnen[170] have shown that *o*-nitroperoxybenzoic acid was more reactive than the *para* isomer, the reactions of *o*-nitroperoxybenzoic acid with compounds (125) and (128) were investigated.

Although the model compound (128) on reaction with *o*-nitroperoxybenzoic acid yielded the expected epoxide (131), the intermediate (125) on reaction under the same conditions did not give the expected epoxide (129). The

resulting product, isolated in 13% yield (the yield was not optimised) was formulated as the cross-conjugated dienone (136). Its mass spectrum showed a molecular ion at *m/z* 384 which upon high resolution measurement gave the



(136)

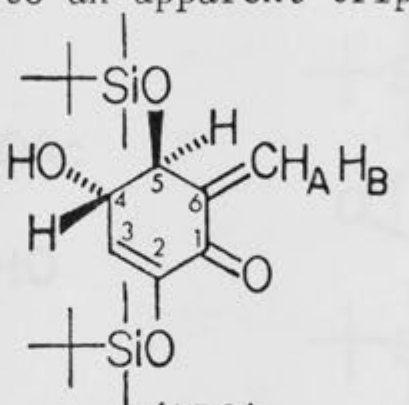
molecular composition of $C_{19}H_{36}O_4Si_2$, indicating the retention of both *t*-butyldimethylsilyl groups. The absence of an infrared absorption at 1740 cm^{-1} and a methyl singlet in the ^1H n.m.r. spectrum at δ 2.0-2.2 suggested that the acetoxyl group had been lost during the formation of the product. The presence of a hydroxyl group was indicated by infrared absorption at 3700-3200 cm^{-1} [101b] and confirmed by an exchangeable proton in the ^1H n.m.r. spectrum.

The α,β-unsaturated carbonyl group, with an α oxygen substituent, was indicated by the ultraviolet maximum at 280 nm.[89b] This absorption shifted to a longer wavelength on addition of sodium hydroxide and this effect was not reversible upon acidification. In addition, the absorption at 280 nm was removed by sodium borohydride, confirming the presence of an α,β-unsaturated carbonyl group.

^{13}C n.m.r. and ^1H n.m.r. studies allowed the assignment of structure (136). The ^{13}C n.m.r. spectrum indicated that there were four olefinic carbons[122c] (δ 152.7, 149.0, 131.3 and 125.2) which on off-resonance proton decoupling appeared as two singlets, a doublet and a triplet respectively, suggesting two quaternary carbons (C-2 and C-6), a tertiary carbon (C-3) and a secondary carbon (the exocyclic methylene carbon). The ^{13}C n.m.r. spectrum also confirmed the presence of a ketone group (δ 189.9) [122b] and indicated that there were two sp^3 carbons possessing an oxygen each (δ 76.8

and 82.1).[122e] Since these sp^3 carbons appeared as two pairs of doublets in the off-resonance proton decoupled spectrum, a proton was attached to each sp^3 carbon.

^{1}H n.m.r. homonuclear decoupling[31f,121g] enabled assignment of the protons in (136). Irradiation at δ 4.34 (H-4 and H-5) caused the broad signal at δ 5.65 (one proton of the exocyclic methylene) to collapse to an unequal intensity doublet recognisable as one half of an AB quartet, and also caused a multiplet at δ 6.08 (H-3 and the second proton of the exocyclic methylene) to collapse to an apparent triplet. Two of the peaks



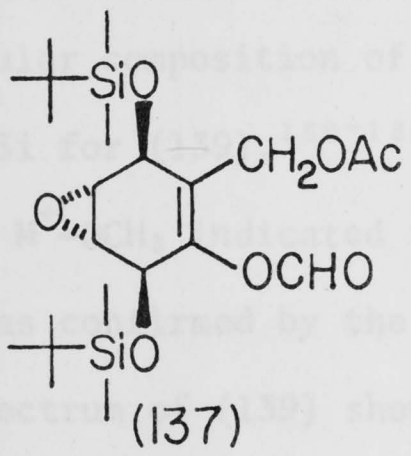
(136)

in the triplet belonged to the AB quartet which was assigned to the exocyclic methylene. The geminal coupling constant $^2J_{AB}$ 2 Hz of the exocyclic methylene protons is in general agreement with the reported geminal coupling constants across sp^2 hybridised carbons (*i.e.* they are much smaller in absolute magnitude than the common range of geminal coupling constants across sp^3 hybridised carbons).[31e,121f]

The addition of a europium shift reagent[121h] tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium(III), [Eu(fod)$_3$], separated the signals and reduced the ^{1}H n.m.r. spectrum to first order, thus enabling the direct measurement of the coupling constants. The exocyclic methylene still exhibited geminal coupling of 2 Hz and long-range allylic coupling of 2 Hz. Inspection of Dreiding models and application of the Karplus equations[36] indicated that the coupling constant $^3J_{4,5}$ 7.5 Hz could only represent a *trans* pseudodiaxial relationship between H-4 and H-5. *Cis* stereochemistry between the hydroxyl and the *t*-butyldimethylsilyloxy groups

could not give rise to such a large coupling constant. The europium shift reagent also enabled the coupling constant between H-3 and H-4 to be measured. H-3 appeared as a doublet with a coupling of 2.7 Hz which indicated that it was adjacent to only one proton, H-4. From the data described, structure (136) was established.

Compound (129) could serve as an intermediate to compound (136) by Baeyer-Villiger oxidation of the aldehyde with *o*-nitroperoxybenzoic acid giving rise to compound (137). Peroxy acids are known to oxidise aldehydes and ketones to yield esters or lactones, in a reaction generally referred to



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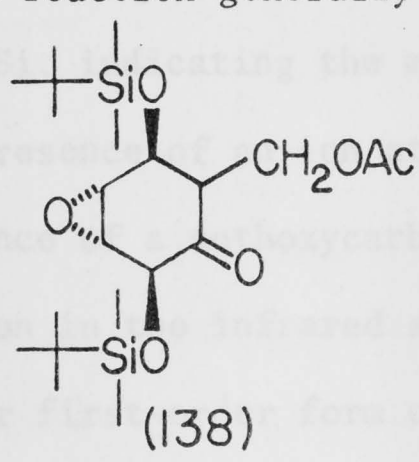
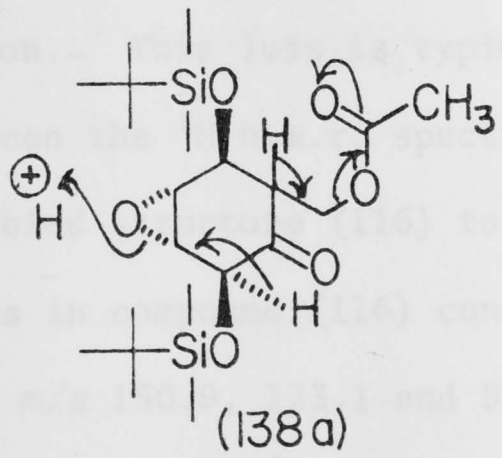


(138)

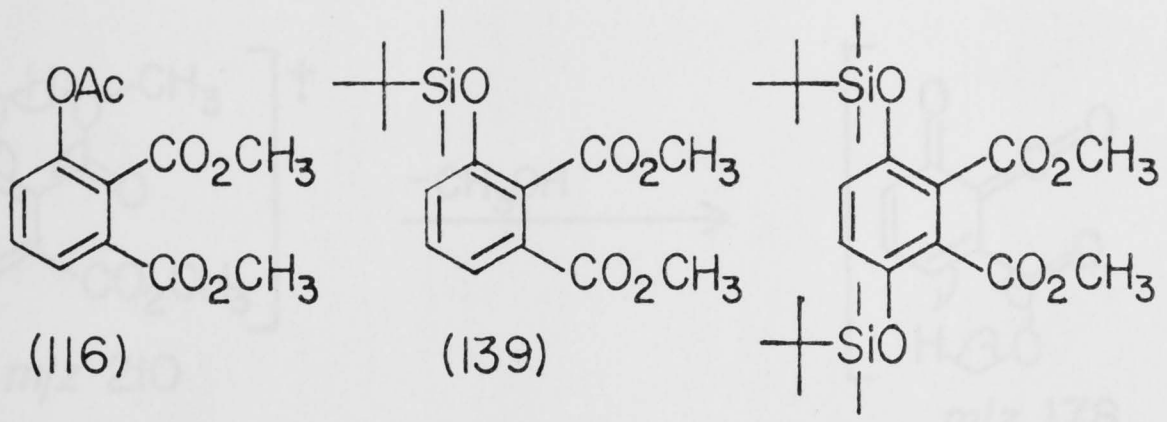
as the Baeyer-Villiger oxidation.[171] Hydrolysis of the formate ester in (137) would give rise to an enol which on subsequent rearrangement would form a ketone (138). Elimination of acetic acid from (138) would give an exocyclic methylene and opening of the epoxide with concurrent loss of H-6 as shown in (138a) would give (136).



(138a)

5.7 The Aromatic Products

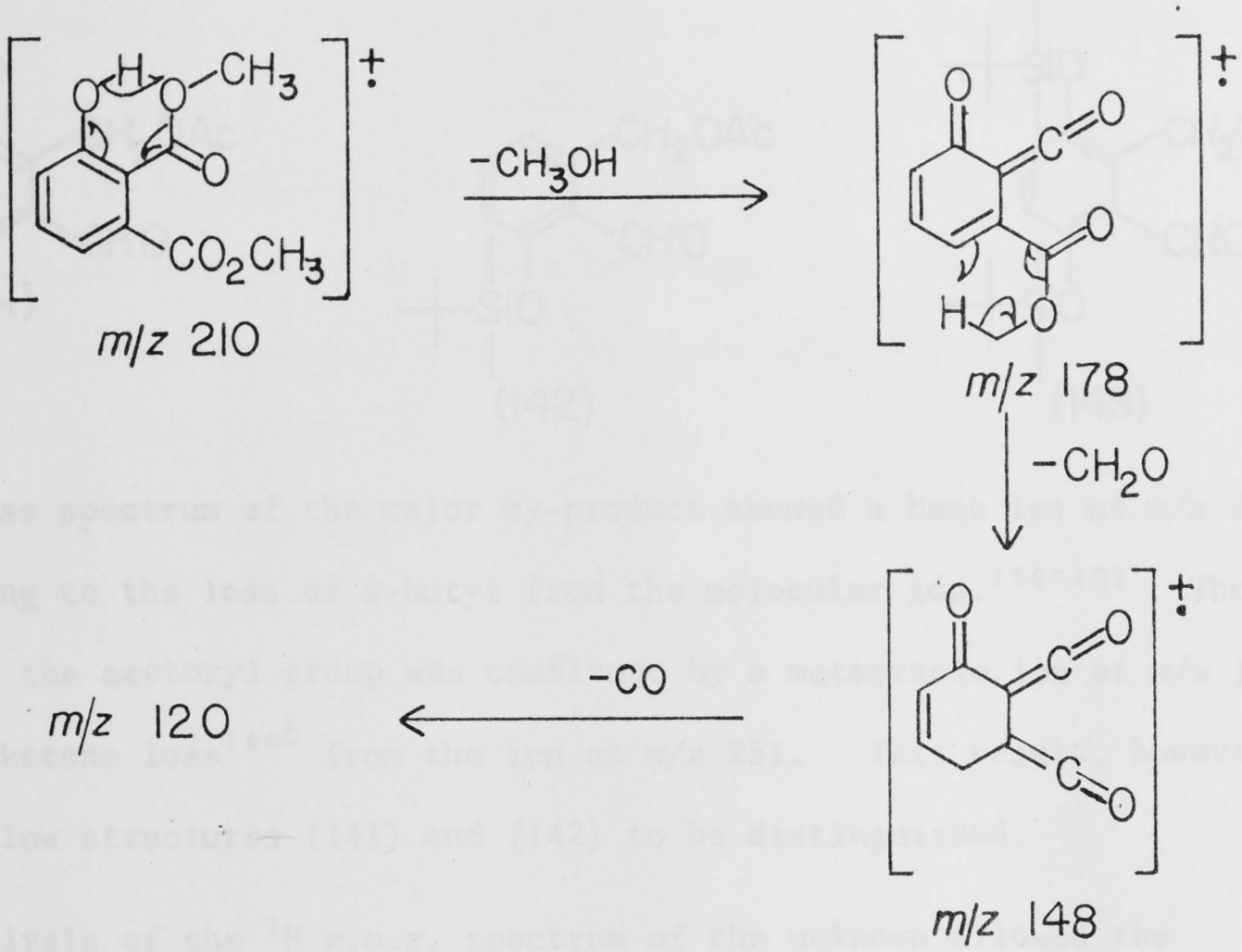
The epoxidation reactions also led to the formation of several aromatic products, some of which were isolated and identified.



(116) (139) (140)

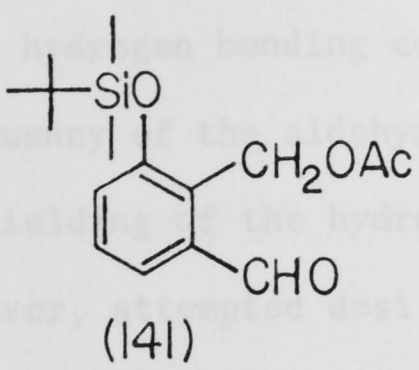
The aromatic compound (139), a by-product from the epoxidation of (128) with *p*-nitroperoxybenzoic acid, showed in its mass spectrum an ion at *m/z* 267 corresponding to $M^+-C(CH_3)_3$. High resolution mass measurement of this ion gave a molecular composition of $C_{12}H_{15}O_5Si$, indicating the molecular formula $C_{16}H_{24}O_5Si$ for (139).[150-152] The presence of an ion at *m/z* 293 corresponding to M^+-OCH_3 indicated the presence of a methoxycarbonyl group in (139) which was confirmed by the absorption in the infrared at 1730 cm^{-1}. The ^1H n.m.r. spectrum of (139) showed a near first-order form of an ABC spin system for the aromatic protons, enabling the direct measurement of coupling constants. The *ortho* couplings of 7.7 Hz and 8 Hz, and the *meta* coupling of 1.2 Hz clearly indicated a 1,2,3-trisubstituted benzene. Other resonances were consistent with the structure (139) for this product which would arise by the elimination of *t*-butyldimethylsilanol from (128).

Compound (116) showed an ion at *m/z* 210 corresponding to the loss of ketene from the molecular ion. This loss is typical of phenyl acetates.[105i] The close similarities between the ^1H n.m.r. spectra of (116) and (139) in the low field region enabled structure (116) to be derived. Characteristic mass spectral fragmentations in compound (116) confirmed the assignment of this structure. Metastables at *m/z* 150.9, 123.1 and 97.3 were observed, which correspond to the losses of methanol[106g], CH_2O and CO[105m,106f] from ions at *m/z* 210, 178 and 148 respectively.



A by-product from epoxidation of (128) with *o*-nitroperoxybenzoic acid was characterised as having the structure (140). The mass spectrum of this compound showed ions at *m/z* 454 and 423 corresponding to a molecular ion and M^+-OCH_3, the latter indicated the presence of a methoxycarbonyl group. High resolution mass measurement of the molecular ion indicated a molecular composition of $C_{22}H_{38}O_6Si_2$. The infrared absorption at 1740 cm^{-1} confirmed the presence of the methoxycarbonyl function. The ^1H n.m.r. spectrum of (140) exhibited symmetry and a 1,2,3,4-tetrasubstituted benzene was indicated by the absence of couplings. Other resonances were consistent with this by-product having the structure (140), which would arise by dehydrogenation of (128).

Other aromatic products having structures (141), 142) and (143) could have been formed as by-products when compound (125) was epoxidised with *p*-nitroperoxybenzoic acid. However, only the major by-product (141) was isolated. A small quantity of (143) was suspected to be present in the mixture but an attempted purification failed. The presence of (142) was indicated by the ^1H n.m.r. spectra in some preparations, but levels were too low for isolation.



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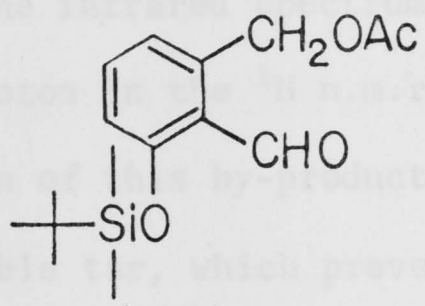

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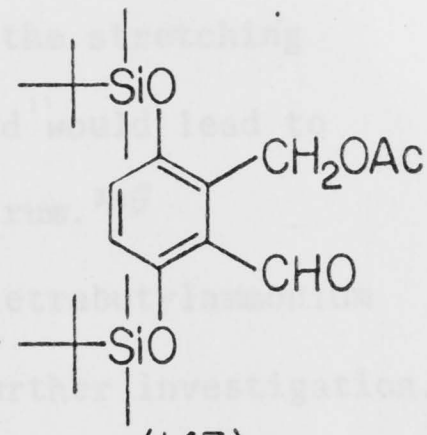


(143)

The mass spectrum of the major by-product showed a base ion at *m/z* 251, corresponding to the loss of *t*-butyl from the molecular ion.[150-152] The presence of the acetoxyl group was confirmed by a metastable ion at *m/z* 174.0 indicating ketene loss[106b] from the ion at *m/z* 251. This result, however, does not allow structures (141) and (142) to be distinguished.

An analysis of the ^{1}H n.m.r. spectrum of the unknown allowed the structure (141) rather than (142) to be tentatively assigned. The aromatic protons of the unknown showed a near first-order ABC type coupling pattern, resulting from a larger chemical shift separation between the aromatic protons due to the close proximity of the electron-withdrawing substituent (CHO), and this would be consistent with structure (141). In agreement, near first-order spectra were observed in compounds (116) and (139) having similar substitution patterns to that of (141). Structure (142) could be discounted since it would be expected to show a complex second-order spectrum as the chemical shift separations between the aromatic protons in (142) were anticipated to be small. In this case, homonuclear decoupling experiments[31f,121g] would not assist in distinguishing between these possible structures because the aldehyde proton in (141) was expected to be weakly coupled to both *o*- and *m*-protons in the aromatic ring.

Desilylation of the by-product tentatively assigned as (141) would have allowed confirmation of its structure. Intramolecular hydrogen bonding cannot occur between the hydroxyl and aldehyde groups in the product derived from desilylation of (141) whereas the opposite is true for compound (142).

Such hydrogen bonding could be detected by a lowering in the stretching frequency of the aldehyde in the infrared spectrum[101f] and would lead to deshielding of the hydroxyl proton in the ^{1}H n.m.r. spectrum.[31g] However, attempted desilylation of this by-product with tetrabutylammonium fluoride[147] led to an intractable tar, which prevented further investigation.

CHAPTER 6

SYNTHESIS OF EUPENOXIDE — WITTIG AND DEPROTECTION REACTIONS

6.1 Introduction

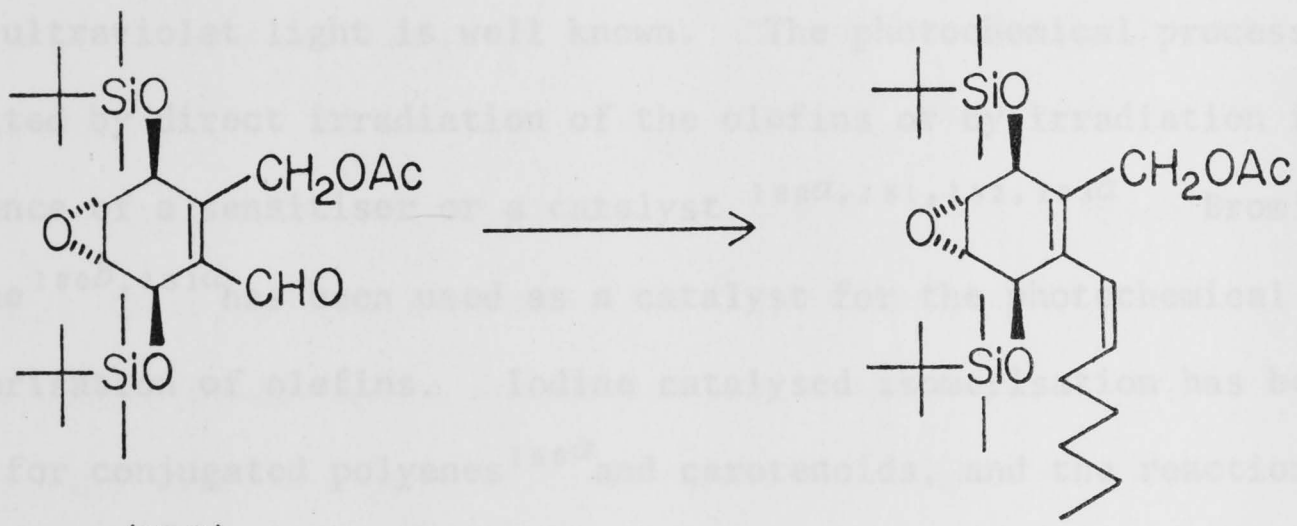
The chain extension in the synthesis of eupenoxide (81) was accomplished through the Wittig carbonyl olefination reaction by means of a phosphorus ylid.[172] This method enabled the acyclic double bond to be formed in a single step under essentially neutral conditions. Other methods of double bond formation require more vigorous conditions or involve longer reaction sequences. For example, Grignard reaction[173] on (129) would result in a hydroxylated side-chain in which the hydroxyl group would need to be modified to make a better leaving group for the subsequent elimination reaction. Although a side-chain containing an *E* double bond could be obtained by this method,[174,175] the longer synthetic sequence made it less attractive.

It has been found in many cases that a phosphorus ylid with an α alkyl substituent (*i.e.* an alkylidenetriphenylphosphorane) reacts rapidly with an α,β-unsaturated ketone or an aromatic aldehyde to give a compound with a *Z* double bond as the major product.[176-179] Double bond formation by Wittig reaction of (129) could result in the formation of a compound possessing a side-chain with the undesired *Z* stereochemistry. If this occurred, then modifications of the standard reaction conditions that have been reported to increase the yield of the *E* isomer[176b] could be investigated. In addition, a *Z* double bond could be photochemically isomerised under neutral conditions to the thermodynamically more stable *E* double bond.[180a]

6.2 Wittig Reaction

Wittig reaction of (129) with *n*-hexylidenetriphenylphosphorane gave a

product (144) (70%) containing a C_7 side-chain. The mass spectrum of (144) showed a weak molecular ion at *m/z* 524, and other significant ions at *m/z* 467 and 464 corresponding to $M^+-C(CH_3)_3$ or $M^+-(CH_2)_3CH_3$ and M^+-CH_3COOH respectively. In the ^1H n.m.r. spectrum of (144), the coupling constant $^3J_{AB}$ 11.5 Hz exhibited by the olefinic protons indicated the Z stereochemistry. This assignment was confirmed by later work which will be discussed subsequently (see § 6.5). All the resonances in the ^1H n.m.r. spectrum could be assigned to structure (144). Since *E* stereochemistry



(129) (144)

for the double bond was required for the synthesis of eupenoxide, modification of the Wittig reaction conditions in order to obtain an *E* double bond or isomerisation of (144) to the corresponding *E* isomer was considered.

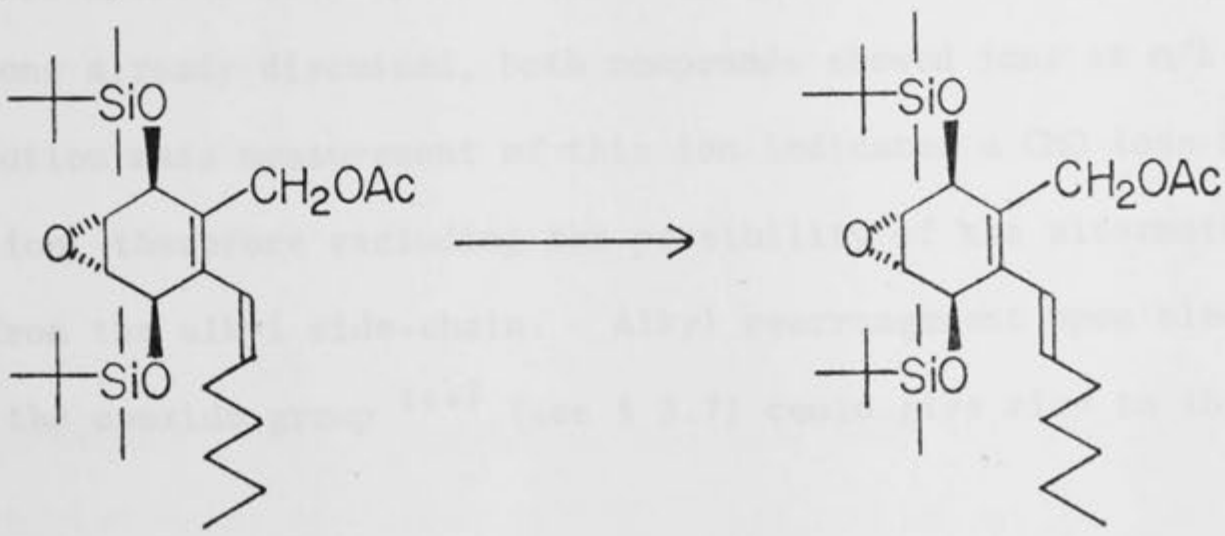
Addition of a lithium salt has been shown to increase the ratio of *E/Z* double bond formation in the Wittig reaction, by shifting the position of the *erythro-threo* equilibrium of the betaine epimers in favour of the *threo* compound. This latter species can then eliminate to form an *E* double bond. However, the equilibration was reported to be too slow to make this modification practical.[177] A possibly superior method makes use of α-metalation of the betaine to create a centre of rapid configurational inversion enabling an *E* double bond to be obtained in high yield.

This modification of the Wittig reaction was not carried out because the stability of the betaine intermediate derived from (129) with the nucleophilic bases used in α-metalation is uncertain. Therefore an alternative approach to the formation of the *E* double bond, by *Z-E* photochemical isomerisation, was considered.

6.3 The Isomerisation of the Double Bond

The ability of olefins to undergo *Z-E* isomerisation upon irradiation with ultraviolet light is well known. The photochemical process can be effected by direct irradiation of the olefins or by irradiation in the presence of a sensitiser or a catalyst.[180a,181,182,183a] Bromine or iodine[180b,181a] has been used as a catalyst for the photochemical *Z-E* isomerisation of olefins. Iodine catalysed isomerisation has been widely used for conjugated polyenes[180a] and carotenoids, and the reaction in general results in a good yield of the thermodynamically more stable product.[181b]

In the presence of iodine the *Z* double bond in (144) was isomerised using pyrex filtered[183b] light sources (sunlight or a tungsten lamp or a medium pressure mercury lamp[183c]). Extensive conversion of the *Z* isomer (144) to the *E* isomer (145) was achieved (70%). However, the isomers were found to be inseparable by the chromatographic techniques investigated. Fortunately, crystallisation of the *E* isomer (145) allowed it to be separated



(144) (145)

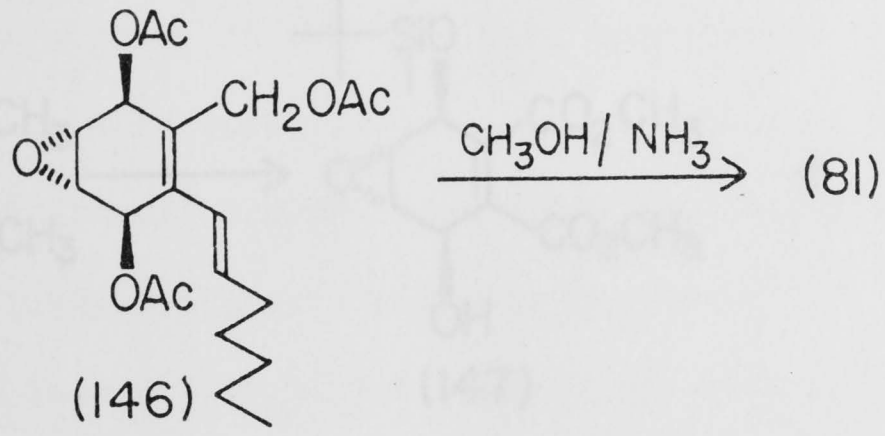
from the *Z* isomer (144). This was the only crystalline compound obtained *en route* to synthetic eupenoxide. Comparison of the ^{1}H n.m.r. and ultraviolet spectra of both isomers established the *Z* and *E* configurations of the double bonds in the respective compounds (144) and (145). The olefinic protons of the *E* isomer (145) resonated at lower field (δ 6.24 and 6.02) and exhibited a larger coupling constant ($^{3}J_{AB}$ = 15 Hz) than those of the *Z* isomer (144) (δ 5.76 and 5.67, $^{3}J_{AB}$ = 11.5 Hz) indicating that the Z → E isomerisation had been achieved. The chemical shifts and the coupling constant of the olefinic protons of (145) are in close agreement with those of eupenoxide (δ 6.29 and 6.11, $^{3}J_{AB}$ 15 Hz).[88] The shift of the ultraviolet absorption, from the end absorption of the *Z* isomer (144) to a maximum at 243 nm (ε 20650) of the *E* isomer (145), confirmed the *Z* and *E* stereochemistry of the double bonds in the respective compounds. The ultraviolet absorption of compound (145) was also in close agreement with that of eupenoxide (240.3 nm, ε 20000). The ultraviolet absorptions of (144) and (145) are in agreement with the general observation that the *Z* isomer of a conjugated olefin often absorbs in the ultraviolet at slightly shorter wavelength and with lower extinction coefficient than the corresponding *E* isomer.[180a] It has been suggested that this shift is caused primarily by steric inhibition of resonance through non-bonded interaction of the *Z* substituents.

The mass spectra of compounds (144) and (145) were very similar. Apart from the ions already discussed, both compounds showed ions at *m/z* 495 (M^{+}–29). High resolution mass measurement of this ion indicated a CHO loss from the molecular ion, therefore excluding the possibility of the alternate ethyl cleavage from the alkyl side-chain. Alkyl rearrangement upon electron impact of the epoxide group [104l] (see § 3.7) could give rise to this ion.

6.4 Removal of the Protecting Groups

Since eupenoxide triacetate (146) could be deacetylated with methanolic

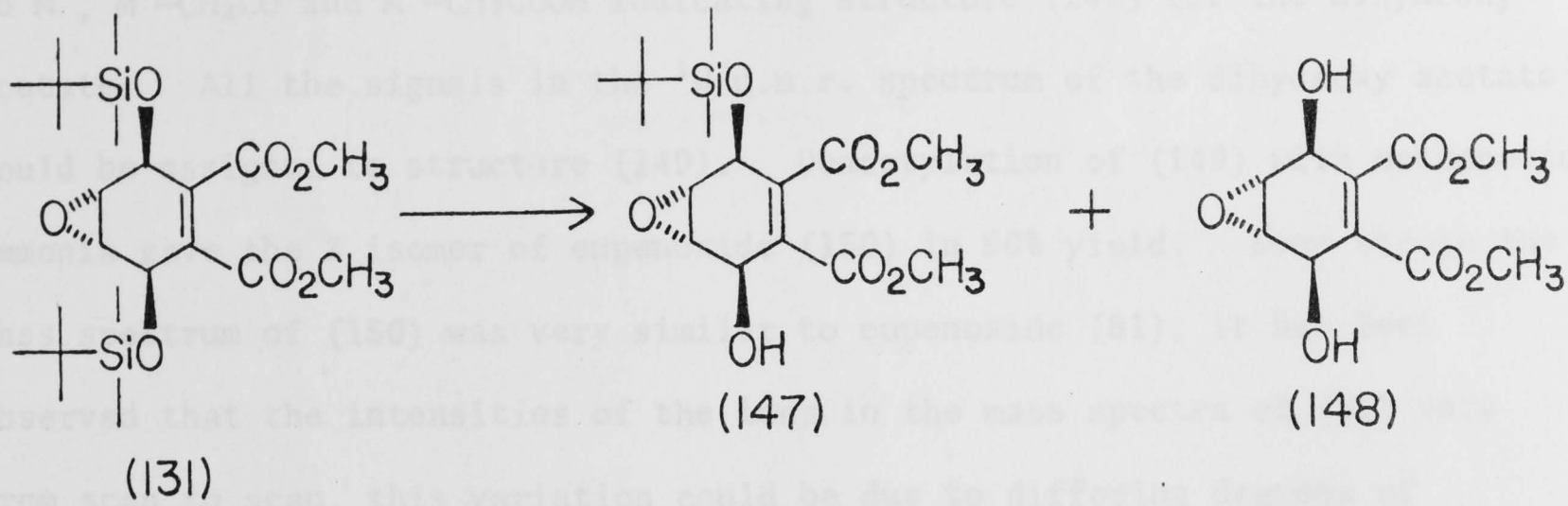
ammonia to yield eupenoxide (81), this method was selected for the removal of the acetoxyl protecting group.



Removal of *t*-butyldimethylsilyl ether protecting groups can in general be achieved by several methods; they can be cleaved with aqueous acetic acid,[146-148] with tetrabutylammonium fluoride in tetrahydrofuran[146-148] or with ferric chloride in acetic anhydride.[149] Eupenoxide (81) or compounds carrying similar functionalities have never been treated with fluoride ions under aprotic conditions, nor with ferric chloride in acetic anhydride, thus the stability of eupenoxide, (144) and (145) under these conditions is uncertain. However, hydrolysis of the acetonide protecting group of the derivatives of eupenoxide (87), (94) and (99) had been successfully achieved with aqueous acetic acid, and therefore desilylation by aqueous acetic acid seemed feasible.

Desilylation by aqueous acetic acid was initially investigated with the model compound (131). Using this method, compound (131) was desilylated to give an alcohol (147) and a diol (148) in 19% and 48% yield respectively. It was anticipated that the 1,4-bis(*t*-butyldimethylsilyl) derivative of eupenoxide would undergo a similar acid hydrolysis and therefore the above method of desilylation was selected for the deprotection of synthetic eupenoxide derivatives.

The presence of hydroxyl groups in compound (148) was confirmed by the infrared absorption at 3500-3200 cm^{-1} [101b] and the ion at *m/z* 226 corresponding to M^+-H_2O[105a] in the mass spectrum. Other than the absence of methyl signals associated with the *t*-butyldimethylsilyl protecting groups (*i.e.* signals between

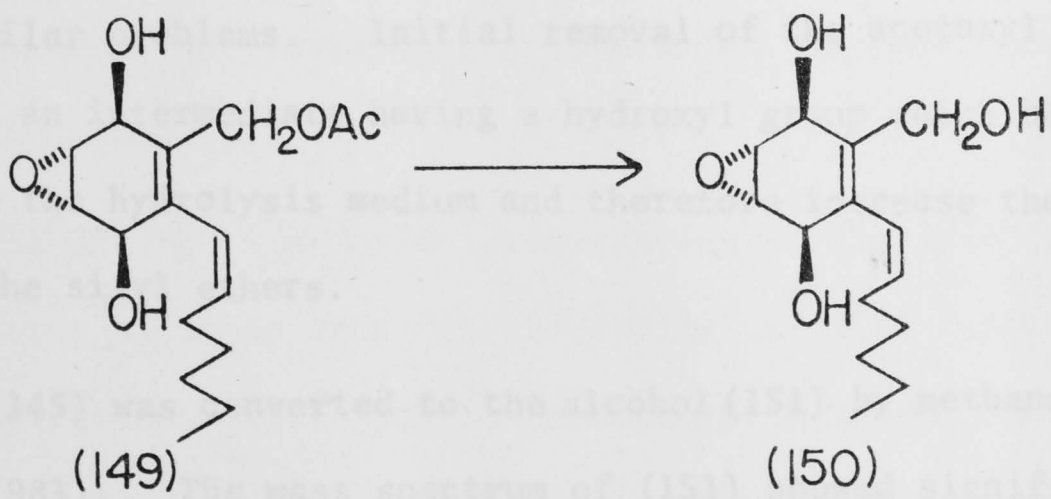


δ 1.0-0.06), the ^{1}H n.m.r. spectrum of (148) was very similar to that of the starting material (131), thus establishing structure (148) for the diol.

The mass spectrum of the alcohol (147) showed ions at *m/z* 327, 301 corresponding to M^+-OCH_3 and $M^+-C(CH_3)_3$ respectively, indicating structure (147) for the alcohol. The presence of a hydroxyl and *t*-butyldimethylsilyloxy groups was indicated by an exchangeable proton and methyl resonances (δ 1.0-0.06). in the ^{1}H n.m.r. spectrum of (147). Other resonances in the ^{1}H n.m.r. spectrum were consistent with structure (147) for the alcohol.

6.5 The Z Isomer of Eupenoxide

To confirm the Z configuration of the double bond in compound (144) and to test the deprotection methods, all the protecting groups in (144) were removed to yield the triol (150) which could be compared with natural eupenoxide. Compound (144) was desilylated by refluxing with tetrahydrofuran/acetic acid/water for two days to give the dihydroxy acetate (149) (57%).



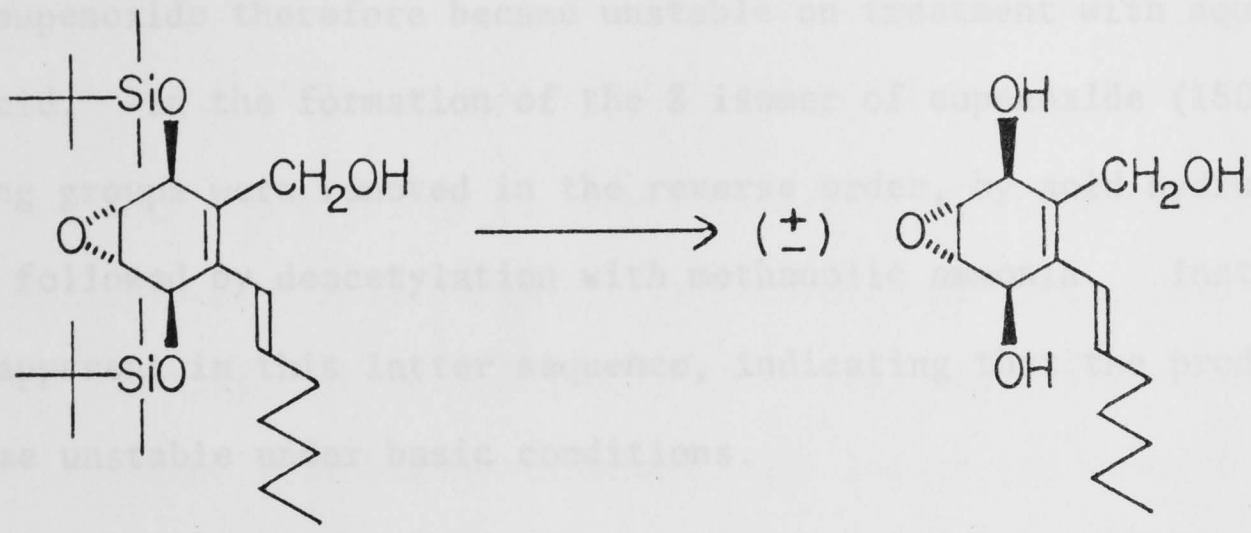
The mass spectrum of (149) showed ions at *m/z* 296, 254 and 236 corresponding to M^+, M^+-CH_2CO and M^+-CH_3COOH indicating structure (149) for the dihydroxy acetate. All the signals in the ^1H n.m.r. spectrum of the dihydroxy acetate could be assigned to structure (149). Deacetylation of (149) with methanolic ammonia gave the Z isomer of eupenoxide (150) in 90% yield. Even though the mass spectrum of (150) was very similar to eupenoxide (81), it has been observed that the intensities of the ions in the mass spectra of (81) vary from scan to scan, this variation could be due to differing degrees of pyrolysis of (81) on flash evaporation from the probe into the ion source of the mass spectrometer. The ^1H n.m.r. spectra of (150) and eupenoxide were very similar, other than in the olefinic proton region; therefore structure (150) was established as the Z isomer of eupenoxide.

Eupenoxide (81) and its Z isomer, however, showed marked differences in their ultraviolet absorption. While eupenoxide showed an ultraviolet maximum at 240.3 nm, the Z isomer of eupenoxide exhibited only end absorption. All three compounds synthesised, having the Z configuration for the double bonds, showed only end absorption which is in agreement with the phenomenon mentioned earlier.

6.6 (±)-Eupenoxide

The desilylation of compound (144) gave only 57% yield of the dihydroxy acetate (149). This could have been due to the insolubility of (144)in the hydrolysis medium, and it seemed likely that desilylation of (145) would suffer from similar problems. Initial removal of the acetoxyl group in (145) would result in an intermediate having a hydroxyl group which should make it more soluble in the hydrolysis medium and therefore increase the rate of hydrolysis of the silyl ethers.

Compound (145) was converted to the alcohol (151) by methanolic ammonia in good yield (98%). The mass spectrum of (151) showed significant ions at



(151) (152)

m/z 482, 467, 464 and 425 corresponding to M^+, M^+-CH_3, M^+-H_2O and $M^+-C(CH_3)_3$. The loss of water from the molecular ion indicated the presence of a hydroxyl group which was confirmed by infrared absorption at 3650-3100 cm^{-1} and an exchangeable proton in the ^1H n.m.r. spectrum. Other resonances in the ^1H n.m.r. spectrum were consistent with the alcohol having structure (151).

Desilylation of (151) with acetic acid/water (4:1) gave a product which appeared initially to be identical to eupenoxide (81) by t.l.c. However, on careful analysis it was found that the natural product showed fluorescence at 366 nm but the synthetic product lacked fluorescence at this wavelength. In addition, the ^1H n.m.r. spectrum of the product in deuterochloroform showed broad peaks which were not recognisable as those of eupenoxide. Detailed t.l.c. analysis of the ^1H n.m.r. sample showed that there were several fractions of higher polarity than natural eupenoxide indicating that extensive decomposition had occurred. Therefore it was necessary to find out if natural eupenoxide was stable under the conditions used to generate (±)-eupenoxide (152).

Treatment of natural eupenoxide with aqueous acetic acid (4:1) under the same conditions as those used for the desilylation of compound (151), followed by preparative t.l.c., gave a product which showed identical t.l.c. and ^1H n.m.r. behaviour to the product obtained by hydrolysis of (151).

Natural eupenoxide therefore became unstable on treatment with aqueous acetic acid. In the formation of the Z isomer of eupenoxide (150), the protecting groups were removed in the reverse order, by acid hydrolysis of (144) followed by deacetylation with methanolic ammonia. Instability was not apparent in this latter sequence, indicating that the product does not become unstable under basic conditions.

Natural eupenoxide, which had become unstable after treatment with aqueous acetic acid, was restabilised by treatment with methanolic ammonia. However, on treatment with methanolic ammonia only 60% of the eupenoxide which was present after acid treatment was recovered. In view of this loss, synthetic eupenoxide (152) was not treated with methanolic ammonia initially, but an attempt was made to obtain all the physical data immediately after the compound was purified. However, even though good ultraviolet and mass spectra were obtained, the ^{1}H n.m.r. spectrum indicated that there was only 70-80% of the required product present. The ultraviolet spectrum of synthetic eupenoxide (152) showed a maximum at 240.7 nm which was comparable with the maximum at 240.3 nm observed in natural eupenoxide. The mass spectrum of the synthetic product showed the same ions as natural eupenoxide (81) but with different intensities. Natural eupenoxide itself exhibited intensity variations in each mass spectrum recorded (§ 6.5). Therefore direct comparison of mass spectral data in this case was not a good method for establishing the identity of the natural and synthetic products.

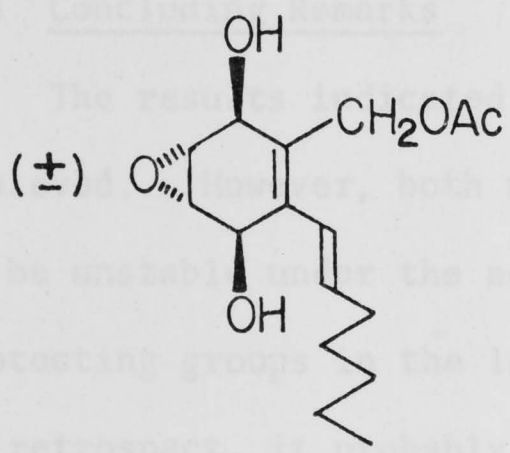
All the remaining synthetic eupenoxide (4 mg) was combined and treated with methanolic ammonia overnight and purified by preparative t.l.c. The ^{1}H n.m.r. spectrum of this material in tetradeuteromethanol was identical with that of natural eupenoxide with the exception of approximately 5% impurities. However, further attempted stabilisation

of the synthetic product (152) to obtain purer compound led to an even smaller quantity of (152) (<1 mg) which precluded further characterisation.

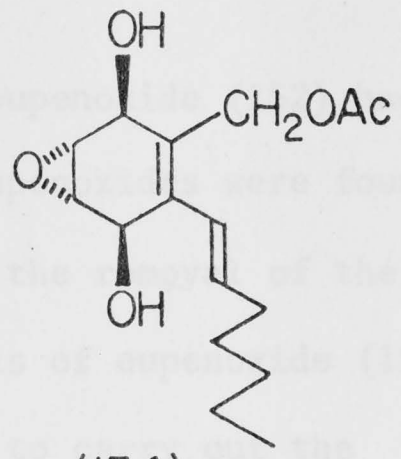
Two possible explanations for the instability of eupenoxide towards acid are as follows. Acidic impurities incorporated during acid treatment could cause eupenoxide to be unstable. The other explanation could be that acid treatment followed by chromatography led to the removal of stabilising impurities present in eupenoxide. Stabilising impurities may have been regenerated on treatment of eupenoxide with methanolic ammonia.

6.7 Eupenoxide monoacetate

Desilylation of (151) led to the formation of another product of lower polarity than eupenoxide in 10% yield. This product was formulated as having structure (153). The mass spectrum of the product showed a



(153)



(154)

molecular ion at *m/z* 296, and other significant ions at 279, 278 and 236 which correspond to the loss of hydroxyl, water[105a] and acetic acid[105b] from the molecular ion. The mass spectrum of this product (153) was virtually identical with that of the synthetic *Z* isomer of eupenoxide monoacetate (149). Initially, this was rather puzzling as the starting material (151) used in the desilylation reaction was free of the bis(*t*-butyldimethylsilyl)eupenoxide acetate (145) as judged by all available physical and spectroscopic data. However, natural eupenoxide on treatment with aqueous acetic acid under the

same conditions also produced compound (154) having an identical t.l.c. and mass spectrum with that of (153). This indicated that compounds (153) and (154) are structurally identical. The infrared spectrum of natural eupenoxide monoacetate (154) showed absorptions at 3600-3100 cm^{-1} and 1740 cm^{-1} indicating the presence of hydroxyl and acetoxyl functions[101b,101c] respectively. Analysis of the ^1H n.m.r. spectrum of (154) enabled its structure and the structure of (153) to be established. Other than the additional methyl resonance at δ 2.03 of the acetoxyl group, and the downfield shift (from δ 4.41 and 4.15 to δ 4.64 and 4.46) and convergence of the AB quartet of the C-2 allylic methylene, the ^1H n.m.r. spectrum of (154) was virtually identical to that of eupenoxide (81), indicating the acetylation at the primary hydroxyl group. This acetate could have arisen by an acid catalysed esterification of the least sterically hindered primary hydroxyl group by acetic acid.

6.8 Concluding Remarks

The results indicated that the synthesis of eupenoxide (152) had been achieved. However, both natural and synthetic eupenoxides were found to be unstable under the acid conditions used for the removal of the silyl protecting groups in the last step of the synthesis of eupenoxide (152). In retrospect, it probably would have been better to carry out the deprotection reactions in the same order as in the formation of the *Z* isomer of eupenoxide. This would involve desilylation of bis(*t*-butyldimethylsilyl)eupenoxide acetate (145), followed by deacetylation to yield (±)-eupenoxide. In this case, (±)-eupenoxide would be formed under basic conditions in which it would be expected to be stable.

To establish the generality of the synthetic method for six-membered carbocyclic compounds possessing *cis*-1,4-dioxygenated substituents, the full scope of the cycloaddition reactions needs to be investigated. The

use of substituted alkenes or acetylenes would enable the formation of cyclohexenes or cyclohexa-1,4-dienes which could be further functionalised to form polyoxygenated cyclohexane and/or cyclohexene derivatives. Many of the cyclohexene oxide antibiotics, such as those in the epoxydon and panepoxydon series are amenable to synthesis by the above method. However, it remains to be seen if the method is more efficient than existing methods. Possibly the most useful features would be the stereospecificity achieved by the cycloaddition reaction and the ease of introduction of side-chains. These features would circumvent the disadvantages of many other syntheses which, in general, are less stereospecific and do not allow facile introduction of side-chains.

EXPERIMENTAL

GENERAL

Melting points were determined on a Kofler microheating stage and are uncorrected. Elemental analyses were carried out by the Microanalytical Unit of the Australian National University. Infrared spectra were recorded on a Perkin-Elmer 257 or a Jasco IRA-1 spectrophotometer. Electronic spectra were recorded on a Cary 118C spectrophotometer. CD spectra were obtained on a Jasco ORD/UV-5 spectrophotometer.

^{13}C and ^{1}H n.m.r. data were obtained on a Varian HA-100 (^{1}H n.m.r.), Jeol Minimar 100 MHz high resolution NMR spectrometer (^{1}H n.m.r.) and Jeol FX-60 high resolution Fourier transform NMR spectrometer (^{1}H and ^{13}C n.m.r.) Chemical shifts are given in ppm downfield from tetramethylsilane internal reference and multiplicities are abbreviated: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Some coupling constants in the ^{1}H n.m.r. spectra were analysed by Mr. M. J. Whittaker, using exact solutions[121b], where possible, and then the parameters were refined with the NMR analysis program, LAOCN-4A[184]. In difficult cases, homonuclear decoupling experiments[121g] were performed to aid the analysis.

Mass spectra were run at 70 eV on an AEI MS-902 or a Varian MAT CH-7 instrument. Accurate mass measurement was obtained from an AEI MS-902 using heptacosa as reference compound. Combined gas liquid chromatographic-mass spectral analyses were carried out on a Varian MAT 111 with a 180 cm×3 mm i.d. glass column containing 2% OV-17 on 80-100 Chromosorb Q. Routine g.l.c. analyses and preparative g.l.c. were carried out on Varian aerograph 1400 and Varian aerograph 90-P instruments respectively, using glass columns containing 2% OV-17 on 80-100 Chromosorb Q.

Ether used for the extraction of eupenoxide was freshly distilled. Low boiling point solvents were fractionated through a vacuum jacketed glass column (100×2 cm) packed with glass helices. Anhydrous ether and tetrahydrofuran were freshly distilled from sodium/benzophenone when required. Chloroform used in the epoxidation reactions was washed with a solution of sodium carbonate, water, dried with potassium carbonate, distilled from phosphorus pentoxide and then used immediately. Light petroleum (30-40°) was used in chromatographic solvents used for preparative t.l.c.

(1R,4S,5R,6S)-2-[(E)-Hept-1'-enyl]-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol

(Eupenoxide) (81)

Eupenicillium species was grown and eupenoxide (81) isolated using the method described by Quinn and Rickards.[88] Purification by preparative t.l.c. on silica gel GF_{254}, dichloromethane/methanol (80:20), gave a colourless viscous *oil (81)* which had spectral properties identical with those described by Quinn and Rickards[88]. λ_{max} (EtOH) 240.4 nm (ε 20000). *m/z* (%) 254(60), 236(50), 223(30), 207(63), 189(30), 183(55), 181(68), 177(85), 147(57), 137(67), 123(85), 107(97), 91(98). ^{1}H n.m.r. δ(CD_3OD) 6.41, H-1', 6.09, H-2', $^3J_{1',2'}$ 16 Hz, $^3J_{2',3'}$ 6 Hz; 4.59, br s, H-1 and H-4; 4.41, 4.15, $^2J_{AB}$ 12 Hz, CH_2OH; 2.40-1.96, m, $CH{=}CH{-}CH_2$; 1.35, br s, $3{\times}CH_2$; 0.90, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(1R,4S,5R,6S)-3-Hydroxymethyl,4-O-isopropylidene-{2-[(E)-hept-1'-enyl]-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol}

(Eupenoxide acetonide) (85)

Eupenoxide acetonide was prepared by the method of Quinn and Rickards.[88] The crude acetonide was chromatographed on a Florisil column using acetone as eluant, and the eluant concentrated under reduced pressure to give a colourless oil (86%). Crystallisation from light petroleum gave colourless *crystals (85)* (m.p. 46-47°) which had properties identical with those of the product reported by Quinn and Rickards.[88] *m/z* (%) 294(1.5), 279(2), 236(26), 219(10), 207(18), 179(100), 147(31), 137(27), 133(52), 123(48), 121(59), 119(44), 109(57), 107(100+). ^{1}H n.m.r. δ($CDCl_3$) 6.02, m, H-1' and H-2'; 4.64, br s, H-1 and H-4; 4.59, 4.39, $^2J_{AB}$ 14 Hz, CH_2O; 3.45, H-6, 3.26, H-5, $^3J_{4,5}$ 4 Hz; 2.70-2.45, br d, $^3J_{1,OH}$ 8 Hz, exchanged in D_2O, OH; 2.30-2.05, m, $CH{=}CH{-}CH_2$; 1.54, 1.42, 2 s, $C(CH_3)_2$; 1.35, br s, $3{\times}CH_2$; 0.92, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(1R,4S,5R,6S)-2-Heptyl-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol (1',2'-Dihydroeupenoxide) (89)

To a solution of eupenoxide (81) (45 mg) in ethanol (5 ml) was added 10% palladium/calcium carbonate (4.5 mg) and the mixture was hydrogenated at 20° for 18 min. After removal of the catalyst by filtration the supernatant was concentrated under reduced pressure. Preparative t.l.c. of the residue on methanol washed silica gel GF_{254}, dichloromethane/methanol (93:7) gave a mixture (40 mg) of 1,2- and 1,4-dihydro reduction products as indicated by signals in the ^1H n.m.r. spectrum (δ 5.5-5.2, m, C=CH). The mixture was further purified by preparative t.l.c. on 10% silver nitrate impregnated silica gel GF_{254}, dichloromethane/methanol (90:10) to give *1',2'-dihydroeupenoxide (89)* (8 mg, 17.5%). *m/z* (%) 256(5), 238(32), 220(37), 209(60), 198(59), 196(61), 179(44), 149(45), 137(100), 123(95), 107(90), 95(100). ^1H n.m.r. δ($CDCl_3$) 4.65, 4.34, 2 br s, H-1 and H-4; 4.54, 4.12, $^2J_{AB}$ 13 Hz, CH_2OH; 3.9-3.5, m, exchanged in D_2O, 3 OH; 3.42, br s, H-5 and H-6; 2.4-2.1, m, $CH=CH-CH_2$; 1.34, br s, $5\times CH_2$; 0.95, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

Attempted Catalytic Hydrogenation followed by Oxidation of Eupenoxide Acetonide (85)

10% Palladium/calcium carbonate (6.6 mg) in 95% ethanol was hydrogenated then compound (85) (47 mg) was added and hydrogenation continued for 1 h at 19.6° and hydrogen (4.73 ml) was consumed. After removal of the catalyst by centrifugation the supernatant was concentrated under reduced pressure to give a colourless viscous *oil* (47 mg, 99%). The ^1H n.m.r. spectrum showed the signals δ 5.8-5.4, m, $C=CH-CH_2$ which indicated the presence of a 1,4-dihydro reduction product (90). However, the mixture could not be separated by 10% silver nitrate impregnated silica gel GF_{254} t.l.c.

The hydrogenated product from the above reaction (55 mg) without further purification, was dissolved in dichloromethane (25 ml), manganese dioxide (550 mg) was added and the mixture was stirred at room temperature for 6 h. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure and subjected to preparative t.l.c. on methanol washed silica gel GF_{254}, ether/light petroleum (1:1) to give a *fraction* (3 mg, 5%) which gave a positive colour test with 2,4-dinitrophenylhydrazine (Found: *m/z* 294.1830 Calc. for $C_{17}H_{26}O_4$: *m/z* 294.1831.) *m/z* 294(35), 279(24), 278(10), 236(7), 235(8), 220(10), 219(10), 207(30), 206(100), 204(15), 190(41), 186(6), 177(12), 175(12), 165(10), 163(10), 161(15), 159(14), 149(25), 147(36), 135(40), 133(70), 131(61), 123(30), 121(40), 119(18), 107(62), 91(34).

(1R,4S,5R,6S)-3-Hydroxymethyl,4-O-isopropylidene-(2-heptyl-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol)

(1',2'-Dihydroeupenoxide acetonide) (86)

A solution of (85) (70 mg) and tris(triphenylphosphine)rhodium(I) chloride (7 mg) in deaerated benzene (25 ml) was stirred under hydrogen at atmospheric pressure for 3 days. The mixture was concentrated under reduced pressure and subjected to preparative t.l.c. on methanol washed silica gel GF_{254} ether/light petroleum (2:1) to give two major overlapping fractions. The more polar fraction was the starting material (23 mg) and the less polar fraction, which became visible with iodine vapour, gave the required product as a colourless viscous *oil (86)* (24 mg, 42% based on unrecovered starting material). (Found: C, 68.62; H, 9.46. $C_{17}H_{28}O_4$ requires C, 68.89; H, 9.52%.) ν_{max} (CCl_4) 3580, 3500-3240 cm^{-1} (OH). *m/z* (%) 296(3), 281(8), 266(1), 238(16), 221(17), 209(17), 208(15), 137(60), 127(20), 125(22), 123(25), 121(24), 111(25), 109(27), 108(27), 107(65). ^1H n.m.r. $\delta(CDCl_3)$ 4.57, br s, H-4; 4.46, 4.30, $^2J_{AB}$ 14.4 Hz, CH_2O; 4.26, br s with sh, appeared sharper on addition of D_2O, H-1; 3.38,

H-6, 3.23, H-5, $^3J_{5,6}$ 3.7 Hz; 2.70-2.25, exchanged in D_2O, OH; 2.35-2.00, m, CH=CH-CH_2; 1.53, 1.39, 2 s, $C(CH_3)_2$; 1.27, br s, 5×CH_2; 0.88, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(4S,5R,6R)-3-Hydroxymethyl,4-O-isopropylidene-(2-heptyl-4-hydroxy-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1-one)

(1-Dehydro-1',2'-dihydroeupenoxide acetonide) (87)

A solution of (86) (24 mg) in dichloromethane (2 ml) was added to a suspension of pyridinium chlorochromate[100] (41 mg) and sodium acetate (8.4 mg) in dichloromethane (3 ml). After stirring the mixture for 1 h, ether (5 ml) was added, the supernatant was decanted, and the residue washed with ether. The supernatant and the washings were combined, passed through a short column of Florisil and eluted with ether. Concentration of the eluant under reduced pressure followed by preparative t.l.c. on silica gel GF_{254} ether/light petroleum (2:1) gave a colourless viscous *oil (87)* (12.5 mg, 52%). (Found: C, 69.46; H, 9.11. $C_{17}H_{26}O_4$ requires C, 69.36; H, 8.90%.) λ_{max} (EtOH) 312 nm (ε51), 249 (7860). ν_{max} (CCl_4) 1676 cm^{-1} (CO). *m/z* (%) 294(28), 279(15), 236(35), 220(22), 219(9), 209(5), 207(15), 191(10), 179(10), 165(12), 153(22), 152(27), 151(27), 137(39), 123(82), 95(40), 91(23), 59(100); metastable 189.4 (294 → 236). ^1H n.m.r. δ($CDCl_3$) 4.79, br s, H-4; 4.52, br s, CH_2O; 3.69, H-6, 3.49, H-5, $^3J_{5,6}$ 3.6 Hz, $^4J_{6,4}$ 1.1 Hz, $^3J_{5,4}$ 0.8 Hz; 2.46 -1.90, m, C=C-CH_2; 1.54, 1.40, 2 s, $C(CH_3)_2$; 1.28, br s, 5×CH_2; 0.88, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(4S,5R,6R)-2-Heptyl-4-hydroxy-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1-one

(1-Dehydro-1',2'-dihydroeupenoxide) (88)

A solution of (87) (10 mg) in glacial acetic acid (1 ml) and water (5 drops) was allowed to stand at room temperature for 4 h. After azeotropic removal of the solvent with toluene under reduced pressure, the residue was subjected to preparative t.l.c. silica gel GF_{254}, dichloro-

methane/methanol (93:7) to give a colourless viscous *oil (88)* (6 mg, 69%). (Found: *m/z* 254.1519. Calc. for $C_{14}H_{22}O_4$: *m/z* 254.1518.) λ_{max} (EtOH) 247.5 nm (ε 7520). ν_{max} (CCl_4) 3660-3200 cm^{-1} (OH), 1680 (CO). *m/z* (%) 254(5), 236(89), 223(28), 207(30), 205(7), 165(33), 151(50), 147(23), 137(25), 135(20), 133(26), 123(59), 121(18), 119(16), 111(21), 81(38), 79(35), 77(30), 71(46), 43(100); metastables 219.3 (254 → 236), 181.6 (236 → 207), 91.7 (165 → 123). ^{1}H n.m.r. δ ($CDCl_3$) 4.92, br s, H-4; 4.52, br s, CH_2OH; 3.78, H-6, 3.52, H-5, $^3J_{5,6}$ 3.6 Hz; 2.60-1.92, m, C=C-CH_2 and 2×OH; 1.28, br s, 5×CH_2; 0.91, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(1S,4R,5S,6R)-2-Hydroxymethyl,1-O-isopropylidene-[4-acetoxy-2-[(E)-hept-1'-enyl]-2-hydroxymethyl-5,6-epoxycyclohex-2-en-1-ol]

(Eupenoxide-1-acetate acetonide) (94)

A solution of compound (85) (370 mg) in anhydrous pyridine (5 ml) and acetic anhydride (1 ml) was allowed to stand at room temperature for 6 h. After concentration under reduced pressure, preparative t.l.c. of the mixture on silica gel GF_{254}, ether/light petroleum (1:1) gave a colourless viscous *oil (94)* (288 mg, 68%). (Found: C, 67.48; H, 8.38. $C_{19}H_{28}O_5$ requires C, 67.83; H, 8.39%.) λ_{max} (EtOH) 240 nm (ε 21330), 237(21590). ν_{max} (CCl_4) 1740 cm^{-1} ($OCOCH_3$). *m/z* (%) 336(9), 321(3), 278(25), 261(3), 260(3), 249(11), 236(40), 233(22), 219(3), 218(18), 208(28), 207(67), 191(23), 190(38), 189(30), 181(41), 179(97), 177(32), 165(38), 164(53), 161(32), 149(39), 147(82), 137(32), 133(80), 123(40), 121(52), 119(65), 109(41), 107(100), 105(51), 43(100+); metastables 230.0 (336 → 278), 200.3 (278 → 236), 181.6 (236 → 207). ^{1}H n.m.r. δ($CDCl_3$) 5.92, H-1', 5.62, H-2', $^3J_{1',2'}$ 16 Hz, $^3J_{2',3'}$ 6.5 Hz; 5.85, br s, H-4; 4.64, s, H-1; 4.64, 4.41, $^2J_{AB}$ 14.6 Hz, CH_2O; 3.29, br s, H-5 and H-6; 2.22-1.94, m, CH=CH-CH_2; 2.09, s, $OCOCH_3$; 1.52, 1.42, 2 s, $C(CH_3)_2$; 1.28, br s, 3×CH_2; 0.89, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(1S,4R,5S,6R)-4-Acetoxy-3-[(E)-hept-1'-enyl]-2-hydroxymethyl-5,6-epoxycyclohex-2-en-1-ol

(Eupenoxide-1-acetate) (95)

A solution of (94) (200 mg) in glacial acetic acid (4 ml) and water (0.5 ml) was allowed to stand at room temperature for 1 h. After azeotropic removal of solvent under reduced pressure with toluene, preparative t.l.c. of the residue on silica gel GF_{254}, dichloromethane/methanol (93:7) gave a colourless viscous *oil (95)* (160 mg, 90%). (Found: *m/z* 296.1623. Calc. for $C_{16}H_{24}O_5$: *m/z* 296.1623.) λ_{max} (EtOH) 240.8 nm. ν_{max} (CCl_4) 3500-3300 cm^{-1} (OH), 1735 ($OCOCH_3$). *m/z* (%) 296(0.5), 278(3), 262(1), 249(18), 236(14), 220(11), 218(55), 207(60), 206(11), 190(24), 189(25), 177(60), 165(100), 161(35), 149(33), 147(95), 137(36), 135(41), 133(57), 123(42), 121(45), 119(83), 107(75), 105(45), 43(100+); metastable 181.4 (234 → 206). ^{1}H n.m.r. δ($CDCl_3$) 6.24, H-1', 5.85, H-2', $^3J_{1',2'}$ 16 Hz, $^3J_{2',3'}$ 6 Hz; 5.76, br s, H-4; 4.68, br s, H-1; 4.59, 4.29, $^2J_{AB}$ 12.8 Hz, CH_2OH; 3.40, br s, H-5 and H-6; 3.2-2.4, m, exchanged in D_2O, 2×OH; 2.33-1.92, m, $CH{=}CH{-}CH_2$; 2.08, s, $OCOCH_3$; 1.30, br s, 3×CH_2; 0.88, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(4R,5S,6S)-4-Acetoxy-3-[(E)-hept-1'-enyl]-2-hydroxymethyl-5,6,-epoxycyclohex-2-en-1-one

(4-Dehydroeupenoxide-1-acetate) (98)

A solution of (95) (120 mg), anhydrous pyridine (1 ml) and *p,p*-dimethoxytrityl chloride (254 mg) in anhydrous dichloromethane (6 ml) was allowed to stand at room temperature for 16 h. After removal of solvent under reduced pressure, the residue was fractionated on a Florisil column, dichloromethane/methanol (93:7). The fractions of lower polarity than (95) were combined, evaporated to dryness and the residue was dried under high vacuum. The dried residue was taken up in anhydrous benzene (200 ml), manganese dioxide (2 g) added and the mixture was stirred at room temperature

for 48 h. Removal of catalyst by filtration followed by concentration of the filtrate under reduced pressure gave a brown residue. Preparative t.l.c. of the residue on silica gel GF_{254}, ether/light petroleum (1:1) gave a fraction containing several overlapping bands which gave a positive colour test with 2,4-dinitrophenylhydrazine. This mixture was directly dissolved in acetic acid (5 ml), saturated with water and allowed to stand at room temperature for 3 h. After azeotropic removal of solvent under reduced pressure with toluene, preparative t.l.c. of the residue on silica gel GF_{254}, dichloromethane/methanol (93:7) gave a very pale yellow viscous *oil (98)* [24 mg, 24% from (95)]. (Found: C, 65.15; H, 7.67. *m/z* 294.1466. Calc. for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53%; *m/z* 294.1467.) λ_{max} (EtOH) 292 nm (ε 19200). ν_{max} (CCl_4) 3620-3240 cm^{-1} (OH), 1745 ($OCOCH_3$), 1668 (CO), 1629 (C=C). *m/z* 234.1254 M^+-CH_3COOH. Calc. for $C_{14}H_{18}O_3$: *m/z* 234.1256, *m/z* 206.1307 M^+-CH_3COOH-CO Calc. for $C_{13}H_{18}O_2$: *m/z* 206.1307, *m/z* 205.1228 M^+-CH_3COOH-CHO. Calc. for $C_{13}H_{17}O_2$: *m/z* 205.1228; *m/z* (%) 294(3), 276(1), 252(2), 234(62), 222(15), 217(5), 207(30), 206(90), 205(74), 177(39), 151(20), 149(53), 147(22), 145(22), 136(82), 135(100), 133(20), 131(57), 123(28), 121(40), 119(29), 108(40), 107(56), 105(20), 55(50), 43(100+). ^{1}H n.m.r. δ($CDCl_3$) 6.49, H-1′, 6.22,H-2′, $^3J_{1',2'}$ 15.9 Hz, $^3J_{2',3'}$ 6.5 Hz; 6.31, br s, H-4; 4.40, br s, CH_2OH; 3.51, H-6, 3.33, H-5, $^3J_{5,6}$ 3.7 Hz, $^4J_{6,4}$ 1.7 Hz, $^3J_{5,4}$ 0.8 Hz; 2.6-1.9, m, 1H exchange in D_2O, CH=CH-CH_2 and OH; 2.11, s, $OCOCH_3$; 1.36, br s, 3×CH_2; 0.92, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(4S,5R,6R)-3-Hydroxymethyl,4-O-isopropylidene-[2-[(E)-hept-1′-enyl]-4-hydroxy-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1-one]

(1-Dehydroeupenoxide acetonide) (99)

To a solution of (85) (100 mg) in dichloromethane (30 ml) was added manganese dioxide (1 g) and the mixture was stirred at room temperature for 24 h. Manganese dioxide was filtered off and washed with dichloro-

methane. The filtrate and the washings were concentrated under reduced pressure and subjected to preparative t.l.c. on silica gel GF_{254}, ether/light petroleum (1:1) to give a pale yellow viscous *oil (99)* (14 mg, 90% based on unrecovered starting material). (Found: *m/z* 292.1675. Calc. for $C_{17}H_{24}O_4$: *m/z* 292.1674.) λ_{max} (EtOH) 216.5 nm (ε 12200), 273(4270). ν_{max} (CCl_4) 1680 cm^{-1} (CO). *m/z* (%) 292(10), 277(2), 234(18), 217(24), 205(27), 192(27), 190(46), 163(31), 149(100), 135(66), 123(22), 121(31), 107(46). ^1H n.m.r. δ($CDCl_3$) 6.08, H-1′, 5.80, H-2′, $^3J_{1',2'}$ 16.5 Hz, $^3J_{2',3'}$ 6.5 Hz; 4.92, br s, H-4; 4.64, br s, CH_2O; 3.69, H-6, 3.48, H-5, $^3J_{5,6}$ 3.5 Hz, $^3J_{5,4}$ 1.1 Hz; 2.34-2.02, m, CH=CH-CH_2; 1.55, s, CCH_3; 1.38, br s, CCH_3 and 3×CH_2; 0.91, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

(4S,5R,6R)-2-[(E)-Hept-1′-enyl]-4-hydroxy-3-hydroxymethyl-5,6-epoxy-cyclohex-2-ene-1-one

(1-Dehydroeupenoxide) (100)

A solution of (99) (50 mg) in glacial acetic acid (1 ml) and water (2 drops) was allowed to stand at room temperature for 4 h. After azeotropic removal of solvent under reduced pressure with toluene, preparative t.l.c. of the residue on silica gel GF_{254}, dichloromethane/methanol (93:7) gave a pale reddish brown viscous *oil (100)* (30 mg, 70%) which readily darkened on storage. (Found: C, 66.74; H, 8.42; *m/z* 252.1362. Calc. for $C_{14}H_{20}O_4$: C, 66.64; H, 7.99%, *m/z* 252.1362.) λ_{max} (EtOH) 266 nm sh (ε 4160), 213(13930). ν_{max} (CCl_4) 3600-3100 cm^{-1} (OH), 1680 (CO). *m/z* (%) 252(30), 236(10), 234(13), 223(8), 221(7), 217(13), 205(89), 177(100), 149(56), 135(40), 123(33), 107(50). ^1H n.m.r. δ($CDCl_3$) 6.12-5.70, m, H-1′ and H-2′; 4.85, br s, H-4; 4.73, 4.46 $^2J_{AB}$ 14.3 Hz, CH_2OH; 3.79, H-6, 3.54, H-5, $^3J_{6,5}$ 3.5 Hz, 2 H exchanged in D_2O, 2×OH; 2.34-1.94, m, CH=CH-CH_2; 1.31, br s, 3×CH_2; 0.87, t, $^3J_{6',7'}$ 6 Hz, CH_2CH_3.

Circular Dichroism (CD)

All CD spectra were carried out at 22 °C in ethanol at concentrations *ca.* 0.01—0.2 mg/ml, cell length 1 cm.

(87): 217 nm ($\Delta\varepsilon$=0), 226(0.79), 237(0), 254(+1.48), 292(0), 344(+0.13), 388(0).

(88): 222 nm ($\Delta\varepsilon$=-1.1), 236(0), 252(+1.54), 288(0), 336(+0.19), 386(0).

(98): 226 nm ($\Delta\varepsilon$=+0.36), 254(0), 275(-0.13), 344(-0.71), 420 (0).

(99): 324 nm ($\Delta\varepsilon$=0), 230(-0.14), 246(0), 269(+1.04), 311(0), 330(-0.15), 390(0).

(100): 215 nm ($\Delta\varepsilon$=-0.91), 229(0), 274(+0.57), 324(+0.12), 342(+0.09), 394(0).

2-(Prop-2'-yn-1'-yloxy)tetrahydropyran (104)

Compound (104) was prepared by acid catalysed condensation of 2,3-dihydropyran and propargyl alcohol according to the known procedure[120]. ν_{max}(neat) 2120 cm^{-1} (C≡C). *m/z* 139(13), 101(7), 85(100). ^{1}H n.m.r. δ($CDCl_3$) 4.8, br s, H-2; 4.23, d, $^4J_{1',3'}$ 2.4 Hz, $OCH_2C{\equiv}C$; 3.96-3.68, m, 1H, 3.62-3.38, m, 1H, CH_2O; 2.46, t, $^4J_{1',3'}$ 2.4 Hz, C≡CH; 2.0-1.4, m, 3×CH_2.

2-(4',4'-Diethoxy-2'-butyn-1'-yloxy)tetrahydropyran (103)

Compound (103) was prepared by the method of Jones and co-workers[120]. ^{1}H n.m.r. δ($CDCl_3$) 5.3, br s, $CH(OEt)_2$; 4.81, br s, H-2; 4.32, br s, $CH_2C{\equiv}C$; 4.0-3.4, m, CH_2O and 2×CH_2CH_3; 2.0-1.4, m, 3×CH_2; 1.23, t, ^3J 7 Hz, 2×CH_2CH_3.

Attempted hydrolysis of (103)

A mixture of compound (103) (4 g) and acetic acid/water (1:1) (25 ml) was stirred at room temperature for 2 days. The mixture was extracted with ether and dried (K_2CO_3). Ether was removed under reduced

pressure and acetic acid was azeotroped off under reduced pressure with toluene. The t.l.c. examination and ^{1}H n.m.r. spectrum of the residue showed numerous products.

2-(1'-Hydroxybut-2'-yn-4'-yloxy)tetrahydropyran (105)

To a stirred solution of (104) (140 g) in tetrahydrofuran (800 ml) cooled to -78° and maintained under a nitrogen atmosphere, was added dropwise *n*-butyllithium in hexane (1 mole, 600 ml). The mixture was allowed to warm to room temperature under continuous stirring. Paraformaldehyde (90 g) was pyrolysed by heating to 180-200° and bubbled through the well stirred reaction mixture with a stream of nitrogen. After stirring the mixture overnight at room temperature, the solvent was removed under reduced pressure and water (600 ml) was added. The mixture was extracted with ether and the ether phase dried (K_2CO_3). Removal of ether under reduced pressure followed by vacuum distillation of the residue gave *compound (105)* (85 g, 80% based on unrecovered starting material, b.p. 110-120°/0.05 mm). (Found: C, 63.10; H, 8.45. $C_9H_{14}O_3$ requires C, 63.51; H, 8.29%.) ν_{max}(neat) 3700-3030 cm^{-1} (OH). *m/z* (%) 169(1.5),115(2), 112(3), 111(3), 101(17), 100(5), 97(6), 85(60), 41(100). ^{1}H n.m.r. δ($CDCl_3$) 4.83, br s, H-2; 4.27, s, $OCH_2C{\equiv}CCH_2O$; 3.98-3.70, 3.68-3.41, 2 m, 2H, CH_2O; 3.23, br s, exchanged in D_2O, OH; 2.0-1.4, m, 3×CH_2. ^{13}C n.m.r. δ($CDCl_3$) 96.75, d, C-2; 84.93, 81.03, 2 br s, C≡C; 61.94, t, C-6; 54.41, 50.65, 2 t, $OCH_2C{\equiv}CCH_2OH$; 30.26, t, C-3; 25.45, t, C-4; 19.09, t, C-5.

2-(1'-Formylprop-2'-yn-3'-yloxy)tetrahydropyran (108)

To a solution of the alcohol (105) (2.5 g) in benzene (470 ml) was added manganese dioxide (30 g) and the mixture was heated under reflux with stirring for 4 h. The catalyst was removed by filtration and washed with benzene then the filtrate and the washings were combined,

concentrated under reduced pressure and purified by preparative t.l.c. silica gel GF_{254}, ether/light petroleum (1:1). Further purification by vacuum distillation gave the *aldehyde (108)* (800 mg, 32%, b.p. 80°/0.1 mm) from which an analytical sample was purified by preparative g.l.c. operating at 185° isothermally. (Found: C, 63.90; H, 7.06. $C_9H_{12}O_3$ requires C, 64.27; H, 7.19%.) (Found: *m/z* 167.0710 M^+-H Calc. for $C_9H_{11}O_3$: *m/z* 167.0708.) λ_{max} (hexane) 375.5 nm (ε 6), 357(45.6), 342(22), 329.5(25.1), 319(23.8), 310 sh (21.7), 258 sh (160.5), 228.4(7250), 220.6(7440), 213(5430). ν_{max}(neat) 2240, 2200 cm^{-1} (C≡C), 1670 (CHO). *m/z* (%) 167(4), 125(0.5), 113(4), 111(1), 110(4), 101(15), 100(20), 97(3), 85(33), 84(40), 83(25), 70(15), 69(17), 68(58), 56(90,19), 55(100), 39(100+). ^{1}H n.m.r. δ($CDCl_3$) 9.24, s, CHO; 4.80, br s, H-2; 4.45, s, $OCH_2C{\equiv}C$; 4.0-3.68, 3.68-3.4, 2 m, CH_2O; 2.0-1.20, m, 3×CH_2. ^{13}C n.m.r. δ($CDCl_3$) 176.22, d, CHO; 97.27, d, C-2; 92.46, s, $C{\equiv}CCHO$; 85.19, s, $C{\equiv}CCHO$; 61.81, t, C-6; 53.76, t, $OCH_2C{\equiv}CCHO$; 30.13, t, C-3; 25.32, t, C-5; 18.83, t, C-4.

2-(1'-Acetoxybut-2'-yn-4'-yloxy)tetrahydropyran (106)

To a stirred solution of the alcohol (105) (85 g) in anhydrous pyridine (124 ml) cooled to 0° was added acetic anhydride (75.5 ml). The mixture was stirred at room temperature overnight, ice (200 g) was added and the mixture extracted with dichloromethane. The extracts were combined, dried (K_2CO_3) and the solvent removed under reduced pressure. Vacuum distillation of the residue gave the *acetate (106)* (98 g, 92.5%) (b.p. 110-112°/0.05 mm). (Found: C, 62.06; H, 7.72. $C_{11}H_{16}O_4$ requires C, 62.25; H, 7.60%). ν_{max}(neat) 1740 cm^{-1} (CO). *m/z* (%) 169(1), 168(1.6), 139(1.4), 111(39), 101(17), 97(7), 86(32), 85(100), 84(95), 83(47), 82(30), 79(30), 55(100), 43(100+). ^{1}H n.m.r. δ($CDCl_3$) 4.76, br s, H-2; 4.66, t, ^4J 1.9 Hz, CH_2OAc; 4.26, br s, $CH_2C{\equiv}CCH_2OAc$; 3.96-3.68, 3.62-3.18, 2 m, CH_2O; 2.06, s, $OCOCH_3$; 2.0-1.4, m, 3×CH_2. ^{13}C n.m.r. δ($CDCl_3$) 169.86, s, $OCOCH_3$; 96.75, d, C-2; 82.85,

79.87, 2 m, C≡C; 61.81, t, C-6; 54.02, 52.20, 2 t, $OCH_2C{\equiv}CCH_2OAc$; 20.65, q, $OCOCH_3$; 30.26, t, C-3; 25.45, t, C-5; 18.96, t, C-4.

4-Acetoxybut-2-ynol (107)

A solution of the acetate (106) (98 g) in 1.0 M acetic acid in methanol (500 ml) was heated under reflux for 3 days. Toluene (500 ml) was added and the mixture was evaporated under reduced pressure. Vacuum distillation of the residue gave the *alcohol (107)* (53 g, 90%, b.p. 93-95°/0.08 mm). (Found: C, 56.53; H, 6.38. $C_6H_8O_3$ requires C, 56.24; H, 6.29%.) ν_{max}(neat) 3700-3100 cm^{-1} (OH), 1740 (CO). *m/z* (%) 128(0.2), 127(0.2), 111(2), 97(4), 86(24), 85(4), 82(21), 71(6), 68(19), 61(9), 57(7), 55(14), 53(3), 52(5), 51(15), 50(9), 43(100). ^{1}H n.m.r. δ($CDCl_3$) 4.68, t, $^5J_{1,4}$ 1.7 Hz, CH_2OAc; 4.27, t, $^5J_{4,1}$ 1.7 Hz, CH_2OH; 2.50-2.24, m, exchanged in D_2O, OH; 2.06, s, $OCOCH_3$. ^{13}C n.m.r. δ($CDCl_3$) 170.90, q s, $OCOCH_3$; 85.58, 79.22, 2 br s, C≡C; 52.46, 50.26, 2 t, CH_2OAc and CH_2OH; 20.65, q, $OCOCH_3$.

4-Acetoxybut-2-ynal (109)

To a solution of the alcohol (107) (8 g) in dichloromethane (1 L) was added manganese dioxide (80 g) and the mixture was stirred at room temperature for 4 days. The catalyst was removed by filtration and washed with dichloromethane. The filtrate and the washings were concentrated under reduced pressure and purified by preparative t.l.c. GF_{254}, ether/light petroleum (1:1). Further purification by molecular distillation gave the *aldehyde (109)* (3 g, 38%, b.p. 30-40°/0.001 mm) from which an analytical sample was obtained by preparative g.l.c. operating at 125° isothermally. (Found: C, 57.12; H, 4.65; *m/z* 126.0319. Calc. for $C_6H_6O_3$: C, 57.14; H, 4.80%; *m/z* 126.0317.) λ_{max} (hexane) 375.0 nm (ε 5.2), 357.5(13.2), 342.3(20.3), 329.5(22.6), 319.0(21.5), 309.5 sh (18.6), 276.5 sh (13.3), 258 sh (98.7), 226(6570), 217.8(7487), 2.215 sh (5108). ν_{max}(neat) 2270, 2200 cm^{-1} (C≡C),

1750 (CO), 1675 (CHO); *m/z* (%) 126(1.5), 125(1), 111(38), 98(2), 97(1.5), 84(100), 70(4.5), 67(3), 66(18), 55(18), 53(13), 50(5), 44(9,4), 43(100+). ^{1}H n.m.r. δ($CDCl_3$) 9.20, s, CHO; 4.84, s, CH_2OAc; 2.10, s, $OCOCH_3$; ^{13}C n.m.r. δ($CDCl_3$) 175.83, d, CHO; 169.73, s, $OCOCH_3$; 89.47, m, C≡C; 51.30, t, CH_2OAc; 20.39, q, $OCOCH_3$.

Attempted Oxidation of Compound (107) with Pyridinium Chlorochromate

A solution of alcohol (107) (52 mg) in anhydrous dichloromethane (2 ml) was added to a mixture of pyridinium chlorochromate[100] (103 mg) and sodium acetate (18 mg) in anhydrous dichloromethane. The mixture was stirred at room temperature for 3 h, anhydrous ether (5 ml) was added. The supernatant was decanted, passed through a short column of Florisil and eluted with ether. Removal of solvent under reduced pressure gave a pale brown residue (34 mg) which showed very little of the required aldehyde in the ^{1}H n.m.r. spectrum.

(E,E)-1,4-bis(Trimethylsilyloxy)buta-1,3-diene (113)

To a stirred solution of the diene (112) (210 mg) in tetrahydrofuran (5 ml), cooled to 0° and maintained under a dry nitrogen atmosphere, was added dropwise methyllithium, 1.7 M in ether (4.2 ml). The mixture was stirred at room temperature for 2 h, then trimethylsilyl chloride (1.1 ml) was added and stirring was continued for 1 h. The mixture was allowed to settle and the supernatant was distilled under reduced pressure to give the *product (113)* (150 mg, 53%, b.p. 120°/0.01 mm) from which an analytical sample was obtained by preparative g.l.c. operating at approx. 180° isothermally. (Found: C, 51.71; H, 9.44; *m/z* 230.1160. Calc. for $C_{10}H_{22}O_2Si_2$: C, 52.12; H, 9.62%; *m/z* 230.1159.) *m/z* (%) 230(65), 215(1), 156(1.5), 75(25), 73(100); metastable 105.8 (230 → 156). ^{1}H n.m.r. δ($CHCl_3$) 6.34, m, H-1 and H-4, 5.56, m, H-2 and H-3, the refined coupling constants are as follows: $^{3}J_{1,2}$ 11.9 Hz, $^{4}J_{1,3}$ 0.6 Hz, $^{3}J_{2,3}$ 11.3 Hz,

$^{5}J_{1,4}$ -0.5 Hz (Error ±0.2 Hz); 0.18, s, 2×Si$(CH_3)_3$. ^{13}C n.m.r. δ($CDCl_3$) 139.86, d, C-1 and C-4; 109.47, d, C-2 and C-3; -0.39, q, 2×OSi$(CH_3)_3$.

This compound decomposes immediately on contact with moisture.

(E,E)-1,4-bis(t-Butyldimethylsilyloxy)buta-1,3-diene (114)

To a stirred solution of the diene (112) (800 mg) in tetrahydrofuran (10 ml), cooled to 0° and maintained under a nitrogen atmosphere, was added dropwise methyllithium, 1.7 M in ether (14 ml). The mixture was heated to reflux and a solution of *t*-butyldimethylsilyl chloride in tetrahydrofuran (10 ml) was added. Refluxing was continued for 1 h, and then a portion of the solvent (approx. 50%) was allowed to distill off. The residue was cooled to room temperature and, with stirring, ether (20 ml) was added, followed by slow addition of water (15 ml). The mixture was extracted with ether and the extract dried (K_2CO_3). Solvent was removed under reduced pressure and other volatile components were removed under reduced pressure at approximately 100°. Vacuum distillation of the residue gave a pale yellow *oil (114)* (1.25 g, 85%, b.p. 170°/0.01 mm) which crystallised in the refrigerator but returned to liquid state at room temperature. The analytical sample was purified by preparative g.l.c. operating at 120-220°, Δ10°/min. (Found: C, 61.27; H, 10.82; *m/z* 314.2098. Calc. for $C_{16}H_{34}O_2Si_2$: C, 61.08; H, 10.89%; *m/z* 314.2098.) λ_{max} (hexane) 210 nm (ε 8300). ν_{max}(neat) 1695 cm^{-1}. *m/z* (%) 314(26), 75(22), 73(100). ^{1}H n.m.r. δ($CHCl_3$) 6.34, m, H-1 and H-4, 5.56, m, H-2 and H-3, the refined coupling constants are as follows: $^{3}J_{1,2}$ 12 Hz, $^{4}J_{1,3}$ 0.7 Hz, $^{5}J_{1,4}$ -0.6 Hz, $^{3}J_{2,3}$ 11.3 Hz; 0.90, s, 18 H, 2×SiC$(CH_3)_3$; 0.12, s, 12 H, 2×Si$(CH_3)_2$.

(E,E)-1,4-Diacetoxybuta-1,3-diene (112)

Compound (112) was prepared by the method of Hill and Carlson[140] and also purchased from Fluka Chemical Company. ^{1}H n.m.r. δ($CDCl_3$) 5.37,

m, H-1 and H-4, 4.95, m, H-2 and H-3, the refined coupling constants are as follows: $^{3}J_{1,2}$ 12.9 Hz, $^{4}J_{1,3}$ 0.9 Hz, $^{3}J_{2,3}$ 11.5 Hz, $^{5}J_{1,4}$ -0.9 Hz (Error ±0.4 Hz); 2.12, s, 2×OCOCH_3. ^{13}C n.m.r. δ($CDCl_3$) 167.39, 2×O$COCH_3$; 137.91, br d, C-1 and C-4; 110.12, br d, C-2 and C-3; 20.52, q, 2×OCOCH_3.

Cycloaddition of compound (112) and compound (108)

A solution of the diene (112) (130 mg) and the dienophile (108) (130 mg) in *m*-xylene (15 ml) was heated under reflux in an atmosphere of nitrogen for 4 days. The mixture was concentrated under reduced pressure, chromatographed on Sephadex LH20, chloroform/methanol (1:1). Fractions which gave a positive colour test with 2,4-dinitrophenylhydrazine were combined, concentrated under reduced pressure and subjected to preparative t.l.c. silica gel GF_{254}, ether/light petroleum (1:1). The two major components both corresponded to starting materials as judged by t.l.c. and a product was isolated as a minor component (5 mg). This product gave a positive colour test[185] for, and also a similar mass spectrum to, *o*-phthalaldehyde. *m/z* (%) 134(65), 105(100), 77(65). The electronic spectrum of the partially purified product was superimposible on that of *o*-phthalaldehyde. The ^{1}H n.m.r. spectra of the product and *o*-phthalaldehyde showed identical peaks in the aromatic and aldehydic regions.

2-Formylbenzylidene diacetate (120)

A mixture of the diene (112) (34 mg) and the dienophile (109) (252 mg) in benzene (0.3 ml) was heated in an evacuated sealed tube at 150° for 24 h. The reaction mixture was extracted with dichloromethane and the combined extracts concentrated under reduced pressure. Preparative t.l.c. silica gel GF_{254}, ether/light petroleum (1:1) of the residue gave the *product (120)* (12 mg, 25%), λ_{max} (EtOH) 321 nm sh (ε 165),

290 sh (1410), 283(1621), 245.5(8607). ν_{max}(neat) 1750 cm^{-1} ($OCOCH_3$), 1700 (CHO). *m/z* 177.0549 M^+-$OCOCH_3$. Calc. for $C_{10}H_9O_3$: *m/z* 177.0552; *m/z* 165.0552 M^+-CH_2CO-CHO. Calc. for $C_9H_9O_3$: *m/z* 165.0552; *m/z* 151.0394 M^+-CH_2CO-CH_3CO. Calc. for $C_8H_7O_3$: *m/z* 151.0395. *m/z* (%) 177(2.5), 176(20), 165(1.5), 151(2), 149(1.5), 135(47), 134(100), 133(40), 105(34), 77(20), 43(95). ^1H n.m.r. δ($CDCl_3$) 10.38, s, CHO; 7.95-786, m, 2H, 7.70-7.46, m, 3H, H-3, H-4, H-5, H-6 and $CH(OAc)_2$; 2.07, s, 2×$OCOCH_3$.

Cycloaddition of compound (114) and compound (108)

A mixture of the diene (114) (35.7 mg) and the dienophile (108) (30 mg) in benzene (25 µl) was heated in an evacuated sealed tube at 110-120° for 3 days. The mixture was subjected to preparative t.l.c., ether/light petroleum (1:2) to give a mixture of products. The major product (8.5 mg) gave a positive colour test[185] for, and also a similar mass spectrum to, *o*-phthalaldehyde. *m/z* (%) 134(66), 105(100), 77(66). The electronic spectrum and the ^1H n.m.r. spectrum in the aromatic and aldehydic regions were identical to those of *o*-phthalaldehyde.

(±)-(3R,6S*)-2-Acetoxymethyl-3,6-bis(trimethylsilyloxy)cyclohexa-1,4-dienecarbaldehyde (121)*

A mixture of the diene (113) (200 mg) and the dienophile (109) (100 mg) was heated in an evacuated sealed tube at 110-120° overnight. A portion of this mixture was distilled at 110-120°/0.03 mm to give a partially purified *product* which showed the following ^1H n.m.r. spectrum. δ($CDCl_3$) 10.02, s, CHO; 5.79, d, 1 Hz spacing, H-4 and H-5; 5.32, 4.82, $^2J_{AB}$ 11 Hz, CH_2OAc; 4.96, d, 2 Hz spacing, H-3 and H-6, 4.74-4.59, m, additional impurities peaks; 2.07, s, $OCOCH_3$; 0.12, 0.09, 0.08, 3 s, 18H, 2×$Si(CH_3)_3$. Analysis by g.l.c.-mass spectrometry at 130-280° Δ10°/min: *peak 1* 134(35), 118(5), 106(80), 105(100), 77(80), 51(30); *peak 2* 194(43), 177(10), 152(64), 135(47), 134(100+), 124(40), 123(34),

121(33), 106(42), 105(65), 96(55), 95(100), 89(31), 78(63), 77(57); *peak 3* 266(6), 251(2), 224(44), 223(50), 209(44), 207(60), 206(57), 135(100+), 134(100+), 105(50), 75(100), 73(100+); *peak 4* 230(43), 158(32), 149(33), 143(27), 129(25), 75(90), 73(100). A small portion was also purified by preparative g.l.c. to give *compound (121)*. *m/z* (%) 356(0.08), 341(0.1), 296(0.5), 281(2), 266(3), 251(2.5), 231(10), 230(46), 224(15), 223(22), 209(14), 207(18), 206(25), 163(10), 135(29), 134(53), 133(20), 177(22), 106(11), 105(28), 77(26), 75(55), 73(97), 43(100).

Methanolysis of compound (121)

Methanol was added to another portion of the mixture from the preparation of (121) and the solution concentrated under reduced pressure and subjected to preparative t.l.c. silica gel GF_{254}, dichloromethane/methanol (100:5) to give the partially purified *product (124)*. ^1H n.m.r. $\delta(CDCl_3)$ 10.02, s, CHO; 6.02, d, 1 Hz spacing, H-4 and H-5; 5.36, 5.06, $^2J_{AB}$ 11 Hz, CH_2OAc; 4.96-4.78, 4.78-4.50, 2 m, H-3 and H-6; 2.12, s, $OCOCH_3$.

(±)-(3R,6S*)-2-Acetoxymethyl-3,6-bis(t-butyldimethylsilyloxy)cyclohexa-1,4-dienecarbaldehyde (125)*

A solution of the diene (114) (105 mg) and the dienophile (109) (190 mg) in benzene (1.5 ml) was heated in an evacuated sealed tube at 115° for 4 days. The mixture was subjected to preparative t.l.c. silica gel PF_{254}, ether/light petroleum (2:1) to give *compound (125)* (93 mg, 68%). (Found: C, 60.05; H, 9.04; *m/z* 440.2414. Calc. for $C_{22}H_{40}O_5Si_2$: C, 59.96; H, 9.15%; *m/z* 440.2414.) λ_{max} (hexane) 332.5 nm (ε 490), 226.5(10900). ν_{max}(neat) 1745 cm^{-1} ($OCOCH_3$), 1694 (CHO). *m/z* (%) 440(0.5), 383(3), 381(4), 365(4), 351(3), 341(7), 325(20), 324(31), 323(100), 315(10), 314(28), 251(100+), 209(100+), 207(18), 181(13), 177(13), 151(12), 117(100+), 75(100+), 43(100+); metastables 272.4

(383 → 323), 174.0 (251 → 209), 156.8 (209 → 181), 135.2 (323 → 209), 109.1 (209 → 151), 54.5 (251 → 117), 48.1 (117 → 75). ^{1}H n.m.r. δ($CHCl_3$) 10.04, s, CHO; δ_A 5.88, δ_B 5.86, H-4 and H-5, δ_C 4.99, δ_D 4.72, H-3 and H-6; the refined coupling constants are tabulated below:

$^3J_{AB}$	10.2 Hz	10.2 Hz
$^3J_{AC}$	1.5 Hz	3.3 Hz
$^4J_{AD}$	-3.3 Hz	-1.5 Hz
$^4J_{BC}$	-3.1 Hz	-1.6 Hz
$^3J_{BD}$	1.6 Hz	3.1 Hz
$^5J_{CD}$	5.3 Hz	5.3 Hz

5.37, 4.83, $^2J_{AB}$ 11 Hz, CH_2OAc; 2.04, s, $OCOCH_3$; 0.91, 0.86, 2 s, 2×$SiC(CH_3)_3$; 0.13, 0.15, 2 s, 2×$Si(CH_3)_2$. ^{13}C n.m.r. δ($CDCl_3$) 191.42, d, CHO; 170.24, s, $OCOCH_3$; 144.80, s, C-1; 137.13, s, C-2; 128.43, 127.52, 2 d, C-4 and C-5; 61.68, 63.89, 2 d, C-3 and C-6; 59.22, t, CH_2OAc; 25.71, q, $OCOCH_3$; 20.78, q, 2×$SiC(CH_3)_3$; 18.05, s, 2×$SiC(CH_3)_3$; -4.29, -3.90, 2 q, 2×$Si(CH_3)_2$.

meso-*Dimethyl 3,6-bis(t-butyldimethylsilyloxy)cyclohexa-1,4-diene-1,2 dicarboxylate (128)*

A solution of (114) (314 mg) and dimethyl acetylenedicarboxylate (280 mg) in benzene (0.8 ml) was heated in an evacuated sealed tube at 115° for 20 h. The mixture was evaporated under reduced pressure and vacuum distilled to give the *product (128)* (335 mg, 75%, b.p. 180°/0.01 mm) which could be further purified by preparative t.l.c. silica gel PF_{254}, ether/light petroleum (1:1). (Found: C, 57.45; H, 8.69. $C_{22}H_{40}O_6Si_2$ requires C, 57.86; H, 8.83%). λ_{max} (hexane) 306.5 nm (ε 588), 282.5(578), 273.5 sh (488). ν_{max}(neat) 1730 cm^{-1} ($COOCH_3$). *m/z* 441(4), 425(0.5), 424(0.4), 409(0.5), 401(14), 400(31), 399(100), 397(5), 393(1), 381(1), 367(12), 341(4), 340(10), 339(34), 314(3), 75(28), 73(46); metastable: 228.0

(399 → 339). ^{1}H n.m.r. δ($CHCl_3$) $\delta_{AA'}$, 5.83, m, H-4 and H-5, $\delta_{XX'}$, 4.87, m, H-3 and H-6; the refined coupling constants are as follows: $^{3}J_{AA'}$, 5.2 Hz, $^{3}J_{AX}$ 2.3 Hz, $^{4}J_{AX'}$, -0.7 Hz, $^{5}J_{XX'}$, 0 Hz; 3.78, s, 2×CO_2CH_3; 0.87, s, 2×$SiC(CH_3)_2$; 0.14, 0.08, 2 s, 2×$Si(CH_3)_3$. ^{13}C n.m.r. δ($CDCl_3$) 167.13, s, 2×CO_2CH_3; 135.58, s, C-1 and C-2; 127.78, d, C-4 and C-6; 63.37, d, C-3 and C-6; 52.07, q, 2×CO_2CH_3; 25.71, q, 2×$SiC(CH_3)_3$; 18.05, s, 2×$SiC(CH_3)_3$; -4.54, -4.16, 2 q, 2×$Si(CH_3)_2$.

meso-*Dimethyl 3,5-diacetoxycyclohexa-1,4-diene-1,2-dicarboxylate (115)*

A solution of the diene (112) (850 mg) and dimethyl acetylenedicarboxylate (1.4 g) was heated in *m*-xylene (15 ml) under reflux for 6 h. After removal of solvent under reduced pressure, the residue was crystallised from cyclohexane/hexane (2:1) to give colourless *crystals (115)* (m.p. 75-80°, lit. 64-67°[141]). The ^{1}H n.m.r. spectrum of the product was identical with the published spectrum of (115).[141] ^{13}C n.m.r. δ($CDCl_3$) 169.73, 165.31, 2 s, 2×CO_2CH_3 and 2×$OCOCH_3$; 134.28, C-1 and C-2; 125.97, C-4 and C-5; 62.85, C-3 and C-6; 52.59, 2×CO_2CH_3; 20.65, 2×$OCOCH_3$.

Dimethyl 3-acetoxyphthalate (116)

To a solution of compound (115) (225 mg) in methanol (5 ml) was added sodium bicarbonate (360 mg), benzonitrile (2.25 ml) and 30% hydrogen peroxide (2.35 ml). The mixture was stirred at room temperature for 3 days and then filtered. The filtrate was evaporated under reduced pressure to give the residue which was subjected to preparative t.l.c. silica gel GF_{254}, dichloromethane/methanol (93:7) to give a colourless *oil (116)* (41 mg, 23%). λ_{max} (MeOH) 303 nm, 235 sh. ν_{max}(neat) 1730 cm^{-1} (CO_2CH_3), ($OCOCH_3$). *m/z* (%) 210(38), 179(54), 178(66), 148(22), 147(19), 120(100), 119(26); metastables

150.9 (210 → 178), 123.1 (178 → 148), 97.3 (148 → 120). ^{1}H n.m.r. δ($CDCl_3$) 6.82-7.42, m, H-4, H-5 and H-6; 3.82, 3.80, 2 s, 2×CO_2CH_3; 1.21, s, $OCOCH_3$.

(±)-(1R,4S*,5R*,6S*)-3-Acetoxymethyl-1,4-bis(t-butyldimethylsilyloxy)-2-formyl-5,6-epoxycyclohex-2-ene (129)*

Method A — Compound (125) (44 mg), *m*-chloroperoxybenzoic acid (25 mg) and 4,4′-thiobis(6-*t*-butyl-4-methylphenol) were heated under reflux in 1,2-dichloroethane (10 ml) for 3 h. After removal of solvent under reduced pressure, the residue was subjected to preparative t.l.c., silica gel PF_{254}, ether/light petroleum (1:1) to give starting material (125) (26 mg, 59%) and a *product (129)* of higher polarity (2 mg, 5%). *m/z* (%) 399(0.08), 369(0.6), 341(0.4), 339(0.8), 327(0.4), 311(0.8), 299(3), 207(100), 75(61), 73(56).

Method B — Compound (125) (44 mg), molybdenum hexacarbonyl (4.5 mg), 4,4′-thiobis(6-*t*-butyl-4-methylphenol) (0.5 mg) and *t*-butylhydroperoxide (47 mg) in benzene (5 ml) were heated under reflux in a nitrogen atmosphere for 6 h. The mixture was concentrated under reduced pressure and purified by preparative t.l.c., firstly on silica gel PF_{254}, ether/light petroleum (1:2), and secondly on neutral alumina F_{254}, using the same solvent, to give *compound (129)* (9 mg, 20%).

Method C — Compound (125) (500 mg), finely powdered *p*-nitroperoxybenzoic acid (500 mg) and 4,4′-thiobis(6-*t*-butyl-4-methylphenol) (4 mg) in chloroform were heated under reflux for 3 days. The mixture was cooled to 0°, filtered and washed with cold chloroform. The filtrate and the washings were concentrated under reduced pressure and subjected to preparative t.l.c., silica gel PF_{254}, ether/light petroleum (1:2) to

give the *compound (129)* (136 mg, 26%). (Found: C, 58.24; H, 8.98. $C_{22}H_{40}O_6Si_2$ requires C, 57.86; H, 8.43%.) λ_{max} (hexane) 229.5 nm (ε 8100). ν_{max}(neat) 1745 cm^{-1} ($OCOCH_3$), 1690 (CHO). *m/z* 399.1653 M^+–57. Calc. for $C_{18}H_{31}O_6Si_2$: *m/z* 399.1658; *m/z* (%) 399(4), 381(5), 339(82), 327(5), 311(8), 267(15), 265(20), 207(44), 179(42), 147(36), 117(74), 75(100), 73(100+), 43(45). ^{1}H n.m.r. δ($CDCl_3$) 10.10, s, CHO; δ_A 5.04, m, H-1, δ_B 4.73, m, H-4, δ_X 3.32, δ_Y 3.26, symmetrical m, H-5 and H-6; the refined coupling constants are tabulated below;

$^5J_{AB}$	±1.6 Hz	±1.3 Hz
$^3J_{AX}$	+2.3 Hz	+1.0 Hz
$^4J_{AY}$	-1.0 Hz	-2.3 Hz
$^4J_{BX}$	-1.0 Hz	-2.3 Hz
$^3J_{BY}$	+2.3 Hz	+1.0 Hz
$^3J_{XY}$	±3.6 Hz	±3.6 Hz

5.35, 4.73, $^2J_{AB}$ 13 Hz, CH_2OAc; 2.08, s, $OCOCH_3$; 0.91, 0.86, 2 s, 2×$SiC(CH_3)_3$; 0.20, s, 9H, 0.10, s, 3H, 2×$Si(CH_3)_2$.

meso-*Dimethyl* t-*3*,t-*6-bis*(t-*butyldimethylsilyloxy*)-r-*4*,c-*5-epoxy-cyclohex-1-ene-1,2-dicarboxylate (131)*

Method A — Compound (128) (46 mg), molybdenum hexacarbonyl (5 mg), 4,4′-thiobis(6-*t*-butyl-4-methylphenol) (0.5 mg) and *t*-butylhydroperoxide (60 mg) in benzene (5 ml), were heated under reflux for 8 h. The mixture was concentrated under reduced pressure and subjected to preparative t.l.c., neutral alumina F_{254}, ether/light petroleum (1:2) to give *compound (131)* (10 mg, 21%) and compound (139) (12 mg, 25%).

Method B — Compound (128) (76) mg), *p*-nitroperoxybenzoic acid (60 mg) and 4,4′-thiobis(6-*t*-butyl-4-methylphenol) (2 mg) in chloro-

form (10 ml) were heated under reflux for 5 days. The mixture was cooled to 0°, filtered, and washed with cold chloroform. The filtrate and the washings were concentrated under reduced pressure and subjected to preparative t.l.c., neutral alumina F_{254}, ether/light petroleum (1:2) to give *compound (131)* (17 mg, 22%).

Method C — Compound (128) (100 mg), *o*-nitroperoxybenzoic acid (120 mg), 4,4′-thiobis(6-*t*-butyl-4-methylphenol) (2 mg) in chloroform (10 ml) were heated under reflux for 3 days. The mixture was cooled to 0°, filtered and washed with cold chloroform. The filtrate and the washings were concentrated under reduced pressure and subjected to preparative t.l.c., silica gel PF_{254}, ether/light petroleum (1:2) to give *compound (131)* (15 mg, 14%) and compound (140) (24 mg, 25%). Compound (131) was a colourless *oil*. (Found: C, 56.33; H, 8.55. $C_{22}H_{40}O_7Si_2$ requires C, 55.90; H, 8.53%.) ν_{max}(neat) 1730 cm^{-1}(CO_2CH_3). *m/z* 457.2081 M^+-CH_3. Calc. for $C_{21}H_{37}O_7Si_2$: *m/z* 457.2077; *m/z* (%) 457(3.5), 417(14), 416(30), 415(100), 399(7), 397(11), 267(10), 127(16), 89(27), 75(24), 73(53). ^{1}H n.m.r. δ($CHCl_3$) 4.65, t, 1.5 Hz spacing, H-3 and H-6; 3.28, t, 1.5 Hz spacing H-4 and H-5, $^3J_{3,4} = {}^4J_{3,5} = \pm 1.5$ Hz, $^5J_{3,6} = {}^3J_{4,5}$ = unknown but the same magnitude and sign; 3.76, s, 2×CO_2CH_3; 0.89, s, 2×$SiC(CH_3)_3$; 0.16, 0.10, 2 s, 2×$Si(CH_3)_2$.

(±)-(4S,5S*)-2,5-bis(t-Butyldimethylsilyloxy)-4-hydroxy-6-methylene-cyclohex-2-en-1-one (136)*

Compound (125) (440 mg), *o*-nitroperoxybenzoic acid (550 mg) and 4,4′-thiobis(6-*t*-butyl-4-methylphenol) (4 mg) in chloroform (50 ml) were heated under reflux for 4 h. The mixture was cooled to 0°,

filtered and washed with cold chloroform. The filtrate and the washings were eluted through a short column of Florisil with ether. The eluant was concentrated under reduced pressure and subjected to preparative t.l.c., firstly on silica gel PF_{254}, ether/light petroleum (1:2), and secondly on neutral alumina F_{254}, ether/light petroleum (1:3) to give compound (129) (10 mg, 2%) and *compound (136)* (50 mg, 13%). (Found: C, 59.66; H, 9.26; *m/z* 384.2151. Calc. for $C_{19}H_{36}O_4Si_2$: C, 59.33; H, 9.43%; *m/z* 384.2152.) λ_{max} (hexane) 279 nm (ε 1930), 244 sh (1300). ν_{max} ($CHCl_3$) 3700-3200 cm^{-1} (OH), 1680 (CO), 1630 (C=C). *m/z* (%) 384(1), 369(5), 227(100), 196(4), 195(9), 167(11), 75(22), 73(57). ^1H n.m.r. δ($CHCl_3$) 6.08, m, 2H, 5.65, m, 1H, H-3 and $C=CH_2$; 4.34, m, H-4 and H-5; 2.64-2.28, m, exchanged in D_2O, OH; 0.96, 0.94, 2 s, $2\times SiC(CH_3)_3$; 0.19, 0.18, 0.16, 0.12, 4 s, $2\times Si(CH_3)_2$. Homonuclear decoupling by irradiating at δ 4.34 caused the signal centred at δ 5.65 to collapse into a doublet and the signal centred at δ 6.08 to collapse into a doublet at δ 6.11 (1H) and a singlet at δ 6.06 (1H). Irradiation at δ 5.65 or δ 6.08 caused the multiplet at δ 4.34 to collapse into a simpler multiplet. ^1H n.m.r. δ[$CDCl_3$+Eu(fod)$_3$] 6.90, d, $^3J_{3,4}$ 2.7 Hz, H-3; 6.53, 5.99, $^2J_{AB}$ 2 Hz, $^4J_{1',5}$ 2 Hz, $C=CH_2$; 4.80-4.62, m, $^3J_{4,3}$ 2.7 Hz, $^3J_{4,5}$ 7.5 Hz, H-4; 4.46-4.30, m, $^3J_{5,4}$ 7.5 Hz, $^4J_{5,1'}$ 2 Hz, H-5; 1.06, 0.99, 2 s, $2\times SiC(CH_3)_2$. ^{13}C n.m.r. δ($CDCl_3$) 189.86, s, CO; 152.72, 148.95, 2 s, C-2, C-6; 131.30, d, C-3; 125.19, t, $C=CH_2$; 82.07, 76.75, 2 d, C-4 and C-5; 30.26, q, $2\times SiC(CH_3)_3$; 22.86, s, $2\times SiC(CH_3)_3$; 4.67, q, $2\times Si(CH_3)_2$.

Dimethyl 3-(t-butyldimethylsilyloxy)phthalate (139)

The aromatic by-product obtained from the preparation of compound (131)

was vacuum distilled (b.p. 160°/0.001 mm) to give a pale yellow *liquid (139)*. (Found: C, 58.93; H, 7.54. $C_{16}H_{24}O_5Si$ requires C, 59.23; H, 7.46%.) λ_{max} (hexane) 292.3 nm (ε 22600), 242 sh (47500). ν_{max} (neat) 1730 cm^{-1} (CO_2CH_3). *m/z* 267.0691 M^+-57. Calc. for $C_{12}H_{15}O_5Si$: 267.0688; *m/z* (%) 309(5), 293(12), 267(100), 237(3), 235(2), 75(1.5), 73(7); metastables 210.4 (267 → 237), 206.8 (267 → 235). ^1H n.m.r. δ($CDCl_3$) δ_A 7.57, δ_B 7.28, δ_C 7.01, $^3J_{AB}$ 7.7 Hz, $^3J_{BC}$ 8.0 Hz, $^4J_{AC}$ 1.2 Hz, H-4, H-5, H-6; 3.92, 3.88, 2 s, 2×CO_2CH_3; 0.98, s, $SiC(CH_3)_3$; 0.20, s, $Si(CH_3)_2$.

Dimethyl 3,6-bis(t-butyldimethylsilyloxy)phthalate (140)

A by-product from the preparation of compound (131) by Method C was isolated as a colourless waxy *solid (140)*. (Found: C, 58.11; H, 8.24; *m/z* 454.2199. Calc. for $C_{22}H_{38}O_6Si_2$: C, 58.11; H, 8.42%; *m/z* 454.2207.) λ_{max} (hexane) 306.5 nm (ε 3500). ν_{max} (CCl_4) 1740 cm^{-1} (CO_2CH_3). *m/z* 397.1506. Calc. for $C_{18}H_{29}O_6Si_2$: *m/z* 397.1502; *m/z* (%) 454(0.15), 453(0.12), 439(3), 423(5), 397(100), 325(2.5), 89(9), 75(2), 73(15), 59(3). ^1H n.m.r. δ($CDCl_3$) 6.75, s, H-5 and H-6; 3.76, s, 2×CO_2CH_3; 0.95, s, 2×$SiC(CH_3)_3$; 0.16, s, 2×$Si(CH_3)_2$.

Aromatic Compound (141)

In the preparation of compound (129) by Method C, an aromatic compound was isolated (10%). The ^1H n.m.r. spectrum of this product was superimposible with that of the major product obtained by Method C when acidic chloroform was used. The product was purified by preparative t.l.c. PF_{254}, ether/light petroleum (1:2), followed by vacuum distillation (b.p. 170°/0.001 mm) to give a pale brownish-yellow *liquid (141)*. λ_{max} (hexane) 307 nm (ε 1840), 249 (5670). ν_{max}(neat) 1740 cm^{-1} ($OCOCH_3$), 1700 (CHO). *m/z* 251.0739 M^+-57. Calc. for $C_{12}H_{15}O_4Si$: *m/z* 251.0739; *m/z* (%) 265(2), 251(100), 209(73), 179(3), 117(92), 75(52), 73(25), 43(29);

metastable 174.0 (251 → 209). ^{1}H n.m.r. δ($CDCl_3$) 10.24, s, CHO; δ_A 7.41, δ_B 7.31, δ_C 7.08, $^3J_{AB}$ 6 Hz, $^3J_{BC}$ 5.9 Hz, $^4J_{AC}$ 1.7 Hz, 3H, aromatic protons; 5.52, s, CH_2OAc; 2.02, s, $OCOCH_3$; 1.01, s, $SiC(CH_3)_3$; 0.13, s, $Si(CH_3)_2$.

(±)-(1R,4S*,5R*,6S*)-3-Acetoxymethyl-1,4-bis(t-butyldimethylsilyloxy)-2-[(Z)-hept-1'-enyl]-5,6-epoxycyclohex-2-ene (144)*

To a suspension of finely powdered triphenylphosphonium bromide (300 mg) in anhydrous ether (25 ml) cooled to 0°, was added *n*-butyllithium, 1.7 M in hexane (0.4 ml). The resulting orange mixture was stirred at room temperature for 3 h. The stirred mixture was cooled to -78°, the aldehyde (129) (126 mg) was added and the mixture allowed to warm to room temperature. Ether (20 ml) was added and the mixture filtered. To the filtrate was added water (20 ml) and the mixture extracted with ether. The ether extract was washed with water until the aqueous phase became neutral, dried ($MgSO_4$), then concentrated under reduced pressure to give a yellow oil. Purification by preparative t.l.c., silica gel PF_{254}, ether/light petroleum (1:3) gave the *product (144)* (90 mg, 70%). (Found: C, 64.27; H, 9.94; *m/z* 524.3350. Calc. for $C_{28}H_{52}O_5Si_2$: C, 64.07; H, 9.99%; *m/z* 524.3353). λ_{max} (hexane) end absorption from 270 nm. ν_{max}(neat) 1745 cm^{-1} ($OCOCH_3$). *m/z* (%) 524(0.2), 509(0.3), 506(0.2), 495(0.2), 467(20), 465(2), 451(3.5), 449(5), 407(25), 393(2), 389(4), 335(13), 328(18), 305(4.5), 304(3), 293(6), 275(22), 259(15), 191(14), 149(20), 147(33), 117(68), 75(68), 73(100). ^{1}H n.m.r. δ($CDCl_3$) 5.76, H-1', 5.67, H-2', $^3J_{1',2'}$ 11.5 Hz, $^3J_{2',3'}$ 7 Hz; 4.67, 4.47, $^2J_{AB}$ 12 Hz, CH_2OAc; 4.64, 4.31, H-1 and H-4; 3.26, br s, H-5 and H-6; 2.28-1.75, m, $CH{=}CH{-}CH_2$; 2.04, s, $OCOCH_3$; 1.27, br s, $3\times CH_2$; 0.86, 0.84, 0.86, 3 s, $2\times SiC(CH_3)_3$ and CH_2CH_3; δ($CHCl_3$) 0.14, 0.12, 0.10, 0.08, 4 s, $2\times Si(CH_3)_2$.

(±)-(1R,4S*,5R*,6S*)-3-Acetoxymethyl-1,4-bis(t-butyldimethylsilyloxy)-2-[(E)-hept-1'-enyl]-5,6-epoxycyclohex-2-ene (145)*

A solution of compound (144) (80 mg) in iodine/hexane (0.1 mg/ml) (25 ml) was cooled to 0° and irradiated with a pyrex filtered medium pressure mercury lamp for 7 h. The mixture was concentrated under reduced pressure and subjected to preparative t.l.c., silica gel PF_{254}, ether/light petroleum (1:3) to give a mixture of *Z,E* isomers in the ratio of 3:1 as indicated by the ^1H n.m.r. spectrum. Crystallisation of the mixture from methanol/water (9:1) gave colourless *needles (145)* (40 mg, 50%, m.p. 60°). (Found: C, 64.05; H, 9.70; *m/z* 524.3352. Calc. for $C_{28}H_{52}O_5Si_2$: C, 64.07; H, 9.99%; *m/z* 524.3353.) λ_{max} 243 nm (ε 20650). ν_{max} (CCl_4) 1745 cm^{-1} ($OCOCH_3$), 1650 (C=C). *m/z* 495.3322. Calc. for $C_{27}H_{51}O_4Si_2$: *m/z* 495.3326; *m/z* 467.2648. Calc. for $C_{24}H_{43}O_5Si_2$: *m/z* 467.2649; *m/z* (%) 524(0.2), 509(0.1), 506(0.15), 495(0.8), 467(0.5), 464(6), 451(3.5), 449(3), 446(2), 415(3), 407(29), 393(4), 379(4), 335(9), 305(15), 293(7), 275(20), 247(3), 219(5), 205(7), 201(8), 191(18), 149(11), 147(32), 117(60), 75(78), 73(100), 59(8). ^1H n.m.r. δ($CHCl_3$) 6.19, H-1', 6.01, H-2', $^3J_{1',2'}$ 15 Hz, $^3J_{2',3'}$ 6 Hz; 4.83, 4.58, $^2J_{AB}$ 12.2 Hz, CH_2OAc; 4.75, 4.63, 2 br s, H-1 and H-4; 3.31, 3.24, 2 br s, $^3J_{5,6}$ 3.8 Hz, H-5 and H-6; 2.26-1.94, m, $CH{=}CH{-}CH_2$; 2.04, s, $OCOCH_3$; 1.54-1.10, m, $3{\times}CH_2$; 0.90, 0.86, 2 s, $2{\times}SiC(CH_3)_3$ and CH_2CH_3; δ($CHCl_3$) 0.16, 0.14, 0.13, 0.06, 4 s, $2{\times}Si(CH_3)_2$.

(±)-(3S,4R*,5S*,6R*)-Dimethyl 3-(t-butyldimethylsilyloxy)-6-hydroxy-4,5-epoxycyclohex-1-ene-1,2-dicarboxylate (147)*

From preparation of compound (148) a partially desilylated *by-product (147)* (3 mg, 19%) was isolated. *m/z* (%) 343(3), 327(6), 301(100), 75(62), 73(25). ^1H n.m.r. δ($CHCl_3$) 4.85, t, 1.5 Hz spacing, H-3 and H-6, 3.27, t, 1.5 Hz spacing, H-4 and H-5; $^3J_{3,4}=^4J_{4,6}=\pm1.5$ Hz, $^5J_{3,6}=^3J_{4,5}$ = unknown, but same magnitude and sign; 3.76, s, $2{\times}CO_2CH_3$; 1.54, br s, exchanged in D_2O, OH; 0.90, s, $SiC(CH_3)_3$; 0.16, 0.10, 2 s, $Si(CH_3)_2$.

meso-Dimethyl t-3,t-6-dihydroxy-r-4,c-5-epoxycyclohex-1-ene-1,2-dicarboxylate (148)

Compound (131) (19 mg) in acetic acid/tetrahydrofuran/water (3:2:1) 6 ml was heated under reflux for 2 days. Toluene (10 ml) was added and the mixture was concentrated under reduced pressure and purified by preparative t.l.c. on silica gel PF_{254}, dichloromethane/methanol (95:5) to give *compound (148)* (6 mg, 48%) (m.p. 144-145° subl.) (Found: C, 49.02; H, 5.07. $C_{10}H_{12}O_7$ requires C, 49.18; H, 5.07%.) ν_{max}(neat) 3500-3200 cm^{-1} (OH), 1730 (CO_2CH_3). *m/z* (%) 213(100), 197(47), 194(87), 169(96), 165(85), 143(99), 142(60), 141(85), 139(85), 59(91). ^1H n.m.r. δ(CD_3OD) 4.72, t, 1.5 Hz spacing, H-3 and H-6; 3.38, t, 1.5 Hz spacing, H-4 and H-5; $^3J_{3,4}=^4J_{4,6}=\pm1.5$ Hz, $^5J_{3,6}=^3J_{4,5}$ = unknown, but the same sign and magnitude; 3.78, s, 2×CO_2CH_3.

(±)-(1R,4S*,5R*,6S*)-3-Acetoxymethyl-2-[(Z)-hept-1'-enyl]-5,6-epoxycyclohex-2-en-1,4-diol (149)*

Compound (144) (8 mg) in acetic acid/tetrahydrofuran/water (3:2:1) (12 ml) was heated under reflux for 2 days. After azeotropic removal of the solvent with toluene, the residue was subjected to preparative t.l.c. silica gel PF_{254}, dichloromethane/methanol (97:3) to give *compound (149)* (2.5 mg, 63%). λ_{max} (EtOH) end absorption from 270 nm. *m/z* (%) 296(5), 279(10), 254(5), 249(10), 236(45), 218(37), 207(47), 190(20), 189(25), 177(33), 165(85), 161(40), 149(50), 147(100). ^1H n.m.r. δ($CDCl_3$) 5.89-5.78, m, $^3J_{1',2'}$ 11.5 Hz, H-1' and H-2'; 4.71, 4.62, 4.58, 3 br s, CH_2OAc, H-1 and H-4 and 2×OH; 3.46, br s, H-5 and H-6; 2.09, s, $OCOCH_3$; 1.92-1.52, m, $CH=CH-CH_2$; 1.25, br s, 3×CH_2; 0.88, br s, CH_2CH_3.

(±)-(1R,4S*,5R*,6S*)-2-[(Z)-hept-1'-enyl]-3-hydroxymethyl-5,6-epoxycyclohex-2-en-1,4-diol [(±)-Z isomer of eupenoxide] (150)*

A stirred solution of compound (149) (2.5 mg) in methanol (2 ml) was cooled to 0° and then saturated with ammonia. The mixture was stirred at room temperature overnight and then evaporated to dryness. Preparative

t.l.c. silica gel GF_{254}, dichloromethane/methanol (93:7) gave *compound (150)* (2 mg, 90%). (Found: *m/z* 254.1517. Calc. for $C_{14}H_{22}O_4$: 254.1518.) λ_{max} (EtOH) end absorption from 265 nm. *m/z* (%) 254(4), 236(6), 223(21), 207(35), 189(25), 177(100), 165(62), 149(55), 147(43), 137(49), 133(58), 123(84), 107(80), 91(86). ^{1}H n.m.r. δ($CDCl_3$) 5.85-5.27, m, $^3J_{1',2'}$ 11.5 Hz, H-1′ and H-2′; 4.75, 4.47, 4.32, 4.18, 3.82, m, CH_2OH, H-1 and H-4; 3.44 br s, H-5 and H-6; 1.95-1.92, m, CH=CH-CH_2, 3×OH; 1.26, br s, 3×CH_2; 0.89, br s, CH_2CH_3.

(±)-(1R,4S*,5R*,6S*)-1,4-bis(t-Butyldimethylsilyloxy)-2-[(E)-hept-1′-enyl]-3-hydroxymethyl-5,6-epoxycyclohex-2-ene (151)*

A stirred solution of the acetate (145) (40 mg) in methanol (4 ml) was cooled to 0° and saturated with ammonia. The mixture was allowed to warm to room temperature and stirring was continued overnight. Removal of the solvent under reduced pressure and preparative t.l.c. silica gel PF_{254}, ether/light petroleum (1:3) gave a colourless *oil (151)* (27 mg, 98% based on recovered starting material). (Found: C, 64.50; H, 10.52. $C_{26}H_{50}O_4Si_2$ requires C, 64.68; H, 10.44%.) λ_{max} (hexane) 242.5 nm (ε 19200). ν_{max} (neat) 3650-3100 cm^{-1} (OH); 1650 (C=C). Found *m/z* 451.3066. Calc. for $C_{25}H_{47}O_3Si_2$: 451.3064. Found *m/z* 425.2542. Calc. for $C_{22}H_{41}O_4Si_2$: 425.2543; *m/z* (%) 482(1.5), 467(0.5), 464(0.3), 451(9), 425(11), 407(4.5), 397(5), 395(2), 333(6), 320(3), 305(4), 293(31), 265(8), 263(5), 179(6), 177(2), 173(3), 149(16), 147(28), 75(60), 73(100). ^{1}H n.m.r. δ($CDCl_3$) 6.24, H-1′, 6.02, H-2′, $^3J_{1',2'}$ 15.5 Hz, $^3J_{2',3'}$ 6 Hz; 4.79, 4.69, 2 br s, H-1 and H-4; 4.26, 4.18, $^2J_{AB}$ 12 Hz, CH_2OH; 3.31, 3.24, $^3J_{5,6}$ 3.6 Hz, H-5 and H-6; 2.28-2.0, m, CH=CH-CH_2; 1.40-1.20, m, exchanged in D_2O, OH; 1.5-1.1, br s, 3×CH_2; 0.91, 0.86, 2 s, 2×$SiC(CH_3)_3$ and CH_2CH_3; δ($CHCl_3$) 0.21, s, 6H, 0.13, s, 3H, 0.08, s, 3H, 2×$Si(CH_3)_2$.

(±)-(1R,4S*,5R*,6S*)-2-[(E)-hept-1'-enyl]-3-hydroxymethyl-5,6-epoxy-cyclohex-2-en-1,4-diol [(±)-Eupenoxide)] (152)*

To a solution of compound (151) (34 mg) in acetic acid (8 ml) was added water (2 ml), and the mixture stirred at 43° overnight. After azeotropic removal of the solvent with toluene, the residue was subjected to preparative t.l.c. silica gel PF_{254}, dichloromethane/methanol (93:7) to give the *product (152)* (17 mg, 99%). (Found: *m/z* 254.1514. Calc. for $C_{14}H_{22}O_4$: 254.1518.) λ_{max} (EtOH) 240.7 nm. *m/z* (%) 254(27), 236(70), 223(15), 218(14), 207(8), 189(22), 183(42), 177(30), 165(92), 147(65), 137(67), 123(86), 107(65), 91(100). A stirred solution of compound (152) (4 mg) in methanol (4 ml) cooled to 0° was saturated with ammonia. The mixture was allowed to warm to room temperature and stirring was continued overnight. The mixture was concentrated under reduced pressure and the residue was subjected to preparative t.l.c. silica gel PF_{254}, dichloromethane/methanol (93:7) to give the *product (152)* (2.5 mg). ^1H n.m.r. $\delta(CD_3OD)$ except for signals due to approximately 5% impurities was superimposable with that of natural eupenoxide.

(±)-(1R,4S*,5R*,6S*)-3-Acetoxymethyl-2-[(E)-hept-1'-enyl]-5,6-epoxy-cyclohex-2-en-1,4-diol [(±)-eupenoxide monoacetate)] (153)*

The by-product obtained from the preparation of compound (152) was a colourless *oil (153)*; *m/z* (%) 296(11), 279(3), 278(4), 249(19), 236(32), 218(33), 207(39), 190(21), 189(23), 177(26), 165(58), 161(28), 147(83), 119(62).

(1R,4S,5R,6S)-3-Acetoxymethyl-2-[(E)-hept-1'-enyl]-5,6-epoxycyclohex-2-en-1,4-diol (154)

To a solution of natural eupenoxide (81) (18 mg) in acetic acid (8 ml) was added water (2 ml) and the mixture was stirred overnight at 43°. After azeotropic removal of the solvent with toluene, the residue was subjected to preparative t.l.c. silica gel PF_{254}, dichloromethane/methanol (93:7) to give

a colourless oil, eupenoxide (**81**) (8 mg, 44%) and a pale brown *oil (154)* (2 mg, 10%). ν_{max} (CCl_4) 3600-3100 cm^{-1} (OH), 1740 ($OCOCH_3$), 1670 (C=C). *m/z* (%) 296(5), 278(8), 249(16), 236(28), 218(49), 207(37), 190(21), 189(22), 177(18), 165(63), 161(27), 147(100), 119(62). ^1H n.m.r. $\delta(CD_3OD)$ 6.36, H-1′, 6.14, H-2′, $^3J_{1',2'}$ 16 Hz, $^3J_{2',3'}$ 6 Hz; 4.90, s, 4.64, 4.46, 2 br s, CH_2OAc, H-1 and H-4; 2.40-1.80, m, $CH{=}CH{-}CH_2$; 2.03, s, $OCOCH_3$; 1.36, br s, $3{\times}CH_2$; 0.90, t, CH_2CH_3. $\delta(C_6D_6)$ 6.54-5.90, m, H-1′ and H-2′; 4.72, 4.55, 2 br s, 4.40-3.60, m, CH_2OAc, H-1, H-4, and $2{\times}OH$; 3.36, br m, H-5 and H-6; 2.30-1.90, m, $CH{=}C{-}CH_2$; 1.67, s, $OCOCH_3$; 1.24, br s, $3{\times}CH_2$; 0.88, t, CH_2CH_3.

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