PARTIAL SYNTHESIS OF NOVEL TRICHOTHECENE MYCOTOXINS

by

Gerard Hugh Thomas

A thesis in part fulfilment for the degree of Ph.D.

Chemistry Department
University of Glasgow
GLASGOW G12 8QQ

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I would also like to thank my colleagues and friends for the innumerable good times shared over the past seven years.

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Thanks also to my mother and my sister Claire for helping in the typing of this thesis.

Finally I would like to dedicate this thesis to my late father for always realising the importance of his son's education.

Summary

The partial syntheses of two novel trichothecene homologues, (13R)-3α,4β,15-triacetoxy-13-methyl-12,13-epoxytrichothec-9-ene (11) and (13S)-3α,4β,15-triacetoxy-13-methyl-12,13-epoxytrichothec-9-ene (12), from the readily available trichothecenes DAS (1) (4β,15-diacetoxy-3α-hydroxy-12,13-epoxytrichothec-9-ene) and MAS (2) (3α,15-dihydroxy-4β-acetoxy-12,13-epoxytrichothec-9-ene) are detailed. These were carried out in order to provide further insight into the mode of action of the trichothecene mycotoxins.

In addition, a model study was conducted which involved the synthesis of the bicyclo[3.2.1]octane epoxy alcohol (96) and the bicyclo[3.2.1]octane epoxy triethylsilyl ethers (110) and (111) from 2-methyl cyclohexanone.

Abbreviations

Ac : acetyl

Bu : butyl

DBU : 1,8-diazabicyclo[5.4.0]undec-7-ene

DEAD : diethylazodicarboxylate

DMAP : 4-dimethylaminopyridine

DMF : N,N-dimethylformamide

DMS : dimethylsulphide

mCPBA : meta-chloroperbenzoic acid

NBS : N-bromosuccinimide

PPTS : pyridinium p-toluene sulphonate

PTSA : p-toluenesulphonic acid

Py : pyridine

TBAF : tetrabutylammonium fluoride

TESCl : triethylsilyl chloride

THF : tetrahydrofuran

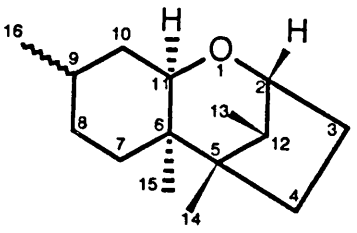
THP : tetrahydropyranyl

TMS : tetramethylsilane

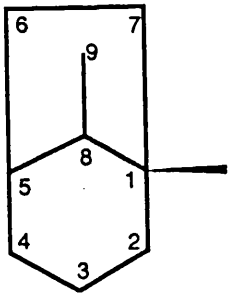
TMSCl : chlorotrimethylsilane

Numbering System

The numbering system of [trichothecene] tricyclic molecules in this thesis follows the conventional trichothecene numbering system as shown.



The model compounds are numbered as indicated.



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1. Introduction

Introduction

Chapter 1.1 12,13-Epoxytrichothecenes

In recent years, people have become increasingly concerned about environmental hazards, including 'chemicals' which they perceive to be dangerous to health. One area of particular concern is chemicals in the food chain - meaning man-made additives and agrochemical residues, rather than the chemicals that are, inevitably, naturally present. In fact many natural chemicals can cause health problems.

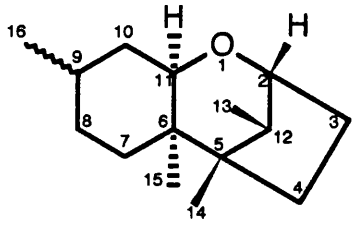
Mycotoxins are the secondary metabolic products of certain fungi that are toxic to man and domestic animals. While not strictly components of foods, but contaminants, the mycotoxins have been of considerable interest to natural product chemists, toxicologists and even the pharmaceutical industry. The contamination of certain foods by this class of compounds can be almost inevitable, caused by the close association of the mycotoxin-producing moulds with the food.

The trichothecene mycotoxins are a group of naturally occurring sesquiterpenoid secondary metabolites produced in the main by species of fungi called Fusarium but a relatively small number are also produced by species of different genera. As a group of mycotoxins the trichothecene compounds have a wide variety of biological

effects which include toxicity to plants and toxic effects on man and animals.

Research in the field of trichothecenes in the last 10 years has been an area of intense interest which cannot be disputed. If one looks at the years 1982 and 1983, there were more than 500 publications and 30 reviews dealing with the subject and no less than 9 research groups with U.S. government funding were pursuing the chemical synthesis of naturally occurring trichothecenes.

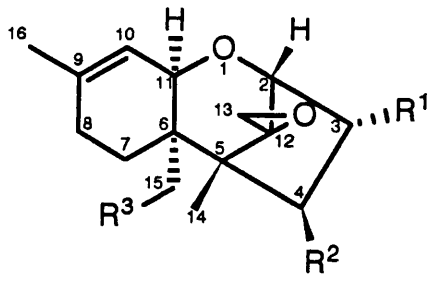
Figure 1



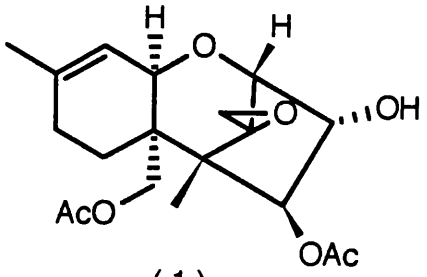
Trichothecane

The name for the structure of the tricyclic skeleton composed of cyclohexane, cyclopentane and 6-membered oxygen-containing ring, and four methyl groups was proposed as "trichothecane" by Godtfredsen[1] et al in 1967 (Figure 1), and since almost all naturally occurring compounds contain an epoxide ring at C-12,13 and a double bond at C-9,10 they are therefore characterised as 12,13-epoxytrichothec-9-enes. The name scirpenes is also used for 12,13-epoxytrichothec-9-enes. The structure and numbering system of the trichothecene skeleton are as shown (Figure 2).

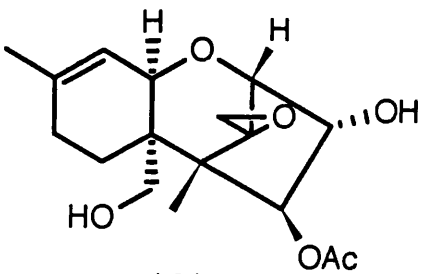
Figure 2



The trichothecenes are divided into two groups depending on the presence or absence of a macrocyclic ring linkage between C-4 and C-15, namely macrocyclic and non-macrocyclic trichothecenes. This thesis will be concerned only with non-macrocyclic trichothecenes and more specifically with the two naturally occurring 12,13-epoxytrichothec-9-enes called DAS (1) and MAS (2) which are the starting materials for the partial synthesis detailed in this thesis and which are obtained in this University by the culturing of Fusarium species.



(1)



(2)

DAS (1), formally known as Anguidine, was the first trichothecene isolated from a fungus of the genus Fusarium scirpi.[2] It is also known as diacetoxyscirpenol,

because it contains one hydroxyl and two acetoxyl groups in the molecule. MAS (2), on the other hand, contains one acetoxyl and two hydroxyl groups in its structure and is thus named monoacetoxyscirpenediol.

The fact that MAS (2) is obtained alongside DAS (1) from culture is unsurprising since results have shown that acylated trichothecenes, produced at an early stage of fungal growth, are deacylated during further growth.[3] The structural distinction is encountered at C-15 where the acetoxyl group of DAS (1) becomes the hydroxyl group of MAS (2).

It is the wide array of diverse biological properties exhibited by this family of mycotoxins which make them of considerable interest from both a health and economic standpoint.

No group of mycotoxins is more important because of their effects on domestic animals and man, with the possible exception of the aflatoxins. As a group the trichothecenes have a diverse biological spectrum which includes antifungal, antibacterial and antiviral activity as well as insecticidal and phytotoxic behaviour.

The trichothecenes have also been implicated in a number of human[4,5] and economically important animal[6,7,8] mycotoxicoses arising from the ingestion of mouldy grain. Potentially the most important and the most extensively studied property of the trichothecenes however, is their cytostatic activity. They are acutely

toxic in whole animal studies; DAS (1) itself exhibits cytopathogenic effects against baby hamster kidney cells at a concentration of 1.5 ng/ml.[9] In the early 1980's DAS (1) also completed phase II clinical trials, conducted by the National Cancer Institute of the United States, against cancer of the colon and breast.[10]

The toxicity of DAS (1) has also been studied in greater detail because of past interest in it as an anti-tumour agent. It has been shown to have significant activity on L-1210 and P-388 leukaemia in mice. Preclinical toxicological studies were performed on dogs and monkeys and significant side-effects were observed. These included vomiting, nausea, diarrhoea, anaemia and feed refusal. Severe skin irritation including inflammation and scabbing was also noticed.

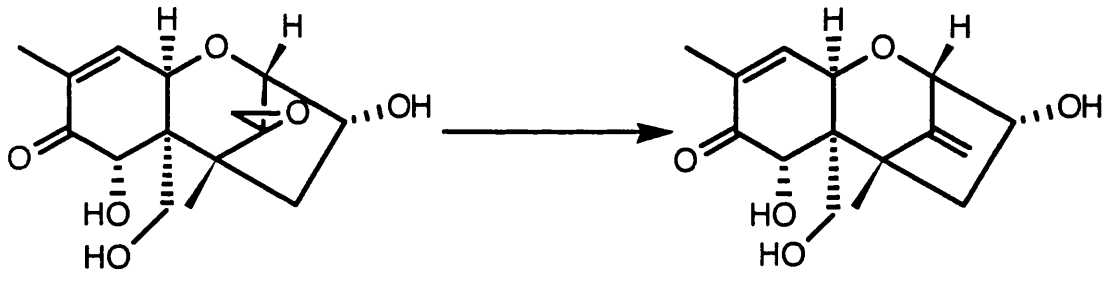
Investigations into the mechanism of action of the trichothecenes[11] have shown that protein and DNA synthesis are inhibited.[12] Surveys which followed these findings clarified that all the 12,13-epoxytrichothecenes possess a potent inhibitory activity on protein synthesis in eukaryotic cells and that the residual groups of functionality for example, on carbons 3, 4 and 15, play an important role in their relative activity.[13]

Although the basis of their mode of action at the molecular level is still unclear, Ueno has presented evidence[14] that the trichothecenes react with thiol residues at the active site of the enzyme peptidyl

transferase. If as suggested trichothecenes act as bio-alkylating agents then they must undergo nucleophilic substitution, the most obvious position for which, is the spiro 12,13-epoxide function. It is thought to be the electrophilic site responsible for this reactivity since removal of it by deoxygenation, nullifies the cytostatic activity.[9]

It was revealed by metabolism studies with rats *in vivo*[15] and with bovine rumen fluid *in vitro*,[16] the predominant biological transformation of the trichothecene deoxynivalenol (3) was deoxygenation to form the 9,12-diene (4) and that this was a possible detoxification route (Scheme 1).[17]

Scheme 1

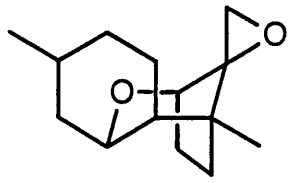


(3) (4)

It is now well-established however, that the epoxide unit is very unreactive under S_N2 conditions.[18] Inspection of the trichothecene structure (Figure 3) shows that steric shielding of C-13 by the C-8 methylene and/or the C-16 methyl group, depending on the conformation of

the cyclohexenyl system, probably accounts for this low reactivity.

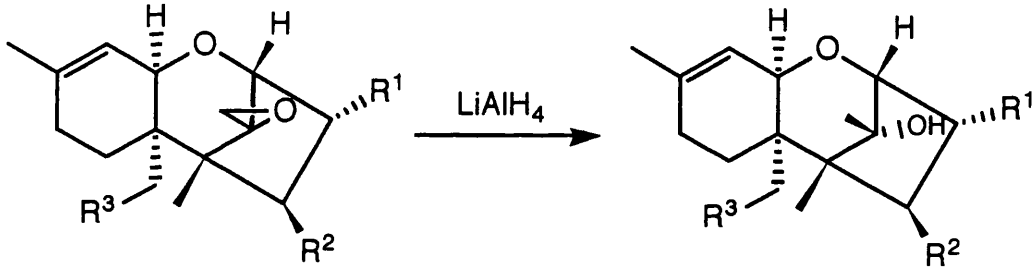
Figure 3



Note : C-15 omitted for clarity

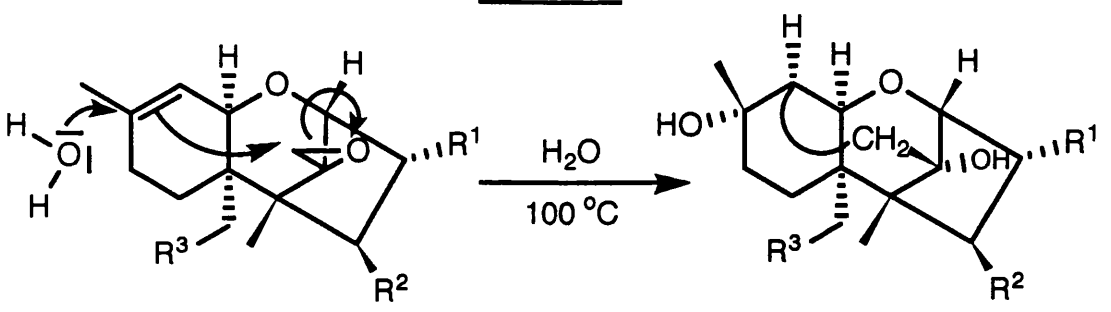
The one general exception to this is the reaction of trichothecenes with $LiAlH_4$ that affords tertiary alcohol derivatives in good yield (Scheme 2).[19] As expected the reductive opening of the epoxide ring gave products which showed very low cytotoxicity.[20]

Scheme 2



Neighbouring group assisted reactions readily occur in the trichothecene series however; prolonged boiling in water gives a hydrate with cleavage of the epoxide ring and formation of a new bond between C-10 and C-13 (Scheme 3).

Scheme 3

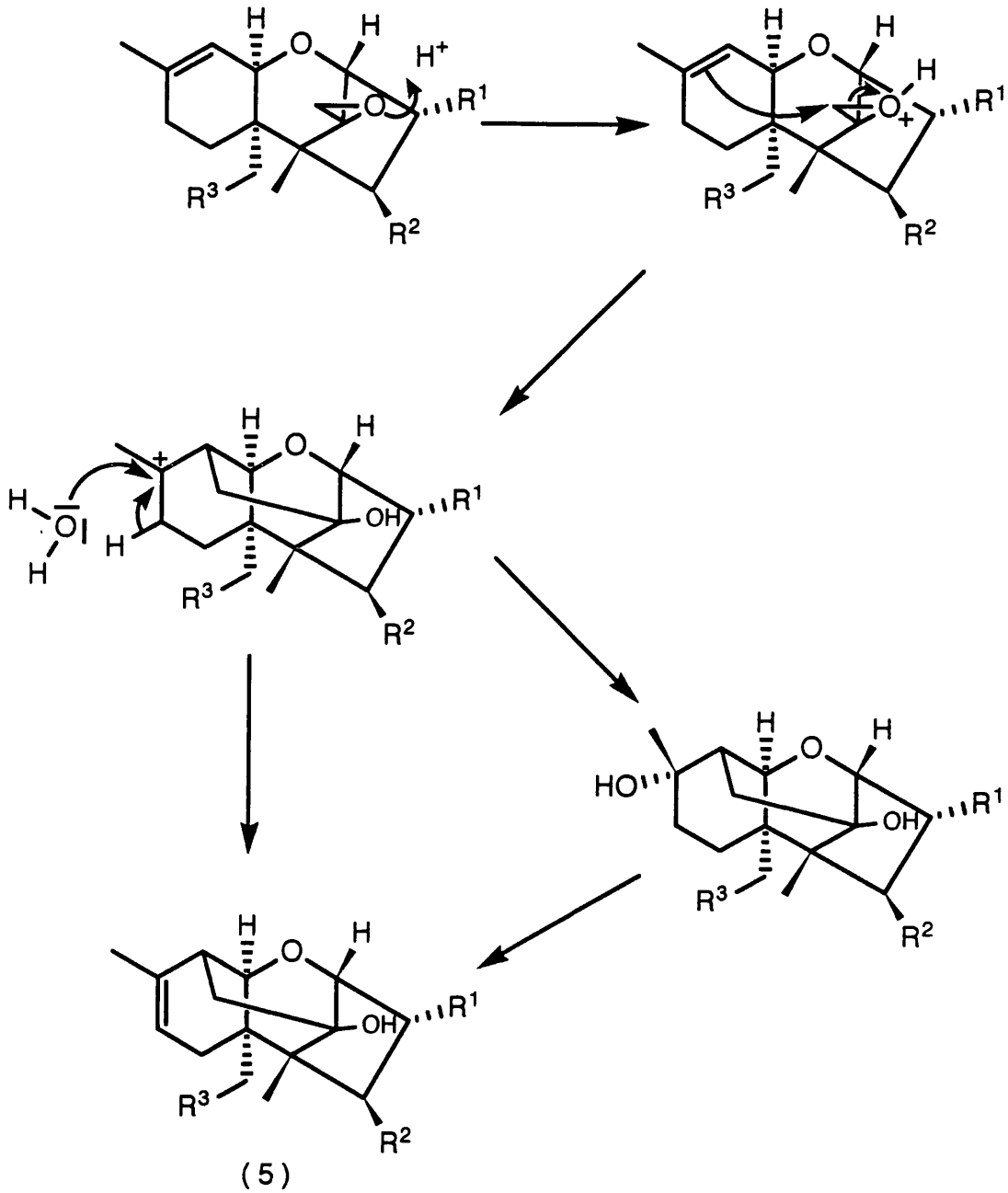


In acidic media, protonation of the epoxide is usually followed by intramolecular rearrangement of the tetracyclic carbon skeleton with the reaction being concluded by the addition of a nucleophile at a more accessible centre.

One such rearrangement occurs readily in water at pH5 at 100°C. This is called the 12,13-epoxy-trichothec-9-ene to 10,13-cyclotrichothecane rearrangement (Scheme 4).[21] Protonation of the spiro epoxide is followed by intramolecular attack by the π electron system of the 9,10 double bond. The reaction is then completed either by the addition of a nucleophile (e.g. OH^-) at the axial 9α position which may subsequently be eliminated, or by the loss of an 8-H proton to give the 8-ene (5) directly. This rearrangement is unaffected by the nature of the substituents R_1, R_2 and R_3 and leads to biologically inactive products.

Under strongly acidic conditions, on the other hand, 12,13-epoxytrichothec-9-enes undergo a facile

Scheme 4

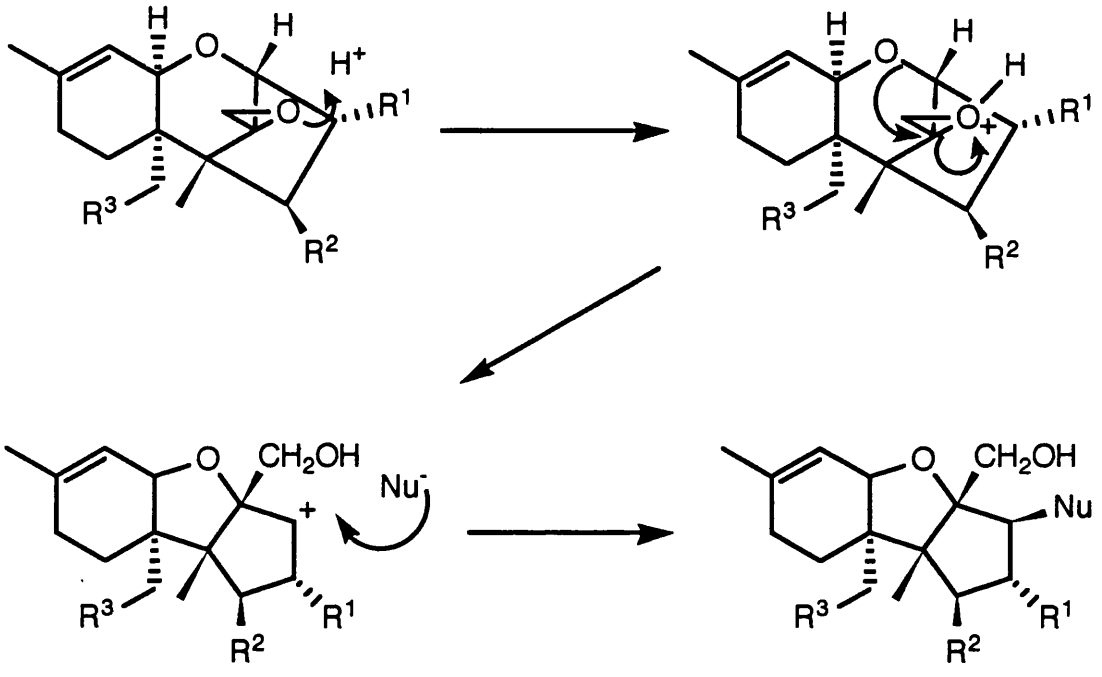


(5)

rearrangement to biologically inactive apo-trichothecenes (Scheme 5).[22] X-Ray studies have shown that the O(1)-C(2) and C(12)-O bonds are approximately co-planar and antiparallel, so protonation of the epoxide, followed by

intramolecular rearrangement via attack by the pyran oxygen at C-12, leads to a contraction of ring B and the generation of a cation at C-2, the nucleophilic capture of which yields the apotrichothecene nucleus (6). Such nucleophilic capture may also be an important step in the manifestation of biological activity. The 9,10 double bond plays no part in this rearrangement unlike the previous one.

Scheme 5

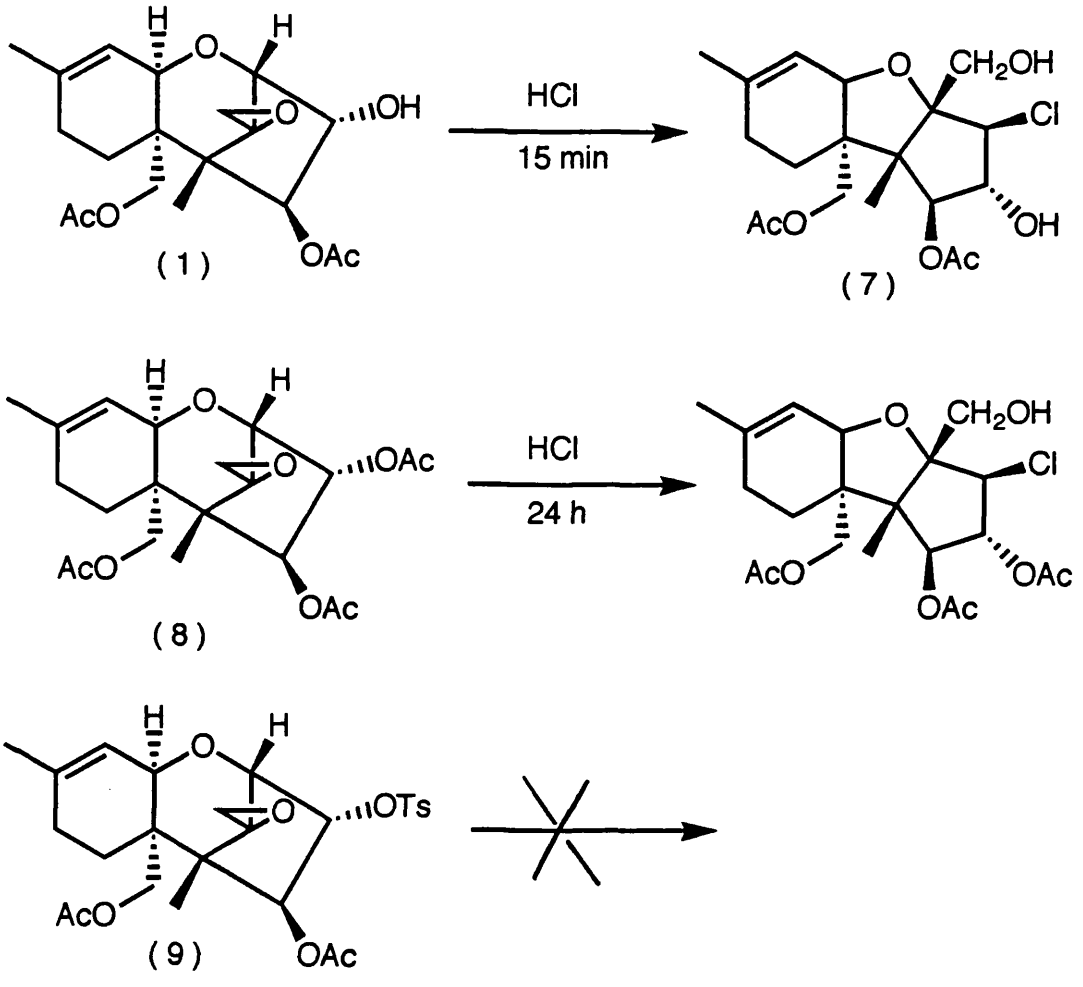


(6)

Rearrangement under these strongly acidic conditions is inhibited by the presence of bulky substituents at the 3α position. DAS (1) is smoothly converted into the apo-derivative (7) by HCl in 15 minutes. 3α,4β,15-Triacetoxy-12,13-epoxytrichothec-9-ene

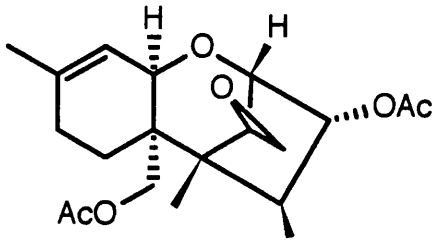
(8) requires 24 hours to undergo the same rearrangement whereas 3α-tosyl,4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (9) is unaffected after 24 hours (Scheme 6). This is probably due to non-bonded interactions between the 3α substituents and the 11-α proton adversely influencing the geometry of the transition state.

Scheme 6



The rationale for proposing such intramolecular rearrangements, however, demands the correct

stereochemistry. Studies of epimeric epoxides[23] derived from ring A-aromatic trichothecene-related compounds demonstrated the necessity for the epoxide oxygen to be anti to ring A for a similar rearrangement to occur.



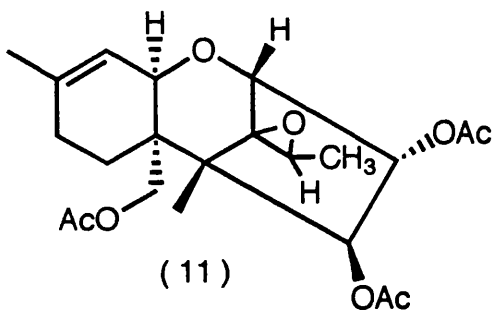
(10)

The synthesis of 3α,4β,15-triacetoxy-12,13-epi-epoxytrichothec-9-ene (10)[24] from the natural trichothecene MAS (2) was one of the first trichothecene analogues that was prepared with the 'unnatural' epoxide configuration, where the epoxide oxygen is syn to ring A. When this was compared to its natural isomer (8), it was shown to be around 270 times less active, and thus essentially non-toxic.[25] This finding emphasises the necessity of the naturally orientated epoxide unit in exhibiting cytostatic activity and fuels the belief that the biological mode of action is related to the mode of chemical reactivity in the acid-catalysed trichothecene to apotrichothecene rearrangement.

In connection with these studies of the metabolism and biological mode of action of trichothecenes, and because it seems to be the first main step in the biological degradation of the trichothecenes

by rumen micro organisms[16] and in mammalian systems,[15] deoxygenation of the epoxide became a synthetic transformation of great interest.

Efficient deoxygenation of the epoxide was seen as a cornerstone reaction, it was to prove crucial in the partial synthesis detailed in this thesis from the point of view of introducing functionality at C-13. The novel trichothecene mycotoxins (11), (12) and (13) which were the target compounds of the present work are closely related synthetic analogues to the natural trichothecenes, differing only in that two of them contain an 'extra' methyl group at C-13 and the other in which the epoxide oxygen was replaced by a different heteroatom, that of nitrogen.



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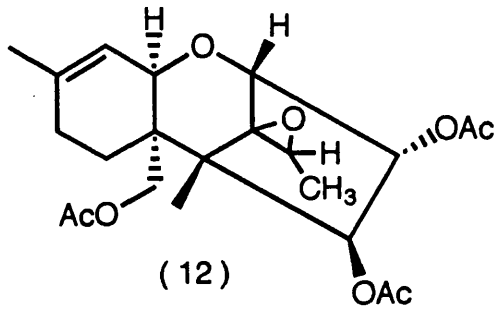

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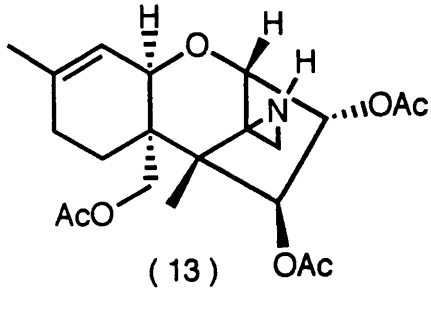


(13)

In order to produce these novel analogues by partial synthesis from the natural trichothecenes DAS (1) and MAS (2) a second extremely important synthetic transformation was that of ozonolysis. Both of these techniques, epoxide deoxygenation and ozonolysis, will be examined in greater detail in subsequent chapters.

A brief examination of the spectroscopic features is helpful in building up a picture of the characteristics of the trichothecene series. The determination of the structures of novel or newly isolated trichothecenes is made very much easier mainly due to the extensive use of nuclear magnetic resonance spectroscopy.

In high field proton NMR the resonances can be clearly assigned using decoupling techniques and because of the rigidity of the trichothecane skeleton, the results from homonuclear n.O.e. experiments also help in assignments. Characteristic features of the proton NMR spectra include an AB quartet arising from the two protons at C-13 of the 12,13 epoxide, and at C-15 where the two protons also give rise to an AB quartet when there is an oxygen containing substituent on that carbon atom, although this signal is sometimes seen as a singlet. A broad doublet is seen corresponding to 10-H but it also shows a small allylic coupling to the signal from the methyl group at C-16.

The rigid nature of five-membered ring C in the trichothecene series ensures that the relevant coupling

constants are affected more by the electronegativity of the substituents than by changes in the dihedral angle. The chemical shift of 2-H is not affected very much by 3α substitution but suffers some deshielding and moves downfield in a 12-ene. Downfield shifts of 14-H protons are also seen in compounds containing a 12-13 double bond.

^{13}C NMR spectroscopy is also an increasingly useful tool in structure determination as more sophisticated and improved techniques are introduced. In the 12,13-epoxytrichothec-9-enes it can be seen that apart from the expected downfield shifts of the carbon that carries the substituent and of the adjacent atoms, the introduction of an OR group produces a number of effects at longer range. These include upfield shifts of C-14 in the presence of a 4β-OR group and of C-7 and C-11 in the presence of a 15-OR group.

It was apparent from early mass spectrometry studies that electron impact mass spectra are complex and that there is no simple fragmentation pattern that will allow either the ready identification of the trichothecene ring system or the distinction between isomeric trichothecenes. The mass spectra normally show fragment peaks resulting from losses of acyl groups and also of water, methyl and hydroxymethyl groups in the higher mass region. Mass spectrometry is therefore not the most useful analytical tool where trichothecenes are concerned.

The trichothecenes are chemically stable

colourless, crystalline, optically active, sparingly soluble in water, but soluble in most organic solvents at room temperature. If the correct safety precautions are observed, with respect to their high toxicity, then the handling of these compounds is relatively straightforward.

Chapter 1.2 Epoxide deoxygenation

The deoxygenation of epoxides to olefins (Figure 4) constitutes a process of importance in both synthesis and structure determination of natural products. A reliable, one-step operationally simple technique for deoxygenating epoxides in high yield under gentle conditions, which would be compatible with certain functionalised systems, was required in the partial synthesis of the novel trichothecenes (11), (12) and (13). Epoxides or oxiranes are also key intermediates in the stereochemical inversion of olefins.

Figure 4

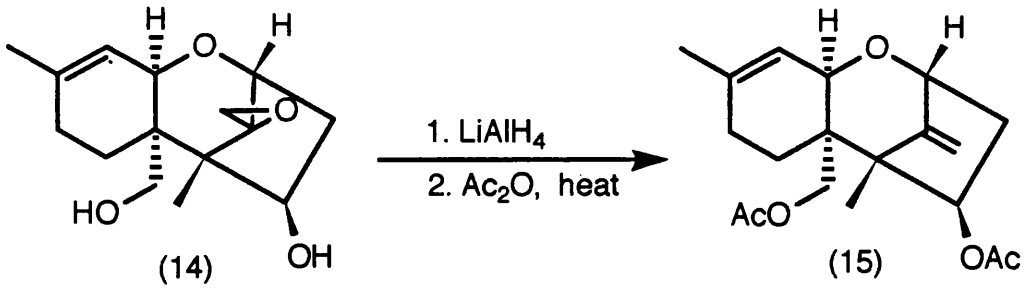
Epoxide deoxygenation or retro-epoxidation is a synthetic transformation of great interest in the trichothecene series since it appears to constitute the first step, except for deacylation and the modification of ester side chains, in their biological degradation both by rumen micro organisms[16] and in mammalian systems.[15]

The classical methods of deoxygenating epoxides include their transformation into iodohydrins and reducing

these with zinc,[26] or treating epoxides with triphenyl phosphine[27] or triethyl phosphite,[28] which proceeds via nucleophilic opening followed by a four centre elimination of an intermediate oxaphosphetane. Alternatively, lithium diphenyl phosphite can be used and the product quaternised with methyl iodide prior to elimination.[29]

The lack of reactivity of the 12,13 epoxide function towards external nucleophilic attack,[18] and its contrasting high lability towards intramolecular rearrangement under acidic conditions, imposes severe constraints on potential epoxide deoxygenating systems.

Scheme 7

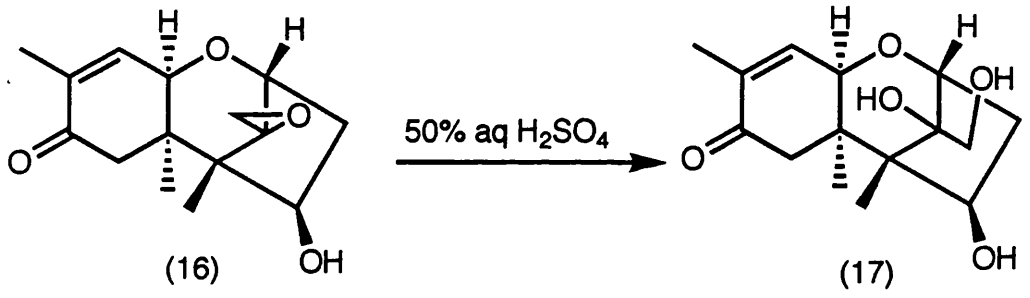


Bimolecular substitution reactions of the trichothecene 12,13 epoxide unit are extremely rare. The first synthesis of a trichothec-9,12-diene was reported by Gutzwiller and co-workers[30] who converted verrucarol (14) into 12,13 deoxyverrucarol diacetate (15) by $LiAlH_4$ reduction followed by acylation of the tertiary alcohol intermediate under forcing conditions (Scheme 7). The elimination reaction, however, proceeded in very poor

yield and so the method is not a very useful one.

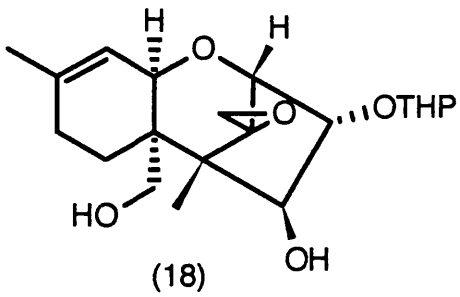
Czechoslovakian workers have reported[31] that hydrolysis of trichothecelone (16) with 50% aqueous H_2SO_4 afforded the corresponding 12,13-diol (17) (Scheme 8). Attempts at reproduction of this by Roush[18] under analogous conditions using DAS (1) afforded only products containing the apotrichothecene nucleus (6).

Scheme 8

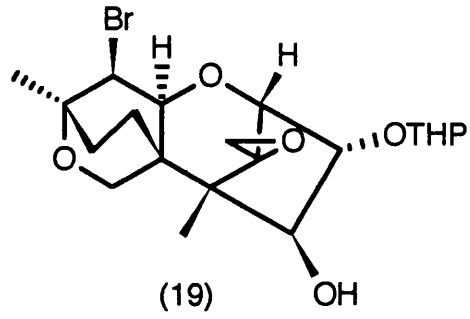


Roush made many attempts to deoxygenate the THP ether (18) and its derived bromo-ether (19) believing that the hydroxymethyl bridge in the latter would help in changing the conformation of ring A thus making the epoxide more accessible to external nucleophilic attack. No reaction was observed however, when (19) was treated with KSeCN in MeOH or EtOH at reflux or DMSO at 100°C.[32] Tamm had previously reported[33] unsuccessful attempts to deoxygenate verrucarol (14) using the same reagent system.

Starting material was recovered when $KSiMe_3$ in HMPT[34] was used. This suggests that the initial attack did not take place since potassium β-alkoxysilanes are



(18)

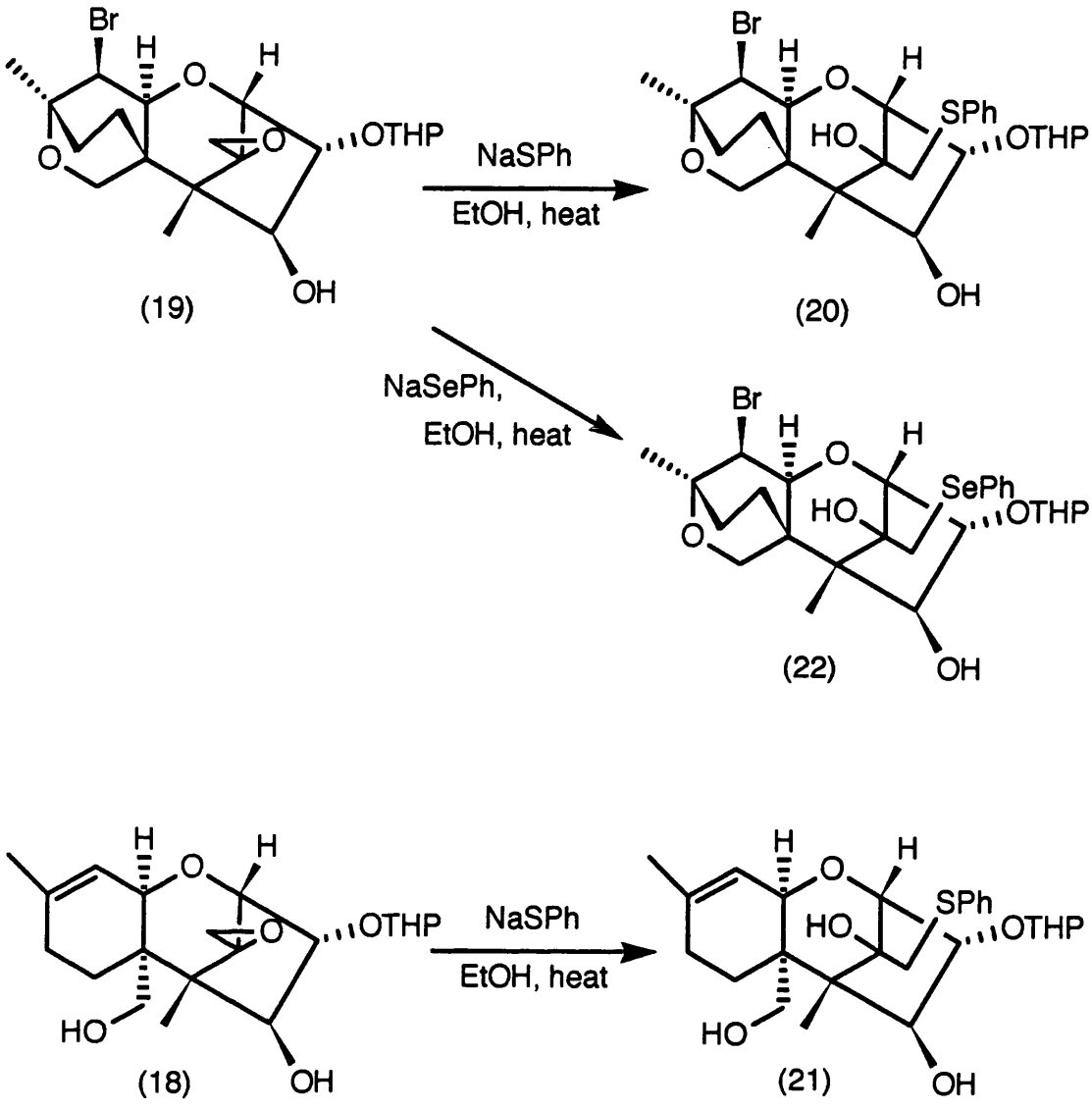


(19)

known to undergo rapid elimination at room temperature. Returned starting material was also obtained when sodium diethyl phosphorotelluroate in EtOH[35] and $Fe(CO)_5$ in tetramethyl urea[36] were used.

A degree of success was achieved by Roush when the bromo-ether (19) was treated with PhSNa in ethanol at reflux (Scheme 9).[18] The reaction was extremely slow, and required an enormous excess of thiophenoxide; PhSeNa in ethanol similarly gave the seleno adduct (22). Under analogous conditions the THP ether (18) was converted into the thiophenyl adduct in slightly lower yield. The adducts (20) and (21) were oxidised using $KHSO_5$ in MeOH in the presence of pH7 phosphate buffer,[37] or with m-CPBA in dichloromethane to give the corresponding sulphones (23) and (24) which were reduced directly using 4-5% Na/Hg amalgam in methanol to give the diene (25) (Scheme 10), the β-elimination occurring with concommitant removal of ring A protection. Interestingly no epoxidation of the 9,10-double bond of (21) was observed when m-CPBA was used as the oxidant. The major byproduct of the

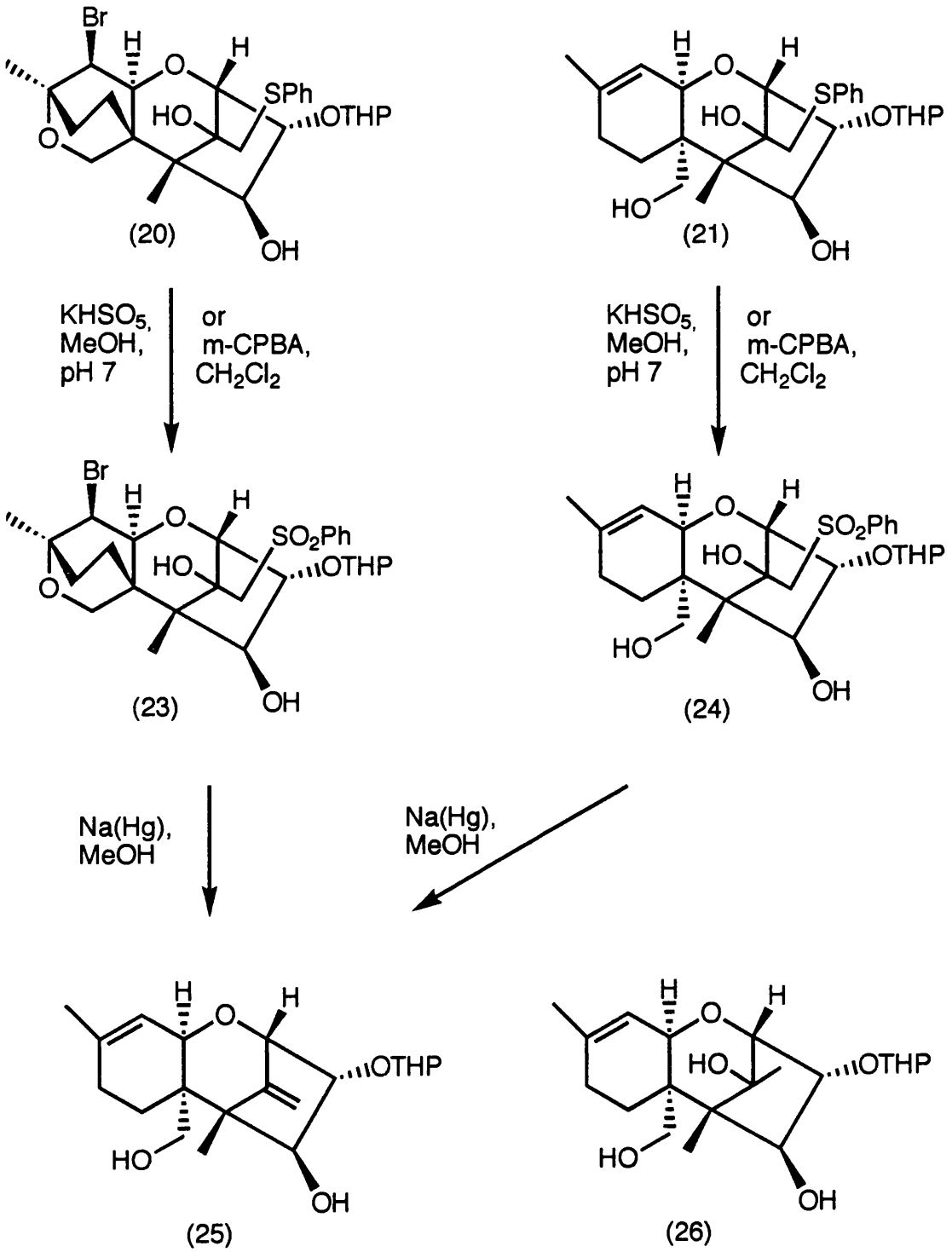
Scheme 9



conversion of (18) or (19) to (25) was the tertiary alcohol (26) presumably resulting from sulphone reductive cleavage without β-elimination of hydroxide.

This three-step procedure has been successfully applied to protected forms of DAS (1) but is incompatible with ester groups, which are common to many

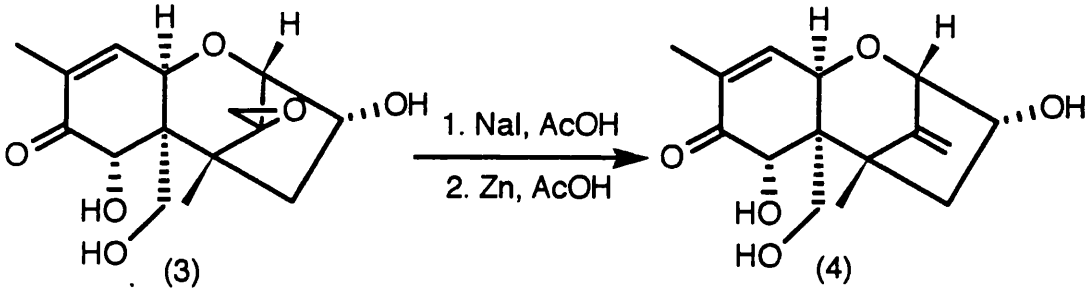
Scheme 10


Br
H
O
H
SPh
HO
OTHP
O
(20)
OH
KHSO5,
MeOH,
pH 7
or
m-CPBA,
CH2Cl2
(21)
(23)
SO2Ph
(24)
Na(Hg),
MeOH
(25)
(26)

trichothecenes. The best overall yields were no better than 60%.

An alternative method of deoxygenation was reported by King and Greenhalgh[38] shortly thereafter. Firstly they attempted refluxing deoxynivalenol (3) in a solution of sodium iodide in acetic acid, before adding powdered zinc, but this furnished only an extremely small quantity of the required diene (4) (Scheme 11).

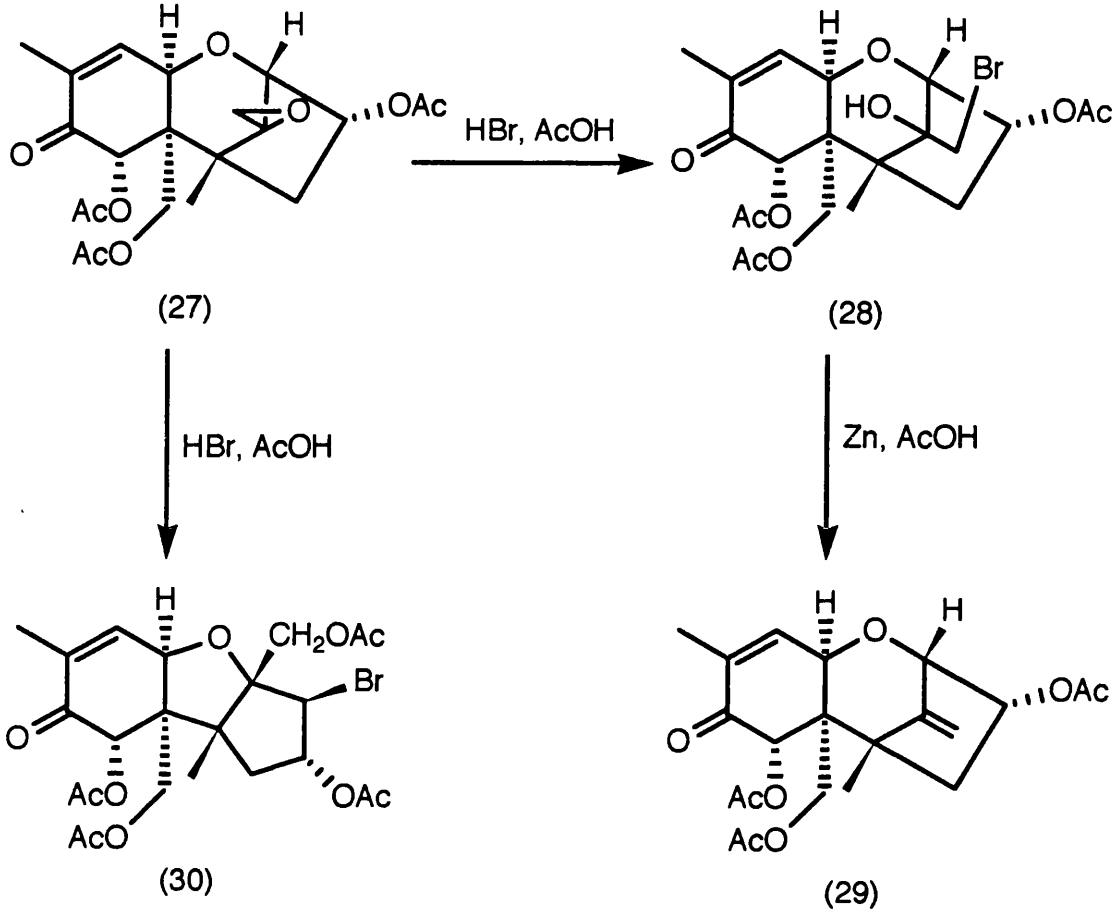
Scheme 11



In order to reduce unwanted side reactions the triacetyl derivative of deoxynivalenol (27) was formed, HBr was substituted for NaI, and the reaction was repeated. This time two compounds were produced (Scheme 12).

The minor product was the desired diene (29), the product of nucleophilic attack by bromide ion at the less highly substituted carbon atom,[39] to give the bromohydrin (28), followed by dehalohydrination using powdered zinc. The predominant species was the product of a skeletal rearrangement, known to occur with trichothecenes under acidic conditions,[40] called an

Scheme 12



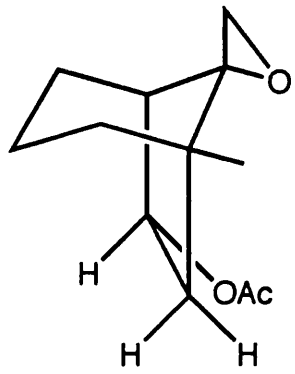
apotrichothecene, which was assigned the structure (30). It was assumed that after rearrangement acid-catalyzed acetylation had taken place at C-13. The pathway to the apo-derivative (30) presumably represents a comparable energy barrier to external nucleophilic attack by bromide ion.

This was also an unsatisfactory method of trichothecene epoxide deoxygenation since the overall yield was less than 9%.

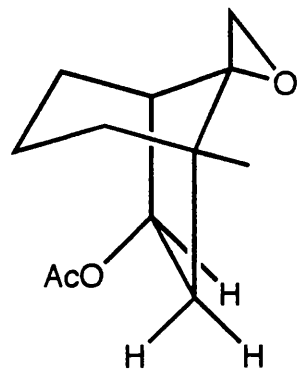
Another possible deoxygenating system was dimethyl diazomalonate (31)[41] with rhodium (II) acetate catalysis which rapidly and cleanly deoxygenates epoxides under neutral conditions without olefin isomerisation or cyclopropanation of, or allylic insertion at, the new π system. A stereospecific deoxygenation which occurred without alkene isomerisation was not an important issue, however, because the trichothecenes contain a terminal 12,13-epoxide unit. Colvin[42] attempted to use this reagent on the bicyclo[3.2.1]octanes (32) and (33), model compounds for the trichothecene series, with only modest success.

$N_2CH(COOMe)_2$

(31)



(32)

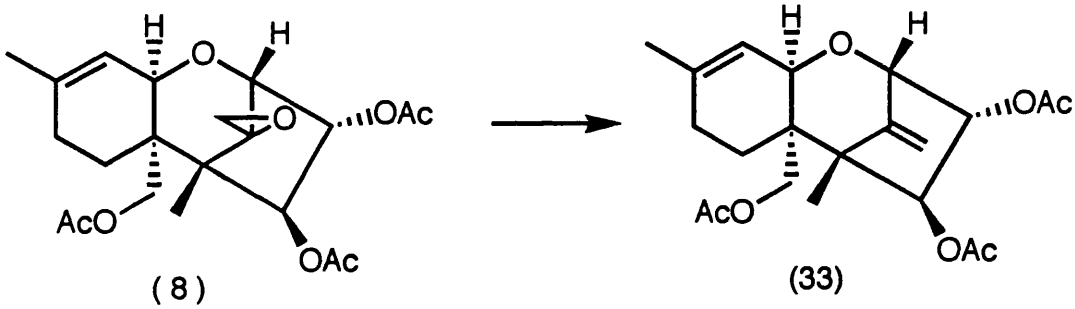


(33)

He turned his attention to lower valent tungsten halides developed by Sharpless et al[43] which, until then, had seen only limited use.[44] The reagent generated from WCl_6 and butyl-lithium deoxygenated the model compounds (32) and (33) in excellent yield. This method was then tested on the trichothecenes themselves.

$3\alpha,4\beta,15$ Triacetoxy-12,13-epoxytrichothec-9-ene (8) was deoxygenated in almost quantitative yield to the diene (33) (Scheme 13) and under the same conditions deoxynivalenol triacetate (27) gave the diene (29). This highly successful method requires two and a half moles of butyl-lithium for every one mole of tungsten hexachloride. Smaller amounts of butyl-lithium can lead to chlorohydrin production, species which are reduced much more slowly than the corresponding epoxides.[45]

Scheme 13

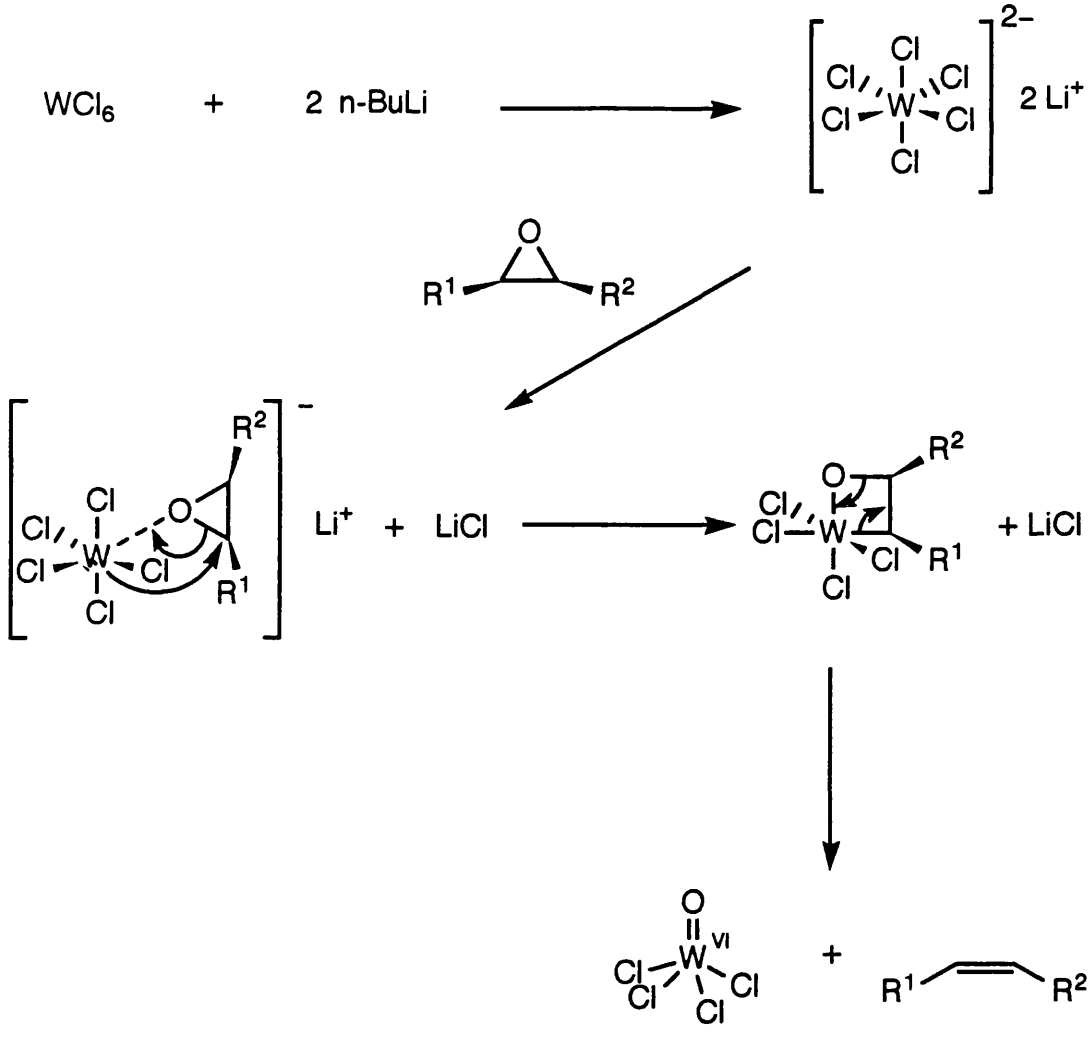


(8) (33)

The mechanism for the reduction of epoxides to olefins using the Sharpless method is stereospecific, the stereochemistry being retained in the product. Reductions with the tungsten reagent occur cleanly and in one step in excellent yields by what Sharpless believes to be a direct frontal approach and extraction of the oxygen atom (Scheme 14).

The arguments presented here involving carbon-metal bonded interactions are inconclusive though. There is as yet no evidence which cannot also be rationalised by

Scheme 14

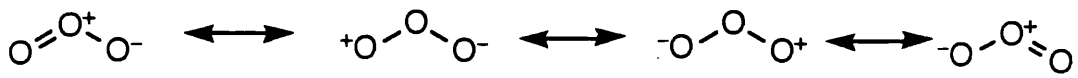


other methods. There may be other ways of explaining how the stereochemistry is retained. The Sharpless deoxygenating system is undoubtedly the reagent of choice for epoxide deoxygenation in the 12,13-epoxytrichothecenes and works well in the partial syntheses detailed in this thesis.

Chapter 1.3 Ozonolysis

In order to understand the reactions of ozone with organic compounds, a knowledge of the structure of the ozone molecule is essential. General agreement on its structure came in 1952, more than 110 years after its discovery. Ozone is a highly reactive allotrope of ordinary atmospheric oxygen in which the molecule is composed of three, rather than two, atoms of oxygen. On the basis of physical properties such as bond lengths, bond angles, negligible paramagnetism and low dipole moment, the structure of the ozone molecule can be described as a resonance hybrid of four canonical forms (Figure 5). From this, it is quite possible then that ozone should be able to function as an electrophile, a nucleophile or a 1,3 dipole.

Figure 5

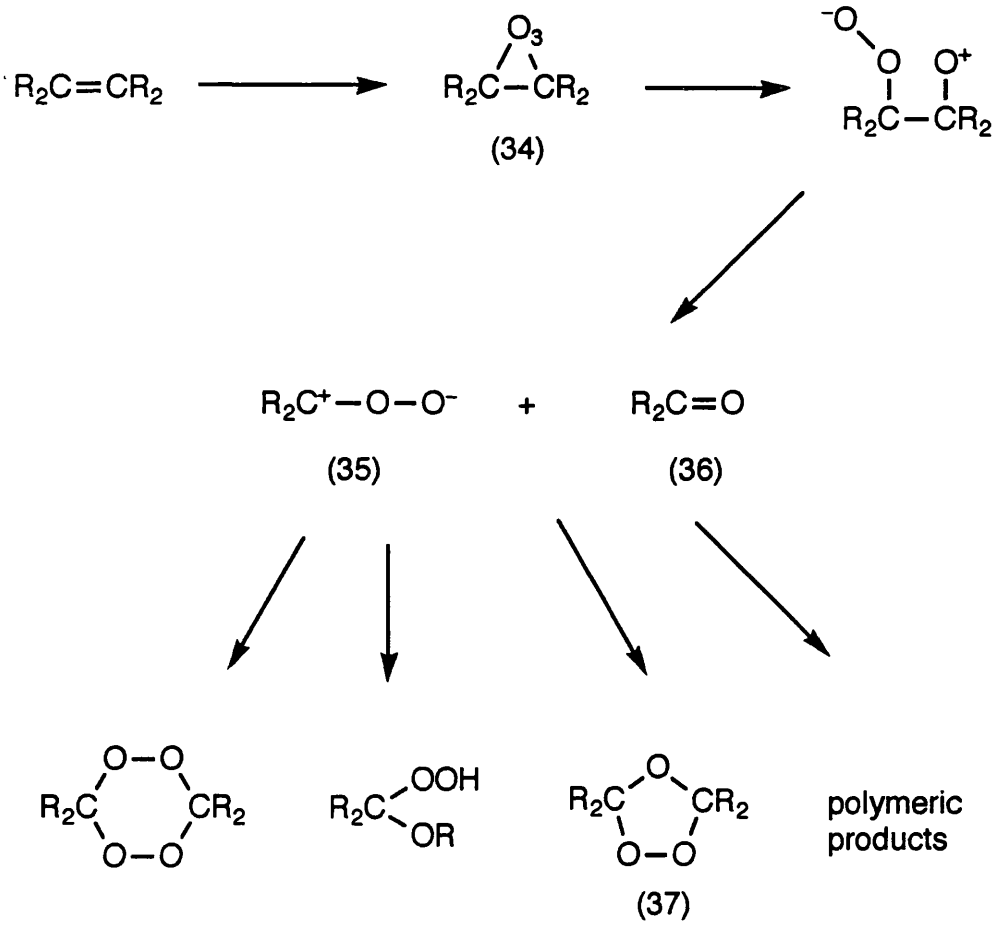


Although present in the stratosphere, ozone is normally generated by electrical discharge through oxygen; it is also highly toxic in concentrations of greater than 0.1 ppm by volume. The understanding that we have of ozone today is mainly due to the contributions of three men, Schonbein,[47] Harries[48] and Criegee.[49]

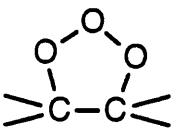
Professor Rudolf Criegee began his work in 1949

studying the reactions of a wide variety of unsaturated compounds with ozone in different types of solvents, publishing his first major paper on the mechanism of ozonolysis in 1953.[50] This was the beginning of an era of great interest in ozone-organic chemistry which culminates in our understanding of the mechanisms of ozonolysis today (Scheme 15).

Scheme 15



The Criegee mechanism undoubtedly set the proper course for future study but at the time several questions remained unanswered. It was necessary to know if an initial adduct (34) existed, what was the structure of the initial adduct , what was the nature of the attack by ozone, and what was the mechanism from this initial adduct to further ozonolysis products.

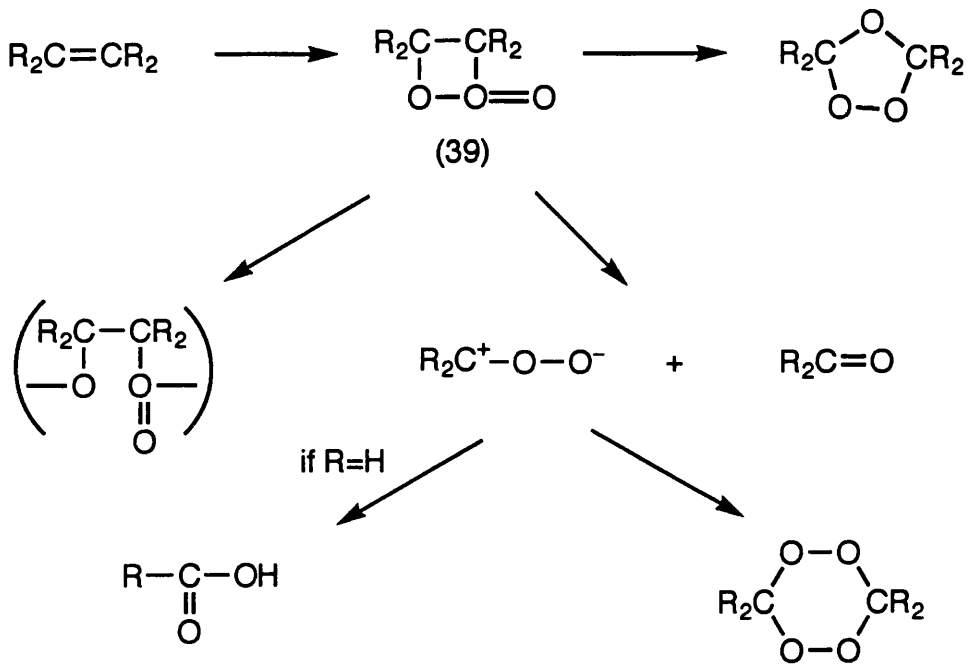


(38)

Before Criegee proposed his mechanism, Harries had assigned a general structure (38) for the initial ozone-olefin adduct which he later called an ozonide.[51] In 1925, Staudinger proposed a more intricate mechanism (Scheme 16).[52] He believed that ozone reacted with olefins to form an unstable initial adduct, an oxy-1,2-dioxetane (39), which he called a 'molozonide'. After these proposals of Staudinger and before the publication of the Criegee mechanism, several mechanisms for the rearrangement of this 'molozonide' were suggested.[53]

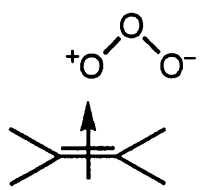
Criegee, however, was left in some doubt as to the structure of the initial adduct, assuming only that it was very unstable and that it cleaved to a zwitterionic intermediate and an aldehyde or ketone. These zwitterions or carbonyl oxides are energy-rich species which have the

Scheme 16

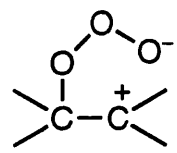


possibility of resonance stabilisation.

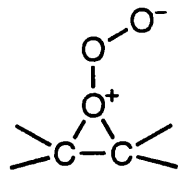
Several other structures were proposed for this initial adduct; these included a π-complex (40), a π-complex (41) and a peroxy epoxide (42).[54]



(40)



(41)

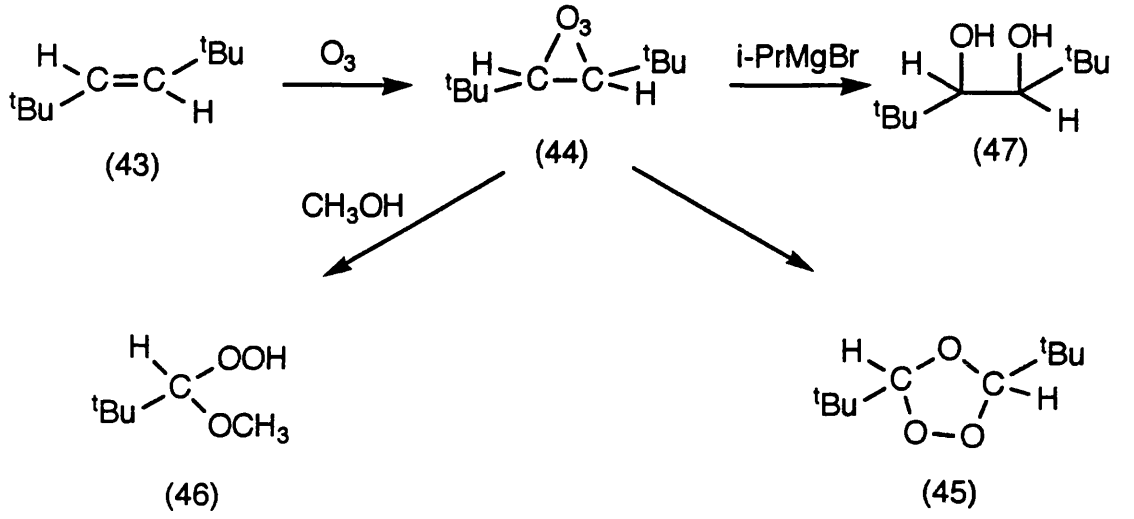


(42)

The actual existence of the initial adduct was finally established in 1959 by Criegee and Schroder.[55] During ozonolysis of trans-1,2-di-tert-butylethylene (43) in pentane at -75°C (Scheme 17), they observed the precipitation of crystalline material which decomposed

when the temperature of the reaction was allowed to rise to -60°C, and evolved 37-39 kcal/mole of heat. Work-up of this reaction mixture gave the expected ozonide (45).

Scheme 17



In order to establish that this crystalline material was indeed the initial adduct (44), and that the σ-bond of the double bond in the starting material was still intact, two reactions were examined.

Firstly, when a cold ether solution of the suspected initial adduct (44) was treated with methanol, the product was the α-methoxy hydroperoxide (46); ozonolysis of the olefin (43) in methanol gave the same result. If, however, the ether solution of the initial adduct was allowed to warm to room temperature before the methanol was added, no methoxy hydroperoxide (46) was obtained. This showed that the primary ozonide had already decomposed and that the Criegee zwitterion had

reacted in other ways before the addition of methanol.

Secondly, reduction of the cold ether solution of the initial adduct with isopropylmagnesium bromide gave a racemic glycol (47), whereas at room temperature, the same reaction failed to give the glycol. This proved the existence of the initial adduct (44) beyond reasonable doubt.

Following this pioneeering work, Greenwood also established the existence of primary ozonides of simpler alkenes by similar techniques.[56]

Although all of these investigations confirmed the existence of the initial ozone-olefin adduct, they did not prove its structure, that is whether it was Staudinger's 'molozonide' (39) or the primary ozonide (38) suggested by Harries.

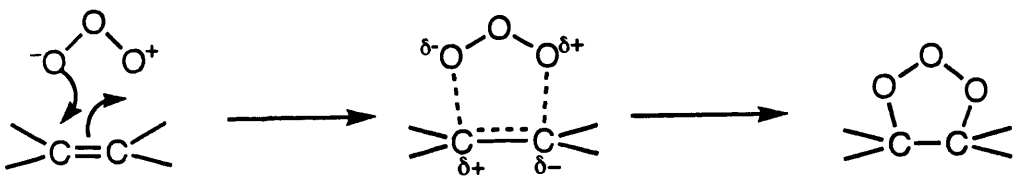
The structure was determined in 1966 by means of low-temperature NMR studies of the ozonolysis reaction mixtures of trans-1,2-di-tert-butylethylene (43) in Freon 11 at -110°C and in d^6-acetone at -95°C.[57] In each case the proton NMR spectrum consisted of two singlet peaks in the expected ratio of 9:1.

When the temperature rose above -60°C, these peaks disappeared and peaks characteristic of the decomposition products appeared. These spectra prove that the primary ozonide is symmetrical in structure, thus eliminating Staudinger's 'molozonide' (39) whose protons would not have been equivalent. The peroxy epoxide can

also be discounted because although both ring protons would give rise to the same type of signals, the chemical shift would be significantly shifted downfield due to the deshielding influence of the positive charge on the neighbouring oxygen atom.

The NMR data alone, however, do not eliminate a symmetrical complex but this data coupled with the reduction of the initial ozonides to glycols establishes Harries primary ozonide (38) beyond all reasonable doubt. There has recently been a definitive characterisation by microwave spectroscopy of the reaction product of ozone and ethylene.[58] Even more remarkable is the recent observation of the van der Waals complex between ozone and ethylene, observed using the same technique.[59]

Figure 6



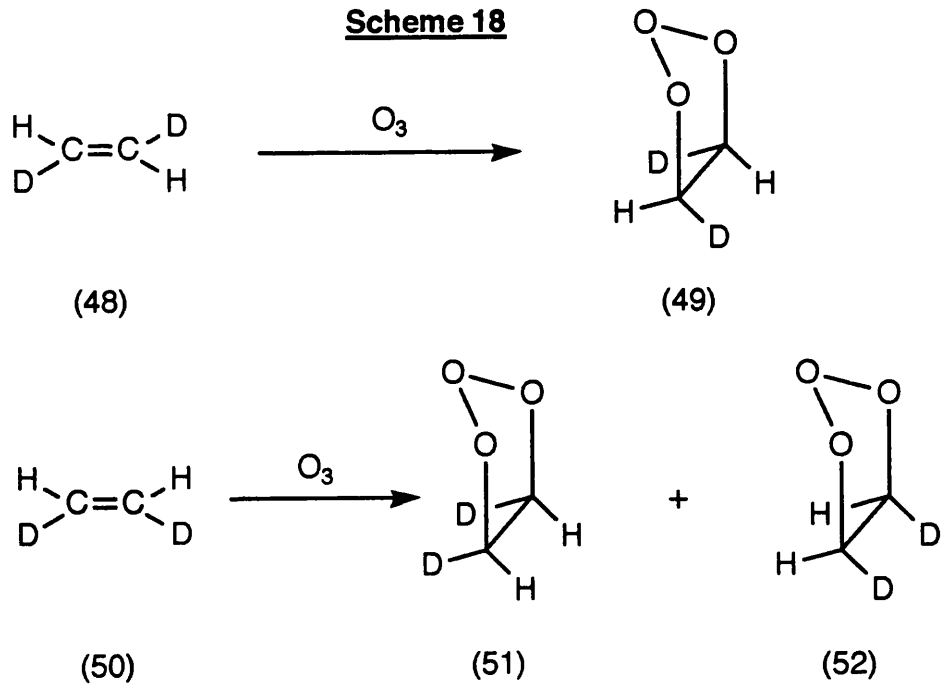
(38)

Attention then turned to determining the nature of the initial ozone attack on an olefin. Before 1965 there was disagreement over whether the process was a one-step, four-centre, concerted process or a two-step addition where the first step involved ozone acting as an electrophile. During the 1960's these arguments ended with Huisgen's classification of ozone as a classical 1,3-

dipole and of the interaction of ozone and an olefinic bond as a 1,3-dipolar cycloaddition (Figure 6).[60]

The stereochemical test for a concerted process for the initial cycloaddition was studied by ozonizing trans- or cis-ethylene-1,2-d_2. The trans species (48) gave exclusively the trans-d_2 primary ozonide (49) whereas the cis isomer (50) gave an equimolar mixture of the endo (51) and exo (52) d_2-forms. This shows that there is no evidence for stereo randomisation about the C=C bond and is consistent with previous studies which infer a concerted cycloaddition (Scheme 18).[61]

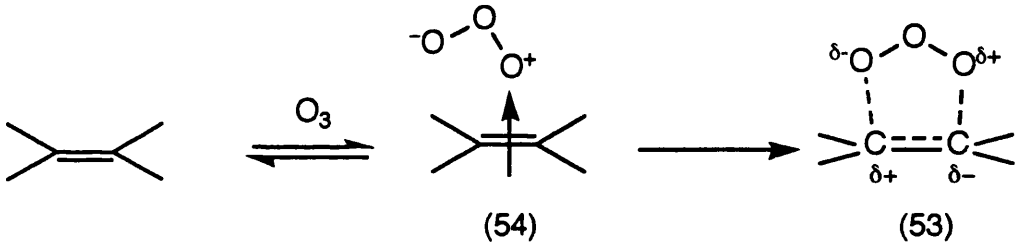
Scheme 18



The primary ozonide is actually formed via a transition state (53) in which the electrophilic oxygen of the ozone molecule is bound to a carbon atom of the double bond more strongly than that of the nucleophilic oxygen

atom. Electron donating and withdrawing groups will respectively stabilise or destabilise the transition state through decreasing or increasing the positive charge on carbon. This semipolar transition state (53) could be attained from the interacting ozone and olefin molecules either via a single-step concerted cycloaddition or via an additional reversible stage in which a π-complex (54) is formed (Scheme 19).

Scheme 19



Kinetic studies clearly show that substituents on the carbons of a double bond increase the rate of ozone attack if they are electron donating and decrease the rate of ozone attack if they are electron withdrawing. This fits much better with the idea of a concerted addition rather than a purely electrophilic attack as the first step of a two-step attack involving a carbonium ion intermediate. The rate of ozonolysis is also influenced by the geometry of the double bond. A trans isomer undergoes ozonolysis faster than a cis isomer especially if the cis substituents are large, such as chloro, carbonyl or phenyl.[62,63,64] This is probably because

during the cycloaddition the carbon atom hybridisation changes from sp^2 to sp^3 in the transition state and, even though the C-C bond length increases, the consequent decrease in bond angle from 120^o to 109^o leads to a significant compression of the van der Waals' radii of the eclipsed cis substituents.[65] This results in a higher activation energy for the cycloaddition of the cis isomer compared with that of the trans isomer. A few exceptions to this have appeared in the literature[66,67] but it is likely that these results were due to solubility differences between the two isomers. Cis and trans olefins of course, give different primary ozonides. The reduction of the primary ozonide from a trans alkene gives a dl (or threo)-1,2-glycol,[55,56] whereas a cis alkene will yield a meso (or erythro)-1,2-glycol, which proves that the primary ozonides are the products of cis addition.

All the evidence indicates that the attack of ozone on an olefinic double bond is a stereospecific, one-step, concerted, 1,3-dipolar cycloaddition, which may involve the initial formation of a reversible π-complex (54).

The term 'ozonide' is used for the 1,2,4-trioxolane structure (38) and this is the major product of ozonolysis if firstly, the solvent is non-nucleophilic and thus non-participating and secondly if the olefin is a mono-, di-, or trisubstituted ethylene or ethylene itself. The second condition can be understood in terms of the

Criegee mechanism[50] which involves ozonide formation by the combination of a carbonyl oxide (35) with a carbonyl moiety (36): since this interaction is a 1,3-dipolar cycloaddition and is influenced by steric and electronic factors, ozonides are not, in general, produced from tetra-substituted olefins. This is because the recombination step involves the carbonyl oxide with a ketone, whose participation in a cycloaddition is much smaller than that of an aldehyde. Dimeric or polymeric peroxides are normally produced in these instances.

There are a few exceptions to this rule however. The ozonolysis of certain cyclic olefins can lead to a favourable intramolecular interaction between the carbonyl oxide and carbonyl moieties to give an ozonide.[68] These are more favourable than intermolecular reactions. Cyclobutenes[69] and cyclopentenes[70] give monomeric ozonides in excellent yields, but if the double bond is present in a six- or higher membered ring only oligomeric products are obtained.

The second exception to the rule is if the carbonyl moiety is a ketone activated by an electron-withdrawing group such as a halogen atom or an ester group: this makes it a better electrophile, and 1,3-dipolar cycloaddition occurs more easily.[71] A keto group can also be reactive if for steric reasons there is a particularly great tendency for its carbonyl carbon to change from sp^2 to sp^3 hybridisation. Some ketones have

proved to be good dipolarophiles towards several carbonyl oxides.[72] Another exception involves ozonolysis of an olefin in the presence of excess ketone.[73]

Ozonides (37) are also much more stable than the older literature would have us believe; most of the early 'explosive' ozonides were not ozonides at all but polymeric peroxides contaminated with such.

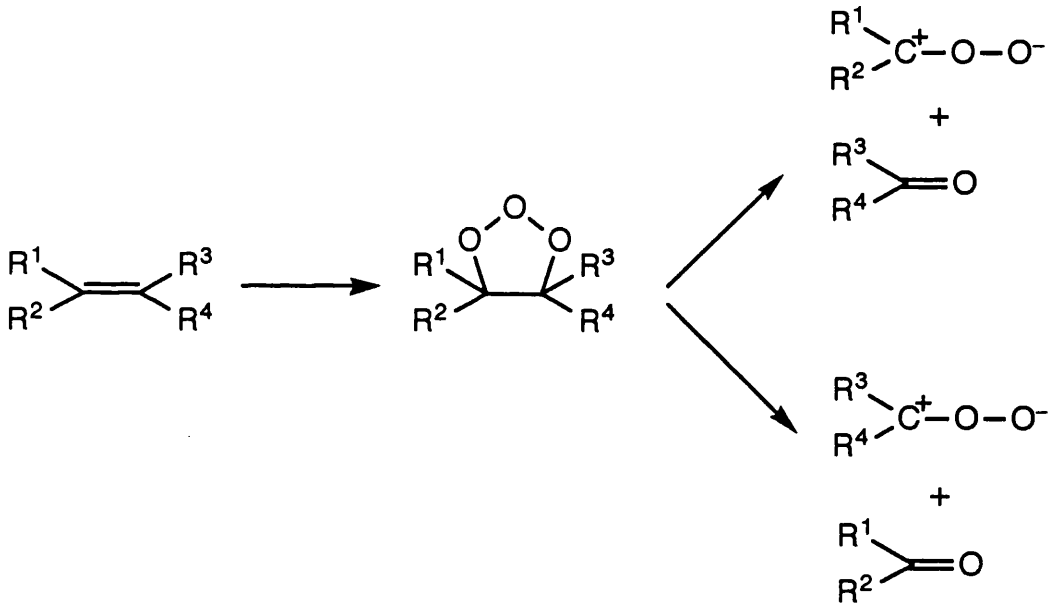
In protic, nucleophilic solvents, the peroxidic ozonolysis products are somewhat different. These solvents participate in the reaction and give α-alkoxy-, acyloxy- and hydroxy alkylhydroperoxides. The most common examples are alkoxy alkylhydroperoxide (46). The alkoxy alkylhydroperoxides are quite stable, have high melting points and are usually produced in good yields. There are fewer examples of acyloxy- or hydroxy alkylhydroperoxides but this is probably due to their reduced stability rather than the Criegee carbonyl oxide being less willing to react with carboxylic acids or water.

α-Methoxy peroxides played an important role determining the mode of decomposition of the primary ozonide. The primary ozonide is low in stability due to the energy rich O-O-O linkage which allows easy rupture of an O-O bond owing to lack of resonance. There is also significant ring strain involved.

Up until the middle of the 1960's, Criegee's mechanism[50] was generally accepted and a fundamental feature of his mechanism is the formation of the carbonyl

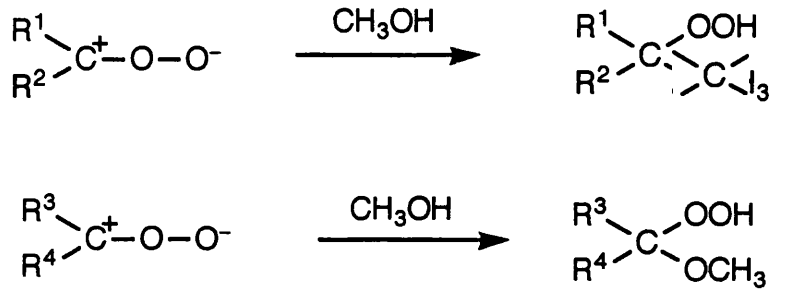
oxide and carbonyl compound as a result of the cleavage of the C-C and then O-O bonds in the 1,2,3-trioxolane ring of the primary ozonide. It is possible to have two pathways for the decomposition of the primary ozonide and thus form two different carbonyl oxide fragments (Scheme 20). It has been shown consistently that the pair of products that are obtained depends mainly on the inductive effects of the substituents.[74] Effects such as temperature, solvent and steric hindrance are negligible and need not be considered.[75]

Scheme 20

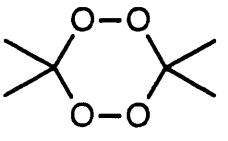


The mode of the cleavage was investigated by analysing the products of the reaction of the carbonyl oxides with methanol. The α-methoxyhydroperoxides which are formed quantitatively correspond directly to the initial carbonyl oxides (Scheme 21).

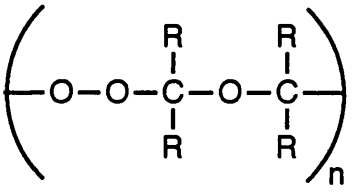
Scheme 21



These studies established that electron donating groups at the double bond favour the formation of a carbonyl oxide as a consequence of the decrease in the local positive charge of the neighbouring carbon atom; correspondingly electron-accepting substituents destabilise the carbonyl oxide (35) and facilitate the formation of the carbonyl moiety (36).



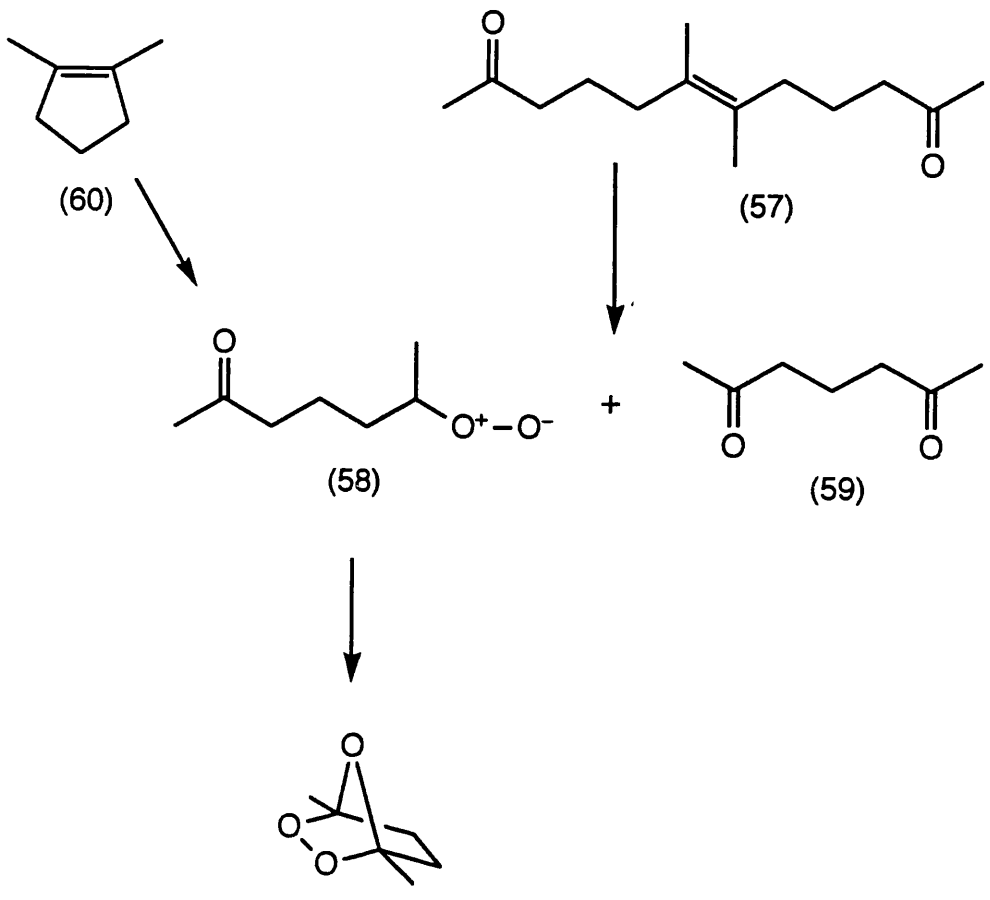
(55)



(56)

The most important and unique concept of Criegee's mechanism[50] is that all known peroxidic ozonolysis products result from various reactions of the key intermediate, the carbonyl oxide (35), and there is a solid basis for this postulate. The strongest evidence for this is that diperoxides (55) and oligomers (56) are products in nonparticipating solvents whereas alkoxy

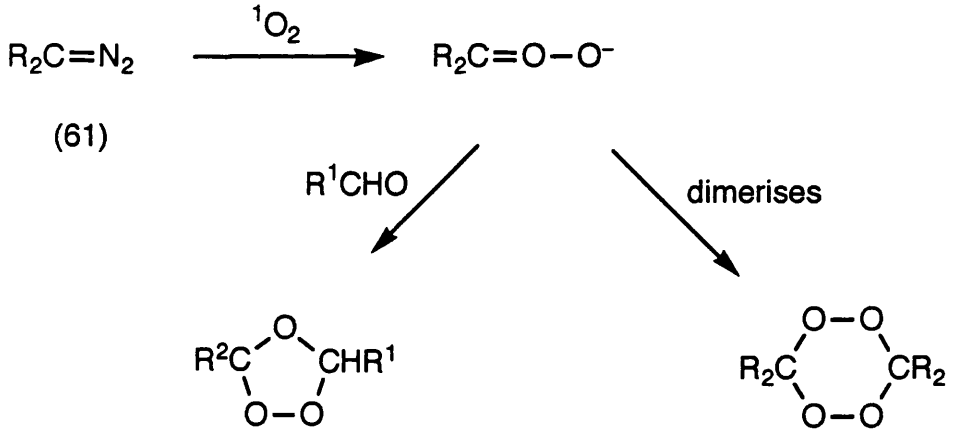
Scheme 22



alkylhydroperoxides (46) are obtained in participating solvents. Other evidence which showed that ozonides are produced by recombination of the carbonyl oxide and aldehyde or ketone moieties was presented by Criegee and co-workers and discussed in a review in 1958.[68] This evidence dealt with the production of 'cross-ozonides' by the ozonolyses of various olefins in the presence of an excess of a reactive aldehyde.[76] Further evidence for the intermediacy of the carbonyl oxide is observed when

olefins bearing keto groups are ozonised and the keto groups interfere in the normal ozonide formation. In the 1950's Lohaus[77] ozonised trans-6,7-dimethyl-6-dodecene-2,11-dione (57), decomposition of the primary ozonide gave the keto-carbonyl oxide (58) and the diketone (59). The carbonyl oxide group can now form an ozonide ring intermolecularly with a keto group of the diketone (59) or one intramolecularly with the keto group in its own molecule. The latter reaction predominates to give the same ozonide as that which is formed from the ozonolysis of 1,2-dimethylcyclopentene (60), thus proving that the same keto-carbonyl oxide was formed in each case. (Scheme 22).

Scheme 23



Other work in support of Criegee's theory came later and was carried out by Murray et al.[78] They synthesised ozonides by oxidation of dialkyl or diaryldiazomethanes (61) in the presence of aldehydes with

singlet oxygen (Scheme 23). This work provided evidence, independant of all ozonolysis results, that ozonides are formed by addition of carbonyl oxides to carbonyl groups. The carbonyl oxide had not been detected spectroscopically until recently when it was observed by I.R.[79] in low temperature matrices and by transient species U.V. techniques in solution.[80]

At around the same time as this convincing evidence was accumulating, other evidence seemed to cast doubt on Criegee's mechanism being the sole pathway to ozonides during ozonolysis of olefins.

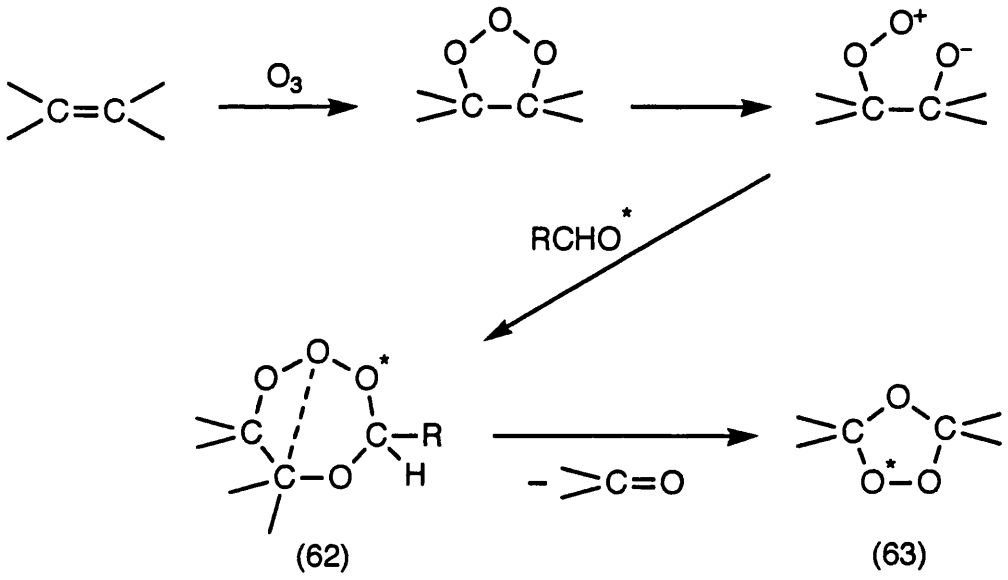
According to Criegee's theory,[50] the geometry of the initial alkene should not have influenced the composition of ozonolysis products because the same intermediate is formed from both cis and trans isomers. Schroder,[81] however, was the first to determine cis- and trans-ozonide ratios accurately and to show a definite stereoselectivity in their formation. He found that trans-1,2-di-tert-butylethylene led almost exclusively to the trans ozonide, while the cis-olefin gave a mixture of 70% of the cis- and 30% of the trans-ozonide. The initial Criegee mechanism could not explain this.

In the light of this gap in Criegee's theory, Bauld, Bailey et al[82] and Kuczkowski et al[83] suggested certain refinements to account for the stereochemistry of ozonide and cross-ozonide formation. The most important of these was that the Criegee carbonyl oxide could exist

in syn and anti configurations with the preference for one depending on the geometry of the primary ozonide. A prerequisite of these requirements is that the primary ozonides are non-planar immediately prior to decomposition and that the preferred conformations of the five-membered ring depend on the size and position of the substituents. There were also certain mechanistic proposals put forward, which were shown to be invalid.[84]

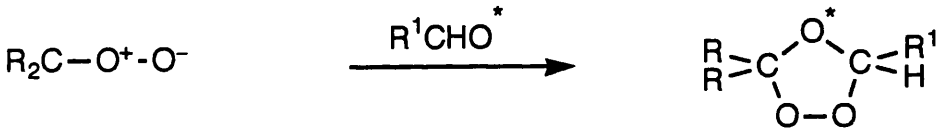
By 1973 the only mechanisms to be given the final test were the Criegee carbonyl oxide mechanism (Scheme 15) with its necessary refinements, and a mechanistic proposal[85] in which the pathway to the ozonide is via a seven-membered cyclic intermediate (62), the product of the attack of an aldehyde molecule on the primary ozonide (Scheme 24).

Scheme 24



It was soon realised that to decide which mechanism actually occurred, ozonolysis of an olefin would have to take place in the presence of excess aldehyde labelled with oxygen-18. The alternative mechanism causes the label to appear in the peroxide bridge of the final ozonide (63) (Scheme 24) whereas in the Criegee carbonyl oxide mechanism the labelled oxygen would be incorporated into the ether bridge (Scheme 25).

Scheme 25

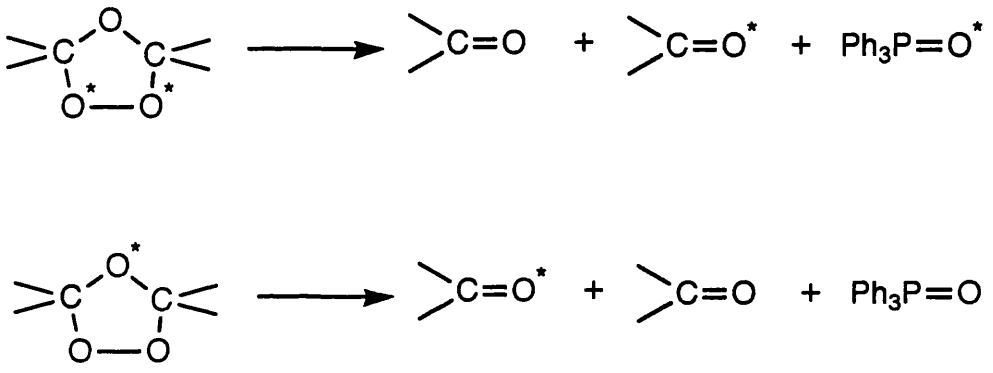


At the outset these isotope studies gave strange results which were attributed to the method of analysis. Mass spectrometry of the derived ozonides directly[86], indirectly after $LiAlH_4$ reduction[87] or together with microwave spectroscopy[88] produced conflicting results.

Gallaher and Kuczkowski,[89] and Higley and Murray[90] showed, in 1976, in a conclusive and unambiguous manner that the alternative mechanism (Scheme 24)[85] made no significant contribution to final ozonide formation. They carried out ^{18}O position determinations on peroxide-labelled and ether-labelled ozonides which were made specifically by photooxidation of diazo compounds; this involved either the use of labelled singlet oxygen or the

combination with a labelled aldehyde. Mass spectrometry as a method of analysis gave misleading fragmentation patterns with certain ozonides,[90] but the results of the triphenylphosphine reductions of these ozonides were shown to be consistently reliable.

Scheme 26



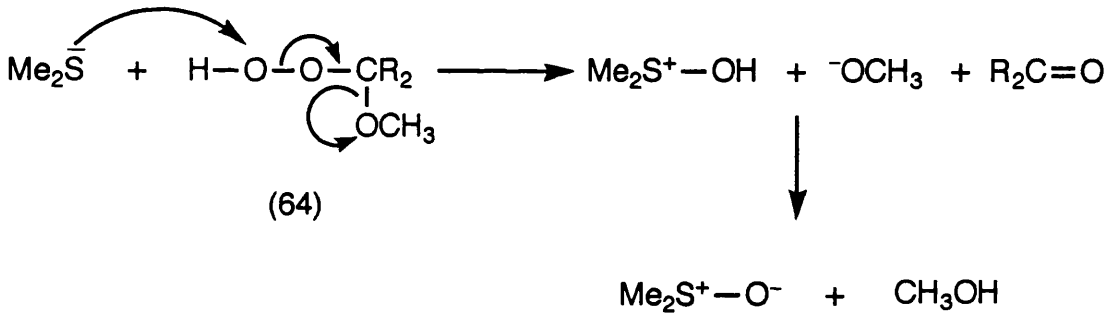
Mass spectrometric analysis of the by-product of the reduction, triphenylphosphine oxide, proved that the attack is directed exclusively at the peroxide oxygen (Scheme 26). This is not surpising as the O-O bond is the most susceptible to nucleophilic attack.

Ozonolysis is a widely utilised reaction because it produces aldehydes, ketones and acids in good yields from olefins. The final piece in the jigsaw is the conversion of ozonides to these non-peroxidic products. These can be formed directly, when normally an aldehyde or ketone is left behind when the accompanying carbonyl oxide dimerises, polymerises or reacts with a participating

solvent. The products from these reactions, as well as any ozonides, must be converted to nonperoxidic products.

The conversion methods can be classified as oxidative, to give carboxylic acids, reductive or hydrolytic, to give aldehydes or ketones, or even thermal or photolytic. Ozonides can be oxidised with oxygen, peracids or hydrogen peroxide to give ketones and/or carboxylic acids, reduced with $LiAlH_4$, BH_3 or H_2 to give alcohols or treated with ammonia, hydrogen and a catalyst to give amines. The majority of reagents used to reduce peroxidic ozonolysis products make a nucleophilic attack on one of the oxygens of the peroxide linkage, which one depends on both electronic and steric factors. Examples of these reducing agents are phosphines[90], phosphites,[91] iodide ion,[92] $LiAlH_4$ and methyl lithium[87] and Grignard reagents.[93]

Scheme 27



(64)

The most common method is ozonolysis in methanol, followed by DMS reduction of the α-methoxy alkylhydroperoxide intermediate (64) (Scheme 27). This

produces ketones and/or aldehyes from olefins both cleanly and in excellent yield.

There is no doubt that great progress has been made in substantiating and building upon the Criegee mechanism and in understanding puzzling aspects of ozonolysis by traditional physical organic techniques and reasoning. The ever advancing sophistication in analysis techniques will no doubt help in the future and the understanding of this fascinating reaction will continue to grow.

2. Discussion

Chapter 2.1

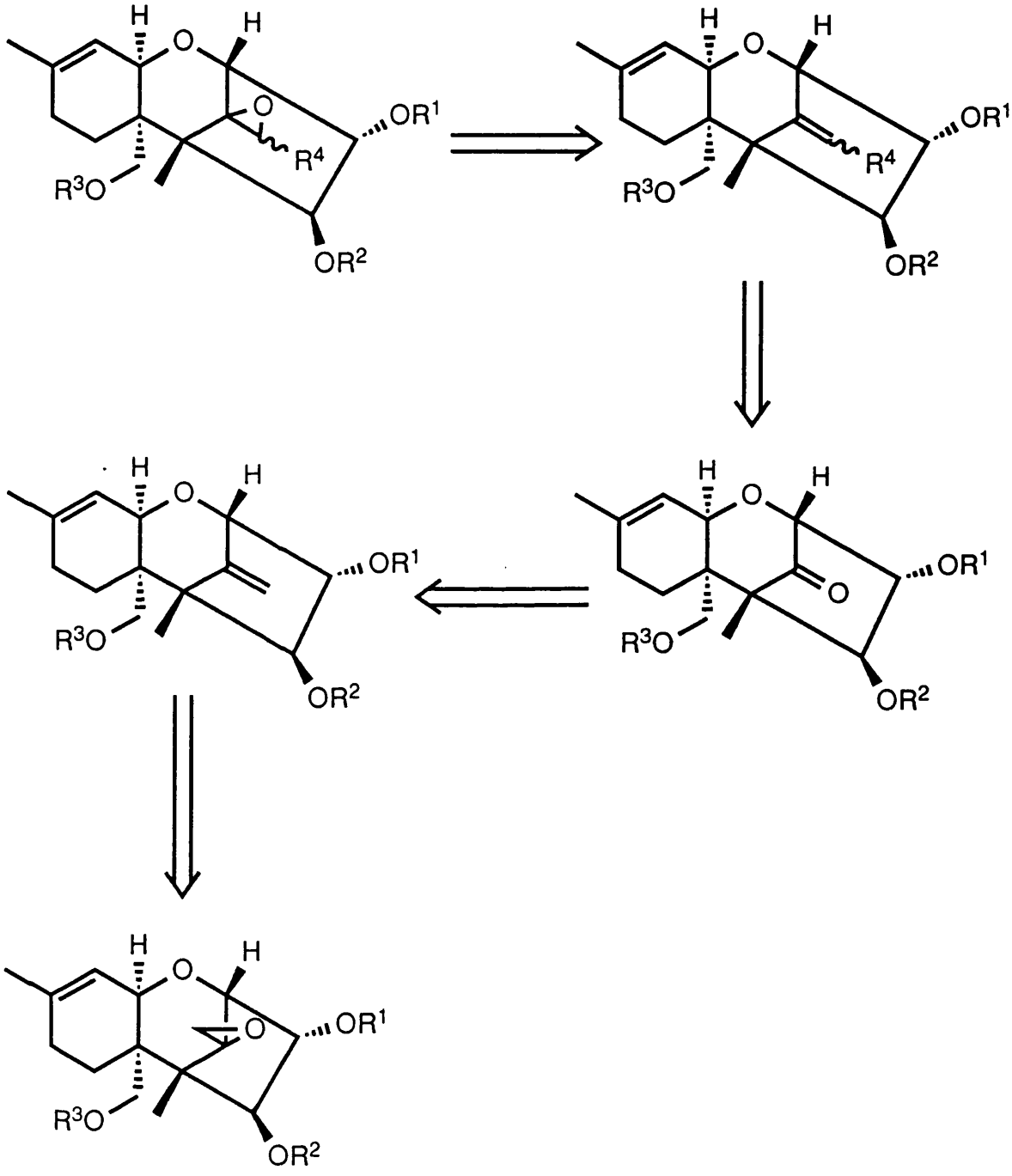
In order to gain a greater insight into the mode of action of the trichothecene mycotoxins and to study their synthetic transformations, the partial synthesis of two novel trichothecenes was attempted. The initial aim was to synthesise (13S)-3α,4β,15-triacetoxy-13-methyl-12,13-epoxytrichothec-9-ene (11) and (13R)-3α,4β,15-triacetoxy-13-methyl-12,13-epoxytrichothec-9-ene (12) from the natural compounds 3α-hydroxy-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (DAS) (1) and 3α,15-dihydroxy-4β-acetoxy-12,13-epoxytrichothec-9-ene (MAS) (2).

These novel target compounds differ from their natural counterparts in that they contain a methyl group at C-13 and have additional ester side chains at C-3 and C-15. C-13 is the unsubstituted carbon of the epoxide ring in the natural compounds (1) and (2). The epoxide ring is essential if biological activity is to be exhibited by the trichothecenes.[20] It has been shown that deoxygenation to the diene (4) is the predominant biological transformation which suggests that the mode of action may involve the epoxide acting as an electrophilic alkylating agent.[17]

Previous studies within the group had already emphasised the necessity not only for the presence of the 12,13 epoxide unit but also of its geometrical requirement to be anti to ring A of the trichothecene, in order to

confer cytotoxic properties. This is detailed in the synthesis of 3α,4β,15-triacetoxy-12,13-epi-epoxytrichothec-9-ene (10).[24]

Scheme 28



The partial synthesis of the two novel unnatural trichothecenes (11) and (12) would therefore be beneficial in providing information on the metabolic fate by examination of their relative biological activities. This would be carried out using human epithelial cells by determining the minimum inhibitory concentration for cell-growth.[25]

A retro-synthetic inspection of the proposed synthesis shows that there are several key synthetic transformations (Scheme 28). The crucial steps include an epoxide deoxygenation, an ozonolysis, a Wittig reaction employing ethylidene triphenylphosphorane and a stereospecific epoxidation. To carry out such synthetic transformations on such a complex multi-functional system, a series of protection and deprotection steps will undoubtedly be required.

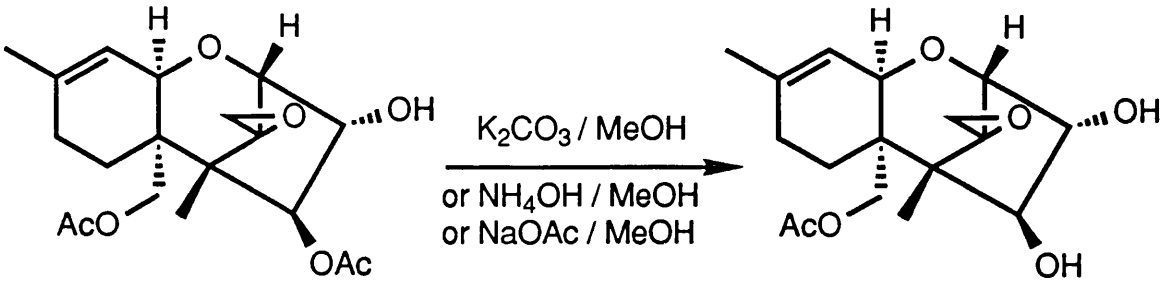
Partial synthesis is obviously a more attractive and less demanding alternative to total synthesis, although several of the naturally occurring trichothecenes have succumbed to total synthesis. There is a limited range of commercially available natural trichothecenes, but these are expensive. Using a suitable Fusarium species (strain C37410-90. Bristol Myers Company) and shake culture methods it was possible to obtain working amounts of the starting materials DAS (1) and MAS (2). The production of these starting materials required the exposure to microbiological techniques including

inoculation of medium, and strict sterilisation of equipment in order to obtain efficient fungal growth. Obtaining a constant temperature for shaking the production culture proved to be an excellent refinement with respect to the yields of DAS (1) and MAS (2) obtained. An incubation period of 7 days led to a greater ratio of DAS, however when left for a further 4 days, equal amounts of the two natural compounds were obtained.

In general the production and isolation of biologically active natural products from fermentations are normally a much more tractable problem than the production and isolation of these compounds from higher plants. The problems associated with large scale collection of plants and the ensuing isolations are often enormous. Fermentation techniques on the other hand can be routine and one can confidently scale-up fermentations with little difficulty.

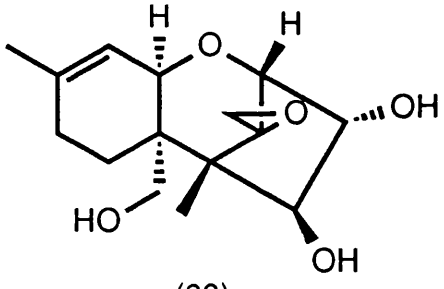
MAS (2) was the preferred starting material for the synthesis as the 15-hydroxyl group was necessary for an early synthetic transformation. There is no quick and simple way of converting DAS (1) to MAS (2) chemically. Hydrolysis of DAS (1) under basic conditions, using potassium carbonate, ammonium hydroxide or sodium acetate in methanol, is highly regioselective but affords almost exclusively the wrong monoacetate, that of $3\alpha,4\beta$-dihydroxy-15-acetoxy-12,13-epoxytrichothec-9-ene (65) (Scheme 29).[94]

Scheme 29



(1) (65)

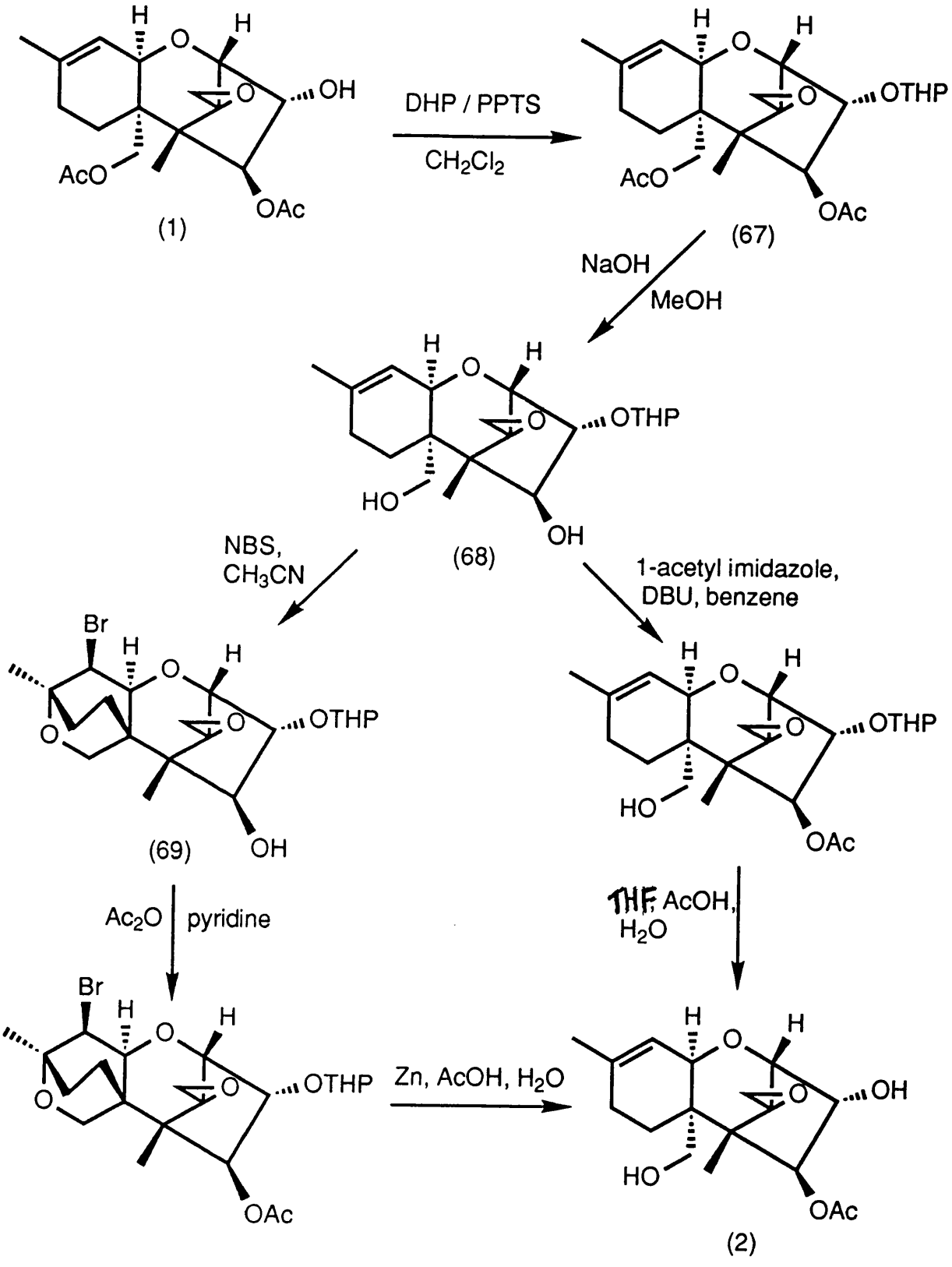
MAS (2) has been prepared directly, though, by treatment of DAS (1) with resting cells of Streptomyces Griseus.[95] Attempted selective acetylation of 3α,4β,15-trihydroxy-12,13-epoxytrichothec-9-ene (66) with various carboxylic acid derivatives gives complicated product mixtures.[96] Two syntheses of MAS (2) from DAS (1) have been reported.[94]



(66)

Treatment of DAS (1) with dihydropyran and PPTS gives the THP ether (67) as a mixture of diastereomers. Hydrolysis using sodium hydroxide in methanol gives the 4β,15-diol (68) which can be selectively acetylated at C-4 using 1-acetylimidazole and DBU. Removal of the 3α-THP protection using a 2 : 1 : 1 mixture of THF, acetic acid

Scheme 30

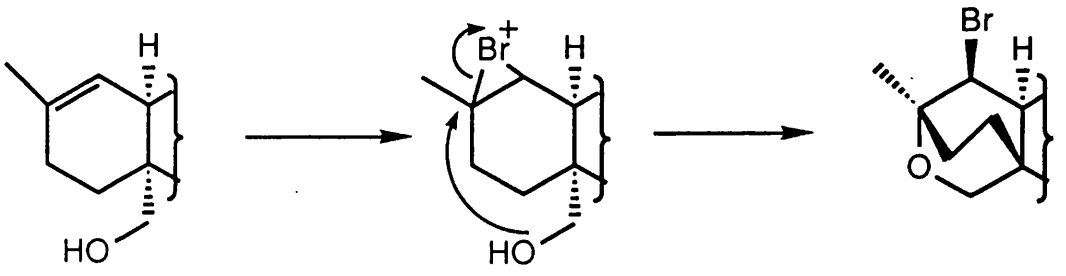

DHP / PPTS
CH_2Cl_2
NaOH
MeOH
NBS,
CH_3CN
1-acetyl imidazole,
DBU, benzene
Ac_2O pyridine
THF, AcOH,
H_2O
Zn, AcOH, H_2O
(1)
(67)
(68)
(69)
(2)

and water completes the conversion (Scheme 30).

Alternatively the C-4 and C-15 hydroxyl groups may be differentiated by making a bromo-ether derivative (69) using NBS in acetonitrile. Acetylation at C-4 using acetic anhydride and pyridine followed by deprotection at C-3 and C-15 by treatment with a zinc-silver couple[97] gives MAS (2) in up to 70% overall yield. The formation of a bromo-ether in ring A of the trichothecenes was to prove extremely useful as an early step in the partial synthesis of (11) and (12).

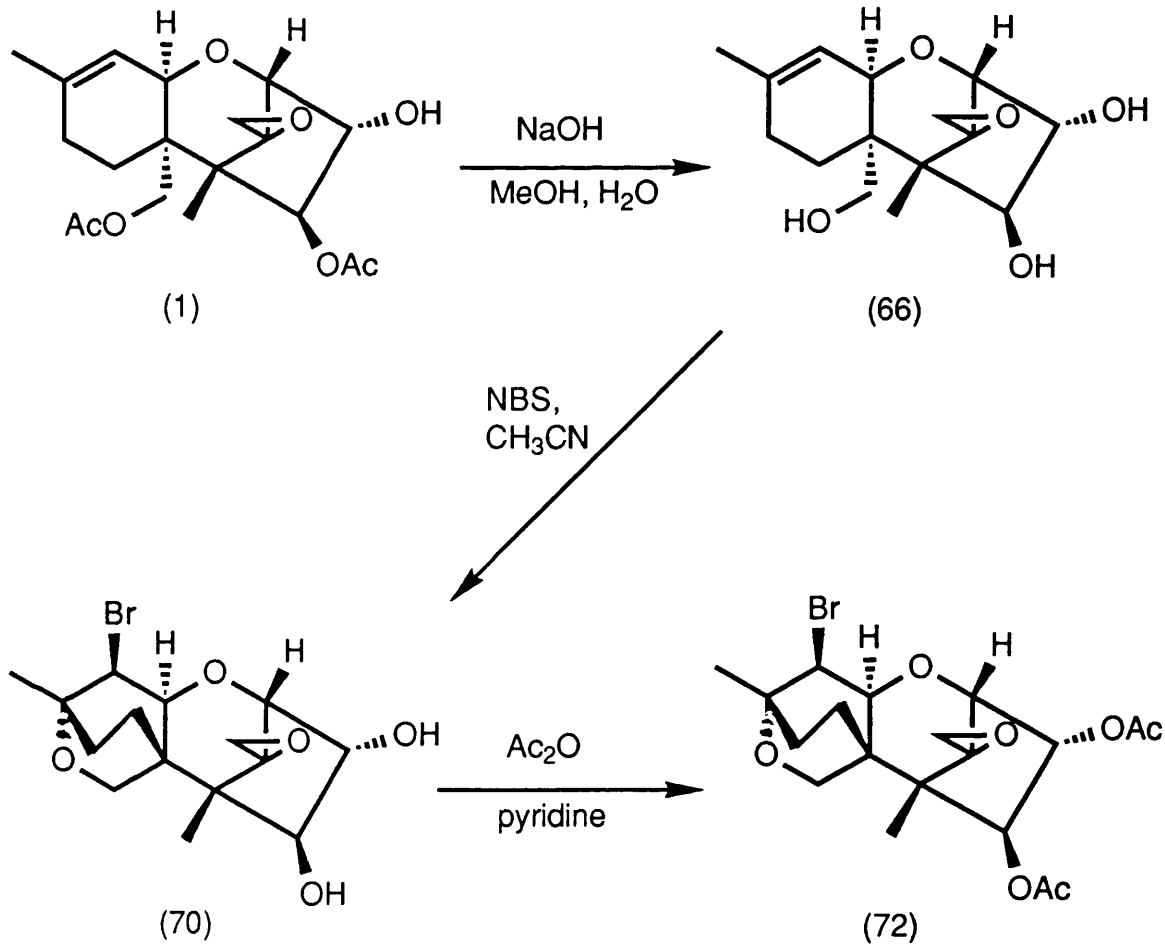
As the ultimate objective of this work was to achieve synthetic manipulation of the 12,13-epoxide moiety, the most obvious way to do this was by deoxygenation of the epoxide followed by oxidative cleavage of the resulting 12-ene. This could not be achieved selectively so it was necessary to protect the 9,10-double bond in some way. Treatment with NBS in acetonitrile gives a cyclic bromonium ion which is then opened intramolecularly by the C-15 hydroxyl group to give an oxabicyclo[2.2.2]bromoether (Scheme 31).

Scheme 31



In order to make use of the amounts of DAS (1) and to avoid making the diastereoisomeric THP ethers, DAS (1) was treated with NaOH in MeOH and H_2O, and then passed through an ion-exchange column filled with Amberlite resin to give scirpenetriol (66). No attempt was made at this point to purify the triol, and the bromoetherification was carried out immediately to give the diol (70) in good yield. Suction chromatography was used to separate the succinimide by-product. Acetylation using acetic

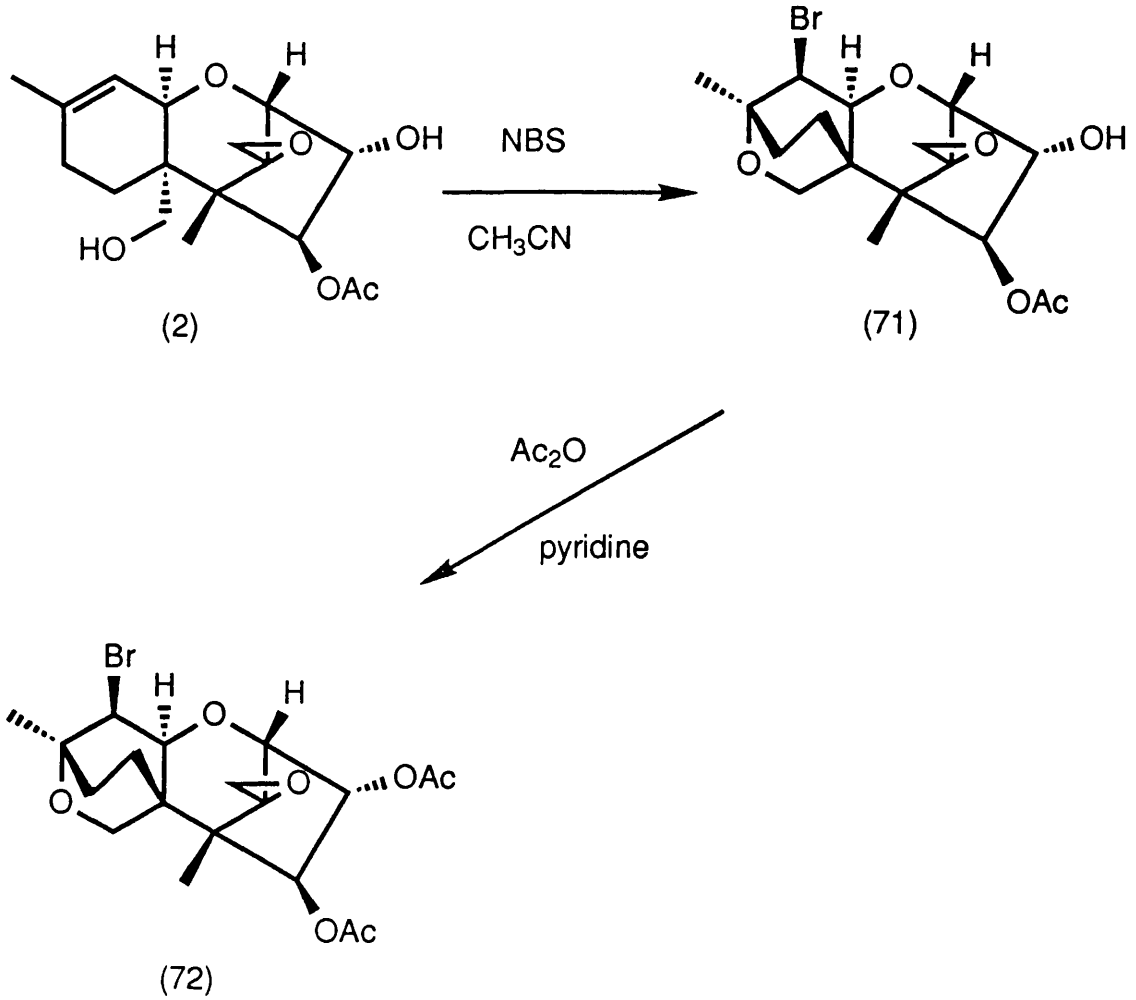
Scheme 32



anhydride and pyridine then gave the 3α,4β diacetate (72), a suitable compound on which to carry out epoxide deoxygenation (Scheme 32).

As MAS (2) already contains a primary hydroxyl group at C-15 there was no need for a hydrolysis step. Bromo-etherification to give (71) followed by acetylation under the usual conditions gave the same 3α,4β diacetate (72), in 86% overall yield (Scheme 33).

Scheme 33

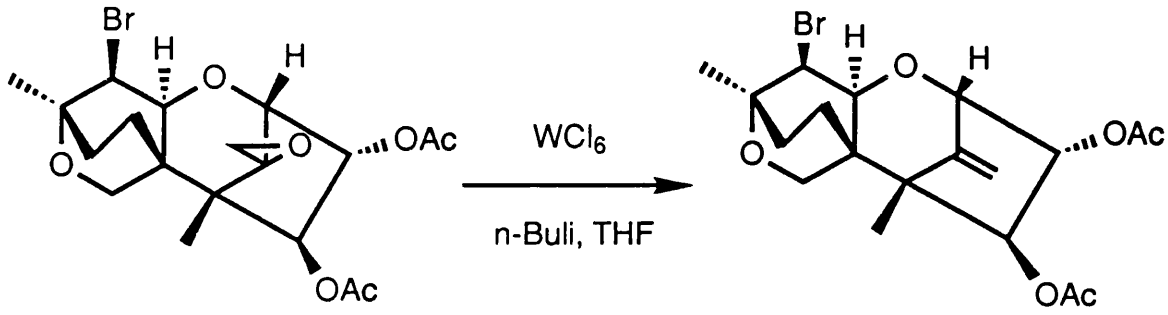


(2) (71) (72)

The next step was a key transformation, that of epoxide deoxygenation using a method devised by Sharpless.[43] This method employs lower valent tungsten halides, and was first used by Colvin[42] in the trichothecene series. In our hands, and at a temperature of -78 °C, before the addition of n-butyllithium, there seemed to be a reaction between the tungsten hexachloride and THF which resulted in the solution turning to a yellow slurry. Whether this was due to the tungsten hexachloride polymerising the THF or a problem in the purity of the THF is unknown. This problem was eradicated by lowering the temperature even further. The temperature was reduced to -196 °C by means of a liquid nitrogen bath and the THF was added at this point to the flask already charged with tungsten hexachloride. The bath was then replaced by one containing dry ice and acetone and n-butyllithium was added almost immediately, greatly reducing the possibility of any side reactions. Care must also be taken to avoid exposure of the tungsten halide to air or moisture. The blue-black crystals can become encrusted with yellow or orange oxides, although slight contamination from these products does not interfere with the deoxygenation.[98] The reaction is carried out at reflux and is generally complete in 4 to 5 hours; operation at room temperature can lead to chlorohydrin formation. In the work-up, if aqueous alkali is used alone, an emulsion is produced. The addition of sodium

tartrate as a chelating agent permits a clean separation of phases and a simple extraction. This reliable one-step technique gave the olefin (73) in 97% yield under mild conditions (Scheme 34). It is also compatible with the ester functionality at C-3 and C-4 and is considered to be significantly superior to the methods of King and Greenhalgh[38] and Roush.[18]

Scheme 34

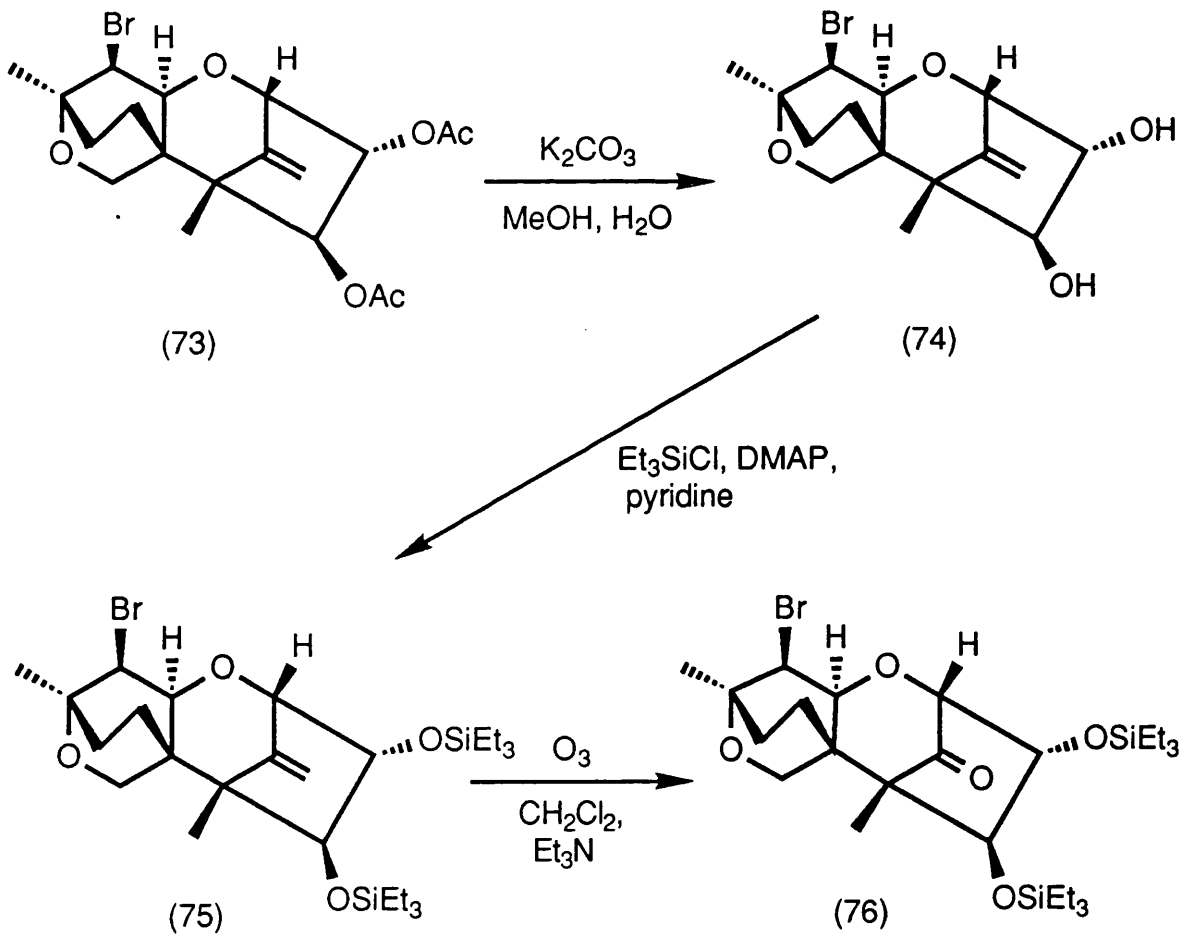


(72) (73)

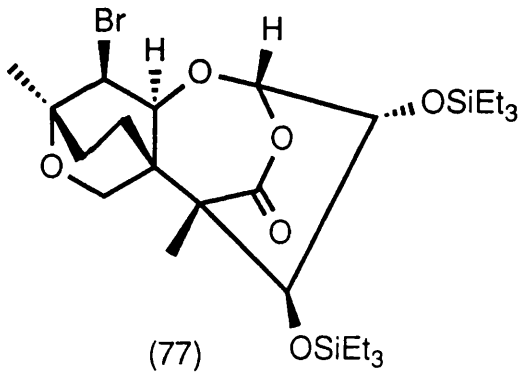
The next planned step in the synthesis was oxidative cleavage of the exomethylene double bond produced by the deoxygenation step. This would then be followed by a Wittig reaction which would introduce the 'extra' methyl group at C-13. The use of ylids would cleave the acetate groups at C-3 and C-4 and if such acetate cleavage were to occur before attack at the ketone then a β-hydroxy ketone would be produced. This could then take part in a retro-aldol reaction, ring C of the trichothecene would be cleaved, and consequently the substrate would be destroyed.

In order to avoid this possibility, the diacetate (73) was hydrolysed to the diol (74) using potassium carbonate in methanol and water. This was then protected as the bistriethylsilyl ether (75), an excellent alcohol protecting group which would remain intact during the forthcoming synthetic transformation. Both the hydrolysis and the silylation occurred in high yield (Scheme 35).

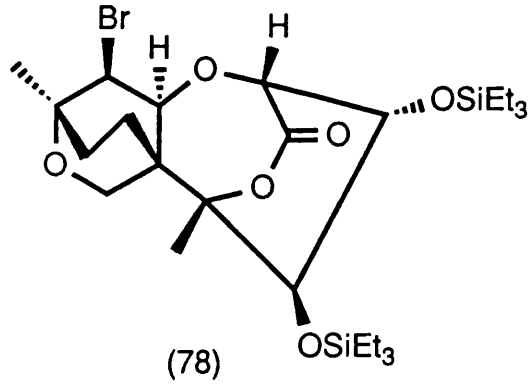
Scheme 35



Ozonolysis oxidatively cleaved the double bond in (75) to give the desired ketone (76). Early attempts at carrying out the reaction in CH_2Cl_2 using Et_3N to reduce the ozonide gave a mixture of products which were difficult to separate. An interesting result was obtained when the ozonide was allowed to warm to room temperature before the addition of the reducing agent. The reaction mixture showed only one spot on t.l.c with a lower polarity than that of the starting olefin. The ^{13}C NMR showed one less carbon than the starting material but no signal indicative of a quaternary ketonic carbon. There was however a signal at 171.4 ppm which seemed to suggest that ester group functionality was present. This indicated that it was possibly one of the two lactones (77) or (78). It was established by ^{1}H NMR that (77) was the reaction product. A doublet appearing at δ 5.52 corresponded to the 2-H proton, shifted downfield as it is now on a carbon adjacent to two oxygen atoms. Attempts at reproducing this result showed that significant amounts of the lactone (78) were evident.



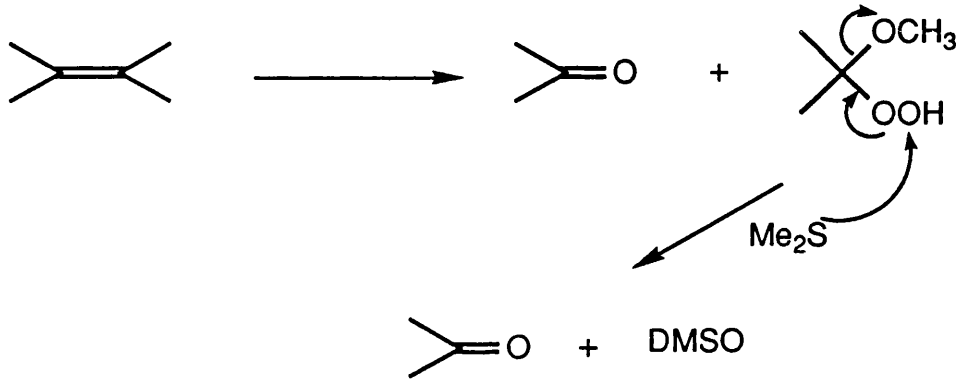
(77)



(78)

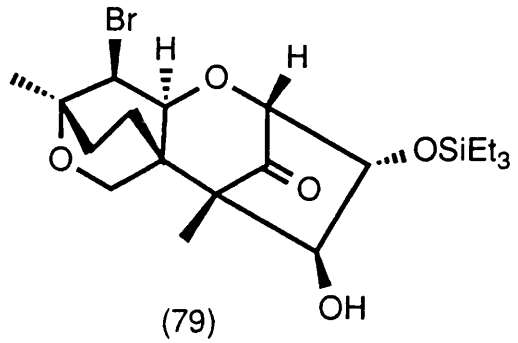
Subsequent attempts, rigorously controlling the temperature at which the reducing agent was added, still seemed to give slight contamination by lactonic products, separation proving tedious and the yield of the desired ketone (76) being no greater than 75% (Scheme 35). It was decided at this stage to attempt this transformation using a more traditional method of ozonolysis.

Scheme 36



This method involved carrying out the reaction in methanol and using DMS as the reductant.[99] The carbonyl oxide moiety produced from the fragmentation of the initial or primary ozonide combines with the protic solvent to give a methyl hydroperoxide which is rapidly and cleanly reduced by dimethyl sulphide (Scheme 36). This method has certain advantages in that the reaction is carried out under neutral conditions, and methanol, DMSO and excess DMS are easily removed. However, attempted ozonolysis under these conditions led to exclusively one monotriethylsilyl ketone. It was not possible to tell

which group had been lost directly from spectroscopy. A small amount of the mono product was acetylated in the usual way. The product of the reaction had a doublet downfield at δ 5.54, indicative of the 4-H proton. Therefore it was the 4β-triethylsilyl protection which was being cleaved during the ozonolysis and the actual reaction product was (79).

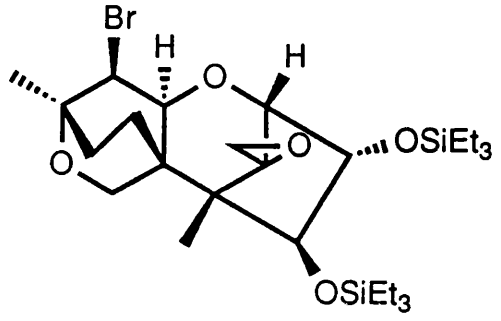


(79)

This method was then adapted: CH_2Cl_2, a non-participating solvent, was used but a three-fold excess of methanol was still added to trap the carbonyl oxide moiety. This method had the desired effect in reducing the 4β-triethylsilyl cleavage but the yield was still no greater than 60%.

By this time the desired ketone (76) could be routinely separated from lactonic products and so the original ozonolysis procedure became the focus of attention once more. It was found using this method that by raising the temperature of the solution containing the ozonide to 0°C, and then immediately adding a three-fold excess of Et_3N, that an acceptable yield of 91% of the

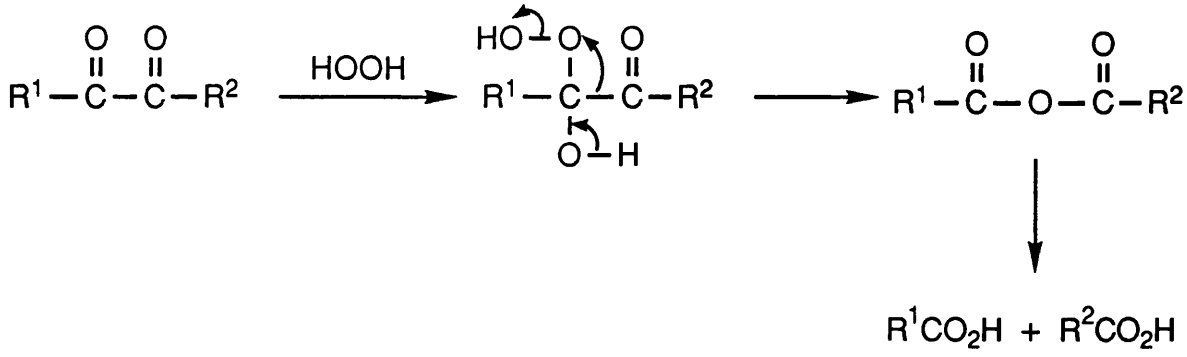
desired ketone (76) could be obtained. This could then be separated from the two lactones (77) and (78) and a third product the epoxide (80) which was present in very small amounts.



(80)

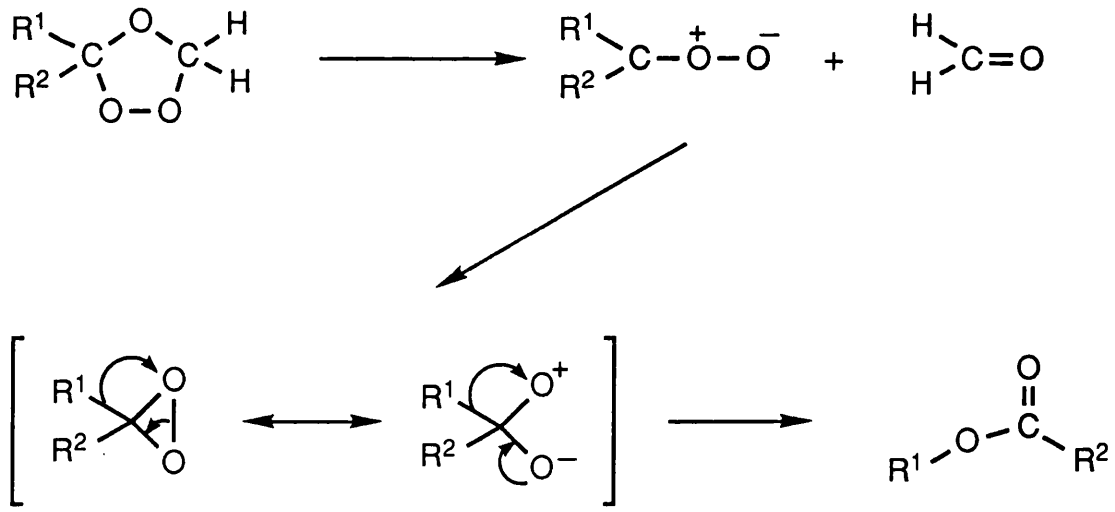
It has been shown[100,101] that several compounds give anomalous products under usual work-up conditions. Very little or none of these unwanted side products was obtained however, when the ozonolyses were carried out at temperatures below -70°C, when overozonation was avoided and when the cold ozonolysis mixtures were immediately reduced. This would seem to indicate that these 'abnormal' ozonolysis products are not the result of rearrangement of an initial ozonide (38) or of a carbonyl oxide moiety (35), since these reactive intermediates should no longer be present before the reductions were carried out. The two anomalous ozonolysis products, lactones (77) and (78), must arise from the rearrangement of a peroxidic ozonolysis product. These are not to be confused with products which originate from oxidation of normal ozonolysis products which can occur as a result of

Scheme 37



overozonation or because an oxidative work-up was used. Indeed many ozonolysis reaction mixtures are treated with hydrogen peroxide[102,103] and it is possible that these 'abnormal' products result from a Baeyer-Villiger oxidation and rearrangement (Scheme 37).[104]

Scheme 38

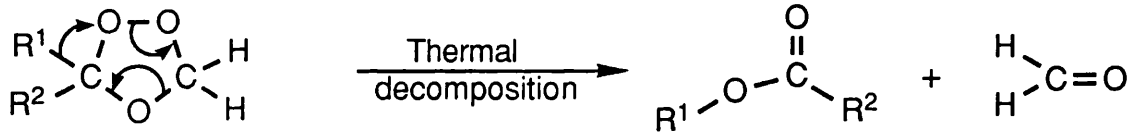


(81)

The abnormal ozonolysis seen in the trichothecene series includes oxidative rupture not only of the double bond but also of an adjacent single carbon-carbon bond. This could possibly be explained in terms of a peroxide rearrangement of a carbonyl oxide which may or may not proceed via a dioxirane intermediate (81) (Scheme 38).

A second, and possibly more likely possibility is thermal decomposition of the ozonide directly to lactonic products (Scheme 39). This has also been used as a complementary method to the Baeyer-Villiger oxidation of hindered ketones.[105]

Scheme 39



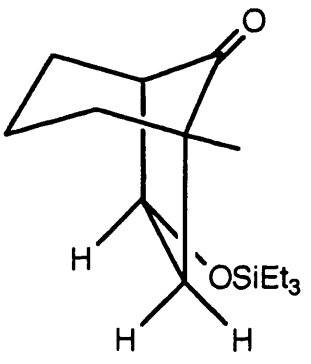
As there are mechanistic similarities to Baeyer-Villiger oxidation, it would not be unreasonable to propose why lactone (77) seems to predominate in all cases since the principle migrating group should be the one best able to sustain a positive charge and as the two possibilities are a tertiary carbonium ion and an oxycarbenium ion, it is the latter which is favoured.

Having obtained what was believed to be an optimal yield for the oxidative cleavage of the 12-ene

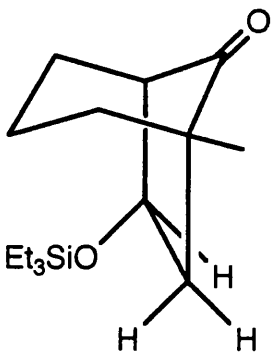
(75), following a series of synthetic transformations which themselves proceeded in good yield, reasonable amounts of the bistriethylsilyl ketone (76) were produced in order to attempt the crucial Wittig reaction using ethylidene triphenyl phosphorane. In order to obtain a working set of experimental conditions and to investigate the ratio of geometric isomers which might be expected from this Wittig reaction, a model study was pursued. This would also help to determine whether subsequent epoxidation proceeded with face stereoselectivity.

Chapter 2.2 Model Studies

The model study used to investigate synthetic transformations within the trichothecene series employed the bicyclo[3.2.1]octanes (81) and (82), which were prepared in racemic form. 2-Methylcyclohexanone (83) was chosen as the commercially available starting material for the synthesis of these target model compounds, which would then be used, in the first instance, to investigate the Wittig reaction.



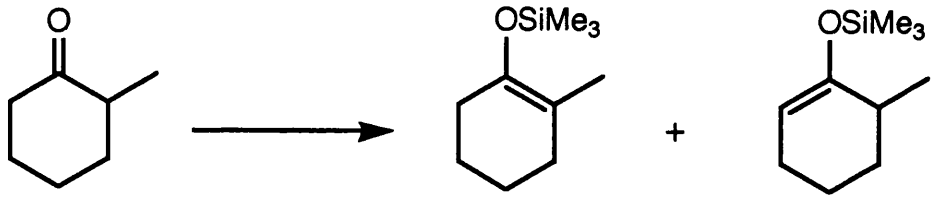
(81)



(82)

As a regiospecific monoalkylation was required as an integral part of building the bicyclic ring system it was decided to use silyl enol ethers to trap the initially produced enolates as isolable derivatives which could be readily regenerated. The starting ketone (83) was converted, using the method of House,[106] into two trimethylsilyl enol ethers (84) and (85) using trimethylsilylchloride, triethylamine and DMF (Scheme 40).

Scheme 40



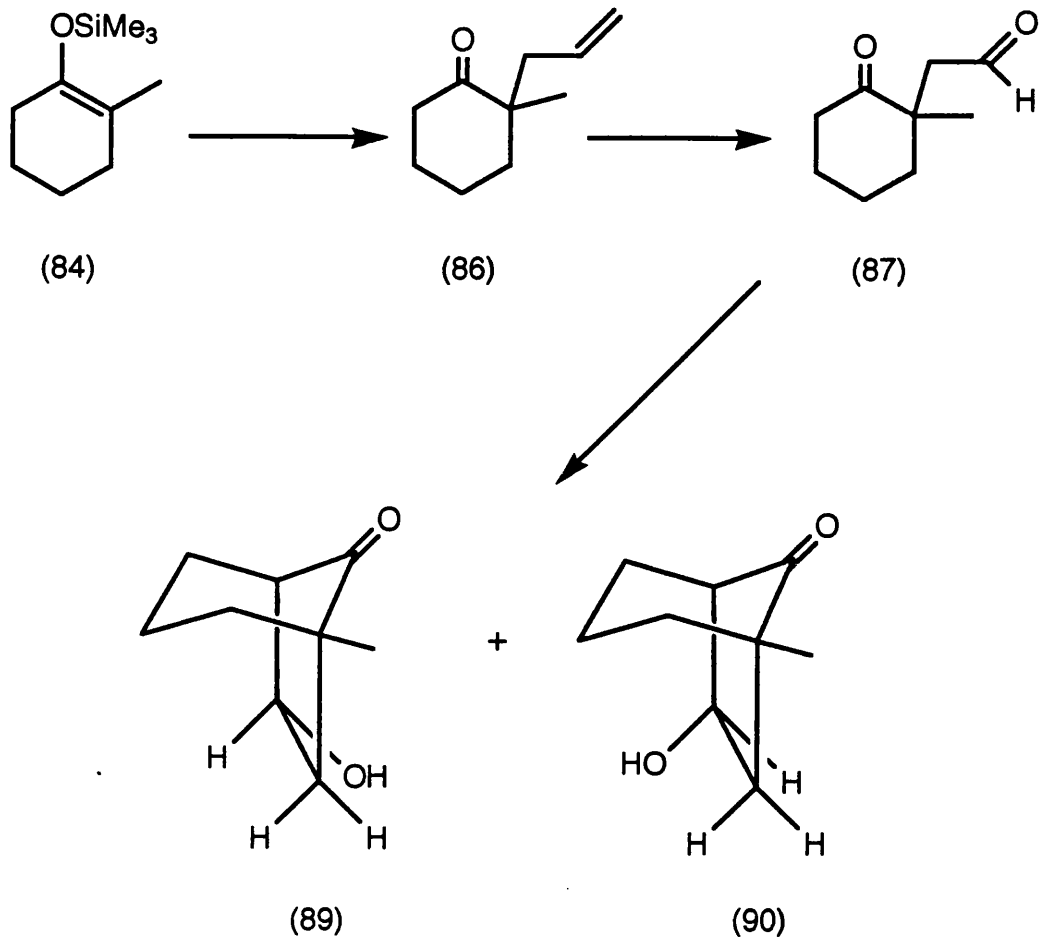
(83) (84) (85)

The major product was as expected the more highly substituted silyl enol ether (84), obtained from the trapping of the enolate formed under the conditions of thermodynamic control. Trimethylsilyl enol ethers are rapidly hydrolysed by water; however, an aqueous work-up was used which allowed satisfactory product isolation. Some of the less highly substituted silyl enol ether (85) was also obtained but this was easily separated from the desired product by vacuum distillation.

The trimethylsilyl enol ether (84) was then readily cleaved by methyl lithium, thus freeing the lithium enolate and giving TMS as the other product, the latter providing a useful internal standard for ^1H NMR investigations of these enolate solutions. The lithium enolate was then allowed to react quickly with allyl bromide to give the allyl ketone (86) (Scheme 41).

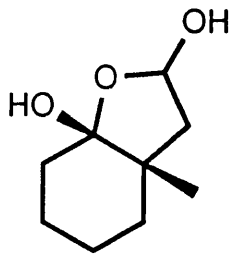
Ozonolysis of the allyl ketone (86) in CH_2Cl_2 followed by reductive work-up using triethylamine gave the keto-aldehyde (87) in good yield. An ethereal solution

Scheme 41



was filtered through a short column of chromatographic silica and concentrated. The resulting pale yellow oil was used immediately in the subsequent aldol reaction. DMS reduction of the ozonolysis product could not be used as the necessary aqueous work-up leads to formation of a bishemiacetal (88).

Intramolecular aldol cyclisation occurred using sodium methoxide in methanol to give two epimeric hydroxy ketones (89) and (90) in modest yield in a ratio of



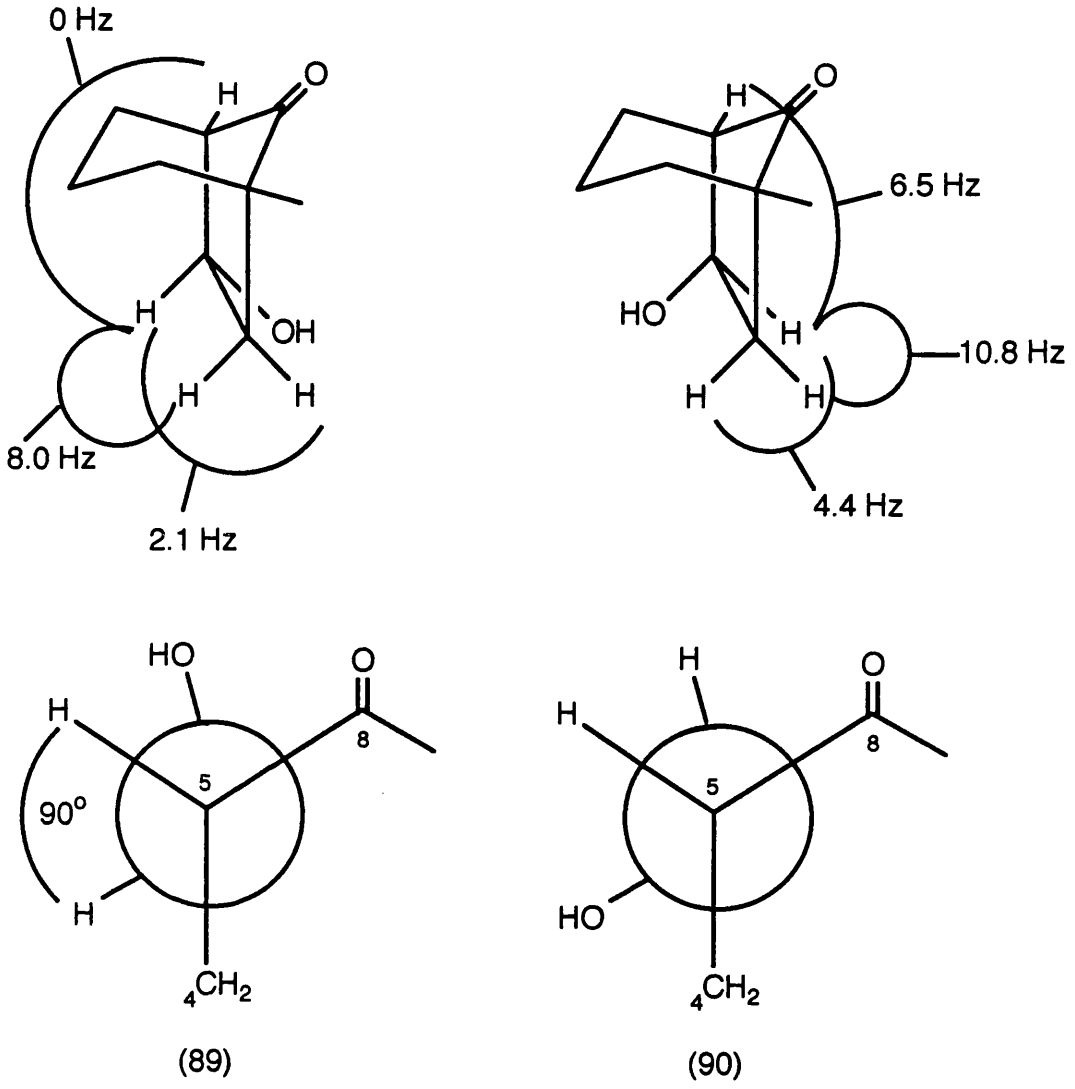
(88)

2.5 : 1 in favour of the β-epimer (89). The β-hydroxy ketone (89), which gave the lower R_f in a solvent system of ethyl acetate and light petroleum (40-60 ^{o}C), could be distinguished from the α-hydroxy ketone by means of their respective proton NMR spectra.

The 6α-H in (89) appears as a doublet of doublets, this is due to an 8.0 Hz coupling to 7α-H and a 2.1 Hz coupling to 7β-H. No splitting is observed from the interaction between 6α-H and 5-H. Examination of molecular models shows that the dihedral angle is around 90^{o}; the Karplus equation[107] approximates that in such cases the coupling constant is zero (Scheme 42).

The 6β-H in (90) appears as a doublet of double doublets, this is due to a 10.8 Hz coupling to 7β-H, a coupling of 6.5 Hz to 7α-H and a 4.4 Hz coupling to the 5-H proton. The downfield shifts of both signals are clearly seen by ^{1}H NMR and so establishing the purity is not a problem. Chromatographic separation is routinely achieved.

Scheme 42

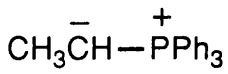


(89) (90)

As acetate protection is sensitive to the conditions of the Wittig reaction, the epimeric hydroxy ketones (89) and (90) were silylated using triethylchlorosilane, triethylamine, DMAP and ether, to furnish the target model compounds (81) and (82).

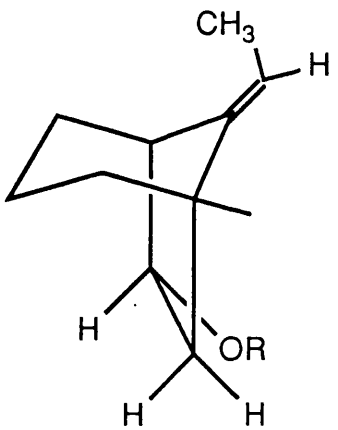
The Wittig reaction was attempted firstly on the β-triethylsilyl ketone (81), as larger amounts of this compound were obtained by the synthesis thus far.

Ethyltriphenylphosphonium bromide was made from triphenylphosphine and bromoethane in toluene in a sealed tube which was heated at a temperature of 105 °C for 16 hours. The ethylidene triphenylphosphorane ylid (91) was made <u>in situ</u> by the reaction of the bromide with n-butyllithium in THF at room temperature, the ketone (81) was added and the resulting solution was refluxed for one hour.

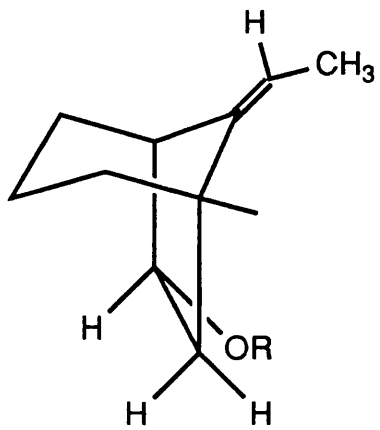


(91)

Interestingly only one product was obtained, where two geometric isomers (92) and (93) may have been expected. The proton NMR spectrum showed that after suction flash chromatography the triethylsilyl group had been cleaved and that it was a 6β-alcohol which was present, so the product was actually (94) or (95). The structure was solved by means of n.O.e. experiments.



(92), R = $SiEt_3$
(94), R = OH



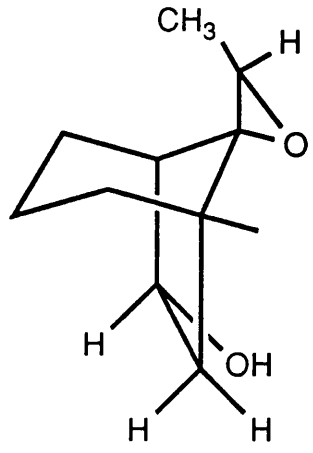
(93), R = $SiEt_3$
(95), R = OH

Unlike the interaction of one magnetic nucleus with another which takes place through the bonds of a molecule and leads to spin-spin coupling, magnetic nuclei also interact through space, but this interaction does not lead to coupling. When one of the nuclei is irradiated at its resonance frequency and the other is detected as a more intense or weaker signal than usual this is called nuclear Overhauser enhancement (n.O.e.). The effect is only noticeable over short distances and falls off rapidly as the inverse of the sixth power of the distance between the two nuclei.

Irradiation of the methyl group at the ring junction (1-Me), leads to an enhancement of the olefinic proton signal but no enhancement of the olefinic methyl group is observed. Equally, irradiation at the resonance frequency of the signal due to the olefinic proton leads to an enhancement of the signal due to the methyl group at the ring junction. On the basis of these results the product was assigned the structure (94).

Having established that the single product from the Wittig reaction on (81) was the alcohol (94), an epoxidation, using m-CPBA as the oxidant, was then performed. Again, only one product was obtained, the epoxide (96). The rigid geometry of the bicyclo[3.2.1]octane ensures that peracid attack occurs from the side of the two-carbon bridge which is the less hindered face. It is also possible that the 6β-hydroxyl

group acts as an H-bonding anchor for the per-acid and is partly responsible for the stereospecificity which is observed.



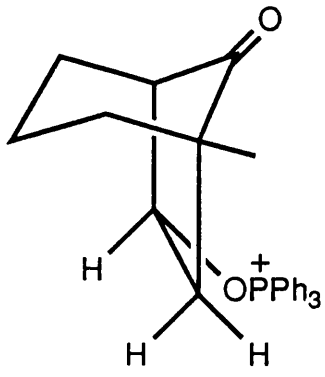
(96)

It was felt at this point that a better model system would be one which had the 6α-triethylsilyl functionality to mimic that of the 3α-triethylsilyl group of (76) which was to undergo the Wittig reaction in the trichothecene series.

In order to generate significant amounts of the ketone (82), a more efficient synthesis of the α-hydroxy ketone (90) was needed. It was decided to do this by converting the β-hydroxy ketone (89) into its epimeric counterpart (90).

One possibility would be to do this directly using DEAD and triphenylphosphine in a Mitsunobu reaction,[108] however the use of hydroxide ion as the nucleophile would almost certainly result in attack at the

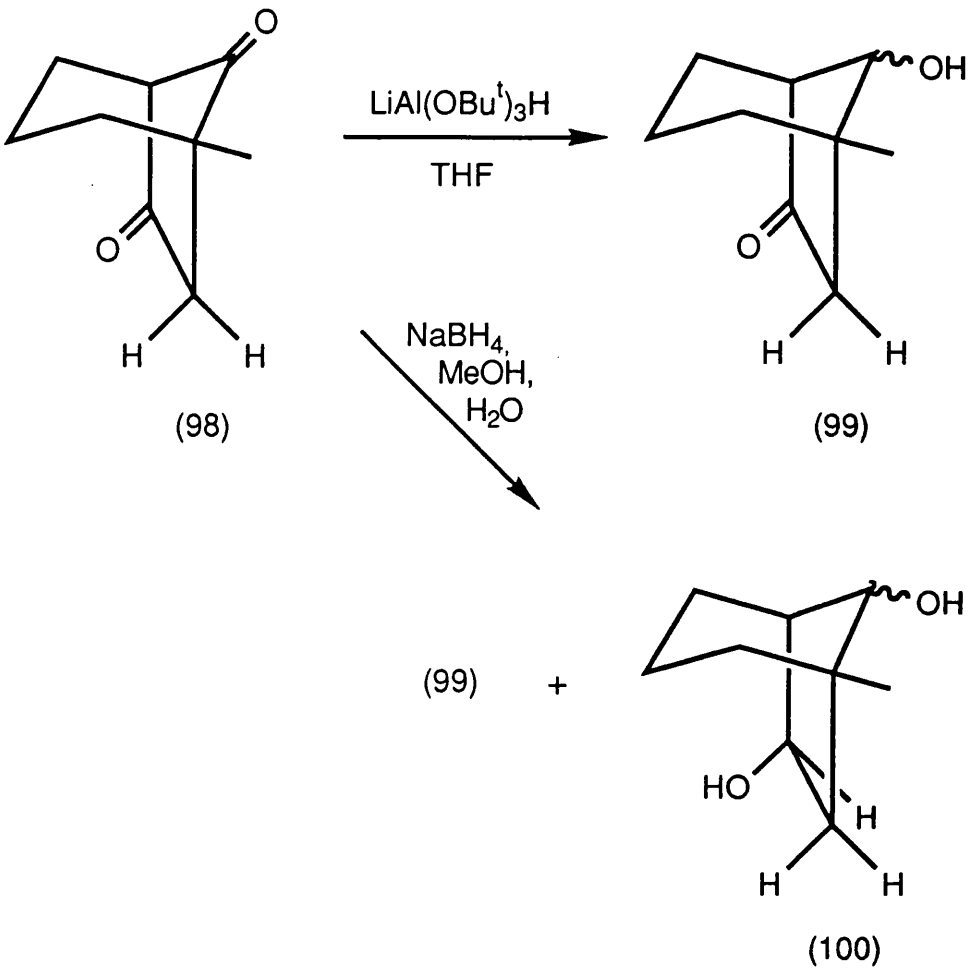
bridgehead carbonyl group and lead to structural rearrangement, before displacement of the alkoxy phosphonium salt (97).



(97)

Prompted by a paper[109] on bridged ring systems involving bicyclo[3.3.1]nonanes in which diones were being reduced both regiospecifically and stereoselectively using lithium hydridotri-t-butoxyaluminate, the same protocol was applied to the bicyclo[3.2.1]octane system. The β-hydroxy ketone (89) was oxidised firstly using the method of Jones[110] to give the dione (98). This was followed by reduction using a slight excess of complex hydride in THF with stirring at room temperature. The ^{1}H NMR spectrum indicated that it was the bridgehead carbonyl which was being reduced to give the keto alcohol (99). Attempts were also made employing $NaBH_4$ at 0°C and at room temperature with the same outcome. These reductions were also accompanied by a small amount of over reduced product (100), which had the 6α-hydroxy configuration (Scheme 43).

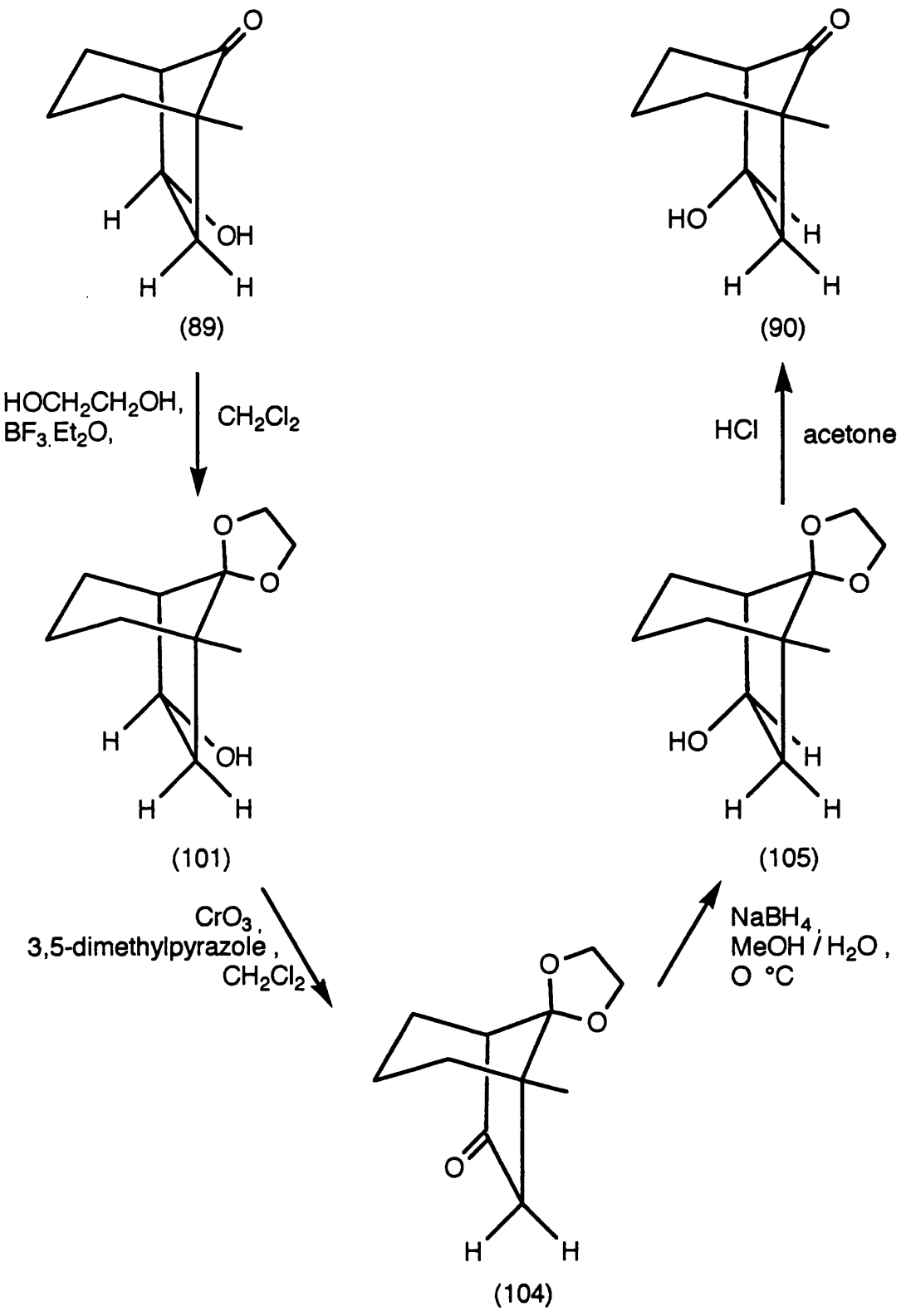
Scheme 43



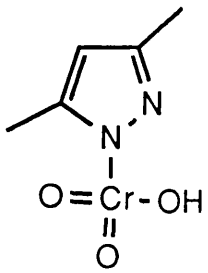
Luche reduction[111] of the dione (98) was also attempted to see if any regioselectivity could be obtained but, as with the complex hydride, the product was found to be the keto-alcohol (99).

It was obvious by this time that the conversion of (89) into (90) could not be achieved by a two-step oxidation-reduction procedure and that protection of the more reactive bridgehead carbonyl group was necessary. The β-hydroxyketone (89) was converted firstly into

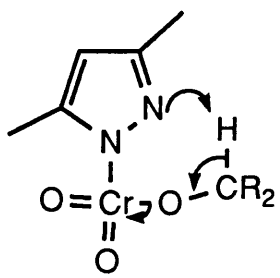
Scheme 44


O
H
OH
H
H
(89)
HOCH2CH2OH,
BF3.Et2O,
CH2Cl2
O
O
H
OH
H
H
(101)
CrO3,
3,5-dimethylpyrazole,
CH2Cl2
O
O
O
H
H
(104)
NaBH4,
MeOH / H2O,
0 °C
O
O
HO
H
H
H
(105)
HCl
acetone
O
HO
H
H
H
(90)

ethylenedioxy derivative (101) in 86% yield using ethylene glycol and $BF_3.Et_2O$ by stirring overnight at room temperature in dichloromethane (Scheme 44). Oxidation could not now employ the method of Jones[110] as the acetal protection would be cleaved under the acidic conditions of this reaction. It was decided to attempt the oxidation under neutral conditions employing the method of Corey and Fleet.[112] The oxidising species is a complex of chromium trioxide and 3,5-dimethylpyrazole. Addition of the pyrazole to a suspension of chromium trioxide in dichloromethane at room temperature gives a dark red solution and after 30 minutes all of the chromium trioxide dissolves. The complex (102) which is formed combines with secondary alcohols to form a chromate ester complex (103), from which oxidation proceeds via a cyclic intramolecular course. This method successfully oxidised the ethylenedioxy derivative (101) with the 6β-alcohol configuration to the ketone (104) in 80% yield .



(102)



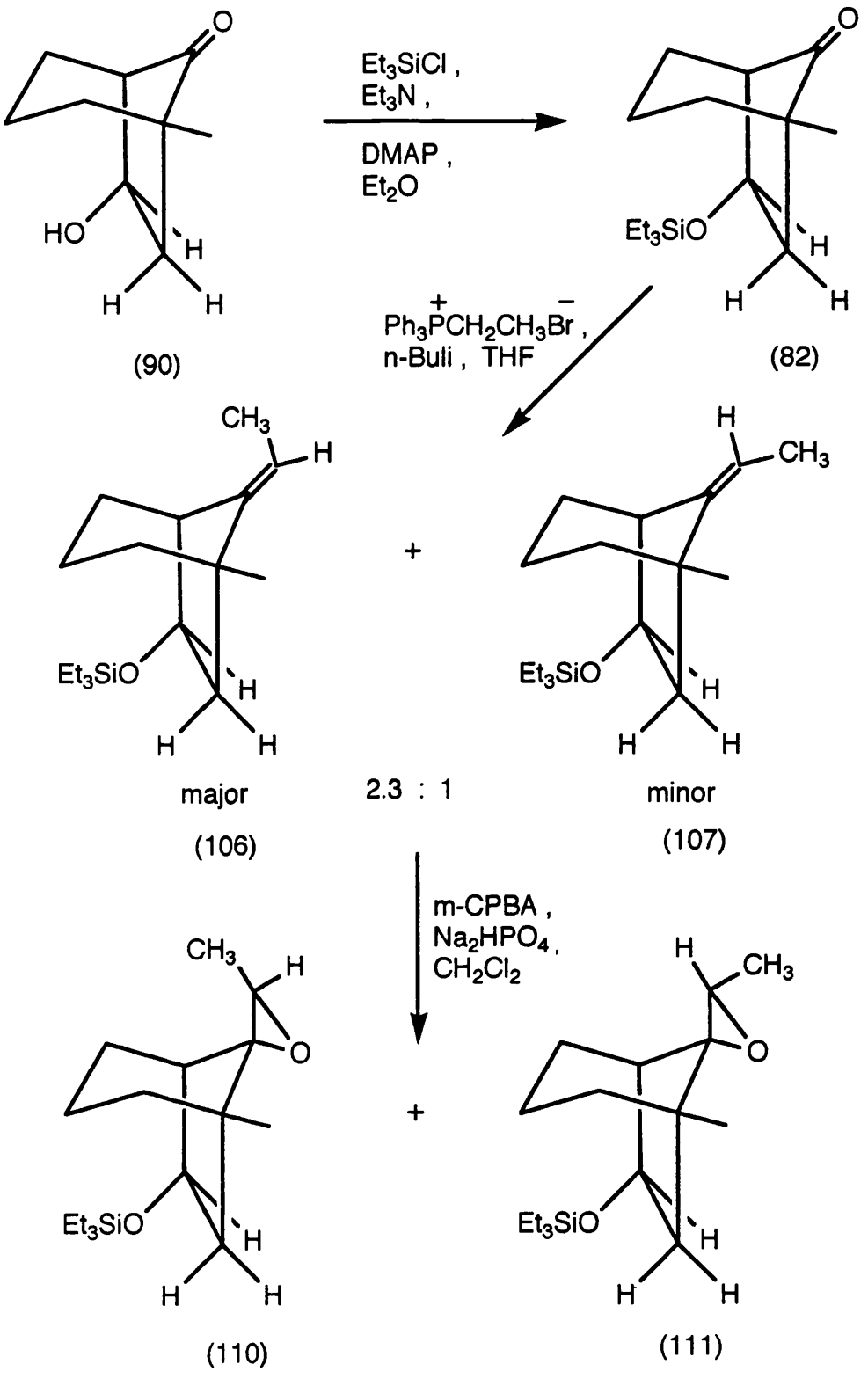
(103)

No problems were anticipated with the following reduction step in terms of stereoselectivity. By analogy with the reduction of the dione (98), using $NaBH_4$ at 0°C in methanol and water, it was noted that the overreduction product, the diol (100), exhibited exclusively the 6α-hydroxy configuration. Reduction of the ketone (104) under analogous conditions gave the ethylenedioxy derivative (105) with 6α-alcohol configuration in 95% yield, together with a trace contaminant which proved to be its 6β-counterpart (101). The final step in this conversion was deprotection of the ketal. This was achieved using a 2% solution of 1 M HCl in acetone with stirring at room temperature for 48 hours, to give the α-hydroxy ketone (90) in essentially quantitative yield. Altogether the conversion from the β-hydroxy ketone (89) to the α-hydroxy ketone (90) proceeded in an overall yield of 65% (Scheme 44).

This four step procedure allowed meaningful amounts of the α-epimer (90) to be prepared. This was then silylated in the same way as before to give the target model compound (82) which would be subjected to the Wittig reaction (Scheme 45).

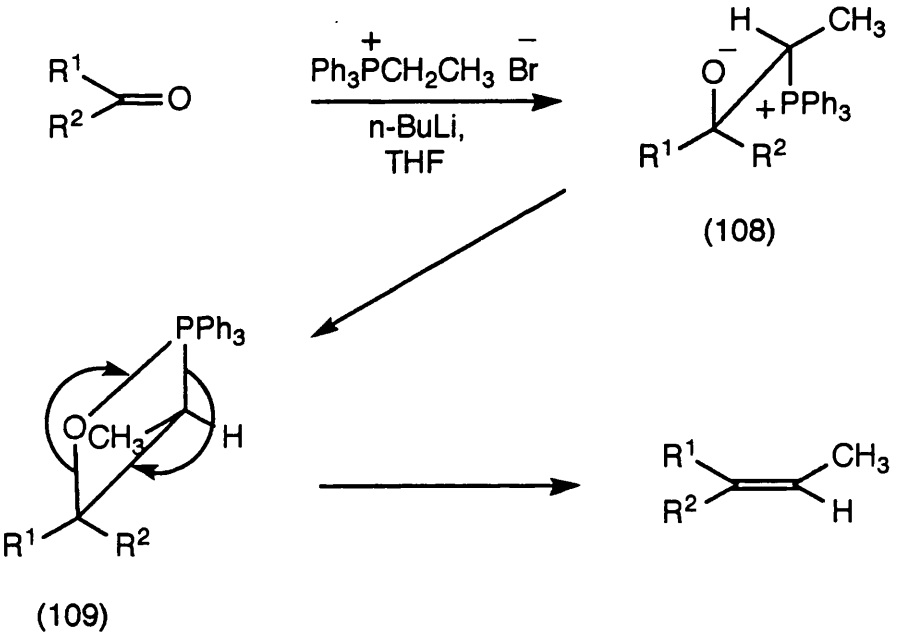
The experimental conditions that were used were identical to those used for the Wittig reaction of the β-triethylsilyl ketone (81). Two geometric isomers (106) and (107) were obtained in a ratio of 2.3 : 1, in a combined yield of 57%.

Scheme 45


Et3SiCl ,
Et3N ,
DMAP ,
Et2O
HO
H
H
H
(90)
Et3SiO
H
H
H
(82)
+
Ph3PCH2CH3Br ,
n-Buli , THF
CH3
H
Et3SiO
H
H
H
major
(106)
+
H
CH3
Et3SiO
H
H
H
minor
(107)
2.3 : 1
m-CPBA ,
Na2HPO4 ,
CH2Cl2
CH3
H
O
Et3SiO
H
H
H
(110)
+
H
CH3
O
Et3SiO
H
H
H
(111)

This is in significant contrast to the result obtained from the same reaction on its epimeric counterpart (81), which produced only one olefinic product. It is possible that a fairly large 6β group, such as triethylsilyl, is having some effect on the approach of the phosphonium ylid (91) and, as a result, only one betaine intermediate (108) is produced. This then leads to only one olefin via the collapse of an oxaphosphetane intermediate (109) (Scheme 46).

Scheme 46



In order to determine which geometric isomer was the major product of the Wittig reaction, n.O.e. experiments were carried out on the mixture of olefins (106) and (107).

In contrast to the β-hydroxy olefin (94), in

which the methyl group of the ring junction could be clearly seen by ^{1}H NMR spectroscopy, in ethers (106) and (107) the methyl group is hidden. Such an overlap of signals does not allow accurate prediction of the chemical shift of this methyl group as one cannot 'see' the signal and therefore cannot irradiate at the precise frequency.

It was possible however to see the proton attached to C-5 of the major isomer clearly. Irradiation of this signal showed a clear enhancement in the doublet due to the olefinic methyl group and a smaller enhancement of the doublet of double doublets corresponding to the 6β-proton. No enhancement was seen in the olefinic proton.

Equally, irradiation of the protons of the olefinic methyl group leads to an enhancement in the signals due to the proton attached to C-5 and additionally to those of the 6β-proton and the olefinic proton. On the basis of this information, the major isomer was assigned the structure (106).

The geometric isomers (106) and (107) were not separated but the mixture was epoxidised using m-CPBA and a basic buffer in order to establish whether two or four epoxides would be produced. As with the β-hydroxy olefin (94), the delivery of oxygen was exclusively from the side of the two carbon bridge to produce a mixture of the two epoxides (110) and (111) which were not separated.

Having completed the objectives of the model

study, work on the bicyclo[3.2.1]octanes was terminated at this point and attention returned to the trichothecene series.

Chapter 2.3 Trichothecene homologue synthesis

The crucial Wittig reaction on the ketone (76) was the next objective in the synthesis of the novel trichothecene homologues (11) and (12).

The reaction conditions were identical to those used in the Wittig reactions carried out in the model study. The ylid was prepared at room temperature and after 45 mins the ketone was added and the resulting mixture was heated under reflux for 1 hour in THF prior to work-up. A 200 MHz ^{1}H NMR was taken of the crude reaction product in order to establish the ratio of geometric isomers. If a purification had been carried out there would have been a danger of altering this ratio by achieving some partial separation and 'throwing away' one of the isomers. The proton NMR indicated that an isomeric ratio of approximately 2 : 1 was obtained. It remained to establish the configuration of the major isomer. This was achieved by means of n.O.e. experiments.

The major through space interactions, as expected from the experiments carried out in the bicyclo [3.2.1]octane series, were those involving the olefinic protons, the olefinic methyl groups, the 2-H protons and the methyl groups of C-14. As we were dealing with two bistriethylsilyl ethers (112) and (113) it was unfortunate that the methyl group at C-14 could not be seen clearly in the upfield region of the spectrum, due to the large

peaks due to the silyl groups.

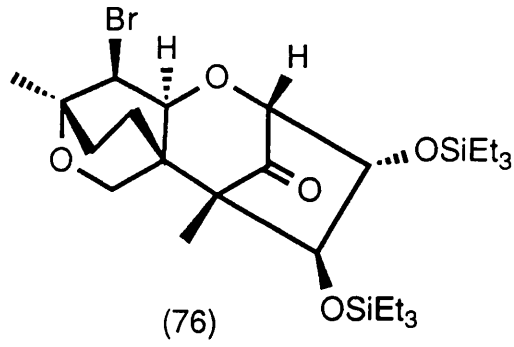
Irradiation of the olefinic protons led to enhancements in the olefinic methyl groups and vice versa, but this does not give any information on the relative configuration. The key to solving the problem lay in the fact that the doublets due to the 2-H protons had significantly different chemical shifts and that these signals could be clearly seen. Irradiation of the 2-H proton of the minor isomer leads to an enhancement in the signal due to the olefinic methyl group of the minor isomer. No enhancement of the signal due to the olefinic proton of the minor isomer was seen. Equally, irradiation of the signal due to the olefinic proton of the minor isomer gave no enhancement in the 2-H proton of the minor isomer. This would seem to indicate that the minor isomer had the structure (113).

Irradiation of the 2-H proton of the major isomer led to an enhancement in the signal due to the olefinic proton of the major isomer. Here, no enhancement of the signal due to the olefinic methyl group of the major isomer was seen. Irradiation of the signal due to the olefinic proton of the major isomer gave a clear enchancement in the 2-H proton of the major isomer.

On the basis of this data, the major isomer was assigned the structure (112) and the minor isomer was assigned the structure (113).

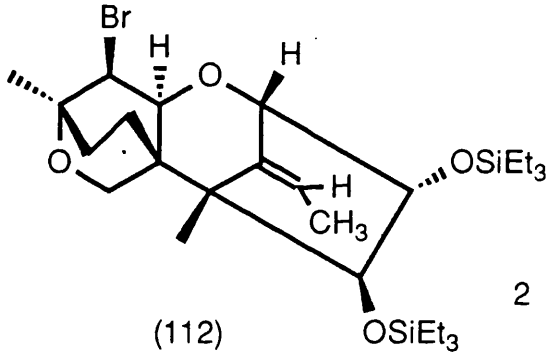
Having established that a 2 : 1 mixture of

Scheme 47



(76)

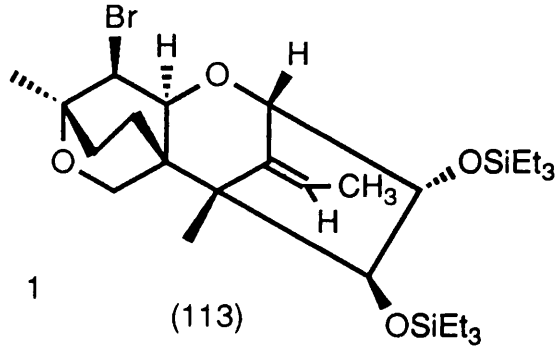
$Ph_3\overset{+}{P}CH_2CH_3\ \overset{-}{Br}$ | n-Buli, THF



(112)

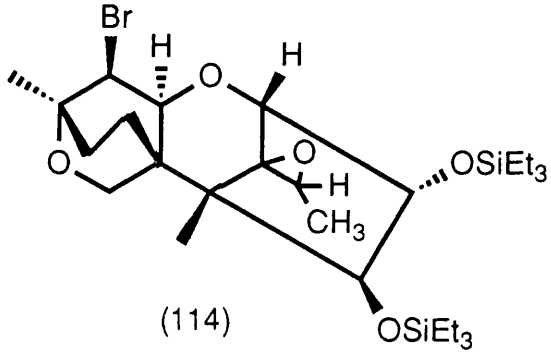
+

2 : 1



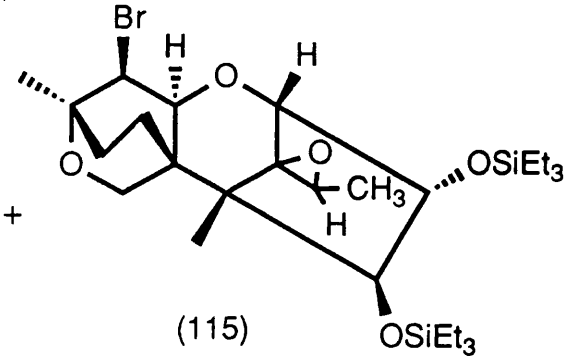
(113)

m-CPBA, Na_2HPO_4, | CH_2Cl_2, 0 °C



(114)

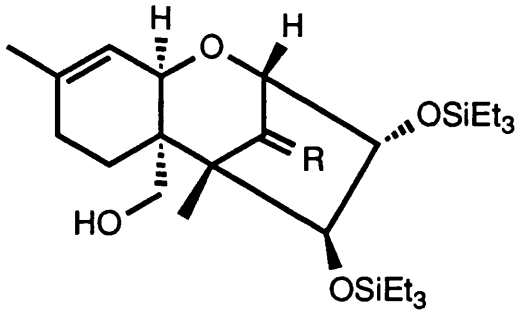
+



(115)

geometric isomers was obtained from the Wittig reaction in moderate yield and that the separation of the two isomers (112) and (113) could not be achieved, epoxidation was performed to determine the stereoselectivity.

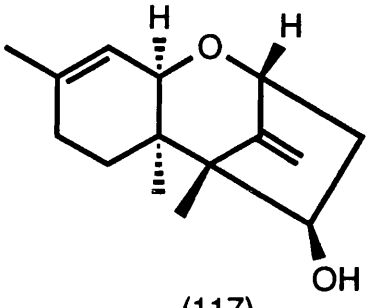
The mixture of olefins (112) and (113) was dissolved in CH_2Cl_2; adding a basic buffer Na_2HPO_4, at room temperature and m-CPBA at 0 ^{o}C with stirring for 1 hour and then overnight gave exclusively the two epoxides (114) and (115) in 86% yield after work-up (Scheme 47). The delivery of oxygen from the per-acid to the mixture of geometric isomers proceeds from the much more accessible exo face of the bicyclo[3.2.1]octane sub unit to give two epoxides rather than four. Both epoxides (114) and (115) have the epoxide oxygen <u>anti</u> to ring A of the trichothecene thus re-establishing their 'natural' configuration.



(116), R = $CHCH_3$

Bromo-ether protection of ring A was still necessary at this stage as the absence of such would have presented a complication. The tricyclic diene (116)

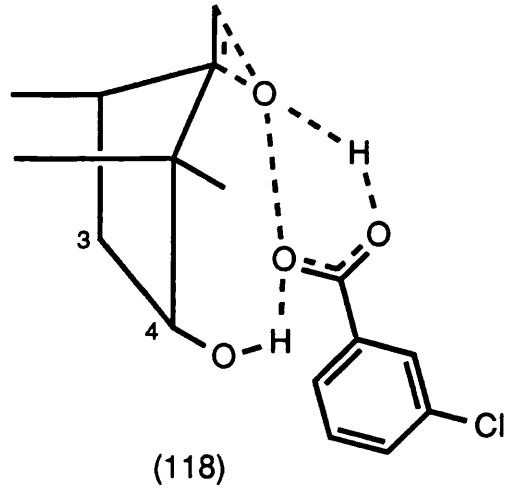
contains two sites vulnerable to epoxidation, both of which are trisubstituted double bonds. Selective epoxidation of the 12,13-double bond is unlikely to be successful in such a system since a 15-OH group can form a H-bonding anchor with the per-acid to epoxidise the 9,10-double bond preferentially.[113]



(117)

The regioselectivity of epoxidation was noted in the synthesis of Trichodermin.[114] The advanced intermediate (117) contains two sites vulnerable to epoxidation. The 9,10-double bond is trisubstituted and was therefore expected to undergo epoxidation at least ten times as fast[115] as the disubstituted methylene 12,13-double bond. However the secondary hydroxyl group at C-4 is directed precisely towards the double bond of the methylene and is thus ideally placed to form a H-bonding anchor for the electrophilic per-acid. This gives rise to a transition state of the type (118).[116] Treatment of (117) with one equivalent of buffered m-CPBA produced the desired 12,13-epoxide exclusively.

Regioselectivity is also obtained in the



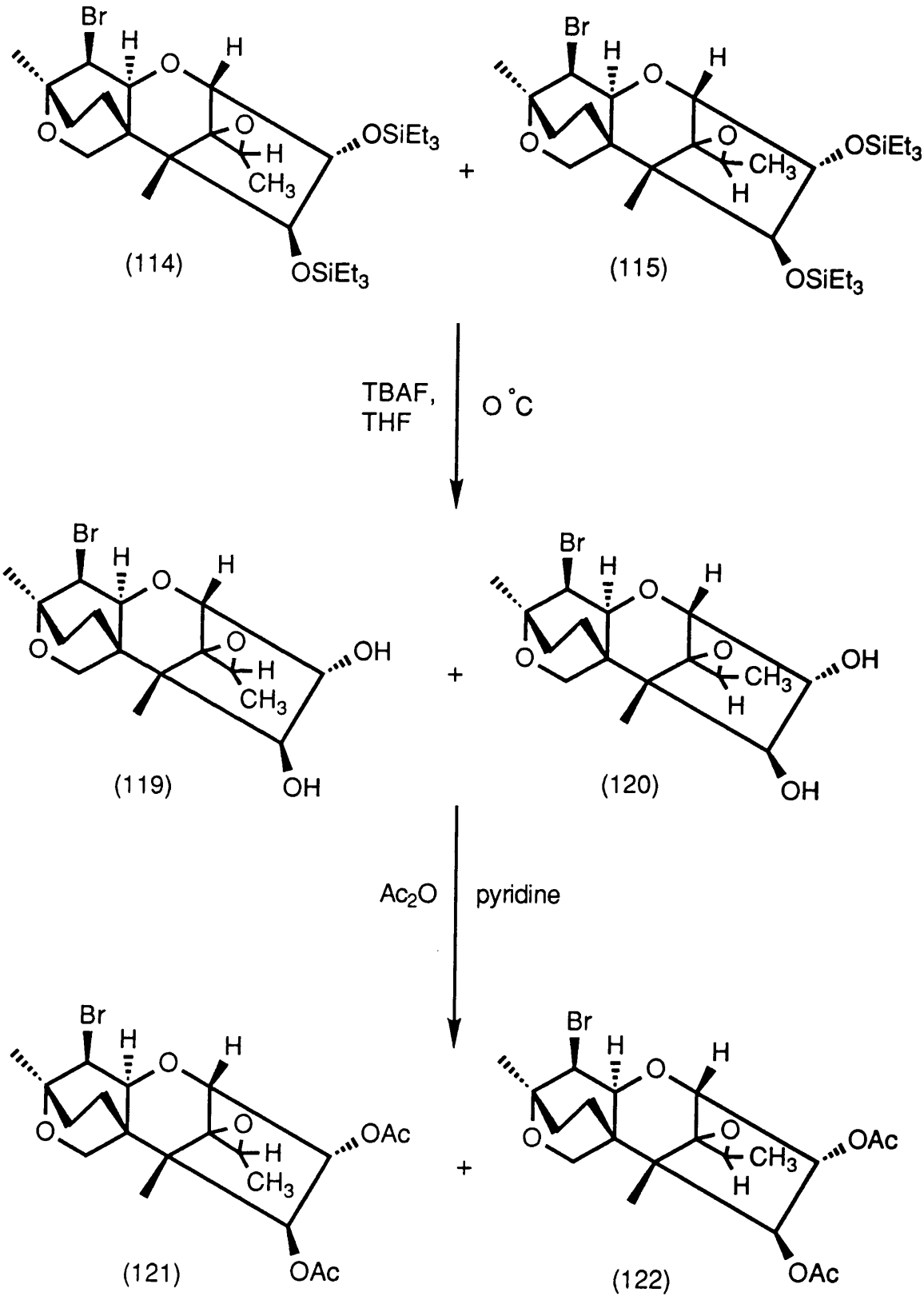
(118)

presence of a 15-acetoxy group.[117] In the absence of a hydroxyl group at C-15 and a β-hydroxyl group at C-4, the 9,10- and 12,13-epoxides have been obtained in the ratio of 1 : 1.[118]

The next stage in the synthesis was the desilylation of the bistriethylsilyl ethers (114) and (115). The sensitivity of the trichothecenes to acid precluded the use of aqueous HF. Fluoride ion-induced cleavage of the triethylsilyl groups was achieved using TBAF,[119] commercially available as a 1 M solution in THF, to give a mixture of epoxy diols (119) and (120) which could not be separated.

Having achieved the hydrolysis of the silyl ether linkage under aprotic conditions to give the diols, the next stage was acetylation to furnish the two epoxy diacetates (121) and (122) in 94% yield (Scheme 48). Deprotection of ring A was not undertaken because the products would have been triols and solubility problems

Scheme 48


Br
H
O
H
OSiEt3
CH3
(114)
(115)
TBAF,
THF
O °C
OH
(119)
(120)
Ac2O
pyridine
OAc
(121)
(122)

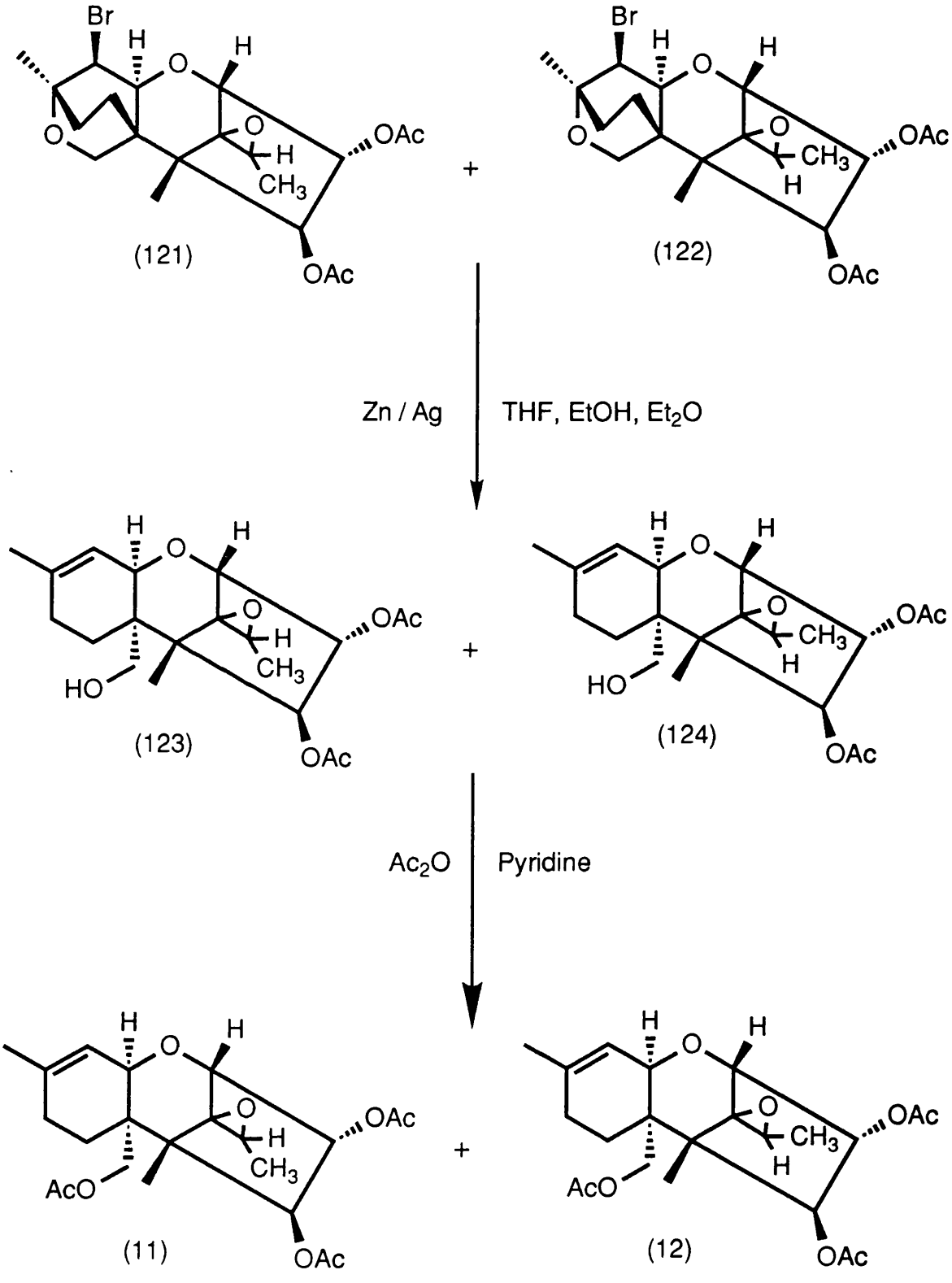
would no doubt have been encountered. Attempts at separating the two diacetates (121) and (122) failed and as the amounts of material being handled were becoming very small it was decided to complete the synthesis of the target compounds (11) and (12) before attempting any further separations.

The next stage in the synthesis was cleavage of the bromo-ether moiety. This was accomplished using a zinc-silver couple.[120] It was noted previously in the synthesis of the trichothecene Calonectrin[121] that other reagents such as zinc dust in DMF, THF or methanol, or magnesium in ether or THF, were ineffective in the reductive regeneration of the trisubstituted 9,10-double bond. The couple was prepared by addition of powdered zinc to a stirred hot solution of silver acetate in acetic acid, followed by successive washes with acetic acid and then ether. This method furnished the alcohols (123) and (124) in 60% yield.

The final step in the synthesis was acetylation of the 15-hydroxyl groups to give the triacetates (11) and (12) (Scheme 49).

Only limited success was achieved in the short time possible for the separation of the two final products, the novel trichothecene mycotoxins (11) and (12). Flash chromatography appeared to show a clean separation of spots on a t.l.c. plate after double elution using 25% ethyl acetate / 75% hexane. 200 MHz ^{1}H NMR

Scheme 49


Br
H
O
H
O
O
H
CH3
OAc
OAc
(121)
+
Br
H
O
H
O
O
CH3
H
OAc
OAc
(122)
Zn / Ag
THF, EtOH, Et2O
H
O
H
O
O
H
CH3
OAc
HO
OAc
(123)
+
H
O
H
O
O
CH3
H
OAc
HO
OAc
(124)
Ac2O
Pyridine
H
O
H
O
O
H
CH3
OAc
AcO
OAc
(11)
+
H
O
H
O
O
CH3
H
OAc
AcO
OAc
(12)

studies on the fraction which appeared to be pure in one isomer still showed that a significant amount of the other isomer remained. This was unsatisfactory for the purposes of testing these compounds. The complete separation of (11) and (12), and indeed the separation of the pairs of diastereomers (121) and (122) and those of (123) and (124) should not present an insurmountable problem if more time and material were available.

Another proposed target by partial synthesis from the trichothecenes DAS (1) and MAS (2) was the aziridine (13). The synthesis of such a compound would also undoubtedly provide important information on the biological mode of action of the trichothecenes and give an even more complete picture of their metabolic fate. Unfortunately the completion of its synthesis from the olefin (73) was not achieved due to lack of time.

It is hoped that future work in the field of trichothecenes may provide answers to questions which remain in all aspects of this field.

3. Experimental

Instrumentation

Melting points were determined on a Kofler hot-stage melting point apparatus and are uncorrected. Bulb to bulb distillations were carried out on a Buchi GKR-50 Kugelrohr. Recorded boiling ranges refer to the indicated air bath temperatures.

^{1}H NMR spectra were recorded on a Bruker WP200SY or Bruker AM200 spectrometer operating at 200 MHz unless stated otherwise. In such cases a Perkin Elmer R32 or Varian EM390 spectrometer operating at 90 MHz was used. All 13C NMR spectra were recorded on the aforementioned Bruker instruments operating at 50 MHz. In all cases, deuteriochloroform was used as solvent with Me_4Si as internal standard. Chemical shifts are reported in parts per million (δ) relative to Me_4Si, using Me_4Si or the δ 7.25 residual chloroform peak and the δ 77 deuteriochloroform peak as internal references for the ^{1}H and ^{13}C NMR respectively. ^{1}H NMR data are reported using the convention : chemical shift, integrated intensity, multiplicity, observed coupling constant (J) in Hz, and assignment.

Infra-red spectra were determined on a Perkin-Elmer 580 spectrometer. Low resolution mass spectra were determined on a VG updated MS 12 spectrometer while high resolution mass spectra were determined on a VG updated MS

902 spectrometer.

Reactions were normally performed in an atmosphere of nitrogen. Tetrahydrofuran (THF) and diethyl ether (ether) were freshly distilled from sodium-benzophenone ketyl. Acetonitrile (MeCN) and dichloromethane (CH_2Cl_2) were distilled from CaH_2 and were stored over molecular sieves. Light petroleum refers to that fraction of petrol which boils between 40 and 60 °C.

4,4-Dimethylaminopyridine (DMAP) was recrystallised from cyclohexane. N-Bromosuccinimide (NBS) was recrystallised fron water and dried <u>in vacuo</u> over P_2O_5. All organic solutions were dried over Na_2SO_4 unless stated otherwise and, after filtration, were concentrated under reduced pressure using a Buchi Rotavapor. Dry-column flash chromatography[122] and flash chromatography[123] refer to techniques described elsewhere.

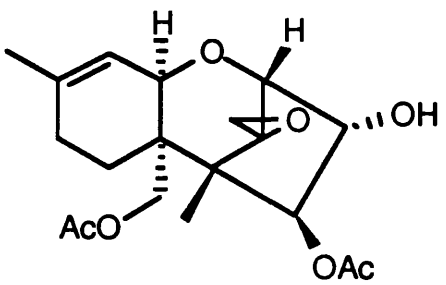
In this thesis all model compounds i.e. all bicyclo[3.2.1]octanes are racemic. All other compounds are derived from natural DAS (1) or MAS (2) and are therefore chiral.

Starting Materials

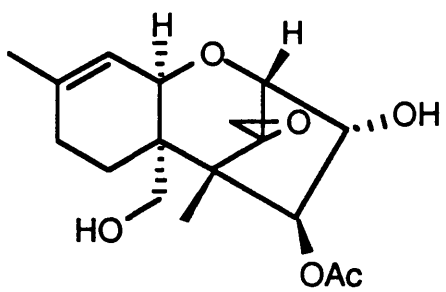
2-Methylcyclohexanone was bought from the Aldrich Chemical Co. and was used without further purification.

DAS (1) and MAS (2) were isolated from culture broths as described on page 101.

3α-Hydroxy-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (1) and 3α,15-Dihydroxy-4β-acetoxy-epoxytrichothec-9-ene (2)



(1)



(2)

Wide-necked conical flasks (500 cm^3) each containing seed medium (100 cm^3) were inoculated with the Fusarium species (strain C37410-90, Bristol-Myers Company) which had been maintained on slants of Sabouraud dextrose agar for 7 days. The seed culture was shaken for 2 days in the dark (27 ^oC, 210 rpm) and was then used to inoculate 36 conical flasks containing production medium (100 cm^3). The production culture was then fermented for 11 days in the dark (27 ^oC, 250 rpm). Continuous extraction of the whole culture (3.6 l) with ethyl acetate, without altering the pH, for 48 h, followed by concentration under reduced pressure, gave a brown, oily residue (3 - 4 g). Purification by dry-column flash chromatography using ethyl acetate-light petroleum as the eluent yielded typically 700 mg each of the title compounds.

Seed Medium : Malt extract (2 g), yeast (2 g), peptone (Oxoid) (2 g), KH_2PO_4 (2 g), $MgSO_4.7H_2O$ (2 g), $FeSO_4.7H_2O$ (0.2 g), NH_4Cl (3 g), glucose (20 g), deionised water (1 l).

Production Medium : $NH_4H_2PO_4$ (1 g), $K_2HPO_4.3H_2O$ (3.93 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (5 g), sucrose (40 g), glycerol (10 g), deionised water (1 l).

3α-Hydroxy-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (1)

ν_{max} (CCl_4) : 3555, 1745 and 1725 $cm.^{-1}$

^1H NMR : 5.47 (1H, dq, J 5.6 and 1.8 Hz, 10-H) 5.14 (1H, d, J 2.9, 4-H), 4.13 (1H, dd, J 4.9 and 2.9 Hz, 3-H), 4.05 (1H, br d, J 5.6, 11-H), 4.14 and 4.0 (2H, ABq, J_{obs} 12.2, 15-H_2), 3.64 (1H, d, J 4.9, 2-H), 3.01 and 2.72 (2H, ABq, J_{obs} 3.9, 13-H_2), 2.10 (3H, s, MeCO), 2.00 (3H, s, MeCO), 2.0 - 1.8 (4H, m, 7-H and 8-H), 1.72 (3H, br s, 16-H), and 0.76 (3H, s, 14-H).

^{13}C NMR : 172.5 (Me<u>C</u>O), 170.5 Me<u>C</u>O) 140.5 (C-9), 118.6 (C-10), 84.8 (C-3), 79.0 (C-4), 78.4 (C-2), 68.0 (C-11), 64.4 (C-12), 63.7 (C-15), 48.8 (C-5), 47.2 (C-13), 44.0 (C-6), 28.0 (C-8), 23.2 (C-16), 21.3 (C-7), 21.0 (<u>Me</u>CO), 21.0 (<u>Me</u>CO), and 6.9 (C-14).

m/z : Found M^+, 306.1459. $C_{19}H_{26}O_7$ - AcOH requires 306.1467.

3α,15-Dihydroxy-4β-acetoxy-12,13-epoxy-trichothec-9-ene (2)

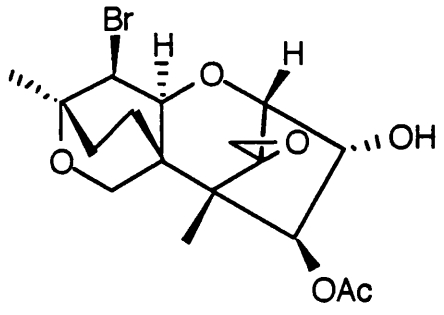
ν_{max} (CCl_4) : 3640, 3500 (br) and 1720 $cm.^{-1}$

^1H NMR : 5.56 (1H, dq, J 5.5 and 1.4 Hz, 10-H) 5.52 (1H, d, J 3.2, 4-H), 4.25 (1H, dd, J 4.9 and 3.2 Hz, 3-H), 4.19 (1H, br d, J 5.5, 11-H), 3.79 and 3.61 (2H, ABq, J_{obs} 12.3, 15-H_2), 3.67 (1H, d, J 4.9, 2-H), 3.05 and 2.77 (2H, ABq, J_{obs} 4.0 Hz, 13-H_2), 2.15 (3H, s, MeCO), 2.10 - 1.95 (4H, m, 7-H and 8-H), 1.72 (3H, br s, 16-H), and 0.84 (3H, s, 14-H).

^{13}C NMR : 173.0 (Me<u>C</u>O), 140.3 (C-9), 118.8 (C-10), 84.4 (C-4), 79.0 (C-2), 77.5 (C-3), 68.0 (C-11), 64.6 (C-12), 62.6 (C-15), 48.7 (C-5), 47.3 (C-13), 44.8 (C-6), 28.0 (C-8), 23.3 (C-16), 21.2 (C-7), 21.0 (<u>Me</u>CO), and 6.6 (C-14).

m/z : Found M^+, 306.1451. $C_{17}H_{24}O_6$ - H_2O requires 306.1467.

4β-Acetoxy-10β-bromo-3α-hydroxy-9α,15;12,13-diepoxytrichothecane (71)



To a solution of 4β-acetoxy-12,13-epoxy-trichothec-9-ene-3α,15-diol (2), (240 mg, 0.74 mmol) in dry MeCN (25 ml) was added NBS (152 mg, 0.85 mmol). The mixture was stirred at 20 °C for 1 hour and then concentrated. Purification of the residue by dry-column flash chromatography using ethyl acetate-light petroleum as the eluent yielded the title compound (271 mg, 91%) as a white amorphous solid.

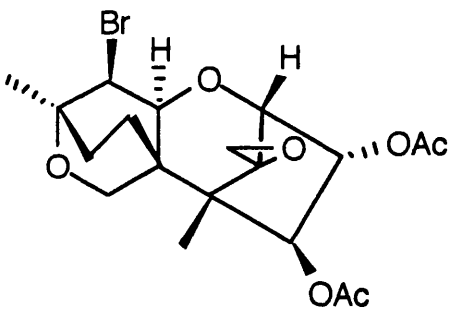
ν_{max} (CCl_4) : 3555 and 1740 $cm.^{-1}$

^1H NMR : 5.04 (1H, d, J 3.2, 4-H), 4.25 (2H, m, 10-H and 11-H), 4.21 (1H, dd, J 5.0 and 3.2 Hz, 3-H), 3.82 (1H, dd, J 9.6 and 2.7 Hz, 15-Hα), 3.79 (1H, d, J 5.0, 2-H), 3.70 (1H, d, J 9.6, 15-Hβ), 3.05 and 2.73 (2H, ABq, J_{obs} 3.8, 13-H_2), 2.3 - 1.4 (4H, m, 7-H and 8-H), 2.10 (3H, s, MeCO), 1.26 (3H, s, 16-H) and 0.61 (3H, s, 14-H).

^{13}C NMR : 172.2 (Me<u>C</u>O), 83.1 (C-3), 79.7 (C-4), 78.1 (C-2), 73.7 (C-9), 68.3 (C-11), 66.0 (C-15), 64.1 (C-12), 54.2 (C-10), 46.4 (C-13), 46.1 (C-5), 41.8 (C-6), 27.9 (C-8), 24.2 (C-16), 20.9 (<u>Me</u>CO), 19.2 (C-7), and 5.8 (C-14).

m/z : Found M^+, 404.0668 and 402.0655. $C_{17}H_{25}BrO_6$ requires M, 404.0659 and 402.0678.

3α,4β-Diacetoxy-10β-bromo-9α,15;12,13-diepoxytrichothecane (72)



Acetic anhydride (4 ml) and pyridine (2 ml) were added to the bromo ether (71) (537 mg, 1.33 mmol) and the resulting solution was stirred overnight at room temperature after which excess of acetic anhydride and pyridine were removed azeotropically under reduced pressure using toluene (3 x 20 ml) and then CCl_4 (3 x 20 ml). Purification by dry-column flash chromatography using ethyl acetate-light petroleum as the eluent yielded the title compound (559 mg, 94%) as a white, low-melting amorphous solid.

$\nu_{max}(CCl_4)$: 1750 $cm.^{-1}$

^1H NMR : 5.54 (1H, d, J 3.6, 4-H), 5.21 (1H, dd, J 4.9 and 3.6 Hz, 3-H), 4.28 (1H, dd, J 8.6 and 1.8 Hz, 11-H), 4.12 (1H, dd, J 8.6 and 2.4 Hz, 10-H), 3.99 (1H, dd, J 9.8 and 2.8 Hz, 15-Hα), 3.96 (1H, d, J 4.9, 2-H), 3.71 (1H, d, J 9.8, 15-Hβ), 3.07 and 2.75 (2H, ABq, J_{obs} 3.9, 13-H_2), 2.3

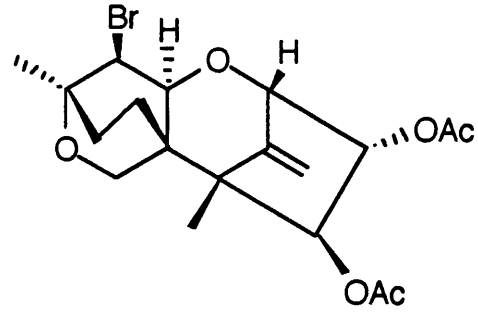
(cont...)

- 1.4 (4H, m, 7-H and 8-H), 2.10 (3H, s, MeCO), 2.09 (3H, s, MeCO), 1.28 (3H, s, 16-H) and 0.55 (3H, s, 14-H).

^{13}C NMR : 170.6 (Me<u>C</u>O), 169.8 (Me<u>C</u>O), 78.4 (C-3), 78.0 (C-4), 77.8 (C-2), 73.7 (C-9), 68.5 (C-11), 66.0 (C-15), 63.8 (C-12), 54.2 (C-10), 46.4 (C-13), 46.1 (C-5), 41.9 (C-6), 27.9 (C-8), 24.2 (C-16), 20.9 (<u>Me</u>CO), 20.7 (<u>Me</u>CO), 19.4 (C-7) and 5.5 (C-14).

m/z : Found M^+, 446.0770 and 444.0794. $C_{19}H_{25}BrO_7$ requires M, 446.0764 and 444.0784.

3α,4β-Diacetoxy-10β-bromo-9α,15-epoxytrichothec-12-ene (73)



To WCl_6 (1.43 g, 3.6 mmol), pre-cooled to -196 °C (liquid nitrogen), was slowly added THF (9.2 ml). After 5 min, n-butyllithium (4.1 ml; 2.21 M in hexane was added, the cooling bath was removed, and the mixture allowed to warm to room temperature with stirring, when it became dark brown and homogeneous. It was then re-cooled to -78 °C, and a solution of the epoxide (72) (535 mg, 1.2 mmol) in THF (15 ml) was added. The cooling bath was removed, and the reaction mixture was heated under reflux for 4 hours. On cooling to room temperature, the mixture was diluted with hexane (20 ml), washed once with an aqueous solution of both NaOH (2 M) and sodium tartrate (1.5 M) (30 ml) and once with water (30 ml). The organic layer was then dried and concentrated in vacuo. Purification by dry-column flash chromatography using ether-light petroleum as the eluent yielded the title compound (540 mg, 97%) as a white crystalline solid (from light petroleum)(m.p. 108-111 °C).

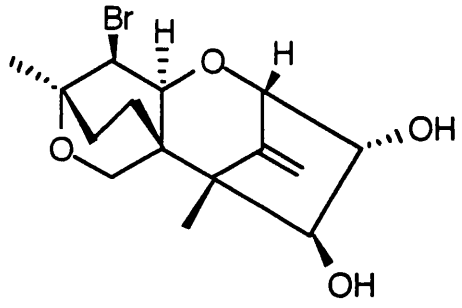
ν_{max} (CCl_4) : 1745 and 910cm.$^{-1}$

^{1}H NMR : 5.53 (1H, d, J 3.5, 4-H), 5.25 (1H, s, 13-Ha), 4.91 (1H, dd, J 5.0 and 3.5 Hz, 3-H), 4.82 (1H, s, 13-Hb), 4.57 (1H, d, J 5.0, 2-H), 4.25 (1H, dd, J 8.6 and 1.6 Hz, 11-H), 4.14 (1H, dd, J 8.6 and 2.4 Hz, 10-H), 3.98 (1H, dd, J 9.7 and 2.7 Hz, 15-Hα), 3.71 (1H, d, J 9.7, 15-Hβ), 2.3 - 1.2 (4H, m, 7-H and 8-H), 2.13 (3H, s, MeCO), 2.04 (3H, s, MeCO), 1.26 (3H, s, 16-H) and 0.79 (3H, s, 14-H).

^{13}C NMR : 170.3 (Me<u>C</u>O), 170.1 (Me<u>C</u>O), 147.3 (C-12), 109.3 (C-13), 78.3 (C-3), 78.1 (C-4), 78.0 (C-2), 73.3 (C-9), 68.2 (C-11), 66.3 (C-15), 54.8 (C-10), 49.1 (C-5), 41.5 (C-6), 27.8 (C-8), 24.2 (C-16), 21.0 (<u>Me</u>CO), 20.7 (<u>Me</u>CO), 18.7 (C-7) and 9.5 (C-14).

m/z : Found M^+, 430.0810 and 428.0827. $C_{19}H_{25}BrO_6$ requires M, 430.0815 and 428.0830.

3α,4β-Dihydroxy-10β-bromo-9α,15-epoxytrichothec-12-ene (74)



Potassium carbonate (2 g) was added to a solution of the diacetate (73) (132 mg, 0.31 mmol) in MeOH (9 ml) and water (1 ml) and the mixture was stirred for 2 hours at room temperature. It was then concentrated and the residue was taken up in water (20 ml) and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were then dried and concentrated in vacuo. Purification of the residue by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded the title compound (95.8 mg, 90%) as a white crystalline solid (from ether-hexane)(m.p. 118-121 °C).

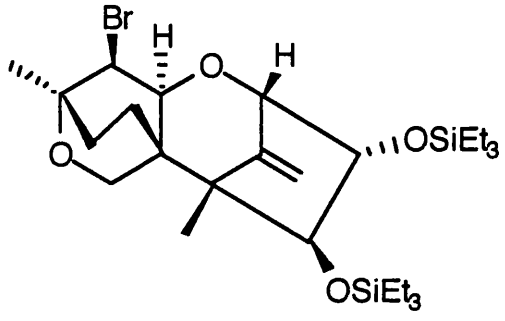
ν_{max} (CCl_4) : 3600 - 3200 (broad) and 910 $cm.^{-1}$

^1H NMR : 5.17 (1H, s, 13-Ha), 4.78 (1H, s, 13-Hb), 4.4 - 3.6 (9H, m, 2-H, 3-H, 4-H, 10-H, 11-H, 15-H, and 2 x OH), 2.25 - 1.25 (4H, m, 7-H and 8-H), 1.25 (3-H, s, 16-H), and 0.86 (3H, s, 14-H).

^{13}C NMR : 148.6 (C-12), 108.7 (C-13), 81.1 (C-3), 79.9 (C-4), 79.5 (C-2), 73.5 (C-9), 68.1 (C-11), 66.5 (C-15), 55.1 (C-10), 49.9 (C-9), 40.8 (C-6), 27.8 (C-8), 24.3 (C-16), 18.7 (C-7), and 10.0 (C-14).

m/z : Found M^+, 346.0596 and 344.0633. $C_{15}H_{21}BrO_4$ requires M, 346.0604 and 344.0624.

3α,4β-Bistriethylsilyloxy-10β-bromo-9α,15-epoxytrichothec-12-ene (75)



To a solution of the diol (74) (210 mg, 0.61 mmol) in pyridine (14 ml) were added chlorotriethylsilane (0.41 ml, 2.44 mmol) and DMAP (30 mg, 0.24 mmol). The mixture was stirred for 24 hours at room temperature before being diluted with dichloromethane (25 ml) and washed with saturated aqueous sodium hydrogen carbonate (25 ml). The organic layer was dried and concentrated under reduced pressure. Purification of the residue by dry-column flash chromatography using ether-hexane as the eluent gave the title compound (300 mg, 86%) as a colourless oil.

ν_{max} (CCl_4) : 1740 and 910 $cm.^{-1}$

^1H NMR : 5.10 (1H, s, 13-Ha), 4.70 (1H, s, 13-Hb), 4.20 (1H, d, J 4.8, 2-H), 4.16 (2H, m, 10-H and 11-H), 4.09 (1H, d, J 2.5, 4-H), 3.80 (1H, dd, J 4.8 and 2.5 Hz, 3-H), 3.72 (2H, br s, 15-H), 2.3-1.2 (4H, m, 7-H and 8-H), 1.25

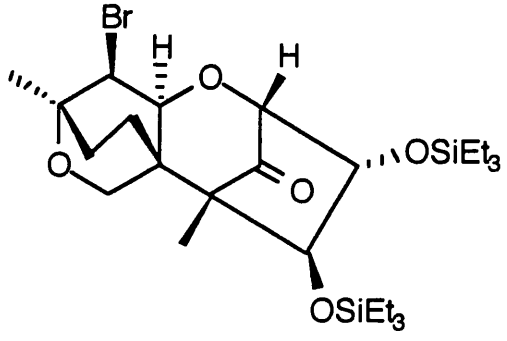
(cont...)

(3H, s, 16-H), 0.95 [18H, 6 x t (overlapping), $C\underline{H}_3CH_2$], 0.77 (3H, s, 14-H), and 0.64 [12H, 6 x q (overlapping), $CH_3C\underline{H}_2$].

^{13}C NMR : 149.7 (C-12), 107.6 (C-13), 83.2 (C-3), 81.6 (C-4) and 80.1 (C-2), 73.3 (C-9), 67.6 (C-11), 66.7 (C-15), 55.0 (C-10), 41.0 (C-6), 27.8 (C-8), 24.4 (C-16), 18.9 (C-7), 10.3 (C-14), 6.9 (2 x $\underline{C}H_3CH_2$), 5.0 and 4.9 (2 x $CH_3\underline{C}H_2$).

m/z : Found M^+, 574.2317 and 572.2342. $C_{27}H_{49}BrO_4Si_2$ requires M, 574.2333 and 572.2353.

3α,4β-Bistriethylsilyloxy-10β-bromo-9α,15-epoxynortrichothecan-12-one (76)



A solution of the alkene (75) (100 mg, 0.17 mmol) in dichloromethane (25 ml) cooled to -78 °C, was treated with an excess of ozone until the pale blue colour of excess ozone appeared. The solution was kept at this temperature for 10 min; if the pale blue colour had discharged by this time, the above procedure was repeated until the pale blue colour persisted. The solution was then purged with nitrogen, to remove excess ozone, and the ozonide was allowed to warm to 0 °C. At this point the ozonide was reduced by the addition of triethylamine (0.076 ml, 0.52 mmol) and the resulting solution was allowed to stir at 0 °C for 1 hour followed by stirring overnight at room temperature. The solution was then concentrated under reduced pressure and ether (25 ml) was added, this was then filtered through a pad of celite and concentrated in vacuo. Purification by dry-column flash chromatography using ether-hexane as the eluent yielded

the title compound (91 mg, 91%) as a white amorphous solid.

ν_{max} (CCl_4) : 1760 $cm.^{-1}$

^1H NMR : 4.4 (1H, dd, J 8.8 and 2.2 Hz, 10-H), 4.26 (1H, d, J 2.6, 4-H), 4.18 (1H, dd, J 8.8 and 1.6 Hz, 11-H), 3.92 (1H, dd, J 5.0 and 2.6 Hz, 3-H), 3.85 (1H, d, J 5.0, 2-H), 3.76 (1H, dd, J 9.2 and 2.4 Hz, 15-H), 3.67 (1H, d, J 9.2, 15-H), 2.3-1.4 (4H, m, 7-H and 8-H), 1.26 (3H, s, 16-H), 0.96 [18H, 6 x t (overlapping), $C\underline{H}_3CH_2$], 0.78 (3H, s, 14-H) and 0.64 [12H, 6 x q (overlapping), $CH_3C\underline{H}_2$].

^{13}C NMR : 211.2 (C-12), 80.0 (C-3), 79.3 (C-4), 79.0 (C-2), 73.8 (C-9), 67.5 (C-11), 65.8 (C-15), 57.2 (C-5), 53.6 (C-10), 47.3 (C-6), 27.5 (C-8), 24.2 (C-16), 19.0 (C-7), 7.0 (C-14), 6.8 (2 x $\underline{C}H_3CH_2$) and 4.9 and 4.8 (2 x $CH_3\underline{C}H_2$).

m/z : Found M^+, 576.2099 and 574.2126. $C_{26}H_{47}BrO_5Si_2$ requires M, 576.2126 and 574.2146.

Small amounts of the major lactone (77) and the minor isomer (78) were also obtained. These were desilylated using TBAF and acetylated before characterisation as their diacetates.

Lactone diacetates :

ν_{max} (CCl_4) : 2920, 1765, 1750, 1235 and 1205 cm.$^{-1}$

m/z : Found M^+ 448.0565 and 446.0585. $C_{18}H_{23}BrO_8$ requires M, 448.0557 and 446.0577.

Major Isomer

^1H NMR : 5.69 (1H, d, J 4.5, 2-H), 5.52 (1H, d, J 3.2, 4-H), 5.21 (1H, dd, J 4.5 and 3.2 Hz, 3-H), 4.43 (1H, dd, J 8.5 and 2.3 Hz, 10-H), 4.33 (1H, dd, J 8.5 and 1.2 Hz, 11-H), 3.79 (1H, d, J 9.7, 15-Hβ), 3.71 (1H, dd, J 9.7 and 2.1 Hz, 15-Hα), 2.4 - 1.5 (4H, m, 7-H and 8-H), 2.17 (3H, s, MeCO), 2.08 (3H, s, MeCO), 1.29 (3H, s, 16-H) and 0.97 (3H, s, 14-H).

^{13}C NMR : 169.8 (Me<u>C</u>O), 169.3 (Me<u>C</u>O), 94.4 (C-2), 73.6 (C-9), 72.0 (C-3), 71.4 (C-4), 71.1 (C-11), 66.0 (C-15), 53.8 (C-10), 48.4 (C-5), 37.8 (C-6), 27.4 (C-8), 24.3 (C-16), 20.8 (<u>Me</u>CO), 20.5 (<u>Me</u>CO), 18.6 (C-7) and 13.4 (C-14).

Minor isomer

^1H NMR : 5.49 (1H, d, J 3.1, 4-H), 5.22 (1H, dd, J 4.2 and 3.1 Hz, 3-H), 4.62 (1H, d, J 4.2, 2-H), 4.43 (1H, dd,

(cont...)

J 8.5 and 2.3 Hz, 10-H), 4.31 (1H, dd, J 8.5 and 1.2 Hz, 11-H), 3.79 (1H, d, J 9.7, 15-Hβ), 3.57 (1H, dd, J 9.7 and 2.0 Hz, 15-Hα), 2.4 - 1.5 (4H, m, 7-H and 8-H), 2.17 (3H, s, MeCO), 2.12 (3H, s, MeCO), 1.29 (3H, s, 16-H) and 1.12 (3H, s, 14-H).

^{13}C NMR : 169.9 (Me<u>C</u>O), 169.8 (Me<u>C</u>O), 84.6 (C-5), 73.7 (C-9), 72.8 (C-3), 72.5 (C-4), 71.6 (C-2), 70.4 (C-11), 66.6 (C-15), 54.1 (C-10), 42.0 (C-6), 27.2 (C-8), 24.3 (C-16), 20.8 (<u>Me</u>CO), 20.5 (<u>Me</u>CO), 17.2 (C-14) and 17.0 (C-7).

Traces of the epoxide (80) were also obtained during ozonolysis.

<u>Epoxide (80)</u>

ν_{max} (CCl_4) : 2955, 2875, 1120 and 1095 cm.$^{-1}$

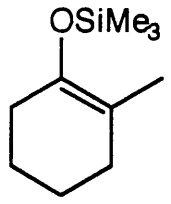
m/z : Found M^+, 590.2282 and 588.1969. $C_{27}H_{49}BrO_5Si_2$ requires M, 590.2282 and 588.1968.

^{1}H NMR : 4.18 (3H, m, 4-H, 10-H and 11-H), 4.14 (1H, dd, J 5.3 and 2.8Hz, 3-H), 3.72 (2H, br s, 15-H), 3.57 (1H, d,

2-H), 2.94 and 2.65 (2H, AB_q, 13-H), 2.3-1.2 (4H, m, 7-H and 8-H), 1.27 (3H, s, 16-H), 0.97 [18H, 6 x t (overlapping), $C\underline{H}_3CH_2$], 0.63 [12H, 6 x q (overlapping), $CH_3C\underline{H}_2$] and 0.56 (3H, s, 14-H).

^{13}C NMR : 82.5 (C-3), 81.9 (C-4), 80.2 (C-2), 73.8 (C-9), 67.8 (C-11), 66.4 (C-15), 64.5 (C-12), 54.4 (C-10), 46.4 (C-5), 45.6 (C-13), 41.6 (C-6), 27.9 (C-8), 24.4 (C-16), 19.4 (C-7), 6.9 (2 x $\underline{C}H_3CH_2$), 6.5 (C-14) and 4.9 (2 x $CH_3\underline{C}H_2$).

1-Trimethylsilyloxy-2-methylcyclohex-1-ene (84)



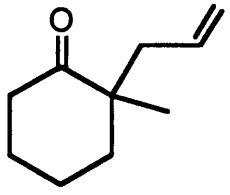
To a solution of triethylamine (83.1 ml, 0.6 mol) in DMF (90 ml) was added a solution of trimethylsilylchloride (38 ml, 0.3 mol) and 2-methyl cyclohexanone (30.3 ml, 0.25 mol). The resulting mixture was heated under reflux (140 °C) with stirring for 48 hours in a nitrogen atmosphere. This was then allowed to cool, diluted with pentane (250 ml) and washed with cold aqueous $NaHCO_3$ (3 x 300 ml), cold aqueous HCl (1.5 M, 50 ml) and then once more with cold aqueous $NaHCO_3$ (150 ml). The organic extract was then dried and concentrated *in vacuo* to give a mixture of silyl enol ethers as a pale yellow oil (34.1 g). Distillaton of the crude residue gave the *title compound* (b. p. 76-78 °C/12mm Hg)(lit.[106] 82-84 °C/16 mm Hg) as a colourless oil (28.9 g, 58%).

ν_{max} (CCl_4) : 2940, 1710, 1250, 1050 and 845 $cm.^{-1}$

^1H NMR : 2.1 - 1.6 (8H, m, 2-H, 3-H, 4-H and 5-H), 1.53 (3H, s, $-C\underline{H}_3$) and 0.15 (9H, s, $-Si(C\underline{H}_3)_3$).

m/z : Found M^+, 184.1286. $C_{10}H_{20}SiO$ requires M, 184.1283.

2-Allyl-2-methylcyclohexanone (86)



Methyl lithium (49.0 ml, 1.5 M in ether) was added with stirring to the silyl enol ether (84), (12.0 g, 65 mmol). After 30 mins the reaction mixture was concentrated in vacuo, and the vacuum was released using nitrogen. Dry THF (70 ml) was then added to the reaction mixture, followed by redistilled allyl bromide (8.65 ml, 71.5 mmol) dropwise with stirring. After 5 mins the reaction mixture was poured onto light petroleum (250 ml). The organic solution was then washed with saturated $NaHCO_3$ (250ml), dried ($MgSO_4$) and concentrated in vacuo. Distillation of the residue yielded the title compound (7.77 g, 78%)(b.p. 78-80 ^{o}C/18 mm Hg) as a colourless oil.

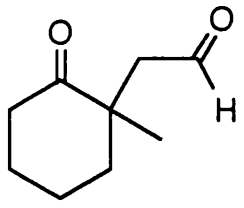
ν_{max} (CCl_4) : 1715, 1650 and 920 $cm.^{-1}$

^1H NMR : 5.53 (1H, m, $-C\underline{H}=CH_2$), 4.85 (2H, m, $-CH=C\underline{H_2}$), 2.3-0.8 (10H, m, 2-H, 3-H, 4-H, 5-H and $-C\underline{H_2}CH=CH_2$) and 0.90 (3H, s, $-C\underline{H_3}$).

^{13}C NMR : 214.5 (C-6), 135.4 (-$\underline{C}H=CH_2$), 117.4 (-$CH=\underline{C}H_2$), 48.0 (C-1), 41.6 (C-5), 38.4 (-$\underline{C}H_2-CH=CH_2$), 38.2 (C-4), 27.0 (C-2), 22.3 (-$\underline{C}H_3$) and 20.7 (C-3).

m/z : Found M^+, 152.1201. $C_{10}H_{16}O$ requires M, 152.1201.

(1-Methyl-2-oxocyclohexyl) ethanal (87)



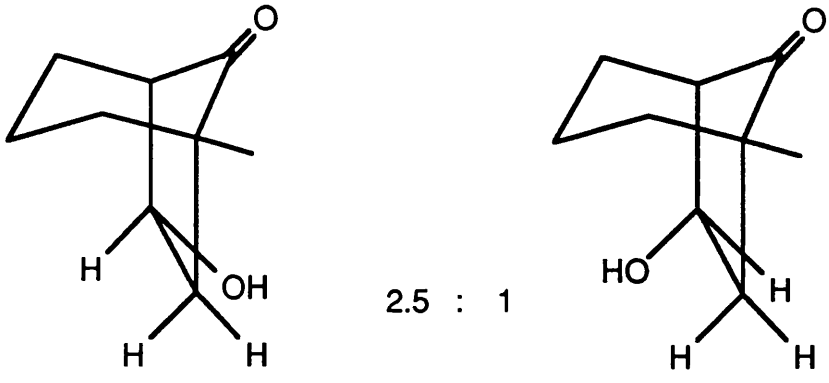
A solution of the allyl ketone (86) (3.84 g, 25.3 mmol) in dichloromethane (35 ml) cooled to -78 °C, was treated with an excess of ozone until the pale blue colour of excess ozone appeared. The solution was kept at this temperature for 10 min; if the pale blue colour had discharged by this time, the above procedure was repeated until the pale blue colour persisted. The solution was then purged with nitrogen to expel excess ozone. At this point the ozonide was reduced by the addition of triethylamine (7.0 ml, 52.8 mmol) and the resulting solution was allowed to stir at -78 °C for 1 hour followed by stirring overnight at room temperature. The solution was then concentrated under reduced pressure and ether (25 ml) was added, this was then filtered through a short column (2 cm) of chromatographic silica and concentrated *in vacuo* to yield the *title compound* (3.43 g, 88%) as a pale yellow oil which was used immediately, without further purification, in the subsequent aldol reaction.

ν_{max} (CCl_4) : 1710, 1450 and 910 $cm.^{-1}$

^1H NMR (90 MHz) : 9.75 (1H, t, J 2.0 Hz, -C<u>H</u>O), 2.6 - 2.3 (4H, m, 6-H and -C<u>H</u>$_2$CHO), 1.9 - 1.6 (8H, m, 2-H, 3-H, 4-H and 5-H) and 1.26 (3H, s, -C<u>H</u>$_3$).

m/z : Found M^+, 154.0984. $C_9H_{14}O_2$ requires M, 154.0994.

(±)-6β-Hydroxy-1-methylbicyclo[3.2.1]octan-8-one (89) and (±)-6α-Hydroxy-1-methylbicyclo[3.2.1]octan-8-one (90)



(89) (90)

A solution of the keto-aldehyde (87) (3.4 g, 22.1 mmol) in redistilled methanol (90 ml) was added to a solution of freshly prepared sodium methoxide [from sodium (3 g) in methanol (200 ml)]. The mixture was heated at reflux for 30 min, allowed to cool and then added to ice water (200 ml). The solution was then concentrated in vacuo to approximately half the original volume and then extracted with ether (3 x 100 ml). The combined organic extracts were then dried and concentrated under reduced pressure. Separation and purification by dry-column flash chromatography using ether-light petroleum as the eluent yielded the title compounds (1.52 g, 45%) as an epimeric mixture in a ratio of 2.5 : 1 in favour of the β-epimer (89).

β-epimer (89)

(±)-1-Methyl-6β-hydroxy-8-oxobicyclo[3.2.1]octane :-

ν_{max} (CCl_4) : 3620, 3450 and 1750 $cm.^{-1}$

^1H NMR : 4.19 (1H, dd, J 8.0 and 2.1 Hz, 6-Hα), 2.93 (1H, br s, -OH), 2.42 (1H, AB_q, J 14.3 and 8.0 Hz, 7-Hα), 2.3 - 1.1 (7H, m, 2-H, 3-H, 4-H, 5-H and 7-Hβ) and 0.98 (3H, s, 1-Me).

^{13}C NMR : 222.3 (C-8), 68.9 (C-6), 55.6 (C-5), 47.8 (C-1), 43.8 (C-7), 43.6 (C-4), 34.8 (C-2), 19.1 (1-Me) and 18.9 (C-3).

m/z : Found M^+, 154.0999. $C_9H_{14}O_2$ requires M, 154.0994.

α-epimer (90)

(±)-1-Methyl-6α-hydroxy-8-oxobicyclo[3.2.1]octane :-

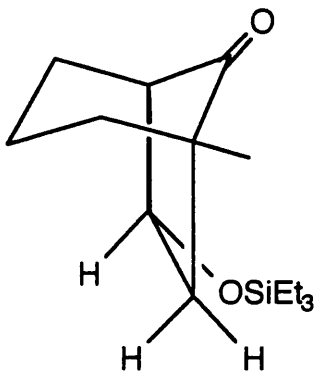
ν_{max} (CCl_4) : 3630 and 1750 $cm.^{-1}$

^1H NMR : 4.46 (1H, ddd, J 10.8, 6.5 and 4.4 Hz, 6-Hβ), 2.45 - 1.50 (9H, m, 2-H, 3-H, 4-H, 5-H and 7-H) and 0.97 (3H, s, 1-Me).

^{13}C NMR : 221.5 (C-8), 65.9 (C-6), 52.0 (C-5), 48.7 (C-1), 45.1 (C-4), 42.2 (C-7), 31.7 (C-2), 19.3 (1-Me) and 19.2 (C-3).

m/z : Found M^+, 154.0998. $C_9H_{14}O_2$ requires M, 154.0994.

(±)-1-Methyl-6β-triethylsilyloxybicyclo[3.2.1]octan-8-one (81)



To a stirred solution of the β-hydroxy ketone (89), (0.312 g, 2.03 mmol) and DMAP (20 mg) in ether (8 ml) was added triethylsilyl chloride (1.0 ml, 5.96 mmol), followed by triethylamine (0.84 ml, 6.0 mmol). The resulting solution was stirred overnight at room temperature before being diluted with ether (20 ml) and washed with saturated aqueous sodium hydrogen carbonate (25 ml). The organic layer was then dried and concentrated in vacuo. Purification of the residue by dry-column flash chromatography using ether-hexane as the eluent yielded the title compound (0.464 g, 85%) as a colourless oil.

ν_{max} (CCl_4) : 1755 $cm.^{-1}$

^1H NMR : 4.11 (1H, dd, J 7.9 and 2.3 Hz, 6-Hα), 2.38 (1H, AB_q, J 13.9 and 7.9 Hz, 7-Hα), 2.2 - 1.4 (8H, m, 2-H, 3-H, 4-H, 5-H and 7-Hβ), 1.00 (3H, s, 1-Me),

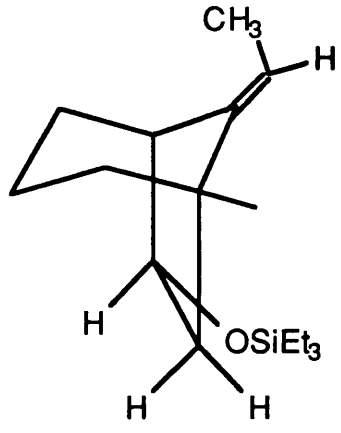
(cont...)

0.89 (9H, 3 x t (overlapping), $C\underline{H}_3CH_2$) and 0.52 (6H, 3 x q (overlapping), $CH_3C\underline{H}_2$).

^{13}C NMR : 221.5 (C-8), 69.5 (C-6), 55.9 (C-5), 47.8 (C-1), 45.2 (C-7), 43.7 (C-4), 34.0 (C-2), 19.2 (1-Me), 19.1 (C-3), 6.6 ($\underline{C}H_3CH_2$) and 4.7 ($CH_3\underline{C}H_2$).

m/z : Found M^+, 268.1847. $C_{15}H_{28}SiO_2$ requires M, 268.1858.

(±)-(E)-1,9-Dimethyl-6β-triethylsilyloxy-bicyclo[3.2.1]oct-8,9-ene (92)



To $Ph_3\overset{+}{P}CH_2CH_3\overset{-}{Br}$ (1.12 g, 3.0 mmol) in THF (4 ml) in a nitrogen atmosphere was added n-BuLi (1.36 ml; 2.21 M in hexane) and the solution was stirred for 45 min at room temperature. The ketone (81) (0.268 g, 1.0 mmol) in THF (2 ml) was then added and the resulting solution was refluxed for 1 h. The reaction mixture was cooled, diluted with water (20 ml) and extracted with Et_2O (3 x 20 ml). The combined organic extracts were then washed once with saturated aqueous ammonium chloride (50 ml) and once with water (30 ml). The organic layer was then dried and concentrated in vacuo. Purification of the residue by dry-column flash chromatography using ether-hexane as the eluent yielded the title compound (3.5 mg, 1%) and the desilylated product (94) (94.9 mg, 57%) as a colourless oil.

(±)-(E)-1,9-Dimethyl-6β-triethylsilyloxy-bicyclo[3.2.1]oct-8,9-ene (92). :

ν_{max} (CCl_4) : 1550(br), 1255, and 1215 cm.$^{-1}$

^{1}H NMR : 5.09 (1H, q, J 6.6, 9-H), 4.08 (1H, dd, J 7.1 and 2.4 Hz, 6-Hα), 1.63 (3H, d, J 6.6, 9-Me), 2.3 - 1.2 (9H, m, 2-H, 3-H, 4-H, 5-H and 7-H), 1.04 (3H, s, -C$\underline{H}_3$), 0.90 [9H, 3 x t (overlapping), C$\underline{H}_3$CH$_2$] and 0.51 [6H, 3 x q (overlapping), CH$_3$C$\underline{H}_2$].

^{13}C NMR : 152.2 (C-8), 107.6 (C-9), 74.5 (C-6), 48.8 (C-7), 47.6 (C-5), 44.1 (C-1), 42.5 (C-4), 31.6 (C-2), 23.0 (9-Me), 20.3 (C-3), 13.5 (-$\underline{C}$H$_3$), 6.8 ($\underline{C}$H$_3$CH$_2$) and 4.8 (CH$_3\underline{C}$H$_2$).

m/z : Found M$^+$, 282.2218. $C_{17}H_{32}SiO$ requires M, 282.2222.

(±)-6β-Hydroxy-9-methyl-bicyclo[3.2.1]oct-8-ene (94). :

ν_{max} (CCl_4) : 3600 and 910 cm.$^{-1}$

^{1}H NMR : 5.16 (1H, q, J 6.7, 9-H), 4.10 (1H, dd, J 7.2 and 2.3 Hz, 6-Hα), 2.55 (1H, br s, -O$\underline{H}$), 2.19 (1H, dd, J 13.9 and 7.2 Hz, 7-H), 1.65 (3H, d, J 6.7, 9-Me), 1.75 - 0.80

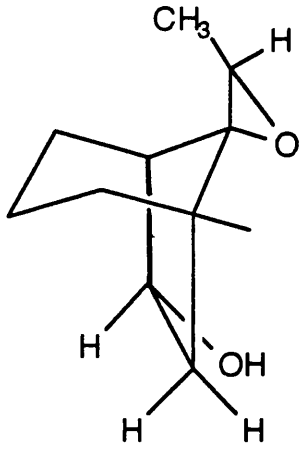
(cont...)

(8H, m, 2-H, 3-H, 4-H, 5-H and 7-Hβ) and 1.05 (3H, s, 1-Me).

^{13}C NMR : 151.4 (C-8), 109.0 (C-9), 74.5 (C-6), 48.4 (C-7), 47.7 (C-5), 44.0 (C-1), 42.2 (C-4), 31.2 (C-2), 23.1 (9-Me), 20.1 (C-3) and 13.6 (1-Me).

m/z : Found M^+, 166.1361. $C_{11}H_{18}O$ requires M, 166.1358.

(±)-(9S)-1,9-Dimethylspiro(bicyclo[3.2.1]octane-exo-8,2'-oxiran)-6β-ol (96)



A mixture of the β-hydroxy alkene (94) (80.0 mg, 0.48 mmol), m-CPBA (166 mg, 0.96 mmol) and Na_2HPO_4 (0.91 g, 6.4 mmol) in dichloromethane (40 ml) was stirred overnight at room temperature. The mixture was then poured into water (25 ml) and extracted thoroughly with ether (3 x 25 ml). The combined organic extracts were washed successively with saturated aqueous sodium hydrogen carbonate and brine and then dried. Concentration under reduced pressure and purification by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded the title compound (52.2 mg, 60%) as a colourless oil.

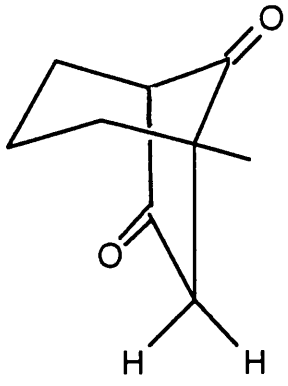
ν_{max} (CCl_4) : 3580 and 1130 $cm.^{-1}$

^{1}H n.m.r. (200 MHz) : 4.07 (1H, dd, J 7.3 and 2.5 Hz, 6-Hα), 3.00 (1H, q, J 5.5, 9-H), 2.22 (1H, dd, J 14.3 and 7.3 Hz, 7-H), 2.05 - 1.25 (9H, m, 2-H, 3-H, 4-H, 5-H, 7-H and -OH), 1.35 (3H, d, J 5.5, 9-Me) and 0.78 (3H, s, 1-Me).

^{13}C n.m.r. (50 MHz) : 75.5 (C-8), 74.3 (C-6), 51.6 (C-9), 48.0 (C-5), 47.7 (C-7), 42.1 (C-1), 38.9 (C-4), 28.6 (C-2), 19.3 (C-2), 18.6 (9-Me) and 15.2 (1-Me).

m/z : Found M^{+}, 182.1321. $C_{11}H_{18}O_2$ requires 182.1307.

(±)-1-Methyl-bicyclo[3.2.1]octane-6,8-dione (98)



To the β-hydroxy ketone (89) (0.281 g, 1.82 mmol) in redistilled acetone (30 ml) cooled to 0 °C was added Jones' reagent[110] (1.25 ml). The supernatant layer was red/brown in colour and remained so. A green precipitate was also formed. After 3 mins the reaction mixture was diluted with water (20 ml) and extracted with EtOAc (3 x 20 ml). Solid NaCl was then added to the aqueous layer and a further extraction with ethyl acetate (20 ml) was carried out. The combined organic extracts were then dried and concentrated in vacuo. Purification by dry-column flash chromatography using ether-hexane as the eluent yielded the title compound (0.181 g, 65%) as a white amorphous solid.

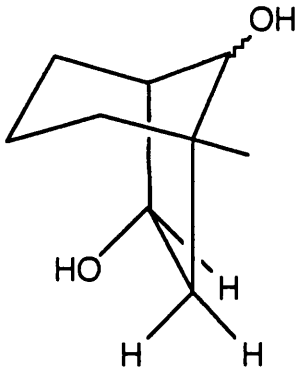
ν_{max} (CCl_4) : 1780 and 1745 $cm.^{-1}$

^1H NMR : 2.9 - 1.5 (9H, m, 2-H, 3-H, 4-H, 5-H and 7-H) and 1.2 (3H, s, 1-Me).

^{13}C NMR : 216.6 (C-8), 210.3 (C-6), 59.5 (C-5), 51.0 (C-7), 50.2 (C-1), 43.6 (C-4), 36.9 (C-2), 18.9 (C-3) and 18.8 (1-Me).

m/z : Found M^+, 152.0833. $C_9H_{12}O_2$ requires M, 152.0837.

(±)-1-Methyl-6α,8-dihydroxy-bicyclo[3.2.1]octane (100)



To a stirred solution of the dione (98) (24.2 mg, 0.16 mmol) in methanol (2 ml) at 0 °C was added $NaBH_4$ (2.6 mg, 0.08 mmol). Stirring was continued for 5 min before the pH was adjusted to neutrality with dilute aqueous HCl and the solution was extracted with ether (3 x 10 ml). The organic extracts were then dried and concentrated in vacuo. Purification by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded the title compound (20.7 mg, 83%) as a white amorphous solid.

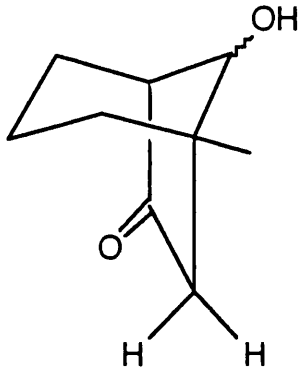
ν_{max} (CCl_4) : 3500 - 3300 (br), 2920, 1080 and 1035cm.$^{-1}$

^{1}H NMR : 4.30 (1H, ddd, J 10.6, 6.1 and 4.5 Hz, 6-Hβ), 3.50 (1H, d, J 4.8, 8-H), 2.2 - 1.0 (11H, m, 2-H, 3-H, 4-H, 5-H, 7-H, 6-OH and 8-OH) and 0.92 (3H, s, 1-Me).

^{13}C NMR : 76.5 (C-8), 69.1 (C-6), 44.2 (C-5), 43.8 (C-7), 40.9 (C-1), 31.3 (C-4), 25.1 (1-Me), 19.1 (C-2) and 18.5 (C-3).

m/z : Found M^+, 156.1144. $C_9H_{16}O_2$ requires M, 156.1150.

(±)-1-Methyl-8-hydroxy-bicyclo[3.2.1]octan-6-one (99)



Procedure A

To the dione (98) (44.6 mg, 0.29 mmol) in THF (2 ml) in a nitrogen atmosphere was added $LiAl(OBu^t)_3H$ (88.1 mg, 0.35 mmol) in THF (2 ml). The resulting mixture was stirred at room temperature for 1h before being diluted with H_2O (20 ml). The solution was then extracted with ether (3 x 20 ml), dried, and concentrated in vacuo. Purification by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded the title compound (36.2 mg, 80%) as a white amorphous solid.

Procedure B

To a stirred solution of the dione (98) (40.4 mg, 0.27 mmol) and $CeCl_3.H_2O$ (98.8 mg, 0.27 mmol) in methanol (2.5 ml) at 0 ^oC was added $NaBH_4$ (3.8 mg, 0.1 mmol). Stirring was continued for 5 min before the pH was adjusted to neutrality with dilute aqueous HCl and the solution was extracted with ether (3 x 20 ml). The

organic extracts were then dried and concentrated *in vacuo*. Purification by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded the *title compound* (26.7 mg, 65%) as a white amorphous solid.

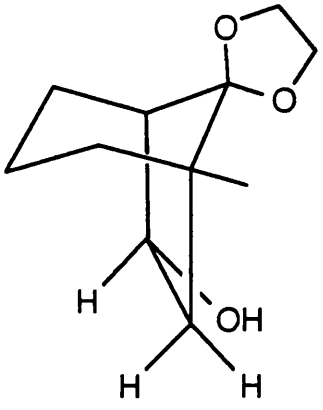
ν_{max} (CCl_4) : 3640, 2960, 2875 and 1755 $cm.^{-1}$

^1H NMR : 3.90 (1H, d, J 5.4, 8-H), 2.4 (1H, br s, -OH), 2.4 - 1.2 (9H, m, 2-H, 3-H, 4-H, 5-H and 7-H) and 1.08 (3H, s, 1-Me).

^{13}C NMR : 218.1 (C-6), 75.4 (C-8), 52.3 (C-5), 50.1 (C-7), 39.7 (C-1), 30.3 (C-4), 24.2 (1-Me), 21.4 (C-2) and 18.7 (C-3).

m/z : Found M^+, 154.0988. $C_9H_{14}O_2$ requires M, 154.0994.

(±)-1-Methyl-6β-hydroxy-8-(1,3-dioxolan-2-yl)-bicyclo[3.2.1]octane (101)



To the β-hydroxy ketone (89), (0.61 g, 3.96 mmol) in dichloromethane (20 ml) in a nitrogen atmosphere was added ethane-1,2-diol (0.26 ml, 4.67 mmol) and freshly distilled $BF_3.Et_2O$ (0.58 ml, 4.72 mmol). The reaction mixture was then stirred overnight at room temperature. A saturated solution of $NaHCO_3$ (25 ml) was then added and the organic layer was separated. The aqueous layer was then extracted further with dichloromethane (3 x 20 ml) and the combined organic layers were then dried. Evaporation of the solvent under reduced pressure followed by purification by dry-column flash chromatography using ether-hexane as the eluent yielded the title compound (0.67 g, 86%) as a colourless oil.

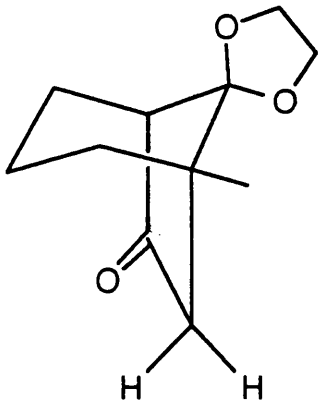
ν_{max} (CCl_4) : 3580cm.$^{-1}$

^{1}H NMR : 3.91 (4H, m, -OC$\underline{H}_2$C$\underline{H}_2$O-), 3.85 (1H, dd, 6-Hα), 2.70 (1H, br s, -O$\underline{H}$), 2.07 (1H, dd, 7-Hα), 1.85-1.0 (8H, m, 2-H, 3-H, 4-H 5-H and 7-Hβ) and 0.87 (3H, s, 1-Me).

^{13}C NMR : 116.9 (C-8), 72.2 (C-6), 65.5 (-O$\underline{C}H_2CH_2O$-), 64.2 (-OCH$_2\underline{C}H_2$O-), 49.0 (C-5), 46.2 (C-7), 44.3 (C-1), 35.9 (C-4), 26.3 (C-2), 19.0 (1-Me) and 18.6 (C-3).

m/z : Found M^+, 198.1260. $C_{11}H_{18}O_3$ requires M, 198.1256.

(±)-1-Methyl-8-(1,3-dioxolan-2-yl)-bicyclo[3.2.1]octan-6-one (104)



To a suspension of CrO_3 (0.20 g, 2.0 mmol) in CH_2Cl_2 (5 ml) at room temperature and under a nitrogen atmosphere was added 3,5-dimethyl pyrazole (0.19 g, 2.0 mmol) in CH_2Cl_2 (2 ml). The mixture was stirred for 30 min during which time the solution became dark red and homogeneous. The β-alcohol (101) (0.20 g, 1.0 mmol) in CH_2Cl_2 (5 ml) was added in one portion and stirring was continued for 4 hours. The mixture was concentrated *in vacuo* and the residue was taken up in Et_2O (25 ml). The ethereal solution was then filtered through celite and concentrated under reduced pressure. The residual oil was diluted with pentane (25 ml) and refiltered through celite before final concentration *in vacuo*. Purification by dry-column flash chromatography using ether-hexane as the eluent yielded the *title compound* (0.158 g, 80%) as a colourless oil.

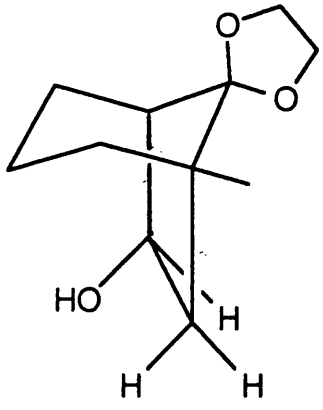
ν_{max} (CCl_4) : 1750 cm.$^{-1}$

^{1}H NMR : 3.91 (4H, m, -OC$\underline{H}_2$C$\underline{H}_2$O-), 2.4 - 1.65 (5H, m, 4-H, 5-H and 7-H), 1.65 - 1.15 (4H, m, 2-H and 3-H) and 1.01 (3H, s, 1-Me).

^{13}C NMR : 216.8 (C-6), 114.1 (C-8), 66.0 (-O$\underline{C}$H$_2$CH$_2$O-), 64.5 (-OCH$_2\underline{C}$H$_2$O-), 53.9 (C-5), 49.2 (C-7), 43.6 (C-1), 35.8 (C-4), 27.4 (C-2), 18.4 (C-3) and 18.3 (1-Me).

m/z : Found M^+, 196.1099. $C_{11}H_{16}O_3$ requires M, 196.1099.

(±)-1-Methyl-6α-hydroxy-8-(1,3-dioxolan-2-yl)-bicyclo[3.2.1]octane (105)



To a solution of the ketone (104) (0.137 g, 0.70 mmol) in methanol (5 ml) and water (2 ml), cooled to 0 °C, was added $NaBH_4$ (53.2 mg, 1.4 mmol). After 10 min the solution was diluted with water (10 ml) and extracted with EtOAc (3 x 20 ml). The combined organic extracts were then dried and concentrated in vacuo. Purification by dry-column flash chromatography using ethyl acetate-hexane as eluent yielded the title compound (0.132 g, 95%) as a colourless oil.

ν_{max} (CCl_4) : 3565 $cm.^{-1}$

^1H NMR : 4.39 (1H, ddd, J 9.4, 6.3 and 4.0 Hz, 6-Hβ), 3.98 (4H, m, $-OCH_2CH_2O-$), 2.2 - 1.1 (9H, m, 2-H, 3-H, 4-H, 5-H and 7-H) and 0.84 (3H, s, 1-Me).

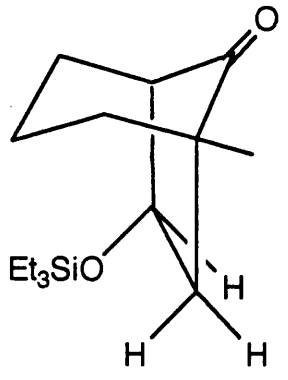
^{13}C NMR : 116.6 (C-8), 69.7 (C-6), 65.4 (-O$\underline{C}H_2CH_2O$-), 64.4 (-OCH$_2\underline{C}H_2$O-), 45.5 (C-5), 43.5 (C-7), 43.0 (C-1), 37.1 (C-4), 23.7 (C-2), 19.3 (1-Me) and 18.8 (C-3).

m/z : Found M^+, 198.1255. $C_{11}H_{18}O_3$ requires M, 198.1256.

To a stirred solution of the α-hydroxy ketal (105) (0.11 g, 0.56 mmol) in acetone (2 ml) was added HCl (2 drops from a Pasteur pipette). The resulting solution was then allowed to stir for 48 h at room temperature before water (10 ml) was added and the solution was extracted with EtOAc (3 x 10 ml). The organic layer was then dried and concentrated <u>in vacuo</u>. Purification by dry column flash chromatography yielded the -hydroxy ketone (90) (0.083 g, 97%) as a white crystalline solid (from ether-light petroleum) (m.p. 92-96 °C).

See p. 126 for characterisation.

(±)-1-Methyl-6α-triethylsilyloxybicyclo[3.2.1]octan-8-one (82)



To a stirred solution of the -hydroxy ketone (90), (98.7 mg, 0.64 mmol) and DMAP (10 mg) in ether (5 ml) was added triethylsilyl chloride (0.42 ml, 2.5 mmol), followed by triethylamine (0.42 ml, 2.5 ml). The resulting solution was stirred overnight at room temperature before being diluted with ether (25 ml) and washed with saturated aqueous sodium hydrogen carbonate (25 ml). The organic layer was then dried and concentrated in vacuo. Purification of the residue by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded the title compound (0.138 g, 80%) as a colourless oil.

ν_{max} (CCl_4) : 1755 $cm.^{-1}$

^1H NMR : 4.35 (1H, ddd, 6-Hβ), 2.5 - 1.2 (9H, m, 2-H, 3-H, 4-H, 5-H and 7-H), 0.93 (3H, s, 1-Me), 0.9 (9H, 3 x t

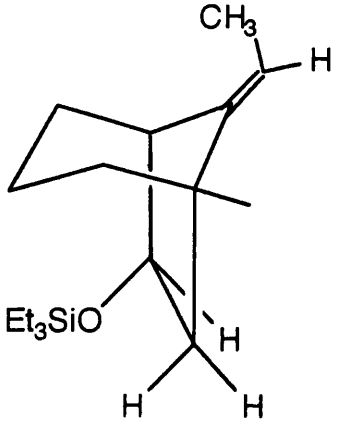
(cont...)

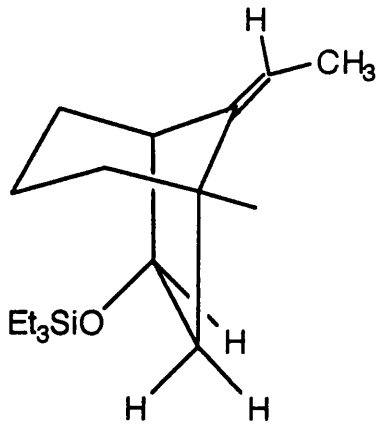
(overlapping), $C\underline{H}_3CH_2$) and 0.62 (6H, 3 x q (overlapping), $CH_3C\underline{H}_2$).

^{13}C NMR : 222.0 (C-8), 65.8 (C-6), 52.6 (C-5), 48.6 (C-1), 45.3 (C-7), 43.7 (C-4), 32.1 (C-2), 19.3 (1-Me), 19.2 (C-3), 6.7 ($\underline{C}H_3CH_2$) and 4.7 ($CH_3\underline{C}H_2$).

m/z : Found M^+, 268.1863. $C_{15}H_{28}SiO_2$ requires M, 268.1858.

(±)-(E)- and (Z)-1,9-Dimethyl-6α-triethylsilyloxy bicyclo[3.2.1]oct-8,9-enes (106) and (107)





2.3 : 1

(106) (107)

To $Ph_3\overset{+}{P}CH_2CH_3\overset{-}{Br}$ (0.57 g, 1.54 mmol) in THF (4 ml) in a nitrogen atmosphere was added n-butyllithium (0.69 ml; 2.21 M in hexane) and the solution was stirred for 45 min at room temperature. The ketone (82) (0.138 g, 0.514 mmol) in THF (6 ml) was then added and the resulting solution was refluxed for 1 h. The reaction mixture was then cooled, diluted with water (20 ml) and then extracted with Et_2O (3 x 20 ml). The organic extracts were then combined, washed once with saturated aqueous ammonium chloride (30 ml) and once with water (30 ml). The organic layer was then dried and concentrated in vacuo. Purification of the residue by dry-column flash chromatography using ether-hexane as the eluent yielded a colourless oil consisting of the title compounds (82.2 mg, 57%) as a mixture of geometric isomers, which were not

separated, in a ratio of 2.3 : 1 in favour of the (E)-isomer (106).

ν_{max} (CCl_4) : 1550 (br), 1110, 1070 and 980 cm.$^{-1}$

m/z : Found M^+, 280.2212. $C_{17}H_{32}SiO$ requires M, 280.2222.

(E)-isomer) (106)

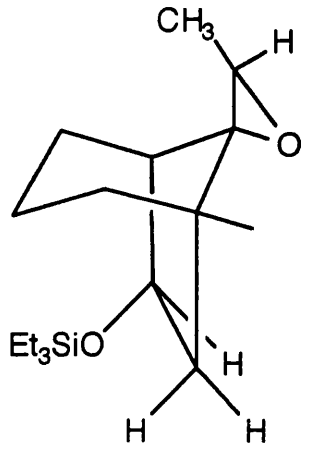
^1H NMR : 4.99 (1H, dq, J 6.7 and 0.7 Hz, 9-H), 4.15 (1H, ddd, J 10.5, 6.3 and 4.1 Hz, 6-Hβ), 2.76 (1H, m, 5-H), 2.2 - 1.3 (8H, m, 2-H, 3-H, 4-H, and 7-H), 1.59 (3H, d, J 6.7, 9-Me), 0.96 (3H, s, 1-Me), 0.95 [9H, 3 x t (overlapping), $C\underline{H}_3CH_2$] and 0.59 [6H, 3 x q (overlapping), $CH_3C\underline{H}_2$].

^{13}C NMR : 153.0 (C-8), 107.5 (C-9), 71.4 (C-6), 46.4 (C-7), 43.9 (C-4), 43.4 (C-5), 42.7 (C-1), 28.8 (C-2), 23.4 (9-Me), 20.2 (C-3), 6.9 (1-Me), 6.8 ($\underline{C}H_3CH_2$) and 4.8 ($CH_3\underline{C}H_2$).

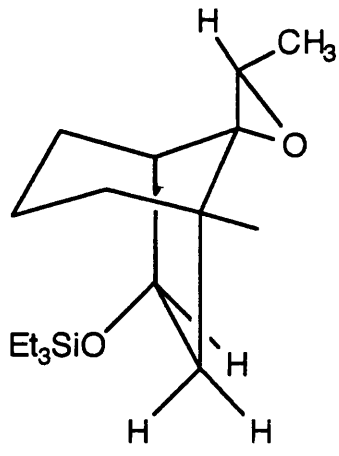
(Z)-isomer (107)

^1H NMR : 5.12 (1H, q, J 7.3, 9-H), 4.20 (1H, ddd, J 10.8, 6.3 and 4.3 Hz, 6-Hβ), 2.27 (1H, m, 5-H), 2.2 - 1.3 (8H, m, 2-H, 3-H, 4-H, and 7-H), 1.67 (3H, d, J 7.2, 9-Me), 0.96 (3H, s, 1-Me), 0.95 [9H, 3 x t (overlapping), $C\underline{H}_3CH_2$] and 0.59 [6H, 3 x q (overlapping), $CH_3C\underline{H}_2$].

(±)-(9S)- and (9R)-1,9-Dimethyl-6α-triethylsilyloxyspiro (bicyclo[3.2.1]octane-exo-8,2'-oxiranes) (110) and (111)



(110)



(111)

A mixture of the α-triethylsilyloxy alkenes (106) and (107) (72.4 mg, 0.26 mmol), m-CPBA (89.3 mg, 0.52 mmol) and Na_2HPO_4 (0.49 g, 3.5 mmol) in dichloromethane (40 ml) was stirred overnight at room temperature. The mixture was then poured into water (20 ml) and extracted thoroughly with ether (3 x 20 ml). The combined organic extracts were washed successively with saturated aqueous sodium hydrogen carbonate and brine and dried. The organic solution was then concentrated under reduced pressure. Purification by dry-column flash chromatography using ether-hexane as the eluent yielded a colourless oil consisting of the title compounds as a mixture of diastereomers which were not separated (69.7 mg, 91%).

ν_{max} (CCl_4) : 1375, 1160, 1110, 1090, 1070 and 880 cm.$^{-1}$

m/z : Found M^+, 296.2177. $C_{17}H_{32}SiO_2$ requires M, 296.2171.

(110)

^1H NMR : 4.50 (1H, ddd, J 10.5, 6.4 and 4.1 Hz, 6-Hβ), 2.96 (1H, q, J 5.5, 9-H), 1.32 (3H, d, J 5.5, 9-Me), 2.30 - 1.40 (9H, m, 2-H, 3-H, 4-H, 5-H, 7-H), 0.92 [9H, 3 x t (overlapping), $C\underline{H}_3CH_2$], 0.68 (3H, s, 1-Me) and 0.57 [6H, 3 x q (overlapping), $CH_3C\underline{H}_2$].

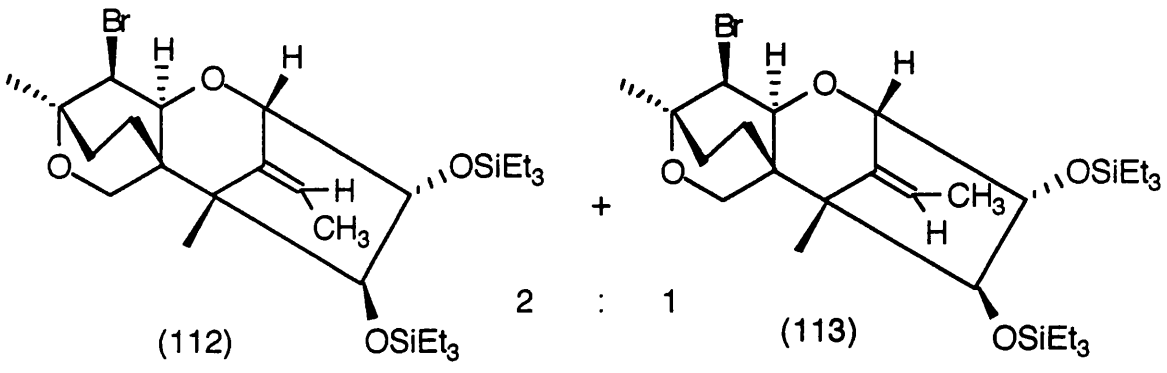
^{13}C NMR : 76.0 (C-8), 70.9 (C-6), 52.4 (C-9), 45.4 (C-7), 44.0 (C-5), 40.4 (C-4), 40.3 (C-1), 26.5 (C-2), 19.4 (C-3), 18.8 (9-Me), 15.2 (1-Me), 6.8 ($\underline{C}H_3CH_2$) and 4.8 ($CH_3\underline{C}H_2$).

(111)

^1H NMR : 4.46 (1H, ddd, 14.7, 6.5 and 4.0 Hz, 6-Hβ), 2.97 (1H, q, J 5.9, 9-H), 2.3 - 1.4 (9H, m, 2-H, 3-H, 4-H, 5-H and 7-H), 1.41 (3H, d, J 5.9, 9-Me), 0.92 [9H, 3 x t (overlapping), $C\underline{H}_3CH_2$], 0.68 (3H, s, 1-Me) and 0.57 [6H, 3 x q (overlapping), $CH_3C\underline{H}_2$].

^{13}C NMR : 75.2 (C-8), 70.0 (C-6), 56.3 (C-9), 50.1 (C-5), 47.7 (C-7), 40.3 (C-1), 40.0 (C-4), 27.4 (C-2), 22.2 (9-Me), 19.7 (C-3), 14.3 (1-Me), 6.8 ($\underline{C}H_3CH_2$) and 4.8 ($CH_3\underline{C}H_2$).

(Z)- and (E)-3α,4β-Bistriethylsilyloxy-10β-bromo-13-methyl-9α,15-epoxytrichothec-12-enes (112) and (113)



To $Ph_3\overset{+}{P}CH_2CH_3\overset{-}{Br}$ (0.66 g, 1.77 mmol) in THF (10 ml) in a nitrogen atmosphere was added n-BuLi (0.63 ml; 2.87 M in hexanes) and the solution was stirred for 45 min at room temperature. The ketone (76) (0.258 g, 0.45 mmol) in THF (6 ml) was then added and the resulting solution was refluxed for 1 h. The reaction mixture was then cooled, diluted with water (20 ml) and then extracted with Et_2O (3 x 20 ml). The organic extracts were then combined, washed once with saturated aqueous ammonium chloride (50 ml) and once with water (30 ml). The organic layer was then dried and concentrated in vacuo. Purification of the residue by dry-column flash chromatography using ether-hexane as the eluent yielded a colourless oil consisting of the title compounds (0.163 g, 62%) as a mixture of geometric isomers, which were not

separated, in a ratio of 2 : 1 in favour of the Z-isomer (112).

ν_{max} (CCl_4) : 2940, 1370, 1290 and 910 $cm.^{-1}$

m/z : Found M^+, 586.0321 and 588.0423. $C_{28}H_{51}BrO_4Si_2$ requires M, 586.0324 and 588.0429.

(Z)-isomer (112)

^1H NMR : 5.67 (1H, q, J 7.3, 13-H), 4.15 (2H, br s, 10-H and 11-H), 4.05 (1H, d, J 2.4, 4-H), 3.99 (1H, d, J 5.0, 2-H), 3.84 (1H, dd, J 5.0 and 2.6 Hz, 3-H), 3.74 (1H, d, J 9.6, 15-H), 3.56 (1H, dd, J 9.3 and 2.7 Hz, 15-H), 2.3 - 1.2 (4H, m, 7-H and 8-H), 1.69 (3H, d, J 7.2, 13-Me), 1.25 (3H, s, 16-H), 0.94 [18H, 6 x t (overlapping), $C\underline{H}_3CH_2$], 0.93 (3H, s, 14-H) and 0.62 [12H, 6 x q (overlapping), $CH_3C\underline{H}_2$].

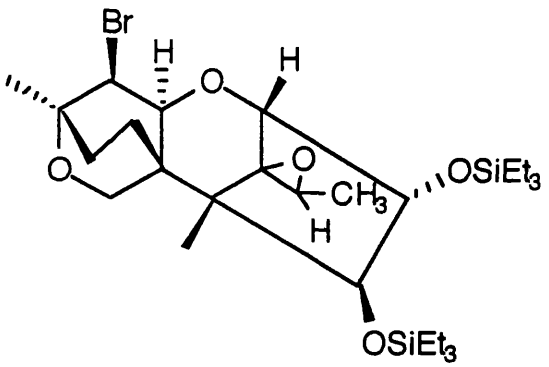
^{13}C NMR : 140.6 (C-12), 121.7 (C-13), 83.3 (C-3), 83.2 (C-4), 82.7 (C-2), 73.4 (C-9), 67.8 (C-11), 66.6 (C-15), 55.3 (C-10), 51.5 (C-5), 42.1 (C-6), 27.9 (C-8), 24.5 (C-16), 18.9 (C-7), 13.9 (13-Me), 12.6 (C-14), 6.9 (2 x $\underline{C}H_3CH_2$), 5.0 and 4.9 (2 x $CH_3\underline{C}H_2$).

(E)-isomer (113)

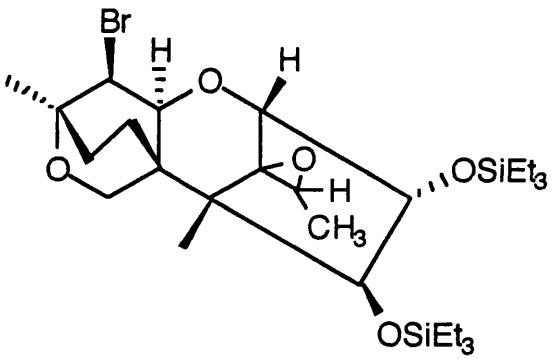
^{1}H NMR : 5.11 (1H, qd, J 6.9 and 0.7 Hz, 13-H), 4.55 (1H, d, J 4.9, 2-H), 4.15 (2H, br s, 10-H and 11-H), 4.06 (1H, d, 4-H), 3.74 (1H, dd, J 4.9 and 2.7 Hz, 3-H), 3.70 (2H, br s, 15-H), 2.3 - 1.2 (4H, m, 7-H and 8-H), 1.72 (3H, d, J 6.2, 13-Me), 1.25 (3H, s, 16-H), 0.94 [18H, 6 x t (overlapping), $C\underline{H}_3CH_2$], 0.93 (3H, s, 14-H) and 0.62 [12H, 6 x q (overlapping), $CH_3C\underline{H}_2$].

^{13}C NMR : 140.1 (C-12), 117.3 (C-13), 83.5 (C-3), 81.9 (C-4), 74.7 (C-2), 73.4 (C-9), 67.6 (C-11), 66.9 (C-15), 55.3 (C-10), 50.0 (C-5), 41.2 (C-6), 27.9 (C-8), 24.5 (C-16), 18.9 (C-7), 13.8 (13-Me), 10.6 (C-14), 6.9 (2 x $\underline{C}H_3CH_2$), 5.0 and 4.9 (2 x $CH_3\underline{C}H_2$).

(13*R*)- and (13*S*)-3α,4β-Bistriethylsilyloxy-10β-bromo-13-methyl-9α,15; 12,13-diepoxytrichothecanes (114) and (115)



(114)



(115)

A mixture of the bistriethylsilyloxy olefins (112) and (113) (0.22 g, 0.374 mmol), m-CPBA (98.5 mg, 0.561 mmol) and Na_2HPO_4 (0.54 g, 3.85 mmol) in dichloromethane (20 ml) was stirred overnight at room temperature. The mixture was then poured into water (20 ml) and extracted thoroughly with ether (3 x 20 ml). The combined organic extracts were washed successively with saturated aqueous sodium hydrogen carbonate and brine and dried. The organic solution was then concentrated under reduced pressure. Purification by dry-column flash chromatography using ether-hexane as the eluent yielded a colourless oil consisting of the title compounds (0.195 g, 86%) as a mixture of diastereoisomers which were not separated.

ν_{max} (CCl_4) : 1125 and 1100 $cm.^{-1}$

m/z : Found $M^{+\cdot}$ 575.2064 and 573.2068.

$C_{28}H_{51}BrO_5Si_2$ - C_2H_5 requires 575.2047 and 573.2067.

(114)

^1H NMR : 4.2 - 4.1 (4H, m, 3-H, 4-H, 10-H and 11-H), 3.71 (2H br s, 15-H_2), 3.40 (1H, d, J 4.6, 2-H), 3.14 (1H, q, J 5.9, 13-H), 1.37 (3H, q, J 5.9, 13-Me), 1.27 (3H, s, 16-H), 0.93 [18H, 6 x t (overlapping), $C\underline{H}_3CH_2$], 0.71 (3H, s, 14-H) and 0.64 [12H, 6 x q (overlapping), $CH_3C\underline{H}_2$].

^{13}C NMR : 83.3 (C-3), 82.3 (C-4), 81.5 (C-2), 73.6 (C-9), 68.3 (C-12), 67.7 (C-11), 66.4 (C-15), 56.6 (C-13), 54.5 (C-10), 47.8 (C-5), 41.4 (C-6), 28.0 (C-8), 24.4 (C-16), 19.8 (C-7), 15.0 (13-Me) and 10.6 (C-14), 6.9 (2 x $\underline{C}H_3CH_2$), 5.0 and 4.9 (2 x $CH_3\underline{C}H_2$).

(115)

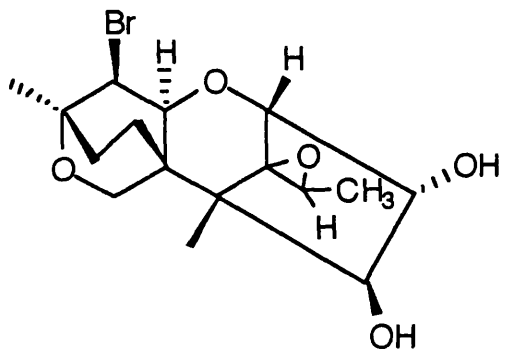
^1H NMR : 4.2 - 4.1 (4H, m, 3-H, 4-H, 10-H and 11-H), 3.71 (2H br s, 15-H_2), 3.40 (1H, d, J 4.6, 2-H), 2.87 (1H, q, J 5.5, 13-H), 1.43 (3H, q, J 5.5, 13-Me), 1.27 (3H, s, 16-H), 0.93 [18H, 6 x t (overlapping), $C\underline{H}_3CH_2$], 0.71 (3H, s, 14-H) and 0.64 [12H, 6 x q (overlapping), $CH_3C\underline{H}_2$].

^{13}C NMR : 82.6 (C-3), 82.3 (C-4), 77.5 (C-2), 73.7 (C-9), 68.4 (C-12), 67.7 (C-11), 66.4 (C-15), 54.7 (C-10), 52.4

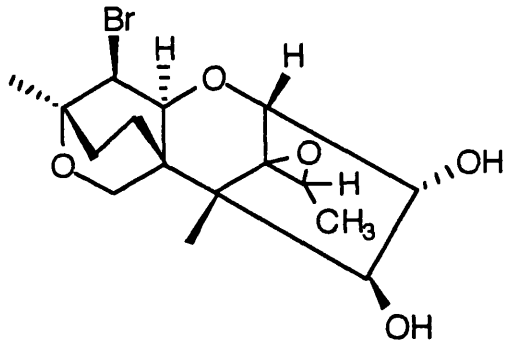
(cont..)

(C-13), 47.2 (C-5), 41.5 (C-6), 28.1 (C-8), 24.4 (C-16), 19.5 (C-7), 14.4 (13-Me) and 6.6 (C-14), 6.9 (2 x $\underline{C}H_3CH_2$), 5.0 and 4.9 (2 x $CH_3\underline{C}H_2$).

(13*R*)- and (13*S*)-3α,4β-Dihydroxy-10β-bromo-13-methyl-9α,15; 12,13-diepoxytrichothecanes (119) and (120)



(119)



(120)

To a flame-dried round bottomed flask with side-arm in a nitrogen atmosphere at 0 °C was added the mixture of epoxides (114) and (115) (0.190 g, 0.315 mmol) in THF (8 ml). TBAF (0.39 ml; 1M in THF) was then added and the resulting solution was stirred for 2 h. The reaction mixture was then diluted with EtOAc (25 ml), washed with brine (25 ml), dried and then concentrated *in vacuo*. Purification of the residue by dry-column flash chromatography using ether-hexane as the eluent yielded a colourless oil consisting of the title compounds (0.108 g, 91%) as a mixture of diastereomers which were not separated.

ν_{max} (CCl_4) : 3360, 1215 and 1065 $cm.^{-1}$

m/z : Found M^+, 317.0576 and 315.0596.

$C_{16}H_{23}BrO_5$-$C_2H_3O_2$ requires 317.05777 and 315.0596.

(119)

^1H NMR : 4.20 (4H, m, 3-H, 4-H, 10-H and 11-H), 3.73 (2H, br s, 15-H_2), 3.57 (1H, d, J 5.0, 2-H), 3.26 (1H, q, J 5.9, 13-H), 2.60 (2H, br s, 2 x OH), 2.3 - 1.5 (4H, m, 7-H and 8-H), 1.40 (3H, d, J 5.9, 13-Me), 1.27 (3H, s, 16-H) and 0.82 (3H, s, 14-H).

^{13}C NMR : 81.8 (C-3), 81.7 (C-4), 79.9 (C-2), 73.7 (C-9), 68.3 (C-12), 68.1 (C-11), 66.1 (C-15), 57.4 (C-13), 54.5 (C-10), 47.9 (C-5), 41.2 (C-6), 28.0 (C-8), 24.3 (C-16), 19.6 (C-7), 14.7 (13-Me) and 10.3 (C-14).

(120)

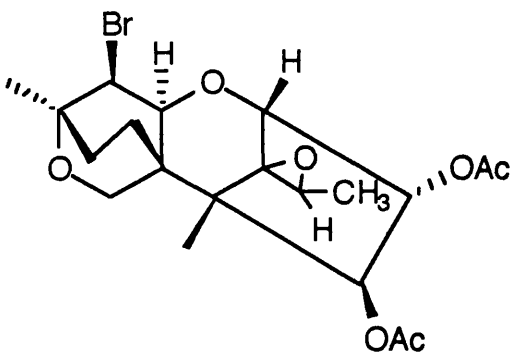
^1H NMR : 4.20 (4H, m, 3-H, 4-H, 10-H and 11-H), 3.92 (1H, d, J 4.8, 2-H), 3.73 (2H, br s, 15-H_2), 2.96 (1H, q, J 5.5, 13-H), 2.60 (2H, br s, 2 x OH), 2.3 - 1.5 (4H, m, 7-H and 8-H), 1.45 (3H, d, J 5.5, 13-Me), 1.27 (3H, s, 16-H) and 0.64 (3H, s, 14-H).

^{13}C NMR : 81.7 (C-3), 80.9 (C-4), 77.0 (C-2), 73.8 (C-9), 68.5 (C-12), 68.1 (C-11), 65.8 (C-15), 53.3

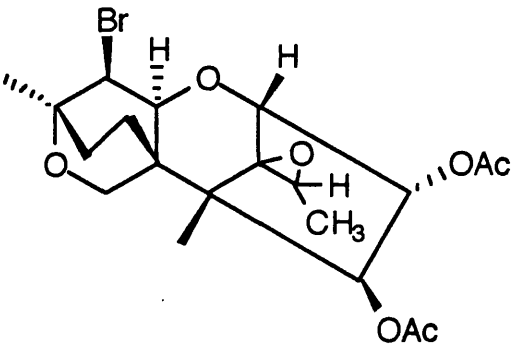
(cont...)

(C-13), 54.6 (C-10), 47.1 (C-5), 41.4 (C-6), 28.0 (C-8), 24.3 (C-16), 19.4 (C-7), 14.2 (13-Me) and 6.1 (C-14).

(13*R*)- and (13*S*)-3α,4β-Diacetoxy-10β-bromo-13-methyl-9α,15; 12,13-diepoxytrichothecanes (121) and (122)



(121)



(122)

Acetic anhydride (4 ml) and pyridine (2 ml) were added to the mixture of diols (119) and (120) (85.2 mg, 0.227 mmol) and the resulting solution was stirred overnight at room temperature after which excess of acetic anhydride and pyridine were removed azeotropically under reduced pressure using toluene (3 x 20 ml) and then CCl_4 (3 x 20 ml). Purification by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded a colourless oil consisting of the title compounds (93.3 mg, 94%) as a mixture of diastereomers which were not separated.

ν_{max} (CCl_4) : 1745 $cm.^{-1}$

m/z : Found M^+, 460.0917 and 458.0930. $C_{20}H_{27}BrO_7$ requires M, 460.0925 and 458.0953.

(121)

^1H NMR : 5.61 (1H, d, J 3.5, 4-H), 5.22 (1H, dd, J 5.0 and 3.5 Hz, 3-H), 4.30 (1H, dd, 8.6 and 1.8 Hz, 11-H), 4.2 - 4.1 (1H, m, 10-H), 3.97 (1H, dd, J 9.6 and 3.0 Hz, 15-Hα), 3.80 (1H, d, J 5.0, 2-H), 3.73 (1H, d, J 9.6, 15-Hβ), 3.29 (1H, q, J 5.9, 13-H), 2.14 (3H, s, MeCO), 2.11 (3H, s, MeCO), 1.43 (3H, d, J 5.9, 13-Me), 1.31 (3H, s, 16-H) and 0.74 (3H, s, 14-H).

^{13}C NMR : 170.7 (Me<u>C</u>O), 169.9 (Me<u>C</u>O), 80.5 (C-3), 79.1 (C-4), 77.6 (C-2), 73.6 (C-9), 68.3 (C-11), 67.5 (C-12), 66.1 (C-15), 57.7 (C-13), 54.3 (C-10), 47.4 (C-5), 41.6 (C-6), 27.9 (C-8), 24.2 (C-16), 20.9 (<u>Me</u>CO), 20.8 (<u>Me</u>CO), 19.7 (C-7), 14.9 (13-Me) and 9.5 (C-14).

(122)

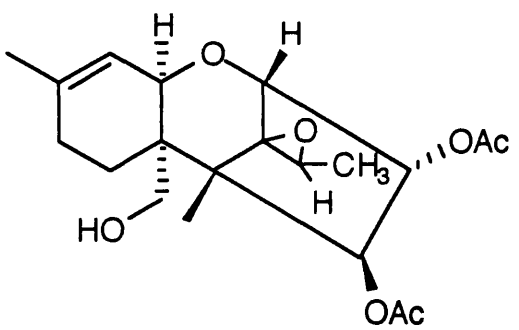
^1H NMR : 5.57 (1H, d, J 3.7, 4-H), 5.22 (1H, dd, 3-H), 4.29 (1H, dd, 8.6 and 1.8 Hz, 11-H), 4.2 - 4.1 (1H, m, 10-H), 3.97 (1H, dd, J 9.6 and 3.0 Hz, 15-Hα), 3.80 (1H, d, J 5.0, 2-H), 3.73 (1H, d, J 9.6, 15-Hβ), 2.98 (1H, q, J 5.5, 13-H), 2.15 (3H, s, MeCO), 2.10 (3H, s, MeCO), 1.48

(cont...)

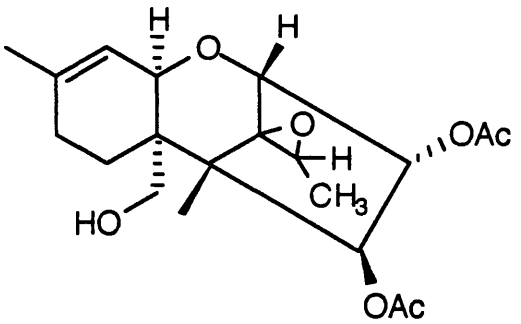
(3H, d, J 5.5, 13-Me), 1.31 (3H, s, 16-H) and 0.57 (3H, s, 14-H).

^{13}C NMR : 171.0 (Me<u>C</u>O), 170.6 (Me<u>C</u>O), 78.3 (C-3), 78.0 (C-4), 75.8 (C-2), 73.8 (C-9), 68.3 (C-11), 67.6 (C-12), 60.3 (C-15), 54.4 (C-10), 53.4 (C-13), 46.6 (C-5), 41.8 (C-6), 28.0 (C-8), 24.2 (C-16), 21.0 (<u>Me</u>CO), 20.8 (<u>Me</u>CO), 19.4 (C-7), 14.2 (13-Me) and 5.6 (C-14).

(13*R*)- and (13*S*)-3α,4β-Diacetoxy-15-hydroxy-13-methyl-12,13-epoxytrichothec-9-enes (123) and (124)



(123)



(124)

Zinc powder (4.16 g) was added in one portion to a stirred, hot suspension of AgOAc (23 mg) in AcOH (23 ml). After 30 secs the AcOH was removed by decantation, and the Zn/Ag couple was washed with AcOH (1 x 10 ml) and ether (5 x 10 ml). Ether (11 ml) was added to the freshly prepared couple, and this was followed by a mixture of the bromo-ethers (121) and (122) (46.3 mg, 0.10 mmol) in THF (21 ml) and EtOH (4 ml). The mixture was heated at 55 °C with stirring for 4 hours, cooled to 20 °C and concentrated in vacuo. The residue was taken up in acetone (20 ml) and filtered through a pad of celite. The filtrate was then concentrated under reduced pressure. Purification by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded a colourless oil consisting of the title compounds

(23.1 mg, 60%) as a mixture of diastereomers which were not separated.

ν_{max} (CCl_4) : 3650, 3510, 1750 and 1725 $cm.^{-1}$

m/z : Found M^+, 380.1811. $C_{20}H_{28}O_7$ requires M, 380.1835.

(123)

^1H NMR : 6.03 (1H, d, J 3.4, 4-H), 5.53 (1H, br d, 10-H), 5.17 (1H, dd, J 4.9 and 3.4 Hz, 3-H), 4.16 (1H, br d, 11-H), 3.82 and 3.64 (2H, AB_q, J_{obs} 12.1, 15-H_2), 3.69 (1H, d, J 4.9, 2-H), 3.67 (1H, br s, -OH), 3.27 (1H, q, J 5.9, 13-H), 2.15 (3H, s, MeCO), 2.13 (3H, s, MeCO), 1.72 (3H, s, 16-H), 1.41 (3H, d, J 5.9, 13-Me) and 0.93 (3H, s, 14-H).

^{13}C NMR : 172.3 (Me<u>C</u>O), 170.1 (Me<u>C</u>O), 140.2 (C-9), 118.8 (C-10), 81.2 (C-3), 79.5 (C-4), 77.4 (C-2), 68.3 (C-12), 67.7 (C-11), 62.6 (C-15), 58.2 (C-13), 50.0 (C-5), 44.7 (C-6), 28.1 (C-8), 23.2 (C-16), 21.7 (C-7), 20.9 (<u>Me</u>CO), 20.9 (<u>Me</u>CO), 14.8 (13-Me) and 10.4 (C-14).

(124)

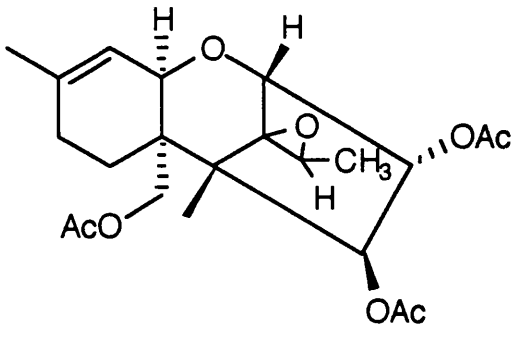
^1H NMR : 6.01 (1H, d, J 3.6, 4-H), 5.53 (1H, br d, 10-H), 5.18 (1H, dd, J 4.7 and 3.6 Hz, 3-H), 4.19 (1H, br d,

(cont...)

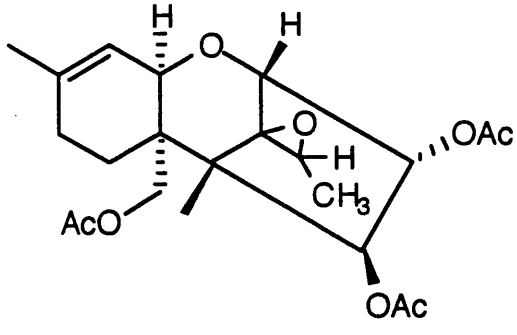
11-H), 4.06 (1H, d, J 4.7, 2-H), 3.80 and 3.64 (2H, AB_q, J_{obs} 12.1, 15-H_2), 3.67 (1H, br s, -OH), 3.00 (1H, q, J 5.7, 13-H), 2.15 (3H, s, MeCO), 2.13 (3H, s, MeCO), 1.72 (3H, s, 16-H), 1.43 (3H, d, J 5.7, 13-Me) and 0.77 (3H, s, 14-H).

^{13}C NMR : 172.3 (Me<u>C</u>O), 170.1 (Me<u>C</u>O), 140.2 (C-9), 118.8 (C-10), 80.5 (C-3), 78.1 (C-4), 74.6 (C-2), 68.3 (C-12), 67.7 (C-11), 62.8 (C-15), 54.2 (C-13), 49.2 (C-5), 44.6 (C-6), 28.1 (C-8), 23.2 (C-16), 21.5 (C-7), 20.9 (<u>Me</u>CO), 20.9 (<u>Me</u>CO), 14.3 (13-Me) and 6.2 (C-14).

(13*R*)- and (13*S*)-3α,4β,15-Triacetoxy-13-methyl-12,13-epoxytrichothec-9-enes (11) and (12)



(11)



(12)

Acetic anhydride (1 ml) and pyridine (0.5 ml) were added to the mixture of 15-hydroxy-3 ,4 -diacetates (123) and (124) (43.8 mg, 0.11 mmol) and the resulting solution was stirred overnight at room temperature after which excess of acetic anhydride and pyridine were removed azeotropically under reduced pressure using toluene (3 x 20 ml) and then CCl_4 (3 x 20 ml). Purification by dry-column flash chromatography using ethyl acetate-hexane as the eluent yielded a colourless oil consisting of the title compounds (39.8 mg, 82%). Partial separation was achieved by flash chromatography using 35% ethyl acetate/ 65% hexane as the solvent system.

ν_{max} (CCl_4) : 2970, 1750, 1370, 1235(br) $cm.^{-1}$

m/z : Found M^+, 379.1761. $C_{22}H_{30}O_8$ - CH_3CO requires 379.1757.

(125)

^1H NMR : 5.78 (1H, d, J 3.2, 4-H), 5.48 (1H, br d, J 5.6, 10-H), 5.14 (1H, dd, J 5.0 and 3.2 Hz, 3-H), 4.21 and 4.03 (2H, AB_q, J_{obs} 12.4, 15-H_2), 3.98 (1H, br d, 11-H), 3.66 (1H, d, J 5.0, 2-H), 3.27 (1H, q, J 5.9, 13-H), 2.15 - 1.65 (4H, m, 7-H and 8-H), 2.12 (3H, s, MeCO), 2.10 (3H, s, MeCO), 2.07 (3H, s, MeCO), 1.71 (3H, br s, 16-H), 1.42 (3H, d, J 5.9, 13-Me) and 0.88 (3H, s, 14-H).

^{13}C NMR : 170.6 (Me<u>C</u>O), 170.6 (Me<u>C</u>O), 169.9 (Me<u>C</u>O), 140.6 (C-9), 118.4 (C-10), 80.4 (C-2), 79.6 (C-3), 78.5 (C-4), 68.1 (C-12), 67.8 (C-11), 63.4 (C-15), 58.0 (C-13), 50.1 (C-5), 43.8 (C-6), 28.0 (C-8), 23.2 (C-16), 21.7 (C-7), 21.0 (<u>Me</u>CO), 20.9 (<u>Me</u>CO), 20.9 (<u>Me</u>CO), 14.7 (13-Me) and 10.7 (C-14).

(126)

^1H NMR : 5.74 (1H, d, J 3.3, 4-H), 5.48 (1H, br d, J 5.6, 10-H), 5.16 (1H, dd, J 4.9 and 3.3 Hz, 3-H), 4.23 and 4.03 (2H, AB_q, J_{obs} 12.1, 15-H_2), 3.98 (1H, br d, 11-H), 3.66 (1H, d, J 4.9, 2-H), 3.01 (1H, q, J 5.5, 13-H), 2.15 - 1.65 (4H, m, 7-H and 8-H), 2.13 (3H, s, MeCO), 2.09 (3H,

(cont...)

s, MeCO), 2.05 (3H, s, MeCO), 1.71 (3H, br s, 16-H), 1.43 (3H, d, J 5.5, 13-Me) and 0.73 (3H, s, 14-H).

^{13}C NMR : 171.1 (Me<u>C</u>O), 170.5 (Me<u>C</u>O), 170.5 (Me<u>C</u>O), 140.7 (C-9), 118.2 (C-10), 78.5 (C-2), 74.7 (C-3), 67.9 (C-4), 67.8 (C-12), 67.7 (C-11), 60.3 (C-15), 54.0 (C-13), 49.4 (C-5), 43.7 (C-6), 28.0 (C-8), 23.2 (C-16), 21.7 (C-7), 21.0 (<u>Me</u>CO), 20.9 (<u>Me</u>CO), 20.9 (<u>Me</u>CO), 14.2 (13-Me) and 6.6 (C-14).

4. References

REFERENCES

1. W.O. Godtfredsen, J.F. Grove, and Ch. Tamm, Helv. Chim. Acta., 1967, **50**, 1666.
2. P.W. Brian, A.W. Grove, J.F. Hemming, D. Lows, and G.L.F. Norris, J. Exp. Bot., 1961, **12**, 1.
3. T. Tatsuno, Y. Morita, H. Tsunoda, and M. Umeda, Chem. Pharm. Bull., 1970, **18**, 1485.
4. Y. Ueno, N. Sato, K. Ishii, K. Sakai, and M. Enomoto, Japan J. Exp. Med., 1972, **42**, 461.
5. L.R. Ember, Chem. Eng. News, 1984, 8.
6. J.R. Bamberg, N.V. Riggs, and F.M. Strong, Tetrahedron, 1968, **24**, 3329.
7. T. Tatsuno, M. Saito, M. Enomoto and H. Tsunoda, Chem. Pharm. Bull., 1968, **16**, 2519.
8. R M. Eppley and W.J. Bailey, Science, 1973, **24**, 758.
9. T.W. Doyle and W.T. Bradner, "Anticancer agents based on natural products" eds. J.M. Cassidy and J.D. Douros, Academic Press, New York, 1980, Chp. 2.
10. S.S. Adler, S. Lowenbraun, B. Birch, R. Jarrell, and J. Garrard, Cancer Treat. Rep., 1984, **68**, 423.
11. Y. Ueno, M. Hosoya, Y. Morita, I. Ueno, and T. Tatsuno, J. Biochem. (Tokyo), 1968, **64**, 479.
12. Y. Ueno, M. Hosoya, and Y. Ishikawa, J. Biochem. (Tokyo), 1969, **66**, 419.
13. Y. Ueno, N. Sato, K. Ishii, K. Sakai, H. Tsunoda and M. Enomoto, Appl. Microbiol., 1973, **25**, 699.

14. Y. Ueno, Pure Appl. Chem. 1977, 49, 1737.

15. T. Yoshizawa, H. Takeda, and T. Ohi, Agric. Biol. Chem., 1983, 47, 2133.

16. R.R. King, R.E. McQueen, D. Levesque, and R. Greenhalgh, J. Agric. Food Chem., 1984, 32, 1181.

17. B.B. Jarvis and E.P. Mazzola, Acc. Chem. Res., 1982, 15, 388.

18. W.R. Roush and S. Russo-Rodriguez, J. Org. Chem., 1985, 50, 5465.

19. A.W. Dawkins and J.F. Grove, J. Chem. Soc. C., 1979, 369.

20. J.F. Grove and P.H. Mortimer, Biochem. Pharmacol., 1969, 18, 1473.

21. J.F. Grove, Nat. Prod. Rep., 1988, 187.

22. J.F. Grove, J. Chem. Soc., Perkin Trans. 1, 1986, 647.

23. D.J. Goldsmith, T.K. John, C.D. Kwong, and G.R. Painter III, J. Org. Chem., 1980, 45, 3989.

24. E.W. Colvin and S. Cameron, J. Chem. Soc., Chem. Commun., 1986, 1642.

25. J. Robb and M. Norval, Appl. Environ. Microbiol., 1983, 46, 948.

26. J.W. Cornforth, R.H. Cornforth, and K.K. Matthew, J. Chem. Soc., 1959, 1, 112.

27. G. Wittig and W. Haag, Chem. Ber., 1955, 88, 1654.

28. C.B. Scott, J. Org. Chem., 1957, 22, 1118.

29. E. Vedejs and P.L. Fuchs, J. Am. Chem. Soc., 1973, 95, 822.

30. J. Gutzwiller, R. Mauli, H.P. Sigg, and Ch. Tamm, Helv. Chim. Acta., 1964, 47, 2234.

31. A. Pribela, P. Lacok, Polnohospodarstvo (S10), 1976, 22, 278.

32. J.M. Behan, R.A.W. Johnston, and M. Wright, J. Chem. Soc., Perkin Trans. 1, 1975, 1216.

33. W. Brietenstein and C. Tamm, Helv. Chim. Acta., 1977, 60, 1522.

34. P.B. Dervan and M.A. Shippey, J. Am. Chem. Soc., 1976, 98, 1265.

35. D.L.J. Clive and S.M. Menchan, J. Org. Chem., 1980, 45, 2347.

36. H. Alper and D. Des Roches, Tetrahedron Lett., 1977, 4155.

37. B.M. Trost and D.P. Curran, Tetrahedron Lett., 1981, 22, 1287.

38. R.R. King and R. Greenhalgh, Can. J. Chem., 1985, 63, 1089.

39. R.E. Parker and N.S. Isaac, Chem. Rev., 1959, 59, 737.

40. J.R. Bamburg and F.M. Strong, "Microbial Toxins", eds. S. Kadis, A. Ciegler, and S.J. Ali, Academic Press, New York, 1971, Vol. V11., pp. 224-227.

41. M.G. Martin and B. Ganem, Tet. Lett., 1984, 25, 251.

42. E.W. Colvin and S. Cameron, J. Chem. Soc., Chem Commun, 1986, 1084.

43. K.B. Sharpless, M.A. Umbreit, M.T. Nieh, and

T.C. Flood, J. Am. Chem. Soc., 1972, **94**, 6538.

44. A. Belanger, D.F. Berney, H.J. Borschberg, R. Brousseau, A. Doutheau, R. Durand, H. Katayama, R. LaPalme, D.M. Leturc, C.C. Liao, F.N. MacLachlan, J.P. Maffrand, F. Marazza, R. Martino, C. Moreau, L. Saint Laurent, R. Saintonge, P. Soucy, L. Ruest, and P. Deslongchamps, Can. J. Chem., 1979, **57**, 3348.

45. N.C.P. Baldwin, B.W. Bycroft, P.M. Dewick, and J.Gilbert, Z. Naturforsch., Teil C, 1986, **41**, 845.

46. K.B. Sharpless, A.Y. Teranishi, and J.E. Backvall, J. Am. Chem. Soc., 1977, **99**, 3125.

47. C.F. Schonbein, C.R. Seances Acad. Sci., 1840, **10**, 706.

48. C.D. Harries, "Untersuchungen Uber das Ozon und Seine Einwirkung auf Organiche Verbindungen", Springer-Verlag, Berlin and New York, 1919.

49. R. Criegee and G. Wenner, Anal. Chem., 1949, **564**, 9.

50. R. Criegee, Anal. Chem., 1953, **583**, 1.

51. C. Harries and F. Evers, Anal. Chem., 1912, **390**, 238.

52. H. Staudinger, Chem. Ber., 1925, **58**, 1088.

53. J.E. Leffler, Chem. Rev., 1949, **45**, 399.

54. R. Criegee, Angew. Chem., Int. Ed. Engl., 1975, **14**, 745.

55. R. Criegee and G. Schroder, Chem. Ber., 1960, **93**, 689.

56. F.L. Greenwood, J. Org. Chem., 1964, **29**, 1321.

57. P.S. Bailey, T.P Carter, Jr., C.M. Fischer, and J.A. Thompson, Can. J. Chem., 1973, **51**, 1278.

58. J.Z. Gillies, C.W. Gillies, R.D. Suenram and F.J. Lovas, J. Am. Chem. Soc., 1988, **110**, 7991.

59. C.W. Gillies, J.Z. Gillies, R.D. Suenram, F.J. Lovas, E. Kraka, and D. Cremer, J. Am. Chem. Soc., 1991, **113**, 2412.

60. R.Huisgen, Angew. Chem., Int. Ed. Engl., 1963, **2**, 565.

61. R.L. Kuczkowski, Chem. Soc. Rev., 1992, **21**, 79.

62. Y.K. Wei and R.J. Cvetanovic, Can. J. Chem., 1963, **41**, 913.

63. E.R. Altwicker and J. Basila, Tetrahedron, 1973, **29**, 1969.

64. M. Tits and A. Bruylants, Bull. Soc. Chim. Belg., 1948, **57**, 50.

65. R. Huisgen, Angew. Chem., Int. Ed. Engl., 1963, **2**, 633.

66. S.D. Razumovskii and G.E. Zaikow, J. Org. Chem. USSR (Engl. Transl.), 1972, **8**, 468.

67. J. Carles and S. Fliszar, Adv. Chem. Ser., 1972, **112**, 35.

68. P.S. Bailey, Chem. Rev., 1958, **58**, 925.

69. R. Criegee and K. Noll, Anal. Chem., 1959, **1**, 625.

70. R. Criegee and G. Lohaus, Chem. Ber., 1952, **86**, 1.

71. R. Criegee, S.S. Bath, and B. von Bornhaupt, Chem. Ber., 1960, **93**, 2891.

72. H. Keul, Chem. Ber., 1975, **108**, 1207.

73. R.W. Murray, P.R. Story, and L.D. Loan, J. Am. Chem Soc., 1965, **87**, 3025.

74. S. Flizar and M. Granger, J. Am. Chem. Soc., 1970, **92**, 3361.

75. S. Flizar, J. Renard, and D. Simon, J. Am. Chem. Soc., 1971, **93**, 6953.

76. R. Criegee, G. Blust, and H. Zincke, Chem. Ber., 1954, **87**, 766.

77. G Lohaus, Chem. Ber., 1954, **87**, 1708.

78. R.W. Murray and G.J. Williams, J. Am. Chem. Soc., 1973, **95**, 3343.

79. O.L. Chapman and T.C. Hess, J. Am. Chem. Soc., 1984, **106**, 1842.

80. H.L. Casul, M. Tanner, N.H. Werstuik, and J.C. Scaiano, J. Am. Chem. Soc., 1985, **107**, 4616.

81. G. Schroder, Chem. Ber., 1962, **95**, 733.

82. N.L. Bauld, J.A. Thompson, C.E. Hudson, and P.S. Bailey, J. Am. Chem. Soc., 1968, **90**, 1822.

83. R.P. Lattimer, R.L. Kuczkowski, and C.W. Gillies, J. Am. Chem. Soc., 1974, **96**, 348.

84. P.R. Story, E.A. Whited, and J.A. Alford, J. Am. Chem. Soc., 1972, **94**, 2143.

85. P.R. Story, R.W. Murray, and R.D. Youssefyeh, J. Am. Chem. Soc., 1966, **88**, 3144.

86. P.R. Story, C.E. Bishop, J.R. Burgess, R.W. Murray, and R.D. Youssefyeh, J. Am. Chem. Soc.,

1968, **90**, 1907.

87. R.P. Lattimer and R.L. Kuczowski, J. Am. Chem. Soc., 1974, **96**, 6205.

88. R.W. Murray and R. Hagen, J. Org. Chem., 1971, **36**, 1103.

89. K.L. Gallaher and R.L. Kuczowski, J. Org. Chem., 1976, **41**, 892.

90. D.P. Higley and R.W. Murray, J. Am. Chem. Soc., 1976, **98**, 4526.

91. J. Carles and S. Fliszar, Can. J. Chem., 1972, **50**, 2552.

92. H. Boardman and G.E. Hulse, J. Am. Chem. Soc., 1953, **75**, 4272.

93. R. Curci and J.O. Edwards, "Organic Peroxides", ed. D. Swern, Wiley (Interscience), New York, 1970, Vol 1, Chp 4, p218.

94. W.R. Roush and S. Russo-Rodriguez, J. Org. Chem., 1985, **50**, 3224.

95. C.A. Claridge and H. Schmitz, Appl. Environ. Microbiol., 1978, **36**, 63.

96. T. Kaneko, H. Schmitz, J.M. Essery, W. Rose, H.G. Howell, F.A. O'Herron, S. Nachfolgen, J. Huflalen, W.T. Bradner, R.A. Partyka, T.W. Doyle, J. Davies, and E. Cundliffe, J. Med. Chem., 1982, **25**, 579.

97. G.A. Kraus, B. Roth, K. Frazier, and M. Shimagaki, J. Am. Chem. Soc., 1982, **104**, 114.

98. K.B. Sharpless and M.A. Umbreit, Org. Synth.,

1981, **60**, 29.

99. J.J. Pappas, W.P. Keavney, E. Gancher, and M. Berger, Tetrahedron Lett., 1966, **36**, 4273.

100. P.S. Bailey, Chem. Ber., 1955, **88**, 795.

101. N.R. Raulins and L.A. Sibert, J. Org. Chem., 1961, **26**, 1382.

102. W.S. Johnson, B. Bannister, and R. Pappo, J. Am. Chem. Soc., 1956, **78**, 6331.

103. T.L. Jacobs and R.B. Brownfield, J. Am. Chem. Soc., 1960, **82**, 4033.

104. C.H. Hassal, Org. React., 1957, **9**, 73.

105. R. Lapalme, H. Jurg Borschberg, P. Soucy, and P. Deslongchamps, Can. J. Chem., 1979, **57**, 3272.

106. H.O. House, L.J. Czuba, M. Gall, and H.D. Olmstead, J. Org. Chem., 1969, **34**, 2324.

107. M. Karplus, J. Am. Chem. Soc., 1963, **85**, 2870.

108. O. Mitsunobu, Synthesis, 1978, 1.

109. J. Martin, W. Parker, B. Shroot, and T. Stewart, J. Chem. Soc.,C1, 1967, 101.

110. K. Bowden, I.M. Heilbron, E.R.H. Jones, and B.C.L. Weedon, J. Chem. Soc., 1946, 39.

111. J.L. Luche and A.L. Gemal, J. Am. Chem. Soc., 1979, **101**, 5848.

112. E.J. Corey and G.W.J. Fleet, Tetrahedron Lett., 1973, 4499.

113. R.H. Schlessinger and R.A. Nugent, J. Am Chem Soc., 1982, **104**, 1116.

114. E.W. Colvin, S. Malchenko, R.A. Raphael, and J.S. Roberts, J. Chem. Soc., Perkin Trans. 1., 1973, 1989.

115. D. Swern, Chem. Rev., 1949, **45**, 49.

116. H.B. Henbest and R.A.L. Wilson, J. Chem. Soc., 1957, 1958.

117. D.W. Brooks, P.G. Grothaus, and H. Mazdiyasni, J. Am. Chem. Soc., 1983, **105**, 4472.

118. N. Masuoka and T. Kamikawa, Tetrahedron Lett., 1976, **3**, 1691.

119. E.J. Corey and B.B. Snider, J. Am. Chem. Soc., 1972, **94**, 2549.

120. J.M. Denis, C. Girard, and J.M. Conia, Synthesis, 1972, 549.

121. G.A. Kraus, B. Roth, K. Frazier, and M. Shimagaki, J. Am. Chem. Soc., 1982, **104**, 1114.

122. L.M. Harwood, Aldrichimica Acta, 1985, **18**, 25.

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