SYNTHETIC STUDIES TOWARDS TRICHOTHECENE MYCOTOXINS

by

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

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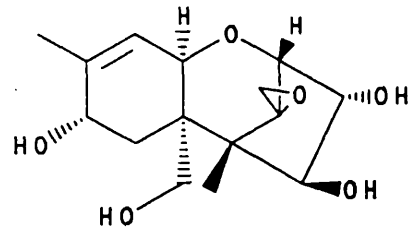
Finally, thanks to Mrs. E. Hughes for her patience in typing this thesis and to Mr. R. Spence who assisted her.

Summary

This thesis describes the first synthesis of a tetrahydroxylated trichothecene mycotoxin, 3α,4β,8α,15-tetrahydroxy-12,13-epoxytrichothec-9-ene (T-2 tetraol) (19), as its tetraacetate (Neosolaniol diacetate) (319) (Scheme 86).



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The synthetic strategy consists of five key elements.

1) formation of the cis fused AB ring system by a Diels-Alder cycloaddition;

2) formation of ring C by an intramolecular aldol reaction;

3) creation of the 3α,4β-diol system of ring C by a stereoselective α-oxygenation/reduction protocol;

4) ring A enone formation *viá* a regiospecific thermodynamically controlled α-selenylation, and

5) regio and stereospecific reduction of this enone to the required α-alcohol.

As part of a concerted effort towards this molecule, this thesis reports a model study aimed at achieving the 3α,4β-diol system of ring C (Scheme 52). The partial synthesis of 8-keto-anguidine (297) described herein (Chapter 2.2) allowed a study (Chapter 2.3) of the stereochemical outcome of the reduction of ring A enones by various reducing agents: it is shown that L-Selectride reduces such enones stereospecifically to 8α-alcohols.

Attempted completion of an approach to deoxynivalenol (6) (Chapter 2.5) is discussed. The termination of this route is attributed to competing intramolecular processes which prevented successful olefination of the ketone (362).

Abbreviations

Ac	:	acetyl
Bu	:	butyl
Bz	:	benzoyl
DABCO	:	1,4-diazabicyclo[2.2.2.]octane
DHP	:	3,4-dihydro-2H-pyran
DIBALH	:	diisobutyl aluminium hydride
DIBALD	:	diisobutyl aluminium deuteride
DMAP	:	4-dimethylaminopyridine
DMF	:	N,N-dimethylformamide
DMS	:	dimethylsulphide
DNA	:	deoxyribonucleic acid
FPP	:	farnesyl pyrophosphate
HMDS	:	1,1,1,3,3,3-hexamethyldisilazane
Imid	:	imidazole
LDA	:	lithium diisopropylamide
mCPBA	:	*meta*-chloroperbenzoic acid
MoOPH	:	molybdenum pentoxide-pyridine-hexamethyl-phosphoramide complex
Ms	:	mesyl
NBS	:	N-bromosuccinimide
NCS	:	N-chlorosuccinimide
PCC	:	pyridinium chloro chromate
PP	:	pyrophosphate
PPTS	:	pyridinium p-toluenesulphonate
PTSA	:	p-toluenesulphonic acid

Py	:	pyridine
TBFA	:	tetrabutylammonium fluoride
TBDMSCL	:	t-butyldimethylchlorosilane
THF	:	tetrahydrofuran
THP	:	tetrahydropyran
TMS	:	trimethylsilyl
TMSCL	:	chlorotrimethylsilane

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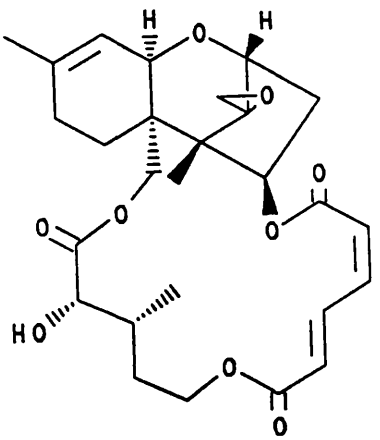
1. Introduction

1.1 Historical Background

In 1946 in the course of an extensive search by Imperial Chemical Industries for new antibiotics, a white crystalline solid was isolated from *Metarrhizium glutinosum*. The compound, named glutinosin, exhibited antifungal activity in some cases at concentrations as low as 0.2μgm per ml and unlike many antifungal antibiotics it appeared to be very stable.

This was the first isolation[1] of a member of the group of closely related fungal sesquiterpenoids - the trichothecenes. Glutinosin was later shown[2] to be a 4:1 mixture of the trichothecenes verrucarin A (1) and verrucarin B (2).



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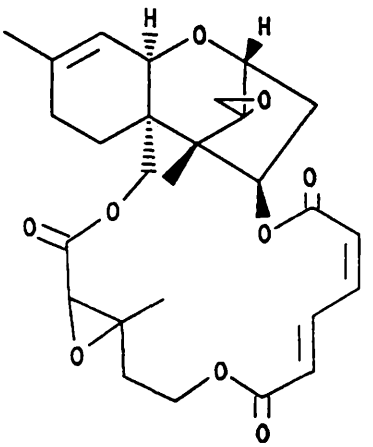

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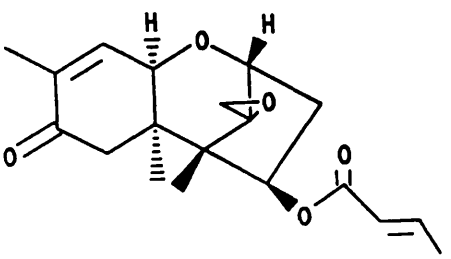
The first trichothecene to be isolated[3] as a single compound was trichothecin (3) from *Trichothecium roseum Link.*



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Since those historic first steps, some 148 trichothecenes have been isolated. All but 25 of them are produced by taxonomically unrelated fungi e.g. *Fusarium*, *Trichoderma*, *Cephalosporium*, *Trichothecium*, *Verticimonosporium* and *Stachybotrys*, making them unique amongst the mycotoxins. For example, the aflatoxins, considered to be the most important of the mycotoxins, are produced by only two, closely related fungi from the *Aspergillus flavus* group.

Of all the known mycotoxins, the trichothecenes are now considered second only to the aflatoxins in economic importance.

Two major factors contribute to this .

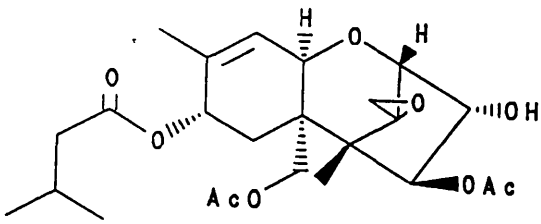
1. The ubiquity of the *Fusarium* genus which produces a variety of secondary metabolites including trichothecenes,

2. The largely adverse biological activity exhibited by the trichothecenes towards mammals.

This biological activity manifests itself in vomiting and feed refusal in animals, diarrhoea, dermal necrosis, inflammation of the gastrointestinal tract, hematological disorders such as an increase in white blood cells, damage to bone marrow, spleen and lymph nodes as well as inhibition of the immunological response.

The role of trichothecenes as the etiological agents in large scale toxicoses was first shown[4] in 1972 by workers at the University of Wisconsin who attributed the deaths of a large number of dairy cattle to ingestion of T-2 toxin (4). This

compound, one of the most toxic members of the series, was found in the mouldy corn on which the cattle had been fed.



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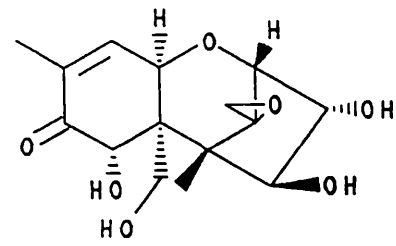

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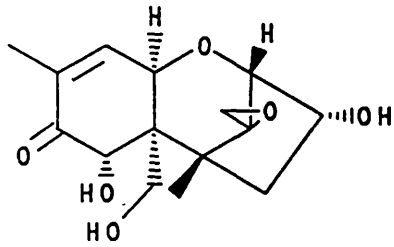

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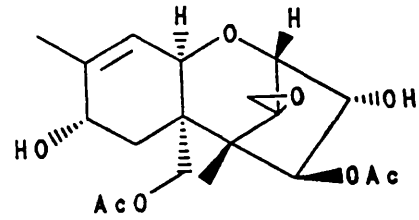


(7)

This discovery implicated the trichothecenes in many other toxicoses. For example, in Japan, Red Mould disease, which occurs sporadically in cereal crops, has been attributed[5] to the presence of the trichothecenes nivalenol (5), deoxynivalenol (6) and their acetylated derivatives.

Outbreaks of alimentary toxic aleukia (septic angina) have been reported in the Soviet Union throughout this century and were thought to be due to a bacterial infection. It is now known[6] that *F. sporotrichoides* was the fungus responsible and Ueno has shown[7] that this fungus can produce T-2 toxin (4), neosolaniol (7) and related trichothecenes.

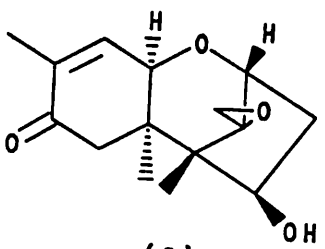
An article assessing the potential use[8] of trichothecenes as

chemical warfare agents was partly inspired by the claims of Alexander Haig who as the then U.S. Secretary of State accused the Soviet Union of using T-2 toxin (4), nivalenol (5) and deoxynivalenol (6) in the so called "Yellow Rain" of Laos and Kampuchea. Haig claimed that high levels of this unnatural combination of trichothecenes in samples of a residue obtained from plants in South East Asia suggested foul play by the Soviet Union. However independent scientists have declared that the U.S. government's analysis was "very definitely qualitative" and even if quantitative it would have given levels of trichothecenes similar to those reported for natural infestations. The supposedly unnatural combination of trichothecenes has been shown[9] to be in fact a natural one and, most embarassingly for the U.S. government, the yellow residue had a marked resemblance to bee faeces.

Much of the present interest in trichothecenes lies in agriculture and farming. Infestation of cereals and the resulting reduction in animal growth keeps interest high both in the isolation of new members of the group and the study of their biological profiles.

1.2 Structure

For approximately ten years after the isolation of trichothecin, the structure of the trichothecenes remained unclear. Freeman and Gill[10] in 1950 correctly concluded that trichothecin was an ester of isocrotonic acid and a ketonic alcohol which they named trichothecolone (8). In 1959 Bohus et al isolated[11] 'antibiotic T' (later to be named[12] crotocin (9)) which on alkaline hydrolysis also appeared to be an ester of isocrotonic acid. The alcohol portion of antibiotic T although not identical to trichothecolone was very similar in chemical and biological behaviour suggesting a close relationship.



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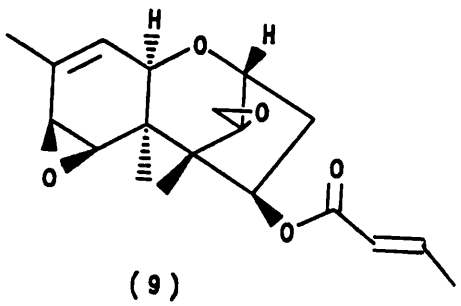

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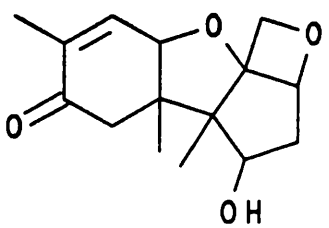
The first major structural breakthrough was achieved independently by Freeman[13], Fishman[14] and Tamm[15] who proposed on the basis of extensive chemical degradation the structures (10) and (11) for the trichothecene nucleus.



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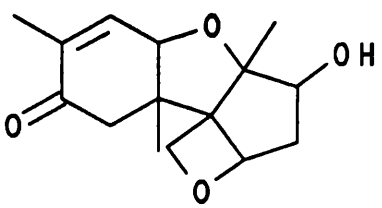
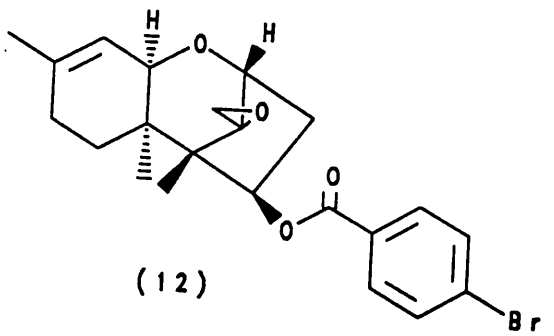


(11)

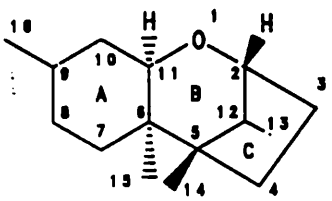
However their structures were shown to be incorrect when in 1964, Abrahamsson reported[16] an X-ray crystallographic analysis of the p-bromobenzoate ester (12) of trichodermol, showing it to have an intriguing tetracyclic nucleus.



(12)

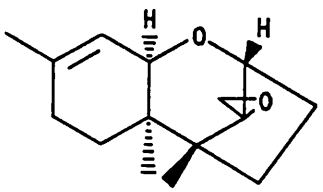
In 1965, McPhail and Sim showed[17] by X-ray analysis that verrucarin A (1) possessed a very closely related structure resulting in modifications to previous proposals.

The name trichothecane, after trichothecin, the first member to be isolated, for the basic nucleus (13) was first proposed[18] by Grove, Tamm and Godtfredsen.



(13)

Almost all trichothecenes are unsaturated at the 9,10-position and also contain a spiro-epoxy function at the 12,13-position and hence are often referred to as 12,13-epoxytrichothec-9-enes(14) or simply as the trichothecenes.



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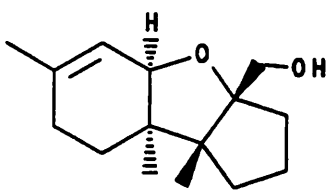
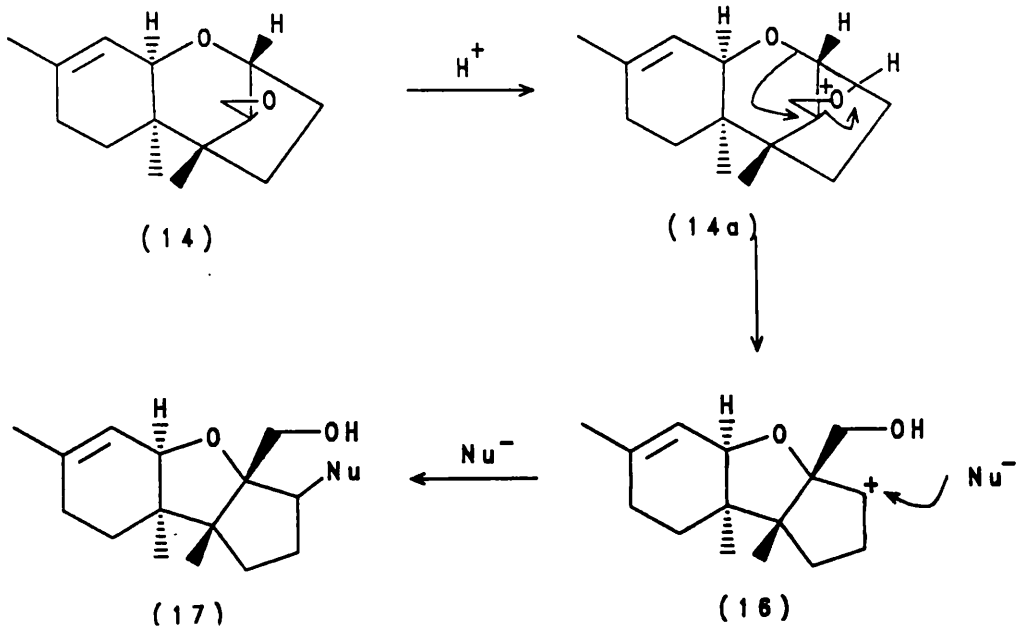
Although the proposed structures (10) and (11) were incorrect, they still have an important place in trichothecene chemistry. Structures of type (15)



(15)

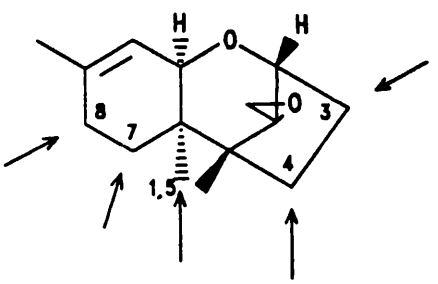
are the biologically inactive apotrichothecenes[19] and are derived from the trichothecenes by an acid catalysed skeletal rearrangement (Scheme 1). Protonation of the epoxide oxygen is followed by nucleophilic attack at C-12 by the pyran oxygen effecting ring contraction to a carbonium ion species (16). This can then be quenched by an external nucleophile giving the apotrichothecene skeleton (17). This rearrangement is important not only in having confused the elucidation of the

trichothecene structure by chemical degradation studies, but also in that it may be of significance in the mechanism of biological action.



Scheme 1

The large number of known trichothecenes is a consequence of the oxygen substitution that can occur as indicated by the arrows.



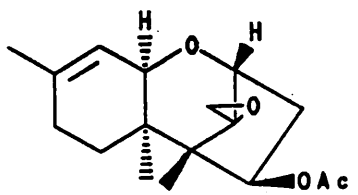
Oxygenation at C7 is the least frequent and the configurations are normally 3α, 4β, 7α, and 8α. This high degree of oxygenation results in wide structural diversity and indeed the trichothecenes can be divided into three major groups based on structural differences. These groups are

1) Non-macrocyclic trichothecenes

2) Macrocyclic trichothecenes

and 3) Trichoverroids.

The first group, the non-macrocyclic trichothecenes, contains those molecules which have been hydroxylated at combinations of positions 3,4,7,8 and 15 and are often simple esters. The simple trichothecenes can be subdivided further on the basis of the level of oxidation at C8 in that this position can be a methylene, e.g. trichodermin (18), an alcohol e.g.T-2 tetraol (19) or a ketone as in deoxynivalenol (6). The 8-keto trichothecenes are considered as a group on their own.



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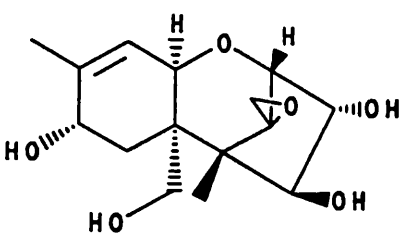

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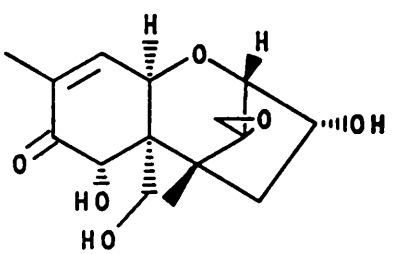

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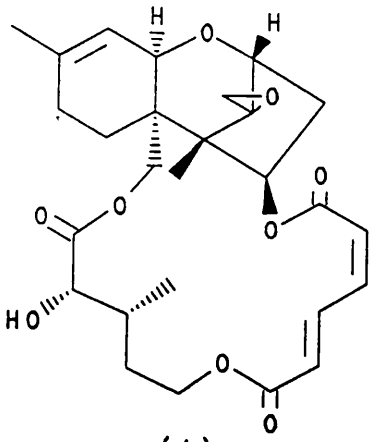

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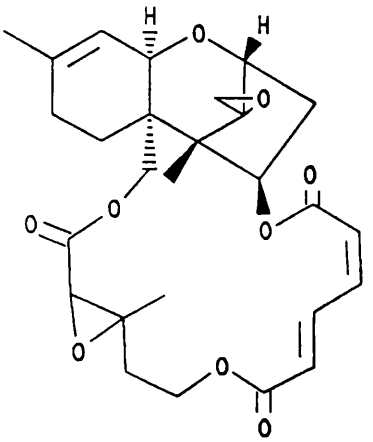

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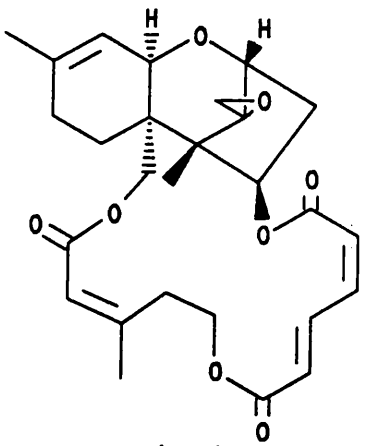

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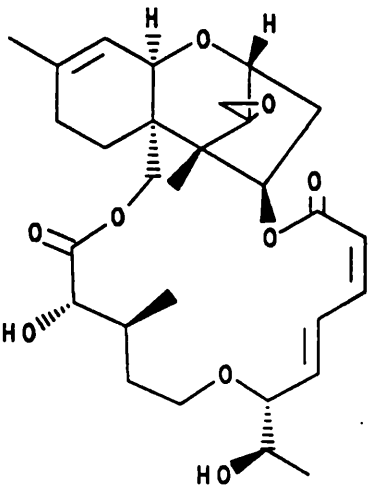

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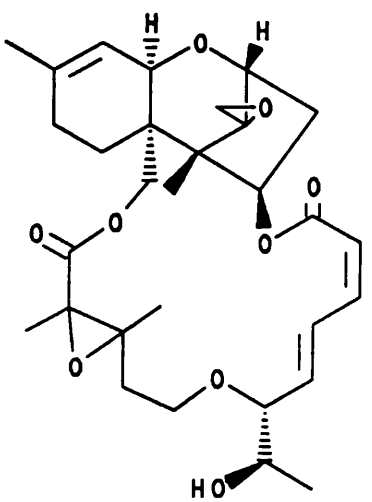

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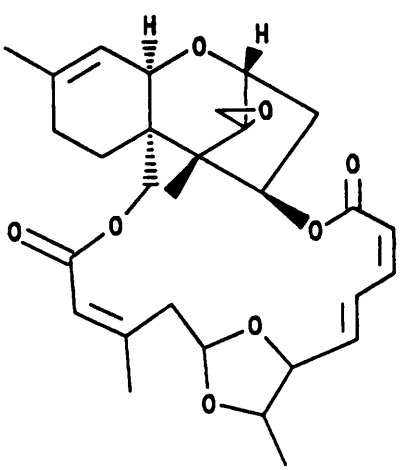

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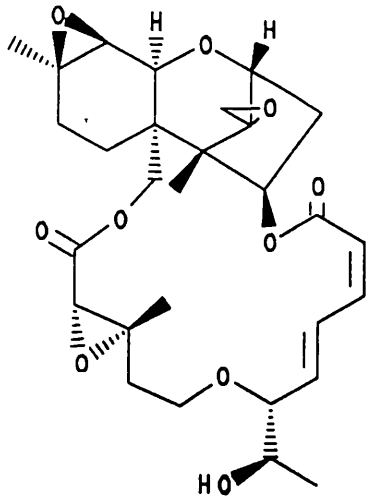

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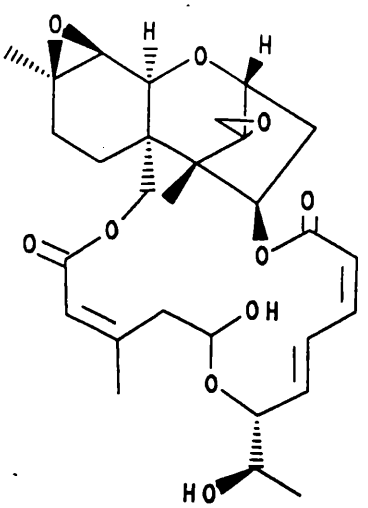

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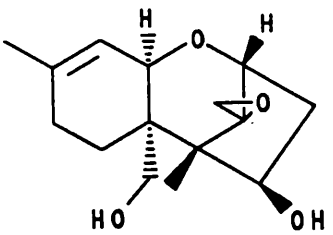

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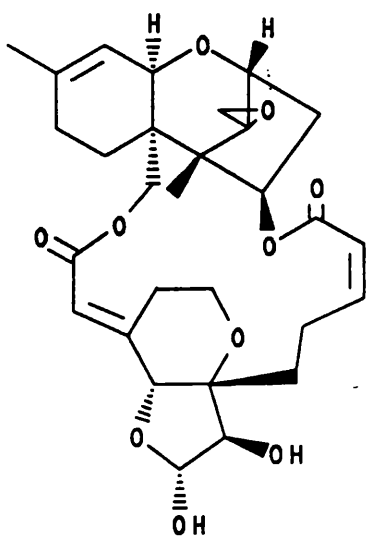

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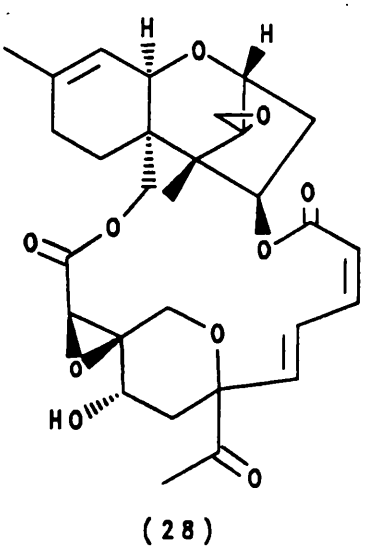
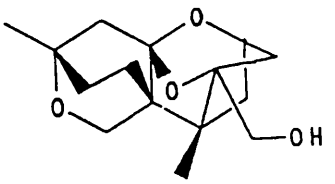


(28)

All of the natural trichothecenes conform to one or other of the above groups. However some contain structural anomalies. For example, some of the baccharenoids have an 8β-hydroxyl instead of the usual 8α-hydroxyl. Two trichothecenes are saturated at the 9,10-position, namely sporol (31) and the macrocyclic roritoxin C (32), derived from verrucarol but which contains a 9β, 10β-epoxide group.



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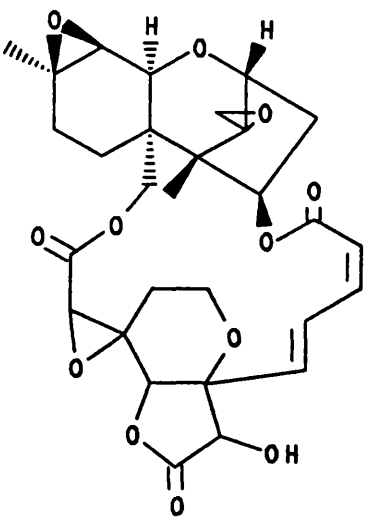
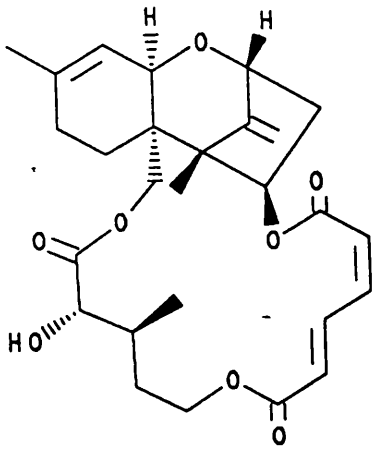
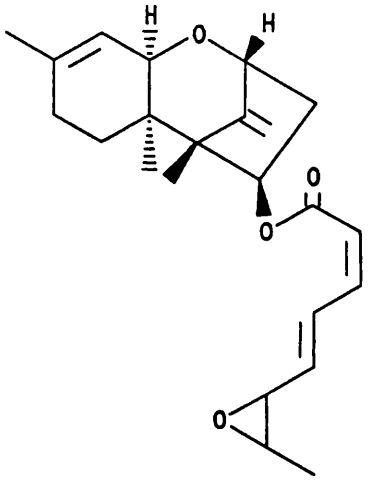


(32)

All but a few natural trichothecenes contain a spiro epoxide group at the 12,13-position, verrucarin K (33a) and 12,13-deoxytrichodermadiene (33b) both of which are products of *Myrothecium verrucaria* do not. The 11,12-ethers of which sporol (31) is an example also lack this important functionality. On the other hand crotocin (9) has the unusual feature of containing two epoxide groups.



(33a)



(33b)

As stated earlier, attempts at elucidating the trichothecene structure by classical chemical degradation proved unsuccessful with structures being determined by either X-ray analysis or by chemical interconversion. Indeed, this latter technique was instrumental in determining the structures of verrucarol (26)[19] and diacetoxyscirpenol (34)[22,23,24].

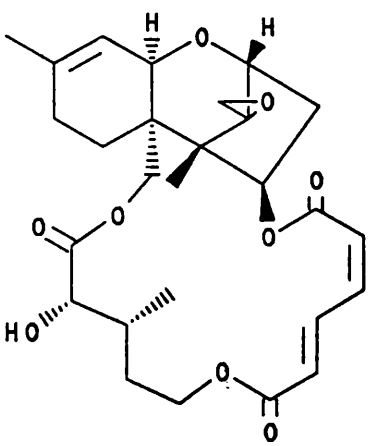
Today, however, due to advances in nmr technology and the rigidity of the trichothecene skeleton which furnishes characteristic chemical shifts and coupling constants as well as observable nuclear Overhauser effects, nmr spectroscopy is the method of choice for structure elucidation. The most characteristic features in the ^{1}H nmr spectra of trichothecenes are the signals for the methylene protons of C-13 which appear as an AB quartet (J ~ 4Hz) in the region 2.7 to 3.1 ppm. If C-15 is oxygenated, the C-15 methylene protons also appear as an AB quartet (J ~ 11-13 Hz) centred at 3.7 ppm as the free alcohol and

at 4.2 ppm when acylated.

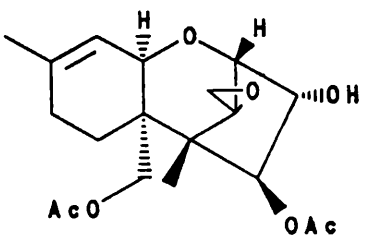
The flexibility of the macrocyclic chains limits the use of nmr in elucidation of their structure, hence X-ray analysis and chemical synthesis[25] are still frequently employed in this area.

1.3 Biology and structure activity relationships

The potent antibiotic activity exhibited by trichothecin stimulated much of the early interest in the trichothecenes. However, the cytotoxic activity shown by most trichothecenes has aroused the greatest interest, especially in the area of chemotherapeutic research. For example, verrucarin A (1) causes inhibition in the growth of mouse tumour cells at concentrations of 0.6ng/ml[26] and is thus one of the most potent cytostatic agents known. Anguidine (34) exhibits cytopathogenic effects against baby hamster kidney cells at a concentration of 1.5ng/ml[27] and has recently been the subject of phase I and II clinical trials by the U.S. National Cancer Institute against breast and colon cancer.



(1)

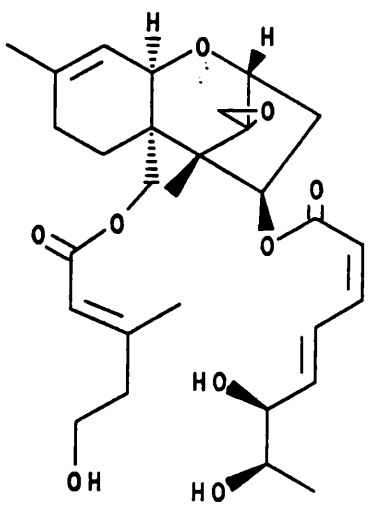


(34)

Unfortunately the trichothecenes, as with many cytostatic agents, are acutely toxic to whole animals. Verrucarin A (1) with an LD_{50} of 0.5mg/kg is one of the most toxic non-nitrogen containing natural products known.

The second group, the macrocyclic trichothecenes are characterised by a di- or tri-lactide linking the C4 and C15 hydroxyl groups. This group can be subdivided into the verrucarins such as verrucarin A (1), verrucarin B (2) and verrucarin J (20), the roridins such as roridin A (21), roridin D (22), and roridin H (23) and finally the baccharins such as baccharin B5 (24) and baccharene (25). Verrucarol (26) is the simple trichothecene most often encountered in the macrocyclic trichothecenes and also included in this group are vertisporin (27) and the satratoxins of which satratoxin F (28) is an example.

The third group, the trichoverroids[20] are structurally intermediate between the simple and the macrocyclic trichothecenes. They contain a side chain on C4 but they lack an ester link to C15 that would make them macrocycles e.g. trichoverrin B (29) and trichoverrol B (30). It would appear[21] however that the trichoverroids are in fact biogenetic precursors of the macrocyclic trichothecenes.



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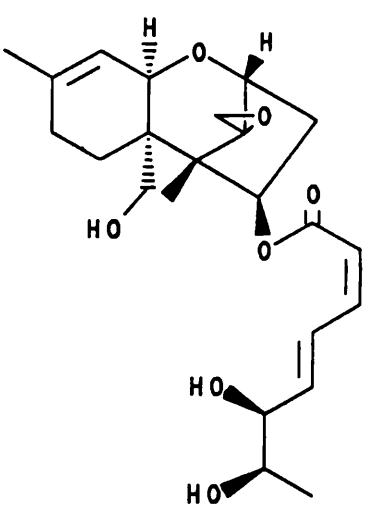


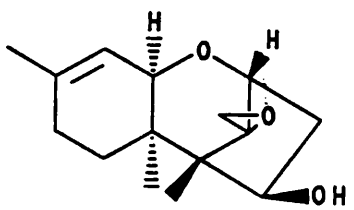
(30)

Studies have shown that both protein and DNA synthesis are inhibited by trichothecenes. It was initially thought that protein synthesis was inhibited only at the initiation stage; work by Tate and Caskey[28], Wei[29] and Hansen and Vaughan[30] indicated that inhibition can occur also at the elongation and termination stages of protein synthesis. The trichothecenes can be subdivided into three groups on the basis of the stage at which they inhibit protein synthesis. The groups are

1) I-type (initiation inhibitors) e.g. the verrucarins, the roridins and highly functionalised simple trichothecenes such as T-2 toxin (4) and deoxynivalenol (6)

2) E-type (elongation inhibitors) e.g. trichodermin (18) and trichothecin (3) i.e. C4 esters

3) T-type (termination inhibitors) e.g. trichodermol (35) and trichodermone (36), i.e., unsubstituted or C4 hydroxylated simple trichothecenes.



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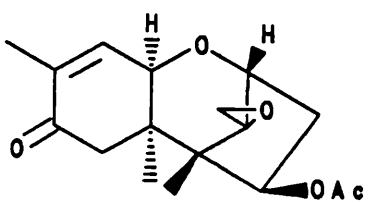
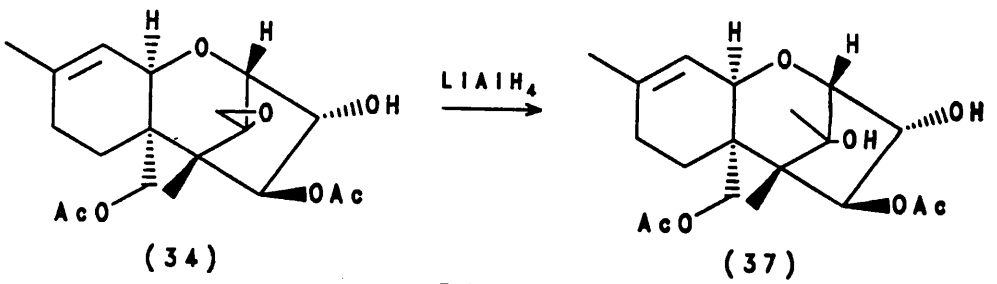


(36)

Evidence[31] suggests that the trichothecenes act as alkylating agents of the thiol residues in the enzyme peptidyl transferase suggesting that the above classification is steric in origin, that is the larger molecules act only at initiation when the ribosome is relatively unhindered by the nascent peptide

chain. As one would expect for a size based classification some degree of overlap is observed.

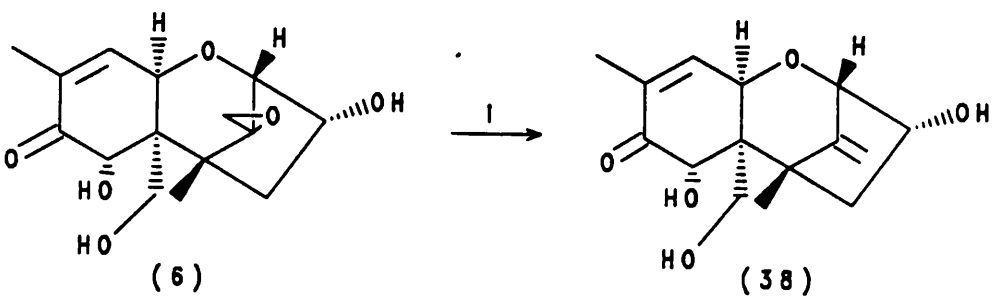
If the trichothecenes do act as biological alkylating agents then they must be susceptible to nucleophilic attack; the most obvious site for this is the spiro-epoxy function. Grove proved that the epoxide is essential for biological activity of the simple trichothecenes by showing[32] that compounds such as (37) (Scheme 2) where the epoxide has been destroyed are devoid of significant biological activity.



(34) (37)

Scheme 2

Furthermore, studies of the metabolism of deoxynivalenol (6) in rats <u>invivo</u>[33] and rumen micro-organisms <u>invitro</u>[34] have shown that deoxygenation of the epoxide to the exomethylene compound (38) (Scheme 3) is the major process and possibly a means of biological detoxification.

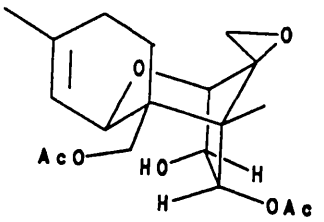


(6) (38)

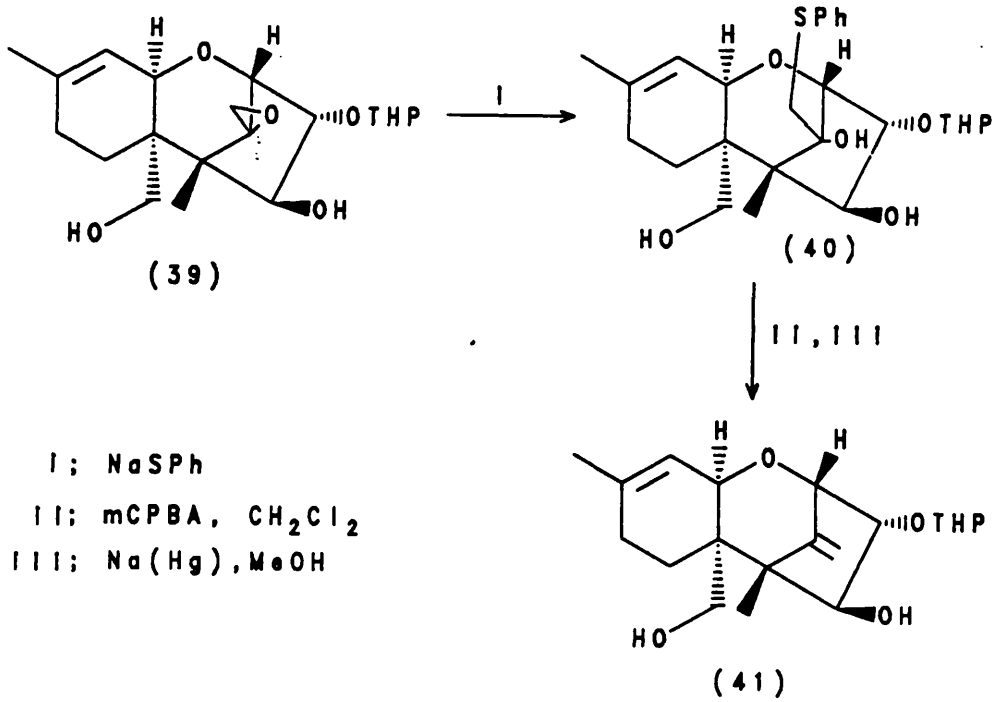
I ; Rumen Micro-organisms

Scheme 3

Examination of the trichothecene structure

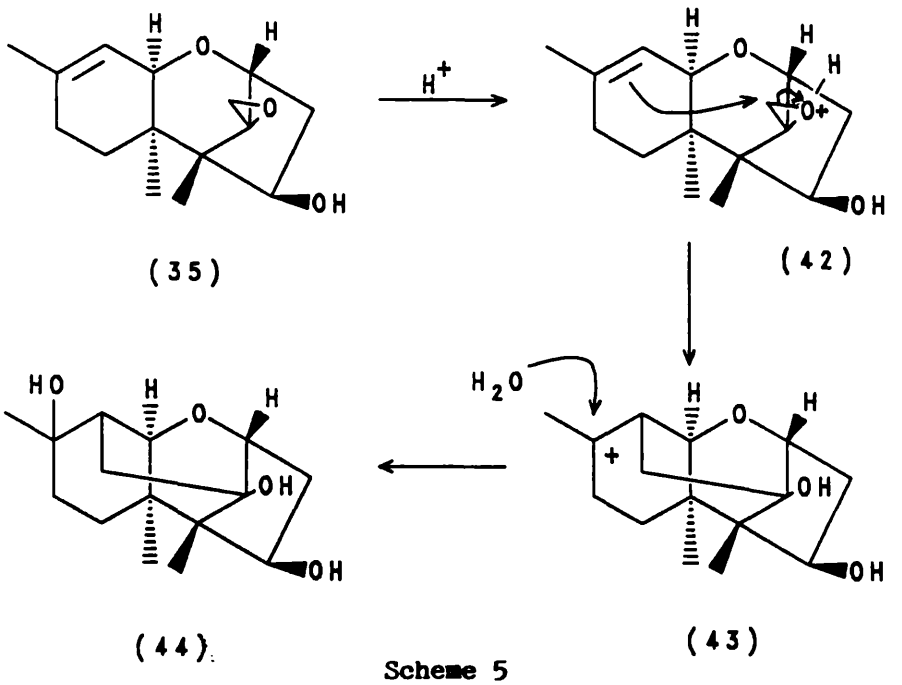


shows that the epoxide is extremely hindered on one side by rings A and B and on the other by the two carbon bridge of ring C; as a consequence bimolecular nucleophilic substitution reactions at the epoxide are very rare. Reduction of the epoxide to a tertiary alcohol e.g. (37) using lithium aluminium hydride[20] and opening the epoxide with sodium benzenethiolate in deoxygenation studies[35] (Scheme 4) appear to be the only noteworthy examples of S_N2 reactions on the epoxide. This suggests that such epoxide ring opening is not the mechanism of action.



Scheme 4

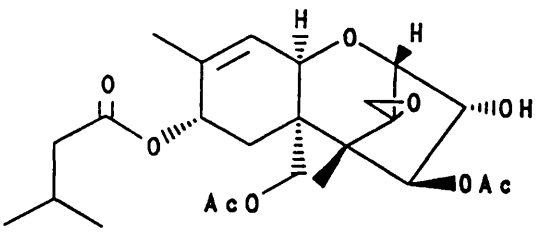
The behaviour of the trichothecenes in acidic media may provide further clues to their mode of action. In mildly acidic conditions, hydration results in the formation[36] of the 10,13-cyclotrichothecene skeleton (44) (Scheme 5). For this to occur the π system of the 9,10-double bond must attack the 12,13-epoxide intramolecularly forming a carbonium ion (43) which is much more accessible to nucleophiles than the 12,13-epoxide.



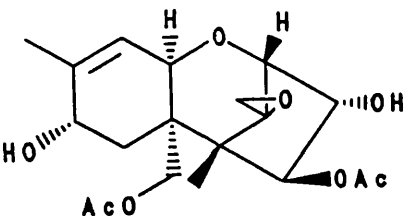
Scheme 5

Another possible mechanism for biological action is skeletal rearrangement to the apotrichothecene system as discussed in chapter 1.2. The apotrichothecene rearrangement occurs in strongly acidic media when the ring A double bond is protonated and therefore non-nucleophilic. Both these rearrangements yield more accessible electrophilic sites for external nucleophiles to attack. However, which if any of these inhibition mechanisms is operational has yet to be proven.

The biological activity of the trichothecenes is high when bulky ester substituents which enhance transport into the cell are present. Ueno[37] divided the trichothecenes into two broad classes A and B. Class A contained polyhydroxylated compounds such as T-2 toxin (4), anguidine (34) and neosolaniol (7), whereas class B contained 8-keto trichothecenes such as deoxynivalenol (6), nivalenol (5) and trichothecin (3). The activity of class A compounds is greater than class B compounds in whole cell preparations while in cell free preparations the trend is reversed, indicating the importance of transport for high activity.



(4)



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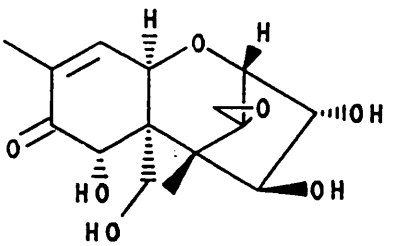
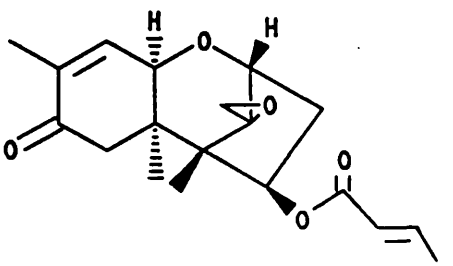


(5)

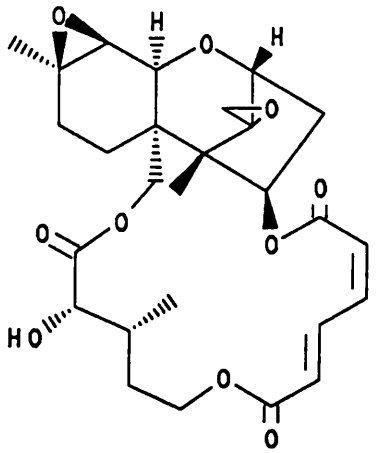


(3)

The 9-ene function is also important since catalytic hydrogenation of the trichothecenes results in a substantial loss of activity[38].

The 9β,10β–epoxybaccharinoids exhibit the greatest <u>invivo</u> activity of all known trichothecenes and indeed the 9β,10β-epoxy

derivatives (45) and (46) of verrucarin A and verrucarin B exhibit significantly higher activity than the original verrucarins[39].



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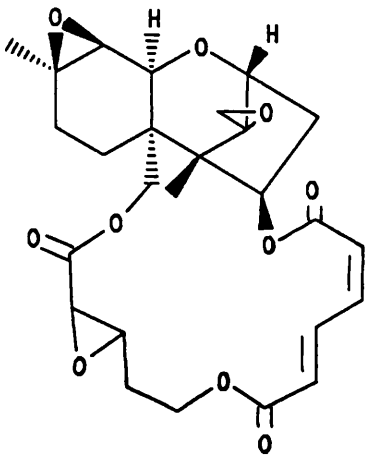
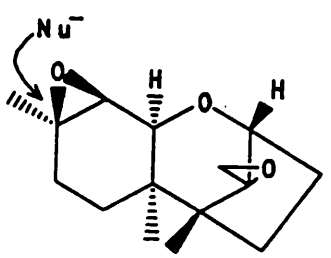


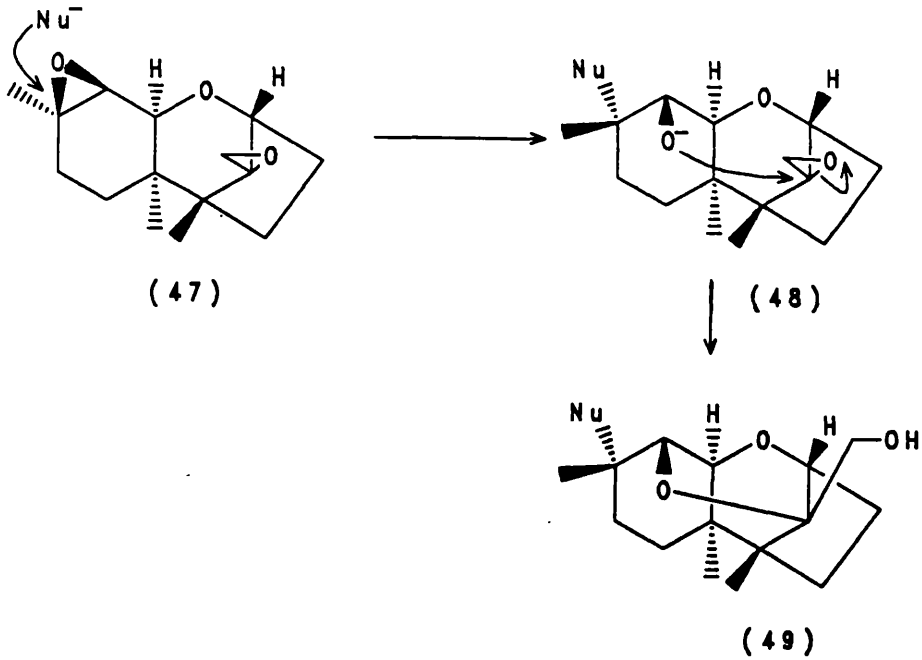
(46)

This finding prompted Grove to investigate[40] the susceptibility of 9β,10β-epoxide derivatives of the non-macrocyclic trichothecenes to nucleophilic attack. He postulated that the delay between administration of the trichothecenes and the first manifestations of toxicoses could be due to the formation of intermediates, possibly 9β,10β-epoxide derivatives (47) formed by the action of a mixed function oxidase on the 9-ene. Alkylation of the thiol residues of peptidyltransferase could be envisioned to occur by



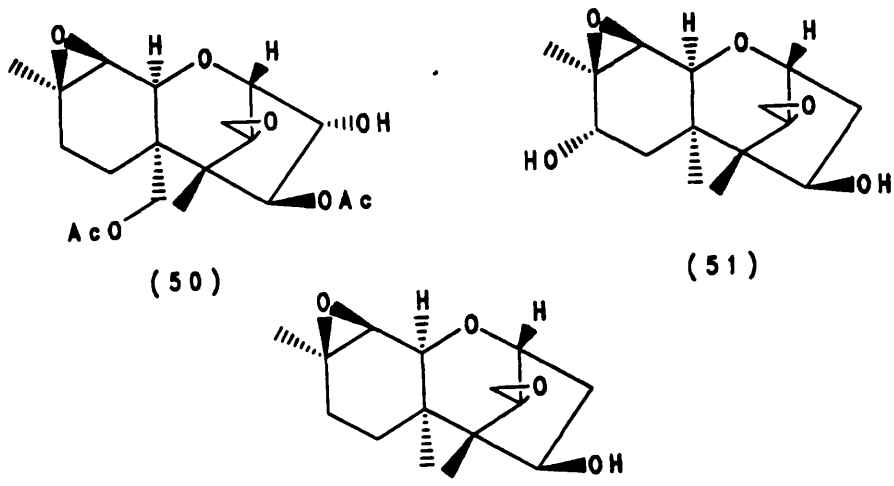
(47)

nucleophilic opening of 9,10-epoxide with concomitant opening of the 12,13-epoxide to give a 10β epoxytrichothecene (49) (Scheme 6).



Scheme 6

His studies showed however that epoxides such as (50), (51) and (52) were susceptible only to internal nucleophilic attack or did not react at all as in the case of the trichodermol derivative (52). Hence, invivo epoxidation is not a mechanism of action of the non-macrocyclic trichothecenes; indeed the 9β,10β-epoxide

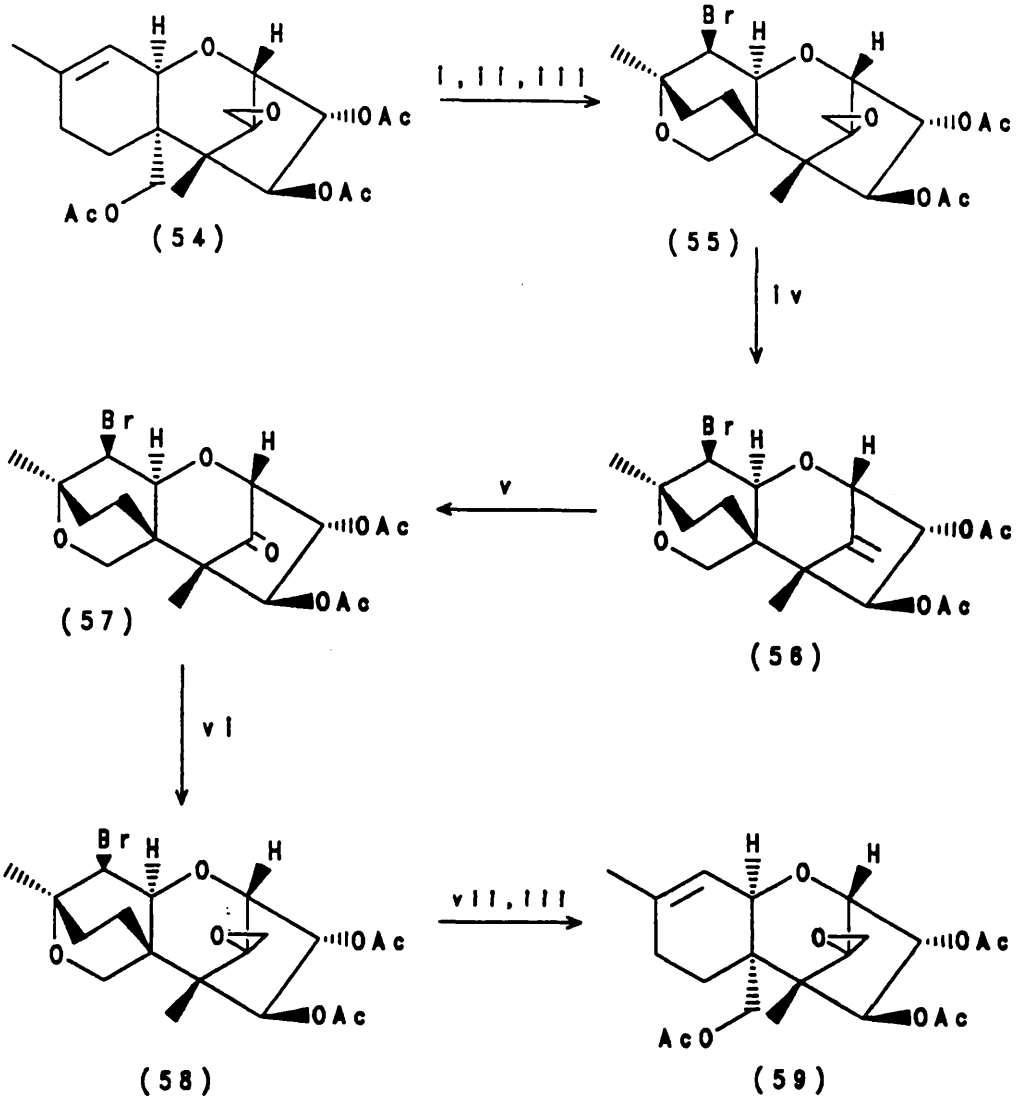


(50) (51)

(52)

derivative (50) of anguidine was less active[41] in a protein inhibition assay than anguidine itself.

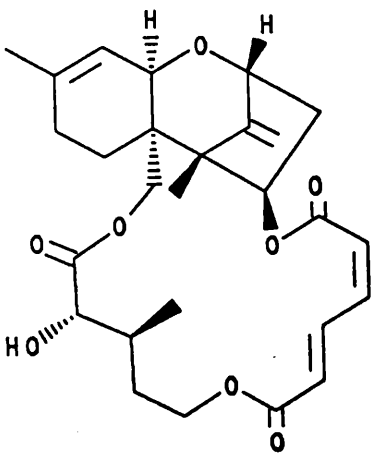
Examination of the mechanism of both the apotrichothecene and the 10,13-cyclotrichothecene rearrangements suggest that not only is the presence of the 12,13-epoxide group essential for biological activity but its stereochemistry must also be important. If the stereochemistry of the epoxide were reversed then the above mechanisms could not occur. Indeed the epi-epoxytrichothecene (59) formed[42] from triacetoxyscirpenol (54) (Scheme 7) retained only 0.4% of the biological activity of (54). The key step in this interconversion was application of the lower valent tungsten deoxygenation system of Sharpless[43] which in one step selectively removed the epoxide oxygen of (55). The epi-epoxide (58) was obtained by treatment of (57) with dimethylsulphonium methylide which attacks the ketone exclusively from the less hindered exo-face of the bicyclo[3,2,1] octane subunit, a fact discovered by Raphael and Colvin during their synthesis[44] of trichodermin (18).



I ; MeOH, K_2CO_3
II ; NBS/CH_3CN
III; Ac_2O/Py/Et_2O
IV; WCl_6/nBuLI/THF
V ; O_3, CH_2Cl_2/Et_3N
VI ; $Me_2\overset{+}{S}\overset{-}{C}H_2$/THF
VII; Zn(Ag)/THF

Scheme 7

Other structural features important for biological activity include a β-configuration of substituents at C4. In the macrocyclic series change of attachment of the macrolide ribbon from C4 to C3 results in complete loss of activity. It is possible that the macrolide ribbon also has cytostatic properties; verrucarin K (33a) with no epoxide group, is still cytotoxic.



(33a)

In conclusion, although much is known of the biology and structure activity relationships of the trichothecenes their mode of action remains unclear.

1.4 Syntheses

Their interesting structures have made the trichothecenes the synthetic targets of a number of groups. The number of chiral centres, at least six, and the varied oxygenation patterns encountered in the trichothecenes have provided formidable challenges to organic chemists over the last two decades.

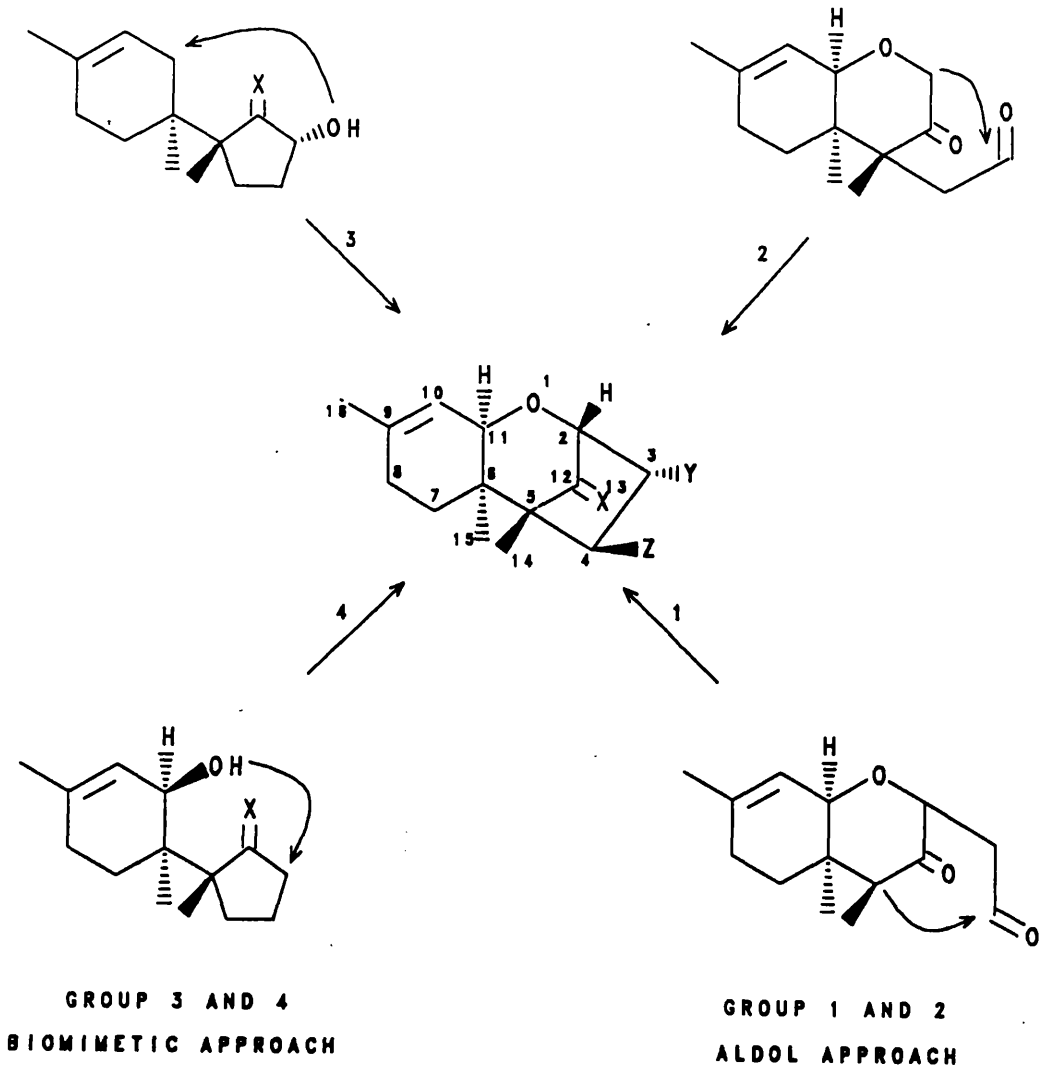
A large number of trichothecenes are available only in submilligram quantities from culture broths; this makes total synthesis and possibly more importantly partial synthesis a necessity for studying their biological profiles.

The salient features of the trichothecene skeleton which have to be overcome for a successful synthesis are

1) Cis fusion of rings A and B
2) Formation of the two carbon bridge of ring C
3) Stereospecific installation of the spiro-epoxide
4) Stereospecific introduction of any hydroxyl functions

Several papers have been published on the synthesis of the macrocyclic and the non-macrocyclic trichothecenes as well as trichothecene models. Since excellent reviews[25,45,46] have been published, and in the interest of brevity and relevance to this thesis, I will review only the syntheses of the non-macrocyclic trichothecenes.

McDougal and Schmuff[45] sub-divided the syntheses of non-macrocylic trichothecenes according to whether the tricyclic skeleton was formed via an aldol or biomimetic approach (Scheme 8).

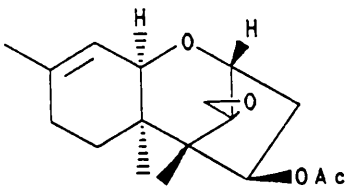


Scheme 8

If the tricyclic nucleus is constructed by formation of bond 1 or 2 then the synthesis belongs to the aldol approaches, groups 1 and 2 respectively. If it is constructed by formation of bond 3 or bond 4 then the synthesis belongs to the biomimetic approaches, groups 3 and 4 respectively.

The first successful synthesis[44] of a trichothecene was of trichodermin (18) and it was achieved by an aldol approach. This approach has also accessed the trichothecene skeleton of 12,13-epoxytrichothec-9-ene (14)[47] and calonectrin (60)[48] as well

as providing the basis of the synthesis[49] of an optically active synthon for calonectrin and of an approach to deoxynivalenol (6)[50].



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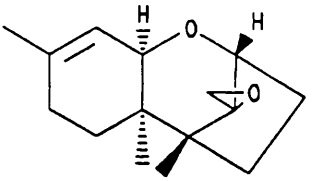

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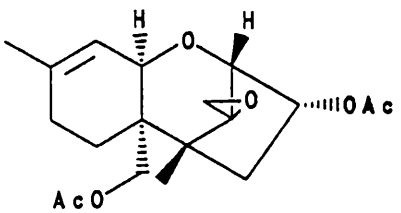

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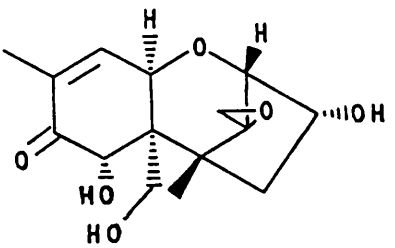
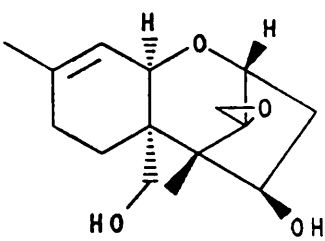


(6)

Syntheses employing a biomimetic approach have received much more attention. 12,13-Epoxytrichothec-9-ene (14) was the first molecule to succumb to total synthesis[51] by this route. Other trichothecenes which have been synthesised by this biomimetic methodology include trichodermin (18)[52], verrucarol (26)[53,54,55], anguidine (34)[56], as well as the 11-epi derivative (61)[55], the 1,3-dioxolane compound (62)[57] and neosporol (63)[58].



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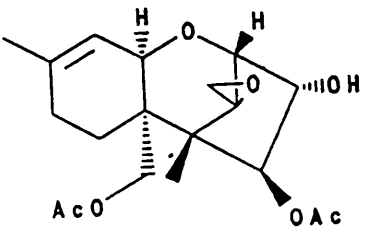

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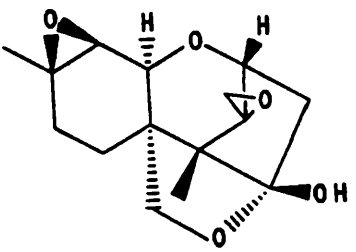

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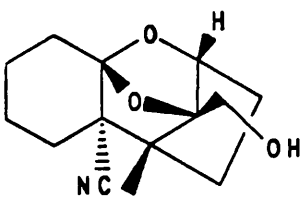

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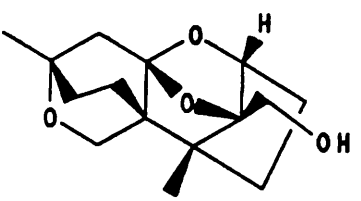


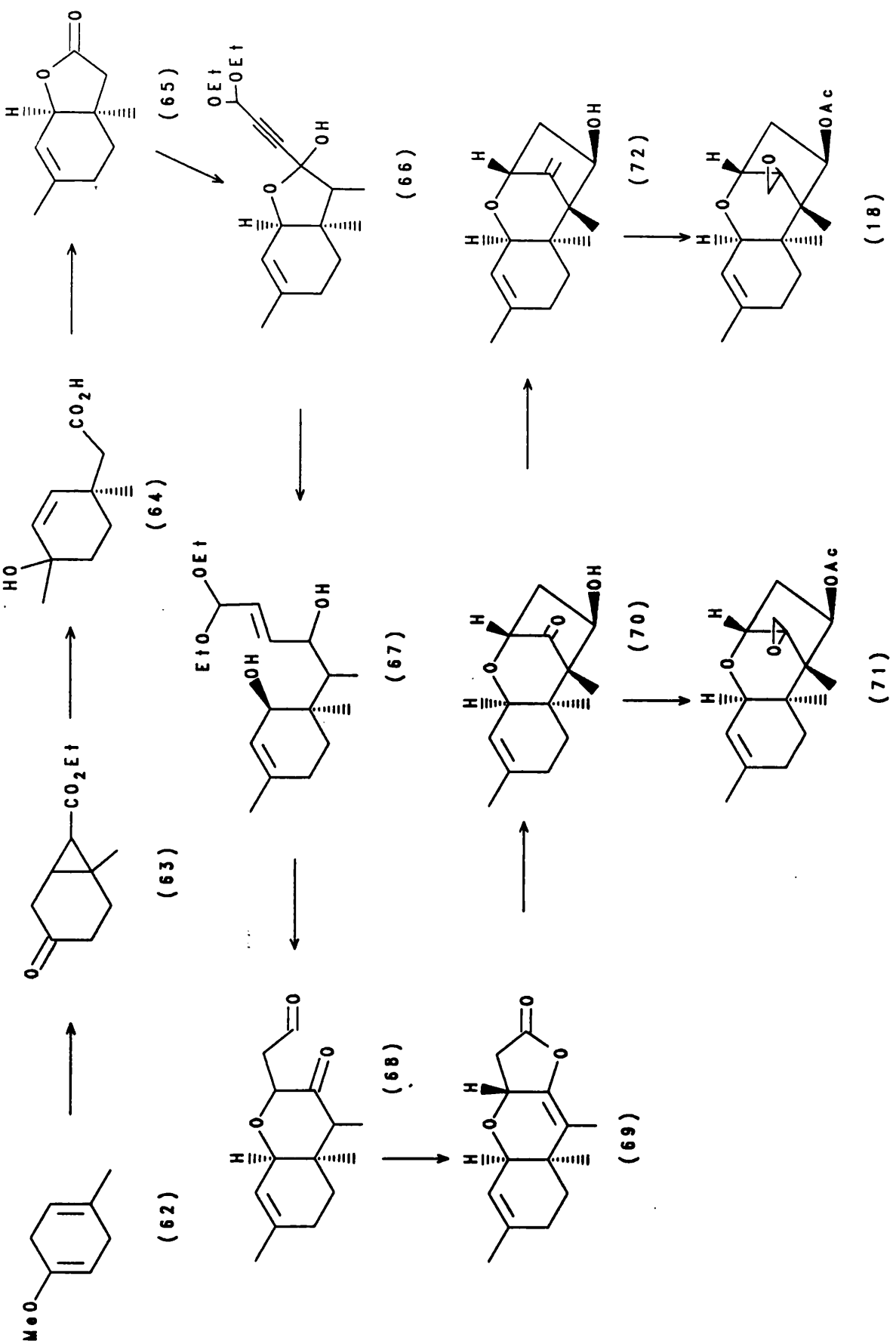
(63)

Group 1 - Aldol Approach

The first trichothecene to yield to total synthesis was trichodermin (18)[44] employing a group 1 aldol approach (Scheme 9). The problem of attaining cis fusion of the cyclohexene and pyran ring was addressed at the earliest possible stage by the synthesis of the cis-fused γ lactone (65). Ring expansion of the lactone ring would ensure cis fusion of the A and B rings of trichodermin.

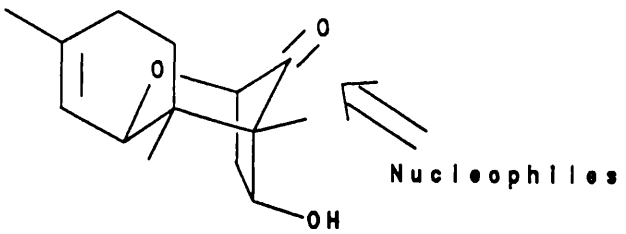
p-Methoxy toluene, the starting material was readily converted into the allylic alcohol (64), acidification of which effected lactonisation to the thermodynamically favoured cis fused γ lactone (65) presumably via an allylic carbonium ion. Having achieved the first major goal of the synthesis attention was turned to elaboration of the lactone to the ketoaldehyde (68) which it was anticipated would undergo an intramolecular aldol cyclisation to furnish the tricyclic skeleton. The pyran ring was introduced via the hemi-acetal (66) which was readily transformed into the trans-acetal (67). Mild acidic hydrolysis of (67) also induced conjugate addition to give a cis fused bicyclic intermediate, selective oxidation of which gave the required keto-aldehyde (68). However, despite much experimentation, keto-aldehyde (68) could not be induced to undergo aldol cyclisation. This problem was circumvented by formation of the enol lactone (69), reduction of which with $LiAl(OBu^t)_3H$ gave the desired aldol product (70), albeit in low yield.

It was thought that treatment of (70) with dimethyl sulphonium methylide would yield trichodermol(18). However the sole product of this reaction was 12,13-epi-trichodermol (71) indicating that the reagent had added to the carbonyl group



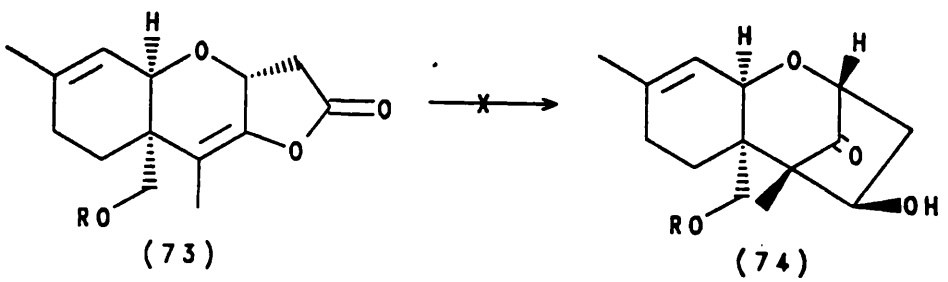
Scheme 9

exclusively from the less hindered face of the bicyclo[3,2,1]octane subunit.



This problem was overcome by Wittig olefination of the ketone to give the exo-methylene compound (72), which by analogy with the epoxidation of the ketone(70), gave on treatment with mCPBA the correct epoxide stereochemistry. Epoxidation occurred regio- selectively, the free C4 alcohol directing the per-acid preferentially to the exo-methylene and not to the ring A olefin. Acetylation of the C4 hydroxyl completed this historic synthesis.

Colvin and Raphael attempted[59] to extend this group 1 aldol approach to verrucarol (26), however the corresponding enol lactone (73) (Scheme 10) defied all attempts to induce reductive cyclisation to give the verrucarol precursor (74).



Scheme 10

This result and the low yield of the crucial cyclisation in the trichodermin synthesis make this approach the least efficient route to the trichothecane skeleton.

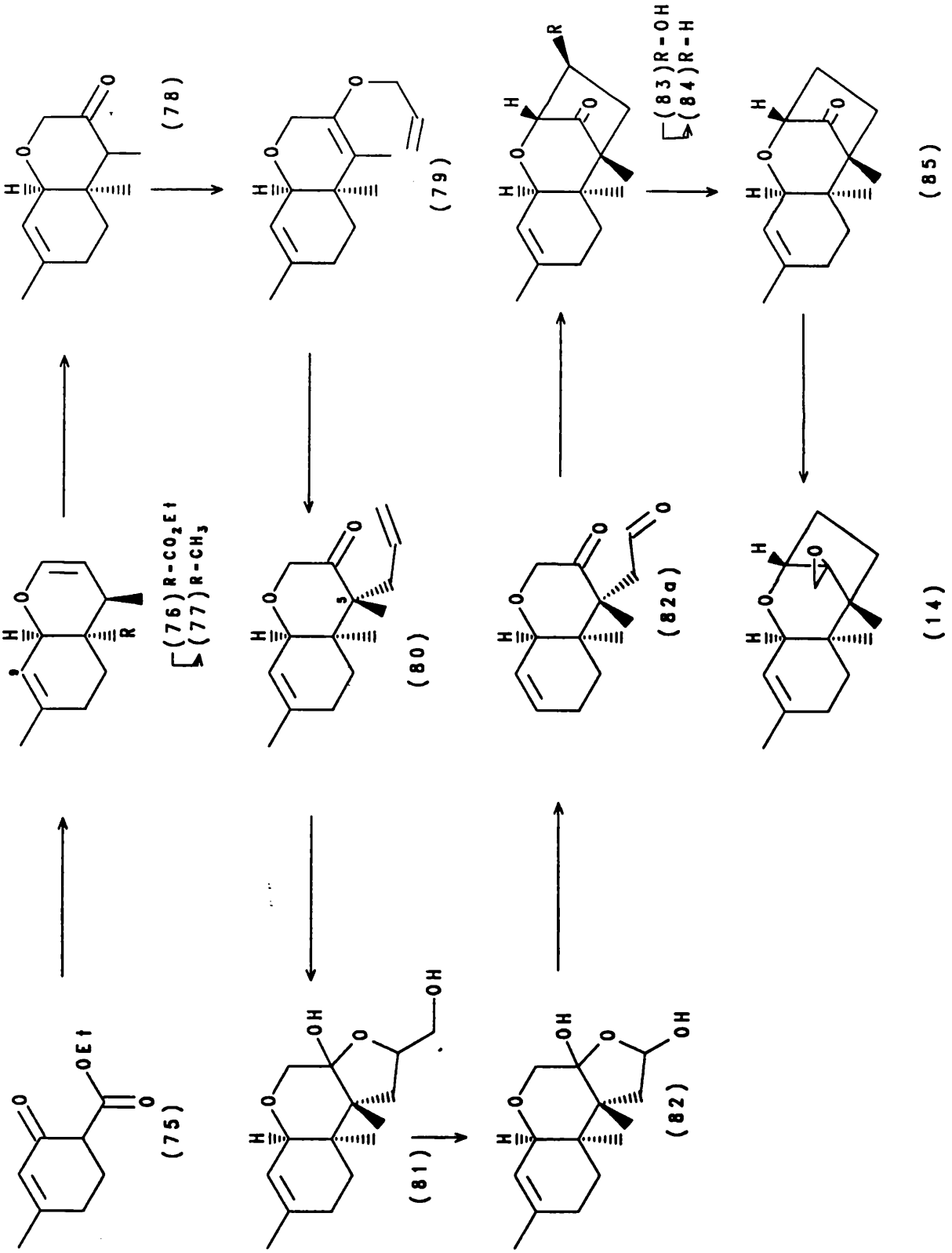
Group 2 - Aldol Approach

The group 2 aldol approach was demonstrated to be a much more efficient route to the trichothecane skeleton by the stereoselective synthesis[47] of the *Trichotheceum roseum* metabolite (±)-12,13-epoxytrichothec-9-ene (14) (Scheme 11).

Cis fusion of the A-B rings was achieved via the pyran (76) derived from the readily available keto-ester (75). Conjugate addition of the anion of (75) to crotonaldehyde followed by protection of the aldehyde as the ethylene acetal and subsequent Meerwein Pondorf reduction resulted in cyclisation to the benzopyran derivative (76); this was shown to be cis fused by comparison of the coupling constants for the C9 proton of (76) with the corresponding (C10) proton of the target (14). Attention then turned to construction of ring C.

Alkylation of the enolate of (78) with allyl bromide gave surprisingly the allyl vinyl ether (79) which underwent Claisen rearrangement to the required allyl-ketone (80), fortuitously as a 2:1 mixture of α and β allyl epimers respectively. Thus the required stereochemistry of C5(trichothecene numbering) had been established stereo-selectively and without the possibility of epimerisation. Oxidative cleavage of the vinyl group using osmium tetroxide and sodium periodate gave the monohydrate (82) which on treatment with sodium methoxide in methanol cyclised to the tricyclic structure (83) as a mixture of C3 epimers in an excellent yield of 90%.

The ketone (85) gave exclusively the wrong epoxide stereochemistry on treatment with dimethylsulphonium methylide in analogy to the trichodermin results. Wittig olefination and epoxidation gave the required compound (14). However the

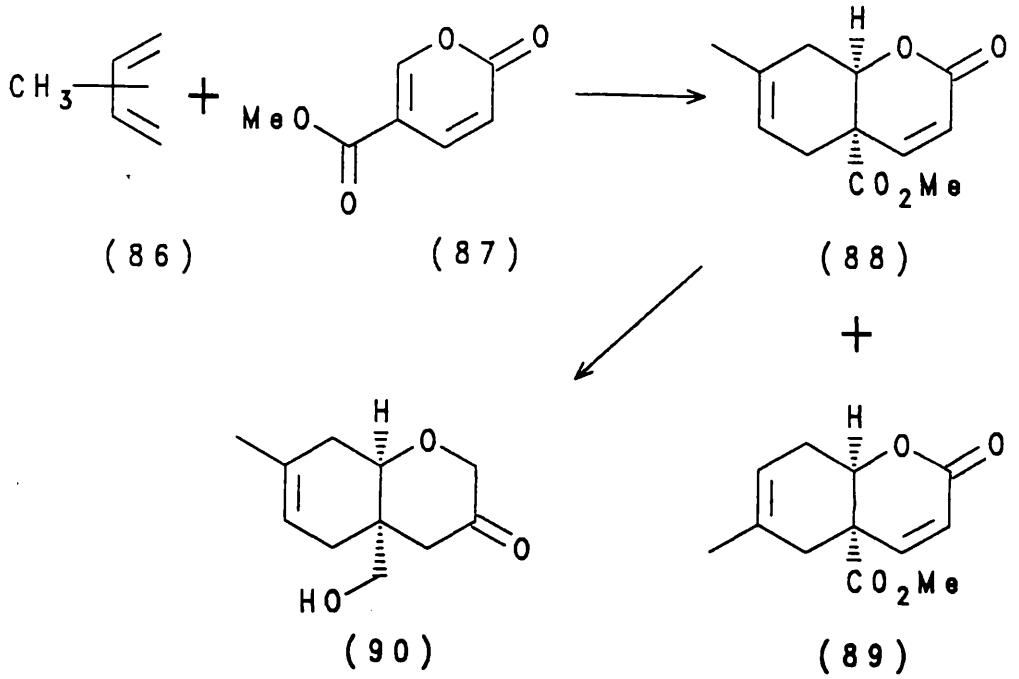


Scheme 11

epoxidation was inefficient (30% with 60% conversion) due to a lack of regioselectivity, a problem that would beset future trichothecene syntheses.

In the synthesis of calonectrin (60)[48], Kraus addressed the problem of forming the crucial B-C junction stereoselectively and also provided novel solutions to the problems of cis fusion of the A and B rings and regioselectivity in epoxidation.

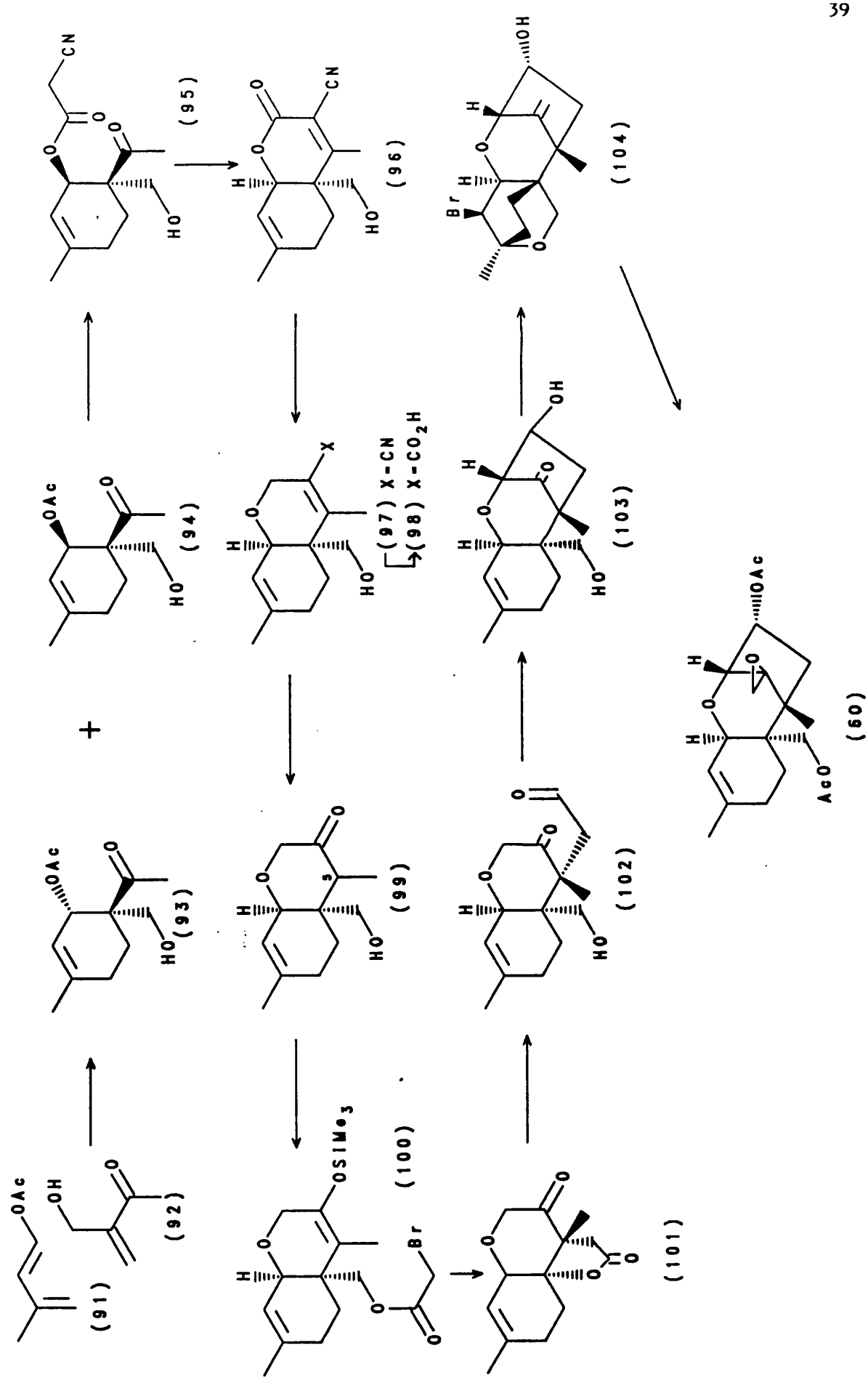
In addressing the cis fusion problem Kraus focussed his attention on a reaction that would give in one step the cis fused bicyclic system with the functionality necessary for elaboration to the AB ring system of verrucarol, namely, Diels Alder cycloaddition. In an earlier approach to verrucarol Kraus found[60] that cycloaddition between isoprene (86) and methyl coumalate (87) under either catalysed or thermal conditions produced an 85:15 mixture of regio-isomers (88) and (89) (Scheme 12) in favour of the desired isomer (88). This synthon for the AB rings of verrucarol could be transformed in 8 steps and 10% yield to the ketone (90) which still required introduction of the 2 carbon bridge, isomerisation of the trisubstituted ring A olefin and formation of the spiro epoxide to complete the synthesis.



Scheme 12

For calonectrin Kraus once again employed[48] a Diels-Alder reaction, however the diene and dienophile were 1-acetoxy-3-methyl butadiene (91) and 3(hydroxymethyl)-3-buten-2-one (92) respectively as in an alternative approach[61] to verrucarol. Lewis acid catalysed cyclo-addition afforded a 3.5:1 diastereo-isomeric mixture of acetoxy-ketones (93) and (94) (Scheme 13). The major adduct (94) not only had the required cis relationship of the acetoxy and methyl ketone for elaboration to ring B, but also had the ring A olefin in the correct position thus eliminating a potentially troublesome olefin isomerisation.

Ring B was introduced by an intramolecular Knoevenagel condensation of the cyano-ester (95) followed by a two step reduction of the lactone to the pyran (97). The cyano group was converted into the acid (98) which underwent a Curtius rearrangement to give the desired ketone (99). Elaboration of this to the keto-aldehyde (102) required a two carbon unit to be

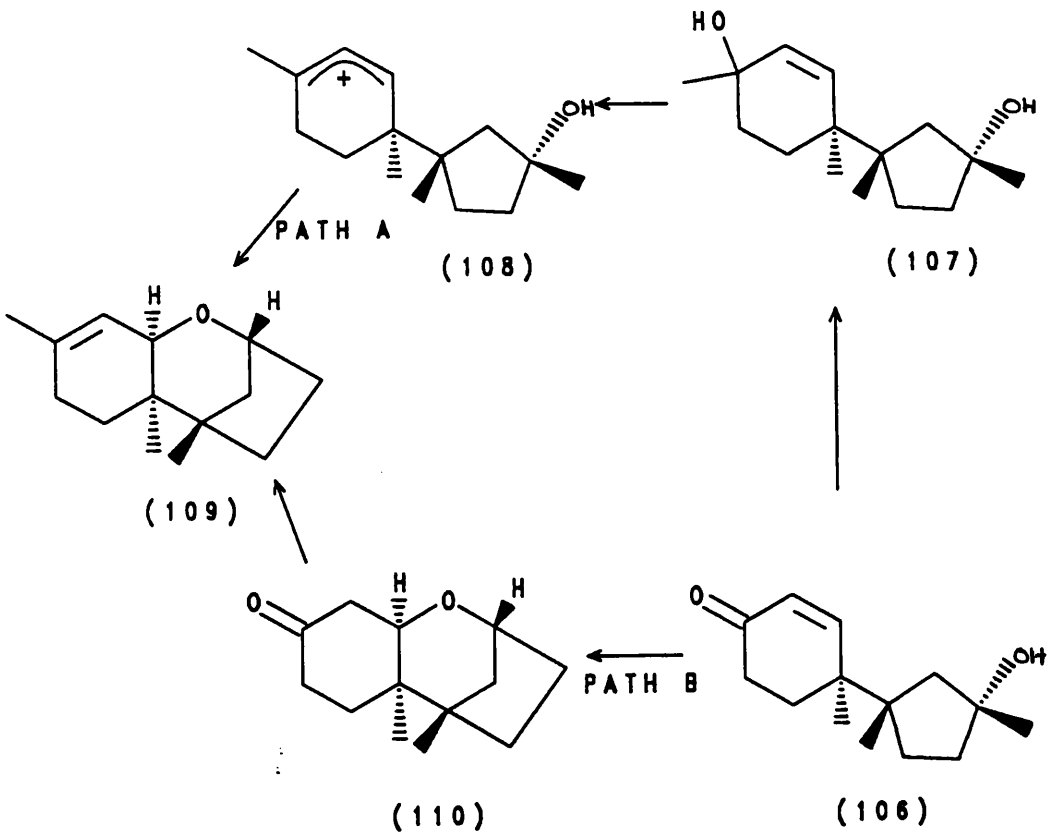


Scheme 13

attached to C5(trichothecene numbering) in the α configuration. Kraus controlled this stereochemistry by an ingenious intramolecular alkylation. The silyl enol ether (100) on treatment with TBAF gave the lactone (101), intramolecular alkylation having occurred at C5 exclusively from the α face. Reduction gave the keto-aldehyde (102) which underwent aldol cyclisation to the tricyclic skeleton as a mixture of C3 epimers. Wittig olefination and an oxidation-reduction sequence provided the correct α configuration at C3. Finally Kraus achieved complete regioselectivity in the epoxidation reaction by protecting the ring A olefin as the bromo-ether (104) and epoxidation of the exomethylene with buffered CF_3CO_3H. Regeneration of the C-9 olefin and acetylation completed this highly efficient and stereoselective synthesis of calonectrin (60).

Group 3 - Biomimetic Approach.

As stated earlier the biomimetic approach and in particular the group 3 approach has received much more attention from synthetic chemists. Cyclisation of the trichodiene analogues (106) and (107) can take place in either of two ways (Scheme 14).



Scheme 14

If cyclisation occurs via an allylic carbonium ion (108) (108) (path A) then the trisubstituted olefin of ring A is produced directly. If on the other hand cyclisation proceeds via a Michael addition (path B) then the ketone at C-9 must be transformed to the C9 olefin. Hence path A which can also start from the enone (106) is regarded as the route of choice. Obviously control of the relative stereochemistry at the two

quaternary centres C5 and C6 is crucial to a successful synthesis.

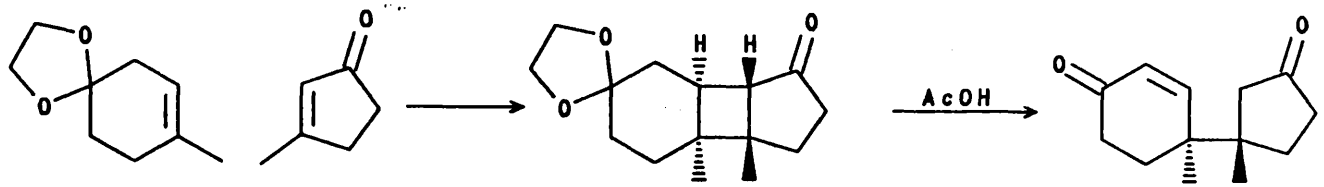
The synthesis[51] of 12,13-epoxytrichothec-9-ene (14) by Masuoka was the first successful group 3 approach to a trichothecene. A model study[62] (Scheme 15) had shown that the diol (111) cyclised stereoselectively to the trichothecanoid skeleton (112) in a similar manner to that proposed for the biosynthesis. Likewise, the diol (120) (Scheme 16) on treatment with acid cyclised in 40% yield to the tricyclic skeleton (121).

The crucial stereochemical control at C5 and C6 was achieved by a [2+2] photo-addition of (113) and (114) which gave as the major product (16%), the cis, anti, cis tricycle (115). This on acid cleavage opened cleanly to the ene dione (116) having the correct relative configuration at C5 and C6. Elaboration to the epimeric alcohols (117) and (118) revealed an interesting result. The α allylic alcohol (118) cyclised by Michael addition to the enone to give the ketone (119) whereas the more abundant β allylic alcohol (117) was recovered unchanged from the reaction mixture. This demonstrated that this approach also requires control of C2 stereochemistry for biomimetic cyclisation to occur. Inversion of the alcohol (117) and Grignard addition to the enone gave the required diol (120) for cyclisation to the tricyclic skeleton.

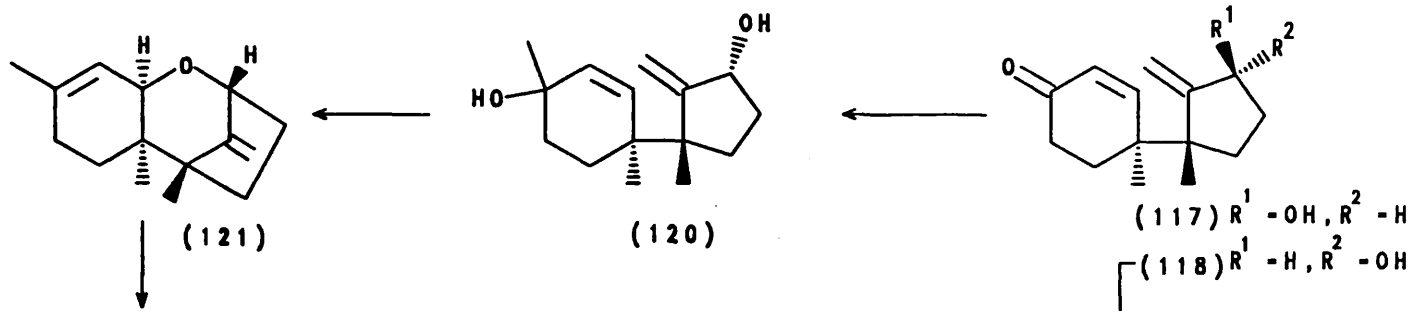
Still's synthesis[52] of trichodermin (18), in which he utilised an ingenious cyclo-addition, ring contraction and fragmentation sequence to provide the crucial intermediate (127)

(111) (112)

Scheme 15



(113) (114) (115) (116)



(117) R^1 -OH, R^2 -H

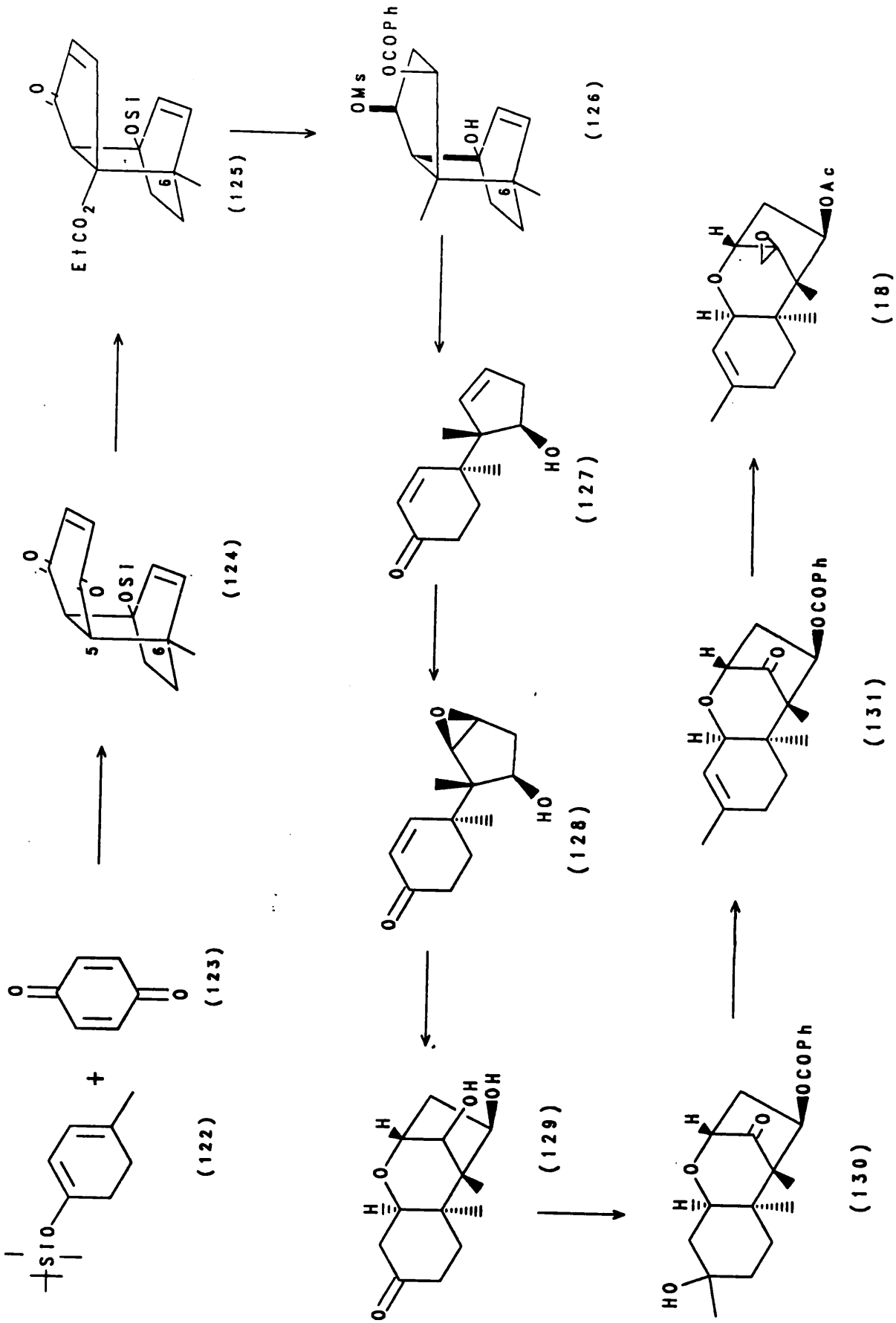
(118) R^1 -H, R^2 -OH

(119) (120) (121) (14)

Scheme 16

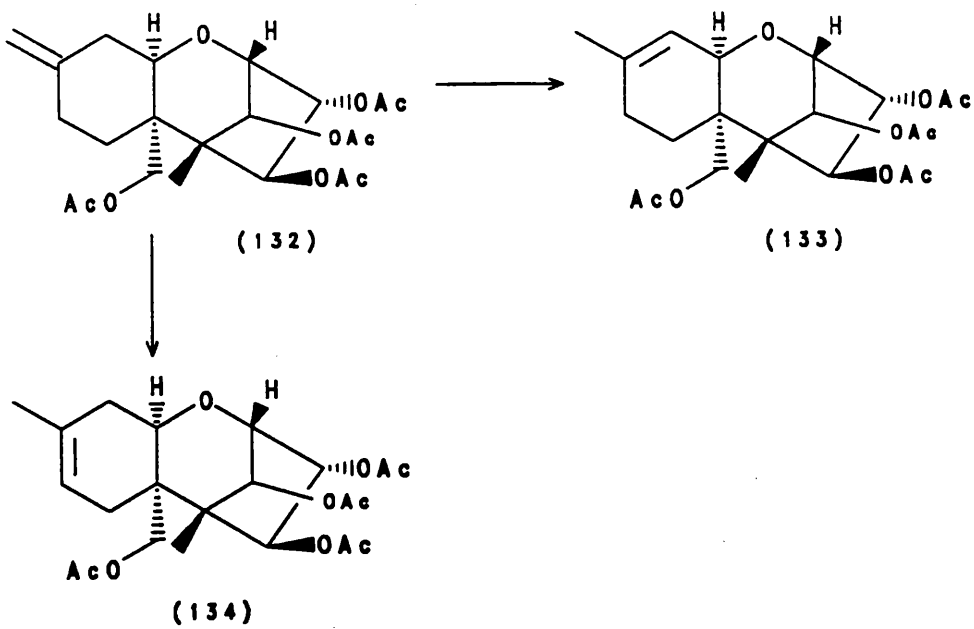
was the second reported synthesis employing a biomimetic approach.

The starting point of the synthesis (Scheme 17) was Diels-Alder reaction of quinone (123) with the silyloxydiene (122) to give the crystalline adduct (124) which possessed the correct relative stereochemistry at C5 and C6. Ring C was introduced by epoxidation and sodium hydroxide induced Herz-Favorski ring contraction which proceeded regiospecifically to the cyclopentenone carboxylic ester (125). This was transformed into the mesylate (126) in 8 steps, including a photochemical deoxygenation, to provide the C5 methyl group. This compound had the required anti-periplanar arrangement of the mesylate and the bond indicated to allow clean anionic fragmentation on treatment with sodium hydride to the alcohol (127) after debenzoylation. Introduction of the C2 α alcohol, necessary for cyclisation, was achieved by a C4 hydroxyl-directed epoxidation of the cyclopentene to give the β epoxide (128). This on exposure to aqueous acid cyclised to the tricyclic skeleton (129) almost certainly via the C2 α alcohol formed by regioselective opening of the epoxide. Elaboration of (129) to trichodermol was achieved without difficulty. One interesting feature in the completion of the synthesis was the dehydration of the keto-alcohol (130) to the required 9-ene (131) and its 8 -ene regioisomer in a 7:1 ratio. Zeigler in an approach[63] to anguidine (34) required isomerisation of the exo-methylene double bond of (132) to the 9-ene (133) (Scheme 18). However the only product isolated was the 8-ene (134). It is apparent therefore that dehydration of (130)



Scheme 17

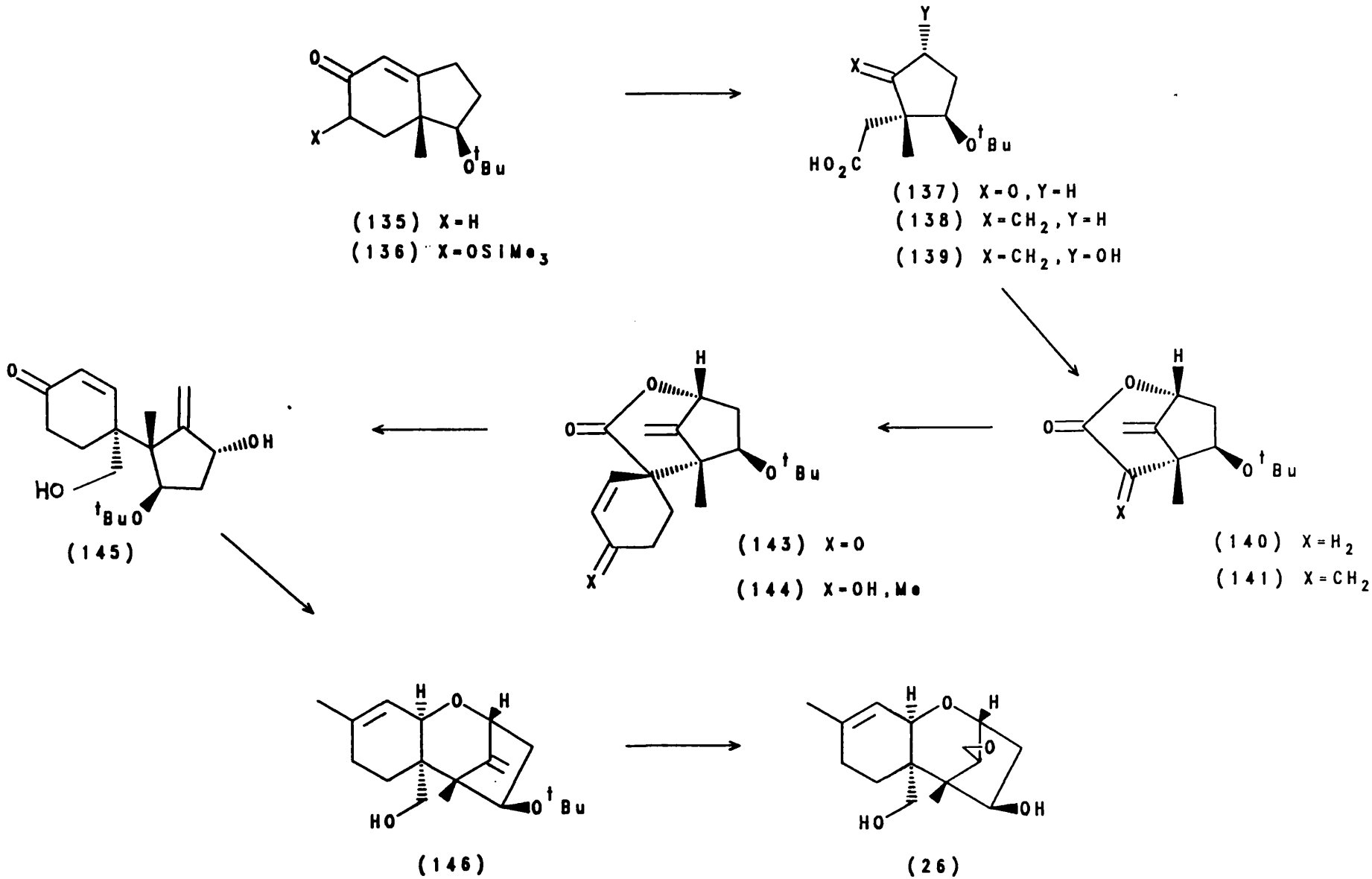
and isomerisation of (132) do not proceed via the same intermediate tertiary carbonium ion.



Scheme 18

Zeigler concluded that one of the reactions may be kinetically controlled while the other is thermodynamically controlled. It has yet to be established which of the two endocyclic olefins is more stable.

Schlessinger and Nugent controlled[54] the stereochemistry of the critical centres in their synthesis of verrucarol (26) by stereoselective construction of the spiro-lactone (144) (Scheme 19). This on aluminium hydride reduction opened to the trichodiene analogue (145) containing the correct stereochemistry for acid catalysed cyclisation via an allylic carbonium ion to the verrucarol precursor (146).


(135) X=H
(136) X=OSIMe3
(137) X=O, Y=H
(138) X=CH2, Y=H
(139) X=CH2, Y=OH
(140) X=H2
(141) X=CH2
(143) X=O
(144) X=OH, Me
(145)
(146)
(26)

Scheme 19

The starting point of the synthesis was the readily available ketone (135) which already contained two features required for verrucarol, namely the C4 hydroxyl and the C14 methyl group, i.e., the relative stereochemistry requiredfor C5 of verrucarol was already in place. Six synthetic steps gave the keto-acid (137). The α allylic alcohol was introduced by Wittig olefination followed by allylic oxidation. Lactonisation of (139) and reaction of the derived lithium enolate with formaldehyde gave directly, the enone (141) a key intermediate in the synthesis.

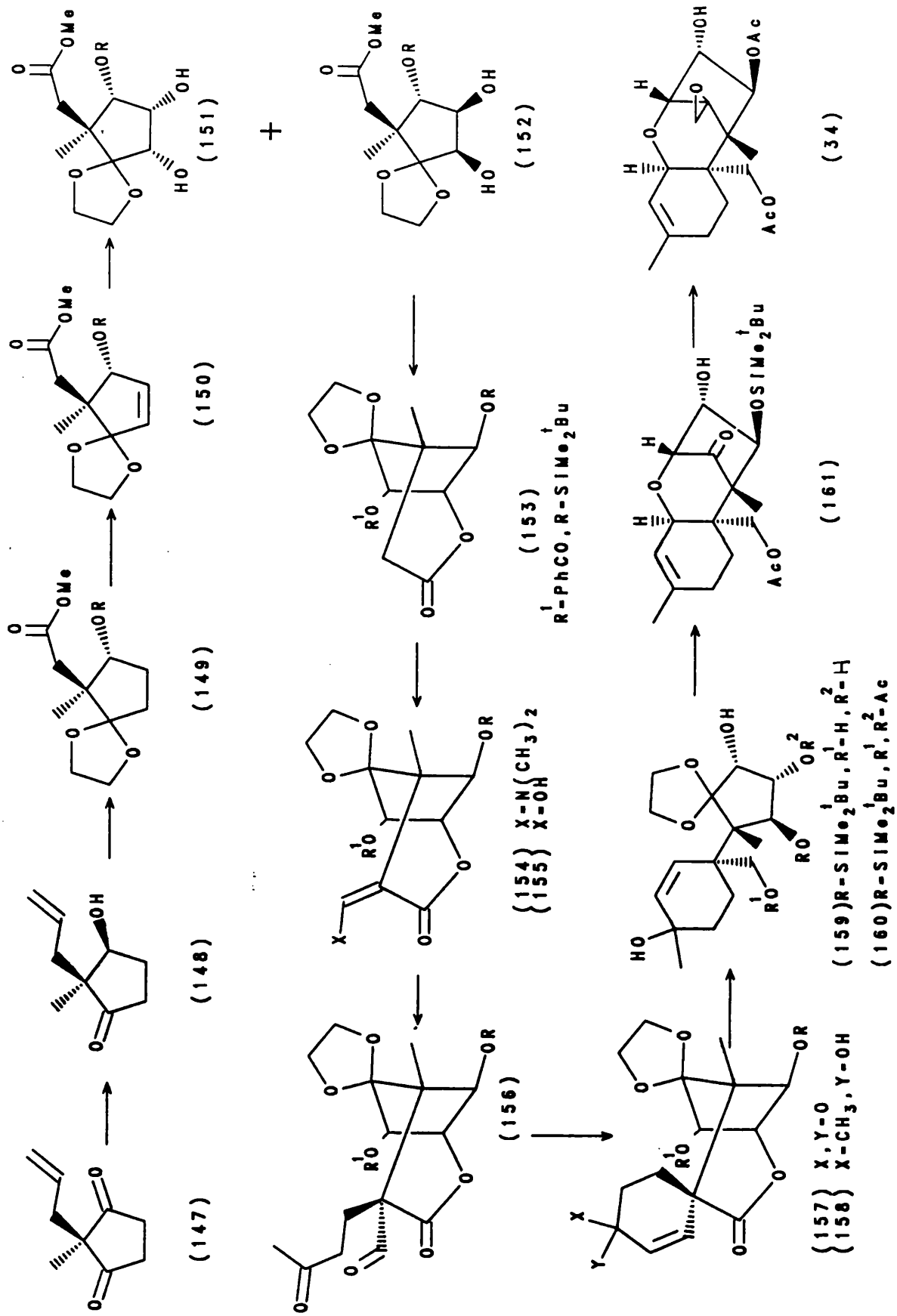
Schlessinger envisioned that [4+2] cycloaddition of (141) with Danishefsky's diene (142) would occur exclusively from the less hindered β face of the lactone to afford the unsaturated ketone (143). Ketone (143) was indeed formed as the sole product in 76% yield thus constructing the C6 junction with high stereo-control. Lactone (144) on $LiAlH_4$ reduction opened to the trichodiene analogue (145) which on exposure to pTSA cyclised to the trichothecene skeleton (146), which was readily converted into verrucarol.

Brook achieved two notable firsts with a synthesis of anguidine (34)[56]; not only was this the first synthesis of a trihydroxylated trichothecene, but it was also enantioselective.

The synthetic plan (Scheme 20) was to add the A ring to a fully functionalised C ring and from there construct the B ring by an intramolecular cyclisation via a ring A allylic carbonium ion.

The cornerstone to the enantioselectivity was the asymmetric microbial reduction of 2-allyl-2-methyl-1,3-cyclopentanedione (147) bakers yeast which provided the chiral starting material (2S,3S)-2-allyl-3-hydroxy-2-methyl-cyclopentanone (148). Inversion of the alcohol was necessary to give the correct configuration for C4 of anguidine.

The diol (152) which had the correct functionality and relative stereochemistry for ring C of anguidine when treated with sodium hydride followed by benzoyl chloride gave the bicyclic lactone (153). Stirring (153) in neat tris(dimethyl) amino methane gave the enamine (154) which on mild acidic hydrolysis gave the hydroxy methylene compound (155). Brooks anticipated that this would add methyl vinyl ketone in an exo manner as reported in a model study by Roush.[63] Indeed Michael addition of (155) to methyl vinyl ketone proceeded exclusively to the desired product (156). Aldol cyclisation and dehydration gave the enone (157) which on treatment with methyl lithium gave the allylic alcohol (158). Reduction of the lactone effected ring opening to the trichodiene analogue (159) which on acylation of the C15 hydroxyl gave the diol (160). This underwent acid catalysed cyclisation via the allylic carbonium ion to the trichothecanoid skeleton (161). Olefination, regioselective epoxidation, desilylation of C4, acetylation to the triacetate and finally selective


(147)
(148)
(149)
(150)
(151)
(152)
(153)
R¹-PhCO, R-SiMe₂ᵗBu
(154) X-N(CH₃)₂
(155) X-OH
(156)
(157) X, Y-O
(158) X-CH₃, Y-OH
(159) R-SiMe₂ᵗBu, R¹-H, R²-H
(160) R-SiMe₂ᵗBu, R¹, R²-Ac
(161)
(34)

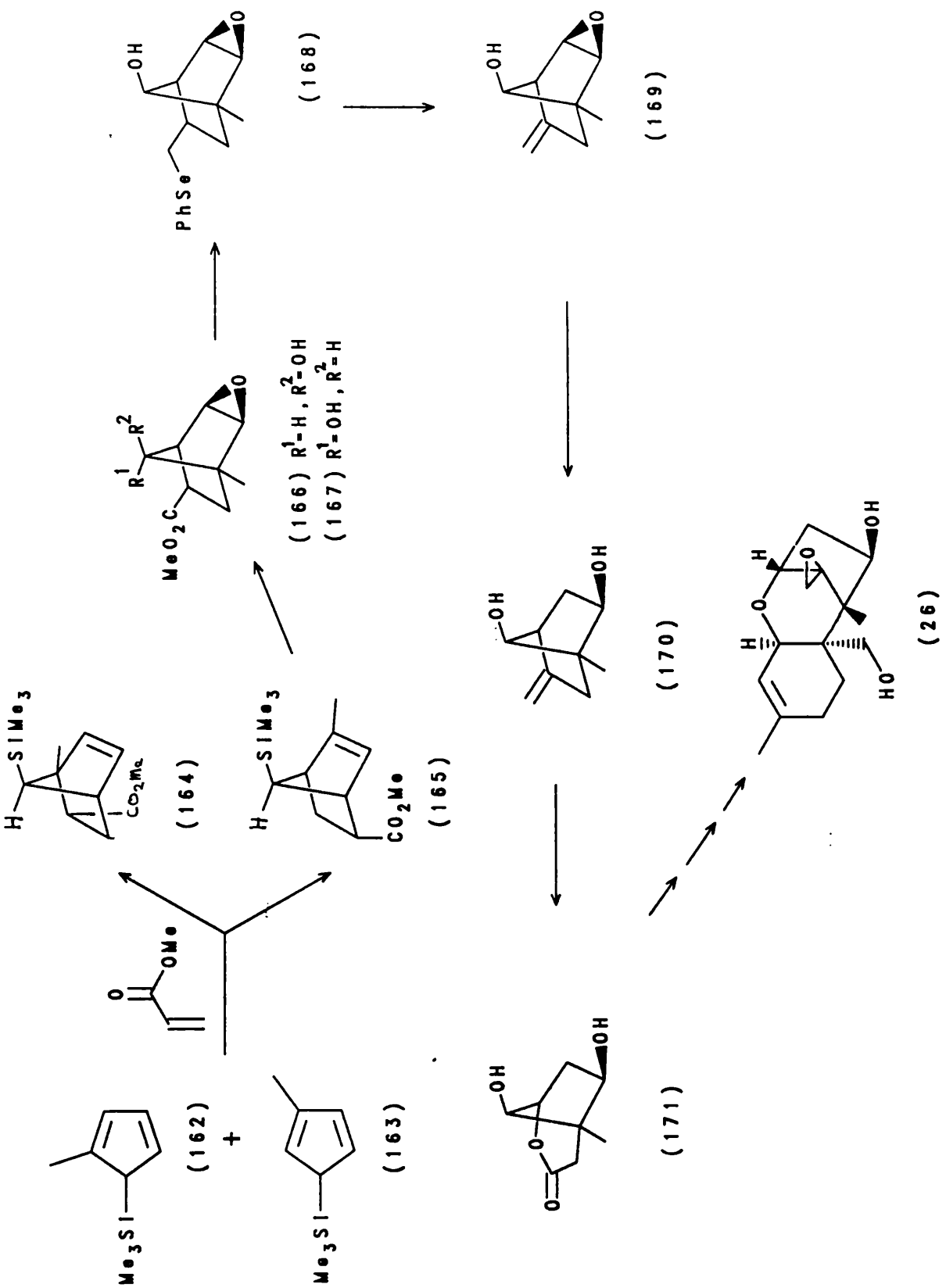
Scheme 20

deacylation of the C3 alcohol gave synthetic anguidine (34) identical in all respects with a natural sample.

In the period 1980-83, Roush described the synthesis of both models[63] and intermediates[64] to the trichothecene skeleton culminating in 1983 with the second total synthesis of verrucarol (26)[53]. This synthesis employed much of the methodology utilised by Schlessinger[54] and so no discussion of the chemistry will be undertaken. However synthesis of the key lactone (171) (Scheme 21) in which a trimethylsilyl group plays a crucial role, merits highlighting.

Firstly, the trimethylsilyl group helped to ensure a high steady state concentration of the methyl-cyclopentadiene isomer (163); Diels-Alder reaction of the isomeric mixture (162) and (163) with methyl acrylate gave in 90% yield a 4:1 mixture of adducts (164) and (165).

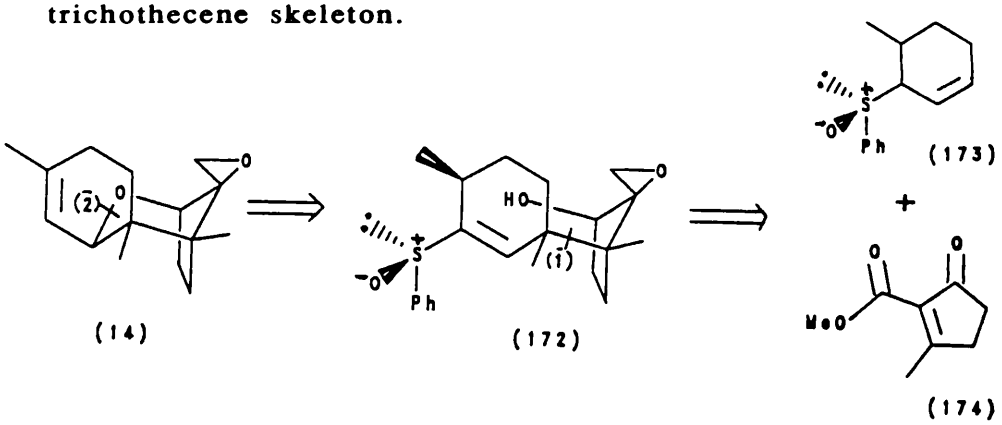
Epoxidation of (165) with mCPBA was sluggish and so the more reactive 3,5-dinitroperbenzoic acid was employed which not only effected epoxidation but also catalysed the trimethylsilyl controlled Wagner-Meerwein rearrangement and further epoxidation to give a mixture of epoxides (166) and (167). Isomer (166) was converted into the selenide (168) which on oxidation and selenoxide elimination gave the exo-methylene-alcohol (169). Treatment of (169) with lithium in ethylenediamine effected regiospecific epoxide opening to give the diol (170) as sole product. Ozonolysis and Baeyer-Villiger oxidation gave the key lactone (171).


Me3Si
(162)
+
(163)
Me3Si
OMe
O
SiMe3
H
CO2Me
(164)
SiMe3
H
CO2Me
(165)
(166) R1=H, R2=OH
(167) R1=OH, R2=H
MeO2C
R1
R2
O
(168)
PhSe
OH
O
(169)
OH
(170)
OH
O
O
(171)
H
O
OH
HO
(26)

Scheme 21

The most recent group 3 synthesis to be reported was Hua's enantioselective total synthesis[65] of (±)-12,13-epoxytrichothec-9-ene (14) and its antipode.

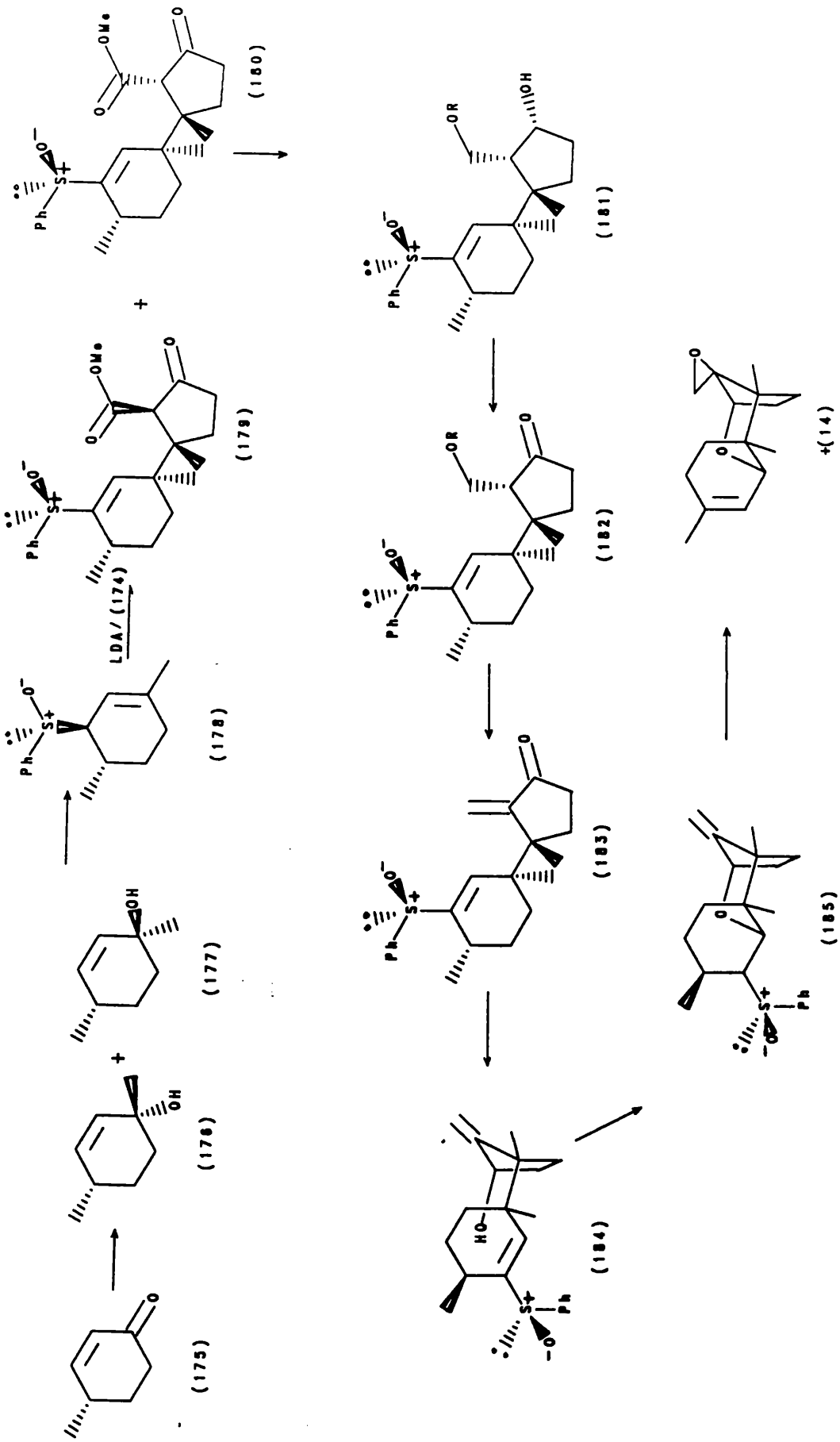
Having studied the enantioselective 1,4 additions of chiral sulphinylallyl anions to cyclic enones he predicted that bond 1 of (172) (Scheme 22) could be formed in this manner between (173) and (174). Intra-molecular Michael addition of the hydroxyl group to the αβ unsaturated sulphoxide of (172) would then form the trichothecene skeleton.



Scheme 22

Synthesis of the chiral sulphoxide (178) (Scheme 23) was achieved in high yield by a sulphenate-sulphoxide[2,3] sigmatropic rearrangement which occurs on addition of benzenesulphenyl chloride to the allylic alcohol (177). The lithium anion of (178) added as predicted in a 1,4 manner to the enone (174) to give a mixture of adducts (179) and (180) in a ratio of 7:93 respectively.

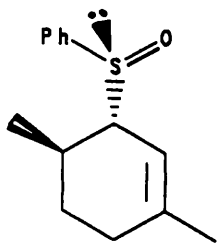
Having constructed a synthon for rings A and C attention then turned to pyran ring formation. The major adduct (180) was converted to the enone (183) which on Luche reduction ($NaBH_4$, $CeCl_3$, $7H_2O$, MeOH) gave a 9:1 mixture of allylic alcohols in favour of the required α alcohol (184). This alcohol cyclised as had been predicted to give the tricyclic ether (185) in 84% yield.



Scheme 23

The target (+) (14) was obtained readily by dehydrosulphenylation using DABCO as base followed by epoxidation.

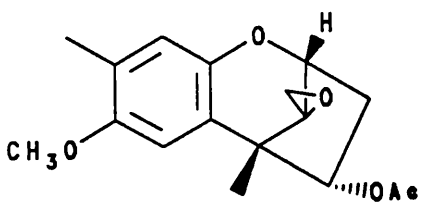
The antipode (-) (14) was synthesised from chiral sulphoxide (186) by the same route.



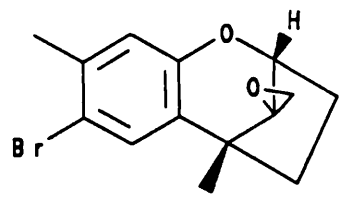
(186)

Group 4 Biomimetic Approach

The group 4 biomimetic approach (Scheme 14) has not been exploited to the same extent as the group 3 approach possibly because the necessary control of C-11 adds further complexity to the synthetic problem. Indeed the first attempt at this approach was the syntheses[66] of the trichothecene models (187) and (188) which having an aromatic A ring have no C-11 stereochemistry.



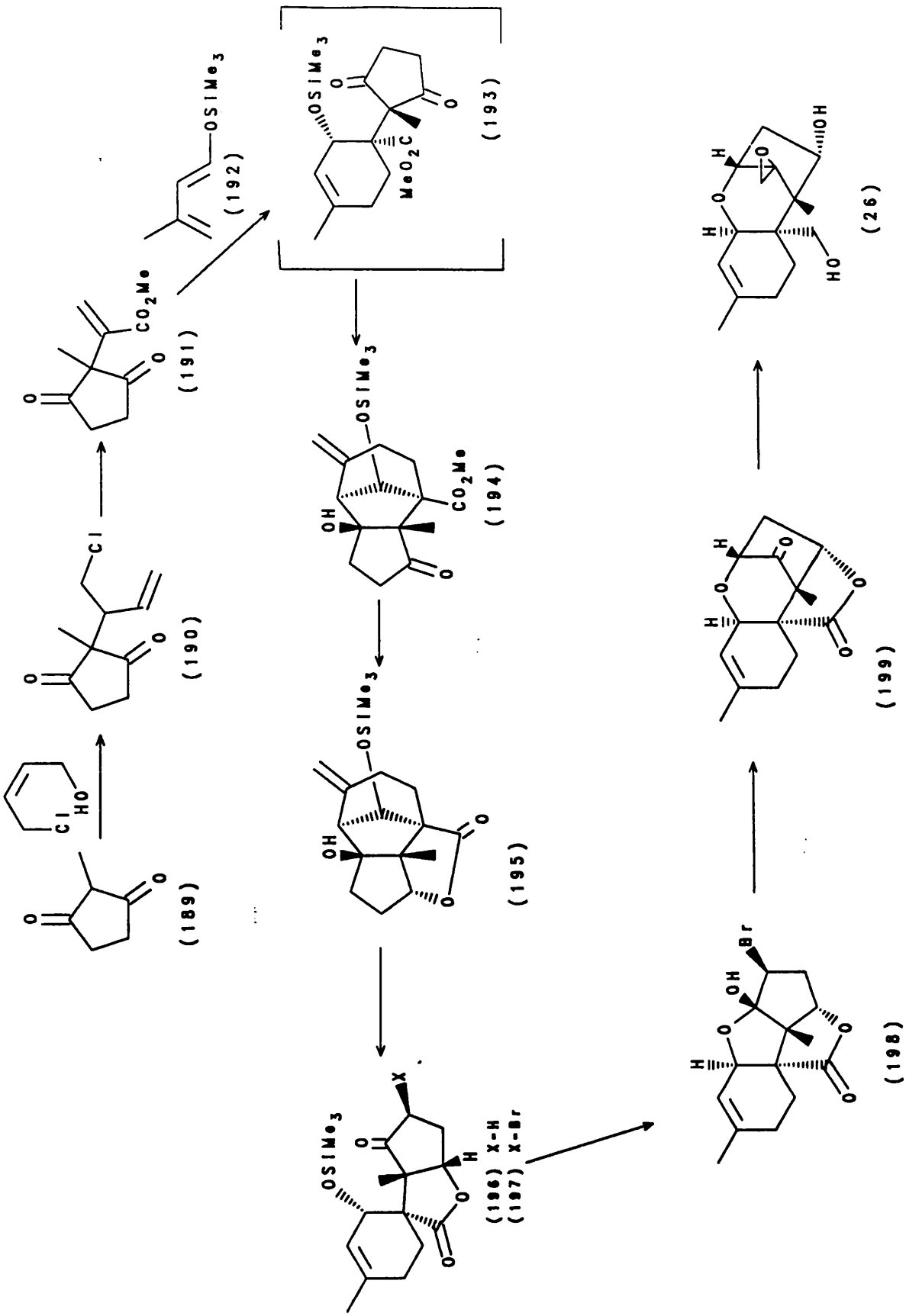
(187)



(188)

Trost and McDougal showed with the synthesis of verrucarol (26)[55] in 1982 that the group 4 approach did offer a viable route to the trichothecenes. To resolve the problem of cis fusion of the AB rings, ring expansion of a cis fused 6,5 ring system (198) to a 6,6 ring system (199) was employed (Scheme 24).

The starting point was the dione (189) which wasreadily converted into the olefin (191). This olefin underwent thermal cyclo addition with the silyloxydiene (192) to give the adduct (193) as the only isolated product. If this adduct was heated further an ene reaction ensued to give the tricycle (194). This proved extremely useful in the differentiation of the two carbonyl groups of (193); analysis revealed that only one of the two diastereotopic carbonyl groups could align itself in the proper orientation for the ene reaction. Reduction of the remaining ketonic group of (194) gave the lactone (195) which on thermolysis



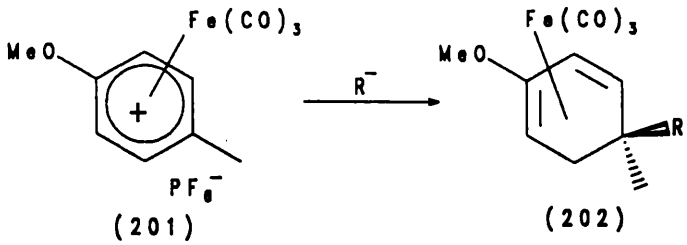
Scheme 24

underwent a retro ene reaction to give the spiro-lactone (196).

Rings A and C were thus synthesised with the correct configuration at C6 and C5 and also with the functionality of C15 and C4 of verrucarol. The remaining synthetic problems were introduction of a leaving group α to the carbonyl and inversion of configuration of C11.

Treatment of the silyl enol ether of (196) with bromine gave the bromo ketone (197). Inversion of configuration at C11 was achieved in a novel manner. Treatment of (197) with trifluoroacetic acid gave the hemi-ketal (198) formed by trapping of the ring A allylic carbonium ion by the lactone hydrate. This compound has the thermodynamically favoured cis fusion of the cyclohexene and tetrahydrofuran required to ensure that expansion to the pyran would give the cis fused trichothecene ring system. Indeed treatment of (198) with fluoride ion induced smooth ring expansion to the tricyclic skeleton (199). The synthesis of verrucarol was completed readily by Wittig olefination, reduction of the lactone, inversion of configuration at C4 and finally regio-selective epoxidation using t-butyl hydroperoxide, in the presence of molybdenum hexacarbonyl as catalyst.

The most recent foray in this area were syntheses of trichodiene (200), 12,13-epoxytrichothec-9-ene (14) and trichodermol (35) by Pearson and O'Brien in 1989.[67] The cyclohexadienyl iron complex (201) (Scheme 25) was used as a ring A precursor; stabilised enolate nucleophiles add to (201) solely at the methyl substituted dienyl terminus and anti to the iron to give diene complexes of general structure (202).

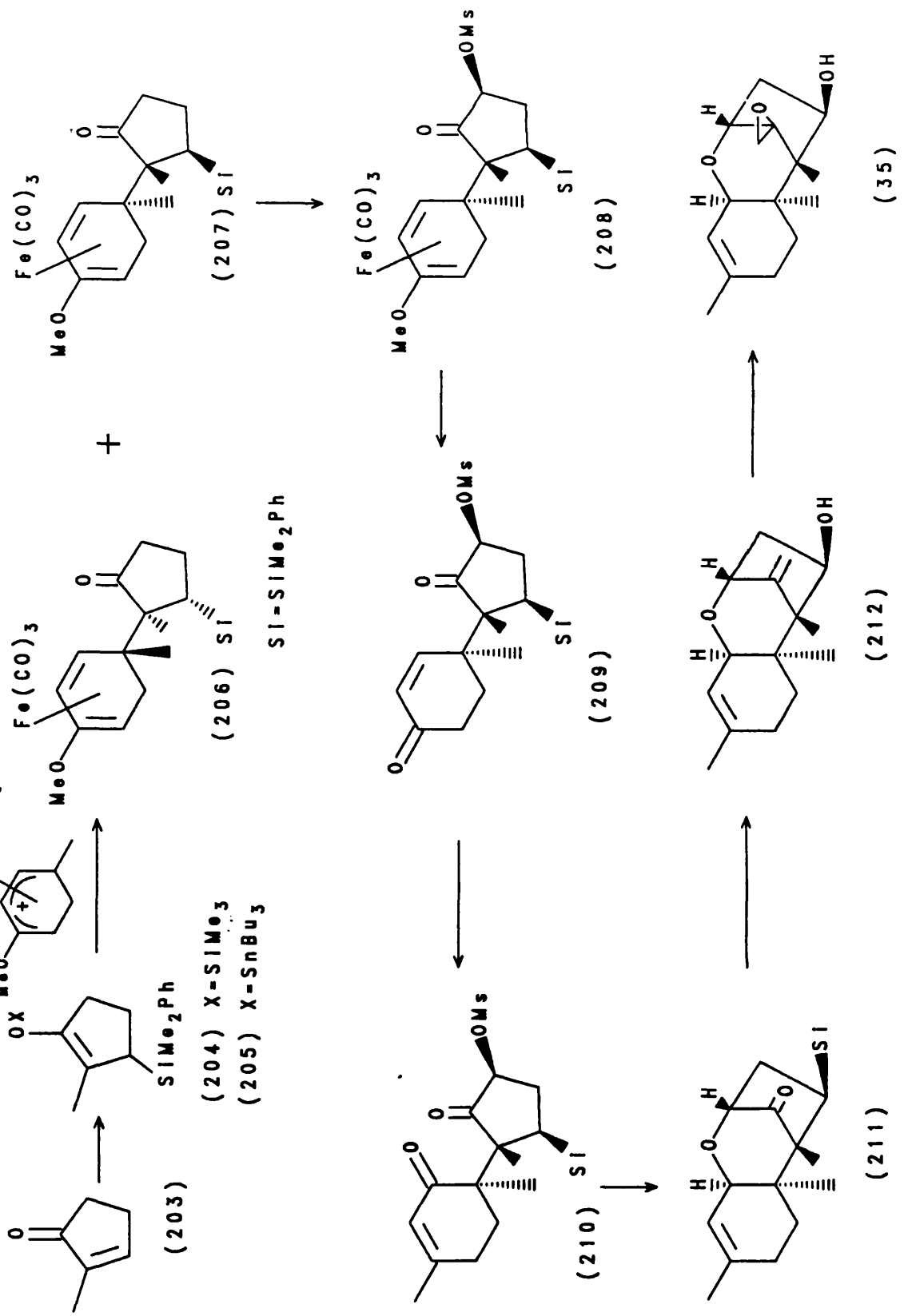


Scheme 25

Trost has shown that π allyl palladium complexes which do not react with lithium enolates or enol-silanes do react smoothly with enol-stannanes and this proved true also for the dienyl iron complex of interest in this synthesis. The principles behind the chemistry reported by this group can be summarised by their synthesis of trichodermol (35) (Scheme 26).

2-Methylcyclopentenone (203), a synthon for ring C, was treated with lithium dimethylphenylsilyl cuprate and the enolate trapped with chlorotrimethylsilane to give the silyl enol ether (204) which could be converted, readily, into the enol stannane (205). This, under carefully controlled conditions reacted with the iron complex to give as a readily separable 1:5 mixture of (206) and (207) with the new bond being stereospecifically trans to the silyl group to give required β configuration of C4. α-Hydroxylation of the ketone (207) surprisingly proceeded stereospecifically on the β face despite the bulky silyl group. Decomplexation followed by Grignard addition to the enone and pyridinium chlorochromate oxidation gave the rearranged enone (210). Careful DIBALH reduction gave the required β configuration at C11 as indicated by the fact that cyclisation to

the tricyclic skeleton (211) occurred spontaneously. Oxidative cleavage of the silyl group as described by Fleming, Wittig olefination and epoxidation gave trichodermol in only 13 steps and an overall yield of 7.7% from the dienyl iron complex (201).


(203)
(204) X-SiMe3
(205) X-SnBu3
SiMe2Ph
Si-SiMe2Ph
(206) Si
(207) Si
(208)
(209)
(210)
(211)
(212)
(35)
Fe(CO)3
MeO
OMs
OH

Scheme 26

1.5 Partial Synthesis

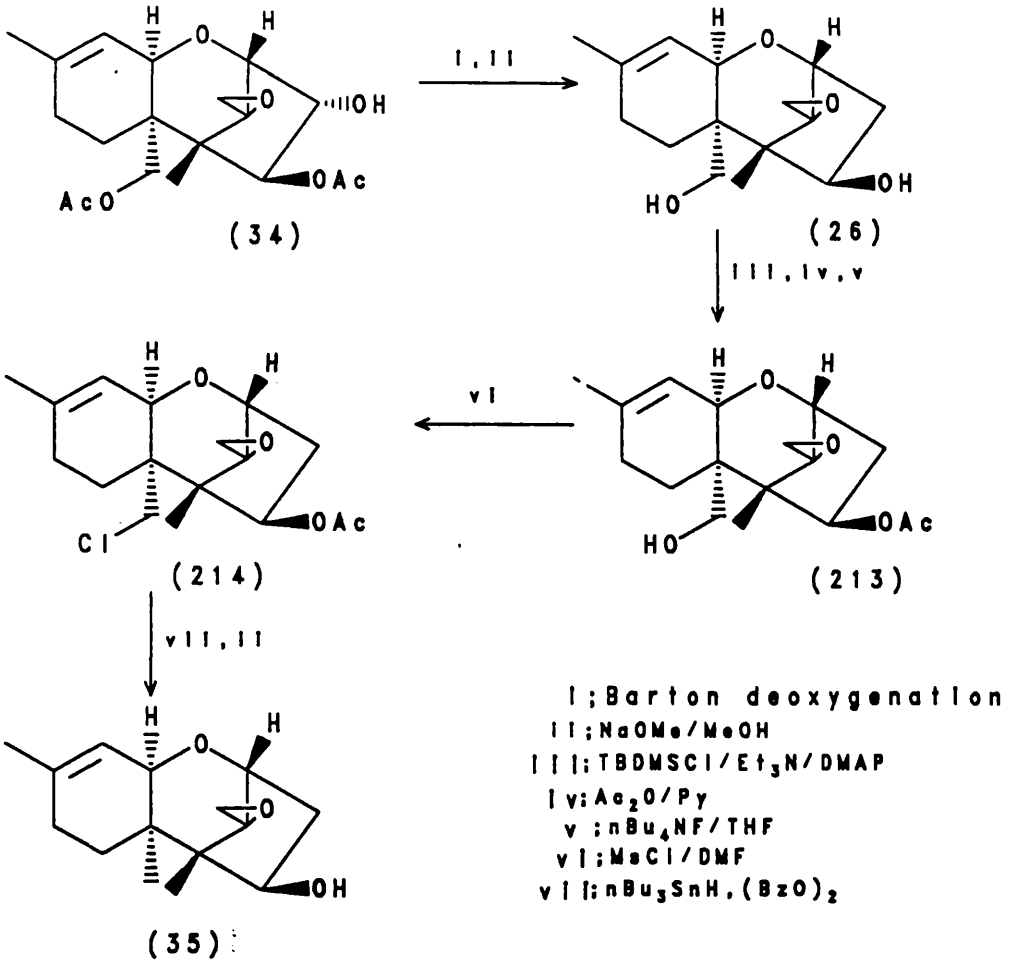
As the majority of trichothecenes can be isolated in only sub-milligram quantities from natural sources, partial synthesis would appear to be essential for obtaining practical amounts of the less abundant trichothecenes.

With the discovery of the trichoverroids[20], Tulshian and Frazer-Reid saw[68] a need to produce their sesquiterpenoid nuclei in order that their biosynthesis and pharmacology could be investigated. The trichothecenes in question, verrucarol (26) and trichodermol (35), are not readily accessible and their isolations elaborate. Anguidine (34), on the other hand, can be obtained directly and in crystalline form from culture broths and was regarded as the ideal starting point for the synthesis of a number of trichothecenes.

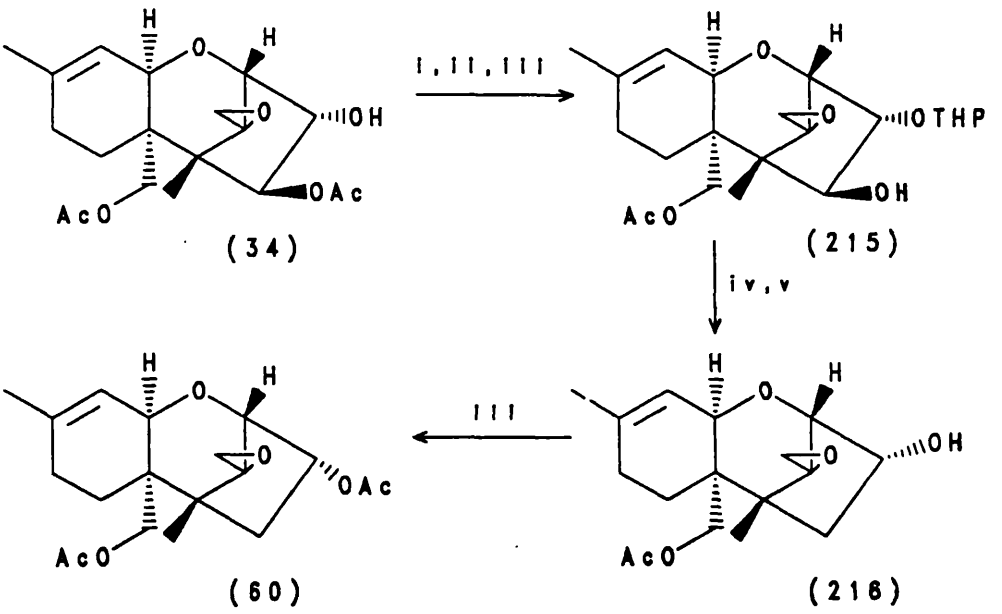
From anguidine (34), Barton deoxygenation and methanolysis gave verrucarol (26) as required (Scheme 27). The C-4 alcohol could then be protected selectively by silylation, acylation and desilylation to give the free primary alcohol (213); transformation into the chloride (214) followed by treatment with Bu_3SnH and finally deacylation gave trichodermol (35) in high yield.

In 1984 Frazer-Reid repeated[69] the synthesis of verrucarol (26) from anguidine(34) employing the phenyl thiocarbonate ester method of deoxygenation of Robin's which gave higher yields of (26).

Tamm and Mohr demonstrated[70] the utility of anguidine in trichothecene partial synthesis in 1984 by the synthesis of calonectrin (60) (Scheme 28). Protection of the C3 hydroxyl as


I, II
III, IV, V
VI
VII, II
(34)
(26)
(214)
(213)
(35)
I ; Barton deoxygenation
II ; NaOMe / MeOH
III ; TBDMSCl / Et3N / DMAP
IV ; Ac2O / Py
V ; nBu4NF / THF
VI ; MeCl / DMF
VII ; nBu3SnH, (BzO)2

Scheme 27



I; DHP, PPTS, CH_2Cl_2
II; NaOH, MeOH
III; Ac_2O, Py, CH_2Cl_2
Iv; $(Imid)_2C=S$, $C_2H_4Cl_2$/ Bu_3SnH
v; PPTS, MeOH

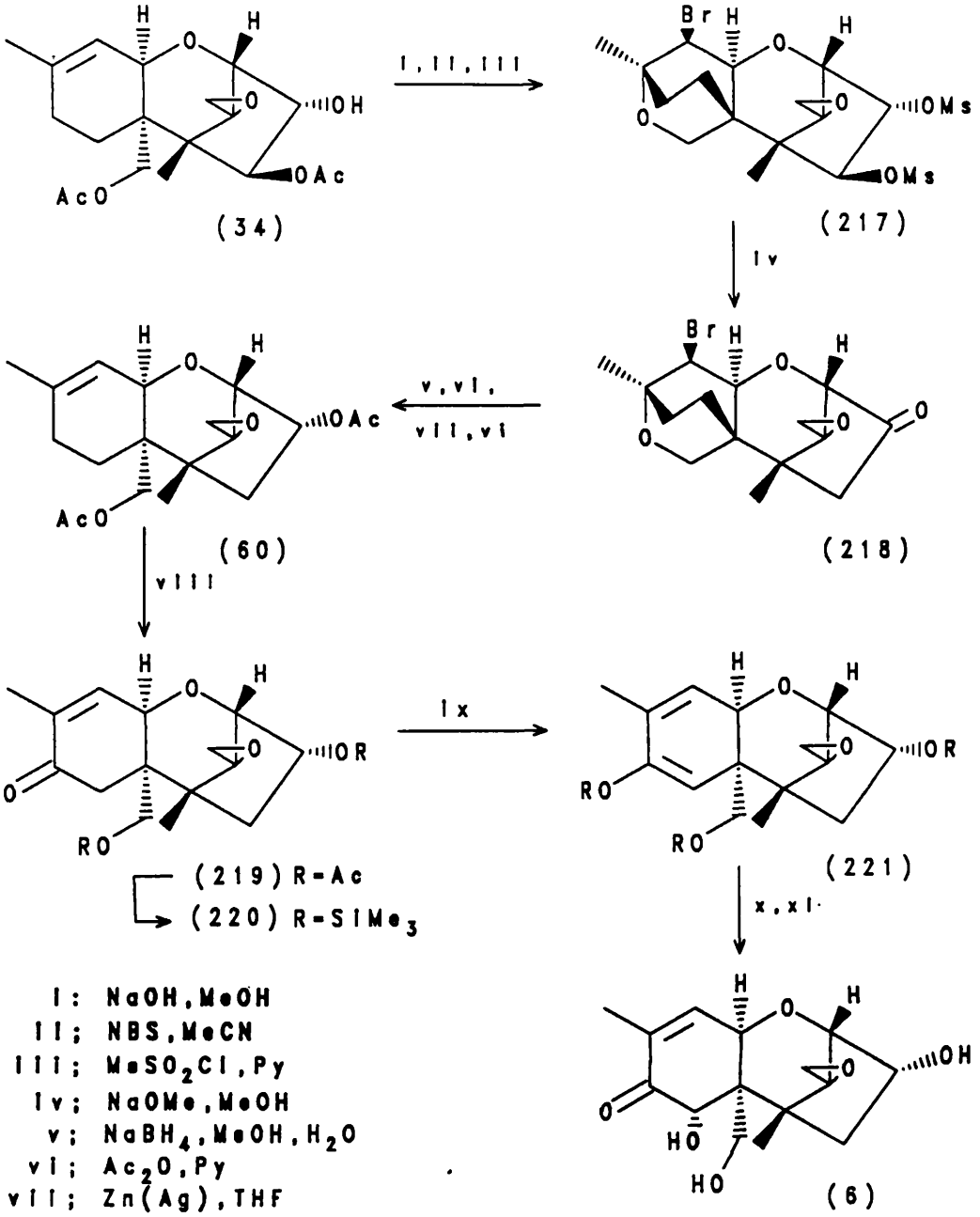
Scheme 28

the THP ether, perdeacylation followed by selective acylation gave the alcohol (215). Treatment of this alcohol with thiocarbonyl diimidazole and reduction with Bu_3SnH gave deacylcalonectrin (216). Acylation gave calonectrin (60) in high overall yield.

Recently Colvin and Cameron[71] illustrated further the utility of anguidine by employing it as the starting material in a synthesis of calonectrin (60) and deoxynivalenol (6) (Scheme 29). Deacylation of anguidine, protection of the ring A olefin as the bromo ether and treatment with methanesulphonyl chloride gave the bis mesylate (217). Refluxing (217) with sodium methoxide in methanol effected regio specific elimination to an unisolated enol mesylate which hydrolysed <u>in situ</u> to the ketone (218). Sodium borohydride reduction of (218) gave the 3α-alcohol, exclusively. Regeneration of the C15 alcohol followed by acylation furnished calonectrin (60) as required in an overall yield of 55%.

Inspection shows that a ketonic group at C8 and an α orientated hydroxyl group at C7 are required to convert calonectrin into deoxynivalenol (6). The first of these functionalisations was achieved by allylic oxidation of (60) with freshly prepared Collin's reagent to give the enone (219). Introduction of the C7 α-alcohol was accomplished as shown. The ketone (220) was converted into the trimethyl silyl enol ether (221). Treatment of this with mCPBA followed by fluoride ion mediated desilylation gave deoxynivalenol (6).

In conclusion it would appear therefore that partial synthesis can provide a viable route to the rarer members of this group.



I: NaOH, MeOH
II; NBS, MeCN
III; $MeSO_2Cl$, Py
IV; NaOMe, MeOH
V; $NaBH_4$, MeOH, H_2O
VI; Ac_2O, Py
VII; Zn(Ag), THF
VIII; $CrO_3.Py_2$, CH_2Cl_2
IX; $LINPr^i_2$, Me_3SiCl, THF
X; mCPBA, hexane
XI; HF, MeCN, H_2O

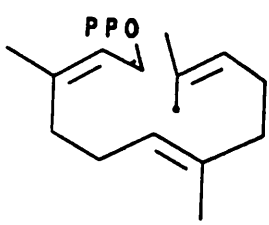
Scheme 29

1.6 Biosynthesis

Since the majority of trichothecene syntheses described herein have employed a biomimetic cyclisation for the successful construction of the tricyclic nucleus, a brief overview of trichothecene biosynthesis is deemed relevant.

The fact that the basic trichothecene nucleus contained fifteen carbons including 3 methyl groups suggested that they were sesquiterpenoids. Indeed [2-^{14}C]mevalonate was incorporated[72] into the trichothecene moiety of trichothecin (3) to the extent of 0.5% with three mevalonate molecules being involved. Experiments that gave a 1% incorporation[73] of [2-^{14}C]mevalonate into diacetoxyscirpenol and a 1.5% incorporation[74] of [^{14}C]farnesyl pyrophosphate into trichothecolone confirmed the earlier suspicions.

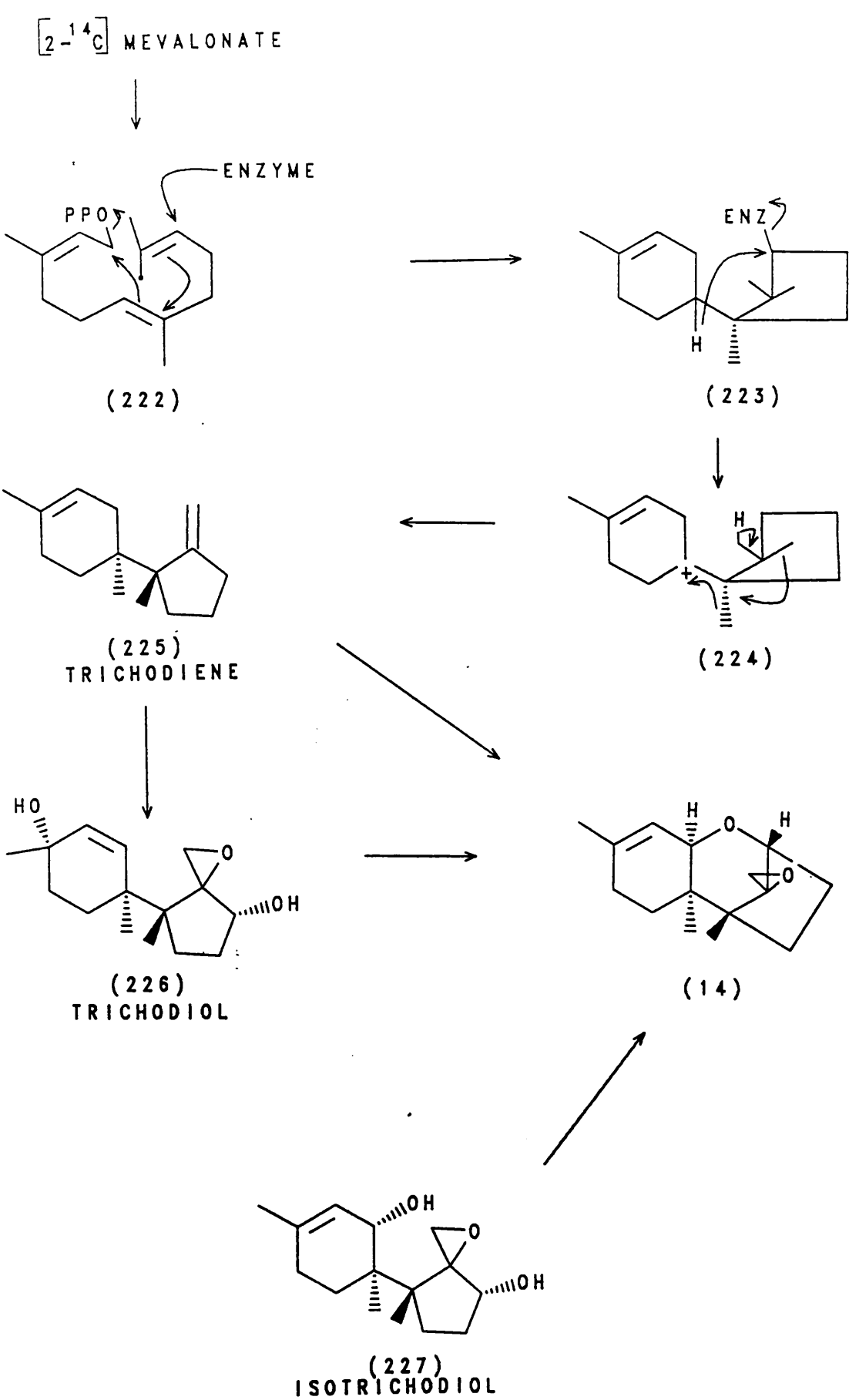
As it was obvious that the trichothecenes were not derived by a straight forward head to tail linkage of isoprene units, the debate turned to the folding sequence of farnesyl pyrophosphate. Hansen showed[75] by ^{13}C nmr that only carbons 4,8 and 14 of trichothecolone displayed any enrichment of ^{13}C on incorporation of[2-^{13}C]mevalonate suggesting that the farnesyl pyrophosphate was coiled as shown, before cyclisation.



This coupled with evidence[76] that a hydride shift from C6 to C10 of farnesyl pyrophosphate occurred during cyclisation supported Hanson's proposed[76c] biogenetic route to the trichothecenes(Scheme 30). Cyclisation is initiated by attack of an enzyme at C10 of FPP, with addition to the central double bond occurring in a cis manner[76d]. Trichodiene (225) which has been isolated from *T.roseum* and is a proven[77] intermediate is formed by a hydride shift from C6 to C10 a double 1,2 methyl group migration[78] and a proton loss.

12,13-Epoxytrichothec-9-ene (14) appears to be a common intermediate in all trichothecene biosyntheses. The trichothecenes being derived from it by hydroxylations and esterifications with mono oxygenase enzymes being responsible for the hydroxylations and epoxidations.

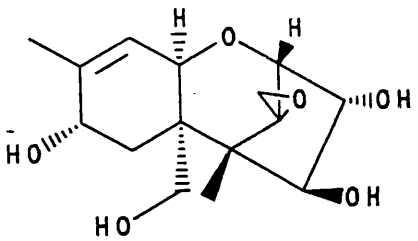
The sequence of events between trichodiene (225) and (14) was until recently unknown. Although trichodiol (226) has been isolated from culture broths its role in the pathway has not been determined. Dewick fed[77] [^{14}C] trichodiene to cultures of *F.culmorum* and isolated the new metabolite isotrichodiol (227) into which trichodiene was incorporated with a specific incorporation of 67%. Further to this, on feeding [^{14}C] isotrichodiol to *F. culmorum* it was incorporated with high specific incorporation into 12,13-epoxytrichothec-9-ene as well as other trichothecenes from *F. culmorum*.


[2-14C] MEVALONATE
ENZYME
PPO
(222)
ENZ
H
(223)
H
(224)
(225)
TRICHODIENE
HO
O
OH
(226)
TRICHODIOL
H
O
H
O
(14)
OH
O
OH
(227)
ISOTRICHODIOL

Scheme 30

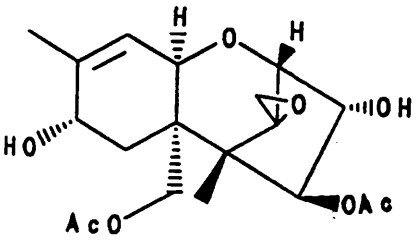
2. Discussion

2.1 Ring C Functionalisation Studies.

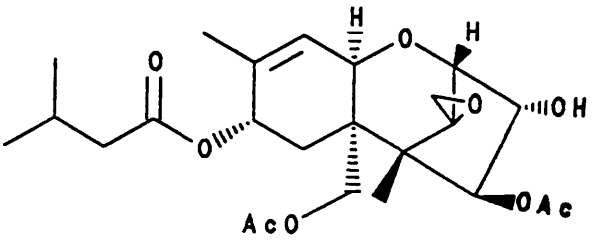


(19)
T-2 TETRAOL

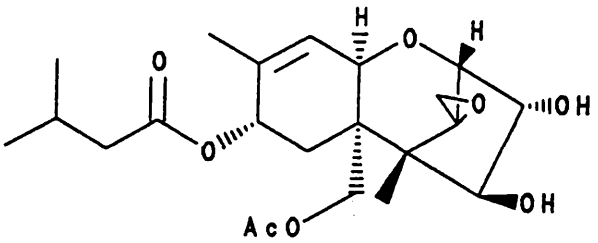
T-2 Tetraol (3α-, 4β-, 8α-, 15-tetrahydroxy-12,13-epoxy trichothec-9-ene) (19) a tetrahydroxylated non-macrocyclic trichothecene, is the parent member of a group of trichothecenes which includes the highly toxic neosolaniol (7), T-2 toxin (4) and HT-2 toxin (228). T-2 tetraol itself is highly toxic and was chosen as one of the targets of our group's study of the synthesis and interconversion of the trichothecene mycotoxins.



(7)
NEOSOLANIOL



(4)
T-2 TOXIN



(228)
HT-2 TOXIN

On examination of the structure of T-2 tetraol the key requirements of the synthesis were defined as:

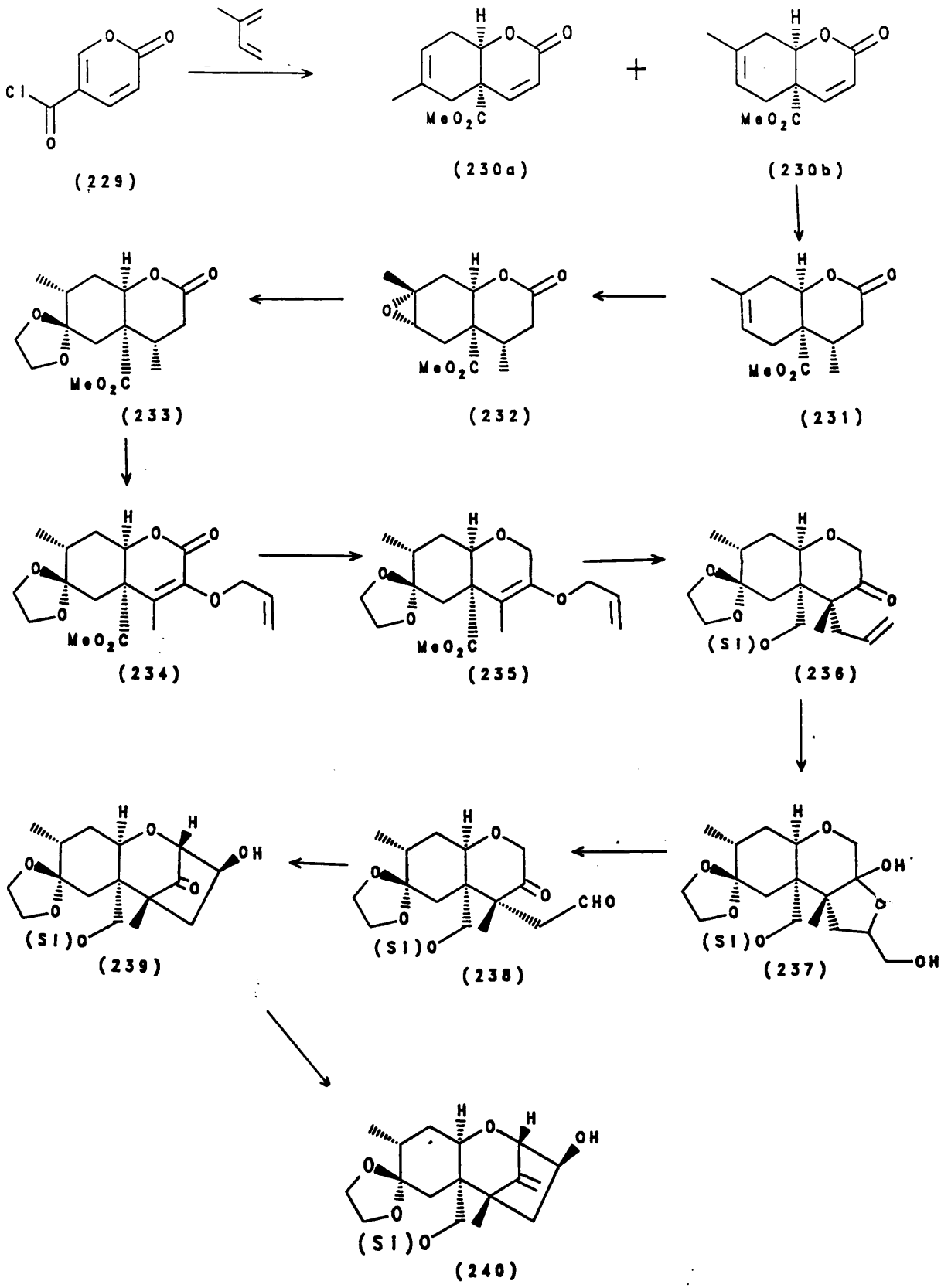
1) construction of the cis fused AB ring system by a Diels Alder reaction.
2) formation of ring C by a group 2 intramolecular aldol cyclisation (see page 29)
3) functionalisation of ring C to a 3α-, 4β- diol, and
4) formation of the 8α- allylic alcohol by base induced epoxide ring opening, or alternatively, formation of an enone function in ring A followed by stereo and regioselective reduction of the enone.

As part of a concerted effort towards T-2 tetraol my brief was to investigate methods for formation of the 3α-, 4β- diol functionality of ring C and to determine which route was the most efficient.

As the synthesis developed, it became apparent that the functionalisation would be performed on the tricyclic alcohol (240) (Scheme 31). Thus any protocol would have to be compatible with the functionality of (240).

The approach to T-2 tetraol formed the basis of F.W. Kerr's Ph.D. thesis and is discussed there in detail. However an overview of this synthesis is relevant here.

Thermally promoted cycloaddition of isoprene with coumalyl chloride (229) (Scheme 31) followed by esterification of the product mixture gave a mixture of adducts (230a) and (230b) in 41% yield with a regioisomeric ratio of 1:4 respectively. The required major adduct (230b) on methyl cuprate addition in the presence of chlorotrimethylsilane gave on acidic work-up the



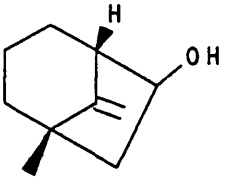
Scheme 31

lactone (231) as a single methyl epimer. Epoxidation with mCPBA gave the epoxide (232), crucial for ring A functionalisation as a single diastereoisomer. Attempted base induced epoxide-allylic alcohol rearrangement[81] was unsuccessful due to intramolecular epoxide opening effected by the lactone enolate. Hence the allylic alcohol would have to be introduced by the alternative route of epoxide rearrangement to the ketone and thence formation and selective reduction of the enone. To this end epoxide rearrangement with a number of catalysts was attempted, with $BF_3.OEt_2$ proving to be the most successful. Indeed it was found that one-pot conversion of (232) into the ketal (233) was effected by stirring the epoxide in ethylene glycol in the presence of $BF_3.OEt_2$. With the functionality necessary for completion of ring A present, attention turned to the construction of ring C; thus the keto-aldehyde (238) was required. Following established methodology[50], hydroxylation α to the lactone, Corey oxidation and O-allylation of the ketolactone in its enol form gave the allyl enol ether (234). Reduction to the lactol then allowed deoxygenation to the pyran (235) by treatment with $BF_3.OEt_2$ and triethylsilane - a remarkably selective deoxygenation considering the other oxygen functionality present. Ester reduction, Claisen rearrangement and silylation gave in high yield a mixture (3.2:1) of (236) and its allyl epimer respectively. Ozonolysis of the allyl group of (236) was unsuccessful; starting material was consumed but the ozonide proved stable to a number of reducing agents. In earlier work Fujimoto obtained the aldehyde (82a) from (80) (Scheme 11) by oxidative cleavage of the olefin with osmium tetroxide and sodium periodate. Surprisingly, similar conditions here stopped at the diol (237). However, ethereal periodic acid cleaved the diol to the aldehyde which cyclised, in the presence of sodium methoxide to the tricyclic skeleton (239), as a mixture of 3β- and 3α-

alcohol epimers in a ratio of 4.2:1 respectively. The major 3β-epimer was protected as its THP ether. Wittig olefination and deprotection then furnished the alcohol (240) on which the ring C oxidative functionalisation would be performed.

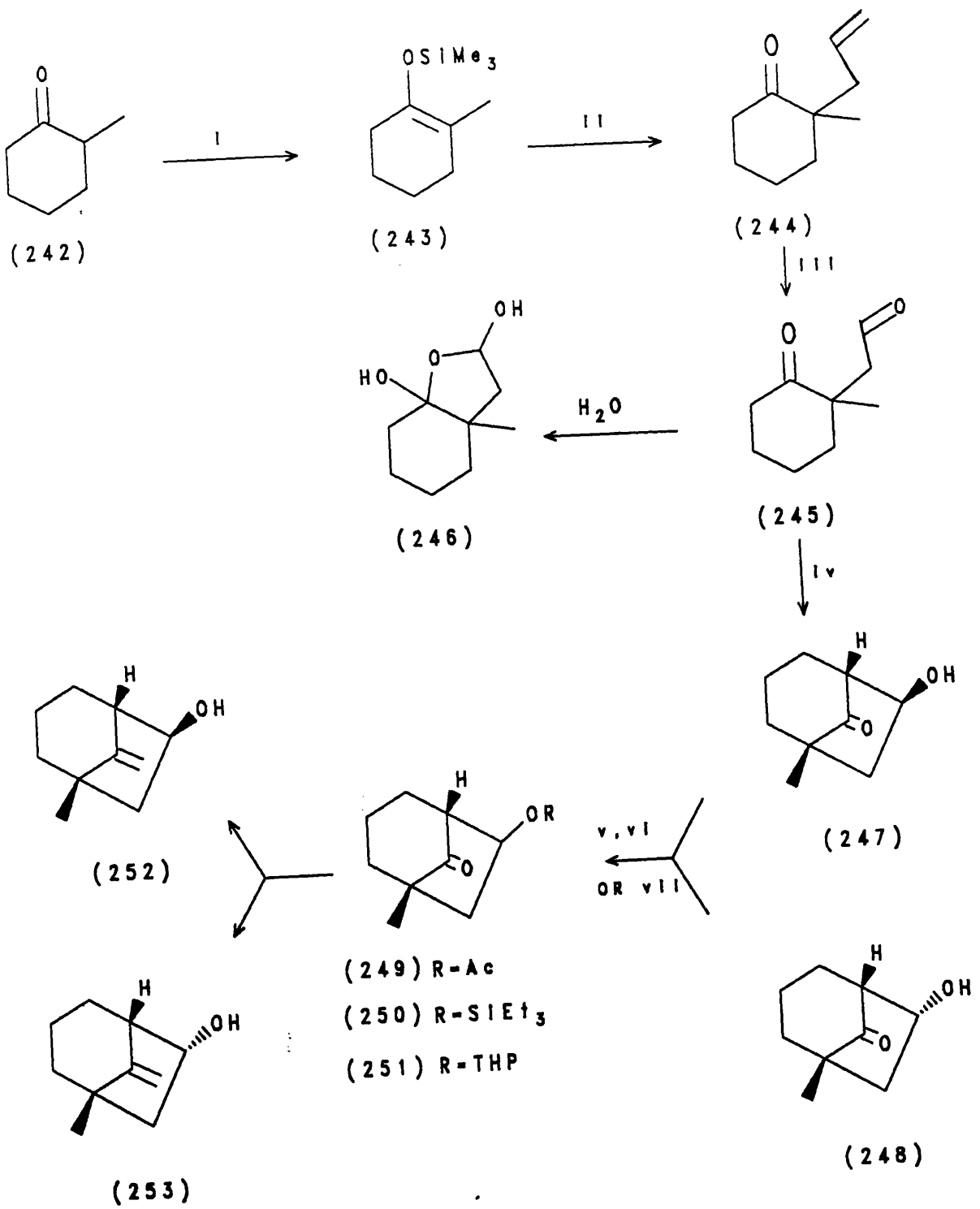
Model Study

As the protocol for ring C functionalisation was being devised simultaneously with the synthesis of (240) (Scheme 31), a model for (240) was required. Earlier deoxygenation studies[82] in the group utilised the bicyclo[3,2,1]octane system (241) as a model for the trichothecene B/C rings; this would be the model employed in this study.



(241)

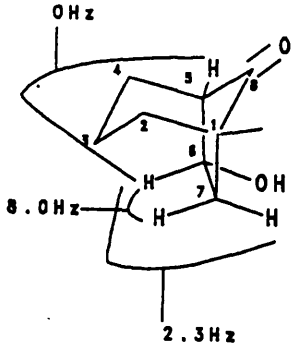
The synthesis of this model compound from 2-methylcyclohexanone (242) is outlined in (Scheme 32). Employment of the conditions of Stork[83] and House[84] produced the thermodynamic silyl enol ether (243) in 75% yield after distillation. When required, the enolate was released with methyl lithium and C-alkylated with allyl-bromide in THF to give the allyl ketone (244) in high yield. Oxidative cleavage of the olefin with ozone employing triethylamine as the ozonide reductant gave the aldehyde (245) which was used immediately after chromatographic purification to minimise material loss by

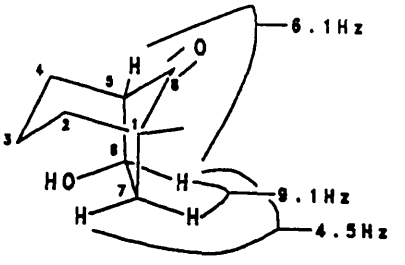


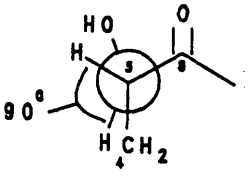
I; Me_3SiCl, Et_3N, DMF
II; MeLi, THF, allyl bromide
III; O_3, CH_2Cl_2, Et_3N
IV; MeONa, MeOH;
V; Ac_2O, Py
VI; Et_3SiCl, Et_3N, DMAP, Et_2O
VII; Dihydropyran, PPTS

Scheme 32

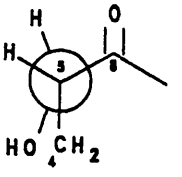
formation of the hydrate (246). Refluxing the aldehyde with sodium methoxide in methanol for 25 minutes effected aldol cyclisation to the bicyclo[3,2,1]octanones (247) and (248) in a ratio of 2:1 in favour of the thermodynamically favoured 6β-epimer (247). The alcohols could be readily separated by dry column flash chromatography[85] and the individual C-6 epimers identified by ^{1}H nmr spectroscopy.







(247)



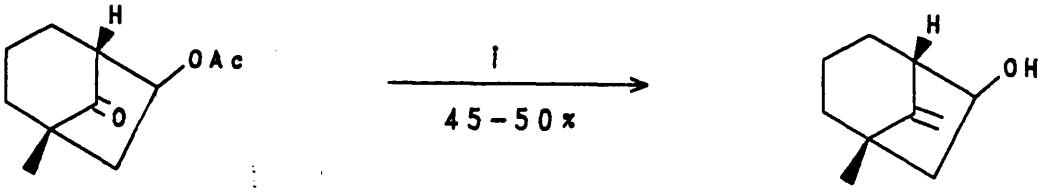
(248)

The more polar C-6β-alcohol epimer (R_f 0.24) was characterised by ^{1}H nmr spectroscopy by a doublet of doublets (J = 8.0 and 2.3 Hz) at 4.20 ppm due to the C-6 proton. Examination of molecular models (see Newman projection, above) revealed that the dihedral angle between the C-6 and C-5 protons was approximately 90° and so by the Karplus equation[86], the coupling constant between them was approximately zero. The doublet of doublets arose by coupling of the C-6 proton to the diastereotopic C-7 protons.

In the less polar C-6α-alcohol epimer (R_f 0.3) the dihedral angle between C-6 and C-5 protons was approximately O° (see Newman

projection above), hence the C-6 proton was seen as a doublet of double doublets (J = 9.1, 6.1 and 4.5 Hz) at 4.42 ppm due to coupling to the C-5 and C-7 protons. The characteristic ^1H nmr signals and coupling constants exhibited by these model compounds proved invaluable in determining the stereochemistry of the target diol.

The final step required for formation of the model compound was Wittig reaction of the ketones (247) and (248) with methylenetriphenylphosphorane. The basic nature of the Wittig reaction made protection of the C-6 alcohol essential to prevent a retro-aldol reaction occurring. The previously chosen[82] protecting group, acetate, gave yields of the required olefin alcohol (241) in the region of 45 to 50% as a mixture of the C6 epimers (252) and (253). The fact that no olefin-acetate was recovered indicated the sensitivity of this protecting group to the Wittig conditions.



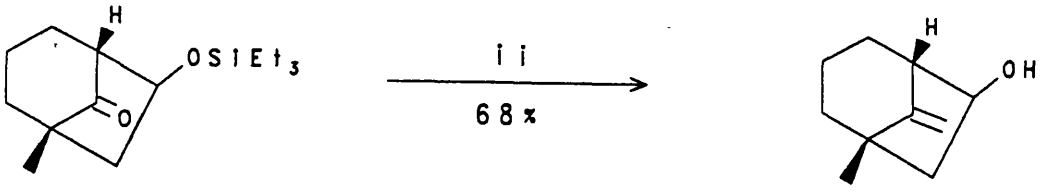
(249) (241)

I, Ph_3PCH_3Br, BuLi, THF.

Scheme 33

The triethylsilyl group proved more stable with up to 68% yields of olefin alcohol being achieved. However, acidic work-up effected only partial desilylation hence treatment with fluoride

ion (HF/MeCN/H_2O) was required to furnish the olefin alcohol.

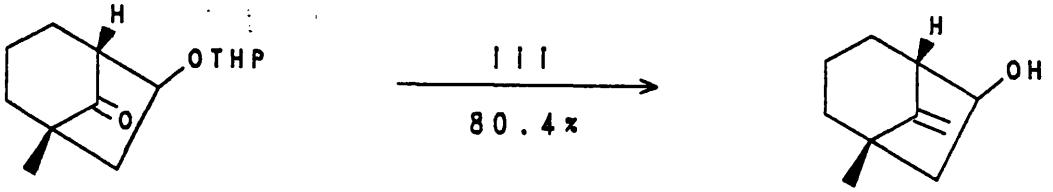


(250) (241)

i , Ph_3PCH_3Br , BuLi , THF/ HF , MeCN , H_2O

Scheme 34

The final protection method tried was tetrahydropyranylation. Quantitative protection of a mixture of alcohols (247) and (248) was achieved by stirring them in neat dihydropyran utilising[87] PPTS as catalyst. Wittig olefination followed by deprotection with ethanol and PPTS gave the required olefin (241) as a readily separable mixture of the olefin alcohols (252) and (253) in 80% yield. Although protection of the alcohol as the THP ether gave an inseparable mixture of diastereoisomers the higher yields achieved in the Wittig reaction-deprotection sequence rendered THP as the protecting group of choice for this model study and subsequently the synthesis of T-2 tetraol.



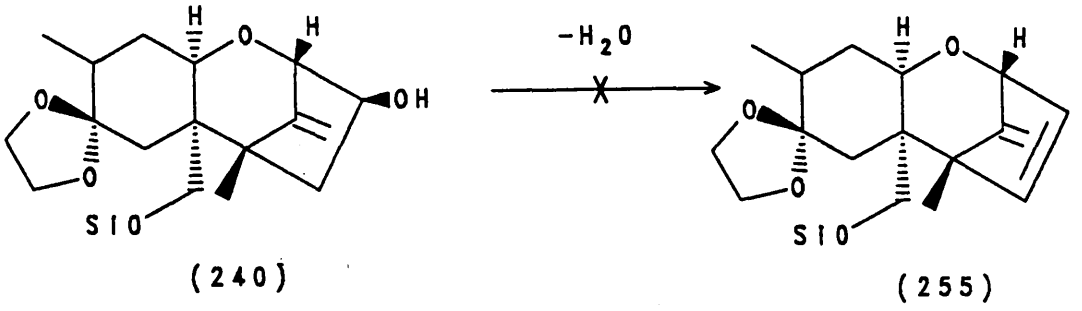
(251) (241)

i , Ph_3PCH_3Br , BuLi , THF/EtOH , PPTS

Scheme 35

Vicinal diols can be produced in a number of ways, the most common routes being dihydroxylation of olefins or, carbonyl α-hydroxylation followed by reduction.

Dihydroxylation of olefins can be effected by a variety of reagents. Alkaline potassium permanganate[88], osmium tetroxide[89] and silver carboxylates in the presence of iodine and water give syn-diols whereas anti-diols are formed on treatment of olefins with silver carboxylates and iodine in anhydrous conditions. Reaction of olefins with hydrogen peroxide and formic acid also furnishes anti-diols. However in the case of the T-2 tetraol precursor (240), necessary dehydration of the alcohol would produce the diene (255) (Scheme 36) for which no chemoselectivity in reactions could be predicted, hence this route to the diol was deemed unsatisfactory.



Scheme 36

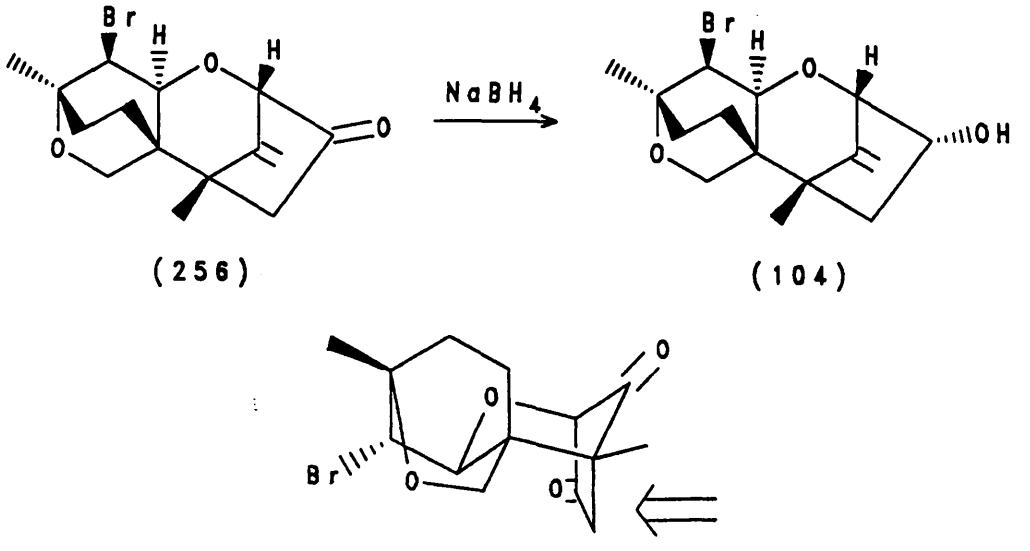
The second possibility, oxidation to the ketone and hydroxylation α to the carbonyl seemed more promising for two reasons:

1) Acyloin formation from ketones has been well documented in the literature:-

Ketones can be α hydroxylated by treatment of their enolates with either the molybdenum pentoxide reagent of Vedejs[91] or by oxidation of their trimethylsilyl enol ethers with a variety of reagents including mCPBA[92], osmium tetroxide and N-methyl-morpholine N-oxide[93], dimethyl dioxirane[94] and N-sulphonyl oxaziridines[95]. Reaction of enamines with molecular oxygen also furnishes[96] acyloins.

2) The stereochemistry of the diol formed by this method could be predicted:-

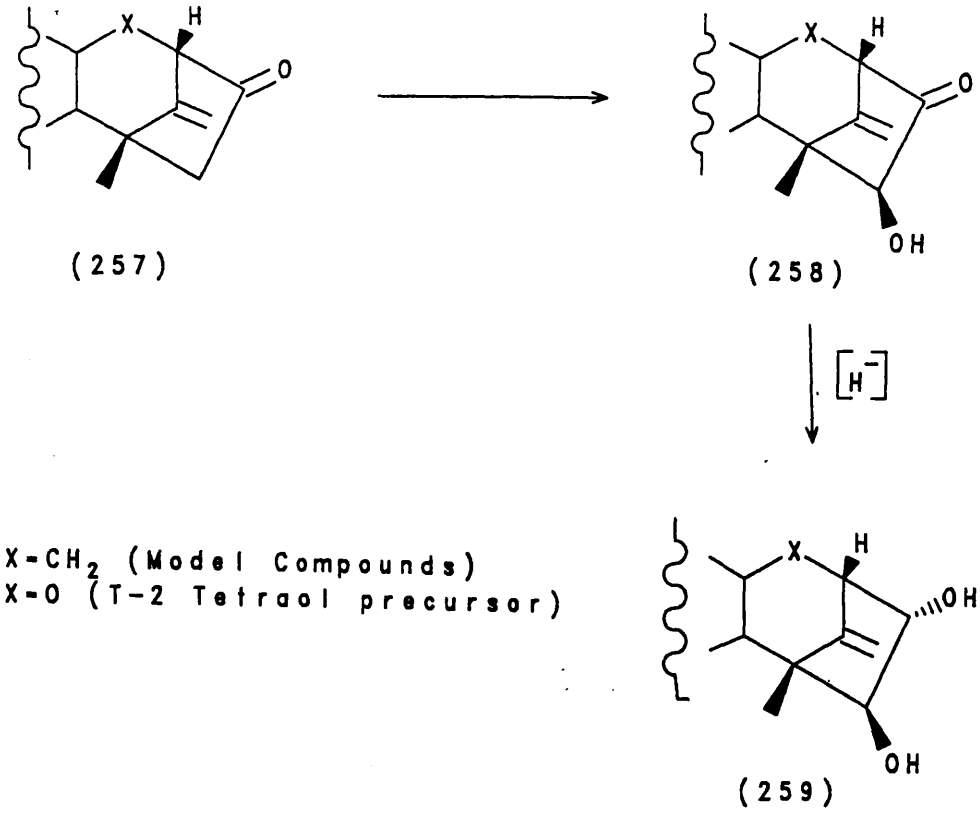
In the synthesis of calonectrin[48], Kraus found that reduction of the ketone (256) (Scheme 37) with sodium borohydride gave exclusively the 3α-alcohol (104).



Scheme 37

Thus reagents attacking the 2 carbon bridge of the trichothecenes appear to do so almost exclusively from the less hindered exo-face. Thus hydroxylation α to the ketone (257) (Scheme 38) should give a β-orientated hydroxyl group; subsequent

borohydride reduction should reduce the carbonyl to an α-orientated hydroxyl group.

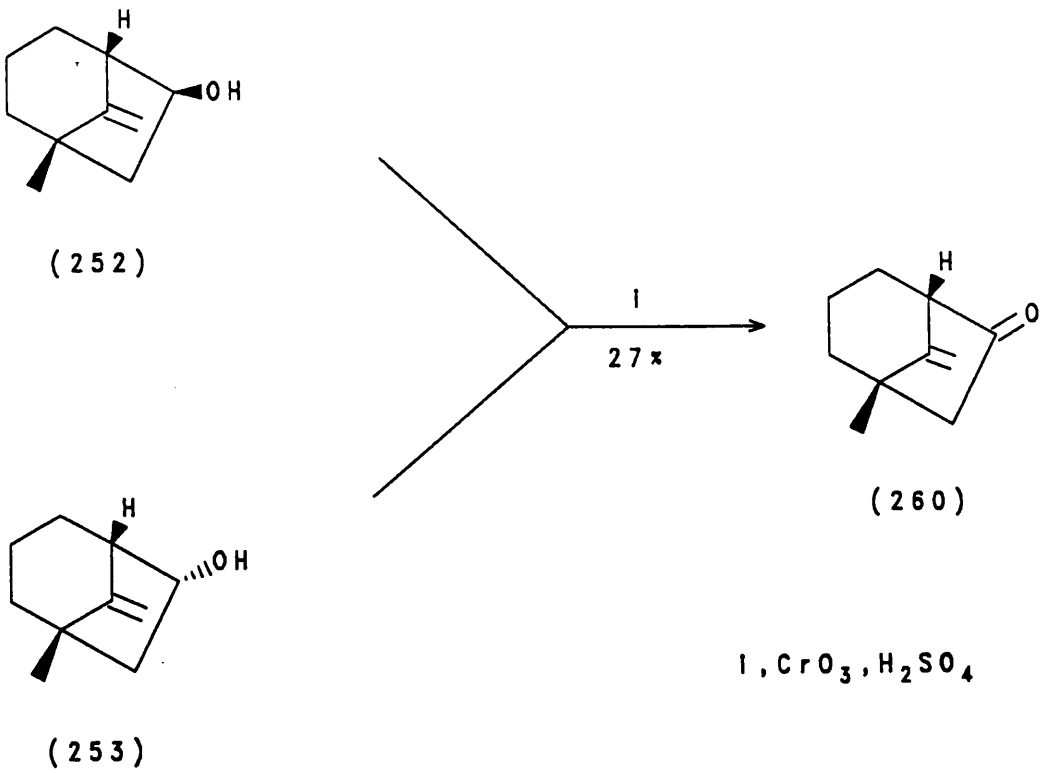


(257) (258) (259)

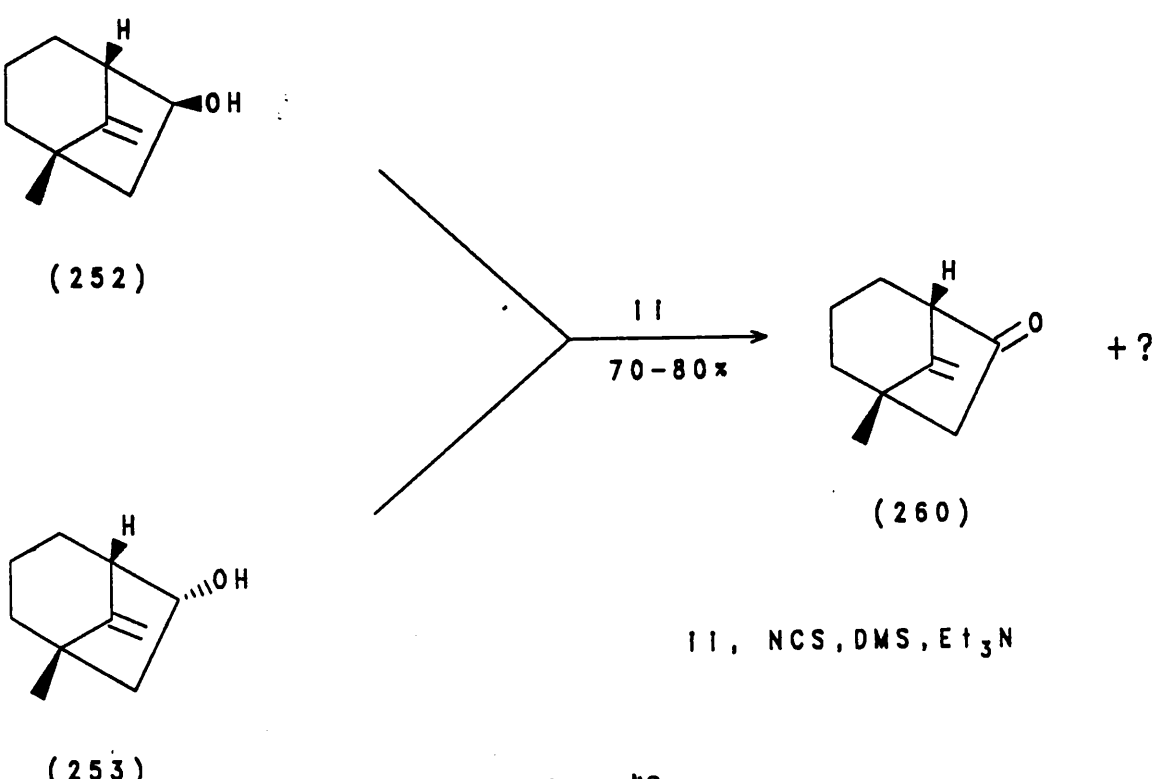
Scheme 38

Oxidation of the alcohols (252) and (253) to the ketone (260) (Scheme 39) was initially performed using Jones reagent[97]. However the yield after column chromatography was only 27%. This and the acidic nature of this oxidation rendered it useless for the functionalisation intended.

The second method of oxidation was that of Corey utilising[98] N-chlorosuccinimide, dimethylsulphide and triethylamine in either toluene or dichloromethane (Scheme 40). This gave yields of ketone of 70-80%. It was apparent however from ^1H nmr spectroscopy that an impurity was present, resonating in the region of 4.5 to 4.7 ppm. However since its polarity was



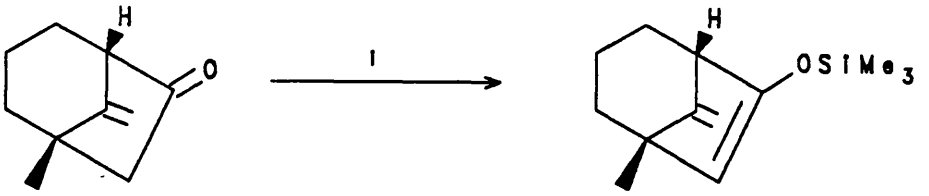
Scheme 39



Scheme 40

very similar to that of the ketone, it could not be removed by column chromatography, preparative t.l.c nor indeed could it be removed by distillation. Since the ketone was the main constituent of the mixture it was decided to continue with the functionalisation in the presence of the impurity.

Initial studies were via the silyl enol ether (261) (Scheme 41) which was formed by deprotonation of the ketone using lithium diisopropylamide and <u>insitu</u> trapping of the enolate with chlorotrimethylsilane as described[99] by Corey.

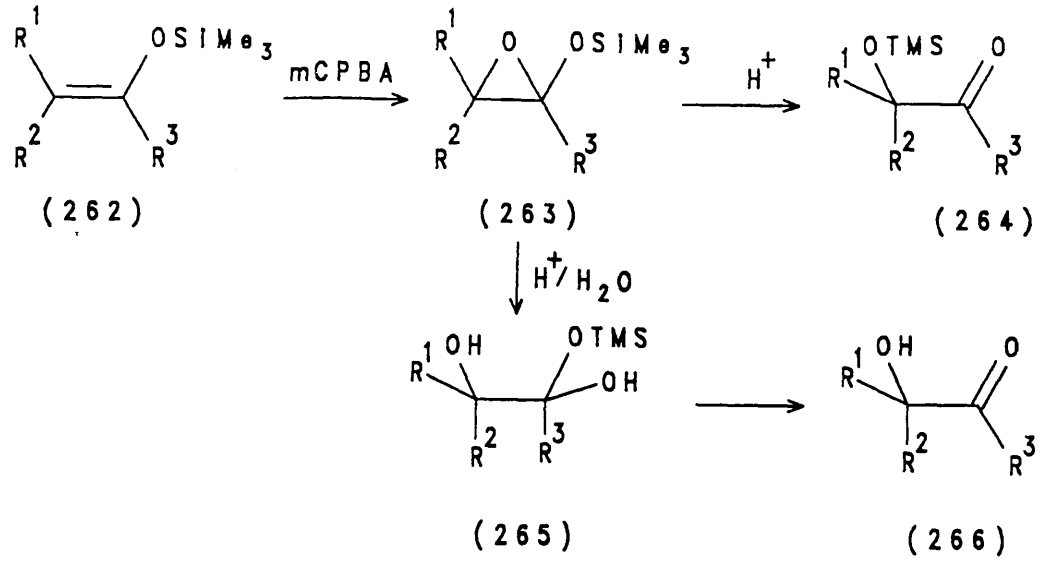


(260) (261)

I ; LDA, TMSCl, Et_3N

Scheme 41

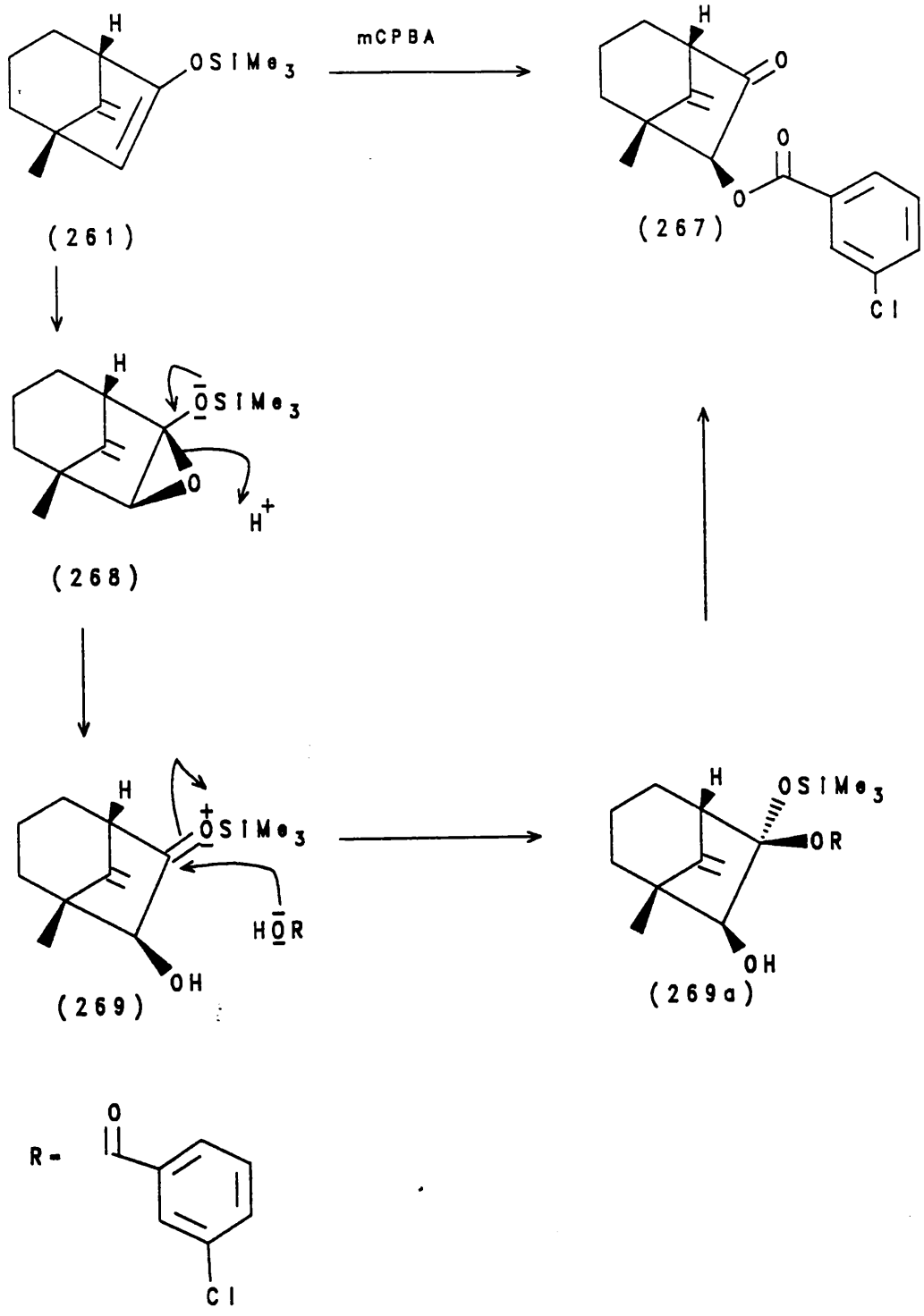
Rubottom has shown[92] that oxidation of silyl enol ethers with mCPBA gives good yields of α hydroxy ketones (Scheme 42). The reaction is believed to proceed by epoxidation of the enol ether (262) to give the silyloxy epoxide (263) which on non aqueous work-up gives the α-trimethylsilyloxy ketone (264). Alternatively aqueous work-up yields the α hydroxy ketone (266) formed via the silyl hemiacetal (265).



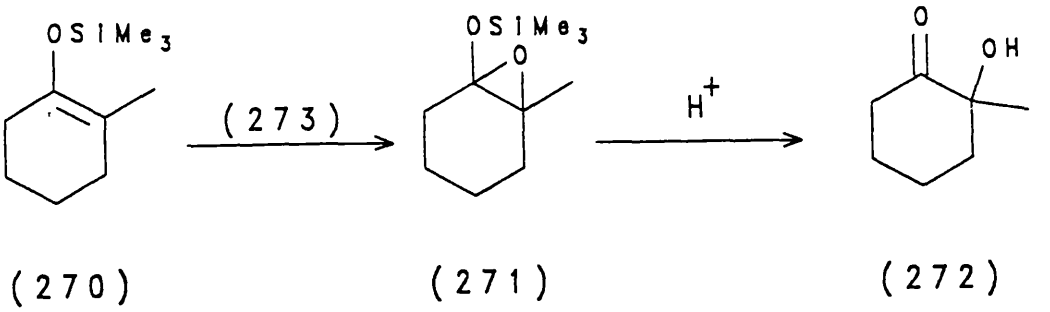
Scheme 42

Addition of a slurry of m-CPBA in hexane to the silyl enol ether (261), followed by heating under reflux for 2 hours gave after chromatographic purification two products of R_f. 0.5 and 0.11 (petroleum ether 40/60). The major product (R_f. 0.5) was not the α hydroxy ketone but appeared to be the benzoate ester (267). (IR: ν maxCCl$_4$: 1760, 1730 and 1665 cm^{-1}. ^{1}H nmr 90 MHz: 7.2 to 7.95 ppm multiplet, 4H; 5.53 ppm, singlet, 1H (C-7H); 5.0 ppm singlet and 4.82 ppm singlet, 2H (C=C$\underline{H}_2$); 3.15 ppm, broad multiplet, 1H (C-5H), and 1.15 ppm singlet 3H (C-9H)). The low resolution mass spectrum showed a parent ion at 304 amu, the molecular weight of (267). Formation of this ester, obtained in 44% yield, could be explained by benzoate ion quenching the oxonium ion (269) (Scheme 43) followed by intramolecular ester exchange. However, methanolysis of this ester gave five discrete compounds indicating that an alternative oxidation was required.

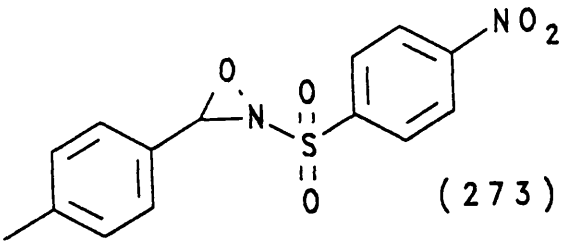
Davis has studied[100] the application of N-sulphonyl oxaziridines in organic synthesis in particular to acyloin formation by oxidation of silyl enol ethers (Scheme 44). The proposed mechanism is similar to that for oxidation with m-CPBA. Indeed, the oxaziridines, being aprotic and neutral reagents allowed isolation of the α-silyloxy epoxide (271), proving its intermediacy in these reactions.



Scheme 43



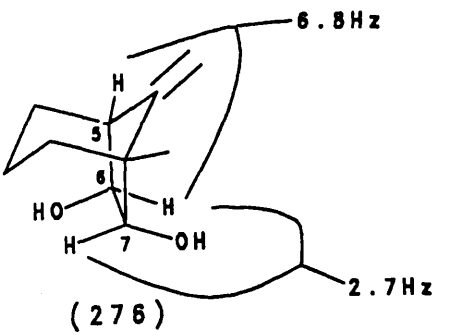
(270) (271) (272)



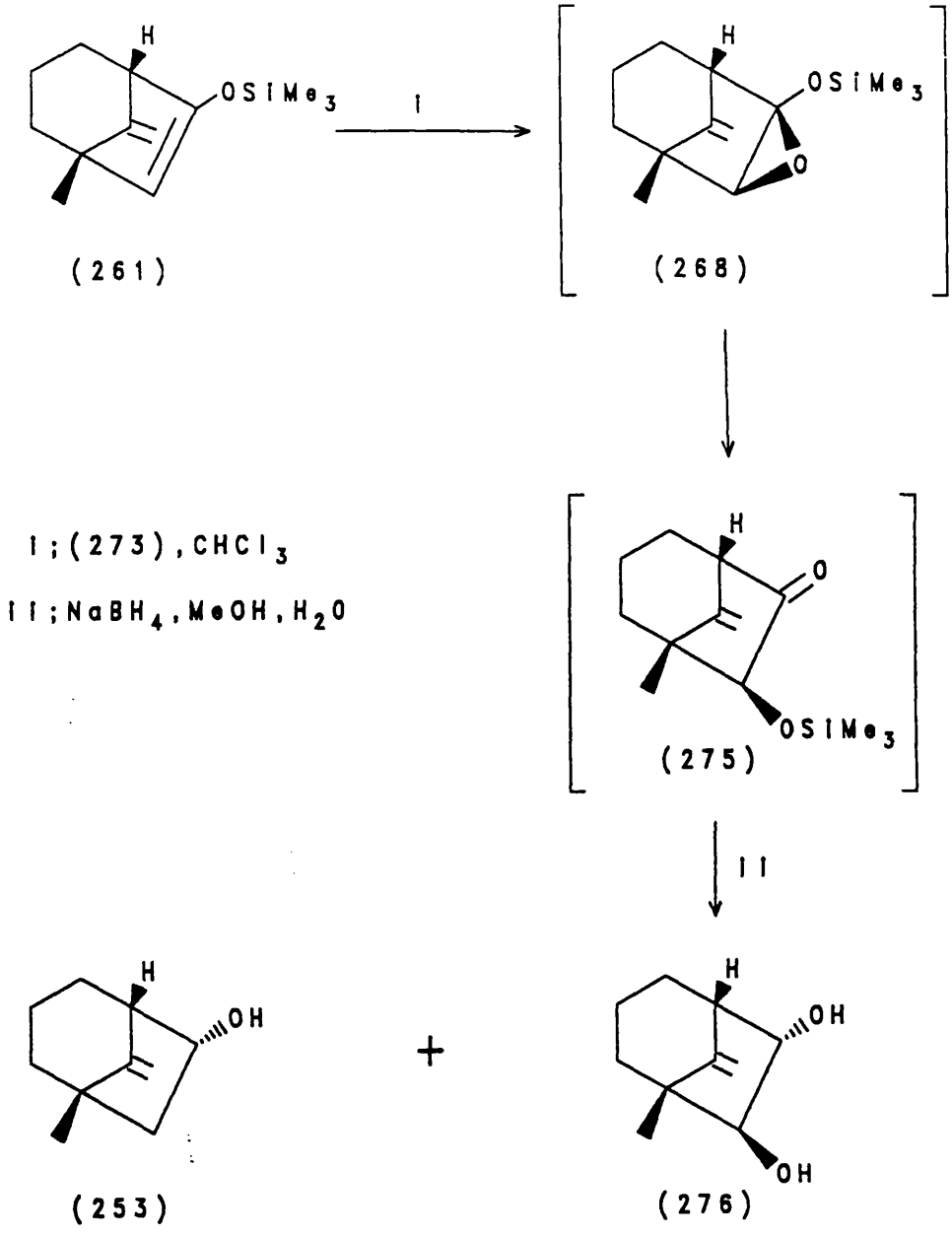
(273)

Scheme 44

Heating a chloroform solution of (261) with 2-p-tolylsulphonyl-3-(p-nitrophenyl) oxaziridine (273) (Scheme 45) at 60°C for two hours followed by concentration gave a yellow orange solid. Pentane extraction, concentration and borohydride reduction of the crude product gave a mixture of seven compounds R_f^s 0.1, 0.26, 0.48, 0.61, 0.88 and 1.0. The compound of R_f 0.26 attracted attention in that on visualising the t.l.c. plate with ceric sulphate solution, it appeared blue, characteristic of olefins in this model series. Isolation gave a solid which recrystalised from chloroform to give white crystals of melting point 142-143°C. ^1H nmr spectroscopy showed that it was indeed the required 6α-, 7β- diol (276) as required.



(276)


H
OSiMe3
I
(261)
(268)
O
I ; (273), CHCl3
II ; NaBH4, MeOH, H2O
(275)
II
OH
+
(253)
(276)

Scheme 45

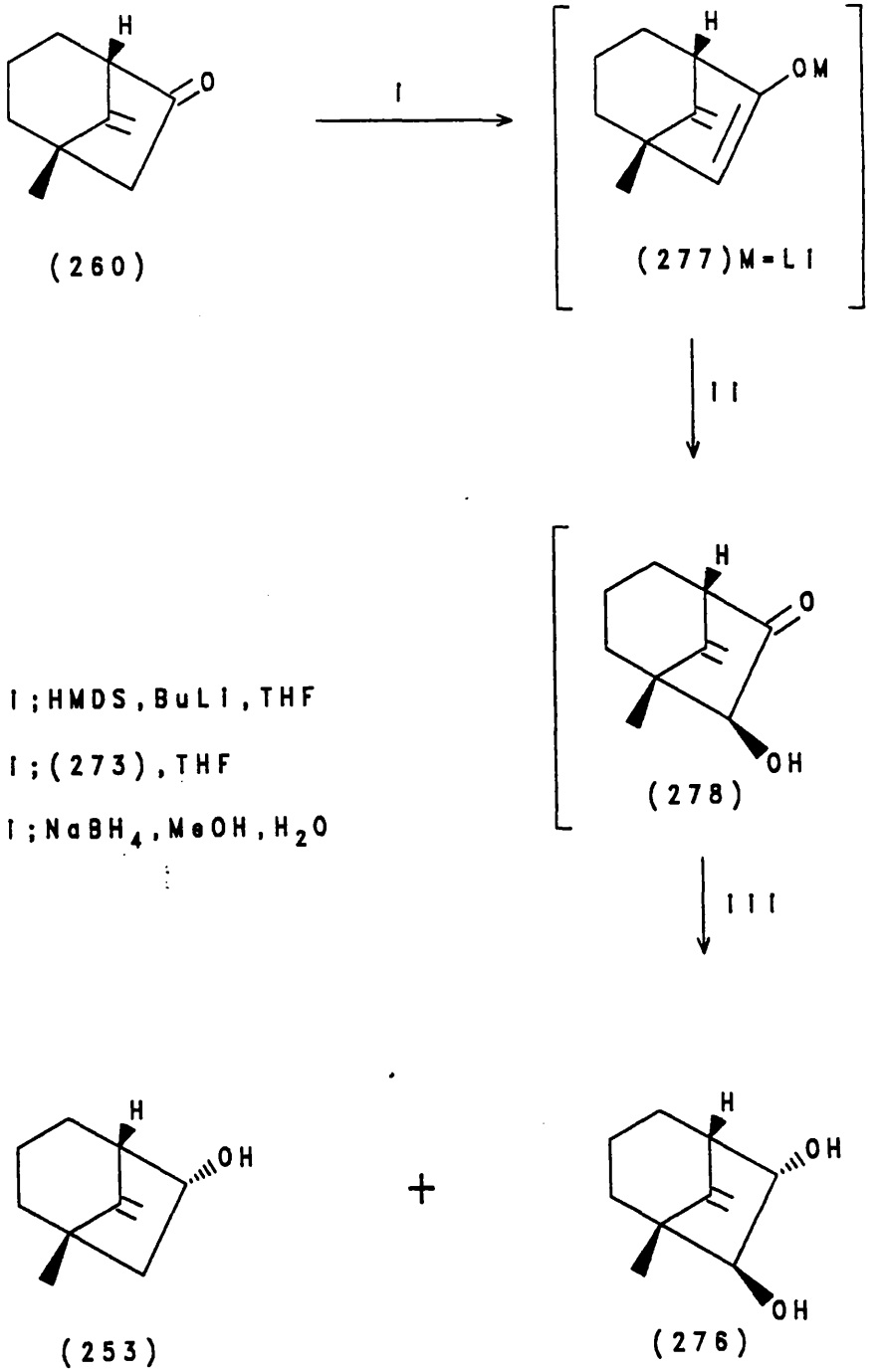
The proton on C7 was seen as a doublet (J = 2.7 Hz) at 3.82 ppm while the proton on C6 was seen as a doublet of doublets (J = 6.8 and 2.7 Hz) at 4.37 ppm. The dihedral angle between the C6 and C7 protons is approximately 60^{o} thus the small trans coupling (2.7 Hz) observed. That the C6 proton is coupled to the C5 proton (J = 6.8 Hz) is indicative that the C6 proton is in a β-orientation by the arguments on page 77 . Thus the diol must have been in the required 6α-, 7β-configurations. Infra red spectroscopy supported this in that on dilution of a carbontetrachloride solution of the diol, the hydrogen bonding stretch at 3450 cm^{-1} disappeared, thus no intramolecular hydrogen bonding was occurring.

The compound of R_f 0.5 was found to be the 6α-alcohol (253) resulting from borohydride reduction of unoxidised ketone; the remainder of the compounds were unidentified.

The 30% yield of diol showed that the required stereocontrolled functionalisation could be achieved. The problem now was one of optimisation. Further attempts at oxidation of the silyl enol ether (261) failed to provide higher yields and so an alternative approach was adopted.

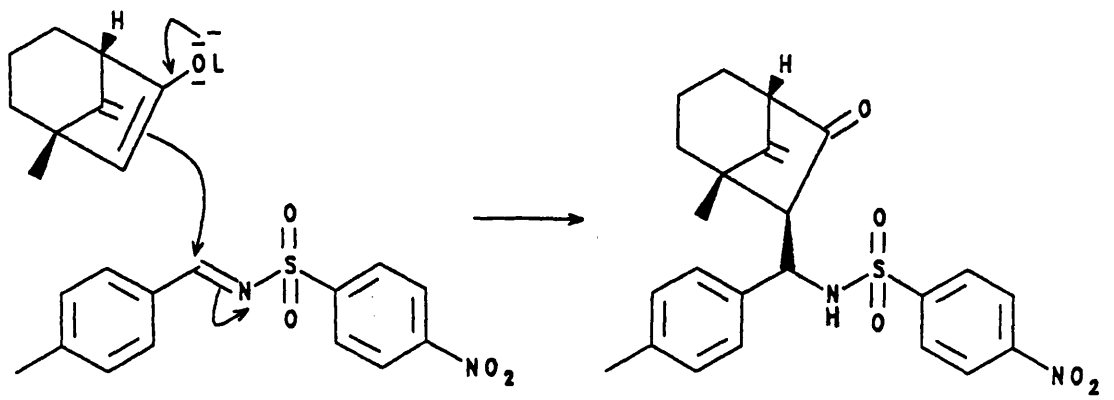
Davis has reported[101] the direct oxidation of enolates with N-sulphonyloxaziridines to be superior to oxidation using the MoOPH reagent; higher yields are obtained and no over-oxidation to 1,2 diketones occurs.

Treatment of (260) with lithium hexamethyldisilazide, (Scheme 46) quenching the so-formed enolate with two equivalents of oxaziridine (273), and reduction of the crude product mixture


H
O
I
H
OM
(260)
(277)M=LI
II
H
O
OH
(278)
I;HMDS,BuLI,THF
II;(273),THF
III;NaBH4,MeOH,H2O
III
H
OH
+
H
OH
OH
(253)
(276)

Scheme 46

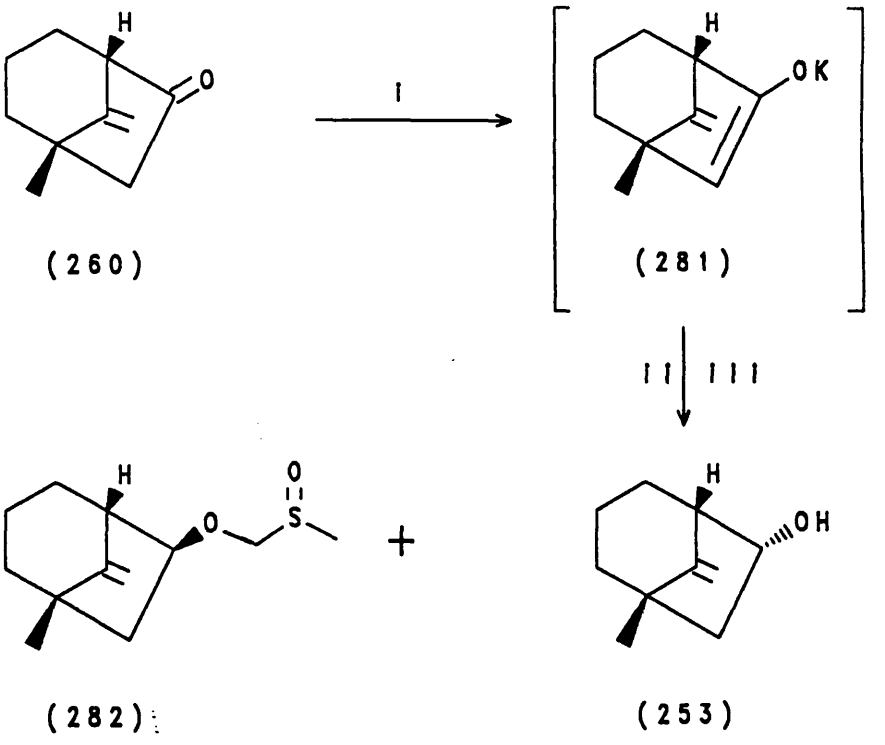
with sodium borohydride gave a 37% yield of diol (276) and a 46% yield of 6α-alcohol (253). Two possible reasons for the low yields and recovery of starting material in this procedure are preferential oxidation of the amine produced in enolate formation and addition of the enolate to the sulphonylimine (279) (Scheme 47). However since two equivalents of the oxaziridine were added, oxidation of the amine should not have been a problem. Further, the adduct (280) was never isolated and indeed generating the more reactive potassium enolate using potassium hexamethyldisilazide failed to increase the yield of diol.



(279) (280)

Scheme 47

Attempted MoOPH oxidation of the potassium enolate provided an explanation to the low yields and recovery of starting material. Following the procedure described by Vedejs, the potassium enolate of (260) was treated with MoOPH (Scheme 48). Work-up and t.l.c. analysis revealed that starting material had apparently been consumed and that a single more polar compound (R_f 0.5) had resulted. Surprisingly, borohydride reduction gave the 6α-alcohol (253) while the compound of R_f 0.5 appeared resistant to borohydride reduction. Isolation of this mysterious compound and characterisation showed that it was derived from the



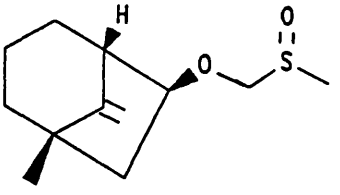
I ; KHMDS (TOLUENE), THF

II ; MoOPH, THF

III ; $NaBH_4$, MeOH, H_2O

Scheme 48

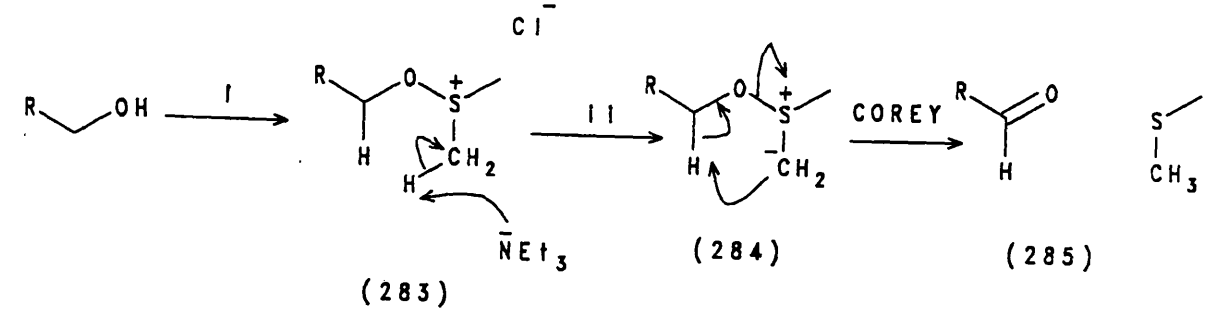
model alcohol (252) but it had an additional methylene and methyl group. Infra red spectroscopy indicated the presence of a sulphoxide (IR:νmax CCl_4: 1040 cm^{-1}) and hence the structure (282) was assigned to this compound.

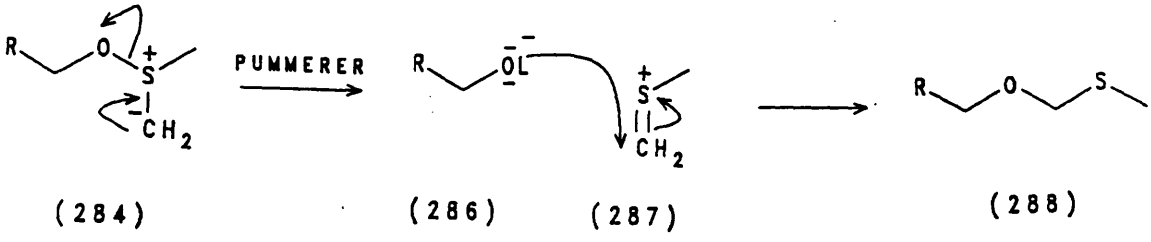


(282)

Corey reported[98] that methyl thiomethyl ethers derived by Pummerer[102] rearrangement of the intermediate ylide (284) (Scheme 49) are side products in the oxidation. It appeared therefore that the methyl thiomethyl ether (282a) was the impurity found in the ketone in this study. This was proven in two ways. The Corey oxidation product was treated[103] with methyl iodide in wet acetone and indeed the alcohol (252) was recovered (Scheme 50). Secondly the Corey oxidation product was reduced by borohydride yielding the 6α-alcohol (253) and the methyl thiomethyl ether (Scheme 51) which was isolated by column chromatography and fully characterised.

The sulphoxide (282) produced in the MoOPH oxidation was merely the product of oxidation of the methylthiomethyl ether (282a).

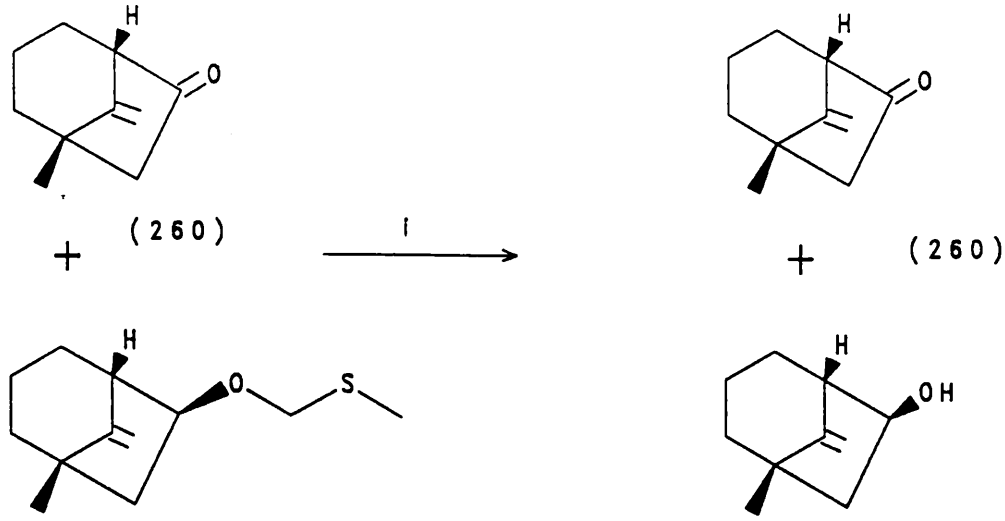




I ; NCS , DMS , CH_2CL_2 (OR TOLUENE)

II ; Et_3N

Scheme 49

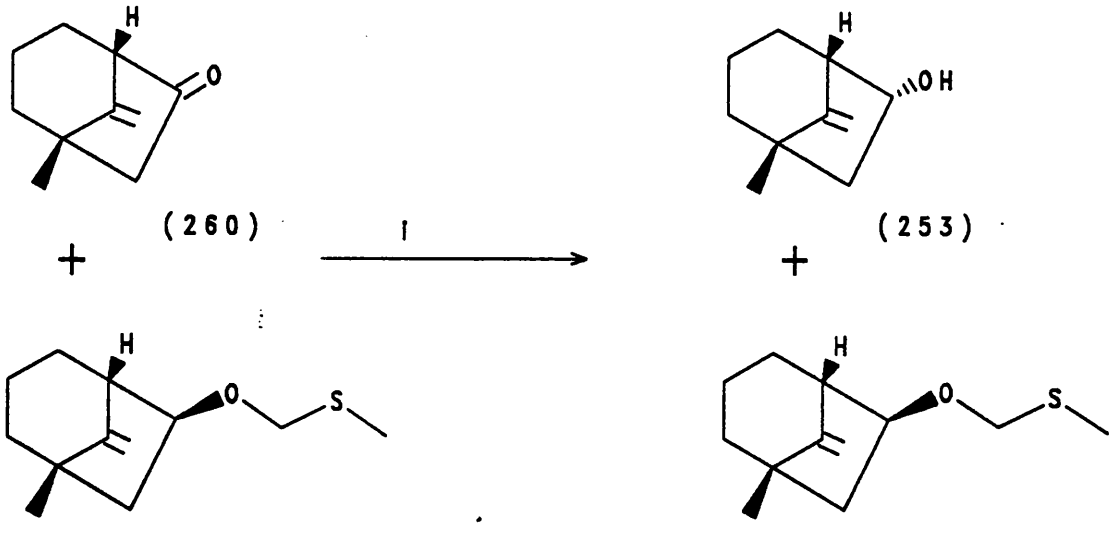


(282a)

(252)

I ; MeI , H_2O , Acetone

Scheme 50



(282a)

(282a)

I ; $NaBH_4$, MeOH , H_2O

Scheme 51

Formation of the Pummerer rearrangement product should be minimised using non-polar solvents, hence the frequent use of toluene in Corey oxidations. However, even when the oxidation was performed in toluene integration of the ^{1}H nmr spectrum of the product mixture indicated that the methyl thiomethyl ether constituted 7% of the mixture. A second anomalous result appeared from the elucidation of the impurity. The ketone (260) did not visualise in t.l.c. analysis by treatment with ceric sulphate, the normal staining reagent for the model compounds. This may have been the reason for the apparent low yields in Jones oxidation, i.e., material was being discarded due to the fact that it was not being visualised on t.l.c. analysis of the column chromatographic fractions.

Indeed, repeating the Jones oxidation and visualising the t.l.c analysis of the chromatographic fractions with water resulted in a 57% yield of pure ketone. However a non acidic oxidation was required for the intended functionalisation. Chromium trioxide-3,5-dimethylpyrazole complex is a mild basic oxidising system[104]; indeed, on stirring the alcohols (252) and (253) in dichloromethane with this complex a 73% yield of pure ketone (260) was obtained.

Using ketone produced by this oxidation, a final attempt at MoOPH hydroxylation was tried. Treatment of the potassium enolate of the ketone with this reagent gave recovery of starting material. Why this reagent failed to deliver an oxygen to the relatively unhindered enolate (281) could not be explained.

Treatment of the potassium enolate of the pure ketone with the oxaziridine (273) gave on work-up a yellowish-orange solid which by t.l.c. analysis contained many polar products. This

crude mixture was acetylated and the resulting solid triturated thoroughly with hexane. On concentration and chromatographic separation the α-acetoxy ketone (289) (Scheme 52) was isolated in 64% yield from the ketone. On repeating this procedure a 69% yield of α-acetoxy ketone was realised, thus a method for the satisfactory introduction of a 7α-orientated oxygen function had been achieved.

To complete the diol formation, borohydride reduction gave the acetoxy-alcohol (290) which on methanolysis gave the desired diol (276). Further treatment of the olefin with mCPBA gave a single epoxide diastereoisomer; presumably the mCPBA attacks the olefin from the less hindered exo face of the molecule giving the epoxide (291).

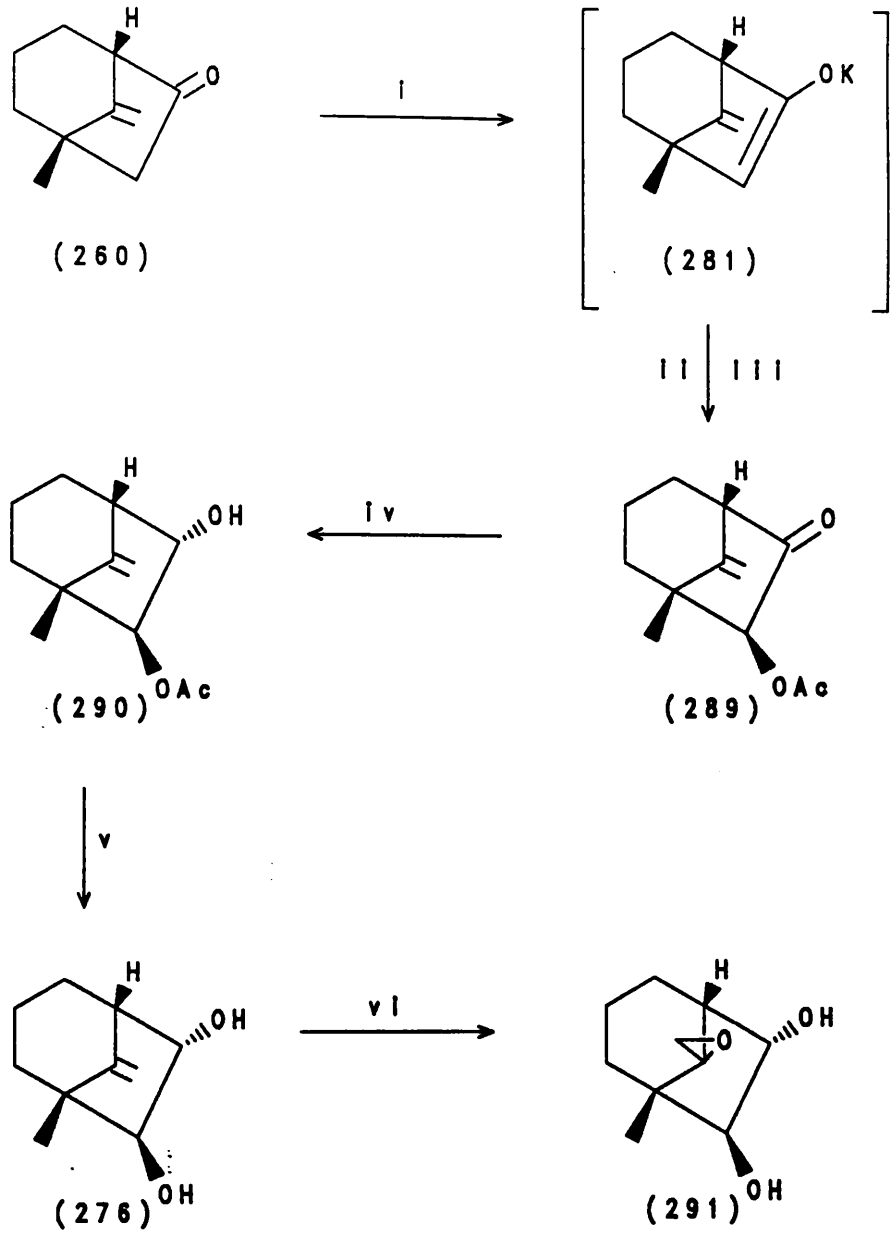
This model study revealed a number of points relevant to the synthesis of T-2 tetraol:-

1) In the Wittig olefination, yields are highest when the tetrahydropyranyl rather than acetyl or triethylsilyl group is used to protect the C3 alcohol.

2) Chromium trioxide-3,5-dimethyl pyrazole is the non acidic oxidising system of choice for C3 oxidation of the T-2 tetraol precursor (239)

3) In that their formation requires an additional synthetic and purification step and that yields of 3α-, 4β-diol from them are low, silyl enol ethers are an inefficient route for ring C formation.

4) Acyloin formation by MoOPH oxidation of the enolate of the bicyclo[3,2,1]octane subunit appears unsatisfactory.



I ; KHMDS(TOLUENE), THF

II ; (273), THF

III ; Ac_2O, Py

Iv ; $NaBH_4$, MeOH, H_2O

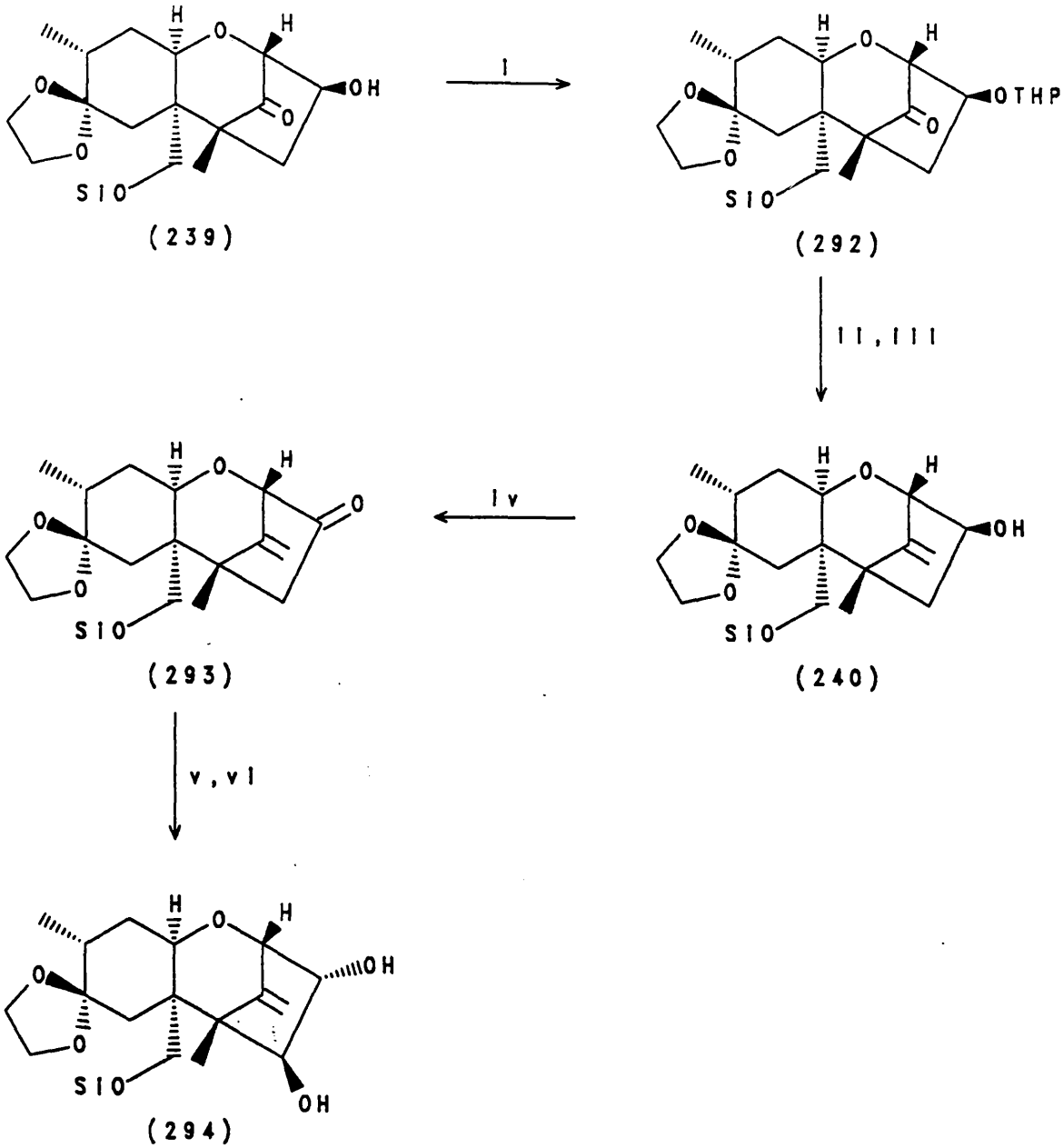
v ; MeOH, K_2CO_3

vI ; mCPBA, CH_2Cl_2, Na_2HPO_4

Scheme 52

and 5) The ring C functionalisation for T-2 tetraol best achieved by oxidation of the C3 ketone-enolate with Davis's N-sulphonyl oxaziridine reagent and subsequent borohydride reduction. By this method, stereoselective functionalisation can be achieved.

All of the above points were taken into consideration in the synthesis of T-2 tetraol, the diol (294) being synthesised from the keto-alcohol (239) by the route shown (Scheme 53).

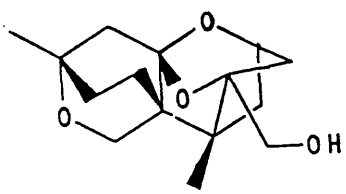


I ; Dihydropyran, PPTS
II ; Ph_3PCH_2, THF
III ; EtOH, PPTS
IV ; CrO_3, 3,5-Dimethylpyrazole, CH_2Cl_2
V ; KHMDS, THF/(273), THF
VI ; $NaBH_4$, MeOH, H_2O

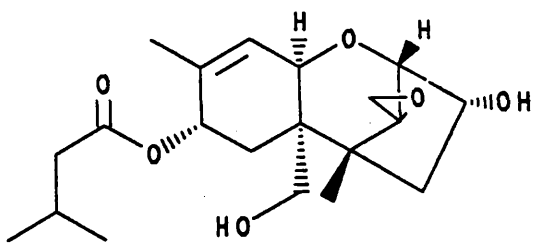
Scheme 53

2.2 8-Keto-anguidine.

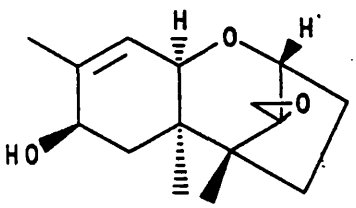
In a study aimed at understanding the role of secondary metabolites in the toxicoses associated with ingestion of *Fusarium* infected grain, Rottinghaus et al isolated[105,106] from *F. sporotichioides* a number of trichothecenes, including sporol (31), sporotrichiol (295), 8β-hydroxytrichothecene (296), 8-oxo-diacetoxyscirpenol (8-keto-anguidine) (297) and 4-propanoyl-HT-2 toxin (298), the first trichothecene to be isolated with a propanoyl ester function.



(31)



(295)



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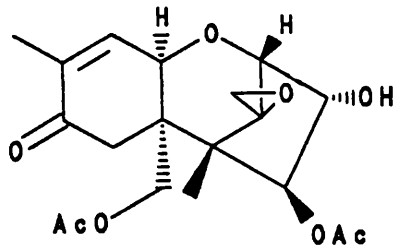
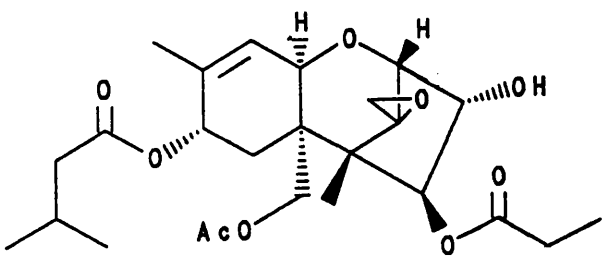
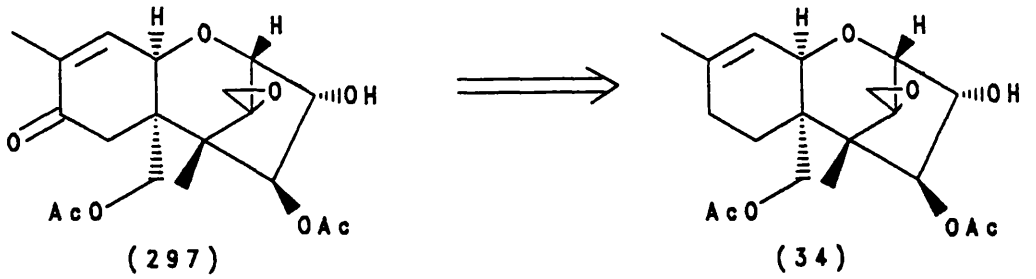


(297)



(298)

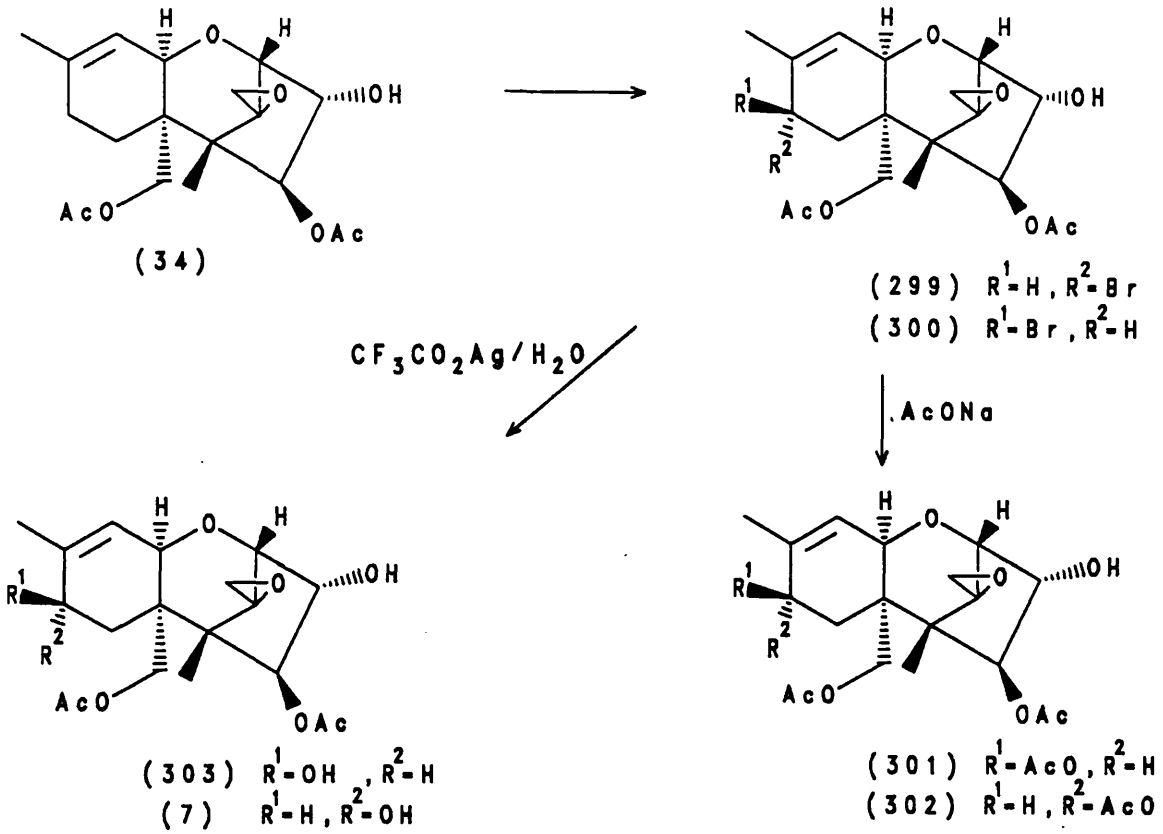
Our interest was aroused by 8-keto-anguidine in that it is a simple analogue of anguidine (34), the starting material for several partial syntheses as described in the introduction. Comparison of anguidine with the title compound indicates that an allylic oxidation of (34) is all that is required for synthesis of (297) (Scheme 54).



Scheme 54

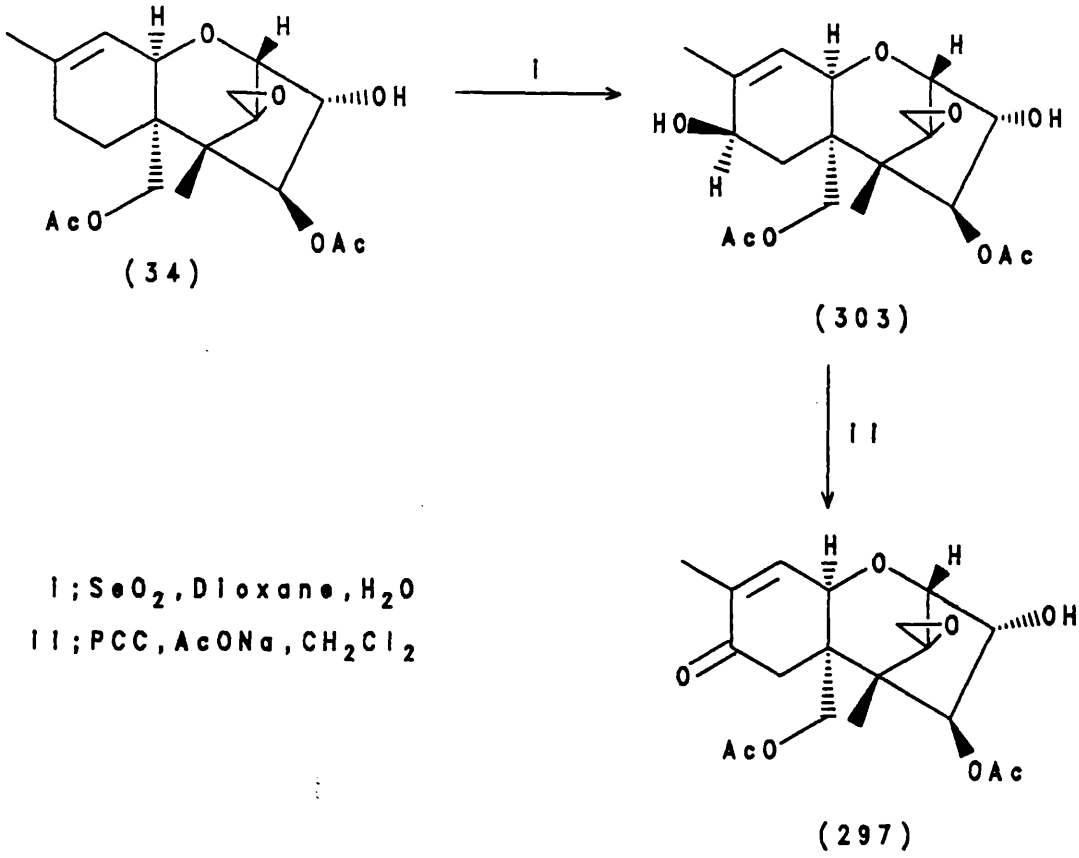
Allylic oxidations of trichothecenes have been reported by several groups. Gorst-Allman et al converted[107] anguidine into neosolaniol mono-acetate(302) and its epimer (301) (Scheme 55) in an overall yield of 45% via the allylic bromides (299) and (300).

Kaneko et al irradiated[27] anguidine in the presence of NBS to produce the bromides (299) and (300) which on treatment with silver trifluoroacetate yielded neosolaniol (7) and its epimer in an overall yield of 24% from anguidine.


(34)
(299) R1=H, R2=Br
(300) R1=Br, R2=H
CF3CO2Ag/H2O
AcONa
(303) R1=OH, R2=H
(7) R1=H, R2=OH
(301) R1=AcO, R2=H
(302) R1=H, R2=AcO

Scheme 55

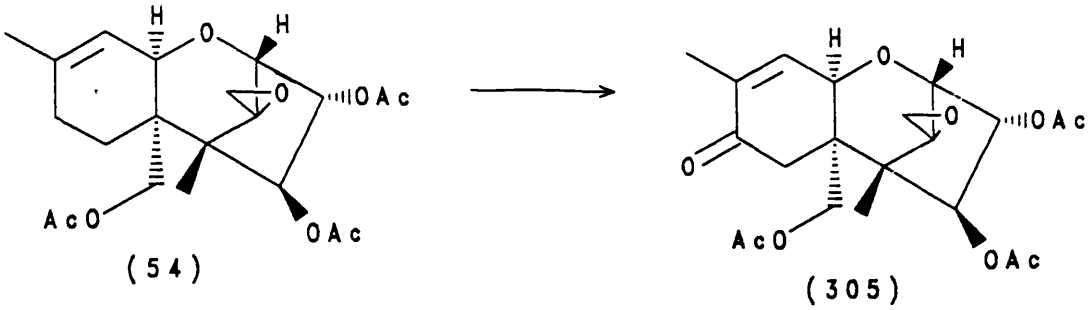
8-Keto-anguidine was in fact synthesised[27] prior to its isolation (Scheme 56). Selenium dioxide oxidation of anguidine afforded the 8β-hydroxy derivative (303) in 39% yield; this on oxidation with PCC gave 8-keto-anguidine (297) in an overall yield of 31%.



(34) (303) (297)

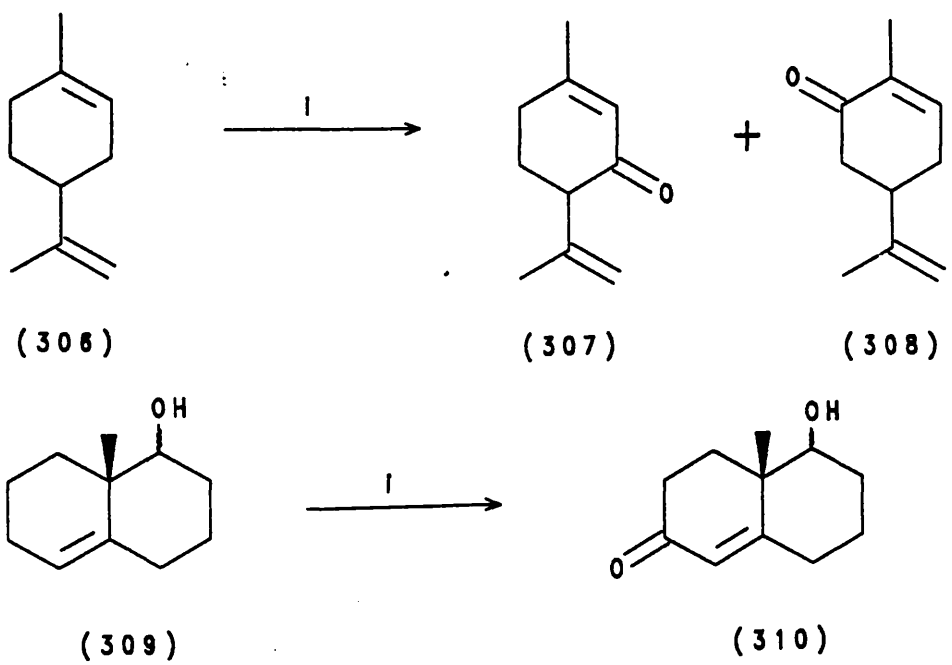
Scheme 56

Oxidation of triacetoxysclrpenol (54) directly to the enone (305) (Scheme 57) has been effected[108] in low yield with t-butyl chromate and, as reported[71] by Colvin and Cameron, in excellent yields of up to 60% by treatment with dipyridine-chromium trioxide. However this 'Collins' oxidation requires[109] a large excess of reagent, long reaction times and an extensive work-up.



Scheme 57

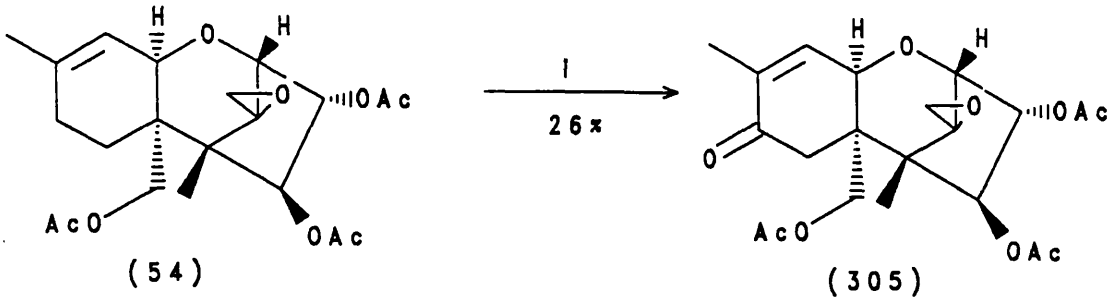
For the synthesis in hand the chromium carbonyl catalysed oxidation of alkenes to enones reported by Pearson appeared much more convenient. For example Pearson found that refluxing (306) (Scheme 58) in acetonitrile for 18 hrs in the presence of a catalytic amount of $Cr(CO)_6$ and 2 equivalents of t-butyl hydroperoxide gave the enones (307) and (308) in 65% yield, with no exocyclic oxidation products being detected. Pearson showed that this oxidation could also be carried out in the presence of certain secondary alcohols by the oxidation of (309) to (310) in 60% yield.



I ; $Cr(CO)_6$, MeCN, ^tBuOOH

Scheme 58

Unfortunately, it was found that even after extensive experimentation on reaction times and equivalents of oxidising agent, improvements on a yield of 26% of (305) from triacetoxyscirpenol (54) could not be achieved.



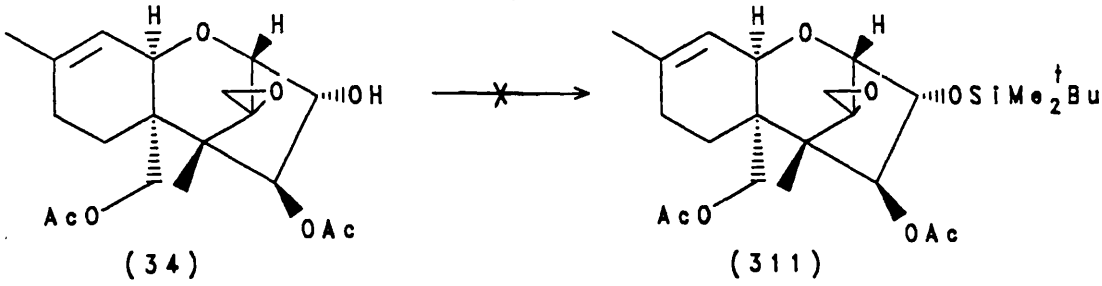
I ; $Cr(CO)_6$, MeCN, ^tBuOOH

Scheme 59

As well as being an excellent reagent for effecting allylic oxidations, dipyridine-chromium trioxide is also an extremely efficient reagent for the oxidation of primary and secondary alcohols.[111] Thus the synthesis of 8-keto-anguidine from diacetoxyscirpenol by oxidation with dipyridine-chromium trioxide required that the 3α-hydroxyl of anguidine be protected. The protecting group had to be one which was stable to the basic conditions of the oxidation and which could be selectively removed in the presence of acetates after the oxidation was completed.

The t-butyldimethylsilyl group seemed to fulfil these criteria. However, stirring anguidine with chloro-t-butyldimethylsilane in the presence of triethylamine and DMAP resulted in recovery of starting material. Indeed even under the most vigorous silylating conditions, i.e., utilising[112] t-butyl-dimethylsilyltriflate and 2,6-lutidine in the presence of 4A molecular sieves, no reaction was observed. It can only be

concluded that such silylation of the 3α-hydroxyl group of the trichothecenes is impossible, probably due to the steric demands on this bulky protecting group.

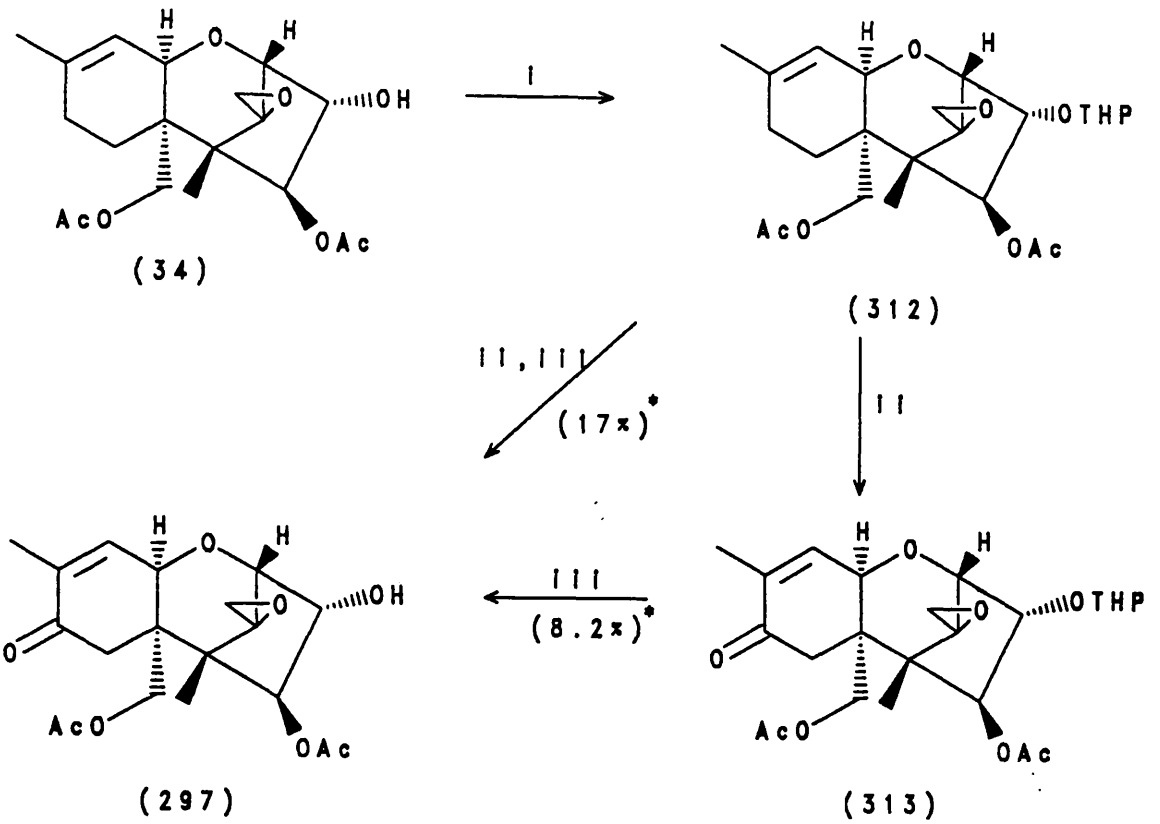


Scheme 60

Tamm employed[70] the tetrahydropyranyl group for protection of the 3α-hydroxyl group of anguidine in the synthesis of calonectrin. Being a base stable acetal, this group seemed to be an ideal choice for protection of the 3α-hydroxyl in this synthesis. Indeed by stirring anguidine in neat dihydropyran in the presence of a catalytic amount of PPTS, near quantitative protection of the 3α-hydroxyl occurred giving the THP-ether (312) (Scheme 61) as a mixture of inseparable diastereomers. Treatment of (312) with freshly prepared dipyridine-chromium trioxide in dichloromethane for five days at room temperature gave in poor yield (10%) an oil which showed in its ^{1}H nmr spectrum a doublet of quartets (J = 5.9 and 1.5 Hz) at 6.57 ppm, a characteristic signal for the C10 proton of trichothecenes possessing an enone function in ring A. Removal of the tetrahydropyranyl group by stirring (313) in ethanol in the presence of catalytic PPTS gave 8-keto-anguidine in a low overall yield of only 8.2%.

In order to eliminate a purification step it was decided that the crude product mixture from the allylic oxidation should be treated with ethanol and PPTS to furnish 8-keto-anguidine

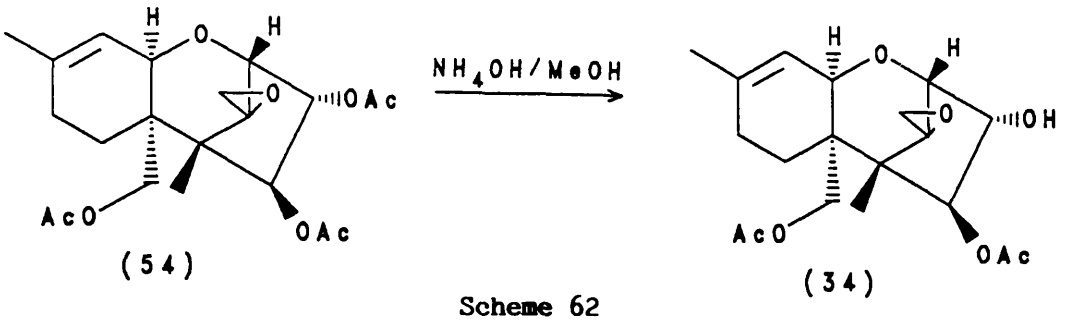
directly. Following the same procedure as previously described but without isolation of (313) an improved but still low yield of 17% of 8-keto-anguidine from (34) was achieved.



Scheme 61

With such poor yields, attention turned to another protecting group for the 3α-hydroxyl group. In the synthesis of anguidine Brooks selectively cleaved[56] the 3α-acetate of (54)

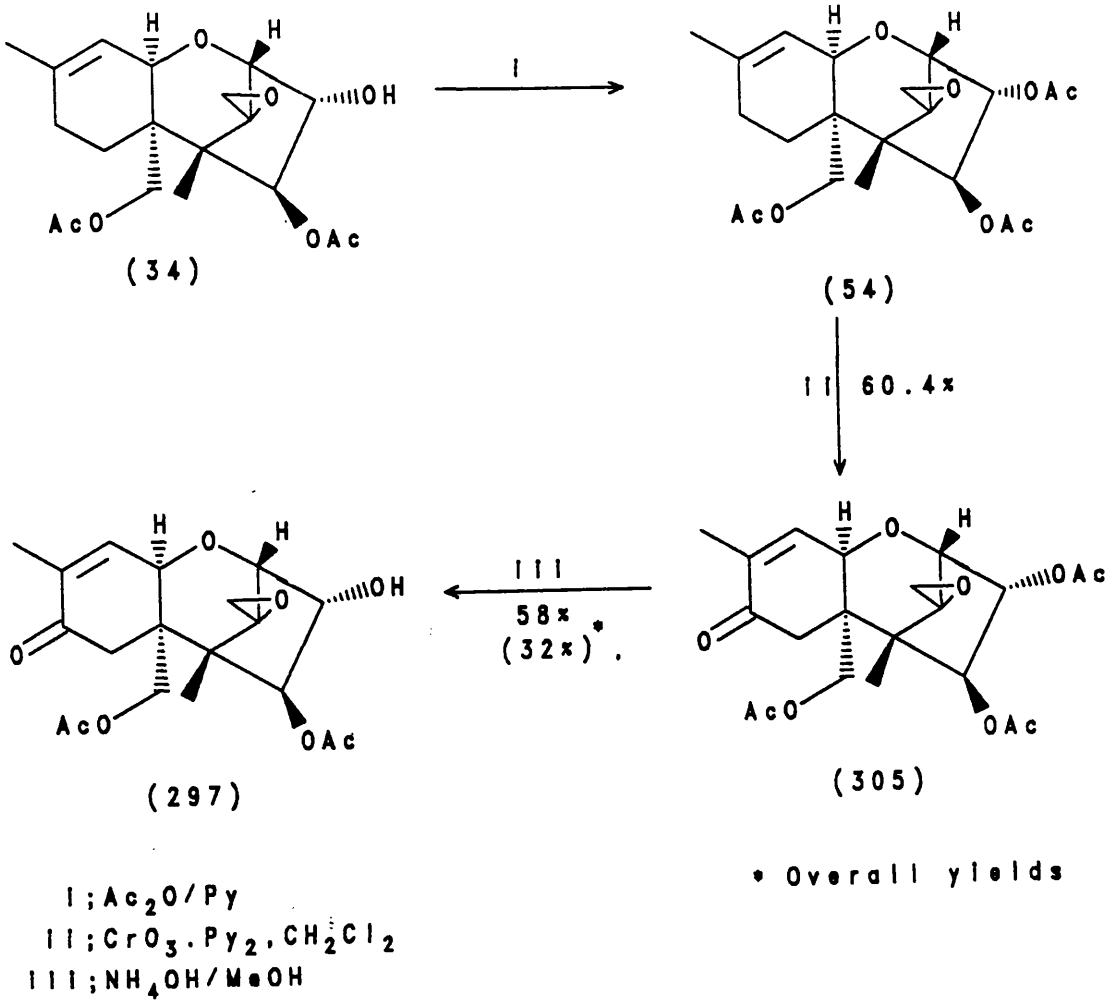
(Scheme 62) in 65% yield by treatment with ammonium hydroxide (2M) in methanol.



(54) (34)

Scheme 62

Thus, acetylation of anguidine furnished the triacetate (54)(Scheme 63) in high yield. Treatment of this with freshly prepared dipyridine-chromium trioxide in dichloromethane gave the enone (305) in 60.4% yield with a 24% recovery of starting material. On the basis of starting material consumed an exceptionally high yield (84%) for the allylic oxidation had been achieved. On stirring this enone in the presence of ammonium hydroxide (2M) in methanol for one hour, t.l.c. analysis revealed that the starting material had been consumed and that two compounds had been formed. The major compound was by t.l.c comparison. 8-keto-anguidine (297) and indeed purification by flash chromatography of the product mixture gave (297) in 58% yield from (305) representing an overall yield of 32%.

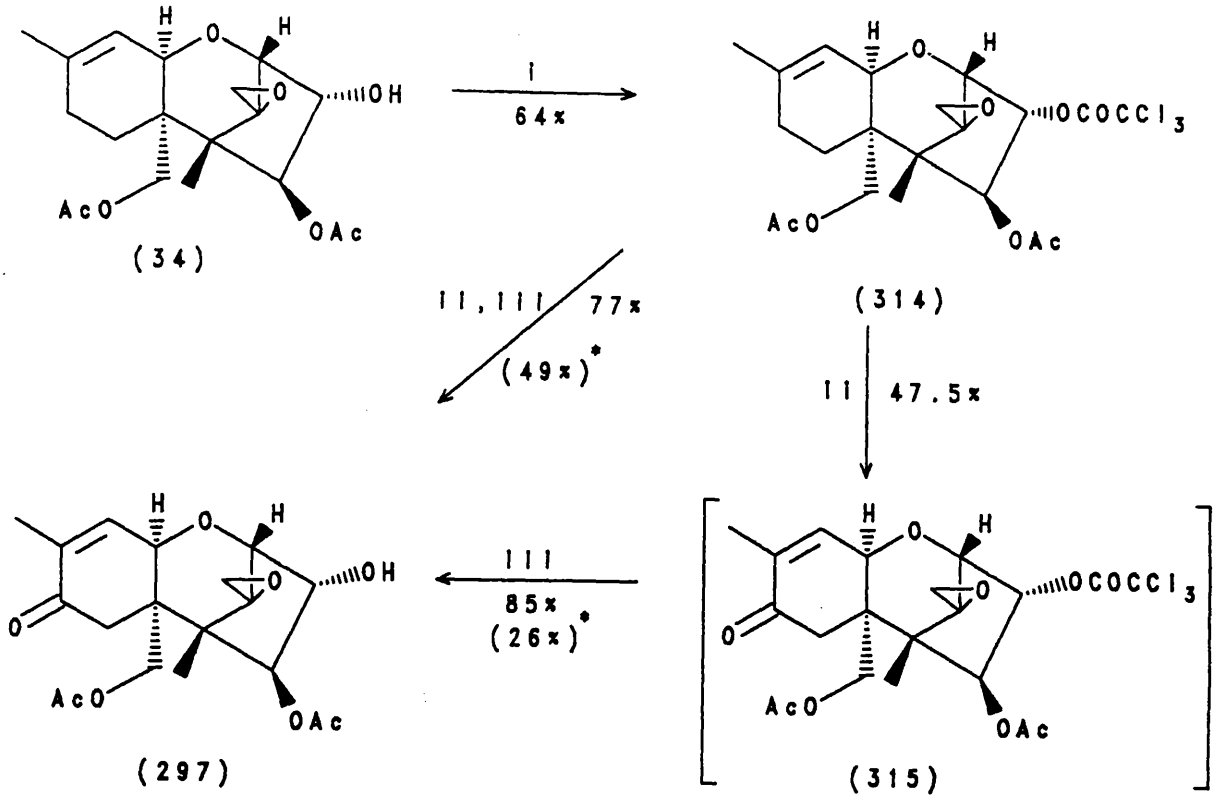

I
II 60.4%
III
58%
(32%)*
(34)
(54)
(305)
(297)
* Overall yields
I ; Ac_2O/Py
II ; $CrO_3.Py_2, CH_2Cl_2$
III ; NH_4OH/MeOH

Scheme 63

The third and final protecting group examined was trichloroacetate, which can be selectively removed in the presence of acetates by treatment with ammonium hydroxide in ethanol.[113] It was thought that in the absence of a good nucleophile, this group should also be relatively stable to the basic conditions encountered in the Collins oxidation.

Stirring anguidine in DMF at room temperature, in the presence of trichloroacetyl chloride, DMAP and triethylamine gave a brown solid which on crystallisation from ether furnished the trichloroacetate(314) (Scheme 64) in 64% yield. Crystallisation was the preferred method of purification in that the trichloroacetyl group appeared unstable to chromatographic purification. Subjection of this compound to Collins oxidation gave, after an aqueous work-up, a 47.5% yield of the crude trichloroacetate enone (315) showing the characteristic doublet of quartets at 6.4 ppm in its ^{1}H nmr spectrum. In the knowledge that the trichloroacetyl group was not stable to column chromatography, this crude material was stirred in dichloromethane in the presence of ethanolic ammonium hydroxide and the reaction followed by t.l.c analysis. After 30 mins, t.l.c analysis indicated consumption of starting material and only one compound to be present. Chromatographic purification gave a clear oil that was characterised as 8-keto anguidine, produced in 85% yield from the crude product of allylic oxidation. It was noted that even on extended reaction times of 3 hrs for trichloroacetate

cleavage no products of acetate cleavage were detected by t.l.c analysis.



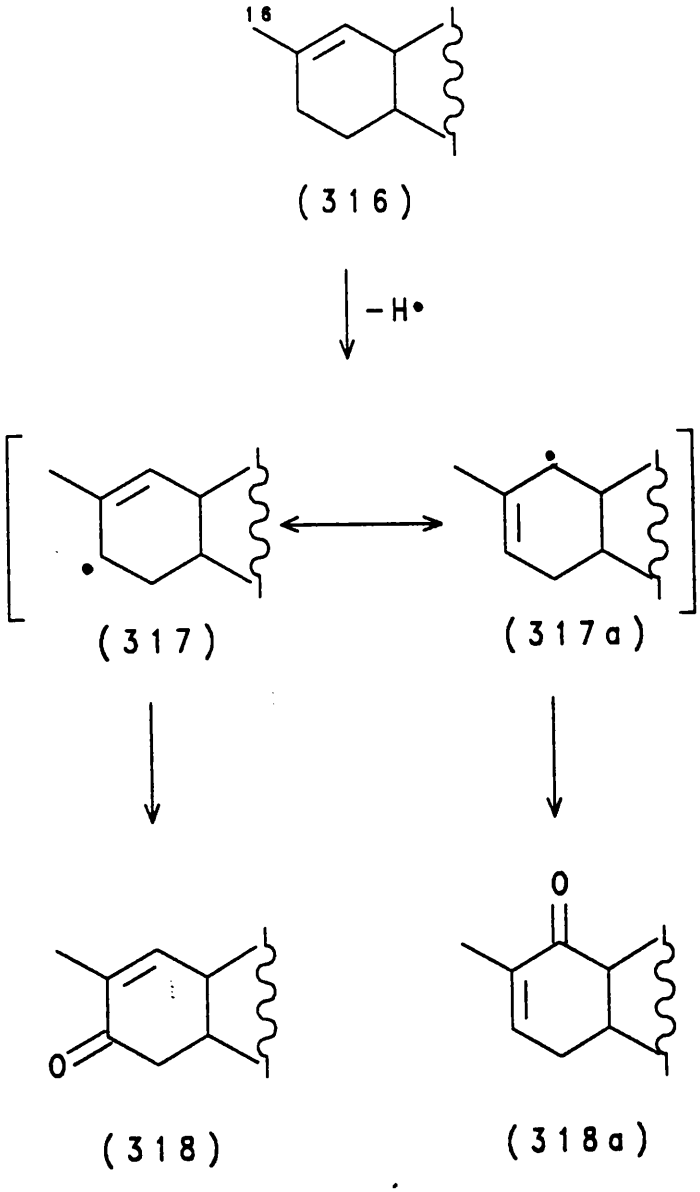
Scheme 64

The extensive work-up associated[109] with the Collins oxidation may have contributed to the low yield, hence a modified work-up was tried. The oxidation was repeated by stirring the trichloroacetate in the presence of a large excess of fresh Collins reagent. Concentration of the reaction mixture, dilution with ether and washing consecutively with aqueous $CuSO_4$, water and brine followed by drying and concentration gave a yellowish oil.

^{1}H nmr Spectrosopy of this oil showed that it was a mixture of the 3α-trichloroacetate (315) and the 3α-alcohol (297). Stirring this mixture in ethanolic ammonium hydroxide gave after column chromatography 8-keto anguidine in a yield of 77.6% from trichloroacetyl anguidine (314). This represented a highly successful allylic oxidation and an overall yield of 49% from anguidine.

This partial synthesis has further demonstrated both the importance of anguidine in trichothecene chemistry and also the superiority of dipyridine-chromium trioxide over other allylic oxidising agents for the formation of ring A enones in the trichothecenes series.

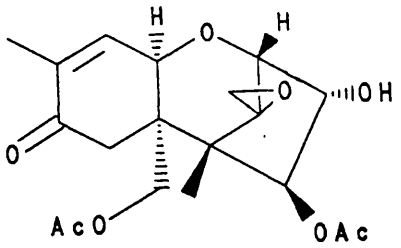
The oxidation is believed to proceed by abstraction of an axial allylic hydrogen to give the allylic radical (317) (Scheme 65) which is subsequently oxidised to the enone (318). It is interesting to note that the isomeric enone (318a) was never isolated and that the C16 methyl group is unaffected by the reagent as oxidation there requires formation of a less stable primary radical.



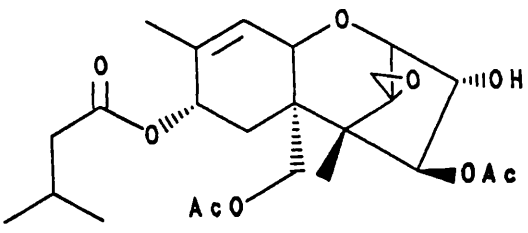
Scheme 65

2.3 Ring A Enone Reduction Studies.

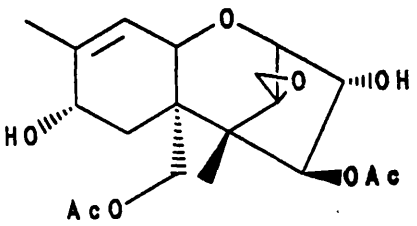
It was obvious, having synthesised 8-ketoanguidine (297) that this allowed access to the series of trichothecenes which possess an 8α-oxygen function such as T-2 toxin (4), neosolaniol (7) and HT-2 toxin (228) merely by stereoselective reduction of the enone.



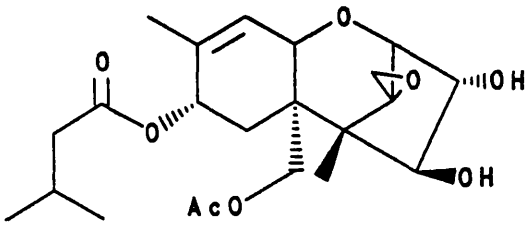
(297)



(4)



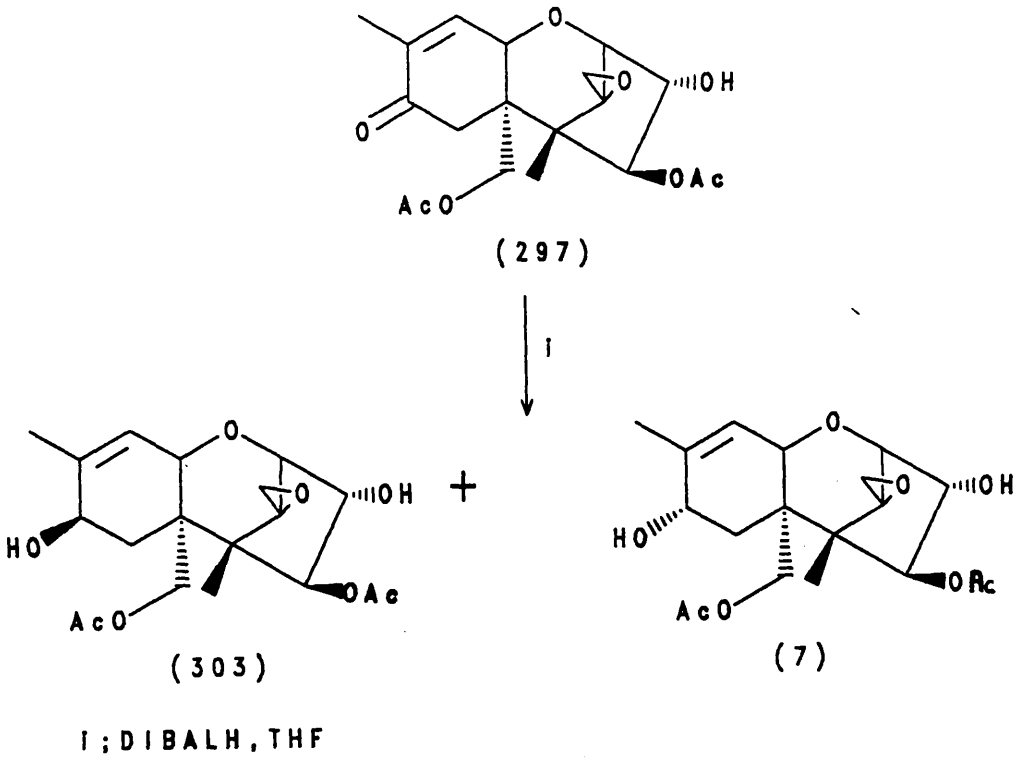
(7)



(228)

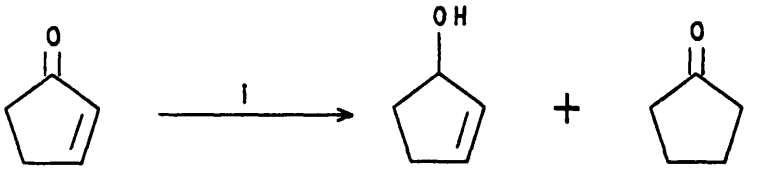
That this reduction could indeed be achieved stereoselectively was shown when Kaneka reported[27] that treatment of the enone (297) with DIBALH produced the 8α- and 8β-alcohols in yields

of 39% and 18% respectively (Scheme 66). This result is in agreement with the fact that the β-face of the molecule is more accessible than the α face due to the C15 acetate.



Scheme 66

The stereochemical outcome of such a reduction was directly relevant to our groups approach to T-2 tetraol (19) in that the 8α-allylic alcohol of this molecule would be introduced by this method. Therefore quantification of the ratio of 8α- and 8β-alcohols produced by reduction of the enone was required as well as experimentation to achieve stereospecificity in the reduction. In that quantification of the ratio of 8α- to 8β-epimers would be determined by gas chromatographic analysis it was deemed prudent to acetylate the crude reduction product in order to make g.c. analysis more practical. Formation of the tetra-acetates was also attractive from the point of view that the 8α-acetate (319)



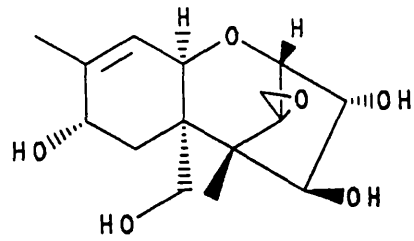
(320) (321) : (322)

97 : 3

I ; $NaBH_4$, $CeCl_3$, MeOH

Scheme 67

was in fact the tetra-acetate of T-2 tetraol, the target of our total synthesis.

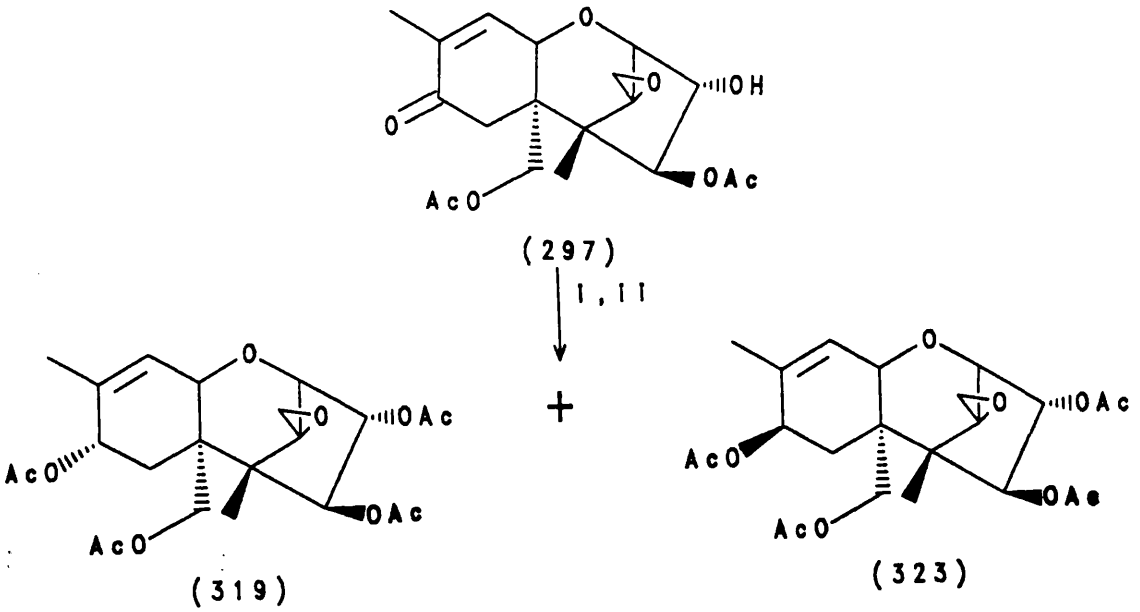


(19)
T-2 TETRAOL

The reduction of enones to allylic alcohols is often complicated by the problem of 1,4 versus 1,2 hydride addition. This can be prevented by the use of sodium borohydride in the presence of lanthanide salts, in particular cerium trichloride.[114] With this reducing system the ratio of 1,2 addition over 1,4 addition to cyclopentenone was 97:3 (Scheme 67). Luche has explained[115] the regioselectivity of this reduction in terms of the Hard-Soft acid and base theory. Kinetic data suggested that the Ce^{3+} was not complexing with the enone but rather catalysing the decomposition of BH_4^- to alkoxyborohydrides, and that it was the alkoxyborohydrides which were effecting the reduction. Alkoxyborohydrides, being 'harder' than borohydride, attack the enone at the hard carbonyl carbon of the enone.

Treatment of the enone (297) with sodium borohydride (Scheme 68) in the presence of 1 equivalent of $CeCl_3$ at -78^oC and acetylation of the crude reaction mixture gave by t.l.c analysis, two compounds of R_fs 0.29 and 0.24, with the less polar compound apparently in excess. Isolation of these two compounds by positive pressure chromatography and comparison of their ^1H nmr spectra with those of the 8α tetraacetate as quoted in the

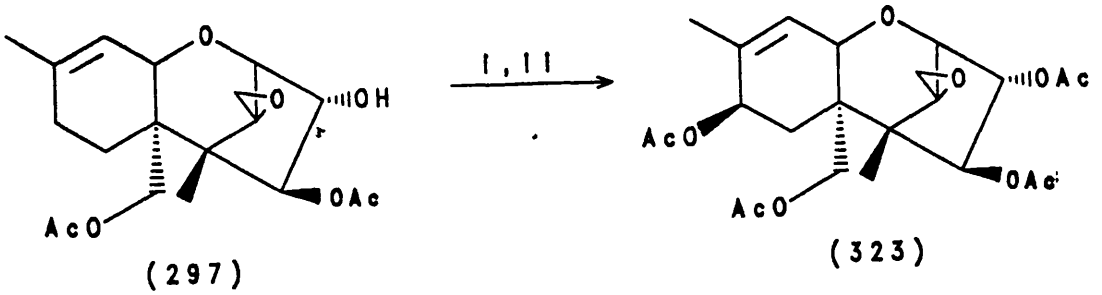
literature[116], suggested that the minor product was the required 8α-compound (319) and that the major product was in fact the unnatural β-epimer (323) isolated in 70% yield.



I ; $NaBH_4$, $CeCl_3$, MeOH
II ; Ac_2O, Py

Scheme 68

This compound was shown to be identical to the compound produced by acetylation of the product of selenium dioxide oxidation of anguidine which gives exclusively the 8β-epimer, the selenium dioxide having effected oxidation of C8 from the more accessible β-face (Scheme 69).



I ; SeO_2, Dioxane, H_2O
II ; Ac_2O, Py

Scheme 69

These epimers (319) and (323) were clearly distinguishable by ^1H nmr spectroscopy in that the 8α-orientated epimer (319) showed in the region of δ5.0 to 5.8 ppm clearly defined signals for the protons on C3 (5.17 ppm, dd 4.9, 3.3 Hz), C8(5.23 ppm, bd; 5.2 Hz), C10(5.73 ppm, dt; 5.8 and 1 Hz) and C4(5.80 ppm, d; 3.3 Hz) whereas the 8β-epimer showed the C8 proton and the C10 protons to be masked by the signals for C3(5.19 dd, J = 4.8 and 3.5 Hz) and C4(5.61 d) respectively.

Having isolated the 8α- and 8β-epimers gas chromatographic analysis of the product mixture of the reduction and acetylation of (297) for various reducing agents could be performed. Using a CPSil 19C B column, the 8β-acetate exhibited a retention time slightly less than that of the 8α-acetate. However base line separation could not be achieved and hence the ratios of 8α- to 8β-acetates quoted herein were determined by measuring peak heights.

Repeating the Luche reduction of the enone (297), the ratio of 8α- to 8β-epimers was found to be 1:16.8 (Table 1) indicating that this reagent had added hydride ion from the more hindered α-face of the molecule. Sodium borohydride attacked the enone preferentially from the opposite β-face to the Luche system giving a ratio of 8α- to 8β-acetates of 1.7:1.

The results of the Luche reduction are in contrast to what one would expect theoretically. If alkoxyborohydrides are indeed the reducing agents in this system then their greater steric bulk compared to BH_4^- would surely result in them attacking the enone with even greater preference than BH_4^- from the less hindered β-face. The stereoselectivity of this reduction system

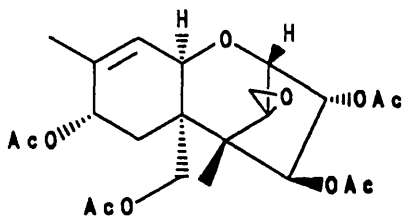
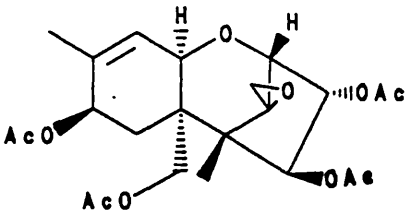
was observed by Luche who found that the presence of Ce^{3+} enhanced the preference for formation of the equatorial alcohol in more than half of the enones studied[115]. In the trichothecene reduction the reaction has also proceeded with preference for the formation of an equatorial alcohol, even when sterically an approach resulting in formation of the axial alcohol would be preferred. Ahn has suggested[117] that the stereoselectivity observed in the reduction of cyclohexanones is related to the hardness of the hydride compound, the harder the reagent the more an axial approach geometry is preferred. This may be an explanation to the observed stereoselectivity in this study.

Repeating the DIBALH reduction reported by Kaneka followed by acetylation and g.c. analysis revealed that indeed the 8α-epimer is formed in excess with a ratio of 8α- to 8β-acetates of 2.5:1 being observed. It was apparent that except for the anomalous result obtained with the Luche reduction, increasing the size of the reducing agent resulted in a greater selectivity for the 8α-epimer, therefore using a bulkier reagent than DIBALH should give even greater selectivity.

Lithium Selectride[118] in many cases gives very high stereoselectivity in the reduction of carbonyl compounds due to its size. Indeed treatment of the enone with 2.5 equivalents of lithium Selectride at -78°C followed by acetylation gave a 72% yield of exclusively the 8α-acetate (319).

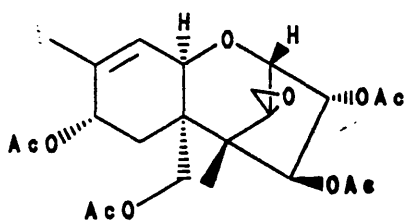
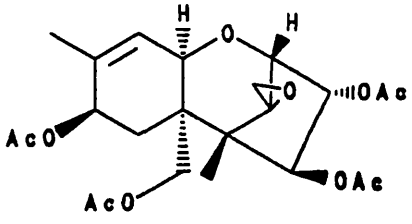
Thus a method had been found for the stereospecific reduction of the ring A enone of the trichothecenes to the natural 8α-allylic alcohol. This method would be utilised in the synthesis of T-2 tetraol. (The results are summarised in Table 1).

TABLE 1 SUMMARY OF GC RESULTS

(297) ⟶  + 

(319) (323)

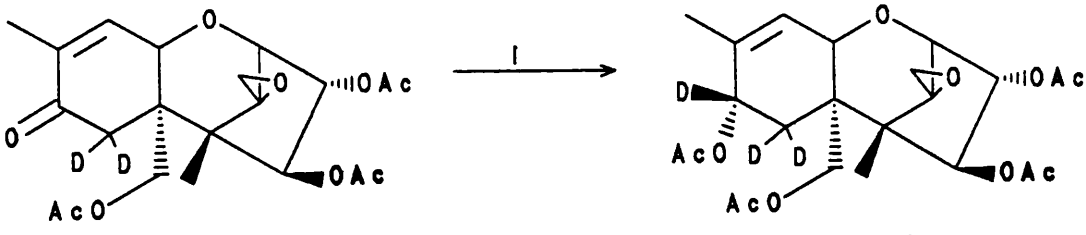
REDUCING AGENT	RATIO (BY G.C.).		
$NaBH_4, CeCl_3, MeOH$	1	:	16.8
$NaBH_4, MeOH$	1.7	:	1
DIBALH	2.5	:	1
L-Selectride	1	:	0

(305) ⟶  + 

(319) (323)

DIBALH	2.1	:	1
L-Selectride	1	:	0

During the course of this investigation Kraus reported[119] that the DIBAL-D reduction of the tri-acetate (324) gave a 56% yield of exclusively the 8α-acetate (325) (Scheme 70). This suggested that the C3 acetate was inducing stereoselectivity, presumably by effecting a conformational change in the molecule that excluded DIBAL reduction from the more hindered α face.



(324) (325)

I ; DIBAL-D

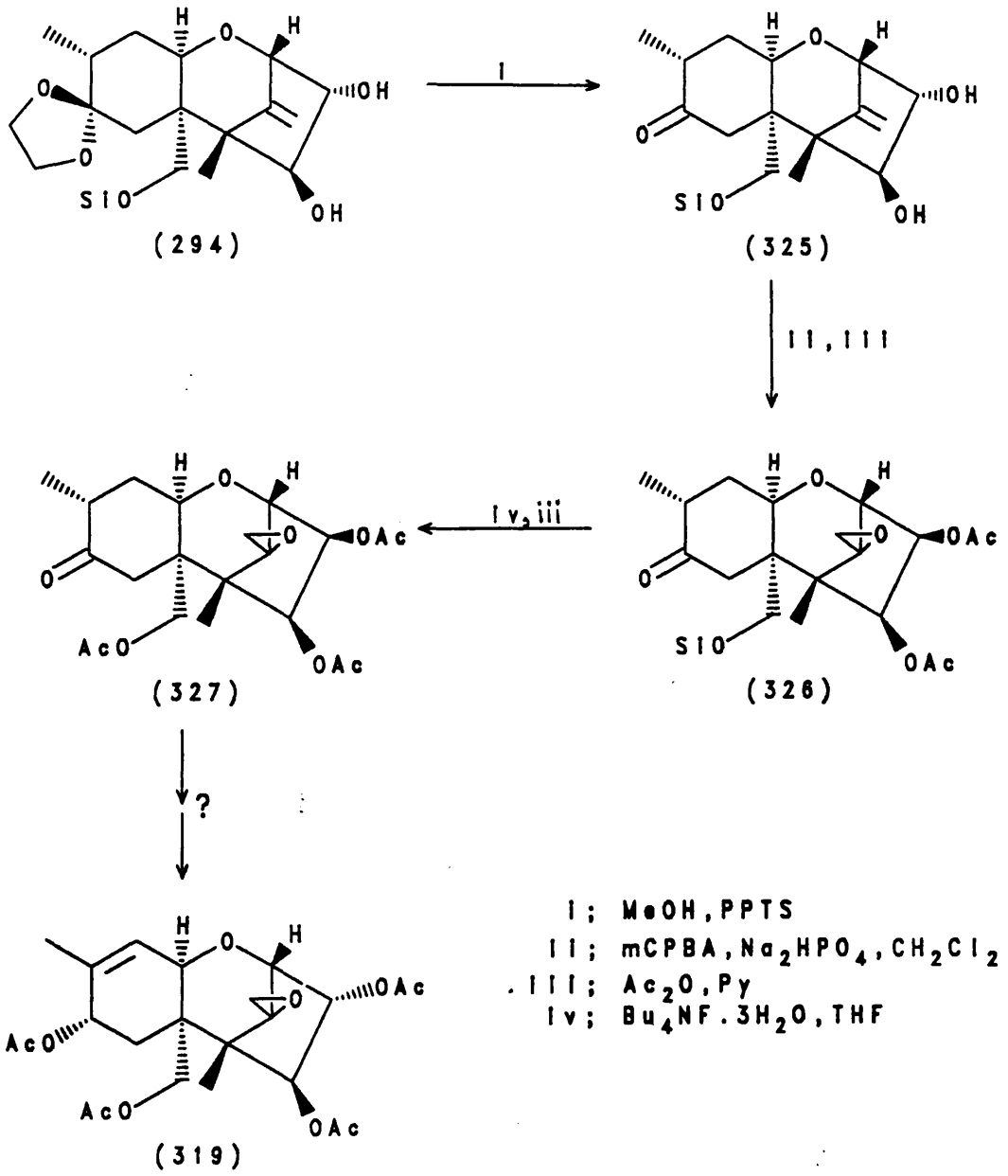
II ; Ac_2O, Py

Scheme 70

However, in our hands DIBAL-H reduction of the triacetate (305) gave by g.c. analysis a 2.1:1 mixture of 8α- and 8β-epimers respectively thus contradicting Kraus's findings. Selectride reduction of the triacetate (305) gave exclusively the 8α-epimer.

2.4 Synthesis of T-2 Tetraol

Having synthesised the olefin diol (294) by the route described in chapter 2.1, F.W. Kerr was able to carry the synthesis forward to the advanced intermediate (327) (Scheme 71).

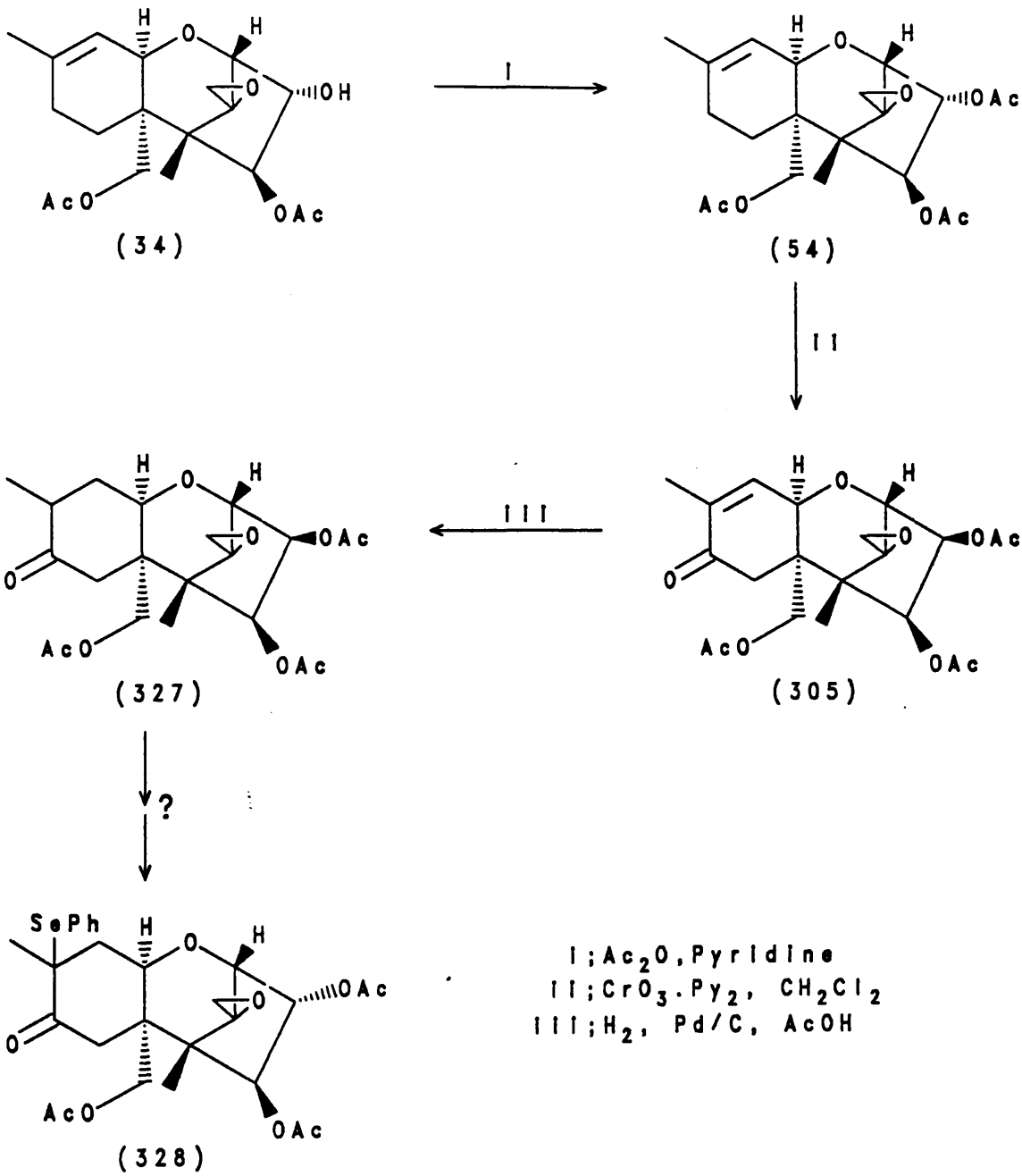


Scheme 71

Deketalisation of (294) with methanol and PPTS, epoxidation with mCPBA and acetylation gave the epoxide (326). Desilylation and acetylation furnished the triacetate (327) at which point his involvement in the synthesis ended. My brief was therefore to complete the synthesis of T-2 tetraol from the intermediate (327).

In that only 7mg of the racemic ketone (327) were available from the synthesis, the first obstacle on the road to T-2 tetraol was that of obtaining sufficient material to complete the synthesis. The ketone (327) could be obtained from isoprene and coumalyl chloride. However as this involved 28 synthetic steps and an inevitable low yield this was deemed impractical. A second, much more attractive route to the ketone was envisioned. This involved a partial synthesis of (327) from readily available anguidine (34) (Scheme 72). Acetylation of anguidine followed by dipyridine chromium trioxide oxidation gave the enone (305) which on palladium-catalysed hydrogenation[120] in acetic acid gave the ketone as a white crystalline solid in an overall yield of 49% from anguidine. This ketone by 200 MHz ^{1}H nmr spectroscopy appeared to be a single C9 epimer as shown by a doublet (J = 6.5 Hz) at 1.0 ppm for the C16 methyl group. It was identical with the ketone synthesised from coumalyl chloride in all respects except that this partially synthesised ketone was homochiral, displaying an optical rotation of $+43.83^{o}$. This partial synthesis therefore provided an effective solution to the first problem.

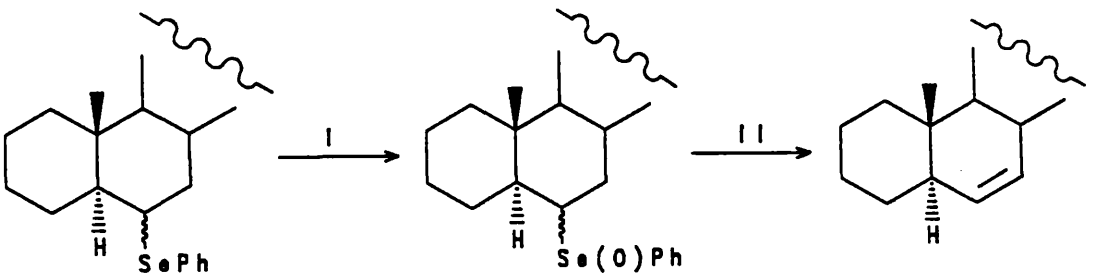
The second problem was formation of the ring A enone which from the work described in chapter 2.3 should, on lithium Selectride reduction, furnish the α– orientated alcohol at C8


H
O
H
OH
O
AcO
OAc
(34)
I
OAc
(54)
II
(305)
III
(327)
?
SePh
(328)
I ; Ac_2O, Pyridine
II ; $CrO_3.Py_2$, CH_2Cl_2
III ; H_2, Pd/C, AcOH

Scheme 72

required for T-2 tetraol.

The formation of enones from ketones has been well documented in the literature, with many of the enone formations involving the β-elimination of oxides and esters of selenium and sulphur substituents, α- to carbonyls. This methodology was developed mainly by Reich[121] and Sharpless[122] following an observation by Jones[123] that during a synthesis of steroidal selenoxides (330) (Scheme 73) the selenoxide decomposed readily to the olefin (331) by a syn elimination mechanism.



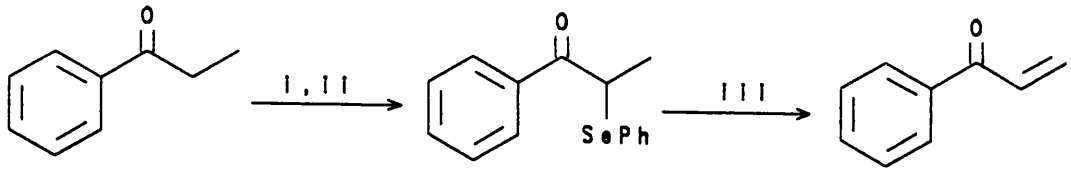
(329) (330) (331)

I ; O_3, CH_2Cl_2 (-78 °C)
II ; CH_2Cl_2 (-78→25 °C)

Scheme 73

In the knowledge that α-seleno derivatives of ketones were formed readily by quenching the ketone enolate with selenohalides, Reich was able to convert, for example, propiophenone (332) to

acrylophenone (334) by oxidation of the selenide (333) with sodium periodate (Scheme 74).

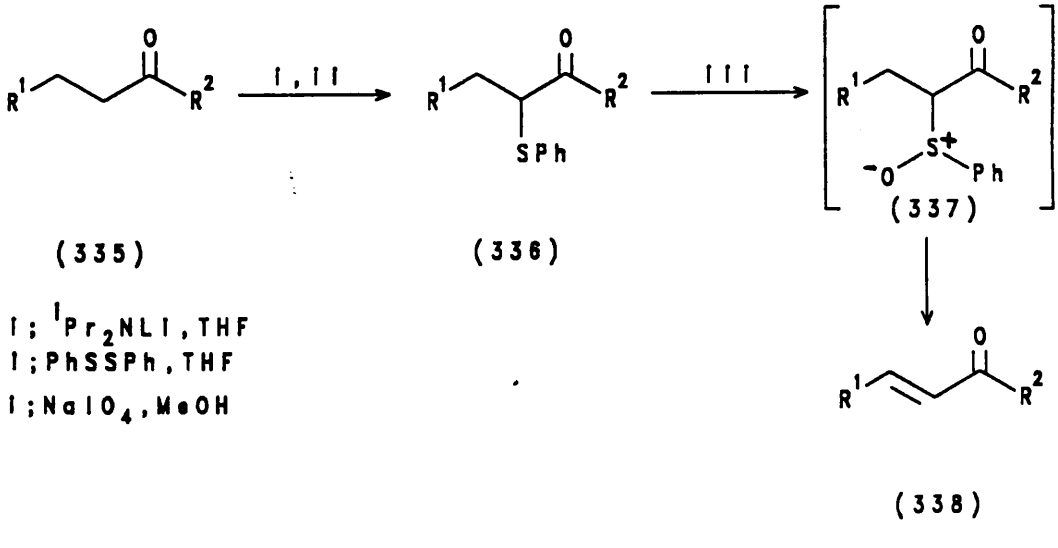


(332) (333) (334)

I; $^{i}Pr_2NLI$, THF(-78° C)
II; PhSeBr, THF(-78 °C)
III; $NaIO_4$, MeOH/H_2O, $NaHCO_3$(20 °C)

Scheme 74

In a related procedure, Trost produced[124] the α,β unsaturated derivatives of a number of ketones by a sulphenylation, oxidation and elimination procedure (Scheme 75).



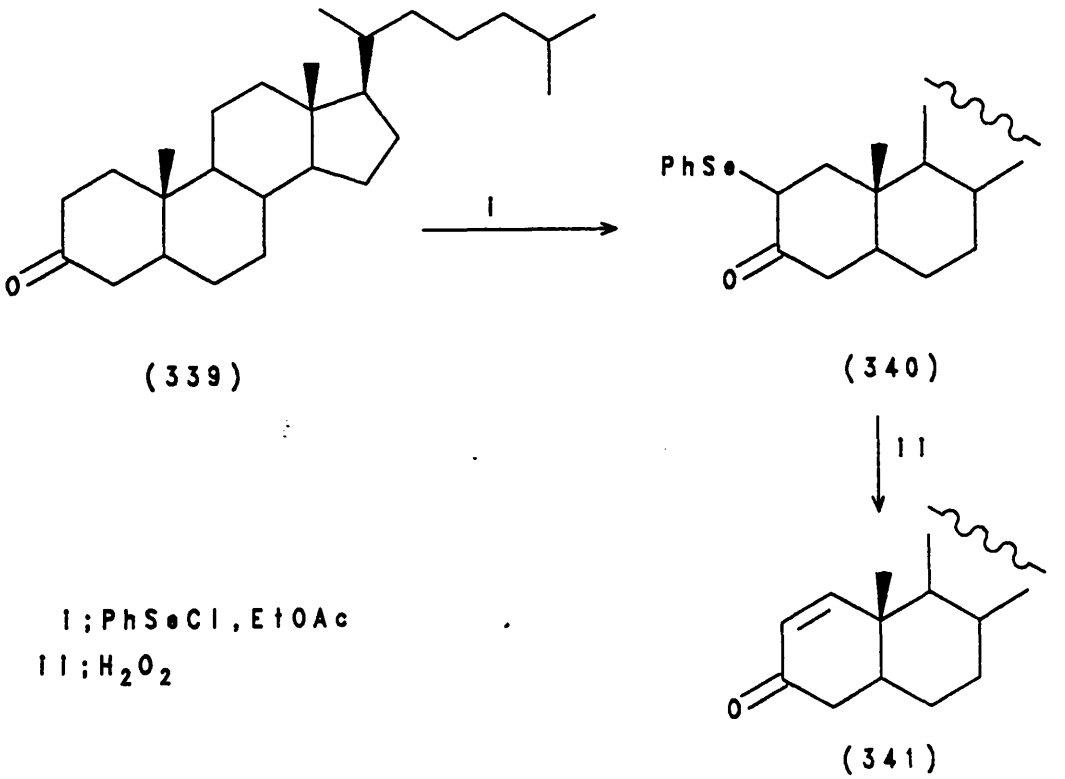
(335) (336) (337) (338)

I; $^{i}Pr_2NLI$, THF
II; PhSSPh, THF
III; $NaIO_4$, MeOH

Scheme 75

With numerous examples of this procedure cited, the problem of enone formation appeared to be one of forming the 9-seleno derivative (328) of the ketone (327) (Scheme 72).

Sharpless had shown[125] that α-selenylation of enolisable ketones could be achieved by simply stirring the ketone (in his example (339) (Scheme 76)) in ethyl acetate in the presence of phenylselenyl chloride. Oxidation of the selenide (340) with hydrogen peroxide gave the unsaturated ketone (341). This simple procedure presumably occurs via the enol form of the ketone and occurs rapidly due to the formation of hydrochloric acid which would catalyse the keto-enol equilibration.

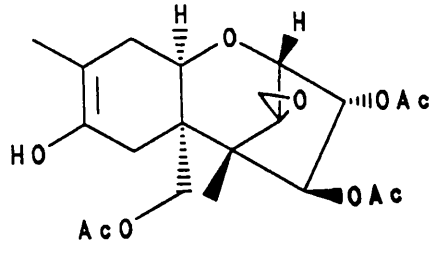


(339) (340) (341)

I ; PhSeCl , EtOAc
II ; H_2O_2

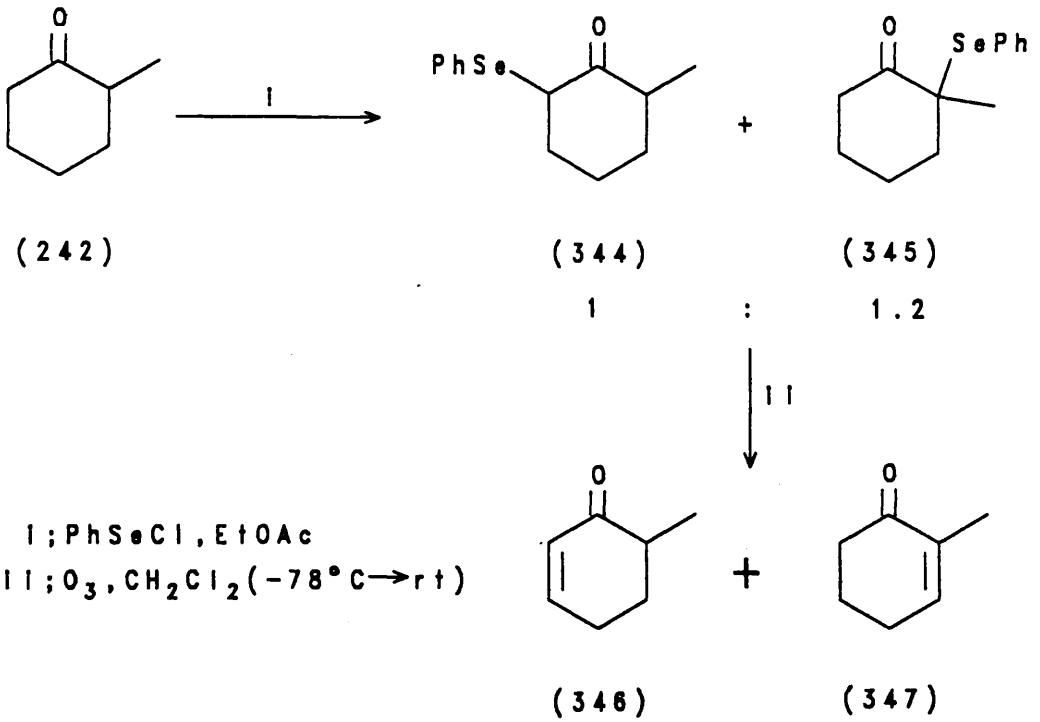
Scheme 76

It was hoped that in the synthesis in hand, selenylation would proceed via the thermodynamically more stable more substituted enol (343) and thus provide some regioselectivity in this step.



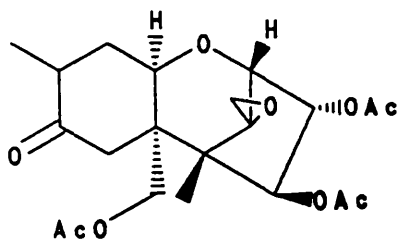
(343)

A simple model study using 2 methyl cyclohexanone (242) did not provide encouraging results (Scheme 77). On stirring 2-methyl cyclohexanone in ethyl acetate in the presence of 1.1 equivalents of phenylselenyl chloride, the deep red solution decolourised in approximately one hour. Examination of the crude product by ^{1}H nmr spectroscopy showed a singlet at 1.4 ppm and was assigned to the methyl group of 2-phenylselenyl-2-methylcyclohexanone (345). However also present was a doublet at 1.05 ppm which was assigned to the methyl group of 6-phenylselenyl-2-methylcyclohexanone (344). Integration of these signals showed a ratio of 1.2:1 in favour of the 2-phenylselenyl derivative (345). Oxidation of the crude product with ozone and distillation gave a mixture of the two endocyclic enones (346) and (347).


O
I
PhSe
O
+
O
SePh
(242)
(344)
(345)
1 : 1.2
II
I ; PhSeCl , EtOAc
II ; O3 , CH2Cl2 (-78°C → rt)
O
O
+
(346)
(347)

Scheme 77

The ^{1}H nmr spectrum of the distillation mixture showed a doublet of triple doublets (J = 10, 4 and 1.2 Hz) at 6.91 ppm and a doublet of triplets (J = 10 and 2 Hz) at 5.97 ppm, these signals being assigned to the C5 and C6 protons respectively of the 5-ene isomer (346). A multiplet resonating at 6.72 ppm was assigned to the C3 proton of the 2-ene isomer (347). Integration of these signals indicated that the desired 2-ene isomer was in only slight excess.

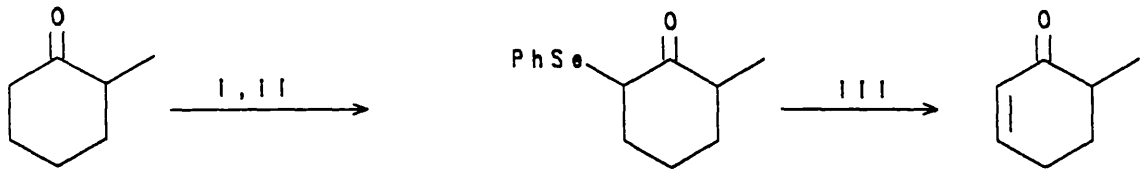


(327)

In spite of this result the ketone (327) was stirred in ethyl acetate in the presence of phenylselenyl chloride, but even after 48 hours the solution remained a deep red colour indicating that the selenyl chloride was unreacted. It appeared that the selenide would have to be formed via the enolate of the ketone. (327). Thermodynamic enolates can be formed either by the equilibration of the kinetic and thermodynamic enolates or by trapping the thermodynamic enolate as the silyl enol ether as described by House and Stork.[82,83] Once again, using 2-methyl cyclohexanone as a model, the first method was attempted.

Lithium diisopropylamide (0.9 equivalents), was added dropwise to a solution of (242) at -78°C and this mixture stirred for 4 hours to allow equilibration. However, on quenching the reaction with phenylselenyl chloride, the product appeared to be exclusively the C6-selenide (344). Indeed oxidation with

hydrogen peroxide gave exclusively the unsubstituted enone (346) (Scheme 78).



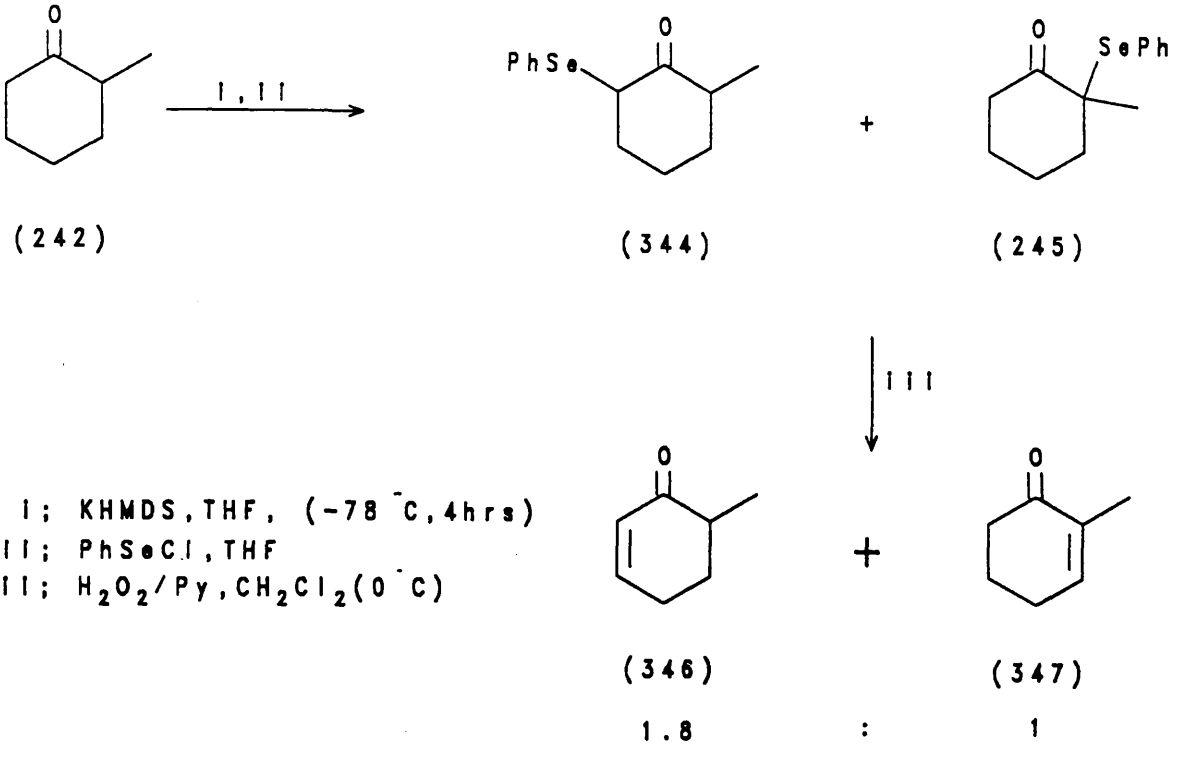
(242) (344) (346)

I; $^{i}Pr_2NLi$, THF(-78°C, 4hrs)
II; PhSeCl, THF
III; H_2O_2/Py, CH_2Cl_2(0°C)

Scheme 78

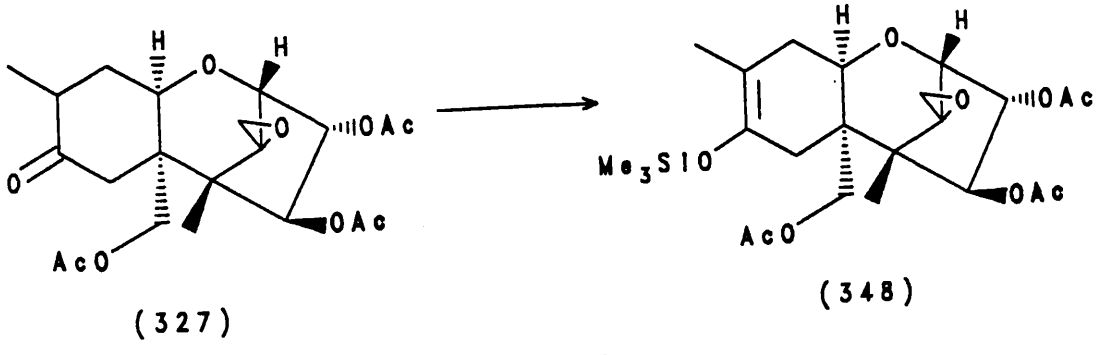
As the potassium enolate is reported to equilibrate more readily than the lithium enolate the above procedure was repeated using potassium hexamethyldisilazide to generate the enolate. (Scheme 79). Selenylation and oxidation gave a product mixture, ^1H nmr spectral analysis of which showed that indeed both

endocyclic enones (346) and (347) were present but in a ratio of 1:1.8 in favour of the undesired unsubstituted enone (346).



Scheme 79

Attention turned to formation of the thermodynamic silyl enol ether (348) of the ketone (327) employing the conditions described by Stork and House, i.e. treating the ketone with triethylamine and chlorotrimethylsilane for 48 hours in refluxing dimethylformamide.



Scheme 80

However, employing the normal isolation procedure; dilution with pentane and washing with aqueous sodium bicarbonate gave a very low recovery of starting material. A greater recovery of starting material was achieved by ethyl acetate extraction of the aqueous washes, indicating the low solubility of the ketone and presumably the silyl enol ether in pentane. Employing diethyl ether as the solvent of extraction in the isolation only starting material was recovered. It was apparent that the polarity of the trichothecenes requires a polar solvent for their extraction from the reaction mixture; however, this facilitates hydrolysis of any silyl enol ether that may be present.

With these disappointing results in mind, attention turned once more to the selenylation procedure of Sharpless. The fact that treatment of the ketone with phenylselenyl chloride gave no reaction suggested that either:

1) The C9 position was too hindered to allow rapid attack by the selenyl halide

or 2) The keto-enol tautomerisation was slow.

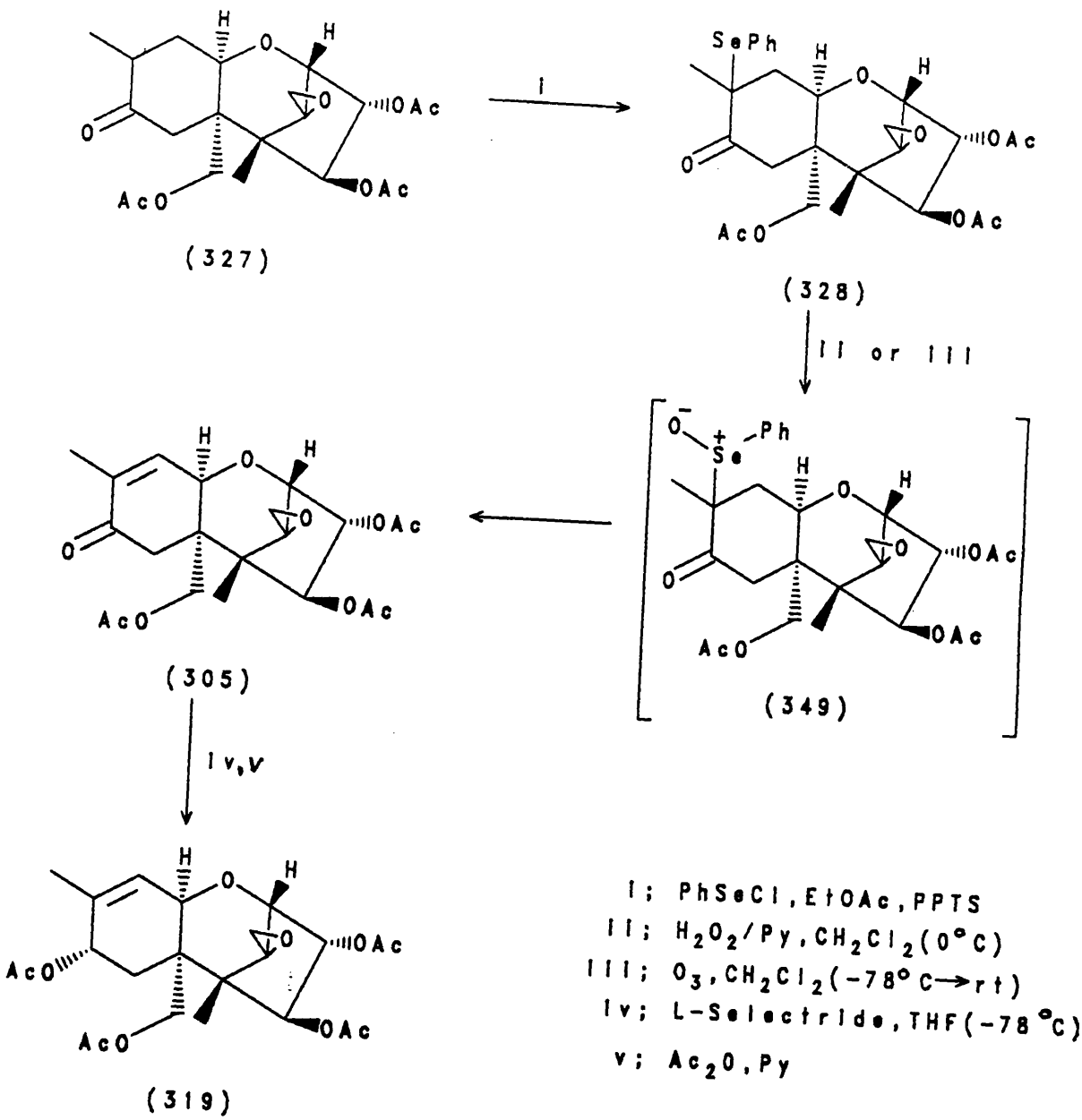
Examination of molecular models provided no obvious evidence for the former, hence the latter was investigated. In that it was perceived that the hydrochloric acid produced in the reaction acts as a catalyst for subsequent selenylations, then possibly, addition of acid to the reaction mixture may catalyse the keto-enol equilibration sufficiently to bring about reaction. Therefore the ketone was stirred in ethyl acetate with fresh phenylselenyl chloride in the presence of a 20% mol equivalent of PPTS and the reaction was followed by t.l.c. analysis; after 19 hours, in addition to the starting material (R_f 0.33) a second spot had appeared (R_f 0.41). After 39 hours stirring it was

apparent that very little starting material remained; however several other compounds in addition to the original product were beginning to appear. The reaction was stopped and the product mixture was subjected to dry column flash chromatographic separation. ^1H nmr spectral analysis of the original product (R_f 0.41) revealed that it was indeed the required phenylselenyl derivative (328) of the ketone, isolated in a yield of 67%.

Due to the formation of other products on prolonged stirring, it was found that to achieve the highest yields of the selenide, the reaction had to be stopped after approximately 40 hours and this unfortunately led to recovery of some starting material. One possible explanation for the appearance of these impurities is that in the acidic conditions of the reaction, apotrichothecene rearrangement of both starting material and product could occur; this possibility was not investigated thoroughly.

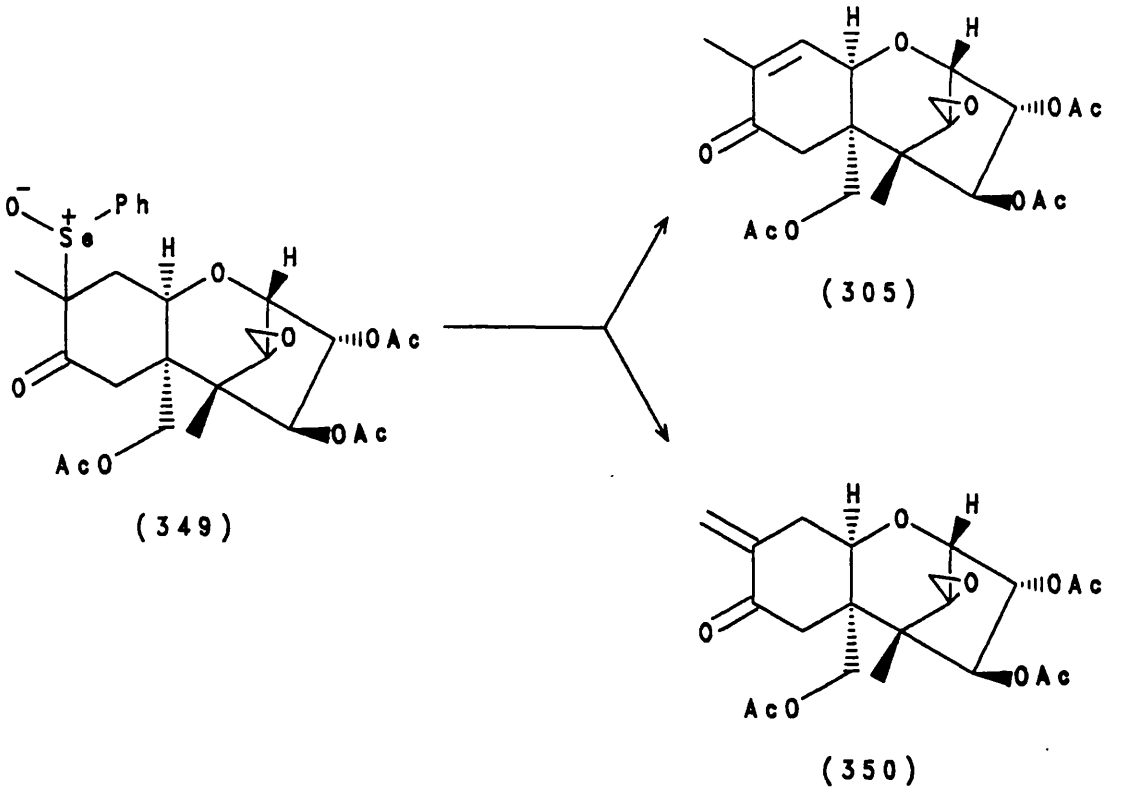
Having introduced the phenylselenyl group with total regioselectivity, all that remained for the formation of the enone was oxidation to the selenoxide and elimination. Indeed treating the selenide with either ozone[123] at -78°C and allowing the selenoxide (349) (Scheme 81) to warm to room temperature, or with hydrogen peroxide and pyridine at O°C, resulted in the formation of the required enone (305) showing in its ^1H nmr spectra at 6.53 ppm the doublet of quartets (J = 5.8 and 1.5 Hz) characteristic of the C10 proton.

It was found that ozone was the oxidant of choice in that it gave a cleaner product mixture than hydrogen peroxide and also the yield of enone obtained using ozone as the oxidant was 69%, compared to 60% for the peroxide oxidation.


(327)
(328)
(349)
(305)
(319)
I; PhSeCl, EtOAc, PPTS
II; H_2O_2/Py, CH_2Cl_2(0°C)
III; O_3, CH_2Cl_2(-78°C→rt)
IV; L-Selectride, THF(-78°C)
V; Ac_2O, Py

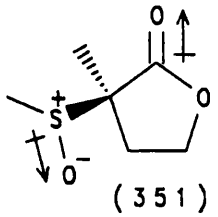
Scheme 81

As this reaction proceeds by a syn-elimination, it is obvious that two possible products could occur i.e. the selenoxide could abstract a proton from either C10 thus giving the required endocyclic olefin (305) or from the methyl group (C16) to give an exocyclic olefin (305) (Scheme 82).

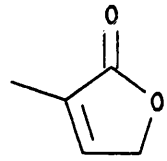


Scheme 82

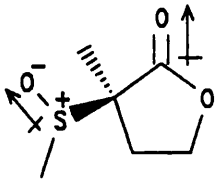
Trost has explained[126] the apparent preference for the former elimination in terms of orientation of the dipoles of the carbonyl and the selenoxide (in Trost's case a sulphoxide (Scheme 83). These dipoles will align themselves in such a way as to minimise their interaction. This suggests that the selenoxide would adopt an orientation in which the oxygen was in proximity to the protons on C10 rather than the methyl group and hence the preference for formation of the endocyclic olefin (305). Of course, other factors such as steric hinderance and the relative



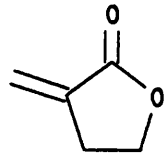
(351)



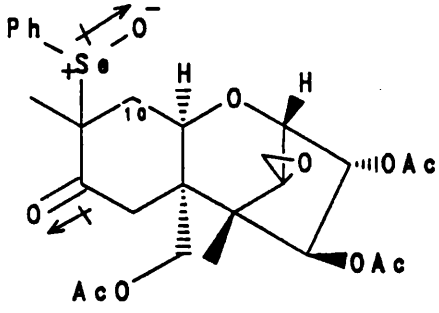
(352)



(351)



(353)



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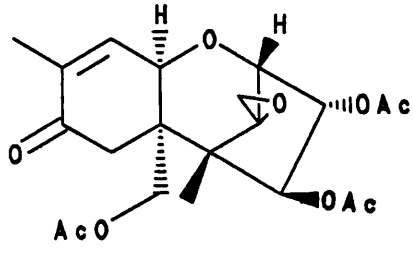
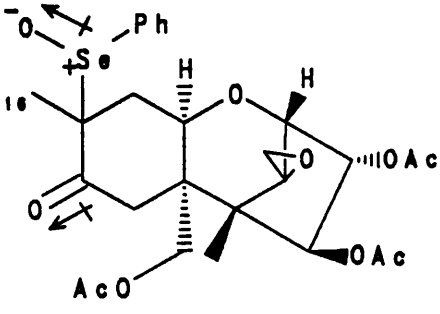
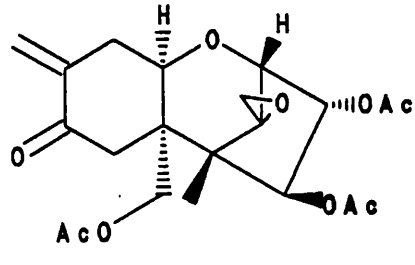


(305)



(349)

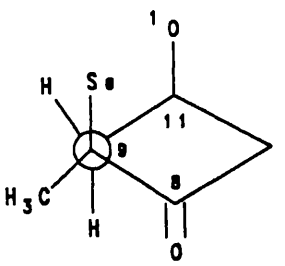


(350)

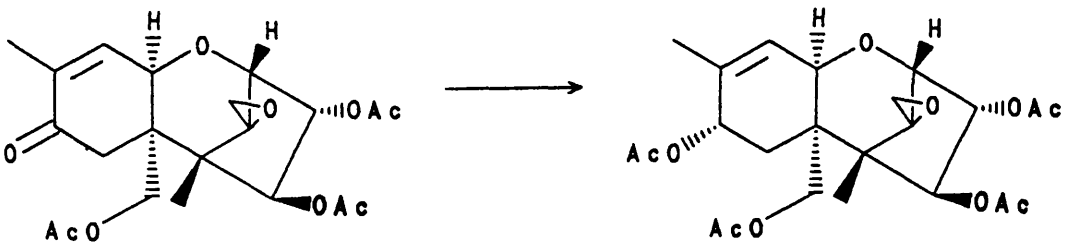
Scheme 83

acidity of the protons being abstracted contribute to the endo-exo ratio.

On the assumption that the selenium attacks the molecule from the more accessible β-face, molecular models revealed that for ring A to adopt a chair conformation the selenium must sit axial to the ring. In this position the selenium is syn to the β-hydrogen of C10. This would favour the formation of an endocyclic olefin.

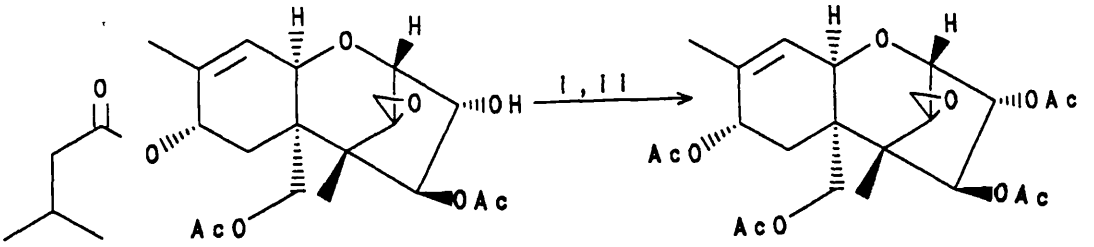


Indeed, the exocyclic olefin was never isolated, and ^1H nmr spectroscopy of the lesser components of the reaction product mixture failed to provide evidence for its existence. Having successfully formed the enone, all that remained for completion of the synthesis was its stereoselective reduction to the 8-α orientated alcohol. The earlier reduction studies of 8-keto anguidine (297) described in chapter 2.3 showed that lithium Selectride reduced the enone of (305) stereo-specifically to the 8α-alcohol. Indeed treatment of (305) with lithium Selectride



Scheme 84

in THF gave, after acetylation of the product mixture, T2 tetraol, as its tetraacetate (neosolaniol diacetate)[127] (319) in 88% yield.



(4) (319)

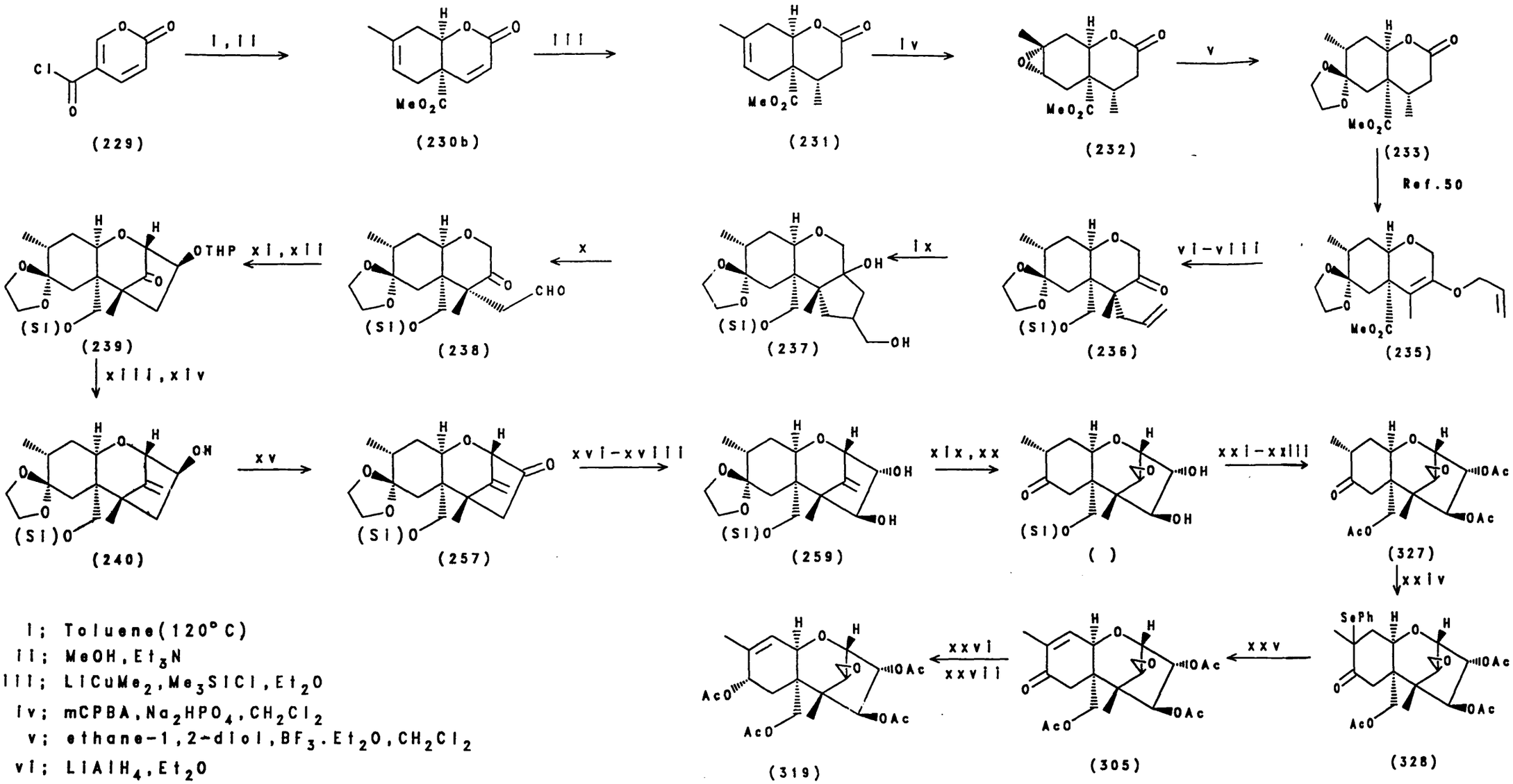
I ; $NH_4OH/MeOH$

II ; Ac_2O, Py

Scheme 85

Professor J.W. Apsimon (Carleton University, Ottawa, Canada) kindly provided a sample of T-2 toxin (4) which allowed authentification of the synthesis. T-2 toxin was subjected to methanolysis followed by per acetylation (Scheme 85). A solid resulted which on recrystallisation from benzene/hexane gave T-2 tetraol tetraacetate (319) as a white crystalline solid. It was found that the products of the synthesis described above and this simple partial synthesis were identical in all respects, including sign and magnitude of optical rotation, and mixed melting point.

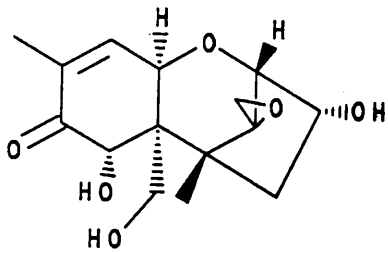
Thus the target of the synthesis, T-2 tetraol tetra-acetate (319) had been reached in 30 steps from isoprene and coumalyl chloride by the route shown (Scheme 86). This work represents the first successful approach to a tetra-hydroxylated trichothecene.



i; Toluene(120°C)
ii; MeOH, Et_3N
iii; $LiCuMe_2$, Me_3SiCl, Et_2O
iv; mCPBA, Na_2HPO_4, CH_2Cl_2
v; ethane-1,2-diol, $BF_3.Et_2O$, CH_2Cl_2
vi; $LiAlH_4$, Et_2O
vii; Toluene, Et_3N(5%)
viii; $Bu^tMe_2SiOSO_2CF_3$, 2,6-lutidine, CH_2Cl_2
ix; $NaIO_4$, OsO_4(cat), Et_2O, H_2O
x; H_5IO_6, Et_2O
xi; NaOMe, MeOH
xii; DHP, PPTS
xiii; $Ph_3P{=}CH_2$, THF
xiv; ethane-1,2-diol, PPTS, CH_2Cl_2
xv; CrO_3. 3,5-dimethylpyrazole, CH_2Cl_2
xvi; KHMDS, toluene, THF
xvii; (273), THF
xviii; $NaBH_4$, MeOH
xix; MeOH, PPTS
xx; mCPBA, Na_2HPO_4, CH_2Cl_2
xxi; Ac_2O, Py, Et_2O
xxii; $Bu_4NF.3H_2O$, THF
xxiii; Ac_2O, Py
xxiv; PhSeCl, PPTS, AcOEt
xxv; O_3, CH_2Cl_2 (-78°C-rt)
xxvi; $LiBu_3BH$, THF (-78°C-rt)
xxvii; Ac_2O, Py

Scheme 86

2.5 Approaches to Deoxynivalenol.

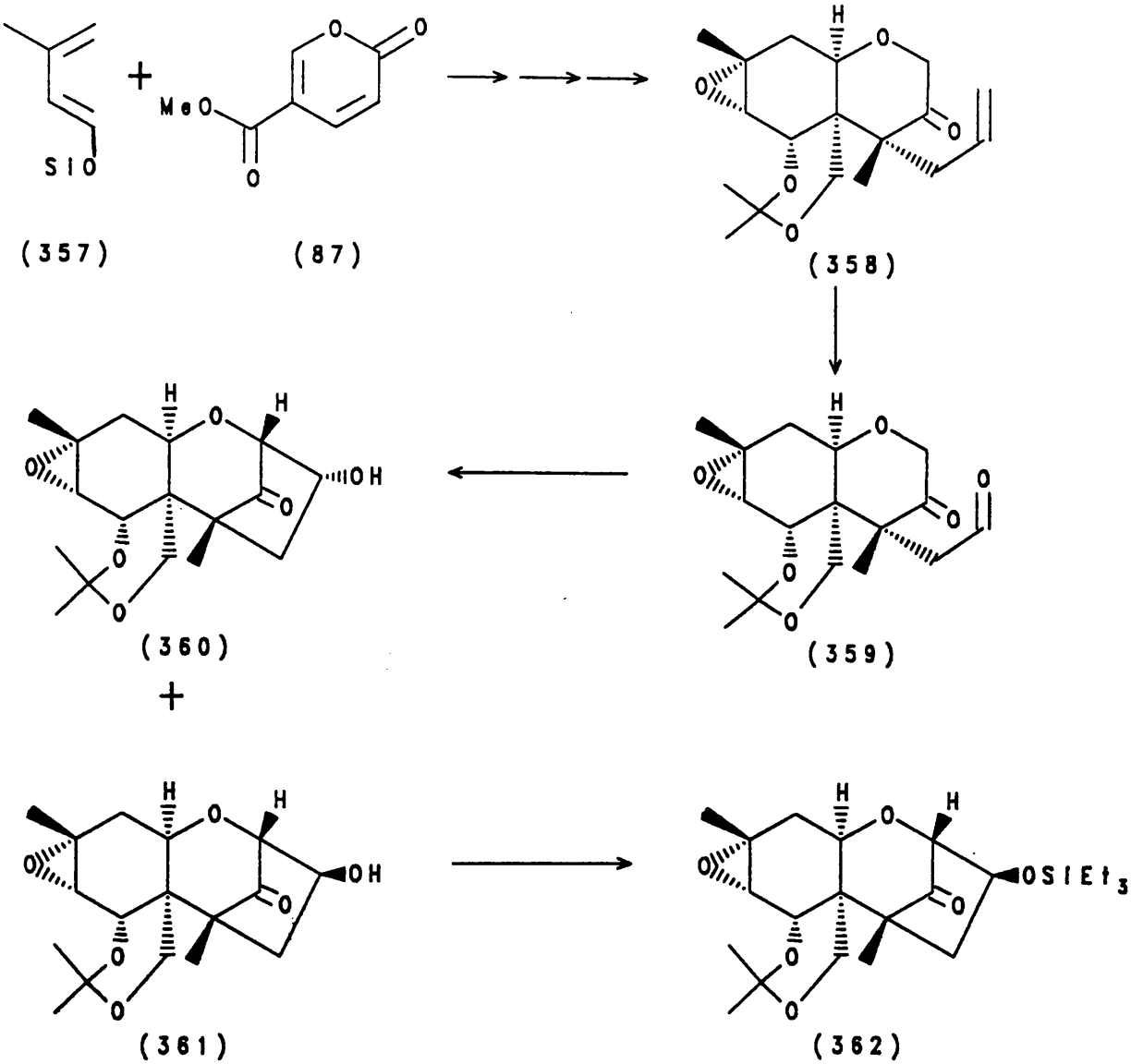


(6)

Deoxynivalenol (6) is a highly oxygenated non-macrocyclic trichothecene of considerable environmental importance. Although it is not one of the more potent trichothecenes, having[128] an LD_{50} of 70mg per kg, it is produced by the ubiquitous *Fusarium roseum* fungus and so is one of the most common members of the group. Ingestion of this toxin results in emesis, hence it is often referred to trivially as vomitoxin. Also, it effects sub-lethal toxicoses and consequently reduced animal growth. Thus being environmentally important and highly oxygenated, deoxynivalenol appeared a challenging target for total synthesis.

The synthesis of the advanced intermediate (358) (Scheme 87) which was initiated by a Diels-Alder reaction of methyl coumalate (87) with the silyloxy diene (357), utilised much of the chemistry described herein for the synthesis of T-2 tetraol and has been

reported previously.[50] Hence, discussion of this work is deemed unnecessary.



Scheme 87

Further work by the group has allowed conversion of this intermediate (358) into the tricyclic system (362). Oxidative cleavage of the olefin with ozone followed by aldol cyclisation utilising methoxide as base gave the tricyclic intermediates (360) and (361) in a ratio of 4:1 in favour of the unnatural β epimer (361). Chromatographic isolation of (361) and protection of the

alcohol as the triethylsilyl ether gave the advanced intermediate (362) at which point my involvement in the synthesis commenced.

Comparison of this intermediate (362) with deoxynivalenol indicated that for completion of the synthesis the following transformations were required:

1) Olefination of the ketone
2) Base induced epoxide-allyl alcohol rearrangement
3) Deprotection and inversion of configuration of the C3 alcohol
4) Selective oxidation of the C8 alcohol
5) Epoxidation of the 12,13 olefin

and 6) Removal of the acetonide.

In the light of the fact that only 120 milligrams of the ketone (362) were available and that time was limited this appeared a rather daunting challenge.

Wittig olefination has been the standard method employed in trichothecene total synthesis to generate the C12 alkene. However in this synthesis, this method of olefination appeared useless[129]; loss of the ring A epoxide ensued on treatment of the ketone with methylenetriphenylphosphorane. An alternative to the Wittig reaction was therefore investigated.

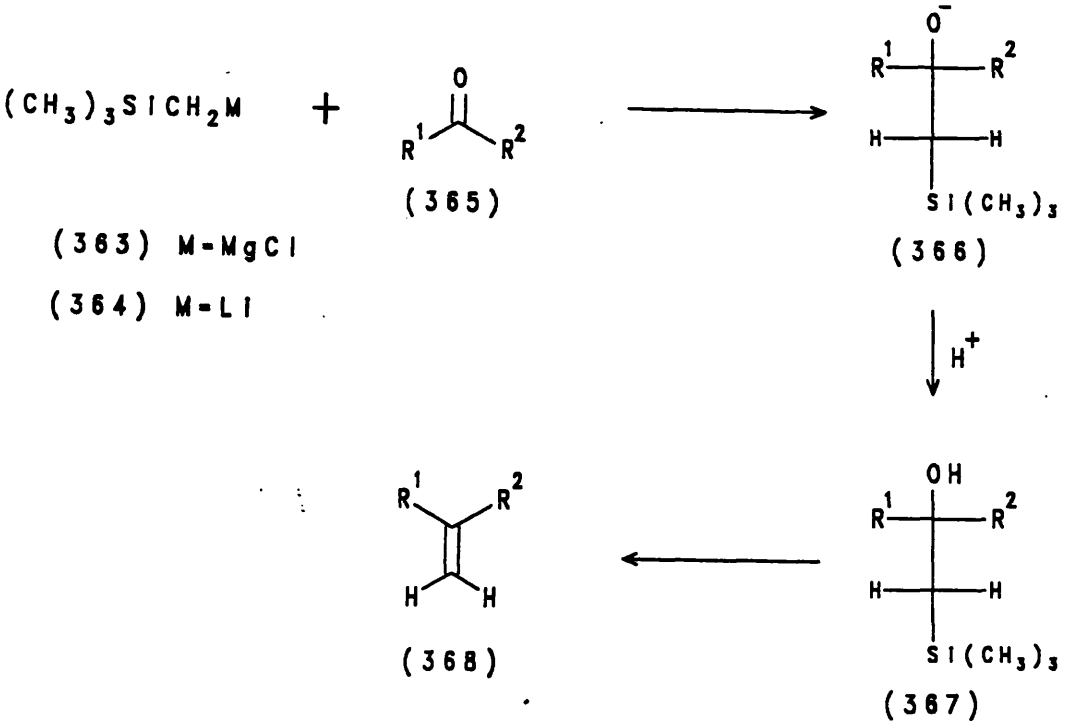
Carbonyl olefination employing silyl substituted organometallic compounds - the Peterson reaction[130] - appeared a promising alternative for three reasons:

1) The by-product of the reaction, hexamethyldisiloxane, is easily removed unlike triphenylphosphine oxide, the by-product of the Wittig reaction.

2) The synthesis of alkenes by Peterson olefination is invariably rapid and occurs at low temperature again in contrast to the Wittig reaction.

and 3) The basicity of the Peterson olefination reagent can be reduced[131] considerably by use of $CeCl_3$ in the reaction.

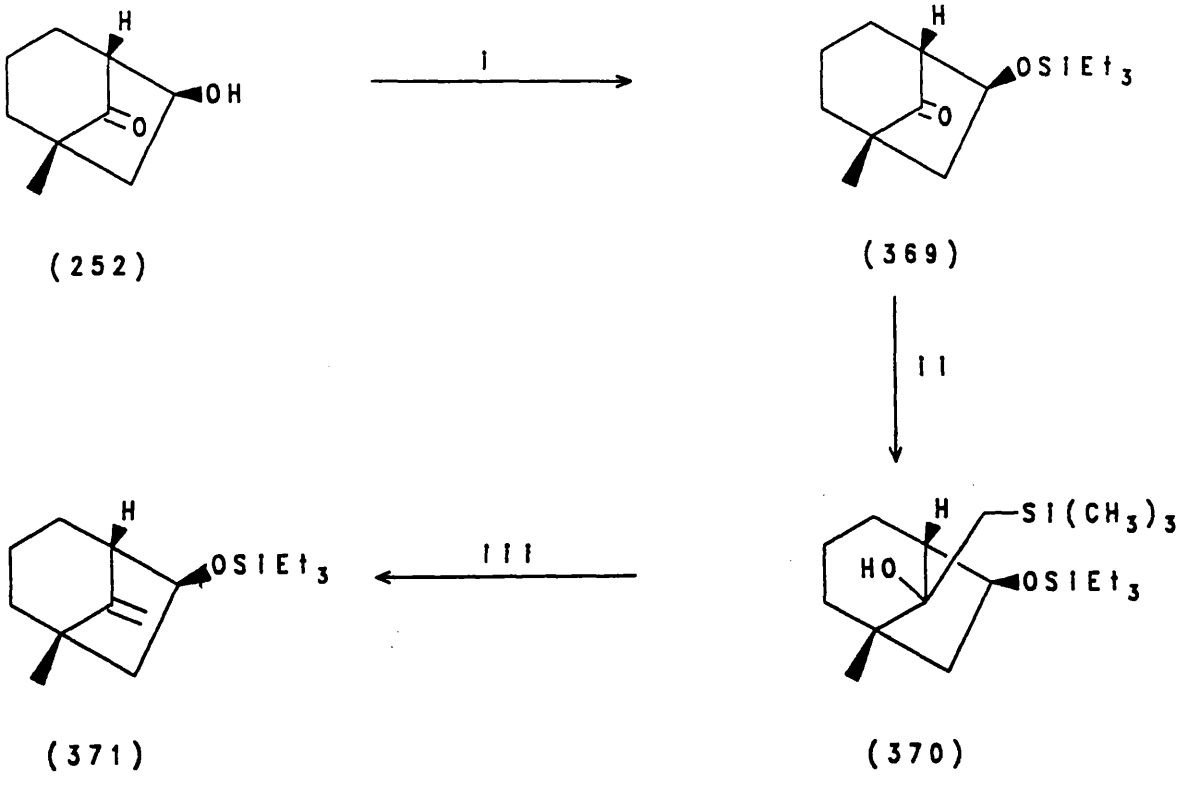
Peterson reported that the β-hydroxysilanes (367) (Scheme 88) required for the olefinations could be synthesised by addition of reagents such as trimethylsilylmethyl-magnesium chloride (363) (M = MgCl) or trimethylsilylmethyl-lithium (364) (M = Li) to the appropriate carbonyl compound followed by protonation.



Scheme 88

Formation of the alkene from the β-hydroxysilane can be effected under both acidic and basic conditions by a number of reagents including KH, NaH, $BF_3.OEt_2$, H_2SO_4 and HF. Although not relevant to the olefination in hand an extremely high degree of stereoselectivity can be achieved by appropriate choice of

reagents for the elimination. For the olefination of (362) a base induced elimination was required due to the acid sensitive functionality present in the molecule. As always, a model study was performed (Scheme 89).



I; Et_3SiCl, DMAP, Et_3N, Et_2O
II; $(CH_3)_3SiCH_2Li$, THF(-78 °C)
III; KHMDS, THF

Scheme 89

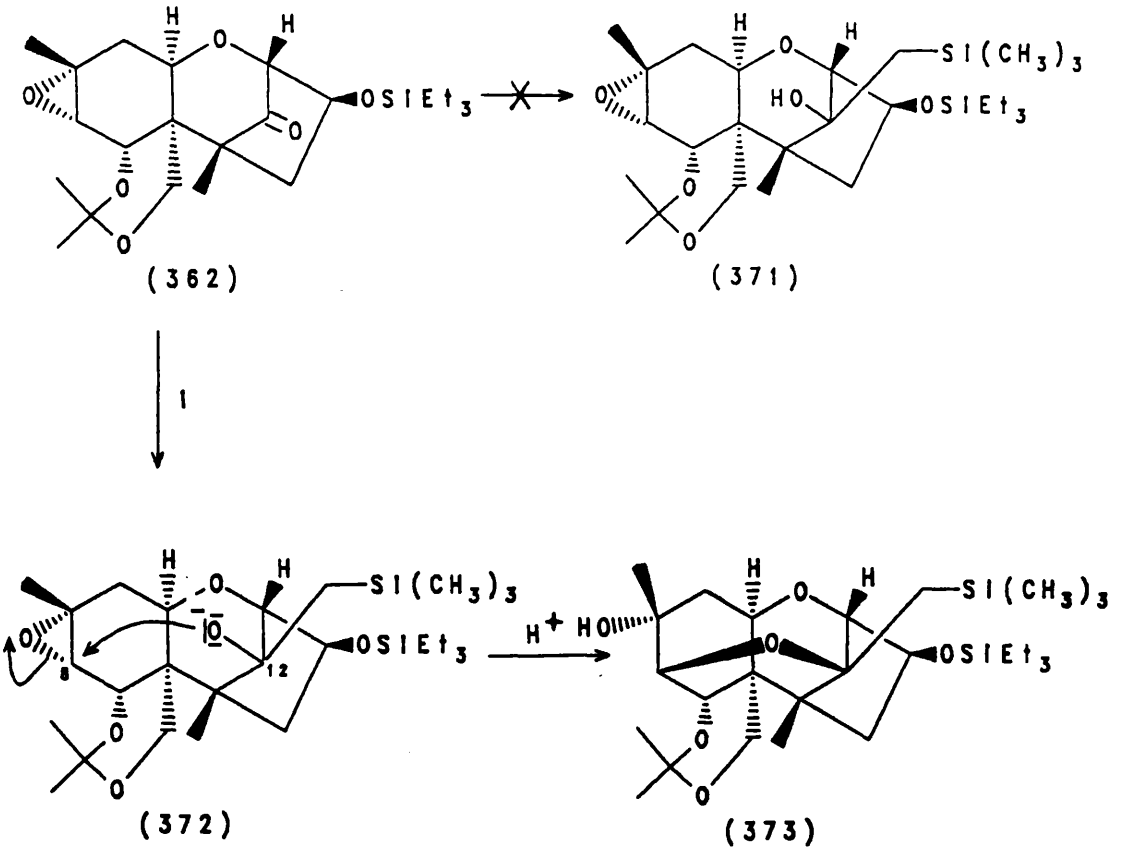
A sample of the model 6β-alcohol (252) was silylated using chlorotriethylsilane, triethylamine and DMAP to give the ketone (369). Treatment of this ketone with trimethylsilylmethyl-lithium at -78°C followed by quenching the reaction with aqueous ammonium chloride gave after chromatographic purification a clear oil, characterised as the required β-hydroxysilane (370) (70% yield). Employing potassium hexamethyldisilazide to generate

the potassium alkoxide, t.l.c. analysis of the reaction indicated that after ten minutes no starting material remained. A single product had formed: on visualising the t.l.c plate with ceric sulphate solution it stained a deep blue colour (characteristic of olefins in the model series).

Isolation and characterisation showed that this compound was indeed the required olefin (371) obtained in 75% yield from the β-hydroxysilane (370).

Turning to the deoxynivalenol precursor (362), initial attempts at addition of trimethylsilylmethyl-lithium to the ketone failed and starting material was recovered unchanged. As these reactions were being performed on a 0.06 mmol scale and only a slight excess of the Peterson reagent was being added to the reaction, residual moisture in the reaction flask may have been the cause of the failure. Hence it was decided to add a large excess of the Peterson reagent to the ketone to overcome this problem - not an elegant solution, but it was effective. A ten-fold excess of the Peterson reagent was added to the ketone stirring at -78^{o}C in THF and the reaction followed by t.l.c. analysis. After 3 hours t.l.c. analysis revealed that the starting material had been consumed and that a single compound had formed. Chromatographic purification of this material gave

a clear oil which by Infra-red analysis was devoid of a carbonyl but instead had an alcohol present.

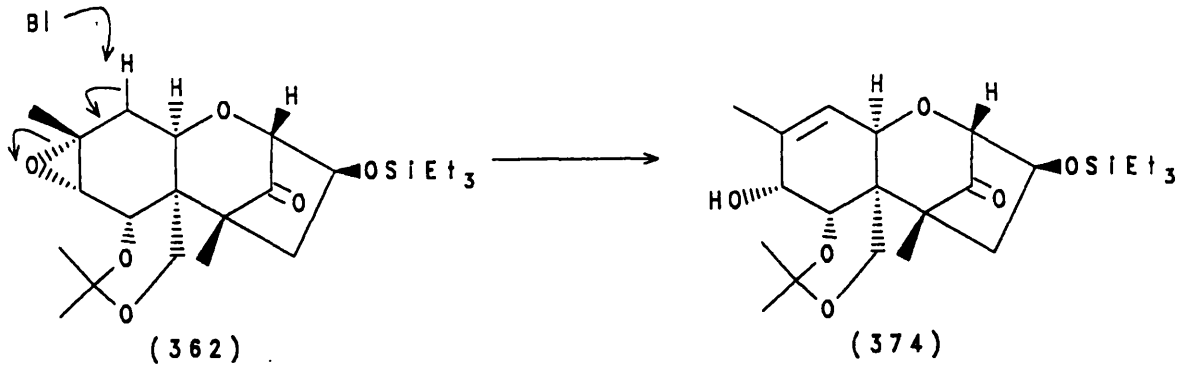


I; $(CH_3)_3SiCH_2Li$, THF(-78°C)

Scheme 90

^1H nmr Spectroscopy revealed however that this compound was not the desired adduct (371) (Scheme 90). Mass spectroscopy as well as ^{13}C nmr and ^1H nmr spectroscopy indicated that the reaction product was structurally a closely related isomer of the desired adduct. However the ^1H nmr spectrum indicated that the epoxide of ring A was no longer present; the signals for the protons of C7(δ 4.27 ppm doublet J = 3.3 Hz) and C8(δ 3.03 ppm doublet J = 3.3 Hz) [values quoted for the starting material (362)] had changed, signifying loss of the epoxide. As the ^1H nmr spectrum was devoid of resonances in the olefinic region the

disappearance of the epoxide by base induced rearrangement to the allylic alcohol (374) (Scheme 91) was ruled out.



Scheme 91

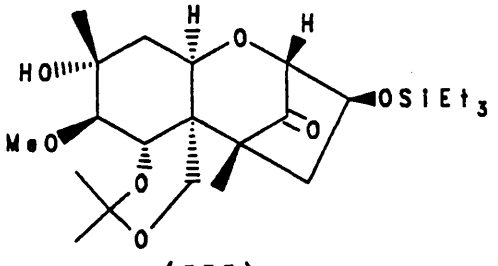
Examination of molecular models of the desired adduct (371) showed that the tertiary alcohol could very readily be brought into proximity with C8. Thus it may have been possible that the lithium alkoxide generated by addition of the Peterson reagent to the ketone effected intramolecular nucleophilic epoxide ring opening to give the adduct (373). Molecular models revealed that such epoxide ring opening produces a structure containing four six-membered rings, three of which readily adopt chair conformations and so may be relatively stable.

Attack at C8 by an alkoxide at C12 results in a trans diaxial opening of the epoxide and is thus stereo-electronically acceptable. Attempted acetylation of the alcohol failed suggesting that the alcohol was indeed tertiary. Although all spectroscopic data was consistent with the proposed structure absolute proof that this was indeed the correct structure could not be found.

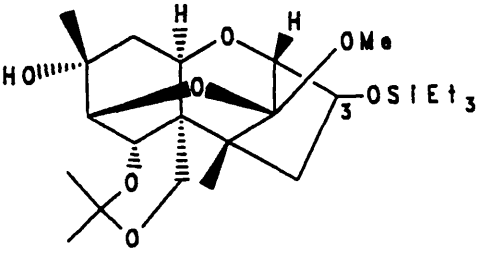
A further piece of circumstantial evidence for this structure came from the aldol reaction employed to construct the tricyclic skeleton. One of the major products of the aldol reaction and silylation of the C3-alcohol was a clear oil which

was initially though to be (375), the product of methoxide opening of the epoxide.

Full characterisation revealed that this oil was in fact an inseparable 4:1 mixture of two compounds which contained methoxy signals in the ^{1}H nmr spectrum. However no carbonyls were present. The clue to the actual structure came from ^{13}C nmr spectroscopy which showed that in addition to the singlet (δ 99.9 ppm) expected for the quaternary acetonide carbon another acetal was present (δ 104 ppm, singlet). The structure (376) was assigned to the compound (the 4:1 mixture of compounds being due to the presence of C3 epimers, with the β epimer predominating). This suggested that intramolecular epoxide ring opening by attack from C12 alkoxides was a favoured process for these compounds.



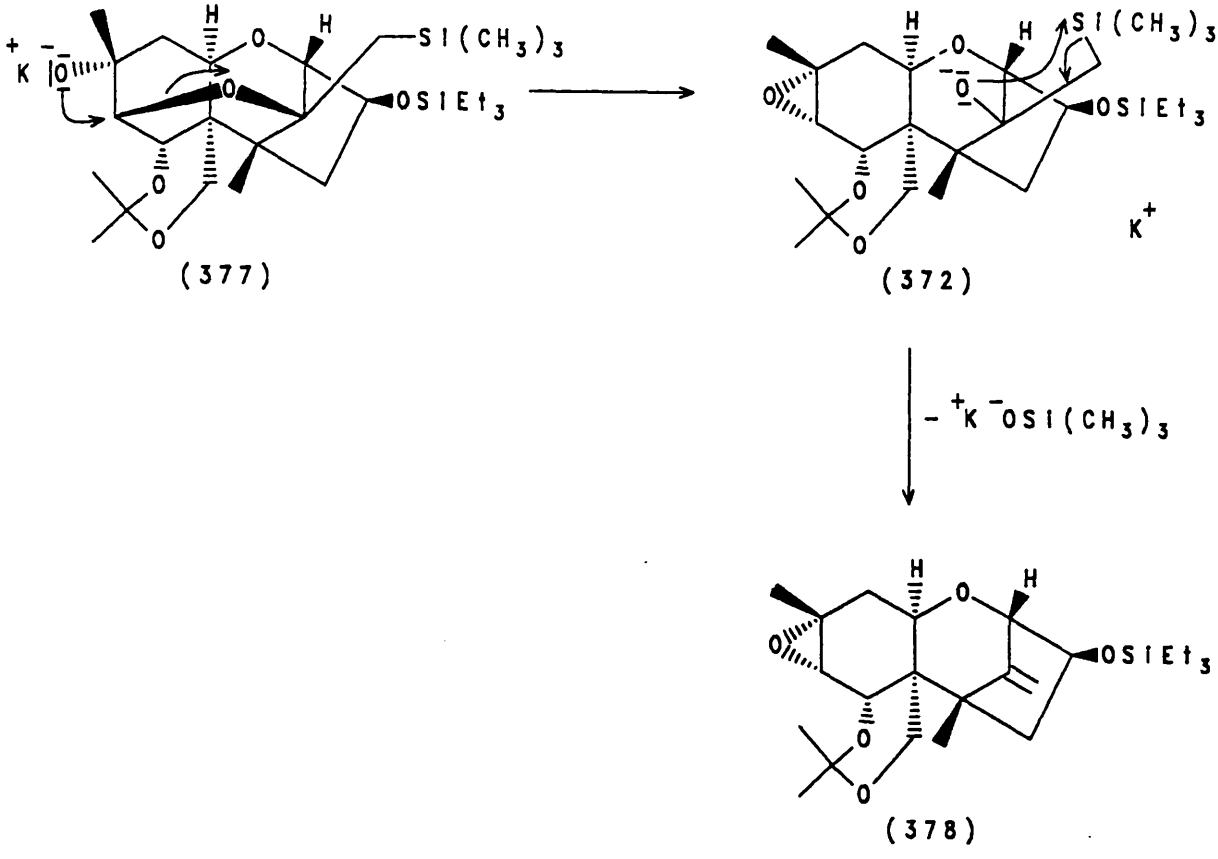
(375)



(376)

It was hoped that the Peterson adduct could still be employed in the synthesis. It was envisioned that formation of the potassium alkoxide (377) (Scheme 92) may result in epoxide reformation with subsequent elimination of $(CH_3)Si\text{-}O^{-}K^{+}$ to yield the required olefin (378). However treatment of the Peterson

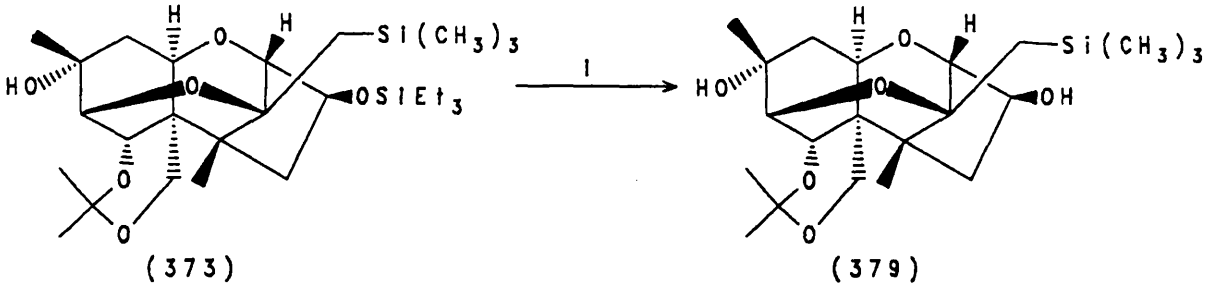
adduct with potassium hexamethyldisilazide in refluxing THF gave only recovery of the starting material.



Scheme 92

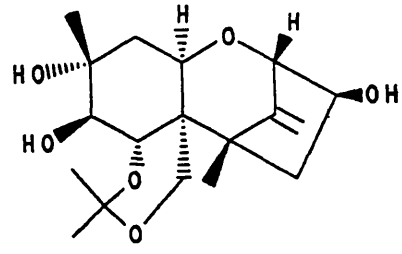
Treatment of the Peterson adduct with tetrabutylammonium fluoride in THF resulted in consumption of the starting material with formation of a single more polar product. Isolation of this product revealed that it was the alcohol (379) (Scheme 93)

resulting from desilylation of C3 and not as it was hoped the triol (381) resulting from desilylation and elimination.



(373) (379)

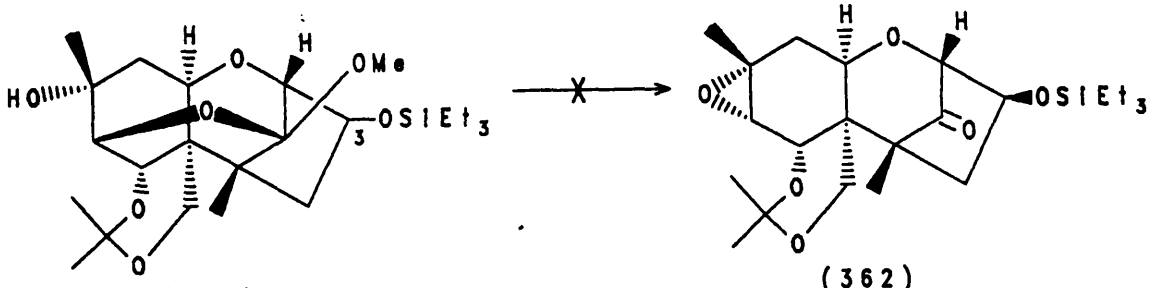
I; TBAF, THF



(381)

Scheme 93

The apparent stability of the structures (373) and (376) was shown further by attempts to regenerate the ketone (362) from the acetal (376) (Scheme 94).

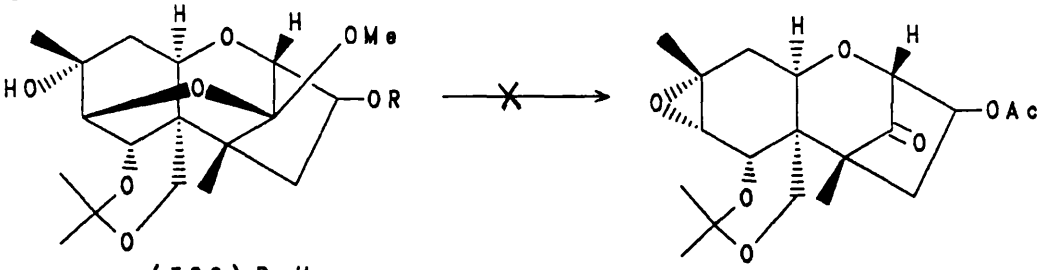


(376) (362)

Scheme 94

Employing a range of conditions including acetone/PPTS, acetone/PPTS/4A Mol.sieves, acetone/pTSA, acetone/pTSA/4A Mol. sieves and finally $BF_3.OEt_2$ both with and without the presence of 4A mol. sieves regeneration of the ketone failed. The only product isolated from these reactions was the alcohol (382)

resulting from desilylation at C3. Acetylating this alcohol, and treating the product with sulphuric acid in acetone also failed to generate the ketone (385).



(382) R-H
(383) R-OAc

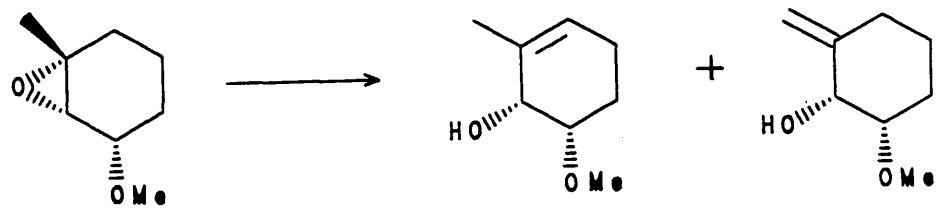
(385)

Scheme 95

The Peterson adduct (373) may be similarly stable and hence its refusal to undergo elimination to the olefin (378) (Scheme 92).

It was evident that the presence of the ring A epoxide was preventing a successful olefination reaction from occurring. Its removal prior to olefination appeared as a possible solution to the problem.

The synthesis was planned such that a base induced epoxide-allyl alcohol rearrangement[80] would be employed to generate the functionality of ring A of deoxynivalenol. Indeed the <u>syn</u> relationship between the epoxide and the adjacent C7 oxygen was deliberately introduced[50] due to the results of an extensive model study. This model study (Scheme 96) revealed that optimum yields of the required allylic alcohol (389) occurred when the epoxide and the adjacent oxygen substituent were <u>syn</u>.



(388)

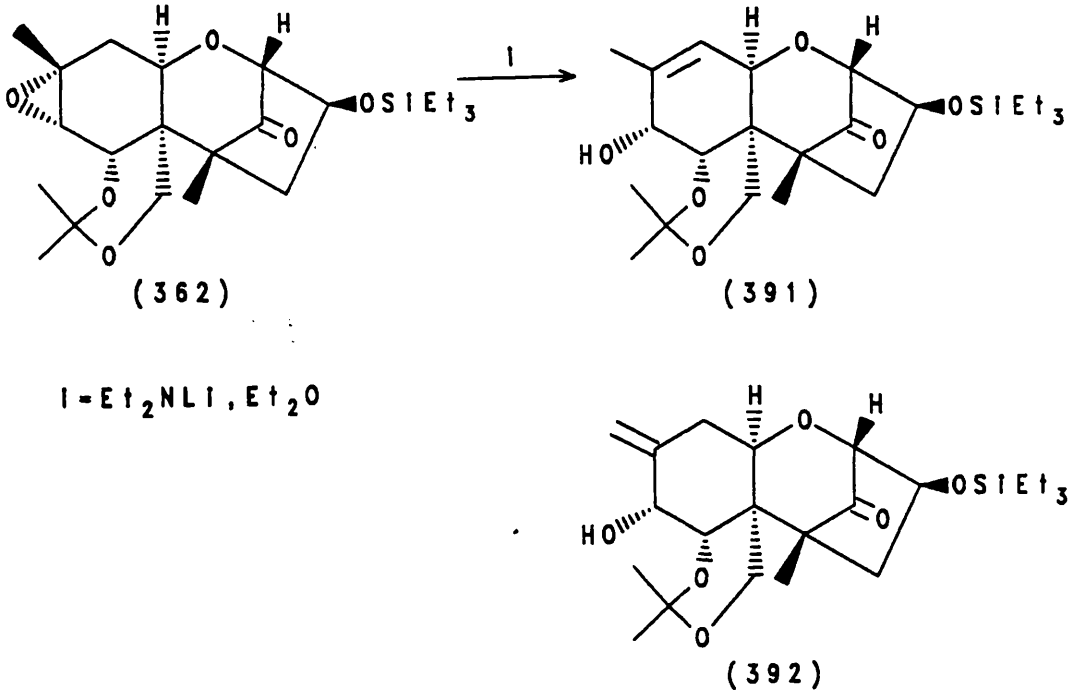
(389)

(390)

5 : 2

Scheme 96

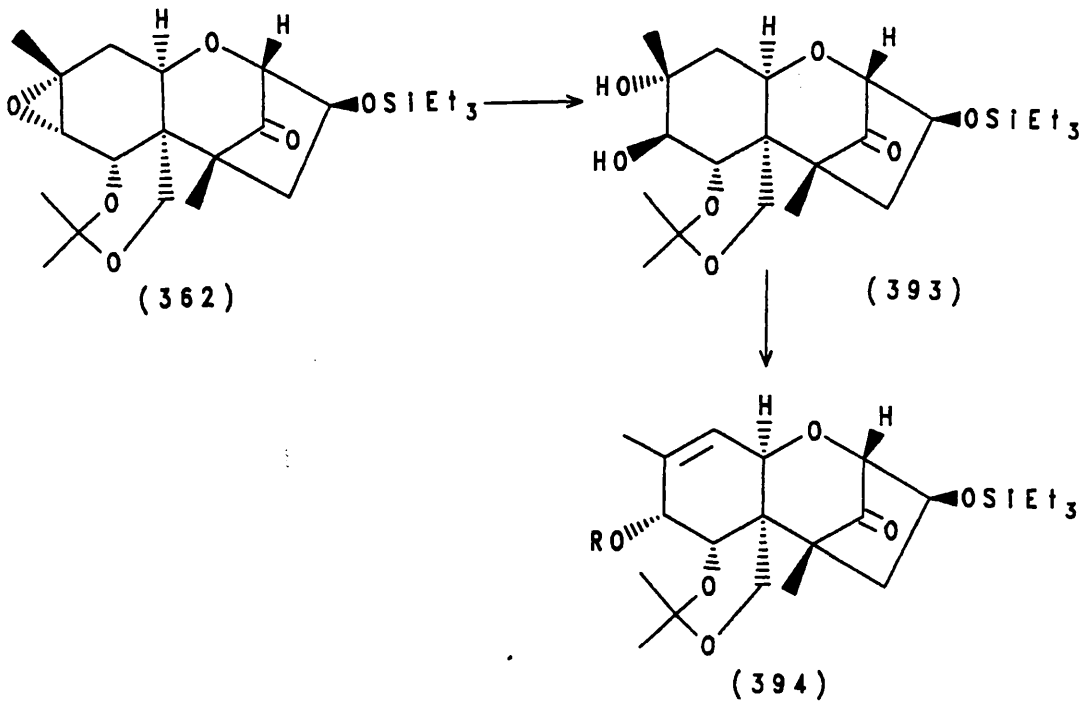
However treatment of the epoxide (362) with lithium diethylamide (Scheme 97) in refluxing diethyl ether gave four discrete products R_fs 0.07, 0.34, 0.48 and 0.52 (1:1, EtOAc/Petrol (40/60)). Chromatographic isolation of these products and ^1H nmr spectral analysis revealed that the major product, that with R_f 0.07 was not the required allylic alcohol (391) nor was it the exomethylene compound (392). ^1H nmr spectroscopic analysis of the compound of R_f 0.34 revealed that it was two compounds. A broad singlet at δ1.85 ppm could possibly have been due to the olefinic methyl group of (391), also resonances at δ5.64 ppm could have been due to the C10 H of (391). However, as there was no hope of isolating the individual components of this mixture full characterisation could not be achieved.



Scheme 97

Repetition of this base induced rearrangement and t.l.c. analysis of the product mixture revealed an identical range of products. Thus with no material or time remaining the synthesis of deoxynivalenol was terminated.

Although unsuccessful, this work revealed that removal of the epoxide prior to olefination of the carbonyl was necessary if a successful synthesis of vomitoxin via the intermediate (362) was to be achieved. One possible route would be epoxide ring opening to the diol (393) (Scheme 98), selective protection of the secondary alcohol followed by dehydration of the tertiary alcohol to the 9-ene (394), for which there is literature precedent.[52]



Scheme 98

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 temperature. ^{1}H n.m.r. spectra were recorded either on a Perkin Elmer R32 spectrometer operating at 90MHz or a Bruker WP 200SY spectrometer operating at 200MHz or a Bruker AM 200 spectrometer operating at 200MHz. ^{13}C n.m.r. spectra were recorded on the aforementioned Bruker instruments operating at 50MHz. Infra red spectra were determined on a Perkin-Elmer 580 spectrometer and optical rotations on a Optical Activity AA-100 auto-digital polarimeter. Low resolution mass spectra were determined on a VG updated MS 12 spectometer while high resolution mass spectra were determined on a VG updated MS 902 spectrometer. Elemental analyses were performed on a Carlo Erba 1106 elemental analyser. Gas-liquid chromatography was performed on a Hewlett Packard 5880A gas chromatograph equipped with a flame ioniser detector. The column used was a 25m x 0.32 mm ID fused silica capillary, CPSil 19cB, with a film thickness of 0.18 μm. Helium was employed as the carrier gas at a flow of 2ml/min.

In this thesis all model compounds i.e. all bicyclo[3,2,1]octanes are racemic, as are compounds (362) (373) (376) (382) and (384) relating to the synthesis of deoxynivalenol. All other compounds are derived from natural anguidine (34) and are therefore chiral. Petroleum ether refers to that fraction of petrol which boils between 40 and 60°C.

Acetylations : Standard product isolation

In the acetylation of alcohols in this thesis, isolation of the crude acetates was carried out as follows.

The reaction mixture was concentrated in vacuo and residual acetic acid and pyridine was removed by azeotropic distillation with toluene (3 x 10 ml) and then CCl_4 (3 x 10 ml).

Dipyridine - Chromium trioxide oxidations : standard product isolation.

The contents of the reaction flask were decanted and the flask was washed with aqueous $NaHCO_3$ (2 x 60ml). The combined aqueous washes were then extracted with Et_2O (2 x 100ml). The ethereal extracts were combined with the decanted reaction mixture and washed with $NaHCO_3$ (6 x 50 ml). The combined $NaHCO_3$ washes were then extracted with Et_2O (2 x 50 ml). All organic extracts were combined and washed sequentially with water (50 ml), HCl (1M, 100ml), aqueous $CuSO_4$ (1 x 100ml) and then brine (1 x 100ml). The organic solution was then dried and concentrated in vacuo.

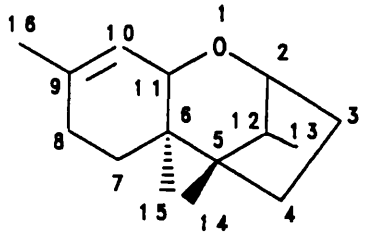
Starting materials.

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

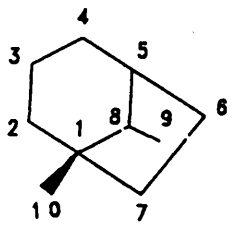
Anguidine (34) was isolated from culture broths as described previously.[71]

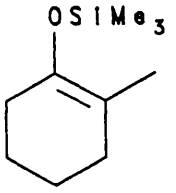
Numbering System

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.





(243)

1-Trimethylsilyloxy-2-methylcyclohex-1-ene (243)

A round bottomed three necked flask was charged with DMF (90ml), chlorotrimethylsilane (38ml, 0.3 moles) and Et_3N (83.1ml, 0.6 moles). To this stirring solution was added 2-methylcyclohexanone (30.3ml, 0.25 moles) and the mixture heated under gentle reflux for 48 hrs.

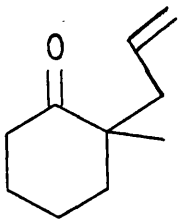
When cool, the resulting solution was diluted with pentane (300ml) and washed sequentially with cold aqueous $NaHCO_3$ (3 x 300ml), cold aqueous HCl (100ml) and $NaHCO_3$ (100ml).

The pentane solution was dried and concentrated to give a yellowish oil which on distillation gave the silyl enol ether (243) (34.1g, yield, 74%) as a clear oil, bp 72-74°C/ 8mm Hg, lit.[83] 82-84°C/ 16mm Hg.

Infra-red: ν max $CHCl_3$: 3010, 2940, 1710, 1250, 1200, 1060 940, 845 and 780 cm^{-1}

Mass Spectrum: Found M^+ : 184.1284 $C_{10}H_{20}OSi$ requires 184.1283 amu

^1H n.m.r, 90MHz ($CDCl_3$) : δ 1.86-2.05 ppm, m, (4H), δ 1.55-1.7ppm, m,(2H), δ 1.53, bs (3H, $C\underline{H}_3$), δ 0.15 ppm, s, (9H,$SiC\underline{H}_3$).



(244)

2-Methyl-2-allyl cyclohexanone (244)

A round bottomed flask equipped with side arm and a reflux condenser was charged with the silyl enol ether (243) (13.1g, 71 mmol). To this was added, dropwise, methyl lithium (1.5M in Et_2O, 49ml, 73.5 mmol) and the solution stirred at room temperature for 0.5 hr. At this point the ether was removed in vacuo and the resulting oil was taken up in THF (50 ml). Allyl bromide (6.6ml, 76 mmol) was then added in one portion and the solution stirred at room temperature for 10 min.

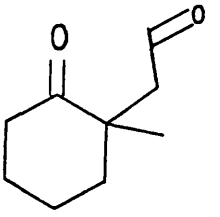
The mixture was diluted with pentane (150ml), washed with aqueous $NaHCO_3$ (3 x 100ml) dried and concentrated.

Distillation gave the allyl ketone (244) (9.4g, 90.4%), as a clear oil, bp 70-72°C/15 mm Hg.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
1	215.0	s	-	-	-	-
(-$\underline{CH}$=CH_2) (8)	133.60	d	5.62	m	-	1H
(CH=$\underline{CH_2}$) (9)	117.66	t	5.01	m	-	1H
2	48.21	s	4.93	m	-	-
6	41.77	t	1.1-2.0	m	-	(8H)
7	38.58	t	2.23	d	6.27	2H
3	38.41	t	1.1-2.0	m	-	(8H)
5	27.21	t	1.1-2.0	m	-	(8H)
C-$\underline{CH_3}$ (10)	22.78	q	0.99	s	-	3H
4	20.66	t	1.1-2.0	m	-	(8H)

<u>Infra-Red</u>: ν_{max} $CHCl_3$: 2930,1702, 1635, 1440, 990, 912 cm^{-1}

<u>Mass Spectrum</u>: Found M^+, 152.1196 $C_{10}H_{16}O$ requires 152.1197



(245)

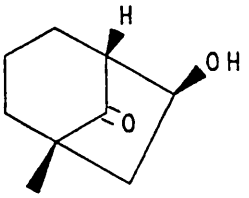
(1-Methyl-2-oxocyclohexyl)Ethanal. (245)

Ozone was bubbled through a solution of the allyl ketone (244) (5.12g, 33 mmol) in CH_2Cl_2 (90ml) at -78°C until the solution turned blue. Excess ozone was removed by purging the solution with nitrogen until the blue colour had disappeared. Et_3N (10.0ml, 72 mmols) was added and the solution stirred and warmed to room temperature overnight.

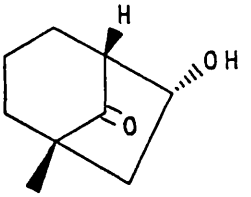
Concentration in vacuo gave a thick yellowish oil which was diluted with Et_2O (80 ml). The ethereal solution was then filtered through a short plug of chromatographic silica gel and concentrated to give the aldehyde (245) (4.38g, 85%) which was used without further purification.

Infra red: ν max CCl_4 : 2930, 1710, 1450, 1375, 1110, 970, 950, 925 and 885 cm^{-1}

^1H n.m.r : 90MHz($CHCl_3$) : δ 9.78 ppm, t, (1H,C<u>H</u>O), δ 1.29 ppm, s, (3H,C<u>H</u>$_3$)



(247)



(248)

1-Methyl-6-hydroxy-8-oxobicyclo[3,2,1]octane (247) and (248)

A round bottom flask fitted with a reflux condenser was charged with MeOH (380ml) and to this sodium (5g, 217 mmols) was added in small lumps. When the sodium had fully reacted, the aldehyde(245) (4.38g, 28.8mmol), in MeOH (120ml) was added and the solution heated under reflux for 35 min.

When cool the solution was poured onto ice and extracted with EtOAc (3 x 250ml), with salting. The organic extracts were dried and concentrated to give the crude product (3.69g).

Purification by dry column flash chromatography gave the keto-alcohol (2.5g, 57%) as a mixture of C6 alcohol epimers in the ratio of 2.5:1, 6β : 6α.

Careful flash chromatography (EtOAc/Pet-ether) allowed separation of the C6 epimers for characterisation.

6β - epimer (247)

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	222.23	s	-	-	-	-
6	69.00	d	4.20	dd	8.0,2.5	1H
5	55.65	d	2.24	bt	2.5	1H
1	47.83	s	-	-	-	-
2	43.77	t	1.4-2.1	m	-	-
7	43.65	t	α (masked)			
			β 2.42	dd	14.3, 8.0	1H
4	33.96	t	1.4-2.1	m	-	
(C-$\underline{C}H_3$) 9	19.13	q	0.99	s	-	3H
3	18.95	t	1.4-2.1	m	-	-
O$\underline{H}$			2.81	bs	-	1H

Infra-Red: ν_{max} $CHCl_3$: 3620, 3440, 2940, 2860, 1750, 1450, 1380, 1080, 1025 cm^{-1}

Mass Spectrum: Found M^+, 154.009 $C_9H_{14}O_2$ requires 154.099

Micro Analysis: C: 70.12 H: 9.12 $C_9H_{14}O_2$ requires
C: 70.08 H: 9.16

Melting Point: 84-86°C (Et_2O/hexane)

6α - epimer (248)

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	222.14	s	-	-	-	-
6	65.66	d	4.42	ddd	9.1, 6.1,4.5	1H
5	52.03	d	2.38	m	-	1H
1	48.61	s	-	-	-	-
2	45.12	t	1.3-2.3			
7	42.02	t	1.3-2.3	m	-	(8H)
4	31.75	t	1.3-2.3			
(C-$\underline{C}H_3$) 9	19.16	q	0.92	s	-	3H
3	19.08	t	1.3-2.3	m	-	(8H)
O$\underline{H}$	-	-	2.68	bs	-	1H

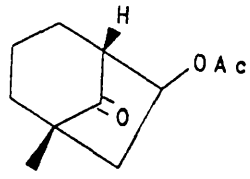
Infra-Red: ν_{max} $CHCl_3$: 3630, 3490, 2960, 2940, 1750, 1095, 1045 cm^{-1}

Mass Spectrum: Found M^+ 154.0980, $C_9H_{14}O_2$ requires 154.099

Micro-Analysis: C: 70.11 H: 9.26

$C_9H_{14}O_2$ requires: C: 70.08 H: 9.16

Melting Point: 98-99°C (Et_2O/hexane)



(249)

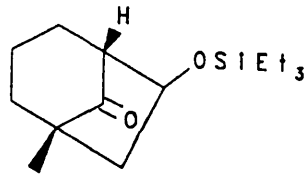
1-Methyl-6-acetoxy-8-oxobicyclo[3,2,1]octane (249)

A round bottomed flask was charged with the keto-alcohols (247) and (248) (0.86g, 5.5 mmol) in pyridine (2.5ml). To this, Ac_2O (3.5ml) was added and the solution stirred at room temperature for 24 h.

Product isolation by the standard procedure and purification by flash chromatography (Et_2O/Pet-ether) gave the keto-acetate (249) (0.95g, 87%) as a clear oil and an inseparable mixture of C-6 diastereomers.

Infra Red: ν max CCl_4 : 2940, 1753, 1743, 1240, 908, 725 and 650 cm^{-1}

Mass spectrum : Found M^+, 196.1080, $C_{11}H_{16}O_3$ requires 196.1099 amu.



(250)

1-Methyl-6-triethylsilyloxy-8-oxobicyclo[3,2,1]octane (250)

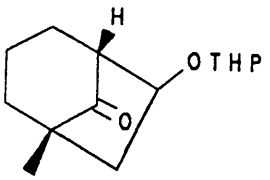
A flame dried round bottomed flask with stirring bar, under nitrogen was charged with DMAP (10mg, 1mmol) and then the keto-alcohols (247) and (248) (2.06g, 13.5 mmol) in Et_2O (15ml). To this stirred solution was added Et_3N (2.02g, 20 mmols) followed by chlorotriethylsilane (3.01g, 20mmols) and the resulting mixture stirred at room temperature for 24 h.

The solution was diluted with Et_2O (50 ml) and washed sequentially with HCl (1M, 10ml) aqueous $NaHCO_3$ solution (10ml) and brine (15ml), then dried and concentrated in vacuo to give a yellowish oil.

Purification by flash chromatography (Et_2O/hexane) gave the triethylsilyl ether (250) (3.5g, 97%) as a clear oil and an inseparable mixture of C6 diastereomers.

Infra Red: ν max $CHCl_3$: 3005, 2900, 1745, 1470, 1420, 1390, 1260, 1245, 1150, 1080, 1020 and 960 cm^{-1}

Mass Spectrum : Found M^+, 268.1853, $C_{15}H_{28}O_2Si$ requires 268.1858



(251)

1-Methyl-6-[(tetrahydro-2H-pyran-2yl)oxy]-8-oxobicyclo-[3,2,1]octane (251)

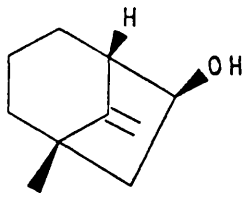
A round bottomed flask fitted with side arm was charged with dihydropyran (3ml, 35mmol) and PPTS (225mg, 0.9mmol) followed by the alcohols (247) and (248) (1.408g, 9.26 mmol) in Et_2O (2ml) and the solution stirred at room temperature. Thin layer chromatographic analysis after 16 hrs revealed that the reaction had reached completion.

The mixture was diluted with Et_2O (100ml) filtered through Celite, washed with H_2O (30ml) and dried (Na_2SO_4).

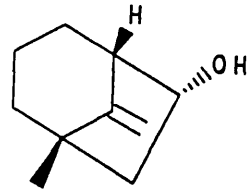
Concentration and purification by flash chromatography (Et_2O/Pet-ether) on neutral alumina gave the THP ether (251) (2.06g, 95%) as an inseparable mixture of diastereomers.

Infra-red ; ν max CCl_4 : 2940, 2875, 1745, 1450, 1375, 1350, 1260, 1200, 1130, 1110, 1075 and 1025 cm^{-1}

Mass Spectrum ; Found M^+, 238.1575, $C_{14}H_{22}O_3$ requires 238.1570



(252)



(253)

1-Methyl-6-hydroxy-8-methylene bicyclo[3.2.1]octane (252) and (253)

Method A : By Wittig reaction on (249)

A round bottomed flask fitted with side arm and reflux condenser was sequentially charged with methyltriphenyl-phosphonium bromide (11.7mg, 33.6 mmol) and dry THF (100ml). To this stirred slurry was added butyl-lithium (2.6M in hexane, 13ml, 33.6 mmol) and stirring continued for 0.5 hr when a solution of keto-acetate (249) (1.10g, 5.6 mmol) in THF (50ml) was added. The mixture was heated under reflux for 1 h, then it was cooled, diluted with H_2O (100ml) and extracted with Et_2O (3 x 100ml). The organic extracts were washed with H_2O (100ml), dilute HCl (100ml) and finally brine (100ml) then dried and concentrated in vacuo.

Purification by dry column flash chromatography (Et_2O-pet. ether) gave the desired olefin (410mg, 45.5%) as a mixture of readily separable C6 epimers.

Method B : By Wittig reaction on (250)

To a slurry of methyltriphenylphosphonium bromide (21.7g, 62 mmol) in THF (110ml) was added butyl-lithium (2.6M in hexane, 23.9ml, 62mmol) and this mixture stirred for 45 min, at which point a solution of the ketone (250) (3.53g, 13mmol) in THF (50ml) was added. This solution was heated under reflux for 1hr hr then cooled, diluted with H_2O (100ml) and extracted with Et_2O (3 x 100ml). The organic extracts were sequentially washed with H_2O (100ml) dilute HCl (100ml) and brine (50ml) then dried and concentrated in vacuo.

The crude product was taken up in CH_3CN (25ml) and to this was added H_2O (25ml) and HF (40%, 3.2g, 64mmol) and the solution stirred overnight when it was basified with K_2CO_3. The mixture was diluted with brine (50ml) and extracted with EtOAc (3 x 100ml) The organic extracts were dried and concentrated in vacuo.

Purification by flash chromatography (Et_2O-pet.ether) gave the desired olefins (252) and (253) (1.1g, 68%).

Method C : By Wittig reaction on (251)

To a slurry of methyltriphenyl phosphonium bromide (13.53g 3.9 mmol) in THF (100ml) was added butyl-lithium (2.4M in hexane, 16.3ml, 39 mmol) and this mixture stirred at room temperature for 45 min, when a solution of the ketone (251) (1.55g, 6.5 mmol) in THF (50ml) was added. This solution was heated under reflux for 1 h, when it was cooled, diluted with H_2O (100ml) and extracted with Et_2O (3 x 100ml). The organic extracts were washed sequentially with H_2O (100ml), dilute HCl (1M)(100ml) and brine (50ml) then dried and concentrated in vacuo.

The crude product was taken up in EtOH (5ml) and to this was added PPTS (250mg, 1 mmol) and the solution stirred at 50°C overnight. Concentration and purification by flash chromatography (Et_2O-pet.ether) gave the desired olefins (252) and (253) (0.796g, 80.4%).

Method D : By cleavage of methylthiomethyl ether (282a)

A round bottomed flask equipped with stirring bar and reflux condenser was charged with a mixture of the methyl thiomethyl ether (282a) and the ketone (260) (94.8mg) in acetone (6ml) and water (1ml). To this was added $NaHCO_3$ (190mg) and methyl iodide (0.25ml). The solution was heated under reflux for 2.5 h and then allowed to cool at which point EtOAc (50ml) was added.

This solution was washed with H_2O (10ml) dried and concentrated. Analysis by thin layer chromatography (EtOAc:pet.ether, 1:6) showed the presence of the 6β- alcohol (252) and also the ketone (260).

Purification by flash chromatography gave the 6β-alcohol (252) (4mg) and pure ketone (260) (46mg).

6β - epimer (252)

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	161.23	s	-	-	-	-
(C=$\underline{C}H_2$) 10	99.49	t	4.73,4.64	s,s	-	2H
6	73.62	d	4.02	d d	7.1,2.2	1H
5	53.38	d	2.41	b m	-	1H
2	48.34	t	-	-	-	-
1	43.76	s	-	-	-	-
7	41.73	t	α 1.3-2.0	m	-	1H
			β 2.3	d d	14, 7	(6H)
4	32.52	t	1.3-2.0	m	-	(6H)
(C_1-$\underline{C}H_3$) 9	22.96	q	1.07	s	-	3H
3	19.99	t	1.3-2.0	m	-	(6H)
O$\underline{H}$	-	-	1.81	b s	-	1H

Infra-Red: ν_{max} CCl_4: 3620, 3595, 2935, 1675, 1445, 1395, 1375, 1125, 1025 and 887 cm^{-1}

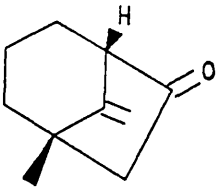
Mass Spectrum: Found M^+, 152.1211 $C_{10}H_{16}O$ requires 152.1200

6α - epimer (253)

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	162.3	s	-	-	-	-
(C=$\underline{C}H_2$) 10	98.76	t	4.62, 4.51	ss	-	2H
6	71.38	d	4.28	ddd	10.4, 6.3, 5	1H
5	48.71	d	2.55	ddd	6.3, 3, 3	1H
2	45.16	t				
7	43.15	t	1.0-2.0	m		(8H)
1	42.7	s	-	-	-	-
4	29.7	t	1.0-2.0	m	-	(8H)
(C_1-$\underline{C}H_3$) 9	23.19	q	1.00	s	-	3H
3	20.27	t	1.0-2.0	m	-	(8H)

Infra-Red: ν_{max} $CHCl_3$: 3630, 2930, 1662, 1445, 1372, 1259, 1235, 1105, 1040, 883 cm^{-1}

Mass Spectrum: Found M^+ 152.1202 $C_{10}H_{16}O$ requires 152.1200



(260)

1-Methyl-6-oxo-8-methylenebicyclo[3,2,1]octane (260)

Method A : By Corey oxidation of the alcohols (252) and (253) in CH_2Cl_2

To a stirred solution of NCS (790mg, 5.9mmol) inCH_2Cl_2 (30ml) at 0^oC was added DMS (0.8ml, 10.8 mmol) and stirring continued for 25 min. The mixture was cooled to -24^oC and a solution of the alcohols (252) and (253) (280mg, 1.8mmol) in CH_2Cl_2 (10ml) added slowly over 10 min. Stirring was continued for 2 h at -24^oC when a solution of Et_3N (0.8ml, 5.7 mmol) in CH_2Cl_2 (2 ml) was added and the mixture stirred for a further 10 min.

The mixture was poured onto Et_2O (60ml) then washed sequentially with HCl (1M) (60ml), H_2O (60ml) and brine (60ml), dried and concentrated in vacuo. Purification by flash chromatography (EtOAc/Pet.ether) gave 220mg (78%) of the ketone (260) R_f. 0.72 contaminated with the methylthiomethyl ether (282a).

Method B : By Corey oxidation of the alcohols (252) and (253) in toluene

Following the procedure described above the olefin alcohols (252) and (253) (283 mg, 1.86 mmol) were oxidised to the ketone (260) by treatment with NCS (379mg, 2.79 mmol) DMS (0.36ml, 4.65 mmol) and Et_3N (0.38ml, 2.79mmol) employing toluene as the solvent. Crude product isolation was as described above.

Purification by dry column flash chromatography (EtOAc/Pet.ether) gave the ketone (260) (215mg, 77%) as a clear oil contaminated with the methylthiomethyl ether (282a) which were visualised in t.l.c. analysis by saturation of the t.l.c plate with water.

Method C : By Jones oxidation of the alcohols (252) and (253)

The alcohols (252) and (253) (300mg, 1.97mmol) in acetone (5ml) were cooled to O^oC and Jones reagent (8N) added dropwise until a red-brown colour remained in the supernatant. The solution was then stirred at O^oC for a further 0.5 h.

The mixture was diluted with aqueous $NaHCO_3$ solution (25ml) and extracted with EtOAc (3 x 25ml). The organic extracts were combined, dried and concentrated in vacuo to give a yellowish oil.

Purification by flash chromatography (EtOAc/Pet.ether) gave 164mg of the ketone (260) (54%) as a clear oil which was visualised in t.l.c. analysis by saturation of the t.l.c. plate with water.

Method D : By oxidation of the alcohols (252) and (253) with chromium trioxide-3,5-dimethylpyrazole complex.

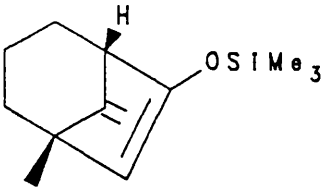
A flame dried flask with side arm under nitrogen was charged with chromium trioxide (500mg, 5.0 mmol), 3,5-dimethyl pyrazole (490mg, 5.0 mmol) and CH_2Cl_2 (25ml). This mixture was stirred at room temperature for 15 min giving a deep-red solution. The alcohols (252) and (253) (236mg, 1.55mmol) in CH_2Cl_2 (5ml) was added rapidly to the solution and this mixture stirred for 1 h at room temperature.

Concentration in vacuo, dilution with Et_2O and filtration through Celite gave a yellowish solution which was concentrated in vacuo, diluted with pentane and filtered through Celite. The resulting clear pentane solution was concentrated in vacuo. Purification by flash chromatography (Et_2O/pet.ether) gave the ketone (260) (168mg, 70.5%) as a clear oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
6	216.99	s	-	-	-	-
8	158.06	s	-	-	-	-
(C=$\underline{C}H_2$) 10	100.68	t	4.70	s	-	2H
5	56.06	d	2.8	b m	-	1H
7	51.83	t	2.0, 2.3	AB q	'obs 18'	
1	42.76	s	-	-	-	-
2	41.25	t	1.0-2.0	m	-	(6H)
4	34.2	t				
(C_1-$\underline{C}H_3$) 9	22.54	q	1.14	s	-	3H
3	16.64	t	1.0-2.0	m	-	(6H)

<u>Infra-Red</u>: ν_{max} CCl_4: 2950, 1745, 1663, 1395, 1115, 1050, 880 cm^{-1}

<u>Mass Spectrum</u>: Found M^+ 150.1053 $C_{10}H_{14}O$ requires 150.1045



(261)

1-Methyl-6-trimethylsilyoxy-8-methylenebicyclo[3,2,1]octan-6-ene (261)

A flame dried round bottomed flask with side arm, under nitrogen was charged with i-Pr_2NH (580 µl, 4.45 mmol) and THF (3ml). This solution was cooled to 0^oC and butyl lithium (2.47M, 1.8ml, 4.45mmol) was added slowly. The resulting solution was stirred for 0.5 h at 0^oC at which point it was cooled further to -78^oC when chlorotrimethylsilane (1.3ml, 10.2 mmol) in THF (3ml) was added dropwise over 5 min. The ketone (260) (311mg, 2 mmol) in THF (3ml) was then added slowly over 2 min and the resulting solution stirred for a further 2 min at which point Et_3N (2ml) was added and this mixture stirred for 3 min.

The cooling bath was removed and the reaction warmed to room temperature at which point petroleum-ether (50ml) was added and the solution washed with H_2O (20ml), dried (Na_2SO_4) and concentrated.

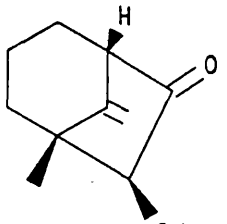
Kugelrohr distillation gave the silyl enol ether (261) (207mg, 45%) as a clear oil (bp. 115^oC 20 mmHg).

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
6	164.8	s	-	-	-	-
8	154.5	s	-	-	-	-
7	106.7	d	4.28	s	-	1H
(C=$\underline{C}H_2$) 10	93.6	t	4.35,4.32	s,s	-	2H
5	51.1	d	2.65	b t	-	1H
1	46.8	s	-	-	-	-
2	37.8	t	0.8-1.8	b m	-	(6H)
4	27.5	t	0.8-1.8	b m	-	(6H)
(C_1-$\underline{C}H_3$) 9	21.8	q	1.06	s	-	3H
3	20.3	t	0.8-1.8	b m	-	(6H)
Si($\underline{C}H_3$)$_3$	0.81	q	0.20	s	-	9H

<u>Infra-Red</u>: ν_{max} CCL_4: 2960, 1675, 1620, 1450, 1440, 1350, 1300, 1250s, 1230, 1175, 1120, 875, 845.

<u>Mass Spectrum</u>: Found M^+ 222.1425

$C_{13}H_{22}OSi$ requires 222.1408 amu.



(289)

1-Methyl-6-oxo-7β-acetoxy-8-methylenebicyclo[3.2.1]octane (289)

A flame-dried flask with side arm under nitrogen at $0^{o}C$ was charged with KHMDS (0.75M in toluene, 4ml, 3 mmol) and THF (5ml). This solution was cooled to $-78^{o}C$ when the ketone (300mg, 2 mmol) in THF (4ml) was added dropwise and the mixture stirred for 0.5 hr. To this pale yellow solution was added 2-p-tolylsulphonyl-3-(p-nitrophenyl)oxaziridine(273) in THF (4ml) dropwise over 3 min and the resulting red-brown solution stirred for 2.25h at $-78^{o}C$.

Saturated NH_4Cl solution (2ml) was added, the cooling bath removed and the mixture warmed to room temperature at which point EtOAc (50ml) was added, and the solution washed with H_2O (2 x 15ml), dried and concentrated in vacuo.

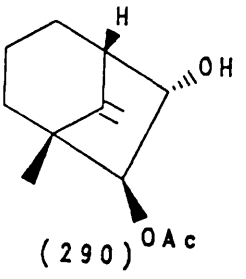
To the resulting orange solid in $CHCl_3$ (10ml) was added Ac_2O (2ml) and pyridine (1ml) and the solution stirred overnight at room temperature.

The crude product was isolated by the standard method and the resulting solid was triturated with petroleum-ether (5 x 25ml), and the petrol extracts combined and concentrated in vacuo. Purification by flash chromatography (EtOAc/Pet.ether) gave the acetate (289) (287 mg, 69%) as a clear oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
($OCOCH_3$)	212.95	s	-	-	-	-
6	169.93	s	-	-	-	-
8	155.39	s	-	-	-	-
($C=CH_2$) 10	103.21	t	4.91,4.82	s s	-	2H
7	79.40	d	5.05	s	-	1H
5	55.74	d	3.04	b m	-	1H
1	46.10	s	-	-	-	-
2	42.03	t	1.2-2.1	m	-	(6H)
4	34.99	t				
($COCH_3$)	20.05	q	2.07	s	-	3H
3	19.68	t	1.2-2.1	m	-	(6H)
(C_1-$\underline{C}H_3$) 9	17.04	q	1.07	s	-	3H

Infra-Red: $\nu_{max}CCl_4$: 2940, 1765, 1750, 1665, 1370, 1225, 1035 and 895 cm^{-1}

Mass Spectrum: Found M^+ 208.1107 $C_{12}H_{16}O$ requires 208.1099



(290)

1-Methyl-6α-hydroxy-7β-acetoxy-8-methylenebicyclo[3,2,1]octane (290)

A round bottomed flask with stirring bar was charged with the ketone (289) (79mg, 0.4mmol) in MeOH (4ml) and water (1ml). The flask was then cooled to $0^{o}C$ and sodium borohydride (40mg, 1.1 mmol) added. This mixture was stirred at $0^{o}C$ for 5 min at which point the cooling bath was removed and the reaction allowed to warm to room temperature over a further 20 min.

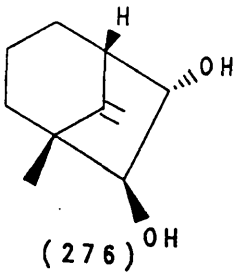
The mixture was diluted with EtOAc (20ml) and washed sequentially with HCl (1M, 5ml), saturated $NaHCO_3$ solution (5ml) and H_2O (15ml) and the organic solution dried and concentrated in vacuo.

Purification by flash chromatography gave the acetoxy alcohol (290) (60mg, 75%) as a colourless oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
($O\underline{C}OCH_3$)	173.02	s	-	-	-	-
8	158.90	s	-	-	-	-
($C=\underline{C}H_2$) 10	100.96	t	4.77,4.61	s,s	-	2H
7	88.17	d	4.49	d	2.7	1H
6	80.32	d	3.87	d d d	6.7, 2.0, 1.9	1H
5	47.17	d	2.74	d d d	6.7, 3.2, 3.0	
1	45.32	s	-	-	-	-
2	42.91	t				
4	28.94	t	1.2-1.9	m	-	(6H)
($OCO\underline{CH}_3$)	21.04	q	2.06	s	-	3H
3	19.47	t	1.2-1.9	m		(6H)
($C_1-\underline{C}H_3$) 9	17.73	q	1.00	s	-	3H
O<u>H</u>	-	-	3.18	d	1.8	1H

<u>Infra-Red</u>: ν_{max} $CHCl_3$: 3560, 2940, 1725, 1670, 1425, 1255, 1078, 1025 and 890 cm^{-1}

<u>Mass Spectrum</u>: Found M^+ 210.1277 $C_{12}H_{18}O_3$ requires 210.1255



(276)

1-Methyl-6α,7β-dihydroxy-8-methylenebicyclo[3,2,1]octane (276)

Method A : By treatment of the silyl enol ether (261) with 2-p-tolylsulphonyl-3-(p-nitrophenyl)oxaziridine (273).

A round bottomed flask equipped with stirring bar and reflux condenser under nitrogen was charged with the silyl enol ether (261) (107mg, 0.48mmol) in $CHCl_3$ (5ml). To this was added 2-p-tolylsulphonyl-3-(p-nitrophenyl)oxaziridine (273) (196mg, 0.64mmol) in $CHCl_3$ (20ml) and the mixture heated to 60°C for 2.5 h.

When cool the reaction mixture was concentrated in vacuo and the resulting solid was triturated with pentane (5 x 10ml). The pentane extracts were combined, filtered and concentrated to give 105 mg of a crude oil.

This oil was dissolved in MeOH (5ml)/H_2O (1ml) and the stirred solution cooled to 0°C. Sodium borohydride (79mg, 2.13mmol) was added and stirring continued at 0°C for 5 min. The cooling bath was removed and the mixture allowed to warm to room temperature over a further 20 min.

EtOAc (100ml) was added and the resulting mixture washed sequentially with HCl (1M, 15ml) aqueous $NaHCO_3$ (10ml) and H_2O

(20ml). The organic extract was dried (Na_2SO_4) filtered and concentrated in vacuo to give 103 mg of crude material.

Purification by flash chromatography (EtOAc/pet.ether) gave the desired diol (276) (22.4mg, 30.2%) as a solid which recrystallised from $CHCl_3$ to give white cubic crystals (m.p. 142-143°C).

Method B : By treatment of the lithium enolate of the ketone (260) with 2-p-tolylsulphonyl-3-(p-nitrophenyl)oxaziridine (273)

A flame-dried round bottomed flask fitted with side arm and a stirring bar, under nitrogen, was charged with hexamethyl-disilazane (210 µl, 0.98 mmol) and THF (3ml). To this solution butyl lithium (1.6M, 620 µ-l, 0.98mmol) was added dropwise and the mixture stirred for 15 min and then cooled to O°C. The ketone (260) (108mg, 0.7mmol) in THF (3ml) was added dropwise to the solution and this mixture was stirred for 30 min at O°C.

On cooling to -78°C the sulphonyloxaziridine (273) (440mg, 1.42 mmol) in THF (6ml) was added slowly over 5 min and the mixture stirred for a further 15 min at which point the reaction was warmed to O°C for 2 min.

Saturated NH_4Cl solution (2ml) was added and the resulting solution extracted with EtOAc (3 x 20ml). The organic extracts were combined, dried and concentrated to give a crude solid (400mg).

Reduction of the solid with sodium borohydride was performed as above using MeOH (5ml)/H_2O (1ml) and sodium borohydride (70mg,

2.05mmol). Isolation of the crude product was carried out as above.

Purification by flash chromatography (EtOAc/pet.ether) gave the diol (276) (41.4mg, 37%) and the 6α-alcohol (253) (46mg, 46%).

Method C : By treatment of the potassium enolate of the ketone (260) with 2-p-tolylsulphonyl-3-(p-nitrophenyl)oxaziridine (273)

A flame dried round bottomed flask with stirring bar and side arm, under nitrogen, was chaged with KHMDS (0.75M in toluene, 1.4ml, 1.1mmol) and THF (3ml). The flask was cooled to -78°C at which point the ketone (260) (95mg, 0.7mmol) in THF (2ml) was added dropwise and this solution stirred for 0.5 h.

The sulphonyloxaziridine (273) (450mg, 1.1mmol) in THF (3ml) was added dropwise and this mixture stirred at -78°C for 20 min. Saturated NH_4Cl (1ml) was added and the solution allowed to warm to room temperature. The solution was then extracted with Et_2O (3 x 20ml), the Et_2O extracts were combined and concentrated to give a yellowish solid.

Reduction of the solid with sodium borohydride was performed as above using MeOH (5ml), H_2O (1ml) and sodium borohydride (75mg, 2.2mmol). Isolation of the crude product was as above.

Purification by flash chromatography (EtOAc/pet.ether) gave the diol (276) (20mg, 19%) as a white solid m.p. 142-143°C.

Method D : By methanolysis of the acetate (290)

A round bottomed flask with stirring bar was charged with

the acetate-alcohol (290) (45mg, 0.21 mmol) in MeOH (2ml). To this solution was added K_2CO_3 (100mg, 0.72 mmol) and this mixture stirred overnight.

The solution was concentrated in vacuo and then diluted with H_2O (10ml). The aqueous solution was extracted with EtOAc (3 x 15 ml) and the organic extracts were combined dried and concentrated to give a white solid.

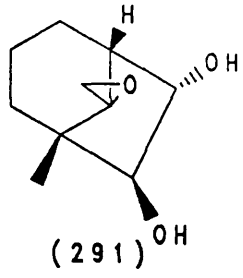
Purification by flash chromatography (EtOAc/pet.ether) gave the diol (276) (28mg, 77%) as a white solid, m.p. 140-142^{o}C.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	159.8	s	-	-	-	-
(C=$\underline{CH_2}$) 10	99.88	t	4.7+4.6	s,s	-	(2H)
6	83.39	d	3.97	dd	6.7, 2.6	1H
7	81.79	d	3.82	d	2.6	1H
5	47.21	d	2.18	ddd	6.7, 3.6	1H
1	45.97	s	-	-	-	-
2	42.33	t				
4	28.47	t	1.1-2.0	m	-	(6H)
3	19.34	t				
(C-$\underline{CH_3}$) 9	17.39	q	0.99	s	-	3H
O$\underline{H}$			2.2	bs		1H

Infra Red: ν_{max} KBr: 3350, 2925, 1668, 1450, 1060, 1025, 885, 740 cm^{-1}

Mass Spectrum: Found M^+ 168.1154 $C_{10}H_{16}O_2$ requires 168.1149

Melting Point: 143-144°C ($CHCl_3$)



(291)

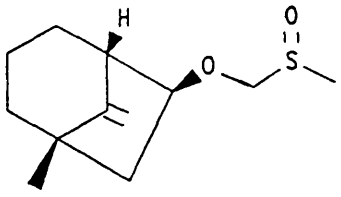
1-Methyl-6α,7β-dihydroxy-8,10-epoxybicyclo[3,2,1]octane (291)

To a round bottomed flask with stirring bar was added the diol (276) (39mg, 0.23mmol) in $CHCl_3$ (7ml) and Na_2HPO_4 (600mg) and cooled to O^oC. A solution of mCPBA (79mg, 0.36mmol) in $CHCl_3$ (3ml) was added dropwise over 2 min and the mixture stirred at O^oC for 1 hr and then at room temperature for a further 3 h. The reaction mixture was diluted with $CHCl_3$ (20ml), filtered through Celite and washed sequentially with aqueous $Na_2S_2O_3$ (2ml), aqueous $NaHCO_3$ (2ml) and brine (2ml). Drying the organics (Na_2SO_4) and concentration in vacuo gave a crude solid (20mg) which on purification by flash chromatography (EtOAc/pet.ether) gave the epoxy-diol (291) (10mg, 23%) as an amorphous solid which resisted recrystallisation.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
6	84.33	d	4.37	d d	6.8, 2.8	1H
7	82.62	d	3.82	d	2.7	
8	71.47	s	-	-	-	-
5	45.24	d				
10	44.55	t	2.64,2.7	ABq	"Obs,4"	2H
1	43.20	s	-	-		-
2	39.22	t				
4	26.00	t	1.0-2.2	m		(6H)
3	16.78	t				
(C_1-$\underline{C}H_3$) 9	13.65	q	0.73	s	-	3H

<u>Infra-Red</u>: ν_{max} $CHCl_3$: 3600, 3430, 3040, 2940, 1260, 850 cm^{-1}

<u>Mass Spectrum</u>: Found M^+ 184.1096 $C_{10}H_{16}O_3$ requires 184.1099



(282)

Attempted MoOPh hydroxylation of the ketone (260)
Formation of the sulphoxide (282).

To a flame dried, round bottomed flask fitted with a Schlenk tube charged with MoOPh (1.0g, 2.35 mmol) under nitrogen, was added KHMDS (0.75M in toluene, 2.5ml, 1.9mmol) in THF (3ml). The flask was cooled to -78°C and the ketone (260)(250mg, 1.66 mmol) (contaminated with the methylthiomethyl ether) in THF (3ml) was added dropwise and stirring at -78°C continued for 45 min.

MoOPH was then added rapidly via the Schlenk tube and vigorous stirring at -78°C continued for 3 h. The cooling bath was removed and the reaction allowed to warm to room temperature overnight.

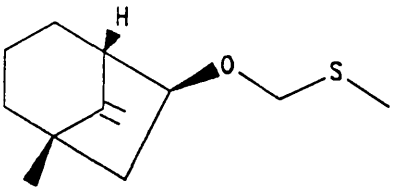
The solution was diluted with H_2O (15ml) and extracted with Et_2O (3 x 10ml). The combined ethereal extracts were washed sequentially with HCl (1M, 5ml) saturated $NaHCO_3$ (5ml) and brine (10ml) then dried and concentrated in vacuo.

The resulting oil was subjected to borohydride reduction following the procedure described on page 184, using MeOH (9ml)/ H_2O (1ml) and sodium borohydride (70mg, 2.0 mmol).

Isolation of the crude product was as described on page . Analysis of this product by t.l.c. indicated that two compounds were present R_f's 0.49 and 0.67. Isolation by flash chromatography (Et_2O/petroleum ether gave the 6α alcohol (253) (185 mg) and the sulphoxide (282) (12mg) as a clear oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	160.09	s	-	-	-	-
(C=$\underline{C}H_2$) 10	99.45	t	4.73,4.65	s,s	-	2H
6	88.37	d	4.35,4.31	d d	7.3, 2.5	1H
(O-$\underline{C}H_2$SO -)	82.72	t	4.41,4.33	ABq	'Obs,12'	2H
5	49.68	d	2.03	bm	-	1H
2	44.73	t	1.2-1.9	m	-	(7H)
1	43.84	s	-	-	-	-
7	41.76	t	α masked			7H
			β 2.18	dd	14.0, 7.3	1H
(SO -$\underline{C}H_3$)	37.79	q	2.86	s	-	3H
4	32.79	t	1.2-1.9	m	-	(7H)
($C_1\underline{C}H_3$) 9	22.81	q	1.09	s	-	3H
3	19.90	t	1.2-1.9	m	-	(7H)

Infra Red: ν_{max} : 2930, 1665, 1445, 1375, 1325, 1145, 1100, 1010 and 448 cm^{-1}



(282a)

1-Methyl-6β-thiomethylmethoxy-8-methylenebicyclo[3,2,1]octane (282a)

A round bottomed flask containing H_2O (700 µl) with a stirring bar was charged with a mixture of the ketone (260) and the methylthiomethyl ether (282a) (241mg) in MeOH (6.3ml) and cooled to 0^oC. To this solution was added sodium borohydride (110mg, 3.14 mmol) and the mixture stirred at 0^oC for 5 min at which point the cooling bath as removed and the solution allowed to warm to room temperature over a further 20 min.

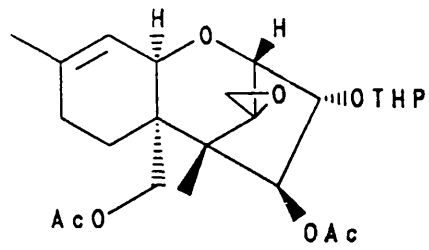
The reaction mixture was diluted with EtOAc (30ml) and washed sequentially with HCl (1M, 5ml), aqueous $NaHCO_3$ (10ml) and brine (10ml). Drying and concentration in vacuo gave an oil which by t.l.c. analysis consisted of two products of R_f 0.32 and 0.64 (EtOAc/pet.ether, 1:6). Isolation by flash chromatography gave the methyl thiomethyl ether (R_f 0.64) (19mg) and the 6α-alcohol (253) (184mg)(R_f 0.32).

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	161.5	s	-	-	-	-
(C=$\underline{CH_2}$) 10	98.9	t	4.62,	s	-	1H
			4.72	s	-	1H
6	77.7	d	4.10	d d	7.5, 2.5	1H
(O-$\underline{CH_2}$-S)	72.5	t	4.56	s	-	2H
5	49.4	d	2.58	bt	-	1H
2	44.9	t	1.2-1.85	m	-	(8H)
1	43.7	s	-	-	-	-
7	42.0	t	α1.2-1.85	m	-	(8H)
			β 2.09	dd	12.5, 7.5	1H
4	33.0	t	1.2-1.85	m	-	(8H)
(S-$\underline{C}H_3$)	22.9	q	2.11	s	-	3H
3	20.1	t	1.2-1.85	m	-	(8H)
(C_1-$\underline{CH_3}$) 9	13.7	q	1.09	s	-	3H

<u>Infra-Red</u>: ν_{max} ($CHCl_3$): 2930, 2870, 2850, 1620, 1440, 1370, 1300, 1270, 1055, 980 cm^{-1}

<u>Mass Spectrum</u>: Found M^+ 212.1231

$C_{12}H_{20}OS$ requires 212.1230 amu



(312)

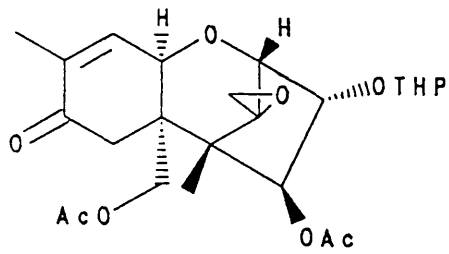
3α-[(Tetrahydro-2H-pyran-2yl)oxy]-4β,15-diacetoxy-12,13-epoxy-trichothec-9-ene (312)

A round bottomed flask with stirring bar was charged with anguidine (34) (160mg, 0.44mmol) and dihydropyran (1.5ml, 1.62g, 19 mmol). To this was added PPTS (15mg, 0.06mmol) and the mixture stirred at room temperature overnight.

The mixture was diluted with Et_2O (30ml), filtered through Celite washed with H_2O (10ml) and dried (Na_2SO_4). Concentration and purification by flash chromatography on neutral alumina gave the THP ether (312) (184 mg, 93.6 %) as a clear oil and an inseparable mixture of diastereomers.

Infra Red: $\tilde{\nu}$ max CCl_4 : 2950, 1740, 1680, 1440, 1365, 1240, 1170, 1125, 1070, 1040 and 980 cm^{-}

Mass Spectrum; Found M^+ 450.2245 $C_{24}H_{34}O_8$ requires 450.2251



(313)

3α-[(Tetrahydro-2H-pyran-2yl)oxy]-4β,15-diacetoxy-8-oxo-12,13-epoxytrichothec-9-ene (313)

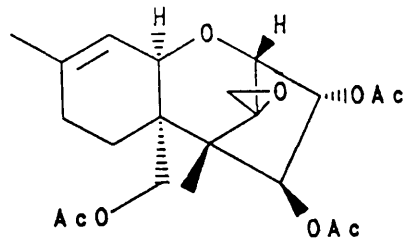
To a flame dried conical flask with stirring bar under nitrogen was added freshly prepared dipyridine chromium trioxide (10g, 38.7mmol) and dry CH_2Cl_2 (30ml). To this was added the tetrahydropyranyl ether (312) (184mg, 0.4mmol) in dry CH_2Cl_2 (10ml) and the mixture stirred for 48 hrs at room temperature.

The CH_2Cl_2 solution was decanted off and the reaction vessel washed with saturated $NaHCO_3$ solution (3 x 60ml). The aqueous washings were combined and extracted with Et_2O (3 x 60ml). The Et_2O extracts were combined with the CH_2Cl_2 extract and the combined organics washed with saturated $NaHCO_3$ solution (6 x 50ml). The combined aqueous washes were then extracted with Et_2O (2 x 50ml). All organic extracts were combined and washed sequentially with HCl (1M, 2 x 50ml), saturated $NaHCO_3$ solution (2 x 50ml), and brine (2 x 100ml) and then dried (Na_2SO_4) and concentrated.

Purification by flash chromatography (EtOAc/pet-ether) gave the enone (313) as a clear viscous oil (19.2mg, 10%) as an inseparable mixture of diastereoisomers.

Infra Red: ν max CCl_4 : 2950, 1745, 1685, 1440, 1365, 1240, 1160, 1125, 1080, 1045, 970 and 910 cm^{-1}

Mass Spectrum: Found M^+ 464.2049 $C_{24}H_{32}O_9$ requires 464.2043



(54)

3α,4β,15-Triacetoxy-12,13-epoxytrichothec-9-ene(54)

A round bottomed flask with stirring bar was charged with anguidine (54) (886 mg, 2.2mmol) in $CHCl_3$ (10ml) and to this solution pyridine (1.5ml) and Ac_2O (2ml) were added and the mixture stirred at room temperature for 24 hours.

Isolation of the crude product in the usual manner gave a brown solid.

Purification by flash chromatography (EtOAc/Petrol-ether) gave the triacetate (54) (902mg, 92%) as a white solid, m.p. 127-128°C.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
$OCOCH_3$	170.45	s	-	-	-	-
$OCOCH_3$	170.45	s	-	-	-	-
$OCOCH_3$	169.86	s	-	-	-	-
9	140.66	s	-	-	-	-
10	118.15	d	5.45	dq	5.6,1.3	1H
4	79.21	d	5.713	d	3.4	1H
3	78.25	d	5.16	dd	4.9,3.8	1H
11	77.44	d	3.95	bd	5.6	1H
2	67.84	d	3.83	d	4.9	1H
15	64.09	t	4.23,4.01	ABq	obs12.3	2H
12	63.38	s	-	-	-	-
6	48.70	s	-	-	-	-
13	47.06	t	3.04,2.77	ABq	obs3.98	2H
5	43.91	s	-	-	-	-
8	27.76	t	masked			
16	23.11	q	1.68	bs	-	3H
7	21.12	t	masked			
$OCOCH_3$	20.92	q	2.12	s	-	3H
$OCOCH_3$	20.79	q	2.07	s	-	3H
$OCOCH_3$	20.75	q	2.03	s	-	-
14	6.46	q	0.73	s	-	3H

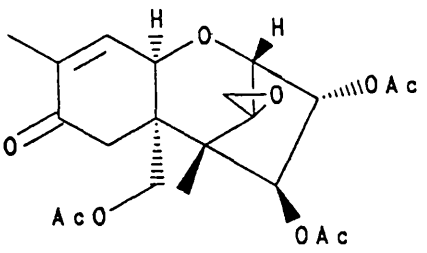
Infra-Red: ν_{max} : 3020, 1740, 1370, 1240, 1200, 1170, 1085, 1060, 1040, 970, 930, 790 and 720 cm^{-1}

Mass Spectrum: Found M^+ 408.1788

$C_{21}H_{28}O_8$ requires 408.1784

Melting Point: 126-128°C (EtOAc/hexane) Lit. Value 124-126°C

$[\alpha]_d^{20}$ +45.2° (c= 1 in AcOEt)



(305)

3α,4β,15-Triacetoxy-8-oxo-12,13-epoxytrichothec-9-ene (305)

Method A : By oxidation of (54) with dipyridine-chromium trioxide complex (Collins oxidation).

An oven dried three necked conical flask with stirring bar, under nitrogen was charged with freshly prepared dipyridine-chromium trioxide complex (18g, 69mmol) and dry CH_2Cl_2 (90ml) and the slurry stirred vigorously at room temperature. Triacetoxy-scirpenol (54) (230mg, 0.56mmol) in CH_2Cl_2 (10ml) was added to this slurry and the mixture stirred at room tempeature for 48 h.

Following the work up described on page , concentration of the organic extracts gave a white solid which was shown to be a mixture of the desired product and starting material by t.l.c. analysis.

Isolation by flask chromatography (EtOAc/pet.ether) gave the enone (305) (240mg, 62%) as a white solid, m.p. 144-146°C (EtOAc/pet.ether) and starting material (54) (44mg, 19%).

Method B : By oxidation of (54) with $Cr(CO)_6$ and t-butylhydroperoxide in acetonitrile

A flame dried round bottomed flask fitted with a reflux condenser, under nitrogen, was charged with chromium hexacarbonyl (55mg, 0.25mmol). Triacetoxyscirpenol (54) (208mg, 0.5mmol) in CH_3CN (10ml) was added, then t-BuOOH (3.5M in toluene, 3.9ml, 13.6mmol) added dropwise, with stirring over 5 min. The reaction mixture was then heated under reflux for 18 h.

When cool, the solution was filtered through Celite then diluted with Et_2O (70ml), washed with H_2O (3 x 10ml) and brine (2 x 20ml) and then dried and concentrated.

Purification by flash chromatography (EtOAc/petroleum ether) gave the desired enone (305) (54.2mg, 26%) m.p. 143-145°C and starting material (54) (96mg, 46%).

Method C : By oxidation of the selenide (328) with ozone

A round bottomed flask was charged with the selenide (328) (26mg, 0.044mmol) in CH_2Cl_2 (5ml) and the solution cooled to -78°C. Ozone was bubbled through this solution until saturation occurred at which point the solution was purged with nitrogen. The cooling bath was removed and the mixture allowed to warm to room temperature.

The solution was concentrated in vacuo to give a yellowish solid which by t.l.c. analysis contained the enone (305) (R_f 0.5). Purification by flash chromatography gave the enone (305) (13mg 69%) as a white solid m.p. 144-146°C.

Method D : By oxidation of the selenide (328) with H_2O_2.

A round bottomed flask with stirring bar under nitrogen was charged with the selenide (328) (61.4mg, 0.1mmol) in CH_2Cl_2 (2ml) and pyridine (26.6µl) and the mixture stirred at room temperature for 1 min. Hydrogen peroxide (30% 34µl, 0.3 mmol) was added dropwise and the solution stirred at room temperature for 1.5 h.

The solution was diluted with Et_2O (30ml) and washed sequentially with H_2O (10ml), saturated $CuSO_4$ (10ml) and H_2O (10ml) then dried and concentrated. Purification by column chromatography (EtOAc/hexane) gave the enone (305) (24.6mg, 55%) as a white solid.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	196.28	s	-	-	-	-
($OC\underline{O}CH_3$)	170.32	s	-	-	-	-
($OC\underline{O}CH_3$)	170.03	s	-	-	-	-
($OC\underline{O}CH_3$)	169.63	s	-	-	-	-
9	138.74	s	-	-	-	-
10	136.35	d	6.53	dq	5.9, 1.53	1H
4	78.15	d	5.69	d	3.3	1H
3	78.05	d	5.20	dd	4.9,3.3	1H
11	77.48	d	4.35	bd	5.9	1H
2	68.16	d	3.92	d	4.9	1H
15	64.23	t	4.3,4.1	ABq	obs 12.5	1H
12	64.07	s	-	-	-	-
6	48.65	s	-	-	-	-
5	47.44	s	-	-	-	-
13	46.70	t	3.07,2.8	ABq	obs3.87	2H
7	38.12	t	2.86,2.42	ABq	obs 15.9	2H
($OCO\underline{C}H_3$)	20.73	q	2.14	s	-	3H
($OCO\underline{C}H_3$)	20.65	q	2.08	s	-	3H
($OCO\underline{C}H_3$)	20.54	q	1.97	s	-	3H
16	15.37	q	1.79		-	3H
14	5.82	q	0.70	s	-	3H

Infra Red: ν_{max} CCl_4: 2980, 1740, 1682, 1368, 1240, 1080, 1045 and 960 cm^{-1}

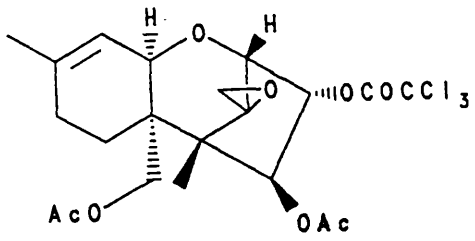
Mass Spectrum: Found M^+ 422.1570 $C_{21}H_{26}O_9$ requires 422.1576

Micro Analysis found: C: 59.77% H: 6.12%

$C_{21}H_{26}O_9$ requires C: 59.69% H: 6.20%

$[\alpha]_D^{18\ C} = +69.7^o$ (c= 1.9 in AcOEt)

Melting Point: 155-157°C (EtOAc/Petroleum-ether),



(314)

3α-Trichloroacetoxy-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (314)

To a flame dried round bottomed flask equipped with stirring bar and side arm under nitrogen was added DMAP (50mg, 0.4 mmol) and anguidine (34) (263mg, 0.72mmol) in CH_2Cl_2 (10ml). The flask was cooled to 0^oC and Et_3N (120µl, 0.84mmol) and trichloroacetyl chloride (180 µl, 1.68 mmol) added and the mixture stirred overnight.

The reaction mixture was diluted with Et_2O (20ml) and washed sequentially with HCl (1M, 5ml), aqueous $NaHCO_3$ (5ml) H_2O (10ml) and brine (10ml). The organic extract was dried and concentrated to give a crude brown solid (417mg).

Recrystallisation (CH_2Cl_2/hexane) gave the trichloroacetate (314) (236mg, 64%) as a white solid, m.p. 201-202^oC.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
($O\underline{C}OCH_3$)	170.53	s	-	-	-	-
($O\underline{C}OCH_3$)	170.18	s	-	-	-	-
($O\underline{C}OCCl_3$)	160.81	s	-	-	-	-
9	140.64	s	-	-	-	-
10	117.99	d	5.38	dq	5.6, 1.3	1H
4	82.83	d	5.93	d	2.9	1H
3	78.53	d	5.19	dd	4.9, 2.9	1H
11	76.74	d	4.03	bd(masked)	-	-
2	67.83	d	4.00	d	4.9	1H
12	64.05	s	-	-	-	-
15	63.36	t	4.23,4.02	ABq	obs13	2H
6	48.95	s	-	-	-	-
13	47.17	t	3.09,2.82	ABq	obs3.9	2H
5	43.81	s	-	-	-	-
8	27.75	t	1.98	m	-	2H
($OCO\underline{C}H_3$)	23.05	q	2.11	s	-	3H
7	21.31	t	masked			
($OCO\underline{C}H_3$)	20.92	q	2.05	s	-	3H
16	20.71	q	1.69	bs	-	3H
14	6.41	q	0.76	s	-	3H

Infra-Red: ν_{max} CCl_4 : 2970, 1775, 1750, 1268, 1245, 1230, 1165, 1085, 1045, 1030 and 970 cm^{-1}

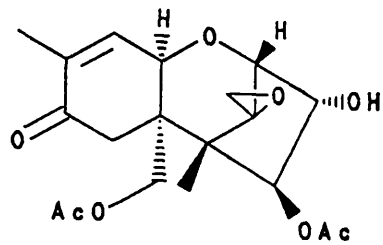
Mass Spectrum: Found 454.0346,

$M^+-CH_3CO_2H$ ($C_{19}H_{21}O_6Cl_3$) requires 454.0336

Micro Analysis : C: 49.42% H: 4.96% Cl: 20.80%

$C_{21}H_{25}O_8Cl_3$ requires C: 49.28% H: 4.92% Cl: 20.78%

Melting Point: 201-202°C (CH_2Cl_2/hexane)



(297)

3α-Hydroxy-4β,15-diacetoxy-8-oxo-12,13-epoxytrichothec-9-ene (8-ketoanguidine) (297)

Method A : By cleavage of the THP ether (313).

A round bottomed flask with stirring bar was charged with the THP-ether (313) (17.7mg, 0.04mmol) in EtOH (2ml). PPTS (7mg, mmol) was added and the solution heated to 30°C and maintained at this temperature for 24 h. T.l.c. analysis revealed that the starting material had been consumed.

The mixture was concentrated in vacuo to give an oil which on purification by positive pressure chromatography (EtOAc:Pet-ether, 3:2) gave as the major product 8-ketoanguidine (297) (10mg, 71%) as a clear oil.

Mehod B : By Collins oxidation of (312) with immediate cleavage of the THP ether

A flame dried, three necked conical flask with stirring bar, under nitrogen was charged with freshly prepared dipyridine-chromium trioxide complex (15g, 49 mmol) and dry CH_2Cl_2 (60ml). The olefin (312) (201mg, 0.43 mmol) in CH_2Cl_2 (15ml) was added and the mixture stirred for 48 h at room temperature.

Isolation of the product by the procedure described on page 159 gave a reddish oil (275mg) that was treated with ethanol (5ml) in the presence of PPTS (40mg, 0.15mmol) at 60°C for 24 hours.

The solution was concentrated in vacuo. Purification by flash chromatography (EtOAc/pet.ether) gave 8-ketoanguidine (297) (30mg, 17%) as a clear oil.

Method C : By Collins oxidation of the trichloroacetate (314)

A flame dried, three necked conical flask with stirring bar, under nitrogen was charged with freshly prepared dipyridine-chromium trioxide complex (18.3g, 70.3 mmol) and dry CH_2Cl_2 (50ml). The trichloroacetate (314) (271mg, 0.54 mmol) in CH_2Cl_2 (20 ml) was added and the mixture stirred at room temperature for 48 hrs.

The solution was diluted with CH_2Cl_2 (60ml) filtered through Celite and concentrated in vacuo. The resulting residue was diluted with Et_2O (60ml), filtered through Celite and washed sequentially with aqueous $CuSO_4$ (20ml), H_2O (10 ml) brine (10ml) and dried and concentrated.

The crude product (297mg) was dissolved in CH_2Cl_2 (10ml) and ethanolic ammonium hydroxide (10% v/v) (1ml) added and the mixture stirred at room temperature for 1 h.

Concentration and purification by flash chromatography (EtOAc/Pet-ether) gave 8-keto-anguidine (297) (156mg, 78%) as a clear oil.

Method D : By cleavage of the 3α-acetate of (305).

(See page for the preparation of (305)).

A round bottomed flask with stirring bar was charged with the enone (305) (20mg, 0.05 mmol) in MeOH (9ml) and to this, NH_4OH (20M, 1ml) was added and the mixture stirred at room temperature for 0.5 hr.

Hydrochloric acid (1M, 1ml) and H_2O (10ml) were added and the solution was extracted with EtOAc (3 x 20ml). The organic extracts were combined, dried and concentrated in vacuo to give an oil which by t.l.c. analysis contained 8-keto anguidine (R_f 0.18).

Isolation by flash chromatography gave 8-keto-anguidine (10.4mg, 58%) as a clear oil.

Method E : By P.C.C. oxidation of the diol (303)

A flame dried flask unde nitrogen was charged with the diol (150mg, 0.39mmol) in CH_2Cl_2 (12ml) and NaOAc (16mg, 0.19 mmol) and freshly prepared P.C.C. (12mg, 0.56 mmol) added. The mixture was stirred at room temperature for 8 h when it was filtered through Celite and concentrated to give an oil.

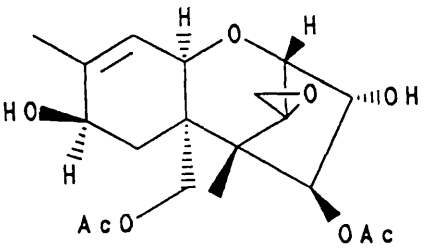
Purification by flash chromatography (EtOAc/hexane) gave the enone (297) (80mg, 53%) as a clear oil and starting material (28mg, 19%).

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	196.66	s	-	-	-	-
($O\underline{C}OCH_3$)	172.46	s	-	-	-	-
($O\underline{C}OCH_3$)	170.12	s	-	-	-	-
9	138.66	s	-	-	-	-
10	136.83	d	6.59	dq	5.9, 1.53	1H
4	83.55	d	5.08	d	2.8	1H
11	78.84	d	4.50	bd	5.9	1H
3	78.21	d	4.22	dd	4.8,2.8	1H
2	68.20	d	3.71	d	4.8	1H
15	64.41	t	4.16,4.08	ABq	obs 12.4	2H
12	64.31	s	-	-	-	-
6	48.66	s	-	-	-	-
5	47.35	s	-	-	-	-
13	46.68	t	3.06,2.79	ABq	obs3.9	2H
7	38.06	t	2.90,2.45	ABq	obs 15.87	2H
($OCO\underline{C}H_3$)	20.92	q	2.12	s	-	3H
($OCO\underline{C}H_3$)	20.59	q	1.96	s	-	3H
16	15.46	q	1.80	bs	-	3H
14	6.19	q	0.78	s	-	3H

Infra-Red: ν_{max} CCl_4 : 3560, 2930, 1750, 1723, 1689, 1370, 1250, 1225, 1160, 1100, 1090, 1050 and 960 cm^{-1}

Mass Spectrum: Found M^+ 380.1455 $C_{19}H_{24}O_8$ requires 380.1471

$[\alpha]_d^{18}$ C =+43.7 (c= 0.27 in EtOAc)



(303)

4β-,15-Acetoxy-3α,8β-hydroxy-12,13-epoxytrichothec-9-ene (303).

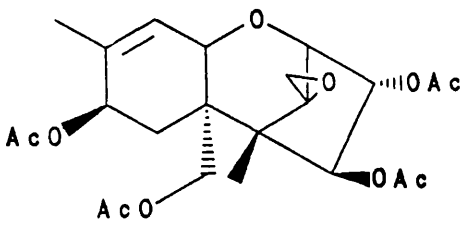
A round bottomed flask fitted with reflux condenser and a stirring bar, was charged with anguidine (34)) (456mg, 1.25mmol) in dioxane (27ml). To this was added SeO_2 (155mg, 1.4mmol) and H_2O (1ml) and the mixture heated under reflux for 24 h.

When cool the mixture was filtered through Celite and concentrated in vacuo. Thin layer chromatographic analysis (EtOAc) indicated the presence of 4 compounds. The compound with R_f 0.32 was isolated by flash chromatography and characterised as the 8β-alcohol (303) (230mg, 48%) as a white solid which did not succumb to recrystallisation.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
($O\underline{C}OCH_3$)	172.43	s	-	-	-	-
($O\underline{C}OCH_3$)	170.42	s	-	-	-	-
9	142.71	s	-	-	-	-
10	120.74	d	5.55	bd	5	1H
4	84.16	d	5.05	d	2.8	1H
11	78.94	d	4.06	bd	5	1H
3	78.02	d	3.98	dd	4.9,2.8	1H
8	68.20	d	masked		-	-
2	67.85	d	3.67	d	4.9	1H
12	64.15	s	-	-	-	-
15	63.72	t	4.16,3.88	ABq	obs 12.4	2H
6	48.83	s	-	-	-	-
13	46.98	t	3.05,2.78	ABq	obs3.84	2H
5	45.91	s	-	-	-	-
7	31.43	t	masked			
($OCO\underline{C}H_3$)	20.94	q	2.11	s	-	3H
($OCO\underline{C}H_3$)	20.94	q	2.02	s	-	3H
16	18.78	q	1.79	bs	-	3H
14	6.75	q	0.78	s	-	3H

<u>Infra-Red</u>: ν_{max} $CHCl_3$: 3600, 3030, 1730, 1370, 1245, 1160, 1110, 1045, 990, 960, 795, 725 and 675 cm^{-1}

<u>Mass Spectrum</u>: Found M^+ 382.1637 $C_{19}H_{26}O_8$ requires 382.1629



(323)

3α,4β,8β,15-Tetra-acetoxy-12,13-epoxytrichothec-9-ene (323)

Method A : By Luche reduction of the enone (297).

To a round bottomed flask with stirring bar was added the enone (297) (26mg, 0.06mmol) in MeOH (4ml) and $CeCl_3.7H_2O$ (44mg, 0.12mmol). The solution was cooled to -78°C at which point sodium borohydride (14mg, 0.37mmol) was added and the solution stirred at -78°C for a further 20 min. Acetone (1ml) was added and the cooling bath was removed and the reaction allowed to warm to room temperature.

The solvent was removed in vacuo, producing a white solid which was dissolved in EtOAc (20ml) and washed with HCl (1M, 2ml) and saturated $NaHCO_3$ (2ml) then dried. Concentration in vacuo gave an oil which was dissolved in $CHCl_3$ (2ml). Pyridine (0.5 ml) and Ac_2O (1ml) were added and the mixture stirred at room temperature overnight. Concentration in vacuo followed isolation of the crude product in the usual manner gave a clear viscous oil. Purification by positive pressure chromatography gave as the major product the 8β-acetate (323) (20mg 70%).

Method B : By acetylation of the diol (304)

A round bottomed flask with stirring bar was charged with the diol (304) (50mg, 0.13mmol). Ac_2O (1ml) and pyridine (0.5ml) and the mixture stirred at room temperature overnight.

The solution was concentrated in vacuo and the crude product isolated in the usual manner. Purification by flash chromatography (EtOAc/Hexane) gave as a white solid, the 8β-acetate (323) (57mg, 94%).

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
($O\underline{C}OCH_3$)	170.59	s	-	-	-	-
($O\underline{C}OCH_3$)	170.59	s	-	-	-	-
($O\underline{C}OCH_3$)	170.59	s	-	-	-	-
($O\underline{C}OCH_3$)	169.87	s	-	-	-	-
9	139.72	s	-	-	-	-
10	121.96	d	5.61	dt	-	1H
4	78.82	d	5.61	masked	-	-
8	78.12	d	5.19	masked	-	-
3	77.60	d	5.19	dd	4.8,3.5	1H
11	70.17	d	3.94	bd	6.11	1H
2	67.54	d	3.88	d	4.8	1H
12	63.92	s	-	-	-	-
15	63.13	t	4.29,4.09	ABq	obs 12.38	2H
6	49.09	s	-	-	-	-
13	46.97	t	3.08,2.79	ABq	obs3.96	2H
5	46.05	s	-	-	-	-
7	27.36	t	2.0	masked	-	-
($OCO\underline{C}H_3$)	21.06	q	2.13	s	-	3H
($OCO\underline{C}H_3$)	20.88	q	2.11	s	-	3H
($OCO\underline{C}H_3$)	20.79	q	2.10	s	-	3H
($OCO\underline{C}H_3$)	20.65	q	2.08	s	-	3H
16	18.65	q	1.71	s	-	3H
14	6.51	q	0.75	s	-	3H

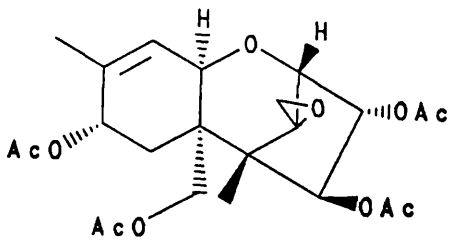
Infra-Red: ν_{max} CCl_4 : 2930, 1742, 1375, 1235, 1120 and 970 cm^{-1}

Mass Spectrum: Found 406.1610,

$M^+-CH_3CO_2H$ ($C_{21}H_{26}O_8$) requires 406.1628

$[\alpha]_D^{13\ C}$ = +76.56 (c= 0.32 in AcOEt)

Melting Point: (benzene/hexane) 147-149°C



(319)

3α,4β,8α,15-Tetra-acetoxy-12,13-epoxytrichothec-9-ene (319)

Method A : From (297) by reduction with L-Selectride followed by acetylation

A flame dried round bottomed flask with stirring bar under nitrogen was charged with 8-keto-anguidine (297) (20mg, 0.053mmol) in THF (2ml). The flask was cooled to -78°C and L-Selectride (1M in THF, 150µl, 0.15mmol) added dropwise. This mixture was stirred at -78°C for a further hour when saturated NH_4Cl (1ml) was added to the solution. The cooling bath was removed and the mixture warmed to room temperature.

The solution was diluted with H_2O (3ml) and extracted with EtOAc (3 x 15ml). The EtOAc extracts were combined, dried and concentrated.

The concentrated product was treated with pyridine (1ml) and Ac_2O (2ml) at room temperature overnight. Product isolation by

the standard method gave a white solid. Gas chromatographic and t.l.c. analysis indicated that it was the 8α-acetate (319) with minor impurities.

Purification by flash chromatography (EtOAc/hexane) gave the 8α-acetate (319) (19mg, 77%) as a white solid, m.p. 182-183°C (benzene/hexane).

Method B : From (305) by reduction with L-Selectride followed by acetylation (Synthesis of T-2-tetraol)

Employing the above procedure the enone (305) (30mg, 0.071 mmol) in THF (3ml) was reduced with L-Selectride (1M in THF, 80µl, 0.08 mmol). The product was acetylated as above.

Purification by flash chromatography (EtOAc/hexane) gave T-2 tetraol tetra-acetate (319) (30mg, 88%) as a white solid m.p. 182-183°C (benzene/hexane).

Method C : From T-2 toxin (4)

A round bottomed flask with stirring bar was charged with T-2 toxin (57.6mg, 0.12mmol) in MeOH (4.5ml). To this solution was added NH_4OH (28%, 0.5ml) and the mixture stirred at room temperature for 48 h. The solution was concentrated in vacuo and diluted with water (1ml). The aqueous solution was extracted with ethyl acetate (3 x 10ml) and the organic extracts combined, dried and concentrated.

The crude product was treated with pyridine (1ml) and Ac_2O (2ml) at room temperature overnight. Crude product isolation in the usual manner gave a yellowish oil. Thin layer chromatographic analysis revealed the presence of two compounds. Isolation of the more polar compound (R_f 0.4) by flash chromatoraphy (EtOAc/hexane) gave a white solid which was characterised as T-2 tetraol tetraacetate (319) (45mg, 78%), m.p. 186-187^oC.
Mixed mp with (319) derived from the ketone (327) = 185-186^oC.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
($OC\underline{O}CH_3$)	170.56	s	-	-	-	-
($OC\underline{O}CH_3$)	170.42	s	-	-	-	-
($OC\underline{O}CH_3$)	170.11	s	-	-	-	-
($OC\underline{O}CH_3$)	169.83	s	-	-	-	-
9	136.40	s	-	-	-	-
10	123.41	d	5.73	dt	5.8,1	1H
4	78.87	d	5.80	d	3.3	1H
8	78.28	d	5.23	bd	5.2	1H
3	77.33	d	5.17	dd	4.9,3.3	1H
11	68.17	d	4.14	bd	5.8	1H
2	67.29	d	3.84	d	4.9	1H
15	64.26	t	4.34,4.09	ABq	obs 12.4	2H
12	64.02	s	-	-	-	-
13	48.55	t	3.05,2.8	ABq	obs3.92	2H
6	47.13	s	-	-	-	-
5	42.93	s	-	-	-	-
7	27.25	t	2.31 + masked	dd	15.08, 5.7	1H
($2xOCOC\underline{H}_3$)	21.02	q	2.14	s	-	3H
			2.09	s	-	3H
($OCOC\underline{H}_3$)	20.84	q	2.04	s	-	3H
($OCOC\underline{H}_3$)	20.79	q	2.01	s	-	3H
16	20.24	q	1.74	bs	-	3H
14	6.62	q	0.73	s	-	3H

Infra-Red: ν_{max} CCl_4 : 2980, 1745, 1370, 1235, 1085, 1060, 1030 and 970 cm^{-1}

Mass Spectrum: Found 406.1627

$M^+ - CH_3CO_2H$ ($C_{21}H_{26}O_8$) requires 406.1628

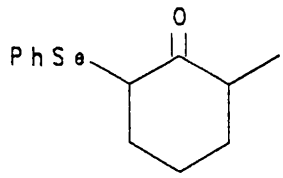
Micro Analysis found C: 59.37% H: 6.45%

$C_{23}H_{30}O_{10}$ requires C: 59.20% H: 6.48%

$[\alpha]^{13\ C}$ = =30° (C= 0.83, EtOAc)

Melting Point: 182-183°C (benzene/hexane),

Lit. Value[133] 178-179°C



(344)

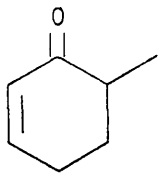
2-Methyl-6-phenylselenyl-6-methyl cyclohexanone (344)

A flame dried round bottomed flask under nitrogen and cooled to $0^{o}C$ was charged with i-Pr_2NH (1.04ml, 7.42mmol) in THF (5ml). To this solution, butyl lithium (2.4M, 3.09ml, 7.42 mmol) was added dropwise and the mixture stirred at $0^{o}C$ for 0.5 hr.

A second flame dried round bottomed flask under nitrogen was charged with 2-methylcyclohexanone (242) (1ml, 8.25mmol) and THF (1ml) and cooled to $-78^{o}C$ and stirred for 2 min. To this cooled solution L.D.A. (0.81M, 9.13ml, 7.42mmol) (prepared as above) was added dropwise over 15 min and the resulting solution stirred for a further 3 h at $-78^{o}C$.

Phenylselenyl chloride (1.57g, 8.25mmol) in THF (3ml) was added rapidly to the solution and the cooling bath was removed. When the mixture attained room temperature Et_2O (50ml) was added and the resulting solution washed with H_2O (2 x 10ml) and brine (10ml) then dried and concentrated to give a yellowish oil (1.95g, 88%) which was used without purification in the following oxidation.

^{1}H nmr 90MHz ($CDCl_3$) : δ 7.6 ppm, m, 2H, δ 7.31 ppm, m, 3H,
δ 1.5 ppm, d, (J=7Hz), (3H,$C\underline{H}_3$)



(346)

2-Methylcyclohex-5-enone (346)

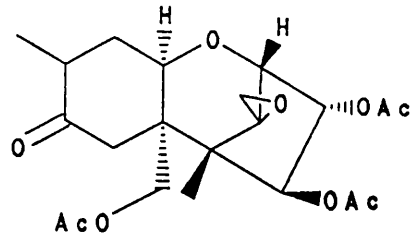
A round bottomed flask under nitrogen was charged with the crude selenide (344) (984mg, 3.61 mmol) in CH_2Cl_2 (20ml). Pyridine (0.9ml, 10.8 mmol) was added to this solution and the mixture stirred at room temperature for 2 min when H_2O_2 (30%, 1.2ml, 10.8mmol) was added dropwise and the resulting solution stirred for a further 1.5 hr.

Dilution with Et_2O (20ml) followed by washing sequentially with H_2O (1 x 10ml) saturated $CuSO_4$ (1 x 5ml) and H_2O (1 x 10ml) then drying and concentration gave a yellowish oil (0.51g). Distillation at reduced pressure gave the enone (346) (220 mg, 54%) as a clear oil (b.p.122°C 18mmHg).

Infra Red ν max CCl_4 : 3040, 2970, 2940, 2860, 1710, 1680, 1450, 1430,1385, 1380, 1215, 1110, 800 and 610 cm^{-1}

Mass Spectrum: Found M^+ 110.0768 $C_7H_{10}O$ requires 110.0772 amu.

^{1}H nmr 90MHz($CDCl_3$) : δ 6.8 ppm d.t, (1H,C5-H), δ 5.85 ppm, d.t, (J=10,4,1.2Hz), 1H, (C6-H) δ 1.0 to 2.4 ppm, m, 5H, δ 1.0, d, (J=7Hz) (3H,C$\underline{H}_3$).



(327)

3α,4β,15-triacetoxy-12,13-epoxytricothecan-8-one (327)

To a round bottomed flask with stirring bar was added the enone (305) (140mg, 0.33mmol) in glacial AcOH (25ml) and Pd/C catalyst (10mg). The flask was evacuated and the vacuum released with hydrogen. The contents of the flask were then stirred for 24 h at room temperature.

The hydrogen atmosphere was removed under vacuum and the vacuum released with air. The solution was filtered through Celite and concentrated in vacuo. Residual AcOH was removed by azeotropic distillation with toluene (3 x 10ml) and then CCl_4 (3 x 10ml) to give the ketone (327) as a white solid (125mg, 89%).

Recrystallisation from EtOAc/hexane gave white cubic crystals, melting point 184-185°C.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	209.7	s	-	-	-	-
(O-$\underline{C}OCH_3$)	170.3	s	-	-	-	-
(O-$\underline{C}OCH_3$)	170.09	s	-	-	-	-
(O-$\underline{C}OCH_3$)	169.78	s	-	-	-	-
4	78.63	d	5.71	d	3.2	1H
3	78.02	d	5.17	dd	4.81, 3.2	1H
2	77.89	d	3.99	d	4.81	1H
11	70.64	d	4.15(masked)		-	-
15	65.07	t	4.08,4.2	ABq	'obs 12.47	2H
12	64.22	s	-	-	-	-
5	50.95	s	-	-	-	-
6	48.35	s	-	-	-	-
13	47.17	t	2.78+3.11	ABq	obs3.92	2H
7	41.55	t	masked	-	-	-
9	38.63	d	2.27	m	-	-
10	36.62	t	masked	-	-	-
(OCO$\underline{C}H_3$)	20.76	q	2.13	s	-	3H
(OCO$\underline{C}H_3$)	20.72	q	2.06	s	-	3H
(OCO$\underline{C}H_3$)	20.68	q	2.00	s	-	3H
16	13.74	q	1.00	d	6.5	3H
14	5.74	q	0.66	s	-	3H

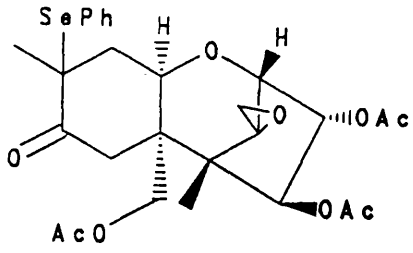
Infra-Red: ν_{max} : 2980, 2940, 1750, 1725, 1370, 1245, 1225, 1095, 1045 and 965 cm^{-1}

Mass Spectrum: Found M^+ 424.1745 $C_{21}H_{28}O_9$ requires 424.1735

$[\alpha]_D^{17.5°C}$ = 43.8 (c= 0.422 in EtOAc)

Micro Analysis found	C: 59.38	H: 6.72
$C_{21}H_{28}O_9$ requires	C: 59.41	H: 6.65

Melting Point: (EtOAc/hexane) : 184-185°C



(328)

3α,4β,15-triacetoxy-9-(phenylselenyl)-12,13-epoxytri-cothecan-8-one(328)

A round bottomed flask with stirring bar under nitrogen was charged with PPTS (10mg, 0.04mmol) and then the ketone (327) (82mg, 0.19mmol) in EtOAc (4ml). To this was added phenylselenyl chloride (43mg, 0.22mmol) in EtOAc (2ml) and the solution stirred at ambient temperature and the reaction course followed by t.l.c. analysis.

When t.l.c. analysis revealed that compounds other than starting material (rf. 0.42) and the product (rf. 0.48) were evident, the solution was diluted with EtOAc (15ml) and washed sequentially with saturated $NaHCO_3$ solution (10ml) and H_2O (10ml). The EtOAc solution was dried and concentrated to give a yellowish oil.

Purification by flash chromatography (EtOAc/hexane) gave the α-selenoketone(328) (84.7mg, 75.6%) as a yellowish oil and starting material (327) (9.7mg, 11.8%).

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	204.95	s	-	-	-	-
(O-$\underline{C}OCH_3$)	170.43	s	-	-	-	-
(O-$\underline{C}OCH_3$)	170.00	s	-	-	-	-
(O-$\underline{C}OCH_3$)	169.74	s	-	-	-	-
(phenyl m-$\underline{C}H$)	137.21	d				
(phenyl p-$\underline{C}H$)	129.29	d	7.2-7.4	m	-	5H
(phenyl o-$\underline{C}H$)	128.78	d				
4	78.25	d	5.61	d	3.4	1H
3	77.85	d	5.26	dd	4.8, 3.4	1H
11	77.64	d	4.19	bm	-	1H
2	70.71	d	4.07	d	4.8	1H
15	65.60	t	4.35,4.09	ABq	obs 12.8	2H
12	64.14	s	-	-	-	-
5	50.98	s	-	-	-	-
6	48.95	s	-	-	-	-
9	48.38	s	-	-	-	-
13	47.27	t	3.25,2.91	ABq	obs 3.85	2H
10	42.42	t	2.57	m	-	2H
7	36.58	t	4.13, masked	ABq	obs 3.74	2H
16	23.92	q	1.21	s	-	3H
(OCO$\underline{C}H_3$)	20.85	q	2.15	s	-	3H
(OCO$\underline{C}H_3$)	20.75	q	2.11	s	-	3H

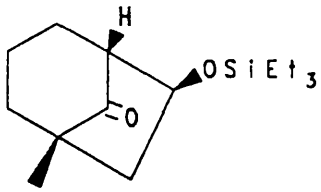
Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
($OCO\underline{C}H_3$)	20.70	q	1.95	s	-	3H
14	5.3	q	0.73	s	-	3H

Infra-Red: ν_{max} CCl_4 : 2980, 1750, 1700, 1370, 1225, 1120, 1090, 1065, 1045, 965 and 690 cm^{-1}

Mass Spectrum: Found M^+ 580.1180

$C_{27}H_{32}O_9Se$ requires 580.1212

$[\alpha]_D^{18°C} = 88^o$ (c 1.082 in EtOAc)



(369)

1-Methyl-6β-triethylsilyloxy-8-oxo-bicyclo[3,2,1]octane (369)

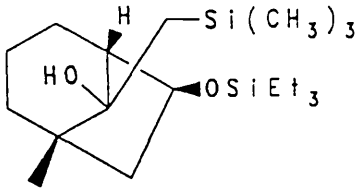
The 6β-alcohol (252) (436mg 2.83 mmol) was silylated by the procedure described on page 169, using DMAP (35mg, 0.28mmol), Et_2O (15ml), chlorotriethylsilane (0.9ml, 4.5mmol) and Et_3N (0.6ml, 4.3mmol).

Product isolation was as described on page 159. Purification by flash chromatography (Et_2O/hexane) gave the ether (369) (740mg, 97%) as a clear oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	221.4	s	-	-	-	-
6	69.4	d	4.09	d d	7.9, 2.7	1
5	55.8	d	2.16	bt	2.7	1
1	47.7	s	-	-	-	-
2	45.1	t	1.1-2.0	m		(7H)
7	43.7	t	α 1.1-2	masked	-	(7H)
			β 2.35	dd	13.3, 7.9	1
4	33.9	t	1.1-2.0	m	-	(7H)
9	19.2	q	0.98	s	-	3
3	19.1	t	1.1-2.0	m	-	(7H)
$Si(CH_2\underline{C}H_3)$	6.6	q	0.87	t	7.7	9
$Si(\underline{C}H_2CH_3)$	4.6	t	0.50	q	7.7	6

Infra-Red: ν_{max} $CHCl_3$: 2960, 2880, 1750, 1455, 1155 cm^{-1}

Mass Spectrum: Found M^+ 268.1849 $C_{15}H_{28}Si$ requires 268.1858



(370)

1-Methyl-6β-triethylsilyloxy-8β-hydroxy-8α-(trimethylsilyl-methyl)bicyclo[3,2,1]octane(370)

A flame dried, round bottomed flask equipped with side arm and stirring bar under nitrogen was charged with trimethylsily-methyl lithium (1.0M, 2ml, 2.0 mmol) and THF (10ml) and cooled to -78°C. To this was added the ketone (269) (358 mg, 1.33mmol) in THF (3ml) dropwise, over 3 min and the mixture stirred at -78°C for 1.25 h. The cooling bath was removed and the solution was allowed to warm to room temperature overnight.

The reaction mixture was poured into a saturated NH_4Cl (3ml) and extracted with EtOAc (3 x 30ml). The organic extracts were combined, dried (Na_2SO_4) and concentrated.

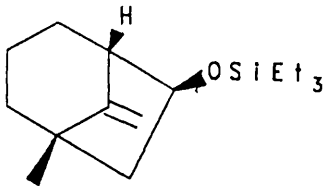
Purification by flash chromatography gave the β-hydroxy-silane (370) (313mg, 70%) as a clear oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	80.46	s	-	-	-	-
6	72.62	d	3.98	dd	8.3, 3.5	1H
5	51.19	d	1.91	bt	-	1H
2	46.81	t	1.15-1.8	m		
1	46.17	s	-	-	-	-
7	33.97	t	α masked			
			β masked		-	-
4	24.49	t	1.15-1.80	m	-	-
3	24.03	t			-	-
(C_1-$\underline{C}H_3$) 9	20.96	q	0.93	s	-	3H
(Si-$\underline{C}H_2$-C_8)	18.79	t	0.88	s	-	2H
($SiCH_2\underline{C}H_3$)	6.87	q	0.94	t	8.1	9H
($Si\underline{C}H_2CH_3$)	4.75	t	0.57	q	8.1	6H
($Si\underline{C}H_3$)	0.98	q	0.06	s	-	9H

Infra-Red: ν_{max} CCl_4 : 3700, 3618, 2961, 1460, 1250, 1083, 1010, 865 and 845 cm^{-1}

Mass Spectrum: Found M^+ 356.2562

$C_{19}H_{40}O_2Si_2$ requires 356.2567



(371)

1-Methyl-6β-triethylsilyloxy-8-methylenebicyclo[3,2,1]-octane (371)

A flame dried round bottomed flask with stirring bar under nitrogen was charged with the β-hydroxysilane (370) (177.1mg, 0.49 mmol) in THF (8ml) and cooled to $0^{o}C$. To this was added KHMDS (0.75M in toluene, 0.9ml, 0.68mmol) dropwise over 3 min and stirring continued for a further 3 min when the cooling bath was removed. After 10 min t.l.c. analysis indicated that the starting material had been consumed.

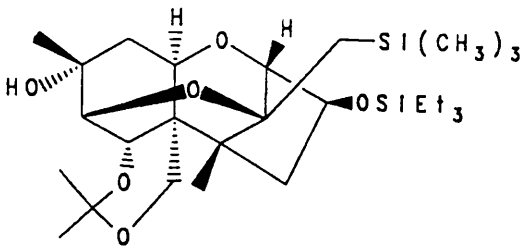
The solution was diluted with Et_2O (15ml) washed with H_2O (2ml) and the organic dried and concentrated to give a clear oil. Purificatin by flash chromatography (et_2O/pet.ether) gave the required olefin (371) (99.7mg, 75%) as a clear oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
8	161.86	s	-	-	-	-
(C=$\underline{C}H_2$) 10	98.52	t	4.69,4.6	s,s	-	2H
6	73.98	d	4.05	d d	7.2, 2.7	1H
5	53.43	d	2.39	bm	-	1H
2	49.06	t				
1	44.08	s	-	-	-	-
7	42.12	t	(β) 2.1	dd	13.5, 7.2	1H
4	33.16	t				
(C_1-$\underline{C}H_3$) 9	22.98	q	1.07	s	-	3H
3	20.202	t				
(Si$CH_2\underline{C}H_3$)	6.81	q	0.93	t	8.1	9H
(Si$\underline{C}H_2CH_3$)	4.78	t	0.57	q	8.1	6H

<u>Infra-Red</u>: ν_{max} CCl_4 : 2950, 1675, 1455, 1375, 1238, 1158, 1148, 1080, 1065 and 885 cm^{-1}

<u>Mass Spectrum</u>: Found 265.1987,

M^+-H($C_{16}H_{29}OSi$) requires 265.1988



(373)

Formation of the hydroxysilane (373):

Attempted Peterson reaction on the ketone (362)

A flame dried round bottomed flask under nitrogen was charged with trimethylsilylmethyl lithium (1M in pentane, 300µl, 0.3mmol) in THF (0.5ml) and then cooled to -78°C. The ketone (362) (9.7mg, 0.024 mmol) in THF (2ml) was added dropwise over 2 min and the mixture stirred for 2 h at -78°C. The cooling bath was removed and the mixture allowed to warm to room temperature at which point t.l.c. analysis revealed that the starting material had been consumed.

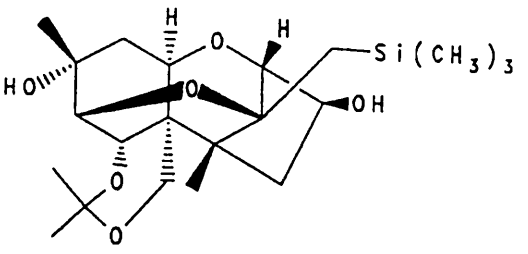
The solution was poured into saturated NH_4Cl (1ml) and the aqueous solution extracted with EtOAc (3 x 15ml). The combined organic extrats were dried (Na_2SO_4) and concentrated to give an opaque oil. Purification by flash chromatography (EtOAc/hexane) gave the adduct (373) (8mg, 68%) as a clear oil.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
$(CH_3)_2\underline{C}(O)_2$	100.11	s	-	-	-	-
9	85.16	s	-	-	-	-
7	83.61	d	4.51	d	6.1	1H
3	77.74	d	3.88	dd	8.5,2.3	1H
8	72.51	d	3.78	d	6	1H
12	72.37	s	-	-	-	-
11	71.88	d	3.64	bm	-	1H
2	66.15	d	3.59	bs	-	1H
15	63.55	t	3.83,3.33	ABq	obs 12.5	2H
6	47.06	s	-	-	-	-
4	43.88	t	2.33(β) α-masked	dd	15.2, 8.5	1H
10	41.74	t	2.13	m	-	2H
5	40.41	s	-	-	-	-
16	27.31	q	1.40	s	-	3H
$(O)_2C(CH_3)\underline{C}H_3$	25.48	q	1.38	s	-	3H
$(O)_2C(CH_3)\underline{C}H_3$	22.82	q	1.36	s	-	3H
$C\text{-}\underline{C}H_2Si$	21.31	t	0.92	s	-	2H
14	18.90	q	1.33	s	-	3H
$Si(CH_2\underline{C}H_3)_3$	6.81	q	0.92	t	8.0	9H
$Si(CH_2\underline{C}H_3)_3$	4.63	t	0.54	q	8.0	6H
$Si(\underline{C}H_3)_3$	0.75	q	0.03	s	-	9H
O<u>H</u>			3.0	bs	-	1H

Infra-Red: ν_{max} CCl_4 : 3560, 3260, 2920, 1460, 1380, 1240, 1225, 1080, 910, 865, 845 and 620 cm^{-1}

Mass Spectrum: Found M^+ 526.3146

$C_{27}H_{50}O_6Si_2$ requires 562.3146



(379)

Formation of the alcohol (379) :

Attempted fluoride ion induced Peterson elimination on the silane (373)

A flame dried, round bottomed flask under nitrogen was charged with the silane (373) (17mg, 0.032mmol) in THF (1ml). To this stirred solution was added tetrabutylammonium fluoride (50mg, 0.16mmol) in THF (1ml) and the mixture stirred at room temperature overnight.

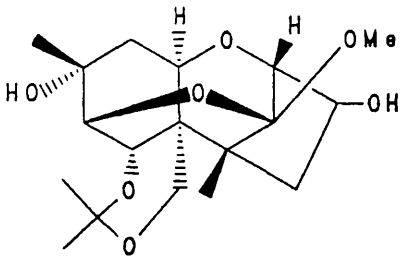
The reaction mixture was diluted with EtOAc (10ml) and washed with H_2O (1ml), dried and concentrated. Purification by flash chromatography gave the diol (379) (11mg, 82%) as a white amorphous solid.

Position	^{13}C		^{1}H			
	δ	m	δ	m	J	I
$(CH_3)\underline{C}(O)_2$	100.12	s	-	-	-	-
9	84.87	s	-	-	-	-
7	83.65	d	4.53	d	5.3	1H
3	77.83	d	4.03	dd	8.8,2.6	1H
8	72.62	d	3.80	d	5.3	1H
12	72.41	s	-	-	-	-
11	71.89	d	3.67	bm	-	1H
2	66.07	d	3.61	bs	-	1H
15	63.45	t	3.85,3.35	ABq	obs12.5	
6	47.28	s	-	-	-	-
4	42.10	t	α masked	-	-	-
			β 2.38	dd	15.5, 8.8	1H
10	41.77	t	2.10	m	-	1H
5	40.21	s	-	-	-	-
16	27.35	q	1.40	s	-	3H
$(O)_2C(CH_3)\underline{C}H_3$	25.54	q	1.38	s	-	3H
$(O)_2C(\underline{C}H_3)CH_3$	22.73	q	1.33	s	-	3H
$C\text{-}\underline{C}H_2\text{-}Si$	21.61	t	1.11,0.96	ABq	obs14.6	2H
14	18.72	q	1.32	s	-	3H
$Si(\underline{C}H_3)_3$	0.81	q	0.04	s	-	9H

Infra-Red: ν_{max} : 3600, 3100, 3050, 1440, 1270, 1175, 1120, 960, 840, 775 and 715 cm^{-1}

Mass Spectrum: Found M^+ 412.2278

$C_{21}H_{36}O_6Si$ requires 412.2275



(382)

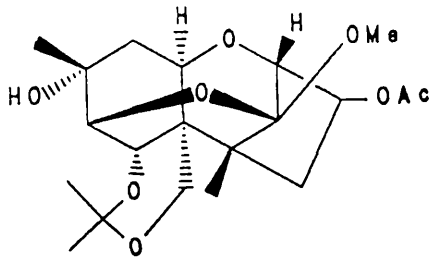
Bridged acetal-alcohol (382)

A flame dried round bottomed flask was charged with the silyl ether (376) (30mg, 0.06 mmol) in THF (2ml). Tetra-butyl ammonium fluoride (30mg, 0.09 mmol) in THF (1ml) was added dropwise and the solution stirred at room temperature overnight.

The solution was diluted with EtOAc (10ml) and washed with H_2O (1ml) then dried and concentrated. Purification by flash chromatography gave the alcohol (382) (17mg, 75%) as an inseparable mixture of C3 diastereomers.

Infra Red; $\tilde{\nu}$ max $CHCl_3$: 3680, 3540, 3010, 1440, 1200, 1120, 930, 790, 760,720, 700, 660 and 510 cm^{-1}

Mass Spectrum: Found M^+ 356.1829, $C_{18}H_{28}O_7$ requires 356.1834 amu.



(383)

Bridged acetal-acetate (383)

A round bottomed flask with stirring bar was charged with the alcohol (382) (as a mixture of C3 diastereomers) (17mg, 0.05mmol) in pyridine (0.5ml). Acetic anhydride (1ml) was added and the mixture stirred at room temperature for 16 h.

Crude product isolation by the standard method and purification by flash chromatography gave the acetate (383) (15 mg, 87%) as a white amorphous solid and an inseparable mixture of diastereomers.

Infra Red: ν max $CHCl_3$: 3680, 3540, 3020, 2940, 1735, 1520, 1460, 1380, 1225, 1200, 1140, 1110, 1070, 1050, 1030, 990, 930, 870, 790, 720, and 660 cm^{-1}

Mass Spectrum : Found M^+. 398.1927, $C_{20}H_{30}O_8$ requires 398.1940.

Preparation of Pyridinium chlorochromate

Hydrochloric acid (6M, 18ml, 0.11mol) was stirred vigorously in a conical flask and chromium trioxide (10g, 0.1mol) added and the mixture stirred for 5 min. The solution was cooled to $0^{o}C$ when pyridine (8ml, 0.1mol) was added dropwise over 10 min. The resulting orange solid was filtered and dried at reduced pressure over P_2O_5 for 1 h. To give 18g, (84%) of dry pyridinium chlorochromate.

Preparation of Dipyridine-chromium trioxide complex

A dried round bottomed flask under nitrogen, fitted with a mechanical stirrer, was charged with pyridine (80ml, 1mol). Chromium trioxide (10g, 0.1mol) added in small portions over 20 min, with vigorous stirring. The resulting solution was stirred for 24 h at room temperature to give a dense brick red solid as a slurry in pyridine.

Filtration under nitrogen, and washing with hexane (1 x 30ml) gave a brick-red solid (25g, 97%) which was used immediately in Collins oxidations.

Enone reductions and g.c. analysis.

G.C. analysis conditions

Gas chromatographic analysis of the tetraacetates (319) and (323) was performed using a CPSil 19C B column with an initial oven temperature of 80°C rising to 205°C at a rate of 30°C/min. The temperature was then raised to 270°C at a rate of 2°C/min.

G.C. Analysis

Reduction of 8-Ketoanguidine by:-

A. Sodium borohydride/cerium trichloride.

A round bottomed flask was charged with 8-ketoanguidine (297) (18mg, 0.05mmol) in MeOH (4ml) containing $CeCl_3.7H_2O$ (44mg, 0.12mmol) and the contents of the flask were cooled to -78°C. Sodium boro-hydride (4.5mg, 0.12 mmol) was added and the resulting solution stirred at -78°C for 20 min when acetone (1ml) was added and the cooling bath removed and the reaction warmed to room temperature.

Ethyl acetate (40ml) was added and the solution was washed with HCl (1M, 5ml) aqueous $NaHCO_3$ (5ml) and H_2O (5ml) then dried and concentrated.

The resulting solid was treated with pyridine (0.5ml) and Ac_2O (1ml) at room temperature for 24 h. Product isolation by the standard method gave a crude product (21mg, 95%) that was subjected to g.c. analysis.

B. Sodium borohydride.

A round bottomed flask was charged with 8-ketoanguidine (297) (10mg, 0.03mmol) in MeOH (2.7ml) and H_2O (0.3ml) and the contents of the flask cooled to O^oC. Sodium borohydride (10mg, 0.26mmol) was added and the solution stirred at O^oC for 5 min when the cooling bath was removed and the mixture warmed to room temperature over 20 min and acetone (1ml) was added.

The solution was diluted with EtOAc (30ml) and washed sequentially with HCl (1M, 3ml) aqueous $NaHCO_3$ (5ml) and H_2O (3ml) then dried and concentrated.

Acetylation as above and product isolation by the standard method gave a crude product (6mg, 53%) that was subjected to g.c. analysis.

C. DIBALH.

A flame dried round bottomed flask under nitrogen was charged with 8-keto anguidine (297) (15mg, 0.04mmol) in THF (5ml) and the contents of the flask were cooled to -78^oC. DIBALH (1M in CH_2Cl_2 160µl, 0.16mmol) was added dropwise and the mixture stirred at -78^oC for a further 4 h when aqueous $NaHCO_3$ (1ml) was added.

On warming to 20^oC the solution was diluted with EtOAc (30ml), washed with water (5ml) and then dried and concentrated.

Acetylation as above gave an opaque oil which was subjected

to preparative t.l.c purification (Et_2O/petroleum ether 3:1) to remove non-polar impurities. The region of silica of R_f 0.15 to 0.55 was extracted with EtOAc and concentrated to give a crude product (12.3mg, 72%) which was subjected to g.c. analysis.

D. Lithium Selectride.

A flame dried flask under nitrogen was charged with 8-keto-anguidine (297) (20mg, 0.05mmol) in THF (2ml) and cooled to -78°C. L-Selectride (1M, 150µl, 0.15mmol) was added dropwise and the solution stirred at -78°C for a further 1 hr, when saturated NH_4Cl (1ml) was added and the cooling bath removed.

When at room temperature the solution was diluted with H_2O (3ml) and extracted with EtOAc (3 x 15ml). The EtOAc extracts were combined dried and concentrated.

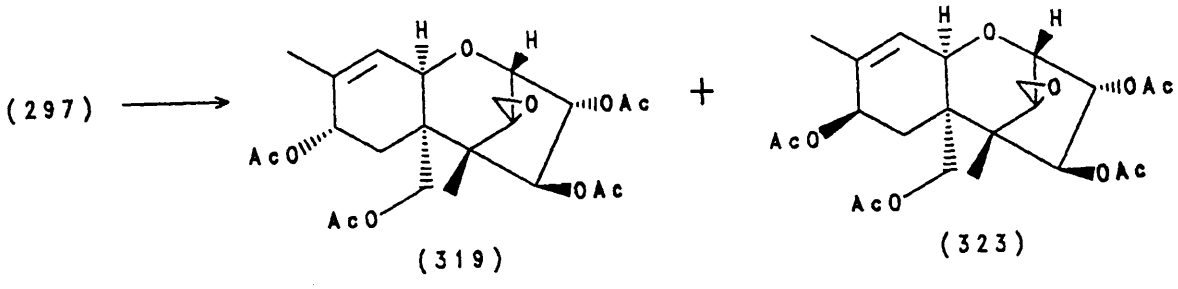
Acetylation as above, followed by concentration in vacuo gave a crude product (22mg, 100%) which was subjected to g.c. analysis.

Reduction of 8-ketotriacetoxyscirpenol (305) by:-

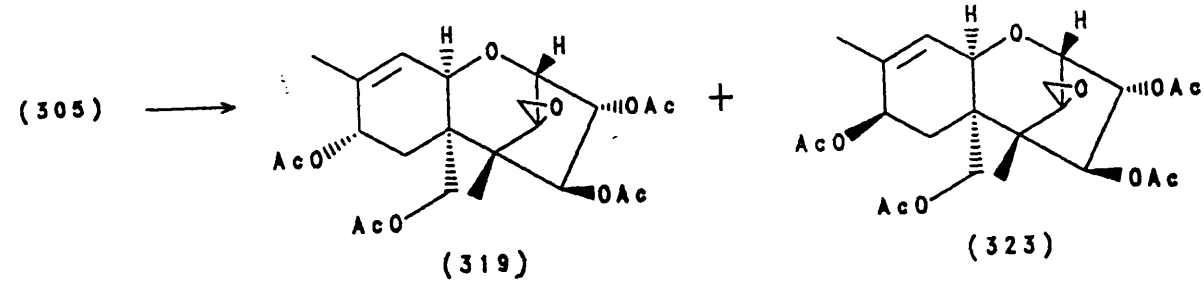
A. Lithium selectride.

Procedure was as above using 8-ketotriacetoxyscirpenol (305) (30mg, 0.07mmol) in THF (3ml) and L-Selectride (1M, 80µl, 0.08mmol). Acetylation and concentration gave a crude product (32mg, 94%) which was subjected to g.c. analysis.

TABLE 1 - SUMMARY OF G C RESULTS



REDUCING AGENT	RATIO (BY G.C.).		
$NaBH_4, CeCl_3, MeOH$	1	:	16.8
$NaBH_4, MeOH$	1.7	:	1
DIBALH	2.5	:	1
L-Selectride	1	:	0



DIBALH	2.1	:	1
L-Selectride	1	:	0

4. References

1. Brian, P.W., and McGowan, J.G. *Nature* 157, 334 (1946).

2. Grove, J.F., *J.Chem.Soc* 810, (1968).

3. Freeman, G.G., and Morrison R.I. *Nature*. 162, 30, (1948).

4. Hsu, I.C., Smalley, E.B., Strong, F.M., and Ribelin, W.E., *Appl. Microbiol.* 24, 684 (1972).

5. (a) Enomoto, M., Saito, M., Tatsuno, T., and Tsunoda, H., *Chem.Pharm.Bull*, 16 2519 (1968); (b) Amakai, K., Enomoto, M Ishikawa, Y., Okubo, K., Saito, M., Tsunoda, H., Ueno, I., and Ueno, Y., *Japan.J, Exp.Med.*, 41 507 (1971); (c) Morooka, N., and Yoshizawa, T., *Agric. Bull. Chem.* 37, 2933, (1973).

6. (a) Kvashnina, E.S., and Sarkisov, A.Ch.,*Publ. Min. Agric., Moscow*, 89 (1948); (b) Sarkisov, A.Ch., Mycotoxicoses Govt. Edit.Agric. Lit., Moscow, p216, (1972).

7. Enomoto, M., Ishii, K., Sakai, K., Sato, N., and Ueno, Y., *Japan. J. Exp. Med.*, 42, 461, (1972).

8. Chemistry in Britain 24^7 657, (1988).

9. Ueno, Y., *Pure.Appl.Chem.*, 49, 1737, (1977).

10. Freeman, G.G and Gill, J.E, *Nature*, 166, 698, (1950).

11. Glaze, E.T., Scheiber, E., Gyimesi, J., Horvath, I., Steczek, K., Szentirmai, A. and Bohus, G., *Nature*, 184, 908, (1959).

12. Gyimesi, J., *Acta. Chim. Hung.*, 45, 323, (1965).

13. Freeman, G.G., Gill, J.E., and Waring, W.S., *J. Chem. Soc.*, 1105, (1959).

14. Fishman, J., Jones, E.R.H., Lowe, G., and Whiting, M.C., *J.Chem.Soc.*, 3498, (1960).

15. Harri, E., Loeffler, W., Sigg, H.P., Stahelin, H., Stoll, Ch., Tamm, Ch., and Wiesinger, D. *Helv.Chim.Acta*. 45, 839, (1962).

16. Abrahamsson, S., and Nilsson B., *Proc. Chem. Soc.*, (London) 188, (1964).

17. McPhail, A.T., and Sim, G.A. *J.Chem.Soc*, 1394, (1966).

18. Godtfredsen, W.O., Grove, J.F., and Tamm, Ch., *Helv. Chim. Acta*, 50, 1666, (1967).

19. Gufzwiller, J., Mauli, R., Sigg, H.P. and Tamm, Ch., *Helv. Chim. Acta*, 47, 2234, (1964).

20. Jarvis, B.B., Midiwo, J.O., Stahly, G.P., Pavanasasivam, G., and Mazola, E.P., *Tetrahedron Lett.*, 787, (1980).

21. Jarvis, B.B., Pavanasasivam, G., Holmlund, G.E., De Silva, T., and Stahly, G.P., *J.Amer.Chem.Soc.* 103, 472, (1981).

22. (a) Grove, J.F. *J.Chem.Soc.C.*, 375, (1970).
(b) Grove, J.F. *J.Chem.Soc.C.*, 378, (1970).

23. Dawkins, A.W., *J.Chem.Soc.,C.*, 116, (1960).

24. Sigg, H.P., Mauli, R., Flury, E., and Hauser, D. *Helv. Chim. Acta.*, 48, 962, (1965).

25. Tamm, Ch., and Jeker, N., *Tetrahedron.*, 45, 2385, (1989) and references within.

26. Harri,E., Loeffler,W., Sigg,H.P., Stahelim,H., Stoll,Ch., Tamm,Ch., and Wiesinger,D., *Helv. Chim. Acta.*, 45, 839, (1962).

27. Kaneko,T., Schmitz,H., Essery,J.M., Rose,W., Howel,H.G., O'Herron,F.A., Nachfolger,S., Huftalen,J., Bradner,W.T., Partyka,R.A., Doyle,T.W., Davies,J., and Cundliffe, E., *J.Med.Chem.*, 25, 579, (1982).

28. Caskey,C.T., and Tate,W.P., *J.Biol.Chem.*, 248, 7970 (1973).

29. Wei, C-M., Hansen, B.S., Vaughan, M.H., and McLaughlin, C.S., *Proc.Nat.Acad.Sci.(USA)*, 71, 713, (1974).

30. Hansen,B.S., and Vaughan,M.H. Jr., *Fed.Proc.Fed.Am.Soc.Exp. Biol.*, 32, 1737, (1973).

31. Ueno.Y., *Pure.App.Chem.*, 49, 1737, (1977).

32. Grove, J.F., and Mortimer, P.H., *Biochem. Pharmacol.*, 18, 1473, (1969).

33. Ohi, T., Takeda, H., and Yoshizawa, T., *Agric. Biol. Chem.*, 47, 2133, (1983), and references therein.

34. Greenhalgh, R., King, R.R., Levesque, D., and McQueen, R., *J. Agric. Food Chem.*, 32, 1181, (1984).

35. Roush, W.R., and Russo-Rodriguez, S., *J.Org.Chem.*, 50, 5465, (1985).

36. Grove, J.F., *J.Chem.Soc.Perkin Trans,1*, 647, (1986).

37. Ueno,Y., Nakajima,M., Sakai,K., Tsunoda,H., and Enomoto, M., *Appl. Microbiol.*, 25, 699, (1973).

38. Doyle, T.W., and Bradner, W.T. in *"Anticancer agents based on Natural Product Models"*, Cassady, J.M. and Douros, J.D., eds. Academic Press: New York, 1980, p43.

39. Curtis, C.R., Jarvis, B.B., and Stahly, G.P., *Cancer. Treat Rep.* 62, 1585, (1978).

40. Grove, J.F., *J.Chem.Soc.Perkin Trans 1*, 115, (1990).

41. Thompson,W.L., and Wannemacher,R.W. *Toxicon*, 24, 985, (1986).

42. Colvin,E.W. and Cameron,S., *J.Chem.Soc.Perkin Trans 1*, 887, (1989).

43. Sharpless,K.B., Flood,T.C., Nieh,M.T., and Umbreit,M.A., *J.Amer.Chem.Soc.*, 94, 6538, (1972).

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

45 McDougall, P.G., and Schmuff, N.R. *Fortschr. Chem. Org. Naturst.*, 47, 153, (1985).

46. Quinkert,G. ed., *Synform*, 4, 2229, (1984).

47. Fujimoto, Y., Yokua, S., Nakamura, T., Morikawa,T., *Tetrahedron Lett.* 2523, (1974).

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

49. Tomioka, K., Sugimori, M., and Koga, K., *Chem. Pharm. Bull*, 35, 906, (1987).

50. Colvin, E.W. and Thom, I.G., *Tetrahedron*, 42, 3137, (1986).

51. Masuoka, N., Kamikawa, T., *Tetrahedron Letters* 1691, (1976).

52. Still, W.C and Tsai, M.-Y., *J.Am.Chem.Soc.* 102 3654, (1980).

53. Roush,W.R., and D'Ambra,T.E. *J.Am.Chem.Soc.* 105, 1058, (1983).

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

55. Trost, B.M., and McDougal P.G. *J.Am.Chem.Soc.* 104, 6110,(1982).

56. Brooks, D.W., Grothaus, P.G. and Muzdiyasni, H., *J.Am.Chem.Soc.*, 105, 4427, (1983).

57. Ziegler, F.E., Nangia, A., and Schulte, G., *J.Am.Chem.Soc.*, 109, 3987, (1987).

58. (a) Ziegler,F.E., Nangia,A., and Tempesta,M.S., *Tetrahedron Lett*, 29, 1665, (1988); (b) Ziegler,F.E., Nangia,A., and Shulte,G., *Tetrahedron Lett*, 29, 1669, (1988).

59. Colvin, E.W., Malchenko, S., Raphael, R.A. and Roberts, J.S. *J.Chem.Soc. Perkin. Trans I*, 658, (1978).

60. Kraus, G.A., and Frazier, K., *J.Org.Chem.* 45, 4820, (1980).

61. Kraus, G.A., and Roth, B. *J.Org. Chem.*, 45, 4825, (1980).

62. Masuoka, N., Kamikawa, T., and Kubota, T., *Chem. Lett.*, 751, (1974).

63. Roush, W.R., and D'Ambra,T.E. *J.Org.Chem.* 45, 3927, (1980).

64. Roush, W.R., and D'Ambra, T.E. *J.Org.Chem.* 46, 5054, (1981).

65. Hua, D.H., Venkataraman,S., Chan-Yu-King,R., and Paukstelis, J.V., *J.Amer.Chem.Soc.*, 110, 4741, (1988).

66. Anderson, W.K. and Lee, G.E., *J.Org.Chem.* 45, 501, (1980).

67. Pearson, A.J. and O'Brien, M.K. *J.Org.Chem*, 54, 4663, (1989).

68. Tulshian,D.B., and Fraser-Reid,B., *Tetrahedron Lett*, 21, 4549, (1980).

69. Esmond,R., Fraser-Reid,B. and Jarvis,B.B., *J.Org.Chem*, 47, 3358, (1982).

70. Jeker,N., Mohr,P., and Tamm,Ch., *Tetrahedron Lett*, 25, 5637, (1984).

71. Colvin,E.W., and Cameron,S., *J.Chem.Soc.Perkin Trans 1*, 887, (1989).

72. James, E.R.H. and Lowe, G., *J.Chem.Soc*, 3959, (1960).

73. Dawkins, A.W. *J.Chem.Soc (C)* 116, (1966).

74. Hanson, J.R. Achilladelis B, *Chem and Industry.*, 1643, (1967).

75. Hanson, J.R., Marten, T., and Siverns, M. *J.Chem.Soc. Perkin Trans I*, 1033, (1974).

76. (a) Achilladelis, B., Adams, P.M. and Hanson, *J.R., Chem. Commun* 511, (1970); (b) Idem. *J.Chem.Soc.Perkin Trans I.*, 1425, (1972); (c) Adams, P.M. and Hanson J.R., *Chem. Commun* 1414, 1971; (d) Arigoni, D. Cane, D.E., Muller, B, and Tamm, Ch. *Helv. Chim. Acta*, 56, 2946, (1973).

77. Hesketh, A.R., Gledhill, L., Marsh, D.C., Bycroft, B.W., Dewick, P.M. and Gilbert, J., *J.Chem.Soc., Chem.Commun.* 1184, (1990) and references within.

78. Ruzicka, *Experientia.*, 9, 375, (1953).

79. Cole, R.J., Dorner,J.W., Cox, R.H., Cunfer,B.M., Cutler,H.G and Stuart,B.P. *J.Nat.Prod.*, 44, 324, (1981).

80. Kerr,F.W., Ph.D. Thesis, Glasgow University, (1989).

81. Rickborn,B., and Thummel, R.P., *J.Org.Chem*, 34, 3583, (1969).

82. Colvin,E.W. and Cameron,S., *J.Chem.Soc.Chem.Commun*, 1084, (1986).

83. Stork,G., and Hudrlik, P.F., *J.Am.Chem.Soc.*, 90, 4462, 4464, (1968).

84. House, H.O., Czuba, L.J., Gall M and Olmstead, H.D., *J. Org. Chem*, 39, 2324, (1969).

85. (a) Harwood, L.M. *Aldrichemica Acta*, 18, 25, (1985); (b) Still, W.C., Kahn, M and Mitra, A., *J. Org. Chem.*, 43, 2923, (1987).

86. Karplus, M., *J. Amer.Chem.Soc*, 85, 2870, (1963).

87. Grieco, P.A., Miyashita, M and Yoshikoshi, A., *J. Org.Chem*, 42, 3772, (1977).

88. Haines, A.H. in Best Synthetic Methods. "Methods for the Oxidation of Organic Compounds" (Alkanes, Alkenes, Alkynes and Arenes) Cpt 3.2.2 page 84 and references within Academic Press, New York, (1985).

89. Schroder, M., *Chem. Rev*, 80, 187, (1980).

90. (a) Woodward, R.B., and Brutcher, F.V., *J.Amer.Chem.Soc*, 80, 209 (1958); (b) Wiberg, K.B., and Saegebarth, K.A., *J.Amer.Chem.Soc*, 79, 6256, (1957).

91. Vedejs, E., Engler, D.A., and Telschow, J.E., *J. Org.Chem*, 43, 188, (1978).

92. Rubottom, G.M., Vasquez, M.A., and Pelegrina, D.R., *Tetrahedron Lett*, 4319, (1974).

93. McCormick, J.B., Tomasik, W., and Johnson, M.W., *Tetrahedron Lett*, 22, 607, (1981).

94. Danishefsky, S.J., and Chenault, H.K., *J. Org. Chem*, 54, 4249, (1989).

95. Davis, F.A., and Sheppard, A.C., *J. Org. Chem*, 52, 954, (1987).

96. (a) Wasserman, H.H., and Lipshute, B.H., *Tetrahedron Lett*, 1731, (1975); (b) Moriarty, R.M., and Hou, K.C., *Tetrahedron Lett*, 25, 691, (1984).

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

98. Corey, E.J., and Kim, C.U., *J. Amer.Chem.Soc*., 94, 7586, (1972).

99. Corey, E.J., and Gross, A.W., *Tetrahedron Lett*, 25, 495, (1984).

100. For a review see: Davis, F.A. *Tetrahedron*, 45, 5703 (1989).

101. Davis,F.A., Vishwakarma,L.C., Billmers,J.G., and Finn,J., *J. Org. Chem*, 49, 3241, (1984).

102. (a) Pummerer, R., *Ber*, 42, 2282, (1909); (b) Johnson, C.R. and Phillips, W.G., *J.Amer.Chem.Soc*, 91, 682, (1969).

103. Pojer, P.M., and Angyal, S.J., *Aust.J.Chem.*, 31, 1031, (1978).

104. Corey, E.J., and Fleet, G.W., *Tetrahedron Lett*, 4499, (1973).

105. Rottinghause, G.E., Corely, D.G., and Tempesta, M.S., *Tetrahedron Lett.* 27, 427, (1986).

106. Rottinghaus, G.E., Corely, D.G., Tempesta, M.S., and Tracey, J.K., *Tetrahedron Lett*, 27, 4133, (1986).

107. Gorst-Allman, C.P., Dillen, J.L.M., and Steyn, P., *S. Afr. J. Chem*, 39, 111, (1986).

108. Sigg, H.P., Mauli, R., Flurry, E., and Hauser, D., *Helv. Chim. Acta*, 48, 962, (1965).

109. Dauben, W.G., Lorber, M., and Fullerton, D.S., *J. Org. Chem* 34, 3587, (1969).

110. Pearson, A.J., Chen, Y.S., Hsu, S.Y., and Ray, T., *Tetrahedron Lett.* 25, 1235, (1984).

111. Collins, J.C., *Tetrahedron Lett*, 3363, (1968).

112. Corey, E.J., Cho, H., Rucker, Ch., and Hau, D.H., *Tetrahedron Lett*, 22, 3455, (1981).

113. Schwarz, V., *Collect. Czech. Chem. Commun.* 27, 2567, (1962).

114. Luche, J.L., *J.Amer.Chem Soc*, 100, 2226, (1978).

115. Luche, J.L., and Gemal, A.L., *J. Amer. Chem. Soc*, 103, 5454, (1981).

116. Savard, M.E., and Greenhalgh, R., *J.Nat.Prod.*, 50, 953, (1987).

117. Huet, J., Maroni-Bernaud, Y., Ahn, N.T., and Seyden-Penn, J *Tetrahedron Lett.* 159, (1976).

118. Brown, H.C., and Krishnamurthy, S., *J.Amer.Chem.Soc*, 94, 7159, (1972).

119. Kraus, G., and Thomas, P.J., *J.Org.Chem*, 53, 1395, (1988).

120. Breitner, E., Roginski, E and Rylander, P.N., *J. Org. Chem*, 24, 1855, (1959).

121. (a) Reich, H.J., Reich, I.L., and Renga, J.M., *J.Amer.Chem. Soc*, 95, 5813, (1973); (b) Reich, H.J. *J.Org.Chem*, 39, 428, (1974); (c) Reich, H.J., Reich, I.L., and Renga, J.M., *J. Org. Chem*, 39, 2134, (1974).

122. (a) Sharpless, K.B., and Lauer, R.F., *J.Amer.Chem.Soc*, 95, 2697, (1973); (b) Sharpless, K.B., Young, M.W., and Lauer, R.F., *Tetrahedron Lett*, 1979, (1973).

123. Jones, D.N., Mundy, D., Whitehouse, R.D. *J.Chem.Soc., Chem.Commun*, 86, (1970).

124. Trost, B.M., Salzman, T.N., and Hiroi, K., *J.Amer.Chem. Soc.*, 98, 4887, (1976).

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

126. (a) Nicolaou, K.C., Seitz, S.P., Sipio, W.J., and Blount, J.F., *J.Amer.Chem.Soc*, 101, 3884, (1979); (b) Nicolaou, K.C, *Tetrahedron*, 37, 4108 (1981).

127. Colvin, E.W., Egan, M.J., and Kerr, F.W. *J.Chem.Soc., Chem Commun.*, 1200, (1990).

128. Cole, R.J., and Cox, R.H., Handbook of Toxic Fungal Metabolites p. 202, Academic Press, New York, (1980).

129. Kelly, A., Postdoctoral work, University of Glasgow, 1986/87

130. Peterson, D.J., *J.Org.Chem*, 33, 780, (1968).

131. Johnson, C.R., and Tait, B.D., *J.Org.Chem.*, 52, 281, (1987).

132. Doddrell, D.M., Pegg, D.T., and Bendall, M.R., *J. Magn. Reson.* 48, 323, (1982).

133. Ishii, K., Pathre, S.V., and Mirocha, C.J., *J.Agric.Food Chem.*,, 26, 649, (1978).