CHEMISTRY   AND   INTERCONVERSION   OF

COMPLEX   TRICHOTHECENES

by

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for the degree of Ph.D.

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CONTENTS

SUMMARY

The trichothecenes are a ubiquitous group of toxic fungal sesquiterpenoids. Previous studies have shown that the 12,13-epoxide present in the trichothecenes may be crucial for biological activity. To gain further insight into the biological role of the epoxide, successful employment of the Sharpless deoxygenation protocol has been achieved after extensive model studies. This procedure gave the 12,13-exomethylene compounds (119) and (121) from the corresponding epoxytrichothecenes, namely triacetoxyscirpene (63) and triacetyldeoxynivalenol (120).

In an extension to this work, Sharpless deoxygenation furnished the key intermediate, olefin (124), which was used to synthesise the 12,13-_epi_-epoxytrichothecene (128) _via_ ozonolysis and reaction of the derived norketone (125) with dimethylsulphonium methylide. Both the Sharpless deoxygenation product (119) and the _epi_-epoxytrichothecene (128) were found to be essentially non-toxic, thus demonstrating for the first time the necessary presence of the epoxide and its stereochemical definition.

Further work has led to methodology for the provision of less readily available trichothecenes. To this end, deoxynivalenol (23) has been successfully synthesised from one of our culture products, anguidine (9), _via_ another naturally occurring trichothecene, calonectrin (5). This methodology involved selective removal of the C-4 oxygen functionality, selective allylic oxidation at C-8, to establish the enone system, and introduction of the C-7 hydroxyl moiety.

ABBREVIATIONS

3–AcDon	3–Acetyldeoxynivalenol
AIBN	Azobisisobutyrylnitrile
DHP	Dihydropyran
DIBAL	Diisobutylaluminium hydride
DMAP	4–($\underline{N}$,$\underline{N}$–Dimethylamino)pyridine
DME	Dimethoxyethane
DMF	$\underline{N}$,$\underline{N}$–Dimethylformamide
DON	Deoxynivalenol
Eu(thd)$_2$	Tris(2,2,6,6–tetramethyl–3,5–heptanedionato) europium
HMPA	Hexamethylphosphoramide
LDA	Lithium Diisopropylamide
MAS	Monoacetoxy(4)scirpenediol
mcpba	$\underline{m}$–Chloroperbenzoic acid
Ms	Methanesulphonyl
NBS	N–Bromosuccinimide
PCC	Pyridinium chlorochromate
PPTS	Pyridinium p–toluenesulphonate
Py	Pyridine
TBDMS	$\underline{t}$–Butyldimethylsilyl
TES	Triethylsilyl
THF	Tetrahydrofuran
THP	Tetrahydropyran
TMS	Trimethylsilyl
Ts	$\underline{p}$–Toluenesulphonyl
pTSA	$\underline{p}$–Toluenesulphonic acid

INTRODUCTION

1.   GENERAL   INTRODUCTION

Since the isolation of penicillin from a fungal mould by Florey and Chain in the late nineteen thirties, the search for other potentially useful mycotoxins has been intense. Because these compounds are the products of secondary metabolism, they often possess very interesting and diverse structures and chemical properties. Such secondary metabolites include the ergot alkaloids, aflatoxins and gliotoxin (plus related epipolythiodioxopiperazines). One of the most important classes of mycotoxin is that comprising the trichothecenes.

The trichothecenes constitute a structurally closely related family of sesquiterpenoids. They are produced by various species of Fungi imperfecti and, in an isolated case, higher plants – although it is believed that the plant further metabolises trichothecenes produced by symbiotic fungi present in the soil. The fact that the trichothecenes are produced by a large number of taxonomically unrelated fungi makes them unique and very important. These fungi can grow on cash crops such as wheat, maize and rice: the production of these mycotoxins is therefore very important from an economic standpoint due to their profound biological activity in both man and domesticated animals.

Intoxication manifests itself in many ways, including severe skin irritation, blood disorders, such as increased numbers of white blood cells, vomiting and food refusal, damage to the haematopoietic tissues in the bone marrow, thymus and lymph nodes, and inhibition of the immune system. At a molecular level they act by inhibiting protein and, occasionally, DNA synthesis.

Although the trichothecenes have been known since 1949, their role in mycotoxicoses was not demonstrated until 1968 by Bamburg et al[1,2]. The Wisconsin Group isolated from mouldy corn a toxic

fungus called <u>Fusarium tricinctum</u>, from which a novel crystalline compound was obtained. This compound was named T-2 Toxin after the strain of <u>Fusarium</u> from which it was isolated. T-2 Toxin was found to be the agent responsible for illness and death in dairy cattle.

Subsequently nivalenol and fusarenon-X, from <u>F. nivale</u> Fn2B, [3,4,5] and deoxynivalenol and ester derivatives, from <u>F. roseum</u> "Graminearum"[6], have been implicated in red-mould disease in Japan. This disease occurs at harvest time in barley and wheat when the temperature is low and humidity high. Intoxication by the infected cereal grains causes the major clinical signs of vomiting, refusal of food, congestion and haemorrhaging of tissues, diarrhoea and finally death.

It is now believed that trichothecenes were responsible for many pre-World War II cases of mycotoxicoses, including the outbreak of dendrochiotoxicoses in horses in Southern Russia in 1937 and cases of alimentary toxic aleukia (ATA) or septic angina in Russia since the 19th century[7-11].

Structurally the trichothecenes consist of a tricyclic skeleton (1), which is numbered in the manner proposed by Godtfredsen <u>et al</u>[12]. Features common to these compounds are the <u>cis</u>-fusion of both rings A and C to the pyran B ring and the presence of the 9,10-double bond and the 12,13-spiro-epoxide. Various sites of oxygenation are encountered, namely, at C-3, C-4, C-7, C-8 and C-15 - hence the large number of trichothecenes that have been isolated. Oxygenation can take the form of alcohols or their simple esters, such as acetates or <u>iso</u>-valerates. The presence of a ketone function of C-8 is quite common and one trichothecene, crotocin (20) possesses a 7,8-epoxide. Some important trichothecenes are shown in SCHEME 1*. In addition to

the presence of simple esters at C-4 and C-5 it is also possible

to have macroyclic esters linking these centres as exemplified

by (29) and (30). Such compounds are not within the scope of this

thesis although they are very important in their own right.

  *A full list of naturally occuring non-macrocyclic trichothecenes

appears in Appendix 1.
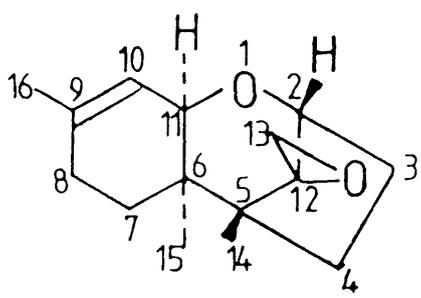
(1)
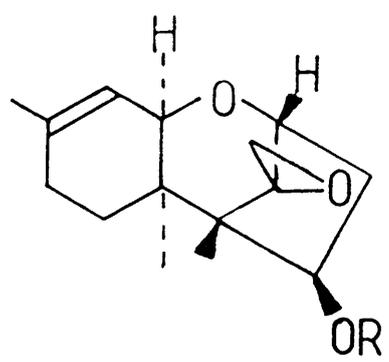
(2) R=H   Trichodermol
(3) R=Ac Trichodermin
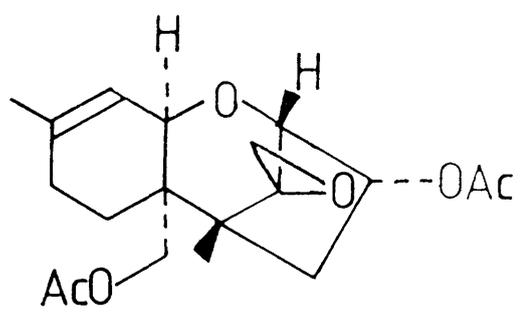
(5) Calonectrin
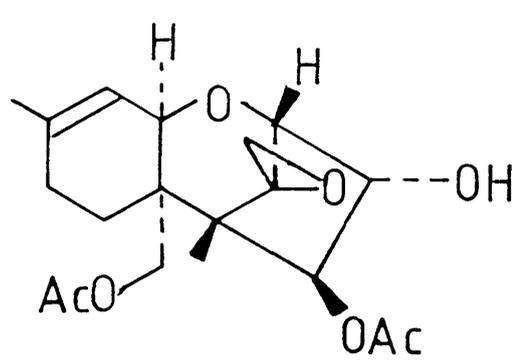
(9) Diacetoxyscirpenol
(Anguidine)
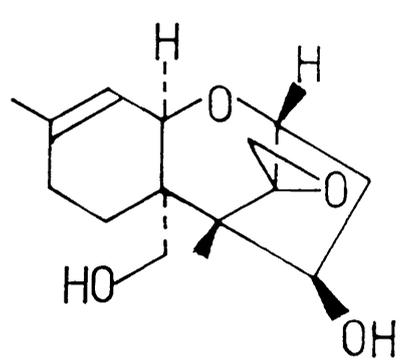
(28) Verrucarol

SCHEME 1a
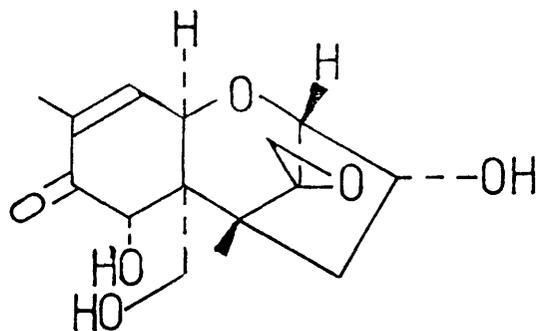
(23) Deoxynivalenol
(Vomitoxin)
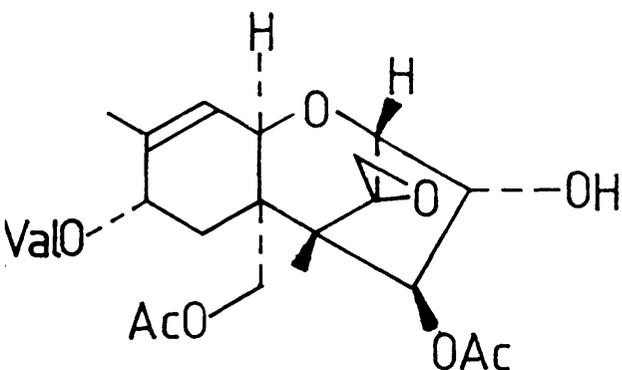
(14) T-2 Toxin
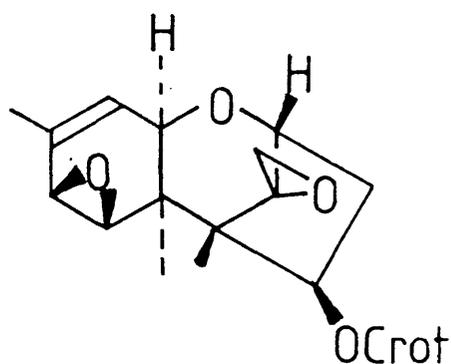
(20) Crotocin

SCHEME 1b
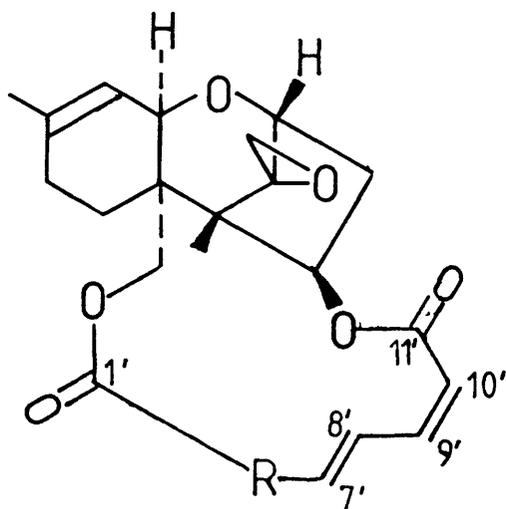
(29) Verrucarin A $R=-CH(OH)CH(CH_3)CH_2CH_2OC-$
                       (S)   (R)

(30) Roridin D $R=-CH-C(CH_3)CH_2CH_2OCHCH(OH)CH_3$

2.   STRUCTURAL ELUCIDATION

Freeman and Morrison[13] isolated the first trichothecene in 1948. It was named trichothecin after the parent fungus, Trichothecium roseum.

Freeman[14] proposed that trichothecin had either structure (31) or (32), with a preference for (31) – a view supported by Fishman[15] who assigned much of the stereochemistry. The main elements of structural evidence were:-

(i)   it was an ester which on hydrolysis gave a ketonic

         alcohol, trichothecolone, and crotonic acid;

(ii)   it was readily hydrogenated using palladium on carbon

         at atmospheric pressure: two moles of hydrogen were

         taken up – one by the ester double bond, the other by

         the parent alcohol double bond;

(iii)   it contained a reactive carbonyl group–hydrazones and

         semicarbazones could be prepared;

(iv)   it was u.v. active at 225 nm suggestive of an

         unsaturated ketone;

(v)   Kuhn–Roth oxidation showed the presence of 3 C–methyl

         groups;

(vi)   i.r. spectroscopy showed bands at 1670 cm^{-1} (α,β–

         unsaturated ketone), 1710 and 1177 (α,β– unsaturated

         ester), 1650 (C=C);

(vii)   reaction with hydrochloric acid gave a chlorohydrin (33):

         (although Freeman undertook x–ray crystallographic analysis

         of (33), no reference was made to the relative stereochemistry

         in trichothecin: in the light of later work the product

         from acid treatment should be (34) – see under Chemistry);
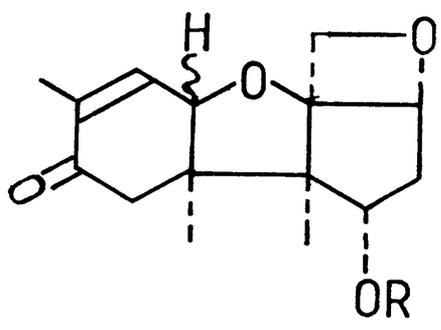
(31)
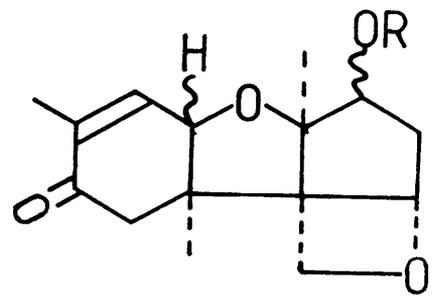
(32)
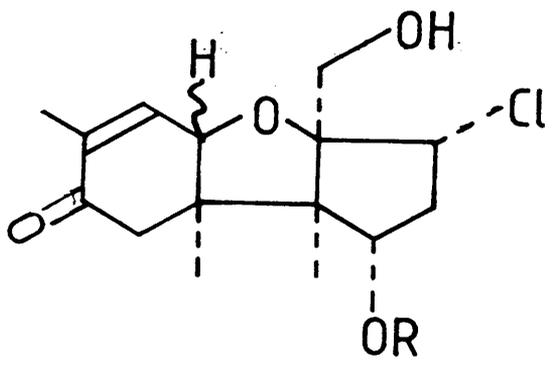
(33)
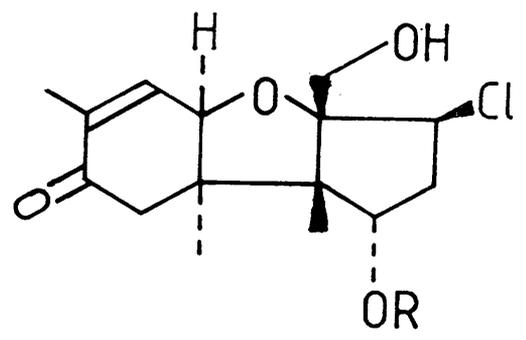
(34)

R=Crotonyl

(viii)   oxidation of trichothecolone gave a diketone, proposed

to be (35), which indicated the presence of a secondary

alcohol in the starting material: the new carbonyl group,

ν_{max} 1735 cm^{-1}, was probably a cyclopentanone;

(ix)   dehydrogenation of fully reduced trichothecolone gave

p̲-xylene, p̲-xylenol, 2,3-dimethylcyclopentanone and

2,3-dimethylcyclopent-2-enone;

(x)   the action of hot alkali on the oxidised trichothecolone

gave p̲-xyloquinone and p̲-xyloquinol as outlined in

SCHEME 2.

While working on trichodermin, Godtfredsen[16,17] arrived at a

similar partial structure to that of trichothecin through a greater

use of ^{1}H n.m.r. spectroscopy. Indeed Godtfredsen showed that

trichodermin and trichothecin were related by vigorous oxidation of

the former to trichothecolone acetate. These findings should have led to

the conclusion that the structure of trichodermin was (36). However,

this structure was in conflict with the observation that reduction of the

hydrolysis product of trichodermin, trichodermol (37), using lithium

aluminium hydride, gave a diol which has a new quaternary methyl group

(by ^{1}H n.m.r. spectroscopy) and which on acetylation and oxidation gave

a monoacetate and monoketone respectively. These results suggested the

presence of a spiro-epoxide moiety in trichodermol – a conclusion supported

by ^{1}H n.m.r. spectroscopy with an observed coupling constant of 4Hz for the

AB spin system at δ 2.79 and 3.09.

The correct structure of trichodermol (2), and hence trichodermin

(3), was finally established by x-ray crystallographic analysis of the

p̲-bromobenzoate of trichodermol[18,19].

(35)
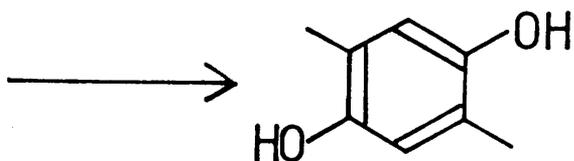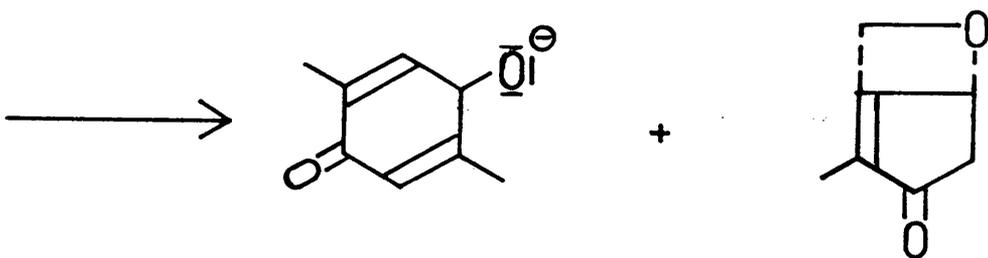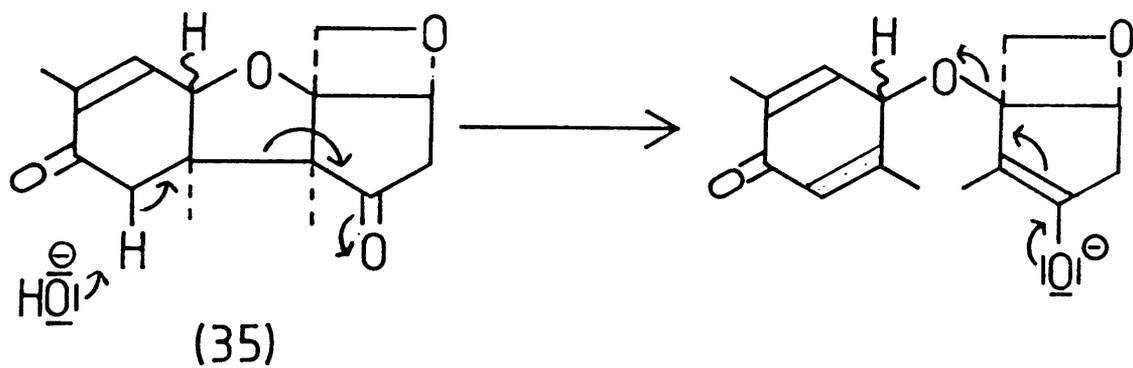
SCHEME 2
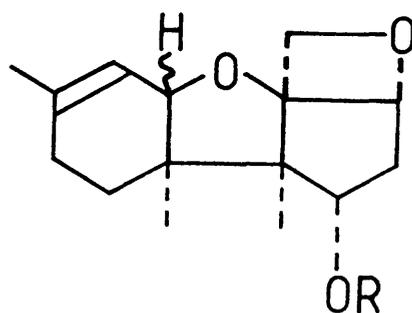
(37) R=H
(36) R=Ac
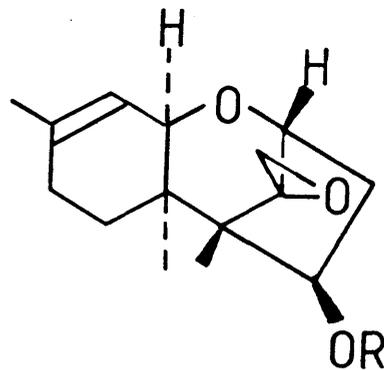
(2) R=H
(3) R=Ac

Based on this basic structure, trichothecin and trichothecolone were reassigned as (22) and (21). Similarly, verrucarol's original structure (39)[20] was reassigned as (28)[16,17,21,22].

In the light of these revised structures the original findings of Fishman[14] and Freeman[15] can be reinterpreted. Observations (i)-(iv) and (ix) are consistent with the correct structure. The oxidation product in (viii) is (38) which still contains the five membered ketone. It is possible for this new ketonic group to be unreactive due to steric congestion by the proximate epoxide and methyl groups. Observations (vii) and (x) can be rationalised on the basic of skeletal rearrangements. These rearrangements will be discussed later.

It should be noted that although Freeman tested for epoxides, using the method of Ross[23], by measurement of the rate of liberation of hydroxyl ions in the presence of sodium thiosulphate, these tests were generally inconclusive. From models it can be seen that the epoxide is highly shielded against attack by external nucleophiles.

Since the early 1960's structure elucidation by chemical degradation has largely given way to ^{1}H and ^{13}C n.m.r. spectroscopic analysis. Some important typical chemical shifts are given in Table 1. (The values for C-3, C-4 and C-15 are for appropriately oxygenated species).
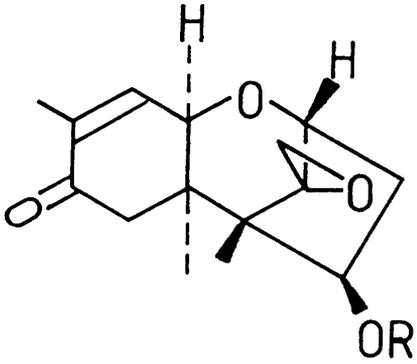
(21) R=H
(22) R=Crotonyl

(38)

(39)

(28)
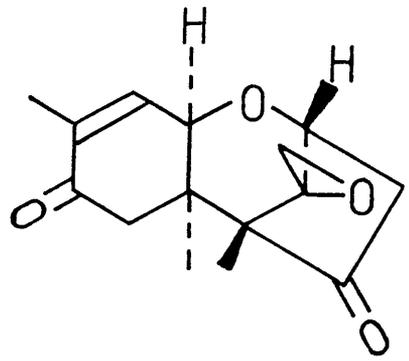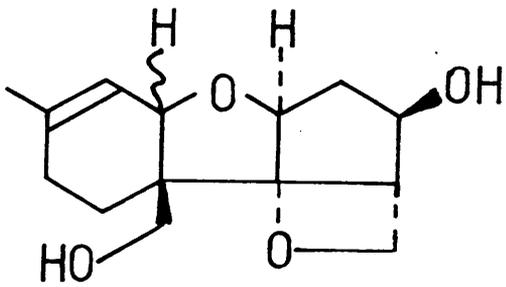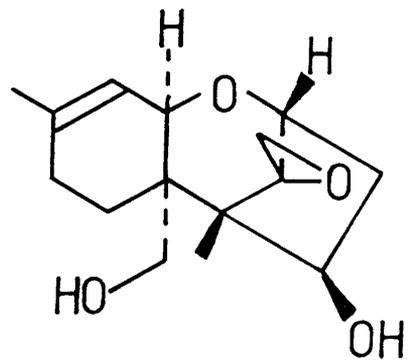
H	δ (ppm)	Comments
2	4.0	
3	4.1	Shifted 1 ppm if acylated
4	4.5	Shifted 1 ppm if acylated
10	5.5	Allylic coupling to H16 Shifted 1 ppm if part of enone
11	4.0–4.5	broad doublet
13	2.9	AB quartet, J = 4 Hz
14	0.8	
15	4.0	Often AB quartet, J = 12–13 Hz
16	1.7	Broadened due to allylic coupling with H 10

TABLE 1

3. CHEMISTRY

3.1 REARRANGEMENTS

Due to the special spatial relationship of the epoxide and the tetrahydropyran oxygen, certain facile rearrangements can occur under suitable acidic or, less commonly, basic conditions to give the apotrichothecene skeleton.

In acid, the mechanism proposed[17] (SCHEME 3) involves initial protonation of the epoxide followed by internal nucleophilic attack by the pyran oxygen to give intermediate oxonium ion (40). This intermediate can then collapse in one of two ways; either by attack by an external nucleophile or by loss of the proton at C-3 leading to a new double bond.

This arrangement is consistent with ^{1}H n.m.r. studies which showed the absence of the epoxide AB quartet and H-2, and the presence of a primary alcohol. This primary alcohol could be oxidised by normal means to the corresponding acid.

The formation of (40) may be important for biological activity, since it has been shown that destruction of the epoxide, for example by lithium aluminium hydride reduction to the tertiary alcohol, renders the molecule inactive[24]. In such cases X^- could denote a growing peptide or DNA chain which would bind to the trichothecene.

Rearrangements under basic conditions are less common and require presence of a C-4 ketone function. For example, trichodermone[17] (41) can be isomerised easily in the presence of sodium carbonate to give the tricyclic enone (42) (SCHEME 4). It should be noted that after opening of the pyran ring via the enol of (27), the subsequent product possesses a tetrahydrofuran ring which arises from "abnormal" epoxide ring opening[25]. A possible explanation of this could be that formation of the tetrahydrofuran ring occurs at a much greater rate than that of the pyran ring to give (43)[26].

X=Cl,Br,I,OH

SCHEME 3

(41)

(43)   (42)
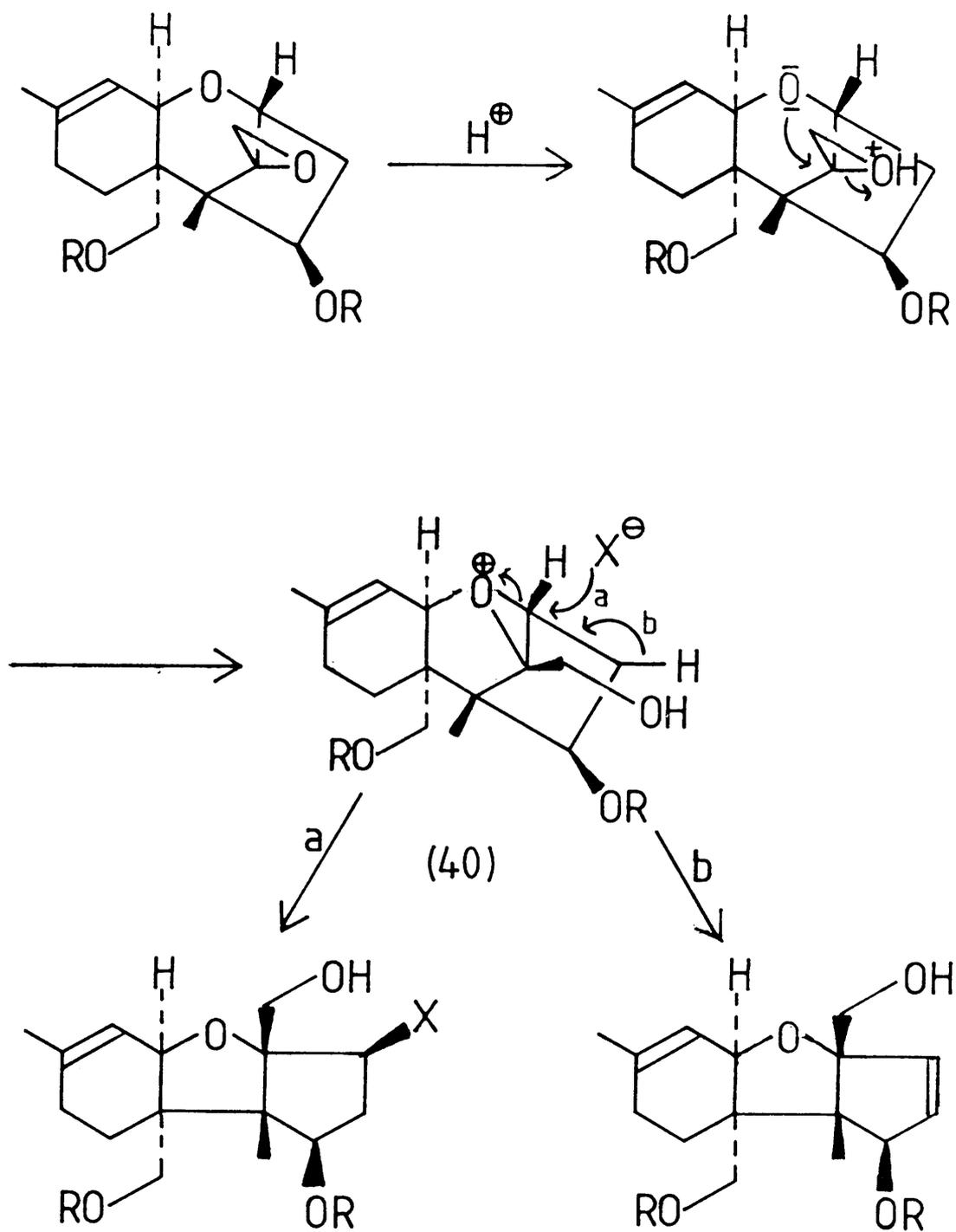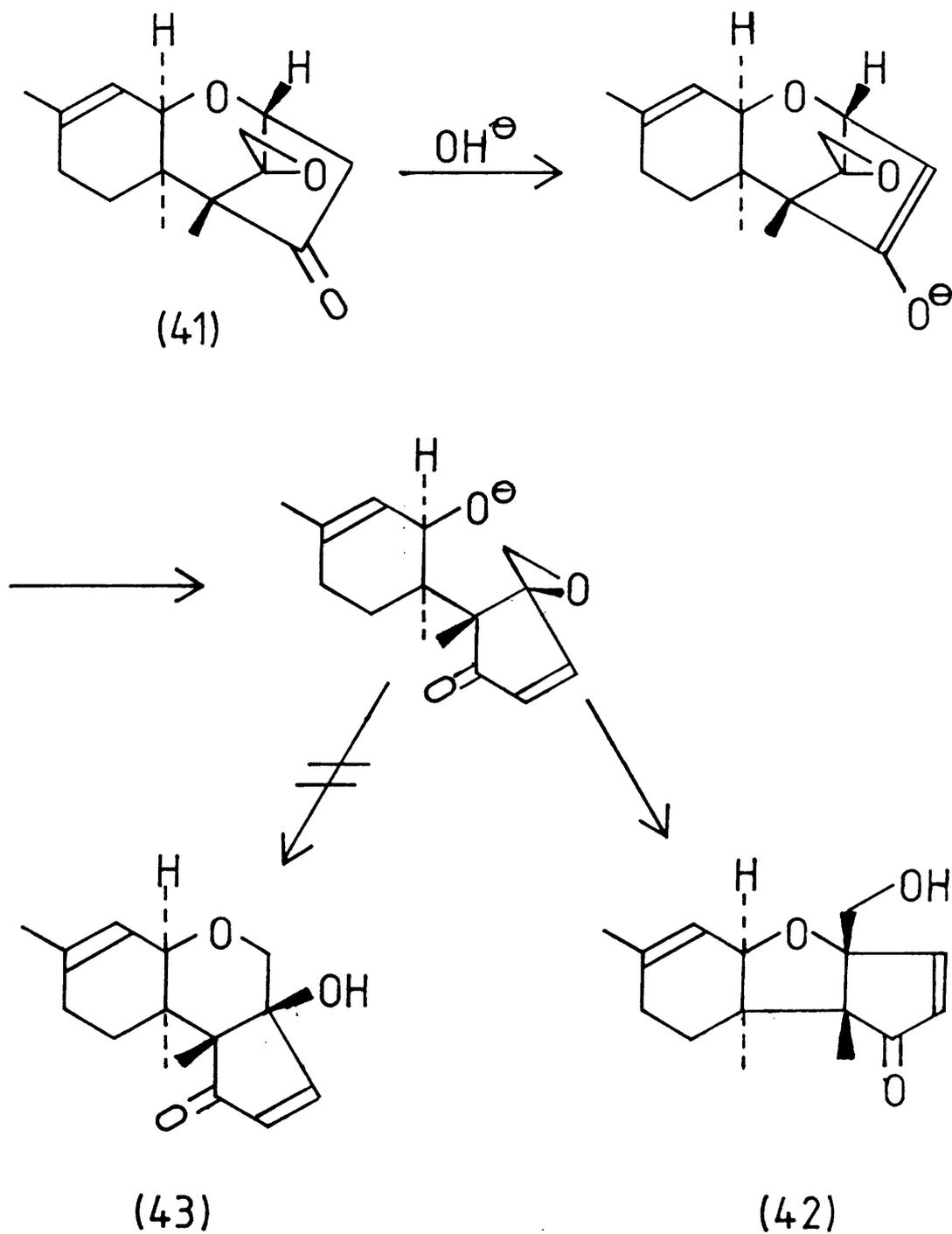
SCHEME 4

3.2 9,10 – DOUBLE BOND

The chemistry of the 9,10–double bond is in the main unremarkable. It can be hydrogenated easily by using palladium on charcoal or calcium carbonate at atmospheric pressure[14,15,17,20,21,22,27]. Reduction leads to diminished biological activity[24], so there may be a steric requirement for the double bond. The double bond can be ozonolysed[27] to give the 9,10–ketone–aldehyde after reduction of the ozonide.

Epoxidation of the double bond can be achieved using m–chloroperbenzoic acid. Orientation of the epoxide can be influenced by the presence of an hydroxyl at C–15. If the C–15 alcohol is esterified, epoxidation occurs from the less hindered β–face of the double bond[28,29]. However, if the C–15 alcohol is free epoxidation can be directed by hydrogen bonding between the alcohol and the peracid to give the α epoxide. In practice a mixture of epoxides results, although the presence of the free C–15 alcohol allows formation of the bridged ether (44) (SCHEME 5).

On prolonged boiling in water the 9,10–double bond can attack the epoxide to give the hydrate (45) (SCHEME 6).

3.3 "PERIPHERAL" CHEMISTRY

In addition to the integral pyran and epoxide oxygen functions, the trichothecenes can be further substituted at C–3,C–4,C–7,C–8 and C–15. Much work has been carried out on these centres including:–

(i)   interconversions of the trichothecenes either by various hydroxyl protection/deprotection methods or by the selective introduction or removal of oxygenation;

(ii)   introduction of "unnatural" esters for biological testing;

(iii)   introduction of totally synthetic macrocyclic side chains linking C–4 and C–15.

(44)

SCHEME 5

SCHEME 6

3.3.1. C — RING AND C-15 HYDROXYL CHEMISTRY

(a) PROTECTION/DEPROTECTION METHODS

Interconversions of trichothecenes are often necessary to facilitate the provision of compounds that are often only available in small amounts from culture. For example, monoacetoxyscirpenol (48) can be made from anguidine (9), a readily available fungal metabolite. Roush[30] employed two methods starting from the diol (46), obtained from anguidine by THP ether formation of the free C-3 alcohol followed by alkaline hydrolysis of the C-4 and C-15 acetates. The first method (SCHEME 7) involved regioselective acetylation at C-4 using 1-acetylimidazole, which gives the monoacetate (47) in 58% yield along with 19% of the diacetate (which can be recycled if desired).

The second, more elegant method (SCHEME 8) involved protection of the C-15 alcohol as the bridged bromo-ether (49) - this permitted acetylation solely at C-4. Suitable deprotection of C-3 and C-15 gave (48). Attempted selective hydrolysis of anguidine under a variety of conditions lead almost exclusively to the C-15 mono-acetate [27,31].

In addition to the various mono- and di-acetates of scirpenetriol that have been prepared, various "unnatural" esters have been created. For example, Kaneko et al[29] prepared chloroacetates, bromoacetates, crotonates and methacrylates, as well as acetates, propionates and butyrates for biological testing.

(b) OXIDATIONS

Oxidations of C-3,C-4 and C-15 alcohols to the respective ketones and aldehydes have been accomplished by various means.

Oxidation at C-15 to the aldehyde proceeds in good yield by the use of dipyridine chromium trioxide (Collins' Reagent)[27] or pyridinium

(9) →(i),(ii)→ (46)

→(iii)→ (47)

→(iv)→ (48)
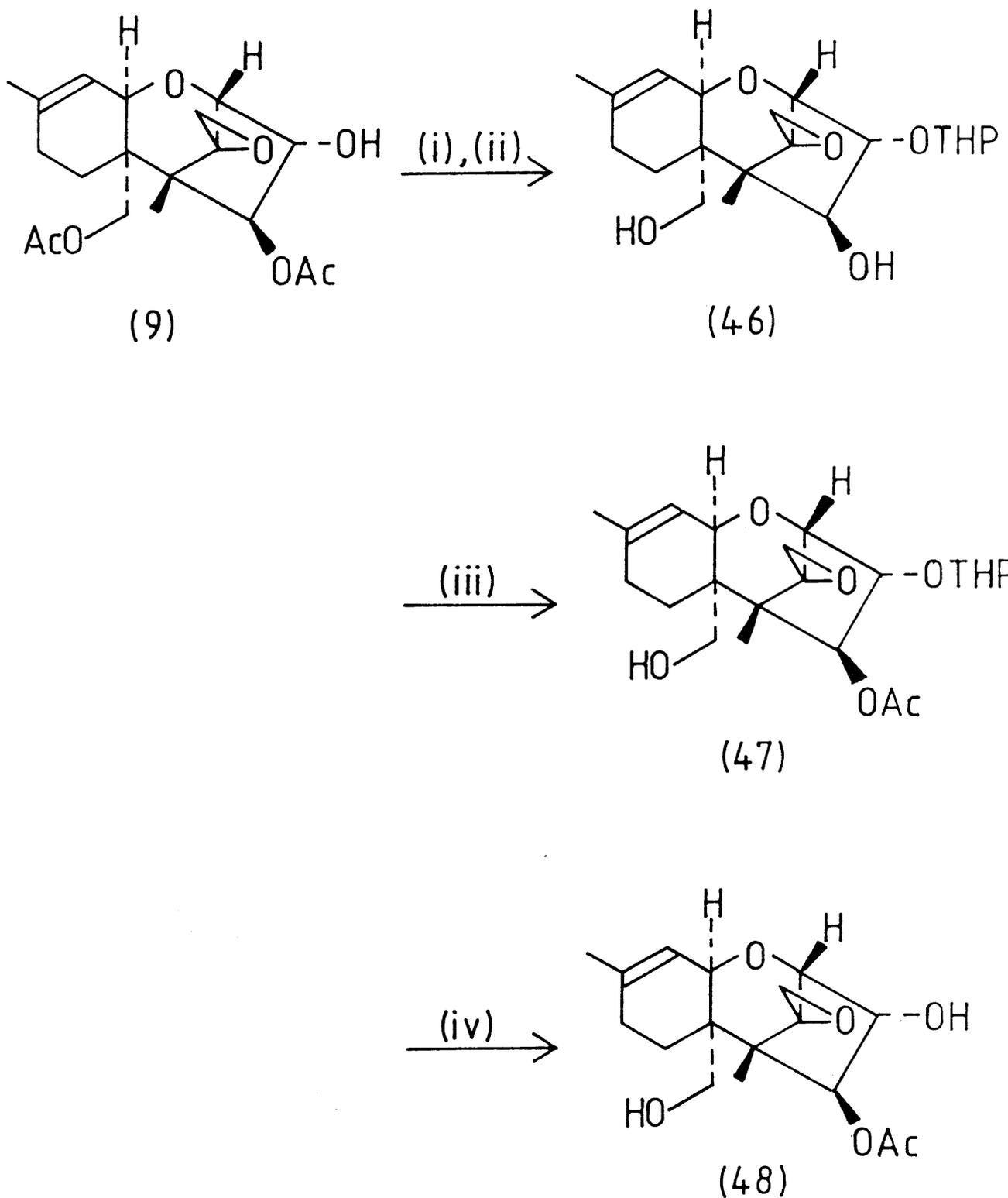
(i) DHP, H$^{\oplus}$; (ii) NaOH, MeOH; (iii) Ac.imid.; (iv) H$^{\oplus}$.

SCHEME 7
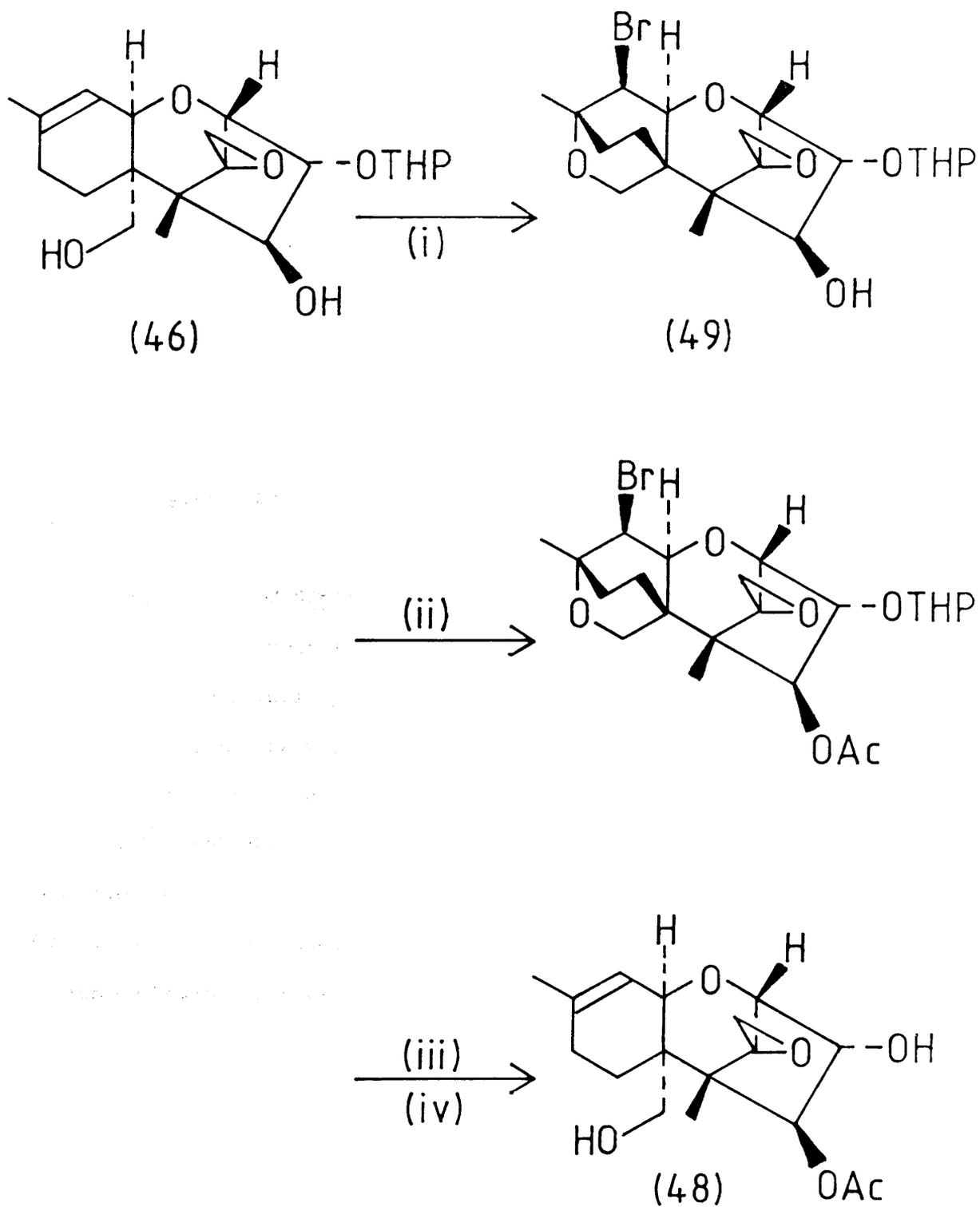
(i)NBS,MeCN;(ii)Ac$_2$O,py.;(iii)Zn(Ag),THF,EtOH,Et$_2$O;
(iv)AcOH,H$_2$O,THF.

SCHEME 8

chlorochromate[29]. As expected, this oxidation can proceed selectively in the presence of the free C-3 or C-4 alcohols. Attempts to oxidise the aldehyde further to the corresponding carboxylic acid under acidic conditions failed[27]. For example, treatment of aldehyde (50) with chromium trioxide in glacial acetic led to epoxide opening by the 9,10-double bond to give (51). However, the C-15 aldehyde has been oxidised by using neutral $KMnO_4$ in aqueous acetone[29].

Sigg et al[27] used chromium trioxide in glacial acetic acid to oxidise C-3 of anguidine. In addition to the desired ketone (52), the aldehydic-acid (53) was also obtained.

Kaneko et al[29] successfully oxidised C-3 and C-4 by Swern oxidation using trifluoroacetic anhydride and dimethyl sulphoxide.

Sigg[27] also oxidised C-4 of (54) in good yield using Jones' reagent to give (55).

(c) REDUCTIVE DEOXYGENATION

Deoxygenation reactions of C-3, C-4 and C-15 are important for ready access to a variety of trichothecenes. For example, deoxygenation at C-3 leads into the verrucarol series, at C-4 to calonectrin, and at C-3 and C-15 to trichodermin.

Sigg[27] published two methods of deoxygenation at C-4. Zinc reduction of the anguidine oxidation product (52) gave the C-3 ketone (57) in poor yield. However, treatment of the bismesylate (56) with sodium methoxide in refluxing methanol gave (57) in good yield Sodium borohydride reduction of this ketone followed by acetylation should give the natural trichothecene calonectrin (5), a compound unknown to Sigg in 1965. Compound (57) has also been synthesised by the action of silver carbonate-Celite reagent[32] (Fetizon oxidation).

Interestingly, although the C-4-acetoxy-C-3-ketone can be reduced to the C-3 ketone, the C-3-mesyl-C-4-ketone could not be reduced to the C-4 ketone[27]. This may be due to the steric hindrance preventing

(50) →(i)→ (51)

(i) CrO₃, AcOH.

(52)

(9) →(i)→ +

(53)

(i) CrO₃, AcOH.

(54)   Jones'   (55)

(52)

(56)

(57)

(58)
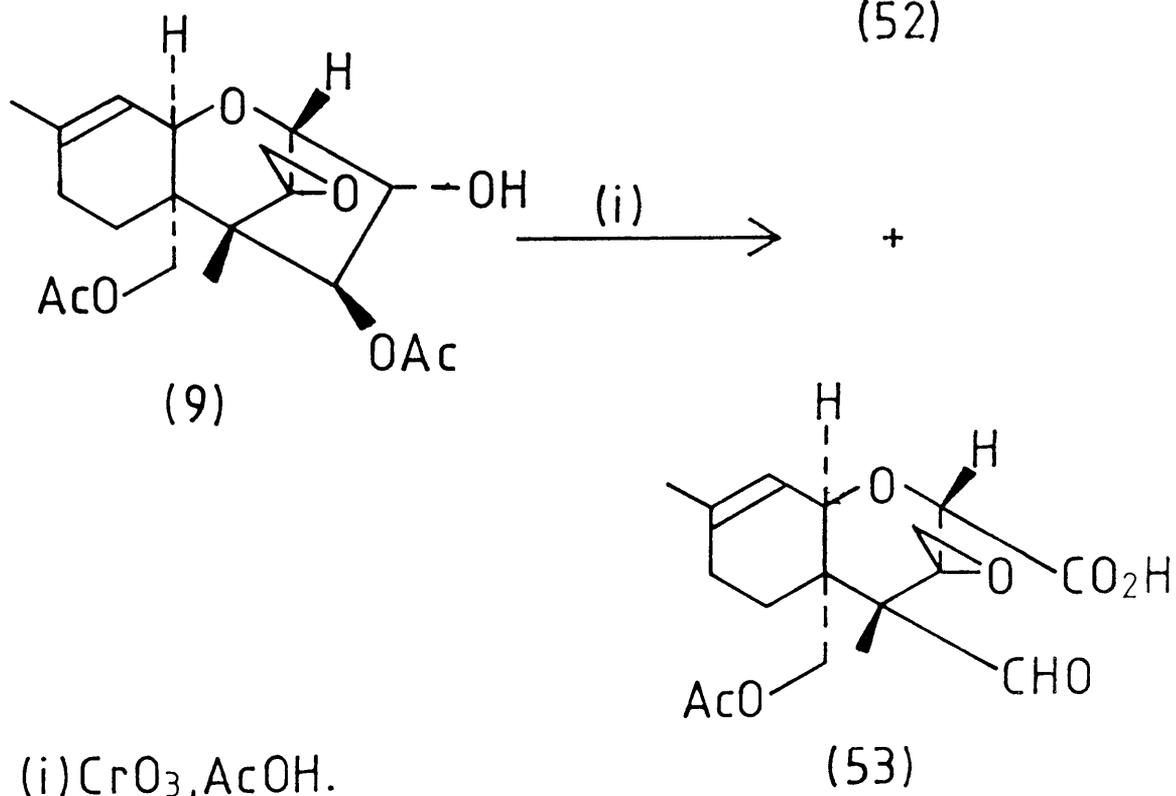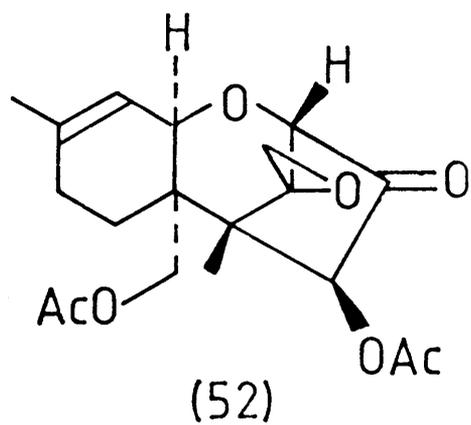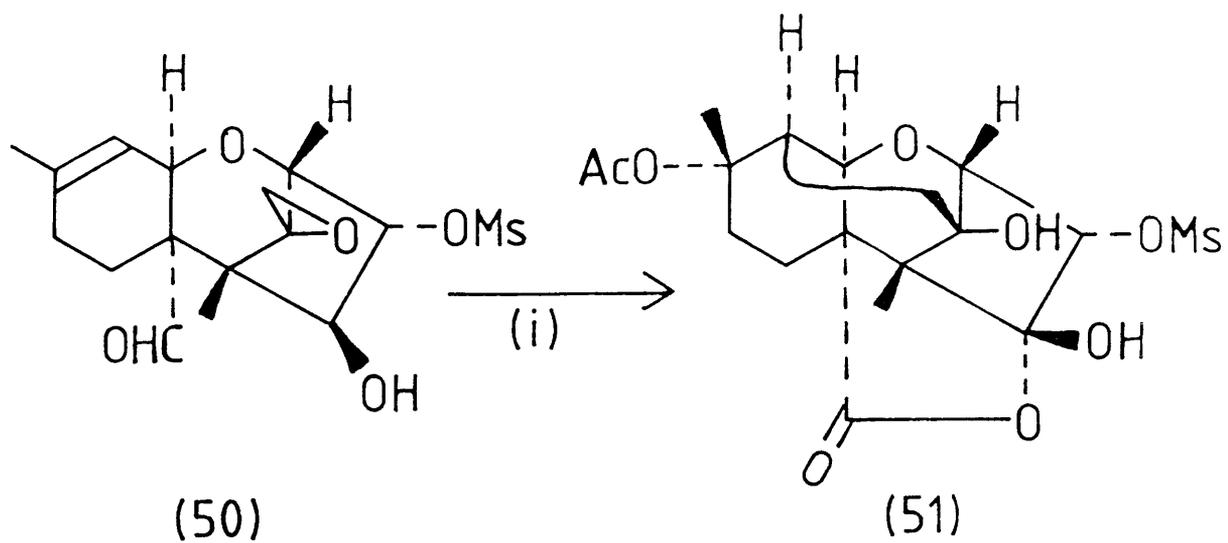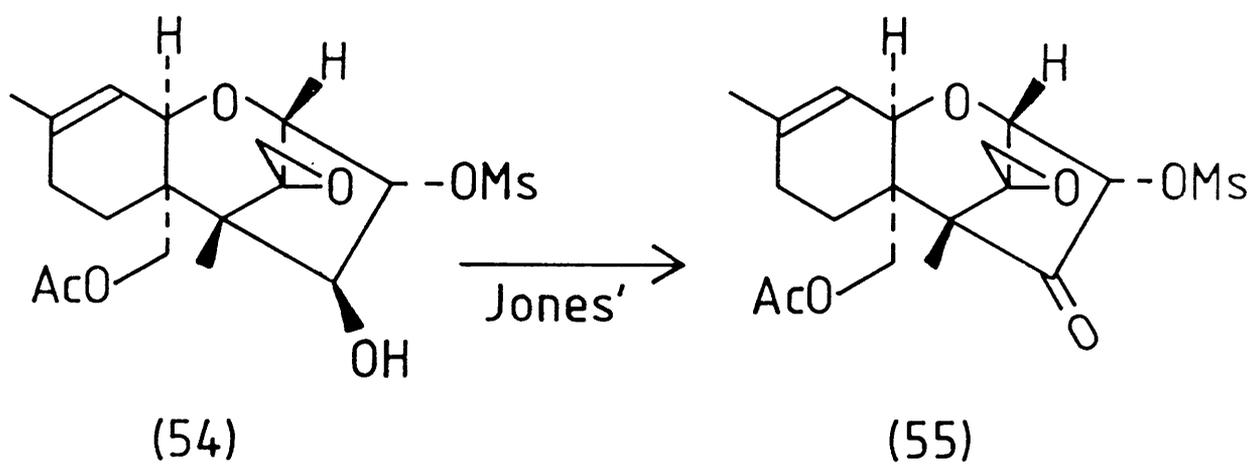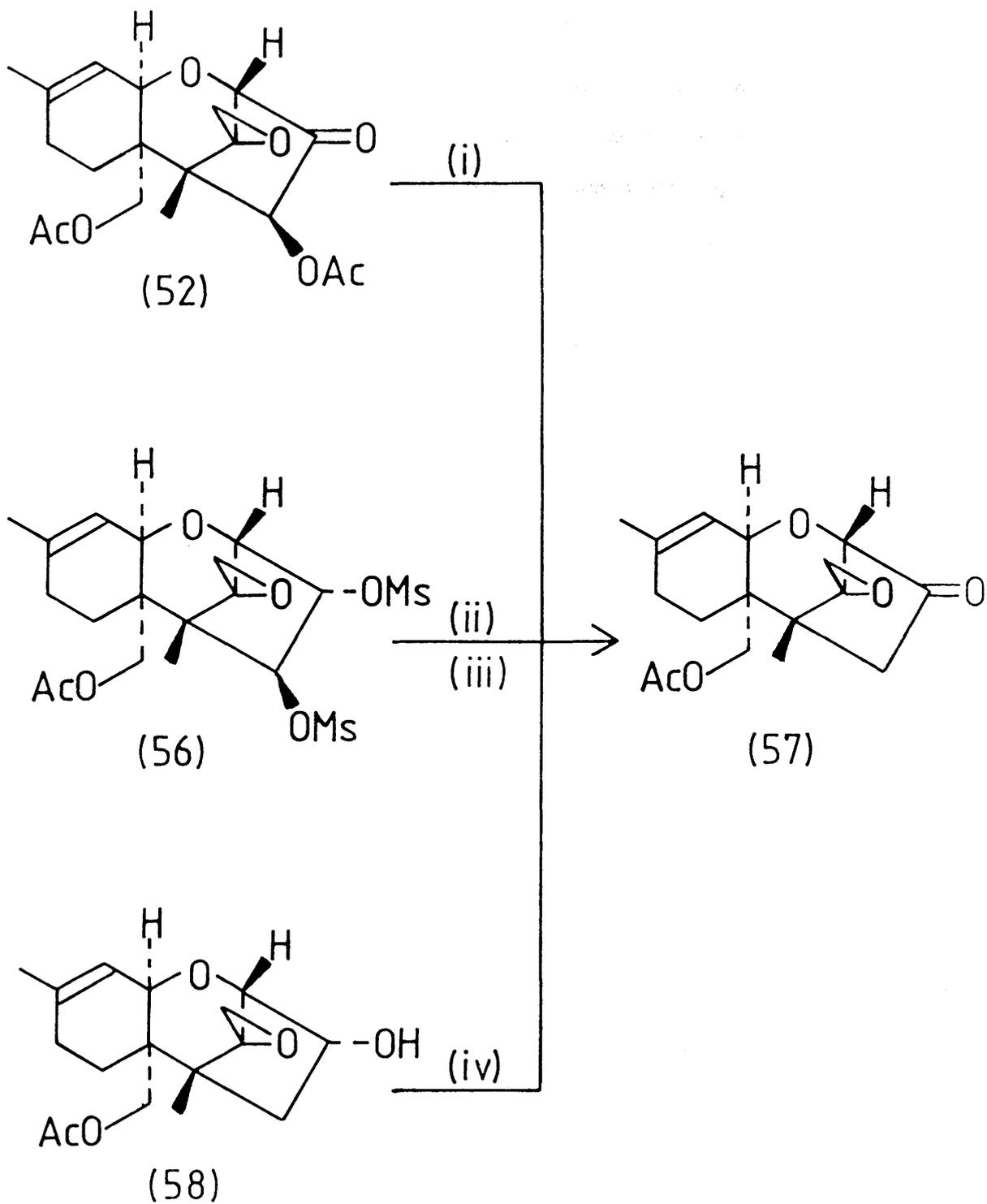
(i)Zn;(ii)NaOMe;(iii)Ac₂O,py.;(iv)AgCO₃/Celite.

access of the large zinc atoms to the relatively inaccessible C-3 substituent.

Other methods for alcohol deoxygenation include conversion of the free hydroxyl into the mesylate, followed by lithium aluminium hydride reduction. However, and as expected, such conditions also result in epoxide ring opening[27].

More recently deoxygenations at C-3 and C-4 have been accomplished successfully by Barton deoxygenation of the appropriate thioester with tributyltin hydride[33]. First to employ such a strategy was Fraser-Reid[34], who synthesised both verrucarol and trichodermol from anguidine.

Conversion of anguidine (9) into verrucarol (28) was straightforward since the C-4 and C-15 hydroxyl groups were already suitably protected as acetates. Thus Barton deoxygenation of anguidine gave diacetyl verrucarol (6), which on methanolysis gave verrucarol in an overall yield of 85%.

Attempts by Fraser-Reid to convert verrucarol (28) into trichodermol (2) _via_ Barton deoxygenation of a suitably protected C-4 derivative (59) resulted only in partial deoxygenation. Deoxygenation at C-15 was ultimately achieved by conversion of (59) into the chloride (60) followed by treatment with tributyltin hydride in the presence of benzoyl peroxide. Suitable deprotection at C-4 then gave trichodermol.

Sigg[27] and Gutzwiller[21] also reduced the C-15 alcohol via the mesylate and derived iodide. Treatment of the C-15 iodo compound with zinc gave the C-15 methyl group (SCHEMES 9 and 10).

Potlock and Tamm have also employed Barton deoxygenation for reductions at C-3 and C-4.

(9)   (i)   →   (6)

(ii)   →   (28)
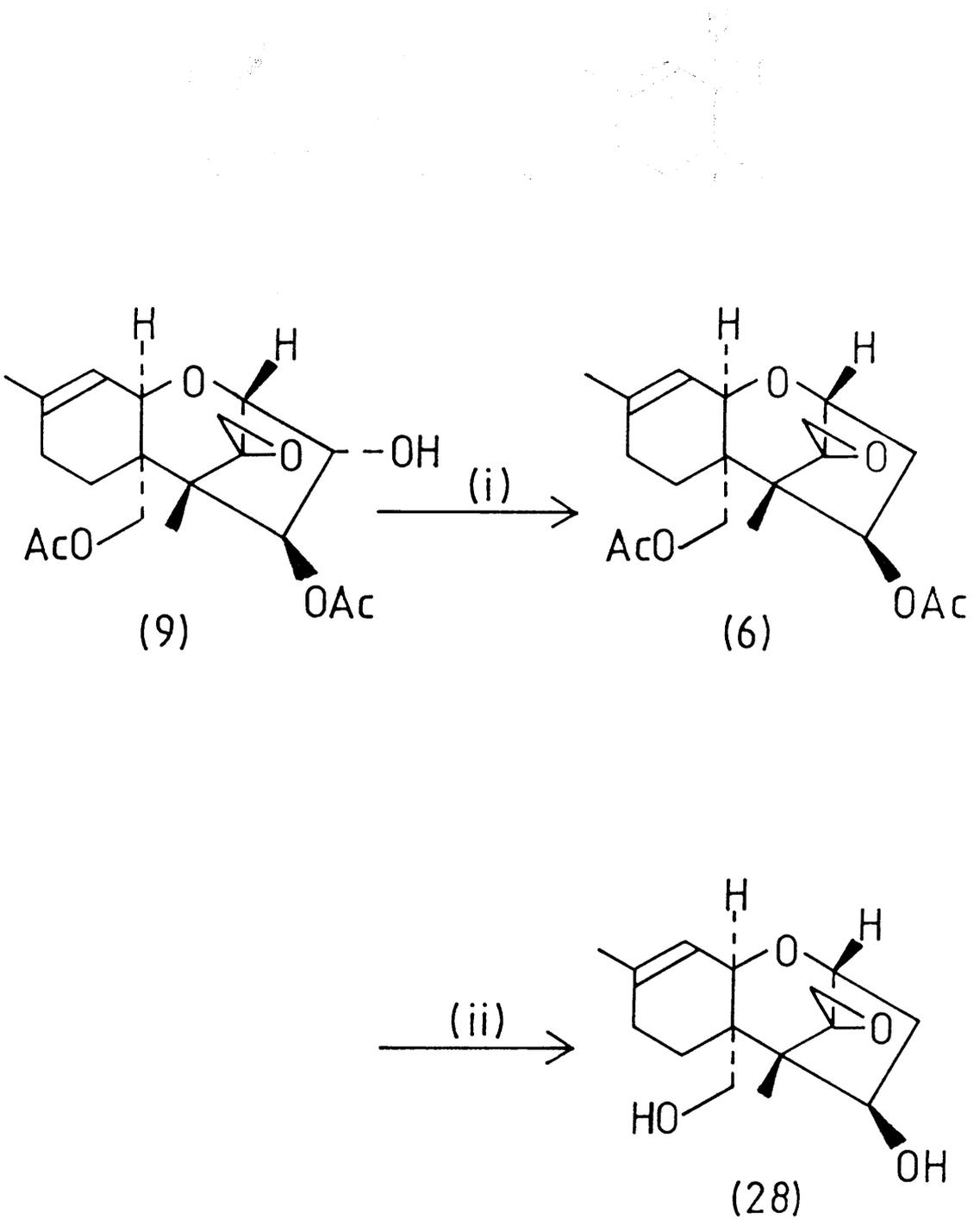
(i)Barton deoxygenation;(ii)NaOMe.

(28) → (59)

R=Ac or TBDMS

(i) → (60) OR

(ii)/(iii) → (2) OH
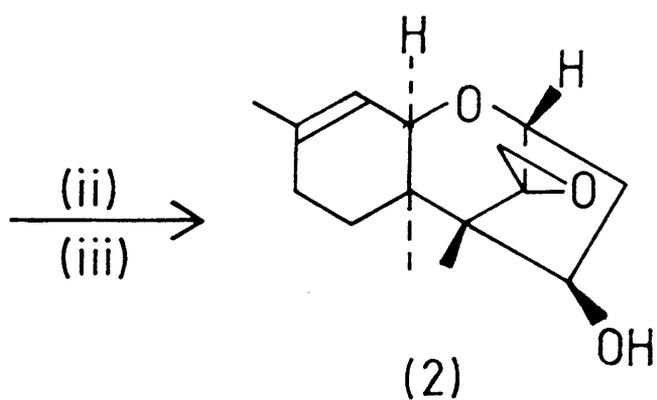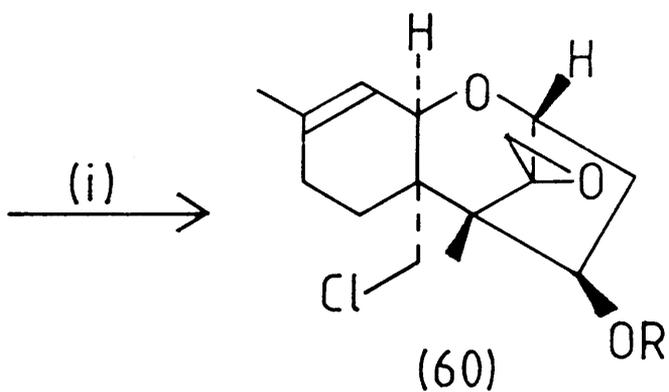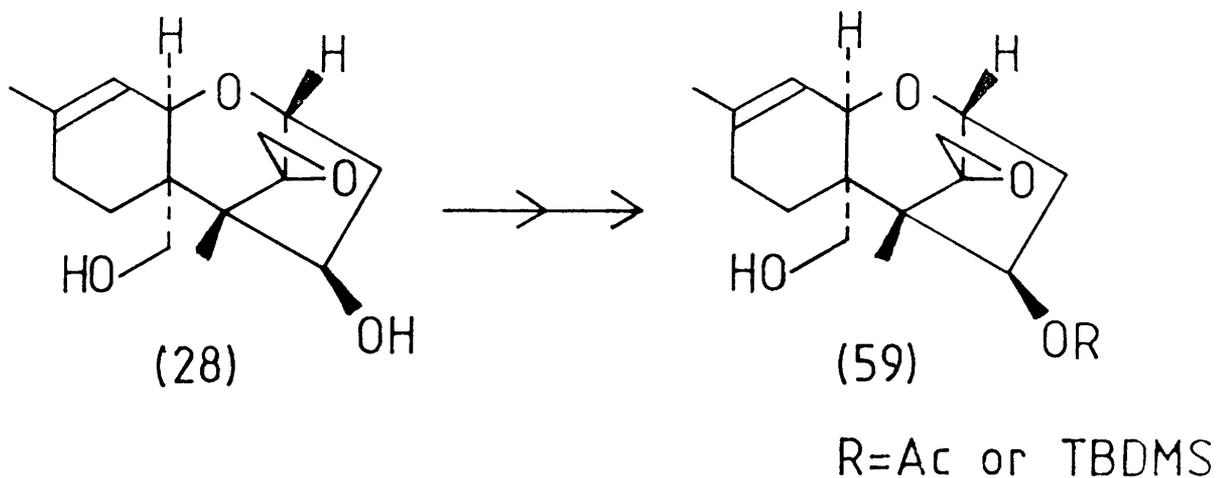
(i) MsCl, DMF, △; (ii) Bu₃SnH, (C₆H₅CO)₂O₂;

(iii) Deprotect.
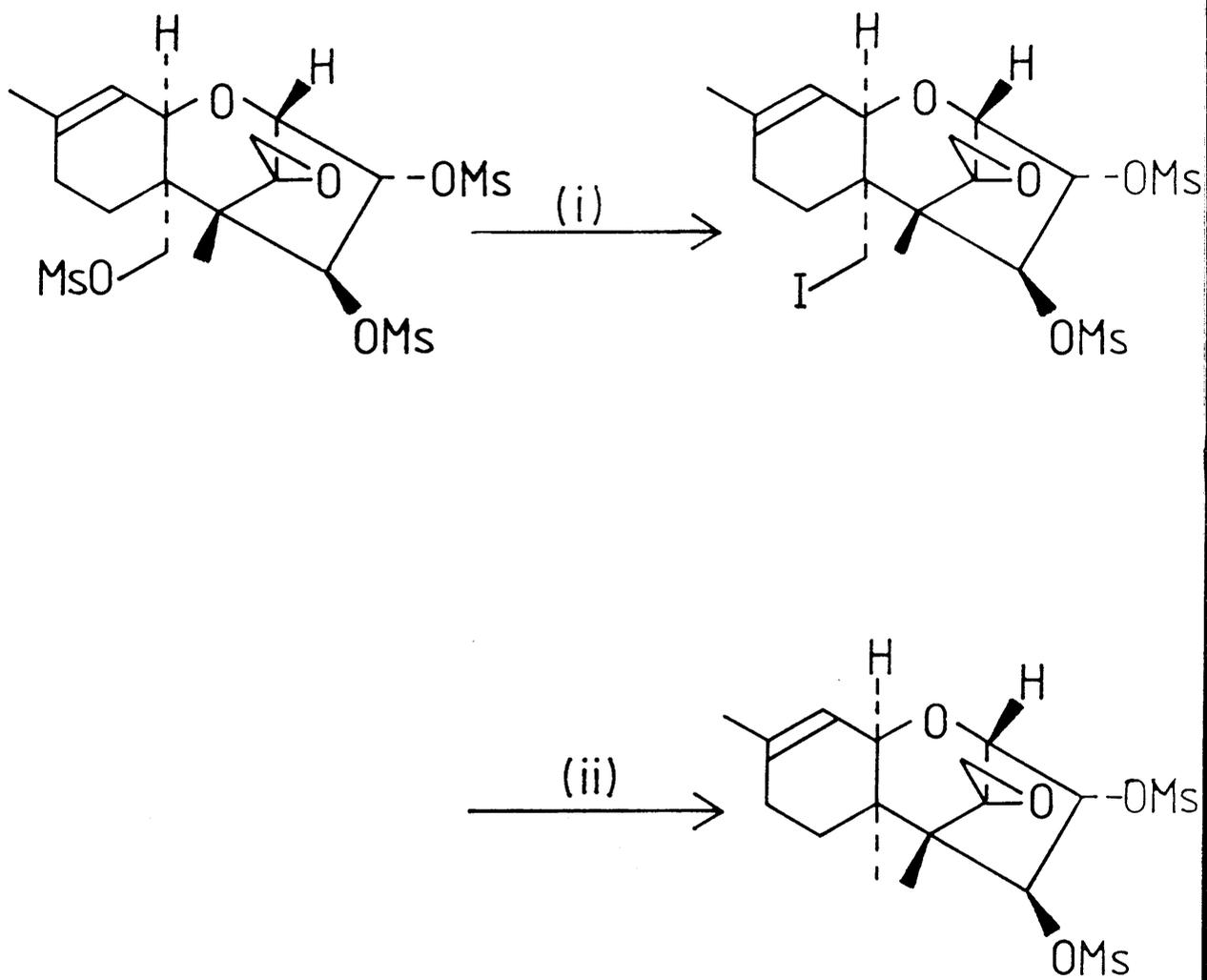
(i) NaI; (ii) Zn.

SCHEME 9
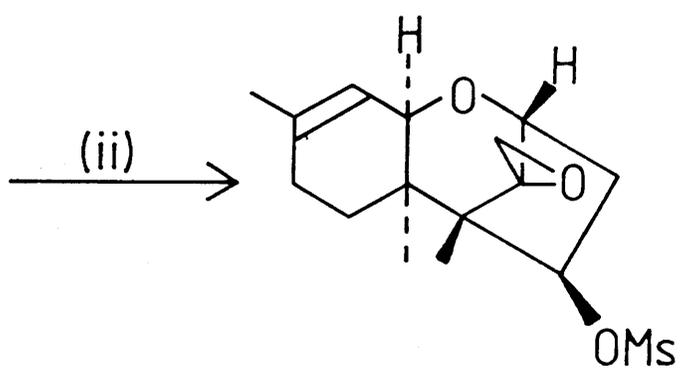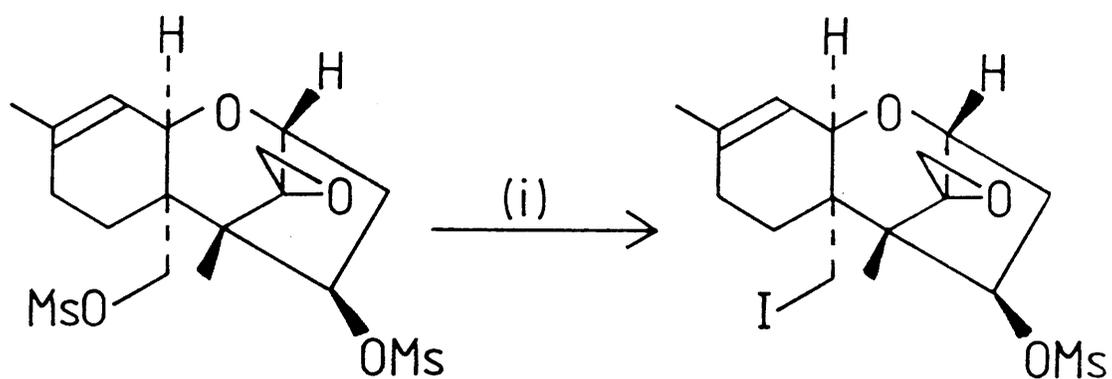
(i) NaI ; (ii) Zn.

SCHEME 10

Potlock[35] synthesised deoxyverrucarol (61) from both verrucarol
(28) and anguidine (9) (SCHEME 11 and 12). Tamm[36] made both
calonectrin (5) and 3-deacetyl calonectrin (62) from anguidine (9)
(SCHEME 13).

3.3.2. A-RING MODIFICATION

Some of the most important trichothecenes have oxygenation at
C-7 or C-8 as in T-2 Toxin (14) or vomitoxin (23) so functionalisation
of the A ring has also received much attention.

Enone (64) was first synthesised by Sigg et al[27] using t-butyl
chromate on triacetoxyscirpene (63) in a yield of less than 20%.
An improvement by Kaneko[29] involved initial allylic oxidation of
anguidine with SeO_2 to give the 3,8-diol (65) which could be oxidised
to the enone (66) using MnO_2 or pyridinium chlorochromate in an overall
yield of 31% (SCHEME 14). The 8β- alcohol is obtained since the β-face
is more accessible to attack by SeO_2.

Gutzwiller[21] treated trichodermyl mesylate (67) similarly with
SeO_2 followed by CrO_3 oxidation to give enone (68) in modest yield.

8α -oxygenated species can be obtained by allylic bromination
using NBS followed by acetolysis. For example, Dillen et al[37] treated
anguidine with NBS in dry dichloromethane to give a 9:2 mixture of
8β- and 8α-bromides (69) and (70) - again with preference for
attack from the less hindered β-face. Treatment of (69) with silver
acetate in refluxing acetic acid gave an approximately equal mixture
of neosolaniol triacetate (72) and its 8β-epimer (71): this last
finding indicates a planar intermediate at C-8 during acetolysis.

Although both SeO_2 allylic oxidation and NBS allylic bromination
are non-stereoselective, the natural 8α-alcohol can be obtained from
its epimer by oxidation to the enone followed by DIBAL reduction[29].

(28)

(i)

(ii)

(iii)
(iv)

(61)
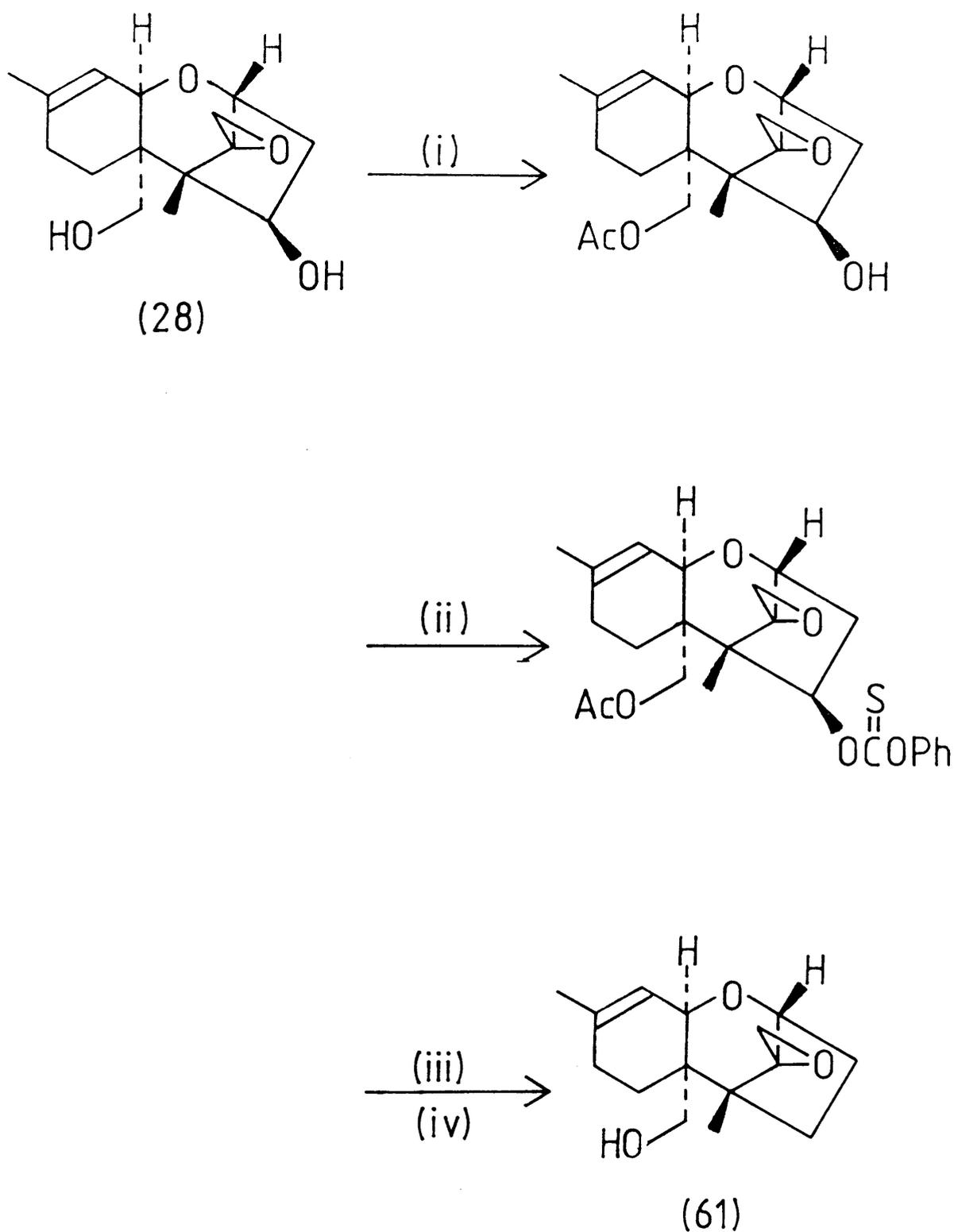
(i) Ac$_2$O, py., CH$_2$Cl$_2$; (ii) PhOC̈Cl, DMAP, CH$_2$Cl$_2$;

(iii) Bu$_3$SnH, AIBN; (iv) K$_2$CO$_3$, MeOH.

SCHEME 11
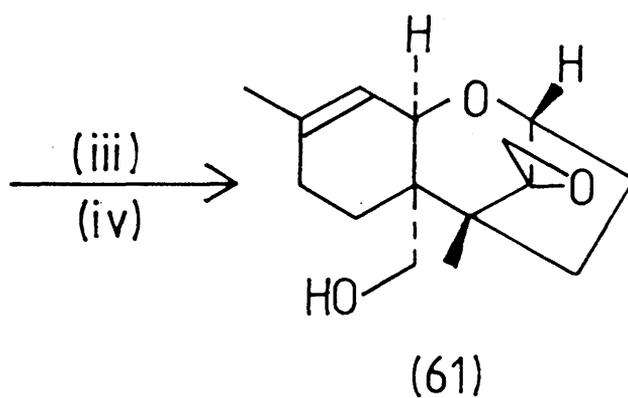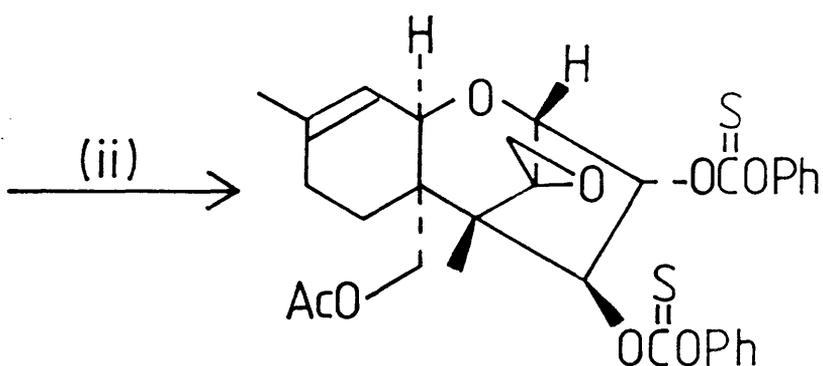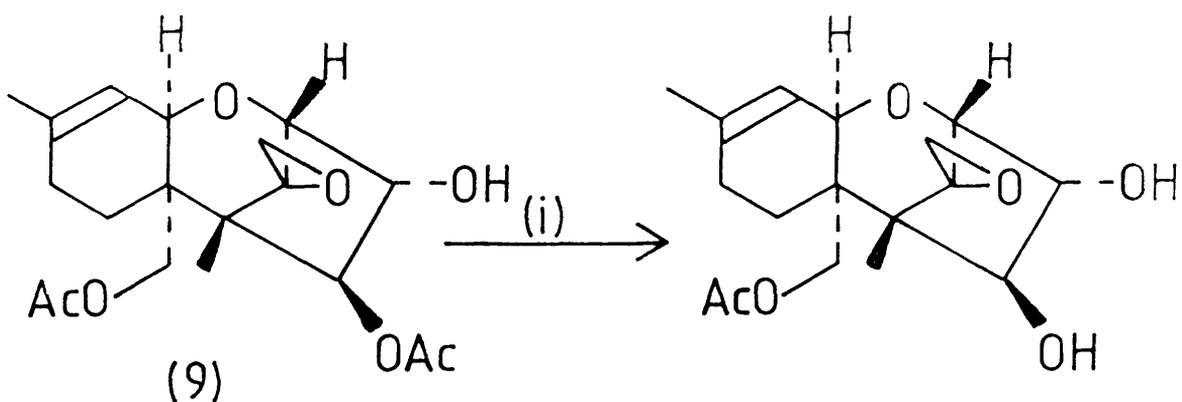
(i)NH$_4$OH,MeOH,H$_2$O;(ii)PhOCCl,DMAP,CH$_2$Cl$_2$;
(iii)Bu$_3$SnH,AIBN;(iv)K$_2$CO$_3$,MeOH.

SCHEME 12
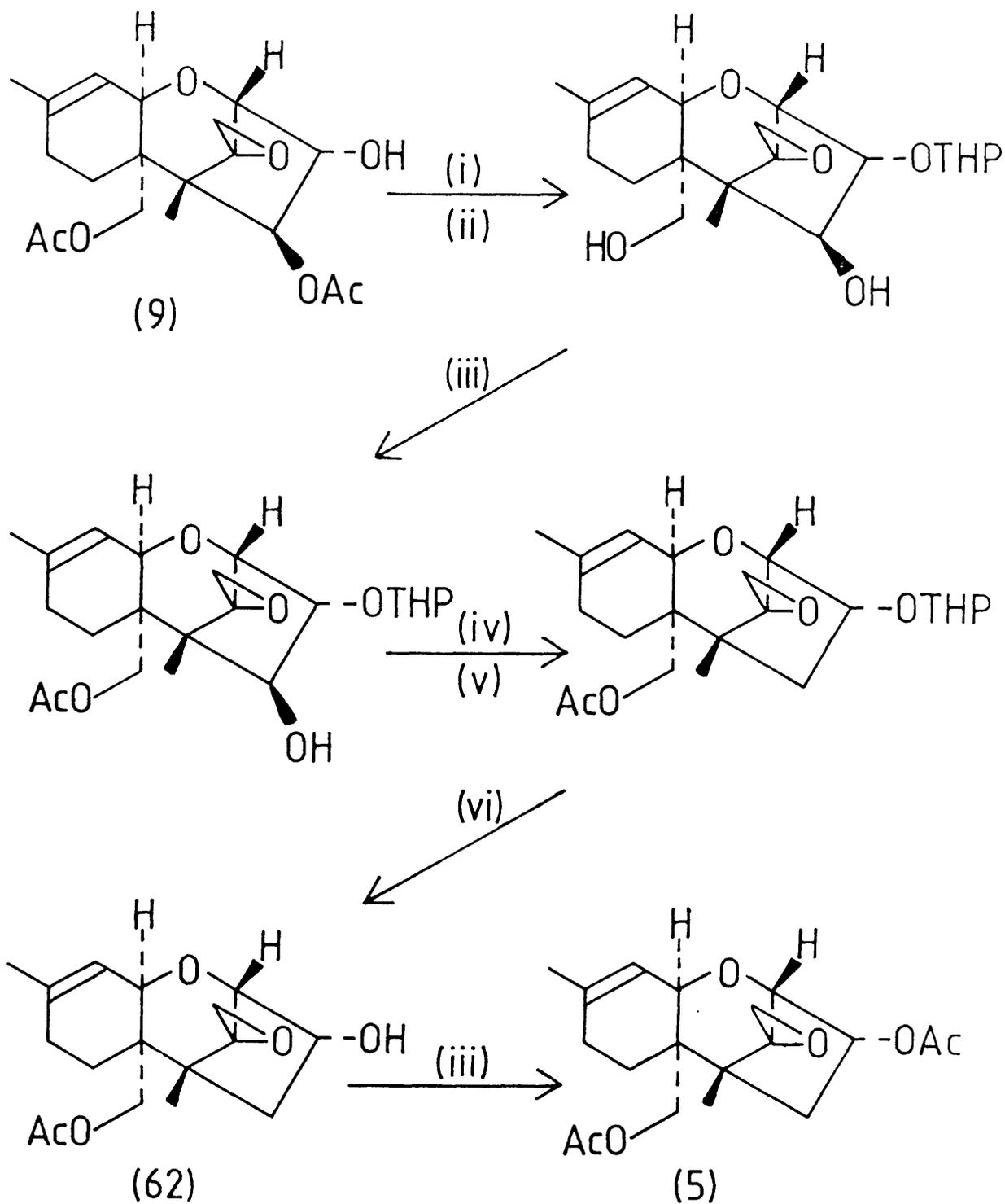
(i) DHP, PPTS, CH_2Cl_2; (ii) NaOH, MeOH;
(iii) Ac_2O, py., CH_2Cl_2; (iv) (Imid.)$_2$C=S, $C_2H_4Cl_2$;
(v) Bu_3SnH, AIBN; (vi) PPTS, MeOH.

SCHEME 13

(14)

(23)

(63) →(i)→ (64)
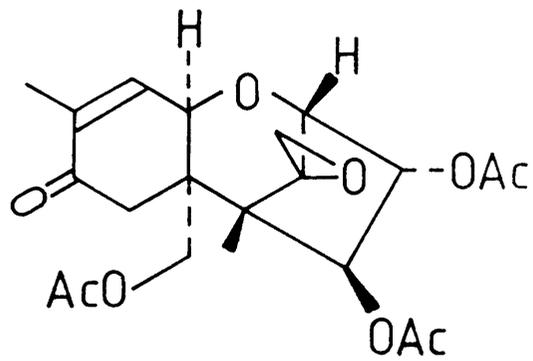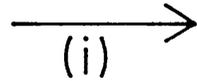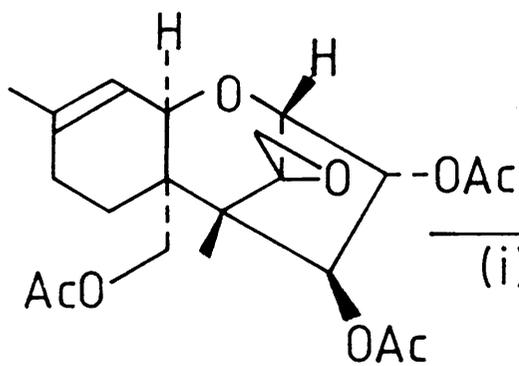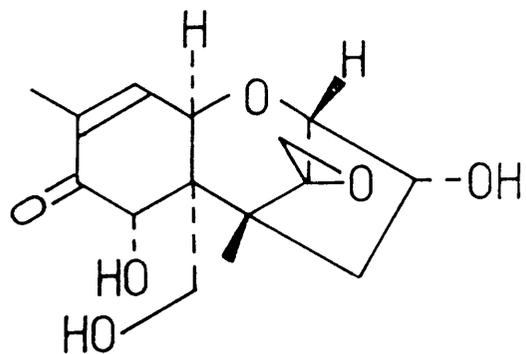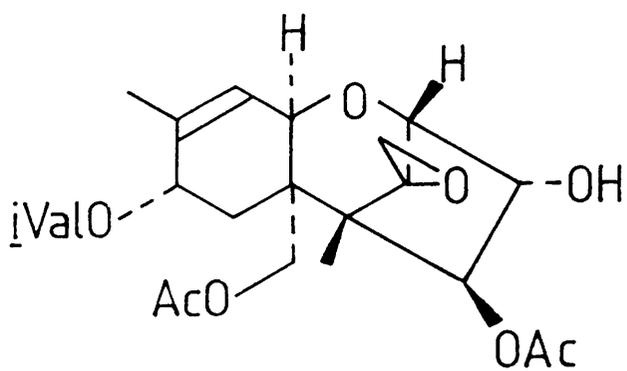
(i) <u>t</u>Butyl chromate
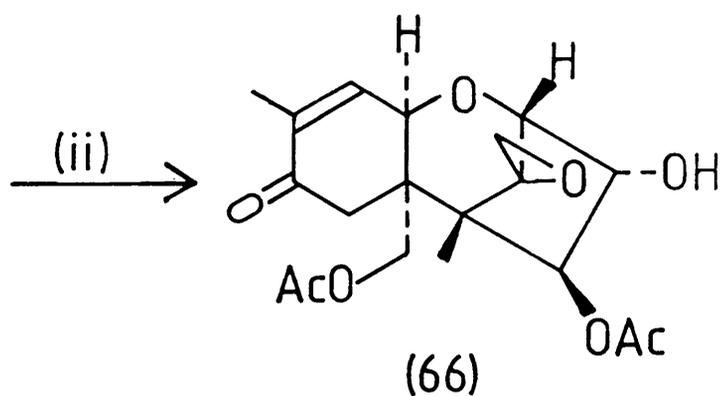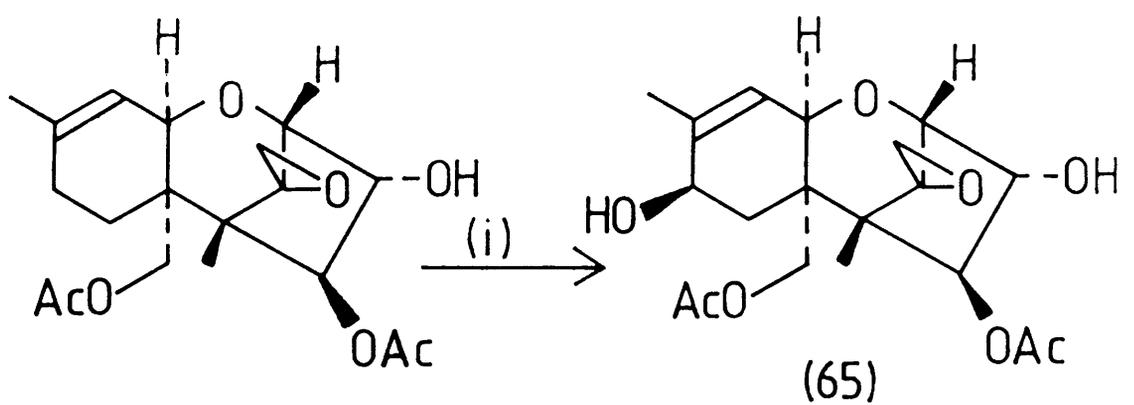
SCHEME 14
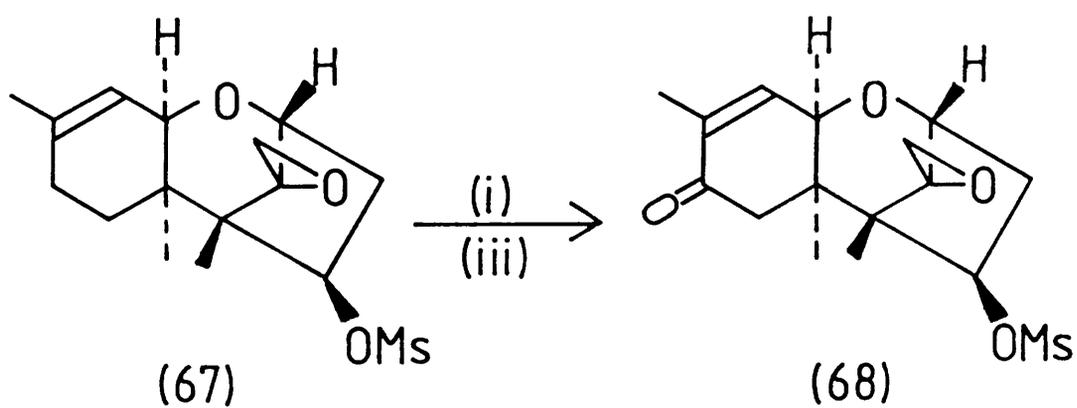
(i) SeO₂ ; (ii) PCC ; (iii) CrO₃.
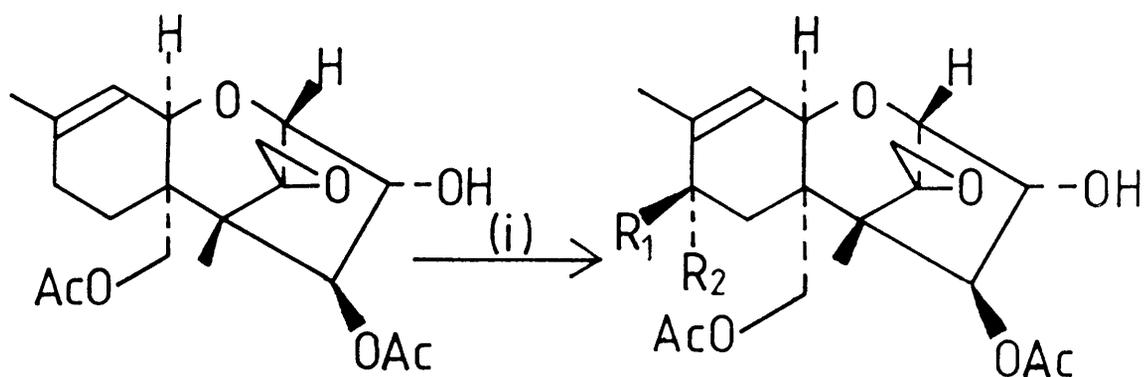
(69) $R_1=Br$ $R_2=H$

(70) $R_1=H$ $R_2=Br$
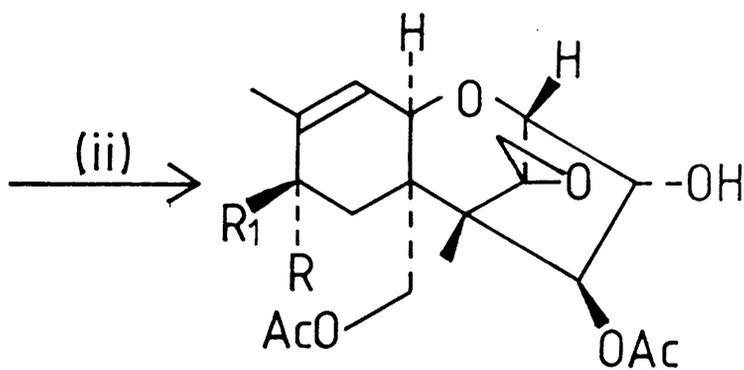
(71) $R_1=OAc$ $R_2=H$

(72) $R_1=H$ $R_2=OAc$

(i) NBS, CH_2Cl_2; (ii) AgOAc, AcOH, $\triangle$.

Synthesis of 8-keto- α -substituted compounds are rare; indeed, there has been to date only one such synthesis reported. Grove[38] treated enone (64) with lead tetra-acetate to give nivalenol tetra-acetate (73) in very poor yield. The tetra-acetate (73) has been reduced back to (44) by Tatsuno et al[39] using zinc.

Another pair of redox reactions have been carried out on nivalenol diacetate (27)[38]. Reduction of the enone using sodium borohydride gave the all α-triol (17). The enone could be reformed in good yield by MnO_2 selective oxidation.

(64)   (i) / (ii)   (73)

(27)   (iii) / (iv)   (17)
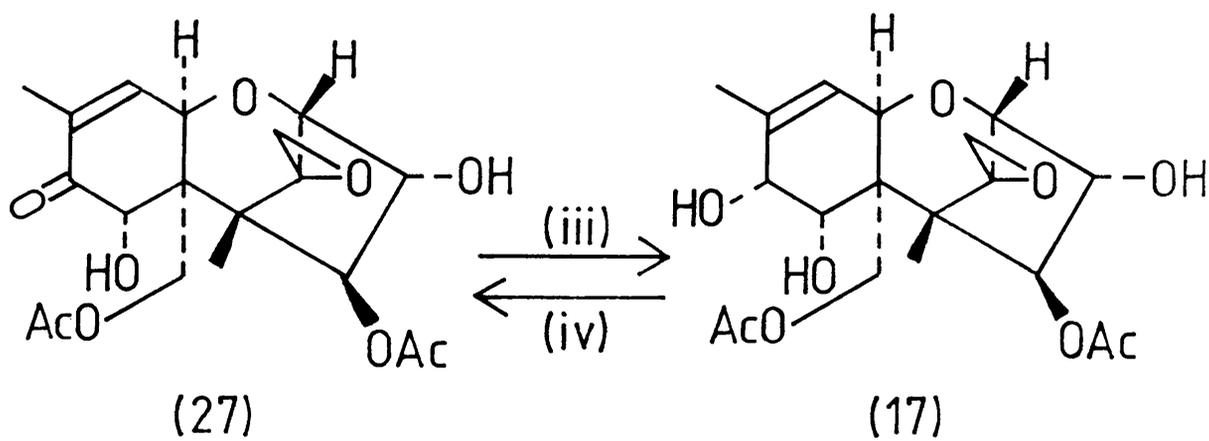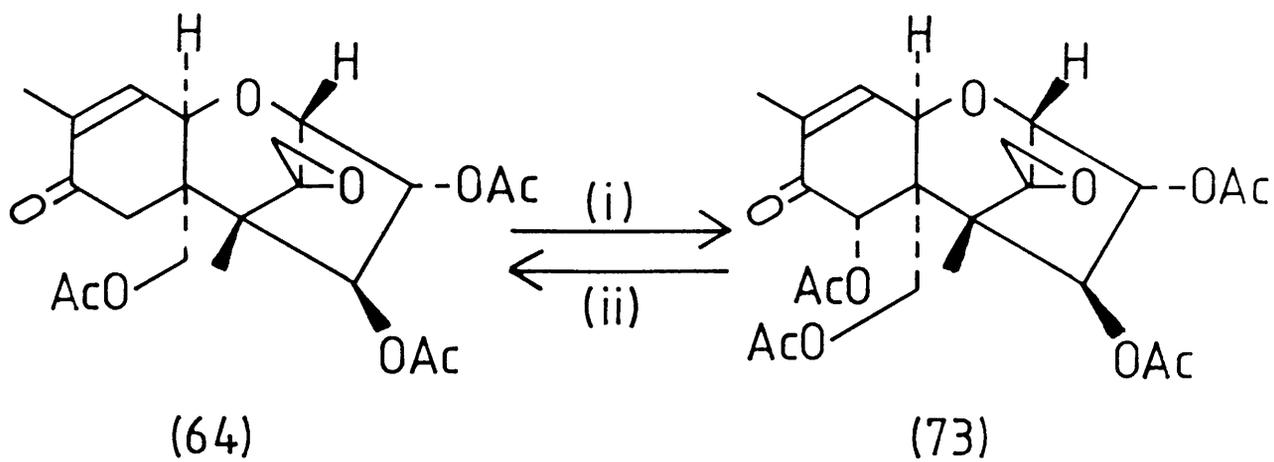
(i) Pb(OAc)$_4$; (ii) Zn.

(iii) NaBH$_4$; (iv) MnO$_2$.

4. TOTAL SYNTHESIS

Due to the complexity of the trichothecene nucleus, total synthesis of these compounds presents a formidable challenge. If successfully achieved, total synthesis would additionally provide a means of obtaining compounds isotopically labelled at specific centres for detailed metabolic studies. With the exception of Roush's recent introduction of a label at C-13 of anguidine[40], such labelled compounds have been only otherwise available by feeding labelled precursors to growing fungi[41].

The disadvantages of total synthesis are that overall yields are low and, with one notable exception, the synthetic targets are obtained in racemic form. In addition, a general route for the provision of a variety of polyoxygenated trichothecenes has yet to be developed.

Although not within the brief of this project, it is important to note various landmarks in the total synthesis of these mycotoxins.

Trichodermin (3) was the first trichothecene to succumb to total synthesis[42]. The key reaction of Colvin et al in forming the basic tricyclic skeleton was intended to be an aldol reaction of keto-aldehyde (74). However, this cyclisation refused to proceed under a wide variety of conditions. This problem was circumvented by oxidation of (74) followed by treatment with acetic anhydride to give the enol lactone (75). C-ring formation was accomplished by reaction of (75) with lithium hydridotri-t-butoxyaluminate which gave (76) in 10% yield. Relatively straightforward chemistry then led to completion of the synthesis of trichothermin (3).

In view of the low yield for the critical cyclisation step in Colvin's synthesis, Still[43] developed an improved synthesis of trichodermol (2) — the hydrolysis product of trichodermin — via a

(74)   (i), (ii)   (75)

(iii)

(76)

(3)
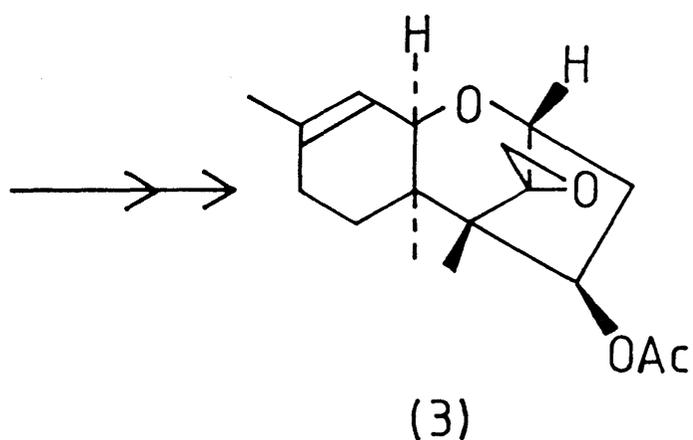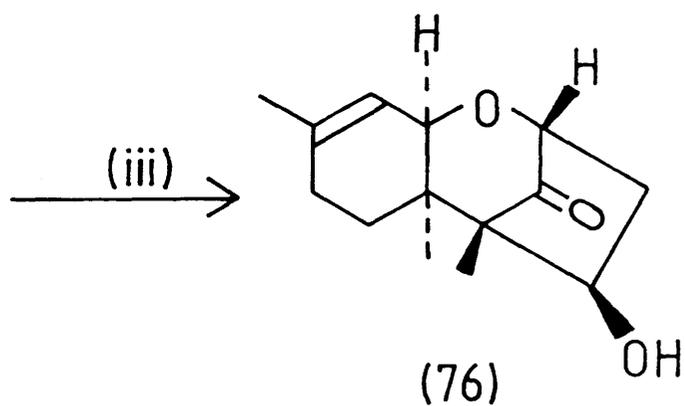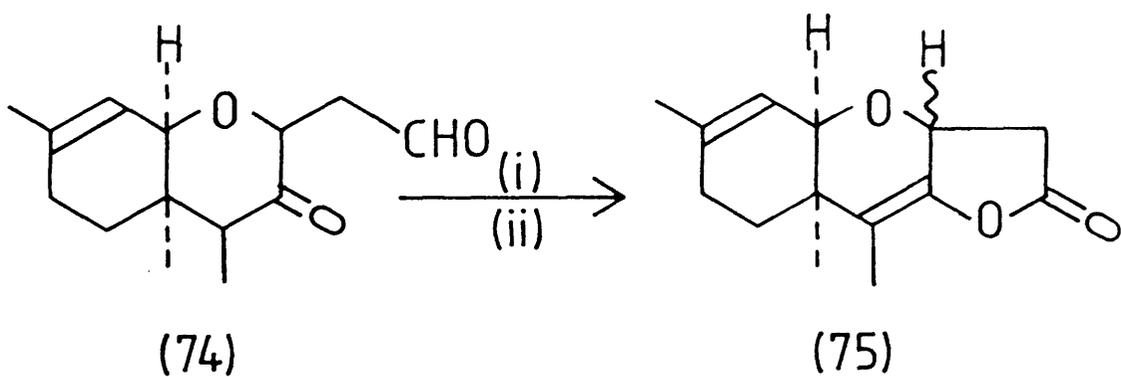
(i) CrO₃, py., H₂O; (ii) Ac₂O, NaOAc;
(iii) LiAlH(OBuᵗ)₃, Et₂O.

(77)   (i)   (78)

(2)
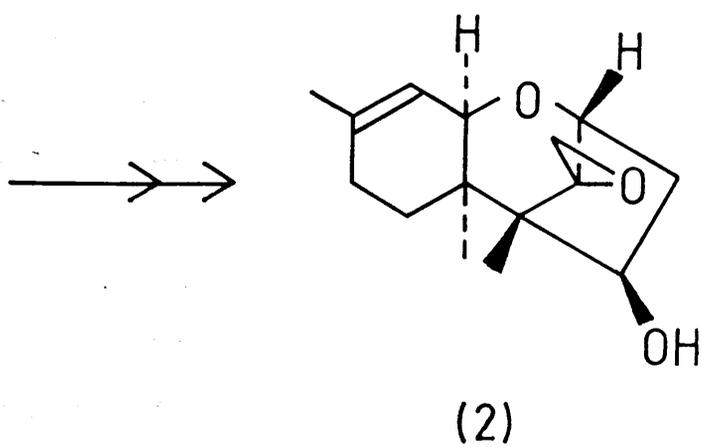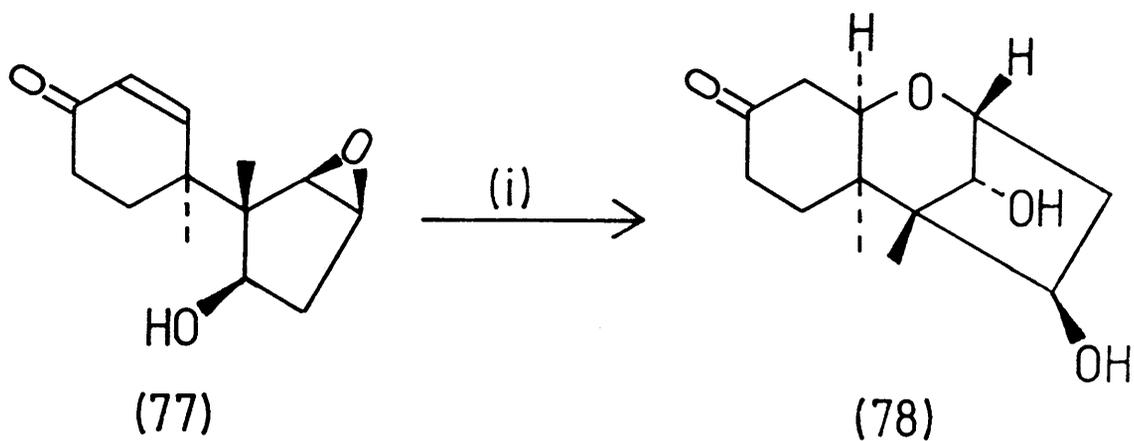
(i) H_2SO_4 (aq.)

biomimetically patterned approach where the key bond-forming reaction was between C-11 and what would become the pyran oxygen. This was accomplished by acid treatment of epoxide (77) to give an intermediate glycol which was trapped by the electrophilic enone double bond to give the tricycle (78).

Due to the importance of macrocylic trichothecenes, the synthesis of the parent alcohol, verrucarol (28), has received much attention.

First to complete the total synthesis of verrucarol was Schlessinger[44] who created the tricylic skeleton by an intra-molecular S_N' reaction on triol (79). On treatment with pTSA (79) underwent cyclisation to the diene (80).

Roush's synthesis of verrucarol[45] also had the C-11-pyran oxygen bond-forming reaction as its cornerstone. Tricycle (82) was formed by trapping of the carbonium ion formed on treatment of the allylic alcohol (81) with PPTS, followed by hydrochloric acid in acetone.

Trost[46] employed a ring expansion strategy in his synthesis of verrucarol. The α-bromohemiacetal (83) was induced to rearrange using tetrabutylammonium bromide, thus establishing the trichothecene skeleton (84) at an early stage of the synthesis. Indeed the only major problem that remained to be overcome was inversion of the stereochemistry at C-4.

Intramolecular aldol cyclisation has been used for the C-2-C-3 bond formation of calonectrin (5) by Kraus[47] and of 12,13-epoxy-trichothec-9-ene (1) by Fujimoto et al[48].

Kraus treated the keto-aldehyde (85) with sodium methoxide in refluxing methanol to give a mixture of C-3 epimeric alcohols (86). The correct stereochemistry was established in a subsequent step by oxidation of the alcohol to the ketone followed by stereo-selective reduction with sodium borohydride.

(79)

(80)

(28)
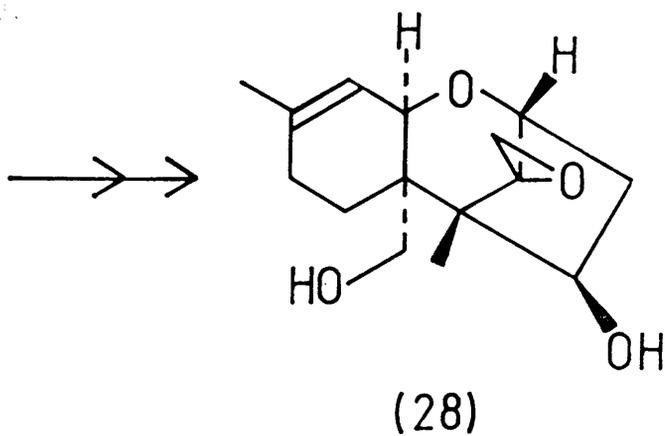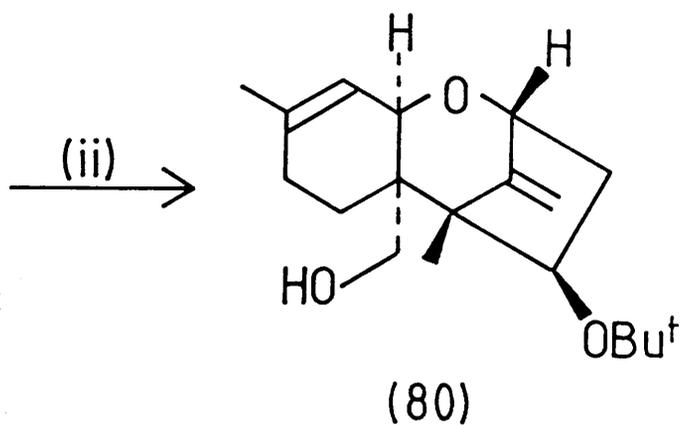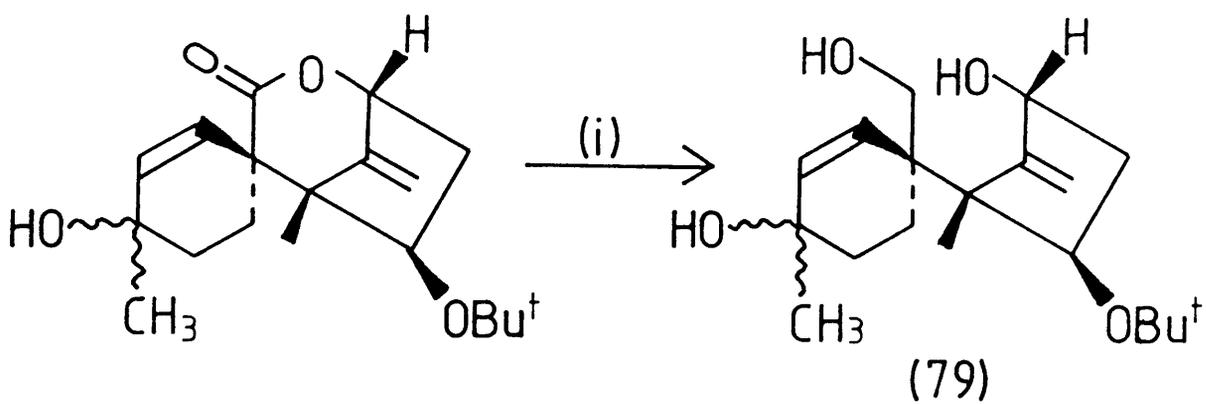
(i) LiAlH₄, DME; (ii) TsOH, CH₂Cl₂.

(81)

(i)
(ii)
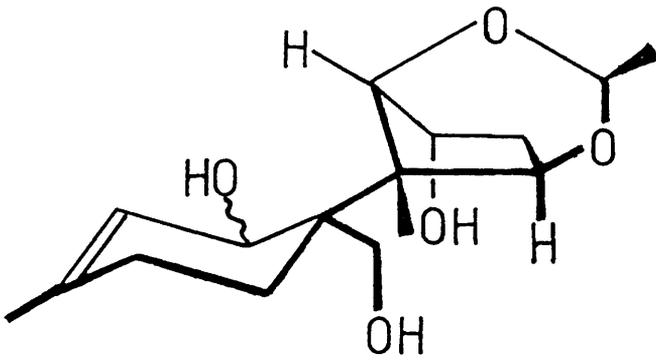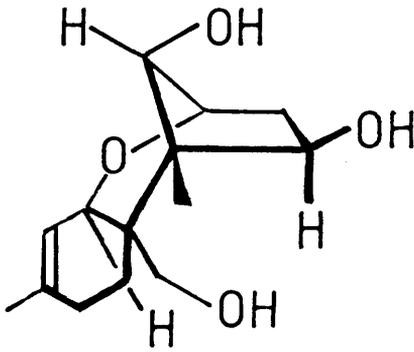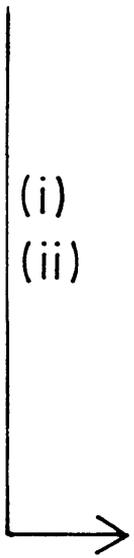
(82)

=
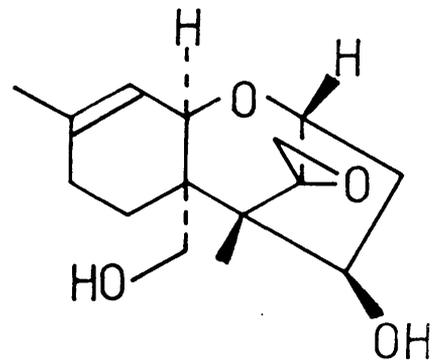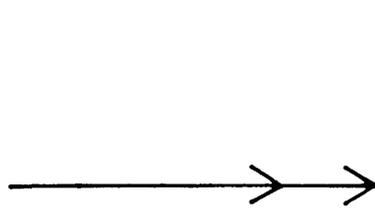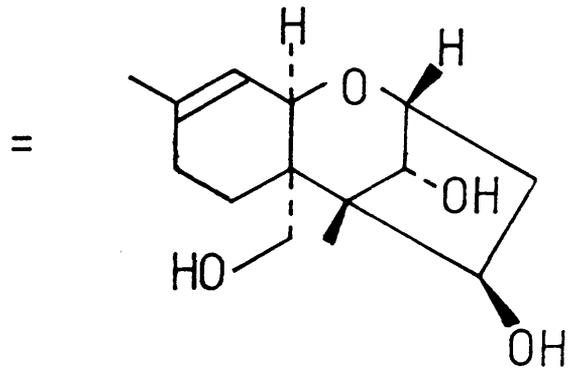
(28)

(i) PPTS, aq. C₆H₆ ; (ii) 1N HCl, acetone.

(83)   (i)   (84)

(28)

(i) Bu₄NBr, THF.

(85) → (i) → (86)

(5)
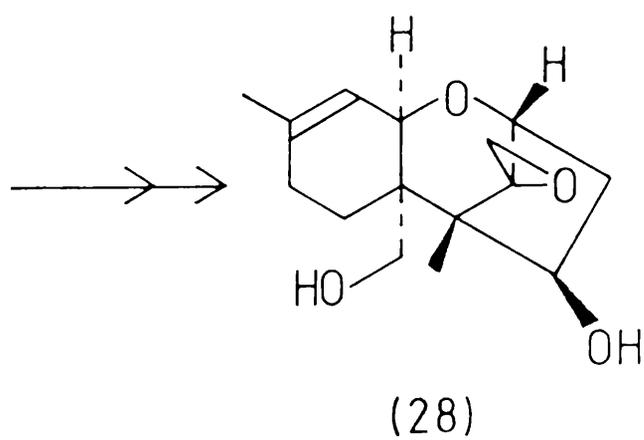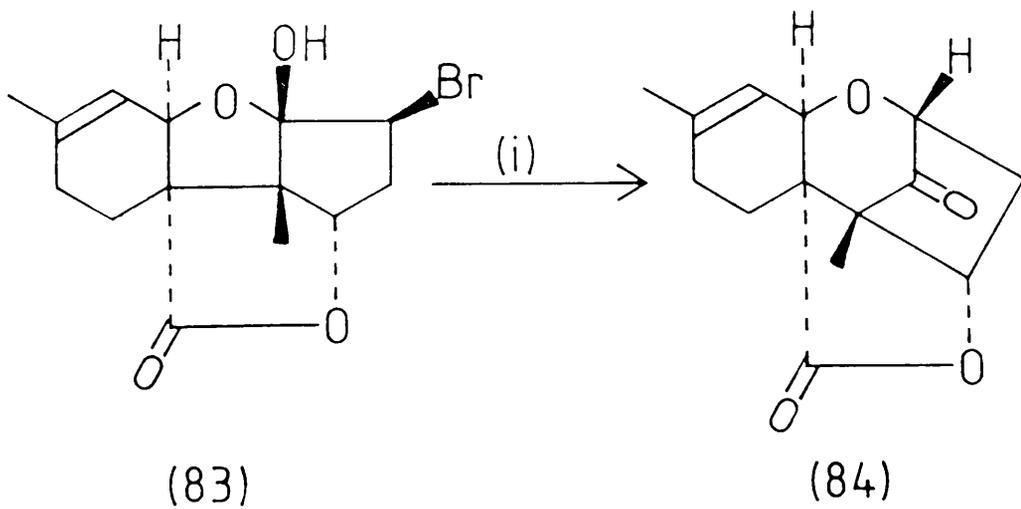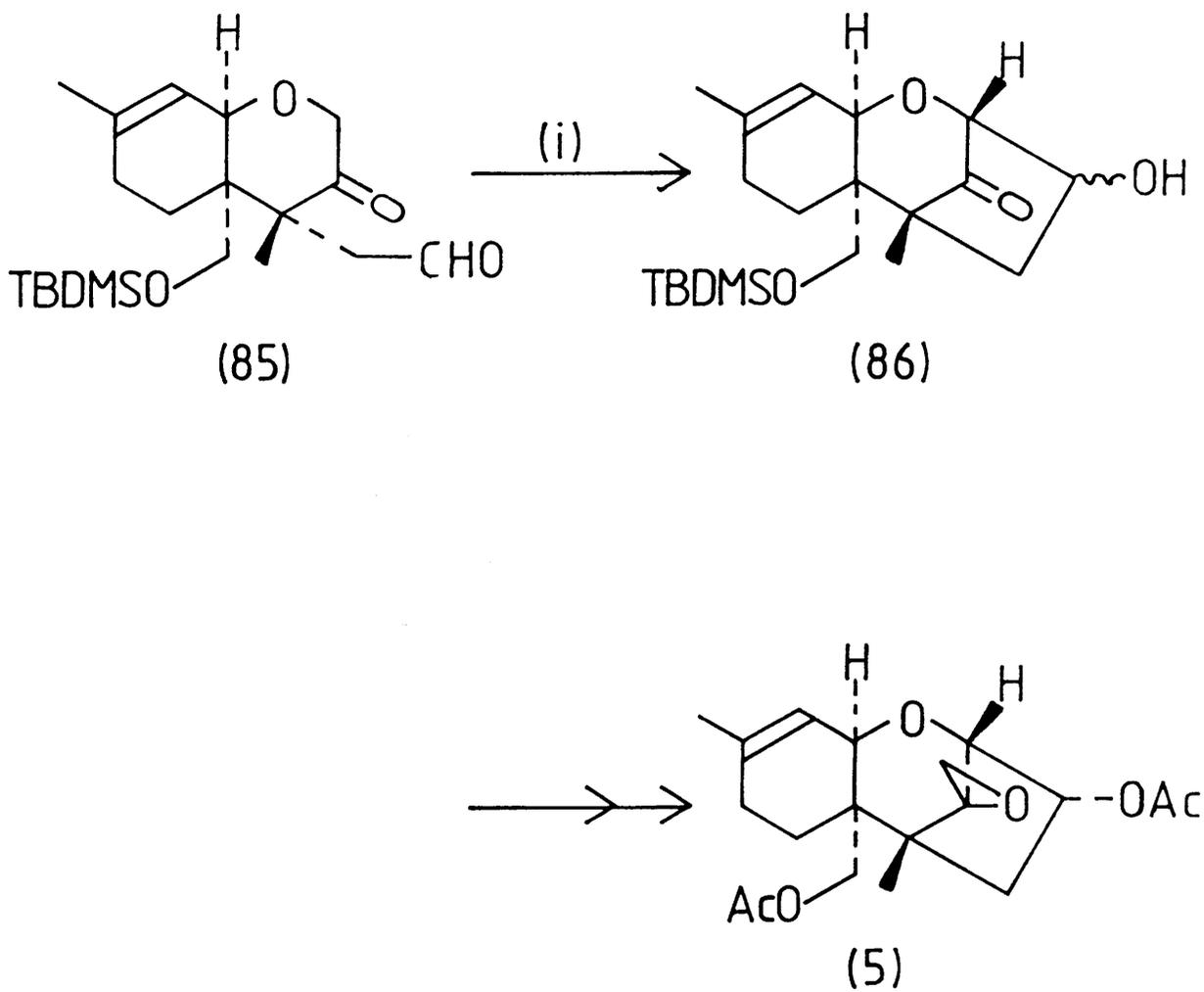
(i) NaOMe, MeOH, △.

Fujimoto treated the keto-aldehyde monohydrate (87) with sodium methoxide to give the tricycle (88). The C-3 alcohol was removed by conversion into the iodide followed by hydrogenolysis with Raney nickel.

A second synthesis of 12,13-epoxytrichothec-9-ene was achieved by Kamikawa et al using a biomimetic approach[49], the basic trichothecene skeleton being obtained upon treatment of the allylic alcohol (89) with acid to give the dione (90).

The only other total synthesis of a trichothecene to date has been that of anguidine (9) by Brooks[50]. This synthesis is unique in two respects; firstly it is the only synthesis of a trioxygenated member of the family to have been achieved and secondly, and perhaps more importantly, it is the only enantioselective total synthesis of a trichothecene. This enantioselectivity was built around the C-ring fragment (92), which was produced by stereoselective reduction of the homomorphic dione (91) using bakers' yeast. Further elaboration led to the cyclisation precursor (93), ring closure of which was effected under acid catalysis to give (94).

(87)   (i)   (88)

(1)

(i) NaOMe, MeOH, △.

(89)   (i) → (90)

(ii) → (1)
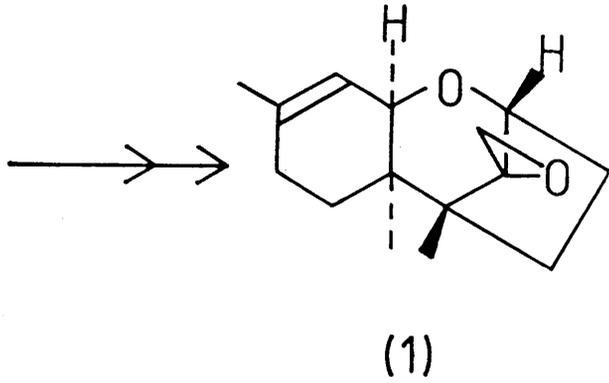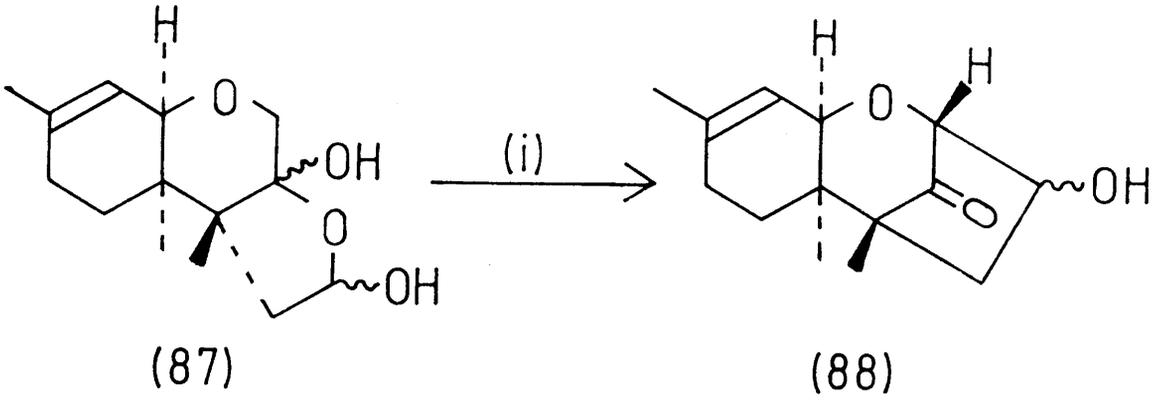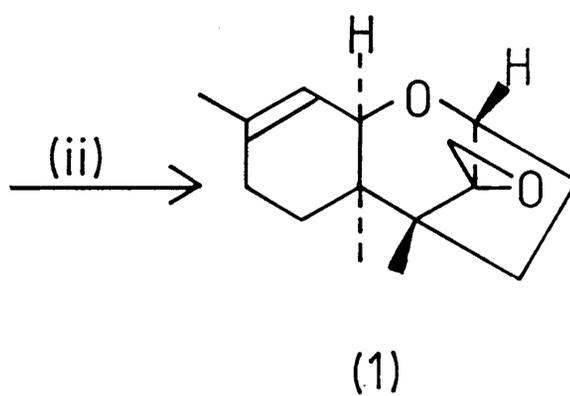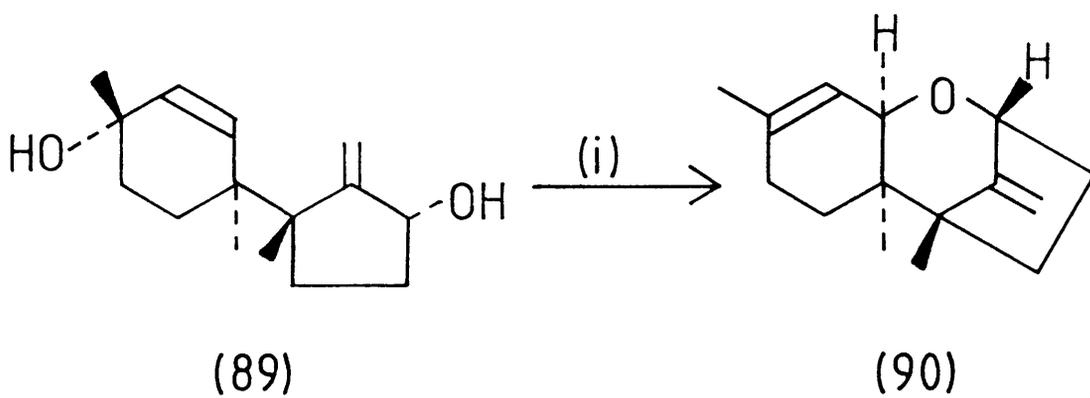
(i) H⁺ ; (ii) mcpba, Na₂HPO₄.

(91)   (i)   (92)

(93)   (ii) (iii)   (94)

(9)
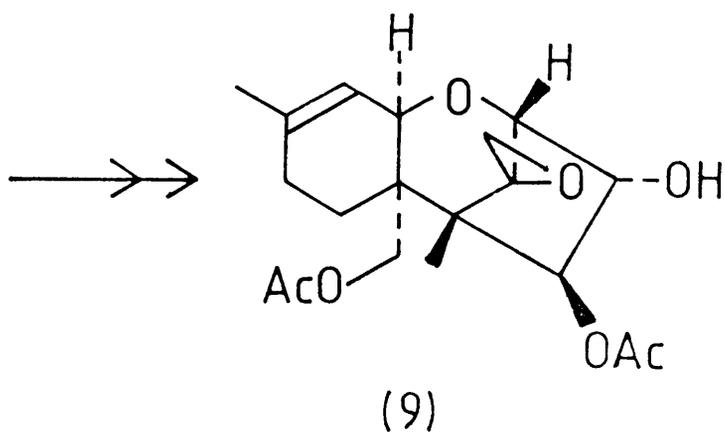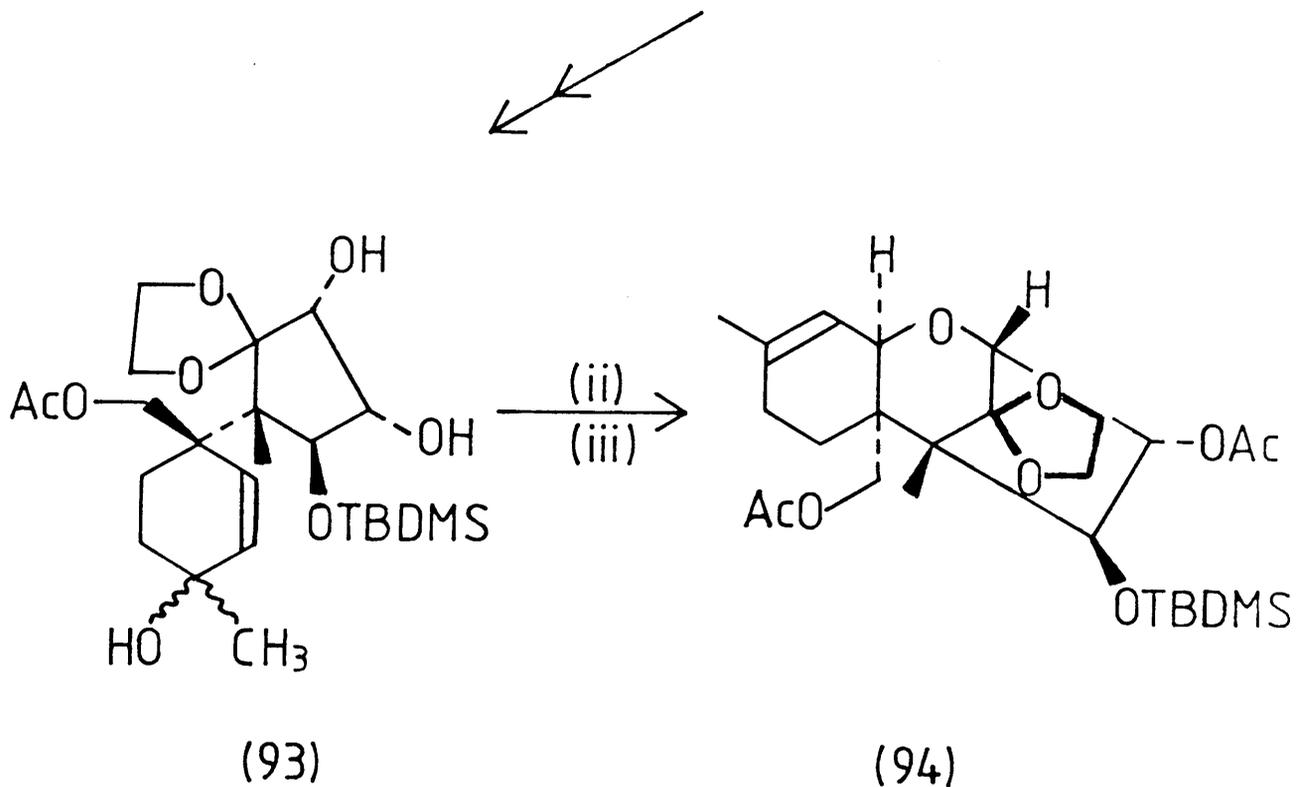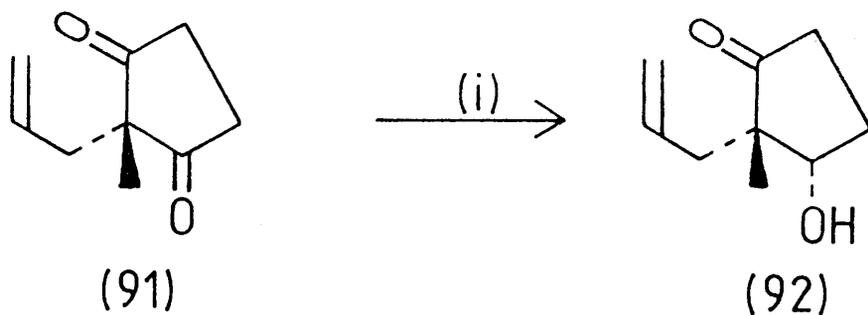
(i)Bakers' yeast;(ii)TsOH,CH₂Cl₂;(iii)Ac₂O,py.

5. BIOSYNTHESIS

The basic trichothecene nucleus contains fifteen carbon atoms, suggesting that it is sesquiterpenoid in nature. This was recognised by the earliest workers in this field; Fishman et al[51] and Jones and Lowe[52] observed that three molecules of [2-^{14}C]-mevalonate were incorporated into trichothecin (22), concluding that a double 1,2-methyl shift had occurred.

Since both groups were using the incorrect apotrichothecene structure in their analysis, a reinterpretation of their findings was carried out by Godtfredsen and Vangedal[17]. They concluded that labelling had occurred at C-4, C-10 and C-14 in trichothecolone (21).

The presence of a label at C-10, however, was later disproven by Achilladelis et al[53,54]. (4R)-[4-^{3}H,2-^{14}C]-Mevalonic acid was fed to Trichoderma sporulosum, when two of the three tritium labels were found to be retained in the isolated trichodermol (2) -- one at C-10, the other at C-2. These results were confirmed by Machida and Nozoe[55] who also selectively isolated C-8 of trichodermol (2) as ^{14}CO$_2$. Both groups' findings support the hypothesis that C-8 and C-10 of the trichothecenes originate from C-2 and C-4 of mevalonate respectively.

Further evidence has also been provided by the work of Achini et al[56], Müller et al[57] and Evans et al[58]. That the trichothecenes were derived from farnesyl pyrophosphate was shown by Hanson and Archiladellis[59]. A 1.5% incorporation of [1-^{14}C] farnesyl pyrophosphate was observed in trichothecin (22) isolated from Trichothecium roseum.

Three possible folding mechanisms have been postulated namely, concerted cyclisation of (95) or of (96), or stepwise cyclisation via a γ-bisabolene derivative (97).

The intermediacy of a γ-bisabolene derivative was disproven by feeding [2-^{3}H,2-^{14}C] geranyl pyrophosphate to Trichothecium roseum[54,50].
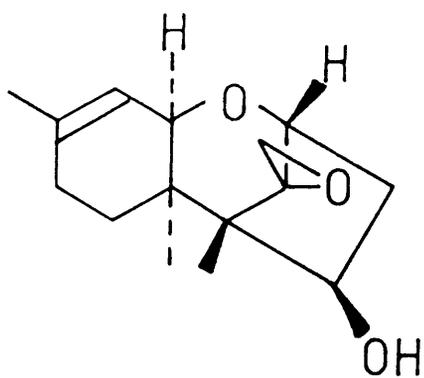
(2)
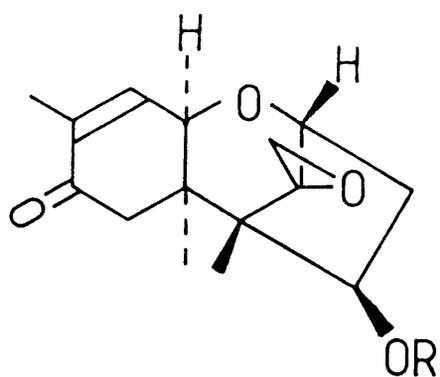
(21) R=H
(22) R=Crotonyl
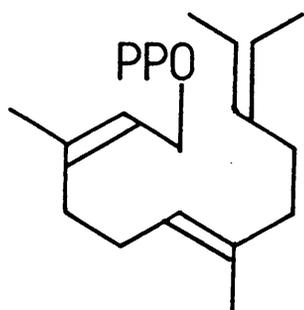
(95)
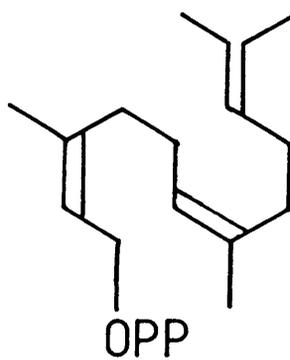
(96)
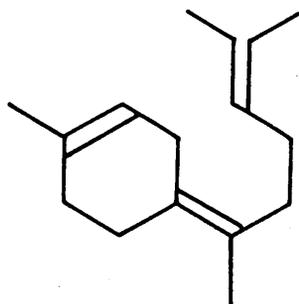
(97)

The culture products trichothecin (22) and trichodermol (2) were found to contain the tritium label at C-2. It can be concluded from this experiment that:-

(i)   the central $[$ 4-^{3}H$]$ mevalonic acid proton is retained;

(ii)   this proton is involved in a hydride shift; and

(iii)   there is no γ-bisabolene intermediate since its formation would necessitate the loss of the isotopic label.

A stepwise cyclisation can be further ruled out by the observation that labelled α-bisabolol, β- and γ-bisabolenes and monocyclofarnesol are incorporated non-specifically and in low yield[61].

Given that the biosynthesis of trichothecenes apparently involves concerted cyclisation of farnesyl pyrophosphate followed by subsequent 1,2-methyl and hydride shifts, the mode of folding has yet to be determined. Feeding $[1-^3\text{H}_2, 2-^{14}\text{C}]$ - and $[2-^3\text{H}, 2-^{14}\text{C}]$ - farnesyl pyrophosphate resulted in the loss of one tritium in the former case and retention in the latter in the isolated trichothecin (22)[53,54]. This indicates a β-folding pattern as in (95) - α-folding should result in no loss of tritium in either case.

The culmination of these labelling studies was the proposed biosynthetic mechanism outlined in SCHEME 15[54,60]. The postulated intermediate trichodiene (98) was isolated from a strain of Trichothecium roseum[62,63] along with trichodiol (99) and 12,13-epoxy-trichothec-9-ene (1). Labelled trichodiene was found to be incorporated into trichothecolone (21) and trichothecene (22)[55,63].

Whether epoxidation of the 12,13-double bond occurs before or after formation of the basic tricycle has yet to be established, since it is conceivable that trichodiol (99) could result from further metabolism of 12,13-epoxy-trichothec-9-ene[55]. The true intermediate

(95)

(98)

(100)

(99)

(101)

(1)
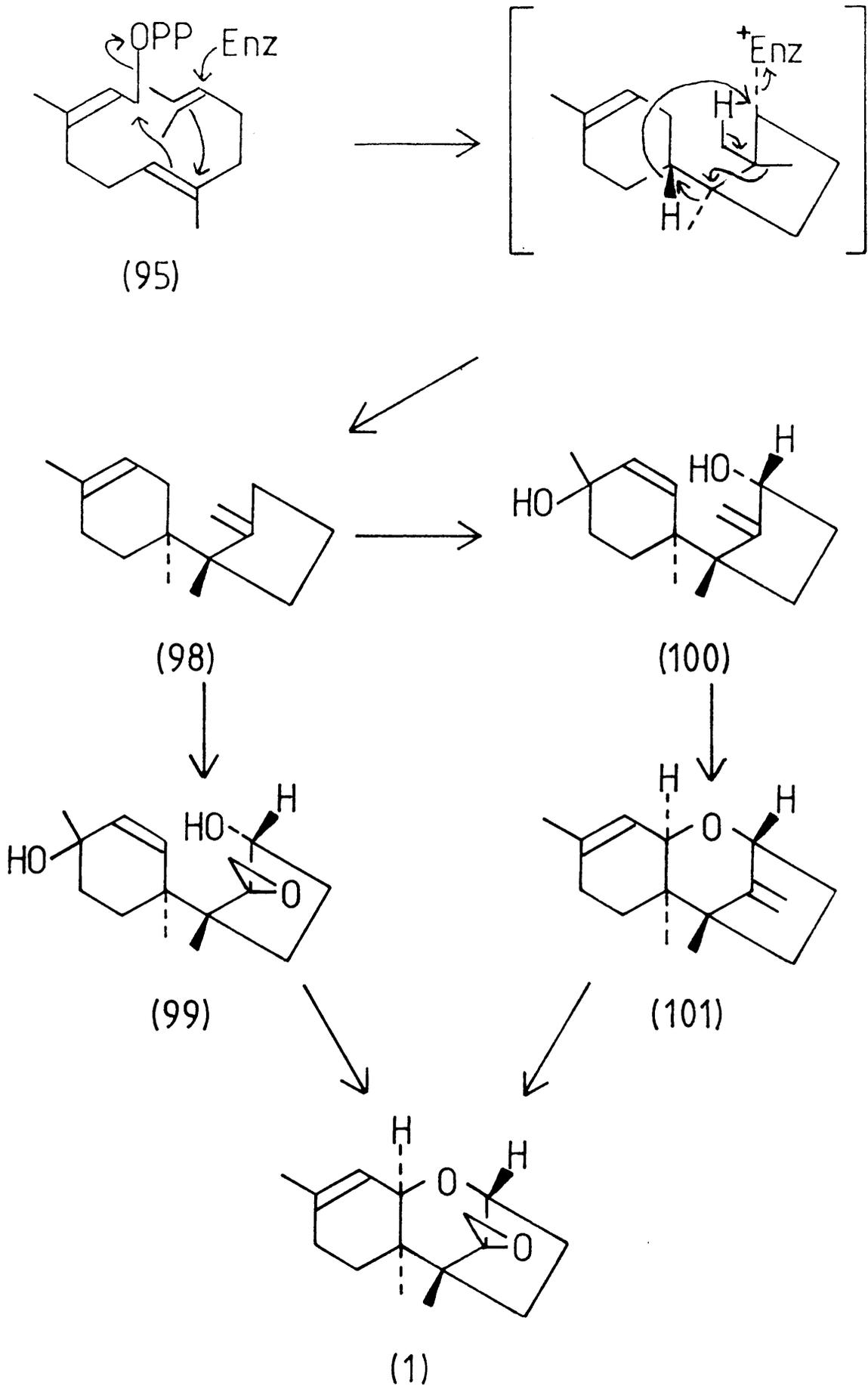
SCHEME 15

could be diol (100) which then cyclises to give (101). Evidence for

this pathway has been provided by the isolation of verrucarin K (102)

by Breitenstein and Tamm in 1977[64]. Even although the cyclisation

step apparently requires 2-cis-farnesyl pyrophosphate, all trans-

farnesol could conceivably act as a precursor, by trans-cis isomerisation.

Overton and Roberts[65], and Suzuki and Marumo[66] working on non-

trichothecene producing culture systems have shown that such

isomerations can occur through a farnesal intermediate. However, Evans

and Hanson[67] found that, although all trans-farnesyl pyrophosphate did

act as a precursor, farnesol itself was not metabolised in a cell-free

culture of Trichothecium roseum. Additionally, they were unable to

detect any radiolabelled farnesal.

An alternative mechanism leading to trichodiene via a cyclopropene

as outlined in SCHEME 16 was suggested by Evans and Hanson[67].

One further noteworthy point concerning cyclisation is that the

two starter methyls of farnesyl pyrophosphate are non-equivalent since

it has been shown by labelling that only the methyl derived from C-2

of mevalonic acid is involved in the migratory process[52,54].

With the basic trichothecene nucleus in place, peripheral

oxygenation occurs. The mechanism of this oxygenation along with more

in-depth consideration of trichothecene biosynthesis are to be found

in Reference 68.

SCHEME 16

(102)
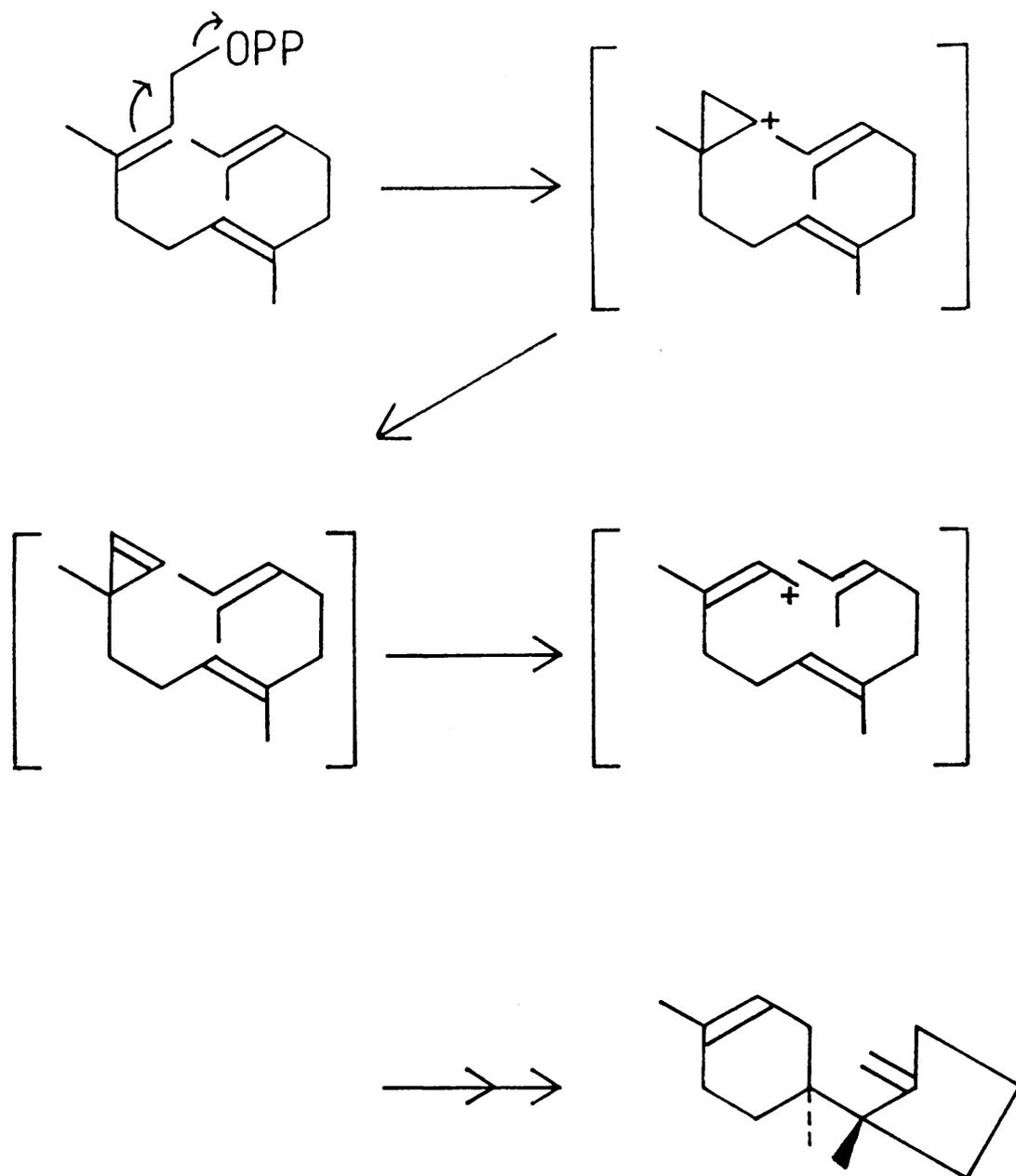
6. STRUCTURE AND BIOLOGICAL ACTIVITY

The trichothecenes have a broad spectrum of biological activity ranging from skin rashes through to serious blood disorders. At a molecular level they act by inhibition of protein synthesis, or less commonly, DNA synthesis. Ueno et al[69] showed that this inhibition was a reversible process since washing the infected cells restored their activity.

The trichothecenes can be sub-divided on the basis of where protein synthesis is inhibited, i.e. on whether they show T-,E- or I-type activity[70]. This classification is very general and there is a large degree of overlap.

T-Type trichothecenes inhibit the termination of protein synthesis and are typically unsubstituted or have a small substituent, e.g. a C-4 alcohol.

Compounds with a C-4 ester are generally E-type - that is they inhibit protein elongation. Trichodermin (3) and trichothecolone (21) are members of this class.

Compounds which inhibit protein initiation are termed I-types. Such compounds tend to have C-15 esters along with C-3 or C-4 esters, Claridge et al[71] tested all of the acetates of scirpenetriol for biological activity and showed that the 15-acetoxydiol (103) to be the most toxic monoacetate.

This classification would appear to depend on the effective size of the trichothecene. Smaller trichothecenes are T-type, intermediate ones are E-type, whereas the larger ones are I-types.

I-types also include T-2 toxin (14), anguidine (9) and also, as one might expect, the macrocyclic diesters.

Although the extent of peripheral substitution is important for relative toxicity, the 12,13-epoxide is vital for any biological activity. This was demonstrated by Grove and Mortimer[24], who tested the lithium alumimium hydride product (104) for toxicity, finding it
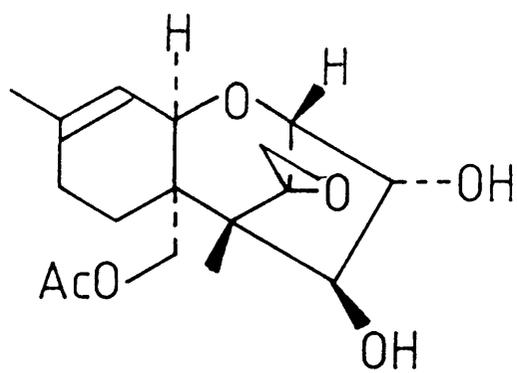
(103)
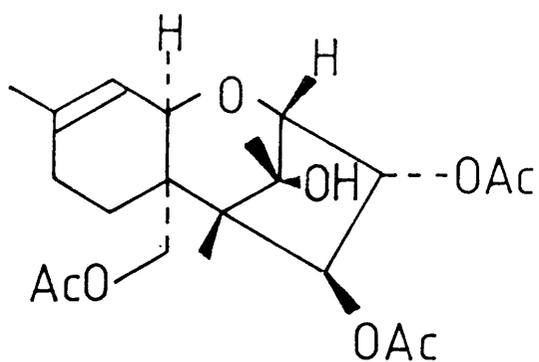
(104)
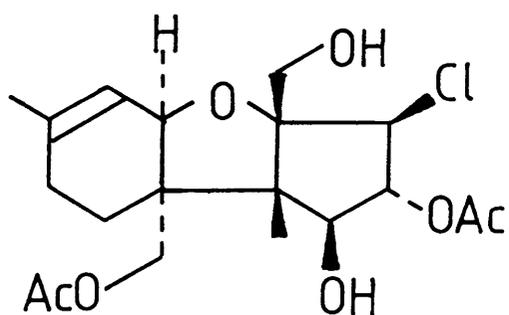
(105)
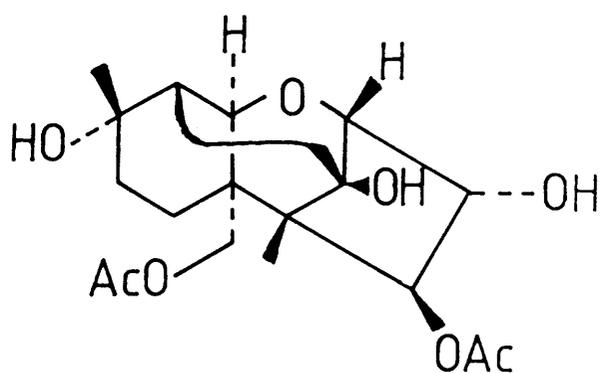
(106)
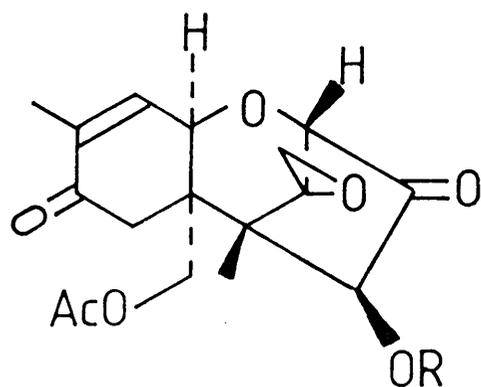
(107) R = Ac
(108) R = ClAc

to be essentially non-toxic. The rearranged apotrichothecene (105) was also shown to be inactive, as was the hydrate (106).

As the epoxide is essential for biological activity, it would seem that an additional process to reversible protein inhibition is in play since any process involving the epoxide is more likely to be irreversible. Such a process may involve ring opening _via_ nucleophilic attack through the 9,10-double bond or through an apotrichothecene intermediate. The latter is possibly more likely since the reduction of the 9,10-double bond[24] results in only a four fold reduction in toxicity suggesting that it has more of a structural role. Also by analogy with the chemical process, apotrichothecene rearrangement is likely to be more facile in biological systems. Direct ring opening by nucleophiles other than hydride does not occur readily due to shielding of the epoxide by the A ring, so this route can be discounted.

In conclusion the 12,13-epoxide and 9,10-double bond are important for biological activity. The more oxygenated compounds tend to be more toxic and esterification, especially at C-15, influences toxicity. In an extension to structure activity relationships, Kaneko _et al_[29] tested a variety of compounds with unnatural oxygenation patterns and ester functions. They found that the diones (107) and (108) were most active in certain tumours.

DISCUSSION

7. TRICHOTHECENE PRECURSORS FROM CULTURE

The starting materials for most of the trichothecene manipulations carried out in this study were obtained from a shake culture of a Fusarium species. Two trichothecenes were obtained, namely, 3α-hydroxy-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (anguidine) (9) and 3α,15-dihydroxy-4β-acetoxy-12,13-epoxytrichothec-9-ene (monoacetoxyscirpenediol, MAS) (48). The yields of these metabolites were high, typically in the range of 250-300 mg/l of each after an 11 day culture time. A shorter culture period of 7 days resulted in more anguidine being isolated, with a corresponding drop in the amount of MAS.

The provision of both anguidine and MAS from culture means that various mono-ols and diols of scirpene can be obtained in a maximum of three steps from one or other metabolite (Table 2). This minimises the amount of functional manipulation that would otherwise be required if only one trichothecene was obtained from culture. For example, Roush[30] has developed two methods to convert anguidine into MAS (SCHEME 7 and 8),* the higher yielding of which requires six steps.

Other trichothecenes used in this study were 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one (deoxynivalenol, DON or, eponymously, vomitoxin) (23) and 3α-acetoxy-7α,15-dihydroxy-12,13-epoxytrichothec-9-en-8-one (3-AcDON) (24), which were generally gifted to us by Dr. J. Gilbert (M.A.F.F., Norwich)

*See Introduction (3.3.1)
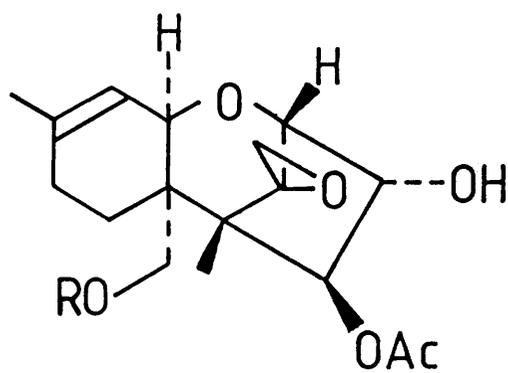
(9)　R=Ac
(48) R=H
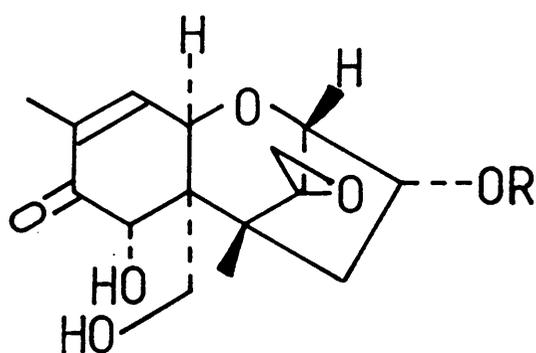
(23) R=H
(24) R=Ac

Starting Material	Desired Mono-ol	Desired Diol	No. of Steps
Anguidine	C-3	-	0
MAS	C-4	-	2
MAS	C-15	-	3
MAS	-	C-3,C-4	2
MAS	-	C-3,C-15	0
Anguidine	-	C-4,C-15	2

TABLE 2

8.   EPOXIDE   DEOXYGENATION

8.1   INTRODUCTION

Trichothecene transformation products such as (104) and (105)

which involve the destruction of the epoxide have been shown to be

devoid of significant biological activity[24] . Limited studies on

deoxynivalenol (23) with rats in vivo[72] and rumen micro-organisms

in vitro[73] have additionally shown that the major biological

transformation, and probably detoxification process, is one of

deoxygenation of the epoxide to the corresponding exomethylene (148).

Since such a biological process is very rare in animals it has been

proposed that the deoxygenation process in rats is actually due to

rumen micro-organisms present in the gut of the rat[74]. When rats

that had been previously fed with antibiotics were then fed with

deoxynivalenol no such transformation was observed.

The first chemical deoxygenation of an epoxytrichothecene was

carried out by Gutzwiller et al[21]. Treatment of verrucarol (28) with

lithium aluminium hydride and then heating the tertiary alcohol

obtained in refluxing acetic anhydride gave diene (149) in a low

overall yield. Such a method could not, of course, be applied to

more complex trichothecenes, such as those which possess keto or

ester functionalities.

Efficient chemical deoxygenation of the epoxide requires a non-

nucleophilic and non-acidic strategy. The epoxide is sterically

hindered to nucleophiles (other than hydride) by the cis-fused

A-ring as can be seen from FIGURE 150. Also rearrangements of the

trichothecenes occur with facility in acidic media to give the

biologically inactive apotrichothecenes (3.1).

Roush[75] attempted nucleophilic ring opening under a wide range

of conditions which included use of KSeCN in methanol, ethanol and

dimethyl sulphoxide, $KSi(CH_3)_3$ in HMPA, $Fe(CO)_6$ in tetramethylurea,

(104)

(105)

(23)

(148)

(28)

(149)

(i) LiAlH$_4$; (ii) Ac$_2$O, △.

(150)

(151)   (i)   (152)

SCHEME 17

(153)
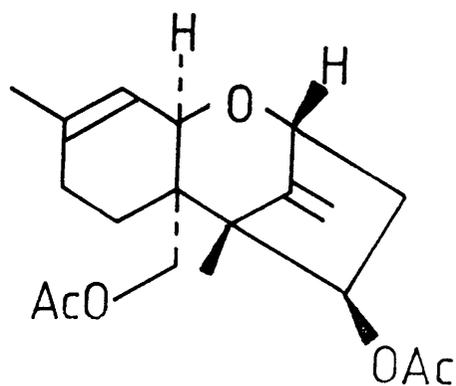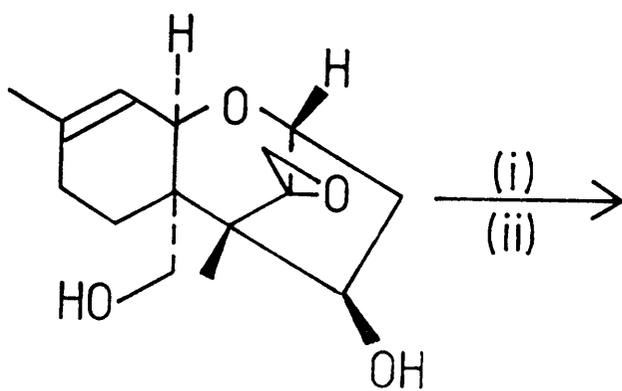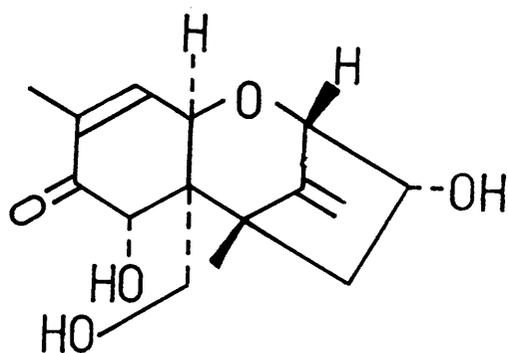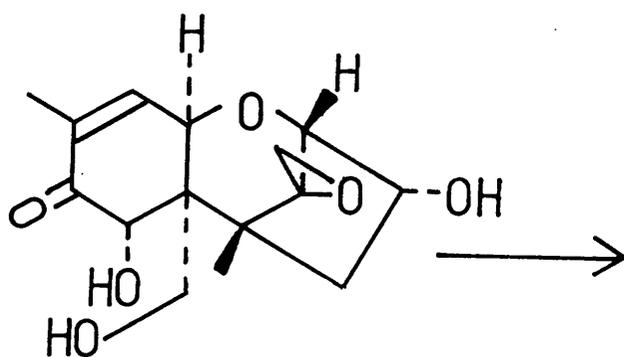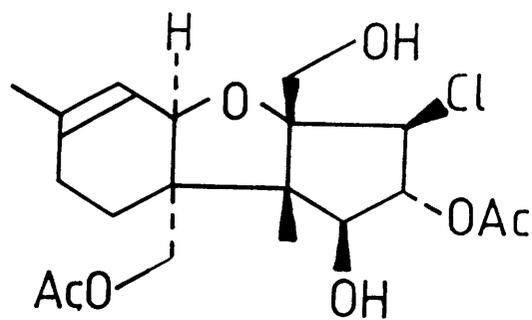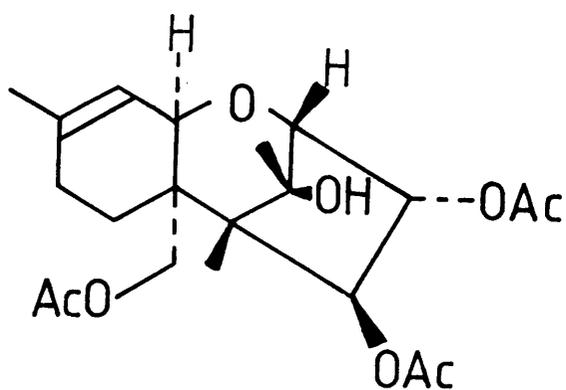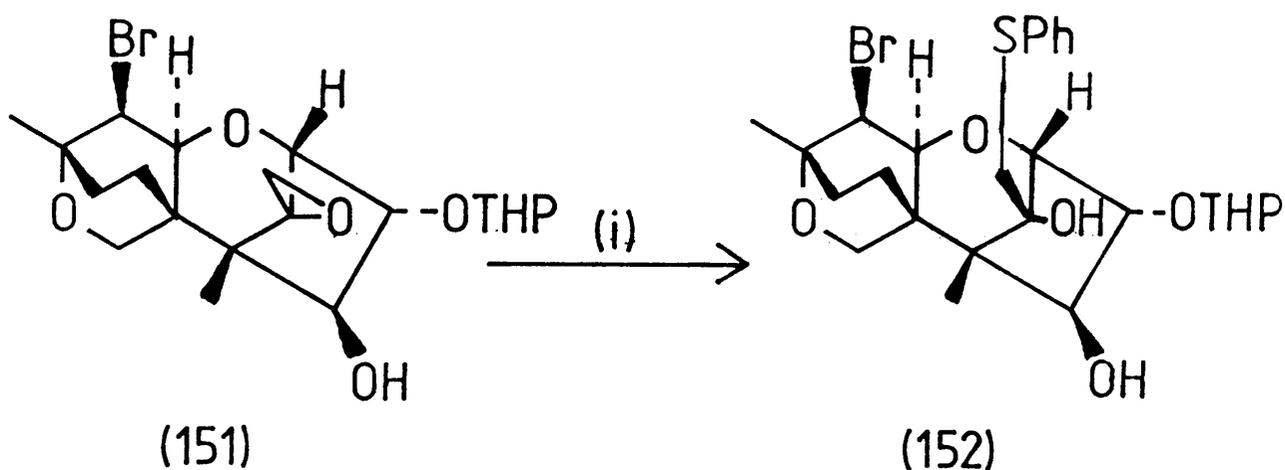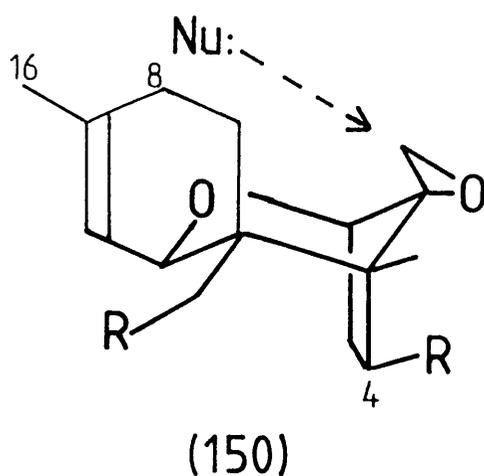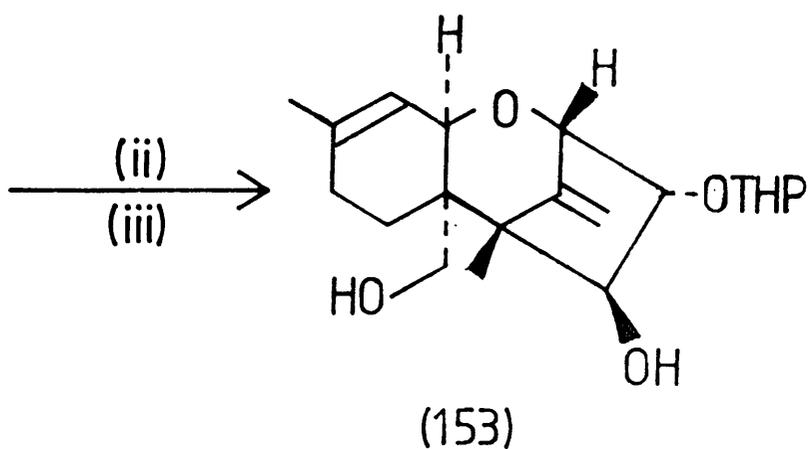
(i) NaSPh, EtOH; (ii) mcpba, CH$_2$Cl$_2$; (iii) Na(Hg), MeOH.

and diethyl phosphorotelluorate, before achieving success with a 40-50 fold excess of sodium benzenethiolate in refluxing ethanol. Deoxygenation of the epoxide moiety in the bromo-ether (151) was accomplished by oxidation of the ring-opened product (152) to the sulphone using m-chloroperbenzoic acid and then reductive treatment of this hydroxysulphone with sodium amalgam to give the alkene (153) in an overall yield of 50% (SCHEME 17). Such an excess of thiolate would be incompatible with ester or enone functions present in more complex trichothecenes: its use is therefore limited to suitably protected substrates.

Deoxygenation via reduction of halohydrins derived from treatment of the epoxide function with mineral acids has been attempted but as expected the sole or major product is that of rearrangement. Treatment of deoxynivalenol triacetate (120) with HBr in acetic acid followed by zinc reduction of the so produced bromohydrin gave the desired diene (121) in only 9% yield, along with 22% of the apotrichothecene (154)[76]. Use of the same protocol by Roush on triacetoxyscirpene (63) gave solely the product of rearrangement[77].

8.2 TRICHOTHECENE RING B/C MODEL CONSTRUCTION

At the outset of this study there was therefore scope for the development of an efficient, mild epoxide deoxygenation method which did not involve the use of nucleophiles nor of acidic media. Rather than use valuable culture products for initial studies, a model compound for the B/C ring system was developed - this being both C-6 acetate epimers of the bicyclo [3,2,1] octanol (117).

The starting point for the synthesis was 2-allyl-2-methyl-cyclohexanone (110) which was made by treatment of the thermodynamic silyl enol ether (109) of 2-methyl-cyclohexanone with methyl lithium followed by allyl bromide in THF. The second ring was established by

(120)

(i)|(ii)

(121)   +   (154)

(63)   ?   (i)
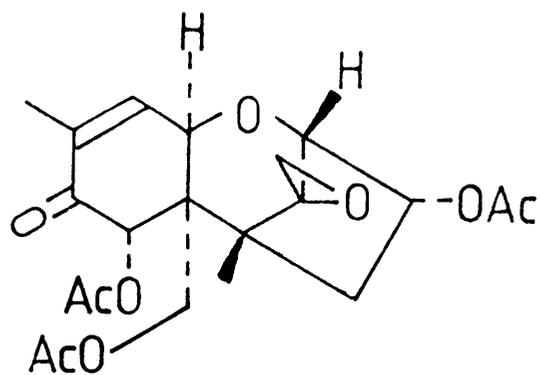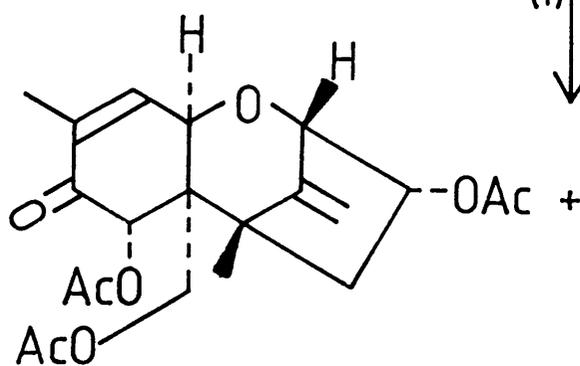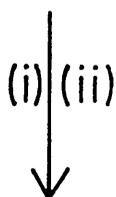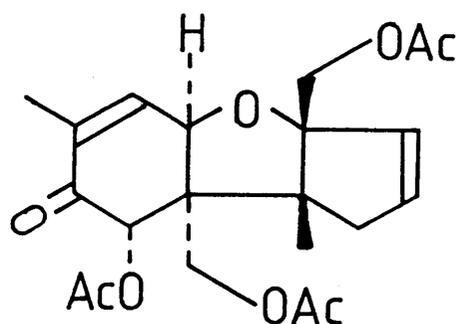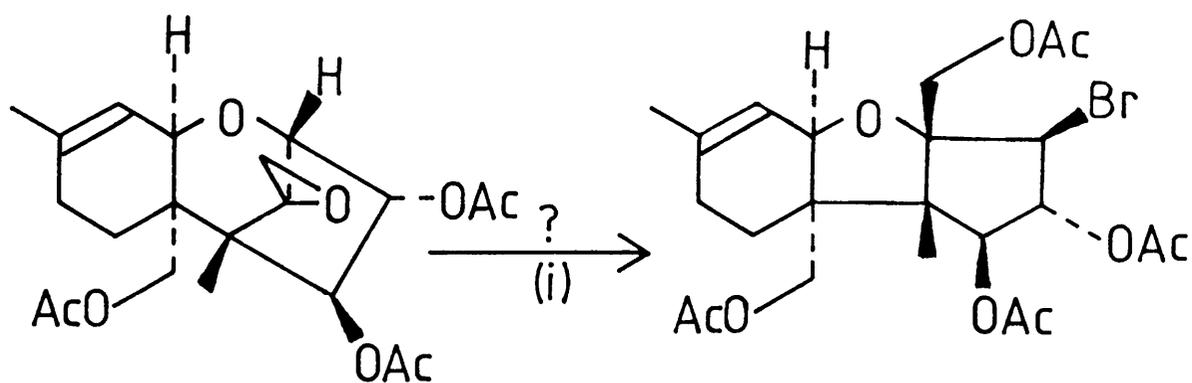
(i) HBr, AcOH; (ii) Zn.

(117a) R₁=H, R₂=OAc → (117a) $R_1=H, R_2=OAc$
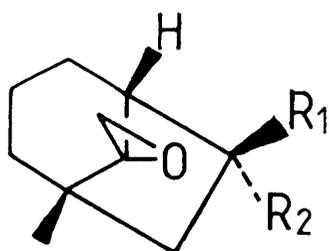
(117a) $R_1=H, R_2=OAc$
(117b) $R_2=H, R_1=OAc$

(109)

(110)

(111)

(155)

(vi)

(112a)   1:2   (112b)
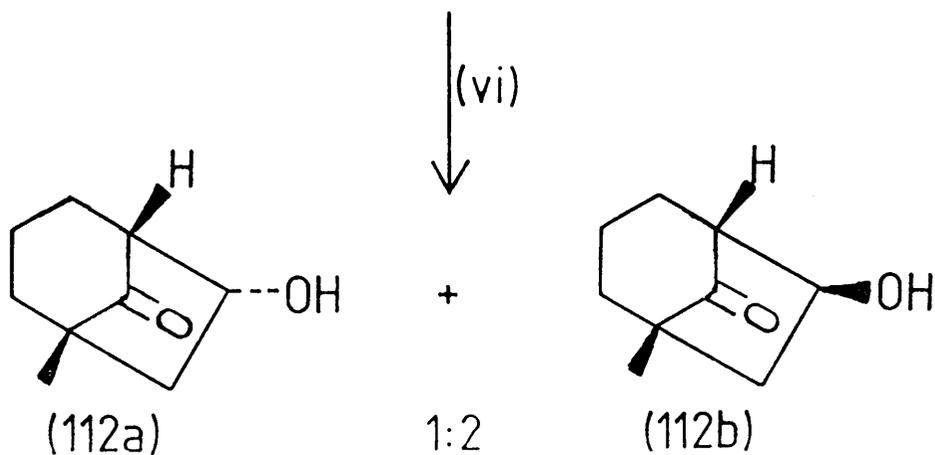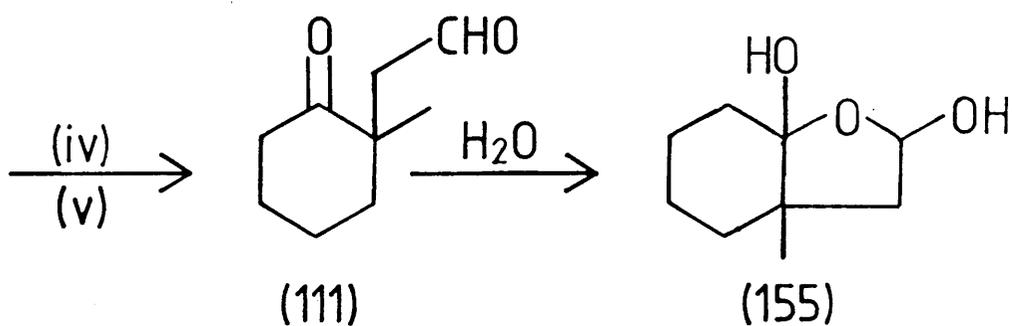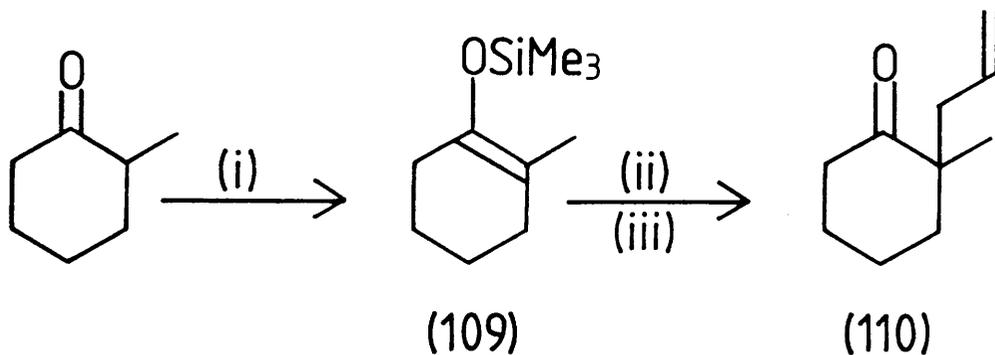
(i) TMSCl, Et₃N, DMF; (ii) MeLi; (iii) CH₂=CHCH₂Br, THF;
(iv) O₃, CH₂Cl₂; (v) Et₃N; (vi) NaOMe, MeOH.

ozonolysis of the vinyl double bond to give the keto–aldehyde (111)

followed by aldol condensation to give an epimeric mixture of

keto–alcohols (112). Reduction of the ozonide necessitated a

non–aqueous work–up since in the presence of moisture the keto–aldehyde

formed a hydrate (155). Formation of such five–membered hydrates is a

very facile process and in our hands its cleavage could not be affected

either by aldol conditions or by distillation. The ozonide could be

reduced by use of triphenylphosphine, dimethyl sulphide or trimethyl

phosphite, but these methods required distillation of the keto–aldehyde

prior to its use. Ozonide reduction using triethylamine proved most

successful: simple filtration of the reaction mixture through a short

column of silica gel and concentration provided the pure keto–aldehyde

ready for the aldol condensation.

Many aldol conditions were tried including use of trichloroacetic

acid or LDA in THF, all of which were unsuccessful[78]. Cyclisation was

ultimately achieved by the use of excess sodium methoxide in refluxing

methanol, the only satisfactory conditions found. This gave a 2:1

epimeric mixture of C–6 β– and α–alcohols. The epimers can be

distinguished on the basis of coupling patterns in their ^{1}H n.m.r.

spectra – the α–epimer gives the expected double–double–doublet at

δ4.45 whereas the β–epimer gives rise to only a double–doublet, no

coupling being observed between H–5 and H–6. From models, it can be

seen that the dihedral angle between H–5 and H–6 is close to 90° in

the β–epimer.

The C–6 epimers can also be distinguished on the basis of their

^{13}C n.m.r. spectra. Stothers and Tan[79] studied the ^{13}C n.m.r. spectra

of a number of bicyclo [3,2,1] octanols including the 6–exo (β) and

6–endo (α) alcohols. They found that the signal of the carbon bearing

the 6–exo alcohol was downfield of the 6–endo epimer: this trend is

supported by the complete series of model compounds used in the present

study (Table 3).

C-6 [13]C n.m.r. shifts of model [3,2,1]bicyclic compounds

	Ketoalcohols (112)	Ketoacetates (113)
α	δ 65.9	68.6
β	69.4	71.7

	Hydroxyolefins (114)	Acetoxyolefins (115)
α	71.3	73.6
β	73.7	76.6

	Epoxyalcohols (116)	Epoxyacetates (117)
α	71.2	73.8
β	74.1	75.2

TABLE   3

C-1, C-5, C-6 and C-8 signals in these compounds were assigned straightforwardly on the basis of expected chemical shifts and off-resonance decoupled spectra. The remaining signals, for the methylenes, appeared as triplets in the off-resonance decoupled spectra. Only one of the triplets was clean. This was assigned to C-7, an assignment confirmed by an upfield shift of the C-7 signal on acetylation, with very little change seen in the signals for the remaining methylenes. The most upfield triplet was assigned to C-3, and C-2 and C-4 were distinguished on the basis of shielding effects from the C-6 alcohol. Stothers and Tan, by comparison of the C-6 octanols and the unsubstituted bicyclooctane, found that the endo epimer had a more pronounced deshielding effect than the exo-epimer on the γ-carbon of the cyclohexane ring, i.e. C-4. The more upfield of the remaining two signals was therefore assigned to C-4.

The observation that C-2, C-3 and C-4 do not give clear triplets in the off-resonance decoupled spectra can be attributed to the fact that the protons on these carbons form a closely coupled system, with second order affects being responsible for broadening and additional splitting[80].

The keto-alcohols (112) decomposed slowly on standing, possibly by a retro-aldol process on the acidic sites on the glassware, so the alcohols were protected as acetates (113) for easy storage and also in preparation for the ensuing Wittig reaction.

Reaction of the separate keto-acetates (113) with excess methylenetriphenyl phosphorane proceeded uneventfully, with accompanying cleavage of the C-6 acetates to give the hydroxyolefins (114). Wittig reaction could also be performed on an epimeric mixture of the starting ketones, since chromatographic separation of the respective hydroxyolefins could be achieved readily. (Easy separation of the bicyclic alcohol derivatives, such as the norketone,

exomethylene and spiro-epoxide is possible. This is not the case with

the corresponding acetates, which possess very similar R_f values).

Epoxidation of the individual hydroxyolefins (114) with

m-chloroperbenzoic acid gave single epoxides as judged by t.l.c.

analysis and [1]H n.m.r. spectroscopy. The orientation of the epoxide

in both cases is that with the epoxide oxygen on the side nearer the

two-carbon bridge, as in (116). This is analogous to Colvin's

synthesis of trichodermin (3)[42], where treatment of alkene (156)

gave the epoxide with the natural trichothecene configuration due

to the directing effect of the free C-3 alcohol. This result was

also expected on the basis of molecular models, which suggested that

attack of the peracid was more likely to occur from the side of the

sterically less hindering two-carbon bridge.

The bicyclic epoxide with the opposite, "unnatural" configuration

(118) was prepared by the action of dimethylsulphonium methylide[81] on

the keto-acetate (113b): the epoxyalcohol obtained was used for

comparison with the "natural" epoxide.

It was thought that the "natural" epoxyalcohol might possess

intramolecular hydrogen bonding from the hydroxyl to the epoxide

oxygen. However i.r. spectroscopic dilution studies did not support

this view.

The best evidence that m-chloroperbenzoic acid epoxidation of

the olefin gives the "natural" epoxide configuration came from [1]H n.m.r.

spectroscopy. The epoxide methylene protons in the "natural" epoxide

occur as a broad singlet at $\delta 2.75$, suggesting that they are in similar

chemical environments. This is in contrast to the "unnatural" epoxide

protons, which are observed as an AB quartet at $\delta 2.9$.

Further evidence came from lanthanide shift studies[82] on both

epoxides. From Table 4 it can be seen that the greatest change in

shifts was observed in the "natural" epoxide. For example, shift

values for H-6 in the natural epoxide ranged from $\delta 4.1$ through

(112a) ⌐ R=H
(113a) ←⌐(i)
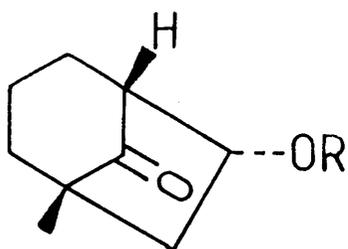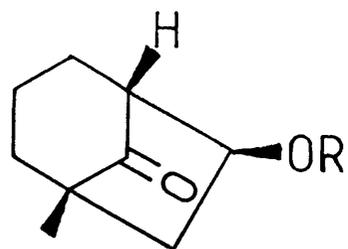
(i)⌐ (112b)
      (113b)

(114a)

(114b)

(116a) ⌐ R=H
(117a) ←⌐(i)   R=Ac
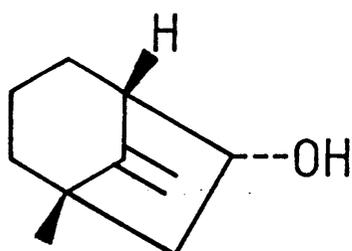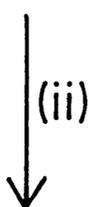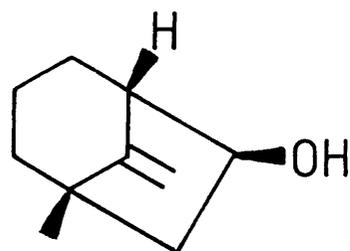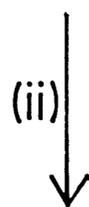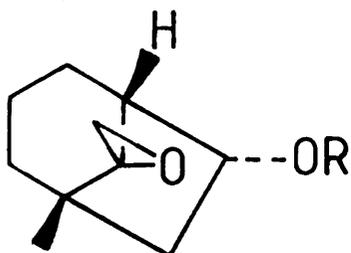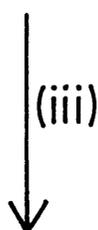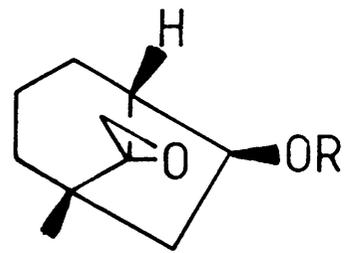
(i)⌐ (116b)
      (117b)

(i) Ac₂O, py.; (ii) Ph₃P=CH₂, THF; (iii) mcpba, CH₂Cl₂, Na₂HPO₄

(156)   (3)

(i) mcpba; (ii) Ac_2O, py.

(116b)   (118)
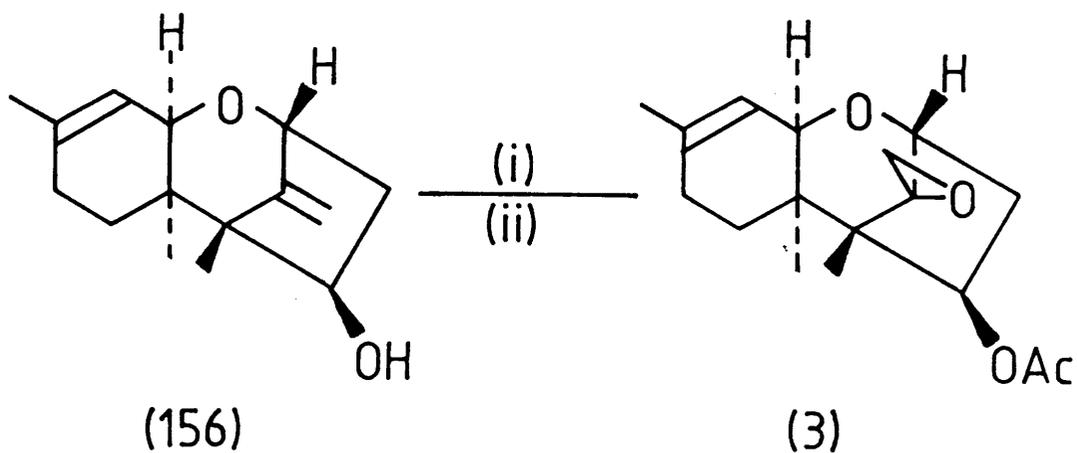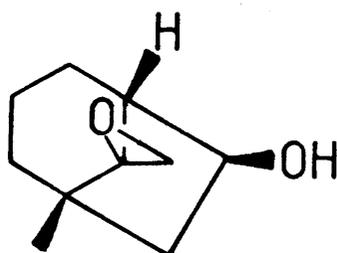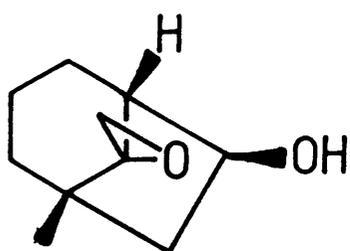
SHIFT   STUDIES

Total shift reagent added (mol.eq.)

	"Natural" epoxide			"Unnatural" epoxide		
	0.0	0.2	0.4	0.0	0.2	0.4
H-5	2.0	6.0	12.8	1.95	4.5	8.0
H-6	4.1	8.75	15.1	4.2	8.2	13.5
H-7	2.2	6.1+4.6	12.15+7.8	2.3	4.6+3.8	8.4+5.7
H-8'	2.75	4.15	6.0	2.9	4.15	5.8
CH_3	0.8	2.1	3.9	0.8	1.55	2.6

Observed Shifts (ppm)

(The shift reagent used in this study was Resolve-Al, $(Eu(thd)_2)$.

100 mg of the compound to be studied was dissolved in $CDCl_3$ and

its 90 MHz [1]H n.m.r. spectrum recorded. The shift reagent was then

mixed with the contents of the n.m.r. tube and the spectrum rerun).

TABLE   4

$\delta 8.75$ to $\delta 15.10$ as against $\delta 4.20$ through $\delta 8.20$ to $\delta 13.50$ for the "unnatural" epoxide. These results suggest that the shift reagent is better chelated with the hydroxyl and epoxide oxygen facing each other, thereby producing a greater change in shift.

As with the keto-alcohols (112) the epoxyalcohols also decompose slowly on standing, so they were separately protected as acetates (117). These acetates were then the starting materials for epoxide deoxygenation studies.

8.3 DEOXYGENATION STUDIES

Attempted deoxygenation of the model epoxides by Ganem's protocol, using dimethyl diazomalonate (DDM) and rhodium (II) acetate catalysis[83], gave unsatisfactory results. This system, under a variety of conditions with benzene or toluene as solvents and varying the amount of catalyst and DDM used, failed to give more than 43% of the desired exomethylene compounds (115). Starting epoxides were also recovered from the reaction mixture, suggesting that the low yield for reaction may be due to steric hindrance of the active species generated from DDM in its approach to the epoxide. By analogy with the decomposition of DDM to malonyl carbene under copper catalysis or irradiation[84], it is likely that the active species for epoxide deoxygenation is also the carbene, which can complex with the epoxide. The intermediate ylid formed, (157), can then collapse to give the olefin and dimethyl oxomalonate - an isolable co-product (SCHEME 18). The low yield for the reaction could be due to self-quenching of the carbene to give the dimer (158). It is interesting to note that the hindered epoxide from adamantylidene-adamantane was recovered unchanged by Ganem.

Attention was then focussed on the WCl_6/nBuLi deoxygenation system developed by Sharpless in 1972[85]. He made low valent tungsten halide derivatives by reacting WCl_6 with either alkyllithiums or lithium iodide in varying stoichiometries as outlined in Table 5. Considering

(117b)   (115b)
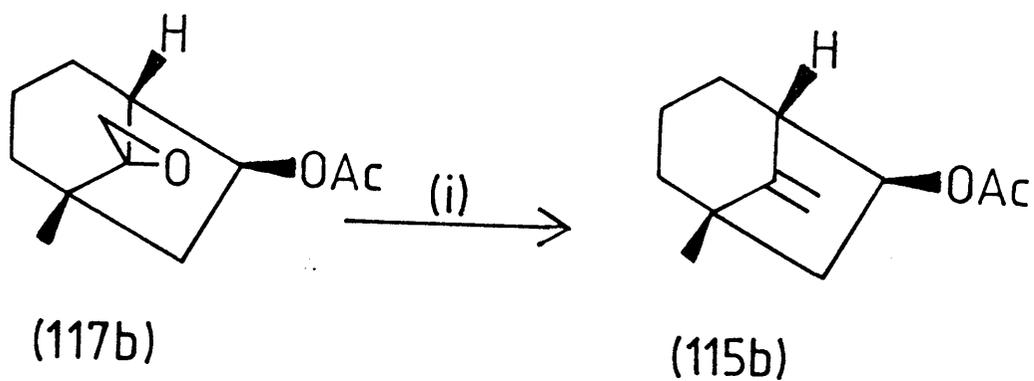
(i) DDM, $Rh_2(OAc)_4$, benzene.

(157)

$E = CO_2Me$

SCHEME 18

(158)

$$WCl_6 + 2RLi \xrightarrow{\text{THF}} \text{I}$$

$$WCl_6 + 3RLi \xrightarrow{\text{THF}} \text{II}$$

$$WCl_6 + 4RLi \xrightarrow{\text{THF}} \text{III}$$

$$WCl_6 + 2Li \text{ dispersion} \xrightarrow{\text{THF}} \text{IV}$$

$$WCl_6 + 3LiI \xrightarrow[\underline{\text{in vacuo, no solvent}}]{130^{\circ}} \text{V}$$

$$WCl_6 + 2LiI \xrightarrow[\underline{\text{in vacuo, no solvent}}]{130^{\circ}} \text{VI}$$
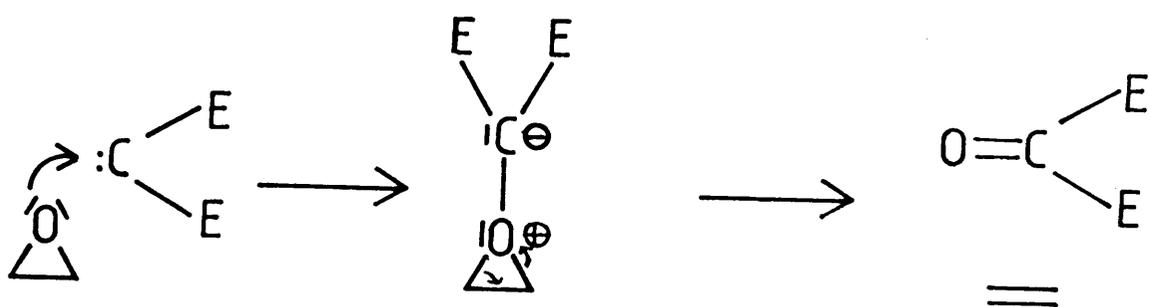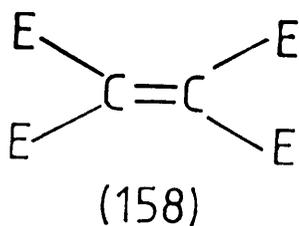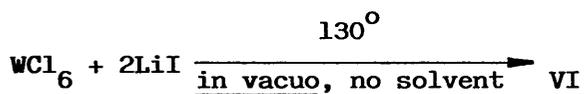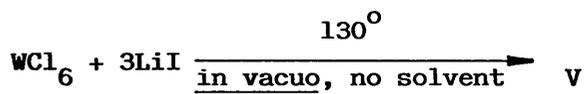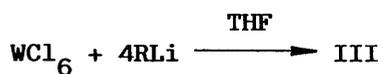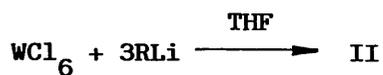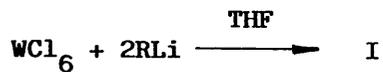
TABLE   5

the reaction of the epoxide with WCl_6/BuLi (1.2) the proposed

mechanism[86] (SCHEME 19) involves co-ordination of the active species

(possibly WCl_4) to the epoxide, followed by insertion of the tungsten

into the epoxide C-O bond and then collapse of the four membered

metallocycle (160) to give the olefin and oxotungsten species (161).

This mechanism explains why epoxides which are sterically hindered

to nucleophilic attack may be successfully deoxygenated. For example,

Deslongchamps[87] successfully deoxygenated the hindered epoxide in (162)

in 95% yield. Interestingly Sharpless also proposes similar

intermediates to (160) for the reverse process, i.e. for the reaction

of chromyl chloride, osmium tetroxide and other oxo metal compounds

with olefins.

If the co-ordinated complex (159) is attacked by chloride ions

present in solution it is possible to form chlorohydrins - here the

low valent tungsten is acting as a Lewis acid. However, the

chlorohydrin is not an intermediate in the deoxygenation process since

reduction of chlorohydrins is a much slower process and also results

in complete loss of stereochemistry. Chlorohydrin formation can be

circumvented either by use of less acidic reagents II, III or IV or

by heating the reaction mixture under reflux.

Lithium aluminium hydride has been used by Fujiwara et al[88]

to generate low valent tungsten species from WCl_6. This system

has been claimed to be as effective as that of Sharpless. Although

not relevant here, Sharpless deoxygenation shows a high degree of

stereoselectivity, mitigating against a radical mechanism.

In the present study, use of WCl_6/nBuLi at room temperature (after

generation of the active deoxygenating species at $- 78^{\circ}C$) on the

β-acetoxyepoxide gave a mixture of the desired olefin (115b) and

the chlorohydrin (163), even after prolonged stirring over three

days. On the other hand, heating the reaction mixture under reflux

for 6 hours gave solely the desired olefin, in over 90% yield for both

(159)

(160)

(161)

SCHEME 19

(162)

$R = CH(OCH_3)_2$

(117b)

(i)
rt

(163)

+

(115b)

(117b)

(i)
reflux

(115b)

(117a)

(i)
reflux

(115a)
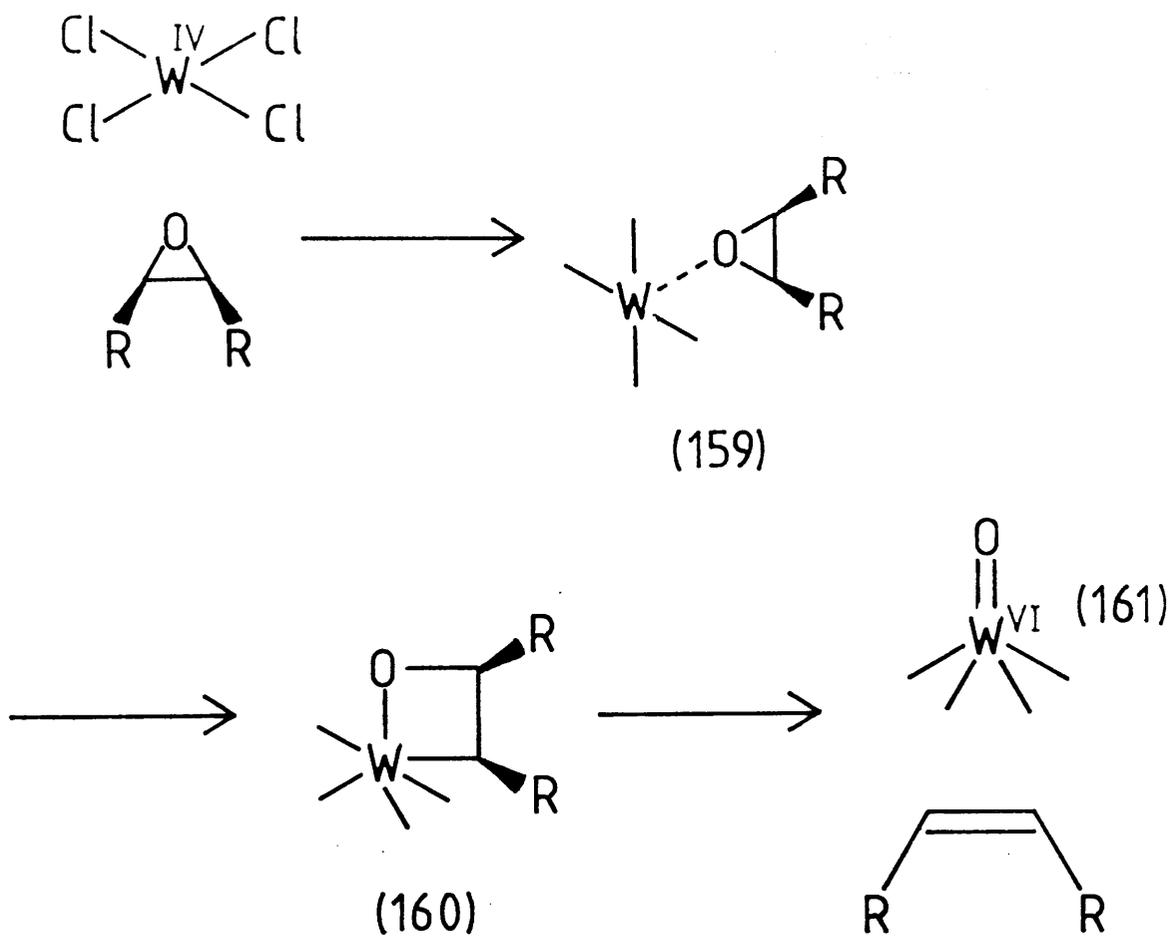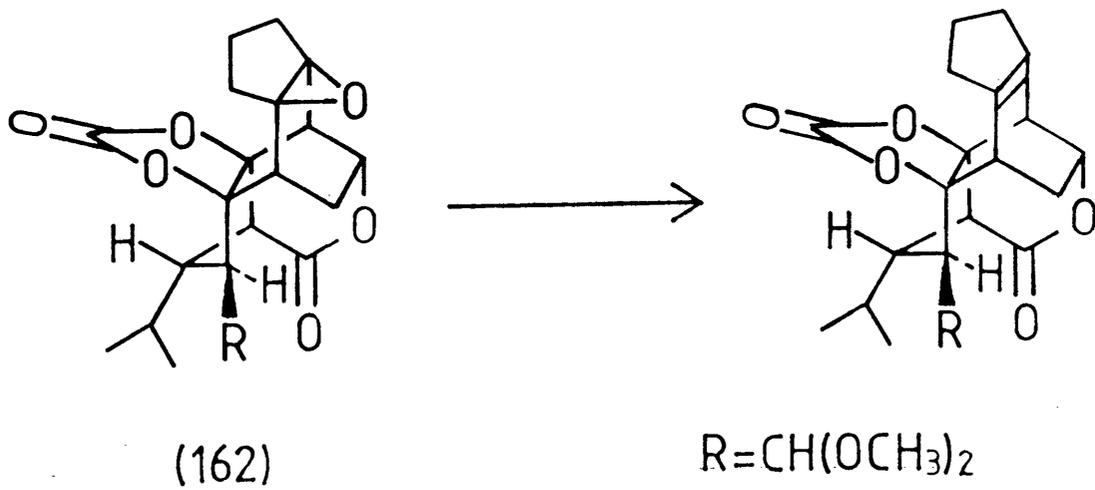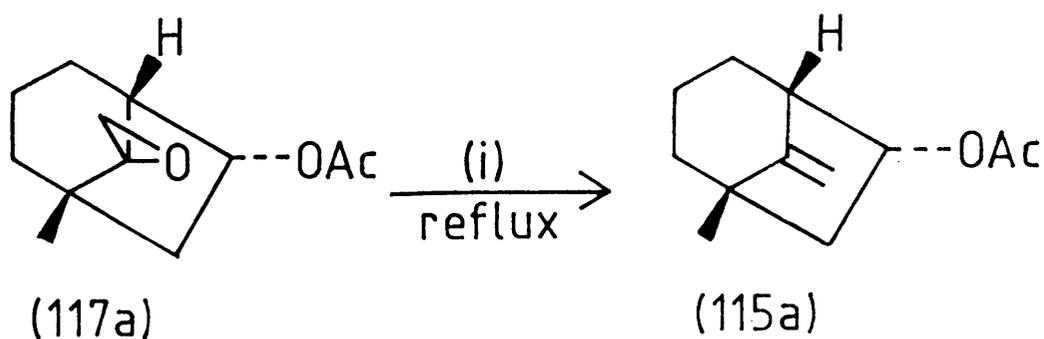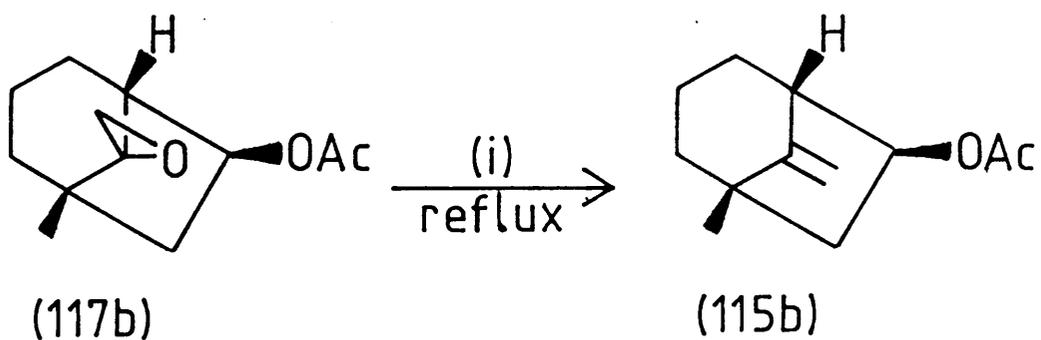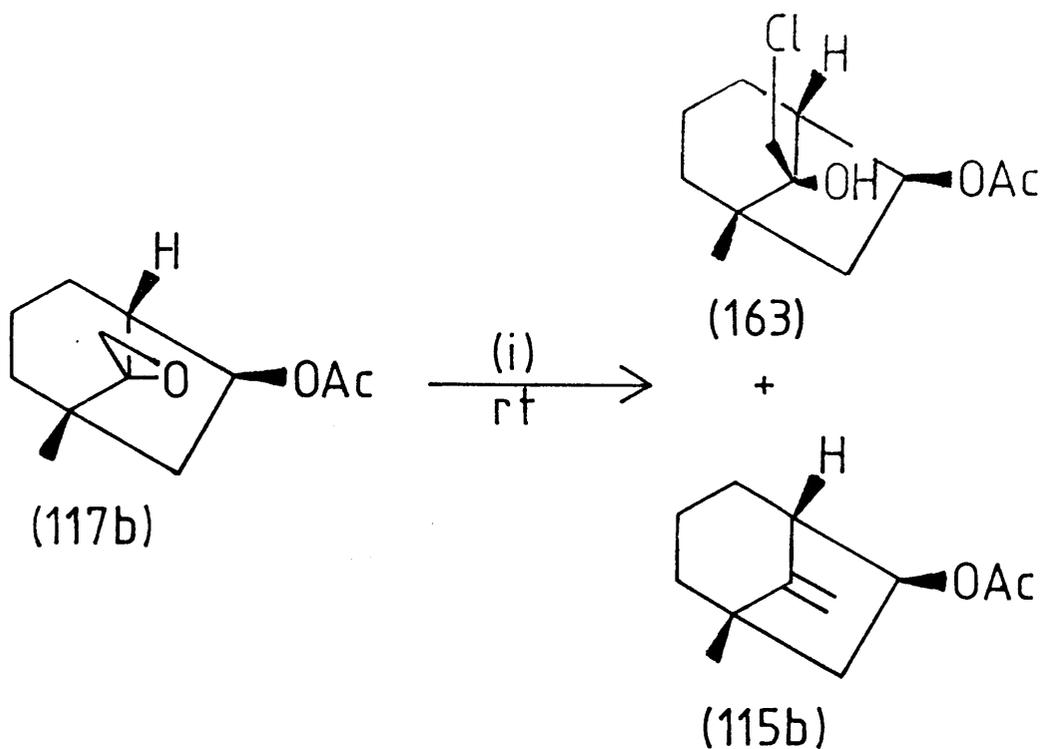
SCHEME 20

(i) nBuLi, WCl₆, THF

epimeric epoxides[89] (SCHEME 20).

Acetylation of either culture product gave the immediate precursor, $3\alpha,4\beta,15$-triacetoxy-12,13-epoxytrichothec-9-ene(63), for Sharpless deoxygenation. Gratifyingly, this procedure gave the desired olefin (119) in near quantitative yield. Similarly, triacetyldeoxynivalenol(120), obtained by acetylation of 3-AcDON, gave diene (121) in a more modest yield of 40% after deoxygenation: this reaction had to be carried out twice in order to effect full consumption of starting material. Chromatographic separation of the starting epoxide and desired diene was not possible since both components had very similar polarity on t.l.c. Subsequent reactions were followed by g.l.c. analysis with the epoxide having the longer retention time. The relatively modest yield for this reaction is a reflection of performing the reaction twice, of the scale of reaction (20–30 mg) and perhaps of the degree of functionality present[89,90].

The diene (119) derived from triacetoxyscirpene was tested for biological activity. This was done by determining the minimum concentration of the diene to inhibit cell division of human epithelial tumour cells (HEp2) using serial dilution[91]. The starting epoxide (63) required a minimum dose of 3.4 mg/ml, whereas diene (119) required 550 mg/ml; in other words, the diene possessed less than 0.7% of the toxicity of the epoxide; it is in essence non-toxic. This finding further demonstrates the necessary presence of the epoxide for full biological activity.

(9) R=Ac
(48) R=H

(63)

(ii) →

(119)

(24)

(iii) →

(120)

(ii) →
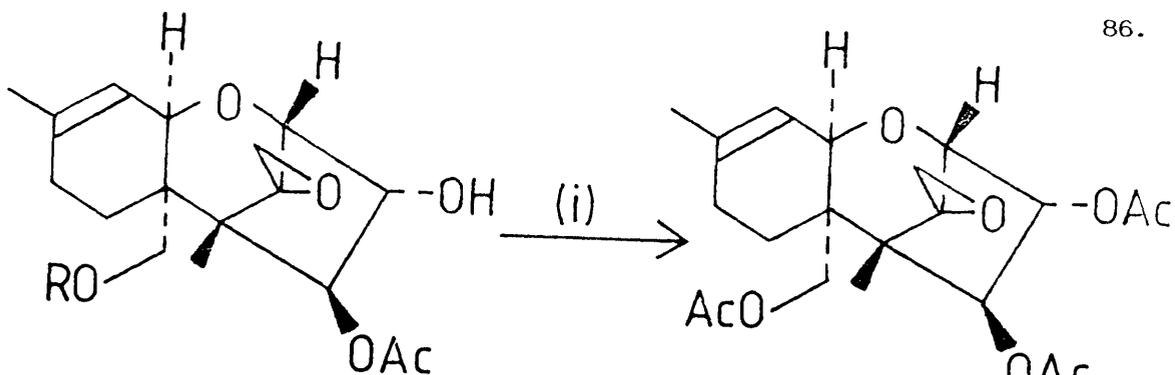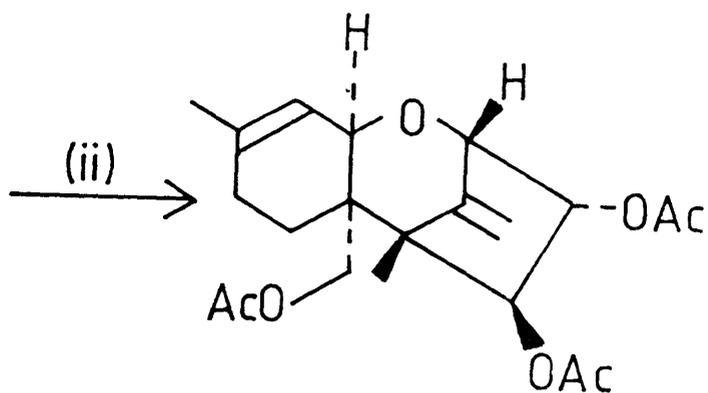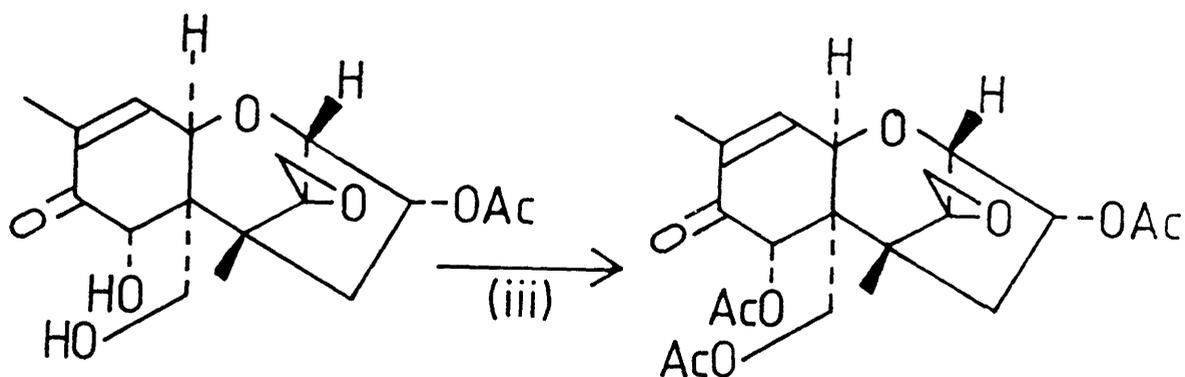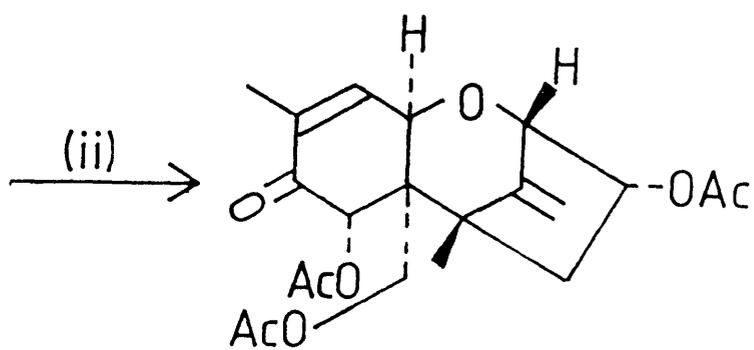
(121)

(i) Ac$_2$O, py.
(ii) nBuLi, WCl$_6$, THF
(iii) Ac$_2$O, Et$_3$N, DMAP

9.   SYNTHESIS OF AN EPI-EPOXYTRICHOTHECENE (128)

To probe further the role of the 12,13-epoxide moiety, it was decided to investigate whether the orientation of the epoxide was critical or whether its mere presence was sufficient for biological activity.   To this end it was decided to synthesise the isomeric epi-epoxide from culture products.

The synthetic plan involved making good use of our deoxygenation protocol on a suitably protected substrate to furnish an exomethylene compound, which could in turn be ozonolysed to the norketone.   The epi-epoxide could then be introduced by treatment of the norketone with dimethylsulphonium methylide.

This plan requires protection of the A-ring double bond from ozonolysis.   It was decided to do this by formation of a bridged bromo-ether using the 9,10-double bond and the unmasked C-15 alcohol [30,44,45,47].   4-acetoxyscirpenediol (48), one of our culture products, is already suitably functionalised.   Treatment of the diol (48) with NBS in dry MeCN gave the bromo-ether (122) in 92% yield.   Formation of the bromo-ether was evidenced in the ^{1}H n.m.r. spectrum by the absence of the olefinic C-10 proton at 5.5 and also by the sharpening and upfield shift of the C-16 methyl signal (δ 1.72 $\rightarrow$ δ 1.26); ^{13}C n.m.r. spectroscopy showed no olefinic carbon signals at 140.3 (C-9) and δ 118.8 (C-10).   In addition, the mass spectrum showed the presence of the parent ion with a doubling of peaks due to the natural isotopes of bromine (^{79}Br and ^{81}Br).

The brominating species is thought to be molecular bromine, which is produced by a fast ionic reaction between NBS and trace amounts of HBr[92].   The mechanism can then proceed by formation of the bridged bromonium ion (164), followed by subsequent intramolecular attack by the free C-15 hydroxyl to give the bridged bromo-ether (122) and HBr.   The HBr produced re-enters reaction 1 to maintain a low, steady-state

[1] HBr + ⟶ + Br₂

(48)

(164)

⟶

+HBr

(122)
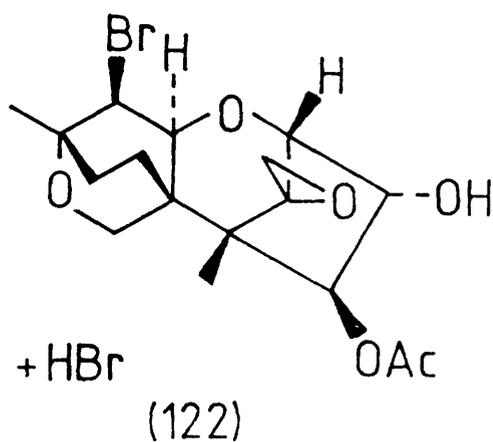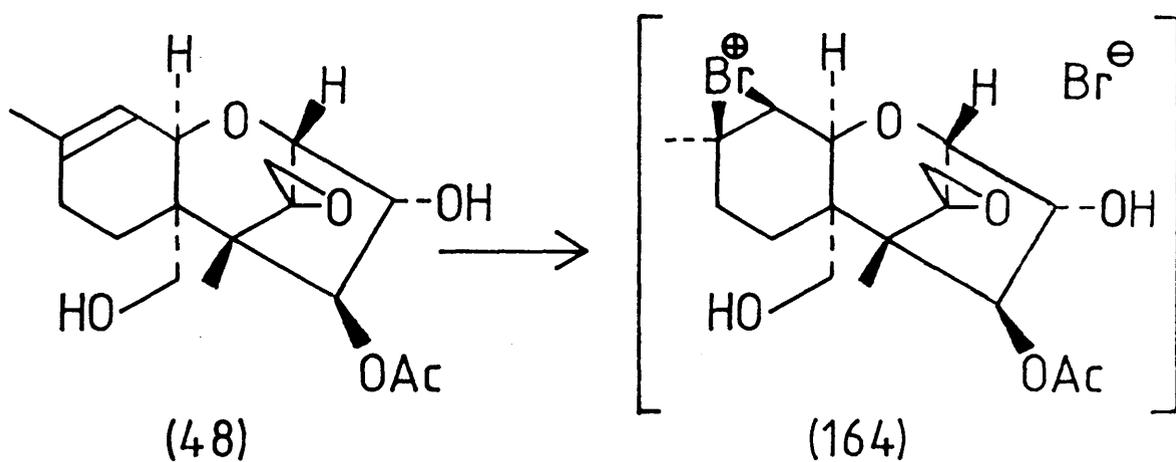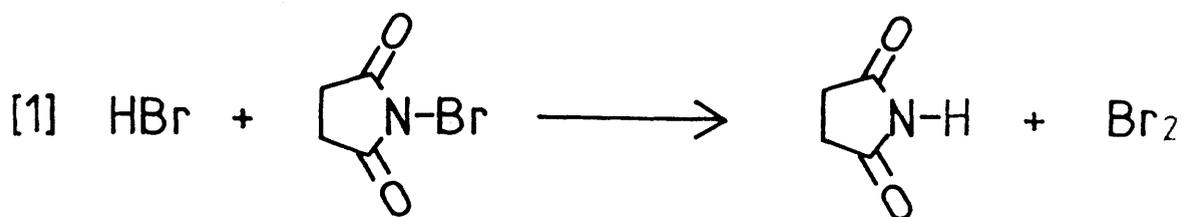
concentration of bromine. Bisbromination does not occur due to the

low standing concentration of Br_2, and in any case, the rate of

bromo–ether formation, being intramolecular, would be expected to be

many orders of magnitude greater.

Treatment of the alcohol (122) with pyridine and acetic anhydride

gave the diacetate (123) in quantitative yield. Sharpless deoxygenation[85]

then gave the desired alkene (124) (δ 5.25 and 4.82, H–13) in a yield of

98%[90]. This reaction further demonstrates the mildness and selectivity

of this deoxygenation procedure. Indeed, this work has shown the system

to be compatible with esters, bromo–ethers, alkenes and enones.

Independent work by Roush[40] has shown that it can also be used with

free hydroxyl groups present if a larger excess of WCl_6 and nBuli is

used. This protocol is not however compatible with the THP ether

protecting group which undergoes cleavage under these conditions.

A literature search revealed that despite the mildness and compatibility

with other functionalities which we have observed, the Sharpless system

has to date had surprisingly little application. In an elegant use of

this system by Roberts et al[94], humulene tris–epoxide (165) could be

selectively deoxygenated to give mono–epoxide (166), in effect

functionalising the least reactive double bond!

Conversion of the exomethylene compound (124) into norketone (125)

required prolonged exposure of the sterically hindered alkene to

ozone – shorter reaction times resulted in mixtures of starting alkene

and norketone. The intermediate ozonide was reduced using triethylamine

to give norketone (125) in 87% yield. I.r. spectroscopy showed the new

carbonyl at 1770 cm^{-1} and the C–12 signal in the ^{13}C n.m.r. spectrum

occurred at δ207.8; both these facts are indicative of a strained,

bridging carbonyl. Interestingly the norketone stains a very distinctive

blue colour in t.l.c. on visualisation with ceric sulphate.

Treatment of the norketone with excess dimethylsulphonium methylide

in the THF[81] gave the epi–epoxydiol (126) in 35% yield, acetate esters

(122) R=H
(123) R=Ac ⟵⎤(i)
                (ii) ⟶

(124)

(165)   (ii) ⟶   (166)

(i)Ac₂O,py.;(ii)nBuLi,WCl₆,THF.

(124) (125)

(i),(ii)

(iv)|(v)   (iii)

(130) (126)

(i)|(ii)   (vi)

(131) (132)

(iii)
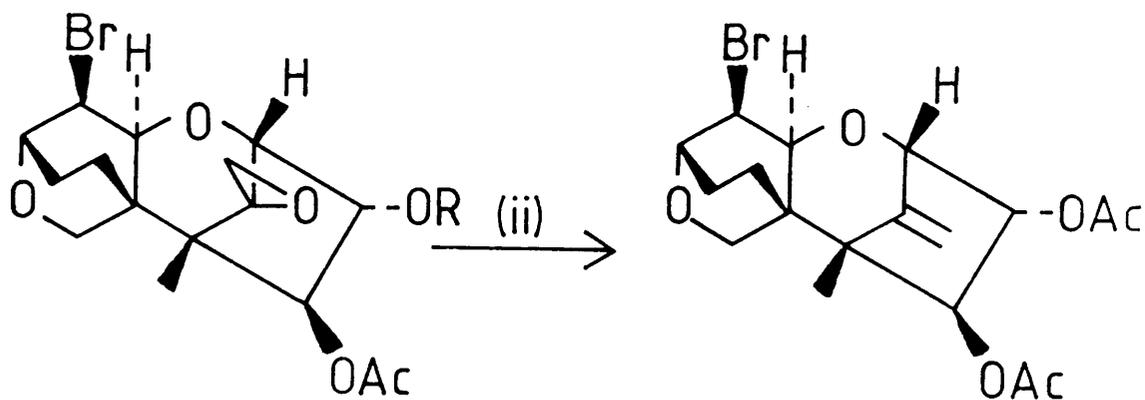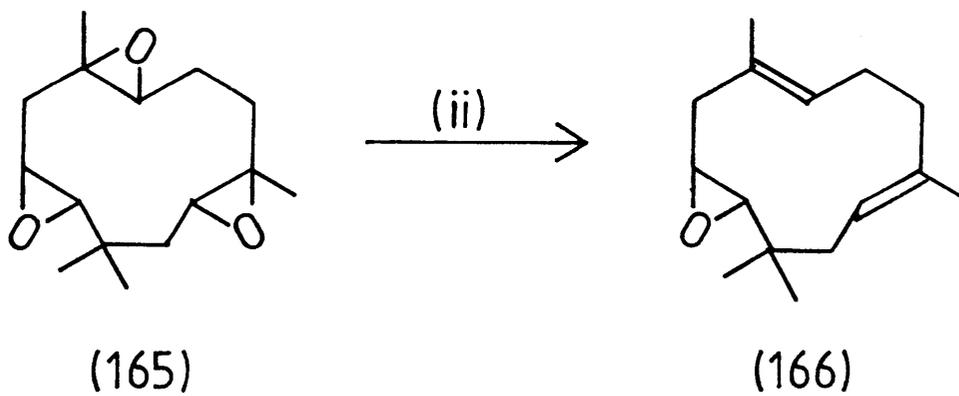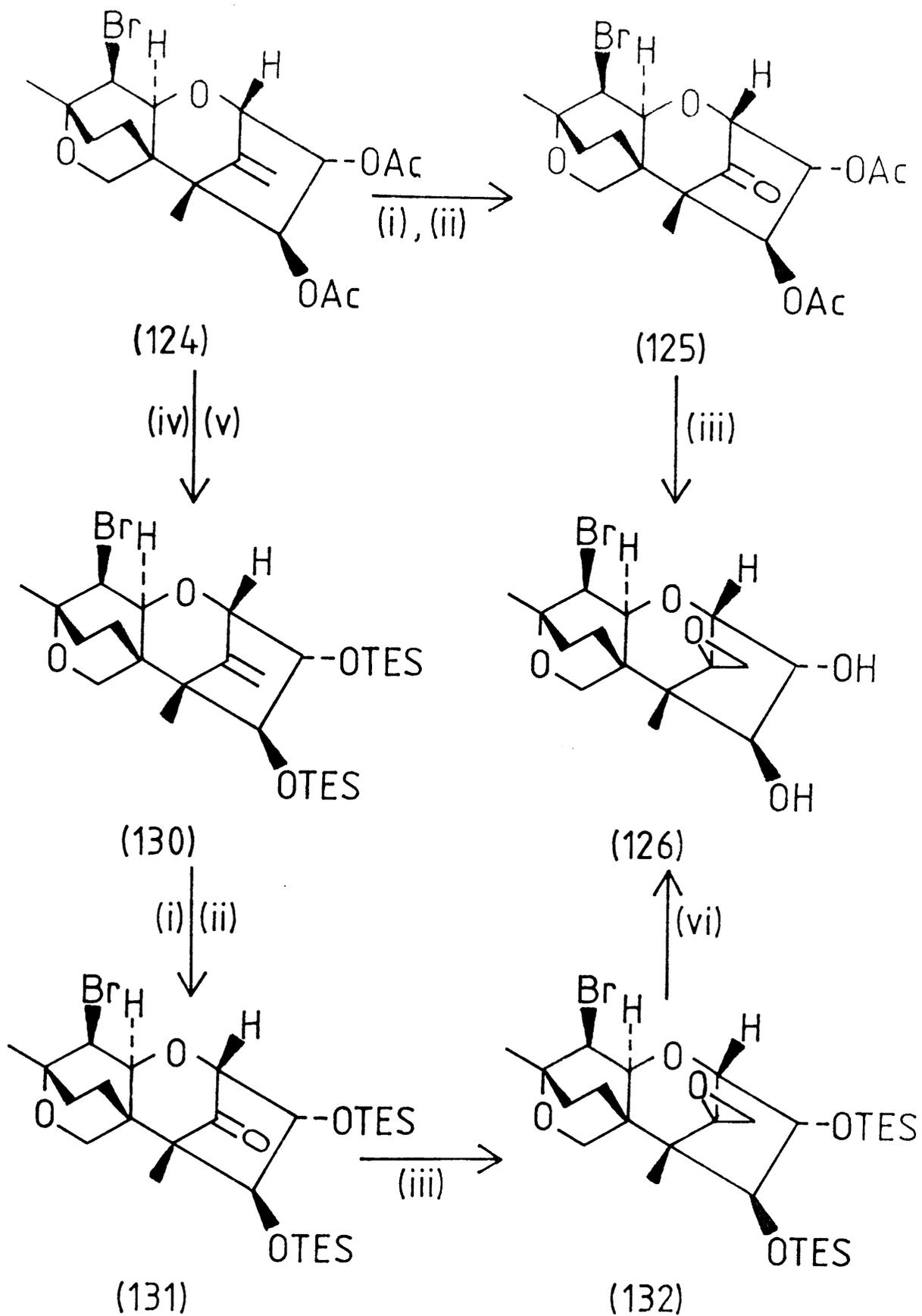
(i) O₃,CH₂Cl₂;(ii)Et₃N;(iii)Me₃S⁺I⁻,nBuLi,THF;
(iv)K₂CO₃,MeOH;(v)TESCl, py. ,DMAP;
(vi)Bu₄NF,THF.

having been cleaved by the ylid. An eight-fold excess of the ylid

was used to ensure that a mixture of C3/C4 acetates and alcohols did

not result. A higher yield and a much cleaner reaction was achieved by

use of triethylsilyl protecting groups rather than acetates. The

triethylsilyl groups were introduced by methanolysis of the Sharpless

deoxygenation product (124) followed by treatment with triethylsilyl

chloride and DMAP in pyridine in an overall yield of 73%. Ozonolysis

of the bissilyloxy olefin (130) gave norketone (131) (^{13}C δ211.2 (C-13).

ν_{max}1765 cm^{-1}) in 84% yield.

Treatment of the norketone with 1.4 equivalents of dimethylsulphonium

methylide[81] gave the desired epoxide (132) in 64% yield. Use of a greater

excess of ylid resulted in the formation of a slightly more polar by-

product. Although not fully characterised this material was believed

to be the thiomethyl alcohol (133) (by M.S.) which arises from attack

on the intermediate betaine (167) by an external nucleophile at the

sulphur-bearing methyl (SCHEME 21), rather than internal attack at the

methylene carbon which would give the epoxide and dimethyl sulphide.

Since the production of this by-product can be eliminated by the use

of less ylid, this suggests that the external nucleophile is the ylid

itself and not the iodide counter ion.

Fluoride ion cleavage of the silyl ethers gave the epi-epoxydiol

(126) identical to that obtained from norketone diacetate.

Completion of the synthesis was relatively straightforward.

Acetylation of diol (126) gave (127). Restoration of the 9,10-double

bond was achieved by treatment of the bromo-ether with a zinc-silver

couple[99]. Finally, acetylation of the unmasked C-15 alcohol gave

triacetate (128)[95].

The epi-epoxytriacetate was slightly more polar and had a higher

melting point than the natural epimer (169-170°C vs 122-3°C). An

inspection of its ^{1}H n.m.r. spectrum showed an upfield shift of the

epoxide protons, perhaps due to shielding by the C-4 acetate or to

(131)

(167)

(132)

(133)

R=OTES

SCHEME 21

(132) → (i) → (126)

(ii) → (127)

(iii), (ii) → (128)
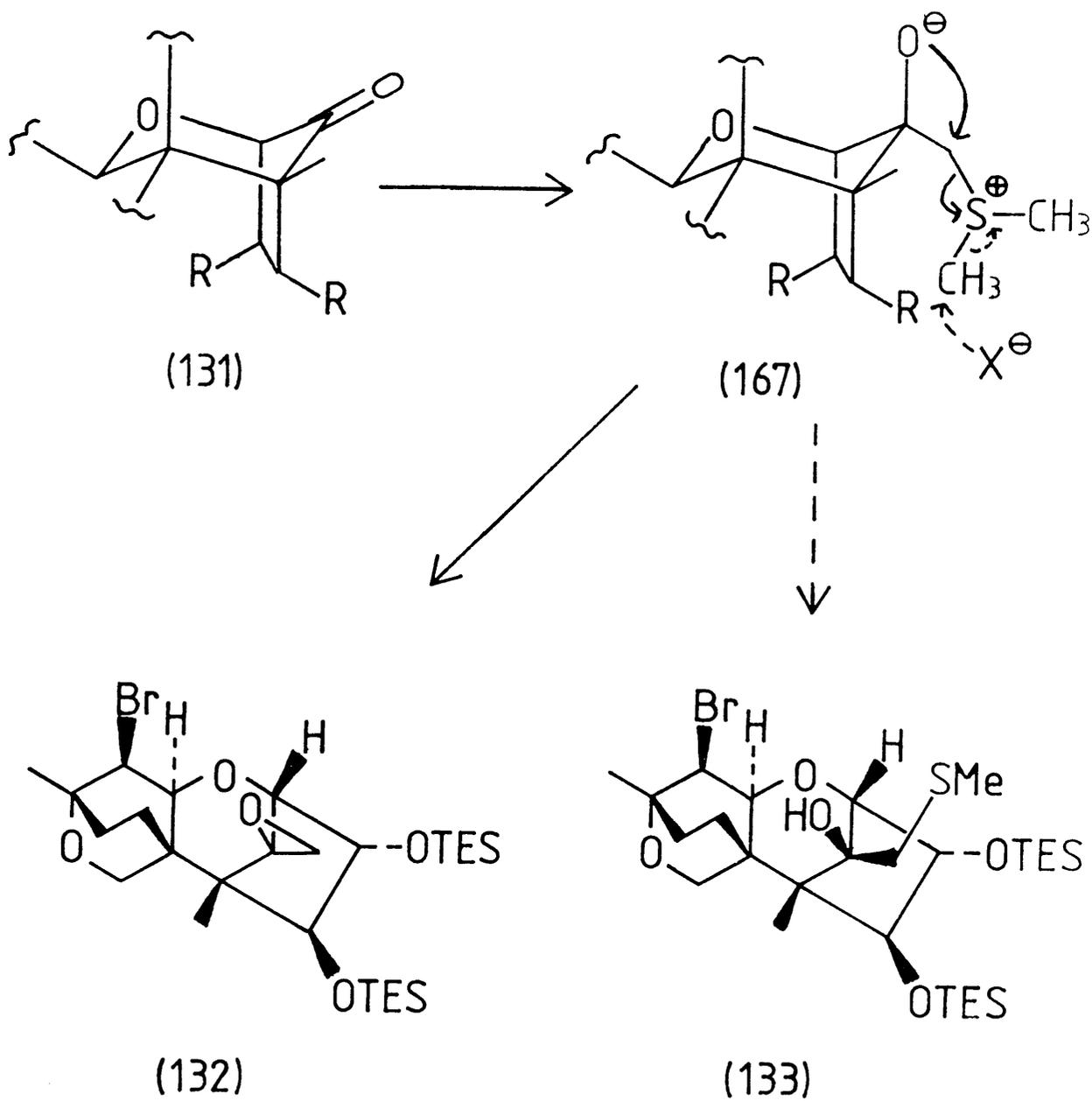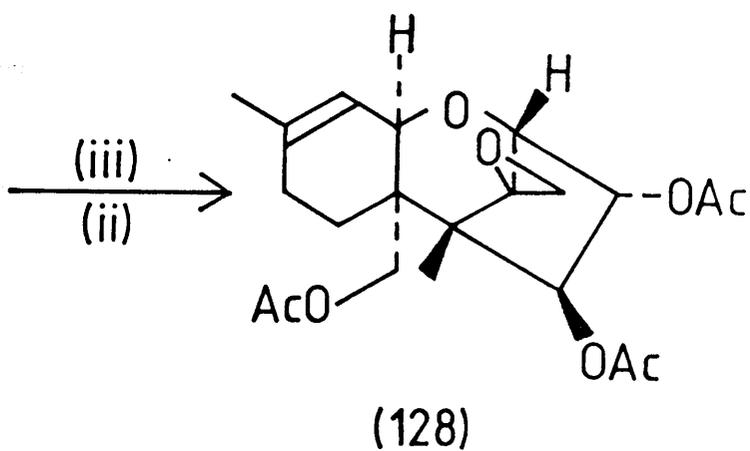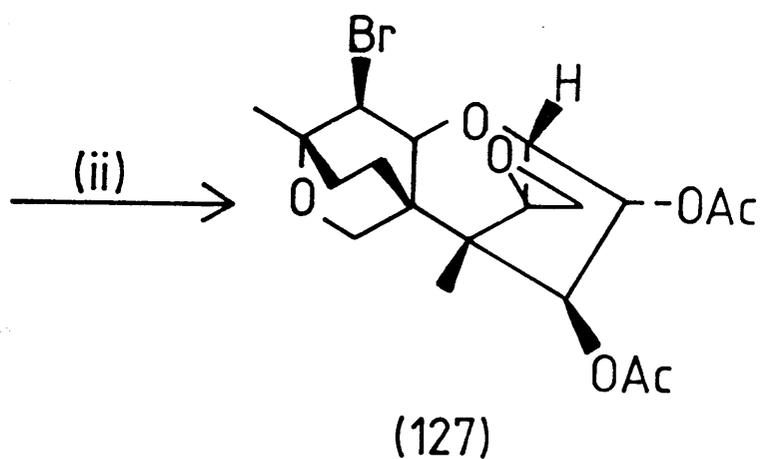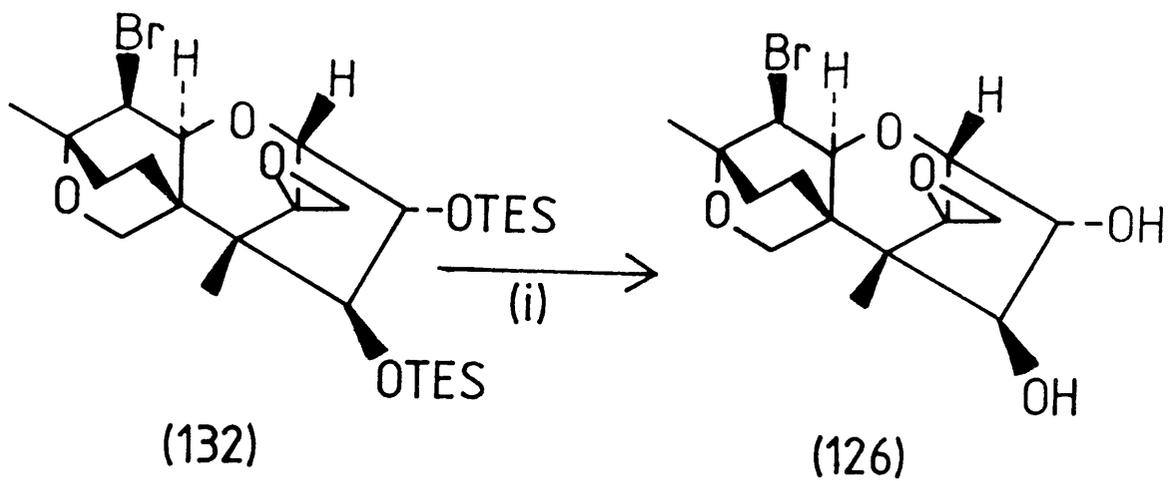
(i) Bu₄NF,THF; (ii) Ac₂O,py.; (iii) Zn(Ag),THF,EtOH,Et₂O

relief of steric congestion from the A—ring. Superimposition of both

[1]H n.m.r. spectra of the respective epoxides (FIGURE A) showed very

little variation in pattern and chemical shift apart from the epoxide

resonances. The configuration of the epoxide ring was confirmed

conclusively by x—ray crystallographic analysis of the triacetate*

(FIGURE B). This clearly shows the epoxide with the oxygen away

from the two—carbon bridge, i.e., the C12—C13 bond is anti—periplanar

to the O(1) — C2 bond.

As already mentioned, the mechanism for the formation of the epoxide

(SCHEME 21) involves nucleophilic attack of the sulphonium ylide at the

carbonyl to give an intermediate betaine (167). An internal displacement

by the alkoxide gives the epoxide. This particular stereochemistry

results from ease of attack at the carbonyl from the less hindered

two—carbon bridge. This is analogous to Colvin's synthesis of

trichodermin[42] where the epoxide with the unnatural stereochemistry (169)

was obtained by treatment of norketone (168) with dimethylsulphonium

methylide.

A recent paper by Volatron and Eisenstein[96] has discussed the

theoretical aspects of this Corey—Chaykovsky reaction and in particular,

why a sulphonium ylid should give the epoxide and a sulphide whereas

a phosphonium ylid gives the Wittig products of an olefin and phosphine

oxide. By use of ab initio calculations they found that both

thermodynamic and kinetic factors were responsible for the products

observed.

For the Wittig reaction, only four—membered covalent rings were

found to be stable intermediates. In the case of phosphorus there were

two isomeric oxaphosphetanes (197) and (198) with the oxygen either in

the apical or equatorial position of phosphorus. Interconversion of

*X—ray crystallographic analysis kindly performed by Dr. A.A. Freer

(Glasgow).

X-ray Structure of Epi-Epoxytrichothecene (128)

FIGURE B

FIGURE A

(168)   (169)

Reactants   (197)   Products

(198)

(170)
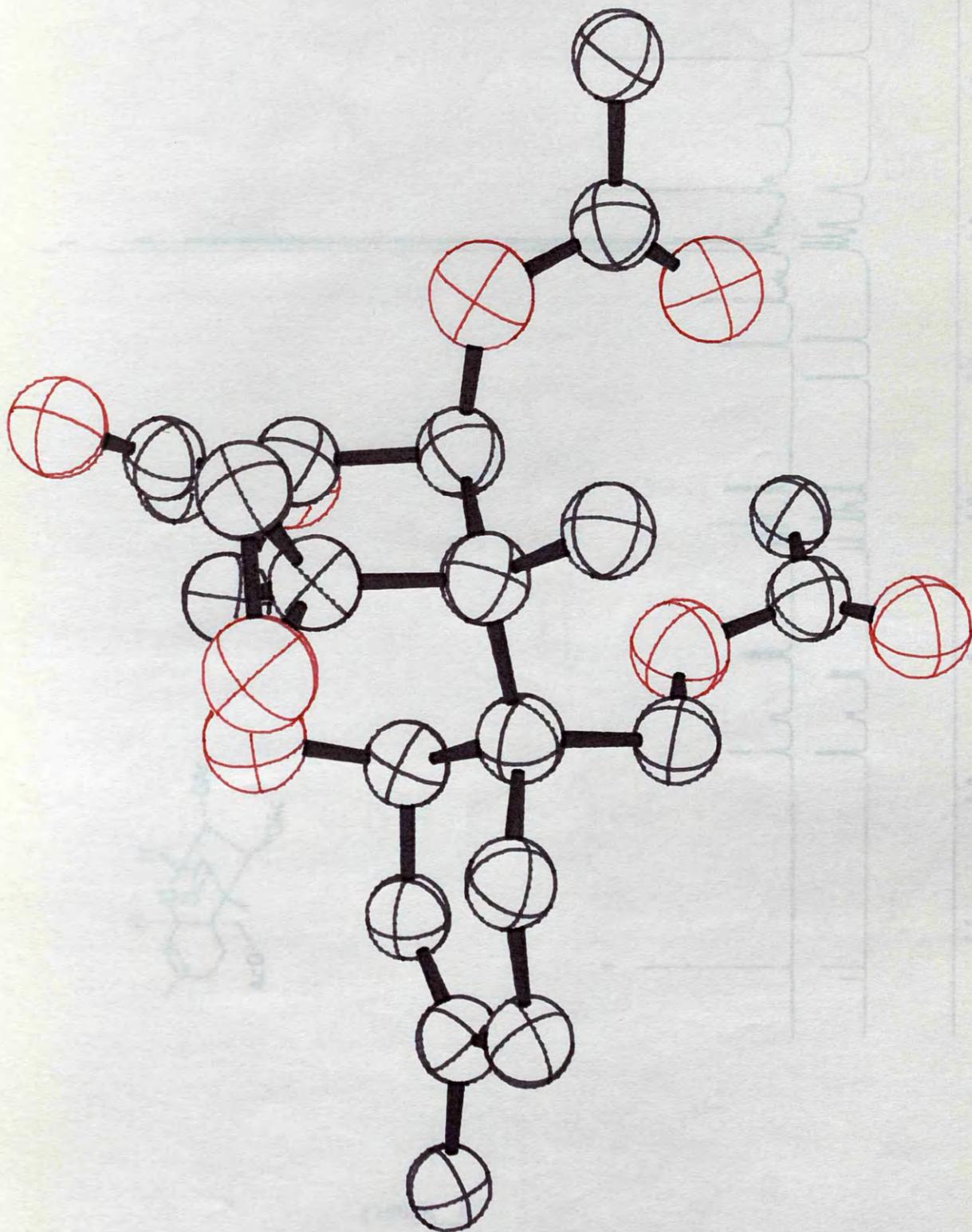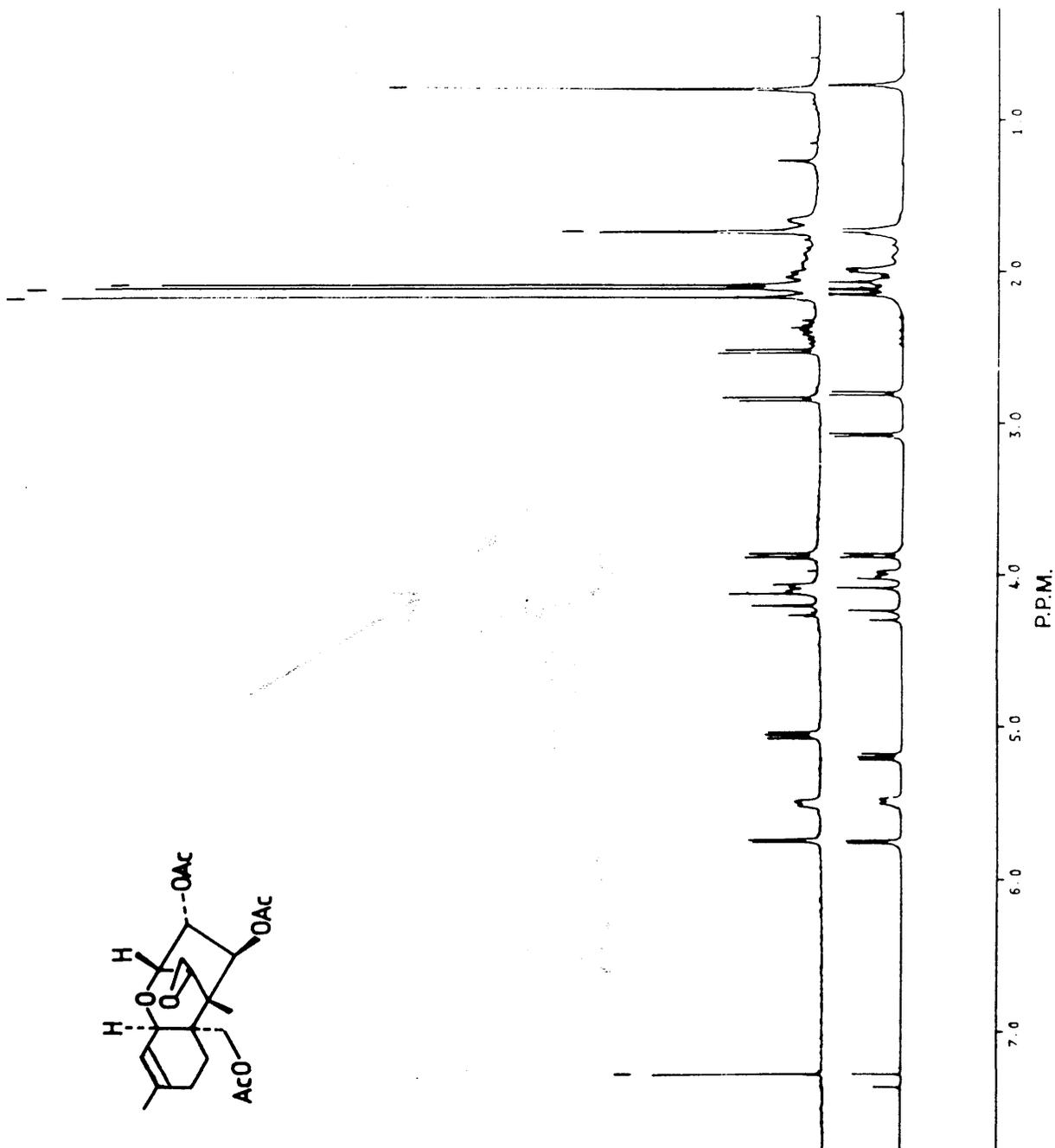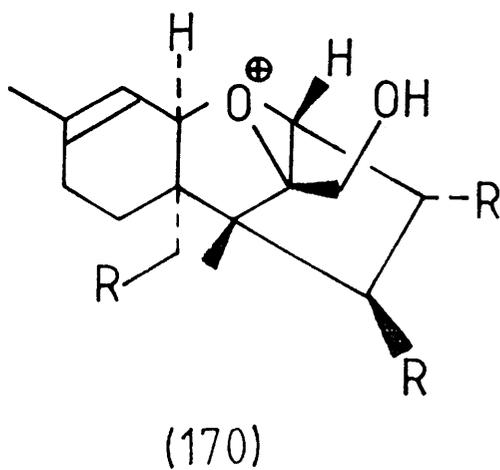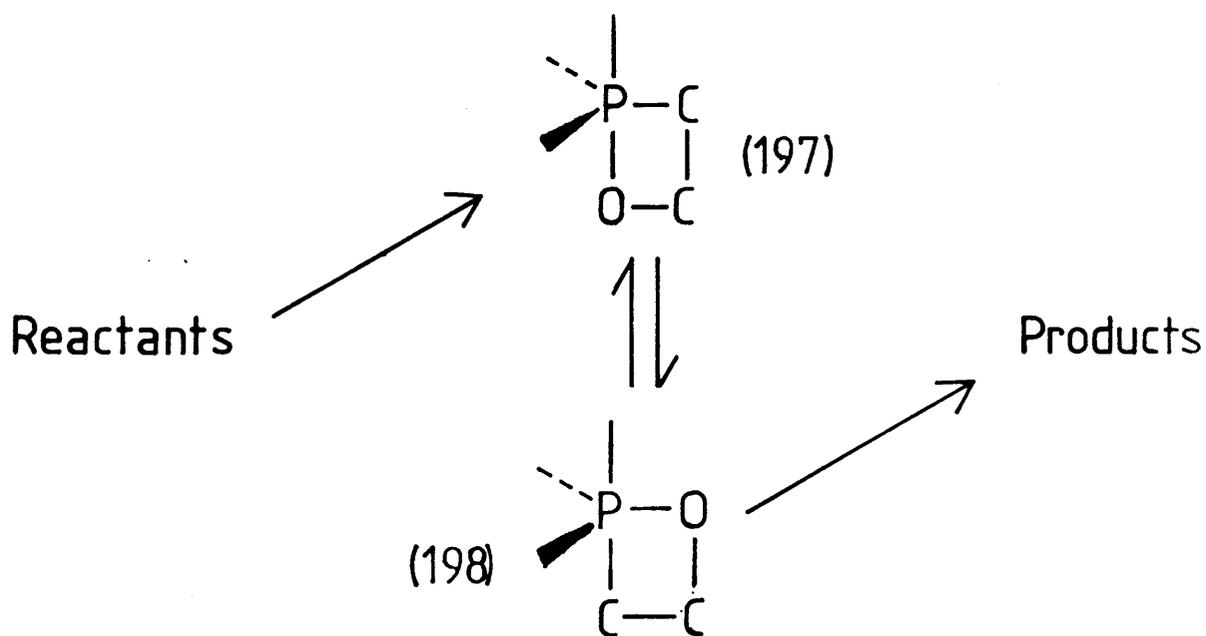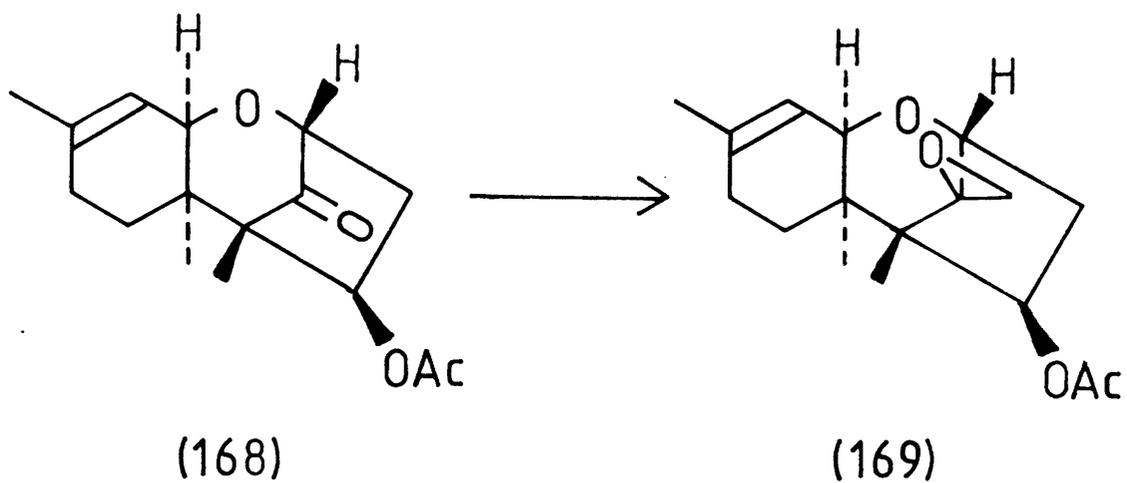
the apical oxaphosphetane to the equatorial oxaphosphetane requires an input of only 3 kcal/mol. This interconversion results in a weakening of the P-C bond, making ring opening to Wittig products easier. Such an interconversion is difficult in the case of sulphur, so kinetically the ease of ring opening of the oxaphosphetanes favours Wittig products.

Thermodynamically, for the sulphonium ylid, formation of the epoxide and sulphide is favoured by 39.1 kcal/mol over the formation of the olefin and sulphoxide. In contrast, for the phosphonium ylid, formation of the olefin and phosphine oxide is favoured by 14.6 kcal/mol over the formation of a epoxide and phosphine.

Deoxygenation of the epoxide to the olefin with phosphine was also shown to be a favourable process, although an input of energy is required to overcome the initial activation barrier. Indeed epoxide deoxygenations with Ph_3P are commonly performed at temperatures in excess of $100^{\circ}C$[97].

The epi-epoxide triacetate (128) was also tested for biological activity by the same method used for the diene (119)[91]. The minimum concentration of the epi-epoxide to inhibit cell division was 920 mg/ml, in other words the epi-epoxide possessed less than 0.4% of the toxicity of the natural isomer. This finding clearly demonstrates the need to have the epoxide in the correct orientation for biological activity. This provides further evidence that the toxicological process may involve intermediate (170) which arises from attack at the epoxide by the pyran oxygen. Such an intermediate is not possible with the epi-epoxide since the geometry is not correct for a S_N2 type of displacement. Additionally, it is suspected that the epi-epoxide may be resistant to apotrichothecene rearrangement[17] for the same reason, i.e., the C-O bond of the pyran ring is no longer anti-periplanar to the epoxide C-O bond.

Independently of this work, Roush[40] has also made use of the Sharpless deoxygenation procedure directly on anguidine (9) to give the exomethylene

(171). Further elaboration of the exomethylene gave the key

intermediate (172). Ozonolysis of the derived C-4 triethyl silyl

ether (173) gave norketone (174) (SCHEME 22). This compound was used

to introduce a radiolabel specifically at C-13 by a Wittig reaction

with $Ph_3P={}^{14}CH_2$ to give labelled (173). Reconstruction of anguidine

was accomplished straighforwardly by desilylation at C-4, followed

by epoxidation of the alkene using m-chloroperbenzoic acid to give

a single epoxide (49). Regeneration of the 9,10-double bond,

acetylation of the diol (46) and hydrolysis of the THP ether gave

radiolabelled anguidine (SCHEME 23). This is the first time that a

trichothecene has been prepared enantiomerically pure and specifically

labelled with ^{14}C.

Norketone (174) also provided the starting point for Roush's

independent synthesis of 12-epi-anguidine (175)[98] by a similar

methodology employed by us.

In addition, he has prepared 12,13-deoxy-12,13-methanoanguidine

(176), with an isosteric cyclopropane ring, from exomethylene (172)

via Simmons-Smith cyclopropanation. No biological data have been

published as yet on the epi-epoxide or cyclopropane.
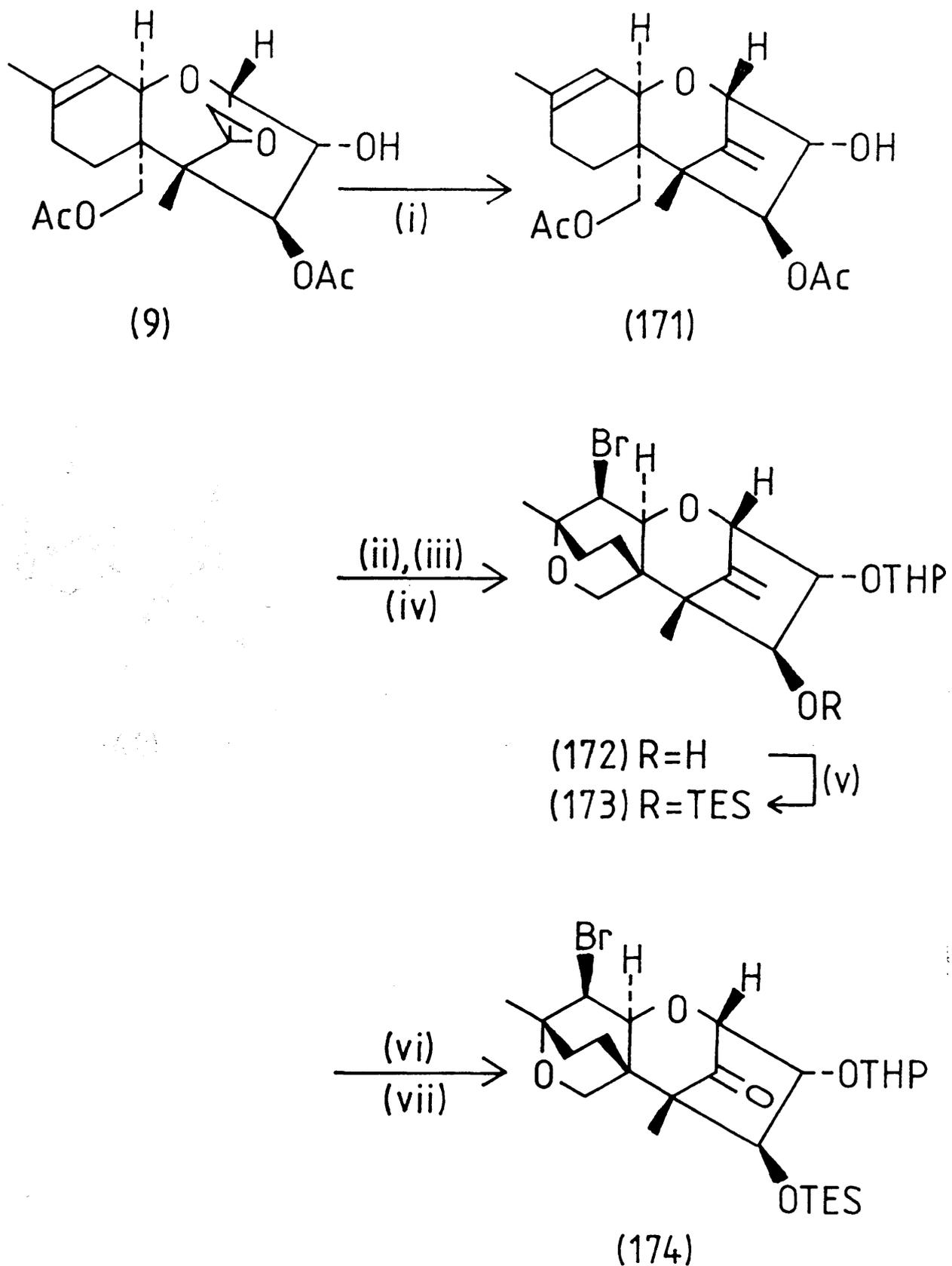
SCHEME 22

(i) nBuLi, WCl$_6$, THF; (ii) DHP, H$^+$; (iii) NaOH;
(iv) NBS, CH$_3$CN, NaHCO$_3$; (v) TESCl, DMAP, py.;
(vi) O$_3$, MeOH; (vii) Me$_2$S.
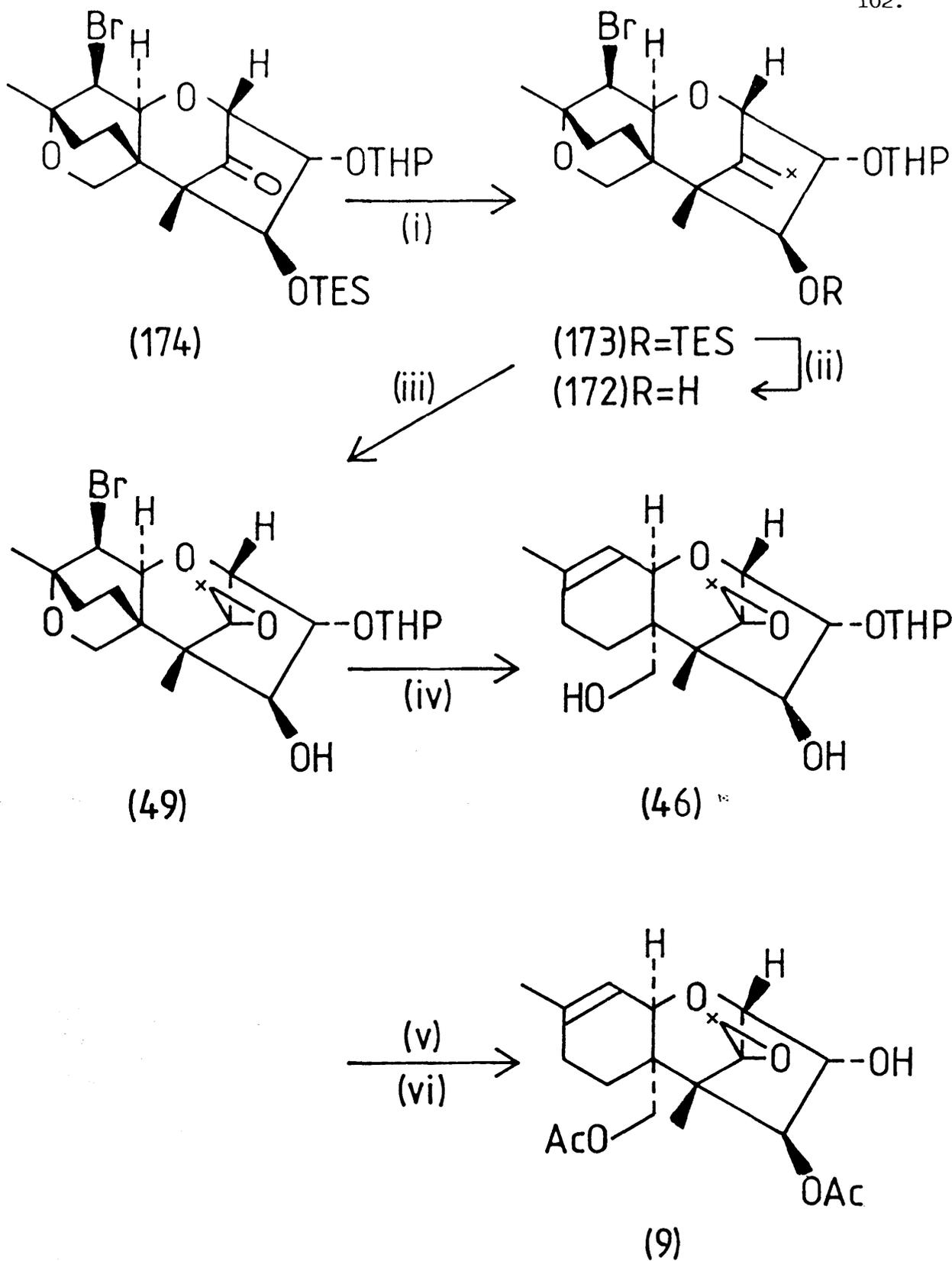
SCHEME 23

(i) Ph₃P=ČH₂,THF;(ii)Bu₄NF,THF;(iii)mcpba,CH₂Cl₂,
Na₂HPO₄;(iv)Zn(Ag),THF,EtOH,Et₂O;
(v)Ac₂O,py.,DMAP;(vi)AcOH,H₂O,THF.

(174)

(175)

(172)

(176)
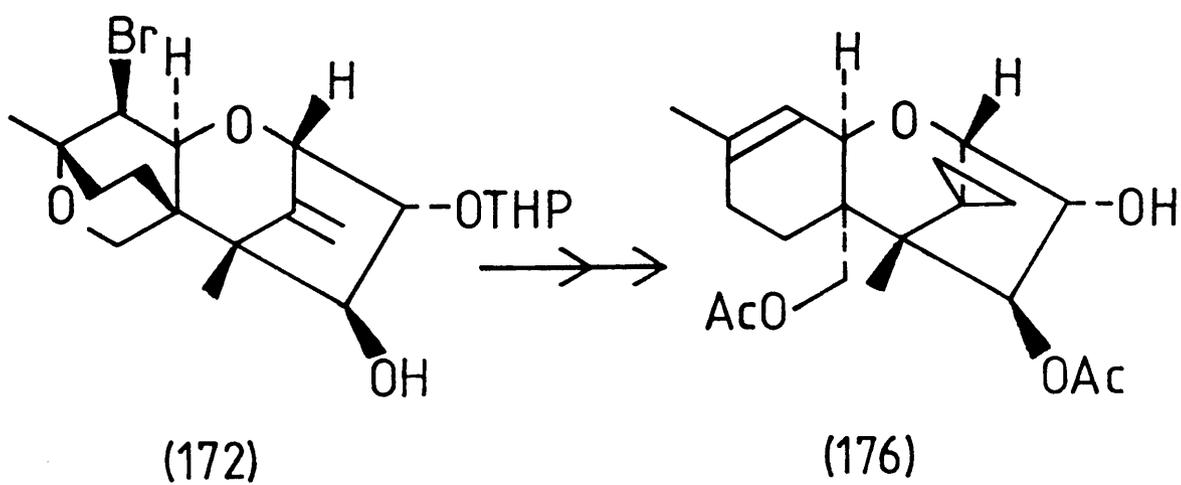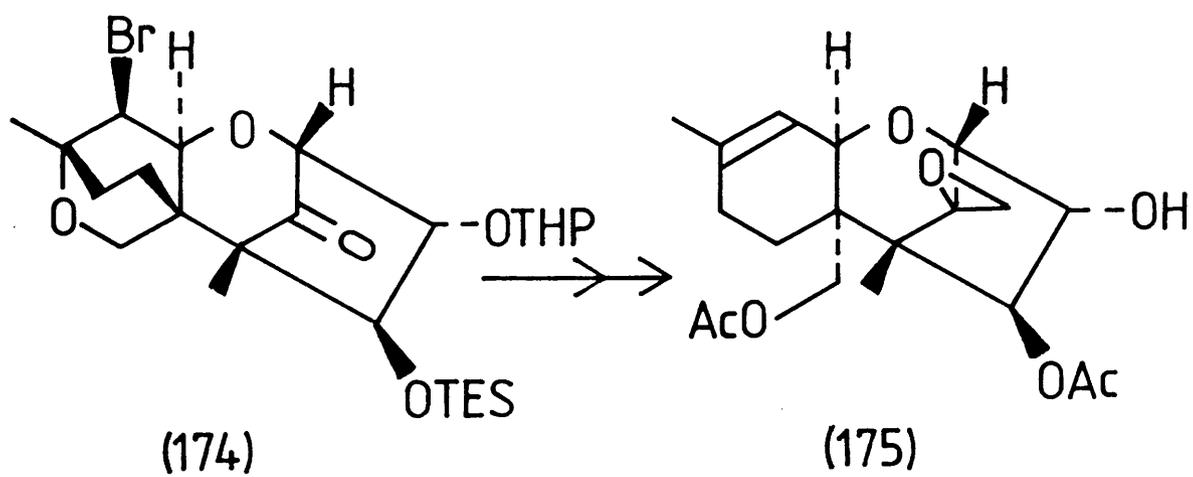
10. TRICHOTHECENE INTERCONVERSIONS

10.1 INTRODUCTION

Although widespread in distribution, provision of some trichothecenes from culture can be low yielding. Partial synthesis, from readily available trichothecenes, is an attractive alternative, carrying with it the additional possibilities of specific isotopic labelling and of analogue synthesis.

Partial synthesis may involve the following elements*:

(i)   use of protecting group chemistry, as illustrated by the conversion of anguidine (9) into 4-acetoxyscirpenediol (48)[30];

(ii)   reductive chemistry involving peripheral oxygenation, as exemplified by the conversion of anguidine into verrucarol (28) <u>via</u> Barton deoxygenation[34];

(iii)   oxidative chemistry to introduce oxygenation at peripheral sites.

Chemical manipulation involving the first two elements has been well documented. However, with the exception of allylic oxidation at C-8, methodology involving the third element has received little attention[21,27,29,37].

It was decided to attempt the synthesis of deoxynivalenol (23) from anguidine: this synthesis proved to involve all three of the above elements. Deoxynivalenol, which is arguably the most important trichothecene in terms of natural contamination of feedstuffs, has yet to succumb to either partial or total synthesis.

The synthetic plan (SCHEME 24) involved reductive elimination of the C-4 oxygen moiety by some means to give calonectrin (5) or a derivative thereof. Having established the correct level of oxygenation in the C-ring, it remained to functionalise the A-ring to give the

*See also Introduction (<u>3.3</u>)

(9)

(48)

(28)

(23)

(5)

(9)
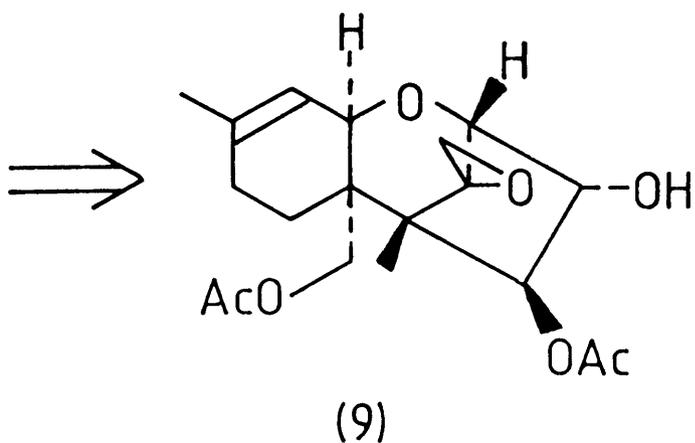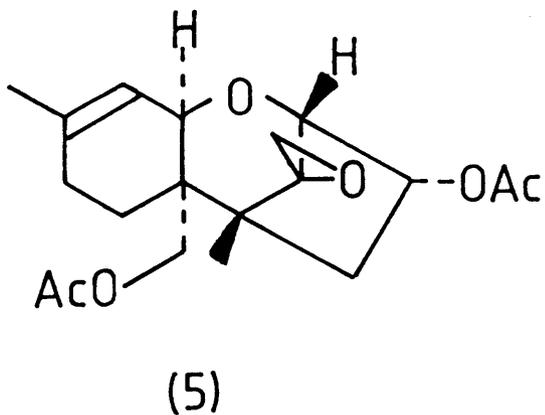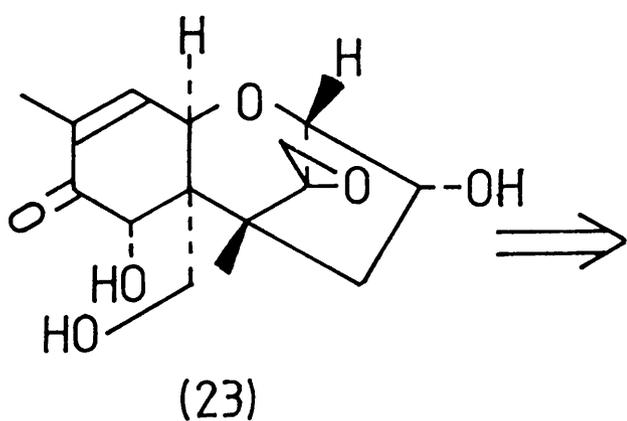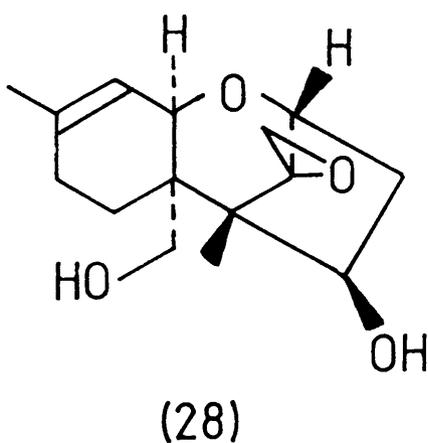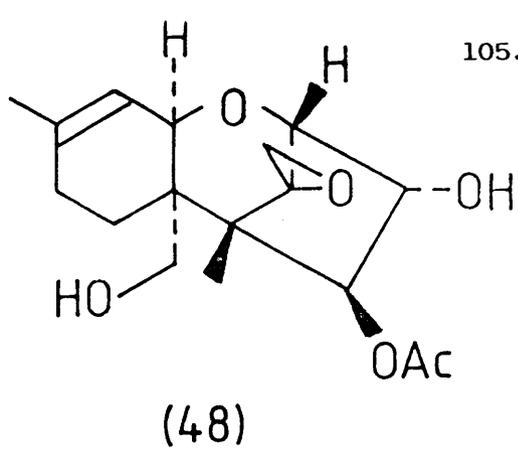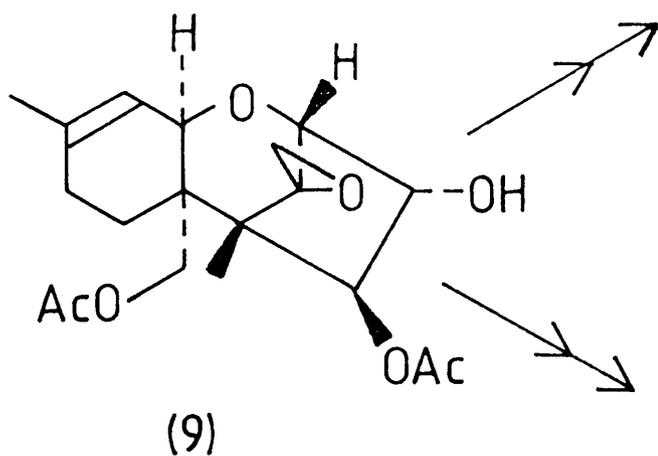
SCHEME 24

correct substitution pattern for deoxynivalenol. This necessitated selective allylic oxidation at C-8 and introduction of the 7α-hydroxyl group.

10.2 SYNTHESIS OF CALONECTRIN

Calonectrin has previously been prepared by total synthesis in racemic form[47] and by conversion, <u>via</u> a Barton deoxygenation procedure, of an anguidine derivative[36].

Tamm's partial synthesis involved formation of the THP ether of the C-3 alcohol of anguidine, followed by saponification of the C-4 and C-15 acetates to give diol (177). Regioselective acetylation of the diol to the C-15 mono-acetate and then reaction with N,N-thio-carbonyldiimidazole gave thiocarbamate (178). Treatment of this thiocarbamate with Bu_3SnH in refluxing dichloromethane gave the C-3 THP derivative of calonectrin (179). Completion of the synthesis was straightforward: THP ether cleavage using pyridinium tosylate, followed by acetylation gave calonectrin in an overall yield of 30% from anguidine. This modest yield is mainly a reflection of the regioselectivity in acetylation and the THP ether cleavage step (SCHEME 25).

By use of 4-acetoxyscirpenediol (48), which can be obtained in good yield from culture, it was envisioned that an improvement on this synthesis could by achieved since 4-acetoxyscirpenediol is already suitably functionalised for easy access to C-4. The C-4-C-5 bistriethylsilyl ether (145) was prepared in near quantitative yield by treatment of the diol with triethylsilyl chloride and DMAP in pyridine. However, selective hydrolysis of the C-4 acetate using K_2CO_3 in aqueous methanol gave a poor yield of the C-4 mono-ol (146) since the triethylsilyl groups were also partially cleaved. Additionally, preliminary studies on C-4 thioester formation using phenylchlorothiocarbonate looked unpromising since the residual acid
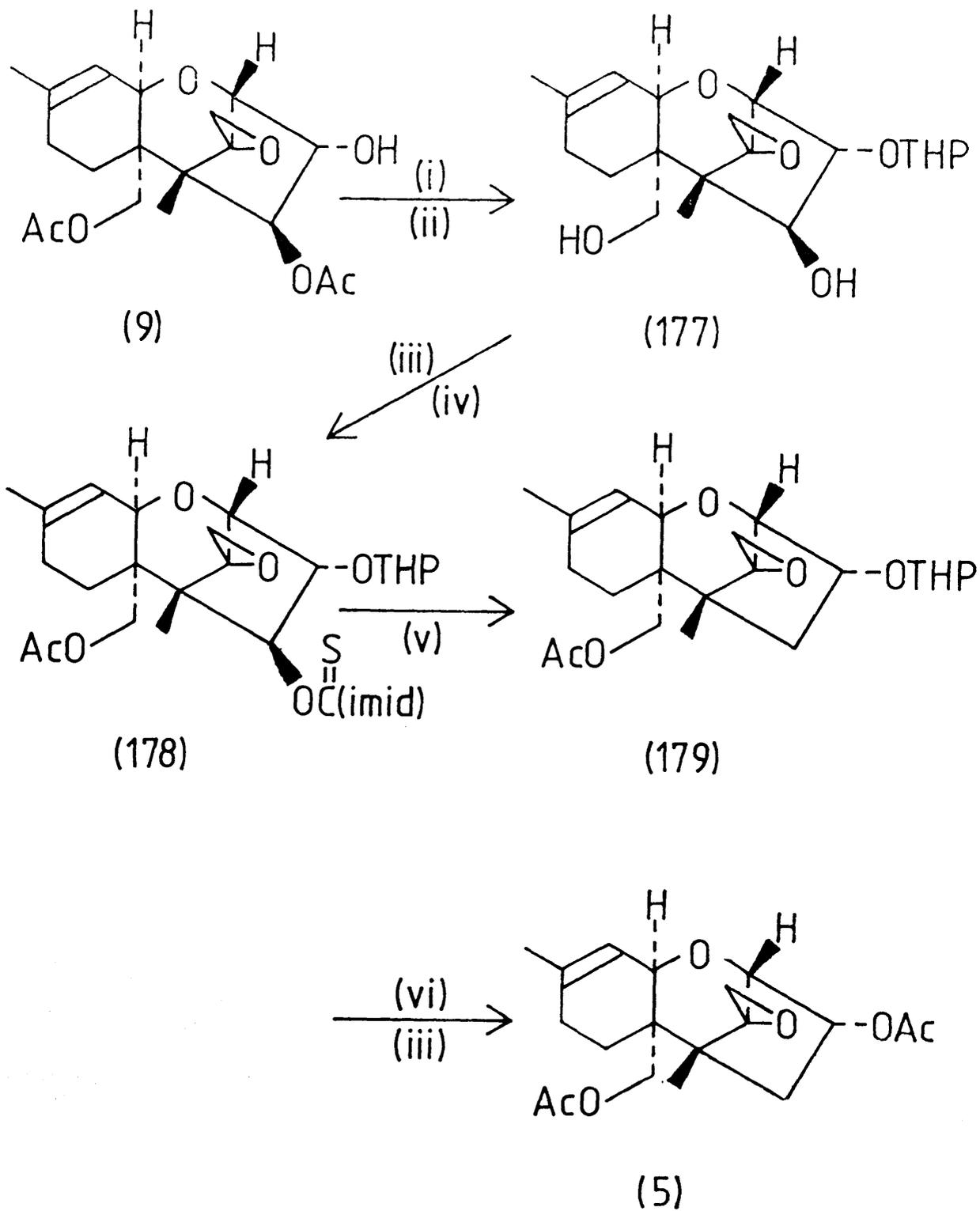
SCHEME 25

(i) DHP, PPTS, CH$_2$Cl$_2$; (ii) NaOH, MeOH;
(iii) Ac$_2$O, py., CH$_2$Cl$_2$; (iv) (imid)$_2$C=S, C$_2$H$_4$Cl$_2$;
(v) Bu$_3$SnH, AIBN; (vi) PPTS, MeOH.
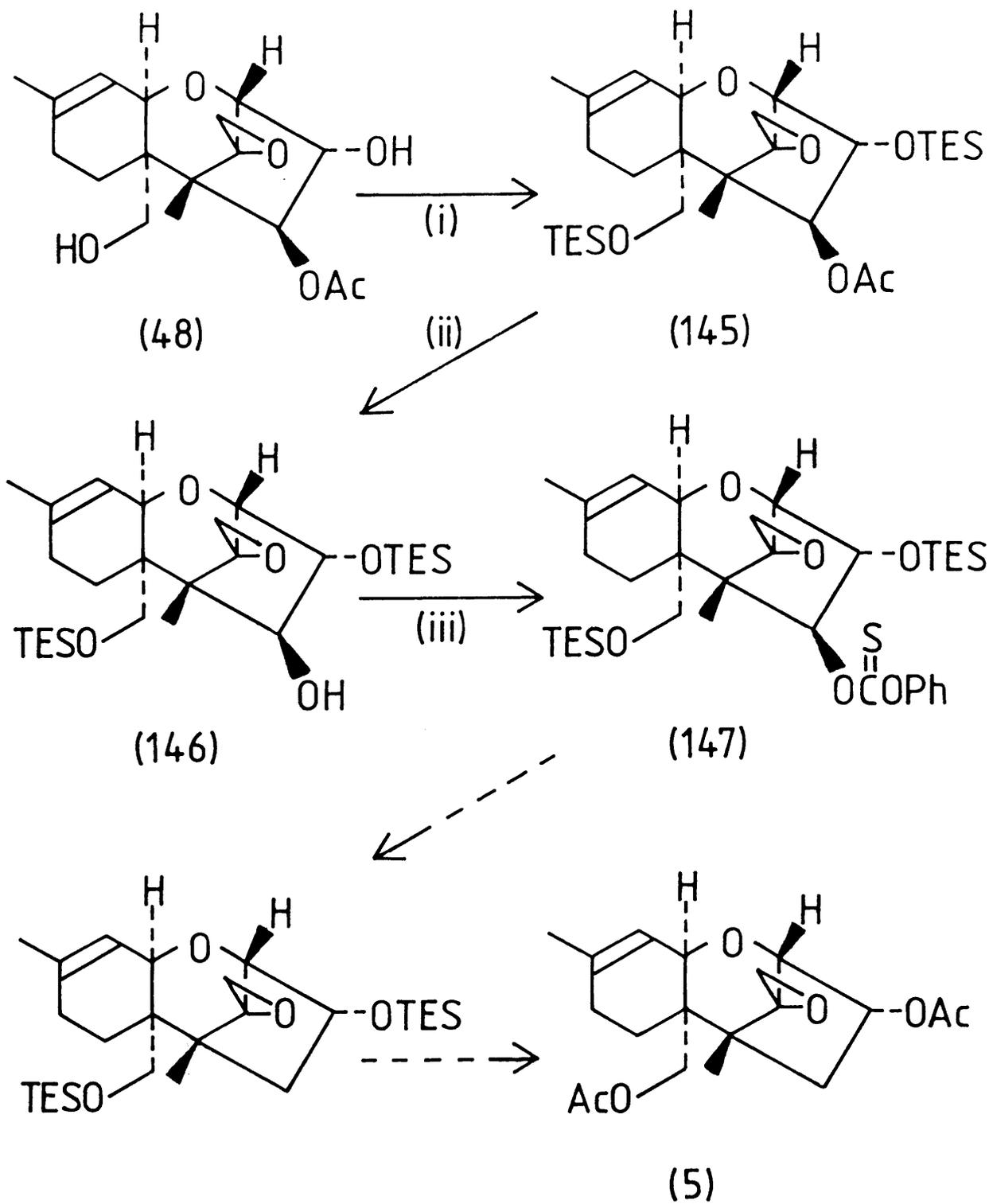
(i) TESCl, DMAP, py.; (ii) K₂CO₃, MeOH;
(iii) PhOCSCl, DMAP, py., CH₂Cl₂

chloride had a similar polarity to its derived thioester (147). These

problems could have certainly been overcome by use of a different

C-3-C-15 protecting group and by use of a different "Barton ester"

eg a thiocarbonyl-imidazole derivative. However, a re-examination

of the literature provided the basis of a novel approach to the synthesis

of calonectrin.

Sigg et al[27] found that upon treatment with sodium methoxide in

refluxing methanol the C-3-C-4 bismesylate (56) gave solely the C-3

ketone (57). In addition, Kraus[47] found that reduction of the C-3

ketone (180) was highly stereoselective, giving the product with

natural C-3 stereochemistry (181), hydride being delivered from the

less hindered exo face of the [3,2,1] bicyclic subunit.

Sigg's observation can be rationalised on the basis of an ElcB

elimination, whereby the more exposed C-3 proton is abstracted selectively

by methoxide ion. The anionic intermediate (182) then collapses to give

the C-3 mesyl enol ether (183), which on in situ hydrolysis gives the

C-3 ketone (57). The rigid ring structure rules out the possibility

of an E2 trans elimination as a trans-coplanar system cannot be attained

Indeed, by analogy with Cristol's work on cis and trans 2,3-dichoro-

norbornanes[100], cis elimination may be more facile than trans elimination.

Additionally, the presence of a α-mesyl group may serve to stabilise

the formation of a carbanion due to its electron-withdrawing nature:

Cristol found that dehydrochlorination of exo-2-p-toluenesulphonyl-

endo-3-chloronorbornane (184) showed a rate enhancement of up to 8

orders of magnitude over the corresponding dichloro derivative (185).

In order to prepare a suitable 3,4 bismesylate it was necessary

to protect selectively the C-15 alcohol. This was readily achieved by

hydrolysis of anguidine (9) to scirpenetriol (7)[27], followed by

treatment of the triol with N-bromosuccinimide in MeCN[30,44,45,47]

to give the bridged bromo-ether diol (134). Treatment of the diol

(56) (i) (ii) → (57)

(180) (iii) → (181)
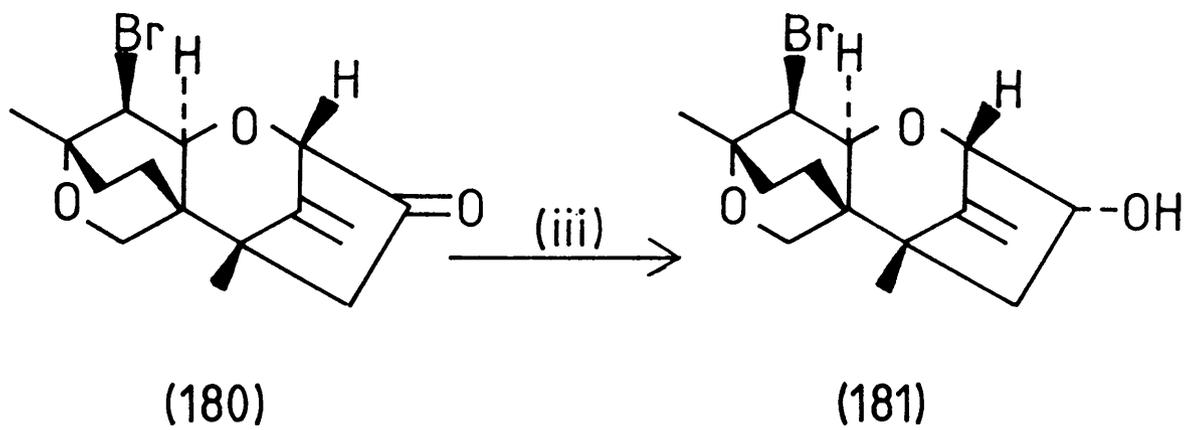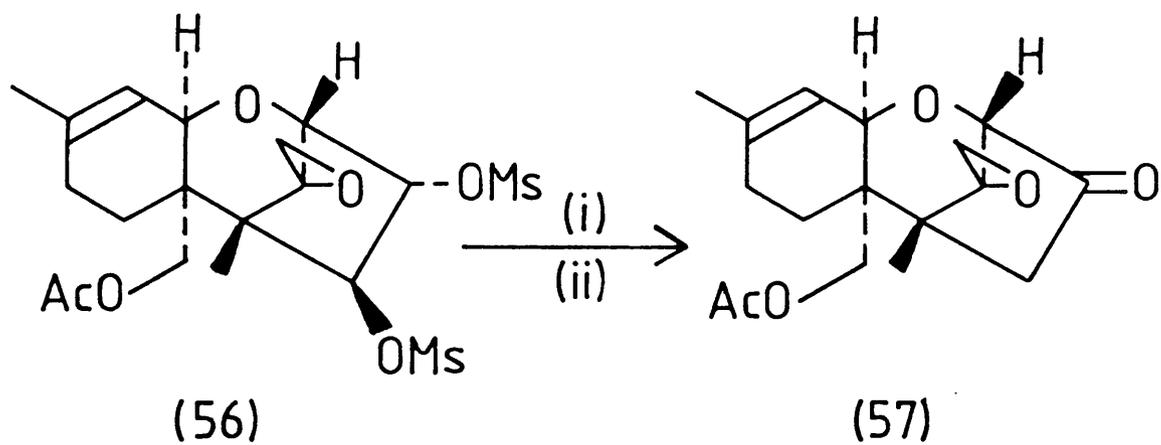
(i) NaOMe, MeOH; (ii) Ac₂O, py.;
(iii) NaBH₄, MeOH.

(56)   (182)   (183)

(57)

(184)   (185)
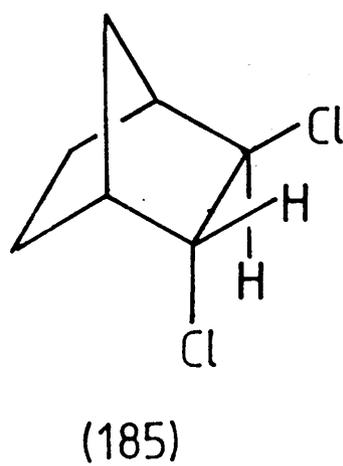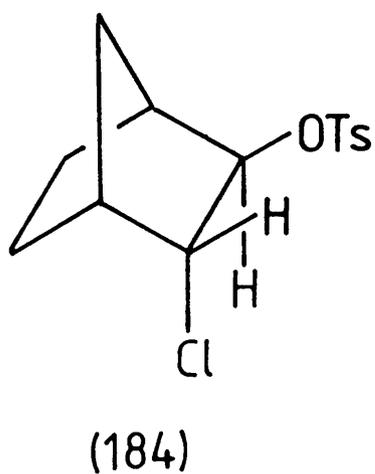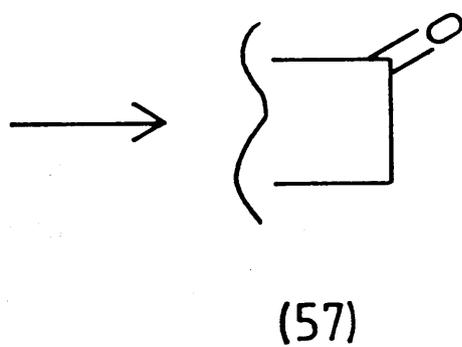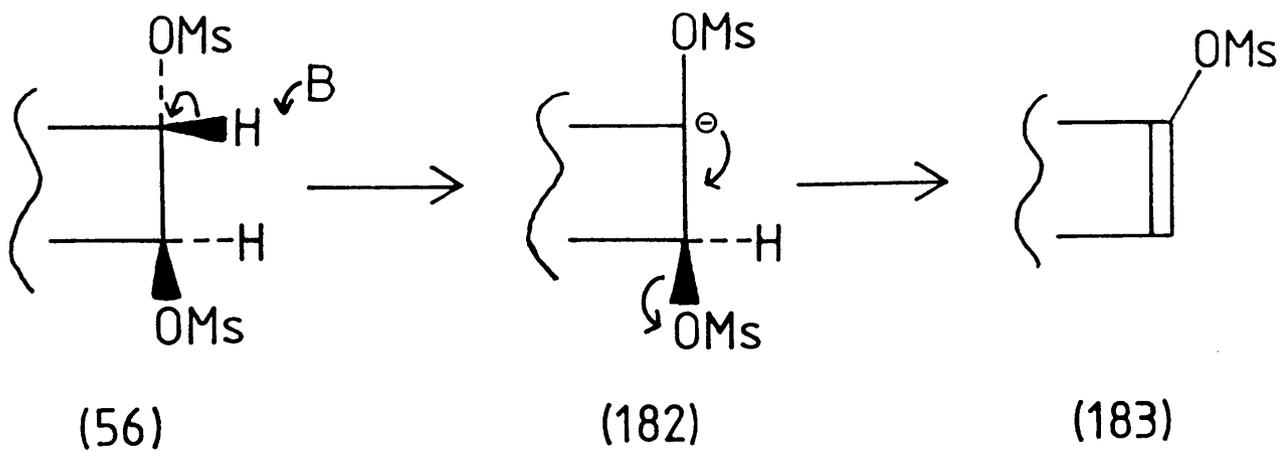
with methanesulphonyl chloride in pyridine[27] gave the bismesylate (135)

in an overall yield of 92% from anguidine.

Treatment of the bismesylate with sodium methoxide in refluxing

methanol gave the expected C-3 ketone (136) in 83% yield (ν_{max} 1770cm^{-1};

^{1}H n.m.r. δ2.25, ABq, J 9 Hz, H-4).

Quantitative and stereoselective reduction of the ketone was achieved

by treatment with NaBH$_4$ in aqueous methanol. The stereochemistry at C-3

was assigned by ^{1}H n.m.r. spectroscopy on the basis of the observed

double-double-doublet at δ5.20 in the derived C-3 acetate (138),

analogous with the published spectrum of calonectrin. Such a spectroscopic

result was also anticipated from the ^{1}H n.m.r. spectra of the

bicyclo[3,2,1]octanols prepared for epoxide deoxygenation studies which

also showed a double-double-doublet for the α-epimers but only a

double-doublet for the β-epimers.

Completion of the synthesis of calonectrin was straighforward.

Acetylation of the borohydride reduction product (137), restoration

of the C-15 alcohol and 9,10-double bond by treatment of the bromoether

with zinc/silver couple[99] and finally acetylation of the C-15 alcohol

gave calonectrin in an overall yield of 55% from culture products.

This material proved to be spectroscopically identical to natural

calonectrin[32].

10.3 SYNTHESIS OF DEOXYNIVALENOL FROM CALONECTRIN

Calonectrin (5) possesses the correct degree of oxygenation in the

C-ring for the synthesis of deoxynivalenol (23). Further elaboration

requires selective allylic oxidation at the C-8 methylene group followed

by introduction of the 7α hydroxy substituent.

Functionalisation at C-8 has been accomplished by treatment of

anguidine with SeO$_2$, which gave the 8β alcohol (65) in 39% yield[29].

Subsequent oxidation of the allylic alcohol using PCC gave the enone

(9)   (7)

(134) R=H
(135) R=Ms   (iii)

(136)

(137) R=H
(138) R=Ac   (vi)

(5)
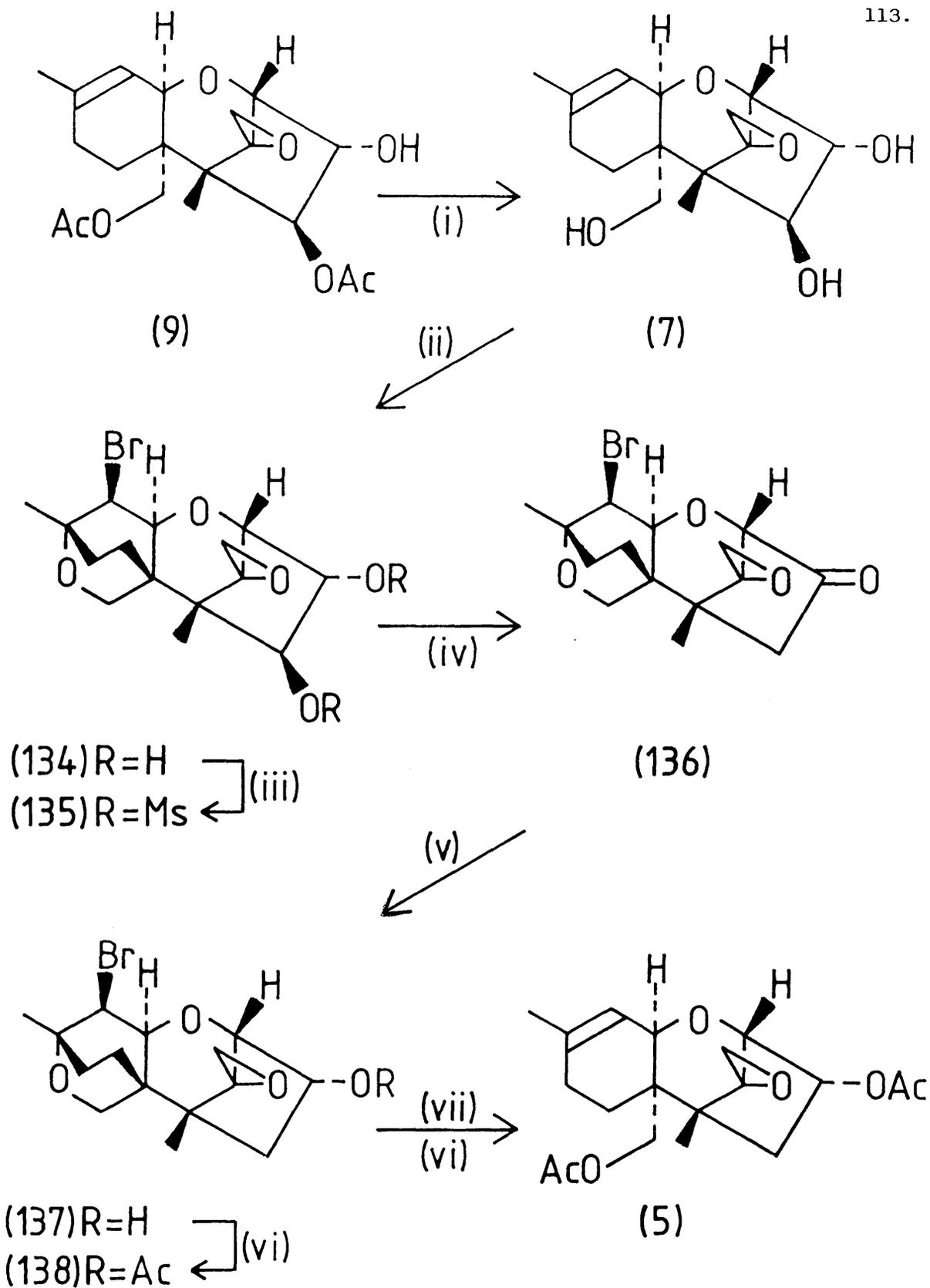
(i)NaOH;(ii)NBS,MeCN;(iii)MsCl,py.;
(iv)NaOMe,MeOH;(v)NaBH₄,MeOH;
(vi)Ac₂O,py.;(vii)Zn(Ag),THF,EtOH,Et₂O.

(5) → (23)

(9) →(i) (65)

→(ii) (66)
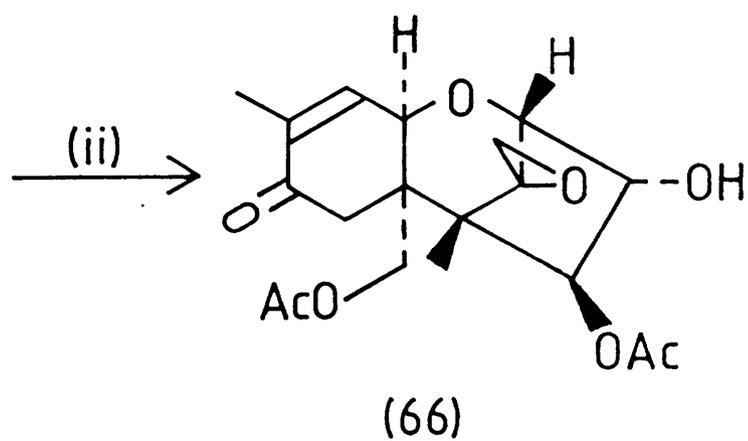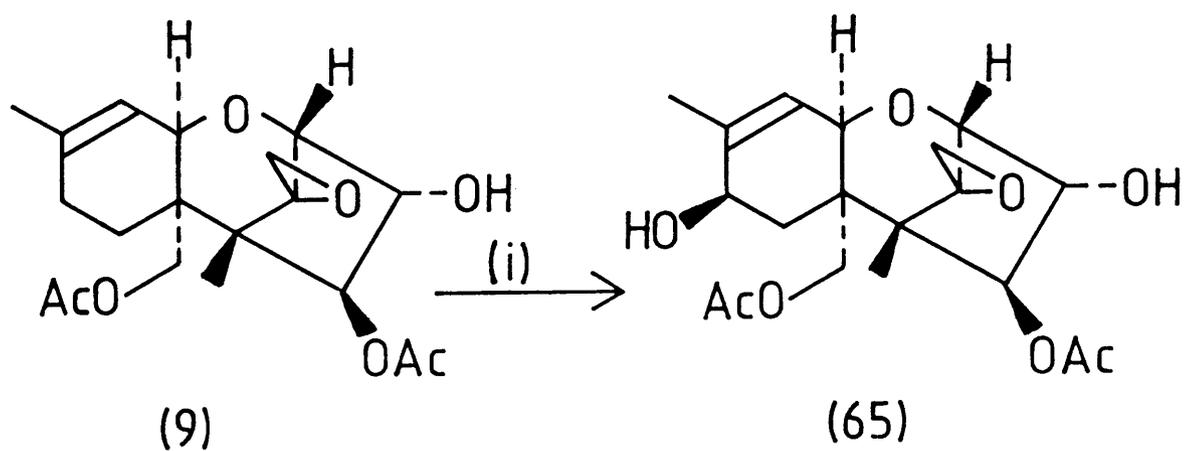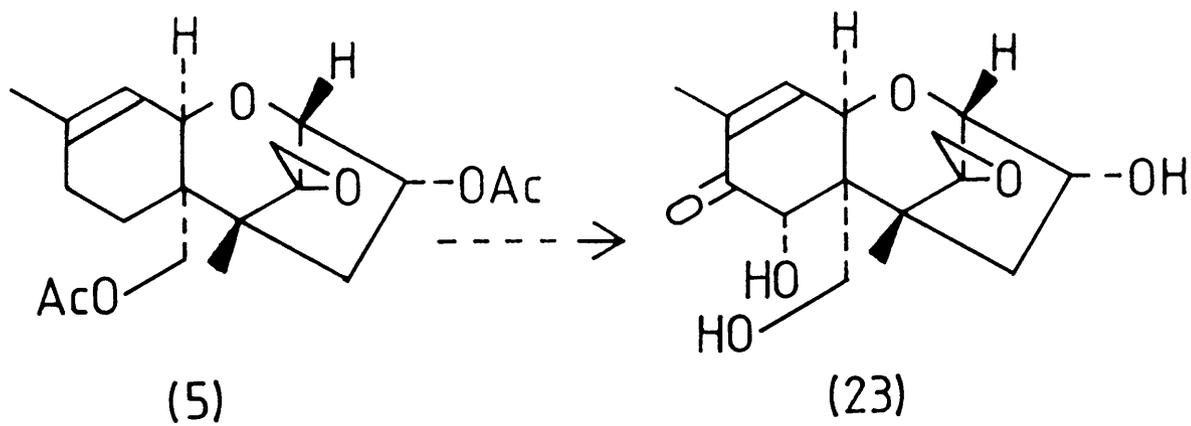
(i)SeO₂;(ii)PCC.

(66) in an overal yield of 31% from anguidine.

Allylic oxidation by treatment of anguidine with N-bromosuccinimide, followed by silver trifluoroacetate has been reported to give a mixture of 8α and 8β alcohols (16,65) in a 24% overall yield[29]. Acetolysis of the C-8 bromo epimers gave the C-8 acetates (71,72) in a 37% overall yield from anguidine[37]. Application of a multistep procedure by these methods on calonectrin would, therefore, be unlikely to give the desired enone in over 35% yield.

It was thus desirable to establish the enone in a single high yielding step. Use of t-butyl chromate on triacetoxyscirpene (63) has been reported to give the enone (64) in under 20% yield[27]. It was decided to investigate other chromium based oxidants, in particular the dipyridine.CrO_3 complex (Collins' reagent)[101]. Treatment of triacetoxy-scirpene with 60 equivalents of freshly prepared Collins' reagent in dry dichloromethane for 4 days gave the enone (64) in an excellent yield of 60%. Similar yields could be obtained upon oxidation of calonectrin to the desired enone (139) (^{1}H n.m.r. δ6.54, dq, J 1.5, 5.8Hz, H-10; λ_{max}^{EtOH} 225 nm (ϵ 8900)). However, on one occasion enone (139) was obtained in 74% after stirring overnight. This suggests that all reagents must be scrupulously dry and that the Collins' reagent should be used as soon as is prepared — even careful storage of the reagent in a desiccator for a few days leads to diminished activity.

The mechanism of this oxidation is believed to involve abstraction of an allylic and axial hydrogen atom to give allylic radical (186). In the case of trichothecene only one hydrogen abstraction is possible — abstraction of a C-16 hydrogen would give a less stable primary radical. The allylic radical can then be oxidised further to the isomeric enones (187) and (188). No trace of (188) was observed. This may be due to the fact that in going to (187) the favourable overlap of the π-system with the C-O σ* orbital would be destroyed in going to (188).

$$(9) \xrightarrow{\text{(i)}} (69)\,R_1=Br\ \ R_2=H$$
$$(70)\,R_1=H\ \ \ R_2=Br$$

(65) $R_1=OH$ $R_2=H$
(16) $R_1=H$ $R_2=OH$

(71) $R_1=OAc$ $R_2=H$
(72) $R_1=H$ $R_2=OAc$
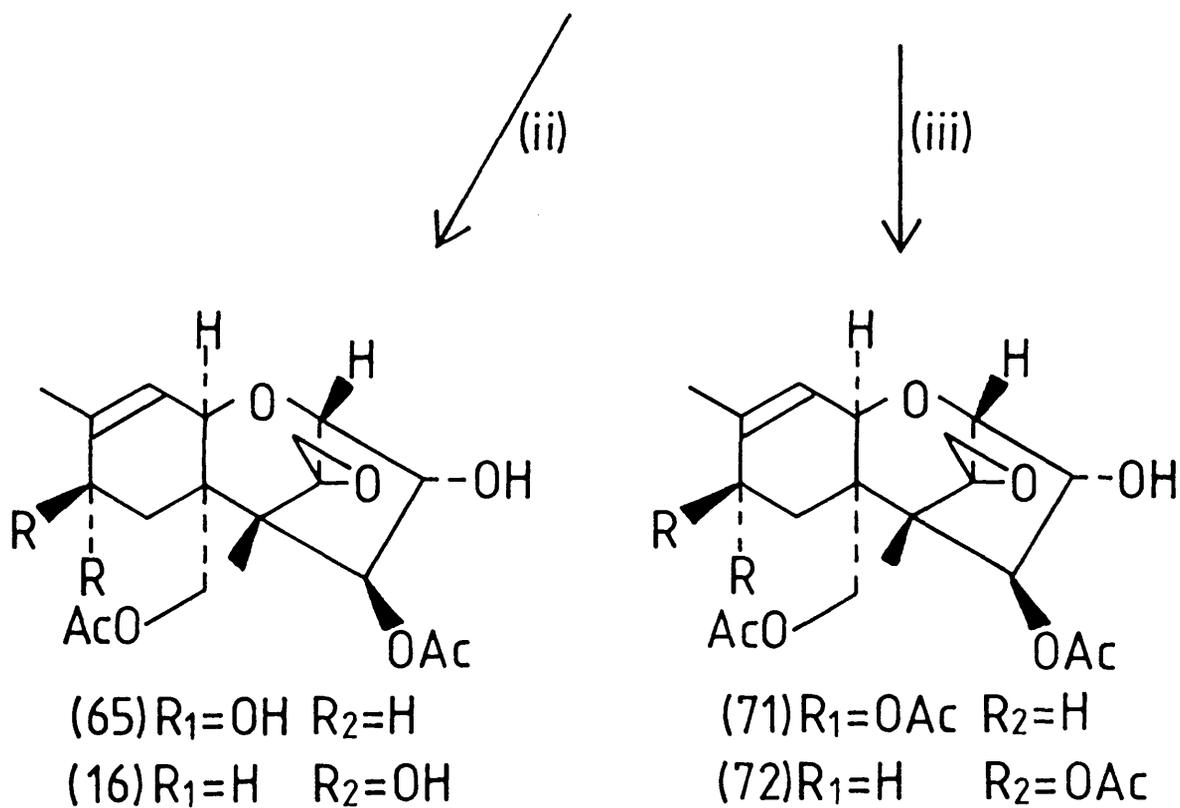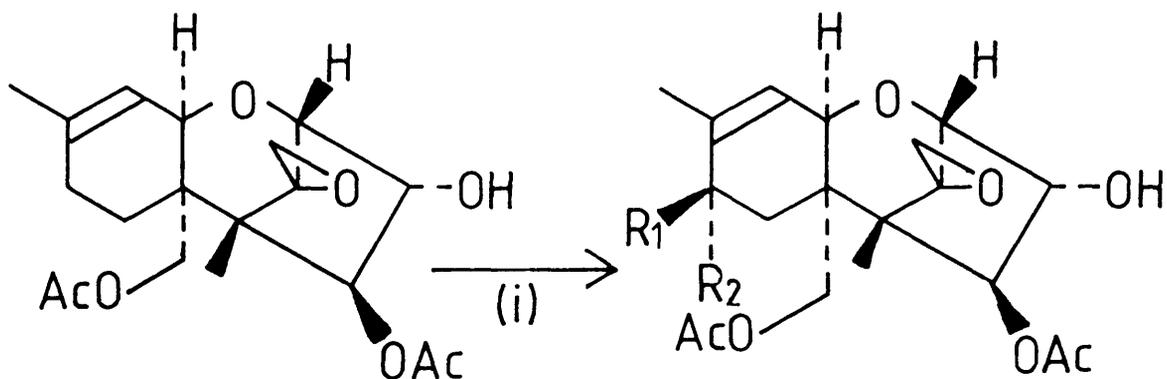
(i) NBS, CH_2Cl_2; (ii) CF_3COOAg; (iii) AgOAc, AcOH.

116.

(63)   (i)   (64)

(5)   (i)   (139)

(i) $py_2.CrO_3, CH_2Cl_2$

(186)

(187)

(188)
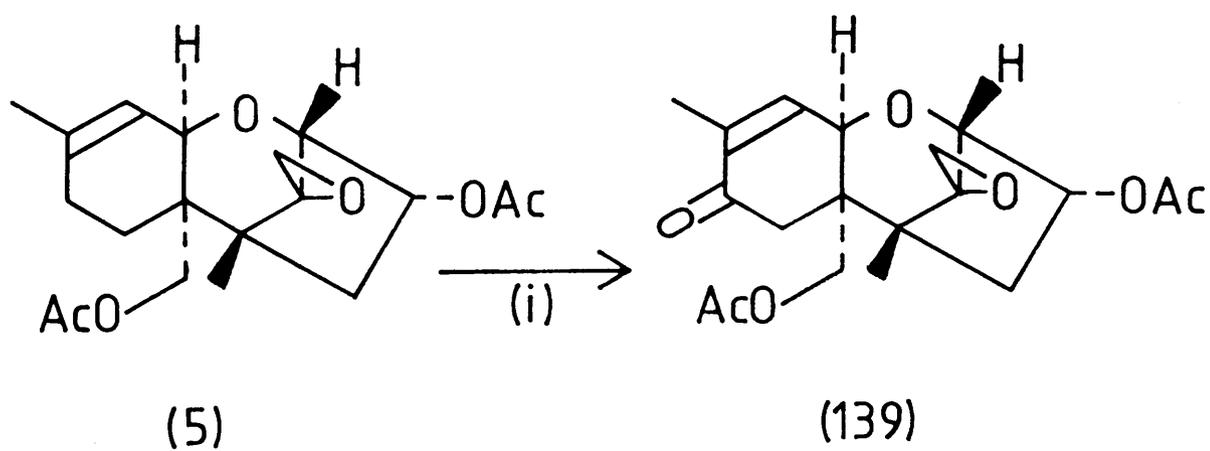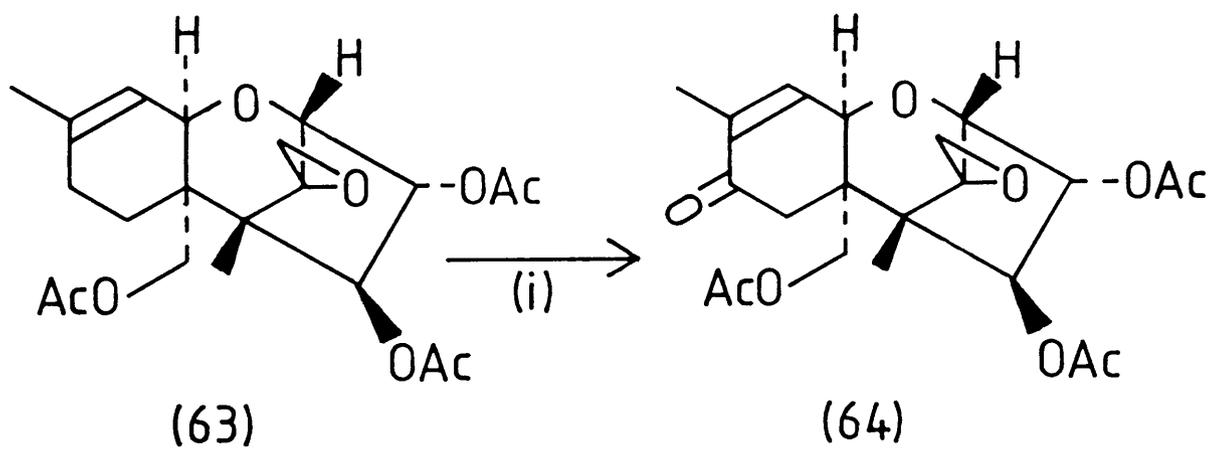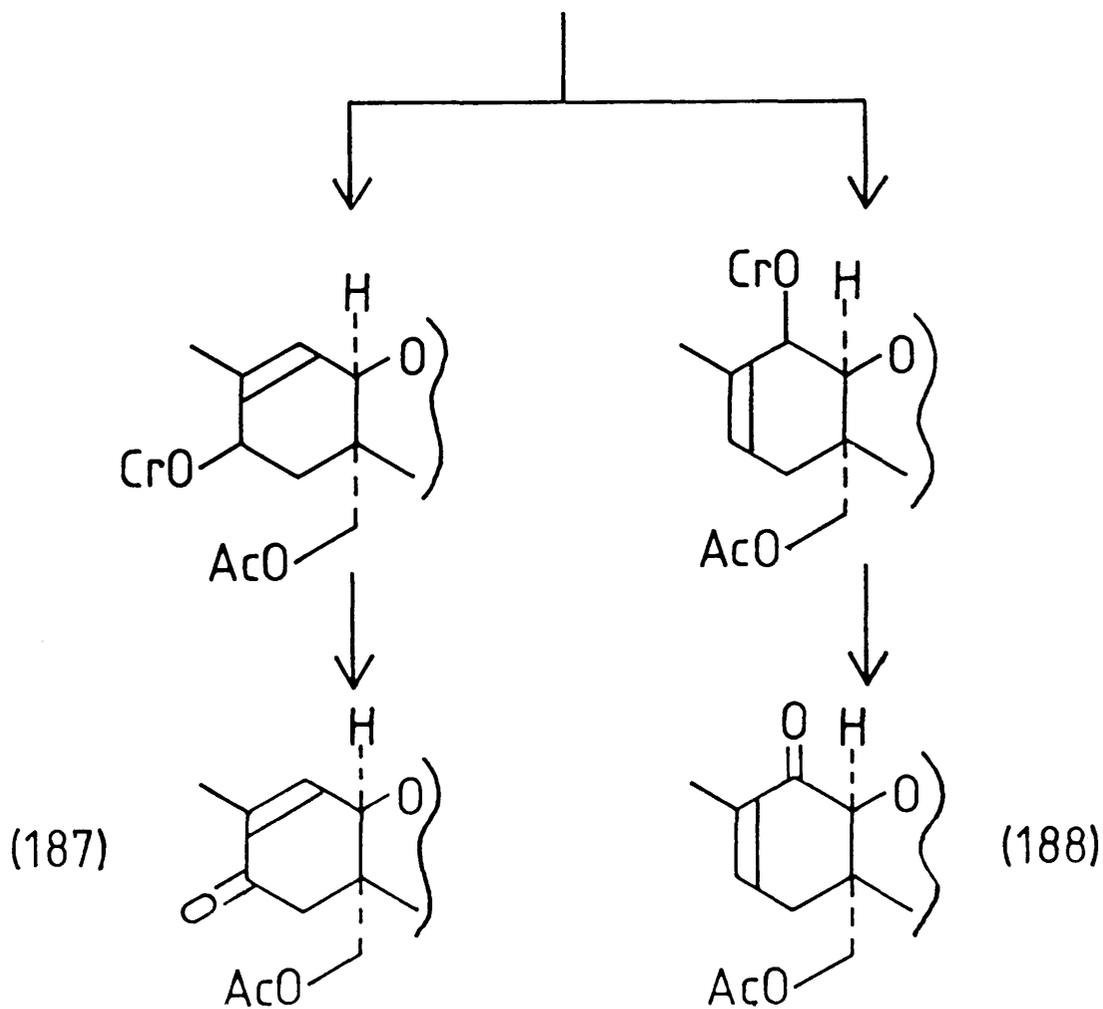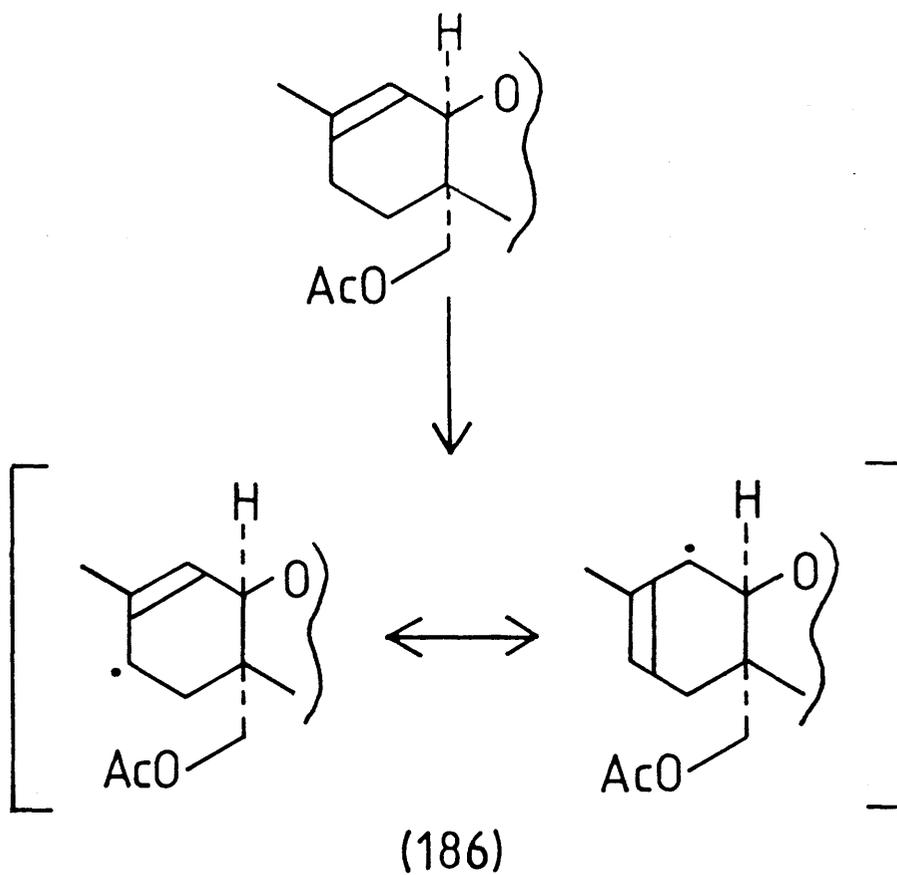
With the A-ring enone now suitably established, completion of the synthesis of deoxynivalenol requires introduction of the 7α hydroxyl moiety. It was decided to do this by conversion of a suitably protected C-3/C-15 species to the silyl enol ether, followed by selective epoxidation with m-chloroperbenzoic acid — a method discovered by Brook[102] and developed by Rubottom[103].

Hydrolysis of the C-3 and C-15 acetates using K_2CO_3 in aqueous methanol, followed by silylation of the diol using trimethylsilyl chloride and pyridine gave the bis-TMS ether (141). Addition of this compound to three equivalents of LDA in THF at $-78°C$, with *in situ* trapping of the derived enolate by TMSCl[104], gave the silyl enol ether (142) (^{1}H n.m.r. δ5.70, br.d , J 6Hz , H-10). Treatment of this silyl enol ether with m-chloroperbenzoic acid in hexane at $-20°C$ gave a 1:2 mixture of the desired 7α-silyloxy ketone (143) (the tris-TMS ether of deoxynivalenol) and the ring opened product (144) in a combined yield of 75%. The products obtained can be rationalised by consideration of the mechanism of this oxidation.

It is believed that epoxidation of the silyl enol ether gives a mixture of α- and β- silyloxyepoxides. The β-silyloxyepoxide predominates due to greater accessibility of the epoxidising agent to the β-face. The respective silyloxyepoxides then undergo ring opening in the presence of a cataytic amount of acid to give the oxocarbenium species (189) and (190). The oxocarbenium species (189), from the α-epoxide, undergoes a facile 1,4-silyl shift to give the desired 7α-silyloxyketone (143). Rather than undergo a facile 1,4-silyl shift, the 7β hydroxyl group in the oxocarbenium species (190), arising from β-face epoxidation, instead attacks the proximate 12,13-epoxide to give the 6-membered ether (144), after loss of the C-8 silyl group in hydrolytic work-up. No 1,4-silyl shift was observed to take place; such a process is further disadvantaged by the rigid ring structure

(139)

(i),(ii)

(141)

(iii)

(142)

(iv)

(143)

1 : 2

(144)
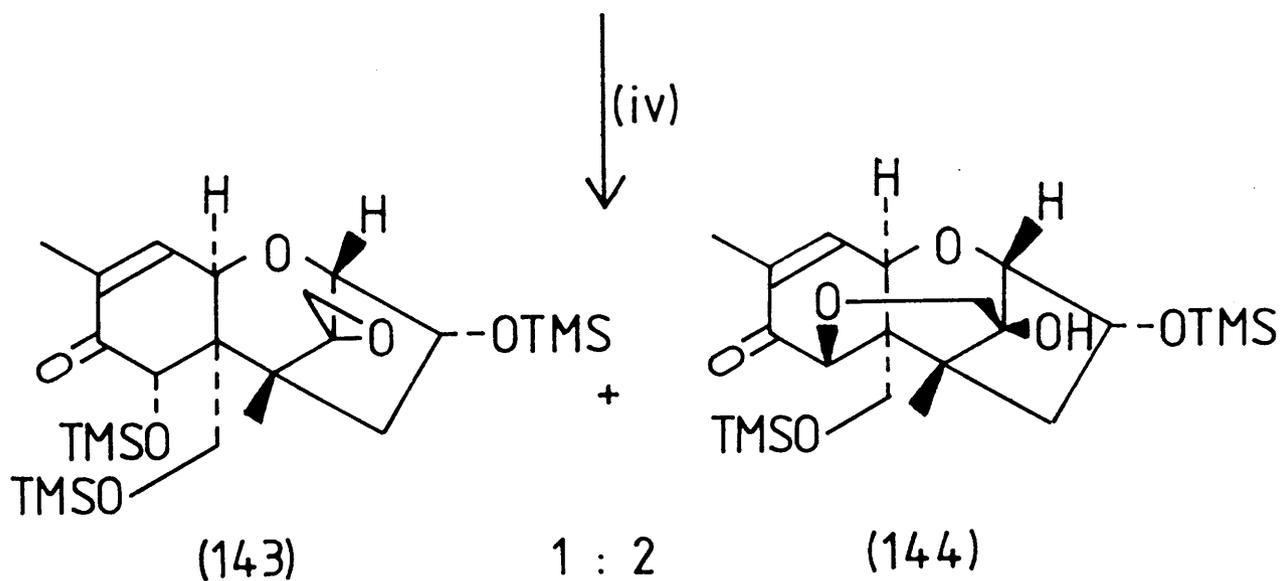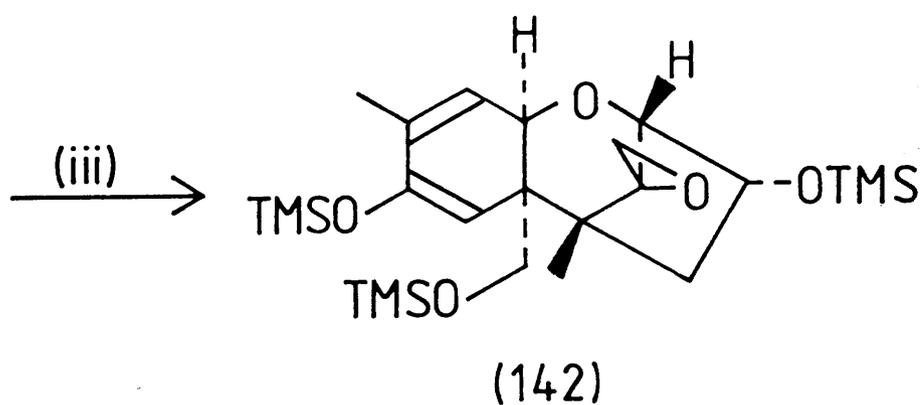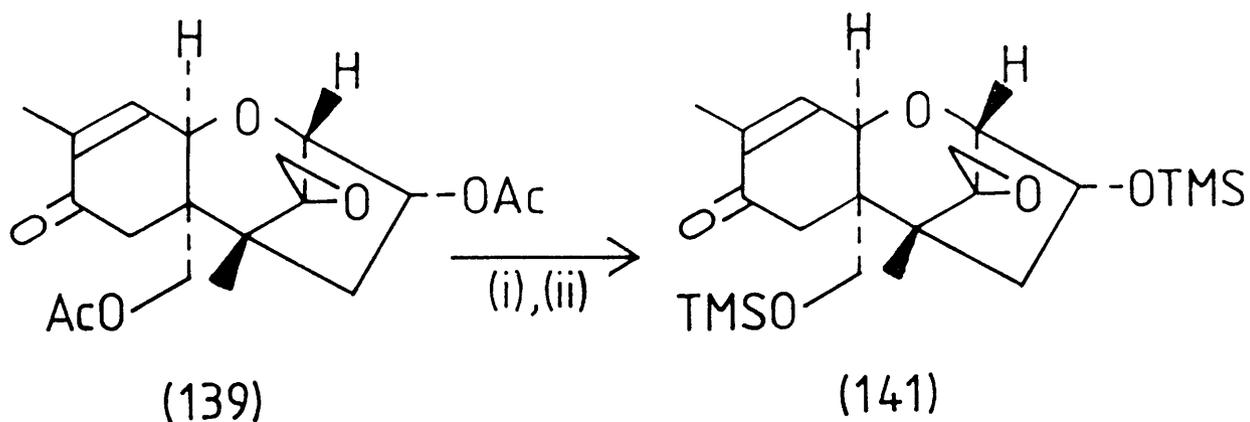
(i)K₂CO₃,MeOH;(ii)TMSCl,py.;
(iii)LDA,TMSCl,THF;(iv)mcpba,hexane.

(189)

(190)

(143)

(144)

(190)
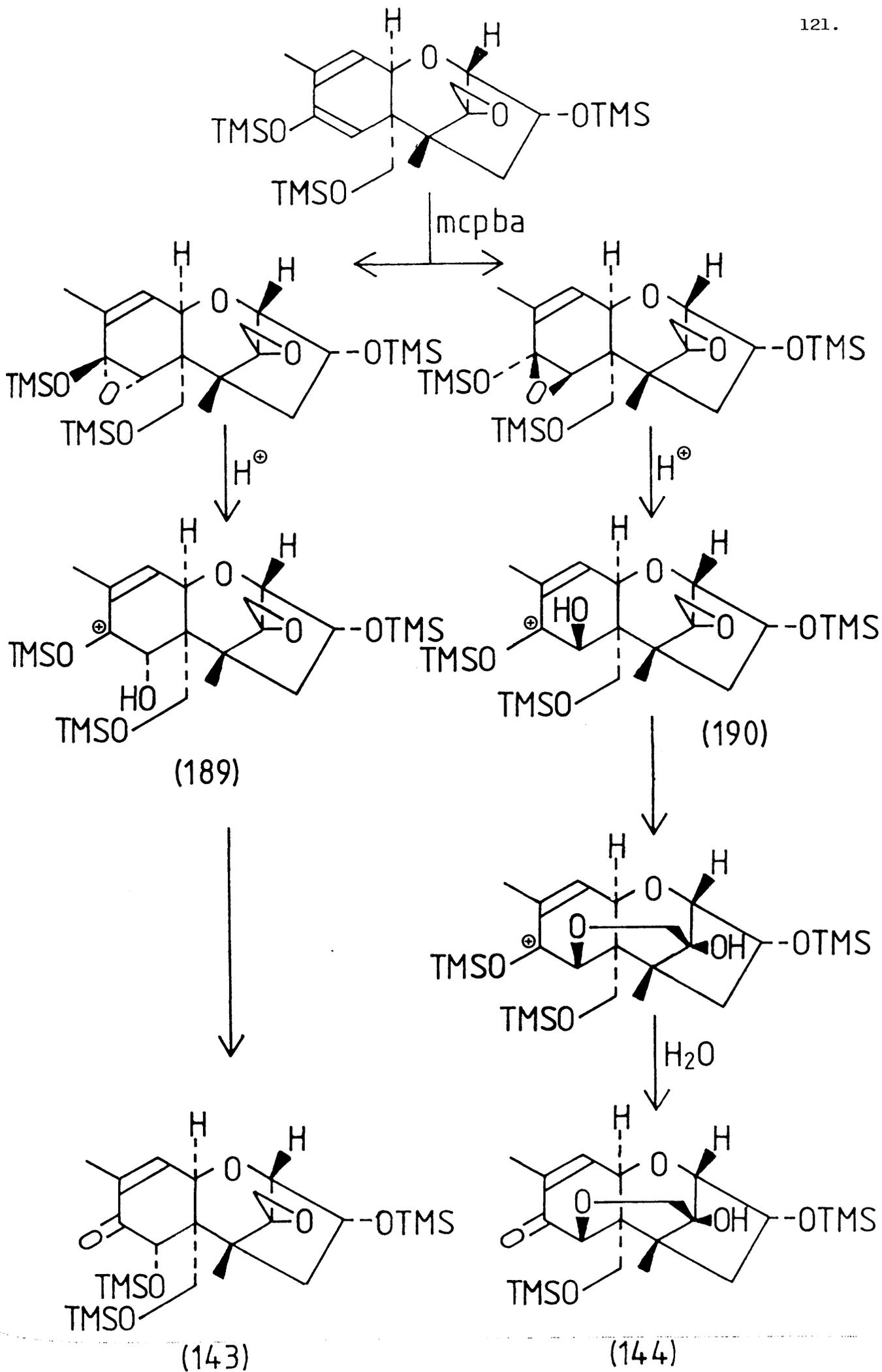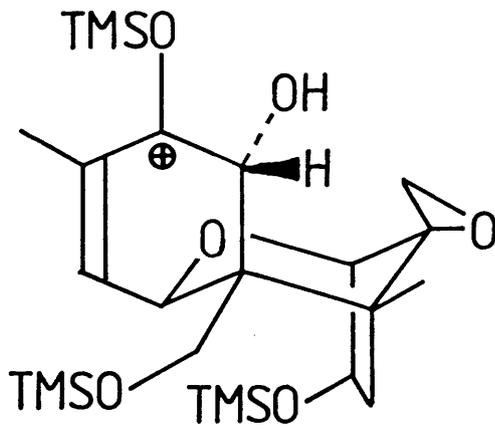
holding the β-hydroxyl too far away from the C-8 silyloxy group for easy migration.

The presence of the pyran ring was evidenced by a [1]H n.m.r. coupling constant of 11.5 Hz and a shift of δ 3.93 and 3.60 for the C-13 AB quartet in comparison with 4-5 Hz and δ3.0-2.5 for the epoxide.

Evidence for the presence of an intermediate silyloxyepoxide has recently been provided independently by Davis and Paquette.

Davis treated the silyl enol ether of isobutyrophenone (191) with 2-(phenylsulphonyl)-3-(p-nitrophenyl) oxaziridine (193) – an aprotic and neutral epoxidising agent – to give the silyloxyepoxide (192) which was stable at room temperature. Rearrangement of the silyloxyepoxide could be effected by treatment with a trace of pTSA[105].

Amazingly, Paquette's silyloxyepoxide (195), obtained upon treatment of (194) with buffered m-chloroperbenzoic acid, was crystalline and therefore amenable to x-ray crystallographic analysis. Rearrangement of the silyloxyepoxide in this case could also be effected by treatment with a catalytic amount of benzoic acid[106].

The synthetic tris-TMS ether of deoxynivalenol (143) proved to be identical by GCMS with a sample prepared from natural deoxynivalenol[107]. Quantitative cleavage of the TMS ether groups in (143) was achieved using HF in aqueous MeCN. The synthetic deoxynivalenol (23) was characterised fully as its triacetate (120)[108,109].

In conclusion an efficient synthesis of calonectrin has been developed and the first synthesis of deoxynivalenol has been achieved. Similar methodology on the triacetoxyscirpene enone (64) should give entry into the neosolaniol series of compounds (196). Additionally enone (64) is suitably functionalised for possible, further elaboration to T-2 Toxin (14). Indeed it should now be possible to obtain a wide range of trichothecenes by partial semi-synthesis. Roush's recent synthesis of C-13 labelled anguidine[40] also means that calonectrin

25

and deoxynivalenol, _inter alia_, can now be synthesised in specifically

radiolabelled form.

(191) → (193) → (192) → pTSA → Ph-C(=O)-C(CH₃)₂-OH

(193) PhSO₂N—CHPh-NO₂-p (with O bridging N and CH, forming oxaziridine)

(194) → (i) → (195)

(195) → (ii) → products
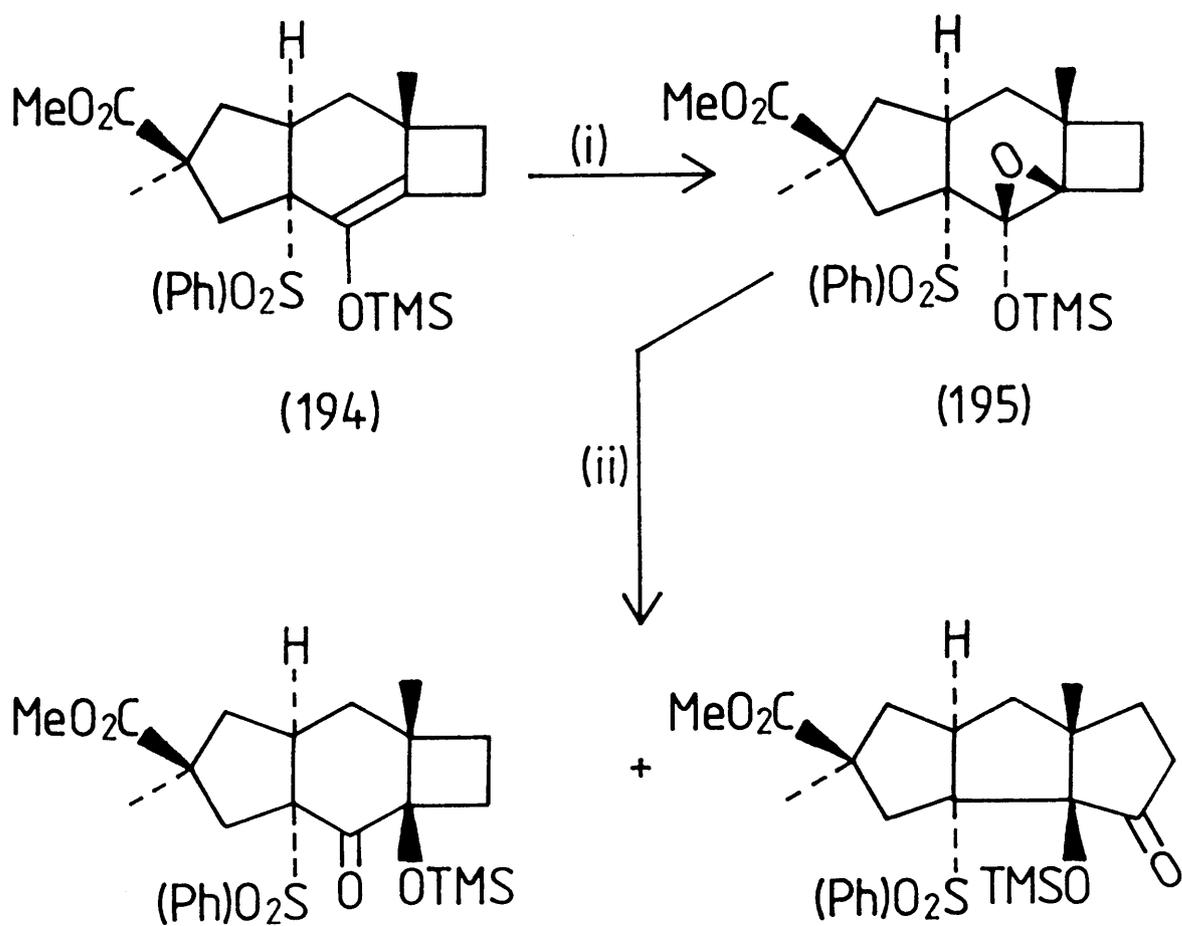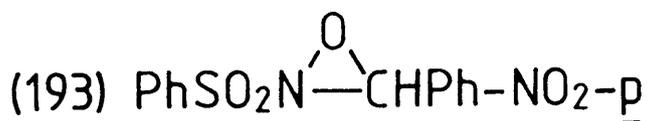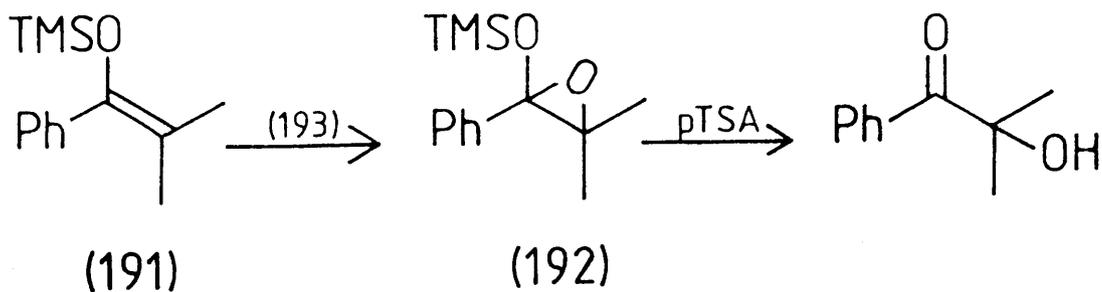
(i) mcpba, buffer; (ii) PhCO₂H, CH₂Cl₂.

(143) → (23)
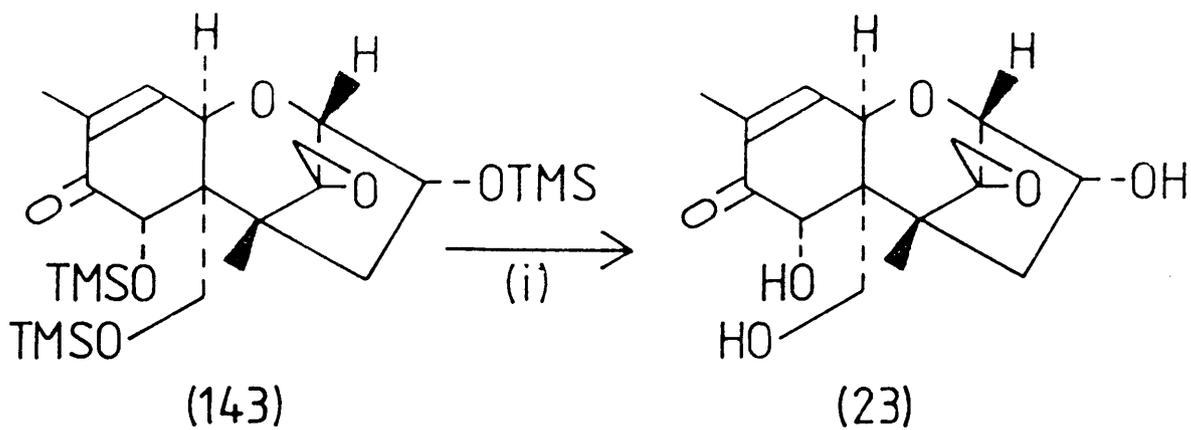
(i) HF, MeCN, H₂O;

$$\text{(i) HF, MeCN, H}_2\text{O;}$$
$$\text{(ii) Ac}_2\text{O, Et}_3\text{N, DMAP,}$$
$$\text{CH}_2\text{Cl}_2.$$

(120)

(64)

(196)

(14)

EXPERIMENTAL
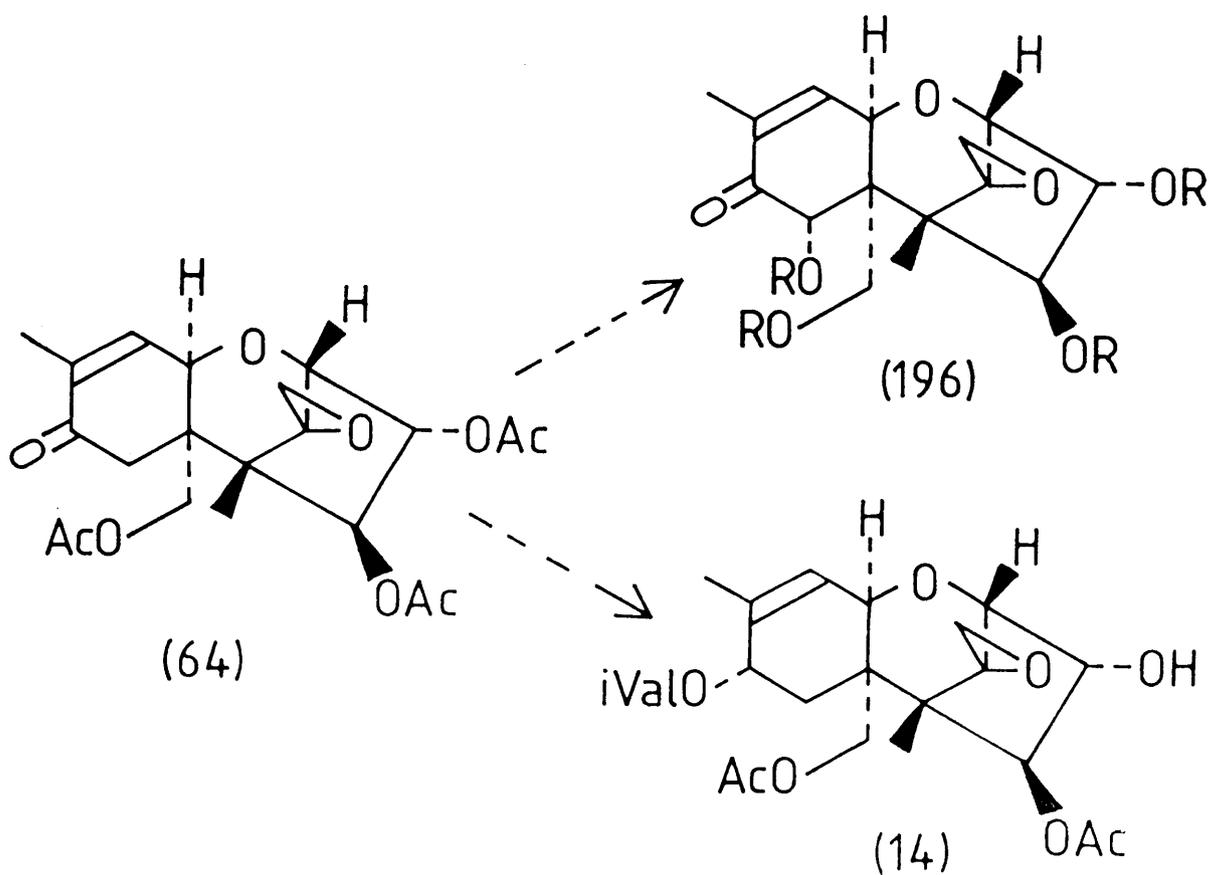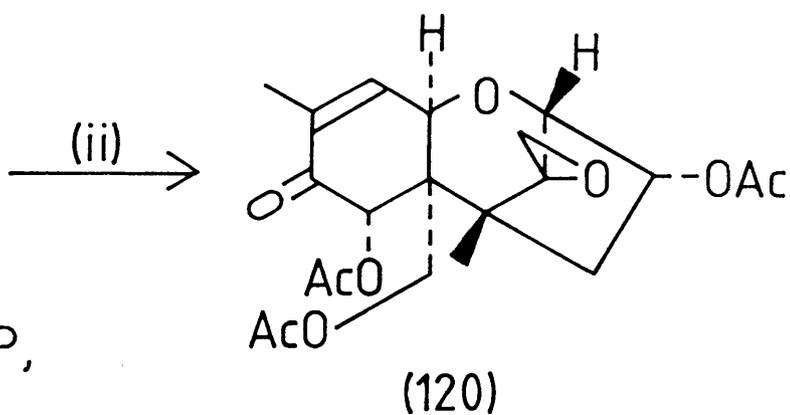
INSTRUMENTATION

Melting points were determined on a Kofler hot stage melting

point apparatus and are uncorrected. Bulb to bulb distillations

were carried out on a Büchi GK3-50 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 90 MHz,

or a Varian XL100 spectrometer operating at 100 MHz or on a Bruker WP

200 SY spectrometer operating at 200 MHz. [13]C n.m.r. spectra were

recorded on either a Varian XL100 spectrometer operating at 25 MHz or

a Bruker WP 200 SY spectrometer operating at 50 MHz. Infra-red spectra

were determined on a Perkin-Elmer 580 spectrometer, ultra-violet

spectra on a Perkin-Elmer 550SE spectrophotometer and optical rotations

on an Optical Activity AA-100 auto-digital polarimeter. Low and high

resolution mass spectra were recorded on VG updated MS 12 and MS 902

instruments respectively. Elemental analyses were performed on a

Carlo Erba 1106 elemental analyser. Instruments and conditions used

for GLC and GCMS studies are described in the relevant experimental

sections.

PURIFICATION OF CHEMICALS

Reactions were usually carried out under an atmosphere of nitrogen.

All solvents and reagents used were analytical grade where possible.

THF and ether were distilled freshly from sodium/benzophenone ketyl.

N,N-Dimethylformamide and acetonitrile were distilled from blue silica

gel and stored over 4Å molecular sieves. Dichloromethane was distilled

from P_2O_5, filtered through Grade I basic alumina and stored over 4Å

molecular sieves. Allyl bromide was distilled from anhydrous $CaCl_2$ and

stored over 4Å molecular sieves. Diisopropylamine was distilled from

KOH and stored over 4Å molecular sieves. Petroleum ether, hexane and

pentane were distilled from CaH and stored over 4Å molecular sieves.

DMAP was recrystallised from cyclohexane. NBS was recrystallised

from water and dried over P_2O_5. Bu_4NF was prepared from its hydrate

by heating at $100^{\circ}C$ on Kugelrohr under high vacuum for 2 hours.

Dry column flash chromatography and flash chromatography refer to

techniques already described [110,111].

SPECTRAL ANALYSIS

Chemical shifts are reported in parts per million (δ) relative

to Me_4Si (0.00 ppm), using Me_4Si or the 7.25 ppm residual chloroform

peak and the 77.0 ppm $CDCl_3$ peak as internal references for ^{1}H and

[13]C n.m.r. spectra respectively. [1]H n.m.r. data are reported using

the following convention: chemical shift [integrated intensity,

multiplicity, s=singlet, d=doublet, t=triplet, q=quartet, coupling

constant (Hz), assignment].

The [1]H n.m.r. signals for H-2, H-3, H-4, H-5 and H-7 protons for

the model compounds generally occur as a multiplet between $\delta2.3$ and

1.5 and are omitted from the experimental section for clarity.

The multiplicities of the 50 MHz ^{13}C spectral resonances were

determined by use of DEPT spectra with pulse angles, $\theta=90^{\circ}$ and

135° [112]. The multiplicities of the 25 MHz ^{13}C spectral resonances

were determined by use of [13]C-^{1}H couplings in the off-resonance

spectra.

GENERAL ACETYLATION PROCEDURE

Unless otherwise stated acetylations were performed by dissolving

the starting alcohol (250 mg) in ether (4 ml) and then adding excess

Ac_2O (2 ml) and pyridine (1 ml). After standing at room temperature

overnight excess Ac_2O and pyridine were removed by azeotroping with

toluene (4x) and then CCl_4 (2x).

NUMBERING SYSTEMS

Numbering of the trichothecenes follows the conventional system as follows:
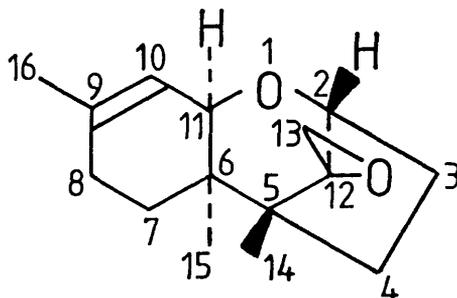
The model bicyclic compounds are numbered thus:
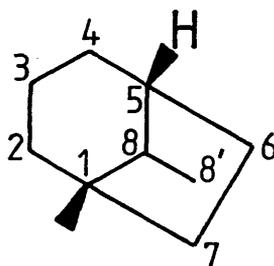
In addition the exo epoxide is defined as that with the 8–8' C–C bond axial, i.e. with the epoxide oxygen on the same side as the 2-carbon bridge. Conversely the endo epoxide is defined as that with the 8–8' C–C bond equatorial.

OSiMe₃ — rendered as structure image.

$$\text{OSiMe}_3$$
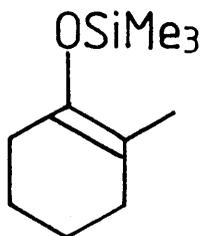
(109)

1-Trimethylsilyloxy-2-methylcyclohex-1-ene (109)

To a solution of TMSCl (38 ml, 0.3 mol) and Et$_3$N (83.1 ml,

0.6 mol) in DMF (90 ml) was added 2-methylcyclohexanone (30.3 ml,

0.25 mol). The resulting mixture was heated under reflux with

stirring for 48 h in a nitrogen atmosphere. It was then allowed

to cool, and diluted with pentane (200 ml) and washed with cold

aqueous NaHCO$_3$ (3 x 300 ml), cold aqueous HCl (1.5 M, 50 ml) and

cold aqueous NaHCO$_3$ (1 x 50 ml). The organic extract was then

dried (Na$_2$SO$_4$) and concentrated in vacuo. Distillation of the

crude residue gave the silyl enol ether (109) as a colourless oil

(37.95 g, 83%), b.p. 66-68°C/10 mmHg, lit.[93] 82-84°C/16 mmHg.

[1]H n.m.r. (CDCl$_3$, 90 MHz) δ 1.90 and 1.50 (11H, m, H-3, H-4, H-5,

H-6 + CH$_3$), 0.05 (9H, s, -OTMS).
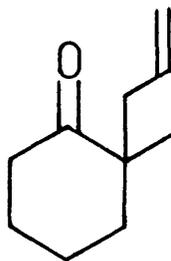
(110)

2-Allyl-2-methylcyclohexanone (110)

MeLi (1.33 M in ether, 57.7 ml, 76.8 mmol) was added with stirring to silyl enol ether (109) (12.8 g, 69.1 mmol). After 0.5 h. the reaction mixture was concentrated in vacuo, the vacuum being released with nitrogen. Dry THF (50 ml) was then added, followed by redistilled allyl bromide (6.5 ml, 76.0 mmol) dropwise with stirring. After 5 m. the reaction mixture was poured on to pentane. The organic layer was washed with saturated $NaHCO_3$, dried (Na_2SO_4) and concentrated in vacuo. Distillation of the residue yielded the allyl ketone (110) (9.52 g, 77%) as a colourless oil, b.p. 78–80°C/ 10 mmHg. ν_{max} (CCl_4) 2970, 2940, 2880, 1720, 1640, 1450, 1380, 1315, 1255, 1130, 920 cm^{-1}

^{1}H n.m.r. $(CDCl_3$, 90 MHz) δ 5.75 (1H, m, $-\underline{CH}=CH_2$), 5.00 (1H, m, $-CH=\underline{CH}_2$), 2.38 (4H, m, H-6 + $-CH_2CH=CH_2$), 1.80 (6H, m, H-3, H-4 + H-5).

Mass spectrum, m/z found: 152.1190 ($C_{10}H_{16}O$ requires 152.1201).
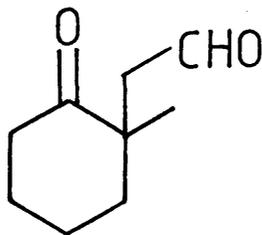
(111)

(1-Methyl-2-oxocyclohexyl)ethanal (111)

Ozone was bubbled through a solution of the allyl ketone (110) (3.97 g, 26.1 mmol) in dry CH_2Cl_2 (50 ml) at $-78^{\circ}C$. On turning blue, the solution was then purged with nitrogen to expel excess ozone. The ozonide was reduced by the addition of Et_3N (6.9 ml, 52.2 mmol) at $-78^{\circ}C$ followed by stirring overnight at room temperature. The solution was then filtered through a short column of chromatographic silica gel and concentrated in vacuo to yield the keto-aldehyde (111) (3.69, 92%) as a pale yellow oil, which was used without further purification.

^{1}H n.m.r. ($CDCl_3$, 90 MHz) δ 9.70 (1H, m, CHO), 2.5 (2H, m, CH_2CHO), 2.4 (2H, m, H-6) 1.25 (3H, s, CH_3).
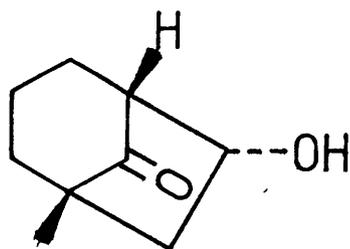
(112a)   (112b)
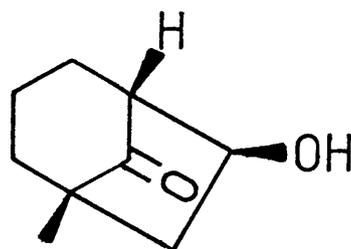
(±) 1-Methyl-6α-hydroxybicyclo[3,2,1]octan-8-one (112a) and

(±) 1-Methyl-6β-hydroxybicyclo[3,2,1]octan-8-one (112b)

A solution of the keto-aldehyde (111) (2.81 g, 0.018 mol) in

methanol (90 ml) was added to a solution of freshly prepared sodium

methoxide (from sodium (2.87 g, 0.124 mol) in methanol (200 ml)).

The mixture was heated under reflux for 0.5 h, cooled and then poured

on to iced water (200 ml). Excess methanol was removed by

concentration in vacuo and the residual, aqueous portion extracted with

ether (3 x 100 ml). The combined organic extracts were dried ($MgSO_4$)

and concentrated in vacuo to give a crude mixture of α- and β-

alcohol epimers in a ratio of 1:2 (by ^{1}H n.m.r. spectroscopy).

Purification by dry column flash chromatography yielded 351 mg (12.5%)

of pure α-alcohol (112a), 760 mg (27%) of pure β-alcohol (112b) along

with 589 mg (21%) of an epimeric mixture – i.e., a total yield of

1.70 g (60.5%).

The α-epimer (112a) was characterised as a white crystalline solid:

m.p. 95-6°C (ether-pet.ether); R_f 0.29   (1:1 EtOAc-pet.ether);

$ν_{max}$ (CCl_4) 3630, 2960, 2930, 2860, 1750, 1500, 1360, 1235 (br),

1095, 1075, 1040, 970, 955, 895 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ4.45 (1H, ddd, J 10,5,5 Hz, H-6);

2.4 (1H, m, H-5), 1.95 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ 221.9 (C-8), 65.9 (C-6), 52.2 (C-5),

48.7 (C-1), 45.2 (C-2), 42.3 (C-7), 31.8 (C-4), 19.3 (C-3 + CH$_3$).

Mass spectrum, $\underline{m}/\underline{z}$ found: 154.0995 (M$^+$, C$_9$H$_{14}$O$_2$ requires

154.0994).

Microanalysis, found: C, 70.20; H, 9.25% (C$_9$H$_{14}$O$_2$ requires

C, 70.10; H, 9.15%).

The β-epimer (112b) was characterised as a white crystalline

solid: mp 69-71°C (ether-pet.ether); R$_f$ 0.22 (1:1 EtOAc-pet.ether);

ν_{max} (CCl$_4$) 3620, 3450 (br), 2940, 2930, 2860, 1750, 1450, 1380,

1255 (br), 1215, 1180, 1155, 1080, 1030, 970, 955 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ4.25 (1H, dd, J 9,4 Hz, H-6),

2.3 (1H, m, H-5), 1.00 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ 221.4 (C-8), 69.4 (C-6), 55.9 (C-5),

47.9 (C-1), 43.9 (C-2), 43.8 (C-7), 34.0 (C-4), 19.1 (C-3 + CH$_3$).

Mass spectrum, $\underline{m}/\underline{z}$ found: 154.1001 (M$^+$, C$_9$H$_{14}$O$_2$ requires

154.0994).

Microanalysis, found: C, 70.07; H, 9.15% (C$_9$H$_{14}$O$_2$ requires

C, 70.10; H, 9.15%).
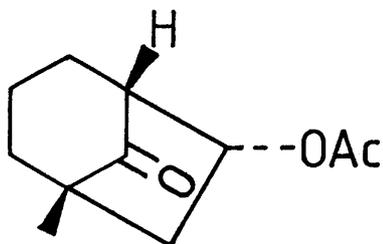
(113a)

(±) 1-Methyl-6α-acetoxybicyclo[3,2,1]octan-8-one (113a)

Keto-alcohol (112a) (270 mg, 1.75 mmol) was acetylated by the normal procedure. Purification by dry column flash chromatography (EtOAc-pet.ether) gave 314 mg (91%) of the keto-acetate (113a) as a colourless oil: bp 85°C (0.5 mmHg, Kugelrohr); R_f 0.44 (1:1 EtOAc-pet.ether); ν_{max} (NaCl) 2960, 2940, 2920, 2860, 1750, 1450, 1380, 1235, 1185, 1140, 1090, 1070, 1035, 980, 910 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ5.2 (1H, ddd, J 10,5,5 Hz, H-6), 2.65 (1H, m, H-5), 2.1 (3H, s, CH$_3$CO), 0.98 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ219.6 (C-8), 170.6 (CH$_3$CO), 68.8 (C-6), 50.0 (C-5), 48.0 (C-1), 45.1 (C-2), 39.4 (C-7), 32.4 (C-4), 20.9 (CH$_3$CO), 19.1 (C-3 + CH$_3$).

Mass spectrum, m/z found: 196.1102 (M$^+$, C$_{11}$H$_{16}$O$_3$ requires 196.1099).

Microanalysis, found: C, 67.19; H, 7.96% (C$_{11}$H$_{16}$O$_3$ requires C, 67.32; H, 8.22%).

(113b)

(±) 1-Methyl-6β -acetoxybicyclo[3,2,1]octan-8-one (113b)

Keto-alcohol (112b) (394 mg, 2.59 mmol) was acetylated by the
normal procedure. Purification by dry column flash chromatography
(EtOAc-pet.ether) gave 440 mg (87%) of the keto-acetate (113b) as a
colourless oil: bp 85°C (0.5 mmHg, Kugelrohr); R_f 0.44 (1:1
EtOAc-pet.ether); ν_{max} (CCl$_4$) 2960, 2940, 2860, 2850, 1750, 1450,
1375, 1360, 1235, 1180, 1150, 1080, 1025, 985 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ5.08 (1H, dd, J 9,4 Hz, H-6),
2.35 (1H, m, H-5), 2.00 (3H, s, CH$_3$CO), 1.02 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ220.2 (C-8), 170.5 (CH$_3$CO),
71.7 (C-6), 52.3 (C-5), 47.6 (C-1), 43.8 (C-2), 40.8 (C-7),
34.0 (C-4), 21.1 (CH$_3$CO), 19.0 (C-3 + CH$_3$).

Mass spectrum, m/z found: 196.1094 (M$^+$, C$_{11}$H$_{16}$O$_3$ requires
196.1099).

Microanalysis, found: C, 67.09: H, 8.31% (C$_{11}$H$_{16}$O$_3$ requires
C, 67.32 H, 8.22%).
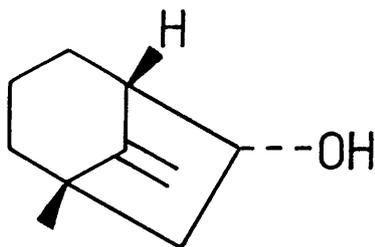
(114a)

(±) 1-Methyl-6α-hydroxy-8-methylenebicyclo[3,2,1]octane (114a)

To a suspension of methyltriphenylphosphonium bromide (1.43 g,

4.0 mmol) in THF (35 ml) was added with stirring and under nitrogen,

a solution of nBuLi (2.2 M in hexane, 2.0 ml, 4.4 mmol). The

mixture was stirred for 10 m. when a solution of the keto-acetate

(113a) (314 mg, 1.6 mmol) in THF (10 ml) was added. The mixture

was then heated under reflux for 1 h. when it was cooled, diluted

with water and extracted with ether. The organic extracts were

washed with dilute HCl and brine, dried (MgSO$_4$) and concentrated

in vacuo. Purification by dry column flash chromatography (ether-

pet.ether) gave 130 mg (53%) of the desired olefin (114a) as a white

crystalline solid: mp 70–72°C (pet.ether); R$_f$ 0.5 (EtOAc–pet.ether);

ν_{max} (CCl$_4$) 3625, 3070, 2950, 2920, 2870, 1660, 1440, 1370, 1230, 1105,

1040, 980, 955, 940 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ4.65 and 4.53 (2H, 2 x s, C=CH$_2$),

4.3 (1H, ddd, J 10,5,5 Hz, H-6), 2.55 (1H, m, H-5), 1.00 (3H, s,

CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ162.3 (C-8), 98.7 (C=CH$_2$), 71.3

(C-6), 48.7 (C-5), 45.2 (C-7), 43.2 (C-2), 42.6 (C-1), 29.7 (C-4),

23.1 (CH$_3$), 20.3 (C-3).

Mass spectrum, m/z found: 152.1204 (M$^+$, C$_{10}$H$_{16}$O requires 152.1201).

Microanalysis, found: C, 78.83; H, 10.72% (C$_{10}$H$_{16}$O requires C, 78.90; H, 10.59%).
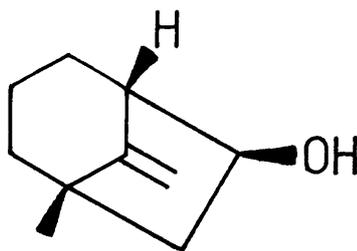
(114b)

(±) 1-Methyl-6β-hydroxy-8-methylenebicyclo[3,2,1]octane (114b)

Olefin (114b) was prepared in an analogous manner to (114a) from keto-acetate (113b) (840 mg, 4.28 mmol) and methyltriphenylphosphonium bromide (3.83 g. 10.71 mmol). Purification by dry column flash chromatography (ether-pet.ether) gave 324 mg (50%) of olefin (114b) as a white crystalline solid: mp 49-52°C (60-80 pet.ether); R_f 0.43 (1:1 EtOAc-pet.ether); ν_{max} (CCl$_4$) 3610, 3580, 3070, 2980, 2930, 2870, 2860, 1665, 1465, 1450, 1445, 1390, 1370, 1120, 1020, 960, 930, 915, 885 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ4.75 and 4.68 (2H, 2 x s, C=CH$_2$), 4.05 (1H, dd, J 8,4 Hz, H-6), 2.4 (1H, m, H-5), 1.08 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ161.4 (C-8), 99.6 (C=CH$_2$), 73.7 (C-6), 53.5 (C-5), 48.5 (C-7), 43.8 (C-1), 41.9 (C-2), 32.7 (C-4), 23.0 (CH$_3$), 20.1 (C-3).

Mass spectrum, m/z found: 152.1197 (M$^+$, C$_{10}$H$_{16}$O requires 152.1201).

Microanalysis, found: C, 78.75; H, 10.65% (C$_{10}$H$_{16}$O requires: C, 78.90; H, 10.59%).
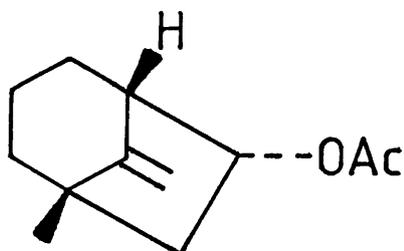
(115a)

(±) 1-Methyl-6α –acetoxy-8-methylenebicyclo[3,2,1]octane (115a)

To a suspension of WCl_6 (439 mg, 1.11 mmol) in THF (4 ml) at

–78°C was added nBuLi (2.58 M in hexane, 1.28 ml, 3.3 mmol). The

cooling bath was removed and the mixture allowed to come to room

temperature when the solution became dark brown and homogeneous.

This solution was temporarily replaced in the cooling bath at –78°C

whereupon epoxide (117a) (117 mg, 0.56 mmol) in THF (6 ml) was added. The

cooling bath was removed, and the reaction mixture was heated under

reflux for 6 h. On cooling to room temperature, it was diluted with

hexane, washed once with a solution 2 M in NaOH and 1.5 M in sodium

tartrate, and once with water. The organic extract was dried ($MgSO_4$)

and concentrated in vacuo. Purification by dry column flash

chromatography (ether–pet.ether) gave 98 mg (91%) of olefin (115a) as

a colourless oil: bp 85°C (1 mm Hg, Kugelrohr); R_f 0.62 (1:1

EtOAc–pet.ether); ν_{max} (CCl_4) 3080, 2960, 2940, 2880, 2860, 2850

1740, 1670, 1470, 1450, 1400, 1375, 1360, 1280, 1250 (br), 1215, 1155,

1105, 1080, 1030, 940, 925, 890 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$, 90 MHz) δ5.05 (1H, ddd, J 10,5,5 Hz, H-6),

4.7 and 4.6 (2H, 2 x s, C=CH$_2$), 2.85 (1H, m, H-5), 2.05 (3H, s, CH$_3$CO), 1.04 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ170.8 (CH$_3$CO), 160.6 (C-8), 99.6 (C=CH$_2$), 73.6 (C-6), 46.5 (C-5), 42.9 (C-2), 42.1 (C-7), 42.0 (C-1), 30.3 (C-4), 22.9 (CH$_3$), 20.9 (CH$_3$CO), 19.9 (C-3).

Mass spectrum, m/z found: 194.1319 (M$^+$, C$_{12}$H$_{18}$O$_2$ requires 194.1307).

Microanalysis, found: C, 74.04; H, 9.36% (C$_{12}$H$_{18}$O$_2$ requires: C, 74.19; H, 9.34%).

Olefin (115a) was identical in all respects to that prepared by acetylation of alcohol (114a) in the normal way (91% yield).
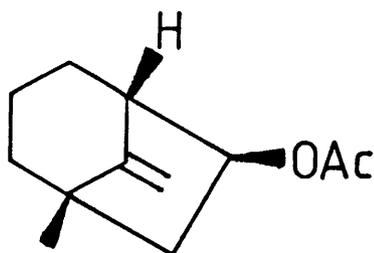
(115b)

(±) 1-Methyl-6β -acetoxy-8-methylenebicyclo[3,2,1]octane (115b)

Olefin (115b) was prepared in an analogous manner to (115a) from epoxyacetate (117b) (77 mg, 0.37 mmol) and WCl_6 (291 mg, 0.73 mmol). Purification by dry column flash chromatography (ether-pet.ether) gave 65 mg (91%) of olefin (115b) as a colourless oil: bp $50°C$ (0.05 mmHg, Kugelrohr); R_f 0.62 (1:1 EtOAc-pet.ether); ν_{max} 3040, 2950, 2930, 2870 2860, 1730, 1670, 1445, 1375, 1360, 1245, 1155, 1145, 1025, 890 cm^{-1}.

^1H n.m.r. ($CDCl_3$, 90 MHz) δ 4.9 (1H, dd, J 9,4 Hz, H-6), 4.7 and 4.6 (2H, 2 x s, $C=CH_2$), 2.5 (1H, m, H-5), 1.95 (3H, s, CH_3CO), 1.04 (3H, s, CH_3).

^{13}C n.m.r. ($CDCl_3$, 25 MHz) δ 170.8 (CH_3CO), 161.0 (C-8), 98.9 ($C=CH_2$), 76.6 (C-6), 50.0 (C-5), 45.0 (C-7), 43.8 (C-1), 42.1 (C-2), 32.8 (C-4), 22.7 (CH_3), 21.4 (CH_3CO), 20.1 (C-3).

Mass spectrum, m/z found: 194.1314 (M^+, $C_{12}H_{18}O_2$ requires C, 194.1307).

Microanalysis, found: C, 74.09; H, 9.63% ($C_{12}H_{18}O_2$ requires C, 74.19; H, 9.34%).

Olefin (115b) was identical in all respects to that prepared by acetylation of alcohol (114b) in the normal way (87% yield).

Use of the above protocol with stirring of the reaction mixture at ambient temperature for 72 hours resulted in production of a 1:1 mixture of the olefin (115b) and chlorohydrin (163).

The chlorohydrin was identified from its [1]H n.m.r. spectrum as follows:

[1]H n.m.r. (CDCl$_3$, 90 MHz) δ 5.03 (1H, dd, J 9, 5 Hz), 4.02 and 3.66 (2H, ABq, J 12 Hz, H-8'), 2.08 (3H, s, CH$_3$CO), 1.06 (3H, s, CH$_3$).
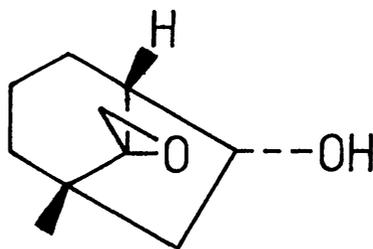
(116a)

(±) Spiro[1-methyl-6α -hydroxybicyclo[3,2,1]octane-exo-8,8'-oxirane]

(116a)

A mixture of the hydroxyolefin (114a) (225 mg, 1.34 mmol),

m-chloroperbenzoic acid (490 mg, 2.41 mmol, assuming 85% purity) and

Na_2HPO_4 (2.28 g) in 70 ml CH_2Cl_2 was stirred overnight at room

temperature. The reaction mixture was then poured into water and

extracted with ether (3x). The combined extracts were washed with

dilute $NaHCO_3$ and then brine, dried ($MgSO_4$) and concentrated

in vacuo. Purification by dry column flash chromatography (ether,

then ether-EtOAc) gave 217 mg (87%) of hydroxyepoxide (116a) as a

colourless oil: bp 120°C (0.1 mm Hg, Kugelrohr); R_f 0.29

(1:1 EtOAc-pet.ether); ν_{max} (CCl_4) 3620, 3500 (br), 3040, 2940,

2880, 2860, 1490, 1465, 1450, 1445, 1375, 1250 (br), 1110, 1090,

1040, 945, 910, 890 cm^{-1}.

^1H n.m.r. ($CDCl_3$, 90 MHz) δ 4.65 (1H, ddd, J 10,5,5 Hz, H-6),

2.72 (2H, s, H-8'), 0.73 (3H, s, CH_3).

^{13}C n.m.r. ($CDCl_3$, 25 MHz) δ 72.6 (C-8), 71.2 (C-6), 46.6 (C-5),

45.8 (C-8'), 44.3 (C-7), 40.2 (C-2), 39.9 (C-1), 26.9 (C-4), 19.6

(C-3), 18.7 (CH_3).

Mass spectrum, m/z found: 168.1148 (M^+, $C_{10}H_{16}O_2$ requires 168.1150).
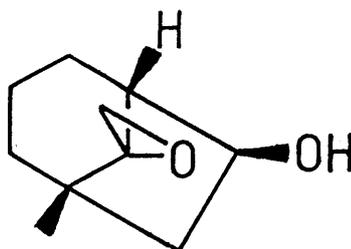
(116b)

($\pm$) Spiro[1-methyl-6β -hydroxybicyclo[3,2,1]octane-exo-8,8'-oxirane]

(116b)

Epoxide (116b) was prepared in an analogous manner to (116a) from

hydroxyolefin (114b) (250 mg, 1.64 mmol), m-chloroperbenzoic acid (540 mg,

2.30 mmol, assuming 85% purity) and Na_2HPO_4 (2.55 g) in CH_2Cl_2 (70 ml).

Purification by dry column flash chromatography (ether, then ether-EtOAc)

gave 242 mg (88%) of hydroxyepoxide (116b) as a white amorphous solid:

mp 95-98°C (40:60 pet.ether); R_f 0.18 (EtOAc-pet.ether) ν_{max} (CCl$_4$)

3600, 3500 (br), 3040, 2940, 2880, 2860, 1470, 1450, 1405, 1375, 1260,

1130, 1115, 1090, 1030, 970, 930, 915, 850 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ4.10 (1H, dd, J 8, 4 Hz, H-6), 2.75

(2H, s, H-8'), 0.80 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ74.1 (C-6), 72.1 (C-8), 50.7 (C-5),

47.6 (C-7), 44.8 (C-8'), 41.6 (C-1), 38.9 (C-2), 29.3 (C-4), 19.3 (C-3),

18.6 (CH$_3$).

Mass spectrum, m/z found: 168.1150 (C$_{10}$H$_{16}$O$_2$ requires 168.1150).

Microanalysis, found: C, 71.39; H, 9.61% (C$_{10}$H$_{16}$O$_2$ requires

C, 71.38; H, 9.59%).
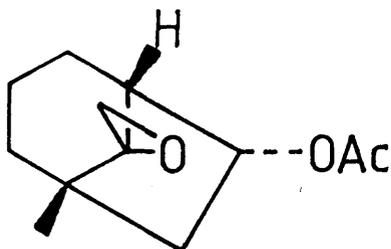
(117a)

(±) Spiro[1-methyl-6α-acetoxybicyclo[3,2,1]octane-exo-8,8'-oxirane] (117a)

Acetylation of hydroxyepoxide (116a) (122 mg, 0.73 mmol) in the normal manner gave 125 mg (82%) of the epoxyacetate (117a) as a colourless oil: bp 110°C (2 mmHg, Kugelrohr); R_f 0.52 (1:1 EtOAc-pet.ether); ν_{max} (CCl$_4$) 3040, 2960, 2940, 2880, 2840, 1740, 1450, 1375, 1360, 1245, 1210, 1110, 1090, 1040, 950, 910 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ 5.33 (1H, ddd, J 9,5,5 Hz, H-6), 2.73 (2H, s, H-8'), 2.08 (3H, s, CH$_3$CO), 0.75 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$ 25 MHz) δ 170.8 (CH$_3$CO), 73.8 (C-6), 71.8 (C-8), 45.7 (C-8'), 44.7 (C-5), 41.4 (C-7), 40.0 (C-2), 39.5 (C-1), 27.4 (C-4), 20.9 (CH$_3$CO), 19.3 (C-3), 18.5 (CH$_3$).

Mass spectrum, m/z found: 210.1254 (C$_{12}$H$_{18}$O$_3$ requires 210.1256).
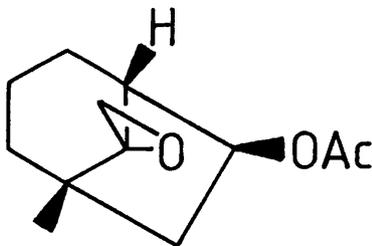
(117b)

(±) Spiro[1-methyl-6β -acetoxybicyclo [3,2,1]octane-exo-8,8'-oxirane]

(117b)

Acetylation of hydroxyepoxide (116b) (153 mg, 0.91 mmol) in the

normal manner gave 155 mg (81%) of the epoxyacetate (117b) as a colourless

oil: bp 100°C (1.0 mmHg, Kugelrohr); R_f 0.52 (1:1 EtOAc-pet.ether);

ν_{max} (NaCl) 3040, 2930, 2870, 2850, 1730, 1460, 1445, 1365, 1295, 1245,

1210, 1165, 1150, 1025, 990, 970, 935, 910, 860, 845 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 90 MHz) δ 5.0 (1H, dd, J 8,4 Hz, H-6), 2.75

(2H, s, H-8'), 2.04 (3H, s, CH$_3$CO), 0.82 (3H, s, CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 25 MHz) δ 170.0 (CH$_3$CO), 75.2 (C-6), 70.5 (C-8),

46.7 (C-5), 43.8 and 43.0 (C-7 + C-8'), 40.6 (C-1), 38.0 (C-2), 28.4

(C-4), 20.3 (CH$_3$CO), 18.3 (C-3), 17.3 (CH$_3$).

Mass spectrum, m/z found: 210.1241 (C$_{12}$H$_{18}$O$_3$ requires 210.1256).

Microanalysis, found: C, 68.61; H, 8.68% (C$_{12}$H$_{18}$O$_3$ requires

C, 68.53; H, 8.63%).
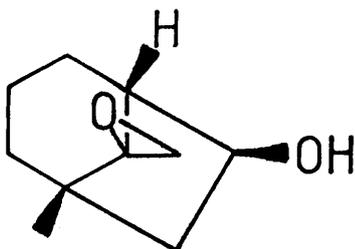
(118)

(±) Spiro[1-methyl-6β -hydroxybicyclo[3,2,1]octane-endo-8,8'
oxirane] (118)

To a stirred suspension of $Me_3S^+I^-$ (833 mg, 4.08 mmol) in THF

(15 ml) under nitrogen was added nBuLi (2.4 M in hexane, 1.91 ml, 4.59

mmol). The solution became clear; it was stirred at $0^\circ C$ for 1 h.

when a solution of ketone (113b) (200 mg, 1.02 mmol) in THF (10 ml)

was added. After stirring for a further hour the mixture was diluted

with ether, washed with saturated NH_4Cl solution and brine, dried

$(MgSO_4)$ and concentrated in vacuo. Purification by dry column flash

chromatography (EtOAc-pet.ether) gave the title compound (118) as a

colourless oil (64 mg, 37%): R_f 0.21 (1:1 EtOAc-pet.ether); ν_{max}

(CCl_4) 3620, 3450, (br), 3050, 2930 (br), 2870, 1490,1465, 1460,

1450, 1440, 1400, 1370, 1355, 1340, 1320, 1260, 1210, 1165, 1140,

1110, 1050, 1020, 960, 900, 855 cm^{-1}.

^1H n.m.r. $(CDCl_3$, 90 MHz) $\delta 4.15$ (1H, dd, J 8.0, 3.5 Hz,.H-6), 2.97

and 2.75 (2H, ABq, J 4.5 Hz, H-8'), 0.74 (3H, s, CH_3).

^{13}C n.m.r. $(CDCl_3$, 25 MHz) $\delta 71.9$ (C-6), 68.7 (C-8), 52.5 (C-8'),

49.1 (C-5), 46.4 (C-7), 39.2 (C-1), 36.0 (C-2), 26.3 (C-4), 20.9 (CH_3)

19.6 (C-3).

Mass spectrum, $\underline{m}/\underline{z}$ found: 168.1149 (M^+, $C_{10}H_{16}O_2$ requires 168.1150).
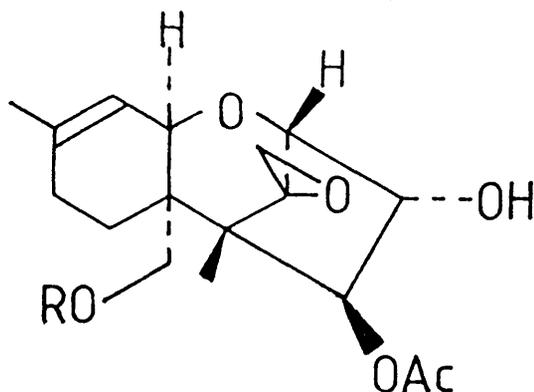
(9) R=Ac   (48) R=H

Culture of Fusarium species

Isolation of 3α-hydroxy-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene

(anguidine, DAS) (9) and 3α,15-dihydroxy-4β-acetoxy-12,13-epoxytrichothec-

9-ene(MAS,4-acetoxyscirpenediol) (48)

Seed Medium	g	Production Medium	g
Malt extract	2	$NH_4H_2PO_4$	1
Yeast	2	$K_2HPO_4 \cdot 3H_2O$	3.93
Peptone (Oxoid)	2	$MgSO_4 \cdot 7H_2O$	0.2
KH_2PO_4	2	NaCl	0.5
$MgSO_4 \cdot 7H_2O$	2	Sucrose	40
$FeSO_4 \cdot 7H_2O$	0.2	Glycerol	10
NH_4Cl	3	Deionised Water	1 L
Glucose	20		
Deionised Water	1 L		

500 ml Conical flasks containing 100 ml of the seed medium were

innoculated with the Fusarium species which had been maintained

on slants of Sabouraud dextrose agar for 7 days. The seed culture

was shaken for 2 days in the dark ($27^\circ C$, 160 rpm) and then was

used to innoculate conical flasks (30-40) containing 100 ml of the

production medium. The production culture was then fermented for 11 days in the dark (27°C, 160 rpm). Continuous EtOAc extraction of the whole culture, without altering the pH, for 24 h. gave a brown oily residue (3-4 g) upon drying and concentration *in vacuo.* Purification by dry column flash chromatography (EtOAc-pet.ether) gave typically 200-300 mg1^{-1} each of anguidine (9) and 4-acetoxyscirpenediol (48).

4-Acetoxyscirpenediol (48) was characterised[30] as a white amorphous solid: $[\alpha]_D^{20}$+9.4 (c 1.3, acetone), lit.$[\alpha]_D^{22}$+10.0 (c 1.2, acetone); R_f 0.08 (1:1 EtOAc-pet.ether), 0.24 (97:3 $CHCl_3$-MeOH); ν_{max} (CCl_4) 3630, 3490 (br), 2960, 2930, 2905, 1720, 1445, 1435, 1375, 1255, 1160, 1105, 1075, 1060, 1045, 1030, 1020, 960, 910 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$, 200 MHz) δ 5.56 (1H, dq, J 5.5, 1.4 Hz, H-10), 5.52 (1H, d, J 3.2 Hz, H-4), 4.25 (1H, dd, J 4.9, 3.2 Hz, H-3), 4.19 (1H, br.d, J 5.5 Hz, H-11), 3.79 and 3.61 (2H, ABq, J 12.3 Hz, H-15), 3.67 (1H, d, J 4.9 Hz, H-2), 3.05 and 2.77 (2H, ABq, J 4.0 Hz, H-13), 2.15 (3H, s, CH_3CO), 2.10-1.96 (4H, m, H-7 + H-8), 1.72 (3H, br.s, H-16), 0.84 (3H, s, H-14).

^{13}C n.m.r. ($CDCl_3$, 50 MHz) δ 173.0 (CH_3CO), 140.3 (C-9), 118.8 (C-10), 84.4 (C-4), 79.0 (C-2), 77.5 (C-3), 68.0 (C-11), 64.6 (C-12), 62.6 (C-15), 48.7 (C-5), 47.3 (C-13), 44.8 (C-6), 28.0 (C-8), 23.3 (C-16), 21.2 (C-7), 21.0 (CH_3CO), 6.6 (C-14).

Mass spectrum, *m/z* found: 306.1467 (M^+-H_2O, $C_{17}H_{22}O_5$ requires 306.1467).

Anguidine (9) was characterised[27,109] as a white crystalline solid: mp 161-3°C (ether), lit. 162-4°C; $[\alpha]_D^{20}$-24.5° (c 1.16, $CHCl_3$), lit. $[\alpha]_D^{24}$-27° (c 1.28, $CHCl_3$); R_f 0.1 (1:1 EtOAc-pet.ether), 0.43 (97:3 $CHCl_3$-MeOH); ν_{max} 3560, 2970, 2920, 1750, 1725, 1450, 1435, 1375, 1365, 1240 (br), 1160, 1110, 1080, 1050, 1040, 990, 960 cm^{-1}.

[1]H n.m.r. (CDCl$_3$, 100 Mz) δ 5.47 (1H, dq, J 5.6, 1.8 Hz, H–10), 5.14 (1H, d, J 2.9 Hz, H–4), 4.13 (1H, dd, J 5.0, 2.9 Hz, H–3), 4.05 (1H, br.d, J 5.0 Hz, H–11), 4.14 and 4.00 (2H, ABq, J 12.2 Hz, H–15), 3.64 (1H, d, J 4.9, H–2), 3.01 and 2.72 (2H, ABq, J 3.9 Hz, H–13), 2.09 (3H, s, CH$_3$CO), 2.00 (3H, s, CH$_3$CO), 2.01–1.80 (4H, m, H–7 + H–8), 1.67 (3H, br.s, H–16), 0.76 (3H, s, H–14).

[13]C n.m.r. (CDCl$_3$, 25 MHz) δ 172.5 (CH$_3$CO), 170.5 (CH$_3$CO), 140.5 (C–9), 118.6 (C–10), 84.8 (C–4), 79.0 (C–2), 78.4 (C–3), 68.0 (C–11), 64.4 (C–12), 63.7 (C–15), 48.8 (C–5), 47.2 (C–13), 44.0 (C–6), 28.0 (C–8), 23.2 (C–16), 21.3 (C–7), 21.0 (2xCH$_3$CO), 6.9 (C–14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 306.1473 (M$^+$–AcOH, C$_{17}$H$_{22}$O$_5$ requires 304.1467

(63)
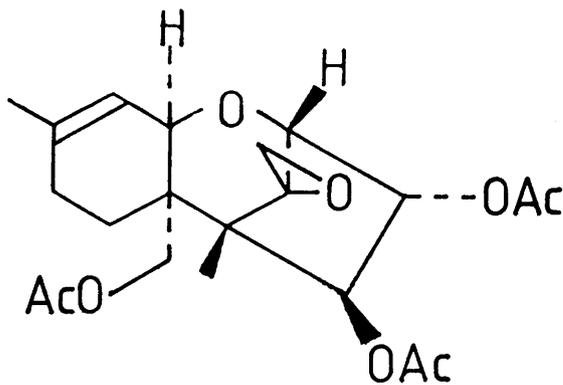
3α,4β,15-Triacetoxy-12,13-epoxytrichothec-9-ene (triacetoxyscirpene) (63)

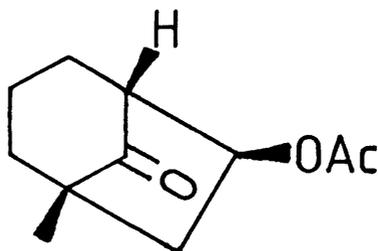Anguidine (9) (315 mg, 0.86 mmol) was acetylated by the usual procedure. Purification by dry column flash chromatography (EtOAc-pet.ether) gave 332 mg (95%) of the triacetate as a white crystalline solid: mp 122-3°C (benzene-n-hexane); $[\alpha]_D^{20}$+36.7 (c 0.99, acetone); lit.[27] $[\alpha]_D^{23}$+44 (c 1.0, acetone); R_f 0.35 (1:1 EtOAc-pet.ether): ν_{max} (CCl$_4$) 2970,2940, 2910, 1750, 1370, 1240, 1230, 1170, 1090, 1060, 1040, 970 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ5.75, (1H, d, J 3.3 Hz, H-4), 5.49 (1H, dq. J 5.6, 1.3 Hz, H-10), 5.19 (1H, dd, J 4.9, 3.3, Hz H-3), 4.26 and 4.06 (2H, ABq, J 12.3 Hz, H-15), 3.99 (1H, br.d, J 5.6 Hz, H-11), 3.87 (1H, d, J 4.9 Hz, H-2), 3.08 and 2.81 (2H, ABq, J 4.0 Hz, H-13), 2.15 (3H, s, CH$_3$CO), 2.11 (3H, s, CH$_3$CO), 2.07 (3H, s, CH$_3$CO), 1.73(3H, br.s, H-16), 0.77 (3H, s, H-14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ170.5 (2 x CH$_3$CO), 170.0 (CH$_3$CO), 140.7 (C-9), 118.2 (C-10), 79.3 (C-2), 78.3 (C-3), 77.5 (C-4), 67.9 (C-11), 64.1 (C-12), 63.4 (C-15), 48.7 (C-5), 47.1 (C-13), 44.0 (C-6), 27.8 (C-8), 23.1 (C-16), 21.2 (C-7), 20.9 (CH$_3$CO), 20.8 (2 x CH$_3$CO),

6.5 (C–14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 348.1588 (M^+–CH_3COOH, $C_{19}H_{24}O_6$ requires 348.1573).

(119)
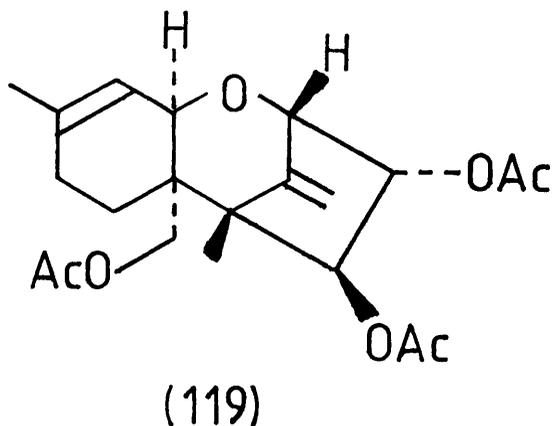
3α ,4β ,15–Triacetoxytrichothec–9,12–diene (119)

To a suspension of WCl_6 (200 mg, 0.504 mmol) in THF (2 ml)

at $-78°C$ was added n.BuLi (2.5 M in hexane, 0.5 ml,1.25 mmol).

The cooling bath was removed and the mixture allowed to come to

room temperature when the solution became dark brown and homogeneous.

This solution was temporarily replaced in the cooling bath whereupon

the epoxide (63) (100 mg, 0.245 mmol) in THF (3 ml) was added. The

reaction mixture was heated under reflux for 6 h. On cooling to

room temperature it was diluted with hexane, washed once with a

solution 2 M in NaOH and 1.5 M in sodium tartrate, and once with

water. The organic extract was dried $(MgSO_4)$ and concentrated

in vacuo. Purification by dry column flash chromatography

(EtOAc–pet.ether) gave 93 mg (97%) of diene (119) as a white

crystalline solid: mp 120–1°C (Et_2O–hexane); $[\alpha]_D^{20}$+2.7°

(c 1.13, $CHCl_3$); R_f 0.40 (1:1 EtOAc–pet.ether); ν_{max} (CCl_4) 2980,

2920, 1750, 1450, 1440, 1370, 1250, 1230, 1085, 1060, 1040, 990, 970,

915 cm^{-1}.

[1]H n.m.r. ($CDCl_3$, 200 MHz) δ 5.75 (1H, d, J 3.1 Hz, H–4), 5.42

(1H, dq, J 5.4,1.4 Hz, H-10) 5.21 (1H, s, H-13a), 4.83 (1H, s, H-13b),

4.81 (1H, dd, J 4.8, 3.1 Hz, H-3), 4.42 (1H, d, J 4.8 Hz, H-2), 4.22

and 4.02 (2H, ABq, J 12.2 Hz, H-15), 4.00 (1H, br.d, J 5.4 Hz, H-11),

2.12 (3H, s, CH_3CO), 2.06 (3H, s, CH_3CO) 2.04 (3H, s, CH_3CO), 1.66

(3H, br.s, H-16), 0.97 (3H, s, H-14).

^{13}C n.m.r. ($CDCl_3$, 50 MHz) δ170.7 (CH_3CO),170.2 (2 x CH_3CO),

149.0 (C-12), 140.3 (C-9), 118.5 (C-10), 109.1 (C-13), 79.4 (C-2),

78.6 (C-3), 77.4 (C-4) 67.6 (C-11), 63.8 (C-15), 52.0 (C-5), 43.8

(C-6), 27.8 (C-8), 23.2 (C-16), 21.0 (CH_3CO), 20.9 (CH_3CO), 20.8

(CH_3CO), 20.5 (C-7), 10.9 (C-14).

Mass spectrum, m/z found: 332.1613 (M^+ - CH_3COOH, $C_{19}H_{24}O_5$

requires 332.1624).

Microanalysis, found: C, 64.26; H, 7.14% ($C_{21}H_{28}O_7$ requires:

C, 64.27; H, 7.19%).

(120)
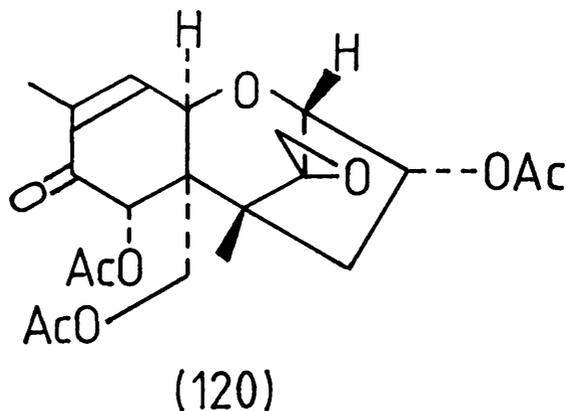
3α,7α,15-Triacetoxy-12,13-epoxytrichothec-9-en-8-one (120)

METHOD 1 (from natural 3-AcDON)

To a solution of 3-acetyldeoxynivalenol (24) (34 mg, 0.101 mmol) in dry CH_2Cl_2 (3 ml) was added excess triethylamine (1.13 ml) and acetic anhydride (0.8 ml) along with a few crystals of DMAP. The mixture was stirred for 2 d. at room temperature when it was diluted with ether, washed once with saturated $NaHCO_3$ solution and once with water. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Purification by flash chromatography (EtOAc-pet.ether) gave 40 mg (94%) of the triacetate (120) as a white crystalline solid: mp 153-7°C (EtOAc-pet.ether) lit.[76] 156-7°C; R_f 0.64 (1:1 EtOAc-pet.ether); ν_{max} (CCl_4) 3010, 2980, 2960, 2930, 1755, 1705, 1450, 1430, 1370, 1225 (br), 1180, 1075, 1060, 1045, 1035, 960 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$, 200 MHz) δ6.53 (1H, dq, J 5.9, 1.6 Hz, H-10), 6.03 (1H, s, H-7), 5.19 (1H, ddd, J 11.0,4.5,4.5 Hz, H-3), 4.72 (1H, d J 5.9 Hz, H-11), 4.31 (2H, ABq, J 12.2 Hz, H-15), 3.88 (1H, d, J 4.5 Hz, H-2), 3.10 and 2.78 (2H, ABq, J 3.5 Hz, H-13), 2.34 (1H, dd, J 15.2, 4.5 Hz, H-4α), 2.18 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 1.88

(3H, s, CH_3CO), 1.82 (3H, br.s, H-16), 0.93 (3H, s, H-14).

^{13}C n.m.r. ($CDCl_3$, 50 MHz) δ 191.9 (C-8), 170.1 (CH_3CO), 170.0 (CH_3CO), 169.6 (CH_3CO), 136.9 (C-9), 136.8 (C-10), 78.7 (C-7), 74.6 (C-2), 70.6 (C-11), 70.2 (C-3), 64.5 (C-12), 62.3 (C-15), 50.0 (C-5), 47.6 (C-13), 45.6 (C-6), 40.7 (C-4), 20.8 (2 x CH_3CO), 20.5 (CH_3CO), 15.3 (C-16), 13.6 (H-14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 422.1557 (M^+, $C_{21}H_{26}O_9$ requires 422.1577).

METHOD 2 (from semi-synthetic deoxynivalenol, (23))

Semi-synthetic deoxynivalenol (23) (24 mg, 0.081 mmol) was acetylated in a similar manner to 3-AcDON (24) using Et_3N (1.35 ml), Ac_2O (1 ml) and CH_2Cl_2 (4 ml) and a few crystals of DMAP. Purification by flash chromatography gave the title compound as a white crystalline solid: mp 152-6°C (EtOAc-pet.ether), lit[76] 156-7°C; $[\alpha]_D^{20}$ +75° (c 0.2, $CHCl_3$); R_f 0.64 (1:1 EtOAc-pet.ether).

Mass spectrum, $\underline{m}/\underline{z}$ found: 422.1551 (M^+, $C_{21}H_{26}O_9$ requires 422.1577).

Microanalysis, found: C, 59.79; H, 6.09% ($C_{21}H_{26}O_9$ requires C, 59.69; H, 6.21%).

This material also possesses identical ^1H and ^{13}C n.m.r., and i.r. spectra to that previously prepared by acetylation of natural 3-AcDON (24).

(121)
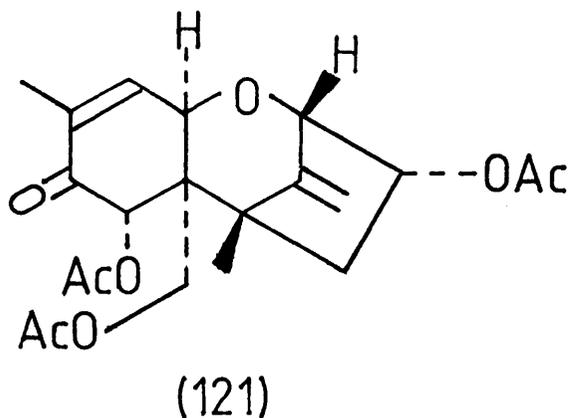
3α ,7α ,15-Triacetoxytrichothec-9,12-dien-8-one (121)

To a suspension of WCl_6 (190 mg, 0.474 mmol) in THF (2 ml) at

$-78^{\circ}C$ was added n.BuLi (2.5 M in hexane 0.5 ml,1.25 mmol). The

cooling bath was removed and the mixture allowed to come to room

temperature when the solution became dark brown and homogeneous.

One half of this solution was taken up in a syringe and added to

a solution of the epoxide (120) (50 mg, 0.118 mmol) in THF (3 ml)

at $-78^{\circ}C$. The reaction mixture was then heated under reflux for

6 h. and on cooling it was diluted with hexane, washed once with a

solution 2 M in NaOH and 1.5 M sodium tartrate, and once with water.

The organic phase was dried $(MgSO_4)$ and concentrated in vacuo.

[1]H n.m.r. showed that the crude material contained desired product

and starting material in a ratio of 3:2 which could not be separated

chromatographically as both compounds had similar R_f's. The crude

mixture was recycled through the above procedure once again.

Purification by flash chromatography (3:2 Et_2O-hexane) gave 19 mg

(40%) of diene (121) as a white solid, attempted recrystallisation

of which under a variety of conditions failed: R_f 0.64 (1:1 EtOAc-

pet.ether); ν_{max} (CCl$_4$) 2960, 2930, 2860, 1750, 1705, 1450, 1370

1225 (br), 1180, 1090, 1070, 1055, 1040, 920 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 6.50 (1H, dq, J 5.7, 1.5 Hz, H–10),

5.87 (1H, s, H–7), 5.28 (1H, s, H–13a), 4.95 (1H, ddd, J 11.5, 4.5,

4.5 Hz, H–3), 4.88 (1H, s, H–13b), 4.69 (1H, br.d, J 5.7 Hz, H–11),

4.49 (1H, d, J 4.5 Hz, H–2), 4.37 and 4.25 (2H, ABq, J 12.0 Hz, H–15),

2.37 (1H, dd, H 14.9, 4.5 Hz, H–4α), 2.21 (3H, s, CH$_3$CO), 2.15 (3H,

s, CH$_3$CO), 1.91 (3H, s, CH$_3$CO), 1.83 (3H, dd, J 1.5, 0.7 Hz, H–16),

1.23 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 192.5 (C–8), 170.2 (CH$_3$CO), 170.0

(CH$_3$CO), 169.0 (CH$_3$CO), 149.9 (C–12), 137.1 (C–9), 136.8 (C–10),

110.0 (C–13), 79.0 (C–7), 74.9 (C–2), 71.0 (C–11), 70.4 (C–3), 62.4

(C–15), 51.1 (C–5), 47.8 (C–6), 41.5 (C–4), 21.0 (CH$_3$CO), 20.9 (CH$_3$CO),

20.6 (CH$_3$CO), 19.1 (C–16), 15.6 (C–14).

Mass spectrum, m/z found: 406.1603 (M$^+$, C$_{21}$H$_{26}$O$_8$ requires

406.1628).

Both the starting epoxide (120) and olefin (121) were

indistinguishable by t.l.c. analysis. Therefore, deoxygenations

were followed by GLC analyses. These were carried out with a

Perkin–Elmer F–11 gas chromatograph equipped with a silanized gas

column (6' x ¼" ID) packed with OV–17 coated onto Gas–Chrom Q,

100–120 mesh. The column was heated to 250°C, and the nitrogen

carrier gas pressure maintained at 14 psi. Under these conditions

epoxide (120) and olefin (121) had retention times of 19 m. and

15 m. respectively.

(122)
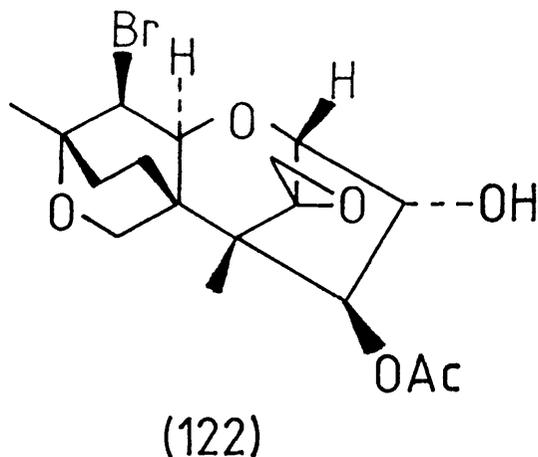
4β –Acetoxy–3α –hydroxy–10β –bromo–9α,15–oxa–12,
13–epoxytrichothecane (122)

To a solution of 4β –acetoxy–3α,15–dihydroxy–12,

13–epoxytrichothec–9–ene (48) (253 mg, 0.78 mmol) in dry

acetonitrile (20 ml) was added recrystallised NBS (146 mg,

0.82 mmol). The mixture was stirred under nitrogen for 1 h.

when it was concentrated under vacuum. Purification by dry

column flash chromatography (EtOAc–pet.ether) gave 288 mg (92%)

of the bromo–ether (122) as a white amorphous solid:

$[\alpha]_D^{20}$–29.4° (c 0.65, CHCl$_3$);   R$_f$ 0.32 (1:1 EtOAc–pet.ether);

ν_{max} (CCl$_4$) 3555, 2980, 2940, 2875, 1740, 1730, 1450, 1375,

1050, 960 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 5.04 (1H, d, J 3.2 Hz, H–4),

4.25 (2H, m, H–10, H–11), 4.21 (1H, dd, J 5.0, 3.2 Hz, H–3),

3.82 (1H, dd, J 9.6, 2.7 Hz, H–15a), 3.79 (1H, d, J 5.0 Hz, H–2),

3.70 (1H, d, J 9.6 Hz, H–15b),   3.05 and 2.73 (2H, ABq, J 3.8 Hz,

H–13), 2.10 (3H, s, CH$_3$CO), 2.20 (1H, dd, J 12.6, 10.0 Hz, H–8β),

2.15–1.95 (1H, m, H–7β), 1.85–1.65 (1H, m, H–8α), 1.60–1.40

(1H, m, H–7α), 1.26 (3H, s, H–16), 0.61 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz), δ 172.2 (CH$_3$CO), 83.1 (C-3),

79.7 (C-4), 78.1 (C-2), 73.7 (C-9), 68.3 (C-11), 66.0 (C-15),

64.1 (C-12), 54.2 (C-10), 46.4 (C-13), 46.1 (C-5), 41.8 (C-6),

27.9 (C-8), 24.2 (C-16), 20.9 (CH$_3$CO), 19.2 (C-7), 5.8 (C-14).

Mass spectrum, m/z found: 404.0668 and 402.0655

(M$^+$, C$_{17}$H$_{23}$O$_6$Br requires 404.0659 and 402.0678).

(123)
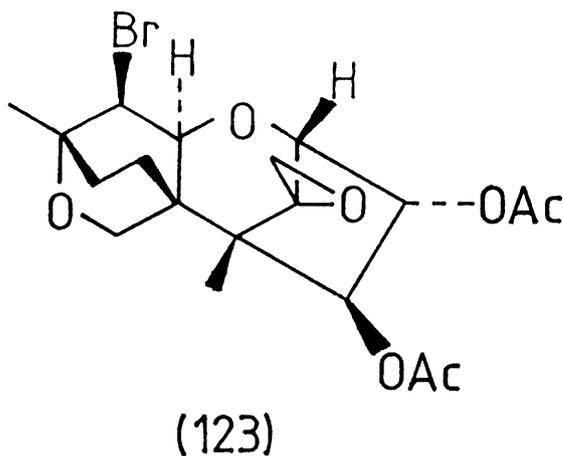
3α,4β-Diacetoxy-10β-bromo-9α,15-oxa-12,13-epoxytrichothecane

(123)

METHOD 1

Acetylation of the bromo-ether (122) (278 mg, 0.625 mmol) by the normal procedure gave 305 mg (99%) of the diacetate (123) as a white low-melting amorphous solid after purification by dry column flash chromatography (EtOAc-pet.ether).

$[\alpha]_D^{20}$ -18.2° (c 1.0, CHCl$_3$); R_f 0.40 (1.1 EtOAc-pet.ether); ν_{max} (CCl$_4$) 2980, 2940, 2875, 1750, 1370, 1240, 1220, 1050 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 2.00 MHz) δ5.57 (1H, d, J 3.6 Hz. H-4), 5.25 (1H, dd, J 4.9, 3.6 Hz, H-3), 4.28 (1H, dd, J 8.6, 1.8 Hz, H-11), 4.12 (1H, dd, J 8.6, 2.4 Hz, H-10), 3.99 (1H, dd, J 9.8, 2.8 Hz, H-15a), 3.96 (1H, d, J 4.9 Hz, H-2) 3.71 (1H, d, J 9.8 Hz, H-15b), 3.07 and 2.75 (2H, ABq, J 3.9 Hz, H-13), 2.30-2.16 (1H, dd, J 12.7, 7.6 Hz, H-8β), 2.10 (3H, s, CH$_3$CO), 2.09 (3H, s, CH$_3$CO), 1.88-1.62 (1H, m, H-7α), 1.60-1.40 (1H, m, H-8α), 1.28 (3H, s, H-16), 0.55 (3H, s, H-14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ170.6 (CH$_3$CO), 169.8 (CH$_3$CO),

78.4 (C-4), 78.0 (C-2), 77.8 (C-3), 73.7 (C-9), 68.5 (C-11),

66.0 (C-15), 63.8 (C-12), 54.2 (C-10), 46.4 (C-13), 46.1 (C-5),

41.9 (C-6), 27.9 (C-8), 24.2 (C-16), 20.9 (CH_3CO), 20.7 (CH_3CO),

19.4 (C-7), 5.5 (C-14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 446.0770 and 444.0794 (M^+,

$C_{19}H_{25}O_7Br$ requires 446.0764 and 444.0784).

METHOD 2 (from anguidine)

To a solution of anguidine (9) (262 mg, 0.72 mmol) in THF

(13.5 ml) and methanol (8.5 ml) was added aqueous sodium hydroxide

(0.4M, 100 ml, 40 mmol). The mixture was stirred for 15 m. when

it was passed through a column packed with Amberlite IR120(H)

ion-exchange resin (10 g). The product was eluted with a MeOH/

water mixture (1:4, 40 ml). After concentration in vacuo the

scirpenetriol obtained was used without further purification.

The crude triol (0.72 mmol, assumed) was dissolved in dry

acetonitrile (21 ml) and to this solution was added recrystallised

NBS (135 mg, 0.76 mmol). The reaction mixture was stirred under

nitrogen for 1 h. when it was concentrated in vacuo. The crude

bromo-ether obtained (134) was acetylated directly by the normal

procedure to give 300 mg (94% overall) of the desired bromo-ether

diacetate (123) after purification by dry column flash chromatography.

This material was identical by [1]H n.m.r. (90 MHz), i.r., m.s. and

t.l.c. to that previously prepared.

(124)
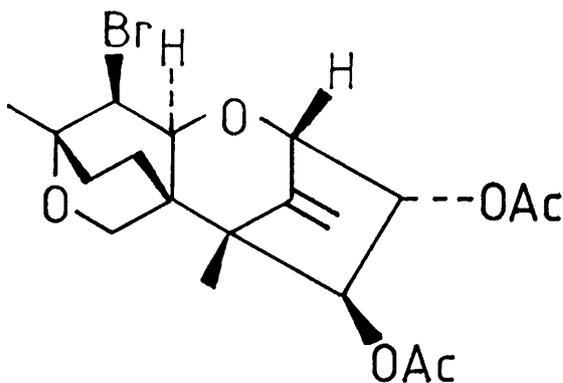
3α,4β–Diacetoxy–10β–bromo–9α–15–oxa–trichothec–12–ene (124)

To a suspension of WCl_6 (802 mg, 2.02 mmol) in THF (5.2 ml) at

–78°C was added n.BuLi (2.4 M in hexane, 2.1 ml, 5.04 mmol). The

cooling bath was removed and the mixture allowed to come to room

temperature when it became dark brown and homogeneous. The solution

was recooled to –78°C whereupon the epoxide (123) (300 mg, 0.67 mmol)

in THF (8.5 ml) was added. After being heated under reflux for 3.5 h.

the reaction mixture was diluted with hexane, washed once with a

solution 2 M in NaOH and 1.5 M in sodium tartrate and once with water.

The organic extract was dried ($MgSO_4$) and concentrated in vacuo.

Purification by dry column flash chromatography (EtOAc–pet.ether)

gave 285 mg (98%) of the desired olefin as a white crystalline solid:

mp 102–106°C (60:80 pet.ether); $[\alpha]_D^{20}$–18.2° (C 0.94, $CHCl_3$); R_f

0.45 (1:1 EtOAc–pet.ether), ν_{max} (CCl_4) 2980, 2940, 2870, 1745, 1370,

1240, 1230, 1100, 1050, 910 cm^{-1}.

[1]H n.m.r. ($CDCl_3$, 200 MHz) δ 5.53 (1H, d, J 3.5 Hz, H–4), 5.25

(1H, s, H–13a), 4.91 (1H, dd, J 5.0, 3.5 Hz, H–3), 4.82 (1H, s, H–13b)

4.57 (1H, d, J 5.0 Hz, H–2), 4.25 (1H, dd, J 8.6, 1.6 Hz, H–11), 4.14

(1H, dd, J 8.6, 2.4 Hz, H–10), 3.98 (1H, dd, J 9.7, 2.7 Hz, H–15a),

3.71 (1H, d, J 9.7 Hz, H–15b), 2.28–2.16 (1H, m, H–8β), 2.13 (3H,

s, CH$_3$CO), 2.04 (3H, s, CH$_3$CO), 1.92–1.65 (2H, m, H–7), 1.42–1.20

(1H, m, H–8α), 1.26 (3H, s, H–16), 0.79 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 170.3 (CH$_3$CO), 170.1 (CH$_3$CO), 147.3

(C–12), 109.3 (C–13), 78.3 (C–2), 78.1 (C–3), 78.1 (C–4), 73.3 (C–9),

68.2 (C–11), 66.3 (C–15), 54.8 (C–10), 49.1 (C–5), 41.5 (C–6), 27.8

(C–8), 24.2 (C–16), 21.0 (CH$_3$CO), 20.7 (CH$_3$CO), 18.7 (C–7), 9.48

(C–14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 430.0810 and 428.0830 (M$^+$, C$_{19}$H$_{25}$O$_6$Br

requires 430.0815 and 428.0835).

(125)
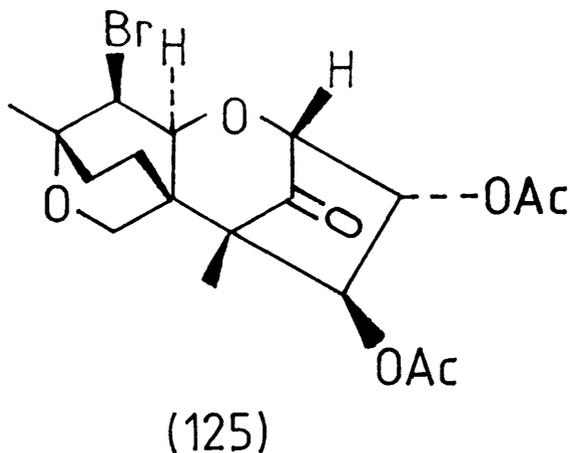
3α,4β –Diacetoxy–10β–bromo–9α,15–oxa–12–oxonortrichothecane (125)

Ozone was bubbled through a solution of olefin (124) (530 mg,

1.24 mmol) in dry CH_2Cl_2 (50 ml) at -78°C until the solution became

blue in colour. The solution was left to stand at $- 78^\circ$C for 15 m.

If the blue colour had disappeared the above procedure was repeated

until the blue colour remained after standing. Nitrogen was then

bubbled through the solution to expel excess ozone. Triethylamine

(0.34 ml, 2.47 mmol) was then added and the mixture stirred overnight

at room temperature. The solution was then filtered through silica

and concentrated in vacuo to give 462 mg (87%) of the norketone (125)

(pure by t.l.c. and [1]H n.m.r.) which could be used without further

purification.

The norketone can be recrystallised from ether–pet.ether:

mp 187–190°C; $[\alpha]_D^{20}$+65° (c 0.36, $CHCl_3$); R_f 0.41 (1:1 EtOAc–pet.

ether); ν_{max} (CCl_4) 2980, 2940, 2875, 1770, 1750, 1450, 1370, 1230,

1215, 1180, 1125, 1095, 1060, 1050, 910 cm^{-1}.

[1]H n.m.r. ($CDCl_3$) δ 5.71 (1H, d, J 3.8 Hz, H–4), 5.08 (1H, dd,

J 5.0, 3.8 Hz, H–3), 4.37 (1H, dd, J 8.6, 2.1 Hz, H–10), 4.27

(1H, dd, J 8.6, 1.6 Hz, H–11), 4.22 (1H, d, J 5.0 Hz, H–2), 4.06

(1H, dd, J 9.5, 2.6 Hz, H–15a), 3.67 (1H, d, J 9.5 Hz, H–15b),

2.3–2.0 (1H, m, H–8β), 2.18 (3H, s, CH_3CO), 2.06 (3H, s, CH_3CO),

1.9–1.5 (3H, m, H–7 and H–8α), 1.28 (3H, s, H–16), 0.76 (3H, s, H–14).

^1C n.m.r. ($CDCl_3$, 50 MHz) δ 207.8 (C–12), 170.0 (CH_3CO), 169.6

(CH_3CO), 77.2, 75.8, 73.8 (C–2, C–3 and C–4), 73.8 (C–9), 68.2 (C–11),

65.4 (C–15), 55.6 (C–5), 53.5 (C–10), 48.2 (C–6), 27.5 (C–8), 24.0

(C–16), 20.8 (CH_3CO) 20.6 (CH_3CO), 18.7 (C–7), 6.4 (C–14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 390.0500 and 388.0524 ($M^+-C_2H_2O$

$C_{16}H_{21}O_6Br$ requires 390.0502 and 388.0522).

Microanalysis, found: C, 50.01; H, 5.38; Br, 18.47% ($C_{18}H_{23}O_7Br$

requires: C, 50.11; H, 5.38; Br, 18.53%).

(126)
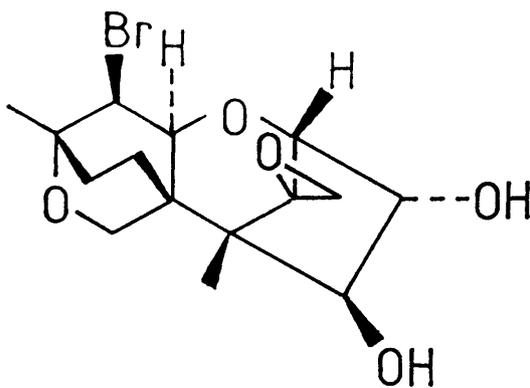
3α,4β–Dihydroxy–10β–bromo–9α,15–oxa–12,13–epiepoxytrichothecane (126)

METHOD 1 (from ketone (125))

To a stirred suspension of Me_3S^+ I^- (560 mg, 2.75 mmol) in THF (11 ml) under nitrogen was added n–BuLi (2.4M in hexane, 1.15 ml, 2.75 mmol). The solution became clear; it was stirred at $0°C$ for 1 h. when a solution of ketone (125) (148 mg, 0.344 mmol) in THF (5.5 ml) was added. After stirring for a further hour the mixture was diluted with ether, washed with saturated NH_4Cl solution and brine, dried ($MgSO_4$) and concentrated in vacuo. Purification by flash chromatography (EtOAc) gave the diol (126) as a white crystalline solid (43 mg, 35%): mp 207–8$°C$ (EtOAc–60–80 pet.ether); $[\alpha]_D^{20}$–51.6$°$ (c 0.51, MeOH); R_f 0.07 (4:1 EtOAc–pet.ether); ν_{max} (KBr) 3600–3200 (br), 2980, 2940, 2910, 2880, 1455, 1385, 1375, 1180, 1120, 1100, 1090, 1060, 950, 870, 840 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$, 200 MHz) δ4.22 (1H, dd, J 8.6, 1.4 Hz, H–11), 4.14 (1H, dd, J 8.6, 1.8 Hz, H–10), 4.05 (1H, d, J 2.9 Hz, H–4) 3.97 (1H, dd, J 4.7, 2.9 Hz, H–3), 3.72 (1H, d, J 9.3 Hz, H–15b), 3.70 (1H, d, J 4.7 Hz, H–2), 3.62 (1H, dd, J 9.3, 1.5 Hz, H–15a), 2.70 and 2.40 (2H, ABq, J 4.5 Hz, H–13) 2.37–2.10 (2H, m, H–7β and H–8β), 1.80–1.45 (2H, m, H–7α and H–8α), 1.22 (3H, s, H–16),

0.56 (3H, s, H-14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 79.6 (C-4), 78.4 (C-3), 78.0

(C-2), 74.0 (C-9), 68.4 (C-11), 66.3 (C-15), 62.6 (C-12), 54.5

(C-10), 45.5 (C-13), 45.2 (C-5), 39.8 (C-6), 28.0 (C-8), 24.3

(C-16), 19.4 (C-7), 7.1 (C-14).

Mass spectrum, m/z found: 362.0545 and 360.0570 (M^+,

$C_{15}H_{21}O_5Br$ requires 362.0553 and 360.0573).

Microanalysis, found: C, 49.83; H, 5.92; Br. 21.91%

$C_{15}H_{21}O_5Br$ requires: C, 49.86; H, 5.86; Br, 22.13%).

METHOD 2 (from bistriethylsilylether (132))

To a solution of the bistriethylsilylether (132) (114 mg,

0.194 mmol) in dry THF (6 ml) at 0°C under nitrogen was added

a solution of $Bu_4N^+F^-$ (1M in THF, 0.78 ml, 0.78 mmol). The

mixture was stirred at 0°C for 2 h. when it was diluted with

EtOAc, washed with brine, dried (MgSO$_4$) and concentrated in vacuo.

Purification by flash chromatography (EtOAc) gave 61 mg (87%) of

the diol (126) as white crystalline solid identical with material

previously prepared (by ^{1}H n.m.r., i.r., m.s., t.l.c).

(127)
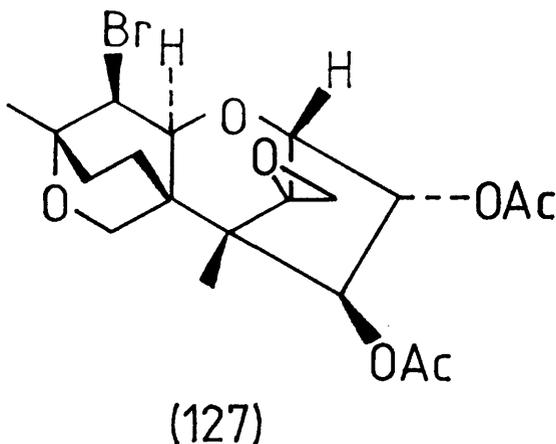
3α,4β-Diacetoxy-10β-bromo-9α,15-oxa-12,13-epiepoxytrichothecane

(127)

Diol (126) (87 mg, 0.24 mmol) was acetylated by the normal

procedure. Purification by flash chromatography (1:1 EtOAc–pet.ether)

gave 103 mg (96%) of the diacetate (127) as a white crystalline solid:

mp 160–161°C (ether–60:80 pet.ether); R_f 0.33 (1:1 EtOAc–pet.ether)

$[\alpha]_D^{20}$ –42.2° (c 0.45, CHCl$_3$); ν_{max} (CCl$_4$) 2990, 2960, 2940, 2880,

1755, 1460, 1375, 1240, 1220, 1110, 1065, 1055, 980 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 5.50 (1H, d, J 3.5 Hz, H–4),

5.12 (1H, dd, J 4.8, 3.5 Hz, H–3), 4.29 (1H, dd, J 8.5, 1.7 Hz,

J–11), 4.19 (1H, dd, J 8.5, 2.2 Hz, H–10), 4.00 (1H, d, J 4.8 Hz,

H–2), 3.93 (1H, dd, J 9.7, 2.8 Hz, H–15a); 3.75 (1H, d, J 9.7 Hz,

H–15b), 2.75 and 2.54 (2H, ABq, J 4.5 Hz, H–13), 2.47–2.17 (2H, m,

H–7β and H–8β), 2.14 (3H, s, CH$_3$CO), 2.07 (3H, s, CH$_3$CO), 1.85–1.45

(2H, m, H–7α and H–8α), 1.28 (3H, s, H–16), 0.54 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 170.0 (2 x CH$_3$CO), 77.7 (C–2),

77.5 (C–3), 75.5 (C–4), 73.9 (C–9), 68.8 (C–11), 66.2 (C–15), 62.3

(C–12), 54.2 (C–10), 45.3 (C–13), 44.6 (C–5), 40.5 (C–6), 28.0 (C–8),

24.3 (C–16), 20.9 (CH$_3$CO), 20.7 (CH$_3$CO), 19.6 (C–7), 7.23 (C–14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 446.0748 and 444.0781 (M^+, $C_{19}H_{25}O_7Br$ requires 446.0764 and 444.0784).

Microanalysis, found: C, 51.14; H, 5.64; Br 17.64% ($C_{19}H_{25}O_7Br$ requires: C, 51.23; H, 5.66; Br, 17.95%).

(128)
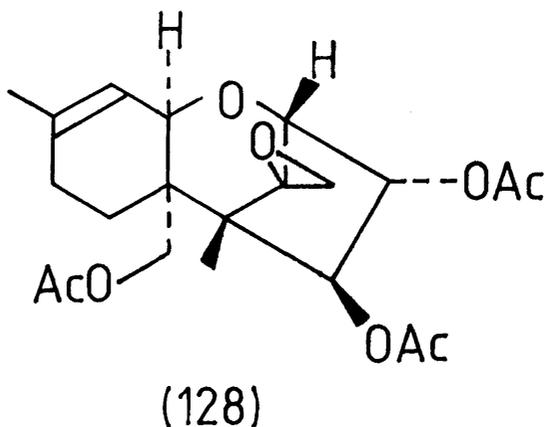
3α,4β,15-Triacetoxy-12,13-_epi_-epoxytrichothec-9-ene (128)

Preparation of Zn/Ag couple

To a stirred, warmed suspension of AgOAc (23 mg) in glacial

acetic acid (23 ml) was added zinc powder (4.16 g) all at once.

After stirring for 30 s. the acetic acid was decanted off and the

Zn/Ag couple rinsed with glacial acetic acid (1x11 ml) and dry

ether (4x11 ml) before use.

Use of Zn/Ag couple

To the freshly prepared Zn/Ag couple was added dry ether

(11 ml) and then the bromo-ether (127) (118 mg, 0.265 mmol) in

THF (21 ml) and ethanol (4 ml). The mixture was stirred at 55°C

for 2 h. and on cooling it was concentrated _in vacuo_. The residue

was taken up in acetone and then filtered through a pad of Celite.

After concentration the crude product was acetylated by the normal

procedure. Purification of the triacetate by flash chromatography

(EtOAc-pet.ether) gave 87 mg (81%) of _epi_-epoxide (128) as a white

crystalline solid: mp 169-170°C (benzene-hexane); R_f 0.33 (1:1 EtOAc-

pet.ether); $[\alpha]_D^{20}$ +7.9^{o} (c 0.81, CHCl$_3$); ν_{max} (CCl$_4$) 2970, 2930,

2910, 1750, 1370, 1240, 1220, 1090, 1060, 1025, 970 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 5.71 (1H, d, J 3.2 Hz, H-4), 5.47 (1H, br.d, H-10), 5.02 (1H, dd, J 4.8, 3.2 Hz, H-3), 4.20 and 4.06 (2H, ABq, J 12.4 Hz, H-15), 4.05 (1H, br.d, J 5.8 Hz, H-11), 3.83 (1H, d, J 4.8Hz, H-2), 2.81 and 2.50 (2H, ABq, J 4.6 Hz, H-13), 2.14 (3H, s, CH$_3$CO), 2.08 (3H, s, CH$_3$CO), 2.06 (3H, s, CH$_3$CO), 1.71 (3H, br.s, H-16), 0.76 (3H, s, H-14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 170.6 (CH$_3$CO), 170.1 (CH$_3$CO), 169.9 (CH$_3$CO), 140.6 (C-9), 118.2 (C-10), 78.5 (C-2), 76.6 (C-3), 76.1 (C-4), 68.1 (C-11), 63.8 (C-15), 62.7 (C-12), 47.1 (C-5) 45.7 (C-13), 42.8 (C-6), 27.8 (C-8), 23.2 (C-16), 21.1 (C-7), 21.0 (CH$_3$CO), 20.9 (CH$_3$CO), 20.7 (CH$_3$CO), 8.13 (C-14).

Mass spectrum, m/z found: 348.1579 (M$^+$-CH$_3$COOH, C$_{19}$H$_{24}$O$_6$ requires 348.1573).

Microanalysis, found: C, 61.76; H, 6.69% (C$_{21}$H$_{28}$O$_8$ requires: C, 61.74; H, 6.91%).

The structure was confirmed by x-ray crystallographic analysis (Appendix 2).

(129)
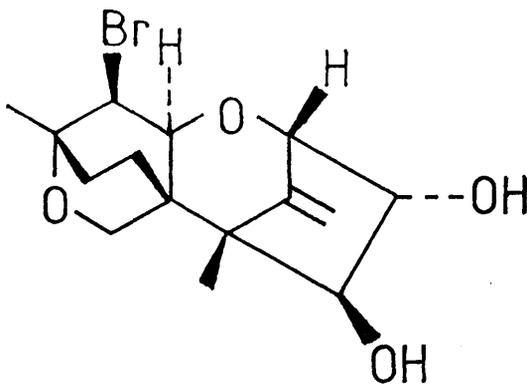
3α ,4β –Dihydroxy–10β –bromo–9α ,15–oxa–trichothec–12–ene (129)

To a solution of the diacetate (124) (151 mg, 0.35 mmol) in methanol (9 ml) and water (1 ml) was added K_2CO_3(2 g). The mixture was stirred for 2 hr. when it was concentrated in vacuo. The concentrate was taken up in water and extracted three times with EtOAc. The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. Purification by flash chromatography (EtOAc) gave 103 mg (85%) of the diol (129) as a white crystalline solid: mp 122-3°C (ether–hexane) $[\alpha]_D^{20}$-34.6° (c 0.78, MeOH); R_f 0.16 (4:1 EtOAc–60:80 pet.ether); ν_{max} (CCl_4) 3600–3200 (br), 2980, 2940, 2880, 1455, 1385, 1220, 1125, 1100, 1060, 910 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ5.17 (1H, s, H–13a), 4.78 (1H, s, 13b), 4.4–3.6 (9H, m, H–2, H–3, H–4, H–10, H–11 and 2xOH), 2.25–2.05, 1.95–1.60, 1.45–1.25, (4H, m, H–7 and H–8), 1.25 (3H, s, H–16), 0.86 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 148.6 (C–12), 108.7 (C–13), 81.08, 79.87, 79.47 (C–2, C–3 and C–4), 73.5 (C–9), 68.1 (C–11),

66.5 (C–15), 55.1 (C–10), 49.9 (C–5), 40.8 (C–6), 27.8 (C–8),

24.3 (C–16), 18.7 (C–7), 10.0 (C–14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 346.0596 and 344.0633 (M^+, $C_{15}H_{21}O_4Br$

requires 346.0604 and 344.0624).

Microanalysis, found: C, 52.19; H, 6.12; Br, 23.35%

($C_{15}H_{21}O_4Br$ requires C 52.19; H, 6.13; Br, 23.15%).

(130)
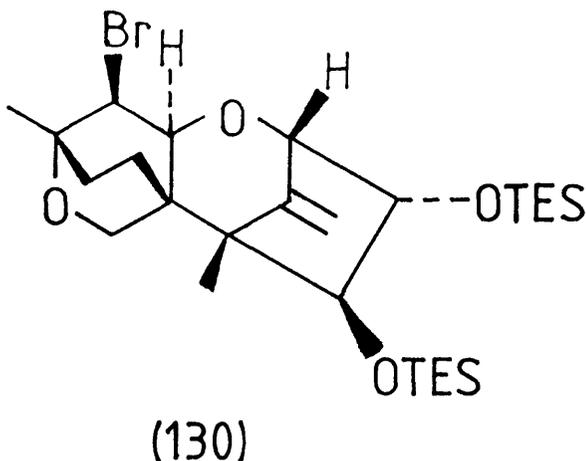
3α,4β-Bistriethylsilyloxy-10β-bromo-9α,15-oxa-trichothec-12-ene

(130)

To a solution of the diol (129) (225 mg, 0.65 mmol) in pyridine

(15 ml) was added DMAP (32 mg, 0.26 mmol) and triethylsilyl chloride

(0.44 ml, 2.62 mmol). The resulting mixture was stirred overnight

under nitrogen then it was diluted with CH_2Cl_2 and washed with

half saturated $NaHCO_3$ solution. The organic extract was dried

(Na_2SO_4) and concentrated in vacuo. Purification by dry column flash

chromatography (EtOAc-pet.ether) gave 327 mg (86%) of the

bistriethylsilylether (130) as a white crystalline solid: mp 51-2°C

(EtOAc/MeOH); $[\alpha]_D^{20}$-33° (c 1.09, $CHCl_3$); R_f 0.75 (1:1 EtOAc

pet.ether); ν_{max} (CCl_4) 2980, 2935, 2910, 2880, 1460, 1410, 1380, 1240,

1110, 1100, 905, 870 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 5.10 (1H, s, H-13a), 4.70 (1H, s,

H-13b), 4.20 (1H, d, J 4.8 Hz, H-2), 4.16 (2H, m, H-10 and H-11),

4.09 (1H, d, J 2.5 Hz, H-4), 3.80 (1H, dd, J 4.8, 2.5 Hz, H-3),

3.72 (2H, br.s, H-15), 2.25-2.10, 1.95-1.60, 1.4-1.2 (4H, m, H-7

and H-8), 1.25 (3H, s, H-16), 0.95 (6H, 2 x t (overlapping), J 8 Hz,

2 x CH_3CH_2Si), 0.77 (3H, s, H-14), 0.64 (4H, 2 x q (overlapping),

J 8 Hz, 2 x CH_3CH_2Si).

^{13}C n.m.r. ($CDCl_3$, 50 MHz) δ 149.7 (C–12), 107.6 (C–13),

83.2, 81.6, 80.1 (C–2, C–3 and C–4), 73.3 (C–9), 67.6 (C–11),

66.7 (C–15), 55.0 (C–10), 50.0 (C–5), 41.0 (C–6), 27.8 (C–8),

24.4 (C–16), 18.9 (C–7), 10.3 (C–14), 6.9 (2 x CH_3CH_2Si), 5.0

and 4.9 (2 x CH_3CH_2Si).

Mass spectrum, m/z found: 574.2317 and 572.2342

(M^+, $C_{27}H_{49}O_4Si_2Br$ requires 574.2333 and 572.2353).

Microanalysis, found: C, 56.33, H, 8.41; Br, 13.45%.

($C_{27}H_{49}O_4Si_2Br$ requires C, 56.52; H, 8.61; Br, 13.94%).

(131)
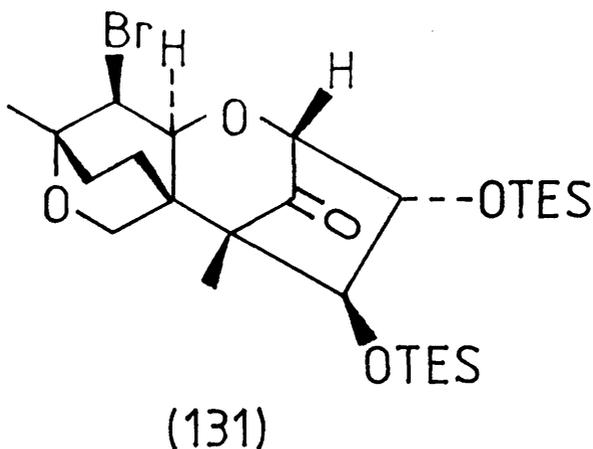
3α,4β-Bistriethylsilyloxy-10β-bromo-9α, 15-oxa-12-oxo-trichothecane

(131)

Ozone was bubbled through a solution of the olefin (130)

(327 mg, 0.57 mmol), in dry CH_2Cl_2(30 ml) at $-78°C$ until the solution

became blue in colour. The solution was left to stand at $-78°C$ for

15 m. If the blue colour had disappeared the above procedure was

repeated until the blue colour remained after standing. Nitrogen

was then bubbled through the solution to expel excess ozone.

Triethylamine (0.25 ml, 1.79 mmol) was added to the solution and the

resulting mixture was left to stir overnight at room temperature.

The solution was then filtered through silica and concentrated in vacuo.

Purification by dry column flash chromatography (EtOAc–pet.ether) gave

275 mg (84%) of norketone (131) as a white crystalline solid: mp 90–91°C

(ether–methanol); $[\alpha]_D^{20}$+33.3° (c 0.63, $CHCl_3$); R_f 0.74 (1:1 EtOAc–

pet.ether); ν_{max} (CCl_4) 2960, 2940, 2920, 2880, 1765, 1460, 1130, 1115,

1100, 1010, 885, 870, 860 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$, 200 MHz) δ4.4 (1H, dd, J 8.8, 2.2 Hz, H–10), 4.26

(1H, d, J 2.6 Hz, H–4), 4.18 (1H, dd, J 8.8, 1.6 Hz, H–11), 3.92

(1H, dd, J 5.0, 2.6 Hz, H-3), 3.85 (1H, d, J 5.0 Hz, H-2), 3.76

(1H, dd, J 9.2, 2.4 Hz, H-15a), 3.67 (1H, d, J 9.2 Hz, H-15b),

2.27-2.10 (1H, m, H-8β), 1.84-1.45 (3H, m, H-8α and H-7), 1.26

(3H, s, H-16), 0.96 (6H, 2 x t (overlapping), J 8.3 Hz, 2 x CH_3CH_2Si),

0.78 (3H, s, H-14), 0.64 (4H, 2 x q (overlapping), J 8.3 Hz,

2 x CH_3CH_2Si).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 211.2 (C-12), 80.0, 79.3, 79.0 (C-2,

C-3, C-4), 73.8 (C-9), 67.5 (C-11), 65.8 (C-15), 57.2 (C-5), 53.6

(C-10), 47.3 (C-6), 27.5 (C-8), 24.2 (C-16), 19.0 (C-7), 7.0 (C-14),

6.8 (2 x CH_3CH_2Si), 4.9 (CH_3CH_2Si), 4.8 (CH_3CH_2Si).

Mass spectrum, $\underline{m}/\underline{z}$ found: 576.2148 and 574.2155 (M^+,

$C_{26}H_{47}O_5Si_2Br$ requires 576.2126 and 574.2146).

Microanalysis, found: C, 54.13; H, 8.13; Br 13.72%

($C_{26}H_{47}O_5Si_2Br$ requires C, 54.24, H, 8.23; Br, 13.89%).

(132)
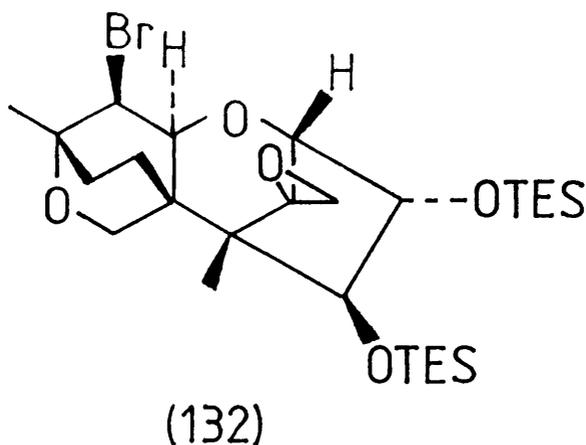
3α,4β-Bistriethylsilyloxy-10β-bromo-9α,15-oxa-12,13-epiepoxytrichothecane (132)

To a suspension of $Me_3S^+I^-$ (141 mg, 0.70 mmol) in THF (10 ml) under nitrogen at $0^\circ C$ was added nBuLi (2.4M in hexane, 0.305 ml, 0.735 mmol). The solution became clear and was left to stir at $0^\circ C$ for 1 hour when a solution of ketone (131) (290 mg, 0.496 mmol) in THF (6 ml) was added. The mixture was left to stir for 1 hour when it was diluted with ether, washed with saturated NH_4Cl solution, dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography (pet.ether then 9:1 pet.ether–EtOAc) gave 191 mg (64%) of the epiepoxide (132) as a colourless oil: $[\alpha]_D^{20}$-27.1° (c 0.35 $CHCl_3$); R_f 0.72 (1:1 EtOAc–pet.ether); ν_{max} (CCl_4) 2955, 2940, 2910, 2880, 1450, 1410, 1380, 1360, 1240, 1200, 1190, 1180, 1165, 1120, 1100, 1075, 1060, 1005, 980, 955, 925, 915, 890, 870, 860 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz)δ4.24 (1H, dd, J 8.7, 2.1 Hz, H-10), 4.15 (1H, dd, J 8.7, 1.5 Hz, H-11), 4.05 (1H, d, J 2.5 Hz, H-4), 3.96 (1H, dd, J 4.8, 2.5 Hz, H-3), 3.75 (1H, d, J 9.1 Hz, H-15a),

3.63 (2H, d + dd, J 4.8 Hz and J 9.1, 2.6 Hz, H–2 and H–15b),

2.66 and 2.38 (2H, ABq, J 4.6 Hz, H–13), 2.42–2.12 (2H, m, H–7β

and H–8β), 1.80–1.61 (1H, m, H–7α), 1.60–1.44 (1H, m, H–8α),

1.25 (3H, s, H–16), 0.95 (6H, 2 x t, CH$_2$CH$_3$), 0.60 (4H, 2 x q,

CH$_2$CH$_3$), 0.55 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ80.9 (C–3), 80.7 (C–4), 79.7

(C–11), 73.8 (C–9), 68.0 (C–2), 66.5 (C–15), 62.6 (C–12), 54.4

(C–10), 45.5 (C–13), 45.4 (C–5), 39.9 (C–6), 28.0 (C–8), 24.5

(C–16), 19.7 (C–7), 7.6 (C–14), 6.8 (2 x CH$_3$CH$_2$), 4.9 (CH$_3$CH$_2$),

4.8 (CH$_3$CH$_2$).

Mass spectrum, m/z found: 561.1891 and 559.1913

(M$^+$–C$_2$H$_5$, C$_{25}$H$_{44}$O$_5$Si$_2$Br requires 561.1891 and 559.1911).

(135)
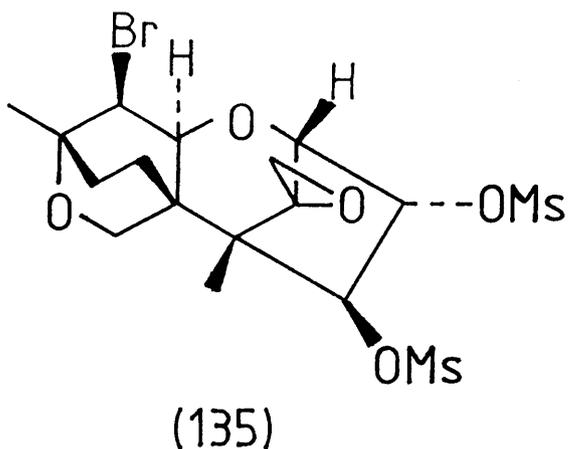
3α,4β-Bismesyloxy-10β-bromo-9α,15-oxa-12,13-epoxytrichothecane

(135)

To a solution of anguidine (9) (374 mg, 1.02 mmol) in THF

(8 ml) and MeOH (5 ml) was added NaOH solution (1M, 10 ml, 10 mmol).

The resulting mixture was stirred for 15 m. then passed through a

column packed with Amberlite IR-120(H) ion exchange resin (10 g) -

the product being eluted with a MeOH-water mixture (1:4).

Concentration in vacuo gave scirpenetriol which was used without

further purification.

To scirpenetriol (1.02 mmol, assumed) in dry CH_3CN (20 ml)

was added recrystallised NBS (213 mg, 1.20 mmol). The mixture

was stirred for 15 m., then concentrated in vacuo. The crude

bromo-ether (134) was used without further purification.

The crude bromo-ether (134) (1.02 mmol) was dissolved in

pyridine (6 ml) and cooled to $0^{\circ}C$. To the solution was added

methanesulphonyl chloride (3 ml) and the resulting mixture was

stoppered and left at $4^{\circ}C$ overnight. The reaction mixture was

then poured on to ice water, acidified with 1N HCl (15 ml) and

then extracted with EtOAc. The organic extracts were dried

(MgSO$_4$) and then concentrated in vacuo. Purification by dry

column flash chromatography (EtOAc–pet.ether) gave 486 mg (92% from

anguidine) of the bismesylate (135) as an off–white amorphous

solid: $[\alpha]_D^{20}$ –17.3 (0.52, CHCl$_3$); R$_f$ 0.28 (1:1 EtOAc–pet.ether);

ν_{max} (CCl$_4$) 2980, 2940, 2880, 1375, 1180, 1050, 1035, 970, 960,

910, 870, 860 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 5.36 (1H, d, J 3.3 Hz, H–4),

5.22 (1H, dd, J 4.9, 3.3 Hz, H–3), 4.25 (1H, dd, J 8.6, 1.7 Hz,

H–11), 4.01 (1H, d, J 4.9 Hz, H–2), 3.99 (1H, dd, J 8.6, 2.3 Hz,

H–10), 3.84 (1H, dd, J 10.0, 2.6 Hz, H–15a), 3.73 (1H, d, J 10.0 Hz,

H–15b), 3.09 and 2.80 (2H, ABq, J 3.8 Hz, H–13), 3.16 (3H, s,

–OSO$_2$CH$_3$), 3.15 (3H, s, –OSO$_2$CH$_3$), 2.22 (1H, dd, J 12.8, 9.8 Hz,

H–8β), 2.15–1.96 (1H, m, H–7β), 1.90–1.70 (1H, m, H–7α), 1.63–

1.46 (1H, m, H–8α), 1.27 (3H, s, H–16), 0.75 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 85.0 (C–4), 82.7 (C–3), 78.5

(C–2), 73.8 (C–9), 68.6 (C–11), 65.5 (C–15), 63.1 (C–12), 53.5

(C–10), 46.7 (C–5), 46.5 (C–13), 42.1 (C–6), 39.1 (–OSO$_2$CH$_3$),

38.1 (–OSO$_2$CH$_3$), 27.7 (C–8), 24.1 (C–16), 19.4 (C–7), 6.6 (C–14).

Mass spectrum, m/z found: 518.0082 and 516.0149 (M$^+$,

C$_{17}$H$_{25}$O$_9$S$_2$Br requires 518.0104 and 516.0124).

(136)
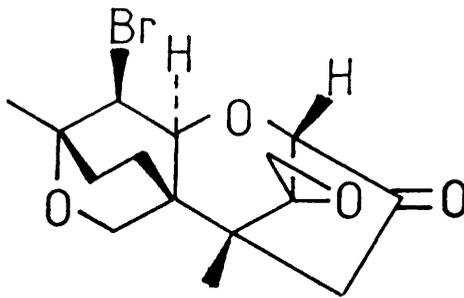
10β-Bromo-9α,15-oxa-3-oxo-12,13-epoxytrichothecane (136)

The bismesylate (135) (214 mg, 0.41 mmol) in methanol (3 ml)

was added to freshly prepared NaOMe in methanol (from 150 mg sodium

in 12 ml methanol). The mixture was heated under reflux for 2 h.

and on cooling was diluted with water, extracted with $CHCl_3$, dried

($MgSO_4$) and concentrated in vacuo. Purification by dry column flash

chromatography (EtOAc-pet.ether) gave 118 mg (83%) of ketone (136)

as a white crystalline solid: mp 92-94°C (hygroscopic; ether-pet.

ether); $[\alpha]_D^{20}$-176° (c 0.33, $CHCl_3$); R_f 0.39 (1:1 EtOAc-pet.ether);

ν_{max} ($CHCl_3$) 2975, 2940, 2875, 1770, 1450, 1380, 1260, 1215, 1200, 1190,

1135, 1100, 1090, 1065, 1050, 1000, 975, 960, 925 cm^{-1}.

[1]H n.m.r. ($CDCl_3$, 200 MHz) δ4.17 (1H, dd, J 8.5, 1.6 Hz, H-11),

3.98 (1H, dd, J 8.5, 2.3 Hz, H-10), 3.76 (1H, d, J 9.4 Hz, H-15b),

3.61 (1H, dd, J 9.4, 2.6 Hz, H-15a), 3.51 (1H, s, H-2), 3.22 and

3.02 (2H, ABq, J 3.8, H-13) 2.59 and 2.25 (2H, ABq, J 9.0 Hz, H-4),

2.25-2.01 (2H, m, H-8), 1.88-1.55 (2H, m, H-7), 1.26 (3H, s, H-16),

0.82 (3H, s, H-14).

[13]C n.m.r. ($CDCl_3$, 50 MHz) δ210.6 (C-3), 80.2 (C-2), 73.4 (C-9),

69.9 (C-11), 66.0 (C-15), 63.6 (C-12), 53.8 (C-10), 48.0 (C-13),

46.4 (C-4), 43.0 (C-5) 41.2 (C-6), 27.7 (C-8), 24.0 (C-16), 18.5

(C-7), 11.0 (C-14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 344.0448 and 342.0471 (M^+,

$C_{15}H_{19}O_4Br$ requires 344.0447 and 342.0467).

Microanalysis, found: C, 52.68; H, 5.67; Br, 23.64%

($C_{15}H_{19}O_4Br$ requires C, 52.47; H, 5.58; Br, 23.29%).

(137)
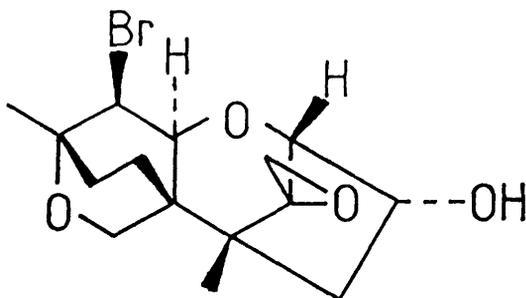
3α-Hydroxy-10β-bromo-9α,15-oxa-12,13-epoxytrichothecane (137)

To a solution of ketone (136) (118 mg, 0.344 mmol) in aqueous

methanol (4 ml water in 10 ml MeOH) at 0°C was added NaBH$_4$

(0.5 g, 13.2 mmol). The mixture was stirred for 15 m. when it was

diluted with EtOAc and washed with water. The aqueous phase was

re-extracted with EtOAc and the combined organic extracts were dried

(MgSO$_4$) and concentrated in vacuo. Purification by dry column flash

chromatography (EtOAc-pet.ether) gave 117 mg (99%) of alcohol (137)

as a white crystalline solid: mp 127-130°C (EtOAc-hexane);

$[\alpha]_D^{20}$-39.0° (c 1.0, CHCl$_3$); R$_f$ 0.12 (1:1 EtOAc-pet.ether);

ν_{max} (CHCl$_3$) 3600, 3050, 2980, 2940, 2880, 1460, 1410, 1380, 1230,

1195, 1180, 1135, 1050, 950, 900 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ4.45 (1H, m, H-3), 4.43 (1H, dd,

J 8.6, 3.2 Hz, H-10), 4.26 (1H, dd, J 8.6, 2.7 Hz, H-11), 3.67 (2H,

br.s, H-15), 3.62 (1H, d, J 4.6 Hz, H-2), 3.08 and 2.79 (2H, ABq,

J 3.9 Hz, H-13), 2.42 (1H, br.d, J 3.3 Hz, H-8β), 2.30 (1H, dd,

J 12.9, 10.0 Hz, H-4α), 2.13-1.90 (3H, m, H-4β, H-7β and -OH),

1.84-1.65 (1H, m, H-8α), 1.60-1.43 (1H, m, H-7α), 1.27 (3H, s, H-16),

0.64 (3H, s, H–14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 80.7 (C–2), 73.7 (C–9). 69.2 (C–3), 68.6 (C–11), 66.6 (C–15), 65.3 (C–12), 55.0 (C–10), 47.8 (C–13), 43.0 (C–5), 40.8 (C–4 and C–6), 28.1 (C–8), 24.3 (C–16), 19.4 (C–7), 11.2 (C–14).

Mass spectrum, m/z found: 346.0599 and 344.0631 (M$^+$, C$_{15}$H$_{21}$O$_4$Br requires: 346.0604 and 344.0624).

Microanalysis, found: C, 52.28; H, 6.15; Br, 23.42% (C$_{15}$H$_{21}$O$_4$Br requires: C, 52.17; H, 6.13; Br, 23.16%).

(138)
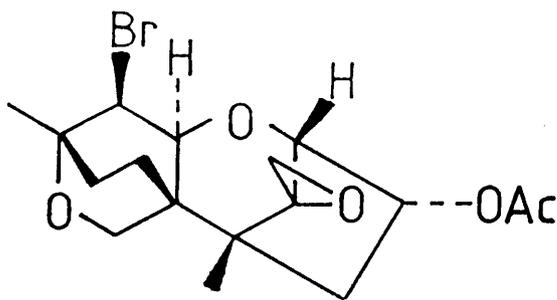
3α-Acetoxy-10β-bromo-9α,15-oxa-12,13-epoxytrichothecane (138)

Alcohol (137) (130 mg, 0.38 mmol) was acetylated in the normal way. Purification by dry column flash chromatography (EtOAc-pet.ether) gave 136 mg (93%) of acetate (138) as a white crystalline solid: mp 119-123°C (benzene-60:80 pet.ether): $[\alpha]_D^{20}$-35.6 (c 0.62, CHCl$_3$); R$_f$ 0.41 (1:1 EtOAc-pet.ether); ν_{max} (CCl$_4$) 2980, 2940, 2880, 1750, 1455, 1385, 1360, 1240, 1210, 1200, 1190, 1135, 1100, 1050, 965 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 5.20 (1H, ddd, J 10.0, 5.0, 5.0 Hz, H-3), 4.25 (2H, br.s, H-10 and H-11), 3.84 (1H, d, J 5.0 Hz, H-2), 3.69 (1H, d, J 9.3 Hz, H-15a), 3.61 (1H, dd, J 9.3, 2.6 Hz, H-15b), 3.09 and 2.80 (2H, ABq, J 3.9 Hz, H-13), 2.10 (3H, s, CH$_3$CO), 2.30-1.35 6H, m, H-4, H-7 and H-8), 1.26 (3H, s, H-16), 0.64 (3H, s, H-14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 170.3 (CH$_3$CO), 79.0 (C-2), 73.8 (C-9), 70.8 (C-3), 68.6 (C-11), 66.5 (C-15), 64.6 (C-12), 54.8 (C-10), 47.7 (C-13), 42.5 (C-5), 40.8 (C-6), 38.2 (C-4), 28.0 (C-8), 24.2 (C-16), 21.0 (CH$_3$CO), 19.0 (C-7), 11.06 (C-14).

Mass spectrum, m/z found: 307.1552 (M$^+$-Br, C$_{17}$H$_{23}$O$_5$ requires 307.1545).

Microanalysis, found: C, 52.93; H, 5.84; Br, 20.60%

($C_{17}H_{23}O_5Br$ requires: C, 52.70; H, 5.99; Br, 20.64%).

(5)
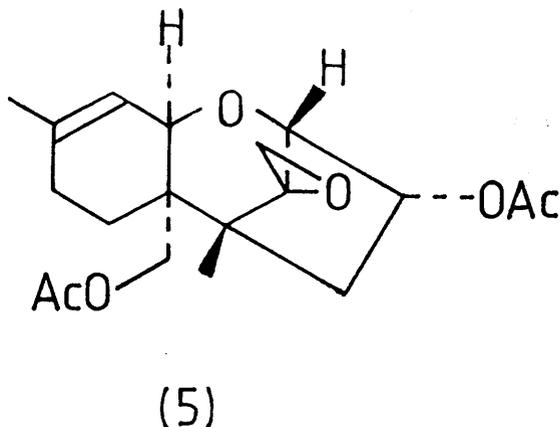
3α,15–Diacetoxy–12,13–epoxytrichothec–9–ene (calonectrin) (5)

Preparation of Zn/Ag couple

To a stirred warmed suspension of AgOAc (118 mg) in glacial acetic acid (120 ml) was added zinc powder (21.5 g) all at once. After stirring for 30 s. the acetic acid was decanted off and the Zn/Ag couple rinsed with glacial acetic acid (1 x 55 ml) and dry ether (4 x 55 ml) before use.

Use of Zn/Ag couple

To the freshly prepared Zn/Ag couple was added dry ether (55 ml) and then the bromo–ether (138) (531 mg, 1.37 mmol) in THF (105 ml) and ethanol (20 ml). The mixture was stirred at 55°C for 1½ h. On cooling it was concentrated in vacuo. The residue was taken up in acetone and filtered through a pad of Celite. After concentration the crude product was acetylated by the normal procedure. Purification by dry column flash chromatography gave 377 mg (79%) of calonectrin (5) as a colourless oil which could not be recrystallised: $[\alpha]_D^{21}$+ 2.9° (c 0.76, CHCl$_3$), lit.[36] $[\alpha]_D^{27}$+ 5.8° ; R_f 0.38

(1:1 EtOAc-pet.ether); ν_{max} (CCl$_4$) 2980, 2940, 2920, 1750, 1450, 1375, 1365, 1240, 1210, 1160, 1060, 1040, 960 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 5.42 (1H, br.d, J 4.4 Hz, H-10), 5.12 (1H, ddd, 9.3, 4.8, 4.8 Hz, H-3), 4.04 and 3.79 (2H, ABq, J 12.2 Hz, H-15), 3.97 (1H, br.d, J 4.4 Hz, H-11), 3.71 (1H, d, J 4.8 Hz, H-2) 3.05 and 2.81 (2H, ABq, J 4.0 Hz, H-13), 2.07 (3H, s, CH$_3$CO), 2.00 (3H, s, CH$_3$CO), 1.68 (3H, br.s, H-16), 0.79 (3H, s, H-14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 170.8 (CH$_3$CO), 170.3 (CH$_3$CO), 140.2 (C-9), 118.8 (C-10), 77.9 (C-2), 71.1 (C-3), 68.0 (C-11), 64.9 (C-12), 63.5 (C-15), 48.4 (C-13), 45.2 (C-5), 42.8 (C-6), 39.2 (C-4), 28.1 (C-8), 23.1 (C-16), 20.9 (CH$_3$CO), 20.9 (CH$_3$CO and C-7), 12.0 (C-14).

Mass spectrum, m/z found: 350.1737 (M$^+$, C$_{19}$H$_{26}$O$_6$ requires 350.1729).

(64)
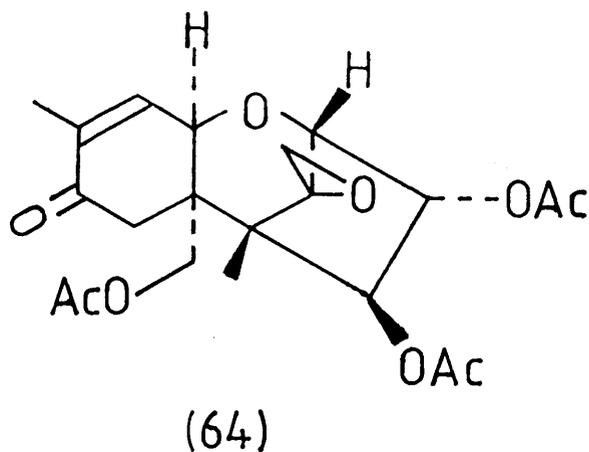
3α,4β,15-Triacetoxy-12,13-epoxytrichothec-9-en-8-one (64)

To a suspension of freshly prepared py_2CrO_3 (4.7g, 18.2 mmol)

in dry CH_2Cl_2 (20 ml) was added a solution of triacetoxyscirpene

(63) (137 mg, 0.33 mmol) in dry CH_2Cl_2 (5 ml) and the resulting

mixture was stirred for 4 d. under nitrogen. Saturated $NaHCO_3$

solution was then added to the mixture to dissolve the solid

residues formed during reaction. The organic layer was separated

and the remaining aqueous layer was carefully extracted with EtOAc.

The combined organic layers were dried ($MgSO_4$) and concentrated

in vacuo. Purification by flash chromatography (1:1 EtOAc-pet.ether)

gave 86 mg (60%) of enone (64) as a white crystalline solid: mp

139–140°C (EtOAc–40:60 pet.ether); $[\alpha]_D^{20}$+80.4° (c 0.56, $CHCl_3$);

R_f 0.30 (1:1 EtOAc–pet.ether); ν_{max} (CCl_4) 2990, 2960, 2930, 1750,

1690, 1450, 1430, 1370, 1240, 1220, 1165, 1085, 1060, 1050, 965 cm^{-1};

λ_{max}^{EtOH} 226 nm (ϵ = 9000).

[1]H n.m.r. ($CDCl_3$, 200 MHz) δ 6.54 (1H, dq, J 5.8, 1.5 Hz, H–10),

5.71 (1H, d, J 3.3 Hz,H–4), 5.22 (1H, dd, J 4.9, 3.3 Hz, H–3), 4.37

(1H, br.d, J 5.8 Hz, H-11), 4.31 and 4.13 (2H, ABq, J 12.5 Hz, H-15),

3.94 (1H, d, J 4.9 Hz, H-2), 3.08 and 2.82 (2H, ABq, J 3.9 Hz, H-13),

2.87 (1H, d, J 15.9 Hz, H-7β), 2.45 (1H, dd, J 15.9, 1.6 Hz, H-7α),

2.16 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 1.99 (3H, s, CH_3CO), 1.82

(3H, dd, J 1.5, 0.7 Hz, H-16), 0.72 (3H, s, H-14).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 196.3 (C-8), 170.0 (2 x CH_3CO), 169.7

(CH_3CO), 138.8 (C-9), 136.4 (C-10), 78.2,(C-2) 78.1 (C-3), 77.6 (C-4),

68.2 (C-11), 64.3 (C-15), 64.1 (C-12), 48.7 (C-5), 47.5 (C-6), 46.8

(C-13), 38.2 (C-7), 20.8 (CH_3CO), 20.7 (CH_3CO), 20.6 (CH_3CO), 15.4 (C-16),

5.9 (C-14).

Mass spectrum, m/z found: 422.1582 (M^+, $C_{21}H_{26}O_9$ requires

422.1577).

Microanalysis, found: C, 59.58: H, 6.12% ($C_{21}H_{26}O_9$ requires

C, 59.69; H, 6.21%).

(139)
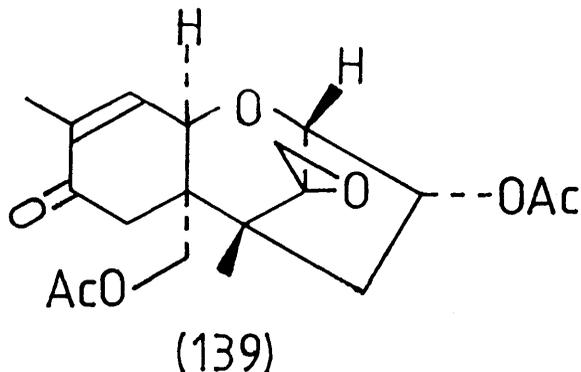
3α,15-Diacetoxy-12,13-epoxytrichothec-9-en-8-one (139)

To a suspension of freshly prepared py_2CrO_3 (12.9 g, 49.9 mmol) in dry CH_2Cl_2 (30 ml) under nitrogen was added a solution of calonectrin (5) (185 mg, 0.53 mmol) in dry CH_2Cl_2 (5 ml) and the resulting mixture stirred for 1 day at room temperature. Saturated $NaHCO_3$ solution was then added to the mixture to dissolve the solid residues formed during reaction. The organic layer was separated and the remaining aqueous phase was carefully extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography gave 141 mg (73%) of enone (139) as a white crystallise solid: mp 139-140°C (ether); $[\alpha]_D^{20}$+61.6 (c 0.54, $CHCl_3$); R_f 0.27 (1:1 EtOAc-pet.ether); ν_{max} (CCl_4) 2980, 2920, 1750, 1680, 1450, 1375, 1365, 1230 (br), 1160 1045, 960 cm^{-1}; λ_{max}^{EtOH} 225 nm (ϵ= 8900).

^{1}H n.m.r. ($CDCl_3$, 200 MHz) δ 6.54 (1H, dq, J 5.8, 1.5 Hz, H-10), 5.21 (1H, ddd, J 10.0, 5.0, 5.0 Hz, H-3), 4.45 (1H, br.d, J 5.8 Hz, H-11), 4.11 and 4.02 (2H, ABq, J 12.0 Hz, H-15), 3.84 (1H, d, J 5.0 Hz, H-2), 3.11 and 2.88 (2H, ABq, J 3.9 Hz, H-13),

2.86 (1H, d, J 15.8 Hz, H-7β), 2.51 (1H, dd, J 15.8, 1.4 Hz, H-7α),

2.30–2.06 (2H, m, H-4), 2.13 (3H, s, CH_3CO), 1.96, (3H, s, CH_3CO),

1.82 (3H, dd, J 1.5, 0.8 Hz, H-16), 0.81 (3H, s, H-14).

^{13}C n.m.r. ($CDCl_3$, 50 MHz) δ197.1 (C-8), 170.3 (CH_3CO), 170.2

(CH_3CO), 138.4 (C-9), 137.1 (C-10), 77.9 (C-2), 70.9 (C-3), 68.6

(C-11), 64.9 (C-12), 64.9 (C-15), 48.2 (C-13), 46.4 (C-5), 45.2 (C-6),

38.4 (C-4), 38.1 (C-7), 20.9 (CH_3CO), 20.6 (CH_3CO), 15.5 (C-16), 11.2

(C-14).

Mass spectrum, $\underline{m}/\underline{z}$ found: 364.1520 (M^+, $C_{19}H_{24}O_7$ requires

364.1522).

Microanalysis, found: C, 62.56; H, 6.78% ($C_{19}H_{24}O_7$ requires

C, 62.61; H, 6.64%).

(140)
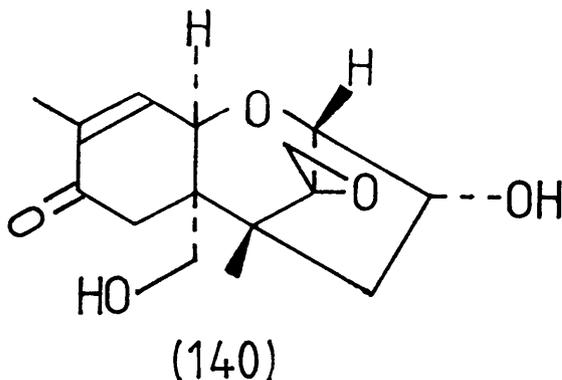
3α,15-Dihydroxy-12,13-epoxytrichothec-9-en-8-one (140)

To the diacetate (139) (81 mg, 0.223 mmol) in wet methanol

(0.5 ml water in 10 ml MeOH) was added K_2CO_3 (0.5 g). The mixture

was stirred for 1 h. when it was concentrated in vacuo. The residue

was taken up in water and extracted thoroughly with EtOAc. The

combined organic layers were dried ($MgSO_4$) and concentrated in vacuo.

Purification by flash chromatography (EtOAc) gave 56 mg (90%) of diol

(140) as a white solid: R_f 0.06 (4:1 EtOAc–pet.ether); ν_{max}

(KBr) 3600–3200 (br), 2960, 2920, 2850, 1680,1660, 1450, 1425, 1380,

1350, 1260, 1165, 1090, 1070, 1060, 1030, 1020, 950, 840 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$ + 1 drop MeOD, 90 MHz) δ 6.65 (1H, br.d,

J 6 Hz, H–10), 4.70 (1H, br.d, J 6 Hz, H–11), 4.50 (1H, ddd, 11, 6,

6 Hz, H–3), 3.65 (2H, br.s, H–15), 3.60 (1H, d, J 6 Hz, H–2), 3.10

and 2.90 (2H, ABq, J 4.0 Hz, H–13), 2.9–2.1 (4H, m, H–4 and H–7),

1.85 (3H, br.s, H–16), 0.85 (3H, s, H–14).

Mass spectrum, m/z found: 280.1317 (M^+, $C_{15}H_{20}O_5$ requires

280.1311).

(141)
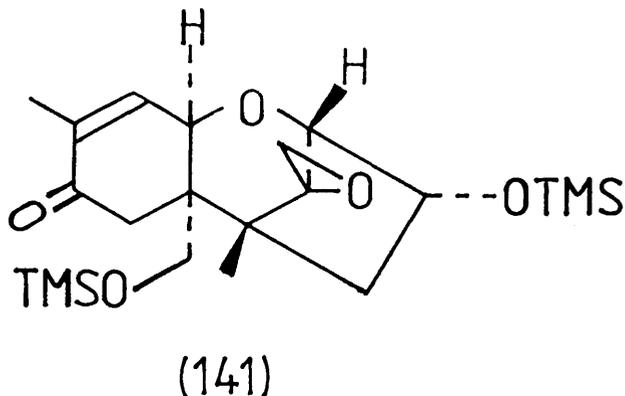
3α,15-Bistrimethylsilyloxy-12,13-epoxytrichothec-9-en-8-one (141)

To the diol (140) (55 mg, 0.196 mmol) in ether (3 ml) and pyridine (6 ml) was added excess TMSCl. The mixture was stirred overnight at room temperature then diluted with ether, washed once with water, dried (Na$_2$SO$_4$) and concentrated _in vacuo_. Purification by flash chromatography (1:9 EtOAc–pet.ether) gave 75 mg (90%) of the bissilylether (141) as a colourless oil: R$_f$ 0.64 (1:1 EtOAc–pet.ether); ν_{max} (CCl$_4$) 2970, 2940, 2920, 1680, 1455, 1370, 1260, 1175, 1150, 1110 (br), 970, 940, 880, 850 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 6.57 (1H, dq, J 5.8, 1.5 Hz, H–10), 4.75 (1H, br.d, J 5.8 Hz, H–11), 4.40 (1H, ddd, J 10.5,4.6, 4.0 Hz, H–3), 3.53 (2H, s, H–15), 3.47 (1H, d, J 4.6 Hz, H–2), 3.05 and 2.84 (2H, ABq, J 4.0 Hz, H–13), 2.78 (1H, d, J 15.9 Hz, H–7β), 2.37 (1H, dd, J 15.9, 1.4 Hz, H–7α), 2.24 (1H, dd, J 14.3, 4.0 Hz, H–4α), 2.04 (1H, dd, J 14.3, 10.5 Hz, H–4β), 1.81 (3H, dd, J 1.4, 0.8 Hz, H–16), 0.77 (3H, s, H–14), 0.15 (9H, s, Si–CH$_3$), 0.01 (9H, s, Si–CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 198.3 (C–8), 138.0 (C–9 + C–10), 79.9 (C–3), 69.5 (C–2), 68.6 (C–11), 66.0 (C–12), 64.1 (C–15),

48.2 (C–13), 47.4 (C–5), 45.2 (C–6), 42.6 (C–4), 38.5 (C–7),

15.5 (C–16), 11.2 (C–14), 0.41 (Si–CH$_3$), –0.99 (Si–CH$_3$).

Mass spectrum, m/z found: 424.2126 (M$^+$, C$_{21}$H$_{36}$O$_5$Si$_2$

requires 424.2111).

(143)
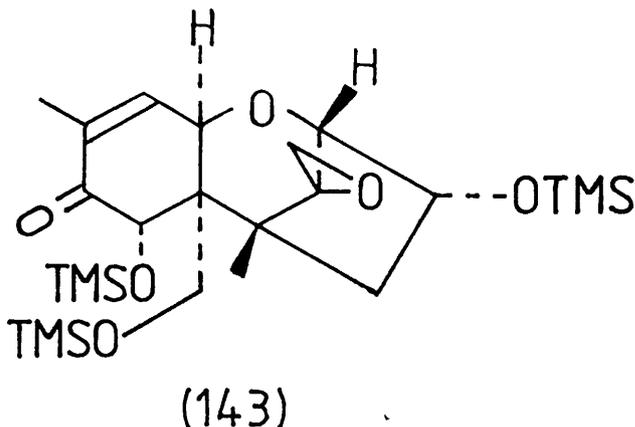
3α,7α,15-Tristrimethylsilyloxy-12,13-epoxytrichothec-9-en-8-one

(143)

A solution of TMSCl (0.17 ml, 1.3 mmol) in THF (2 ml) cooled

to -78°C was added to freshly prepared LDA (0.36 M in THF, 0.94 ml,

0.33 mmol) at -78°C. To this solution was added enone (141) (46 mg,

0.11 mmol) in THF (2 ml). The reaction was quenched after 1 m. by

addition of Et₃N (0.5 ml). The reaction mixture was diluted with

pet.ether, washed once with water, dried (Na₂SO₄) and concentrated

in vacuo to give enol ether (142).

^{1}H n.m.r. (CCl₄, 90 MHz) δ 5.70 (1H, br.d, J 6.0 Hz, H-10),

4.60 (1H, br.s, H-7), 4.45 (1H, br.d, J 6.0 Hz, H-11), 4.25 (1H,

ddd, J 10.0, 5.0, 5.0 Hz, H-3), 3.50 and 3.20 (2H, ABq, J 12 Hz,

H-15), 3.10 (1H, d, J 6.0 Hz, H-2), 2.75 (2H, ABq, J 4.0 Hz, H-13),

2.60-1.80 (2H, m, H-4), 1.9 (3H, br.s, H-16), 0.85 (3H, s, H-14).

To the crude silyl enol ether (142) (0.11 mmol, assumed) in

hexane (8 ml) at -15°C was added mcpba (80%, 30 mg, 0.14 mmol).

After stirring at -15°C for 30 m, the solution was then warmed at

30°C for 2 h, after which time it was concentrated in vacuo.

Purification by flash chromatography (1:9 EtOAc–pet.ether) gave

14 mg (25%) of the desired α–silyloxyketone (143) along with 24 mg

(50%) of the ring opened product (144).

The α–silyloxyketone (143) was obtained as a colourless oil:

R_f 0.70 (1:1 EtOAc–pet.ether); ν_{max} (CCl$_4$) 2955, 2920, 2900, 1695,

1260, 1250, 1175, 1160, 1100, 1035, 960, 900, 870, 850 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 6.50 (1H, dq, J 6.0, 1.4 Hz, H–10),

4.96 (1H, br.d, J 6.0 Hz, H–11), 4.38 (1H, ddd, J 10.8, 4.3, 4.3 Hz,

H–3), 3.75 and 3.67 (2H, ABq, J 10.7 Hz, H–15), 3.53 (1H, d, J 4.3

Hz, H–2), 3.08 and 2.99 (2H, ABq, J 4.5 Hz, H–13), 2.38 (1H, dd,

J 14.5, 4.3 Hz, H–4α), 1.95 (1H, dd, J 14.5, 10.8 Hz, H–4β), 1.80

(3H, dd, J 1.4, 0.7 Hz, H–16), 1.00 (3H, s, H–14), 0.16 (9H, s, Si–CH$_3$),

0.15 (9H, s, Si–CH$_3$), 0.00 (9H, s, Si–CH$_3$), 4.91 (1H, s, H–7).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 199.1 (C–8), 137.2 (C–10), 136.3

(C–9), 80.85 (C–7), 76.8 (C–2), 70.6 (C–11), 69.3 (C–3), 65.8 (C–12),

61.0 (C–15), 52.6 (C–5), 47.2 (C–13), 45.8 (C–6) 45.2 (C–4), 15.5

(C–16), 14.0 (C–14), 1.31 (Si–CH$_3$), 0.09 (Si–CH$_3$), –0.95 (Si–CH$_3$).

Mass spectrum, m/z found: 512.2453 (M$^+$, C$_{24}$H$_{44}$O$_6$Si$_3$ requires

512.2446).

The ring–opened product was also obtained as a colourless oil

R_f 0.51 (1:1 EtOAc–pet.ether); ν_{max} (CCl$_4$) 3600, 3450 (br), 2960,

2930, 2910, 2880, 1700, 1450, 1390, 1370, 1265, 1255, 1175, 1140,

1100 (br), 1050, 980, 970, 945, 875, 845 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 6.62 (1H, dq, J 5.7, 1.5 Hz, H–10),

4.52 (1H, ddd, J 10.3, 4.5, 4.5 Hz, H–3), 4.42 (1H, br.d, J 5.7,

H–11), 4.06 (1H, d, J 2.1 Hz, H–7), 3.93 (1H, d, J 11.5 Hz, H–13a),

3.87 (1H, d, J 4.5 Hz, H–2), 3.60 (1H, d, J 11.5 Hz, H–13b), 3.53

and 3.36 (2H, ABq, J 11.0 Hz, H–15), 2.14 (1H, dd, J 14.5, 10.2 Hz,

H–4β), 1.94 (1H, dd, J 14.5, 4.3 Hz, H–4α), 1.84 (3H, dd, J 1.5,

0.7 Hz, H–16), 1.12 (3H, s, H–14), 0.13 (9H, s, Si–CH$_3$), –0.02

(9H, s, Si–CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 194.6 (C–8), 139.2 (C–10), 135.6

(C–9), 83.0 (C–7), 75.7 (C–2), 74.6 (C–12), 70.1 (C–11), 68.6 (C–3),

66.8 (C–13), 61.9 (C–15), 45.7 (C–5), 45.5 (C–6), 41.9 (C–4), 15.7

(C–16), 12.2 (C–14), 0.14 (Si–CH$_3$), –1.14 (Si–CH$_3$).

Mass spectrum, $\underline{m}/\underline{z}$ found: 440.2041 (M$^+$, C$_{21}$H$_{36}$O$_6$Si$_2$ requires

440.2050).

The semi–synthetic tris–TMS ether (143) was identical by both

GLC and GCMS analyses with a sample prepared by silylation of

natural deoxynivalenol (23) using Regisil 323[107] – a commercially

available mixture of bis–trimethylsilyltrifluoroacetamide,

trimethylchlorosilane and trimethylsilylimidazole.

Capillary column GLC was performed with a Hewlett–Packard

5880A gas chromatograph equipped with SE–54 and CP Sil 5 CB fused–

silica capillary columns (25 m x 0.32 mm ID) and Grob–type injectors

operated in split mode (50:1). Both columns were temperature

programmed to operate at 230°C; the helium carrier and make–up

gas flow rates were 3 ml/min and 25 ml/min for both columns

respectively.

The respective retention times for the tris–TMS ether on these

columns were 24.2 m and 19.3 m.

GCMS was carried out with an LKB 9000 instrument fitted with

a DB–1 fused–silica capillary column, 60 m x 0.32 mm ID and a

falling needle injector. The helium carrier and make–up gas flow

rates were 7 ml/min (measured at ambient temperature) and 25 ml/min

respectively. The column was operated at a temperature of $210^{\circ}C$.

Mass spectra (22 eV) were recorded under electron-impact conditions;

accelerating voltage, 3.5 kV; filament current, 4A; trap current,

60 μA; source and separator temperatures, $270^{\circ}C$. Both the semi-

synthetic and naturally derived tris-TMS ethers possessed identical

mass spectral profiles.

(23)
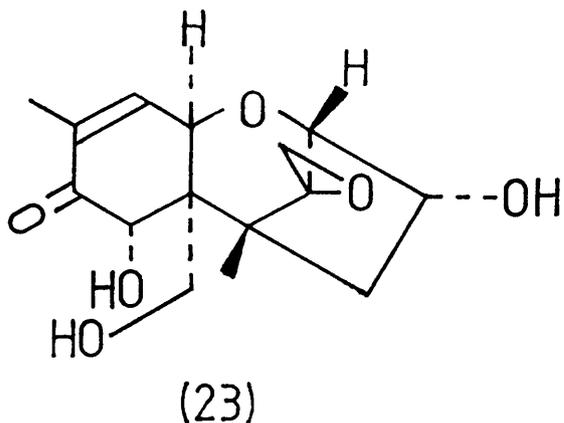
3α,7α,15-Trihydroxy-12,13-epoxytrichothec-9-en-8-one (23)

To the tristrimethylsilyl ether (143) (25 mg, 0.048 mmol) in aqueous

MeCN (1 ml H_2O and 1 ml MeCN) was added 3 drops of 40% HF. After

30 m. K_2CO_3 (0.5 g) was added. The reaction mixture was diluted with

brine and the aqueous phase was extracted with EtOAc (4 x 15 ml).

The combined organic layers were dried ($MgSO_4$) and concentrated

in vacuo. Purification by flash chromatography gave 15 mg (100%)

of the triol (23) as a white solid: R_f 0.11 (4:1 EtOAc-pet.ether).

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ 6.57 (1H, m, H-10), 4.80 (2H, s +

br.d, H-7 + H-11), 4.51 (1H, ddd, J 10.6, 4.5, 4.5 Hz, H-3), 3.88

and 3.71 (2H, ABq, J 11.7 Hz, H-15), 3.61 (1H, d, J 4.5 Hz, H-2),

3.15 and 3.07 (2H, ABq, J 4.3, H-13), 2.20 (1H, dd, J 10.6, 4.5 Hz,

H-4β), 2.05 (1H, dd, J 14.7, 10.6 Hz, H-4α), 1.68 (3H, dd, J 1.3,

0.6 Hz, H-16), 1.11 (3H, s, H-14).

Mass spectrum, m/z found: 296.1250 (M$^+$, $C_{15}H_{20}O_6$ requires

296.1250).

This material was fully characterised as the triacetate (120).

(145)
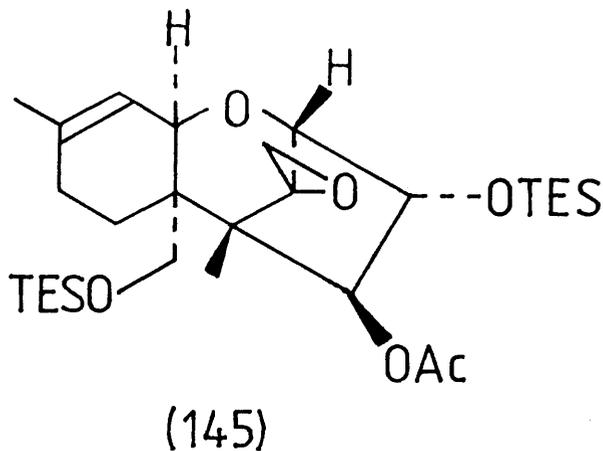
3α,15-Bis(triethylsilyloxy)-4β-acetoxy-12,13-epoxytrichothec-9-ene

(145)

To a stirred solution of 4-acetoxyscirpenediol (48) (202 mg,

0.62 mmol) and DMAP (31 mg, 0.25 mmol) in dry pyridine (15 ml) was

added triethylsilyl chloride (0.44 ml, 2.63 mmol). The mixture was

stirred overnight at room temperature. It was then diluted with

CH_2Cl_2 (100 ml) and washed with half-saturated $NaHCO_3$ (40 ml). The

organic extract was dried (Na_2SO_3) and concentrated in vacuo.

Purification by dry column flash chromatography (EtOAc-pet.ether)

gave the bis-TES ether as a colourless oil: R_f 0.62 (1:1 EtOAc-

pet.ether); ν_{max} (CCl_4) 2955, 2910, 2880, 1740, 1460, 1415, 1370,

1240, 1175, 1170, 1120, 1085, 1020, 1010, 970, 930, 855 cm^{-1}.

^{1}H n.m.r. ($CDCl_3$, 200 MHz) δ5.48 (1H, d, J 3.1 Hz, H-4),

5.41 (1H, br.d, J 5.5 Hz, H-10), 4.26 (1H, dd, J 4.9, 3.1 Hz, H-3),

3.98 and 3.41 (2H, ABq, J 10.7 Hz, H-15), 3.94 (1H, br.d, J 5.5 Hz,

H-11), 3.45 (1H, d, J 4.9 Hz, H-2), 2.98 and 2.74 (2H, ABq, J 4.1

Hz, H-13), 2.05-1.75 (4H, m, H-7 + H-8), 1.70 (3H, s, H-16), 1.00-

0.85 (18H, m, $SiCH_2CH_3$), 0.72 (3H, s, H-14), 0.65-0.45 (12H, m,

SiCH$_2$CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 170.4 (CH$_3$CO), 140.9 (C-9), 118.7

(C-10), 83.9 (C-4), 79.6 (C-2), 77.7 (C-3), 68.2 (C-11), 65.0 (C-12),

61.6 (C-15), 49.1 (C-5), 47.0 (C-13), 45.9 (C-6), 28.4 (C-8), 23.3

C-16), 20.8 (CH$_3$CO), 20.6 (C-7), 6.8, 6.7 and 6.5 (C-14 + 2 x

SiCH$_2$CH$_3$), 4.5 (SiCH$_2$CH$_3$), 4.1 (SiCH$_2$CH$_3$).

Mass spectrum, <u>m</u>/<u>z</u> found: 524.3002 (M$^+$-C$_2$H$_4$, C$_{27}$H$_{48}$O$_6$Si$_2$

requires 524.2989).

(146)
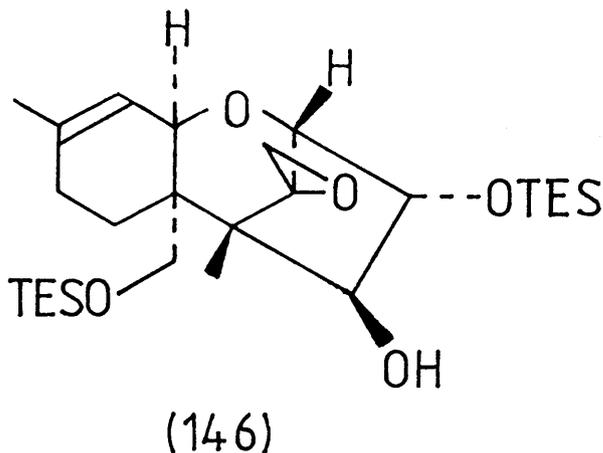
3α,15-Bistriethylsilyloxy-4β-hydroxy-12,13-epoxytrichothec-9-ene (146)

To the mono-acetate (145) (881 mg, 1.6 mmol) in wet methanol (7 ml water in 70 ml MeOH) was added K_2CO_3 (6 g). The mixture was stirred for 4 d. when it was concentrated in vacuo. The residue was taken up in water and extracted thoroughly with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Purification by dry column flash chromatography (EtOAc-pet.ether) gave 319 mg (40%) of the C-4 mono-ol (146) as a colourless oil: R_f 0.39 (1:1 EtOAc-pet. ether); ν_{max} (CCl_4) 3600, 3450 (br), 2960, 2915, 2880, 1460, 1450, 1415, 1380, 1250, 1220, 1130, 1115, 1080, 1040, 1015, 960, 930, 910, 850 cm^{-1}.

^{1}H n.m.r. (CDCl$_3$, 200 MHz) δ5.48 (1H, br.d, J 5.5 Hz, H-10), 4.50 (1H, br.s, H-3)*, 4.18 (1H, br.s, H-4)*, 3.91 (1H, br.d, J 5.5 Hz, H-11), 3.66 and 3.43 (2H, ABq, H 10.9 Hz, H-15), 3.58 (1H, d, J 4.9 Hz, H-2), 3.00 and 2.73 (2H, ABq, J 3.9 Hz, H-13), 2.2-1.8 (4H, m, H-7 + H-8), 1.69 (3H, br.s, H-16), 0.92 (18H, 2xt (overlapping), J 7.9 Hz, SiCH$_2$CH$_3$), 0.84 (3H, s, H-14), 0.55 (12H, 2 x q (overlapping), J 7.9 Hz, Si$_2$CH$_2$CH$_3$).

^{13}C n.m.r. (CDCl$_3$, 50 MHz) δ 140.6 (C-9), 118.7 (C-10), 81.9 (C-4), 80.7 (C-2), 78.9 (C-3), 68.2 (C-11), 65.1 (C-12), 62.1 (C-15) 49.0 (C-5),

47.0 (C-13), 44.6 (C-6), 28.1 (C-8), 23.2 (C-16), 21.9 (C-7), 6.8

(C-14 + 2 x $SiCH_2CH_3$), 4.2 (2 x $SiCH_2CH_3$).

Mass spectrum (M.S.12), $\underline{m}/\underline{z}$ found 396.1 ($M^+-(CH_3CH_2)_3Si+1$,

$C_{21}H_{36}O_5Si$ requires 396.2).

In addition to the title compound, 119 mg of a more polar compound

was obtained. This compound upon acetylation gave triacetoxyscirpene

(63). The more polar compound was thus believed to be scirpenetriol

(7) (26% yield).

*These assignments were made on the basis of the 90 MHz ^{1}H n.m.r.

spectrum which showed the signal at δ4.5 to be a doublet (J 3.5 Hz)

and the signal at δ4.18 to be a double-doublet (J 5.5, 3.5 Hz).

APPENDIX 1

	R$_1$	R$_2$	R$_3$	
1	H	H	H	12,13 epoxytrichothec–9–ene
2	H	OH	H	Trichodermol (Roridin C)
3	H	OAc	H	Trichodermin
4	OH	H	OAc	15–Deacetylcalonectrin
5	OAc	H	OAc	Calonectrin
6	OAc	OAc	H	Di–O–acetylverrucarol
7	OH	OH	OH	Scirpenetriol
8	OAc	OH	OH	Monoacetoxyscirpenol
9	OAc	OAc	OH	Diacetoxyscirpenol
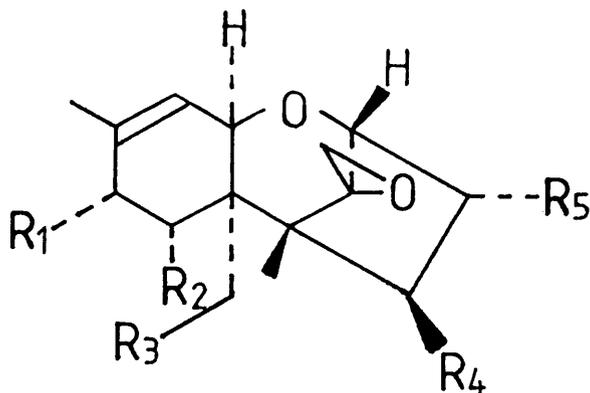
	R_1	R_2	R_3	R_4	R_5	
10	H	OH	OAc	OAc	OH	7-Hydroxydiacetoxy- scirpenol
11	OH	H	H	OH	H	——
12	OH	H	OH	OH	OH	T-2 Tetraol
13	O^{i}Val	H	OAc	OH	OH	HT-2 Toxin
14	O^{i}Val	H	OAc	OAc	OH	T-2 Toxin
15	O^{i}Val	H	OAc	OAc	OAc	Acetyl T-2 Toxin
16	OH	H	OAc	OAc	OH	Solaniol (Neosolaniol)
17	OH	OH	OAc	OAc	OH	7,8 Dihydroxy- diacetoxyscirpenol
18	OAc	OH	OAc	OAc	OH	——
19	OAc	H	OH	OAc	OH	——

i-Val = $-COCH_2CH(CH_3)_2$
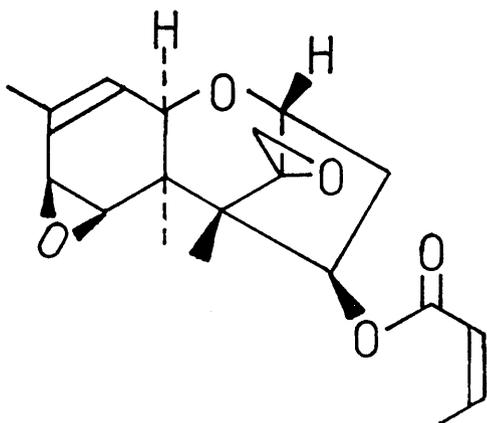
(20) Crotocin
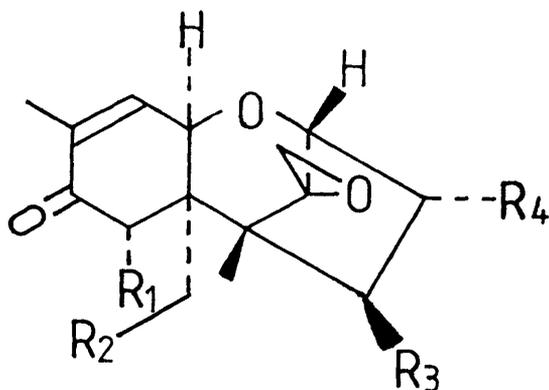
	R₁	R₂	R₃	R₄	
21	H	H	OH	H	Trichothecolone
22	H	H	OCrot	H	Trichothecin
23	OH	OH	H	OH	Vomitoxin (Deoxynivalenol)
24	OH	OH	H	OAc	Deoxynivalenol monoacetate
25	OH	OH	OH	OH	Nivalenol
26	OH	OH	OAc	OH	Fusarenon-X
27	OH	OAc	OAc	OH	Nivalenol diacetate

Crot = -COCH=CHCH₃

APPENDIX 2

X-ray crystallographic analysis of 3α ,4β ,15-triacetoxy-12,13-epi-

epoxytrichothec-9-ene (128)

Crystal data:

$C_{21}H_{28}O_8$, $\underline{M}_r$ = 408.5, orthorhombic, $P2_12_12_1$, a = 9.375(1),

b = 13.682(2), c = 16.207(2) $\overset{\circ}{A}$, V = 2078.6 $\overset{\circ}{A}{}^3$, Z = 4, D_m = 1.30 gcm^{-3},

Cu-K$_\alpha$, λ = 1.5418$\overset{\circ}{A}$, μ = 7.61 cm^{-1}, F(000) = 872, T = 291 K, final

R = 0.053 for 2343 observed reflections.

A large, cube-shaped, colourless crystal measuring $\underline{ca}$ 1.1 mm

x 0.8 mm x 0.7 mm was used in data collection on an Enraf Nonius

CAD-4 diffractometer. 2435 independent reflections collected with

a θ limit of 70°. Structure solved by direct phasing techniques

using MITHRIL (Gilmore, 1984). Full-matrix least-squares on F of

co-ordinates and anisotropic thermal parameters for non-H atoms

converged to R and WR of 0.053 and 0.078. H-atom co-ordinates

from difference Fourier synthesis were included, but not refined

in final two cycles of full-matrix least-squares. 2343 independent

reflections, I$\geqslant$3σ_I, used.

ATOMIC CO-ORDINATES (Å x 10^4)

	X/A	Y/B	Z/C	U
O(1)	.38546	.01108	.79307	.035
O(2)	.24226(19)	.19035(12)	.76327(11)	.036
O(3)	.3406(3)	.2058(2)	.63385(1)	.064
O(4)	.07218(19)	.07736(16)	.75470(12)	.043
O(5)	.1883(3)	.1223(3)	.8698(2)	.070
O(6)	.1108(3)	.0849(2)	.9831(1)	.051
O(7)	.0586(3)	.0576(2)	1.0770(2)	.079
O(8)	.2110(3)	.1655(2)	.7202(1)	.057
C(2)	.2803(3)	.0135(2)	.7304(1)	.036
C(3)	.1767(3)	.1003(2)	.7365(2)	.034
C(4)	.0620(3)	.0692(2)	.7986(2)	.036
C(5)	.0949(3)	.0409(2)	.8211(2)	.038
C(6)	.2052(3)	.0457(2)	.8945(1)	.033
C(7)	.2652(3)	.1511(2)	.9043(2)	.043
C(8)	.3832(3)	.1609(2)	.9691(2)	.048
C(9)	.4830(3)	.0762(2)	.9725(2)	.045
C(10)	.4584(3)	.0067(2)	.9312(2)	.040
C(11)	.3321(3)	.0234(2)	.8762(1)	.032
C(12)	.1725(3)	.0677(2)	.7411(2)	.041
C(13)	.1096(4)	.1149(3)	.6688(2)	.062
C(14)	.0376(4)	.1024(2)	.8368(3)	.058
C(15)	.1367(3)	.0187(2)	.9774(2)	.043
C(16)	.6159(5)	.0900(3)	1.0228(2)	.066
C(17)	.3247(3)	.2360(2)	.7066(2)	.040
C(18)	.3925(3)	.3256(2)	.7416(2)	.051
C(19)	.1888(3)	.1059(2)	.7974(2)	.048
C(20)	.3153(3)	.1112(3)	.7416(3)	.059
C(21)	.0067(4)	.1132(3)	1.0353(2)	.057
C(22)	.0072(6)	.2222(3)	1.0349(3)	.088

ANISOTROPIC THERMAL PARAMETERS ($\mathring{A}^2$)

	U11	U22	U33	U12	U13	U23
O(1)	.0317(9)	.0410	.0313	.0002	.0054	.0003
O(2)	.0409(9)	.0298(7)	.0369(8)	-.0045(7)	-.0035(7)	.0019(6)
O(3)	.080(2)	.069(1)	.042(1)	-.030(1)	.013(1)	.000(1)
O(4)	.0308(8)	.0534(11)	.0463(10)	.0024(7)	-.0013(7)	.0032(8)
O(5)	.052(1)	.098(2)	.061(1)	.014(1)	.018(1)	.004(1)
O(6)	.069(1)	.039(1)	.047(1)	.006(1)	.017(1)	.000(1)
O(7)	.091(2)	.072(2)	.073(2)	.009(2)	.042(2)	-.001(1)
O(8)	.081(2)	.035(1)	.056(1)	.002(1)	.003(1)	-.012(1)
C(2)	.038(1)	.037(1)	.031(1)	-.004(1)	.003(1)	.002(1)
C(3)	.034(1)	.034(1)	.033(1)	.001(1)	.000(1)	.000(1)
C(4)	.031(1)	.037(1)	.041(1)	-.005(1)	-.001(1)	.003(1)
C(5)	.036(1)	.034(1)	.044(1)	-.002(1)	.005(1)	-.002(1)
C(6)	.035(1)	.031(1)	.034(1)	-.007(1)	.002(1)	-.002(1)
C(7)	.053(1)	.030(1)	.046(1)	-.008(1)	.005(1)	.011(1)
C(8)	.054(1)	.043(1)	.046(1)	.001(1)	.000(1)	.003(1)
C(9)	.049(1)	.049(1)	.036(1)	.001(1)	.004(1)	.000(1)
C(10)	.038(1)	.042(1)	.040(1)	.005(2)	-.002(1)	.002(1)
C(11)	.035(1)	.032(1)	.029(1)	-.011(2)	-.002(1)	.004(1)
C(12)	.046(1)	.034(1)	.042(1)	-.016(1)	-.017(2)	-.012(1)
C(13)	.077(2)	.052(2)	.058(2)	-.003(1)	.002(1)	-.011(1)
C(14)	.045(1)	.049(2)	.080(2)	-.012(2)	.015(2)	.005(1)
C(15)	.051(1)	.038(1)	.041(1)	-.004(1)	-.016(1)	.008(2)
C(16)	.067(2)	.081(2)	.052(2)	.012(1)	-.002(1)	.009(1)
C(17)	.038(1)	.045(1)	.040(1)	-.001(1)	-.002(1)	.007(1)
C(18)	.044(1)	.045(1)	.063(2)	-.000(1)	.008(1)	.017(1)
C(19)	.035(1)	.048(1)	.063(2)	.018(2)	.003(1)	.016(2)
C(20)	.032(1)	.056(2)	.088(2)	.026(3)	-.014(1)	.003(1)
C(21)	.073(2)	.057(2)	.040(1)		.036(3)	-.001(2)
C(22)	.139(4)	.054(2)	.071(2)			

BOND LENGTHS (Å)

Bond	Length	Bond	Length
O(1) - C(11)	1.447(3)	O(1) - C(2)	1.416(3)
O(2) - C(17)	1.354(4)	O(2) - C(3)	1.443(3)
O(4) - C(4)	1.450(3)	O(3) - C(17)	1.187(4)
O(5) - C(19)	1.194(5)	O(4) - C(19)	1.352(4)
O(6) - C(21)	1.348(5)	O(6) - C(15)	1.440(4)
O(8) - C(12)	1.429(4)	O(7) - C(21)	1.188(5)
C(2) - C(3)	1.537(4)	O(8) - C(13)	1.446(5)
C(3) - C(4)	1.533(4)	C(2) - C(12)	1.512(4)
C(5) - C(6)	1.577(4)	C(4) - C(5)	1.580(4)
C(5) - C(14)	1.522(5)	C(5) - C(12)	1.532(4)
C(6) - C(11)	1.548(4)	C(6) - C(7)	1.556(4)
C(7) - C(8)	1.532(5)	C(6) - C(15)	1.534(4)
C(9) - C(10)	1.337(4)	C(8) - C(9)	1.490(5)
C(10) - C(11)	1.499(4)	C(9) - C(16)	1.502(6)
C(17) - C(18)	1.494(5)	C(12) - C(13)	1.462(5)
C(21) - C(22)	1.498(6)	C(19) - C(20)	1.494(5)

BOND ANGLES (°)

Atom 1		Atom 2		Atom 3	Angle
C(17)	–	O(2)	–	C(3)	115.7(2)
C(21)	–	O(6)	–	C(15)	116.4(3)
C(3)	–	C(2)	–	O(1)	114.3(2)
C(12)	–	C(2)	–	C(3)	97.9(2)
C(4)	–	C(3)	–	C(2)	109.8(2)
C(3)	–	C(4)	–	C(3)	105.4(2)
C(5)	–	C(4)	–	C(3)	106.3(2)
C(12)	–	C(5)	–	C(4)	97.2(2)
C(12)	–	C(5)	–	C(6)	108.5(3)
C(14)	–	C(5)	–	C(12)	113.4(3)
C(11)	–	C(6)	–	C(5)	109.5(2)
C(11)	–	C(6)	–	C(7)	107.9(3)
C(15)	–	C(6)	–	C(11)	110.0(2)
C(9)	–	C(8)	–	C(7)	114.2(3)
C(16)	–	C(9)	–	C(8)	116.3(3)
C(11)	–	C(10)	–	C(9)	124.3(3)
C(10)	–	C(11)	–	O(1)	105.2(2)
C(2)	–	C(12)	–	C(8)	119.3(3)
C(13)	–	C(12)	–	C(8)	60.0(3)
C(13)	–	C(12)	–	C(2)	120.1(3)
C(12)	–	C(13)	–	C(8)	58.9(2)
O(3)	–	C(17)	–	O(2)	122.8(3)
C(18)	–	C(17)	–	O(3)	125.9(3)
C(20)	–	C(19)	–	C(4)	110.2(3)
O(7)	–	C(21)	–	O(6)	123.1(4)
C(22)	–	C(21)	–	O(7)	126.5(4)

Atom 1		Atom 2		Atom 3	Angle
C(11)	–	O(1)	–	C(2)	115.1(2)
C(19)	–	O(4)	–	C(4)	118.2(3)
C(13)	–	O(8)	–	C(12)	61.1(2)
C(12)	–	C(2)	–	O(1)	111.4(2)
C(2)	–	C(3)	–	C(2)	114.2(2)
C(4)	–	C(3)	–	C(4)	105.7(2)
C(5)	–	C(4)	–	C(4)	110.9(3)
C(14)	–	C(5)	–	C(4)	110.0(2)
C(14)	–	C(5)	–	C(6)	114.0(3)
C(7)	–	C(5)	–	C(5)	112.7(3)
C(15)	–	C(6)	–	C(5)	110.6(2)
C(15)	–	C(6)	–	C(7)	112.1(3)
C(8)	–	C(6)	–	C(6)	106.5(2)
C(10)	–	C(7)	–	C(8)	114.3(3)
C(16)	–	C(9)	–	O(10)	122.2(3)
C(6)	–	C(9)	–	C(11)	121.5(3)
C(10)	–	C(11)	–	C(8)	111.9(2)
C(5)	–	C(11)	–	C(8)	113.6(2)
C(5)	–	C(12)	–	O(2)	123.1(3)
C(13)	–	C(12)	–	C(5)	103.8(3)
C(6)	–	C(12)	–	C(6)	126.4(3)
C(18)	–	C(15)	–	O(2)	111.3(3)
C(5)	–	C(17)	–	C(4)	111.3(3)
C(20)	–	C(19)	–	O(5)	123.7(3)
C(22)	–	C(19)	–	C(6)	126.1(3)
	–	C(21)	–		110.3(4)

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