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BY

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SYNTHESIS OF TRICHOTHECENE PUTATIVE PRECURSORS

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My thesis is dedicated to my parents, Costas and Vasiliki Nikolakakis, whose support and encouragement was invaluable for the successful completion of this project.

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL NAME
EPT	12,13-Epoxytrichothec-9-ene
AIBN	2,2'-azobis(2-methylpropionitrile)
3-ADN	3-Acetyldeoxynivalenol
3-ATD	3-Apotrichodiol
DON	4-Deoxynivalenol
4-DOV	4-Deoxyverrucarol
4-DMAP	4-Dimethylaminopyridine
Ac	Acetate
Ac_2O	Acetic anhydride
ATO	Apotrichool
$CDCl_3$	Chloroform-D
D	Deuterium
DHP	Dihydropyran
DIBALH	Diisobutylaluminum hydride
DMF	Dimethylformamide
EtOAc	Ethyl acetate
FPP	Farnesyl pyrophosphate
PP	Pyrophosphate
HPLC	High performance liquid chromatography
IR	Infrared
i-PrOH	Iso-propanol
ITD	Isotrichodermin
KPL	Kinetic Pulse Labeling

MeOH	Methanol
MVL	Mevalonate
n-BuLi	n-Butyllithium
NMR	Nuclear Magnetic Resonance
NOED	Nuclear overhauser effect difference
Ph	Phenyl
Pyr	Pyridine
PCC	Pyridinium chlorochromate
PPTS	Pyridinium para-toluene sulfonate
SOL	Sambucinol
TBDMSi	t-Butyldimethylsilyl
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
$n\text{-}Bu_3SnH$	tri-n-Butyltin hydride
TDN	Trichodiene
TES	Triethylsilyl
T	Tritium
UV	Ultraviolet
Val	$C(O)CH_2CH(CH_3)_2$

LIST OF CHEMICAL STRUCTURES

RESUMÉ EN FRANÇAIS

Les trichothécènes sont des sesquiterpenes. Le composé intermediaire dans la biosynthèse des trichothécènes est le 6,7-trans farnesyl pyrophosphate qui provient de la condensation de trois molecules de mevalonate. Les trichothécènes sont isolés à partir de sources naturelles mais en majorité ils proviennent de certaines souches de Fungi Imperfecti des genres Fusarium, Myrothecium, Trichothecium, Cephalosporium, Trichoderma et Verticimonosporium. Ces sesquiterpenes ont un squelette tricyclique avec une double liaison entre les carbones 9 et 10 et un époxide entre les positions 12 et 13.

L'importance allouée aux trichothécènes est due à leurs qualités biologiques et physiologiques. Ils servent sommes agents fungicides, antibiotiques, antileucémiques, phytotoxiques and neurotoxiques. Récemment la recherche de la biosynthèse des métabolites de Fusarium culmorum et Fusarium graminearum a été augmentée a cause du danger que représentent les toxines qui sont produites dans les grains et dans le maïs. La menace est surtout présente dans l'industrie agricole car l'empoisonnement des animaux de ferme, par ces toxines, cause le rejet de l'alimentation et en conséquence la perte de poids.

En cultures liquides, les métabolites majeurs de Fusarium culmorum, sont le 3-acétyldeoxynivalenole et le sambucinole. Presque rien n'est connu de la biosynthèse de ces métabolites sauf que le 6,7-trans farnesyl pyrophosphate est

incorporé dans trichodiene. Trichodiene est le composé bicyclique qui provient de l'isomerization et de la cyclization du 6,7-trans farnesyl pyrophosphate. Les mechanismes responsables de la conversion du 6,7-trans farnesyl pyrophosphate à trichodiene sont bien connues. Plusieurs chercheurs ont montré en utilisant des composés radioactifs, que le trichodiene est incorporé dans les trichothécènes. Zamir et al. (1989) ont récemment confirmé en utilisant des radioisotopes stables, que le trichodiene bicyclique est le précurseur des trichothécènes tricycliques.

Afin de préparer un mechanisme, plusieurs précurseurs sont nécessaires pour élucider la biosynthèse des trichothécènes à partir du trichodiene. Certains métabolites ont été isolés en petites quantités par HPLC à partir de milieux du cultures de Fusarium culmorum. Par exemple, le 12,13-époxytrichothécène, l'isotrichodermine et l'apotrichoole ont été identifiés. Zamir et al. ont montré que l'EPT et l'ITD sont des précurseurs de l'ADN et que l'apotrichool est le précurseur du SOL. Pour confirmer les résultats qui ont été obtenus par des expériences utilisant des précurseurs radioactifs, il est nécessaire de démontrer l'incorporation de chaque composé marqué par des radioisotopes stables dans les métabolites majeurs du 3-ADN et du SOL. Par cette voie, la présence de l'isotope dans l'acétyldeoxynivalenole ou le sambucinole peut être confirmé et identifié par la spectroscopie RMN.

Donc pour réaliser ces expériences il est nécessaire d'avoir les composés en question en grande quantité et marqués au

deutérium. Les manipulations chimiques des différents groupes fonctionnels de trichothécènes permettent la préparation facile de métabolites mineurs en grande quantité, à partir d'autres métabolites qui sont abondants comme l'anguidine ou le verrucarole.

C'est la première fois que l'isotrichodermine a été synthétisé en grande quantité, à partir uniquement de l'anguidine en dix étapes avec de hauts rendements. Le 12,13-époxytrichothécène, le composé clé entre trichodiene et les métabolites plus complexes du trichothécène, a été egalement synthétisé en dix étapes seulement et en grande quantité à partir du verrucarole. Antérieurement à ce present travail, l'isotrichodermine n'a jamais été synthétisé. Le 12,13-époxytrichothécène a été obtenu à partir de plusieurs synthèses inefficaces et à faibles rendements. Le 12,13-époxytrichothécène a été utilisé comme materiel de départ pour la synthèse de trois apotrichothécènes: l'apotrichoole, le 2ã-apotrichodiol et le 2ß-apotrichodiol. A l'aide de ces apotrichothécènes synthétiques il sera possible de comprendre la biosynthèse du SOL car l'apotrichoole semble être le précurseur du SOL. D'autre part l'EPT et l'ITD synthétiques permettrons d'élucider la biosynthèse de l'ADN.

SUMMARY

3-Acetyldeoxynivalenol and sambucinol are the major metabolites isolated from liquid cultures of Fusarium culmorum. To elucidate their biosynthesis, putative precursors were synthesized, radiolabeled with deuterium and tritium. For the first time Isotrichodermin was synthesized in large amounts from anguidine, in only ten high yield reactions. 12,13-Epoxytrichothecene, the elusive key link between trichodiene and the more complex trichothecene metabolites, was also synthesized in only ten steps and in large amounts from verrucarol. Prior to this Isotrichodermin had never been synthesized. 12,13-Epoxytrichothecene had been obtained via inefficient total syntheses in numerous low yield reactions. To understand the novel isolations of two apotrichothecene metabolites from culture broths of Fusarium culmorum, 12,13-Epoxytrichothecene was used as the starting material for the synthesis of three apotrichothecenes, namely apotrichool, 2ã-apotrichodiol and 2ß-apotrichodiol. Chemical manipulation of the different functional groups of trichothecenes, allows the facile preparation of otherwise minor metabolites, in large quantities, from other more readily available ones, such as anguidine or verrucarol.

INTRODUCTION

Trichothecenes are tricyclic sesquiterpenes. They are produced by certain species of Fungi Imperfecti, of the genera, Myrothecium, Cephalosporium, and Fusarium. In liquid cultures of Fusarium culmorum the major metabolites are known to be 3-ADN and SOL. In the literature, mechanisms are known by which 6,7-trans farnesyl pyrophosphate isomerizes and then cyclizes into trichodiene, the bicyclic precursor of the trichothecenes. Very little is known, however, about what kind of transformations trichodiene undergoes to form the more complex trichothecenes. There exists a large gap between trichodiene and 3-ADN and SOL. Many precursors have been isolated in culture broths of Fusarium culmorum. Among those which have been isolated via radiolabeling experiments are ITD, EPT and apotrichool. These experiments indicate that ITD and EPT are precursors of 3-ADN, while apotrichool is only a precursor of SOL. To confirm these results, the putative precursors must be labeled with deuterium and subsequently fed individually to liquid cultures of Fusarium culmorum. The isolation of labeled 3-ADN and/or SOL would confirm the above preliminary results obtained in Dr. Zamir's laboratory.

Isotrichodermin was prepared from the more readily available trichothecene anguidine isolated from culture broths of Fusarium sambucinum. An amelioration of the existing procedure for the production of verrucarol, provided high yields of verrucarol from culture broths of Myrothecium verrucaria.

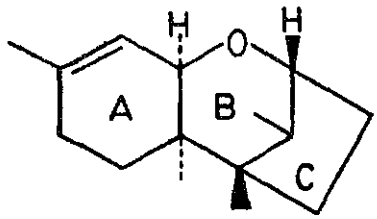
Verrucarol was used as the starting material for the synthesis of 12,13-epoxytrichothecene which was in turn used for the preparation of the apotrichothecenes: apotrichool, 2ã-apotrichodiol and 2ß-apotrichodiol. All the compounds synthesized were specifically labeled with deuterium at various positions.

The feeding experiments of these synthesized compounds to cultures of Fusarium culmorum will in the future, provide the answers necessary to understand the transformation of trichodiene to the more complex trichothecenes.

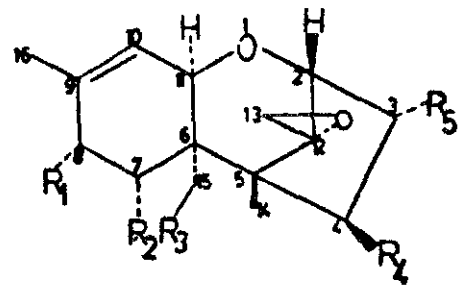
LITERATURE REVIEW

1. INTRODUCTION

The trichothecenes are isolated from natural sources, mainly from certain species of Fungi Imperfecti of the genera Fusarium, Myrothecium, Trichothecium, Cephalosporium, Trichoderma and Verticimonosporium (Ueno, 1983; Lacety, 1985; Cole and Cox, 1981). A total of 148 trichothecenes have been isolated (Grove, 1988). This group of fungal secondary metabolites includes non-macrocyclic as well as macrocyclic members, which are derived from a common bicyclic sesquiterpene skeleton. They all share the basic trichothecane (1) tricyclic skeleton depicted in figure 1. Most naturally occurring trichothecenes have a double bond



(1)



(2)

Figure 1

between C-9 and C-10 and contain a 12,13-epoxide group. The 12,13-epoxytrichothec-9-ene (2) with its appropriate stereochemistry and numbering system is also shown in figure 1. In addition, the macrocyclic trichothecenes contain a chain of di- or tri-esters linking the C-15 alcohols to those at C-4. Base catalyzed hydrolysis of these verrucarins and roridins yields the same alcohol, verrucarol.

The current surge in the research of trichothecenes is due to their biological and physiological properties. They are antifungal, antibiotic, antileukemic (cytotoxic), phytotoxic and neurotoxic agents (Tamm, 1974). Various authors have written excellent reviews on the trichothecenes (Tamm and Breitenstein, 1980; Cane, 1981; Cordell, 1976; Ong, 1982) and their biological properties (Bamburg, 1983; Ueno, 1983; Jarvis and Mazzola, 1982).

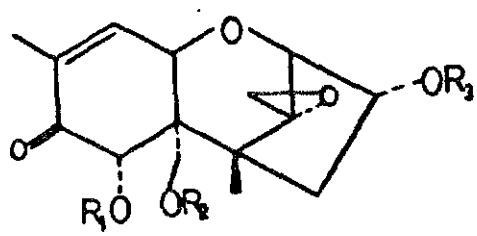
Most recently, attention has shifted to the study of the metabolites isolated from Fusarium species, mainly Fusarium culmorum and Fusarium graminearum because of the real threat that they pose to the agricultural industry. The most economically injurious of these metabolites is 4-deoxynivalenol (vomitoxin) (DON) (3) (Figure 2), because of its contamination of corn and cereal grains. Consequently, it is the cause of mycotoxicoses (feed refusal, emesis and weight loss) of farm animals (Pathre and Mirocha, 1979; Vesonder and Hesseltine, 1981; Mirocha, 1984; Ueno, 1977a; Mirocha et al., 1977).

Humans may also be affected by these toxins upon consumption of infected grains. Moreover the trichothecenes, T-2 toxin (fusariotoxin) and nivalenol have recently been implicated in the "Yellow Rain" controversy, allegedly used against the populations in Southeast Asia (Mirocha et al., 1983; Ashton et al., 1983; Ember, 1984). Therefore, trichothecenes may adversely affect both humans and animals. The most striking biological effects displayed by the trichothecenes are their toxicity and

their anti-leukemic activities in man and animals (Doyle and Bradner, 1980; Bamburg and Strong, 1971; Smalley and Strong, 1974; Ueno, 1983). The anti-leukemic activity in the naturally occurring trichothec-9-enes is associated with the presence of the 12,13-epoxy linkage and derivatives lacking this functionality are inactive (Grove and Mortimer, 1969). Thus this biological activity may reside in the sesquiterpene backbone, as in the case of anguidine which has been shown to possess anti-tumor activites (Penta, 1973). In the case of the macrocyclic trichothecenes, examination of the various roridins and verrucarins indicates that there is a key role enjoyed by the heavily functionalized di- and tri-esters, which connect the C-4 and C-15 hydroxyl groups of verrucarol, profoundly influencing the anti-tumor activity (Bamburg and Strong, 1971). The mode of action of the trichothecenes is presumed to occur through attachment to eukaryotic polysomes and ribosomes followed by inactivation of the ribosomal cycle, consequently inhibiting protein synthesis in eukaryotic cells (Ueno, 1977b; Ueno et al., 1969; Ueno et al., 1968).

A few of these toxic mycotoxins are trichothecin isolated from Trichothecium roseum (Freeman et al., 1959; Fishman et al., 1960; Godtfredsen and Vangedal, 1965), verrucarins and roridins isolated from Myrothecium species (Traxler et al., 1970), diacetoxyscirpenol and acetates of nivalenol isolated from Fusarium species (Dawkins et al., 1965;

Dawkins, 1966; Flury et al., 1965; Sigg et al., 1965; Tidd, 1967; Tatsuno et al., 1969; Grove, 1970).



(3) $R_1=R_2=R_3=H$
(17) $R_1=R_3=H$; $R_2=Ac$
(18) $R_1=R_2=H$; $R_3=Ac$

Figure 2

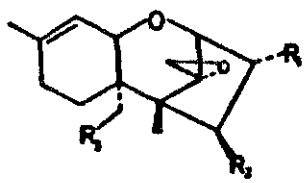
2. TOTAL AND PARTIAL SYNTHESES OF PERTINENT TRICHOTHECENES

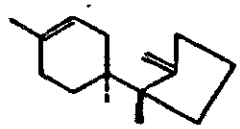
The trichothecenes arouse the curiosity of chemists for a number of reasons. Because of their aforementioned physiological properties it would be interesting to subject these toxic metabolites to chemical modifications in order to render them less toxic. Accordingly, anguidine was subjected to various modification reactions in the hope that its derivatives would be less toxic and yet more potent in terms of their antitumor activites (Kaneko et al., 1982).

From the chemical point of view, trichothecenes are interesting structures because they contain many different functionalities. Consequently, researchers have extensively investigated the chemistry of important trichothecenes such as verrucarol and anguidine (Dawkins et al., 1965; Dawkins, 1966;

Flury et al., 1965; Sigg et al., 1965; Müller et al., 1975).

Because many of these mycotoxins are not produced in sufficient amounts from culture broths for chemical and biochemical investigations, researchers have performed the total synthesis of pertinent trichothecenes. Until now the total synthesis of verrucarol (4) (Schlessinger and Nugent, 1982; Roush and D'Ambra, 1983; Trost et al., 1984), trichodermin (5) (Colvin et al., 1973), trichodermol (6) (Still and Tsai, 1980), anguidine (7) (Brooks et al., 1983), calonectrin (8) (Kraus et al., 1982), 12,13-epoxytrichothec-9-ene (9) (Fujimoto et al., 1974; Masuoka and Kamikawa, 1976; Hua et al., 1988; Fujimoto et al., 1981) and trichodiene (10) (Welch et al., 1980; Suda, 1982; Schlessinger and Schultz, 1983; Harding and Clement, 1983; Kraus and Thomas, 1986) have been achieved (Figure 3).





(10)

(4) R_2=R_3=OH; R_1=H; Verrucarol
(5) R_1=R_3=H; R_2=OAc; Trichodermin
(6) R_1=R_3=H; R_2=OH; Trichodermol
(7) R_2=R_3=OAc; R_1=OH; Anguidine
(8) R_1=R_3=OAc; R_2=H; Calonectrin
(9) R_1=R_2=R_3=H; 12,13-EPT
(11) R_1=R_2=H; R_3=OH; 4-DOV
(14) R_1=R_3=OH; R_2=OAc; 4ß-Acetoxy-scirpene-3ã, 15-diol
(32) R_1=R_2=R_3=OAc; Triacetate

Figure 3

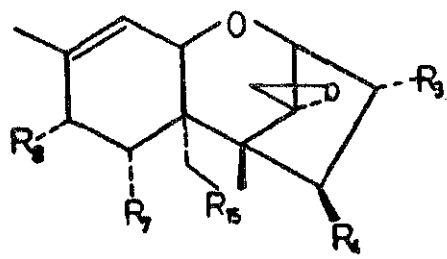
The tedious and elaborate procedure of any total synthesis can be avoided, when it is possible to transform more abundantly available mycotoxins to less abundant ones. Anguidine

is one such readily available trichothecene (Dawkins, 1966; Sigg et al., 1965) and hence has been targeted as the starting material for numerous chemical transformations. Tulshian and Fraser-Reid (1980) transformed anguidine (7) into verrucarol (4) which was in turn converted to trichodermol (6). Shuda et al. (1984) used both verrucarol (4) and anguidine (7) as starting materials to synthesize 4-deoxyverrucarol (11) hoping to obtain a derivative less toxic than verrucarol. The above chemical transformations involve appropriate deoxygenations at C-3, C-4, and C-15. Employing the Barton deoxygenation procedure (Barton and McCombie, 1975) or variations thereof, coupled with the appropriate protective group chemistry, led to the deoxygenation of the alcohol at position C-3 of anguidine to provide verrucarol. Applying the procedure to the alcohol functionality at C-4 of verrucarol effects its transformation to 4-deoxyverrucarol. On the other hand, if the deoxygenation procedure is applied to both alcohol functionalities simultaneously, anguidine can be directly converted to 4-deoxyverrucarol. Jeker, Mohr and Tamm (1984) accomplished the partial synthesis of calonectrin (8) from anguidine (7) by applying the Barton deoxygenation procedure to the oxygen functionality at C-4 of anguidine. Thus the simple seven step conversion of anguidine into calonectrin provided gram quantities of a substance that is otherwise produced in minute quantities in culture broths.

The Barton deoxygenation procedure does not apply to

primary alcohols and thus the oxygen functionality at C-15 must be removed in a different manner, namely by chloride formation and reductive elimination with a tin hydride (Tulshian and Fraser-Reid, 1980).

Anderson et al. (1987) showed the conversion of T-2 toxin (12) and neosolaniol (13) into anguidine (Figure 4). This deoxygenation at C-8 was accomplished by ß-bromide formation and subsequent reductive elimination with tin-hydride.



(12) R_3=OH;R_4=R_{15}=OAc;R_7=H;R_8=OVal

(13) R_3=R_8=OH; R_7=H; R_4=R_{15}=OAc

(33) R_4=R_8=OH; R_3=R_7=R_{15}=H

(34) R_3=R_7=R_8=OH; R_4=R_{15}=OAc

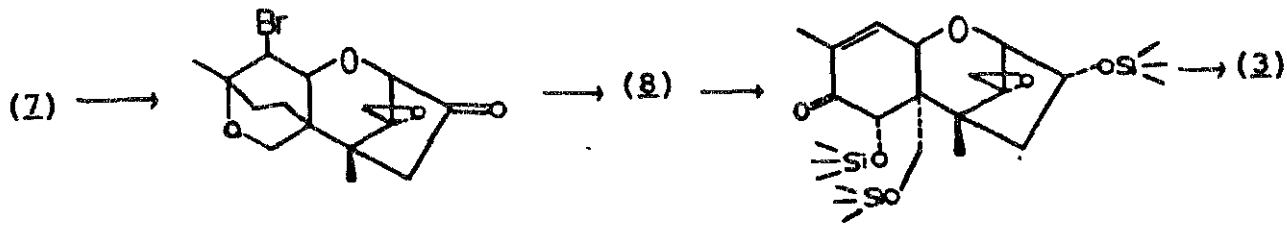
(36) R_4=R_8=OAc; R_3=R_7=R_{15}=H

Figure 4

Roush and Russo-Rodriguez (1985) performed the partial synthesis of 4ß-acetoxyscirpene-3ã,15-diol (14) (Figure 3) from anguidine by simple protective group manipulations. This provided gram quantities of the desired compound which was required to elucidate the metabolism of anguidine by strains of Fusarium roseum and Fusarium sulphureum.

In view of the recent importance of 4-deoxynivalenol Colvin and Cameron (1988) were the first to partially synthesize deoxynivalenol from anguidine. This partial synthesis was even

more impressive because a total synthesis of 4-deoxynivalenol has yet to be realized. The investigators chose to initiate the partial synthesis of DON from calonectrin which they also had partially synthesized from anguidine via ketone (15) (Figure 5) (a different route to calonectrin from that of Jeker, Mohr and Tamm, 1984). Oxidation of calonectrin at position C-8 followed by the succesive manipulation of the different functional groups provided 4-DON via ether (16).



(15) (16)

Figure 5

Grove et al. (1988) provided a two step partial synthesis of 15-acetyl-4-deoxynivalenol (17) from 3-acetyl-deoxynivalenol (3-ADN) (18) (Figure 2) by hydrolysis of the latter and selective acetylation of the resultant DON (3). The importance of this synthesis lies in the fact that heretoforth the most common trichothecenone produced in minute quantities by strains of Fusarium graminearum isolated from Eastern North American grain (Miller et al., 1983), was now available in gram quantities.

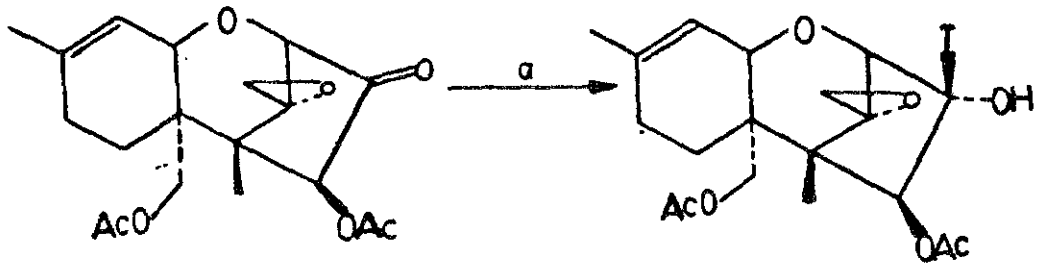
The benefits of partial syntheses are important. The

methodology can potentially be applied to any trichothecene which is not readily available. This type of chemical transformation is especially suited for the incorporation of radiolabels. Biochemical metabolism studies require radiolabeled precursors. Intermediates labeled with ^{14}C or ^{3}H obtained through biosynthesis (Hagler et al., 1981; Wallace et al., 1977; Zamir and Devor 1987; Zamir et al., 1987a; Zamir et al., 1987b) are isolated in very small amounts. Moreover, if their isolation is successful it provides compounds with very low specific activities thereby rendering them insufficient for further in vivo studies.

The total synthesis of optically active trichothecenes entails a laborious multistep process. Up to date only two total syntheses of enantiomeric trichothecenes exist. One is the total synthesis of enantiomeric anguidine (Brooks et al., 1983) and the second is the enantioselective total synthesis of (+)-12,13-epoxytrichothec-9-ene (EPT) (Hua et al., 1988). Unfortunately, the single total synthesis having incorporated ^{14}C is that of racemic [13-^{14}C]-12,13-epoxytrichothec-9-ene (Fujimoto et al., 1981), obtained after fifteen labor intensive steps.

Thus partial synthesis is ideally suited for radiolabeling since only natural products are used as starting materials. Pertinent radiolabeled compounds can be obtained in high yields and with high specific activities. Roush (Caggiano and Roush, unpublished results) prepared [^{3}H]-(7a) from the 3-

keto anguidine (19) (Figure 6).[1] Tritium atoms can be easily introduced by reduction of trichothecen-3-ones with the tritiated analog of sodium borohydride.



(19) (7a)

a: NaB^3H_4, i-PrOH

Figure 6

Roush and Russo-Rodriguez (1987) were the first to prepare through partial synthesis an enantiomerically pure ^{14}C-trichothecene, with high specific activity.[2] The twelve step synthesis provided skeletally labeled [13-^{14}C]-anguidine in 30% yield, with a specific activity of 19,2 mCi/mmol. C-12,13 epoxide deoxygenation of anguidine using a tungsten reagent discovered by

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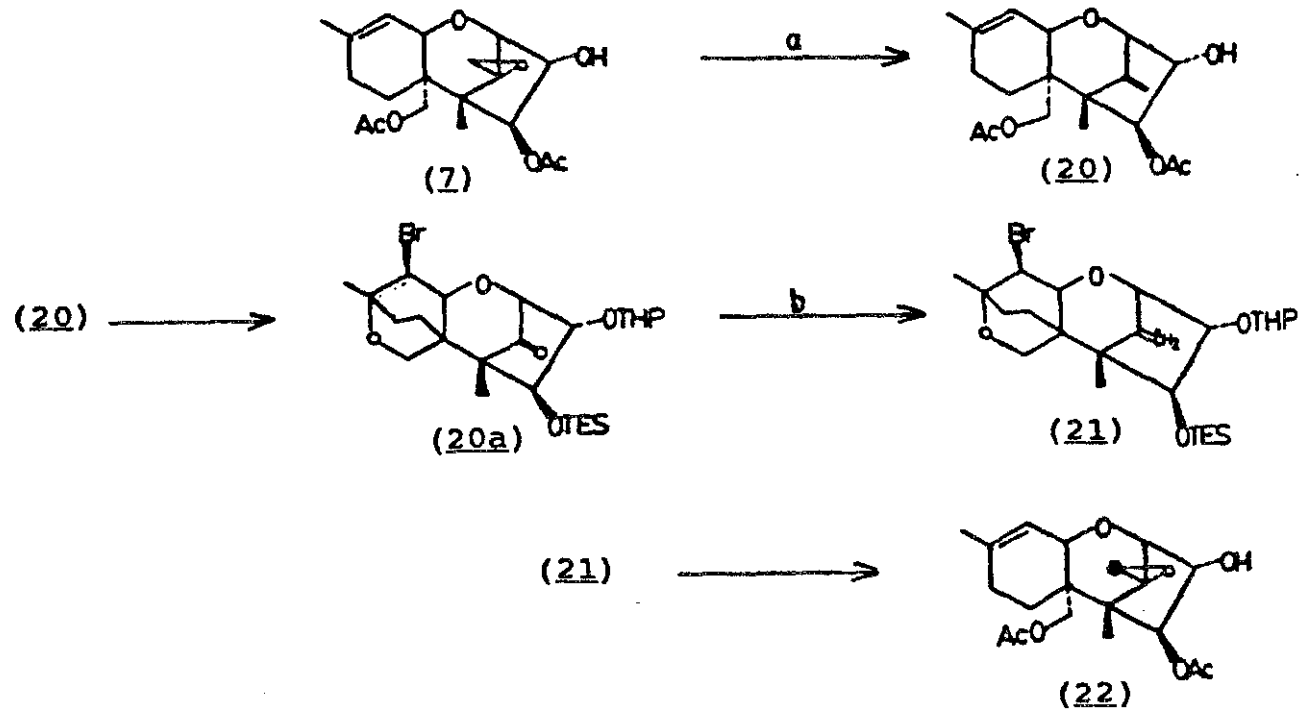
[1] The procedure for the preparation of [^3H]-7a was supplied by Dr. T.W. Doyle of Bristol Laboratories from unpublished results. (See reference (7) in Roush and Russo- Rodriguez, 1987).

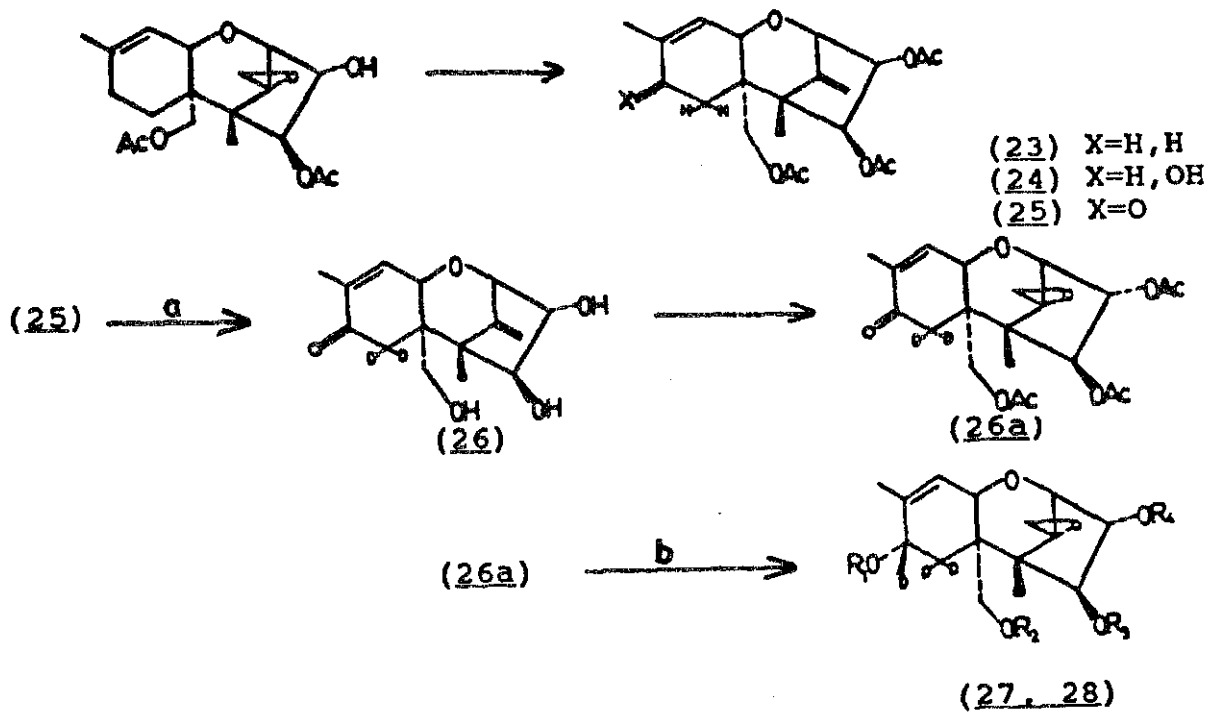
[2] Prior to this, radiolabels such as ^2H and ^{14}C have been introduced into trichothecenes but only through acylation of oxygen functionalities with radiolabeled acetic anhydride. Refer to: Wei and Guan, 1976; Michaud et al., 1988. Although these radiolabeled compounds may be utilized as internal standards for the analysis of mycotoxins, they are incompatible for metabolism studies since the label is easily lost by hydrolysis of the acetate, in vivo.

Sharpless (Sharpless et al., 1972; Umbreit and Sharpless, 1981; Colvin and Cameron, 1986; Colvin and Cameron, 1987) and developed by Colvin for the trichothecenes, provided diene (20) (Figure 7) which was oxidized (after the introduction of certain protective groups), to ketone (20a). (20a) plays the key role in the synthesis since a Wittig reaction between this ketone and ^{14}C methylenetriphenylphosphorane (Ph_3P : $^{14}CH_2$) permitted the incorporation of ^{14}C at position 13 of the resultant diene (21). Subsequent epoxide formation and functional group manipulations provided 13-^{14}C anguidine (22) from cold anguidine. This novel synthesis can be extended to other members of the trichothecene mycotoxins.



a: WCl_6, n-BuLi, THF, 50C, 97%; b: Ph_3P:$^{14}CH_2$, THF, 0°C-reflux; 58-61%

Figure 7



(a)CD_3ONa, CD_3OD; (b)(1)DIBAL-D, (2)Ac_2O,pyr,4-DMAP.

Figure 8

Kraus and Thomas (1988) have successfully incorporated three deuterium molecules into the T-2 toxin metabolite neosolaniol (13) (Figure 4), thus paving the way to the preparation of ^3H-labeled trichothecenes through partial synthesis. Once again the readily available mycotoxin anguidine was used as the starting material. Epoxide deoxygenation of acetylated anguidine provided diene (23) (Figure 8). A mixture of diastereomeric allylic alcohols (24) was produced when (23) was subjected to a selenium dioxide oxygenation at C-8. After PCC

oxidation of (24), two of the three deuterium labels were introduced into ketone (25) by deuterium exchange. The resultant dideuteriated trichothecene (26), was acetylated, epoxidized and reduced with DIBAL-^{2}H to produce the trideuteriated tetraacetate (27) (R_1=R_2=R_3=R_4=OAc) of neosolaniol (28) (R_1=R_4=OH; R_2=R_3=OAc) after acetylation.

3. THE APOTRICHOTHECENES AND THEIR CHEMISTRY

Recently, Zamir et al (Zamir and Devor, 1987; Zamir et al., 1987a), proved the structure of a new trichothecene, the 3-apotrichodiol (3-ATD) (29) isolated for the first time in their lab from cultures of Fusarium culmorum.[3] By NMR experiments (29) (Figure 9) was shown to contain a trans junction between rings A and B. This result is significant because it proves unequivocally that (29) is indeed a natural product and not a

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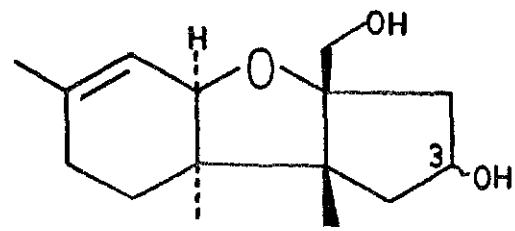
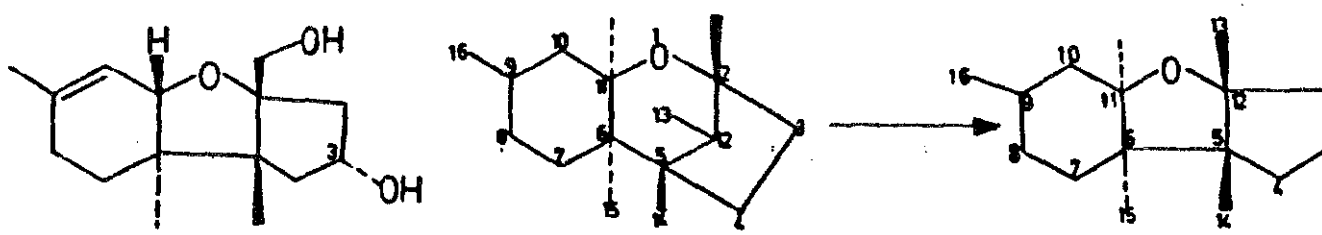
[3] ApSimon and Greenhalgh reported at a symposium the isolation of two C-3 diastereomeric alcohols (i) and (ii) containing a cis junction between rings A and B. Unfortunately, although the structures were said to have been proven by x-ray crystallography, the data was not shown and neither has it been published (ApSimon , 1986; Greenhalgh et al., 1986a).



i, ii

rearrangement product from the non-enzymatic acid catalyzed hydrolysis of a trichothecene. The most common rearrangement product of the trichothecane skeleton (1) is called apotrichothecane (30) and is shown in figure 9 with its appropriate numbering sequence and stereochemistry, both derived from that of trichothecane. Apotrichothecanes derived by chemical rearrangements contain a cis junction between rings A and B. The novel discovery of (29) paves the way to a whole new series of metabolites and promises to lead to new biosynthetic pathways.



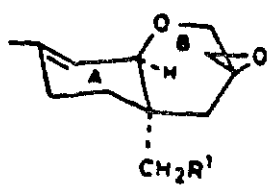
(29) (1) (30)

Figure 9

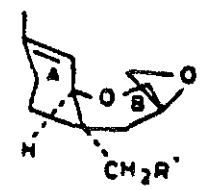
The chemistry of the apotrichothecanes has been investigated by Muller and Tamm (1975). The acid and base catalyzed rearrangements of the apotrichothecanes have been extensively studied (Godtfredsen and Vangedal, 1965; Grove, 1985; Grove, 1986). The bulk of the chemistry concerns the 12,13-epoxide because of the key role it maintains with respect to biological activity. The 12,13-epoxide is protected from rearside

nucleophilic attack from external anions, by shielding from ring A and by the rigid oxabicyclo [3,2,1] octane system of rings B and C. This shielding is reflected by the fact that verrucarol (4) and trichodermol (6) are unaffected when treated with hot dilute alkali. On the other hand, the epoxide is susceptible to acid, wherein protonation of the epoxide causes intramolecular rearrangement and addition of a nucleophile at a more accessible center.

There are two types of acid rearrangements of the trichothecenes: the 10,13-cyclotrichothecane rearrangement and the apotrichothecene rearrangement (Grove, 1986). In the former, the epoxide is protonated and then is atacked by the 9,10 double bond (Figure 11). Finally a nucleophile will attack the newly formed positive center. In order to facilitate the attack of the protonated epoxide by the 9-ene, the trichothecene must pass from the one half-chair conformation (31A) to the alternative half-chair conformation (31B) of the cis-fused A/B rings. This transformation is dependent upon the nature of the substituents of ring A, but not of ring C (Figure 10). Thus verrucarol (4),



(31A)



(31B)

Figure 10

trichodermin (5), calonectrin (8) and the triacetate (32) (Figure 3) will all rearrange to their appropriate 10,13-cyclotrichothecane when boiled in water (ph 5) for six hours (standard conditions). Thus the various substitution patterns on ring C and on the C-15 position have no effect on this type of rearrangement, because they do not interfere in the transformation of conformation A to B.

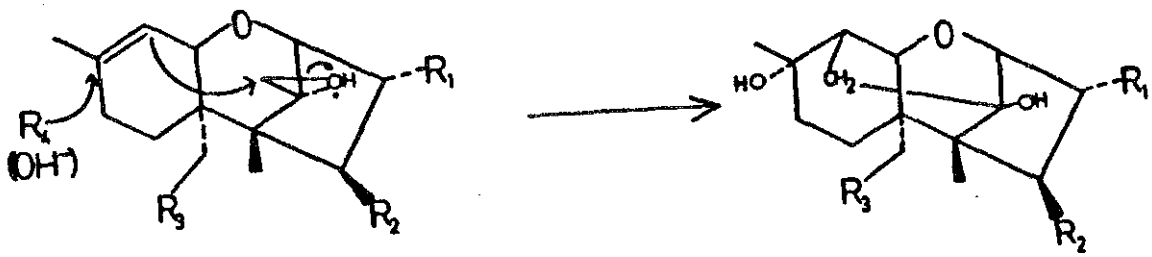


Figure 11

There is an unfavorable effect of a bulky group on position 8ã and 7ã on the energetics of the conformational change (31A) to (31B). Deoxynivalenol (3) (Figure 2) does not undergo this type of rearrangement because of the 8-keto group which hinders the conformational change and which also decreases the nucleophilicity of the 9,10 double bond. Similarly, trichothecodiol (33) and the triol (34) (Figure 4), because of the 7ã and 8ã oxygen functionalities, do not readily undergo the rearrangement, whereas T-2 toxin (12) (Figure 4), containing an 8ã-O-valerate functionality is resistant to the rearrangement.

In strongly acidic media the 10,13-cyclotrichothecane rearrangement is prevented probably because of the resultant

protonation of the 9-ene thus decreasing its nucleophilicity. Instead, the protonated epoxide is attacked by the oxygen ether linkage of ring B. This is then followed by a nucleophilic attack on C-2 upon which rearrangement to the apotrichothec-9-ene skeleton (35) occurs (Figure 12), where the R_4 substituent depends on the acidic conditions utilized. When 10M HCl (or hydrogen chloride gas in a non-polar solvent thereby giving a cleaner product) is used, R_4=Cl. When concentrated trifluoroacetic acid is used or aqueous H_2SO_4 is used, R_4=OH.

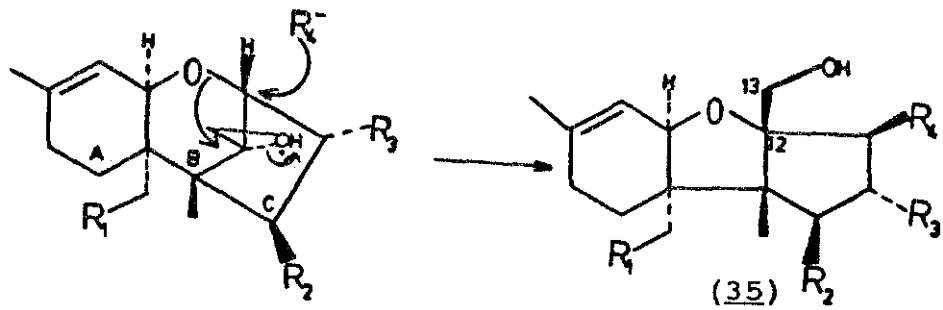


Figure 12

Overall, the apotrichothec-9-ene rearrangement is deterred by bulky substituents at positions 3ã and 7â but is not hindered by 8ã-OH or 8ã-OR substituents. Anguidine (7) (Figure 3) readily undergoes this rearrangement but when the 3ã oxygen functionality is replaced by an acetate, as is the case for the triacetate (32) (Figure 3), the reaction time is increased from 15 minutes to 24 hours. Trichothecodiol (33) and diacetate (36) (Figure 4) containing an 8ã-OH and 8ã-O-acetate functionality respectively, readily undergo the rearrangement. Triol (34) (Figure 4) is completely resistant to this rearrangement because

it is hindered by the 7â-OH functionality.

Because the 12,13-epoxide is protected from rearside nucleophilic attack, only trichothec-9-ene-8-ones undergo a number of interesting reactions in basic media (Figure 13). Their reactivity towards base is facilitated by the 8-ketone functionality which enables the formation of a carbanion at position 7. Therefore in strongly basic media a 7,13-cyclotrichothecene (38) product is obtained from intramolecular attack of the carbanion on the epoxide (Grove, 1985) of trichothecolone (37). A similar reaction occurs with the 7ß-8ß-epoxide crotocol (39) leading to the 7,13-epoxytrichothecene (40).

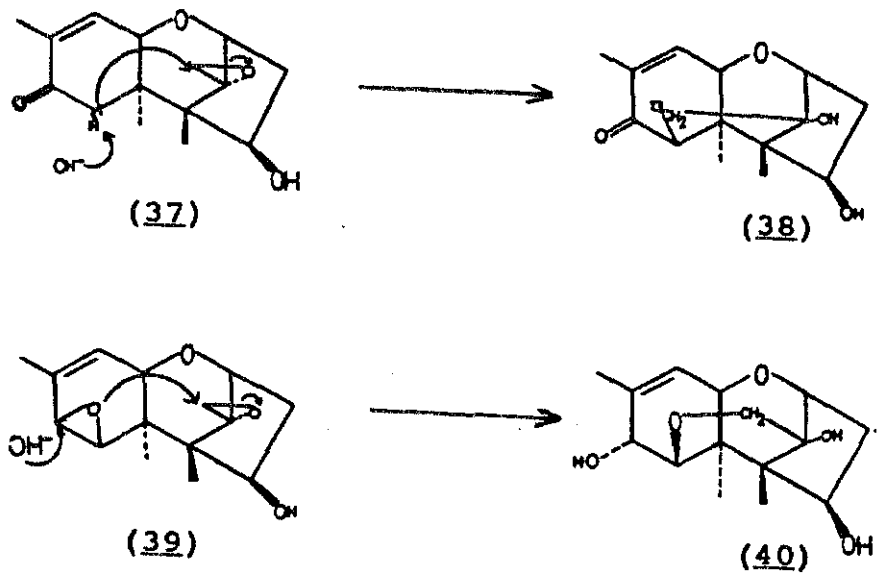
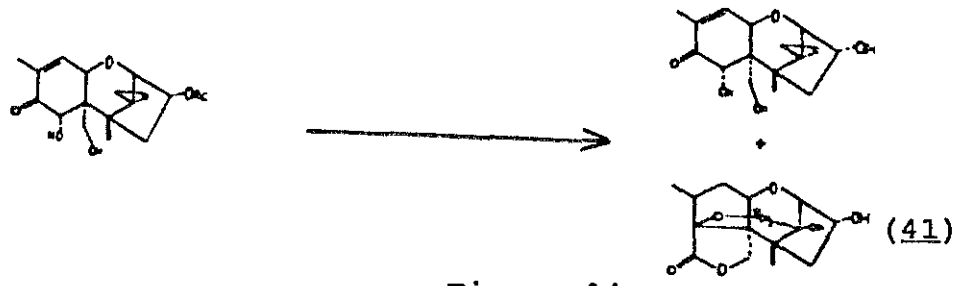


Figure 13

Hydrolysis of 3-acetyl-4-deoxynivalenol, in 0.1 M NaOH for 18 hours at room temperature yields 90-95% of 4-deoxynivalenol and 5-10% of an impurity which has been identified

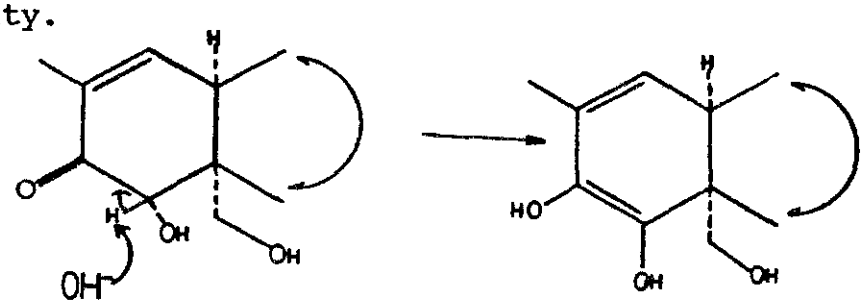
as the A-nortrichothecane (41) (Figure 14) (Grove, 1985). Rings B and C of this product remain intact but the 12,13-epoxide has been opened to give a tertiary hydroxy. The 9,10 double bond appears to have been reduced and the C=O of the 8-ketone has been incorporated in the ÿ-lactone. Both CH_2OR groups characterized by the NMR spectrum were held within rings which numbered five in



(41)

Figure 14

all. The mechanism for the formation of this ÿ-lactone is demonstrated in its entirety by Grove (1985). The crucial precursor in this rearrangement is the dienediol (42) species formed (Figure 15). Its formation is made possible by the 8-ketone whose presence causes the 7ß-H to be acidic and therefore more susceptible to abstraction by a base. Hydrolysis of 3-acetyl-4-deoxynivalenol in 0.1 M NaOH at room temperature for 1.5 hours yields 100% DON therefore eliminating the production of this impurity.

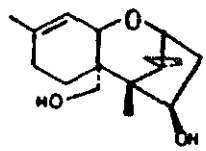


(42)

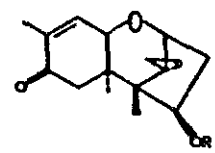
Figure 15

4. THE BIOSYNTHESIS OF 3-OXYTRICHOTHECENES AND 4-OXYTRICHOTHECENES

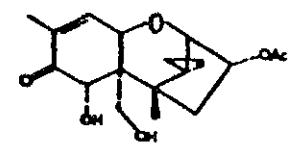
Research into the biosynthesis of trichothecenes was conducted predominantly via studies on Myrothecium species, Trichothecium roseum and Trichoderma sporulosum, producers of the 4-oxytrichothecenes, verrucarol (4), trichothecin (43) and trichodermin (5) (Figure 16), respectively. 3-Oxytrichothecenes (trichothecenes with a hydroxy function at C-3) such as calonectrin (8) and more recently 3-acetyldeoxynivalenol (18) both metabolites of Fusarium culmorum, have also been investigated.



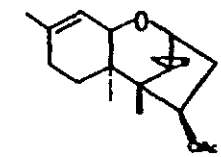
(4)



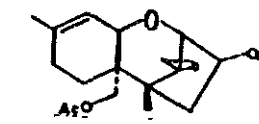
(43) R=C(O)CH=CHCH$_3$
(44) R=H



(18)



(5)



(8)

Figure 16

Fishman et al. (1959) were the first to observe the incorporation of three 2-^{14}C mevalonate molecules into trichothecolone (44) (obtained after basic hydrolysis of trichothecin (43)). At that time, the true structure of trichothecolone had not been correctly established (Godtfredsen and Vangedal, 1965). Later studies initiated by Achilladelis et al. (1970; 1972) and confirmed by Machida and Nozoe (1972a) correctly traced the C-2 of mevalonate to C-4, C-14 and C-8 of

the trichothecenes, and not C-10 as had been incorrectly established by Fishman et al. Hanson (Hanson et al., 1974) fed [2-^{13}C] mevalonate to Trichothecium roseum and isolated trichothecolone (Figure 17). From ^{13}C NMR spectra, enrichment was observed only for C-4, C-8 and C-14 thereby confirming unambiguously the results of Achilladelis et al. (1970; 1972) and defenitively proving the sesquiterpene nature of the trichothecenes.

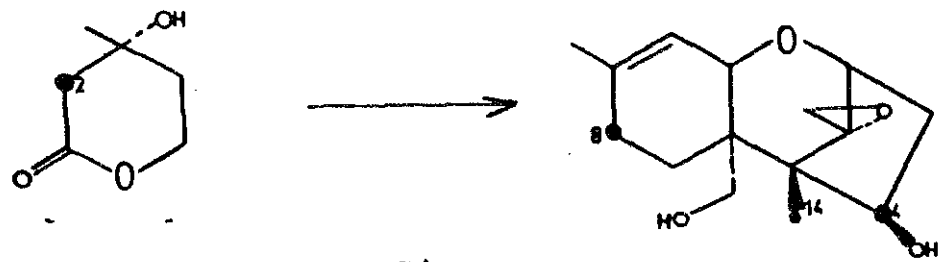


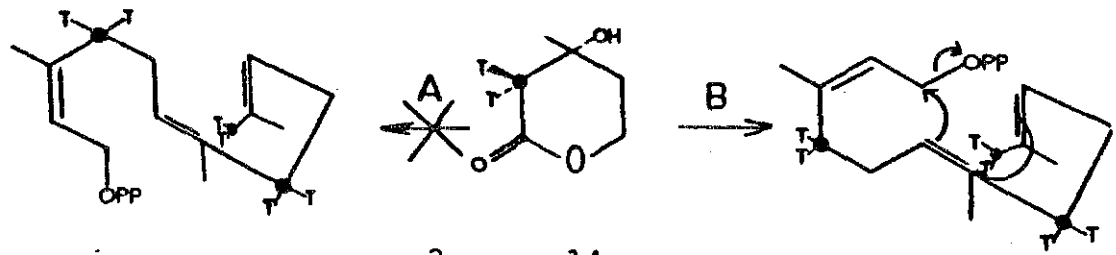
Figure 17

For a detailed review on the biosynthesis of trichothecenes see Tamm and Breitenstein (1980) and Cordell (1976).

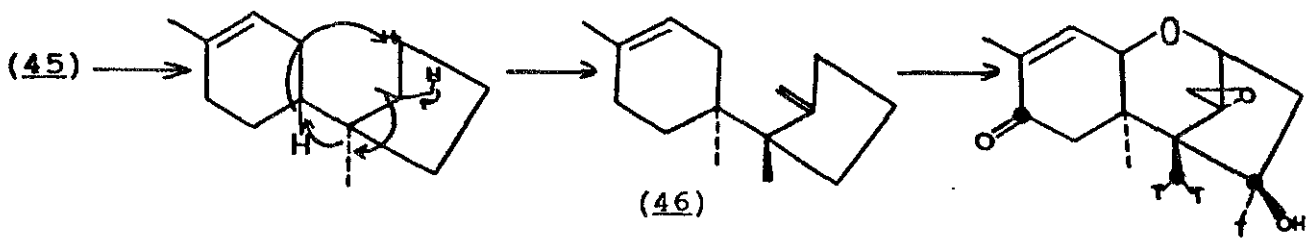
6,7-Trans farnesyl pyrophosphate (45), obtained from the condensation of three units of mevalonate, acts as the intermediate in the biosynthesis of the trichothecenes. Hanson et al. (1974) proved unambiguously the preference of [2-^{13}C] mevalonate derived trichothecolone for the "B" folding of farnesyl pyrophosphate but not for the "A" folding (Figure 18). Via the former pattern only positions C-4, C-8 and C-14 show ^{13}C enrichment by ^{13}C NMR as would be expected.

Farnesyl pyrophosphate is incorporated into

trichothecolone via the bicyclic precursor trichodiene (TDN) (46) which was first isolated from Trichothecium roseum and then structurally characterized by Nozoe and Machida (1970a; 1970b; 1972). This cyclization involves a 1,4-hydride shift from C-6 to C-10 of farnesyl pyrophosphate and two 1,2-methyl shifts. The methyl group at position C-11 of farnesyl pyrophosphate (45) corresponding to C-2 of mevalonate, migrates to C-7 thereby causing the second migration from position C-7 to C-6 (Figure 18).



[2-$^{3}H_2$][2-^{14}C] MVL (45)



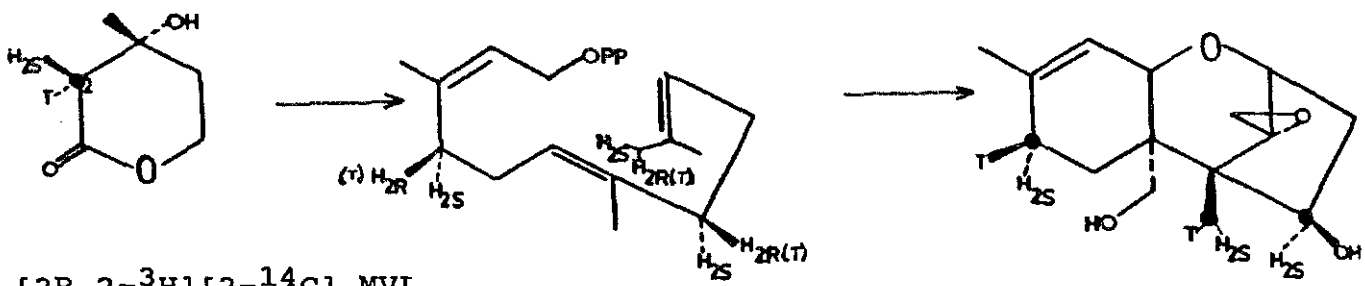
(46)

Figure 18

The first 1,2-methyl group migration (thereby implying the occurrence of the second migration), was proven by Adams and Hanson (1970) and Achilladelis et al (1970; 1972). The 1,4-hydride shift was proven by Hanson et al. (Achilladelis et al., 1970; Achilladelis et al., 1972; Adams and Hanson, 1971) and Arigoni et al. (1973).

The last stages of the biosynthetic sequence after trichodiene, involve a series of hydroxylations which lead to the formation of the different trichothecenes. To determine the stereochemistry of the hydroxylation at C-4, [2-$^{3}H_2$] [2-^{14}C] mevalonate was fed to Myrothecium species (Achini et al., 1971; Müller et al., 1975). The verrucarol isolated retained five tritium labels. In order to determine the stereochemistry of the C-4 hydroxylation, two separate feeding experiments were conducted with [2R, 2-^{3}H] [2-^{14}C] mevalonate and [2S, 2-^{3}H] [2-^{14}C] mevalonate (Figure 19).

Thus in verrucarol, the hydroxy group at C-4 replaces a pro-2R mevalonate hydrogen atom. Hydroxylation at C-4 occurs with an overall retention of configuration, that is the newly formed C-O bond possesses the same relative stereochemistry as the C-H bond replaced.

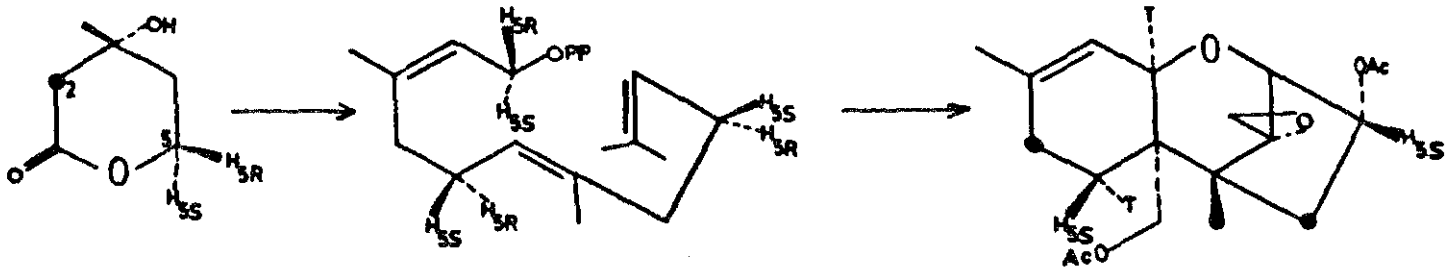


[2R,2-^{3}H][2-^{14}C] MVL

Figure 19

Calonectrin (8), the 3-oxytrichothecene, derived from [5-$^{3}H_2$] [2-^{14}C] mevalonate contains four possible tritium labels. When it is derived from [5R, 5-^{3}H] [2-^{14}C] mevalonate (Evans et al., 1974), calonectrin exhibits the presence of two tritium

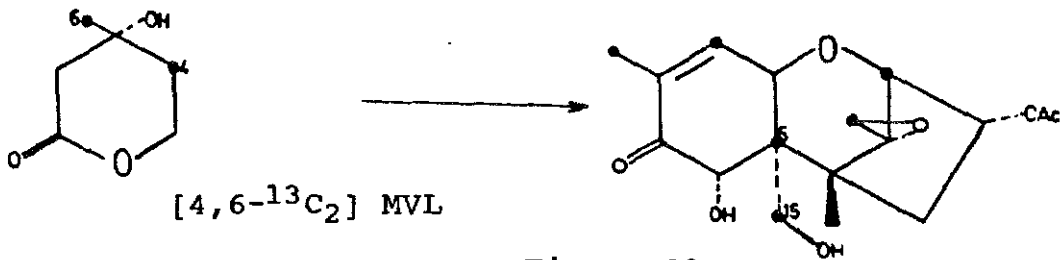
labels (Figure 20). Thus hydroxylation at C-3 replaces a pro-5R mevalonate hydrogen atom also with retention of configuration.



[5R,5-^{3}H][2-^{14}C] MVL

Figure 20

The biosynthesis of the exceptional 3-oxytrichothecene 3-ADN (18) has been investigated by Zamir et al (Zamir, 1986; Zamir et al., 1987b) using ^{13}C double labeled mevalonates and stereospecifically deuterium labeled mevalonates. The distribution of label was established with the use of [2,4-$^{13}C_2$] mevalonate. The enrichment sites as located by ^{13}C NMR spectra fit the expected pattern of 4-oxytrichothecenes derived from mevalonate. The 3-ADN derived from the feeding of [4,6-$^{13}C_2$] mevalonate showed ^{13}C-^{13}C direct coupling between C-6 and C-15 (3-ADN numbering), thus substantiating the 1,2-methyl shift of C-6 to C-4 of mevalonate (Figure 21).



[4,6-$^{13}C_2$] MVL

Figure 21

Cultures of Fusarium culmorum were fed with [3,4-$^{13}C_2$] mevalonate. Direct coupling in 3-ADN between C-2 and C-12, C-5 and C-6, and C-9 and C-10 is proof of the "B" folding of farnesyl pyrophosphate, similar to that for 4-oxytrichothecenes. To determine the stereochemistry of oxygenation of 3-ADN, three separate feedings of [3RS, 5R, 5-^{2}H], [3RS, 5S, 5-^{2}H] and [3RS, 5-$^{2}H_2$] mevalonates were conducted in cultures of Fusarium culmorum. Thus hydroxylation of 3-ADN at positions C-3 and C-7 replaced a pro-5R mevalonate hydrogen atom. Hydroxylation at C-11 of 3-ADN replaced a pro-5S mevalonate hydrogen atom. All oxygenations occurred with retention of configuration.

The exceptional value of these last studies is found in the nature of the radiolabeling. Unlike radioisotope labeling which requires chemical degradations to locate the label, stable isotopes can be located without ambiguity by ^{13}C or ^{2}H NMR spectroscopy.

MATERIALS AND METHODS

1. MATERIALS

Proton ^{1}H-NMR spectra were measured at 200 or 300 MHz on a Varian XL-300 spectrometer at ambient temperature (22°C). The samples (2-5mg) were dissolved in $CDCl_3$. Chemical shifts are reported in õ units using the 7.262 ppm resonance of residual chloroform as internal reference. Infrared spectra were measured on a Perkin-Elmer Model 683 infrared spectrophotometer calibrated with the 1601 cm^{-1} absorption of polystyrene, in chloroform solutions. Infrared spectra are reported in wave numbers. Melting points were obtained on a Buchi 510 melting point apparatus.

All reactions were conducted in flame or oven dried glassware under dry argon atmosphere. The following solvents were purified before use: CH_2Cl_2 and DMF were distilled from CaH_2, toluene was distilled from P_2O_5, MeOH was distilled from Mg metal and THF was distilled from lithium aluminum hydride.

Analytical thin layer chromatography was performed using E. Merck glass supported silica gel 60 (F254, 0.25 mm) plates. The compounds were visualized using a cerique sulfate/ ammonium molybdate solution.[4] Column chromatography was accomplished by the "flash chromatography" method of Still (Still et al., 1978) (E. Merck silica gel 60, 230-400 mesh).

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[4] For one liter of developing agent: 100 ml H_2SO_4 concentrated; 900 ml water, 25 g ammonium molybdate, 10 g $(CeSO_4)_2$ (cerique sulfate).

2. ANGUIDINE TO $^{2}H_2$-ISOTRICHODERMIN (FIGURE 23)

2.1. 3-O-THP-Anguidine (47)

A solution of anguidine (0.80 g; 0.0022 mol) in 15 ml of CH_2Cl_2 at room temperature was treated with dihydropyran (0.44 ml; 0.0048 mol) and PPTS (0.086 g; 0.34 mmol) over a four day period. The mixture was diluted in 200 ml CH_2Cl_2 and washed successively with 1x25 ml saturated $NaHCO_3$ solution and 2x25 ml brine to pH 7. The solution was dried ($MgSO_4$) and filtered, and the volatiles were removed 'in vacuo'. The thick crude sirup obtained was chromatographed (3 cm x 21 cm) using 35% ethyl acetate in hexane. This gave 0.98 g (100%) of a thick sirup as a mixture of diastereomers. Rf (40% EtOAc:Hexane); 0.31. IR($CHCl_3$), 3005, 2945, 2975, 1747, 1740, 1690, 1455, 1390, 1265 cm^{-1}. ^{1}H-NMR; õ(ppm). H-2 [d, 1H, 3.769,3.7, $J_{2,3}$ = 5.0] // H-3 [dd, 1H, 4.348 ($J_{2,3}$ = 4.8, $J_{3,4}$ = 3.3),4.269 ($J_{2,3}$ = 5.0, $J_{3,4}$ = 3.3)] // H-4 [d, 1H, 5.697, 5.631, $J_{3,4}$ = 3.3] // H-10 [broad, d, m, 1H, 5.499, 5.488] //H-11 [broad, d, 1H, 4.135 ($J_{10,11}$ = 6.35), 4.113 ($J_{10,11}$ = 6.5)] // H-13A [d, 1H, 3.052 (J_{AB} = 4.0), 3.043 (J_{AB} = 3.96)] // H-13B [d, 1H, 2.781 (J_{AB} = 4.03), 2.779 (J_{AB} = 4.1)] // H-14 [s, 3H, 0.742, 0.735] // H-15A [d, 1H, 4.354 (J_{AB} = 12.3), 4.325 (J_{AB} = 12.1)] // H-15B [d, 1H, 4.069 (J_{AB} = 12.3), 4.045 (J_{AB} = 12.3)] // H-16 [s, 3H, 1.721] // C-15-O-acetate [s, 3H, 2.062, 2.060] // C-4-O-acetate [s, 3H, 2.109, 2.104] // O-THP H_2, [4.954 (J = 2.9),4.693 (J = 5.4, 2.2)]

2.2. 3-O-THP-Scirpenetriol (48)

A solution of 3-OTHP-anguidine (0.98 g; 0.0022 mol) in 178 ml MeOH was treated with anhydrous K_2CO_3 (1.95 g; 0.014 mol). The reaction mixture was stirred at room temperature for three hours. The methanol was evaporated in vacuo. The residue was diluted with 200 ml of chloroform. The organic layer was washed to neutrality with 2x25 ml H_2O and 2x25 ml brine. Since some product passed into the aqueous layer it was extracted with 2x100 ml EtOAc. The combined organic layers were dried ($MgSO_4$), filtered and evaporated in vacuo. The crude sirup was chromatographed (3 cm x 20 cm) using 75% EtOAc in hexane. This gave 0.80 g (100%) of a thick oil. Rf (75% EtOAc:Hexane);0.21. IR($CHCl_3$), 3630, 3600-3300 broad band, 3005, 2945, 1675, 1440, 1385, 1240, 1200, 1125 cm^{-1}. ^{1}H-NMR; δ(ppm). H-2 [d, 1H, 3.734 ($J_{3,2}$ = 4.89), 3.703 ($J_{3,2}$ = 4.76)] // H-3 [dd, 1H, 4.091 ($J_{3,2}$ = 4.95, $J_{3,4}$ = 3.1), 4.064 ($J_{3,2}$ = 4.9, $J_{3,4}$ = 3.3)] // H-4 [d(sharp), 1H, 4.414 ($J_{4,3}$ = 3.0), d,(broad), 4.437 ($J_{4,3}$ = 3.0)] // H-10 [broad, 1H, 5.504, 5.49] // H-11 [d, 1H, 3.902 ($J_{10,11}$ = 5.0), 3.964 ($J_{10,11}$ = 5.1)] // H-13A [d, 1H, 3.026 (J_{AB} = 4.02), 3.021 (J_{AB} = 4.0)] // H-13B [d, 1H, 2.774 (J_{AB} = 4.03), 2.755 (J_{AB} = 4.0)] // H-14 [s,3H, 0.928, 0.907] // H-15A [d, 1H, 3.80, 4.00] // H-15B [d, 1H, 3.55] // H-16 [s, broad, 1.732] // O-THP H_2, [4.953 (J = 3.0), 4.697 (J = 5.43; 2.6)]

2.3. 3-O-THP,15-O-Acetyl-scirpenetriol (49)

A solution of 3-O-THP-scirpenetriol (0.80 g; 0.0022 mol) in 48 ml CH_2Cl_2 was treated with pyridine (1.25 ml; 0.016

mol) and acetic anhydride (0.31 ml; 0.0033 mol). The reaction mixture was stirred for 40 hours at room temperature after which more acetic anhydride (0.090 ml; 0.95 mmol) was added, and stirring was continued for a further 24 hours. The reaction mixture was diluted with 200 ml of $CHCl_3$ and washed successively with 1x30 ml 10% HCl, 1x30 ml saturated $NaHCO_3$ and 1x30 ml brine to neutrality. The aqueous layer was extracted with 2x100 ml EtOAc. The organic layers were dried ($MgSO_4$), filtered and evaporated in vacuo. The crude sirup was chromatographed (3 cm x 19 cm) using 50% EtOAc in hexane to elute the desired product and 100% EtOAc to elute the residual starting material. This gave 0.14 g of recovered starting material and 0.66 g (90%) of the 3-O-THP,15-O-acetyl-scirpenetriol as crystals (melting point: 153-155°C from hexane). Rf (55% EtOAc:Hexane);0.21. IR($CHCl_3$), 3575, 3600-3380 broad band, 3005, 2945, 1745, 1685, 1480, 1455, 1260 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.745, 3.711, $J_{2,3}$ = 5.0] // H-3 [dd, 1H, 4.095 ($J_{3,2}$ = 5.0, $J_{3,4}$ = 3.0), 4.052 ($J_{3,2}$ = 4.7, $J_{3,4}$ = 3.06)] // H-4 [bd, 4.295, 4.309, $J_{3,4}$ = 2.7, $J_{4,OH}$ = 7.9] // H-10 [broad, 1H, 5.488] // H-11 [overlapping with H-15B, 1H, 4.208, 4.166] // H-13A [d, 1H, 3.034 (J_{AB} = 4.02), 3.028 (J_{AB} = 3.91)] // H-13B [d, 1H, 2.773 (J_{AB} = 4.12), 2.752 (J_{AB} = 4.05)] // H-14 [s, 3H, 0.849, 0.823] // H-15A [d, 1H, 4.187 (J_{AB} = 12.6)] // H-15B [d, 1H, 3.894 (J_{AB} = 12.3)] // H-16 [s, 3H, 1.718] // C-15-O-acetate [s, 3H, 2.063, 2.058] // O-THP H_2, [4.954 (t, J = 2.9), 4.693 (dd, J = 2.2, J = 5.4)

2.4. 3-O-THP,15-O-acetylscirpenetriol-4-N,N-thiocarbonyl-imidazolide (50)

A solution of (49) (0.66 g; 0.0016 mol) in 21 ml CH_2Cl_2 was treated with N,N-thiocarbonyldiimidazole (0.69 g; 0.0039 mol) and was refluxed at 54°C for 16 hours. The cooled solution was diluted with 200 ml of CH_2Cl_2 and washed successively with 1x25 ml 1 N HCl, 1x25 ml saturated $NaHCO_3$ solution and 1x25 ml brine to neutrality. The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. The crude oil obtained was chromatographed (3 cm x 19 cm) using 70% EtOAc in Hexane. This gave 0.77 g (92%) of the desired compound as an oil. Rf (70% EtOAc:Hexane);0.31. IR($CHCl_3$), 3160, 3130, 3005, 2970, 2945, 1745, 1685, 1540, 1475, 1400, 1260. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.861, $J_{2,3}$ = 4.9] // H-3 [dd, 1H, 4.492 ($J_{2,3}$ = 4.8, $J_{3,4}$ = 2.8), 4.425 ($J_{2,3}$ = 4.9, $J_{3,4}$ = 2.7)] // H-4 [d, 1H, 6.436 ($J_{4,3}$ = 2.8), 6.410 ($J_{4,3}$ = 2.7] // H-10 [broad, 1H, 5.53 ($J_{10,11}$ = 5.6), 5.50 ($J_{10,11}$ = 5.5) // H-11 [broad, 1H, 4.17] // H-13A [d, 1H, 3.117 ($J_{A,B}$ = 4.15), 3.099 ($J_{A,B}$ = 4.1)] // H-13B [d, 1H, 2.823 ($J_{A,B}$ = 4.03)] // H-14 [s, 3H, 0.848, 0.830] // H-15A [d, 1H, 4.557 ($J_{A,B}$ = 12.2), 4.442 ($J_{A,B}$ = 12.9)] // H-15B [d, 1H, 4.047 ($J_{A,B}$ = 12.45), 4.041 ($J_{A,B}$ = 12.3)] // H-16 [s, 3H, 1.741] // C-15-O-Ac [s, 3H, 2.061] // O-THP-H_2, [t, 4.811 (J = 3.1), 4.745 (J = 3.3)] // H_6,A [broad, 3.95, 3.72] // H_6,B [3.49, 3.41]

2.5. 3-O-THP-4-^{2}H-3-Deacetylcalonectrin (51)

A solution of the thiocarbonylimidazolide (50) (0.77 g; 0.0015 mol) in 182 ml toluene was treated with AIBN (0.071 g; 0.43 mmol) and heated to 80°C. Freshly prepared n-Bu_3Sn^2H (1.6

ml; 0.0059 mol) was added rapidly via syringe and the solution was stirred at 80°C for 30 minutes. The reaction mixture was cooled and the toluene was removed in vacuo. The residue was chromatographed (3 cm x 17 cm) using 30% EtOAc in hexane. This gave 0.49 g (84%) of an oil which slowly crystallized, (m.p.: 120.5-122.5°C from petroleum ether), at cold temperatures. Rf (30% EtOAC:Hexane);0.30. IR($CHCl_3$), 3005, 2960, 2870, 2855, 1740, 1685, 1475, 1390, 1260 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d,1H, 3.651 ($J_{2,3}$ = 4.64), 3.646 ($J_{2,3}$ = 4.4)] // H-3 [t, 1H, 4.268 ($J_{2,3}$ = $J_{3,4}$ = 4.4), 4.458 $J_{2,3}$ = $J_{3,4}$ = 4.3)] // ^{2}H-4 [deuterium NMR, 1-D, 1.99)] // H-10 [bd, 1H, 5.497 ($J_{10,11}$ = 5.6), 5.453 ($J_{10,11}$ = 5.4)] // H-11 [broad, d, 1H, 4.206 ($J_{10,11}$ = 5.6), 4.22 (overlapping)] // H-13A [d, 1H, 3.073 (J = 4.0), 3.062 (J = 4.0)] // H-13B [d, 1H, 2.841 (J = 4.0), 2.838 (J = 4.1)] // H-14 [s, 3H, 0.804, 0.813] // H-15A [d, 1H, 4.130 ($J_{A,B}$ = 12.1), 4.119 ($J_{A,B}$ = 12.1)] // H-15B [d, 1H, 3.830 ($J_{A,B}$ = 12.2), 3.835 ($J_{A,B}$ = 12.1)] // H-16 [s, 3H, 1.719] // H-15-O-Ac [s, 3H, 2.047] // H_2, [t, 1H, 4.760 (J = 3.0), 4.704 (J = 3.0)].

2.6. 3,15-Deacetyl-3-O-THP-4-^{2}H-calonectrin (52)

A solution of 3-deacetyl-3-O-THP-4-^{2}H-calonectrin (0.49 g; 0.0012 mol) in 52 ml MeOH was treated with anhydrous K_2CO_3 (1.2 g; 0.0087 mol) and stirred for 16 hours at room temperature. The reaction mixture was evaporated to dryness in vacuo. The residue obtained was diluted with 200 ml $CHCl_3$ and washed to neutrality with 4x30 ml brine. The aqueous layer was extracted 1x100 ml with $CHCl_3$. The organic layer was dried

($MgSO_4$), filtered and evaporated in vacuo. The sirup obtained was chromatographed (3 cm x 17 cm) using 40% EtOAc in Hexane. This gave 0.357 g (82%) of the desired compound as a thick foamy sirup. Rf (30% EtOAc:Hexane);0.15. IR($CHCl_3$), 3630, 3005, 2945, 1680, 1455, 1375, 1245 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [two d overlapping, 1H, 3.632 ($J_{3,2}$ = 4.76)] // H-3 [2xt, 1H, 4.450 ($J_{2,3}$ = $J_{3,4}$ = 4.3), 4.240 ($J_{2,3}$ = $J_{3,4}$ = 4.4)] // ^{2}H-4 [deuterium NMR, 2.086] // H-10 [bd, 1H, 5.501 ($J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.4), 5.450 ($J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.4)] // H-11 [broad d, 1H, 4.138] // H-13A [d, 1H, 3.063 ($J_{A,B}$ = 4.1), 3.053 ($J_{A,B}$ = 4.0)] // H-13B [d, 1H, 2.843 ($J_{A,B}$ = 4.1), 2.839 ($J_{A,B}$ = 4.1)] // H-14 [s, 3H, 0.894, 0.900] // H-15A [broad d, 1H, 3.711 ($J_{A,B}$ = 13.3)] // H-15B [overlapping with $H_{6'}$, 3.5-3.4] // H-16 [s, 3H, 1.726] // $H_{2'}$ [2t, 4.757 (J = 3.1), 4.690 (J = 2.8)].

2.7. Chloride (53)

The 3,15-deacetyl-3-O-THP-4-^{2}H-calonectrin obtained (0.281 g; 0.799 mmol) was divided into three portions. Each portion was reacted individually as a precautionary measure. Thus a solution of (84) (0.092 g; 0.26 mmol) in 8 ml DMF was treated with imidazole (0.081 g; 0.0012 mol) and was cooled to -40°C in a dry ice/acetone bath. To the cooled solution was added via syringe, dropwise SO_2Cl_2 (0.042 ml; 0.52 mmol). The solution was stirred at -40°C for 30 minutes, then the dry ice/acetone bath was removed and the solution was allowed to come to room temperature for 30 minutes. Imidazole (0.073 g; 0.0011 mol) was then added, the solution was once again cooled to -40°C and more

SO_2Cl_2 (0.042 ml; 0.52 mmol) was added, dropwise, via syringe. The bath was removed and the reaction mixture was allowed to equilibrate to room temperature and was stirred as such for 15 hours. The reaction mixtures were diluted in 200 ml EtOAc and washed with 2x25 ml brine and 1x25 ml H_2O. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo. The residual sirup obtained was chromatographed (15 mm x 17 cm) using 20% EtOAc in hexane. This gave 0.244 g (83%) of the desired compound as an oil. Rf (25%EtOAc:Hexane);0.29. IR($CHCl_3$), 3005, 1680, 1465, 1455, 1375, 1245 cm^{-1}.^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.651 ($J_{2,3}$ = 4.6), 3.645 ($J_{2,3}$ = 4.4)] // H-3 [t, 1H, 4.464 ($J_{3,4}$ = $J_{2,3}$ = 4.4), 4.253 $J_{3,4}$ = $J_{2,3}$ = 4.4)] // ^{2}H-4 [D-NMR, 2.144] // H-10 [d, 1H, 5.453 ($J_{10,11}$ = 5.5), 5.404 ($J_{10,11}$ = 5.5)] // H-11 [broad, 1H, 4.143, 4.130] // H-13A [d, 1H, 3.063 ($J_{A,B}$ = 4.1), 3.055 (J, = 4.05)] // H-13B [d, 1H, 2.850 ($J_{A,B}$ = 4.1), 2.846 ($J_{A,B}$ = 4.1)] // H-14 [s, 3H, 0.960, 0.954] // H-15A [d, 1H, 3.608 ($J_{A,B}$ = 12.2), 3.601 ($J_{A,B}$ = 12.4)] // H-15B [d, 1H, 3.422 ($J_{A,B}$ = 12.0), 3.398 ($J_{A,B}$ = 12.2)] // H-16 [broad s, 3H, 1.739] // $H_{2'}$ [t, 1H, 4.759 ($J_{2',3'}$ = 3.2), 4.680].

2.8. 3-Deacetyl-3-O-THP-$^2H_2$-isotrichodermin

A solution (0.244 g; 0.660 mmol) of the above chloride (53) in 52 ml toluene was treated with AIBN (0.029 g; 0.18 mmol) and then heated to 80°C. To the reaction mixture was then added freshly prepared n-Bu_3Sn^2H (0.89 ml; 0.0033 mol) rapidly via syringe. The solution was heated at 80°C for 18 hours. Additional amounts of AIBN (0.028 g; 0.17 mmol) and n-Bu_3Sn^2H (0.89 ml;

0.0033 mol) were added and stirring was resumed for a further 48 hours at 80°C. The toluene was evaporated and the residue was chromatographed (15 mm x 15 cm) using 20% EtOAc in Hexane, to give an oil 0.228 g (100%). Rf (25% EtOAc:Hexane);0.29. IR($CHCl_3$), 3005, 2960, 1680, 1465, 1455, 1445, 1375, 1245 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.648 ($J_{2,3}$ = 4.6)] // H-3 [t, 1H, 4.468 ($J_{2,3}$ = $J_{3,4}$ = 4.4), 4.258 ($J_{2,3}$ = $J_{3,4}$ = 4.5)] // ^{2}H-4 [deuterium NMR, 2.009] // H-10 [d, 1H, 5.494 ($J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.4), 5.445 ($J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.5) // H-11 [broad d, 1H, 4.217 (J = 6.3), 4.196 (J = 6.3)] // H-13A [d, 1H, 3.062 $J_{A,B}$ = 3.84), 3.050 ($J_{A,B}$ = 3.5)] // H-13B [d, 1H, 2.837 ($J_{A,B}$ = 4.04), 2.834 ($J_{A,B}$ = 4.1)] // H-14 [s, 3H, 0.739, 0.734] // H-15 (broad singlets, 2H, overlapping), ^{2}H-15 [deuterium NMR, 0.800] // H-16 [s, 3H, 1.714] // H_2, [t, 1H, 4.781 (J = 3.0), 4.716 (J = 4.1)].

2.9. 3-Deacetyl-$^2H_2$-isotrichodermin

A solution of the 3-deacetyl-3-O-THP-$^2H_2$-ITD obtained from the previous step, (0.228 g; 0.678 mmol) in 11 ml of MeOH was treated with PPTS (0.019g; 0.076 mmol). The solution was stirred at 60°C for 9 hours. The methanol was evaporated and the resulting residue was chromatographed (15 mm x 15 cm) using 50% EtOAc in hexane. The resulting product 0.133 g (78%) was crystalline (m.p.: 151-152°C from petroleum ether). Rf (50% EtOAc: Hexane);0.24. IR($CHCl_3$), 3600(broad), 3010, 2975, 2920, 1680, 1455, 1380, 1200 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.510 ($J_{2,3}$ = 4.6)] // H-3 [q, 1H, 4.451 ($J_{2,3}$ = $J_{3,4}$ = $J_{3,OH}$ = 4.5)]

// H-4 [overlapping with H-7A, H-8A,B, 1H, (2.05-1.87)] // H-10 [dq, 1h, 5.492 ($J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.5)] // H-11 [broad, d, 1H, 4.088 ($J_{10,11}$ = 5.5)] // H-13A [d, 1H, 3.078 ($J_{A,B}$ = 4.0)] // H-13B [d, 1H, 2.850 ($J_{A,B}$ = 4.0)] // H-14 [s, 3H, 0.747] // H-15 [broad, s, 2H, 0.801] // H-16 [broad, s, 3H, 1.730] // OH [2.134 ($J_{OH,3}$ = 4.9)] // Deuterium NMR, D-4 [2.059], D-15 [0.815].

2.10. $^{2}H_2$-Isotrichodermin (54)

A solution of the above alcohol (0.133 g; 0.527 mmol) in 4 ml of acetic anhydride was treated with pyridine (0.40 ml; 0.0050 mol). The reaction mixture was stirred at room temperature for 18 hours. The solution was diluted with 100 ml of CH_2Cl_2 and was washed successively with 2x15 ml saturated $NaHCO_3$ solution , 1x15 ml 10% HCl, 1x15 ml saturated $NaHCO_3$ solution and 2x15 ml H_2O to neutrality. The organic layer was dried ($MgSO_4$), filtered and evaporated. The residue was chromatographed (15 mm x 15 cm) using 25% EtOAc in hexane. This gave $^{2}H_2$-isotrichodermin 0.144 g (93%) as white crystals (m.p.: 93-95°C from pentane). Rf (25% EtOAc:Hexane);0.30. IR($CHCl_3$), 3005, 2975, 2945, 2915, 1740, 1680, 1440, 1380, 1250 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 3.743 ($J_{2,3}$ = 4.6)] // H-3 [t, 1H, 5.167 ($J_{2,3}$ = $J_{3,4}$ = 4.5)] // H4 [d, 1H, 2.060 ($J_{3,4}$ = 4.8)] // H-10 [dq, 1H, 5.466 ($J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.47)] // H-11 [broad, d, 1H, 3.982 ($J_{10,11}$ = 5.56)] // H-13A [d, 1H, 3.091 ($J_{A,B}$ = 4.0)] // H-13B [d, 1H, 2.858 ($J_{A,B}$ = 4.0)] // H-14 [s, 3H, 0.759] // H-15 [broad, s, 2H, 0.799] // H-16 [s, 3H, 1.721] // OAc [s, 3H, 2.132] // deuterium NMR: D-4 [2.142], D-15 [0.825].

3. VERRUCAROL TO $^2H_4$-EPOXYTRICHOTHECENE (FIGURE 25)

3.1. Production of Verrucarol (Jarvis et al., 1984)[5]

Myrothecium verrucaria slants were a gift from Dr. Jarvis (from the University of Maryland). Seed cultures were prepared as follows: a 1.5% Sabouraud Dextrose agar slant, of M. verrucaria covered with a thick growth that was rich in spores, (previously stored at 4°C) was homogenized in 100 ml of sterile deionized water for one minute using a Polytron homogenizer (Brinkman Instruments, Rexdale, Ontario). Inoculation of seed cultures was accomplished by adding 15 ml of the homogenate to each of two 2 liter Erlenmeyer flasks all containing 300 ml of seed medium. The seed medium consisted of a sterile solution of 9.5 g of glucose and 6 ml of corn steep liquor in 600 ml of deionized water. These seed cultures were incubated for 30 hours at 28°C and at 220 rpm in a New Brunswick G-25 rotary shaker coupled to a model RF-10 frigid flow bath circulator (New Brunswick Scientific Co., Inc., Edison, N.J.) At this time, 35 ml portions of this seed culture were added to each of fourteen 2 liter Erlenmeyer flasks all containing 350 ml of a sterile production medium containing $NH_4H_2PO_4$ (1.0 g), K_2HPO_4 (3.0 g), NaCl (5.0 g), $MgSO_4$-$7H_2O$ (0.2 g), sucrose (40.0 g) and glycerol

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[5] The procedure that has been applied to the production of verrucarol (Jarvis et al., 1984) has been somewhat modified in order to enhance the yield of verrucarol, which has been increased from that previously reported.

(10 ml) per one liter of deionized water. The flasks were shaken for seven days at 28°C and at 220 rpm in a model G-25 shaker. At this time, each flask was treated with 0.35 g sodium azide and 100 ml of XAD-7 resin (Rohm and Haas) and shaking was continued for another 5 h.

The medium containing the mycelium-resin mixture was filtered through a Büchner funnel equipped with a Whatman no. 1 filter paper. To expediate the filtration process only the contents of two 2 liter flasks were filtered per filter paper. The mixture of mycelium and resin was washed with acetone (5 liters), soaked in fresh acetone (1 liter) overnight and filtered. Both filtrates obtained were combined. This aqueous acetone extract (6 liters) was concentrated in vacuo. The resulting aqueous portion (approximately 800 ml) was saturated with NaCl and then extracted with EtOAc (1x300 ml, 2x200 ml, 1x300 ml), dried ($MgSO_4$) and concentrated in vacuo to give 14 g of an oil.

After filtration, the mycelium from the recovered mycelium-resin mixture, was separated from the resin by soaking in 5 liters of water, stirring vigorously and decanting. The dense resin settles faster thus facilitating the removal of the mycelium. To thoroughly remove the mycelium from the resin, this process was repeated twice. The recovered resin was stored for further use after soaking in 3 liters of 1% sodium azide overnight.

To a solution of the above crude oil (14 g) in 150ml

MeOH was added 6 g of NaOH dissolved in 35 ml of water. The solution was allowed to stand at room temperature for 15 hours. The aqueous solution was washed with hexane (3x100 ml), neutralized with 5% HCl (140 ml) and then concentrated by rotary evaporation. To the resulting aqueous mixture was added a saturated solution of Na_2CO_3 (150 ml). The solution was saturated with NaCl and then extracted with EtOAc (6x100 ml). The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness to obtain 3.7 g of an oil which was chromatographed (3 cm x 22 cm) using 70% EtOAc in hexane. The resulting oil obtained was dissolved in ether and hexane was added until the solution became turbid. The solution was allowed to stand overnight at 4°C to allow complete crystallization of verrucarol. Upon filtration this gave 0.75 g of pure crystals (m.p.: 154-155°C) (literature value: 155-156°C) and 0.30 g of impure verrucarol as an oil. Rf (100% EtOAc);0.25.

3.2. 15-Acetoxyverrucarol (56)

To a solution of verrucarol (0.50 g; 0.0019 mol) in 23 ml of CH_2Cl_2 was added pyridine (0.86 ml; 0.011 mol) and acetic anhydride (0.210 ml; 0.0022 mol). The solution was stirred at room temperature for 40 hours at which time more acetic anhydride (0.030 ml; 0.32 mmol) was added. The solution was stirred for a further 24 hours. The reaction mixture was diluted with $CHCl_3$ to 200 ml and washed successively with 1x30 ml 10% HCl, 1x30 ml saturated $NaHCO_3$ solution and 1x30 ml of brine to neutrality. The aqueous layer was extracted with EtOAc (2x100 ml). The organic

layers were dried ($MgSO_4$), filtered and evaporated to dryness. The residue was chromatographed (3 cm x 20 cm) using 65% EtOAc in hexane to elute the desired compound and 100% EtOAc to elute the recovered verrucarol (0.14 g). 15-Acetoxyverrucarol 0.41 g (98%) was obtained as white needle-like crystals (m.p.: 145.5-147°C from ether-hexane). Rf (100% EtOAc);0.44. IR($CHCl_3$), 3600, 3570, 3021, 3000, 2965, 2935, 2900, 2840, 1730, 1670, 1470, 1445, 1365, 1240 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.828 (J = 5.4)] // H-3A [dd, 1H, 2.589 ($J_{A,B}$ = 15.75, $J_{3A,4}$ = 7.47, $J_{3A,2}$ = 0] // H-3B [ddd, 1H, 1.922 ($J_{A,B}$ = 15.74, $J_{3B,4}$ = 3.0, $J_{3B,2}$ = 5.4)] // H-4 [broad, d, 1H, 4.481 ($J_{4,3A}$ = 7.39, $J_{4,3B}$ = 2.75)] // H-10 [dq, 1H, 5.4 J = 5.6, J = 1.4] // H-11 [broad, d, 1H, 3.600 (J = 5.94)] // H-13A [d, 1H, 3.113, J = 3.9] // H-13B [d, 2.803, J = 3.9] // H-14 [s, 3H, 0.870] // H-15A [d, 1H, 4.126, $J_{A,B}$ = 12.23] // H-15B [d, 1H, 3.920, $J_{A,B}$ = 12.33] // H-16 [s, 3H, 1.706] // OAc [s, 3H, 2.036].

3.3. 4-Ketone-15-acetoxyverrucarol.

A solution of 15-acetoxyverrucarol (56) (0.57 g; 0.0018 mol) in 74 ml of CH_2Cl_2 was cooled to 0°C in an ice-water bath. To the chilled solution was added PCC (1.60 g; 0.0074 mol). The solution was stirred at 0°C for 10 minutes, the ice-water bath was removed and the reaction mixture was stirred at room temperature for 2 hours. The mixture was filtered through a column of dry silica gel (3 cm x 15 cm). The product was eluted with 250 ml EtOAc. The volatiles were removed in vacuo and the residue chromatographed (3 cm x 15 cm) using 35% EtOAc in hexane.

This gave 0.42 g (74%) of the 4-ketone-15-acetoxyverrucarol as a dense yellow sirup. Rf (35% EtOAc:Hexane);0.27. IR($CHCl_3$), 3020, 3000, 2965, 2935, 2905, 2850, 1740, 1670, 1470, 1445, 1390, 1370, 1240 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 4.097 ($J_{2,3B}$ = 4.15)] // H-3A [d, 1H, 2.6 ($J_{A,B}$ = 18.3)] // H-3B [dd, 1H, 2.6 ($J_{A,B}$ = 19.2, $J_{3B,2}$ = 4.5)] // H-10 [dq, 1H, 5.402 ($J_{10,11}$ = 4.2, $J_{10,Me}$ = 1.2)] // H-11 [broad, d, 1H, 3.783 ($J_{10,11}$ = 6.8)] // H-13A [d, 1H, 3.243 ($J_{A,B}$ = 3.9)] // H-13B [d, 1H, 2.955 ($J_{A,B}$ = 3.9)] // H-14 [s, 3H, 0.889] // H-15A [d, 1H, 3.979 ($J_{A,B}$ = 12.15)] // H-15B [d, 1H, 3.816 ($J_{A,B}$ = 12.2)] // H-16 [s, 3H, 1.723] // OAc [s, 3H, 2.044].

3.4. 4-^{2}H-15-Acetoxyverrucarol (57)

A solution of 4-ketone-15-acetoxyverucarol (0.42 g; 0.0014 mol) in 46 ml of MeOH was cooled to 0°C in an ice-water bath. To the chilled solution was added NaB^2H_4 (0.17 g; 0.0041 mol) and the reaction mixture was stirred at 0°C for 30 minutes at which time the reaction was complete. The solution was diluted to 200 ml with CH_2Cl_2 and washed to neutrality with 25 ml portions of brine. The organic layer was dried ($MgSO_4$), filtered and evaporated. The crystalline residue was chromatographed (3 cm x 15 cm) using 40% EtOAc in hexane. This gave 0.36 g (85%) of 15-acetoxyverrucarol deuterated at position C-4 as white needle-like crystals (m.p.: 204-206°C from ether/hexane). Rf (50% EtOAc:Hexane);0.34. IR($CHCl_3$), 3605, 3580-3300 broad band, 3020, 3000, 2965, 2935, 2905, 2835, 1715, 1670, 1475, 1440, 1385, 1250 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.656, J = 5.5] // H-3A [dd,

1H, 2.507 ($J_{A,B}$ = 15.2, $J_{3A,2}$ = 5.66] // H-10 [dq, 1H, 5.439 ($J_{10,11}$ = 5.55, $J_{10,Me}$ = 1.5)] // H-11 [broad, d, 1H, 4.188 ($J_{11,10}$ = 5.42)] // H-13A [d, 1H, 3.046, J = 4.0] // H-13B [d, 1H, 2.773, J = 4.0] // H-14 [s, 3H, 0.880] // H-15A [d, 1H, 5,228, $J_{A,B}$ = 11.96] // H-15B [d, 1H, 3.788, $J_{A,B}$ = 11.99] // H-16 [s, 3H, 1.711] // OAc [s, 3H, 2.041]

3.5. 4-^{2}H-4-phenylthionocarbonate-15-Acetoxyverrucarol (58)

A solution of 4-^{2}H-15-acetoxyverrucarol (57) (0.36 g; 0.0012 mol) in 41 ml CH_2Cl_2 containing pyridine (0.44 ml; 0.0055 mol) and 4-DMAP (0.16 g; 0.0013 mol) was treated with phenylchlorothionocarbonate (0.38 g; 0.0027 mol). The reaction mixture was stirred at room temperature for 19 hours. By TLC analysis it was evident, at this time, that some starting material remained. More phenylchlorothionocarbonate (0.38 ml; 0.0027 mol) was added and stirring was resumed for a further 24 hours at room temperature. The reaction mixture was diluted to 200 ml with CH_2Cl_2 and was washed successively with 1x30 ml 10% HCl, 1x30 ml saturated $NaHCO_3$ solution and 1x30 ml brine to pH 7. The organic layer was dried ($MgSO_4$), filtered and evaporated. The residual oil was chromatographed (3 cm x 19 cm) using 20% EtOAc in hexane. This gave 0.46g (89%) of the desired compound as a foam. Rf (25% EtOAc:Hexane);0.23. IR($CHCl_3$), 3020, 3000, 2965, 2935, 2900, 2835, 1725, 1670, 1585, 1485, 1435, 1385, 1370, 1240, 690 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.743, J = 5.36] // H-3A [dd, 1H, 2.796 ($J_{A,B}$ = 15.7, $J_{3A,2}$ = 5.64)] // H-3B [d, 1H, 2.008, $J_{A,B}$ = 15.5] // H-10 [broad, dq, 1H, 5.439 ($J_{10,11}$ = 4.48,

$J_{10,Me}$ = 1.15)] // H-11 [broad, d, 1H, 4.128, $J_{11,10}$ = 4.67] // H-13A [d, 1H, 3.107, $J_{A,B}$ = 3.93] // H-13B [d, 1H, 2.846, $J_{A,B}$ = 3.97] // H-14 [s, 3H, 1.020] // H-15A [d, 1H, 4.808, J = 12.3] // H-15B [d, 1H, 3.883, J = 12.35] // H-16 [broad, s, 3H, 1.707] // OAc [s, 3H, 2.033] // Phenyl protons [multiplet, 5H, 7.2].

3.6. 15-Acetoxy-4$^{2}H_2$-4-deoxyverrucarol (59)

The thionocarbonate (58) (0.46 g; 0.0010 mol) dissolved in 133 ml toluene was treated with AIBN (0.046 g; 0.28 mmol) and the solution was heated to 80°C. At this time, n-$Bu_3Sn^{2}H$ (1.7 ml; 0.0063 mol) freshly prepared, was added to the heated solution rapidly via syringe. The mixture was stirred for 16 hours at 80°C. It was then cooled, evaporated to remove the toluene and the residue was chromatographed (3 cm x 19 cm), eluting first with 500 ml petroleum ether and then with 20% EtOAc in hexane. This gave 0.30 g (100%) of a very viscous colourless oil. Rf(25% EtOAc:Hexane);0,24. IR($CHCl_3$), 3020, 3000, 2955, 2905, 2865, 1725, 1670, 1445, 1375, 1245 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.708, $J_{2,3}$ = 4.9] // H-10 [dq, 1H, 5.410, $J_{10,11}$ = 5.4] // H-11 [d, broad, 1H, 3.699, $J_{10,11}$ = 5.29] // H-13A [d, 1H, 3.153, $J_{A,B}$ = 4.1] // H-13B [d, 1H, 2.873, $J_{A,B}$ = 4.1] // H-14 [s, 3H, 0.820] // H-15A [d, 1H, 4.085, $J_{A,B}$ = 12.2] // H-15B [d, 1H, 3.838, $J_{A,B}$ = 12.1] // H-16 [s, 3H, 1.698] // OAc [s, 3H, 2.036] // Deuterium NMR: ^{2}H-4, 2D, 2.158, 2.621.

3.7. 4-$^{2}H_2$-4-Deoxyverrucarol (60)

Acetate (59) (0.30 g; 0.0010 mol) was dissolved in 35 ml MeOH at room temperature. Anhydrous K_2CO_3 (0.86g; 0.0062 mol)

was added and the solution was stirred at room temperature for 24 hours. The methanol was evaporated and the residue was diluted to 150 ml with $CHCl_3$. The organic layer was washed with 1x20 ml H_2O and 3x20 ml brine to neutrality. The aqueous layer was extracted with 2x75 ml EtOAc. The combined organic layers were dried ($MgSO_4$), filtered and evaporated. The crystalline residue was chromatographed (3 cm x 15 cm) using 35% EtOAc in hexane. This gave 0.21 g (82%) of (60) as white crystals (m.p.: 122-122.5°C from petroleum ether) (literature value: 122-123°C). Rf (35% EtOAc:Hexane);0.26. IR($CHCl_3$), 3620, 3580-3340 broad band, 3020, 3000, 2960, 2935, 2905, 1670, 1445, 1375 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.713, $J_{2,3B}$ = 5.00] // H-3B [dd, 1H, 1.78] // H-10 [dq, 1H, 5.4, $J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.4] // H-11 [d, 1H, 3.673, $J_{10,11}$ = 5.6] // H-13A [d, 1H, 3.166, $J_{A,B}$ = 4.07] // H-13B [d, 1H, 2.895, $J_{A,B}$ = 4.09] // H-14 [s, 3H, 0.926] // H-15A [d, 1H, 3.711, $J_{A,B}$ = 12.2] // H-15B [d, 1H, 3.491, $J_{A,B}$ = 12.6] // H-16 [broad s, 3H, 1.730] // Deuterium NMR: ^{2}H-4: 2D, 2.234, 1.635.

3.8. Oxidation of 4-$^2H_2$-4-deoxyverrucarol

A solution of 4-$^2H_2$-4-deoxyverrucarol (0.21 g; 0.83 mmol) dissolved in 28 ml CH_2Cl_2 was cooled to 0C in an ice-water bath. At this temperature PCC (0.73 g; 0.0034 mol) was added to the solution and the reaction mixture was stirred for 1.5 hours at 0°C then at ambient temperature for 30 minutes. The mixture was filtered through a column (15 mm x 15 cm) of dry silica gel and then eluted with 100 ml EtOAc. The volatiles were removed in vacuo and the crystalline residue was chromatographed

(15 mm x 16 cm) using 25% EtOAc in Hexane. This gave 0.20 g (96%) of white flaky crystals (m.p.: 114-116°C from petroleum ether). Rf (25% EtOAc:Hexane);0.25. IR($CHCl_3$), 3020, 3000, 2960, 2930, 2905, 2860, 2720, 1710, 1670, 1440, 1375 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.746, $J_{2,3}$ = 3.45] // H-10 [dq, 1H, 5.544 ($J_{10,11}$ = 5.43, $J_{10,Me}$ = 1.5)] // H-11 [broad, d, 1H, 4.513, $J_{10,11}$ = 5.58] // H-13A [d, 1H, 3.170, $J_{A,B}$ = 4.02] // H-13B [d, 1H, 2.922, $J_{A,B}$ = 4.02] // H-14 [s, 3H, 0.917] // H-15 [s, 1H, 9.608] // H-16 [broad, s, 3H, 1.673].

3.9. 15-^{2}H-4-$^2H_2$-4-Deoxyverrucarol (61)

To a solution of the above aldehyde (0.20 g; 0.80 mmol) dissolved in 24 ml of MeOH was added at 0°C NaB^2H_4 (0.10 g; 0.0024 mol). The solution was stirred for 15 minutes at 0C. It was then diluted to 150 ml with $CHCl_3$ and washed with 1x20 ml H_2O and 3x20 ml brine to neutrality. The aqueous layer was extracted with 2x75 ml EtOAc. The organic layers were dried ($MgSO_4$), filtered and evaporated. The crystalline residue was chromatographed (15 mm x 15 cm) using 35% EtOAc in hexane to give 0.18 g (89%) of the desired compound, as white crystals (m.p.: 121-122°C from petroleum ether). Rf (35% EtOAc:Hexane);0.26. IR($CHCl_3$), 3620, 3580-3340 broad band, 3020, 3000, 2960, 2935, 2905, 2860, 1670, 1440, 1375, 1235 cm^{-1}. ^{H}-NMR; õ(ppm): H-2 [d, 1H, 3.707, J = 4.98] // H-3B [d, 1H, 1.824, J = 15.0] // H-10 [dq, 1H, 5.436, $J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.4] // H-11 [d, 1H, 3.670, $J_{10,11}$ = 6.9] // H-13A [d, 1H, 3.161, $J_{A,B}$ = 4.09] // H-13B [d, 1H, 2.890, $J_{A,B}$ = 4.07] // H-14 [s, 3H, 0.926] // H-15

[broad, s, 1H, 3.681] // H-16 [broad, s, 3H, 1.715] // Deuterium NMR, [2H-4, 2D, 2.227, 1.628], [^{2}H-15, 1D (two isomers, major: 3.485, minor: 3.701)]

3.10. 15-^{2}H-15-Chloro-4^{2}H$_2$-4-deoxyverrucarol (62)

A solution of alcohol (61) (0.18 g; 0.71 mmol) in 22.5 ml DMF was treated with imidazole (0.22 g; 0.0032 mol) and subsequently cooled to -40 C in an acetone /dry ice bath. Subsequently SO_2Cl_2 (0.115 ml; 1.43 mmol) was added dropwise, via syringe. The solution was stirred at -40°C for 30 minutes, then at room temperature for 30 minutes. More imidazole (0.19 g; 0.0028 mol) was added and the solution once again was cooled to -40°C and more SO_2Cl_2 (0.115 ml; 1.43 mmol) was added. The solution was allowed to come to room temperature and was stirred as such for 15 hours. The reaction mixture was diluted to 100 ml with EtOAc and washed with 2x15 ml brine and 1x15 ml water. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo. The residual sirup obtained was chromatographed (15 mm x 15 cm) using 15% EtOAc in hexane. This gave 0.156 g (81%) of the desired compound as an oil. Rf (20% EtOAc:Hexane);0.25. IR($CHCl_3$), 3000, 2960, 2940, 2910, 2860, 1670, 1445, 1380 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.727, $J_{2,3}$ = 5.0] // H-3A [1H, 2.010] // H3B [d, 1H, 1.841, $J_{A,B}$ = 15.1] // H-10 [dq, 1H, 5.387, $J_{10,11}$ = 6.0, $J_{10,Me}$ = 1.4] // H-11 [broad, d, 1H, 3.644, $J_{10,11}$ = 6.0] // H-13A [d, 1H, 3.165, $J_{A,B}$ = 4.15] // H-13B [d, 1H, 2.906, $J_{A,B}$ = 4.10] // H-14 [s, 3H, 0.986] // H-15 (two isomers, 1H), [minor, s, 3.584] [major, s, 3.407] // H-16 [broad, s, 3H,

1.740] // Deuterium NMR: ^{2}H-4 [2D, 2.104, 1.902], ^{2}H-15 [1D, two isomers, 3.591 major, 3.422 minor].

3.11. $^2H_4$-12,13-Epoxytrichothecene (EPT) (63)

A solution of chloride (62) (0.156 g; 0.574 mmol) and AIBN (0.028 g; 0.17 mmol) in 45 ml toluene was heated at 80°C. At this time the solution was treated with freshly prepared n-Bu_3Sn^2H (0.930 ml; 3.45 mmol) rapidly via syringe. The solution was stirred at 80°C for 15 hours. The reaction mixture was then cooled, the toluene was evaporated in vacuo and the residue was chromatographed (15 mm x 15 cm) using 15% EtOAc in hexane. This gave 0.137 g (100%) of the desired compound as a viscous sirup that slowly crystallized when allowed to stand at 4°C (m.p.: 60-62.5°C from pentane). Rf (20% EtOAc:Hexane);0.32. IR(CHCl3), 3000, 2960, 2920, 2870, 1670, 1440, 1370 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.700, $J_{2,3}$ = 4.9] // H-10 [dq, 1H, 5.400, $J_{10,11}$ = 5.4, $J_{10,Me}$ = 1.4] // H-11 [broad, d, 1H, 3.682] // H-13A [d, 1H, 3.139, $J_{A,B}$ = 4.2] // H-13B [d, 1H, 2.8, $J_{A,B}$ = 4.2] // H-14 [s, 3H, 0.742] // H-15 [very weak broad quintet, 0.777, $J_{H,D}$ = 2.0] // H-16 [s, 3H, 1.690] // Deuterium NMR: ^{2}H-4: [2D, 2.106, 1.554] // H-15 [2D, 0.790, $J_{D,H}$ = 2.0].

4. $^2H_4$-12,13-EPOXYTRICHOTHECENE TO DEUTERATED APOTRICHOOL (FIGURE 26)

4.1. $^2H_4$-Chlorohydrin (64)

$^2H_4$-12,13-epoxytrichothecene (63) (0.22 g; 0.92 mmol) was treated with HCl in MeOH (3.8 ml, conc. HCl:MeOH (1:10)) at

room temperature for one hour. To the reaction mixture was added 15 ml of a saturated NaCl solution and the aqueous solution was extracted with 2x75 ml ether. The combined organic layer was washed to neutrality with 3x15 ml brine, dried ($MgSO_4$), filtered and evaporated to yield a crystalline residue which was chromatographed (15 mm x 14 cm) using 15% EtOAc in hexane. This gave 0.193 g (76%) of the desired chlorohydrin as crystals (m.p.: 66-67°C) (recrystallization was achieved by solubilizing in a minimum amount of pentane then cooling the solution in dry ice). Rf (20% EtOAc:Hexane);0.28. IR($CHCl_3$), 3585, 3005, 2975, 2940, 2880, 1670, 1450, 1385, 1000 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [t, 1H, 4.331, $J_{2,3A} = J_{2,3B} = 4.9$] // H-3A [broad, dd, 1H, overlapping with "OH", 2.093 ($J_{A,B} = 13.6$, $J_{3A,2} = 5.4$)] // H-3B [dd, overlapping with H-8B, 1H, 1.920] // H-10 [d, multiplet, 1H, 5.508] // H-11 [d, broad, 1H, 3.751 ($J_{10,11} = 5.25$)] // H-13A [dd, broad, 1H, 3.950 ($J_{A,B} = 12.0$, $J_{A,OH} = 6.6$)] // H-13B [dd, broad, 1H, 3.828 ($J_{A,B} = 11.82$, $J_{B,OH} = 4.3$)] // H-14 [s, 3H, 1.131] // H-15 [broad, s, 1H, 0.798] // H-16 [broad, s, 3H, 1.715] // Deuterium NMR: ^{2}H-4 [2D, 2.086, 1.690], ^{2}H-15 [2D, 0.807].

4.2. $^2H_4$-Apotrichool (65)

A solution of $^2H_4$-chlorohydrin (0.193 g; 0.702 mmol) and AIBN (0.031 g; 0.19 mmol) in 58 ml toluene was heated to 80°C. At this time n-Bu_3SnH (1.1 ml; 0.0041 mol) was added rapidly via syringe. The solution was stirred at 80°C for three hours, subsequently cooled and then the toluene was evaporated in

vacuo. The residue was chromatographed (15 mm x 14 cm) using 25% EtOAc in hexane. This gave 0.168 g (100%) of the desired compound as a sirop. Rf (25% EtOAc:Hexane);0.22. IR($CHCl_3$), 3600, 3010, 2960, 2935, 2880, 2860, 1670, 1465, 1450, 1385, 1000 cm^{-1}. ^1H-NMR; õ(ppm): H-10 [d, multiplet, 1H, 5.575] // H-11 [broad, d, 1H, 3.807, $J_{10,11}$ = 5.08] // H-13A [dd, 1H, 3.609 ($J_{A,B}$ = 11.06, $J_{A,OH}$ = 4.63)] // H-13B [dd, 1H, 3.427 ($J_{A,B}$ = 11.5, $J_{B,OH}$ = 6.95)] // H-14 [s, 3H, 0.952] // H-15 [s, 1H, 0.747] // H-16 [broad, s, 3H, 1.717] // Deuterium NMR:^2H-4 [2D, 1.848, s, 1.244, s], ^2H-15 [2D, 0.753 $J_{H,D}$ = 2.0].

5. $^2H_4$-12,13-EPOXYTRICHOTHECENE TO DEUTERATED ã and ß-APOTRICHODIOL (FIGURE 27)

5.1. $^2H_4$-2ß-Apotrichodiol (66)

A solution of deuterated EPT (63) (0.53 g; 0.0022 mol) in 11 ml MeOH was treated with 22 ml of a 0.25 M H_2SO_4 solution, at room temperature for 16 hours. The solution was neutralized with a saturated solution of $NaHCO_3$, and extracted with 2x150 ml $CHCl_3$. The organic layer was washed with 1x25 ml brine, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was chromatographed (3 cm x 15 cm) using 75% EtOAc in hexane, to give 0.433 g (76%) of the desired compound as a viscous sirup. Rf (70% EtOAc:Hexane); 0.39. IR($CHCl_3$), 3700-3200 broad band, 3005, 2965, 2940, 2880, 1675, 1450, 1385, 1250, 1045, 1000 cm^{-1}. ^1H-NMR; õ(ppm): H-2 [dd, 1H, 4.182 ($J_{2,3B}$ = 9.76, $J_{2,3A}$ = 6.5)] // H-3A [dd, 1H, 1.987 ($J_{A,B}$ = 12.3, $J_{3A,2}$ = 6.3)] // H-3B [broad, t, 1H,

1.614] // H-10 [dq, 1H, 5.541 ($J_{10,11}$ = 5.03, $J_{10,Me}$ = 1.4)] // H-11 [broad, d, 1H, 3.788 ($J_{10,11}$ = 5.98)] // H-13A,B [broad, s, 2H, 3.811] // H-14 [s, 3H, 0.967] // H-15 [broad, s, 0.742] // H-16 [broad, s, 3H, 1.723] // CH_2OH, 3.384, C-2-OH, 2.7. Deuterium NMR: ^{2}H-4 [2D, 1.766, s, 1.294, s], ^{2}H-15 [2D, 0.745, J = 1.85].

5.2. $^2H_4$-13-Acetoxy-2ß-apotrichodiol (67)

A solution of $^2H_4$-2ß-apotrichodiol (0.433 g; 0.00169 mol) in 20 ml of CH_2Cl_2 was treated with pyridine (0.8 ml; 0.01 mol) and then with acetic anhydride (0.185 ml; 0.00196 mol) at room temperature for 40 hours. At this time more acetic anhydride (0.054 ml; 0.57 mmol) was added and stirring was resumed for an additional 24 hours after which TLC analysis using 50% EtOAc in hexane showed a mixture of the starting material and three additional products. The solution was diluted to 200 ml with $CHCl_3$ and then washed successively with 1x25 ml 10% HCl, 1x25 ml saturated $NaHCO_3$ solution and 1x25 ml brine. The combined aqueous layers were extracted with 1x100 ml EtOAc. The organic layers were combined, dried ($MgSO_4$), filtered and evaporated in vacuo. Flash chromatography (3 cm x 16 cm) using 40% EtOAc in hexane afforded: the diacetate of 2ß-apotrichodiol (68) as a sirup: yield of 0.074 g (15%), Rf (50% EtOAc:Hexane);0.60 / the reverse isomer (69) of the desired product as a sirup: yield of 0.060 g (14%), Rf (50% EtOAc:Hexane);0.41 / the desired crystalline compound (67): yield 0.227 g (54%), Rf (50% EtOAc:Hexane);0.30, (m.p.: 91-93°C from petroleum ether) / and finally the starting material 0.069 g, Rf (50%

EtOAc:Hexane);0.22. IR($CHCl_3$), (67): 3620, 3580-3300 broad band, 3005, 2960, 2935, 2875, 1735, 1675, 1465, 1455, 1385, 1250, 990 cm^{-1}. (68): 3010, 2965, 2940, 2880, 1735, 1675, 1450, 1385, 1375, 1255, 990 cm^{-1}. (69): 3610 broad, 3010, 2970, 2940, 2880, 1735, 1675, 1450, 1375, 1250 cm^{-1}. ^{1}H-NMR; õ(ppm): (67): H-2 [t, 1H, 4.123, $J_{2,3}$ = 5.49] // H-3A [dd, 1H, 1.886 ($J_{A,B}$ = 12.8, $J_{A,2}$= 5.0)] // H-3B [dd, 1H, 1.647 ($J_{A,B}$ = 12.8, $J_{B,2}$ = 6.0)] // H-10 [dq, 1H, 5.549 ($J_{10,11}$ = 5.2, $J_{10,Me}$ = 1.4)] // H-11 [broad, d, 1H, 3.747 ($J_{10,11}$ = 5.32] // H-13A [d, 1H, 4.486, $J_{A,B}$ = 11.7] // H-13B [d, 1H, 4.204, $J_{A,B}$ = 11.7] // H-14 [s, 3H, 1.014] // H-15 [broad, s, 1H, 0.768] // H-16 [broad, s, 3H, 1.712] // C-13-OAc [s, 3H, 2.112] // OH [broad, s, 2.426]. (68): H-2 [t, 1H, 5.159 ($J_{2,3A}$ = $J_{2,3B}$ = 5.15 = 5.13)] // H-3A [dd, 1H, 1.925 ($J_{A,B}$ = 13.2, $J_{A,2}$ = 5.1)] // H-3B [dd, 1H, 1.641 ($J_{A,B}$ = 13.2, $J_{B,2}$ = 5.2)] // H-10 [broad, multiplet, 1H, 5.550] // H-11 [broad, d, 1H, 3.765, $J_{10,11}$ = 5.1] // H-13A [d, 1H, 4.372, $J_{A,B}$ = 11.6] // H-13B [d, 1H, 4.120, $J_{A,B}$ = 11.2] // H-14 [s, 3H, 1.038] // H-15 [broad, s, 1H, 0.785] // H-16 [broad, s, 3H, 1.702] // OAc [two singlets, 2.037, 3H, 1.996, 3H] // Deuterium NMR: ^{2}H-4 [2D, 2.037, 1.443] // ^{2}H-15 [2D, 0.804] // (69) H-2 [dd, 1H, 5.101 ($J_{2,3}$ = 7.8, $J_{2,3}$ = 5.6)] // H-10 [d, multiplet, 1H, 5.532 ($J_{10,11}$ = 5.2, $J_{10,Me}$ = 1.3)] // H-11 [broad, d, 1H, 3.792, J = 5.4] // H-13A [1H, 3.727] // H-13B [1H, 3.710] // H-14 [s, 3H, 1.039] // H-15 [broad, s, 0.765] // H-16 [s, 3H, 1.713] // OAc [s, 3H, 2.071].

5.3. $^{2}H_4$-13-Acetoxy-2-keto-apotrichodiol.

A solution of 13-acetoxy-2ß-apotrichodiol (67) (0.340 g; 0.0011mol) in 45 ml CH_2Cl_2 was cooled to 0°C and was treated with PCC (0.98 g; 0.0045 mol). The reaction mixture was stirred at 0°C for 10 minutes at which time it was warmed to room temperature and stirred for 2.5 hours. The mixture was filtered through a column (3 cm x 15 cm) of dry silica gel, rinsed with CH_2Cl_2 and eluted with 25% EtOAc in hexane. This gave 0.28 g (83%) of the desired compound as a sirup. Rf(25% EtOAc:Hexane); 0.24. IR($CHCl_3$), 3020, 2970, 2940, 2920, 2880, 1750, 1675, 1465, 1450, 1375, 1250, 1045, 990 cm^{-1}. ^{1}H-NMR; ô(ppm): H-3B [d, 1H, 2.399, $J_{A,B}$ = 18.8] // H-10 [d, multiplet, 1H, 5.535, $J_{10,11}$ = 5.1] // H-11 [broad, d, 1H, 3.798, $J_{10,11}$ = 5.3] // H-13A [d, 1H, 4.366, $J_{A,B}$ = 11.3] // H-13B [d, 1H, 4.137, $J_{A,B}$ = 11.2] // H-14 [s, 3H, 1.130] // H-15 [broad, s, 1H, 0.815, $J_{H,D}$ = 1.7] // H-16 [broad, s, 3H, 1.723] // OAc [s, 3H, 2.004] // Deuterium NMR: ^{2}H-4 [2D, singlets, 1.905, 1.587] // ^{2}H-15 [2D, 0.826].

5.4. $^{2}H_4$-13-Acetoxy-2ã-apotrichodiol (70)

A solution of the previously prepared ketone (0.28 g; 0.94 mmol) in 31 ml MeOH was cooled to 0°C, and was treated with $NaBH_4$ (0.11 g; 0.0029 mol) at 0°C for 15 minutes. TLC analysis showed the production of two distinct products. The reaction mixture was diluted with $CHCl_3$ to 200 ml and washed to neutrality with a saturated solution of NaCl. The volatiles were evaporated in vacuo. Flash chromatography (3 cm x 15 cm) with 35% EtOAc in hexane afforded 0.238 g (84%) of the desired product as a sirup.

Rf(35% EtOAc:Hexane); 0.24. The residue was further eluted with 50% EtOAc in hexane to obtain a crystalline compound (0.028 g; 10% yield) which was identified as the reverse isomer (67). IR($CHCl_3$), 3620-3400 broad band, 3010, 2960, 2940, 2880, 1735, 1675, 1450, 1385, 1250, 1045, 990 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [quintet, 1H, 3.963 ($J_{2,3A} = J_{2,OH} = 2.3$, $J_{2,3B} = 4.6$] // H-10 [dq, 1H, 5.55] // H-11 [broad, d, 1H, 3.920, $J_{10,11} = 5.31$] // H-13A [d, 1H, 4.085, $J_{A,B} = 11.7$] // H-13B [d, 1H, 4.027, $J_{A,B} = 11.7$] // H-14 [s, 3H, 0.932] // H-15 [broad, s, 1H, 0.760] // H-16 [broad, s, 3H, 1.728] // OAc [s, 3H, 2.064] // OH [s, 2.865] // Deuterium NMR: ^{2}H-4 [2D, singlets, 2.094, 1.186] // ^{2}H-15 [2D, 0.783].

5.5. $^2H_4$-2â-Apotrichodiol (71)

A solution of monoacetate (70) (0.253 g; 0.848 mmol) in 28 ml dry MeOH was treated with anhydrous K_2CO_3 (0.67 g; 0.0048 mol) at room temperature for 16 hours. The MeOH was evaporated, 30 ml of a saturated NaCl solution was added to the residue and the mixture was extracted with $CHCl_3$ (2x100 ml). The $CHCl_3$ layer was washed with 1x30 ml brine. The volatiles were dried ($MgSO_4$), filtered and evaporated. The residue was chromatographed (15 mm x 16 cm) using 65% EtOAc in Hexane. This gave 0.205 g (94%) of a viscous sirup. Rf(70% EtOAc:Hexane) 0.33; IR($CHCl_3$), 3660-3300 broad band, 3010, 2965, 2940, 2885, 1675, 1450, 1385, 1250, 1000 cm^{-1}.^{1}H-NMR; õ(ppm): H-2 [t, 4.016 ($J_{2,3B} = 7.2$, $J_{2,3A} = J_{2,OH} = 2.3$)] // H-3 [d, 2H, 1.98, 1.713] // H-10 [d, multiplet, 1H, 5.582, $J_{10,11} = 5.2$] // H-11 [broad, d, 1H, 3.930, $J_{10,11} = 5.4$]

// H-13A [dd, 1H, 3.704 ($J_{A,B}$ = 12.2, $J_{A,OH}$ = 4.4)] // H-13B [dd, 1H, 3.398 ($J_{A,B}$ = 11.7, $J_{B,OH}$ = 8.1)] // H-14 [s, 3H, 0.917] // H-15 [broad, s, 1H, 0.768] // H-16 [broad, s, 3H, 1.746] // OH_2 [d, 2.805, $J_{H2,OH2}$ = 2.5] // OH-13 [dd, 1.71] // Deuterium NMR: ^{2}H-4 [two singlets, 2D, 2.085, 1.162] // ^{2}H-15 [2D, 0.782].

6. VERRUCAROL TO TRITIATED EPT, ATO, 2ß-ATD AND 2ã-ATD (FIGURE 29).

6.1. 15-Acetoxyverrucarol-4-thionocarbonate (74)

The preparation of (74) from 15-acetoxyverrucarol (56) was identical to that described for (58) from (57) (Figure 25). The IR spectrum of compound (74) is similar to that of phenylchlorothionocarbonate (58). The yields of compounds (74)-(83) are similar to those of their non-tritiated equivalents. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.743, J = 5.36] // H-3A [dd, 1H, 2.796 ($J_{A,B}$ = 15.7, $J_{A,2A}$ = 5.64)] // H-3B [d, 1H, 2.008 ($J_{A,B}$ = 15.5)] // H-10 [broad, dq, 1H, 5.439 ($J_{10,11}$ = 4.48, $J_{10,Me}$ = 1.15)] // H-11 [broad, d, 1H, 4.128, $J_{11,10}$ = 4.67] // H-13A [d, 1H, 3.107, $J_{A,B}$ = 3.93] // H-13B [d, 1H, 2.846, $J_{A,B}$ = 3.97] // H-14 [s, 3H, 1.020] // H-15A [d, 1H, 4.808, J = 12.30] // H-15B [d, 1H, 3.883, J = 12.35] // H-16 [broad, s, 3H, 1.707] // OAc [s, 3H, 2.033].

6.2. 15-Acetoxy-4-deoxyverrucarol (75a)

The preparation of (75a) from (74) was the same as that described for (59) from (58) except the n-Bu_3Sn^2H was replaced by n-Bu_3SnH. The IR spectrum of (75a) is similar to that of (59).

6.3. 4-Deoxyverrucarol (75b)

The preparation of (75b) from (75a) was the same as that for (60) from (59). The IR spectrum of (75b) is similar to that of (60). ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.713, J = 5.63] // H-4A [ddd, 1H, 2.230 ($J_{A,B}$ = 13.3, J = 10.47, J = 4.13)] // H-4B [ddd, 1H, 1.633 ($J_{A,B}$ = 13.12, J = 12.15, J = 4.94)] // H-10 [dq, 1H, 5.441, ($J_{10,11}$ = 5.46, $J_{10,Me}$ = $J_{10,8}$ = 1.4 = 1.35)] // H-11 [broad, d, 1H, 3.669, J = 5.31)] // H-13A [dd, 1H, 3.166, (J = 4.08, J = 0.54)] // H-13B [dd, 1H, 2.895, J = 4.09, J = 0.61)] // H-14 [s, 3H, 0.927] // H-15A [dd, 1H, 3.715, $J_{A,B}$ = 11.77, J_{OH} = 5.63)] // H-15B [dd, 1H, 3.489, $J_{A,B}$ = 11.76, J_{OH} = 6.09] // H-16 [multiplet, 3H, 1.719].

6.4. 15-^{3}H-4-Deoxyverrucarol (76a)

Alcohol (75b) was oxidized following the procedure for the oxidation of alcohol (60). The aldehyde (0.22 g; 0.89 mmol) was dissolved in 26 ml MeOH and the solution was cooled to 0°C. NaB^3H_4 (100mCi, 15.0 Ci/mmol) in 0.2 ml of ethanol was added and stirring at 0°C was continued for 1 hour. The reduction was completed with the addition of non-tritiated $NaBH_4$ (0.10 g; 0.0026 mol) and stirring for 15 minutes. The solution was diluted to 150 ml with $CHCl_3$, and then washed with 1x20 ml H_2O and 3x20 ml brine to neutrality. The aqueous layer was extracted with 2x75 ml EtOAc. The volatiles were dried ($MgSO_4$), filtered and evaporated. The crystalline residue was used as such for the succeeding reaction.

6.5. 15-^{3}H-15-Chloro-4-Deoxyverrucarol (76b)

The preparation of (76b) from (76a) was the same as that of (62) from (61).

6.6. 15-^{3}H-12,13-Epoxytrichothecene (77)

The preparation of (77) from (76b) was the same as that of (63) from (96) except for the replacement of n-Bu_3Sn^2H by n-Bu_3SnH. Specific Activity: 6.44 x 10^8 dpm/mmol.

6.7. 15-^{3}H-Chlorohydrin (78)

The preparation of (78) from (77) was the same as that of (64) from (63) (Figure 26).

6.8. 15-^{3}H-Apotricho-ol (79)

The preparation of (79) from (78) was the same as that of (65) from (64). Specific Activity: 5.20 x 10^8 dpm/mmol.

6.9. 15-^{3}H-2ß-Apotrichodiol (80)

The preparation of (80) from (77) was identical to that of (66) from (63) (Figure 27). Specific activity: 5.90 x 10^8 dpm/mmol.

6.10. 15-^{3}H-13-Acetoxy-2ß-Apotrichodiol (81)

The preparation of (81) from (80) was identical to that of (67) from (66).

6.11. 15-^{3}H-13-Acetoxy-2ã-Apotrichodiol (82)

Alcohol (81) was oxidized following the procedure used for the oxidation of (67). The 2-ketone obtained was reduced to obtain (82) similarly to the preparation of (70) from the corresponding 2-keto compound.

6.12. 15-^{3}H-2ã-Apotrichodiol (83)

The preparation of (83) from (82) was identical to that of (71) from (70). Specific Activity: 5.79 x 10^{8} dpm/mmol.

7. VERRUCAROL TO DEUTERATED AND TRITIATED TRICHODERMOL (FIGURE 30).

7.1. 15-Acetoxy-4-t-butyldimethylsilyloxyverrucarol (84)

To a solution of 15-acetoxyverrucarol (56) (0.47 g; 0.0015 mol) in 53 ml THF was added imidazole (1.03 g; 0.0151 mol) and t-butyldimethylsilyl chloride (1.15 g; 0.00763 mol). The solution was seen to produce a white precipitate. It was heated at 60°C for 111 hours at which time a considerable amount of the starting material had not reacted. More imidazole (1.03 g; 0.0151 mol) and t-butyldimethylsilylchloride (1.15 g; 0.00763 mol) were added. The solution was stirred for an additional 24 hours after which the reaction was still not complete. Thus the solution was allowed to stir for a further 96 hours to completion. The reaction mixture was diluted in 200 ml ether and the solution was washed successively with 1x25 ml 10% HCl, 1x25 ml saturated $NaHCO_3$ solution and brine to neutrality. The volatiles were dried ($MgSO_4$), filtered and evaporated to dryness. The residue was chromatographed (3 cm x 18 cm) using 15% EtOAc in Hexane to give 0.64 g (100%) of the desired silyl compound as a sirup. Rf (25% EtOAc:Hexane); 0.36. IR($CHCl_3$), 3005, 2950, 2925, 2855, 1730, 1670, 1470, 1460, 1370, 1250, 840 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.761, $J_{2,3}$ = 5.1] // H-3A [dd, 1H, 2.394

($J_{A,B}$ = 14.95, $J_{3A,4}$ = 7.1)] // H-3B [ddd, 1H, 1.933, $J_{A,B}$ = 15.0, $J_{3B,4}$ = 3.3, $J_{3B,2}$ = 5.2)] // H-4 [dd, 4.452, ($J_{4,3}$ = 7.1, $J_{4,3}$ = 3.3)] // H-11 [d, broad, 1H, 3.527, J = 5.9] // H-13A [d, 1H, 3.031, $J_{A,B}$ = 4.16] // H-13B [d, 1H, 2.745, $J_{A,B}$ = 4.16] // H-14 [s, 3H, 0.775] // H-15A [d, 1H, 4.276, $J_{A,B}$ = 12.25] // H-15B [d, 1H, 3.841, $J_{A,B}$ = 12.25] // H-16 [s, broad, 3H, 1.695] // Si-Me_2 [two singlets, 3H, 3H, 0.054, 0.074] // Si-t-butyl [s, 0.889] // OAc [s, 3H, 2.055].

7.2. 4-t-Butyldimethylsilyloxyverrucarol (85)

A solution of silyl (84) (0.64 g; 0.0015 mol) in 48 ml dry MeOH was treated with anhydrous K_2CO_3 (1.19 g; 0.00861 mol) at room temperature for 16 hours, at which time not all of the starting material had been reacted. More K_2CO_3 (0.42 g; 0.0030 mol) was added, and stirring was resumed for a further 3.5 hours after which the reaction was complete. The MeOH was evaporated and the residue was diluted with $CHCl_3$. The mixture was washed successively with 1x25 ml water and 2x25 ml brine. The water layer was extracted with 2x100 ml EtOAc. The organic layers were combined, dried ($MgSO_4$), filtered and evaporated. The residue was chromatographed (3 cm x 15 cm) using 25% EtOAc in hexane to give 0.56 g (97%) of the desired alcohol as a white crystal (m.p.: 152.5-153°C from petroleum ether). Rf (25% EtOAc:Hexane); 0.23. IR($CHCl_3$), 3620, 3000, 2950, 2925, 2855, 1670, 1470, 1460, 1260, 840 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.755, $J_{2,3}$ = 5.23] // H-3A [dd, 1H, 2.376 ($J_{A,B}$ = 14.85, $J_{3A,4}$ = 7.2)] // H-3B [ddd, 1H, 1.917 ($J_{A,B}$ = 14.9, $J_{3B,4}$ = 3.3, $J_{3B,2}$ = 5.2)] // H-4 [dd, 1H,

4.598, ($J_{4,3}$ = 7.2, $J_{4,3}$ = 3.25)] // H-10 [dq, 1H, 5.423, $J_{10,11}$ = 5.5, $J_{10,Me}$ = 1.5] // H-11 [broad, d, 1H, 3.560, $J_{10,11}$ = 5.87] // H-13A [d, 1H,. 3.028, $J_{A,B}$ = 4.16] // H-13B [d, 1H, 2.748, $J_{A,B}$ = 4.21] // H-14 [s, 3H, 0.858] // H-15A [dd, 1H, 3.744 ($J_{A,B}$ = 12.0, $J_{15A,OH}$ = 4.3] // H-15B [dd, 1H, 3.530 ($J_{A,B}$ = 12.0, $J_{15B,OH}$ = 6.2)] // H-16 [broad, s, 3H, 0.713] // Si-t-butyl [s, 9H, 0.896] // Si-Me_2 [s, 6H, 0.082].

7.3. Oxidation of 4-t-butyldimethylsilyloxyverrucarol (86)

Alcohol (85) (0.56 g; 0.0015 mol) was dissolved in 49 ml CH_2Cl_2. The solution was cooled to 0°C and was treated with PCC (1.27 g; 0.00589 mol) at 0°C for 10 minutes. At this time the solution was allowed to warm to room temperature and was stirred for 1 hour 20 minutes at which time the reaction was complete. The mixture was filtered through a dry silica gel column (3 cm x 13 cm), rinsed with CH_2Cl_2 and eluted with EtOAc. Evaporation of the solvent to dryness was followed by flash chromatography (3 cm x 15 cm) using 15% EtOAc in hexane. This gave pure aldehyde with a yield of 0.47 g (84%) as white needle-like crystals (m.p.: 87-89°C from petroleum ether). Rf (20% EtOAc:Hexane); 0.30. IR($CHCl_3$), 3000, 2950, 2925, 2850, 1715, 1470, 1460, 1260, 840 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.792, $J_{2,3}$ = 5.25] // H-3A [dd, 1H, 2.525 ($J_{3A,4}$ = 7.16, $J_{A,B}$ = 15.1)] // H-3B [ddd, 1H, 1.944 ($J_{3B,2}$ = 5.3, $J_{3B,4}$ = 3.3, $J_{A,B}$ = 15.1)] // H-4 [dd, 1H, 4.380 ($J_{3A,4}$ = 7.15, $J_{3B,4}$ = 3.3)] // H-10 [broad, dq, 1H, 5.498, $J_{10,11}$ = 5.5] // H-11 [broad, d, 1H, 4.254, $J_{10,11}$ = 5.46] // H-13A [d, 1H, 3.050, $J_{A,B}$ = 4.08] // H-13B [d, 1H, 2.803, $J_{A,B}$ =

3.9] // H-14 [s, 3H, 0.902] // H-15-CHO [s, 1H, 9.647] // H-16 [s, 3H, 1.665] // Si-Me_2 [s, 3H, 3H, 0.046, 0.008] // Si-t-butyl [s, 9H, 0.868].

7.4. 15-^{2}H-4-t-Butyldimethylsilyloxyverrucarol (87a)

To a solution of aldehyde (86) (0.47 g; 0.0012 mol) in 37 ml MeOH at 0°C was added NaB^2H_4 (0.16 g; 0.0038 mol). The solution was stirred for 15 minutes at 0°C. The reaction mixture was diluted to 200 ml with $CHCl_3$, and washed with 1x25 ml water and 2x25 ml brine. The aqueous layer was extracted with 2x100 ml EtOAc. The organic layers were combined, dried ($MgSO_4$), filtered and evaporated. The residue was chromatographed (3 cm x 15 cm) using 25% EtOAc in hexane. This gave the desired alcohol as white needle-like crystals with a yield of 0.41 g (87%) (m.p.: 151.5-152°C from petroleum ether). Rf (25% EtOAc:Hexane); 0.23. IR($CHCl_3$), 3620, 3000, 2950, 2925, 2850, 1670, 1470, 1460, 1260, 840 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.762, $J_{2,3}$ = 5.2] // H-3A [dd, 1H, 2.378 ($J_{A,B}$ = 14.9, $J_{3A,4}$ = 7.14)] // H-3B [ddd, 1H, 1.923 ($J_{A,B}$ = 14.9, $J_{3B,2}$ = 5.3, $J_{3B,4}$ = 3.3)] // H-4 [dd, 1H, 4.596, $J_{3,4}$ = 3.3, $J_{3,4}$ = 7.2] // H-10 [broad, d, 1H, 5.428, $J_{10,11}$ = 6.4] // H-11 [broad, d, 1H, 3.565, $J_{10,11}$ = 5.4] // H-13A [d, 1H, 3.037, $J_{A,B}$ = 4.24] // H-13B [d, 1H, 2.756, $J_{A,B}$ = 4.24] // H-14 [s, 3H, 0.862] // H-15A [d, 1H, major, 3.725, $J_{15,OH}$ = 4.0] // H-16 [broad, s, 3H, 1.720] // Si-t-butyl [s, 9H, 0.901] // Si-Me_2 [s, 3H, 3H, 0.051, 0.086] // OH [1.217] // Deuterium NMR: H-15A [1D, 3.711, small] // H-15B [1D, 3.518, large].

7.5. 15-^{3}H-4-t-Butyldimethylsilyloxyverrucarol (87b)

Aldehyde (86) (0.081 g; 0.21 mmol) was dissolved in 6.5 ml MeOH. The solution was cooled to 0C and subsequently $NaB^{3}H_4$ (65mCi in 130 l of ethanol) was added. At this temperature the solution was allowed to stir for one hour. At this time the reduction was completed by the addition of $NaBH_4$ (0.024 g; 0.63 mmol) and stirring for 15 minutes. The reaction mixture was diluted with 100 ml $CHCl_3$, and washed with brine to neutrality. The residue was chromatographed (15 mm x 13 cm) with 25% EtOAc in hexane to yield 0.067 g (82%) of the desired tritiated alcohol. Rf (25% EtOAc:Hexane); 0.23.

7.6. 15-^{2}H-15-Chloro-4-t-butyldimethylsilyloxyverrucarol (88a)

The deuterated alcohol (87a) (0,41 g; 0.0011 mol) was dissolved in 34 ml DMF and was treated with imidazole (0.33 g; 0.0048 mol) and cooled to -40°C. At this temperature SO_2Cl_2 (0.173 ml; 0.00215 mol) was added dropwise via syringe. The mixture was stirred at -40°C for 30 minutes then at room temperature for 30 minutes. More imidazole (0.29 g; 0.0043 mol) was added. The solution was cooled to -40°C and more SO_2Cl_2 (0.173 ml; 0.00215 ml) was added. After the addition, the solution was warmed to room temperature and was stirred for 16 hours. The reaction mixture was then diluted with EtOAc (200 ml) and washed with 3x25 ml brine. The volatiles were dried ($MgSO_4$), filtered and evaporated to dryness. The residue was chromatographed (3 cm x 15 cm) using 15% EtOAc in hexane to yield a sirup of 0.42 g (98%). Rf (20% EtOAc:Hexane); 0.34. IR($CHCl_3$),

3000, 2950, 2925, 2850, 1670, 1470, 1460, 840 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.778, $J_{2,3B}$ = 5.2] // H-3A [dd, 1H, 2.393 ($J_{3A,2}$ = 0, $J_{3A,4}$ = 7.25)] // H-3B [overlap, 1H, 2.00] // H-4 [dd, 1H, 4.444 ($J_{3A,4}$ = 7.2, $J_{3B,4}$ = 3.3] // H-10 [broad, d, 1H, 5.366, $J_{10,11}$ = 5.2] // H-11 [broad, d, 1H, 3.490, $J_{10,11}$ = 5.2] // H-13A [d, 1H, 3.039, $J_{A,B}$ = 4.2] // H-13B [d, 1H, 2.767, $J_{A,B}$ = 4.2] // H-14 [s, 3H, 0.925] // H-15B [s, 1H, 3.431] // H-16 [s, 3H, 1.73] // Si-t-butyl [s, 9H, 0.905] // Si-Me_2 [s, 3H, 3H, 0.098, 0.095] // Deuterium NMR: ^{2}H-15 [1D, 3.529].

7.7. 15-^{3}H-15-Chloro-4-t-butyldimethylsilyloxyverrucarol (88b)

The preparation of (88b) from (87b) was identical to that of (88a).

7.8. 15-$^{2}H_2$-t-Butyldimethylsilyloxytrichodermol (89a)

Chloride (88a) (0.42 g; 0.0010 mol) was dissolved in 95 ml toluene. The addition of AIBN (0.049 g; 0.30 mmol) was followed by heating the reaction mixture to 80°C. At this time n-$Bu_3Sn^{2}H$ was added rapidly via syringe and the solution was stirred at 80°C for 4 hours. The solution was then cooled, the toluene was evaporated and the residue was chromatographed (3 cm x 15 cm) using 10% EtOAc in hexane. This yielded 0.39 g (100%) of a viscous sirup. Rf (15% EtOAc:Hexane); 0.20. IR($CHCl_3$), 3000, 2955, 2930, 2860, 1670, 1470, 1460, 1260, 840 cm^{-1}. ^{1}H-NMR; δ(ppm): H-2 [d, 1H, 3.770, $J_{3,2}$ = 5.24] // H-3A [dd, 1H, 2.413 ($J_{A,B}$ = 14.89, $J_{3A,4}$ = 7.2] // H-3B [ddd, 1H, 1.924, ($J_{A,B}$ = 14.9, $J_{3B,4}$ = 3.4, $J_{3B,2}$ = 5.3] // H-4 [dd, 1H, 4.377 ($J_{4,3A}$ = 7.2, $J_{4,3}$ = 3.3] // H-10 [broad, d, 1H, 5.387, J = 5.4] // H-11

[broad, d, 1H, 3.497, J = 5.3] // H-13A [d, 1H, 3.033, $J_{A,B}$ = 4.15] // H-13B [d, 1H, 2.759, $J_{,B}$ = 4.15] // H-14 [s, 3H, 0.719] // H-16 [broad, s, 3H, 1.701] //Si-Me_2 [two singlets, 3H, 3H, 0.074, 0.089] // Si-t-butyl [s, 9H, 0.902] // Deuterium NMR: ^{2}H-15 [2D, decoupled 0.802, coupled, $J_{H,D}$ = 1.52].

7.9. 15-^{3}H-t-Butylsilyloxytrichodermol (89b)

The preparation of (89b) from (88b) was similar to that of (89a) except that n-Bu_3Sn^2H was replaced by n-Bu_3SnH.

7.10 . 15-$^2H_2$-Trichodermol (90)

The silyl (89a) (0.39 g; 0.0011 mol) was dissolved in 35 ml THF and treated with a 1.0 M solution of tetra-n-butylammonium fluoride in THF (4.0 ml; 0.0040 mol). The reaction mixture was heated to 60°C and stirred for 1 hour. The solution was diluted in EtOAc (200 ml) and washed with 3x25 ml brine. The organic layer was dried ($MgSO_4$), filtered and evaporated. The residue was chromatographed (3 cm x 15 cm) using 60% EtOAc in hexane. This gave 0.22 g (82%) of deuterated trichodermol crystals (m.p.: 112-114°C from petroleum ether). Rf (70% EtOAc:Hexane); 0.34. IR($CHCl_3$), 3600, 3580, 3000, 2955, 2940, 2870, 1670, 1445, 1380, 1065, 960 cm^{-1}. ^{1}H-NMR; õ(ppm): H-2 [d, 1H, 3.827, $J_{2,3B}$ = 5.37] // H-3A [dd, 1H, 2.613 ($J_{A,B}$ = 15.6, $J_{3A,4}$ = 7.45] // H-3B [ddd, overlapping, 1H, 1.908] // H-4 [ddd, 1H, 4.329, ($J_{3A,4}$ = 7.5, $J_{3B,4}$ = 3.0, $J_{4,OH}$ = 10.0)] // H-10 [broad, dq, 1H, 5.390, $J_{10,11}$ = 5.6, $J_{10,Me}$ = 1.4)] // H-11 [broad, d, 1H, 3.505, $J_{10,11}$ = 5.5] // H-13A [d, 1H, 3.107, $J_{A,B}$ = 3.9] // H-13B [d, 1H, 2.811, $J_{A,}$ = 3.9] // H-14 [s, 3H, 0.800]

// H-15 [very small] // H-16 [s, 3H, 1.706] // Deuterium NMR: ^{2}H-15 [2D, 0.828, coupled D, $J_{H,D}$ = 1.9].

7.11. 15-^{3}H-Trichodermol (91)

The preparation of (91) from (89b) was identical to that of (90). Specific Activity: 8.88 x 10^{5} dpm/mmol.

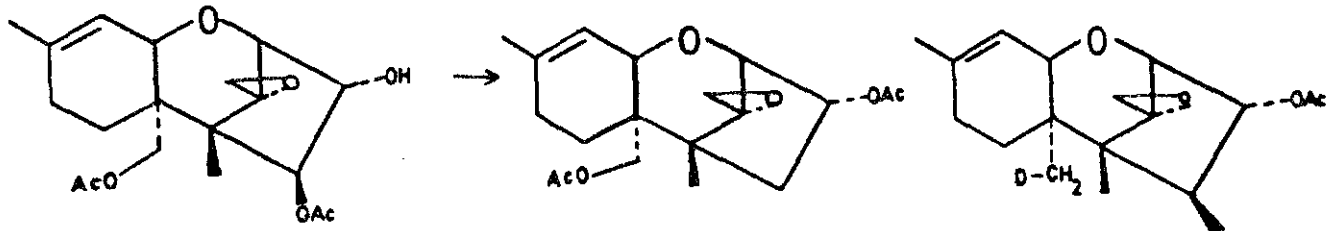
RESULTS

1. ANGUIDINE TO ISOTRICHODERMIN.

During the course of our laboratory's study of the biosynthesis of the mycotoxins sambucinol and 3-acetyl-4-deoxynivalenol, the need arose for the synthesis of a minor metabolite isotrichodermin (ITD). Furthermore, we were in need of the aforementioned compound labeled with deuterium, the stable isotope of hydrogen. The partial synthesis of ITD (54), from anguidine, is reported below.

Close examination of $^{2}H_{2}$-ITD (54) leads to the obvious conclusion that a particular starting material must be chosen such as to provide the appropriate functional group and accompanying stereochemistry, at position C-3. Referring to Figure 22, both anguidine (7) and calonectrin (8) have the appropriate oxygen functionality at C-3. Owing to the greater availability of anguidine (thanks to the generous supply from Dr. T.W. Doyle of Bristol Laboritories) it was deemed more efficient to use anguidine (7) as the basis of the synthesis. Comparing $^{2}H_{2}$-ITD to anguidine , it is apparent that it is necessary to remove both oxygen functionalities, at positions 4 and 15, from anguidine with the incorporation of two deuterium atoms. The first crucial step is the removal of the oxygen functionality from position C-4, upon which a compound similar to calonectrin is obtained. The total synthesis of calonectrin has been reported (Kraus et al., 1982) but it is a laborious process (minimum of 25 steps). Its partial synthesis from anguidine is also known

(Jeker et al., 1984) and is used here to synthesize deuterated 3-O-THP-3-deacetylcalonectrin (51) which is in turn transformed to the desired compound $^2H_2$-ITD.



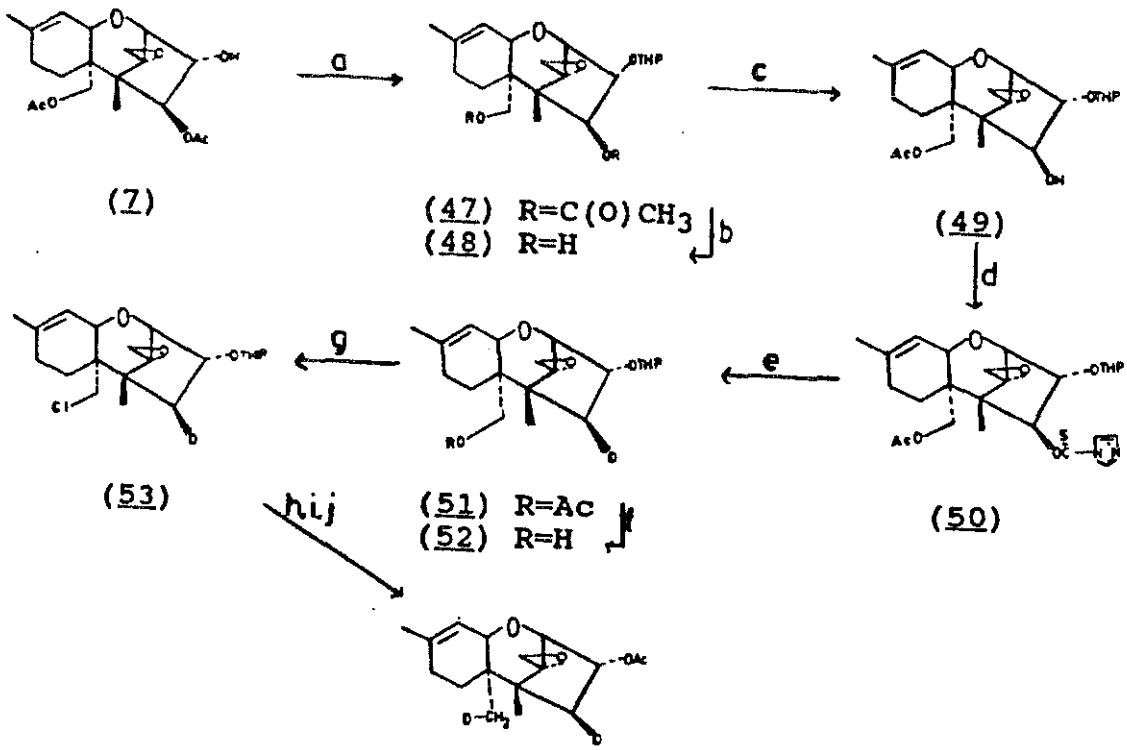
(7) (8) (54)

Figure 22

The oxygen functionality at C-3 of anguidine must be protected against further reactions at positions C-4 and C-15. This was accomplished by the reaction of anguidine with dihydropyran and a trace of the mild acid catalyst PPTS (pyridinium para-toluenesulfonate) (Kaneko et al., 1982; Miyashita et al., 1977). The tetrahydropyranyl diastereomers (47) (Figure 23), were obtained in 100% yield. Separation of these diastereomers was not attempted since it was not deemed necessary for the scope of the synthesis. Furthermore the Rf's of these diastereomers under the conditions used are the same thus this mixture does not interfere with the rest of the synthesis.

In order to accomplish the removal of the oxygen at C-4, diastereomers (47) had to be selectively hydrolyzed. Upon hydrolysis with 1 M NH_4OH-MeOH (Sigg et al., 1965), a mixture of compounds were obtained, thus it was deemed more efficient to completely hydrolyze both acetates as was done by Jeker, Mohr and

Tamm (1984), and to follow the procedure applied to verrucarol, by Tulshian and Fraser-Reid (1980), in order to selectively acetylate the C-15 alcohol. Hydrolysis was achieved by anhydrous K_2CO_3 in MeOH (Schuda et al., 1984) to obtain diastereomers (48) in 100% yield. Subsequent selective acetylation of (48) using acetic anhydride/ pyridine in dichloromethane, gave the mono-acetate diastereomers (49) in 90% yield and some recovered starting material (48).



(a)PPTS,DHP,CH_2Cl_2; (b)K_2CO_3,MeOH; (c)Ac_2O,pyr,CH_2Cl_2; (d)N,N-thiocarbonyldiimidazole,CH_2Cl_2; (e)n-Bu_3SnD,AIBN,toluene; (f)K_2CO_3,MeOH; (g)SO_2Cl_2,imidazole,DMF (-40-25C); (h)n-Bu_3SnD,AIBN,toluene; (i)PPTS,MeOH; (j)Ac_2O,pyr.

Figure 23

The deoxygenation at C-4 was accomplished by the Barton-deoxygenation reaction (Barton and McCombie, 1975). Reaction of diastereomers (49) with N,N-thiocarbonyldiimidazole in dichloromethane yielded the thiocarbonyl-imidazole derivative in 92% yield. Subsequent reduction with tri-n-butyltin deuteride (Kuivila and Beumel, 1961) in toluene treated with the radical initiator AIBN gave diastereomers (51) in 84% yield. The tin deuteride is extremely unstable and must be used immediately following its preparation. Because of the use of tri-n-butyltindeuteride and not its hydride analog an interesting result was observed. From the NOED technique (nuclear overhauser effect difference spectra) the preirradiation of the methyl group (C-15) yielded an increase in intensity on the C-4ã proton. Thus the deuterium atom is seen to occupy the ß position and not the ã at C-4. Thus the stannane reduction of the thiocarbonyl-imidazole occurs with a retention of configuration. Due to the radical nature of the reaction it is usually not stereoselective, but because the ã face of radical C-4 is more sterically hindered than the ß face, the incoming deuterium radical favors the ß face of position C-4 rather than the more hindered ã face. This result is in accord with the mechanism of such reductions as studied by Conway and Nagel (Patroni and Stick, 1979; Fuller and Stick, 1980). The final delivery of the deuterium atom to the intermediate radical occurs mainly from the less hindered face of the system. Basic hydrolysis of (51), which closely resembles calonectrin (8) [the 3-O-acetate group is replaced by the 3-O-THP

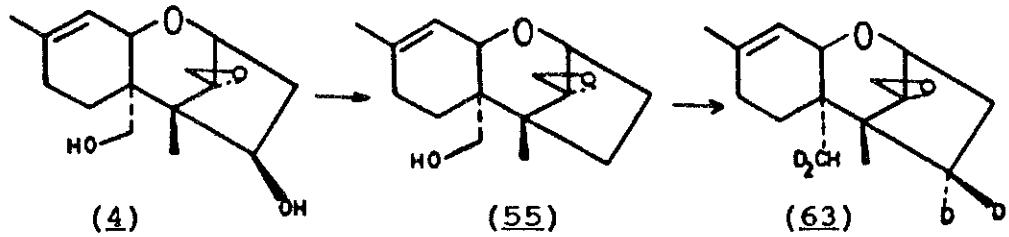
group], in K_2CO_3/MeOH yielded (52) in 82% yield. The chlorination of this alcohol was a crucial step. Since the Barton oxygenation is only applicable to secondary alcohols a different method had to be applied for the deoxygenation of this primary alcohol of C-15. In the literature, Tulshian and Fraser-Reid (1980) reacted the primary 15-OH group of verrucarol with methanesulfonyl chloride in DMF at 70°C to obtain the appropriate chloride which they then reduced with tri-n-butyltin hydride. Deciding to apply this procedure to diastereomers (52) gave one major compound but NMR analysis showed that it was not the expected product. On the contrary, under these conditions, the epoxide was hydrolyzed and an aldehyde was formed. Thus a different method had to be used, one not reported before in the literature.

Reacting diastereomers (52) with sulfuryl chloride in anhydrous pyridine at 0°C (Arita et al., 1972), gave the desired compound in 52% yield. Unfortunately, this reaction was not very reproducible and thus a more efficient method needed to be used. Thus diastereomers (52) were reacted with SO_2Cl_2 in the presence of the base imidazole in DMF at -40°C (Hanessian and Vatele, 1981). This was a two step reaction as followed by TLC. The alcohol first reacted with the SO_2Cl_2 to give a chlorosulfate ester which was not isolated because it quickly reacted with the available chloride ions in the solution, to give the desired alkyl halide. The yield of this reaction was 83%. The chloride (53) was then reduced with tri-n-butyltin deuteride in toluene

(AIBN), in 100% yield, in order to incorporate the second and final label into the compound. Cleavage of the THP ether using the acid catalyst PPTS in MeOH (Jeker et al., 1984; Miyashita, et al., 1977) gave isotrichodermol in 78% yield. Acetylation with acetic anhydride/pyridine then gave the desired final compound $^{2}H_{2}$-ITD (54) in 93% yield.

2. VERRUCAROL TO 12,13-EPOXYTRICHOTHECENE.

In order to more efficiently elucidate the biosynthesis of the mycotoxins sambucinol and 3-acetyldeoxynivalenol, the synthesis of deuterium labeled 12,13-EPT (63) was very important. From a close examination of figure 24, it is evident that 12,13-EPT (63) can be synthesized from 4-deoxyverrucarol (4-DOV) (55) by deoxygenation at position C-15. This latter compound was in turn synthesized by Schuda et al (1984) from verrucarol. Thus the synthesis of EPT from the starting material, verrucarol, up to 4-DOV (55) will be similar to that known in the literature except for a few changes to accomodate the incorporation of deuterium. Verrucarol (Jarvis et al., 1984)[5] was obtained by the basic hydrolysis of crude mixtures of roridins and verrucarins produced by a Fungi Imperfecti, Myrothecium verrucaria, followed by flash chromatography (Still et al., 1978).



(4) (55) (63)

Figure 24

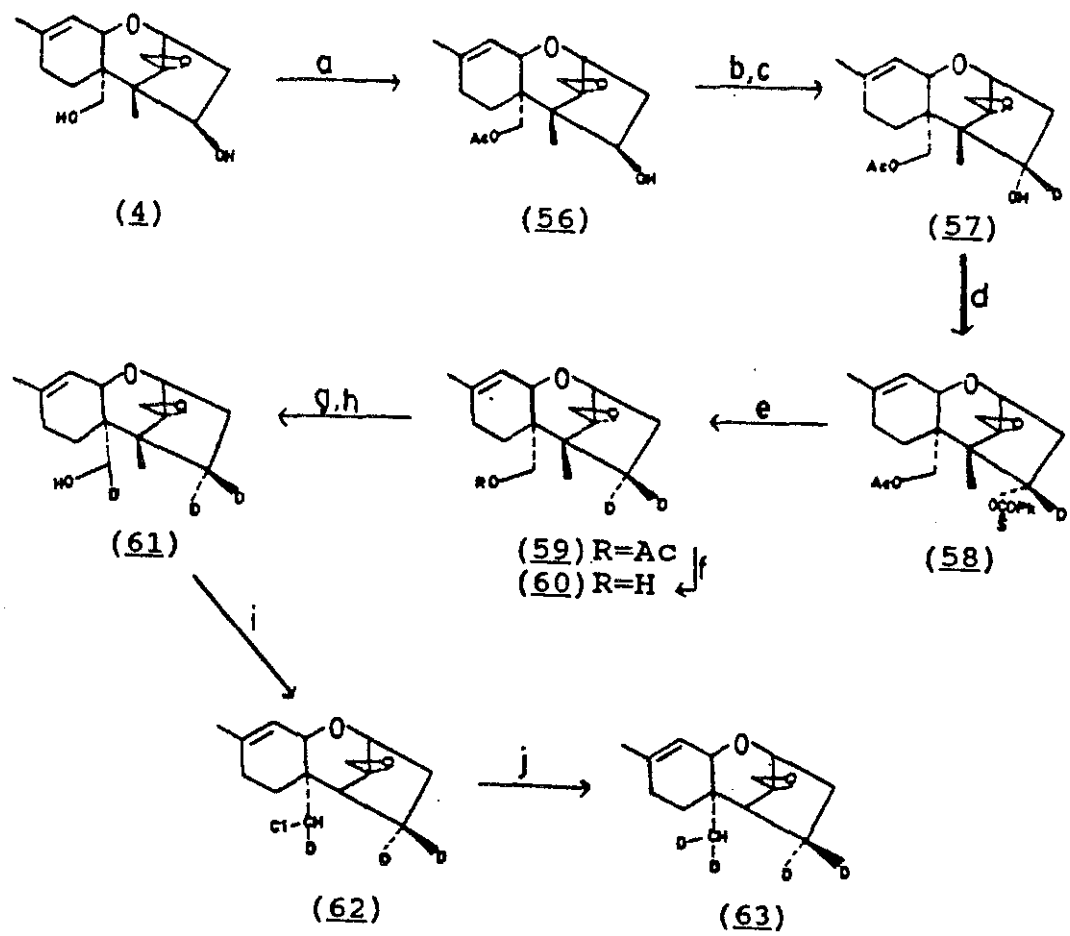
Applying Fraser-Reid's selective acylation of the C-15 primary alcohol of verrucarol, verrucarol (4) (see Figure 25) was treated with acetic anhydride/pyridine in CH_2Cl_2 to yield (56) in 98% and some recovered verrucarol. The first deuterium label was introduced at position C-4 by oxidation of (56) with PCC (Corey and Suggs, 1975; Müller and Tamm, 1975) in dichloromethane to yield the ketone in 74% yield and subsequent reduction with NaB^2H_4 in MeOH at 0°C to yield the alcohol (57) in 85% yield. Reductions of ketones with $NaBH_4$ or NaB^2H_4 are frequently stereoselective. Compound (57) was obtained as the major isomer with traces of the initial alcohol (56). The two isomers differ considerably in their physical properties and their NMR spectra. The melting point of (56) is 145.5-147°C and that of (57) is 204-206°C. By TLC analysis (57) is less polar than (56), this is probably due to the greater amount of hydrogen bonding within the molecule between the C-4α-OH and C-15 acetate in (57). In (56) the C-4β-OH is not as close to the C-15 acetate thus being freer and consequently giving a larger polarity to the molecule. The stereoselectivity of the reduction reaction can be

attributed to the greater steric hindrance of the ã face of the planar C-4 ketone produced. The incoming deuterium nucleophile will attack the planar C-4 ketone from the less sterically hindered ß face.

Alcohol (57) was deoxygenated using a variation of the Barton deoxygenation (Barton and McCombie, 1975) procedure. Treatment of (57) with phenylchlorothionocarbonate (Schuda et al., 1984), [easily prepared (88%) by the reaction of sodium phenoxide with thiophosgene in aqueous chloroform (Miyazaki, 1957) (two-phase)], pyridine/4(dimethylamino)pyridine in dichloromethane according to the Robins variation (Robins and Wilson, 1981) of the standard procedure, gave the thiocarbonate (58) in 89% yield. This was reduced with tri-n-butyltindeuteride in toluene (AIBN) in order to complete the deoxygenation procedure and concurrently to introduce the second label of deuterium into the molecule. 15-Acetoxy-4-$^{2}H_{2}$-4-deoxyverrucarol (59) was obtained in 100% yield. Hydrolysis of the C-15 acetate with K_2CO_3/MeOH afforded 4-$^{2}H_{2}$-4-deoxyverrucarol (60) in 82% yield. The third label of deuterium was introduced by the oxidation of the C-15-OH of (60) with PCC in CH_2Cl_2 (Corey and Suggs, 1975; Müller and Tamm, 1975) to give the aldehyde in 96% yield, and subsequent reduction with $NaB^{2}H_4$ in MeOH at 0°C to afford deuterated alcohol (61) in 89% yield.

Alcohol (61) was chlorinated following the SO_2Cl_2-imidazole procedure (1:2 ratio) (Hanessian and Vatele, 1981). Alcohol (61) was treated with sulfuryl chloride and imidazole in

DMF at -40°C. This was a two step reaction. First the alcohol reacted with the sulfuryl chloride to form a chlorosulfonate ester. This then quickly reacted with a chloride ion in the solution to provide the desired alkyl halide (62) in 81% yield. Chloride (62) was reduced with tri-n-butyltin deuteride in toluene (AIBN) to afford $^2H_4$-EPT in 100% yield.

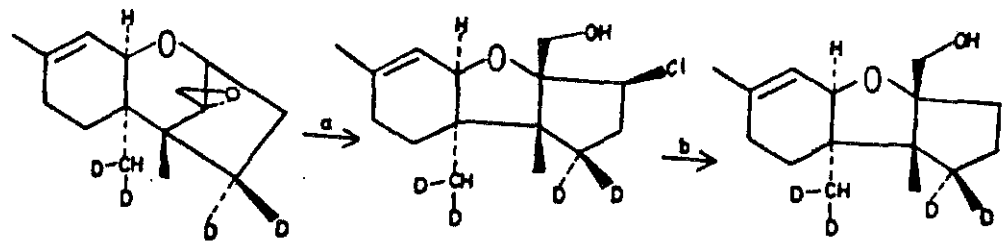


(a) Ac_2O/pyr, CH_2Cl_2; (b) PCC, CH_2Cl_2; (c) $NaBD_4$, MeOH, 0C; (d) PhOC(S)Cl, pyr, 4-DMAP, CH_2Cl_2; (e) n-Bu_3SnD, AIBN, toluene; (f) K_2CO_3, MeOH; (g) PCC, CH_2Cl_2; (h) $NaBD_4$, MeOH, 0C; (i) SO_2Cl_2, imidazole, DMF(-40-25C); (j) n-Bu_3SnD, AIBN, toluene.

Figure 25

3. 12,13-EPOXYTRICHOTHECENE TO APOTRICHOOL

When the trichothecene $^{2}H_4$-EPT (63) (Figure 26) is exposed to strong acidic conditions, intramolecular rearrangement of the carbon skeleton occurs. Treating EPT (63) with concentrated HCl in MeOH (1:10 solution), at room temperature, resulted in its rearrangement to the chlorohydrin (64) in 76% yield (Machida and Nozoe, 1972a). Comparing this chlorohydrin (64) to the desired final product (65), it is apparent that reduction of the chloride moiety will provide the apotricho-ol (ATO) (65). Reduction was achieved with tri-n-butyltinhydride in toluene (AIBN) at 80°C with 100% efficiency. In both the chlorohydrin and the apotrichool the junction between rings A and B is cis and not trans as in the ATO obtained from natural sources.



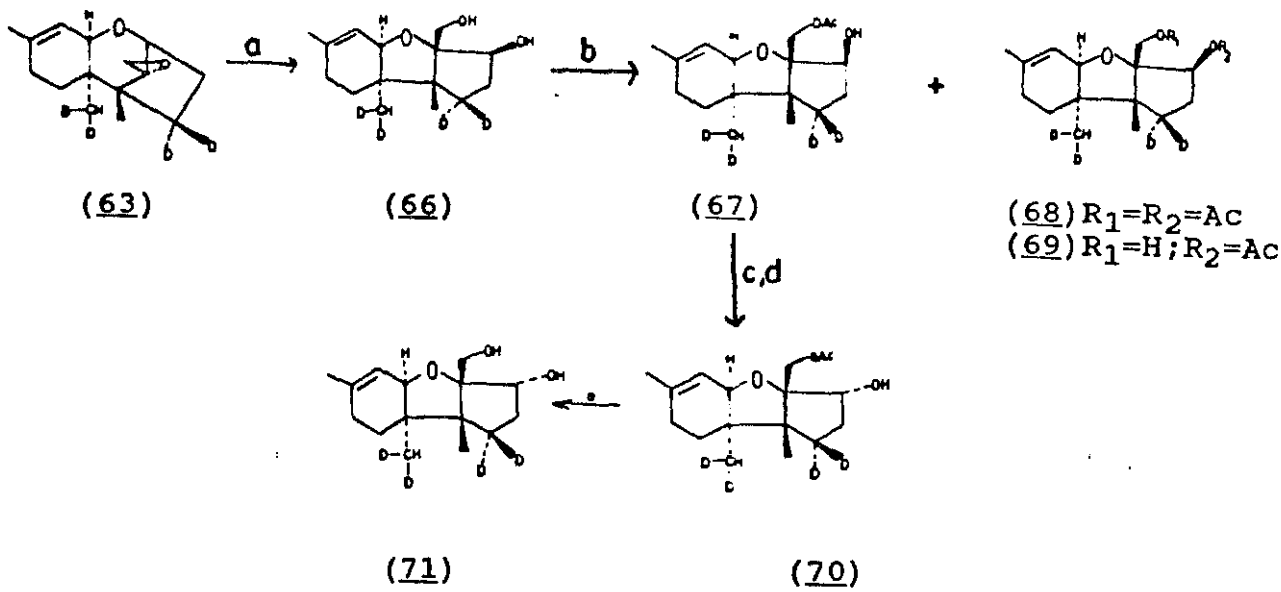
(63) (64) (65)

(a) concentrated HCl:MeOH (1:10); (b) n-Bu_3SnH, AIBN, toluene, 80C

Figure 26

4. 12,13-EPOXYTRICHOTHECENE TO 2ß-APOTRICHODIOL TO 2ã-APOTRICHODIOL

When EPT (63) was exposed to a 0.25 M H_2SO_4 solution in MeOH (2:1) at room temperature (Grove, 1988), the appropriate rearranged product was obtained -- 2ß-apotrichodiol (2ß-ATD) (66), (Figure 27), in 76% yield. Hydrolysis of EPT using a mixture of trifluoroacetic acid in H_2O (1:1) (Godtfredsen and Vangedal; 1965) also provides the desired product but in a smaller yield (50%) since the reaction is not as clean and more side products are generated.



(a) H_2SO_4 (0.25M), MeOH (2:1); (b) Ac_2O, pyr, CH_2Cl_2; (c) PCC, CH_2Cl_2; (d) $NaBH_4$, MeOH, 0C; (e) K_2CO_3, MeOH.

Figure 27

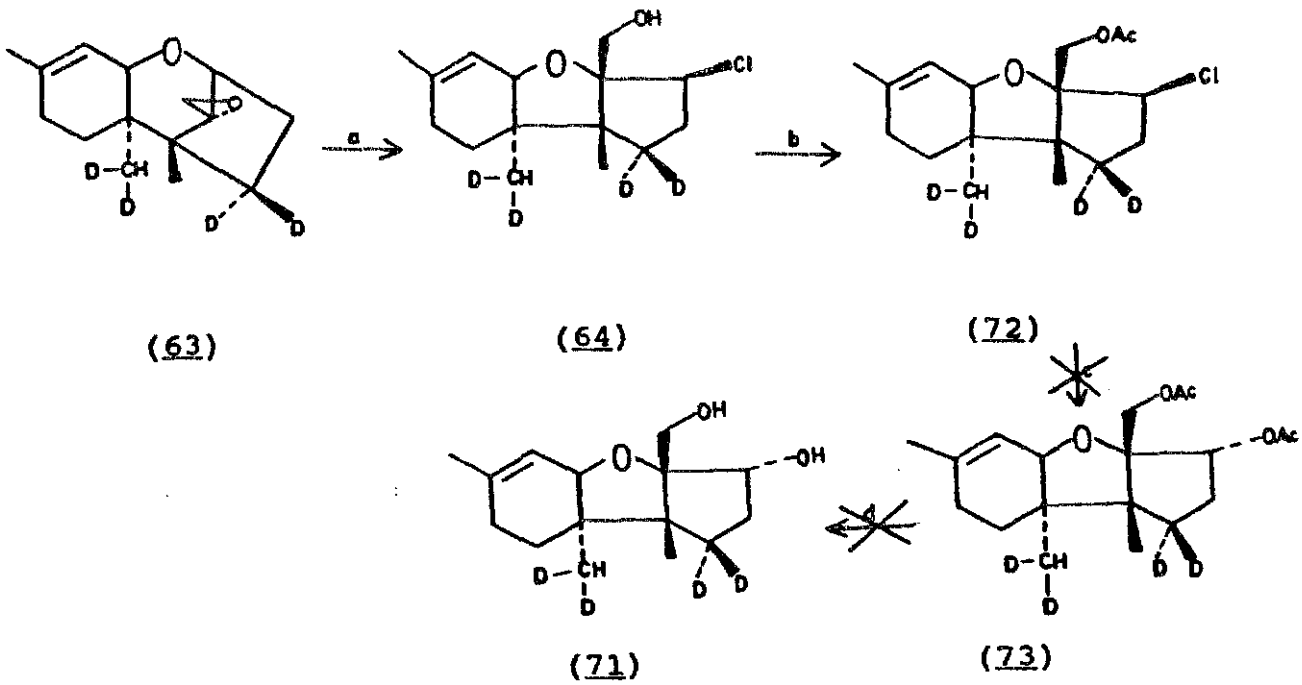
The ã isomer of 2ß-apotrichodiol cannot be obtained directly from EPT. The first method employed (Figure 28) used the

principle that alkyl halides are transformed to their corresponding alcohols, by reacting with silver acetate, with an inversion of configuration at that center. Thus the primary alcohol of chlorohydrin (64) was protected in order that the 2ß-Cl functionality be transformed to its 2ã-O-acetate (73) which would have been hydrolyzed to afford the 2ã-ATD (71). Unfortunately the conditions used (silver acetate in glacial acetic acid) (Rodunz and Schneider, 1985) provided the 2ß-O-acetate (68). Hence the reaction took place with retention of configuration. From the NOED spectrum preirradiation of H-11 causes an enhancement in intensity for H-2 therefore establishing their cis relationship. Consequently, the acetate at C-2 occupies the ß position. This pathway was not pursued further.

The 2ß-ATD (66) (Figure 27) was instead selectively acetylated (Schuda et al., 1984) when treated with acetic anhydride/pyridine in dichloromethane at room temperature. TLC analysis showed a mixture of the starting material (66) and three additional products. By NMR these were characterized as (67), the desired protected acetate in 54% yield; as compound (68) the diacetate of 2ß-ATD (66) obtained in 15% yield; and as compound (69), the isomer of acetate (67) obtained in 14% yield. The starting material (66) was obtained in 14% yield.

The protected alcohol (67) was then oxidized with PCC in dichloromethane (Corey and Suggs, 1975; Müller and Tamm, 1975) at room temperature to afford the 2-ketone in 83% yield, which was reduced with $NaBH_4$ in MeOH at 0°C to afford the desired

monoacetylated 2ã-OH (70) in 84% yield and a small amount of the original ß-alcohol (67) in 10% yield. Once again the reduction of the 2-ketone occurs stereoselectively because the end product is less sterically hindered since the C-13-CH_2OAc group is trans to the 2ã-OH whereas in compound (67) the molecule is more hindered since the C-13-CH_2OAc is cis to the 2ß-OH. Reduction of the 2-ketone allows the molecule to assume a less hindered conformation. Finally deprotection of acetate (70) with anhydrous K_2CO_3 in MeOH at room temperature afforded the desired 2ã-ATD (71) (Schuda et al., 1984) in 94% yield.



(63) (64) (72)

(71) (73)

(a)concentrated HCl:MeOH(1:10); (b)Ac_2O,pyr; (c)AgOAc,acetic acid; (d)K_2CO_3,MeOH.

Figure 28

5. VERRUCAROL TO ^{3}H-EPT to ^{3}H-ATO TO ^{3}H-2ß-ATD TO ^{3}H-2ã-ATD.

It is obvious that the compounds to be prepared 15-^{3}H-epoxytrichothecene (EPT) (77), (Figure 29), 15-^{3}H-apotrichool (ATO) (79), 15-^{3}H-2ß-apotrichodiol (2ß-ATD) (80) and 15-^{3}H-2ã-apotrichodiol (2ã-ATD) (83) have the same characteristics as their deuterated counterparts, $^{2}H_4$-EPT (63), $^{2}H_4$-ATO (65), $^{2}H_4$-2ß-ATD (66) and $^{2}H_4$-2ã-ATD (71), respectively. From TLC analysis the Rf of each member of the corresponding pairs are the same.

In order to obtain tritiated EPT (77), the procedure used for the preparation of deuterated EPT (63) had to be slightly modified. Since there was no need for the introduction of any deuterium atoms (only one tritium label was required), 15-acetoxyverrucarol (56) (Figure 29) was treated directly with phenylchlorothionocarbonate in the presence of pyridine and 4-dimethylaminopyridine in dichloromethane. The thionocarbonate (74) obtained has similar physical properties to that of the reverse isomer (58) (Figure 25). They are both foamy sirups. The difference among them lies in their polarities. By TLC analysis, the Rf(35% EtOAc:Hexane) of (74) is 0.32 and that of (58) is 0.38. Thus (74) is more polar. The thionocarbonate on the ã side of C-4 (58) is shielded more by the 15-OAc and the A/B rings than that of the ß side of C-4 (74) hence making compound (58) less polar (larger Rf). The NMR spectra of each compound is slightly different. Furthermore the preparation of (74) from its original alcohol (56) proceeds more quickly; less reagent is

needed because the 4ß-OH is less sterically hindered than the 4ã-OH of (57).

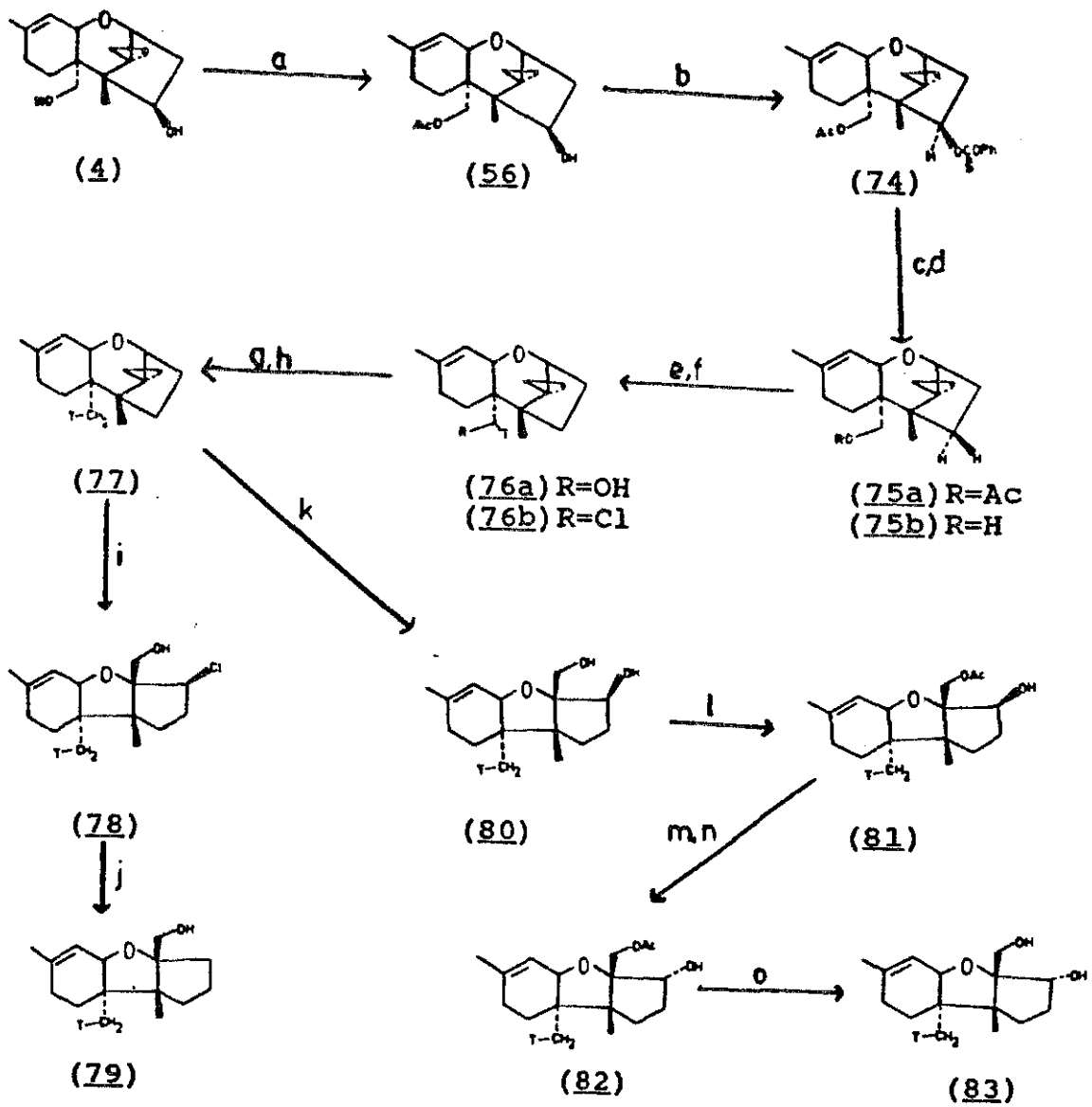
The thionocarbonate (74) was reduced in toluene (AIBN) with the tri-n-butyltin hydride and not the tri-n-butyltin-deuteride as was used for (58). Hydrolysis of (75a) with K_2CO_3 in MeOH provided the alcohol (75b) which was oxidized with PCC in dichloromethane (Corey and Suggs, 1975; Müller and Tamm, 1975). The resulting aldehyde was reduced with NaB^3H_4 in MeOH to introduce the tritium label and then subsequently with $NaBH_4$ to complete the reduction. The resultant alcohol (76a) was used to prepare tritiated EPT (77) which was used as the starting material for the syntheses of tritiated ATO (79), 2ß-ATD (80) and 2ã-ATD (83). The procedure for the aforementioned syntheses was the same for the tritiated compounds as that for their deuterated counterparts.

6. VERRUCAROL TO TRICHODERMOL

The final compound to be synthesized was trichodermol in its deuterated and tritiated forms. Cold (unlabeled) trichodermol has been synthesized by Tulshian and Fraser-Reid (1980). This procedure was slightly modified in order to incorporate the deuterium and tritium labels.

Once again verrucarol (4) (Figure 30) was selectively acetylated using acetic anhydride/pyridine in dichloromethane at room temperature (Schuda et al., 1984). The product 15-

acetoxyverrucarol (56) was treated with imidazole and t-butyldimethylsilyl chloride in refluxing THF to yield the

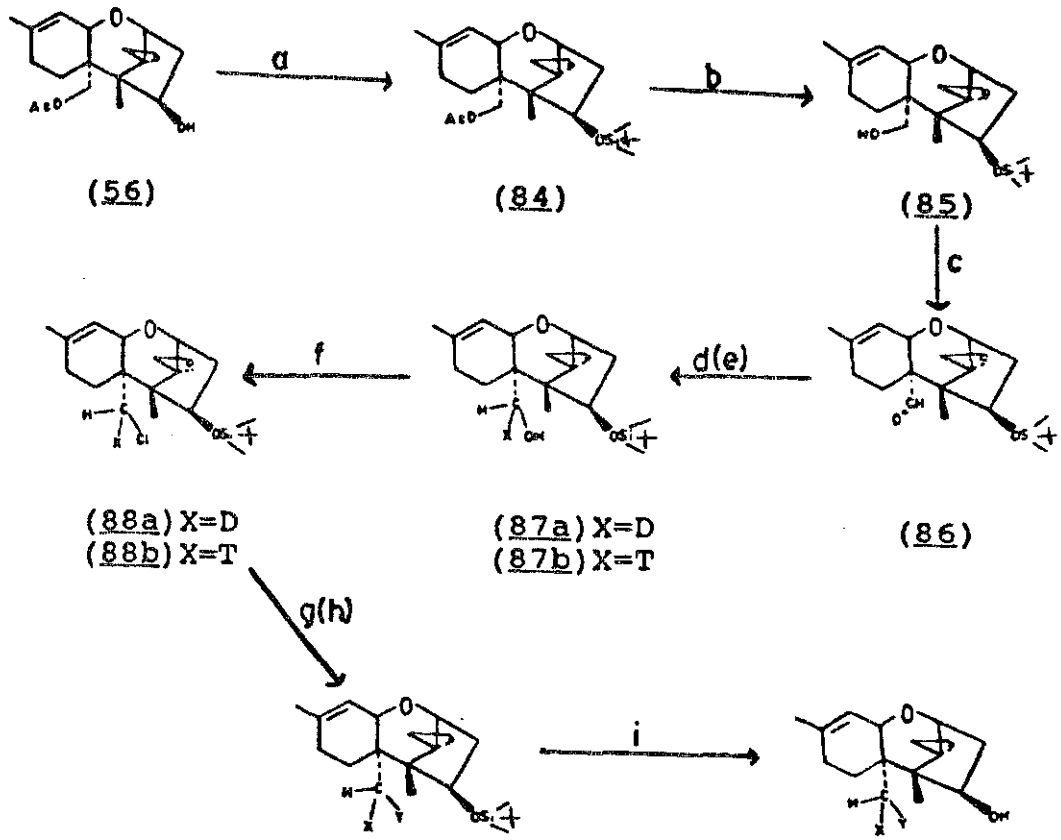


(a) Ac_2O, pyr, CH_2Cl_2; (b) phenylchlorothionocarbonate, pyr, 4-DMAP, CH_2Cl_2; (c) n-Bu_3SnH, AIBN, toluene, 80C; (d) K_2CO_3, MeOH; (e) PCC, CH_2Cl_2; (f) NaB^3H_4, $NaBH_4$, MeOH; (g) SO_2Cl_2, imidazole, DMF(-40C-25C); (h) n-Bu_3SnH, AIBN, toluene, 80C; (i) concentrated HCl:methanol (1:10); (j) n-Bu_3SnH, AIBN, toluene, 80C; (k) H_2SO_4 (0.25M), MeOH (2:1); (l) Ac_2O, pyr, CH_2Cl_2; (m) PCC, CH_2Cl_2; (n) $NaBH_4$, MeOH, 0C; (o) K_2CO_3, MeOH.

Figure 29

compound (84) in 100% yield. Deacetylation of (84) with K_2CO_3 in MeOH (Schuda et al., 1984) at room temperature provided alcohol (85) (97%), oxidation of which, with PCC in dichloromethane (Corey and Suggs, 1975; Müller and Tamm, 1975) at room temperature, afforded aldehyde (86) (84%). Reduction of the aldehyde with NaB^2H_4 in MeOH at 0°C afforded the deuterated alcohol (87a) (87%), whereas reduction with NaB^3H_4 in MeOH yielded the tritiated alcohol (87b) (82%). Both of the above alcohols were treated with SO_2Cl_2 in DMF, to which had been added imidazole, at -40°C (Hanessian and Vatele, 1981) to provide chlorides (88a) (98%) and (88b). Chlorination of alcohols (87a,87b) with methanesulfonyl chloride in DMF at 70°C as was done by Tulshian and Fraser-Reid (1980), resulted in the hydrolysis of the epoxide, consequently such acidic conditions were not employed. Silylated trichodermol (89a) (100%) was obtained by the reduction of chloride (88a) with tri-n-butyltindeuteride in toluene (AIBN) to incorporate the second deuterium atom, whereas protected trichodermol (89b) was obtained when alcohol (88b) was reduced with tri-n-butyltinhydride in toluene (AIBN).

Compounds (89a) and (89b) were treated with tetra-n-butylammonium fluoride in refluxing THF (Tulshian and Fraser-Reid, 1980) to yield deuterated trichodermol (90) (82%) and tritiated trichodermol (91), respectively.



(a) TBDMSi-Cl, imidazole, THF; (b) K_2CO_3, MeOH; (c) PCC, CH_2Cl_2; (d) NaB^2H_4, MeOH, 0C; (e) NaB^3H_4, $NaBH_4$, MeOH, 0C; (f) SO_2Cl_2, imidazole, DMF, (-40-25C); (g) n-Bu_3SnD, AIBN, toluene, 80C; (h) n-Bu_3SnH, AIBN, toluene, 80C; (i) tetra-n-butylammonium fluoride, THF.

Figure 30

DISCUSSION

1. LARGE PRECURSORS TO 3-ADN AND SOL

Because of their fungal infection of grains, attention has recently been turned to the study of the metabolites from liquid cultures of Fusarium species such as Fusarium culmorum and Fusarium graminearum. There has been an array of trichothecene structures now identified in Fusarium culmorum by Greenhalgh and coworkers (Greenhalgh et al., 1986a; Greenhalgh et al., 1986b; Greenhalgh et al., 1984a) and Baldwin et al (1987). Among these are EPT (9), ITD (95), 3-deacetylisotrichodermin (where the acetate of ITD has been hydrolyzed) and calonectrin (8) which are all minor metabolites and 3-acetyldeoxynivalenol (18) and SOL (99) which are the major metabolites.

Zamir and coworkers have been interested in the isolation and characterization of the major precursors to 3-ADN and SOL, so as to elucidate their biosynthesis. For this purpose they have employed the technique of kinetic pulse labeling (KPL) (Zamir, 1980; Wilson and Calvin, 1955; Bassham et al., 1953), which involves the feeding of an early radiolabeled precursor to liquid cultures of Fusarium culmorum and the subsequent analysis of the sequential appearance, that is, at different time intervals, of the newly biosynthesized radiolabeled metabolites. These metabolites are detected by HPLC via UV and radioactivity detectors.

The KPL technique has facilitated the detection of two types of metabolites from radioactive HPLC tracings, putative

intermediates or precursors to 3-ADN and SOL and dead end metabolites. The putative intermediates are those metabolites which are degraded with time as the major end products of Fusarium culmorum, 3-ADN and SOL, are seen to accumulate. Furthermore they are formed before the end products are produced. Dead end metabolites on the other hand, accumulate while 3-ADN and SOL are being produced.

Dr. Zamir has formulated a biogenetic scheme to explain the production of 3-ADN and SOL in culture broths of Fusarium culmorum (Figure 31). As can be inferred from the scheme, the precursors to 3-ADN (18) are proposed to be ITD (95), EPT (9) and trichodiol (94). The proposed precursors to SOL (99) are pre-SOL (98), EPT and trichodiol from one of two pathways. From the second pathway, ATO (96), 2-ATD (97) and pre-SOL are plausible intermediates in the biosynthesis of SOL.

The three suggested precursors trichodiene (46), trichodiol (94) and EPT (9) have been implicated in the biosynthesis of 4-oxytrichothecenes. Trichodiene was first isolated in the culture broth of Trichothecium roseum by Nozoe and Machida (Nozoe and Machida, 1970a; Nozoe and Machida, 1972) and then characterized by the same authors. Trichodiol-A (100) (Figure 32) which was first isolated by Nozoe and Machida (1970b) was later proven to be an artifact produced from trichodiol (94) (Nozoe and Machida, 1972) (Figure 32) during alkali hydrolysis. 12,13-epoxytrichothec-9-ene (EPT) (9) was first isolated by Machida and Nozoe (1972a) and has been reported by Baldwin et al.

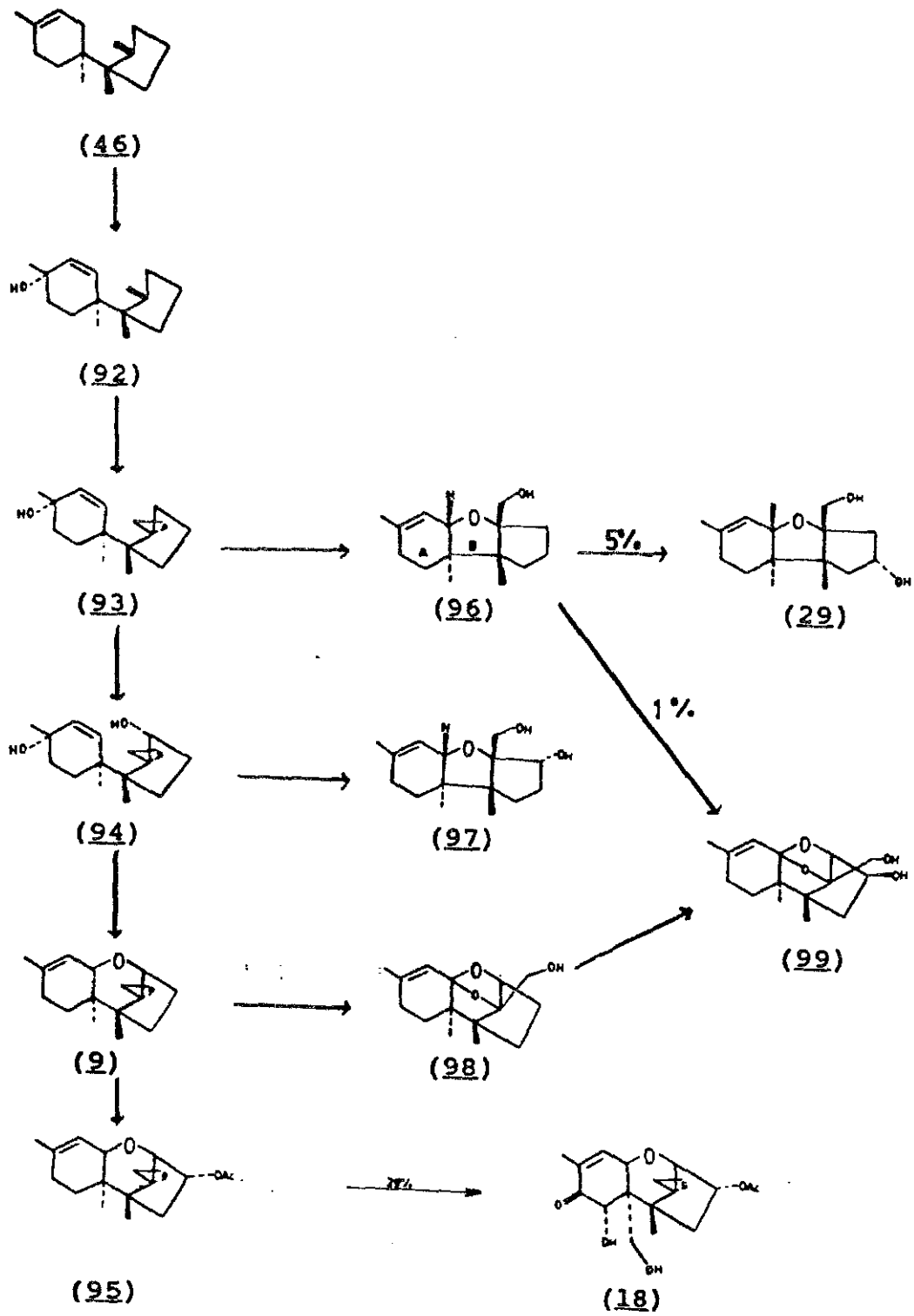
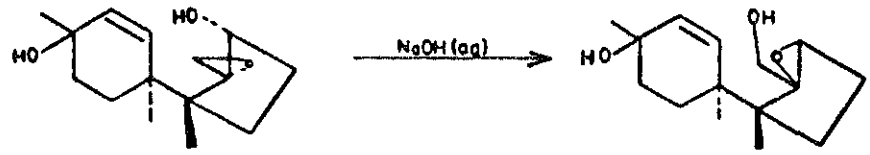


Figure 31

(1987) in Fusarium culmorum cultures. It was suggested by the same authors that trichodiene was incorporated into trichothecin via precursors, trichodiol (94) and EPT (9).

Indeed Machida and Nozoe (1972b) proved that trichodienewas a precursor of trichothecin via biogenetic precursor studies of T. roseum. Trichodiene was radiolabeled chemically with tritium at the olefinic methyl group (C-16) of the cyclohexene moiety. It was then added to a growing culture of Trichothecium roseum, and subsequently trichodiene was incorporated into trichothecolone (3.2%). Upon saponification two more metabolites were isolated, 12,13-epoxytrichothecene (9) and trichodiol-A (100) (Figure 32). Measurement of the radioactivity from preparative TLC of the mother liquor showed that trichodiene was incorporated 0.6% in the former and 2% in the latter. The incorporation of trichodiene into the trichothecenes was confirmed by Hanson et al (Evans et al., 1973; Evans and Hanson, 1976). Labeled trichodiene prepared from [5-$^{3}H_2$] [2-^{14}C] mevalonate was fed to a growing culture of Trichothecium roseum. A 5.3% incorporation of the precursor into trichothecin was reported. The above results indicate that trichodiene is probably a precursor to trichothecin. However none of the investigators tried to locate the exact position of the label through degradation studies, therefore these experimental results are ambiguous.



(94) (100)

Figure 32

Recently though Zamir et al (1989) were the first to prove conclusively that trichodiene is indeed a precursor to 3-ADN and SOL. 3-ADN and SOL isolated from Fusarium culmorum cultures fed with deuterium labeled trichodiene, were indeed themselves labeled with deuterium. The exact position of the label was detected by ^{2}H-NMR spectroscopy.

To give credence to the biogenetic scheme proposed by Dr. Zamir each step must be proven. Zamir et al already have a strong indication of the validity of these steps by radiolabeled intermediates . The use of stable isotope labeled precursors will ensure unambiguously, the location of the position of the incorporation into 3-ADN and/or SOL through NMR spectroscopy.

2. BIOGENETIC RATIONALE FOR THE SYNTHESIS OF PUTATIVE PRECURSORS

2.1. Partial Synthesis of ITD.

Using the KPL technique a compound behaving like a putative precursor was purified to constant activity and then refed to Fusarium culmorum. The isolated 3-ADN and SOL were

purified to constant activity. The compound showed an incorportation of 29% only into 3-ADN. SOL was unlabeled. Appropriate incubations corresponding to the timing prescribed by the KPL technique, allowed for the isolation and characterization of the cold compound corresponding to the radiolabeled precursor. This compound was identified as isotrichodermin (ITD) (95), a structure originally reported and characterized by Greenhalgh et al (1984b; 1986b).

Deuterium labeled isotrichodermin is necessary to unambiguously prove that it is incorporated into 3-ADN (29%) as indicated by the KPL technique. The proof lies only in the deuterium NMR spectrum of 3-ADN following its isolation from culture broths of Fusarium culmorum fed with deuterated ITD. Zamir and coworkers attempted to isolate a sufficient quantity of deuterated ITD from culture broths of Fusarium culmorum fed with stereospecifically deuterium labeled mevalonate. Successive attempts to accumulate necessary quantities of deuterated ITD required for further feeding experiments to isolate deuterated 3-ADN, failed. Evidently ITD is quickly metabolized to 3-ADN and thus it does not remain in the medium in sufficient quantities.

Hence, ITD had to be synthesized. Deuterated ITD was readily obtained (ten steps) for the first time, from the readily available trichothecene, anguidine, in large quantities. This partial synthesis was well suited for the incorporation of two deuterium atoms. The preparation of deuterated ITD is new since it has never been synthesized before, radiolabeled or otherwise.

It can now be obtained relatively easily in large quantities from readily available anguidine.

3-ADN isolated from Fusarium culmorum liquid cultures fed with synthetic $^{2}H_{2}$-ITD (54) must show an incorporation of two deuterium atoms one each at positions C-4 and C-15. Two distinct signals from the ^{2}H- NMR spectrum will corroborate the results obtained with the KPL technique.

2.2. Partial Synthesis of EPT.

It must be stressed that figure 31, depicts a plausible biogenetic scheme where not all of the steps have been substantiated by the KPL technique. It remains to be proven whether EPT (9) is indeed a precursor to 3-ADN and/or SOL.

The isolation of EPT is of considerale significance. It has been suggested as a key link between trichodiene and the more complex trichothecene structures (Tamm and Breitenstein, 1980). From figure 31, a series of hydroxylations of TDN (46) lead to the epoxytrichothec-9-ene skeleton (9), namely: abstraction of the pro-11S of trichodiene (46), with concurrent hydroxylation at C-9 leads to compound (92), which upon epoxidation of its 12,13-ene affords compound (93) which is transformed to trichodiol after hydroxyltion occurs at C-2. Trichodiol (94) then cyclizes to EPT (9). Whether or not EPT is indeed a link between trichodiene and the rest of the more complex trichothecenes depends on whether EPT is incorporated

into both 3-ADN and SOL. If it is then this belief will have been confirmed. On the other hand, if EPT is incorporated into one and not the other of these major metabolites, then some other precursor must necessarily be the key link. EPT has been demonstrated to be produced from labeled trichodiene in Trichothecium rosem (Machida and Nozoe, 1972b) but as yet there is little direct evidence for its further metabolism.

The isolation of sufficient quantities of EPT, required for further feeding experiments, is lengthy and quite inefficient. Only trace amounts of EPT are isolated from numerous liters of culture broth of Fusarium culmorum. On the other hand, chemical synthesis of EPT avoids this inadequate procedure and provides the required trichothecene in large quantities.

$^{2}H_4$-EPT was obtained in high yields after ten steps from verrucarol which is itself readily available from culture broths of Myrothecium verrucaria, in large quantities, especially after the original production procedure was modified (Jarvis et al., 1984).[5]

Deuterium NMR spectra of isolated 3-ADN and SOL from Fusarium culmorum cultures fed with $^{2}H_4$-EPT (63) would be expected to show, if indeed the incorporation were positive, the incorporation of four deuterium atoms into SOL and 3-ADN, two of which would would be located at positions C-4 and two at positions C-15.

Prior to the present synthesis , 12,13-EPT had been obtained via total syntheses only (Fujimoto et al., 1974; Masuoka

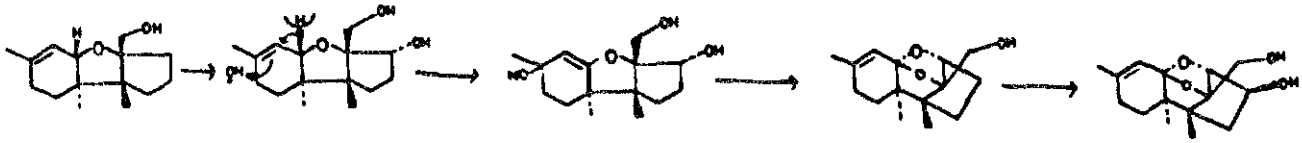
and Kamikawa, 1976; Hua et al., 1988; Fujimoto et al., 1981). Now, for the first time 12,13-EPT could be obtained readily from verrucarol in just ten controlled steps , the advantage being that EPT is now available in large amounts either radiolabeled or cold. Thus the tediousness and inefficiency of total synthesis can be avoided.

2.3. Partial Synthesis of ATO, 2ã-ATD and 2ß-ATD.

Another intermediate detected by the KPL technique, used by Zamir et al, was apotricho-ol (96) (ATO). The compound was characterized by the usual methods and was shown to belong to the apotrichothecene series of mycotoxins. ATO (96) is the second apotrichothecene (after 3-ATD) isolated from natural sources. Its structure is similar to that of 3-ATD (29). It also exhibits a trans junction between rings A and B. From radiolabeled incorporation experiments there is a strong indication that ATO (96) is a putative precursor in the biosynthesis of SOL. SOL isolated from Fusarium culmorum cultures fed with radiolabeled ATO (96) showed a 1% incorporation of the label. (Radiolabeled ATO was also incorporated 5% into the dead end metabolite 3-ATD).

Referring to figure 31, to obtain SOL from ATO, ATO must first be hydroxylated at C-2 with the appropriate stereochemistrty as to afford 2ã-ATD (97). Abstraction of the C-11 ß hydrogen of 2ã-ATD yields compound (101) (Figure 33). The

2ã-OH of this precursor will then attack position C-11 to yield pre-SOL, hydroxylation of which at C-3 will yield SOL with the appropriate stereochemistry as proven by Mohr et al (1984).



ATO 2-ATD (101) pre-SOL SOL

Figure 33

Apotricho-ol (96) and 2-ATD (Figure 31) cannot be prepared, from EPT, synthetically with the correct trans junction between rings A and B. EPT will rearrange to the apotrichothecene structure when exposed to acid. Unfortunately, this rearrangement gives a cis junction between rings A and B and not the required trans junction as in the natural apotrichothecenes, ATO (96) and 3-ATD (29). Thus the 1% incorporation of ATO (96) into SOL as indicated through the KPL technique cannot be substantiated, but the compounds ATO, 2ß-ATD and 2ã-ATD, labeled with deuterium and tritium will be used in feeding experiments by Zamir et al, in order to determine their incorproration into SOL.

If $^{2}H_{4}$-ATO is shown to be incorporated into SOL then it must mean that the particular configuration enjoyed by the C-11 hydrogen, which exists cis in the synthetic ATO but trans in the natural isomer, is inconsequential in the transformation of ATO

to SOL. This would imply that the enzyme responsible for the abstraction of the hydrogen at C-11 of 2-ATD is non-specific.

One more condition must be met if ATO is to be transformed to SOL. If the proposed scheme (Figure 33) is correct, then the hydroxylation of ATO at C-2 must necessarily give 2ã-ATD. The opposite ß hydroxy stereochemistry would prevent the cyclization of compound (101) to pre-SOL and eventually to SOL. Therefore if and only if the aforementioned enzyme is non-specific then $^{2}H_4$-2ã-ATD would be incorporated into SOL because the second condition will have been met. $^{2}H_4$-2ß-ATD should not be incorporated into SOL since the hydroxy orientation is incorrect.

2.4. Synthesis of $^{2}H_2$-Trichodermol.

The known trichodermol synthesis from anguidine was modified for the incorporation of the deuterium labels. Once again large amounts of an important trichothecene was easily prepared so that it may be used in biosynthetic studies of Myrothecium verrucaria and Fusarium culmorum.

CONCLUSION

Since large amounts of precursors are required to be fed to Fusarium culmorum cultures to prove certain steps of the biosynthetic scheme proposed by Dr. Zamir, it was necessary to synthesize chemically the two major metabolites ITD and EPT. Isotrichodermin was readily prepared in ten steps from the more readily available trichothecene, anguidine. It had never been prepared before yet after chemically manipulating the different funtional groups of anguidine, ITD was obtained in large amounts. 12,13-Epoxytrichothecene had been prepared before via several total syntheses. Unlike total syntheses however, which can be elaborate, thus time consuming not to mention inefficient, 12,13-EPT was prepared from the more abundant verrucarol. Thus EPT is now available in large amounts. Both ITD and EPT will serve as precursors to 3-ADN and SOL in different feeding experiments of Fusarium culmorum.

The apotrichothecenes synthesized will serve as learning tools since they may shed some light into the mechanism involved for the biosynthesis of SOL.

The chemistry involved in the preparation of the aforementioned compounds is inherently interesting since it demonstrates the complexity of the trichothecene structures. On the other hand, the syntheses will be more valuable once the feeding of ITD, EPT and the apotrichothecenes will have been done since any results obtained will expand our knowledge of the route by which trichodiene is incorporated into the more complex trichothecenes. It remains to be seen if EPT is indeed the key

link between trichodiene and the more complex trichothecenes. Thus slowly the pieces of the trichothecene puzzle are falling together. The synthetic work is the means by which the end will be achieved.

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