

TRICHOHECANES - A REVIEW

Chi Wi Ong

University Chemical Laboratory, Lensfield Road,
Cambridge CB2 1EW, U.K.

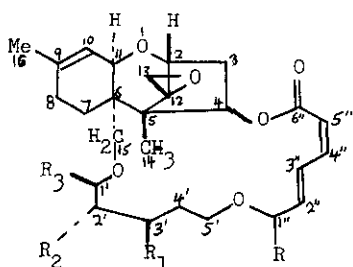
Abstract - The trichothecanes are a family of polyoxygenated sesquiterpenes showing a number of interesting pharmacological properties, i) antibiotic, ii) antifungal, iii) antiviral, iv) insecticidal and v) cytotoxic and cytostatic activity, the most common examples being trichodermin and verrucarol. Recent trichothecanes synthesis have been reviewed, including our contribution in the total synthesis of 12,13-Epoxy-14-methoxy-trichothecene using dienyl-Fe(CO)₃ complexes.

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- I. Introduction
- II. Structural Elucidations
- III. Chemical Transformations
- IV. Total Synthesis
- V. Biosynthesis
- VI. Mode of Action and Biological Properties

I. INTRODUCTION

The macrocyclic trichothecene esters (verrucarins and roridins derivatives) are secondary metabolites of the soil fungi imperfecti which belongs to the order of Moniliales, family Tuberculariaceae, and of numerous species, example Myrothecium, Trichothecium and Fusarium. These are the most toxic non-nitrogen natural products known, containing only C, H, and O. They are interesting compounds not only for chemical reasons, but also because of the vast remarkable biological properties, antibiotic, antifungal, antiviral, insecticidal, cytotoxic and cytostatic activities.^{1a, 1b} They are colourless, crystalline, optically active solids, sparingly soluble in water, but soluble in all common organic solvents at room temperature. These macrocyclic trichothecene esters are sesquiterpenes characterized by the presence of a 12,13-epoxy trichothecene ring system.



Verrucarin A - $R = O$, $R_1 = CH_3$, $R_2 = OH$, $R_3 = O$

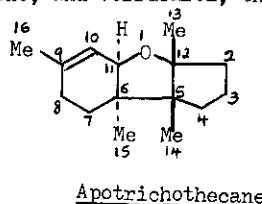
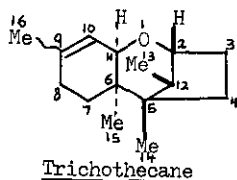
2'-Dehydro-Verrucarin A - $R = O$, $R_1 = CH_3$, $R_2 = R_3 = O$

Verrucarin B - $R = O$, $R_1 = R_2 = O$, $R_3 = O$

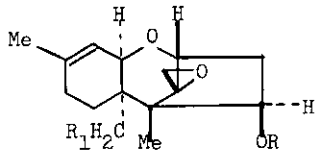
Roridin A - $R = (CH)OHCH_3$, $R_1 = CH_3$, $R_2 = OH$, $R_3 = O$

Roridin D - $R = (CH)OHCH_3$, $R_1 = R_2 = O$, $R_3 = O$

Most macrocyclic trichothecene esters yield the same tricyclic epoxy sesquiterpenoid upon base catalysed hydrolysis (example verrucarins and roridins give verrucarol). To date approximately 47 naturally occurring derivatives are known. In view of the growing number of fungal metabolites which are esters of sesquiterpene alcohols possessing the same tricyclic structure as found in trichodermol (roridin C), trichothecolone, and verrucarol, the name



TRICHOHECANE with a particular numbering was introduced for the basic skeleton. The corresponding rearranged skeleton is called APOTRICHOTHECANE. According to this nomenclature, verrucarol is to be named 4β -15-dihydro-12,13-epoxy- Δ^9 -trichothecene and roridin C (trichodermol), 4β -hydroxy-12,13-epoxy- Δ^9 -trichothecene.²



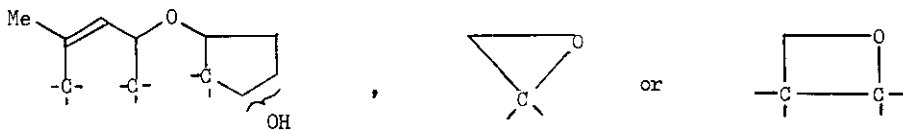
Trichodermol	R = R ₁ = H
Trichodermin	R = OAc, R ₁ = H
Verrucarol	R = H, R ₁ = OH

Despite the ability to isolate these compounds, the world's supply is low and insufficient at present for extensive testing. Moreover, reliable analytical methods are not available making it difficult to evaluate cause and effect relationships among toxins. This, together with their complicated structures have made their total synthesis a significant challenge. The first total synthesis towards this end has been achieved by Colvin, Raphael and co-workers in 1971 with the synthesis of (±) trichodermol. ³

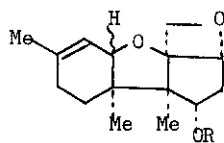
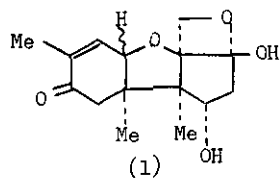
II. STRUCTURE ELUCIDATIONS ⁴

Structure elucidation of the macrocyclic trichothecene esters has been carried out mainly on the common chromophoric part of the molecule, the 12,13-epoxytrichothecene ring system, namely trichodermol (also trichodermin) and verrucarol, and their derivatives.

Trichodermin, the acetylated chromophore of trichodermol contains carbon, hydrogen and oxygen only and elementary analysis and molecular weight determination correspond well with the formula C₁₇H₂₄O₄. The probable partial structures from combined n.m.r. evidence with the other spectroscopic and chemical informations were as follows :-



During this period, another fungal metabolite of *Trichothecium roseum*, called trichothecolone has been assigned the structure (1) by two different groups, Freeman and co-workers in 1959 ⁵ and Fishman and co-workers in 1960. ⁶



(2) R = Ac

(3) R = H

The similarity of the n.m.r. , IR-spectra and many reactions that proceed analogously makes it tempting to try to correlate a suitable structure for trichodermol with a corresponding derivative of trichothecolone. From further studies, provided the structure of trichothecolone (1) is correct, trichodermin and trichodermol should be represented as (2) and (3).

On the other hand certain reactions of trichodermin and its derivatives were difficult to interpret in the light of structures (2) and (3). Lithium aluminium hydride reduction of trichodermol produced a diol, $C_{15}H_{24}O_3$ (m.p. $148^{\circ}C$); the n.m.r. spectrum shows the presence of a quaternary methyl group (s, δ 1.47) more than in the spectrum of trichodermol (3). The AB system (d, δ 2.79 and δ 3.09) assigned to the methylene group in the four membered oxide ring was absent in the diol. Also the new hydroxy group is tertiary, suggesting that trichodermin contains an epoxide group ($\begin{matrix} \text{C}^{\text{H}}-\text{O} \\ \diagup \quad \diagdown \\ \text{C} \end{matrix}$). Because of this disagreement, structure (2) was discarded as the correct structure for trichodermin.

X-ray crystallography of the p-bromobenzoate derivative of trichodermol by Abrahamsson and Nilsson ⁷ revealed the presence of an epoxide ring and has rigorously established the compound as structure (4a). The structure of trichodermin and trichodermol is thus assigned as (4b) and (4c).

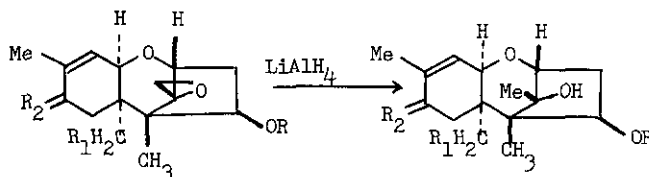
(4a) R = p-bromobenzoyl, $R_1 = H$, $R_2 = 2H$

(4b) R = Ac, $R_1 = H$, $R_2 = 2H$

(4c) R = $R_1 = H$, $R_2 = 2H$

(4d) R = H, $R_1 = H$, $R_2 = O$

(4e) R = H, $R_1 = OH$, $R_2 = 2H$

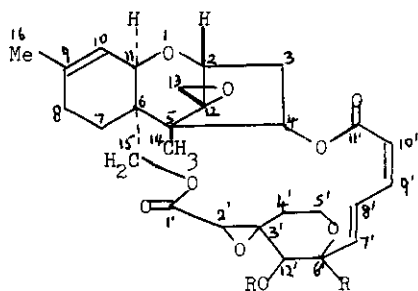


The structure of verrucarol has been proved by Gutzwiller and co-workers⁸ to be (4e) and he has successfully correlated verrucarol and trichodermol and unambiguously established the location of the primary hydroxy group.

Spectroscopic methods have thus whenever possible replaced the difficult and time consuming degradation studies for structural elucidation.

The characteristic ^1H n.m.r. spectra have played a significant role in the structural determination of those new trichothecane group of fungal metabolites isolated. With the availability of ^{13}C n.m.r. , an analysis of their n.m.r. could now play an additional role for structural elucidation. The application of ^{13}C n.m.r. (normally together with the ^1H n.m.r.) has been illustrated with the structural elucidation of Satratoxin F and G using these methods,⁹ together with other spectroscopic data available. The ^1H n.m.r. and ^{13}C n.m.r. of Satratoxin F and G were observed to be quite similar to those of roridin E, and assigned as structure shown below, having the tricyclic trichothecane chromophore.

Satratoxin



^{13}C n.m.r. has also been extensively used today in the studies of biosynthetic pathways. The ^{13}C n.m.r. of verrucarol has been assigned by Ch. Tamm and co-worker in 1975¹⁰ and that of trichothecin and trichodermol by Tim Marten and co-workers in 1974.¹¹

¹³C n.m.r. of various compounds

	<u>Verrucarol</u>	<u>Trichothecin</u>	<u>Trichodermol</u>	<u>Satratoxin G</u>
C-1				
C-2	78.5 d	70.1 d	70.8 d	79.3 d
C-3	39.5 t	36.95 t	40.2 d	34.4 t
C-4	74.0 d	73.35 d	74.2 d	73.7 d
C-5	48.7 s	49.0 s	49.6 s	49.3 s
C-6	43.7 s	43.7 s	40.4 s	43.3 s
C-7	20.9 t	42.1 t	24.85 t	20.2 t
C-8	28.0 t	198.5 s	28.4 t	27.5 t
C-9	140.4 s	138.0 s	140.4 s	140.3 s
C-10	118.7 d	137.2 d	119.4 d	118.8 d
C-11	66.4 d	79.5 d	79.3 d	68.1 d
C-12	65.6 s	65.5 s	66.2 s	65.4 s
C-13	47.4 t	47.4 t	47.9 t	48.1 t
C-14	6.8 q	5.7 q	6.4 q	8.0 q
C-15	62.1 t	15.1 q	16.0 q	64.9 t
C-16	22.9 q	18.4 q	23.4 q	23.3 q

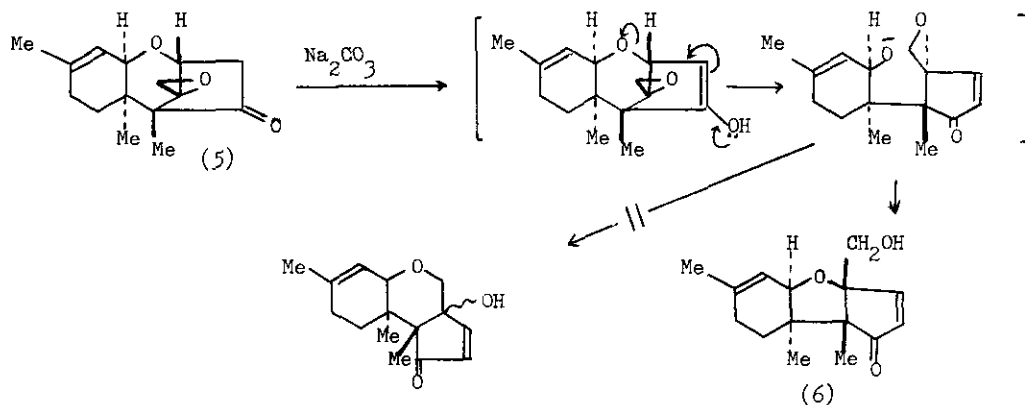
III. CHEMICAL TRANSFORMATIONS ^{1b,4,12}

A predominant feature of the tricyclic 12,13-epoxytrichothecene and its derivatives is the fact that the epoxide under certain circumstances is susceptible to intramolecular nucleophilic attack accompanied by skeletal rearrangements into the apotrichothecane skeleton.

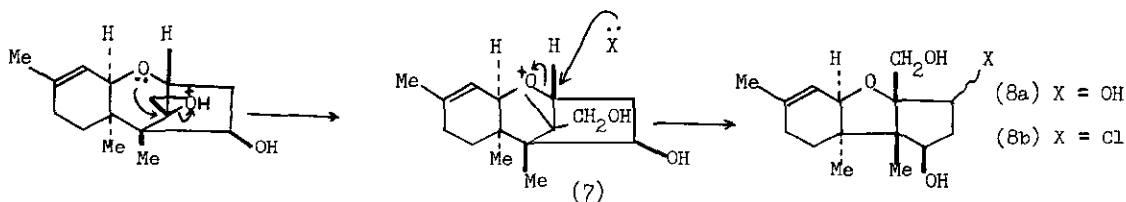
Base Catalysed Rearrangement- Trichodermol, as well as trichothecolone and verrucarol are stable to hot dilute alkali and their stability, in part is attributed to shielding of the epoxide ring from a nucleophilic attack from the rear by external anions.

In contrast, trichodermone (5) isomerises smoothly on treatment with sodium carbonate

by enolization of the ketone followed by opening of the tetrahydropyran ring to give the rearranged product (6)

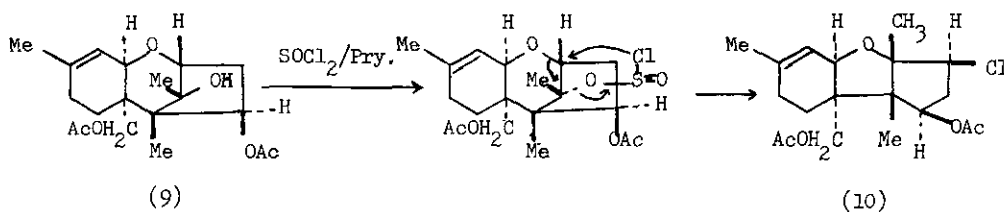


Acid Catalysed Rearrangement - Treatment with dilute sulphuric acid or dilute trifluoroacetic acid of trichodermol gives the rearranged triol, $C_{15}H_{24}O_4$ (8a). The acid catalysed rearrangement is initiated by protonation of the epoxide, followed by the internal nucleophilic attack of the tetrahydropyran oxygen on the protonated epoxide, leading to the oxonium ion (7). The oxonium ion is then cleaved with an external anion, in this case hydroxide to give the triol (8a). When dilute hydrochloric acid is used, the external anion is the



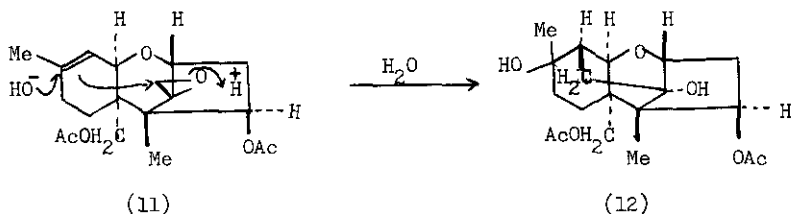
chloride, giving rise to (8b).

Rearrangement of Other Trichothecene Derivatives - Treatment of di-O-acetyldihydroverrucarol (9) with thionyl chloride in pyridine effects rearrangement to the apotriconothecane derivative (10) instead of the expected dehydrated product. This rearrangement is assumed to



occur via the chlorosulphite.⁸

Di-O-acetylverrucarol (11) on boiling with water also rearranges to give (12).



IV. TOTAL SYNTHESIS

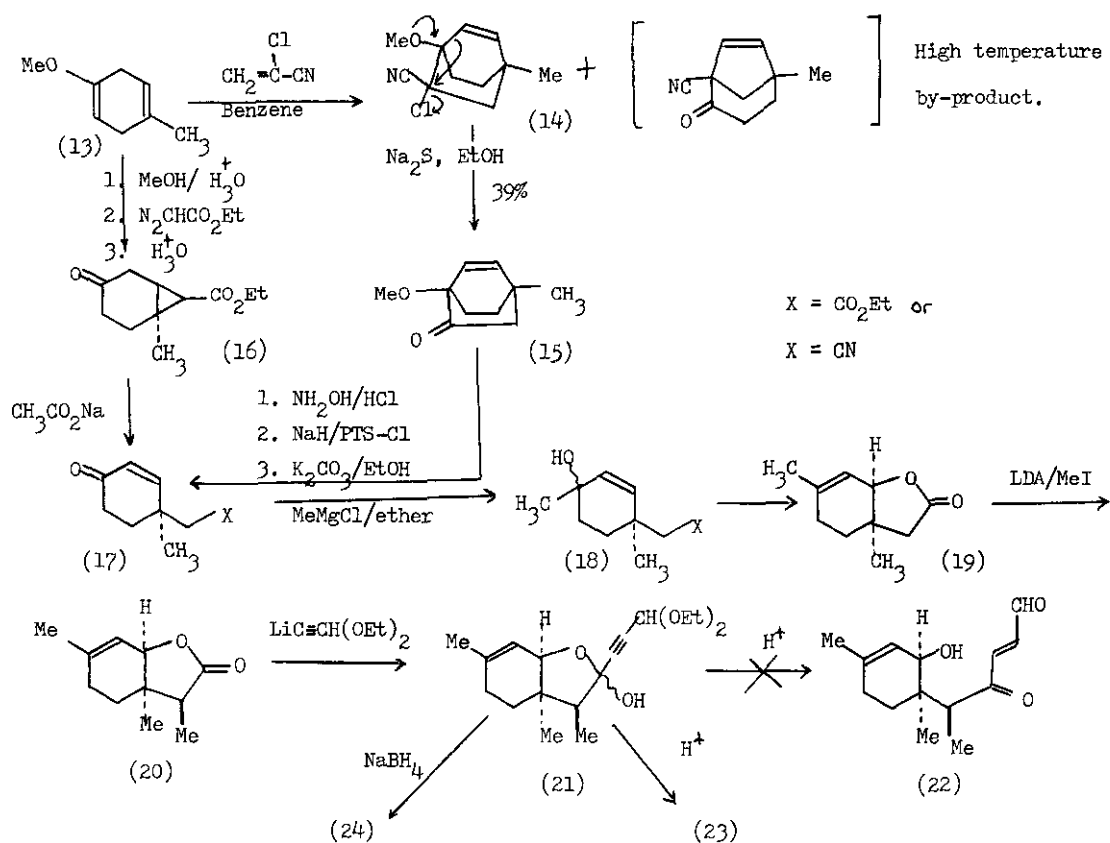
The total synthesis of the macrocyclic trichothecene esters has been centred on the two major chromophores, trichodermol (also trichodermin) and verrucarol since their structures were revealed in 1964.⁷ The total synthesis of trichodermin and trichodermol was achieved in 1971 by Colvin, Raphael and co-workers.³ The high complexity of the macrocyclic trichothecene esters containing an intricate concentration of ether-ester-olefin-alcohol functionalities (verrucarins and roridins derivatives) present very difficult targets for their total synthesis and this rather formidable challenge has not been met till the present time. The failure in all attempts since 1978 to synthesize the chromophore verrucarol itself lies the major setback. Verrucarol contained the C-4 and C-15 hydroxy groups in the tricyclic backbone required for the completion of the macrocyclic ring in the macrocyclic trichothecene esters total synthesis. A more successful study towards the total synthesis of verrucarol has been achieved recently in 1980 with the synthesis of 13,14-dinor-15-hydroxytrichothec-9-ene (76) by Rousch and Ambra¹³ containing the required C-15 hydroxy group of the tricyclic backbone, but lacking the epoxide ring at C-12 and the hydroxy group at C-4. This should hopefully inspire other research groups to synthesize verrucarol itself.

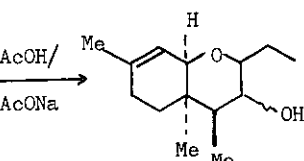
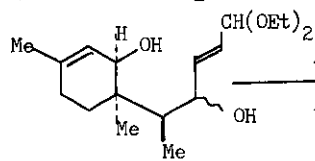
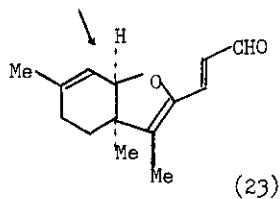
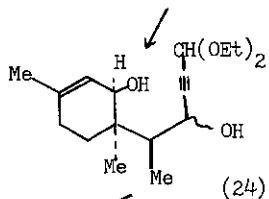
The total synthesis of the tricyclic trichothecene backbone can be divided into two different strategies: 1) the method of Colvin, Raphael and co-workers^{3,14} in which rings A and B are formed first followed by the construction of ring C, through various chemical transformations and rearrangements, and 2) the biomimetic approach in which ring A and C are formed

first followed by ring B formation by cyclization.

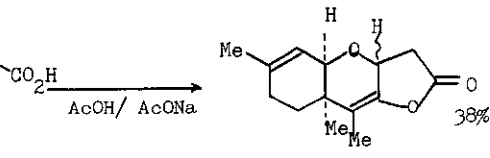
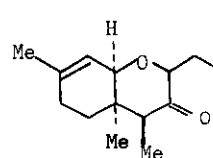
The first total synthesis of trichodermin was achieved by Colvin, Raphael and co-workers³ in 1971. It was based on the observation by M.S. Newman and co-worker¹⁵ in 1945 that the *cis* isomer of a fused bicyclic γ -lactone is the thermodynamically favoured one, this being the required stereochemistry for the ring A and B junction in trichodermin. This synthetic route is illustrated in Scheme 1. The key intermediate, 4-hydroxy-5,6,9-trimethyl-2-oxabicyclo-[4.4.0.] deca-4,9-dien-3-yl-acetic lactone (28) was synthesized from the readily available 4-methoxy-1-methylcyclohexa-1,4-diene in 15 steps (most steps being low yielding). Treatment of (28) with lithium hydridotri-*t*-butoxyaluminate gives two products, the keto aldehyde (29) and the crystalline tricyclic Keto-alcohol (30), formed in low yield through intra-molecular cyclization followed by an internal aldolisation.

Scheme 1 The first total synthesis of (\pm) - trichodermin¹³

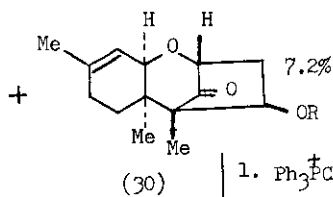
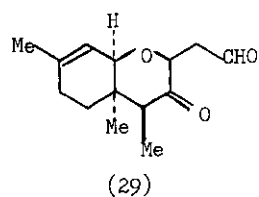




1. Cornforth Oxidation
 CrO_3 / pyridine
 2. Jones Oxidation

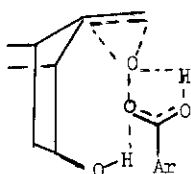
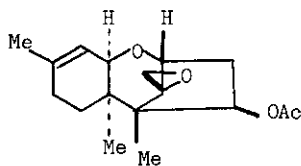


other $\xrightarrow{\text{LiAlH}(\text{OEt})_3}$



R = H
 R = OAc after acetylation.

1. $\text{Ph}_3\text{C}^+\text{CH}_2\text{Br}^-$ / base 46%
 2. MCPBA 50%
 3. Ac_2O / pyridine 77%



Epoxidation intermediate.

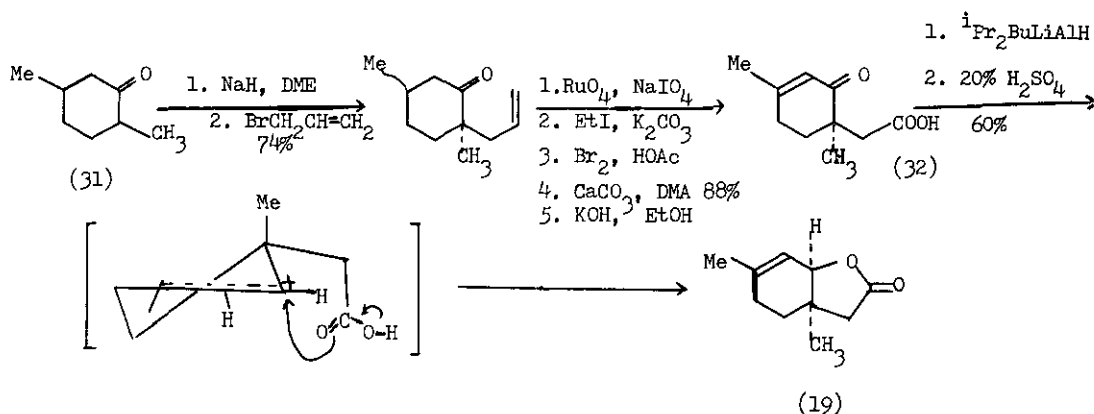
Ar = m-Cl-C₆H₄-

The tricyclic keto alcohol (30) can be readily converted into trichodermin in three steps i) conversion of the tricyclic ketone into the corresponding methylene compound by reaction with methylenetriphenylphosphorane which causes de-acetylation followed by ii) epoxidation of this methylene function and finally iii) re-acetylation. The desired regio and stereo-selectivity of epoxidation was achieved with the aid of the secondary hydroxy group present in (30) which is directed precisely towards the double bond of the methylene group and is thus ideally placed to form a hydrogen bonding 'anchor' for the electrophilic peroxy acid. The 9,10-double bond is left untouched using the appropriate condition (meta-chloroperbenzoic acid, disodium hydrogen phosphate buffer).

The key internal aldolisation step to establish the C-4 and C-5 bond in much less than 10% yield of the tricyclic trichothecene skeleton (30) makes it clear that further work on trichothecene synthesis should be undertaken.

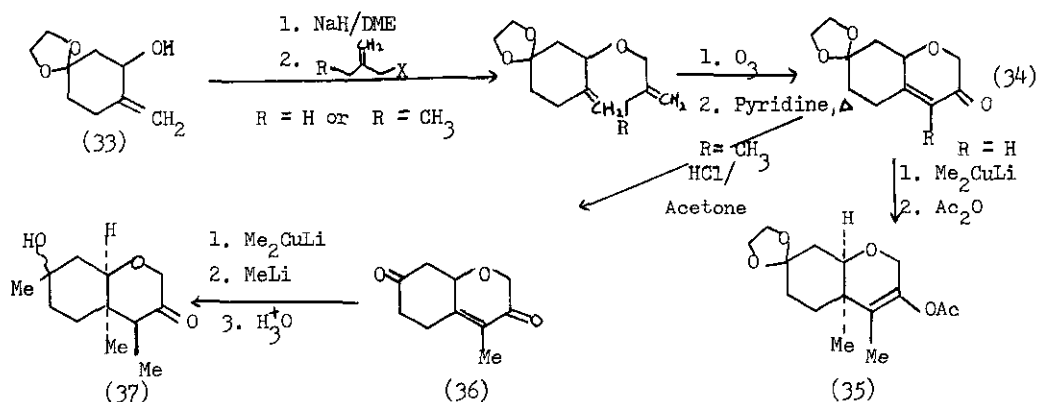
A variety of synthesis that followed utilised routes parallel to the one developed by Colvin, Raphael and co-workers in 1973.¹⁴ Various advanced intermediates in the total synthesis of trichodermin by Colvin, Raphael and co-workers in 1971³ and 1973¹⁴ became the target of synthesis themselves since they are produced in low yield and involved numerous steps.

Welch and Wang in 1972¹⁶ aimed at the synthesis of the intermediate (19) used in the trichodermin synthesis by Colvin, Raphael and co-workers. This intermediate (19) is easily synthesized from the readily available 1-oxo-2,5-dimethylcyclohexane (31) in yield higher than 60%. Reduction of the ketone in compound (32) by lithium diisopropylbutyl aluminium hydride followed by acid catalysed cyclization gave the required intermediate (19).

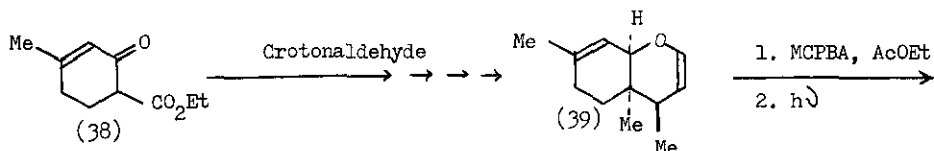


The lactonization proceeds by an allylic carbonium ion intermediate in which the cyclization is highly regio and stereo-selective to give the desired cis-fused γ -lactone (19).

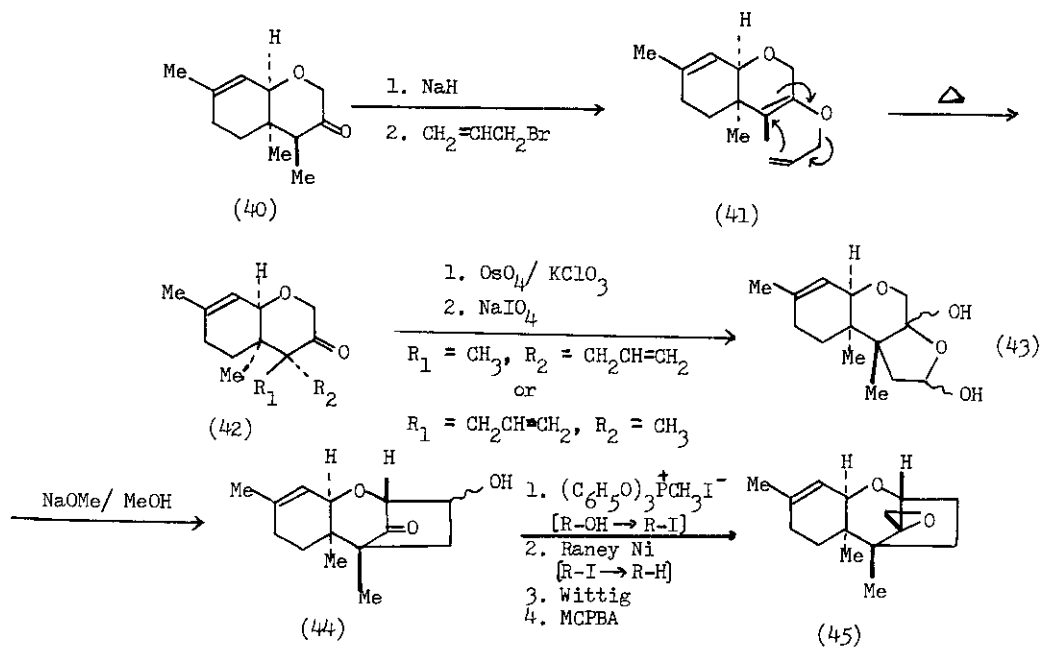
D.J. Goldsmith and co-workers in 1973¹⁷ synthesized the cis ketol (37) and proposed it as a synthetically useful intermediate towards trichothecene synthesis. The desired stereo-selective introduction of the cis angular methyl in the diketone (36) was accomplished using lithium dimethylcuprate.



Yasuo Fujimoto and co-workers in 1974¹⁸ reported the stereoselective total synthesis of (\pm)-12,13-epoxytrichotec-9-ene, isolated from the metabolite of *Trichothecium roseum* by Nozoe and Machida.¹⁹ The cis-fused pyrane derivative (39), containing ring A and B of trichothecene was formed from the keto ester (38) with crotonaldehyde, which was subsequently converted into (40). Alkylation of (40) with allyl bromide did not provide the expected C-alkylation product in this case, but gave an unexpected O-alkylation product (41). Claisen type rearrangement of (41) was accomplished by heating to give (42). The keto-aldehyde monohydrate (43) was cyclised with sodium methoxide to give a diastereomeric mixture of the tricyclic ketol (44) in yield of 90%. This rearrangement to form the C-2 and C-3 bond between the B and C ring of the tricyclic trichothecene skeleton is very much higher

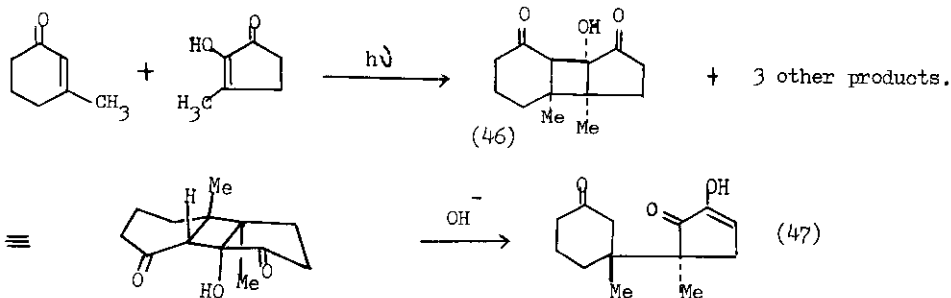


yielding than in Colvin, Raphael and co-workers rearrangement of (28) to form the C-4 and



C-5 bond. (44) was finally converted into (\pm)-epoxytrichothec-9-ene in reasonable yield.

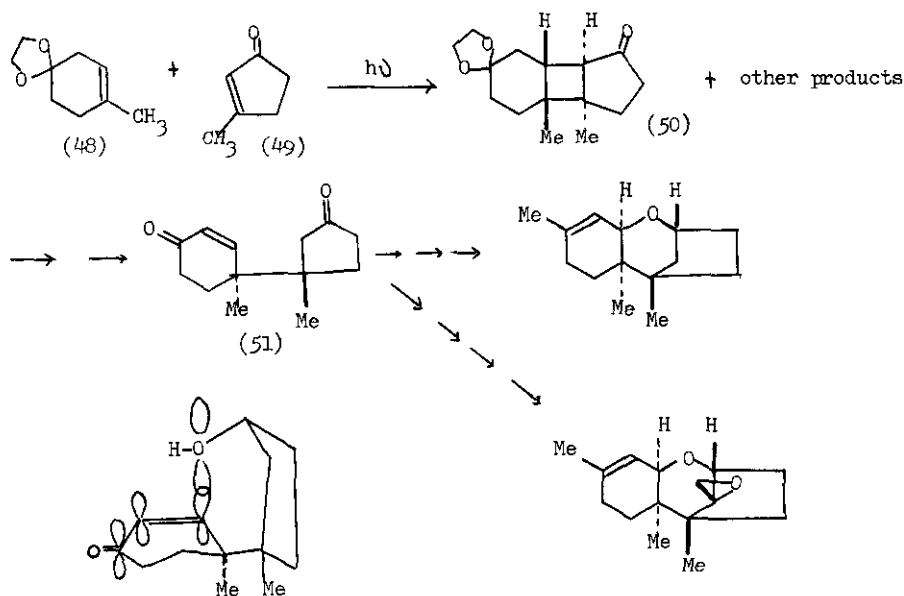
The tricyclic trichothecene skeleton synthesis branched at this point into the biogenetic approach involving the construction of ring A and C first, followed by a subsequent $\text{S}_{\text{N}}2^*$ intramolecular cyclization to form ring B. This approach has been inspired by Yanakawa and co-workers in 1973²⁰ who reported the photoaddition reaction between 3-methylcyclohexenone



and 2-hydroxy-3-methylcyclopentenone for the synthesis of trichodiene (101).

The head to head photocycloaddition reaction between 3-methylcyclohexenone and 2-hydroxy-3-methylcyclopentenone gave (46), with three other products in very low yield, followed by retro-aldol cleavage of the cyclobutane ring yielded the desired vicinal dimethyl compound (47), which could be converted into the required trichodiene (101).

The next total synthesis towards the tricyclic trichothecene metabolites utilising the photocycloaddition reaction of Yamakawa and co-workers²⁰ has been extensively studied by Kamikawa and co-worker²¹ with the synthesis of 13-nortrichotec-9(10)-ene in 1974 and 12,13-epoxy-trichotec-9-ene in 1976,²² its first successful biomimetic synthesis. Both these syntheses began with the (2 + 2) photocycloaddition of 4-methylcyclohex-3-en-1-one ethylene ketal (48) and 3-methylcyclopent-2-en-1-one (49) to give the required crystalline adduct (50) in 16% yield followed by conversion into the common enedione intermediate (51). The success in the formation of the cyclic ether ring B for the tricyclic trichothecene skeleton is highly dependent on the stereospecific cyclization that results as a consequence of

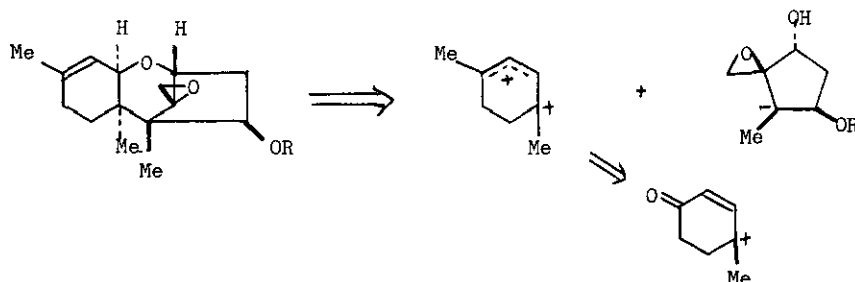


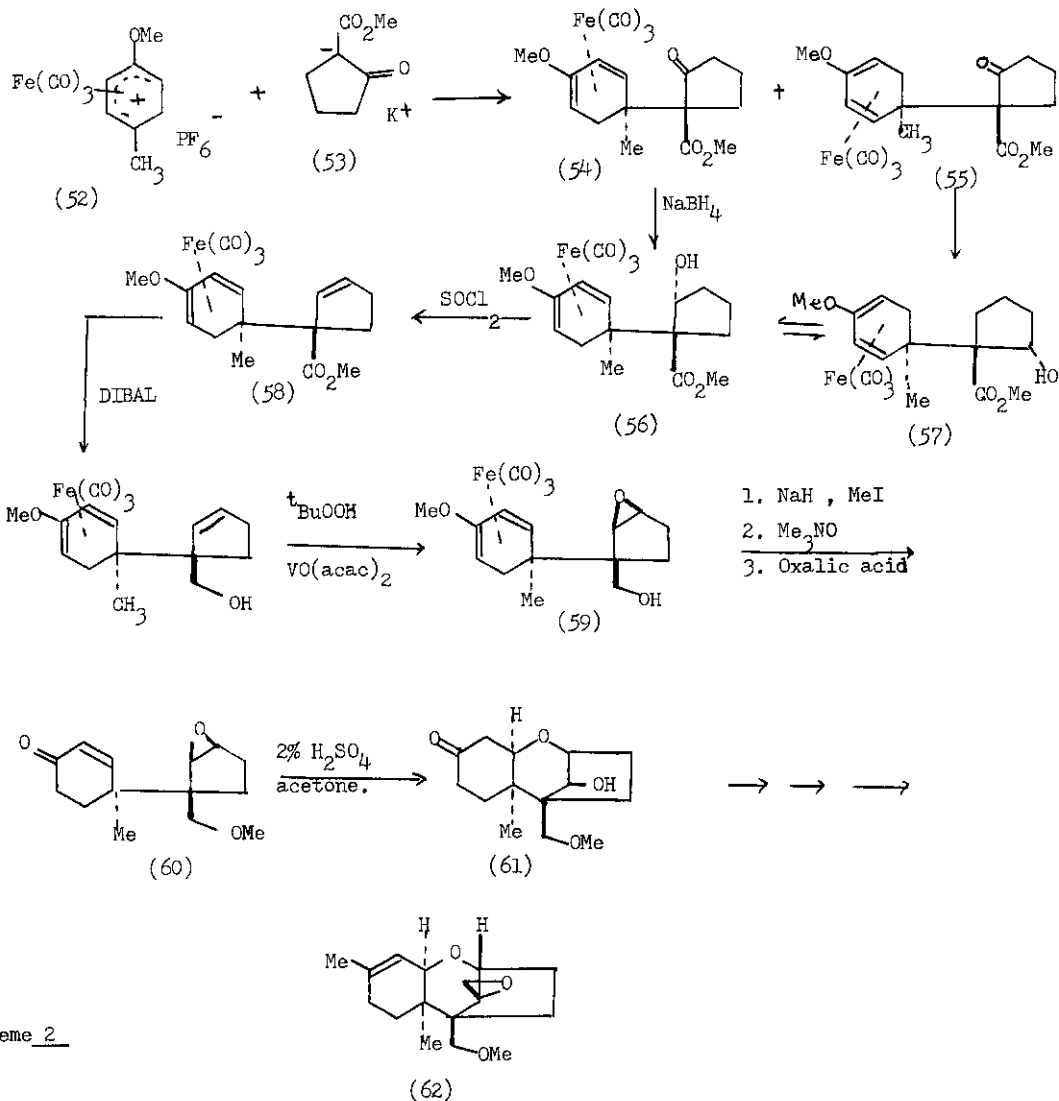
favourable orbital overlap during the addition (see intermediate) between the π -orbital of the $C=C$ bond and the p-orbital of the oxygen.

The synthesis of the polyoxygenated sesquiterpenes, trichodermol (also trichodermin) and the analogues described so far have several setbacks, mainly the low yield of certain key steps and the lack of total stereocontrol, an important criterion for modern synthesis. It is thus still a challenge for the synthetic chemist to find a convenient and high yielding preparation of these sesquiterpenes. An alternative source of these sesquiterpenes is required for a more thorough study of their mode of biological actions in which so little is known today.

Pearson in 1980²³ used retrosynthetic analysis towards the problem of trichothecene synthesis, which involved the disconnection as indicated in scheme 2. The approach using this scheme would require a reversal of polarity at the γ -position of the appropriate cyclohexenone and this exactly corresponds to the well known behavior of tricarbonyliron derivatives of the 2-methoxycyclohexadienyl cation (52). Methyl-2-cyclopentane-carboxylate (53) has been used as the cyclopentane synthon for ring C in the tricyclic trichothecene skeleton. Reaction of (52) and (53) gave rise to a diastereoisomeric mixtures of tricarbonyl[methyl 1-(2-5-4-methoxy-1-methylcyclohexa-2,4-dienyl)-2-oxocyclopentane-carboxylate] iron (54) and (55), which contain the A and C ring of the tricyclic trichothecene skeleton. This synthetic strategy could offer a very flexible approach for the synthesis of a wide variety of trichothecene analogues by using different substituted cyclopentane synthons as nucleophiles.

Using the method of Pearson²³ we have achieved the total synthesis of 12,13-epoxy-14-methoxytrichothecene (62) in this laboratory in a regio and stereoselective manner.^{24,25} Sodium borohydride reduction of (54) and the undesired isomer (55) gave the hydroxy ester (56) and (57) respectively, as single epimers in 100% yield. Treatment of (57) with 4-toluenesulphonic acid in dichloromethane at room temperature resulted in its conversion into an equimolar mixture of (56) and (57). In boiling dichloromethane, the required (56) becomes predominant (70-75%). Thus we are able to obtain (56) in yield in excess of 80% from the salt (52). Conversion of (56) into the alkene (58), and stereospecifically into the protected epoxy alcohol (59) was readily achieved in high yield as shown.²⁶ Removal of the iron moiety and enol ether hydrolysis gave the enone (60) which was converted into (61), now an advanced intermediate possessing the trichothecane ring system. Further elaboration of this compound into the final product, 12,13-epoxy-14-methoxytrichothecene was achieved using standard methods: i) methylation of the ketone using methyl lithium, ii) oxidation of the secondary alcohol, iii) dehydration of the tertiary alcohol, iv) Wittig reaction using methyltriphenylphosphonium bromide and v) epoxidation.

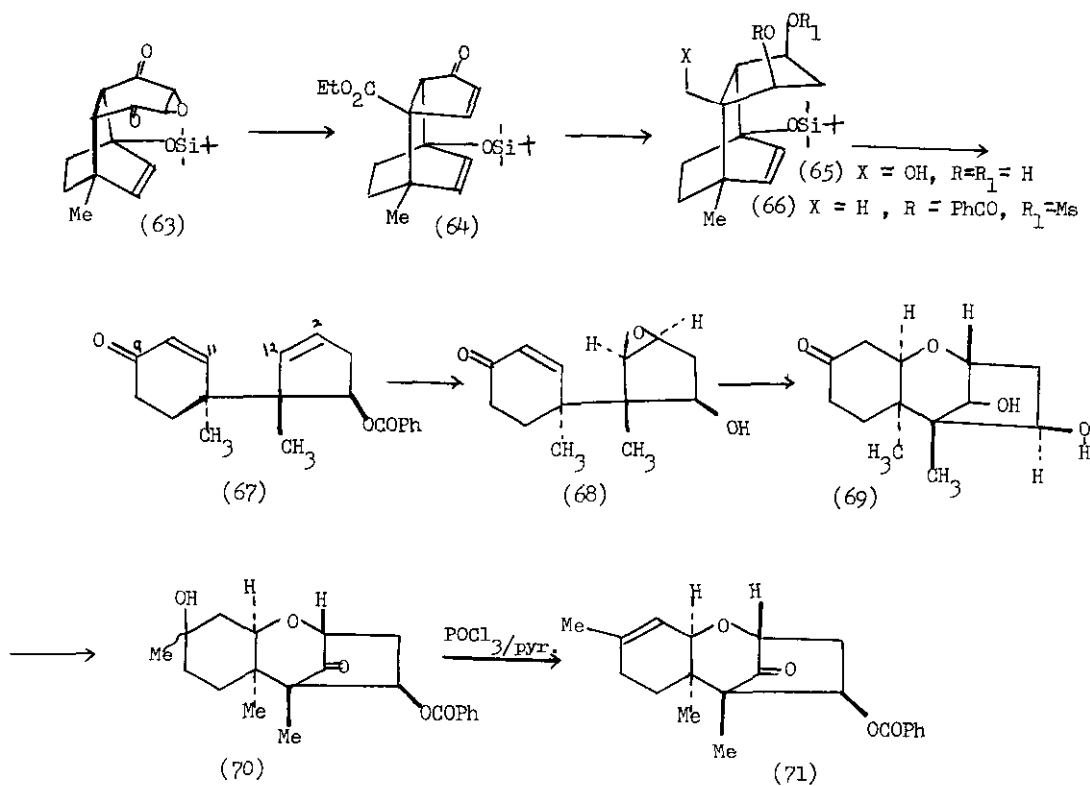




Scheme 2

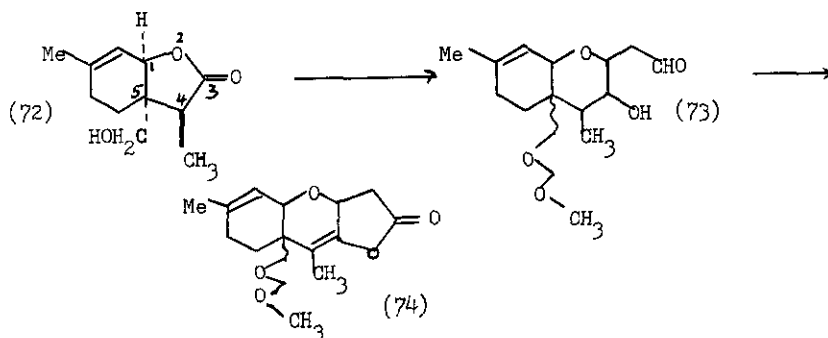
A convenient and high yielding preparation of (*f*)-trichodermol was reported recently by Still and co-workers²⁷ in 1980 along a biomimetic line of approach. Regio and stereocontrol are observed throughout the synthesis. Herz-Favorskii ring contraction²⁸ of (63) proceeded regiospecifically to give the crystalline cyclopentanonecarboxylic ester (64). The C-4 hydroxy group was then introduced stereospecifically by epoxidation followed by dissolving metal reduction to give the triol (65), which was then finally converted into (66). Anionic fragmentation then proceeds cleanly by deprotonation with sodium hydride to yield (67). Stereoselective trans-hydroxylation of C-2 and C-12 olefin in (67) required for trichodermol

synthesis was achieved using a hydroxyl directed epoxidation (t BuOOH, VO(acac)₂) of (67) to give the β -epoxide (68). Acid catalysed glycol formation gave the expected inversion at C-2 with direct formation of the bridged tricyclic intermediate (69), having all the required stereochemistry and functionality for transformation into trichodermol. It should be noted that (70) undergoes regioselective dehydration using phosphoryl oxychloride to give a 7:1 mixture of olefins, the major isomer being required for the synthesis of trichodermol (4c).



The polyoxygenated sesquiterpene, verrucarol, has not been totally ignored by chemist and is a great synthetic challenge yet to be met. Attempts towards the synthesis of verrucarol began to appear in 1978. All synthetic approaches during this period followed routes parallel to those of Colvin's, Raphael's and co-workers trichodermol synthesis.¹⁴ The synthetic attempt to verrucarol by Raphael and co-workers in 1978 is illustrated in scheme 2

The only difference between the synthetic approach towards verrucarol compared to trichodermol is the presence of the hydroxymethylene function at C-5 in (72) instead of the usual methyl group. (72) is converted into the advanced intermediate (73) through (74). It was predicted that aldol cyclization to establish the C-4 and C-5 bond to give the tricyclic intermediate for verrucarol synthesis should proceed smoothly as before in the trichodermol synthesis.

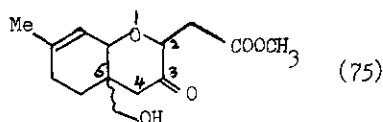


Scheme 3

Before the final attempt towards verrucarol synthesis had been achieved by Raphael and co-workers²⁹, various independent research groups became actively involved in the synthesis of various advanced intermediates.

Using a Diels-Alder reaction, Barry B. Snider and co-worker in 1978³⁰ synthesized the intermediate (72) in a much higher yield than the previously achieved.

At the same time Trost and co-worker in 1978³¹ published a synthetic strategy towards verrucarins with the synthesis of the intermediate (75), lacking the methyl group at C-4.



Extensive studies have been made to methylate the C-4 position without success.

But the intermediate (74) of Raphael and co-workers²⁹ did not undergo the analogous transformation as in the synthesis of trichodermol. Variation of the reduction process gave no trace of the required tricyclic product required for further transformation into verrucarol.

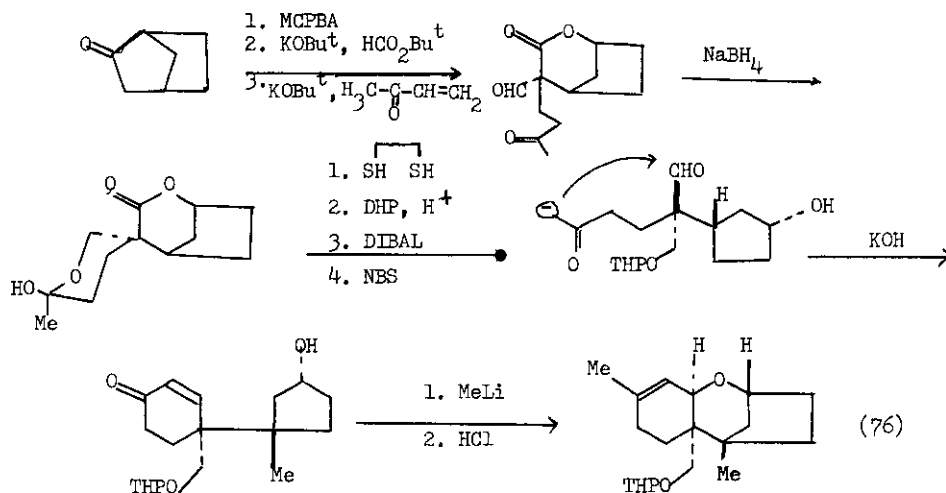
All the attempts toward verrucarol total synthesis so far have thus failed. No alternative approach for the transformation of (74) into verrucarol has yet been found. A more

successful study towards the total synthesis of verrucarol has recently been achieved

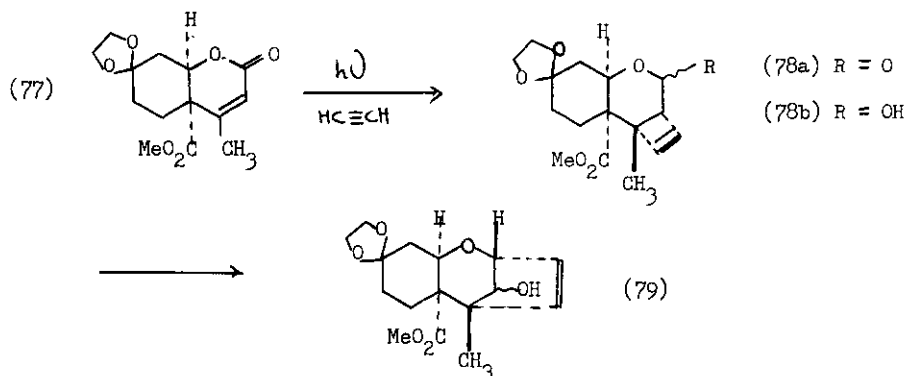
with the synthesis of 13,14-dinor-15-hydroxytrichotec-9-ene by Rousch and Ambra in 1980.¹³

It should be noted that this strategy reverted back to the biomimetic approach (scheme 4). It should also be noted that this molecule (76) lacks the functionalities at C-4 and C-12 required in verrucarol, and necessary for its biological activities. The introduction of the C-4 and C-12 functionalities using this synthetic strategy will be difficult to achieve for the total synthesis of verrucarol.

Scheme 4

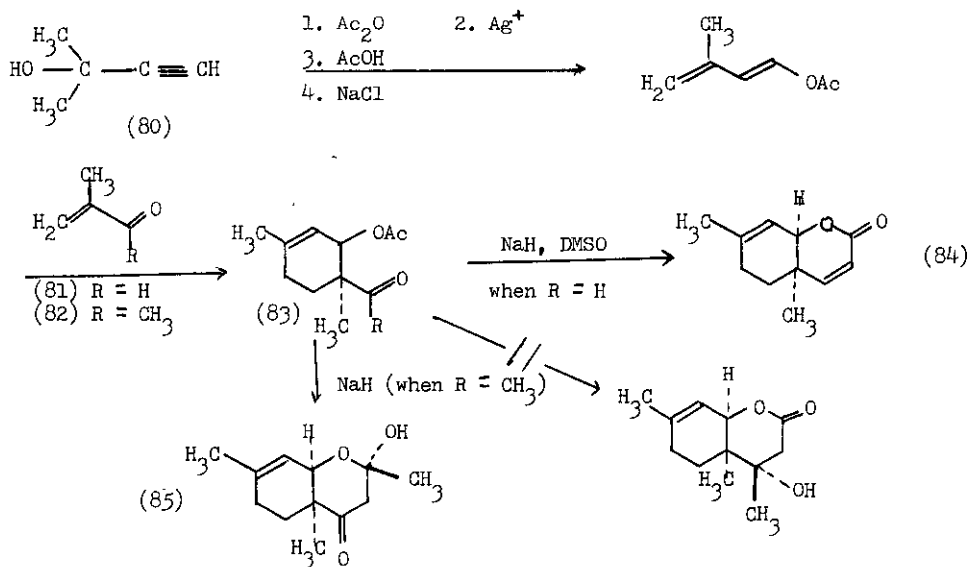


A more novel synthesis into the tricyclic nucleus of verrucarol has been achieved by White and co-workers in 1981.³² The crucial steps in this synthesis required a photochemical cycloaddition of acetylene to (77) and a rearrangement of the cyclobutenyl carbinol formed (78b) to give the cyclopentenol as the key step in the construction of ring C to give (79). Stereocontrolled synthesis of (79) makes it a potentially useful intermediate for the synthesis of functionalized trichothecoid system, including verrucarol, scirpenol derivatives and anguidine.



There remains a need for the development of more general synthetic methodology which would provide sufficient flexibility to be applied to a wide range of trichothecane structures or their analogues.

Stanley, Miller and co-workers in 1981³³ converted 2-methylbut-3-yn-2-ol (80) into the lactone (84) and (85) using a Diels-Alder reaction with either (81) or (82). The fused structures (84) and (85) have reactive functionalities in the heterocyclic ring and should allow a number of approaches to selected trichothecin by the addition of ring C and the C-12, C-13 epoxide ring in turn. No total synthesis has yet been reported using this methodology. Also, nucleophilic addition to the formyl group of the intermediate (83) should give rise to a variety of different substitution patterns at the C-5 center of trichothecane structure. But the carbonyl group was found to be too hindered for any reaction of nucleophiles to occur, reducing the synthetic flexibility of possible trichothecane analogues.

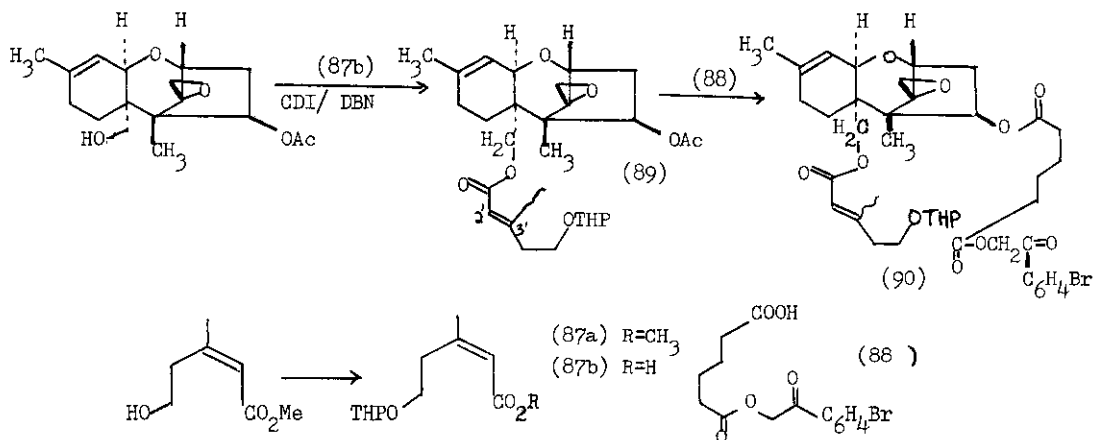


Impressive gains in the synthetic methodology relating to the tricyclic backbone have been illustrated, but by contrast there have been very few reports concerning the components of the macrocyclic ribbon. This is due to the unsuccessful synthetic approaches towards the total synthesis of verrucarol which prevented further progress in the total synthesis of the macrocyclic trichothecene esters.

The inability to synthesize verrucarol itself has not stopped chemists from developing appropriate methodology for the formation of the macrocyclic ribbon using verrucarol from naturally occurring sources. This macrocyclic ribbon with the intricate concentration of

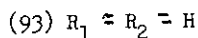
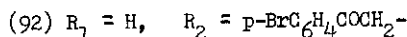
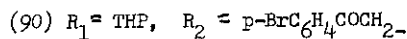
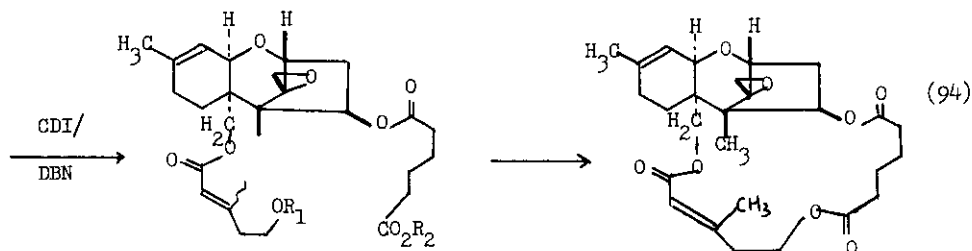
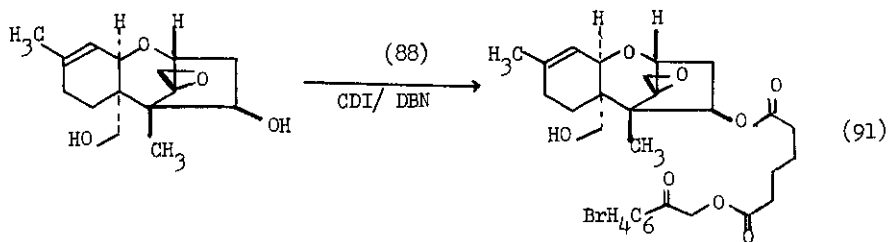
ether-ester-olefin-alcohol functionalities connected to the C-4 and C-15 hydroxy group are found to elicit profound biological effects and variation of these macrocyclic rings could provide an interesting biological study .

The first partial synthesis of a macrocyclic trichothecene esters from the sesquiterpene alcohol, verrucarol, was carried out by Tamm and co-worker in 1978³⁴ with the synthesis of tetrahydroverrucarin J . There are two preparative methods for the advanced intermediate (90), containing the two requisite side chains. Both these methods were based on the finding that the secondary hydroxy group of C-4 proved to be more reactive than the primary hydroxy group in verrucarol. Protection of verrucarol as the 4-O-acetyl derivative and condensation of the primary hydroxy group with (Z)-3-methyl-5-(tetrahydro-2-pyranyl)oxy-2-pentenic acid (87b) using N,N'-carbonyldiimidazol (CDI) with 1,5-Diazabicyclo-4,3,0-non-5-ene (DBN) as catalyst gave an isomeric mixture of E/Z (89) at the 2', 3' double bond. Deacetylation, followed by condensation of the C-4 secondary hydroxy group with adipic acid-mono(p-bromophenacyl ester) (88) gives the required advanced isomeric E/Z intermediate (90).

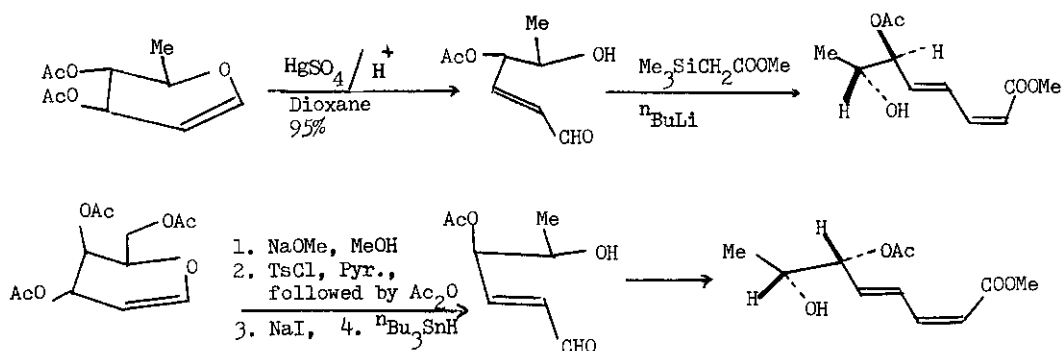


The second method involved the direct condensation of (88) with the more reactive secondary hydroxy group in verrucarol using CDI and DBN as catalyst to give (91). Further condensation of (87b) with (91) gave the advanced E/Z intermediate (90). In this case no protection and deprotection will be required.

(90) was then converted into E/Z (93) which can be lactonized with di-(Z-pyridyl)disulphide and triphenylphosphine to give the required tetrahydroverrucarin J of the E configuration, (94), as shown in the n.m.r. .



The discovery of the role played by the trichoverrins (104) and (105) in the biosynthesis of the macrocyclic trichothecenes (see page 26) suggests another viable synthetic route for the conversion of verrucarol to the highly biologically active macrocyclic trichothecenes using trichoverrins.^{35,36} Synthetic methodology using this biomimetic approach is now slowly



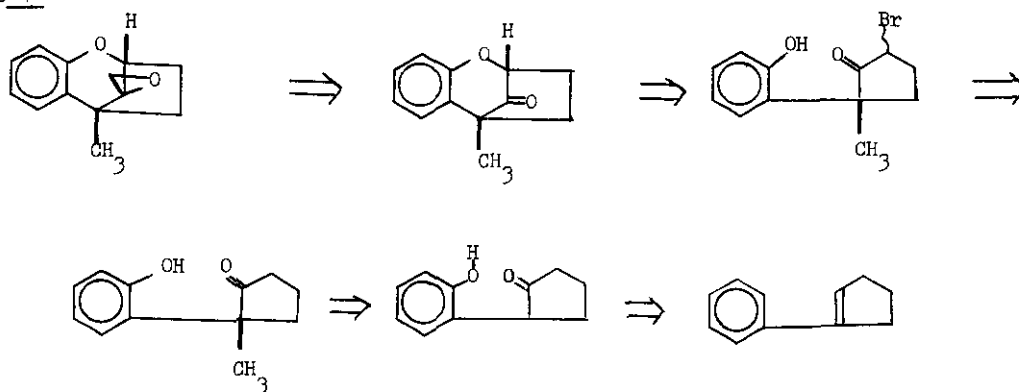
emerging. Reid and co-worker³⁷ in 1981 synthesized the C-4 octadienic ester of the trichoverrins from D-glucose.

A variety of synthetic analogues of the trichothecenes which possess an aromatic ring have been reported by Anderson and co-workers.^{38,39} They were prepared by routes parallel to

that developed along the biomimetic approaches. 6,9-Bisnormethyl-8-methoxy-12,13-epoxy-6,8,10-trichothetriene (98) was synthesized by Anderson and co-worker³⁸ using the retrosynthetic analysis presented in scheme 4. Reaction of sodium p-methoxyphenoxide with 3-chlorocyclopentene gave 3-(p-methoxy-phenyloxy)cyclopentene (95) which undergoes Claisen rearrangement to give 2-(3'-cyclopentenyl)-4-methoxyphenol (96).

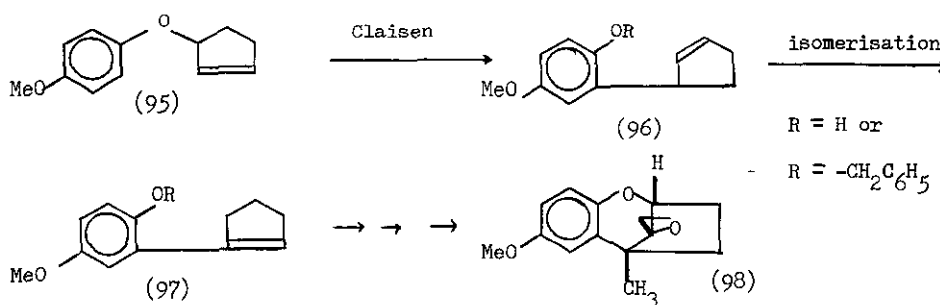
Protection of the phenol and isomerization of the cyclopentene double bond were achieved by

Scheme 4



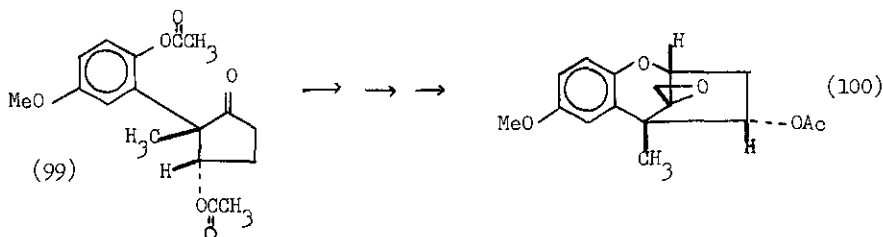
treatment with potassium t-butoxide in dimethyl sulphoxide and water to give the requisite intermediate, 2-(1'-cyclopentenyl)-1-benzyloxy-4-methoxy benzene (97). (97) was transformed into the aromatic trichothecane (98) using the route presented in scheme 4.

A further synthetic analogue of the aromatic trichothecane with a functionalised C-4



position, 15,16-dinor-4 α -acetoxy-8-methoxy-6,8,10-trichothecatriene 12,13-epoxide (100) was achieved by the same authors in 1980.³⁹ (100) was prepared from 2 α -(2-acetoxy-5-methoxyphenyl)-2 β -methyl-3 α -acetoxy-cyclopentanone (99) in three steps, bromination, DBN induced cyclization, and spiroepoxidation. This analogue (100) has a closer resemblance to the naturally occurring trichothecanes.

The authors also reported that these A-ring aromatic trichothecane analogues possess significant in vivo antileukemic activity. This should inspire other chemists to synthesize more trichothecane analogues.

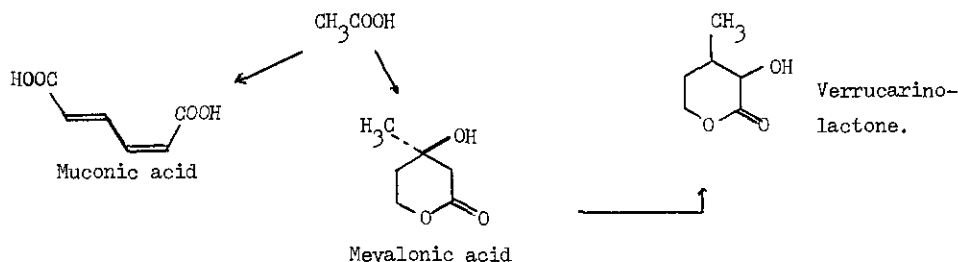


V. BIOSYNTHESIS 1b,40

The biosynthetic pathway has been investigated by the feeding experiments of some likely ^{14}C and ^3H labelled precursors to growing culture of *Myrothecium*.

Acetate, the simplest two carbon unit was found to be incorporated into the trichothecene toxins, and the distribution of radioactivity suggested that 12 acetate units make up verrucarin A and of these, 6 acetate units are located in the verrucurool skeleta; and 3 each in the verrucarinolactone and cis, trans muconic acid which made up the macrocyclic ribbon.

The acetate units that make up the trichothecane skeleta were first transformed into

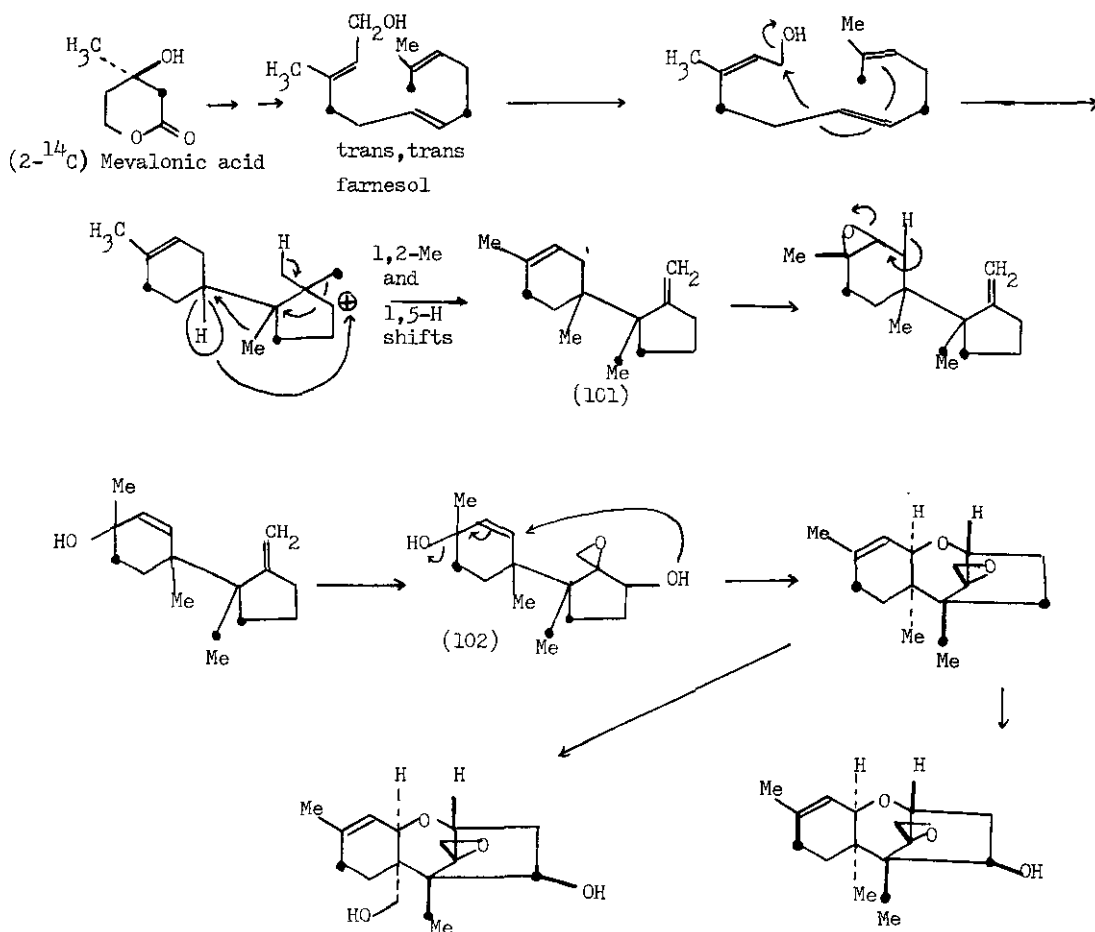


mevalonates. Studies with medium containing (2- ^{14}C)-mevalonate revealed that 3 molecules of mevalonate were incorporated into trichothecin to form the initially labelled trans,trans farnesol, confirming the sesquiterpene nature of the trichothecane skeleta.

The role of farnesyl pyrophosphate as a precursor of the trichothecene nucleus was confirmed by incorporating (2-¹⁴C)-farnesyl pyrophosphate into the trichothecene nucleus.⁴¹

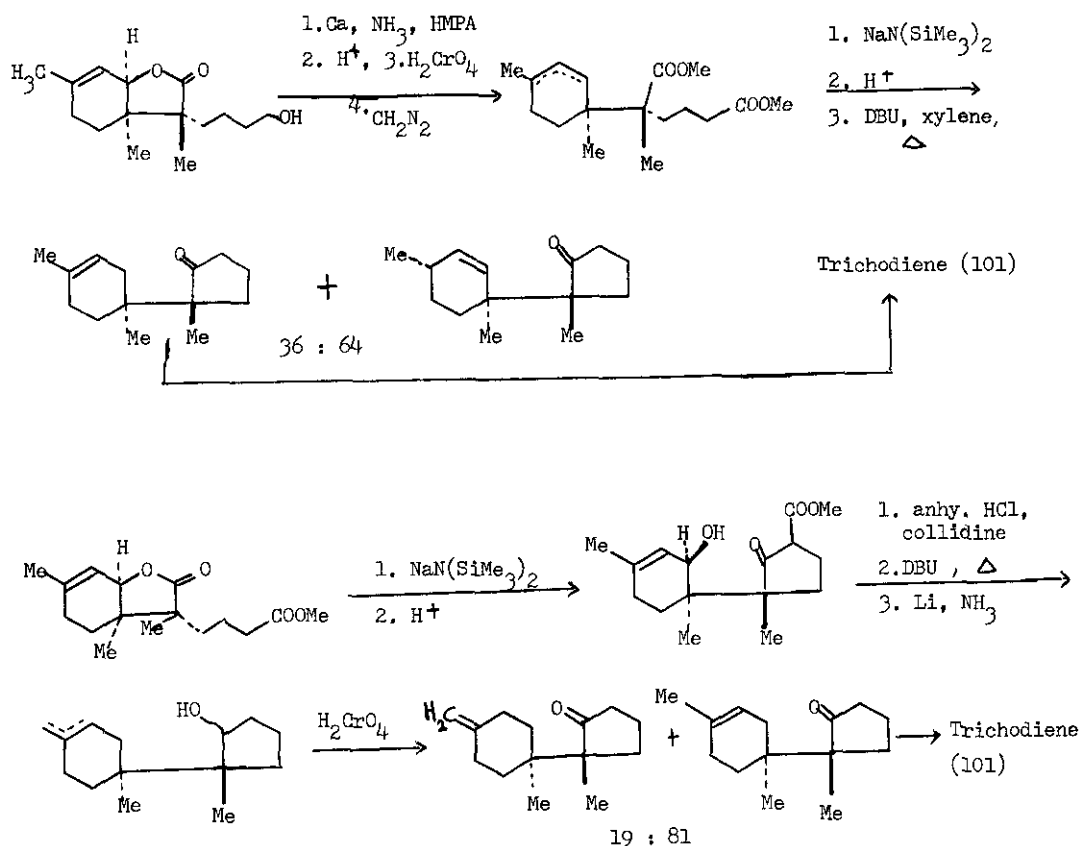
Biogenetically, the trichothecene ring system arises by the cyclization of farnesyl pyrophosphate, followed by two 1,2-methyl shifts and a 1,5-hydrogen shift to give the intermediate trichodiene (101).⁴²

It has been proposed by Muller and Tamm⁴³ that the formation of trichothecene from trichodiene then proceeds through trichodiol (102), an epoxide intermediate. The recent detection of baccharin strongly favours this epoxide intermediate.

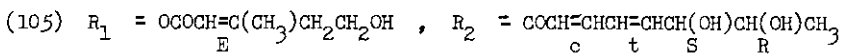
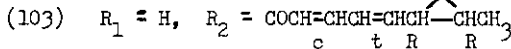
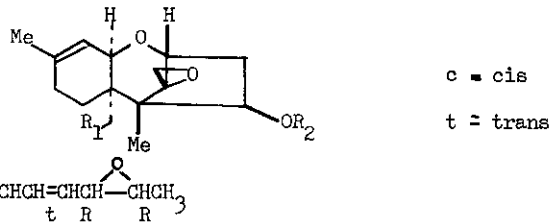


The biosynthetic sequence between trichodiene and trichothecene is still unclear, but the postulation of Muller and Tamm⁴³ has been favoured. In order to have a better understanding, the ready availability of labelled trichodiene for feeding experiments is needed. This should help to elucidate the complete biosynthetic sequence of trichothecene.

Recently, the stereoselective total synthesis of (+)-trichodiene (101), the advance biosynthetic intermediate of trichothecene has been achieved by Welch and co-workers in 1980,⁴⁴ as illustrated by the scheme below.



The biosynthetic pathway of the macrocyclic trichothecene has now slowly been uncovered. Jarvis and co-workers^{35,36} in 1981 reported the isolation of a series of new trichothecenes, namely trichodermadiene (103) and trichoverrin A (104) and B (105) which are unique in that they possess all the necessary elements to be macrocyclic and yet are non-macrocyclic.



These compounds will help to shed more light on the biosynthetic pathway towards macrocyclic trichothecene esters. Trichoverrins A (104) and B (105) appear as likely candidates for precursors to roridin E and/or isororidin E since ring closure and dehydration of trichoverrins A and/or B would lead to these compounds. Experimental studies by incubation of 100mg each of trichoverrins A and B with a resting culture of *M. verrucaria* was found to give 12mg of verrucarins A, 5mg of verrucarins B and an appreciable amount of roridin A and isororidin E, plus recovered trichoverrins. These experiments strongly suggest that trichoverrins lie along the biosynthetic pathway to the macrocyclic trichothecene esters.

VI. MODE OF ACTION AND BIOLOGICAL PROPERTIES. ⁴⁵

The first indication that members of this class of compounds, the macrocyclic trichothecene esters might possess some utility as antitumor agents came from the reports of Harri and co-workers in 1962 ⁴⁶ on the activity of verrucarins and roridins in mouse tumor cell tissue culture experiments. Since these initial reports, a majority of trichothecenes have been reported to possess cytotoxic activity in tissue culture. As a group, trichothecenes exhibit a very broad spectrum of activities, including radiomimetic (cytotoxic and cytostatic), dermatological, phytotoxic, insecticidal and hematological effects.

The mechanism of action of the trichothecenes appeared to be the effect on the synthesis of macromolecules (mainly proteins) in both culture cells and cell free system. ⁴⁷ On the basis of their effect on protein synthesis and their structure, the compounds were divided into two broad classes by Ueno and co-workers in 1973 ⁴⁸: those having multiple hydroxy function as class A, example, T-2 toxin (106), HT-2 toxin (107), neosolaniol (108) and anguidine (109); and those bearing an 8-keto function as class B, example, nivalenol (110),

fusarenon-X (111), and trichothecin. The class A compounds are more potent in effecting protein synthesis, cytotoxic and polysomal breakup compared to the class B compounds in whole cell preparations. This illustrates the importance of the transport system involved, which favours the class A compounds.

Protein synthesis takes place in three different phases, i) initiation, ii) elongation, iii) termination. Trichothecenes can effect any one of these phases of protein synthesis. On this basis, the trichothecenes can be further divided into three classes, i) initiation inhibitors or the I-type, example nivalenol, T-2 toxin and verrucaric acid; ii) elongation inhibitors or the E-type, example trichodermin and trichothecin; and iii) termination inhibitors or the T-type, example trichodermin and 4-epitrichodermin.

The structural activity relationship for the I-type and E-type activities were defined by Wei and McLaughlin.⁴⁹ Substitution of the 4 β -position by an acylated alcohol is necessary for E-type activity. Substitution at C-15 on the α -face by an acylated alcohol exhibits I-type activity. There is no clear cut division between I-type and E-type activity. It has been demonstrated by various groups^{50,51} that the expression of E-type or I-type activity is dependent upon the concentration at which the compound is given.

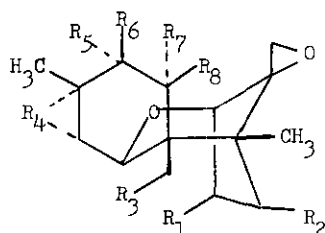
It has also been established that certain trichothecenes, especially anguidine showed significant activity on L-1210 and P-388 leukemia in mice. Anguidine has been extensively studied for its antileukemic activity and is currently under clinical trial.

Structure Activity Relationship - The 12,13-epoxide function in the chromophore of trichothecenes is essential for activity. Removal of the epoxide ring by reduction using lithium aluminium hydride^{1b} has led to a complete loss of activity. Also it is evident that conversion of the 9,10-double bond to an epoxide results in greatly enhanced activity. Similarly, a great increase in activity was also seen on the introduction of an 8-hydroxy function. However, the increase in activity is accompanied by a loss in potency.

Variation in the macrocyclic ribbon of the trichothecene macrocyclic esters occurs for most part at the 2', 3', 4' and 7' positions. The presence of a double bond between the 2' and 3' positions confers a greater potency and activity than when the 2' position is hydroxylated. Acetylation of the 2'-hydroxyl group results in a slight increase of potency.

A systematic study using one or more of the naturally occurring trichothecenes and the macrocyclic trichothecene esters as a starting point and varying the substitution pattern at all accessible positions should be carried out and would probably be most useful in establishing the structure activity relationships. This could lead to an optimal drug for

use against neoplastic diseases in man.



	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
T-2 toxin (106)	OH	OAc	OAc	-	O-i-Val	H	H	H
HT-2 toxin (107)	OH	OH	OAc	-	O-i-Val	H	H	H
Neosolaniol (108)	OH	OAc	OAc	-	OH	H	H	H
Anguidine (109)	OH	OAc	OAc	-	H	H	H	H
Nivalenol (110)	OH	OH	OH	-	<u>O</u>		H	OH
Fusarenon-X (111)	OH	OAc	OH	-	O		H	OH
Trichothecin	H	OCot	H	-	O		H	OH

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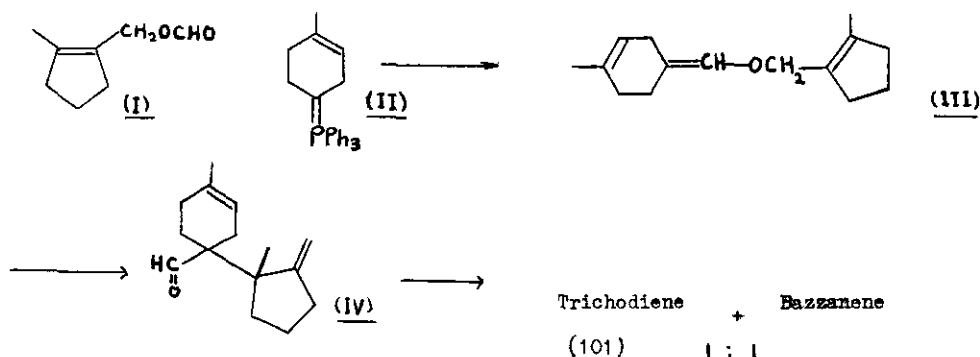
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Addendum

1. (±)-Trichodiene and (±)-Bazzanene Total Synthesis.

(Minoru Suda, Tet. Letts., 1982, 427.)

The sesquiterpene hydrocarbons, bazzanene and trichodiene were synthesized in three steps from a substituted allyl formate (I) by i) reaction with the corresponding phosphonium ylid (II) produced the vinyl ether (III), ii) Claisen rearrangement to give the aldehyde (IV) of a 1 : 1 mixture of the two diastereomers and, iii) Wolff-Kishner reduction produced the desired trichodiene and bazzanene as a 1 : 1 mixture.

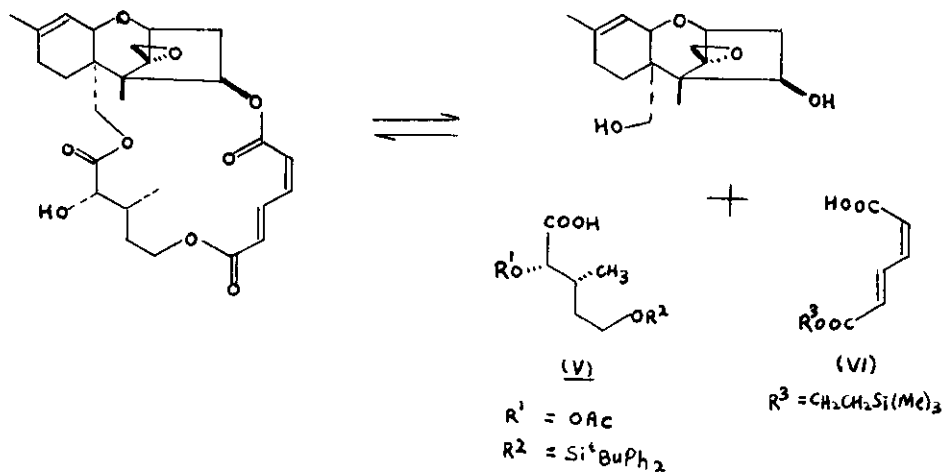


2. Verrucarin A Partial Synthesis

(W. C. Still and H. Ohmizu, J. Org. Chem., 1981, 46, 5242.)

This is the first partial synthesis of a naturally occurring macrocyclic trichothecane ester, verrucarin A, from verrucarol.

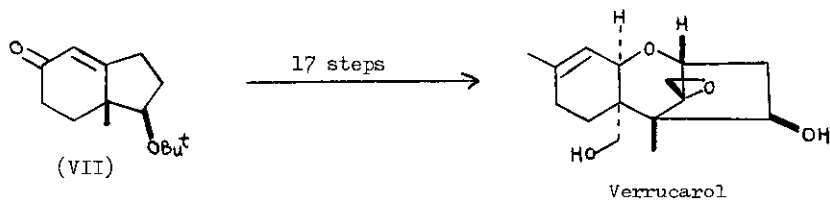
Selective esterifications of verrucarol by the verrucarinic acid (V) and muconic acid (VI) were accomplished by the DCC method. Under appropriate conditions, only the primary alcohol was esterified with verrucarinic acid. The more reactive muconic acid then reacted cleanly to the secondary alcohol under the same condition. Ring closure to form the macrocyclic 'ribbon' gave the synthetic verrucarin A.



Total Synthesis of Racemic Verrucarol.

The first total synthesis of racemic verrucarol has been achieved by R.H. Schlessinger and co-worker recently in 1982 (J. Am. Chem. Soc., 1982, 104, 1116).

The racemic verrucarol was obtained in 3.4% overall yield from the readily available ketonic substance (VII) in seventeen steps.



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