Trichodermin, a New Sesquiterpene Antibiotic*

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The antifungal antibiotic trichodermin, $C_{17}H_{24}O_4$, which is the acetate of a sesquiterpene alcohol trichodermol, $C_{15}H_{22}O_3$, has been correlated with trichothecin. The structure based upon the previously proposed trichothecin structure (Ia) was incompatible with certain reactions, and trichodermol-p-bromobenzoate was therefore subjected to an X-ray crystallographic analysis which revealed that trichodermol represents a new sesquiterpene type (IIIc). Based upon the trichodermin structure a revised formula of trichothecin (IIId) is proposed. The relationship of trichodermin (IIIb) to the verrucarines and roridines is discussed. Some unusual rearrangements of the trichodermol ring system are described, and its biogenesis shortly discussed.

From the culture fluid of a strain of *Trichoderma viride*, isolated from a soil sample collected in New Guinea, a hitherto unrecorded antifungal antibiotic called trichodermin has been isolated.[1] The active compound was extracted from the clarified broth with light petroleum, and purified by chromatography over neutral alumina. Originally, trichodermin was isolated in the form of a colourless, viscous oil, which, however, after standing for several weeks crystallized to yield crystals of m.p. 45—46°. It is a neutral compound, sparingly soluble in water, but soluble in all common organic solvents at room temperature. The antibiotic is active against a variety of pathogenic fungi, including *Candida albicans*, and inhibits in low concentrations the growth of various cell types in tissue culture.

FUNCTIONAL GROUPS AND PARTIAL STRUCTURE

Trichodermin contains carbon, hydrogen and oxygen only. The elementary analysis and the molecular weight determination (Rast) correspond well with the formula $C_{17}H_{24}O_4$. The IR-spectrum contains bands at 1730 (CO), 1682 (C=C, trisubstituted), 1245 and 1225 (acetate), and 1085 cm^{-1} (C—O—C), whereas no hydroxyl bands could be detected. The ultraviolet spectrum (λ_{max}^{EtOH} *ca.* 200 mμ (ε 2400)) shows the presence of an isolated double bond.

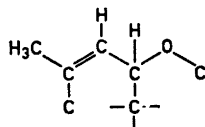* For a preliminary communication see *Proc. Chem. Soc.* **1964** 188.

Alkaline hydrolysis of trichodermin gave an alcohol, trichodermol,* $C_{15}H_{22}O_3$ (m.p. 118°), the IR-spectrum of which contains no bands in the carbonyl region. On acetylation (acetic anhydride-pyridine) trichodermol was reconverted into its progenitor in high yield indicating that no rearrangements have taken place during the saponification.

Trichodermol on catalytic hydrogenation over palladium on calcium carbonate took up one mole of hydrogen. The resulting dihydrotrichodermol, $C_{15}H_{24}O_3$, (m.p. 92—93°) shows no selective absorption in the ultraviolet, and consequently does not contain double bonds resistant to hydrogenation. Since no carbonyl groups are present it follows from the empirical formula that trichodermin contains a tetracyclic ring system.

Oxidation of trichodermol with chromium(VI)-oxide in 95 % acetic acid gave a ketone, trichodermone, $C_{15}H_{20}O_3$ (m.p. 154—155°), the IR-spectrum of which contains a strong band at 1735 cm^{-1} (five-ring ketone) indicating that the hydroxyl group in trichodermol is secondary and located in a five-membered ring.
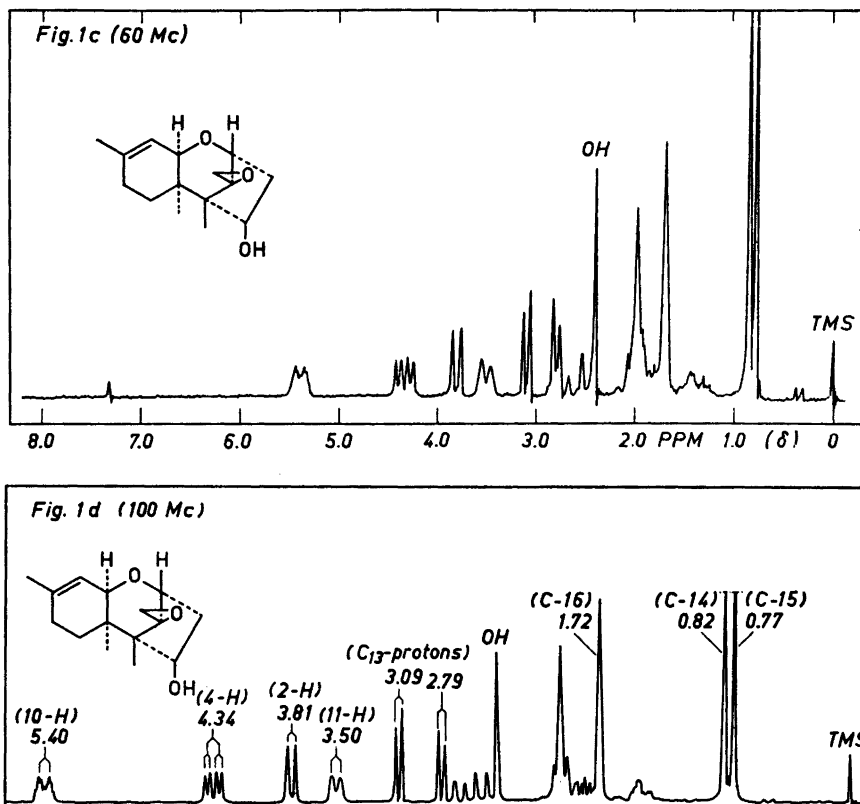
A study of the NMR-spectra of trichodermin, trichodermol, and trichodermone gave valuable information.** The spectra of trichodermol and trichodermin in deuterochloroform were recorded at 60 and 100 Mc, (Fig. 1, a—d). The two singlets at δ 0.77 and δ 0.82 in the spectrum of trichodermol reveal the presence of two quaternary methyl groups, and the singlet at δ 1.70 can be attributed to a methyl group attached to a carbon-carbon double bond. The AB-system consisting of two doublets centered at δ 2.79 and δ 3.09 (J_{AB} 4 c.p.s.) is characteristic of the grouping $\overset{CH_2-O}{>C<}$ [3] but could possibly also be due to the methylene group in an oxetane ring of the type: $-\overset{\overset{CH_2-O}{|}}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-$ although the magnitude of the coupling constant favors the first possibility.[4]

Of the three O—C—H signals (δ 3.50, δ 3.81, and δ 4.34) the quartet at δ 4.34 is due to the proton at the hydroxylbearing carbon atom since this signal in the spectrum of trichodermin is shifted to a lower field (δ 5.58) and has disappeared in the spectrum of trichodermone. It will be noted that only one vinylic proton is present (signal at δ 5.40) which implies that the double bond in trichodermol is trisubstituted. Spin-spin decoupling shows that its complex pattern is due to long range coupling with the methyl group at δ 1.70 since irradiation of the latter causes the signal to collapse into a doublet. The

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Fig. 1a (60 Mc)

Fig. 1b (100 Mc)

neighboring proton is evidently that which is responsible for the doublet at δ 3.50 and the following situation is therefore suggested.

The six protons in the trichodermol spectrum which have not been accounted for are all in the region δ 1.3 to 2.6.

By combining the NMR-evidence with the spectroscopic and chemical information it was possible with a reasonable degree of probability to establish the following partial structure:

CORRELATION WITH TRICHOTHECIN

This partial structure suggested a relationship to trichothecin,[5] an antifungal metabolite of *Trichothecium roseum* L. Trichothecin, the structure of which has been studied by Freeman, Gill, and Waring [6] and by Fishman,

Fig. 1c (60 Mc)

Fig. 1d (100 Mc)

Jones, Lowe, and Whiting [7] is the isocrotonyl ester of an alcohol trichothecolone, which has been assigned structure Ib.[6,7] A remarkable feature in this structure is the presence of a four-membered oxide ring, not previously encountered in natural products. It will be noted, that structure Ib contains all the features deduced above for trichodermol with the modification that a keto group is present allylic to the double bond. Since the empirical formulae show that trichothecolone contains one oxygen atom more and two hydrogen atoms less than trichodermol, and since many reactions of the two compounds proceed analogously (vide infra) it was tempting to try to correlate a suitable derivative of trichodermol with the corresponding derivative of trichothecolone by introduction of a keto group in allylic position to the double bond. This was at first attempted with trichodermone, and actually vigorous chromium-(VI)-oxide oxidation of this compound gave a 5 % yield of a substance, which in all respects was identical with an authentic sample of trichothecodione,[6]* the ketone corresponding to trichothecolone. In order to determine whether

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* Trichothecodione was prepared according to Freeman et al.[6] from a sample of trichothecin, kindly submitted by Dr. Freeman, I.C.I., Ayrshire.
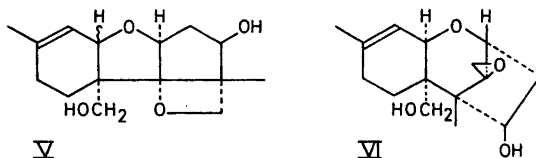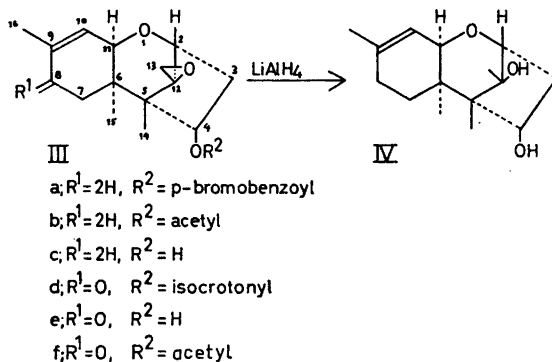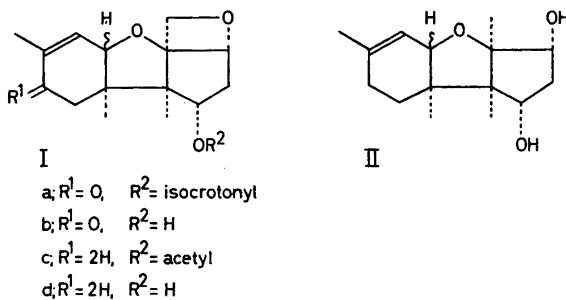
the hydroxyl groups in trichodermol and trichothecolone have the same or the opposite orientation trichodermin was subsequently oxidized in a similar manner. In addition to unchanged starting material and two unidentified acids, $C_{16}H_{22}O_6$ and $C_{15}H_{18}O_4$, a small amount of trichothecolone acetate could be isolated after chromatography of the reaction mixture. As a consequence of these oxidations the structures of trichodermin and trichodermol should be represented by formulae Ic and Id provided that structure Ia is correct and that no rearrangements have taken place during the oxidations.

The similarity of the NMR- and IR-spectra of corresponding members of the two series of compounds and the analogous course of many reactions seem to exclude the possibility of such rearrangements. On the other hand, certain reactions of trichodermin and its derivatives were difficult to interpret in the light of structure Ic, the major difficulty in accepting this structure being the outcome of the $LiAlH_4$-reduction of trichodermol: The reaction product, $C_{15}H_{24}O_3$, (m.p. 148°) was a diol, the NMR-spectrum of which shows the presence of one quaternary methyl group (singlet at δ 1.47) more than in the spectrum of trichodermol. The AB-system (doublets at δ 2.79 and δ 3.09) in the spectrum of the latter, which had been assigned the methylene group in the four-membered oxide ring, was absent in the spectrum of the diol, and this compound should consequently be represented by structure II. The fact that acetylation of the diol gave a mono-acetate and chromium(VI)-oxide oxidation a monoketone indicates, however, that the new hydroxyl group is tertiary and suggests that trichodermin contains the grouping $\overset{CH_2-O}{\underset{\diagdown}{>}C<}$. Because of this and other disagreements with structure Ic an appropriate derivative of trichodermol namely the p-bromobenzoate,* was subjected to an X-ray crystallographic analysis through the courtesy of Dr. Abrahamsson. The crystallographic work [8] revealed the presence of an epoxide ring, and has rigorously established that this compound has the constitution and configuration, apart from absolute configuration, depicted in IIIa. The structures of trichodermin and trichodermol are consequently IIIb and IIIc, respectively, and the diol, obtained on $LiAlH_4$-reduction of trichodermol, must evidently be represented by IV.

Because of the transformations of trichodermone into trichothecodione and of trichodermin into trichothecolone acetate, and because of the close analogy between the spectral and chemical behaviour of corresponding derivatives of trichodermin and trichothecin (*vide infra*) the structures of trichothecin and trichothecolone should be revised into IIId and IIIe, respectively.

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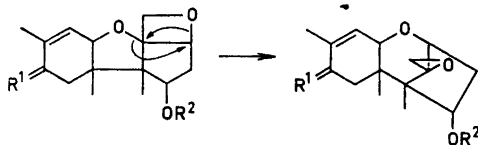
* The fact that alkaline hydrolysis of the p-bromobenzoate gave a high yield of trichodermol excludes that rearrangements have taken place during its preparation.

I

a; R^1 = O, R^2 = isocrotonyl
b; R^1 = O, R^2 = H
c; R^1 = 2H, R^2 = acetyl
d; R^1 = 2H, R^2 = H

II

III

a; R^1 = 2H, R^2 = p-bromobenzoyl
b; R^1 = 2H, R^2 = acetyl
c; R^1 = 2H, R^2 = H
d; R^1 = O, R^2 = isocrotonyl
e; R^1 = O, R^2 = H
f; R^1 = O, R^2 = acetyl

LiAlH₄ →

IV

V

VI

It should be noted that formula III, which at first glance seems rather different from I, formally may be derived from the latter by interchanging two C—O bonds as indicated above, when the stereochemistry is left out of account.

RELATIONSHIP TO VERRUCAROL

The ring system in trichodermin and trichothecin represents a new sesquiterpene type, which seems to be fairly widespread in nature. Thus, certain members of the group of antifungal and cytostatic antibiotics called roridanes and verrucarines,[2] which are formed by various strains of *Myrothecium roridum* and *Myrothecium verrucaria*, contain this ring system. The

most important and best investigated member of this group, verrucarin A,*
$C_{27}H_{34}O_9$, is a neutral compound which upon alkaline hydrolysis is cleaved
into three fragments: A sesquiterpene alcohol, verrucarol, $C_{15}H_{22}O_4$, cis,trans-
muconic acid, and a lactone, verrucarinolactone which has been shown to
be the δ-lactone of trans-α, δ-dihydroxy-β-methylvaleric acid.[9],[10] Structure V
has recently been proposed for verrucarol,[11] but because of the identity between
trichodermol and roridine C (a minor metabolite of a strain of Myrothecium
roridum, which also produces verrucarin A) it was suggested in the preliminary
communication corresponding to this paper that verrucarol contains the
same ring system as trichodermin and, on the basis of the data reported
by Gutzwiller and Tamm,[11] formula VI was proposed for this compound.
This structure has now been proved by Gutzwiller et al.[12]** who have succeeded
in correlating verrucarol and trichodermol and unambigously established the
location of the primary hydroxyl group.

In addition to verrucarin A numerous other verrucarines and roridines
contain verrucarol as a part of the molecule.[13]

## REARRANGEMENTS OF THE TRICHODERMIN RING SYSTEM

A predominant feature of the chemistry of trichodermin and its derivatives
is the fact that the epoxide under certain circumstances is susceptible to
intramolecular nucleophilic attacks accompanied by skeletal rearrangements.
This feature is also found in other compounds containing the same ring system,
such as trichothecin [6],[7] and verrucarol,[12] and has undoubtedly complicated
the elucidation of the structures of these. In the following some of these
reactions will be discussed.

1. "Neotrichodermone". Inspection of a model of trichodermol reveals
that the epoxide is strongly shielded against nucleophilic attack from the rear
by external anions. This shielding is reflected in the fact that trichodermol
as well as trichothecolone [6] and verrucarol [12] are unaffected by hot dilute alkali.
In contrast, trichodermone (VIIa) isomerizes smoothly on treatment with
sodium carbonate to an α,β-unsaturated ketone containing a primary hydroxyl
group. This behaviour is analogous with the formation of neotrichothecodione
from trichothecodione under similar conditions. On the basis of a series of
reliable experiments and interpretations neotrichothecodione has been assigned
structure VIIIb,***[6],[7] and "neotrichodermone" should consequently be re-
presented by VIIIa. This assignment is supported by the NMR-spectrum
(Fig. 2) which shows the presence of two vinylic protons (doublets centered
at δ 6.30 and δ 7.38 J_{AB} 6 c.p.s.) not present in trichodermone, while the
AB-system corresponding to the C_{13}-methylene group (doublets at δ 2.79 and
δ 3.09) as well as the signal due to the C_2-proton (doublet at δ 3.81) in the
spectrum of trichodermone are absent.

* The antibiotic muconomycin A [9] seems to be identical with verrucarin A.
** We are indebted¡ to Dr. H. P. Sigg, Sandoz A.G., Basel, for sending us the manuscript of
this paper prior to its publication.
*** The stereochemistry shown in VIII, and XI, which differs from that originally proposed,[7]
is a natural consequence of the established stereochemistry of trichodermol-p-bromobenzoate.
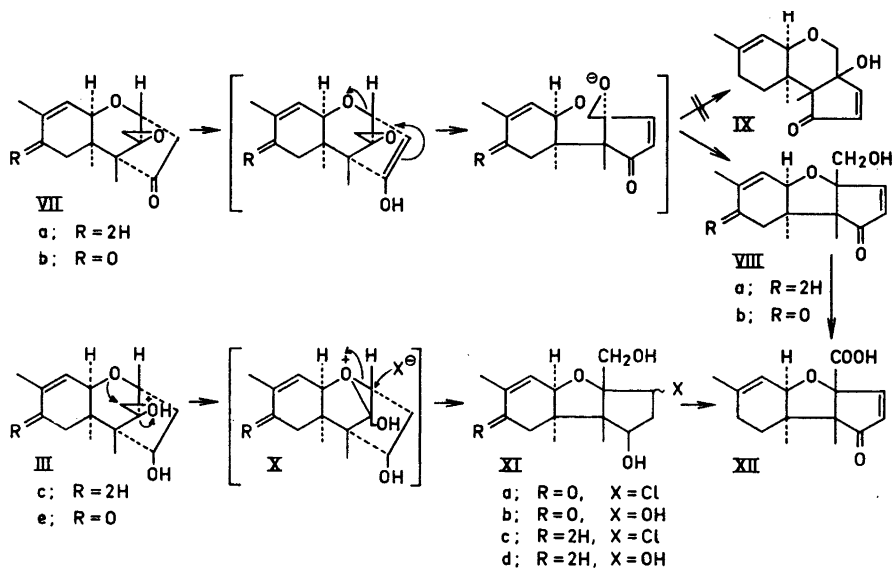
A plausible mechanism for the conversion of trichodermone into neotrichodermone is an initial opening of the tetrahydropyrane ring, facilitated through enolization of the ketone, followed by an internal nucleophilic attack on the epoxide ring (VII → VIII).

The direction of the epoxide opening in this reaction is abnormal[14] (a normal opening would lead to compound IX), and since inspection of models does not offer a satisfactory steric explanation of this phenomenon the reason must be that the formation of the tetrahydrofurane ring in VIII proceeds with a much greater velocity than the formation of the tetrahydropyrane
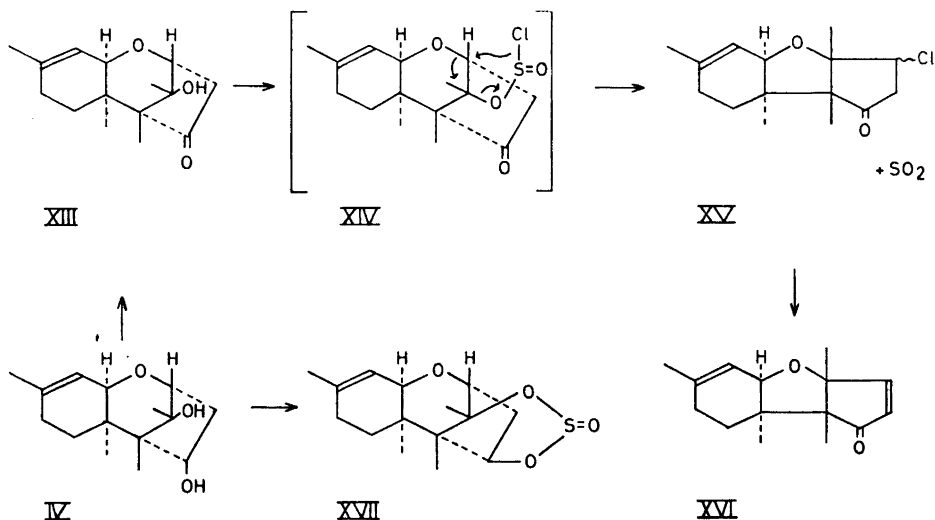

Fig.2 (60 Mc)
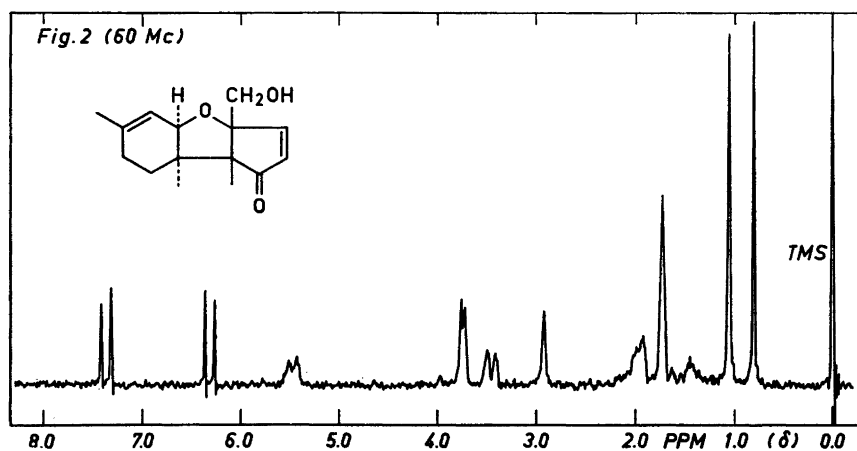
ring in IX. That tetrahydrofurane rings are formed faster than corresponding tetrahydropyrane rings in similar reactions is well known.[15]

2. *"Trichodermol chlorohydrin" and "trichodermol glycol"*. Trichodermol and its esters are rapidly attacked by strong acids with opening of the oxirane ring. Thus, treatment of trichodermol with dilute hydrochloric acid yields a chlorohydrin, $C_{15}H_{23}ClO_3$, which by analogy with the corresponding compound formed from trichothecolone is called trichodermol chlorohydrin. When dilute sulfuric acid, or better dilute trifluoroacetic acid, is substituted for hydrochloric acid a triol, $C_{15}H_{24}O_4$, trichodermol glycol, results. Trichothecolone chlorohydrin and trichothecolone glycol have been assigned formulae XIa and XIb, respectively,[6] and both compounds have been interrelated with neotrichothecodione.[6,7] The assignment of the analogous structures XIc and XId to trichodermol chlorohydrin and trichodermol glycol, respectively, is consistent with their NMR-spectra which show the absence of the epoxide as well as of the C_2-proton and the presence of a $-CH_2OH$ group. Oxidation of XIc affords the acid XII, which also is obtained on oxidation of neotrichodermone.

It must be assumed that these acid-catalyzed rearrangements are initiated by protonation of the epoxide, and that the protonized epoxide subsequently is subjected to *internal* nucleophilic attack by the tetrahydropyrane oxygen, leading to the oxonium ion X which then by *external* anions is cleaved with formation of XI.

3. *Other rearrangements.* When the ketone XIII, obtained by chromium(VI)-oxyde oxidation of the diol IV, is treated with thionylchloride in pyridine a chloroketone $C_{15}H_{21}ClO_2$ is obtained which readily eliminates hydrogen chloride with formation of an α,β-unsaturated ketone $C_{15}H_{20}O_2$.

The NMR-spectrum of this compound is very similar to that of neotrichodermone, but shows the presence of an additional quaternary methyl group and the absence of the $-CH_2OH$ group in neotrichodermone. The

ultraviolet spectrum is practically identical with that of neotrichodermone, and the compound must obviously be represented by XVI. This rearrangement, which has a certain resemblance to the rearrangement leading to trichodermol chlorohydrin, may be rationalized by assuming an internal nucleophilic attack on the chlorosulfite XIV by the tetrahydropyrane oxygen.*

It is remarkable that the profound rearrangements discussed under 1, 2, and 3, which are accomplished under fundamentally different experimental conditions all lead to compounds containing the same basic ring system.

Biogenesis. The biogenesis of trichothecin has been studied by Jones and Lowe,[16] who found that 1-^{14}C-acetate as well as 2-^{14}C-mevalonic lactone were

* Treatment of the diol IV with thionylchloride in pyridine gave the cyclosulfite XVII in accordance with the *cis* arrangement of the two hydroxyl groups in IV.

incorporated, the latter more efficiently than the former. When 2-^{14}C-MVL was used it could be demonstrated that all of the radioactivity resided in the trichothecolone moiety of the molecule, and on the basis of a series of reliable degradations it was deduced that the radioactivity was distributed as indicated in formula XVIII. The revision of the formula does not influence these conclusions, and it seems justifiable to assume that the distribution in the light of the revised formula is as shown in formula XIX. This distribution, which, as outlined, is in accordance with the Ružicka biogenetic isoprene rule,[17] must of course be expected to be found in trichodermin, verrucarol, and other sesquiterpenes containing the same ring system.

The steric aspects of the biosynthesis of trichothecin has been discussed by Ružicka [17] in terms of formula XVIII. It was pointed out that when starting from one of the two geometric isomers of bisabolen with the side chain in the chair-type folding (XX) it was possible via bridged carbonium ions to arrive at the labelling and stereochemistry depicted in XVIII.

In view of the revised formula of trichothecolone it is necessary to assume a boat-type folding of the side chain in bisabolen (XXI) to obtain a compound with the correct stereochemistry and labelling (XIX).

EXPERIMENTAL

All m.p. are corrected. Optical rotations, unless otherwise stated, were measured in chloroform ($c = 1$) and UV-spectra in 96 % ethanol solution. The IR-spectra were obtained with a Perkin-Elmer Model 21 spectrophotometer with a sodium chloride prism. NMR-spectra were obtained with Varian HR-100 (100 Mc) and Varian A-60 (60—Mc) high resolution spectrometers. Deuteriochloroform was used as solvent. The line positions are given in δ-values and with tetramethylsilane as internal reference. For characterization of the signals the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Numbers in parentheses represent the number of protons found by integration. Microanalyses were by Mr. G. Cornali and Mr. W. Egger.

Isolation of trichodermin (IIIb). Clarified fermentation broth (4.0 l) containing about 2.5 g trichodermin (determined by the agar cup method using *Candida albicans* as test organism) was adjusted to pH 7.5 and extracted with light petroleum (b.p. 40—60°) (1 + 0.5 l). The combined extracts were washed with water, dried, and evaporated to a syrup. This syrup (2.12 g) was dissolved in pentane (10 ml) and passed through a column of neutral alumina (10 g) which was eluted with a further 20 ml of pentane. The combined eluates were evaporated to yield trichodermin (1.60 g) as a colourless, viscous oil $[\alpha]_D^{20}$ —11°, b.p. 110°/0.05 mm Hg. After standing for several weeks this oil crystallized to yield colourless crystals of m.p 45—46°. In subsequent runs a pure crystalline product, m.p. 45—46°, was obtained directly by cooling the concentrated eluate (containing about 20 % of trichodermin) slowly to about —50° while seeding. Trichodermin is readily soluble in all common organic solvents at room temperature, but can be recrystallized from pentane by cooling with dry ice. M.p. 45—46°. $[\alpha]_D^{20}$ —10.2°. UV: λ_{max} 205 mμ (ε 2400). IR (KBr): 1730 (CO), 1682 (C=C), 1225 and 1245 (acetate), and 1085 cm^{-1} (C—O—C). NMR-spectrum, see Fig. 1. (Found: C 69.97; H 8.36; acetyl 14.61. Calc. for $C_{17}H_{24}O_4$: C 69.83; H 8.27; acetyl 14.4).

Trichodermol (IIIc). A solution of trichodermin (IIIb, 1.5 g) in a mixture of ethanol (10 ml) and 2 N aqueous sodium hydroxide (3 ml) was refluxed for 1 h. After cooling, most of the ethanol was removed under reduced pressure, and the residue was extracted with ether (25 ml). The extract was washed with water, dried, and evaporated *in vacuo*. The residue crystallized from light petroleum to yield 1.2 g of trichodermol, m.p. 116—117°. One recrystallization from ether-hexane gave the analytical specimen, m.p.

117.5—118°. $[\alpha]_D^{20}$ —33.5°. IR: no bands in the carbonyl region. NMR-spectrum, see Fig. 1. (Found: C 72.18; H 8.88. Calc. for $C_{15}H_{22}O_3$: C 71.97; H 8.86).

Acetylation of trichodermol. Trichodermol (IIIc; 5.0 g) was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (5 ml). After standing at room temp. for 20 h water (50 ml) was added, and the mixture was extracted with light petroleum (2 × 20 ml). The combined extracts were washed with water, dried, and evaporated to a syrup, which again was dissolved in light petroleum and evaporated. This was repeated until all the pyridine was removed. Finally, the residue was dissolved in pentane (20 ml). The solution was cooled to —50° with seeding. The crystalline precipitate was collected and washed on the filter with cold (about —50°) pentane to yield 5.0 g, m.p. 45—46° alone or in admixture with authentic trichodermin. The IR-spectra were identical.

Dihydrotrichodermol. A solution of trichodermol (Ic; 2.50 g) in 96 % ethanol (30 ml) was shaken at room temperature under 1 atm hydrogen in the presence of 5 % palladium on calcium carbonate (1.25 g). In 60 min, 260 ml of hydrogen was absorbed and the consumption ceased. The catalyst was removed, and the filtrate evaporated to dryness *in vacuo.* The residue crystallized from pentane to afford 1.72 g, m.p. 86—90°. Recrystallization from methanol-water raised the m.p. to 91.5—92.5°. UV: No selective absorption above 200 mμ. (Found: C 71.32; H 9.62. Calc. for $C_{15}H_{24}O_3$: C 71.39; H 9.59).

Trichodermone (VIIa). To a solution of trichodermol (IIIc; 10.0 g) in acetic acid (20 ml) 10 % chromium(VI)oxide in 95 % acetic acid (50 ml) was added in one portion. After standing for 15 min, water (200 ml) was added, and upon scratching the product crystallized to yield 5.70 g, m.p. 151—153°. One recrystallization from ether-hexane gave the analytical sample. m.p. 154—155°. $[\alpha]_D^{20}$ —42.3°. UV: λ_{max} 205 mμ (ε 2800). IR (KBr): 1735 cm^{-1} (five-ring ketone). NMR-spectrum: $\delta = 5.24$/m (1); 3.88/t (1); 3.52/d (1); 3.06/d (1) and 2.80/d (1) $J_{AB} = 4$ cps; 2.47/d (2); 1.70 + 0.78 + 0.73/3s (each 3). (Found: C 72.43; H 8.17. Calc. for $C_{15}H_{20}O_3$: C 72.55; H 8.12).

Chromium(VI)-oxide oxidation of trichodermone (VIIa) into trichothecodione (VIIb). To a solution of trichodermone (VIIa; 4.0 g) in acetic acid (40 ml) 10 % chromium(VI)-oxide in 95 % acetic acid (50 ml) was added. The mixture was heated to 50°, whereafter an exothermic reaction set in, and the temperature rose to 65°. After standing for 1 h, water (500 ml) was added and the mixture extracted with ether (3 × 100 ml). The combined extracts were washed with aqueous sodium bicarbonate followed by water, dried, and evaporated to a faintly yellow syrup (1.0 g), which upon treatment with ether yielded 240 mg of crystalline material, m.p. 190—205°. Three recrystallizations from ether raised the m.p. to 216—218°, and this was not depressed on admixture with authentic trichothecodione. The infrared and ultraviolet spectra of the two compounds were identical. (Found: C 68.66; H 6.96. Calc. for $C_{15}H_{18}O_4$: C 68.68; H 6.92).

Chromium(VI)-oxide oxidation of trichodermin (IIIb) into trichothecolone acetate (IIIf). To a solution of trichodermin (IIIb; 9.0 g) in acetic acid (40 ml) was added 10 % chromium(VI)-oxide in 95 % acetic acid (150 ml) in three portions. After standing overnight the green solution was concentrated *in vacuo* to about 50 ml. Water (300 ml) was added and the mixture extracted with ether (3 × 100 ml). The combined extracts were washed successively with water, aqueous sodium bicarbonate, and water, dried, and evaporated to yield 2.6 g of a pale yellow oil. Thin layer chromatography of this oil (adsorbent: silica gel G; solvent system: ethyl acetate-cyclohexane (1:1)) revealed the presence of unchanged trichodermin and trichothecolone acetate in almost equal amounts in addition to other, more polar compounds. The oil was chromatographed over silica gel (75 g; 100 mesh). Elution with benzene-ether (95:5) afforded a series of fractions of which the first ones contained trichodermin, the following mixtures of trichodermin and trichothecolone acetate. Finally, fractions containing trichothecolone acetate only were obtained. These were combined and evaporated. The residue (250 mg) crystallized from methanol to yield 72 mg of trichothecolone acetate, m.p. 140—144°. Recrystallization from methanol raised the melting point to 145—146° alone or in admixture with authentic trichothecolone acetate. The IR-spectra were identical.

The more polar compounds, which according to thin layer chromatography were present in the crude product, were eluted with benzene-ether (80:20). The fractions containing the least polar of the two main components were combined and evaporated to yield, after recrystallization of the residue from ether, 140 mg of an acid called "acid A", while the fractions containing the more polar component, after evaporation and recrystallization of the residue from ether gave 200 mg of an acid called "acid B".

"Acid A" M.p. 206—207° (decomp.). UV: λ_{max} 225 mμ (ε 7700). IR (KBr): 1753, 1670 and 1580 cm^{-1}. (Found: C 68.62; H 6.98. Calc. for $C_{15}H_{18}O_4$: C 68.68; H 6.92).

The methyl ester of "acid A" was obtained by treating "acid A" with ethereal diazo-methane. M.p. 127°. UV: λ_{max} 225 mμ (ε 8000). IR (KBr): 1760, 1715, 1672, and 1586 cm^{-1}. NMR-spectrum: $\delta = 7.53/d$ (1) and 6.32/d (1) J_{AB} = 6 cps; 5.52/m (1); 3.75/s (3); 3.63/d (1); 1.74 + 1.17 + 0.87/3s (each 3). (Found: C 69.48; H 7.33. Calc. for $C_{16}H_{20}O_4$: C 69.54; H 7.30).

"Acid B" M.p. 139.5—140°. UV: λ_{max} 204 mμ (ε 4000). IR (KBr): 1750, 1725, 1672, and 1640 cm^{-1}. (Found: C 61.87; H 7.15. Calc. for $C_{16}H_{22}O_5$: C 61.92; H 7.15).

The methyl ester of "acid B", obtained by treating "acid B" with ethereal diazomethane, was an oil. IR (CCl$_4$): 1765, 1745, and 1675 cm^{-1}. NMR-spectrum: $\delta = 5.62/t$ (2); 4.63/d (1); 3.67/s (3); 2.07 + 1.78 + 1.18 + 0.92/4s (each 3). (Found: C 62.98; H 7.44. Calc. for $C_{17}H_{24}O_5$: C 62.95; H 7.46).

LiAlH$_4$-reduction of trichodermol(IIIc) to the diol IV. A solution of trichodermol (15.0 g) in dry dioxane (25 ml) was added drop by drop to a solution of LiAlH$_4$ (3 g) in ether (100 ml). After the addition was completed the mixture was refluxed for 3 h. Excess of LiAlH$_4$ was destroyed by addition of ethyl acetate, and sufficient water was thereafter added to hydrolyze the product complex with formation of a granular mass (lithium aluminate) which was removed by filtration and washed with ether. The combined filtrate and washings were washed with water, dried, and evaporated to dryness. The residue crystallized with ether to yield 14.0 g, m.p. 147—148°. Recrystallization from ether raised the m.p. to 147.5—148.5°. $[\alpha]_D^{20}$ —30°. NMR-spectrum: $\delta = 5.30/m$ (1), 4.27/q (1), 3.93/d (1); 3.40/d (1); 1.70 + 1.47 + 1.03 + 0.83/4s (each 3). (Found: C 71.27; H 9.55. Calc. for $C_{15}H_{24}O_3$: C 71.39; H 9.59).

Acetylation of the diol IV. A solution of IV (500 mg) in a mixture of acetic anhydride (5 ml) and pyridine (5 ml) was heated on the steam bath for 2 h. After cooling and addition of water (40 ml) the product crystallized on scratching to yield 250 mg, m.p. 114—116°. Recrystallization from methanol-water raised the m.p. to 117—117,5°. $[\alpha]_D^{20}$ + 5.3° IR (KBr): 3470 and 3540 (OH), 1710 (CO), 1675 (C=C), and 1265 cm^{-1} (acetate). (Found: C 69.26; H 8.96; acetyl 14.62. Calc. for $C_{17}H_{26}O_4$: C 69.36; H 8.90; acetyl 14.6).

Oxidation of the diol IV into the ketol XIII. To a solution of IV (500 mg) in acetic acid (5 ml) was added 10 % chromium(VI)oxide (5 ml). After standing for 15 min, water (100 ml) was added, and the resulting solution was extracted with ether (3 × 75 ml). The combined extracts were washed successively with water, aqueous sodium bicarbonate, and water, dried and evaporated *in vacuo*. The residue crystallized from methanol-water to yield 200 mg, m.p. 174—175°. Recrystallization from ether-hexane raised the m.p. to 175—176°. $[\alpha]_D^{20}$ —51.3°. IR (KBr): 3475 (OH), and 1730 cm^{-1} (five-ring ketone). (Found: C 71.94; H 8.85. Calc. for $C_{15}H_{22}O_3$: C 71.97; H 8.86).

Trichodermol p-bromobenzoate (IIIa). Trichodermol (2.0 g) was dissolved in a mixture of dry pyridine (10 ml) and p-bromobenzoylchloride (2.5 g). After standing for 2 h at room temp. water (50 ml) was added to precipitate an oil which shortly after solidified. Filtration, washing with water, and drying gave the crude product which was treated with dioxane (25 ml). Undissolved material was removed by filtration to yield 250 mg, m.p. 218—220° (p-bromobenzoic anhydride). Addition of water to the filtrate gave 2.3 g of trichodermol-p-bromobenzoate, m.p. 159—164°. Recrystallization from methanol raised the m.p. to 161—163°. The crystals for the X-ray investigation were obtained from acetonitrile, m.p. 161—163°. $[\alpha]_D^{20}$ —55.3°. (Found: C 60.79; H 5.86; Br. 18.84. Calc. for $C_{22}H_{25}BrO_4$: C 61.0; H 5.81; Br 18.5).

Hydrolysis of trichodermol p-bromobenzoate (IIIa). A mixture of IIIa (200 mg), methanol (5 ml), and 2 N aqueous NaOH was refluxed for 1 h. Water (10 ml) was added, and the mixture extracted with ether (3 × 10 ml). The combined extracts were washed with water, dried, and evaporated to leave a crystalline residue (95 mg) which, after recrystallization from ether-hexane, afforded 78 mg, m.p. 116—117°, alone or in admixture with authentic trichodermol. The IR-spectra were identical.

Neotrichodermone (VIIIa). Trichodermone (1.0 g) was dissolved in methanol (35 ml) at 50°, and anhydrous sodium carbonate (1.0 g) in water (35 ml) added. The solution was heated to 60° for 10 min, and the methanol then removed *in vacuo*. Addition of water (30 ml) to the residual solution caused neotrichodermone to precipitate as an oil which was extracted with ether (2 × 20 ml). The combined extracts were washed with water, dried, and evaporated to dryness. The residue (1.0 g) crystallized from hexane to yield

550 mg of neotrichodermone, m.p. 97—98°. Recrystallization from hexane afforded 400 mg, m.p. 97.5—98.5°. $[\alpha]_D^{20}$ —29.2°. UV: λ_{max} 204 mμ (ε 10 200), $\lambda_{shoulder}$ 215 mμ. IR (KBr): 3480 and 3410 (OH); 1690 (CO); 1650 (shoulder) and 1595 cm^{-1} (C=C). NMR-spectrum; see Fig. 2. (Found: C 72.60; H 8.13. Calc. for $C_{15}H_{20}O_3$: C 72.55; H 8.12).

Trichodermol chlorohydrin (XIc). A solution of trichodermol (1.0 g) in a mixture of methanol (5 ml) and conc. hydrochloric acid (0.5 ml) was left at room temp. for 15 min. Addition of water (15 ml) caused trichodermol chlorohydrin (800 mg; m.p. 156—160°) to precipitate. Recrystallization from ether raised the m.p. to 161—163°, and this was not changed by further recrystallizations from the same solvent. $[\alpha]_D^{20}$ + 13.3°. NMR-spectrum: δ = 5.47/m (1); 4.11/q (1); 3.68/d (1); 1.72 + 1.10 + 0.90/3s (each 3). (Found: C 62.88; H 8.15; Cl 11.74. Calc. for $C_{15}H_{23}ClO_3$: C 62.80; H 8.08; Cl 12.36).

Trichodermol glycol (XId). Trichodermol (IIIc) (5.0 g) was dissolved in a mixture of trifluoroacetic acid (25 ml) and water (25 ml). After standing overnight at room temp. the solution was evaporated to dryness *in vacuo*. The residue crystallized from ether to afford 3.2 g of trichodermol glycol, m.p. 140—142°. Recrystallization from benzene gave 2.6 g, m.p. 143.5—144.5°. $[\alpha]_D^{20}$ + 2.4°. NMR-spectrum: δ = 5.50/m (1); 4.08/m (2); 3.77/s (2); 3.70/d (1); 1.72 + 0.97 + 0.88/3s (each 3). (Found: C 66.89; H 9.06. Calc. for $C_{15}H_{24}O_4$: C 67.13; H 9.02).

Acid XII. a. From trichodermol chlorohydrin. To a solution of trichodermol chloro-hydrin (2.0 g) in acetic acid (10 ml) was added 10 % chromium(VI)-oxide in 95 % acetic acid (30 ml) in one portion. The temperature was allowed to rise to 40°, and was maintained here by cooling with water. After about 30 min the evolution of heat ceased and the mixture was left for 2 h at room temp. Water (150 ml) was added and the mixture extracted with ether (3 × 50 ml). The combined extracts were washed with water and then extracted with aqueous sodium bicarbonate (20 ml). The aqueous extract was acidified to precipitate an oil which was extracted with ether (2 × 10 ml). The ethereal extract was washed with water, dried, and evaporated to leave an oil (900 mg) which was chromatographed over silica gel (100 mesh; 50 g). Elution with ether-hexane (1:5) gave 650 mg of an oil which crystallized from ether-pentane to afford 450 mg of XII, m.p. 144—145°. Recrystallization from the same solvents did not change the m.p. $[\alpha]_D^{20}$ —9.9°. UV: λ_{max} 208 mμ (ε 7900). IR (KBr): 1760, 1715, 1675, and 1599 cm^{-1}. (Found: C 68.60; H 6.90. Calc. for $C_{15}H_{18}O_4$: C 68.68; H 6.92).

b. From neotrichodermone. Neotrichodermone (1.0 g), when oxidized in the same manner as described under a. above, gave 670 mg of an acidic fraction which was chromatographed over silica gel (100 mesh; 25 g). Elution with ether-hexane (1:5) gave 230 mg of crystals, m.p. 144—145° alone or in admixture with the product obtained under a. above. The IR-spectra were identical.

Conversion of XIII to the chloroketone XV. Thionylchloride (1.5 ml) was added drop by drop to a solution of XIII (650 mg) in dry pyridine (10 ml) at —30°. After the addition, the mixture was kept at —20° for 30 min, then heated to room temp. and poured on ice. The crystalline precipitate, which formed, was collected, washed with water, and dried to yield 460 mg, m.p. 111—114°. The crude product, thus obtained, was treated with hot ether. After removal of some undissolved material by filtration, the filtrate was evaporated *in vacuo* and the residue recrystallized from methanol to yield 215 mg, m.p. 114.5—115°. IR (KBr): 1745 and 1675 cm^{-1}. (Found: C 67.03; H 7.83; Cl 13.17. Calc. for $C_{15}H_{21}ClO_2$: C 67.02; H 7.88; Cl 13.19).

Dehydrochlorination of XV with formation of XVI. XV (500 mg) was refluxed with collidine (5 ml) for 5 min. After cooling, water (25 ml) and acetic acid (3 ml) were added and the mixture extracted with ether (2 × 25 ml). The combined extracts were washed with water, dried, and evaporated to yield 450 mg of an oil which was dissolved in pentane (5 ml). Cooling of this solution in a dry-ice bath afforded 350 mg of crystals, m.p. 90—94°, raised by recrystallization from methanol-water to 92—94°. The analytical specimen, which was obtained from pentane, had m.p. 94.5—95.5°. $[\alpha]_D^{20}$ —44.0°. IR (KBr): 1700 and 1605 cm^{-1}. NMR-spectrum: δ = 6.98/d (1) and 6.06/d (1) J_{AB} = 5.5 cps; 5.32/m (1); 3.23/d (1); 1.70 + 1.32 + 0.92 + 0.73/4s (each 3). (Found: C 77.50; H 8.77. Calc. for $C_{15}H_{20}O_2$: C 77.55; H 8.68).

Cyclosulfite XVII. Thionylchloride (0.3 ml) was added drop by drop to a solution of IV (250 mg) in dry pyridine (2 ml) at 0°. After standing for 2 h at 0° the mixture was poured on ice. The crystalline precipitate, which formed, was collected, washed with

water, and dried to yield 250 mg, m.p. 117—120°. Recrystallization from pentane gave 170 mg, m.p. 122—124°. IR (KBr): 1680, 1215, and 1205 cm^{-1}. (Found: C 60.32; H 7.39; S 10.51. Calc. for $C_{15}H_{22}O_4S$: C 60.39; H 7.43; S 10.73).

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