783. *The Chemistry and Stereochemistry of Trichothecin.*

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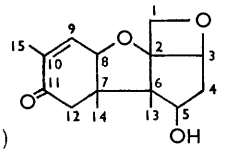
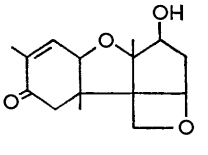
The chemistry of trichothecolone, the sesquiterpenoid hydrolysis-product of the fungal metabolite trichothecin, has been further examined. In particular, direct evidence for structure (I) has been obtained by alkali-induced scission of the diketone (XIII) into 2,5-dimethylcyclohexane-1,4-dione (XV) and 2-methyl-3-oxocyclopent-1-enecarboxylic acid (XVI). The rearrangement of trichothecolone in alkaline solution to isotrichothecolone has been formulated, and another rearrangement product, allodihydrotrichothecolone, has been prepared by reduction with zinc and alkali. The establishment of its structure, and a consideration of the mechanism by which it must be formed, have led to almost complete elucidation of the stereochemistry of trichothecolone (XVIII) and hence of trichothecin.

The chemistry of trichothecin,[1] an antifungal metabolite of *Trichothecium roseum* Link, has been extensively studied by Freeman, Gill, and Waring.[2] When their investigations were discontinued it became possible for us, through the courtesy of Imperial Chemical Industries Limited, to tackle the intriguing structural problem. Trichothecin is the isocrotonyl ester of a keto-alcohol, trichothecolone,[3] and, as has now been indicated by Freeman, Gill, and Waring,[2] of the two formulæ (I) and (Ia) for trichothecolone, the former is compatible with the greater proportion of the reactions. At the time we commenced work on this problem there appeared to be little to choose between the two structures. This paper describes observations, experiments, and interpretations which finally prove the correctness of (I) and in addition, largely elucidate its stereochemistry.



(I)



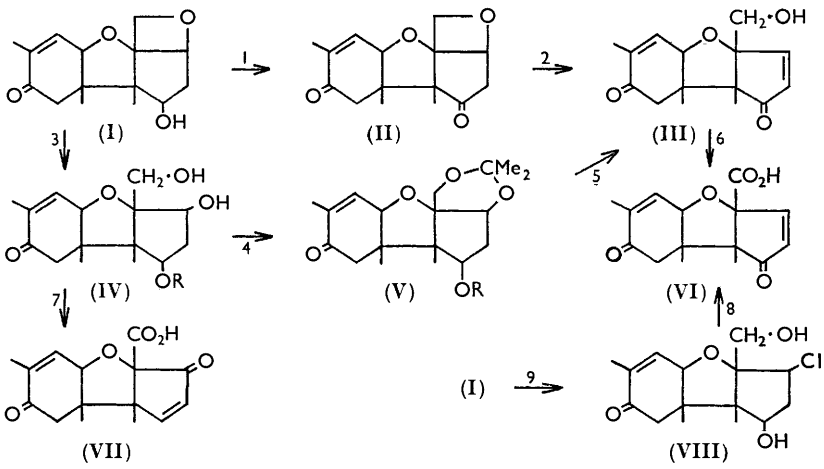
(Ia)

The most significant features of the chemistry of trichothecolone elucidated by Freeman *et al.*[2] were: (*a*) its dehydrogenation to C_7 and C_8 fragments in low yield; (*b*) a variety of ring-opening reactions of a trimethylene oxide system fused to a hydroxycyclopentane ring; and (*c*) the apparent fission of neotrichothecodione (III) into isolable C_8 fractions, *p*-xylo-quinone and -quinol, and a C_7 residue which could not be isolated. Of these, the group of reactions under (*a*) requires no further discussion, and the formulation of groups (*b*) and (*c*) was given as the simplest, if not the only, explanation of the facts. However, a fourth group of observations, (*d*), the isomerisation of trichothecolone itself with alkali to isotrichothecolone, was left unexplained. Our initial aims were therefore to provide more rigorous proof of the interpretations of reactions in groups (*b*) and (*c*), to choose between structures (I) and (Ia), and to explain the isomerisation (*d*). In attempting the last a further set of transformations (*e*) was discovered, based upon zinc–alkali reduction of trichothecolone, and it is these which led to the stereochemical conclusions referred to above.

In the addition of the elements of hydrogen chloride to trichothecolone, *ca.* 25 kcal. $mole^{-1}$ are evolved; it followed directly [2] that a strained system such as a three- or four-membered oxide ring must be present. The choice of the latter depended upon the reaction scheme below; [2] briefly, acid (VI) which was not decarboxylated by heat, could be obtained

[1] Freeman and Morrison, *Nature*, 1948, **162**, 30; *Biochem. J.*, 1949, **44**, 1.
[2] Freeman, Gill, and Waring, *J.*, 1959, 1105.
[3] Freeman and Gill, *Nature*, 1950, **166**, 698.

from trichothecolone (I) either *via* the chlorohydrin (VIII) or *via* neotrichothecodione (III). Initial ring-opening of trichothecolone with sulphuric acid gave a trihydroxy-ketone (IV; R = H) (" trichothecolone glycol ") which, after similar oxidation steps, led to an isomeric acid (VII) which was not decarboxylated. This formulation required an *ad hoc* assumption that the oxidation of the trihydroxy-ketone involves more or less exclusively the 3-hydroxyl group,[3] although in trichothecolone itself the group at $C_{(5)}$ is readily oxidised. The difference between the diketo-acids (VI) and (VII) and the analogous dihydro- and tetrahydro-acids might, alternatively, be explained by skeletal rearrangement in one case when the oxide ring was opened under acidic conditions. Proof of the proposed reaction scheme thus necessitated the interrelation of the two sets of transformation products (*e.g.*, III and IV), and this was achieved in the following way. The trihydroxy-ketone (IV; R = H) and the parent " trichothecin glycol " (IV; R = isocrotonyl) both gave isopropylidene derivatives, and the trichothecin derivative (V; R = isocrotonyl) was converted into the isopropylidene derivative (V; R = H) containing a 5-hydroxy-group by mild alkaline hydrolysis. The secondary alcohol group of the latter was oxidised and the isopropylidene group then removed by hot aqueous acetic acid, yielding neotrichothecodione (III).



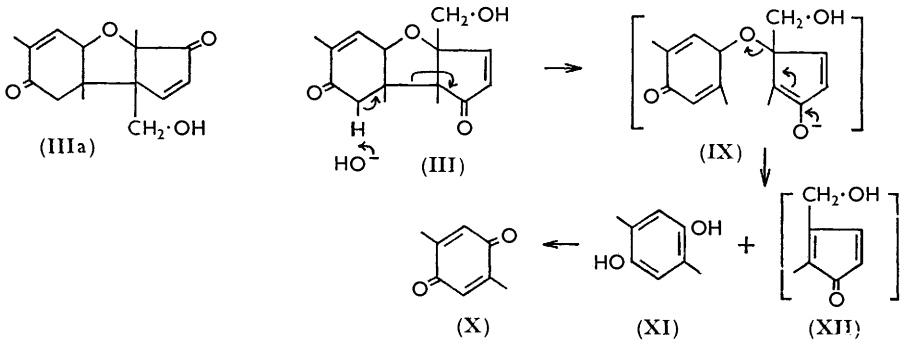
Reagents: 1, CrO_3–$COMe_2$. 2, Na_2CO_3. 3. Dil. H_2SO_4.
4, $COMe_2$–H^+. 5, Alkali; CrO_3–Pyr; AcOH. 6, CrO_3–$COMe_2$.
7, CrO_3. 8, CrO_3. 9, HCl.

Evidence given below implies that the C–O bonds at positions 2 and 3 are *trans*; this explains the remarkable difference in the reactivity of the trimethylene oxide ring towards basic and acidic conditions, as after protonation, fission of the $C_{(3)}$–O bond would be aided by participation of the tetrahydrofuran oxygen atom. The new substituents at $C_{(3)}$ in (IV) and (VIII) would then enter with retention of configuration; this in its turn explains the impossibility of regenerating the four-membered ring by treating compound (VIII) with alkali. The chlorohydrin was recovered in high yields from experiments in which it was heated under reflux with 10% methanolic sodium methoxide or potassium hydroxide.

As a four-membered oxide ring is almost unknown among natural products, more direct evidence for its presence in trichothecin was desirable. Trichothecolone, and all its derivatives in which chemical evidence suggested the retention of the oxide ring, show a strong infrared absorption band at 960—964 $cm.^{-1}$, similar to that exhibited by *trans*-CH=CH– groupings (obviously not present), but otherwise rarely encountered. Such bands are, however, also characteristic of four-membered rings, although they are variable in position (*e.g.*, 890 $cm.^{-1}$ for 3α,5α-epoxycholestane, and 970—980 $cm.^{-1}$ for simple

trimethylene oxides). They overlap the observed range for tetrahydrofurans (960—1150 cm.$^{-1}$) but not that for ethylene oxides (790—850 cm.$^{-1}$).[4,5] In view of this evidence the straightforward interpretation [2] of reactions (*b*) therefore seems justified.

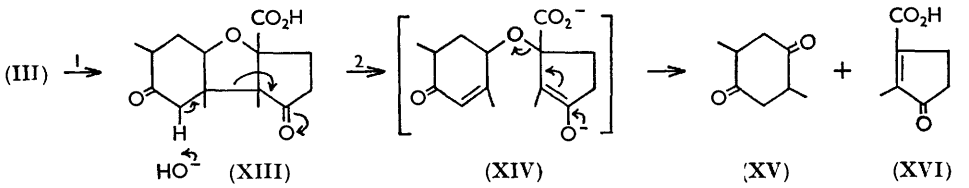
Freeman, Gill, and Waring [2] discovered that treatment of the diketone (II) with hot aqueous alkali gave a mixture of *p*-xyloquinone (X) and the quinol (XI). No fragment corresponding to the remaining portion of the molecule could be isolated. The same products were given by neotrichothecodione (III), which was obviously an intermediate in this transformation, and by the acid (VI) and other compounds in which the 5-hydroxyl group had been oxidised. They were not obtained from trichothecolone itself, which gave instead isotrichothecolone under more drastic conditions (see below). These observations did not in themselves permit a choice between structures (I) and (Ia), as the derived formulæ for neotrichothecodione, (III) and (IIIa), were respectively a 1,5-diketone and a vinylogous 1,5-diketone, and either could undergo Michael cleavage. In either case the immediate product (*e.g.*, IX) of such a reaction would be expected to undergo a β-elimination to give *p*-xyloquinol and a derivative (XII) of cyclopentadienone which would not survive the reaction conditions.



In order to interpret this complex and ambiguous reaction it was necessary to see whether fission would take place with a saturated derivative of neotrichothecodione (III), as on the alternative formulation (IIIa) Michael fission would then be impossible. Maintenance of non-oxidising conditions would allow the isolation of the C_8 fragment without subsequent change, and the C_7 residue, now a cyclopentenone rather than a cyclopentadienone, should also survive. By treatment of acid (XIII) with alkali, Freeman, Gill, and Waring [2] had already obtained a compound $C_8H_{12}O_2$, m. p. 82—84°, which reduced Tollens's reagent. According to the above mechanism this reaction should give 2,5-dimethylcyclohexane-1,4-dione (non-reducing). Re-investigation under controlled conditions gave the more stable of the two known stereoisomers [6] of the cyclohexanedione (XV), which when pure had m. p. 90—91°, did not reduce Tollens's reagent, and showed no optical activity. An acidic product was also isolated and was shown to be 2-methyl-3-oxocyclopent-1-enecarboxylic acid (XVI), identical with a sample derived by degradation of picrotoxin.[7] The yields of these two compounds left no doubt that they had arisen from different parts of the molecule, confirming the proposed mechanism (XIII ⟶ XIV ⟶ XV and XVI) and proving that formula (I) and not (Ia) was the structure of trichothecolone. The formation of optically inactive products from a compound with five asymmetric centres is noteworthy.

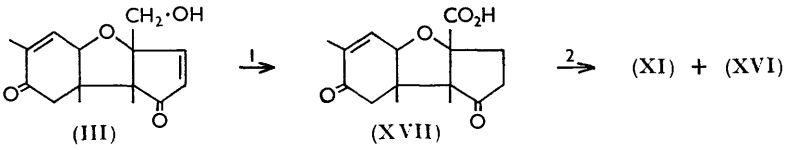
Partial hydrogenation of neotrichothecodione (III) and oxidation of the product by chromic acid gave acid (XVII). Alkali cleavage of this acid gave, as predicted, *p*-xyloquinol (XI) and 2-methyl-3-oxocyclopent-1-enecarboxylic acid (XVI) together with a new

[4] Bellamy, " Infra-red Spectra of Complex Molecules," Methuen, London, 1958, p. 118.
[5] Henbest, Millward, and Nicholls, personal communication.
[6] Baeyer, *Ber.*, 1892, **25**, 2122; Zelinsky and Naumow, *Ber.*, 1898, **31**, 3206.
[7] Sutter and Schlittler, *Helv. Chim. Acta*, 1949, **32**, 1860.



Reagents: 1, CrO_3–$COMe_2$; Pd–H_2. 2, Alkali.

C_{15} acid (XXV). The structure and mechanism of formation of this minor reaction product are considered below.



Reagents: 1, Pd–H_2; CrO_3. 2, Alkali.

The carbon–carbon bond which is broken in the reversed Michael reaction is severely weakened by the compression energy associated with the hexasubstituted ethane structure. It can be estimated [8] that the difference in energy between the eclipsed and the staggered conformation of hexamethylethane is 10—15 kcal. mole^{-1} and the release of compression energy in breaking the bond will of course be somewhat greater. (It is assumed in this discussion that the two five-membered rings are *cis*-fused; this will later be shown to be true.) The release of this compression energy is considered to be the main driving force of the reversed Michael reaction.

Structure (I) for trichothecolone was further confirmed by the value (3·6) for the pK_a of acid (XIII), as expected for an α-alkoxy-acid.

The presence of an α-methyl-αβ-unsaturated ketone grouping in trichothecolone (I) has now been directly demonstrated by treating the trihydroxy-ketone (IV; R = H) with a solution of periodate containing a small amount of permanganate.[9] Acetic acid (1 mol.) was the only volatile acid produced.

At the outset of our studies we had hoped that further investigation of the chemistry of isotrichothecolone, produced [2] by the action of hot aqueous alkali on trichothecolone (I), would be of help with the major problem. No structure, including stereochemistry, for trichothecin would be acceptable unless it allowed satisfactory explanation of the mode of formation and the structure of this isomer. As it became apparent that the isomerisation involved complex structural changes, we tried to isolate some intermediate compounds. It seemed possible that oxidation–reduction might be taking place and so alkali-treatment in the presence of zinc was investigated, with the result that a new product was readily isolated. Although this proved to be only a very distant relative of isotrichothecolone, nevertheless the elucidation of its structure resulted in the almost complete determination of the stereochemistry of trichothecin.

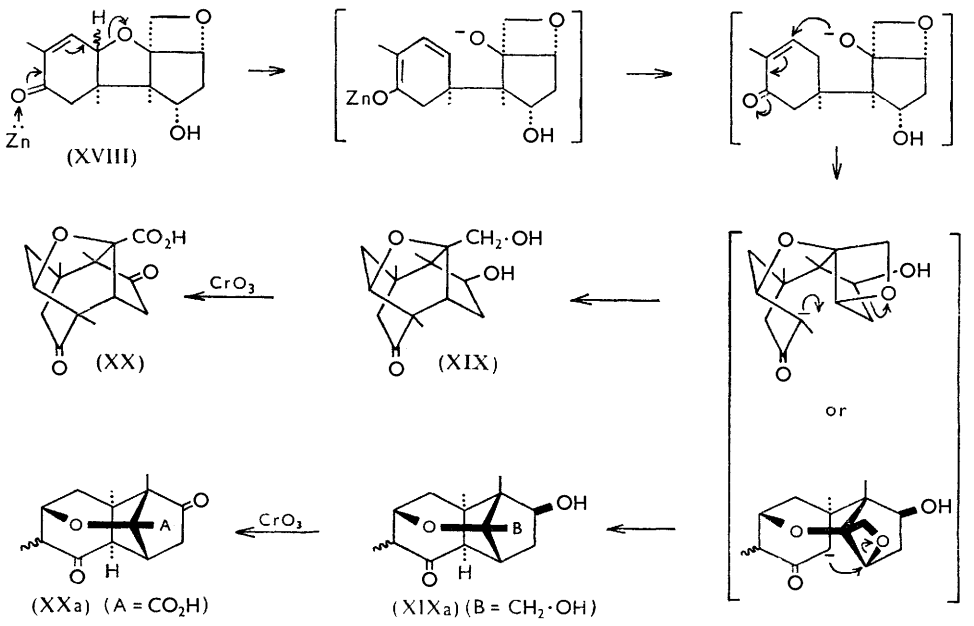
Allodihydrotrichothecolone (XIX), the zinc–alkali reduction product, contained two hydrogen atoms more than the parent, and the infrared spectrum indicated the presence of an isolated keto-group on a six-membered ring. The destruction of the αβ-unsaturated ketone system of trichothecolone is an essential part of this zinc–alkali reaction since under similar conditions dihydrotrichothecolone is merely converted into the dihydroiso-compound.[2]

Acetylation indicated two hydroxyl groups, the remaining oxygen atom being presumably ethereal; and oxidation gave a diketo-acid (XX), so that the hydroxyl groups must be severally primary and secondary. No unsaturation could be detected (no hydrogen

[8] Pitzer, *Discuss. Faraday Soc.*, 1951, **10**, 66 and references there cited.
[9] Lemieux and von Rudloff, *Canad. J. Chem.*, 1955, **33**, 1701.

uptake; no ethylenic C–H deformation bands; little absorption near 2000 Å; no colour with tetranitromethane). The compound must therefore be tetracyclic and, in view of the oxygen functionality, at least tricarbocyclic; that is, in its formation a new carbocyclic ring must have been formed.

In such a cyclisation, occurring under hydrolytic conditions, the most plausible electrophilic centre seemed to be $C_{(3)}$ of the trimethylene oxide ring (the 960 cm.$^{-1}$ band is in fact absent in allodihydrotrichothecolone) while the possible nucleophilic centres were $C_{(10)}$ or $C_{(12)}$ (see p. 3948). The transformation may be formulated as in the annexed scheme (XVIII) ⟶ (XIX) or (XIXa).



Examples are available of the reductive cleavage of a γ-alkoxy-αβ-unsaturated ketone [10] and, since there is no tertiary hydroxyl group in the allodihydrotrichothecolone, addition of the tertiary hydroxyl anion to the unsaturated centre in the β-position to the carbonyl group seems probable. This process saturates the double bond and reorientates ring A so that, *if* (and only if) trichothecolone possesses the stereochemistry (XVIII), the enolate anion at $C_{(10)}$ or $C_{(12)}$ would be suitably placed for a rear-side attack on the four-membered oxide ring at $C_{(3)}$, thus generating a primary hydroxyl group. This mechanism leads to formula (XIX) or (XIXa) for allodihydrotrichothecolone, which account for all its properties. It has also established the relative configurations of four of the asymmetric centres and that of a fifth can be deduced from the fact that the two hydroxyl groups are strongly hydrogen-bonded and form a cyclic carbonate [11] when treated with diethyl carbonate and a trace of sodium. The hydroxyl groups must therefore be *cis* as indicated and, working backwards, the stereochemistry * of trichothecolone has to be (XVIII) in which only the configuration of one centre is indeterminate.

The chemical properties of allodihydrotrichothecolone were consistent with these formulæ, but did not distinguish between them. The derived keto-acid (XX or XXa) had pK_a 3·6, as expected for an α-alkoxy-acid, and the new ketone group was part of a five-membered ring (infrared evidence) and adjacent to a methylene group (benzylidene

* *I.e.*, the relative configurations; absolute configurations are not yet known.

[10] Woodward, Bader, Bickel, Frey, and Kierstead, *Tetrahedron*, 1958, **2**, 1; Woodward, Sondheimer, Taub, Heusler, and McLamore, *J. Amer. Chem. Soc.*, 1952, **74**, 4225.

[11] Carothers and Van Natta, *J. Amer. Chem. Soc.*, 1930, **52**, 314.

derivative; no such derivative could be obtained from allodihydrotrichothecolone). Quantitative bromination [12] of allodihydrotrichothecolone and of the derived keto-acid resulted in the consumption of one and two mol. respectively, a bromodiacetyl derivative, hydrolysable to bromoallodihydrotrichothecolone, being obtained in the former case. On the basis of formulæ (XIX) and (XX) uptake values of two and four mol. would be expected, and (XIXa) and (XXa) would suggest the uptake of one and three mol., as the bridgehead hydrogen atom cannot enolise (Bredt's rule). Since the polybromo-ketones which would result from complete substitution of $\cdot CH_2 \cdot CO \cdot$ groupings would suffer appreciable steric strain, lower values are not surprising.

The nuclear magnetic resonance spectrum of the methyl ester of the derived diketo-acid (for which we are grateful to Dr. L M. Jackman) allowed an easy choice of structure (XX), and hence of (XIX). It included, in addition to bands not yet assigned, three equally intense bands (τ = 8·93, 9·01, and 9·08 in chloroform [13] calibrated against tetramethylsilane) due to quaternary methyl groups; the alternative structure (XXa) would require the splitting of one of these bands by interaction with the adjacent C–H grouping.

It is now appropriate to consider further the chemistry of isotrichothecolone.[2] The acetylation experiments of Freeman, Gill, and Waring [2] were re-investigated and isotrichothecolone was found to contain two hydroxyl groups, giving a di- as well as a monoacetate. It also gave a bis(ethyl carbonate) and not a cyclic carbonate.[11] Chromic acid oxidation gave isotrichothecodione, one hydroxyl group remaining unchanged and the second generating a carbonyl group in a five-membered ring. This and the relative ease of acetylation supported the conclusion that the isomer contains a secondary and a tertiary hydroxyl group. The infrared absorption spectrum under high resolution indicated that there was no intramolecular hydrogen-bonding of either of the hydroxyl groups. The presence of an α-methyl-αβ-unsaturated ketone system in isotrichothecolone was confirmed by the isolation of acetic acid (1 mol.) as the only volatile acid on oxidation with periodate–permanganate.[9] The dihydro-derivative showed no unsaturation, so that it is again necessary to postulate a tetracyclic, tricarbocyclic structure. Freeman and his collaborators [2] were also able to show that dihydrotrichothecolone underwent rearrangement analogous to that of trichothecolone with hot aqueous alkali, to give a dihydroiso-compound which could also be obtained by catalytic hydrogenation of isotrichothecolone. The double bond is therefore not involved in the rearrangement for which the following mechanism, leading to (XXII) for isotrichothecolone, can now be proposed.

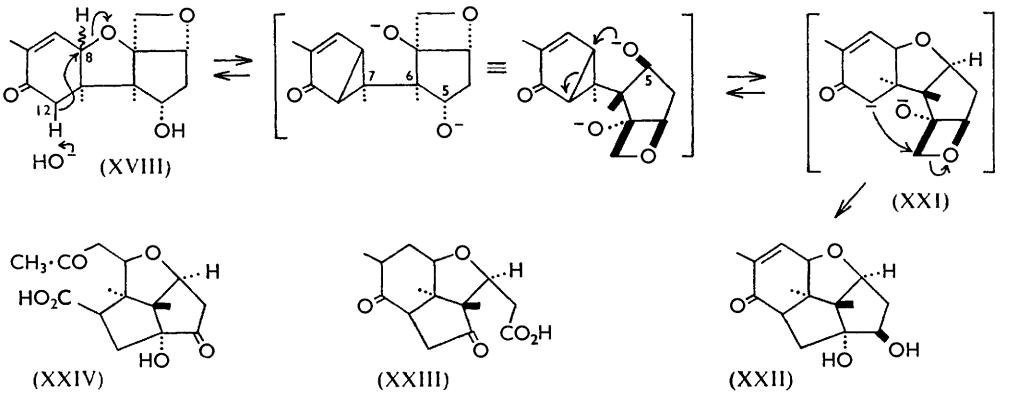
The first step involves the displacement of the ether-oxygen atom (from $C_{(8)}$) by the enolate carbanion ($C_{(12)}$). There is a close analogy for this in the reaction of 4-hydroxycyclohexanone toluene-*p*-sulphonate with base to give bicyclo[3,1,0]hexan-2-one.[14] Now, although the alkoxide anion is not such a good leaving group as the toluene-*p*-sulphonate anion, considerable non-bonded strain energy is removed since the transition state can approach the staggered conformation about the 6,7-bond. The anion of the secondary hydroxyl group at $C_{(5)}$ in trichothecolone can now add to the cyclopropane ring, and the resulting carbanion is suitably located to attack the four-membered oxide ring at $C_{(1)}$ with the formation of a new carbocyclic ring and generation of a secondary hydroxyl group.

Consideration of non-bonded interactions and strain energies in (XVIII) and (XXI) indicates that the equilibrium between them may well slightly favour (XVIII), so that it must be the attack on the four-membered oxide ring which renders the reaction irreversible. This would explain why the trihydroxy-ketone (IV; R = H) is unchanged after similar treatment.

In an attempt to confirm structure (XXII), isotrichothecolone was treated with periodate, but no uptake was observed. However, although *trans*-cyclopentane-1,2-diol

[12] Barnes, Barton, Cole, Fawcett, and Thomas, *J.*, 1953, 571.
[13] Tiers, *J. Phys. Chem.*, 1958, **62**, 1151.
[14] Nelson and Mortimer, *J. Org. Chem.*, 1957, **22**, 1146.

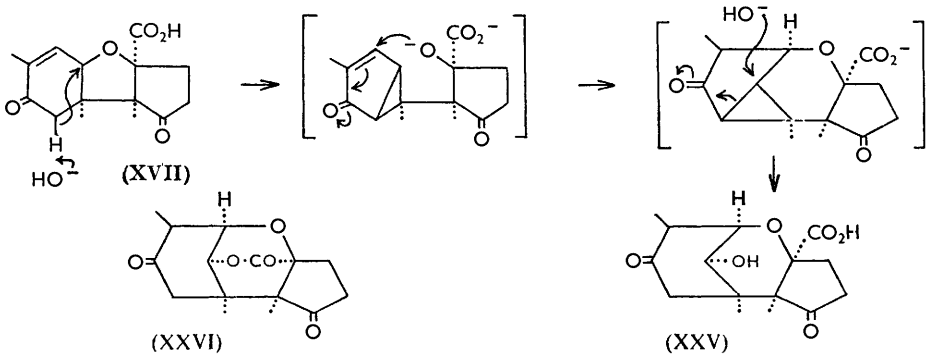
has been shown to react slowly with periodate,[15] a *trans*-cyclopentane-1,2-diol system held rigid by fusion with other ring systems is completely inert.[16] Reduction of isotrichothecodione with sodium borohydride gave a mixture of triols (presumably including some with a *cis*-orientated α-glycol grouping) which showed no carbonyl function in the



infrared spectrum. Catalytic hydrogenation of this mixture followed by treatment with periodate–permanganate [9] gave an acidic mixture ($\nu_{max.}$ at 1740 cm.$^{-1}$, indicating a carbonyl group in a 5-membered ring). Chromic acid oxidation of this gave a crystalline diketo-acid (XXIII) with the keto-groups in five- and six-membered rings ($\nu_{max.}$ 1740 and 1706 cm.$^{-1}$). This series of reactions provides strong confirmation of the existence of a *trans*-1,2-diol system in isotrichothecolone and of the presence of a *new* five-membered ring fused to that originally present in the parent compound.

Quantitative bromination experiments on dihydrotrichothecolone glycol and the corresponding iso-compound showed that the former consumed three mol. of bromine and the latter only two. This confirmed the presence of further substitution adjacent to the carbonyl function in isotrichothecolone.

A few additional minor points in the chemistry of trichothecin have also been elucidated. Freeman and his collaborators [2] found that chromic acid oxidation of one form of dihydrotrichothecolone gave an acid $C_{15}H_{22}O_6$, m. p. 167°. On the basis of its infrared spectrum ($\nu_{max.}$ 1742, 1710 and typical carboxyl absorption in the 3000—2500 cm.$^{-1}$ region) it can tentatively be assigned the structure (XXIV).



The minor acidic product (XXV) (p. 3951) obtained by alkali cleavage of acid (XVII) has the molecular formula $C_{15}H_{20}O_6$, so that the elements of water have been added, and its ultraviolet absorption spectrum reveals the disappearance of the αβ-unsaturated

[15] Criegee, Büchner, and Walther, *Ber.*, 1940, **73**, 571.
[16] Dimler, Davis, and Hilbert, *J. Amer. Chem. Soc.*, 1946, **68**, 1377; Alexander, Dimler, and Mehltretter, *ibid.*, 1951, **73**, 4658.

ketone system. This acid readily formed a lactone (XXVI) at its melting point, but it could be regenerated therefrom either by acid or by alkali. The most probable structure and mechanism of formation of this acid and its lactone are as shown, (XVII) ⟶ (XXV).

It remains only to explain the general character of the chemistry of trichothecin. This is dominated by two features: the tendency of the $C_{(6)}$–$C_{(7)}$ bond to split or to rotate, which is a consequence of the heavy substitution on these atoms, and the tendency of the four-membered ring to resist *external* nucleophilic attack, as a result of hindrance from the heavy substitution at $C_{(2)}$. This resistance favours *internal* nucleophilic attack by centres suitably located, and results in the complex rearrangements observed.

Experimental

M. p.s were determined on a Kofler block and are corrected. Peter Spence's alumina (grade H) was used for chromatography. Light petroleum refers to the fraction with b. p. 60—80°. Ultraviolet spectra were determined for ethanol solutions with a Cary recording spectrophotometer model 14M. Infrared absorption spectra were obtained for chloroform solutions on a Perkin–Elmer model 21 spectrophotometer. Dissociation constants were determined for aqueous solutions on a Cambridge pH indicator with a calomel reference electrode and a sealed glass electrode.

Dihydroneotrichothecodione.—Neotrichothecodione [2] (III) (1·72 g.) was dissolved in ethanol and hydrogenated over palladium–calcium carbonate. When 1 mol. of hydrogen (146 c.c. at N.T.P.) had been absorbed, the product was isolated. Crystallisation from benzene–light petroleum gave the *dihydro-compound* as prisms (1·43 g.), m. p. 173—176° (Found: C, 67·7; H, 7·7. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6%), $\lambda_{max.}$ 2270 Å (ε 6850).

Acid (XVII).—Dihydroneotrichothecodione (0·1 g.) was dissolved in acetone (15 c.c.), and 8N-chromic acid reagent [prepared from chromium trioxide (267 g.), concentrated sulphuric acid (230 c.c.), and water (400 c.c.), and made up to 1 l.] was added dropwise with shaking until a permanent orange-brown colour was obtained. After working-up through ether, the *acid* (70 mg.) crystallised from benzene–hexane as prisms, m. p. 196—199° (Found: C, 64·7; H, 6·4. $C_{15}H_{18}O_5$ requires C, 64·7; H, 6·5%), $\lambda_{max.}$ 2270 Å (ε 7500).

The *methyl ester* formed prisms (from hexane), m. p. 118—120° (Found: C, 65·4; H, 7·0. $C_{16}H_{20}O_5$ requires C, 65·7; H, 6·9%).

Hydrogenation of the acid in ethanol over 10% palladium–calcium carbonate gave a saturated acid, m. p. 183—186°, which did not depress the m. p. of the saturated acid (m. p. 184—185°) prepared by the method of Freeman *et al.*[2] The infrared spectra were also identical.

The overall yield (18%) [2] of (XVII) from trichothecolone has been improved to 57%, principally by the use of the chromic acid–acetone technique.

Alkali Fission of Acid (XVII).—The acid (300 mg.) was refluxed under nitrogen in 10% aqueous sodium hydroxide (15 c.c.) for 2½ min. The mixture was rapidly cooled, and then acidified, and the product was isolated with ether; a portion was found readily to sublimeat 85°.

The residue, *acid* (XXV) (110 mg.), was crystallised from ether and had double m. p. 165°, 212—215° (after resolidification) (see below), pK_a 4·25 (Found: C, 60·2; H, 6·6%; equiv., 296. $C_{15}H_{20}O_6$ requires C, 60·8; H, 6·8%; equiv., 296).

Acid (XXV), on melting or on subliming at 185°, gave a *lactone* (XXVI), m. p. 212—215° (Found: C, 64·7; H, 6·4. $C_{15}H_{18}O_5$ requires C, 64·7; H, 6·5%). The acid was regenerated by acid or alkaline hydrolysis.

The sublimed material was separated into two fractions by means of sodium hydrogen carbonate solution. The insoluble fraction (60 mg.), m. p. 209—210° (from benzene), $\lambda_{max.}$ 2170 (ε 5400) and 2930 Å (ε 3450), was identified as *p*-xyloquinol by comparison with an authentic specimen. The soluble fraction, isolated with ether from the acidified aqueous layer, was obtained from benzene–hexane as plates (70 mg.) (changing to needles at 120°), m. p. 172—174° (Found: C, 59·8; H, 5·8. Calc. for $C_7H_8O_3$: C, 60·0; H, 5·8%), $\lambda_{max.}$ 2430 Å (ε 12,500). This material was identical with authentic 2-methyl-3-oxocyclopent-1-enecarboxylic acid,[7] kindly supplied by Dr. E. Schlittler. The methyl ester gave a 2,4-dinitrophenylhydrazone, m. p. 206—208° (lit., 202—203°) (Found: C, 50·3; H, 4·6. Calc. for $C_{14}H_{14}O_6N_4$: C, 50·3; H, 4·2%).

Alkali-fission of Acid (XVII).—The acid (300 mg.) was added to hot 10% aqueous sodium hydroxide (15 c.c.) which had previously been boiled in nitrogen for 15 min. The solution was refluxed for 2½ min. under nitrogen, cooled, acidified with dilute sulphuric acid, and extracted

with ether. The acidic product was separated by washing with saturated sodium hydrogen carbonate solution. The ether solution was dried (Na_2SO_4), the solvent carefully fractionated, and the residual solid (110 mg.) adsorbed on deactivated alumina. Elution with benzene–light petroleum (1 : 1) and crystallisation of the eluate (93 mg.) from light petroleum gave needles of 2,5-dimethylcyclohexane-1,4-dione, m. p. 89—90°, mixed m. p. 90—91° with an authentic synthetic specimen of the *trans*-isomer (see below) (Found: C, 68·4; H, 8·5. Calc. for $C_8H_{12}O_2$: C, 68·5; H, 8·6%), $\lambda_{max.}$ 2500 Å (ε 168). The infrared spectra were also identical.

The bicarbonate extract yielded an acidic portion (120 mg.), m. p. 164—169°, which on recrystallisation from benzene–hexane gave 2-methyl-3-oxocyclopent-1-enecarboxylic acid as prisms, m. p. 170—172°.

2,5-*Dimethylcyclohexane*-1,4-*dione* (With J. S. Stephenson).—Finely cut lithium (0·35 g.) was added to a solution of *p*-xyloquinol dimethyl ether (1·07 g.) in ethanol (2·4 g.), liquid ammonia (24 c.c.), and ether (20 c.c.). The mixture was stirred for 5 min. and ethanol was added until the solution became colourless. The ammonia was allowed to evaporate, and the solution diluted with water and extracted with ether. After removal of the solvent the enol ether (0·91 g.) crystallised from light petroleum, with m. p. 35—36°. An ethereal solution of this material (0·65 g. in 50 c.c.) was stirred with concentrated hydrochloric acid (0·2 c.c.) for 2 hr. at 20°. The mixture was neutralised with sodium hydrogen carbonate, and the ethereal solution dried and evaporated to give a mixture of diones (0·6 g.) which was adsorbed from light petroleum–benzene (2 : 1; 15 c.c.) on deactivated alumina (Peter Spence's grade "H" deactivated with 5% of 10% acetic acid; 100 g.). Elution with the same solvent gave the *cis*-dione (0·07 g.), needles from light petroleum, m. p. 118—118·5° (Found: C, 68·6; H, 8·6. Calc. for $C_8H_{12}O_2$: C, 68·5; H, 8·6%), followed by the *trans*-dione (0·40 g.), m. p. 91—92° (Found: C, 68·6; H, 8·3%) (cf. lit.,[6] m. p. 115—117° and 93° respectively).

A solution of the *cis*-dione (70 mg.) in water (20 c.c.) was refluxed under nitrogen for 15 min. Potassium hydroxide (2·0 g.) was then added and heating continued for 30 min. The solution was cooled and the product (60 mg.) extracted with chloroform. Crystallisation from light petroleum afforded *trans*-dione (50 mg.; m. p. 91°) whilst subsequent chromatography of the total product gave *cis*-dione (8 mg.) together with the *trans*-isomer (50 mg.) (thus showing that chromatography does not materially affect the composition of the mixture). Repetition of this experiment without taking precautions against oxidation led to *p*-xyloquinone as the only isolable product.

Isopropylidene Derivative (V) *of Trihydroxy-ketone* (IV; R = isocrotonyl).—Trichothecin (1 g.) was refluxed for 2 hr. in 0·1N-hydrochloric acid (150 c.c.). The cold solution was extracted with chloroform, and the combined extracts were distilled. The residue, without purification, was refluxed for 48 hr. in "AnalaR" acetone (50 c.c.) containing toluene-*p*-sulphonic acid (0·05 g.). The solution was neutralised with anhydrous potassium carbonate, and the acetone removed by distillation. Isolated *via* chloroform, the *derivative* crystallised from methanol in plates, m. p. 140—142° (Found: C, 68·2; H, 7·8. $C_{22}H_{30}O_6$ requires C, 67·7; H, 7·7%).

Isopropylidene Derivative (V) *of Trihydroxy-ketone* (IV; R = H).—(*a*) The trihydroxy-ketone [2] (IV; R = H) (0·11 g.), "AnalaR" acetone (25 c.c.), and toluene-*p*-sulphonic acid (0·02 g.) were refluxed for 20 hr. The solution was neutralised with sodium carbonate, acetone was removed by distillation, and the product isolated with ether. The *derivative* crystallised from benzene–hexane in plates, m. p. 182—183·5° (Found: C, 67·5; H, 8·2. $C_{18}H_{26}O_5$ requires C, 67·1; H, 8·1%).

(*b*) The trihydroxy-ketone isopropylidene derivative (V; R = isocrotonyl) (0·2 g.) was refluxed with 5% methanolic potassium hydroxide for 2 hr. Isolation in the usual way gave the derivative (V; R = H) (0·13 g.), identified by mixed m. p. and infrared spectrum.

Isopropylidene Derivative of "Tricothecodione Glycol."—The trihydroxy-ketone isopropylidene derivative (V; R = H) (0·07 g.) in pyridine (1 c.c.) was added to the complex prepared from pyridine (1 c.c.) and chromium trioxide (0·35 g.) and kept at 20° for 4 days. Isolation *via* ether and crystallisation from benzene–hexane gave *trichothecodione glycol isopropylidene derivative* (0·05 g.), m. p. 142—143° with previous softening (Found: 67·6; H, 7·5. $C_{18}H_{24}O_5$ requires C, 67·5; H, 7·6%), $\lambda_{max.}$ 2290 Å (ε 7400).

This derivative (0·025 g.) was heated for 2 hr. on the steam-bath with 90% acetic acid. Isolation in the usual way gave neotrichothecodione [2] (III), m. p. 165—166°, undepressed on admixture with an authentic specimen and with an identical infrared spectrum.

Permanganate-catalysed Periodate Oxidation of the Trihydroxy-ketone (IV; R = H).—The

trihydroxy-ketone (IV; R = H) (0·14 g., 0·5 mmole) was dissolved in, and made up to 200 c.c. with, a stock solution of Lemieux's reagent [9] [containing anhydrous potassium carbonate (7·5 mmole), sodium periodate (20 mmole), and potassium permanganate (0·3 mmole) per l.]. The reagent and the reaction mixture were titrated for periodate. After 20 hr. the equivalent of 1 mole of trihydroxy-ketone (IV; R = H) had reduced 2·95 moles; after 70 hr., 3·5 moles; after 5 days, 3·7 moles; and after 7 days, 3·85 moles of periodate.

An aliquot part of the solution (50 c.c.) was acidified and concentrated to a small volume. The distillate was treated with 0·1N-sodium hydroxide and required 1·54 c.c. for neutralisation (theor. for 1 mol. of acetic acid, 1·50 c.c.). The solution was evaporated to about 1 c.c. Paper chromatography [17] of a drop of this solution indicated the presence of formic or acetic acid. The remainder of the solution was evaporated to dryness and refluxed for 1 hr. with ethanol (0·5 c.c.) and 4-phenylphenacyl bromide (30 mg.). Recrystallisation of the product from ethanol gave 4-phenylphenacyl acetate, m. p. and mixed m. p. 109—111°.

Allodihydrotrichothecolone (XIX).—(a) *From trichothecin.* To boiling N-sodium hydroxide (50 c.c.) was added zinc dust (5 g.), followed by trichothecin (1·0 g.). When the organic layer had dissolved (45 min.) the mixture was cooled, filtered, and continuously extracted with ether for 20 hr. The product crystallised from the ether in prisms (0·5 g.), changing crystalline form at 241—242° and thereafter melting up to a temperature of *ca.* 290° (Found: C, 67·4; H, 8·4. $C_{15}H_{22}O_4$ requires C, 67·6; H, 8·3%), $\nu_{max.}$ 3605, 3540, 1710 cm.$^{-1}$. R.D. in methanol (*c* 0·054): $[\alpha]^{31}$ (*7000* Å), +50°; (*5890*), +83°; (*3100*), +3400°; (*2750*), −3400°; (*2700*), −3060°. Allodihydrotrichothecolone cannot be catalytically hydrogenated at atmospheric pressure and gives no colour with tetranitromethane.

(b) *From trichothecolone.* When trichothecolone (1·0 g.) was treated as described above, allodihydrotrichothecolone (0·59 g.) was isolated after continuous ether-extraction.

Allodihydrotrichothecolone Diacetate.—Allodihydrotrichothecolone (25 mg.) was refluxed with acetic anhydride (5 c.c.) and sodium acetate (50 mg.) for 4 hr. The *diacetate* was isolated and slowly crystallised from hexane; it had m. p. 70—73° (Found: C, 65·7; H, 7·8; Ac, 28·1. $C_{19}H_{26}O_6$ requires C, 65·1; H, 7·5; 2Ac, 24·6%), $\nu_{max.}$ 1740, 1712 cm.$^{-1}$.

Allodihydrotrichothecolone Carbonate.—Allodihydrotrichothecolone (40 mg.), diethyl carbonate (300 mg.), and sodium (*ca.* 1 mg.) were heated at 120° for 3 hr.[11] Crystals separated on cooling and these when crystallised from ethanol gave the *carbonate* as prisms, m. p. 270—271° (Found: C, 65·7; H, 7·1. $C_{16}H_{20}O_5$ requires C, 65·7; H, 6·9%), $\nu_{max.}$ 1770, 1720 cm.$^{-1}$.

Diketo-acid (XX) *from Allodihydrotrichothecolone.*—Allodihydrotrichothecolone (2·0 g.) was dissolved in " AnalaR " acetone (400 c.c.) and treated with 8N-chromic acid reagent (see above) slowly and with shaking until an orange colour persisted. After 15 min. the mixture was diluted with water (400 c.c.) and extracted with chloroform (10 × 200 c.c.). The extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue (2·0 g.), m. p. 240—245° (slight decomp.), on recrystallisation from benzene gave the *acid* as stout needles, m. p. 243—245° (slight decomp.), pK_a 3·6 (Found: C, 64·9; H, 6·6. $C_{15}H_{18}O_5$ requires C, 64·7; H, 6·5%). R.D. in methanol (*c* 0·0595): $[\alpha]^{31}$ (*7000* Å), +20°; (*5890*), +30°; (*3150*), +1400°; (*2850*), −1530°. $\nu_{max.}$ 1740 and 1708 cm.$^{-1}$ together with typical $-CO_2H$ absorption in the 3000—2500 cm.$^{-1}$ region.

Bromination of Allodihydrotrichothecolone.—Allodihydrotrichothecolone (306·4 mg.) was dissolved in the brominating reagent [12] (10 c.c.) [bromine (*ca.* 20 g.) and constant-boiling hydrobromic acid (0·1 c.c.) were made up to 100 c.c. with glacial acetic acid] and kept at 40°. Bromine estimations revealed that after 1 day 0·99 mol. and after 2 days, 1·09 mol. had been consumed.

The reaction mixture was evaporated at 15 mm. and the *bromo-diacetate* crystallised from ether to give prisms (0·26 g.), m. p. 159—160° (Found: C, 53·4; H, 5·8; Br, 19·0. $C_{19}H_{25}BrO_6$ requires C, 53·1; H, 5·9; Br, 18·6%), $\nu_{max.}$ 1740 cm.$^{-1}$.

The bromo-ester (0·3 g.) was dissolved in N-methanolic sodium hydroxide (25 c.c.) and kept at 20° for 2 days. The solution was diluted with water (100 c.c.), the methanol removed at 20°/15 mm., and the residual aqueous solution continuously extracted with ether for 20 hr. Removal of solvent and crystallisation from benzene–ethyl acetate gave the *bromo-ketone* as prisms, changing crystalline form at 210—212° and melting at 230° (decomp.) (Found: C, 52·3; H, 6·1; Br, 23·2. $C_{15}H_{21}BrO_4$ requires C, 52·2; H, 6·1; Br, 23·1%). R.D. in methanol (*c* 0·0565): $[\alpha]^{30}$ (*7000* Å), +44°; (*5890*), +64°, (*5000—4500*, broad peak) +88°; (*3430*) +335°;

[17] Block, Durrum, and Zweig, " A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press, New York, N.Y., 1955, p. 157.

(*3370*, shoulder) +315°; (*2950*) −290°; (*2920*) −255°; (*2900*) −290°; (*2800*) +285°. $\nu_{max.}$ 1725 cm.$^{-1}$.

Treatment of the bromo-diacetate with zinc dust and acetic acid followed by hydrolysis with N-methanolic potassium hydroxide gave allodihydrotrichothecolone, identified by m. p., mixed m. p., and infrared spectrum.

Bromination of Diketo-acid (XX).—The diketo-acid (XX) (326·9 mg.) was dissolved in the brominating reagent [12] (see above; 10 c.c.) and kept at 40°. Bromine estimations revealed that after 1 day 1·80 mol. and after 2 days 2·06 mol. had been consumed.

The reaction mixture was evaporated at 15 mm. and the *dibromo-derivative* crystallised from ether (210 mg.); it had m. p. *ca.* 260° (decomp.) (Found: C, 41·5; H, 4·0; Br, 36·1. $C_{15}H_{16}Br_2O_5$ requires C, 41·3; H, 3·7; Br, 36·6%). R.D. in methanol (*c* 0·0825): $[\alpha]^{31}$ (*7000* Å), −80°; (*5890*), −80°; (*3480*), −1220°; (*2900* shoulder) +1550°; (*2750*), +2200°. $\nu_{max.}$ 1740 cm.$^{-1}$.

Benzylidene Derivative of Diketo-acid (XX).—The acid (100 mg.) was dissolved in N-alcoholic sodium hydroxide (20 c.c.) containing benzaldehyde (200 mg.). After 3 days at 20° the mixture was treated with dilute acid; isolation *via* chloroform gave the *benzylidene derivative* which crystallised from benzene–hexane in pale yellow needles (120 mg.), m. p. 237—238° (Found: C, 72·4; H, 5·8. $C_{22}H_{22}O_5$ requires C, 72·1; H, 6·0%), $\lambda_{max.}$ 2250 (ε 9000), 2300 (ε 9000), and 3070 Å (ε 24,000).

Isotrichothecolone Diacetate.—Isotrichothecolone (100 mg.) was refluxed for 4 hr. with sodium acetate (300 mg.) and acetic anhydride (3 c.c.). After evaporation under reduced pressure the residue was taken up in chloroform, and the solution washed with sodium hydrogen carbonate solution, dried and evaporated. The *diacetate* crystallised from hexane in needles (65 mg.), m. p. 155—157° (Found: C, 65·2; H, 7·2; Ac, 23·7. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·9; 2Ac, 24·7%), $\lambda_{max.}$ 2290 Å (ε 10,700).

Isotrichothecolone Bis(ethyl Carbonate).—Isotrichothecolone (40 mg.), diethyl carbonate (300 mg.) and sodium (*ca.* 1 mg.) were heated at 120° for 6 hr.[11] The diethyl carbonate was removed and the residue treated with ethanol and a drop of water. The product crystallised slowly, and recrystallisation from benzene–light petroleum gave the *bis(ethyl carbonate)*, m. p. 168—172° (Found: C, 61·9; H, 7·0. $C_{21}H_{28}O_8$ requires C, 61·8; H, 6·9%), $\nu_{max.}$ 1750 and 1678 cm.$^{-1}$.

Permanganate-catalysed Periodate Oxidation of Isotrichothecolone.—Isotrichothecolone (132 mg., 0·5 mmole) was dissolved in, and made up to 200 c.c. with, a stock solution of Lemieux's reagent [9] (see above). The reagent and the reaction mixture were titrated for periodate. After 20 hr. the equivalent of 1 mole of isotrichothecolone had consumed 4·75 moles; after 70 hr., 5·6 moles; after 5 days, 5·8 moles; and after 7 days, 5·9 moles of periodate.

An aliquot part of the solution (50 c.c.) was acidified and the solution evaporated. The distillate required 1·54 c.c. of 0·1N-sodium hydroxide for neutralisation (theor. for 1 mol. of acetic acid, 1·50 c.c.). The acid was identified as acetic acid (as above) by paper chromatography [17] and preparation of the 4-phenylphenacyl ester, m. p. and mixed m. p. 108—110°.

Diketo-acid (XXIII) *from Isotrichothecolone.*—Isotrichothecodione (1·5 g.) in ethanol (30 c.c.) was treated with sodium borohydride (0·6 g.) and kept at 20° for 40 hr. Acetone (2 c.c.) was added, followed by 2N-sulphuric acid (10 c.c.) and water (50 c.c.). The ethanol was removed at 20°/20 mm. and the aqueous solution continuously extracted with ether for 2 days. Removal of the ether yielded the triol mixture (1·4 g.) which partially crystallised in contact with benzene (m. p. 60—85°); the infrared spectrum showed the absence of any carbonyl function.

The triol mixture in ethanol (100 c.c.) was hydrogenated over 10% palladium–norite (0·2 g.). Filtration and removal of solvent gave a residue which was treated with Lemieux's reagent [9] (1 l.; see above) at 20° for 2 days. The reaction mixture was continuously extracted with ether for 2 days; the residue (0·8 g.) obtained on removal of the ether showed no carbonyl absorption in its infrared spectrum. The aqueous solution was acidified and continuously extracted with ether for 2 days. The viscous product (210 mg.; $\nu_{max.}$ 1740, 1710 cm.$^{-1}$ and typical CO_2H absorption in 3000—2500 cm.$^{-1}$ region) in "AnalaR" acetone (50 c.c.) was oxidised with the 8N-chromic acid reagent. The product, isolated in the usual way, crystallised slowly from benzene, giving the *diketo-acid* (XXIII), m. p. 104—109° (Found: C, 64·4; H, 6·9%; equiv., 288. $C_{15}H_{20}O_5$ requires C, 64·3; H, 7·2%; equiv. for monobasic acid, 280), pK_a 4·3, $\nu_{max.}$ 1740, 1725, 1706, and typical CO_2H absorption in the 3000—2500 cm.$^{-1}$ region.

Bromination of "Dihydrotrichothecolone Glycol."—Dihydrotrichothecolone glycol [2] (271·4 mg.) was dissolved in the brominating reagent [12] (10 c.c.; see above) and kept at 40°. After 1 day the bromine contents of the reagent and reaction mixture were determined. The equivalent of 1 mole of dihydrotrichothecolone glycol had consumed 3·06 moles, and after 2 days, 3·10 moles of bromine had been consumed.

Bromination of Dihydroisotrichothecolone.—Isotrichothecolone (565·7 mg.) in ethanol (50 c.c.) was hydrogenated over 5% palladium–norite (200 mg.). The mixture was filtered quantitatively, the solvent removed, and the residue treated with the brominating reagent [12] (10 c.c.; see above) and kept at 40°. After 1 day 1·75 mol. and after 2 days 2·05 mol. had been consumed.

On cooling of the solution, the *dibromo-ketone* (480 mg.) crystallised in fine needles, m. p. 235—236° (Found: C, 43·9; H, 4·8; Br, 34·1. $C_{17}H_{22}Br_2O_5$ requires C, 43·8; H, 4·8; Br, 34·3%), $\nu_{max.}$ 3600, 1740, and 1716 cm.$^{-1}$.

The dibromodihydroisotrichothecolone monoacetate (70 mg.) was dissolved in glacial acetic acid (20 c.c.), and zinc dust (0·3 g.) was added. The mixture was stirred for 5 min., filtered, and washed with acetone. Removal of solvent and crystallisation from benzene–light petroleum gave acetyldihydroisotrichothecolone, m. p. 120—122° (lit.,[2] 123—124°).

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