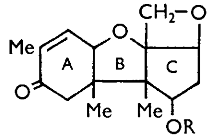
224. *The Structure of Trichothecin and its Hydrolysis Products.*

By G. G. Freeman, J. E. Gill, and W. S. Waring.

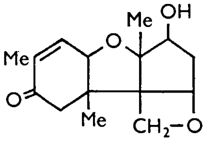
Trichothecin is the *iso*crotonic ester of the ketonic alcohol, trichothecolone. On the basis of its properties and those of degradation products, obtained by oxidation, alkaline fission, and dehydrogenation, the alternative structures (I; R=H) and (II), of which the former is preferred, are proposed for trichothecolone. On treatment with hot potassium hydroxide, trichothecolone gives an isomer, *iso*trichothecolone. Some properties and derivatives of the latter are described.

Trichothecin is an antifungal substance, produced by the fungus *Trichothecium roseum* Link.[1] Some of its chemical and biological properties have been described.[2-4] It is an ester, $C_{19}H_{24}O_5$, giving [5] on hydrolysis *iso*crotonic acid and a ketonic alcohol, trichothecolone, $C_{15}H_{20}O_4$. Trichothecin was readily hydrogenated at atmospheric pressure with palladium–charcoal, two mols. of hydrogen being absorbed to give the non-crystalline tetrahydrotrichothecin. This uptake was accounted for by saturation of one ethylenic double bond in the *iso*crotonic ester group and a second ethylenic double bond in the trichothecolone part of the molecule. Trichothecin contained a reactive carbonyl group, which was retained in tetrahydrotrichothecin. Kuhn–Roth determinations showed that trichothecin has at least three *C*-methyl groups. No methoxyl group was detected. The infrared spectrum of trichothecin had bands at 1670 (αβ-unsaturated ketone), 1710 and 1177 (αβ-unsaturated ester), and 1650 cm.$^{-1}$ (attributed to ethylenic unsaturation). Absence of a band in the region of 3400 cm.$^{-1}$ indicated absence of hydroxyl groups which was confirmed by indifference of trichothecin, even under the most vigorous conditions, to acetic anhydride or benzoyl chloride. There was no indication of benzenoid absorption in the spectrum.

(I)



Trichothecolone
when R = H



(II)

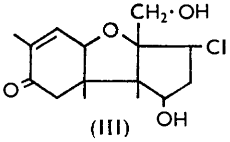
Trichothecin was readily hydrolysed with cold methanolic potassium hydroxide to trichothecolone (98% yield) and β-methoxybutyric acid (presumably formed by addition of methanol to *iso*crotonic acid; Purdie and Marshall [6] showed that ethyl crotonate on treatment with sodium methoxide gave sodium β-methoxybutyrate). Hydrolysis of trichothecin with boiling aqueous 0·5N-sodium hydroxide gave *iso*crotonic acid and *iso*trichothecolone, isomeric with trichothecolone. The functional groups of trichothecin (I; R = CHMe:CH·CO) were thus shown to be two ethylenic double bonds, a ketonic group, and an ester group, which account for three of the five oxygen atoms in the molecule.

Trichothecin reacted exothermally with concentrated hydrochloric acid to form a chlorohydrin $C_{19}H_{25}O_5Cl$, m. p. 132·5°; the infrared spectrum of this showed a band associated with a hydroxyl group whose presence was confirmed by quantitative acetylation though the acetyl derivative of the chlorohydrin was not obtained crystalline. The chlorohydrin was still ketonic. Hydrolysis of this chlorohydrin with methanolic potassium hydroxide at room temperature gave trichothecolone chlorohydrin (III), m. p. 139—140°, also formed by the action of 10N-hydrochloric acid on trichothecolone. This indicated that

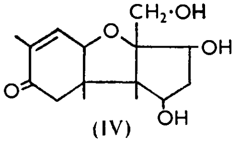
---

[1] Freeman and Morrison, *Nature*, 1948, **162**, 30.
[2] *Idem, Biochem. J.*, 1949, **44**, 1.
[3] *Idem, J. Gen. Microbiol.*, 1949, **3**, 60.
[4] Freeman, *ibid.*, 1955, **12**, 213.
[5] Freeman and Gill, *Nature*, 1950, **166**, 698.
[6] Purdie and Marshall, *J.*, 1891, 468.

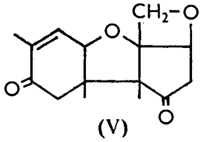
the hydrochloric acid had reacted with the trichothecolone part of the molecule, opening an ether linkage with formation of an alcoholic group. Trichothecin chlorohydrin was not formed when dry hydrogen chloride was passed into solutions of trichothecin in ether or chloroform, and trichothecin did not produce hydroxyl ions when warmed with sodium thiosulphate in 50% acetone,[7] which are normal reactions of epoxides. Trichothecin bromohydrin $C_{19}H_{25}O_5Br$ and trichothecolone bromohydrin $C_{15}H_{21}O_4Br$ were prepared in analogous ways.



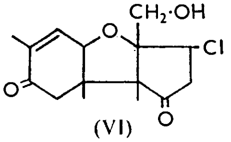
(III)
Trichothecolone chlorohydrin



(IV)
Trichothecolone glycol



(V)
Trichothecodione



(VI)
Trichothecodione chlorohydrin

When trichothecin was boiled in 0·1N-hydrochloric acid, addition of water took place, giving a glycol, $C_{19}H_{26}O_6$, hydrolysed by cold alkali to trichothecolone glycol (IV), which was also obtained from trichothecolone. Quantitative acetylation indicated that there were three hydroxyl groups present in trichothecolone glycol and it is assumed that two of these have been formed by opening of an oxygen ring. Trichothecolone glycol adsorbed one mol. of hydrogen at ordinary temperature to form dihydrotrichothecolone glycol which was also prepared by the action of boiling 0·5N-sulphuric acid on dihydrotrichothecolone. Trichothecolone glycol was recovered unchanged after one hour's boiling in 15% aqueous potassium hydroxide. Acetylation of trichothecolone glycol gave a non-crystalline triacetyl derivative. The glycols from trichothecin and trichothecolone were both ketonic.

Hydrogenation of trichothecin chlorohydrin at ordinary pressure gave the non-crystalline tetrahydrotrichothecin chlorohydrin which on oxidation with chromic acid gave a chloro-acid, $C_{19}H_{27}O_6Cl$, with no loss of carbon. The chloro-acid no longer contained a hydroxyl group, but was still ketonic, formed an amide, and sublimed *in vacuo* unchanged. Oxidation of trichothecin chlorohydrin with chromic acid gave an acid, $C_{19}H_{23}O_6Cl$, which on hydrogenation gave the crystalline chloro-acid, $C_{19}H_{27}O_6Cl$, described above.

$$\begin{tikzcd}
 & & \begin{array}{c}\text{Tetrahydro-derivative}\\ C_{19}H_{29}O_6Cl\end{array} \arrow[dr, "CrO_3"] & \\
\begin{array}{c}\text{Trichothecin}\\ C_{19}H_{24}O_5\end{array} \arrow[r, "HCl"] & \begin{array}{c}\text{Trichothecin chlorohydrin}\\ C_{19}H_{25}O_5Cl\end{array} \arrow[ur, "H_2"] \arrow[dr, "CrO_3"'] & & \begin{array}{c}\text{Crystalline chloro-acid}\\ C_{19}H_{27}O_6Cl\end{array} \\
 & & \begin{array}{c}\text{Acid}\\ C_{19}H_{23}O_6Cl\end{array} \arrow[ur, "H_2"'] &
\end{tikzcd}$$

In a similar way, trichothecin bromohydrin was hydrogenated to non-crystalline tetrahydrotrichothecin bromohydrin, and this was oxidised to the corresponding saturated bromo-acid, $C_{19}H_{27}O_6Br$. These reactions with concentrated hydrochloric and hydrobromic acid can be explained by assuming that trichothecin contained an oxide ring which was easily opened by strong acids with the formation of a hydroxymethyl group. Oxidation of the latter then resulted in the formation of a carboxylic acid with no loss of carbon. Tetrahydrotrichothecin also reacted with concentrated hydrochloric acid. Cold alkaline hydrolysis of the saturated chloro-acid $C_{19}H_{27}O_6Cl$ gave a hydroxy-chloro-acid, m. p. 199°, $C_{15}H_{21}O_5Cl$.

Trichothecolone.—The keto-alcohol trichothecolone, $C_{15}H_{20}O_4$, contains at least two

---

[7] Ross, *J.*, 1950, 2257.

C-methyl groups (Kuhn–Roth) and one active hydrogen (hydroxyl group). Its infrared spectrum has bands at 1650 (C:C), 1670 (C:C·CO) and 3510 cm.$^{-1}$ (OH) but no benzenoid absorption. Its carbonyl group is reactive (semicarbazone; deep orange 2 : 4-dinitrophenylhydrazone). It readily gave an acetyl derivative, which formed a 2 : 4-dinitrophenylhydrazone and regenerated trichothecolone on hydrolysis at room temperature. The infrared spectrum of acetyltrichothecolone contained no OH band, indicating that normal esterification had occurred. Cold alkaline hydrolysis of trichothecin 2 : 4-dinitrophenylhydrazone yielded trichothecolone 2 : 4-dinitrophenylhydrazone, identical with that prepared from the alcohol.

In presence of palladium–charcoal trichothecolone rapidly absorbed one mol. of hydrogen, the product still being ketonic (semicarbazone and 2 : 4-dinitrophenylhydrazone). The ease of hydrogenation precluded hydrogenolysis and this was supported by the formation of acetyldihydrotrichothecolone (no infrared OH band). The wave number of the carbonyl group in the infrared spectrum of dihydrotrichothecolone was 1695 cm.$^{-1}$ and that of trichothecolone was 1670 cm.$^{-1}$, the change indicating loss of αβ-conjugation. As described below, a similar difference was observed for the six-membered ring ketonic groups of trichothecodione and dihydrotrichothecodione. The ultraviolet absorptions of trichothecin, trichothecolone, and *iso*trichothecolone (see Table), when compared with Woodward's[8] observations on αβ-unsaturated ketones [monosubstituted (α or β) 2250 ± 50 Å; disubstituted (αβ or ββ) 2390 Å; trisubstituted (αββ) 2540 Å], suggest that trichothecolone and *iso*trichothecolone are α- or β-monosubstituted αβ-unsaturated ketones. Hydrogenation caused the disappearance of the bands, *e.g.*, tetrahydrotrichothecin and dihydrotrichothecolone showed no high-intensity absorption. In the spectrum of trichothecolone semicarbazone there was a displacement of the band to longer wavelength (by 410 Å) and an increase in intensity (by ε 12,000). This behaviour was characteristic of αβ-unsaturated ketones.[9]

	$\lambda_{max.}$ (Å)	ε	$\lambda_{max.}$ (Å)	ε
	In hexane		In MeOH	
Trichothecin	2170	18,000	2150	19,000
Trichothecolone	2240	8000	2260	8000
*iso*Trichothecolone	2220	9000	2300	8050

A number of esters of trichothecolone was prepared by treating the alcohol with the acid anhydrides in pyridine, but *iso*crotonyltrichothecolone was not obtained. Some biological properties of these esters have been reported.[4] Hydrogenation of butyryltrichothecolone gave an oil, presumed to be butyryldihydrotrichothecolone, which formed a microcrystalline semicarbazone identical with that prepared from the non-crystalline tetrahydrotrichothecin.

Trichothecolone gave a negative result in the haloform reaction, indicating the absence of the CH_3·CO group. Both trichothecolone and *iso*trichothecolone reacted as primary and secondary alcohols with nitric–chromic acid reagent[10] which did not distinguish between the two compounds. Trichothecolone did not react with phthalic anhydride in benzene or toluene, as expected of a primary alcohol. Ozonolysis of trichothecolone in chloroform gave acetic acid (0·58 mol. by titration; isolated as *p*-phenylphenacyl acetate) and acetaldehyde but no other characterisable products. Oxidations of trichothecolone, *iso*trichothecolone, or dihydrotrichothecolone with potassium permanganate gave only water-soluble products, including oxalic acid. Oxidation of tetrahydrotrichothecodiol with potassium permanganate gave 0·95 mol. of acetic acid.

Oxidation Products of Trichothecolone.—Oxidation of trichothecolone and dihydro trichothecolone with chromic acid gave compounds containing two hydrogen atoms less. The product, trichothecodione (V), from the former gave a mono-semicarbazone, a mono-2 : 4-dinitrophenylhydrazone, and in small yield a bis-2 : 4-dinitrophenylhydrazone.

---

[8] Woodward, *J. Amer. Chem. Soc.*, 1941, **63**, 1123.
[9] Evans and Gillam, *J.*, 1943, 565.
[10] Fearon and Mitchell, *Analyst*, 1932, **57**, 372.

Dihydrotrichothecolone gave on oxidation dihydrotrichothecodione which formed a mono-2 : 4-dinitrophenylhydrazone, and was also prepared by hydrogenation of trichothecodione. The infrared spectra of these diones did not contain the hydroxyl band (3510 cm.$^{-1}$) of trichothecolone. The bands at 1670 cm.$^{-1}$ for trichothecodione and at 1710 cm.$^{-1}$ for dihydrotrichothecodione, attributed to the original ketonic group, were present, together with a new band at 1735 cm.$^{-1}$ due to a new carbonyl. This was consistent with the presence of a secondary alcoholic group in trichothecolone and its dihydro-derivative. The difference in frequency between the two carbonyl groups in trichothecodione indicated that the hydroxyl group in trichothecolone was not attached to the same ring as the carbonyl group. The frequency of the carbonyl group at 1735 cm.$^{-1}$ is of the order of that of an unconjugated, five-membered ring ketone.

The second carbonyl group in trichothecodione was relatively unreactive to ketonic reagents. The ultraviolet absorption showed a maximum at 2250 Å (ε 8600) and its semicarbazone a maximum at 2680 Å (ε 21,700), characteristic of an $\alpha\beta$-unsaturated ketone, and this suggested that the original carbonyl of trichothecolone was the more reactive one in trichothecodione. Trichothecodione did not give a pyrrole derivative on reaction with ammonia, a colour with ferric chloride, or a copper complex. Thus the characteristic reactions of $\alpha\beta$- and $\alpha\gamma$-diketones were absent.

Trichothecodione with cold 10N-hydrochloric acid yielded a chlorohydrin (VI) in a way analogous to that of trichothecin, so the oxide bridge is intact in the dione. The chlorohydrin was ketonic. Similarly dihydrotrichothecodione in 10N-hydrochloric acid formed a crystalline chlorohydrin in 34% yield. Trichothecolone chlorohydrin with aqueous chromic acid gave trichothecodione chlorohydrin, which was isolated as the dinitrophenylhydrazone.

Oxidation of the hydroxy-chloro-acid $C_{15}H_{21}O_5Cl$ (see above) of dihydrotrichothecodione chlorohydrin, and of dihydrotrichothecolone chlorohydrin with chromic acid, each gave a product, $C_{15}H_{18}O_5$ (XIX), isolated as 2 : 4-dinitrophenylhydrazones which were shown to be identical by mixed m. p. and comparison of their *X*-ray powder photographs.

Dehydrogenation of Tetrahydrotrichothecodiol.—As a preliminary to dehydrogenation, trichothecolone was hydrogenated at 100°/100 atm. in the presence of Raney nickel. The colourless amorphous product, tetrahydrotrichothecodiol $C_{15}H_{26}O_4$, did not react with Brady's reagent and carbonyl absorption was absent from its infrared spectrum. Tetrahydrotrichothecodiol has three active hydrogen atoms and analysis of its acetyl derivative indicated the presence of three acetyl groups, although the results were not conclusive. These observations suggested that hydrogenolysis had occurred. Estimation of the *C*-methyl content gave a slightly higher value than that obtained for trichothecolone.

Tetrahydrotrichothecodiol was unchanged by palladium–charcoal in boiling 1-methylnaphthalene for 6 hr. but in absence of solvent decomposition commenced at 140°. The volatile products were collected by distillation up to 300°. Further decomposition products were obtained by distillation when the residue was heated *in vacuo*, and by extracting the residue with solvents, respectively. The products consisted of water (2·75 mols.), *p*-xylene, and two ketones, $C_7H_{10}O$ and $C_7H_{12}O$. Reduction of a mixture of the ketones gave a saturated ketone $C_5H_6Me_2O$, which did not form a dibenzylidene derivative and hence was not 3 : 4-dimethyl*cyclo*pentanone; its 2 : 4-dinitrophenylhydrazone and semicarbazone were identical with those of 2 : 3-dimethyl*cyclo*pentanone (mixed m. p.; infrared spectrum). 2 : 4-, 3 : 4-, and 2 : 5-Dimethyl*cyclo*pentanone were prepared and gave different 2 : 4-dinitrophenylhydrazones.

2 : 3-Dimethyl*cyclo*pent-2-enone [11] gave a semicarbazone and 2 : 4-dinitrophenylhydrazone identical with those of our ketone $C_7H_{10}O$. The absorption maximum of authentic 2 : 3-dimethyl*cyclo*pent-2-enone was at 2335 ± 10 Å; that of the degradation product (2290 ± 10 Å) may have been shifted by impurities.

---

[11] Frank, Armstrong, Kwiatek, and Price, *J. Amer. Chem. Soc.*, 1948, **70**, 1379.

The oil which was obtained by solvent extraction of the residue from the dehydrogenation was separated into two fractions by steam-distillation. The infrared spectrum of the steam-volatile fraction indicated hydroxyl, carbonyl, and ethylenic groups. Fractionation by distillation gave *p*-xylenol. Attempts to aromatise the higher-boiling fraction by bromination and dehydrobromination, and by dehydrogenation, gave a compound which still contained traces of hydroxyl and carbonyl groups but was probably identical with or very similar to the product (described below) obtained by reduction of dihydrotrichothecolone with phosphorus and hydriodic acid.

The following observations are of importance in interpretation of the dehydrogenation experiments: (1) The reaction was carried out under relatively mild conditions; although the temperature was finally raised to 300°, the bulk of the product distilled at 140—220°. (2) The yields of *p*-xylene and 2 : 3-dimethyl*cyclo*pentanone were such that their formation by breakdown of a single ring cannot be excluded. (3) The infrared spectrum of trichothecodione indicates the presence of a *cyclo*pentanone nucleus. (4) The bulk of the oxygen in the starting material was accounted for by formation of approximately three mols. of water and of the *cyclo*pentanones. (5) The fifteen carbon atoms of tetrahydrotrichothecodiol were accounted for on a qualitative, though not a quantitative basis, by those present in the products (*p*-xylene and dimethyl*cyclo*pentanone). (6) Tetrahydrotrichothecodiol probably contained three *C*-methyl groups (Found: 13·0. Required for 3*C*-Me, 16·7%), whereas the dehydrogenation products contained four *C*-methyl groups. Taken as a whole this evidence strongly suggests that the precursors of *p*-xylene and of 2 : 3-dimethyl*cyclo*pentanone exist as independent rings in tetrahydrotrichothecodiol and that a five-membered carbon ring, substituted by a hydroxyl group (in tetrahydrotrichothecodiol) and a keto-group (in trichothecodione), gives rise to the *cyclo*pentanone isolated.

Dehydration-dehydrogenation of *cyclo*hexanol has been reported to occur in the presence of various catalysts such as platinum,[12] nickel–aluminium, rhenium, silica gel,[13] and copper chromite [14] with production of benzene, phenol, and *cyclo*hexanone. Analogous reactions leading to *p*-xylene and *p*-xylenol from a substituted *cyclo*hexanol system seem probable in the dehydrogenation of tetrahydrotrichothecodiol. The position of the carbonyl band in the infrared spectrum of trichothecolone (1670 cm.$^{-1}$) indicates that it is present in a six-membered ring; isolation of *p*-xylenol from the dehydrogenation of tetrahydrotrichothecodiol furnishes supporting evidence of the presence of a C_6 ring in the latter compound. The low temperature of the reaction precludes isomerisation of the methyl groups, and this is supported by the isolation of pure *p*-xylene; a mixture of xylenes would be expected if isomerisation had occurred.

High-pressure hydrogenation of trichothecolone glycol and dehydrogenation of the product, under the conditions used for tetrahydrotrichothecodiol, also gave *p*-xylene, 2 : 3-dimethyl*cyclo*pentanone, and 2 : 3-dimethyl*cyclo*pent-2-enone. *p*-Xylene and the same *cyclo*pentenone were also isolated by dehydrogenation of the hydrogenated neutral compound (VII) obtained by oxidation of trichothecolone glycol with aqueous chromic acid (see below). High-pressure hydrogenation of an acid (XX) prepared by oxidation of the primary alcohol group in *neo*trichothecodione (see below) and hydrogenation of the ethylenic bonds, followed by the usual dehydrogenation, further gave *p*-xylenol and 2-methyl*cyclo*pentanone after hydrogenation of a ketonic mixture (cf. Scheme 3): isolation of 2-methyl*cyclo*pentanone in this experiment supports the interpretation (below) that the methylene group of the methyleneoxy-bridge in trichothecolone is in the β-position with respect to the hydroxyl group, and appears as the 3-methyl group in 2 : 3-dimethyl*cyclo*pentanone. The carboxyl group of the acid (XX) is assumed to have been lost by decarboxylation under the conditions of the experiment. Dehydrogenation of the product of high-pressure hydrogenation of the saturated acid (XI) occurred only at a very high

---

[12] Adkins, Richards, and Davis, *ibid.*, 1941, **63**, 1320.

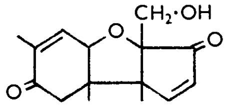
[13] Rollett and Maurer-Stroh, *Ber.*, 1940, **73**, 740.

[14] Hurd, Greengard, and Roe, *J. Amer. Chem. Soc.*, 1939, **61**, 3359.

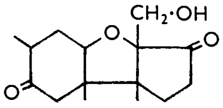
temperature; *p*-xylenol and a mixture of 2- with some 3-methyl*cyclo*pentanone were obtained after hydrogenation at atmospheric pressure.

Alkaline Fission of Trichothecodione.—When trichothecodione was heated for 3 min. with one equivalent of sodium hydroxide *neo*trichothecodione (XVI) (see below) was isolated (62% yield). Boiling for 2 min. with an excess of 10% sodium hydroxide solution gave *p*-xyloquinone (10—15%) and *p*-xyloquinol (25%) (under similar conditions it was found that about 90% of authentic *p*-xyloquinone was irrecoverable). These products were consistent with the hypothesis that trichothecolone contains a six-membered ring having two methyl groups or potential methyl groups in the *para*-position to each other and also two oxygen atoms in the *para*-position to each other. Dehydrogenation and infrared evidence suggested that one of these oxygen atoms was in the form of a keto-group. Certain other derivatives of trichothecin gave small amounts of *p*-xyloquinone when heated with an excess of sodium hydroxide, *e.g.*, trichothecin chlorohydrin (5%), *neo*-trichothecodione (5%), the chloro-acid $C_{19}H_{27}O_6Cl$ (8%), and the acid (XVIII) (6%) (see below). The formation of *p*-xyloquinone from the latter acid confirmed the findings and was in agreement with the deductions drawn from the dehydrogenation of acid (XX), *viz.*, that the methyleneoxy-bridge was attached to that part of the molecule which gave rise to 2 : 3-dimethyl*cyclo*pentanone. No quinone was detected when acid (X) or ketone (XIII) was heated with aqueous sodium hydroxide. The acid (XX), $C_{15}H_{16}O_5$, decomposed in hot dilute sodium hydroxide solution giving a compound $C_8H_{12}O_2$. This product gave slight reduction of Tollens's reagent at room temperature. A purer specimen, free from reducing properties towards Tollens's reagent, has since been obtained by other workers.[15]

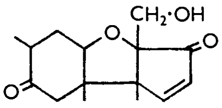
Further C_{15} and C_{14} Oxidation Products of Trichothecolone Derivatives.—Oxidation of trichothecolone glycol (IV) with aqueous chromic acid at room temperature gave a neutral compound (VII) (42% yield), $\lambda_{max.}$ 2270 Å (ε 13,500), $\nu_{max.}$ 3440 (OH) and 1710 and 1680 cm.$^{-1}$



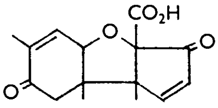
(VII)



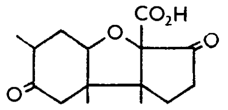
(VIII)



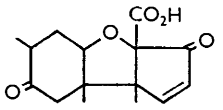
(IX)



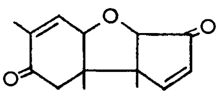
(X)



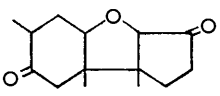
(XI)



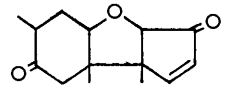
(XII)



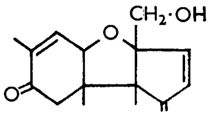
(XIII)



(XIV)

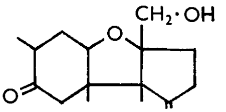


(XV)

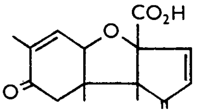


(XVI)

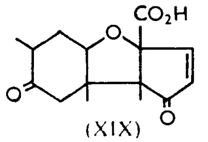
*neo*Trichothecodione



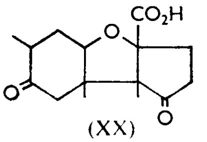
(XVII)



(XVIII)



(XIX)



(XX)

(both >CO), which formed an orange mono-2 : 4-dinitrophenylhydrazone and a mono-acetate. The acetate showed no infrared hydroxyl band but an ester group and two bands at 1680 cm.$^{-1}$ and 1750 cm.$^{-1}$ and regenerated the compound (VII) on alkaline hydrolysis in good yield. On hydrogenation in the presence of palladium–charcoal

---

[15] Fishman, Jones, and Whiting, personal communication, 1957.

compound (VII) absorbed two mols. of hydrogen, giving a compound (VIII), which formed a yellow mono-2 : 4-dinitrophenylhydrazone. When dihydrotrichothecolone glycol was oxidised with aqueous chromic acid an unsaturated diketone (IX) was obtained, having an ultraviolet absorption maximum at 2190 Å (ε 6250), forming an orange mono-2 : 4-dinitrophenylhydrazone, and giving on hydrogenation the ketone (VIII).

Formation of compound (VII) by aqueous chromic acid from trichothecolone glycol (IV) may be explained on the basis of (*a*) oxidation, to a keto-group, of the secondary hydroxyl group formed in ring C by addition of water to trichothecolone, and (*b*) loss of water from the αβ-position to the new ketonic group. Quantitative acetylation of trichothecolone glycol indicated the presence of three hydroxyl groups whereas acetylation of compound (VII) showed only one hydroxyl group per molecule. The hydrogenation of compound (VII) to (VIII), and the increased intensity in the ultraviolet absorption spectrum of (VII), suggest that a new ethylenic link has been formed during oxidation, probably in conjugation with the new carbonyl group. This is supported by the formation of compound (IX) on oxidation of dihydrotrichothecolone glycol. The facts that compound (IX) can be hydrogenated to (VIII) and that the former shows definite ultraviolet absorption whereas dihydrotrichothecolone glycol has no high-intensity absorption are further evidence in favour of new unsaturation. The three neutral compounds (VII, VIII, IX) were oxidised to acids by the action of chromic acid in acetic acid. Oxidation of the compound $C_{15}H_{18}O_4$ (VII) gave the acid $C_{15}H_{16}O_5$ (X) (obtained as a monohydrate). Its ultraviolet absorption showed a maximum at 2270 Å (ε 12,500). Oxidation of the compound (VIII) $C_{15}H_{22}O_4$ gave the acid $C_{15}H_{20}O_5$ (XI), and of the compound (IX) $C_{15}H_{20}O_4$ gave the acid $C_{15}H_{18}O_5$ (XII).

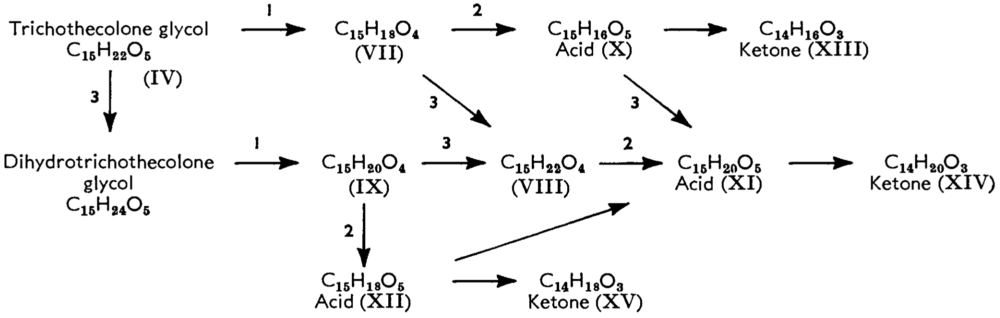
The acid-catalysed opening of the oxygen bridge in trichothecin has been shown to give rise to a primary hydroxyl group which is present in trichothecolone glycol (IV) and in (VII, VIII, and IX). Although stable to cold aqueous chromic acid the primary hydroxyl group is attacked by chromic acid in acetic acid (a very much faster reaction than in water; cf. Cohen and Westheimer [16]) with the formation of acids (X, XI, and XII). These three acids lost carbon dioxide on melting and gave crystalline neutral ketones. Acid (X) gave the ketone $C_{14}H_{16}O_3$ (XIII); acid (XI) gave the ketone $C_{14}H_{20}O_3$ (XIV); and acid $C_{15}H_{18}O_5$ (XII) gave the ketone $C_{14}H_{18}O_3$ (XV). The behaviour of the acids on melting was suggestive of β-keto-acids. Their failure to react with aqueous ferric chloride suggests that they may be αα-disubstituted β-keto-acids. The semicarbazone of ketone (XIV) crystallised from chloroform, and apparently held solvent of crystallisation; the analytical figures agreed better with those for the bis- than the mono-derivative. The dinitrophenylhydrazone of (XIV) also gave analytical figures in closer agreement with those for a bis- than a mono-derivative. The inter-relationship of the acids and ketones from trichothecolone is summarised in Scheme 1.

Isomers of Trichothecodione and Dihydrotrichothecodione.—When trichothecodione (V) was warmed with ammonia or sodium carbonate solution, or with one equivalent of sodium hydroxide for a short time, isomerisation occurred yielding *neo*trichothecodione (XVI). Similarly, dihydrotrichothecodione yielded *neo*dihydrotrichothecodione. Both *neo*-compounds gave mono-2 : 4-dinitrophenylhydrazones. It seemed probable that the isomerism involved the opening of a methyleneoxy-bridge and the elimination of an oxygen atom from the β-position to a carbonyl group. In support of this it was found that *neo*trichothecodione contained a hydroxyl group (as shown by quantitative acetylation and infrared absorption spectrum). The infrared spectra of these compounds showed strong absorption due to a hydroxyl group, and to ketonic bands at 1700 cm.$^{-1}$ and 1720 cm.$^{-1}$ respectively. *neo*Trichothecodione was found to possess two ethylenic double bonds, and *neo*dihydrotrichothecodione one such bond, by quantitative hydrogenation, both giving the same compound (XVII), $C_{15}H_{22}O_4$, thus confirming the presence of a new ethylenic bond in these two compounds. The intensity of the ultraviolet absorption of

[16] Cohen and Westheimer, *J. Amer. Chem. Soc.*, 1952, **74**, 4387.

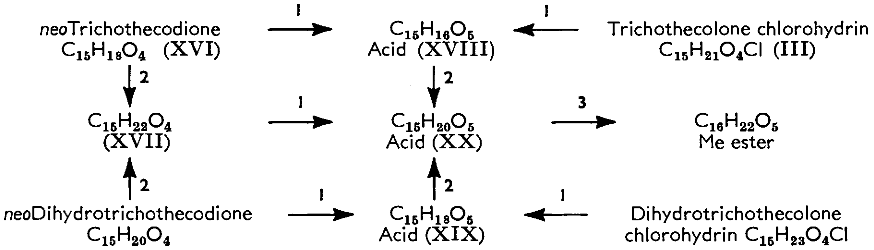
*neo*trichothecodione ($\lambda_{max.}$ 2200 Å, ε 12,910) was almost double that of trichothecodione ($\lambda_{max.}$ 2250 Å, ε 8600), which indicated that the new ethylenic bond in *neo*trichothecodione was conjugated with a keto-group. In further support of the hypothesis, *neo*trichothecodione contained a new hydroxyl group, being oxidised by chromic acid to an acid (XVIII), $C_{15}H_{16}O_5$ (Scheme 2). Similarly *neo*dihydrotrichothecodione was oxidised to an acid (XIX), $C_{15}H_{18}O_5$. These acids were hydrogenated to the same saturated acid (XX), $C_{15}H_{20}O_5$, proving that a new ethylenic double bond had been introduced during the isomerisation of trichothecodione. Oxidation of the compound (XVII) with chromic acid gave the acid (XX). The acids (XVIII—XX) were not decarboxylated on melting, and thus differed from those obtained from the trichothecolone glycol series. The β-elimination of water involved when the methyleneoxy-bridge was opened during the alkaline isomerisation of trichothecodione, was paralleled by the β-elimination of hydrogen chloride from trichothecolone chlorohydrin (in which the methyleneoxy-bridge was already open) when this was oxidised with chromic acid. The products of oxidation of the chlorohydrins were identical with those obtained from the corresponding derivatives in the *neo*trichothecodione series. Thus oxidation of trichothecolone chlorohydrin (III) yielded acid (XVIII), and oxidation of dihydrotrichothecolone chlorohydrin yielded acid (XIX). Both these acids were hydrogenated to acid (XX), identical with that obtained from the *neo*trichothecodione series. Acid (XIX) gave an orange 2 : 4-dinitrophenylhydrazone, and acid (XX) gave a crystalline methyl ester.

SCHEME 1. *Oxidation products of trichothecolone glycol.*



Reagents: 1, CrO_3–H_2O; 2, CrO_3–AcOH; 3, hydrogen.

SCHEME 2. *Oxidation products of* neo*trichothecodione.*



Reagents: 1, CrO_3; 2, hydrogen; 3, CH_2N_2.

Tests for an Oxide Ring.—According to their rate of liberation of hydroxyl ions in the presence of sodium thiosulphate (cf. Ross[7]), trichothecin and its derivatives may be divided into three classes. Trichothecolone, dihydrotrichothecolone, trichothecodione, dihydrotrichothecodione, *neo*trichothecodione, and *neo*dihydrotrichothecodione gave typical rapid, positive reactions, indicative of the presence of an oxide ring. In the case of trichothecolone, when the pink colour of phenolphthalein was continuously discharged with acetic acid, 0·52 equivalent of labile oxygen was accounted for in 7 hours' heating. Compounds of the second group slowly developed weak pink colours; these included acetyltrichothecolone, butyryltrichothecolone, *iso*trichothecolone, trichothecolone glycol, and trichothecolone chlorohydrin. Negative reactions were given by trichothecin, trichothecin glycol, trichothecin chlorohydrin, crotonyltrichothecolone, and acid (XI). Comparative experiments with reference compounds containing 3—6-membered oxide rings are detailed on p. 1128.

The negative reaction obtained with trichothecin may be explained by its ease of hydrolysis with alkali, so that the liberated hydroxyl ions reacted with the ester group and never reached a sufficiently high concentration to be detected by the indicator. Support for this view is afforded by glycidyl acetate whose normal strongly positive reaction is suppressed by the presence of methyl oxalate (cold) or ethyl acetate (on warming). The strongly positive reactions of *neo*trichothecodione (XVI) and *neo*dihydrotrichothecodione, in which the methyleneoxy-bridge is absent, may be due to the presence of a *cyclo*pentenone system. The same explanation is suggested for the weakly positive reaction of compound (VII), which contains a different *cyclo*pentenone structure.

According to Nicholson [17] the ring strain associated with five-, four-, and three-membered saturated rings containing one oxygen atom is 6, 22, and 27 kcal. mole^{-1}, respectively (corresponding figures for carbon rings are 6, 26, and 27 kcal. mole^{-1}). Although not enough compounds have been examined to make it clear how far these values are constant, the heat of reaction of trichothecolone and hydrochloric acid was determined to provide some evidence of the nature of the oxygen ring in this compound. In case the bond reaction was not exactly neutral the reaction of epichlorohydrin with hydrochloric acid was examined: the heat of reaction in this case (29 kcal. mole^{-1}) was in agreement with the above generalisation. In duplicate experiments the heat of reaction of trichothecolone with hydrochloric acid was found to be (sample 1) 26·5, 28·9, (sample 2) 23·2, 22·9 kcal. mole^{-1}. This did not distinguish between a three- and a four-membered ring for trichothecolone but the structure appeared to contain one or the other. That the result for trichothecolone is lower than for epichlorohydrin suggests that a four-membered ring is present.

Miscellaneous Reactions of Trichothecolone and its Derivatives.—A small yield of *p*-xyloquinone was obtained when trichothecolone was boiled with aqueous chromic acid.

Boiling hydriodic acid and red phosphorus converted dihydrotrichothecolone into a liquid, b. p. 230—240°, $n_D^{21\cdot5}$ 1·5252, and a similar product was obtained by dehydrogenation of tetrahydrotrichothecodiol. The properties of these products were in reasonable agreement with those of a hydrocarbon, $C_{15}H_{28}$, which showed no aromatic absorption in its infrared spectrum.

None of the compounds of the trichothecin or trichothecolone series gave normal αβ-glycol reactions with periodate. Trichothecolone glycol was, however, slowly oxidised to compound (VII).

Trichothecodione and *neo*trichothecodione both reduced Fehling's solution and Tollens's reagent.

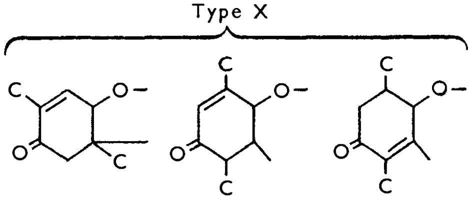
Structure of Trichothecolone.—The infrared spectrum of trichothecolone (band at 1670 cm.$^{-1}$) shows the presence of an αβ-unsaturated ketone group in a six-membered ring, and the ultraviolet spectrum (max. at 2260 Å, ε 8000) conforms to this type with either 0 or 1 alkyl substituent on the ethylenic bond and an oxygen atom attached to the γ-carbon atom. Trichothecolone is a secondary alcohol which on oxidation gives trichothecodione having a new, relatively unreactive carbonyl group (1735 cm.$^{-1}$) in a different ring from the other; so the original ketonic and hydroxyl groups of trichothecolone are in different rings. Trichothecin contains an easily opened oxygen bridge, giving with concentrated hydrochloric acid a chlorohydrin which contains a new hydroxyl group that is oxidised to an acid without loss of carbon. The bridge is therefore a methyleneoxy-bridge,

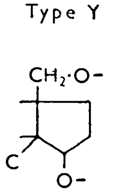
---

[17] Nicholson, personal communication, 1952.

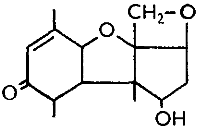
$-CH_2{\cdot}O- \longrightarrow -CH_2{\cdot}OH\ Cl^-$. Three of the oxygen atoms of trichothecolone are thus accounted for. The fourth is assumed to be in an ether group. The action of hot dilute acids on trichothecolone opens the methyleneoxy-bridge with addition of water and gives trichothecolone glycol ($-CH_2{\cdot}O- \longrightarrow -CH_2{\cdot}OH\ HO-$), which is oxidised in stages and, without loss of carbon, gives rise to acids which are decarboxylated at their m. p.s. Dilute alkali isomerises trichothecodione to *neo*trichothecodione in which a new hydroxyl group and ethylenic double bond are present. This isomerism is interpreted as a β-elimination of the oxygen atom of the methyleneoxy-bridge, after opening of this ring. Oxidation of *neo*trichothecodione gives, with no loss of carbon, a series of acids, identical with those obtained by oxidation of the trichothecolone chlorohydrin series.

Dehydrogenation of fully reduced trichothecolone gave *p*-xylene, *p*-xylenol, 2 : 3-dimethyl*cyclo*pentanone, and 2 : 3-dimethyl*cyclo*pent-2-enone. The action of hot alkali on trichothecodione yielded *p*-xyloquinone and *p*-xyloquinol; *p*-xyloquinone was obtained by oxidation of trichothecolone with hot chromic acid. It thus seems probable that the keto-group in trichothecolone is present in a *cyclo*hex-2-enone ring to which are attached two methyl groups or potential methyl groups in the *para*-position to each other, and an oxygen atom also *para* to the ketonic group. Dehydrogenation of acid (XX), which had been obtained by opening the oxygen bridge in trichothecolone (either *via* trichothecolone chlorohydrin or *neo*trichothecodione), and oxidation of the primary alcoholic group so formed to carboxyl, gave not 2 : 3-dimethyl*cyclo*pentanone, but 2-methyl*cyclo*pentanone together with *p*-xylenol: isolation of 2-methyl*cyclo*pentanone in this experiment supports the interpretation that the methylene group of the methyleneoxy-bridge is in the β-position with respect to the hydroxyl group. The carboxyl group of acid (XX) is assumed to have been lost by decarboxylation.

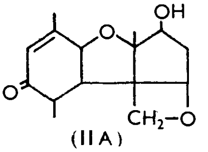




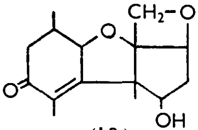
The degradation fragments can thus be arranged to conform to the skeletal types (X) and (Y).* These two together comprise all the carbon and oxygen atoms in trichothecolone; suitable junctions of them yield six possible structural formulæ, (I; R = H) ,(II), (IA and B), (IIA and B), for trichothecolone. Of these, formula (I) is preferred for the following reasons.



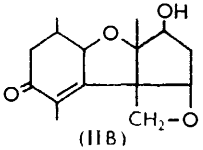
(IA)



(IIA)



(IB)



(IIB)

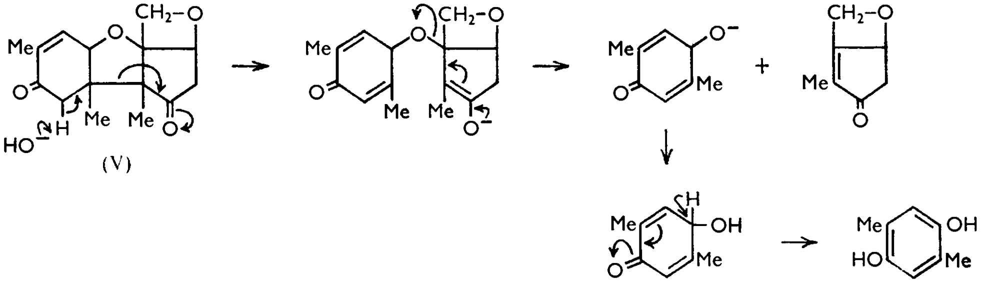
That tetrahydrotrichothecolone with potassium permanganate gave 0·95 mol. of acetic acid is in better agreement with formulæ (I) and (II) than with the other four. Substances (IA and B) and (IIA and B) would be expected to yield a fifteen-carbon compound containing an aromatic six-membered ring on mild dehydrogenation, yet this did not occur when trichothecolone derivatives were treated with hydriodic acid, and this is further evidence in favour of formulæ (I) and (II), with a quaternary carbon atom in the six-membered ring.

The easy breakdown of trichothecodione by alkali, involving rupture of a C–C bond, is almost certainly due to alkaline cleavage of a 1 : 5-diketone, followed by β-elimination of the substituted alkoxy-group, the mechanism being as in the annexed formulæ. The

---

* We are indebted to a Referee for constructive help in elucidation of various possibilities.

four-membered oxide ring may be opened before, during, or after the 1 : 5-cleavage, but this would not affect the formation of *p*-xyloquinol. The trichothecodione corresponding to (IA) would also be a 1 : 5-diketone and alkaline cleavage should likewise yield *p*-xyloquinol. On the other hand, the trichothecodione from (IB) would contain a vinylogous 1 : 3-diketone system and would probably not undergo alkaline cleavage at room temperature. Structure (IB) can also be excluded because it should absorb at about 2480 Å (cf. Dorfman [18]), whereas the observed absorption in methanol is at 2260 Å (the calculated value on the same basis is 2300 Å). The trichothecodiones corresponding to (II) and (IIA) contain 1 : 6-diketone systems, and that from (IIB) contains the vinylogue of a 1 : 4-diketone system. Compounds with the structures (IA) and (IIA) would be expected to be readily converted, probably by aerial oxidation under alkaline conditions, into a C_{15} phenol: this reaction has never been observed with trichothecolone derivatives. Structures (IB) and (IIB) have highly substituted ethylenic double bonds, which might be expected to undergo hydrogenation only with difficulty, whereas trichothecolone and its derivatives were readily hydrogenated. Thus, only structure (I) is left in agreement with all the facts.



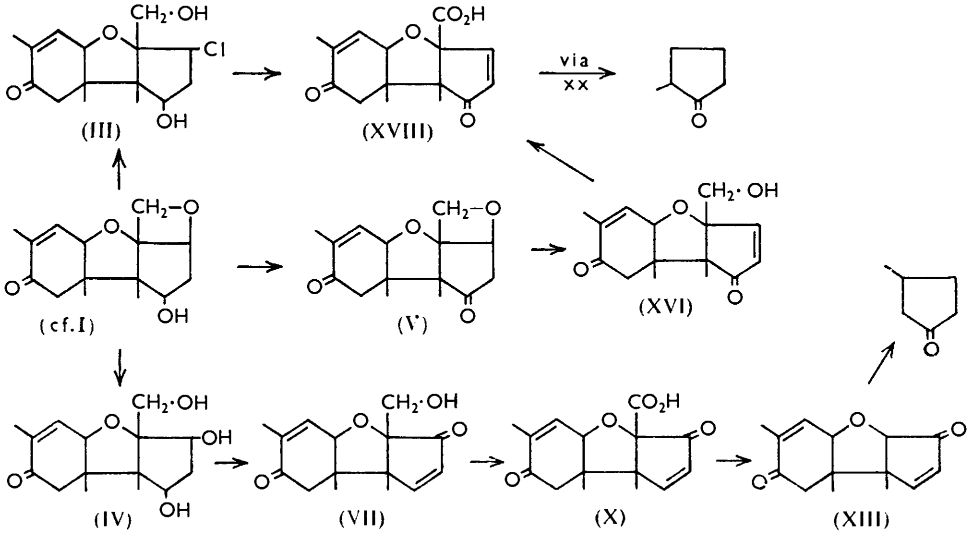
The difference in properties between the trichothecolone chlorohydrin and the trichothecolone glycol series is believed to depend upon the oxidation of different hydroxyl groups in the two series. Trichothecolone glycol gives a series of acids which are decarboxylated on melting, whereas trichothecolone chlorohydrin gives a different series which are not decarboxylated on melting but are identical with the acids obtained in the *neo*trichothecodione series. On dehydrogenation, acid (XX) (derived from the trichothecolone chlorohydrin series) gave 2-methyl*cyclo*pentanone presumably by the route shown in Scheme 3. Under similar conditions, the acid (XI) (from trichothecolone glycol) would be expected to yield 3-methyl*cyclo*pentanone (cf. Scheme 3). Dehydrogenation of this acid, however, required the high temperature of 350° and yielded a mixture of 2- and 3-methyl*cyclo*pentanone. Since our experimental work was completed, Fishman, Jones, and Whiting [15] have accumulated evidence based on alkaline fission of diketonic derivatives into C_7 and C_8 fragments, that confirms the structures of the acids and ketones derived from trichothecolone glycol and chlorohydrin, respectively, on the basis of formula (I). They have also confirmed the view that different hydroxyl groups are involved in the oxidation of trichothecolone chlorohydrin and of trichothecolone glycol (cf. Scheme 3).

iso*Trichothecolone.*—*iso*Trichothecolone is obtained by isomerisation of trichothecolone or directly from trichothecin with hot caustic alkali. It contains at least two *C*-methyl groups but two active hydrogen atoms. A hydroxyl group is denoted by an infrared band at 3400 cm.$^{-1}$ (cf. trichothecolone, 3510 cm.$^{-1}$; see Figure). It forms a mono-2 : 4-dinitrophenylhydrazone and a mono-*p*-nitrobenzoate, m. p. 195—196°. It is indifferent to concentrated hydrochloric acid (or hot 30% aqueous sodium hydroxide), which suggests that the methyleneoxy-bridge is absent in *iso*trichothecolone and is involved in the isomerisation of trichothecolone to *iso*trichothecolone. In an attempt to dehydrate *iso*trichothecolone with phosphorus oxychloride in pyridine a phosphorus-containing

---

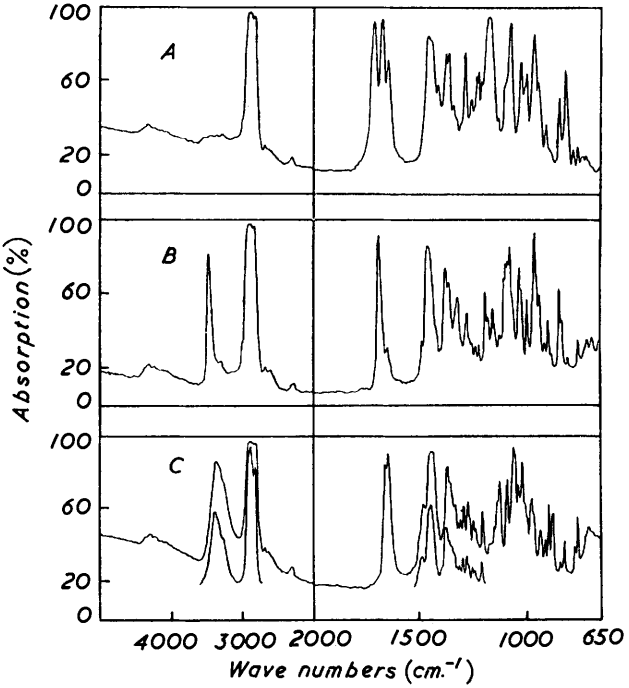
[18] Dorfman, *Chem. Rev.*, 1953, **53**, 73.

SCHEME 3. *Routes to ketonic dehydrogenation products from trichothecolone chlorohydrin and glycol.*



crystalline product, $C_{15}H_{18}O_5PCl$, was obtained, whose infrared spectrum showed the absence of hydroxyl groups, but a ketonic band at 1667 cm.$^{-1}$.

Infrared spectra of trichothecin (*A*), *trichothecolone* (*B*), *and* iso*trichothecolone* (*C*) (*solids, mulled*).



In the presence of palladium–charcoal, *iso*trichothecolone at atmospheric pressure very rapidly absorbed one mol. of hydrogen, giving dihydro*iso*trichothecolone (which is dimorphic), identical with that obtained by alkaline isomerisation of dihydrotrichothecolone. Thus *iso*trichothecolone retains the ethylenic bond of trichothecolone, which is not concerned in or necessary to the conversion of trichothecolone into its isomeride.

*iso*Trichothecolone formed a monoacetyl derivative when heated with an excess of acetic anhydride in pyridine or with glacial acetic acid alone, and hydrolysis of this acetyl derivative regenerated *iso*trichothecolone. A different acetylation product, obtained by means of acetic anhydride and sodium acetate, has been shown at Oxford [15] to be a diacetate. Monoacetyl*iso*trichothecolone absorbs 1 mol. of hydrogen (palladium–charcoal), the product giving dihydro*iso*trichothecolone on hydrolysis and being obtained also by acetylation of dihydro*iso*trichothecolone.

Oxidation Products of iso*Trichothecolone.*—Oxidation of *iso*trichothecolone with aqueous chromic acid at room temperature gave *iso*trichothecodione having infrared bands at 3390 (OH), 1667 (original $>$CO), and 1721 cm.$^{-1}$ (new $>$CO). This dione was unchanged by cold 10N-hydrochloric acid or boiling 2N-sulphuric acid, and on hydrogenation (palladium–charcoal) gave dihydro*iso*trichothecodione [infrared band at 3400 (OH) and 1706 cm.$^{-1}$ ($>$CO)]. Oxidation of dihydro*iso*trichothecolone with aqueous chromic acid at room temperature gave an acid $C_{15}H_{22}O_7$, which was not decarboxylated on melting and this acid was obtained also from dihydro*iso*trichothecodione by chromic acid in acetic acid.

The isomerisation of trichothecolone to *iso*trichothecolone clearly involves the methyleneoxy-group and the range of structural possibilities is limited by the experiments described above. The present evidence is, however, insufficient for proposal of a final structural formula for *iso*trichothecolone.

EXPERIMENTAL

Trichothecin was prepared as described by Freeman and Morrison.[2] Analytical work was carried out by Drs. Weiler and Strauss, and in the Analytical Laboratories of the Dyestuffs and Nobel Divisions of Imperial Chemical Industries Limited. The infrared spectra of solid specimens, in " Nujol " between rock-salt plates, were measured in the double-beam recording infrared spectrometer described by Tetlow *et al.*[19] The bulk of this work was done in 1949—53 and some of the observations, including those reproduced as the Figure, were recently confirmed by measurements in a Hilger H800 recording double-beam infrared spectrometer. Owing to the variations in the thickness of the specimens used, it was not possible to compare the intensities in the spectrum of one compound with those of another. Cells, constructed from rock-salt plates, were used for liquid specimens.

Trichothecin gave *C*-Me values 13·8, 14·4, 13·1% (Calc. for $C_{19}H_{24}O_5$: 3-*C*Me, 13·5%. Other analytical data have been reported [5]) and $\lambda_{max.}$ 2170 Å (ε 18,000) in hexane. The *semicarbazone*, crystallised from methanol or aqueous methanol, had m. p. 243—244° (decomp.) (Found: C, 61·3; H, 7·0; N, 11·3. $C_{20}H_{27}O_5N_3$ requires C, 61·7; H, 6·9; N, 10·8%).

10N-Hydrochloric acid (0·15 ml.) was added to trichothecin (0·15 g.) and 2 : 4-dinitrophenylhydrazine (0·1 g.) in boiling ethanol (7·5 ml.). The 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol as pale orange-coloured needles (0·15 g.), m. p. 203° (corr.) (Found: C, 58·6, 58·7; H, 5·2, 5·2; N, 10·5, 10·8. $C_{25}H_{28}O_8N_4$ requires C, 58·6; H, 5·5; N, 10·9%).

Hydrolysis of Trichothecin 2 : 4-*Dinitrophenylhydrazone.*—Trichothecin 2 : 4-dinitrophenylhydrazone (0·4 g.), in methanolic N-potassium hydroxide (16 ml.), was set aside at room temperature for 17 hr. The methanol was removed *in vacuo* and the dark brown residue extracted with chloroform. The solvent was removed and the residue crystallised from ethanol as deep orange rods (0·37 g.), m. p. 263—265° (corr.) alone or mixed with trichothecolone 2 : 4-dinitrophenylhydrazone (see below).

Tetrahydrotrichothecin.—Trichothecin (0·5 g.) in methanol at 22° in the presence of palladium–charcoal absorbed 78 ml. of hydrogen ($2H_2$ = 68 ml. at N.T.P.). Removal of the catalyst and distillation of the methanol *in vacuo* left the product as a colourless syrup.

This tetrahydrotrichothecin (0·18 g.) gave the *semicarbazone* (0·2 g.), m. p. 220° (decomp.), as a thick gel from methanol (Found: C, 60·2, 60·1; H, 7·85, 7·8; N, 10·95, 10·7. $C_{20}H_{31}O_5N_3$ requires C 61·1; H 7·9; N 10·7%); $\lambda_{max.}$ in methanol, 2280 Å (ε 14,000).

Trichothecolone.—Trichothecin (1·0 g.) in cold methanolic N-potassium hydroxide (40 ml.)

---

[19] Tetlow, McAuslan, Brimley, and Price, *J. Sci. Instr.*, 1951, **28**, 161.

was kept at room temperature for 17 hr. Water (30 ml.) was added and the methanol removed under reduced pressure below 35°. The aqueous residue was extracted with chloroform. Removal of the chloroform left colourless *trichothecolone* (0·776 g.), which crystallised from benzene–light petroleum (2 : 1) in long colourless needles, m. p. 183—184° (corr.) soluble in water and most organic solvents except light petroleum [Found: C, 67·9, 68·8, 68·9; H, 7·5, 7·3, 7·3; *C*-Me 10·6 10·0; active H, 0·47; *M* (Rast), 244, 248. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6; 2*C*-Me, 11·4; 1 active H, 0·37%; *M*, 264], $[\alpha]_D^{19\cdot5}$ +22·5° (*c* 1 in chloroform), $[\alpha]_D^{18}$ +17° (*c* 1 in ethanol), $\lambda_{max.}$ (in methanol), 2260 Å (ε 8000). The chlororm-extracted aqueous solution was acidified with sulphuric acid and extracted with chloroform, evaporation of which left colourless liquid β-methoxybutyric acid (0·26 g.) (Freeman and Gill[5]).

Acetic anhydride (2 ml.) and trichothecolone (0·25 g.) in pyridine (3 ml.) gave, in 4 days at room temperature, the *acetyl derivative* as prisms (0·274 g.) (from methanol), m. p. 148—149° (corr.) (Found: C, 66·4; H, 7·0; Ac, 19·8. $C_{17}H_{22}O_5$ requires C, 66·7; H, 7·2; 1Ac, 14·1%), $\lambda_{max.}$ 2270 Å (ε 8000) in MeOH.

Acetyltrichothecolone (0·3 g.), on hydrolysis with methanolic potassium hydroxide, gave trichothecolone (0·23 g.).

Trichothecolone 2 : 4-*dinitrophenylhydrazone* was prepared by Brady and Elsmie's method[20] and crystallised from chloroform–ethanol (1 : 3) as deep orange rods, m. p. 261—262° (corr.) (Found: C, 56·2; H, 5·6; N, 11·9, 11·9. $C_{21}H_{24}O_7N_4$ requires C, 56·7; H, 5·4; N, 12·6%).

Acetyltrichothecolone 2 : 4-*dinitrophenylhydrazone* crystallised from chloroform–ethanol (1 : 3) as orange needles, m. p. 251—252° (corr.) (Found: C, 56·2, 56·5; H, 5·3, 5·2; N, 12·0, 11·8; Ac, 19·3. $C_{23}H_{26}O_8N_4$ requires C, 56·8; H, 5·3; N, 11·5; 1Ac, 8·9%).

Trichothecolone (0·264 g.) gave a *semicarbazone* (0·341 g.), m. p. 250—251°, colourless needles from methanol (Found: C, 59·4; H, 7·25; N, 12·9. $C_{16}H_{23}O_4N_3$ requires C, 59·8; H, 7·2; N, 13·1%), $\lambda_{max.}$ (in methanol), 2670 Å (ε 20,000).

Dihydrotrichothecolone.—Hydrogenation of trichothecolone (3·0 g.) at atmospheric pressure led to uptake of one mol. of hydrogen. *Dihydrotrichothecolone* crystallised from benzene–light petroleum (1 : 1) as colourless rods (2·65 g.), m. p. 178—179° (corr.) (Found: C, 67·5, 67·6; H, 8·0, 8·3. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3%), identical with a specimen prepared by hydrolysis of tetrahydrotrichothecin.[5]

Dihydrotrichothecolone 2 : 4-dinitrophenylhydrazone crystallised from ethanol as orange needles, m. p. 170—171° (corr.). The *semicarbazone*, prepared by the usual method, crystallised as needles (from methanol), m. p. 258—259° (decomp.) (Found: C, 59·5; H, 7·5; N, 13·25. $C_{16}H_{25}O_4N_3$ requires C, 59·4; H, 7·7; N, 13·0%), $\lambda_{max.}$ (in methanol) 2290 Å (ε 13,000).

Dihydrotrichothecolone (0·2 g.) was acetylated by pyridine–acetic anhydride, and *acetyl-dihydrotrichothecolone* crystallised from toluene–light petroleum (1 : 1) as prisms (0·19 g.), m. p. 126·5—127·5° (corr.) (Found: C, 66·0; H, 8·0; Ac, 14·5. $C_{17}H_{24}O_5$ requires C, 66·2; H, 7·9; 1Ac, 14·0%).

Butyryltrichothecolone.—To a solution of trichothecolone (0·264 g.) in dry pyridine (3 ml.), cooled in ice, was added butyric anhydride (2 ml.). The *ester* (0·21 g.) crystallised from aqueous methanol as prisms, m. p. 105—106° (Found: C, 68·25; H, 7·5. $C_{19}H_{26}O_5$ requires C, 68·3; H, 7·8%), $\lambda_{max.}$ 2220 Å (ε 8000) in hexane.

Hydrogenation. Butyryltrichothecolone (0·11 g.) in methanol was shaken with hydrogen in the presence of Adams catalyst for 1 hr. The oily product (butyryldihydrotrichothecolone) was converted into the *semicarbazone* which separated from hot methanol as a gelatinous mass (0·125 g.), m. p. 220° (decomp.) (Found: C, 61·2; H, 7·75; N, 11·1. $C_{20}H_{31}O_5N_3$ requires C 61·1; H, 7·9; N, 10·7%). *X*-Ray powder photographs of this and tetrahydrotrichothecin semicarbazone showed them both to be microcrystalline and identical.

Crotonyltrichothecolone.—Trichothecolone (0·264 g.) was treated with pyridine (3 ml.) and crotonic anhydride (2 ml.). The sticky *ester* (0·372 g.), after being washed with light petroleum (b. p. 40—60°), crystallised from methanol in cream-coloured needles (0·142 g.), m. p. 117—118° (Found: C, 68·3; H, 7·0. $C_{19}H_{24}O_5$ requires C, 68·7; H, 7·2%), $\lambda_{max.}$ 2170 Å (ε 11,000) in hexane.

Ozonolysis of Trichothecolone.—Ozonised oxygen was passed into a solution of trichothecolone (0·25 g.) in dry chloroform (20 ml.) for 4 hr. at 0°. Removal of the solvent under reduced pressure left a colourless amorphous residue which was treated with water (25 ml.) at room temperature for 20 hr. The aqueous solution was distilled. A portion of the distillate, treated

---

[20] Brady and Elsmie, *Analyst*, 1926, **51**, 77.

with Brady's reagent, gave a small precipitate of acetaldehyde-2 : 4-dinitrophenylhydrazone, m. p. 142—144°, undepressed on admixture with an authentic specimen (m. p. 148—149°). The acidity of the distillate was equivalent to 0·58 mol. of acid, and the acid was identified as its *p*-phenylphenacyl ester, m. p. 108·5—110°, undepressed on admixture with *p*-phenylphenacyl acetate.

Trichothecodione.—A 5% solution of chromic anhydride in 95% acetic acid (40 ml.) was added to trichothecolone (2·0 g.) in glacial acetic acid (5 ml.). A slight initial rise in temperature was controlled by cooling in ice-water, and the mixture was then set aside for 1 hr. Pouring it into cold water (200 ml.) gave crystalline *trichothecodione*. This recrystallised from aqueous ethanol as colourless rods (1·45 g.), m. p. 218—219° (corr.) [Found: C, 69·0; H, 7·0%; *M* (Rast), 232, 244. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%; *M*, 262], readily soluble in most solvents except water and light petroleum.

The dione (0·1 g.) was treated in ethanol (1 ml.) with Brady's reagent (70 ml.). The 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol as deep orange plates (0·13 g.), m. p. 220—221° (corr.) (Found: C, 57·6, 57·2; H, 5·1, 4·9; N, 12·3, 12·4. $C_{21}H_{22}O_7N_4$ requires C, 57·0; H, 5·0; N, 12·7%). A second preparation by this method gave a *form* (orange plates), m. p. 143° (corr.). On further heating this resolidified and melted at 220—221° (Found: C, 56·7; H, 5·1; N, 12·6, 13·0%). The infrared spectra of the two hydrazones were identical; they showed a band at 1740 cm.$^{-1}$, whilst the band at 1670 cm.$^{-1}$ was absent, indicating that the 2 : 4-dinitrophenylhydrazine had reacted with the original ketonic group. The lower-melting compound is believed to be am etastable form.

Trichothecodione (1·0 g.) was boiled with 2 : 4-dinitrophenylhydrazine (1·5 g.) in ethanol (50 ml.) and 10N-hydrochloric acid (1·5 ml.) for 2 hr. A reddish-brown precipitate (0·946 g.) was filtered off from the boiling solution, and had m. p. 212°. Extraction of this product with boiling ethanol and chloroform left a red solid which was insoluble in all common solvents, except acetone. It was extracted with boiling acetone, and the solution concentrated to crystallisation, to obtain a deep red *bis*-2 : 4-*dinitrophenylhydrazone* (0·025 g.), which darkened at 200° but did not melt below 300° (Found: C, 47·6; H, 3·5; N, 20·2; Cl, 0. $C_{27}H_{26}O_{10}N_8$ requires C, 52·1; H, 4·2; N, 18·0%).

The *monosemicarbazone* crystallised from aqueous methanol and had m. p. 227° (decomp.) (Found: C, 59·9; H, 6·5; N, 13·0. $C_{16}H_{21}O_4N_3$ requires C, 60·2; H, 6·6; N, 13·2%), $\lambda_{max.}$ (in methanol), 2680 Å (ε 21,700).

Dihydrotrichothecodione.—(*a*) Oxidation of dihydrotrichothecolone (2·5 g.) with chromic acid in acetic acid gave colourless rods of *dihydrotrichothecodione* (1·97 g.) (from aqueous ethanol), m. p. 164—164·5° (corr.) (Found: C, 68·3, 68·0; H, 7·5, 7·5. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6%).

(*b*) Trichothecodione (0·25 g.) in ethanol (50 ml.) was hydrogenated at atmospheric pressure with 10% palladium–charcoal (0·2 g.). The theoretical quantity of hydrogen was absorbed in 30 minutes, giving crystals (0·245 g.), m. p. 161—162° undepressed on admixture with the preceding material.

Dihydrotrichothecodione mono-2 : 4-*dinitrophenylhydrazone* crystallised from ethanol as yellow needles, m. p. 225—226° (corr.) (Found: C, 56·9, 56·5; H, 5·5, 5·4; N, 12·8, 13·3. $C_{21}H_{24}O_7N_4$ requires C, 56·8; H, 5·4; N, 12·6%).

Trichothecin Chlorohydrin.—Trichothecin (0·25 g.) was triturated with 10N-hydrochloric acid (2·5 ml.), and ethanol (1 ml.) added to obtain complete solution. On stirring, the *chlorohydrin* crystallised. It recrystallised from dilute acetic acid as colourless rods (0·183 g.), m. p. 132·5° (corr.) (Found: C, 61·8; H, 6·8; Cl, 9·4; active H, 0·43. $C_{19}H_{25}O_5Cl$ requires C, 61·9; H, 6·8; Cl, 9·6; 1 active H, 0·33%). Quantitative acetylation indicated the presence of one hydroxyl group per molecule, and the infrared spectrum confirmed the presence of carbonyl, ester, and hydroxyl groups. This compound was not obtained by passing dry hydrogen chloride into a solution of trichothecin in dry ether or chloroform.

The acetyl derivative of trichothecin chlorohydrin was amorphous and melted at 43—53° (Found: C, 59·7; H, 7·1; Cl, 9·8; Ac, 14·3. Calc. for $C_{21}H_{27}O_6Cl$: C, 61·4; H, 6·3; Cl, 8·9; Ac, 10·5%). *Trichothecin chlorohydrin* 2 : 4-*dinitrophenylhydrazone* was obtained as orange crystals, m. p. 205° (corr.) (Found: C, 54·4; H, 5·2; N, 10·9. $C_{25}H_{29}O_8N_4Cl$ requires C, 54·7; H, 5·3; N, 10·7%). *Trichothecin chlorohydrin* 4-*chloro*-2-*nitrophenylhydrazone* crystallised from acetic acid as bright red needles, m. p. 187—188° (Found: C, 55·5; H, 5·3; N, 7·4. $C_{25}H_{29}O_6N_3Cl_2$ requires C, 55·8; H, 5·4; N, 7·8%).

Tetrahydrotrichothecin Chlorohydrin.—Trichothecin chlorohydrin (0·184 g.) in methanol was shaken with hydrogen in the presence of palladium–charcoal. 2 Mols. of hydrogen were absorbed in 5 min. The tetrahydro-derivative was isolated as a glass (0·145 g.).

Reduction of Trichothecin Chlorohydrin with Zinc and Acetic Acid.—Trichothecin chlorohydrin (0·3 g.) in glacial acetic acid (15 ml.) and zinc dust (0·9 g.) were heated on the water-bath for 7 hr. The *product* crystallised from methanol as colourless needles (0·047 g.), m. p. 166—167° (Found: C, 61·8; H, 7·2; Cl, 9·2. $C_{19}H_{27}O_5Cl$ requires C, 61·5; H, 7·3; Cl, 9·6%). The infrared absorption spectrum showed no carbonyl band, but bands due to hydroxyl and ester were present.

Trichothecolone Chlorohydrin.—(*a*) *By hydrolysis of trichothecin chlorohydrin.* Hydrolysis of trichothecin chlorohydrin (0·726 g.) with methanolic potassium hydroxide gave *trichothecolone chlorohydrin* (0·70 g.) as colourless needles (from ether), m. p. 139—140° (corr.) (Found: C, 60·2, 60·0; H, 7·2, 7·0; Cl, 11·7. $C_{15}H_{21}O_4Cl$ requires C, 59·9; H, 7·0; Cl, 11·8%). The liquid acid (0·097 g.), b. p. 210°, present in the aqueous alkaline fraction was isolated as *p*-phenylphenacyl β-methoxybutyrate, m. p. 60° (cf. Drake and Bronitsky [21]).

(*b*) *By reaction of trichothecolone with aqueous hydrochloric acid.* Trichothecolone (2 g.) dissolved rapidly in 10N-hydrochloric acid (2 ml.) with evolution of heat. The residue obtained by evaporation crystallised from benzene–light petroleum (1 : 1) as needles, m. p. 133—134° (corr.) undepressed on admixture with the chlorohydrin described above.

Trichothecolone chlorohydrin (0·3 g.) gave the *semicarbazone* (0·254 g.) as prisms (from methanol), m. p. 214° (decomp.) (Found: C, 52·4; H, 6·5; N, 11·5. $C_{15}H_{24}O_4N_3Cl$ requires C, 52·1; H, 6·9; N, 12·1%). The 2 : 4-*dinitrophenylhydrazone* was prepared by Brady's method and crystallised from methanol in orange-red crystals, m. p. 278—279° (decomp.) (Found: C, 52·3; H, 5·3; N, 11·8; Cl, 6·6. $C_{21}H_{25}O_7N_4Cl$ requires C, 52·5; H, 5·2; N, 11·7; Cl, 7·4%). The 4-*chloro*-2-*nitrophenylhydrazone* crystallised from acetic acid in short red-brown prisms, m. p. 242—243° (decomp.) (Found: C, 53·9; H, 5·5; N, 8·0. $C_{21}H_{25}O_5N_3Cl_2$ requires C, 53·6; H, 5·3; N, 8·9%), and the 4-*bromo*-2-*nitrophenylhydrazone* from acetic acid as orange prisms, m. p. 231—232° (decomp.) (Found: C, 49·4; H, 4·9; N, 7·8. $C_{21}H_{25}O_5N_3ClBr$ requires C, 49·0; H, 4·9; N, 8·2%).

Hydrogenation of Trichothecolone Chlorohydrin.—Trichothecolone chlorohydrin (0·25 g.) in ethanol (20 ml.) was hydrogenated at atmospheric pressure in the presence of Adams catalyst (0·2 g.). Reduction was rapid, the total uptake of hydrogen being 60 ml. (N.T.P.) ($2H_2$ = 37 ml.). The product (0·251 g.) separated from benzene–light petroleum (1 : 1) as a white amorphous solid (0·192 g.), m. p. 164—165° (corr.). It gave no reaction with Brady's reagent.

Trichothecin Bromohydrin.—Trichothecin (0·5 g.) and concentrated hydrobromic acid (5 ml.) gave colourless needles of *trichothecin bromohydrin* (0·45 g.), m. p. 140°, from dilute acetic acid (Found: C, 53·6; H, 5·9. $C_{19}H_{25}O_5Br$ requires C, 55·2; H, 6·0%). The corresponding tetrahydro-derivative was a thick gum.

Trichothecolone Bromohydrin.—Hydrolysis of trichothecin bromohydrin (1·0 g.) gave *trichothecolone bromohydrin* (0·9 g.), needles (from benzene), m. p. 129—130° (Found: C, 52·5; H, 6·1. $C_{15}H_{21}O_4Br$ requires C, 52·2; H, 6·1%). This gave a *semicarbazone*, prisms (from aqueous methanol), darkening at 240° without melting (Found: C, 46·6; H, 5·9; N, 10·4. $C_{15}H_{24}O_4N_3Br$ requires C, 46·2; H, 6·2; N, 10·8%), and a 4-*chloro*-2-*nitrophenylhydrazone*, brown-red prisms (from acetic acid), m. p. 226—227° (decomp.) (Found: C, 48·7; H, 4·8; N, 8·1. $C_{21}H_{25}O_5N_3ClBr$ requires C, 49·0; H, 4·9; N, 8·2%).

Trichothecin Glycol.—A solution of trichothecin (0·635 g.) in 0·1N-hydrochloric acid (1500 ml.) was heated at 100° for 2 hr. The hydrochloric acid was neutralised to pH 6·0 by 2N-sodium hydroxide, and the solution concentrated to 130 ml. under reduced pressure. *Trichothecin glycol* crystallised as colourless prisms (0·374 g.), m. p. 146·5° (corr.) [Found: C, 64·9, 65·1; H, 7·5, 7·4%; *M* (Rast), 338, 330. $C_{19}H_{26}O_6$ requires C, 65·1; H, 7·4%; *M*, 350]. This formed a 2 : 4-*dinitrophenylhydrazone* by Brady's method, which crystallised from aqueous alcohol in reddish-orange rods, m. p. 216° (corr.) (Found: C, 56·8, 56·7; H, 5·6, 5·5; N, 9·9, 9·7. $C_{25}H_{30}O_9N_4$ requires C, 56·6; H, 5·7; N, 10·6%).

Trichothecolone Glycol.—(a) *From trichothecin glycol.* Hydrolysis of trichothecin glycol (4·23 g.) with methanolic potassium hydroxide gave colourless prisms, m. p. 193—194° (corr.), (3·15 g.) from acetone. The m. p. of the product was undepressed on admixture with trichothecolone glycol (m. p. 193°) prepared as in (b).

---

[21] Drake and Bronitsky, *J. Amer. Chem. Soc.*, 1930, **52**, 3715.

(b) *From trichothecolone.* Trichothecolone (5·0 g.) in 0·5N-sulphuric acid (500 ml.) was heated under reflux for 2 hr. The solution was cooled and made neutral to Congo Red by barium carbonate (60 g.). The filtrate was evaporated to dryness and the residue crystallised from ethanol as colourless prismatic crystals of *trichothecolone glycol* (4·3 g.), m. p. 193—194° (corr.) [Found: C, 63·5; H, 7·8; *C*-Me, 12·0%; *M* (Rast), 310, 305. $C_{15}H_{22}O_5$ requires C, 63·8; H, 7·8; 3*C*-Me, 15·9%; *M*, 282], $\lambda_{max.}$ (in methanol) 2260 Å (ε 6500).

(c) Trichothecolone glycol was also prepared by treatment of trichothecolone with 50% (w/w) sulphuric acid at room temperature for 24 hr.

The pale red *glycol* 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol; at 189° it softened and changed crystalline form to long needles which melted at 273° (corr.) (Found: C, 54·3, 54·6; H, 5·7, 5·6; N, 12·2, 12·3. $C_{21}H_{26}O_8N_4$ requires C, 54·5; H, 5·6; N, 12·1%).

Trichothecolone glycol (0·1 g.) with acetic anhydride–pyridine gave a syrupy *triacetate* (0·135 g.) (Found: C, 61·6, 61·8; H, 7·5, 7·3; Ac, 33·3. $C_{21}H_{28}O_8$ requires C, 61·7; H, 6·9; 3Ac, 31·6%).

Dihydrotrichothecolone Glycol.—(*a*) Trichothecolone glycol (0·5 g.) in ethanol (12 ml.) was hydrogenated at atmospheric pressure with palladium–charcoal as catalyst. The product (0·5 g.) crystallised from ethanol in colourless prisms, m. p. 216—217°.

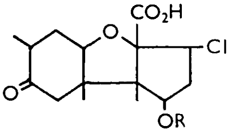
(*b*) Dihydrotrichothecolone (1·0 g.) in 0·5N-sulphuric acid (100 ml.) was heated under reflux for 2 hr. and gave the *glycol* (0·72 g.) which, crystallised from ethanol, had m. p. 215° (Found: C, 63·2, 63·3; H, 8·5, 8·5. $C_{15}H_{24}O_5$ requires C, 63·4; H, 8·5%).

Reaction of Trichothecodione with Acids: Trichothecodione Chlorohydrin.—Trichothecodione (0·45 g.) was triturated with concentrated hydrochloric acid (5 ml.), and the *chlorohydrin* precipitated from the solution by dilution with water (25 ml.). Recrystallisation from ethanol gave colourless, rectangular prisms (0·204 g.), m. p. 165° (corr.) (Found: C, 60·3; H, 6·4; Cl, 11·4. $C_{15}H_{19}O_4Cl$ requires C, 60·3; H, 6·4; Cl, 11·9%). Its 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol as orange needles, m. p. 213° (decomp.) (corr.) (Found: C, 52·5; H, 5·1; N, 11·6; Cl, 7·3. $C_{21}H_{23}O_7N_4Cl$ requires C, 52·7; H, 4·8; N, 11·7; Cl, 7·4%). An identical hydrazone was obtained by oxidation of trichothecolone chlorohydrin (0·5 g.) with 5% aqueous chromic acid (10 ml.).

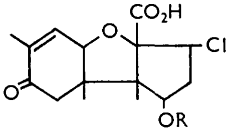
Dihydrotrichothecodione Chlorohydrin.—Dihydrotrichothecodione (0·5 g.) reacted similarly with acid, to give the *chlorohydrin* (0·195 g.), m. p. 174—176° (decomp.) (corr.) (Found: C, 60·4; H, 7·2; Cl, 10·8. $C_{15}H_{21}O_4Cl$ requires C, 59·9; H, 7·0; Cl, 11·8%).

Oxidation of Trichothecin Halogenohydrins to Acids.—(a) *Tetrahydrotrichothecin chlorohydrin.* Tetrahydrotrichothecin chlorohydrin (1·0 g.) was dissolved in glacial acetic acid (5 ml.) and the solution divided into 5 equal parts. Each aliquot part (1 ml.) was treated with 5% chromic acid in glacial acetic acid (6 ml.) and the temperature allowed to rise to 32°. After 1 hr. the combined solutions were poured into water (200 ml.) and extracted with chloroform. The chloroform solution was extracted with sodium hydrogen carbonate solution, and acidification of the alkaline extract precipitated an *acid* (XXI; R = Pr·CO) which crystallised from aqueous methanol in colourless needles (0·574 g.), m. p. 171—172° (Found: C, 59·5, 59·1, 59·0; H, 7·1, 7·2, 7·0; Cl, 9·4, 9·2%; equiv., 398. $C_{19}H_{27}O_6Cl$ requires C, 59·0; H, 7·0; Cl, 9·2%; equiv., 386·5). The acid gave no colour with ferric chloride, absorbed no hydrogen in the presence of palladium–charcoal, and had no high-intensity absorption. It gave a 2 : 4-*dinitrophenylhydrazone* as bright yellow prisms, m. p. 125°, from benzene (Found: C, 52·6; H, 5·2; N, 9·6. $C_{25}H_{31}O_9N_4Cl$ requires C, 52·9; H, 5·5; N, 9·9%).

The C_{19} chloro-acid (0·055 g.) was heated in thionyl chloride (1 ml.) at 80° for 30 min. Excess of thionyl chloride was removed *in vacuo*, and the residue dissolved in ether and treated with dry ammonia. The *amide* (0·045 g.) crystallised from aqueous methanol in prisms (0·016 g.), m. p. 165—166° (Found: N, 3·3. $C_{19}H_{28}O_5NCl$ requires N, 3·6%).



(XXI)



(XXII)

Hydrolysis of the C_{19} chloro-acid (0·45 g.) with sodium hydroxide gave a C_{15} *acid* (XXI; R = H) (0·388 g.) which crystallised from benzene as colourless needles, m. p. 198—199° (Found, after drying at 130°: C, 57·8; H, 7·0; Cl, 9·9. $C_{15}H_{21}O_5Cl$ requires C, 56·9; H, 6·6; Cl, 11·2%).

(b) *Trichothecin chlorohydrin.* In a similar way trichothecin chlorohydrin (0·2 g.) was oxidised with chromic acid in acetic acid and gave an *acid* (XXII; R = CHMe:CH·CO) (0·115 g.), m. p. 85—90° (Found: C, 58·3; H, 6·2; Cl, 9·8%; equiv., 389. $C_{19}H_{23}O_6Cl$ requires C, 59·6; H, 6·0; Cl, 9·3%; equiv., 382·5). The acid (0·115 g.) was hydrogenated at atmospheric pressure in the presence of palladium–charcoal, giving colourless needles (0·082 g.), m. p. 169—170°, of acid (XXI; R = Pr·CO).

(c) *Tetrahydrotrichothecin bromohydrin.* Similarly, tetrahydrotrichothecin bromohydrin (0·24 g.) gave a C_{19} *bromo-acid* (0·125 g.) as colourless needles (from aqueous methanol), m. p. 176—177° (Found: C, 52·9; H, 5·9. $C_{19}H_{27}O_6Br$ requires C, 52·9; H, 6·3%).

Tetrahydrotrichothecodiol.—Trichothecolone (4·0 g.) in absolute ethanol (100 ml.) was hydrogenated for 6 hr. at 100°/100 atm. in hydrogen with Raney nickel (2 g.). The catalyst was removed and the alcoholic solution evaporated under reduced pressure, leaving a colourless amorphous *alcohol* (4·0 g.) (Found: C, 66·5, 66·6; H, 10·4, 10·0; active H, 1·1; *C*-Me, 13·0, 12·7. $C_{15}H_{26}O_4$ requires C, 66·7; H, 9·7; 3 active H, 1·1; 3*C*-Me, 16·7%). The product did not form a dinitrophenylhydrazone on treatment with Brady's reagent, and its infrared spectrum showed that a ketonic group was absent.

Acetylation of this tetrahydrotrichothecodiol gave an amorphous (?)triacetate [Found: C, 66·0, 65·7; H, 8·9, 8·9; Ac, 27·1. Calc. for $C_{21}H_{32}O_7$ (triacetate): C, 63·7; H, 8·1; Ac, 32·6. Calc. for $C_{19}H_{30}O_6$ (diacetate): C, 64·4; H, 8·5; Ac, 24·3%].

Dehydrogenation of Tetrahydrotrichothecodiol.—Tetrahydrotrichothecodiol (16 g.), mixed with 10% palladium–charcoal (16 g.), was placed in a 250 c.c. flask connected to a downward condenser. The receiver was cooled in ice, and the effluent gases were passed through 0·2N-barium hydroxide. The flask was heated (metal-bath) at a rate of about 2°/minute from 100° to 300° which was maintained for 4 hr. Distillation commenced at about 140°, became fairly rapid at 170°, and was slow at 200°. Distillates at atmospheric and 12 mm. pressure were collected. The residue in the flask was extracted successively with ether, acetone, and chloroform. The following fractions were collected: (*A*) Distillate at atmospheric pressure (7·414 g.) which consisted of (1) upper layer, greenish-yellow oil (3·615 g.) and (2) lower aqueous layer, colourless (3·715 g.). (*B*) Distillate at 12 mm., yellow oil (0·538 g.), containing a trace of water. (*C*) Ether-extract of flask residue, yellowish-brown oil (2·02 g.). (*D*) Combined acetone and chloroform extracts of flask residue, yellowish-brown oil (0·96 g.). (*E*) Washings from condenser and receiver (1·193 g.), containing an aqueous layer (0·1 g.). Total recovery was 12·125 g. (75·8%). Less than 0·1 mol. of carbon dioxide (0·19 g.) was recovered from the effluent gases.

Fraction (A2). The water content of the aqueous layer was determined by Fischer's method (Found: 78·6%, *i.e.*, 2·96 g., 2·754 mol.). The aqueous layer contained a mixture of ketones which were present in larger amounts and were isolated from the upper oily layer.

Fraction (A1). (i) The non-aqueous fraction (A1) (1·0 g.) was heated in 9 : 1 (v/v) ethanol–glacial acetic acid (10 ml.) with Girard T reagent (carbohydrazidotrimethylammonium chloride) (1·0 g.) for 1 hr., the ethanol removed at atmospheric pressure (distillate fraction *X*), and the non-volatile residue dissolved in water (25 ml.) and extracted with ether. The extract contained an oil (0·043 g.), b. p. 130—140°, which was found to be a mixture of a ketone and an oil. The aqueous solution after extraction with ether was acidified with N-hydrochloric acid, heated under reflux for 30 min., and extracted with ether. The residue from the extract (0·6515 g.) was separated into fractions (*a*) b. p. 34—40° (0·1075 g.), (*b*) b. p. 40—120° (0·123 g.), and (*c*) b. p. 120—134° (0·105 g.). Subfraction (*c*), with Brady's reagent, gave 2 : 3-*dimethyl*cyclo*pent-2-enone* 2 : 4-*dinitrophenylhydrazone* which crystallised from glacial acetic acid as red needles, m. p. 227—228° (corr.) (Found: C, 53·4; H, 4·7; N, 19·2; *C*-Me, 9·8. $C_{13}H_{14}O_4N_4$ requires C, 53·8; H, 4·8; N, 19·3; two *C*-Me, 10·3%). Subfraction (*b*) gave 2 : 3-*dimethyl*cyclo*pentanone* 2 : 4-*dinitrophenylhydrazone*, deep orange coloured plates (from ethanol), m. p. 156—157° (corr.) (Found: 53·4; H, 5·2; N, 19·5; *C*-Me, 10·3. $C_{13}H_{16}O_4N_4$ requires C, 53·4; H, 5·5; N, 19·2; 2*C*-Me, 10·3%).

The distillate fraction *X* was diluted with five volumes of water, and the resulting suspension extracted with ether. The residue from the ether-extract was distilled, giving a colourless mobile liquid (0·21 g.), b. p. 136—138° (Found: C, 90·2, 90·0; H, 9·7, 9·8. Calc. for C_8H_{10}: C, 90·6; H, 9·4%), n_D^{20} (Leitz–Jelley refractometer) 1·490 ± 0·001; *p*-xylene has b. p. 138·5° and n_D^{20} 1·494. The infrared spectrum of the hydrocarbon was identical with that of *p*-xylene over the range 2·5—13 μ. Nitration gave 2 : 3 : 5-trinitro-*p*-xylene, m. p. and mixed m. p. 138—139° (corr.) (Found: C, 40·2, 40·3; H, 3·3, 3·2; N, 17·1, 17·1. Calc. for $C_8H_7O_6N_3$: C, 39·8; H, 2·9; N, 17·4%).

(ii) A further quantity of fraction (A1) was treated with Girard T reagent as above, and the ketonic fraction separated. The latter (0·1 g.) was hydrogenated in the presence of 10% palladium–charcoal at atmospheric pressure. The product gave a 2 : 4-dinitrophenylhydrazone (by Brady's method) which crystallised from ethanol as orange plates, m. p. 156—157° (corr.) (Found: C, 53·4, 53·4; H, 5·6, 5·5; N, 19·2; *C*-Me, 10·3%). The hydrazone was identical with that of the *cyclo*pentanone isolated from fraction (*b*) as above. The *semicarbazone* crystallised from aqueous alcohol as rods, m. p. 206—207° (corr.) (Found: C, 56·5; H, 8·7; N, 25·2. $C_8H_{15}ON_3$ requires C, 56·8; H, 8·9; N, 24·8%). None of the m. p.s of the semicarbazones of 2 : 3-, 2 : 4-, and 2 : 5-dimethyl*cyclo*pentanones quoted in the literature corresponded to that of the semicarbazone of the unknown saturated ketone. Authentic 2 : 3-dimethyl*cyclo*pentanone was prepared by the method of Staudinger and Ruzicka,[22] and its semicarbazone had m. p. 206—207° (corr.) alone or mixed with the above semicarbazone (Dutta [23] records m. p. 179—180°). Authentic 2 : 3-dimethyl*cyclo*pentanone 2 : 4-dinitrophenylhydrazone melted at 156—157° (corr.) and the m. p. was undepressed on admixture with the above hydrazone. The infrared spectrum of the saturated ketone was identical with that of authentic 2 : 3-dimethyl*cyclo*pentanone.

(iii) *Identification of the unsaturated ketone with 2 : 3-dimethyl*cyclo*pent-2-enone.* 2 : 3-Dimethyl*cyclo*pent-2-enone was prepared by the method of Frank *et al.*[11] Its semicarbazone, m. p. 248—249° (decomp.), agreed satisfactorily in its properties with that of Frank *et al.* (m. p. 247—250°, decomp.). The m. p. of the 2 : 4-dinitrophenylhydrazone (m. p. 227—228°) was undepressed on admixture with the dinitrophenylhydrazone of the above unsaturated ketone (m. p. 227—228°). The ultraviolet absorption spectrum of 2 : 3-dimethyl*cyclo*pent-2-enone had maximum at 2335 Å (2290 Å for the above unsaturated ketone, which may have contained a trace of *p*-xylene).

Fractions (C) *and* (D). Fractions (*C*) and (*D*) were combined and steam-distilled. The volatile component was separated into fractions (i) b. p. 70—120°/10 mm. and (ii) b. p. 120—160°/10 mm. Fraction (i) was separated into fractions (i*a*) b. p. 200—220° (17% of steam-volatile oil) and (i*b*) b. p. 220—270°. Fraction (i*a*) contained a small quantity of 2 : 3-dimethyl*cyclo*pent-2-enone (isolated as 2 : 4-dinitrophenylhydrazone), and a hydroxylic compound which was difficultly soluble in sodium hydroxide solution and gave no colour reaction with ferric chloride and formed a 3 : 5-dinitrobenzoate, m. p. 131—134° (corr.) (Found: C, 57·7, 57·7; H, 3·9, 4·1; N, 8·4, 8·2; *C*-Me, 5·9. Calc. for $C_{15}H_{12}O_6N_2$: C, 57·0; H, 3·8; N, 8·9; 2*C*-Me, 9·5%). A mixed m. p. with authentic *p*-xylyl 3 : 5-dinitrobenzoate (m. p. 137°) was at 134—136°. The phenylurethane, m. p. 158—162° (corr.), did not depress the m. p. of authentic *p*-xylyl phenylurethane (m. p. 164°).

The carbonyl group in fraction (ii) (Found: C, 81·6; H, 9·7%) was reduced with lithium aluminium hydride, and the product dehydrogenated with an equal weight of 10% palladium–charcoal at 300° for 7 hr. Extraction of the product with ether gave a straw-coloured oil, b. p. 220—260°, in 40% yield (Found: C, 84·8; H, 9·8%), n_D^{16} 1·5174. The product had properties similar to those of the steam-volatile oil obtained by reduction of dihydrotrichothecolone with phosphorus and hydriodic acid.

Pyrolysis of Tetrahydrotrichothecodiol.—This diol (4·0 g.) and activated charcoal (4 g.) (undried) were heated at 300° for 2 hr. The distillate consisted of an oil (0·4 g.) and an aqueous layer (1·64 g.) containing 78·7% of water. The residue was extracted with solvents, giving a viscous oil (1·8 g.). The aqueous layer (1·64 g.) gave a 2 : 4-dinitrophenylhydrazone as red needles (from acetic acid), m. p. 225—226° (corr.) (0·04 g.), undepressed on admixture with 2 : 3-dimethyl*cyclo*pent-2-enone 2 : 4-dinitrophenylhydrazone (m. p. 227—228°). The infrared spectrum of the oil (0·4 g.) indicated the presence of *p*-xylene, together with a ketone.

High-pressure Hydrogenation of Trichothecolone Glycol (IV) *and Dehydrogenation of the Product.*—Trichothecolone glycol (5·5 g.) was hydrogenated at 100°/100 atm. in hydrogen for 6 hr. in the presence of Raney nickel. The amorphous product contained no ketonic group. The alcohol (5·6 g.) was dehydrogenated with 10% palladium–charcoal (6 g.) as described above. The distillate (2·866 g.) consisted of an oil (0·633 g.) and an aqueous layer (2·077 g.), containing 86·2% of water (5·05 mols.). The oil was fractionated with Girard T reagent, and *p*-xylene (0·27 g.) and a mixture of saturated and unsaturated ketones were isolated. Hydrogenation of the ketonic mixture gave 2 : 3-dimethyl*cyclo*pentanone.

---

[22] Staudinger and Ruzicka, *Helv. Chim. Acta*, 1924, **7**, 245.

[23] Dutta, *Science and Culture*, 1945, **10**, 355.

High-pressure Hydrogenation of Compound (VII) *and Dehydrogenation of the Product.*—Compound (VII) (4·6 g.) was hydrogenated at 100°/100 atm.; the product was a colourless, amorphous solid free from carbonyl groups. Dehydrogenation was carried out in the usual manner. The distillate (2·53 g.) consisted of a lower layer (1·49 g.), containing water (1·257 g.) (4 mols.) and an oil. *p*-Xylene and 2 : 3-dimethyl*cyclo*pent-2-enone were identified as components of the oil.

Dehydrogenation of the Saturated Acid (XX).—Acid (XX) (8·0 g.) was heated with 10% palladium–charcoal (8 g.). Distillation began at 250° and was very slow; the temperature was slowly raised to 340° and kept thereat for 4 hr. The distillate consisted of a lower layer (1·326 g.) containing water (1·127 g., 2·2 mols.), and a colourless upper layer (0·652 g.). Extraction of the residue in the distillation flask gave a yellow oil (1·6 g.), from which *p*-xylenol (0·8 g.) was isolated. The upper ketonic layer of distillate (0·2 g.) was hydrogenated at atmospheric pressure over palladium–charcoal. The product on distillation gave a colourless liquid (0·13 g.), b. p. 140°, from which a 2 : 4-dinitrophenylhydrazone, m. p. 161—162° (corr.), was obtained as golden needles from ethanol. The m. p. of the hydrazone was undepressed on admixture with authentic 2-methyl*cyclo*pentanone 2 : 4-dinitrophenylhydrazone (m. p. 160—161°) (Found: C, 52·8; H, 5·5; N, 19·3; *C*-Me, 7·6, 7·3. Calc. for $C_{12}H_{14}O_4N_4$: C, 51·8; H, 5·0; N, 20·1; *C*-Me, 5·4%). The *cyclo*pentanone also gave a semicarbazone, m. p. 180—181° (corr.), undepressed on admixture with authentic 2-methyl*cyclo*pentanone semicarbazone (m. p. 179—180°) (Found: C, 54·5; H, 8·6. Calc. for $C_{17}H_{13}ON_3$: C, 54·2; H, 8·4%). A mixed m. p. with 3-methyl*cyclo*pentanone semicarbazone (m. p. 180—181°) was 169°. The infrared spectrum of the ketone was identical with that of 2-methyl*cyclo*pentanone and differed from those of 3-methyl*cyclo*pentanone and the dimethyl*cyclo*pentanones described above. The spectrum of the dehydrogenation products showed no trace of an aromatic hydrocarbon.

Dehydrogenation of the Saturated Acid (XI).—Acid (XI) (10 g.) and 10% palladium–charcoal (10 g.) were heated as described above. Liberation of volatile products was not apparent until the bath-temperature reached 300° and distillation did not take place at a satisfactory rate until the temperature was raised to 370°. This temperature was maintained for 3 hr. The residue was extracted with ether, chloroform, and acetone. The distillate consisted of an oil (0·38 g.) and a lower layer (1·418 g.) containing water (1·316 g., 2·05 mols.). Hydrogenation of the oil (0·3 g.) at atmospheric pressure and distillation of the product gave a colourless liquid (0·15 g.), b. p. 140—150°; the latter gave a 2 : 4-dinitrophenylhydrazone which crystallised from ethanol in orange-yellow plates, m. p. 149—154° (corr.). Repeated recrystallisation, washing with hot light petroleum or chromatography on activated alumina, all failed to raise the m. p. of the hydrazone (Found: C, 52·4, 52·4; H, 5·0, 5·2; N, 20·1. Calc. for $C_{12}H_{14}O_4N_4$: C, 51·8; H, 5·0; N, 20·1%). A mixed m. p. with authentic 2-methyl*cyclo*pentanone 2 : 4-dinitrophenylhydrazone (m. p. 160—161°) was 155—158°. A mixed m. p. with 2 : 3-dimethyl*cyclo*pentanone 2 : 4-dinitrophenylhydrazone was 144—146°. Similarly, the semicarbazone, m. p. 169—172° (corr.) (from water), was not obtained pure. A mixed m. p. with authentic 2-methyl*cyclo*pentanone semicarbazone (m. p. 179—180°) was 171—176°, and with authentic 3-methyl*cyclo*pentanone semicarbazone (m. p. 183—185°) was 168—170°. The infrared spectrum of the unknown ketone showed bands characteristic of both 2- and 3-methyl*cyclo*pentanone, and it was concluded that a mixture of the two ketones had been obtained in which 2-methyl*cyclo*pentanone predominated. *p*-Xylenol (0·16 g.) was isolated from extracts of the still residue.

Alkaline Breakdown of Trichothecodione, etc.—(a) p-*Xyloquinone from trichothecodione.* When trichothecodione (0·15 g.) and 10% sodium hydroxide solution (15 ml.) were boiled under reflux, the solution darkened rapidly. After 5 min. the solution was cooled and extracted with chloroform. The extract (0·03 g.) on sublimation at 60°/20 mm. gave lemon-yellow crystals, which sintered at 110° and melted at 120—121° (Found: C, 69·9; H, 5·7. Calc. for $C_8H_8O_2$: C, 70·6; H, 5·9%) (mixed m. p. with *p*-xyloquinone undepressed), $\lambda_{\text{max.}}$ (in hexane) 2490 and 2560 Å (ε 21,400 and 19,000 respectively). For *p*-xyloquinone in hexane, Braude[24] gives maxima at 2500 and 2560 Å (ε 22,200 and 21,000 respectively). Comparison of the infrared spectrum of the sample with that of authentic *p*-xyloquinone showed them to be identical.

(b) p-*Xyloquinol from trichothecodione.* Trichothecodione (0·5 g.) and 10% sodium hydroxide solution (15 ml.) were boiled for 2 min. *p*-Xyloquinol (0·051 g.) was isolated from the products after acidification; it had m. p. and mixed m. p. 208—209° (Found: C, 69·6; H, 7·4. Calc. for $C_8H_{10}O_2$: C, 69·6; H, 7·25%).

---

[24] Braude, *J.*, 1945, 490.

(c) *Acid* (XX). Acid (XX) (approx. 1 g.) was boiled in N-sodium hydroxide until a brown colour developed. Extraction of the alkaline solution with ether gave colourless plates (0·03 g.), m. p. 82—84°. This *substance* sublimed readily *in vacuo* at 100° (Found: C, 68·1; H, 8·6. $C_8H_{12}O_2$ requires C, 68·5; H, 8·6%).

Further C_{15} *and* C_{14} *Oxidation Products of Trichothecolone Derivatives.*—(i) *Compound* (VII). (*a*) Trichothecolone glycol (0·9 g.) was dissolved in 5% aqueous chromic acid solution (20 ml.); after a short time a *ketone* (0·323 g.) separated. After recrystallisation from methanol it melted at 228—229° [Found: C, 68·6, 68·3; H, 6·7, 6·9; *C*-Me, 14·5; active H, 0·51%; *M* (by *X*-ray method), 259. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9; 2*C*-Me, 11·5; 3*C*-Me, 17·2; 1 active H, 0·38%; *M*, 262].

(*b*) Trichothecolone glycol (1·0 g.) and periodic acid (0·7 g.) in water (40 ml.) were kept at room temperature in the dark for 8 days. Fern-like crystals separated, and an estimation of the excess of periodic acid showed that it had been almost completely consumed. The product (0·09 g.) crystallised from ethanol in colourless elongated prisms, m. p. 236—237° (corr.) (Found: C, 68·55; H, 6·5; active H, 0·45; *C*-Me, 15·0%) (the compound sublimed unaltered at 200°/0·1 mm.), identical (mixed m. p. and infrared spectra) with that obtained by method (*a*).

The 2 : 4-*dinitrophenylhydrazone* of the ketone (VII) crystallised from acetic acid in orange prisms, m. p. 302° (decomp.) (Found: C, 56·7; H, 4·8; N, 12·4. $C_{21}H_{22}O_7N_4$ requires C, 57·0; H, 5·0; N, 12·7%). The ketone (0·1 g.) with acetic anhydride gave a *monoacetate* (0·1 g.), m. p. 133°, prisms (from methanol) (Found: C, 67·4, 67·2; H, 6·5, 6·7; Ac, 18·3. $C_{17}H_{20}O_5$ requires C, 67·0; H, 6·6; 1Ac, 14·2%), $\lambda_{max.}$ (in methanol) 2290 Å (ε 12,750).

Compound (VII) (0·21 g.) absorbed hydrogen (50 ml.) in 5 min. when shaken in the presence of palladium–charcoal. The product was a *ketone* (VIII) (0·205 g.), m. p. 165—166°, needles (from methanol) (Found: C, 67·8, 67·4; H, 8·2, 8·3. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3%), whose yellow 2 : 4-*dinitrophenylhydrazone* (from methanol) had m. p. 266° (decomp.) (Found: C, 56·4; H, 5·5; N, 12·6. $C_{21}H_{26}O_7N_4$ requires C, 56·5; H, 5·8; N, 12·6%).

(ii) *Compound* (IX). Dihydrotrichothecolone glycol (1·0 g.) with aqueous chromic acid, as described above, gave a *ketone* (IX) (0·5 g.), m. p. 185—186°, needles (from benzene) (Found: C, 68·5, 68·2; H, 7·4, 7·6. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·5%), whose orange 2 : 4-*dinitrophenylhydrazone*, crystallised from acetic acid, had m. p. 264—265° (Found: C, 55·7, 55·4; H, 5·6, 5·2; N, 12·1. $C_{21}H_{24}O_7N_4$ requires C, 57·7; H, 5·4; N, 12·6%).

(iii) *Acid* (XI). Oxidation of compound (VIII) (0·15 g.) in glacial acetic acid (1 ml.) with 5% chromic acid in acetic acid (5 ml.) at 34° yielded *acid* (XI) (0·04 g.), m. p. 184—185° (decomp.), as prisms from hot water (Found: C, 64·2; H, 7·1%; equiv., 291. $C_{15}H_{20}O_5$ requires C, 64·3; H, 7·1%; equiv., 280). The infrared absorption spectrum showed absorption between 2500 and 3200 cm.$^{-1}$, confirming the presence of a carboxyl group, and bands in the carbonyl region at 1685, 1722 (weak), and 1750 cm.$^{-1}$. This acid was also prepared by hydrogenation of acid (X) and of acid (XII) (described below).

(iv) *Acid* (X). Compound (VII) (0·45 g.) was oxidised with chromic acid in acetic acid at 33°. The product, *acid* (X) (0·146 g.), m. p. 187—188° (decomp.), crystallised from hot water in needles (Found: C, 61·2, 61·3; H, 6·0, 5·7. $C_{15}H_{16}O_5,H_2O$ requires C, 61·2; H, 6·1%). A sample after 2·5 hours' drying at 120° became opaque (Found: C, 64·7; H, 5·7. $C_{15}H_{16}O_5$ requires C, 65·2; H, 5·8%), $\lambda_{max.}$ (in methanol) 2270 Å (ε 12,500), $\nu_{max.}$ 3530 (OH-hydrate), 2700—2800 (CO_2H), and CO bands at 1720 and 1675 cm.$^{-1}$. The acid gave no colour with ferric chloride.

The preceding hydrate (0·18 g.) in methanol was shaken with hydrogen in the presence of palladium–charcoal, 40 ml. of hydrogen being absorbed at 21° during 5 min. ($2H_2$ = 31 ml. at N.T.P.). The product, acid (XI) (0·14 g.) crystallised from hot water as needles, m. p. 182—183° (decomp.) (Found: C, 63·9; H, 6·9%). A mixed m. p. with acid (XI) obtained by oxidation of compound (VIII) was undepressed.

(v) *Acid* (XII). On oxidation with chromic acid in acetic acid, compound (IX) (0·2 g.) gave *acid* (XII) as *hydrate* (0·093 g.), which crystallised from hot water as prisms, m. p. 205—206° (decomp.) (Found: C, 60·4, 60·7; H, 6·4, 6·4. $C_{15}H_{18}O_5,H_2O$ requires C, 60·8; H, 6·7%. Found, after drying at 100°: C, 64·6; H, 6·7. $C_{15}H_{18}O_5$ requires C, 64·8; H, 6·5%). The acid gave no colour with ferric chloride.

Acid (XII) (0·1 g.) in methanol absorbed hydrogen (13 ml.) in 5 min. in the presence of palladium–charcoal at 17°. On recrystallisation from water the product formed prisms of acid

(XI) (0·06 g.), m. p. 184—185° (decomp.) (Found: C, 64·0, 64·0; H, 7·4, 7·0%). A mixed m. p. with acid (XI) prepared by oxidation of (VIII) was undepressed. The acid gave no colour with ferric chloride.

Ketone (XIII)*: Decarboxylation of Acid* (X).—Acid (X) (0·223 g.), when heated at 210° for 3 min., melted and effervesced vigorously, losing carbon dioxide. The melt solidified on cooling, and after trituration with ether it was obtained as a colourless insoluble powder (0·138 g.). *Ketone* (XIII) sublimed at 150—160°/20 mm. as plates, m. p. 132—133° (Found: 72·2, 71·8; H, 7·0, 6·6. $C_{14}H_{16}O_3$ requires C, 72·4; H, 6·9%), $\lambda_{max.}$ (in methanol) 2230 Å (ε 11,500). The infrared absorption spectrum showed the absence of bands due to carboxyl groups, and the presence of two carbonyl bands at 1720 and 1680 cm.$^{-1}$. Ketone (XIII) formed a 2 : 4-dinitrophenylhydrazone which was obtained as orange needles, m. p. 255—256° (decomp.).

Ketone (XIV)*: Decarboxylation of Acid* (XI).—On decarboxylation, acid (XI) (0·5 g.) gave *ketone* (XIV) (0·177 g.) as needles, m. p. 110—112° [from light petroleum (b. p. 60—80°)] (Found: C, 70·9; H, 8·9. $C_{14}H_{20}O_3$ requires C, 71·2; H, 8·5%), $\nu_{max.}$ (>CO) 1710 and 1740 cm.$^{-1}$. This ketone (XIV) (0·1 g.) gave prisms (from chloroform) of a *bis-semicarbazone* (0·07 g.), m. p. 230° (decomp.), which gave a weakly positive Beilstein halogen test [Found: C, 47·8, 48·7; H, 6·8, 7·0; N, 19·5, 19·7. $(C_{16}H_{26}O_3N_6)_2,CHCl_3$ requires C, 48·3; H, 6·5; N, 20·5%], and a deep orange *bis*-2 : 4-*dinitrophenylhydrazone*, m. p. 250—252° (decomp.) (Found: C, 53·8, 54·2; H, 5·2, 5·4; N, 17·2, 16·8. $C_{26}H_{28}O_9N_8$ requires C, 52·4; H, 4·7; N, 18·8. Calc. for $C_{20}H_{24}O_6N_4$: C, 57·7; H, 5·8; N, 13·5%).

Ketone (XV)*: Decarboxylation of Acid* (XII).—Acid (XII) (0·07 g.) was decarboxylated by heating at 220°. The product (0·026 g.) was *ketone* (XV), which was purified by sublimation at 140—150°/0·6 mm. and obtained as needles, m. p. 158—160° (Found: C, 71·9, 72·0; H, 7·7, 7·8. $C_{14}H_{18}O_3$ requires C, 71·8; H, 7·7%).

neo*Trichothecodione*.—(*a*) Trichothecodione (0·25 g.) was heated on a water-bath for 1 hr. with concentrated ammonia solution (7 ml.; *d* 0·880). The dark brown solution was cooled and extracted with chloroform. Evaporation of the chloroform left a dark solid (0·19 g.) which was dissolved in benzene (10 ml.) and decolorised by boiling it with charcoal. neo*Trichothecodione* (XVI) was precipitated by addition of light petroleum and obtained as irregular cream-coloured crystals, m. p. 165—166° (corr.), which were nitrogen-free [Found: C, 68·4, 68·4; H, 6·6, 6·8%; *M* (Rast), 257. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%; *M*, 262].

(*b*) Trichothecodione (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 (20 ml.) to the residual solution caused *neo*trichothecodione to separate as colourless prisms (0·971 g.), m. p. 168—169° (corr.).

(*c*) Trichothecodione (0·130 g.) was dissolved in boiling methanol (6 ml.), and 0·1N-sodium hydroxide (5 ml., one equiv.) added. The solution was heated under reflux for 3 min., then cooled, water (5 ml.) was added, and the whole extracted with chloroform. Removal of the solvent gave *neo*trichothecodione (0·08 g.), m. p. 162—163°. Estimation of ethylenic double bonds by hydrogenation showed that two were present.

neo*Trichothecodione* 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol in yellow needles, m. p. 268° (corr.) (Found: C, 57·2, 57·4; H, 5·0, 5·2; N, 12·5, 12·6. $C_{21}H_{22}O_7N_4$ requires C, 57·0; H, 5·0; N, 12·7%).

neo*Dihydrotrichothecodione*.—Similar treatment of dihydrotrichothecodione (1·0 g.) with sodium carbonate yielded the neo*dihydro-dione* (0·96 g.), which recrystallised from benzene–light petroleum as colourless needles, m. p. 136—136·5° (corr.) (Found: C, 68·5, 68·4; H, 7·5, 7·5. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6%), $\lambda_{max.}$ (in methanol), 2150 Å (ε 7280). It gave an orange *mono*-2 : 4-*dinitrophenylhydrazone* (from ethanol), m. p. 245° (Found: C, 56·6, 56·5; H, 5·4, 5·4; N, 12·7, 12·2. $C_{21}H_{24}O_7N_4$ requires C, 56·8; H, 5·4; N, 12·6%).

Reaction of Dihydrotrichothecodione with Dilute Hydrochloric Acid.—Dihydrotrichothecodione (0·1 g.) in N-hydrochloric acid (3 ml.) was boiled for 30 min. Extraction with chloroform gave a soft colourless product (0·1 g.) which was precipitated from benzene solution by addition of light petroleum as an amorphous mass which slowly crystallised on long standing, giving conglomerates of crystals, m. p. 108—110° (corr.). A mixed m. p. with *neo*dihydrotrichothecodione (m. p. 135°) was 120—121°.

Oxidation of neo*Trichothecodione and Trichothecolone Chlorohydrin: Acid* (XVIII).—(*a*) On oxidation with chromic acid in acetic acid by the method described above, *neo*trichothecodione (0·5 g.) gave *acid* (XVIII) as colourless prisms (from benzene–light petroleum) (0·35 g.), m. p.

173—174° (corr.) (Found: C, 64·8, 64·7; H, 5·9, 5·7%; equiv., 273. $C_{15}H_{16}O_5$ requires C, 65·2; H, 5·8%; equiv., 276). The acid was not decarboxylated on melting. If the acid was isolated by extraction into sodium hydrogen carbonate solution and precipitated by dilute sulphuric acid, the product was obtained as a hydrate, m. p. 115—116° with previous sintering, undepressed on admixture with acid (XVIII) prepared by oxidation of trichothecolone chlorohydrin (described below). *X*-Ray photographs confirmed that these two acids were identical. The hydrated acid crystallised from hot water in colourless needles, which sintered about 105° and melted 115—116°. A sample dried at 100° had m. p. 173—174°, unchanged after recrystallisation from benzene.

(*b*) In a similar way, trichothecolone chlorohydrin (0·2 g.) gave acid (XVIII) as needles (from aqueous methanol) (0·197 g.), m. p. 115—116° (Found: C, 60·9, 60·9; H, 6·4, 5·9. $C_{15}H_{16}O_5,H_2O$ requires C, 61·2; H, 6·1%), $\lambda_{max.}$ (in methanol) 2220 Å (ε 12,220), $\nu_{max.}$ 3480 s (hydrate), ~2500 (CO_2H) and 1740, 1708, and 1670 cm.$^{-1}$ (>CO, last three).

Acid (XIX).—(a) *By oxidation of dihydrotrichothecolone chlorohydrin.* The corresponding oxidation product from dihydrotrichothecolone chlorohydrin (0·3 g.) was large colourless plates of an unstable *hydrate*, m. p. 53—54° (Found: C, 59·7; H, 6·7. $C_{15}H_{18}O_5,H_2O$ requires C, 60·8; H, 6·8%). When kept at room temperature or dried at 42°, the crystals lost water and became opaque, giving anhydrous *acid* (XIX) which crystallised from benzene–light petroleum (b. p. 40—60°) as needles, m. p. 163—164°. The acid was not decarboxylated on melting, but on cooling the melt resolidified and then remelted at 198° (Found: C, 65·0, 64·5; H, 6·4, 6·5. $C_{15}H_{18}O_5$ requires C, 64·8; H, 6·5%), $\lambda_{max.}$ (in methanol), 2170 Å (ε 7670).

(b) *By oxidation of* neo*dihydrotrichothecodione.* The same acid (0·092 g.) was obtained by oxidation of *neo*dihydrotrichothecodione (0·2 g.) as needles (from benzene–light petroleum), m. p. 161·5—162·5° (corr.), which resolidified and remelted at 208° (corr.) (Found: C, 64·8; H, 6·4%).

Acid (XIX) was recovered unchanged after 2 minutes' heating at 210—220° and distilled at 200°/0·05 mm.

The anhydrous acid, prepared by oxidation of dihydrotrichothecolone chlorohydrin, gave the 2 : 4-*dinitrophenylhydrazone*, orange-yellow needles (from methanol), m. p. 254—255° (decomp.) (Found: N, 12·2. $C_{21}H_{22}O_8N_4$ requires N, 12·2%). Mixed m. p.s of the hydrazones (m. p. 251—252°) obtained by oxidation of the hydroxy-chloro-acid (XXI; R = H) from dihydrotrichothecodione chlorohydrin and from dihydrotrichothecolone chlorohydrin were undepressed.

Dihydrotrichothecodione chlorohydrin (0·2 g.) was oxidised with chromic acid in acetic acid, and the product treated with Brady's reagent. The preceding 2 : 4-dinitrophenylhydrazone (0·065 g.), orange prisms (from methanol), m. p. 251—252° (decomp.) (Found: C, 54·8; H, 4·9; Cl, 0. $C_{21}H_{22}O_8N_4$ requires C, 55·0; H, 4·8%), was again obtained.

Similarly the hydroxy-chloro-acid (XXI; R = H) (0·086 g.), prepared by oxidation of tetrahydrotrichothecin chlorohydrin and hydrolysis of the product, was oxidised with aqueous chromic acid. The crude product (0·028 g.) gave once more the same 2 : 4-dinitrophenylhydrazone, orange prisms (from methanol), m. p. 251—252° (decomp.) (0·012 g.).

X-Ray powder photographs of the hydrazones prepared by the three methods, described above, confirmed their identity.

Acid (XX).—(a) *By hydrogenation of acid* (XVIII). Acid (XVIII) (0·177 g.) (prepared from *neo*trichothecodione) absorbed hydrogen (28 ml.) at 22° in the presence of palladium–charcoal in 5 min. The product crystallised from benzene, giving colourless prisms of acid (XX), m. p. 187—188° {mixed m. p. 183—184° with (XX) (prepared from dihydrotrichothecolone chlorohydrin) (m. p. 183—184°). Mixed m. p. 187—188° with (XX) [prepared by oxidation of (XVII)], m. p. 187—189°}. The infrared absorption spectrum showed carbonyl bands at 1700 and 1730 cm.$^{-1}$. Acid (XVIII) (0·193 g.) (prepared from trichothecolone chlorohydrin) absorbed hydrogen (26 ml.) at 21° in 5 min. in the presence of palladium-charcoal, giving *acid* (XX), prisms (from benzene), m. p. 185—186° (0·072 g.) (Found: C, 64·1, 64·2; H, 6·8, 6·9. $C_{15}H_{20}O_5$ requires C, 64·3; H, 7·1%). The acid did not decompose on melting and gave no colour with ferric chloride.

(b) *By hydrogenation of acid* (XIX). Similarly, acid (XIX) (0·2 g.) (prepared from *neo*dihydrotrichothecodione) absorbed hydrogen (23 ml.) in 2 min. at 21° in the presence of palladium–charcoal. The product crystallised from benzene in prisms m. p. 187—188°; the mixed m. p. with acid (XX) [prepared from (XVIII)], m. p. 187—188°, was undepressed.

(c) *By oxidation of compound* (XVII). Compound (XVII) (0·15 g.) was oxidised with chromic acid in acetic acid, giving acid (XX) (0·072 g.) as prisms, m. p. 187—189° (from benzene) (Found: C, 63·9, 64·1; H, 7·0, 7·5%), mixed m. p. 185—187° with (XX) [prepared by hydrogenation of (XVIII) from the trichothecolone chlorohydrin series], m. p. 185—186°.

Compound (XVII).—(a) *By hydrogenation of* neo*trichothecodione.* *neo*Trichothecodione (0·2 g.) in methanol absorbed hydrogen (33 ml.) at 21° in 5 min. in the presence of palladium–charcoal, *i.e.*, 31 ml. at N.T.P. ($2H_2$ = 34 ml. at N.T.P.). *Ketone* (XVII) crystallised from methanol and melted at 192—193° (Found: C, 67·3, 67·5; H, 8·5, 8·4. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3%).

(b) *By hydrogenation of* neo*dihydrotrichothecodione.* Similarly, *neo*dihydrotrichothecodione (0·2 g.) absorbed hydrogen (14 ml.) in 5 min. at 23° (*i.e.*, 13 ml. at N.T.P.; H_2 = 17 ml.). The product (XVII) crystallised in prisms, m. p. 192—193°, undepressed on admixture with the product of hydrogenation of *neo*trichothecodione. The infrared spectrum showed a strong hydroxyl band, and carbonyl bands at 1722 and 1690 cm.$^{-1}$.

Methyl Ester of Acid (XX).—The acid (0·5 g.) was esterified with diazomethane. The *ester* crystallised from light petroleum (b. p. 60—80°) as prisms, m. p. 101—102° (Found: C, 65·0, 65·1; H, 7·2, 7·6. $C_{16}H_{22}O_5$ requires C, 65·3; H, 7·5%). The infrared spectrum showed a broad band of carbonyl absorption (which may be double) at about 1725 cm.$^{-1}$.

Tests for Oxide Ring.—*Group* 1. The compounds of this group gave a strong positive reaction with the reagent at 60—70° in, usually, 10—15 min. The red colour due to the indicator rapidly increased in intensity with the time of heating. The times recorded are approximate. Compound (VII) gave a yellow solution in 30 min., and before hydroxyl ions were liberated. There was a marked difference between the behaviour of this compound and compounds of the *neo*trichothecodione series.

Compound	Time for colour to develop (min.)	Compound	Time for colour to develop (min.)
Trichothecolone	10	*neo*Trichothecodione	5
Dihydrotrichothecolone	15	*neo*Dihydrotrichothecodione	20
Trichothecodione	10	Compound (VII)	120
Dihydrotrichothecodione	10		

Group 2. The compounds of this group gave only weak pink colours, which did not increase in intensity, and in most cases faded. These weak colours were very slow to develop.

Compound	Time for colour to develop
Acetyltrichothecolone	20 min. (fades)
Butyryltrichothecolone	60 min. (fades after 2 hr.)
*iso*Trichothecolone	1—2 hr.
Trichothecolone glycol	2 hr. (extremely faint)
Trichothecolone chlorohydrin	1 hr. (fades after 2 hr.)

Group 3. The compounds of this group gave no colours even after many hours' heating.

Compound	Time heated (hr.)	Compound	Time heated (hr.)
Tricothecin	5	Trichothecin chlorohydrin	2
Trichothecin glycol	2	Crotonyltrichothecolone	3

Comparative experiments were carried out under similar conditions with oxide rings of various sizes, with the following results:

Three-membered oxide rings:	
Propylene oxide	Pink immediately in the cold.
Phorone dioxide	Pink after 3 min. in the cold, or immediately at 50°.
Et *cyclo*hexane*spiro*-2-oxiran-3-carboxylate	No colour after 3 hr. at 70°.
Glycidyl acetate	Pink colour immediately in the cold.
Four-membered oxide rings:	
3 : 3-Dimethyloxetan	No colour after 3 hr. at 70°.
3 : 3-Diacetoxymethyloxetan	No colour after 3 hr. at 70°.
Trimethylene oxide	Pink after 2 min. at 70°; pale pink after 20 min. in the cold.
Five-membered oxide rings:	
Tetrahydrofuran	Extremely weak colour after 40 min. at 70°; fades after 2 hr.
Six-membered oxide rings:	
Tetrahydropyran	No colour after 3 hr. at 70°.
Dihydropyran	Extremely weak colour after 3 hr. at 70°.

Heat of Reaction of Trichothecolone with Hydrochloric Acid (*by* T. COTTRELL, R. A. HAMILTON, and R. P. TAUBINGER).—The reaction between trichothecolone and hydrochloric acid involves the breaking of an ether-type C–O link: in terms of bonds the reaction is: $\text{C–O} + \text{H–Cl} \longrightarrow \text{C–Cl} + \text{H–O}$. Consideration of bond-energy terms suggests that this reaction will be about thermally neutral, and it therefore seems reasonable to believe that any heat evolved in the reaction of trichothecolone with hydrochloric acid will be due to ring rupture and thus will give an indication of ring strain. In case the bond reaction itself might not be exactly neutral, the reaction of epichlorohydrin with hydrochloric acid was examined for comparison. Measurements were carried out by using a conventional Dewar vessel calorimeter, the heat capacity of the system being determined by measuring the temperature rise on dilution of 37% hydrochloric acid. The experimental accuracy was probably about ±5%. Three measurements were made for epichlorohydrin, giving $-\Delta H$ for the reaction:

$$\text{epichlorohydrin } (l) + \text{HCl } (37\%, \text{aq.}) \longrightarrow \text{Products} \quad . \quad . \quad . \quad . \quad (1)$$

as 27·8, 29·4, and 29·5 kcal. mole^{-1} respectively. Thus $-\Delta H$ (1) = 29 kcal. mole^{-1}. Initially two measurements of $-\Delta H$ for the reaction:

$$\text{Trichothecolone } (s) + \text{HCl } (37\% \text{ aq.}) \longrightarrow \text{Products} \quad . \quad . \quad . \quad . \quad (2)$$

gave 26·5 and 28·9 kcal. mole^{-1}. Two further measurements on another specimen of trichothecolone gave 23·2 and 22·9 kcal. mole^{-1}. There appears to have been a difference between the two samples, so that the value of $-\Delta H$ (2), 25 kcal. mole^{-1}, is subject to an unknown uncertainty. These results are in good agreement with the expected range of values, considering that unknown heats of solution and fusion are involved in the measured quantities.

Miscellaneous Reactions of Trichothecolone and its Derivatives.—*Reduction of dihydrotrichothecolone with phosphorus and hydriodic acid.* Dihydrotrichothecolone (2·0 g.), red phosphorus (0·5 g.), and hydriodic acid (35 ml.; *d* 1·7) were heated under reflux for 42 hr. Within a short time an immiscible oil was refluxing in the condenser. The mixture was cooled, diluted with water, and extracted with ether. The extract was washed successively with 5% sodium hydroxide solution, sodium thiosulphate solution, and water, and the solvent was then removed. The residue was steam-distilled and the pale yellow oil in the distillate extracted with ether. Removal of the solvent left a mobile oil (0·3 g.) which sublimed at 100°/20 mm. and had a b. p. (Siwoloboff's method) between 230° and 240° (yield 0·135 g.). Most preparations contained a trace of halogen, but a sample distilled over sodium gave a liquid, n_D^{22} 1·5130, free from halogen (Found: C, 86·6; H, 10·9%). Another sample, $n_D^{21.5}$ 1·5252, examined on a mass spectrograph, contained a fraction of molecular weight 206, this being the mass : charge ratio of the heaviest abundant ion present. The product showed no aromatic absorption bands in its infrared spectrum.

Reduction of trichothecin with Adams catalyst. Trichothecin (1·0 g.) in methanol absorbed hydrogen (200 ml.) at 21° during 30 min. in the presence of Adams catalyst ($3H_2$ = 202 ml.). The product, a viscous oil, was hydrolysed with 6% methanolic potassium hydroxide (40 ml.). Chloroform extraction gave a *compound* (0·868 g.) which crystallised from benzene in colourless prisms (0·212 g.), m. p. 138° (Found: C, 67·4, 67·2; H, 9·2, 9·0. $C_{15}H_{24}O_4$ requires C, 67·2; H, 9·0%). The infrared absorption spectrum showed no carbonyl groups, but two bands were present at the hydroxyl frequency.

Permanganate oxidations of trichothecolone derivatives. Acetic acid was isolated on alkaline permanganate oxidation of trichothecolone glycol; oxalic acid from the oxidation of *iso*trichothecolone and dihydro*iso*trichothecolone. Oxidation of dihydrotrichothecolone with acid permanganate gave a small yield of dihydrotrichothecodione.

X-*Ray Examination of Halogenated Derivatives* (*by* Dr. J. A. JARVIS).—The following crystalline derivatives of trichothecin were submitted to *X*-ray examination:

(1) C_{19}-bromo acid. Orthorhombic: a = 9·0, b = 9·9, c = 22·1 Å. Space group $P2_12_12_1$, 4 molecules per cell.

(2) C_{19}-chloro-acid (XXI; R = Pr·CO); isomorphous with above.

(3) Trichothecin bromohydrin; monoclinic; a = 20·4, b = 6·1, c = 16·3 Å, β = 96°, space group $A2$, 4 molecules per cell.

(4) Trichothecin chlorohydrin; material recrystallised from aqueous alcohol or aqueous acetic acid was not isomorphous with the bromohydrin.

Zero-layer Weissenberg photographs have been taken of the isomorphous C_{19} acids about their *a* and *b* axes. Comparison of Patterson projections for the two compounds gave co-ordinates of halogen atoms. By using these co-ordinates it was possible to calculate values of $F_{Br} - F_{Cl}$ and hence the signs of many structure amplitudes. Study of the *a* and *b* axis projections of the C_{19} chloro-acid reduced the residual *R* to 22%. The 4-chloro-2-nitrophenylhydrazones of trichothecolone chloro- and bromo-hydrins were isomorphous and had the following cell dimensions: chloro-compound, *a* 20·56, *b* 7·09, *c* 7·90 Å, β 98·5°; bromo-compound, *a* 20·83, *b* 7·09, *c* 7·88 Å, β 98·5°.

iso*Trichothecolone*.—iso*Trichothecolone* was formed from trichothecolone in practically quantitative yields, or directly from trichothecin, by the action of hot aqueous or alcoholic sodium or barium hydroxides. Trichothecolone (2·5 g.)was completely isomerised after 30 min. in boiling N-sodium hydroxide (50 ml.) and ethanol (30 ml.). *iso*Trichothecolone crystallised in colourless needles [from benzene–light petroleum (1 : 1)], m. p. 184—185° (corr.), depressed to 154° on admixture with trichothecolone [m. p. 183—184° (corr.)] [Found: C, 68·0, 67·9; H, 7·6, 7·5; *C*-Me, 10·7; active H, 0·64%; *M* (Rast), 293, 279. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6; 2*C*-Me, 11·4; 3*C*-Me, 17·0; 2 active H, 0·76%; *M*, 264]. *iso*Trichothecolone had $[\alpha]_D^{17·5}$ −38° (*c* 1 in ethanol), $\lambda_{max.}$ (in methanol), 2300 Å (ε 8050).

Its 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol as deep orange needles, m. p. 252° (corr.) (Found: C, 55·8; H, 5·6; N, 12·5, 12·5. $C_{21}H_{24}O_7N_4$ requires C, 56·9; H, 5·4; N, 12·6%), and the *mono-*p*-nitrobenzoate* from *iso*trichothecolone (1·5 g.) separated from benzene–light petroleum as an amorphous solid (1·27 g.), m. p. 195—196° (corr.) (Found: C, 63·8, 63·6; H, 5·7, 5·5; N, 3·6, 3·7. $C_{22}H_{23}O_7N$ requires C, 63·9; H, 5·6; N, 3·4%).

*Dihydro*iso*trichothecolone*.—(*a*) *iso*Trichothecolone (0·2 g.) was hydrogenated in ethanol (25 ml.) at 1 atm. with 10% palladium–charcoal (0·2 g.). Absorption of hydrogen was complete in 2 min. (17·5 ml. at N.T.P.; $1H_2$ = 17 ml.). The crude product (0·206 g.) was extracted with benzene; the residue was crystalline *dihydro*iso*trichothecolone* (0·1 g.) which recrystallised from chloroform–light petroleum (1 : 4) as colourless rods, m. p. 199—201° (corr.) [Found: C, 66·8; H, 8·1%; *M* (Rast), 250. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3%; *M*, 266], $[\alpha]_D^{20}$ −1·2° ± 0·5° (*c* 3·9 in acetic acid). Evaporation of the benzene extract gave a crystalline residue, m. p. 130—132° (corr.), undepressed on admixture with dihydro*iso*trichothecolone (m. p. 132°) obtained by the action of hot alkali on dihydrotrichothecolone or by hydrolysis of hydrogenated acetyl*iso*trichothecolone (Found: C, 67·3, 67·4; H, 8·2, 8·4%), $[\alpha]_D^{20}$ −114° (*c* 3·8 in acetic acid). The infrared spectra of the above specimens of dihydro*iso*trichothecolone were identical in their principal bands, but differed very slightly in detail in the 8—13 μ region.

(*b*) Dihydrotrichothecolone (0·25 g.) was heated with methanolic potassium hydroxide (12 ml.). The product (0·244 g.) crystallised from benzene–light petroleum (1 : 1) as a new form (prisms), m. p. 130—132° (corr.) (Found: C, 67·3, 67·4; H, 8·2, 8·4%).

Dihydro*iso*trichothecolone, m. p. 132°, was recovered unchanged after 2 hours' boiling in 0·1N-hydrochloric acid. The same 2 : 4-*dinitrophenylhydrazone* was prepared from both forms and crystallised as pale orange needles from ethanol and had m. p. 185—186° (Found: C, 55·3, 55·4; H, 6·2, 6·0; N, 11·5, 11·8. $C_{21}H_{26}O_7N_4$ requires C, 56·5; H, 5·8; N, 12·5%), $[\alpha]_D^{19·5}$ −347° (*c* 0·5 in acetic acid).

Dihydro*iso*trichothecolone, m. p. 199—201° (0·5 g.), was heated under reflux with N-sodium hydroxide (25 ml.) for 3 hr.; the product isolated by extraction with chloroform (0·46 g.) had m. p. 128—131°, undepressed on admixture with dihydro*iso*trichothecolone, m. p. 132°. It had $[\alpha]_D^{16·5}$ −113° (*c* 1·3 in ethanol).

Dihydro*iso*trichothecolone, m. p. 199—201° (0·4 g.), was dissolved in ethanol (25 ml.), and concentrated hydrochloric acid (5 ml.) added to bring the normality of the solution to 2N. The solution was kept at room temperature for 4 hr., then it had $[\alpha]_D^{18·5}$ −116° (*c* 1·3 in 2N-ethanolic hydrochloric acid), virtually identical with that of dihydro*iso*trichothecolone, m. p. 132°.

*Monoacetyl*iso*trichothecolone*.—*iso*Trichothecolone was heated with acetic anhydride in pyridine for 30 min. The *acetate* crystallised from benzene–light petroleum (1 : 1) in colourless needles, m. p. 185—186° (corr.) (Found: C, 66·5; H, 7·3; Ac, 18·8, 18·3. $C_{17}H_{22}O_5$ requires C, 66·6; H, 7·2; 1Ac, 14·1%). It was also obtained by heating *iso*trichothecolone under reflux for 30 hr. with glacial acetic acid. It had infrared bands at 3400 (OH), 1650 (C⁚C), 1750 cm.$^{-1}$ (ester CO), but the band at 1667 cm.$^{-1}$, due to the original ketonic group, was absent.

*Hydrogenation of Monoacetyl*iso*trichothecolone*.—The acetate was hydrogenated at 1 atm.

with 10% palladium–charcoal, with uptake of one mol. of hydrogen. *Acetyldihydro*iso*trichothecolone* crystallised from benzene–light petroleum (1 : 4) in colourless needles containing benzene of crystallisation, had m. p. 123—124° (corr.) (Found: C, 71·9; H, 8·0. $C_{17}H_{24}O_5,C_6H_6$ requires C, 71·6; H, 7·8%). Hydrolysis of the acetyl derivative with methanolic potassium hydroxide gave dihydro*iso*trichothecolone, m. p. 126—130°, undepressed on admixture with dihydro*iso*trichothecolone of m. p. 132° prepared by the action of hot alcoholic potassium hydroxide on dihydrotrichothecolone. Acetyldihydro*iso*trichothecolone was also prepared from either form of dihydro*iso*trichothecolone by acetic anhydride in pyridine. The product crystallised as small aggregates [from benzene–light petroleum (2 : 1)], m. p. 120—121°, undepressed on admixture with acetyldihydro*iso*trichothecolone (m. p. 123—124°) prepared by hydrogenation of acetyl*iso*trichothecolone.

*Diacetyl*iso*trichothecolone.*—*iso*Trichothecolone (1·0 g.) was heated under reflux for 4 hr. with acetic anhydride (5 ml.) and sodium acetate (1 g.). The product crystallised from benzene–light petroleum as long colourless rods (1·05 g.), m. p. 161·5—162·5° (corr.) [mixed m. p. 136° with monoacetyl*iso*trichothecolone (m. p. 185—186°)] (Found: C, 67·1, 67·2, 65·5, 65·7; H, 7·3, 7·2, 7·1, 7·0; Ac, 18·6, 18·1, 20·0, 21·7, 23·8. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·9; 2Ac, 24·7%). The infrared spectrum had max. at 1667 (>CO) and 1742 cm.$^{-1}$ (ester) but no hydroxyl absorption. This compound is believed to be identical with diacetyl*iso*trichothecolone, m. p. 155—157°, obtained on vigorous acetylation by Fishman *et al.*[15] Its 2 : 4-*dinitrophenylhydrazone* separated from ethanol as an amorphous solid, m. p. 155° (corr.) (Found: C, 55·2, 55·6; H, 5·5, 5·5; N, 11·4, 10·9. $C_{25}H_{28}O_9N_4$ requires C, 56·8; H, 5·4; N, 10·6%).

*Diacetyldihydro*iso*trichothecolone.*—Under similar conditions dihydro*iso*trichothecolone, m. p. 130—132° (0·8 g.), gave the *diacetate*, m. p. 154—155°, needles from aqueous ethanol (Found: C, 63·3, 63·3; H, 7·3, 7·7; Ac, 31·0, 32·0. $C_{19}H_{26}O_6$ requires C, 65·1; H, 7·5; 2Ac, 24·6%).

Reaction of iso*Trichothecolone with Phosphorus Oxychloride.*—Freshly distilled phosphorus oxychloride (1 ml.) was added to *iso*trichothecolone (0·5 g.) in pyridine (15 ml.). After 20 hr. at room temperature the mixture was diluted with water (100 ml.), and the precipitate collected. Crystallisation from ethanol gave the *product* as colourless needles (0·21 g.) which darkened at 198° but did not give a sharp m. p. (Found: C, 52·3, 52·3; H, 5·3, 5·2; Cl, 10·3, 10·1; P, 9·0. $C_{15}H_{18}O_5PCl$ requires C, 52·3; H, 5·2; Cl, 10·3; P, 9·0%).

iso*Trichothecodione.*—*iso*Trichothecolone dissolved readily in 5% aqueous chromic acid and gradually a colourless crystalline precipitate, m. p. 190—191° (corr.), was obtained. iso*Trichothecodione* crystallised from benzene in colourless rods (Found: C, 68·3; H, 7·0. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%), $[\alpha]_D^{18}$ −28·8° (*c* 3·0 in ethanol) (*c* 3·0), absorbing 1 mol. of hydrogen. Further oxidation of *iso*trichothecodione with chromic acid in acetic acid gave only a trace of an acid, m. p. 207—209° (corr.), the bulk of the original material being recovered unchanged. *iso*Trichothecodione dissolved readily in a small volume of concentrated hydrochloric acid and was precipitated unchanged on addition of water.

*Acetyl*iso*trichothecodione*, crystallised from water, had m. p. 184—185° (corr.) (Found: C, 67·2, 67·5; H, 6·6, 6·7; Ac, 13·7. $C_{17}H_{20}O_5$ requires C, 67·1; H, 6·6; Ac, 14·1%), and the 2 : 4-dinitrophenylhydrazone, prepared in low yield by Allen's method, had m. p. 194—195° (corr.).

*Dihydro*iso*trichothecodione.*—*iso*Trichothecodione (0·5 g.) in ethanol was hydrogenated at 1 atm. with palladium–charcoal: 1 mol. of hydrogen was absorbed. *Dihydro*iso*trichothecodione* crystallised from benzene as prisms (0·41 g.), m. p. 176—178° (corr.) (Found: C 68·1, 68·2; H, 7·2, 7·5. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6%).

*Oxidation of Dihydro*iso*trichothecolone.*—Dihydro*iso*trichothecolone (m. p. 132°; 0·5 g.) was treated with chromic acid in acetic acid as previously described. The product crystallised from benzene in compact crystals, m. p. 166—167° (corr.) (Found: C, 57·9, 57·6; H, 7·3, 7·2%; equiv., 310, 306. $C_{15}H_{20}O_6,H_2O$ requires C, 57·3; H, 7·0%; *M*, 314). The *acid* was not decarboxylated at the m. p. With acetic anhydride and pyridine it gave a syrupy *acetate* (Found: C, 60·0, 59·0, 59·3; H, 6·6, 6·6, 6·5; Ac, 11·2. $C_{17}H_{22}O_7$ requires C, 60·4; H, 6·5; Ac, 12·7%). The orange 2 : 4-*dinitrophenylhydrazone* of the acid had m. p. 137° (Found: C, 51·1, 50·9; H, 4·9, 5·3; N, 11·7, 11·8. $C_{21}H_{26}O_{10}N_4$ requires C, 51·0; H, 5·3; N, 11·3%).

*Oxidation of Dihydro*iso*trichothecodione.*—The hydrogenation product of *iso*trichothecodione (1·5 g.) in acetic acid (3 ml.) was added to 5% aqueous chromic acid (60 ml.), and the mixture kept at room temperature for 4 days. Extraction with ether gave a colourless acidic solid which crystallised from benzene in compact crystals (1·28 g.), m. p. 162—163° (corr.), undepressed on admixture with the acid $C_{15}H_{20}O_6,H_2O$, m. p. 166—167°, described above.

We are grateful to Professor E. R. H. Jones, F.R.S., Dr. M. C. Whiting, and Dr. J. Fishman for their interest and for permission to refer to their unpublished results.

RESEARCH DEPARTMENT, IMPERIAL CHEMICAL INDUSTRIES LIMITED,
NOBEL DIVISION, STEVENSTON, AYRSHIRE.
RESEARCH DEPARTMENT, IMPERIAL CHEMICAL INDUSTRIES LIMITED,
PHARMACEUTICALS DIVISION, ALDERLEY PARK,
MACCLESFIELD, CHESHIRE.

[*Received, August* 20*th*, 1958.]