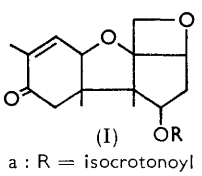
## 784. *The Biogenesis of Trichothecin.*

By E. R. H. Jones and G. Lowe.

The antifungal metabolite trichothecin (Ia) has been obtained in labelled forms from cultures of the fungus *Trichothecium roseum* Link grown on media containing sodium [1-$^{14}$C]acetate and [2-$^{14}$C]mevalonic lactone (II). By degradation of these products it has been shown that the trichothecolone moiety (Ib) is biogenetically sesquiterpenoid in origin, a double 1,2-methyl group migration being involved, whereas the isocrotonate portion is derived rather directly from acetate units.

The structure of trichothecin, an antifungal metabolite of *Trichothecium roseum* Link, has been established [1,2] as (Ia). Although the $C_{15}$ moiety, trichothecolone (Ib), contains three methyl groups, its carbon skeleton cannot be derived by the classical head-to-tail linking of three isopentane units. However, the structure (Ib) does obey the Ruzicka biogenetic isoprene rule,[3] *i.e.*, a normal sesquiterpenoid skeleton can be achieved by a 1,3- or double 1,2-methyl group migration. It was therefore of interest to ascertain whether or not the biochemical "isoprene unit," mevalonic acid [4] (II), is utilised as a major source of its carbon skeleton. Added interest was aroused when during this work it was revealed that rosenonolactone,[5] a metabolite of the same fungus but structurally unrelated to trichothecin, readily incorporates labelled mevalonic acid and that the labelled carbon atoms are located at the expected positions in the molecule.[6]



(I) OR

a : R = isocrotonoyl

b : R = H

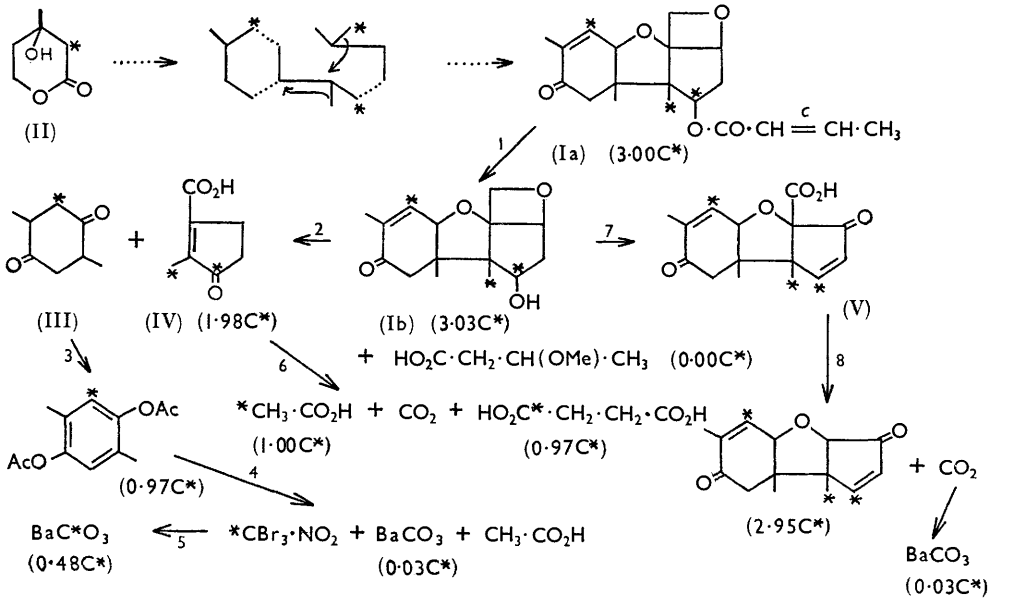
[1] Freeman, Gill, and Waring, *J.*, 1959, 1105.
[2] Fishman, Jones, Lowe, and Whiting, preceding paper.
[3] Ruzicka, *Experientia*, 1953, **9**, 357; *Proc. Chem. Soc.*, 1959, 341.
[4] Wolf, Hoffmann, Aldrich, Skeggs, Wright, and Folkers, *J. Amer. Chem. Soc.*, 1956, **78**, 4499; "CIBA Symposium on Terpene and Sterol Biosynthesis," Churchill, London, 1959.
[5] Harris, Robertson, and Whalley, *J.*, 1958, 1799.
[6] Birch, Rickards, Smith, Harris, and Whalley, *Proc. Chem. Soc.*, 1958, 223; Britt and Arigoni, *ibid.*, 1958, 224.

Sodium [1-$^{14}$C]acetate was incorporated into trichothecin (Ia) to the extent of about 0·2%. Hydrolysis showed that about 95% of the activity was in the isocrotonate moiety and only about 5% in the trichothecolone (Ib).

The observation [7] that in mycophenolic acid the extent of incorporation of acetate into the nucleus (shown to be derived directly from acetate) and into the isoprenoid side-chain was the same, must therefore, as expected, be fortuitous.

[2-$^{14}$C]Mevalonic lactone (II) was incorporated into trichothecin (Ia) to the extent of about 0·5%. The distribution of the radioactivity was found by degradation to be as shown diagrammatically. It is noteworthy that no activity was found in the isocrotonate moiety, indicating, as has been previously observed,[7] that mevalonic acid is an irreversible precursor of the isopentane unit (isopentenyl pyrophosphate [8]) used in terpene biosynthesis.

Degradation of the trichothecolone by the procedure already described [1,2] gave 2,5-dimethylcyclohexane-1,4-dione (III) and 2-methyl-3-oxo-cyclopent-1-enecarboxylic acid (IV) having relative molar activities (*i.e.*, counts/100 sec. × *M*) in the ratio 1 : 2. [Owing to its volatility the activity of the diketone (III) had to be computed from that of the diacetyl-*p*-xyloquinol.] Since trichothecolone contains fifteen carbon atoms it is reasonable to assume that it contains three active carbon atoms and that the above two fragments derived from it contain one and two active carbon atoms respectively. By degradation of the acid (IV) with Lemieux's reagent [9] (periodate containing a trace of permanganate), succinic and acetic acid were isolated, the latter as its 4-phenylphenacyl ester. The presence of one active carbon atom in each of these acids permitted the conclusion that the active methyl group had migrated and that a double 1,2-methyl shift and not a single 1,3-methyl shift had occurred. This result was confirmed by an



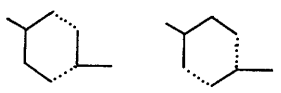

Reagents: 1, Alkali. 2, $CrO_3$–$COMe_2$; $Na_2CO_3$; $CrO_3$–$COMe_2$; Pd–$H_2$; Alkali. 3, $Ac_2O$–*p*-$C_6H_4Me{\cdot}SO_3H$; "Quinone." 4, $HNO_3$; $Ba(OBr)_2$. 5, Van Slyke–Folch oxidn. 6, $KIO_4$–$KMnO_4$. 7, $H^+$; $CrO_3$–$H_2O$; $CrO_3$–AcOH. 8, Heat.

alternative degradation (see diagram) that involved the decarboxylation of the β-keto-acid (V) obtained by stepwise oxidation of trichothecolone glycol [1] and isolation of the

[7] Birch, English, Massy-Westropp, and Smith, *J.*, 1958, 369.
[8] Lynen, Eggerer, Henning, and Kessel, *Angew. Chem.*, 1958, **70**, 738.
[9] Lemieux and von Rudloff, *Canad. J. Chem.*, 1955, **33**, 1701.

carbon dioxide as barium carbonate, which was found to be inactive. This result was, of course, to be expected on mechanistic grounds and in the light of recent observations of double 1,2-methyl migrations in the biogenesis of steroids and triterpenes.[10,11]

The cyclisation of the six-membered ring of trichothecolone could presumably occur in two ways (VIa and b). In order to distinguish between them it was necessary to degrade the 2,5-dimethylcyclohexane-1,4-dione (III). The dienol diacetate was prepared and dehydrogenated with tetrachloro-1,8-diphenoquinone [12] to diacetyl-*p*-xyloquinol. Nitration gave the dinitro-derivative which was degraded with barium hypobromite [13] to barium carbonate, bromopicrin, and acetic acid. The bromopicrin was oxidised to carbon dioxide, converted into barium carbonate, and found to contain the active carbon atom, indicating that the six-membered ring in trichothecin was formed as in (VIa). This result provides yet another illustration of the linking rôle of the carbon atom adjacent to the carboxyl group in mevalonic acid in the head-to-tail fusion of the isopentane units.[6,14]



(a) (VI) (b)

## Experimental

*Radioactive Assay.*—Specimens were assayed for radioactivity as " infinitely thick " solid samples of standard geometry and 1 sq. cm. cross-sectional area.[15] Counting rates were corrected for background; all other sources of error proved to be negligible. Counting equipment consisted of an EKCO automatic scaler of type N530C in conjunction with a probe unit and Mullard Geiger–Müller tube of type MX123. The counts per 100 sec. were determined by recording the time required for $10^5$ counts to be aggregated. Hence the statistical counting error had a 99·7% probability of being less than 1%.[16] Estimates of absolute activity (for determining incorporation) were obtained by comparison with a disc of radioactive poly(methyl methacrylate) (Amersham) of nominal specific activity 0·1 μc/g., counted under identical geometrical conditions. All specimens where possible were recrystallised to constant activity.

*Isolation of* [$^{14}$C]*Trichothecin.*—(a) *Trichothecium roseum* Link was grown under the conditions already described.[17] Preliminary experiments had shown that trichothecin was produced steadily from the time of inoculation. Accordingly sodium [1-$^{14}$C]acetate (0·08 mc; 1·3 mg.) was distributed evenly between 2 flasks each containing 750 c.c. of medium, before inoculation. After 28 days' growth the trichothecin was isolated and diluted with inactive trichothecin (1·0 g.) (Found: relative molar activity × $10^{-2}$, 1100). The yield in terms of incorporation of radioactivity was about 0·2%.

(b) [2-$^{14}$C]Mevalonic lactone (0·1 mc; 21·6 mg.) was similarly distributed between two flasks, each containing 750 c.c. of medium, before inoculation. After 28 days' growth the trichothecin was isolated and diluted with inactive trichothecin (15·0 g.) (Found: relative molar activity × $10^{-2}$, 171). The yield in terms of incorporation of radioactivity was about 0·5%.

*Hydrolysis of Trichothecin (from Sodium* [1-$^{14}$C]*Acetate).*—Treatment of the trichothecin with N-methanolic potassium hydroxide [1] gave trichothecolone (Found: relative molar activity × $10^{-2}$, 71) and β-methoxybutyric acid isolated as its 4-phenylphenacyl ester (Found: relative molar activity × $10^{-2}$, 1050).

*Hydrolysis of Trichothecin (from* [2-$^{14}$C]*Mevalonic Lactone).*—Treatment of trichothecin with N-methanolic potassium hydroxide solution [1] gave trichothecolone (Found: relative molar activity × $10^{-2}$, 173; 3C* require 171) and β-methoxybutyric acid isolated as its 4-phenylphenacyl ester (Found: relative molar activity × $10^{-2}$, 0·2).

*Degradation of Trichothecolone (from* [2-$^{14}$C]*Mevalonic Lactone).*—(*a*) The following sequence of reactions has previously been described in detail.[1,2] The radioactive trichothecolone (5·0 g.)

[10] Cornforth, Cornforth, Pelter, Horning, and Popjak, *Proc. Chem. Soc.*, 1958, 112.
[11] Maudgal, Tchen, and Bloch, *J. Amer. Chem. Soc.*, 1958, **80**, 2589.
[12] Braude, Brook, and Linstead, *J.*, 1954, 3569.
[13] Baddiley, Ehrensvard, Klein, Reio, and Saluste, *J. Biol. Chem.*, 1950, **183**, 777.
[14] Amdur, Rilling, and Bloch, *J. Amer. Chem. Soc.*, 1957, **79**, 2646; Birch, Rickards, and Smith, *Proc. Chem. Soc.*, 1958, 192.
[15] Popjak, *Biochem. J.*, 1950, **46**, 560.
[16] Faires and Parks, " Radioisotope Laboratory Techniques," Newnes, London, 1958, 153.
[17] Freeman and Morrison, *Biochem. J.*, 1949, **44**, 1; Freeman, *J. Gen. Microbiol.*, 1955, **12**, 213.

was oxidised to trichothecodione which was isomerised with base to neotrichothecodione. Oxidation of neotrichothecodione followed by catalytic hydrogenation gave an acid (3·1 g., crude) which was cleaved with alkali [2] to give 2,5-dimethylcyclohexane-1,4-dione (0·78 g.) (too volatile for satisfactory counting) and 2-methyl-3-oxocyclopent-1-enecarboxylic acid (0·51 g.) (Found: relative molar activity × $10^{-2}$, 113; 2C* require 114)

(*b*) The following sequence of reactions has previously been described in detail.[1] Trichothecolone (3·0 g.) was treated with acid, and the resulting " trichothecolone glycol " oxidised stepwise (see diagram) to the β-keto-acid (0·4 g.). Decarboxylation of this gave the diketone (Found: relative molar activity × $10^{-2}$, 168; 3C* require 171) and carbon dioxide collected as barium carbonate (Found: relative molar activity × $10^{-2}$, 0·2).

*Degradation of* 2,5-*Dimethylcyclohexane*-1,4-*dione*.—The diketone (0·78 g.), acetic anhydride (20 c.c.), and toluene-*p*-sulphonic acid (0·4 g.) were heated at 130° in an oil-bath for 4 hr. The solution was poured into water and extracted with ether, and the extracts were washed with sodium carbonate solution, water, dried ($Na_2SO_4$), and evaporated. The residue crystallised from ethanol in needles, m. p. 136—137°, but this m. p. was depressed to 122—128° on admixture with 2,5-dimethylquinol diacetate (m. p. 134—135°; prepared by treatment of *p*-xyloquinone with zinc dust, acetic acid, and acetic anhydride [18]). The crude product in benzene (20 c.c.) was refluxed for 8 hr. with tetrachloro-1,8-diphenoquinone [12] (2·5 g.). The cooled mixture was filtered and purified by chromatography on alumina from benzene. The evaporated eluate (0·39 g.) crystallised, and recrystallisation from ethanol gave 2,5-dimethylquinol diacetate, m. p. and mixed m. p. 134—135° (Found: relative molar activity × $10^{-2}$, 55; 1C* requires 57).

The diacetate was treated at 0° with about a 5-fold excess of fuming nitric acid (*d* 1·5). After 1 hr., ice-water (20 c.c.) was added and the mixture extracted with ether. The combined extracts were washed with water, dried ($Na_2SO_4$), and evaporated at 20°. The 2,5-*dimethyl*-3,6-*dinitroquinol diacetate* crystallised from methanol in yellow plates, decomp *ca.* 100° without melting (Found: C, 46·7; H, 3·9. $C_{12}H_{12}O_8N_2$ requires C, 46·2; H, 3·9%).

The dinitro-compound (0·26 g.) was treated with a carbonate-free solution of barium hydroxide (20 c.c., containing *ca.* 1 g. of barium hydroxide) at 20°. After 2 hr. bromine (0·2 c.c.) was added and after a further 1 hr. the mixture was acidified and the liberated carbon dioxide collected as barium carbonate (0·21 g.) and washed with carbonate-free distilled water (Found: relative molar activity × $10^{-2}$, 0·2).

The residual aqueous mixture was basified and the bromopicrin (0·30 g.) isolated by steam-distillation. Van Slyke–Folch [19] oxidation gave carbon dioxide which was collected as barium carbonate (0·09 g.) and washed with carbonate-free distilled water (Found: relative molar activity × $10^{-2}$, 27·5, ½C* requires 28·5).

*Degradation of* 2-*Methyl*-3-*oxocyclopent*-1-*enecarboxylic Acid*.—The pure acid (0·30 g.) was dissolved in Lemieux's reagent [9] (1 l.) [1 l. of reagent contained anhydrous potassium carbonate (7·5 mmoles), sodium periodate (20 mmoles) and potassium permanganate (0·3 mmole)], and kept at 20° for 4 days. The solution was acidified and continuously extracted with ether for 4 days. The ether was carefully removed through a column and the residue triturated with ether. The ethereal solution was decanted and the colourless solid residue washed with ether, to give pure succinic acid (0·09 g.), m. p. and mixed m. p. 185° (Found: relative molar activity × $10^{-2}$, 57; 1C* requires 57).

The ether washings were combined and the ether carefully removed by fractional distillation. The residue yielded acetic 4-phenylphenacyl ester (0·04 g.), m. p. and mixed m. p. 109—111° (Found: relative molar activity × $10^{-2}$, 55; 1C* requires 57).

For acknowledgments, see preceding paper.

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, December* 31*st*, 1959.]

[18] Smith and Opie, *J. Org. Chem.*, 1941, **6**, 427.
[19] See Calvin, Heidelberger, Reid, Tolbert, and Yankwich, " Isotopic Carbon," Wiley, New York, N.Y., 1949, p. 92.