Studies in Terpenoid Biosynthesis. Part XI.[1] Stereochemistry of Some Stages in Trichothecane Biosynthesis

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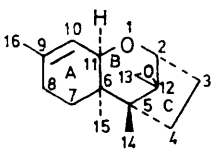
The *exo* C-4β hydrogen atom of 15-acetoxy-12,13-epoxytrichothec-9-en-3-one (5) has been shown to exchange and this has been used to show that this hydrogen atom in calonectrin is derived from a 2-*pro-R* mevalonoid hydrogen. Hydroxylation at C-3 in calonectrin biosynthesis and at C-4 in trichothecolone has been shown to proceed with retention of configuration.

THE trichothecanes are a group of fungal sesquiterpenoid metabolites possessing the common carbon skeleton (1) [2] but differing in their oxygenation pattern. Some metabolites such as trichothecin (2) [3] and trichodermol (3) [4]



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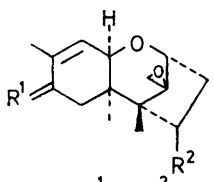
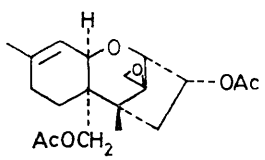


(2) R^1 = O, R^2 = O·CO·CH=CH·CH_3
(3) R^1 = H_2, R^2 = OH



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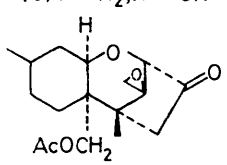


(5)

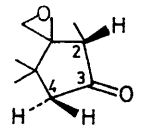
possess a β-oxygen substituent at C-4 whilst others such as calonectrin (4) [5] possess an α-oxygen substituent at C-3. It was our objective to determine the stereochemistry of the mevalonoid labels at these centres through a knowledge of the stereochemistry of enolization of ring C ketones. Bearing in mind that simple hydroxylations commonly proceed with retention of configuration, we then wished to correlate this with the known [6] chirality of mevalonoid labels in farnesyl pyrophosphate which is a precursor of this series.

Calonectrin (4) was converted into the C-3 ketone (5) by selective hydrolysis with 0·04N-aqueous methanolic sodium hydroxide and then oxidation with the silver carbonate–Celite reagent.[5] The C-4 proton resonances in the n.m.r. spectrum of this compound were distinguishable; C(4α)H τ 7·06 and C(4β)H τ 7·69 ($J_{4\alpha,4\beta}$ 19 Hz). The latter was identified by the long-range coupling (J 1 Hz) to the C-2 proton (τ 6·63) with which it possesses a W type relationship (6), and this is the '*exo*' hydrogen which would be expected [7] to exchange more rapidly with deuterium than the C-4 *endo*-proton. The *exo*-proton underwent exchange with deuterium in the presence of 0·01N-sodium hydroxide in [$^2H_6$]acetone–deuterium oxide (1 : 1). The product (*m/e* 307) lacked the resonance at τ 7·69 whilst those at τ 7·06 and 6·63 collapsed to singlets. Hence the stereochemistry of enolization at C-4 is known.

This centre is derived [8] from C-2 of mevalonate. The best time of feeding mevalonate to *Calonectria nivalis* (*Fusarium culmorum*) to obtain maximum incorporation into calonectrin (4) was found by trial experiments to be after 3 days growth. In separate experiments (2*R*)-[2-^{3}H,2-^{14}C]- and (2*S*)-[2-^{3}H,2-^{14}C]-mevalonic acid were

[1] Part X, R. Evans, J. R. Hanson, and L. J. Mulheirn, *J.C.S. Perkin I*, 1973, 753.
[2] W. O. Godtfredsen, J. F. Grove, and Ch. Tamm, *Helv. Chim. Acta*, 1967, **50**, 1666.
[3] J. Fishman, E. R. H. Jones, G. Lowe, and M. C. Whiting, *J. Chem. Soc.*, 1960, 3948.
[4] W. O. Godtfredsen and S. Vangedal, *Acta Chem. Scand.*, 1965, **19**, 1088.
[5] D. Gardner, A. T. Glen, and W. B. Turner, *J.C.S. Perkin I*, 1972, 2576.
[6] G. Popjak and J. W. Cornforth, *Biochem. J.*, 1966, **101**, 553.
[7] *Cf.* A. F. Thomas, R. A. Schneider, and J. Meinwald, *J. Amer. Chem. Soc.*, 1967, **89**, 68.
[8] E. R. H. Jones and G. Lowe, *J. Chem. Soc.*, 1960, 3959.

fed to the fungus. The calonectrin was isolated in each case and converted into the ketone (5) which was then subjected to the exchange reaction. The results are



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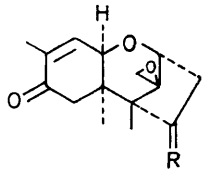


(7) R = β-OH, α-H
(8) R = O

tabulated (see Table 1) from which it may be seen that a 2-*pro*-*R*-mevalonoid hydrogen atom is located at the C-4β position.

The location of a C-2 mevalonoid hydrogen atom at C-8 in the trichothecanes was established by oxidizing calonectrin to 8-oxocalonectrin with chromium trioxide in acetic acid. The product of this oxidation showed $\lambda_{max.}$ 246 nm (ε 5·6 × 10^3). In the n.m.r. spectrum the C-13 proton resonances remained at τ 7·10 and 6·85 (*J* 4 Hz) whilst the C-10 proton resonance had shifted downfield to τ 3·43 ($J_{10,11}$ 6 Hz). The C-7 proton resonances appeared at τ 7·50 and 7·11 (*J* 15 Hz). The ^3H : ^{14}C ratio of calonectrin from the (2*S*)-[2-^{3}H,2-^{14}C]-mevalonate incubation, dropped from 7·7 : 1 to 4·8 : 1 consistent with the loss of one label.

The (2*R*)-[2-^{3}H-]- and (2*S*)-[2-^{3}H,2-^{14}H]-mevalonates were then fed to *Trichothecium roseum* and the trichothecolone (7) was isolated. A preliminary experiment was also conducted [9] with trichodermol (3) produced by *Trichoderma sporulosum* although the incorporation was low. These results are given in Table 1. The departure from whole numbers in the incorporation of [2-^{3}H]-mevalonoid labels is probably due to the reversible activity of prenyl isomerase.[10] The metabolites were oxidized with chromium trioxide to the corresponding C-4 ketones. The products from the (2*R*) feeds showed only a slight drop in activity whilst the trichothecadione (8) from the (2*S*) feed showed a loss of half the activity. Thus hydroxylation has involved the replacement of a 2-*pro*-*R* mevalonoid label. This is in accord with similar work that has been carried out [11] on the related metabolite, verrucarol.

Attempts to determine the stereochemistry of enolization at C-3 and C-7 in trichothecodione (8) and trichothecolone (7) were unsuccessful. Unfortunately trichothecodione decomposed under basic conditions whilst trichothecolone readily gave [7-$^2H_2$]trichothecolone (M^+ − H_2O, 248 for $C_{15}H_{18}D_2O_4$, no resonances at τ 7·72 and 7·09) and [7-^{2}H]isotrichothecolone (M^+ 265). Nevertheless (5*R*)-[5-^{3}H,2-^{14}C]mevalonic acid, which would be expected to introduce a label at these centres, was fed to *Trichothecium roseum* and to *Fusarium culmorum*. These results (Table 2) implied that hydroxylation at C-3 in calonectrin had replaced a 5-*pro*-*R*-mevalonoid hydrogen atom and that, from the exchange reaction, a 5-*pro*-*R*-mevalonoid hydrogen atom was located at C-7 in trichothecolone.

A number of conclusions can be drawn from these results. First a simple folding of farnesyl pyrophosphate (9) would afford the ring C labelling pattern (10). Our

TABLE 1

Incorporation of [2-^{3}H]mevalonates into the trichothecanes

	(2*R*)-[2-^{3}H,2-^{14}C]Mevalonate		(2*S*)-[2-^{3}H,2-^{14}C]Mevalonate	
	^3H : ^{14}C Ratio	Corresponding no. of labels	^3H : ^{14}C Ratio	Corresponding no. of labels
(a) Initial ratio	9·9 : 1	3·0	8·55 : 1	3·0
Calonectrin	9·03 : 1	2·75	7·7 : 1	2·70
% Incorporation	1·1		0·9	
3-Deacetylcalonectrin	8·7 : 1	2·64	7·3 : 1	2·56
15-Acetoxy-12,13-epoxytrichothec-9-en-3-one				
Before exchange	8·15 : 1	2·47	7·3 : 1	2·56
After exchange	6·3 : 1	1·90	7·1 : 1	2·50
(b) Initial ratio	6·85 : 1	3·0	5·8 : 1	3·0
Trichothecolone *	2·45 : 1	1·04	3·0 : 1	1·6
% Incorporation	0·55		0·55	
Trichothecodione	2·1 : 1	0·92	1·6 : 1	0·82
(c) Initial ratio	8·28 : 1	3·0		
Trichodermol	4·99 : 1	1·81		
% Incorporation	0·05			
Trichodermone	4·32 : 1	1·57		

* Trichothecolone is more readily purified than trichothecin.

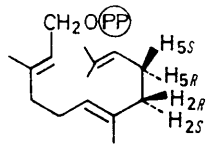
TABLE 2

Incorporation of (5*R*)-[5-^{3}H]mevalonate into the trichothecanes

	^3H : ^{14}C Ratio	Corresponding no. of labels
Initial ratio	8·4 : 1	3·0
Calonectrin	5·3 : 1	1·93
15-Acetoxy-12,13-epoxy-trichothec-9-en-3-one	5·15 : 1	1·83
Trichothecolone		
Before exchange	8·6 : 1	3·07
After exchange	6·5 : 1	2·3

[9] P. M. Adams and J. R. Hanson, *Chem. Comm.*, 1970, 1569.
[10] B. W. Agranoff, H. Eggerer, U. Henning, and F. Lynen, *J. Biol. Chem.*, 1960, **235**, 326.
[11] R. Achini, B. Muller, and Ch. Tamm, *Chem. Comm.*, 1971, 404.

results with the 2-*pro*-*R*- and 2-*pro*-*S*-labelled mevalonates in calonectrin are in accord with this. Secondly hydroxylation at C-3 and C-4 has proceeded with retention of configuration. Thirdly all three 5-*pro*-*R*-mevalonoid tritium atoms of all *trans*-farnesyl pyrophosphate are retained in the formation of the trichothecane skeleton. It has been shown [12] that one [5-$^3H_2$]-mevalonoid label is replaced by non-mevalonoid hydrogen at C-1 when all-*trans*-farnesyl pyrophosphate is converted into the 2-*cis*-isomer and into trichodiene, the hydrocarbon precursor of trichodiene. This result shows that the hydrogen atom at C-1 of farnesyl pyrophosphate which is introduced [12] on the isomerization of the C-2 double bond, is lost again during the subsequent steps, possibly during the formation of trichodiol (11).[13]



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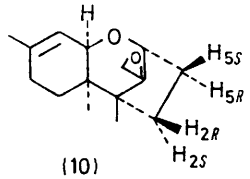

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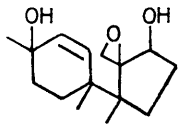

(11)

EXPERIMENTAL

General experimental details have been described previously.[14]

Deuterium Exchange Reactions.—(a) 15-Acetoxy-12,13-epoxytrichothec-9-en-3-one had m.p. 177—178° (lit.,[5] 169—171°) (Found: C, 66·2; H, 7·2. Calc. for $C_{17}H_{22}O_5$: C, 66·6; H, 7·2%). The ketone (25 mg) was shaken with [$^2H_6$]acetone (1 ml), deuterium oxide (1 ml), and 1N-sodium hydroxide (20 μl) for 18 h. The solvent was partially removed under reduced pressure and the aqueous phase extracted with chloroform. The extract was dried and the solvent was evaporated to give [4β-^{2}H]-15-acetoxy-12,13-epoxytrichothec-9-en-3-one, m.p. 177° (Found: *m/e*, 307. $C_{17}H_{21}DO_5$ requires *M*, 307). The n.m.r. spectrum lacked the resonance at τ 7·69 whilst the resonance at τ 7·1 was a broad singlet and that at 6·63 a sharp singlet.

(b) Trichothecolone (60 mg) in [$^2H_6$]acetone (3 ml) was treated with a solution of sodium (345 mg) in deuterium oxide (15 ml) at room temperature for 5 h. The solution was extracted with chloroform and the extract was washed with water and dried. The solvent was evaporated to give [7-$^2H_2$]trichothecolone which crystallized from ethyl acetate–light petroleum as needles, m.p. 183—184° (Found: $M^+ - H_2O$, *m/e* 248. $C_{15}H_{18}D_2O_4$ requires $M^+ - H_2O$, 248). The mother liquors afforded [7-^{2}H]isotrichothecolone, m.p. 185—187° (lit.,[3] 184—185°) (Found: C, 67·8; H, 7·8%; *m/e*, 265. $C_{15}H_{19}DO_4$ requires C, 68·0; H, 7·9%; *M*, 265), τ 9·10 (3H, s, 14-H), 8·95 (3H, s, 15-H), 8·18 (3H, d, *J* 1·5 Hz, 16-H), 6·33 (1H, d, *J* 6·5 Hz, 11-H), 6·06 (1H, d, *J* 4·5 Hz, 2-H), 5·91 (1H, m, 4-H), and 3·24 (1H, dd, $J_{10,11}$ 6·5, $J_{10,16}$ 1·5 Hz, 10-H).

Preparation of Calonectrin from the Labelled Mevalonic Acids.—*Calonectria nivalis* (*Fusarium culmorum*) CMI 14764, ICI culture 1731, was grown on surface culture on a Czapek–Dox medium.[5]

(a) (2R)-[2-^{3}H,2-^{14}C]*Mevalonic acid incubation.* The mevalonate (^{3}H : ^{14}C, 9·9 : 1; ^{14}C, 25 μCi) in ethanol (500 μl) was fed to five Thomson bottles of *F. culmorum* after 3 days growth. After a further 8 days, the broth was extracted with chloroform to give a gum which was purified by p.l.c. on silica in 40% ethyl acetate–light petroleum. The calonectrin (30 mg) was obtained from the band R_F 0·6. It had m.p. 84° [^{3}H : ^{14}C, 9·03 : 1; ^{14}C, 2·4 × 10^4 d.p.m. (disint. min^{-1}) mg^{-1}; 1·1% incorporation].

(b) (2S)-[2-^{3}H,2-^{14}C]*Mevalonic acid incubation.* The mevalonate (^{3}H : ^{14}C, 8·55 : 1; ^{14}C, 25 μCi) in ethanol (500 μl) was fed to five Thomson bottles of *F. culmorum* after 3 days growth. After a further 8 days, the calonectrin (60 mg) was isolated as above. It had m.p. 84° (^{3}H : ^{14}C, 7·7 : 1; ^{14}C, 8·8 × 10^3 d.p.m. mg^{-1}; 0·9% incorporation).

(c) (5R)-[5-^{3}H,2-^{14}C]*Mevalonic acid incubation.* The mevalonate (^{3}H : ^{14}C, 8·4 : 1; ^{14}C, 25 μCi) in ethanol (1 ml) was fed to two Thomson bottles of *F. culmorum* after 3 days growth. After a further 7 days, the calonectrin (20 mg) was isolated as above. It had m.p. 84° (^{3}H : ^{14}C, 5·24 : 1; ^{14}C, 2·2 × 10^4 d.p.m. mg^{-1}; 1·3% incorporation).

Degradation of Calonectrin.—(a) Calonectrin (16 mg) (^{3}H : ^{14}C, 9·03 : 1) {from the (2*R*)-[2-^{3}H,2-^{14}C]mevalonate incubation} was mixed with unlabelled material (48 mg) and dissolved in methanol (2 ml). The solution was treated with 0·1N-sodium hydroxide (1·34 ml) at 0° and left for 30 min. Water (5 ml) was then added and the solution was extracted with ether. The extract was dried and the solvent evaporated to give a gum which was crystallized from acetone–light petroleum to give 3-deacetylcalonectrin (60 mg) as prisms, m.p. 144—145° (lit.,[5] 144—145°) (^{3}H : ^{14}C, 8·7 : 1; ^{14}C, 5·0 × 10^3 d.p.m. mg^{-1}). The alcohol (58 mg) was heated under reflux in toluene (15 ml) with silver carbonate–Celite (1·5 g) for 1 h. 15-Acetoxy-12,13-epoxytrichothec-9-en-3-one (55 mg) crystallized from acetone–light petroleum as prisms, m.p. 176—178° (^{3}H : ^{14}C, 8·15 : 1; ^{14}C, 5·1 × 10^3 d.p.m. mg^{-1}). The ketone (30 mg) in aqueous acetone (10 ml) was treated with 1N-sodium hydroxide (25 μl) for 18 h. The ketone was recovered as described previously. It had m.p. 176—177° (^{3}H : ^{14}C, 6·3 : 1; ^{14}C, 5·0 × 10^3 d.p.m. mg^{-1}).

(b) Calonectrin (32 mg) (^{3}H : ^{14}C, 7·7 : 1) {from the (2*S*)-[2-^{3}H,2-^{14}C]mevalonate incubation} was mixed with unlabelled material (32 mg) and converted into 3-deacetylcalonectrin (60 mg) as described above. This had m.p. 144—145° (^{3}H : ^{14}C 7·3 : 1; ^{14}C, 4·3 × 10^3 d.p.m. mg^{-1}). 15-Acetoxy-12,13-epoxytrichothec-9-en-3-one (50 mg) was obtained as above. It had m.p. 176—178° (^{3}H : ^{14}C, 7·3 : 1; ^{14}C, 4·3 × 10^3 d.p.m. mg^{-1}). The ketone (30 mg) in aqueous acetone (10 ml) was treated with 1N-sodium hydroxide (25 μl) and set aside for 18 h. The ketone was recovered as described previously. It had m.p. 176—177°, (^{3}H : ^{14}C, 7·1 : 1; ^{14}C, 4·3 × 10^3 d.p.m. mg^{-1}).

[12] K. H. Overton and F. M. Roberts, *J.C.S. Chem. Comm.*, 1973, 378; R. Evans, J. R. Hanson, and A. M. Holtom, *ibid.*, p. 465.

[13] Y. Machida and S. Nozoe, *Tetrahedron*, 1972, **28** 5113.

[14] J. R. Hanson J. Hawker, and A. F. White, *J.C.S. Perkin I*, 1972, 1892.

(c) Calonectrin (20 mg) (^3H : ^{14}C, 5·3 : 1) {from the (5*R*)-[5-^3H,2-^{14}C]mevalonate incubation} was mixed with unlabelled material (30 mg) and converted into 3-deacetylcalonectrin (45 mg) as described above. This had m.p. 144—145° (^3H : ^{14}C, 5·24 : 1; ^{14}C, 8 × 10^3 d.p.m. mg^{-1}). The alcohol was oxidized to 15-acetoxy-12,13-epoxytrichothec-9-en-3-one (40 mg) as described above. This had m.p. 176—177° (^3H : ^{14}C, 5·15 : 1; ^{14}C, 8 × 10^3 d.p.m. mg^{-1})

3α,15-*Diacetoxy*-12,13-*epoxytrichothec*-9-*en*-8-*one*.—A solution (310 μl) of 10% chromium trioxide in 95% acetic acid was added to calonectrin (35 mg) in acetic acid (500 μl) and the mixture was stirred for 3 h at 50°. Water (2 ml) was added and the solution was extracted with chloroform. The extract was washed with aqueous sodium hydrogen carbonate, dried, and the solvent was evaporated to give a gum which was chromatographed by p.l.c. on silica in 35% ethyl acetate–light petroleum. The plate was developed 5 times, and a band at R_F 0·6 gave 3α,15-*diacetoxy*-12,13-*epoxytrichothec*-9-*en*-8-*one* (8 mg) which crystallized from ethyl acetate–light petroleum as needles, m.p. 140—141°, $[\alpha]_D^{20}$ +55° (*c* 0·15) (Found: C, 62·0; H, 6·75. $C_{19}H_{24}O_7$ requires C, 62·6; H, 6·6%), $\lambda_{max.}$ 246 nm (ε 5·6 × 10^3), $\nu_{max.}$ 1740, 1675, 1240, and 955 cm^{-1}, τ 9·17 (3H, s, 14-H), 8·15br (3H, s, 16-H), 8·03, 7·85 (each 3H, s, 3-OAc, 15-OAc), 7·50 (1H, dd, $J_{7\alpha,7\beta}$ 15, $J_{15,7\beta}$ 1 Hz, 7β-H), 7·11 (1H, d, *J* 15 Hz, 7α-H), 7·10, 6·85 (2H, ABq, *J* 4 Hz, 13-H), 6·13 (1H, d, *J* 4 Hz, 2-H), 5·90 (2H, s, 15-H), 5·52 (1H, d, *J* 6 Hz, 11-H), 4·76 (1H, m, 3-H), and 3·43 (1H, dd, $J_{10,11}$ 6, $J_{10.16}$ 1·5 Hz, 10-H).

Under similar conditions calonectrin (28·7 mg) {^3H : ^{14}C, 7·7 : 1, from the (2*S*)-[2-^3H,2-^{14}C]mevalonate incubation} mixed with unlabelled material (71·3 mg) gave 3α,15-diacetoxy-12,13-epoxytrichothec-9-en-8-one (15 mg), m.p. 140—141° (^3H : ^{14}C, 4·8 : 1; ^{14}C, 2·6 × 10^3 d.p.m. mg^{-1}).

Preparation of Trichothecolone from the Labelled Mevalonic Acids.—Trichothecium roseum (IFO 6157) was cultured as described previously.[15]

(a) (2R)-[2-^3H,2-^{14}C]*Mevalonic acid incubation*. (2*R*)-[2-^3H,2-^{14}C]Mevalonic acid (^3H : ^{14}C, 6·85 : 1; ^{14}C, 25 μCi) in ethanol (200 μl) was fed to two Thomson bottles of *Trichothecium roseum* after 3 days growth. After a further 5 days, the broth was extracted with chloroform to give trichothecin (70 mg) which crystallized from light petroleum as needles, m.p. 116—117° (lit.,[3] 117—118°) (^3H : ^{14}C, 2·60 : 1; ^{14}C, 3·7 × 10^3 d.p.m. mg^{-1}; 0·55% incorporation). The trichothecin in methanol (0·5 ml) was treated with 1N-potassium hydroxide in methanol (2·8 ml) at 0° for 2 h. The solution was diluted with water and the trichothecolone recovered in chloroform. Trichothecolone crystallized from ethyl acetate–light petroleum as needles, m.p. 183—184° (lit.,[3] 183—184°) (^3H : ^{14}C, 2·45 : 1; ^{14}C, 4·5 × 10^3 d.p.m. mg^{-1}).

(b) (2S)-[2-^3H,2-^{14}C]*Mevalonic acid incubation*. (2*S*)-[2-^3H,2-^{14}C]Mevalonic acid (^3H : ^{14}C, 5·8 : 1; ^{14}C, 25 μCi) in ethanol (200 μl) was fed to two Thomson bottles of *T. roseum* after 3 days growth. After a further 5 days the broth was extracted with chloroform and the trichothecin (70 mg) was isolated. It had m.p. 117—118° (^3H : ^{14}C, 3·07 : 1; ^{14}C, 3·7 × 10^3 d.p.m. mg^{-1}; 0·55% incorporation) and was converted into trichothecolone, m.p. 183—184° (^3H : ^{14}C, 3·01 : 1; ^{14}C, 4·5 × 10^3 d.p.m. mg^{-1}).

(c) (5R)-[5-^3H,2-^{14}C]*Mevalonic acid incubation*. (5*R*)-[5-^3H,2-^{14}C]Mevalonic acid (^3H : ^{14}C, 8·4 : 1; ^{14}C, 25 μCi) in ethanol (1·5 ml) was fed to three Thomson bottles of *T. roseum* after 3 days growth. After a further 7 days the broth was extracted with chloroform and the trichothecin (70 mg) was isolated. It had m.p. 117—118° (^3H : ^{14}C, 8·7 : 1; ^{14}C, 2·7 × 10^3 d.p.m. mg^{-1}; 0·5% incorporation) and was converted into trichothecolone, m.p. 183—184° (^3H : ^{14}C, 8·6 : 1; ^{14}C, 3·6 × 10^3 d.p.m. mg^{-1}).

Degradation of the Trichothecolone.—(a) Trichothecolone (25 mg) {^3H : ^{14}C 2·45 : 1, from the (2*R*)-[2-^3H,2-^{14}C]mevalonate incubation} was mixed with unlabelled material (25 mg) in acetone (0·5 ml). The 8N-chromium trioxide reagent (50 μl) was added and the mixture was stirred for 30 min. Methanol (1 drop) was added and the solution was diluted with water. The organic product was recovered in ethyl acetate. Trichothecodione (41 mg) crystallized from ethyl acetate–light petroleum as needles, m.p. 218—219° (lit.,[3] 218—219°) (^3H : ^{14}C, 2·1 : 1; ^{14}C, 2·2 × 10^3 d.p.m. mg^{-1}).

(b) Under similar conditions the trichothecolone (44 mg) from the (2*S*)-[2-^3H,2-^{14}C]mevalonate incubation, gave trichothecodione (36 mg), m.p. 218—219° (^3H : ^{14}C, 1·55 : 1; ^{14}C, 4·0 × 10^3 d.p.m. mg^{-1}).

(c) Trichothecolone (30 mg) {^3H : ^{14}C, 8·6 : 1, from the (5*R*)-[5-^3H,2-^{14}C]mevalonate incubation} in acetone (2 ml) was treated with a solution of sodium hydroxide (150 mg) in water (2 ml) for 36 h. The solution was diluted with water and then extracted with chloroform to afford trichothecolone, m.p. 183—184° (^3H : ^{14}C, 6·5 : 1; ^{14}C, 3·4 × 10^3 d.p.m. mg^{-1}).

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