Fusicoccin. Part IV.[1] The Structure of Fusicoccin J †

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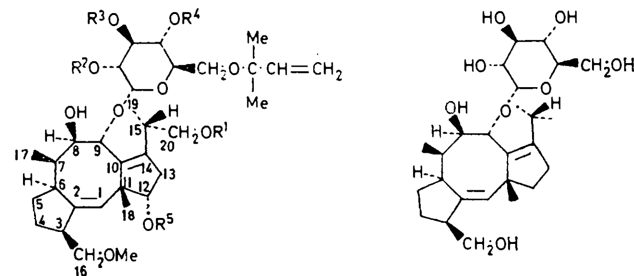
The structure (III) of fusicoccin J, a minor glucoside produced by *Fusicoccum amygdali*, has been determined by its synthesis from fusicoccin (Ia). Feeding experiments have established that fusicoccin J can act as an efficient precursor of fusicoccin. This suggests that the new fusicoccin is a late intermediate on the biosynthetic pathway.

THE fungus *Fusicoccum amygdali* Del., a wilting pathogen of peach and almond trees in Southern Europe, when grown in submerged culture, produces a number of structurally related phytotoxic metabolites.[2] The structure of the parent member of this family, fusicoccin (Ia), which is responsible for most of the phytotoxic action of the culture filtrates, has been established independently by Italian workers [3] and by us.[4,5]

A number of related fusicoccin derivatives (Ib—h) have also been isolated from the culture filtrates and their structures determined by spectroscopic and other methods.[6,7] These have been shown to form when fusicoccin is incubated, at ambient temperature, at the pH of the culture filtrate during the production phase and probably arise non-enzymically during the fermentations.

In addition to the above metabolites, we have detected several other compounds in the culture filtrate, which show colour reactions similar to those of fusicoccin and incorporate activity from [2-^{14}C]mevalonic acid. The structure of one of these compounds, fusicoccin H (II), was recently established by us,[1] by degradative studies and chemical correlation with fusicoccin (Ia). We now present evidence that establishes the structure (III) for another of these metabolites, which we have called fusicoccin J for convenience, and show that the compound can serve as a biogenetic precursor of fusicoccin.

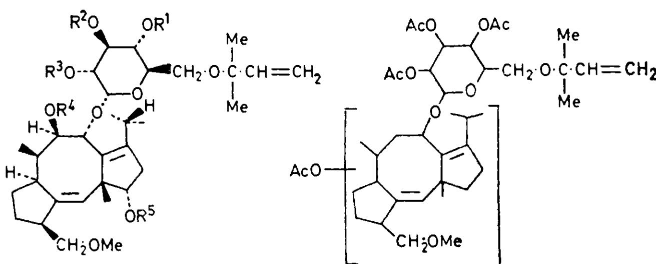
At the outset of this work only 1 mg of fusicoccin J penta-acetate (V), obtained by chromatographic separation of the acetylated culture fluid extract, was available. In its mass spectrum, the highest mass peak was observed at *m/e* 732, which, as became apparent later, results from the loss of acetic acid from the molecular ion. Although this *m/e* value is now known to be correct for $M^+ - CH_2CO_2H$, it was, at the time, only provisionally assigned because of the difficulty in counting across the void region at the higher end of the spectrum. The spectrum clearly showed the presence of four acetoxy-groups and the glycopyranose unit. Three of the



(I) (II)

a; $R^1 = R^3 = Ac$, $R^2 = R^4 = R^5 = H$
b; $R^1 = R^2 = Ac$, $R^3 = R^4 = R^5 = H$
c; $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = H$
d; $R^1 = Ac$, $R^{2-5} = H$
e; $R^{1-5} = H$
f; $R^2 = Ac$, $R^1, R^{3-5} = H$
g; $R^3 = Ac$, $R^1 = R^2 = R^4 = R^5 = H$
h; $R^4 = Ac$, $R^{1-3}, R^5 = H$
i; $R^{1-5} = Ac$
j; $R^1 = R^5 = Ac$, $R^2 = R^3 = R^4 = H$
k; $R^1 = R^5 = Ac$, $R^2 = R^3 = R^4 = Bz$
l; $R^1 = Tps$, $R^{2-5} = H$
m; $R^1 = R^3 = Ac$, $R^2 = R^4 = H$, $R^5 = Tps$
n; $R^1 = R^5 = Tps$, $R^2 = R^3 = R^4 = H$

Tps = 2,4,6-tri-isopropylphenylsulphonyl



(III) $R^{1-5} = H$
(IV) $R^{1-3} = R^5 = Ac$, $R^4 = H$
(V) $R^{1-5} = Ac$

(VI)

acetoxy-groups were carried by the glycopyranose unit, as indicated by the characteristic glycopyranose triacetate fragmentation pattern: *m/e* 289, 229, 169, and 109.[5] The fourth acetoxy-group was carried by the ion

† The previously isolated fusicoccins have been given the letters A—H for convenience. We have omitted the use of the letters I in the series to avoid confusion with formulae numbers and have therefore called the new isolate fusicoccin J.

[1] Part III, K. D. Barrow, D. H. R. Barton, Sir Ernst Chain, U.F.W. Ohnsorge, and R. P. Sharma, *J. Chem. Soc.* (*C*), 1973, 1590.

[2] A. Ballio, E. B. Chain, P. de Leo, B. F. Erlanger, M. Mauri, and A. Tonolo, *Nature*, 1964, **203**, 297.

[3] A. Ballio, M. Bufani, C. G. Casinovi, S. Cerrini, W. Fedeli, R. Pellicciari, B. Santurbano, and A. Vaciago, *Experientia*, 1968, **24**, 631.

[4] (*a*) K. D. Barrow, D. H. R. Barton, E. B. Chain, U. F. W. Ohnsorge, and R. Thomas, *Chem. Comm.*, 1968, 1198; (*b*) E. Hough, M. B. Hursthouse, S. Neidle, and D. Rogers, *Chem. Comm.*, 1968, 1197; (*c*) K. D. Barrow, D. H. R. Barton, Sir Ernst Chain, U. F. W. Ohnsorge, and R. Thomas, *J. Chem. Soc.* (*C*), 1971, 1265.

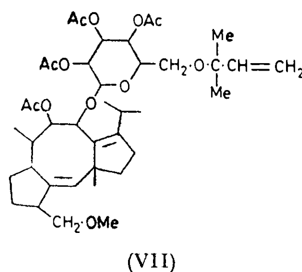
[5] K. D. Barrow, D. H. R. Barton, Sir Ernst Chain, C. Conlay, T. C. Smale, R. Thomas, and E. S. Waight, *J. Chem. Soc.* (*C*), 1971, 1259.

[6] A. Ballio, C. G. Casinovi, G. Randazzo, and C. Rossi, *Experientia*, 1970, **26**, 349.

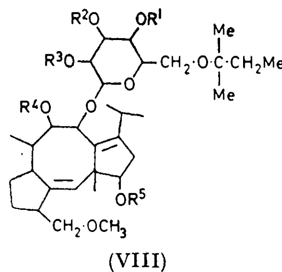
[7] A. Ballio, C. G. Casinovi, M. Framon-dino, G. Grandolini, F. Merichini, G. Randazzo, and C. Rossi, *Experientia*, 1972, **28**, 126.

at *m*/*e* 374 which fragmented into that at *m*/*e* 314 (374 − CH_3CO_2H). The base peak at *m*/*e* 69 indicated the presence of an isopentenyl unit. The mass spectrum of the product obtained from catalytic hydrogenation of the acetylated isolate showed the highest mass peak at two mass units higher than that recorded for the unhydrogenated derivative, with the base peak at *m*/*e* 71 (C_5 unit).

It was at first assumed that the acetylated isolate had the same carbon skeleton as fusicoccin and contained

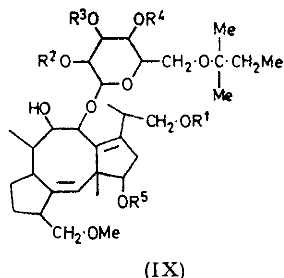


(VII)

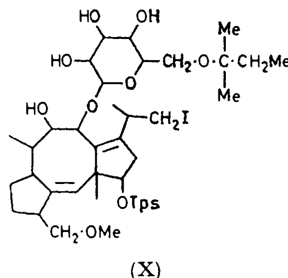


(VIII)

a; R^{1-5} = H
b; R^{1-5} = Ac



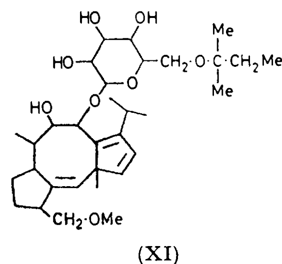
(IX)



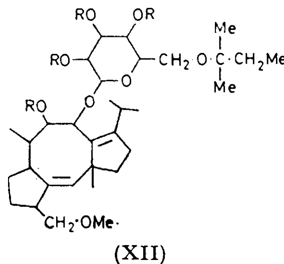
(X)

a; R^1 = R^5 = Ac, R^{2-4} = H
b, R^1 = R^5 = Ac, R^{2-4} = Bz
c; R^{1-5} = H
d; R^1 = R^5 = Ac, R^{2-4} = Thp
e; R^1 = R^5 = H, R^{2-4} = Thp
f; R^1 = R^5 = Tps, R^{2-4} = Thp
g; R^1 = R^5 = Tps, R^{2-4} = H
h; R^1 = Tps, R^{2-4} = R^5 = H

Thp = tetrahydropyran-2-yl



(XI)



(XII)

a; R = H
b; R = Ac

only four acetoxy-groups. If the positions of the glycosidic linkage and methoxy-substituent (inferred from the mass spectra) and the sites of unsaturation were also the same, then the partial structure (VI) would be possible. Although this part structure would represent 734 mass units, two units higher than that recorded for the acetylated isolate, it was felt at the time that the discrepancy could be accounted for by the aforementioned difficulty in accurately assigning the *m*/*e* values to the highest peaks in the mass spectra of the two derivatives. On biogenetic grounds and in view of the oxygenation pattern of fusicoccin H (II), it was reasoned that structure (VII) was plausible. The synthesis of the dihydro-derivative (XIIb), containing the less acid-sensitive dihydro-C_5 unit,[5] was therefore attempted from the readily available fusicoccin (Ia).

Treatment of the penta-acetate (Ii) [obtained from (Ia) by acetylation [5]] with aqueous methanolic sodium hydrogen carbonate gave a mixture of products from which the diacetate (Ij), $C_{36}H_{56}O_{12}$, was isolated. In its mass spectrum this showed a molecular ion at *m*/*e* 680, and a peak at *m*/*e* 450 was attributed to the diacetyl aglycone ion, which successively fragmented to ions at *m*/*e* 390 and 330 by loss of acetic acid. Our aim was to synthesise the bis-2,4,6-tri-isopropylbenzenesulphonate (IXg) from (Ij). It appeared that the tribenzoate (IXb) might undergo selective deacetylation and this was therefore prepared by treatment of the dihydro-acetate (IXa), obtained from (Ij) by catalytic hydrogenation,[5] with benzoyl chloride in pyridine. However, attempted selective deacetylation with sodium carbonate and piperidine was unsuccessful.

A successful approach was made as follows. Treatment of the dihydro-diacetate (IXa) with dihydropyran in toluene containing concentrated hydrochloric acid at 80° gave the tristetrahydropyranyl ether (IXd) which was deacetylated with aqueous methanolic sodium hydroxide to give (IXe). Reaction of (IXe) with several equivalents of 2,4,6-tri-isopropylbenzenesulphonyl chloride in pyridine at room temperature for 104 h, followed by removal of the tetrahydropyranyl (Thp) groups with aqueous hydrochloric acid afforded a mixture of mono- and di-Tps derivatives in the ratio 1 : 5. The di-Tps derivative (IXg) was isolated by preparative t.l.c. and was characterised by spectra only, $\lambda_{max.}$ 281 nm (ε 3700), τ 2·82 and 2·92 (each 2H, s, aromatic). Treatment of (IXg) with sodium iodide in acetone afforded the mono-Tps iodide (X), $C_{47}H_{73}IO_{11}S$, $\lambda_{max.}$ 281 nm (ε 1900), τ 2·90 (2H, s, aromatic).

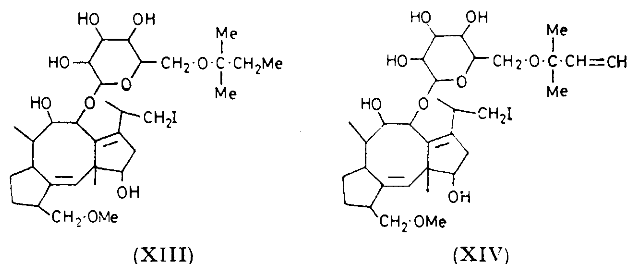
In an alternative synthesis of the mono-Tps iodide (X), fusicoccin (Ia) was treated with 2,4,6-tri-isopropylbenzenesulphonyl chloride in pyridine for 3 months to give the mono-Tps diacetate (Im), $C_{51}H_{78}O_{14}S$. In its n.m.r. spectrum (Im) showed a two-proton singlet at τ 2·88 indicating that only one hydroxy-group had been substituted. Deacetylation of (Im) followed by retreatment with 2,4,6-tri-isopropylbenzenesulphonyl chloride in pyridine gave the bis-sulphonate (In). The latter was hydrogenated over Adams catalyst in ethyl acetate to give the di-Tps dihydro-derivative (IXg), characterised by spectra only. Reaction of (IXg) with sodium iodide in acetone afforded the mono-Tps iodide (X), identical with that produced by the aforementioned route. This confirmed the structural assignment of (X). Reaction of (X) with sodium borohydride in dimethyl sulphoxide at 140° for 3 days generated the desired isopropyl system from the primary iodide group and led to

the non-reductive elimination of the Tps function, as expected,[1] furnishing the cyclopentadiene (XI), $C_{37}H_{52}O_8$, $\lambda_{max.}$ 270 nm (ε 1200). The abnormally low intensity of u.v. absorption has precedent.[1]

On catalytic hydrogenation over 10% palladium–strontium carbonate, (XI) absorbed 1 mol. equiv. of hydrogen to give a single product (XIIa) which had lost the cyclopentadiene absorption at $\lambda_{max.}$ 270 nm in the u.v. spectrum. The compound had also lost the two olefinic cyclopentadiene signals which were observed as a singlet at τ 3·86 in the n.m.r. spectrum of (XI). The product (XIIa) was characterised by acetylation to the tetra-acetate (XIIb). The mass spectrum of (XIIb) showed the molecular ion at *m/e* 734 ($C_{40}H_{62}O_{12}$) and was clearly different from that of the dihydro-acetylated derivative of the new isolate. The t.l.c. mobilities of the two compounds were also different. Furthermore, t.l.c. of acetylated and hydrogenated extracts of cultures of the fungus showed no sign of a compound identical with (XIIb).

While the above synthesis was being conducted, more substantial amounts of the new metabolite were isolated and more detailed physical data obtained. The penta-acetate (V) was isolated in slightly impure form by column and preparative layer chromatography. Deacetylation of (V) with aqueous methanolic sodium hydroxide followed by preparative t.l.c. on silver nitrate–impregnated Kieselgel GF_{254} afforded pure (III). T.l.c. comparisons indicated that (III), rather than an acetylated derivative, was the naturally occurring metabolite. The molecular formula of fusicoccin J was established as $C_{32}H_{52}O_9$. In its mass spectrum the molecular ion was observed at *m/e* 580. The aglycone ion (*m/e* 350) appeared at 16 mass units lower than that in the mass spectrum of deacetylfusicoccin (Ie).[4] The same ion was also seen in the mass spectrum of dihydrofusicoccin J (VIIIa), $C_{32}H_{54}O_9$, obtained from (III) by catalytic hydrogenation. Fusicoccin J therefore contained one less hydroxy-group than (Ie). The n.m.r. spectrum of (III) showed the presence of a methoxy-group (τ 6·70), three secondary methyls [τ 8·96 (d, *J* 6 Hz), 9·04 (d, *J* 6 Hz), and 9·17 (d, *J* 7 Hz)] and three tertiary methyls [τ 8·82 (s) and 8·76 (6H, s)] and the characteristic one-proton double doublet, centered at τ 4·27, due to the proton X of the ABX system of the isopentyl unit.[4]

On the basis of these spectral observations structure (III) seemed most likely for fusicoccin J. The synthesis of dihydrofusicoccin J (VIIIa), was therefore undertaken. Reaction of the tri-Thp ether (IXe), prepared as described earlier, with 2,4,6-tri-isopropylbenzenesulphonyl chloride in pyridine for a shorter reaction period than for the preparation of (IXf), followed by acidic treatment, afforded mainly the monosulphonate (IXh), $C_{47}H_{76}SO_{12}$, $\lambda_{max.}$ 281 nm (ε 2400). The n.m.r. spectrum showed the signal for the two aromatic protons as a singlet at τ 2·86. Reaction of (IXh) with sodium iodide in acetone gave the iodide (XIII), $C_{32}H_{51}IO_9$, which gave a positive Beilstein test. Reduction of (XIII) with sodium borohydride in dimethyl sulphoxide gave (VIIIa), $C_{32}H_{54}O_9$, whose mass spectrum and chromatographic properties were identical with those of dihydrofusicoccin J. Acetylation of (VIIIa) at 80° gave the penta-acetate

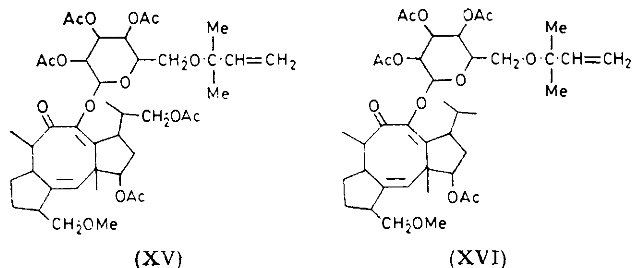


(VIIIb), $C_{42}H_{64}O_{14}$ which was also chromatographically homogeneous with the dihydro-acetylated derivative of the isolate obtained at the beginning of this work. The mass spectrum of the latter was identical with that of (VIIIb) except that the spectrum of (VIIIb) showed a very weak molecular ion at *m/e* 794.

Although the dihydrofusicoccin (VIIIa) formed from the new metabolite crystallised, the synthetic compound could not be obtained in crystalline form. A good comparison could not therefore be made. Despite the risk of losing the isopentenyl unit during acidic treatment, the synthesis of fusicoccin J was successfully achieved from (Ia). Treatment of (Ia) successively with (i) dihydropyran and hydrochloric acid in toluene, (ii) sodium hydroxide, (iii) 2,4,6-tri-isopropylbenzenesulphonyl chloride in pyridine, and (iv) aqueous ethanolic hydrochloric acid, afforded the monosulphonate (II), $C_{47}H_{74}SO_{12}$, $\lambda_{max.}$ 281 nm (ε 1900). The two aromatic protons in the n.m.r. spectrum gave rise to a singlet at τ 2·85, and the one-proton double doublet at τ 4·20 (*J* 10 and 18 Hz) confirmed that the isopentenyl unit had survived the acidic treatment. Reaction of (II) with sodium iodide in acetone containing triethylamine gave the iodide (XIV), $C_{32}H_{49}IO_9$, $\lambda_{max.}$ 224 nm (ε 7400) and 259 nm (1800). In the absence of triethylamine, lower yields of (XIV) were obtained, presumably owing to traces of acidity in the sodium iodide. Reduction of (XIV) with sodium borohydride in dimethyl sulphoxide for 20 min, monitored by t.l.c., furnished (III), which was separated from unchanged (XIV) by preparative t.l.c. Use of a longer reaction time generated an uncharacterised compound and gave poor yields of (III). The i.r., n.m.r., and mass spectra and optical rotations of synthetic and natural (III) were identical. A mixed m.p. showed no depression. This correlation establishes the constitution and absolute configuration of fusicoccin J.

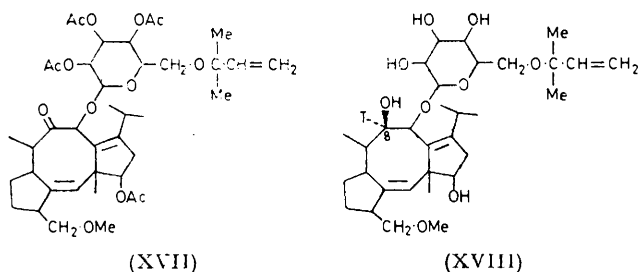
Our attention was next turned to the role of fusicoccin J as a possible intermediate in fusicoccin biosynthesis. In earlier studies[1] fusicoccin H had been successfully labelled at C-8 with tritium, and the resulting label in the biosynthesised fusicoccin had been removed by oxidation of its triacetate (Ii) to the αβ-unsaturated ketone (XV). We employed the same labelling method. Acetylation of fusicoccin J (III) at 2° afforded the tetra-acetate (IV), which was characterised by spectral data only. The n.m.r. spectrum showed 4 acetate signals and the i.r.

spectrum confirmed that the secondary hydroxy-group at C-8 had not been acetylated: $\nu_{max.}$ 3570 cm^{-1} (OH). To avoid isomerising the double bond to give the αβ-unsaturated ketone (XVI), the tetra-acetate (IV) was



(XV) (XVI)

treated with Jones reagent [8] at −5° for only 2 min. The product showed only end absorption in the u.v. spectrum, and presumably consisted of a mixture of unchanged (IV) and the desired ketone (XVII). This mixture was



(XVII) (XVIII)

reduced with lithium borotriti-ide prepared from lithium borohydride and tritiated water.[9] It was assumed from a similar experiment in the fusicoccin H series [1] that the reduction product had the correct stereochemistry at C-8 (β-OH). The reduction product was deacetylated with aqueous methanolic sodium hydroxide to afford [8-^{3}H]fusicoccin J (XVIII), which crystallised without further purification, with a specific activity of $9{\cdot}69 \times 10^5$ disint. min^{-1} mg^{-1}. Two feeding experiments, conducted in parallel, showed incorporations of 20·1 and 20·6% into fusicoccin. The labelled fusicoccin (Ia) was converted into its triacetate (Ii), oxidation of which gave the αβ-unsaturated ketone (XV) with a loss of 89% of the label.

These results demonstrate that fusicoccin J can act as an efficient biogenetic precursor of fusicoccin. Comparison of the incorporation efficiencies of fusicoccin J (*ca.* 20%) with those of fusicoccin H (*ca.* 2%) into fusicoccin suggest that the former is a later intermediate on the biosynthetic pathway to fusicoccin, as expected. The final stages in the biosynthesis must therefore involve the specific hydroxylation of C-20 and the acetylation of the primary hydroxy-group so formed, as well as acetylation in the glucoside residue.

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage apparatus. I.r. spectra were recorded either for solutions in chloroform or for Nujol mulls on a Unicam SP 200 spectrophotometer. U.v. spectra were recorded on a Unicam SP 800 spectrometer. N.m.r. spectra were recorded for solutions in deuteriochloroform on a Varian HA-100 spectrometer. Mass spectra were taken at 70 eV on an A.E.I. MS9 spectrometer. Rotations were measured for solutions in chloroform on a Perkin-Elmer 141 polarimeter. Radioactivity measurements were carried out with a Beckman LS-200B liquid scintillation counter by use of a liquid scintillator comprising butyl PBD (6 g) and naphthalene (50 g) in toluene (1 l). T.l.c. and preparative layer chromatography (p.l.c.) were performed on Kieselgel GF_{254}, activated at 110° for $1\frac{1}{2}$ h.

Isolation of Fusicoccin J Penta-acetate (V).—A chloroform extract of the culture filtrate of *Fusicoccum amygdali* Del. was concentrated and fractionated on a silica gel MFC column. Elution with chloroform–propan-2-ol (95 : 5 v/v) afforded fractions rich in fusicoccin (Ia). These were combined and the fusicoccin was crystallised. A portion of the mother liquor concentrate (96 mg) was acetylated with acetic anhydride in pyridine at 85° for 24 h. From the multicomponent product, an oily compound (1 mg), later identified as fusicoccin J penta-acetate (V) was isolated by p.l.c. [elution with ethyl acetate–light petroleum (b.p. 40—60°) (3 : 7 v/v)]. The mass spectrum showed the highest recorded peak at *m/e* 732 (16%) with significant peaks at *m/e* 672 (4%), 663 (1), 603 (1), 442 (8), 417 (5), 374 (19), 373 (18), 357 (21), 314 (33), 313 (33), 289 (99), 229 (80), 169 (74), 109 (53), and 69 (100). Hydrogenation of the new fusicoccin penta-acetate (V) (1 mg) in ethyl acetate over Adams catalyst gave the dihydro-derivative (VIIIb). The mass spectrum showed the highest peak at *m/e* 734 with other peaks at *m/e* 674, 663, 603, 444, 417, 374, 373, 357, 314, 289, 229, 169, 109, and 71.

Isolation of Fusicoccin J (III).—The mother liquor concentrate (26 g) obtained from the crystallisation of fusicoccin (Ia) from chromatography fractions rich in (III) was acetylated under nitrogen with acetic anhydride in pyridine at 70° for 2 days. Fractionation of the acetylated material on a column of silica gel MFC (1150 g) [light petroleum (b.p. 40—60°)–ethyl acetate (7 : 3 v/v) as eluant] afforded fractions rich in fusicoccin J penta-acetate, which were further purified by p.l.c. (same solvent mixture). The product (610 mg) resisted all attempts at crystallisation and was deacetylated with aqueous 2N-sodium hydroxide (4 ml) in methanol (20 ml) at 23° overnight. The mixture was diluted with water and extracted with ether (3 × 50 ml). The combined extracts were washed with water, dried, and evaporated. The residue was purified by p.l.c. on Kieselgel GF_{254} impregnated with silver nitrate (5%) [chloroform–propan-2-ol (9 : 1 v/v) as eluant]. The band at R_F *ca.* 0·4 afforded *fusicoccin J* (III) which crystallised from ether as needles (300 mg), m.p. 194—203° (decomp.) (204—206° under nitrogen in a sealed tube); $[\alpha]_D$ +24° (*c* 0·54); $\nu_{max.}$ (KBr) 3420 (OH), 1645, 1460, 1385, 1350, 1275, 1240, and 1150 cm^{-1}; τ 4·27 (1H, dd, *J* 10 and 18 Hz), 6·70 (3H, s), 8·76 (6H, s), 8·82 (3H, s), 8·96 (3H, d, *J* 6 Hz), 9·04 (3H, d, *J* 6 Hz), and 9·17 (3H, d, *J* 7 Hz); *m/e* 580 (M^+, 0·5%), 350 (15), 332 (26), 259 (20), 207 (15), 151 (23), 109 (38), and 69 (100) (Found: C, 65·9; H, 9·1. $C_{32}H_{52}O_9$ requires C, 66·2; H, 9·0%).

Note added 14th November, 1974. The structures assigned to fusicoccins H and J have been independently established by Ballio and his collaborators.[10,11]

[8] A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *J. Chem. Soc.*, 1953, 2548.
[9] R. H. Cornforth, *Tetrahedron*, 1970, **26**, 4635.
[10] A. Ballio, C. G. Casinovi, M. Framondino, G. Grandolini, G. Randazzo, and C. Rossi, *Experientia*, 1972, **28**, 1150.
[11] A. Ballio, C. G. Casinovi, V. D'Alessio, G. Grandolini, G. Randazzo, and C. Rossi, *Experientia*, 1974, **30**, 844.

Dihydrofusicoccin J (VIIIa).—Fusicoccin J (III) (50 mg), which was isolated from the culture extracts, was hydrogenated (1 mol. equiv. uptake) over Adams catalyst to give dihydrofusicoccin J (VIIIa) (50 mg), which crystallised from ether–light petroleum (b.p. 40—60°) as needles, m.p. 106—108°, $[\alpha]_D^{21}$ +22° (*c* 0·9); $\nu_{max.}$ 3500 (OH), 1475, 1400, 1380, and 1055 cm^{-1}; *m/e* 582 (1%, M^+), 350 (23), 332 (29), 314 (9), 259 (10), 207 (19), 151 (28), 109 (31), and 71 (100).

The Diacetate (Ij).—To a solution of tri-*O*-acetylfusicoccin (Ii) (602 mg) in methanol (75 ml) was added aqueous 0·2M-sodium carbonate (15 ml) and the solution was kept at 35° during 1½ h. The mixture was diluted with water (250 ml) and extracted with ether (3 × 50 ml). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by p.l.c. with chloroform–propan-2-ol (95 : 5) as eluant to give the *diacetate* (Ij) (210 mg), which crystallised as needles from diethyl ether, m.p. 99—100°; $[\alpha]_D^{20}$ +31° (*c* 0·3); $\nu_{max.}$ 3475, 2970, 1730, 1380, 1245, and 1050 cm^{-1}; τ 4·24 (1H, dd, *J* 10 and 18 Hz), 6·70 (3H, s), 7·93 (3H, s), 8·05 (3H, s), 8·77 (6H, s), 8·80 (3H, s), 8·92 (3H, d, *J* 6·5 Hz), and 9·15 (3H, d, *J* 7 Hz); *m/e* 680 (M^+), 450, 390, 330, and 69 (Found: C, 63·3; H, 8·2. $C_{36}H_{56}O_{12}$ requires C, 63·5; H, 8·3%).

The Tribenzoates (Ik) *and* (IXb).—The diacetate (Ij) (68 mg) in pyridine (3 ml) was treated with benzoyl chloride (500 mg) at room temperature overnight. After the usual work-up, the product was purified by p.l.c. [chloroform–propan-2-ol (97·5 : 2·5) as eluant] to give the oily tribenzoate (Ik), $[\alpha]_D^{22}$ +36° (*c* 0·5); $\nu_{max.}$ 3510 and 1730 cm^{-1}; $\lambda_{max.}$ 231 (ε 54,400), 275 (5700), and 282 (4500); τ 2·16 (6H, m), 2·72 (9H, m), 4·20 (1H, dd, *J* 10 and 18 Hz), 6·71 (3H, s), 7·93 (3H, s), 8·04 (3H, s), 8·73 (3H, s), 9·03 (6H, s), 9·20 (3H, d, *J* 6·5 Hz), and 9·25 (3H, d, *J* 6·5 Hz).

When the experiment was repeated on the dihydro-diacetate (IXa) the oily tribenzoate (IXb) was obtained (Found: C, 68·7; H, 7·0. Calc. for $C_{57}H_{68}O_{15}$: C, 68·9; H, 6·9%).

The Dihydro-diacetate (IXa).—The diacetate (Ij) (575 mg) in ethyl acetate was hydrogenated over Adams catalyst, at 22° and atmospheric pressure to give the *dihydro-diacetate* (IXa) (480 mg), which crystallised as needles from ether, m.p. 75°; $[\alpha]_D$ +23° (*c* 0·6); $\nu_{max.}$ 3450 and 1730 cm^{-1}; τ 6·71 (3H, s), 7·94 (3H, s), 8·06 (3H, s), 8·78 (3H, s), 8·91 (6H, s), 8·93 (3H, d, *J* 5 Hz), 9·14 (3H, d, *J* 7 Hz), and 9·18 (3H, d, *J* 7 Hz); *m/e* 682 (M^+), 450, 390, 330, and 71 (Found: C, 63·4; H, 8·3. $C_{36}H_{58}O_{12}$ requires C, 63·5; H, 8·3%).

Deacetyldihydrodi-Tps-fusicoccin (IXg).—A solution of the dihydro-diacetate (IXa) (297 mg), concentrated hydrochloric acid (1 drop), and dihydropyran (10 ml) in toluene was stirred, under nitrogen, at 80° overnight. The mixture was evaporated to dryness to give the oily tri-Thp ether (IXd). The latter was deacetylated with aqueous 2N-sodium hydroxide (2 ml) in methanol (10 ml) at 23° overnight. The mixture was diluted with water (50 ml) and extracted with ether (3 × 15 ml). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give the deacetylated tri-Thp ether (IXe). To a solution of (IXe) in pyridine was added 2,4,6-tri-isopropylbenzenesulphonyl chloride (2·1 g) and the mixture was kept at 23° for 104 h. Excess of ethanol was added and the solvents were evaporated off under reduced pressure to give an oily residue. The residue was dissolved in ethanol (75 ml) and treated with 2·4N-hydrochloric acid at 30° during 16 h. The mixture was diluted with water and extracted with ether (3 × 30 ml). The combined extracts were washed with aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated. The residue contained both the mono- and di-Tps derivatives. P.l.c. [ethyl acetate–light petroleum (b.p. 40—60°) (1 : 5) followed by re-elution with chloroform–propan-2-ol (95 : 5)] afforded the oily deacetyldihydrodi-Tps-fusicoccin (IXg) (115 mg), $[\alpha]_D^{21}$ +12° (*c* 0·9); $\nu_{max.}$ 3530, 3000, 1605, 1475, and 1440 cm^{-1}; $\lambda_{max.}$ 281 nm (ε 3700); τ 2·86 (2H, s), 2·92 (2H, s), 6·75 (3H, s), 8·73 (6H, s), 8·77 (18H, d, *J* 7 Hz), 8·78 (3H, d, *J* 7 Hz), 8·82 (18H, d, *J* 7 Hz), 8·86 (3H, s), 9·02 (3H, d, *J* 7 Hz), and 9·14 (3H, t, *J* 7 Hz).

The Mono-Tps Iodide (X).—The di-Tps derivative (IXg) (87 mg) and sodium iodide (1·5 g) were heated under reflux in acetone (15 ml), with stirring, for 16 h. The mixture was diluted with water and extracted with ether; the extracts were washed with water and dried. Evaporation furnished a residue which was purified by p.l.c. [chloroform–propan-2-ol (95 : 5)] to give the iodo-Tps compound (X) (44 mg), which crystallised as needles from ether–petroleum (b.p. 40—60°); m.p. 102—103°; $[\alpha]_D^{18}$ −49° (*c* 0·9); $\nu_{max.}$ 3450, 1605, 1475, and 1440 cm^{-1}; $\lambda_{max.}$ 281 nm (ε 1900); τ 2·90 (2H, s), 6·74 (3H, s), 8·74 (6H, s), 8·77 (12H, d, *J* 7 Hz), 8·84 (6H, d, *J* 7 Hz), 8·87 (3H, s), 8·98 (3H, d, *J* 7 Hz), 9·16 (3H, t, *J* 7 Hz), and 9·22 (3H, d, *J* 6·5 Hz).

Alternative Synthesis of the Mono-Tps Iodide (X).—(i) *The mono-Tps diacetate* (Im). A mixture of fusicoccin (1 g) and 2,4,6-tri-isopropylbenzenesulphonyl chloride (1·5 g) in pyridine was stirred at room temperature during 3 months. Excess of ethanol was added and the solvents were removed *in vacuo*. The residue was purified by p.l.c. [chloroform–propan-2-ol (*ca.* 95 : 5)] to give the *mono-Tps diacetate* (Im), which crystallised as needles (891 mg) from methanol, m.p. 92—93°; $[\alpha]_D^{22}$ +8° (*c* 0·9); $\nu_{max.}$ 3500, 1725, 1650, and 1605 cm^{-1}; $\lambda_{max.}$ 281 nm (ε 2500); τ 2·88 (2H, s), 4·22 (1H, dd, *J* 10 and 18 Hz), 6·73 (3H, s), 7·90 (3H, s), 7·96 (3H, s), 8·73 (9H, s), 8·78 (18H, d, *J* 7 Hz), 8·98 (3H, d, *J* 6·5 Hz), and 9·21 (3H, d, *J* 7 Hz) (Found: C, 64·8; H, 8·3; S, 3·3. $C_{51}H_{78}O_{14}S$ requires C, 64·5; H, 8·2; S, 3·4%).

(ii) *The mono-Tps iodide* (X). The mono-Tps diacetate (Im) (450 mg) was deacetylated with aqueous 2N-sodium hydroxide–methanol (1 : 4 v/v) at room temperature, overnight. Dilution with water, extraction with ether (3 × 50 ml), and evaporation of the combined extracts gave the corresponding deacetyl compound. The latter was treated with 2,4,6-tri-isopropylbenzenesulphonyl chloride (1 g) in pyridine at 24° for 6 days. After the usual work-up the product was purified by p.l.c. [chloroform–propan-2-ol (95 : 5)] to give the bis-sulphonate (In), which was not characterised. Hydrogenation of (In) (350 mg) in ethyl acetate over Adams catalyst afforded the oily di-Tps derivative (IXg) (243 mg) as the sole product, $[\alpha]_D^{20}$ +21° (*c* 0·2); $\lambda_{max.}$ 281 nm (ε 4800); τ 2·77 (2H, s) and 2·90 (2H, s). The di-Tps derivative (IXg) (124 mg) was treated with sodium iodide (1 g) in acetone (19 ml) under reflux for 18 h. The usual work-up gave the mono-Tps iodide (X) (87 mg), m.p. 101—103°, identical with (X) prepared as described above.

Reduction of the Iodo-Tps Derivative (X) *with Borohydride; the Cyclopentadiene* (XI).—The iodo-Tps derivative (X) (140 mg) in dimethyl sulphoxide (20 ml) was stirred with sodium borohydride (1 g) at 140° during 3 days. The mixture was cooled, added to water, and extracted with ether (3 × 50 ml). The combined extracts were washed with water, dried, and evaporated under reduced pressure. The residue was purified by p.l.c. [chloroform–propan-2-ol (95 : 5)], affording the oily *cyclopentadiene* (XI), $[\alpha]_D^{20}$ +53° (*c* 0·1); $\nu_{max.}$ 3420,

1705, and 1470 cm^{-1}; $\lambda_{max.}$ 270 nm (ε 1200); τ 3·86 (2H, s), 6·70 (3H, s), 8·76 (6H, s), 8·92 (6H, d, *J* 6·5 Hz), 9·10 (3H, d, *J* 6·5 Hz), and 9·14 (3H, t, *J* 7 Hz); *m/e* 564 (M^+) (Found: C, 67·9; H, 9·5. $C_{32}H_{52}O_8$ requires C, 68·0; H, 9·3%).

Hydrogenation of the Cyclopentadiene (XI).—The cyclopentadiene (XI) (14 mg) in ethyl acetate (2 ml) was hydrogenated (1 mol. equiv. uptake) over 10% palladium–strontium carbonate (10 mg) at 22° and atmospheric pressure to give a single, oily product (14 mg). The latter showed only end absorption in the u.v. spectrum and the two olefinic cyclopentadiene proton signals at τ 3·86 in its n.m.r. spectrum. This dihydro-compound (14 mg) in pyridine (0·3 ml) and acetic anhydride (0·5 ml) was set aside at 22° for 4 days. The mixture was worked up as usual. The product was purified by p.l.c. [light petroleum (b.p. 40—60°)–ethyl acetate (7 : 3)] to give the oily tetra-acetate (XIIb) (15 mg); *m/e* 734 (3%) (M^+, $C_{40}H_{62}O_{12}$), 378 (12), 360 (8), 359 (11), 358 (11), 315 (17), 300 (28), 289 (44), 256 (22), 229 (28), 169 (28), 151 (28), 109 (26), and 71 (100). T.l.c. comparison of (XIIb) with the acetylated isolate showed that the two compounds had different mobilities.

The Dihydro-Tps Derivative (IXh).—To the dihydrodiacetate (IXa) (1 g) in dihydropyran (30 ml) and toluene (10 ml) was added concentrated hydrochloric acid (2 drops). The mixture was stirred at 70° for 18 h, under nitrogen, then evaporated to dryness under reduced pressure, and the residue was deacetylated with aqueous 2N-sodium hydroxide (5 ml) in methanol (25 ml) at 23° for 18 h. The mixture was worked up as usual. The product was dissolved in pyridine and treated with 2,4,6-tri-isopropylbenzenesulphonyl chloride (6 g) at 23° for 69 h. Excess of ethanol was added and the solvents were evaporated off under reduced pressure. The residue was dissolved in ethanol (150 ml) and treated with aqueous 2·4N-hydrochloric acid at 30° for 18 h. The mixture was worked up and the product purified as described for (IXg) to give the oily *dihydro-Tps derivative* (IXh) (786 mg), $[\alpha]_D^{21}$ +49° (*c* 1·8); $\nu_{max.}$ 3500, 3000, 1605, 1475, 1440, and 1355 cm^{-1}; $\lambda_{max.}$ 281 nm (ε 2400); τ 2·86 (2H, s), 6·71 (3H, s), 8·72 (18H, d, *J* 7 Hz), 8·75 (6H, s), 8·86 (6H, s), 8·97 (3H, d, *J* 6·5 Hz), 9·12 (3H, d, *J* 6·5 Hz), and 9·17 (3H, t, *J* 7 Hz) (Found: C, 65·1; H, 8·5; S, 3·6. $C_{47}H_{76}SO_{12}$ requires C, 65·4; H, 8·7; S, 3·7%).

The Iodide (XIII).—The dihydro-Tps derivative (IXh) (415 mg) and sodium iodide (1·5 g) were heated under reflux in acetone (15 ml) for 18 h. The mixture was worked up and the product purified by p.l.c., as described for (X), to give the *iodide* (XIII), which crystallised as needles (276 mg) from ether, m.p. 137—140°; $[\alpha]_D^{20}$ +4° (*c* 0·7); $\lambda_{max.}$ 224 (ε 7500) and 259 (1600) (Found: C, 54·3; H, 7·4. $C_{32}H_{51}IO_9$ requires C, 54·4; H, 7·2%).

Reduction of the Iodide (XIII) *with Borohydride; Dihydrofusicoccin J* (VIIIa).—The iodide (XIII) (61 mg) in dimethyl sulphoxide (23 ml) was stirred with sodium borohydride (200 mg) at 100° for 30 min. The mixture was diluted with water and extracted with ether (3 × 50 ml). The extracts were washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated. The residue was purified by triple-elution p.l.c. [chloroform–propan-2-ol (97 : 3)] to give dihydrofusicoccin J (VIIIa) (30 mg) which resisted all attempts at crystallisation; *m/e* 582 (1%) (M^+, $C_{40}H_{62}O_{12}$), 350 (29), 332 (34), 314 (10), 259 (13), 207 (20), 151 (27), 109 (31), and 71 (100).

Dihydrofusicoccin J Penta-acetate (VIIIb).—Synthetic dihydrofusicoccin J (VIIIa) (30 mg) was acetylated with acetic anhydride (1 ml) in pyridine (0·5 ml), under nitrogen, at 70° for 18 h. The product was purified by p.l.c. [light petroleum (b.p. 40—60°)–ethyl acetate (7 : 3)] to give the oily dihydrofusicoccin J penta-acetate (VIIIb) (32 mg); $[\alpha]_D^{22}$ +18° (*c* 2·8); $\nu_{max.}$ 1740, 1370, 1240, and 1045 cm^{-1}; τ 6·78 (3H, s), 8·03 (6H, s), 8·06 (3H, s), 8·11 (6H, s), 8·79 (6H, d, *J* 7 Hz), 8·96 (9H, s), 9·14 (3H, d, *J* 7 Hz), and 9·18 (3H, t, *J* 7 Hz). The mass spectrum was identical with that of the acetylated dihydro-derivative of the new metabolite except that a weak molecular ion was observed at *m/e* 794 ($C_{42}H_{66}O_{14}$). The t.l.c. mobilities and the i.r. spectra of synthetic and natural (VIIIb) were identical.

The Tps Derivative (Il).—Fusicoccin (Ia) (500 mg), concentrated hydrochloric acid (2 drops), and dihydropyran (10 ml) in toluene (5 ml) were stirred at 23° for 5 days and then at 65° for 7 h. After the usual work-up, the product was deacetylated with aqueous 2N-sodium hydroxide (2 ml) in methanol (13 ml) at 22° overnight. The product in pyridine was stirred with 2,4,6-tri-isopropylbenzenesulphonyl chloride (800 mg) at 22° for 19 days. The mixture was worked up as described in the preparation of (IXg). The pyridine-free product was dissolved in ethanol (75 ml) and treated with 2·4N-hydrochloric acid at 30° for 17 h. After the usual work-up, the residue was purified by p.l.c. as described above to give the oily *Tps derivative* (Il) (197 mg), $[\alpha]_D^{20}$ +57° (*c* 1·2); $\nu_{max.}$ 3500, 1605, 1475, 1435, 1385, 1355, and 1270 cm^{-1}; $\lambda_{max.}$ 281 nm (ε 1900); τ 2·85 (2H, s), 4·20 (1H, dd, *J* 10 and 18 Hz), 6·69 (3H, s), 8·76 (18H, d, *J* 7 Hz), 8·76 (6H, s), 8·98 (3H, d, *J* 6·5 Hz), and 9·13 (3H, d, *J* 7 H) (Found: C, 65·3; H, 8·5; S, 3·6. $C_{47}H_{74}SO_{12}$ requires C, 65·4; H, 8·7; S, 3·7%).

The Iodide (XIV).—The Tps derivative (Il) (306 mg), sodium iodide (1·5 g), and triethylamine (1 ml) were heated under reflux in acetone (15 ml) for 24 h. The mixture was worked up and the product purified by p.l.c. as described for (X) to give the *iodide* (XIV) which crystallised as needles (211 mg) from ether, m.p. 157°, $[\alpha]_D^{20}$ +5° (*c* 0·8); $\nu_{max.}$ (KBr) 3450, 1645, 1465, 1385, 1370, and 1260 cm^{-1}; $\lambda_{max.}$ 224 (ε 7400) and 259 nm (1800); τ 4·25 (1H, dd, *J* 10 and 18 Hz), 6·70 (3H, s), 8·76 (6H, s), 8·83 (3H, s), 8·85 (3H, d, *J* 6·5 Hz), 9·16 (3H, d, *J* 7 Hz) (Found: C, 54·6; H, 7·2; I, 17·7. $C_{32}H_{49}IO_9$ requires C, 54·4; H, 7·2; I, 18·0%).

Reduction of the Iodide (XIV) *with Borohydride; Fusicoccin J*.—The iodide (XIV) (782 mg) and sodium borohydride (1 g) in dimethyl sulphoxide (23 ml) were stirred at 100° for 23 min. The mixture was quenched with aqueous sodium hydrogen carbonate (200 ml) and extracted with ether (3 × 50 ml). The combined extracts were washed with water, dried, and evaporated. The residue was purified by triple-elution p.l.c. [chloroform–propan-2-ol (95 : 5 v/v)] to give unchanged (XIV) (166 mg) and fusicoccin J, which crystallised from ether as needles (391 mg), m.p. 202—204° (under nitrogen in a sealed tube), $[\alpha]_D^{20}$ +24° (*c* 0·6). The t.l.c. mobility and i.r., u.v., and mass spectra were identical with those of the new isolate, and a mixed m.p. showed no depression (Found: C, 66·1; H, 9·0. Calc. for $C_{32}H_{52}O_9$: C, 66·2; H, 9·0%).

[8-^{3}H]*Fusicoccin J* (XVIII).—Fusicoccin J (III) (187 mg) was acetylated with pyridine–acetic anhydride overnight at 2°. The usual work-up afforded the oily tetra-acetate (IV) (218 mg), $\nu_{max.}$ 3570 (OH), 1750 (C=O), and 1645 cm^{-1}; τ 4·32 (1H, dd, *J* 10 and 17·5 Hz), 6·70 (3H, s), 7·94 (3H, s), 8·03 (3H, s), 8·06 (3H, s), 8·07 (3H, s), 8·80 (9H, s), 8·87 (3H, d, *J* 6·5 Hz), 8·97 (3H, d, *J* 6·5 Hz), and 9·19 (3H, d, *J* 6·5 Hz). This compound was not characterised further and was used in the next reaction. To a solution of the tetra-acetate

(IV) (76 mg) in acetone (5 ml) at $-5°$ was added Jones reagent (0·5 ml); the mixture was shaken for 2 min at $-5°$, poured into ice–water, and extracted with ether. The extracts were washed with water, dried, and evaporated to dryness. The residue, presumably containing the ketone (XVII), was dissolved in dry tetrahydrofuran (7 ml). The latter was added to a solution of lithium borohydride (170 mg) in tetrahydrofuran (10 ml) that had been treated with tritiated water (75 µl; 6 Ci ml^{-1}) for $1\frac{1}{2}$ h under reflux. The mixture was heated under reflux for 15 h. Aqueous 2N-sodium hydroxide (5 ml) and methanol (5 ml) were added and the mixture was kept at 22° for 7 h, diluted with saturated aqueous sodium chloride (50 ml), and extracted with ether (2 × 20 ml). The extracts were combined, washed with more brine (20 ml), dried, and evaporated under reduced pressure. The residue was repeatedly crystallised from ether to constant specific activity to furnish [8-^{3}H]-fusicoccin J (17·6 mg), $9{\cdot}69 \times 10^5$ disint. min^{-1} mg^{-1}.

Feeding Experiments.—Fusicoccum amygdali Del. was grown in shaken flasks (500 ml) in medium (100 ml) of the following composition: glucose (3·0%), $NaNO_3$ (0·33%), KH_2PO_4 (2·0%), KCl (0·05%), $MgSO_4,7H_2O$ (0·05%), $FeSO_4,7H_2O$ (0·001%), soya bean meal (0·2%), and tap water to volume. The flasks were incubated at 24°. After 27 h [8-^{3}H]fusicoccin J in aqueous ethanol (1 ml; 7 : 3 v/v) was added. The flasks were harvested after 5 and 7 days and the mycelium was filtered off. The culture filtrate was extracted with isobutyl methyl ketone (3 × 100 ml). The combined extracts were washed with water and evaporated under reduced pressure. The residue was dissolved in methanol (25 ml) and treated with aqueous 2N-sodium hydroxide (5 ml) at 23° overnight. The mixture was diluted with water and extracted with isobutyl methyl ketone (3 × 30 ml). The combined extracts were evaporated. The residue was purified by p.l.c. [chloroform–propan-2-ol] to give deacetylfusicoccin (Ie), which was repeatedly crystallised to constant specific activity ($9{\cdot}69 \times 10^5$ disint. min^{-1} mg^{-1}) from acetone–light petroleum (b.p. 40—60°) (1 : 1 v/v). The results are given in the Table.

Incorporations of [8-^{3}H]fusicoccin J (XVIII) into fusicoccin

Feeding expt.	Wt. of (XVIII) fed (mg)	Activity fed (disint. min^{-1})	Yield of (Ii) (mg)	Specific activity of (Ii) (disint. min^{-1} mg^{-1})	Incorporation (%)
1	1·07 †	$1{\cdot}04 \times 10^6$	11	$1{\cdot}89 \times 10^4$	20·1
2	3·61 ‡	$3{\cdot}50 \times 10^6$	17·6	$4{\cdot}10 \times 10^4$	20·6

† Fed to a 100 ml culture and harvested after 5 days. ‡ Fed to 2 × 100 ml cultures and harvested after 7 days.

Specificity of Incorporation.—The combined sample of labelled deacetylfusicoccin (22·4 mg) was acetylated at 2° overnight to give triacetylfusicoccin (Ii) (28 mg), m.p. 116° (lit.,[5] 116—117°), which was crystallised to a constant specific activity of $3{\cdot}07 \times 10^6$ distint. min^{-1} $mmol^{-1}$. The triacetate (Ii) (28 mg) was oxidised with Jones reagent to furnish the αβ-unsaturated ketone (XV) (21 mg), m.p. 136—139°, $[\alpha]_D$ +80° (*c* 1·8 (lit.,[4c] m.p. 137—138°; $[\alpha]_D$ +80°). The latter was crystallised to a constant specific activity of $3{\cdot}37 \times 10^3$ distint. min^{-1} $mmol^{-1}$, corresponding to 89% loss of label.

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