Fusicoccin. Part III.[1] The Structure of Fusicoccin H

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The structure (IIa) of fusicoccin H, a minor phytotoxic glycoside produced by *Fusicoccum amydali*, has been determined by degradative studies and by chemical correlation with the known fusicoccin series. Feeding experiments have shown that fusicoccin H can act as a precursor of fusicoccin. This strongly suggests that fusicoccin is a diterpenoid and not a degraded sesterterpenoid.

CULTURES of *Fusicoccum amydali* Del., a fungus responsible for a wilting disease of peach and almond trees in Southern Europe, have been shown to produce phytotoxic metabolites.[2] The structure of the major metabolite, 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.[1,4]

Besides fusicoccin (Ia), the culture filtrates contain a number of related metabolites (Ib—e, h—j) which are also formed when fusicoccin is incubated at room temperature at the pH of the culture filtrate during the production phase. These products probably arise non-enzymatically from fusicoccin in the course of the fermentation. Their structures have recently been established by spectroscopic and other methods.[5,6]

We have detected the presence in the culture filtrate of several compounds other than those already characterised. These show colour reactions similar to those of fusicoccin and incorporate activity from [2-^{14}C]mevalonic acid. Here we present evidence that establishes the structure (IIa) for one of these metabolites, conveniently termed fusicoccin H, and show that this compound can act as a biogenetic precursor of fusicoccin.

The molecular formula of fusicoccin H (IIa) was established as $C_{26}H_{42}O_8$. On acetylation a hexa-acetate (IIb), $C_{38}H_{54}O_{14}$, was obtained. This showed no hydroxy-absorption in the i.r. spectrum. The mass

[1] Part II, K. D. Barrow, D. H. R. Barton, Sir Ernst Chain, U. F. W. Ohnsorge, and R. Thomas, *J. Chem. Soc.* (*C*), 1971, 1265.

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

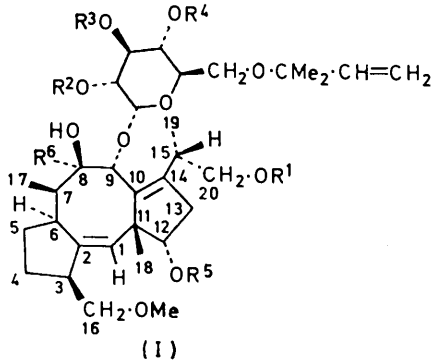
[3] A. Ballio, M. Bufani, C. G. Casinovi, S. Cerrini, W. Fedeli, R. Pellicciari, B. Santurbano, and A. Vaciago, *Experientia*, 1968, **24**, 631.

[4] 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.

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

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

spectrum showed the molecular ion at *m/e* 734 and other major peaks at 614, 386, 343, 331, 271, 169, and 109. The peaks at *m/e* 331, 271, 169, and 109 are characteristic of the fragmentation pattern of a tetra-*O*-acetylglucosyl derivative.[7] The presence of glucose was confirmed by

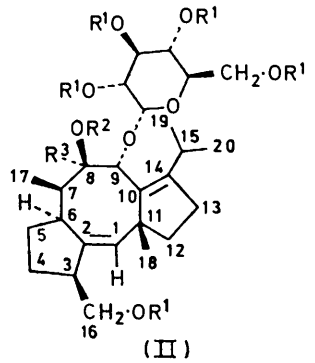


(I)

a; $R^1 = R^3 = Ac$, $R^2 = R^4 = R^5 = R^6 = H$
b; $R^1 = R^2 = Ac$, $R^3 = R^4 = R^5 = R^6 = H$
c; $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = R^6 = H$
d; $R^1 = Ac$, $R^{2-6} = H$
e; $R^{1-6} = H$
f; $R^{1-5} = Ac$, $R^6 = H$
g; $R^{1-5} = H$, $R^6 = {}^2H$
h; $R^2 = Ac$, $R^1, R^{3-6} = H$
i; $R^3 = Ac$, $R^1, R^2, R^{4-6} = H$
j; $R^4 = Ac$, $R^{1-3}, R^5, R^6 = H$

hydrolysing fusicoccin H with acid and assaying the hydrolysate with glucose oxidase.[8] The n.m.r. spectrum of the hexa-acetate (IIb) showed the presence of six acetoxy-groups, one tertiary (τ 8·75) and three secondary methyl groups [τ 8·82 (3H, d, J 7 Hz), 8·9 (3H, d, J 6·5 Hz), and 9·06 (3H, d, J 6·5 Hz)], and the absence of any signals for the methxoy- and isopentenyl substituents that are shown by fusicoccin and its derivatives of structure type (I). Fusicoccin H therefore has six acetylatable hydroxy-groups, four on the glucose unit and two on the aglycone; as there are two more oxygen atoms involved in the sugar ring and the glycosidic linkage the functionality of all eight oxygen atoms in fusicoccin H is accounted for. It was assumed on the basis of spectral data that fusicoccin H has the same carbon skeleton as fusicoccin. If the position of the glycosidic linkage and the sites of unsaturation were also the same then the partial structure (III) could be envisaged.

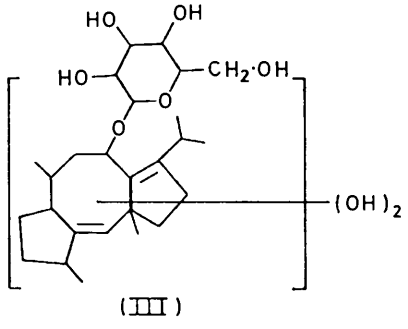
To remove the sugar, fusicoccin H was treated successively with periodate, borohydride, and acid,[9] but only a complex mixture of products was obtained. The alternative procedure, involving treatment of the periodate reaction product with strong base,[10] yielded the aglycone (IVa), $C_{20}H_{32}O_3$, in an overall yield of 40%. The aglycone (IVa) formed an isopropylidene derivative (IVb), $C_{23}H_{36}O_3$, which showed hydroxy-absorption in the i.r. spectrum at 3450 cm^{-1}. The n.m.r. spectrum displayed the olefinic proton at C-1 as a triplet showing only long-range coupling [τ 4·58 (J 1·7 Hz)]. A doublet (J 9·5 Hz) (H-9) and a double doublet (J 9·5 and 4·5 Hz) (H-8) were assigned to the allylic α-glycol unit on the basis of comparison with the corresponding fusicoccin derivative. The signals at τ 8·68 (3H, s), 9·02 (6H, d, J 6·5 Hz), and 9·1 (3H, d, J 6·5 Hz) account for one



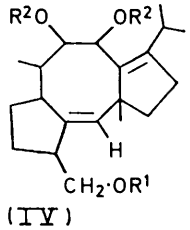
(II)

a; $R^1 = R^2 = R^3 = H$
b; $R^1 = R^2 = Ac$, $R^3 = H$
c; $R^2 = R^3 = H$, $R^1 = Ac$
d; $R^1 = R^2 = H$, $R^3 = {}^3H$

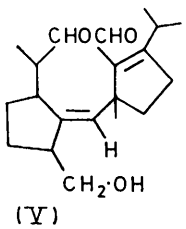
tertiary and three secondary methyl groups in the molecule. The presence of the allylic α-glycol unit was confirmed by periodate oxidation of the aglycone (IVa),



(III)



(IV)



(V)

a; $R^1 = R^2 = H$
b; $R^1 = H$, $R^2R^2 = CMe_2$
c; $R^1 = Me$, $R^2R^2 = CMe_2$

giving the $\alpha\beta$-unsaturated aldehyde (V), $\nu_{max.}$ 1720 and 1660 cm^{-1}, $\lambda_{max.}$ 250 nm (ε 4000). It remained to decide

[7] K. Biemann, D. C. De Jongh, and H. K. Schnoes, *J. Amer. Chem. Soc.*, 1963, **85**, 1763; T. C. Smale and E. S. Waight, *Chem. Comm.*, 1966, 680.

[8] A. St. G. Hugget and D. A. Nixon, *The Lancet*, 1957, **2**, 368.

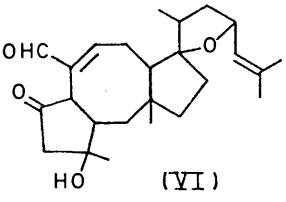
[9] M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 4970.

[10] J. J. Dugan and P. de Mayo, *Canad. J. Chem.*, 1965, **43**, 2033.

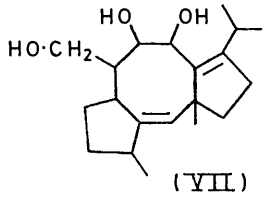
the position of the remaining hydroxy-group. As signals for only four methyl groups are visible in the n.m.r. spectrum of fusicoccin H and its derivatives, the hydroxy-group must be placed on one of the five methyl groups shown in the part structure (III). It cannot be placed on a tertiary methyl group, as such a signal is clearly visible in the n.m.r. spectrum. Its primary nature is further indicated by a two-proton doublet (J 7·5 Hz) at τ 6·46 in the n.m.r. spectrum of the acetonide (IVb).

Examination of the structures of fusicoccin and of the ophiobolins [11] [the related sesterterpenoids possessing the same system, *e.g.* ophiobolin A (VI)] would allow on biogenetic grounds the structures (VII), (VIII), and (IVa). Structures (VIII) and (IVa) contain homoallylic alcohol systems, a structural feature also present in fusicoccin. As fusicoccin H and its aglycone are more acid-sensitive than dihydrofusicoccin (vinyl group saturated) we favoured structure (IVa) and ascribed the sensitivity to acid to the homoallylic alcohol system at C-16. The acetonide from fusicoccin H was available only in very small amounts and therfore chemical correlation with the known fusicoccin aglycone was undertaken.

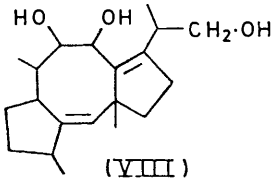
Reaction of the fusicoccin acetonide (IXa) with several equivalents of toluene-*p*-sulphonyl chloride in pyridine gave mixtures of the monotosylate (IXb) and the ditosylate (IXc). A larger excess and long reaction time (4 days) gave the ditosylate in good yield. Since lithium aluminium hydride reduction of the ditosylate resulted in a complex mixture of products a stepwise removal of the oxygen functions was attempted. Treatment of the



(VI)

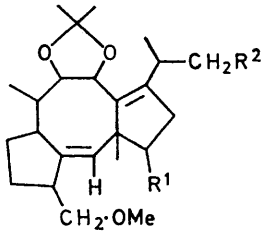


(VII)



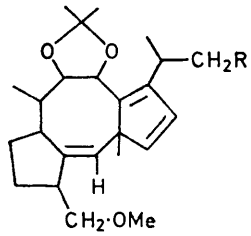
(VIII)

ditosylate (IXc) with sodium iodide in acetone afforded a mixture of one minor and one major product. The major product was an oil and was identified as the iodo-tosylate (IXd) by spectra only. The n.m.r. spectrum showed the presence of one tosyl group [τ 2·2 (2H, d, J 9 Hz), 2·6 (2H, d, J 9 Hz), and 7·6 (3H, s)] and a double doublet at τ 5·15 (1H, dd, J 2 and 2·5 Hz) assigned to the proton at C-12, the carbon bearing the tosyloxy-group. The presence of iodine was shown by a positive Beilstein test. The minor component (Xa), $C_{24}H_{35}IO_3$, displayed u.v. absorption [$\lambda_{max.}$ 272 nm (ε 2500)] attributed to the cyclopentadiene system. No n.m.r. signals for the tosyl group were present and a broad two-proton singlet at τ 3·8 had appeared. Surprisingly, the two olefinic protons of the cyclopentadiene system have the same



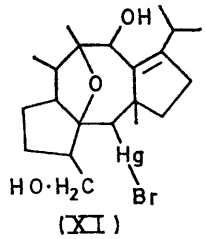
(IX)

a; $R^1 = R^2 = OH$
b; $R^1 = OH$, $R^2 = OTs$
c; $R^1 = R^2 = OTs$
d; $R^1 = OTs$, $R^2 = I$



(X)

a; R = I
b; R = H



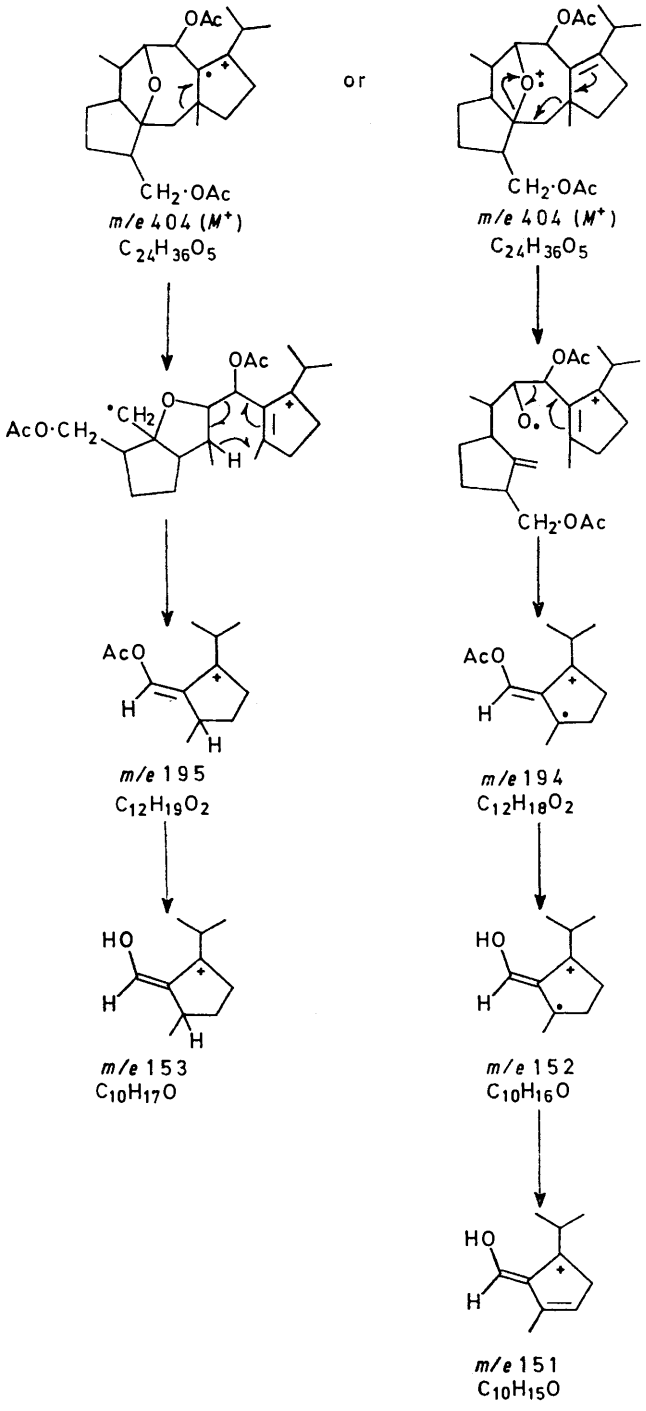
(XI)

chemical shift. The iodo-tosylate (IXd) was reduced with sodium borohydride in dimethyl sulphoxide and one major product (Xb), $C_{24}H_{36}O_3$, was obtained. The u.v. spectrum showed the presence of the cyclopentadiene system [$\lambda_{max.}$ 272 nm (ε 2000)] and the n.m.r. spectrum showed the olefinic protons at τ 3·84 (2H, broad s) and six methyl groups, τ 8·5 (3H, s), 8·62 (3H, s), 8·76 (3H, s), 8·92 (3H, d, J 7 Hz), 8·96 (3H, d, J 7 Hz), and 9·08 (3H, d, J 7 Hz). The primary iodide system had been reductively removed and the tosyl group eliminated. The same cyclopentadiene (Xb) was also obtained when (Xa) was treated with borohydride under the same conditions. Hydrogenation of (Xb) over palladium–strontium carbonate led to the uptake of 1 mol. equiv. of hydrogen and furnished (IVc), $C_{24}H_{38}O_3$.

Methylation of the acetonide (IVb), derived from fusicoccin H, with methyl iodide and sodium hydride in *NN*-dimethylacetamide gave the same derivative (IVc). The i.r., n.m.r., and mass spectra and optical rotations of the two samples were identical, and a mixed m.p. showed no depression. This correlation establishes the constitution and absolute configuration of the fusicoccin H aglycone. It remained to determine the point of attachment of the glucose unit. Fusicoccin H consumed 2 mol. equiv. of periodate with oxidation of the glucosyl grouping. Therefore one of the vicinal hydroxy-groups (at C-8 and C-9) provides the glucosidic linkage. To decide between these, the method used for fusicoccin (Ia) was followed.[1] The aglycone (IVa) was converted into the mercuribromide derivative (XI), and the crude product was reduced with sodium borohydride to the cyclic ether (XIIa), $C_{20}H_{32}O_3$. The cyclic ether (XIIa) formed a di-

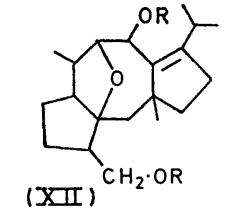
[11] K. Tsuda, S. Nozoe, M. Morisaki, K. Hirai, A. Itai, S. Okuda, L. Canonica, A. Fiecchi, M. Galli Kienle, and A. Scala, *Tetrahedron Letters*, 1967, 3369.

acetate (XIIb), and the characteristic pattern shown in the mass spectrum (Scheme 1) locates the primary

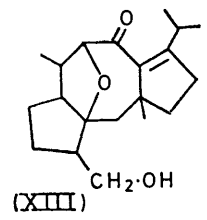


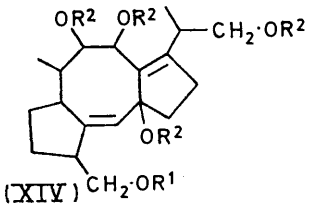
SCHEME 1

hydroxy-grouping in the ' left-hand side ' of the molecule. Oxidation of the cyclic ether (XIIa) with manganese dioxide gave the αβ-unsaturated ketone (XIII), $C_{20}H_{30}O_3$, $\lambda_{max.}$ 255 nm (ε 7600), $\nu_{max.}$ 1660 cm^{-1}. It is evident therefore that the C-8 hydroxy-group has been involved in the cyclic ether formation. The same cyclic ether (XIIa) was obtained when fusicoccin H was subjected to successive treatment with mercuric ions, borohydride, periodate, and strong base. Hence the glucose unit must be attached to the C-9 hydroxy-group. The α-configuration of the sugar linkage was assigned from the coupling constant of the anomeric proton [τ 4·5 (1H, d, *J* 5 Hz)] in the hexa-acetate (IIb).

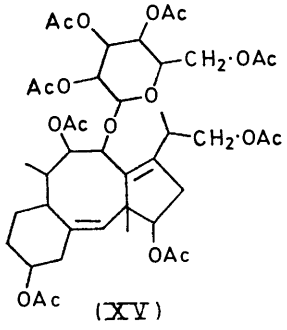


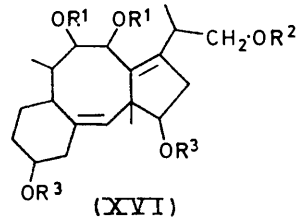
a; R = H
b; R = Ac

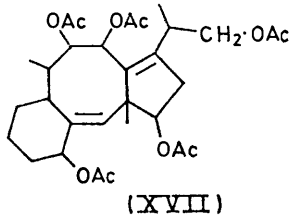




a; R^1 = Me, R^2 = Ac
b; R^1 = R^2 = Ac
c; R^1 = Me, R^2 = H







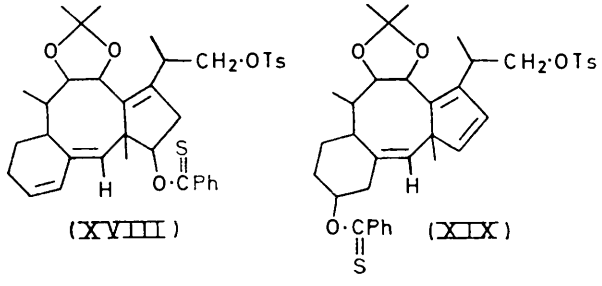
a; R^1 = R^2 = R^3 = Ac
b; R^1 = R^2 = R^3 = H
c; R^1R^1 = CMe_2, R^2 = R^3 = H
d; R^1R^1 = CMe_2, R^2 = Ts, R^3 = H
e; R^1R^1 = CMe_2, R^2 = Ts, R^3 = CSPh

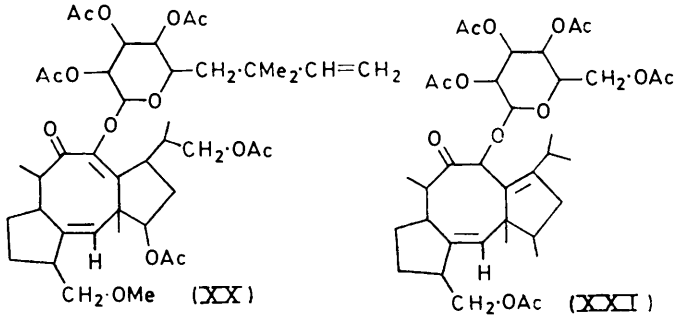
Considerable effort was spent in attempting to remove the methoxy-group from compounds of the readily available fusicoccin series to produce derivatives of fusicoccin H which are only obtainable in small quantities. Positive results were only obtained by the reaction of boron trifluoride–acetic anhydride [12] with fusicoccin triacetate (If) and fusicoccin aglycone tetra-acetate (XIVa). The product (XV), $C_{42}H_{58}O_{18}$, obtained from fusicoccin triacetate (If), showed no OH absorption in the i.r. spectrum. The n.m.r. spectrum indicated that the methoxy- and isopentenyl groups had been lost and showed the presence of six acetoxy-groups. The mass spectrum, which exhibited a molecular ion at *m/e* 850 and peaks at *m/e* 331, 271, 169, and 109, confirmed the presence of a tetra-*O*-acetylglycosyl system in the mole-

[12] C. R. Narayanan and K. N. Iyer, *J. Org. Chem.*, 1965, **30**, 1734.

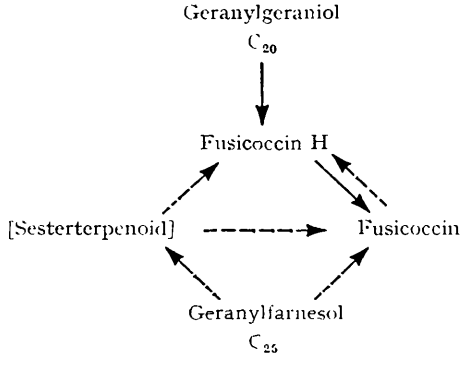
cule. The product (XVIa), $C_{30}H_{42}O_{10}$, produced under similar conditions from the fusicoccin aglycone tetra-acetate (XIVa) showed five acetoxy-signals in the n.m.r. spectrum and the signals for the *C*-methyl groups were at the same position as in the starting material, *i.e.* τ 8·86 (3H, d, *J* 6·5 Hz), 8·89 (3H, s), and 8·92 (3H, d, *J* 6·5 Hz). No methoxy-signal was present and the only other striking difference between the n.m.r. spectra of the starting material and the product was the appearance of a new acetoxy-group and a broad signal for one proton at τ 5·4, suggesting the presence of this new acetoxy-group on a six-membered ring. On the basis of this evidence structure (XIVb) was ruled out and it appeared that there had been a skeletal rearrangement producing either (XVIa) or (XVII). The chemical shift of the broad signal at τ 5·4 favoured (XVIa), as a signal at lower field would be expected in the case of an allylic acetoxy-group, but definite chemical proof was required. Hydrolysis of the penta-acetate (XVIa) produced the rearranged aglycone (XVIb), $C_{20}H_{32}O_5$, which was converted into the acetonide (XVIc), $C_{23}H_{36}O_5$. Treatment of this acetonide (XVIc) with a large excess of active manganese dioxide gave no detectable reaction and the starting material was recovered unchanged. However the failure of the manganese dioxide oxidation could perhaps be attributed to a hindered allylic hydroxy-group. An allylic alcohol is readily distinguished from a homoallylic alcohol by irradiation of the thionobenzoate. The former will yield a thiolbenzoate, the latter a diene.[13] The rearranged acetonide (XVIc) was converted into the monotosylate (XVId) and this on treatment with thiobenzoyl chloride yielded the bisthionobenzoate (XVIe). Irradiation of this bisthionobenzoate (XVIe) with a medium-pressure mercury lamp yielded a single product (XVIII), $C_{37}H_{44}O_6S_2$, in 80% yield. The compound had no carbonyl absorption in the i.r. spectrum and so the possibility of thiolbenzoate formation was ruled out. One thionobenzoate group had been lost, giving (XVIII) or (XIX). The n.m.r. spectrum showed a doublet at τ 3·15 (1H, *J* 10 Hz) and a broad multiplet at τ 3·95 (1H), suggesting that the thionobenzoate on the six-membered ring had been lost to give (XVIII). If the thionobenzoate group on the cyclopentane ring had been eliminated, then the product would be expected to show the broad singlet (2H) similar to that observed in the case of the cyclopentadiene (Xb) or two double doublets for the two olefinic protons. The product obtained from the BF_3–Ac_2O reaction of fusicoccin aglycone tetra-acetate (XIVa) therefore has the structure (XVIa). The product (XV) obtained from reaction of fusicoccin triacetate (If) was converted into the rearranged aglycone (XVIb) by successive treatment with base, sodium periodate, and strong base.

Having established the structure of fusicoccin H we turned our attention to the role of this C_{20} glycoside as a possible biogenetic precursor of fusicoccin. Fusicoccin H itself could be conceived to have been formed from geranylgeraniol pyrophosphate by a cyclisation analogous to that producing the ophiobolins from geranylfarnesol pyrophosphate.[14] However the possibility of fusicoccin being a degraded sesterterpenoid could not be ruled out.





Scheme 2 indicates some of the possibilities for the biosynthesis of the fusicoccins.



SCHEME 2

It is doubtful that fusicoccin could act as a precursor of fusicoccin H, as fusicoccin shows more extensive hydroxylation. Even the generation of fusicoccin H from a sesterterpenoid is unlikely as this would necessitate a *reductive* fission of a carbon–carbon bond. Therefore if fusicoccin H were to act as a precursor of fusicoccin there would be strong evidence that fusicoccin is a diterpenoid, not a degraded sesterterpenoid.

[13] S. Achmatowicz, D. H. R. Barton, P. D. Magnus, G. A. Poulton, and P. J. West, *Chem. Comm.*, 1971, 1014; D. H. R. Barton, M. Bolton, P. D. Magnus, P. J. West, G. Porter, and J. Wirz, *J.C.S. Chem. Comm.*, 1972, 632.

[14] L. Canonica, A. Fiecchi, M. G. Kienle, B. M. Ranzi, A. Scala, T. Salvatori, and E. Pella, *Tetrahedron Letters*, 1967, 3371.

To test fusicoccin H as a possible precursor it had to be labelled at a position that would allow a convenient specific degradation of the fusicoccin obtained. Position 8 was chosen for this purpose because a tritium label at C-8 would be easily removed on oxidation of the fusicoccin triacetate (If) to the αβ-unsaturated ketone (XX). To introduce tritium at C-8 it was necessary to oxidise the 8-OH to give the βγ-unsaturated derivative analogous to (XX). Reduction with a labelled (^{3}H) hydride reagent would then be expected to give the desired product. We have previously reported that when fusicoccin triacetate (If) was oxidised with Jones reagent [15] at 0 °C for 5 min the product was the αβ-unsaturated ketone (XX). It was anticipated that if the reaction were carried out under controlled conditions then the migration of the double bond could be avoided. In practice it was found necessary to limit the reaction to produce only a small amount of oxidised material to avoid this isomerisation. Trial experiments were again carried out in the more readily available fusicoccin series. Oxidation of the triacetate (If) at −5 °C for 2 min gave a product which showed i.r. absorption at 3500 (OH) and 1745 cm^{-1} (C=O) and only end absorption in the u.v. spectrum. Reduction of this crude product with sodium borodeuteride gave a product that was converted into the aglycone (XIVc) in the way previously described. The acetonide (IXa) formed from the aglycone was shown by mass spectrometry to contain 15% excess of deuterium, presumably at C-8. The formation of the known acetonide (IXa) also showed that the reduction product had the correct stereochemistry (β-OH at C-8).

Acetylation of fusicoccin H (IIa) at room temperature afforded a mixture of the penta-acetate (IIc) and the hexa-acetate (IIb). The penta-acetate (IIc) was oxidised with Jones reagent at −5 °C for 2 min, yielding a product that showed only end absorption in the u.v. spectrum and was presumed to be a mixture of the starting material and the ketone (XXI). This product was reduced with lithium borotritide prepared by reaction of lithium borohydride with tritiated water.[16] The reduction product was purified by preparative t.l.c., diluted with inactive fusicoccin H, and crystallised to constant specific activity. Fusisoccin H of activity 6—12 × 10^5 disint. min^{-1} μmol^{-1} was produced in several preparations. Successive feeding experiments showed 1·25, 2·3, and 2·1% incorporations into fusicoccin. Oxidation of the labelled fusicoccin triacetate (If) to the αβ-unsaturated ketone (XX) resulted in 90% loss of activity. The remaining 10% could be located at C-7 and result from enolisation of the ketone under the reducing conditions. Confirmatory evidence for the specific incorporation of the label was obtained by comparing the molar activities of the acetonide derivative (IXa) of the aglycone and deacetylfusicoccin (Ie). These were found to be 685 and 667 disint. min^{-1} μmol^{-1}, respectively.

These results demonstrate that fusicoccin H can act as an efficient biogenetic precursor of fusicoccin and therefore strongly suggest that fusicoccin is a diterpenoid.

[15] A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *J. Chem. Soc.*, 1953, 2548.
[16] R. H. Cornforth, *Tetrahedron*, 1970, **26**, 4635.

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage apparatus. U.v. spectra were recorded on a Unicam SP 800 spectrometer. I.r. spectra were recorded on a Unicam SP 200 spectrophotometer for solutions in chloroform or Nujol mulls. N.m.r. spectra were recorded for solutions in deuteriochloroform on a Varian HA100 spectrometer. Mass spectra were taken at 70 eV on an A.E.I. MS9 spectrometer. Rotations, unless otherwise stated, were measured for solutions in chloroform on a Perkin-Elmer 141 polarimeter. Radioactive measurements were carried out in a Beckman LS-200B liquid scintillation spectrometer using a modified Bray's scintillant.

Fusicoccin H (IIa).—The concentrate (200 g) of a butyl acetate extract of the culture filtrate from a 400 l fermentation of *Fusicoccum amydali* Del. was dissolved in chloroform and chromatographed on a Florisil column (80 × 6 cm, 1500 g). Elution with chloroform yielded fractions (20 × 200 ml) that were rejected, 5% methanol in chloroform (25 × 200 ml fractions) yielded fusicoccin and its acetyl derivatives, 7% methanol in chloroform afforded minor unidentified fusicoccins (32 × 200 ml fractions) and 10% methanol in chloroform gave fractions (25 × 200 ml) enriched in the most polar metabolite fusicoccin H (IIa), R_F 0·3 on silica gel GF_{254} [chloroform–propan-2-ol (8 : 2 v/v)]. Repetitive preparative t.l.c. on silica gel HF_{254} (same solvent system) afforded a chromatographically homogeneous semi-solid that crystallised on trituration with benzene to afford *fusicoccin H monohydrate* (IIa) as prisms, m.p. 125°, $[\alpha]_D^{23}$ +20° (*c* 0·25 in EtOH), $\nu_{max.}$ 3400 and 1020 cm^{-1}, only end absorption in the u.v. spectrum; τ 8·68 (3H, s), 8·94 (6H, d, *J* 7 Hz), and 9·16 (3H, d, *J* 6·5 Hz); *m/e* 482 (M^+), 320 (M^+ − glucose), 287, 259 (Found: C, 62·2; H, 8·7. $C_{26}H_{42}O_8,H_2O$ requires C, 62·4; H, 8·8%). Analytical samples were dried at 50° and 0·1 mmHg for 48 h.

Fusicoccin H Hexa-acetate (IIb).—Fusicoccin H was treated with pyridine–acetic anhydride at 85° for 24 h. The usual work-up afforded *fusicoccin H hexa-acetate* (IIb) as prisms, m.p. 75° [from light petroleum (b.p. 60—80°)], $[\alpha]_D^{23}$ +15° (*c* 0·2), $\nu_{max.}$ 1745 (C=O), 1240, and 1040 cm^{-1}; τ 7·92 (3H, s), 7·96 (3H, s), 8·02 (6H, s), 8·04 (3H, s), 8·06 (3H, s), 8·78 (3H, s), 8·82 (3H, d, *J* 7 Hz), 8·92 (3H, d, *J* 6·5 Hz), and 9·06 (3H, d, *J* 6·5 Hz); *m/e* 734 (M^+), 674, 614, 386, 343, 331, 271, 169, and 109 (Found: C, 62·1; H, 7·4. $C_{38}H_{54}O_{14}$ requires C, 62·1; H, 7·35%).

Quantitative Periodate Oxidation of Fusicoccin H.—Fusicoccin H (IIa) (20 mg) in water (10 ml) and a little methanol was treated with 0·02M-sodium periodate (10 ml). The volume was adjusted to 50 ml and the solution was kept in the dark at room temperature. Samples (5 ml) were titrated at intervals with 0·05M-sodium thiosulphate. Reaction was complete in 24 h; 2·1 mol. equiv. of periodate had then been consumed.

Estimation of Glucose in Fusicoccin H.—Fusicoccin H (IIa) (4 mg) was added to 2N-hydrochloric acid (3 ml) and enough methanol to effect dissolution. The solution was heated under reflux for 2 h, cooled to room temperature, and neutralised with sodium hydroxide. Assay for glucose by a modified glucose oxidase procedure [8] indicated a 75% yield of glucose. A similar yield of glucose was obtained from fusicoccin in a parallel experiment run under identical conditions.

Fusicoccin H Aglycone (IVa).—Sodium periodate (250 mg) in water (1 ml) was diluted with methanol (2 ml). Fusicoccin H (100 mg) in methanol (2 ml) was added and the mixture was kept in the dark at room temperature. After 2 days it was diluted with water (50 ml) and extracted with chloroform (5 × 50 ml). Evaporation of the combined extracts yielded a foam (70 mg). This, in absolute ethanol (20 ml) containing potassium hydroxide (1 g), was heated gently under reflux in an atmosphere of nitrogen (t.l.c. control). After 1 h all the starting material had disappeared and one major product had formed. The solution was cooled to room temperature, diluted with water, and acidified with acetic acid. It was then extracted with chloroform (5 × 50 ml) and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by preparative t.l.c. on silica gel HF_{254} [chloroform–propan-2-ol (100 : 10 v/v)]. The product (IVa) (25 mg) was not crystalline and was used as such in further reactions. It was characterised only by its mass spectrum: *m/e* 320 (M^+), 302, 287, 259, 229, 191, and 151.

Fusicoccin H Aglycone Acetonide (IVb).—The aglycone (IVa) (20 mg) in acetone (5 ml) containing a crystal of toluene-*p*-sulphonic acid was left overnight. The solution was passed through a short column of alumina (grade V; 1·5 × 10 cm) and the column was washed with acetone (50 ml). Evaporation of the solvent and crystallisation from light petroleum (b.p. 60—80°)–acetone gave the *aglycone acetonide* (IVb) as needles, m.p. 105°, $[\alpha]_D^{23}$ −27° (*c* 0·375), $\nu_{max.}$ 3450 (OH), 1775, 1060, and 1020 cm^{-1}; τ 4·58 (1H, t, *J* 1·7 Hz), 5·88 (1H, d, *J* 9·5 Hz), 6·02 (1H, dd, *J* 9·5 and 4·5 Hz), 6·46 (2H, d, *J* 7·5 Hz), 8·52 (3H, s), 8·68 (3H, s), 8·86 (3H, s), 9·02 (3H, d, *J* 6·5 Hz), and 9·1 (3H, d, *J* 6·5 Hz); *m/e* 360 (M^+), 345, 302, 287, 259, and 191 (Found: C, 76·55; H, 10·1. $C_{23}H_{36}O_3$ requires C, 76·65; H, 10·0%).

The Acetonide Methyl Ether (IVc).—Sodium hydride (30 mg; 60% suspension in oil) was added to a stirred solution of the aglycone acetonide (IVb) (6 mg) in *NN*-dimethylacetamide (1 ml). After 30 min methyl iodide (0·5 ml) was added and stirring continued for 5 h. The mixture was poured onto water and extracted with chloroform (5 × 50 ml); the combined extracts were washed with water, dried, and evaporated. High vacuum was necessary to remove the last traces of dimethylacetamide. The product was purified by preparative t.l.c. on silica gel GF_{254} with light petroleum (b.p. 40—60°)–ethylacetate (7 : 1 v/v) as eluant. Crystallisation of the product from acetone–light petroleum (b.p. 40—60°) afforded the *acetonide methyl ether* (IVc) (6 mg) as needles, m.p. 125°, $[\alpha]_D^{23}$ −11° (*c* 0·2), $\nu_{max.}$ 1170, 1125, 1090, and 1060 cm^{-1}; τ 4·58 (1H, t, *J* 1·8 Hz), 5·78 (1H, d, *J* 9·5 Hz), 6·02 (1H, dd, *J* 9·5 and 4·5 Hz), 6·68 (3H, s), 8·52 (3H, s), 8·66 (3H, s), 8·78 (3H, s), 8·94 (3H, d, *J* 7 Hz), 8·98 (3H, d, *J* 7 Hz), and 9·1 (3H, d, *J* 7 Hz); *m/e* 374 (M^+), 359, 301, 278, 223, 205, and 149 (Found: C, 76·9; H, 10·05. $C_{24}H_{38}O_3$ requires C, 77·0; H, 10·15%).

The Cyclic Ether (XIIa).—The aglycone (IVa) (20 mg) was added to a suspension of mercury(II) acetate (50 mg) in methanol–water (2 : 1; 1·5 ml) and the mixture was stirred overnight, added to a saturated solution of potassium bromide (50 ml), and extracted with chloroform (5 × 50 ml). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by preparative t.l.c. in chloroform–propan-2-ol (9 : 1). The major product was dissolved in methanol (5 ml), and 2N-sodium hydroxide (2 ml) was added, followed by sodium borohydride (20 mg). The mixture was stirred for 30 min, diluted with water (50 ml), acidified with acetic acid, and extracted with chloroform (5 × 40 ml). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by preparative t.l.c. [chloroform–propan-2-ol (95 : 5 v/v)] affording the cyclic ether (XIIa) as an oil, $[\alpha]_D^{23}$ +11° (*c* 0·26), $\nu_{max.}$ 3500, 1100, 1025, and 1000 cm^{-1}; τ 5·42 (1H, d, *J* 5 Hz), 8·78 (3H, s), 9·02 (6H, d, *J* 7 Hz), and 9·08 (3H, d, *J* 6·5 Hz) (Found: M^+, 320·2350. Calc. for $C_{20}H_{32}O_3$: *M*, 320·2351).

Preparation of the Cyclic Ether (XIIa) *from Fusicoccin H.*—Fusicoccin H (100 mg) was dissolved in methanol (2 ml) and added to mercury(II) acetate (200 mg) in water–methanol (1 : 6; 3·5 ml). The mixture was stirred overnight and then added to a 50% saturated solution of potassium bromide (5 ml). A solution of sodium borohydride (100 mg) in 2N-sodium hydroxide (20 ml) was then added; the mixture was neutralised with acetic acid and extracted with chloroform (5 × 50 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was taken up in methanol (5 ml) and treated with sodium periodate (200 mg) in water (2 ml). After 24 h the mixture was worked up as usual and the crude product was heated under gentle reflux for 1 h with 5% potassium hydroxide in absolute ethanol (10 ml). The usual work-up followed by preparative t.l.c. afforded the cyclic ether (XIIa) (20 mg), $[\alpha]_D^{23}$ +11° (*c* 0·115). The t.l.c. mobilities of the two preparations were the same and the i.r., n.m.r., and mass spectra were identical.

The Cyclic Ether Diacetate (XIIb).—The cyclic ether (XIIa) (10 mg) was acetylated with acetic anhydride–pyridine at room temperature. Work-up followed by preparative t.l.c. gave the diacetate (XIIb) (10 mg) as an oil, *m/e* 404 (M^+), 195, 194, 153, 152, 151, 135, and 134 (Found: M^+, 404·2564. Calc. for $C_{24}H_{36}O_5$: *M*, 404·2562). Other mass measurements were as follows: *m/e* 195·1378 (Calc. for $C_{12}H_{19}O_2$: 195·1385), 194·1313 (Calc. for $C_{12}H_{18}O_2$: 194·1307), 153·1267 (Calc. for $C_{10}H_{17}O$: 153·1279), 152·1188 (Calc. for $C_{10}H_{16}O$: 152·1201), 151·1123 (Calc. for $C_{10}H_{15}O$: 151·123), 135·1163 (Calc. for $C_{10}H_{15}$: 135·1173), and 134·1083 (Calc. for $C_{10}H_{14}$: 134·1095).

Manganese Dioxide Oxidation of the Cyclic Ether (XIIa).—The cyclic ether (XIIa) (5 mg) in chloroform (2 ml) was stirred with an excess of active manganese dioxide for 4 h. The manganese dioxide was then filtered off and washed with chloroform, and the combined filtrate and washings were evaporated. The residue was purified by preparative t.l.c. yielding the ketone (XIII) as an oil, $\nu_{max.}$ 3500 (OH) and 1650 cm^{-1} (C=O); $\lambda_{max.}$ 255 nm (ε 7600); *m/e* 318 (M^+) and 151 (Found: M^+, 318·2200. Calc. for $C_{20}H_{30}O_3$: *M*, 318·2195).

Periodate Oxidation of the Aglycone (IVa).—Sodium periodate (20 mg) in water (1 ml) was added to the aglycone (IVa) (10 mg) in methanol (2 ml). The mixture was kept at room temperature in the dark; after 4 h it was diluted with water (20 ml) and extracted with chloroform (5 × 30 ml). The combined extracts were washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by preparative t.l.c. and afforded the dialdehyde (V) as an oil that was characterised only by its i.r. and u.v. spectra: $\nu_{max.}$ 3500 (OH), 1720, and 1660 cm^{-1} (C=O); $\lambda_{max.}$ 250 nm (ε 4000).

The Ditosylate (IXc).—A solution of the acetonide (IXa) (100 mg) in pyridine (0·75 ml) was cooled and toluene-*p*-sulphonyl chloride (500 mg) was added at such a rate that the temperature did not exceed 4°. The mixture was then

left at room temperature and the reaction was monitored by t.l.c. After 4 days the reaction was complete and the usual work-up gave a solid which crystallised from acetone–light petroleum (b.p. 40—60°) affording the *ditosylate* (IXc) as needles, m.p. 143—145°, $[\alpha]_D^{23}$ +14° (*c* 0·3), $\nu_{max.}$ 1600, 1193, 1110, and 1060 cm^{-1}; τ 2·2 (2H, d, *J* 9 Hz), 2·3 (2H, d, *J* 9 Hz), 2·8 (4H, d, *J* 9 Hz), 4·95 (1H, t, *J* 1·8 Hz), 5·55 (1H, d, *J* 10 Hz), 6·74 (3H, s), 7·62 (6H, s), 8·68 (3H, s), 8·78 (3H, s), 9·05 (3H, d, *J* 7 Hz), and 9·12 (3H, d, *J* 7 Hz) (Found: C, 64·05; H, 6·75. $C_{38}H_{50}O_9S_2$ requires C, 63·85; H, 7·0%).

The Monotosylate (IXb).—The acetonide (IXa) (50 mg) in pyridine (4 ml) was treated with toluene-*p*-sulphonyl chloride (90 mg) and left at room temperature overnight. After the usual work-up the major component was separated on preparative t.l.c. and the *monotosylate* (IXb) thus obtained crystallised from light petroleum (b.p. 40—60°) as needles, m.p. 167°, $[\alpha]_D^{27}$ +66° (*c* 0·404), $\nu_{max.}$ 3400 (OH), 1600, 1196, 1180, 1060, and 960 cm^{-1}; τ 2·22 (2H, d, *J* 9 Hz), 2·6 (2H, d, *J* 9 Hz), 4·74 (1H, t, *J* 1·7 Hz), 5·85 (1H, d, *J* 10 Hz), 6·65 (3H, s), 7·52 (3H, s), 8·65 (3H, s), 8·72 (3H, s), 8·88 (3H, s), 8·94 (3H, d, *J* 7 Hz), and 9·15 (3H, d, *J* 7 Hz) (Found: C, 66·4; H, 7·65. $C_{31}H_{44}O_7S$ requires C, 66·45; H, 7·85%).

Reaction of the Ditosylate (IXc) *with Sodium Iodide.*—The ditosylate (IXc) (100 mg) and sodium iodide (1·6 g) were heated under reflux in acetone (15 ml) with stirring for 48 h. The mixture was added to water and extracted with chloroform; the extracts were washed with water and dried. Evaporation furnished a residue that was separated by preparative t.l.c. into a major and a minor component. The major component (IXd) (50 mg) gave a positive Beilstein test and the n.m.r. spectrum showed τ 2·2 (2H, d, *J* 9 Hz), 2·6 (2H, d, *J* 9 Hz), 4·78 (1H, t, *J* 1·7 Hz), 5·15 (1H, dd, *J* 2 and 2·5 Hz), 5·35 (1H, d, *J* 10 Hz), 6·5 (3H, s), 6·85 (2H, m), 7·6 (3H, s), 8·42 (3H, s), 8·64 (3H, s), 8·82 (3H, s), 8·9 (3H, d, *J* 7 Hz), and 9·08 (3H, d, *J* 7 Hz). The minor component, the *iodide* (Xa) (10 mg) crystallised from acetone–light petroleum (b.p. 60—80°) as fine needles, m.p. 128°, $[\alpha]_D^{23}$ +150° (*c* 0·11), $\lambda_{max.}$ 272 nm (ε 2500); τ 3·80 (2H, s), 4·54 (1H, t, *J* 1·7 Hz), 5·5 (1H, d, *J* 10 Hz), 6·05 (1H, dd, *J* 10 and 5·5 Hz), 6·65 (3H, s), 8·45 (3H, s), 8·6 (3H, s), 8·72 (3H, s), 8·75 (3H, d, *J* 7 Hz), and 9·05 (3H, d, *J* 7 Hz); *m/e* 498 (M^+), 440, 372, 371, 313, 243, and 211 (Found: C, 57·8; H, 6·95. $C_{24}H_{35}IO_3$ requires C, 57·85; H, 7·05%).

Reduction of the Iodo-tosylate (IXd) *with Borohydride; the Cyclopentadiene* (Xb).—The iodo-tosylate (IXd) (125 mg) in dimethyl sulphoxide (7 ml) was added to sodium borohydride (75 mg) in dimethyl sulphoxide (3 ml) with stirring. The temperature was slowly raised to 100° and the reaction was continued for 27 h. After cooling to room temperature the mixture was added to ice–water and extracted with ether (5 × 50 ml). The combined extracts were washed with water, dried, and evaporated under reduced pressure. The residue was purified by preparative t.l.c. [light petroleum (b.p. 40—60°)–ethyl acetate (5 : 1 v/v)] and afforded a major product that crystallised from acetone–light petroleum (b.p. 60—80°) to give the *cyclopentadiene* (Xb) as needles, m.p. 110°, $[\alpha]_D^{23}$ +72° (*c* 0·124), $\nu_{max.}$ 1175, 1065, 1035, 1000, and 900 cm^{-1}; $\lambda_{max.}$ 272 nm (ε 2000); τ 3·8 (2H, s), 4·58 (1H, t, *J* 1·7 Hz), 5·58 (1H, d, *J* 10 Hz), 5·96 (1H, dd, *J* 10 and 5·5 Hz), 6·68 (3H, s), 8·5 (3H, s), 8·62 (3H, s), 8·76 (3H, s), 8·92 (3H, d, *J* 7 Hz), 8·96 (3H, d, *J* 7 Hz), and 9·08 (3H, d, *J* 7 Hz); *m/e* 372 (M^+), 357, 299, 269, and 211 (Found: C, 77·3; H, 9·55. $C_{24}H_{36}O_3$ requires C, 77·4; H, 9·7%).

Reduction of the Iodide (Xa).—The iodide (Xa) (10 mg) was reduced with sodium borohydride in dimethyl sulphoxide as already described. The usual work-up gave the product (Xb) which crystallised from light petroleum (b.p. 60—80°)–acetone as fine needles, m.p. 110°; a mixed m.p. determination with the product obtained in the foregoing experiment showed no depression.

The Acetonide (IVc).—The cyclopentadiene (Xb) (7 mg) in ethanol (10 ml) was hydrogenated over 10% palladium–barium carbonate (uptake 1 mol. equiv. in 2 h). The catalyst was filtered off and washed with chloroform. Evaporation of the combined filtrate and washings gave a solid residue that crystallised from acetone–light petroleum (b.p. 40—60°) yielding the acetonide (IVc) as needles, m.p. 125°, $[\alpha]_D^{23}$ −12·5° (*c* 0·6) (Found: C, 76·9; H, 9·95. Calc. for $C_{24}H_{38}O_3$: C, 77·0; H, 10·15%), identical (mixed m.p., i.r., n.m.r., and mass spectra) with the derivative (IVc) made from fusicoccin H.

The Octa-acetate (XV).—Boron trifluoride–ether complex (1·5 ml) was added to a solution of fusicoccin triacetate (If) (50 mg) in acetic anhydride (2 ml) and dry ether (3 ml) held at 0°. After 24 h at 4 °C the mixture was poured into ice–water (50 ml) and, after a few hours, extracted with ether (5 × 50 ml). The combined extracts were washed with saturated sodium hydrogen carbonate solution (5 × 20 ml) and water (3 × 50 ml), dried (Na_2SO_4), and evaporated. The residue crystallised from ethyl acetate affording the *octa-acetate* (XV) (40 mg) as large prisms, m.p. 228°, $[\alpha]_D^{23}$ −12° (*c* 0·235); $\nu_{max.}$ 1735 (C=O), 1250, and 1040 cm^{-1}; τ 7·98 (12H, s), 8·02 (3H, s), 8·06 (6H, s), 8·10 (3H, s), 8·85 (3H, s), 8·89 (3H, d, *J* 7 Hz), and 9·02 (3H, d, *J* 7 Hz); *m/e* 850 (M^+), 790, 730, 670, 610, 400, 382, 340, 331, 271, 169, and 109 (Found: C, 59·45; H, 6·7. $C_{42}H_{58}O_{18}$ requires C, 59·3; H, 6·8%).

The Penta-acetate (XVIa).—Fusicoccin aglycone tetra-acetate (XIVa) (50 mg) was treated with boron trifluoride–ether complex and acetic anhydride under the conditions just described. After a similar work-up the residue obtained crystallised from light petroleum (b.p. 40—60°) yielding the *penta-acetate* (XVa) (42 mg) as needles, m.p. 104°, $[\alpha]_D^{23}$ −96° (*c* 1·04); $\nu_{max.}$ 1725, 1250, and 1050 cm^{-1}; τ 4·58 (1H, d, *J* 10·5 Hz), 4·86 (1H, dd, *J* 10·5 and 5 Hz), 4·94 (1H, t, *J* 1·7 Hz), 5·14 (1H, t, *J* 6 Hz), 5·4br (1H, $W_{\frac{1}{2}}$ 14 Hz), 5·95 (1H, dd, *J* 6·5 and 10 Hz), 6·12 (1H, dd, *J* 6·5 and 10 Hz), 7·38 (1H, dd, *J* 6 and 15 Hz), 7·66 (1H, dd, *J* 6 and 15 Hz), 7·98 (3H, s), 8·02 (3H, s), 8·04 (6H, s), 8·06, (3H, s), 8·86 (3H, d, *J* 6·5 Hz), 8·89 (3H, s), and 8·92 (3H, d, *J* 6·5 Hz); *m/e* 562 (M^+), 502, 442, 400, 382, 367, 340, 322, 307, 297, 280, and 262 (Found: C, 64·05; H, 7·4. $C_{30}H_{42}O_{10}$ requires C, 64·05; H, 7·45%).

Correlation of the Rearranged Products (XV) *and* (XVIa).—The octa-acetate (XV) (100 mg) was hydrolysed with 10% potassium hydroxide in ethanol, and the product was treated successively with sodium periodate and potassium hydroxide as described earlier for the preparation of fusicoccin H aglycone (IVa). The crude preparation was acetylated with acetic anhydride–pyridine yielding the penta-acetate (XVIa) as needles, m.p. and mixed m.p. 104°.

The Rearranged Aglycone (XVIb).—The penta-acetate (XVIa) (50 mg) in methanol (5 ml) was treated with 10% potassium hydroxide in methanol (2 ml) and left overnight at room temperature. After the usual work-up the product crystallised from light petroleum (b.p. 60—80°) giving the

rearranged aglycone (XVIb) (30 mg) as prisms, m.p. 130°, $[\alpha]_D^{23}$ −40° (*c* 0·2); $\nu_{max.}$ 3500 (OH) and 1120 cm^{-1}; *m/e* 352 (M^+) (Found: C, 67·85; H, 8·9. $C_{20}H_{32}O_5$ requires C, 68·2; H, 9·1%).

The Rearranged Aglycone Acetonide (XVIc).—The rearranged aglycone (XVIb) (50 mg) was treated overnight with acetone–toluene-*p*-sulphonic acid. The product crystallised from acetone–light petroleum (b.p. 60—80°) affording the *rearranged aglycone acetonide* (XVIc) (40 mg) as prisms, m.p. 110°, $[\alpha]_D^{23}$ −22° (*c* 0·2); $\nu_{max.}$ 3500 (OH) and 1050 cm^{-1}; τ 4·85 (1H, t, *J* 1·7 Hz), 5·72 (1H, d, *J* 10 Hz), 6·02 (1H, dd, *J* 10 and 5·5 Hz), 8·48 (3H, s), 8·68 (3H, s), 8·85 (3H, s), 8·98 (3H, d, *J* 6·5 Hz), and 9·02 (3H, d, *J* 7 Hz); *m/e* 392 (M^+), 304, and 286 (Found: C, 69·9; H, 9·75. $C_{23}H_{36}O_5$ requires C, 70·4; H, 9·2%).

The Tosyl Acetonide (XVId).—The acetonide (XVIc) (100 mg) in dry pyridine was cooled and toluene-*p*-sulphonyl chloride (200 mg) was added at such a rate that the temperature did not rise above 4°. After 8 h at 4 °C all the starting material had reacted and the usual work-up gave a residue that was purified by preparative t.l.c., developed in chloroform–propan-2-ol (9 : 1). The major component crystallised from light petroleum (b.p. 60—80°) affording the *tosyl acetonide* (XVId) as needles, m.p. 75°, $[\alpha]_D^{23}$ +18° (*c* 0·1); $\nu_{max.}$ 3500 (OH), 1600, 1198, 1180, 1100, and 1060 cm^{-1}; τ 2·2 (2H, d, *J* 9 Hz), 2·6 (2H, d, *J* 9 Hz), 4·85 (1H, t, *J* 1·7 Hz), 5·8 (1H, d, *J* 10 Hz), 7·62 (3H, s), 8·48 (3H, s), 8·55 (3H, s), 8·6 (3H, s), 8·88 (3H, d, *J* 7 Hz), and 9·05 (3H, d, *J* 7 Hz); *m/e* 546 (M^+), 374, 356, 316, 298, and 280 (Found: C, 65·8; H, 7·95. $C_{30}H_{42}O_7S$ requires C, 65·95; H, 7·7%).

The Bisthionobenzoate (XVIe).—The monotosylate (XVId) (50 mg) in pyridine (0·25 ml) was cooled in an ice-bath under nitrogen and thiobenzoyl chloride (0·2 ml) was added. The mixture was kept overnight at room temperature in the dark, then poured into water and extracted with dichloromethane (5 × 100 ml). The combined extracts were washed with water, dried, and evaporated. The residue was purified by preparative t.l.c., developed with light petroleum–ethyl acetate (1 : 4). The major product crystallised from cyclohexane yielding the solvated *bisthionobenzoate* (XVIe) (40 mg) as yellow needles, m.p. 115°, $[\alpha]_D^{23}$ +140°; $\nu_{max.}$ 1600, 1270, 1245, 1190, 1180, and 1100 cm^{-1}; $\lambda_{max.}$ 285 nm (ε 22,000) and 253 nm (ε 19,000); τ 2·0—2·8 (14H, m), 4·2 (1H, t, *J* 1·7 Hz), 7·62 (3H, s), 8·58 (3H, s), 8·68 (3H, s), 8·88 (3H, s), 8·85 (3H, d, *J* 6·5 Hz), and 8·95 (3H, d, *J* 6·5 Hz); *m/e* 510 (M^+ − $2C_6H_5CSOH$) 452, 402, 338, 295, and 280 (Found: C, 69·0; H, 7·15. $C_{44}H_{50}O_7S_3,C_6H_{12}$ requires C, 69·0; H, 7·1%). Analytical samples were dried at 70 °C for 48 h and showed the presence of 1 mol. equiv. of cyclohexane in the n.m.r. spectrum.

Photoreaction of the Bisthionobenzoate (XVIe).—The bisthionobenzoate (XVIe) (20 mg) was dissolved in methylene chloride (200 ml) in a two-necked Pyrex flask fitted with a drying tube and a nitrogen inlet. The flask was cooled to −75° with a solid carbon dioxide–acetone bath and irradiated with a medium-pressure mercury lamp. The reaction was monitored by t.l.c. and was complete in 30 min. The solvent was removed under reduced pressure and the residue purified by preparative t.l.c., developed with light petroleum (b.p. 40—60°)–ethyl acetate (5 : 1). The major product crystallised from light petroleum (b.p. 60—80°) affording the *diene* (XVIII) (14 mg) as yellow needles, m.p. 170°, $[\alpha]_D^{23}$ −35°; $\nu_{max.}$ 1600, 1240, 1190, 1180, 960, 920, and 850 cm^{-1}; $\lambda_{max.}$ (cyclohexane) 283 (ε 6000), 255 (11,000), 240 (14,000) and 235 nm (16,000); τ 2·1—2·8 (9H, m), 3·15 (1H, d, *J* 10 Hz), 3·95 (1H, m), 4·75 (1H, t, *J* 1·7 Hz), 7·5 (3H, s), 8·55 (3H, s), 8·65 (3H, s), 8·75 (3H, s), 8·9 (3H, d, *J* 6·5 Hz), and 9·05 (3H, d, *J* 6·5 Hz); *m/e* 648 (M^+), 510, 452, 424, and 339 (Found: C, 68·35; H, 7·0. $C_{37}H_{44}O_6S_2$ requires C, 68·5; 6·8%).

[8-^{3}H]*Fusicoccin H* (IId).—Fusicoccin H (IIa) (25 mg) was acetylated with pyridine–acetic anhydride overnight at 4°. The usual work-up gave the penta-acetate (IIc) as a chromatographically homogeneous oil, $\nu_{max.}$ 3500 (OH) and 1740 cm^{-1} (C=O); τ 7·90 (3H, s), 7·92 (3H, s), 8·02 (6H, s) and 8·04 (3H, s); *m/e* 692 (M^+). This compound was not characterised further and was used directly in the next reaction. The penta-acetate (IIc) (28 mg) was dissolved in acetone (2 ml) and cooled to −5 °C. Jones reagent (0·2 ml) was added and the mixture was shaken for 2 min at −5 °C. An excess of isopropyl alcohol was then added and the mixture was poured into water and extracted with ether; the extracts were washed with dilute aqueous sodium hydrogen carbonate and water, and dried. Evaporation left a residue, presumably containing the ketone (XXI), $\nu_{max.}$ 1745 and 3500 cm^{-1}. The residue was dissolved in dry tetrahydrofuran (THF) and added to a solution of lithium borohydride (100 mg) in THF (10 ml) that had been treated with tritiated water (0·05 ml; *ca.* 10 Ci in 2·3 ml) for 1 h under reflux. The mixture was heated under reflux overnight then cooled to room temperature, and 10% potassium hydroxide solution (30 ml) was added carefully. After 4 h the mixture was acidified with acetic acid and extracted with butanol. Evaporation of the extract furnished crude [8-^{3}H]fusicoccin H, which was purified by repetitive preparative t.l.c. as described earlier. Fusicoccin H of the following specific activities was obtained in successive experiments: (i) $1·2 \times 10^6$, (ii) 1×10^6, (iii) 2×10^6 disint. min^{-1} mg^{-1}.

Feeding Experiments.—Fusicoccum amydali Del. was grown in shaken flasks [500 ml; 100 ml medium 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]. After incubation for 24 h, [8-^{3}H]-fusicoccin (1 mg) dissolved in water–ethanol (1 ml; 7 : 3) was added aseptically to each of two flasks. The flasks were harvested after 4 days and the mycelium was filtered off. The culture filtrate was extracted several times with chloroform; the combined extracts were evaporated and the residue was treated with 10% potassium hydroxide for 12 h at room temperature. Work-up furnished deacetylfusicoccin (IIe), which was crystallised to constant specific activity of 1000 disint. min^{-1} mg^{-1}, which corresponds to 1·25% incorporation. Two repetitions of this experiment but purification of fusicoccin triacetate (If) showed in corporations of 2·3 and 2·1%, respectively.

Specificity of Incorporation.—The labelled fusicoccin triacetate obtained by feeding [8-^{3}H]fusicoccin H was oxidised with Jones reagent to produce the αβ-unsaturated ketone (XX) which was crystallised to constant specific activity. The results are given in the Table.

Incorporations of [8-^{3}H]fusicoccin H into fusicoccin

Feeding expt.	Incorporation (%)	Specific activity * of fusicoccin derivative	Specific activity * of unsaturated ketone (XX)	Loss of activity (%)
1	1·3	740	75	92
2	2·1	1120	90	92
3	2·3	2054	102	95

* In disint. min^{-1} mg^{-1}.

The deacetylfusicoccin (Ie) obtained from another experiment, specific molar activity 667 disint. min^{-1} μmol^{-1}, was degraded to the aglycone (XIVc) as described. This was converted into the acetonide (IXa) and recrystallised to constant specific activity, 1680 disint. min^{-1} mg^{-1} (685 disint. min^{-1} μmol^{-1}).

Stereochemistry of the Reduction of the αβ-Unsaturated Ketone (XX).—Fusicoccin triacetate (If) was oxidised with Jones reagent as described for fusicoccin H penta-acetate (IIc). The product was reduced with sodium borodeuteride (98% ^2H) in ethanol for 12 h. The product was treated successively with ethanolic 10% potassium hydroxide, sodium periodate, sodium borohydride, and acid to yield the aglycone (XIVc), that was converted into the acetonide (IXa), m.p. 149—150°. The mass spectrum of this acetonide showed that it contained 15% excess of deuterium and that only one ^2H atom per molecule was present.

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