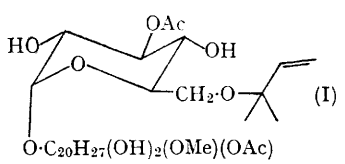
Fusicoccin: Characterisation of the Oxygen Substituents

By K. D. BARROW, D. H. R. BARTON, E. B. CHAIN,* C. CONLAY, T. V. SMALE, R. THOMAS, and E. S. WAIGHT

(Departments of Biochemistry and Chemistry, Imperial College of Science and Technology, London S.W.7)

FUSICOCCIN[1] (fusicoccin A) is a highly phytotoxic metabolite of *Fusicoccum amygdali* which has been assigned the molecular formula $C_{38}H_{58}O_{13}$. It was shown to yield one molecule of glucose on acid hydrolysis and to form dihydrofusicoccin on catalytic hydrogenation. The n.m.r. and microanalytical evidence indicated one methoxy- and two acetoxy-groups. We now report further results which permit the characterisation of the oxygen substituents as shown in the partial structure of fusicoccin (I).



(I)

The above formula was based on mass spectral evidence (supported by an *X*-ray *M*-estimation) in which the highest mass peak (*m/e* 704) was attributed to dehydration of the parent ion $C_{38}H_{58}O_{13}$ (M^+ 722). Subsequent spectra have shown a minor peak at *m/e* 722 which appeared to fragment into three principal segments, corresponding to the aglycone $C_{23}H_{36}O_6$ (*m/e* 408); a diacetylglucosyl fragment $C_{10}H_{15}O_7$ (*m/e* 247), accompanied by more intense ions at *m/e* 205 (loss of CH_2CO) and 145 (205—$MeCO_2H$); and the base peak at *m/e* 69 (C_5H_9).

The mass spectrum of deacetylfusicoccin, $C_{32}H_{52}O_{10}$ (M^+ 596), m.p. 126—127°, aglycone ion *m/e* 366, appeared to indicate the loss of three acetyl groups in contrast to the two observed in the n.m.r. (100 MHz) spectrum ($CDCl_3$) of fusicoccin crystallized from either benzene or aqueous methanol. Supporting evidence for the presence of two acetoxy-groups in fusicoccin is provided by examination of the mass spectrum of the acetylation product. Acetylation with acetic anhydride in pyridine gave a product, $C_{42}H_{62}O_{15}$ (M^+ 806), m.p.

116—117°, which was found to contain five acetoxy-groups (n.m.r. in $CDCl_3$), while on acetylation with [$^2H_6$]acetic anhydride, the parent ion was shifted by 9 m.u. to *m/e* 815; this requires the addition of three acetyl groups.

Both deacetylfusicoccin and triacetylfusicoccin showed moderately intense parent ions, in contrast to the very small peak at *m/e* 722 in the fusicoccin mass spectrum, which however, exhibited a more intense ion at *m/e* 680. It is probable that the minor *m/e* 722 ion requiring three acetate groups, and the associated diacetylglucosyl ion at *m/e* 247, arise through a thermal reaction prior to ionisation of the parent compound (M = 680). Subsequent discussion is consequently based on the revised molecular formula of fusicoccin, $C_{36}H_{56}O_{12}$, containing two acetoxy-groups shared between the glucosyl unit, [which yields the prominent ions at *m/e* 205 ($C_8H_{13}O_6$) and *m/e* 145 (205 − AcOH)], and the aglycone fragment at *m/e* 405.

This distribution of acetoxy-substituents is supported by the mass spectrum of triacetylfusicoccin, two extra acetates appearing on the sugar residue giving rise to a characteristic glucopyranose triacetate ion series[2] at *m/e* 289, 229, 169, and 109, while the other is present in the algycone fragment (*m/e* 450). Triacetylfusicoccin retains one hydroxy-group (*m/e* 788, $M^+ - H_2O$), ν_{max} (Nujol) 3550 cm.$^{-1}$.

Fusicoccin is unstable in aqueous methanol at 30° outside the pH range 2—6·5, giving rise to a series of products of which five were isolated by preparative t.l.c. and characterised. Between pH 7 and pH 9 two major products are formed, namely compound B, whose mass spectrum closely resembles that of fusicoccin, indicating that it may be an isomer, and compound C, $C_{34}H_{54}O_{11}$ (M^+ 638), a monodeacetyl fusicoccin formed by hydrolysis of the glucose acetyl substituent, since the aglycone ion (*m/e* 408) is retained. Above pH 9 the aglycone acetyl group is lost to give compound D (deacetylfusicoccin), $C_{32}H_{52}O_{10}$ (M^+ 596). At pH 2, two other products are formed, compound E, $C_{29}H_{46}O_{11}$ (M^+ 570) containing a single acetyl substituent on the aglycone, n.m.r. ($CDCl_3$), τ 7·86, aglycone ion *m/e* 408, and its deacetylation product, compound F, $C_{27}H_{44}O_{10}$ (M^+ 528), aglycone ion *m/e* 366. Compounds B, C, and D are present in culture filtrates of *F. amygdali*, but since this is normally between pH 7 and 8, they probably arise to some extent by spontaneous degradation of fusicoccin.

The base peak C_5H_9 (*m/e* 69) characteristic of fusicoccin and compounds B, C, and D is absent in compounds E and F and is attributed to an acid-labile C_5H_9-ether substituent of the glucose, since compound F forms a hexa-acetate $C_{39}H_{56}O_{16}$ (M^+ 780), the mass spectrum of which shows a characteristic tetra-acetylglucosyl fragmentation series[2] at *m/e* 331, 271, 169, and 109. Although compound F is apparently an unsubstituted glucoside, it proved to be resistant to enzymic hydrolysis with snail digestive juice (a crude mixture containing active α- and β-glucosidases) or emulsin (β-glucosidase). The assignment of an α-glycoside is strongly indicated by the n.m.r. spectrum ($CDCl_3$) of dihydrofusicoccin in view of the small coupling constant (J 3·7 c./sec.) of the anomeric proton,[3] τ 4·98.

A prominent n.m.r. signal fusicoccin, τ 8·75 (6H, s) is absent in compound F and is assigned to a *gem*-dimethyl group in the acid-labile olefinic C_5H_9 group which is consequently formulated as a 1,1-dimethylallyl ether. The X-proton of the expected vinyl ABX system in fusicoccin, τ 4·24 (1H, q), is absent in dihydrofusicoccin for which the base peak is shifted to *m/e* 71 (C_5H_{11}). This in turn requires the presence of an ethyl group in dihydrofusicoccin, which is seen at τ 9·18 (3H, t, J 7 c./sec.) and 8·50 (2H, q, J 7 c./sec.), both multiplets collapsing to singlets on double irradiation at the appropriate frequencies. On degrading fusicoccin to compound F by shaking for 6 days at room temperature with a mixture of N-HCl and CCl_4 (1 : 1, v/v), the resulting CCl_4 solution showed n.m.r. signals consistent with a mixture of acetic acid (τ 7·94), 1,1-dimethylallyl alcohol (τ 8·75, s, isopropyl protons), and its dehydration product, isoprene (τ 8·18, s, Me protons plus 7 characteristic olefinic signals).

The presence of these two C_5-compounds was confirmed by g.l.c. (kindly carried out by Dr. D. Blythin).

Although fusicoccin is resistant to oxidation with periodate, deacetylfusicoccin consumes two mole equivalents. The product, $C_{31}H_{48}O_9$ (M^+ 564), which still exhibits the characteristic aglycone ions, is therefore a glucopyranoside unsubstituted at C-2, C-3, and C-4 and consequently carrying the ether substituent at C-6. This also places the fusicoccin glucose acetoxy-group at C-3. Deacetylfusicoccin aglycone, $C_{21}H_{34}O_5$ (M^+ 366), m.p. 161—162°, $[\alpha]_D^{20}$ −3° ($CHCl_3$), is obtained by treatment of the periodate oxidation product either with base[4] (30 — 40% yield) or through borohydride reduction followed by acid hydrolysis[5] (65 — 80% yield).

The apparent isoprenoid character of fusicoccin was confirmed biosynthetically through the incorporation of DL-[2-^{14}C]mevalonic acid lactone (2% efficiency), which labelled both the C_5-ether and the aglycone moiety.

These conclusions are in agreement with the

independent structural studies recently described by Ballio *et al.*[6]

The fusicoccin used in these investigations was produced in the fermentation pilot plant of the Biochemistry Department.

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