The Structures of Isofusicoccin and Allofusicoccin

Besides fusicoccin (I)[1-3], a highly phytotoxic compound[4], culture filtrates of *Fusicoccum amygdali* Del. contain a number of by-products[5]. Four of them are also formed when fusicoccin is incubated at room temperature at the same pH of the culture brew during the production phase, and might arise non-enzymically from fusicoccin in the course of the fermentation[6]. In a previous paper[5], the structure of two of these compounds, namely monodeacetylfusicoccin (II) and dideacetylfusicoccin (III), were firmly established, whereas a third product (isofusicoccin) was shown to be an isomer of fusicoccin, probably formed through migration of the *O*-acetyl group carried at C-3′ of the glucose moiety[7].

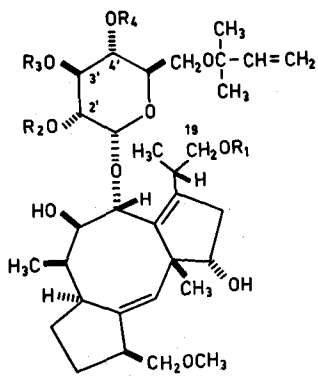
The present report describes the characterization of the fourth compound as a second isomer of fusicoccin (allofusicoccin), and illustrates the evidence on which structures IV and V are assigned to allofusicoccin and isofusicoccin respectively.

Isofusicoccin was prepared as reported in a previous paper[5]. Allofusicoccin was obtained by chromatographic fractionation of the residue left in ethyl acetate after crystallization of fusicoccin, as previously described[5]. In particular, repeated fractionations on silica gel columns (Kieselgel S-HR, Macherey and Nagel) of F II[5] yielded chromatographically pure allofusicoccin, which was eventually obtained as a partially crystalline substance by precipitation with light petroleum (b.p. 30–50 °C) from an acetone solution. By essentially the same procedures small amounts of pure allofusicoccin have been isolated from mixtures (also containing fusicoccin and isofusicoccin) obtained on incubation at room temperature of equal volumes of fusicoccin dissolved in benzene or ethyl acetate (2%, w/v) and of 0.2 *M* aqueous sodium bicarbonate (pH 8.9).

Allofusicoccin[9], m.p. 83–86°, $[\alpha]_D^{25}$ + 35 (c = 1.26), has the molecular formula $C_{36}H_{56}O_{12}$ (M^+ 680). The IR-spectrum in the region 850–3700 cm^{-1} was superimposable upon that of fusicoccin, whereas it was different in the range 500–850 cm^{-1}. The NMR-spectrum clearly indicated the same features observed in fusicoccin and isofusicoccin, namely a vinyl on a quaternary carbon, an olefinic proton on a trisubstituted double bond, 1 *O*-Me, 2 *O*-Ac, 2 secondary and 3 tertiary C-Me groups. The mass spectrum was also very similar to that of fusicoccin, showing the molecular ion at *m/e* 680 and characteristic ions at *m/e* 408 (aglycone), 205 (monoacetylglucosyl), 69 ($C_5H_9^+$) and 43 (CH_3CO^+). The close relationship with fusicoccin and isofusicoccin was further evidenced by formation of triacetylfusicoccin[2] on acetylation, and dideacetylfusicoccin[5] on deacetylation. As with isofusicoccin, and at variance with fusicoccin, allofusicoccin was oxidizable with periodate.

Therefore, both isofusicoccin and allofusicoccin must differ from fusicoccin only for the sites of acetylation. As shown by mass spectra, the 2 fusicoccin isomers carry one acetoxy group on the sugar moiety and the second on the aglycone. The location of the latter on C-19 follows from considerations identical to those previously reported for the acetylaglycone[2] and for monodeacetylfusicoccin[5]. As a consequence the difference must concern the site of esterification on glucose only, which must be C-2′ for one isomer and C-4′ for the other. Unambiguous assignement of the acetylated position for each isomer was attained through NMR- and NMDR-spectra. Allofusicoccin dissolved in d_6-acetone showed a *dd* centred at 4.65 δ (1 H) with splittings (3.5 and 10 Hz) consistent with 1′ (eq) 2′ (ax) and 2′ (ax) 3′ (ax) couplings, as expected for an α-glucopyranoside esterified at C-2′. The *dd* collapsed to a *d* centred at 4.65 δ (J = 10 Hz) on irradiation of the *d* at 4.99 δ (J = 3.6 Hz, anomeric proton) and to a *d* centred at 4.70 δ (J = 3.5 Hz) on irradiation at 3.96 δ (which therefore must correspond to the chemical shift of CH-3′). Thus, allofusicoccin has structure IV differing from fusicoccin for the location of one acetoxy group on C-2′ instead of C-3′.

A 220 MHz NMR-spectrum[10] of isofusicoccin in $CDCl_3$ shows a *t* (J = 10 Hz, 1 H) centred at 4.85 δ, as compared to a similar signal at 4.96 δ for fusicoccin. The NMR-spectrum (100 MHz at 60°) of a d_6-acetone solution of dihydroisofusicoccin[11] shows a partially resolved *q* centred at 4.75 δ characterized by (*ax*) (*ax*), (*ax*) (*ax*) coupling constants (J = 10 and 10 Hz). This is unaffected on irradiation at 3.54 δ, which decouples the anomeric proton (4.96 δ, *d*, J = 3.5 Hz), but collapses to a *s* on



I R_1, R_3 = $COCH_3$; R_2, R_4 = H
II R_1 = $COCH_3$; R_2, R_3, R_4 = H
III R_1, R_2, R_3, R_4 = H
IV R_1, R_2 = $COCH_3$; R_3, R_4 = H
V R_1, R_4 = $COCH_3$; R_2, R_3 = H
VI R_2 = $COCH_3$; R_1, R_3, R_4 = H
VII R_4 = $COCH_3$; R_1, R_2, R_3 = H
VIII R_3 = $COCH_3$; R_1, R_2, R_4 = H

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[7] Similar results have been recently reported by BARROW et al.[8].
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[9] UV-spectra were recorded on ethanolic solutions with a Beckman DK-2 spectrophotometer: all compounds showed only final absorption. IR-spectra were recorded in KBr pellets with a Beckman IR-9 spectrophotometer. NMR-spectra were recorded on a Varian HA-100 apparatus (unless otherwise stated) with TMS as an internal reference. Optical rotations were measured with a Perkin Elmer 141 polarimeter on ethanolic solutions. Mass spectra were recorded on an A. E. I. MS-902 spectrometer. Melting points are uncorrected. All compounds gave satisfactory elementary analyses.
[10] We are grateful to Dr. L. PAOLILLO for the 220 MHz spectra of fusicoccin and isofusicoccin.

irradiation at 3.91 δ (a value compatible with CH-3′ and CH-5′) which in turn has no effect on the *d* of CH-1′. One can therefore conclude that isofusicoccin is the fusicoccin isomer carrying one acetoxy group on C-4′ (V).

The ready migration of the acetoxy group of fusicoccin from C-3′ to C-2′ and C-4′ prompted an investigation of the behaviour of the 2 isomers IV and V on incubation at pH 8.9. Thin layer chromatography showed that each isomer was interconverted into the other two. In particular IV gave at first I and later V, thus suggesting an intramolecular shift, possibly through the formation of a labile *ortho*ester. In accordance, V gave first I and later IV. Migration from C-2′ and C-3′ to C-4′ was faster than from C-4′ to C-3′ and from C-3′ to C-2′. The behaviour of fusicoccin and its isomers is paralleled by the corresponding compounds lacking the *O*-acetyl at C-19, namely monodeacetylallofusicoccin (VI) and monodeacetylisofusicoccin (VII), which are minor metabolites of *F. amygdali* to be described in a separate paper[12], and 19-monodeacetylfusicoccin (VIII), prepared by isomerization at pH 8.9 of either VI or VII. The latter compound has molecular formula $C_{34}H_{54}O_{11}$ (M^+ 638), is not oxidizable with periodate and after crystallization from cyclohexane-ethyl acetate has m.p. 112–114° and $[\alpha]_D^{25}$ + 36.6 (c = 0.42). The mass spectra of VI, VII and VIII are nearly identical to each other and show, besides the parent ion, characteristic ions at *m/e* 366 (deacetylaglycone), 205 (monoacetylglucosyl), 69 ($C_5H_9^+$) and 43 (CH_3CO^+)[13]. Their structures are inferred from NMR- and NMDR-spectra, which yield the same type of information about the position of the acetoxy group as discussed above in the case of compounds IV and V.

Migration of the *O*-acetyl group on the glucose moiety has also been observed with the dihydroderivatives of I, IV, V, VI, VII, VIII, all prepared by catalytic hydrogenation with Pd on $BaSO_4$. Dihydrofusicoccin[2,8] and dihydroisofusicoccin[11] have already been described; the characterization of the other 4 dihydroderivatives will be reported elsewhere.

Rearrangements of acetyl groups in carbohydrates have been well known for a long time, but they usually take place under conditions stronger than those used for the above compounds. For this reason the 3 monoacetyl derivatives of methyl-6-trityl-α-D-glucopyranoside have been synthetized and submitted to the mild treatment used for fusicoccin and related compounds. Again, ready migration of the acetyl group took place[14], thus indicating that the rearrangements observed in the fusicoccin series are not influenced by the nature of the aglycone[15].

Riassunto. L'allofusicoccina e l'isofusicoccina, due isomeri della fusicoccina isolati dai brodi di coltura di *Fusicoccum amygdali* Del., differiscono dalla fusicoccina solamente per la posizione del gruppo acetossilico sul residuo del glucosio; nella prima questo è sul C-2′ e nella seconda sul C-4′. Fusicoccina, allofusicoccina e isofusicoccina, nonchè i loro 19-deacetilderivati ed i sei corrispondenti diidroderivati, si interconvertono a pH leggermente alcalino a temperatura ambiente.

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[13] Like fusicoccin[2,8], compound VIII gave also transacetylation ions at *m/e* 680 and 722.
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[15] This work was supported in part by the Italian Research Council (CNR). The technical assistance of Drs. F. Maietta and M. Scotto Lavinia is gratefully acknowledged.
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Isolation and Structure of Raucaffrinoline — A New Alkaloid from *Rauwolfia caffra* Sonder

In a reinvestigation of *Rauwolfia caffra* Sonder, an apparently new indolenine alkaloid, provisionally named raucaffrinoline, was isolated from the basic fraction of the alcoholic extractive of fresh undried root bark of the plant. Carefully avoiding any contact with strong alkali and acids or excessive heat, the alcoholic extractive was worked up in the following way: The alcoholic extracts were freed of the solvent in vacuo, the residue thus obtained was digested with hot water and distributed into aqueous and ethyl acetate layers. A small quantity of non-basic material did not go into either of these solvents and was neglected. The well cooled aqueous fraction was saturated with sodium chloride, and the water-insoluble hydrochloride formed was filtered out. The filterate was basified with 10% ammonia and repeatedly extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried over anhydrous sodium sulphate and filtered. On keeping overnight, the solution gave a crystallisate which on recrystallisation from a mixture of moist ethyl acetate and methanol (5:1) yielded raucaffricine[1–3]. The mother liquor of raucaffricine was freed of the solvent in vacuo and the residue was exhaustively extracted with hot benzene. The benzene solution of the base was passed through a column of alumina (Aluminium oxide 'Woelm' activity grade I, M. Woelm-Eschweger; Fabrik Chemisch-Pharmazeutischer Präparate) and eluted with benzene which yielded perakine[4] m.p. 186–189 °C. The column was subsequently eluted with a mixture of benzene and ethyl acetate (1:1) which yielded a glassy mass on removal of the solvent in vacuo. It crystallized from methylene dichloride in prismatic rods melting at

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