560 g of caterpillars were put in a round 2 liter 24/40 flask, killed with powdered dry ice, and the flask connected to a trap cooled with dry ice; the system was evacuated to 0.05 mm and kept at that pressure during 48 h in order to dry completely the larvae. The vapor trapped in the dry ice trap was extracted with hexane, evaporated and the oily residue (200 mg) analized directly by NMR, showing the spectrum that the composition of the mixture was the same as found before[1] (57:43) of isobutyric and 2-methyl butyric acids. The dried bodies were ground with chloroform in a Waring blendor and allowed to reflux for 4 h. The extract was filtered and evaporated to dryness in a rotavapor and the oily residue (57 g) was chromatographed on silica gel (250 g). The fractions eluted with benzene (13 g) showed by thin layer chromatography, a mixture of compounds, that were chromatographed on 350 g of alumina. From the fractions eluted with hexane, crystallized a white solid (90 mg), m.p. 52–53°, that resulted to be a mixture of 3 saturated hydrocarbons, $C_{25}H_{52}$, $C_{27}H_{56}$ and $C_{29}H_{60}$, characterized by IR-, NMR- and mass-spectrometry. From the fractions eluted with benzene, crystallized a white solid (60 mg), m.p. 82°, that on mass-spectrometry showed 4 molecular ions, M^+: 336, 364, 392 and 420 that had allylic protons in 3.6 ppm (δ) as seen in its NMR-spectrum. The elution of the column with benzene-ethyl acetate 90:10, a complex mixture of triglycerides was obtained (2 g) and 50 mg of a crystalline solid, m.p. 146°, that was characterized as cholesterol by comparison of its IR-, NMR-, mass spectra and its chromatographic (TLC) behavior with an authentic sample of cholesterol.

Finally, the fractions eluted with ethyl acetate yielded a mixture of 7 g of 2 monounsatured acids, M^+: 256 and 284 and palmitic and stearic acids in a approximately 25% each.

Resumen. Se describe la naturaleza de algunos de los componentes principales que se encuentran en la oruga *Baronia brevicornis S.*

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The Structure of Three Isomers of Monodeacetylfusicoccin

Thin layer chromatography of culture filtrates of *Fusicoccum amygdali* Del. has shown[1] that fusicoccin (I), the main phytotoxic compound produced by this phytopathogenic microorganism[2–4], is consistently accompanied by a series of co-metabolites. The major by-products are isofusicoccin (II), monodeacetylfusicoccin (III) and dideacetylfusicoccin (IV), which are also formed, together with allofusicoccin (V), on incubation of fusicoccin at neutral or slightly alkaline pH values[1]. Their structural identification has been reported in previous papers[1,5].

Further work concerned with the isolation of minor by-products and with the structure determination of three of them is described here.

Extensive chromatographic fractionations on silica gel columns (Kieselgel S-HR, Macherey and Nagel) of the residue left in ethylacetate after crystallization of fusicoccin showed that some of the spots detected on thin layer chromatograms (indicated in a previous paper[1] as F-II, F-III..., F-VI, in order of decreasing mobilities) were due to more than one compound. In particular, the following results were obtained: F-II was a mixture of allofusicoccin (F-II/1) and minute amounts of a new compound (F-II/2); F-III and F-IV contained only isofusicoccin and monodeacetylfusicoccin, respectively; material initially behaving as spot F-V gave 3 groups of homogeneous fractions (F-V/1, F-V/2, F-V/4) and a 4th group corresponding to a mixture of a new compound (F-V/3, present only in traces) with F-V/2 and F-V/4; F-VI corresponded to a single substance; F-VII was entirely composed of dideacetylfusicoccin; furthermore, a new substance (F-VIII) was detected in a group of fractions eluted after F-VII. Compounds F-V/1, F-V/2, F-V/4, F-VI and F-VIII were isolated as pure substances and submitted to a detailed study. All of them are D-glucosides, as shown by the positive test with glucose oxidase after acid hydrolysis, and are oxidizable with periodate.

Compounds F-V/2, F-V/4 and F-VI yielded triacetylfusicoccin (VI) on acetylation[6] and dideacetylfusicoccin (IV) on deacetylation with alkali, thus indicating that their structures are very closely related to fusicoccin. Their NMR-spectra[7] demonstrated, besides other features characteristic of fusicoccin and related compounds, the presence of a single *O*-Ac group. Their mass spectra showed that they had molecular formula $C_{34}H_{54}O_{11}$ (M^+ 638), also confirmed by elementary analyses. Ions at *m/e* 366 (aglycone) and 205 (monoacetylglucosyl) in the mass spectra of F-V/2 and F-VI located in both compounds the *O*-Ac on the sugar moiety. A strong ion at *m/e* 408 in the mass spectrum of F-V/4 indicated that this substance carries the *O*-Ac group on the aglycone. The esterification site in each of the 3 above-mentioned monoacetates was defined by NMR- and NMDR-spectroscopy.

Compound F-V/2, m.p. 87–90° and $[\alpha]_D^{25}$ + 15.0 (c = 0.65), in d_6-acetone (60°) shows a *dd* (1H) centred at 4.70 δ with (eq) (ax) and (ax) (ax) couplings (J = 4 and 9 Hz), which collapses to a *d* on irradiation at 4.91 δ (1H, *d*, J = 4 Hz: anomeric proton, partially overlapping the

[1] A. BALLIO, C. G. CASINOVI, G. RANDAZZO and C. ROSSI, Experientia *26*, 349 (1970).

[2] A. BALLIO, E. B. CHAIN, P. DE LEO, B. F. ERLANGER, M. MAURI and A. TONOLO, Nature, Lond. *203*, 297 (1964).

[3] A. BALLIO, M. BRUFANI, C. G. CASINOVI, S. CERRINI, W. FEDELI, M. PELLICCIARI, B. SANTURBANO and A. VACIAGO, Experientia *24*, 631 (1968).

[4] K. D. BARROW, D. H. R. BARTON, Sir Ernst CHAIN, U. F. W. OHNSORGE and R. THOMAS, J. chem. Soc. (C) *1971*, 1265.

[5] A. BALLIO, C. G. CASINOVI, M. FRAMONDINO, G. GRANDOLINI, F. MENICHINI, G. RANDAZZO and C. ROSSI, Experientia *28*, 126 (1972).

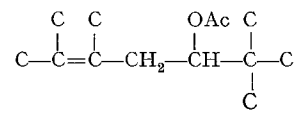
[6] All acetylations were performed with acetic anhydride-pyridine at room temperature.

[7] NMR-spectra were recorded on a Varian HA-100 apparatus with TMS as internal reference. Optical rotations were measured with a Perkin-Elmer 141 polarimeter on ethanolic solutions. Mass spectra were recorded on an AEI MS-902 spectrometer. Melting points are uncorrected. All compounds gave satisfactory elementary analyses.

AB part of the ABX system due to CH_2=CH–). Therefore the *dd* can be attributed to the C*H*(2′) OAc and compound F-V/2 must thus correspond to monodeacetylallofusicoccin (VII).

Compound F-VI was crystallized from ethylacetate and had m.p. 211–213° and $[\alpha]_D^{25}$ + 8.0 (c = 0.50). The dihydroderivative (M^+ 640), prepared by catalytic hydrogenation[8] of F-VI, was used for NMR- and NMDR-spectroscopy; a solution in $CDCl_3$ (26°) showed the anomeric proton as a *d* (J = 3.5 Hz) at 5.12 δ, coupled to a proton centred at 3.69 δ (overlapped by other resonances) a frequency which does not affect the *t* (1H, J = 9 Hz) resonating at 4.86 δ; the latter collapses to a broad *s* on irradiation around 3.85 δ, a value compatible with C*H*-3′ and C*H*-5′. Compound F-VI is thus monodeacetylisofusicoccin (VIII). As reported in a previous paper[5], compounds F-V/2 and F-VI are interconvertible on incubation at slightly alkaline pH values, both giving also rise to the third isomer (IX).

Compound F-V/4 was crystallized from aceton and had m.p. 186–188° and $[\alpha]_D^{25}$ + 11.5 (c = 0.49). In d_6-acetone (60°) it shows the signal expected for the C*H*-OAc at 4.91 δ as a *m* partially overlapping the AB part of the ABX system due to CH_2=CH– and clearly affected on irradiation at 2.33 δ. This value corresponds to the centre of the AB part (2 out of 8 lines are covered by the signals of acetone) of an ABX system which can be attributed to the following partial structure:



This was best evidenced in the dihydroderivative[8] of F-V/4, which in d_5-pyridine shows separate signals for the anomeric proton (5.44 δ, *d*, J = 3.5 Hz, collapsing to a *s* upon irradiation at 4.05 δ) and for the acetylated function (1H, 5.27 δ, *dd*, collapsing to a *d*, J = 5.0 Hz upon irradiation at 2.37 δ and J = 3.5 Hz upon irradiation at 2.88 δ; the latter two frequencies correspond each to the center of a four line system, which gives rise to a *d*, J = 16 Hz, upon irradiation at 5.27 δ). Therefore, compound F-V/4 is 12-*O*-acetyl-dideacetylfusicoccin (X).

Compounds F-V/1 andF-VIII have, respectively, 1 and 2 oxygen atoms less than fusicoccin in the aglycone moiety. Evidence so far obtained suggests that they correspond to XI and XII, respectively, but further work is still necessary to confirm unambiguously these structural assignments. Work concerning compounds F-II/2 and F-V/3 is also in progress[9].

Riassunto. Si dimostra che tre metaboliti secondari di *Fusicoccum amygdali* Del., presenti in piccola quantità nelle acque madri di cristallizzazione della fusicoccina, corrispondono a monodeacetilallofusicoccina (VI), monodeacetilisofusicoccina (VIII) e 12-*O*-acetil-dideacetilfusicoccina (X).

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I R_1, R_3=$COCH_3$; R_2, R_4, R_5=H

II R_1, R_4=$COCH_3$; R_2, R_3, R_5=H

III R_1=$COCH_3$; R_2, R_3, R_4, R_5=H

IV R_1, R_2, R_3, R_4, R_5=H

V R_1, R_2=$COCH_3$; R_3, R_4, R_5=H

VI R_1, R_2, R_3, R_4, R_5=$COCH_3$

VII R_2=$COCH_3$; R_1, R_3, R_4, R_5=H

VIII R_4=$COCH_3$; R_1, R_2, R_3, R_5=H

IX R_3=$COCH_3$; R_1, R_2, R_4, R_5=H

X R_5=$COCH_3$; R_1, R_2, R_3, R_4=H

XI R′= $-C(CH_3)_2-CH=CH_2$ R″=OH

XII R′=H R″=H

[8] Catalytic hydrogenations were performed with Pd on $BaSO_4$ at room temperature.

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