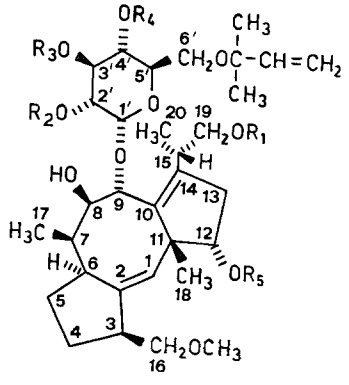
SPECIALIA

12-*O*-Acetylfusicoccin and 12-*O*-Acetylisofusicoccin, two New Minor Metabolites of *Fusicoccum amygdali* Del.

In previous papers[1–4] we have shown that culture filtrates of the phytotoxic fungus *Fusicoccum amygdali* Del. contain small amounts of compounds which differ only in the acetylation pattern from the main toxic metabolite called fusicoccin (I)[5–7]. Two of them, namely allofusicoccin (II)[3] and isofusicoccin (III)[2,3], are isomers of fusicoccin having *O*-Ac groups respectively in 2′ and 19, and in 4′ and 19; another five contain a single *O*-Ac group placed either in the aglycone (IV[1,2] and V[4]), or in the glucose unit (VI[4], VII[4] and VIII[8]). Finally, dideacetylfusicoccin (IX)[1,2] has also been isolated from the same source[9].

We now report the ocurrence in the culture filtrates of *F. amygdali* of 2 *O*-triacetates which correspond to 12-*O*-acetyl-fusicoccin (X) and 12-*O*-acetylisofusicoccin (XI).

Methods. NMR-spectra were recorded on a Varian HA-100 apparatus with TMS as internal reference. Rotations were measured for solutions in chloroform on a Perkin-Elmer 141 polarimeter. Mass spectra were recorded on an AEI MS-902 spectrometer operated at 70 eV. Melting points are uncorrected. The 2 triacetates were isolated by repeated column and thin layer chromatography on silica gel of appropriate fractions obtained by chromatographic fractionation of the residue left in ethyl acetate after crystallization of fusicoccin[1,2].



	R_1	R_2	R_3	R_4	R_5
I	Ac	H	Ac	H	H
II	Ac	Ac	H	H	H
III	Ac	H	H	Ac	H
IV	Ac	H	H	H	H
V	H	H	H	H	Ac
VI	H	Ac	H	H	H
VII	H	H	H	Ac	H
VIII	H	H	Ac	H	H
IX	H	H	H	H	H
X	Ac	H	Ac	H	Ac
XI	Ac	H	H	Ac	Ac
XII	Ac	Ac	Ac	Ac	Ac

Results and discussion. Compound X was obtained from fractions eluted slightly ahead of fusicoccin; it was crystallized from ethyl ether-light petroleum (40–70°), had m.p. 82–83° and $[\alpha]_D^{25}$ + 30.0° (c = 0.9). Compound XI corresponded to the substance indicated as F-II/2 in a previous paper[4]; it was crystallized from ethyl ether-light petroleum (40–70°), had m.p. 59° and $[\alpha]_D^{25}$ + 35.1° (c = 1.4). Both products yield triacetylfusicoccin (XII)[6] on acetylation with acetic anhydride-pyridine at room temperature, and dideacetylfusicoccin (IX)[2] on deacetylation with alkali; therefore, they differ from fusicoccin only for the position and/or the number of *O*-Ac groups. Their NMR-spectra (in $CDCl_3$) demonstrate, besides other features characteristic of fusicoccin and related compounds, the presence of 3 *O*-Ac groups. Their mass spectra show them to be isomers with molecular formula $C_{38}H_{58}O_{13}$ (M^+ 722); ions at *m/e* 405 and 205 locate in both compounds 2 *O*-Ac groups in the aglycone and 1 in the sugar moiety. The formation of triacetylfusicoccin (XII) on acetylation rules out the C-8 hydroxy group as a site of esterification; consequently both compounds carry *O*-Ac groups on C-12 and C-19 of the aglycone moiety and their difference must reside in the position of the *O*-Ac on the glucose unit. As compound X, at variance with compound XI, is not oxidized by periodate, the *O*-Ac must be placed on C-3′ in X and either on C-2′ or C-4′ in XI. NMDR data indicate that the third *O*-Ac in XI is on C-4′: in fact, on irradiation around 3.90 δ (a value compatible with C*H*-3′ and C*H*-5′) a *t* (J = 9 Hz, 1 H) centred at 4.72 δ collapses to a broad *s*; multiplicity and splitting are incompatible with C*H*(2)OAc and compare quite well with values found for C*H*(4′)OAc in isofusicoccin (III)[3] and monodeacetylisofusicoccin (IV)[4].

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[8] Compound VIII, previously reported as a product of alkaline isomerization of either VI or VII[3], has now been also isolated from culture filtrates of *F. amygdali*. It is eluted from silica gel columns between isofusicoccin and 19-deoxydideacetylfusicoccin[9].
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As previously oberved with other compounds of the fusicoccin series *O*-acetylated in the glucose moiety[3], compounds X and XI are interconvertible at basic pH values. The phytotoxicity of both compounds (determined by Professor A. GRANITI, University of Bari) is lower than that of fusicoccin[10].

Riassunto. Due nuovi metaboliti minori del fungo fitopatogeno *Fusicoccum amygdali* Del. vengono identificati come 12-*O*-acetilfusicoccina (X) e 12-*O*-acetilisofusicoccina (XI).

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[10] This work was supported in part by the Italian Research Council (CNR).

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Isolation of β-Ecdysone (20-Hydroxyecdysone) from the Parasitic Nematode *Ascaris lumbricoides*

The growth of nematodes involves a series of moults[1,2] similar to those marking the successive stages of arthropod development. It is generally accepted that these two widely divergent groups are likely to have evolved from a common ancestral stock. Thus it is of especial interest to establish whether moulting in nematodes is controlled by steroidal moulting hormones like the arthropod ecdysones, or by any similar steroids.

Free moulting hormones occur in insects at such low concentrations that large amounts of tissue are required for satisfactory isolation and characterization of the active substances. The highest concentrations of ecdysones appear in insects at the time of moulting (about 500 μg/kg), whereas during intermoult periods the amounts are much less (about 2 μg/kg)[3]. In the case of nematodes similar concentrations might be expected if ecdysones have a hormonal role.

The parasitic nematode *Ascaris lumbricoides* can be obtained in relatively large numbers from the intestines of pigs. Adults of both sexes, but of unknown age from the final moult, were thoroughly washed in water after collection. Pooled samples were stored frozen until analyzed for the presence of ecdysones. All equipment used for the subsequent extraction and purification procedures was carefully cleaned to avoid contamination of the *Ascaris* extracts with ecdysones of plant origin. The animals (15.5 kg) were thawed overnight and minced into ethanol (70 l). After 24 h at about 20 °C the alcohol was decanted and the residue extracted 3 times further with ethanol (50 l). The combined ethanol extracts were evaporated to an aqueous ethanol extract (11 l) which was twice extracted with hexane (1 l) to remove lipids. The aqueous ethanol phase was then concentrated to 3.5 l, water (3 l) added and the mixture extracted twice with *n*-butanol (3 l). The butanol extracts were combined, evaporated to dryness and partitioned between chloroform (2 l), methanol (3 l) and water (2 l) (2 tube separation). The combined methanol layers were evaporated to an aqueous residue of 750 ml, potassium hydrogen carbonate (20 g) added and the mixture extracted 3 times with an equal volume of butanol. The butanol extracts were in turn washed with an equal volume of water and the combined butanol extracts evaporated to an oily residue (5.6 g). Exploratory studies with this material indicated that ecdysones, if present, were too low in concentration to be detectable with the *Calliphora* bioassay[4]. Therefore to select fractions in the subsequent fractionation procedures which might contain β-ecdysone, tritium labelled β-ecdysone (370×10^3 cpm, 0.185 μg) was added. The labelled β-ecdysone was prepared by injecting [23, 23, 24, 24,-$^3H_4$]-α-ecdysone[5] into 3rd instar larvae of *Calliphora stygia* at the time of puparium formation and isolating the labelled β-ecdysone formed[6].

Further fractionation of the butanol extract by reversed phase partition chromatography[7] afforded a β-ecdysone fraction (64 mg) which was chromatographed on a column of silica gel (10 g, 15% water) made up and eluted with 96% ethanol-chloroform (14:86). The fractions containing the radioactivity were combined and chromatographed on CM-Sephadex[7]. It was then found on plotting the UV-absorption (at 254 nm) and the radioactivity against elution volume that the peak due to the radioactivity coincided with the peak due to UV-absorption. When the peak fractions were chromatographed on a column of silica gel (10 g, 15% water) made up and eluted with 96% ethanol-chloroform (10:90) the UV- and radioactivity curves again coincided closely. From the intensity of the UV-absorption λ *max* 242 nm in water) it was estimated that a total of 4.5 μg of ecdysone was present. When the material isolated was dissolved in 400 μg of water and bioassayed[4] in *C. stygia*, 73% sclerotization was found. These data indicate that a moulting hormone active substance, almost certainly β-ecdysone is present in the extract. Inokosterone, the only other known animal ecdysone with similar chromatographic properties is much less active in the *Calliphora* test[8]. α-Ecdysone could not be detected in the *Ascaris* extracts after similar fractionation steps.

While it is thus established that the *Ascaris* sample contained β-ecdysone, the amount isolated was exceed iongly small (0.3 μg/kg) and is about 1/10 the amount isolated from crayfish at an intermoult stage[9]. It is

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