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THE BIOSYNTHETIC ORIGIN OF FUSICOCCIN HYDROXYISOPROPYL GROUP†

A. EVIDENTE* and G. RANDAZZO

Istituto de Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone, 16, 80134 Napoli, Italy

and

A. BALLIO

Istituto di Chimica Biologica, Università di Roma, p. le A. Moro, 5, 00185 Roma, Italy

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Abstract—The biosynthetic origin of the hydroxyisopropyl group of fusicoccin **1** has been determined. Two out of the eight tritium mevalonoid atoms retained in **1** following incorporation of MVA-[2-$^3H_2$, 2-^{14}C] have been located at C-20; whilst none has been found in the C-19 methylene group.

Studies on fusicoccin[1] **1** biosynthesis, performed by a British[2] and an Italian[3] group, have (a) demonstrated that fusicoccin is a diterpenoid, not a rearranged sesterterpenoid, (b) elucidated the mechanism of geranylgeranyl pyrophosphate cyclization implying consecutive 1,2 - hydride shifts, (c) discriminated minor fusicoccins that are true intermediates in the biosynthetic pathway from those which represent shunt products.

The biosynthetic origin of HOH_2C-19 and H_3C-20, corresponding to the terminal methyl groups of the prenyl pyrophosphate, has remained an open question; in this paper we show that the methyl group is entirely derived from C-2 of mevalonic acid.

RESULTS AND DISCUSSION

A previous study[3a] had demonstrated that six out of the eight tritium atoms retained in **1**, following incorporation of MVA-[2-$^3H_2$, 2-^{14}C], are located in the aglycone moiety. In particular, one tritium was found at C-8[3a] and one at C-12;[3b] according to the isoprenoid rule, two are expected to be present at C-4 and two at either C-19 or C-20. Labelled **1**, prepared by feeding cultures of *Fusicoccum amygdali* Del. with MVA-[2-$^3H_2$, 2-^{14}C], was diluted with inactive material and chemically degraded to give the derivatives reported in Scheme 1. In two independent experiments, **1** was transformed, through known reactions,[1] into the aglycone and the corresponding 8,9 - isopropylidene derivative **2**.

In a first experiment, **2** was oxidized, by pyridinium chlorochromate, to the rather unstable 12,19 - dioxoderivative **3** which on reduction by $NaBH_4$ afforded a mixture of **2** and its 12-epimer **4**; this was fractionated by column chromatography and the epimers separately counted as 12,19 - dimesyl derivatives **6**[3b] and **5**, respectively (Scheme 1, route a).

The ^3H : ^{14}C ratios of **5** and **6** (Table 1) are consistent with the loss of one tritium atom on oxidation of **2**; the loss is from C-12, since it was previously demonstrated[3b] that the 19-tritylderivative of **2**,[1c] prepared from fusicoccin biosynthesized from MVA-[2-$^3H_2$, 2-^{14}C], loses one ^3H when it reacts with pyridinium chlorochromate to afford the corresponding 12-oxo-compound.[3b] In conclusion the presence of ^3H at C-19 is ruled out.

In a second experiment, **1** was converted, through known reactions,[3b] into the tetraene **7**. Oxidation of **7** with SeO_2 gave compound **8** (14% yield) which afford a monoacetate **10**. Spectroscopic data of compounds **8** and **10** allow the assignment of a 20-hydroxytetraene structure to **8**. Treatment of **8** with activated MnO_2 resulted in its quantitative conversion to the α,β-unsaturated aldehyde **9** (Scheme 1, route b). As shown in Table 1, 1.05 tritium atoms are lost on passing from **6** to **9**. This result is in reasonable agreement with the theoretical value of $1\frac{1}{3}$. In fact, hydroxylation of the C-20 methyl results in the statistical removal of $\frac{2}{3}$ of a tritium atom and the subsequent oxidation of HOH_2C-20 to the aldehyde causes a further 50% loss of $1\frac{1}{3}$ atoms.

The results of the degradations clearly show that two of the hydrogen atoms at C-20, and none at C-19, are derived from C-2 of mevalonate. Thus, the terminal methyl groups of the geranylgeranyl pyrophosphate retain their individuality in the formation of **1**, as demonstrated for other polycyclic diterpenes,[4] sesquiterpenes[5] and triterpenes.[6]

EXPERIMENTAL

General methods. Melting points are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter in $CHCl_3$ solutions. IR spectra were recorded on a Perkin–Elmer

Table 1. Radioactivity (^3H) retained in some derivatives prepared from fusicoccin isolated from cultures of *Fusicoccum amygdali* Del. supplemented with MVA - [2 - $^3H_2$, 2 - ^{14}C]

Compound	Experiment	$^3H/^{14}C$	Atom ratio
2	1	6.45	6.00*
5	1	5.33	4.96
6	1	5.14	4.78
6	2	4.45	6.00*
9	2	3.67	4.95

*Theoretical values based on previous results.[3a]

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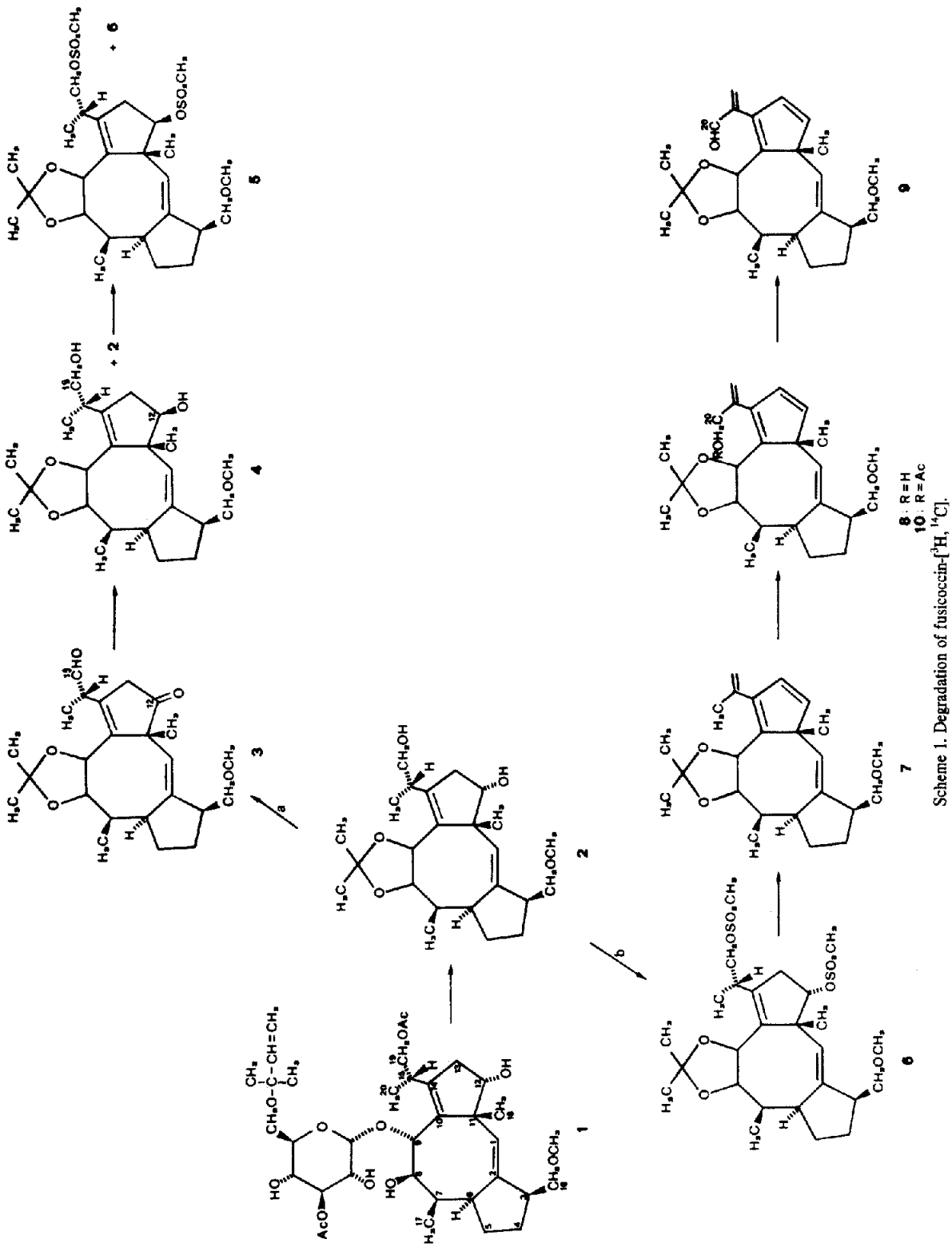

Scheme 1. Degradation of fusicoccin-[^{3}H, ^{14}C].

399 instrument for solutions in $CHCl_3$. UV spectra were measured for solutions in CH_3CN or EtOH on a Varian Cary 210 spectrophotometer. ^1H NMR spectra were recorded at 270 MHz on a Bruker spectrometer in $CDCl_3$ using TMS as internal standard; chemical shifts are in ppm. Mass spectra were recorded on an AEI-902 mass spectrometer at 70 eV. Analytical and preparative TLC were performed on silica gel plates (Merck, Kieselgel 60 F_{254} 0.25 and 2 mm, respectively) and spots were developed by spraying with a 10% H_2SO_4 solution in MeOH and then heating at 105°C. Column chromatography was carried out on silica gel (Merck, Kielselgel 60 0.063–0.2 mm).

Feeding and product isolation. Fermentations were carried out according to a published procedure[7] scaled down to 500 ml Erlenmeyer flasks containing 100 ml of medium. Specific activities of the labelled precursors were the highest commercially available (Amersham). Labelled **1**, prepared from two independent fermentations and separately used for experiments 1 and 2, was extracted with $CHCl_3$, diluted with unlabelled **1** and purified by column chromatography to give about 1220 and 187 dpm mg^{-1} (batch 1), 952 and 215 dpm mg^{-1} (batch 2) for ^3H and ^{14}C, respectively. Radioactivity measurements were carried out with a Beckman LS 8100 liquid scintillation counter, using a solution containing 6.5 g of PPO, 130 mg of POPOP and 104 g of naphthalene in 1000 ml of toluene-dioxane (1:1 v/v) as scintillant. All compounds were crystallized to constant specific activity.

12,19 - *dioxo* - 8,9 - *isopropylideneaglycone of fusicoccin* **3**. Pyridinium chlorochromate[8] (4.73 g) was added to a soln of **2** (150 mg) in dry CH_2Cl_2 (10 ml); the mixture was stirred at room temp. and monitored by TLC ($CHCl_3$-isopropanol 95:5 v/v). After 4 h, when all the starting material had reacted, the reaction was stopped with anhydrous EtO_2 (20 ml). The mixture was filtered through a short SiO_2 column which was then washed with the same solvent. The combined filtrates and washings were evaporated under reduced pressure. Purification of the residue by preparative TLC ($CHCl_3$-isopropanol 95:5 v/v) afforded an oil (103 mg, 68% yield) which crystallized from pet. ether (b.p. 40–70°): m.p. 115–118°; $[\alpha]_D^{25} = -42.3$ ($c = 0.95$); UV (CH_3CN), $\lambda_{max}(\epsilon)$: 294 nm (640); IR, ν_{max}: 2740, 1750, 1730 cm^{-1}; ^1H NMR δ: 0.81 (3H, d, $J = 7$ Hz, 20-Me), 1.1 (3H, d, $J = 7$ Hz, 17-Me), 1.20 (3H, s, 18-Me), 1.27 and 1.39 (3H each, s, two Me of isopropylidene group), 2.45 (1H, d, $J = 24$ Hz, 13-H), 2.87 (1H, d, $J = 24$ Hz, 13-H), 3.20 (3H, s, OMe), 3.80 (1H, dd, $J = 10$ and 5 Hz, 8-H), 4.15 (1H, m, 15-H; 1H, m, 9-H; by ^1H NMDR), 5.25 (1H, t, $J = 1.5$ Hz, 1-H) and 9.4 (1H, s, 19-H); MS *m/e*: 402 (25%, M^+), 373 (50), 344 (50), 315 (100) and 283 (81).

12 - epi -8,9 - *isopropylideneaglycone of fusicoccin* **4**. Compound **3** (103 mg) in MeOH (10 ml) was stirred with $NaBH_4$ (206 mg) at room temp. TLC ($CHCl_3$-isopropanol 95:5 v/v) showed that after 30 min all the starting material had been reduced. After decomposition with 0.1 *N* HCl of borohydride excess, the mixture was diluted with water (200 ml) and extracted with CH_2Cl_2 (3 × 60 ml). The combined extracts were washed with water and dried (Na_2SO_4). Evaporation of the solvent gave an oily residue consisting of **2** and its 12-epiderivative **4**. Column chromatography of the mixture eluted with $CHCl_3$-isopropanol 95:5 v/v afforded **2** (29 mg, 33% yield) followed by **4** (48 mg, 47%) yield); the latter crystallized from Et_2O-*n*-hexane: m.p. 199–200°; $[\alpha]_D^{25} = -33.9$ ($c = 4.41$); UV (EtOH), λ_{max}: < 220 nm; IR, ν_{max}: 3450, 1600 cm^{-1}; ^1H NMR, δ: 0.92 (3H, d, $J = 7$ Hz, 17-Me), 0.96 (3H, d, $J = 7$ Hz, 20-Me), 1.12 (3H, s, 18-Me), 1.35 and 1.54 (3H each, s, two Me of isopropylidene group), 2.11 (1H, dd, $J =$ 18 and 6 Hz, 13-H), 2.57 (1H, dd, $J = 18$ and 6 Hz, 13-H), 3.35 (3H, s, OMe), 3.55 (1H, m, 15-H), 3.95 (1H, m, 8-H; 1H, m, 12-H; by ^1H NMDR), 4.18 (1H, d, $J = 9$ Hz, 9-H), and 5.54 (1H, t, $J =$ 1.5 Hz, 1-H); MS *m/e*: 406 (5.4%, M^+), 391 (1.5), 388 (3.6), 348 (13), 330 (31) and 310 (100).

12 - epi - 12,19 - *dimesyl derivative of* 8,9 - *isopropylideneaglycone of fusicoccin* **5**. The compound **5** was prepared from 48 mg of **4** according to a reported procedure.[3b] The reaction afforded 67 mg of a solid (99% yield) which crystallized from Me_2CO-pet. ether (b.p. 40–70°): m.p. 158–159°; $[\alpha]_D^{25} = -14.6$ ($c = 5.13$); UV (CH_3CN), λ_{max}: < 220 nm; IR, ν_{max}: 3450, 1600 cm^{-1}; ^1H NMR, δ: 0.94 (3H, d, $J = 7$ Hz, 17-Me), 1.12 (3H, d, $J = 7$ Hz, 20-Me), 2.46 (1H, dd, $J = 15$ and 6 Hz, 13-H), 2.82 (1H, dd, $J = 15$ and 6 Hz, 13-H), 3.04 (3H, s, O_2SMe), 3.06 (3H, s, O_2SMe), 3.39 (3H, s, OMe), 3.92 (1H, dd, $J = 10$ and 6 Hz, 8-H), 4.77 (1H, dd, $J =$ 6 Hz, 12-H). The other proton resonances are very similar to those of **6**; MS, *m/e*: 562 (8.8%, M^+), 547 (1.6), 530 (0.8), 504 (3.5), 472 (8.8), 466 (26), 451 (13) and 79 (100).

20 - *hydroxytetraene* **8**. A soln of **7** (310 mg) in EtOH (51 ml) was heated under reflux with SeO_2 (993 mg); the reaction was followed by TLC (*n*-hexane - EtOAc 7:3 v/v). After 3.5 h the mixture was poured into H_2O (250 ml) and then extracted with Et_2O (3 × 100 ml). The combined extracts, after washing with a satured $NaHCO_3$ solution followed by water, were dried (Na_2SO_4). Evaporation of the solvent gave a brown oil containing at least four chromatography distinguishable compounds. Column chromatography (*n*-hexane-EtOAc 7:3 v/v) of the mixture separated the main component from unreacted material and minor reaction products. Further purification by preparative TLC, eluted with same solvent, afforded a crystalline solid (44 mg, 14% yield): m.p. 86–88°; $[\alpha]_D^{25} = +120.6$ ($c = 1.24$); UV (EtOH), λ_{max} (ϵ): 274 nm (1987); IR, ν_{max}: 3420, 1610 cm^{-1}; ^1H NMR, δ: 0.95 (3H, d, $J = 7$ Hz, 17-Me), 1.36 (3H, s, 18-Me), 1.39 and 1.47 (3H each, s, two Me of the isopropylidene group), 2.06 (1H, d, $J = 7$ and 4.4 Hz 7-H), 2.64 (1H, m, 3-H; 1H, m, 6-H; by ^1H NMDR), 3.36 (3H, s, OMe), 4.13 (1H, dd, $J = 9.6$ and 4.4 Hz, 8-H), 4.22 (2H, m, 20-H), 4.48 (1H, d, $J = 9.6$ Hz, 9-H), 5.06 (1H, d, $J = 1.8$ Hz, 19-H), 5.27 (1H, d, $J = 1.8$ Hz, 19-H), 5.52 (1H, t, $J = 1.8$ Hz, 1-H), 6.06 and 6.27 (1H-12 and 1H-13, d, $J = 5.5$ Hz); MS, *m/e*: 386 (94%, M^+), 368 (26), 343 (28), 328 (47) and 311 (100).

20 - *oxotetraene* **9**. The compound **8** (44 mg) in dry *n*-hexane (35 ml) containing active MnO_2 (250 mg) was stirred at room temp. The reaction was monitored by TLC (*n*-hexane-EtOAc 7:3 v/v); after 4 h all **8** was reacted. The mixture was filtered through Celite and this was washed with *n*-hexane (300 ml). The oily residue left after evaporation of the combined filtrates was purified by preparative TLC (*n*-hexane-EtOAc 7:3 v/v) to afford a crystalline solid (41 mg, 94% yield) which was recrystallized from *n*-hexane: m.p. 104–105°; $[\alpha]_D^{25} = +42.2$ ($c = 0.45$); UV, (EtOH), λ_{max} (ϵ): 274 nm (2057); IR, ν_{max}: 1705 cm^{-1}; ^1H NMR, δ: 1.27 and 1.37 (3H each, s, two Me of the isopropylidene group), 1.35 (3H, s, 18-Me), 2.62 (1H, m, 3-H; 1H, m, 6-H; by ^1H NMDR), 3.84 (1H, dd, $J = 9.9$ and 4.4 Hz, 8-H), 4.48 (1H, d, 9.9 Hz, 9-H), 6.25 (1H, d, $J = 1.8$ Hz, 19-H), 6.45 (1H, d, $J =$ 1.8 Hz, 19-H), 9.62 (1H, s, 20-H); the other proton resonances are very similar to those of **8**; MS, *m/e*: 384 (59%, M^+), 339 (41), 327 (100) and 281 (35).

20 - *acetyltetraene* **10**. Acetylation of **8** (4.6 mg) with $(CH_3CO)_2O$/Py afforded the 20-acetyl derivative; the usual work-up gave an oil (5.2 mg): IR, ν_{max}: 1725 cm^{-1}; ^1H NMR, δ: 2.05 (3H, s, OCMe), 4.76 (2H, m, 20-H), 5.30 (1H, d, $J = 1.8$ Hz, 19-H), 5.34 (1H, d, $J = 1.8$ Hz, 19-H); the other proton resonances are very similar to those of **8**.

Nomenclature - 12,19 - *dioxo* - 8,9 - *isopropylideneaglycone of fusicoccin* **3**. Dicyclopenta [3,4:6,7] cycloocta [1,2 - d] - 1,3 - dioxole - 4 - ethanal - 6(3a*H*) - one - 5,6a,8,9,10,10a,11,11a - octahydro - 8 - (methoxymethyl) - β,2,2,6a,11 - pentamethyl. [3aR - [3aα, 4(S*),6aβ,8β,10aα,11β,11aβ]]. 20 - *oxotetraene* **9**: Dicyclopenta [3,4:6,7] cycloocta [1,2 - d] - 1,3 - dioxole - 3a,6a,8,9,10,10a,11,11a - octahydro - 8 - (methoxymethyl) - 2,2,6a,11 - tetramethyl - 4 - (1 - al - ethenyl). [3aR[3aα,(S*),6aβ,8β,10aα,11β,11aβ].]

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