COMMUNICATION

Isolation of (+)-fusicocca-2,10(14)-diene, a 5-8-5 tricyclic diterpene hydrocarbon biosynthetically related to the fusicoccin aglycon from *Fusicoccum amygdali* and confirmation of its structure by total synthesis

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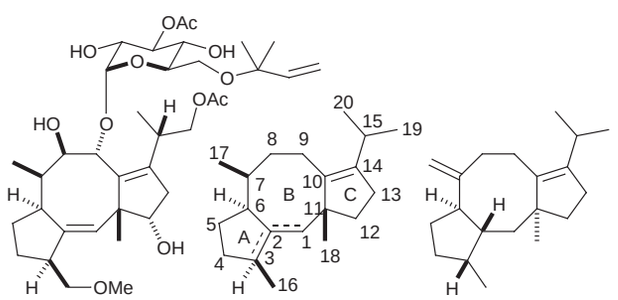
(+)-Fusicocca-2,10(14)-diene, a double bond isomer of the putative fusicoccin biosynthetic intermediate, *i.e.*, fusicocca-1,10(14)-diene, was isolated for the first time as a main hydrocarbon constituent from the fusicoccin-producing fungus *Fusicoccum amygdali*, and its stereostructure including absolute configuration was unambiguously confirmed by total synthesis.

Fusicoccin A (**1**),[1] a fusicoccane-diterpene glucoside from the fungus *Fusicoccum amygdali*, possesses potent H^+-ATPase activating activity,[2] whose action of mechanism has recently been demonstrated by binding with a 14-3-3 protein in plant signal transductions.[3] Fusicoccane is one of the tricyclic diterpenes having a fused 5-8-5 ring system. Fusicoccin biosynthesis has been investigated in detail using [3-^{13}C,4-$^{2}H_2$]mevalonolactone, suggesting the presence of fusicocca-1,10(14)-diene (**2**) as its



fusicoccin A (**1**) **2** : Δ^1, **3** : Δ^2 **4**

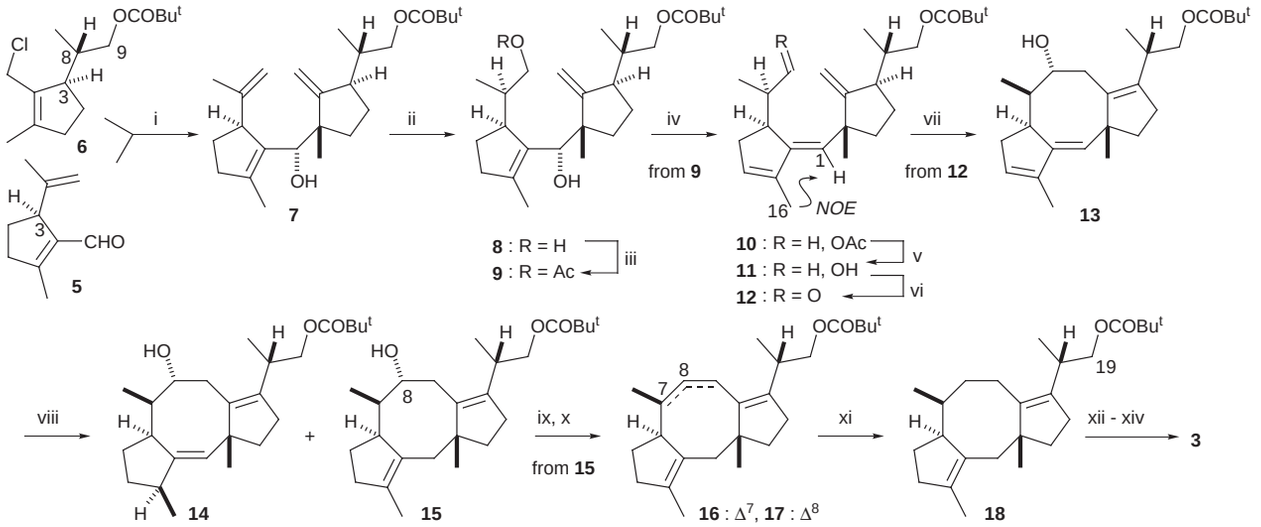
biosynthetic hydrocarbon intermediate.[4] No fusicoccane hydrocarbons, however, have been isolated from this fungus or from other natural sources, although cycloaraneosene (**4**), which has the antipodal configuration at the quaternary carbon (C-11) of the fusicoccane framework, has been isolated.[5] It is quite important to identify the initially-formed fusicoccane hydrocarbon in the biosynthesis of fusicoccins especially for cloning of the cDNA encoding fusicoccane synthase as a fungal diterpene cyclase.[6] We report here the first isolation of fusicocca-2,10(14)-diene (**3**), a double bond isomer of **2**, from *F. amygdali* and its stereocontrolled synthesis from two optically active iridoid derivatives.

The fungus strain F6† was cultured at 25 °C for 5 days in constantly shaken 500 cm^3 Sakaguchi flasks each containing 100 cm^3 medium of 8.0% commercial sugar, 1.0% corn steep liquor, 0.5% peptone and 0.5% NaCl. Mycelia obtained from 40 flasks were treated with acetone, and the aqueous acetone solution obtained by filtration was concentrated *in vacuo* and then extracted with EtOAc at pH 9.0. The residue of the EtOAc extract was dissolved in acetonitrile and partitioned between acetonitrile and hexane to yield a pale yellow oil (750 mg) from the latter. This oil was carefully separated by silica gel flash chromatography using hexane as the eluent; fusicoccane hydrocarbons were monitored on TLC with an authentic sample of fusicocc-2-ene synthesized previously.[7] The main terpene-like hydrocarbon was isolated as a colorless oil {2.9 mg, $[a]_D^{20}$ +20.0 (*c* 0.16, $CHCl_3$)}. It exhibited a molecular ion peak at *m/z* 272.2460 ($C_{20}H_{32}$ requires 272.2504) in the mass spectrum [*m/z* 272 (M^+, 5%), 229 (10), 135 (100), 122 (63) and 95 (41)] and showed characteristic ^{1}H NMR (400 MHz, $CDCl_3$) signals at δ_H 0.86 (d, 3H, *J* 7 Hz, H-17), 0.92 and 0.98 (d, each 3H, *J* 7 Hz, H-19/20), 0.93 (s, 3H, H-18), 1.60 (br s, 3H, H-16), 1.95 and 2.30 (ABq, 2H, *J* 13 Hz, H-1), *ca.* 2.49 (m, 1H, H-6) and 2.62 (septet, 1H, *J* 7 Hz, H-15), indicating that the structure of this hydrocarbon ‡ is compatible with fusicocca-2,10(14)-diene (**3**).

To obtain concrete proof of the structure including absolute configuration, the total synthesis of **3** was carried out independently. At first, similar to our earlier studies,[8] the C_{10}-synthon for the A-ring, (3*S*)-irida-1,8-dien-7-al (**5**),[9] was condensed with another C_{10}-synthon for the C-ring, (3*R*,8*S*)-7-chloro-9-(trimethylacetoxy)irid-1-ene (**6**),[9] which carries an additional functional group at C-9 (C-19 in the fusicoccane framework) for the purpose of obtaining isotope-labelled compounds of **2** and **3** in future, by use of low-valent chromium species[10] in DMF to give the desired condensate **7**. The site- and stereo-selective hydroboration of **7** gave **8** and the subsequent protection of its primary hydroxy group led to **9**. Dehydration of the allylic secondary hydroxy group at C-1 of **9** cleanly gave **10** by treatment with BF_3·OEt_2 in THF. The requisite *E*-configuration of the C1–C2 double bond was confirmed by a nuclear Overhauser effect from the C-16 allylic methyl to the C-1 olefinic proton. Deprotection and oxidation of the C-8 hydroxy group gave aldehyde **12** *via* **11**. Intramolecular ene-reaction on **12** proceeded smoothly to give the desired tricyclic fusicoccane derivative **13** catalyzed by H^+. Catalytic hydrogen-

† This fungus produced large amounts (60–120 μg ml^{-1}) of fusicoccins A and J in the culture broth.

‡ ^{13}C NMR (100 MHz, $CDCl_3$) data for **3**: δ_C 15.50 (CH_3), 20.98 (CH_3), 21.24 (CH_3), 21.31 (CH_3), 21.33 (CH_2), 22.69 (CH_2), 26.69 (CH_3), 26.75 (CH_2), 27.04 (CH), 29.95 (CH), 32.27 (CH_2), 36.71 (CH_2), 38.60 (CH_2), 39.35 (CH_2), 51.65 (C), 54.69 (CH), 132.14 (C), 137.19 (C), 139.66 (C) and 140.50 (C).



Scheme 1 *Reagents and yields*: i, $CrCl_3$–LAH, DMF, 67%, ii, BBN then H_2O_2–3 M NaOH, THF; iii, Ac_2O–py, 77% from **7**; iv, BF_3·OEt_2, THF, 95%; v, K_2CO_3, MeOH; vi, $(ClCO)_2$, DMSO then Et_3N, CH_2Cl_2, 90% from **10**; vii, AcCl, wet CH_2Cl_2, 84%; viii, H_2–Ir black, Bu^{t}OH, **14** (67%) and **15** (27%); ix, MsCl, py; x, LiBr, Li_2CO_3, DMF, 86% from **15**; xi, H_2–Ir black, Bu^{t}OH, 89%; xii, LAH, THF; xiii, MsCl, py; xiv, $LiBEt_3H$, THF, 89% from **18**

ation of the conjugated diene moiety of **13** using iridium black as catalyst resulted in the formation of two positional isomers, **14** and **15**, which were easily separated§ by silica gel column chromatography. Hydrogenation of a mixture of the trienes, **16** and **17**, obtained from **15** by dehydrative treatments on its C-8 hydroxy group, afforded **18** as the sole product. Finally, the hydroxy group at C-19 was removed by $LiBEt_3H$ reduction of the corresponding mesylate to complete the total synthesis of **3**, which was identical with natural **3** in all physicochemical properties including optical rotation {$[\alpha]_D^{20}$ +20.3 (*c* 0.80, $CHCl_3$)}.

Thus, the structure of the main hydrocarbon constituent from *F. amygdali* was confirmed to be **3**. Although it appears possible, **3** should not be an artifact arising from **2** during the isolation processes, because (1) fusicoccins A and J[11] were isolated stably together with **3**, (2) no double bond isomers of fusicoccins have been isolated yet even as artifacts and (3) **14** and **15** are easily separable and stable compounds on a silica gel column. The possibility of the isomerization of **2** to **3** *in vivo* cannot be denied, however, and, therefore, isolation of **3** does not necessarily mean that this is the biosynthetic intermediate. Compound **2** is apparently an alternative as has been proposed. Efforts on the synthesis of **2** from the promising precursor **14** are currently under way. Since isotope-labelled compounds of **2** and **3** will be obtained by manipulation of the additional functional group at C-19, their incorporation experiments into fusicoccins by the fungus should resolve the ambiguity.

Experimental

Intramolecular ene-reaction forming the 8-membered ring

A solution of **12** (206 mg, 0.533 mmol) in CH_2Cl_2 (20 cm^3) was treated with a catalytic amount of HCl, generated *in situ* by addition of AcCl (*ca*. 5 mg), at room temperature for 7 h. The reaction was quenched by addition of aqueous $NaHCO_3$ and the organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on a silica gel column (hexane–EtOAc 5:1) to give the colorless oily **13** {172 mg, 0.445 mmol, 83.5%, $[\alpha]_D^{25}$ −63.8 (*c* 7.96, $CHCl_3$)}.

§ In spite of being simple double bond isomers, **14** and **15** were well separated on a silica gel column probably due to the existence of the hydroxy group on the eight-membered ring (at C-8); *i.e.*, the eight-membered ring conformations in **14** and **15** may be different from each other.

Iridium black-catalyzed hydrogenation

Into a solution of **13** (172 mg, 0.445 mmol) in Bu^{t}OH (6 cm^3) was added iridium black (85 mg), and the mixture was stirred for 20 h under H_2 atmosphere. The suspension was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc 15:1 then 10:1) to afford two colorless oily products, **15** {46 mg, 0.118 mmol, 26.5%, $[\alpha]_D^{25}$ +26.8 (*c* 1.31, $CHCl_3$), R_f 0.22 on TLC (hexane–EtOAc 10:1)} and **14** {115 mg, 0.296 mmol, 66.5%, $[\alpha]_D^{25}$ +30.7 (*c* 3.26, $CHCl_3$), R_f 0.14 on TLC (hexane–EtOAc 10:1)}.

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