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# Transformation of fusicocca-2,10(14)-dien-8β-ol into fusicoccin J by the fusicoccin-producing fungus, *Phomopsis (Fusicoccum) amygdali*. Support for the intermediacy of fusicocca-2,10(14)-diene in the fusicoccin biosynthesis

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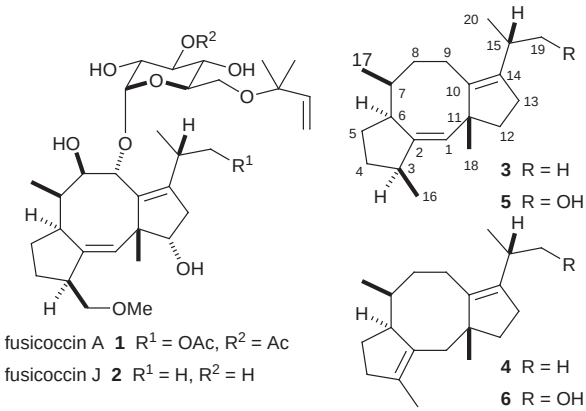
**(+)-Fusicocca-2,10(14)-diene, isolated recently by the authors, is most likely a genuine hydrocarbon intermediate in the biosynthesis of fusicoccin, as its 8β-hydroxy derivative is smoothly and efficiently converted into fusicoccin J by the fusicoccin-producing fungus, *Phomopsis (Fusicoccum) amygdali* F6.**

Fusicoccin A (**1**)[1] and J (**2**)[2] and their congeners, produced by the phytopathogenic fungus *Phomopsis (Fusicoccum) amygdali*, possess potent $H^+$–ATPase activating activity.[3] Their




action is at the center of interest, since the fusicoccin-binding 14-3-3 proteins are regarded as key proteins in intracellular signal transductions in both plant and animal cells.[4]

Earlier studies on the fusicoccin biosynthesis have postulated fusicocca-1,10(14)-diene **3** as the hydrocarbon intermediate.[5] However, we have recently isolated fusicocca-2,10(14)-diene **4**, a double bond isomer of **3**, from *P. amygdali* F6† as a main hydrocarbon constituent.[6] This fact called our attention to the initially-forming fusicoccane hydrocarbon in the fusicoccin biosynthesis. To clarify the true hydrocarbon intermediate, feeding experiments of synthetic derivatives of both **3** and **4** have been carried out. The results reported here strongly suggest that **4**, not **3**, is the genuine hydrocarbon.

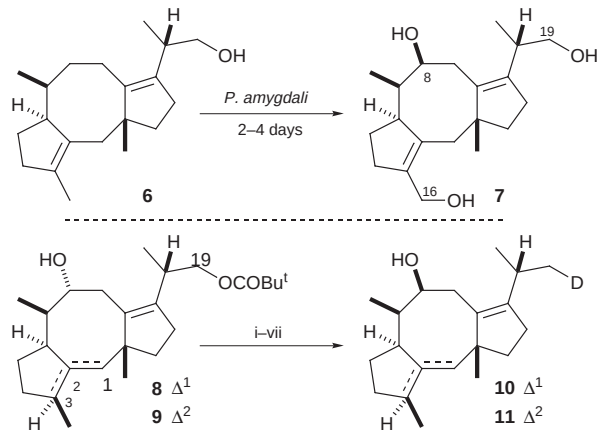
For feeding experiments of synthetic intermediary substrates, we chose hydrophilic monohydroxylated derivatives of **3** and **4**, which should have suitable solubility in the culture medium. As a preliminary study, feeding experiments (*vide infra*) using fusicocca-1,10(14)-dien-19-ol **5** and its isomer **6**[6] were carried out. Feeding of **5** showed no significant formation of the corresponding metabolites and the production of fusicoccins fell off clearly as monitored on TLC with a control mixture of metabolites, indicating that **5** seemed to perturb the biological processes. On the other hand, **6** gave a new metabolite {$[\alpha]_D^{22}$ + 35.8 (*c* 0.30, $CHCl_3$)}, whose structure was elucidated as fusicocca-2,10(14)-diene-8β,16,19-triol **7** from its spectral data (Scheme 1).‡

These results led the authors to suspect that 8β-hydroxylation at the non-allylic position occurs at an early stage of the biosynthesis. Therefore, we turned our attention to the transformations of the 8β-hydroxylated derivatives of **3** and **4**. The 8α-hydroxy-substituents of **8** and **9**[6] were epimerized by an oxidation–reduction process. Then, the 19-hydroxy group was removed by $LiEt_3BD$ reduction of the corresponding mesylate providing the deuterated compounds **10** and **11**.

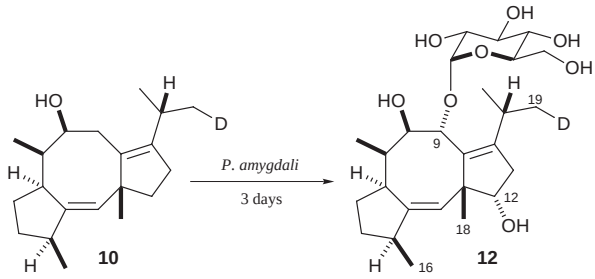
A solution of 60 mg of **10** or **11** in a small amount of EtOH was added equally (10 mg each) to six cultures of the fungus strain F6 which had been growing for 2 or 3 days at 25 °C.§ After additional cultivation for 3 days, the combined culture filtrate was extracted with EtOAc at pH 9.0.

From the feeding experiment of **10**, which has the 1,10(14)-diene system typical for fusicoccin, 50 mg of **2** and 13 mg of a new non-natural metabolite {mp 126–127 °C, $[\alpha]_D^{22}$ + 16.6 (*c* 0.41, $CHCl_3$)} were obtained. The structure of the latter was elucidated to be 16-demethoxy-6′-*O*-de-*tert*-pentenyl [19-$^2H_1$]fusicoccin J (**12**) from its spectral data (Scheme 2).

The molecular formula is $C_{26}H_{41}DO_8$; FAB MS: *m/z* 506.2844 [(M + Na)$^+$, $C_{26}H_{41}DO_8Na$ requires, 506.2840]. $^1H$ and $^{13}C$ NMR ($CDCl_3$–$CD_3OD$ 5 : 1, +40 °C, 600 and 150 MHz, respectively) signals are fully assignable by 2D measurements. Diagnostic signals are as follows; $\delta_H$ 1.02 (2H, br d, *J* 6.8; H19, deuterated methyl), 1.09 (3H, d, *J* 7.0; H16), 1.18 (3H, s; H18), 3.18 (sextet, *J* 6.8; H15), 3.46 (dd, *J* 9.5, 9.3; H4′), 3.50 (dd, *J* 9.5, 3.8; H2′), 3.63 (ddd, *J* 9.5, 3.5, 3.3; H5′), 3.67 (dd, *J* 11.7, 3.5; H6′), 3.72 (t, *J* 6.2; H12), 3.75 (dd, *J* 9.5, 9.3; H3′), 3.77 (dd,




**Scheme 1** *Reagents and conditions*: i, $(ClCO)_2$, DMSO then $Et_3N$, $CH_2Cl_2$ ($\Delta^1$, 86%; $\Delta^2$, 97%); ii, DIBAL-H, THF [$\Delta^1$, 24% (**8**, 42%); $\Delta^2$, 52% (**9**, 15%)]; iii, TBDMSCl, imidazole, $CH_2Cl_2$; iv, LAH, THF; v, MsCl, Py (3 steps, $\Delta^1$, 83%; $\Delta^2$, 73%); vi, $LiEt_3BD$, THF; vii, $Bu_4NF$, THF (2 steps, $\Delta^1$, 87%; $\Delta^2$, 86%).




**Scheme 2**

*J* 11.7, 3.3; H6′), 3.80 (d, *J* 9.9; H9), 3.92 (dd, *J* 9.9, 4.6; H8), 4.95 (d, *J* 3.8; H1′) and 5.20 (t, *J* 1.8; H1); $\delta_C$ 19.73 (C16), 21.01 (t, C19, deuterated carbon), 61.83 (C6′), 70.59 (C4′), 71.82 (C5′), 72.37 (C2′), 73.90 (C3′), 77.55 (C9), 78.62 (C8), 80.22 (C12) and 101.87 (C1′). These data clearly reveal that **12** was generated only from feeded **10**; the C9 and C12 positions were hydroxylated; α-glucosidation occurred at C9. The C16 methyl was preserved intact. The stereochemistry of the hydroxylated positions was confirmed with NOE enhancements of H9 and H12 upon irradiation of H18. The glucosidic linkage between C1′ and C9 was deduced from HMBC correlations of C9/H1′ and C1′/H9. Thus, although **12** is a fusicoccin type-glucoside, it lacks the hydroxylation of its C16-methyl group. The fusicoccin J (**2**) obtained in this feeding experiment was natural; no deuterated substance was detected by NMR spectroscopy. Identification of **12** suggests that 12α-hydroxylation of fusicoccin precedes the prenylation of its glucosyl moiety.

The result obtained from the feeding experiment using **11** was more striking and conclusive for the elucidation of the biosynthetic pathway of **2**. Fusicoccin J (**2**, 51 mg) isolated from the culture broth of this experiment was found to have a remarkable amount of the deuterated substance (**2**-*d*). Incorporation of **11** into **2**-*d* was easily recognized by $^1$H, $^{13}$C NMR ($CDCl_3$, 600 and 150 MHz, respectively) and mass spectra. The distinguishable signals between **2** and **2**-*d* are $\delta_H$ 1.04 (2H, br d, *J* 6.8; **2**-*d* H19, deuterated methyl)/1.06 (3H, d, *J* 6.8; **2**-H19) and 3.169 (sextet, *J* 6.8; **2**-*d* H15)/3.174 (septet, *J* 6.8; **2** H15); $\delta_C$ 20.44 (**2**-*d* C20)/20.47 (**2** C20), 20.98 (t, **2**-*d* C19, deuterated carbon)/21.27 (**2** C19) and 27.78 (**2**-*d* C15)/27.86 (**2** C15). In the FAB mass spectrum, the ratios of **2** $(M + H)^+$/**2**-*d* $(M + H)^+$ and **2** $(M + Na)^+$/**2**-*d* $(M + Na)^+$ are close to 1 : 1; *m/z* 581 (7.9%)/582 (7.9%) and 603 (6.3%)/604 (6.7%); FAB-MS: *m/z* 603.3513 ($C_{32}H_{52}O_9Na$ requires, 603.3509) and 604.3577 ($C_{32}H_{51}DO_9Na$ requires, 604.3572). At least 40% of fusicoccin J was derived from the feeded substrate **11**, the double bond of which had originally been located at the C2–C3 position.

Thus, these results clearly demonstrate that the C16 methyl has to be allylic for its conversion into the methoxymethyl group. Therefore **4**, actually isolated from mycelia of the fungus, is most likely the genuine hydrocarbon intermediate in the biosynthetic pathway of fusicoccin.

Our interests are currently focused on the identification of non-deuterated **11** from the fungus and on the chemical mechanism of the double bond isomerization occurring during the conversion of **11** to **2**. The transformation from **10** to **12** by the fungus can be regarded as the creation of an 'artificial fusicoccin' from a '*pseudo*-biosynthetic intermediate'. Efforts on this line are also in progress.

## Notes and references

† The fungus strain F6 produces fusicoccin J as a main metabolite (*ca*. 120 μg $ml^{-1}$) in the culture medium indicated below.
‡ Details will be reported in a full paper.
§ The composition of the culture medium was as follows; 8.0% commercial sugar, 1.0% corn steep liquor, 0.5% peptone and 0.5% NaCl in 100 ml of deionized water .

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