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Microbial Transformation of the Diterpene 7-*epi*-Foliol by *Fusarium fujikuroi*

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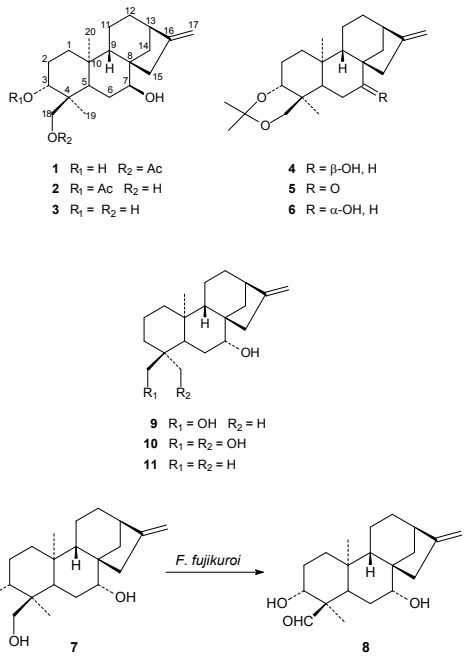
The incubation of 3α,7α,18-trihydroxy-*ent*-kaur-16-ene (7-*epi*-foliol) with the fungus *Fusarium fujikuroi* gave 3α,7α,18-trihydroxy-*ent*-kaur-16-en-18-al as the sole product. The biotransformation of other 7α- or 7β-hydroxy derivatives had led to the oxidation of C-19, which is a main step in the biosynthesis of gibberellins and kaurenolides. Now, the presence of the 3α-hydroxyl impedes that oxidation, which is directed to the adjacent C-18 hydroxymethyl forming the corresponding aldehyde.

Keywords: *Fusarium fujikuroi*, Biotransformations, Diterpenes, *ent*-Kaur-16-enes, 7-*epi*-Foliol, 18-Oxo-7-*epi*-foliol.

During the last several years we have been interested in the microbiological transformation of *ent*-kaurenes and other tetracyclic diterpenes by the fungus *Fusarium fujikuroi* (*Gibberella fujikuroi)*. The main aims of these works have been to determine the specificity in the substrate of the enzymes involved in the biosynthesis of gibberellins, to prepare new gibberellin analogues, and to study the biosynthetic pathway of these plant hormones [1-3]. Thus, we have carried out the biotransformation by this fungus of 7α-hydroxyditerpenes such as 7α-hydroxy-*ent*-kaur-16-ene and 7α-hydroxy-*ent*-atis-15-ene [4,5] and 3α-hydroxy derivatives such as 3α-hydroxy-*ent*-kaur-16-ene, 3α,18-dihydroxy-*ent*-kaur-16-ene and 3α,15β-dihydroxy-*ent*-kaur-16-ene [6,7]. Now, to complete these works and compare results we report herein on the biotransformation of 7-*epi*-foliol (3α,7α,18-trihydroxy-*ent*-kaur-16-ene) (**7**) by *F. fujikuroi*.

The biotransformation by *F. fujikuroi* was carried out in the presence of AMO 1618, a compound that inhibits the formation of endogenous *ent*-kaur-16-ene without perturbing the post-kaurene metabolism [8,9], thus facilitating the analysis of the products formed. The substrate **7** was prepared in the following way: Hydrolysis of a linearol (**1**) and sidol (**2**) mixture afforded foliol (**3**) [10]. Its acetonide (**4**) was oxidized with pyridinium dichromate in DMF affording the 7-oxo derivative **5** [6,11]. Reduction of this compound with sodium borohydride in ethanol gave the corresponding 7α-hydroxy-3α,18-acetonide (**6**), which, by acid hydrolysis, led to 3α,7α,18-trihydroxy-*ent*-kaur-16-ene (**7**).

The incubation of the substrate **7** afforded a single biotransformation product **8**, in low yield (Scheme 1). The structure of 3α,7α-dihydroxy-*ent*-kaur-16-en-18-al (**8**) was assigned to this metabolite on the basis of the following considerations. Its high resolution mass spectrum was in accordance with the molecular formula $C_{20}H_{30}O_3$ (*m/z* 318.2195), which pointed to the loss of two hydrogen atoms during the fermentation. Comparison of its ^{1}H-NMR spectrum with that of the substrate **7** showed the disappearance of the AB system of the primary alcohol and the presence of a new singlet at δ 9.40. The chemical shift and shape of



Scheme 1. Biotransformation of 7-*epi*-foliol (**7**)

the latter signal is typical of the hydrogen of an aldehyde group. Analogously, the ^{13}C NMR spectrum (Table 1) revealed the disappearance of the hydroxymethylene group and the presence of a new carbon doublet at δ 206.5, due to the resonance of the new oxo group. Therefore, we assigned to this metabolite the structure 3α,7α-dihydroxy-*ent*-kaur-16-en-18-al (**8**), which was confirmed by its 2D NMR spectroscopic data. Thus, in the HMBC experiment, correlations of H-5 with C-1, C-6, C-7, C-9, C-19 and C-20, of H-9 with C-20, and of C-18 with H-5 and H-19 were observed.

This biotransformation of 7-*epi*-foliol (**7**) led us to the following conclusions:

Table 1:[13]C NMR spectroscopic data of compounds **7** and **8**.

Carbon	7	8	Carbon	7	8
1	38.2	38.1	11	18.2	18.2
2	26.9	26.4	12	33.5	33.5
3	75.8	72.1	13	43.2	43.2
4	41.8	n.o.	14	38.5	38.5
5	46.2	45.1	15	43.2	43.2
6	29.2	31.4	16	154.7	153,6
7	74.9	74.2	17	103.7	103.9
8	49.6	49.7.	18	71.0	206.5
9	55.0	54.8	19	11.5	9.0
10	38.8	38.7	20	18.1	18.1

n.o. = Not observed

1. This work confirms that a hydroxyl group at C-3(α) impedes the oxidation at C-19, which is characteristic of the biosynthesis of gibberellins and kaurenolides [6]. While this fact could be due to steric hindrance, the absence also of this oxidation due to the presence of a 15α-hydroxyl group [12,13] can be attributed to a true inhibition of the enzyme involved therein.

2. Oxidations at C-18 had also been observed in biotransformations of 6β-hydroxy derivatives of *ent*-kaur-16-ene and *ent*-atis-16-ene by *F. fujikuroi* [5,11]. In these cases the C-19 oxidation is inhibited by the presence of the 6β-alcohol. Probably in these cases and now in the incubation of **7** the same enzyme, *ent*-kaurene oxidase, which catalyzes the oxidation of the C-19 methyl to acid in the biosynthesis pathway of gibberellins [14], is responsible for the C-18 oxidation.

3. In the biotransformation of 7α-hydroxy-*ent*-kaur-16-ene (*epi*-candol A) (**11**) the oxidation of C-19 is produced to the acid level. The 7α-hydroxyl is then oxidized to form an oxo group [4]. Now, in the incubation of 7-*epi*-foliol (**7**) this last oxidation does not occur, which suggests that the presence of an acid group at C-19 is a prerequisite for the oxidation of the 7α-alcohol to take place.

Experimental

General procedures: ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution at 500.13 and 125.03 MHz, respectively, with a Bruker AMX-500 spectrometer. Mass spectra were taken at 70 eV (probe) in a Micromass Autospec spectrometer. HPLC was performed using a Beckman System Gold 125P. Purification by HPLC was achieved using a silica gel column (Ultrasphere Si 5μm, 10 x 250 mm). Dry CC was made on silica gel Merck 0.040-0.063 mm.

Microorganism: The fungal strain *Gibberella fujikuroi* MP-C IMI 58289, renamed *Fusarium fujikuroi* [15], was a gift from Prof. J. R. Hanson, School of Chemistry (University of Sussex, UK).

Preparation of 3α,7α,18-trihydroxy-ent-kaur-16-ene (7)

Hydrolysis of linearol (1) and sidol (2): A mixture of **1** and **2** (1 g) was stirred in a 5% methanolic KOH solution (15 mL) for 12 h at room temp. The solvent was removed under vacuum and the residue neutralized with HCl solution (5%). Extraction with EtOAc in the usual way afforded foliol (3α,7β,18-trihydroxy-*ent*-kaur-16-ene) (**3**) (802 mg) [10]; ^{1}H NMR and MS data, see [16]; ^{13}C NMR data, see [17].

Foliol acetonide (4) and its 7-oxo-derivative (5): The preparation of these compounds has been described [6,11].

Foliol acetonide (**4**)
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.04 (3H, *s*, H-19), 1.07 (3H, *s*, H-20), 1.14 (1H, dd, J = 11.3 and 5.1 Hz, H 14), 1.41 and 1.43 (each 3H, s, H-22 and H-23), 1.83 (1H, d, *J* = 11.3 Hz, H-14),1.91 (1H, dt, *J* = 13.3 and 3.4 Hz, H-1α),2.24 (2H, br s, H-15), 2.69 (1H, br s, H-13), 3.44 and 3.50 (each 1H, d, *J* = 10.6 Hz, H-18), 3.55 (1H, dd, *J* = 11.8 and 3.9 Hz, H-3), 3.58 (1H, br t, *J* = 2.8 Hz, H-7), 4.79 and 4.83 (each 1H, s, H-17).
EIMS *m/z* (rel. int.): 360 $[M]^+$ (3), 345 (98), 302 (7), 284 (12), 273 (28), 267 (38), 254 (20), 239 (22), 233 (19).
HREIMS: *m/z* 360.2671 $[M]^+$ (calcd for $C_{23}H_{36}O_3$ 360.2664).

7-Oxo-acetonide (**5**)
NMR and MS data, see [11].

Reduction of the 7-oxo-3α,18-acetonide (5): The 7-oxo-derivative **5** (260 mg) in methanol (20 mL) was treated with sodium borohydride (28 mg) and stirred for 1 h at room temperature. The mixture was poured over water and extracted with EtOAc in the usual way to afford **6** (252 mg).
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (1H, m, H-5), 0.98 (1H, m, H-1β), 1.04 (3H, s, H-19), 1.07 (1H, s, H-9), 1.08 (3H, s, H-20), 1.41 and 1.42 (each 3H, s, H-22 and H-23), 1.90 (1H, dt, *J* = 13.5 and 3.6 Hz, H-1α), 1.92 (1H, br d, *J* = 16.7 Hz, H-15β), 2.64 (1H, dt, *J* = 16.7 and 2.7 Hz, H-15α), 2.69 (1H, br s, H-13), 3.40 and 3.53 (each 1H, d, *J* = 10.4 Hz, H-18), 3.44 (1H, m, overlapped with H-3, H-7), 3.47 (1H, dd, *J* = 11.6 and 4.2 Hz, H-3), 4.76 and 4.83 (each 1H, br s, H-17).
EIMS *m/z* (rel.int.): 360 $[M]^+$ (1), 345 (100), 302 (14), 290 (6), 284 (28), 272 (19), 267 (27), 254 (15), 239 (15), 233 (14), 227 (15), 211 (13), 199 (11), 189 (10).
HREIMS: *m/z* 360.2649 $[M]^+$ (calcd for $C_{23}H_{36}O_3$ 360.2664).

7-epi-Foliol (7): The acetonide **6** (250 mg) in methanol (20 mL) was stirred with a 0.25% methanolic HCl solution (5 mL) for 5 h at room temp. The reaction mixture was poured over water and extracted with EtOAc in the usual way to afford 7-*epi*-foliol (3α,7α,18-trihydroxy-*ent*-kaur-16-ene) (**7**) (212 mg).
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (3H, s, H-19), 1.04 (2H, m, H-5 and H-9), 1.08 (3H, s, H-20), 1.84 (1H, dt, *J* = 13.1 and 3.5 Hz, H-1α), 1.92 (1H, br d, *J* = 16.9 Hz, H-15β), 2.64 (1H, dt, *J* = 16.8 and 2.7 Hz, H-15α), 2.69 (1H, br s, H-13), 3.40 and 3.71 (each 1H, d, *J* = 10.2 Hz, H-18), 3.46 (1H, dd, *J* = 11.4 and 4.2 Hz, H-7), 3.62 (1H, t, *J* = 9.6 Hz, H-3), 4.76 and 4.83 (each 1H, br s, H-17).
EIMS *m/z* (rel. int.): 320 $[M]^+$ (1), 302 (3), 284 (15), 271 (16), 253 (17) 243 (2), 232 (10), 217 (7).
HREIMS: *m/z* $[M]^+$ 320.2339 (calcd for $C_{20}H_{32}O_3$ 320.2351).

Incubation of 3α,7α,18-trihydroxy-ent-kaur-16-ene (7): The fungus *F. fujikuroi*, inhibited with 5 x 10^{-5} M AMO 1618 (2'-isopropyl-4'-[trimethyl-ammonium chloride]-5'-methylphenyl piperidine-1-carboxylate), was grown In shake culture at 25ºC for 2 days in 80 conical flasks (250 mL), each containing 50 mL of sterile medium comprising (per dm^3) glucose (80 g), NH_4NO_3 (0.48 g), KH_2PO_4 (5 g), $MgSO_4$ (1 g), and a solution of trace elements (2 mL). This solution contained (per 100 mL) $Co(NO_3)_2$ (0.01 g), $CuSO_4$ (0.015 g), $ZnSO_4$ (0.16 g), $MnSO_4$ (0.01 g), and $(NH_4)_6Mo_7O_{24}$ (0.01 g). The substrate (190 mg) dissolved in EtOH (16 mL) and Tween 80 (3 drops) was evenly distributed between the flasks and the incubation allowed to continue for a further 6 days. The broth was filtered and the culture filtrate extracted with EtOAc. The mycelium was treated with liquid nitrogen, crushed in a mortar and extracted also with EtOAc. Usual work-up gave acid (406 mg) and neutral (911 mg) fractions. Chromatography of the latter on a silica gel column, using a light petroleum-EtOAc gradient, afforded starting material (**7**) (141 mg) and

3α,7α-dihydroxy-*ent*-kaur-16-en-18-al (**8**) (1 mg). Biotransformed products were not obtained in the acid fraction.

3α,7α-Dihydroxy-*ent*-kaur-16-en-18-al (**8**)
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.97 (1H, td, *J* = 13.1 and 3.8 Hz, H-1β), 1.09 (6H, s, H-19 and H-20), 1.27 (2H, m, H-6 and H-14), 1.39 (1H, dd, J = 12.7 and 1.5 Hz, H-5), 1.64 (1H, m, H-2), 1.77 (1H, m, H-2), 1.90 (1H, dt, *J* = 13.1 and 3.4 Hz, H-1α), 1.94 (1H, br d, *J* = 16.9 Hz, H-15β), 2.67 (1H, dt, *J* = 16.9 and 2.8 Hz, H-15α), 2.71 (1H, br s, H-13), 3.51 (1H, dd, *J* = 11.4 and 4.5 Hz, H-7), 3.74 (1H, dd, *J* = 11.5 and 4.6 Hz, H-3), 4.79 and 4.85 (each 1H, br s, H-17), 9.40 (1H, s, H-18).
EIMS *m/z* (rel. int.): 318 $[M]^+$ (100), 300 (27), 282 (55), 275 (9), 271 (13), 267 (11), 253 (20), 243 (10), 225 (8), 201 (26), 164 (25), 149 (39), 121 (74).
HREIMS: *m/z* 318.2207 $[M]^+$ (calcd for $C_{20}H_{30}O_3$ 318.2195).

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