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MICROBIAL TRANSFORMATIONS OF 6β-ACETOXYEUDESMENES BY *CURVULARIA LUNATA*

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ABSTRACT.—Microbial transformation of 6β-acetoxyeudesmanols and 6β-acetoxyeudesmanones has been carried out with a *Curvularia lunata* strain. 6β-Acetoxyeudesm-3-en-1β-ol [**1**] and 6β-acetoxyeudesm-4(15)-en-1β-ol [**2**] remained unaltered after 7 days incubation. Incubation of 6β-acetoxyeudesm-3-en-1-one [**3**] and 6β-acetoxyeudesm-4(15)-en-1-one [**4**] resulted in 1α-hydroxyl compounds as principal products, along with minor quantities of 4α- and 4β-hydroxyeudesm-2-en-1-one from **3** and 6β-acetoxyeudesm-4(15)-ene-1α,2α-diol from **4**.

We are carrying out a series of systematic biotransformations of 6β-acetoxyeudesmanes (1) with hydroxylating fungi as part of a wide program of sesquiterpenoid (2) and diterpenoid (3–7) biotransformations. We have used *Curvularia lunata* (Dematiaceae) strain CECT 2130 to obtain 6β-eudesmanolides (1). *C. lunata* introduced a hydroxyl group into the isopropyl moiety of 6β-acetoxy-1β,4β-dihydroxyeudesmane, which can then be used to obtain 6β-eudesmanolides (1). Thus we have incubated a number of natural products isolated from *Sideritis* (8) and their oxidation derivatives at C-1 with *C. lunata* in order to discover the relationship between the structure of substrate and the biotransformation activity of the fungus. The sesquiterpene eudesmanes hydroxylated at C-1 can be transformed to other sesquiterpene skeletons such as guaianes, pseudoguaianes, and elemanes.

MATERIAL AND METHODS

PHYSICAL ANALYSES.—Measurements of nmr spectra (300 MHz ^{1}H and 75.47 MHz ^{13}C) were made in $CDCl_3$ (which also provided the lock signal) in a Bruker AM-300 spectrometer, equipped with process controller and array processor. The assignments of ^{13}C chemical shifts were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. Monodimensional nOe difference experiments were made by irradiation for 4 sec in series of 8 scans, on-resonance and off-resonance alternately. Ir spectra were recorded on a Nicolet 20SX FT-IR spectrometer. Eims (70 eV) was carried out with a Hewlett-Packard 5988A spectrometer. Elemental analyses were made in a Perkin-Elmer 240C analyzer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20°. Si gel Scharlau 60 (less than 0.06 mm) was used for flash chromatography. CH_2Cl_2 containing increasing amounts of Me_2CO was used as eluent. Analytical plates (Si gel, Merck G) were rendered visible by spraying with H_2SO_4, followed by heating to 120°.

ISOLATION OF EUDESMENES **1** AND **2**.—6β-Acetoxyeudesm-3-en-1β-ol [**1**] and 6β-acetoxyeudesm-4(15)-en-1β-ol [**2**] were isolated from *Sideritis varoi* ssp. *cuatrecasasii* (8).

OXIDATION OF EUDESMENES **1** AND **2**.—6β-Acetoxyeudesm-3-en-1β-ol [**1**] (200 mg) was oxidized with pyridinium dichromate (9) (250 mg) for 12 h at room temperature. Chromatography on a Si gel column yielded 6β-acetoxyeudesm-3-en-1-one [**3**] (8) (120 mg, 60%). The 6β-acetoxyeudesm-4(15)-en-1β-ol [**2**] (300 mg) was also oxidized with pyridinium dichromate (375 mg) under the same conditions. Chromatography on a Si gel column yielded 6β-acetoxyeudesm-4(15)-en-1-one [**4**] (8) (173 mg, 58%).

ORGANISM, MEDIA, AND CULTURE CONDITIONS.—*C. lunata* CECT 2130 came from the Colección Española de Cultivos Tipo, Departamento de Microbiología, Facultad de Ciencias, Universidad de Valencia, Spain, and was kept in YEPGA medium, containing yeast extract (1%), peptone (1%), glucose (2%), and agar (2%) in H_2O at pH 5. In all the transformation experiments a medium of peptone (0.1%), yeast

extract (0.1%), beef extract (0.1%), and glucose (0.5%) in H_2O at pH 5.7 was used. Erlenmeyer flasks (250 ml) containing 60 ml of medium were inoculated with a dense suspension of *C. lunata*. The cultures were incubated with shaking (150 rpm) at 28° for 6 days, after which substrates **1**, **2**, **3**, and **4** in EtOH were added.

BIOTRANSFORMATION OF SUBSTRATE **3**.—Substrate **3** (120 mg) was dissolved in EtOH (2.5 ml), distributed among 5 Erlenmeyer-flask cultures, and incubated for 3 days, after which the cultures were filtered and pooled; the cells were washed with H_2O and the liquid was saturated with NaCl and extracted twice with CH_2Cl_2. Both extracts were pooled, dried with anhydrous $MgSO_4$, and evaporated at 40° in vacuo to give a mixture of compounds (83 mg) (Scheme 1). This mixture was chromatographed on a Si gel column to obtain 34 mg of starting material **3**, 25 mg of metabolite **5** (6β-acetoxyeudesm-3-en-1α-ol), 12 mg of metabolite **6** (6β-acetoxy-4α-hydroxyeudesm-2-en-1-one), and 12 mg of metabolite **7** (6β-acetoxy-4β-hydroxyeudesm-2-en-1-one).

6β-ACETOXYEUDESM-3-EN-1α-OL [**5**].—[α]D +70° (*c* = 1, $CHCl_3$); ir ν max cm^{-1} 3442, 1734, 1643, 1243; ^{1}H nmr (δ) 5.72 (1H, m, W½ = 7 Hz, H-6), 5.29 (1H, m, W½ = 10 Hz, H-3), 3.27 (1H, br d, *J* = 3.7 Hz, H-1), 2.29 (1H, m, W½ = 10 Hz, H-5), 2.00 (3H, s, AcO group), 1.69 (3H, br s, Me-15), 0.93 (3H, s, Me-14), 1.00 and 0.86 (3H each, d, *J* = 6.5 Hz, Me-12 and Me-13); ^{13}C nmr see Table 1; *m/z* (%) $[M]^+$ 280 (4), 220 (10), 205 (3), 202 (44), 187 (11), 159 (100), 145 (11), 131 (15). Found C 73.1, H 9.95; $C_{17}H_{28}O_3$ requires C 72.82, H 10.06%.

6β-ACETOXY-4α-HYDROXYEUDESM-2-EN-1-ONE [**6**].—[α]D +6° (c = 0.5, $CHCl_3$); ir ν max cm^{-1} 3426, 1735, 1676, 1242; ^{1}H nmr (δ) 6.62 (1H, d, *J* = 10.5 Hz, H-3), 5.85 (1H, m, W½ = 4 Hz, H-6), 5.80 (1H, d, *J* = 10.5 Hz, H-2), 2.08 (3H, s, AcO group), 1.38 and 1.35 (3H each, s, Me-14 and Me-15), 0.96 and 0.88 (3H each, d, *J* = 6.5 Hz, Me-12 and Me-13); ^{13}C nmr see Table 1; *m/z* (%) $[M]^+$ 294 (4), 279 (7), 234 (7), 219 (10), 191 (20), 165 (10), 149 (9), 137 (100), 98 (31), 81 (51). Found C 69.5, H 9.1; $C_{17}H_{26}O_4$ requires C 69.36, H 8.90%.

6β-ACETOXY-4β-HYDROXYEUDESM-2-EN-1-ONE [**7**].—[α]D +17° (*c* = 0.5, $CHCl_3$); ir ν max cm^{-1} 3442, 1735, 1677, 1248; ^{1}H nmr (δ) 6.55 (1H, d, *J* = 10.5 Hz, H-3), 5.89 (1H, d, *J* = 10.5 Hz, H-2), 5.86 (1H, m, W½ = 4 Hz, H-6), 2.10 (3H, s, AcO group), 1.61 and 1.50 (3H each, s, Me-14 and Me-15), 0.99 and 0.97 (3H each, d, *J* = 6.5 Hz, Me-12 and Me-13); ^{13}C nmr see Table 1; *m/z* (%) $[M]^+$ 294 (2), 279 (5), 234 (4), 219 (6), 191 (16), 163 (10), 149 (12), 137 (71), 98 (40), 81 (100). Found C 69.2, H 9.0; $C_{17}H_{26}O_4$ requires C 69.36, H 8.90%.

TABLE 1. ^{13}C-nmr Chemical Shifts (δ) of Compounds **1**, **3**, **4**, **5**, **6**, **7**, **8**, and **9**.

Carbon	Compound							
	1	**3**	**4**	**5**	**6**	**7**	**8**	**9**
C-1	76.81	213.55	214.55	74.56	195.37	207.50	75.85	78.61
C-2	32.14	31.61	32.73	31.75	154.94	150.97	29.41	68.29
C-3	121.33	120.01	33.80	119.23	125.20	125.53	30.82	39.86
C-4	133.49	134.31	142.77	133.77	70.42	69.25	146.60	143.85
C-5	50.76	48.95	50.81	43.97	54.11	49.92	46.08	45.05
C-6	71.23	72.15	71.23	71.60	69.40	69.51	71.83	71.38
C-7	49.16	49.26	49.63	49.33	49.63	49.34	50.06	49.97
C-8	20.42	19.97	20.24	20.65	20.59	20.83	20.52	20.09
C-9	35.56	38.43	37.66	33.85	35.82	34.06	33.95	33.94
C-10	37.86	47.02	48.39	37.58		44.50	40.27	39.50
C-11	28.74	28.54	28.26	28.80	28.51	29.29	28.30	28.30
C-12	20.68[a]	20.47[a]	20.39[a]	21.02[a]	20.82[a]	21.10[a]	20.73[a]	20.50[a]
C-13	20.22[a]	20.23[a]	20.31[a]	20.28[a]	20.49[a]	20.71[a]	20.47[a]	20.04[a]
C-14	11.90	18.49	21.44	19.30	21.61	21.55	21.53	21.49
C-15	21.46	21.55	110.59	21.53	23.83	28.88	107.40	109.44
CH_3CO	22.03	22.03	21.81	22.05	21.85	21.71	21.93	21.89
MeCO	170.18	170.02	170.53	170.19	171.27		170.78	170.73

[a]Values in the same column may be interchanged.

BIOTRANSFORMATION OF SUBSTRATE **4**.—Substrate **4** (170 mg) was dissolved in EtOH (3 ml), distributed among 6 Erlenmeyer flask cultures, and incubated for 7 days, after which the cultures were processed as indicated above for biotransformation of substrate **3**, to give a mixture (88 mg) which was chromatographed on a Si gel column to obtain 20 mg of starting material **4**, 51 mg of metabolite **8** [6β-acetoxyeudesm-4(15)-en-1α-ol] and 17 mg of metabolite **9** [6β-acetoxyeudesm-4(15)-ene-1α,2α-diol] (Scheme 2).

6β-ACETOXYEUDESM-4(15)-EN-1α-OL [**8**].—$[\alpha]_D$ +67° (c = 1, $CHCl_3$); ir ν max cm^{-1} 3446, 1732, 1643, 1241; ^1H nmr (δ) 5.63 (1H, m, W½ = 4 Hz, H-6), 4.74 and 4.56 (1H each, s, H_2-15), 3.35 (1H, m, W½ = 7 Hz, H-1), 2.43 (1H, br s, H-5), 2.02 (3H, s, AcO group), 0.92 (3H, s, Me-14), 0.85 and 0.99 (3H each, d, J = 6.5 Hz, Me-12 and Me-13); ^{13}C nmr see Table 1; *m/z* (%) $[M-60]^+$ 220 (6), 202 (55), 187 (14), 159 (100), 145 (12), 131 (14), 117 (31), 105 (25), 91 (39). Found C 72.8, H 10.1; $C_{17}H_{28}O_3$ requires C 72.82, H 10.06%.

6β-ACETOXYEUDESM-4(15)-EN-1α,2α-DIOL [**9**].—$[\alpha]_D$ +24° (c = 1, $CHCl_3$); ir ν max cm^{-1} 3430, 1730, 1646, 1235; ^1H nmr (δ) 5.66 (1H, m, W½ = 4 Hz, H-6), 4.82 and 4.65 (1H each, s, H_2-15), 3.93 (1H, ddd, $J_{2,3}$ = 8.6, $J_{1,2}$ = 2.9 Hz, H-2), 3.32 (1H, d, J = 2.9 Hz, H-1), 2.40 (1H, br s, H-5), 2.02 (3H, s, AcO group), 0.91 (3H, s, Me-14), 0.99 and 0.85 (3H each, d, J = 6.5 Hz, Me-12 and Me-13); ^{13}C nmr see Table 1; *m/z* (%) $[M]^+$ 296 (1), 278 (1), 236 (6), 218 (39), 203 (15), 175 (38), 157 (100), 119 (13), 91 (16). Found C 69.1, H 9.5; $C_{17}H_{28}O_4$ requires C 68.89, H 9.52%.

EPOXIDATION OF SUBSTRATE **3**.—Substrate **3** (20 mg) was dissolved in $CHCl_3$ (2 ml) and epoxidized with MCPBA (50 mg) at room temperature for 48 h. After cc a compound (16 mg, 82%) identical to metabolite **6** was isolated.

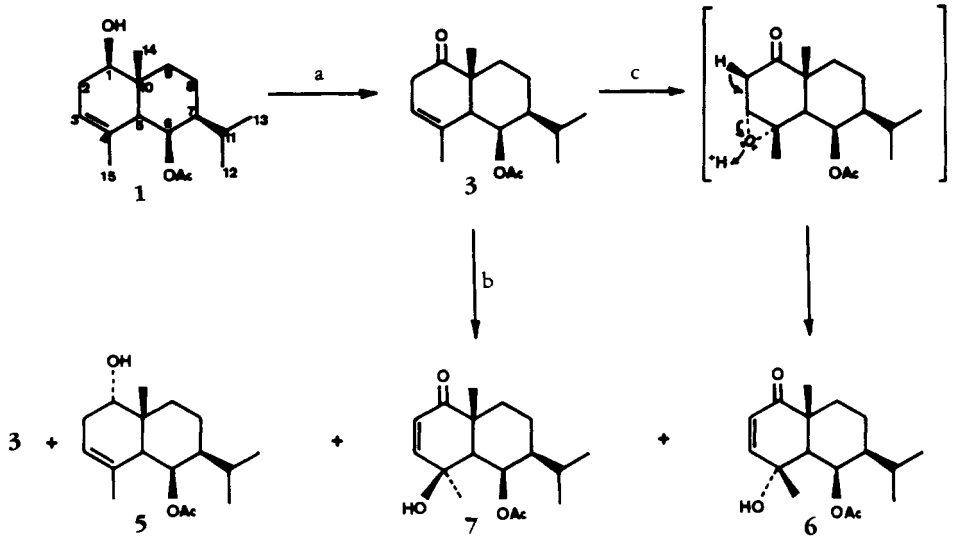
RESULTS AND DISCUSSION

6β-Acetoxyeudesm-3-en-1β-ol [**1**] (8) was incubated with *C. lunata* for 7 days, after which the substrate **1** was recovered more or less unaltered. Substrate **1** is not very stable; thus incubating it for more than 7 days is not very practical. Its *exo*-isomer **2** affords the same problem, and it was given the same treatment. We have described elsewhere (1) the incubation of 6β-acetoxyeudesma-1β,4β-diol with *C. lunata* which after 12 days produces 12- and 13-hydroxyl derivatives. It is possible that substrates **1** and **2** might be biotransformed during such a period of incubation. Nevertheless, their instability makes such a process infeasible.

We have observed that deoxyvulgarine [**10**] is readily biotransformed by *Rhizopus nigricans* and *Aspergillus ochraceous* while vulgarine [**11**] remains unaffected. For this reason we have oxidized the hydroxyl group at C-1 of substrates **1** and **2** to give products **3** and **4**, respectively [see Experimental and Arias *et al.* (2)]. Ketone **3** is structurally similar in ring A to deoxyvulgarine [**10**].

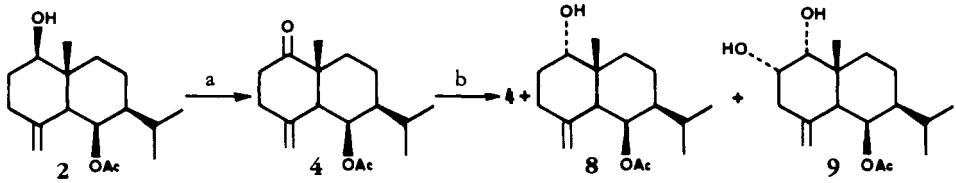
Incubation of substrate **3** with *C. lunata* for 3 days gave the metabolites **5** (21%), **6** (10%), and **7** (10%). Recovery of the unaltered substrate **3** was 28%. The main metabolite **5** isolated from this incubation resulted from reduction of the ketone group presumably from the β face, which is difficult to access by chemical means. The *S* configuration at C-1 could easily be deduced from the signal in the ^1H-nmr spectrum (δ 3.27, 1H, br d, J = 3.7 Hz). Furthermore, the reduction at C-1 and the configuration of the new hydroxyl group could also be confirmed by a comparison of the ^{13}C-nmr spectra of compound **1** and metabolite **5** (Table 1), in which the γ-syn effect is observed for C-5 in metabolite **5** ($\Delta\delta = -6.79$). We have also observed a similar reduction of the keto group at C-1 of deoxyvulgarin [**10**] by *A. ochraceous* (2). In addition *C. lunata* transformed substrate **3** to the Δ^2-4α-hydroxyl **6** and the Δ^2-4β-hydroxyl **7** derivatives.

Some nOe experiments were performed to determine the configuration at C-4 of metabolites **6** and **7**. Irradiation of the signal for H-6 in metabolite **7** produced a considerable nOe on Me-15 (ca. 10%), on H-5 (ca. 12%), and on one of the two methyl groups of the isopropyl moiety. However, irradiation at the H-6 signal metabolite **6** produced no nOe at Me-15.



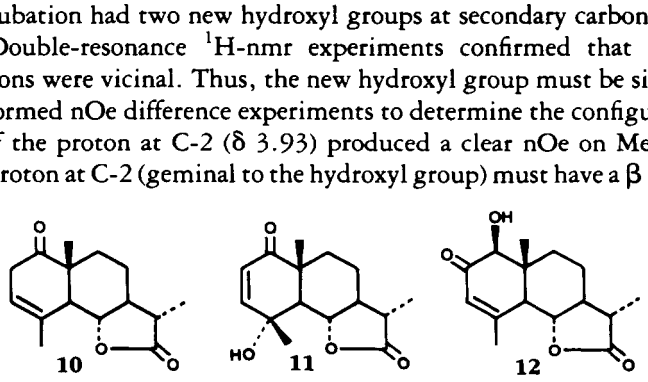
SCHEME 1. Microbial transformation of substrate **3** by *Curvularia lunata* and chemical correlation of metabolite **6**. a, Pyridinium dichromate; b, *C. lunata* (3 days); c, MCPBA.

We have previously shown (2) that when *A. ochraceous* transforms deoxyvulgarin [**10**] an α-epoxide is produced, which evolves into the Δ^2-4α-hydroxyl derivative. When substrate **3** was epoxidized with MCPBA, only metabolite **6** was obtained. In all probability *C. lunata* epoxidizes both faces of the double bond of substrate **3** to give both 4-hydroxyl epimer metabolites **6** and **7**.



SCHEME 2. Microbial transformation of substrate **4** by *Curvularia lunata*. a, Pyridinium dichromate; b, *C. lunata* (7 days).

After incubating the more stable substrate **4** with *C. lunata* for 7 days, 12% of the substrate was recovered. The principal metabolite produced was the 1α-hydroxyl derivative **8** (30%), which is also an *S* alcohol. Another metabolite, **9** (10%), isolated from this incubation had two new hydroxyl groups at secondary carbons (ms, ^{1}H and ^{13}C nmr). Double-resonance ^{1}H-nmr experiments confirmed that both carbinol methine protons were vicinal. Thus, the new hydroxyl group must be situated at C-2. We also performed nOe difference experiments to determine the configuration at C-2. Irradiation of the proton at C-2 (δ 3.93) produced a clear nOe on Me-14 (ca. 5%). Hence, this proton at C-2 (geminal to the hydroxyl group) must have a β configuration.



We may conclude that as with *A. ochraceous* and *R. nigricans* (2), *C. lunata* reduces the keto group at C-1 to give alcohols with an *S* configuration (10). Moreover, the incubation of deoxyvulgarin [**10**] with *R. nigricans* produces a 1β-acyloin **12**, for the synthesis of which we have postulated an α-hydroxylation (2) at C-2 to produce an intermediate acyloin, which evolves into the final acyloin via a Marker-Lawson rearrangement. We believe that the α hydroxylation at C-2 discussed in this paper occurs in a similar way, but that in this case a previous reduction of the hydroxyl group at C-1 of substrate **4** occurs. Thus, a keto group at C-1 and a double bond in ring A seem to direct biotransformations towards ring A (2). On the other hand, when the substrate does not contain a double bond in ring A biotransformation by these fungi is directed towards the isopropyl group of the eudesmanes. Presently, we are engaged in a wider series of biotransformation experiments to confirm this structure-activity relationship.

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