# Microbial Transformations of 6β-Acetoxyeudesmenes by Curvularia lunata

**ARTICLE** *in* JOURNAL OF NATURAL PRODUCTS · MARCH 1990

Impact Factor: 3.8 · DOI: 10.1021/np50068a023



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*Journal of Natural Products*
*Vol. 53, No. 2, pp. 436-440, Mar-Apr 1990*

# 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 $\nu$ 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 $\nu$ 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.

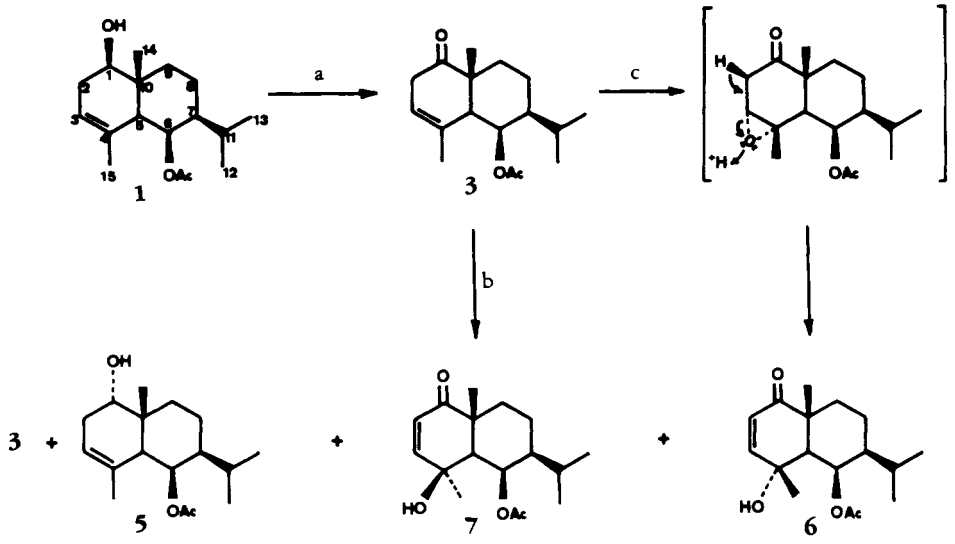
## RESULTS AND DISCUSSION

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**].

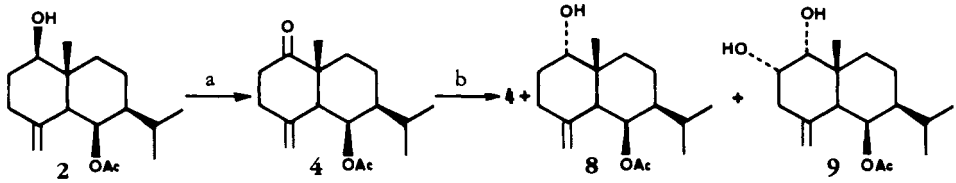
Incubation of substrate **3** with *C. lunata* for 3 days gave the metabolites **5** (21%), **6** (10%), and **7** (10%). Recovery of the unaltered substrate **3** was 28%. The main metabolite **5** isolated from this incubation resulted from reduction of the ketone group presumably from the β face, 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 $\Delta^2$-4α-hydroxyl **6** and the $\Delta^2$-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.



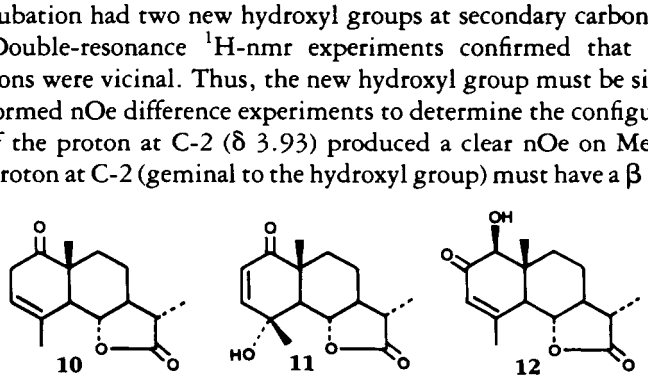
SCHEME 1. Microbial transformation of substrate **3** by *Curvularia lunata* and chemical correlation of metabolite **6**. a, Pyridinium dichromate; b, *C. lunata* (3 days); c, MCPBA.

We have previously shown (2) that when *A. ochraceous* transforms deoxyvulgarin [**10**] an α-epoxide is produced, which evolves into the $\Delta^2$-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.

ACKNOWLEDGMENTS

This work was supported by a grant from the Comisión Asesora de Investigación Científica y Técnica to "Servicio de Análisis y Determinación de Estructuras" and "Servicio de Traducción" (Servicios Técnicos, Universidad de Granada, Spain).

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*Received 6 October 1989*