Microbial Transformations of Sesquiterpenoids: Conversion of Deoxyvulgarin by *Rhizopus nigricans* and *Aspergillus ochraceous*

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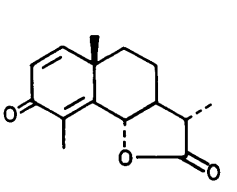
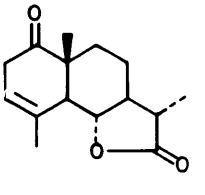
Microbial transformation of the sesquiterpene lactone deoxyvulgarin (**2**) has been carried out with *Aspergillus ochraceous* and *Rhizopus nigricans* cultures. *A. ochraceous* converted deoxyvulgarin (**2**) into vulgarin (**3**) and 11,13-dihydrodouglanin (**4**). *R. nigricans* transformed deoxyvulgarin (**2**) into vulgarin (**3**), erivanin (**6**), and 1β-hydroxy-2-oxoeudesm-3-en-6,13-olide (**7**). Vulgarin (**3**) was obtained chemically by epoxidation of deoxyvulgarin (**2**) in a one-step process in virtually quantitative yield. A pathway proposed for the conversion of deoxyvulgarin (**2**) into the more functionalized metabolites is discussed.

Research into new routes or synthetic transformations in order to obtain sesquiterpene lactones is currently increasing.[1] This research is motivated to some extent by the varied biological activity[2] of these compounds. The sesquiterpene lactone santonin (**1**) has been used as starting material in the preparation of several eudesmanolides *via* acetal derivatives of the ketone (**2**) which were obtained from santonin (**1**) in low yield. Since decalinic compounds with an equatorial hydroxy group at C-1 can be rearranged to perhydroazulenes,[3] there is increasing interest in substances with such characteristics.[4]

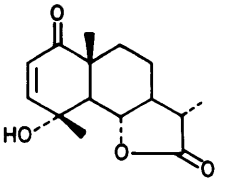
Surprisingly, few microbial transformations have been reported in this field (α-santonin, germacrene, kessyl alcohol, α-cyperotundone, guaioxide), despite which specific chemical transformations have occasionally been achieved with a high degree of selectivity.[5] The microbial conversions of the sesquiterpene lactone costunolide by *Aspergillus niger*, *Cunninghamella echinulata*, and *Fusarium oxysporum* are noteworthy.[1f] We now report on some microbial transformations of substrate (**2**) in order to obtain several eudesmanolides with different hydroxylation sites.



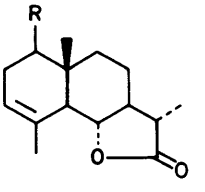
(**1**)



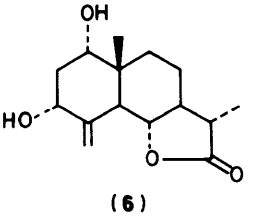
(**2**)



(**3**)



(**4**) R = α-OH
(**5**) R = β-OH



(**6**)

Results and Discussion

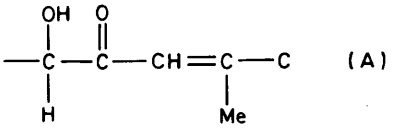
Vulgarin (**3**) is a very abundant sesquiterpene lactone in *Artemisia canariensis* Lees.[6] Attempts at microbial transformation of this lactone with *Aspergillus ochraceous* cultures, in experiments performed by us, were unsuccessful. However, deoxyvulgarin (**2**), a compound readily obtained from vulgarin (**3**), was totally metabolized by incubation for 30 h with *A. ochraceous* cultures, after which incubation was stopped. Proceeding as described in the Experimental section, we isolated two metabolites. The less polar was identified as 11,13-dihydrodouglanin (**4**),[7] and the more polar as vulgarin (**3**) on both occasions by direct comparison with authentic samples. Compound (**4**) can be obtained chemically as a minor component, in addition to its 1β-epimer, 11,13-dihydrosantamarin (**5**), by reduction of deoxyvulgarin (**2**) with metallic hydrides [$NaBH_4$, $Zn(BH_4)_2$ or $LiAlH(Bu^tO)_3$.[1a,1g]

Five metabolites were isolated from submerged cultures of *Rhizopus nigricans*. Two of them were identified as the aforementioned 11,13-dihydrodouglanin (**4**) and vulgarin (**3**). The third and most abundant compound was optically active and had the molecular formula $C_{15}H_{22}O_4$. The ^1H n.m.r. spectrum showed signals for an exocyclic methylene and two protons geminal to hydroxy groups. The metabolite had physical and spectral data consistent with those reported for erivanin (**6**), a eudesmanolide isolated from *Artemisia fragans*[8] Willd. var. erivanica Bess and later from *Tanacetum balsamita*.[9]

Compound (**6**) was definitively identified by direct comparison of its spectral data with those of authentic erivanin.

A further compound isolated from this incubation had the

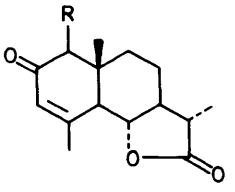
molecular formula $C_{15}H_{20}O_4$ (high-resolution m.s.). The u.v. and ^1H n.m.r. spectra are consistent with those reported for an elemanolide (**9**)[10] having a chromophoric system such as (A) (see Experimental section) [$\lambda_{max.}$ 236 nm; $\nu_{max.}$ 1 665 and 1 620 cm^{-1}; δ_H 6.08 (1 H, s, $W_{\frac{1}{2}}$ 6 Hz), 3.96 (1 H, s), and 2.20 (3 H, s)]. This substance is formulated as having structure (**7**).



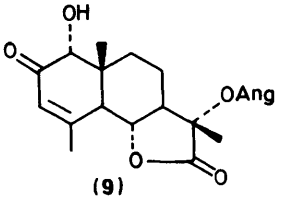
(A)

The signal of 5-H_α in this compound, δ_H 2.71 (1 H, d, J 11 Hz), presented the same chemical shift in C_6D_6 solution, which excluded a *syn*-diaxial relationship between this proton and the 1-OH.[11]

Oxidation of the elemanolide (**10**), obtained in a solvolytic reaction of the triflate (**11**),* with sodium metaperiodate and a catalytic amount of OsO_4, afforded compound (**8**). In this substance, the signal of the 5-H_α (δ_H 3.06, 1 H, d, J 10 Hz) appears paramagnetically shifted (0.35 p.p.m.) with respect to that arising from the same proton in isomer (**7**). Considering that the attack of the reagent on compound (**10**) must be carried out on the α face of the molecule as a consequence of the strong steric hindrance caused by the two β-axial angular methyls present, the observed shift suggests a *syn*-diaxial orientation between 5-H_α and the hydroxy group at C-1. On the other hand, the signal generated by the proton geminal to the hydroxy group appears diamagnetically shifted (0.55 p.p.m.) with respect to that of compound (**7**). On the basis of an inspection of Dreiding models it was deduced that the OH group in compound (**8**) must be α-oriented. Both compounds must be epimeric at C-1 and compound (**7**) is 1β-hydroxy-2-oxoeudesm-3-en-6α,12α-olide, with the 1-OH in the more stable equatorial configuration.

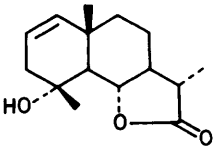


(**7**) R = β-OH
(**8**) R = α-OH

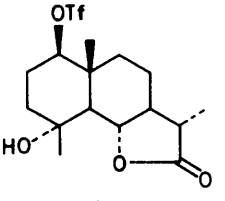


(**9**)

Ang = COC(Me)=CHMe

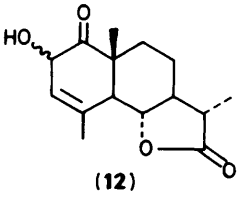


(**10**)



(**11**)

Tf = CF_3SO_2



(**12**)

Microbial transformation of deoxyvulgarin (**2**) to vulgarin (**3**) might be accomplished by α-epoxidation of the double bond and rearrangement of the oxirane moiety. Evidence to this effect was provided by the chemical transformation achieved in the course of this work. The epoxidation of deoxyvulgarin with *m*-chloroperbenzoic acid (MCPBA) yielded vulgarin quantitatively. Deoxyvulgarin has been used as a chemical precursor of vulgarin, but limited yields were obtained by processes in which the keto group at C-1 was masked as its 1,1-ethylenedioxy derivative before osmylation[1f] or epoxidation,[1g] after which the keto group was restored by treatment in acid media. This one-step conversion of deoxyvulgarin into vulgarin confirms our hypothesis of the pathway proposed for the microbial conversion and provides an improvement for this step in the chemical synthesis of vulgarin.

As described above, both *A. ochraceous* and *R. nigricans* cultures reduced the above ketone group at C-1 of substrate (**2**) to give 1α-alcohols, in agreement with a previous report that microbial reduction of ketone groups yields alcohols with an *S* configuration.[5c] The formation of erivanin (**6**) can be explained as a result of epoxidation of 11,13-dihydrodouglanin (**4**)[12] and later evolution of the oxirane ring. The acyloin (**7**) could be obtained by α-hydroxylation of substrate (**2**) to produce acyloin (**12**), which would evolve to (**7**) *via* a Marker–Lawson rearrangement. There are several precedents for microbial oxidation of 17-keto steroids to give 16-keto-17-hydroxy derivatives.[13] On the other hand, the rearrangement of 16α-hydroxy-17-keto steroids to 17α-hydroxy-16-keto steroids has also been carried out by chemical methods.[14]

A fifth metabolite could not be identified due to the minuscule amount obtained.

Experimental

M.p.s were determined on a Kofler block and are uncorrected. ^1H N.m.r. spectra were measured at 90 MHz in $CDCl_3$ with $SiMe_4$ as internal standard. I.r. spectra were recorded on a Perkin-Elmer 681 spectrophotometer. U.v. spectra were obtained on a Perkin-Elmer 402 spectrophotometer. Electron-impact mass spectroscopy was carried out on a VG Micromass ZAB-2F spectrometer. Silica gel, Merck 7729 (less than 0.08 nm) was used for flash chromatography. CH_2Cl_2 containing increasing amounts of acetone was used as eluant. Analytical plates (silica gel Merck G) were visualized by spraying with H_2SO_4–AcOH, followed by heating for 5 min. All the aforementioned products behaved like pure substances in t.l.c., with the exception of compound (**7**). The identity of the remainder has been confirmed by direct comparison with authentic samples (m.p., mixed m.p., and spectral data).

Isolation of Vulgarin (**3**).—The vulgarin (**3**) used in this work was isolated from *Artemisia canariensis* Lees, as described in ref. 6.

Preparation of Deoxyvulgarin (**2**).—Vulgarin (**3**) (1 g) was treated with Zn–AcOH as indicated in ref. 15, to give deoxyvulgarin (**2**) (850 mg).

Organisms, Media, and Culture Conditions.—Rhizopus nigricans (CECT 2672) and *Aspergillus ochraceous* (CECT 2069) from the Colección Española de Cultivos Tipo were used

* It should be noted that this reaction also afforded the guaianolides compressanolide and dihydromicheliolide; M. Ogura, G. A. Cordell, and N. R. Farnsworth, *Phytochemistry*, 1978, **17**, 957, owing to cationic skeletal rearrangement *via* triflate (**11**)

in these studies.* Medium YEPGA containing 1% yeast extract, 1% peptone, 2% glucose, 2% agar, pH 5, was used for storage of *R. nigricans* and *A. ochraceous*. In all transformation experiments, a medium of the following composition was used: 0.1% peptone, 0.1% corn skep, 0.1% beef extract, and 0.5% glucose in water. Erlenmeyer flasks (250 ml) containing 100 ml of medium were inoculated with a dense suspension of *R. nigricans* or *A. ochraceous*. Incubations were maintained at 28 °C with gyratory shaking (120 r.p.m.) for 6 days, after which substrates in EtOH were added.

Fermentation of Deoxyvulgarin (**2**) *with* A. ochraceous *Cultures*.—Substrate (**2**) (300 mg) was dissolved in EtOH (6 ml), distributed among 6 Erlenmeyer flask cultures, and incubated for 30 h, after which the cultures were filtered and pooled; the cells were washed twice with water. The liquid was saturated with NaCl and extracted with CH_2Cl_2. Both extracts were dried with $MgSO_4$ and evaporated at 43 °C under reduced pressure to give a mixture of compounds (212 mg). The mixture was chromatographed over silica gel. Elution of the column with CH_2Cl_2–$(CH_3)_2CO$ (2%) yielded 11,13-dihydrodouglanin (**4**) (90 mg, 33%), m.p. 139 °C (from acetone–hexane); $[\alpha]_D^{20}$ +119° (*c* 1.85 $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 600 (OH), 1 760 (γ-lactone), and 1 640 cm^{-1} (double bond); δ_H 5.33 (1 H, m, $W_{\frac{1}{2}}$ 9 Hz, 3-H), 4.05 (1 H, dd, *J* 11 and 9 Hz, 6-H), 3.40 (1 H, d, *J* 4 Hz, 1-H), 1.88 (3 H, br s, 4-Me), 1.22 (3 H, d, *J* 7 Hz, 11-Me), and 0.83 (3 H, s, 10-Me); *m/z* 250 (M^+, 2%) and 232 (100).

Continued elution of the column with CH_2Cl_2–$(CH_3)_2CO$ (5%) yielded vulgarin (**3**) (28 mg, 9%), m.p. 176—177 °C, $[\alpha]_D^{20}$ +39° (*c* 0.5 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 600 (OH), 1 780 (γ-lactone), and 1 675 cm^{-1} (α,β-unsaturated ketone); $\lambda_{max.}$ 215 nm (EtOH); δ_H 6.61 (1 H, d, *J* 10 Hz, 3-H), 5.90 (1 H, d, *J* 10 Hz, 2-H), 4.25 (1 H, dd, *J* 9 and 10 Hz, 6-H), 2.36 (1 H, d, *J* 10 Hz, 5-H), 1.55 (3 H, s, 4-Me), 1.28 (3 H, d, *J* 7 Hz, 11-Me), and 1.23 (3 H, s, 10-Me).

Fermentation of Deoxyvulgarin (**2**) *with* Rhizopus nigricans *Cultures*.—Substrate (**2**) (300 mg) was dissolved in EtOH (6 ml), distributed among 6 Erlenmeyer flasks, and incubated for 30 h, after which the cultures were processed as indicated above for *A. ochraceous* fermentation, to give a mixture (209 mg), which was chromatographed on silica gel.

Elution of the column with CH_2Cl_2–$(CH_3)_2CO$ (5%) yielded 1β-*hydroxy*-2-*oxo*-5α,11βH-*eudesm*-3-*en*-6α,12-*olide* (**7**) (23 mg, 8%) m.p. 130—132 °C; $\nu_{max.}$($CHCl_3$) 3 460 (OH), 1 770 (OCO), 1 665 (α,β-unsaturated ketone), and 1 620 (conjugated double bond); $\lambda_{max.}$(EtOH) 236 (ε 5 600); δ_H 6.08 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz, 3-H), 4.03 (1 H, dd, *J* 11 and 9 Hz, 6-H), 3.96 (1 H, s), 2.71 (1 H, d, *J* 11 Hz, 5-H), 2.20 (3 H, d, *J* 1.5 Hz, 4-Me), 1.27 (3 H, d, *J* 7 Hz, 11-Me), and 0.85 (3 H, s, 10-Me) (Found: M^+, 264.1348. $C_{15}H_{20}O_4$ requires *M*, 264.1359); *m/z* 249.1096 (*M* − 15) and 246.1268 (*M* − 18, 14%).

Further elution with the same eluant yielded vulgarin (**3**) (10 mg, 3%), characterized by direct comparison. The use of CH_2Cl_2–$(CH_3)_2CO$ (2:1) afforded erivanin (**6**) (40 mg, 14%), m.p. 202—203 °C (from $CHCl_3$–n-hexane); $[\alpha]_D^{20}$ +104° (*c* 0.2 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 610 and 3 540 (OH), 3 050 (C=CH_2), 1 770 (OCO), and 1 645 cm^{-1} (C=C); δ_H 5.19 and 5.04 (1 H each, s, C=CH_2), 4.42 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3-H), 4.07 (1 H, dd, *J* 11 and 9 Hz, 6-H), 3.38 (1 H, m, $W_{\frac{1}{2}}$ 9 Hz, 1-H), 3.04 (1 H, d, *J* 11 Hz, 5-H), 1.22 (3 H, d, *J* 7 Hz, 11-Me), and 0.80 (3 H, s, 10-Me); *m/z* 266 (M^+, 9%), 248 (90), 233 (42), and 145 (100).

4α-*Hydroxy*-5α,6β,7α,11βH-*eudesm*-1-*en*-6,12-*olide* (**10**).—To a stirred solution of 1β,4α-dihydroxy-5α,6β,7α,11βH-eudesman-6,12-olide (tetrahydrovulgarin) (1 g) in dry pyridine (40 ml) was added dropwise trifluoromethanesulphonic anhydride (2.3 g). The mixture was left for 48 h in a refrigerator, then poured into ice–water and extracted with ethyl acetate. The extract was washed (water, saturated aq. $NaHCO_3$, and saturated aq. NaCl), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was chromatographed over silica gel [n-hexane–ethyl acetate (5:2)] to give *compound* (**10**) (300 mg, 30%) as an oil, $\nu_{max.}$($CHCl_3$) 3 560 (OH) and 1 770 cm^{-1} (γ-lactone); δ_H 5.50 (2 H, s, 1- and 2-H), 4.22 (1 H, dd, *J* 11 and 9 Hz, 6-H), 1.40 (3 H, s, 15-H_3), 1.27 (3 H, d, *J* 7 Hz, 13-H_3), and 1.08 (3 H, s, 14-H_3) [Found: M^+, 250.1580 (11%). $C_{15}H_{22}O_3$ requires *M*, 250.1569]; *m/z* 235.1359 (*M* − 15, 88%) and 166 (100).

Further compounds eluted were 4α-hydroxy-5α,6β,7α,11βH-guaia-1(10)-en-6,12-olide (dihydromicheliolide) (50 mg, 5%) as an oil, $\nu_{max.}$($CHCl_3$) 3 560 (OH) and 1 770 cm^{-1} (γ-lactone); δ_H 3.80 (1 H, dd, *J* 10 and 9 Hz, 6-H), 1.70 (3 H, s, 14-H_3), 1.30 (3 H, s, 15-H_3), and 1.25 (3 H, d, *J* 7 Hz, 13-H_3); *m/z* 250 (M^+); and 4α-hydroxy-1α,5α,6β,7α,11βH-guaia-10(14)-en-6,12-olide (compressanolide), as an oil, $\nu_{max.}$($CHCl_3$) 3 560 (OH), 1 760 (γ-lactone), and 1 640 cm^{-1} (CH_2); δ_H 4.94 (2 H, br s, 14-H_2), 4.10 (1 H, dd, *J* 11 and 9 Hz, 6-H), 1.29 (3 H, s, 15-H_3), and 1.21 (2 H, d, *J* 7 Hz, 13-H_2); *m/z* 250 (M^+) and 232 (*M* − 18).

1α-*Hydroxy*-2-*oxo*-5α,11βH-*eudesm*-3-*en*-6α,12-*olide* (**8**).—A solution of compound (**10**) (234 mg) in dioxane–water (3:1) (8 ml) was oxidized with $NaIO_4$ (416 mg) and a catalytic amount of OsO_4. The periodate was added in small proportions during 15 min to the stirred solution. After 1.5 h at room temperature the solution was poured into cold water and extracted with $CHCl_3$. The extract was washed with water, dried, and evaporated to dryness. Chromatography on silica gel with n-hexane–AcOEt (3:1) yielded the enone (**8**) (66 mg, 28%) as a liquid, $\nu_{max.}$($CHCl_3$) 3 580 and 3 340 (OH), 1 770 (γ-lactone), 1 660 (C=O conjugated), and 1 610 cm^{-1} (conjugated double bond); $\lambda_{max.}$(EtOH) 230 nm (log ε 4.15); δ_H 5.90 (1 H, br s, $W_{\frac{1}{2}}$ 6 Hz, 3-H), 4.00 (1 H, dd, *J* 10 and 9 Hz, 6-H), 3.48 (1 H, s, 1-H), 2.16 (3 H, br s, *J* 2 Hz, 4-Me), 1.24 (3 H, d, *J* 7 Hz, 11-Me), and 0.93 (3 H, s, 10-Me) (Found: M^+, 264.1360. Calc. for $C_{15}H_{20}O_4$: *M*, 264.1359); *m/z* 246.1227 (*M* − 18).

Epoxidation of Deoxyvulgarin (**2**).—Deoxyvulgarin (**2**) (250 mg) was dissolved in $CHCl_3$ (20 ml) and epoxidized with MCPBA (300 mg) at room temp. for 8 h. The reaction product was chromatographed over silica gel. Elution with CH_2Cl_2–$(CH_3)_2CO$ (5%) gave only vulgarin (**3**) (237 mg).

Acknowledgements

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