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MICROBIAL TRANSFORMATIONS OF 6α- AND 6β-EUDESMANOLIDES BY *RHIZOPUS NIGRICANS* CULTURES

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ABSTRACT.—Microbial transformations of 6α-eudesmanolides and 6β-eudesmanolides were accomplished using the hydroxylating fungus *Rhizopus nigricans*. The eudesmane skeleton was functionalized principally at positions C-1, C-4, and C-8. Hydroxylation yields depended on the configuration of the hydroxyl group at C-3, the methyl group at C-4, and the lactone ring in the substrates. Several metabolites with hydroxylation at C-9 and C-11 were also obtained with 6α-eudesmanolide substrates. An inversion of configuration at C-3 may occur when the substrates have an axial hydroxyl group at this carbon. Hydroxylation at C-4 was observed only when the substrate had a 4β-methyl group.

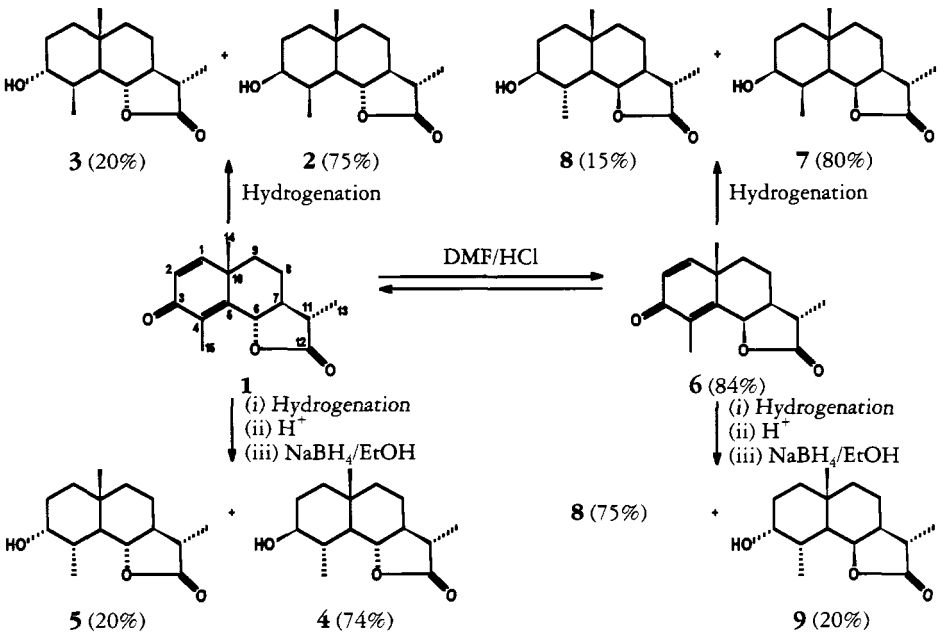
Sesquiterpene lactones with a eudesmane-6α,12-olide structure are abundant in nature and the chemistry (1–7) and biotransformation (8–14) of some have been studied extensively. However, 6β-eudesmanolides are scarce and have rarely been studied. As a continuation of our studies on the systematic biotransformation of terpenes (15–20), we present the results of microbial transformations of some 6α-and 6β-eudesmanolides using the fungus *Rhizopus nigricans*. Bioconversion permits access to remote positions on the molecules and is an alternative to chemical routes. The data obtained in this work, together with previous findings (17,18), help to establish the relationship between the structures of eudesmanolide substrates and the hydroxylating activity of this fungus. We describe herein a series of biotransformations on commercial α-santonin. These conversions have yielded eudesmanolides hydroxylated at C-1 that can be chemically transformed into other sesquiterpene skeletons. We have also obtained 6-eudesmanolides with hydroxylation at C-8 which can be converted to 8-eudesmanolides with either an 8α- or 8β-configuration.

RESULTS AND DISCUSSION

The hydrogenation of α-santonin [**1**] was described many years ago (21,22). We reduced commercial α-santonin [**1**] (H_2/PtO_2), yielding products **2** (21) (75%) and **3** (21) (20%) (Scheme 1). ^1H- and ^{13}C-nmr spectra of **2** indicated that the hydroxyl group in this compound was located at C-3 and the methyl group was at C-4, both with a β-configuration. Therefore, **2** was formed by hydrogenation of the A ring on the α-face of the molecule. Product **3** exhibited a C-3 geminal proton signal (ddd, $J_1=J_2=J_3=2.8$ Hz), which indicated that it was equatorial. The methyl group at C-4 in this compound [**3**] had a β-configuration ($J=7.6$ Hz).

Partial hydrogenation of **1**, followed by treatment with acid and $NaBH_4$ reduction gave rise to compounds **4** (23) (74%) and **5** (23) (20%) (Scheme 1). These products are epimers at C-3 with the methyl group at C-4α. The compound with a 3β-hydroxyl configuration was again the main product.

Epimerization of α-santonin [**1**] at C-6 to 6β-santonin [**6**] was carried out as previously described (17) (Scheme 1). Catalytic hydrogenation of **6** with H_2/PtO_2 yielded the previously described products **7** (80%) (18) and **8** (15%) (17).



SCHEME 1. Starting materials **2**, **4**, **5**, **7**, and **9** obtained from (−)-α-santonin [**1**].

Hydrogenation of the A ring in 6β-santonin [**6**] with H_2/Pt/C gave a C-3 carbonyl compound that was epimerized at C-4 in acidic medium (17). Finally, $NaBH_4$ reduction of this ketone yielded compounds **8** (75%) and **9** (20%) (17). Nmr experiments indicated that **9** had a 3α-hydroxyl group and a 4α-methyl group.

3β-Hydroxy-4α,5α,11β-H-eudesman-6α,12-olide [**2**] was incubated with *Rhizopus nigricans* for 12 days to give the unaltered substrate **2** (8%), metabolite **10** (32%), metabolite **11** (18%), and a mixture of more polar metabolites (Scheme 2). Acetylation of this mixture of metabolites gave the products **12** (4%), **13** (7%), **14** (10%), and **15** (2%). The cims of **10** showed a molecular peak at *m/z* 269 ($[M+1]^+$), indicating that **10** had a new hydroxyl group compatible with a signal at δ 3.37 (1H, dd, J_1=11.5 Hz, J_2=4.2 Hz) in its ^{1}H-nmr spectrum. On the basis of this signal and the ^{13}C-nmr spectrum of **10**, we propose that the hydroxylation occurred at C-1. The new hydroxyl-group configuration was confirmed by nOe experiments. Irradiation at H-3 produced a nOe effect on H-1, thus metabolite **10** was a 1β-hydroxyl derivative.

The second metabolite [**11**] of this incubation also had a cims molecular peak at *m/z* 269. The C-15 methyl group signal of **11** was converted to a singlet at δ 1.25 (3H, s), and the H-3 signal was at δ 3.52 (1H, dd, J_1=11.8 Hz, J_2=4.6 Hz). Therefore, the new hydroxyl was at C-4, a conclusion corroborated by its ^{13}C-nmr spectrum, where a new oxygenated carbon (δ 75.0) was detected. Several nOe difference experiments were performed to determine the configuration of **11** at C-4. Irradiation at δ 4.05 (H-6β) produced a nOe on the C-15 methyl group, and vice versa, indicating an α-configuration.

Product **12** was identified as the acetate of **2**. Acetate **13** corresponded to the C-3 monoacetate of metabolite **11**. Compound **14** had a cims molecular peak of *m/z* 353, which indicated that it was a diacetate of **2**. Geminal protons of the two acetoxyl groups appeared in its ^{1}H-nmr spectrum at δ 4.81 (1H, ddd, J_1=10.7 Hz, J_2=J_3=5.2 Hz, H-3α) and δ 5.08 (1H, ddd, J_1=15.1 Hz, J_2=10.9 Hz, J_3=4.2 Hz), respectively. These observations, together with the ^{13}C-nmr spectrum, indicated that the new acetoxyl group was at C-8 with an 8*S* configuration. This 8α-arrangement of the acetoxyl group

was confirmed by nOe experiments between the H-8, H-11, and Me-14 protons. The last product [**15**], obtained in low yield, had a single acetoxyl group at C-3 and a new hydroxyl group. Its ^{1}H-nmr spectrum showed two singlet methyl groups at δ 1.05 and δ 1.24; the chemical shift and the multiplicity of these methyl group signals indicated that the new hydroxyl group was situated at C-11. This conclusion was confirmed by the presence of a new quaternary oxygenated carbon in its ^{13}C-nmr spectrum.

Incubation of substrate **4** with *Rhizopus nigricans* for 10 days gave metabolites **16** (40%), **17** (7.5%), **18** (16%), and **19** (13%) (Scheme 2). The main metabolite [**16**] isolated from this biotransformation showed ^{1}H-nmr signals at δ 3.37 (1H, dd, J_1=11.9 Hz, J_2=4.4 Hz). An α-effect for C-1 and β-effects for C-2 and C-10 were observed in its ^{13}C-nmr spectrum. Therefore, metabolite **16** had a new equatorial hydroxyl group at C-1.

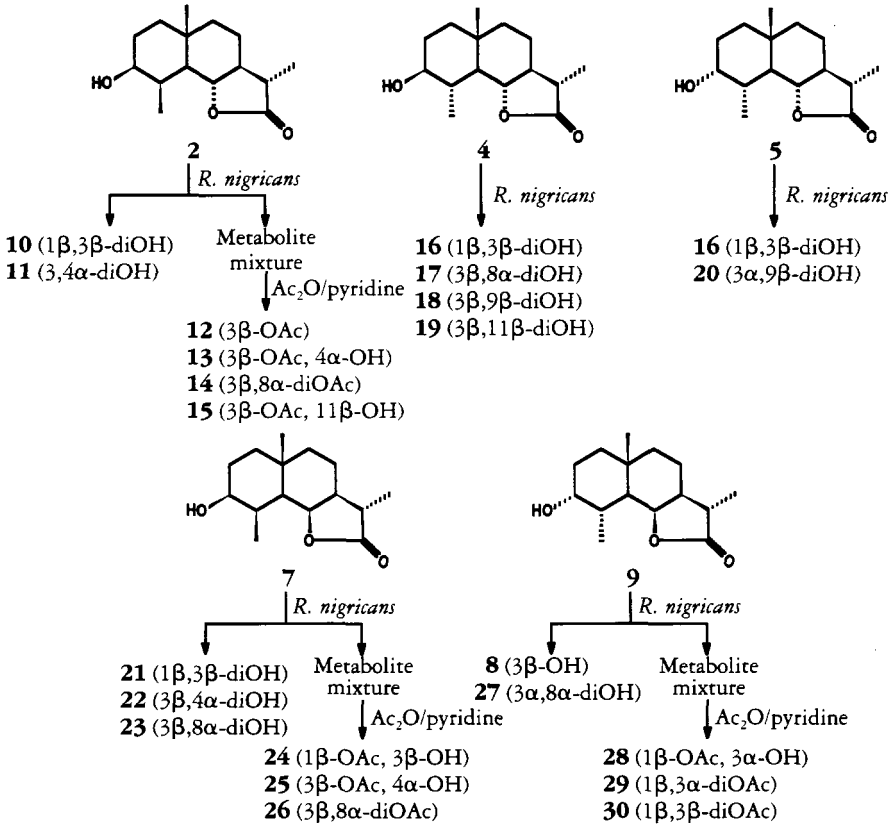
Metabolite **17** gave a cims molecular peak of *m/z* 269; its ^{1}H-nmr spectrum showed a signal at δ 3.94 (1H, ddd, J_1=14.9 Hz, J_2=10.6 Hz, J_3=4.4 Hz) due to an axial proton geminal to a new hydroxyl group at C-8. This hydroxylation was confirmed by the ^{13}C-nmr data: an α-effect on C-8 (Δδ +45.6), β-effects on C-7 (Δδ +6.1) and C-9 (Δδ +10.5), and γ-effects on C-6 (Δδ −2.8) and C-10 (Δδ −0.5) were observed.

The third metabolite [**18**] obtained from this incubation had spectroscopic data very similar to those of metabolite **16**. The ^{1}H-nmr spectrum of **18** indicated that it had an axial proton geminal to a new hydroxyl group (δ 3.37, 1H, dd, J_1=10.2 Hz, J_2=4.2 Hz). The multiplicity and coupling constants of this signal indicated that the new hydroxyl group was at C-9 with a 9β-configuration. ^{13}C-Nmr data of **18** corroborated this conclusion; thus, an α-effect on C-9 (Δδ +37.3), β-effects on C-8 (Δδ +6.8) and C-10 (Δδ +4.7), and γ-effects on C-1 (Δδ −4.0), C-5 (Δδ −2.2), C-7 (Δδ −2.7) and C-14 (Δδ −3.8) were noted.

The last metabolite obtained from this biotransformation [**19**] had a cims molecular peak of *m/z* 269, which indicated that substrate **4** had again been hydroxylated by the fungus. The ^{1}H-nmr spectrum of **19** indicated that the new hydroxyl group was introduced at a tertiary carbon. The hydroxyl group was therefore expected at C-4 or C-11. Comparison of the ^{13}C-nmr chemical shifts of C-11, C-6, C-7, and C-8 to those of substrate **4** showed that the new hydroxyl group was at C-11. Finally, the OH-11 configuration was determined by several nOe experiments. Irradiation on the C-13 methyl group produced a nOe effect with H-6β and vice versa; therefore the hydroxyl group at C-11 had an 11β-configuration.

When substrate **5** was incubated with *Rhizopus nigricans* for 10 days, only two metabolites (**16**, 30%, and **20**, 17%) were isolated. The first metabolite of this biotransformation had physical and spectroscopic properties identical to those of metabolite **16**, isolated previously from the incubation of substrate **4**. Therefore, the microorganism had functionalized at C-1 and epimerized at C-3. Metabolite **20** was a dihydroxylated eudesmanolide (molecular mass 268 daltons). Comparison of the ^{13}C-nmr spectrum of compound **5** and metabolite **20** showed that the new functionalization was at C-9. On the basis of these observations, the structure 3α,9β-dihydroxy-4β,5α,11β-H-eudesman-6α,12-olide was proposed for metabolite **20**.

Biotransformation of substrate **7** with *Rhizopus nigricans* for 12 days gave metabolites **21** (16%), **22** (20%), **23** (12%), and a mixture of more polar metabolites (Scheme 2). This mixture was acetylated, and products **24** (4%), **25** (10%), and **26** (5%) were isolated. Metabolite **21** showed spectroscopic behavior that indicated the presence of a new hydroxyl group at C-1, with a 1β configuration (δ 3.21, 1H, dd, J_1=11.5 Hz, J_2=4.3 Hz). The physical and spectroscopic data of metabolite **22** (4α-hydroxyl derivative) were consistent with those reported previously for this compound (18). The



SCHEME 2. Biotransformation results of substrates **2**, **4**, **5**, **7**, and **9**.

^{1}H-nmr spectrum of metabolite **23** showed a new signal of a geminal hydroxyl group proton (δ 3.53, 1H, ddd, J_1=12.0 Hz, J_2=9.9 Hz, J_3=4.2 Hz). The multiplicity and the coupling constants of this signal indicated that the new hydroxylation was situated at C-2 or at C-8. A comparison of the ^{13}C-nmr chemical shifts of metabolite **23** to those of substrate **7** revealed that this compound had an 8α-hydroxyl group (β-effects on C-7 and C-9, Δδ +9.2 and +10.3, respectively). As shown in Scheme 2, metabolites **24**, **25**, and **26** were the acetylated derivatives of **21**, **22**, and **23**, respectively, and were identified by their physical and spectroscopic data.

Finally, we carried out the bioconversion of the 6β-eudesmanolide **9** with *Rhizopus nigricans* for 7 days. Metabolites **8** (17%), **27** (31%), and a mixture of additional metabolites were isolated. Acetylation of this mixture gave the acetylated derivatives **28** (4%), **29** (4%), and **30** (17%). The first metabolite isolated from this biotransformation had physical and spectroscopic properties identical to those of **8**. This metabolite was formed by epimerization of substrate **9** at C-3. The structure of metabolite **27** was deduced by comparison of its spectroscopic data with those described for product **23**, which confirmed a new hydroxylation at C-8 with an 8α-configuration.

Product **28** had a cims that indicated a new acetoxyl group (molecular peak of *m/z* 311). Taking into account the previously described results, and the ^{1}H- and ^{13}C-nmr spectra of **28**, we could establish that this metabolite had a 1β-acetoxyl group that was due to a 1β-hydroxylation of substrate **9** by the fungus and subsequent partial acetylation by chemical means. The diacetylated compound **29** was identified as the acetoxyl derivative of **28**. Product **30** had spectroscopic data indicating that it was a

diacetoxyl eudesmanolide that could be formed by epimerization at C-3 of the hydroxyl group in this position of substrate **9**, and subsequent 1β-hydroxylation.

Several conclusions can be drawn from the above results of the 6α- and 6β-eudesmanolide biotransformations with *Rhizopus nigricans* and from our previously published results (17,18). Thus, *Rhizopus nigricans* functionalizes the C-1, C-4, and C-8 eudesmane skeleton positions, although the yields depend on the C-3, C-4, and C-6 substituent arrangements. Epimerization at C-3 was observed when the hydroxyl group at this position had an α-configuration. If the substrate was a 6α-eudesmanolide, minor hydroxylations at C-9 and C-11 were observed. The microorganism *Rhizopus nigricans* hydroxylated at C-4 only when the methyl group at this position was axial (β-configuration). Some of these biotransformations give acceptable yields of C-1 or C-8 hydroxylated products, which are adequate starting materials to attempt the biogenetic formation of guaianolides and 8,12-eudesmanolides.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined using a Kofler (Reichter) apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 20°. Ir spectra were recorded on a Perkin-Elmer 983G spectrometer or on a Nicolet 20SX Ft-ir spectrometer. Ms were determined with ci (CH_4) using a Hewlett-Packard 5988A mass spectrometer. Elemental analyses were made in a Perkin-Elmer 240C analyzer. Nmr spectra (400.13 MHz ^{1}H, 300.13 MHz ^{1}H, and 100.62 MHz ^{13}C) were recorded in $CDCl_3$ (which also provided the lock signal) on Bruker ARX-400 and Bruker AM-300 spectrometers. The assignments of ^{13}C-nmr chemical shifts were done with the aid of DEPT experiments using a flip angle of 135°. Si gel SDS 60A CC (40–60 μm) was used for flash chromatography. CH_2Cl_2 or $CHCl_3$ containing increasing amounts of Me_2CO were used as the eluent. Analytical tlc plates (Si gel, Merck 60 G) were visualized by spraying with H_2SO_4/AcOH, followed by heating to 120°. The starting material was (−)-α-santonin (99%, Aldrich Chemical Company).

ORGANISM, MEDIA, AND CULTURE CONDITIONS.—*Rhizopus nigricans* CECT 2072 was obtained from the Coleccion Española de Cultivos Tipo, Departamento de Microbiologia, 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 80 ml of medium were inoculated with a dense suspension of *Rhizopus nigricans*. The cultures were incubated by shaking (150 rpm) at 28° from 6 days, after which substrates **2**, **4**, **5**, **7**, and **9** in EtOH (1%) were added.

CATALYTIC HYDROGENATION OF (−)-α-SANTONIN [**1**].—(−)-α-Santonin (2 g) was dissolved in EtOH (30 ml) and hydrogenated with H_2 (4 atmospheres) on PtO_2 (300 mg) for 5 h at room temperature. Si gel cc yielded 3β-hydroxy-4α,5α,11β-H-eudesman-6α,12-olide [**2**] (21) (1.53 g, 75%) and 3α-hydroxy-4α,5α,11β-H-eudesman-6α,12-olide [**3**] (21) (412 mg, 20%).

3β-Hydroxy-4α,5α,11β-H-eudesman-6α,12-olide [**2**].—Mp 125°; $[\alpha]^{25}D$ −62° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3460, 1767, 1237 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 3.95 (1H, dd, $J_{6,5}$=11.6 Hz, $J_{6,7}$=9.9 Hz, H-6β), 3.72 (1H, ddd, $J_{3,2\beta}$=11.0 Hz, $J_{3,4\alpha}$=$J_{3,2\alpha}$=5.3 Hz, H-3α), 2.39 (1H, ddq, $J_{4\alpha,15}$=7.5 Hz, $J_{4\alpha,3}$=$J_{4\alpha,5}$=5.3 Hz, H-4α), 2.33 (1H, dq, $J_{11,7}$=12.2 Hz, $J_{11,13}$=6.9 Hz, H-11β), 1.19 (3H, d, $J_{13,11}$=6.9 Hz, Me-13), 1.01 (3H, s, Me-14), 0.96 (3H, d, $J_{15,4}$=7.5 Hz, Me-15); ^{13}C nmr ($CDCl_3$) δ 40.2 (C-1), 25.9 (C-2), 73.0 (C-3), 34.4 (C-4), 49.7 (C-5), 80.1 (C-6), 53.6 (C-7), 23.5 (C-8), 43.3 (C-9), 35.9 (C-10), 41.9 (C-11), 178.7 (C-12), 12.6 (C-13), 21.1 (C-14), 8.8 (C-15); cims (CH_4) *m/z* 253 $[M+1]^+$ (50), 235 $[M+1-18]^+$ (100); *anal.*, found C 71.3, H 9.6; $C_{15}H_{24}O_3$ requires C 71.38, H 9.59%.

3α-Hydroxy-4α,5α,11β-H-eudesman-6α,12-olide [**3**].—Mp 116–118°, $[\alpha]^{25}D$ −54° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3460, 1767 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 3.93 (1H, dd, $J_{6,5}$=11.6 Hz, $J_{6,7}$=10.0 Hz, H-6β), 3.85 (1H, ddd, $J_{3,4\alpha}$=$J_{3,2\alpha}$=$J_{3,2\beta}$=2.8 Hz, H-3β), 2.32 (1H, dq, $J_{11,7}$=12.4 Hz, $J_{11,13}$=6.9 Hz, H-11β), 2.10 (1H, m, H-4α), 2.03 (1H, dd, $J_{5,6}$=11.6 Hz, $J_{5,4}$=5.0 Hz, H-5α), 1.20 (3H, d, $J_{11,13}$=6.9 Hz, Me-13), 1.02 (3H, s, Me-14), 0.99 (3H, d, $J_{15,4}$=7.6 Hz, Me-15); cims (CH_4) *m/z* 253 $[M+1]^+$ (49), 235 $[M+1-18]^+$ (100); *anal.*, found C 71.4, H 9.6; $C_{15}H_{24}O_3$ requires C 71.38, H 9.59%.

COMPOUNDS **4** AND **5** FROM (−)-α-SANTONIN [**1**].—A solution of (−)-α-santonin (2 g) in CH_2Cl_2 (60 ml) was partially hydrogenated with H_2 (4–5 atmospheres) for 5 h on Pt-charcoal. Then, the reaction mixture was filtered and concentrated. $CHCl_3$ (50 ml) and several drops of aqueous 2 N HCl were added and the

mixture allowed to stand for 7 days at room temperature. The reaction mixture was concentrated, dissolved in EtOH (200 ml), $NaBH_4$ (735 mg) added, and the solution stirred for 2 h at 0°. Finally the reaction was treated with dilute HCl, H_2O, and extracted thoroughly with CH_2Cl_2. The $CHCl_3$ solubles were chromatographed on Si gel and products **4** (23) (1.52 g, 74%) and **5** (23) (406 mg, 20%) were isolated.

CONVERSION OF (−)-α-SANTONIN [**1**] TO (−)-6-*EPI*-α-SANTONIN [**6**] BY THE ISHIKAWA PROCEDURE.—(−)-α-Santonin (5 g) was dissolved in anhydrous *N,N'*-dimethylformamide (50 ml) containing anhydrous HCl (5%). The solution was heated for 3 h at 85°. The mixture was stirred at room temperature for 12 h, then diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous NaCl, saturated aqueous $NaHCO_3$, and water. The solvent was removed under reduced pressure. Chromatography over Si gel yielded (−)-6-*epi*-α-santonin (**6**, 4.2 g, 84%) (17).

CATALYTIC HYDROGENATION OF (−)-6-*EPI*-α-SANTONIN [**6**].—A solution of (−)-6-*epi*-α-santonin (1.5 g) in CH_2Cl_2 (50 ml) was hydrogenated for 6 h with H_2 (4–5 atmospheres) on PtO_2 (0.25 g). The reaction mixture was filtered and concentrated *in vacuo*. Chromatography on a Si gel column yielded **7** (18) (1.23 g, 80%) and **8** (17) (230 mg, 15%).

COMPOUNDS **8** AND **9** FROM (−)-6-*EPI*-α-SANTONIN [**6**].—A solution of (−)-6-*epi*-α-santonin (2.5 g) in CH_2Cl_2 (80 ml) was partially hydrogenated with H_2 (4–5 atmospheres) for 6 h on Pt-charcoal. The reaction mixture was dissolved in $CHCl_3$ (60 ml) and several drops of a 2 N HCl solution were added. The reaction mixture was shaken vigorously at room temperature for 3 days. The resultant mixture was treated with EtOH (300 ml), with $NaBH_4$ (900 mg) added, keeping the reaction for 2 h at 0°. The reaction was then treated with dilute HCl, diluted with H_2O, and extracted with CH_2Cl_2. The solvent was removed under reduced pressure and chromatographed on a Si gel column yielding **8** (17) (1.92 g, 75%) and **9** (17) (511 mg, 20%).

BIOTRANSFORMATION OF 3β-HYDROXY-4α,5α,11β-H-EUDESMAN-6α,12-OLIDE [**2**].—Substrate **2** (725 mg) was dissolved in EtOH (15 ml), distributed among 15 Erlenmeyer-flask cultures and incubated for 12 days after which the cultures were filtered and pooled; the cells were washed thoroughly with H_2O and the liquid was saturated with NaCl and extracted twice with CH_2Cl_2. Extracts were pooled, dried (Na_2SO_4), and evaporated at 40° *in vacuo* to give a mixture of compounds (695 mg). This mixture was chromatographed on a Si gel column to obtain 60 mg (8%) of starting material, 250 mg (32%) of metabolite **10**, 140 mg (18%) of metabolite **11**, and 245 mg of a polar mixture, which was dissolved in Ac_2O/pyridine (1:2) and maintained for 12 h at room temperature. The reaction mixture was diluted with H_2O, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4. Chromatography on Si gel yielded the acetoxy derivatives **12** (35 mg, 4%), **13** (60 mg, 7%), **14** (100 mg, 10%), and **15** (20 mg, 2%). All yields are given with respect to substrate **2**.

1β,3β-Dihydroxy-4α,5α,11β-H-eudesman-6α,12-olide [**10**].—Mp 195°; $[\alpha]^{25}D$ +12° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3424, 1768 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.06 (1H, dd, $J_{6,5}$=11.4 Hz, $J_{6,7}$=10.0 Hz, H-6β), 3.82 (1H, ddd, $J_{3,2\beta}$=10.2 Hz, $J_{3,4\alpha}$=5.7 Hz, $J_{3,2\alpha}$=4.6 Hz, H-3α), 3.37 (1H, dd, $J_{1\alpha,2\beta}$=11.5 Hz, $J_{1\alpha,2\alpha}$=4.2 Hz, H-1α), 2.38 (1H, ddq, $J_{4\alpha,5}$=13.7 Hz, $J_{4\alpha,15}$=7.5 Hz, $J_{4\alpha,3\alpha}$=6.8 Hz, H-4α), 2.32 (1H, dq, $J_{11,7}$=13.9 Hz, $J_{11,13}$=7.1 Hz, H-11β), 1.22 (3H, d, $J_{13,11}$=7.1 Hz, Me-13), 1.02 (3H, s, Me-14), 0.98 (3H, d, $J_{15,4}$=7.5 Hz, Me-15); ^{13}C nmr ($CDCl_3$) δ 77.6 (C-1), 35.2 (C-2), 70.6 (C-3), 34.3 (C-4), 47.6 (C-5), 79.5 (C-6), 53.7 (C-7), 23.3 (C-8), 39.7 (C-9), 41.3 (C-10), 42.0 (C-11), 179.5 (C-12), 12.6 (C-13), 15.2 (C-14), 8.7 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (28), 251 $[M+1-18]^+$ (100), 233 $[M+1-36]^+$ (70); *anal.*, found C 67.0, H 9.0; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

3β,4α-Dihydroxy-5α,11β-H-eudesman-6α,12-olide [**11**].—Syrup; $[\alpha]^{25}D$ +13° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3464, 1781 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.05 (1H, dd, $J_{6,5}$=11.3 Hz, $J_{6,7}$=10.4 Hz, H-6β), 3.52 (1H, dd, $J_{3,2\beta}$=11.8 Hz, $J_{3,2\alpha}$=4.6 Hz, H-3α), 2.26 (1H, dq, $J_{11,7}$=12.5 Hz, $J_{11,13}$=6.9 Hz, H-11β), 1.18 (3H, d, $J_{13,11}$=6.9 Hz, Me-13), 1.25 (3H, s, Me-15), 0.96 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 39.1 (C-1), 26.0 (C-2), 77.4 (C-3), 75.0 (C-4), 55.6 (C-5), 80.6 (C-6), 53.2 (C-7), 23.3 (C-8), 42.9 (C-9), 37.6 (C-10), 40.4 (C-11), 178.3 (C-12), 12.3 (C-13), 19.9 (C-14), 17.8 (C-15); cims (CH_3) *m/z* 269 $[M+1]^+$ (15), 251 $[M+1-18]^+$ (100), 233 $[M+1-36]^+$ (35); *anal.*, found C 67.0, H 8.9; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

3β-Acetoxy-4α,5α,11β-H-eudesman-6α,12-olide [**12**].—Mp 205°; $[\alpha]^{25}D$ +23° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 1766, 1734 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.79 (1H, ddd, $J_{3,2\beta}$=11.7 Hz, $J_{3,4\alpha}$=10.5 Hz, $J_{3,2\alpha}$=5.2 Hz, H-3α), 3.92 (1H, dd, $J_{6,5}$=11.4 Hz, $J_{6,7}$=9.9 Hz, H-6β), 2.50 (1H, ddd, $J_{4\alpha,3}$=12.5 Hz, $J_{4\alpha,15}$=7.4 Hz, $J_{4\alpha,5}$=5.1 Hz, H-4α), 2.31 (1H, dq, $J_{11,7}$=12.2 Hz, $J_{11,13}$=6.9 Hz, H-11β), 2.01 (3H, s, Me-AcO), 1.19 (3H, d, $J_{13,11}$=6.9 Hz, Me-13), 1.03 (3H, s, Me-14), 0.96 (3H, d, $J_{15,4}$=7.4 Hz, Me-15); ^{13}C nmr ($CDCl_3$) δ 39.8 (C-1), 22.6 (C-2), 75.1 (C-3), 31.7 (C-4), 49.5 (C-5), 79.7 (C-6), 53.6 (C-7), 23.5 (C-8), 43.2 (C-9), 35.9 (C-10), 41.8 (C-11), 179.4 (C-12), 12.6 (C-13), 21.1 (C-14), 9.4 (C-15), 170.3 (Me*C*O), 21.3 (*Me*CO); cims (CH_4) *m/z* 295 $[M+1]^+$ (10), 235 $[M+1-60]^+$ (100); *anal.*, found C 69.3, H 8.9; $C_{17}H_{26}O_4$ requires C 69.34, H 8.91%.

3β-Acetoxy-4α-hydroxy-5α,11β-H-eudesman-6α,12-olide [**13**].—Mp 197°; $[\alpha]^{25}D$ +14.5° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3565, 1781, 1736 cm^{-1}; ^1H nmr ($CHCl_3$) δ 4.74 (1H, ddd, $J_{3,2\beta}$=11.5 Hz, $J_{3,2\alpha}=J_{3,4\alpha}$=4.9 Hz, H-3α), 4.05 (1H, dd, $J_{6,5}$=11.3 Hz, $J_{6,7}$=10.3 Hz, H-6β), 2.26 (1H, dq, $J_{11,7}$=12.5 Hz, $J_{11,13}$=6.9 Hz, H-11β), 2.06 (3H, s, Me-AcO), 1.20 (3H, d, $J_{13,11}$=6.9 Hz, Me-13), 1.33 (3H, s, Me-15), 0.99 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 39.1 (C-1), 23.5 (C-2), 78.8 (C-3), 73.5 (C-4), 56.3 (C-5), 80.4 (C-6), 53.3 (C-7), 24.8 (C-8), 42.8 (C-9), 37.4 (C-10), 40.5 (C-11), 178.2 (C-12), 12.5 (C-13), 21.4 (C-14), 19.0 (C-15), 170.7 (Me*C*O), 19.9 (*Me*CO); cims (CH_4) *m/z* 311 $[M+1]^+$ (42), 293 $[M+1-18]^+$ (76), 251 $[M+1-60]^+$ (100); *anal.*, found C 65.8, H 8.5; $C_{17}H_{26}O_5$ requires C 65.77, H 8.45%.

3β,8α-Diacetoxy-4α,5α,11β-H-eudesman-6α,12-olide [**14**].—Mp 203°, $[\alpha]^{25}D$ +12° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 1780, 1733, 1712 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.08 (1H, ddd, $J_{8\beta,7}$=15.1 Hz, $J_{8\beta,9\alpha}$=10.9 Hz, $J_{8\beta,9\beta}$=4.2 Hz, H-8β), 4.81 (1H, ddd, $J_{3,2\beta}$=10.7 Hz, $J_{3,2\alpha}=J_{3,4\alpha}$=5.2 Hz, H-3α), 4.00 (1H, dd, $J_{6,5}$=11.2 Hz, $J_{6,7}$=10.9 Hz, H-6β), 2.55 (1H, dq, $J_{11,7}$=12.5 Hz, $J_{11,13}$=6.9 Hz, H-11β), 2.04 (3H, s, Me-AcO), 2.03 (3H, s, Me-AcO), 1.22 (3H, d, $J_{13,11}$=6.9 Hz, Me-13), 1.11 (3H, s, Me-14), 0.96 (3H, d, $J_{15,4}$=7.5 Hz, Me-15); ^{13}C nmr ($CDCl_3$) δ 39.4 (C-1), 22.3 (C-2), 74.7 (C-3), 31.6 (C-4), 49.0 (C-5), 76.8 (C-6), 57.1 (C-7), 70.2 (C-8), 49.1 (C-9), 35.1 (C-10), 41.2 (C-11), 178.3 (C-12), 14.0 (C-13), 21.8 (C-14), 9.4 (C-15), 170.4 (Me*C*O), 170.4 (Me*C*O), 21.3 (*Me*CO), 21.1 (*Me*CO); cims (CH_4) *m/z* 353 $[M+1]^+$ (29), 293 $[M+1-60]^+$ (64), 233 $[M+1-120]^+$ (100); *anal.*, found C 64.7, H 7.9; $C_{19}H_{28}O_6$ requires C 64.74, H 8.01%.

3β-Acetoxy-11β-hydroxy-4α,5α-H-eudesman-6α,12-olide [**15**].—Syrup; $[\alpha]^{25}D$ +13° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3449, 1783, 1734 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.80 (1H, ddd, $J_{3,2\beta}$=10.3 Hz, $J_{3,2\alpha}=J_{3,4\alpha}$=4.9 Hz, H-3α), 4.43 (1H, dd, $J_{6,5}$=11.7 Hz, $J_{6,7}$=10.0 Hz, H-6β), 2.03 (3H, s, Me-AcO), 1.24 (3H, s, Me-13), 1.05 (3H, s, Me-14), 1.00 (3H, d, $J_{15,4}$ =7.4 Hz, Me-15); ^{13}C nmr ($CDCl_3$) δ 39.9 (C-1), 22.6 (C-2), 75.1 (C-3), 31.8 (C-4), 49.8 (C-5), 78.3 (C-6), 55.9 (C-7), 18.3 (C-8), 43.0 (C-9), 35.9 (C-10), 74.2 (C-11), 177.8 (C-12), 21.8 (C-13), 21.0 (C-14), 9.5 (C-15), 170.5 (Me*C*O), 21.3 (*Me*CO); cims (CH_4) *m/z* 311 $[M+1]^+$ (7), 233 $[M+1-78]^+$ (56); *anal.*, found C 65.6, H 8.3; $C_{17}H_{26}O_5$ requires C 65.77, H 8.45%.

BIOTRANSFORMATION OF 3β-HYDROXY-4β,5α,11β-H-EUDESMAN-6α,12-OLIDE [**4**].—Substrate **4** (800 mg) was dissolved in EtOH (10 ml), distributed among 18 Erlenmeyer-flask cultures and incubated for 10 days, after which the cultures were processed as indicated above for the biotransformation of substrate **2** to give a mixture (785 mg) that was chromatographed on a Si gel column to obtain 72 mg (8.5%) of starting material, 344 mg (40%) of metabolite **16**, 64 mg (7.5%) of metabolite **17**, 136 mg (16%) of metabolite **18**, and 120 mg (13%) of metabolite **19**.

1β,3β-Dihydroxy-4β,5α,11-H-eudesman-6α,12-olide [**16**].—Syrup; $[\alpha]^{25}D$ +39° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3408, 1759 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.92 (1H, dd, $J_{6,5}=J_{6,7}$=10.5 Hz, H-6β), 3.37 (1H, dd, $J_{1\alpha,2\beta}$=11.9 Hz, $J_{1\alpha,2\alpha}$=4.4 Hz, H-1α), 3.19 (1H, ddd, $J_{3,2\beta}$=11.4 Hz, $J_{3,4\alpha}$=9.8 Hz, $J_{3,2\alpha}$=5.1 Hz, H-3α), 2.21 (1H, dq, $J_{11,7}$=12.3 Hz, $J_{11,13}$=6.9 Hz, H-11β), 1.19 (3H, d, $J_{13,11}$= 6.9 Hz, Me-13), 1.19 (3H, d, $J_{15,4}$=6.4 Hz, Me-15), 0.95 (3H, s , Me-14); ^{13}C nmr ($CDCl_3$) δ 73.4 (C-1), 36.8 (C-2), 76.1 (C-3), 38.5 (C-4), 50.3 (C-5), 82.7 (C-6), 53.7 (C-7), 23.1 (C-8), 39.2 (C-9), 42.1 (C-10), 40.7 (C-11), 179.4 (C-12), 12.3 (C-13), 12.5 (C-14), 16.4 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (28), 251 $[M+1-18]^+$ (26), 233 $[M+1-36]^+$ (47); *anal.*, found C 67.2, H 9.1; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

3β,8α-Dihydroxy-4β,5α,11β-H-eudesman-6α,12-olide [**17**].—Mp 201°, $[\alpha]^{25}D$ −18° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3417, 1761 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.94 (1H, ddd, $J_{8\beta,7}$=14.9 Hz, $J_{8\beta,9\alpha}$=10.6 Hz, $J_{8\beta,9\beta}$=4.4 Hz, H-8β), 3.83 (1H, dd, $J_{6\beta,7}=J_{6\beta,5}$=10.8 Hz, H-6β), 3.15 (1H, ddd, $J_{3,2\beta}$=10.9 Hz, $J_{3,4\alpha}$=9.6 Hz, $J_{3,2\alpha}$=5.1 Hz, H-3α), 2.48 (1H, dq, $J_{11,7}$=13.8 Hz, $J_{11,13}$=6.9 Hz, H-11β), 1.36 (3H, d, $J_{13,11}$=6.9 Hz, Me-13), 1.20 (3H, d, $J_{15,4}$=6.3 Hz, Me-15), 0.98 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 39.7 (C-1), 29.9 (C-2), 76.0 (C-3), 38.6 (C-4), 51.8 (C-5), 80.6 (C-6), 59.8 (C-7), 68.9 (C-8), 51.3 (C-9), 36.3 (C-10), 40.6 (C-11), 179.1 (C-12), 14.5 (C-13), 16.5 (C-14), 20.0 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (46), 251 $[M+1-18]^+$ (69), 233 $[M+1-36]^+$ (100); *anal.*, found C 67.1, H 8.9; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

3β,9β-Dihydroxy-4β,5α,11β-H-eudesman-6α,12-olide [**18**].—Mp 194°; $[\alpha]^{25}D$ +74° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3419, 1768 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.81 (1H, dd, $J_{6,5}=J_{6,7}$=10.5 Hz, H-6β), 3.37 (1H, dd, $J_{9,8\beta}$=10.2 Hz, $J_{9,8\alpha}$=4.2 Hz, H-9α), 3.12 (1H, ddd, $J_{3,2\beta}$=11.3 Hz, $J_{3,4\alpha}$=9.8 Hz, $J_{3,2\alpha}$=4.7 Hz, H-3α), 2.26 (1H, dq, $J_{11,7}$=12.6 Hz, $J_{11,13}$=6.8 Hz, H-11β), 1.20 (3H, d, $J_{15,4}$=6.4 Hz, Me-15), 1.19 (3H, d, $J_{13,11}$=6.8 Hz, Me-13), 0.97 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 36.0 (C-1), 31.6 (C-2), 75.8 (C-3), 38.4 (C-4), 50.3 (C-5), 82.4 (C-6), 51.0 (C-7), 30.1 (C-8), 78.1 (C-9), 41.5 (C-10), 40.5 (C-11), 179.1 (C-12), 12.5 (C-13), 12.8 (C-14), 16.5 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (35), 251 $[M+1-18]^+$ (47), 233 $[M+1-36]^+$ (100); *anal.*, found C 67.1, H 9.0; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

3β,11β-Dihydroxy-4β,5α,11β-H-eudesman-6α,12-olide [**19**].—Mp 187°; $[\alpha]^{25}D$ +62° (*c*=1, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 4.29 (1H, dd, $J_{6,5}$=11.0 Hz, $J_{6,7}$=10.1 Hz, H-6β), 3.13 (1H, ddd, $J_{3,2\beta}$=10.9 Hz, $J_{3,4\alpha}$=9.9 Hz, $J_{3,2\alpha}$=5.0 Hz, H-3α), 1.42 (3H, s, Me-13), 1.20 (3H, d, $J_{15,4}$=6.4 Hz, Me-15), 0.99 (3H, s,

Me-14); ^{13}C nmr ($CDCl_3$) δ 40.0 (C-1), 30.4 (C-2), 76.2 (C-3), 39.0 (C-4), 52.8 (C-5), 82.0 (C-6), 56.0 (C-7), 17.9 (C-8), 40.7 (C-9), 36.9 (C-10), 77.3 (C-11), 179.5 (C-12), 21.6 (C-13), 18.8 (C-14), 16.7 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (38), 251 $[M+1-18]^+$ (65), 233 $[M+1-36]^+$ (100); *anal.*, found C 67.2, H 9.0; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

BIOTRANSFORMATION OF 3α-HYDROXY-4β,5α,11β-H-EUDESMAN-6α,12-OLIDE [**5**].—Substrate **5** (220 mg) was dissolved in EtOH (4 ml), distributed among four Erlenmeyer-flask cultures and incubated for 10 days, after which the cultures were processed as indicated above for the biotransformation of substrate **2**. Chromatography on Si gel yielded 33 mg (15%) of starting material, 55 mg (30%) of metabolite **16**, which was also isolated in the biotransformation of substrate **4**, and 40 mg (17%) of metabolite **20**.

3α,9β-*Dihydroxy*-4β,5α,11β-*H*-*eudesman*-6α,12-*olide* [**20**].—Syrup; $[\alpha]^{25}D$ +9° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3443, 1762 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 3.78 (1H, dd, $J_{6,5}$=11.0 Hz, $J_{6,7}$=10.0 Hz, H-6β), 3.76 (1H, m, H-3β), 3.47 (1H, dd, $J_{9,8\beta}$=10.6 Hz, $J_{9,8\alpha}$=4.5 Hz, H-9α), 2.25 (1H, dq, $J_{11,7}$=12.2 Hz, $J_{11,13}$=6.9 Hz, H-11β), 1.19 (3H, d, $J_{13,11}$=6.9 Hz, Me-13), 1.15 (3H, d, $J_{15,4}$=6.8 Hz, Me-15), 0.95 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 31.5 (C-1), 28.5 (C-2), 71.9 (C-3), 34.8 (C-4), 45.1 (C-5), 82.3 (C-6), 50.2 (C-7), 31.3 (C-8), 78.2 (C-9), 41.7 (C-10), 40.7 (C-11), 179.4 (C-12), 12.5 (C-13), 11.8 (C-14), 18.0 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (20), 251 $[M+1-18]^+$ (25), 233 $[M+1-36]^+$ (61); *anal.*, found C 67.2, H 9.0; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

BIOTRANSFORMATION OF 3β-HYDROXY-4α,5α,11β-H-EUDESMAN-6β,12-OLIDE [**7**].—Substrate **7** (1 g) was dissolved in EtOH (20 ml), distributed among 20 Erlenmeyer-flask cultures and incubated for 12 days, after which the cultures were processed as indicated above for the biotransformation of substrate **2**. The resulting mixture (800 mg) was chromatographed on a Si gel column to obtain 100 mg (10%) of starting material, 165 mg (16%) of metabolite **21**, 110 mg (20%) of metabolite **22** (18), 130 mg (12%) of metabolite **23**, and 60 mg of a polar mixture which was dissolved in Ac_2O/pyridine (1:2) and maintained for 10 h at room temperature. The reaction mixture was diluted with H_2O, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4. Cc on Si gel yielded acetoxy derivatives **24** (4%), **25** (10%), and **26** (18) (5%). All yields are given with respect to substrate **7**.

1β,3β-*Dihydroxy*-4α,5α,11β-*H*-*eudesman*-6β,12-*olide* [**21**].—Syrup; $[\alpha]^{25}D$ −77.5° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3373, 1768 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 4.60 (1H, dd, $J_{6,7}$=4.1 Hz, $J_{6,5}$=3.0 Hz, H-6α), 3.78 (1H, ddd, $J_{3,2\beta}$=11.7 Hz, $J_{3,2\alpha}$=5.2 Hz, $J_{3,4\alpha}$=4.5 Hz, H-3α), 3.21 (1H, dd, $J_{1\alpha,2\beta}$=11.5 Hz, $J_{1\alpha,2\alpha}$=4.3 Hz, H-1α), 2.32 (1H, q, $J_{11,13}$=7.6 Hz, H-11β), 1.27 (3H, d, $J_{13,11}$=7.6 Hz, Me-13), 1.19 (3H, d, $J_{15,4}$=7.4 Hz, Me-15), 1.06 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 78.0 (C-1), 35.5 (C-2), 71.3 (C-3), 40.2 (C-4), 45.2 (C-5), 82.2 (C-6), 43.7 (C-7), 23.8 (C-8), 38.5 (C-9), 37.7 (C-10), 43.5 (C-11), 180.2 (C-12), 14.1 (C-13), 15.4 (C-14), 9.7 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (21), 251 $[M+1-18]^+$ (100), 233 $[M+1-36]^+$ (69); *anal.*, found C 67.1, H 8.9; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

3β,8α-*Dihydroxy*-4α,5α,11β-*H*-*eudesman*-6β,12-*olide* [**23**].—Mp 149°; $[\alpha]^{25}D$ −60.0° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3585, 1753 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 4.70 (1H, dd, $J_{6,5}$=2.9 Hz, $J_{6,7}$=4.5 Hz, H-6α), 3.68 (1H, ddd, $J_{3,2\beta}$=9.6 Hz, $J_{3,2\alpha}$=$J_{3,4\alpha}$=4.7 Hz, H-3α), 3.53 (1H, ddd, $J_{8\beta,7}$=12.0 Hz, $J_{8\beta,9\alpha}$=9.9 Hz, $J_{8\beta,9\beta}$=4.2 Hz, H-8β), 2.69 (1H, q, $J_{11,13}$=7.6 Hz, H-11β), 1.86 (1H, dd, $J_{7,8\beta}$=9.9 Hz, $J_{7,6}$=4.5 Hz, H-7), 1.25 (3H, d, $J_{13,11}$=7.6 Hz, Me-13), 1.11 (3H, d, $J_{15,4}$=7.4 Hz, Me-15), 1.06 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 41.4 (C-1), 26.2 (C-2), 74.1 (C-3), 41.6 (C-4), 47.7 (C-5), 84.3 (C-6), 52.7 (C-7), 67.6 (C-8), 52.5 (C-9), 34.5 (C-10), 40.6 (C-11), 180.1 (C-12), 14.4 (C-13), 22.5 (C-14), 10.3 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (33), 251 $[M+1-18]^+$ (100), 233 $[M+1-36]^+$ (87); *anal.*, found C 67.0, H 8.9; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

1β-*Acetoxy*-3β-*hydroxy*-4α,5α,11β-*H*-*eudesman*-6β,12-*olide* [**24**].—Syrup; $[\alpha]^{25}D$ −88.6° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3461, 1768, 1728 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 4.67 (1H, dd, $J_{1\alpha,2\beta}$=11.3 Hz, $J_{1\alpha,2\alpha}$=4.3 Hz, H-1α), 4.61 (1H, dd, $J_{6,7}$=4.3 Hz, $J_{6,5}$=2.8 Hz, H-6α), 3.84 (1H, ddd, $J_{3,2\beta}$=11.3 Hz, $J_{3,2\alpha}$=$J_{3,4\alpha}$=5.1 Hz, H-3α), 2.03 (3H, s, Me-AcO), 1.27 (1H, q, $J_{11,13}$=7.6 Hz, H-11β), 1.22 (3H, d, $J_{15,4}$=7.4 Hz, Me-15), 1.13 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 78.7 (C-1), 29.8 (C-2), 70.9 (C-3), 40.0 (C-4), 45.1 (C-5), 82.0 (C-6), 43.5 (C-7), 23.6 (C-8), 38.3 (C-9), 36.9 (C-10), 43.5 (C-11), 180.3 (C-12), 14.0 (C-13), 16.4 (C-14), 9.7 (C-15), 170.1 (*MeCO*), 21.2 (*MeCO*); cims (CH_4) *m/z* 311 $[M+1]^+$ (48), 293 $[M+1-18]^+$ (24), 251 $[M+1-60]^+$ (100), 233 $[M+1-78]^+$ (87); *anal.*, found C 65.8, H 8.5; $C_{17}H_{26}O_5$ requires C 65.77, H 8.45%.

3β-*Acetoxy*-4α-*hydroxy*-5α,11β-*H*-*eudesman*-6β,12-*olide* [**25**].—Mp 174°; $[\alpha]^{25}D$ −39° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3478, 1770 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 5.15 (1H, dd, $J_{6,7\alpha}$=4.0 Hz, $J_{6,5}$=2.6 Hz, H-6α), 4.61 (1H, dd, $J_{3,2\beta}$=11.9 Hz, $J_{3,2\alpha}$=$J_{3,4\alpha}$=4.7 Hz, H-3α), 2.30 (1H, q, $J_{11,13}$=7.6 Hz, H-11β), 2.10 (3H, s, Me-AcO), 1.43 (3H, s, Me-15), 1.28 (3H, d, $J_{13,11}$=7.6 Hz, Me-13), 1.06 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 39.4 (C-1), 23.8 (C-2), 82.0 (C-3), 74.2 (C-4), 53.6 (C-5), 75.7 (C-6), 43.6 (C-7), 25.2 (C-8), 42.4 (C-9),

33.5 (C-10), 43.1 (C-11), 180.5 (C-12), 14.1 (C-13), 20.4 (C-14), 19.4 (C-15), 171.8 (Me*CO*), 21.4 (*Me*CO); cims (CH_4) *m/z* 311 $[M+1]^+$ (15), 293 $[M+1-18]^+$ (100), 251 $[M+1-60]^+$ (81), 233 $[M+1-78]^+$ (46); *anal.*, found C 65.7, H 8.5; $C_{17}H_{26}O_5$ requires C 65.77, H 8.4%.

BIOTRANSFORMATION OF 3α-HYDROXY-4β,5α,11β-H-EUDESMAN-6β,12-OLIDE [**9**].—Substrate **9** (450 mg) was dissolved in EtOH (10 ml), distributed among 10 Erlenmeyer-flask cultures and incubated for 7 days, after which the cultures were throughly processed as indicated above for the biotransformation of substrate **2**. This yielded a mixture (420 mg) that was chromatographed on Si gel to afford 40 mg (9%) of starting material, 75 mg (17%) of product **8**, 150 mg (31%) of metabolite **27**, and 200 mg of a polar mixture that was dissolved in Ac_2O-pyridine (1:2) and maintained for 14 h at room temperature. Chromatography on Si gel yielded 40 mg (4%) of product **28**, 40 mg (4%) of product **29**, and 100 mg (17%) of product **30**. All yields are given with respect to substrate **9**.

3α,8α-Dihydroxy-4β,5α,11β-H-eudesman-6β,12-olide [**27**].—Mp 174°; $[\alpha]^{25}D$ −36° (*c*=1, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 4.72 (1H, dd, $J_{6,7}$=5.2 Hz, $J_{6,5\alpha}$=3.7 Hz, H-6α), 3.90 (1H, dd, J_3=2.6 Hz, J_1=J_2=2.7 Hz, H-3β), 3.70 (1H, ddd, $J_{8,7}$=12 Hz, $J_{8,9\alpha}$=10.1 Hz, $J_{8,9\beta}$=4.5 Hz, H-8β), 2.82 (1H, q, $J_{11,13}$=7.7 Hz, H-11β), 1.34 (3H, d, $J_{13,11}$=7.7 Hz, Me-13), 1.07 (3H, d, $J_{15,4}$=6.8 Hz, Me-15), 0.97 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) δ 36.0 (C-1), 28.8 (C-2), 71.9 (C-3), 33.1 (C-4), 42.9 (C-5), 79.1 (C-6), 51.3 (C-7), 68.1 (C-8), 48.9 (C-9), 34.6 (C-10), 42.1 (C-11), 180.3 (C-12), 15.0 (C-13), 18.6 (C-14), 15.5 (C-15); cims (CH_4) *m/z* 269 $[M+1]^+$ (16), 251 $[M+1-18]^+$ (89), 233 $[M+1-36]^+$ (100); *anal.*, found C 67.0, H 9.0; $C_{15}H_{24}O_4$ requires C 67.12, H 9.02%.

1β-Acetoxy-3α-hydroxy-4β,5α,11β-H-eudesman-6α,12-olide [**28**].—Mp 152°; $[\alpha]^{25}D$ −50° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 3496, 1767, 1737 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.86 (1H, dd, $J_{1,2\alpha}$=11.7 Hz, $J_{1,2\beta}$=5.0 Hz, H-1α), 4.60 (1H, dd, $J_{6,5\alpha}$=$J_{6,7}$=6.9 Hz, H-6α), 3.98 (1H, dd, J_3=2.7 Hz, J_1=J_2=2.8 Hz, H-3β), 2.35 (1H, q, $J_{11,13}$=7.7 Hz, H-11β), 2.01 (3H, s, Me-AcO), 1.28 (3H, d, $J_{13,11}$=7.7 Hz, Me-13), 1.07 (3H, d, $J_{15,4}$=6.8 Hz, Me-15), 1.00 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$) 77.4 (C-1), 34.8 (C-2), 71.3 (C-3), 32.9 (C-4), 41.9 (C-5), 76.3 (C-6), 41.8 (C-7), 23.1 (C-8), 34.8 (C-9), 37.1 (C-10), 44.5 (C-11), 180.4 (C-12), 14.6 (C-13), 12.6 (C-14), 14.9 (C-15), 170.8 (Me*CO*), 21.3 (*Me*CO); cims (CH_4) *m/z* 311 $[M+1]^+$ (16), 251 $[M+1-60]^+$ (56); *anal.*, found C 65.6, H 8.4; $C_{17}H_{26}O_5$ requires C 65.77, H 8.45%.

1β,3α-Diacetoxy-4β,5α,11β-H-eudesman-6α,12-olide [**29**].—Mp 175°; $[\alpha]^{25}D$ −26° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 1771, 1736 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.14 (1H, dd, J_1=J_2=3.0 Hz, J_3=2.9 Hz, H-3β), 4.77 (1H, dd, $J_{1,2\alpha}$=11.7 Hz, $J_{1,2\beta}$=4.9 Hz, H-1α), 4.60 (1H, dd, $J_{6,5\alpha}$=$J_{6,7}$=4.0 Hz, H-6α), 2.38 (1H, q, $J_{11,13}$=7.5 Hz, H-11), 2.07 (3H, s, Me-AcO), 2.01 (3H, s, Me-AcO), 1.29 (3H, d, $J_{13,11}$=7.5 Hz, Me-13), 1.00 (3H, s, Me-14), 0.98 (3H, d, $J_{15,4}$=6.7 Hz, Me-15); ^{13}C nmr ($CDCl_3$) δ 76.8 (C-1), 31.7 (C-2), 73.6 (C-3), 31.7 (C-4), 43.2 (C-5), 76.0 (C-6), 41.9 (C-7), 23.0 (C-8), 34.7 (C-9), 37.0 (C-10), 44.5 (C-11), 180.1 (C-12), 14.4 (C-13), 12.6 (C-14), 14.6 (C-15), 170.6 (Me*CO*), 170.5 (Me*CO*), 21.2 (*Me*CO), 21.2 (*Me*CO); cims (CH_4) *m/z* 353 $[M+1]^+$ (57), 293 $[M+1-60]^+$ (76), 233 $[M+1-120]^+$ (100); *anal.*, found C 64.7, H 8.0; $C_{19}H_{28}O_6$ requires C 64.74, H 8.01%.

1β,3β-Diacetoxy-4β,5α,11β-H-eudesman-6β,12-olide [**30**].—Syrup; $[\alpha]^{25}D$ −15° (*c*=1, $CHCl_3$); ir ν max ($CHCl_3$) 1771, 1739 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.70 (1H, dd, $J_{6,5\alpha}$=$J_{6,7}$=4.0 Hz, H-6α), 4.53 (1H, dd, $J_{1,2\alpha}$=12.2 Hz, $J_{1,2\beta}$=4.6 Hz, H-1α), 4.51 (1H, dd, J_1=J_2=12.1 Hz, J_3=5.4 Hz, H-3α), 2.38 (1H, q, $J_{11,13}$=7.7 Hz, H-11β), 2.16 (3H, s, Me-AcO), 2.01 (3H, s, Me-AcO), 1.29 (3H, d, $J_{13,11}$=7.7 Hz, Me-13), 1.03 (3H, s, Me-14), 0.97 (3H, d, $J_{15,4\beta}$=6.4 Hz, Me-15); ^{13}C nmr ($CDCl_3$) δ 76.9 (C-1), 32.2 (C-2), 74.6 (C-3), 33.3 (C-4), 45.9 (C-5), 75.0 (C-6), 41.7 (C-7), 22.7 (C-8), 34.6 (C-9), 36.4 (C-10), 44.2 (C-11), 179.9 (C-12), 14.3 (C-13), 13.3 (C-14), 14.4 (C-15), 170.4 (Me*CO*), 170.2 (Me*CO*), 21.0 (*Me*CO), 20.9 (*Me*CO); cims (CH_4) *m/z* 353 $[M+1]^+$ (29), 293 $[M+1-60]^+$ (100), 233 $[M+1-120]^+$ (65); *anal.*, found C 64.6, H 8.0; $C_{19}H_{28}O_6$ requires C 64.74, H 8.01%.

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