# Chemical-Microbiological Synthesis of Cryptomeridiol Derivatives by *Gliocladium roseum*: Semisynthesis of 11-Hydroxyeudesmanolides

Andrés García-Granados,* María C. Gutiérrez, Andrés Parra, and Francisco Rivas

*Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Granada, 18071-Granada, Spain*

*Received December 12, 2001*

Biotransformations of 4α- and 4β-hydroxyeudesmane derivatives by the filamentous fungus *Gliocladium roseum* were achieved. Hydroxylation at C-11 was the main action of this microorganism, producing new cryptomeridiol (**12** and **14**) and 4-*epi*-cryptomeridiol derivatives (**6** and **7**), respectively, in good yields. The biotransformation activity of *G. roseum* toward 4β-hydroxyeudesmane was focused on the isopropyl moiety, but more scattered on the 4α-hydroxylated derivative, acting in both the "A" and "B" rings and the isopropyl group of the molecule. Semisyntheses of 11-hydroxyeudesmanolides from the isolated 11,12-dihydroxylated metabolites were also accomplished and used in assigning the stereochemistry of hydroxylation.

*Gliocladium roseum* CECT 2733, the anamorphic form of *Nectria ochroleuca* IMI 40022, is a filamentous fungus that has been used previously to hydroxylate the sesquiterpene patchoulol,[1] to biotransform a derivative of the diterpene varodiol, to produce an *ent*-Ambrox derivative,[2] and to carry out the bioconversion of a 4β-hydroxyeudesmane derivative.[3] Eudesmane sesquiterpenes are common in nature,[4,5] and in the past decade, several biotransformations of these kinds of compounds have been carried out to acquire products difficult to achieve by chemical means.[6–16] Cryptomeridiol is a natural sesquiterpene diol[17] that exerts a potent antispasmodic effect on the isolated rabbit ileum,[18] and 4-*epi*-cryptomeridiol is also a natural compound.[19]

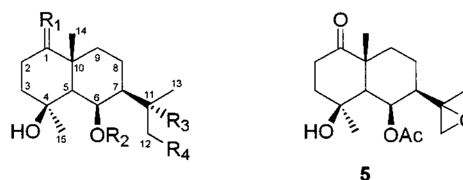
Sesquiterpene lactones show a wide variety of remarkable biological activities, but many of them present strong cytotoxic properties, apparently due to the α,β-unsaturated lactone group.[20–23] Some authors suspect that 11-hydroxysesquiterpenolides are the precursors of those compounds, and thus the unsaturated system could be released in small quantities, thereby attenuating the cytotoxic effect.[24]

In previous papers[25,26] we reported the incubations of several 4β-hydroxyeudesmane compounds by the filamentous fungi *Curvularia lunata* ATCC 12017 and *Rhizopus nigricans* ATCC 10404. The action of these microorganisms was directed mainly toward the isopropyl moiety; specifically, *C. lunata* was active toward C-12 and *R. nigricans* to C-11. Some of the best results were achieved on the 6β-acetoxy-1-keto derivative. Also we have described the incubations of some 4α-hydroxylated derivatives,[25,26] but with poor transformation results. Therefore, in this study we have chosen 6β-acetoxy-1-keto derivatives that are 4β- or 4α-hydroxylated to carry out several biotransformations with the microorganism *G. roseum*.

## Results and Discussion

6β-Acetoxy-4β-hydroxyeudesman-1-one (**1**) and its 4α-epimer compound (**2**) were obtained respectively, by Jones' oxidation at C-1, from the natural compounds **3**[27] and **4**.[28] Incubation of substrate **1** with *G. roseum* for 9 days yielded the metabolites **5** (3%), **6** (6%), **7** (58%), and **8** (4%).

Metabolite **5** had a molecular formula of $C_{17}H_{26}O_5$, which indicated the presence of an additional oxygen atom and




**1**: $R_1$=O, $R_2$=Ac, $R_3$=$R_4$=H

**3**: $R_1$=β-OH,α-H, $R_2$=Ac, $R_3$=$R_4$=H

**6**: $R_1$=O, $R_2$=$R_4$=H, $R_3$=OH

**7**: $R_1$=O, $R_2$=Ac, $R_3$=OH, $R_4$=H

**8**: $R_1$=O, $R_2$=H, $R_3$=$R_4$=OH

the absence of two hydrogen atoms in the molecule. Its $^1$H NMR spectrum revealed two signals of an AB system at $\delta$ 2.69 and 2.47 (each 1H, d, $J = 4.9$ Hz), characteristic of two geminal protons of an epoxide group. The absence of the signal for one of the methyls of the isopropyl group indicated that this epoxide group was situated at C-11 and C-12 in the molecule, this being confirmed by the analysis of its $^{13}$C NMR spectrum. In conclusion, metabolite **5** was 6β-acetoxy-11,12-epoxy-4β-hydroxyeudesman-1-one. Due to the absence of a hydrogen atom at C-11 and the limited quantity available of this metabolite (**5**), we could not establish the configuration at this carbon.

Analysis of the spectral data of **6** suggested the insertion of a new hydroxyl group at C-11 and deacetylation at C-6. This metabolite, 4β,6β,11-trihydroxyeudesman-1-one (**6**), was a 4-*epi*-cryptomeridiol[19] derivative, which was previously obtained from the incubation of a eudesmane derivative with *R. nigricans*.[26]

The main metabolite isolated (**7**) had a molecular formula of $C_{17}H_{28}O_5$, which indicated the presence of an additional hydroxyl group in the molecule. The β-effects on C-7, C-12, and C-13 detected in its $^{13}$C NMR spectrum indicated that hydroxylation had occurred at C-11, establishing its structure as 6β-acetoxy-4β,11-dihydroxyeudesman-1-one. This major metabolite (**7**) was also a 4-*epi*-cryptomeridiol[19] derivative, obtained from incubation of substrate **1** with *R. nigricans*.[26] The difference between metabolites **6** and **7** is the presence of an acetoxyl group at C-6 in the latter. Therefore, **7** should have eluted in the chromatographic separation before **6**, but this did not occur

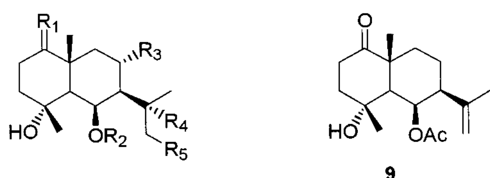
* To whom correspondence should be addressed. Tel/fax: +34-958-243364. E-mail: agarcia@ugr.es.

10.1021/np010631m CCC: $22.00 © 2002 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/03/2002

perhaps due to a reduction in the expected polarity attributable to the formation of two hydrogen bonds between the three hydroxyl groups present in metabolite **6**.

The molecular formula ($C_{15}H_{26}O_5$) of the last metabolite isolated (**8**) agreed with the presence of two new hydroxyl groups in the molecule and with the absence of an acetoxyl group at C-6. Comparisons of the $^1$H NMR data of **8** with those of **6** revealed the presence of an AB system, centered at $\delta$ 3.67 and 3.32 ($J = 11.1$ Hz), and the absence of one of the methyl signals of the isopropyl group. Therefore, these hydroxyl groups should be positioned at C-11 and C-12. Consequently, metabolite **8** is 4$\beta$,6$\beta$,11,12-tetrahydroxyeudesman-1-one. The configuration at C-11 was determined as "*S*" by the conversion of **8** into the corresponding 11-hydroxy-6$\beta$,12-eudesmanolide (see below).

Incubation of substrate **2** with *G. roseum* for 15 days yielded the metabolites **9** (5%), **10** (9%), **11** (19%), **12** (32%), and **13** (4%) and a mixture of metabolites, from which, after treatment with $LiAlH_4$, product **14** (4%) was isolated.




**2**: R1=O, R2=Ac, R3=R4=R5=H

**4**: R1=β-OH,α-H, R2=Ac, R3=R4=R5=H

**10**: R1=α-OH,β-H, R2=Ac, R3=R4=R5=H

**11**: R1=O, R2=Ac, R3=OH, R4=R5=H

**12**: R1=O, R2=Ac, R4=OH, R3=R5=H

**13**: R1=O, R2=R3=H, R4=OH, R5=OAc

**14**: R1=β-OH,α-H, R2=R5=H, R3=R4=OH

The IR spectrum of metabolite **9** indicated the presence of a carbon−carbon double bond (3080 and 1645 cm$^{-1}$), and its $^1$H NMR data confirmed a 2-propenyl group ($\delta$ 4.82, 1H, dd, $J = 1.5$, 1.5 Hz; $\delta$ 4.69, 1H, br s; $\delta$ 1.76, 3H, br s) in the molecule. Therefore, a double bond between carbons 11 and 12 had been formed, probably by a dehydration reaction from an 11-hydroxyl compound. Metabolite **9** was therefore 6$\beta$-acetoxy-4α-hydroxyeudesm-11-en-1-one.

Metabolite **10** was the result of the stereoselective reduction of the keto group at C-1 of **1** from the $\beta$ face, giving a (1*S*)-hydroxylated derivative, as is usual in enzymatic reductions,[29] although this is difficult to achieve by chemical means. The "*S*" configuration at C-1 could easily be deduced from the signal in the $^1$H NMR spectrum ($\delta$ 3.29, 1H, dd, $J = 3.0$, 3.0 Hz), with coupling constants corresponding to an equatorial proton. Thus, metabolite **10** had the structure 6$\beta$-acetoxy-1α,4α-dihydroxyeudesmane.

The third metabolite isolated (**11**) had a molecular formula of $C_{17}H_{28}O_5$, which indicated the presence of an additional hydroxyl group in the molecule. In its $^1$H NMR spectrum a signal appeared at $\delta$ 4.12 (1H, ddd, $J = 11.2$, 11.2, 4.1 Hz) due to an axial proton. The position of the new functional group at C-8 was determined by the $\beta$-effects found on the adjacent carbons (C-7 and C-9). Therefore, metabolite **11** was 6$\beta$-acetoxy-4α,8α-dihydroxyeudesman-1-one.

Metabolite **12** had the same molecular formula as **11**, and the spectral data suggested a new hydroxyl group at C-11. This major metabolite (**12**) was therefore the cryptomeridiol[17] derivative 6$\beta$-acetoxy-1-oxocryptomeridiol.

The molecular formula ($C_{17}H_{28}O_6$) of **13** indicated that the microorganism had introduced two hydroxyl groups in the molecule. The spectral data of **13** confirmed that the new hydroxyl groups were situated at C-11 and C-12 and that a rearrangement of the acetoxyl group at C-6 to the hydroxyl group at C-12 had taken place. Again, the configuration at C-11 was determined as "*S*" by the conversion of **13** into the corresponding 11-hydroxy-6$\beta$,12-eudesmanolide (see below).

Finally, we could not separate a mixture of additional metabolites. Treatment of this mixture with $LiAlH_4$ enabled us to isolate product **14**, in which reduction of the keto group at C-1 from the α-face (producing a 1$\beta$-alcohol) and the deacetylation of the acetoxyl group at C-6 had taken place. Product **14** showed spectral data that, by comparison with those of metabolites **11** and **12**, positioned the new hydroxyl groups at C-8 and C-11. Hence, the structure of **14** was 1$\beta$,6$\beta$,8α-trihydroxycryptomeridiol.

In summary, *G. roseum* directed its principal action on this type of substrate toward carbon 11, producing 4α,11- and 4$\beta$,11-dihydroxyeudesmane derivatives (cryptomeridiol[17] and 4-*epi*-cryptomeridiol[19] derivatives). The activity of this microorganism on 4$\beta$-hydroxyeudesmane (**1**) was focused on the isopropyl moiety, but was more scattered for the 4α-hydroxy derivative (**2**), acting in both the "A" and "B" rings and the isopropyl group of the molecule. The biotransformations carried out by both *G. roseum* and *R. nigricans*[26] on the 4$\beta$-hydroxylated substrate (**1**) yielded the 11-hydroxy derivative (**7**) as the main metabolite, although the former also produced an 11,12-dihydroxylated derivative (**8**), which may be the precursor of an 11-hydroxyeudesmanolide. On the other hand, the main action on the substrate (**1**) by *C. lunata*[25] was the reduction of the carbonyl group at C-1, also producing a limited quantity of 12-hydroxylated derivatives. In addition, *G. roseum* effected a more efficient bioconversion of a 4α-hydroxylated eudesmane (**2**) than that achieved by *C. lunata*[25] or *R. nigricans*[26] with some 4α-hydroxylated eudesmane analogues.

Ley et al. have reported[30] that vicinal diols undergo glycol cleavage reactions with tetrapropylammonium perruthenate (TPAP) to give a carbonyl group. In the present study, we have investigated the reactions of 11,12-diol eudesmanes, with a third hydroxyl group at C-6, with TPAP. Instead of undergoing glycolytic cleavage, these substrates are oxidized to α-hydroxyl lactones. Starting from the 11,12-dihydroxylated metabolites, isolated in the biotransformation processes, we have thereby accomplished the semisyntheses of 11-hydroxyeudesmanolides (Figure 1).

Treatment of metabolite **8** with TPAP gave 4$\beta$,11α-dihydroxy-1-oxoeudesman-6$\beta$,12-olide (**15**). The configuration at C-11 was determined by a 2D NOESY experiment, due to the absence of a hydrogen atom in this carbon. This experiment demonstrated that the protons situated at C-8 and C-13 were spatially close, these results being compatible with an "11*S*" configuration for lactone **15** (Figure 2).

$LiAlH_4$ treatment of metabolite **13** led to product **16**, which had undergone reduction of the keto group at C-1 and deacetylation of the acetoxyl group at C-12, as shown by spectral data. Therefore, **16** was 1$\beta$,4α,6$\beta$,11,12-pentahydroxyeudesmane. Polyalcohol derivative (**16**) was converted into the 11-hydroxyeudesmanolide **17** by reaction with TPAP. This product (**17**) was the result of the oxidation of the hydroxyl groups situated at C-1 and C-12, followed by a lactonization reaction. Consequently, **17** was 4α,11α-dihydroxy-1-oxoeudesman-6$\beta$,12-olide. The configuration at C-11, and therefore of its precursor (**16**), was

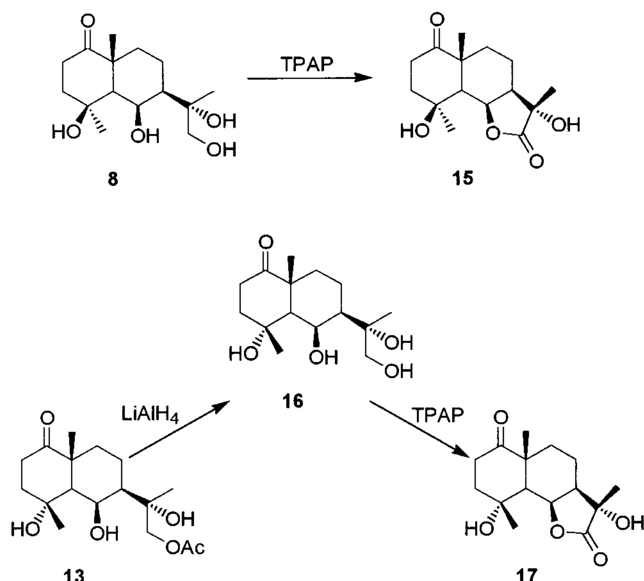



**Figure 1.** Semisyntheses of 4$\beta$- and 4$\alpha$,11-dihydroxyeudesmanolides.

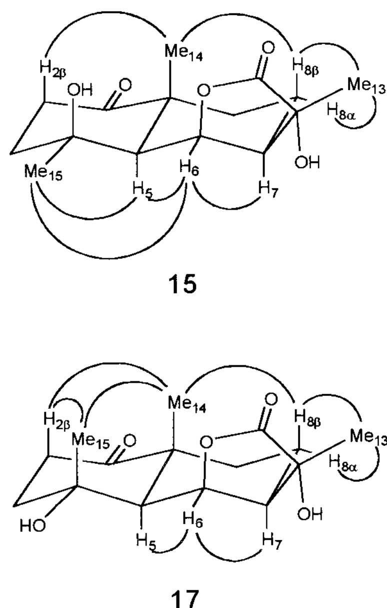



**Figure 2.** Observed NOEs for eudesmanolides **15** and **17**.

again established as "*S*" by the NOE effects observed between protons of C-8 and C-13 (see Figure 2).

## Experimental Section

**General Experimental Procedures.** Measurements of NMR spectra (300.13 MHz $^{1}H$ and 75.47 MHz $^{13}C$) were made in $CDCl_3$, $CD_3COCD_3$, or $CD_3OD$ (which also provided the lock signal) with Bruker spectrometers (AM-300, ARX-400, and AMX-500). 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°. One-dimensional NOE difference experiments were made by irradiation for 4 s in series of eight scans with alternation between on- and off-resonance. Bruker's programs were used for COSY (GSMF), NOESY (TPFI), and C/H correlation (HMQC). IR spectra were recorded on a Nicolet 20SX FT-IR spectrometer. High-resolution mass spectra were made by LSIMS (FAB) ionization mode with a MICROMASS AUTOSPEC-Q spectrometer (EBE geometry). Uncorrected melting points were determined using a Kofler (Reichter) apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 25 °C. Silica gel Scharlau 60 (40−60 $\mu$m) was used for flash chromatography. $CH_2Cl_2$ or $CHCl_3$ containing increasing amounts of $Me_2CO$ or MeOH was used as eluent. Analytical plates (silica gel, Merck 60 G) were rendered visible by spraying with $H_2SO_4$−AcOH, followed by heating to 120 °C. The identity of compounds **3** and **4** was confirmed by direct comparison with the authentic samples (IR, MS, NMR, etc.). Compounds **1** and **2** were obtained by oxidation of **3** and **4**, respectively, with Jones' reagent (see Supporting Information).

**Organism, Media, and Culture Conditions.** *Gliocladium roseum* CECT 2733 was obtained 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 transformation experiments a BEM medium containing 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 the corresponding microorganism. The cultures were incubated by shaking (150 rpm) at 28 °C for 6 days, after which the substrates **1** and **2** (5−10%) in EtOH were added.

**Biotransformation of 1.** Substrate **1** (450 mg) was dissolved in EtOH (7 mL), distributed among seven Erlenmeyer flask cultures of *G. roseum* and incubated for 9 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 $CH_2Cl_2$. Both extracts were pooled, dried with anhydrous $Na_2SO_4$, and evaporated at 40 °C in a vacuum to give a mixture of compounds. This mixture was chromatographed on a silica gel column to obtain 42 mg (9%) of starting material **1**, 12 mg (3%) of 6$\beta$-acetoxy-11,12-epoxy-4$\beta$-hydroxyeudesman-1-one (**5**), 24 mg (6%) of 4$\beta$,6$\beta$,11-trihydroxyeudesman-1-one (**6**),[26] 276 mg (58%) of 6$\beta$-acetoxy-4$\beta$,11-dihydroxyeudesman-1-one (**7**),[26] and 18 mg (4%) of (11S)-4$\beta$,6$\beta$,11,12-tetrahydroxyeudesman-1-one (**8**).

**6$\beta$-Acetoxy-11,12-epoxy-4$\beta$-hydroxyeudesman-1-one (5)**: colorless syrup; $[\alpha]_D$ +26° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3477, 1735, 1708, 1243 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.80 (1H, br s, H-6), 3.10 (1H, ddd, $J$ = 14.1, 14.1, 6.1 Hz, H-2$\beta$), 2.69, 2.47 (each 1H, d, $J$ = 4.9 Hz, H-12), 2.07 (3H, s, $CH_3CO$), 1.52 (3H, s, H-14), 1.45 (3H, s, H-15), 1.35 (3H, s, H-13); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 215.2 (s, C-1), 170.8 (s, $CH_3CO$), 71.7 (s, C-4), 70.2 (d, C-6), 56.8 (s, C-11), 53.5 (d, C-5), 51.3 (t, C-12), 48.1 (s, C-10), 47.2 (d, C-7), 42.1 (t, C-3), 34.3 and 33.7 (t, C-2 and C-9), 29.3 (q, C-15), 21.8 (q, $CH_3CO$), 21.2 and 21.1 (q, C-13 and C-14), 17.6 (t, C-8); HRLSIMS *m*/*z* 311.1862 $[M + 1]^+$ (calcd for $C_{17}H_{27}O_5$, 311.1858).

**(11S)-4$\beta$,6$\beta$,11,12-Tetrahydroxyeudesman-1-one (8)**: colorless syrup; $[\alpha]_D$ +31° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3385, 1704 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 4.75 (1H, br s, H-6), 3.67, 3.32 (each 1H, d, $J$ = 11.1 Hz, H-12), 3.11 (1H, ddd, $J$ = 13.7, 13.7, 6.3 Hz, H-2$\beta$), 1.61 (3H, s, H-14), 1.39 (3H, s, H-15), 1.28 (3H, s, H-13); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 216.0 (s, C-1), 74.3 (s, C-11), 73.1 (s, C-4), 68.9 (d, C-6), 66.8 (t, C-12), 52.7 (d, C-5), 50.4 (d, C-7), 47.7 (s, C-10), 41.0 (t, C-3), 34.6 and 34.5 (t, C-2 and C-9), 29.8 (q, C-15), 25.4 (q, C-13), 22.1 (q, C-14), 17.9 (t, C-8); HRLSIMS *m*/*z* 287.1854 $[M + 1]^+$ (calcd for $C_{15}H_{27}O_5$, 287.1858).

**Biotransformation of 2.** Substrate **2** (450 mg) was dissolved in EtOH (7 mL), distributed among seven Erlenmeyer flask cultures of *G. roseum* and incubated for 15 days, after which the cultures were processed as indicated above for the biotransformation of **1**, to give a mixture, which was chromatographed on a silica gel column to obtain 58 mg (13%) of starting material **2**, 22 mg (5%) of 6$\beta$-acetoxy-4$\alpha$-hydroxyeudesm-11-en-1-one (**9**), 43 mg (9%) of 6$\beta$-acetoxy-1$\alpha$,4$\alpha$-dihydroxyeudesmane (**10**), 92 mg (19%) of 6$\beta$-acetoxy-4$\alpha$,8$\alpha$-dihydroxyeudesman-1-one (**11**), 150 mg (32%) of 6$\beta$-acetoxy-4$\alpha$,11-dihydroxyeudesman-1-one (**12**), 18 mg (4%) of (11S)-12-acetoxy-4$\alpha$,6$\beta$,11-trihydroxyeudesman-1-one (**13**), and a mixture of metabolites, from which, after treatment with $LiAlH_4$, 1$\beta$,4$\alpha$,6$\beta$,8$\alpha$,11-pentahydroxyeudesmane (**14**, 19 mg, 4% of overall yield) was isolated.

**6β-Acetoxy-4α-hydroxyeudesm-11-en-1-one (9)**: colorless syrup; $[\alpha]_D$ −8° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3418, 3080, 1740, 1711, 1645, 1240 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.76 (1H, br s, H-6), 4.82, 4.69 (each 1H, br s, H-12), 2.60 (1H, ddd, *J* = 15.5, 11.5, 5.4 Hz, H-2β), 2.42 (1H, ddd, *J* = 15.5, 6.2, 4.8 Hz, H-2α), 2.01 (3H, s, C*H*$_3$CO), 1.87 (1H, d, *J* = 2.0 Hz, H-5), 1.76 (3H, br s, H-13), 1.39 (3H, s, H-14 or H-15), 1.34 (3H, s, H-14 or H-15); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 214.8 (s, C-1), 171.1 (s, $CH_3$*C*O), 145.7 (s, C-11), 111.3 (t, C-12), 70.8 (s, C-4), 69.0 (d, C-6), 54.4 (d, C-5), 49.9 (d, C-7), 46.9 (s, C-10), 40.5 (t, C-3), 35.5 and 35.3 (t, C-2 and C-9), 25.1 (q, C-15), 23.0 (q, C-13), 21.5 (q, *C*$H_3$CO), 21.0 (t, C-8), 20.8 (q, C-14); HRLSIMS *m*/*z* 317.2095 [M + 23]$^+$ (calcd for $C_{17}H_{26}O_4Na$, 317.2093).

**6β-Acetoxy-1α,4α-dihydroxyeudesmane (10)**: colorless syrup; $[\alpha]_D$ +23° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3453, 1734, 1716, 1247 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.81 (1H, br s, H-6), 3.29 (1H, dd, *J* = 3.0,3.0 Hz, H-1β), 2.06 (3H, s, C*H*$_3$CO), 1.19 (3H, s, H-14 or H-15), 1.11 (3H, s, H-14 or H-15), 0.89 (3H, d, *J* = 6.4 Hz, H-12 or H-13), 0.87 (3H, d, *J* = 6.4 Hz, H-12 or H-13); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 172.3 (s, $CH_3$*C*O), 74.4 (d, C-1), 71.7 (s, C-4), 70.0 (d, C-6), 49.8 (d, C-5 and C-7), 39.3 (s, C-10), 37.3 and 37.1 (t, C-3 and C-9), 28.8 (d, C-11), 27.1 (t, C-2), 24.7 (q, C-15), 22.0 (q, *C*$H_3$CO), 21.7 and 21.4 (q, C-12 and C-13), 20.7 (t, C-8), 20.7 (q, C-14); HRLSIMS *m*/*z* 321.2056 [M + 23]$^+$ (calcd for $C_{17}H_{30}O_4Na$, 321.2042).

**6β-Acetoxy-4α,8α-dihydroxyeudesman-1-one (11)**: colorless syrup; $[\alpha]_D$ +47° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3543, 3463, 1719, 1691, 1259 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.85 (1H, dd, *J* = 2.9, 2.2 Hz, H-6), 4.12 (1H, ddd, *J* = 11.2, 11,2, 4.1 Hz, H-8), 2.65 (1H, ddd, *J* = 15.6, 13.5, 6.0 Hz, H-2β), 2.37 (1H, ddd, *J* = 15.6, 4.5, 4.1 Hz, H-2α), 2.20 (1H, dd, *J* = 12.9, 4.1 Hz, H-9β), 2.09 (3H, s, C*H*$_3$CO), 1.92 (1H, ddd, *J* = 13.5, 6.0, 4.1 Hz, H-3α), 1.80 (1H, ddd, *J* = 13.5, 13.5, 4.5 Hz, H-3β), 1.76 (1H, d, *J* = 2.2 Hz, H-5), 1.45 (1H, dd, *J* = 12.9, 11.2 Hz, H-9α), 1.38 (6H, s, H-14 and H-15) 1.02 (3H, d, *J* = 7.2 Hz, H-12 or H-13), 0.96 (3H, d, *J* = 7.2 Hz, H-12 or H-13); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 212.8 (s, C-1), 172.2 (s, $CH_3$*C*O), 72.1 (d, C-6), 70.4 (s, C-4), 65.8 (d, C-8), 55.1 (d, C-5), 53.5 (d, C-7), 48.2 (s, C-10), 45.9 (t, C-9), 41.4 (t, C-3), 35.2 (t, C-2), 27.5 (d, C-11), 24.2 (q, C-15), 22.3 (q, *C*$H_3$CO), 21.6 (q, C-12), 20.6 (q, C-14), 19.6 (C-13); HRLSIMS *m*/*z* 335.1830 [M + 23]$^+$ (calcd for $C_{17}H_{28}O_5Na$, 335.1834).

**6β-Acetoxy-4α,11-dihydroxyeudesman-1-one (12)**: colorless syrup; $[\alpha]_D$ +28° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3429, 1707, 1251 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.92 (1H, br s, H-6), 2.59 (1H, ddd, *J* = 15.6, 12.0, 5.4 Hz, H-2β), 2.39 (1H, ddd, *J* = 15.6, 5.7, 4.8 Hz, H-2α), 2.10 (3H, s, C*H*$_3$CO), 1.93 (1H, ddd, *J* = 13.5, 3.3, 3.3 Hz, H-9β), 1.76 (1H, d, *J* = 1.9 Hz, H-5), 1.38 (3H, s, H-12 or H-13), 1.34 (3H, s, H-12 or H-13), 1.19 (3H, s, H-14 or H-15), 1.10 (3H, s, H-14 or H-15); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 214.4 (s, C-1), 172.6 (s, $CH_3$*C*O), 71.6 (s, C-11), 70.3 (s, C-4), 69.9 (d, C-6), 55.0 (d, C-5), 52.4 (d, C-7), 46.9 (s, C-10), 40.5 (t, C-3), 35.9 and 35.1 (t, C-2 and C-9), 29.2 and 26.7 (q, C-12 and C-13), 24.6 (q, C-15), 22.1 (q, *C*$H_3$CO), 20.7 (q, C-14), 18.9 (t, C-8); HRLSIMS *m*/*z* 335.1832 [M + 23]$^+$ (calcd for $C_{17}H_{28}O_5Na$, 335.1834).

**(11*S*)-12-Acetoxy-4α,6β,11-trihydroxyeudesman-1-one (13)**: colorless syrup; $[\alpha]_D$ +13° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3410, 1706, 1242 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 4.84 (1H, br s, H-6), 4.16, 3.92 (each 1H, d, *J* = 11.2 Hz, H-12), 2.65 (1H, ddd, *J* = 15.2, 11.8, 5.2 Hz, H-2β), 2.36 (1H, ddd, *J* = 15.2, 6.3, 5.2 Hz, H-2α), 2.10 (3H, s, C*H*$_3$CO), 1.70 (3H, s, H-13), 1.49 (1H, d, *J* = 2.0 Hz, H-5), 1.43 (3H, s, H-14 or H-15), 1.40 (3H, s, H-14 or 3H-15); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 215.5 (s, C-1), 171.3 (s, $CH_3$*C*O), 74.8 (s, C-11), 71.7 (s, C-4), 70.0 (t, C-12), 68.0 (d, C-6), 55.5 (d, C-5), 47.4 (d, C-7), 46.8 (s, C-10), 41.4 (t, C-3), 35.7 and 35.5 (t, C-2 and C-9), 26.2 (q, C-15), 23.8 (q, C-13), 21.0 (q, C-14 and *C*$H_3$CO), 16.7 (t, C-8); HRLSIMS *m*/*z* 351.1778 [M + 23]$^+$ (calcd for $C_{17}H_{28}O_6Na$, 351.1784).

**1β,4α,6β,8α,11-Pentahydroxyeudesmane (14)**: colorless solid; mp 132−134 °C; $[\alpha]_D$ −3° (*c* 1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3405, 1143 $cm^{-1}$; $^1H$ NMR ($CDCl_3$/$CD_3COCD_3$, 300 MHz) δ 4.74 (1H, dd, *J* = 2.1, 2.1 Hz, H-6), 4.30 (1H, ddd, *J* = 11.1, 11.1, 4.1 Hz, H-8), 3.20 (1H, dd, *J* = 9.2, 5.3 Hz, H-1), 2.17 (1H, dd, *J* = 12.1, 4.1 Hz, H-9β), 1.39 (3H, s, H-12 or H-13), 1.38 (3H, s, H-12 or H-13), 1.26 (3H, s, H-14), 1.19 (3H, s, H-15); $^{13}C$ NMR ($CDCl_3$/$CD_3COCD_3$, 75 MHz) δ 79.8 (d, C-1), 74.0 (s, C-11), 71.7 (s, C-4), 68.1 (d, C-6), 66.2 (d, C-8), 58.7 (d, C-7), 56.0 (d, C-5), 51.7 (t, C-9), 42.7 (t, C-3), 40.7 (s, C-10), 28.8 (t, C-2), 28.6 and 27.3 (q, C-12 and C-13), 24.9 (q, C-15), 15.6 (q, C-14); HRLSIMS *m*/*z* 311.1821 [M + 23]$^+$ (calcd for $C_{15}H_{28}O_5$-Na, 311.1834).

**Oxidative Lactonization of 8.** Solid TPAP (tetrapropylammonium perruthenate, 3 mg) was added in a single portion to a stirred mixture of (11S)-4β,6β,11,12-tetrahydroxyeudesman-1-one (**8**, 15 mg), NMO (4-methylmorpholine *N*-oxide, 10 mg), and activated powdered molecular sieves (10 mg) in dry $CH_2Cl_2$ (3 mL) at room temperature under an argon atmosphere. On completion, the reaction mixture was concentrated under vacuum. Purification by column chromatography on silica gel yielded 12 mg of 4β,11α-dihydroxy-1-oxoeudesman-6β,12-olide (**15**, 81%) as a white solid: mp 220−222 °C; $[\alpha]_D$ +19° (*c* 1, $CHCl_3$); IR (KBr) $\nu_{max}$ 3471, 1756, 1689, 1183 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.24 (1H, dd, *J* = 4.3, 2.9 Hz, H-6), 3.21 (1H, ddd, *J* = 14.0, 14.0, 6.4 Hz, H-2β), 1.58 (1H, d, *J* = 2.9 Hz, H-5), 1.44 (3H, s, H-13), 1.43 (3H, s, H-14), 1.41 (3H, s, H-15); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 214.3 (s, C-1), 176.4 (s, C-12), 77.9 (d, C-6), 77.3 (s, C-11), 71.4 (s, C-4), 50.8 (d, C-5), 46.6 (s, C-10), 45.6 (d, C-7), 40.3 (t, C-3), 34.4 (t, C-2), 32.1 (t, C-9), 29.1 (q, C-15), 20.9 and 19.0 (q, C-13 and C-14), 18.3 (t, C-8); HRLSIMS *m*/*z* 305.1355 [M + 23]$^+$ (calcd for $C_{15}H_{22}O_5Na$, 305.1365).

**Reduction of 13.** Metabolite **13** (18 mg) was added to a solution (0.5 mL) of $LiAlH_4$ in THF (1 M). The mixture was stirred and heated for 10 min at 50 °C. Saturated aqueous ether was then added, and the reaction mixture was extracted with $CH_2Cl_2$. The organic layer was dried over anhydrous $Na_2$-$SO_4$ and evaporated to dryness. Chromatography on a silica gel column yielded 13 mg (82%) of (11*S*)-1β,4α,6β,11,12-pentahydroxyeudesmane (**16**) as a solid: mp 81−83 °C; $[\alpha]_D$ +11° (*c* 1, $CH_3OH$); IR (KBr) $\nu_{max}$ 3398, 1258, 1039 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 300 MHz) δ 4.61 (1H, br s, H-6), 3.43, 3.29 (each 1H, d, *J* = 11.3 Hz, H-12), 3.08 (1H, dd, *J* = 10.3, 4.8 Hz, H-1), 1.85 (1H, ddd, *J* = 12.7, 3.4, 3.4 Hz, H-9β), 1.73 (1H, dddd, *J* = 13.2, 13.2, 13.2, 3.4 Hz, H-8β), 1.35 (3H, s, H-13), 1.18 (3H, s, H-14 or H-15), 1.09 (3H, s, H-14 or H-15); $^{13}C$ NMR ($CD_3OD$, 75 MHz) δ 80.1 (d, C-1), 74.9 (s, C-11), 72.1 (s, C-4), 67.2 (t, C-12), 66.0 (d, C-6), 55.7 (d, C-5), 49.3 (d, C-7), 41.9 (t, C-9), 41.2 (t, C-3), 39.2 (s, C-10), 28.3 (t, C-2), 24.3 and 23.8 (q, C-13 and C-15), 17.3 (t, C-8), 14.5 (q, C-14); HRLSIMS *m*/*z* 311.1840 [M + 23]$^+$ (calcd for $C_{15}H_{28}O_5Na$, 311,1834).

**Oxidative Lactonization of 16.** To a stirred mixture of (11S)-1β,4α,6β,11,12-pentahydroxyeudesmane (**16**, 13 mg), NMO (4-methylmorpholine *N*-oxide, 8 mg), and activated powdered molecular sieves (8 mg) in dry $CH_2Cl_2$ (3 mL) was added solid TPAP (2.5 mg) in a single portion. The mixture was maintained for 1 h at room temperature under an argon atmosphere, after which the reaction mixture was concentrated to dryness. The residue was chromatographed over silica gel, yielding 10 mg (82%) of 4α,11α-dihydroxy-1-oxoeudesman-6β,12-olide (**17**) as a white solid: mp 228−230 °C; $[\alpha]_D$ −9° (*c* 1, $CHCl_3$); IR (KBr) $\nu_{max}$ 3436, 1754, 1696, 1178 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 5.37 (1H, dd, *J* = 3.8, 2.5 Hz, H-6), 2.69 (1H, ddd, *J* = 15.4, 11.5, 5.2 Hz, H-2β), 2.44 (1H, ddd, *J* = 15.4, 6.4, 4.8 Hz, H-2α), 1.85 (1H, d, *J* = 2.5 Hz, H-5), 1.67 (3H, s, H-15), 1.42 (3H, s, H-13), 1.23 (3H, s, H-14); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 214.0 (s, C-1), 177.4 (s, C-12), 77.3 (d, C-6), 75.2 (s, C-11), 71.2 (s, C-4), 52.7 (d, C-5), 46.2 (d, C-7), 45.2 (s, C-10), 40.9 (t, C-3), 35.2 (t, C-2), 33.6 (t, C-9), 26.3 (q, C-15), 19.8 and 19.0 (q, C-13 and C-14), 18.6 (t, C-8); HRLSIMS *m*/*z* 305.1365 [M + 23]$^+$ (calcd for $C_{15}H_{22}O_5Na$, 305.1365).

**Acknowledgment.** This work was financially supported by grants from the Comisión Interministerial de Ciencia y Tecnología (PM98-0213) and the Consejería de Educación y Ciencia de la Junta de Andalucía (FQM 0139). We thank David Nesbitt for reviewing the English in the manuscript.

**Supporting Information Available:** Details concerning the isolation of compounds **3** and **4** and preparation of **1** and **2**. Spectral data of **6** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP010631M