

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

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Biotransformations of 4 α - and 4 β -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 4 β -hydroxyeudesmane was focused on the isopropyl moiety, but more scattered on the 4 α -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 4 β -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 diol¹⁷ that exerts a potent antispasmodic effect on the isolated rabbit ileum,¹⁸ and 4-*epi*-cryptomeridiol is also a natural compound.¹⁹

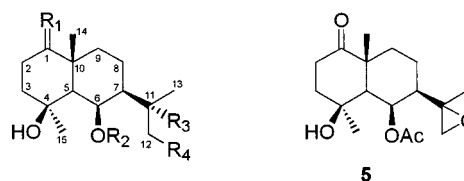
Sesquiterpene lactones show a wide variety of remarkable biological activities, but many of them present strong cytotoxic properties, apparently due to the α,β -unsaturated lactone group.^{20–23} Some authors suspect that 11-hydroxy-sesquiterpenolides are the precursors of those compounds, and thus the 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 4 β -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 6 β -acetoxy-1-keto derivative. Also we have described the incubations of some 4 α -hydroxylated derivatives,^{25,26} but with poor transformation results. Therefore, in this study we have chosen 6 β -acetoxy-1-keto derivatives that are 4 β - or 4 α -hydroxylated to carry out several biotransformations with the microorganism *G. roseum*.

Results and Discussion

6 β -Acetoxy-4 β -hydroxyeudesman-1-one (**1**) and its 4 α -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 C₁₇H₂₆O₅, which indicated the presence of an additional oxygen atom and



- 1: R₁=O, R₂=Ac, R₃=R₄=H
 3: R₁= β -OH, α -H, R₂=Ac, R₃=R₄=H
 6: R₁=O, R₂=R₄=H, R₃=OH
 7: R₁=O, R₂=Ac, R₃=OH, R₄=H
 8: R₁=O, R₂=H, R₃=R₄=OH

the absence of two hydrogen atoms in the molecule. Its ¹H NMR spectrum revealed two signals of an AB system at δ 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 ¹³C NMR spectrum. In conclusion, metabolite **5** was 6 β -acetoxy-11,12-epoxy-4 β -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, 4 β ,6 β ,11-trihydroxyeudesman-1-one (**6**), was a 4-*epi*-cryptomeridiol¹⁹ derivative, which was previously obtained from the incubation of a eudesmane derivative with *R. nigricans*.²⁶

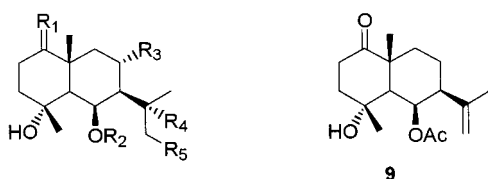
The main metabolite isolated (**7**) had a molecular formula of C₁₇H₂₈O₅, which indicated the presence of an additional hydroxyl group in the molecule. The β -effects on C-7, C-12, and C-13 detected in its ¹³C NMR spectrum indicated that hydroxylation had occurred at C-11, establishing its structure as 6 β -acetoxy-4 β ,11-dihydroxyeudesman-1-one. This major metabolite (**7**) was also a 4-*epi*-cryptomeridiol¹⁹ derivative, obtained from incubation of substrate **1** with *R. nigricans*.²⁶ The difference between metabolites **6** and **7** is the presence of an acetoxy group at C-6 in the latter. Therefore, **7** should have eluted in the chromatographic separation before **6**, but this did not occur

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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 acetoxy group at C-6. Comparisons of the 1H NMR data of **8** with those of **6** revealed the presence of an AB system, centered at δ 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 β ,6 β ,11,12-tetrahydroxy-eudesman-1-one. The configuration at C-11 was determined as "S" by the conversion of **8** into the corresponding 11-hydroxy-6 β ,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: $R_1=O$, $R_2=Ac$, $R_3=R_4=R_5=H$

4: $R_1=\beta-OH, \alpha-H$, $R_2=Ac$, $R_3=R_4=R_5=H$

10: $R_1=\alpha-OH, \beta-H$, $R_2=Ac$, $R_3=R_4=R_5=H$

11: $R_1=O$, $R_2=Ac$, $R_3=OH$, $R_4=R_5=H$

12: $R_1=O$, $R_2=Ac$, $R_4=OH$, $R_3=R_5=H$

13: $R_1=O$, $R_2=R_3=H$, $R_4=OH$, $R_5=OAc$

14: $R_1=\beta-OH, \alpha-H$, $R_2=R_5=H$, $R_3=R_4=OH$

The IR spectrum of metabolite **9** indicated the presence of a carbon-carbon double bond (3080 and 1645 cm^{-1}), and its 1H NMR data confirmed a 2-propenyl group (δ 4.82, 1H, dd, $J = 1.5, 1.5$ Hz; δ 4.69, 1H, br s; δ 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 β -acetoxy-4 α -hydroxyeudesman-11-en-1-one.

Metabolite **10** was the result of the stereoselective reduction of the keto group at C-1 of **1** from the β 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 1H NMR spectrum (δ 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 β -acetoxy-1 α ,4 α -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 1H NMR spectrum a signal appeared at δ 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 β -effects found on the adjacent carbons (C-7 and C-9). Therefore, metabolite **11** was 6 β -acetoxy-4 α ,8 α -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 cryptomeridiol¹⁷ derivative 6 β -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 acetoxy 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 β ,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 β -alcohol) and the deacetylation of the acetoxy 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 β ,6 β ,8 α -trihydroxycryptomeridiol.

In summary, *G. roseum* directed its principal action on this type of substrate toward carbon 11, producing 4 α ,11- and 4 β ,11-dihydroxyeudesmane derivatives (cryptomeridiol¹⁷ and 4-*epi*-cryptomeridiol¹⁹ derivatives). The activity of this microorganism on 4 β -hydroxyeudesmane (**1**) was focused on the isopropyl moiety, but was more scattered for the 4 α -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 β -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 4 α -hydroxylated eudesmane (**2**) than that achieved by *C. lunata*²⁵ or *R. nigricans*²⁶ with some 4 α -hydroxylated eudesmane analogues.

Ley et al. have reported³⁰ that vicinal diols undergo glycol cleavage reactions with tetrapropylammonium per-ruthenate (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 β ,11 α -dihydroxy-1-oxoeudesman-6 β ,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 acetoxy group at C-12, as shown by spectral data. Therefore, **16** was 1 β ,4 α ,6 β ,11,12-pentahydroxyeudesmane. Polyol 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 4 α ,11 α -dihydroxy-1-oxoeudesman-6 β ,12-olide. The configuration at C-11, and therefore of its precursor (**16**), was

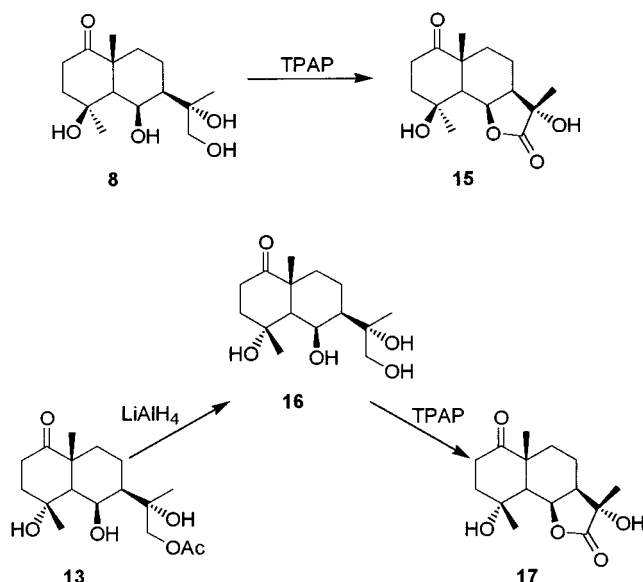


Figure 1. Semisyntheses of 4β- and 4α,11-dihydroxyeudesmanolides.

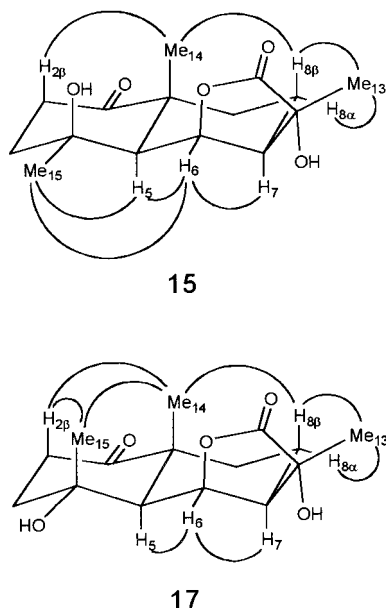


Figure 2. Observed NOEs for eudesmanolides 15 and 17.

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 ¹H and 75.47 MHz ¹³C) were made in CDCl₃, CD₃COCD₃, or CD₃OD (which also provided the lock signal) with Bruker spectrometers (AM-300, ARX-400, and AMX-500). The assignments of ¹³C 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 μm) was used for flash chromatography. CH₂Cl₂ or CHCl₃ containing increasing amounts of Me₂CO or MeOH was used as eluent. Analytical plates (silica gel, Merck 60 G) were rendered visible by spraying with H₂SO₄-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 H₂O 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 H₂O 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 H₂O, and the liquid was saturated with NaCl and extracted twice with CH₂Cl₂. Both extracts were pooled, dried with anhydrous Na₂SO₄, 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 6β-acetoxy-11,12-epoxy-4β-hydroxyeudesman-1-one (5), 24 mg (6%) of 4β,6β,11-trihydroxyeudesman-1-one (6),²⁶ 276 mg (58%) of 6β-acetoxy-4β,11-dihydroxyeudesman-1-one (7),²⁶ and 18 mg (4%) of (11S)-4β,6β,11,12-tetrahydroxyeudesman-1-one (8).

6β-Acetoxy-11,12-epoxy-4β-hydroxyeudesman-1-one (5): colorless syrup; [α]_D +26° (c 1, CHCl₃); IR (CHCl₃) ν_{max} 3477, 1735, 1708, 1243 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 5.80 (1H, br s, H-6), 3.10 (1H, ddd, J = 14.1, 14.1, 6.1 Hz, H-2β), 2.69, 2.47 (each 1H, d, J = 4.9 Hz, H-12), 2.07 (3H, s, CH₃CO), 1.52 (3H, s, H-14), 1.45 (3H, s, H-15), 1.35 (3H, s, H-13); ¹³C NMR (CDCl₃, 75 MHz) δ 215.2 (s, C-1), 170.8 (s, CH₃CO), 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, CH₃CO), 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 C₁₇H₂₇O₅, 311.1858).

(11S)-4β,6β,11,12-Tetrahydroxyeudesman-1-one (8): colorless syrup; [α]_D +31° (c 1, CHCl₃); IR (CHCl₃) ν_{max} 3385, 1704 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 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-2β), 1.61 (3H, s, H-14), 1.39 (3H, s, H-15), 1.28 (3H, s, H-13); ¹³C NMR (CDCl₃, 75 MHz) δ 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 C₁₅H₂₇O₅, 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 6β-acetoxy-4α-hydroxyeudesman-11-en-1-one (9), 43 mg (9%) of 6β-acetoxy-1α,4α-dihydroxyeudesman-1-one (10), 92 mg (19%) of 6β-acetoxy-4α,8α-dihydroxyeudesman-1-one (11), 150 mg (32%) of 6β-acetoxy-4α,11-dihydroxyeudesman-1-one (12), 18 mg (4%) of (11S)-12-acetoxy-4α,6β,11-trihydroxyeudesman-1-one (13), and a mixture of metabolites, from which, after treatment with LiAlH₄, 1β,4α,6β,8α,11-pentahydroxyeudesman-1-one (14, 19 mg, 4% of overall yield) was isolated.

6 β -Acetoxy-4 α -hydroxyeudesm-11-en-1-one (9): colorless syrup; $[\alpha]_D -8^\circ$ (*c* 1, CHCl₃); IR (CHCl₃) ν_{\max} 3418, 3080, 1740, 1711, 1645, 1240 cm⁻¹; ¹H NMR (CDCl₃, 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-2 β), 2.42 (1H, ddd, *J* = 15.5, 6.2, 4.8 Hz, H-2 α), 2.01 (3H, s, CH₃CO), 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); ¹³C NMR (CDCl₃, 75 MHz) δ 214.8 (s, C-1), 171.1 (s, CH₃CO), 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, CH₃CO), 21.0 (t, C-8), 20.8 (q, C-14); HRLSIMS *m/z* 317.2095 [M + 23]⁺ (calcd for C₁₇H₂₆O₄Na, 317.2093).

6 β -Acetoxy-1 α ,4 α -dihydroxyeudesmane (10): colorless syrup; $[\alpha]_D +23^\circ$ (*c* 1, CHCl₃); IR (CHCl₃) ν_{\max} 3453, 1734, 1716, 1247 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 5.81 (1H, br s, H-6), 3.29 (1H, dd, *J* = 3.0, 3.0 Hz, H-1 β), 2.06 (3H, s, CH₃CO), 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); ¹³C NMR (CDCl₃, 75 MHz) δ 172.3 (s, CH₃CO), 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, CH₃CO), 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 C₁₇H₃₀O₄Na, 321.2042).

6 β -Acetoxy-4 α ,8 α -dihydroxyeudesman-1-one (11): colorless syrup; $[\alpha]_D +47^\circ$ (*c* 1, CHCl₃); IR (CHCl₃) ν_{\max} 3543, 3463, 1719, 1691, 1259 cm⁻¹; ¹H NMR (CDCl₃, 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-2 β), 2.37 (1H, ddd, *J* = 15.6, 4.5, 4.1 Hz, H-2 α), 2.20 (1H, dd, *J* = 12.9, 4.1 Hz, H-9 β), 2.09 (3H, s, CH₃CO), 1.92 (1H, ddd, *J* = 13.5, 6.0, 4.1 Hz, H-3 α), 1.80 (1H, ddd, *J* = 13.5, 13.5, 4.5 Hz, H-3 β), 1.76 (1H, d, *J* = 2.2 Hz, H-5), 1.45 (1H, dd, *J* = 12.9, 11.2 Hz, H-9 α), 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); ¹³C NMR (CDCl₃, 75 MHz) δ 212.8 (s, C-1), 172.2 (s, CH₃CO), 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, CH₃CO), 21.6 (q, C-12), 20.6 (q, C-14), 19.6 (C-13); HRLSIMS *m/z* 335.1830 [M + 23]⁺ (calcd for C₁₇H₂₈O₅Na, 335.1834).

6 β -Acetoxy-4 α ,11-dihydroxyeudesman-1-one (12): colorless syrup; $[\alpha]_D +28^\circ$ (*c* 1, CHCl₃); IR (CHCl₃) ν_{\max} 3429, 1707, 1251 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 5.92 (1H, br s, H-6), 2.59 (1H, ddd, *J* = 15.6, 12.0, 5.4 Hz, H-2 β), 2.39 (1H, ddd, *J* = 15.6, 5.7, 4.8 Hz, H-2 α), 2.10 (3H, s, CH₃CO), 1.93 (1H, ddd, *J* = 13.5, 3.3, 3.3 Hz, H-9 β), 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); ¹³C NMR (CDCl₃, 75 MHz) δ 214.4 (s, C-1), 172.6 (s, CH₃CO), 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, CH₃CO), 20.7 (q, C-14), 18.9 (t, C-8); HRLSIMS *m/z* 335.1832 [M + 23]⁺ (calcd for C₁₇H₂₈O₅Na, 335.1834).

(11S)-12-Acetoxy-4 α ,6 β ,11-trihydroxyeudesman-1-one (13): colorless syrup; $[\alpha]_D +13^\circ$ (*c* 1, CHCl₃); IR (CHCl₃) ν_{\max} 3410, 1706, 1242 cm⁻¹; ¹H NMR (CDCl₃, 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-2 β), 2.36 (1H, ddd, *J* = 15.2, 6.3, 5.2 Hz, H-2 α), 2.10 (3H, s, CH₃CO), 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 H-15); ¹³C NMR (CDCl₃, 75 MHz) δ 215.5 (s, C-1), 171.3 (s, CH₃CO), 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 CH₃CO), 16.7 (t, C-8); HRLSIMS *m/z* 351.1778 [M + 23]⁺ (calcd for C₁₇H₂₈O₆Na, 351.1784).

1 β ,4 α ,6 β ,8 α ,11-Pentahydroxyeudesmane (14): colorless solid; mp 132–134 °C; $[\alpha]_D -3^\circ$ (*c* 1, CHCl₃); IR (CHCl₃) ν_{\max} 3405, 1143 cm⁻¹; ¹H NMR (CDCl₃/CD₃COCD₃, 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-9 β), 1.39 (3H, s, H-12 or H-13), 1.38 (3H, s, H-12 or H-13), 1.26 (3H, s, H-13), 1.19 (3H, s, H-15); ¹³C NMR (CDCl₃/CD₃COCD₃, 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 C₁₅H₂₈O₅-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)-4 β ,6 β ,11,12-tetrahydroxyeudesman-1-one (**8**, 15 mg), NMO (4-methylmorpholine *N*-oxide, 10 mg), and activated powdered molecular sieves (10 mg) in dry CH₂Cl₂ (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 4 β ,11 α -dihydroxy-1-oxoeudesman-6 β ,12-olide (**15**, 81%) as a white solid: mp 220–222 °C; $[\alpha]_D +19^\circ$ (*c* 1, CHCl₃); IR (KBr) ν_{\max} 3471, 1756, 1689, 1183 cm⁻¹; ¹H NMR (CDCl₃, 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-2 β), 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); ¹³C NMR (CDCl₃, 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 C₁₅H₂₂O₅Na, 305.1365).

Reduction of 13. Metabolite **13** (18 mg) was added to a solution (0.5 mL) of LiAlH₄ 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 CH₂Cl₂. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. Chromatography on a silica gel column yielded 13 mg (82%) of (11S)-1 β ,4 α ,6 β ,11,12-pentahydroxyeudesmane (**16**) as a solid: mp 81–83 °C; $[\alpha]_D +11^\circ$ (*c* 1, CH₃OH); IR (KBr) ν_{\max} 3398, 1258, 1039 cm⁻¹; ¹H NMR (CD₃OD, 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-9 β), 1.73 (1H, dddd, *J* = 13.2, 13.2, 13.2, 3.4 Hz, H-8 β), 1.35 (3H, s, H-13), 1.18 (3H, s, H-14 or H-15), 1.09 (3H, s, H-14 or H-15); ¹³C NMR (CD₃OD, 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 C₁₅H₂₈O₅Na, 311.1834).

Oxidative Lactonization of 16. To a stirred mixture of (11S)-1 β ,4 α ,6 β ,11,12-pentahydroxyeudesmane (**16**, 13 mg), NMO (4-methylmorpholine *N*-oxide, 8 mg), and activated powdered molecular sieves (8 mg) in dry CH₂Cl₂ (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 4 α ,11 α -dihydroxy-1-oxoeudesman-6 β ,12-olide (**17**) as a white solid: mp 228–230 °C; $[\alpha]_D -9^\circ$ (*c* 1, CHCl₃); IR (KBr) ν_{\max} 3436, 1754, 1696, 1178 cm⁻¹; ¹H NMR (CDCl₃, 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-2 β), 2.44 (1H, ddd, *J* = 15.4, 6.4, 4.8 Hz, H-2 α), 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); ¹³C NMR (CDCl₃, 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 C₁₅H₂₂O₅Na, 305.1365).

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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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