Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis B: Enzymatic 27 (2004) 133–138
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Biotransformation of a 4α-hydroxylated eudesmane with *Exserohilum halodes* Chemo-enzymatic synthesis of cryptomeridiol and 6-*epi*-colartin derivatives

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

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

Received 27 March 2003; received in revised form 9 October 2003; accepted 14 October 2003

Abstract

Microbial transformation of a 4α-hydroxylated eudesmane by *Exserohilum halodes* has been achieved. Regioselective hydroxylations in both "A" and "B" rings, on C-2 and C-8, were detected, but the main hydroxylating action was directed to the isopropyl moiety and cryptomeridiol analogues were isolated. Semi-synthesis of two sesquiterpenolides, 6-*epi*-colartin derivatives, were accomplished from the significant metabolites hydroxylated at C-12.

Keywords: Whole-cell biotransformation; Microbial hydroxylation; Sesquiterpene lactones; *Exserohilum halodes*

1. Introduction

During recent years the use of biocatalysis for the selective transformation of synthetic and natural products has increased. The relatively mild conditions, the environmental safety and, mainly, the high selectivity of these biocatalytic processes are the principal advantages. Notable attention has been paid to filamentous fungi, since they are capable of catalysing the regio- and stereoselective hydroxylation [1,2] of non-activated carbon centres that permit the access to a great variety of natural organic compounds, a difficult challenge by classical chemical means [3].

Sesquiterpenoids are widely distributed in Nature [4,5]. Particularly, sesquiterpene lactones are a large group of compounds with noteworthy biological and therapeutic activities [6,7] (e.g. anti-inflammatory [8], anti-tumoral [9–12], and anti-microbial [13–15]), and their biogenesis and chemical reactivity have been extensively studied [16]. On the other hand, the microbial hydroxylation of terpenoids has been also investigated, as they represent an inexpensive source of substrates for the preparation of asymmetric building blocks to synthesise several compounds [17].

Previous papers have reported the successful biotransformations of sesquiterpene derivatives using different hydroxylating fungi and the chemical-microbiological syntheses of 6β-sesquiterpenolides from biohydroxylated metabolites [18–21]. These lactones are scarce but useful compounds, since their synthesis offers an attractive approach to their biogenetic study. Microbial transformations of some 4α-hydroxylated derivatives using the hydroxylating fungi *Curvularia lunata* [18], *Rhizopus nigricans* [19] and *Gliocladium roseum* [21] have been studied, and the interesting semi-synthesis of a 4α,11-dihydroxyeudesmanolide has also been described [21]. In the present work, the biocatalysis of a 4α-hydroxylated eudesmane with the filamentous fungus *Exserohilum halodes* (CECT 2716), the anamorphous form of *Setosphaeria rostrata* (IMI 76563), was tested and the chemical-microbiological synthesis of two 6β-sesquiterpene lactones with a structure of 1β-hydroxy-6-*epi*-colartin [22] and 1β-hydroxy-6,11-di*epi*-colartin was accomplished (Table 1).

* Corresponding author. Tel.: +34-958-24-33-64; fax: +34-958-24-33-64.
E-mail address: agarcia@ugr.es (A. García-Granados).

1381-1177/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2003.10.003

Table 1
^{13}C NMR chemical shifts

C	1	3	4	6	7	9	10	13
1	214.6	213.2	217.7	214.4	214.7	80.3	80.5	79.5
2	35.4	34.6	69.5	35.4	35.4	71.1	28.8	28.8
3	41.1	41.2	44.3	41.1	40.8	47.0	41.5	38.5
4	70.5	70.5	71.4	70.5	70.7	70.6	72.4	71.8
5	55.0	55.0	51.7	55.1	54.8	55.2	56.0	53.2
6	70.3	67.6	69.8	70.0	71.1	69.2	69.1	76.4
7	49.2	51.7	50.2	44.7	43.2	49.5	48.8	40.9
8	20.6	70.3	20.2	20.8	20.0	20.2	17.5	18.4
9	36.1	42.6	36.3	36.0	36.0	42.3	42.4	42.0
10	46.9	46.1	45.0	47.0	46.9	38.9	39.6	38.4
11	28.7	25.2	28.3	36.2	36.2	28.8	38.5	41.2
12	21.3[a]	22.8[a]	21.8[a]	65.7	66.1	21.3[a]	64.7	179.6
13	20.8[a]	21.1[a]	20.9[a]	15.4	14.8	20.7[a]	14.8	9.2
14	20.6	20.3	20.4	20.9	20.9	15.7	17.4	14.3
15	24.5	24.5	27.4	24.5	24.7	26.4	25.3	25.5
*C*H$_3$CO	21.8	21.8	21.8	21.9	21.9	21.9		
CH$_3$*C*O	172.1	171.7	170.6	172.1	172.1	172.5		

[a] Values may be interchanged.

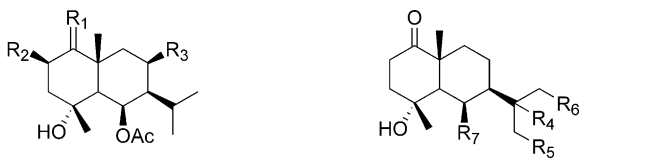
2. Results and discussion

2.1. Biotransformation of 6β-acetoxy-4α-hydroxyeudesman-1-one (1)

6β-Acetoxy-4α-hydroxyeudesman-1-one (**1**) [21] was obtained from the oxidation at C-1 of the natural compound **2** [23] by treatment with Jones' reagent. Biotransformation of **1** with *Exserohilum halodes* for 6 days gave the metabolites **3** (4%), **4** (22%), **5** (28%), **6** (6%), **7** (6%) and **8** (8%), besides some 15% of unaltered substrate (**1**) (Fig. 1).

The first metabolite isolated (**3**) had a formula ($C_{17}H_{28}O_5$) which suggested the presence of an additional hydroxyl group in the molecule. The β-effects on C-7 and C-9 and the γ-effects on C-6, C-10 and C-11, detected in its ^{13}C NMR spectrum, positioned the new hydroxyl group at C-8. The stereochemical arrangement of this new functional group was deduced from the shape of the signal of H-8 in its ^{1}H NMR spectrum. This signal at δ: 4.25 (1H, m, $w_{1/2} = 10.0$ Hz) was due to a geminal proton to an axial hydroxyl group. Therefore, metabolite **3** was 6β-acetoxy-4α,8β-dihydroxyeudesman-1-one. The C-8 epimer was isolated in the biotransformation of the same substrate (**1**) by *G. roseum* [21].

Metabolite **4** showed the same molecular formula as the above metabolite (**3**). On comparing the ^{13}C NMR data of this metabolite (**4**) with those of the substrate **1**, we observed β-effects on C-1 and C-3, a γ-anti-effect on C-4 and a γ-gauche effect on C-10. Moreover, the presence, in the ^{1}H NMR spectrum of this metabolite (**4**), of a signal at δ: 4.52 ($J_{2,3\beta} = 13.9$, $J_{2,3\alpha} = 5.7$ Hz) due to a geminal axial proton to a hydroxyl group, and the absence of the characteristic H-2 signal, placed this hydroxyl group on C-2 in an equatorial arrangement. The absolute configuration of this new functional group was unequivocally determined by the stereochemical reduction of carbonyl group on C-1. This process occurred by the less sterically hindered face and, consequently, gave a 1β-hydroxyl derivative (**9**). In its ^{1}H NMR spectrum the corresponding H-2 signal appeared at δ: 4.12 (ddd, $J_{2,1} = J_{2,3\alpha} = J_{2,3\beta} = 3.5$ Hz) with coupling constants consistent with an equatorial disposition, the hydroxyl group being on C-2 in an axial arrangement. These results could be explained assuming the "A" ring of metabolite **4** in a "half-boat" conformation. Consequently, product **9** was



1 R_1= O; R_2= R_3= H
2 R_1= α-H, β-OH; R_2= R_3= H
3 R_1= O; R_2= H; R_3= OH
4 R_1= O; R_2= OH; R_3= H
9 R_1= α-H, β-OH; R_2= OH; R_3= H

5 R_4= OH; R_5= H; R_6= H; R_7= OAc
6 R_4= H; R_5= OH; R_6= H; R_7= OAc
7 R_4= H; R_5= H; R_6= OH; R_7= OAc
8 R_4= OH; R_5= OAc; R_6= H; R_7= OH

Fig. 1. Structures of compounds **1**–**9**.

6β-acetoxy-1β,2β,4α-trihydroxyeudesmane, and metabolite **4** was, hence, 6β-acetoxy-2β,4α-dihydroxyeudesman-1-one.

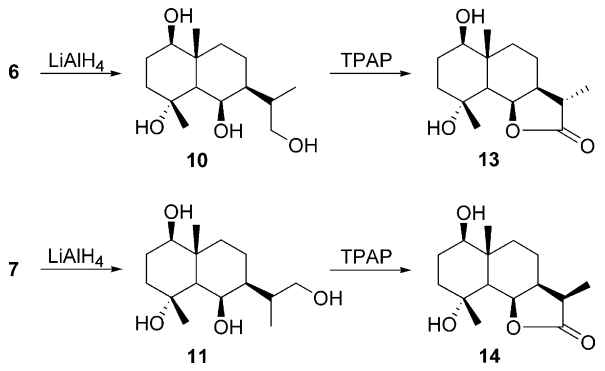
The molecular formula of the major metabolite isolated (**5**) indicated, as seen in previous metabolites, the presence of an additional hydroxyl group. In accordance with the β-effects produced on C-7, C-12 and C-13 (^{13}C NMR spectrum) and the presence of two deshielded methyl singlet signals (^{1}H NMR spectrum), the microbial hydroxylation took place on C-11. This metabolite was a 1-oxocryptomeridiol derivative, with a structure of 6β-acetoxy-4α,11-dihydroxyeudesman-1-one, and was previously achieved from the incubation of substrate **1** by *G. roseum* [21].

The spectral data of metabolites **6** and **7** were similar. Their molecular formulas also indicated an additional oxygen atom in the molecule. The spectroscopic behaviour of these compounds (see Section 4) indicated that hydroxylations, respectively, occur at C-12 and C-13. Both metabolites, which were epimers at C-11, had a structure of 6β-acetoxy-4α,12-dihydroxyeudesman-1-one. Their absolute configuration was determined as "11*R*" for **6** and as "11*S*" for **7** by their respective conversion into their corresponding 6β,12-eudesmanolides (see below).

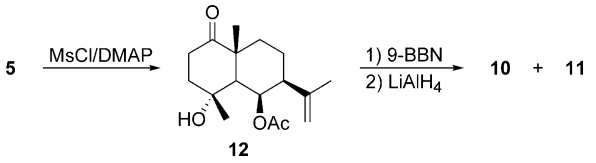
The most polar metabolite isolated (**8**) had a molecular formula ($C_{17}H_{28}O_6$) that suggested the presence of two new hydroxyl groups in the molecule. These functional groups were positioned at C-11 and C-12 by their spectral data analysis, which also confirmed the rearrangement of the acetoxyl group at C-6 to the hydroxyl group at C-12. This compound was previously isolated in the biotransformation of **1** by *G. roseum* [21] and was (11*S*)-12-acetoxy-4α,6β,11-trihydroxyeudesman-1-one.

2.2. Synthesis of 6-epi-colartin derivatives

Respective treatment of metabolites **6** and **7** with $LiAlH_4$ gave the corresponding 1β,4α,6β,12-tetrahydroxyeudesmane derivatives (**10** and **11**) by the hydrolysis of their acetoxyl groups at C-6 and the stereoselective reduction of their carbonyl groups on C-1 (Scheme 1). On the other hand, both polyhydroxylated compounds (**10** and **11**) were also



Scheme 1. Semi-syntheses of 6-*epi*-colartin derivatives (**13** and **14**).



Scheme 2. Semi-synthesis of 1β,4α,6β,12-tetrahydroxyeudesmanes (**10** and **11**).

synthesised from the major metabolite (**5**), an 11-hydroxyl derivative. First, the hydroxyl group at C-11 was dehydrated through the formation of the corresponding mesylate derivative, using 4-dimethylaminopyridine (DMAP) and methanesulfonyl chloride [24] giving the elimination product (**12**) with the less-substituted double bond (Scheme 2). Their subsequent hydroboration followed by treatment with $LiAlH_4$ gave a mixture from which compounds **10** and **11** were separated as a result of the hydration on both faces of the double bond, the stereoselective reduction of the carbonyl group at C-1 and the hydrolysis of the acetoxyl group at C-6. Oxidative lactonization of product **10** with tetrapropylammonium perruthenate (TPAP) [25] gave product **13**, and, in a similar way, product **11** led to product **14** (Scheme 1). Both epimers (**13** and **14**) were 6-*epi*-colartin derivatives with a structure of 1β,4α-dihydroxyeudesman-6β,12-olide. The C-11 configurations were assigned from the corresponding coupling constants of the H-11 proton signals of their ^{1}H NMR spectra. Thus, the H-11 signal of lactone **13** (δ: 2.74, dq, $J_{11,13} = J_{11,7} = 6.9$ Hz) indicated a dihedral angle between H-7 and H-11 of approximately 40°. However, for lactone **14**, no coupling was observed between H-7 and H-11 (δ: 2.31, q, $J_{11,13} = 7.7$ Hz), which indicated that the dihedral angle was nearly 90° for these protons. We assigned a "11*R*" configuration to lactone **13** (1β-hydroxy-6,11-di*epi*-colartin), and consequently to their precursors **6** and **10**, and a "11*S*" configuration to lactone **14** (1β-hydroxy-6-*epi*-colartin), as well as compounds **7** and **11**. Lactone **14** had been previously synthesised from vulgarin [22] and the comparison of their spectral data confirmed the "11*S*" configuration.

3. Conclusions

In summary, the principal hydroxylating action of *E. halodes* was directed towards the isopropyl moiety, mainly on C-11 (producing cryptomeridiol derivatives), as previously described in biotransformation of **1** by *G. roseum* [21]. Moreover, the remarkable biohydroxylation on C-12 enabled the synthesis of two 4α-hydroxy-6β,12-eudesmanolides (6-*epi*-colartin derivatives), epimers at C-11. This synthesis was also carried out from the main 11-hydroxylated metabolite. In addition, a 11,12-hydroxyl eudesmane derivative, which had been previously used to semi-synthesise a new 11-hydroxy-6β,12-eudesmanolide, was again isolated, but with better yields. On the other hand, microbial actions in

both the "A" and "B" rings (on C-2 and C-8, respectively) were also detected. In this case, the 8-hydroxyl derivative was an epimer of a compound previously isolated in the biotransformation of the same substrate by *G. roseum*. The present work again confirms the use of biohydroxylating catalysis as a promising tool to achieve the semi-synthesis of sesquiterpenolides from their sesquiterpene precursors.

4. Experimental

4.1. General Experimental Procedures

Measurements of NMR spectra (300.13 MHz ^{1}H and 75.47 MHz ^{13}C) were made in $CDCl_3$ (which also provided the lock signal) with BRUKER spectrometers (AM-300 and ARX-400). 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°. Bruker's programs were used for C/H correlation (HMQC). IR spectra were recorded on a Nicolet 20SX FT-IR spectrometer. High-resolution mass spectra were made by LSIMS ionization mode with a MICROMASS AUTOSPEC-Q spectrometer. Uncorrected melting points were determined using a Kofler (Reichter) apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 25 °C. Scharlau 60 silica gel (40–60 μm) was used for flash chromatography. CH_2Cl_2, $CHCl_3$ or hexane containing increasing amounts of Me_2CO, MeOH or 2-propanol were used as eluents. 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 1, 2, 5, 8, 11, 12 and 14 was confirmed by direct comparison with the authentic samples (IR, MS, NMR, etc.).

4.2. Isolation of 6β-acetoxy-1β,4α-dihydroxyeudesmane (2)

6β-acetoxy-1β,4α-dihydroxyeudesmane was isolated from *Sideritis varoi* subsp. *oriensis* [23].

4.3. Oxidation of 6β-acetoxy-1β,4α-dihydroxyeudesmane (2)

Jones' reagent was added dropwise to a stirred solution of 6β-acetoxy-1β,4α-dihydroxyeudesmane (**2**, 300 mg) in acetone at 0 °C until an orange-brown colour persisted (30 min), following the oxidation by TLC. MeOH was then added and the reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Chromatography on a silica-gel column yielded 6β-acetoxy-4α-hydroxyeudesman-1-one (**1**, 265 mg, 89%) [23].

4.4. Organism, Media and Culture Conditions

Exserohilum halodes (CECT 2716), the anamorphous form of *Setosphaeria rostrata* (IMI 76563), 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 substrates **1** (5–10%) in EtOH was added.

4.5. Biotransformation of 6β-acetoxy-4α-hydroxyeudesman-1-one (1)

Substrate **1** (250 mg) was dissolved in EtOH (4 ml), distributed among 4 Erlenmeyer flask cultures of *E. halodes* and incubated for 6 days, after which the cultures were filtered and pooled. The cells were washed thoroughly with water 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 vacuum to give a mixture of compounds. This mixture was chromatographed on a silica-gel column to obtain 38 mg (15%) of starting material **1**; 11 mg (4%) of 6β-acetoxy-4α,8β-dihydroxyeudesman-1-one (**3**); 58 mg (22%) of 6β-acetoxy-2β,4α-dihydroxyeudesman-1-one (**4**); 73 mg (28%) of 6β-acetoxy-4α,11-dihydroxyeudesman-1-one (**5**) [21]; 16 mg (6%) of (11*R*)-6β-acetoxy-4α,12-dihydroxyeudesman-1-one (**6**); 16 mg (6%) of (11*S*)-6β-acetoxy-4α,12-dihydroxyeudesman-1-one (**7**); 21 mg (8%) of (11*S*)-12-acetoxy-4α,6β,11-trihydroxyeudesman-1-one (**8**) [21].

4.5.1. 6β-acetoxy-4α,8β-dihydroxyeudesman-1-one (3)

Colourless syrup; $[\alpha]_D$ +14° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3524, 3440, 1705, 1690, 1266 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.93 (1H, dd, $J_{6,7} = 4.0$, $J_{6,5} = 2.1$ Hz, H-6), 4.25 (1H, m, $w_{1/2} = 10.0$ Hz, H-8), 2.72 (1H, ddd, $J_{2\alpha,2\beta} = 15.6$, $J_{2\alpha,3\beta} = 13.3$, $J_{2\alpha,3\alpha} = 5.9$ Hz, H-2α), 2.35 (1H, ddd, $J_{2\beta,2\alpha} = 15.6$, $J_{2\beta,3\alpha} = J_{2\beta,3\beta} = 4.6$ Hz, H-2β), 2.27 (1H, dd, $J_{9\beta,9\alpha} = 15.0$, $J_{9\beta,8} = 3.1$ Hz, H-9β), 2.14 (3H, s, acetoxyl group), 1.96 (1H, ddd, $J_{3\alpha,3\beta} = 13.3$, $J_{3\alpha,2\alpha} = 5.9$, $J_{3\alpha,2\beta} = 4.6$ Hz, H-3α), 1.83 (1H, ddd, $J_{3\beta,3\alpha} = J_{3\beta,2\alpha} = 13.3$, $J_{3\beta,2\beta} = 4.6$ Hz, H-3β), 1.77 (1H, d, $J_{5,6} = 2.1$ Hz, H-5), 1.54 and 1.43 (3H each, s, 3H-14 and 3H-15), 1.01 and 0.97 (3H each, d, $J = 6.6$ Hz, 3H-12 and 3H-13); HRLSIMS *m/z* 335.1832 [M+23]$^+$ (cacld. for $C_{17}H_{28}O_5Na$, 335.1834).

4.5.2. 6β-acetoxy-2β,4α-dihydroxyeudesman-1-one (4)

Colourless syrup; $[\alpha]_D$ −71° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3448, 1734, 1713, 1241 cm^{-1}; ^{1}H NMR ($CDCl_3$,

300 MHz) δ: 5.75 (1H, brs, H-6), 4.52 (1H, dd, $J_{2,3\beta} = 13.9$, $J_{2,3\alpha} = 5.7$ Hz, H-2), 2.23 (1H, dd, $J_{3\alpha,3\beta} = 14.3$, $J_{3\alpha,2} = 5.7$ Hz, H-3α), 2.04 (3H, s, acetoxyl group), 1.96 (1H, ddd, $J_{9\beta,9\alpha} = 13.3$, $J_{9\beta,8\alpha} = J_{9\beta,8\beta} = 3.2$ Hz, H-9β), 1.74 (1H, dd, $J_{3\beta,3\alpha} = 14.3$, $J_{3\beta,2} = 13.9$ Hz, H-3β), 1.53 (1H, dddd, $J_{8\beta,7} = J_{8\beta,8\alpha} = J_{8\beta,9\alpha} = 13.3$, $J_{8\beta,9\beta} = 3.2$ Hz, H-8β), 1.33 and 1.28 (3H each, s, 3H-14 and 3H-15), 0.98 and 0.95 (3H each, d, $J = 6.6$ Hz, 3H-12 and 3H-13); HRLSIMS *m/z* 335.1830 $[M+23]^+$ (calcd. for $C_{17}H_{28}O_5Na$, 335.1834).

4.5.3. (11R)-6β-acetoxy-4α,12-dihydroxyeudesman-1-one (6)

Colourless syrup; $[\alpha]_D$ +18° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3428, 1734, 1708, 1243 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.75 (1H, br s, H-6), 3.65 and 3.56 (1H each, part AB of an ABX system, $J_{A,B} = 10.7$, $J_{A,X} = 3.6$ Hz, $J_{B,X} = 5.2$ Hz, 2H-12), 2.62 (1H, ddd, $J_{2\alpha,2\beta} = 15.6$, $J_{2\alpha,3\beta} = 13.0$, $J_{2\alpha,3\alpha} = 5.8$ Hz, H-2α), 2.37 (1H, ddd, $J_{2\beta,2\alpha} = 15.6$, $J_{2\beta,3\beta} = J_{2\beta,3\alpha} = 4.7$ Hz, H-2β), 2.10 (3H, s, acetoxyl group), 1.77 (1H, d, $J_{5,6} = 2.0$ Hz, H-5), 1.40 and 1.32 (3H each, s, 3H-14 and 3H-15), 0.98 (3H, d, $J = 6.4$ Hz, 3H-13); HRLSIMS *m/z* 335.1838 $[M + 23]^+$ (calcd. for $C_{17}H_{28}O_5Na$, 335.1834).

4.5.4. (11S)-6β-acetoxy-4α,12-dihydroxyeudesman-1-one (7)

Colourless syrup; $[\alpha]_D$ −1° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3404, 1732, 1708, 1243 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.76 (1H, br s, H-6), 3.55 (2H, d, $J_{12,11} = 4.6$ Hz, 2H-12), 2.59 (1H, ddd, $J_{2\alpha,2\beta} = 15.5$, $J_{2\alpha,3\beta} = 12.1$, $J_{2\alpha,3\alpha} = 5.5$ Hz, H-2α), 2.39 (1H, ddd, $J_{2\beta,2\alpha} = 15.5$, $J_{2\beta,3\beta} = J_{2\beta,3\alpha} = 5.2$ Hz, H-2β), 2.10 (3H, s, acetoxyl group), 1.81 (1H, d, $J_{5,6} = 2.0$ Hz, H-5), 1.38 and 1.32 (3H each, s, 3H-14 and 3H-15), 0.92 (3H, d, $J = 6.5$ Hz, 3H-13); HRLSIMS *m/z* 335.1840 $[M + 23]^+$ (calcd. for $C_{17}H_{28}O_5Na$, 335.1834).

4.6. Reduction of metabolite 4

Ten milligram of $NaBH_4$ were carefully added to an ethanol solution (2.5 ml) of 6β-acetoxy-2β,4α-dihydroxyeudesman-1-one (**4**, 20 mg). The mixture was stirred at room temperature and the reaction was followed by TLC. After completion, diluted HCl was added and this mixture was extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Chromatography on a silica-gel column yielded 18 mg (89%) of 6β-acetoxy-1β,2β,4α-trihydroxyeudesmane (**9**) Colourless syrup; $[\alpha]_D$ +8° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3432, 1715, 1252 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.76 (1H, br s, H-6), 4.12 (1H, ddd, $J_{2,1} = J_{2,3\alpha} = J_{2,3\beta} = 3.5$ Hz, H-2), 3.21 (1H, d, $J_{1,2} = 3.5$ Hz, H-1), 2.07 (3H, s, acetoxyl group), 1.99 (1H, ddd, $J_{9\beta,9\alpha} = 12.8$, $J_{9\beta,8\alpha} = J_{9\beta,8\beta} = 3.3$ Hz, H-9β), 1.46 (1H, ddd, $J_{8\beta,8\alpha} = J_{8\beta,9\alpha} = 13.1$, $J_{8\beta,9\beta} = 3.3$ Hz, H-8β), 1.39 and 1.29 (3H each, s, 3H-14 and 3H-15), 1.37 (1H, d, $J_{5,6} = 2.1$ Hz, H-5), 0.89 and 0.88 (3H each, d, $J = 6.7$ Hz, 3H-12 and 3H-13); HRLSIMS *m/z* 337.1986 $[M + 23]^+$ (calcd. for $C_{17}H_{30}O_5Na$, 337.1992).

4.7. Reduction and saponification of metabolite 6

After the addition of 16 mg of (11*R*)-6β-acetoxy-4α,12-dihydroxyeudesman-1-one (**6**) to a solution (0.5 ml) of $LiAlH_4$ in THF (1M), 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_2SO_4 and evaporated to dryness. Chromatography on a silica-gel column afforded 12 mg (86%) of (11*R*)-1β,4α,6β,12-tetrahydroxyeudesmane (**10**) colourless solid: mp 85–87 °C; $[\alpha]_D$ −15° (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3398, 1077, 1033 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 4.39 (1H, br s, H-6), 3.55 and 3.50 (1H each, part AB of an ABX system, $J_{A,B} = 10.8$, $J_{A,X} = 7.9$, $J_{B,X} = 4.9$ Hz, 2H-12), 3.18 (1H, dd, $J_{1,2\beta} = 10.7$, $J_{1,2\alpha} = 4.2$ Hz, H-1), 1.50 (3H, s, 3H-15), 1.19 (3H, s, 3H-14), 1.18 (1H, d, $J_{5,6} = 2.9$ Hz, H-5), 0.94 (3H, d, $J_{13,11} = 7.1$ Hz, 3H-13); HRLSIMS *m/z* 273.2074 $[M+1]^+$ (calcd. for $C_{15}H_{29}O_4$ 273.2066).

4.8. Reduction and saponification of metabolite 7

This reaction was carried out as described before for the reduction and saponification of metabolite **6**. Metabolite **7** (16 mg) provided 81% (11 mg) of (11*S*)-1β,4α,6β,12-tetrahydroxyeudesmane (**11**) [22].

4.9. Dehydration of metabolite 5

To a stirred solution of 6β-acetoxy-4α,11-dihydroxyeudesman-1-one (**5**, 50 mg) in CH_2Cl_2 (3 ml), Et_3N (0.2 ml) and DMAP (2 mg) were added. The reaction mixture was cooled to 0 °C, and methanesulfonyl chloride (0.1 ml) was added dropwise and the reaction was stirred for 2 h at room temperature. Then, crushed ice was added and the mixture was stirred for 1 h and afterwards extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. Flash column chromatography over silica gel gave 30 mg (64%) of 6β-acetoxy-4α-hydroxyeudesm-11-en-1-one (**12**) [21].

4.10. Hydroboration of product 12

After 25 mg of 6β-acetoxy-4α-hydroxyeudesm-11-en-1-one (**12**) were added to a solution (0.5 ml) of 9-BBN in THF (0.5 M), the mixture was stirred for 2 h at room temperature in an argon atmosphere, after which ethanol (0.6 ml), a 6 N solution of NaOH (0.2 ml), and H_2O_2 (30%) (0.4 ml) were added, and this mixture was heated for 1 h at 50°C. The mixture was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude residue was

chromatographed over silica gel yielding 12 mg (54%) of **10** and 7 mg (30%) of **11**.

4.11. Oxidative lactonization of product *10*

Solid TPAP (3 mg) was added in a single portion to a stirred mixture of (11*R*)-1β,4α,6β,12-tetrahydroxyeudesmane (**10**, 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 in an argon atmosphere. On completion, the reaction mixture was concentrated in vacuum. Purification by column chromatography on silica gel yielded 12 mg of (11*R*)-1β,4α-dihydroxyeudesman-6β,12-olide (**13**, 83%) as a white solid: mp 76–78 °C; IR (KBr) ν_{max} 3415, 1735, 1168 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ:4.87 (1H, dd, $J_{6,7} = 3.9$, $J_{6,5} = 2.6$ Hz, H-6), 3.27 (1H, dd, $J_{1,2\beta} = 10.5$, $J_{1,2\alpha} = 4.1$ Hz, H-1α), 2.74 (1H, dq, $J_{11,7} = J_{11,13} = 6.9$ Hz, H-11), 2.26 (1H, dddd $J_{7,8\beta} = 12.0$, $J_{7,11} = 6.9$, $J_{7,8\alpha} = 6.1$, $J_{7,6} = 3.9$ Hz, H-7), 1.96 (1H, ddd, $J_{9\beta,9\alpha} = 12.6$, $J_{9\beta,8\alpha} = J_{9\beta,8\beta} = 3.3$ Hz, H-9β), 1.47 (3H, s, 3H-15), 1.17 (3H, d, $J_{13,11} = 6.9$ Hz, 3H-13), 1.04 (3H, s, 3H-14); HRL-SIMS *m/z* 291.1565 $[M + 23]^+$ (calcd. for $C_{15}H_{24}O_4Na$, 291.1572).

4.12. Oxidative lactonization of product *11*

This reaction was carried out as described for the oxidative lactonization of product **10**. Compound **11** (15 mg) supplied 86% (13 mg) of (11*S*)-1β,4α-dihydroxyeudesman-6β,12-olide (**14**) [22].

Acknowledgements

This work was financially supported by grants from the Comisión Interministerial de Ciencia y Tecnología (PM98-0213), the Consejería de Educación y Ciencia de la Junta de Andalucía (FQM 0139) and a pre-doctoral fellowship from the Ministerio de Educación, Cultura y Deporte. We thank David Nesbitt for reviewing the English in the manuscript.

References

[1] H.L. Holland, Stereoselective hydroxylation reactions, in: R.N. Patel (Eds.), Stereoselective Biocatalysis, Marcel Dekker, New York, 2000, pp. 131–152.
[2] H.L. Holland, H.K. Weber, Curr. Opin. Biotechnol. 11 (2000) 547–553.
[3] W.A. Duetz, J.B. van Beilen, B. Witholt, Curr. Opin. Biotechnol. 12 (2000) 419–425.
[4] B.M. Fraga, Nat. Prod. Reprod. 19 (2002) 650–672, and references therein.
[5] J. Buckingham (Ed.), Dictionary of Natural Products on CD-ROM, Version 6.1, Chapman and Hall, London, UK, 1998.
[6] R.J. Marles, L. Pazos-Sanou, C.M. Compadre, J.M. Pezzuto, E. Boszyk, J.T. Arnason, in: J.T. Arnason, R. Mata, J.T. Romeo (Eds.), Sesquiterpene Lactones revisited: Recent developments in the Assessment of Biological Activities and Structure Relationships, Phytochemistry of Medicinal Plants, vol. 29, Plenum Press, New York, 1995, pp. 333–356.
[7] M. Robles, M. Aregullin, J. West, E. Rodríguez, Planta Med. 61 (1995) 199–203.
[8] S. Heptinstall, L. Willianson, A. White, J.R.A. Mitchell, Lancet (1985) 1071.
[9] I.H. May, K. Lee, E.C. Mar, C.O. Starnes, J. Med. Chem. 20 (1977) 333–336.
[10] K.H. Lee, Y. Imakura, D. Sims, A.T. McPhail, K.D. Onan, Phytochemistry 16 (1977) 393–395.
[11] N. Gören, H.J. Woerdenbag, C. Bozok-Johansson, Planta Med. 62 (1996) 419–422.
[12] J.Y. Cho, J. Park, S.E. Yoo, K.U. Baik, J.H. Jung, J. Lee, M.H. Park, Planta Med. 64 (1998) 594–597.
[13] N. Gören, J. Jakupovic, S. Topal, Phytochemistry 29 (1990) 1467–1469.
[14] N.H. Fischer, T. Lu, C.L. Cantrell, J. Castañeda-Acosta, L. Quijano, S.G. Franzblau, Phytochemistry 49 (1998) 559–564.
[15] C.L. Cantrell, I.S. Núñez, J. Castañeda-Acosta, M. Foroozesh, F.R. Fronczek, S.G. Franzblau, N.H. Fischer, J. Nat. Prod. 61 (1998) 1181–1186.
[16] N.H. Fischer, E.J. Olivier, H.D. Fischer, in: W. Herz, H. Grisebach, G.W. Kirby (Eds.), The Biogenesis and Chemistry of Sesquiterpene Lactones, vol. 38, Fortschrittder Chemie Organischer Naturstoffe, Springer, New York, 1979, pp. 47–390 and references therein.
[17] R. Azerad, Regio- and stereoselective microbial hydroxylation of terpenoid compounds, in: R.N. Patel (Eds.), Stereoselective Biocatalysis, Marcel Dekker, New York, 2000, pp. 153–180.
[18] A. García-Granados, A. Martínez, F. Rivas, M.E. Onorato, J.M. Arias, Tetrahedron 47 (1991) 91–102.
[19] A. García-Granados, A. Martínez, A. Parra, F. Rivas, M.E. Onorato, J.M. Arias, Tetrahedron 49 (1993) 1091–1102.
[20] A. García-Granados, M.C. Gutiérrez, A. Martínez, F. Rivas, Tetrahedron 54 (1998) 3311–3320.
[21] A. García-Granados, M.C. Gutiérrez, A. Parra, F. Rivas, J. Nat. Prod. 65 (2002) 1011–1015.
[22] J.L. Breton, J.J. Cejudo, A. García-Granados, A. Parra, F. Rivas, Tetrahedron 50 (1994) 2917–2928.
[23] A. García-Granados, A. Martínez, A. Molina, M.E. Onorato, Phytochemistry 25 (1986) 2171–2173.
[24] J.S. Yadav, S.V. Mysorekar, Synth. Commun. 19 (1989) 1057–1060.
[25] S.V. Ley, J. Norman, W.P. Griffith, S.P. Marsden, Synthesis, 1994, pp. 639–666, and references therein.