Tetrahedron Vol. 49, No 5, pp. 1091-1102, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00
Pergamon Press Ltd

Chemical-Microbiological Synthesis of 6ß-Eudesmanolides from 11-Hydroxyl Derivatives obtained by *Rhizopus nigricans* cultures: Synthesis of 6ß-Dendroserins.

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(*Received in UK 27 July* 1992)

Key Words: Biotransformation; 6ß-eudesmanolides; 11-hydroxyeudesmanes; *Rhizopus nigricans*

Abstract: The relationship between the structure of the substrates and the action of the fungus *Rhizopus nigricans* has been studied in order to obtain, in the highest possible yield, 11-hydroxyl derivatives which were dehydrated to the "exo" position, hydroborated with 9-BBN and oxidized with $RuH_2(Ph_3P)_4$ to give 11-R and 11-S-6-*epi*-dendroserin. In the course of the biotransformation processes, 8α-hydroxyl and 7α-hydroxyl derivatives were also obtained, as well as the oxidation of the hydroxyl groups at C-1, an unusual process with *Rhizopus nigricans*. We have proved that the eudesmanones are more easily hydroxylated at C-11 than the hydroxyeudesmane compounds.

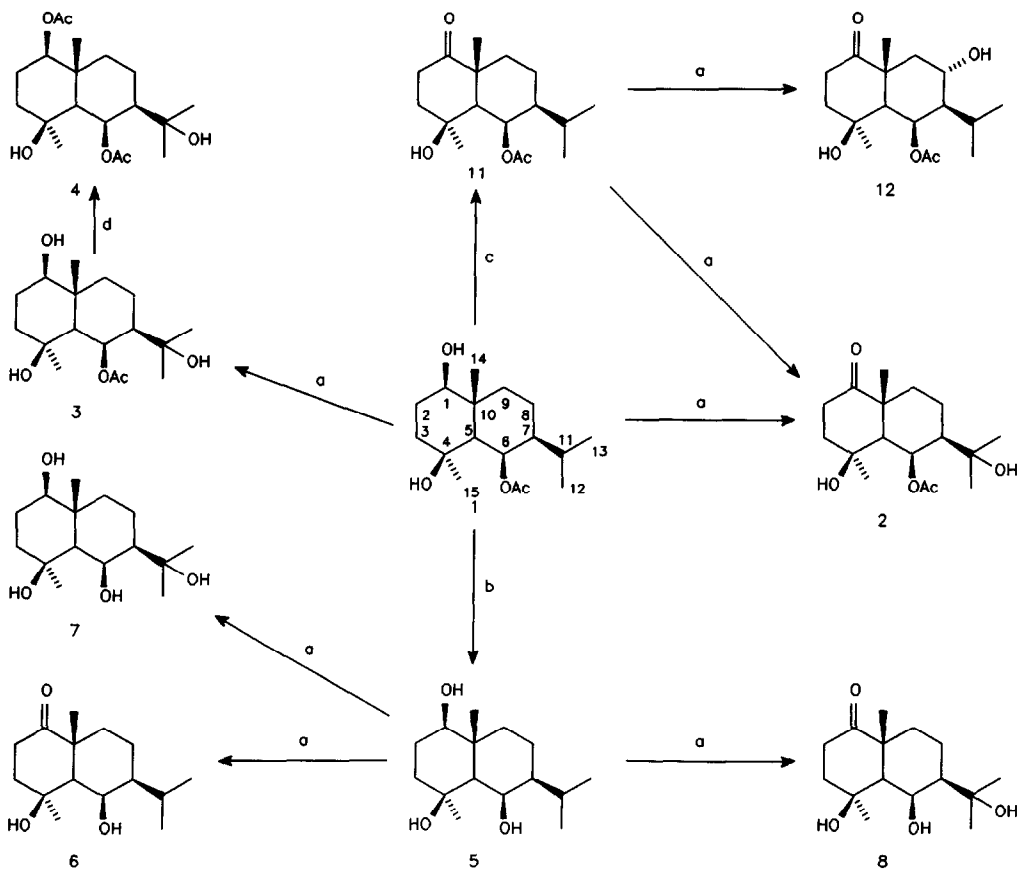
INTRODUCTION

Two strategies can be used to obtain sesquiterpene lactones: transformations in the rest of the molecule of a sesquiterpene lactone, or an approach involving synthesis pathways to obtain the lactone group from a non-lactone sesquiterpene compound. To obtain sesquiterpene-6,12-lactones it is necessary to have some kind of functional group at C-6 and C-12, or in some adjacent position, which makes it possible to obtain a hydroxyl group at C-6 and a carboxyl group at C-12.

Biogenetically speaking, it seems to be accepted that the carboxyl group at C-12 arises from a hydroxyl group at C-12, via an epoxide intermediate or an enzymatically controlled reaction of singlet oxygen to give an allylic hydroperoxide, which evolves to an aldehyde and then to a carboxylic acid[1-3]. The hydroxylation at C-12 of eudesmane compounds has been achieved by us[4,5], and this hydroxyl group has then been oxidized with $RuH_2(Ph_3P)_4$ to give, in this case, 6ß-eudesmanolides. Although the results in these studies were of interest, the yields were limited. Hence, we have also obtained 6ß-eudesmanolides by functionalization at C-11 with the fungus *Rhizopus nigricans*[6]. This kind of product was then dehydrated and subsequently hydroborated to obtain 12-hydroxyl derivatives, which were then chemically transformed to the corresponding 6ß,12-lactones, as mentioned above.

6ß-lactone compounds are scarce in the nature. Nevertheless, they are of interest because of their role as possible intermediates in some biogenetical pathways[7]. We have also communicated a method to epimerize 6α-sesquiterpene lactones[8], more abundant in nature, at C-6, and thus to obtain the corresponding 6ß-sesquiterpene lactones. We now report a more extensive study of the relationship between the structure of the sesquiterpene compounds and the action of the fungus *Rhizopus nigricans* to obtain 11-hydroxyl derivatives in high yield, which were then chemically transformed to the eudesman-6ß,12-olide compounds.

RESULTS AND DISCUSSION

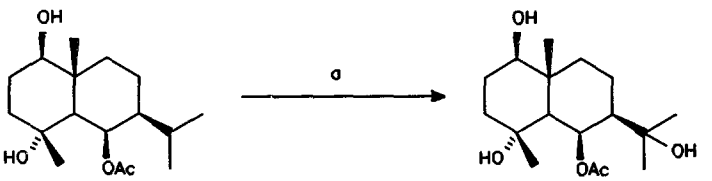


SCHEME 1: a: *Rhizopus nigricans*; b: KOH/MeOH/H_2O; c: CrO_3/H_2SO_4; d: Ac_2O/Py

The incubation of 6ß-acetoxy-1ß,4ß-dihydroxyeudesmane (**1**)[9] with *Rhizopus nigricans* for 7 days yielded a mixture of metabolites (57%) from which metabolite **2** (70% of the mixture) was isolated. A more polar (TLC) metabolite (**3**, 30% of the mixture) and substrate (**1**, 21%) were also isolated. The spectroscopic

behaviour of metabolite **2** indicated that the original hydroxyl group at C-1 was oxidized by *Rhizopus nigricans*. The presence of this keto group at C-1 produced considerable deshielding of the axial proton at C-2 (see Experimental). Moreover, a new hydroxyl group seemed to be located at C-11, because the doublet signals of the methyl groups at C-11 in the substrate (**1**) had become singlet signals in this metabolite (**2**). Hydroxylation was confirmed by comparison of the ^{13}C-NMR spectra of these compounds (see Table I). The acetylation of metabolite **3** yielded the diacetate **4**, which conserved the original hydroxyl group (now acetylated) at C-1, and presented a hydroxyl group at C-11. Thus, *Rhizopus nigricans* hydroxylated substrate **1** with an overall yield of 57%.

Deacetylation of substrate **1** gave product **5**[9], which was also incubated with *R. nigricans* cultures for 7 days, after which a large amount of substrate **5** (62%) was recovered unaltered. In this case, oxidation at C-1 and hydroxylation at C-11 was also noted. Thus, metabolite **6** (5%) was the result of oxidation at C-1, metabolite **7** (14%) was the result of hydroxylation at C-11 and metabolite **8** (5%) was the result of both processes: oxidation at C-1 and hydroxylation at C-11. The trihydroxylated substrate (**5**) gave worse results than the 6-acetoxy compound (**1**), although the biotransformation processes were parallel. The C-4 epimer compound of substrate **1** (product **9**)[10] likewise failed to give good results. Thus, after 7 days of incubation with *R. nigricans*, the 11-hydroxyl derivative **10** (18%) was obtained, but a large amount (59%) of substrate **9** was also recovered unaltered.



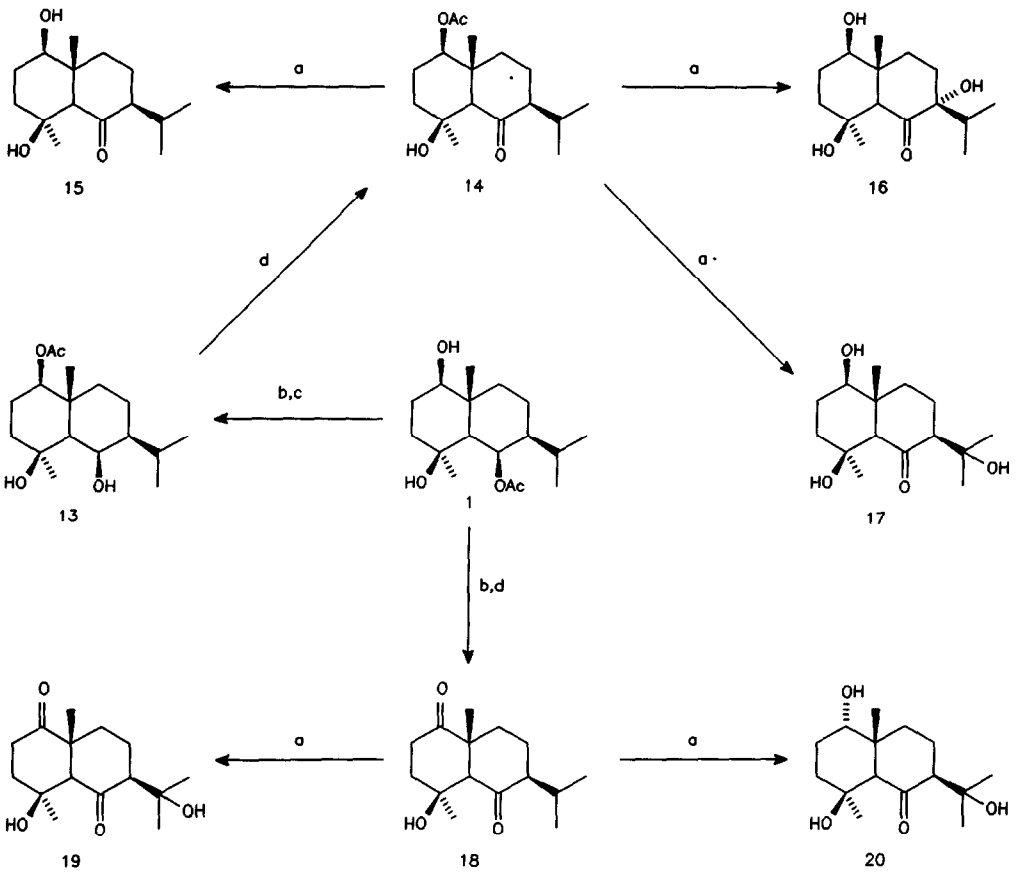
9 10

SCHEME 2: a: *Rhizopus nigricans*

At this moment, the metabolite obtained at the highest yield was **2** (40%), which, besides the hydroxyl group at C-11, also had a keto group at C-1. To increase the yield of hydroxylation at C-11, we incubated substrate **11**[9], which has a keto group at C-1, with *R. nigricans*. In this case, almost all of substrate **11** was metabolized after 3 days (only a small amount (6%) was recovered), and a considerable amount of metabolite **2** (72%) was isolated. Another metabolite (**12**, 15%) was also isolated. Although this metabolite (**12**) was not hydroxylated at C-11, it showed an interesting hydroxylation at C-8, which could be used to attempt the synthesis of 8,12-lactones. The position and configuration of the carbon with the new hydroxylation were deduced from the ^{13}C-NMR chemical shifts of metabolite **12** and the coupling constants of H-8 in the ^{1}H-NMR spectrum. The signals of this proton (δ 4.18, ddd, $J_1 = J_2 = 11.1$, $J_3 = 4.5$ Hz) were according to those calculated for this configuration.

Substrate **11**, which has a keto group at C-1, was more efficiently biotransformed than substrates **1** and **5**. Hence, we saponified substrate **1**, and obtained the trihydroxyl derivative **5**, which was acetylated at C-1 (product **13**)[9] and then oxidized to give the 6-keto derivative **14**[5]. Incubation of this product (**14**) with *R. nigricans* for 3 days produced only a limited quantity of unaltered substrate **14** (17%). Metabolites **15** (55%), **16** (5%) and **17** (14%) were also isolated. Metabolite **15** was the result of the deacetylation of substrate **14**.

We also observed that the other metabolites (**16** and **17**) were also deacetylated at C-1 and hydroxylated at C-7 (metabolite **16**) and at C-11 (metabolite **17**) respectively. As the first action of *R. nigricans* was the deacetylation at C-1, the controls done during this biotransformation indicated that after three days, the substrate was almost entirely metabolized. However, a longer process would probably give greater yields of the trihydroxylated metabolites.

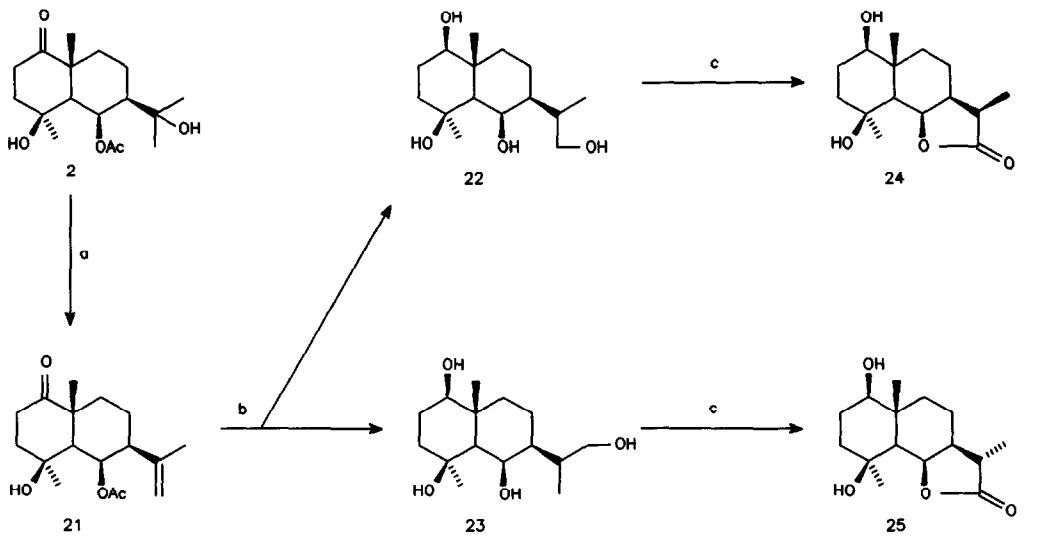


SCHEME 3: a: *Rhizopus nigricans*; b: KOH/MeOH/H_2O; c: Ac_2O/Py; d: CrO_3/H_2SO_4

We also incubated the 1,6-diketo derivative **18**[5], obtained by oxidation of **5**. This diketo derivative (**18**) was very efficiently biotransformed by *R. nigricans* for 4 days, after which only metabolites with hydroxylation at C-11 were isolated. Thus the 11-hydroxyl derivative (**19**) of substrate **18** was isolated as the minor product (32%). The main metabolite **20** (53%), besides the hydroxyl group at C-11, also has another hydroxyl group at a secondary carbon and conserves one of the two keto groups initially present in the substrate (**18**). The keto group reduced by *R. nigricans* was situated at C-1, giving an S-alcohol, as is usual in enzymatic reductions[11].

Although both metabolites can be easily separated by a chromatographic process, it is possible to oxidize both metabolites to obtain only one product (**19**) at a high yield (85 %).

To transform 11-hydroxyl derivatives into 12-hydroxyl derivatives, we used metabolite **2**, although it was available at a lower yield (72 %) than metabolite **19** (theorical yield 85 %), because the process of dehydration to produce the *exo*-isomer with 4-dimethylaminopyridine and mesyl chloride (DMAP/MsCl)[12] was favoured. With metabolite **19**, we needed to reduce the keto group and to protect the resulting hydroxyl group before dehydration. In the case of metabolite **2**, direct dehydration with DMAP/MsCl yielded product **21** (73 %) and the product of dehydration towards C-7 was not detected.

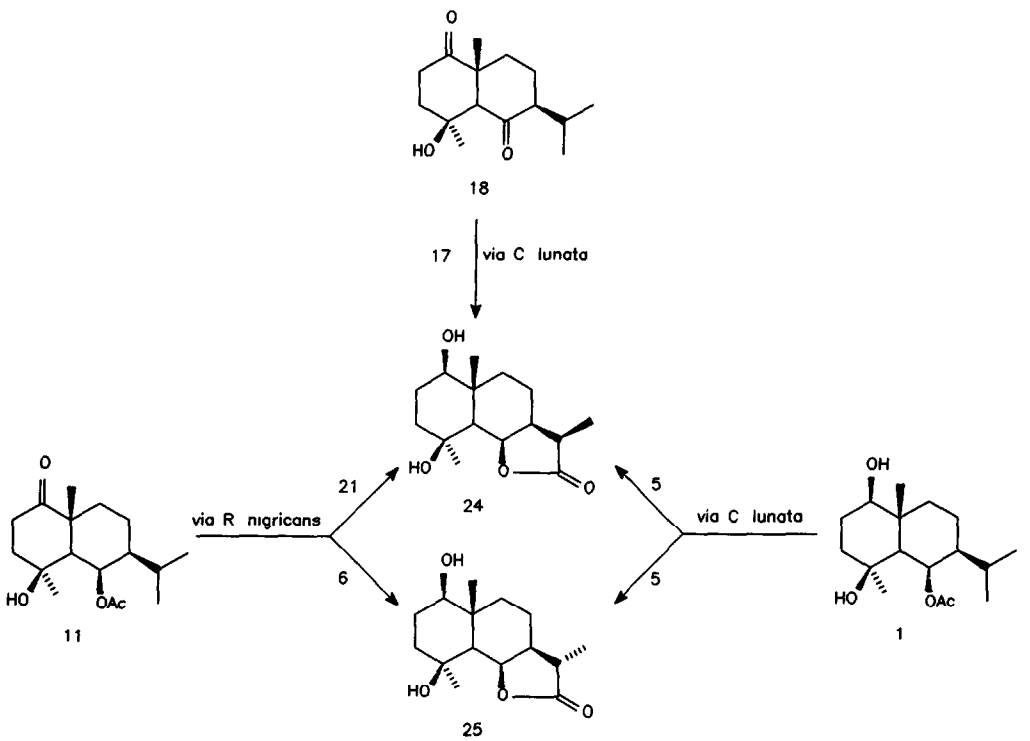


SCHEME 4: a: DMAP/MsCl; b: 9-BBN/THF; c: $RuH_2(Ph_3P)_4$

Product **21** was efficiently hydroborated with 9-borabicyclo[3.3.1]nonane (9-BBN)[13]. This reagent was chosen among several boranes because in terminal olefins, the inductive effect of an α-methyl substituent reportedly increases the electron availability in the double bond[14], and 9-BBN is very sensitive to this electron availability. As 9-BBN does not present marked steric hindrance, this electron contribution is decisive. On the other hand, disiamylborane (Sia_2BH) produces much greater steric congestion. Thus, 9-BBN reacts 40 times faster than Sia_2BH with terminal α-methyl olefins[14]. However, it is sufficiently voluminous to reduce the keto group at C-1 only on the α-face.

The hydroboration of product **21** gave the tetrahydroxyl derivatives **22** (65 %) and **23** (18 %), products described by us in a previous paper[6]. Compound **22**, the result of hydroboration on the Re face of C-11 in product **21**, was the main product obtained. We attempted to calculate the energies of the transition states in the hydroboration process, however, our calculations were not according to the experimental results. Nevertheless, this procedure allowed us to obtain both configurations at C-11, although the 11-R configuration predominated.

Product **22** was oxidized with $RuH_2(Ph_3P)_4$[15] to obtain the 6ß-eudesmanolide **24** (62%, 11-R-6-*epi*-dendroserin)[16]; in a similar way, product **23** was oxidized to give the 6ß-eudesmanolide **25** (61%, 11-S-6-*epi*-dendroserin)[16]. Bearing in mind overall yields, we observed that via hydroxylation at C-12 by *Curvularia lunata*[5] (and the corresponding oxidations), 5% and 17% of the 11R-eudesmanolide **24** from products **1** and **18**, and 5% the 11S-eudesmanolide **25** from product **1** were obtained. However, through hydroxylation at C-11 by *R. nigricans*, 21% of the 11R-eudesmanolide **24** and 6% of the 11S-eudesmanolide from product **11** were obtained.



SCHEME 5: Comparative results with the fungus *Rhizopus nigricans* (11-hydroxyl derivatives) and *Curvularia lunata* (12-hydroxyl derivatives).

Therefore we conclude 6ß,12-eudesmanolides are more efficiently obtained through biotransformations with *R. nigricans* to produce 11-hydroxyl derivatives, than by direct hydroxylation at C-12 with *C. lunata*. This procedure constitutes a suitable method for obtaining 6ß,12-sesquiterpenolides from non-lactone sesquiterpenes hydroxylated at C-6.

TABLE I

	2	6	7	8	10	12
C- 1	215.45	216.32	79.91	216.31	80.28	214.40
C- 2	34.33[a]	34.53	27.05	34.29[a]	28.57	33.99
C- 3	42.31	41.04	39.71[a]	40.97	41.49[a]	42.41
C- 4	71.69	72.75	73.29	72.73	71.14	71.40
C- 5	53.87	52.95	52.05	52.78	55.45	53.79
C- 6	70.61	70.32	69.23	69.29	69.34	72.23
C- 7	52.53	49.18	49.49	49.05	52.91	53.74
C- 8	19.02	20.46	16.86	16.78	18.96	66.29
C- 9	34.48[a]	34.53	39.09[a]	34.58[a]	41.72[a]	44.36
C-10	48.28	47.63	38.52	47.52	39.53	49.15
C-11	72.02	29.57	73.72	74.12	72.07	27.40
C-12	29.10[b]	21.92[a]	28.98[b]	29.26[b]	29.13[b]	22.16[a]
C-13	27.11[b]	21.07[a]	28.83[b]	28.85[b]	27.30[b]	20.10[a]
C-14	21.33	20.78	14.51	21.85	14.78	20.03
C-15	29.34	28.75	30.07	29.49	24.63	29.34
MeCOO	22.11				22.39	22.09
MeCOO	172.15				172.97	171.30

TABLE II

	16	17	19	20	21
C- 1	78.76	78.44	213.08	73.33	215.41
C- 2	26.83	26.59[a]	32.73[a]	25.25	34.26[a]
C- 3	37.82	37.71	39.48	32.47	41.61
C- 4	69.95	69.73	69.54	70.12	71.75
C- 5	56.50	62.81	63.54	57.71	53.24
C- 6	216.00	219.41	217.23	221.24	70.32
C- 7	80.34	60.34	60.04	60.26	48.89
C- 8	33.17[a]	26.26[a]	24.86	25.71	21.37
C- 9	33.61[a]	37.71	33.68[a]	34.70	34.39[a]
C-10	46.79	47.03	53.74	46.24	48.15
C-11	31.46	71.18	70.97	71.14	145.44
C-12	16.99[b]	28.61[b]	28.50[b]	28.71[a]	111.58
C-13	16.67[b]	25.95[b]	26.02[b]	26.11[a]	22.97
C-14	12.43	12.92	19.53	19.38	21.22
C-15	29.83	29.93	29.16	30.27	29.27
MeCOO					21.51
MeCOO					170.61

[a] or [b] (These values could be interchanged)

EXPERIMENTAL

Measurements of NMR spectra (300 MHz ^1H and 75.47 MHz ^{13}C) were made in $CDCl_3$ (which also provided the lock signal) in a Bruker AM-300 spectrometer equipped with a process controller and an array processor. 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 COSY (45°), NOESY, CONOESY (90°), C/H correlation and J-RESOLVED. Ir spectra were recorded on a Nicolet 20SX FT-IR spectrometer. Mass spectra were determined with CI (methane) or EI (70 eV) in a Hewlett-Packard 5988A spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20°. Silica gel SDS 60 A CC (40-60 μm) was used for flash chromatography. CH_2Cl_2 containing increasing amounts of Me_2CO was used as the eluent. Analytical plates (silica gel, Merck 60 G) were rendered visible by spraying with H_2SO_4-AcOH, followed by heating to 120°. The identity of compounds **1**, **4**, **5**, **9**, **11**, **13**, **14**, **15**, **18**, **22**, **23**, **24** and **25** were confirmed by direct comparison with authentic samples (IR, MS, NMR, etc.).

***Isolation of 6β-acetoxy-1β,4β-dihydroxyeudesmane* (1)**

6β-acetoxy-1β,4β-dihydroxyeudesmane (**1**) was isolated from *Sideritis varoi* subsp. *cuatrecasasii*[9].

***Saponification of eudesmane* 1**

6β-acetoxy-1β,4β-dihydroxyeudesmane (**1**) (1.25 g) was dissolved in MeOH/H_2O (70%) (80 ml) containing KOH (5%) (4 g) and refluxed for 1 h. The reaction mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated in vacuum. Chromatography on a silica gel column yielded 1β,4β,6β-trihydroxyeudesmane (**5**)[9] (953 mg, 89%).

***Isolation of 6β-acetoxy-1β,4α-dihydroxyeudesmane* (9)**

6β-acetoxy-1β,4α-dihydroxyeudesmane (**9**) was isolated from *Sideritis varoi* subsp. *oriensis*[10].

***Oxidation of eudesmane* 1**

Jones reagent was added dropwise to a stirred solution of 6β-acetoxy-1β,4β-dihydroxyeudesmane (**1**) (185 mg) in acetone at 0° until an orange-brown colour persisted. Methanol was then added and the reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 6β-acetoxy-4β-hydroxyeudesman-1-one (**11**)[9] (165 mg, 90%).

***Acetylation of 1β,4β,6β-trihydroxyeudesmane* (5)**

1β,4β,6β-trihydroxyeudesmane (**5**) (410 mg) was dissolved in Ac_2O/Py (1:2) (30 ml) and kept at 0° for 2 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with saturated SO_4HK aqueous and dried with anhydrous Na_2SO_4. Chromatography over silica gel yielded 1β-acetoxy-4β,6β-dihydroxyeudesmane (**13**)[9] (420 mg, 88%).

***Oxidation of 1β-acetoxy-4β,6β-dihydroxyeudesmane* (13)**

1β-acetoxy-4β,6β-dihydroxyeudesmane (**13**) (420 mg) was dissolved in acetone (10 ml) and oxidized with Jones reagent for 30 min at room temperature. After chromatography 1β-acetoxy-4β-hydroxyeudesman-6-one (**14**)[5] (379 mg, 91%) was isolated.

***Oxidation of 1β,4β,6β-trihydroxyeudesmane* (5)**

1β,4β,6β-trihydroxyeudesmane (**5**) (350 mg) was also oxidized with Jones reagent under the same conditions as for product **13**. Chromatography on a silica gel column yielded 4β-hydroxyeudesmane-1,6-dione (**18**)[5] (313 mg, 90%).

Organism, media and culture conditions

Rhizopus nigricans CECT 2072 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 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 *R. nigricans*. The cultures were incubated by shaking (150 rpm) at 28° for 6 days, after which substrates **1**, **5**, **9**, **11**, **14** and **18** in EtOH were added.

***Biotransformation of substrate* 1**

Substrate **1** (120 mg) was dissolved in EtOH (3 ml), distributed among 3 Erlenmeyer-flask cultures and incubated for 7 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° in vacuum to give a mixture of compounds (96 mg). This mixture was chromatographed on a silica gel column to obtain 25 mg of starting material **1**, 50 mg (40%) of 6ß-acetoxy-4ß,11-dihydroxyeudesman-1-one (**2**); syrup; $[\alpha]_D$= +47° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3464, 1734, 1707 and 1258 cm^{-1}; ^1H nmr (δ) 5.86 (1H, bs, H-6), 3.08 (1H, ddd, $J_{2ax,2eq}$= $J_{2ax,3ax}$= 14.1, $J_{2ax,3eq}$= 6.1 Hz, H-2ß), 2.06 (3H, s, AcO group), 1.53 (3H, s, 3H-14), 1.40 (3H, s, 3H-15), 1.33 (1H, d, $J_{5,6}$= 1.1 Hz, H-5) and 1.18 and 1.12 (3H each, s, 3H-12 and 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 313 (67), 295 (100), 253 (12), 235 (56), 217 (20); and 21 mg (17%) of a polar metabolite (**3**), which was dissolved in Ac_2O/Py (1:2) (3 ml) and refluxed for 2 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with saturated SO_4HK aqueous and dried with anhydrous $NaSO_4$. Chromatography on a silica gel column yielded 21 mg (88%) of 1β,6β-diacetoxy-4β,11-dihydroxyeudesmane (**4**)[5].

***Biotransformation of substrate* 5**

Substrate **5** (172 mg) was dissolved in EtOH (4 ml), distributed among 4 Erlenmeyer flask cultures and incubated for 7 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**. The resulting mixture (149 mg) was chromatographed on a silica gel column to obtain 106 mg of starting material **5**, 8 mg (5%) of 4β,6β-dihydroxyeudesman-1-one (**6**); syrup; $[\alpha]_D$= +44° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3397 and 1701 cm^{-1}; ^1H nmr (δ) 4.55 (1H, m, $w_{½}$= 6 Hz, H-6), 3.15 (1H, ddd, $J_{2ax,2eq}$= $J_{2ax,3ax}$= 14.1, $J_{2ax,3eq}$= 6.1 Hz, H-2ß), 1.58 and 1.36 (3H each, s, 3H-14 and 3H-15), 1.25 (1H, d, $J_{5,6}$= 1.8 Hz, H-5) and 0.98 and 0.96 (3H each, d, J= 6.7 Hz, 3H-12 and 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 255 (65), 237 (100), 219 (51); 26 mg (14%) of 1β,4ß,6β,11-tetrahydroxyeudesmane (**7**); syrup; $[\alpha]_D$= +6° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3343 cm^{-1}; ^1H nmr (δ) 4.77 (1H, dd, $J_{6,7}$= $J_{6,5}$= 2.7 Hz, H-6), 3.16 (1H, dd, $J_{1,2ax}$= 11.7, $J_{1,2eq}$= 4.0 Hz, H-1) and 1.40, 1.34, 1.31 and 1.25 (3H each, s, 3H-12, 3H-13, 3H-14 and 3H-15); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 273 (4), 255 (17), 237 (100), 219 (48); and 9 mg (5%) of 4ß,6ß,11-trihydroxyeudesman-1-one (**8**); syrup; $[\alpha]_D$= +34° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3384 and 1705 cm^{-1}; ^1H nmr (δ) 4.81 (1H, dd, $J_{6,7}$= $J_{6,5}$= 2.0 Hz, H-6), 3.16 (1H, ddd, $J_{2ax,2eq}$= $J_{2ax,3ax}$= 14.0, $J_{2ax,3eq}$= 6.1 Hz, H-2ß), 1.63 and 1.41 (3H each, s, 3H-14 and 3H-15) and 1.38 and 1.27

(3H each, s, 3H-12 and 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 271 (81), 253 (31), 235 (100), 217 (13).

***Biotransformation of substrate* 9**

Substrate **9** (120 mg) was dissolved in EtOH (3 ml), distributed among 3 Erlenmeyer flask cultures, and incubated for 7 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**. Chromatography on a silica gel column yielded 6ß-acetoxy-1ß,4α,11-trihydroxyeudesmane (**10**) (23 mg, 18%); syrup; $[\alpha]_D = +2°$ ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3419, 1714 and 1261 cm^{-1}; ^1H nmr (δ) 5.92 (1H, bs, H-6), 3.26 (1H, dd, $J_{1,2ax} = 10.6$, $J_{1,2eq} = 4.2$ Hz, H-1), 1.18 (6H) and 1.13 and 1.12 (3H each) (s, 3H-12, 3H-13, 3H-14 and 3H-15); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 315 (1), 297 (100), 279 (27), 237 (74), 219 (46), 201 (7).

***Biotransformation of substrate* 11**

Substrate **11** (161 mg) was dissolved in EtOH (4 ml), distributed among 4 Erlenmeyer flask cultures and incubated for 3 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**. The resulting mixture (158 mg) was chromatographed on a silica gel column to yield 10 mg of starting material **11**, 26 mg (15%) of 6ß-acetoxy-4ß,8α-dihydroxyeudesman-1-one (**12**); syrup; $[\alpha]_D = +69°$ ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3464, 1736, 1711 and 1252 cm^{-1}; ^1H nmr (δ) 5.78 (1H, dd, $J_{6,7} = J_{6,5} = 2.0$ Hz, H-6), 4.18 (1H, ddd, $J_{8ax,7} = J_{8ax,9ax} = 11.1$, $J_{8ax,9eq} = 4.5$ Hz, H-8ß), 3.09 (1H, ddd, $J_{2ax,2eq} = J_{2ax,3ax} = 14.1$, $J_{2ax,3eq} = 6.1$ Hz, H-2ß), 2.03 (3H, s, AcO group), 1.56 and 1.47 (3H each, s, 3H-14 and 3H-15), 1.37 (1H, d, $J_{5,6} = 2.0$ Hz, H-5) and 1.03 and 0.95 (3H each, d, J= 7.1 Hz, 3H-12 and 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 313 (39), 295 (100), 253 (14), 235 (67), 217 (33); and 122 mg (72%) of metabolite **2**, which was also isolated in the biotransformation of substrate **1**.

***Biotransformation of substrate* 14**

Substrate **14** (375 mg) was dissolved in EtOH (7 ml, 7 Erlenmeyer flask cultures) and incubated for 3 days, after which the cultures were thoroughly processed as indicated above for the biotransformation of substrate **1**. This yielded a mixture (312 mg) which was chomatographed over silica gel to yield 65 mg of starting material **14**, 180 mg (55%) of 1β,4β-dihydroxyeudesman-6-one (**15**)[5]; 18 mg (5%) of 1β,4β,7α-trihydroxyeudesman-6-one (**16**); syrup; $[\alpha]_D = +78°$ ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3442 and 1696 cm^{-1}; ^1H nmr (δ) 3.51 (1H, dd, $J_{1,2ax} = 11.6$, $J_{1,2eq} = 4.1$ Hz, H-1), 3.13 (1H, s, H-5), 1.15 and 0.96 (3H each, s, 3H-14 and 3H-15) and 0.93 and 0.92 (3H each, d, J= 6.8 Hz, 3H-12 and 3H-13); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 271 (7), 253 (59), 235 (100), 217 (8); and 49 mg (14%) of 1β,4β,11-dihydroxyeudesman-6-one (**17**); syrup; $[\alpha]_D = +24°$ ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3436 and 1689 cm^{-1}; ^1H nmr (δ) 3.39 (1H, dd, $J_{1,2ax} = 11.6$, $J_{1,2eq} = 4.1$ Hz, H-1), 2.44 (1H, ddd, $J_{7,8ax} = 12.7$, $J_{7,8eq} = 6.5$, $J_{7,5(W)} = 0.8$ Hz, H-7), 2.18 (1H, d, $J_{5,7(W)} = 0.8$ Hz, H-5), 1.22 and 1.19 (3H each, s, 3H-12 and 3H-13) and 1.16 and 0.96 (3H each, s, 3H-14 and 3H-15); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 271 (16), 253 (64), 235 (100).

***Biotransformation of substrate* 18**

Substrate **18** (311 mg) was dissolved in EtOH (6 ml, 6 Erlenmeyer flask cultures) and incubated for 4 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**. This gave a mixture (282 mg) which was chomatographed on a silica gel column to obtain 105 mg (32%) of 4β,11-dihydroxyeudesmane-1,6-dione (**19**); syrup; $[\alpha]_D = +61°$ ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3527 and 1700 cm^{-1}; ^1H nmr (δ) 3.03 (1H, ddd, $J_{2ax,2eq} = J_{2ax,3ax} = 14.2$, $J_{2ax,3eq} = 6.0$ Hz, H-2ß), 2.55 (1H, s, H-5), 2.39

(1H, dd, $J_{7,8ax}$= 12.2, $J_{7,8eq}$= 7.3 Hz, H-7), 1.24 and 1.23 (3H each, s, 3H-14 and 3H-15) and 1.21 and 1.18 (3H each, s, 3H-12 and 3H-13); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 269 (49), 251 (100), 233 (63); and 177 mg (53%) of 1α,4β,11-trihydroxyeudesman-6-one (**20**); syrup; $[\alpha]_D$= +50° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3443 and 1684 cm^{-1}; ^{1}H nmr (δ) 3.51 (1H, dd, $J_{1,2ax}$= $J_{1,2eq}$= 2.8 Hz, H-1), 2.64 (1H, bs, H-5), 2.41 (1H, ddd, $J_{7,8ax}$= 12.7, $J_{7,8eq}$= 6.3, $J_{7,5(W)}$= 1.1 Hz, H-7), 1.23 and 1.18 (3H each, s, 3H-12 and 3H-13), 1.19 (3H, s, 3H-15) and 1.00 (3H, s, 3H-14); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 271 (38), 253 (90), 235 (100), 217 (23).

***Dehydration of product* 2**

To a stirred solution of 6ß-acetoxy-4ß,11-dihydroxyeudesman-1-one (**2**, 100 mg) in CH_2Cl_2 (3 ml) we added Et_3N (0.2 ml) and DMAP[12] (5 mg). The mixture was cooled to 0°, and methanesulfonyl chloride (0.1 ml) was added dropwise. The mixture was stirred for 2 h at room temperature, then crushed ice was added and the mixture stirred for 1 h, after which it was extracted with CH_2Cl_2 (3x10 ml). The organic extracts were combined, washed with water, dried with anhydrous Na_2SO_4 and concentrated in vacuum. Chromatography of the residue over silica gel yielded 69 mg (73%) of 6ß-acetoxy-4ß-hydroxyeudesm-11-en-1-one (**21**); syrup; $[\alpha]_D$= +47° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3481, 1735, 1711 and 1248 cm^{-1}; ^{1}H nmr (δ) 5.68 (1H, bs, H-6), 4.83 and 4.72 (1H each, s, 2H-12), 3.11 (1H, ddd, $J_{2ax,2eq}$= $J_{2ax,3ax}$= 14.1, $J_{2ax,3eq}$= 6.2 Hz, H-2ß), 2.02 (3H, s, AcO group), 1.75 (3H, bs, 3H-13), 1.55 (3H, s, 3H-14) and 1.40 (3H, s, 3H-15); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 295 (100), 277 (51), 235 (34), 217 (59).

***Hydroboration of product* 21**

60 mg of 6ß-acetoxy-4ß-hydroxyeudesm-11-en-1-one (**21**) was dissolved in dried THF. To this solution, 1 ml of 9-BBN[13] in THF (0.5 M) was added. The mixture was stirred for 2 h at room temperature under argon atmosphere, then ethanol (0.6 ml), a 6 N solution of NaOH (0.2 ml) and H_2O_2 (30%) (0.4 ml) were added, and the mixture was heated for 1 h at 50°. Then the mixture was extracted with CH_2Cl_2, dried with anhydrous Na_2SO_4 and concentrated in vacuum. The residue was chromatographed over silica gel yielding 36 mg (65%) of 1β,4β,6β,12-tetrahydroxyeudesm-11(R)-ane (**22**)[5] and 10 mg (18%) of 1β,4β,6β,12-tetrahydroxyeudesm-11(S)-ane (**23**)[5].

***Lactonization of product* 22**

1β,4β,6β,12-tetrahydroxyeudesm-11(R)-ane (**22**) (36 mg, 0.13 mmol) was dissolved in toluene (0.5 ml) and acetone was added (0.02 ml, 0.3 mmol). $RuH_2(PPh_3)_4$[15] (2.2 mg, 2x10^{-3} mmol) was added to the solution, and the mixture was kept in a closed tube at 180° under argon atmosphere for 6 h. The reaction mixture was cooled before the tube was opened, and then concentrated in vacuum. Chromatography on a silica gel column yielded 1β,4β-dihydroxy-5α,7α,11α-H-eudesman-6ß,12-olide (**24**)[5] (22 mg, 62%).

***Lactonization of product* 23**

1β,4β,6β,12-tetrahydroxyeudesm-11(S)-ane (**23**) (10 mg, 0.04 mmol) was dissolved in toluene (0.5 ml), and acetone (0.02 ml, 0.3 mmol) and $RuH_2(PPh_3)_4$[15] (2.2 mg, 2x10^{-3} mmol) were added under the same working conditions as for product **22**. Chromatography over silica gel yielded 1β,4β-dihydroxy-5α,7α,11β-H-eudesman-6ß,12-olide (**25**)[5] (6 mg, 61%).

ACKNOWLEDGEMENTS

This work was supported by a grant from the Dirección General de Investigación Científica y Técnica. We thank Karen Shashok for her assistance in the translation of the text.

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