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CHEMICAL-MICROBIOLOGICAL SYNTHESIS OF 6β-EUDESMANOLIDES BY *Curvularia lunata* CULTURES FROM EUDESMANES WITH FUNCTIONS AT C-1 AND C-6.

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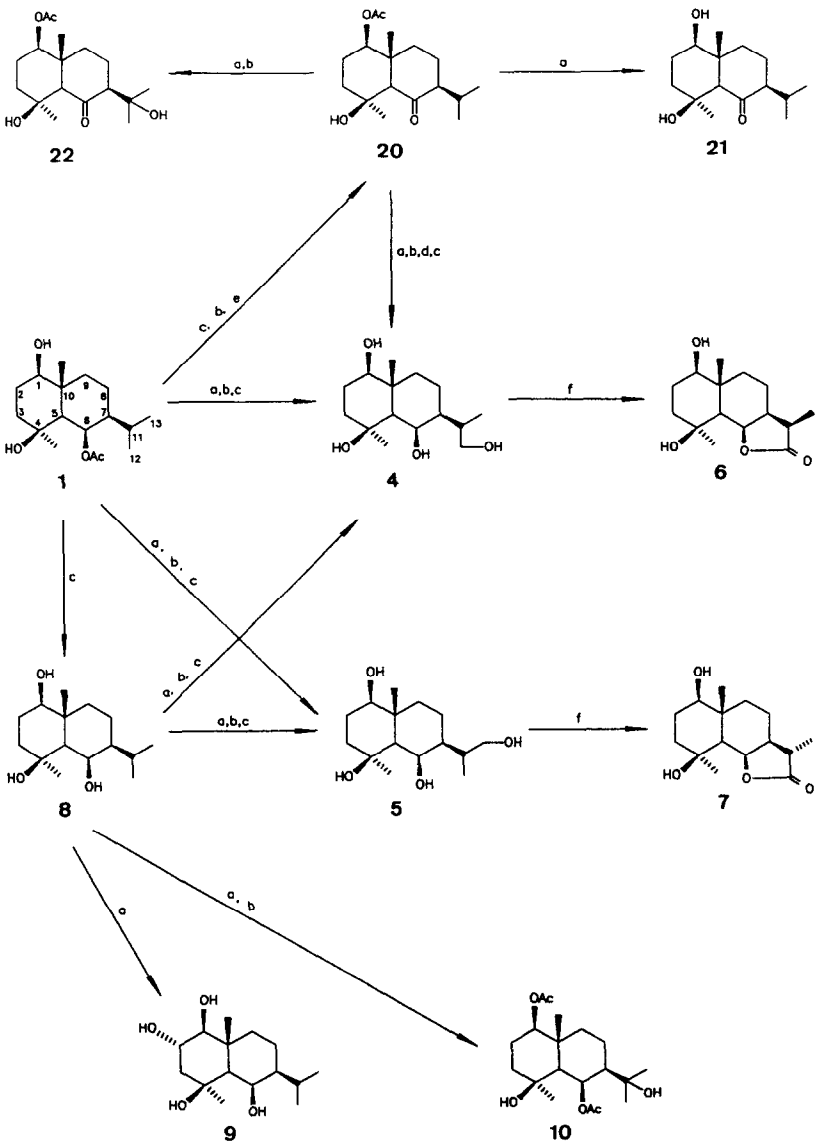
SUMMARY.- The biotransformation of several 1,6-difunctionalized eudesmanes was carried out with *Curvularia lunata* cultures in order to obtain 11-R and 11-S 12-hydroxy derivatives, which were oxidized with $RuH_2(Ph_3P)_4$ to give 11-R and 11-S-6βeudesmanolides. The best results were achieved by biotransformation of the 1,6-diketo compound, which allowed us to obtain a considerable yield of 11-R-eudesmanolides.

INTRODUCTION

We have performed a series of biotransformations of diterpenes [1] and sesquiterpenes [2] including 6α-eudesmanolides [3] and 6ß-eudesmanolides [4]. Now, we attempt to obtain compounds with a hydroxyl group at C-12, as this hydroxylation allows us to obtain 11-R and 11-S-6ß,12-eudesmanolides [5]. Biogenetically speaking, it seems to be accepted that C-12 hydroxylation occurs initially in the formation of sesquiterpene lactones [6-8], *via* an epoxide intermediate or enzymatically controlled reaction of singlet oxygen to give allylic hydroperoxides, which evolves to carboxylic acid *via* aldehyde. Posterior enzymatically-mediated hydroxylation at C-6 or C-8 (and lactonization) give the respective sesquiterpenolides. In this paper, we present a chemical-microbiological alternative pathway with starting materials bearing functions at C-6, which are hydroxylated at C-12 by *Curvularia lunata*, and subsequently chemically transformed in a one-step process to 6,12-eudesmanolides. On incubating different substrates with different functions at C-1 and C-6, we also observed the influence of the configuration at C-4 in the kind and yield of the products thus obtained.

RESULTS AND DISCUSSION

The incubation of 6ß-acetoxy-1ß,4ß-dihydroxyeudesmane (**1**) [9] with *C. lunata* for 12 days gave a mixture of polar (TLC) metabolites (23%) which were acetylated and chromatographed to give triacetates **2** (1ß,6ß,12-triacetoxy-4ß-hydroxyeudesm-11(R)-ane, 51% of the polar mixture) and **3** (1ß,6ß,12-triacetoxy-4ß-hydroxyeudesm-11(S)-ane, 49%). The spectroscopic behavior of these products (see Table I and Experimental) indicated that hydroxylations at C-12 and C-13 (isopropyl moiety of substrate **1**) took place, these products (**2** and **3**) being epimers at C-11.



SCHEME I: a: Curvularia lunata; b: Ac_2O-Py; c: KOH alc.; d: H_4BNa; e: Jones reagent; f: $RuH_2(Ph_3P)_4$

We attempted to directly determine the configuration at C-11 of **2** and **3**. However, n.O.e.difference experiments failed to provide unequivocal data. Hence, triacetates **2** and **3** were saponified to give **4** and **5** respectively, which were then selectively oxidized with $RuH_2(Ph_3P)_4$ [10] to produce the lactones **6**

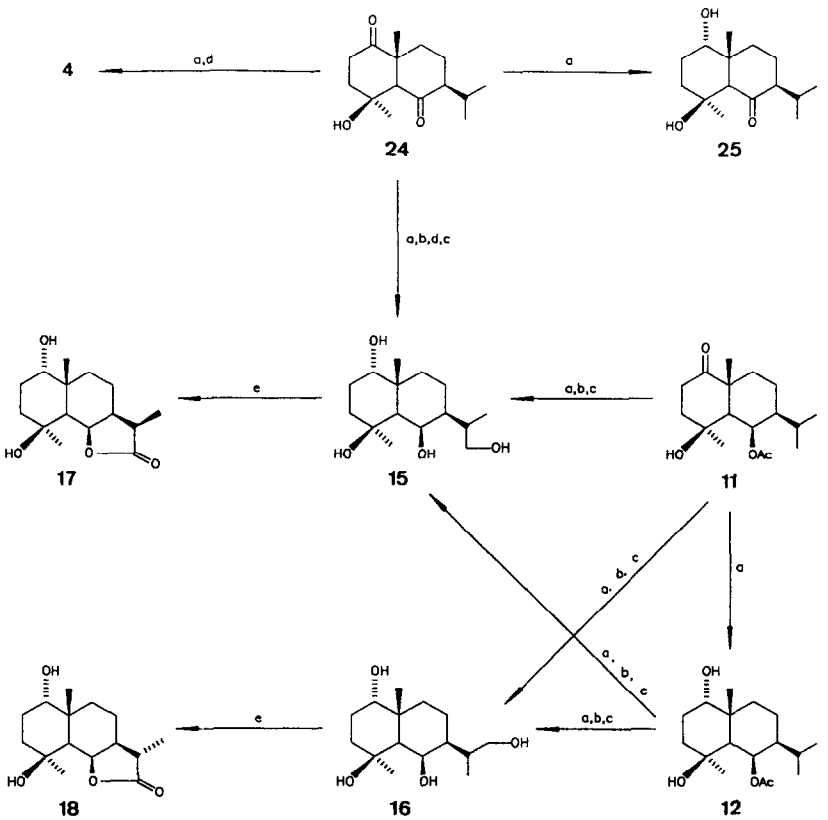
(43%) and **7** (41%) respectively. The structures and configurations at C-11 of **6** and **7** were deduced from mono and bidimensional NMR experiments. Thus, the proton signal of H-11 of lactone **6** (δ 2.77, dq, $J_{7,11} = J_{11,13} = 7.1$ Hz) indicated a dihedral angle H-7/H-11 of approx. 30°. However, no coupling was observed between H-7 and H-11, which indicated that the dihedral angle was nearly 90° for these protons. 13C NMR data of **6** and **7** (see Table I) were also in accordance with the C-11 configurations noted above. Thus, a great γ-effect on C-8 (and *vice versa* on C-13) was observed in the case of **6** (11-R configuration). NOESY experiments also confirmed these configurations, showing a dipolar correlation between H-6 and H-11 for the lactone **6** and H-6/H-13 and H-7/H-13 for the lactone **7**. These data allowed us to assign the configuration at C-11 the triacetates to **2** (11-R) and **3** (11-S).

Although this is a valid chemical-microbiological pathway for obtaining 6ß,12-eudesmanolides, the yield is limited (approx. 3% for each lactones). Hence, we have incubated another substrates to attempt higher yields.

Saponification of **1** gave **8** (90%, [9]) which was incubated for 12 days with *C. lunata*. This incubation gave worse results than those described above for incubation of **1**. Unaltered substrate **8** was recovered (79%) and a small quantity (2%) of a polar metabolite (**9**) was isolated as well as a mixture from which, after acetylation, three products were isolated (**10**, 55%, **2**, 24% and **3**, 21% of the mixture). Metabolite **9** showed a new hydroxylation at C-2 (see Table I and Experimental). Irradiation in the signal of the geminal proton of the new hydroxyl group, (δ 4.00, ddd, $J_{2,3ax} = 11.2$, $J_{2,1} = 9.2$, $J_{2,3eq} = 5.1$ Hz) transformed the new signal of H-1 (δ 2.94, d, $J_{1,2} = 9.2$ Hz) into a singlet signal. The coupling constants noted above clearly indicated that the new hydroxyl group at C-2 was α (equatorial). The incubation of this new substrate **8** produced a limited yield of hydroxylations at C-12. Elsewere, a new product (**10**) was isolated after the acetylation described above. The spectroscopic data from **10** indicated that a new hydroxyl group at C-11 was introduced by *C. lunata* on substrate **8**.

Oxidation of **1** with Jones' reagent gave **11**, which was incubated for 12 days with *C. lunata* after which substrate **11** (43%), a new metabolite (**12**, 42%) and a mixture of more polar metabolites (10%) were isolated. This last mixture was then acetylated to give **13** (1α,12-diacetoxy-4ß,6ß-dihydroxy-eudesm-11(R)-ane, 51% of the mixture) and **14** (1α,12-diacetoxy-4ß,6ß-dihydroxyeudesm-11(S)-ane, 49%). Metabolite **12** showed a molecular weight in accordance with the reduction of the keto group at C-1, which was confirmed spectroscopically (see Table II and Experimental). As microbial reduction of a keto group normally gives S-alcohols [11], thus the reduction of a similar keto group in eudesmanolides [3] and eudesmenes [1] gave 1S-alcohols. The new 1α-hydroxyl group produced clear γ-syn effects on C-3 ($\Delta\delta$ = -4.83) and C-5 ($\Delta\delta$ = -3.26). These reductions produced 1S-alcohols, which are very difficult to obtain by chemical means.

The acetates **13** and **14** seem to be the result of the reduction of substrate **11** to give 1α-hydroxy derivatives, such as described for **12**, and posterior hydroxylation at C-12 to give two derivative epimers at C-11. Saponification of **13** and **14** gave respectively **15** and **16**. These products were compared to the 12-hydroxy derivatives **4** and **5**, obtained from incubation of **1**. The spectroscopic evidences indicated (see Tables I and II and Experimental) that product **15** was the epimer at C-1 of **4**, which has a 11-R configuration. On the other hand, product **16** was an epimer at C-1 of **5** (11-S). These configurations at C-11 were confirmed by the formation of the respective lactones (**17**, 63% and **18**, 63%) by oxidation with $RuH_2(Ph_3P)_4$ [10], whose configuration at C-11 was determined as described for lactones **4** and **5**. The better yields in the last oxidations of **15** and **16** may be due to the lower quantity of acetone used as a hydrogen acceptor (see Experimental). The overall yield of lactones **17** and **18** from substrate **11** (2.5% for each one) was duplicated by incubation of **12** for 12 days, which also proved that the first action of microorganism was the reduction of the keto group at C-1.

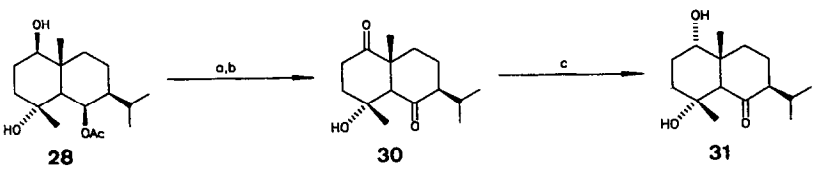


SCHEME II: a: Curvularia lunata; b: Ac_2O-Py; c: KOH alc.; d: H_4BNa; e: $RuH_2(Ph_3P)_4$

To further study the relationship between structure of substrate and the action of microorganism, product **1** was saponified (to give **8**), acetylated at 0° (to give **19**, 1ß-acetoxy-4ß,6ß-dihydroxyeudesmane) and oxidized with Jones' reagent at room temperature to give **20**, which was incubated with *C. lunata* for 3 days. The controls of the aqueous phase in the Erlenmeyer flasks of the incubation indicated that the whole of substrate **20** was biotransformed. However, when the process was stopped and the aqueous phase and mycelium were extracted (see Experimental) 50% of unaltered substrate **20** was isolated, as well as metabolite **21** (31%) and a mixture of more polar metabolites (15%). Metabolite **21** was the result of deacetylation at C-1 of substrate **20**, which must be due to the microorganism, as such deacetylation was not observed after similar times and conditions of culture without fungus. The acetylation of the polar metabolites gave the monoacetate **22** (21% of the mixture) and the diacetate **23** (1ß,12-diacetoxy-4ß-hydroxyeudesm-11(R)-an-6-one, 79%). The spectroscopic study of **22** (see Table III an Experimental) indicated that, as for product **10** described above, a new tertiary hydroxyl group was introduced at C-11. On the other hand, the main acetate **23** was the result of hydroxylation at C-12 of substrate **20**, as can be deduced from the comparison of MS, 1H and 13C NMR of diacetate **23** and substrate **20**. However, the determination of its configuration at C-11 was not possible until the chemical transformation of **23** to product **4** was carried out. Thus, the reduction of **23** with H_4BNa and subsequent basic treatment gave **4** (79%). The overall yield of **4** from substrate **20** was 8%, with only 3 days of incubation. However, only 11-R derivatives are obtained in this process.

The best results from these incubations were obtained by biotransformation of substrate **24**, also obtained from **1** (see Experimental). This 1,6-diketo compound (**24**) was incubated for 4 days with *C. lunata*, after which only a small quantity of substrate was isolated (7%). However, considerable amounts of metabolites **25** (1α,4ß-dihydroxyeudesman-6-one, 28%), **26** (4ß,12dihydroxyeudesm-11(R)-ane-1,6-dione, 32%) and **27** (1α,12-diacetoxy-4ßhydroxyeudesm-11(R)-an-6-one, 11%, isolated after acetylation) were now isolated. Metabolite **25** was the result of the regio- and stereoselective reduction of the keto group at C-1 of substrate **24** to give the 1S-hydroxyl derivative. Its structure was easely deducted by comparison of its spectroscopic data with those of substrate **24** and its C-1 epimer, compound **21** (see Tables II and III). The main metabolite isolated from this incubation (**26**, 32%) was the result of hydroxylation at C-12 of substrate **24**. Its structure was determinated by correlation (nearly quantitative reduction with H_4BNa) with tetrol **4**. In this incubation the production of the tetrol **4**, the precursor of 6,12-lactones, was considerable, although only the lactone with 11-R configuration could be obtained. Moreover, the diacetate **27** mentioned above was the result of selective reduction, as occurred for metabolite **25**, and hydroxylation at C-12 (to give the 11-R configuration). Thus, saponification and subsequent reduction of **27** gave **15**.

Some 4α-hydroxyl analogues of the substrates mentioned above were also incubated with *C. lunata* cultures. However, the microorganism was unable to hydroxylate at C-12 . Thus incubation of substrate **28** with *C. lunata* for 14 days was unsuccessful, leading to the recovery of unaltered substrate. Treatment of **28** with H_4AlLi to cleave the acetoxy group at C-6 [12] gave the triol **29** (1ß,4α,6ß-trihydroxyeudesmane), which was then oxidized, giving the diketo compound **30**. This was incubated for 5 days, after which the substrate was totally transformed. The main result of this process was metabolite **31** (64%), which had an 1S-hydroxyl group as the only modification of the substrate (**30**). Another single metabolite isolated from this incubation (15%) was shown to be unstable, and its structure could not be determined.



28 30 31

SCHEME III: a: H_4AlLi-THF; b: Jones reagent; c: Curvularia lunata

In conclusions for this type of compound only substrates with 4ß-hydroxyl groups were transformed to 12-hydroxyl compounds. Substrates with a keto group at C-6 were more efficient, although more selective, in the production of 12-hydroxyl compounds with the 11-R configuration only. Hence, while all these substrates are suitable for obtaining 6ß-lactones, substrate **24**, which had keto groups at C-1 and C-6, proved to be the most efficiently biotransformed substrate.

TABLA I

	2	3	4	5	6	7	8	9	10	11
C- 1	81.50	81.54	79.96	80.10	79.41	79.38	80.06	84.67	81.40	215.77
C- 2	23.50	23.49	27.07	27.13	27.29	27.20	27.01	68.19	23.47	34.38[a]
C- 3	40.61	40.78	39.55[a]	39.57[a]	38.64	38.66	39.55[a]	47.27	40.65[a]	42.22
C- 4	71.75	71.74	73.07	72.86	71.20	71.28	73.02	74.41	71.97	71.57
C- 5	53.03	53.15	52.27	51.90	50.29	50.15	52.19	52.28	53.20	53.87
C- 6	70.60	69.58	71.42	68.14	77.85	77.38	69.06	69.07	69.90	71.16
C- 7	44.21	45.31	48.49	48.63	40.18	42.46	49.58	49.43	52.88	49.28
C- 8	20.06	20.81	16.88	22.04	18.28	23.32	20.50	20.20	19.09	21.10
C- 9	39.12	39.00	39.49[a]	39.34[a]	36.58	36.79	39.37[a]	39.39	39.38[a]	34.42[a]
C-10	38.71	38.69	39.22	38.96	38.23	37.99	38.69	---	38.89	48.05
C-11	33.85	33.67	38.68	37.91	40.55	43.44	30.14	30.30	72.02	29.23
C-12	67.64	67.41	64.37	66.47	178.66	179.49	21.11[b]	21.14[b]	29.28[b]	21.10
C-13	14.80	15.80	17.82	16.15	9.11	14.84	20.82[b]	20.78[b]	26.85[b]	21.10
C-14	14.72	14.70	14.70	14.54	14.29	14.24	14.55	15.64	15.05	20.63
C-15	28.82	29.82	30.11	30.24	29.62	29.63	28.78	28.79	29.92	28.85
MeCOO	21.03	21.00							21.33	21.74
MeCOO	21.32	21.32							22.18	
MeCOO	21.81	21.77								
MeCOO	170.95	170.90							170.96	171.59
MeCOO	170.95	171.33							172.27	
MeCOO	171.32	171.33								

TABLA II

	12	13	14	15	16	17	18	19	20	21
C- 1	75.92	78.17	78.14	75.99	75.99	78.64	78.11	81.31	79.69	78.70
C- 2	25.01	22.62	22.57	25.27	25.31	25.76	25.64	23.61	23.15	26.97
C- 3	35.99[a]	35.66	35.90	35.16	34.82	34.24	34.21[a]	39.20[a]	37.84[a]	38.10[a]
C- 4	72.36	73.22	73.46	73.90	73.62	71.86	71.88	72.85	69.53	69.73
C- 5	49.68	46.93	47.16	48.68	48.70	44.29	44.18	52.27	62.24	62.40
C- 6	70.95	68.75	69.88	71.71	68.15	75.09	75.04	68.87	216.05	216.85
C- 7	46.93	45.91	45.90	46.01	45.59	40.63[a]	42.73	49.49	57.95	58.09
C- 8	21.01	20.68	19.45	17.97	22.30	18.53	23.51	20.30	26.61	26.75
C- 9	36.11[a]	36.39	36.40	36.68	36.70	34.24	34.37[a]	39.09[a]	37.45[a]	37.71[a]
C-10	38.54	37.15	37.28	38.37	38.10	37.38	37.10	37.82	45.47	46.85
C-11	30.06	34.03	34.59	38.96	38.04	40.48[a]	43.43	30.01	26.06	26.07
C-12	21.43[b]	68.58	68.26	64.42	66.17	178.87	179.65	21.03[b]	21.46[b]	21.55[b]
C-13	21.33[b]	15.99	16.38	16.97	16.21	9.13	14.72	20.76[b]	18.67[b]	18.72[b]
C-14	20.59	21.76	21.85	22.27	22.07	20.91	20.80	15.67	14.02	12.92
C-15	28.87	30.35	30.33	30.37	30.41	29.82	29.79	28.69	29.94	30.00
MeCOO	21.82	21.12	21.11					21.28	21.24	
MeCOO		21.37	21.38							
MeCOO	171.45	170.59	170.56					171.10	170.80	
MeCOO		171.55	171.69							

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

TABLA III

	22	23	24	25	26	27	29	30	31
C- 1	79.54	79.58	213.76	73.60	213.59	75.85	80.53	211.96	72.47
C- 2	23.12	23.17	33.13[a]	25.31	33.11[a]	26.63	28.73	34.27[a]	26.93
C- 3	37.60[a]	37.85[a]	39.64	33.76	39.58	35.10[a]	41.26	39.46	33.90[a]
C- 4	69.58	69.54	69.64	70.11	69.58	69.84	72.41	69.97	71.24
C- 5	62.79	62.38	63.28	57.12	63.29	58.13	55.85	65.11	60.06
C- 6	218.69	214.94	214.95	218.45	215.91	216.00	66.84	212.20	214.87
C- 7	60.37	54.42	57.71	58.10	54.62	54.60	50.39	55.67	55.71
C- 8	25.97	27.23	25.74	26.49	25.04	22.41	20.34	21.39	21.34
C- 9	37.53[a]	37.50[a]	33.86[a]	35.09	33.80[a]	33.34[a]	42.51	35.15[a]	35.89[a]
C-10	45.75	45.60	53.79	45.93	53.89	44.69	39.09	50.76	43.47
C-11	71.09	31.28	26.15	26.08	33.48	31.28	28.90	23.71	24.07
C-12	28.70[b]	66.62	21.44[b]	21.59[b]	64.96	66.68	21.13[b]	20.71[b]	20.87[b]
C-13	26.06[b]	15.82	18.70[b]	18.80[b]	15.47	15.84	20.70[b]	18.10[b]	17.97[b]
C-14	14.07	14.11	19.65	19.47	19.70	19.43	14.70	20.45	20.65
C-15	29.94	29.97	29.31	30.35	29.33	30.29	25.42	25.69	25.45
MeCOO	21.27	21.04				21.06			
MeCOO		21.30				21.33			
MeCOO	170.82	170.86				170.31			
MeCOO		171.23				171.19			

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

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°. Monodimensional n.O.e.-difference experiments were made by irradiation for 4 s in series of 8 scans with alternate on-resonance and off-resonance. 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°. Si 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 (Si gel, Merck 60 G) were rendered visible by spraying with H_2SO_4-AcOH, followed by heating to 120°. The identity of compounds **1**, **8**, **11**, **19** [9], **28** and **29** [12] have been 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**) (1.3 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 vacuo. Chromatography on a Si gel column yielded 1β,4β,6β-trihydroxyeudesmane (**8**) (994 mg, 90%) [9].

OXIDATION OF EUDESMANE **1**.- Jones reagent was added dropwise to a stirred solution of 6β-acetoxy-1β,4β-dihydroxyeudesmane (**1**) (500 mg) in acetone at 0° until an orange-brown color persisted [13]. 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 Si gel column yielded 6β-acetoxy-4β-hydroxyeudesman-1-one (**11**) (446 mg, 90%) [9].

ACETYLATION OF 1β,4β,6β-TRIHYDROXYEUDESMANE (**8**).- 1β,4β,6β-trihydroxyeudesmane (**8**) (500 mg) was dissolved in Ac_2O/Py (1:2) (30 ml) and maintained at 0° for 2 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with sat. SO_4HK aq. and dried with anhydrous Na_2SO_4. Chromatography over Si gel yielded 1β-acetoxy-4β,6β-dihydroxyeudesmane (**19**) (518 mg, 89%) [9].

OXIDATION OF 1β-ACETOXY-4β,6β-DIHYDROXYEUDESMANE (**19**).- 1β-acetoxy-4β,6β-dihydroxyeudesmane (**19**) (515 mg) was dissolved in acetone (10 ml) and oxidized with Jones reagent for 30 min. at room tempetature. After CC 1β-acetoxy-4β-hydroxyeudesman-6-one (**20**) (465 mg, 91%) was isolated; syrup; $[\alpha]_D$= +62.4° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3513, 1731, 1694 and 1246 cm^{-1}; ^1H nmr (δ) 4.67 (1H, dd, $J_{1,2ax}$= 11.8, $J_{1,2eq}$= 4.1 Hz, H-1), 2.26 (1H, s, H-5), 2.02 (3H, s, AcO group), 1.25 and 1.02 (3H each, s, 3H-14 and 3H-15), 0.88 and 0.84 (3H each, d, J= 6.7 Hz, 3H-12 and 3H-13); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 297 (40), 279 (76), 237 (100), 219 (88).

OXIDATION OF 1β,4β,6β-TRIHYDROXYEUDESMANE (**8**).- 1β,4β,6β-trihydroxyeudesmane (**8**) (400 mg) was also oxidized with Jones reagent under the same conditions as for product **19**. Chromatography on a Si gel column yielded 4β-hydroxyeudesmane-1,6-dione (**24**) (352 mg, 89%); syrup; $[\alpha]_D$= +67.1° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3527, 1712 and 1705 cm^{-1}; ^1H nmr (δ) 3.07 (1H, ddd, $J_{2ax,2eq}$= $J_{2ax,3ax}$= 14.2, $J_{2ax,3eq}$= 6.0 Hz, H-2β), 2.58 (1H, s, H-5), 1.25 and 1.24 (3H each, s, 3H-14 and 3H-15), 0.91 and 0.87 (3H each, d, J= 6.6 Hz, 3H-12 and 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 253 (80), 235 (100).

ISOLATION OF 6β-ACETOXY-1β,4α-DIHYDROXYEUDESMANE (**28**).- 6β-acetoxy-1β,4α-dihydroxyeudesmane (**28**) was isolated from *Sideritis varoi subsp. oriensis* [12].

SAPONIFICATION OF EUDESMANE **28**.- H_4AlLi (100 mg) was added to a solution of 6β-acetoxy-1β,4α-dihydroxyeudesmane (**28**) (300 mg) in anhydrous THF (60 ml). The solution was refluxed for 2 h. The reaction mixture was cooled, poured into water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Chromatography over Si gel yielded 1β,4α,6β-trihydroxyeudesmane (**29**) (224 mg, 87%) [12].

OXIDATION OF 1β,4α,6β-TRIHYDROXYEUDESMANE (**29**).- 1β,4α,6β-trihydroxyeudesmane (**29**) (210 mg) was oxidized with Jones reagent under the same conditions as for product **19**. Chromatography on a Si gel column yielded 4α-hydroxyeudesmane-1,6-dione (**30**) (183 mg, 89%); syrup; $[\alpha]_D$= -4.8° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3487 and 1706 cm^{-1}; ^1H nmr (δ) 2.67 (1H, s, H-5), 2.66 (1H, ddd, $J_{2ax,2eq}$= $J_{2ax,3ax}$= 15.0, $J_{2ax,3eq}$= 6.3 Hz, H-2β), 1.70 and 1.08 (3H each, s, 3H-14 and 3H-15), 0.91 and 0.85 (3H each, d, J= 6.9 Hz, 3H-12 and 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 253 (18), 235 (100).

ORGANISM, MEDIA AND CULTURE CONDITIONS.- *Curvularia lunata* CECT 2130 came 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 the transformation experiments a medium of peptone (0.1%), yeast extract (0.1%), beef extract (0.1%) and glucose (0.5%) in H_2O at pH 5.7 was used. Erlenmeyer flasks (250 ml) containing 80 ml of medium were inoculated with a dense suspension of *C. lunata*. The cultures were incubated with shaking (150 rpm) at 28° for 6 days, after which substrates **1**, **8**, **11**, **12**, **20**, **24**, **28** and **30** in EtOH were added.

BIOTRANSFORMATION OF SUBSTRATE 1.- Substrate **1** (345 mg) was dissolved in EtOH (7 ml), distributed among 7 Erlenmeyer-flask cultures, and incubated for 12 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 vacuo to give a mixture of compounds (296 mg) (Scheme 1). This mixture was chromatographed on a Si gel column to obtain 214 mg of starting material **1** and 82 mg (23%) of a polar mixture, which was dissolved in Ac_2O/Py (1:2) (6 ml) and refluxed for 2 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with sat. SO_4HK aq. and dried with anhydrous $NaSO_4$. Chromatography on a Si gel column yielded firstly 1β,6β,12-triacetoxy-4β-hydroxyeudesm-11(R)-ane (**2**) (46 mg, 51% of the polar mixture); syrup; $[\alpha]_D$= +43.5° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3505, 1731 and 1246 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 5.66 (1H, m, $w_{½}$= 3.7 Hz, H-6), 4.45 (1H, dd, $J_{1,2ax}$= 11.9, $J_{1,2eq}$= 3.6 Hz, H-1), 4.04 and 3.95 (1H each, part AB of an ABX system, J_{AB}= 11.0, J_{AX}= 4.9, J_{BX}= 6.3 Hz, 2H-12), 2.06, 2.04 and 2.02 (3H each, s, AcO groups), 1.34 (6H, s, 3H-14 and 3H-15) and 0.90 (3H, d, J= 6.5 Hz, 3H-13); ^{13}C nmr see table I; ms, m/z(%): [M-120] 278 (20), 218 (21), 160 (32). Further elution yielded 1β,6β,12-triacetoxy-4β-hydroxyeudesm-11(S)-ane (**3**) (44 mg, 49% of the polar mixture); syrup; $[\alpha]_D$= +27.3° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3504, 1727 and 1246 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 5.66 (1H, dd, $J_{5,6}$= $J_{6,7}$= 1.9 Hz, H-6), 4.44 (1H, dd, $J_{1,2ax}$= 11.9, $J_{1,2eq}$= 3.6 Hz, H-1), 4.12 and 3.92 (1H each, part AB of an ABX system, J_{AB}= 11.0, J_{AX}= 3.7, J_{BX}= 6.2 Hz, 2H-12), 2.04 (6H, s, AcO groups), 2.02 (3H, s, AcO group), 1.38 and 1.34 (3H each, s, 3H-14 and 3H-15) and 1.00 (3H, d, J= 6.5 Hz, 3H-13); ^{13}C nmr see table I; ms, m/z(%): [M-120] 278 (22), 218 (25), 160 (36).

SAPONIFICATION OF PRODUCT **2**.- 1β,6β,12-triacetoxy-4β-hydroxyeudesm-11(R)-ane (**2**) (43 mg) was dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) (0.2 g), with the same working conditions as for product **1**. Chromatography on a Si gel column yielded 1β,4β,6β,12-tetrahydroxyeudesm-11(R)-ane (**4**) (26 mg, 88%): syrup; $[\alpha]_D$= +7.1° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3332 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.34 (1H, sa, H-6) 3.57 and 3.49 (1H each, part AB of an ABX system, J_{AB}= 10.8, J_{AX}= 8.8, J_{BX}= 3.7 Hz, 2H-12), 3.18 (1H, dd, $J_{1,2ax}$= 11.7, $J_{1,2eq}$= 3.7 Hz, H- 1), 1.33 and 1.29 (3H each, s, 3H-14 and 3H-15) and 0.96 (3H, d, J= 7.2 Hz, 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 273 (6), 255 (47), 237 (100), 219 (37), 201 (5).

SAPONIFICATION OF PRODUCT **3**.- 1β,6β,12-triacetoxy-4β-hydroxyeudesm-11(S)-ane (**3**) (40 mg) was dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) (0.2 g), with the same working conditions as for product **2**. Chromatography over Si gel yielded 1β,4β,6β,12-tetrahydroxyeudesm-11(S)-ane (**5**) (25 mg, 91%); syrup; $[\alpha]_D$= +14.2° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3349 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.53 (1H, sa, H-6), 3.71 and 3.60 (1H each, part AB of an ABX system, J_{AB}= 10.6, J_{AX}= 2.2, J_{BX}= 7.2 Hz, 2H-12), 3.18 (1H, dd, $J_{1,2ax}$= 11.6, $J_{1,2eq}$= 4.0 Hz, H-1), 1.33 and 1.27 (3H each, s, 3H-14 and 3H-15) and 0.97 (3H, d, J= 7.1 Hz, 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 273 (3), 255 (39), 237 (100), 219 (40), 201 (4).

LACTONIZATION OF PRODUCT **4**.- 1β,4β,6β,12-tetrahydroxyeudesm-11(R)-ane (**4**) (26 mg, 0.1 mmol) was dissolved in toluene (0.5 ml) and acetone was added (0.02 ml, 0.3 mmol). $RuH_2(PPh_3)_4$ [10] (2.2 mg, $2x10^{-3}$ mmol) was added to the solution and kept in a closed tube at 180° under argon atmosphere for 6 h. The reaction mixture was cooled before opening the tube, and then concentrated in vacuo. Chromatography on a Si gel column yielded 1β,4β-dihydroxy-6α,11αH-eudesman-6,12-olide (**6**) (11 mg, 43%); syrup; $[\alpha]_D$= -41.8° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3302, 1767 and 1161 cm^{-1}; ^1H nmr (δ) 4.74 (1H, dd, $J_{6,7}$= $J_{5,6}$= 3.7 Hz, H-6), 3.22 (1H, dd, $J_{1,2ax}$= 11.6, $J_{1,2eq}$= 4.2 Hz, H-1), 2.77 (1H, dq, $J_{11,7}$= $J_{11,13}$= 7.1 Hz, H-11), 1.28 (3H, s, 3H-15), 1.20 (3H, d, $J_{13,11}$= 7.1 Hz, 3H-13) and 1.16 (3H, s, 3H- 14); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 269 (9), 251 (100), 233 (52).

LACTONIZATION OF PRODUCT **5**.- 1β,4β,6β,12-tetrahydroxyeudesm-11(S)-ane (**5**) (25 mg, 0.09 mmol) was dissolved in toluene (0.5 ml) and acetone (0.02 ml, 0.3 mmol) and $RuH_2(PPh_3)_4$ [10] (2.2 mg, $2x10^{-3}$ mmol) were added under the same working conditions as for product **4**. Chromatography over Si gel yielded 1β,4β-dihydroxy-6α,11βH-eudesman-6,12-olide (**7**) (10 mg, 41%); syrup; $[\alpha]_D$= -41.1° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3314, 1766 and 1156 cm^{-1}; ^1H nmr (δ) 4.94 (1H, dd, $J_{6,7}$= 4.7, $J_{5,6}$= 3.3 Hz, H-6), 3.20 (1H, dd, $J_{1,2ax}$= 11.6, $J_{1,2eq}$= 4.2 Hz, H-1), 2.38 (1H, q, $J_{11,13}$= 7.7 Hz, H-11), 1.32 (3H, d, $J_{13,11}$= 7.7 Hz, 3H-13), 1.29 (3H, s, 3H-15) and 1.17 (3H, s, 3H-14); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 269 (5), 251 (100), 233 (36).

BIOTRANSFORMATION OF SUBSTRATE **8**.- Substrate **8** (300 mg) was dissolved in EtOH (6 ml), distributed among 6 Erlenmeyer flask cultures and incubated for 12 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**, to give a mixture (280 mg) which was chromatographed on a Si gel column to obtain 238 mg of starting material **8**, 7.5 mg (2%) of 1β,2α,4β,6β-tetrahydroxyeudesmane (**9**); syrup; $[\alpha]_D$= +3.4° ($CHCl_3$, c 0.5); ir μ_{max} ($CHCl_3$): 3353 cm^{-1}; ^1H nmr (δ) 4.53 (1H, dd, $J_{6,7}$= $J_{6,5}$= 2.6 Hz, H-6), 4.00 (1H, ddd, $J_{2,3ax}$= 11.2, $J_{2,1}$= 9.2, $J_{2,3eq}$= 5.1 Hz, H-2), 2.94 (1H, d, $J_{1,2}$= 9.2 Hz, H-1), 1.34 and 1.31 (3H each, s, 3H-14 and 3H-15), 0.99 and 0.96 (3H each, d, J= 6.7 Hz, 3H-12 and 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 273 (1), 255 (43), 237 (100), 219 (84), 201 (5); and 34 mg (11%) of a polar mixture, which was acetylated with Ac_2O/Py (1:2) (3 ml) under the same conditions as described above for the polar mixture. Chromatography on a Si gel column yielded three products: 1β,6β-diacetoxy-4β,11-dihydroxy-eudesmane (**10**) (19 mg, 55% of the mixture); syrup; $[\alpha]_D$= +28.2° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3468, 1731 and 1245 cm^{-1}; ^1H nmr (δ) 5.87 (1H, sa, H-6), 4.45 (1H, dd, $J_{1,2ax}$= 11.8, $J_{1,2eq}$= 3.7 Hz, H-1), 2.06 and 2.05 (3H each, s, AcO groups), 1.37 and 1.32 (3H each, s, 3H-14 and 3H-15), 1.19 and 1.11 (3H each, s, 3H-12 and 3H-13); ^{13}C nmr see table I; ms, m/z(%): $[M+1]^+$ 357 (3), 339 (100), 279 (24), 237 (43), 219 (62); and products **2** (24% of the mixture) and **3** (21% of the mixture).

BIOTRANSFORMATION OF SUBSTRATE **11**.- Substrate **11** (440 mg) was dissolved in EtOH (9 ml), distributed among 9 Erlenmeyer flask cultures and incubated for 12 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**, to give a mixture (421 mg) which was chromatographed on a Si gel column to yield 188 mg of starting material **11**, 185 mg (42%) of 6β-acetoxy-1α,4β-dihydroxyeudesmane (**12**); syrup; $[\alpha]_D$= +25.0° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3373, 1712 and 1266 cm^{-1}; ^1H nmr (δ) 5.72 (1H, m, $w_{½}$= 4 Hz, H-6), 3.81 (1H, m, $w_{½}$= 8 Hz, H-1), 2.00 (3H, s, AcO group), 1.35 and 1.24 (3H each, s, 3H-14 and 3H-15), 0.88 and 0.86 (3H each, d, J= 6.5 Hz, 3H-12 and 3H-13); ^{13}C nmr see table II; ms, m/z(‰): [M-15] 283 (2), 223 (18), 220 (29), 205 (97); and 48 mg (10%) of a polar mixture, which was acetylated with Ac_2O/Py (1:2) (3 ml) at room temperature for 2 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with sat. SO_4HK aq. and dried with anhydrous $NaSO_4$. Chromatography on a Si gel column yielded two products: 1α,12-diacetoxy-4β,6β-dihydroxyeudesm-11(R)-ane (**13**) (25 mg, 51% of the mixture); syrup; $[\alpha]_D$= +31.2° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3462, 1730 and 1245 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.56 (2H, m, $w_{½}$= 13 Hz, H-1 and H-6), 4.27 and 3.86 (1H each, part AB of an ABX system, J_{AB}= 10.5, J_{AX}= 4.5, J_{BX}= 7.5 Hz, 2H-12), 2.07 and 2.05 (3H each, s, AcO groups), 1.39 and 1.30 (3H each, s, 3H-14 and 3H-15) and 1.00 (3H, d, J= 6.5 Hz, 3H-13); ^{13}C nmr see table II; ms, m/z(%): [M-15] 341 (1), 323 (5), 278 (9), 263 (12), 221 (9), 218 (41), 203 (67); and 1α,12-diacetoxy-

4β,6β-dihydroxyeudesm-11(S)-ane (**14**) (24 mg, 49% of the mixture); syrup; $[\alpha]_D$= +25.0° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3454, 1731 and 1246 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.57 (1H, m, $w_{½}$= 8 Hz, H-1), 4.51 (1H, m, $w_{½}$= 8 Hz, H-6), 4.33 and 3.85 (1H each, part AB of an ABX system, J_{AB}= 10.5, J_{AX}= 3.6, J_{BX}= 7.5 Hz, 2H-12), 2.07 and 2.06 (3H each, s, AcO group), 1.41 and 1.34 (3H each, s, 3H-14 and 3H-15) and 1.06 (3H, d, J= 6.5 Hz, 3H-13); ^{13}C nmr see table II; ms, m/z(%): [M-15] 341 (3), 323 (9), 278 (10), 263 (18), 218 (30), 203 (94).

SAPONIFICATION OF PRODUCT **13**.- 1α,12-diacetoxy-4β,6β-dihydroxyeudesm-11(R)-ane (**13**) (25 mg) was dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) (0.2 g) under the same working conditions as for product **1**. Chromatography over Si gel yielded 1α,4β,6β,12-tetrahydroxyeudesm-11(R)-ane (**15**) (16 mg, 84%); syrup; $[\alpha]_D$= +5.2° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3336 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.40 (1H, sa, H-6), 3.55 and 3.44 (1H each, part AB of an ABX system, J_{AB}= 10.8, J_{AX}= 9.1, J_{BX}= 3.6 Hz, 2H-12), 3.34 (1H, m, $w_{½}$= 6.0 Hz, H-1), 1.34 and 1.32 (3H each, s, 3H-14 and 3H-15) and 0.94 (3H, d, J= 7.1 Hz, 3H-13); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 273 (4), 255 (16), 237 (30), 219 (100), 201 (9).

SAPONIFICATION OF PRODUCT **14**.- 1α,12-diacetoxy-4β,6β-dihydroxyeudesm-11(S)-ane (**14**) (24 mg) was dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) (0.2 g) under the same working conditions as for product **1**. Chromatography on a Si gel column yielded 1α,4β,6β,12-tetrahydroxyeudesm-11(S)-ane (**16**) (16 mg, 87%); syrup; $[\alpha]_D$= +9.6° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3332 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.60 (1H, dd, $J_{6,7}$= $J_{6,5}$= 2.5 Hz, H-6), 3.68 and 3.58 (1H each, part AB of an ABX system, J_{AB}= 10.7, J_{AX}= 2.3, J_{BX}= 7.1 Hz, 2H-12), 3.35 (1H, dd, $J_{1,2ax}$= $J_{1,2eq}$= 2.8 Hz, H-1), 1.34 and 1.30 (3H each, s, 3H-14 and 3H-15) and 0.97 (3H, d, J= 7.1 Hz, 3H-13); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 273 (5), 255 (11), 237 (30), 219 (100), 201 (7).

LACTONIZATION OF PRODUCT **15**.- 1α,4β,6β,12-tetrahydroxyeudesm-11(R)-ane (**15**) (16 mg, 0.06 mmol) was dissolved in toluene (0.5 ml) and traces of acetone and $RuH_2(PPh_3)_4$ [10] (1.3 mg, $1.2x10^{-3}$ mmol) were added under the same working conditions as for product **4**. Chromatography on a Si gel column yielded 1α,4β-dihydroxy-6α,11αH-eudesman-6,12-olide (**17**) (10 mg, 63%); syrup; $[\alpha]_D$= -21.4° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3445, 1773 and 1159 cm^{-1}; ^1H nmr (δ) 4.82 (1H, dd, $J_{6,7}$= $J_{6,5}$= 3.5 Hz, H-6), 3.41 (1H, dd, $J_{1,2ax}$= $J_{1,2eq}$= 2.8 Hz, H-1), 2.75 (1H, dq, $J_{11,7}$= $J_{11,13}$= 7.1 Hz, H-11), 1.84 (1H, d, $J_{5,6}$= 3.5 Hz, H-5), 1.30 (3H, s, 3H-15), 1.19 (3H, s, 3H-14) and 1.18 (3H, d, $J_{13,11}$= 7.1 Hz, 3H-13); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 269 (7), 251 (30), 233 (100).

LACTONIZATION OF PRODUCT **16**.- 1α,4β,6β,12-tetrahydroxyeudesm-11(S)-ane (**16**) (16 mg, 0.06 mmol) was dissolved in toluene (0.5 ml) and traces of acetone and $RuH_2(PPh_3)_4$ [10] (1.3 mg, $1.2x10^{-3}$ mmol) were added under the same working conditions as for product **4**. Chromatography over Si gel yielded 1α,4β-dihydroxy-6α,11βH-eudesman-6,12-olide (**18**) (10 mg, 63%); syrup; $[\alpha]_D$= -21.8° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3460, 1773 and 1154 cm^{-1}; ^{1} nmr (δ) 5.03 (1H, dd, $J_{6,7}$= 4.5, $J_{6,5}$= 3.5 Hz, H-6), 3.40 (1H, dd, $J_{1,2ax}$= $J_{1,2eq}$= 2.8 Hz, H-1), 2.36 (1H, q, $J_{11,13}$= 7.7 Hz, H-11), 2.02 (1H, ddd, $J_{7,8ax}$= 12.0, $J_{7,8eq}$= 7.1, $J_{7,6}$= 4.5 Hz, H-7), 1.80 (1H, d, $J_{5,6}$= 3.5 Hz, H-5), 1.32 (3H, s, 3H-15), 1.31 (3H, d, $J_{13,11}$= 7.7 Hz, 3H-13) and 1.20 (3H, s, 3H-14); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 269 (4), 251 (25), 233 (100).

BIOTRANSFORMATION OF SUBSTRATE **12**.- 6β-acetoxy-1α,4β-dihydroxyeudesmane (**12**) (170 mg) was dissolved in EtOH (4ml), distributed among 4 Erlenmeyer flask cultures, and incubated for 12 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**, to give a mixture (152 mg) which was chromatographed on a Si gel column to obtain 116 mg of starting material **12** and 36 mg (21%) of a polar mixture, which was acetylated with Ac_2O/Py (1:2) (3 ml) under the same conditions as described above for the polar mixture. Chomatography on a Si gel column yielded product **13** (19 mg, 50% of the mixture) and product **14** (19 mg, 50% of the mixture).

BIOTRANSFORMATION OF SUBSTRATE **20**.- Substrate **20** (465 mg) was dissolved in EtOH (10 ml, 10 Erlenmeyer flask cultures) and incubated for 3 days, after which the cultures thoroughly processed as indicated above for the biotransformation of substrate **1**, to give a mixture (421 mg) which was chomatographed over Si gel to yield 235 mg of starting material **20**, 124 mg (31%) of 1β,4β-dihydroxyeudesman-6-one (**21**); syrup; $[\alpha]_D$= +34.1° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3440 and 1691 cm^{-1}; ^1H nmr (δ) 3.41 (1H, dd, $J_{1,2ax}$= 11.6, $J_{1,2eq}$= 4.1 Hz, H-1), 2.19 (1H, s, H-5), 1.15 and 0.94 (3H each, s, 3H-14 and 3H-15), 0.89 and 0.85 (3H each, d, J= 6.5 Hz, 3H-12 and 3H-13); ^{13}C nmr see table II; ms, m/z(%): $[M+1]^+$ 255 (16), 237 (100), 219 (7); and 62 mg (15%) of a mixture of metabolites, 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 sat. SO_4HK aq. and dried with $NaSO_4$. Chomatography on a Si gel column yielded two products: 1β-acetoxy-4β,11-dihydroxyeudesman-6-one (**22**) (14 mg, 21% of the mixture); syrup; $[\alpha]_D$= +38.8° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3531, 1730, 1692 and 1242 cm^{-1}; ^1H nmr (δ) 4.69 (1H, dd, $J_{1,2ax}$= 11.8, $J_{1,2eq}$= 4.2 Hz, H-1), 2.46 (1H, ddd, $J_{7,8ax}$= 12.7, $J_{7,8eq}$= 6.4, $J_{7,5W}$= 1.0 Hz, H-7), 2.29 (1H, sa, H-5), 2.05 (3H, s, AcO group), 1.26 and 1.08 (3H each, s, 3H-14 and 3H-15) and 1.21 (6H, s, 3H-12 and 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 313 (28), 295 (65), 277 (16), 253 (38), 235 (100), 217 (11); and 1β,12-diacetoxy-4β-hydroxyeudesm-11(R)-an-6-one (**23**) (54 mg, 79% of the mixture); syrup; $[\alpha]_D$= +53.5° ($CHCl_3$, c 1); ir

μ_{max} ($CHCl_3$): 3530, 1735, 1697 and 1243 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.71 (1H, dd, $J_{1,2ax}$= 11.8, $J_{1,2eq}$= 4.1 Hz, H-1), 4.06 and 3.98 (1H each, part AB of an ABX system, J_{AB}= 10.9, J_{AX}= 5.0, J_{BX}= 6.1 Hz, 2H-12), 2.41 (1H, ddd, $J_{7,8ax}$= 12.7, $J_{7,8eq}$= $J_{7,11}$= 6.7 Hz, H-7), 2.30 (1H, s, H-5), 2.18 (1H, dddq, $J_{11,12}$= 5.0, $J_{11,12'}$= 6.1, $J_{11,7}$= $J_{11,13}$= 6.7 Hz, H-11), 2.05 and 2.04 (3H each, s, AcO groups), 1.18 and 1.05 (3H each, s, 3H-14 and 3H-15) and 0.99 (3H, d, $J_{13,11}$= 6.7 Hz, 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 355 (8), 337 (48), 295 (68), 277 (100), 235 (18).

REDUCTION AND SAPONIFICATION OF PRODUCT **23**.- H_4BNa (10 mg) was added to a stirred solution of 1β,12-diacetoxy-4β-hydroxyeudesm-11(R)-an-6-one (**23**) (54 mg) in EtOH (4 ml) at room temperature for 30 min. The reaction mixture was acidified with dil. HCl, extracted with CH_2Cl_2, dried with $NaSO_4$ and concentrated in vacuo. It was then dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) (0.2 g) under the same working conditions as for product **1**. Chomatography on a Si gel column yielded product **4** (33 mg, 79%).

BIOTRANSFORMATION OF SUBSTRATE **24**.- Substrate **24** (340 mg) was dissolved in EtOH (7 ml, 7 Erlenmeyer flask cultures) and incubated for 4 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**, to give a mixture (283 mg) which was chomatographed on a Si gel column to obtain 23 mg of starting material **24**, 95 mg (28%) of 1α,4β-dihydroxyeudesman-6-one (**25**); syrup; $[\alpha]_D$= +21.3° ($CHCl_3$, c 1); ir μ_{max} (CHC_3): 3444 and 1690 cm^{-1}; ^1H nmr (δ) 3.52 (1H, dd, $J_{1,2ax}$= $J_{1,2eq}$= 3.0 Hz, H-1), 2.64 (1H, s, H-5), 1.18 (3H, s, 3H-15), 0.98 (3H, s, 3H-14), 0.88 and 0.85 (3H each, d, J= 6.6 Hz, 3H-12 and 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 255 (9), 237 (100), 219 (45); 117 mg (32%) of 4β,12-dihydroxyeudesm-11(R)-ane-1,6-dione (**26**); syrup; $[\alpha]_D$= +8.5° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3454 and 1711 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 3.59 and 3.51 (1H each, part AB of an ABX system, J_{AB}= 10.7, J_{AX}= 5.3, J_{BX}= 6.7 Hz, 2H-12), 3.07 (1H, ddd, $J_{2ax,2eq}$= $J_{2ax,3ax}$= 14.2, $J_{2ax,3eq}$= 6.0 Hz, H-2β), 2.62 (1H, d, $J_{5,7(W)}$= 1.0 Hz, H-5), 2.43 (1H, dddd, $J_{7,8ax}$= 11.9, $J_{7,8eq}$= 6.5, $J_{7,11}$= 5.3, $J_{7,5(W)}$= 1.0 Hz, H-7), 1.27 and 1.26 (3H each, s, 3H-14 and 3H-15) and 0.95 (3H, d, J= 7.0 Hz, 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 269 (29), 251 (100), 233 (25); and 48 mg (13%) of a polar metabolite, which was acetylated with Ac_2O/Py (1:2) (3 ml) under the same conditions as described above for acetylation. Chromatography on a Si gel column yielded 1α,12-diacetoxy-4β-hydroxyeudesm-11(R)-an-6-one (**27**) (53 mg, 84%); syrup; $[\alpha]_D$= +45.9° ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3527, 1739, 1695 and 1243 cm^{-1}; ^1H nmr (measured at the spectral map) (δ) 4.77 (1H, dd, J_1= 3.2, J_2= 2.4 Hz, H-1), 4.06 and 3.99 (1H each, part AB of an ABX system, J_{AB}= 10.9, J_{AX}= 5.1, J_{BX}= 6.2 Hz, 2H-12), 2.57 (1H, sa, H-5), 2.39 (1H, dddd, $J_{7,8ax}$= 12.6, $J_{7,8eq}$= $J_{7,11}$= 6.3, $J_{7,5(W)}$= 1.0 Hz, H-7), 2.12 and 2.04 (3H each, s, AcO groups), 1.22 and 1.07 (3H each, s, 3H-14 and 3H-15) and 0.99 (3H, d, J= 6.8 Hz, 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 355 (5), 337 (78), 295 (81), 277 (100), 235 (33), 217 (20).

REDUCTION OF PRODUCT **26**.- H_4BNa (20 mg) was added to a stirred solution of 4β,12-dihydroxyeudesm-11(R)-ane-1,6-dione (**26**) (117 mg) in EtOH (6 ml) under the same conditions as described above for the reduction, yielding after CC a compound (104 mg,88%) identical to metabolite **4**.

REDUCTION AND SAPONIFICATION OF PRODUCT **27**.- H_4BNa (10 mg) was added to a stirred solution of 1α,12-diacetoxy-4β-hydroxyeudesm-11(R)-an-6-one (**27**) (53 mg) in EtOH (4 ml) under the same conditions as for the above reduction. It was then dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) (0.2 g) under the same working conditions as described above for saponification. Chromatography on a Si gel column yielded a compound (32 mg, 79%) identical to metabolite **15**.

BIOTRANSFORMATION OF SUBSTRATE **28**.- Substrate **28** (250 mg) was dissolved in EtOH (5 ml, 5 Erlenmeyer flask cultures) and incubated for 14 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**. This process yielded 228 mg of starting material **28** only.

BIOTRANSFORMATION OF SUBSTRATE **30**.- Substrate **30** (180 mg) was dissolved in EtOH (4 ml), distributed among 4 Erlenmeyer flask cultures, and incubated for 5 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**. Chromatography on a Si gel column yielded 1α,4α-dihydroxyeudesman-6-one (**31**) (117 mg, 64%); syrup; $[\alpha]_D$= +18.3 ($CHCl_3$, c 1); ir μ_{max} ($CHCl_3$): 3434, 1690 cm^{-1}; ^1H nmr (δ) 3.35 (1H, dd, $J_{1,2ax}$= $J_{1,2eq}$= 2.3 Hz, H-1), 2.69 (1H, d, $J_{5,7(W)}$= 1.0 Hz, H-5), 1.39 and 0.79 (3H each, s, 3H-14 and 3H-15), 0.83 and 0.76 (3H each, d, J= 6.9 Hz, 3H-12 and 3H-13); ^{13}C nmr see table III; ms, m/z(%): $[M+1]^+$ 255 (4), 237 (100), 219 (65).

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