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Biotransformation of 6β-Eudesmanolides Functionalized at C-3 with *Curvularia lunata* and *Rhizopus nigricans* Cultures

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Abstract: *A series of biotransformations of 6β-santonin and its derivatives with functions at C-3, were carried out with Curvularia lunata and Rhizopus nigricans cultures Rhizopus nigricans was more active in the biotransformation process against these substrates The biotransformation of 6β-santonin yielded its 2α-hydroxy-1,2-dihydro derivative The biotransformation of ketones at C-3 obtained by partial or total hydrogenation of double bonds in ring A led to 3S alcohols Incubation of the 3S-hydroxyl-4S-13S- 6β-eudesmanolide with Rhizopus nigricans produced epimerization at C-4 and hydroxylation at C-8, C-1 or C-4, in decreasing order Thus epimerization is probably produced with the participation of the hydroxyl group at C-3 Microbial functionalization at C-8 can provide access to the synthesis of 8,12-eudesmanolides*

INTRODUCTION

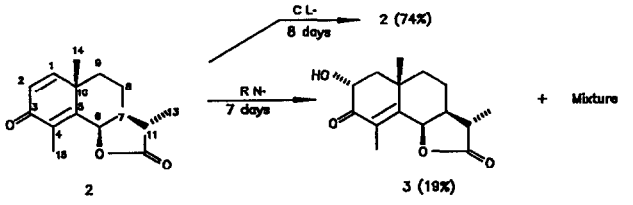
Sesquiterpene lactones are compounds whose chemistry[1], photochemistry[2] and bioformation processes[3,4,5] have been extensively studied Although small in size, the presence or absence of certain functional groups is decisive in their stereochemistry and reactivity[6]. On the other hand, eudesmanolide compounds are biogenetical and chemical precursors of a wider range of sesquiterpene lactones[7], and hence their synthesis can provide access to the formation of other groups of sesquiterpene lactones. The eudesman-6α,12-olides are the most abundant compounds which have been studied chemically and biogenetically. However, the group of eudesman-6ß,12-olides are scarce in nature and have rarely been studied, although they are probably the biogenetical precursors of pseudoguaianolides and elemanolides[7].

Bioconversion of sesquiterpenes is actually accepted as an advisable method, in combination with the adequate chemical procedures, to semisynthesis of products of interest[8]. In this paper, we present a series of biotransformations of eudesman-6ß,12-olides with functions at C-3, which can be obtained from commercial α-santonin[9] in order to establish a structure-biotransformation relationship with hydroxylating fungi (*Curvularia lunata* and *Rhizopus nigricans*) and thus obtain a series of products, some

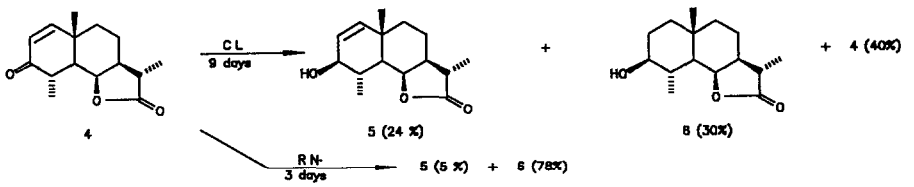
of which may be suitable starting material for further chemical or chemical-microbiological processes

RESULTS AND DISCUSSION

The 6ß-santonin (**2**) was obtained from commercial α-santonin (**1**) as described[9,10]. The incubation of **2** with *Curvularia lunata* was unsuccessful, and **2** was recovered unaltered after 8 days. However, the incubation of **2** with *Rhizopus nigricans* culture gave, after a 7 day incubation, the α-ketal **3** (20%). The configuration at C-2 can be deduced from its ^{13}C and ^{1}H nmr spectra. This type of α,ß-unsaturated ketone is photosensitive and unstable[2]



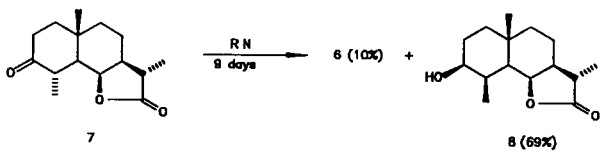
The partial reduction of 6ß-santonin (**2**) produced 6ß-11,13-dihydrotuberiferin (**4**)[9] which was incubated with *C. lunata* and *R. nigricans* cultures. Part of this substrate (40%) was recovered unaltered after a 9 day incubation with *C. lunata*, which also produced metabolites **5** (24%) and **6** (3%). Substrate **4** was totally biotransformed with *R. nigricans* for 3 days, which also gave the metabolites **5** and **6** with a yield of 5% and 78% respectively. As can be seen, the yields with these microorganisms were inverse, and *R. nigricans* totally metabolized **4** Metabolites **5** and **6** can be readily obtained by chemical procedures[9]. However the formation of **3** by these media is problematic



Substrate **7** (6ß-artepaulin) was obtained by catalytic hydrogenation of 6ß-santonin (**2**) Incubation for 9 days with *R. nigricans* culture, which, as described above, was more active against this type of substances than *C lunata*, gave the alcohol **6** (10%)[9] and the novel alcohol **8** (69%) This metabolite **8** had no ketone character and hence the functional group present at C-3 in substrate **7** was reduced. The geminal proton to this new hydroxyl group was axial, as can be seen in the ^{1}H nmr spectrum (δ 3 70, J_1= 11 6, J_2=J_3= 4 70 Hz) but J_2 and J_3 values indicated that the configuration at C-4 was inverted Likewise, a clear γ-syn effect on C-2 with respect to **6** ($\Delta\delta$= 6 67) can be observed in the ^{13}C nmr spectrum The chemical shift of C-15 was in accordance with this axial disposition (δ= 9.73 in **8** and δ= 14 42 in **6**) Several n O e experiments confirmed this new configuration at C-4 in substance **8** Principally, the irradiation at δ 3 70 (H-3) produced a great n O e on H-4 (δ 2 18), but not on the C-15 methyl group

The epimerization at C-4 gave the least thermodynamically stable metabolite **8**, which has an axial methyl group at C-4 It is of interest to determine whether this epimerization occurred before or after

the reduction of the keto group at C-3, and whether the functional group at C-3 participates in this process For these purposes, we incubated alcohol **6** for 9 days with *Rhizopus nigricans* culture, after which a minuscule amount of substrate **6** (10%) was recovered unaltered. Moreover, the epimer at C-4 (metabolite **8**, 17%) was also obtained, which indicated that the reduction of **7** at C-3 may have

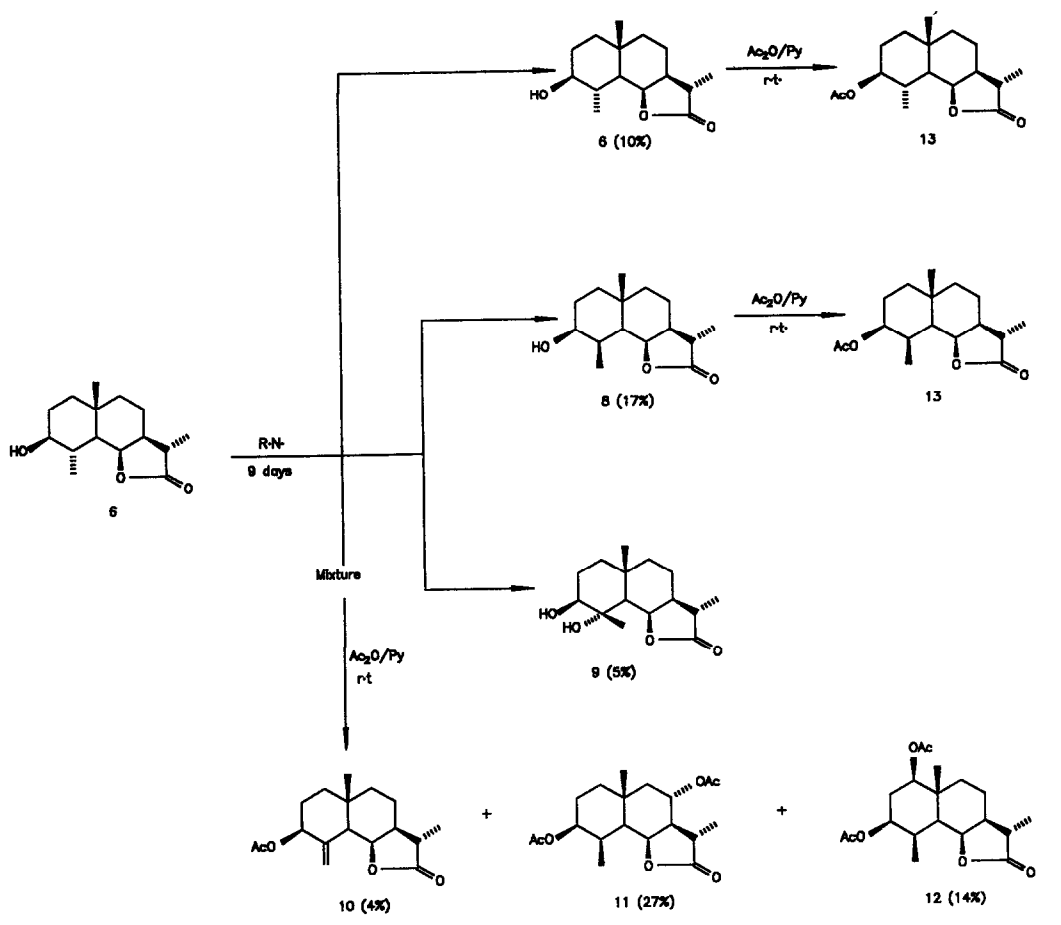


occurred before epimerization at C-4 The incubation of **6** afforded a further metabolite **9** (5%) which showed a new hydroxylation at C-4, as the proton signal at C-3 was a double doublet and a new oxygenated carbon (δ 75.57) was detected in its ^{13}C nmr spectrum (see tables I and II). Several n.O.e. difference experiments were performed to determine the configuration at C-4 of **9**. Irradiation at δ 5.11 (H-6) produced no n.O.e effect on the C-14 methyl group and *vice versa*. Thus, the new hydroxyl group was α.

The acetylation of a mixture of metabolites that was difficult to resolve gave acetates **10** (4%), **11** (27%) and **12** (14%), which are yields relative to substrate **6**. The acetate **10** showed two ethylene protons and carbons (see tables I and II) and its structure can readily be deduced to be the result of the formation of a C-4/C-15 exocyclic methylene group. Product **11** was the acetate of the main metabolite isolated from this incubation. The structure of **11** was determined after acetylation of **8** (to give **13**) and **6** (to give **14**) by comparing its ^{13}C nmr data with those of other acetates of metabolites with more numerous functions Thus, it can be seen that the configuration at C-4 for **11** and **13** was the same (4αH) Moreover, compound **11** had another acetoxy function at C-8, as can be deduced from the study of its ^{1}H and ^{13}C nmr spectra, and we assigned an 8S-configuration after considering the two axial-axial coupling constants of the proton at C-8 (see table I) However, a considerable δ-syn effect on C-11 was observed ($\Delta\ \delta$ = -7 0) (see Table II) which may indicate a ß-disposition of the acetoxy group at C-8 After considering the structure of **11** in Dreiding models, a similar distance (approx. 2 5 A) was observed between the hydrogen at C-11 and oxygen at C-8 for both configurations. A series of n.O e. difference experiments were carried out in acetate **11** to establish the overall stereochemistry of this compound Irradiation at H-8 produced an n O e effect on the H-11 and C-14 methyl groups, confirming a ß-disposition for all these groups Irradiation at H-3 increased the signals of H-4 and H-5, which indicated the α-disposition of these three protons. Finally, irradiation at 3H of the C-14 methyl group produced a clear n O e effect on H-8. These experiments proved the overall stereochemistry of acetate **11** This 8 -hydroxylation introduced by *Rhizopus nigricans* had the inverse configuration of those obtained by the same microorganism on a substrate oxygenated at C-1 instead of C-3[9]. Thus, the stereochemistry of the functionalization at C-8 in this type of lactones seems to be controlled by the functions present in ring A

Diacetate **12** also had a 4R-configuration as can be deduced from comparison of its ^{1}H and ^{13}C nmr data with those of acetates **13** and **14** In addition to the epimerization at C-4, *R. nigricans* hydroxylated on C-1 or C-9 The ^{13}C nmr data of **12** did not allow us to unequivocally assign this structure because some overlapping of chemical shifts was observed between pairs of carbons in both possible structures However, n O.e difference experiments were also decisive to determine the structure of **12**. Thus, irradiation at H-3 produced n O.e. on H-1 and H-5 Therefore, we conclude that the new functionalization in **12** was 1ß- hydroxyl group, subsequently acetylated by chemical means.

As can be seen, all metabolites isolated from the incubation of substrate **6** were epimerized at C-4 (with the exception of **10**, which presents an exocyclic double bond) *R. nigricans* probably reduced at



the C-3 substrates **4** and **7** before epimerization of **6** at C-4

Processes of epimerization of saturated carbon are uncommon with the exception of the α-carbon with respect of an enolizable carbonyl group to give the most thermodynamically stable epimer

In this case, the participation of the 3ß-hydroxyl group could be postulated after enzymatic abstraction of the 4α-proton to give an oxirane-like group on the ß-side, which could be enzymatically reduced to result in epimerization at C-4, hydroxylation at C-4 to give **9**, or loss of a proton from C-15 to give **10**

Biotransformation of 6β-eudesmanolides

TABLE I

	2	3	4	5	6	7	8	9	10	11	12	13	14
H-1	6 74 d	----	6 63 d	5 48 Q_{AB}	----	----	----	----	----	----	----	----	----
H- 1α	----		----	----		1 66 ddd					4 5 dd		
H- 1β	----		----	----							----		
H-2	6 23 d	----	5 83 d	5 48 Q_{AB}	----	----	----	----	----	----	----	----	----
H- 2α	----	----	----	----		2 39 ddd							
H- 2β	----	4 17 dd	----	----		2 58 ddd							
H- 3α	----	----	----	3 80 d	3 06 ddd	----	3 70 ddd	3 43 dd	5 11 ddd	4 72 ddd	4 83 ddd	4 75 ddd	4 41 ddd
H- 4α	----	----	----	----	----	----	2 17 ddq	----	----	2 38 ddq	2 38 ddq		----
H- 4β	----	----	2 74 dq	1 96 ddq		2 74 dq	----	----	----	----	----	----	
H- 5α	----	----	1 72 dd	----	1 27 dd				1 95 dd		1 39 dd		
H- 6α	5 52 d	5 38d	4 68 dd	4 69 dd	4 62 dd	4 62 dd	4 53 dd	5 11 dd	4 76 dd	4 62 dd	4 58 dd	4 51 dd	4 67 dd
H-7	2 15 ddd		2 04 ddd			1 98 ddd	1 98 ddd	1 96 ddd					
H- 8α	1 86 dddd					1 75 dddd				----	1 66 dddd		
H- 8β										4 82 ddd			
H- 9α													
H- 9β						1 57 ddd					1 57 ddd		
H-11β	2 52 q	2 49 q	2 40 q	2 36 q	2 29 q	2 39 q	2 29 q	2 32 q	2 32 q	2 51 q	2 29 q	2 29 q	2 36 q
3H-13	1 37 d	1 35 d	1 31 d	1 29 d	1 28 d	1 29 d	1 25 d	1 29 d	1 27 d	1 26 d	1 25 d	1 24 d	1 28 d
3H-14	2 03 s	1 23 s	1 17 s	1 05 s	0 94 s	1 84 s	1 05 s	1 05 s	0 89 s	1 16 s	1 13 s	1 08 s	0 97 s
3H-15	1 26 s	1 99 s	1 25 d	1 18 d	1 07 d	1 12 d	1 17 d	1 41 s	----	1 13 d	1 16 d	1 15 d	
2H-15	----	----	----	----	----	----	----	----	5 25 dd	----	----	----	----
									5 04 dd				
3H-AcO	----	----	----	----	----	----	----	----	2 14 s	2 02 s	2 02 s	2 03 s	2 05 s
3H-AcO	----	----	----	----	----	----	----	----	----	2 01 s	2 02 s	----	----

J(Hz) 2 and 3 6α,7= 5 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 11β, 13= 7 6 2, 4 and 5 1,2= 9 8 2, 4 and 7 7,8α= 6 7, 7,8β= 11 6 2, 7 and 12 8α,8β= 13 6, 8α,9α= 3 6, 8α,9β= 3 6 3 1α,2= 12 1, 1β,2= 6 8 4 and 5 5α,6α= 4 4, 6α,7= 4 5 4, 5 and 7 4β,5α= 12 8 4 and 6 4β,15= 6 7 5 3,4β= 8 8 5, 7 and 14 4β,15= 6 5 6 2α,3= 5, 2β,3= 10 5, 3,4β= 10 5 6, 7, 8,9, 10, 12, 13 and 14 6α,7= 4 6 and 14 5α,6α= 3 8 7 1α,2β= 14 1, 1β,2β= 6 6, 2α,1α= 5, 2α,1β= 5, 2α,1β= 5, 2α,2β= 13, 5α,6α= 3 3 7 and 12 8β,9β= 6 6, 9α,9β= 13 2 8 2α,3= 4 7, 2β,3= 11 6, 3,4α= 4 7, 4α,5α= 2 7 8 and 9 7,8α= 6 3, 7,8β= 12 8, 10 and 13 5α,6α= 2.9. 8 and 13 4α,15= 7.4. 9. 2α,3=4.5, 2β,3= 12, 5α,6α= 2.5 10. 2α,3= 5 2, 2β,3= 11 3, 3,15= 1 8 11 2α,3= 4 8, 2β,3= 12, 3,4α= 4 8, 6α,7= 4 5, 8,9α= 10 5, 8,9β= 4 2 11 and 12 4α,5α= 3 4, 4α,15= 7 4, 5α,6α= 2 9 12 1,2α= 4 68, 1,2β= 11 5, 2α 3= 5 1, 2β,3= 12 1, 3,4α= 5 1, 7,8α= 6 4 13 2α,3= 4 6, 2β,3= 12 2, 3,4α= 12.2, 3,4α= 4 6 14 2α,3= 5 2, 2β,3= 11, 3,4β= 11

TABLE II

	2	3	4	5	6	7	8	9	10	11	12	13	14
C- 1	157 30	47 21	159 30	141 02	40 40	41 76	40 89	40 25	40 55	40 10	78 28	40 63	39 80
C- 2	125 82	68 81	126 22	127 30	30 94	38 02	24 27	23 80	29 05	22 04	28 36	24 27	26 96
C- 3	186 01	200 60	201 43	76 16	76 92	211 85	74 40	79 83	74.46	76 07	72 63	76 40	76 78
C- 4	137 50	133 26	40 46	35 98	36 88	42 99	40 41	75 57	143 21	40 48	36 85	37 40	33 81
C- 5	148 52	152 28	48 43	47 19	48 97	50 39	47 62	52 98	49 87	46 92	44 59	47 26	48 99
C- 6	76 20	76 67	76 45	76 74	77 10	77 01	82 97	75 92	78..15	82 48	81 71	82 60	78 57
C- 7	42 42	43 94	41 48	42 05	42 20	41 78	43 47	43 77	42 76	48 92	43 39	43 43	42 10
C- 8	27 01	24 04	23 47	23 75	23 60	23 55	26 34	27 65	23 68	70 63	23 48	24 20	23 55
C- 9	34 44	35 81	35 71	36 61	39 24	38 55	42 19	42 54	38 57	47 14	38 24	42 14	39 04
C-10	39 16	36 08	35 26	34 49	32 32	32 58	32 58	33 76	34 93	33..89	37 06	32 67	32 23
C-11	43 82	44 04	44 41	44 57	44 52	44 49	43 61	43 08	43 69	36 57	43 44	43 57	44 49
C-12	179 43	179 46	179 79	180 44	180 54	179 91	180 73	180 56	180 40	179 07	180 13	180 58	180 38
C-13	14 70	14 79	14 65	14 75	14 64	14 61	14 12	14 15	13 91	14 26	13 98	14 07	14 64
C-14	24 84	28 63	19 16	21 60	18 58	18 08	21 44	20..63	18..26	21..63	16 34	21 35	18 45
C-15	11 02	12 18	11 30	15 00	14 42	10 85	9 73	18 30	107 23	10 45	10 47	10 58	14 39
COOME									170 30	170 57	170 51	170 64	171 00
COOME										170 47	170 20		
$COOCH_3$									21 24	21 26	21 08	21 35	21 35
$COOCH_3$										21 26	21 08		

EXPERIMENTAL

Melting points (Kofler apparatus) are uncorrected. The nmr spectra were obtained with a Bruker AM-300 spectrometer equipped with a process controller and an "array processor" Samples were dissolved in $CDCl_3$ Bruker programs were used for COSY (45°) NOESY, C/H correlation and CONOESY (90°) experiments Monodimensional nOe-difference experiments were performed by irradiation for 4 s in series of 8 scans, with alternate on-resonance and off- resonance. Distortionless enhancement by polarization transfer (DEPT) was achieved with a "flip angle" of 135°. The optical rotations were measured on a Perkin-Elmer 240 polarimeter. IR spectra were recorded in a FT-IR-Nicolet 20SXB spectrophotometer. Mass spectra were carried out on a Hewlett-Packard 5988-A spectrometer with chemical ionization.Silica gel, Merck 7729 (less than 0.08 mm) was used for flash chromatography. CH_2Cl_2 containing increasing amounts of acetone was used as the eluent. Analytical plates (silica gel Merck G) were visualized by spraying with H_2SO_4-AcOH, followed by heating for 5 min at 120°C

Preparation of 6β-santonin (**2**)

(-)-α-Santonin (**1**) (1 g) was epimerized by Ishikawa's procedure[9] to give (-)-6-epi-α-santonin (**2**) (600 mg).

Preparation of 6β-artepaulin (**7**)

6ß-Santonin (**2**) (1 g) was hydrogenated with H_2 on Pt/charcoal to give 6ß-artepaulin[9] (**7**) (500 mg).

Preparation of 3β-hydroxy-tetrahydrofrulanolide (**6**)

3ß-Hydroxy-tetrahydrofrulanolide (**6**) (200 mg) was prepared as described[9]

Media and culture conditions

Medium YEPGA containing 1% yeast extract, 1% peptone, 2% glucose, 2% agar, pH 5, was used for storage of *Rhizopus nigricans* and *Curvularia lunata* In all transformation experiments the medium was 0 1 % peptone, 0.1 % corn skep, 0 1 % beef extract, and 0.5 % glucose in water. Erlenmeyer flasks (250 ml) containing 100 ml of medium were inoculated with a dense suspension of *Rhizopus nigricans* or *Curvularia lunata*. Incubations were maintained at 28 °C with gyratory shaking (120 r p.m.), after which substrates dissolved in EtOH were added The cultures were filtered and pooled and the cells were washed twice with water The liquid was saturated with NaCl and extracted with CH_2Cl_2

General procedure for acetylation

The product was dissolved in a mixture of Ac_2O/py (1 2) and stirred at room temperature for 6 h. the reaction product was poured into cold H_2O and extracted with CH_2Cl_2, washed with diluted HCl and $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and evaporated at reduced pressure.

Incubation of 6β-santonin (**2**) *with* Curvularia lunata *cultures*

Substrate (**2**) (100 mg) was dissolved in EtOH (2 ml) distributed between 2 Erlenmeyer flask cultures, and incubated for 8 days, after which the cultures were processed as indicated above, to give 74 mg of the starting material (**2**).

Incubation of 6β-santonin (**2**) *with* Rhizopus nigricans *cultures*

Substrate (**2**) (100 mg) was dissolved in EtOH (2 ml), distributed between 2 Erlenmeyer flask cultures and incubated for 7 days Incubation yielded a mixture (60 mg) which was chromatographed. The first band gave 2α-hydroxy-3-oxo-6αH,11ßH-eudesm-4,5- en-6,12-olide (**3**) (20 mg, 19%); syrup; $[\alpha]_D$ - 97.1° ($CHCl_3$, c 1), ir ν_{max} ($CHCl_3$) 3200 and 1760 cm^{-1} ^1H nmr· see table I, ^{13}C nmr: see Table II,ms,

m/z (%)· 265 (M^++1) Further elution yielded an inseparable mixture (30 mg).

Incubation of 6β-11,13-dihydro-tuberiferine (**4**) *with* Curvularia lunata *cultures*

Substrate (**4**) (100 mg) was incubated for 9 days to give a mixture (80 mg) which was chromatographed The first run gave starting material 6ß-11,13-dihydro-tuberiferine (**4**) (40 mg, 40%). Successive elutions of the column yielded 3ß-hydroxy-5,6αH,4,11ßH-eudesm-1-en-6,12-olide (**5**) (25 mg, 24%), syrup; $[\alpha]_D$- 90° ($CHCl_3$, c 1), ir $_{max}$ ($CHCl_3$). 3175 and 1754 cm-1, ^{1}H nmr : see Table I; ^{13}C nmr· see Table II, m/z· 251 (M^+1+1, 54%), 250 (M^+, 7%), 233 (M^+-17)(100). Further elution gave 3ß-hydroxy- 5,6αH,4,11ßH-eudesman-6,12-olide (**6**) (3 mg, 3%), syrup, $[\alpha]_D$ - 121.4 ° ($CHCl_3$, c 1); ir ν_{max} ($CHCl_3$) 3241 and 1755 cm -1, ^{1}H nmr : see Table I, ^{13}C nmr see Table II; m/z: 253 (M^++1, 7%), 252 (M^+, 44%), 234 (M^+-17) (100)

Incubation of 6β-11,13-dihydro-tuberiferine (**4**) *with* Rhizopus nigricans *cultures*

Substrate (**4**) (100 mg) was incubated for 3 days to give a mixture (90 mg) which was chromatographed to produce 3ß-hydroxy- 5,6αH,4,11ßH-eudesm-1-en-6,12-olide (**5**) (5 mg, 5%) and 3ß-hydroxy-5,6αH,4,11ßH-eudesman-6,12-olide (**6**) (80 mg, 78%).

Incubation of 6β-artepaulin (**7**) *with* Rhizopus nigricans *cultures*

Substrate (**7**) (200 mg) was dissolved in EtOH (4 ml), distributed among 4 Erlenmeyer flask cultures and incubated for 9 days. After cultures were processed, a mixture (180 mg) was obtained and chromatographed Elution of the column gave 3ß-hydroxy-4,5,6αH,11ßH- eudesman-6,12-olide (**8**) (140 mg,69%); syrup; $[\alpha]_D$ -15.2° ($CHCl_3$, c 1); ir ν_{max} ($CHCl_3$): 3500 and 1710 cm-1; ^{1}H nmr : see Table I, ^{13}C nmr see Table II; ms, m/z (%). 253 (M^++1)(27), 235 (M^+-17)(100) and 3ß-hydroxy-5,6αH,4,11ßH-eudesman-6,12-olide (**6**) (20 mg, 10%)

Incubation of 3β-hydroxy-tetrahydro-frulanolide (**6**) *with* Rhizopus nigricans *cultures*

Substrate (**6**) (200 mg) was dissolved in EtOH (4 ml), distributed among 4 Erlenmeyer flask cultures and incubated for 9 days. Cultures were processed and a mixture was obtained and chromatographed over silica gel. The first run yielded starting substrate 3ß-hydroxy-5,6αH,4,11ßH-eudesman-6,12-olide (**6**) (10 mg, 10%). The second band gave 3ß-hydroxy-4,5,6αH,11ßH-eudesman-6,12-olide (**8**) (17 mg, 17%). Further elution yielded 3ß,4α-dihydroxy-5,6αH,11ßH- eudesman-6,12-olide (**9**) (5 mg, 5%); syrup; $[\alpha]_D$ - 11.2° ($CHCl_3$, c 1); ir $_{max}$ 3600 and 1762 cm-1; ^{1}H nmr see Table I, ^{13}C nmr see Table II;ms, m/z (%). 269 (M^++1) (100) Continued elution gave a polar mixture which was acetylated at room temperature with Ac_2O/py for 2 h and chromatographed over silica gel After chromatography, the first band gave 3ß-acetoxy-5,6αH,11ßH-eudesm-4(15)-en-6,12-olide (**10**) (10 mg, 4%); mp 181- 183 °C; $[\alpha]_D$ - 40.3° ($CHCl_3$, c 1); ir $_{max}$ ($CHCl_3$) 3050 and 1760 cm-1; ^{1}H nmr · see Table I, ^{13}C nmr see Table II;ms, m/z (%): 293 (M^+1+1) (12), 232 (100), 233 (59).The second band yielded 3ß,8α-diacetoxy-4,5,6αH,11ßH-eudesman-6,12-olide (**11**) (76 mg, 27%), mp 130-132 °C; $[\alpha]_D$ -60.5° ($CHCl_3$, c 1); ir $_{max}$ ($CHCl_3$): 1765 cm-1, ^{1}H nmr see Table I; ^{13}C nmr see Table II, ms,m/z (%) 353 (M^++1) (47), 293 (100), 233 (83). The last run afforded 1ß,3ß-diacetoxy -4,5,6αH,11ßH- eudesman-6,12-olide (**12**) (40 mg, 14%); mp 118-119 °C, $[\alpha]_D$ -20 1° ($CHCl_3$, c 1), ir ν_{max} ($CHCl_3$)· 1762 cm-1; ^{1}H nmr . see Table I; ^{13}C nmr: see Table II, ms,m/z (%) 353 (M^++1) (66), 293 (92), 233 (100)

Acetylation of **6**

Product **6** (20 mg) was treated as in the general procedure. After column chromatography 3ß-acetoxy-5,6αH,4,11ßH- eudesman-6,12-olide (**14**) was isolated (21 mg, 90%); mp 206-207 °C; $[\alpha]_D$ -92° ($CHCl_3$, c 1), ir ν_{max} ($CHCl_3$): 1720 cm-1, ^{1}H nmr see Table I, ^{13}C nmr see Table II, ms, m/z (%) 295 (M^++1) (100).

Acetylation of **8**

Product **8** (50 mg) was treated as in the general procedure. After column chromatography 3ß-acetoxy- 4,5,6αH,11ßH- eudesman-6,12-olide (**13**) was isolated (55 mg, 90%); mp 190-192 °C; $[\alpha]_D$ -30° ($CHCl_3$, c 1); ^{13}C nmr· see table II; ms, m/z (%)· 295 ($M^+ + 1$) (100).

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