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Chemical, Enzymatic and Microbiological Synthesis of 8,12-Eudesmanolides: Synthesis of Sivasinolide and Yomogin Analogues

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Abstract: Conversion of α-santonin into 8,12-eudesmanolides was accomplished by chemical, enzymatic and microbiological means. Catalytic hydrogenation of α-santonin gave a mixture of hexahydro derivatives, which were sequentially isolated by lipase-catalysed selective acylation. Reduction of the 6α-lactone ring, chemical acetylation and enzymatic selective deacetylation gave an eudesmane with a hydroxymethylene group at C-12, which was hydroxylated at C-8 by *Rhizopus nigricans*. Finally lactonization with TPAP allowed us to obtain 8,12-eudesmanolides. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

A number of sesquiterpenoid lactones possessing the eudesmane skeleton are found in several plants. Owing to the presence of many diverse functional groups, the most abundant, α-santonin, has been extensively used as a starting material to obtain several naturally occurring terpenoid compounds.[1] Sesquiterpene lactones are formed from an initial hydroxylation at C-12, subsequent enzymatic hydroxylation at C-6 or C-8 and lactonization.[2] In previous papers we reported the synthesis of 6β-eudesmanolides by chemical and/or microbiological means from 6α- and 6β-sesquiterpene lactones more abundant in nature.[3,4] Elsewhere we have described the bioconversion of different eudesmanolides by the fungus *Rhizopus nigricans* and the formation of C-8 hydroxylated derivatives which could be converted into 8,12-eudesmanolides.[5,6] We now report a way to obtain these 8,12-eudesmanolides from α-santonin by a combination of chemical, *in vitro* enzymatic and microbiological means. Application of the enzymatic transesterification method in organic solvents, described by Klibanov[7] and Whitesides,[8] prompted us to investigate the enzymatic acetylation/deacetylation in some hydroxylated eudesmanes. These lipase-catalysed selective transesterifications were carried out with lipases from *Candida antarctica* (CAL),[9] *Mucor miehei* (MML)[9] and porcine pancreas (PPL),[10] and helped to prepare appropriate eudesmanic substrates for incubation with hydroxylating fungi.

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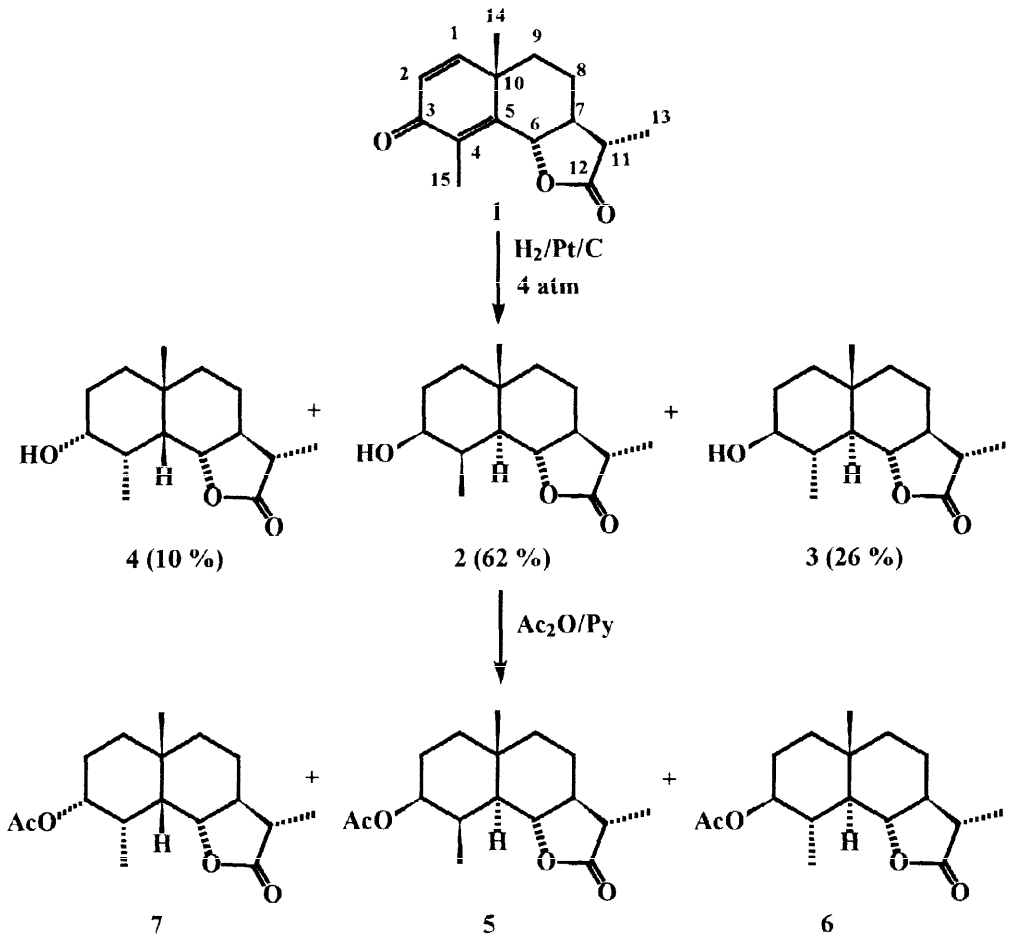
RESULTS AND DISCUSSION

Catalytic hydrogenation of α-santonin (**1**) gave the hexahydro derivatives **2**[6] (62 %), **3**[6] (26 %) and **4** (10 %) (aprox. yields measured by PMR). Quantitative acetylation of this mixture with Ac_2O/Py gave the corresponding acetoxy derivatives **5**, **6** and **7**. The isolation of these products (**2**, **3** and **4**) or their respective acetates (**5**, **6** and **7**) by chromatographic methods was very difficult.

Scheme 1



Moreover, for preparative purposes, selective acetylation was almost impossible to achieve following classical chemical procedures, but enzyme-catalysed acetylation was a viable alternative. To determine the optimal conditions of the enzymatic reactions, we previously tested the biocatalyst, reaction time and enzyme/substrate relationship. We used CAL, PPL and MML as enzymes, vinyl acetate (VA) as solvent and acylating agent and a mixture of **2**, **3**, and **4** as the substrate. The results obtained employing different enzyme/substrate relationships and different reaction times are summarized in Table 1. It is remarkable that PPL, MML and CAL acted in a diasteroselective and different manner.

Table 1. Enzyme-Catalysed Acetylation of **2**, **3** and **4** with PPL, MML and CAL.

Enzyme	Enzyme/Substrate	Time (h)	(% **5**)[a]	(% **6**)[a]	(% **7**)[a]
PPL	2/1	4	n.d.	n.d.	60
	3.5/1	24	n.d.	n.d.	80
	5/1	72	n.d.	n.d.	100
MML	2/1	4	n.d.	n.d.	30
	3.5/1	24	n.d.	n.d.	80
	5/1	72	n.d.	n.d.	100
CAL	2/1	4	n.d.	70	60
	3.5/1	24	n.d.	95	90
	6/1	24	n.d.	100	100

[a]Determined by ^{1}H- and ^{13}C-NMR.

n.d. = not detected.

As it can be seen from Table 1, none of the lipase acetylated product **2** (to give **5**) and complete acetylation of **4** (to give **7**) was obtained with PPL or MML when the enzyme/substrate relationship was 5/1 and the reaction time was 72 hours. Treatment of **2**, **3**, and **4** with CAL (6/1 for 24 hours) yielded products **3** and **4** totally acetylated (to give **6** and **7** respectively) and product **2** was recovered unaltered.

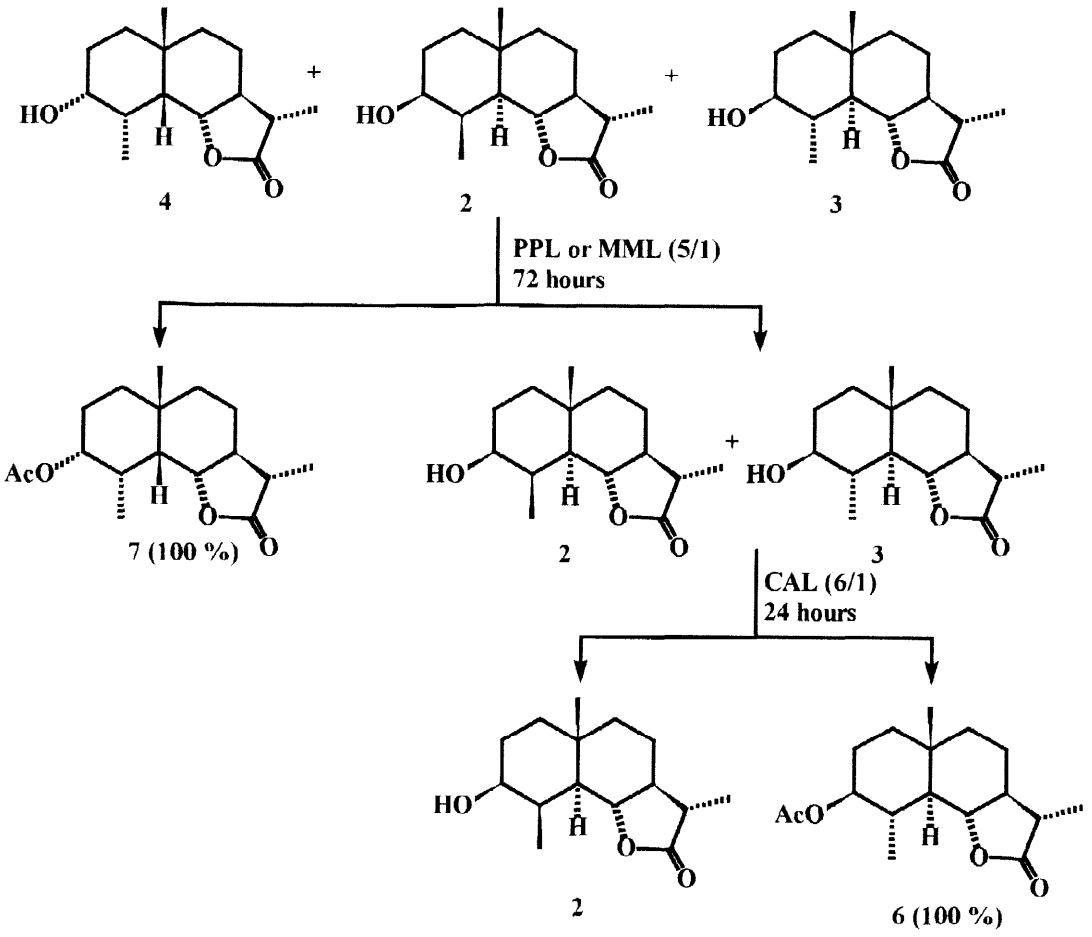
Based on this preliminary study we have established a sequential general procedure to isolate and to identify compounds **2**, **3** and **4** by diasteroselective acetylation of the mixture with PPL or MML and later with CAL (Scheme 2).

Treatment of **2**, **3** and **4** in VA with PPL or MML (5/1) for 72 hours produced complete acetylation of product **4** to give the acetoxy derivative **7** which was then separated from **2** and **3** by chromatography. The mixture of **2** and **3** was then treated with VA and CAL (6/1) for 24 hours being product **3** completely acetylated to give **6**. Thus, we separated acetoxy derivative **6** from unaltered original hydroxyeudesmanolide **2** by chromatography. By chemical acetylation of **2** the corresponding acetoxy derivative **5** was obtained and by deacetylation of acetates **6** and **7** the respective hydroxyeudesmanolides **3** and **4** were formed (see Experimental).

The structures of compounds **2**-**7** were deduced from their spectroscopic properties. Thus, the main product of the reduction of santonin **2**[6] was formed by catalytic hydrogenation of the A ring on the α-face of santonin. Compound **3**[6] was also obtained by partial hydrogenation of the A ring on the α-face and subsequent epimerization at C-4 by keto-enol tautomerization between a carbonyl group at C-3 and the enolizable proton at C-4. Both **2** and **3** were 3β-hydroxyl eudesmanolides epimers at C-4 but they had a *trans*-decalin

stereochemistry because the hydrogenation of C-4/C-5 double bond occurs on the less hindered α-face so H-5 has an α-disposition. However, minor product **4** was the result of the hydrogenation of α-santonin by the β-face obtaining a 5-*epi*-H-eudesmane skeleton. Stereochemical differences between 5α-H-eusdemanolide (**2** and **3**) and 5β-H-eudesmanolide (**4**) were principally detected by ^{13}C-NMR chemical shifts of C-9 and C-14 (see Experimental).

Scheme 2

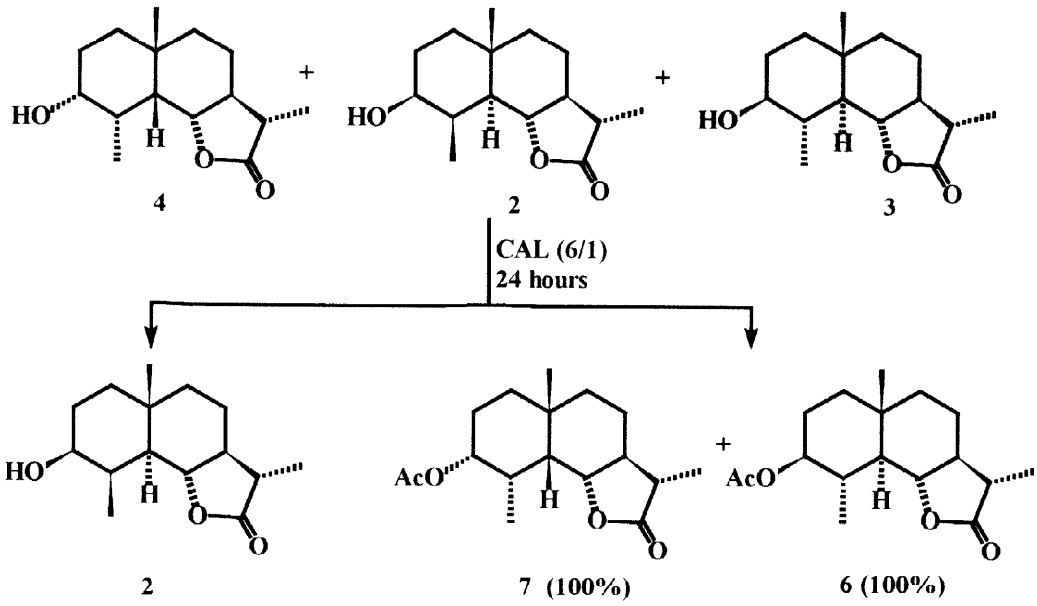


In conclusion, we have shown that both PPL and MML lipases have total preference towards 3-OH-sesquiterpenolides with *cis*-decalin stereochemistry (product **4**) whereas CAL showed an overwhelming selectivity towards products with 5α-H- (product **3**) or 5β-H-disposition (compound **4**) but always with a 4β-H-configuration.

Since it was already known that CAL acetylated **3** and **4** without affecting **2**, and to obtain a large amount of this product pure, we treated the starting mixture of **2**, **3** and **4** in VA with CAL (6/1) for 24 hours. So

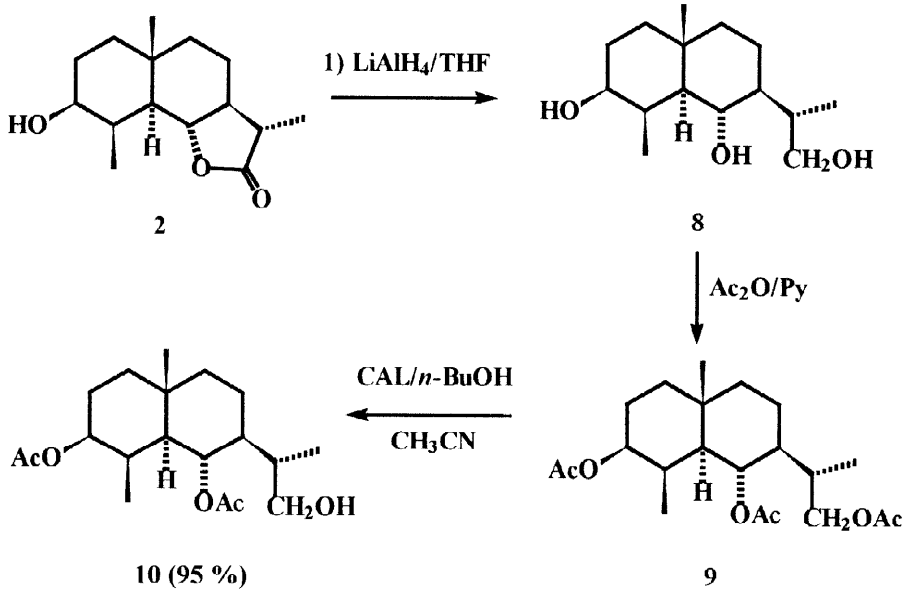
selective acetylation of **3** and **4** gave 3β-acetoxy derivatives **6** and **7**, and the separation of unaltered 3β-hydroxyeudesmanolide **2** was easily carried out (Scheme 3).

Scheme 3



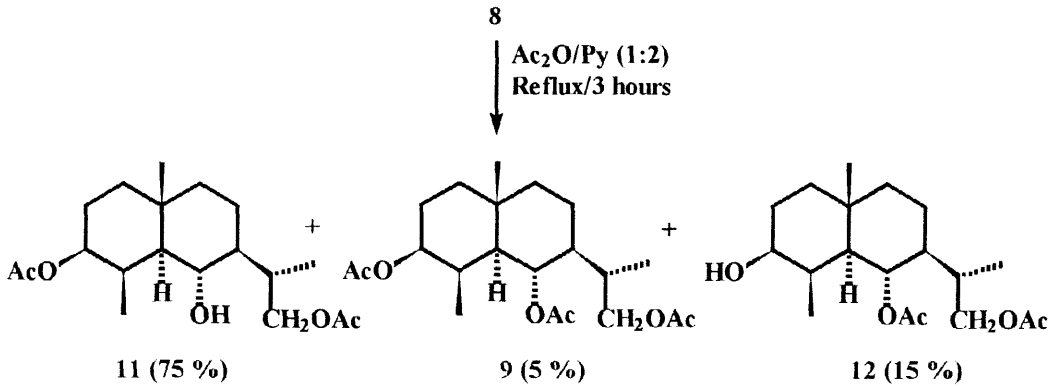
Product **2** was then treated with $LiAlH_4$ to obtain triol **8**,[4] which was acetylated to give triacetate **9**.[4] Regioselective enzymatic deacetylation of **9** was also tested with different solvents (MeOH, dioxane and acetonitrile, etc) and the best yields (95 % 12-deacetylation) of the 3,6-diacetoxy derivative **10** were obtained using CAL as enzyme, *n*-butanol as the nucleophile and acetonitrile as the solvent (Scheme 4). The site of deacetylation was easily established by direct comparison of the ^{1}H- and ^{13}C-NMR data of **9** and **10** (see Experimental).

Scheme 4



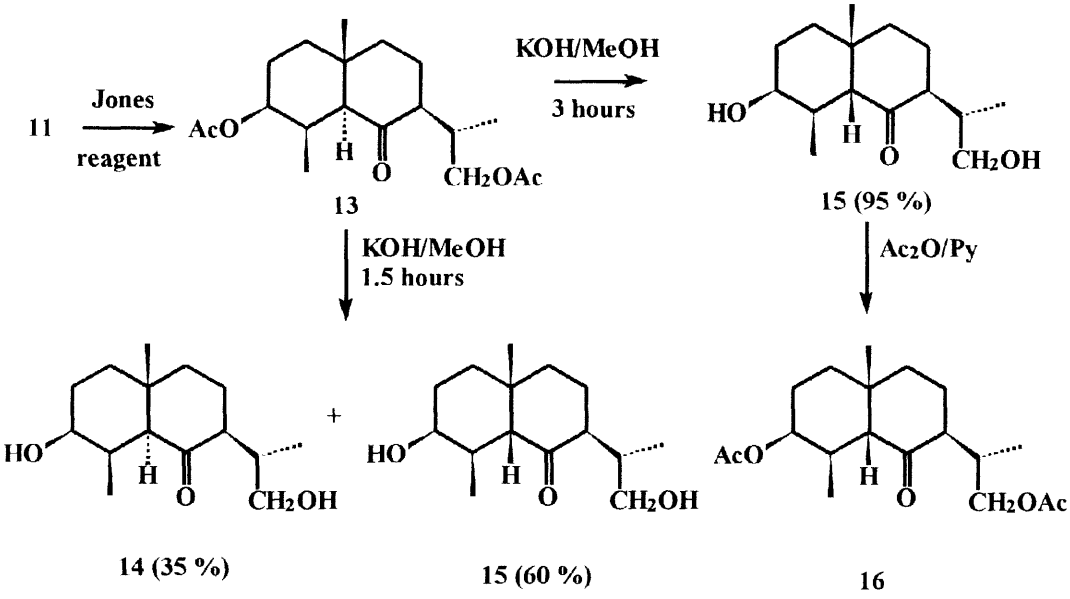
To obtain appropriate substrates to be incubated with fungi and thus to attempt the semisynthesis of interesting natural products, new chemical reactions on triol **8** were carried out. Controlled acetylation of **8** gave **11** (75 %), **12** (15 %) and **9** (5 %)[4] (Scheme 5). Oxidation of diacetate **11** with Jones' reagent gave 6-keto compound **13**.[4]

Scheme 5



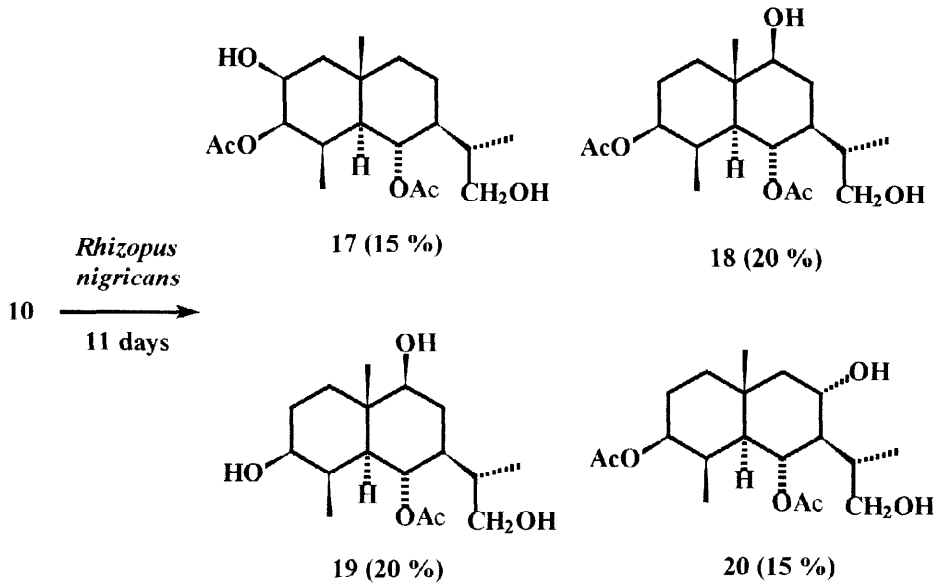
When this 3β,12-diacetoxy *trans*-decalone **13** was treated with KOH/MeOH for 1.5 hours it was equilibrated to the *cis* isomer (Scheme 6). In addition the deacetylation occurred and thus, 3β,12-dihydroxy *trans*-decalone (**14**, 35 %) and 3β,12-dihydroxy *cis*-decalone (**15**, 60 %) were obtained (Scheme 6). If this basic medium was maintained for 3 hours the isomerization to the *cis* isomer was almost completed and the subsequent chemical acetylation of **15** yielded 3β,12-diacetoxy *cis*-6-decalone **16**. The differences in the stereochemistry between **14** and **15** were easily elucidated from spectroscopic data (see Experimental). Again the isomerization occurs due to an enolizable proton at C-5 by an enol-keto interconversion. These experimental results were in accordance with the values of steric energies calculated[11] for **14** (30.3 Kcal/mol) and for **15** (26.1 Kcal/mol).

Scheme 6



3β,6α-Diacetoxy-12-hydroxy-4α,5α,7α,11β-H-eudesmane **10** was incubated with *Rhizopus nigricans* for 11 days to give metabolites **17** (15 %), **18** (20 %), **19** (20 %), **20** (15 %) and unaltered substrate **10** (20 %) (Scheme 7).

Scheme 7



The first metabolite from this incubation (**17**) had a molecular peak of m/z 356, consistent with the presence of an additional oxygen. The ^{1}H-NMR spectrum of **17** showed a new signal of a geminal hydroxy group proton (δ 4.13, 1H, ddd, J_1=J_2=2.9 Hz; J_3=3.3 Hz). The multiplicity and coupling constants of this signal indicated that the site of hydroxylation was at C-2 or C-8. A comparison of the ^{13}C-NMR chemical shifts of metabolite **17** to those substrate **10** revealed that this compound had a new 2β-hydroxyl group. This was also confirmed by the simplification of the H-3α signal which appears now as a double doublet (δ 4.76, 1H, dd, J_1=3.4 Hz; J_2=6.2 Hz). Metabolite **18** also contained an additional oxygen, as was deduced from its molecular mass (356). Its ^{1}H-NMR spectrum showed a new signal at δ 3.27 (1H, dd, J_1=4.3 Hz; J_2=11.4 Hz), and detailed study of the ^{13}C-NMR data indicated that the new hydroxyl group was equatorial at C-9. Metabolite **19** was the result of equatorial hydroxylation of substrate **10** at C-9 and deacetylation at C-3. The structure of **19** was supported by analysis of its ^{1}H- and ^{13}C-NMR spectra. Therefore, the first action of *Rhizopus nigricans* on substrate **10** was 9β-hydroxylation and subsequent deacetylation at C-3. The last metabolite obtained from this biotransformation (**20**) had a molecular peak of m/z 356, which suggested that substrate **10** had again been hydroxylated by the fungus. The ^{1}H-NMR spectrum of **20** showed that the new hydroxyl group was introduced at C-8 since its geminal proton was situated at 3.76 (1H, J_1=4.7; Hz J_2=J_3=9.8 Hz) as a double double doublet. Based on multiplicity and coupling constants of this signal and the ^{13}C-NMR

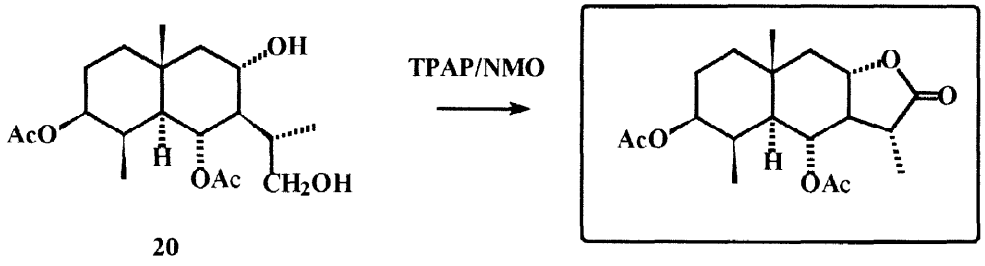
data of **20**, it was deduced that this compound had structure of 3β,6α-diacetoxy-8α,12-dihydroxy-4α,5α,7α,11β-H-eudesmane.

Substrate **10** was also incubated with *Gliocadium roseum* for 11 days to give the previously known triol **8** (1 %), metabolite **21** which was identified as 6α-acetoxy-3β,12-dihydroxy-4α,5α,7α,11β-H-eudesmane and a high amount of unaltered substrate **10** (85 %). As it can be seen from the results of the bioconversion of **10** with *Gliocadium roseum*, this fungus directed its action towards the deacetylation at C-3 (to give metabolite **21**) or at C-3 and C-6 (to give trihydroxy derivative **8**).

To test the behaviour of *Rhizopus nigricans* and *Gliocadium roseum* with another type of sesquiterpene compounds, we incubated substrate **16**, which had a structure of *cis*-decalin, but these biotransformations gave very low yields of products of little interest. In the light of these results we can conclude that these 5β-H-eudesmanes are not appropriate substrates to be incubated with these fungi.

Finally, 3β,6α-diacetoxy-8α,12-dihydroxy-4α,5α,7α,11β-H-eudesmane **20**, obtained by 8α-hydroxylation of substrate **10** with *Rhizopus nigricans*, was oxidised with TPAP/NMO[4] to obtain 3β,6α-diacetoxy-4α,5α,7α,11β-H-eudesman-8α,12-olide **22**, which was the result of the oxidation of the hydroxyl group at C-12 and later lactonization towards C-8 (Scheme 8).

Scheme 8



20 **22 (90%)**

The structure of **22** was established by its ^{1}H- and ^{13}C-NMR data and it is an analogue of natural 8,12-eudesmanolides such as yomogin isolated from *Artemisia*[12] and sivasinolide from *Tanacetum*.[13] We are currently preparing new substrates that can be hydroxylated at C-8 by *Rhizopus nigricans*, and are also testing other hydroxylating fungi to increase the yields of 8-hydroxylation and thus to enhance the formation of 8,12-eudesmanolides.

EXPERIMENTAL

Measurements of NMR spectra (300.13 MHz and 400.13 MHz ^1H and 75.47 MHz ^{13}C) were made in $CDCl_3$ and $(CD_3)_2SO$ (which also provided the lock signal) using BRUKER AM-300 or ARX-400 spectrometers. 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°) and C/H correlation. Monodimensional n.O.e.-difference experiments were made by irradiation for 4 seconds in series of 8 scans. Ir spectra were recorded on a Nicolet 20SX FT-IR spectrometer. Mass spectra were determined with CI (methane) in a Hewlett-Packard 5988A spectrometer. High resolution mass spectra were made by LSIMS (FAB) ionization mode in a MICROMASS AUTOSPEC-Q spectrometer (EBE geometry). Mps were determined using a Kofler (Reichter) apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20°. Silica gel Scharlau 60 (40-60 μm) was used for flash chromatography. CH_2Cl_2 or $CHCl_3$ containing increasing amounts of Me_2CO were used as eluents. Analytical plates (silica gel, Merck 60 G) were rendered visible by spraying with H_2SO_4-AcOH, followed by heating to 120°. Lipase (type II, crude) from porcine pancreas (PPL) (190 units/mg protein) was purchased from Aldrich. Lipozyme (MML) (lipase IM-60 from *Mucor miehei* in the immobilized form on a microporous anion exchange resin) and *Candida antarctica* lipase (Novozym 435 acrylic resin supported lipase produced by a host organism *Aspergillus oryzae*, after transfer of the genetic coding for lipase B from *Candida antarctica*) were generous gifts of *Novo Nordisk Bioindustrial Group*.

Catalytic hydrogenation of α-santonin (1). A solution of product **1** (3-oxo-11β-H-eudesm-1,4-dien-6α,12-olide, 1 g) in CH_2Cl_2 (50 mL) was hydrogenated for 1.5 h with H_2 (4 atm) on Pt/C (10 %). The reaction mixture was filtered and the solvent evaporated at reduced pressure, obtaining a residue containing products $\mathbf{2}^6$ (62 %), $\mathbf{3}^6$ (26 %) y **4** (10 %) (aprox. yields measured by PMR).

Chemical acetylation of products 2, 3 and 4. A mixture of products **2**, **3** and **4** (200 mg) was dissolved in Ac_2O/Py (1:2) (12 mL) and refluxed for 2 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4, obtaining a residue containing products **5**, **6** y **7** (same proportions as indicated above for the hydroxylated products).

Enzymatic acetylation of products 2, 3 and 4 with PPL or MML. Porcine pancreatic lipase (1g) or *Mucor miehei* lipase (1 g) was added to a solution of a mixture of **2**, **3**, and **4** (200 mg) in vinyl acetate (14 mL). The suspension was shaken on an orbital shaker (180 rpm) at 40°C for 72 hours. The reaction was terminated by filtration of the enzyme and the products were isolated by flash chromatography yielding the mixture of products **2** and **3** (180 mg) and 23 mg (100%) of 3α-acetoxy-4β,5β,7α,11β-H-eudesman-6α,12-olide (**7**); colourless solid, mp 91 °C; $[\alpha]_D$= +54° ($CHCl_3$, c 1); IR (film): 1777, 1733 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): 4.74 (1H, ddd, J_1=3.4 Hz; J_2=4.5 Hz; J_3=12.0 Hz, H-3β), 4.26 (1H, dd, J_1=6.3 Hz; J_2=11.5 Hz, H-6β), 2.42 (m, H-11β),

2.03 (3H, s, AcO group), 1.22 (3H, d, J=7.3 Hz, 3H-13), 1.08 (3H, d, J=5.3 Hz, 3H-15) and 1.03 (3H, s, 3H-14); ^{1}C NMR ($CDCl_3$): δ 12.7 (C-15), 13.5 (C-13), 21.4 (*Me*CO), 22.3 (C-8), 23.8 (C-2), 30.3 (C-14), 32.3 (C-4), 34.5 (C-9), 34.7 (C-10), 40.0 (C-1), 42.0 (C-11), 43.8 (C-5), 46.1 (C-7), 75.8 (C-3), 82.0 (C-6), 170.4 (Me*CO*) and 179.5 (C-12); HRLSIMS, *m/z*: $[M+Na]^+$ 317.1727 ($C_{17}H_{26}O_4Na$ 317.1729, PPM 0.5).

Enzymatic acetylation of products 2 and 3 with CAL. *Candida antarctica* lipase (600 mg) was added to a solution of a mixture of **2** and **3** (100 mg) in vinyl acetate (7 mL). The suspension was shaken on an orbital shaker (180 rpm) at 40°C for 24 h. The reaction was terminated by filtration of the enzyme and the products were isolated by flash chromatography yielding 70 mg of 3β-hydroxy-4α,5α,7α,11β-H-eudesman-6α,12-olide (**2**)[6] and 51 mg (100%) of 3β-acetoxy-4β,5α,7α,11β-H-eudesman-6α,12-olide (**6**); colourless solid, mp 110 °C; $[\alpha]_D$= +54° ($CHCl_3$, c 1); IR (film): 1774, 1733 and 1245 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.36 (1H, ddd, J_1=5.2 Hz; J_2=J_3=10.8Hz, H-3α), 3.81 (1H, dd, J_1=J_2=10.5 Hz, H-6β), 2.19 (1H, dq, J_1=6.9 Hz; J_2=12.1 Hz, H-11β), 2.03 (3H, s, AcO group), 1.17 (3H, d, J=6.9 Hz, 3H-13), 1.04 (3H, d, J=6.3 Hz, 3H-15) and 0.97 (3H, s, 3H-14); ^{1}C NMR ($CDCl_3$): δ 12.5 (C-13), 16.6 (C-14), 18.7 (C-15), 21.3 (*Me*CO), 23.3 (C-8), 26.7 (C-2), 35.9 (C-4), 36.7 (C-10), 39.5 (C-1), 40.6 (C-9), 40.7 (C-11), 52.6 (C-5), 53.6 (C-7), 78.0 (C-3), 83.1 (C-6), 170.8 (Me*CO*) and 179.4 (C-12); HRLSIMS, *m/z*: $[M+Na]^+$ 317.1728 ($C_{17}H_{26}O_4Na$ 317.1729, PPM 0.1).

Chemical acetylation of product 2. Product **2** (50 mg) 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 aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4. Chromatography over silica gel yielded 55 mg (95 %) of 3β-acetoxy-4α,5α,7α,11β-H-eudesman-6α,12-olide (**5**); colourless solid, mp 115°C; $[\alpha]_D$= +14° ($CHCl_3$, c 1); IR (film): 1762, 1730 and 1247 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.78 (1H, ddd, J_1= J_2=5.12 Hz; J_3=11.9 Hz, H-3α), 3.91 (1H, dd, J_1=9.9 Hz; J_3=11.4 Hz, H-6β), 2.50 (m, H-11β), 2.00 (3H, s, AcO group), 1.18 (3H, d, J=6.9 Hz, 3H-13), 1.02 (3H, s, 3H-14) and 0.96 (3H, d, J=7.4 Hz, 3H-15); ^{1}C NMR ($CDCl_3$): δ 9.4 (C-15), 12.6 (C-13), 21.0 (C-14), 21.2 (*Me*CO), 22.6 (C-2), 23.5 (C-8), 31.7 (C-4), 35.9 (C-10), 39.9 (C-1), 41.8 (C-11), 43.2 (C-9), 49.6 (C-5), 53.6 (C-7), 75.1 (C-3), 79.7 (C-6), 170.2 (Me*CO*) and 179.2 (C-12); HRLSIMS, *m/z*: $[M+Na]^+$ 317.1733 ($C_{17}H_{26}O_4Na$ 317.1729, PPM –1.4).

Saponification of product 6. 3β-Acetoxy-4β,5α,7α,11β-H-eudesman-6α,12-olide (**6**, 20 mg) was dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) and maintained at 40-50°C for 2 h. The reaction mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 16 mg (95%) of 3β-hydroxy-4β,5α,7α,11β-H-eudesman-6α,12-olide (**3**).[6]

Saponification of product 7. 3α-Acetoxy-4β,5β,7α,11β-H-eudesman-6α,12-olide (**7**, 20 mg) was dissolved in MeOH/H_2O (70%) (4 ml) containing KOH (5%) and maintained at 40-50°C for 2 h. The reaction mixture was

extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 16 mg (95%) of 3α-hydroxy-4β,5β,7α,11β-H-eudesman-6α,12-olide (**4**); colourless solid, mp 93°C; $[\alpha]_D$= +25° ($CHCl_3$, c 1); IR (film): 3435 and 1771 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.25 (1H, dd, J_1=6.2 Hz; J_2=11.5 Hz, H-6β), 3.66 (1H, ddd, J_1=3.3 Hz; J_2=4.7 Hz; J_3=11.4 Hz, H-3β), 1.19 (3H, d, J=6.7 Hz, 3H-13), 1.05 (3H, d, J=7.3 Hz, 3H-15) and 1.00 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 12.3 (C-15), 12.6 (C-13), 23.8 (C-8), 25.3 (C-2), 30.3 (C-14), 34.5 (C-9), 34.6 (C-10), 35.1 (C-4), 40.3 (C-1), 42.1 (C-11), 44.1 (C-5), 46.1 (C-7), 73.5 (C-3), 82.4 (C-6) and 179.6 (C-12); HRLSIMS, *m/z*: $[M+Na]^+$ 275.1626 ($C_{15}H_{24}O_3Na$ 275.1623, PPM -1.2).

Enzymatic acetylation of products 2, 3 and 4 with CAL. *Candida antarctica* lipase (6 g) was added to a solution of a mixture of **2**, **3** and **4** (1 g) in vinyl acetate (70 mL). The suspension was shaken on an orbital shaker (180 rpm) at 40°C for 24 h. The reaction was finished by filtration of the enzyme and the products were isolated by flash chromatography yielding 303 mg (100%) of product **6**, 117 mg (100%) of product 7 and 620 mg of product **2**.

Reduction of product 2. 820 mg of product **2** were dissolved in 50 mL of dry THF and 10 mL of a solution of $LiAlH_4$ in THF (1 M) were added. The reaction mixture was maintained at 50°C for 1.5 h, and diluted with aqueous ether, extracted with CH_2Cl_2, dried with anhydrous Na_2SO_4 and evaporated to dryness. Chromatography over silica gel yielded 785 mg (95%) of 3β,6α,12-trihydroxy-4α,5α,7α,11β-H-eudesmane (**8**); colourless solid, mp 174 °C; $[\alpha]_D$= 0° (MeOH, c 1); IR (film): 3350 cm^{-1}; ^1H NMR ($(CD_3)_2SO$): δ 3.41 (1H, m, H-3α), 3.25 (3H, m, H-6β y 2H-12), 2.32 (1H, m, H-11β), 2.14 (1H, m, H-4α), 0.79 (3H, s, 3H-14, Me group), 0.76 (3H, d, J=7.3 Hz, Me group) and 0.74 (3H, d, J=6.8 Hz, Me group); ^1C NMR ($(CD_3)_2SO$): δ 8.2 (C-15), 11.0 (C-13), 19.1 (C-8), 20.5 (C-14), 25.6 (C-2), 33.2 (C-11), 33.6 (C-4), 33.7 (C-10), 40.0 (C-1), 43.1 (C-9), 46.1 (C-5), 51.8 (C-7), 65.2 (C-12), 66.0 (C-6) and 72.5 (C-3); HRLSIMS, *m/z*: $[M+Na]^+$ 279.1936 ($C_{15}H_{28}O_3Na$ 279.1936, PPM 0.2).

Total chemical acetylation of triol 8. 3β,6α,12-trihydroxy-4α,5α,7α,11β-H-eudesmane (**8**, 140 mg) was dissolved in Ac_2O/Py (1:2) (9 mL) and refluxed for 3 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4. Chromatography over silica gel yielded 200 mg (95%) of 3β,6α,12-triacetoxy-4α,5α,7α,11β-H-eudesmane (**9**).[4]

Enzymatic deacetylation of product 9 with CAL. *Candida antarctica* lipase (500 mg) was added to a solution of **9** (100 mg) in acetonitrile (5 mL) and *n*-butanol (0.5 mL). The suspension was shaken on an orbital shaker (180 rpm) at 40°C for 24 hours. The reaction was terminated by filtration of the enzyme and the products were isolated by flash chromatography yielding 84 mg (95%) of 3β,6α-diacetoxy-12-hydroxy-4α,5α,7α,11β-H-

eudesmane (**10**); syrup; $[\alpha]_D$= -69° ($CHCl_3$, c 1); IR (film): 3489, 1732 and 1244 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.05 (1H, dd, $J_1=J_2=10.6$ Hz, H-6β), 4.71 (1H, ddd, $J_1=J_2=4.8$ Hz; $J_3=12.1$ Hz, H-3α), 3.42 (2H, d, J=7.2 Hz, 2H-12), 2.00 (3H, s, AcO group), 1.99 (3H, s, AcO group), 0.91 (3H, s, 3H-14), 0.83 (3H, d, J=7.2 Hz, 3H-13) and 0.83 (3H, d, J=7.2 Hz, 3H-15); ^1C NMR ($CDCl_3$): δ 9.2 (C-15), 11.0 (C-13), 19.2 (C-8), 20.6 (C-14), 21.1 (*Me*CO), 21.4 (*Me*CO), 22.4 (C-2), 31.4 (C-11), 34.6 (C-10), 34.7 (C-4), 39.8 (C-1), 42.9 (C-9), 44.0 (C-5), 50.3 (C-7), 66.4 (C-12), 70.9 (C-6), 76.3 (C-3), 170.6 (Me*C*O) and 170.8 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 363.2147 ($C_{19}H_{32}O_5Na$ 363.2147, PPM 0.0).

Controlled chemical acetylation of triol 8. 3β,6α,12-Trihydroxy-4α,5α,7α,11β-H-eudesmane (**8**, 1 g) was dissolved in Ac_2O/Py (1:2) (60 mL) and stirred for 12 h at room temperature. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4. Chromatography over silica gel yielded 74 mg (5%) of 3β,6α,12-triacetoxy-4α,5α,7α,11β-H-eudesmane (**9**), 199 mg (15%) of 6α,12-diacetoxy-3β-hydroxy-4α,5α,7α,11β-H-eudesmane (**12**)[4] and 996 mg (75%) of 3β,12-diacetoxy-6α-hydroxy-4α,5α,7α,11β-H-eudesmane (**11**).[4]

Oxidation of product 11. Jones' reagent was added dropwise to a stirred solution of 3β,12-diacetoxy-6α-hydroxy-4α,5α,7α,11β-H-eudesmane (**11**, 400 mg) in acetone at 0°C 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 anhydrous Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 358 (90%) of 3β,12-diacetoxy-4α,5α,7α,11β-H-eudesman-6-one (**13**).[4]

Saponification and epimerization of product 13. 3β,12-Diacetoxy-4α,5α,7α,11β-H-eudesman-6-one (**13**, 80 mg) was dissolved in MeOH/H_2O (70%) (8 mL) containing KOH (5%) and maintained at 40-50°C for 1.5 h. The reaction mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 20 mg (35%) of 3β,12-dihydroxy-4α,5α,7α,11β-H-eudesman-6-one (**14**); syrup; $[\alpha]_D$= -38 ($CHCl_3$, c 0.2); IR (film): 3427 and 1697 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.61 (1H, ddd, $J_1=J_2=5.2$ Hz; $J_3=11.1$ Hz, H-3α), 3.55 (1H, dd, $J_1=5.4$ Hz; $J_2=10.7$ Hz, 1H-12), 3.41 (1H, dd, $J_1=7.9$ Hz; $J_2=10.7$ Hz, 1H-12), 1.09 (3H, d, J=7.1 Hz, Me group), 0.89 (3H, s, Me group) and 0.86 (3H, d, J=6.9 Hz, Me group); ^{13}C NMR ($CDCl_3$): δ 9.5 (C-15), 13.1 (C-13), 21.8 (C-14), 23.1 (C-8), 25.6 (C-2), 33.9 (C-4), 33.9 (C-11), 38.6 (C-10), 39.6 (C-1), 42.7 (C-9), 50.8 (C-5), 58.8 (C-7), 66.0 (C-12), 73.7 (C-3) and 212.1 (C-6); HRLSIMS, *m/z*, $[M+Na]^+$ 277.1769 ($C_{15}H_{26}O_3$ Na 277.1780, PPM 3.7); and 36 mg (60%) of 3β,12-dihydroxy-4α,5β,7α,11β-H-eudesman-6-one (**15**); syrup; $[\alpha]_D$= -62° ($CHCl_3$, c 1); IR (film): 3396 and 1692 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.78 (1H, ddd, $J_1=J_2=2.5$ Hz; $J_3=5.2$ Hz, H-3α), 3.46 (1H, dd, $J_1=10.7$ Hz; $J_2=21.4$ Hz, 1H-12), 3.44 (1H, dd, $J_1=10.7$ Hz; $J_2=22.5$ Hz, 1H-12), 2.50 (1H, ddd, $J_1=J_2=6.2$ Hz; $J_3=13.0$ Hz, H-7α), 0.87 (3H, d, J=7.0 Hz, Me group), 0.83 (3H, s, Me group) and 0.79 (3H, d, J=6.6 Hz, Me group); ^{13}C NMR

($CDCl_3$): δ 13.7 (C-15), 16.0 (C-13), 25.5 (C-8), 27.6 (C-14), 28.7 (C-2), 29.6 (C-1), 32.4 (C-9), 34.1 (C-11), 35.6 (C-4), 37.9 (C-10), 46.6 (C-5), 60.2 (C-7), 66.3 (C-12), 69.9 (C-3) and 216.6 (C-6); HRLSIMS, *m/z*, $[M+Na]^+$ 277.1779 ($C_{15}H_{26}O_3$ Na 277.1780, PPM 0.3). When this deacetylation reaction was carried out under the previously described conditions but for 3 h, only product **15** (95 %) was obtained.

Chemical acetylation of product 15. Product **15** (20 mg) was dissolved in Ac_2O/Py (1:2) (2 mL) and refluxed for 2 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4. Chromatography over silica gel yielded 25 mg (95%) of 3β,12-diacetoxy-4α,5β,7α,11β-H-eudesman-6-one (**16**); syrup; $[\alpha]_D$= +1° ($CHCl_3$, c 1); IR (film): 1737, 1703 and 1244 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.93 (1H, m, H-3α), 3.95 (1H, s, 1H-12), 3.93 (1H, s, 1H-12), 2.40 (1H, ddd, J_1=J_2=6.1 Hz; J_3=12.5 Hz, H-7α), 2.00 (3H, s, AcO group), 1.98 (3H, s, AcO group), 0.87 (3H, d, J=6.9 Hz, Me group), 0.84 (3H, s, Me group) and 0.68 (3H, d, J=6.4Hz, Me group); ^{13}C NMR ($CDCl_3$): δ 13.6 (C-15), 15.6 (C-13), 20.9 (*Me*CO), 21.1 (*Me*CO), 24.9 (C-8), 25.8 (C-2), 27.4 (C-14), 29.4 (C-1), 30.6 (C-11), 33.0 (C-9), 34.2 (C-4), 37.7 (C-10), 45.6 (C-5), 60.8 (C-7), 67.6 (C-12), 72.2 (C-3), 170.6 (Me*CO*), 171.1 (Me*CO*) and 214.0 (C-6); HRLSIMS, *m/z*, $[M+Na]^+$ 361.1989 ($C_{19}H_{30}O_5Na$ 361.1991, PPM 0.4).

Organism, media and culture conditions. *Rhizopus nigricans* and *Gliocadium roseum* CECT 2072 were obtained from the Colección Española de Cultivos Tipo, Departamento de Microbiología, Facultad de Ciencias, Universidad de Valencia, Spain, and were 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* or *G. roseum*. The cultures were incubated by shaking (150 rpm) at 28° for 6 days, after which substrates **1** and **2** in EtOH were added.

Biotransformation of substrate 10 with Rhizopus nigricans. Substrate **10** (335 mg) was dissolved in EtOH (8mL), distributed among 8 Erlenmeyer-flask cultures and incubated for 11 days, after which the cultures were filtered and pooled; the cells were washed thoroughly with water and the liquid was saturated with NaCl and extracted twice with CH_2Cl_2. Both extracts were pooled, dried with anhydrous Na_2SO_4, and evaporated at 40°C in vacuum to give a mixture of compounds. This mixture was chromatographed on a silica gel column to obtain 67 mg (20%) of starting material (**10**), 53 mg (15%) of 3β,6α-diacetoxy-2β,12-dihydroxy-4α,5α,7α,11β-H-eudesmane (**17**); syrup; $[\alpha]_D$= -44° ($CHCl_3$, c 1); IR (film): 3458, 1730 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.20 (1H, dd, J_1=J_2=10.6 Hz, H-6β), 4.76 (1H, dd, J_1=3.4 Hz; J_2=6.2 Hz; H-3α), 4.13 (1H, ddd, J_1=J_2=2.9 Hz; J_3=3.3 Hz, H-2α), 3.48 (1H, s, 1H-12), 3.46 (1H, s, 1H-12), 2.12 (3H, s, AcO group), 2.04 (3H, s, AcO group), 1.24 (3H, s, 3H-14), 1.10 (3H, d, J=7.6 Hz, Me group) and 0.89 (3H, d, J=6.9 Hz, Me group); ^{13}C

NMR ($CDCl_3$): δ 10.6 (C-15), 11.1 (C-13), 18.8 (C-8), 21.1 (*Me*CO), 21.2 (*Me*CO), 21.6 (C-14), 31.1 (C-11), 34.2 (C-10), 34.9 (C-4), 43.5 (C-9), 44.2 (C-5), 45.3 (C-1), 50.6 (C-7), 66.9 (C-12), 69.9 (C-2), 71.0 (C-6), 76.9 (C-3), 170.0 (Me*CO*) and 170.8 (Me*CO*); HRLSIMS, *m/z*, $[M+Na]^+$ 379.2097 ($C_{19}H_{32}O_6Na$ 379.2097, PPM -0.2); 70 mg (20%) of 3β,6α-diacetoxy-9β,12-dihydroxy-4α,5α,7α,11β-H-eudesmane (**18**); colourless solid, mp 137°C; $[\alpha]_D$= -66° ($CHCl_3$, c 1); IR (film): 3441, 1734 and 1243 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.09 (1H, dd, $J_1=J_2$=10.8 Hz, H-6β), 4.74 (1H, ddd, $J_1=J_2$=5.2 Hz; J_3=10.5 Hz, H-3α), 3.49 (1H, dd, J_1=6.7 Hz; J_2=10.5 Hz, 1H-12), 3.44 (1H, dd, J_1=7.8 Hz; J_2=10.5 Hz, 1H-12), 3.27 (1H, dd, J_1=4.3 Hz; J_2=11.4 Hz, H-9α), 2.04 (3H, s, AcO group), 2.02 (3H, s, AcO group), 0.94 (3H, s, 3H-14), 0.91 (3H, d, J=7.4 Hz, Me group) and 0.88 (3H, d, J=6.9 Hz, Me group); ^{13}C NMR ($CDCl_3$): δ 9.7 (C-15), 11.0 (C-13), 13.5 (C-14), 21.0 (*Me*CO), 21.3 (*Me*CO), 22.0 (C-2), 28.1 (C-8), 31.5 (C-11), 34.7 (C-4), 36.3 (C-1), 38.7 (C-10), 41.6 (C-5), 48.9 (C-7), 66.2 (C-12), 70.1 (C-6), 76.1 (C-3), 78.8 (C-9), 170.5 (Me*CO*) and 170.7 (Me*CO*); HRLSIMS, *m/z*, $[M+Na]^+$ 379.2102 ($C_{19}H_{32}O_6Na$ 379.2097, PPM –1.4); 62 mg (20%) of 6α-acetoxy-3β,9β,12-trihydroxy-4α,5α,7α,11β-H-eudesmane (**19**); colourless solid, mp 133°C; $[\alpha]_D$= -18° ($CHCl_3$, c 0.3); IR (film): 3383, 1732 and 1241 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.12 (1H, dd, $J_1=J_2$=10.8 Hz, H-6β), 3.65 (1H, ddd, $J_1=J_2$=6.6 Hz; J_3=9.3 Hz, H-3α), 3.48 (2H, m, 2H-12), 3.27 (1H, dd, J_1=4.3 Hz; J_2=11.4 Hz, H-9α), 2.05 (3H, s, AcO group), 0.93 (3H, s, 3H-14), 0.92 (3H, d, J=7.4 Hz, Me group), 0.89 (3H, d, J=7.0 Hz, Me group); ^{13}C NMR ($CDCl_3$): δ 8.8 (C-15), 11.0 (C-13), 13.5 (C-14), 21.1 (*Me*CO), 25.5 (C-2), 27.8 (C-8), 34.1 (C-11), 34.5 (C-4), 36.4 (C-1), 38.8 (C-10), 41.4 (C-5), 49.0 (C-7), 66.1 (C-12), 70.2 (C-6), 73.8 (C-3), 78.9 (C-9) and 170.7 (Me*CO*); HRLSIMS, *m/z*, $[M+Na]^+$ 337.1992 ($C_{17}H_{30}O_5Na$ 337.1991, PPM –0.4); and 54 mg (15%) of 3β,6α-diacetoxy-8α,12-dihydroxy-4α,5α,7α,11β-H-eudesmane (**20**); syrup; $[\alpha]_D$= -42° ($CHCl_3$, c 0.5); IR (film): 3304, 1735 and 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.06 (1H, dd, $J_1=J_2$=11.0 Hz, H-6β), 4.75 (1H, ddd, $J_1=J_2$=4.7 Hz; J_3=9.8 Hz, H-3α), 3.76 (1H, ddd, J_1= 4.7 Hz; $J_2=J_3$=10.8 Hz, H-8β), 3.71 (1H, dd, J_1=5.3 Hz; J_2=11.0 Hz, 1H-12), 3.61 (1H, dd, J_1=2.8 Hz; J_2=10.8 Hz, 1H-12), 2.05 (3H, s, AcO group), 2.03 (3H, s, AcO group), 1.04 (3H, d, J=7.2 Hz, Me group), 0.95 (3H, s, 3H-14), 0.84 (3H, d, J=7.4 Hz, Me group); ^{13}C NMR ($CDCl_3$): δ 9.1 (C-15), 10.6 (C-13), 21.0 (*Me*CO), 21.3 (*Me*CO), 21.3 (C-14), 22.1 (C-2), 31.6 (C-11), 33.2 (C-10), 34.0 (C-4), 39.6 (C-1), 49.8 (C-5), 52.1 (C-9), 54.5 (C-7), 64.3 (C-8), 67.9 (C-12), 70.8 (C-6), 76.1 (C-3), 170.5 (Me*CO*) and 170.8 (Me*CO*); HRLSIMS, *m/z*, $[M+Na]^+$ 379.2080 ($C_{19}H_{32}O_6Na$ 379.2097, PPM 4.3).

Biotransformation of substrate 10 with Giocadium roseum. Substrate **10** (420 mg) was dissolved in EtOH (10 mL), distributed among 10 Erlenmeyer flask cultures and incubated for 11 days, after which the cultures were processed as indicated above for the biotransformation of substrate **10** with *Rhizopus nigricans*. The resulting mixture was chromatographed on a silica gel column to obtain 358 mg (85%) of starting material (**10**), 3 mg (1%) of 3β,6α,12-trihydroxy-4α,5α,7α,11β-H-eudesmane (**8**) and 37 mg (10%) of 6α-acetoxy-3β,12-dihydroxy-4α,5α,7α,11β-H-eudesmane (**21**); syrup; $[\alpha]_D$= -41° ($CHCl_3$, c 1); IR (film): 3364, 1727 and

1243 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.09 (1H, dd, $J_1=J_2=10.8$ Hz, H-6β), 3.63 (1H, ddd, $J_1=J_2=5.0$ Hz; $J_3=11.3$ Hz, H-3α), 3.46 (1H, s, 1H-12), 3.44 (1H, s, 1H-12), 2.02 (3H, s, AcO group), 0.91 (3H, s, 3H-14), 0.86 (3H, d, J=6.9 Hz, Me group) and 0.85 (3H, d, J=7.4 Hz, Me group); ^{13}C NMR ($CDCl_3$): δ 8.4 (C-15), 11.1 (C-13), 19.4 (C-8), 20.7 (C-14), 21.0 (*Me*CO), 26.0 (C-2), 34.2 (C-11), 34.7 (C-10), 34.9 (C-4), 40.3 (C-1), 43.1 (C-9), 44.2 (C-5), 50.8 (C-7), 66.5 (C-12), 71.3 (C-6), 74.1 (C-3), and 170.7 (Me*CO*); HRLSIMS, *m/z*, $[M+Na]^+$ 321.2040 ($C_{17}H_{30}O_4Na$ 321.2042, PPM 0.6).

Lactonization of product 20. Solid TPAP (tetrapropylammonium perruthenate, 3 mg) was added in a single portion to a stirred mixture of product **20** (15 mg), NMO (4-methylmorpholine N-oxide, 15 mg) and activated powdered molecular sieves (15 mg) in dry CH_2Cl_2 (3 mL) at room temperature under argon atmosphere. On completion, the reaction mixture was concentrated in a vacuum. Purification by column chromatography on silica gel yielded 13 mg (90%) of 3β,6α-diacetoxy-4α,5α,7α,11β-H-eudesman-8α,12-olide (**22**); syrup; $[\alpha]_D$= -53 ° ($CHCl_3$, c 0.3); IR (film): 1785, 1732 and 1242 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.22 (1H, dd, $J_1=J_2=10.7$ Hz, H-6β), 4.76 (1H, ddd, $J_1=J_2=4.8$ Hz; $J_3=10.0$ Hz, H-3α), 4.31 (1H, ddd, $J_1=4.0$ Hz; $J_2=J_3=11.8$ Hz, H-8β), 2.64 (1H, dq, $J_1=J_2=7.6$ Hz, H-11β), 2.07 (3H, s, AcO group), 2.04 (3H, s, AcO group), 1.23 (3H, d, J=7.6 Hz, 3H-13), 1.07 (3H, s, 3H-14) and 0.88 (3H, d, J=7.5 Hz, 3H-15); ^{13}C NMR ($CDCl_3$): δ 9.7 (C-15), 9.7 (C-13), 21.0 (*Me*CO), 21.4 (*Me*CO), 22.1 (C-2), 22.3 (C-14), 30.7 (C-4), 35.3 (C-10), 37.6 (C-11), 39.3 (C-1), 46.6 (C-9), 51.5 (C-5), 54.1 (C-7), 66.9 (C-8), 75.3 (C-3), 75.4 (C-6), 170.1 (Me*CO*), 170.5 (Me*CO*) and 178.8 (C-12); HRLSIMS, *m/z*, $[M+Na]^+$ 375.1797 ($C_{19}H_{28}O_6Na$ 375.1784, PPM –3.6).

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9. Lipase from *Candida antarctica* (CAL) and *Mucor miehei* (MML) were generously supplied by Novo-Nordisk A/S.
10. Porcine pancreatic lipase (PPL) was purchased from Aldrich Chemical Co.
11. Program PC-Model for Windows available from Serena Software, P.O. Box 3076, Bloomington, IN 47402-3076.
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