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Chemical Semisynthesis and Biotransformation with *Rhizopus nigricans* of Several Sesquiterpenes: Obtention of New 1α- and 2α-Hydroxyselinane Derivatives

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Abstract—Starting with the natural product vulgarin, isolated from *Artemisia canariensis*, several acetylated, acetonated and oxidized polyhydroxylated eudesmanes and eudesmenes were semisynthesized. Some of these derivatives were biotransformed with the fungus *Rhizopus nigricans* and thus metabolites with new hydroxylation, reduction and/or deacetylation were isolated. Incubation of 1β,6α-diacetoxy-12-hydroxy-5α,11β-*H*-eudesm-4(15)-ene gave a 2-hydroxyselinane derivative in high yield (60%). Microbiological transformations of 1-oxo- and 6-oxoeudesmanes gave other useful hydroxyselinane derivatives in high proportions as the result of a stereoselective reduction of the carbonyl groups at these positions by the fungus on the β-face. Moreover, *R. nigricans* gave occasionally, regioselective deacetylated and/or hydrolyzed isopropylidene compounds.

Introduction

Eudesmane sesquiterpene are common in nature[1,2] and they possess remarkable biological properties,[3–6] particularly antimicrobial, antimalarial, antifeedant, cell-growth-inhibiting and plant-growth-regulating activities. They are often used as starting materials for the semisynthesis of other versatile products.[7–11] At present, biotransformation with hydroxylating fungi or with isolated enzymes constitutes a potent tool in organic synthesis,[12–17] since it permits access to remote positions on the molecule and thus bioconversion offers a useful alternative to chemical methods. In a previous work, we reported the biotransformation of several eudesmanolides from α-santonin[13] and different actions of the microorganisms on the eudesmane skeleton. These results have helped us to establish a relationship between the activities of fungi and the structure as well as the functionalization of eudesmanolide substrates. Moreover, in a recent paper,[18] we converted 6α,12-eudesmanolides into 8α,12-eudesmanolides by using chemical, enzymatic and microbiological procedures; thus, by biotransformation, we produced an 8α hydroxylated derivative which was lactonized to form the 8α,12-eudesmanolides.

In the present work, starting with the natural product vulgarin, isolated from *Artemisia canariensis*,[19] we obtained several polyhydroxylated eudesmanes, which we protected through acetylation by chemical means.[20] Moreover, from these hydroxylated eudesmanes with some protected positions, we have now prepared oxidized, acetonated and acetylated derivatives by chemical and enzymatic procedures. Furthermore, we described a series of biotransformations of certain appropriate eudesmane substrates, using *Rhizopus nigricans*. From these bioconversions, we have isolated metabolites in which stereoselective hydroxylation as well as reduction and regioselective deacetylations were observed. Thus, a C-1α hydroxyl group was achieved by microbial stereoselective reduction of a carbonyl group on this position, whereas a C-2α hydroxyl group appeared through a direct microbial hydroxylation. This approach led us to 1α- and 2α-hydroxyselinanes which, due their above-mentioned activities, have been attracting considerable attention.

Results and Discussion

The 4α-hydroxy-1-oxo-5α,11β-*H*-eudesm-2-en-6α,12-olide (vulgarin, **1**), a common sesquiterpene lactone in the genus *Artemisia*, is very abundant in *A. canariensis*.[19] Catalytic hydrogenation of **1** with H_2/Pt-C followed by reduction with $LiAlH_4$/THF gave its tetrahydroxy derivative **2**,[20] which, treated with Ac_2O/pyridine at room temperature, gave the 1,12-diacetoxy derivative **3**.[20] A similar treatment of **2** at 0°C yielded principally the 12-acetoxy derivative **4**.

Keywords: vulgarin; eudesmane; selinane; enzyme; lipase; biotransformation; fungi.
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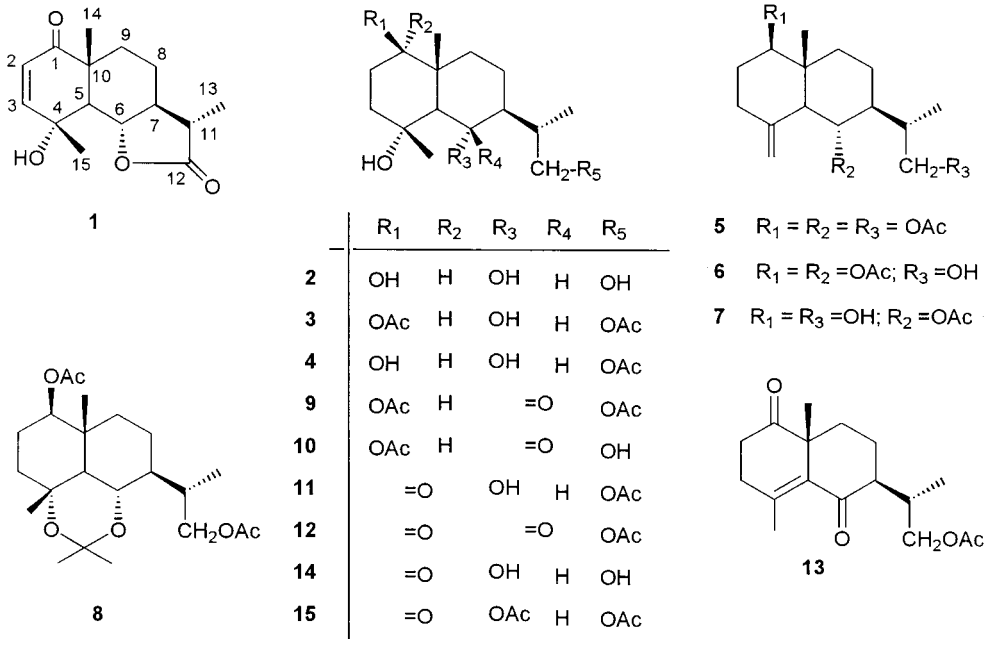

Figure 1. Structures of compounds **1**–**15**.

Acetylation of **2** with Ac_2O/Py at reflux gave the triacetoxy-4(15)-eudesmene derivative **5** in high yield as result of a dehydration between the hydroxyl group at C-4 and a C-15 proton in the acetylating medium. Regioselective enzymatic deacetylation of **5** with *Candida antarctica* lipase (CAL)[21] as a biocatalyst, *n*-butanol as a nucleophile, and acetonitrile as a solvent provided compounds **6** (80%) and **7** (10%). The site of deacetylation was easily determined by direct comparison of ^1H and ^{13}C NMR data of **5**–**7**. The major product **6** was the 1,6-diacetoxy derivative and it was the result of deacetylation at the primary alcohol (C-12) while the minor compound, **7**, had only an acetoxy group at C-6.

Acetonation of **3** with 2,2-dimethoxypropane yielded acetonide **8**, a new substrate for the following biotransformations. Oxidation of **3** with Jones' reagent gave the 6-oxo derivative **9**, which was partially deacetylated at C-12 and the monoacetoxy derivative **10** was obtained. Starting with 12-acetoxy derivative **4**, we also obtained a number of 1-oxo derivatives that were appropriate substrates to be later biotransformed with *R. nigricans*. Thus, the oxidation of **4** with Jones' reagent at 0°C for 45 min yielded 1-oxo derivative **11**, and when this oxidation treatment was maintained for 3 h, the 1,6-dioxo derivative **12** (45%) and the corresponding C-4/C-5 dehydrated compound **13** (45%) were

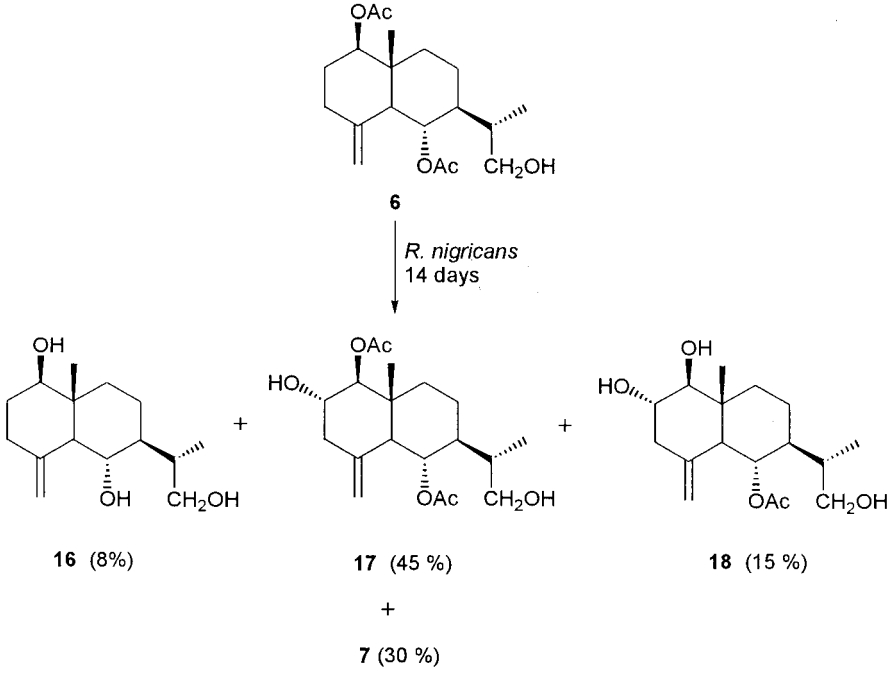

Figure 2. Biotransformation of substrate **6** with *R. nigricans*.

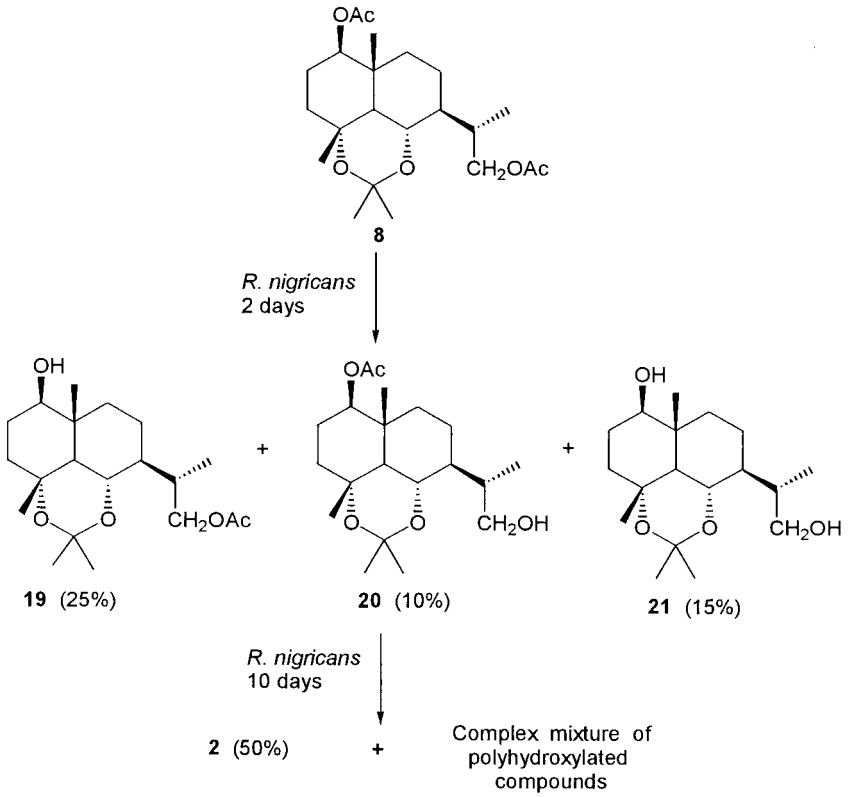

Figure 3. Biotransformation of substrate **8** with *R. nigricans*.

isolated. On the other hand, saponification of **11** gave 4α,6α,12-trihydroxy-5α,11β-*H*-eudesm-1-one (**14**) and chemical acetylation of this substrate (**11**) provided the diacetoxy derivative **15**, which are appropriate substrates for the following incubations. Structures of compounds **1**–**15** are summarized in Fig. 1.

Biotransformation of substrate **6** with *R. nigricans* for 14 days gave metabolites **7** (30%), **16** (8%), **17** (45%) and **18** (15%) (Fig. 2). The first metabolite of this biotransformation had physical and spectroscopic properties identical to those of metabolite **7**, derived previously from the enzymatic deacetylation with CAL at C-1 and C-12 of the triacetoxyeudesmene **5**. This compound (**7**) was now formed in this incubation by regioselective deacetylation by the fungus at C-1 from the substrate **6**. Metabolite **16** had a molecular ion peak of *m/z* 254 and its ^{1}H and ^{13}C NMR spectra showed no signal for the acetoxy group. Therefore, product **16** was the result of a double microbiological deacetylation of substrate **6**, and the structure of 1β,6α,12-trihydroxy-5α,11β-*H*-eudesm-4(15)-ene was proposed for **16**. The main metabolite (**17**) isolated from this biotransformation, had a high resolution mass spectrum with a molecular ion peak of *m/z* 354, indicating that substrate **6** had been hydroxylated by *R. nigricans*. Moreover, the ^{1}H NMR spectrum of **17** showed that H-1 was now a doublet (δ 4.64, 1H, *J*=9.6 Hz) and that, at 3.68 ppm, there was a new signal (1H, ddd, J_1=5.5 Hz, J_2=9.6 Hz, J_3=11.3 Hz). Based on these observations and the ^{13}C NMR spectrum of **17** (new oxygenated methine at 70.6 ppm), we concluded that the new hydroxyl group was situated at C-2 with an α-disposition. The new hydroxyl configuration was established by the H-2 coupling constant with H-1α (approximately axial–axial value, *J*=9.6 Hz) and the α, β and γ effects of this hydroxyl group on the chemical shifts of the C-2, C-1 or C-3 and, C-4 or C-10 atoms, respectively. Metabolite **18** also had a new hydroxyl group of which the geminal proton resonated at δ 3.30 (1H, ddd, J_1=5.4 Hz, J_2=9.0 Hz, J_3=11.1 Hz). The proton geminal to the C-1 hydroxyl group appeared in compound **18** also as a doublet (3.18 ppm, 1H, *J*=9.0 Hz) but it was more shielded than in product **17**. These spectroscopic observations and the comparison of the ^{13}C NMR spectra for substrate **6** and metabolite **18** indicated a double action of *R. nigricans* on substrate **6**, a C-2α hydroxylation and a C-1 deacetylation.

To test the behaviour of *R. nigricans* with an isopropylidene eudesmane derivative as a substrate, we incubated compound **8** with this fungus for 2 days. From this incubation, products **19** (25%), **20** (10%) and **21** (15%) and unaltered substrate **8** were isolated (Fig. 3). Metabolites **19** and **20** showed identical molecular ion peaks of *m/z* 354, indicating a loss of an acetoxy group from **8** at C-1 or C-12, respectively. However, metabolite **21** had a molecular mass of *m/z* 312, and therefore it was the double-deacetylated compound. From these results, corroborated by the ^{1}H and ^{13}C NMR spectra of **19**–**21**, we deduced that, in this case, the fungus partially or totally deacetylated the substrate **8** during the first few days of biotransformation. When maintained for 10 days, this biotransformation produced a complex mixture of polyhydroxylated compounds. From this mixture, we isolated the previously known tetrol **2**, the appearance of which in

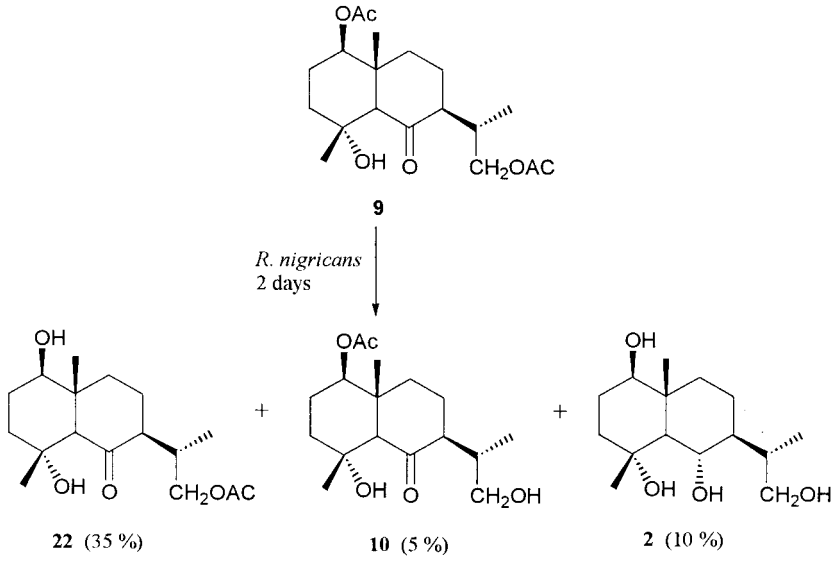

Figure 4. Biotransformation of substrate **9** with *R. nigricans*.

this incubation was due to a double deacetylation and an opening of isopropylidenedioxy ring in substrate **8**.

Afterwards, to determine the action of the microorganism on the eudesmane skeleton when a ketone group was situated at C-6 or C-1, we incubated two oxoeudesmanes (compounds **9** and **15**) with *R. nigricans*. Incubation of the 6-oxo derivative, **9**, for 2 days with this fungus yielded metabolites **2** (10%), **10** (5%) and **22** (35%) (Fig. 4). The first metabolite isolated from this incubation had physical and spectroscopic properties identical to those of tetrol **2**. Therefore, compound **2** was formed by reduction of the ketone group at C-6 by the fungus, on the β-face, to give a 6α-hydroxyl group. The second metabolite isolated, which coincided in its spectroscopic characteristics with the previously semisynthesized product **10**, arose from the regioselective deacetylation at C-12 by *R. nigricans*. On the other hand, the last metabolite, **22**, had an identical molecular ion peak to **10** (*m/z* 312) but now, in its ^{1}H NMR spectrum, the H-1 signal was significantly shielded (δ 3.49, 1H, dd, J_1=4.3 Hz; J_2=10.4 Hz), indicating that this position had been regioselectively deacetylated by the fungus.

The last bioconversion carried out with *R. nigricans* as the fungus and the 1-oxo derivative **15** as the substrate gave metabolites **11** (25%), **14** (10%), **23** (10%), **24** (25%) and **25** (15%) (Fig. 5). The structures of first two metabolites were determined by comparison of their physical and spectroscopic properties with those of the previous compounds **11** and **14**. These products were the result of a partial (at C-6, product **11**) or total (at C-6 and C-12, product

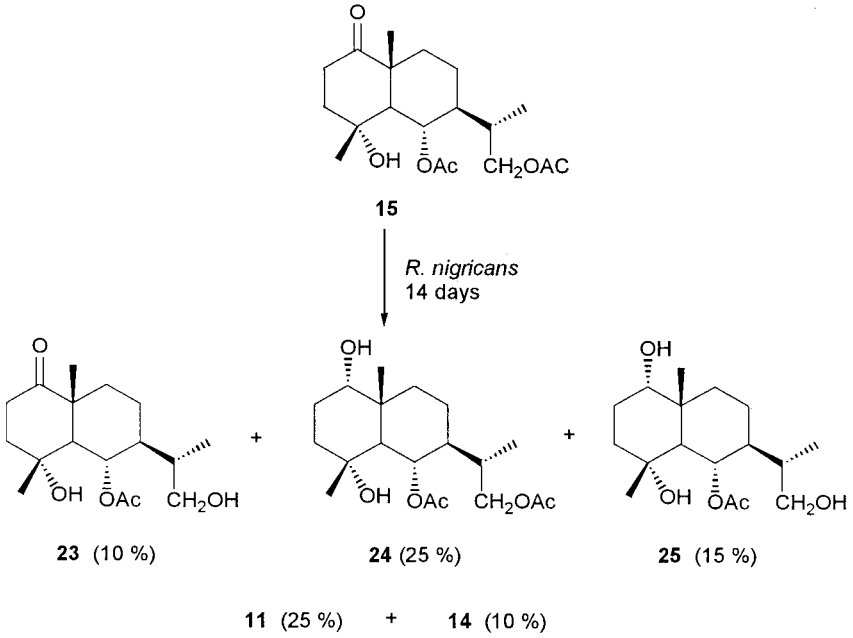

Figure 5. Biotransformation of substrate **15** with *R. nigricans*.

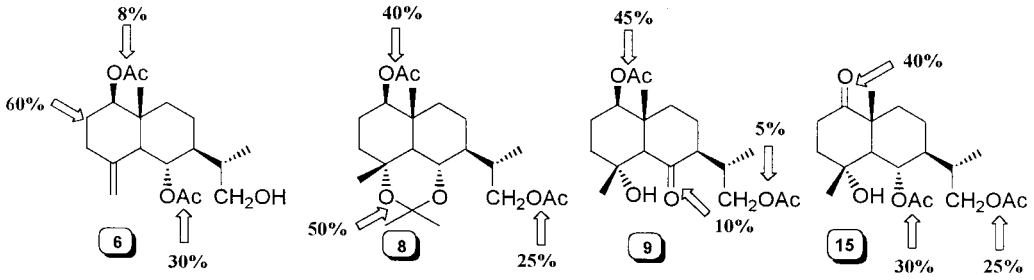

Figure 6. Summarized results of the biotransformation of substrates **6**, **8**, **9** and **15** with *R. nigricans*.

14) deacetylation of the substrate **15** by the fungus. Metabolite **23** had the same molecular mass as **11** (*m/z* 312), indicating that it was the result of a new deacetylation at C-12 from substrate **15**. This deduction was corroborated by the shielding at the 2H-12 signals in the ^{1}H NMR spectrum of **23** (δ 3.93 and δ 3.86 for **15** and δ 3.50 and δ 3.44 for **23**). Metabolite **24** possessed spectra that were very different from that of substrate **15**, and thus, its HRMS showed a molecular ion peak (*m/z* 356) two units larger than those of **15** (*m/z* 354). Moreover, metabolite **24** had no ketone character (no signal of a carbonyl group in its ^{13}C NMR spectra) and hence the carbonyl group present at C-1 in substrate **15** was reduced by the microorganism to give a hydroxyl group. The geminal proton to the new hydroxyl group was equatorial, as can be seen in the ^{1}H NMR spectrum (δ 3.35, 1H, dd, J_1=J_2=3.5 Hz). In this signal, the J_1 and J_2 values indicated that the microbial reduction had occurred on the β-face, giving a (*S*)-hydroxyl group at C-1. This configuration at C-1 was also confirmed by the α and γ effects of the equatorial hydroxyl group in this position. Thus, by comparing the ^{13}C NMR spectra of **24** (1α-OH) and **4** (1β-OH), we could discern, above all, a sharply different α-effect on C-1 (δ 78.6 for **4** and δ 73.9 for **24**) and opposite γ-effects for C-3 and C-5 (40.4 and 56.0 ppm for **4** and 36.0 and 51.2 ppm for **24**) and C-14 (δ 14.0 for **4** and δ 19.7 for **24**). Finally, metabolite **25** also showed a geminal proton 1(*S*)-hydroxyl signal (δ 3.35, 1H, dd, J_1=J_2=3.5 Hz), but it had a molecular ion peak of *m/s* 314, and similar α and γ effects to those of **24** were detected in its ^{13}C NMR spectrum. On the basis of these results, we conclude that, in this case, there was a double action of the microorganism on substrate **15**, a regioselective deacetylation at C-12 and a C-1 reduction on the β-face.

In addition, to ascertain that the deacetylation process was due to the microorganism and not to the medium, the substrates **6**, **8**, **9** and **15** were maintained in similar incubation media, but now without the fungus, at pH 5.7, for several days. The results of these control experiments were studied periodically by TLC and, after 8 days, no deacetylated product was detected. Only in the case of substrate **8**, did we isolate a small quantity of product **3** as the result of the opening of the isopropylidenedioxy ring by acid medium (as it is indicated in Fig. 3, when the biotransformation of substrate **8** was maintained for 10 days). Therefore, we conclude that the hydrolysis of these acetoxy derivatives did not take place in the biotransformation medium alone but rather they occur through a microbial process.

Conclusions

Several conclusions can be drawn from the above biotransformation results, enabling us to establish a relationship between the structure of the substrate and the action and site where the fungal enzymes act (Fig. 6). Thus, when the substrate was a C-4/C-15 eudesmene, *R. nigricans* hydroxylated C-2 on the α-face in high yield (60%), forming a C-2α hydroxyl derivative. Moreover, *R. nigricans* provided a polyhydroxylated compound in high yield by opening the isopropylidenedioxy ring between C-4 and C-6 of eudesmane. Both 1- and 6-oxo eudesmanes were reduced by this fungus on the β-face, but the ketone group at C-1 was more reduced than the one at C-6 and thus a high proportion (40%) of C-1α hydroxyl derivative was obtained. In all cases, there was a regioselective microbial deacetylation at the C-1, C-6 and/or C-12 positions of the eudesmane skeleton. Therefore, starting of the abundant natural product vulgarin, and by combining both microbial and chemical methods, we produced several attractive hydroxyselinane derivatives.

Experimental

General

Measurements of NMR spectra (300.13 MHz ^{1}H 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. 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). Melting points were determined using a Kofler (Reichter) apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at 25°C. 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°C. *C. antarctica* lipase (CAL)[21] (Novozym 435 acrylic resin supported lipase

produced by the host organism *Aspergillus oryzae*, after transfer of the genetic coding for lipase B from *C. antarctica*) was generously donated by Novo Nordisk Bioindustrial Group.

Catalytic hydrogenation of vulgarin (1). Vulgarin (**1**) (4α-hydroxy-1-oxo-5α,11β-*H*-eudesm-2-en-6α,12-olide, 3 g) was hydrogenated with H_2 (4 atm) on Pt/C and reduced with $LiAlH_4$ in THF to give 1β,4α,6α,12-tetrahydroxy-5α,11β-*H*-eudesmane (**2**) (2.58 g).[20]

Acetylation at room temperature of 2. 1β,4α,6α,12-Tetrahydroxy-5α,11β-*H*-eudesmane (**2**, 1 g) treated with Ac_2O/Py. Chromatography over silica gel yielded 1β,12-diacetoxy-4α,6α-dihydroxy-5α,11β-*H*-eudesmane (**3**) (1.17 g).[20]

Cold acetylation of 2. 1β,4α,6α,12-Tetrahydroxy-5α,11β-*H*-eudesmane (**2**, 1 g) was dissolved in Ac_2O/Py (1:2) (60 mL) and stirred for 6 h at 0°C. 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 325 mg (25%) of 1β,12-diacetoxy-4α,6α-dihydroxy-5α,11β-*H*-eudesmane (**3**) and 805 mg (70%) of 12-acetoxy-1β,4α,6α-trihydroxy-5α,11β-*H*-eudesmane (**4**); colourless solid; mp 110–112°C; $[\alpha]_D^{25}=-16$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3347, 1737, 1243, 1067 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.96 (1H, dd, $J_1=7.3$ Hz; $J_2=10.8$ Hz, H-12), 3.89 (1H, dd, $J_1=7.1$ Hz; $J_2=10.8$ Hz, H-12), 3.85 (1H, dd, $J_1=J_2=10.3$ Hz, H-6β), 3.27 (1H, dd, $J_1=4.3$ Hz; $J_2=10.6$ Hz, H-1α), 2.02 (3H, s, AcO group), 1.30 (3H, s, 3H-15), 0.86 (3H, d, $J=7.0$ Hz, 3H-13), 0.81 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 11.1 (C-13), 14.0 (C-14), 19.1 (C-8), 21.1 (*Me*CO), 23.5 (C-15), 28.1 (C-2), 30.4 (C-11), 39.1 (C-9), 40.3 (C-10), 40.4 (C-3), 46.8 (C-7), 56.0 (C-5), 68.3 (C-12), 69.8 (C-6), 73.7 (C-4), 78.6 (C-1), 171.6 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 337.1996 ($C_{17}H_{30}O_5Na$, 337.1991, PPM −1.5).

Acetylation at reflux of 2. 1β,4α,6α,12-Tetrahydroxy-5α,11β-*H*-eudesmane (**2**, 600 mg) was dissolved in Ac_2O/Py (1:2) (36 mL) and stirred for 4 h at reflux. 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 670 mg (80%) of 1β,6α,12-triacetoxy-5α,11β-*H*-eudesm-4(15)-ene (**5**); colourless syrup; $[\alpha]_D^{25}=2$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 1734, 1652, 1238 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.03 (1H, dd, $J_1=J_2=10.5$ Hz, H-6β), 4.77 (1H, bs, H-15), 4.63 (1H, dd, $J_1=4.8$ Hz; $J_2=11.7$ Hz, H-1α), 4.51 (1H, bs, H-15), 3.87 (1H, dd, $J_1=7.2$ Hz; $J_2=10.9$ Hz, H-12), 3.83 (1H, dd, $J_1=7.8$ Hz; $J_2=10.9$ Hz, H-12), 1.99 (3H, s, AcO group), 1.97 (3H, s, AcO group), 1.93 (3H, s, AcO group), 0.85 (3H, d, $J=6.9$ Hz, 3H-13), 0.75 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 11.0 (C-13), 12.6 (C-14), 18.4 (C-8), 20.9 (*Me*CO), 21.1 (*Me*CO), 21.1 (*Me*CO), 28.6 (C-2), 30.9 (C-11), 34.6 (C-9), 35.4 (C-3), 41.0 (C-10), 43.6 (C-7), 53.4 (C-5), 67.4 (C-12), 69.5 (C-6), 80.0 (C-1), 108.2 (C-15), 143.8 (C-4), 170.0 (Me*C*O), 170.6 (Me*C*O), 171.1 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 403.2099 ($C_{21}H_{32}O_6Na$, 403.2096, PPM −0.7).

Enzymatic deacetylation of 5 with CAL. *C. antarctica* lipase (3 g) was added to a solution of **5** (500 mg) in acetonitrile (25 mL) and *n*-butanol (2.5 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 356 mg (80%) of 1β,6α-diacetoxy-12-hydroxy-5α,11β-*H*-eudesm-4(15)-ene (**6**); colourless syrup; $[\alpha]_D^{25}=0$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3450, 3087, 1730, 1238 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.10 (1H, dd, $J_1=J_2=10.5$ Hz, H-6β), 4.81 (1H, bs, H-15), 4.57 (1H, bs, H-15), 4.66 (1H, dd, $J_1=4.8$ Hz; $J_2=11.7$ Hz, H-1α), 3.48 and 3.45 (2H, AB collapsed system, 2H-12), 2.02 (3H, s, AcO group), 1.98 (3H, s, AcO group), 0.87 (3H, d, $J=6.9$ Hz, 3H-13), 0.80 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 11.0 (C-13), 12.7 (C-14), 18.5 (C-8), 21.3 (*Me*CO), 21.3 (*Me*CO), 28.7 (C-2), 34.5 (C-11), 34.8 (C-9), 35.6 (C-3), 41.2 (C-10), 43.3 (C-7), 53.6 (C-5), 66.4 (C-12), 69.8 (C-6), 80.2 (C-1), 108.3 (C-15), 144.0 (C-4), 170.8 (Me*C*O), 171.3 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 361.1991 ($C_{19}H_{30}O_5Na$, 361.1991, PPM −0.1); and 39 mg (10%) of 6α-acetoxy-1β,12-dihydroxy-5α,11β-*H*-eudesm-4(15)-ene (**7**); colourless solid; mp 109–111°C; $[\alpha]_D^{25}=0$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3405, 3087, 1728, 1252 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.11 (1H, dd, $J_1=J_2=10.5$ Hz, H-6β), 4.79 (1H, bs, H-15), 4.54 (1H, bs, H-15), 3.48 (1H, dd, $J_1=6.7$ Hz; $J_2=11.0$ Hz, H-12), 3.52 (1H, dd, $J_1=7.8$ Hz; $J_2=11.0$ Hz, H-12), 3.42 (1H, dd, $J_1=4.7$ Hz; $J_2=11.6$ Hz, H-1α), 1.98 (3H, s, AcO group), 0.88 (3H, d, $J=6.9$ Hz, 3H-13), 0.72 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 11.0 (C-13), 11.7 (C-14), 18.6 (C-8), 21.3 (*Me*CO), 32.2 (C-2), 34.6 (C-11), 35.1 (C-9), 35.9 (C-3), 42.2 (C-10), 43.3 (C-7), 53.5 (C-5), 66.4 (C-12), 70.2 (C-6), 79.1 (C-1), 107.9 (C-15), 144.6 (C-4), 171.3 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 319.1886 ($C_{17}H_{28}O_4Na$, 319.1885, PPM −0.3).

Acetonation of 3. Product **3** (1β,12-diacetoxy-4α,6α-dihydroxy-5α,11β-*H*-eudesmane, 600 mg) was treated with 2,2-dimethoxypropane (60 mL) and a catalytic amount of pyridinium toluene-4-sulfonate at reflux for 3.5 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 600 mg (90%) of 1β,12-diacetoxy-4α,6α-isopropylidenedioxy-5α,11β-*H*-eudesmane (**8**); colourless solid; mp 75–77°C; $[\alpha]_D^{25}=-37$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 1739, 1240 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.55 (1H, dd, $J_1=4.9$ Hz; $J_2=11.1$ Hz, H-1α), 3.97 (1H, dd, $J_1=7.3$ Hz; $J_2=11.2$ Hz, H-12), 3.93 (1H, dd, $J_1=7.3$ Hz; $J_2=11.2$ Hz, H-12), 3.74 (1H, dd, $J_1=J_2=10.0$ Hz, H-6β), 2.03 (3H, s, AcO group), 2.00 (3H, s, AcO group), 1.40 and 1.43 (3H each, s, Me groups of isoproyliden group), 1.30 (3H, s, 3H-15), 0.88 (3H, s, 3H-14), 0.87 (3H, d, $J=7.1$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 12.0 (C-13), 15.6 (C-14), 19.0 (C-8), 21.1 (*Me*CO), 21.2 (*Me*CO), 24.2 (C-15), 24.7 (C-2), 26.1 (C-11), 31.3 and 32.3 (Me groups of isoproyliden group), 37.4 (C-10), 39.8 (C-3), 39.8 (C-9), 44.2 (C-7), 52.1 (C-5), 65.0 (C-6), 68.2 (C-12), 72.2 (C-4), 80.6 (C-1), 98.4 (quaternary C of isopropylidene group), 170.7 (Me*C*O), 171.3 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 419.2403 ($C_{22}H_{36}O_6Na$, 419.2409, PPM 1.6).

Oxidation at C-6 of 3. Jones' reagent was added dropwise to a stirred solution of 1β,12-diacetoxy-4α,6α-dihydroxy-

5α,11β-*H*-eudesmane (**3**, 500 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 447 mg (90%) of 1β,12-diacetoxy-4α-hydroxy-5α,11β-*H*-eudesman-6-one (**9**); colourless syrup; $[\alpha]_D^{25}=19$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 1737, 1239 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.72 (1H, dd, $J_1=4.8$ Hz; $J_2=11.0$ Hz, H-1α), 3.96 (1H, dd, $J_1=6.2$ Hz; $J_2=10.9$ Hz, H-12), 3.88 (1H, dd, $J_1=8.1$ Hz; $J_2=10.9$ Hz, H-12), 2.40 (1H, s, H-5α), 2.05 (3H, s, AcO group), 2.04 (3H, s, AcO group), 1.49 (3H, s, 3H-15), 0.92 (3H, s, 3H-14), 0.86 (3H, d, $J=7.0$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 12.7 (C-13), 16.3 (C-14), 21.0 (*Me*CO), 21.2 (*Me*CO), 21.5 (C-8), 24.1 (C-15), 24.8 (C-2), 30.2 (C-11), 38.2 (C-9), 38.7 (C-3), 42.6 (C-10), 50.8 (C-7), 64.9 (C-5), 66.7 (C-12), 70.5 (C-4), 79.2 (C-1), 170.7 (Me*C*O), 171.2 (Me*C*O), 211.8 (C-6); HRLSIMS, *m/z*: $[M+Na]^+$ 377.1944 ($C_{19}H_{30}O_6Na$, 377.1940, PPM −1.1).

Partial saponification of 9. 1β,12-Diacetoxy-4α-hydroxy-5α,11β-*H*-eudesman-6-one (**9**, 50 mg) was dissolved in MeOH/H_2O (70%) (4 mL) containing KOH (5%) and maintained at 0°C for 3 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 40 mg (90%) of 1β-acetoxy-4α,12-dihydroxy-5α,11β-*H*-eudesman-6-one (**10**); colourless syrup; $[\alpha]_D^{25}=24$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3452, 1738, 1241 cm^{-1}; ^1H ($CDCl_3$): δ 4.70 (1H, dd, $J_1=4.7$ Hz; $J_2=10.9$ Hz, H-1α), 3.52 (1H, dd, $J_1=5.7$ Hz; $J_2=10.6$ Hz, H-12), 3.39 (1H, dd, $J_1=8.2$ Hz; $J_2=10.6$ Hz, H-12), 2.40 (1H, s, H-5α), 2.03 (3H, s, AcO group), 1.48 (3H, s, 3H-15), 0.90 (3H, s, 3H-14), 0.82 (3H, d, $J=6.9$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 12.7 (C-13), 16.2 (C-14), 21.2 (*Me*CO), 21.8 (C-8), 24.0 (C-15), 24.8 (C-2), 33.5 (C-11), 38.2 (C-3), 38.8 (C-9), 42.7 (C-10), 50.8 (C-7), 64.9 (C-5), 65.5 (C-12), 70.5 (C-4), 79.2 (C-1), 170.7 (Me*C*O), 213.1 (C-6); HRLSIMS, *m/z*: $[M+Na]^+$ 335.1837 ($C_{17}H_{28}O_5Na$, 335.1834, PPM −0.9).

Oxidation at C-1 of 4. Jones' reagent was added dropwise to a stirred solution of 12-acetoxy-1β,4α,6α-trihydroxy-5α,11β-*H*-eudesmane (**4**, 900 mg) in acetone at 0°C until an orange-brown colour persisted (45 min), following the monooxidation by TLC. 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 715 mg (80%) of 12-acetoxy-4α,6α-dihydroxy-5α,11β-*H*-eudesman-1-one (**11**); colourless syrup; $[\alpha]_D^{25}=-67$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3402, 1737, 1711, 1239 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.96 (1H, dd, $J_1=7.5$ Hz; $J_2=10.9$ Hz, H-12), 3.93 (1H, dd, $J_1=7.1$ Hz; $J_2=10.9$ Hz, H-12), 3.86 (1H, dd, $J_1=J_2=10.2$ Hz, H-6β), 2.04 (3H, s, AcO group), 1.49 (3H, s, 3H-15), 1.03 (3H, s, 3H-14), 0.89 (3H, d, $J=7.1$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 11.2 (C-13), 18.8 (C-8), 18.9 (C-14), 21.1 (*Me*CO), 25.7 (C-15), 30.6 (C-11), 34.6 (C-3), 34.8 (C-2), 37.5 (C-9), 46.4 (C-7), 46.7 (C-10), 55.3 (C-5), 68.0 (C-12), 69.4 (C-6), 72.1 (C-4), 171.5 (Me*C*O), 215.6 (C-1); HRLSIMS, *m/z*: $[M+Na]^+$ 335.1826 ($C_{17}H_{28}O_5Na$, 335.1834, PPM 2.4).

Oxidation at C-1 and C-6 of 4. Jones' reagent was added dropwise to a stirred solution of 12-acetoxy-1β,4α,6α-trihydroxy-5α,11β-*H*-eudesmane (**4**, 50 mg) in acetone at 0°C until an orange-brown colour persisted (3 h). 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 22 mg (45%) of 12-acetoxy-4α-hydroxy-5α,11β-*H*-eudesman-1,6-dione (**12**); colourless syrup; $[\alpha]_D^{25}=4$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3537, 1737, 1709, 1243 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.97 (1H, dd, $J_1=6.1$ Hz; $J_2=10.9$ Hz, H-12), 3.88 (1H, dd, $J_1=7.9$ Hz; $J_2=10.9$ Hz, H-12), 2.71 (1H, s, H-5α), 2.02 (3H, s, AcO group), 1.70 (3H, s, 3H-15), 1.09 (3H, s, 3H-14), 0.87 (3H, d, $J=7.0$ Hz, 3H-13). ^{13}C NMR ($CDCl_3$): δ 12.8 (C-13), 20.4 (C-14), 21.0 (*Me*CO), 21.2 (C-8), 23.7 (C-15), 30.2 (C-11), 34.0 (C-2), 35.1 (C-3), 39.4 (C-9), 50.6 (C-7), 51.9 (C-10), 65.1 (C-5), 66.7 (C-12), 69.9 (C-4), 171.1 (Me*C*O), 211.4 (C-1), 211.6 (C-6); HRLSIMS, *m/z*: $[M+Na]^+$ 333.1672 ($C_{17}H_{26}O_5Na$, 333.1678, PPM 1.7) and 17 mg (44%) of 12-acetoxy-11β-*H*-eudesm-4-en-1,6-dione (**13**); colourless syrup; $[\alpha]_D^{25}=11$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 1738, 1718, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.01 (1H, dd, $J_1=5.6$ Hz; $J_2=11.2$ Hz, H-12), 3.95 (1H, dd, $J_1=7.5$ Hz; $J_2=11.2$ Hz, H-12), 2.03 (3H, s, AcO group), 1.81 (3H, s, 3H-15), 1.14 (3H, s, 3H-14), 0.92 (3H, d, $J=7.0$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 12.9 (C-13), 20.6 (C-14), 21.0 (C-8), 21.2 (*Me*CO), 23.9 (C-15), 30.6 (C-11), 32.6 (C-3), 32.6 (C-2), 35.1 (C-9), 49.8 (C-10), 52.0 (C-7), 67.2 (C-12), 136.7 (C-4), 138.5 (C-5), 171.2 (Me*C*O), 204.8 (C-6), 213.2 (C-1); HRLSIMS, *m/z*: $[M+Na]^+$ 315.1571 ($C_{17}H_{24}O_4Na$, 315.1572, PPM 0.5).

Saponification of 11. 12-Acetoxy-4α,6α-dihydroxy-5α,11β-*H*-eudesman-1-one (**11**, 35 mg) was dissolved in MeOH/H_2O (70%) (3 mL) containing KOH (5%) and maintained at 0°C for 1 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 24 mg (80%) of 4α,6α,12-trihydroxy-5α,11β-*H*-eudesman-1-one (**14**); colourless solid; mp 131–133°C; $[\alpha]_D^{25}=-66$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3345, 1710, 1011 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.89 (1H, dd, $J_1=J_2=10.2$ Hz, H-6β), 3.64 (1H, dd, $J_1=4.8$ Hz; $J_2=10.6$ Hz, H-12), 3.50 (1H, dd, $J_1=7.9$ Hz; $J_2=10.6$ Hz, H-12), 1.50 (3H, s, 3H-15), 1.05 (3H, s, 3H-14), 0.92 (3H, d, $J=7.1$ Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 13.4 (C-13), 18.9 (C-14), 21.1 (C-8), 25.7 (C-15), 34.8 (C-2), 34.9 (C-9), 36.1 (C-11), 37.5 (C-3), 46.7 (C-10), 47.1 (C-7), 55.5 (C-5), 66.5 (C-12), 70.6 (C-6), 72.2 (C-4), 215.9 (C-1); HRLSIMS, *m/z*: $[M+Na]^+$ 293.1730 ($C_{15}H_{26}O_4Na$, 293.1729, PPM −0.5).

Acetylation of 14. 4α,6α,12-Trihydroxy-5α,11β-*H*-eudesman-1-one (**14**, 900 mg) was dissolved in Ac_2O/Py (1:2) (48 mL) and stirred for 92 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 945 mg (80%) of 6α,12-diacetoxy-4α-hydroxy-5α,11β-*H*-eudesman-1-one (**15**); colourless solid; mp 43–45°C; $[\alpha]_D^{25}=-50$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3588, 1736, 1240 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.24 (1H, dd,

$J_1=J_2=10.6$ Hz, H-6β), 3.93 (1H, dd, $J_1=6.8$ Hz; $J_2=10.9$ Hz, H-12), 3.86 (1H, dd, $J_1=8.1$ Hz; $J_2=10.9$ Hz, H-12), 2.14 (3H, s, AcO group), 2.05 (3H, s, AcO group), 1.24 (3H, s, 3H-15), 1.09 (3H, s, 3H-14), 0.90 (3H, d, $J=6.9$ Hz, 3H-13); ^{13}C ($CDCl_3$): δ 11.1 (C-13), 18.6 (C-8),18.7 (C-14), 21.1 (*Me*CO), 21.8 (*Me*CO), 23.5 (C-15), 30.7 (C-11), 34.5 (C-2), 34.8 (C-3), 37.1 (C-9), 44.8 (C-7), 47.1 (C-10), 55.1 (C-5), 67.4 (C-12), 71.4 (C-4), 72.7 (C-6), 170.2 (Me*C*O), 171.1 (Me*C*O), 215.0 (C-1); HRLSIMS, *m/z*: $[M+Na]^+$ 337.1942 ($C_{19}H_{30}O_6Na$, 377.1940, PPM −0.5).

Organism, media and culture conditions. *R. nigricans* was obtained from the Colección Española de Cultivos Tipo, Departamento de Microbiología, Facultad de Ciencias, Universidad de Valencia, Spain, and was kept in YEPGA medium containing yeast extract (1%), peptone (1%), glucose (2%) and agar (2%) in H_2O at pH 5. In all transformation experiments a medium of peptone (0.1%), yeast extract (0.1%), beef extract (0.1%) and glucose (0.5%) in H_2O at pH 5.7 was used. Erlenmeyer flasks (250 mL) containing 80 mL of medium were inoculated with a dense suspension of *R. nigricans*. The cultures were incubated by shaking (150 rpm) at 28°C for 6 days, after which the different substrates in EtOH were added.

Biotransformation of 6. Substrate **6** (320 mg) was dissolved in EtOH (12 mL), distributed among six Erlenmeyer-flask cultures and incubated for 14 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 84 mg (30%) of 6α-acetoxy-1β,12-dihydroxy-5α,11β-*H*-eudesm-4(15)-ene (**7**), 19 mg (8%) of 1β,6α,12-trihydroxy-5α,11β-*H*-eudesm-4(15)-ene (**16**); colourless solid; mp 142–144°C; $[\alpha]_D^{25}=16$ (MeOH, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3310, 1674, 1032 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.02 (1H, bs, H-15), 4.73 (1H, bs, H-15), 3.76 (1H, dd, $J_1=J_2=9.7$ Hz, H-6β), 3.64 (1H, dd, $J_1=5.5$ Hz; $J_2=10.8$ Hz, H-12), 3.50 (1H, dd, $J_1=7.1$ Hz; $J_2=10.8$ Hz, H-12), 3.42 (1H, dd, $J_1=4.6$ Hz; $J_2=11.5$ Hz, H-1α), 0.92 (3H, d, $J=7.0$ Hz, 3H-13), 0.70 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 11.6 (C-13), 13.0 (C-14), 20.7 (C-8), 32.0 (C-2), 35.1 (C-9), 36.2 (C-3), 36.6 (C-11), 41.6 (C-10), 44.9 (C-7), 55.9 (C-5), 67.0 (C-12), 67.6 (C-6), 79.0 (C-1), 108.0 (C-15), 146.2 (C-4); HRLSIMS, *m/z*: $[M+Na]^+$ 277.1774 ($C_{15}H_{26}O_3Na$, 277.1780, PPM 2.1); 150 mg (45%) of 1β,6α-diacetoxy-2α,12-dihydroxy-5α,11β-*H*-eudesm-4(15)-ene (**17**); colourless syrup; $[\alpha]_D^{25}=4$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3434, 1730, 1653, 1238 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.10 (1H, dd, $J_1=J_2=10.5$ Hz, H-6β), 4.90 (1H, bs, H-15), 4.64 (1H, d, $J=9.6$ Hz, H-1α), 4.66 (1H, bs, H-15), 3.68 (1H, ddd, $J_1=5.5$ Hz; $J_2=9.6$ Hz; $J_3=11.3$ Hz, H-2β), 3.47 (1H, dd, $J_1=6.8$ Hz; $J_2=10.6$ Hz, H-12), 3.43 (1H, dd, $J_1=8.0$ Hz; $J_2=10.6$ Hz, H-12), 2.64 (1H, dd, $J_1=5.5$ Hz; $J_2=12.5$ Hz, H-3β), 2.11 (3H, s, AcO group), 1.98 (3H, s, AcO group), 0.84 (3H, d, $J=6.9$ Hz, 3H-13), 0.78 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 10.9 (C-13), 13.5 (C-14), 18.1 (C-8), 21.1 (*Me*CO), 21.2 (*Me*CO), 34.3 (C-11), 35.5 (C-9), 40.6 (C-10), 43.0 (C-7), 44.4 (C-3), 53.5 (C-5), 66.1 (C-12), 69.7 (C-6), 70.6 (C-2), 84.5 (C-1), 110.5 (C-15), 141.3 (C-4), 171.3 (Me*C*O), 172.0 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 377.1939 ($C_{19}H_{30}O_6Na$, 377.1940, PPM 0.1) and 45 mg (15%) of 6α-acetoxy-1β,2α,12-trihydroxy-5α,11β-*H*-eudesm-4(15)-ene (**18**); colourless syrup $[\alpha]_D^{25}=5$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3430, 1730, 1650, 1240 cm^{-1}; ^1H NMR ($(CD_3)_2SO$): δ 4.95 (1H, dd, $J_1=J_2=10.6$ Hz, H-6β), 4.77 (1H, bs, H-15), 4.45 (1H, bs, H-15), 3.30 (1H, ddd, $J_1=5.4$ Hz; $J_2=9.0$ Hz; $J_3=11.1$ Hz, H-2β), 3.24 (1H, dd, $J_1=6.7$ Hz; $J_2=10.7$ Hz, H-12), 3.18 (1H, dd, $J_1=7.9$ Hz; $J_2=10.7$ Hz, H-12), 2.92 (1H, d, $J=9.0$ Hz, H-1α), 2.37 (1H, dd, $J_1=5.4$ Hz; $J_2=12.2$ Hz, H-3β), 2.01 (1H, d, $J=10.5$ Hz, H-5α), 1.92 (3H, s, AcO group), 0.71 (3H, d, $J=6.9$ Hz, 3H-13), 0.59 (3H, s, 3H-14); ^{13}C NMR ($(CD_3)_2SO$): δ 10.8 (C-13), 12.3 (C-14), 17.6 (C-8), 20.8 (*Me*CO), 33.6 (C-11), 35.4 (C-9), 40.5 (C-10), 42.3 (C-7), 43.8 (C-3), 52.9 (C-5), 64.2 (C-12), 69.6 (C-6), 70.7 (C-2), 82.2 (C-1), 107.7 (C-15), 143.7 (C-4), 170.4 (*MeCO*); HRLSIMS, *m/z*: $[M+Na]^+$ 335.1731 ($C_{19}H_{30}O_6Na$, 377.1730, PPM −0.5).

Biotransformation of 8. Substrate **8** (500 mg) was dissolved in EtOH (10 mL), distributed among 10 Erlenmeyer-flask cultures and incubated for 2 days, after which eight Erlenmeyer-flasks 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 90 mg (25%) of 12-acetoxy-1β-hydroxy-4α,6α-isopropylidenedioxy-5α,11β-*H*-eudesmane (**19**); colourless syrup; $[\alpha]_D^{25}=-40$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3469, 1738, 1246, 1192 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.99 (1H, dd, $J_1=7.0$ Hz; $J_2=10.7$ Hz, H-12), 3.95 (1H, dd, $J_1=7.5$ Hz; $J_2=10.7$ Hz, H-12), 3.76 (1H, dd, $J_1=J_2=9.9$ Hz, H-6β), 3.33 (1H, dd, $J_1=4.7$ Hz; $J_2=10.8$ Hz, H-1α), 2.04 (3H, s, AcO group), 1.44 (3H, s, 3H-15), 1.39 and 1.31 (3H each, s, Me groups of isopropylidene group), 0.89 (3H, d, $J=7.0$ Hz, 3H-13), 0.82 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 12.1 (C-13), 14.7 (C-14), 19.3 (C-8), 21.1 (*Me*CO), 24.2 (C-15), 26.1 (C-11), 28.3 (C-2), 31.4 and 32.4 (Me groups of isopropylidene group), 38.2 (C-10), 39.9 (C-9), 40.0 (C-3), 44.4 (C-7), 52.1 (C-5), 65.2 (C-6), 68.4 (C-12), 72.5 (C-4), 79.5 (C-1), 98.4 (quaternary C of isopropylidene group), 171.5 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 377.2295 ($C_{20}H_{34}O_5Na$, 377.2304, PPM 2.5); 43 mg (10%) of 1β-acetoxy-12-hydroxy-4α,6α-isopropylidenedioxy-5α,11β-*H*-eudesmane (**20**); colourless solid; mp 64–66°C; $[\alpha]_D^{25}=-30$ ($CHCl_3$, *c* 0.2); IR ($CHCl_3$) ν_{max}: 3448, 1738, 1241, 1195 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.56 (1H, dd, $J_1=4.9$ Hz; $J_2=11.1$ Hz, H-1α), 3.82 (1H, dd, $J_1=J_2=9.8$ Hz, H-6β), 3.69 (1H, dd, $J_1=4.6$ Hz; $J_2=11.1$ Hz, H-12), 3.41 (1H, dd, $J_1=4.7$ Hz; $J_2=11.1$ Hz, H-12), 2.02 (3H, s, AcO group), 1.39 and 1.31 (3H each, s, Me groups of isopropylidene group), 1.24 (3H, s, 3H-15), 0.97 (3H, d, $J=6.9$ Hz, 3H-13), 0.89 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 14.9 (C-13), 15.5 (C-14), 21.2 (*Me*CO), 22.2 (C-8), 24.1 (C-15), 24.7 (C-2), 25.8 (C-11), 32.3 (Me group of isopropylidene group), 37.2 (C-10), 38.6 (Me group of isopropylidene group), 39.7 (C-9), 39.8 (C-3), 44.0 (C-7), 52.3 (C-5), 66.6 (C-12), 67.3 (C-6), 72.4 (C-4), 80.6 (C-1), 98.6 (quaternary C of

isopropylidene group), 170.7 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 377.2310 ($C_{20}H_{34}O_5Na$, 377.2304, PPM −1.5); and 57 mg (15%) of 1β,12-dihydroxy-4α,6α-isopropylidenedioxy-5α,11β-*H*-eudesmane (**21**); colourless solid; mp 141–143°C; $[\alpha]_D^{25}=-43$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3609, 1253, 1195 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.82 (1H, dd, $J_1=J_2=9.8$ Hz, H-6β), 3.69 (1H, dd, $J_1=4.6$ Hz; $J_2=11.1$ Hz, H-12), 3.41 (1H, dd, $J_1=4.7$ Hz; $J_2=11.1$ Hz, H-12), 3.33 (1H, dd, $J_1=4.7$ Hz; $J_2=10.8$ Hz, H-1α), 1.49 (3H, s, 3H-15), 1.40 and 1.33 (3H each, s, Me groups of isopropylidene group), 0.97 (3H, d, *J*=6.9 Hz, 3H-13), 0.81 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 14.6 (C-13), 14.9 (C-14), 22.3 (C-8), 24.1 (C-15), 25.8 (C-11), 28.2 (C-2), 32.4 (Me group of isopropylidene group), 38.0 (C-10), 38.5 (Me group of isopropylidene group), 39.9 (C-9), 40.0 (C-3), 44.0 (C-7), 52.2 (C-5), 66.6 (C-12), 67.5 (C-6), 72.7 (C-4), 79.5 (C-1), 98.5 (quaternary C of isopropylidene group); HRLSIMS, *m/z*: $[M+Na]^+$ 335.2201 ($C_{18}H_{32}O_4Na$, 335.2198, PPM −0.7). Two Erlenmeyer-flasks of this incubation were maintained for 10 days and, working in a similar manner, tetrol **2** (40 mg, 50%) and a complex mixture of polyhydroxylated products were obtained.

Biotransformation of 9. Substrate **9** (719 mg) was dissolved in EtOH (20 mL), distributed among 20 Erlenmeyer-flask cultures and incubated for 14 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 32 mg (5%) of 1β-acetoxy-4α,12-dihydroxy-5α,11β-*H*-eudesman-6-one (**10**); 60 mg (10%) of 1β,4α,6α,12-tetrahydroxy-5α,11β-*H*-eudesmane (**2**) and 222 mg (35%) of 12-acetoxy-1β,4α-dihydroxy-5α,11β-*H*-eudesman-6-one (**22**); colourless solid; mp 93–95°C; $[\alpha]_D^{25}=-12$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3447, 1737, 1240 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.95 (1H, dd, $J_1=6.3$ Hz; $J_2=10.9$ Hz, H-12), 3.89 (1H, dd, $J_1=7.9$ Hz; $J_2=10.9$ Hz, H-12), 3.49 (1H, dd, $J_1=4.3$ Hz; $J_2=10.4$ Hz, H-1α), 2.32 (1H, s, H-5α), 2.03 (3H, s, AcO group), 1.46 (3H, s, 3H-15), 0.86 (3H, d, *J*=7.0 Hz, 3H-13), 0.84 (3H, s, 3H-14); ^{13}C NMR ($CDCl_3$): δ 12.7 (C-13), 15.2 (C-14), 21.0 (*Me*CO), 21.7 (C-8), 24.0 (C-15), 28.4 (C-2), 30.1 (C-11), 38.4 (C-9), 38.9 (C-3), 43.7 (C-10), 50.8 (C-7), 64.9 (C-5), 66.8 (C-12), 70.7 (C-4), 78.1 (C-1), 171.2 (Me*C*O), 212.4 (C-6); HRLSIMS, *m/z*: $[M+Na]^+$ 335.1825 ($C_{17}H_{28}O_5Na$, 335.1834, PPM 2.9).

Biotransformation of 15. Substrate **15** (360 mg) was dissolved in EtOH (16 mL), distributed among eight Erlenmeyer-flask cultures and incubated for 14 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 80 mg (25%) of 12-acetoxy-4α,6α-dihydroxy-5α,11β-*H*-eudesman-1-one (**11**), 28 mg (10%) of 4α,6α,12-trihydroxy-5α,11β-*H*-eudesman-1-one (**14**), 30 mg (10%) of 6α-acetoxy-4α,12-dihydroxy-5α,11β-*H*-eudesman-1-one (**23**); colourless solid; mp 82–84°C; $[\alpha]_D^{25}=-68$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3434, 1712, 1242 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.26 (1H, dd, $J_1=J_2=10.7$ Hz, H-6β), 3.50 (1H, dd, $J_1=6.3$ Hz; $J_2=10.3$ Hz, H-12), 3.44 (1H, dd, $J_1=8.0$ Hz; $J_2=10.3$ Hz, H-12), 2.14 (3H, s, AcO group), 1.29 (3H, s, 3H-15), 1.08 (3H, s, 3H-14), 0.86 (3H, d, *J*=6.9 Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 11.0 (C-13), 18.5 (C-8), 18.7 (C-14), 21.8 (*Me*CO), 26.5 (C-15), 34.0 (C-11), 34.6 (C-2), 34.8 (C-3), 37.1 (C-9), 44.3 (C-7), 47.1 (C-10), 55.2 (C-5), 66.0 (C-12), 71.3 (C-4), 72.9 (C-6), 170.1 (Me*C*O), 215.3 (C-1); HRLSIMS, *m/z*: $[M+Na]^+$ 335.1831 ($C_{17}H_{28}O_5Na$, 335.1834, PPM 1.0); 92 mg (25%) of 6α,12-diacetoxy-1α,4α-dihydroxy-5α,11β-*H*-eudesmane (**24**); colourless syrup; $[\alpha]_D^{25}=-3$ ($CHCl_3$, *c* 1); IR ($CHCl_3$) ν_{max}: 3460, 1730, 1245 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.30 (1H, dd, $J_1=J_2=10.6$ Hz, H-6β), 3.92 (1H, dd, $J_1=7.1$ Hz; $J_2=10.8$ Hz, H-12), 3.87 (1H, dd, $J_1=8.0$ Hz; $J_2=10.8$ Hz, H-12), 3.35 (1H, dd, $J_1=J_2=3.5$ Hz, H-1β), 2.08 (3H, s, AcO group), 2.04 (3H, s, AcO group), 1.21 (3H, s, 3H-15), 0.91 (3H, s, 3H-14), 0.88 (3H, d, *J*=6.9 Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 11.1 (C-13), 18.8 (C-8), 19.7 (C-14), 21.1 (*Me*CO), 21.8 (*Me*CO), 23.8 (C-15), 26.6 (C-2), 30.7 (C-11), 36.0 (C-3), 36.2 (C-9), 40.8 (C-10), 44.6 (C-7), 51.2 (C-5), 67.5 (C-12), 73.0 (C-6), 73.9 (C-1), 78.8 (C-4), 170.4 (Me*C*O), 171.2 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 379.2092 ($C_{19}H_{32}O_6Na$, 379.2097, PPM 1.3); and 48 mg (15%) of 6α-acetoxy-1α,4α,12-trihydroxy-5α,11β-*H*-eudesmane (**25**); colourless solid; mp 156–158°C; $[\alpha]_D^{25}=-10$ ($CHCl_3$, *c* 0.5); IR ($CHCl_3$) ν_{max}: 3395, 1727, 1246 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.32 (1H, dd, $J_1=J_2=10.8$ Hz, H-6β), 3.49 (1H, dd, $J_1=7.2$ Hz; $J_2=11.0$ Hz, H-12), 3.46 (1H, dd, $J_1=7.6$ Hz; $J_2=11.0$ Hz, H-12), 3.35 (1H, dd, $J_1=J_2=3.5$ Hz, H-1β), 2.09 (3H, s, AcO group), 1.22 (3H, s, 3H-15), 0.92 (3H, s, 3H-14), 0.87 (3H, d, *J*=6.9 Hz, 3H-13); ^{13}C NMR ($CDCl_3$): δ 11.1 (C-13), 18.9 (C-8), 19.8 (C-14), 21.9 (*Me*CO), 23.8 (C-15), 26.6 (C-2), 34.2 (C-11), 36.0 (C-9), 36.3 (C-3), 40.8 (C-10), 44.3 (C-7), 51.3 (C-5), 66.4 (C-12), 72.4 (C-4), 73.3 (C-6), 74.0 (C-1), 170.4 (Me*C*O); HRLSIMS, *m/z*: $[M+Na]^+$ 337.1996 ($C_{17}H_{30}O_5Na$, 337.1991, PPM −1.4).

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