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Chemical-Microbiological Semisynthesis of *enantio*-Ambrox® Derivatives

Andrés García-Granados*, Antonio Martínez, Raquel Quirós

Instituto de Biotecnología, Grupo de Investigación en Biotecnología y Química de Productos Naturales, Departamento de Química Orgánica. Facultad de Ciencias, Universidad de Granada.Tel. 34-58-243364. E-mail: agarcia@goliat.ugr.es

Antonio Luis Extremera

Departamento de Microbiología, Facultad de Ciencias, Universidad de Granada,

18071-GRANADA. (Spain).

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Abstract: *enantio*-3-Hydroxyambrox derivatives were obtained through combined chemical and microbiological methods from *ent*-12-oxo-13-*epi*-manoyl oxides. Regioselective and stereoselective reduction of the carbonyl group at C-3 of *ent*-3,12-dioxo-13-epi-manoyl oxide was carried out with Baker's yeast. Regioselective acetylation of *ent*-3,12-dihydroxy-13-epi-manoyl oxide was accomplished with *Candida cylindracea* lipase or Novozym 435 and vinyl acetate. Microbial biotransformation of *ent*-3β-acetoxy-12-oxo-13-*epi*-manoyl oxide with *Nectria ochroleuca* yielded new *ent*-1β-hydroxy- and *ent*-7β-hydroxy derivatives. Baeyer-Villiger oxidation at C-12 was observed in the biotransformation with baker's yeast and *G. roseun* to obtain norambreinolide lactones which were converted in to *enantio*-Ambrox® derivatives.

INTRODUCTION

The usefulness of enzymes and microorganisms in organic synthesis has been reported in excellent reviews[1-5] and books[6-11] published in recent years. The synthesis of natural products which possess certain stereochemistry difficult to obtain with exclusively chemical methods is one of the fields in which biotransformation processes are of great interest. The manipulation of isolated enzymes is comparable to that of classic catalysts, and one of its greatest advantages is in organic synthesis, especially when the enzymes conserve their activity in organic solvents.[12-14] The most useful enzymes available are lipases which do not require cofactors for activity. Porcine pancreas lipase (PPL), *Candida antarctica* lipase (CAL), *Candida cylindracea* lipase (CCL) and *Pseudomonas* lipases (PSL), have been the most frequently used in esterification processes in organic solvents.[12-14] The regioselectivity of these enzymes can be exploited in polyfunctionalized compounds, because the modification of only one of the identical functional groups in a molecule make it possible to manipulate a specific position without the need to use protective groups. This is a considerable advantage with regard to strictly chemical procedures. For synthetic purposes, in microbiological transformations, the microorganism is used as a bag of enzymes for transformations of substrates that are not necessarily natural. The microorganism most often used to reduce carbonyl groups is *Saccharomyces cerevisiae* (baker's yeast), which is the best alternative to the use of oxidoreductases. Moreover *S. Cerevisiae* acts on a variety of substrates.[15-18] Particularly interesting is the regioselective reduction of polyketones, in which the yeast reduces a carbonyl group without affecting other groups in the molecule. Most of the reductions carried out with yeasts and many microorganisms follow Prelog's rule[19].The transfer of hydrogen is carried out on the Re face of the prochiral ketone, giving hydroxyl groups with an S configuration.

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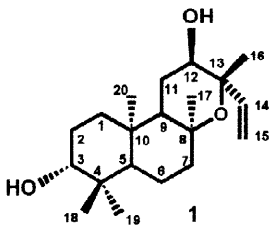
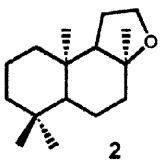
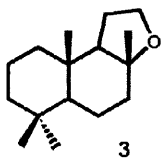
In this article we describe a biotransformation series with baker's yeast, filamentous fungi and commercial lipases. These transformations, combined with appropriate chemical processes, can be of great use for the semisynthesis of new products. Starting from *ent-3β,12α*-dihydroxy-13-*epi*-manoyl oxide[20] (varodiol, **1**), isolated from *Sideritis varoi* subspecie *cuatrecasasii*,[21] we now report a way to obtain new 3-hydroxyderivatives of *ent*-Ambrox® (**2**) by combining chemical and biotransformation procedures. 3-Hydroxyderivatives of Ambrox® (**3**) were obtained in biotransformation processes from Ambrox® with the microorganims *Cladosporium oxysporum*[22] and *Cefalosporium aphidicola*.[23]







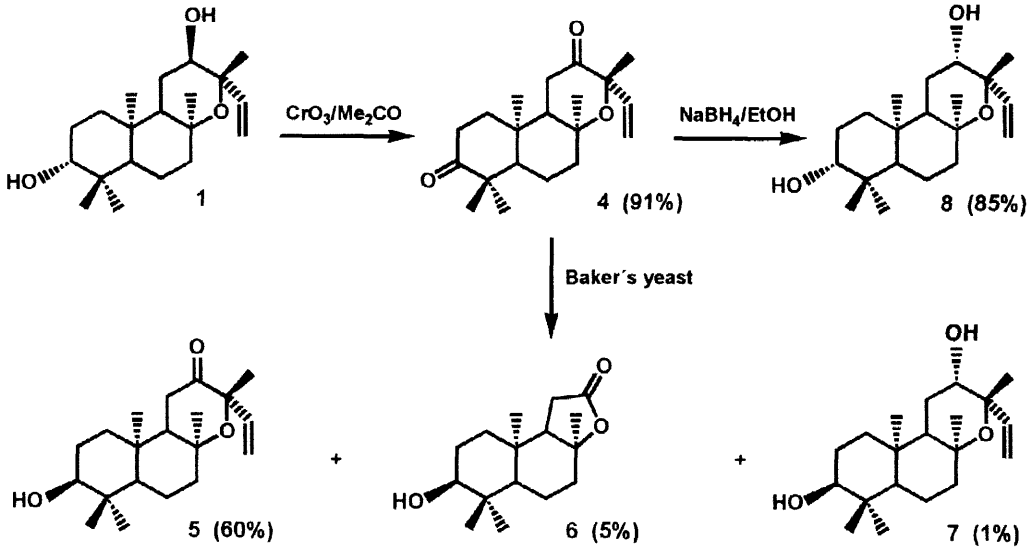
RESULTS AND DISCUSSION

Oxidation of varodiol (**1**) with Jones' reagent[24] gave 3,12-dioxoderivative[25] (varodione **4**, 91%). The biotransformation of diketone (**4**) with baker's yeast[26] for 6 days yielded metabolites **5** (60%), **6** (5%) and **7** (1%) (Scheme 1). The molecular formula of metabolite **5** was $C_{20}H_{32}O_3$. The IR spectrum of metabolite **5** showed carbonyl and hydroxyl group bands (1710 and 3487 cm^{-1} respectively). Its ^{1}H-NMR spectrum contained a signal at 3.41 ppm (t, *J* 2.9 Hz), which indicated the reduction of the carbonyl group at C-3 by the *ent*-β face to give an axial (S-configuration) hydroxyl group in this position. The position and stereochemistry of reduction were confirmed by comparison of the ^{13}C-NMR spectra of metabolite **5**, varodione **4**[25] and its C-3 epimer compound **1**).[27] A strong γ-effect of the axial hydroxyl group on C-3 was observed at C-1 and C-5 in metabolite **5**. In addition, disappearance of the γ-gauche effect at C-19 in metabolite **5** was observed. Thus, metabolite **5** obtained in this biotransformation possessed a structure of *ent*-3α-hydroxy-12-oxo-13-*epi*-manoyl oxide as a result of regioselective and stereoselective reduction of the carbonyl group at C-3 by baker's yeast on the *ent*-β face. However, the chemical reduction of varodione **4** with $NaBH_4$ led to reduction of carbonyl groups at C-3 and C-12 by the *ent*-α face to give *ent*-3ß,12β-dihydroxy-13-*epi*-manoyl oxide (**8** , 85%).[27]

Metabolite **6** exhibited a molecular peak of *m/z* 266 ($C_{16}H_{26}O_3$) , implying a loss of C_4H_6 from metabolite **5**. The IR spectrum of metabolite **6** showed a hydroxyl group band (3462 cm^{-1}) and a lactone group band (1769 and 1230 cm^{-1}). In its ^{1}H-NMR spectrum the typical ABX system of vinyl protons was not observed. Moreover only four signals for methyl singlets (δ 1.31, 0.94, 0.89 and 0.83) were observed, along with a signal at 3.43 corresponding to the equatorial proton geminal to the hydroxyl group at C-3, and signals attributable to an ABX system of protons at C-11 and at C-9. Chemical shifts of protons at C-11 indicated the proximity of a carbonyl group at C-12. In the ^{13}C-NMR spectrum of metabolite **6** we observed the disappearance of four signals of carbon atoms, the presence of a signal for a lactone group (δ 176.9, C-12), and a signal for a tertiary oxygenated carbon atom (δ 86.4, C-8). All considered, metabolite **6** was the result of loss of the 13-16 carbon atoms from substrate **4** and the presence of a lactone group in the C ring. Thus, the structure of metabolite **6** was *ent*-3α-hydroxy-13,14,15,16-tetranor-12,8α-labdanolide (*ent*-3α-hydroxysclareolide). The formation of lactone **6** was probably due to the Baeyer-Villiger oxidation of diketone **4** catalyzed by baker's yeast, to give lactone **9** (Scheme 2). This lactone **9** was hydrolyzed to acid **10** in the biotransformation conditions, and then lactonized to **give 11** which was reduced by

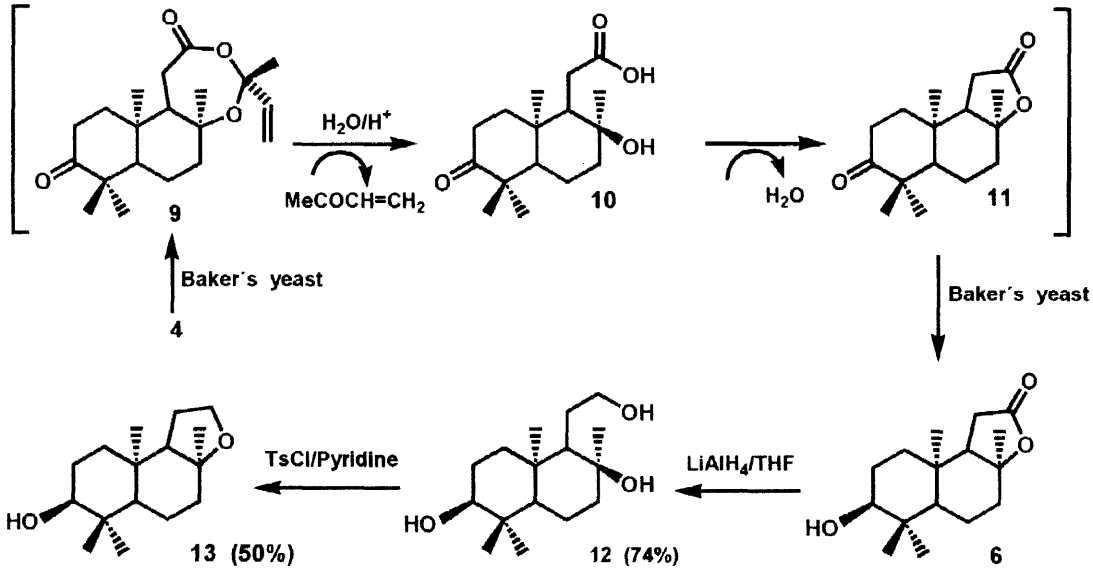
baker's yeast on the carbonyl group at C-3 to give lactone **6**. The action of baker's yeast on the major metabolite **5** was ruled out, and this metabolite **5** was recovered unaltered after its biotransfomation with baker's yeast.

Scheme 1



The last metabolite (7) isolated in this biotransformation showed a strong hydroxyl group band (3409 cm^{-1}) in its IR spectrum. A signal corresponding to an equatorial proton at C-3 (δ 3.41) appeared in its ^{1}H-NMR spectrum together with a new signal at 3.51 ppm (dd, *J* 11.0, 4.7 Hz) attributable to an equatorial proton, geminal to the hydroxyl group at C-12. The structure of metabolite 7 was *ent*-3α,12β-dihydroxy-13-*epi*-manoyl oxide.

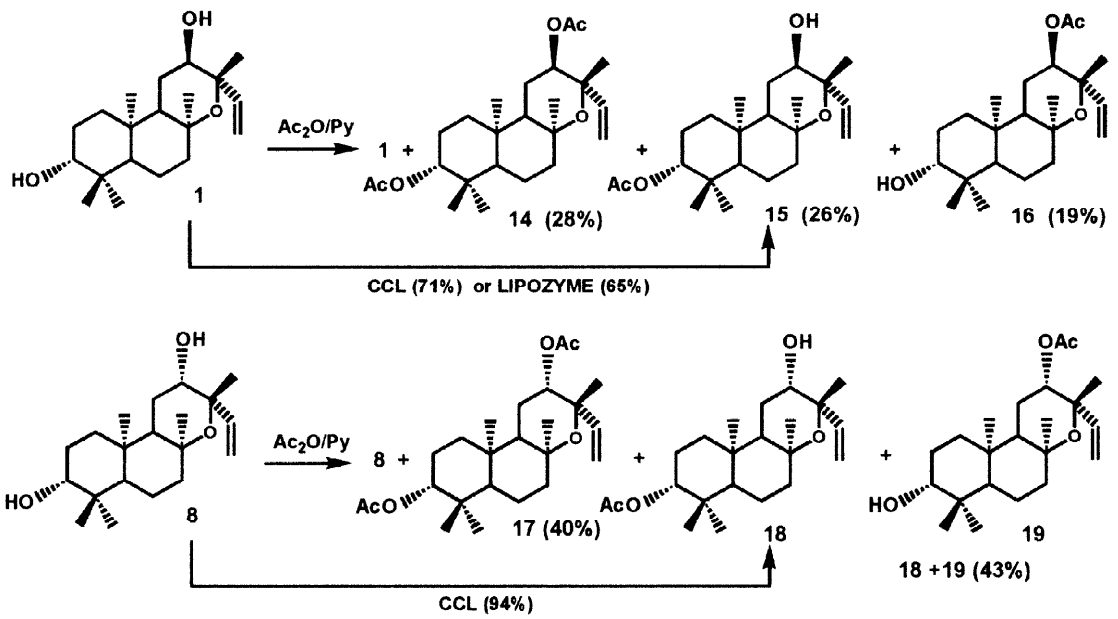
Scheme 2



Lactone **6**, obtained in the biotransformation of varodione **4** with baker's yeast, allowed us to obtain new *ent*-Ambrox derivatives (Scheme 2). Reduction of lactone **6** with $LiAlH_4$ gave triol **12** (74%), which was treated with TsCl/pyridine to yield product **13** (50%, molecular formula $C_{16}H_{28}O_2$). In its ^{1}H-NMR spectrum, signals of

protons geminals to the oxygenated function at C-11 were observed. In its ^{13}C-NMR spectrum we confirmed the existence of an oxygenated methylenic carbon atom (65.1 ppm). The ^{1}H NMR data and ^{13}C NMR were compatible with the structure of *ent*-8α,12-epoxy-3α-hydroxy-13,14,15,16-tetranorlabdane (*ent*-3α-hydroxyambrox).

Scheme 3



Acetylation of varodiol (**1**) with Ac_2O/Py previously published,[25] produced a diacetoxyderivative (**14**, 28%), 3-acetoxyderivative **15** (26%) and 12-acetoxyderivative **16** (19%). To determine the possible regioselectivity of the acetylation of varodiol (**1**) with lipases, we undertook a series of biotransformations of this compound with several commercial lipases and vinyl acetate (see Experimental). The results of these biotransformations appear in Table 1. Porcine pancreas lipase[28] (PPL) and Novozym 435[29] were inactive in these transformations. However, regioselective acetylation of the hydroxyl group at C-3 with *Candida cylindracea* lipase[28] (CCL) and Lipozyme IM[29] (71% and 65% respectively) was observed (Scheme 3).

Table 1. Enzyme-Catalysed Acetylation of productos (**1**) and (**8**) with lipases and vinyl acetate

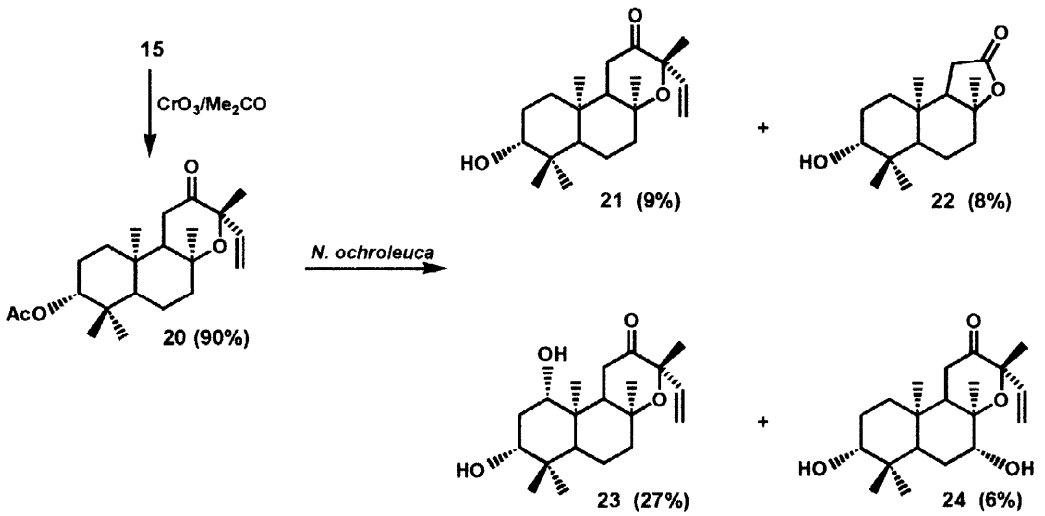
Substrate	Lipase	Time (days)	% Conversion
1	*Candida cylindracea* lipase (CCL)	5	71
1	porcine pancreas lipase (PPL)	5	0
1	*Novozym 435*	2	0
1	*Lipozyme IM*	2	65
8	CCL	2	85
8	PPL	5	0

Acetylation of 12-*epi*-varodiol (**8**) with Ac_2O/pyridine gave *ent*-3ß,12ß-diacetoxy-13-*epi*-manoyl oxide (**17**, 40%) and a mixture of *ent*-3ß-acetoxy-12ß-hydroxy-13-*epi*-manoyl oxide (**18**) and *ent*-12ß-acetoxy-3ß-hydroxy-13-*epi*-manoyl oxide (**19**) (43%) (Scheme 3). The results of the biotransformation of substrate **8** with lipases are show in Table 1. Regioselective acetylation of the hydroxyl group at C-3 was now also observed with

CCL (85%). These results indicate that the configuration of C-12 did not influence the regioselective acetylation with CCL. Moreover, the yield of *ent*-3β-acetoxy derivatives was increased with regard to chemical acetylation. Mixtures of acetylation products were not observed, therefore greater ease of separation of the products was achieved. According to these results, we were able to obtain a greater quantity of monoacetate **15** by biotransformation with CCL and vinyl acetate for 5 days. Oxidation of monoacetate **15** with Jones's reagent[24] gave *ent*-3ß-acetoxy-12-oxo-13-*epi*-manoyl oxide (**20**) which was incubated with *Nectria ochroleuca*.

The biotransformation of substrate **20** with *Nectria ochroleuca* for 7 days produced metabolites **21** (9%), **22** (8%), **23** (27%) and **24** (6%), (Scheme 4). Metabolite **21** was the result of the deacetylation of substrate **20**. The molecular formulae of metabolites **22** and **6** were the same and their spectroscopic data were similar. A signal at δ 3.25 (*dd*, *J* 10.9, 5.3 Hz) due to an axial proton at C-3 was observed in the ^{1}H-NMR spectrum of metabolite **22**. A signal correspon ding to a carbon atom (176.6 ppm) of the lactone group in the ^{13}C-NMR spectrum of metabolite **22** was observed. Oxidation of metabolites **6** and **22** with Jones' reagent yielded the same 3-oxo-derivative **11** (Scheme 5). Therefore metabolite **22** was *ent*-3β-hydroxy-13,14,15,16-tetranor-12,8-labdanolide (*ent*-3β-hydroxysclareolida). The formation of lactone **22**, epimer at C-3 of lactone **6**, probably occurred through a process similar to that described previously for lactone **6** (Scheme 2).

Scheme 4



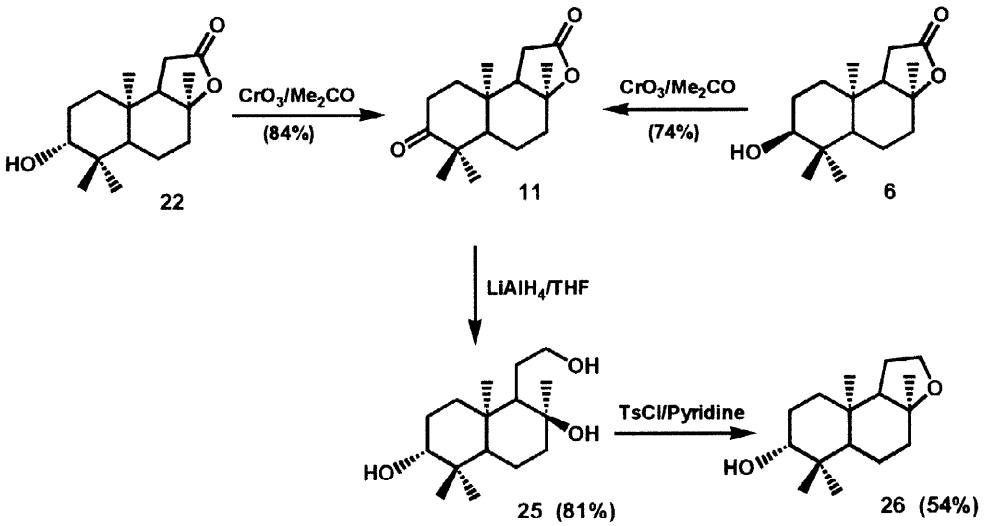
A new signal at 3.25 ppm due to an axial proton geminal to the hydroxyl group at C-1 or C-7 was observed as double doblet in the ^{1}H RMN spectrum of metabolite **23**. The stereochemistry of the hydroxyl group was deduced from the vecinal *J* values (12.0 and 4.4 Hz). This equatorial hydroxylation was located at C-1 by the observation of deshielding of C-11 (Δδ=+3.4), β-effects at C-2 (Δδ=+10.7) and C-10 (Δδ=+5.8), γ-gauche effect at C-20 (Δδ= - 4.3) and low γ-effects at C-3 (Δδ=-2.9) and C-5 (Δδ= -2.0) when the ^{13}C-NMR spectra of metabolites **23** and **21** were compared. Thus, metabolite **23** was *ent*-1β,3 β-dihydroxy-12-oxo-13-*epi*-manoyl oxide.

The last metabolite (**24**) isolated from this incubation presented spectroscopic data similar to those of metabolite **23**. A signal for an axial proton at C-3 (3.23 ppm) was observed, together with an other signal at 3.67 ppm in its ^{1}HNMR spectrum.This latter signal is a double doblet, with vecinal J values of 11.8 and 4.8 Hz, due to an axial proton geminal to a hydroxyl group. As the spectroscopic data for metabolite **24** differed from those of metabolite **23** (with hydroxylation at C-1), the new hydroxylation must be located on C-7, which was confirmed

by comparison of ^{13}C-NMR data of the metabolites **21** and **24**. Thus, we observed an α-effect at C-7 (Δδ = +37.7), β-effects at C-6 (Δδ= +7.1) and C-8 (Δδ= +3.7) and γ-effects at C-5 (Δδ = -1.6), C-9 (Δδ = -2.0) and C-17 (Δδ =-6.6). Hence we deduce the structure *ent*-3β,7β-dihydroxy-12-oxo-13-*epi*-manoyl oxide for metabolite **24**.

The isolation of lactone **22** from microbial transformation with *G. roseum* allowed us to obtain, as described above for lactone **6**, a new derivative of *ent*-Ambrox® (**2**) (Scheme 5). Reduction with $LiAlH_4$ of ketolactone **11**, obtained from **6** and **11**, gave triol **25** (81%), which was treated with TsCl/pyridine to give *ent*-3β-hydroxyambrox (**26**, 54%). The combination of biotransformation processes and chemical reactions allow us to obtain new 3-hydroxyderivatives of *ent*-Ambrox® with both configurations on C-3 (compounds **13** and **26**). The procedure described here constitutes a new method for the chemical-microbiological semisynthesis of these products from *ent*-12-oxo-manoyl oxides.

Scheme 5



EXPERIMENTAL

Measurements of NMR spectra were made in $CDCl_3$ using a Bruker AM-300 spectrometer. Assignments of ^{13}C chemical shifts were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. IR spectra were recorder on a Nicolet 20SX FT-IR spectrometer. High resolution mass spectra were made by LSIMS (FAB) ionization mode in a MICROMASS VG-AUTOSPEC-Q spectrometer (EBE geometry). Mps were determined using a Kofler (Reichter) apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 240 polarimeter at 20 °C. Silica gel Scharlau 60 (40-60 μm) was used for flash chromatograpy. CH_2Cl_2 with increasing amounts of Me_2CO was used as eluent; analytical plates (silica gel Merck G) were rendered visible by spraying with H_2SO_4-HOAc-H_2O, followed by heating at 120°C.

***Isolation of ent-3β,12α-dihydroxy-13-epi-manoyl oxide* (1).** *ent*-3β,12α-Dihydroxy-13-*epi*-manoyl oxide (varodiol, **1**) was isolated from *Sideritis varoi* subspecie *cuatrecasasii*.[21] The identity of compound **1** was confirmed by direct comparison with an authentic sample (IR, MS and NMR).

Oxidation of varodiol (1). Jones's reagent[24] was added dropwise to a stirred solution of varodiol (**1**, 2 g) in acetone (50 ml) at 0°C until an orange-brown color persisted. MeOH (1 ml) was then added and the reaction

mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded 1.8 g of *ent*-3,12 dioxo-13-*epi*-manoyl oxide[21] (**4**, varodione, 91%).

Organism, media and culture conditions. The microorganisms used in these biotransformations were *Saccharomyces cerevisiae* (baker's yeast)[26] and *Nectria ochroleuca*.[26] Medium YEPGA containing 1% yeast extract, 1% peptone, 2% glucose, 2% agar, at pH 5 was used for storage of *N. ochroleuca*. In all biotransformations experiments a medium containing 0.1% peptone, 0.1% yeast ext., 0.1% beef ext. and 0.5% glucose at pH 5.7 in H_2O was used. Erlenmeyer flasks (250 ml) containing 80 ml of medium were inoculated with a suspension of the corresponding microorganism. Incubations were maintained at 28° with gyratory shaking (150 rpm) for 6 days after which the substrates were added, in quantities indicated in each biotransformation, as solution in EtOH.

Biotransformation of varodione (2) with baker's yeast. 1.190 mg of varodione (**2**) were dissolved in EtOH (15 ml) distributed among 6 Erlenmeyer-flask cultures each containing 7 g of baker's yeast (*Saccharomyces cerevisiae).* The incubation was maintained for 6 days after which the cultures were centrifugated (15 min, 2500 rpm) and the supernatant was saturated with NaCl and extracted with CH_2Cl_2. The organic extracts were pooled, dried over anhydrous Na_2SO_4 and evaporated at 40° in vacuum to give a mixture of compounds which was chromatographed on a silica gel column to obtain 360 mg of substrate (**2**) (30 %), 730 mg of *ent*-3α-hydroxy-12-oxo-13-*epi*-manoyl oxide (**5**, 60 %) as a colourless solid, m.p. 123-125°C; $[\alpha]_D$ -68 (*c* 0.5, $CHCl_3$); ν_{max} (KBr) 3487, 3088, 1710, 1637, 1086, 1000 and 928 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.04 (1H, dd, *J* 17.5, 10.8 Hz , H-14), 5.13 (1H, dd, *J* 17.5, 1.2 Hz) and 5.02 (1H, dd, *J* 10.8, 1.2 Hz) (2H-15), 3.41 (1H, t , *J* 2.9 Hz, H-3), 2.47 (1H, dd, *J* 18.5, 5.9 Hz) and 2.30 (1H, dd, *J* 18.5, 13.1 Hz) (2H-11), 1.98 (1H, dd, *J* 13.1, 5.9 Hz ,H-9) , 1.29 and 1.15 (3H each, s, 3H-16 and 3H-17), 0.95, 0.81 and 0.76 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 14.5 (C-20), 19.4 (C-6), 22.0 (C-19), 22.2 (C-17), 25.1 (C-2), 28.3 and 28.6 (C-16 and C-18), 31.7 (C-1), 33.6 (C-11), 36.7 (C-10), 37.4 (C-4), 42.0 (C-7), 48.5 (C-5), 54.1 (C-9), 75.5 (C-8), 75.8 (C-3), 81.8 (C-13), 113.2 (C-15), 142.3 (C-14), 211.8 (C-12); HRLSIMS, *m/z*, MNa^+, found 343.2237. $C_{20}H_{32}O_3Na$ requires 343.2249; 52 mg of *ent*-3α-hydroxy-13,14.15,16-tetranor-12,8α-labdanolide (*ent*-3α-hydroxysclareolide) (**6**, 5%) as a colourless solid, m.p. 120-122°C; $[\alpha]_D$ -15 (*c* 0.33, $CHCl_3$); ν_{max} (KBr) 3462, 1769, 1230 and 1061 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.43 (1H, t, *J* 2.7 Hz, H-3), 2.38 (1H, dd, *J* 16.2, 14.8 Hz) and 2.22 (1H, dd, *J* 16.3, 6.5 Hz) (2H-11), 2.03 (1H, dd, *J* 4.8, 6.5 Hz, H-9), 1.31 (3H, s, H-13), 0.94, 0.89 and 0.83 (3H each, s, 3H-14, 3H-15 and 3H-16); δ_C (75.47 MHz, $CDCl_3$) 15.0 (C-20), 20.3 (C-6), 21.5 and 21.6 (C-17 and C-19), 25.0 (C-2), 28.2 (C-18), 28.7 (C-11), 32.7 (C-1), 35.9 (C-10), 37.5 (C-4), 38.7 (C-7), 48.9 (C-5), 58.7 (C-9), 75.9 (C-3), 86.4 (C-8), 176.9 (C-12); HRLSIMS, *m/z*, MNa^+, found 289.1780. $C_{16}H_{26}O_3Na$ requires 289.1780; and 12 mg of *ent*-3α,12β-dihydroxy-13-*epi*-manoyl oxide (**7**, 1%) as a syrup; $[\alpha]_D$ -26 (*c* 1, $CHCl_3$); ν_{max} (film) 3409, 3084, 1708, 1069 and 995 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.303 (1H, dd, *J* 17.7, 11.3 Hz, H-14), 5.42 (1H, dd, *J* 17.7, 1.4 Hz) and 5.23 (1H, dd, *J* 11.3, 1.4 Hz) (2 H-15), 3.51 (1H, dd , *J* 11.0, 4.7 Hz, H-12), 3.41 (1H, t, *J* 2.9 Hz, H-3), 1.34 and 1.23 (3H each , s, 3H-16 and 3H-17), 0.93, 0.81 and 0.77 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 15.9 (C-20), 19.7 (C-6), 21.8 (C-19), 25.2 and 25.3 (C-2 and C-11), 25.5 (C-17), 28.0 (C-18), 28.3 (C-16), 32.5 (C-1), 36.7 (C-10), 37.6 (C-4), 42.4 (C-7), 57.4 (C-9), 48.8 (C-5), 75.9 (C-13), 76.1 (C-3), 76.3 (C-8), 77.6 (C-12), 117.2 (C-15), 140.4 (C-14); HRLSIMS, *m/z*, MNa^+, found 345.2405. $C_{20}H_{34}O_3Na$ requires 345. 2406.

Reduction of varodione (2). 500 mg of varodione (**2**) was dissolved in EtOH (15 ml) and $NaBH_4$ (50 mg) was added. The mixture was maintained for 30 min at room temperature after which the reaction mixture was treated with HCl (10%) and extracted with CH_2Cl_2. The organic layer was dried with anhydrous Na_2SO_4 and concentrated *in vacuo* to give 430 mg of **8** (85 %) with identical spectroscopic data that published for *ent*-3β,12β-dihydroxy-13-*epi*-manoyl oxide.[27]

Incubation of ketol 5 with baker's yeast. Ketol **5** (170 mg) was dissolved in EtOH (6 ml) and distributed between 2 Erlenmeyer-flask cultures each containing 7 g of baker's yeast. After 10 days **5** was recovered unaltered.

Obtention of ent-3α-hydroxyambrox (13) from lactone 6. 20 mg of lactone **6** was dissolved in anhydrous THF (2 ml) and a 1M solution of $LiAlH_4$ in THF was added. The reaction mixture was refluxed for 30 min., quenched by adition of aqueous ether followed by water and extracted with CH_2Cl_2. The organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness. Chromatography on silica gel yielded 15 mg of *ent*-3α,8α,12-trihydroxy-13,14,15,16-tetranorlabdane (**12**, 74%) as a colourless solid, m.p. 168-170 °C; $[\alpha]_D$ +25 (*c* 0.5, $CHCl_3$); ν_{max} ($CHCl_3$) 3352 and 1072 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.79 (1H, ddd, *J* 10.1, 4.4, 4.4 Hz) and 3.48 (1H; ddd, *J* 10.1, 8.8, 5.1 Hz) (2H-12), 3.40 (1H, t, *J* 2.8 Hz, H-3), 1.18 (3H, s, 3H-17), 0.95, 0.81, 0.80 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 15.0 (C-20), 20.2 (C-6), 21.3 and 21.7 (C-17 and C-19), 22.0 (C-11), 25.2 (C-2), 28.6 (C-18), 32.4 (C-1), 37.6 (C-4), 38.8 (C-10), 44.4 (C-7), 48.4 (C-5), 58.9 (C-9), 64.2 (C-12), 75.9 (C-3), 76.7 (C-8); HRLSIMS, *m/z*, MNa^+, found 293.2098. $C_{16}H_{30}O_3Na$ requires 293.2093. Triol **12** (13 mg) was dissolved in pyridine (1 ml) and TsCl (15 mg) was added. The reaction mixture was stirred for 12 h at room temperature, then crushed ice was added and the mixture was extracted with CH_2Cl_2. The organic layer was washed with aqueous $KHSO_4$, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was chromatographed over silica gel yielding 5 mg of triol **(12,** 38%) and 6 mg of *ent*-8α,12-epoxy-3α -hydroxy-13,14,15,16-tetranorlabdane (*ent*-3α-hydroxyambrox, **13**, 50%) as a colourless solid, m.p. 143-145 °C; $[\alpha]_D$ +39 (*c* 0.5, $CHCl_3$); ν_{max} ($CHCl_3$) 3431 and 1071 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.91 (1H, m) and 3.82 (1H, c, *J* 8.1 Hz) (2H-12), 3.43 (1H, t, *J* 2.7 Hz, H-3), 1.08 (3H, s, 3H-17), 0.95, 0.85 and 0.84 (3H each , s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 15.0 (C-20), 20.4 (C-6), 21.3 and 21.7 (C-17 and C-19), 22.7 (C-11), 25.2 (C-2), 28.6 (C-18), 33.2 (C-1), 36.1 (C-10), 37.5 (C-4), 39.8 (C-7), 49.7 (C-5), 59.8 (C-9), 65.1 (C-12), 76.4 (C-3), 80.0 (C-8); HRLSIMS, *m/z*, MH^+, found 253.2162. $C_{16}H_{29}O_2$ requires 253.2168.

General procedure for acetylation with lipases. The compound to acetylate was dissolved in vinyl acetate and lipase was added in the quantities indicated in each experiment. The biotransformation was maintained by shaking (180 rpm) at 35°C.The reaction was monitored by thin layer chromatography. When reaction was complete, the mixture was filtered and the lipase obtained was washed with CH_2Cl_2. The organic layer was concentrated in vacuum and the mixture obtained was purified on a silica gel column. Lipases used in these biotransformations were *Candida cylindracea* lipase (CCL)[28] porcine pancreas lipase[28] (PPL), *Novozym 435*[29] and *Lipozyme IM.*[29]

Biotransformations of varodiol (1) with CCL and PPL Varodiol (**1**, 120 mg) was dissolved in vinyl acetate (15 ml) and *Candida cylindracea* lipase (500 mg) was added. The biotransformation was maintained for 5 days to give 96 mg of monoacetate **15** (71%).

Biotransformation of varodiol (1) with PPL. Varodiol (**1**, 100 mg) was dissolved in vinyl acetate (10 ml) and porcine pancreas lipase (500 mg) was added. After 5 days the substrate **1** was recovered unaltered.

Biotransformation of varodiol (1) with Novozym 435. Varodiol (**1**, 100 mg) was dissolved in vinyl acetate (10 ml) and *Novozym 435* (500 mg) was added. After 24 h the substrate **1** was recovered unaltered.

Biotransformation of varodiol (1) with Lipozyme IM. Varodiol (**1**, 100 mg) was dissolved in vinyl acetate (10 ml) and *Lipozyme IM* (500 mg) was added. The biotransformation was maintained for 24 h to give 74 mg of monoacetate **15** (65%).

Acetylation of diol 8. 200 mg of diol **8** were dissolved in pyridine (6 ml) and Ac_2O (3 ml). The reaction was stirred for 4 h. at 0°C, after which the reaction mixture was diluted with cold water (25 ml), extracted with CH_2Cl_2 , washed with saturated aquous $KHSO_4$ and dried over anhydrous Na_2SO_4. The solvent was evaporated to give a mixture of compounds with was chromatographed on silica gel yielding starting material **8** (10 mg, 5 %), *ent*-3β,12β-diacetoxy-13-*epi*-manoyl oxide (**17**) (100 mg, 40 %) as a syrup; $[\alpha]_D$= -53° (*c* 1 $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 6.21 (1H, dd, *J* 17.9, 11.4 Hz, H-14), 5.41 (1H, dd, *J* 17.9, 1.2 Hz) and 5.13 (1H, dd, *J* 11.4, 1.2 Hz (2H-15), 4.70 (1H, dd, *J* 11.4, 4.7 Hz, H-12), 4.46 (1H, dd, *J* 11.3, 4.9 Hz, H-3), 2.03 and 2.01 (3H each, s, AcO groups), 1.22 (6H, s, 3H-16 and 3H-17), 0.84, 0.81 and 0.77 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 16.2 and 16.4 (C-19 and C-20), 19.5 (C-6), 21.4 (MeCO groups), 22.2 (C-11), 23.6 (C-2), 25.3 (C-17), 28.1 and 28.9 (C-16 and C-18), 36.6 (C-10), 37.2 (C-1), 37.8 (C-4), 42.1 (C-7), 55.1 (C-5), 57.2 (C-9), 74.6 (C-13), 76.0 (C-8), 78.5 (C-3), 80.5 (C-12), 115.6 (C-15), 141.5 (C-14), 170.2 and 170.9 (MeCO groups); HRLSIMS, *m/z* MNa^+, found 429.2607. $C_{24}H_{38}O_5Na$ requires 429.2617; and a mixture of *ent*-3β-acetoxy-12β-hydroxy-13-*epi*-manoyl oxide (**18**) and *ent*-12β-acetoxy-3β-hydroxy-13-*epi*-manoyl oxide (**19**) (97 mg, 43%).

Biotransformation of diol 8 with CCL. Diol **8** (100 mg) was dissolved in vinyl acetate (10 ml) and *Candida cylindracea* lipase (200 mg) was added. The biotransformation was maintained for 2 days to give 96 mg of *ent*-3β-acetoxy-12β-hydroxy-13-*epi*-manoyl oxide (**18**, 85%) as a colourless solid, m.p. 119-122 °C; $[\alpha]_D$= -78° (*c* 1, $CHCl_3$); ν_{max}(KBr) 3457, 3080, 1731 and 1247 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.28 (1H, dd, *J* 17.9, 11.3 Hz, H-14), 5.41 (1H, d, *J* 17.8 Hz) and 5.22 (1H, d, *J* 17.8 Hz) (2H-15), 4.45 (1H, dd, *J* 11.3, 4.5 Hz, H-3); 3.49(1H, dd, *J* 11.4, 4.6 Hz, H-12), 2.20 (3H, s, AcO group), 1.33 and 1.22 (3H each, s, 3H-16 and 3H-17), 0.83, 0.81, 0.78 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 16.2 and 16.3 (C-19 and C-20), 19.5 (C-6), 21.4 (MeCO), 23.6 (C-11), 25.3 (C-2), 25.5 (C-17), 28.0, 28.1, (C-18, C-16), 36.6 (C-10), 37.3 (C-1), 37.8 (C-4), 42.2 (C-7), 55.2 (C-5), 57.6 (C-9), 75.3 (C-13), 76.3 (C-8), 77.6 (C-3), 80.7 (C-12), 117.3 (C-15), 140.3 (C-14), 171.1 (MeCO); HRLSIMS, *m/z*, MNa^+, found 387.2512. $C_{22}H_{36}O_4Na$ requires 387.2511.

Biotransformation of diol 8 with PPL. Diol **8** (100 mg) was dissolved in vinyl acetate (10 ml) and porcine pancreas lipase (200 mg) was added. After 5 days the substrate **3** was recovered unaltered.

Obtention of ketone 20. Varodiol **1** (3 g) was dissolved in vinyl acetate (200 ml) and *Candida cylindracea lipase* (5 g) was added. The biotransformation was maintained for 5 days to give 2.4 g of monoacetate **15** (71%) and 600 mg of substrate **1** (20 %). Monoacetate **15** (600 mg) was dissolved in acetone (25 ml) and oxidized with

Jones's reagent as indicate for varodiol (**1**) to give 540 mg (90%) of **20** with identical spectroscopic data that *ent*-3β-acetoxy-12-oxo-13-*epi*-manoyl oxide previously obtained by us.[24]

Biotransformation of acetate 20 with Nectria ochroleuca. Substrate **20** (280 mg) was dissolved in EtOH (6 ml), distributed among 7 Erlenmeyer-flask cultures of *N. ochroleuca* and incubated for 7 days, after which the cultures were filtered and pooled and cells were washed thoroughly with water and the liquid was saturated with NaCl and extracted with CH_2Cl_2. Both extracts were pooled, dried with anhydrous Na_2SO_4, and evaporated at 40° in vacuum to give a mixture of compounds. This mixture was chromatographed on a silica gel column to obtain 109 mg of starting material **20** (39 %), 22 mg of *ent*-3β-hydroxy-12-oxo-13-*epi*-manoyl oxide (**21**, 9%) as a syrup; $[\alpha]_D$ -66 (*c* 1, $CHCl_3$); ν_{max} (film) 3470, 3089, 1712, 1638, 1083, 990 and 923 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.11 (1H, dd, *J* 17.5, 10.9 Hz, H-14), 5.14 (1H, dd , *J* 17.5, 1.1 Hz) and 5.04 (1H, dd , *J* 10.8, 1.1 Hz) (2H-15), 3.23 (1H, dd *J* 11.1, 4.8 Hz, H-3), 2.46 (1H, dd , *J* 17.9, 6.0 Hz) and 2.33 (1H, dd , *J* 17.9,13.0 Hz) (2H-11), 1.85 (1H, dd, *J* 13.0, 6.0 Hz, H-9), 1.31, 1.18 (3H each, s, 3H-16 and 3H-17), 1.02, 0.79 and 0.78 (3H each, s, 3H-18, 3H-19 and 3H-20; δ_C (75.47 MHz, $CDCl_3$) 14.8 (C-20), 15.5 (C-19), 19.5 (C-6), 22.2(C-17), 27.1(C-2), 28.2 (C-18), 28.7 (C-16), 33.8 (C-11), 36.7 (C-10), 37.0 (C-1), 38.7 (C-4), 42.1 (C-7), 54.6 (C-5), 55.0 (C-9), 75.4 (C-8), 78.7 (C-3), 82.0 (C-13), 113.2 (C-15), 142.4 (C-14), 211.3 (C-12); HRLSIMS, *m/z*, MNa^+, found 343.2237. $C_{20}H_{32}O_3Na$ requires 343.2249; 20 mg of *ent*-3β-hydroxy-13,14,15,16-tetranor-12,8α-labdanolide (*ent*-3β-hydroxysclareolide, **22,** 8%) as a syrup; ν_{max} (film) 3457, 1771, 1239 and 1035 cm^{-1}; δ_H (300 MHz, $CDCl_3$)3.25 (1H, dd , *J* 10.9, 5.3 Hz, H-3), 2.41 (1H, dd , *J* 16.2, 14.7 Hz) and 2.25 (1H, dd, *J* 16.2, 6.5 Hz) (2H-11), 1.90 (1H, dd , *J* 14.7, 6.5 Hz, H-9); 1.33 (3H, s, 3H-17), 1.00, 0.91 and 0.80 (3H each, s , 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 15.1 and 15.2 (C-19 and C-20), 20.4 (C-6), 21.6 (C-17), 26.9 (C-2), 28.0 (C-18), 28.8 (C-11), 35.9 (C-10), 37.8 (C-1), 38.5 (C-7), 38.9 (C-4), 55.4 (C-5), 59.0 (C-9), 78.7 (C-3), 86.2 (C-8), 176.6 (C-12); HRLSIMS, *m/z*, MNa^+, found 289.1780. $C_{16}H_{26}O_3Na$ requires 289.1780; 70 mg of *ent*-1β,3β-dihydroxy-12-oxo-13-*epi*-manoyl oxide (**23**, 27%) as a syrup; $[\alpha]_D$ -49 (*c* 2, $CHCl_3$); ν_{max} (film) 3460, 3090, 1702, 1642, 1093, 994 and 927 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.11 (1H, dd, *J* 17.4, 10.8 Hz, H-14); 5.17 (1H, dd, *J* 17.4, 1.3 Hz) and 5.03 (1H, dd, *J* 10.8, 1.3 Hz) (2H-15), 3.45 (1H, dd, *J* 11.4, 4.5 Hz, H-1), 3.25 (1H, dd, *J* 12.0, 4.4 Hz, H-3); 3.11 (1H, dd , *J* 19.2, 6.2 Hz, H_{eq}-11), 2.55 (1H, dd, *J* 19.3, 12.9 Hz, H_{ax}11), 2.03 (1H, dd, *J* 12.9, 6.20 Hz, H-9), 1.28 and 1.12 (3H each, s, 3H-16 and 3H-17), 0.96, 0.82 and 0.74 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 10.5 (C-20), 15.3 (C-19), 19.0 (C-6), 22.0 (C-17), 28.0 (C-18), 28.7 (C-16), 37.2 (C-11), 37.8 (C-2), 38.8 (C-4), 41.9 (C-7), 42.5 (C-10), 52.6 (C-5), 54.3 (C-9),75.5 (C-8), 75.8 (C-3), 77.8 (C-1), 81.4 (C-13), 113.3 (C-15), 142.2 (C-14), 212.8 (C-12); HRLSIMS, *m/z*, MNa^+, found 359.2197. $C_{20}H_{32}O_4Na$ requires 359.2198; and 16 mg of *ent*-3β,7β-dihydroxy-12-oxo-13-*epi*-manoyl oxide (**24,** 6%) as a syrup; $[\alpha]_D$ -37 (*c* 1, $CHCl_3$); ν_{max} (film) 3455, 3056. 1713 and 924 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.08 (1H, part X of an ABX system, $J_{AX}+J_{BX}$=28.4 Hz, H-14), 5.13 and 5.04 (part AB of an ABX system, 2H-15), 3.67 (1H, dd, *J* 11.8, 4.6 Hz, H-7), 3.23 (1H, dd, *J* 11.7, 4.9 Hz, H-3); 1.30, 1.18, 1.02, 0.80 and 0.79 (3H each, s, 3H-16, 3H-17, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 14.9 (C-20), 15.6 (C-17), 16.2 (C-19), 26.6 (C-6), 27.0 (C-2), 28.2 (C-18), 28.5 (C-16), 33.4 (C-11), 36.6 (C-1), 37.1 (C-10), 38.8 (C-4), 53.0 (C-5), 53.0 (C-9), 78.5 (C-3), 79.1 (C-8), 79.8 (C-7), 81.9 (C-13), 113.4 (C-15), 142.1 (C-4), 209.7 (C-12); HRLSIMS, *m/z*, MH^+, found 337.2366. $C_{20}H_{33}O_4$ requires 337.2379.

Oxidation of lactones 6 and 22. 15 mg of lactone **6** was dissolved in 1 ml of Me_2CO and oxidized with Jones's reagent as indicate for varodiol (**1**). Chromatography on a silica gel column yielded 11 mg of lactone **11**

(74%). 12 mg of lactone **22** was dissolved in 2 ml of Me_2CO and oxidized with Jones's reagent. Chromatography on a silica gel column yielded 10 mg of *ent*-3-oxosclareolida (**11**, 84%) as a syrup; ν_{max} ($CHCl_3$) 1777, 1739 cm^{-1}; δ_H (300 MHz, $CDCl_3$)2.6-2.4 (3H, superposed signals, 2H-2 and H-11), 2.28 (1H, dd , *J* 16.2, 6.5 Hz, H-11), 1.98 (1H, dd , *J* 14.7, 6.5 Hz, H-9), 1.37 (3H, s, 3H-17), 1.12,1.05 and 1.02 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 14.6 (C-20), 20.8 (C-19), 21.2 (C-17), 21.6 (C-6), 26.8 (C-18), 28.7 (C-11), 33.5 (C-2), 35.7 (C-10), 37.8, and 37.9, (C-1 and C-7), 47.4 (C-4), 54.5 (C-5), 58.3 (C-9), 85.7 (C-8), 175.9 (C-12), 215.5 (C-3); HRLSIMS, *m/z*, MNa^+, found 287.1628. $C_{16}H_{24}O_3Na$ requires 287.1623.

Obtention of ent-3β-hydroxyambrox (26) from ketolactone 11. 18 mg of ketolactone **11** was dissolved in 5 ml of THF and 2 ml of $LiAlH_4$ (1M) in THF were added. The mixture was maintained for 60 min at reflux after which was quenched by adition of aqueous ether followed by water and extracted with CH_2Cl_2. The organic phase was dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. Chromatography on a silica gel column yielded 15 mg of *ent*-3β,8α,12-trihydroxy-13,14,15,16-tetranorlabdane (**25**, 81%) as a colourless solid, m.p. 197-198°C; $[\alpha]_D$ +8 (*c* 0.25 $CHCl_3$); ν_{max} (KBr) 3303 and 1039 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.79 (1H, ddd, *J* 10.1, 4.4, 4.4 Hz) and 3.47 (1H, ddd, *J* 10.1, 9.2, 4.7 Hz) (2H-12), 3.22 (1H, dd, *J* 11.1, 4.8 Hz, H-3), 1.20 (3H, s, 3H-17), 0.99, 0.81, 0.76 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 15.4 and 15.5 (C-19 and C-20), 20.3 (C-6), 24.7 (C-17), 27.2 (C-2), 28.3 (C-18), 29.9 (C-11), 37.7 (C-1), 38.8 and 38.9 (C-4 and C-10), 44.4 (C-7), 55.1 (C-5), 58.9 (C-9), 64.2 (C-12), 73.0 (C-8), 78.8 (C-3); HRLSIMS, *m/z*, MNa^+, found 293.2092. $C_{16}H_{30}O_3Na$ requires 293.2093. 10 mg of triol **25** was dissolved in 1 ml of pyridine and 15 mg of TsCl were added. The mixture was maintained during 72 h at 25 °C. Then cold water was added to the mixture which was extracted with CH_2Cl_2, washed with aqueous $KHSO_4$ and water. The organic solution was dried and evaporated at 40°C in vacuum. The mixture was chromatographed on a silica gel column to obtain 3 mg of triol **25** and 5 mg of *ent*-8α,12-epoxy-3β-hydroxy-13,14,15,16-tetranorlabdane (*ent*-3β-hydroxyambrox, **26**, 54 %) as a colourless solid, m.p. 142-145 °C; $[\alpha]_D$ +190 (*c* 1, $CHCl_3$); ν_{max} ($CHCl_3$) 3422, 1043 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 3.91 (1H, m) and 3.81 (1H; c, *J* 8.4 Hz) (2H-12), 3.23 (1H, dd, *J* 9.9, 6.3 Hz, H-3), 1.08 (3H, s, 3H-17), 1.00, 0.84 and 0.80 (3H each, s, 3H-18, 3H-19 and 3H-20); δ_C (75.47 MHz, $CDCl_3$) 15.2 and 15.3 (C-19 and C-20), 20.5 (C-6), 21.2 (C-17), 22.8 (C-11), 27.3 (C-2), 28.4 (C-18), 36.1 (C-10), 38.3 (C-1), 38.9 (C-4), 39.7 (C-7), 56.2 (C-5), 60.2 (C-9), 65.0 (C-12), 79.2 (C-3), 79.9 (C-8); HRLSIMS, *m/z*, MNa^+, found 275.1979. $C_{16}H_{28}O_2Na$ requires 275.1987.

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29 Novozym 435 is an immobilized preparation of a thermostable lipase derived from a selected strain of *Candida antartica*. Lipozyme IM is an immobilized lipase preparation derived from a selected strain of *Mucor miehei*. These lipases were generously supplied by Novo-Nordisk Bioindustrial S.A, Madrid, Spain.