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Microbial Transformation of Diterpenes: Hydroxylation of Sclareol, Manool and Derivatives by *Mucor plumbeus*

Gérard Aranda*, Mohammed Samir El Kortbi, Jean-Yves Lallemand,

Laboratoire de Synthèse Organique, URA 1308 associée au CNRS, Ecole Polytechnique, 91128- Palaiseau, France

Alain Neuman,

Laboratoire de Chimie Bio-organique Structurale, U.F.R.Biomédicale, rue M.Cachin, 93012- Bobigny, France

Abderrahmane Hammoumi, Isabelle Facon and Robert Azerad

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA 400 asociée au CNRS, Université René Descartes, 45 rue des Saints-Pères, 75270- Paris Cedex 06, France.

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Key Words: Mucor plumbeus; microbiological transformation; diterpenes; sclareol and manool derivatives; structural determination.

Abstract: Microbial transformation of several labd-14-ene derivatives has been carried out with a *Mucor plumbeus* strain. Sclareol (**1**) (labd-14-en-8α,13β-diol) affords quantitatively a mixture of triols from which the labd-14-en-3β,8α,13β-triol (**2**) is obtained in high yield. Incubation of other labdane derivatives, including manool (**6**) [labd-8(17),14-dien-13β-ol], the corresponding 7α-hydroxy derivative (**10**), manoyl oxide (**14**) or sclareolide (**15**) with the same microorganism results in a mixture of various new hydroxylated and keto- derivatives.

INTRODUCTION

As part of a program about biotransformation of easily accessible natural or synthetic terpenes by microorganisms, in order to obtain homochiral intermediates for hemisynthesis of biologically relevant molecules, we have carried out a study of the biotransformation of labdane-derived terpenoids by filamentous fungi. Several examples of microbial transformations in this structural class have been previously described. Soil bacterial isolates JTS-162 and JTS-131 have been found to transform *cis*-abienol and sclareol into several new labdanoid compounds [1,2]: the pathway for such conversions involved essentially hydroxylation and further oxidation of one of the methyl groups in position -18. A *Scopuloriopsis* species H/134, out of 263 soil fungal isolates tested, has been described [3] to hydroxylate in very low yields 7-deacetyl-1,9-dideoxyforskolin either at 1-α and 9-α positions to give forskolin, or at 2-β position; other hydroxylated products were isolated, and particularly a 3β-hydroxy derivative [4], depending on slight modifications of the structure of the substrate. Similar results, but in higher yields, have been claimed using *Mortierella isabellina* ATCC 160074 [5] or *Neurospora crassa* ATCC 10336 [6,7]. At last, several recent reports [8-14] about the biotransformation of *ent*-13-*epi*-manoyl oxides and related diterpenes by *Rhizopus nigricans*, *Gibberella fujikuroi* or *Curvularia lunata* have shown a large variety of hydroxylation patterns, including 1β, 3β, 6β, 7α, 11β, 12α, 12β and C-20 positions.

The fungus *Mucor plumbeus*, which is currently used in our laboratory for the regioselective introduction of hydroxy groups into polycyclic enones [15], has been recently used as a powerful and selective hydroxylating agent for sclareol **1**, a diterpenoid which is easily obtained from clary sage (*Salvia sclarea* Linn.)[16], affording in high yield a 3β-hydroxylated derivative [17]. After submission of these results for publication, a similar report using another fungal strain was recently published [18]. We now report here detailed results of these experiments and their extension using as substrates the commercially available manool **6** [labd-8(17),14-dien-13β-ol], which is isolated from wood oil of the yellow pine, *Dacrydium biforme* [19] ; various derived diterpenes such as the corresponding 7α-hydroxymanool **9** [20], manoyl oxide epimers **12** [21] or sclareolide **13** [16,21], respectively obtained from manool **6** or sclareol **1** have been also tested as substrates for biotransformation by the same microorganism.

RESULTS AND DISCUSSION

A preliminary screening among numerous commonly used microorganisms showed a widespread ability to transform sclareol **1** into a small number of more polar products easily separated by thin layer chromatography (Table 1). One main product was observed in most cases and *Mucor plumbeus*.ATCC 4740 was selected as the faster metabolizing strain and used systematically throughout this work.

Table 1. Detection by TLC of products obtained from selected fungal strains incubated with sclareol (0.5 $g.L^{-1}$). Solvent: diethyl ether- methanol (9:1).

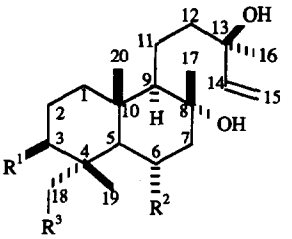
		Products detected [a]			
Strains	incubation (days)[b]	sclareol **1**[c]	**2**[c]	**3+4**[c]	others (number)[d]
Aspergillus alliaceus NRRL 2315	7	++	+++	±	-
Aspergillus niger	2	++	+++	±	+(4)
Aspergillus ochraceus ATCC 1009	6	++	++	++	-
Cunninghamella echinulata NRRL 3655	1	±	+++	+++	±(5)
Cunninghamella elegans	4	+++	-	±	-
Curvularia lunata NRRL 2380	3	-	++++	+	±(3)
Mortierella isabellina MMP 108	6	-	+++	++	±(4)
Mortierella ramanniana MMP 117	7	++	+++	+	±(3)
Mucor plumbeus ATCC 4740	2	-	+++	++	-
Rhizopus arrhizus ATCC 11145	7	-	+	+++	±(3)
Sporotrichum exile QM 1250	3	+++	+	+	±(1)

[a] detection by spraying 10% phosphomolybdic acid in ethanol and heating on a hot plate.
[b] time of incubation corresponding to the total disappearance of sclareol or to unchanged substrate-products pattern.
[c] R_f: **1**, 0.9; **2**, 0.8; **3+4**, 0.7.
[d] including products with higher R_f than **2** (probably keto-products)

Bioconversion of sclareol

When sclareol **1** was incubated at 0.3-0.5 $g.L^{-1}$ concentrations, either with a growing culture of *Mucor plumbeus* ATCC 4740 or with a washed biomass of the same microorganism in a buffered solution, a nearly complete conversion of the terpene [22] occurred affording after extraction a mixture of three triols **2, 3** and **4** in a quantitative yield. The extent of the conversion was essentially dependent on the presence of detergent (Tween 80), added at 0.1% concentration in the incubation medium. The major triol **2** (3β-hydroxy sclareol) was isolated by silica gel column chromatography in a 84% optimised yield. This triol was recovered unchanged on oxidation with $BaMnO_4$ or $AgCO_3$/celite, indicating a low reactivity of the hindered newly introduced hydroxy group.

The ^1H- and ^{13}C-NMR chemical shifts of sclareol **1** have been reported unambiguously [23]. The ^1H-NMR spectrum of triol **2** was characterized by a one hydrogen signal centered at 3.15 ppm and represented by a four lines splitting pattern with J_1= 9.5 and J_2= 6.5 Hz, in agreement with a hydroxylated position at C-1, C-3, C-6 or C-7, adjacent to only one CH_2 group. The ^{13}C-NMR spectrum confirmed the presence of a new hydroxylated carbon atom at δ = 79.6 ppm (C-1 or C-3) [24,25] and the invariance of the side chain. Analysis of the substituent



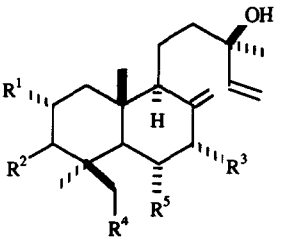
1 : $R^1=R^2=R^3=H$

2 : $R^1=OH, R^2=R^3=H$

3 : $R^1=R^3=H, R^2=OH$

4 : $R^1=R^2=H, R^3=OH$

5 : $R^1=R^2=H, R^3=OC(C_6H_5)_3$



6: $R^1=R^2=R^3=R^4=R^5=H$

7: $R^1=OH, R^2=R^3=R^4=R^5=H$

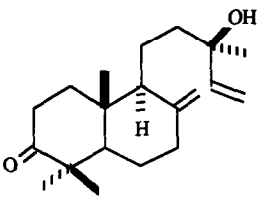
8: $R^1=R^3=R^4=R^5=H, R^2=OH$

9: $R^1=R^2=R^4=R^5=H, R^3=OH$

10: $R^1=R^4=R^5=H, R^2=R^3=OH$

11: $R^1=R^2=R^4=H, R^3=R^5=OH$

12: $R^1=R^2=R^5=H, R^3=R^4=OH$



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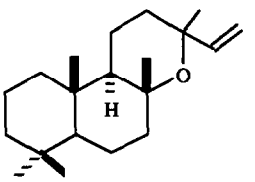

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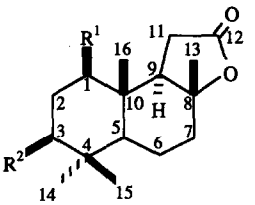
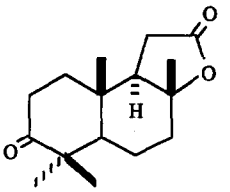


15: $R^1=R^2=H$

16: $R^1=H, R^2=OH$

17: $R^1=OH, R^2=H$



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effects in the ^{13}C NMR spectrum (absence of downfield shift of the C-20 methyl group) and of the 2D-^1H,^{13}C correlations showed that the hydroxylation probably affected C-3 and not C-1. However, some discrepancies were pointed out when comparing the described coupling constants of H-3α in **2** (9.5 and 6.5 Hz) and in some corresponding 3β-hydroxycompounds earlier quoted in the literature [4,9]; others [10,25] were very similar to that actually found and attributed to an ax- eq + eq-eq coupling pattern. Ultimately, the X-ray diffraction pattern of **2** was in accordance with a crystalline structure corresponding to a 3β-hydroxy derivative of sclareol; figure 1 shows an ORTEP view of the molecule, with thermal ellipsoids at the 50% probability level. Analysis of the geometrical parameters confirmed the β-position of the hydroxyl group at C-3 and indicated that the C(11)-C(15) chain adopts an elongated *anti* conformation with a tilted double bond C(14)-C(15) and with dihedral angles around C(11)-C(12) = 178°, C(12)-C(13) = -177° and C(13)-C(14) = 114°

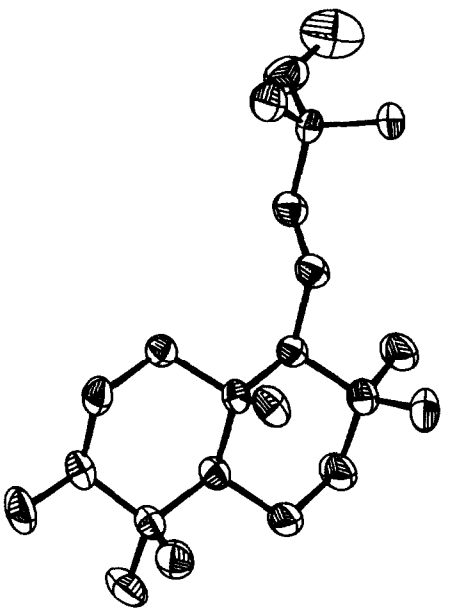

Fig.1. Computer generated perpective drawing obtained from the crystal coordinates of 3β-hydroxy sclareol **2**

The minor triols **3** (6α-OH) and **4** (18-OH) obtained in a 1:1 ratio (13% total yield) could not be separated by silica gel chromatography; however, pure triol **3** was obtained by fractional crystallization. ^{1}H-NMR of **3** was characterized by a double triplet (one hydrogen) centered at 3.75 ppm (J_1= 3.6, J_2= 11.2 Hz) corresponding to a C*H*(OH) axial hydrogen coupled with two axial and one equatorial hydrogen atoms, thus located at positions C-6 or C-11. The chemical shift and the coupling constants observed are in agreement with a 6α-hydroxy derivative of sclareol, similar to larixol, the 6α-OH derivative of manool [26] (δ 6β-H= 3.67 ppm, J_1=5, J_2= 10 Hz). No interaction of the 6-hydroxy group with the C-20 methyl group (δ = 0.84 ppm) was apparent, confirming an equatorial 6α-position. On the contrary, the 18- and 19-methyl signals are shifted downfield by the deshielding effect of the 6α-hydroxy group. Analysis of the ^{13}C-NMR spectrum showed the invariance of the C-11 to C-16 side chain atoms and the expected downfield shift of C-5 and C-7.

Spectroscopic data of triol **4** were deduced from a mixture of **3** and **4**, indicating again the presence of a new hydroxy group, but as a primary alcohol. However, the triol **4** remained unaffected in a tentative of etherification with trityl chloride in the usual conditions. Using a recently described procedure [27], the trityl derivative **5** could be prepared and separated, albeit the tritylation reaction was uncomplete. The spectroscopic data of **5** verified ^{1}H-NMR attributions of the starting triol **4**. However, it was impossible to effect detritylation with usual reagents, particularly $ZnBr_2$ in various solvents [28]. On the other hand, subsequent dehydration invariably occurred when using trifluoroacetic acid in chloroform, even at low temperatures. Fortunately, a pure sample of triol **4** was isolated by repeated extraction of the incubation mixture and mycelium with methylene chloride. The ^{13}C-NMR spectrum of the triol **4** was characterized by the presence of a CH_2OH group (δ= 72.1 ppm), replacing one of the gem-dimethyl groups; furthermore, in comparison with related 18(α)- or 19(β)-hydroxylated diterpenic homologous structures, the ^{13}C-chemical shift of the CH_2OH group fits better with a 18(α)-OH (about 72 ppm) than a 19(β)-OH (about 65 ppm) structure [1,2,29,30]; correspondingly, the vicinal methyl group resonance (δ= 17.9 ppm) is in agreement with a 19(β)-CH_3, characterized by a chemical shift of about 18 ppm like for example in 18-hydroxymanool (torreferol) [1,31] rather than the 27 ppm shift of the 18(α)-methyl group of agathadiol or methyl agatholate [32] and 12-O-methyl podocarpol [33].

While 3β-hydroxylation is not exceptional in the microbial oxidation of cyclic diterpenoids [6-8,25], the high yield observed for the previously unknown 3β-hydroxy sclareol is nearly unprecedented in common terpenoid conversions [34]. Only small amounts of otherwise hydroxylated derivatives (6α- and 18-) are additionally formed, without any significant degradation of the side chain [35], probably prevented by the presence of a C-16 methyl branch.

Bioconversion of manool

Using the same incubation conditions (substrate added to the culture medium), manool **6** was about 90% converted in 5-6 days and afforded a mixture of ketonic material (4%) from which the major (about 80%) 3-keto derivative **13** was tentatively identified by ^{1}H- and ^{13}C-NMR spectroscopy, comparatively with a similar earlier

described 3-keto compound derived from sclareol [23]. The diols **7** and **8** were isolated in 16.6 and 6.6% respective yields, together with the more polar triol **10** (14.2%), resulting from a double hydroxylation reaction. The diol **8** displays a doublet of doublets centered at δ 3.26 ppm (J= 11.2 and 4.7 Hz) which corresponds to a 3α-axial C*H*(OH) splitted by one axial and one equatorial adjacent hydrogens. Such spectroscopic features are in agreement with those previously described for the 3β-hydroxy derivative of forskolin [4] and are similar to the data shown above for 3β-hydroxy sclareol **2**; diol **8** must hence be 3β-hydroxy manool.

The ^{1}H-NMR spectrum of the diol **7** was characterized by a one hydrogen-signal centered at δ 3.86 ppm and represented by a nine line splitting pattern. Since C-2 is the unique position with four adjacent hydrogens, it can be inferred that the newly introduced hydroxy group is 2α-equatorial and that the observed triple triplet originates from the equivalent coupling of the 2β-axial hydrogen with vicinal axial and equatorial 1- and 3-hydrogens ($J_{2\beta,1\alpha}= J_{2\beta,3\alpha}=$ 11.5 Hz, $J_{2\beta,1\beta}= J_{2\beta,3\beta}=$ 4.0 Hz). The 2α-hydroxylation of **7** is rather unexpected, by comparison with the results previously obtained with sclareol. However abietic acid has been found to give 2α-hydroxy metabolites upon incubation with *Mortierella isabellina* [5]; on the other hand, *M.isabellina* and *Neurospora crassa* are also efficient microorganisms for the 2α-hydroxylation of 1,9-dideoxyforskolin [6,7]. Contrarily to sclareol, no significant hydroxylation of the 18 (19)-methyl groups was observed.

The bioconversion of manool by *M.plumbeus* is less productive than the bioconversion of sclareol by the same microorganism. While the functionalization at C-3 (about 25%) remains favoured, the low recovery of products does not only result from separation problems, but probably also reflects an extensive degradation of the substrate and products by the microorganism in long time incubations.

Bioconversion of 7α-hydroxymanool ***9***

Diol **9**, which was easily obtained from manool by oxidation [36], was completely converted by incubation in the culture medium with *M.plumbeus* ATCC 4740. This diol afforded again, as a major product (22%), the (3β-hydroxy)triol **10**, precedently obtained by double microbial hydroxylation of manool. Moreover, another hydroxylated product, identified as a 6α,7α,13β-triol **11** was isolated in substantial amount (26%) together with a minor product isolated as oil (2%), identified as a 19(β)-hydroxy derivative **12**. These products were acompanied by a complex mixture of keto-derivatives (26%), unseparated by thin layer chromatography.

Triol **11** was, *inter alia*, characterized by a ^{13}C-NMR chemical shift corresponding to a new CHOH group at 79.1 ppm, possibly at C-1 or C-3 (see above). Both hydroxylation positions must be characterized in ^{1}H-NMR by a doublet of doublets. The ^{1}H,^{13}C-correlation spectra showed that the secondary hydroxyl group at 79.1 ppm corresponded to a doublet at 4.11 ppm (J= 3.4 Hz), effectively present in the ^{1}H-NMR spectrum of triol **11**, together with a doublet of doublets at 3.70 ppm (J= 11.5 and 3.4 Hz). These splitting patterns are consistent respectively with ax.6β- and eq.7β-C*H*(OH), where the 7β-H (originally a broad triplet in diol **10**) becomes a doublet, while the 6β-H is splitted by two adjacent protons (eq.7β-H and ax.5α-H). Furthermore, 5α-proton appears in the spectrum as a doublet (J= 11.5 Hz).

The minor triol **12** corresponds to a 19(β)-hydroxymethyl derivative characterized by ^{13}C-NMR chemical shifts for 19-CH_2OH and 18-CH_3 at 65.5 and 27 ppm respectively. Comparatively to triol **4**, C-3 and C-18 are shifted upfield, while C-5 is shifted downfield as previously predicted [33]. The ^{1}H-NMR spectrum of **12** is characterized by an AB system (3.74-3.77 and 3.42-3.67 ppm, $J_{AB}=$ 10.8 Hz) representative of the 19-CH_2OH group, shifted downfield comparatively to the 18(α)-CH_2OH of triol **4**. The ketones were tentatively identified as 3-keto derivatives, with ^{13}C-NMR chemical shifts at 217.1 and 216.8 ppm and IR absorption at 1709 cm^{-1}.

Bioconversion of manoyl oxide **14**

Quite surprinsingly, manoyl oxide **14** was rapidly (2 days) and completely metabolized by a culture of *M.plumbeus* ATCC 4740 and it was not possible to extract significant amount of any hydroxylation product.

Bioconversion of sclareolide **15**

The lactonic labdane derivative **15** [21] afforded by incubation with *M.plumbeus* in the culture medium and after complete disappearing of the substrate (2 days), the 3β-hydroxy and the 3-keto derivatives **16** and **18** (respectively 7.9 and 3.2%); a third minor compound (2.5%) was additionally isolated and tentatively identified

as the 1β-hydroxy compound **17.** This compound differed from the known 3α-hydroxy lactone recently isolated from *Salvia aethiopis* [37] ; the hydrogen atom geminal to the newly introduced hydroxy group showed a doublet of doublets at δ 3.28 ppm (J= 5.5 and 10.5 Hz) indicative of an axial hydrogen at C-1, C-3 or C-7 positions. The 79 ppm resonance of the -CHOH- carbon may be attributed to either a 1β- [24] or 3β-equatorial hydroxyl groups, while a 1α-hydroxyl group is characterized by a 75.5 ppm signal [38]. The *syn*-γ effect on C-5 and C-9, without any significant effect on the quaternary hydroxylated C-8, again strongly suggests a 1β-OH and not a 7β-OH substitution, as confirmed by 2D-^{1}H,^{13}C-NMR correlation spectra, where the 7β-H is localized as a 11α-H overlapping signal at 2.38 ppm. However, the reasons why the ^{1}H-NMR signals of 11-H_2 are shifted downfield, with J_{BX}= 0, and the ^{13}C-NMR downfield shift of the 13-CH_3 are not obvious. Apparently, such an introduction of an OH group in the 1β- position may induce large framework deformations. Other studies with the help of radiocrystallographic methods should be necessary.

The recovery of hydroxylated compounds from the fast and complete bioconversion of the lactone **13** was particularly low and indicative of an extensive metabolism of the molecule, probably favoured by the initial opening of the lactone structure and the subsequent attack of the side chain. In spite of several assays in different conditions, where the bioconversion was stopped at early stages, it was not possible to isolate any other intermediate than the 1- and the 3- oxidized compounds.

Bioconversion of 3β-hydroxysclareol **2**

In a tentative to obtain overhydroxylated derivatives of sclareol, the precedently obtained 3β-hydroxylated compound **2** was incubated again during seven days with a culture of *M.plumbeus* ATCC 4740. The triol **2** remained unaffected. Only a small fraction (about 3%) of a ketonic material (probably the 3-keto derivative [23] deriving from oxidation of 3-hydroxysclareol) was isolated. Moreover, about 15% of the starting material was recovered in the form of an esterification product with fatty acid, possibly resulting from a transesterification reaction with Tween 80 during incubation and/or isolation procedures.

This result confirms the high conversion yields previously observed with sclareol, and the inertness of the primary hydroxylation product, in contrast with the active degradation metabolism affecting other substrates.

CONCLUSION

The results described in this paper show that *Mucor plumbeus* may be considered as a versatile microbial tool for the regio- and stereoselective introduction of hydroxy groups into cyclic diterpenes of the labdane family; Beside 3β- position which is highly favoured in sclareol, 1β-, 2α, 6α-, 7α-, 18- or 19- positions may be hydroxylated. However, while the metabolism of sclareol is nearly limited to a unique hydroxylation reaction, other derivatives are deeply metabolized and the yield of recovered hydroxylated terpenes is sometimes much lower. It is possible that the presence of an additional exocyclic double bond in manool, or a lactone structure in sclareolide, or an ether bridge in manoyl oxide, enhances dramatically the occurrence of degradative reactions in the microorganism used. Nevertheless, sclareol, manool and their derivatives probably remain molecules of choice for further bioconversion studies, actually in progress with appropriate fungal isolates.

EXPERIMENTAL

General.

Melting points were determined on a Reichert microscope hot plate and are uncorrected. IR spectra were recorded in CCl_4 solution on a Perkin-Elmer 399 spectrometer. ^{1}H-NMR spectra were recorded at 200 and 400 MHz on Bruker AC 200 FT or AM 400 FT spectrometers. NMR samples were prepared in $CDCl_3$ or CD_3OD containing 1% TMS as internal reference. ^{13}C-NMR spectra were recorded at 50.3 or 100.6 MHz respectively and assignments were made by polarization transfer using a DEPT135 sequence. Full assignments of ^{1}H-spectra, and specially assignments of individual chemical shifts of the methyl groups were obtained using 2D-^{1}H,^{13}C heteronuclear correlation analyses. Elemental analysis of crystalline samples were performed at the Central Microanalysis Laboratory, C.N.R.S., Gif-sur-Yvette, France. Optical rotations were measured in 1 dm-

cells on a Perkin -Elmer 241 spectropolarimeter. High resolution mass spectrometry (HR-MS) was performed on a Bruker CMS.47 FT-ICR spectrometer. Fast atomic bombardment (FAB-MS) mass spectra were obtained with a Kratos MS-80 RF spectrometer and chemical ionization (CI) spectra with a modified AEI MS-9 spectrometer [39]. Analytical thin layer chromatography (TLC) on 0.25 mm Merk silicagel $60F_{254}$ precoated plates was routinely used to monitor bioconversions. Silicagel Merk H_{60} was used for column chromatography.

Starting materials.

Sclareol **1** [22] was a gift from Synarome (France). Manool **6** was purchased from Westchem Industries Ltd, Dunedin, New Zealand, as a crude brown oil, affording pure manool in 84% yield after silica gel chromatography. 7α-Hydroxy manool **9** was obtained by oxidation of manool with SeO_2 and *t*-butyl hydroperoxide in benzene solution (85% yield) following a described procedure [36]: M.p. 124.5-125.5°C; IR (CCl_4) cm^{-1}: 3620, 3600-3100, 3080, 2940, 1100, 920 and 905; ^{1}H-NMR, δ ppm ($CDCl_3$): 5.88 (14-H, dd, $J_{14,15}$= 17.5 Hz, $J_{14,15'}$= 10.7 Hz), 5.20 and 5.04 (15-H_2, 2 dd, $J_{15,15'}$= 1.15 Hz), 5.02 and 4.60 (17-H_2, 2 br.s, ΔW= 5 and 3 Hz), 4.35 (7β-H, br.t, ΔW= 3 Hz), 1.26 (16-CH_3, s), 0.86, 0.78 and 0.63 (18-, 19- and 20-CH_3, 3s); ^{13}C-NMR, δ ppm ($CDCl_3$): 149.7(C-8), 145.5(C-14), 111.6(C-15), 109.6(C-17), 74.1(C-7), 73.7(C-13), 51.2(C-9), 47.2(C-5), 42.2(C-3), 40.9(C-12), 40.1(C-10), 38.8(C-1), 33.4(C-18), 33.2(C-4), 31.0(C-6), 28.1(C-16), 21.6(C-19), 19.4(C-2), 17.4(C-11), 13.5(C-20), in correct agreement with [20]. Manoyl oxide **14** was prepared from sclareol [21] as a colorless oil [mixture of 13-epimers (2:1)]; spectroscopic data were in agreement with those already reported [20,23]. Sclareolide **15** was obtained from sclareol following a described procedure [21]: M.p.126-127°C; ^{1}H-NMR, δ ppm ($CDCl_3$): 2.46-2.12 (11β-H and 11α–H, AB part of an ABX system with 9α-H, J_{AB}= 15.0 Hz, J_{AX}= 14.7 Hz, J_{BX}= 6.0 Hz), 1.29, 0.87, 0.84 and 0.80 (13-, 16-, 14- and 15-CH_3, 4s); ^{13}C-NMR spectrum ($CDCl_3$) was in good agreement with published data [37] excepted for the assignments of C-7 and C-11; new assignments (δ= 38.8 and 28.7 ppm respectively) are based on correlations by 2D-^{1}H,^{13}C-NMR.

Microorganisms, culture and incubation conditions.

Mucor plumbeus ATCC 4740, obtained from the American Type Culture Collection (Rockville, Md, USA) was maintained and sporulated on agar slants (Glucose, 20 g; pancreatic peptone, 5g; yeast extract, 5g; malt extract, 5g; Bacto-agar, 20g in water, 1L). 250 ml Erlenmeyer flasks, containing 100 ml of a liquid medium (Corn steep liquor, 10g; K_2HPO_4, 2g; KH_2PO_4, 1g; $NaNO_3$, 2g; KCl, 0.5g; $MgSO_4,7H_2O$, 0.5g; $FeSO_4,7H_2O$, 0.02g; Glucose, 30g in water, 1L.) were inoculated with a freshly collected spore suspension in water and incubated at 27°C with orbital shaking (250 r.p.m.). After full growth of the mycelium (48-72 hours), substrate (25-50 mg) was dispensed to each flask as a solution in ethanol (0.5-1 ml) and tween 80 (0.1 ml).and the incubation continued in the same conditions during 2-6 days. In other experiments quoted as "washed mycelium incubations", the microrganism was grown in the same culture medium in a 7 L helix-aerated Biolafitte fermentor at 27°C during 48-60 hours; the mycelium (as 0.5-1 mm pellets) was recovered by filtration, washed repeatedly with water, then suspended again (at a concentration corresponding to about 20 g dry weight. L^{-1} in a 0.1 M potassium phosphate buffer, pH 7.0 which was distributed to final 500 ml volumes into 2 L conical flasks. The substrate (0.5 $g.L^{-1}$) was added and incubations were performed as described above.

Other microorganisms, used for the screening experiments in similar culture conditions were obtained from various origins: MMP, Mycotheque of the Museum d'Histoire Naturelle, Paris (France), NRRL, Northern Utilization Research and Development Division, Peoria (Illinois, USA); QM, Quartermaster Research and Development Center, Natick (Massachusetts, USA); no identification number, local isolate.

Recovery and purification of biotransformation products

The incubation medias were filtered. The mycelium was washed with water then kept in methanol. Filtrate and washings were pooled and extracted continuously with diethyl ether during 2-3 days for convenience. However, when the amount of starting material in incubations was higher than 300-400 mg, longer and complementary extractions of the incubation mixture with methylene chloride were necessary. After removal of the solvents *in vacuo*, the yellow residue was dissolved in methylene chloride and washed with cold 5% NaOH

then with water until neutral. The alkaline extract, after acidification, only afforded fatty acids. The methanolic extract of the mycelium contained generally at most 1-2% of the terpenic compounds and the major part of the detergent and was usually discarded. Washed extract was deposited on a silica gel column and eluted stepwise with cyclohexane containing increasing amounts of diethyl ether, then with diethyl ether containing ethyl acetate (up to 35%). Fractions homogeneous on thin layer plates were pooled and submitted to crystallization.

Biotransformation of sclareol ***1***.

In several incubations in the culture medium at concentrations up to 0.5 $g.L^{-1}$, in the presence of Tween 80, sclareol was generally 92-99% consumed in 2-4 days. The corresponding yields of isolated triols **2** and of the mixture **3** + **4** amounted respectively to 52-84% and 46-11%. A small fraction (2-4%), where epi-sclareol derivatives were predominant [22] was also isolated. Overall recovery ranged between 62 to 97%. Washed mycelium incubations in buffer solution afforded very similar results.with an unchanged ratio of triol **2** to **3**+**4**.

3β-hydroxy sclareol (labd-14-en-3β,8α,13β-triol) **2**. Mp 169-171°C (from EtOAc-pentane); $[\alpha]_D^{21}$= -9.7°, $[\alpha]_{578}^{21}$= -10°, $[\alpha]_{546}^{21}$= -11.3°, $[\alpha]_{436}^{21}$= -19.6°, $[\alpha]_{365}^{21}$= -31.1° (c 1.9, MeOH); anal.for $C_{20}H_{36}O_3$, calc. C% 74.02, H% 11.18, found C% 73.99, H% 11.15; ^{1}H-NMR, δ ppm (CD_3OD): 5.92 (14-H, dd, $J_{14,15}$= 17.5 Hz, $J_{14,15'}$= 10.5 Hz), 5.19 and 5.00 (15-H_2,2dd, $J_{15,15'}$=1.7 Hz), 3.15 (3α-H,dd, J=9.5 and 6.5 Hz), 1.23, 1.11, 0.96, 0.82, 0.75 (16-, 17-, 18-, 20- and 19-CH_3, 5s); ^{13}C-NMR, δ ppm (CD_3OD): 146.8(C-14), 111.7(C-15), 79.6(C-3), 75.0(C-8), 74.4(C-13), 62.6(C-9), 56.6(C-5), 46.5(C-12), 45.1(C-7), 40.3(C-10), 39.9(C-4), 39.4(C-1), 27.6(C-2), 27.4(C-16), 24.0(C-17), 21.2(C-6), 20.7(C-11), 16.1(C-19 and C-20); HRMS for $C_{20}H_{34}O_2$ (M-H_2O), calc.306.2666, found 306.2687; MS (CI, NH_3): 342$(MNH_4)^+$, 324(342-$H_2O)^+$, 306(324-$H_2O)^+$, 289(306-$NH_3)^+$, 271(289-$H_2O)^+$.

6α-hydroxy sclareol (labd-14-en-6α,8α,13β-triol) **3**. Mp 152-154°C (from CH_2Cl_2-AcOEt); $[\alpha]_D^{21}$= +21°, $[\alpha]_{578}^{21}$= +21.95°, $[\alpha]_{546}^{21}$= +24.8°, $[\alpha]_{436}^{21}$= +41.7°, $[\alpha]_{365}^{21}$= +64.15° (c 1.64, MeOH); anal.for $C_{20}H_{36}O_3$, calc. C% 74.02, H% 11.18, found C% 74.42, H% 11.15; ^{1}H-NMR, δ ppm (CD_3OD): 5.91 (14-H, dd, $J_{14,15}$=17.4 Hz, $J_{14,15'}$= 10.5 Hz), 5.20 and 5.00 (15-H_2, 2dd, $J_{15,15'}$=1.8 Hz), 3.75 (6β–H, ddd, $J_{6\beta,5\alpha}$ and $J_{6\beta,7\alpha}$=11.2 Hz, $J_{6\beta,7\beta}$=3.6 Hz), 2.05 (7β-H, dd, $J_{7\beta,7\alpha}$=16.4 Hz, $J_{7\beta,6\beta}$=3.6 Hz), 1.22, 1.15, 1.14, 0.98 and 0.84 (16-, 17-, 18-, 19- and 20-CH_3, 5s); ^{13}C-NMR, δ ppm (CD_3OD): 146.7(C-14), 111.6(C-15), 74.7(C-8), 74.4(C-13), 69.4(C-6), 62.2(C-9), 62.1(C-5), 55.5(C-7), 46.4(C-12), 45.2(C-3), 41.2(C-1), 40.8(C-10), 37.2(C-18), 34.7(C-4), 27.4(C-16), 25.2(C-17), 20.7(C-11), 22.5(C-19), 19.3(C-2), 17.1(C-20); MS (CI, NH_3): 342$(MNH_4)^+$, 324(342-$H_2O)^+$, 306(324-$H_2O)^+$, 289(306-$NH_3)^+$, 271(289-$H_2O)^+$.

18(α)-hydroxy sclareol (labd-14-en-,8α,13β,18-triol) **4**. Mp.97-99°C (from acetone-pentane); $[\alpha]_D^{21}$= +2.6°, $[\alpha]_{578}^{21}$= +2.7°, $[\alpha]_{546}^{21}$= +2.9°, $[\alpha]_{436}^{21}$= +4.8°, $[\alpha]_{365}^{21}$= +7.0° (c 2.06, MeOH); ^{1}H-NMR, δ ppm (CD_3OD): 5.92 (14-H, dd, $J_{14,15}$=17.5 Hz, $J_{14,15'}$= 10.5 Hz), 5.20 and 5.00 (15-H_2, 2dd, $J_{15,15'}$=1.5 Hz), 3.38, 3.32- 3.04, 2.98 (18-CH_2OH, AB system, J_{AB}= 11.0 Hz), 1.22, 1.10, 0.84 and 0.70 (16-, 17-, 19- and 20-CH_3, 4s); ^{13}C-NMR, δ ppm (CD_3OD): 146.7(C-14), 111.6(C-15), 75.1(C-8), 74.4(C-13), 72.1(C-18), 62.8(C-9), 50.3(C-5), 46.6(C-12), 44.8(C-7), 40.7(C-1), 40.4(C-10), 38.6(C-4), 36.5(C-3), 27.3(C-16), 24.0(C-17), 21.1(C-6), 20.7(C-11), 18.9(C-2), 17.9(C-19), 16.5(C-20); HRMS for $C_{19}H_{31}O_2$ [M-(H_2O+CH_3)], calc.291.2289, found 291.2318. MS: 291 (48) [M-(H_2O+CH_3)]$^+$, 273 (33) [291-H_2O]$^+$, 257 (20), 177 (66), 161 (49), 147 (66), 133 (81), 119 (95), 107 (95), 95 (92) and 81 (100).

18(α)-hydroxy sclareol-18-O-trityl ether **5**. The trityl derivative **5** was obtained by treating a mixture of triols **3** and **4** following a recently described procedure [27]. To 126 mg of the triols mixture (approximately 6:4) dissolved in acetonitrile (5 ml) were added successively, under nitrogen and with magnetic stirring, triphenylcarbonium tetrafluoroborate (128 mg) and 2,6-di-t-butyl-4-methyl-pyridine (90 mg). After 2 days at room temperature, CH_2Cl_2 (6 ml) was added; the solution was washed with water (10 ml), 5% HCl (10 ml), and water (10 ml)) and dried over K_2CO_3. After removal of the solvents in vacuo, the partially crystallized residue (282 mg) was chromatographed on silica gel under nitrogen pressure. The trityl derivative **5** (37 mg) and

a mixture of triols **3** + **4** (94 mg) were isolated. **5**: Mp=84-86°C (from Et_2O-pentane); $[\alpha]_D^{21}$= +24°, $[\alpha]_{578}^{21}$= +25°, $[\alpha]_{546}^{21}$ = +28.3°, $[\alpha]_{436}^{21}$= +48.2°, $[\alpha]_{365}^{21}$= -72.2° (c 1.41, MeOH); IR (CCl_4) cm^{-1}: 3640, 3320, 2945, 1460, 1135 and 930; ^{1}H-NMR, δ ppm ($CDCl_3$): 7.47-7.20 (ArH, m), 5.95 (14-H, dd, $J_{14,15}$= 17.5 Hz, $J_{14,15'}$= 10.5 Hz), 5.22 and 5.04 (15-H_2, 2dd, $J_{15,15'}$= 1.5 Hz), 2.78, 2.74 and 2.71, 2.67 (CH_2-O-trityl, AB system, J_{AB}= 8.8 Hz), 1.25, 1.09, 0.77 and 0.63 (16-, 17-, 19- and 20-CH_3); ^{13}C-NMR, δ ppm ($CDCl_3$): 146.3(C-14), 144.4, 128.9, 127.8, 126.9(Arom.C), 111.3(C-15), 86.0 (Ar_3-C), 74.9(C-8), 73.7(C-13), 70.7(C-18), 61.9(C-9), 49.7(C-5), 45.2(C-12 and C-7), 39.6(C-1), 37.8(C-10), 39.3(C-4), 36.5(C-3), 27.3(C-16), 24.2(C-17), 20.0(C-6), 18.1(C-11), 19.2(C-2), 18.1(C-19), 16.0(C-20).

X-Ray diffraction analysis of 3β-hydroxysclareol **2.** X-ray diffraction experiments were performed on a Philips PW1100 automatic four-circle diffractometer operating with the Cu-Ka radiation (λ= 1.5418 Å) monochromated by graphite. A crystal approximately 0.5 x 0.3 x 0.3 mm was obtained from methanol. The orientation matrix of the crystal was calculated from the angular settings of 25 randomly distributed reflections in the range 10°<θ>25° and refined by least-squares procedure. No significant decomposition was found during the data collection and no correction was applied. The reflections were scanned over a 1.2° angle width at a speed of 0.03°.s^{-1} and for each reflection, the background was deduced from two stationary measurements on both sides of the reflection. The intensities, measured up to θ = 68°, were reduced to **F** structural factors by means of standard Lorentz and polarization corrections and considered as observed above the 2σ background level. No absorption correction was applied. The system is monoclinic, space group C2 with a=23.983(5); b=6.318(3); c=17.299(3) Å; b= 127.18(6)° and Z=4. The structure (Figure 1), found as a 1:1 methanol solvate, was deduced from direct methods [40] and refined with anisotropic thermal factors to R= Σ|Fo-Fc|/Σ|Fo|= 7.5% for 1886 reflections.

Bioconversion of manool **6.**

In three incubations in the culture medium, the average conversion of manool (0.3 g L^{-1}) was 90% in 5-7 days; the following products were successively eluted from the column: ketone material (3.8%), 3β-hydroxy manool **8** (6.6%), 2α-hydroxy manool **7** (16.6%) and 3β, 7α-dihydroxy manool **10** (14.2%)

2α-hydroxy manool [labd-8(17),14-dien-2α,13β-diol] **7.** Mp 145-147°C (from CH_2Cl_2-pentane); $[\alpha]_D^{21}$= -4.2°, $[\alpha]_{578}^{21}$= -4.5°, $[\alpha]_{546}^{21}$ = -5.7°, $[\alpha]_{436}^{21}$= -15.7°, $[\alpha]_{365}^{21}$= -45.4° (c 1.48, MeOH); anal. for $C_{20}H_{34}O_2$, calc.C%= 78.37, H%= 11.19, found C%= 78.64, H%= 11.21; IR (CCl_4) cm^{-1}: 3612, 3300, 2940, 1094, 1038, 925 and 905; ^{1}H-NMR, δ ppm ($CDCl_3$): 5.90 (14-H, dd, $J_{14,15}$= 17.3 Hz, $J_{14,15'}$= 10.7 Hz), 5.21 and 5.06 (15-H_2, 2 dd, $J_{15,15'}$= 1.3 Hz), 4.84 and 4.52 (17-H_2, 2d, J= 1.3 Hz), 3.86 (2β-H, dddd, $J_{2\beta,1\alpha}$= $J_{2\beta,3\alpha}$= 11.5 Hz, $J_{2\beta,1\beta}$= $J_{2\beta,3\beta}$= 4.0 Hz), 2.40 (7β-H, ddd, $J_{7\alpha,7\beta}$= 12.5 Hz and J= 2-2.5 Hz), 1.27 (16-CH_3, s), 0.93, 0.83 and 0.71 (18-, 19- and 20-CH_3, 3s); ^{13}C-NMR, δ ppm ($CDCl_3$): 148.0(C-8), 145.3(C-14), 111.3(C-15), 107.4(C-17), 73.7(C-13), 65.8(C-2), 57.3(C-9), 55.2(C-5), 51.3(C-3), 48.4(C-1), 41.5(C-12), 41.4(C-10), 38.2(C-7), 35.2(C-4), 33.8(C-18), 28.1(C-16), 24.1(C-6), 22.8(C-19), 18.1(C-11), 15.5(C-20); HRMS for $C_{19}H_{31}O_2$ (M-15), calc.291.2318, found 291.2315; FAB-MS: 437 [M+Na+Thiogly.]$^+$, 379, 329 [M+Na]$^+$, 271 [(M+H)-$2H_20$]$^+$.

3β-hydroxy manool [labd-8(17),14-dien-3β,13β-diol] **8.** Mp 111-113°C (from Et_2O-pentane); $[\alpha]_D^{21}$= +17.7°, $[\alpha]_{578}^{21}$=+18.4°, $[\alpha]_{546}^{21}$= +20.9°, $[\alpha]_{436}^{21}$= +34.6°, $[\alpha]_{365}^{21}$= +51.5° (c 1.5, MeOH); IR (CCl_4) cm^{-1}: 3600-3300, 1640, 925, 892; ^{1}H-NMR, δ ppm ($CDCl_3$): 5.90 (14-H, dd, $J_{14,15}$= 17.5 Hz, $J_{14,15'}$= 10.75 Hz), 5.20 and 5.05 (15-H_2, 2 br.d,ΔW= 3 Hz), 4.81 and 4.45 (17-H_2, 2 br.s, ΔW= 3 Hz), 3.26 (3α-H, dd, $J_{3\alpha,2\beta}$= 11.2 Hz, $J_{3\alpha,2\alpha}$= 4.7 Hz), 2.41 (7α-H, ddd, $J_{7\alpha,7\beta}$= 12.0 Hz and J= 2 and 2.5 Hz), 1.26 (16-CH_3, s.), 0.98, 0.76 and 0.67 (18-, 19- and 20-CH_3, 3s); ^{13}C-NMR, δ ppm ($CDCl_3$): 148.2(C-8), 145.2(C-14), 111.8(C-15), 106.9(C-17), 78.9(C-3), 73.7(C-13), 57.0(C-9), 54.8(C-5), 41.4(C-12), 39.6(C-10), 39.2(C-4), 38.3(C-7), 37.2(C-1), 28.4(C-18), 28.2(C-16), 28.0(C-2), 24.1(C-6), 17.9(C-11), 15.5(C-19), 14.6(C-20); HRMS for $C_{19}H_{31}O_2$ (M-15), calc. 291.2318, found 291.2470; FAB-MS: 437 [M+Na+Thiogly.]$^+$, 379, 329 [M+Na]$^+$, 271 [(M+H)-$2H_20$]$^+$.

3β, 7α-dihydroxy manool [labd-8(17),14-dien-3β,7α,13β–triol] **10.** Mp 166.5-168.5°C (from EtOAc-pentane); $[\alpha]_D^{21}$= -18.9°, $[\alpha]_{578}^{21}$= -19.8°, $[\alpha]_{546}^{21}$ = -23.0°, $[\alpha]_{436}^{21}$= -44.1°, $[\alpha]_{365}^{21}$= -79.1° (c 1.438, MeOH); anal. for $C_{20}H_{34}O_3$, calc.C%= 74.48, H%= 10.63, found C%= 74.37, H%= 10.72; IR (CCl_4) cm^{-1}: 3600-3300, 1640, 925, 892; ^{1}H-NMR, δ ppm (CD_3OD): 5.89 (14-H, dd, $J_{14,15}$= 17.4 Hz, $J_{14,15'}$= 10.75 Hz), 5.19 and 5.03 (15-H_2, 2 dd, $J_{15,15'}$= 1.6 Hz), 5.0 and 4.63 (17-H_2, 2 br.s, J= 1.3 Hz), 4.29 (7β-H, br.t, ΔW= 4 Hz), 3.21 (3α-H, dd, $J_{3\alpha,2\beta}$= 10.1 Hz, $J_{3\alpha,2\alpha}$= 5.9 Hz), 1.23 (16-CH_3, s), 0.96, 0.75 and 0.67 (18-, 19- and 20-CH_3, 3s); ^{13}C-NMR, δ ppm (CD_3OD): 150.9(C-8), 146.4(C-14), 112.0(C-15), 110.1(C-17), 79.6(C-3), 74.7(C-7), 74.2(C-13), 52.3(C-9), 48.6(C-5), 42.0(C-12), 40.7(C-10), 39.7(C-4), 38.3(C-1), 31.7(C-6), 28.6(C-2 and C-18), 28.0(C-16), 18.6(C-11), 16.0(C-19), 14.0(C-20); FAB-MS: 455 $[M+Na+Thiogly.]^+$, 379, 347 $[M+Na]^+$, 289 $[(M+H)-2H_2O]^+$, 271 $[(M+H)-3H_2O]^+$.

3-keto manool [labd-8(17),14-dien-3-oxo-13β–ol] **13.** Oil, purified from the ketone fraction; IR (CCl_4) cm^{-1}: 3609, 3083, 2934, 2855, 1708, 1455, 1137, 1090, 923, 895. ^{1}H-NMR, δ ppm ($CDCl_3$): 5.92 (14-H,dd,$J_{14,15}$ = 17.5 Hz,$J_{14,15'}$=10.7 Hz), 5.22 and 5.09 (15-H_2, 2dd,$J_{15,15'}$=1.3 Hz), 4.90 and 4.58 (17-H_2,2d,J=1.3 Hz), 1.28 (16-CH_3, s), 1.09, 1.02 and 0.86 (18-, 19- and 20-CH_3, 3s). ^{13}C-NMR, δ ppm ($CDCl_3$): 217.1(C-3), 147.4(C-8), 145.1(C-14), 112.0(C-15), 107.7(C-17), 73.7(C-13), 56.3(C-9), 55.4(C-5), 47.9(C-4), 41.3(C-12), 39.6(C-10), 38.0(C-1), 37.7(C-7), 34.9(C-2), 28.3(C-16), 26.1(C-18), 25.2(C-6), 21.6(C-19), 18.3(C-11), 14.1(C-20); HRMS for $C_{19}H_{27}O$ [M-(18+15)], calc.271.2088, found 271.2056; MS (CI, NH_3): 305 $(MH)^+$, 287 $(MH-18)^+$. MS: 271 (88) $[M-(H_2O+CH_3)]^+$, 149 (68), 133 (68), 119 (64), 107 (100) and 93 (69).

Bioconversion of 7α-hydroxy manool **9.**

7α-hydroxy manool (0.6 $g.L^{-1}$) was fully converted by incubation (4 days) in the culture medium. The following products were successively eluted from the column: 6α,7α-dihydroxy manool **11** (26%), a mixture of keto derivatives (26%), 19(β),7α-dihydroxy manool **12** (2%) and 3β,7α-dihydroxy manool **10** (22%).

6α,7α-dihydroxy manool [labd-8(17),14-dien-6α,7α,13β–triol] **11.** Mp.66-68°C (from acetone-pentane); $[\alpha]_D^{21}$= +5.2°, $[\alpha]_{578}^{21}$= +5.3°, $[\alpha]_{546}^{21}$ = +5.6°, $[\alpha]_{436}^{21}$= +5.0°, $[\alpha]_{365}^{21}$= -1.5° (c 1.835, MeOH); ^{1}H-NMR, δ ppm (CD_3OD): 5.78 (14-H, dd, $J_{14,15}$= 17.5 Hz, $J_{14,15'}$= 10.5 Hz), 5.18 and 5.02 (15-H_2, 2dd, $J_{15,15'}$= 1.7 Hz), 5.08 and 4.68 (17-H_2, 2br.s., Δw= 3.5 Hz), 4.11 (7β-H, d, J= 3.4 Hz), 3.70 (6β-H,dd, $J_{6\beta,5\alpha}$= 11.5 Hz, $J_{6\beta,7\beta}$= 3.4 Hz), 2.04 (9α-H,m), 1.59 (5α-H, d, J= 11.5 Hz), 1.23, 1.14, 0.99 and 0.69 (16-,18-,19- and 20-CH_3, 4s). ^{13}C-NMR, δ ppm (CD_3OD): 149.9(C-8), 146.3(C-14), 112.1(C-15), 111.4(C-17), 79.1(C-7), 74.2(C-6), 73.9(C-13), 53.0(C-5), 51.7(C-9), 45.1(C-3), 42.0(C-12), 39.9(C-1 and C-10), 37.1(C-18), 34.4(C-4), 27.9(C-16), 22.5(C-19), 20.2(C-2), 18.7(C-11), 15.8(C-20); HRMS for $C_{19}H_{29}O_2$ (M-33), calc. 289.2162, found 289.2260; MS: 289 (2) $[M-CH_3]^+$, 271 (9) $[289-H_2O]^+$, 243 (8), 205 (59), 187 (72), 161 (67), 145 (78), 123 (85), 109 (70), 95 (96) and 82 (100).

19,7α-dihydroxy manool [labd-8(17),14-dien-19,7α,13β–triol] **12.** isolated as oil: ^{1}H-NMR, δ ppm ($CDCl_3$): 5.90 (14-H, dd, $J_{14,15}$= 17.3 Hz, $J_{14,15'}$= 10.7 Hz), 5.20 and 5.06 (15-H_2, 2dd, $J_{15,15'}$= 1.2 Hz), 5.04 and 4.63 (17-H_2, 2br.s., Δw= 3.2 Hz), 4.36 (7β-H, t, J= 2.9 Hz), 3.77, 3.72- 3.42, 3.37 (19-H_2, AB system, J_{AB}= 10.8 Hz), 1.97 (9α-H, dt, $J_{9\alpha,11\beta}$= 12.5 Hz, $J_{9\alpha,11\alpha}$= 2.5 Hz), 1.28 (16-CH_3, s), 0.99 (18-CH_3, s), 0.64 (20-CH_3, s). ^{13}C-NMR, δ ppm ($CDCl_3$): 149.6(C-8), 145.3(C-14), 111.9(C-15), 110.1(C-17), 74.1(C-7), 73.7(C-13), 65.5(C-19), 51.6(C-9), 48.6(C-5), 41.1(C-12), 40.2(C-4), 39.1(C-3), 38.5(C-1 and C-10), 35.9(C-6), 28.2(C-16), 27.0(C-18), 19.2(C-2), 17.7(C-11), 14.3(C-20).

Bioconversion of sclareolide **15.**

Sclareolide (0.25 $g.L^{-1}$) incubated in the culture medium disappeared completely in 2 days; the following products were successively eluted from the column: 3-ketone **18** (3.2%), 3β-hydroxysclareolide **16** (7.9%) and 1β-hydroxysclareolide **17** (2.5%).

3β-hydroxy sclareolide (7β-hydroxy-dodecahydro-3a,6,6,9a-tetramethyl-naphtho-[2,1-b]furan-1-one) **16.** Mp=

161-163°C (from Et_2O-pentane); $[\alpha]_D^{21}$= +37.8°, $[\alpha]_{578}^{21}$= +39.6°, $[\alpha]_{546}^{21}$ = +45.6°, $[\alpha]_{436}^{21}$= +83.4°, $[\alpha]_{365}^{21}$= +141.5° (c 2.47, MeOH); IR (CCl_4) cm^{-1}: 3620, 2930, 2865, 1776, 1385, 1122, 1085 and 930; ^{1}H-NMR, δ ppm ($CDCl_3$): 3.27 (3α-H, dd, $J_{3\alpha,2\alpha}$=10.75 Hz, $J_{3\alpha,2\beta}$=5.95 Hz), 2.52 to 2.19 (11-H_2, m, AB part of an ABX syst.with 9α-H,J_{AB}=16.1 Hz,J_{AX}=14.7 Hz,J_{BX}=6.5 Hz), 2.10 (7β-H, dt,$J_{7\beta,7\alpha}$=11.6 Hz,$J_{7\beta,6\alpha}$= $J_{7\beta,6\beta}$= 3.2 Hz), 1.93 (9α-H, dd, J=14.7 and 6.5 Hz), 1.35 (13-CH_3, s), 1.02; 0.93 and 0.82 (14-, 15- and 16-CH_3, 3s); ^{13}C-NMR, δ ppm ($CDCl_3$): 176.7(C-12), 86.3(C-8), 78.7(C-3), 58.9(C-9), 55.3(C-5), 38.9(C-4), 38.5(C-7), 37.7(C-1), 35.8(C-10), 28.8(C-11), 27.9(C-14), 26.9(C-2), 21.6(C-13), 20.3(C-6), 15.2(C-15 and C-16); HRMS for $C_{16}H_{26}O_3$, calc. 266.1876, found 266.1934; MS: 266 (13) $[M]^+$, 251 (34) $[M-15]^+$, 233 (43) $[M-(15+18)]^+$, 204 (30), 189 (26), 173 (24), 161 (50), 135 (71),134 (72), 121 (100), 97 (100).

1β-hydroxy sclareolide (9β-hydroxy-dodecahydro-3a,6,6,9a-tetramethyl-naphtho-[2,1-b]furan-1-one) **17**. Mp= 173-175°C (from Et_2O-pentane); $[\alpha]_D^{21}$= -21.2°, $[\alpha]_{578}^{21}$= -21.8°, $[\alpha]_{546}^{21}$ = -25.0°, $[\alpha]_{436}^{21}$= -44.3°, $[\alpha]_{365}^{21}$= -77.4° (c 0.444, MeOH); IR (CCl_4) cm^{-1}: 3610, 2920, 2850, 1775, 1122 and 1090; ^{1}H-NMR, δ ppm ($CDCl_3$): 3.28 (1α-H, dd, $J_{1\alpha,2\alpha}$= 10.5 Hz, $J_{1\alpha,2\beta}$= 5.5 Hz), 2.84 to 2.36 (11-H_2, m, AB part of an ABX system with 9α-H, J_{AB}= 18 Hz, J_{AX}= 7.8 Hz, J_{BX}=0), 2.38 (7β-H, m, overlapping with 11α-H); 1.78 (9α-H, d, $J_{9\alpha,11\beta}$= 7.8 Hz), 1.34 (13-CH_3, s), 1.05, 0.93 and 0.86 (14-, 15- and 16-CH_3, 3s); ^{13}C-NMR, δ ppm ($CDCl_3$): 177.4(C-12), 85.5(C-8), 79.0(C-1), 54.7(C-9), 51.2(C-5), 38.8(C-3), 36.0(C-10), 35.2(C-7), 32.6(C-11), 32.0(C-4), 30.0(C-13), 28.7(C-14), 26.8(C-2), 18.3(C-6), 16.0(C-15), 14.8(C-16); HRMS for $C_{16}H_{26}O_3$, calc. 266.1876, found 266.1927; MS: 251 (37) $[M-15]^+$, 233 (24) $[M-(15+18)]^+$, 173 (20), 152 (95),134 (87), 119 (47), 107 (100), 93 (60).

3-keto sclareolide (7-oxo-dodecahydro-3a,6,6,9a-tetramethyl-naphtho-[2,1-b]furan-1-one) **18**. Mp 184-186°C (from Et_2O-pentane); $[\alpha]_D^{21}$= +66.6°, $[\alpha]_{578}^{21}$= +69.6°, $[\alpha]_{546}^{21}$= +79.6°, $[\alpha]_{436}^{21}$= +148.9°, $[\alpha]_{365}^{21}$= +268.3° (c 0.58, MeOH); IR (CCl_4) cm^{-1}: 2920, 1782, 1705, 1120, 1090; ^{1}H-NMR, δ ppm ($CDCl_3$): 2.6-2.3 (11-H_2, m, AB part of an ABX syst.with 9α-H, overlapping with 2-H_2,J_{AB}=15.7 Hz,J_{AX}=15.0 Hz,J_{BX} =6.0 Hz), 2.15 (7β-H, dt,$J_{7\beta,7\alpha}$=11.0,$J_{7\beta,6\alpha}$=$J_{7\beta,6\beta}$=2.5 Hz), 2.01 (9α-H, dd,J=15 and 6 Hz), 1.40 (13-CH_3, s), 1.14, 1.07 and 1.04 (14, 15 and 16-CH_3, 3s); ^{13}C-NMR, δ ppm ($CDCl_3$): 215.7(C-3), 176.1(C-12), 85.8(C-8), 58.3(C-9), 54.5(C-5), 47.5(C-4), 37.9(C-7), 37.8(C-1), 35.7(C-10), 33.5(C-2), 28.7(C-11), 26.8(C-14), 21.6(C-6), 21.2(C-13), 20.8(C-15), 14.6(C-16); HRMS for $C_{16}H_{24}O_3$, calc. 264.1799, found 264.1722; MS: 264 (100) $[M]^+$, 249 (24) $[M-15]^+$, 231 (23) $[M-(15+18)]^+$, 149 (26), 139 (36), 121 (26), 107 (35).

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