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BIOTRANSFORMATION OF *ENT*-6α-ACETOXY- AND *ENT*-6-KETOMANOYL OXIDES WITH *RHIZOPUS NIGRICANS* AND *CURVULARIA LUNATA* CULTURES

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Key Word Index—*Rhizopus nigricans*; *Curvularia lunata*; biotransformation; diterpenes; *ent*-manoyl oxides.

Abstract—Microbial transformation of *ent*-16,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide by *Rhizopus nigricans* produced *ent*-11β-hydroxy, *ent*-20-hydroxy and *ent*-3β-hydroxy derivatives. Biotransformation of *ent*-16,18-dihydroxy-6-oxomanoyl oxide gave *ent*-11β-hydroxy and *ent*-3β-hydroxy derivatives. However, biotransformations of these substrates with *Curvularia lunata* produced only *ent*-11β-hydroxy derivatives. Incubation of *ent*-18-hydroxy-6-oxo-13-*epi*-manoyl oxide with *R. nigricans* produced 14,15-epoxy derivatives with and without hydroxylation at C-3, whereas microbial transformation of its epimer at C-13 gave *ent*-11β-hydroxylated derivatives.

INTRODUCTION

In recent years, microbial biotransformation processes have been used to introduce hydroxyl groups at difficult positions on diterpenoid compounds [1]. Chemical-microbiological methods constitute an alternative way to obtain new polyoxygenated compounds from abundant natural products. Highly functionalized manoyl oxides, mainly forskolin (**1**), have shown interesting biological properties [2, 3]. We are currently assaying an extensive series of chemical-microbiological routes of semisynthesis of differently functionalized manoyl oxides of the *enantio* series with both configurations at C-13. In previous papers we reported the biotransformations of *ent*-manoyl oxides functionalized at C-16 and C-18 with *Rhizopus nigricans* [4–6], *Curvularia lunata* [7, 8], *Fusarium moniliforme* [9] and *Cunninghamella elegans* [9]. Some of the manoyl oxides obtained show interesting biological activities [6, 8]. To complete these studies, we now describe the biotransformation with *Rhizopus nigricans* and *Curvularia lunata* of other *ent*-manoyl oxides epimers at C-13 functionalized at C-6, C-16 and C-18 or C-6 and C-18.

The starting material was andalusol (**2**) [10]. Acetylation of this product produced 6,18-diacetylandalusol (**3**) [11]. Treatment of product **3** with MCPBA yielded *ent*-manoyl oxide epimers at C-13 with a hydroxyl group at C-16 (**4** and **5**) [5]. On the other hand, treatment of product **3** with $TiCl_4$ produced *ent*-manoyl oxides without functionalization at C-16 (**6** and **7**) [12]. We have studied the influence of these structural differences on the action of the microorganisms.

RESULTS AND DISCUSSION

The biotransformation of diacetate **6** by *Rhizopus nigricans* over a period of 3 days produced only 6% of one metabolite, **8**. Comparison of its ^{1}H NMR spectrum with that of the substrate (**6**) showed that the typical ABX system had disappeared from the vinyl protons; three signals were seen to be due to protons geminal to an oxygen atom: δ 3.06 (1H, *dd*, $J_1 = 4.5$, $J_2 = 3.0$ Hz), 2.78 (1H, *dd*, $J_1 = J_2 = 4.5$ Hz) and 2.48 (1H, *dd*, $J_1 = 4.5$, $J_2 = 3.0$ Hz). These data indicated that the microorganism had accomplished the epoxidation of the double bond of the substrate (**6**) to give 14,15-epoxy derivative; this process was confirmed by the ^{13}C NMR spectrum. The configuration at C-14 could not be determined from the available data. Metabolite **8** is, therefore, a(13R)-*ent*-6α,18-diacetoxy-8α,13;14ξ,15-diepoxylabdane.

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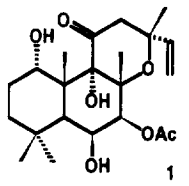

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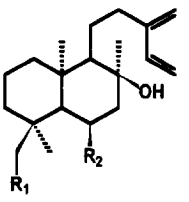
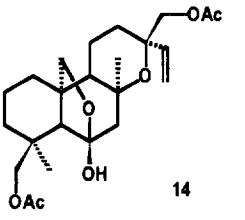


	R_1	R_2
2	OH	OH
3	OAc	OAc



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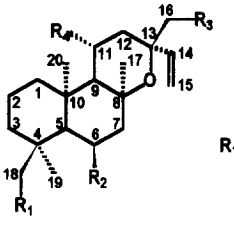
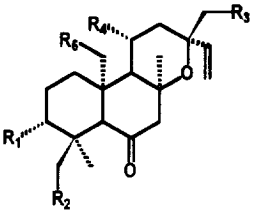
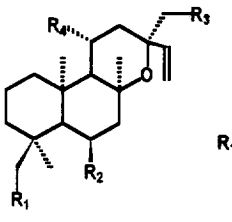
	R_1	R_2	R_3	R_4
4	OAc	OAc	OH	H
6	OAc	OAc	H	H
26	OH	OH	OH	H
28	OH	OH	H	H

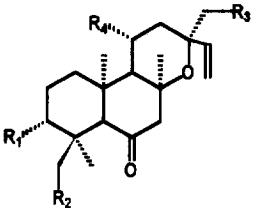




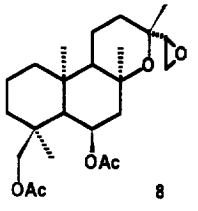
	R_1	R_2	R_3	R_4	R_5
10	H	OH	OH	H	H
11	H	OAc	OAc	H	H
12	H	OAc	OAc	OH	H
13	H	OAc	OAc	H	OAc
15	OH	OH	OH	H	H
16	OAc	OAc	OAc	H	H
20	H	OAc	H	H	H

	R_1	R_2	R_3	R_4
5	OAc	OAc	OH	H
7	OAc	OAc	H	H
9	OAc	OAc	H	OH
27	OH	OH	OH	H
29	OH	OH	H	H





	R_1	R_2	R_3	R_4
17	H	OH	OH	H
18	H	OH	OH	OH
19	OH	OH	OH	H
23	H	OAc	H	H
24	H	OAc	H	OH
25	H	OH	H	OH



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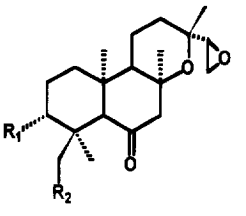

	R_1	R_2
21	H	OAc
22	OH	OAc

The biotransformation of diacetate **7** by *Rhizopus nigricans* over a 3 day period gave 29% of metabolite **9**. The IR spectrum of this metabolite showed a hydroxyl group band (3510 cm^{-1}). In the ^1H NMR spectrum a signal of a proton geminal to a hydroxyl group was observed at δ 4.46 (1H, *ddd*, $J_1 = J_2 = 5.8$, $J_3 = 3.9$ Hz). In addition, three of the signals of methyl groups were affected when we compared the ^1H NMR spectra of the substrate (**7**) and its metabolite (**9**). These data are compatible with the *ent*-11β-hydroxylation [5, 6] of the substrate, which was confirmed by comparison of the ^{13}C NMR spectra of **7** and **9** (Table 1). Thus, metabolite **9** was identified as *ent*-6α,18-diacetoxy-11β-hydroxymanoyl oxide.

A mixture of metabolites was isolated following the incubation of ketodiol **10**, obtained from compound **4** (see Experimental), with *Rhizopus nigricans* for 8 days. Acetylation of this mixture for chromatographic separation produced, in addition to an acetylated derivative **11** of compound **10**, products **12** (4%), **13** (8%) and **14** (6%). Also, a more polar metabolite (**15**) (13%) was isolated. In the ^1H NMR spectrum of compound **12** a new signal appeared at δ 4.58 (1H, *ddd*, $J_1 = 3.6$, $J_2 = 3.1$, $J_3 = 2.2$ Hz), representing an equatorial proton coupled with three protons. We also observed that two of the methyl groups and the vinyl proton of H-14 had been deshielded. This is compatible with an axial arrangement for a hydroxyl group at C-11, which was clearly confirmed on comparing the ^{13}C NMR spectra of compounds **11** and **12**. In the spectrum of compound **12** the signal of the methylene carbon had disappeared, and a new signal corresponding to an oxygenated methine carbon was seen. The chemical shift of this signal indicated that the hydroxyl group was probably at C-11. A β effect at C-12 ($\Delta\delta = +8.2$) and γ effects at C-8 and C-13 ($\Delta\delta = -0.7$ and -1.5, respectively) were observed. Also the C-14, C-17 and C-20 signals were deshieded ($\Delta\delta = +1.3$, $+2.5$, $+1.3$, respectively), in agreement with an axial hydroxylation at C-11. Therefore, com-

Table 1. ^{13}C NMR spectral data of compounds **6–16**

C	6	7	8	9	10	11	12	13	14	15	16
1	38.5	38.3	38.6	38.6	39.4	39.3	38.6	34.7	37.1	38.3	37.8
2	17.4	17.4	17.5	17.4	17.7	17.5	17.3	18.0	18.2	26.0	22.9
3	36.9	36.9	37.0	37.0	36.0	36.0	35.9	35.8	36.3	72.9	73.0
4	37.4	37.7	37.6[a]	36.5[a]	37.0	35.6	35.7	35.3	†	41.4	39.3[a]
5	52.1	52.3	52.3	53.1	61.2	60.8	61.5	59.9[a]	59.8	59.5	58.3[b]
6	70.4	70.6	70.6	70.9	210.1	208.4	208.2	207.0	82.8	208.5	206.9
7	49.3	49.5	49.3	50.8	59.7	59.6	61.0	59.8	57.4	59.7	59.7
8	74.7	74.0	74.2	73.9	78.6	78.2	77.5	77.8	77.3	78.6	78.2
9	57.9	54.8	57.3	55.9	58.5	58.5	58.6	59.0[a]	58.4	58.3	58.8[b]
10	36.3	36.4	36.5[a]	38.5[a]	40.1	40.1	40.3	43.2	50.0	39.9	39.8[a]
11	16.0	15.5	16.8	65.2	16.0	15.9	64.7	17.6	19.2	16.0	16.0
12	34.4	35.0	31.8	44.3	28.1	29.7	37.9	30.9	33.3	27.8	29.7
13	73.4	73.5	72.0	72.9	77.2	75.2	73.7	75.5	75.0	77.4	75.4
14	147.1	147.5	58.8	147.5	143.1	142.4	143.7	142.4	140.0	142.8	142.4
15	109.7	110.5	46.0	110.8	114.3	114.3	115.3	114.5	115.6	114.4	114.5
16	32.4	28.9	28.6	30.0	69.8	71.2[a]	71.1[a]	71.3[b]	70.1	69.4	71.2
17	25.0	26.7	23.9	28.8	24.6	24.5	27.0	23.9	22.8	24.5	24.5
18	74.1	74.3	74.3	74.5	71.6	71.7[a]	71.8[a]	71.6[b]	72.6	66.9	65.2
19	17.6	17.8	17.8	17.9	17.6[a]	17.6[b]	17.5	17.9	17.4	11.6	13.2
20	17.2	16.8	17.3	18.6	17.3[a]	17.1[b]	18.4	63.9	74.9	17.4	17.4
MeCOO	21.5	21.7	21.7	21.8		21.0	21.0	21.1	21.1		21.1
—	20.9	21.1	21.1	21.1		21.0	20.9	20.9	21.0		21.0
MeCOO	171.0	171.2	171.3	171.3		170.9	171.0	170.9	171.2		170.8
—	170.1	170.2	170.3	170.3		170.8	170.7	170.3	171.0		170.6

The ^{13}C chemical shifts are given in δ values (ppm) relative to $CDCl_3$ signals.
[a,b]Values bearing the same superscript may be interchanged.
†Not detected.

pound **12** had the structure of *ent*-16,18-diacetoxy-11β-hydroxy-6-oxo-13-*epi*-manoyl oxide. The ^1H NMR spectrum of compound **13** showed signals of three acetoxy groups (δ 2.06, 2.04 and 2.00) and three AB systems produced by the geminal protons to these acetoxy groups. Two of these AB systems were due to the protons at C-16 and C-18 and the third AB system (δ 4.38 and 3.70, 2H, J = 12.3 Hz) was produced by the geminal protons of a new oxygenated function introduced by the microorganism in one of the methyl groups of the substrate (**10**). On comparing the ^{13}C NMR spectra of compounds **11** and **13** we concluded that the new oxygenated function was found at C-20: β effect at C-10 ($\Delta\delta$ = +3.1), γ at C-1 ($\Delta\delta$ = −4.6) and δ at C-11 ($\Delta\delta$ = +1.7). These data were compatible with the findings for this type of hydroxylation at C-20 [4, 5]. Thus, compound **13** was assigned as *ent*-16,18,20-triacetoxy-6-oxo-13-*epi*-manoyl oxide. Compound **14** had a molecular peak of 436 (*m/z* 437 $[M+1]^+$, CIMS) compatible with a molecular formula of $C_{24}H_{36}O_7$. Its ^1H NMR spectrum contained only two methyl signals. As in compound **13**, one of the methyls of the substrate had been functionalized. This function was not acetylated and the signals of the C-5 and C-7 protons, compared with those of the diacetate **11**, appeared modified. We thus assumed that the hydroxyl group introduced by the microorganism at C-20, under conditions of biotransformation, with a weakly acid pH (5.7), could react with the carbonyl group at C-6 to give a cyclic hemiketal. This assumption was supported by the presence in its ^1H NMR spectrum of an ABX system due to H_2-20 and H-5, with long-distance coupling between the two (J = 1.8 Hz) confirmed by double resonance experiments. The ^{13}C NMR spectra of compounds **11** and **14** confirmed these assumptions, since the ^{13}C NMR spectrum of compound **14** contained a signal indicating a totally substituted dioxygenated carbon (δ 82.8), attributed to C-6, and no signal attributable to the carbonyl carbon. Therefore, compound **14** was assigned as *ent*-16,18-diacetoxy-6β,20-epoxy-6α-hydroxy-13-*epi*-manoyl oxide. The ^1H NMR spectrum of the more polar metabolite (**15**) had a new signal at δ 3.58 (1H, *m*, $W_{1/2}$ = 12.0 Hz) due to a proton geminal to a hydroxyl group. As this signal partially overlapped another, we acetylated this metabolite, obtaining triacetate **16**, in whose spectrum the signal of the geminal proton to the secondary acetoxy group was clearly visible at δ 4.64. This signal corresponded to an axial proton coupled with two other protons (*dd*, J_1 = 11.4, J_2 = 4.3 Hz). The equatorial acetoxy group was thought to be located at C-1, C-3 or C-12. Comparisons of the ^{13}C NMR spectra for compounds **10** and **15** showed that the oxygenated function

was located at C-3. Therefore metabolite **15** was assigned as *ent*-3β,16,18-trihydroxy-6-oxo-13-*epi*-manoyl oxide.

Ketodiol **17**, the epimer at C-13 of compound **10**, was obtained from compound **5** (see Experimental). Biotransformation with *R. nigricans* for 8 days gave two metabolites. Some spectroscopic data of the major metabolite (**18**) (47%) suggested that the microorganism had introduced a hydroxyl group at C-11 (δ_H 4.55 as *ddd*, $J_1 = J_2 = 6.9$, $J_3 = 4.6$ Hz). The ^{13}C NMR spectrum confirmed that metabolite was *ent*-11β,16,18-trihydroxy-6-oxomanoyl oxide. The other metabolite (**19**, 12%) isolated had a ^1H NMR spectrum which, compared with that of substrate (**17**), presented a new signal due to an axial proton geminal to a hydroxyl group at δ 3.53 (*dd*, $J_1 = 10.8$, $J_2 = 4.8$ Hz). The ^{13}C NMR spectra of compounds **17** and **19** indicated an equatorial oxygenated function at C-3. Therefore, metabolite **19** was assigned as *ent*-3β,16,18-trihydroxy-6-oxomanoyl oxide.

The biotransformation of substrate **20**, obtained from compound **6** (see Experimental), by *R. nigricans* for 60 hr gave a mixture of metabolites. After chromatographic separation, two metabolites **21** (3%) and **22** (21%) were isolated. The ^1H NMR spectrum of the first metabolite (**21**) contained a group of signals corresponding to the protons of an epoxide ring, produced by the epoxidation of the double bond of substrate (**20**) by the microorganism. Thus, we could assign the structure of (13R)-*ent*-18-acetoxy-6-oxo-8α,13;14ξ,15-diepoxylabdane to metabolite **21**. Metabolite **22** had a ^1H NMR spectrum very similar to that of compound **21**, except for the presence at δ 3.21 of a signal (1H, *dd*, $J_1 = 9.8$, $J_2 = 6.0$ Hz) of an axial proton geminal to the hydroxyl group at C-3. Therefore, metabolite **22** was (13R)-*ent*-18-acetoxy-3β-hydroxy-6-oxo-8α,13;14ξ,15-diepoxylabdane.

Incubation of substrate **23**, obtained from compound **7** (see Experimental), with *R. nigricans* for 60 hr gave a mixture of metabolites (**24**, 31%) and (**25**, 25%). The first metabolite (**24**) had a hydroxyl group at C-11: its ^1H NMR spectrum showed a signal at δ 4.58 identical to those observed in the spectra of metabolites **9** and **18**. The ^{13}C NMR spectrum confimed the structure of metabolite **24** as *ent*-18-acetoxy-11β-hydroxy-6-oxomanoyl oxide. The most polar metabolite (**25**) was the saponification product at C-18 of metabolite **24**, assigned as *ent*-11β,18-dihydroxy-6-oxomanoyl oxide.

The two compounds (**10** and **17**) that had given the best yields of biotransformation products with *R. nigricans* were also incubated with the microorganism *Curvularia lunata*. The biotransformation of ketodiol **10** with *C. lunata* for 8 days gave two compounds which were acetylated and identified as diacetates **11** (15%) and **12** (35%), isolated after incubation of compound **10** with *R. nigricans*.

The biotransformation of substrate **17** by *C. lunata* for 8 days gave 14% of a more polar metabolite whose spectroscopic data indicated that it was *ent*-11β,16,18-trihydroxymanoyl oxide (**18**), isolated from the incubation of substrate **17** with *R. nigricans*.

These biotransformations showed that *R. nigricans* produced the greatest variety of metabolites, whereas *C. lunata* acted mainly on C-11. Furthermore, we observed that substrates with a 6-keto group were biotransformed more efficiently than those with an acetoxy group in this position. With respect to *ent*-11β-hydroxylated compounds, in substrates with an *ent*-13-*normal* configuration the greatest yields were obtained with *R. nigricans*, whereas in substrates with an *ent*-13-*epi* configuration they were obtained with *C. lunata*. The production of *ent*-11β-hydroxylated compounds in acceptable yields will permit subsequent studies of the synthesis of highly functionalized manoyl oxides of biological interest.

EXPERIMENTAL

Mps: uncorr.; NMR: 300 MHz (^1H) and 75.47 MHz (^{13}C), $CDCl_3$ (which also provided the lock signal) in 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°. CI (CH_4)-MS: Hewlett-Packard 5988 A spectrometer. Specific rotations: 20°; CC: silica gel SDS 60 A CC, CH_2Cl_2 with increasing amounts of Me_2CO was used as the eluent; TLC: silica gel (Merck G), visualization by spraying with H_2SO_4–HOAc–H_2O, followed by heating at 120°.

Starting material. ent-6α, 8α,18-Trihydroxylabda-13(16), 14-diene (**2**, andalusol) was isolated from *Sideritis foetens* collected in September 1991 near Alcolea (Almeria) [10]. Acetylation of 12 g of andalusol (**2**) yielded 11.5 g of *ent*-6α,18-diacetoxy-8α-hydroxylabda-13(16),14-diene (**3**, diacetylandalusol) [11].

Cyclization of diacetate **3** *with MCPBA*. The Diacetate (**3**) (6 g) was dissolved in $CHCl_3$ (150 ml) and MCPBA (3.4 g) was added. The reaction mixt. was stirred for 24 hr at 0°, diluted with $CHCl_3$ (100 ml) and washed successively with aq. Fe_2SO_4, aq. $NaHCO_3$ and H_2O. The organic soln was dried over dry $MgSO_4$ and evapd to dryness. After CC on silica gel with 10% of $AgNO_3$, starting material (**3**, 1.7, 28%), *ent*-6α,18-diacetoxy-16-hydroxy-*epi*-manoyl oxide [5] (**4**, 2.2 g, 35%) and *ent*-6α,18-diacetoxy-16-hydroxymanoyl oxide [5] (**5**, 1.8 g, 29%) were isolated.

Cyclization of diacetate **3** *with* $TiCl_4$. Diacetate **3** (6 g) was dissolved in dry CH_2Cl_2 (350 ml). The mixt. was cooled to −78° and kept under Ar gas. Then, $TiCl_4$ (4 ml) in dry CH_2Cl_2 (6 ml) (previously cooled) was added. The mixt. was stirred at −78° for 1 hr, and pyridine (200 ml), previously cooled, was added. This mixt. was allowed to reach room temp., treated with aq. $KHSO_4$ and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over dry $MgSO_4$ and concd *in vacuo*. After CC on silica gel with 10% of $AgNO_3$ starting material (**3**, 2.3 g, 38%), *ent*-6α,18-diacetoxy-13-*epi*-manoyl oxide [12] (**6**, 2.1,

35%) and of *ent*-6α,18-diacetoxymanoyl oxide [12] (**7**, 785 mg 13%) were isolated.

ent-6α,18-*Diacetoxy*-13-epi-*manoyl oxide* (**6**). ^{1}H NMR (300 MHz): δ 5.89 (1H, *dd*, part X of an AB system, $J_{AX}+J_{BX}$ = 28.9 Hz, H-14), 4.97 (1H, *ddd*, $J_1 = J_2$ = 11.3, J_3 = 3.9 Hz, H-6), 4.91–4.80 (2H, part AB of an ABX system, 2H-15), 3.96 and 3.52 (2H, AB system, J = 10.8 Hz, 2H-18), 2.00 and 1.91 (3H each, *s*, AcO groups), 1.24 (3H, *s*, 3H-17), 1.06 (3H, *s*, 3H-16), 0.77 and 0.76 (3H each, *s*, 3H-19 and 3H-20); ^{13}C NMR (75.47 MHz): Table 1.

ent-6α,18-*Diacetoxymanoyl oxide* (**7**). ^{1}H NMR (300 MHz): δ 5.83 (1H, *dd*, J_1 = 17.3, J_2 = 10.7 Hz, H-14), 5.10 (1H, *dd*, J_1 = 17.3 Hz, J_2 = 1.5 Hz) and 4.89 (1H, *dd*, J_1 = 10.7 Hz, J_2 = 1.5 Hz) (2H-15), 5.03 (1H, *ddd*, $J_1 = J_2$ = 11.3 Hz, J_3 = 3.9 Hz, H-6), 3.99 and 3.61 (2H, AB system, J = 10.8 Hz, 2H-18), 2.04 and 1.97 (3H each, *s*, AcO groups), 1.36 (3H, *s*, 3H-17), 1.21 (3H, *s*, 3H-16), 0.87 (3H, *s*, 3H-19) and 0.82 (3H, *s*, 3H-20); ^{13}C NMR (75.47 MHz): Table 1.

Production of substrate **10** *from diacetate* **4**. Diacetate **4** (2 g) was dissolved in MeOH–H_2O (70%, 100 ml) containing KOH (5%) and refluxed for 1 hr. The reaction mixt. was diluted with H_2O (100 ml), neutralized with HCl (2 M) and extracted with CH_2Cl_2. The organic layer was dried over dry $MgSO_4$ and conc *in vacuo*. After CC, *ent*-6α, 16, 18-trihydroxy-13-*epi*-manoyl oxide [13] (**26**, 1.5 g, 94%) was isolated. Triol **26** (1 g) was dissolved in dry CH_2Cl_2 (80 ml) and PDC (1035 mg) was added [14]. The reaction mixt. was stirred for 2 hr at room temp., then filtered and chromatographed to give of *ent*-16,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide (**10**, 650 mg, 65%). Mp 126–129°; $[\alpha]_D$ −39° ($CHCl_3$; *c* 2); IR ν_{max} cm^{-1}: 4316, 3089, 1643, 1705, 1095 and 1043; ^{1}H NMR: δ 5.87 (1H, *dd*, part X of an AB system, $J_{AX}+J_{BX}$ = 29.2 Hz, H-14), 5.15 and 5.09 (2H, part AB of an ABX system, 2H-15), 3.54 and 3.12 (2H, AB system, J = 10.4 Hz, 2H-18), 3.33 and 3.02 (2H, AB system, J = 11.0 Hz, 2H-16), 2.61 and 2.40 (2H, AB system, J = 11.4 Hz, 2H-7), 2.55 (1H, *s*, H-5), 1.20 (3H, *s*, 3H-17), 1.09 (3H, *s*, 3H-19) and 0.78 (3H, *s*, 3H-20); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M+1]^+$ 337 (48), 319 (30), 301 (11).

Production of substrate **17** *from diacetate* **5**. Diacetate **5** (1.7 g) was dissolved in MeOH–H_2O (70%, 85 ml) containing KOH (5%) and refluxed for 1 hr. Operating in the same working conditions as for the saponification of diacetate **4** and after CC *ent*-6α,16,18-trihydroxymanoyl oxide [10] (**27**, 1.24 g 91%) was isolated. Triol **27** (1.2 g) was dissolved in dry CH_2Cl_2 (120 ml) and PDC (1.4 g) was added. Operating under the same working conditions as for oxidation of triol **26** *ent*-16,18-dihydroxy-6-oxomanoyl oxide [10] (**17**, 845 mg, 71%) was isolated.

Production of compound **20** *from diacetate* **6**. Diacetate **6** (1.5 g) was saponified under the same working conditions as for diacetate **4**, yielding 1.15 g of *ent*-6α,18-dihydroxy-13-*epi*-manoyl oxide (**28**, 97%). Mp 145–147°. $[\alpha]_D$ −56° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3304, 3089, 1637, 1094, 1064, 980; ^{1}H NMR: δ 5.97 (1H, *dd*, part X of an AB system, $J_{AX}+J_{BX}$ = 29.0 Hz, H-14), 4.98–4.88 (2H, part AB of an ABX system, 2H-15), 3.80 (1H, *ddd*, $J_1 = J_2$ = 10.7, J_3 = 4.0 Hz, H-6), 3.50 and 3.07 (2H, AB system, J = 11.0 Hz, 2H-18), 1.24 (3H, *s*, 3H-17), 1.11 (3H, *s*, 3H-16), 0.86 (3H, *s*, 3H-18) and 0.74 (3H, *s*, 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 323 (1), 305 (80), 287 (100). Compound **28** (950 mg) was dissolved in dry CH_2Cl_2 (80 ml) and oxidized with PDC (1.4 g) for 2 hr at room temp. The mixt. obtained was acetylated with Ac_2O–Py (10 : 5 ml) for 12 hr at room temp. to give *ent*-18-acetoxy-6-oxo-13-*epi*-manoyl oxide (**20**, 700 mg, 65%). Gum; $[\alpha]_D$ −26° ($CHCl_3$, *c* 1); IR ν_{max} cm^{-1}: 3089, 1739, 1709, 1638, 1236, 1092, 1036, 972 and 914; ^{1}H NMR: δ 5.92 (1H, part X of an AB system, $J_{AX}+J_{BX}$ = 29.0 Hz, H-14), 4.98–4.88 (2H, *dd*, part AB of an ABX system, H-15), 3.93 and 3.71 (2H, AB system, J = 10.7 Hz, 2H-18), 2.49 and 2.35 (2H, AB system, J = 11.3 Hz, 2H-7), 2.43 (1H, *s*, H-5), 1.98 (3H, *s*, AcO group), 1.14 (3H, *s*) and 1.12 (6H, *s*) (3H-16, 3H-17 and 3H-19) and 0.74 (3H, *s*, 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 363 (8), 303 (100), 285 (16).

Production of compound **23** *from diacetate* **7**. Diacetate **7** (400 mg) was saponified under the same working conditions as for diacetate **6**. After purification of the reaction mixt., 310 mg of *ent*-6α,18-dihydroxymanoyl oxide (**29**, 98%) were isolated; Gum; $[\alpha]_D$ −48° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3296, 3088, 1641, 1123, 1062, 920; ^{1}H NMR: δ 5.81 (1H, *dd*, J_1 = 17.7, J_2 = 10.7 Hz, H-14), 5.10 (1H, *dd*, J_1 = 17.4 Hz, J_2 = 1.5 Hz) and 4.89 (1H, *dd*, J_1 = 10.7 Hz, J_2 = 1.5 Hz) (2H-15), 3.76 (1H, *ddd*, $J_1 = J_2$ = 10.9 Hz, J_3 = 3.9 Hz, H-6), 3.43 and 3.07 (2H, AB system, J = 10.9 Hz, 2H-18), 1.29 (3H, *s*, 3H-17), 1.21 (3H, *s*, 3H-16), 0.86 (3H, *s*, 3H-19) and 0.78 (3H, *s*, 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 323 (3), 305 (38), 287 (100). Compound **29** (300 mg) was dissolved in dry CH_2Cl_2 (40 ml) and oxidized with PDC (500 mg) for 2 hr. The mixt. obtained was acetylated with Ac_2O–Py (6 : 3 ml) to give 225 mg of *ent*-18-acetoxy-6-oxomanoyl oxide (**23**, 67%): Gum; $[\alpha]_D$ −19 ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3089, 1739, 1709, 1237 and 1643; ^{1}H NMR: δ 5.86 (1H, *dd*, J_1 = 17.4, J_2 = 10.8 Hz, H-14), 5.12 (1H, *dd*, J_1 = 17.4 Hz, J_2 = 1.4 Hz) and 4.93 (1H, *dd*, J_1 = 10.8 Hz, J_2 = 1.4 Hz) (2H-15), 3.93 and 3.73 (2H, AB system, J = 10.7 Hz, 2H-18), 2.54 and 2.42 (2H, AB system, J = 11.5 Hz, 2H-7), 2.42 (1H, *s*, H-5), 1.99 (3H, *s*, AcO group), 1.26 and 1.24 (3H each, *s*, 3H-17 and 3H-16), 1.15 (3H, *s*, 3H-19) and 0.81 (3H, *s*, 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 363 (8), 303 (100), 285 (19).

Organism, media and culture conditions. Rhizopus nigricans CECT 2072 (ATCC 10404) and *Curvularia lunata* CECT 2130 (ATCC 12017) were obtained from the Colección Española de Cultivos Tipo, Departamento de Microbiología, Universidad de Valencia, Spain. Medium YEPGA containing 1% yeast extract,

Table 2. ^{13}C NMR spectral data of compounds **17**–**25**, **28** and **29**

C	17*	18	19	20	21	22	23	24	25	28	29
1	38.5	38.6	37.5	39.4	39.2	38.0	39.0	38.7	38.8	39.1[a]	38.7[a]
2	17.2	17.5	26.7[a]	17.5	17.6	25.5	17.5	17.4	17.6	18.0	17.9
3	35.6	35.9	74.3	36.1	36.1	71.0	36.0	36.1	36.1	38.5[a]	38.3[a]
4	36.6	37.0	41.2	35.6	35.7	40.8	35.6[a]	35.8	37.1	37.5	37.6
5	60.7	61.9	60.0	60.7	60.7	58.9	60.7	61.5	62.2	57.7	57.4
6	209.8	209.9	208.2	208.7	208.6	207.5	208.7	208.5	210.3	67.5	67.2
7	59.9	61.0	60.3	59.9	59.6	59.6	60.0	61.3	61.4	53.5	53.4
8	76.4	76.6	77.5[b]	77.9	†	77.1	77.2	76.9	76.9	73.6[a]	73.6[b]
9	52.4	55.6	52.7	58.7	57.7	57.5	56.6	56.9	56.5	57.0	54.2
10	39.9	40.6	40.0	40.1	40.2	39.9	40.2	40.5	40.5	38.3	38.2
11	14.8	64.1	15.1	16.6	17.2	17.2	16.0	65.1	65.2	16.1	15.5
12	26.4	38.2	26.1[a]	34.4	30.7	30.6	35.7[a]	44.4	44.3	34.7	35.0
13	77.3	77.4	77.7[b]	74.3	72.4	72.4	74.2	73.3	73.3	75.1	74.7[b]
14	143.0	142.9	143.2	146.6	57.3	57.1	147.3	147.4	147.3	147.3	147.5
15	114.3	114.9	114.7	110.3	47.5	47.5	110.6	110.8	111.0	109.9	110.7
16	68.2	68.5	68.4	32.5	29.1	29.0	27.9	29.3	29.5	32.6	28.9
17	26.2	28.4	26.4	24.4	24.6	24.4	25.8	28.1	28.3	25.3	26.9
18	71.2	71.9	68.4	71.7	71.7	66.2	71.7	72.0	72.1	74.9	74.8
19	17.5	17.8	11.4	17.6	17.8	12.0	17.6	17.7	17.7	17.7[b]	17.7
20	16.3	19.0	16.6	17.1	17.1	17.1	16.9	18.7	18.8	17.5[b]	16.8
MeCOO				21.0	21.0	21.0	21.0	21.0			
MeCOO				170.9	171.8	171.8	170.9	171.0			

The ^{13}C chemical shifts are given in δ values (ppm) relative to $CDCl_3$ signals.
*See ref. [10].
[a,b]Values bearing the same superscript may be interchanged.
†Not observed.

1% peptone, 2% glucose, 2% agar, at pH 5 was used for storage of microorganisms. In the transformation expts, a medium containing 0.1% peptone, 0.1% yeast extract, 0.1% beef extract and 0.5% glucose at pH 5.7 in H_2O was used. Conical flasks (250 ml) containing 80 ml of medium were inoculated with a dense suspension of the appropriate microorganism. Incubations were carried at 28° with gyratory shaking (150 rpm) for 6 days after which the substrates in EtOH were added.

Recovery and purification of metabolites. Cultures were filtered and pooled, and the cells washed (×2) with H_2O. The liquid was satd with NaCl and extracted with CH_2Cl_2. These extracts were dried over dry $MgSO_4$, and evapd *in vacuo*. The mixt. of compounds was chromatographed on silica gel.

Biotransformation of substrate **6** *by* R. nigricans. Substrate **6** (300 mg) was dissolved in EtOH (6 ml) and incubated for 3 days to obtain, after CC, 186 mg of substrate **6** (62%) and 20 mg of (13R)-*ent*-6α,18-diacetoxy-8α,13;14ξ,15-diepoxylabdane (**8**, 6%) as a gum: $[\alpha]_D$ −50° ($CHCl_3$; *c* 0.9); IR ν_{max} cm^{-1}: 1735, 1244, 1102, 870, 820; ^1H NMR: δ 5.08 (1H, *ddd*, $J_1 = J_2 = 11.4$, $J_3 = 4.0$ Hz, H-6), 4.04 and 3.60 (2H, AB system, $J = 10.8$ Hz, 2H-18), 3.06 (1H, *dd*, $J_1 = 4.5$, $J_2 = 3.0$ Hz), 2.78 (1H, *dd*, $J_1 = J_2 = 4.5$ Hz) and 2.48 (1H, *dd*, $J_1 = 4.5$, $J_2 = 3.0$ Hz) (H-14 and 2H-15), 2.07 and 1.99 (3H each, *s*, AcO groups), 1.50 (3H, *s*, 3H-16), 1.09 (3H, *s*, 3H-17), 0.88 and 0.84 (3H each, *s*, 3H-19 and 3H-20); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M+1]^+$ 423 (1), 363 (100), 347 (41), 303 (19).

Biotransformation of substrate **7** *by* R. nigricans. Substrate **7** (100 mg) was dissolved in EtOH (2 ml) and incubated for 3 days to obtain, after CC, 38 mg of substrate **7** (38%) and 30 mg of *ent*-6α,18-diacetoxy-11β-hydroxymanoyl oxide (**9**, 29%) as a gum: $[\alpha]_D$ −114° ($CHCl_3$; *c* 0.5); IR ν_{max} cm^{-1}: 3510, 1735, 1643, 1238, 1030 and 916; ^1H NMR: δ 5.84 (1H, *dd*, $J_1 = 17.4$, $J_2 = 10.7$ Hz, H-14), 5.16 (1H, *ddd*, $J_1 = J_2 = 11.3$ Hz, $J_3 = 4.1$ Hz, H-6); 5.12 (1H, *dd*, $J_1 = 17.4$ Hz, $J_2 = 1.4$ Hz) and 4.93 (1H, *dd*, $J_1 = 10.7$ Hz, $J_2 = 1.4$ Hz) (2H-15), 4.46 (1H, *ddd*, $J_1 = J_2 = 5.8$ Hz, $J_3 = 3.9$ Hz, H-11), 4.01 and 3.64 (2H, AB system, $J = 10.8$ Hz, 2H-18), 2.06 and 2.00 (3H each, *s*, AcO groups), 1.70 (3H, *s*, 3H-17), 1.40 (3H, *s*, 3H-16), 1.27 (3H, *s*, 3H-19), 0.87 (3H, *s*, 3H-20); ^{13}C NMR: Table 1; CI-MS, *m/z* (rel. int.): $[M+1]^+$ 423 (1), 405 (4), 363 (48), 345 (22), 295 (100).

Biotransformation of substrate **10** *by* R. nigricans. Substrate **10** (230 mg) was dissolved in 5 ml of EtOH and distributed among 5 conical flask cultures of *R. nigricans* and incubated for 8 days. After CC, 115 mg of substrate **10** (50%) was recovered and 80 mg of a mixt. of metabolites were acetylated with Ac_2O–pyridine (0.8 : 1.5 ml) at reflux for 4 hr to obtain 10

mg of *ent*-16,18-diacetoxy-6-oxo-13-*epi*-manoyl oxide (**11**, 3%), 15 mg of *ent*-16,18-diacetoxy-6-oxo-11β-hydroxy-13-*epi*-manoyl oxide (**12**, 4%), 26 mg of *ent*-16,18,20-triacetoxy-6-oxo-13-*epi*-manoyl oxide (**13**, 8%) and 18 mg of *ent*-16,18-diacetoxy-6β,20-epoxy-6α-hydroxy-13-*epi*-manoyl oxide (**14**, 6%). In this incubation 32 mg of *ent*-3β,16,18-trihydroxy-6-oxo-13-*epi*-manoyl oxide (**15**, 13%) were also isolated.

ent-16,18-*Diacetoxy*-6-*oxo*-13-epi-*manoyl oxide* (**11**). Gum; $[\alpha]_D$ −34° ($CHCl_3$; *c* 2); IR ν_{max} cm^{-1}: 3084, 1741, 1711, 1643, 1238, 1040, 984 and 926; ^1H NMR: δ 5.88 (1H, *dd*, part X of an AB system, $J_{AX}+J_{BX}$ = 29.0 Hz, H-14), 5.13 and 5.06 (2H, part AB of an ABX system, 2H-15), 3.94 and 3.72 (2H, AB system, *J* = 10.7 Hz, 2H-18), 3.81 (2H, *s*, 2H-16), 2.49 and 2.37 (2H, AB system, *J* = 11.4 Hz, 2H-7), 2.42 (1H, *s*, H-5) 2.02 and 2.00 (3H each, *s*, AcO groups), 1.16 and 1.13 (3H each, *s*, 3H-17 and 3H-19) and 0.75 (3H, *s*, 3H-20); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M+1]^+$ 421 (10), 361 (100), 301 (11).

ent-16,18-*Diacetoxy*-11β-*hydroxy*-6-*oxo*-13-epi-*manoyl oxide* (**12**). Mp 136–140°; $[\alpha]_D$ −45° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3522, 3088, 1738, 1244, 1103, 988; ^1H NMR: δ 6.12 (1H, *dd*, part X of an AB system, $J_{AX}+J_{BX}$ = 29.1 Hz, H-14), 5.21 and 5.15 (2H, part AB of an ABX system, 2H-15), 4.58 (1H, *ddd*, J_1 = 3.6, J_2 = 3.1, J_3 = 2.2 Hz, H-11), 3.91 and 3.70 (2H, AB system, *J* = 10.7 Hz, 2H-18), 3.84 and 3.75 (2H, AB system, *J* = 11.0 Hz, 2H-16), 2.44 and 2.32 (2H, AB system, *J* = 11.8 Hz, 2H-7), 2.32 (1H, *s*, H-5), 2.26 (1H, *dd*, J_1 = 14.7, J_2 = 3.1 Hz) and 1.85 (1H, *dd*, J_1 = 14.7, J_2 = 3.6 Hz) (2H-12), 2.01 and 1.98 (3H each, *s*, AcO groups), 1.72 (1H, *d*, *J* = 2.2 Hz, H-9), 1.51 (3H, *s*, 3H-17) and 1.16 and 1.12 (3H each, *s*, 3H-19 and 3H-20); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M+1]^+$ 437 (50), 419 (32), 377 (100), 359 (65), 317 (7), 299 (13).

ent-16,18,20-*Triacetoxy*-6-*oxo*-13-epi-*manoyl oxide* (**13**). Gum; $[\alpha]_D$ −24° ($CHCl_3$; *c* 0.5); IR ν_{max} cm^{-1}: 3089, 1740, 1235 and 1039; ^1H NMR: δ 5.91 (1H, part X of an ABX system, $J_{AX}+J_{BX}$ = 28.9 Hz, H-14), 5.19–5.08 (2H, part AB of an ABX system, 2H-15), 4.38 and 3.70 (2H, AB system, *J* = 12.3 Hz, 2H-20), 3.97 and 3.78 (2H, AB system, *J* = 10.7 Hz, 2H-18), 3.84 (2H, *s*, 2H-16), 2.57 (1H, *s*, H-5), 2.51 (2H, *s*, 2H-7), 2.06, 2.04 and 2.00 (3H, *s*, AcO groups), 1.26 (3H, *s*, 3H-17) and 1.15 (3H, *s*, 3H-19); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M+1]^+$ 479 (42), 419 (100), 359 (80), 299 (11).

ent-16,18-*diacetoxy*-6β,20-*epoxy*-6α-*hydroxy*-13-epi-*manoyl oxide* (**14**). Gum; $[\alpha]_D$ −12° ($CHCl_3$; *c* 0.5); IR ν_{max} cm^{-1}: 3456, 3088, 1737, 1240, 1039; ^1H NMR: δ 5.82 (1H, *dd*, J_1 = 17.3, J_2 = 10.8 Hz, H-14), 5.37 (1H, *dd*, J_1 = 17.3, J_2 = 1.0 Hz) and 5.26 (1H, *dd*, J_1 = 10.8, J_2 = 1.0 Hz) (2H-15), 4.36 and 3.97 (2H, AB system, *J* = 10.6 Hz, 2H-18), 4.15 and 4.00 (2H, AB system, *J* = 11.3 Hz, 2H-16), 4.10 (1H, *d*, *J* = 8.9 Hz) and 3.54 (1H, *dd*, J_1 = 8.9, J_2 = 1.8 Hz) (2H-20), 2.48 (2H, *s*, 2H-7), 2.40 (1H, *d*, *J* = 1.8 Hz, H-5), 2.08 and 2.03 (3H, *s*, AcO groups), 1.26 (3H, *s*, 3H-17) and 1.13 (3H, *s*, 3H-19); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M+1]^+$ 437 (21), 419 (11), 377 (100), 359 (10).

ent-3β,16,18-*Trihydroxy*-6-*oxo*-13-epi-*manoyl oxide* (**15**). Gum; $[\alpha]_D$ −11° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3404, 3089, 1706, 1452, 1388, 1274 and 1046; ^1H NMR: δ 5.84 (1H, *dd*, part X of an AB system, $J_{AX}+J_{BX}$ = 29.1 Hz, H-14), 5.15–5.06 (2H, part AB of an ABX system, 2H-15), 3.58 (1H, *m*, $W_{1/2}$ = 12.0 Hz, H-3), 3.54 and 3.47 (2H, AB system, *J* = 10.8 Hz, 2H-18), 3.31 and 2.97 (2H, AB system, *J* = 11.2 Hz, 2H-16), 2.65 and 2.35 (2H, AB system, *J* = 11.3 Hz, 2H-7), 2.43 (1H, *s*, H-5), 1.18 (3H, *s*, 3H-17), 1.05 (3H, *s*, 3H-19), 0.78 (3H, *s*, 3H-20); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M+1]^+$ 353 (30), 335 (100), 317 (26).

Acetylation of metabolite **15**. Metabolite **15** (15 mg) was acetylated with Ac_2O–pyridine (0.5 : 1 ml) for 24 hr at room temp. to give 13 mg of *ent*-3β,16,18-triacetoxy-6-oxo-13-*epi*-manoyl oxide (**16**, 65%) as a gum: $[\alpha]_D$ −24° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3089, 1741, 1243 and 1044; ^1H NMR: δ 5.90 (1H, part X of an AB system, $J_{AX}+J_{BX}$ = 28.9 Hz, H-14), 5.31-5.09 (2H, part AB of an ABX system, 2H-15), 4.64 (1H, *dd*, J_1 = 11.4, J_2 = 4.3 Hz, H-3), 3.92 (2H, *s*, 2H-16), 3.86 and 3.82 (2H, AB system, *J* = 10.9 Hz, 2H-18), 2.58 (1H, *s*, H-5), 2.52 and 2.42 (2H, AB system, *J* = 11.5 Hz, 2H-7), 2.05, 2.02 and 2.01 (3H each, *s*, AcO groups), 1.19 (3H, *s*, 3H-17), 1.17 (3H, *s*, 3H-19) and 0.81 (3H, *s*, 3H-20); ^{13}C NMR: Table 1; CI-MS, *m/z* (rel. int.): $[M+1]^+$ 479 (76), 419 (100) 359 (88).

Biotransformation of substrate **17** *by* R. nigricans. Substrate **17** (350 mg) was dissolved in EtOH (7 ml) and distributed among 7 conical flask cultures and incubated for 8 days. After CC, substrate **17** (76 mg, 22%), *ent*-11β,16,18-trihydroxy-6-oxomanoyl oxide (**18**, 172 mg, 47%) and *ent*-3β,16,18-trihydroxy-6-oxomanoyl oxide (**19**, 45 mg, 12%) were obtained.

ent-11β,16,18-*Trihydroxy*-6-*oxomanoyl oxide* (**18**). Mp 225–227; $[\alpha]_D$ −42° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3417, 3089, 1700, 1042 and 927; ^1H NMR: δ 5.81 (1H, *dd*, J_1 = 17.4, J_2 = 10.8 Hz, H-14), 5.25 (1H, *dd*, J_1 = 17.4, J_2 = 1.4 Hz) and 5.15 (1H, *dd*, J_1 = 10.8, J_2 = 1.4 Hz) (2H-15), 4.55 (1H, *ddd*, J_1 = J_2 = 6.9, J_3 = 4.6 Hz, H-11), 3.51 and 3.11 (2H, AB system, *J* = 10.5 Hz, 2H-18), 3.48 and 3.38 (2H, AB system, *J* = 11.30 Hz, 2H-16), 2.66 and 2.48 (2H, AB system, *J* = 12.5 Hz, 2H-7), 2.45 (1H, *s*, H-5), 2.08 (1H, *d*, *J* = 4.6 Hz, H-9), 1.55 (3H, *s*, 3H-17) and 1.19 and 1.13 (3H each, *s*, 3H-19 and 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 353 (100), 335 (38), 317 (25), 299 (6).

ent-3β,16,18-*Trihydroxy*-6-*oxomanoyl oxide* (**19**). Gum; $[\alpha]_D$ −16° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3395, 3079, 1705 and 1049; ^1H NMR: δ 5.83 (1H, J_1 = 17.4, J_2 = 10.8 Hz, H-14), 5.26 (1H, *d*, *J* = 17.4 Hz) and 5.16 (1H, *d*, *J* = 10.8 Hz) (2H-15), 3.68 and 3.42 (2H, AB system, *J* = 10.2 Hz, 2H-18), 3.53 (1H, *dd*, J_1 = 10.8, J_2 = 4.8 Hz, H-3), 3.30 (2H, *s*, 2H-16), 2.64 and 2.47 (2H, AB system, *J* = 11.9 Hz, 2H-7), 2.33 (1H, *s*, H-5), 1.24 (3H, *s*, 3H-17), 1.13 (3H, *s*, 3H-19),

0.84 (3H, *s*, 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 353 (59), 335 (100), 317 (22), 299 (3).

Biotransformation of substrate **20** *by* R. nigricans. Substrate **20** (220 mg) was dissolved in EtOH (6 ml), distributed between 6 conical flask cultures, and incubated for 60 hr. After CC, 130 mg of substrate (**20**, 59%), 7 mg of (13R)-*ent*-18-acetoxy-6-oxo-8α,13;14ξ,15-diepoxylabdane (**21**, 3%) and 50 mg of (13R)-*ent*-18-acetoxy-3β-hydroxy-6-oxo-8α,13;14ξ,15-diepoxylabdane (**22**, 21%) were obtained.

(13R)-ent-18-*Acetoxy*-6-*oxo*-8α,13;14ξ,15-*di*-*epoxylabdane* (**21**). Gum; $[\alpha]_D$ −8° ($CHCl_3$; *c* 0.5); IR ν_{max} cm^{-1}: 1736, 1239 and 1033; ^{1}H NMR: δ 3.97 and 3.76 (2H, AB system, *J* = 10.8 Hz, 2H-18), 2.89 (1H, *dd*, J_1 = 3.8, J_2 = 2.8 Hz), 2.82 (1H, dd, J_1 = 4.7, J_2 = 3.8 Hz) and 2.77 (1H, *dd*, J_1 = 4.7, J_2 = 2.8 Hz) (H-14 and 2H-15), 2.57 and 2.44 (2H, AB system, *J* = 11.5 Hz, 2H-7), 2.48 (1H, *s*, H-5), 2.03 (3H, *s*, AcO group), 1.24 (6H, *s*, 3H-16 and 3H-17), 1.18 and 0.80 (3H each, *s*, 3H-19 and 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 379 (8), 319 (100), 301 (5).

(13R)-ent-18-*Acetoxy*-3β-*hydroxy*-6-*oxo*-8α,13; 14ξ,15-*diepoxylabdane* (**22**). Mp 166–168°; $[\alpha]_D$ +25° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3464, 1712, 1241 and 1031; ^{1}H NMR: δ 4.29 and 3.90 (2H, AB system, *J* = 11.4 Hz, 2H-18), 3.21 (1H, *dd*, J_1 = 9.8, J_2 = 6.0 Hz, H-3); 2.88 (1H, *dd*, J_1 = 3.8, J_2 = 2.7 Hz), 2.79 (1H, *dd*, J_1 = 4.7, J_2 = 3.8 Hz) and 2.74 (1H, *dd*, J_1 = 4.7, J_2 = 2.7 Hz) (H-14 and 2H-15), 2.55 and 2.43 (2H, AB system, *J* = 11.5 Hz, 2H-7), 2.38 (1H, *s*, H-5), 2.55 and 2.43 (2H, AB system, *J* = 11.5 Hz, 2H-7), 2.38 (1H, *s*, H-5), 2.04 (3H, *s*, AcO group), 1.22 and 1.21 (3H each, *s*, 3H-16 and 3H-17), 1.07 and 0.78 (3H each, *s*, 3H-19 and 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 395 (19), 377 (22), 335 (100), 317 (21).

Biotransformation of substrate **23** *by* R. nigricans. Substrate **23** (110 mg) was dissolved in EtOH (2 ml) and distributed between 2 conical flask cultures and incubated for 60 hr. After CC, 18 mg of substrate (**23**, 16%), 36 mg of *ent*-18-acetoxy-11β-hydroxy-6-oxomanoyl oxide (**24**, 31%) and 26 mg of *ent*-11β,18-dihydroxy-6-oxomanoyl oxide (**25**, 25%) were isolated.

ent-18-*Acetoxy*-11β-*hydroxy*-6-*oxomanoyl oxide* (**24**). Gum; $[\alpha]_D$ −37° ($CHCl_3$; *c* 1); IR ν_{max} cm^{-1}: 3509, 3089, 1737, 1708, 1643, 1238, 1036 and 918; ^{1}H NMR: δ 5.87 (1H, *dd*, J_1 = 17.3, J_2 = 10.7 Hz, H-14), 5.14 (1H, *dd*, J_1 = 17.3, J_2 = 1.4 Hz) and 4.95 (1H, *dd*, J_1 = 10.7, J_2 = 1.4 Hz) (2H-15), 4.58 (1H, *ddd*, J_1 = 5.7, J_2 = 5.3, J_3 = 3.6 Hz, H-11), 3.94 and 3.75 (2H, AB system, *J* = 10.7 Hz, 2H-18), 2.54 and 2.45 (2H, AB system, *J* = 12.3 Hz, 2H-7), 2.34 (1H, *s*, H-5), 2.05 (1H, *dd*, J_1 = 14.8, J_2 = 5.7 Hz) and 1.91 (1H, *dd*, J_1 = 14.8, J_2 = 5.3 Hz) (2H-12), 2.00 (3H, *s*, AcO group), 1.93 (1H, *d*, *J* = 3.6 Hz, H-9), 1.59 (3H, *s*, H-16), 1.44 (3H, *s*, 3H-17), 1.21 and 1.19 (3H each, *s*, 3H-19 and 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 379 (10), 361 (5), 319 (100), 301 (8).

ent-11β,18-*Dihydroxy*-6-*oxomanoyl oxide* (**25**). Mp 157–159°; $[\alpha]_D$ −24° ($CHCl_3$; *c* 0.5); IR ν_{max} cm^{-1}: 3428, 3089, 1702, 1638, 1244 and 1039; ^{1}H NMR: δ 5.87 (1H, *dd*, J_1 = 17.4, J_2 = 10.7 Hz, H-14), 5.14 (1H, *dd*, J_1 = 17.4, J_2 = 1.2 Hz) and 4.96 (1H, *dd*, J_1 = 10.7, J_2 = 1.2 Hz) (2H-15), 4.59 (1H, *ddd*, J_1 = 5.7, J_2 = 5.3, J_3 = 3.8 Hz, H-11), 3.52 and 3.14 (2H, AB system, *J* = 10.5 Hz, 2H-18), 2.63 and 2.48 (2H, AB system, *J* = 12.0 Hz, 2H-7), 2.46 (1H, *s*, H-5), 2.09 (1H, *dd*, J_1 = 14.9, J_2 = 5.7 Hz) and 1.92 (1H, *dd*, J_1 = 14.9, J_2 = 5.3 Hz) (2H-12), 2.00 (1H, *d*, *J* = 3.8 Hz, H-9), 1.60 (3H, *s*, 3H-16), 1.44 (3H, *s*, 3H-17), 1.20 and 1.16 (3H-19 and 3H-20); ^{13}C NMR: Table 2; CI-MS *m/z* (rel. int.): $[M+1]^+$ 337 (100), 319 (44), 301 (24).

Biotransformation of substrate **10** *by* C. lunata. Substrate **10** (250 mg) was dissolved in EtOH (5 ml), distributed among 5 conical flask cultures of *C. lunata* and incubated for 8 days. After CC, substrate **10** (60 mg, 18%) and 172 mg of a mixt. of metabolites were obtained. This mixt. was acetylated with Ac_2O–pyridine (2:4 ml) at room temp. for 2 hr. to give 46 mg of diacetate **11** (15%) and 115 mg of *ent*-16,18-diacetoxy-11β-hydroxy-6-oxo-13-*epi*-manoyl oxide (**12**, 35%).

Biotransformation of substrate **17** *by* C. lunata. Substrate **17** (200 mg) was dissolved in EtOH (4 ml) and distributed among 4 conical flask cultures and incubated for 8 days. After CC, diol **17** (145 mg, 72%) and *ent*-6-oxo-11β,16,18-trihydroxymanoyl oxide (**18**, 30 mg, 14%) were obtained.

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