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PREPARATION OF POLYOXYGENATED *ENT*-13-*EPI*-MANOYL OXIDES BY CHEMICAL–MICROBIOLOGICAL SEMISYNTHESES

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Key Word Index—Biotransformation; *ent*-13-*epi*-manoyl oxides; *ent*-labdanes; filamentous fungi.

Abstract—The biotransformation of *ent*-3β-hydroxy-13-*epi*-manoyl oxide with *Fusarium moniliforme* gave *ent*-7β, 11α-dihydroxy, *ent*-7β,12α-dihydroxy derivatives and the corresponding products oxidized at C-3. The biotransformation of *ent*-3-oxo-13-*epi*-manoyl oxide with *Fusarium moniliforme* gave *ent*-1β-hydroxy and Δ^1-derivatives. The incubation of *ent*-3,12-dioxo-13-*epi*-manoyl oxide with *Fusarium moniliforme* gave *ent*-11α-hydroxy, *ent*-1β-hydroxy and *ent*-7β,11α-dihydroxy derivatives, and with *Cunninghamella elegans* gave the 14,15-dihydroxy derivative. The biotransformation of *ent*-3β,12β-dihydroxy-13-*epi*-manoyl oxide with *Rhizopus nigricans* gave the *ent*-7α-hydroxy derivative.

INTRODUCTION

Polyfunctionalized manoyl oxides present interesting biological properties, forskolin being the most interesting product [1]. Biotransformation processes with microorganisms can be used to introduce hydroxyl groups at difficult positions on the substrates. Such processes have been used to obtain forskolin analogues from deoxy forskolin derivatives [2–6]. Biotransformations of *ent*-13-*epi*-manoyl oxides with the fungi *Gibberella fujikuroi* [7], *Curvularia lunata* [8, 9] and *Rhizopus nigricans* [10, 11] also sometimes yield products with biological activity [9, 12].

Using *ent*-13-*epi*-manoyl oxides isolated from Andalusian *Sideritis* species, we have accomplished a series of chemical transformations to obtain derivatives that present certain structural variations. Oxidation of *ent*-3β-hydroxy-13-*epi*-manoyl oxide (ribenol, **1**) [13] and *ent*-3β,12α-dihydroxy-13-*epi*-manoyl oxide (varodiol, **2**) [14] yields *ent*-3-oxo-13-*epi*-manoyl oxide (ribenone, **3**) [13] and *ent*-3,12-dioxo-13-*epi*-manoyl oxide (**4**) [14], respectively. Reduction of diketone **4** takes place exclusively on the *ent*-α face, to give the *ent*-3β,12β-dihydroxy derivative **5** [14]. The *ent*-13-*epi*-manoyl oxides **1**, **3**–**5** were used as substrates in different biotransformations with various filamentous fungi, to study the influence of these microorganisms on these transformations. In this work we used the fungi *Cunninghamella elegans*, *Fusarium moniliforme* and *Rhizopus nigricans*, to complete an earlier biotransformation study of substrates **1**, **3**–**5** with the microorganism *Curvularia lunata* [8, 9].

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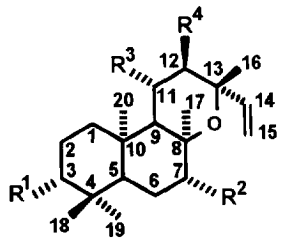
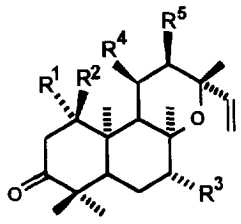
RESULTS AND DISCUSSION

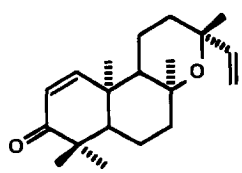
After biotransformation of substrate **1** with *Fusarium moniliforme* for 14 days, 62% was recovered unaltered, together with the metabolites **6**–**8**, and a mixture of polar products, which was acetylated to give products **9** and **10**.

The IR spectrum of the first metabolite isolated (**6**) showed bands representing hydroxyl (3436 cm^{-1}) and carbonyl groups. In its ^1H NMR spectrum, we observed two signals at δ4.23 (1H, *ddd*, J = 9.9, 8.9, 4.5 Hz) and 3.57 (1H, *dd*, J = 11.7, 4.1 Hz), caused by geminal axial protons to the hydroxyl groups. There was no signal from the geminal proton to the hydroxyl group on C-3 (δ3.20 in the substrate **1**). In the ^{13}C NMR spectrum of metabolite **6** (Table 1), we noted a signal at δ217.3, which confirmed that metabolite **6** possessed a carbonyl group on C-3. Comparison of the ^{13}C NMR spectra of ribenone (**3**) and of metabolite **6** indicated hydroxylations on C-7 and C-11 (see Table 2). Therefore, metabolite **6** is considered to be *ent*-7β,11α-dihydroxy-3-oxo-13-*epi*-manoyl oxide.

The IR spectrum of the second metabolite (**7**) also showed a carbonyl group (1698 cm^{-1}) and in its ^1H NMR signal of H-3 geminal to hydroxyl group is not detected. Two double doublets appeared at δ4.07 (J = 3.2, 3.2 Hz) and at 3.61 (J = 11.5, 4.0 Hz), caused by geminal protons to hydroxyl groups. The ^{13}C NMR spectra of ribenone (**3**) and metabolite **7** confirmed the existence of the keto group on C-3 (δ216.9), the existence of an equatorial hydroxyl group on C-7, and a second axial hydroxylation on C-12 (see Table 2). Therefore, metabolite **7** is considered to be *ent*-7β,12α-dihydroxy-3-oxo-13-*epi*-manoyl oxide.







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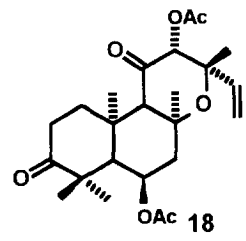
	R^1	R^2	R^3	R^4
1	OH	H	H	H
2	OH	H	H	OH
8	OH	OH	H	OH
9	OAc	OAc	H	OAc
10	OAc	OAc	OAc	H

	R^1	R^2	R^3	R^4	R^5
3	H	H	H	H	H
6	H	H	OH	OH	H
7	H	H	OH	H	OH
11	OH	H	H	H	H
13	H	OH	H	H	H



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The CI-mass spectrum of the third metabolite isolated (**8**) contained an ion at *m/z* 339 $[M + 1]^+$ compatible with dihydroxylation of substrate **1**. In its IR spectrum we noted an intense band owing to a hydroxyl group (3413 cm^{-1}), and its ^1H NMR spectrum displayed three geminal proton signals to hydroxyl groups. One of these signals, δ3.21 (1H, *dd*, J = 11.2, 4.9 Hz) was assigned to the proton on C-3, while the other two signals appeared as two double doublets at δ4.06 (J = 3.5, 3.5 Hz) and δ3.57 (J = 11.5, 4.6 Hz). These data, together with those from ^{13}C NMR (see Tables 1 and 2), suggest that as in metabolite **7**, the microorganism has introduced an axial hydroxylation on C-11 and another equatorial hydroxylation on C-7.

Acetylation of the polar metabolite fraction led to the isolation of the triacetoxy derivatives **9** and **10**. Product **9** had some spectroscopic data identical to those of the acetylation product of metabolite **8**; therefore it is considered to be *ent*-3β,7β,12α-triacetoxy-13-*epi*-manoyl oxide. The ^1H NMR spectrum of the triacetoxy derivative **10** showed two geminal proton signals to acetoxy groups, identical to those observed in the spectrum of metabolite **9** (δ4.45, *dd*, J = 11.5, 4.8 Hz, H-3 and δ4.80, *dd*, J = 11.6, 4.7 Hz, H-7). We also observed a third signal at δ5.25 (*ddd*, J = 9.0, 5.5, 4.1 Hz) owing to a geminal axial proton to the acetoxy group on C-11. The ^{13}C NMR data (see Table 1) for this product (**10**) were compatible with the structure of *ent*-3β,7β,11α-triacetoxy-13-*epi*-manoyl oxide.

The results obtained in this biotransformation can be compared with those described previously. With *Curvularia lunata* we obtained products with axial hydroxylations on C-1 (4%), C-6 (20%) or C-11 (2%) [8]. However, with *Gibberella fujikuroi*, hydroxylations were geared toward the C ring, producing monohydroxylations on C-11 (6.5%) and C-12 (6%), or dihydroxylation on C-11 and C-12 (4%), in all the cases on the *ent*-α face [7]. The results obtained in the present work indicate that *Fusarium moniliforme* functionalizes largely on C-7 on the *ent*-β face (21%), and thereafter introduces hydroxyl groups either on C-12 (16%) or on C-11 (5%), on the *ent*-α face. We have also observed that products 3,7,11- or 3,7,12-trihydroxylated were oxidized on C-3 (4 and 5%, respectively).

Biotransformation of substrate **3** with *Fusarium moniliforme* left 50% of the substrate (**3**) unaltered, and produced two metabolites (**11**, 4.7% and **12**, 10%). The ^1H NMR spectrum of metabolite **11**, compared with that of ribenone (**3**), indicated a new signal at δ3.95 owing to a geminal proton to a hydroxyl group, which was coupled with other two protons (J = 6.7, 3.7 Hz). Furthermore, the signals of the protons of C-2 in product **3** were modified in product **11**, appearing as two double doublets (δ2.97, J = 12.7, 6.7 Hz and δ2.31, J = 12.7, 3.7 Hz). Thus, these two signals, together with that of the geminal proton to a hydroxyl group, which appeared at δ3.95, constitute an ABX system; this is compatible only with hydroxylation on C-1. In view of the data for *ent*-1α-hydroxy-3-oxo-13-*epi*-manoyl oxide (**13**), obtained in a previous biotransformation from this substrate (**3**) with *Curvularia lunata* [9], we can assign the structure of *ent*-1β-hydroxy-3-oxo-13-*epi*-manoyl oxide to metabolite **11**. The spectroscopic data for metabolite **12** coincided with those for α,β-insaturated ketone, isolated in a previous incubation of substrate **3** with *Curvularia lunata* [9], and probably formed by dehydration of metabolite **11**.

In a previous biotransformation study with ribenone (**3**), we proved that *Curvularia lunata* produced an *ent*-6β-hydroxy (6%) and another *ent*-1α-hydroxy derivatives (7%), which subsequently evolved toward an α,β-unsaturated system in the A ring (5%). We also observed reduction of the keto group (3%), as well as hydroxylation on C-11 (1%) [9]. The biotransformation of this substrate (**3**) with *Fusarium moniliforme* produced a 1β-

Table 1. ^{13}C NMR spectral data of **1**, **3–10**, **14–16**, **19**, **20**, **22** and **24**

C	**1***	**3†**	**4†**	**5***	**6**	**7**	**8**	**9**	**10**	**14**	**15**	**16**	**19**	**20**	**22**	**24**
1	37.8	38.2	37.3	37.6	39.9	37.4	37.1	36.7	36.7	39.5	39.1	77.9	39.6	77.3	37.4	36.6
2	27.4	33.8	33.7	27.3	33.9	33.6	27.2	23.4	23.4	33.9	33.7	45.1	27.6	38.1	27.3	33.6
3	78.9	217.2	216.3	78.9	217.3	216.9	78.7	80.3	80.1	217.3	216.7	214.0	78.5	75.8	78.7	213.9
4	38.9	47.2	47.3	38.9	47.5	47.0	38.9	37.7	37.8	47.6	47.4	47.3	39.0	38.9	38.4	47.5
5	55.4	54.6	54.5	55.1	52.2	52.4	53.3	52.9	52.4	54.5	52.1	51.1	55.3	52.8	46.4	54.3
6	19.5	20.7	20.7	19.7	27.4	27.8	26.7	25.1	25.1	20.7	27.5	20.2	19.6	19.3	24.7	36.4
7	43.0	42.1	41.3	42.3	79.4	79.4	80.4	81.1	81.4	42.1	78.8	41.1	43.0	42.2	72.7	206.2
8	75.9	75.5	75.1	76.4	79.8	79.1	79.4	77.3	76.3	76.8	80.1	‡	‡	76.1	77.3[a]	80.0
9	58.4	57.6	54.0	57.7	65.1	46.5	47.3	48.3	57.3	61.1	59.6	53.8	61.4	58.1	50.0	54.2
10	36.3	36.4	36.5	36.7	38.3	36.0	36.4	35.7	37.7	38.6	39.0	42.3	39.4	42.8	37.4	36.7
11	16.0	16.3	33.8	25.3	60.7	23.7	23.4	21.2	67.3	72.2	71.5	36.7	70.2	28.2	24.7	33.6
12	35.0	34.8	210.6	77.6	45.2	68.6	68.8	70.8	38.8	210.8	210.2	212.2	83.0	77.7	77.4	208.3
13	73.4	73.6	82.2	75.8	74.6	76.3	76.1	74.8	72.7	82.4	82.5	81.6	‡	75.8	77.2[a]	83.5
14	147.5	147.3	142.3	140.3	147.2	146.5	146.7	146.4	146.8	143.0	142.4	142.0	141.0	140.5	139.4	141.5
15	109.8	109.7	113.3	117.3	110.5	111.2	111.1	111.0	110.6	113.3	113.6	113.4	117.2	117.1	117.8	114.1
16	32.7	32.6	28.6	28.1[a]	32.2	27.0[a]	26.8	26.9	30.8	28.0	27.9	28.7	29.1	28.1	27.8[b]	28.4
17	24.1	23.3	21.9	25.5	19.0	18.4	17.9	19.1	21.6	21.0	17.1	21.8	26.8	25.6	24.9	20.5
18	28.0	26.6	26.6	28.0[a]	27.3	26.9[a]	28.1	27.9	28.1	27.3	27.3	26.8	28.3	27.9	27.7[b]	25.9
19	15.2	20.8	21.3	15.2	20.9	20.8	15.3	15.8	16.5	20.8	21.0	21.0	15.3	14.9	15.2	21.1
20	16.0	15.5	14.3	16.1	16.4	15.7	16.1	16.3	16.5	15.3	15.5	9.8	16.6	12.0	15.7	13.7
MeCO								21.5	21.8							
								21.3	21.4							
								21.1	21.4							
								171.0	171.0							
MeCO								170.9	170.6							
								170.8	170.5							

^{13}C chemical shifts are given in δ values (ppm) relative to $CDCl_3$ signals.

*See ref. [14].

†See ref. [9].

[a,b]Values bearing the same superscript may be interchanged.

‡Not observed.

Table 2. Substituent chemical shifts ($\Delta\delta$)* for **6–8**, **14**, **15** and **22**

C	δ(**6**)–δ(**3**)†	δ(**7**)–δ(**3**)‡	δ(**8**)–δ(**1**)‡	δ(**14**)–δ(**4**)§	δ(**15**)–δ(**14**)‖	δ(**22**)–δ(**5**)¶
1	+ 1.7	− 0.8	− 0.7	+ 2.2	− 0.4	− 0.2
5	− 2.4	− 2.2	− 2.1	0.0	− 2.4	− 8.7
6	+ 6.7	+ 7.1	+ 7.2	0.0	+ 6.8	+ 5.0
7	+ 37.3	+ 37.3	+ 37.4	+ 0.8	+ 36.7	+ 30.4
8	+ 4.3	+ 3.6	+ 3.5	+ 1.7	+ 3.3	+ 0.9
9	+ 7.5	− 11.1	− 11.1	+ 7.1	− 1.5	− 7.7
10	+ 1.9	− 0.4	− 0.1	+ 2.1	+ 0.4	+ 0.7
11	+ 44.4	+ 7.4	+ 7.4	+ 38.4	− 0.7	− 0.6
12	+ 10.4	+ 33.8	+ 33.8	+ 0.2	− 0.6	− 0.2
13	+ 1.0	+ 2.7	+ 2.7	+ 0.2	+ 0.1	+ 1.4
17	− 4.3	− 4.9	− 6.2	− 0.9	− 3.9	− 0.6

*The numbers given are the chemical shift differences for corresponding carbon atoms.
†Substituent effects of two equatorial hydroxyl groups at C-7 and C-11.
‡Substituent effects of equatorial OH-7 and axial OH-12 groups.
§Substituent effects of equatorial OH-11 group.
‖Substituent effects of equatorial OH-7 group.
¶Substituent effects of axial OH-7 group.

hydroxylated compound that became an α,β-unsaturated system in the A ring (10%).

The biotransformation of substrate **4** with *Fusarium moniliforme* for seven days left 63% of the substrate unaltered, and gave rise to three metabolites (**14–16**) and a mixture of polar products, from which products **17** and **18** were isolated after acetylation.

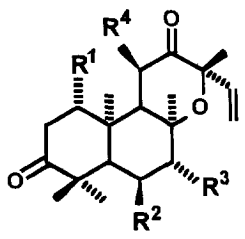
The first metabolite (**14**) had a peak at *m/z* (335 [M + 1]$^+$ in its CI-mass spectrum and a band representing a hydroxyl group (3466 cm^{-1}) in its IR spectrum, which indicated that a hydroxyl group had been introduced on substrate **4**. Its ^{1}H NMR spectrum showed a signal at δ4.54 (1H, *d*, J = 12.8 Hz), which was a result of its high chemical shift and multiplicity, indicating equatorial hydroxylation on C-11. The ^{13}C NMR spectrum of **14**, compared with that of substrate **4**, confirmed the equatorial hydroxylation on C-11 (see Table 2). Reduction of metabolite **14** gave a triol (**19**, 80%), whose spectroscopic data indicated that this product is *ent*-3β,11α,12β-trihydroxy-13-*epi*-manoyl oxide, produced by reduction on the *ent*-α face of the carbonyl groups on C-3 and on C-12 of metabolite **14**. Therefore, the structure of **14** was established to be *ent*-11α-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide.

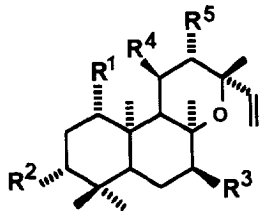
The ^{1}H NMR spectrum of metabolite **15** showed a signal representing the geminal axial proton to the hydroxyl group on C-11 (δ4.63, *d*, J = 12.7 Hz). Furthermore, a double doublet appeared at δ3.64 (J = 11.7, 4.0 Hz), produced by a geminal proton to the hydroxyl group on C-7. Comparison of the ^{13}C NMR data for metabolites **14** and **15** (see Table 2), showed that the structure of *ent*-7β,11α-dihydroxy-3,12-dioxo-13-*epi*-manoyl oxide could be assigned to metabolite **15**.

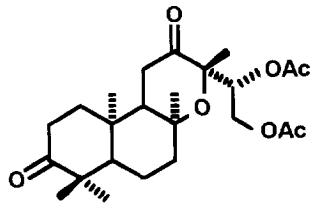
The third metabolite (**16**) had mass and IR spectra similar to those of metabolite **14**. Its ^{1}H NMR spectrum showed a signal at 3.86 (*dd*, J = 7.9, 6.9 Hz) owing to a geminal axial proton to a hydroxy group, which was coupled to two protons at δ2.78 and 2.50; this was confirmed by double resonance experiments. These data suggest the existence of an equatorial hydroxy group on C-1, as was found in metabolite **11**. The ^{13}C NMR spectra of metabolite **16** and substrate **4** showed an α-effect on C-1 ($\Delta\delta$ = + 40.56), β-effects on C-2 ($\Delta\delta$ = + 11.49) and on C-10 ($\Delta\delta$ = + 5.86), γ-effects on C-5 ($\Delta\delta$ = − 3.44) and on C-20 ($\Delta\delta$ = − 4.48), and deshielding on C-11 ($\Delta\delta$ = + 2.84). Thus, metabolite **16** is considered to be *ent*-1β-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide. Reduction of this metabolite (**16**) occurred, like in metabolite **14**, on the *ent*-α face, giving rise to *ent*-1β,3β,12β-trihydroxy-13-*epi*-manoyl oxide (**20**).

After acetylation of the polar metabolites, two diacetoxy derivatives (**17** and **18**) with very similar spectroscopic data were isolated. The ^{1}H NMR spectra of both products (**17** and **18**) showed signals representing geminal axial protons to acetoxy on C-6 at δ4.94 (*ddd*, J = 11.5, 11.5, 4.0 Hz) and at δ4.91 (*ddd*, J = 11.3, 11.3, 4.4 Hz). The spectrum of product **17** also had a doublet at δ5.59 (J = 13.2 Hz), indicating an acetoxy group on C-11. However, the spectrum of product **18** showed two singlets at δ5.38 (H-12) and δ2.88 (H-9), which were compatible with an acetoxy group on C-12 and a carbonyl group on C-11. On the basis of this data we assign the structures of *ent*-6α,11α-diacetoxy-3,12-dioxo-13-*epi*-manoyl oxide to product **17** and *ent*-6α,12β-diacetoxy-3,11-dioxo-13-*epi*-manoyl oxide to product **18**.

After biotransformation of substrate **4** with *Cunninghamella elegans*, 32% of the substrate was recovered unaltered, together with a polar metabolite that was acetylated to give (14*R*)-*ent*-14,15-diacetoxy-8α,13(*S*)-epoxylabda-3,12-dione (**21**, 24%); this was isolated in a previous biotransformation of substrate **4** (21%) with *Curvularia lunata* [9]. However, the biotransformation with *F. moniliforme* afforded *ent*-11α-hydroxy (7%), *ent*-1β-hydroxy (9%), *ent*-7β,11α-dihydroxy (9%) derivatives.







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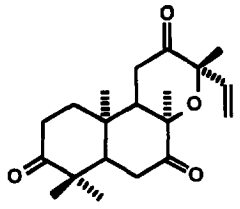
	R^1	R^2	R^3	R^4
4	H	H	H	H
14	H	H	H	OH
15	H	H	OH	OH
16	OH	H	H	H
17	H	OAc	H	OAc

	R^1	R^2	R^3	R^4	R^5
5	H	OH	H	H	OH
19	H	OH	H	OH	OH
20	OH	OH	H	H	OH
22	H	OH	OH	H	OH
23	H	OAc	H	H	OAc



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This fungus also functionalized C-6 and C-11 on the *ent*-α face (1%).

The biotransformation of substrate **5** with *Rhizopus nigricans* for nine days left 31% of the substrate (**5**) unaltered and produced metabolite **22** (5%) and a mixture of polar metabolites that was acetylated to give only one diacetoxy derivative (**23**, 3.8%). Compared with that of substrate **5**, the ^{1}H NMR spectrum of metabolite **22** showed a new signal of a geminal equatorial proton to a hydroxy group (δ3.63, *dd*, J = 2.7, 2.7 Hz). This hydroxylation was found on C-7, since the chemical correlation of this metabolite (**22**) with the trioxo derivative **24**, obtained from oxidation of metabolite **8**, was accomplished. Comparison of the ^{13}C NMR data for substrate **5** and metabolite **22** indicated an axial arrangement of the hydroxyl group on C-7 (see Table 2). These data are compatible with the structure of *ent*-3β,7α,12β-trihydroxy-13-*epi*-manoyl oxide. From acetylation of the mixture of polar metabolites we obtained product **23**, whose spectroscopic data indicated that was a diacetoxy derivative of metabolite **22**, whose structure is *ent*-3β,12β-diacetoxy-7α-hydroxy-13-*epi*-manoyl oxide.

We had previously studied the biotransformation of the 3-acetoxy derivative of product **5** with *Curvularia lunata*, and obtained product **5** (75%) and a product hydroxylated on C-6 on the *ent*-β face (9%) [9]. When biotransformation of substrate **5** was performed with *Rhizopus nigricans* hydroxylation took place on C-7, but on the *ent*-α face (9%). These results indicate that the adequate combination of chemical and microbiological processes can be useful to obtain new polyfunctionalized manoyl oxides which can be subjected to various biological activity tests.

EXPERIMENTAL

Mps: uncorr.; NMR: 300 MHz ^{1}H and 75.47 MHz ^{13}C, $CDCl_3$ soln (which also provided the lock signal). 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: Perkin-Elmer 983 G spectrometer. CI (methane) MS: Hewlett-Packard 5988 A spectrometer. Specific rotations: Perkin-Elmer 240 polarimeter at 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°.

Isolation of starting materials. The *ent*-3β-hydroxy-13-*epi*-manoyl oxide (ribenol, **1**) was isolated from *Sideritis varoi* [13]. The *ent*-3β,12α-dihydroxy-13-*epi*-manoyl oxide (varodiol, **2**) was isolated from *Sideritis varoi* ssp. *cuatrecasasii* [14].

Oxidation of ribenol (**1**). Ribenol (**1**) (500 mg) was dissolved in Me_2CO and treated with Jones' reagent at 0° until an orange–brown colour persisted [15]. MeOH was then added and the reaction mixt. was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with aq. $NaHCO_3$, dried over dry $MgSO_4$ and evapd to dryness. After CC 380 mg of *ent*-3-oxo-13-*epi*-manoyl oxide (ribenone, **3**, 77%) was isolated [13].

Oxidation of varodiol (**2**). Varodiol (**2**) (800 mg) was oxidized as indicated for ribenol (**1**) to give 720 mg of *ent*-3,12-dioxo-13-*epi*-manoyl oxide (**4**, 91%) [14].

Reduction of product (**4**). Product (**4**) (500 mg) was dissolved in EtOH (10 ml) and $NaBH_4$ (30 mg) was added. The reaction was maintained for 15 min at room temp., after which the reaction mixt. was treated with aq. HCl, extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concd *in vacuo* to give 430 mg of *ent*-3β,12β-dihydroxy-13-*epi*-manoyl oxide (**5**, 85%) [14].

Organism, media and culture conditions. Rhizopus nigricans (2072), *Cunninghamella elegans* (2123) and *Fusarium moniliforme* (2152) were obtained from the Colección Española de Cultivos Tipo (CECT), Departamento de Micobiología, Universidad de Valencia, Spain. Medium YEPGA containing 1% yeast ext., 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 ext., 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 dense suspension of corresponding microorganism. Incubations were maintained 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 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 at 40° *in vacuo*. The mixt. of compounds was chromatographed on silica gel.

Biotransformation of ribenol (**1**) *with* Fusarium moniliforme. Substrate **1** (400 mg) was dissolved in 5 ml of EtOH and distributed among 5 conical flask cultures of *Fusarium moniliforme*, and incubated for 14 days. After CC 250 mg of substrate **1** (62%), 18 mg of *ent*-7β,11α-dihydroxy-3-oxo-13-*epi*-manoyl oxide (**6**, 4%), 22 mg of *ent*-7β,12α-dihydroxy-3-oxo-13-*epi*-manoyl oxide (**7**, 5%), 30 mg of *ent*-3β,7β,12α-trihydroxy-13-*epi*-manoyl oxide (**8**, 7%) and a polar mixt. of compounds (40 mg) were isolated. Acetylation of this polar mixt. (pyridine–Ac_2O, 2:1 at room temp. for 24 hr) gave 25 mg of *ent*-3β,7β,12α-triacetoxy-13-*epi*-manoyl oxide (**9**, 4% with respect of substrate **1**) and 6 mg of *ent*-3β,7β,11α-triacetoxy-13-*epi*-manoyl oxide (**10**, 1% with respect of substrate **1**).

ent-7β,11α-*Dihydroxy*-3-*oxo*-13-epi-*manoyl oxide* (**6**). Gum; $[\alpha]_D$ − 35° ($CHCl_3$, *c* 1); IR ν_{max} cm^{-1}: 3436, 3080, 1698, 1638, 1121, 917; ^1H NMR: δ5.95 (1H, *dd*, *J* = 17.7, 10.9 Hz, H-14); 5.07 (1H, *d*, *J* = 17.7 Hz) and 4.96 (1H, *d*, *J* = 10.9 Hz) (2H-15); 4.23 (1H, *ddd*, *J* = 9.9, 8.9, 4.5 Hz, H-11); 3.57 (1H, *dd*, *J* = 11.7, 4.1 Hz, H-7); 1.22, 1.21, 1.10, 1.04 and 0.99 (3H each, *s*); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 337 (100), 319 (50), 301 (49), 283 (16).

ent-7β,12α-*Dihydroxy*-3-*oxo*-13-epi-*manoyl oxide* (**7**). Mp 171–173°; $[\alpha]_D$ − 57° ($CHCl_3$; *c* 1); IR, ν_{max} cm^{-1}: 3437, 3080, 1698, 1638, 1083, 985, 916; ^1H NMR: δ6.52 (1H, *dd*, *J* = 18.2, 11.4 Hz, H-14); 4.99 (1H, *d*, *J* = 11.4 Hz) and 4.95 (1H, *d*, *J* = 18.2 Hz) (2H-15); 4.07 (1H, *dd*, *J* = 3.2, 3.2, H-12); 3.61 (1H, *dd*, *J* = 11.5, 4.0 Hz, H-7); 2.48 (1H, *d*, *J* = 8.6 Hz); 2.45 (1H, *d*, *J* = 8.6 Hz, 2H-2); 1.22, 1.17, 1.07, 0.99 and 0.81 (3H each *s*); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 337 (44), 319 (100), 301 (38).

ent-3β,7β,12α-*Trihydroxy*-13-epi-*manoyl oxide* (**8**). Mp 152–153°; $[\alpha]_D$ − 32° (EtOH; *c* 1); IR, ν_{max} cm^{-1}: 3413, 3079, 1080, 982; ^1H NMR: δ6.02 (1H, *dd*, *J* = 18.1, 11.4 Hz, H-14); 4.98 (1H, *d*, *J* = 11.4 Hz) and 4.95 (1H, *d*, *J* = 18.1 Hz) (2H-15); 4.06 (1H, *dd*, *J* = 3.5, 3.5 Hz, H-12); 3.57 (1H, *dd*, *J* = 11.5, 4.6 Hz, H-7); 3.21 (1H, *dd*, *J* = 11.2, 4.9 Hz, H-3); 1.2, 1.17, 0.98, 0.76, 0.75 (3H each, *s*); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 339 (2), 321 (100), 303 (44), 285 (18).

ent-3β,7β,12α-*Triacetoxy*-13-epi-*manoyl oxide* (**9**). Mp 110–113°; $[\alpha]_D$ − 68° ($CHCl_3$; *c* 1); IR, ν_{max} cm^{-1}: 3078, 1736, 1243, 1028; ^1H NMR: δ5.95 (1H, *dd*, *J* = 18.2, 11.4 Hz, H-14); 5.28 (1H, *dd*, *J* = 3.0, 3.0 Hz, H-12); 5.01 (1H, *d*, *J* = 18.2 Hz) and 4.97 (1H, *d*, *J* = 11.4 Hz) (2H-15); 4.81 (1H, *dd*, *J* = 11.4, 4.8 Hz, H-7); 4.45 (1H, *dd*, *J* = 11.5, 4.7 Hz, H-3); 2.11, 2.06 and 2.03 (3H each, *s*, AcO); 1.25 (3H, *s*, 3H-17), 1.01 (3H, *s*, 3H-16), 0.85 (3H, *s*, 3H-18), 0.81 (3H, *s*, 3H-20) and 0.75 (3H, *s*, 3H-19); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 465 (12.5), 405 (95), 345 (100), 285 (28).

ent-3β,7β,11α-*Triacetoxy*-13-epi-*manoyl oxide* (**10**). Gum; IR, ν_{max} cm^{-1}: 3090, 1734, 1241, 1029; ^1H NMR: δ5.81 (1H, *dd*, *J* = 17.4, 10.8 Hz, H-14); 5.25 (1H, *ddd*, *J* = 9.0, 5.5 4.1 Hz, H-11); 5.15 (1H, *dd*, *J* = 17.4, 1.4 Hz) and 4.88 (1H, *dd*, *J* = 10.8, 1.4 Hz) (2H-15); 4.80 (1H, *dd*, *J* = 11.6, 4.7 Hz, H-7); 4.45 (1H, *dd*, *J* = 11.5, 4.8 Hz, H-3); 2.33 (1H, *dd*, *J* = 15, 5.5 Hz) and 1.65 (1H, *dd*, *J* = 15, 4.1 Hz) (2H-12); 2.06, 2.03 and 2.02 (3H each, *s*, AcO); 1.72 (1H, *d*, *J* = 9.0 Hz, H-9); 1.26, 1.21, 0.91, 0.88 and 0.83 (3H each, *s*); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 465 (33), 405 (64), 345 (82), 285 (100).

Acetylation of metabolite **8**. Metabolite **8** (15 mg) was acetylated with Ac_2O–pyridine (0.5:1) for 24 hr at room temp. to give 11 mg of *ent*-3β,7β,12α-triacetoxy-13-*epi*-manoyl oxide (**9**, 53%).

Biotransformation of **3** *with* Fusarium moniliforme. Substrate **3** (40 mg) was dissolved in EtOH (1 ml) and incubated for 8 days to give a mixt. of metabolites 29 mg). After CC substrate **3** (20 mg, 50%), *ent*-1β-hydroxy-3-oxo-13-*epi*-manoyl oxide (**11**, 2 mg, 5%) and *ent*-3-oxo-8α, 13(*R*)epoxylabda-1,14-diene (**12**, 4 mg, 10%) were isolated [9].

ent-1β-*Hydroxy*-3-*oxo*-13-epi-*manoyl oxide* (**11**). Gum; ^1H NMR: δ5.99 (1H, *dd*, $J_{AX} + J_{BX}$ = 28 Hz, H-14), 5.1–4.9 (2H, 2H-15); 3.95 (1H, *dd*, *J* = 6.7, 3.7 Hz, H-1); 2.97 (1H, *dd*, *J* = 12.7, 6.7 Hz, H_{ax} − 2); 2.31 (1H, *dd*, *J* = 12.7, 3.7 Hz, H_{eq} − 2); 1.25, 1.13, 1.06, 1.02 and 0.77 (3H each, *s*).

Biotransformation of **4** *with* Fusarium moniliforme. Substrate **4** (400 mg) was dissolved in EtOH (5 ml), distributed among 5 conical flask cultures and incubated for 7 days to give a mixt. of metabolites (390 mg). After CC substrate **4** (253 mg, 63%), *ent*-11α-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide (**14**, 30 mg, 7%), *ent*-7β,11α-dihydroxy-3,12-dioxo-13-*epi*-manoyl oxide (**15**, 40 mg, 9%), *ent*-1β-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide (**16**, 38 mg, 9%) and a polar mixt. were isolated. Acetylation of this polar mixt. (pyridine–Ac_2O, 2:1 at room temp. for 24 hr) gave 6 mg *ent*-6α,11α-diacetoxy-3,12-dioxo-13-*epi*-manoyl oxide (**17**, 1% with respect to substrate **4**) and 5 mg *ent*-6α,12β-diacetoxy-3,11-dioxo-13-*epi*-manoyl oxide (**18**, 1% with respect to substrate **4**).

ent-11α-*Hydroxy*-3,12-*dioxo*-13-epi-*manoyl oxide* (**14**). Mp 107–109°; $[\alpha]_D$ − 73° ($CHCl_3$; *c* 1); IR, ν_{max} cm^{-1}: 3466, 3085, 1705, 1123, 922; ^1H NMR: δ6.04 (1H, *dd*, *J* = 17.9, 10.9 Hz, H-14), 5.05 (1H, *d*, *J* = 10.9 Hz) and 4.86 (1H, *d*, *J* = 17.9 Hz) (2H-15); 4.54 (1H, *d*, *J* = 12.8 Hz, H-11); 1.75 (1H, *d*, *J* = 12.8 Hz, H-9); 1.45, 1.39, 1.11, 1.06 and 1.05 (3H each, *s*); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 335 (100), 317 (32), 265 (89).

ent-7β,11α-*Dihydroxy*-3,12-*dioxo*-13-epi-*manoyl oxide* (**15**). Mp 124–126°; $[\alpha]_D$ − 89° ($CHCl_3$; *c* 1); IR,

ν_{max} cm^{-1}: 3465, 3079, 1706, 1049, 1010, 972, 929; ^1H NMR: δ6.02 (1H, *dd*, *J* = 17.8, 10.9 Hz, H-14), 5.07 (1H, *d*, *J* = 10.9 Hz) and 4.87 (1H, *d*, *J* = 17.9 Hz) (2H-15); 4.63 (1H, *d*, *J* = 12.7 Hz, H-11); 3.64 (1H, *dd*, *J* = 11.7, 4.0 Hz, H-7); 1.71 (1H, *d*, *J* = 12.7 Hz, H-9); 1.41 (3H, *s*, 3H-17), 1.36 (3H, *s*, 3H-16), 1.09 (3H, *s*, 3H-18), 1.05 (3H, *s*, 3H-19) and 1.03 (3H, *s*, 3H-20); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 351 (100), 333 (58), 315 (23).

ent-1β-*Hydroxy*-3,12-*dioxo*-13-epi-*manoyl oxide* (**16**). Gum; $[\alpha]_D$ − 86° ($CHCl_3$; *c* 1); IR, ν_{max} cm^{-1}: 3466, 3080, 1706, 1087, 1000, 925; ^1H NMR: δ6.14 (1H, *dd*, *J* = 17.5, 10.8 Hz, H-14), 5.19 (1H, *dd*, *J* = 17.5, 1.2 Hz) and 5.07 (1H, *dd*, *J* = 10.8, 1.2 Hz) (2H-15); 3.86 (1H, *dd*, *J* = 7.9, 6.9 Hz, H-1); 3.09 (1H, *dd*, *J* = 18.9, 5.9 Hz) and 2.59 (1H, *dd*, *J* = 18.9, 13.0 Hz) (2H-11); 2.78 (1H, *dd*, *J* = 15.3, 6.9 Hz) and 2.50 (1H, *dd*, *J* = 15.3, 7.9 Hz) (2H-2); 2.11 (1H, *dd*, *J* = 13.0, 5.9 Hz, H-9); 1.32 (3H, *s*, 3H-16), 1.2 (3H, *s*, 3H-17), 1.1 (3H, *s*, 3H-18), 1.05 (3H, *s*, 3H-19) and 0.92 (3H, *s*, 3H-20); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 335 (14), 317 (100), 299 (43).

ent-6α,11α-*Diacetoxy*-3,12-*dioxo*-13-epi-*manoyl oxide* (**17**). Mp 140–142°; IR ν_{max} cm^{-1}: 1735, 1230, 1027; ^1H NMR: δ5.98 (1H, *dd*, *J* = 17.9, 10.9 Hz, H-14); 5.59 (1H, *d*, *J* = 13.2 Hz, H-11); 5.08 (1H, *d*, *J* = 10.9 Hz) and 5.04 (1H, *d*, *J* = 17.9 Hz) (2H-15); 4.94 (1H, *ddd*, *J* = 11.5, 11.5, 4.0 Hz, H-6); 2.17 and 2.07 (3H each, *s*, AcO); 1.53, 1.35, 1.25, 1.07 and 0.82 (3H each, *s*); CI-MS *m/z* (rel. int.): $[M + 1]^+$ 435 (14), 375 (31), 315 (100).

ent-6α,12β-*Diacetoxy*-3,11-*dioxo*-13-epi-*manoyl oxide* (**18**). Gum; IR ν_{max} cm^{-1}: 1737, 1708, 1227, 1025; ^1H NMR: δ5.89 (1H, *dd*, *J* = 17.8, 11.2 Hz, H-14); 5.38 (1H, *s*, H-12); 5.25 (1H, *d*, *J* = 17.8 Hz) and 5.19 (1H, *d*, *J* = 11.2 Hz) (2H-15); 4.91 (1H, *ddd*, *J* = 11.3, 11.3, 4.4 Hz, H-6); 2.88 (1H, *s*, H-9); 2.19 and 2.06 (3H each, *s*, AcO); 1.48, 1.25, 1.24, 1.06 and 1.01 (3H each, s); CI-MS *m/z* (rel. int.): $[M + 1]^+$ 435 (33), 375 (100), 315 (22).

Reduction of metabolite **14**. Metabolite **14** (15 mg) was dissolved in EtOH (2 ml), and $NaBH_4$ (5 mg) was added. The reaction mixt. was maintained for 1 hr at room temp. After CC 12 mg of *ent*-3β,11α,12β-trihydroxy-13-*epi*-manoyl oxide (**19**, 80%) was obtained as a gum: IR, ν_{max} cm^{-1}: 3384, 1122, 1039; ^1H NMR: δ6.25 (1H, *dd*, *J* = 17.8, 11.3 Hz, H-14), 5.44 (1H, *dd*, *J* = 17.8, 1.3 Hz) and 5.22 (1H, *dd*, *J* = 11.3, 1.3 Hz) (2H-15); 4.01 (1H, *dd*, *J* = 10.8, 8.9 Hz, H-11); 3.32 (1H, *d*, *J* = 8.9 Hz, H-12); 3.22 (1H, *dd*, *J* = 10.5, 5.9 Hz, H-3); 2.48 (1H, *ddd*, *J* = 13.6, 6.8, 3.4 Hz, H-2 axial); 1.37, 1.30, 0.98, 0.97 and 0.77 (3H each, *s*); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 339 (7), 321 (70), 303 (100), 285 (31).

Reaction of metabolite **16**. Metabolite **16** (10 mg) was dissolved in EtOH (2 ml) and $NaBH_4$ (2 mg) was added. The reaction was maintained for 1 hr at room temp. After CC 8 mg of *ent*-1β,3β,12β-trihydroxy-13-*epi*-manoyl oxide (**20**, 79%) was obtained as a solid: mp 160–163°; IR ν_{max} cm^{-1}: 3297, 1075, 1041, 994; ^1H NMR: δ6.26 (1H, *dd*, *J* = 17.9, 11.3 Hz, H-14), 5.34 (1H, *dd*, *J* = 17.9, 1.7 Hz) and 5.08 (1H, *dd*, *J* = 11.3, 1.7 Hz) (2H-15); 3.46 (1H, *dd*, *J* = 11.4, 4.6 Hz, H-12); 3.43 (1H, *dd*, *J* = 11.2, 4.6 Hz, H-3); 3.18 (1H, *dd*, *J* = 12.0, 4.5 Hz, H-1); 1.18, 1.16, 0.88, 0.78 and 0.67 (3H each, *s*); ^{13}C NMR: Table 1; CI-MS *m/z* (rel. int.): $[M + 1]^+$ 339 (3), 321 (44), 303 (100), 285 (53).

Biotransformation of **4** *with* Cunninghamella elegans. Substrate **4** (50 mg) was dissolved in 1 ml of EtOH and incubated for 6 days to obtain 48 mg of a mixt. of compounds. After CC, **4** (17 mg, 32%) and a polar mixt. were isolated. Acetylation of this polar mixt. (pyridine–Ac_2O, 2:1, for 28 hr at room temp.) gave 16 mg of 14(*R*)-*ent*-14,15-diacetoxy-8α,13(*S*)-epoxylabda-3,12-dione (**21**, 11%) [9].

Biotransformation of **5** *with* Rhizopus nigricans. Substrate **5** (200 mg) dissolved in 15 ml of EtOH and distributed among 10 conical flasks for 9 days. After CC, 62 mg of **5** (31%), 10 mg of *ent*-3β,7α,12β-trihydroxy-13-*epi*-manoyl oxide (**22**, 5%) and 45 mg of a polar mixt. were isolated. Acetylation of this polar mixt. (pyridine–Ac_2O 2:1 for 24 hr at room temp.) gave 10 mg of *ent*-3β,12β-diacetoxy-7α-hydroxy-13-*epi*-manoyl oxide (**23**, 4% with respect to **5**).

ent-3β,7α,12β-*Trihydroxy*-13-epi-*manoyl oxide* (**22**). Mp 145–147°; $[\alpha]_D$ − 6° ($CHCl_3$; *c* 0.5) IR ν_{max} cm^{-1}: 3395, 3080, 1069, 1036; ^1H NMR: δ6.26 (1H, *dd*, *J* = 17.7 Hz, 11.2 Hz, H-14), 5.43 (1H, *dd*, *J* = 17.7, 1.3 Hz) and 5.27 (1H, *dd*, *J* = 11.2, 1.3 Hz) (2H-15); 3.63 (1H, *dd*, *J* = 2.7, 2.7 Hz, H-7); 3.49 (1H, *dd*, *J* = 10.9, 4.7 Hz, H-12); 3.26 (1H, *dd*, *J* = 10.9, 4.8 Hz, H-3); 1.37, 1.24, 0.97, 0.78 and 0.75 (3H each, *s*); ^{13}C NMR: Table 1; CIMS *m/z* (rel. int.): $[M + 1]^+$ 339 (2), 321 (73), 303 (100), 285 (16).

ent-3β,12α-*Diacetoxy*-7α-*hydroxy*-13-epi-*manoyl oxide* (**23**). Mp 128–130°; $[\alpha]_D$ − 21° ($CHCl_3$; *c* 0.5); IR, ν_{max} cm^{-1}: 3350, 1736, 1239, 1035; ^1H NMR: δ6.19 (1H, *dd*, *J* = 17.8, 11.3 Hz, H-14), 5.43 (1H, *dd*, *J* = 17.8, 1.2 Hz) and 5.18 (1H, *dd*, *J* = 11.3, 1.2 Hz) (2H-15); 4.75(1H, *dd*, *J* = 11.2, 4.7 Hz, H-12); 4.51 (1H, *dd*, *J* = 11.4, 4.8, H-3); 3.63 (1H, *dd*, *J* = 1.7, 1.7 Hz, H-7); 2.03 and 2.02 (3H each, *s*, AcO); 1.26, 1.22, 0.85, 0.81 and 0.78 (3H each, *s*); CI-MS *m/z* (rel. int.): $[M + 1]^+$ 423 (9), 405 (38), 363 (78), 345 (100), 303 (13), 285 (16).

Oxidation of metabolite **8**. Metabolite **8** (5 mg) was dissolved in Me_2CO and was treated with Jones' reagent at 0° until an orange–brown colour persisted. The reaction was stirred for 1.5 hr. MeOH was then added and the reaction mixt. was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with aq. $NaHCO_3$, dried over $MgSO_4$ and evapd to dryness. After CC, 4 mg of *ent*-3,7,12-trioxo-13-*epi*-manoyl oxide (**24**, 81%) was isolated: mp 165–168°; $[\alpha]_D$ + 0.8° ($CHCl_3$; *c* 0.5); IR ν_{max} cm^{-1}: 2924, 1705, 1082; ^1H NMR: δ6.11 (1H, *dd*, *J*: 17.3, 10.8 Hz, H-14), 5.20 (1H, *d*, *J* = 17.3 Hz) and 5.09 (1H, *d*, *J* = 10.8 Hz) (2H-15); 1.45, 1.41. 1.17, 1.10 and 1.07 (3H each, *s*); ^{13}C NMR: Table 1.

Oxidation of **22**. Metabolite **22** (3 mg) was dissolved in Me_2CO and oxidized under the same conditions described above for **8**. After CC, 2.9 mg of *ent*-3,7,12-trioxo-13-*epi*-manoyl oxide (**24**, 98%) was isolated.

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