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Biotransformations of *ent*-18-acetoxy-6-ketomanoyl oxides epimers at C-13 with filamentous fungi

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Abstract

Two *ent*-18-acetoxy-6-oxomanoyl oxides, epimers at C-13, have been prepared from *ent*-6α,8α,18-trihydroxylabda-13(16),14-diene (andalusol), isolated from *Sideritis foetens*, by means of several chemical pathways and a regioselective acylation with *Candida cylindracea* lipase (CCL). Biotransformation of these 13-epimeric *ent*-manoyl oxides by *Fusarium moniliforme* and *Neurospora crassa* produced mainly *ent*-1β- or *ent*-11α-hydroxylations, as well as their deacetylated derivatives, in both epimers. In addition, with the 13-*epi* substrate *N. crassa* originated other minor hydroxylations by the *ent*-α face at C-1 or at C-12, whereas an *ent*-11β-hydroxyl group, probably originated by reduction of an 11-oxo derivative also isolated, was achieved with the 13-*normal* substrate.

Keywords: *Fusarium moniliforme*; *Neurospora crassa*; Biotransformation; *ent*-Manoyl oxides; Diterpenoids; Filamentous fungi; Biohydroxylation

1. Introduction

Regio- and stereoselective hydroxylation of non-activated carbon atoms is a very useful methodology in organic chemistry (Azerad, 2001; Holland, 1999; Li and Chang, 2004), and, as these processes are difficult to carry out by chemical means, whole-cells fermentation is the procedure most often employed in such fungal hydroxylation (Lehman and Stewart, 2001; Ishige et al., 2005). The main problem for the biohydroxylation of a certain substrate at a specific position is to find the appropriate microorganism, therefore customarily; one of the most widely used techniques is screening with several fungal strains. In this context, the microbial transformation of *ent*-manoyl oxides – labdane-type diterpenoids – by filamentous fungi constitutes one line of our research. We are currently exploring an extensive series of chemical-microbiological pathways to semi-synthesise diversely functionalized *ent*-manoyl oxides, with both configurations at C-13. These biotransformation processes are used to introduce hydroxyl groups, at positions difficult to achieve by classical chemical methods, onto substrates. The main interest of biotransformation of manoyl oxides is to produce new poly-functionalized compounds, due to the wide variety of biological properties described for these compounds, including, anti-inflammatory (Alcaraz et al., 1989), anti-hypertensive (Tandon et al., 1992), anti-leishmanial (García-Granados et al., 1997a), antibacterial (Demetzos et al., 1998), enzyme stimulation (García-Granados et al., 1994a,b, 1995a), cytotoxic (Chaichantipyuth et al., 2005; Demetzos et al., 1994; Dimas et al., 1999, 2001; Konishi et al., 1998), phytotoxic (Rivero-Cruz et al., 2000) and insecticidal (Ybarra et al., 2005).

In previous papers, we have reported the incubations of several *ent*-manoyl oxides epimers at C-13, with functions at C-6, C-16 and C-18 or C-6 and C-16, with the filamentous fungi *Rhizopus nigricans* and *Curvularia lunata* yielding

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new poly-oxygenated *ent*-manoyl oxides (Arias et al., 1988; García-Granados et al., 1990, 1995a,b, 1997b). In the present work, the fungi *Fusarium moniliforme* (CECT 2152) – a synonym of *Fusarium verticillioides* (EAN 337) and the anamorphic form of *Gibberella fujikuroi* (ATCC 12616) – and *Neurospora crassa* (CECT 2261, ATCC 10336) are used to complete the earlier biotransformation studies and obtain new highly oxygenated *ent*-manoyl oxides analogues of *ent*-forskolin.

2. Results and discussion

The phytochemical study of *Sideritis foetens* Clem. ex Lag. revealed an abundant diterpenic content with structures of *ent*-labda-13(16),14-diene (García-Alvarez and Rodríguez, 1980; García-Granados et al., 1994a). In an earlier paper, a method was described for the in vitro micropropagation of this plant (García-Granados et al., 1994a). These *ent*-labdadienic diterpenoids can be transformed into manoyl oxides with or without a 16-hydroxyl group (Amate et al., 1991; Arias et al., 1988; García-Granados et al., 1990, 1997b). The starting material used in this work was andalusol (**1**). Acetylation of this compound yielded 6,18-diacetylandalusol (**2**) (López et al., 1977). Treatment of this diacetate (**2**) with $TiCl_4$ produced *ent*-manoyl oxides, epimers at C-13, without functionalization at C-16 (**3** and **4**). Chemical deacetylation of **3** and **4** gave diols **5** and **6**, respectively. Regioselective acetylation of these diols (**5** and **6**) with *Candida cylindracea* lipase (CCL) rendered monoacetyl derivatives at C-18 (**7** and **8**, respectively) in high yield. This enzymatic acetylation improved the results of chemical acetylation, so that acetylation with Ac_2O/Pyridine of the epimeric diols (**5** and **6**) yielded diacetates **3** and **4**, monoacetates **7** and **8**, and the acetyl derivatives at C-6 (**9** and **10**), respectively. The individual oxidation of monoacetates **7** and **8** with Jones' reagent produced the corresponding 6-oxo derivatives (**11** and **12**), which were used as substrates in the biotransformation processes. The overall yields of these compounds (**11**, 80%; **12**, 80%) from diols **5** and **6** were considerably higher than those obtained using chemical acetylation (38% and 34%, respectively). These yields were also superior to those achieved in another chemical procedure (**11**, 65%; **12**, 67%), based on the oxidation of diols **5** and **6** at C-6 with pyridinium dichromate (PDC), and subsequent chemical acetylation (García-Granados et al., 1997b).

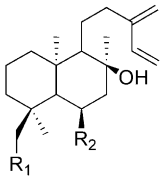
Biotransformation of substrate **11** with *F. moniliforme* produced the deacetylated metabolites **13** (17%), **14** (16%) and **15** (6%). The first metabolite (**13**) was the result of the sole deacetylation of substrate **11**. The molecular formula of metabolite **14** ($C_{20}H_{32}O_4$) suggested the presence of an additional hydroxyl group in the molecule. This hydroxyl group was in an equatorial arrangement, pointed out by the signal of the geminal axial proton (δ 3.61, *dd*, $J = 10.0$, 5.3 Hz), in its ^{1}H NMR spectrum. The position of this hydroxyl group was determined by comparing the ^{13}C NMR spectra of **13** and **14**, the δ-effect on C-11 ($\Delta\delta = +2.5$) being significant due to the spatial proximity of this carbon atom and the equatorial hydroxyl group at C-1, and confirmed by HMBC experiments (cross-peak signals between H-2, H-5, H-9, 3H-20, and C-1). Therefore, metabolite **14** was *ent*-1β,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide. Metabolite **15** had the same molecular formula ($C_{20}H_{32}O_4$) of **14** and its ^{1}H NMR spectrum showed a signal at δ 4.21 (*ddd*, $J = 9.5$, 7.7, 4.8 Hz) due to a geminal proton to a hydroxyl group, that could only be situated at C-11 in this molecule. The arrangement of this hydroxyl group was deduced from the chemical shift and the coupling constants of H-11, which were comparable to those observed for other *ent*-13-*epi*-manoyl oxide derivatives containing an *ent*-11α-hydroyl group (Konishi et al., 1996; Fraga et al., 1999). The position and the stereochemistry of this hydroxylation were confirmed by the analysis of the ^{13}C NMR data of **13** and **15** (δ-effect at C-1 of +2.1), and by HMBC experiments (correlations of C-11 with H-9 and H-12, and of H-11 with C-9, C-10 and C-13). Consequently, metabolite **15** was *ent*-11α,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide.

The biotransformation of substrate **11** with *N. crassa* gave the same metabolites (**13**, 18%; **14**, 22%; and **15**, 9%), previously isolated from the biotransformation of this substrate (**11**) by *F. moniliforme*, together with **16** (3%), **17** (10%), **18** (2%), and **19** (2%). Spectral data of **16** and **17** revealed that these metabolites were the 18-acetyl derivatives of **14** and **15**, respectively, as a consequence of the direct biohydroxylation at C-1 (*ent*-β) or C-11 (*ent*-α) of substrate (**11**). Metabolites **18** and **19** were again 18-deacetylated compounds. In the ^{1}H NMR spectrum of **18** appeared a signal at 3.70 ppm as a broad singlet, similar to that observed for an axial hydroxyl group at C-1 (Fraga et al., 1998a). This *ent*-1α-hydroxylation was confirmed from the analysis of the ^{13}C NMR of **13** and **18**, particularly by the strong γ-gauche effects on C-3 ($\Delta\delta = -6.8$), C-5 ($\Delta\delta = -6.56$) and C-9 ($\Delta\delta = -8.55$), suggesting that the hydroxyl group adopted an axial disposition at C-1. Thus, metabolite **18** was *ent*-1α,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide. In the ^{1}H NMR spectrum of **19**, the equatorial geminal proton to a hydroxyl group appeared at δ 4.14 (*dd*, $J = 3.3$, 3.3 Hz), this hydroxyl group being located at C-3 or C-12. The ^{13}C NMR strong γ-gauche effects on C-9 ($\Delta\delta = -8.75$) and C-16 ($\Delta\delta = -5.6$) positioned, in an axial arrangement, the hydroxyl group at C-12. Therefore, **19** had a structure of *ent*-12α,18-dihydroxy-6-oxo-13-*epi*-manoyl oxide.

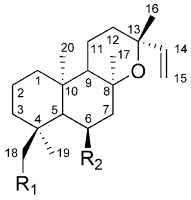
Biotransformation of substrate **12** – the 13-epimer compound of **11** – with *F. moniliforme* produced metabolites **20** (21%), **21** (10%), and **22** (9%), in which the first action of this fungus was again the deacetylation of the substrate (**12**). In this way, metabolite **20** was the 18-deacetyl derivative of **12**, whereas the molecular formula of **21** and **22** ($C_{20}H_{32}O_4$) indicated that this microorganism had introduced an additional hydroxyl group into the molecule, respectively. In the ^{1}H NMR spectrum of **21**, the signal

of a geminal proton to a hydroxyl group appeared partially overlapped with that of the hydroxymethylene group at C-18. For this reason, acetylation of **21** was carried out, giving diacetate **23**, in which the signal of the ^{1}H NMR spectrum at δ 4.70 (*dd*, $J = 10.6$, 4.9 Hz) was clearly distinguished. These coupling constants were compatible with an equatorial oxygenated function located at C-1, C-3 or C-12, the position of which was determined by the ^{13}C NMR and HMBC data of **21**, similar to those of **14**, indicating that this metabolite (**21**) had a structure of *ent*-1β,18-dihydroxy-6-oxo-manoyl oxide. The multiplicity of the signal at δ 4.01 (*ddd*) – present in the ^{1}H NMR spectrum of **22** – pointed out that the additional hydroxyl group should be located only at C-11. This chemical shift (δ 4.02) suggested an *ent*-11α- arrangement of this hydroxyl group, since the 11-epimer derivative (**24**) (García-Granados et al., 1997b), with an *ent*-11β-hydroxyl group, showed a signal at δ 4.59. In addition, the *ent*-11β-hydroxylation of **24** deshielded the signals of the hydrogen atoms on C-16, C-17 and C-20, due to the spatial proximity of these methyl groups, which did not happen in **22**. In conclusion, the structure of **22** was *ent*-11α,18-dihydroxy-6-oxo-manoyl oxide. In this metabolite (**22**), the coupling constants of H-11 with H-9 ($J = 7.8$ Hz) and 2H-12 ($J = 6.0$, 1.4 Hz) were not typical for an axial arrangement of this hydrogen atom. This was attributable to a "twist-boat" conformation of the C ring of the molecule, as has been described for other *ent*-11α-manoyl oxides (Fraga et al., 1998a; Zhou et al., 1995), instead of the usual "chair" conformation.

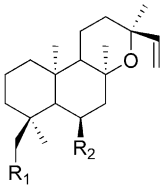
Biotransformation of substrate **12** by *N. crassa* produced the same metabolites **20** (17%), **21** (9%), and **22** (27%), previously isolated from that of substrate **12** with *F. moniliforme*, as well as the *ent*-11β-hydroxy derivatives **24** (12%) and **25** (2%), achieved from the incubation of **12** with *R. nigricans* (García-Granados et al., 1997b). Furthermore, two new metabolites **26** (6%) and **27** (3%), were isolated. The spectral data of **26** showed that this compound was the 18-acetyl derivative of **22**, and had a structure of *ent*-18-acetoxy-11α-hydroxy-6-oxo-manoyl oxide. Metabolites **25** and **26** are epimers at C-11, which was confirmed by oxidation of both compounds to give the same 11-oxo derivative **28**. Metabolite **27** showed similar spectral data to those of **28**, revealing the presence of an 11-oxo group in the molecule. This was confirmed by a chemical correlation (acetylation) with **28**, and consequently **27** was *ent*-18-hydroxy-6,11-dioxo-manoyl oxide.



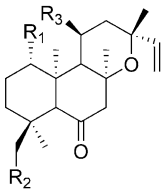
1: $R_1 = R_2 = OH$
2: $R_1 = R_2 = OAc$



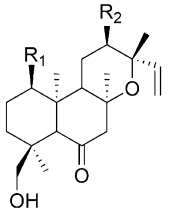
3: $R_1 = R_2 = OAc$
5: $R_1 = R_2 = OH$
7: $R_1 = OAc$, $R_2 = OH$
9: $R_1 = OH$, $R_2 = OAc$



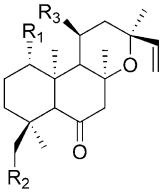
4: $R_1 = R_2 = OAc$
6: $R_1 = R_2 = OH$
8: $R_1 = OAc$, $R_2 = OH$
10: $R_1 = OH$, $R_2 = OAc$



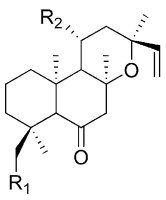
11: $R_1 = H$, $R_2 = OAc$, $R_3 = H$
13: $R_1 = H$, $R_2 = OH$, $R_3 = H$
14: $R_1 = R_2 = OH$, $R_3 = H$
15: $R_1 = H$, $R_2 = R_3 = OH$
16: $R_1 = OH$, $R_2 = OAc$, $R_3 = H$
17: $R_1 = H$, $R_2 = OAc$, $R_3 = OH$



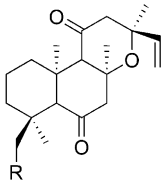
18: $R_1 = OH$, $R_2 = H$
19: $R_1 = H$, $R_2 = OH$



12: $R_1 = H$, $R_2 = OAc$, $R_3 = H$
20: $R_1 = H$, $R_2 = OH$, $R_3 = H$
21: $R_1 = R_2 = OH$, $R_3 = H$
22: $R_1 = H$, $R_2 = R_3 = OH$
23: $R_1 = R_2 = OAc$, $R_3 = H$
26: $R_1 = H$, $R_2 = OAc$, $R_3 = OH$



24: $R_1 = R_2 = OH$
25: $R_1 = OAc$, $R_2 = OH$



27: R = OH
28: R = OAc

3. Conclusions

(a) Regioselective acetylation of 6,18-dihydroxymanoyl oxides with CCL and vinyl acetate produces 18-acetyl derivatives in high yield, avoiding the formation of mixtures of mono- and diacetylated compounds.

(b) The action of *F. moniliforme* on epimers **11** and **12** was the same, regardless of the configuration at C-13, rendering the deacetylation of substrates and subsequent hydroxylation at C-1, by *ent*-β face (16% and 10%, respectively), or at C-11, by the other face of the molecule (6% and 9%, respectively). This parallel action contrasts with that of *R. nigricans*, which introduces an *ent*-3β-hydroxyl group and an epoxy group at the double bond (21%) in **11**, but an *ent*-11β-hydroxyl group (56%) in **12**.

(c) The main action of *N. crassa* and *F. moniliforme* with the epimeric compounds **11** and **12** is similar, yielding the same functionalizations (*ent*-1β- or *ent*-11α-hydroxyl groups), independently of the configuration at C-13 of substrates. However, *N. crassa* also produced other metabolites, giving, from **11**, hydroxylations at C-1 or at C-12, both by the *ent*-α face; while from **12**, *ent*-11β-hydroxy and 11-oxo derivatives were derived.

(d) *F. moniliforme* and *R. nigricans* give hydroxylations at C-11 with different stereoselectivity. The biohydroxylation by *F. moniliforme* takes place by the *ent*-α face, while with *R. nigricans* occurs by the *ent*-β face. *N. crassa* is less stereoselective, yielding both isomers, together with an 11-oxo derivative.

(e) The major biohydroxylations at C-1 or at C-11, in an equatorial arrangement, are achieved in these biotransformations with the same stereochemistry in several manoyl oxides isolated from natural sources, as *Excoecaria agallocha* (Konishi et al., 1996) and *Croton oblongifolius* (Chaichantipyuth et al., 2005). In addition, *ent*-1β-hydroxylations have been produced in biotransformations of manoyl oxides by *F. moniliforme*, *Gliocladium roseum*, *Curvularia lunata*, and *R. nigricans* (García-Granados et al., 1995a,b,c, 1999, 2004), whereas equatorial 1- or 11-hydroxylations have been achieved by *G. fujikuroi*, *M. plumbeus*, and *Cunninghamella elegans* (Fraga et al., 1989, 1998a,b, 1999, 2001; García-Granados et al., 1995b).

4. Experimental

4.1. General experimental procedures

Measurements of NMR spectra (300.13 MHz ^{1}H and 75.47 MHz ^{13}C) were made in $CDCl_3$ (which also provided the lock signal), in a Bruker AM-300 spectrometer. Assignments of ^{13}C chemical shifts (Tables 1 and 2) were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. Several programs were used for HMQC, HMBC and NOE experiments. IR spectra were recorded on a MATTSON SATELLITE FT-IR spectrometer. High-resolution mass spectra were made by LSIMS (FAB) ionization mode in a MICROMASS AUTOSPEC-Q spectrometer (EBE geometry). Mps were

Table 1
^{13}C NMR chemical shifts of *ent*-13-*epi*-manoyl oxides

C	δ_C								
	7	**9**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
1	38.9	38.8	39.5	79.3	41.6	79.2	41.6	69.9	39.2
2	17.7	17.8	17.7	29.1	17.9	29.0	17.8	25.3	17.7
3	37.2	37.9	36.1	34.2	35.9	34.3	35.9	29.31	36.1
4	37.4*	37.6*	37.0	36.7	37.2	35.4	36.0	37.1	37.1
5	55.3	51.1	61.5	59.7	60.9	59.6	60.4	54.9	61.7
6	68.4	73.7	210.6	209.9	210.2	208.4	208.6	211.4	210.3
7	54.2	49.3	60.0	59.9	60.0	59.8	59.9	60.0	59.9
8	73.6	73.6	78.1	77.6	78.3	77.5	78.4	77.9	77.1
9	58.1	57.8	58.6	59.3	63.4	59.6	63.7	50.1	49.9
10	37.1*	37.9*	40.2	45.2	42.2	45.3	42.2	43.8	39.7
11	16.2	16.1	16.9	19.0	65.6	19.0	65.6	16.4	24.4
12	34.7	34.6	34.6	34.6	44.6	34.6	44.7	34.2	68.8
13	75.3	71.1	74.4	73.9	74.4	74.0	74.5	74.4	–
14	147.4	147.2	146.7	146.6	146.9	146.6	146.9	146.8	146.4
15	109.8	109.9	110.4	110.4	110.7	110.5	110.7	110.4	110.4
16	32.6	32.5	32.5	32.5	31.8	32.5	31.9	32.5	26.9
17	25.4	25.1	24.6	24.6	26.3	24.6	26.2	24.9	24.9
18	75.0	74.7	71.7	70.7	71.8	71.2	71.9	71.8	71.8
19	17.7	18.0	17.6	17.3	17.9	17.4	17.9	17.9	17.6
20	17.5	17.2	17.3	13.1	17.7	13.1	17.6	17.5	17.3
CH_3COO	21.3	21.7				21.0	21.1		
CH_3COO	171.5	169.4				170.9	171.0		

Values bearing an asterisk may be interchanged.

determined using a Kofler (Reichert) apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 431 polarimeter at 25 °C. Silica-gel (40–60 μm) was used for flash chromatography. CH_2Cl_2 with increasing amounts of Me_2CO was used as eluent. Analytical plates were rendered visible by spraying with H_2SO_4–HOAc–H_2O, followed by heating to 120 °C.

4.2. Acetylation of *1*

ent-6α,8α,18-Trihydroxylabda-13(16),14-diene (andalusol, **1**) was isolated from *S. foetens*, collected near Alcolea (Almería) (García-Granados et al., 1994a). Andalusol **1** (5 g) was dissolved in pyridine (100 ml) and Ac_2O (50 ml). The reaction was maintained for 24 h at room temperature, after which the reaction mixture was diluted with cold H_2O, extracted with CH_2Cl_2, washed with satd. aq. $KHSO_4$ and dried over dry Na_2SO_4. The solvent was evapd. to give a mixture of compounds that was chromatographed on silica gel to give 4.5 g of *ent*-6α,18-diacetoxy-8α-hydroxylabda-13(16),14-diene (diacetylandalusol, **2**, 71%) (López et al., 1977).

4.3. Cyclization of diacetate *2* with $TiCl_4$

Diacetate **2** (4.5 g) was dissolved in dry CH_2Cl_2 (150 ml), and was cooled to −78 °C and kept under Ar gas, then, 15 ml of a solution 0.1 M of $TiCl_4$ in CH_2Cl_2 (previously cooled) were added. This mixture was stirred for 3 h and pyridine (100 ml), previously cooled, was then added. When the reaction mixture reached room temperature, it was treated with aq. $KHSO_4$ and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over dry Na_2SO_4. After CC on silica gel with 10% of $AgNO_3$, starting material (**2**, 1 g, 22%), *ent*-6α,18-diacetoxy-13-*epi*-manoyl oxide (**3**, 1.6 g, 36%) (García-Granados et al., 1997b), and *ent*-6α,18-diacetoxymanoyl oxide (**4**, 1.7 g, 38%) (García-Granados et al., 1997b), were isolated.

Table 2
^{13}C NMR chemical shifts of *ent*-manoyl oxides

C	δ_C							
	8	**10**	**20**	**21**	**22**	**26**	**27**	**28**
1	38.5	38.4	39.1	79.1	40.0	40.0	39.4	39.3
2	17.6	17.8	17.6	28.9	17.6	17.4	17.5	17.3
3	37.0	37.8	36.1	34.2	35.9	35.8	35.9	36.0
4	37.6*	37.8	37.0	36.7	37.0	36.0	37.0	35.8
5	55.3	60.0	61.4	59.6	60.9	60.8	59.8	60.0
6	68.4	71.2	210.6	210.0	209.9	208.3	208.5	207.1
7	54.3	49.5	60.2	60.0	60.1	60.1	59.4	59.3
8	74.3	73.6*	77.3	76.8	76.5	76.5	79.3	79.3
9	54.9	54.2	56.0	56.2	62.7	62.9	66.5	66.7
10	37.2*	37.8	40.3	45.2	40.8	40.8	40.1	40.0
11	15.6	15.5	16.0	18.5	65.0	64.9	205.9	205.7
12	35.2	34.7	35.5	35.2	43.4	43.5	50.3	50.4
13	73.6	73.9*	74.3	74.0	74.1	74.2	75.7	75.8
14	147.7	147.4	147.3	147.4	147.7	147.8	146.3	146.3
15	110.5	110.6	110.7	110.8	112.7	112.5	112.7	112.6
16	27.0	26.8	28.2	28.6	31.4	31.4	31.1	30.9
17	29.0	29.1	26.0	26.1	28.2	28.1	28.8	28.7
18	75.1	73.8	71.7	70.7	71.6	72.0	71.6	71.9
19	17.7	18.1	17.6	17.4	17.9	17.8	18.0	17.8
20	16.9	16.7	16.9	12.7	17.7	17.7	17.3	17.2
CH_3COO	21.3	21.8				21.0		21.0
CH$_3$*C*OO	171.6	169.4				171.0		171.0

Values bearing an asterisk may be interchanged.

4.4. Production of substrate *11* from diacetate *3*

Diacetate **3** (1.6 g) was dissolved in MeOH–H_2O (70%, 50 ml) containing KOH (5%) and refluxed for 1 h. The reaction mixture was diluted with H_2O (50 ml) and extracted with CH_2Cl_2. The organic layer was dried over dry Na_2SO_4 and evaporated in vacuum, isolating 1.2 g of *ent*-6α,18-dihydroxy-13-*epi*-manoyl oxide (**5**, 95%) (García-Granados et al., 1997b). Diol **5** (1 g) was dissolved in vinyl acetate (100 ml) and *C. cylindracea* lipase (2 g) was added. The biotransformation was carried out at 35 °C with gyratory shaking (180 rpm). After 2 h, the reaction mixture was filtered and the lipase was washed with CH_2Cl_2. This organic layer was concentrated in vacuum, and after CC, *ent*-18-acetoxy-6α-hydroxy-13-*epi*-manoyl oxide (**7**, 1.1 g, 97%) was isolated. Monoacetate **7** (1.1 g) was dissolved in acetone (25 ml) and treated with Jones' reagent at 0 °C until an orange-brown colour persisted. MeOH was then added and the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with aq. $NaHCO_3$, dried over dry Na_2SO_4 and evaporated to dryness. After CC, *ent*-18-acetoxy-6-oxo-13-*epi*-manoyl oxide (**11**, 910 mg, 83%) was isolated (García-Granados et al., 1997b).

4.5. *ent*-18-Acetoxy-6α-hydroxy-13-epi-manoyl oxide (*7*)

White solid, mp 115–8 °C; $[\alpha]_D$ − 46.8° ($CHCl_3$; *c* 1), IR ν_{max}^{KBr} cm^{-1}: 3460, 3089, 1732, 1638, 1251, 1065, 1035, 912; ^1H NMR ($CDCl_3$): δ 5.97 (1H, *dd*, *J* = 17.9, 11.1 Hz, H-14), 4.94 (1H, *d*, *J* = 17.9 Hz) and 4.89 (1H, *d*, *J* = 11.1 Hz) (2H-15), 4.46 and 3.79 (2H, AB system, *J* = 10.6 Hz, 2H-18), 3.77 (1H, *ddd*, *J* = 11.0, 11.0, 3.8 Hz, H-6), 2.05 (3H, s, AcO group), 1.25 (3H, *s*, 3H-17), 1.11 (3H, *s*, 3H-16), 0.95 and 0.77 (3H each, *s*, 3H-19 and 3H-20); HRLSIMS *m*/*z*: 387.2518 $[M + Na]^+$ (calcd for $C_{22}H_{36}O_4Na$, 387.2511).

4.6. Acetylation of diol *5*

Diol **5** (200 mg) was acetylated with Ac_2O-pyridine (2:4 ml) at 0 °C for 2 h to give diacetate **3** (46 mg, 18%), monoacetate **7** (104 mg, 46%), *ent*-6α-acetoxy-18-hydroxy-13-*epi*-manoyl oxide (**9**, 127 mg, 12%) and diol **5** (28 mg, 14%).

4.7. *ent*-6α-Acetoxy-18-hydroxy-13-epi-manoyl oxide (*9*)

White solid, mp 106–8 °C; $[\alpha]_D$ − 39.2° ($CHCl_3$; *c* 1), IR ν_{max}^{KBr} cm^{-1}: 3400, 3089, 1736, 1235 and 1027; ^1H

NMR ($CDCl_3$): δ 5.93 (1H, *dd*, $J = 17.9$, 11.0 Hz, H-14), 5.06 (1H, *ddd*, $J = 11.4$, 11.4, 4.2 Hz, H-6), 4.92 (1H, *d*, $J = 17.9$ Hz) and 4.88 (1H, *d*, $J = 11.0$ Hz) (2H-15), 3.44 and 3.19 (2H, AB system, $J = 11.5$ Hz, 2H-18), 1.99 (3H, *s*, AcO group), 1.27 (3H, *s*, 3H-17), 1.07 (3H, *s*, 3H-16), 0.79 (3H, *s*, 3H-19), 0.69 (3H, *s*, 3H-20); HRLSIMS *m/z*: 387.2513 $[M + Na]^+$ (calcd for $C_{22}H_{36}O_4Na$, 387.2511).

4.8. Achievement of substrate *12* from diacetate *4*

Diacetate **4** (1.7 g) was dissolved in MeOH–H_2O (70%, 50 ml) containing KOH (5%) and refluxed for 1 h. Operating in the same working conditions as for the saponification of diacetate **3**, *ent*-6α,18-dihydroxymanoyl oxide (**6**, 1.3 g, 96%) (García-Granados et al., 1997b) was isolated. This diol (**6**, 1.1 g) was dissolved in vinyl acetate (100 ml) and *C. cylindracea* lipase (2 g) was added. The biotransformation was maintained for 2 h to give *ent*-18-acetoxy-6α-hydroxymanoyl oxide (**8**, 1.2 g, 97%). Monoacetate **8** (1.2 g) was dissolved in acetone (25 ml) and oxidized with Jones' reagent, as indicate for monoacetate **7**, to give *ent*-18-acetoxy-6-oxomanoyl oxide (**12**, 980 mg, 82%) (García-Granados et al., 1997b).

4.9. ent-18-Acetoxy-6α-hydroxymanoyl oxide (*8*)

White solid, mp 108–110 °C; $[\alpha]_D - 50.0°$ ($CHCl_3$; *c* 1), IR ν_{max}^{KBr} cm^{-1}: 3470, 3085, 1735, 1251 and 1033 ^1H NMR ($CDCl_3$): δ 5.85 (1H, *dd*, $J = 17.4$, 10.7 Hz, H-14), 5.12 (1H, *dd*, $J = 17.4$, 1.4 Hz) and 4.91 (1H, *dd*,$J = 10.7$, 1.4 Hz) (2H-15), 4.46 and 3.80 (2H, AB system, $J = 10.7$ Hz, 2H-18), 3.79 (1H, *ddd*, $J = 10.9$, 10.9, 3.8 Hz, H-6), 2.06 (3H, *s*, AcO group), 1.32 (3H, *s*, 3H-17), 1.24 (3H, *s*, 3H-16), 0.97 (3H, *s*, 3H-19), 0.83 (3H, *s*, 3H-20); HRLSIMS *m/z*: 387.2516 $[M + Na]^+$ (calcd for $C_{22}H_{36}O_4Na$, 387.2511).

4.10. Acetylation of diol *6*

Diol **6** (200 mg) was acetylated with Ac_2O-pyridine (2:4 ml) at 0 °C for 2 h to achieve diacetate **4** (40 mg, 16%), monoacetate **8** (92 mg, 41%), *ent*-6α-acetoxy-18-hydroxymanoyl oxide (**10**, 25 mg, 11%) and **6** (26 mg, 13%).

4.11. ent-6α-Acetoxy-18-hydroxymanoyl oxide (*10*)

Syrup; $[\alpha]_D - 68.1°$ ($CHCl_3$; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3469, 3089, 1736, 1643, 1238, 1130; ^1H NMR ($CDCl_3$): δ 5.82 (1H, *dd*, $J = 17.4$, 10.7 Hz, H-14), 5.10 (1H, *dd*, $J = 17.4$, 1.5 Hz) and 4.89 (1H, *dd*, $J = 10.7$, 1.5 Hz) (2H-15), 5.08 (1H, *ddd*, $J = 11.5$, 11.5, 4.2 Hz, H-6), 3.46 and 2.88 (2H, AB system, $J = 11.5$ Hz, 2H-18), 2.02 (3H, *s*, AcO group), 1.35 (3H, *s*, 3H-17), 1.21 (3H, *s*, 3H-16), 0.87 (3H, *s*, 3H-19), 0.72 (3H, *s*, 3H-20); HRLSIMS *m/z*: 387.2512 $[M + Na]^+$ (calcd for $C_{22}H_{36}O_4Na$, 387.2511).

4.12. Organism, media and culture conditions

F. moniliforme CECT 2152 (EAN 337) and *N. crassa* CECT 2261 (ATCC 10336) were provided from the Colección Española de Cultivos Tipo, Departamento de Microbiología, Universidad de Valencia, Spain. Medium YEPGA containing 1% yeast extract, 1% peptone, 2% glucose, 2% agar (at pH 5), was used for storage of microorganisms. In all transformations experiments, 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. Erlenmeyer flasks (250 ml) containing 90 ml of medium were inoculated with a dense suspension of the corresponding microorganism. Incubations were maintained at 28 °C with gyratory shaking (150 rpm) for 6 days, after which the substrates in EtOH were added.

4.13. Recovery and purification of metabolites

Cultures were filtered and pooled, and cells were washed thoroughly with water and the liquid was saturated with NaCl and extracted twice with CH_2Cl_2. Both extracts were pooled, dried with dry Na_2SO_4, and evaporated at 40 °C in vacuum. The mixture of metabolites was chromatographed on a silica gel column.

4.14. Biotransformation of *11* by F. moniliforme

Substrate **11** (100 mg) was dissolved in EtOH (2 ml), distributed among 2 Erlenmeyer-flask cultures (*F. moniliforme*) and incubated for 6 days, after which the cultures were processed as indicated above, to obtain starting material (**11**, 18 mg, 18%), **13** (15 mg, 17%), **14** (15 mg, 16%) and **15** (6 mg, 6%).

4.15. ent-18-Hydroxy-6-oxo-13-epi-manoyl oxide (*13*)

White solid, mp 110–112 °C; $[\alpha]_D - 30.7°$ ($CHCl_3$; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3426, 3081, 1708, 1092, 971, 913; ^1H NMR ($CDCl_3$): δ 5.94 (1H, *dd*, $J = 17.9$, 11.0 Hz, H-14), 4.97 (1H, *d*, $J = 17.9$ Hz) and 4.92 (1H,*d*, $J = 11$ Hz) (2H-15), 3.51 and 3.12 (2H, AB system, $J = 10.5$ Hz, 2H-18), 2.60 and 2.39 (2H, AB system, $J = 11.3$ Hz, 2H-7), 2.53 (1H, *s*, H-5), 2.27 (1H, *dd*, $J = 10.2$, 3.3 Hz, H_{eq}-12), 1.35 (1H, *ddd*, $J = 13.1$, 13.1, 4.0 Hz, H_{ax}-3), 1.84 (1H, *dd*, $J = 11.1$, 3.1 Hz, H-9) 1.17 (3H, *s*, 3H-17), 1.13 (3H, *s*, 3H-16), 1.09 (3H, *s*, 3H-19) and 0.76 (3H, *s*, 3H-20); HRLSIMS *m/z*: 343,2251 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2249).

4.16. ent-1β,18-Dihydroxy-6-oxo-13-epi-manoyl oxide (*14*)

White solid, mp 129–131 °C; $[\alpha]_D - 26.2°$ ($CHCl_3$; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3412, 3081, 1705, 1072, 969, 915; ^1H NMR ($CDCl_3$): δ 5.94 (1H, *dd*, $J = 17.9$, 11.1 Hz, H-14), 4.99 (1H,*d*, $J = 17.9$ Hz) and 4.93 (1H,*d*,$J = 11.1$ Hz) (2H-15), 3.61 (1H, *dd*, $J = 10.0$, 5.3 Hz, H-1), 3.55 and

3.11 (2H, AB system, $J = 10.3$ Hz, 2H-18), 2.60 and 2.39 (2H, AB system, $J = 11.2$ Hz, 2H-7), 2.56 (1H, *s*, H-5), 2.27 (1H, *dd*, $J = 10.2$, 3.3 Hz, H_{eq}-12), 2.00 (1H, *dd*, $J = 11.5$, 2.6 Hz, H-9), 1.18 (3H, *s*, 3H-17), 1.13 (3H, *s*, 3H-16), 1.08 (3H, *s*, 3H-19) and 0.81 (3H, *s*, 3H-20); HRLSIMS *m/z*: 359.2196 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

4.17. *ent*-11α,18-Dihydroxy-6-oxo-13-*epi*-manoyl oxide (*15*)

White solid, mp 133–135 °C; $[\alpha]_D$ − 30.5° (EtOH; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3377, 3086, 1712, 1042, 970, 916; ^1H NMR ($CDCl_3$): δ 5.93 (1H, *dd*, $J = 17.7$, 11.0 Hz, H-14), 5.09 (1H,*d*, $J = 17.7$ Hz) and 4.96 (1H, *d*, $J = 11.0$ Hz) (2H-15), 4.21 (1H, *ddd*, $J = 9.5$, 7.7, 4.8 Hz, H-11), 3.56 and 3.12 (2H, AB system, $J = 10.4$ Hz, 2H-18), 2.68 and 2.38 (2H, AB system, $J = 11.3$ Hz, 2H-7), 2.64 (1H, *s*, H-5), 2.48 (1H, *dd*, $J = 13.9$, 4.8 Hz) and 1.65 (1H, *dd*, $J = 13.9$, 7.7 Hz) (2H-12), 1.97 (1H, *d*, $J = 9.5$ Hz, H-9), 1.28 (3H, *s*, 3H-16), 1.18 (3H, *s*, 3H-17), 1.13 (3H, *s*, 3H-19) and 0.98 (3H, *s*, 3H-20); HRLSIMS *m/z*: 359.2200 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

4.18. Biotransformation of *11* by *N. crassa*

Substrate **11** (250 mg) was dissolved in EtOH (5 ml), distributed among 5 Erlenmeyer-flask cultures (*N. crassa*) and incubated for 6 days, isolating starting material **11** (30 mg, 12%), **16** (8 mg, 3%), **17** (25 mg, 10%), **13** (40 mg, 18%), **14** (50 mg, 22%), 1**5** (20 mg, 9%), **18** (5 mg, 2%), and **19** (5 mg, 2%).

4.19. *ent*-18-Acetoxy-1β-hydroxy-6-oxo-13-*epi*-manoyl oxide (*16*)

Syrup; $[\alpha]_D$ − 19.5° ($CHCl_3$; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3078, 1738, 1712, 1240, 1036, 971, 915; ^1H NMR ($CDCl_3$): δ 5.94 (1H, *dd*, $J = 18.0$, 11.1 Hz, H-14), 4.98 (1H,*d*, $J = 18.0$ Hz) and 4.93 (1H,*d*, $J = 11.1$ Hz) (2H-15), 3.93 and 3.74 (2H, AB system, $J = 10.8$ Hz, 2H-18), 3.61 (1H, *dd*, $J = 10.5$, 5.2 Hz, H-1) 2.51 and 2.39 (2H, AB system, $J = 11.2$ Hz, 2H-7), 2.45 (1H, *s*, H-5), 2.15 (1H, *dd*, $J = 10.3$, 3.3 Hz, H_{eq}-12) 2.02 (3H, *s*, AcO), 1.97 (1H, *dd*, $J = 11.5$, 2.6, H-9), 1.18 (3H, *s*, 3H-17), 1.15 (3H, *s*, 3H-16), 1.14 (3H, *s*, 3H-19) and 0.80 (3H, *s*, 3H-20); HRLSIMS *m/z*: 401.2308 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_5Na$, 401.2304).

4.20. *ent*-18-Acetoxy-11α- hydroxy-6-oxo-13-*epi*-manoyl oxide (*17*)

Syrup; $[\alpha]_D$ − 19.8° ($CHCl_3$; *c* 0.5); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3090, 1737, 1712, 1240, 1039, 967, 916; ^1H NMR ($CDCl_3$): δ 5.94 (1H, *dd*, $J = 17.7$, 11.0 Hz, H-14), 5.09 (1H, *d*, $J = 17.7$ Hz) and 4.96 (1H, *d*, $J = 11.0$ Hz) (2H-15), 4.22 (1H, *ddd*, $J = 9.5$, 7.9, 4.7 Hz, H-11), 3.97 and 3.73 (2H, AB system, $J = 10.7$ Hz, 2H-18), 2.59 and 2.37 (2H, AB system, $J = 11.2$ Hz, 2H-7), 2.55 (1H, *s*, H-5), 2.50 (1H, *dd*, $J = 13.8$, 4.7 Hz) and 1.67 (1H, *dd*, $J = 13.8$, 7.9 Hz) (2H-12), 2.03 (3H, *s*, AcO), 1.94 (1H, *d*, $J = 9.5$ Hz, H-9), 1.29 (3H, *s*, 3H-16), 1.19 (3H, *s*, 3H-19), 1.18 (3H, *s*, 3H-19) and 0.98 (3H, *s*, 3H-20); HRLSIMS *m/z*: 401.2312 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_5Na$, 401.2304).

4.21. *ent*-1α,18-Dihydroxy-6-oxo-13-*epi*-manoyl oxide (*18*)

Syrup; $[\alpha]_D$ − 34.8° ($CHCl_3$; *c* 0.5); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3427, 3083, 1702, 1074, 1037, 963, 914; ^1H NMR ($CDCl_3$): δ 5.96 (1H, *dd*, $J = 18.0$, 11.1 Hz, H-14), 4.99 (1H, *d*, $J = 18.0$ Hz) and 4.94 (1H, *d*, $J = 11.1$ Hz) (2H-15), 3.70 (1H, *bs*, H-1), 3.54 and 3.19 (2H, AB system, $J = 10.5$ Hz, 2H-18), 2.92 (1H, *s*, H-5), 2.60 and 2.42 (2H, AB system, $J = 11.5$ Hz, 2H-7), 1.18 (3H, *s*, 3H-17), 1.16 (3H, *s*, 3H-16), 1.12 (3H, *s*, 3H-19) and 0.79 (3H, *s*, 3H-20); HRLSIMS *m/z*: 359.2197 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

4.22. *ent*-12α,18-Dihydroxy-6-oxo-13-*epi*-manoyl oxide (*19*)

Syrup; $[\alpha]_D$ − 35.2° ($CHCl_3$; *c* 0.3); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3399, 3084, 1704, 1079, 1035, 975, 915; ^1H NMR ($CDCl_3$): δ 6.03 (1H, *dd*, $J = 18.2$, 11.5 Hz, H-14), 5.00 (1H, *d*,$J = 11.5$ Hz) and 4.98 (1H, *d*, $J = 18.2$ Hz) (2H-15), 4.14 (1H, *dd*, $J = 3.3$, 3.3 Hz, H-12), 3.53 and 3.14 (2H, AB system, $J = 10.4$ Hz, 2H-18), 2.66 and 2.42 (2H, AB system, $J = 11.4$ Hz, 2H-7), 2.60 (1H, *s*, H-5), 1.21 and 1.20 (3H each, *s*, 3H-16 and H-17), 1.10 (3H, *s*, 3H-19) and 0.79 (3H, *s*, 3H-20); HRLSIMS *m/z*: 359.2199 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

4.23. Biotransformation of *12* by *F. moniliforme*

Substrate **12** (120 mg) was dissolved in EtOH (3 ml), distributed among 3 Erlenmeyer-flask cultures (*F. moniliforme*) and incubated for 6 days, after which the cultures were processed as indicated above for other biotransformation processes to obtain starting material **12** (25 mg, 23%), **20** (22 mg, 21%), **21** (11 mg, 10%) and **22** (10 mg, 9%).

4.24. *ent*-18-Hydroxy-6-oxo-manoyl oxide (*20*)

Syrup; $[\alpha]_D$ − 20.7° ($CHCl_3$; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3439, 3086, 1708, 917; ^1H NMR ($CDCl_3$): δ 5.86 (1H, *dd*, $J = 17.4$, 10.7 Hz H-14), 5.13 (1H, *dd*, $J = 17.4$, 1.4 Hz) and 4.93 (1H, *dd*, $J = 10.7$, 1.4 Hz) (2H-15), 3.51 and 3.11 (2H, AB system, $J = 10.5$ Hz, 2H-18), 2.64 and 2.45 (2H, AB system, $J = 11.6$ Hz, 2H-7), 2.52 (1H, *s*, H-5), 1.97 (1H, *dd*, $J = 11.7$, 3.4 Hz H-9), 1.26 (3H, *s*, 3H-16), 1.25 (3H, *s*, 3H-17), 1.09 (3H, *s*, 3H-19) and 0.82 (3H, *s*, 3H-20); HRLSIMS *m/z*: 321.2425 $[M + H]^+$ (calcd for $C_{20}H_{33}O_3$, 321.2430).

*4.25. ent-1β,18-Dihydroxy-6-oxo-manoyl oxide (**21**)*

White solid, mp 94–96 °C; $[\alpha]_D$ − 8.8° ($CHCl_3$; *c* 1), IR ν_{max}^{KBr} cm^{-1}: 3438, 3086, 170, 918; ^1H NMR ($CDCl_3$), δ 5.87 (1H, *dd*, *J* = 17.3, 10.8 Hz, H-14), 5.14 (1H, *dd*, *J* = 17.3, 1.4 Hz) and 4.94 (1H, *dd*, *J* = 10.8, 1.4 Hz) (2H-15), 3.56 (1H, partially overlapped with the part A of the AB system of 2H-18, H-1) 3.54 and 3.10 (2H, AB system, *J* = 10.4 Hz, 2H-18), 2.64 and 2.44 (2H, AB system, *J* = 11.5 Hz, 2H-7), 2.54 (1H, *s*, H-5), 2.14 (1H, *dd*, *J* = 11.1, 4.3 Hz, H-9), 1.25 (3H, *s*, 3H-17), 1.24 (3H, *s*, 3H-16), 1.09 (3H, *s*, 3H-19) and 0.86 (3H, *s*, 3H-20); HRLSIMS *m*/*z*: 337.2388 $[M + H]^+$ (calcd for $C_{20}H_{33}O_4$, 337.2379).

*4.26. ent-11α,18-Dihydroxy-6-oxo-manoyl oxide (**22**)*

White solid, mp 117–118 °C; $[\alpha]_D$ + 0.5° ($CHCl_3$; *c* 1), IR ν_{max}^{KBr} cm^{-1}: 3431, 3087, 1704, 921; ^1H NMR ($CDCl_3$), δ 6.04 (1H, *dd*, *J* = 17.1, 10.6 Hz, H-14), 5.45 (1H, *dd*, *J* = 17.1, 1.6 Hz) and 5.10 (1H, *dd*, *J* = 10.6, 1.6 Hz) (2H-15), 4.01 (1H, *ddd*, *J* = 7.8, 6.0, 1.4 Hz, H-11), 3.54 and 3.11 (2H, AB system, *J* = 10.4 Hz, 2H-18), 2.72 and 2.47 (2H, AB system, *J* = 11.6 Hz, 2H-7), 2.58 (1H, *s*, H-5); 2.36 (1H, *dd*, *J* = 15.5, 6.0 Hz) and 1,97 (1H, *dd*, *J* = 15.5, 1.4 Hz) (2H-12), 2.13 (1H, *d*, *J* = 7.8 Hz, H-9), 1.24 (3H, *s*, 3H-17), 1.20 (3H, *s*, 3H-16), 1.11 (3H, *s*, 3H-19) and 0.91 (3H, *s*, 3H-20); HRLSIMS *m*/*z*: 337.2369 $[M + H]^+$ (calcd for $C_{20}H_{33}O_4$, 337.2379).

*4.27. Acetylation of **21***

Metabolite **21** (11 mg) was dissolved in pyridine (0.5 ml) and Ac_2O (0.25 ml) for 24 h at room temperature, yielding 9 mg of *ent*-1β,18-diacetoxy-6-oxo-manoyl oxide (**26**, 64%).

*4.28. ent-1β,18-Diacetoxy-6-oxo-manoyl oxide (**26**)*

Syrup; $[\alpha]_D$ − 2.95° ($CHCl_3$; *c* 0.3); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3084, 1738, 1717, 1239, 918; ^1H NMR ($CDCl_3$), δ 5.89 (1H, *dd*, *J* = 17.3, 10.7 Hz, H-14), 5.17 (1H, *dd*, *J* = 17.3, 1.4 Hz) and 4.99 (1H, *dd*, *J* = 10.7, 1.4 Hz) (2H-15), 4.70 (1H, *dd*, *J* = 10.6, 4.9Hz, H-1), 3.95 and 3.76 (2H, AB system, *J* = 10.9 Hz, 2H-18), 2.03 (6H, *s*, AcO groups), 1.26, 1.23, 1.21 and 0.98 (3H each, *s*, 3H-16, 3H-17, 3H-19 and 3H-20); HRLSIMS *m*/*z*: 421.2588 $[M + H]^+$ (calcd for $C_{24}H_{37}O_6$, 421.2590).

*4.29. Biotransformation of **12** by N. crassa*

Substrate **12** (300 mg) was dissolved in EtOH (5 ml), distributed among 5 Erlenmeyer-flask cultures (*N. crassa*) and incubated for 6 days. Chromatographic separation of the mixture of metabolites yielded starting material **12** (50 mg, 13%), **20** (60 mg, 17%), **21** (35 mg, 9%), **22** (100 mg, 27%), **24** (45 mg, 12%), **25** (10 mg, 2%) (García-Granados et al., 1997b), **26** (26 mg, 6%) and **27** (11 mg, 3%).

*4.30. ent-18-Acetoxy-11α-hydroxy-6-oxo-manoyl oxide (**26**)*

Syrup; $[\alpha]_D$ − 3.4° ($CHCl_3$; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490, 3087, 1738, 1643, 1240, 1039, 984, 921; ^1H NMR ($CDCl_3$): δ 6.09 (1H, *dd*, *J* = 17.1, 10.6 Hz, H-14), 5.41 (1H, *dd*, *J* = 17.1, 1.7 Hz) and 5.14 (1H, *dd*, *J* = 10.6, 1.7 Hz) (2H-15), 4.05 (1H, *ddd*, *J* = 7.8, 5.9, 1.8 Hz, H-11), 3.97 and 3.78 (2H, AB system, *J* = 10.7 Hz, 2H-18), 2.66 and 2.50 (2H, AB system, *J* = 11.8 Hz, 2H-7), 2.48 (1H, *s*, H-5), 2.38 (1H, *dd*, *J* = 15.4, 5.9 Hz) and 1.98 (1H, *dd*, *J* = 15.4, 1.8 Hz) (2H-12), 2.12 (1H, *d*, *J* = 7.8 Hz, H-9), 2.04 (3H, *s*, AcO group), 1.27, 1.24, 1.21 and 0.93 (3H each, *s*, 3H-16, 3H-17, 3H-19 and 3H-20); HRLSIMS *m*/*z*: 401.2303 $[M + Na]^+$ (calcd for $C_{22}H_{34}O_5Na$, 401.2304).

*4.31. ent-18-Hydroxy-6,11-dioxo-manoyl oxide (**27**)*

Syrup; $[\alpha]_D$ + 30.0° ($CHCl_3$; *c* 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3457, 3088, 1712, 1037, 986, 918; ^1H NMR ($CDCl_3$): δ 5.99 (1H, *dd*, *J* = 17.3, 10.7 Hz, H-14), 5.24 (1H, *dd*, *J* = 17.3, 0.6 Hz) and 5.11 (1H, *dd*, *J* = 10.7, 0.6 Hz) (2H-15), 3.56 and 3.10 (2H, AB system, *J* = 10.4 Hz, 2H-18), 3.24 (1H, *s*, H-9), 2.89 and 2.58 (2H, AB system, *J* = 12.0 Hz, 2H-7), 2.72 and 2.61 (2H, AB system, *J* = 18.0 Hz, 2H-12), 2.52 (1H, *s*, H-5), 1.30 (6H, *s*, 3H-16 and 3H-17), 1.12 and 1.06 (3H each, *s*, 3H-19 and 3H-20); HRLSIMS *m*/*z*: 357.2050 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

*4.32. Oxidation of **25** and **26***

Metabolites **25** (5 mg) and **26** (5 mg) were dissolved in Me_2CO (2 ml) and oxidized with Jones' reagent to obtain *ent*-18-acetoxy-6,11-dioxo-manoyl oxide (**28**, 8 mg of overall yield).

*4.33. ent-18-Acetoxy-6,11-dioxomanoyl oxide (**28**)*

Syrup; $[\alpha]_D$ 1.15° ($CHCl_3$; *c* 0.5); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3055, 1719, 1244, 926; ^1H NMR ($CDCl_3$), δ 6.00 (1H, *dd*, *J* = 17.3, 10.7 Hz, H-14), 5.26 (1H, *dd*, *J* = 17.3, 0.9 Hz) and 5.12 (1H, *dd*, *J* = 10.7, 0.9 Hz) (2H-15), 3.94 and 3.72 (2H, AB system, *J* = 10.8 Hz, 2H-18), 3.21 (1H, *s*, H-9), 2.80 and 2.58 (2H, AB system, *J* = 12.0 Hz, 2H-7), 2.34 (1H, *s*, H-5), 2.72 and 2.60 (2H, AB system, *J* = 17.8 Hz, 2H-12), 2.03 (3H, *s*, AcO group), 1.30, 1.29, 1.20 and 1.05 (3H each, *s*, 3H-16, 3H-17, 3H-19 and 3H-20); HRLSIMS *m*/*z*: 399.2141 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_5Na$, 399.2147).

*4.34. Enzymatic acetylation of **27***

Metabolite **27** (5 mg) was dissolved in vinyl acetate (5 ml) and *C. cylindracea* lipase (20 mg) was added. After CC, 4 mg of **28** were obtained.

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