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Biotransformation of *ent*-13-*epi*-manoyl oxides difunctionalized at C-3 and C-12 by filamentous fungi

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Abstract

Biotransformation of *ent*-3β,12α-dihydroxy-13-*epi*-manoyl oxide with *Fusarium moniliforme* gave the regioselective oxidation of the hydroxyl group at C-3 and the *ent*-7β-hydroxylation. The action of *Gliocladium roseum* in the 3,12-diketoderivative originated monohydroxylations at C-1 and C-7, both by the *ent*-β face, while *Rhizopus nigricans* produced hydroxylation at C-7 or C-18, epoxidation of the double bond, reduction of the keto group at C-3, and combined actions as biohydroxylation at C-2/epoxidation of the double bond and hydroxylation at C-7/reduction of the keto group at C-3. In the *ent*-3-hydroxy-12-keto epimers, *G. roseum* originated monohydroxylations at C-1 and C-7 and *R. nigricans* originated the oxidation at C-3 as a major transformation, epoxidation of double bond and hydroxylation at C-2. Finally, in the *ent*-3β-hydroxy epimer *R. nigricans* also originated minor hydroxylations at C-1, C-6, C-7 and C-20 and *F. moniliforme* produced an hydroxylation at C-7 and a dihydroxylation at C-7/C-11.

Keywords: Filamentous fungi; Biotransformation; Diterpenoids; *ent*-Manoyl oxides; Biohydroxylation

1. Introduction

Biotransformation is today considered to be a routine economically and ecologically competitive technology by the synthetic organic chemists, in search of new production routes for fine chemical, pharmaceutical and agrochemical compounds (Huisman et al., 2002; Laumen et al., 2002; Patel, 2001, 2002; Rasor et al., 2001; Straathof et al., 2002). The selectivity and mildness of the biotransformations can make these processes superior to similar, chemical-based methods (Davis et al., 2001; Loughlin, 2000; Roberts, 2000; Thomas et al., 2002; Zaks, 2001). From the different transformations catalysed by enzymatic systems, the selective hydroxylation of non-activated carbon atoms is particularly interesting, because this transformation is difficult to achieve by classical chemical methods. These biohydroxylations have been developed mainly in the fields of the steroids and terpenoids because of the industrial demand of the end products. Problems with substrate acceptance, undesired side reactions, selectivity and prediction of the hydroxylation position all hamper the general synthetic utility of the biohydroxylation. Biotransformations are typically carried out using either whole cells or isolated enzymes. Although in recent years some enzymes responsible for fungal hydroxylation have been isolated, whole-cell fermentation is the technique most often employed in fungal hydroxylation (Lehman et al., 2001; Urlacher and Schmid, 2002; Van Beilen et al., 2003). A great difficulty for the biohydroxylation of a certain substrate is to find the appropriate microorganism, so that, traditionally, one of the most widely used techniques is the screening with different fungal strains. In this context, the microbial transformation of *ent*-manoyl oxides—labdane-type diterpenoids—by filamentous fungi constitutes one of the aims of the present study. These biotransformation processes have been used to introduce hydroxyl groups, at positions difficult to achieve by chemical means, on the substrates. The objective was to produce new bioactive, highly oxygenated *ent*-manoyl oxide analogues of *ent*-forskolin. In

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previous papers, we reported the incubation of several *ent*-manoyl oxides, with functions at C-3 or at C-3 and C-12, with the filamentous fungi *Curvularia lunata*, *Cunninghamella elegans*, *Fusarium moniliforme*, *Rhizopus nigricans* and *Gliocladium roseum*, yielding, in some cases, products with biological activity (García-Granados et al., 1990, 1994, 1995a, 1999). New *ent*-manoyl oxides also were obtained by biotransformation of some *ent*-13-*epi*-manoyl oxides by the fungus *Gibberella fujikuroi* (Fraga et al., 1989, 1999, 2001, 2003). In the present work, we used the fungi *Fusarium moniliforme* CECT 2152, a synonym of *Fusarium verticilliodes* (EAN 337), *Rhizopus nigricans* CECT 2672, a synonym of *Rhizopus stolonifer* (ATCC 10404) and *Gliocladium roseum* CECT 2733, the anamorphic form of *Nectria ochroleuca* (ATCC 10523), to complete the earlier biotransformation studies.

2. Results and discussion

ent-3β,12α-Dihydroxy-13-*epi*-manoyl oxide (varodiol, **1**) was isolated from *Sideritis varoi* (Algarra et al., 1983). Biotransformation of substrate **1** with *Fusarium moniliforme* gave metabolites **2** (16%) and **3** (35%), and substrate **1** (37%). Metabolite **2**, the result of the regioselective oxidation of the hydroxyl group at C-3 in substrate **1**, presented spectroscopic data identical to those of *ent*-12α-hydroxy-3-oxo-13-*epi*-manoyl oxide obtained in the biotransformation of ribenone **4** with *Gibberella fujikuroi* (Fraga et al., 1999). Metabolite **3** was identified as *ent*-3β,7β,12α-trihydroxy-13-*epi*-manoyl oxide, previously obtained in the biotransformation of ribenone **4** with *F. moniliforme* (García-Granados et al., 1995a).

Oxidation of diol **1** gave *ent*-3,12-dioxo-13-*epi*-manoyl oxide (varodione, **5**), which was incubated with *Gliocladium roseum* to give **6** (7%) and **7** (19%). Metabolite **6** had a molecular formula $C_{20}H_{30}O_4$, which indicated the presence of an additional hydroxyl group in the molecule. Its ^{1}H NMR spectrum revealed a signal at δ 3.72 (*dd*, $J = 10.5$, 4.4 Hz) due to an axial geminal proton to a hydroxyl group. The position of the new function was established by oxidation to give **8** (García-Granados et al., 1995a), and therefore, the structure of **6** was determined as *ent*-7β-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide. The second metabolite (**7**), with a structure of *ent*-1β-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide, has been previously isolated in the biotransformation of diketone **5** with *F. moniliforme* (García-Granados et al., 1995a).

Biotransformation of varodione **5** with *Rhizopus nigricans* originated metabolites **6** (4%), **9** (5%), **10** (6%), **11** (3%), **12** (13%), **13** (14%), **14** (10%) and a polar mixture of compounds, from which, after acetylation, diacetate **15** (2%) was obtained.

Metabolites **9** and **10** had the molecular formula $C_{20}H_{30}O_4$, which indicated the presence of an additional oxygen atom. In their ^{1}H NMR spectra, three double doublet signals of geminal protons to a 14,15-epoxy group were observed (δ 3.29, 2.72 and 2.67 for metabolite **9** and δ 3.10, 2.75 and 2.63 for metabolite **10**). These epoxide groups were confirmed by their ^{13}C NMR data. Both metabolites (**9** and **10**) were epimers at C-14 with a structure of *ent*-3,12-dioxo-8α,13;14,15-diepoxylabdane. The configuration at C-14 for metabolites **9** and **10** was established by chemical correlation with the epoxy derivatives **16** and **17**, achieved by epoxidation of metabolite **2** with MCPBA. These compounds (**16** and **17**) had been previously obtained in the biotransformation of ribenone by *Mucor plumbeus* (Fraga et al., 2001), and their stereochemistry unequivocally determined, "14*S*" being for the epoxy derivative **16** and "14*R*" for **17**. Oxidation of **16** gave compound **9** ((13*S*,14*S*)-*ent*-3,12-dioxo-8α,13;14,15-diepoxylabdane) while identical oxidation of **17** gave compound **10** ((13*S*,14*R*)-*ent*-3,12-dioxo-8α,13;14,15-diepoxylabdane). Metabolite **11** was *ent*-3α-hydroxy-12-oxo-13-*epi*-manoyl oxide, and was previously obtained from the incubation of **5** with baker's yeast (García-Granados et al., 1999).

Compounds **12** and **13** had the same molecular formula ($C_{20}H_{30}O_5$) and very similar spectroscopic data. Their ^{1}H NMR spectra showed three double-doublet signals, analogous to those observed for metabolites **9** and **10**, which correspond to the geminal protons to the epoxy group on C-14 and C-15. In addition, a deshielding signal at 4.52 ppm (1H, *dd*, $J = 12.5$, 6.6 Hz) due to a geminal proton to an equatorial hydroxyl group at C-2 was noted. The α- and β-effects detected in their ^{13}C NMR spectra (Table 1) confirmed this position. Thus, metabolite **12** was (13*S*,14*S*)-*ent*-2α-hydroxy-3,12-dioxo-8α,13;14,15-diepoxylabdane and **13** (13*S*,14*R*)-*ent*-2α-hydroxy-3,12-dioxo-8α,13;14,15-diepoxylabdane.

The molecular formula of **14** indicated the presence of an additional hydroxyl group in the molecule. Comparisons of the ^{1}H NMR data of this metabolite (**14**) with those of substrate **5** revealed the existence of two signals, forming an AB system (δ 3.71 and 3.39, $J = 11.3$ Hz), and the absence of one of the methyl signals. The α-effect on C-18, the β-effect on C-4 and the γ-effect on C-19 positioned the new hydroxyl group at C-18, which was confirmed by comparison of the ^{13}C NMR data with those of triol **18**—obtained by reduction of metabolite **14**—and those previously published for triol **19** (Arias et al., 1988). These compounds showed similar chemical shifts values for C-3, C-4, C-5, C-18 and C-19, which was in accordance with a structure of *ent*-3β,12β,18-trihydroxy-13-*epi*-manoyl oxide for **18**, and consequently metabolite **14** was identified as *ent*-18-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide.

Diacetate **15** presented in its ^{1}H NMR spectrum two signals of geminal hydrogens to an acetoxyl group at δ

Table 1
^{13}C NMR chemical shifts

C	9	10	12	13	14	15	18	20	21	26	30
1	37.4	37.3	47.5	47.5	37.0	32.2	37.4	32.5	77.0	37.0	31.7
2	33.7[a]	33.7[a]	68.8	68.9	33.8	22.5	26.9	22.6	37.2	27.0	23.5
3	216.2	216.4	215.5	215.5	217.4	77.2	77.6	77.8	74.7	78.7	79.9
4	47.4	47.4	47.6	47.6	36.3	—	42.0	36.7	37.4	38.8	37.6
5	54.7	54.7	57.0	57.1	53.7	47.5	49.7	49.9	47.5	52.9	55.5
6	20.8	20.8	20.2	20.2	20.1	25.0	19.6	19.3	19.4	19.6	19.1
7	41.4	41.4	41.5	41.7	41.2	80.3	42.1	42.0	42.0	42.0	42.2
8	74.9	74.8	74.8	74.8	75.1	—	77.3	75.5	75.7	75.2	75.2
9	52.3	54.4	52.5	54.7	48.1	53.2	57.7	54.5	54.1	54.9[a]	52.2
10	36.5	36.5	37.1	37.2	52.5	36.7	36.6	36.7	42.5	36.7	39.9
11	33.3[a]	35.5[a]	33.3	35.4	34.9	33.1	25.3	33.7	36.1	33.3	36.2
12	211.4	208.4	211.0	207.9	210.7	210.9	76.2	211.2	213.0	212.1	210.1
13	78.6	81.0	78.5	81.0	82.1	81.6	75.6	81.9	81.4	78.5	82.6
14	54.9	57.1	54.7	57.0	142.2	141.8	140.3	142.5	142.2	55.0[a]	142.7
15	43.1	45.5	43.0	45.5	113.4	113.3	117.3	113.2	113.2	43.2	113.1
16	24.9	24.0	24.8	24.0	28.6	29.1	28.0	28.6	28.7	24.7	28.7
17	21.8[b]	21.8[b]	22.3	22.4	22.1	16.7	25.4	22.3	22.1[a]	22.1	22.3
18	26.5	26.6	24.9	24.9	66.7	27.8	71.5	27.9	28.0	28.2	28.9
19	21.4[b]	21.2[b]	21.5	21.4	16.8	21.6	11.2	21.6	21.9[a]	15.5	17.0
20	14.2	14.6	15.3	15.5	14.5	14.5	16.4	14.5	10.3	14.7	63.2

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

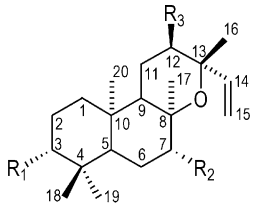
4.96 (*dd*, $J=11.8$, 4.8 Hz) and at δ 4.67 (*t*, $J=2.9$ Hz), due to axial and equatorial protons, respectively. The comparison of the ^{13}C NMR spectrum of **15** with that of **20**, obtained by acetylation of metabolite **11**, revealed that the microorganism had reduced the keto group at C-3 to give an axial hydroxyl group, as in metabolite **11**, and had introduced a hydroxyl group at C-7, which were then acetylated. Consequently, compound **15** was *ent*-3α,7β-diacetoxy-12-oxo-13-*epi*-manoyl oxide.

The regioselective and stereoselective reduction of the carbonyl group at C-3 of varodione **5** with baker's yeast gave ketol **11** as the main metabolite (García-Granados et al., 1999). Biotransformation of this ketol (**11**) with *R. nigricans* for 3 days yielded metabolite **5** (7%) (varodione), and the previously isolated metabolites in the above biotransformation of varodione (**5**) with *R. nigricans*, **9** (11%), **10** (9%), **12** (11%) and **13** (10%).

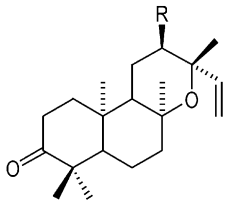
The incubation of the same ketol (**11**) with *G. roseum* for 13 days produced **21** (32%) and a mixture of more polar metabolites, which was acetylated, yielding diacetate **15** (4%), previously obtained in the biotransformation of varodione (**5**) with *R. nigricans*, and compound **22** (3%). The molecular formula of metabolite **21** ($C_{20}H_{32}O_4$) suggested the presence of an additional hydroxyl group in the molecule. Its ^{1}H NMR spectrum confirmed this proposal with the presence of a new signal due to a geminal axial proton to a hydroxyl group at δ 3.88 (*dd*, $J=11.4$, 4.7 Hz). The β-effect on C-2 ($\Delta\delta=+12.1$), the γ-effect on C-20 ($\Delta\delta=-4.2$), and the δ-effect on C-11 ($\Delta\delta=+2.5$), positioned this hydroxyl group at C-1, establishing its structure as *ent*-1β,3α-dihydroxy-12-oxo-13-*epi*-manoyl oxide (**21**). The IR spectrum of compound **22** showed hydroxyl, acetoxyl and carbonyl group bands. Comparison of the ^{1}H NMR spectrum of this compound with that of **20** revealed the chemical acetylation only on the hydroxyl group at C-3, and the presence of a new signal at δ 4.49, due to an axial geminal proton to a hydroxyl group. The position of this alcohol was determined by analysing the ^{1}H NMR chemical shifts of H-17, H-19 and H-20 in both compounds. The signals of these protons for **22** were more deshielded (1.45, 1.27 and 1.18 ppm, respectively) than those for compound **20** (1.17, 0.86 or 0.85 and 0.78 ppm, respectively), due to the 1,3-diaxial interactions produced by a hydroxyl group located at C-6, as in other *ent*-6β-hydroxyderivatives (García-Granados et al., 1990, 1995b). Thus, **22** was identified as *ent*-3α-acetoxy-6β-hydroxy-12-oxo-13-*epi*-manoyl oxide.

Treatment of varodiol (**1**) with *CCL* (lipase of *Candida cylindracea*) originated the regioselective acetylation of the hydroxyl group at C-3 to give *ent*-3β-acetoxy-12α-hydroxy-13-*epi*-manoyl oxide (**23**) (71%). Oxidation of this monoacetate (**23**) yielded compound **24**, which was then hydrolysed to give *ent*-3β-hydroxy-12-oxo-13-*epi*-manoyl oxide (**25**) (García-Granados et al., 1999). The biotransformation of this substrate (**25**) by *R. nigricans* over a period of 6 days produced metabolites **9** (6%), **10** (5%), **12** (11%), **13** (9%), **26** (6%), **27** (1%), **28** (9%), together with a more polar mixture of metabolites which was acetylated to give compounds **29** (1%), **30** (8%) and **31** (1%).

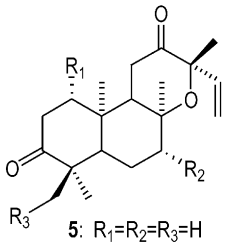
The ^{1}H NMR spectrum of metabolite **26** revealed the presence of three double-doublets signals characteristic of a 14,15-epoxide group. The stereochemistry at C-14 of this function was determined by comparison with the spectral data of **10**, being "*R*" and resulting in a structure of (13*S*,14*R*)-*ent*-3β-hydroxy-12-oxo-8α,13;14,15-diepoxylabdane. The ^{1}H NMR spectrum of metabolite **27** showed a signal at δ 3.91 that corresponded to an axial geminal proton to a hydroxyl group, coupled with two axial protons ($J=11.0$, 11.0 Hz) and an equatorial one ($J=3.9$ Hz). In this compound the equatorial hydroxylation can be located only at C-6. Thus, **27** was identified as *ent*-3β,6α,-dihydroxy-12-oxo-13-*epi*-manoyl oxide. Metabolite **28** presented spectroscopic data identical to those of *ent*-3β,7β-dihydroxy-12-oxo-13-*epi*-manoyl oxide, previously obtained in the biotransformation of compound **24** with *Nectria ochroleuca* (García-Granados et al., 1999). Compound **29** was the diacetate of **28**. In the ^{1}H NMR spectrum of compound **30**, only four signals of methyl groups and an AB system signal with doublets centred at δ 4.58 and 4.07 ($J=12.4$ Hz) were detected, indicating that one of methyl groups of substrate (**25**) was functionalized by the microorganism. This oxygenated functional group was positioned at C-20 by comparison of the ^{13}C NMR spectra of diacetate **30** and monoacetate **24**, and confirmed by other bibliographical data (Arias et al., 1988;



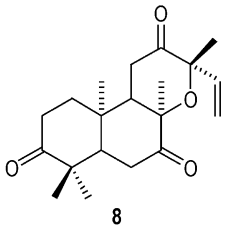
1: R_1=R_3=OH, R_2=H
3: R_1=R_2=R_3=OH
23: R_1=OAc, R_2=H, R_3=OH
34: R_1=OH, R_2=R_3=H



2: R =OH
4: R = H



5: R_1=R_2=R_3=H
6: R_1=R_3=H, R_2=OH
7: R_1=OH, R_2=R_3=H
14: R_1=R_2=H, R_3=OH



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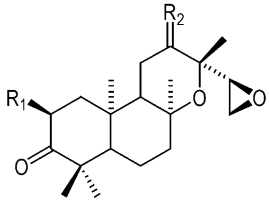
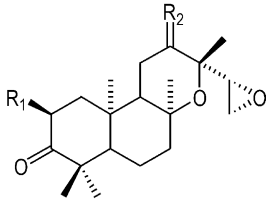
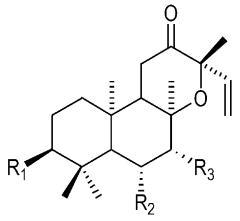


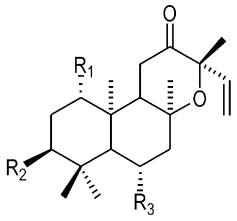
9: R_1 =H, R_2=O
12: R_1 =OH, R_2=O
16: R_1 =H, R_2=*ent*-α-OH,H



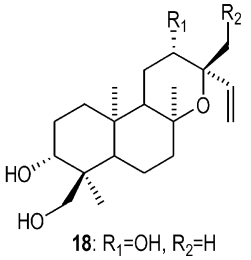
10: R_1 =H, R_2=O
13: R_1 =OH, R_2=O
17: R_1 =H, R_2=*ent*-α-OH,H



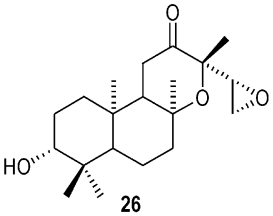
11: R_1=OH, R_2=R_3=H
15: R_1=R_3=OAc, R_2=H
20: R_1=OAc, R_2= R_3=H



21: R_1=R_2=OH, R_3=H
22: R_1=H, R_2=OAc, R_3=OH



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



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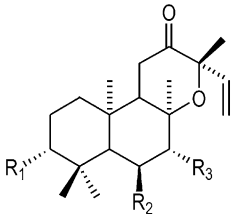
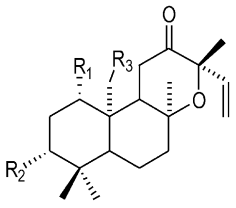
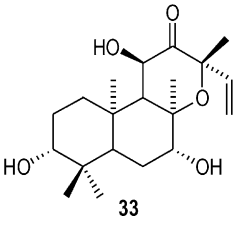


24: R_1=OAc, R_2= R_3=H
25: R_1=OH, R_2= R_3=H
27: R_1=R_3=OH, R_3=H,
28: R_1=R_3=OH, R_2=H
29: R_1=R_3=OAc, R_2=H



30: R_1=H, R_2=R_3=OAc
31: R_1=R_2=OAc, R_3=H
32: R_1=R_2=OH, R_3=H



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García-Granados et al., 1997). Thus, compound **30** was identified as *ent*-3β,20-diacetoxy-12-oxo-13-*epi*-manoyl oxide. Compound **31** had in its ^{1}H NMR spectrum a signal at δ 4.72 (*dd*, J = 11.5, 4.7 Hz) originated by a geminal proton to an acetoxyl group that could be located at C-1 or C-7. The first position was confirmed by acetylation of an *ent*-1β,3β-dihydroxy derivative (**32**), isolated in the biotransformation of compound **24** by *N. ochroleuca* (García-Granados et al., 1999), which proved to be identical with **31**. Therefore, this compound had the structure of *ent*-1β,3β-diacetoxy-12-oxo-13-*epi*-manoyl oxide (**31**).

The biotransformation of **25** with *F. moniliforme* gave unaltered substrate **25** (60%), and the metabolites **28** (8%), isolated in the biotransformation of ketol **25** with *R. nigricans*, and **33** (7%). This metabolite (**33**) had a molecular formula of $C_{20}H_{32}O_5$, indicating that two new oxygen atoms were introduced in the biotransformation process. In its ^{1}H NMR spectrum, as in that of **28**, appeared two signals of the equatorial geminal protons

to the hydroxyl groups at C-3 and C-7, together with a new signal at 4.59 ppm (*d*, $J = 12.4$ Hz) originated by an axial geminal proton to a hydroxyl group at C-11. This *ent*-11α-hydroxylation was also observed in the biotransformation of varodione (**5**) with *F. moniliforme* (García-Granados et al., 1995a). Hence, the structure of this compound was determined as *ent*-3β,7β,11α-trihydroxy-12-oxo-13-*epi*-manoyl oxide (**33**).

3. Conclusions

a). The regioselective oxidation of varodiol **1** with *F. moniliforme* gave better yields for compound **2** (16%) than did biohydroxylation at C-12 of ribenone (**4**) by *G. fujikuroi* (6%) (Fraga et al., 1999). The *ent*-7β-hydroxylation (35%) of varodiol (**1**) with *F. moniliforme* was improved with regard to the corresponding biohydroxylation of ribenol (**34**) with the same microorganism (7%), indicating that a second oxygenated function at C-12 seems to accelerate the action of the microorganism.

b). The biotransformation of varodione (**5**) by *G. roseum* was focussed only on two monohydroxylations at C-1 (19%), while *F. moniliforme* produced only 9% (García-Granados et al., 1995a), and at C-7 (7%), both by the *ent*-β face. Whereas *R. nigricans* showed lower selectivity with biohydroxylations at C-2, C-7 and C-18, reduction of the carbonyl group at C-3, and epoxidations of the double bond. *G. roseum* and *F. moniliforme* have in common the non-functionalization of the double bond of the molecule, as was also observed with *R. nigricans*, and in previous biotransformations with *Curvularia lunata* (García-Granados et al., 1994) and *Cunninghamella elegans* (García-Granados et al., 1995a).

c). In the biotransformation processes by *R. nigricans* of the epimer ketols **11** and **25**, the main action of the microorganism was the oxidation at C-3 and epoxidation of the double bond of the molecule, with or without hydroxylation at C-2. In addition, with substrate **25**, *R. nigricans* also originated minor hydroxylations at C-1, C-6, C-7 and C-20. The configuration at C-3 of ketols **11** and **25** (García-Granados et al., 1999) does not appear to influence the action of *G. roseum*, producing monohydroxylations at C-1 and C-7 in both substrates. The action of *F. moniliforme* on ketol **25** was focused at C-7, producing *ent*-7β-hydroxyl and *ent*-7β,11α-dihydroxyl derivatives.

d). The hydroxylations at non-activated positions of the carbocyclic system of this type of compounds are difficult to achieve by classical chemical means, this being tenable by microbial transformation, selecting the microorganism and the functional groups of the diterpene molecule. Some of the isolated metabolites have similar structures to several remarkable compounds, such as forskolin (Bhat et al., 1977), hamachilobenes (Toyota et al., 1988), natural ptychantins (Hashimoto et al., 1994; Wu et al., 2001), etc., but with a skeleton of the *enantio* series.

4. Experimental

Melting points were determined using a Kofler (Reichter) apparatus and are uncorr. Measurements of NMR spectra (300.13 MHz, ^{1}H, and 75.47 MHz, ^{13}C) were made in $CDCl_3$ in a Bruker AM-300 spectrometer. Assignments of ^{13}C chemical shifts (Table 1) were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. IR spectra were recorder on a Nicolet 20SX FT-IR spectrometer. High-resolution mass spectra were made by LSIMS (FAB) ionization mode in a MICROMASS AUTOSPEC-Q spectrometer (EBE geometry). Optical rotations were measured on a Perkin-Elmer 240 polarimeter at 25 °C. Silica gel Scharlau 60 (40–60 μm) was used for flash chromatography. CH_2Cl_2 with increasing amounts of Me_2CO was used as eluent. Analytical plates (silica gel, Merck 60 G) rendered visible by spraying with H_2SO_4–HOAc–H_2O, followed by heating at 120 °C. Varodiol (**1**) used as starting material in these biotransformations was isolated from *Sideritis varoi* (Algarra et al., 1983).

4.1. Organism, media and culture conditions

Rhizopus nigricans CECT 2672 (ATCC 10404), *Glyocladium roseum* CECT 2733 (ATCC 10523) and *Fusarium moniliforme* CECT 2152 (EAN 337) were obtained 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 ext., 0.1% beef ext. and 0.5% glucose at pH 5.7 in H_2O was used. Erlenmeyer flasks (250 ml) containing 80 ml of medium were inoculated with a dense suspension of the corresponding microorganism. Incubations were maintained at 28 °C with gyratory shaking (150 rpm) for 6 days after which the substrates (5–10%) in EtOH were added.

4.2. 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 mixt. of compounds obtained was chromatographed on silica gel column.

4.3. Biotransformation of *1* by *F. moniliforme*

Substrate **1** (150 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 **1** (56 mg, 37%), *ent*-12α-hydroxy-3-oxo-13-*epi*-manoyl oxide (**2**, 24 mg, 16%) (Fraga et al., 1999), and *ent*-3β,7β,12α-trihydroxy-13-*epi*-manoyl oxide (**3**, 55 mg, 35%) (García-Granados et al., 1995a).

4.4. Oxidation of *1*

Jones's reagent was added dropwise to a stirred soln. of varodiol (**1**, 500 mg) in Me_2CO (20 ml) at 0 °C until an orange-brown colours persisted (15 min), following the oxidation by TLC. MeOH was them added, 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 Na_2SO_4 and evaporated to dryness. Chromatography on a silica gel column yielded varodione (**5**, 450 mg, 91%) (García-Granados et al., 1994).

4.5. Biotransformation of *5* by *G. roseum*

Substrate **5** (90 mg) was dissolved in EtOH (2 ml), distributed among 2 Erlenmeyer-flask cultures (*G. roseum*) and incubated for 7 days, after which the cultures were processed as indicated above to obtain starting material **5** (50 mg, 56%); *ent*-7β-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide (**6**, 7 mg, 7%): syrup; $[\alpha]_D$ −28° ($CHCl_3$; *c* 0.5); IR ν_{max} ($CHCl_3$), 3466, 3080, 1705, 1085, 1000 and 925 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.09 (1H, *dd*, *J* = 13.1, 6.3 Hz, H-14), 5.12 (1H, *dd*, *J* = 13.1, 0.8 Hz) and 5.07 (1H, *dd*, *J* = 6.3, 0.8 Hz) (2H-15), 3.72 (1H, *dd*, *J* = 10.5, 4.4 Hz, H-7), 1.32, 1.24 (3H each, *s*, 3H-16 and 3H-17), 1.13, 1.05 and 0.91 (3H each, *s*, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 335.2219 $[M+H]^+$ (calc. for $C_{20}H_{31}O_4$, 335.2222); and *ent*-1β-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide (**7**, 18 mg, 19%) (García-Granados et al., 1995a).

4.6. Oxidation of *6*

Metabolite **6** (7 mg) was dissolved in Me_2CO (2 ml) and oxidized with Jones' reagent to obtain *ent*-3,7,12-trioxo-13-*epi*-manoyl oxide (**8**, 5 mg, 72%) (García-Granados et al., 1995a).

4.7. Biotransformation of *5* by *R. nigricans*

Substrate **5** (350 mg) was dissolved in EtOH (10 ml), distributed among 10 Erlenmeyer-flask cultures (*R. nigricans*) and incubated for 6 days, after which the cultures were processed as indicated above to obtain starting material **5** (110 mg, 31%); *ent*-7β-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide (**6**, 14 mg, 4%); (13*S*,14*S*)-*ent*-3,12-dioxo-8α,13;14,15-diepoxylabdane (**9**, 19 mg, 5%): syrup; $[\alpha]_D$ −34° ($CHCl_3$; *c* 0.5); IR ν_{max} ($CHCl_3$), 1709, 1098 and 1078 cm^{-1}; ^1H NMR ($CDCl_3$), δ 3.29 (1H, *dd*, *J* = 4.0, 2.8 Hz, H-14), 2.72 (1H, *dd*, *J* = 5.5, 2.8 Hz) and 2.67 (1H, *dd*, *J* = 5.5, 4.0 Hz) (2H-15), 2.52 (1H, *dd*, *J* = 19.1, 6.5 Hz, H_{eq}-11), 2.37 (1H, *dd*, *J* = 19.1, 12.7 Hz, H_{ax}-11), 2.00 (1H, *dd*, *J* = 12.7, 6.5 Hz, H-9), 1.27 and 1.16 (3H each, *s*, 3H-16 and 3H-17), 1.11, 1.04 and 0.94 (3H each, *s*, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 357.2055 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2042); (13*S*,14*R*)-*ent*-3,12-dioxo-8α,13;14,15-diepoxylabdane (**10**, 21 mg, 6%): syrup; IR ν_{max} ($CHCl_3$), 1710, 1115 and 1093 cm^{-1}; ^1H NMR ($CDCl_3$), δ 3.10 (1H, *dd*, *J* = 4.3, 2.8 Hz, H-14), 2.75 (1H, *dd*, *J* = 4.8, 4.3 Hz) and 2.63 (1H, *dd*, *J* = 4.8, 2.8 Hz) (2H-15), 1.53 and 1.30 (3H each, *s*, 3H-16 and 3H-17), 1.11, 1.05 and 0.94 (3H each, *s*, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 357.2040 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2042); *ent*-3α-hydroxy-12-oxo-13-*epi*-manoyl oxide (**11**, 10 mg, 3%) (García-Granados et al., 1999); (13*S*,14*S*)-*ent*-2α-hydroxy-3,12-dioxo-8α,13;14,15-diepoxylabdane (**12**, 52 mg, 13%): syrup; IR ν_{max} ($CHCl_3$), 3456, 1705, 1105 and 1090 cm^{-1}; ^1H NMR ($CDCl_3$), δ 4.52 (1H, *dd*, *J* = 12.5, 6.6 Hz, H-2), (1H, *dd*, *J* = 4.0, 2.8 Hz, H-14), 2.70 (1H, *dd*, *J* = 5.5, 2.8 Hz) and 2.66 (1H, *dd*, *J* = 5.5, 4.0 Hz) (2H-15), 2.55 (1H, *dd*, *J* = 19.1, 7.1 Hz, H_{eq}-11), 2.40 (1H, *dd*, *J* = 19.1, 12.6 Hz, H_{ax}-11), 2.00 (1H, *dd*, *J* = 12.6, 7.1 Hz, H-9), 1.24, 1.18, 1.16, 1.14 and 1.11 (3H each, *s*, 3H-16, 3H-17, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 351.2169 $[M+H]^+$ (calc. for $C_{20}H_{31}O_5$, 351.2172); (13*S*,14*R*)-*ent*-2α-hydroxy-3,12-dioxo-8α,13;14,15-diepoxylabdane (**13**, 56 mg, 14%): syrup; IR ν_{max} ($CHCl_3$), 3466, 1712, 1110 and 1096 cm^{-1}; ^1H NMR ($CDCl_3$), δ 4.52 (1H, *dd*, *J* = 12.5, 6.6 Hz, H-2), 3.09 (1H, *dd*, *J* = 4.3, 2.8 Hz, H-14), 2.76 (1H, *dd*, *J* = 4.7, 4.3 Hz) and 2.63 (1H, *dd*, *J* = 4.7, 2.8 Hz) (2H-15), 2.50 (1H, *dd*, *J* = 16.9, 13.6 Hz, H_{ax}-11), 2.40 (1H, *dd*, *J* = 16.9, 5.0 Hz, H_{eq}-11), 1.77 (1H, *dd*, *J* = 13.6, J_2 = 5.0 Hz, H-9), 1.53 and 1.27 (3H each, *s*, 3H-16 and 3H-17), 1.18, 1.16 and 1.11 (3H each, *s*, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 351.2176 $[M+H]^+$ (calc. for $C_{20}H_{31}O_5$, 351.2172); *ent*-18-hydroxy-3,12-dioxo-13-*epi*-manoyl oxide (**14**, 35 mg, 10%): syrup; $[\alpha]_D$ −87° ($CHCl_3$, *c* 1); IR ν_{max} ($CHCl_3$), 3460, 3080, 1707, 1090, 1000 and 925 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.10 (1H, *dd*, *J* = 17.5, 10.8 Hz, H-14), 5.15 (1H, *dd*, *J* = 17.5, 1.0 Hz) and 5.06 (1H, *dd*, *J* = 10.8, 1.0 Hz) (2H-15), 3.71 (1H, *d*, *J* = 11.3 Hz) and 3.39 (1H, *d*, *J* = 11.3 Hz) (2H-18), 2.61 (1H, *ddd*, *J* = 16.5, 13.0, 6.9 Hz, H-2), 2.51 (1H, *dd*, *J* = 17.8, 6.2 Hz, H_{eq}-11), 2.39 (1H, *dd*, *J* = 17.8, 12.9 Hz, H_{ax}-11), 2.35 (1H, *ddd*, *J* = 16.5, 5.8, 2.6 Hz, H-3), 2.00 (1H, *dd*, *J* = 12.9, 6.1 Hz, H-9); 1.31 and 1.24 (3H each, *s*, 3H-16 and 3H-17), 1.00 and 0.98 (3H each, *s*, 3H-19

and 3H-20); HR-LSIMS *m/z*: 357.2043 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 357.2042); together with a mixture which was acetylated to give *ent*-3α,7β-diacetoxy-12-oxo-13-*epi*-manoyl oxide (**15**, 10 mg, 2% of overall yield): syrup; $[\alpha]_D$ −2° ($CHCl_3$, *c* 0.5); IR ν_{max} ($CHCl_3$), 3085, 1736, 1243 and 926 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.15 (1H, *dd*, *J* = 17.4, 10.8 Hz, H-14), 5.16 (1H, *dd*, *J* = 17.4, 1.4 Hz) and 5.04 (1H, *dd*, *J* = 10.8, 1.4 Hz) (2H-15), 4.96 (1H, *dd*, *J* = 11.8, 4.8 Hz, H-7), 4.67 (1H, *t*, *J* = 2.9 Hz, H-3), 2.10 and 2.08 (3H each, *s*, AcO group), 1.29 and 1.25 (3H each, *s*, 3H-16 and 3H-17), 1.19, 0.89 and 0.83 (3H each, *s*, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m/z*: 421.2588 $[M+H]^+$ (calcd for $C_{24}H_{37}O_6$, 421.2590).

4.8. Epoxidation of 2

Metabolite **2** (20 mg) was dissolved in $CHCl_3$ (3 ml) and MCPBA (15 mg) was added. After 24 h at 0 °C the mixt. was diluted with $CHCl_3$ (20 ml) and washed with aq. $FeSO_4$, $NaHCO_3$ and H_2O. The organic layer was dried with dry Na_2SO_4, concentrated in vacuum and chromatographed on a silica gel column to give (13*S*,14*S*)-*ent*-12α-hydroxy-3-oxo-8α,13;14,15-diepoxylabdane (**16**, 5 mg, 24%) and (13*S*,14*R*)-*ent*-12α-hydroxy-3-oxo-8α,13;14,15-diepoxylabdane (**17**, 4 mg, 19%) (Fraga, 2001).

4.9. Oxidation of diepoxylabdane 16

Diepoxylabdane **16** (5 mg) was dissolved in Me_2CO (1 ml) and oxidized with Jones' reagent to give (13*S*,14*S*)-*ent*-3,12-dioxo-8α,13;14,15-diepoxylabdane (**9**, 4 mg, 80%).

4.10. Oxidation of diepoxylabdane 17

Diepoxylabdane **17** (4 mg) was dissolved in Me_2CO (1 ml) and oxidized with Jones' reagent to give (13*S*,14*R*)-*ent*-3,12-dioxo-8α,13;14,15-diepoxylabdane (**10**, 3 mg, 75%).

4.11. Reduction of 14

Metabolite **14** (10 mg) was dissolved in EtOH (5 ml) and $NaBH_4$ (5 mg) was added. The mixture was maintained for 2 h at room temp. after which the reaction mixture was treated with HCl (10%) and extracted with CH_2Cl_2 in the usual way to give *ent*-3β,12β,18-trihydroxy-13-*epi*-manoyl oxide (**18**, 8 mg, 80%): syrup; $[\alpha]_D$ −45° ($CHCl_3$, *c* 0.5); IR ν_{max} ($CHCl_3$), 3446, 3081, 1050, 990 and 915 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.28 (1H, *dd*, *J* = 17.8, 11.2 Hz, H-14), 5.42 (1H, *dd*, *J* = 17.8, 1.2 Hz) and 5.24 (1H, *dd*, *J* = 11.2, 1.2 Hz) (2H-15), 3.70 (1H, *d*, *J* = 11.3 Hz) and 3.40 (1H, *d*, *J* = 10.3 Hz) (2H-18), 3.64 (1H, *dd*, *J* = 10.7, 5.3 Hz, H-3), 3.49 (1H, *dd*, *J* = 11.5, 4.6 Hz, H-12), 1.34 and 1.24 (3H each, *s*, 3H-16 and 3H-17), 0.84 and 0.81 (3H each, *s*, 3H-19 and 3H-20); HR-LSIMS *m/z*: 361.2351 $[M+Na]^+$ (calc. for $C_{20}H_{32}O_4Na$, 361.2355).

4.12. Acetylation of 11

Metabolite **11** (10 mg) was dissolved in pyridine (3 ml) and Ac_2O (1.5 ml). The reaction was maintained for 72 h at room temp., and extracted in the usual way. The solvent was evapd. to give a mixt. of compounds which was chromatographed on silica gel to give *ent*-3α-acetoxy-12-oxo-13-*epi*-manoyl oxide (**20**, 8 mg, 71%): white solid, mp: 143–145 °C; IR ν_{max} (KBr), 3088, 1732, 1716, 1639, 1245, 1087, 991 and 924 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.09 (1H, *dd*, *J* = 17.6, 10.8, H-14), 5.12 (1H, *dd*, *J* = 17.6, 1.1 Hz) and 5.07 (1H, *dd*, *J* = 10.8, 1.1 Hz) (2H-15); 4.64 (1H, *t*, *J* = 2.8 Hz, H-3), 2.45 (1H, *dd*, *J* = 17.8, 5.9 Hz, H_{eq}-11), 2.32 (1H, *dd*, *J* = 17.8, 13.1 Hz, H_{ax}-11), 2.05 (3H, *s*, AcO group), 1.31 and 1.17 (3H each, *s*, 3H-16 and 3H-17), 0.86 and 0.85 (3H each, *s*, 3H-18 and 3H-19), 0.78 (3H, *s*, 3H-20); HR-LSIMS *m/z*: 385.2354 $[M+Na]^+$ (calc. for $C_{22}H_{34}O_4Na$, 385.2355).

4.13. Semisynthesis of ketol 11 and biotransformation by R. nigricans

Ketol **11** was previously obtained in the biotransformation of varodione **5** with baker's yeast (García-Granados et al., 1999). Ketol **11** (100 mg) was dissolved in EtOH (2 ml), distributed among 2 Erlenmeyer-flask cultures (*R. nigricans*) and incubated for 3 days, after which the cultures were processed as indicated above to give starting material **11** (30 mg, 40%); varodione (**5**, 7 mg, 7%); **9** (12 mg, 11%); **10** (10 mg, 9%); **12** (12 mg, 11%); and **13** (11 mg, 10%).

4.14. Biotransformation of 11 by G. roseum

Substrate **11** (100 mg) was dissolved in EtOH (3 ml), distributed among 2 Erlenmeyer-flask cultures (*G. roseum*) and incubated for 13 days, after which the cultures were processed as indicated above to obtain starting material (**11**, 58 mg, 58%) and *ent*-1β,3α-dihydroxy-12-oxo-13-*epi*-manoyl oxide (**21**, 34 mg, 32%): white solid, mp: 138–140 °C; $[\alpha]_D$ −73° ($CHCl_3$, *c* 0.5); IR ν_{max} (KBr), 3433, 3091, 1704, 1640, 1091, 996 and 925 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.13 (1H, *dd*, *J* = 17.4, 10.8 Hz, H-14), 5.18 (1H, *dd*, *J* = 17.4, 1.3 Hz) and 5.03 (1H, *dd*, *J* = 10.8, 1.3 Hz) (2H-15), 3.88 (1H, *dd*, *J* = 11.4, 4.7 Hz, H-1), 3.50 (1H, *t*, *J* = 2.3 Hz, H-3), 3.12 (1H, *dd*, *J* = 19.2, 6.1 Hz, H_{eq}-11), 2.58 (1H, *dd*, *J* = 19.2, 6.1 Hz, H_{ax}-11), 2.18 (1H, *dd*, *J* = 12.9, 6.1 Hz, H-9), 1.30 and 1.13 (3H each, *s*, 3H-16 and 3H-17), 0.94, 0.83 and 0.82 (3H each, *s*, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m/z*: 359.2197 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198); together with a mixture of

polar metabolites which was acetylated to give **15** (5 mg, 4% of overall yield) and *ent*-3α-acetoxy-6β-hydroxy-12-oxo-13-*epi*-manoyl oxide (**22**, 4 mg, 3% of overall yield): syrup; IR ν_{max} ($CHCl_3$), 3500, 3090, 1728, 1716, 1245, 990 and 925 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.14 (1H, *dd*, $J=17.6$, 10.9 Hz, H-14), 5.16 (1H, *dd*, $J=17.6$, 1.1 Hz) and 5.06 (1H, *dd*, $J=10.9$, 1.1 Hz) (2H-15), 4.66 (1H, *t*, $J=2.8$ Hz, H-3), 4.49 (1H, *m*, $W_{1/2}=8.3$ Hz, H-6), 2.07 (3H, *s*, AcO group), 1.45 (3H, *s*, 3H-17), 1.34 (3H, *s*, 3H-16), 1.27 (3H, *s*, 3H-19), 1.18 (3H, *s*, 3H-20), and 0.97 (3H, *s*, 3H-18); HR-LSIMS *m*/*z*: 401.2305 $[M+Na]^+$ (calcd for $C_{22}H_{34}O_5Na$, 401.2304).

4.15. Semisynthesis of ent-3β-hydroxy-12-oxo-13-epi-manoyl oxide (25)

ent-3β-Hydroxy-12-oxo-13-*epi*-manoyl oxide (**25**) was obtained from monoacetate **23**, which had been obtained in the regioselective acylation of varodiol (**1**) with *Candida cylindracea* lipase and vinyl acetate. Monoacetate **23** (500 mg) was dissolved in acetone (20 ml) and oxidized with Jones' reagent as indicate for varodiol (**1**) to give *ent*-3β-acetoxy-12-oxo-13-*epi*-manoyl oxide (**24**, 460 mg, 93%). Product **24** (300 mg) was dissolved in MeOH/H_2O (70%) (10 ml) containing KOH (5%) (0.5 g). The mixture reaction was maintained for 12 h at room temp. The reaction mixture was diluted with H_2O, extracted in the usual way and chromatographed on a silica gel column to yield ketol **25** (240 mg, 90%) (García-Granados et al., 1999).

4.16. Biotransformation of 25 with R. nigricans

Substrate **25** (200 mg) was dissolved in EtOH (5 ml), distributed among 5 Erlenmeyer-flask cultures (*R. nigricans*) and incubated for 6 days, after which the cultures were processed as indicated above to obtain starting material (**25**, 58 mg, 29%); **9** (12 mg, 6%); **10** (10 mg, 5%); **12** (24 mg, 11%); **13** (20 mg, 9%); (13*S*,14*R*)-*ent*-3β-hydroxy-12-oxo-8α,13;14,15-diepoxy-labdane (**26**, 13 mg, 6%): syrup; $[\alpha]_D$ −36° ($CHCl_3$, *c* 0.5); IR ν_{max} ($CHCl_3$), 3470, 1712, 1127, 1097, 1068 and 1039 cm^{-1}; ^1H NMR ($CDCl_3$), δ 3.26 (1H, *dd*, $J=4.0$, 2.8 Hz, H-14), 3.23 (1H, *dd*, $J=11.3$, 4.8 Hz, H-3), 2.70 (1H, *dd*, $J=5.5$, 2.8 Hz) and 2.65 (1H, *dd*, $J=5.5$, 4.0 Hz) (2H-15), 2.48 (1H, *dd*, $J=19.1$, 6.8 Hz, H_{eq}-11), 2.30 (1H, *dd*, $J=19.1$, 12.9 Hz, H_{ax}-11), 1.91 (1H, *dd* $J=12.9$, 6.8 Hz, H-9), 1.25 and 1.11 (3H each, *s*, 3H-16 and 3H-17), 1.00, 0.79 and 0.77 (3H each, *s*, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 359.2208 $[M+Na]^+$ (calc. for $C_{20}H_{32}O_4Na$, 359.2198); *ent*-3β,6α-dihydroxy-12-oxo-13-*epi*-manoyl oxide (**27**, 3 mg, 1%): syrup; $[\alpha]_D$ −11.3° ($CHCl_3$, *c* 0.3); IR ν_{max} ($CHCl_3$), 3436, 3414, 3090, 1711 and 923 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.12 (1H, *dd*, $J=17.5$, 10.8 Hz, H-14), 5.18 (1H, *dd*, $J=17.5$, 1.1 Hz) and 5.06 (1H, *dd*, $J=10.8$, 1.1 Hz) (2H-15), 3.21 (1H, *dd*, $J=11.6$, 4.8 Hz, H-3), 3.91 (1H, *ddd*, $J=11.0$, 11.0, 3.9 Hz, H-6), 1.33, 1.31, 1.22, 0.97 and 0.83 (3H each, *s*, 3H-16, 3H-17, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 359.2190 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198); *ent*-3β,7β-dihydroxy-12-oxo-13-*epi*-manoyl oxide (**28**, 19 mg, 9%) (García-Granados et al., 1999); together with a mixture which was acetylated to give *ent*-3β,7β-diacetoxy-12-oxo-13-*epi*-manoyl oxide (**29**, 5 mg, 1% of overall yield): syrup; $[\alpha]_D$ −1.2° ($CHCl_3$, *c* 0.3); IR ν_{max} ($CHCl_3$), 3060, 1736, 1242 and 926 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.14 (1H, *dd*, $J=17.4$, 10.8 Hz, H-14), 5.16 (1H, *dd*, $J=17.4$, 1.4 Hz) and 5.04 (1H, *dd*, $J=10.8$, 1.4 Hz) (2H-15), 4.50 (1H, *dd*, $J=11.7$, 4.7 Hz, H-3), 4.90 (1H, *dd*, $J=11.6$, 4.9 Hz, H-7), 2.10 and 2.04 (3H each, *s*, AcO group), 1.27, 1.17, 0.90, 0.85 and 0.84 (3H each, *s*, 3H-16, 3H-17, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 421.2596 $[M+H]^+$ (calc. for $C_{24}H_{37}O_6$, 421.2590); *ent*-3β,20-diacetoxy-12-oxo-13-*epi*-manoyl oxide (**30**, 21 mg, 8% of overall yield): syrup; $[\alpha]_D$−13.1° ($CHCl_3$, *c* 0.5); IR ν_{max} ($CHCl_3$), 3082, 1738, 1240 and 924 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.07 (1H, *dd*, $J=17.6$, 10.9 Hz, H-14); 5.07 (1H, *d*, $J=17.6$ Hz) and 5.02 (1H, *d*, $J=10.9$ Hz) (2H-15), 2.81 (1H, *dd*, $J=17.4$, 14.1 Hz, H_{ax}-11), 2.61 (1H, *dd*, $J=17.4$, 4.5 Hz, H_{eq}-11), 4.58 and 4.07 (2H, AB system, $J=12.4$ Hz, 2H-20), 4.50 (1H, *dd*, $J=11.8$, 4.7 Hz, H-3), 1.33, 1.31, 0.91 and 0.88 (3H-16, 3H-17, 3H-18 and 3H-19); HR-LSIMS *m*/*z*: 421.2595 $[M+H]^+$ (calc. for $C_{24}H_{37}O_6$, 421.2590); and *ent*-1β,3β-diacetoxy-12-oxo-13-*epi*-manoyl oxide (**31**, 3 mg, 1% of overall yield): syrup; $[\alpha]_D$ −2.95° ($CHCl_3$, *c* 0.5); IR ν_{max} ($CHCl_3$), 3087, 1740, 1713, 1246 and 925 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.19 (1H, *dd*, $J=17.6$, 10.8 Hz, H-14), 5.26 (1H, *dd*, $J=17.6$, 1.7 Hz) and 5.08 (1H, *dd*, $J=10.8$, 1.7 Hz) (2H-15), 4.72 (1H, *dd*, $J=11.5$, 4.7 Hz, H-1), 4.62 (1H, *dd*, $J=12.3$, 4.6 Hz, H-3), 2.03 and 2.03 (3H each, *s*, AcO group), 1.29, 1.08, 0.97, 0.89 and 0.86 (3H each, *s*, 3H-16, 3H-17, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m*/*z*: 421.2590 $[M+H]^+$ (calcd for $C_{24}H_{37}O_6$, 421.2590).

4.17. Acetylation of 28

Metabolite **28** (10 mg) was dissolved in pyridine (0.5 ml) and Ac_2O (0.25 ml). The reaction was stirred for 24 h. at room temp. The reaction mixture was processed as indicated for acetylation of metabolite **11** yielding **29** (9 mg, 72%).

4.18. Acetylation of 32

ent-1β,3β-Dihydroxy-12-oxo-13-*epi*-manoyl oxide (**32**, 20 mg), previously isolated from the biotransformation of product **24** with *N. ochroleuca* (García-Granados et al., 1999), was dissolved in pyridine (1 ml) and Ac_2O (0.5 ml). The reaction was stirred for 24 h at room

temp., and then processed as indicated for acetylation of **11** to give **31** (18 mg, 72%).

4.19. Biotransformation of 25 by F. moniliforme

Substrate **25** (50 mg) was dissolved in EtOH (1.5 ml), distributed among 1 Erlenmeyer-flask cultures (*F. moniliforme*) and incubated for 6 days, after which the cultures were processed as indicated above to obtain starting substrate (**25**, 30 mg, 60%); **28** (4 mg, 8%); and *ent*-3β,7β,11α-trihydroxy-12-oxo-13-*epi*-manoyl oxide (**33**, 4 mg, 7%): syrup; $[\alpha]_D$ −1.5° ($CHCl_3$, *c* 0.5); IR ν_{max} ($CHCl_3$), 3453, 3082, 1720 and 927 cm^{-1}; ^1H NMR ($CDCl_3$), δ 6.02 (1H, *dd*, *J* = 18.0, 10.9 Hz, H-14), 5.06 (1H, *d*, *J* = 10.9 Hz) and 4.87 (1H, *d*, *J* = 18.0 Hz) (2H-15), 4.59 (1H, *d*, *J* = 12.4 Hz, H-11), 3.60 (1H, *dd*, *J* = 11.3, 3.4 Hz, H-7), 3.21 (1H, *dd*, *J* = 11.4, 5.5 Hz, H-3), 1.60 (1H, *d*, *J* = 12.4, H-9), 1.39, 1.37, 1.02, 1.01 and 0.80 (3H each, *s*, 3H-16, 3H-17, 3H-18, 3H-19 and 3H-20); HR-LSIMS *m/z*: 353.2322 $[M+H]^+$ (calcd for $C_{20}H_{33}O_5$, 353.2328).

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