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Biotransformation of the diterpene ribenone by *Mucor plumbeus*

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Abstract—The microbiological transformation of the diterpene ribenone (3-oxo-ent-13-epi-manoyl oxide) by the fungus *Mucor plumbeus* has been studied. Epoxidation of the vinyl group constitutes the main reaction and there exists a preference for hydroxylation at C-1(α), C-6(α or β) and C-12(β) and, to a lesser degree, at C-7(α) and C-11(β). Other observed reactions were the reduction of the 3-oxo group to a 3β-alcohol and the formation of metabolites with a 1,2-double bond. In this work the stereochemistry at C-14 of the diterpenes excoecarins A, B and C has been revised on the basis of X-ray data.

1. Introduction

The biotransformation of diterpenoids with fungi has been one of the goals of our studies in recent years. In the microbiological transformation of manoyl oxide derivatives we have used two microorganisms: *Gibberella fujikuroi*, a fungus possessing a high substrate specificity, and *Mucor plumbeus*, a fungus with a low specificity. We have studied the incubation of manoyl oxide derivatives, such as jhanol and jhanidiol, with both fungi.[1,2] In addition we have also examined the biotransformation of compounds of the enantio series, such as ribenone (**1**),[3] ribenol (**2**) and 19-hydroxy-*ent*-13-*epi*-manoyl oxide[4] with *G. fujikuroi*. In this work we describe the results of the biotransformation of ribenone with *M. plumbeus*. The purpose was to obtain substances with a functionality similar to that of the bioactive diterpene forskolin[5] (**3**), but with a skeleton of the enantio series. The fungus *M. plumbeus* has been used previously for microbiological transformations, for example with sesquiterpenes possessing the aromadendrane,[6] cedrane,[7] guaiane[8] and patchoulane[9] skeletons, and with diterpenes of the labdane type.[10–12] In this last case, the main reaction observed was the hydroxylation of ring A.

2. Results and Discussion

The substrate used was ribenone (**1**), which had been isolated from *Sideritis canariensis*.[13] Its biotransformation by *M. plumbeus* led to the isolation of the metabolites **4**–**18** and **21**–**28**. The substances **4**, **6**, **8**, **9**, **11** and **28** had been obtained in the incubation of **1** with *G. fujikuroi*,[3] and were identified by direct comparison. Compound **5** possesses the molecular formula $C_{20}H_{30}O_3$. Its ^{1}H NMR spectrum showed, in comparison with that of the substrate, the presence of two new vinylic hydrogens at δ 5.88 and 7.13 (J=10.2 Hz). The chemical shifts of these protons indicated that this new double bond introduced into the molecule must be conjugated with the 3-oxo group. Another signal observed in the spectrum of **5** was a proton geminal to a new hydroxyl group at δ 4.19 as a broad singlet, which could be situated at C-7 (α) or C-12 (β). ^{13}C NMR data of this compound and of its acetate **5a** (Table 1) permitted the alcoholic function to be assigned to C-12 (β). Thus the structure of this substance was determined as 12β-hydroxy-3-oxo-1-en-*ent*-13-*epi*-manoyl oxide (**5**). This compound **5** must be formed from **7**, which was also isolated from this biotransformation.

Table 1. ^{13}C NMR data of compounds **2a**, **5**, **5a**, **7**, **10** and **12**–**14**

Carbon	2a	5	5a	7	10	12	13	14
1	37.3	157.1	156.8	77.4	37.6	40.9	40.3	37.8
2	23.8	125.9	126.1	45.1	32.5	34.2	33.9	33.7
3	80.8	205.0	205.4	215.2	218.5	216.3	215.5	216.8
4	37.4	44.6	44.7	47.1	47.0	49.3	48.9	47.0
5	55.3	53.4	53.3	50.9	58.8	56.8	56.8	52.3
6	16.0	20.2	20.2	20.5	68.3	69.5	69.4	27.5
7	42.9	41.7	41.6	41.3	51.9	50.7	50.3	79.8
8	75.7	76.4[a]	75.3[a]	75.8	74.9	74.9	75.1	78.7
9	58.1	44.1	44.8	49.2	47.9	58.6	49.7	55.4
10	36.4	38.7	38.6	41.9	36.2	36.7	36.1	36.5
11	19.4	23.7	21.7	25.6	23.9	16.5	23.8	15.9
12	34.7	68.9	71.1	69.0	68.7	34.8	68.8	34.5
13	73.5	76.2[a]	76.2[a]	76.0	76.2	73.4	75.9	73.5
14	147.5	146.7	146.2	146.7	146.6	147.5	147.0	146.8
15	109.6	110.9	111.2	110.8	110.9	109.5	110.4	110.1
16	32.6	26.7	27.0	26.9	26.9	32.8	27.0	32.5
17	23.6	24.3	22.7	23.5	24.2	24.5	24.5	17.9
18	27.9	27.6	27.6	27.4	31.7	24.9	25.1	26.6
19	16.3	21.1	21.2	20.0	19.2	23.5	23.4	20.9
20	15.9	18.9	18.8	11.0	16.8	16.2	16.4	15.6

[a] These values can be interchanged.

Keywords: microbial reactions; X-ray crystal structures; epoxidation; terpenes and terpenoids.

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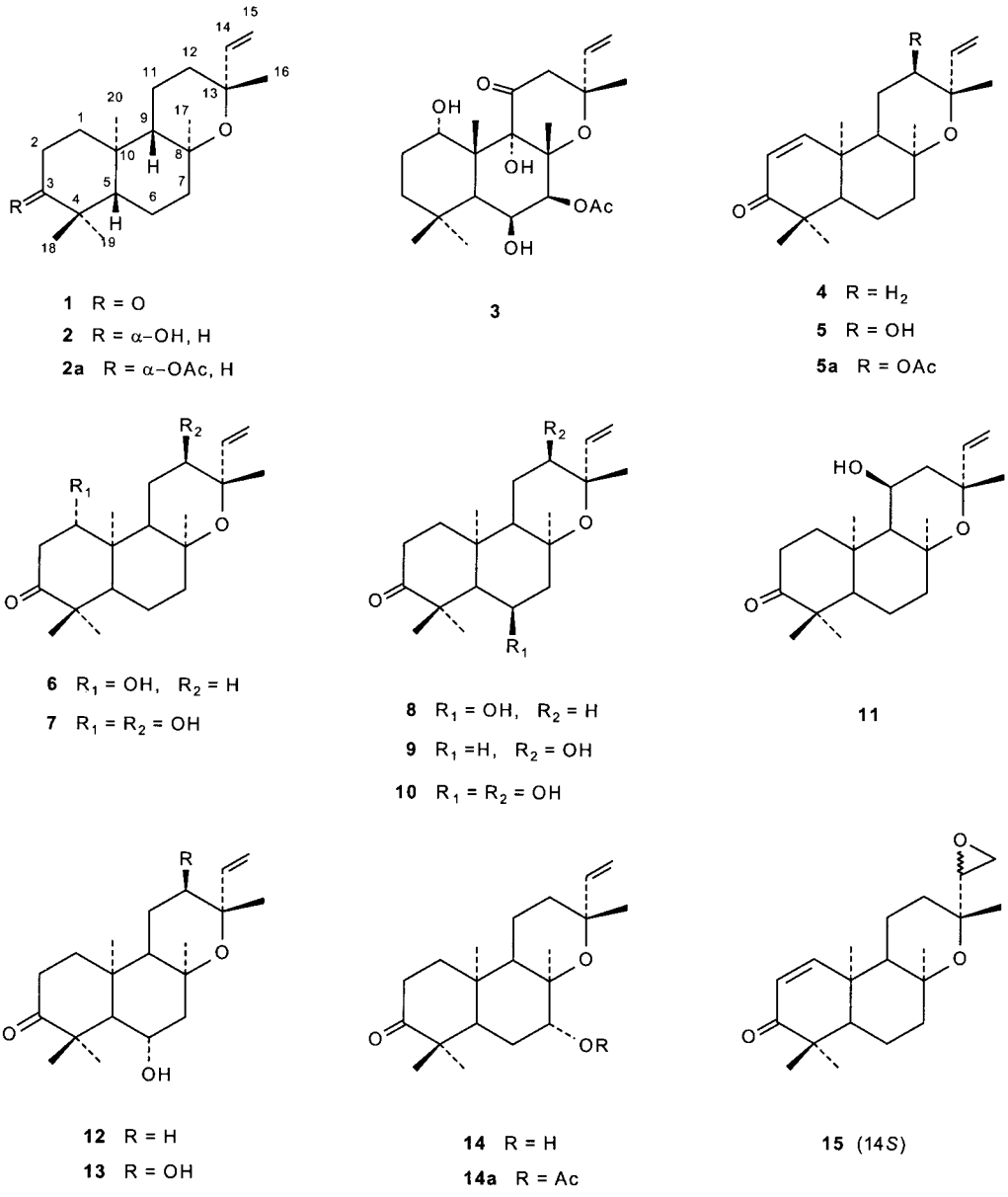


Scheme 1.

The structure of **7** was determined as 1α,12β-dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide on the basis of the following considerations: The high resolution MS did not show the molecular ion, but gave a fragment at *m/z* 318.2208 ($C_{20}H_{30}O_3$), formed by loss of a water molecule. Its ^{1}H NMR spectrum showed the signal of the 1β-hydrogen as a double doublet at δ 3.98 (*J*=7.8 and 5.0 Hz). The relatively low value of the greater of these coupling constants (7.8 Hz), compared with that observed in a normal chair (10–12 Hz), indicated that the corresponding geminal alcohol must be located at C-1 (α) in ring A, which is a deformed chair. Moreover, the chemical shift of the 11α-hydrogen, δ 2.27, is characteristic of compounds with a 1α-hydroxyl group. The second alcohol, with the geminal hydrogen resonating at δ 4.11 (t, *J*=3.3 Hz), was placed at C-12(β) considering 2D NMR data, which also permitted us to unambiguously assign the ^{13}C NMR spectrum of this compound (Table 1).

Another metabolite obtained from this incubation was 6α-hydroxy-ribenone (**12**). In its high resolution MS the molecular ion appeared at *m/z* 320.2360 indicating that the molecular formula of this substance was $C_{20}H_{32}O_3$. The new oxygen introduced into the molecule during the fermentation must be a part of a secondary hydroxyl group, because in its ^{1}H NMR spectrum there appears a proton geminal to a new alcoholic group at δ 4.50 as a triplet (*J*=3 Hz). Double irradiation experiments showed the existence of a –CH–CHOH–CH_2– group, indicating the presence of an equatorial hydrogen geminal to a hydroxyl at C-6(α) or C-11(α). The first position was assigned on the basis of the ^{13}C NMR spectrum (Table 1). Therefore, its structure must be 6α-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**12**). This product was identical with one obtained in the biotransformation of ribenone (**1**) by *Curvularia lunata*.[14]

Compound **14**, an isomer of **12**, showed the resonance of the

Table 2. ^{13}C NMR data of compounds **14a**–**18**, **21**, **23** and **24** (n.o. not observed)

Carbon	14a	15	16	17	18	21	23	24
1	37.6	157.3	39.3	40.1	77.8	40.5	37.7	37.4
2	33.5	125.9	33.9	33.9	45.1	34.0	33.7	33.4
3	216.6	n.o.	216.9	217.5	214.6	n.o.	217.1	216.9
4	46.8	44.7	47.6	47.6	47.1	48.8	47.2	47.2
5	51.9	53.3	55.1	54.9	50.9	56.7	54.6	54.6
6	26.2	20.2	20.8	20.9	20.5	69.2	20.9	20.9
7	80.6	41.9	42.6	42.4	41.4	50.3	41.7	41.9
8	n.o.	75.0	74.8	75.8	74.4	73.8	74.7	74.6
9	55.9	51.6	61.5	61.9	57.0	57.5	48.9	49.6
10	36.1	39.1	37.6	37.9	42.3	36.6	35.9	36.1
11	15.9	16.7	65.0	65.7	18.5	16.6	25.4	24.9
12	34.2	30.8	40.7	41.3	31.0	30.9	66.8	66.0
13	73.3	71.7	71.8	72.8	71.3	71.2	75.1	74.1
14	147.2	57.5	58.1	58.8	57.4	57.4	58.5	57.6
15	109.6	47.3	45.4	45.7	47.3	47.4	46.2	46.3
16	32.4	29.1	28.1	28.4	29.0	29.2	23.1	24.4
17	18.5	24.2	25.8	23.8	23.4	24.6	22.4	22.4
18	26.8	27.7	26.6	26.9	27.4	25.0	26.7	26.7
19	20.7	21.3	21.1	20.9	20.0	23.5	20.9	21.0
20	15.4	18.7	15.7	15.9	10.8	16.2	15.4	14.9

proton geminal to the alcoholic group as a double doublet at δ 3.58 with coupling constants of 11.6 and 4.3 Hz. These couplings are characteristic of an axial hydrogen geminal to an alcoholic group at C-7(α) or C-12(β). The first position was chosen considering its ^{13}C NMR spectrum and a 2D NMR study (COSY, NOESY, HMQC and HMBC) of the acetate **14a** (Table 1). Thus, the structure of 7α-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**14**) was given to this metabolite (Scheme 1).

Other compounds isolated from this incubation were the pair of isomers **10** and **13**, which have the molecular formula $C_{20}H_{32}O_4$. Both are formed during feeding by the introduction of two new hydroxyl groups into the substrate **1**. In both substances the position of the alcoholic groups were assigned at C-12 and C-6 by a study of their ^{13}C NMR data (Table 1). The stereochemistry at C-12 for the hydroxyl group was determined as β-axial in both metabolites, because its geminal hydrogen appears in the NMR spectrum of **10** and **13** at δ 4.13 (t, J=3.7 Hz) and 4.15 (t, J=3.5 Hz), respectively. The stereochemistry of the second alcohol group at the C-6 position of **10** and **13** was determined as β-equatorial and α-axial, respectively, considering that the 6(α)-H and the 6(β)-H resonate at δ 3.86 (td, J=11.6 and 4.4 Hz) and 4.52 (t, J=3 Hz), respectively. Therefore, the structures of these compounds were determined as 6β,12β-dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**10**) and 6α,12β-dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**13**).

A series of epoxy derivatives were also formed in this fermentation by epoxidation of the 14,15-double bond. The least polar of these compounds was **15**. Its MS showed the molecular ion at m/z 318.2198 corresponding to a molecular formula $C_{20}H_{30}O_3$. The presence of the α,β-unsaturated ketone on ring A was determined considering the same arguments given above for **4**, and by the absorbances at 1670 cm^{-1} and 227 nm observed in the IR and UV spectra, respectively. The epoxidation of the 14,15-double bond of the substrate during the incubation was indicated in the ^1H NMR spectrum of **15** by the appearance of three double doublets at δ 2.82, 2.86 and 2.95, due to the two H-15 and H-14, respectively, and by the disappearance of the corresponding vinylic hydrogens. Therefore, the structure of this compound was determined as 3-oxo-1-en-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**15**). On the other hand, the assignment of the position of the alcoholic groups at 1α in **18**, 6α in **21** and 7α in **25** was made on the basis of the same reasons given above for **7**, **13** and **14**, respectively, and consequently we will not repeat them. The stereochemistry at C-14 in all these metabolites was given considering the reasons described below

The structures 12β-hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**23**) and 12β-hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**24**) were assigned to another two isomers isolated from this feeding on the basis of the following considerations: The molecular formula $C_{20}H_{32}O_4$ was determined by high resolution MS. Their ^{13}C NMR data (Table 2) permitted the assignment of the alcohol, which had been introduced in the incubation at C-12 in both metabolites. In their ^1H NMR spectra, both showed the presence of the hydrogens of a 14,15-epoxide and of the proton geminal to the hydroxyl group. This last signal appears in **23** and **24** at δ 3.71 (br d, J=4.4 Hz) and 3.89 (br d, J=4.0 Hz), respectively. These coupling constants indicated a β-axial stereochemistry for the alcohol at C-12 for both metabolites, and these in consequence must be epimeric at C-14. Moreover, a nOe effect was observed between H-12 and the H-16 methyl in the NOESY spectrum. These ^1H NMR data and molecular mechanics methods indicated that the lowest energy conformation for ring C was a chair in both substances. The structures of these metabolites were chemically confirmed by epoxidation of **9** with *m*-chloroperbenzoic acid, which led to the compounds **23** and **24** and the lactones **29** and **30**. The last two substances were formed from **23** and **24**, respectively, by a Baeyer–Villiger reaction.

The stereochemistry at C-14 in the epoxides isolated from this feeding was assigned on the basis of the following considerations: Konishi et al.[15] have isolated epoxy-derivatives of this type from *Excoecaria agallocha*. In their study, the stereochemistry of excoecarin A (14*R*,15-epoxy-ribenone), excoecarin B (14*S*,15-epoxy-ribenone) and excoecarin C (14*R*,15-epoxy-ribenol) was assigned combining molecular mechanics calculations and the observation of a long-range coupling in the 14*S* derivative, between one of the H-15 and the H-16 methyls, and a nOe effect between the same hydrogens in the 14*R*-derivative. However, we could not detect this long-range coupling in the ^1H NMR spectra of our epoxy-derivatives. Thus, since we considered that the stereochemistry of these compounds was not clear we prepared the 14*R*- and 14*S*-stereoisomers of 14,15-epoxy-ribenol acetate, by epoxidation of ribenol acetate (**2a**), and then the structure of **32a** was determined by single-crystal X-ray diffraction. Fig. 1 shows a computer-generated perspective drawing[16] of the final X-ray model of compound **32a**, which possesses a 14S-stereochemistry. The bond lengths and the bond angles are within the usual ranges. The two cyclohexane and the tetrahydropyrane rings are in a chair conformation. The hydrolysis of **32a** led to a substance that was spectroscopically identical with excoecarin C, to which the 14*R* stereochemistry had been assigned.[15] Thus, in consequence, excocaerin C must

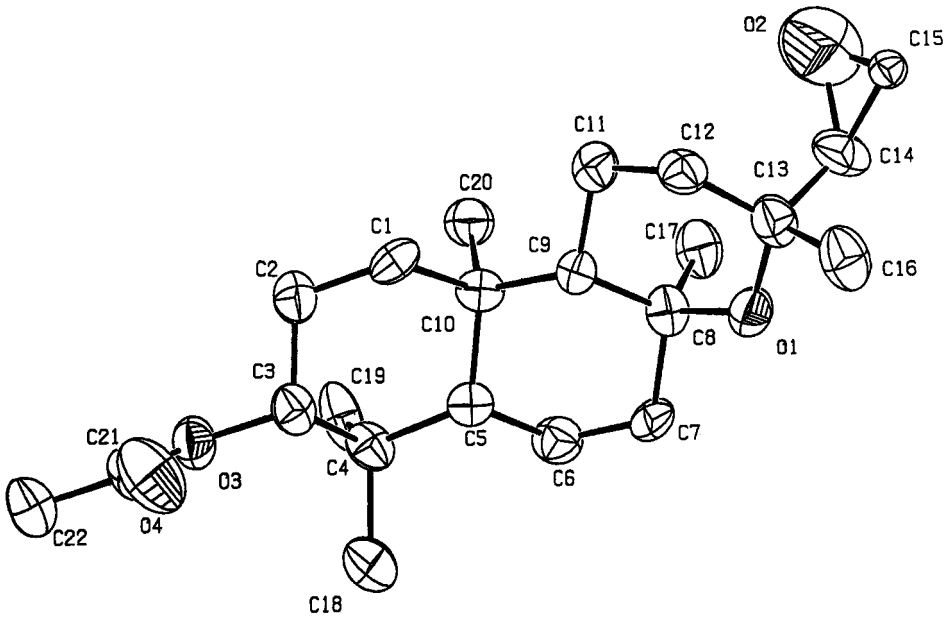


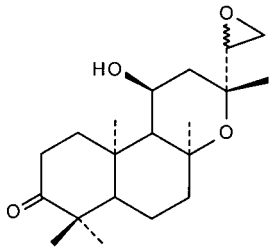
Figure 1. ORTEP drawing of **32a** (displacement ellipsoids are drawn at 40% probability level).

be a 14*S* derivative (**32**) and not 14*R*. Moreover, the stereochemistry at this carbon in excoecarins A and B must also be revised to 14*S* (**19**) and 14*R* (**20**), respectively, because in the original work these compounds had been related to excoecarin C (**32**) (Scheme 2).

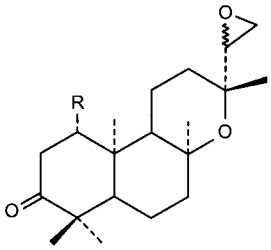
We have noted NMR differences between the 14*R*- and 14*S*-epoxy derivatives, concretely between the two epoxyacetates **31a** and **32a**, the pair of epimeric compounds **16** and **17**, in **23** and **24**, and also in those reported for excoecarins A and B, that now permitted the stereochemistry at C-14 in the epoxides of this type isolated from this feeding to be resolved using only NMR data. These differences, taking for example **23** and **24**, are: (a) the NOESY spectrum of the 14*S*-derivative showed a nOe effect between H-15 and H-17, whilst in the 14*R* derivative this effect was not observed. (b) In the epoxide **24**, which possesses a 14*R* stereochemistry, the H-17 methyl resonates at a lower field (δ 1.42) in comparison with that of the unepoxidated compound **9** (δ 1.23)[3], whilst in **23**, with a 14*S* stereochemistry, it is practically not affected (δ 1.27). (c) The deshielding of the H-17 methyl in the 14*R* derivative **24** is accompanied by another deshielding of H-14 (δ 3.11) and by the shielding of one of the H-15 (δ 2.56) in this compound, in comparison with the respective hydrogens in the 14*S*-derivative **23** (δ 2.81 and 3.03, respectively). These differences in the resonances of H-14, H-15 and H-17 between the 14*R*- and 14*S*-stereoisomers were also observed in all the 14,15-epoxides described in this work and permitted them to be assigned the C-14 stereochemistry. In consequence, this is a facile and direct procedure for the assignment of the sterochemistry at C-14 in 14,15-epoxy-13-epi-manoyl oxides.

Another two stereoisomers with structures **16** and **17** and molecular formula $C_{20}H_{32}O_4$ were isolated from this feeding. The molecular ion was not observed in the MS of either, a fragment appearing at *m/z* 321, which is formed by loss of a methyl group. ^{13}C NMR data (Table 2) permitted the C-11 position to be assigned to this alcohol in both metabolites. Their ^1H NMR spectra showed a proton geminal to a hydroxyl group with similar chemical shifts and coupling constants. Thus, this last signal appears in **16** and **17** as a double triplet at δ 4.13 (J=9.1 and 5.4 Hz) and as a broad multiplet at δ 4.29, respectively. In this latter case, double resonance experiments on **17** allowed observation of the couplings of this carbinol with the protons of the C-12 methylene at δ 1.34 and 1.85 (J=10 and 5.0 Hz) and with that of the C-9 methine at δ 1.36 (J=10.2 Hz). These facts indicated a β-equatorial stereochemistry for the alcohol in both substances. Moreover, a nOe effect was observed between H-11 and the methyl groups at C-8 and C-10 in the NOESY spectra of both compounds. These resonance studies indicated that the low energy conformation of ring C was a chair, which was confirmed by molecular mechanics calculations. Thus, these two metabolites must be stereoisomers at C-14, with the structures 11β-hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**16**) and 11β-hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**17**). The stereochemistry at C-14 was determined in the same way as described above for **23** and **24**.

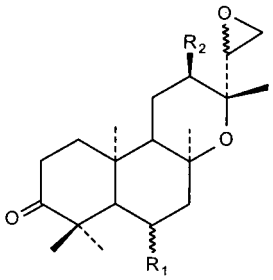
Compound **22** and the acetate **26a**, which have been identified considering their ^1H NMR data, were present as traces, contaminating fractions of **16** and of the acetate **25a**, respectively. Finally, a diol **27** was isolated in the form of its diacetate **27a** by acetylation and chromatography of the fractions containing it. Its mass spectrum does not show the molecular ion, but at *m/z* 407.2418 ($C_{23}H_{35}O_6$) a fragment ion was observed, which is formed by loss of a methyl group. Thus, the molecular formula of the corresponding diol must be $C_{20}H_{34}O_4$. The presence of the 14,15 epoxide was observed in the ^1H NMR spectrum of **27a** as three double doublets at δ 2.81 (J=4.6 and 3.6 Hz), 2.85 (J=4.6 and 3.0 Hz) and 2.97 (1H, dd, J=3.6 and 3.0 Hz), due to H-15 and H-14, respectively. In this spectrum, resonances of protons geminal to two acetoxyl groups at 4.67 (t, J=3.0 Hz) and 4.79 (t, J=4.1 Hz) were also



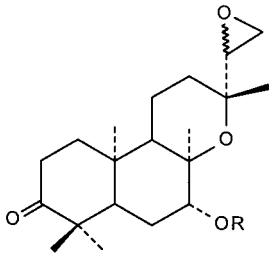
16 (14*S*)
17 (14*R*)



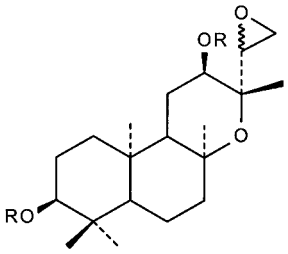
18 R = OH (14*S*)
19 R = H (14*S*)
20 R = H (14*R*)



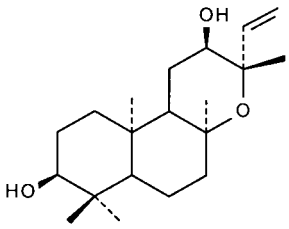
21 R_1 = α-OH, R_2 = H (14*S*)
22 R_1 = β-OH, R_2 = H (14*S*)
23 R_1 =H, R_2 = OH (14*S*)
24 R_1 =H, R_2 = OH (14*R*)



25 R = H (14*R*)
25a R = Ac (14*R*)
26 R = H (14*S*)
26a R = Ac (14*S*)



27 R = H (14*S*)
27a R = Ac (14*S*)



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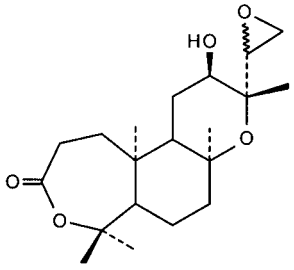
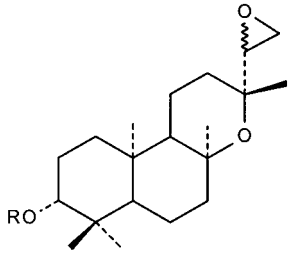


29 (14*S*)
30 (14*R*)



31 R = H (14*R*)
31a R = Ac (14*R*)
32 R = H (14*S*)
32a R = Ac (14*S*)

Scheme 2.

observed, which, considering its molecular formula, indicated that the 3-oxo group of ribenone (**1**) had been reduced to an hydroxyl group during the biotransformation, and that a new hydroxyl group had also been introduced into the molecule. ^{13}C NMR data (Table 3) and 2D NMR studies of the diacetate **27a** (COSY, NOESY, HMQC and HMBC) confirmed the presence of a C-3 acetoxyl and permitted the C-12 position to be assigned to the second acetate group. On the other hand, considering the coupling constants of the geminal hydrogen, an axial stereochemistry was assigned to both acetoxyl groups. The chemical shifts of H-14 (δ 2.97) and the two H-15 (δ 2.81 and 2.85) indicated the 14*S* stereochemistry of the epoxy group, which was confirmed by the nOe effect observed between the two H-15 and H-17 (see above). Therefore, the structure 3β,12β-dihydroxy-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**27**) was assigned to the corresponding diol. An analogous bioreduction of the 3-oxo group of ribenone (**1**) to the 3β-alcohol has been observed in its biotransformation with both *C. lunata*[14] and *G. fujikuroi*,[3] whilst the borohydride reduction of the same group afforded the α-alcohol.[3]

3. Conclusions

In addition to the structure revision of excoecarins A–C and to the ^{1}H NMR method described for the assignment of the stereochemistry at C-14 in this type of diterpene, several

Table 3. ^{13}C NMR spectroscopic data of compounds **25**, **25a**, **27a** and **29**–**32a**

Carbon	25	25a	27a	29	30	31a	32	32a
1	37.6	37.5	32.5	37.1	37.9	37.2	37.4	37.1
2	33.6	33.9	22.6[a]	31.8	31.9	23.6	27.2	23.5
3	215.7	215.6	77.8	174.4	174.6	80.8	78.8	80.7
4	47.0	47.0	36.0	85.5	85.6	37.8	38.8	37.8
5	52.3	52.0	49.5[b]	53.2	52.8	55.4	55.2	55.3
6	27.6	26.4	19.3	25.3	25.8	19.5	19.6	19.5
7	79.8	80.6	42.2	41.4	41.2	42.8	42.8	42.7
8	78.0	76.0.	75.1	74.1	74.7	75.0	74.8	74.7
9	54.6	55.3	50.2[b]	50.2	49.8	57.7	57.2	57.1
10	36.4	36.2	36.6	38.8	38.5	36.5	36.6	36.5
11	16.5	16.6	22.5[a]	24.4	24.7	16.7	16.5	16.5
12	31.7	31.5	68.8	66.1	66.8	32.0	30.9	30.9
13	71.8	71.7	72.9	73.9	74.6	71.7	71.3	71.3
14	58.6	58.8	57.1	57.4	58.5	58.9	57.6	57.6
15	45.7	45.9	46.4	46.2	46.3	45.9	47.5	47.4
16	28.5	28.6	24.3	22.3	21.6	28.0	29.2	29.1
17	16.6	17.3	22.5	23.6	23.1	22.7	23.9	24.0
18	26.7	26.8	27.7	31.4	31.1	28.6	28.0	28.0
19	20.9	20.9	21.4	26.3	26.7	16.3	15.6	16.4
20	15.4	15.3	14.9	16.6	17.4	15.8	15.2	15.7

[a] These values can be interchanged.
[b] These values can be interchanged.

consequences may be deduced from the results obtained in this biotransformation of ribenone (**1**) by *M. plumbeus*: (1) The epoxidation of the vinyl group constitutes the main reaction and there exists a preference for hydroxylation at C-1(α) (**6**, **7** and **18**), C-6(α or β) (**8**, **10**, **12**, **13**, **21** and **22**), and C-12(β) (**5**, **7**, **9**, **10,** **13**, **23**, **24**, **27** and **28**) and, to a lesser degree, at C-7(α) (**14**, **25** and **26**) and C-11(β) (**11**, **16** and **17**). (2) The epoxidations produced in this biotransformation are not stereospecific. This lack of stereospecificity has also been observed in the chemical epoxidation of this type of compounds or in its occurrence in plants, for example in excoecarins A and B, **19** and **20**, previously cited.[15] In all these cases the main stereoisomer obtained was 14*S*. (3) The hydroxylations produced with this fungus are not very regioselective, permitting the functionalization of all the methylene groups of ribenone, except C-2, which, on the other hand, is the most chemically accessible. (4) The metabolites with a 1,2-double bond (**5** and **15**) are probably formed by dehydration of 1α-hydroxy-derivatives. (5) Another observed reaction was the reduction of the 3-oxo group to a 3β-alcohol (**27** and **28**), which is also produced in the biotransformation of **1** by *G. fujikuroi*. The chemical reduction of the 3-oxo group formed the 3α-alcohol.

4. Experimental

4.1. General procedures

Mps were determined with a Reichert Thermovar apparatus and are uncorrected. IR and UV spectra were recorded in a Perkin–Elmer 1600 FT and a Varian Cary 1E spectrophotometer, respectively. ^1H NMR spectra were recorded in $CDCl_3$ solutions at 200.13 and 500.13 MHz, with a Bruker AC-200 or a Bruker AMX2-500 spectrometer, respectively. ^{13}C NMR spectra were run in $CDCl_3$ at 50.32 MHz, with a Bruker AC-200. Chemical shifts are given in ppm (δ). Mass spectra were taken at 70 eV (probe) in a Shimadzu Q2000, and HRMS in a Micromass Autospec spectrometer. Conformations of minimum energy were determined by computational methods employing the *Hyperchem* program from Hypercube. Dry column chromatographies were made on Si gel Merck 0.02–0.063 mm. The fungal strain was *Mucor plumbeus* CMI 116688 and was a gift from Dr J. R. Hanson, School of Chemistry, Physics and Environmental Sciences, University of Sussex, UK.

4.2. Incubation procedure and isolation of products

The fungus *M. plumbeus* was grown in shake cultures at 25°C in conical flasks (250 mL), each containing 50 mL of a sterile medium comprising (per L) glucose (80 g), NH_4NO_3 (0.48 g), KH_2PO_4 (5 g), $MgSO_4$ (1 g) and trace elements solution containing (per 100 mL) $Co(NO_3)_2$ (0.01 g), $CuSO_4$ (0.015 g), $ZnSO_4$ (0.16 g), $MnSO_4$ (0.01 g) and $(NH_4)_6Mo_7O_{24}$ (0.01 g). The substrate (260 mg) dissolved in EtOH (5.2 mL) was equally distributed among the 26 conical flasks after one day of growth. After a further six days, the fermentation was harvested. The mycelium was filtered, and the culture filtrate was extracted with EtOAc. The extract was dried over Na_2SO_4, the solvent evaporated, and the residue chromatographed on a Si gel column using a petroleum ether–EtOAc gradient, giving: starting material (**1**) (5 mg), 3-oxo-1-en-*ent*-13-*epi*-manoyl oxide (**4**) (3 mg), 3-oxo-1-en-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**15**) (2 mg), 6α-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**12**) (0.8 mg), 7α-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**14**) (1.7 mg), 1α-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**6**) (20 mg), 6α-hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**21**) (1.7 mg), 11β-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**11**) (5 mg), 6β-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**8**) (1.3 mg), 12β-hydroxy-3-oxo-1-en-*ent*-13-*epi*-manoyl oxide (**5**) (1.2 mg), 12β-hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**9**) (16 mg), 1α-hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**18**) (4.2 mg), 11β-hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**16**) (1.4 mg), 6β-hydroxy-3-oxo-14*S*,15-*ent*-13-*epi*-manoyl oxide (**22**) (traces), 11β-hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**17**) (1.8 mg), 7α-hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**25**) (0.6 mg), 7α-hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**26**) (traces), 6β,12β-dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**10**) (1 mg), 6α,12β-dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**13**) (1.3 mg), 12β-hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**23**) (3.2 mg), 12β-hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**24**) (2.9 mg), 3β,12β-dihydroxy-*ent*-13-*epi*-manoyl oxide (**28**) (13 mg), 3β,12β-dihydroxy-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (**27**) and 1α,12β-dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (**7**) (16 mg). Compound **27** was identified as the 3β,12β-diacetate **27a** (0.8 mg) by acetylation and chromatography of the fraction containing it.

4.2.1. 12β-Hydroxy-3-oxo-1-en-*ent*-13-*epi*-manoyl oxide (5). Colourless gum; ^1H NMR (500 MHz) δ: 1.00 (3H, s, H-20), 1.09 (3H, s, H-19), 1.16 (3H, s, H-18), 1.22 (3H, s, H-16), 1.33 (3H, s, H-17), 4.19 (1H, br s, H-12), 5.01 (1H, d, J=18.1 Hz, H-15), 5.03 (1H, d, J=11.5 Hz, H-15), 5.88 (1H, d, J=10.2 Hz, H-1), 6.10 (1H, dd, J=18.1, 11.5 Hz, H-14), 7.13 (1H, d, J=10.2 Hz, H-2); EIMS *m/z* (rel. int.):

318 $[M]^+$ (1), 303 (4), 300 (10), 285 (4), 248 (66), 233 (4), 205 (68); $[M]^+$ at *m/z* 318.2190, $C_{20}H_{30}O_3$ requires 318.2194.

4.2.2. Acetate (5a). IR (film) ν_{max} 2930, 1740, 1670, 1460, 1380, 1240, 1080 cm^{-1}; UV (EtOH) λ_{max} 226 nm (ϵ=4.99×10^3), ^{1}H NMR (500 MHz) δ: 0.97 (3H, s, H-20), 1.08 (3H, s, H-19), 1.13 (3H, s, H-18), 1.17 (3H, s, H-16), 1.33 (3H, s, H-17), 2.14 (3H, s), 5.05 (1H, d, *J*=11.4 Hz, H-15), 5.11 (1H, d, *J*=18.2 Hz, H-15), 5.44 (1H, t, *J*=3.0 Hz, H-12), 5.88 (1H, d, *J*=10.1 Hz, H-1), 65.06 (1H, dd, *J*=18.2, 11.4 Hz, H-14), 6.98 (1H, d, *J*=10.1 Hz, H-2); EIMS *m/z* (rel. int.): 360 $[M]^+$ (1), 345 (3), 333 (1), 300 (2), 290 (3), 285 (5), 248 (40), 230 (14), 215 (8), 204 (100); $[M]^+$ at *m/z* 360.2283, $C_{22}H_{32}O_4$ requires 360.2300.

4.2.3. 1α,12β-Dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (7). Colourless oil; IR (film) ν_{max} 3420, 2980, 2940, 2870, 1700, 1460, 1390, 1290, 1240, 1090 cm^{-1}; ^{1}H NMR (500 MHz) δ: 0.80 (3H,s, H-20), 1.03 (3H, s, H-19), 1.07 (3H, s, H-18), 1.20 (3H, s, H-16), 1.28 (3H, s, H-17), 1.50 and 1.81 (each 1H, m, H-7), 1.68 (1H, m, H-6), 1.89 (1H, dd, *J*=13.0, 2.4 Hz, H-9), 2.02 (1H, td, *J*=13.8, 2.8 Hz, H-11), 2.27 (1H, ddd, *J*=13.8, 4.0, 2.6 Hz, H-11), 2.36 (1H, dd, *J*=14.9, 5.0 Hz, H-2), 2.96 (1H, dd, *J*=14.9, 7.8 Hz, H-2), 3.98 (1H, dd, 7.8, 5.0 Hz, H-1), 4.11 (1H, t, *J*=3.3 Hz, H-12), 4.97 (1H, d, *J*=18.2, H-15), 4.99 (1H, d, *J*= 11.4 Hz, H-15), 6.07 (1H, dd, *J*=18.2, 11.4 Hz, H-14); EIMS *m/z* (rel. int.): 318 $[M-H_2O]^+$ (15), 300 (66), 285 (16), 257 (7), 248 (21), 235 (7), 223 (10), 205 (33); $[M-H_2O]^+$ at *m/z* 318.2208, $C_{20}H_{30}O_3$ requires 318.2194.

4.2.4. 6β,12β-Dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (10). Colourless prisms, mp 88–91°C (benzene–EtOAc); ^{1}H NMR (500 MHz) δ: 0.65 (3H, s, H-20), 1.21 (3H, s, H-16), 1.28 (3H, s, H-17), 1.34 (3H, s, H-19), 1.57 (3H, s, H-18), 1.68 (1H, dd, *J*=11.4, 3.7 Hz, H-11), 1.79 (1H, d, *J*=3.7 Hz, H-11), 2.11 (1H, dd, *J*=11.6, 4.4 Hz, H-7), 2.31 (1H, m, H-2), 2.74 (1H, m, H-2), 3.86 (1H, td, 11.6, 4.4 Hz, H-6), 4.13 (1H, t, *J*=3.7 Hz, H-12), 4.97 (1H, d, *J*=18.2, H-15), 5.01 (1H, d, *J*=11.6 Hz, H-15), 6.07 (1H, dd, *J*=18.2, 11.6 Hz, H-14); EIMS *m/z* (rel. int.): 336 $[M]^+$ (3), 318 (2), 301 (1), 266 (13), 248 (10), 223 (77), 205 (66); $[M]^+$ at *m/z* 336.2300, $C_{20}H_{32}O_4$ requires 336.2300.

4.2.5. 6α-Hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (12). ^{1}H NMR (500 MHz) δ: 1.13 (3H, s, H-18), 1.16 (3H, s, H-16), 1.33 (3H, s, H-20), 1.40 (3H, s, H-19), 1.57 (3H, s, H-17), 1.35 (1H, d, *J*=3.3 Hz, H-5), 1.66 (1H, dd, *J*=15.1, 3.3, H-7), 1.95 (1H, ddd, *J*=6.2, 2.9 Hz, H-1), 2.85 (1H, td, *J*=14.6, 6.2 Hz, H-2), 4.50 (1H, t, *J*=3 Hz, H-6), 4.95 (1H, d, *J*=11.0 Hz, H-15), 4.99 (1H, d, *J*=17.8 Hz, H-15), 6.04 (1H, dd, *J*=17.8, 11.0 Hz, H-14); EIMS *m/z* (rel. int.): 320 $[M]^+$ (1), 305 (100), 287 (18), 269 (24), 250 (6), 235 (1), 232 (2), 217 (100), 199 (10); $[M]^+$ at *m/z* 320.2360, $C_{20}H_{32}O_3$ requires 320.2351.

4.2.6. 6α,12β-Dihydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (13). Colourless gum; ^{1}H NMR (500 MHz) δ: 1.17 (3H, s, H-18), 1.19 (3H, s, H-16), 1.35 (3H, s, H-20), 1.41 (3H, s, H-19), 1.60 (3H, s, H-17), 1.37 (1H, d, *J*=3.0 Hz, H-5), 1.71 (1H, dd, *J*=14.0, 2.9 Hz, H-7), 1.91 (1H, m, H-1), 2.00 (1H, dd, *J*=14.0, 6.0 Hz, H-7), 2.03 (1H, dd, *J*=13.8, 3.5 Hz, H-11), 2.31 (1H, m, H-2), 2.84 (1H, dd, *J*=14.3, 6.4 Hz, H-2), 4.15 (1H, t, *J*=3.5 Hz, H-12), 4.52 (1H, t, *J*=6.0, 3.0 Hz, H-6), 4.98 (1H, d, *J*=17.5 Hz, H-15), 5.01 (1H, d, *J*=11.2 Hz, H-15), 6.09 (1H, dd, *J*=17.5 Hz, H-15), 5.01 (1H, d, *J*=11.2 Hz, H-15), 6.09 (1H, dd, *J*=17.5, 11.2 Hz, H-14; EIMS *m/z* (rel. int.): 336 $[M]^+$ (1), 321 (2), 318 (6), 303 (3), 300 (3), 293 (14), 285 (4), 248 (7), 223 (81), 205 (88); $[M]^+$ at *m/z* 336.2299, $C_{20}H_{32}O_4$ requires 336.2300.

4.2.7. 7α-Hydroxy-3-oxo-*ent*-13-*epi*-manoyl oxide (14). Colourless gum; ^{1}H NMR (500 MHz) δ: 0.84 (3H, s, H-20), 1.03 (3H, s, H-19), 1.11 (3H, s, H-18), 1.13 (3H, s, H-16), 1.23 (3H, s, H-17), 1.48 (1H, m, H-6), 1.77 (1H, ddd, *J*=13.0, 4.3, 3.1 Hz, H-6), 3.58 (1H, dd, *J*=11.6, 4.3 Hz, H-7), 4.96 (1H, d, *J*=11.4 Hz, H-15), 4.99 (1H, d, *J*=18.2 Hz, H-15), 5.97 (1H, dd, *J*=18.2, 11.4 Hz, H-14); EIMS *m/z* (rel. int.): 320 $[M]^+$ (2), 305 (80), 302 (1) 287 (28), 275 (5), 269 (10), 250 (6), 235 (9), 217 (8); $[M]^+$ at *m/z* 336.2310, $C_{20}H_{32}O_3$ requires 336.2300.

4.2.8. Acetate (14a). ^{1}H NMR (500 MHz) δ: 0.83 (3H, s, H-20), 1.01 (3H, s, H-18), 1.08 (3H, s, H-19), 1.10 (3H, s, H-16), 1.28 (3H, s, H-17), 1.45 (2H, complex signal, H-1 and H-6), 1.77 (1H, dt, *J*=12.0, 4.5 Hz, H-6), 1.84 (1H, m, H-1), 2.09 (3H, s), 2.50 (1H, dd, *J*=8.6, 6.1 Hz, H-2), 4.77 (1H, dd, *J*=12.0, 4.5 Hz, H-7), 4.93 (1H, d, *J*=11.0 Hz, H-15), 4.96 (1H, d, *J*=18.0 Hz, H-15), 5.96 (1H, dd, *J*=18.0, 11.0 Hz, H-14); EIMS *m/z* (rel. int.): 362 $[M]^+$ (3), 347 (100), 305 (14), 287 (93), 277 (5), 269 (52), 253 (18), 232 (27), 217 (33), 203 (23),; $[M]^+$ at *m/z* 362.2468, $C_{22}H_{34}O_4$ requires 362.2457.

4.2.9. 3-Oxo-1-en-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (15). Colourless gum; IR (film) ν_{max} 2930, 1735, 1670, 1460, 1380, 1260, 1110, 1080 cm^{-1}; UV (EtOH) λ_{max} 227 nm (ϵ=6.44×10^3) ^{1}H NMR (500 MHz) δ: 1.01 (3H, s, H-20), 1.09 (3H, s, H-19), 1.17 (3H, s, H-18), 1.24 (3H, s, H-16), 1.36 (3H, s, H-17), 2.82 (1H, dd, *J*=4.6, 2.9 Hz, H-15), 2.86 (1H, dd, *J*=4.6, 3.9 Hz, H-15), 2.95 (1H, dd, *J*=3.9, 2.9 Hz, H-14), 5.88 (1H, d, *J*=10.2 Hz, H-1), 7.07 (1H, d, *J*=10.2 Hz, H-2); EIMS *m/z* (rel. int.): 318 [M]+ (1), 303 (23), 275 (100), 257 (50), 217 (31), 199(35); $[M]^+$ at *m/z* 318.2198, $C_{20}H_{30}O_3$ requires 318.2194.

4.2.10. 11β-Hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (16). ^{1}H NMR (500 MHz) δ: 1.01 (3H, s, H-20), 1.03 (3H, s, H-19), 1.09 (3H, s, H-18), 1.23 (3H, s, H-17), 1.31 (3H, s, H-16), 1.46 (2H, m, H-9 and H-12β), 1.78 (1H, m, H-1), 2.21 (1H, dd, *J*=14.6, 5.4 Hz, H-12α), 2.24 (1H, m, H-1), 2.42 (1H, ddd, *J*=15.8, 7.3, 3.9 Hz, H-2α), 2.51 (1H, ddd, *J*=15.8, 10.4, 7.4 Hz, H-2β), 2.72 (1H, dd, *J*=5.0, 4.1 Hz, H-15), 2.81(2H, m, H-14 and H-15), 4.13 (1H, dt, *J*=9.1, 5.4 Hz, H-11); EIMS *m/z* (rel. int.): 321 $[M-CH_3]^+$ (6), 303 (3), 293 (100), 275 (68), 257 (37), 235 (15), 217 (12), 207 (10), 201 (6), 199 (10); $[M-CH_3]^+$ at *m/z* 321.2064, $C_{19}H_{29}O_4$ requires 321.2065.

4.2.11. 11β-Hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (17). Colourless gum; ^{1}H NMR (500 MHz) δ: 1.04 (3H, s, H-20), 1.06 (3H, s, H-19), 1.12 (3H, s, H-18), 1.24 (3H, s, H-16), 1.42 (3H, s, H-17), 1.35 (2H, m, H-9 and H-12β), 1.74 (1H, m, H-1), 1.85 (1H, dd, 13.2, 5.0 Hz, H-12α), 2.46 (1H, m, H-2), 2.48 (2H, m, H-1 and H-2),

2.56 (1H, dd, J=4.4, 2.9 Hz, H-15), 2.83 (1H, t, J=4.4 Hz, H-15), 3.04 (1H, dd, J=4.4, 2.9 Hz, H-14), 4.29 (1H, br m, $W_{1/2}$=30 Hz, H-11); EIMS m/z (rel. int.): 336 $[M]^+$ (1), 321 (7), 303 (9), 293 (100), 275 (51), 257 (20), 235 (14), 217 (11), 201 (5); $[M]^+$ at m/z 336.2298, $C_{20}H_{32}O_4$ requires 336.2300.

4.2.12. 1α-Hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (18). White needles, mp 134–135°C (petroleum ether–EtOAc), $[\alpha]_D$=−52 (c 0.21, $CHCl_3$); ^{1}H NMR (500 MHz) δ: 0.81 (3H, s, H-20), 1.04 (3H, s, H-19), 1.09 (3H, s, H-18), 1.22 (3H, s, H-16), 1.32 (3H, s, H-17), 1.36 (1H, dt, J=12.5, 5.7 Hz, H-12), 1.44 (1H, dd, J=12.2, 2.4 Hz, H-9), 1.47 (3H, br m, H-5, H-6 and H-7), 1.49 (1H, m, H-11α), 1.61 (1H, m, H-12), 1.69 (1H, m, H-6), 1.83 (1H, m, H-7), 2.06 (1H, m; H-11β), 2.35 (1H, dd, J=14.8, 4.9 Hz, H-2β), 2.81 (2H, m, H-15), 2.92 (1H, t, J=3 Hz, H-14), 2.97 (1H, dd, J=14.8, 7.8 Hz, H-2α), 3.94 (1H, dd, J=7.8, 4.9 Hz, H-1); EIMS m/z (rel. int.): 321 $[M-CH_3]^+$ (1), 303 (2), 293 (56), 275 (100), 257 (34), 217 811), 201 (7), 199 (10); $[M-CH_3]^+$ at m/z 321.2063, $C_{19}H_{29}O_4$ requires 321.2065.

4.2.13. 6α-Hydroxy-3-oxo-14*S*,15-*ent*-13-*epi*-manoyl oxide (21). Colourless gum; ^{1}H NMR (500 MHz) δ: 1.15 (3H, s, H-18), 1.19 (3H, s, H-16), 1.28 (1H, d, J=3.0 Hz, H-5), 1.33 (3H, s, H-20), 1.39 (3H, s, H-19), 1.59 (3H, s, H-17), 1.68 (1H, dd, J=14.0, 3.0 Hz, H-7), 1.89 (1H, ddd, J=14.0, 6.1, 2.8 Hz, H-2), 2.00 (1H, dd, J=14, 3.0 Hz, H-7), 2.25 (1H, ddd, 14, 4.5, 2.8 Hz, H-1), 2.81 (3H, m, 2H-15 and H-2), 2.92 (1H, t, J=3.2 Hz, H-14), 4.49 (1H, t, J=3.0 Hz, H-6); EIMS m/z (rel. int.): 336 $[M]^+$ (1), 321 (22), 303 (8), 293 (100), 275 (40), 257 (64), 215 (15), 199 (18); $[M]^+$ at m/z 336.2310, $C_{20}H_{32}O_4$ requires 336.2300).

4.2.14. 6β-Hydroxy-3-oxo-14*S*,15-*ent*-13-*epi*-manoyl oxide (22). This compound was observed contaminating a fraction of **15**, ^{1}H NMR (500 MHz) δ: 0.67 (3H, s, H-20), 1.23 (3H, s, H-16), 1.26 (3H, s, H-19), 1.29 (3H, s, H-17), 1.32 (3H, s, H-18), 2.10 (1H, dd, J=11.5, 3.9 Hz, H-7α), 2.75 (1H, dd, J=4.8, 3.0 Hz, H-15), 2.80 (1H, t, J=4.0 Hz, H-14), 2.89 (1H, t, J=3.0 Hz, H-14), 3.82 (1H, br m, H-6).

4.2.15. 12β-Hydroxy-3-oxo-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (23). White needles, mp 142–145°C (petroleum ether–CH_2Cl_2), $[\alpha]_D$=−46 (c 0.16, $CHCl_3$); ^{1}H NMR (500 MHz) δ: 0.89 (3H, s, H-20), 1.03 (3H, s, H-19), 1.10 (3H, s, H-18), 1.26 (3H, s, H-16), 1.27 (3H, s, H-17), 1.64 (1H, m, H-11), 1.87 (1H, dd, J=13.0, 5.0 Hz, H-11), 2.43 (1H, m, H-2β), 2.51 (1H, m, H-2α), 2.81 (1H, dd, 4.6, 4.0 Hz, H-15), 2.86 (1H, dd, J=4.6, 2.9 Hz, H-15), 3.03 (1H, dd, J=4.0, 2.9 Hz, H-14), 3.71 (1H, br d, J=4.4 Hz, H-12); EIMS m/z (rel. int.): 336 $[M]^+$ (1), 321 (5), 318 (1), 303 (1), 293 (53), 275 (12), 257 (7), 249 (21), 235 (2), 206 (100); $[M]^+$ at m/z 336.2295, $C_{20}H_{32}O_4$ requires 336.2300).

4.2.16. 12β-Hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (24). Colourless needles, mp 178–180°C (petroleum ether–CH_2Cl_2), $[\alpha]_D$=−42 (c 0.21, $CHCl_3$); ^{1}H NMR (500 MHz) δ: 0.88 (3H, s, H-20), 1.01 (3H, s, H-19), 1.08 (3H, s, H-18), 1.14 (3H, s, H-16), 1.42 (3H, s, H-17), 2.03 (1H, td, J=14 and 4 Hz, H-11α), 2.44 (1H, m, H-2β), 2.52 (1H, m, H-2α), 2.56 (1H, dd, J=4.4, 2.9 Hz, H-15), 2.82 (1H, t, J=4.4 Hz, H-15), 3.11 (1H, dd, J=4.4, 2.9 Hz, H-14), 3.89 (1H, br d, J=4.0 Hz, H-12); EIMS m/z (rel. int.): 336 $[M]^+$ (1), 321 (6), 318 (2), 303 (1), 293 (47), 275 (10), 257 (5), 249 (18), 235 (2), 217 (2), 206 (100), 191 (51); $[M]^+$ at m/z 336.2295, $C_{20}H_{32}O_4$ requires 336.2300.

4.2.17. 7α-Hydroxy-3-oxo-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (25). ^{1}H NMR (500 MHz) δ: 0.90 (3H, s, H-20), 1.05 (3H, s, H-19), 1.11 (6H, s, H-16 and H-18), 1.44 (3H, s, H-17), 1.52 and 1.79 (each 1H, m, H-6), 2.51 (3H, m, H-15 and H-2), 2.82 (1H, t, J=4.0 Hz, H-15), 3.09 (1H, t, J=4.0 Hz, H-14), 3.63 (1H, dd, J=11.5, 4.3 Hz, H-11); EIMS m/z (rel. int.): 336 [M]+ (1), 321 (2), 293 (100), 275 (32), 257 (15), 217 (8), 199 (7); $[M]^+$ at m/z 336.2308, $C_{20}H_{32}O_4$ requires 336.2300.

4.2.18. Acetate (25a). ^{1}H NMR (500 MHz) δ: 0.87 (3H, s, H-20), 1.03 (3H, s, H-19), 1.07 (3H, s, H-16), 1.11 (3H, s, H-18), 1.49 (3H, s, H-17), 1.47 and 1.84 (each 1H, m, H-1), 1.74 (1H, dd, J=12.6, 3.0 Hz, H-5), 1.78 (2H, m, H-6), 2.48 (3H, m, 2H-15 and H-2), 2.81 (1H, t, J=4.0 Hz, H-15), 3.07 (1H, t, J=4.0 Hz, H-14), 4.85 (1H, dd, J=11.7, 4.6 Hz, H-7), EIMS m/z (rel. int.): 378 [M]+ (1), 335 (78), 275 (100), 257 (83), 239 (6), 217 (21), 199 (14); $[M]^+$ at m/z 378.2408, $C_{22}H_{34}O_5$ requires 378.2406.

4.2.19. 7α-Acetoxy-14*S*,15-epoxy-*ent*-13-epi-manoil oxide (26a). This compound was observed contaminating **24a**, ^{1}H NMR (500 MHz) δ: 0.86 (3H, s, H-20), 1.02 (3H, s, H-19), 1.11 (3H, s, H-18), 1.18 (3H, s, H-16), 1.35 (3H, s, H-17), 2.77 (1H, dd, J=4.9, 2.8 Hz, H-15), 2.81 (1H, t, J=4.9 Hz, H-15), 2.87 (1H, t, J=2.8 Hz, H-14), 4.85 (1H, dd, J= 11.7, 4.6 Hz, H-7).

4.2.20. 3β,12β-Diacetoxy-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (27a). White prisms, mp 184–186°C (petroleum ether–EtOAc), $[\alpha]_D$=−38 (c 0.04, $CHCl_3$); ^{1}H NMR (500 MHz) δ: 0.79 (3H, s, H-20), 0.87 (3H, s, H-18), 0.89 (3H, s, H-19), 1.12 and 1.35 (each 1H, m, H-1), 1.20 (3H, s, H-16), 1.31 (3H, s, H-17), 1.51 (1H, dd, J=11.8, 2.4 Hz, H-5), 1.64 (1H, m, H-2), 1.67 (1H, dd, J=13.0, 4.1 Hz, H-11), 1.73 (1H, dd, J=12.5, 3.0 Hz, H-9), 1.79 (1H, dd, J=13.0, 4.1 Hz, H-11), 1.84 and 1.87 (each 1H, m, H-7), 1.90 (1H, m, H-2), 2.09 and 2.11 (each 3H, s), 2.81 (1H, dd, J=4.6, 3.6 Hz, H-15), 2.85 (1H, dd, J=4.6, 3.0 Hz, H-15), 2.97 (1H, dd, J=3.6, 3.0 Hz, H-14), 4.67 (1H, t, J=3.0 Hz, H-3), 4.67 (1H, t, J=3.0 Hz, H-3), 4.79 (1H, t, J=4.1 Hz, H-12); EIMS m/z (rel. int.): 407 $[M-CH_3]^+$ (9), 379 (16), 347 (6), 319 (64), 302 (5), 259 (46), 250 (20), 241 (52), 201 (10); $[M-CH_3]^+$ at m/z 407.2418, $C_{23}H_{35}O_6$ requires 407.2433.

4.2.21. Epoxidation of 9. Compound **9** (15 mg) in $CHCl_3$ (2 mL) was treated with *m*-chloroperbenzoic acid (11 mg) at 0°C for 6 h and washed with a saturated solution of $NaHCO_3$. Usual work-up and chromatograpy of the residue, eluting with petrol–EtOAc (1:1), afforded **23**, **24**, **29** and **30**. The first two compounds were identical with those obtained in this incubation.

4.2.22. Compound 29. ^{1}H NMR (500 MHz) δ: 1.01 (3H, s, H-20), 1.26 (3H, s, H-16), 1.29 (3H, s, H-17), 1.41 (3H, s,

H-19), 1.49 (3H, s, H-18), 2.59 (1H, dq, J=15, 11.1, 7.1, 3.4 Hz, H-2β), 1.90 (1H, dd, J=12, 3.5 Hz, H-5), 2.67 (1H, dq, J=15.5, 15, 11.1, 4.4 Hz, H-2α), 2.81 (1H, t, J=4.5 Hz; H-15), 2.85 (1H, dd, J=4.5, 3.2 Hz, H-15), 3.02 (1H, t, J=3.2 Hz, H-14), 3.70 (1H, br s, H-12); EIMS m/z (rel. int.): 352 $[M]^+$ (2), 337 (4), 334 (4), 316 (2), 309 (24), 291 (19), 223 (36), 154 (38), 164 (100), 149 (47); $[M]^+$ at m/z 352.2249, $C_{20}H_{32}O_5$ requires 352.2250.

4.2.23. Compound 30. White needles, mp 138–140°C (petroleum ether–EtOAc), $[\alpha]_D$=−35 (c 0.26, $CHCl_3$); IR (film) ν_{max} 3460, 2930, 2860, 1720, 1455, 1380, 1295, 1255, 1110 cm^{-1}; ^1H NMR (500 MHz) δ: 0.98 (3H, s, H-20), 1.11(3H, s, H-16), 1.38 (3H, s, H-17), 1.42 (3H, s, H-19), 1.46 (3H, s, H-18), 2.53 (1H, m, H-2β), 2.55 (1H, t, J= 4.3, Hz, H-15), 2.83 (1H, t, J=4.4 Hz, H-15), 3.10 (1H, t, J=3.0 Hz), 3.38 (1H, t, J=3.3 Hz); EIMS m/z (rel. int.): 352 $[M]^+$ (1), 337 (5), 309 (17), 291 (14), 223 (31), 164 (100), 149 (40); $[M]^+$ at m/z 352.2200, $C_{20}H_{32}O_5$ requires 352.2250.

4.2.24. Epoxidation of ribenol acetate. Compound **2a** (40 mg) was epoxidised as described above for **9** giving a mixture of the two stereoisomers at C-14 (34.5 mg). Chromatography of the mixture by recycled HPLC, using as solvent n-hexane–EtOAc (8:2), afforded (**32a**) (9 mg) and (**31a**) (2 mg).

4.2.25. 3α-Acetoxy-14*R*,15-epoxy-*ent*-13-*epi*-manoyl oxide (31a). This compound was obtained slightly contaminated by (**32a**), ^1H NMR (500 MHz) δ: 0.80 (3H, s, H-20), 0.83 (3H, s, H-19), 0.87 (3H, s, H-18), 1.01 (1H, dd, J=12.1, 2.1 Hz, H-5), 1.10 (3H, s, H-16), 1.14 (1H, dd, J=12.4, 2.6 Hz, H-9), 1.40 (3H, s, H-17), 1.50 (1H, dt, J=13.3, 3.7 Hz, H-12), (1.82 (1H, dt, J=12, 3.1 Hz, H-7), 2.04 (3H, s), 2.49 (1H, dd, J=4.7, 3.1 Hz, H-15), 2.78 (1H, t, J=4.2 Hz, H-15), 3.09 (1H, t, J=3.6 Hz, H-14), 4.47 (1H, dt, J=12, 4.7 Hz, H-3).

4.2.26. 3α-Acetoxy-14*S*,15-epoxy-*ent*-13-*epi*-manoyl oxide (32a). Colourless needles, mp 200–201 °C, (petroleum ether–EtOAc), $[\alpha]_D$=−28 (c 0.15, $CHCl_3$); IR (film) ν_{max} 2945, 1730, 1480, 1455, 1365, 1250, 1105, 1020, 985; ^1H NMR (500 MHz) δ: 0.76 (3H, s, H-20), 0.82 (3H, s, H-19), 0.85 (3H, s, H-18), 1.01 (1H, dd, J=12.2, 2.4 Hz, H-5), 1.06 (1H, td, J=13.6, 3.6 Hz, H-1β), 1.19 (3H, s, H-16), 1.28 (3H, s, H-17), 1.42 (1H, td, J=12.8, 3.6 Hz, H-7β), 1.47 (1H, m, H-11), 1.80 (1H, dt, J=12.1, 3.3 Hz, H-7α), 2.77 (1H, dd, J=4.8, 3.2 Hz, H-15), 2.79 (1H, t, J=4.7, H-15), 2.89 (1H, t, J=3.1 Hz, H-14), 4.45 (1H, dd, J=11.7, 5.0 Hz, H-3); EIMS m/z (rel. int.): 364 $[M]^+$ (0.2), 349 (7), 321 (56), 261 (86), 243 (100), 203 (24), 201 (32), 191 (12), 189 (11), 187 (17), 173 (9), 147 (9), 135 (52), 121 (19); $[M]^+$ at m/z 364.2613, $C_{22}H_{36}O_4$ requires 364.2614.

4.2.27. Hydrolysis of 32a. Compound **32a** (5 mg) in methanol (3 mL) was stirred with aq. sat. potassium carbonate (1.5 mL) for 6 h at 40°C. The methanol was eliminated at vacuum, water was added and the mixture extracted with chloroform, in the usual way, giving **32** (3 mg): mp 179–180°C (petroleum ether–EtOAc) (lit.[14] 183–185°C), $[\alpha]_D$= −11 (c 0.81, $CHCl_3$) (lit.[14] −30.7); ^1H NMR (500 MHz) δ: 0.75 (3H, s, H-20), 0.76 (3H, s, H-19), 0.92 (1H, dd, J=11.8, 2.4 Hz, H-5), 0.98 (1H, td, J=12.5, 3.8 Hz, H-1), 1.19 (3H, s, H-16), 1.26 (3H, s, H-17), 1.40 (3H, dt, J=13.2, 3.6 Hz, H-7β), 1.48 (1H, m, H-11), 1.81 (1H, dt, J=12.3, 3.2 Hz, H-7α), 2.78 (1H, dd, J=4.8, 2.9 Hz, H-15), 2.80 (1H, t, J=3.8 Hz, H-15), 2.90 (1H, t; J=3.1 Hz, H-14), 3.21 (1H, J=11.5, 4.0 Hz, H-3); EIMS m/z (rel. int.): 322 (0.2), 307 (6), 279 (50), 261 (61), 243 (100), 203 (25), 189 (29), 187 (30), 173 (16), 159 (20), 147 (21), 135 (77), 121 (45). $[M]^+$ at m/z 322.2521, $C_{20}H_{34}O_3$ requires 322.2508.

4.2.28. Crystallographic data for compound 32a. Colourless needles, $C_{22}H_{36}O_4$, M_w 364.5, orthorhombic, space group $P2_12_12_1$, a=7.330(2) Å, b=11.083(2) Å, c= 25.311(5) Å, V=2056.2(8) Å^3, Z=4, D_c=1.18 g cm^{-3}, $F(000)$=800, μ(Mo Kα)=0.079 mm^{-1}.

The intensity data of all unique reflections within θ range 2.5–26.3° were collected at 273 K in an Enraf–Nonius CAD4 diffractometer, using Mo Kα radiations and graphite monochromator. Three standard reflections monitored every 2 h of X-ray exposure showed not significative intensity variation. A total of 1610 unique reflections were recorded, of which 759 were considered as observed under $Fo>4\sigma Fo$. The intensities were corrected for Lorentz and polarisation factors, but no absorption correction was made. The structure was solved by direct methods using SHELXS86,[17] refinement was performed with SHELXL93,[18] using full-matrix least squares with anisotropic thermal parameters for non-H atoms (the C-15 was kept isotropic). The H-atoms were placed in calculated positions and added to the refinement as fixed isotropic contribution. The refinement has converged at R_1=6.17% and wR_2=14.37% with a goodness of fit on F^2 of 1.007, the largest peak on the final difference map was 0.345 e/Å.^{3} Crystallographic data of **32a**, including atomic coordinates, have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (Fax: +44-(0)1223-336033, e-mail: deposit@ccdc.cam.ac.uk).

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