Microbiological Transformation of Manoyl Oxide Derivatives by *Mucor plumbeus*

Braulio M. Fraga,*,† Pedro González,‡ Ricardo Guillermo,‡ and Melchor G. Hernández†

Instituto de Productos Naturales y Agrobiología, CSIC, P.O. Box 195, La Laguna, 38206-Tenerife, Canary Islands, Spain, and Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Tenerife, Spain

Received April 7, 1998

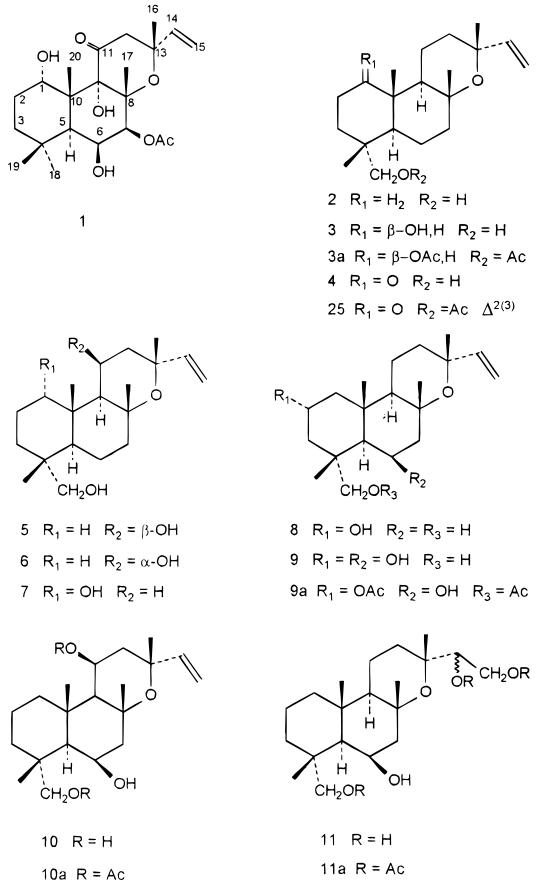
Biotransformations of jhanol (18-hydroxymanoyl oxide) (**2**), jhanidiol (1β,18-dihydroxymanoyl oxide) (**3**), and 1-oxo-jhanol (1-oxo-18-hydroxymanoyl oxide) (**4**) by the fungus *Mucor plumbeus* have been studied. In the incubation of **2** there exists a preference for hydroxylation at C-2(α) (**8**) and C-6(β) (**9**−**11**) and, to a lesser degree, at C-1(α) (**7**), C-11(α) (**6**), and C-11(β) (**5** and **10**). In the second substrate (**3**), the presence of a 1β-hydroxyl group inhibits 6β- or 11-hydroxylation. Epoxidation of the vinyl group constitutes the main reaction, with the positions 2α (**14**) and 3β (**15**) being hydroxylated. In the incubation of **4**, there was a preference for 6β-hydroxylation (**21**) or epoxidation of the vinyl group (**22**). Other hydroxylations observed were at the 2α (**19**), 2β (**20**), 3α (**23**), 3β (**24**), and 11β (**18**) positions.

In a continuation of studies of the microbiological transformation of diterpenoids with fungi we have examined the biotransformation of three manoyl oxide derivatives with *Mucor plumbeus* (Mucoraceae), a fungus with a low substrate specificity. The purpose was to obtain substances with functionality similar to that of forskolin[1] (**1**). In previous work we studied incubation of the same compounds with *Gibberella fujikuroi*,[2] a fungus that, despite possessing high substrate specificity, produces an enantiomeric derivative of manoyl oxide. The fungus *M. plumbeus* has been used previously for biotransformations, for example, with sesquiterpenes possessing the cedrane[3] and aromadendrane[4] skeletons and with diterpenes of the labdane type.[5−7] In the latter case, the main compounds isolated were hydroxylated at ring A.

Results and Discussion

The substrates used were jhanol (**2**), jhanidiol (**3**), and 1-oxo-jhanol (**4**). The first two diterpenes had been isolated from *Eupatorium jhanii*,[8] a plant that grows in the Andean region of Venezuela, and the last was a synthetic sample obtained chemically from **3** or microbiologically from **2**.[2] The incubation of **2** led to the isolation of compounds **5**−**11**. The substances **5**−**7** had been obtained in the incubation of this compound with *G. fujikuroi*[2] and were identified by direct comparison.

The structure of 2α,18-dihydroxymanoyl oxide (**8**) was given to one of the metabolites, on the basis of the following considerations: HRMS showed a peak at *m*/*z* 307.2273, formed by the loss of a methyl group from the molecular ion. Thus, the molecular formula of this product was $C_{20}H_{34}O_3$, indicating that a new oxygen had been introduced into the molecule of **2**. This oxygen function was a secondary alcohol, because in the ^{1}H NMR spectrum a proton geminal to a hydroxyl group appeared as a triplet of triplets at δ 3.99. The multiplicity of this resonance can only be explained by a 2β-hydrogen, with two adjacent methylene groups, in which the triplet of triplets arises from the equivalent coupling observed with H-1(α) and H-3(α) (J = 11.2 Hz) and with H-1(β) and H-3(β) (J = 4.5 Hz). Thus, the alcohol must be situated at C-2 with an α-stereochemistry, a conclusion that was confirmed by the ^{13}C NMR data (Table 1).



Another compound obtained in this feeding was **9**. Its HRMS was in accordance with the molecular formula $C_{20}H_{34}O_4$, indicating that two new oxygen atoms had been introduced into **2** during the fermentation. The ^{1}H NMR spectrum showed two protons geminal to hydroxylic functions. One of these resonated at δ 4.08 as a triplet of triplets, in a manner similar to that observed in **7**, which indicated that this hydroxyl must be situated at the 2α position. The hydrogen geminal to the second alcohol group

* To whom correspondence should be addressed. Tel.: 34-22251728. Fax: 34-22260135. E-mail: bmfraga@ipna.csic.es.
† Instituto de Productos Naturales.
‡ Instituto Universitario de Bio-Orgánica.

10.1021/np980133m CCC: $15.00
Published on Web 08/05/1998

Table 1. ^{13}C NMR Data of Compounds **8**, **9**, **9a**, **10a**, **12**, and **13a**

carbon	**8**	**9**	**9a**	**10a**	**12**	**13a**
1	47.6	49.7	45.8	37.7	79.3	81.2
2	65.0	65.1	68.0	17.7	29.1	24.2
3	44.5	46.6	42.8	40.7	33.1[a]	33.3
4	38.4	38.4	38.4	37.3[a]	37.3	36.1
5	48.6	51.0	51.2	52.8	48.1	48.8
6	19.3	68.1	68.4	68.6	19.5	19.3
7	42.6	50.3	50.7	52.3	42.4	42.4
8	74.9	74.5	74.1	73.8	65.0	74.3
9	55.6	56.3	56.6	56.7	58.0	57.3
10	39.0	39.7	38.4	37.8[a]	42.6	41.3
11	15.4	15.4	15.4	66.8	17.7	17.5
12	35.6	35.8	35.8	40.7	33.4[a]	36.3
13	73.4	73.4	73.3	72.0	70.9	71.7
14	147.6	147.8	147.8	147.5	59.9	43.2
15	110.4	110.4	110.2	110.6	43.9	61.3
16	28.3	28.3	28.3	28.8[b]	24.8[b]	27.7
17	25.5	26.5	26.4	28.1[b]	24.2[b]	25.1
18	71.2	71.5	72.0	72.9	71.4	71.8
19	18.1	20.4	20.1	19.5	16.8	16.9
20	16.9	18.3	18.2	18.2	11.9	13.1

[a,b] These values may be interchanged.

Table 2. ^{13}C NMR Data of Compounds **14a**, **16**, **16a**, **17**, **20**, and **23**

carbon	**14a**	**16**	**16a**	**17**	**20**	**23**
1	78.4	79.1	81.2	215.7	217.0	211.0
2	29.2	29.2	24.1	35.4	71.3	43.5
3	70.2	33.2[a]	33.3	35.7	39.7	75.9
4	40.3	37.3	36.1	37.4	36.7	42.5
5	45.6	48.0	48.8	49.4[a]	43.5	48.6
6	18.6	19.4	19.3	20.1	19.7	19.4
7	42.6	42.5	42.1	41.4	42.1	41.7
8	74.3	75.8	74.7	75.7	75.0	74.5
9	53.8	58.4	56.5	50.4[a]	48.5	49.0
10	41.1	42.7	41.3	50.9	48.8	52.1
11	17.3	17.5	17.1	16.9	16.7	17.4
12	34.0	33.4[a]	31.8	33.1	35.3	36.3
13	73.3	71.3	73.1	70.5	73.7	73.8
14	147.1	76.7	77.2	77.0	147.4	147.7
15	111.0	63.3	63.4	63.2	110.6	110.4
16	29.5	24.7[b]	25.3[a]	24.7[b]	28.6	27.9
17	25.9	24.4[b]	25.2[a]	24.5[b]	25.9	25.2
18	64.9	71.3	71.8	70.3	70.9	70.6
19	12.8	16.8	16.7	18.8	20.6	15.3
20	12.5	12.1	13.1	15.3	17.1	11.8

[a,b] These values may be interchanged.

appeared as a broad singlet at δ 4.48. This could be explained by the presence of this hydroxyl group at various positions such as C-1(α), C-3(α), C-6(β), or C-12(α). The C-6(β) position was indicated in light of the ^{13}C NMR data (Table 1) and then confirmed by acetylation of this compound, leading to the 2α,18-diacetate (**9a**). The 6β-hydroxyl is stereochemically hindered, which in this type of molecule only occurs at this position. Hence, the structure of this metabolite was resolved as 2α,6β,18-trihydroxymanoyl oxide (**9**).

The more polar metabolites **10** and **11** were obtained as their acetates by acetylation and chromatography of the fraction containing them. The structure of 6β,11β,18-trihydroxymanoyl oxide (**10**) was assigned to the first alcohol. The molecular formula of its 11β,18-diacetate (**10a**) was $C_{24}H_{38}O_6$, indicating that the parent triol **10** was $C_{20}H_{34}O_4$. Its ^1H NMR spectrum showed a proton geminal to a hydroxyl group at δ 4.37 as a broad singlet. The chemical shift and multiplicity of the resonance were similar to those observed for the 6α-H in compound **9a**, indicating the existence in **10a** of a 6β-alcohol that was not acetylated under usual conditions. A proton geminal to an acetoxy group, which was assigned to C-11(β), also appeared in this spectrum. It resonated as a double doublet at δ 5.54. Double resonance experiments permitted observation of the couplings of this carbinol with the hydrogens of the C-12 methylene (J = 4.5 Hz) and with that of the C-9 methine (J = 3.2 Hz). These ^1H NMR data were similar to those observed for 11β-acetoxymanoyl oxide.[9,10] Finally, the structure of the diacetate (**10a**) was confirmed by assignment of its ^{13}C NMR spectrum (Table 1).

The second metabolite, also obtained as the acetate, was 14ξ,15,18-triacetate (**11a**), which had a molecular formula of $C_{26}H_{42}O_8$, indicating that the compound obtained in the feeding was the tetraol **11**. Thus, the nonacetylated hydroxyl was assigned to the 6β position for the reason given previously for **9** and **10**. In the ^1H NMR spectrum, the disappearance of the double bond and the observation of three hydrogens situated on two adjacent carbon-bearing alcohols indicated that one of the alcohols was primary and the other secondary. These facts permitted us to assign the remaining two hydroxyls to C-14 and C-15, with the stereochemistry at C-14 remaining undetermined.

The incubation of jhanidiol (**3**) with *M. plumbeus* led to the isolation of **12**−**16**. Compound **15** had been obtained in the biotransformation of this same substance (**3**) by *G. fujikuroi*.[2] The least polar metabolite isolated from the feeding of **3** was the epoxide **12**, which was a single stereoisomer. The ^1H NMR spectrum of this metabolite showed no vinylic hydrogens but had those indicative of an epoxide at C-14,C-15. The two H-15 protons resonated as two double doublets at δ 2.68 (J = 5.1, 2.9 Hz) and 2.71 (J = 5.1, 3.9 Hz), while H-14 appeared as a double doublet at δ 2.87 (J = 3.9, 2.9 Hz). Consequently, the structure 1β,18-dihydroxy-14ξ,15-epoxymanoyl oxide (**12**) was assigned. Epoxidation of **3** with *m*-chloroperbenzoic acid led to a mixture of stereoisomers in a 6:4 ratio. The pure stereoisomer obtained in the biotransformation was identical with that formed, in a lesser proportion, in the chemical epoxidation.

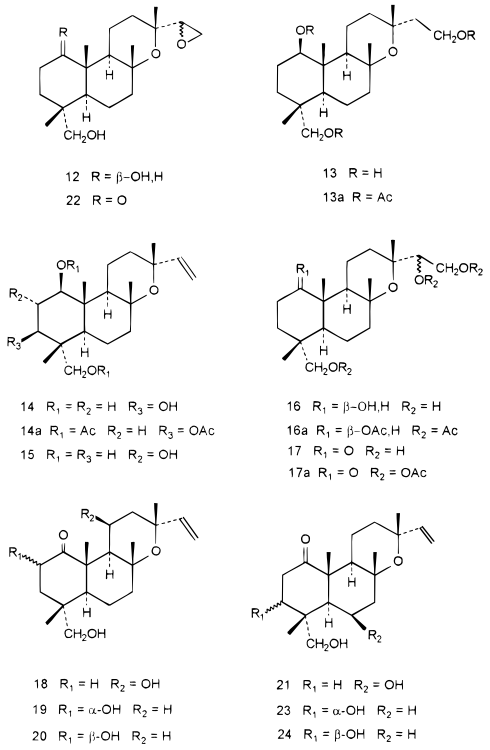
The metabolite **13**, obtained in this feeding of **3**, was isolated together with **14** as their triacetates **13a** and **14a** by acetylation and chromatography of the fractions containing them. Structure **13a** was assigned by comparing its ^1H NMR spectrum with that of **3a**.[8] The hydrogens of the double bond disappeared, being substituted by those of a $-CH_2-CH_2OAc$ group. The two protons of this 15-acetoxymethylene group resonated as a triplet at δ 4.19 (J = 7.3 Hz). This structure was confirmed by assignment of the ^{13}C NMR spectrum (Table 1), thus, the parent triol must be 1β,15,18-trihydroxymanoyl oxide (**13**).

The molecular formula of triacetate **14a** ($C_{26}H_{40}O_7$), corresponding to an alcohol $C_{20}H_{34}O_4$, indicated that a new oxygen had been introduced. This must be a secondary alcohol, because a new hydrogen geminal to an acetoxy group appeared in the ^1H NMR spectrum of **14a**, resonating as a double doublet at δ 4.91 (J = 12.3, 4.8 Hz). These data are characteristic of a proton geminal to an β-equatorial acetate at C-3, C-7, or C-12. In light of the ^{13}C NMR spectrum (Table 2), the acetoxy group was placed at C-3β. Therefore, the structure of 1β,3β,18-trihydroxymanoyl oxide (**14**) was assigned to the corresponding triol.

The most polar metabolite (**16**) did not yield a molecular ion in its MS, but gave one at m/z 323.2240 ($C_{19}H_{31}O_4$) formed by loss of water and a methyl group. The two oxygen atoms introduced during the feeding must be situated at C-14 and C-15, because the hydrogen signals of the double bond disappeared in its ^1H NMR spectrum, being substituted by those of another three coupled protons, now on carbon-bearing oxygens. These signals were over-

lapped at δ 3.70 and were resolved in the corresponding spectrum of the triacetate **16a**, which showed the H-14 as a double doublet at δ 5.00 (J = 9.0, 2.5 Hz) and the two H-15 signals as another pair of double doublets at δ 4.09 (J = 11.9, 9.0 Hz) and 4.43 (J = 11.9, 2.5 Hz). Hence, the corresponding alcohol possessed the structure 1β,14ξ,15,-18-tetrahydroxymanoyl oxide (**16**). Assignments of the ^{13}C NMR spectra of **16** and **16a** are given in Table 2.

Incubation of 1-oxo-18-hydroxymanoyl oxide (**4**) led to compounds **17**−**25**. Metabolites **18**, **19**, **21**, **22**, and **24** had been previously obtained in the biotransformation of **4** with the fungus *G. fujikuroi*.[2] The least polar product was **20**, which had the molecular formula $C_{20}H_{32}O_4$. Its ^{1}H NMR spectrum showed the resonance of a hydrogen geminal to a new hydroxyl group, which was assigned to C-2 in light of the disappearance in this spectrum of the characteristic methylene group adjacent to the 1-oxo group and assignment of the ^{13}C NMR data (Table 2). The stereochemistry assigned to this hydroxyl group was determined as follows. The most stable conformation of ring A, obtained using molecular mechanics calculations, was a boat, showing a difference of about 6 kcal/mol from that of a chair. Therefore, the observed coupling constants between H-2 and the two H-3, 14.1 and 6.1 Hz, indicated a β-equatorial stereochemistry for the hydroxyl group. On the other hand, the spectroscopic data of this product were similar to those of its 2α-epimer (**19**), obtained in this feeding and also in the incubation of **4** with *G. fujikuroi*.[2] Consequently, the structure of 1-oxo-2β,18-dihydroxymanoyl oxide (**20**) was given to this compound.



Another substance isolated was **23**, which was an isomer of **24**. Comparing the ^{1}H NMR spectrum of **23** with that of the substrate **4**, a new hydrogen geminal to a hydroxyl group appeared. This alcohol was assigned to C-3(α) on the basis of the following considerations: (a) the least energy conformation was determined as a boat by molecular mechanics calculations; and (b) the 3β-proton resonated as a double doublet at δ 3.93 (J = 12, 5.2 Hz), indicating an axial stereochemistry. This hydrogen is coupled with the characteristic signals of H-2, which are deshielded by the adjacent 1-oxo group. The location of the hydroxyl group was confirmed by the formation of an α,β-unsaturated carbonyl group during treatment with Ac_2O−pyridine, which led to the dehydrated product **25**. Thus, the structure of 1-oxo-3α,18-dihydroxymanoyl oxide (**23**) was given to this substance. The assignment of its ^{13}C NMR spectrum is given in Table 2.

Finally, the structure of 1-oxo-14,15,18-trihydroxymanoyl oxide (**17**) was assigned to the most polar metabolite on the basis of the following considerations. The peak at m/z 321.2062, observed in its MS, was formed from the molecular ion by loss of water and a methyl group. This compound gave a triacetate (**17a**), the ^{1}H NMR spectrum of which showed signals of a −CH(OAc)−CH_2OAc group. Thus, the two H-15 protons appeared as a pair of double doublets at δ 4.14 (J = 11.7, 9.0 Hz) and 4.46 (J = 11.7, 2.4 Hz), while the H-14 resonated as another double doublet at δ 4.99 (J = 9.0, 2.4 Hz). The corresponding alcohol **17** is formed in the incubation by epoxidation of the vinyl group to give **22** and opening of the oxirane ring by nucleophilic attack of water from the culture medium.

The results of the biotransformation of jhanol (**2**) indicated that there is a preference for hydroxylation at C-2(α) or C-6(β) and, to a lesser extent, at C-1(α) or C-11(α or β), while the presence of a 1β-hydroxyl group in jhanidiol (**3**) inhibits the 6β- or the 11-hydroxylation, the epoxidation of the vinyl group appearing as the main reaction. Moreover, the positions 2α and 3β were also hydroxylated. In the case of the 1-oxo-jhanol (**4**), there exists preference for 6β-hydroxylation or epoxidation of the vinyl group. Other hydroxylations observed were at the 2α, 2β, 3α, 3β, or 11β positions.

Experimental Section

General Experimental Procedures. Melting points 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. ^{1}H 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, and the ^{13}C NMR were run at 50.32 MHz, with a Bruker AC-200 and are reported in parts per million (δ). MS 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 *Chem X* program of Chemical Design. Dry column chromatographies were made on Si gel Merck 0.02−0.063 mm.

Organism. The fungal strain was *Mucor plumbeus* CMI 116688 and was a gift from Dr. J.R. Hanson, School of Chemistry and Molecular Sciences (University of Sussex).

Incubation Experiments. The fungus *M. plumbeus* was grown in shake culture 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 (2 mL). The trace-elements solution contained (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 dissolved in EtOH was evenly distributed between flasks after 1 day of growth. After a further 6 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.

Incubation of Jhanol (2): The substrate (**2**, 300 mg) in 26 conical flasks was incubated as above. Chromatography of the extract on Si gel gave: 11β,18-dihydroxymanoyl oxide (**5**) (5 mg); 11α,18-dihydroxymanoyl oxide (**6**) (2 mg); 1α,18-

dihydroxymanoyl oxide (**7**) (3 mg); 2α,18-dihydroxymanoyl oxide (**8**) (60 mg); 2α,6β,18-trihydroxymanoyl oxide (**9**) (32 mg); 6β,11β,18-trihydroxymanoyl oxide (**10**) (2 mg); and 6β,14ξ,-15,18-tetrahydroxymanoyl oxide (**11**) (10 mg). The two last compounds (**10**) and (**11**) were identified as the 11β,18-diacetate (**10a**) and the 14ξ,15,18-triacetate (**11a**), by acetylation and chromatography of the fractions containing them.

2α,18-Dihydroxymanoyl oxide (8): colorless crystals (petroleum ether−EtOAc); mp 158−160 °C; ^{1}H NMR (500 MHz) δ 0.79, 0,87, 1.28, 1.30 (each 3H, s), 3.15, 3.43 (each 1H, d, J = 10.9 Hz, H-18), 3.99 (1H, tt, J = 11.2, 4.5 Hz, H-2), 4.92 (1H; dd, J = 10.7, 1.0 Hz, H-15), 5.14 (1H, dd, J = 17.4, 1.0 Hz, H-15), 5.87 (1H, dd, J = 17.4, 10.7 Hz, H-14); EIMS m/z (rel int) 307 $[M - CH_3]^+$ (63), 289 (17), 271 (18), 259 (13), 241 (16), 224 (7), 206 (20), 189 (25); HREIMS m/z 307.2273 $[M - CH_3]^+$ (calcd for $C_{19}H_{31}O_3$, 307.2271).

2α,6β,18-Trihydroxymanoyl oxide (9): ^{1}H NMR (500 MHz) δ 1.18, 1.21, 1.30, 1.57 (each 3H, s), 3.27, 3.54 (each 1H, d, J = 10.8 Hz, H-18), 4.08 (1H, tt, 11.2, 4.4 Hz, H-2), 4.48 (1H, br s, H-6), 4.91 (1H, dd, J = 10.7, 1.4 Hz, H-14), 5.11 (1H, dd, J = 17.4, 1.5 Hz, H-15), 5.85 (1H, dd, J = 17.4, 10.7 Hz, H-15); EIMS m/z (rel int) 338 $[M]^+$ (0.5), 323 (100), 305 (24), 287 (19), 269 (27), 257 (18), 239 (9), 237 (14), 222(17), 207(13), 204(14), 199(13); HREIMS m/z 338.2453 $[M]^+$ (calcd for $C_{20}H_{34}O_4$, 338.2457).

2α,18-Diacetate (9a): colorless crystal (petroleum ether−EtOAc), mp 158−160 °C; ^{1}H NMR (200 MHz) δ 1.14 (1H, d, J = 2.7 Hz, H-5), 1.28, 1.29, 1.31 1.58 (each 3H, s), 1.67 (1H, dd, J = 13.6, 4.6 Hz, H-7), 2.01 (1H, dd, J = 13.6, 2.7 Hz, H-7), 2.03, 2.08 (each 3H, s), 3.78, 4.03 (each 1H, d, J = 11.1 Hz, H-18), 4.36 (1H, br s, H-6), 4.93 (1H, dd, J = 10.7, 1.5 Hz, H-15), 5.12 (1H, dd, J = 17.4, 1.5 Hz, H-15), 5.21 (1H, m, H-2), 5.87 (1H, dd, J = 17.4, 10.7 Hz, H-14); EIMS m/z (rel int) 422 $[M]^+$ (1), 407 (97), 389 (8), 347 (14), 329 (15), 287 (33), 269 (54), 264 (13), 251 (19), 239 (6), 217 (10), 204 (13), 199 (41); HREIMS m/z 422.2677 $[M]^+$ (calcd for $C_{24}H_{38}O_6$, 422.2668).

6β,11β,18-Trihydroxymanoyl oxide (10): obtained as its 11β,18-diacetate (**10a**) by acetylation at room temperature and chromatography of the fraction containing it.

11β,18-Diacetate (10a): ^{1}H NMR (500 MHz) δ 1.23, 1.40, 1.43, 1.89 (each 3H, s), 1.52 (1H, d, J = 3.2 Hz, H-9), 1.94 (2 H, d, J = 4.5 Hz, H-12), 2.07, 2.10 (each 3H, s), 3.71, 4.00 (each 1H, d, J = 11.1 Hz, H-18), 4.37 (1H, br s, H-6), 4.94 (1H, d, J = 10.7 Hz, H-15), 5.13 (1H, d, J = 17.3 Hz, H-15), 5.54 (1H, dd, J = 4.5, 3.2 Hz, H-11), 5.85 (1H, dd, J = 17.4, 10.7 Hz, H-14). EIMS m/z (rel int) 422 $[M]^+$ (1), 407 (4), 389 (5), 347 (53), 329 (19), 302 (6), 287 (16), 275 (5), 269 (19), 251 (7), 231 (11), 217 (7), 199 (13); HREIMS m/z $[M]^+$ 422.2655 (calcd for $C_{24}H_{38}O_6$, 422.2668).

6β,14ξ,15,18-Tetrahydroxymanoyl oxide (11): obtained in the form of the triacetate **11a** by acetylation at room temperature and chromatographic separation.

14ξ,15,18-Triacetate (11a): ^{1}H NMR (200 MHz) δ 1.13, 1.19, 1.26, 1.54 (each 3H, s), 2.03, 2.09, 2.10 (each 3H, s), 3.73, 4.00 (each 1H, d, J = 11.1 Hz, H-18), 4.12 (1H, dd, J = 11.7, 9.2 Hz, H-15), 4.31 (1H, br s, H-6), 4.43 (1H, dd, J = 11.7, 2.5 Hz, H-15), 5.00 (1H, dd, J = 9.2, 2.5 Hz, H-14); EIMS m/z (rel int) 482 $[M]^+$ (0.5), 467 (1), 449 (1), 422 (2), 407 (3), 389 (1), 379 (6), 337 (14), 319 (9), 275 (5), 259 (74), 241 (26), 201 (30); HREIMS m/z $[M]^+$ 482.2871 (calcd for $C_{26}H_{42}O_8$, 482.2879).

Incubation of Jhanidiol (3). The substrate (230 mg) in 28 conical flasks was incubated as described above. The extract was chromatographed on Si gel affording: starting material (90 mg); 1β,18-dihydroxy-14ξ,15-epoxymanoyl oxide (**12**) (9 mg); 1β,15,18-trihydroxymanoyl oxide (**13**); 1β,3β,18-trihydroxymanoyl oxide (**14**); 1β,2α,18-trihydroxymanoyl oxide (**15**) (3 mg); and 1β,14ξ,15,18-tetrahydroxymanoyl oxide (**16**) (8 mg). Compounds **13** and **14** were identified as their triacetates **13a** (2 mg) and **14a** (2 mg), respectively.

1β,18-Dihydroxy-14ξ,15-epoxymanoyl oxide (epoxyjhanidiol) (12): ^{1}H NMR (200 MHz) δ 0.74, 0.89, 1.23, 1.32 (each 3H, s), 2.68 (1H, dd, J = 5.1, 2.9 Hz, H-15), 2.71 (1H, dd, J = 5.1, 3.9 Hz, H-15), 2.87 (1H, dd, J = 3.9, 2.9 Hz, H-14), 3.12, 3.43 (each 1H, d, J = 11.0 Hz, H-18), 3.45 (1H, m, H-1); EIMS m/z (rel int) 338 $[M]^+$ (1), 323 (14), 307 (3), 305 (4), 295 (47), 277 (24), 259 (59), 247 (100), 241 (14), 229 (28), 219 (7), 199 (6); HREIMS m/z $[M]^+$ 338.2473 (calcd for $C_{20}H_{34}O_4$, 338.2457).

1β,15,18-Trihydroxymanoyl oxide (13): obtained in the form of its triacetate **16** by acetylation in the usual way and chromatography of the fractions containing it.

Triacetate 13a: ^{1}H NMR (200 MHz) δ 0.83, 0.99, 1.22, 1.27 (each 3H, s), 2.02, 2.04, 2.10 (each 3H, s), 3.65, 3.84 (each 1H, d, J = 11.0 Hz, H-18), 4.19 (1H, t, J = 7.3 Hz, H-15), 4.62 (1H, dd, J = 6.6, 4.4 Hz, H-1), EIMS m/z (rel int) 451 $[M - CH_3]^+$ (7), 391 (15), 379 (44), 331 (19), 319 (22), 301 (44), 259 (100), 253 (15), 241 (75), 201 (54), 199 (9); HREIMS m/z $[M - CH_3]^+$ 451.2697 (calcd for $C_{25}H_{39}O_7$, 451.2695).

1β,3β,18-Trihydroxymanoyl oxide (14): isolated as its triacetate **14a** by acetylation and chromatographic separation.

Triacetate 14a: ^{1}H NMR (200 MHz) δ 0.84, 1.03, 1.23, 1.30 (each 3H, s), 2.01 (6 H, s), 2.09 (3H, s), 3.74 (2 H, s, H-18), 4.70 (1H, dd, J = 11.4, 4.8 Hz, H-1), 4.91 (1H, dd, J = 12.3, 4.8 Hz, H-3), 4.97 (each 1H, dd, J = 10.7, 1.5 Hz, H-15), 5.15 (1H, dd, J = 17.2, 1.5 Hz, H-15), 5.87 (1H, dd, J = 17.2, 10.7 Hz, H-14); EIMS m/z (rel int) 464 $[M]^+$ (1), 449 (100), 389 (21), 329 (55), 275 (10), 269 (59), 215 (6), 213 (10), 199 (40), HREIMS m/z $[M]^+$ 464.2753 (calcd for $C_{26}H_{40}O_7$, 464.2774).

1β,14ξ,15,18-Tetrahydroxymanoyl oxide (16): ^{1}H NMR (200 MHz) δ 0.73, 1.19, 1.26, 1.32 (each 3H, s), 3.11, 3.42 (each 1H, d, J = 10.8 Hz, H-18), 3.45 (1H, overlapped with H-18, H-1), 3.70 (3H, m, H-14, H-15); EIMS m/z (rel int) 323 $[M - CH_3 - H_2O]^+$ (5), 307 (4), 295 (25), 277 (24), 259 (52), 247 (100), 241 (15), 229 (28), 201 (20), 189 (26); HREIMS m/z $[M - CH_3 - H_2O]^+$ 323.2240 (calcd for $C_{19}H_{31}O_4$, 323.2222).

Tetraacetate 16a: ^{1}H NMR (200 MHz) δ 0.83, 0.98, 1.23, 1.27 (each 3H, s), 2.02, 2.03 (each 3H, s), 2.11 (6 H, s), 3.65, 3.85 (each 1H, d, J = 11.1 Hz, H-18), 4.09 (1H, dd, J = 11.9, 9.0 Hz, H-15), 4.43 (1H, dd, J = 11.9, 2.5 Hz, H-15), 4.61 (1H, br s, H-1), 5.00 (1H, dd, J = 9.0, 2.5 Hz, H-14); EIMS m/z (rel int) 464 $[M - AcOH]^+$ (1), 449 (1), 407 (1), 404 (3), 389 (3), 379 (18), 319 (15), 301 (12), 277 (6), 259 (100), 241 (39), 229 (11), 201 (39); HREIMS m/z $[M - AcOH]^+$ 464.2758 (calcd for $C_{26}H_{40}O_7$, 464.2774).

Epoxidation of Jhanidiol (3). Compound **3** (30 mg) in $CHCl_3$ (5 mL) was treated with *m*-chloroperbenzoic acid (20 mg) for 24 h at room temperature. Usual workup afforded a mixture of the diastereomeric 14,15-epoxides (4:6), which were inseparable by chromatography.

Incubation of 1-Oxo-18-hydroxymanoyl Oxide (4). The substrate **4** (200 mg) in 21 conical flasks was incubated as above. Chromatography of the extract gave starting material (80 mg); 1-oxo-11β,18-dihydroxymanoyl oxide (**18**) (4 mg); 1-oxo-2α,18-dihydroxymanoyl oxide (**19**) (2 mg); 1-oxo-2β,18-dihydroxymanoyl oxide (**20**) (1 mg); 1-oxo-6β,18-dihydroxymanoyl oxide (**21**) (11 mg); 1-oxo-14ξ,15-epoxy-18-hydroxymanoyl oxide (**22**) (2 mg); 1-oxo-3β,18-dihydroxymanoyl oxide (**24**) (2 mg); 1-oxo-3α,18-dihydroxymanoyl oxide (**23**) (2 mg), and 1-oxo-14,15,18-trihydroxy manoyl oxide (**17**) (7 mg).

1-Oxo-2β,18-dihydroxymanoyl oxide (20): colorless crystal (petroleum ether−EtOAc), mp 106−107 °C; IR ($CHCl_3$) ν_{max} 3460, 1700 cm^{-1}; ^{1}H NMR (200 MHz) δ 0.88, 1.16, 1.29, 1.38 (each 3H, s), 1.49 (1H, dd, J = 14.1, 13.8 Hz, H-3), 2.29 (1H, dd, J = 13.8, 6.1 Hz, H-3), 3.38, 3.63 (each 1H, d, J = 10.5 Hz, H-18), 4.68 (1H, dd, J = 14.1, 6.1 Hz, H-2), 4.92 (1H, dd, J = 10.7, 1.5 Hz, H-15), 5.13 (1H, dd, J = 17.2, 1.5 Hz, H-15), 5.84 (1H, dd, J = 17.2, 10.7 Hz, H-14); EIMS m/z (rel int) 336 $[M]^+$ (4), 321 (100), 303 (61), 291 (6), 285 (12), 273 (7), 266 (5), 255 (9), 238 (19), 223 (15), 207 (12), 203 (8), 197 (5); HREIMS m/z $[M]^+$ 336.2305 (calcd for $C_{20}H_{32}O_4$, 336.2300).

1-Oxo-3α,18-dihydroxymanoyl oxide (23): colorless crystal (petroleum ether−EtOAc), mp 152−154 °C; ^{1}H NMR (200 MHz) δ 1.07, 1.23, 1.31, 1.35 (each 3H, s), 2.43 (1H, dd, J = 12.0 5.2 Hz, H-2), 3.09 (1H, t, J = 12.0 Hz, H-2), 3.43, 3.74 (each 1H, d, J = 10.2 Hz, H-18), 3.93 (1H, dd, J = 12, 5.2 Hz, H-3), 4.93 (1H, dd, J = 10.7, 1.4 Hz, H-15), 5.14 (1H, dd, J = 17.3, 1.4 Hz, H-15), 5.88 (1H, dd, J = 17.3, 10.7 Hz, H-14); EIMS m/z (rel int) 336 $[M]^+$ (1), 321 (100), 309 (3), 303 (18), 285 (20), 279 (9), 273 (7), 267 (6), 255 (15), 238 (12), 233 (6),

215 (8), 203 (11), 199 (2); HREIMS $[M]^+$ *m/z* 336.2305 (calcd for $C_{20}H_{32}O_4$, 336.2300).

Compound 25. Treatment of **23** with $Ac_2O-C_5H_5N$ (2:1) at room temperature for 12 h and chromatography over Si gel afforded the dehydrated compound **25**: UV ($CHCl_3$) λ_{max} 242 nm; ^1H NMR (200 MHz) δ 1.09, 1.18, 1.34, 1.39 (each 3H, s), 2.04 (3H, s), 3.86, 3.99 (each 1H, d, J = 11.2 Hz, H-18), 4.94 (1H, dd, J = 10.6, 1.4 Hz, H-15), 5.16 (1H, dd, J = 17.2, 1.4 Hz, H-15), 5.83, 6.33 (each 1H, d, J = 10.2 Hz, H-2 and H-3, respectively), 5.90 (1H, dd, J = 17.2, 10.6 Hz, H-14); EIMS *m/z* (rel int) 360 $[M]^+$ (1), 345 (100), 333 (5), 327 (4), 290 (3), 275 (8), 267 (19), 262 (15), 215 (6), 211 (10), 199 (2); HREIMS *m/z* $[M]^+$ 360.2297 (calcd for $C_{22}H_{32}O_4$, 360.2300).

1-Oxo-14,15,18-trihydroxymanoyl oxide (17): colorless crystal (petroleum ether−EtOAc), mp 159−161 °C; IR ($CHCl_3$) ν_{max} 3430, 1700 cm^{-1}; ^1H NMR (500 MHz) δ 0.97, 1.19, 1.27, 1.35 (each 3H, s), 1.99 (1H, ddd, J = 13.8, 10.5, 5.3 Hz, H-3), 2.31 (1H, ddd, J = 14.0, 6.7, 5.3 Hz, H-2), 2.81 (1H, ddd, J = 14.0, 10.5, 5.5 Hz, H-2), 3.23, 3.46 (each 1H, d, J = 10.7 Hz, H-18), 3.66 (2 H, signals overlapped, H-14 and H-15), 3.80 (1H, dd, J = 11.3, 5.1 Hz, H-15); EIMS *m/z* (rel int) 321 $[M - CH_3 - H_2O]^+$ (2), 305 (3), 293 (79), 275 (20), 257 (38), 245 (85), 227 (9), 217 (8), 201 (17), 199 (23); *m/z* $[M - CH_3 - H_2O]^+$ 321.2062 (calcd for $C_{19}H_{29}O_4$, 321.2065).

Triacetate (17a): ^1H NMR (200 MHz) δ 1.03, 1.18, 1.25, 1.30 (each 3H, s), 3.73, 3.87 (1H, d, J = 11.1 Hz, H-18), 4.14 (1H, dd, J = 11.7, 9.0 Hz, H-15), 4.46 (1H, dd, J = 11.7, 2.4 Hz, H-15), 4.99 (1H, dd, J = 9.0, 2.4 Hz, H-14); EIMS *m/z* (rel int) 465 $[M - CH_3]^+$ (1), 405 (1), 387 (2), 360 (1), 345 (3), 335 (100), 317 (7), 257 (92), 239 (17), 199 (52); HREIMS *m/z* $[M - CH_3]^+$ 465.2482 (calcd for $C_{25}H_{37}O_8$, 465.2488).

Acknowledgment. This work has been supported by the DGES, Ministry of Education and Culture, Spain.

References and Notes

(1) Bhat, S. V.; Bajwa, B. S.; Dornauer, H.; De Souza, N. J.; Fehlhaber, H. W. *Tetrahedron Lett.* **1977**, *16*, 1913−1917.
(2) Fraga, B. M.; González, P.; Guillermo, R.; Hernández, M. G. *Tetrahedron* **1998**, *54*, 6159−6168.
(3) Fraga, B. M.; Guillermo, R.; Hanson, J. R.; Truneh, A. *Phytochemistry* **1996**, *42*, 1583−1586.
(4) Guillermo, R.; Hanson, J. R.; Truneh, A. *J. Chem. Res.* **1997**, 28−29.
(5) Aranda, G.; Hammoumi, A.; Azerad, R.; Lallemand, J. Y. *Tetrahedron Lett.* **1991**, *32*, 1783−1786.
(6) Aranda, G.; El Kortbi, M. S.; Lallemand, J. Y.; Neuman, A.; Hammoumi, A.; Facon, I.; Azerad, R. *Tetrahedron* **1991**, *47*, 8339−8350.
(7) Hoffmann, J.; Fraga, B. M. *Phytochemistry* **1993**, *33*, 827−830.
(8) González, A. G.; Arteaga, J. M.; Bretón, J. L.; Fraga, B. M. *Phytochemistry* **1977**, *16*, 107−110.
(9) De Pascual-Teresa, *J.*; San Feliciano, A.; Del Corral, M. J. *Anal. Quim.* **1975**, *71*, 110−111.
(10) Topcu, G.; Tan, N.; Ulubelen, A.; Sun, D.; Watson, W. H. *Phytochemistry* **1995**, *40*, 501−504.

NP980133M