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The Biotransformation of Manoyl Oxide Derivatives by *Gibberella fujikuroi*: The Fungal Epimerization of an Alcohol Group

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Abstract: The microbiological transformations of jhanol (18-hydroxymanoyl oxide) and jhanidiol (1β,18-dihydroxymanoyl oxide) with the fungus *Gibberella fujikuroi* have been studied. In the biotransformation of the first compound there exists a preference for hydroxylation at C-1(α) and in lower yield at C-11(α or β), while in that of the second, which possesses a 1β-hydroxylation, the main reaction observed is the oxidation of this 1β-hydroxyl to an oxo group. This product is then mainly reduced to the 1α-alcohol or, in minor yield, hydroxylated at C-11(β), C-6(β) and C-2(α). The fungal epimerization of a hydroxyl group, as occurs in the biotransformation of the 1β-alcohol of jhanidiol to the 1α-alcohol, is a very rare process.

During the past few years we have been interested in the study of the microbiological transformation of diterpenes by *Gibberella fujikuroi*. This fungus produces the gibberellins and kaurenolides, which are diterpenoid metabolites derived from *ent*-kaur-16-ene.[1] In previous work we have reported that although 13-*epi*-*ent*-manoyl oxide is a final metabolite of a biosynthetic branch in *G. fujikuroi*, some of its derivatives, with a greater polarity, are transformed by this fungus.[2] The aim of the present work was to study the biotransformation of manoyl oxide derivatives of the *normal* series, to compare with the results previously obtained in the incubation of the enantio series. It should be noted that the diterpenes produced by *G. fujikuroi* are of the enantio series, and that those of the normal series are not biotransformed along the gibberellin pathway.[3,4] On the other hand, the incubation of manoyl oxide derivatives with this fungus may lead to compounds with a functionalization similar to that of forskolin (**1**),[5] a diterpene with interesting biological properties.

The substrates were jhanol (**2**) and jhanidiol (**3**), which had been isolated from *Eupatorium jhanii*,[6] a plant which grows in the Venezuelan Andes region. As a consequence of the results of the feeding of **3**, the reincubation of one of the metabolites formed, 1-oxo-jhanol (**13**), was also carried out.

The incubations with the fungus were carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene without disturbing post-kaurene metabolism, which facilitates the

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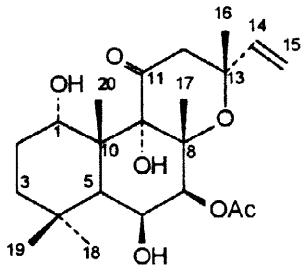
isolation of the metabolites which are formed.[7,8] The fermentations were harvested after eight days, and the broth and mycelium extracts were combined. Chromatography of the extract of the incubation of jhanol (**2**) gave the metabolites **6-9**.

The least polar substance **6** showed in its HR mass spectrum a peak at *m/z* 307.2267, which is formed by loss of a methyl from the molecular ion. Thus the molecular formula of this compound was $C_{20}H_{34}O_3$, which indicated that a new oxygen had been introduced into the molecule during the biotransformation. This oxygen function must be a secondary alcohol, because in the ^{1}H NMR spectrum, a new geminal proton to a hydroxyl group appears at δ 4.48 as a doublet of a double doublets, with coupling constants of 5.9, 5.5 and 3.8 Hz. The form of the signal was characteristic of the geminal hydrogen to an axial alcohol situated at C-6 or C-11. Considering the ^{13}C NMR spectrum of **6** (table 1), which was assigned by comparison with that of **2**, the C-11(β) position was chosen for the new hydroxyl group. Therefore, the structure of 11β,18-dihydroxy-manoyl oxide (**6**) was assigned to this substance. A related compound, 11β-hydroxymanoyl oxide, with similar NMR data for the 11-CHOH, has been isolated from *Juniperus oxycedrus*[9] and *Salvia candidissima*.[10]

The second product isolated from the incubation (**7**) was isomeric with compound **6**. The MS and the ^{13}C NMR spectrum (table 1) of both compounds were very similar. The assignment of the latter indicated that both products were epimeric at C-11 and, consequently, the α-stereochemistry was given to the new alcohol introduced into the molecule. Moreover, in the ^{1}H NMR spectrum of **7** the observed shifts of the hydrogens of the vinyl group with respect to those of **6** indicated that the alcohol must be situated also at C-11, but with the α-stereochemistry. This spectrum also showed the geminal proton to the new hydroxyl group as a broad triplet, at δ 3.94 ($W_{1/2}$ = 13.8 Hz). Double resonance experiments permitted observation of the couplings of this carbinol with the protons of the C-12 methylene at δ 1.96 and 2.38 (J = 1 and 6 Hz) and with that of the C-9 methine at 1.54 (J = 7.8 Hz). These data were similar to those described for 11α-hydroxymanoyl oxide, isolated from *Kyllinga erecta*,[11] indicating that the conformation of ring C was not a true chair, as was observed in its 11β-epimer.[10] Thus, compound **7** possesses the structure of 11α,18-dihydroxymanoyl oxide.

The structure 1α,18-dihydroxymanoyl oxide (**9**) was assigned to another compound isolated from this feeding. Its MS indicated that it was isomeric with **6** and **7**, while its ^{1}H NMR spectrum showed the geminal hydrogen to the new hydroxyl group was a broad singlet at δ 3.59. The form of resonance permitted us to assign this alcohol to C-1, C-3 or C-12, with an α-axial stereochemistry. The ^{13}C NMR spectrum pointed to the C-1 position and this was confirmed by chemical methods. Thus, the partial acetylation of **9** led to the diacetate **10** and the 18-monoacetate **11**. The latter was oxidized with Jones reagent to give the corresponding oxo-derivative **12**. This compound was identical with one obtained in the oxidation of the monoacetate **4**, which had been prepared by partial acetylation of jhanidiol (**3**).[6]



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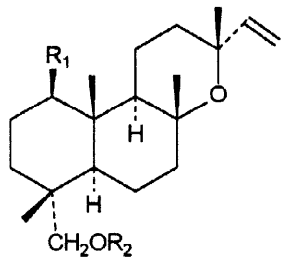
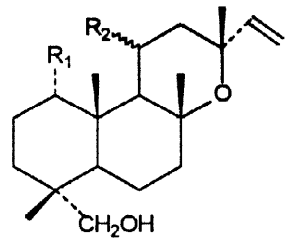


2 $R_1 = R_2 = H$

3 $R_1 = OH$ $R_2 = H$

4 $R_1 = OH$ $R_2 = Ac$

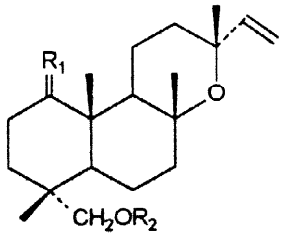
5 $R_1 = OAc$ $R_2 = Ac$



6 $R_1 = H$ $R_2 = \beta$-OH

7 $R_1 = H$ $R_2 = \alpha$-OH

8 $R_1 = OH$ $R_2 = \alpha$-OH



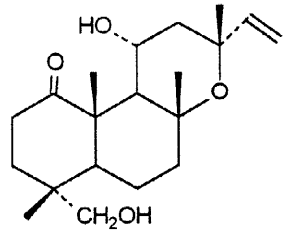
9 $R_1 = \alpha$-OH $R_2 = H$

10 $R_1 = \alpha$-OAc,H $R_2 = Ac$

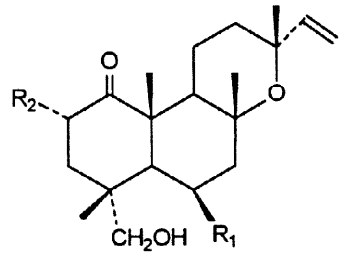
11 $R_1 = \alpha$-OH,H $R_2 = Ac$

12 $R_1 = O$ $R_2 = Ac$

13 $R_1 = O$ $R_2 = H$

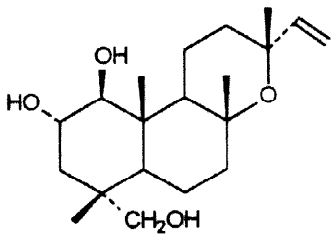


14

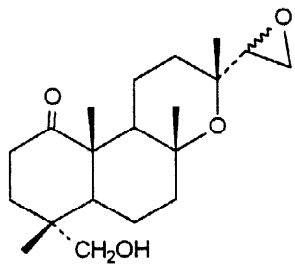


15 $R_1 = OH$ $R_2 = H$

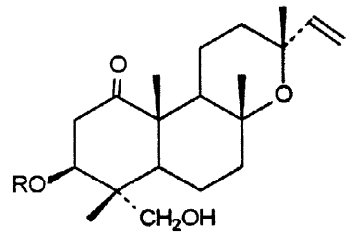
16 $R_1 = H$ $R_2 = OH$



17



18



19 R = H

20 R = Ac

The most polar compound isolated from this fermentation was the triol **8**. As with the majority of these compounds, the molecular ion was not observed in the mass spectrum, because they easily lose the methyl at C-13 giving a peak at $[M-15]^+$, in this case at *m/z* 323.2255. Thus, the molecular ion should correspond to a molecular formula $C_{20}H_{34}O_4$. In comparison with the substrate **2**, this compound has two new oxygen atoms, which have been introduced into the molecule as two secondary alcohols. Its ^1H NMR spectrum showed the geminal protons to these hydroxyls at δ 3.67 (br s) and 4.12 (br t). These chemical shifts and couplings are

similar to those observed in the spectra of compounds **7** and **9**, also isolated from this feeding, indicating that this product must have the structure of 1α,11α,18-trihydroxymanoyl oxide (**8**). This was confirmed by assignment of the ^{13}C NMR spectrum (table 1).

The incubation of jhanidiol (**3**) led to the isolation of substances **9** and **13-17**. The least polar compound obtained in this fermentation was **13** which was isolated in good yield. It analyzed for $C_{20}H_{32}O_3$ indicating that this compound had a molecular weight two units lower in comparison with that of the substrate. Since its IR spectrum showed the presence of a carbonyl group at 1700 cm^{-1} and in the ^1H NMR spectrum the geminal proton to the hydroxyl group had disappeared, we believed that the hydroxyl group at C-1 had been oxidized to an oxo group and assigned it the structure of 1-oxo-18-hydroxymanoyl oxide (**13**). This fact was confirmed by hydrolysis of **12**, that led to **13**, sodium borohydride reduction of which gave **9**.

Compound **9**, which was obtained in the feeding of jhanol (**1**) by hydroxylation at C-1(α) (see above), has also been isolated in this incubation. Now it has been formed via the enzymatic oxidation of **3**, which gave **13**, followed by stereospecific reduction to give the 1α-alcohol. This reduction was in accordance with Prelog's rule.[12] The entire oxidation-reduction process, which implies inversion of configuration at C-1, is very rare in microbiological transformations.

The third compound obtained in this biotransformation was **14**, to which the structure of 1-oxo-11α,18-dihydroxymanoyl oxide was given. Its high resolution mass spectrum was in accordance with the molecular formula $C_{20}H_{32}O_4$, indicating that a new oxygen atom had been introduced into the molecule. Only a geminal proton to a hydroxyl group appears in the ^1H NMR spectrum. This has a chemical shift (δ 4.25) and a form of resonance, a multiplet, which was different from those of the 1α-hydrogen in the substrate (**3**). This indicated that the secondary hydroxyl group of **3** had been oxidized to an oxo group. On the other hand, the coupling constants of the geminal hydrogen to the alcohol group (9.7, 8.5 and 6.6 Hz), clearly observed by irradiations at δ 1.70 (H-9), 2.09 (H-12) and 2.25 (H-12), indicated that this must be at C-6(α) or C-11(α). The assignment of the ^1H NMR and ^{13}C NMR spectra permitted us to choose the C-11(α) position.

A fourth product was isolated in the feeding of jhanidiol and assigned the structure **15**. Its ^1H NMR spectrum was similar to that of **14**, of which it is an isomer, but the geminal hydrogen to the alcoholic group now appeared as a broad singlet at δ 4.50. The form of this signal indicated an axial stereochemistry for the hydroxyl group. The position chosen for this secondary hydroxyl was C-6(β) on the basis of the ^{13}C NMR spectrum. Thus, the structure assigned to this compound was 1-oxo-6β,18-dihydroxymanoyl oxide (**15**).

Another substance which was obtained in this feeding was **16**. It was isomeric with **14** and **15**. As in these compounds, the oxidation to an oxo group at C-1 had also taken place, however now the hydroxylation was at C-2(α). Thus, the ^1H NMR spectrum showed a geminal hydrogen to a secondary alcohol at 4.79 (dd,

Table 1. ^{13}C NMR data of compounds **2**, **3** and **5-10**

C	2	3	5	6	7	8	9	10
1	38.4	79.3	80.8	38.7	39.4	70.3	70.4	72.8
2	17.8	28.9	23.8	17.8	17.7	24.0	24.8	21.6
3	35.2	33.4	33.0	35.2	35.2	28.5	28.3	29.5
4	36.8	37.2	35.7	37.6[a]	37.6[a]	37,6	37.4	36.2
5	49.5	48.1	48.6	50.2	49.5	41.8	41.4	44.1
6	19.6	19.5	19.1	19.9	19.8	19.4	19.4	19.6
7	42.8	42.8	42.4	44.3	43.0[b]	42.7[a]	42.5	42.5
8	74.9	74.9	74.2	74.7	74.3	74.5	75.3	75.0
9	55.5	55.2	53.5	56.6	62.9	54.1	47.9	47.4
10	37.5	42.6	40.9	37.7[a]	37.8[a]	41.6	40.7	40.0
11	15.2	18.5	17.1	65.3	64.9	64.7	14.9	14.9
12	35.6	34.9	33.6	44.3	43.3[b]	42.8[a]	35.4	34.7
13	73.2	73.3	72.9	72.4	73.1	72.8	73.4	73.5
14	147.7	147.8	146.9	147.7	148.2	147.8	147.8	147.8
15	110.3	110.4	110.6	110.6	112.4	112.6	110.3	110.4
16	28.4	29.0	29.4	29.7	31.7	31.6	28.3	29.0
17	25.4	25.8	25.8	27.5	27.8	27.7	25.6	25.7
18	71.9	71.4	71.6	72.1	72.0	72.0	71.6	72.9
19	17.1	16.9	16.6	17.6	17.3	17.0	17.1	17.0
20	15.7	11.6	12.4	17.3	16.5	17.0	16.5	15.9

Table 2. ^{13}C NMR data of compounds **12-19**

C	12	13	14	15	16	17	18	19
1	214.4	216.0	220.0	215.4	215.3	84.5	215.8	212.9
2	34.7	35.4	30.9	35.0	68.2	69.8	35.4	43.6
3	35.9[a]	35.5	37.5	39.0	48.1	41.6	35.7	80.6
4	35.6	37.3	36.1	38.8	38.1	38.4	37.4	41.1
5	48.8[b]	48.9[a]	46.6	53.1	51.8.	48.1	49.6[a]	45.9
6	19.8	20.3	20.6	69.7	19.8	19.3	20.2	19.5
7	41.2	41.7	42.9	49.3	41.6	42.8	41.3	41.7
8	74.1	74.8	75.5	74.2	74.4	74.8	74.9	74.7
9	49.4[b]	49.5[a]	49.1	49.3	48.9	55.4	50.3[a]	49.0
10	50.5	51.1	51.2	52.2	51.3	42.8	51.1	52.0
11	17.0	17.5	65.1	17.6	17.4	18.4	17.0	17.4
12	35.2[a]	36.2	40.1	36.5	36.1	34.9	33.6	36.4
13	73.1	73.7	73.3	73.7	73.8	73.4	71.2	73.7
14	147.3	147.7	146.3	148.0	147.5	147.7	60.1	147.7
15	109.6	110.4	111.7	110.2	110.4	110.5	43.8	110.4
16	27.5	28.5	28.3	28.1	28.0	29.1	24.5[b]	27.9
17	24.6	25.2	32.1	26.3	25.3	25.9	24.4[b]	25.3
18	70.5	70.5	70.7	71.3	70.0	71.2	70.5	69.8
19	18.6	18.9	18.5	20.1	18.3	17.8	18.8	17.6
20	14.8	15.3	20.6	16.6	15.4	13.1	15.4	14.2

[a,b] These values can be interchanged.

J = 12 and 7.1 Hz). This relatively high value of the chemical shift and the disappearance of the coupling of the C-2 protons, observed in **14** and **15**, pointed to the C-2 position, and this was later confirmed by ^{13}C NMR data (table 2). Thus, the structure of 1-oxo-2α,18-dihydroxymanoyl oxide (**16**) was assigned to this product.

To the most polar metabolite the structure of 1β,2α,18-trihydroxymanoyl oxide (**17**) was given. It showed in the HRMS a peak at *m/z* 323.2195, which was formed by loss of a methyl group from the molecular ion. Thus the molecular formula of this compound was $C_{20}H_{34}O_4$, indicating that during the feeding an oxygen atom had been introduced into the molecule of jhanidiol. A new geminal proton appeared in the ^1H NMR spectrum at δ 3.74, as a doublet of a double doublets, with coupling constants of 11.2, 8.9 and 5.7 Hz. When this hydrogen was irradiated, in a double resonance experiment, the signal of a doublet at δ 3.12 of H-1(α) collapsed to a singlet. Alternatively, when this proton was irradiated the doublet of double doublets signal was transformed into a double doublet (J = 11.2 and 5.7 Hz). These facts showed that the new hydroxyl group was situated at C-2(α).

Finally, we decided to do a refeeding of compound **13**, which had been isolated in the incubation of jhanidiol (**3**). The aim of this work were to confirm the fungal reduction of **13** to give the alcohol **9**, to identify the minor compounds obtained and gain further biosynthetic information on some of the hydroxylations produced. In this fermentation, compounds **9**, **14-16**, also obtained in the feeding of jhanidiol (**3**), and two minor products, **18** and **19**, were isolated.

The first of these two substances was identified as the 14ξ,15-epoxy of 1-oxo-18-hydroxymanoyl oxide (**18**). In its ^1H NMR spectrum the hydrogen of the double bond had disappeared, being replaced by those geminal to the oxirane ring. Its mass and ^{13}C NMR spectra were also in accordance with the structure given. The second compound was formed by the introduction of a hydroxyl group at C-3(β) and assigned the structure of 1-oxo-3β,18-dihydroxymanoyl oxide (**19**). In its ^1H NMR spectrum the geminal hydrogen to the hydroxyl group appears as a triplet at δ 4.13 (J = 3.8 Hz), showing the same coupling with another two protons at δ 2.20 and 3.35, which were identified as the two H-2 by double resonance experiments. The most stable conformation of ring A, obtained by using molecular mechanics calculations, was a boat. Finally, its structure was confirmed by assignment of the ^{13}C NMR spectrum (table 2).

Several conclusions can be deduced from the microbiological transformations described in this work. 1) The biotransformation of jhanol (**2**) and jhanidiol (**3**) indicated that these compounds are not transformed by the fungus as in the gibberellin pathway. Thus, the first possible step, the oxidation at C-19 is not produced. 2) In the biotransformation of jhanol there is a preference for the hydroxylation at C-1(α), and in lower yield at C-11(α or β). 3) In that of jhanidiol (**3**), which possesses a 1β-hydroxylation, the main reaction observed is the oxidation of this 1β-hydroxyl to an oxo group. This product is then mainly reduced to the 1α-alcohol or, in a lesser yield, hydroxylated at C-11(α), C-6(β) or C-2(α). 4) The oxidation of the 1β-hydroxyl to the

corresponding oxo group has not been observed in the biosynthesis of gibberellins or kaurenolides. 5) The subsequent stereospecific reduction of the oxo group to give the 1α-alcohol is also interesting. We had observed the enzymatic reduction by *G. fujikuroi* of the double bond of an α,β-unsaturated carbonyl,[13] but not that of an oxo group. The reduction is probably due to the addition of hydride from an NADH-depending enzyme and its stereospecificity is the same as that observed in the sodium borohydride reduction. 6) This oxidation-reduction process, the epimerization of an alcohol by the enzymatic system of a fungus, is a very unusual microbiological transformation. 6) The results of the incubation of 1-oxo-jhanol (**13**) confirmed the results of the incubation of jhanidiol (**3**) and showed that the 1α,18-diol (**9**) is a final metabolite, indicating also that in the formation of the 1α,11α,18-triol (**8**), resulting from the incubation of jhanol (**2**), the hydroxylation at C-11(α) to give the 18,11α-diol (**7**) is prior to that of C-1(α). 7) The non-isolation of the 1β,2α,18-triol (**17**) in the incubation of 1-oxo-jhanol (**13**), also indicated, that **17** is directly formed by 2α-hydroxylation of jhanidiol (**3**), and excludes an alternative way via **13**.

EXPERIMENTAL

Mps were determined with a Reichert Thermovar apparatus and are uncorrected. IR spectra were obtained in $CHCl_3$ on a Perkin Elmer 1600 FT spectrophotometer. ^1H NMR spectra were recorded in $CDCl_3$ at 200.1 and 500.1 MHz, with a Bruker AC-200 or a Bruker AMX2-500 spectrometers, respectively, and the ^{13}C NMR were run at 50.3 MHz, with a Bruker AC-200. Low- and high-resolution mass spectra were obtained on a Shimadzu QP-2000 and Micromass Autospec spectrometer, respectively. Column chromatography was performed with silica gel Merck (0.05-0.2 mm). Conformations of minimum energy were determined by computational methods employing the *Chem X* program of Chemical Design. The fungus strain was *Gibberella fujikuroi* (IMI 58289) from the International Mycological Institute, Englefield Green, Egham, Surrey, U.K.

Incubation procedure. The fungus *G. fujikuroi* inhibited with 5×10^{-5} M AMO 1618 was grown on shake culture at 25 °C for two days in conical flasks (250 ml.), each containing 50 ml of sterile medium.[14] The substrate dissolved in EtOH (15 ml) was equally distributed between the flasks and the incubation allowed to continue for a further six days. The broth was filtered and extracted with EtOAc. The mycelium was treated with liquid nitrogen, crushed with a mortar and extracted with EtOAc. Both extracts were combined, dried and concentrated.

Incubation of jhanol (1). The substrate (240 mg) in 77 conical flasks was incubated as above. Chromatography of the extract on silica gel, eluting with petrol-EtOAc mixtures, gave starting material **2** (120 mg), 11β,18-dihydroxymanoyl-oxide (**6**) (7 mg), 11α,18-dihydroxymanoyl-oxide (**7**) (6 mg), 1α,18-dihydroxy-manoyl-oxide (**9**) (22 mg) and 1α,11α,18-trihydroxymanoyl-oxide (**8**) (4 mg).

11β,18-Dihydroxymanoyl oxide (6). $[M - CH_3]^+$ at *m/z* 307.2267, $C_{19}H_{31}O_3$ requires 307.2273; ^1H NMR (500 MHz) δ 0.79, 1.22, 1.44 and 1.62 (each 3H, s), 1.39 (1H, d, J = 3.8 Hz, H-9), 1.86 (1H, dd, J = 14.2 and 5.5 Hz, H-12), 2.00 (1H, dd, J = 14.2 and 5.9 Hz, H-12), 3.11 and 3.42 (1H each, d, J = 10.9 Hz, H-18), 4.48 (1H, ddd, J = 5.9, 5.5 and 3.8 Hz, H-11), 4.92 (1H, dd, J = 10.7 and 1.4 Hz, H-15), 5.13 (1H, dd, J = 17.4 and 1.4 Hz, H-15), 5.86 (1H, dd, J = 17.4 and 10.7 Hz, H-14); EIMS *m/z* (rel. int.): 307 $[M- CH_3]^+$ (48), 289 (58), 271 (29), 259 (19), 241 (21), 239 (100), 221 (12), 209 (40), 201 (13).

11α,18-Dihydroxymanoyl oxide (7). $[M-CH_3]^+$ at *m/z* 307.2280, $C_{19}H_{31}O_3$ requires 307.2273; ^1H NMR (500 MHz) δ: 0.77, 0.92, 1.22 and 1.30 (each 3H, s), 1.54 (1H, d, J = 7.8 Hz, H-9), 1.96 (1H, dd, J =

15.4 and 1 Hz, H-12), 2.38 (1H, dd, J = 15.4 and 6.0 Hz, H-12), 3.13 and 3.45 (each 1H, d, J = 10.8 Hz, H-18), 3.94 (1H, br t, $W_{1/2}$ = 14 Hz, H-11), 5.08 (1H, dd, J = 10.6 and 1.9 Hz, H-15), 5.44 (1H, dd, J = 17 and 1.9 Hz, H-15), 6.03 (1H, dd, J = 17 and 10.6 Hz, H-14); EIMS *m/z* (rel. int.): 307 $[M\text{-}CH_3]^+$ (100), 289 (64), 271 (34), 259 (32), 253 (7), 241 (12), 237 (9), 209 (7), 201 (20).

1α,11α,18-Trihydroxymanoyl oxide (8). $[M\text{-}CH_3]^+$ at *m/z* 323.2225, $C_{19}H_{31}O_4$ requires 323.2222; ^1H NMR (500 MHz) δ: 0.80, 0.91, 1.24 and 1.30 (each 3H, s), 1.94 (1H, dd, J = 16.0 and 1.0 Hz, H-12), 2.15 (1H, d, J = 8 Hz, H-9), 2.37 (1H, dd, J = 15.6 and 5.9 Hz, H-12), 3.19 and 3.47 (1H each, d, J = 10.9 Hz, H-18), 3.67 (1H, br. s, H-1), 4.12 (1H, br t, H-11), 5.08 (1H, dd, J = 10.5 and 1.7 Hz, H-15), 5.44 (1H, dd, J = 17.1 and 1.7 Hz, H-15), 6.05 (1H, dd, J = 17.1 and 10.5 Hz, H-14); EIMS *m/z* (rel. int.):323 $[M\text{-}CH_3]^+$ (58), 305 (28), 287 (15), 271 (3), 269 (3), 257 (6), 253 (6), 235 (63), 205 (17), 187 (14).

1α,18-Dihydroxymanoyl oxide (9). $[M\text{-}CH_3]^+$ at *m/z* 307.2261, $C_{19}H_{31}O_3$ requires 307.2273; ^1H NMR (200 MHz) δ: 0.78, 0.86, 1.29 and 1.32 (each 3H, s), 3.15 and 3.46 (each 1H, d, J = 10.8 Hz, H-18), 3.59 (1H, br. s, H-1), 4.94 (1H, dd, J = 10.6 and 1.5 Hz, H-15), 5.14 (1H, dd, J = 17.4 and 1.5 Hz, H-15), 5.90 (1H, dd, J = 17.4 and 10.6 Hz, H-14); EIMS *m/z* (rel. int.): 307 $[M\text{-}CH_3]^+$ (100), 290 (6), 289 (27), 271 (6), 259 (28), 241 (11), 224 (19), 203 (17), 193 (21), 189 (73).

Acetylation of 9. Treatment of **9** (10 mg) with Ac_2O-C_5H_5N (2:1) at room temperature for 12 h and chromatography over silica gel, eluting with petroleum ether- EtOAc (9:1), afforded the **diacetate (10)** (4 mg): $[M]^+$ at *m/z* 406.2717, $C_{24}H_{38}O_5$ requires 406.2719. ^1H NMR (200 MHz) δ: 0.87, 0.90, 1.26 and 1.31 (each 3H, s), 2.07 and 2.09 (3H each, s), 3.75 and 3.87 (each 1H, d, J = 10.8 Hz, H-18), 4.68 (1H, br. s, H-1), 4.94 (1H, dd, J = 10.6 and 1.5 Hz, H-15), 5.14 (1H, dd, 17.4 and 1.5 Hz, H-15), 5.90 (1H, dd, J = 17.6 and 10.6 Hz, H-14); EIMS *m/z* (rel. int.): 391 $[M\text{-}CH_3]^+$ (100), 349 (11), 331 (56), 271 (48), 253 (35), 201 (62), 199 (12). Further elution gave the **monoacetate (11)** (6 mg): ^1H NMR (200 MHz) δ: 0.84 (6H, s), 1.30, and 1.32 (each 3H, s), 2.08 (3H, s), 3.60 (1H, br. s, H-1), 3.70 and 3.87 (each 1H, d, J = 11 Hz, H-18), 4.94 (1H, dd, J = 10.7 and 1.5 Hz, H-15), 5.14 (1H, dd, J = 17.3 and 1.5 Hz, H-15), 5.90 (1H, dd, J = 17.3 and 10.7 Hz, H-14); EIMS *m/z* (rel. int.): 349 $[M\text{-}CH_3]^+$ (100), 331 (26), 271 (33), 253 (23), 203 (30), 201 (66), 199 (11).

1-Oxo-18-acetoxymanoyl oxide (12). Monoacetate **11** (6 mg) in Me_2CO (3 ml) was treated dropwise with a slight excess of Jones reagent, and left at room temperature for 10 min. MeOH was added to destroy the excess of reagent. The mixture was poured into water and worked up. The solvent was evaporated and the residue chromatographed over silica gel. Elution with petroleum ether-EtOAc (9:1) gave **12**: Found C, 72.83 %; H, 9.62 %; $C_{22}H_{34}O_4$ requires C, 72.89 %; H, 9.45 %; ^1H NMR (200 MHz) δ: 1.04, 1.21, 1.31 and 1.35 (each 3H, s), 2.07 (3H, s), 2.27 (1H, ddd, J = 14.1, 7.0 and 5.1 Hz, H-2), 2.79 (1H, ddd, J = 14.1, 10.2 and 5.7 Hz, H-2), 3.74 and 3.89 (each 1H, d, J = 11 Hz, H-18), 4.94 (1H, dd, J = 10.7 and 1.3 Hz, H-15), 5.14 (1H, dd, J = 17.3 and 1.3 Hz, H-15), 5.89 (1H, dd, J = 17.3 and 10.7 Hz, H-14); EIMS *m/z* (rel. int.): 362 $[M]^+$ (1), 347 (100), 269 (39), 264 (27), 257 (6), 251 (5), 217 (4), 213 (5), 201 (6), 199 (27).

Incubation of jhanidiol (3) The substrate **3** (210 mg) in 75 conical flasks was incubated with *G. fujikuroi* as described above. The metabolites were separated by chromatography on silica gel, eluting with petrol-EtOAc mixtures, to give starting material (82 mg), 1-oxo-18-hydroxymanoyl oxide (**13**) (46 mg), 1-oxo-11β,18-dihydroxymanoyl oxide (**14**) (4 mg), 1α,18-dihydroxymanoyl oxide (**9**) (7 mg), 1-oxo-2α,18-dihydroxymanoyl oxide (**16**) (2 mg), 1-oxo-6β,18-dihydroxymanoyl oxide (**15**) (2 mg) and 1β,2α,18-trihydroxymanoyl oxide (**17**) (2 mg).

1-Oxo-18-hydroxymanoyl oxide (13). M.p. 153-154 °; IR (ν_{max}, cm^{-1}): 3630, 3440, 3000, 2940, 2870, 1700, 1470, 1380, 1110, 1080, 1035; Found C, 75.35 %; H, 10.18 %. $C_{20}H_{32}O_3$ requires C, 74.96 %; H, 10.06 %; ^1H NMR (200 MHz) δ: 0.98, 1.21, 1.30 and 1.35 (each 3H, s), 2.23 (1H, ddd, J = 14, 7 and 5.1

Hz, H-2), 2.75 (1H, ddd, J = 14, 10.2 and 5.5 Hz, H-2), 3.24 and 3.46 (each 1H, d, J = 10.7 Hz, H-18), 4.92 (1H, dd, J = 10.7 and 1.5 Hz, H-15), 5.14 (1H, dd, J = 17.2 and 1.5 Hz, H-15), 5.87 (1H, dd, J = 17.2 and 10.7 Hz, H-14); EIMS *m/z* (rel. int.): 305 $[M-CH_3]^+$ (100), 287 (21), 269 (25), 257 (42), 222 (16), 201 (26), 199 (16).

Hydrolysis of 12. The monoacetate **12** (310 mg) was treated with methanolic KOH (5%) solution (12 ml) at room temperature overnight. Usual work-up and chromatography with petroleum ether-EtOAc (9:1) gave 1-oxo-18-hydroxymanoyl oxide (**13**) (250 mg), identical with that obtained in the feeding of jhanidiol (**3**).

Reduction of 13. The 1-oxo derivative **13** (22 mg) in methanol was treated at room temp. and with stirring with sodium borohydride (7 mg) for 3 h. The mixture was poured into water (20 ml), acidified with diluted hydrochloric acid and extracted with ethyl acetate. The solvent was evaporated under vacuum and the crude product chromatographed over silica gel, eluting with light petroleum-EtOAc (1:1), to give **9** (20 mg).

1-Oxo-11α,18-dihydroxymanoyl oxide (14). M.p. 142-145 °C; IR (ν_{max}, cm^{-1}): 3620, 3450, 2930, 1690, 1470, 1370, 1110, 1070. $[M]^+$ at *m/z* 336.2301, $C_{20}H_{32}O_4$ requires 336.2300. ^1H NMR (200 MHz) δ: 0.93, 1.25, 1.46 and 1.54 (each 3 H, s), 1.70 (1H, d, J = 6.6 Hz, H-9), 2.09 (1H, dd, J = 14.2 and 9.7 Hz, H-12), 2.25 (1H, dd, J = 14.2 and 8.5 Hz, H-12), 2.38 (1H, dt, J = 14.2, 4.3 and 3.4 Hz, H-2), 2.74 (1H, td, J =14.2, 14.2 and 4.9 Hz, H-2), 3.34 and 3.53 (each 1H, d, J = 10.5 Hz, H-18), 4.25 (1H, m, H-11), 4.93 (1H, dd, J = 10.6 and 1.7 Hz, H-15), 5.17 (1H, dd, J = 17.2 and 1.7 Hz, H-15), 5.83 (1H, dd, J = 17.2 and 10.6 Hz, H-14); EIMS *m/z* (rel. int.): 336 $[M]^+$ (9), 321 (46), 318 (11), 303 (48), 291 (6), 285 (5), 253 (33), 233 (15), 223 (5), 219 (9), 203 (100), 191 (32).

1-Oxo-6β,18-dihydroxymanoyl oxide (15). M.p. 179-181 °C; IR (ν_{max}, cm^{-1}): 3614, 3420, 2930, 1700; $[M-CH_3]^+$ at *m/z* 321.2065, $C_{19}H_{29}O_4$ requires 321.2050. ^1H NMR (200 MHz) δ: 1.34, 1.42, 1.54 and 1.65 (each 3H, s), 2.17 (1H, ddd, J = 12.8, 8.4, 4 Hz, H-2), 3.09 (1H, ddd, J = 13.1, 7.0 and 6.0 Hz, H-2), 3.35 and 3.57 (each 1H, d, J = 10.7 Hz, H-18), 4.50 (1H, br. s, H-6), 4.92 (1H, dd, J = 10.7 and 1.5 Hz, H-15), 5.13 (1H, dd, J = 17.4 and 1.5 Hz, H-15), 5.88 (1H, dd, J = 17.4 and 10.7 Hz, H-14); EIMS *m/z* (rel. int.): 321 $[M-CH_3]^+$ (100), 318 (4), 303 (22), 285 (12), 255 (7), 251 (13), 233 (13), 215 (18), 201 (8), 197 (14).

1-Oxo-2α,18-dihydroxymanoyl oxide (16). $[M-CH_3]^+$ at *m/z* 321.2065, $C_{19}H_{29}O_4$ requires 321.2055. ^1H NMR (200 MHz) δ: 1.05, 1.27, 1.32 and 1.36 (each 3H, s), 2.11 (1H, dd, J = 12.9, 7.1 Hz, H-3), 3.17 and 3.42 (each 1H, d, J = 10.8 Hz, H-18), 4.79 (1H, dd, J = 12 and 7.1 Hz, H-2), 4.96 (1H, dd, J = 10.7, 1.4 Hz, H-15), 5.17 (1H, dd, J = 17.4 and 1.4 Hz, H-15), 5.90 (1H, dd, J = 17.4 and 10.7 Hz, H-14); EIMS *m/z* (rel. int.): 321 $[M-CH_3]^+$ (100), 303 (19), 285 (13), 273 (15), 255 (10), 238 (7), 215 (11), 203 (7), 197 (9).

1β,2α,18-Trihydroxymanoyl oxide (17). $[M-CH_3]^+$ at *m/z* 323.2195. $C_{19}H_{31}O_4$ requires 323.2195; ^1H NMR (200 MHz) δ: 0.82, 0.96, 1.26 and 1.31 (each 3H, s), 3.12 (1H, d, J = 8.9 Hz, H-1), 3.15 and 3.45 (each 1H, d, J = 11.0 Hz, H-18), 3.74 (1H, ddd, J = 11.2, 8.9 and 5.7 Hz, H-2), 4.94 (1H, dd, J = 10.7 and 1.5 Hz, H-15), 5.16 (1H, dd, J = 17.2 and 1.5 Hz, H-15), 5.89 (1H, dd, J = 17.2 and 10.7 Hz, H-14); EIMS *m/z* (rel. int.): 323 $[M-CH_3]^+$ (16), 305 (9), 287 (8), 269 (9), 257 (12), 245 (8), 222 (6), 199 (15).

Incubation of 1-oxo-18-hydroxymanoyl oxide (13). The substrate **13** (300 mg), which was obtained in the fermentation of jhanidiol (**3**) and also synthesized, was incubated with *G. fujikuroi* in 80 conical flasks as described above. The metabolites were separated by chromatography on silica gel, eluting with petrol-EtOAc mixtures, to give starting material (110 mg), 1-oxo-11β,18-dihydroxymanoyl oxide (**14**) (8 mg), 1α,18-dihydroxymanoyl oxide (**9**) (30 mg), 1-oxo-2α,18-dihydroxymanoyl oxide (**16**) (3 mg), 1-oxo-6β,18-dihydroxymanoyl oxide (**15**) (4 mg), 1-oxo-14ξ,15-epoxy-18-hydroxymanoyl oxide (**18**) (2 mg) and 1-oxo-3α,18-dihydroxymanoyl oxide (**19**) (1.5 mg).

1-Oxo-14ξ,15-epoxy-18-hydroxymanoyl oxide (18). M.p. 191-193° C; IR (ν_{max}, cm^{-1}): 3680, 3450, 2930, 1700, 1110 $[M-CH_3]^+$ at *m/z* 321.2079, $C_{19}H_{29}O_4$ requires 321.2065; ^1H NMR (200 MHz) δ: 0.98, 1.20, 1.33 and 159 (each 3H, s), 2.30 (1H, ddd, J = 13.8, 6.6 and 5.2 Hz, H-2), 2.66 (1H, dd, J = 5.2 and 4 Hz, H-15), 2.75 (1H, dd, J = 5.2 and 2.8 Hz, H-15), 2.82 (1H, ddd, J = 13.8, 10.6 and 5.8 Hz, H-2), 2.86 (1H, dd, J = 4 and 2.8 Hz, H-14), 3.25 and 3.46 (each 1H, d, J = 10.6 Hz, H-18); EIMS *m/z* (rel. int.): 321 $[M-CH_3]^+$ (5), 305 (2), 303 (2), 293 (100), 275 (14), 257 (25), 245 (58), 227 84), 217 (7), 201(9), 199 (13).

1-Oxo-3β,18-dihydroxymanoyl oxide (19). $[M-CH_3]^+$ at *m/z* 321.2070. $C_{19}H_{29}O_4$ requires 321.2065; ^1H NMR (200 MHz) δ: 0.96, 1.23, 1.32 and 1.37 (each 3H, s), 2.20 (1H, dd, J = 13.8 and 3.8 Hz, H-2), 3.35 (1H, dd, J = 13.8 and 3.8 Hz, H-2), 3.53 (2 H, s, H-18), 4.13 (1H, t, J = 3.8 Hz, H-3), 4.94 (1H, dd, J = 10.7 and 1.5 Hz, H-15), 5.16 (1H, dd, J = 17.3 and 1.5 Hz, H-15), 5.89 (1H, dd, J = 17.3 and 10.6 Hz, H-14); EIMS *m/z* (rel. int.): 321 $[M-CH_3]^+$ (100), 309 (3), 303 (11), 285 (13), 273 (19), 255 (10), 251 (5), 238 (22), 215 (6), 203 (6), 199 (4). **Diacetate (20)** ^1H NMR (200 MHz) δ: 1.20, 1.23, 1.32 and 1.35 (each 3H, s), 2.01 and 2.02 (each 3H, s), 2.30 (1H, dd, J = 14.3 and 3.9 Hz, H-2), 3.21 (1H, dd, J = 14.3 and 3.9 Hz, H-2), 3.88 and 3.96 (each 1H, d, J = 10.4 Hz, H-18), 4.94 (1H, dd, J = 10.7 and 1.5 Hz, H-15), 5.17 (1H, dd, J = 17.4 and 1.5 Hz, H-15), 5.18 (1H, t, J = 3.9 Hz, H-3), 5.89 (1H, dd, J = 17.4 and 10.6 Hz, H-14); EIMS *m/z* (rel. int.): 420 $[M]^+$ (1), 405 (60), 345 (60), 327 (12), 322 (11), 285 (6), 275 (7), 267 (35), 187 (22).

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