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Microbial Transformation of 18-Hydroxy-9,13-*epi-ent*-pimara-7,15-diene by *Gibberella fujikuroi*

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The incubation of the diterpene 18-dihydroxy-9,13-*epi-ent*-pimara-7,15-diene (**3**) with the fungus *Gibberella fujikuroi* gave 14 metabolites, **4** and **6–18**. The carbons functionalized were the C-20 methyl and all the secondaries, except C-12. The main reaction observed was the epoxidation of the 7,8-double bond, which rearranged to form 7-keto derivatives, such as **10–17**, or the allylic alcohol **18**. Compound **9** was the only one obtained in which the 7,8-double bond of the substrate remained unaltered. This work confirms that, in the feeding of this type of diterpene with this fungus, the oxidation at C-19, typical of the biosynthesis of gibberellins from *ent*-kaur-16-ene, is inhibited.

In previous work we have described the biotransformation of two 9-*epi-ent*-pimarane diterpenes, **1** and **2**, by the fungus *Gibberella fujikuroi*.^{1,2} Although compounds with this skeleton are not produced naturally by this fungus, it has been proposed that *G. fujikuroi* forms an *ent*-pimarane carbonium ion as an intermediate in the production of *ent*-kaur-16-ene, a precursor of the gibberellin plant hormones.³

Continuing with this work, we describe here results obtained from the incubation of 18-hydroxy-9,13-*epi-ent*-pimara-7,15-diene (**3**) with *G. fujikuroi*. This substrate was chosen with the aim of determining whether a change in the orientation of the isopropenyl group at C-13 has any effect on the results of the fermentation, considering that now in **3** the spatial position of the 13,14-double bond is more comparable to that of the *ent*-kaur-16-ene double bond.

Results and Discussion

The substrate **3** was isolated from *Calceolaria glandulosa*, an uncommon taxon that grows on the coastal hills of Central Chile.⁴ Other species of this genus also contain diterpenes of this type.

The fermentation of *G. fujikuroi* was carried out in the presence of AMO 1618, a substance that inhibits the production of *ent*-kaur-16-ene, without affecting the post-kaurane metabolism.^{5,6} The incubation was harvested after 6 days, and the broth and mycelium extracts were combined. Chromatography of the extract gave the metabolites **4** and **6–18**. Compound **17** was isolated as its acetate **17a**, by acetylation and chromatography of the fraction containing it. High-resolution MS of compound **4** led to its structural formula, C₂₀H₃₂O₂, indicating that during the incubation an oxygen atom had been introduced into the molecule. The resonance of a hydrogen at δ 2.93 as a doublet ($J = 4.8$) and the disappearance of the vinylic H-7 indicated that the new oxygen must form a part of an oxirane ring. Thus structure **4** was assigned to this compound, which was confirmed by 2D NMR data. The α -stereochemistry assigned to the epoxide group was based on the fact that the chemical shift and the form of

resonance observed were practically identical with the epoxide formed in our incubation of **1** and **2**, in which both possess the α -orientation.^{1,2} On the other hand, the chemical epoxidation of the substrate with *m*-chloroperbenzoic acid led to the main compound **4** and to the minor one **5**, the first of which was identical with the metabolite obtained in the biotransformation.

The structure 9 β ,18-dihydroxy-7 α ,8 α -epoxy-13-*epi-ent*-pimara-15-ene (**6**) was assigned to a product, which was probably formed by hydroxylation of the metabolite **4**. This compound showed in the high-resolution MS spectrum a molecular ion at m/z 320.2347, corresponding to C₂₀H₃₂O₃. In the ¹H NMR spectrum H-7 resonated as a double doublet ($J = 5.5$ and 1.6 Hz) at δ 3.08, and in the ¹³C NMR spectrum C-7 appeared at δ 64.0 and C-9 at 74.9. Complete spectral assignment of this spectrum (Table 1) was carried out using 2D NMR experiments (COSY, NOESY, HSQC, and HMBC) including the location of the new hydroxyl group at C-9. The α -stereochemistry assigned to this alcohol was made considering the deshielding of H-14(α), which resonated at δ 2.26 in comparison with 1.81 in **4**.

Metabolite **7** (C₂₀H₃₂O₄) was related to **6**, with the exception that **7** has a additional oxygen atom in the form of a secondary hydroxyl group. The ¹H NMR spectrum of **7** showed the resonance of 14 α -H at δ 2.26, unchanged from the spectrum of **6**, indicating an α -stereochemistry for the hydroxyl group at C-9. The proton geminal to the new secondary alcohol resonated as a double doublet at δ 3.82 ($J = 11.3$ and 4 Hz), indicating the presence of an equatorial hydroxyl group at C-1(α), C-3(α), or C-12(β). The C-1(α) position was chosen from the comparison of the ¹³C NMR spectra of **6** and **7** and was confirmed in the HMBC spectrum, where a correlation between C-1 and C-20 was observed. Other proof of the existence of the hydroxyl group at C-1 was the hydrogen bond observed between the C-1 and C-9 hydroxyl groups, the protons of which resonated as singlets at δ 2.68 and 2.27, respectively. Thus, the structure of this metabolite was determined to be **7**.

The structures assigned to **6** and **7**, both which possess a 9 α -hydroxyl group, indicated that the β -stereochemistry assigned by us to two metabolites obtained in the biotransformation of **1** and **2** with *G. fujikuroi*^{1,2} must be revised to the corresponding 9 α -alcohols, **19** and **20**, respectively.

The HRMS of compound **8** showed the molecular ion at m/z 336.2300, corresponding to the molecular formula

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Chart 1

$C_{20}H_{32}O_4$. Its 1H NMR spectrum showed the characteristic resonances of H-1 and H-7 observed in **7**; however, now the signal of H-7 appeared as a clear doublet at δ 3.19, with a coupling of 5 Hz. This can be explained by the presence also in the molecule of an α -axial hydroxyl group at C-6, whose geminal proton resonates at δ 4.36 as a triplet ($J = 5$ Hz). This was confirmed by double resonance experiments with irradiation of H-6, collapsing the doublets at δ 1.29 (H-5) and 3.19 (H-7) to singlets. Therefore, the structure **8** was assigned to this compound and confirmed by assignment of the ^{13}C NMR spectrum using 2D NMR data (Table 1).

Compound **9** was the only metabolite obtained from this fermentation in which the 6,7-double bond of the substrate was unaltered. Its molecular formula, $C_{20}H_{32}O_3$, was determined by HRMS (m/z 320.2344). The two oxygens introduced into the molecule during the incubation were in the form of two secondary hydroxyl groups, which were assigned to C-6(β) and C-14(α) on the basis of the following information. The proton geminal to the hydroxyl at C-6 appeared as a double doublet at δ 4.18 ($J = 10.4$ and 1.6 Hz), while H-7 resonates as a broad singlet at 5.42. This pattern indicated a *trans*-diaxial coupling between H-6 and H-5. The hydrogen geminal to the alcohol at C-14 appears at δ 3.75 as a clear singlet. The α -stereochemistry for this hydroxyl group was assigned considering the γ -effects observed in the ^{13}C NMR spectrum with C-12 and C-17 and

not with C-15, in comparison with the corresponding spectrum of the substrate **3**, whose assignment⁴ (Table 1) has now been confirmed using 2D NMR methods.

Several metabolites obtained from this incubation possessed a 7-oxo group, which is produced by the opening of a 7 α ,8 α -epoxy group. The simplest of these compounds was **10** ($C_{20}H_{32}O_2$), which is formed from **4**. The 1H NMR spectrum, in comparison with that of the substrate **3**, showed the disappearance of the 7,8-double bond. This was now replaced by a 7-oxo group, which was confirmed in the ^{13}C NMR spectrum by the resonance of the C-7 at δ 215.3. In this and other compounds described later, the stereochemistry of H-8 must be β , because the neutralization of the carbocation at C-8, formed by opening of the α -oxirane ring, must occur on the β -face. This was confirmed by the 1H NMR spectrum, which showed the resonance of H-8 as a triple doublet at δ 2.47 ($J = 11.7$ and 3.2 Hz), indicating a *trans* relationship with H-9. Thus, the structure of this metabolite was determined as 18-hydroxy-7-oxo-9,13-*epi*-ent-pimara-15-ene (**10**).

Another compound isolated from this fermentation was the metabolite **11**. In its HRMS the molecular ion appeared at m/z 320.2350 ($C_{20}H_{32}O_3$), which meant that this compound possesses two oxygens more than the substrate **3**. The presence of a carbonyl group was supported by an absorbance in the IR spectrum, as well as a resonance at δ 216.2 in the ^{13}C NMR spectrum. The absence of signal

Chart 2

Table 1. ^{13}C NMR Data for Compounds 3–9

C	3	4	4a	5	6	7	8	9	9a
1	36.3	37.4 ^b	36.8 ^b	38.7	32.6	74.3	73.6	37.3	37.3
2	18.0	17.5	17.5	17.9	17.3	25.3	26.7	17.9	17.6
3	36.5	36.3 ^b	36.9	36.8	35.8	33.5	36.9	38.3	37.7
4	37.4	37.5	36.4	33.9	38.3	37.6	38.0	37.8	35.9
5	37.1	35.2	36.0	32.7	37.8	37.6	45.3	46.1	40.2
6	23.6	22.7 ^a	22.6 ^a	23.2	21.7	21.3	66.9	67.5	71.3
7	119.0	60.8	60.8	59.3	64.0	62.8	62.9	124.7	124.4
8	137.0	60.7	60.7	61.9	63.0	62.3	64.4	141.0	137.8
9	53.1	49.0	48.7	50.7	74.9	77.2	41.6	48.0	46.7
10	34.9	35.5	35.6	37.0	41.1	44.6	40.0	36.0	36.1
11	25.3	22.4 ^a	22.8 ^a	22.1	28.9	28.9	22.8	24.5	24.1
12	39.4	37.0 ^b	36.8 ^b	35.3	32.6	32.3	36.0	32.5	33.2
13	38.9	38.3	38.5	39.1	38.3	38.2	38.5	42.9	42.1
14	49.6	48.2	48.3	49.0	44.0	43.3	48.0	80.4	80.7
15	145.8	144.9	145.0	147.6	144.6	144.5	144.5	143.8	142.4
16	111.2	112.3	112.3	108.9	112.6	112.6	112.6	112.7	113.3
17	29.7	29.9	30.2	30.6	29.9	29.9	30.1	24.5	24.5
18	72.5	71.7	72.5	72.0	71.9	70.9	71.8	75.4	74.2
19	18.4	17.5	17.6	18.9	18.2	17.9	20.8	18.4	18.8
20	22.7	24.5	24.3	25.9	16.3	10.4	20.3	23.0	23.0

^{a,b} These values can be interchanged.

for the 7,8-double bond in the ^1H NMR spectrum permitted us to assign this oxygen function at C-7. The second oxygen atom must form part of a tertiary hydroxyl group, as hydrogens geminal to a hydroxyl groups were not observed in the ^1H NMR spectrum, while a tetrasubstituted carbon bearing an oxygen atom was observed at δ 78.5 in its ^{13}C NMR spectrum. This signal was assigned to C-8 because, in the ^1H NMR spectrum, the two H-14 resonated as a pair

Table 2. ^{13}C NMR Data for Compounds 10–16 and 18

C	10	11	12	12a	13	14	15	16	18
1	35.4	35.3	34.1 ^a	33.9 ^a	34.3 ^a	73.3	44.9	36.9	35.9
2	18.5	17.6	18.8	18.5	17.9	27.9	65.4	27.6	19.0
3	34.9	34.7	34.6 ^a	34.9 ^a	34.5 ^a	33.1	44.2	75.3	34.9
4	37.7	37.3	37.2	37.2	35.2	37.5	38.7	42.2	38.0
5	39.8	36.3	40.8	41.9	44.1	38.5	38.9	40.2	43.4
6	37.3	35.6	36.9 ^b	37.0 ^b	35.1	37.7 ^a	36.9 ^a	37.4	27.8
7	215.3	216.2	214.8	210.2	201.2	214.7	214.5	214.0	73.7
8	45.9	78.5	43.4	43.6	130.6	48.2	45.9	45.6	142.2
9	52.6	57.4	55.2	52.3	162.8	54.6	52.6	51.5	52.8
10	35.8	35.0	37.8	36.7 ^c	39.9	41.3	35.8	35.8	38.6
11	24.6	20.6	68.5	71.7	67.3	26.2	24.9	24.5	66.8
12	38.1	33.3	47.9	41.8	44.9	38.2 ^a	37.9 ^a	38.0	45.7
13	36.4	37.9	37.8	36.8 ^c	37.8	36.9	36.4	36.4	39.9
14	37.5	42.9	36.9 ^b	36.3 ^b	34.8	38.1 ^a	37.4 ^a	33.4	133.0
15	145.9	148.7	144.9	144.2	144.9	146.3	145.7	145.8	146.1
16	112.7	109.1	113.1	113.7	111.8	112.6	112.8	112.9	113.6
17	31.3	29.3	31.2	31.0	27.8	31.2	31.3	31.4	28.8
18	71.6	71.4	71.5	72.7	71.1	71.1	71.1	70.4	72.0
19	18.2	18.0	18.2	18.0	17.5	18.3	19.1	12.0	18.1
20	25.1	23.6	25.4	25.0	19.9	18.5	26.1	24.9	25.1

^{a,b,c} These values can be interchanged.

of doublets centered at δ 1.06 and 1.67 ($J = 13.8$ Hz) and, moreover, the H-8 does not appear, which resonates in 10 as a triple doublet at δ 2.47. In the MS, the cleavage of the 6,7 and 9,10 bond produces two fragments at m/z 165 and 123, which are characteristic of 7-oxo-8-hydroxy derivatives.⁷ The location of the hydroxyl group at C-8 was confirmed by study of 2D NMR data. In the HMBC spectrum a correlation was observed between the H-14 at δ 1.06 and the C-8 at δ 78.5. The β -stereochemistry for the 8-OH was determined considering that the value of the resonance of the H-9 at δ 1.60, in comparison with δ 1.02 in 10, is more typical of a *trans* relationship with 8-OH than of a *cis* relationship. Therefore, the structure of 8 β ,18-dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (11) was assigned to this metabolite.

Compound 12, in comparison with 10, possesses one additional oxygen ($\text{C}_{20}\text{H}_{32}\text{O}_3$), which forms part of a secondary alcohol group. This was supported by the ^1H NMR spectrum with a signal of a proton geminal to a hydroxyl group at δ 4.00 as a triple doublet ($J = 10.6$ and 4.6). The coupling and form of resonance indicated that the hydroxyl group must be equatorial and located at C-11(α), where the H-11 has the same axial coupling (10.6 Hz) with H-9 and H-12(α). This structure was confirmed by assignment of its ^{13}C NMR spectrum and that of its diacetate, 12a (Table 2).

The metabolite, to which the structure 13 was assigned, showed two units less in its HRMS (m/z 318.2204) in comparison with 12. This was the result of a new double bond, which must be conjugated with the 7-oxo group, because absorptions in the IR and UV spectra appeared at 1650 cm^{-1} and 247 nm, respectively. Moreover, this unsaturation must be in the C-8,C-9 position, because no new vinylic hydrogens were observed in the ^1H NMR spectrum. The secondary alcohol was located at C-11 considering its ^{13}C NMR spectrum, which was assigned on the basis of the 2D NMR data. In the HMBC spectrum correlations were observed between H-11 and C-8 and C-9 and between H-14 and C-8, C-9, C-12, C-13, and C-15. The α -stereochemistry for this alcohol was assigned considering the resonance of its geminal proton, which appears at δ 4.62 as a triplet ($J = 6$ Hz). In its least energy conformation, determined by MM+ molecular mechanics calculations, this coupling constant is in accordance with an α -axial hydroxyl. Therefore, the structure of this compound was determined to be 11 α ,18-dihydroxy-7-oxo-13-*epi-ent*-pimara-8(9),15-diene (13).

Elmer 1600 FT and a Varian Cary 1E spectrometer, respectively. ^1H NMR spectra were taken 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 125.03 MHz in a Bruker AMX2-500, except those of **9** and **9a**, which were recorded at 50 MHz in a Bruker AC-200. MS and HRMS were taken at 70 eV (probe) in a Micromass Autospec spectrometer. Dry column chromatography was performed on Merck Si gel (0.02–0.063 mm). Molecular mechanics calculations were carried out with the program Hyperchem 7.0 (Hypercube).

Organism. The fungal strain was *Gibberella fujikuroi* IMI 58289 and was a gift from Dr. J. R. Hanson, School of Chemistry, Physics and Environmental Science (University of Sussex, UK).

Incubation Experiment. The fungus *G. fujikuroi*, inhibited with 5×10^{-5} M AMO 1618, was grown in shake culture in 75 conical flasks (250 mL) each containing sterile medium (50 mL) at 25 °C for 1 day.⁹ The substrate **3** (256 mg), dissolved in EtOH (16 mL) with Tween (2 drops), was distributed equally between the flasks and the incubation allowed to continue for a further 6 days.

Extraction and Isolation. The broth was filtered, adjusted to pH 2 with dilute HCl, and extracted with EtOAc. The mycelia were treated with liquid N_2 , crushed in a mortar, and extracted with EtOAc. The two extracts were combined, dried, and concentrated. Chromatography of the residue on silica gel, eluting with petrol–EtOAc mixtures, gave starting material (**3**) (47 mg), 18-hydroxy-7 α ,8 α -epoxy-9,13-*epi-ent*-pimara-15-ene (**4**) (1 mg), 8 β ,18-dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (**11**) (6 mg), 9 α ,18-dihydroxy-7 α ,8 α -epoxy-13-*epi-ent*-pimara-15-ene (**6**) (12 mg), 18-hydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (**10**) (2 mg), 11 α ,18-dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (**12**) (3 mg), 11 α ,18-dihydroxy-13-*epi-ent*-pimara-8(9),15-diene (**13**) (4 mg), 6 β ,14 α ,18-trihydroxy-9,13-*epi-ent*-pimara-7,15-diene (**9**) (4.4 mg), 1 α ,9 α ,18-trihydroxy-7 α ,8 α -epoxy-13-*epi-ent*-pimara-15-ene (**7**) (3.2 mg), 1 α ,18-dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (**14**) (7 mg), 7 α ,11 α ,18-trihydroxy-9,13-*epi-ent*-pimara-8(14),15-diene (**18**) (9 mg), 2 β ,18-dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (**15**) (1.2 mg), 18,20-dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (**17**) (0.6 mg), 1 α ,6 α ,18-trihydroxy-7 α ,8 α -epoxy-9,13-*epi-ent*-pimara-15-ene (**8**) (1.4 mg), and 3 α ,18-dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (**16**) (1 mg).

18-Hydroxy-7 α ,8 α -epoxy-9,13-*epi-ent*-pimara-15-ene (4**):** ^1H NMR (500 MHz) δ 0.77 (3H, s, H-19), 1.00 (3H, s, H-17), 1.10 (3H, s, H-20), 1.14 (1H, dd, J = 13.2, 2.8 Hz, H-14 β), 1.64 (1H, m, H-1), 1.81 (3H, m, 2H-6 and H-14 α), 2.93 (1H, d, J = 4.8, H-7), 3.08 and 3.31 (each 1H, d, J = 11 Hz, H-18), 4.98 (1H, dd, J = 17.5, 1.2 Hz, H-16), 5.01 (1H, dd, J = 10.9, 1.2 Hz, H-16), 5.85 (1H, dd, J = 17.5, 10.9 Hz, H-15); EIMS m/z 304 [M]⁺ (19), 289 (25), 286 (33), 273 (31), 271 (31), 268 (48), 255 (44), 253 (85), 239 (39), 211 (20), 203 (19), 199 (38), 185 (46), 169 (31); HREIMS m/z 304.2399 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_2$, 304.2902).

Acetate **4a:** ^1H NMR (500 MHz) δ 0.85 (H-19), 0.99 (3H, s, H-17), 1.03 (1H, dd, J = 12.2 Hz, H-12), 1.10 (3H, s, H-20), 1.13 (1H, dd, J = 13, 2.8 Hz, H-14), 1.60 (1H, ddt, J = Hz, H-2), 2.04 (1H, s, OAc), 2.91 (1H, m, H-2), 2.04 (3H, s, OAc), 2.92 (1H, d, J = 5, H-7), 3.54 and 3.86 (each 3H, d, J = 11 Hz, H-18), 4.97 (1H, d, J = 18, 1.2 Hz, H-16), 5.00 (1H, d, J = 11 Hz, H-16), 5.85 (1H, dd, J = 18, 11 Hz, H-15); EIMS m/z 346 [M]⁺ (74), 331 (100), 304 (30), 286 (14), 271 (83), 255 (21), 253 (15), 243 (7), 223 (7), 203 (15), 189 (11), 177 (16), 163 (30); HREIMS m/z 346.2510 (calcd for $\text{C}_{22}\text{H}_{34}\text{O}_3$, 346.2508).

9 α ,18-Dihydroxy-7 α ,8 α -epoxy-13-*epi-ent*-pimara-15-ene (6**):** ^1H NMR (500 MHz) δ 0.83 (3H, s, H-19), 0.85 (1H, dd, J = 13, 2.7 Hz, H-14 β), 1.14 (3H, s, H-17), 1.26 (3H, s, H-20), 2.26 (1H, d, J = 13 Hz, H-14 α), 3.08 (1H, dd, J = 5.5, 1.6 Hz, H-7), 3.09 and 3.31 (each 1H, d, J = 11 Hz, H-18), 4.99 (1H, dd, J = 17.6, 1.3 Hz, H-16), 5.04 (1H, dd, J = 10.9, 1.3 Hz, H-16), 5.83 (1H, dd, J = 17.6, 10.9 Hz, H-15); EIMS m/z 320 [M]⁺ (6), 302 (17), 287 (15), 284 (36), 269 (38), 255

(11), 243 (11), 227 (8), 215 (8), 181 (26); HREIMS m/z 320.2347 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

1 α ,9 α ,18-Trihydroxy-7 α ,8 α -epoxy-13-*epi-ent*-pimara-15-ene (7**):** ^1H NMR (500 MHz) δ 0.83 (3H, s, H-19), 0.88 (1H, dd, J = 13, 2.8 Hz, H-14 β), 1.04 (3H, s, H-17), 1.25 (3H, s, H-20), 1.28 (1H, dt, J = 13, 3.4 Hz, H-3 α), 1.58 (1H, td, J = 13, 4 Hz, H-3 β), 2.05 (1H, dt, J = 13 Hz, 4 Hz, H-11 β), 2.26 (1H, d, J = 13 Hz, H-14 α), 2.27 (1H, s, HO–C-9), 2.68 (1H, s, HO–C-1), 3.06 (1H, d, J = 5.7 Hz, H-7), 3.08 and 3.30 (each 1H, d, J = 10.8 Hz, H-18), 3.82 (1H, dd, J = 11.3, 4 Hz, H-1), 5.03 (1H, d, J = 17, 1 Hz, H-16), 5.05 (1H, d, J = 11.3, 1 Hz, H-15), 5.84 (1H, dd, J = 17, 11.3 Hz, H-14); EIMS m/z 336 [M]⁺ (1), 321 (2), 318 (5), 307 (5), 303 (4), 300 (6), 287 (27), 269 (15), 228 (33), 221 (22), 203 (10), 189 (9), 180 (14), 175 (34), 167 (55), 156 (51); HREIMS m/z 336.2304 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_4$, 336.2301).

1 α ,6 α ,18-Trihydroxy-7 α ,8 α -epoxy-9,13-*epi-ent*-pimara-15-ene (8**):** ^1H NMR (500 MHz) δ 1.04 (3H, s, H-17), 1.24 (3H, s, H-19), 1.28 (3H, s, H-20), 1.29 (1H, d, J = 5 Hz, H-5), 1.90 (1H, d, J = 13 Hz, H-14), 1.93 (1H, br d, J = 6.7 Hz, H-9), 3.19 (1H, d, J = 5 Hz, H-7), 3.29 and 3.62 (each 1H, d, J = 11 Hz, H-18), 3.49 (1H, dd, J = 11.5, 4 Hz, H-1), 4.36 (1H, t, J = 5 Hz, H-6), 5.00 (1H, d, J = 17.6 Hz, H-16), 5.03 (1H, d, J = 11 Hz, H-16), 5.81 (1H, dd, J = 17.6, 11 Hz, H-15); EIMS m/z 336 [M]⁺ (16), 318 (15), 305 (16), 300 (16), 287 (20), 269 (32), 241 (14), 215 (20), 203 (26), 189 (20), 183 (28), 159 (21), 149 (31), 145 (26); HREIMS m/z 336.2300 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_4$, 336.2301).

6 β ,14 α ,18-Trihydroxy-9,13-*epi-ent*-pimara-7,15-diene (9**):** ^1H NMR (200 MHz) δ 0.98 (3H, s, H-19), 1.05 (3H, s, H-20), 1.09 (3H, s, H-17), 3.18 and 3.35 (each 1H, d, J = 11 Hz, H-18), 3.75 (1H, s, H-14), 4.18 (1H, dd, J = 10.4, 1.6 Hz, H-6), 5.02 (1H, d, J = 10.8 Hz, H-16), 5.04 (1H, d, J = 17.8 Hz, H-16), 5.42 (1H, br s, H-7), 5.82 (1H, dd, J = 17.8, 10.8 Hz, H-15); EIMS m/z 320 [M]⁺ (36), 305 (54), 289 (57), 287 (34), 271 (20), 269 (13), 262 (13), 253 (6), 243 (10), 221 (14), 219 (11), 203 (15), 189 (18), 167 (19), 151 (28); HREIMS m/z 320.2344 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

Triacetate **9a:** ^1H NMR (500 MHz) δ 0.97, 0.98, and 1.01 (each 3H, s), 1.81 (1H, d, J = 10 Hz, H-5), 2.05 and 2.06 (each 3H, s, –OAc), 3.68 and 3.92 (each 1H, d, J = 11 Hz, H-18), 4.97 (1H, s, H-14), 5.06 (1H, dd, J = 17.4, 1.1 Hz, H-16), 5.07 (1H, dd, J = 11.2 Hz, 1.1 Hz, H-16), 5.39 (1H, d, J = 10 Hz, H-6), 5.41 (1H, br s, H-7), 5.84 (1H, dd, J = 17.4, 11.2 Hz, H-15); EIMS m/z 446 [M]⁺ (0.2), 386 (6), 344 (57), 326 (27), 311 (12), 284 (51), 269 (35), 266 (19), 251 (100), 245 (26), 223 (16), 211 (20), 197 (46), 185 (51), 183 (48); HREIMS m/z 446.2580 (calcd for $\text{C}_{26}\text{H}_{38}\text{O}_6$, 404.2668).

18-Hydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (10**):** ^1H NMR (500 MHz) δ 0.82 (3H, s, H-19), 0.94 (3H, s, H-20), 0.97 (3H, s, H-17), 1.02 (1H, dt, J = 12.4, 3.3 Hz, H-9), 1.15 (1H, td, J = 13, 3.6 Hz, H-12 α), 1.20 (1H, dd, J = 14, 11.7 Hz, H-14 α), 1.81 (1H, ddd, J = 13, 5.8, 2.8 Hz, H-12 β), 1.95 (1H, dt, J = 14, 3.5 Hz, H-14 β), 2.12 (1H, d, J = 13 Hz, H-5), 2.17 (1H, dd, J = 16.2, 13 Hz, H-6 α), 2.29 (1H, dd, J = 16.2, 2.6 Hz, H-6 β), 2.47 (1H, td, J = 11.7, 3.2 Hz, H-8), 3.12 and 3.32 (each 1H, d, J = 10.1 Hz, H-18), 4.94 (1H, dd, J = 17.4, 1.2 Hz, H-16), 5.03 (1H, dd, J = 10.9, 1.2 Hz, H-16), 5.67 (1H, dd, J = 17.4, 10.9 Hz, H-15); EIMS m/z 304 [M]⁺ (12), 286 (71), 279 (12), 273 (62), 257 (37), 243 (8), 218 (10), 203 (14), 191 (10), 177 (18), 164 (22), 149 (55), 136 (38), 123 (77), 121 (77), 109 (86), 107 (80); HREIMS m/z 304.2384 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_2$, 304.2402).

8 β ,18-Dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (11**):** ^1H NMR (500 MHz) δ 0.87 (3H, s, H-19), 0.93 (3H, s, H-20), 1.06 and 1.67 (each 1H, d, J = 13.8 Hz, H-14), 1.18 (3H, s, H-17), 1.60 (2H, m, H-2 and H-9), 2.28 (1H, dd, J = 11, 8.8 Hz, H-5), 2.44 (2H, m, H-6), 3.11 and 3.27 (each 1H, d, J = 10.5 Hz, H-18), 4.87 (1H, dd, J = 17.4, 1.2 Hz, H-16), 4.89 (1H, dd, J = 10.7, 1.2 Hz, H-16), 5.84 (1H, dd, J = 17.4, 10.7 Hz, H-15); EIMS m/z 320 [M]⁺ (14), 305 (17), 302 (15), 292 (11), 287 (13), 277 (77), 271 (16), 269 (10), 259 (10), 243 (24), 225 (16), 215 (27), 189 (16), 181 (33), 165 (52), 138 (21), 123 (100), 109 (82); HREIMS m/z 320.2350 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

11 α ,18-Dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (12): IR (film) $\nu_{\max}$ 3400, 2930, 1704, 1455, 1380 cm^{-1} ; ^1H NMR (500 MHz) δ 0.82 (3H, s, H-19), 1.02 (3H, s, H-20), 1.17 (3H, s, H-17), 2.20 (1H, dd, J = 18.6, 13.7 Hz, H-6 α), 2.31 (1H, dd, J = 18.6, 3.1 Hz, H-6 β), 3.08 and 3.35 (each 1H, d, J = 11 Hz, H-18), 4.00 (1H, td, J = 10.6, 4.6 Hz, H-11), 4.96 (1H, d, J = 17.4 Hz, H-16), 4.89 (1H, d, J = 10.7 Hz, H-16), 5.84 (1H, dd, J = 17.4, 10.7 Hz, H-15); EIMS m/z 320 $[\text{M}]^+$ (13), 305 (9), 302 (12), 287 (27), 271 (76), 253 (17), 179 (40), 161 (45), 147 (20), 143 (56), 135 (31), 133 (25), 121 (65), 109 (74); HREIMS m/z 320.2338 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

Diacetate 12a: ^1H NMR (500 MHz) δ 0.91 (3H, s, H-19), 1.00 (3H, s, H-20), 1.20 (3H, s, H-17), 2.05 and 2.06 (each 3H, s, -OAc), 3.63 and 3.79 (each 1H, d, J = 11 Hz, H-18), 5.10 (1H, td, J = 10.7, 4.6 Hz, H-11), 5.13 (1H, d, J = 17.4 Hz, H-16), 4.89 (1H, d, J = 10.9 Hz, H-16), 5.84 (1H, dd, J = 17.7, 10.9 Hz, H-15); EIMS m/z 404 $[\text{M}]^+$ (28), 344 (93), 331 (11), 329 (12), 300 (10), 284 (100), 271 (79), 253 (20), 215 (21), 189 (20), 181 (55), 167 (50), 163 (30), 149 (49), 135 (29), 133 (19), 121 (75), 109 (55), 107 (49); HREIMS m/z 404.2551 (calcd for $\text{C}_{24}\text{H}_{36}\text{O}_5$, 404.2562).

11 α ,18-Dihydroxy-7-oxo-13-*epi-ent*-pimara-8(9),15-diene (13): IR (film) $\nu_{\max}$ 3610, 2920, 1650, 1460, 1380, 1045, 920 cm^{-1} ; UV (EtOH) $\lambda_{\max}$ 247 nm; ^1H NMR (500 MHz) δ 0.90 (3H, s, H-19), 1.08 (3H, s, H-17), 1.38 (3H, s, H-20), 1.50 and 1.98 (1H, dd, J = 13.5 and 6 Hz, H-12), 2.17 (1H, dd, J = 18, 2.1 Hz, H-14 α), 2.26 (1H, d, J = 18 Hz, H-14 β), 2.39 (2H, m, H-6), 3.12 and 3.38 (each 1H, d, J = 11 Hz, H-18), 4.62 (1H, t, J = 6 Hz, H-11), 4.88 (1H, d, J = 17.5 Hz, H-16), 4.94 (1H, d, J = 10.7 Hz, H-16), 5.69 (1H, dd, J = 17.5, 10.7 Hz, H-15); EIMS m/z 318 $[\text{M}]^+$ (100), 303 (6), 300 (6), 290 (6), 285 (4), 272 (3), 269 (7), 199 (10), 179 (31), 164 (28), 145 (10), 129 (13), 121 (16), 117 (14), 105 (22); HREIMS m/z 318.2204 (calcd for $\text{C}_{20}\text{H}_{30}\text{O}_3$, 318.2194).

1 α ,18-Dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (14): ^1H NMR (500 MHz) δ 0.81 (3H, s, H-19), 0.86 (3H, s, H-20), 1.02 (3H, s, H-17), 1.09 (1H, td, J = 11, 2.5 Hz, H-9), 1.33 (1H, dt, J = 13, 3 Hz, H-3 α), 2.09 (1H, ddd, J = 11, 5.5, 2.7 Hz, H-11), 2.36 (1H, td, J = 11 Hz, 2 Hz, H-8), 3.14 and 3.30 (each 1H, d, J = 11 Hz, H-18), 3.63 (1H, t, J = 9 Hz, H-1), 4.98 (1H, d, J = 17.5 Hz, H-16), 5.05 (1H, d, J = 11 Hz, H-16), 5.68 (1H, dd, J = 17.5, 11 Hz, H-15); EIMS m/z 320 $[\text{M}]^+$ (100), 302 (27), 289 (38), 271 (46), 253 (20), 243 (11), 205 (20), 164 (32), 159 (20), 149 (33), 133 (28), 121 (59), 107 (68); HREIMS m/z 320.2351 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

2 β ,18-Dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (15): ^1H NMR (500 MHz) δ 0.87 (3H, s, H-19), 0.98 (3H, s, H-20), 0.99 (3H, s, H-17), 1.00 (1H, td, J = 11, 3.5 Hz, H-9), 1.97 (1H, dt, J = 14, 3 Hz, H-12), 2.28 (1H, m, H-6), 2.45 (1H, td, J = 11.6, 3 Hz, H-8), 3.14 and 3.33 (1H, d, J = 10.4 Hz, H-18), 4.93 (1H, d, J = 17.7, H-16), 4.00 (1H, br m, $W_{1/2}$ = 24 Hz, H-2), 5.02 (1H, d, J = 11 Hz, H-16), 5.64 (1H, dd, J = 17.7, 11 Hz, H-15); EIMS m/z 320 $[\text{M}]^+$ (7), 302 (98), 289 (11), 284 (35), 271 (64), 257 (21), 253 (13), 243 (14), 231 (37), 189 (29), 175 (14), 162 (28), 149 (51), 133 (22), 121 (76), 107 (100); HREIMS m/z 320.2358 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

3 α ,18-Dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (16): ^1H NMR (500 MHz) δ 0.89 (3H, s, H-19), 0.95 (3H, s, H-20), 0.96 (3H, s, H-17), 0.99 (1H, td, J = 12, 3.5 Hz, H-9), 1.13 (1H, td, J = 13.5, 3.5 Hz, H-12 α), 1.17 (1H, dd, J = 14, 12 Hz, H-14 α), 1.41 (1H, ddd, J = 16, 12, 3.5 Hz, H-11 β), 1.75 (1H, ddd, J = 13.5, 5, 2 Hz, H-12 β), 1.90 (1H, dd, J = 13, 5 Hz, H-5), 1.98 (1H, dt, J = 14, 5.6 Hz, H-14 β), 2.16 (1H, dd, J = 18, 5 Hz, H-6 β), 2.25 (1H, dd, J = 18, 13 Hz, H-6 α), 2.39 (1H, td, J = 12, 3.1 Hz, H-8), 3.36 and 3.62 (each 1H, d, J = 10.5 Hz, H-18), 3.68 (1H, dd, J = 11, 5 Hz, H-3), 4.93 (1H, d, J = 17.7, H-16), 5.03 (1H, d, J = 10.6 Hz, H-16), 5.65 (1H, dd, J = 17.7, 10.6 Hz, H-15); EIMS m/z 320 $[\text{M}]^+$ (7), 302 (26), 290 (43), 289 (36), 271 (36), 253 (17), 233 (26), 215 (21), 189 (21), 149 (30), 133 (27), 121 (55), 107 (86); HREIMS m/z 320.2342 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

18,20-Dihydroxy-7-oxo-9,13-*epi-ent*-pimara-15-ene (17): 17 was obtained as its diacetate 17a by acetylation of the fractions containing it: ^1H NMR (500 MHz) δ 0.93 (3H, s, H-19), 0.98 (3H, s, H-17), 1.82 (1H, ddd, J = 14, 6, 3 Hz, H-12 β), 1.99 (1H, dt, J = 16 Hz, 3 Hz, H-14 β), 2.03 and 2.06 (each 3H, s), 2.19 (1H, dd, J = 13, 5.5 Hz, H-5), 2.27 (1H, dd, J = 19, 5.5 Hz, H-6 β), 2.35 (1H, dd, J = 19, 13 Hz, H-6 α), 2.45 (1H, td, J = 11.5, 3 Hz, H-8), 3.64 and 3.80 (each 1H, d, J = 10.8 Hz, H-18), 3.95 and 4.20 (each 1H, d, J = 11.2 Hz, H-20), 4.94 (1H, d, J = 17.8 Hz, H-16), 5.04 (1H, d, J = 11, H-16), 5.64 (1H, dd, J = 17.8, 11 Hz, H-15); EIMS m/z 404 $[\text{M}]^+$ (46), 376 (7), 344 (20), 331 (15), 302 (6), 284 (36), 271 (100), 253 (66), 243 (11), 225 (16), 201 (5), 189 (16), 159 (17), 149 (58), 133 (24), 121 (37), 107 (44); HREIMS m/z 404.2569 (calcd for $\text{C}_{24}\text{H}_{36}\text{O}_5$, 404.2562).

7 α ,11 α ,18-Trihydroxy-9,13-*epi-ent*-pimara-8(14),15-diene (18): ^1H NMR (500 MHz) δ 0.80 (3H, s, H-19), 1.04 (1H, dd, J = 13, 1.6 Hz, H-5), 1.06 (3H, s, H-20), 1.30 (3H, s, H-17), 1.72 (2H, m, H-2 and H-12 β), 1.82 (1H, dt, J = 12.4, 3.6 Hz, H-1 α), 1.99 (1H, ddd, J = 13.4, 9, 2 Hz, H-6 β), 2.23 (1H, dd, J = 8.5, 2.4 Hz, H-9), 3.10 and 3.34 (each 1H, d, J = 11 Hz, H-18), 4.03 (1H, td, J = 12, 8.5, 4 Hz, H-11), 4.31 (1H, dd, J = 9, 7.3 Hz, H-7), 4.83 (1H, dd, J = 17.5, 1.3 Hz, H-16), 4.99 (1H, dd, J = 11, 1.3 Hz, H-16), 5.40 (1H, s, H-14), 5.74 (1H, dd, J = 17.5, 11 Hz, H-15); EIMS m/z 320 $[\text{M}]^+$ (1), 302 (12), 284 (7), 271 (7), 253 (8), 151 (36), 123 (100); HREIMS m/z 320.2345 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3$, 320.2351).

Epoxidation of 3. Compound 3 (40 mg) in CH_2Cl_2 and Na_2HPO_4 (80 mg) in water (2 mL) was treated with *m*-chloroperbenzoic acid (32 mg) at 0 °C for 24 h with stirring. The reaction mixture was diluted with more solvent and washed with NaHCO_3 . The organic layer was evaporated and the residue chromatographed, eluting with petrol-EtOAc (8:2) to give 18-hydroxy-7 α ,8 α -epoxy-9,13-*epi-ent*-pimara-15-ene (4) (28 mg), which was identical with the metabolite obtained from the incubation. Further elution afforded 18-hydroxy-7 β ,8 β -epoxy-9,13-*epi-ent*-pimara-15-ene (5) (3 mg).

Compound 5: ^1H NMR (500 MHz) δ 0.82 (3H, s, H-19), 0.98 (3H, s, H-17), 0.97 (3H, s, H-20), 0.98 (3H, s, H-20), 1.21 (1H, dd, J = 12.8, 2.6 Hz, H-14), 1.75 (1H, d, J = 12.8 Hz, H-14), 1.83 (1H, m, H-6), 1.99 (1H, ddd, J = 13.7, 5.2, 2.6 Hz, H-1), 3.08 and 3.31 (each 1H, d, J = 10.9 Hz, H-18), 3.10 (1H, s, H-7), 4.92 (1H, s, H-15), 4.93 (1H, d, J = 17 Hz, H-16), 4.94 (1H, d, J = 10.7 Hz, H-16), 6.15 (1H, dd, J = 17, 10.7 Hz, H-15); EIMS m/z 304 $[\text{M}]^+$ (16), 289 (21), 273 (39), 255 (100), 241 (16), 227 (7), 199 (12), 187 (15), 185 (13), 173 (20), 161 (24), 159 (25), 147 (38); HREIMS m/z 304.2414 (calcd for $\text{C}_{20}\text{H}_{32}\text{O}_2$, 304.2402).

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References and Notes

- (1) Fraga, B. M.; González, P.; Hernández, M. G.; Chamy, M. C.; Garbarino, J. A. *Phytochemistry* **1998**, *47*, 211–215.
- (2) Fraga, B. M.; Hernández, M. G.; González, P.; Chamy, M. C.; Garbarino, J. A. *Phytochemistry* **2000**, *53*, 395–399.
- (3) Bearder, J. R. In *The Biochemistry and Physiology of Gibberellins*; Crozier, A., Ed.; Praeger: New York, 1983; Vol. 1, p 251.
- (4) Piovano, M.; Gambaro, V.; Chamy, M. C.; Garbarino, J. A.; Nicoletti, M.; Guilhem, J.; Pascard, C. *Phytochemistry* **1988**, *27*, 1145–1149.
- (5) Dennis, D. T.; Upper, C. D.; West, C. A. *Plant Physiol.* **1965**, *40*, 948–952.
- (6) Cross, B. E.; Myers, P. L. *Phytochemistry* **1969**, *8*, 79–93.
- (7) Pinto, A. C.; Silva, A. J. R.; Mayer, L. M. U.; Braz, R. *Phytochemistry* **1979**, *18*, 2036–2037.
- (8) Seale, M. H.; Bearder, J. R.; Down, G. H.; Hutchison, M.; MacMillan, J.; Phinney, B. O. *Phytochemistry* **1982**, *21*, 1279–1287.
- (9) Hanson, J. R.; Hawker, J.; White, A. F. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1982–1896.

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