Biotransformation of Two *ent*-Pimara-9(11),15-diene Derivatives by *Gibberella fujikuroi*

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The incubation of 19-hydroxy-13-*epi-ent*-pimara-9(11),15-diene (**4**) with *Gibberella fujikuroi* gave 8α,19-dihydroxy-9α,11α-epoxy-13-*epi-ent*-pimara-15-ene (**6**), 7-oxo-11α,19-dihydroxy-13-*epi-ent*-pimara-8(9),15-diene (**7**), 7-oxo-11*β*,19-dihydroxy-13-*epi-ent*-pimara-8(9),15-diene (**9**), and 8α,19-dihydroxy-9α,11α:15,16-diepoxy-13-*epi-ent*-pimarane (**11**), while the feeding of 13-*epi-ent*-pimara-9(11),15-diene-19-oic acid (**5**) with this fungus afforded 1-oxo-2α,9α-dihydroxy-13-*epi-ent*-pimara-11,15-dien-19-oic acid (**13**), 1-oxo-2*β*,9α-dihydroxy-13-*epi-ent*-pimara-11,15-dien-19-oic acid (**14**), 13-*epi-ent*-pimara-9(11),15-dien-1,19-dioic acid 1,2-lactone (**15**), and 1-oxo-12*β*-hydroxy-13-*epi-ent*-pimara-9(11),15-dien-19-oic acid (**16**). In both biotransformations, the main reaction was the epoxidation of the 9(11)-double bond, followed by rearrangement to afford allylic alcohols. The formation of lactone **15** represents the first time that a Baeyer−Villiger oxidation has been observed in a microbiological transformation with this fungus.

In previous work, we have carried out the microbiological transformation of the *ent*-pimara-7,15-diene diterpenes **1**−**3** by the fungus *Gibberella fujikuroi*.[1−3] In these biotransformations, C-19 oxidation did not occur, and the main reaction was the epoxidation of the 7,8-double bond, followed by rearrangement to afford 7-oxo derivatives. We have now extended our studies to the biotransformations of 13-*epi-ent*-pimar-15-ene derivatives with a 9(11)-double bond, such as **4** and **5**, with a view to determine, in this type of situation, the effects of the position change of the double bond and the influence of the oxidation level at C-19.

Results and Discussion

The substrates **4** and **5** were isolated from *Calceolaria polifolia*, a plant that grows on the coastal hills of central Chile.[4] Their incubations with the fungus *G. fujikuroi* were carried out in the presence of the inhibitor AMO 1618, a substance that blocks the production of *ent*-kaur-16-ene without affecting the postkaurene metabolism.[5,6] The fermentations were harvested after six days, and the combined broth and mycelium extracts were chromatographed on silica gel.

The biotransformation of 19-hydroxy-13-*epi-ent*-pimara-9(11),15-diene (**4**) led to the isolation of the metabolites **6**, **7**, **9**, and **11**, in order of increasing polarity. The HRMS of **6** was in accordance with the molecular formula, $C_{20}H_{32}O_3$, indicating that two new oxygens have been introduced into the molecule. Its ^{1}H NMR spectrum did not show an olefinic proton at C-11. Instead, a doublet appeared at δ 3.73 ($J = 5.9$ Hz), assigned to the geminal hydrogen at C-11 of a 9α,11α-epoxide. The α-orientation of this group was assigned considering the NOE observed between H-11 and H-16. The other oxygen must be from a tertiary alcohol group because there were no further new signals in the ^{1}H NMR spectrum, while in the ^{13}C NMR spectrum a singlet appeared at δ 72.3, which was located on C-8 from the 2D NMR experiments. The configuration of this alcohol was assigned after examination of the NOESY spectrum, where a correlation between H-15 and H-7*β* was observed, which indicated an α-orientation for this hydroxy group, axial for C-15 and H-7*β*, and equatorial for the C-17 methyl. The equatorial position of C-17 explains its abnormal downfield shift in the carbon spectrum, δ 30.3 in **6** versus 22.0 in **4**.[7] A 8*β*-hydroxyl group in **6** would involve a boat ring B and an equatorial H-7*β*, without any NOE with H-15. Thus, the structure of this compound was determined as 8α,19-dihydroxy-9α,11α-epoxy-13-*epi-ent*-pimara-15-ene (**6**).

The metabolites **7**, **9**, and **11** were identified as their acetates **8**, **10**, and **12**, respectively, by acetylation of fractions containing them. The HRMS of **8** showed the molecular formula $C_{24}H_{34}O_5$, which indicated the elemental composition $C_{20}H_{30}O_3$ for the corresponding alcohol, **7**. Thus, two new oxygens were incorporated into the substrate during the incubation, one of them as a hydroxyl group. The geminal hydrogen to the acetoxy group in **8** resonated at δ 5.69 (t, $J = 6$ Hz), indicating that it must be situated between a tetrasubstituted carbon and a methylene group. This acetate was assigned to C-11α after scrutiny of the 2D NMR spectra. The α-configuration was assigned considering the NOE effect observed between H-11*β* and H-1α. The second oxygen introduced in the molecule was an oxo group assigned to C-7, which resonated in the ^{13}C NMR spectrum of **8** as a singlet at δ 200.0. Another two singlets at 132.9 and 158.4 ppm were due to a new 8,9-double bond, which is conjugated with the 7-oxo group. This was confirmed by absorptions in the IR and UV spectra at 1670 cm^{-1} and 247 nm, respectively. In the HMBC experiment, the main correlations were H-11 with C-8 and C-9, and the two H-14 with C-8, C-9, C-12, C-13, and C-15. All these spectroscopic data of **8** are in accordance with the structure 7-oxo-11α,19-dihydroxy-13-*epi-ent*-pimara-8,15-diene (**7**) for the corresponding diol.

Compound **9** was also studied as its diacetate, **10**, which was isomeric with **8**. Both showed a similar ^{1}H NMR spectrum, where the main differences were the chemical shift and coupling of the geminal hydrogen to the acetoxy group, δ 5.53 (brs) in **10**. The IR spectrum showed, as in **8**, the absorption of an α,*β*-unsaturated oxo group. The ^{13}C NMR spectra were also similar, with the exception of the signals belonging to the C ring. The study of the 2D NMR spectra of **10** led us to assign the structure of the corresponding diol as 7-oxo-11*β*,19-dihydroxy-13-*epi-ent*-pimara-8,15-diene (**9**), the C-11 epimer of **7**. Thus, in the NOESY experiment of **10**, cross-peaks of H-11α with H-20, H-6α with H-19, and H-20 with H-19 were observed, while HMBC correlations appeared for H-20 with C-1, C-5, C-9, and C-10, H-18 with C-5 and C-19, H-14 with C-8, and H-19 with C-3.

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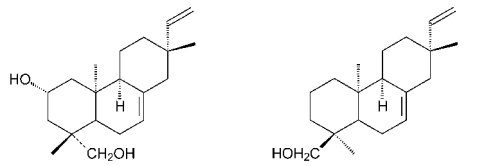

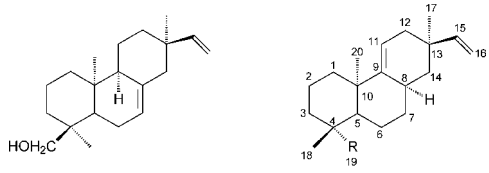

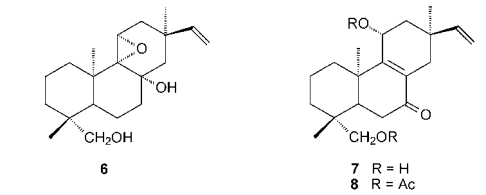

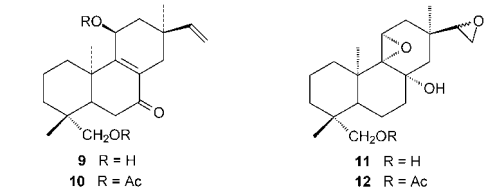

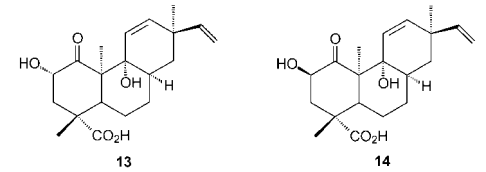

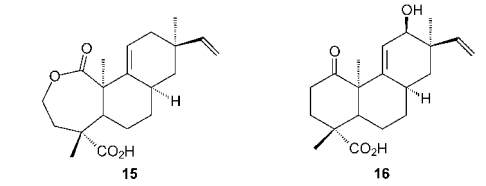

The most polar metabolite, **11**, was identified as its monoacetate **12**. Its HRMS did not show the molecular ion. Instead, a fragment at *m/z* 360.2291($C_{22}H_{32}O_4$), corresponding to the loss of water, was observed, indicating that three new oxygens were present in the molecule. No vinylic protons appeared in its ^{1}H NMR spectrum, because the exocyclic double bond was epoxidized. Thus, a double doublet at δ 2.45 (J = 4.5 and 2.9 Hz), a broad singlet at 2.66, and a triplet at 2.79 (J = 4.5 Hz) were assigned to H-16a, H-15, and H-16b, respectively. A new oxirane doublet at δ 3.58 (J = 5.4 Hz), similar to the one in **6**, was due to H-11 and showed a NOE effect with H-16. Here, the C-17 methyl is also equatorial, considering its carbon resonance at δ 27.4 versus 22.0 in **4**.[7] The ^{13}C NMR spectrum showed a singlet at δ 71.8, which was due to the carbon of a tertiary hydroxyl group at C-8α. The configuration of this center was assigned considering the NOE effect observed between the hydroxylic proton and H-20. Other 2D NMR experiments led to the establishment of the structure 8α,19-dihydroxy-9α,11α:15,16-diepoxy-13-*epi*-*ent*-pimarane (**11**) for this metabolite. The configuration of the epoxy group at C-15 remains undetermined.

The second incubated substrate with a 9(11)-double bond was 13-*epi*-*ent*-pimara-9(11),15-dien-19-oic acid (**5**), which led to the isolation of four metabolites, **13**−**16**. The first of these, **13**, showed in its HRMS a molecular ion at *m/z* 348.1983 ($C_{20}H_{28}O_5$), indicating that three new oxygens were introduced into the molecule. One of them, in the form of an oxo group resonating at δ 217.3, was located at C-1, because in the HMBC experiment this carbon showed correlations with H-3β and H-20. In the ^{1}H NMR spectrum, signals from the exocyclic double bond could be observed, together with resonances at δ 5.64 (dd, J = 10.3 and 1.0 Hz) and 6.67 (d, J = 10.3 Hz), which correspond to an endocyclic double bond situated between two tetrasubstituted carbons. The 11,12-position was assigned to the endocyclic double bond considering that the signal at δ 6.67, of the olefinic hydrogen, showed a correlation with C-13 in the HMBC experiment. In the proton spectrum also appeared a signal at δ 4.49 (ddd, J = 14.0, 6.0, 2.5 Hz), which is due to a geminal proton to a hydroxy group. Its downfield shift and the correlations observed in the HMBC experiment led us to assign it to C-2. The hydroxyl group has an α-orientation, because NOE effects of H-2β with H-3β, H-5, and H-18 were observed. The large coupling constant indicated that ring A should be a twisted boat, probably favored by a hydrogen bond between the hydrogen of the alcohol and the oxygen of the oxo group at C-1. The third new oxygen of the molecule must be a tertiary hydroxyl group, because there are no relevant signals in the ^{1}H NMR spectrum, and in the ^{13}C NMR spectrum a singlet carbon could be observed at δ 74.4, which showed correlations with H-12 and H-20 in the HMBC experiment. Thus, this alcohol was assigned to C-9 with an α-configuration, considering the relatively high value of the resonance of H-8, δ 1.83, which is typical of a *cis* relationship between H-8 and OH-9α. This product (**13**) can be formed from a 9α,11α-epoxide by opening of the oxirane ring and concomitant formation of an 11,12-double bond. Two metabolites, **6** and **11**, formed in the biotransformation of **4**, possess a 9α,11α-epoxy group. Consequently, the structure of this product was determined as 1-oxo-2α,9α-dihydroxy-13-*epi*-*ent*-pimara-11,15-dien-19-oic acid (**13**).

Compound **14** was an isomer of **13**, showing the same molecular formula and similarities in its ^{13}C NMR spectrum, such as a singlet at δ 217.0 assigned also to C-1. In its ^{1}H NMR spectrum, signals at δ 5.98 (d, J = 10.2 Hz) and 5.56 (dd, J = 10.2 and 1.6 Hz) were assigned to the hydrogens of the 11,12-double bond. Another resonance of this spectrum was a double doublet, partially overlapped with the signals from the exocyclic double bond, at δ 4.96 (J = 12.8 and 6.8 Hz) due to a geminal hydrogen to an alcohol group in C-2β. In the COSY experiment, the H-2α signal showed coupling with both H-3β and H-3α, δ 1.29 (12.8 Hz) and 2.81 (6.8 Hz), respectively, while in the NOESY spectrum, correlations of H-2α with H-3α and H-20 were observed. The third oxygen was due to an α-hydroxyl group at C-9, based on the same reasoning used above for **13**. In addition, the resonances of H-8 in **13** and **14** are very similar, at δ 1.83 and 1.78, respectively. Moreover, the chemical shifts of the H-20 methyl are also similar in both metabolites, δ 1.15 and 1.19, respectively Thus, the structure of **14**, an epimer at C-2 of **13**, was assigned as 1-oxo-2β,9α-dihydroxy-13-*epi*-*ent*-pimara-11,15-dien-19-oic acid.

The third metabolite (**15**) obtained in this incubation gave the molecular formula $C_{20}H_{28}O_4$, showing that, during the fermentation, two hydrogens were lost and two oxygens were incorporated into the molecule of the substrate. In its ^{1}H NMR spectrum, proton signals of the exocyclic and endocyclic double bonds could be observed, which were not affected during incubation. In the ^{13}C NMR spectrum appeared two singlets at δ 180.6 and 181.8, with the latter due to the C-19 acid present in the substrate, showing HMBC correlations with H-18 and H-5. The first was assigned to C-1, showing connectivity with H-20 in this experiment. Its ^{13}C NMR spectrum indicated that it belongs to the oxo group of an acid, ester, or lactone. Examination of the ^{1}H NMR spectrum showed that it was due to a lactone, because two hydrogens of an oxymethylene group appeared at δ 4.20 (q, J = 8.0 Hz) and 4.28

Table 1. ^{13}C NMR Data of Compounds **4**−**6**, **8**, **10**, and **12**−**16**

carbon	4	5	6	8	10	12	13	14	15	16
1	37.2	37.6	35.5	35.5	34.1	35.5	217.3	217.0	180.6	216.5
2	18.7	19.6	17.8	18.1	18.1	18.1	71.3	69.5	64.7	36.7
3	35.5	38.0	35.1	35.8	35.9	36.2	38.4	47.4	34.1	31.8
4	39.5	44.3	38.9	34.7	36.6	37.5	43.0	43.8	47.0	43.4
5	54.3	55.0	53.1	51.3	49.6	54.1	42.4	50.4	46.2	52.1
6	21.9	23.5	18.3	35.2	35.5	17.7	23.2	23.5	25.4	23.0
7	36.5	36.3	38.0	200.0	199.0	38.6	28.8	27.8	33.4	35.6
8	31.1	31.3	72.3	132.9	133.4	71.8	33.6	34.0	31.5	30.7
9	149.3	148.4	69.4	158.4	157.5	69.3	74.4	72.5	141.7	145.2
10	38.9	39.7	38.5	36.9	40.0	38.6	52.6	54.8	53.0	53.4
11	112.7	114.2	61.9	69.2	66.1	61.6	128.4	128.8	119.5	119.1
12	37.6	37.4	34.3	39.7	41.6	29.8	139.6	139.3	37.3	70.8
13	34.7	34.6	37.1	39.6	33.2	33.4	38.9	39.2	34.5	39.6
14	42.5	42.3	50.0	34.0	33.6	48.5	37.0	36.9	41.5	34.5
15	150.1	150.2	147.0	145.2	146.0	57.6	147.3	147.5	149.3	145.2
16	109.3	109.2	110.9	111.8	109.8	47.2	109.2	110.8	109.8	114.0
17	22.0	21.9	30.3	26.9	29.0	27.4	25.1	25.0	22.0	22.0
18	26.8	28.9	27.0	27.0	26.6	27.7	27.0	27.4	21.4	26.6
19	65.0	184.3	65.2	66.9	66.7	67.0	178.2	179.3	181.8	179.2
20	21.9	19.6	18.4	19.3	19.4	18.4	16.3	14.6	19.3	19.2

(td, $J = 8.0$ and 4.8 Hz). These two protons gave correlations with C-1 in the HMBC experiment and coupling with H-3 in the COSY spectrum, so they were assigned to C-2. This lactone group comes from a Baeyer−Villiger oxidation that took place on a 1-oxo derivative, previously formed in the fermentation. We therefore assigned the structure 13-*epi*-*ent*-pimara-9(11),15-dien-1,19-dioic acid 1,2-lactone to this metabolite (**15**).

The most polar compound isolated in this biotransformation was **16**. The molecular ion in the HRMS at *m*/*z* 332.1999 was in accordance with the molecular formula $C_{20}H_{28}O_4$, isomeric with **15**. In its ^{13}C NMR spectrum, the resonance of an oxo group at δ 216.5 could be observed, which was assigned to C-1 for the same reasons given above for **13**. The ^1H NMR spectrum showed a doublet at δ 3.69 ($J = 5.9$ Hz) of a geminal hydrogen to a secondary hydroxy group, which was allocated between a tetrasubstituted carbon and a methine group. The COSY experiment showed vicinal coupling of this hydrogen with H-11, and in the HMBC spectrum, correlations of this proton were observed with C-14 and C-17. In consequence, we assigned this alcohol to the C-12β position. The β-stereochemistry was accorded on the following basis: Correlations in the NOESY experiment were observed of H-8 with H-17 and H-20, indicating a conformation with an axial orientation for C-17. Molecular mechanics calculations were supportive of this, showing that in 12α- and 12β-hydroxy derivatives, the most stable conformation is that where C-17 is axial. Taking this into account, the observed coupling constant of H-12 with H-11 (5.9 Hz) was in accordance with a β-orientation for this alcohol group. The calculated value for a β-hydroxy group was 5.6 Hz, while for an α-hydroxy substituent it was 1.8 Hz.[8] Furthermore, NOE effects of H-12 with H-11, H-15, and H-17 were observed, confirming the β-configuration of this alcohol at C-12. Therefore, the structure 1-oxo-12β-hydroxy-13-*epi*-*ent*-pimara-9(11),15-dien-19-oic acid (**16**) was given to this compound.

From these microbiological transformations performed in this investigation the following conclusions were made:

1. The oxidation of C-19, which is characteristic of the biosynthetic pathway of gibberellins, is inhibited in the alcohol **4**, confirming earlier results obtained in biotransformations of other diterpenes with an *ent*-pimar-15-ene skeleton.[1−3]

2. The main reaction, in the incubations of **4** and **5** with *G. fujikuroi*, was the epoxidation of the 9(11)-double bond, followed by rearrangement to afford allylic alcohols. A 7,8-epoxidation was observed earlier in the biotransformation of the *ent*-pimara-7,15-diene derivatives **1**−**3** with this fungus, but in these cases the rearrangement led to 7-oxo derivatives. On the other hand, it is probable that the enzyme responsible for the epoxidation is the same as that producing the 6,7-epoxidation of *ent*-kaur-6,16-dien-19-oic acid in the biosynthesis of kaurenolides in *G. fujikuroi*.

3. There are also differences in the results of the biotransformations of **4** and **5**. Thus, in the incubation of the acid **5**, a double oxidation at C-1 is produced, forming an oxo group, followed by hydroxylation at C-2, either α or β, to give **13** or **14**, or by a Baeyer−Villiger oxidation to afford the lactone **15**, while in the feeding of alcohol **4**, two 7-oxo derivatives, **7** and **9**, were obtained.

4. The formation of a 1-oxo-2-hydroxy group in the biotransformation of **5** is very interesting, being similar to that produced in the incubation of some 7-oxo-*ent*-kaur-16-ene derivatives to give the corresponding 7-oxo-6β-hydroxy derivatives.[9] We have indicated in previous work the similar results obtained in the biotransformation of 7-oxo-*ent*-kaur-16-ene derivatives and 3-oxo-13-*epi*-*ent*-manoyl oxide (ribenone) by *G. fujkuroi* giving *seco*-acids in rings B and A, respectively.[10] Now, a comparable equivalence of both rings is also observed between the *ent*-kaur-16-ene and 13-*epi*-*ent*-pimara-9(11),15-diene skeletons, which can be explained considering the similarity of the *ent*-pimaradiene and *ent*-manoyl oxide carbon frameworks.

5. A 1α-hydroxy derivative should be the precursor of the 1-oxo-group in all the metabolites obtained in the biotransformation of **5**, because compounds hydroxylated at this position, and with the same stereochemistry, have been isolated in the fermentation of **3**.[3]

6. Allylic hydroxylation of an endocyclic double bond has resulted from the microbiological transformation by *G. fujikuroi* of the *ent*-pimaradienes **1**−**3**[1−3] and now has been observed with the substrate **5**. An analogous oxidation had also been observed in the incubation of 19-hydroxy-*ent*-kaur-2,16-diene by this fungus.[11]

7. The formation of the lactone **15** from the incubation of **5** represents the first time that a Baeyer−Villiger oxidation has been observed in a microbiological transformation with the fungus *G. fujikuroi*. It is also worth mentioning that this bio-oxidation takes place at the less substituted carbon, while the same reaction using chemical reagents occurs at the more substituted position.

Experimental Section

General Experimental Procedures. IR spectra were taken in a Perkin-Elmer Spectrum BX FT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500.13 and 125.77 MHz, respectively, with a Bruker AMX2-500 spectrometer. Chemical shifts are given in ppm (δ). Mass spectra were taken at 70 eV (probe) in a Micromass Autospec spectrometer. Semipreparative HPLC was carried out with a Beckman System Gold 125P. Dry column chromatography was performed on Merck 0.02−0.063 mm silica gel. Conformations of minimum energy were determined by computational methods employing the Hyperchem 7.1 program of Hypercube. The substrates

4 and **5** were isolated from *Calceolaria polifolia*, a plant that grows on the coastal hills of central Chile.[4]

Incubation and Isolation Procedures. *Gibberella fujikuroi* MP-C (*Fusarium fujikuroi*) IMI 58289, inhibited with 5×10^{-5} M AMO 1618, was grown in shaking culture at 25 °C for 2 days in 65−75 conical flasks (250 mL), each containing sterile medium (50 mL).[12] The substrate was dissolved in EtOH (13−15 mL) and distributed equally between the flasks, and the incubation was allowed to continue for a further 6 days. The broth was separated from the mycelium by filtration, and both were extracted with EtOAc. The extracts were combined and chromatographed on silica gel using as eluent a petroleum ether−EtOAc gradient. Some mixtures of products were acetylated, with Ac_2O−pyridine at room temperature, to reduce their polarity, thus facilitating their separation by column chromatography. Other mixtures were resolved by HPLC on an Ultrasphere silica gel 5 μm column (1 × 25 cm), eluting with mixtures of isocratic *n*-hexane−EtOAc at 3 mL/min.

Biotransformation of 19-Hydroxy-13-*epi*-*ent*-pimara-9(11),15-diene (4). The incubation of **4** (300 mg) afforded, from the neutral fraction, starting material (34 mg), 8α,19-dihydroxy-9α,11α-epoxy-13-*epi*-*ent*-pimara-15-ene (**6**) (9 mg), 7-oxo-11α,19-dihydroxy-13-*epi*-*ent*-pimara-8(9),15-diene (**7**), isolated as its 11,19-diacetate (**8**) (4 mg), 7-oxo-11β,19-dihydroxy-13-*epi*-*ent*-pimara-8(9),15-diene (**9**), isolated as its 11,19-diacetate (**10**) (2 mg), and 8α,19-dihydroxy-9α,11α:15,16-diepoxy-13-*epi*-*ent*-pimarane (**11**), identified as its 19-monoacetate (**12**) (6 mg).

8α,19-Dihydroxy-9α,11α-epoxy-13-*epi*-*ent*-pimara-15-ene (6). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (1H, tdd, J = 13.6, 4.3, 1.0 Hz, H-3β), 0.91 (3H, s, H-17), 0.97 (3H, s, H-18), 1.01 (1H, m, H-1), 1.11 (1H, dd, J = 12.9, 2.2 Hz, H-5), 1.17 (3H, s, H-20), 1.34 (1H, m, H-1), 1.39 (1H, dd, J = 13.5, 2.2 Hz, H-14), 1.43 (1H, d, J = 13.5 Hz, H-14), 1.51 (1H, qt, J = 13.6, 3.2 Hz, H-2α), 1.58 (1H, d, J = 15.5 Hz, H-12α), 1.62 (1H, m, H-7α), 1.66 (1H, qd, J = 12.9, 3.4 Hz, H-6α), 1.84 (1H, brd, J = 13.6 Hz, H-3α), 1.97 (1H, td, J = 12.9, 4.8 Hz, H-7β), 2.17 (1H, ddd, J = 15.5, 5.9, 2.4 Hz, H-12β), 3.46 (1H, dd, J = 10.9, 1.0 Hz, H-19), 3.76 (1H, d, J = 10.9 Hz, H-19), 3.73 (1H, d, J = 5.9 Hz, H-11), 4.85 (1H, d, J = 17.7 Hz, H-16), 5.01 (1H, d, J = 10.8 Hz, H-16), 5.67 (1H, dd, J = 17.7, 10.8 Hz, H-15); ^{13}C NMR, see Table 1; EIMS *m*/*z* 320 $[M]^+$ (13), 302 (14), 287 (9), 271 (25), 259 (19), 253 (15), 223 (24), 209 (33), 205 (57), 193 (68), 187 (47), 175 (47), 147 (70); HREIMS *m*/*z* 320.2327 (calcd for $C_{20}H_{32}O_3$, 320.2351).

7-Oxo-11α,19-dihydroxy-13-*epi*-*ent*-pimara-8(9),15-diene (7). This compound was obtained as its diacetate **8** by acetylation of the fractions containing it: IR (film) ν_{max} 2935, 1735, 1670, 1373, 1232, 1030, 1020 cm^{-1}; UV (EtOH) λ_{max} 247 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.98 (3H, s, H-18), 1.03 (3H, s, H-17), 1.08 (1H, td, J = 13.8, 4.0 Hz, H-3β), 1.30 (3H, s, H-20), 1.35 (1H, td, J = 12.9, 3.9 Hz, H-1β), 1.53 and 2.01 (each 1H, dd, J = 13.6, 6.0 Hz, H-12), 1.78 (1 H, dd, J = 13.9, 4.0 Hz, H-5), 2.07 and 2.08 (each 3H, s, OAc), 2.22 and 2.27 (each 1H, d, J = 14.3 Hz, H-14), 2.57 (1H, dd, J = 18.0, 13.9 Hz, H-6α), 2.65 (1H, dd, J = 18.0, 4.0 Hz, H-6β), 4.00 and 4.28 (each 1H, d, J = 11.2 Hz, H-19), 4.91 (1H, d, J = 17.5 Hz, H-16), 4.95 (1H, d, J = 10.8 Hz, H-16), 5.69 (1H, t, J = 6.0 Hz, H-11), 5.72 (1H, dd, J = 17.5, 10.8 Hz, H-15); ^{13}C NMR, see Table 1; EIMS *m*/*z* 402 $[M]^+$ (9), 361 (23), 360 (100), 327 (28), 318 (9), 292 (10), 269 (38), 267 (13), 203 (10), 187 (11), 171 (18), 164 (19), 161 (27), 160 (50); HREIMS *m*/*z* 402.2393 (calcd for $C_{24}H_{34}O_5$, 402.2406).

7-Oxo-11β,19-dihydroxy-13-*epi*-*ent*-pimara-8(9),15-diene (9). This product was obtained as its diacetate **10** by acetylation of the fractions containing it: IR (film) ν_{max} 2935, 1735, 1671, 1373, 1235, 1020 cm^{-1}; UV (EtOH) λ_{max} 244 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.00 (3H, s, H-18), 1.05 (3H, s, H-17), 1.12 (3H, s, H-20), 1.75 (1H, brd, J = 13.5 Hz, H-3), 2.00 and 2.07 (each 3H, s, OAc), 2.49 (1H, dd, J = 18.0, 14.8 Hz, H-6α), 2.65 (1H, dd, J = 18.5, 2.0 Hz, H-14), 2.70 (1H, dd, J = 18.0, 3.7 Hz, H-6β), 3.98 and 4.26 (each 1H, d, J = 11.1 Hz, H-19), 4.83 (1H, dd, J = 17.0, 1.0 Hz, H-16), 4.86 (1H, dd, J = 11.4, 1.0 Hz, H-16), 5.53 (1H, brs, H-11), 5.87 (1H, dd, J = 17.0, 11.4 Hz, H-15); ^{13}C NMR, see Table 1; EIMS *m*/*z* 402 $[M]^+$ (6), 361 (7), 360 (33), 342 (42), 327 (19), 318 (4), 269 (24), 267 (14), 203 (5), 199 (8), 185 (12), 171 (32), 160 (100); HREIMS *m*/*z* 402.2403 (calcd for $C_{24}H_{34}O_5$, 402.2406).

8α,19-Dihydroxy-9α,11α:15,16-diepoxy-13-*epi*-*ent*-pimarane (11). This compound was obtained as its monoacetate **12** by acetylation of the fractions containing it: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.96 (3H, s, H-18), 0.98 (3H, s, H-17), 1.04 (1H, dd, J = 12.0, 2.0 Hz, H-5), 1.54 (3H, s, H-20), 2.04 (3H, s, OAc), 2.45 (1H, dd, J = 4.5, 2.9 Hz, H-16), 2.66 (1H, brs, H-15), 2.79 (1H, t, J = 4.5 Hz, H-16), 3.58 (1H, d, J = 5.4 Hz, H-11), 3.94 and 4.26 (each 1H, d, J = 11.1 Hz, H-19); ^{13}C NMR, see Table 1; EIMS *m*/*z* 360 $[M - H_2O]^+$ (31), 345 (11), 342 (14), 330 (14), 317 (9), 303 (53), 287 (18), 268 (26), 251 (21), 205 (36), 191 (39), 173 (30), 147 (49), 131 (43); HREIMS *m*/*z* 360.2291 (calcd for $C_{22}H_{32}O_4$, 360.2301).

Biotransformation of 13-*epi*-*ent*-Pimara-9(11),15-dien-19-oic acid (5). The incubation of **5** (305 mg) afforded starting material (6 mg), 1-oxo-2α,9α-dihydroxy-13-*epi*-*ent*-pimara-11,15-dien-19-oic acid (**13**) (0.6 mg), 1-oxo-2β,9α-dihydroxy-13-*epi*-*ent*-pimara-11,15-dien-19-oic acid (**14**) (1.0 mg), 13-*epi*-*ent*-pimara-9(11),15-dien-1,19-dioic acid 1,2-lactone (**15**) (1.5 mg), and 1-oxo-12β-hydroxy-13-*epi*-*ent*-pimara-9(11),15-dien-19-oic acid (**16**) (1.0 mg).

1-Oxo-2α,9α-dihydroxy-13-*epi*-*ent*-pimara-11,15-dien-19-oic acid (13). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.07 (3H, s, H-17), 1.15 (3H, s, H-20), 1.52 (3H, s, H-18), 1.83 (1H, m, H-8), 1.95 (1H, dd, J = 13.1, 3.0 Hz, H-6β), 2.01 (1H, dd, J = 14.0, 6.0 Hz, H-3β), 2.35 (1H, t, J =14.0 Hz, H-3α), 2.70 (1H, dd, J = 12.2, 3.0 Hz, H-5), 3.65 (1H, d, J = 2.5 Hz, −OH), 4.49 (1H, ddd, J = 14.0, 6.0, 2.5 Hz, H-2), 4.92 (1H, d, J = 10.5 Hz, H-16), 4.95 (1H, d, J = 17.5 Hz, H-16), 5.64 (1H, dd, J = 10.3, 1.0 Hz, H-12), 5.76 (1H, dd, J = 17.5, 10.5 Hz, H-15), 6.67 (1H, d, J = 10.3 Hz, H-11); ^{13}C NMR, see Table 1; EIMS *m*/*z* 348 $[M]^+$ (6), 330 (8), 315 (5), 297 (7), 286 (17), 285 (14), 253 (9), 218 (8), 194 (11), 179 (11), 163 (38), 150 (32), 149 (72), 148 (22), 137 (34), 136 (100); HREIMS *m*/*z* 348.1983 (calcd for $C_{20}H_{28}O_5$, 348.1937).

1-Oxo-2β,9α-dihydroxy-13-*epi*-*ent*-pimara-11,15-dien-19-oic acid (14). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.06 (3H, s, H-17), 1.17 (1H, dd, J = 13.0, 3.2 Hz, H-14α), 1.19 (3H, s, H-20), 1.29 (1H, t, J = 12.8 Hz, H-3β), 1.36 (3H, s, H-18), 1.51 (1H, qd, J = 13.0, 4.0 Hz, H-7β), 1.78 (1H, tt, J = 13.0, 3.2 Hz, H-8), 1.93 (1H, dq, J = 13.0, 4.0 Hz, H-6β), 1.97 (1H, t, J = 13.0 Hz, H-14β), 2.04 (1H, qd, J = 13.0, 4.0 Hz, H-6α), 2.35 (1H, dd, J = 13.0, 4.0 Hz, H-5), 2.81 (1H, dd, J = 12.8, 6.8 Hz, H-3α), 2.94 (1H, brs, OH), 3.65 (1H, brs, OH), 4.93 (1H, dd, J = 10.6, 1.0 Hz, H-16), 4.96 (1H, dd, J = 12.8, 6.8 Hz, H-2), 5.00 (1H, dd, J = 17.4, 1.0 Hz, H-16), 5.56 (1H, dd, J = 10.2, 1.6 Hz, H-12), 5.81 (1H, dd, J = 17.4, 10.6 Hz, H-15), 5.98 (1H, d, J = 10.2 Hz, H-11); ^{13}C NMR, see Table 1; EIMS *m*/*z* 348 $[M]^+$ (8), 330 (20), 315 (23), 304 (9), 297 (12), 286 (42), 285 (24), 267 (7), 253 (20), 194 (15), 187 (11), 163 (38), 149 (90), 136 (100); HREIMS *m*/*z* 348.1946 (calcd for $C_{20}H_{28}O_5$, 348.1937).

13-*epi*-*ent*-Pimara-9(11),15-dien-1,19-dioic acid 1,2-lactone (15). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.92 (3H, s, H-17), 1.20 (1H, m, H-14), 1.27 (3H, s, H-18), 1.37 (3H, s, H-20), 1.52 (2H, m, H-14 and H-16), 1.63 (1H, ddd, J = 13.0, 3.5 Hz, H-6α), 1.74 (1H, ddt, J = 17.5, 6.0, 2.0 Hz, H-12), 1.83 (1H, ddd, J = 13.0, 3.5 Hz, H-7), 1.91 (1H, ddd, J = 13.0, 8.0, 4.8 Hz, H-3), 2.09 (1H, dd, J = 17.5, 2.0 Hz, H-12), 2.17 (1H, m, H-8), 2.43 (1H, dt, J = 13.0, 8.0 Hz, H-3), 2.76 (1H, dd, J = 13.0, 3.5 Hz, H-5), 4.20 (1H, q, J = 8.0 Hz, H-2), 4.28 (1H, td, J = 8.0, 4.8 Hz, H-2), 4.89 (1H, dd, J = 10.7, 1.1 Hz, H-16), 4.93 (1H, dd, J = 17.5, 1.1 Hz, H-16), 5.36 (1H, dt, J = 6.0, 2.0 Hz, H-11), 5.78 (1H, dd, J = 17.5, 10.7 Hz, H-15); ^{13}C NMR, see Table 1; EIMS *m*/*z* 332 $[M]^+$ (0.6), 317 (2), 314 (2), 287 (3), 286 (3), 254 (4), 233 (8), 188 (14), 187 (38), 157 (6), 145 (15), 131 (20); HREIMS *m*/*z* 332.1987 (calcd for $C_{20}H_{28}O_4$, 332.1988).

1-Oxo-12β-hydroxy-13-*epi*-*ent*-pimara-9(11),15-dien-19-oic acid (16). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.92 (3H, s, H-17), 1.19 (1H, m, H-7), 1.27 (3H, s, H-20), 1.37 (3H, s, H-18), 1.78 (1H, ddd, J = 13.5, 5.8, 4.2 Hz, H-3α), 1.96 (1H, m, H-6β), 2.43 (1H, dt, J = 13.5, 4.2 Hz, H-2β), 2.54 (1H, td, J = 13.5, 4.2 Hz, H-3β), 2.66 (1H, td, J = 13.5, 5.8 Hz, H-2α), 3.69 (1H, d, J = 5.9 Hz, H-12), 5.09 (1H, dd, J = 17.7, 1.0 Hz, H-16), 5.16 (1H, dd, J = 10.9, 1.0 Hz, H-16), 5.60 (1H, d, J = 5.9 Hz, H-11), 5.87 (1H, dd, J = 17.7, 10.9 Hz, H-15); EIMS *m*/*z* 332 $[M]^+$ (9), 314 (100), 299 (12), 271 (30), 264 (70), 253 (30), 251 (28), 235 (25), 210 (25), 195 (30), 186 (33), 171 (48), 169 (58), 143 (47); HREIMS *m*/*z* 332.1999 (calcd for $C_{20}H_{28}O_4$, 332.1988).

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