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

Braulio M. Fraga,*,† Pedro González,‡ Melchor G. Hernández,† María C. Chamy,§ and Juan A. Garbarino§

Instituto de Productos Naturales y Agrobiología, CSIC, P.O. Box 195, 38206-La Laguna, Tenerife, Canary Islands, Spain, Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Tenerife, Spain, and Departamento de Química, Universidad Técnica Federico Santa María, P.O. Box 110-V, Valparaíso, Chile

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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*-pimarene 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-kaurene 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, $C_{20}H_{32}O_2$, 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 9β,18-dihydroxy-7α,8α-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 $C_{20}H_{32}O_3$. In the ^{1}H NMR spectrum H-7 resonated as a double doublet (J = 5.5 and 1.6 Hz) at δ 3.08, and in the ^{13}C 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** ($C_{20}H_{32}O_4$) was related to **6**, with the exception that **7** has a additional oxygen atom in the form of a secondary hydroxyl group. The ^{1}H NMR spectrum of **7** showed the resonance of 14α-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 ^{13}C 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 9α-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 9α-alcohols, **19** and **20**, respectively.

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

* To whom correspondence should be addressed. Tel: 34-922-251728. Fax: 34-922-260135. E-mail: bmfraga@ipna.csic.es.
† Instituto de Productos Naturales y Agrobiología, CSIC.
‡ Instituto de Bio-Orgánica, Universidad de La Laguna.
§ Departamento de Química, Universidad Federico Santa María.

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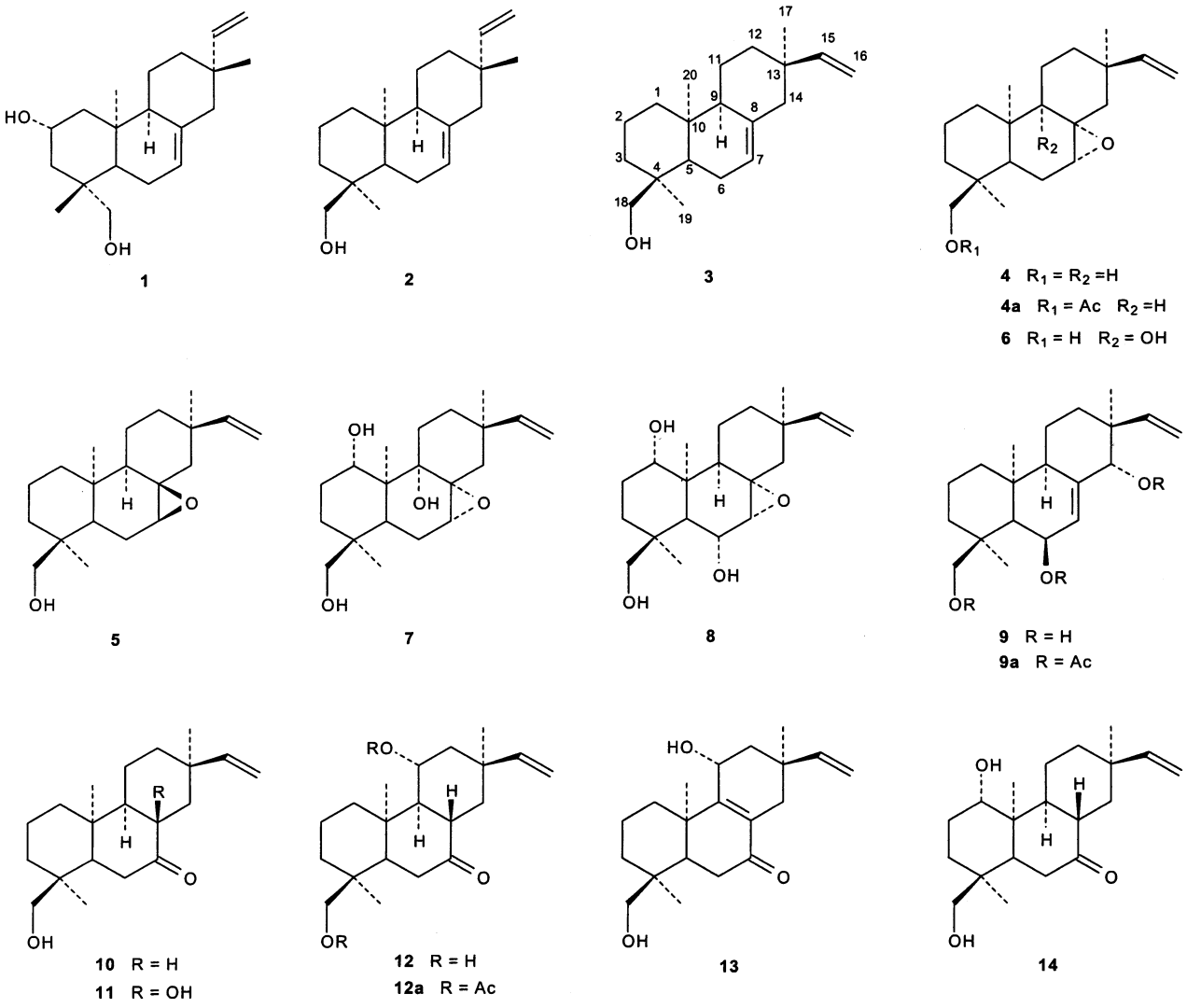
Chart 1



$C_{20}H_{32}O_4$. Its ^{1}H 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 assignment[4] (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 7α,8α-epoxy group. The simplest of these compounds was **10** ($C_{20}H_{32}O_2$), which is formed from **4**. The ^{1}H 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 ^{1}H 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

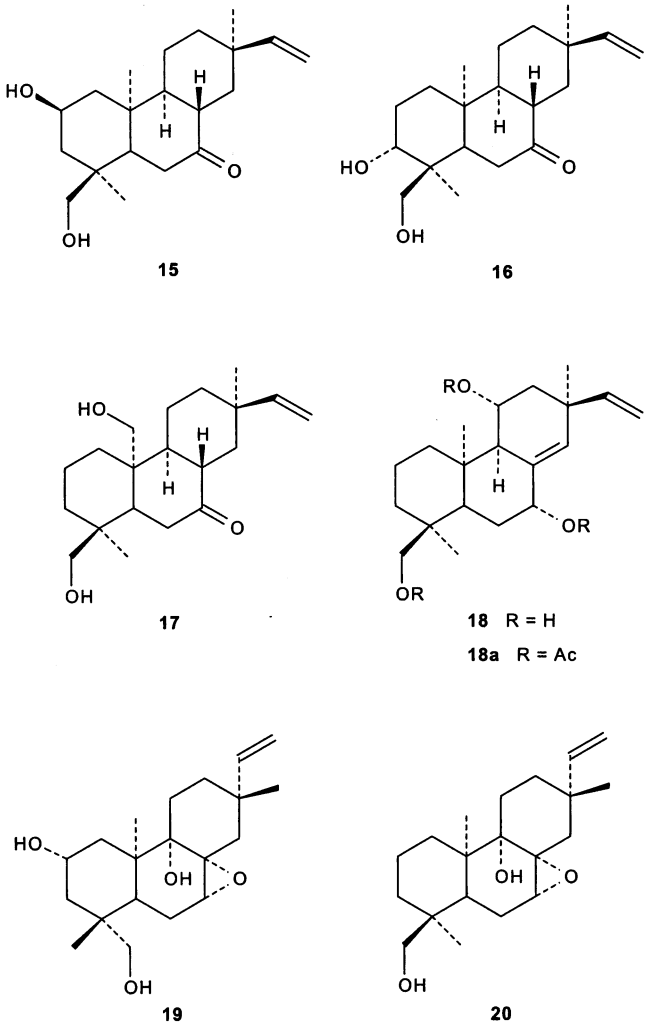


Table 1. ^{13}C NMR Data for Compounds **3**−**9**

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 ^{1}H 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 ^{1}H 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 ^{1}H 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	36.9b	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 8β,-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 oyxgen ($C_{20}H_{32}O_3$), which forms part of a secondary alcohol group. This was supported by the ^{1}H 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 ^{1}H 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 11α,18-dihydroxy-7-oxo-13-*epi*-*ent*-pimara-8(9),15-diene (**13**).

Another metabolite obtained in this feeding was 1α,18-dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (**14**). HRMS showed a molecular ion at *m*/*z* 320.2351 for a molecular formula of $C_{20}H_{32}O_3$. The new oxygen of the molecule must be a part of a secondary alcoholic group, because its ^{1}H NMR spectrum showed the proton geminal to a new hydroxyl group at δ 3.63 as a triplet (J = 9 Hz). This coupling points to an equatorial hydroxyl group at C-1(α), C-3(α), or C-12(β). The first position was chosen by assignment of the ^{1}H and ^{13}C NMR spectra on the basis of 2D NMR studies. Thus, in the HMBC spectrum correlations were observed between H-20 and C-1 and between H-1 and C-20, C-10, and C-9.

A compound isomeric with **14** and with a similar ^{1}H NMR spectrum was **15**. The main difference between **14** and **15** was the resonance of the hydrogen geminal to the secondary hydroxyl group, which now appeared as a broad multiplet at δ 4.00 ($W_{1/2}$ = 24 Hz), and the resonance of the corresponding methine carbon at δ 65.4, which is a relatively low value. These data indicated the existence of a $-CH_2-CHOH-CH_2-$ group, pointing to C-2(β) for the location of this alcohol. This fact was confirmed by assignment of the proton and carbon spectra using 2D NMR data (COSY, HSQC, and HMBC). Therefore, the structure of this metabolite was determined to be 2β,18-dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (**15**).

Another substance, with a 7-oxo group, obtained in this feeding, was **16**. As with other compounds of this type, it was formed by rearrangement of a 6α,7β-epoxidic function. In the 7-oxo metabolites the resonances of H-8 and the two H-6 appear at a relatively high field: in this case, the first as a triple doublet at δ 2.39 (J = 12 and 3.1 Hz) and the last hydrogens as two double doublets at δ 2.16 (J = 18 and 5 Hz) and 2.25 (J = 18 and 13 Hz), the equatorial and axial hydrogens, respectively. The additional oxygen, introduced into the molecule in the form of a hydroxyl group, was assigned to C-3(α). Stereochemistry of the hydroxyl was established by observing that the proton geminal to the hydroxyl appeared in the NMR spectrum as a double doublet at δ 3.68 (J = 11 and 5 Hz), indicating an equatorial stereochemistry for this alcohol. The proton resonance of the hydroxymethylene group was affected by the presence of the 3α-OH, H-18 resonating at δ 3.36 and 3.62, in comparison with 3.12 and 3.32 in the metabolite **10**. This effect was also observed in the carbon resonance of C-18 at 70.4 in **16** and at 71.6 in **10**. Thus, the structure of this compound was determined to be 3α,18-dihydroxy-7-oxo-13-*epi*-*ent*-pimara-15-ene (**16**). This was confirmed by assignments of the ^{1}H and ^{13}C NMR spectra using 2D NMR data. Thus, cross-peaks of H-18 with C-3 and of H-3 with C-18 were observed in the HMBC spectrum.

Metabolite **17** was the only one in which the hydroxylation of a methyl group was observed. It was obtained as the diacetate **17a** by acetylation of a fraction containing it. Its high-resolution mass spectrum showed the molecular ion at *m*/*z* 404.2569 ($C_{24}H_{36}O_5$). As a result of the small quantity isolated, structure determination was limited to ^{1}H NMR data. The existence of a 7-oxo function was deduced from the resonance of H-8, a triple doublet at δ 2.45 (J = 11.5 and 3 Hz), typical of structures such as **10**, **12**, and **14**−**16**. The resonance of the new acetoxymethylene group appears as a pair of doublets at δ 3.95 and 4.20. Of the three possible locations for this acetoxy group, C-17, C-19, and C-20, the first was discarded considering that the resonance of the 15,16-double bond protons and H-12 and H-14 were similar to that observed in compound **10**. The resonance of H-18, similar to that of the diacetate **12a** (δ 3.63 and 3.79), indicated that C-19 was not functionalized, and consequently the acetate group must be located at C-20. This was confirmed because H-9 was not observed at δ 0.98−1.00, the position of resonance characteristic of other 7-oxo derivatives such as **10**. Moreover, a NOE effect was not observed in the NOESY spectrum between the hydrogens of the two acetoxymethylene groups. Thus, the structure of the corresponding alcohol was determined to be 18,20-dihydroxy-7-oxo-13-epi-*ent*-pimara-15-ene (**17**).

Finally, we describe metabolite **18**, the only allylic alcohol produced in the biotransformation by rearrangement of a 7,8-epoxide. Its HRMS was in accordance with the molecular formula $C_{20}H_{32}O_3$. In the ^{1}H NMR spectrum the resonance of the hydrogen of the 7(8)-double bond of the substrate does not appear; instead there is a new singlet at δ 5.40, indicating the existence of an 8(14)-double bond. The spectrum shows two geminal hydrogens which correspond to two alcohol groups at δ 4.03 (td, J = 12, 8.5 and 4 Hz) and 4.31 (dd, J = 9 and 7.3 Hz), indicating that the two new oxygens of the molecule are part of two secondary alcohols. The resonance of the first was typical of the proton geminal to an 11α-alcohol, while that of the second is characteristic of a hydroxyl group situated at C-1(α), C-3(α), or C-7(α). Hydroxylations at either C-1 or C-3 were discarded by comparing the NMR data of other compounds obtained in this fermentation and hydroxylated at C-1 or C-3. Furthermore, in the NOESY spectrum cross-peaks were observed between H-7β and H-6β, and between H-18 and H-6β, confirming a 7α-equatorial stereochemistry in a chair-deformed ring B. This was also the lowest energy conformation found by molecular mechanics (MM+) calculations. Thus, the structure 7α,11α,18-trihydroxy-13-*epi*-*ent*-pimara-8(14),15-diene (**18**) was assigned to this metabolite and confirmed by a 2D NMR study. The H-14 showed, in the HMBC spectrum, correlations with C-17, C-13, C-12, C-9, and C-7, while H-7 correlated with C-9 and C-14.

From results of this microbiological transformation with *G fujikuroi*, the following conclusions can be made.

1. The oxidation of C-19, which is characteristic of the biosynthetic pathway of gibberellins,[3] is inhibited in this type of compound, confirming earlier results obtained in the biotransformations[1,2] of **1** and **2**.

2. The main reaction observed was the epoxidation of the 7,8-double bond of the substrate. This result was also analogous to that obtained in the incubation of **1** and **2**. It is probable that the enzyme responsible for the epoxidation is the same one that produced the 6,7-epoxidation of *ent*-kaur-6,16-dien-19-oic acid in the biosynthesis of kaurenolides in *G. fujikuroi*.[8]

3. The formation of 6(β)-, 9(α)-, and C-14(α)-hydroxy derivatives was observed in this feeding and also in the incubation of **2**.

4. The hydroxylations at C-1(α), C-11(α), and C-20, observed in the incubation of **3**, are the characteristic reactions of this microbiological transformation, because 1-, 11-, and 20-alcohols had not been obtained in the feeding of **1** or **2**. Thus, this appears to be the main effect produced by the stereochemical change of the isopropylidene group in the substrate **3** as compared to **1** and **2**. On the other hand, these hydroxyls are in the same spatial region, and consequently it is probable that their formation is due to the same enzyme.

Experimental Section

General Experimental Procedures. Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. IR and UV spectra were recorded in a Perkin-

Elmer 1600 FT and a Varian Cary 1E spectrometer, respectively. ^{1}H 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 chromatographiy 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-7α,8α-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), 9α,18-dihydroxy-7α,8α-epoxy-13-*epi*-*ent*-pimara-15-ene (**6**) (12 mg), 18-hydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (**10**) (2 mg), 11α,18-dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (**12**) (3 mg), 11α,18-dihydroxy-13-*epi*-*ent*-pimara-8(9),15-diene (**13**) (4 mg), 6*β*,14α,18-trihydroxy-9,13-*epi*-*ent*-pimara-7,15-diene (**9**) (4.4 mg), 1α,9α,18-trihydroxy-7α,8α-epoxy-13-*epi*-*ent*-pimara-15-ene (**7**) (3.2 mg), 1α,18-dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (**14**) (7 mg), 7α,11α,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), 1α,6α,18-trihydroxy-7α,8α-epoxy-9,13-*epi*-*ent*-pimara-15-ene (**8**) (1.4 mg), and 3α,18-dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (**16**) (1 mg).

18-Hydroxy-7α,8α-epoxy-9,13-*epi*-*ent*-pimara-15-ene (4): ^{1}H 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-14α), 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 (93), 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 $C_{20}H_{32}O_2$, 304.2902).

Acetate 4a: ^{1}H 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 $C_{22}H_{34}O_3$, 346.2508).

9α,18-Dihydroxy-7α,8α-epoxy-13-*epi*-*ent*-pimara-15-ene (6): ^{1}H 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-14α), 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 $C_{20}H_{32}O_3$, 320.2351).

1α,9α,18-Trihydroxy-7α,8α-epoxy-13-*epi*-*ent*-pimara-15-ene (7): ^{1}H 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-3α), 1.58 (1H, td, J = 13, 4 Hz, H-3*β*), 2.05 (1H, td, J = 13 Hz, 4 Hz, H-11*β*), 2.26 (1H, d, J = 13 Hz, H-14α), 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 $C_{20}H_{32}O_4$, 336.2301).

1α,6α,18-Trihydroxy-7α,8α-epoxy-9,13-*epi*-*ent*-pimara-15-ene (8): ^{1}H 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 $C_{20}H_{32}O_4$, 336.2301).

6*β*,14α,18-Trihydroxy-9,13-*epi*-*ent*-pimara-7,15-diene (9): ^{1}H 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 $C_{20}H_{32}O_3$, 320.2351).

Triacetate 9a: ^{1}H 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 $C_{26}H_{38}O_6$, 404.2668).

18-Hydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (10): ^{1}H 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-12α), 1.20 (1H, dd, J = 14, 11.7 Hz, H-14α), 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-6α), 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 $C_{20}H_{32}O_2$, 304.2402).

8*β*,18-Dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (11): ^{1}H 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 $C_{20}H_{32}O_3$, 320.2351).

11α,18-Dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (12): IR (film) ν_{max} 3400, 2930, 1704, 1455, 1380 cm^{-1}; ^{1}H 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-6α), 2.31 (1H, dd, J = 18.6, 3.1 Hz, H-6β), 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 [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 $C_{20}H_{32}O_3$, 320.2351).

Diacetate 12a: ^{1}H 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 [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 $C_{24}H_{36}O_5$, 404.2562).

11α,18-Dihydroxy-7-oxo-13-*epi*-*ent*-pimara-8(9),15-diene (13): IR (film) ν_{max} 3610, 2920, 1650, 1460, 1380, 1045, 920 cm^{-1}; UV (EtOH) λ_{max} 247 nm; ^{1}H 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-14α), 2.26 (1H, d, J = 18 Hz, H-14β), 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 [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 $C_{20}H_{30}O_3$, 318.2194).

1α,18-Dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (14): ^{1}H 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-3α), 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 [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 $C_{20}H_{32}O_3$, 320.2351).

2β,18-Dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (15): ^{1}H 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 [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 $C_{20}H_{32}O_3$, 320.2351).

3α,18-Dihydroxy-7-oxo-9,13-*epi*-*ent*-pimara-15-ene (16): ^{1}H 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-12α), 1.17 (1H, dd, J = 14, 12 Hz, H-14α), 1.41 (1H, ddd, J = 16, 12, 3.5 Hz, H-11β), 1.75 (1H, ddd, J = 13.5, 5, 2 Hz, H-12β), 1.90 (1H, dd, J = 13, 5 Hz, H-5), 1.98 (1H, dt, J = 14, 5.6 Hz, H-14β), 2.16 (1H, dd, J = 18, 5 Hz, H-6β), 2.25 (1H, dd, J = 18, 13 Hz, H-6α), 2.39 (1H, td (1H, 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 [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 $C_{20}H_{32}O_3$, 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: ^{1}H 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-12β), 1.99 (1H, dt, J = 16 Hz, 3 Hz, H-14β), 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-6β), 2.35 (1H, dd, J = 19, 13 Hz, H-6α), 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 [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 $C_{24}H_{36}O_5$, 404.2562).

7α,11α,18-Trihydroxy-9,13-*epi*-*ent*-pimara-8(14),15-diene (18): ^{1}H 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-12β), 1.82 (1H, dt, J = 12.4, 3.6 Hz, H-1α), 1.99 (1H, ddd, J = 13.4, 9, 2 Hz, H-6β), 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 [M]$^+$ (1), 302 (12), 284 (7), 271 (7), 253 (8), 151 (36), 123 (100); HREIMS m/z 320.2345 (calcd for $C_{20}H_{32}O_3$, 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-7α,8α-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-7β,8β-epoxy-9,13-*epi*-*ent*-pimara-15-ene (**5**) (3 mg).

Compound 5: ^{1}H NMR (500 MHz) δ 0.82 (3H, s, H-19), 0.98 (3H, s, H-17), 0.97 (3H, s, H-17), 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 [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 $C_{20}H_{32}O_2$, 304.2402).

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