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THE MICROBIOLOGICAL TRANSFORMATION OF A 9-*EPI-ENT*-PIMARADIENE DITERPENE BY *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; microbiological transformation; diterpenes; 9-*epi-ent*-pimarenes; epoxidation.

Abstract—Incubation of 2α,19-dihydroxy-9-*epi-ent*-pimara-7,15-diene with the fungus *Gibberella fujikuroi* gave the compounds 2α,19-dihydroxy-7α,8α-epoxy-9-*epi-ent*-pimara-15-ene, 2α,19-dihydroxy-7-oxo-9-*epi-ent*-pimara-15-ene, 2α,19-dihydroxy-7-oxo-*ent*-pimara-8(9),15-diene, 2α,6β,19-trihydroxy-7α,8α-epoxy-9-*epi-ent*-pimara-15-ene, 2α,9β,19-trihydroxy-7α,8α-epoxy-*ent*-pimara-15-ene, 2α,7α,8β,19-tetrahydroxy-9-*epi-ent*-pimara-15-ene and 2α,7α,9β,19-tetrahydroxy-*ent*-pimara-15-ene. Oxidation of C-19, which is characteristic of the biosynthetic pathway of gibberellins, was not observed. © 1997 Elsevier Science Ltd

INTRODUCTION

In continuation of our studies on the microbiological transformation of diterpenes by the fungus *Gibberella fujikuroi* [1–3], we report here on the biotransformation of a 9-*epi-ent*-pimarene derivative by the fungus. Compounds with this skeleton have not been isolated from *G. fujikuroi*, but in the formation of *ent*-kaur-16-ene, a precursor of the gibberellins produced by the fungus, an *ent*-pimarane carbonium ion has been proposed as an intermediate [4]. Therefore, the study of the biotransformation of **1** was of particular interest.

RESULTS AND DISCUSSION

The substrate (**1**) was isolated from *Calceolaria hypericina*, a plant that grows in the hills of Central Chile (in the original paper, the structure of **1** is drawn incorrectly) [5]. Other species of this genus are also characterised by containing diterpenes of this type.

Incubation of the fungus with **1** was carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene without disturbing post-kaurene metabolism [6, 7]. The fermentation was carried out for a period of 6 days, and the combined broth and mycelium extract separated into neutral and acid fractions. Compounds **3**, **4**, **6**–**9** and **11** were isolated from the neutral fraction. The last compound was obtained as the triacetate **12** by acetylation and chromatography of the fraction containing it. No metabolites were isolated from the acidic fraction.

The HR mass spectrum of the least polar substance **3** was in accordance with the formula $C_{20}H_{32}O_3$, possessing one oxygen more than the substrate **1**. The ^1H NMR spectrum contained the signals observed in the spectrum of the substrate, such as the hydrogens at C-2, C-15, C-16 and C-19, but not that due to the vinylic H-7, which was replaced by a signal for a geminal hydrogen to a new oxygen function (δ 2.96, *d*, $J = 5.6$ Hz). This indicated that the double bond in **2** had been transformed into an oxirane ring in the incubation. Thus, structure **3** was assigned to this biotransformed product, which was confirmed by the ^{13}C NMR data (Table 1) and by chemical means. Epoxidation with *m*-chloroperbenzoic acid of **1** led to a monoepoxide, which was identical with the metabolite **3**.

The α-stereochemistry of the epoxide was determined as follows: (a) The calculated coupling constants of H-7 with the two H-6 protons in the conformer of less energy with the α- and β-epoxide functions were 6.6 and 1.2 Hz for the first, and 5.4 and 3.1 for the second. Thus, the form of the observed signal, a sharp doublet, was more in accord with the α-stereochemistry than with the β; (b) The same epoxide was obtained by both, microbiological and chemical methods, and this indicated that the stereospecificity of the reaction was probably due to one of the faces being sterically hindered. In a study of the molecule

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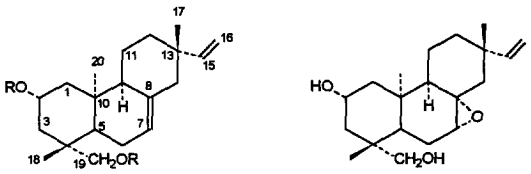

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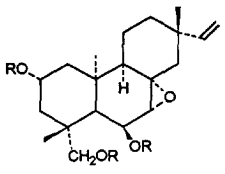

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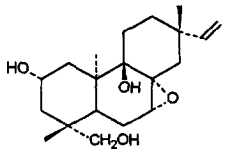

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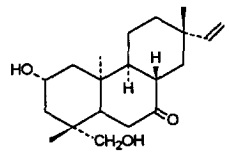

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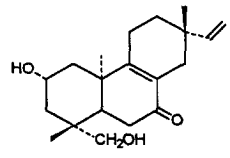

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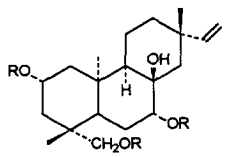

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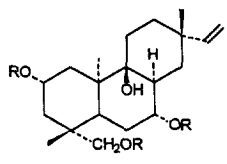

11 R = H
12 R = Ac

Structure 1

of the substrate **1** it can be seen that the 7,8-double bond is more hindered by the β-methyl on C-13 than by the α-methyl over C-10. Thus α-epoxidation is more favourable. The conformation of **1** considered was that of least energy, which was similar to that of an analogous substance determined by X-ray analysis [8]; (c) The structures of the metabolites **9** and **11**, described below and derived from **3**, can only be explained as being derived from an α-epoxide.

Structure **4** was assigned to another metabolite obtained with molecular formula $C_{20}H_{32}O_4$. Its ^1H NMR spectrum was very similar to that of **3**, except that a geminal proton to a new hydroxyl group appeared at δ 4.42, as a doublet ($J = 10.5$ Hz), and that the H-7 geminal to the oxirane ring resonated as a singlet. This indicated that the new alcoholic group must be at C-6 with a β-stereochemistry, with it's geminal proton forming a 90° angle with H-7. Thus, the doublet observed was due to the coupling between H-6(α) and H-5. This last proton resonates at δ 1.38. The ^1C NMR spectrum (Table 1) also confirmed the proposed structure.

Another compound obtained in the biotransformation was **6**, which is isomeric with **4**. The hydroxyl group was also allylic to the oxirane ring, but was now at C-9 rather than at C-6. This position was assigned by the ^{13}C NMR (Table 1), which eliminated the alternative C-5 location for this tertiary hydroxyl group. The β-stereochemistry followed from the fact that hydroxylation must occur on the less hindered β-face of the molecule, as also occurs in **4**.

Compound **7** analysed for $C_{20}H_{32}O_3$, which indicated that it was an isomer of **3**, but neither the vinylic H-7 of the substrate nor the geminal proton to the oxirane appeared in its ^1H NMR spectrum as in **3**.

Table 1. ^{13}C NMR data of compounds **1–8**, **10** and **12**

C	1*	2*	3	4	5	6	7	8	10	12
1	45.0	41.4	44.8	45.7	42.9	42.0	45.4	44.3	41.0	36.9
2	64.7	68.4	64.1	64.0	67.5	64.0	65.6	64.4	68.5	68.2
3	45.9	41.8	46.5†	47.2	43.6	44.0	45.8	44.8	42.3	40.6
4	38.7	38.6	39.6	40.0	38.5†	39.9	38.5	39.9	38.5	38.2
5	43.8	43.8	42.1	48.5†	46.5	45.2	47.7	49.5	46.3	43.1
6	25.3	25.2	22.0	67.5	68.9	21.2	37.9†	32.9	24.1	27.0†
7	119.7	119.4	60.2	66.2	61.7	63.0	217.4	199.1	75.7	74.5
8	136.8	136.6	60.8	61.2	60.4	63.1	53.2	128.9	74.4	38.2
9	53.2	52.9	48.7	48.7†	49.5	74.9	45.3	164.3	55.8	75.9
10	39.7	38.0	37.6‡	38.1	37.1	43.3	40.5	40.9	39.1	42.6
11	23.1	23.0	22.6	22.6	22.6	28.8	24.7	22.7	20.8	27.4†
12	37.7	37.5	36.1	36.0	36.2	32.2	38.5	33.4	36.7	32.3
13	37.0	36.7	37.9‡	38.1	38.3†	37.9	36.6	34.1	36.6	36.1
14	47.9	47.7	46.3†	48.5	45.2	42.2	38.0†	35.2	47.1	35.1
15	150.3	150.1	148.9	148.9	148.6	148.6	151.5	147.0	150.5	145.0
16	109.3	109.4	109.8	109.9	110.1	110.1	109.9	110.8	109.4	112.8
17	21.8	20.8	22.1	22.2	21.9	21.7	22.4	24.6	23.0	31.3
18	27.1	27.7	26.9	30.1	28.8	27.5	28.0	26.4	27.6	27.6
19	66.1	67.1	65.0	68.2	68.2	64.9	66.5	65.7	66.9	66.9
20	23.9	23.6	26.1	26.6	27.0	18.2	26.1	19.6	25.9	17.8

* Taken from ref. [1].
†,‡ These values can be interchanged.

This, and the presence of a carbonyl function in the molecule (IR 1705 cm^{-1}; ^{13}C NMR δ 217.4), permitted the structure **7** to be assigned to this product. The stereochemistry at C-8 reflects the thermodynamically more stable structure.

Metabolite **8** showed a molecular ion in the high resolution mass spectrum at *m/z* 318.2190, which indicated that it possessed two hydrogens less than **7**. Its IR and UV spectrum showed absorptions of an α,β-unsaturated ketone. In the ^1H NMR spectrum no proton signals for an endocyclic double bond were present, indicating that this must be tetrasubstituted. Thus, this compound had to have a C-7 carbonyl group and a C-8, C-9 double bond, and the remainder of the functions over other carbons must be those present in the substrate. Structure **8** was confirmed by assignment of the ^{13}C NMR spectrum (Table 1). A substance with similar B and C rings has been isolated from *Helianthus strumosus* [9].

Compound **9** was assigned the molecular formula $C_{20}H_{34}O_4$. Of the four oxygens of the molecule, two were present in the substrate. Its ^1H NMR spectrum, with respect to that of **1**, showed the presence of a geminal hydrogen to a new hydroxyl group (δ 3.89, *dd*, J = 10.5 and 5 Hz) and the disappearance of the 7,8-double bond. The other oxygen was part of a tertiary hydroxyl group, which was located over a carbon resonating at δ 74.4. These data led to the assignment of structure **9** to this product, where the stereochemistry assigned to C-7 and C-8 reflected the fact that its precursor must be the epoxide **3**. Thus, the opening of the oxirane ring forms the 7α-alcohol and a carbon ion at C-8, which is neutralised with a hydroxyl group of water, which enters by the β-face. The structure was confirmed by the ^{13}C NMR spectrum, which showed carbon resonances of ring C analogous to those of similar substances [10, 11].

Finally, compound **11** was obtained as the triacetate **12** by acetylation and chromatography of the fractions containing it. This compound was isomeric with the triacetate **10** obtained from **9** and we have assigned to it the provisional structure **12**. Characteristic of this compound is the resonance of the geminal proton to the 7α-hydroxyl which appears as a triplet of doublets at δ 3.73, with coupling constants of 10.3 Hz, with H-6(α) and H-8(α), and 5.2 Hz, with H-6(β). Its structure is also in accordance with the ^{13}C NMR spectrum (Table 1). The introduction of H-8 in this compound alters the spatial disposition of the C-13 substituents affecting their carbon resonances. Thus, the 17-methyl changes from δ 22.1 in **3** to δ 31.3 in **12** [12, 13]. This compound can also be formed by opening of the oxirane ring of **3**, formation of a carbon ion at C-8, migration of the hydrogen from C-9 and neutralization of this carbon with a hydroxyl group entering by the β-face. Compounds **9** and **11**, which were obtained in relatively small amounts, could be artefacts produced in the acidification step during the extraction procedure.

The results show that the main compound (**3**), obtained in the biotransformation, is formed by epoxidation of the 7,8-double bond of the substrate. This is followed by allylic hydroxylation at either C-6(β) or C-9(β) to form **4** and **6**, respectively. The alternative route, whereby hydroxylation precedes epoxidation, can not be discounted.

Hydroxylations at C-6, C-7 or epoxidation of a 6,7-double bond are features of the *ent*-kaur-16-ene derivatives produced during the biosynthesis in this fungus of the gibberellins, the kaurenolides and other metabolites. It now seems probable that the same enzymes are involved in the formation of the metabolites obtained in this study. Another interesting aspect, also observed in this biotransformation, is that the C-19 alcohol was not oxidized to the corresponding aldehyde and acid. These oxidations are also characteristic steps in the biosynthesis of gibberellins and kaurenolides [4].

EXPERIMENTAL

General. Mps: uncorr; IR and UV: $CHCl_3$ and EtOH, respectively; ^1H NMR: 200 and 500 MHz in $CDCl_3$, unless stated otherwise; ^{13}C NMR: 50.3 MHz in $CDCl_3$, except that of **7**, which was run in CD_3OD; MS: direct inlet, 70 eV. Conformations of minimum energy and calculated coupling constants were determined by computational methods employing the Chem X program.

Incubation experiments. Gibberella fujikuroi (IMI 58289), inhibited with 5×10^{-5} M AMO 1618, was grown in shake culture at 25° for 1 day in 70 conical flasks (250 ml) each containing sterile medium (50 ml) [14]. The substrate **1** (280 mg) in EtOH (13–15 ml) was distributed equally between the flasks and the incubation allowed to continue for a further 6 days. The broth was filtered, adjusted to pH 2 with dilute HCl, and extracted with EtOAc. The mycelium was treated with liquid N_2, crushed in a mortar and extracted with EtOAc. The two extracts were combined and sepd into acidic and neutral frs with $NaHCO_3$. The acidic fr. was methylated with CH_2N_2.

The neutral frn was chromatographed on silica gel eluting with petrol–EtOAc mixts giving: starting material (**1**) (47 mg), 2α,19-dihydroxy-7α,8α-epoxy-9-*epi*-*ent*-pimara-15-ene (**3**) (35 mg), 2α,19-dihydroxy-7-oxo-9-*epi*-*ent*-pimara-15-ene (**7**) (65 mg), 2α,19-dihydroy-7-oxo-*ent*-pimara-8(9),15-diene (**8**) (5 mg), 2α,6β,19-trihydroxy-7α,8α-epoxy-9-*epi*-*ent*-pimara-15-ene (**4**) (14 mg), 2α,9β,19-trihydroxy-7α,8α-epoxy-*ent*-pimara-15-ene (**6**) (30 mg), 2α,7α,8β,19-tetrahydroxy-9-*epi*-*ent*-pimara-15-ene (**9**) (3 mg) and 2α,7α,9β,19-tetrahydroxy-*ent*-pimara-15-ene (**11**) (7 mg). The last compound was identified in the 2α,7α-19-triacetate form (**12**) by acetylation and chromatography of the fr. containing it.

2α,19-*Dihydroxy*-7α,8α-*epoxy*-9-epi-ent-*pimara*-15-*ene* (**3**). $[M]^+$ at *m/z* 320.2347, $C_{20}H_{32}O_3$ requires 320.2351; ^1H NMR (200 MHz): δ 1.00, 1.04 and 1.12 (each 3H, *s*), 2.96 (1H, *d*, J = 5.6 Hz, H-7), 3.49 and

3.68 (each 1H, *d*, $J = 11$ Hz, H-19), 3.92 (1H, *m*, H-2), 4.89 (1H, *dd*, $J = 10.6$ and 1 Hz, H-16), 4.93 (1H, *dd*, $J = 17.4$ and 1 Hz, H-16), 5.76 (1H, *q*, $J = 10.6$ and 17.4 Hz, H-15); EIMS *m/z* (rel. int.): 320 $[M]^+$ (11), 305 (22), 287 (25), 271 (11), 257 (12), 255 (3), 247 (7), 243 (7), 229 (11), 305 (22), 287 (25), 271 (11), 257 (12), 255 (3), 247 (7), 243 (7), 229 (11), 197 (14).

2α,19-*Dihydroxy*-7-*oxo*-9-epi-ent-*pimara*-15-*ene* (**7**). Mp 196–198°; IR ν_{max} cm^{-1}: 3605, 3420, 3010, 2360, 1705, 1470, 1375, 1030; Found: C, 74.64%; H, 10.26%; $C_{20}H_{32}O_3$ require C, 74.96%; H, 10.06%; ^{1}H NMR (200 MHz): δ 0.98, 0.99 and 1.08 (each 3H, *s*), 3.53 and 3.68 (each 1H, *d*, $J = 11$ Hz, H-19), 3.99 (1H, *m*, H-2), 4.89 (1H, *dd*, $J = 10.7$ and 1 Hz, H-16), 4.96 (1H, *dd*, $J = 17.4$ and 1 Hz, H-16) and 5.80 (1H, *dd*, $J = 10.7$ and 17.4 Hz, H-15); EIMS *m/z* (rel. int.): 320 $[M]^+$ (9), 302 (7), 290 (6), 289 (5), 272 (16), 271 (16), 257 (7), 243 (9), 187 (7), 183 (12), 164 (29), 161 (10), 151 (18), 149 (20), 133 (19), 123 (40), 121 (83), 119 (30), 107 (67).

2α,19-*Dihydroxy*-7-oxo-ent-*pimara*-8(9),15-*diene* (**8**). UV λ_{max} nm^{-1}: 246; IR ν_{max} cm^{-1}: 3580, 3430, 3015, 2855, 1730, 1650, 1610, 1460, 1370, 1260, 1165, 1085, 1032; $[M]^+$ at *m/z* 318.2190. $C_{20}H_{30}O_3$ requires 318.2195; ^{1}H NMR (200 MHz): δ 0.96, 1.06 and 1.11 (each 3H, *s*), 3.57 and 3.75 (each 1H, *d*, $J = 11$ Hz, H-19), 4.00 (1H, *m*, H-2), 4.91 (2H, *m*, H-16), 5.75 (1H, *dd*, $J = 10.7$ and 17.4); EIMS *m/z* (rel. int.): 318 $[M]^+$ (3), 303 (3), 300 (19), 285 (7), 277 (4), 269 (14), 259 (13), 245 (6), 241 (15), 213 (7), 199 (12).

2α,6β-19-*Trihydroxy*-7α,8α-*epoxy*-9-epi-ent-*pimara*-15-*ene* (**4**). $[M]^+$ at *m/z* 336.2301. $C_{20}H_{32}O_4$ requires 336.2300; ^{1}H NMR (200 MHz): δ 1.08, 1.19 and 1.30 (each 3H, *s*), 1.38 (1H, *d*, $J = 10.5$ Hz, H-5), 2.95 (1H, *s*, H-7), 3.61 and 3.96 (each 1H, *d*, $J = 11$ Hz, H-19), 3.91 (1H, *m*, H-2), 4.42 (1H, *d*, $J = 10.5$ Hz, H-6), 4.90 (1H, *dd*, $J = 10.6$ and 1 Hz, H-16), 4.95 (1H, *dd*, $J = 17.4$ and 1 Hz) and 5.79 (1H, *dd*, $J = 17.5$ and 10.6, H-15); EIMS *m/z* (rel. int.): 336 $[M]^+$ (2), 321 (3), 318 (3), 305 (11), 300 (6), 287 (6), 285 (10), 273 (3), 269 (5), 257 (2), 251 (4), 201 (5), 195 (4), 185 (67).

Triacetate (**5**). $[M]^+$ at *m/z* 462.2646. $C_{26}H_{38}O_7$ requires 462.2617; ^{1}H NMR (200 MHz): δ 1.05 (6H, *s*), 1.27 (3H, *s*), 1.68 (1H, *d*, $J = 10.0$ Hz, H-5), 2.02, 2.09 and 2.10 (each 3H, *s*), 2.72 (1H, *s*, H-7), 4.15 and 4.22 (each 1H, *d*, $J = 11$ Hz, H-19), 4.91 (1H, *dd*, $J = 10.6$ and 1 Hz, H-16), 4.93 (1H, *dd*, $J = 17.4$ and 1 Hz, H-16), 5.57 (1H, *d*, $J = 10$ Hz, H-6), 5.79 (1H, *dd*, $J = 17.4$ and 10.6 Hz, H-15); EIMS *m/z* (rel. int.): 462 $[M]^+$ (4), 420 (2), 402 (4), 387 (4), 360 (10), 342 (8), 327 (8), 300 (6), 282 (8), 269 (18), 253 (10), 251 (14), 241 (8), 222 (8), 199 (6).

2α,9β,19-*Trihydroxy*-7α,8α-*epoxy*-ent-*pimara*-15-*ene* (**6**). $[M]^+$ at *m/z* 336.2302. $C_{20}H_{32}O_4$ requires 336.2300; ^{1}H NMR (200 MHz): δ 1.03, 1.08 and 1.16 (each 3H, *s*), 3.13 (1H, *d*, $J = 5.6$ Hz, H-7), 3.52 and 3.75 (each 1H, *d*, $J = 11$ Hz, H-19), 3.88 (1H, *m*, H-2), 4.91 (1H, *dd*, $J = 10.6$ and 1 Hz, H-16), 4.96 (1H, *dd*, $J = 17.4$ and 1 Hz, H-16), 5.81 (1H, *dd*, $J = 17.4$ and 10.6 Hz, H-15); EIMS *m/z* (rel. int.): 336 $[M]^+$ (1), 318 (6), 305 (15), 300 (10), 287 (30), 269 (33), 251 (18), 241 (28), 215 (55), 197 (23).

2α,7α,8β,19-*Tetrahydroxy*-9-epi-ent-*pimara*-15-*ene* (**9**). $[M]^+$ at *m/z* 338.2452. $C_{20}H_{34}O_4$ requires 338.2457; ^{1}H NMR (200 MHz): δ 1.08, 1.11 and 1.31 (each 3H, *s*), 3.48 and 3.72 (each 1H, *d*, $J = 11$ Hz, H-19), 3.89 (1H, *dd*, $J = 10.5$ and 5 Hz, H-7), 4.02 (1H, *m*, H-2), 4.86 (1H, *dd*, $J = 10.6$ and 1 Hz, H-16), 4.93 (1H, *dd*, $J = 17.4$ and 1 Hz, H-16), 5.77 (1H, *dd*, $J = 17.4$ and 10.6 Hz, H-15); EIMS *m/z* (rel. int.): 338 $[M]^+$ (7), 320 (13), 305 (2), 302 (3), 289 (10), 271 (20), 253 (8), 247 (13), 227 (8), 199 (31).

2α,7α,19-*Triacetate* (**10**). $[M-C_2H_2O]^+$ at *m/z* 422.2672. $C_{24}H_{38}O_6$ requires 422.2668; ^{1}H NMR (200 MHz): δ 0.99, 1.05 and 1.40 (each 3H, *s*), 2.02, 2.06 and 2.17 (each 3H, *s*), 3.96 and 4.16 (each 1H, *d*, $J = 11$ Hz, H-19), 4.85 (1H, *dd*, $J = 10.6$ and 1 Hz, H-16), 4.16 (1H, *dd*, $J = 17.4$ and 1, H-16), 5.08 (1H, *m*, H-2), 5.09 (1H, *dd*, $J = 10.5$ and 5 Hz, H-7), 5.72 (1H, *dd*, $J = 17.4$ and 10.6, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ 1.00, 1.05 and 1.53 (each 3H, *s*), 1.66, 1.76 and 1.80 (each 3H, *s*), 4.07 and 4.44 (each 1H, *d*, $J = 11$ Hz, H-19), 4.93 (1H, *d*, $J = 10.6$ Hz, H-16) and 4.96 (1H, *d*, $J = 17.4$, H-16), 5.17 (1H, *dd*, $J = 10.5$ and 5 Hz, H-7), 5.41 (1H, *m*, H-2), 5.73 (1H, *dd*, $J = 17.4$ and 10.6 Hz, H-15); EIMS *m/z* (rel. int.): 422 $[M-C_2H_2O]^+$ (12), 404 (8), 389 (4), 362 (4), 344 (8), 329 (6), 326 (8), 320 (4), 302 (4), 284 (10), 271 (16), 266 (8), 253 (24), 243 (10), 239 (6), 199 (6).

2α,7α,19-*Triacetoxy*-9β-*hydroxy*-ent-*pimara*-15-*ene* (**12**). Obtained by acetylation and chromatography of the fr. containing the corresponding alcohol, $[M-HOAc]^+$ at *m/z* 404.2575. $C_{24}H_{36}O_5$ requires 404.2562; ^{1}H NMR (200 MHz): δ 0.97 (3H, *s*), 1.05 (6H, *s*), 2.02 (3H, *s*), 2.07 (6H, *s*), 3.95 and 4.15 (each 1H, *d*, $J = 11$ Hz, H-19), 4.73 (1H, *td*, $J = 10.3$ and 5.2 Hz, H-7), 4.98 (1H, *m*, H-2), 4.99 (1H, *dd*, $J = 17.4$ and 1.2 Hz, H-16), 5.09 (1H, *dd*, $J = 10.8$ and 1.2 Hz, H-16), 5.68 (1H, *dd*, $J = 17.4$ and 10.8 Hz, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ 0.84, 0.94 and 0.96 (each 3H, *s*), 1.73 (3H, *s*), 1.81 (6H, *s*), 3.97 and 4.32 (each 1H, *d*, $J = 11$ Hz, H-19), 5.03 (1H, *dd*, $J = 17.4$ and 1.2 Hz, H-16), 5.09 (1H, *dd*, $J = 10.8$ and 1.2 Hz, H-16), 5.25 (2H, *m*, H-2 and H-7), 5.63 (1H, *dd*, $J = 17.4$ and 10.8 Hz, H-15).

Epoxidation experiments. (a) Compound **1** (23 mg) in CH_2Cl_2 (5 ml) was treated with MCPBA (20 mg) at room temp. for 12 hr, after which the reaction mixt. was diluted with more solvent and washed with $NaHCO_3$. The organic layer was evapd and the residue chromatographed, eluting with petrol–EtOAc (7:3), to give **3** (9 mg). Further elution afforded an inseparable mixt. (12 mg) of the two diastereomeric epoxides at C-15, in 1:1 ratio. (b) The epoxide **3** was epoxidated, under the same conditions, affording the same mixt. of diepoxides obtained above, which confirmed that the difference between these two diepoxides was the stereochemistry at C-15.

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