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The biotransformation of 18-hydroxy-9-*epi*-*ent*-pimara-7,15-diene by *Gibberella fujikuroi*

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In the memory of the late Professor Joaquin de Pascual-Teresa (1915–1998), University of Salamanca, Spain.

Abstract

Incubation of 18-hydroxy-9-*epi*-*ent*-pimara-7,15-diene with the fungus *Gibberella fujikuroi* gave the compounds 18-hydroxy-7α,8α-epoxy-9-*epi*-*ent*-pimara-15-ene, 18-hydroxy-7-oxo-*ent*-pimara-15-ene, 6β,18-dihydroxy-7α,8α-epoxy-9-*epi*-*ent*-pimara-15-ene, 9β,18-dihydroxy-7α,8α-epoxy-*ent*-pimara-15-ene and 6β,14α,18-trihydroxy-9-*epi*-*ent*-pimara-7,15-diene. Oxidation of C-19, which is characteristic of the biosynthesis pathway of the gibberellins is not produced.

Keywords: *Gibberella fujikuroi*; Microbiological transformation; Diterpenes; 18-Hydroxy-9-*epi*-*ent*-pimara-7,15-diene; Hydroxylation; Epoxidation

1. Introduction

The gibberellins are diterpenoids with plant growth regulating properties, which are commercially produced by fermentation of the fungus *Gibberella fujikuroi*. Since in the formation of *ent*-kaur-16-ene, precursor of the gibberellins, an *ent*-pimarane carbonium ion has been proposed as an intermediate (Bearder, 1983), we believe the biotransformation of compounds with this carbon framework are of particular interest. In a previous study, we described the microbiological transformation of a 9-*epi*-*ent*-pimaradiene diterpene (**1**) by this fungus (Fraga, González, Hernández, Chamy & Garbarino, 1998).

Continuing with this study we report here on the results obtained in the incubation of 18-hydroxy-9-*epi*-*ent*-pimara-7,15-diene by *G. fujikuroi*. This work was initiated with the main aim of determining whether a spatial change in the orientation of the hydroxymethylene group at C-4, from axial (C-19) in **1** to equatorial (C-18) in **2**, has any effect on the results of the incubation, taking into account that 19-hydroxy-*ent*-kaur-16-ene is an intermediate in the biosynthesis of gibberellins (Bearder, 1983).

2. Results and discussion

The substrate (**2**) was isolated from *Calceolaria petioalaris*, a plant that grows in Central Chile (Silva, Chamy, Piovano & Garbarino, 1993). A chemotaxonomical characteristic of this genus is that it contains diterpenes of the *ent*-pimaradiene type.

The incubation with *G. fujikuroi* was carried out in the presence of the inhibitor AMO 1618, a compound that hinders the formation of *ent*-kaur-16-ene without affecting the post-kaurene metabolism (Dennis, Upper

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Table 1
^{13}C-NMR data of compounds **2**–**4**, **6**, **7** and **10**–**12**

C	2	3	4	6	7	10	11	12
1	37.7	37.0	36.9	37.4	37.3[a]	32.5	37.3	37.3
2	18.1	17.6	17.4	18.4	17.2	17.2	18.0	17.6
3	36.4	36.4	36.8	36.6	37.4[a]	36.3	38.4	37.7
4	37.4	37.6	36.5	36.1	36.6	36.7	37.8	36.1[a]
5	37.1	35.2	36.3	41.6	45.3	38.6	46.2[a]	40.4
6	23.7	22.4[a]	22.4	35.6[a]	69.5	21.8	67.5	71.3
7	119.4	60.2	60.1	214.4	62.0	63.5	125.8	124.6
8	136.9	60.7	60.5	52.5	60.5	62.8	139.8	137.8
9	53.1	49.0	48.8	45.2	49.3	74.9	47.7[a]	47.1
10	34.8	35.7	35.7	35.7	35.3	41.2	36.1	36.0[a]
11	25.0	22.5[a]	22.7	24.0	22.4	28.6	24.1	23.9
12	36.3	36.3	36.3	35.5[a]	36.2	32.3	29.5	29.3
13	38.8	38.0	38.0	36.7	38.3	37.8	43.2	41.7
14	48.0	46.8	46.7	37.8	45.3	42.3	79.5	80.5
15	150.3	149.2	149.1	150.1	148.8	148.6	145.7	144.9
16	109.2	109.6	109.7	109.5	109.9	110.0	114.5	112.4
17	21.9	22.2	21.9	22.1	21.8	21.3	22.9[b]	22.7[b]
18	72.3	71.7	72.7	73.1	74.0	72.4	75.4	74.2
19	18.4	17.7	17.6	18.2	18.3	18.2	18.5	18.8
20	22.6	24.5	24.4	25.0	25.9	16.3	23.0[b]	23.1[b]

[a,b] These values can be interchanged.

& West, 1965; Cross & Myers, 1969), which favours the isolation of the biotransformed products. The incubation was carried out for a period of 6 days, and the combined broth and mycelium extract separated into neutral and acid fractions. The neutral fraction was chromatographed and the substances **3**, **7**, **9** and **11** were isolated. Compound **5** was obtained as acetate **6** by acetylation and chromatography of the fraction containing it.

The structural formula of compound **3** was determined as $C_{20}H_{32}O_2$ from its high resolution MS. This fact indicated that an oxygen atom was introduced into the molecule during the incubation. This oxygen must form part of an oxirane ring, because in the ^{1}H-NMR spectrum of this metabolite the vinylic H-7 was not observed, having been replaced by a hydrogen geminal to a new oxygen function (δ 2.96, *d*, J = 5.1 Hz). Thus, structure **3** was assigned to this product, which was confirmed by 2D NMR data (COSY, HMQC and HMBC) and by chemical methods. Epoxidation of **2** with *m*-chloroperbenzoic acid afforded the monoepoxide **3** and a mixture of two diepoxides, diastereomeric at C-15, in 1:1 ratio.

The stereochemistry of the oxirane ring was assigned considering the following points: (a) The form of resonance of H-7, a sharp doublet, is more in accordance with an α-stereochemistry than with a β. Thus, the calculated coupling constants of this hydrogen with the two H-6, were 6.8 and 1.2 Hz in the α-epoxide, and 5.3 and 1.2 Hz in the β-epoxide. (b) The α-epoxidation is more favourable. Thus, the same epoxide was obtained by both microbiological and chemical means, which was probably due to the fact that one of the faces was sterically hindered. In the less energy conformation of substrate **2**, it can be seen that the 7,8-double bond is more hindered by the C-17 methyl(β) than by the C-20 methyl(α). (c) The chemical shift and form of resonance of H-7 were practically identical with those observed in the ^{1}H-NMR spectrum of the 7α,8α-epoxide of **1** (Fraga et al., 1998).

Another compound identified in this biotransformation was **5**, which was obtained, as stated above, in acetate form **6**. Its molecular formula is $C_{22}H_{34}O_3$, which indicated a $C_{20}H_{32}O_2$ for the corresponding alcohol, being isomeric with **3**. The ^{1}H-NMR spectrum of **6** was very different from the acetate of **2** (Silva et al., 1993). Thus, neither the vinylic H-7 of the substrate nor the hydrogen geminal to the oxirane was observed. These data, the absorption of a carbonyl group at 1710 cm^{-1} and the assignment of its ^{13}C-NMR spectrum (Table 1) permitted us to assign the structure of this acetate as **6**. Thus, the corresponding alcohol must be 18-hydroxy-7-oxo-9-*epi*-*ent*-pimara-15-ene (**5**).

Compound **7** possesses the molecular formula $C_{20}H_{32}O_3$. Its ^{1}H-NMR spectrum was very similar to that of **3**, except that the resonance of a geminal hydrogen to a new hydroxyl group appeared at δ 4.19 (*d*, J = 9 Hz) which was assigned to C-6 considering that now the signal of the H-7 resonates as a singlet and that of H-5 as a doublet (δ 1.54, *d*, J = 9 Hz). This indicated that the novel hydroxyl group at C-6 must have a β-stereochemistry, with the geminal proton forming a 90° angle with H-7.

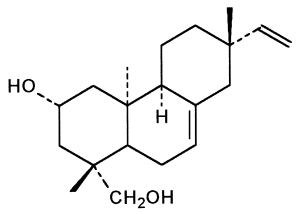
Structure **9** was given to another metabolite, which was isomeric with **7**. This compound also had an oxirane ring, but now the alcohol group was tertiary at C-9. This position was placed considering the ^{13}C-NMR spectrum of its monoacetate **10** (Table 1). The β-stereochemistry was assigned to this alcohol group considering that the hydroxylation at C-9 must have occurred by the β-face due to the presence of the α-methyl on C-10.

Finally, compound **11** showed a molecular ion in the high resolution MS at 320.2359 corresponding to a molecular formula $C_{20}H_{32}O_3$, which indicated that two oxygen atoms had been introduced in the molecule of the substrate during the incubation. This was the only metabolite obtained, which retains the C-7, C-8 double bond of the substrate. In its ^{1}H-NMR spectrum the H-7 appeared at δ 5.51, as a singlet, which indicated that one of the new hydroxyl groups must be located at C-6(β) with its geminal hydrogen forming a 90° angle with H-7, in an analogous manner to that observed in **7**. This geminal proton resonates as a doublet at δ 4.24 (J = 9.6 Hz) by coupling with H-5. The other oxygen introduced into the molecule forms a part of another hydroxyl group, because a geminal

proton appears as a singlet at δ 3.70, which permitted its assignment at C-14. Its α-stereochemistry was given considering the following point: Comparison of the ^{13}C-NMR spectra of **2** and **11** showed that γ-gauche effects were observed between the hydroxyl group at C-14(α) and C-12 and C-15, and not with the C-17 methyl (Pinto, Epifanio, Pizzolatti, Rezende & Silva, 1992). The non-formation of the 7α,8α-epoxide of **11** may be due to steric hindrance produced by the presence of the 14α-alcohol.

The results of this biotransformation with *G. fujikuroi* indicated: (a) The main reactions observed were the epoxidation of the 7,8-double bond of the substrate and the allylic hydroxylation at either C-6(β) or C-9(β). (b) The reactions observed in this feeding are similar to those produced in the incubation of **1** with this fungus, which implies that the replacement of a 2α- and a 19-alcohol (**1**) by a 18-alcohol (**2**) has had limited effects on the results of these biotransformations. This indicates a lack of specificity of the enzymes involved in these processes.

We think that probably the enzyme that epoxidises the 6,7-double bond of *ent*-kaur-6,16-dien-19-oic acid in the biosynthesis pathway of the kaurenolides in *G. fujikuroi* may also be responsible for the 7,8-epoxidation produced in the microbiological transformations of these *ent*-pimaradiene derivatives. It must be remembered that this enzyme epoxidises such different substrates as *ent*-kaur-6,16-dien-19-oic acid (Beale, Bearder, Down, Hutchison, Macmillan & Phinney, 1982), 3α,18-dihydroxy-*ent*-kaur-6,16-diene (Fraga, González, González, Hanson, Hernández & Hitchcock, 1982) and 3α-hydroxy-*ent*-kaur-6,16-diene (Díaz, Fraga & Hernández, 1989).



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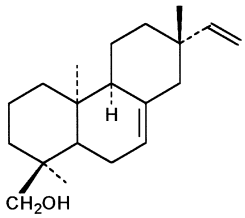

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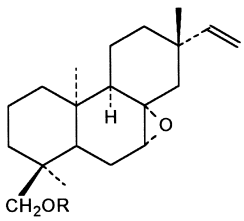

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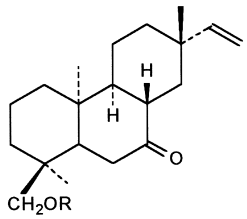

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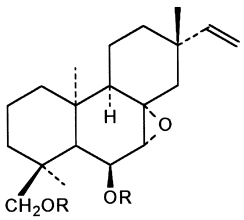

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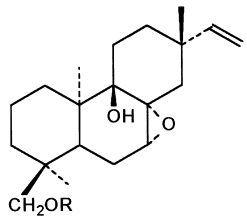

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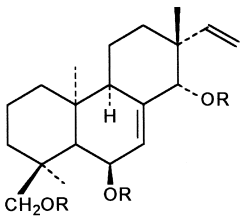

11 R = H
12 R = Ac

3. Experimental

3.1. General

Mps: uncorrec.; IR were taken in the film; ^{1}H-NMR: 200 and 500 MHz in $CDCl_3$, unless stated otherwise; ^{13}C-NMR: 50.3 MHz in $CDCl_3$; MS: direct inlet., 70 eV. Conformations of minimum energy and calculated coupling constants were determined by computational methods employing the Chem X program.

3.2. Incubation experiments

Gibberella fujikuroi (IMI 58289) was grown in shake culture at 25°C in the presence of 5×10^{-5} M AMO 1618 for 1 day in 55 conical flasks (250 ml) each containing sterile medium (50 ml) (Hanson, Hawker & White, 1972). The substrate **2** (153 mg) in EtOH (10 ml) was distributed equally between the flasks and the incubation allowed to continue for further 6 days.

3.3. Isolation of the metabolites

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 separated into acidic and neutral fractions with $NaHCO_3$. The acidic fraction was methylated with CH_2N_2.

The neutral fraction was chromatographed on silica gel, eluting with petrol–EtOAc (9:1), gave starting

material (**2**) (68 mg), 18-hydroxy-7α,8α-epoxy-9-*epi*-*ent*-pimara-15-ene (**3**) (20 mg), 9β,18-dihydroxy-7α,8α-epoxy-*ent*-pimara-15-ene (**9**) (7 mg) and 18-hydroxy-7-oxo-*ent*-pimara-15-ene (**5**). Further elution with petrol–EtOAc (8:2) afforded 6β,18-dihydroxy-7α,8α-epoxy-9-*epi*-*ent*-pimara-15-ene (**7**) (8 mg) and 6β,14α,18-trihydroxy-9-*epi*-*ent*-pimara-7,15-diene (**11**) (13 mg). Compound **5** was identified as acetate **6** (6 mg) by acetylation and chromatography of the fraction containing it. No metabolites were isolated from the acidic fraction.

*3.3.1. 18-Hydroxy-7α,8α-epoxy-9-epi-ent-pimara-15-ene (**3**)*

$[M]^+$ at *m/z* 304.2397. $C_{20}H_{32}O_2$ requires 304.2402; ^{1}H-NMR (500 MHz): δ 0.81, 1.02 and 1.14 (each 3H, *s*), 2.96 (1H, *d*, *J* = 5.1 Hz, H-7), 3.12 and 3.37 (each 1H, *d*, *J* = 10.7 Hz, H-18), 4.88 (1H, *dd*, *J* = 10.7 and 1.1 Hz, H-16), 4.93 (1H, *dd*, *J* = 17.5 and 1.1 Hz, H-16), 5.79 (1H, *dd*, *J* = 17.5 and 10.7 Hz, H-15); EIMS *m/z* (rel. int.): 304 $[M]^+$ (19), 289 (67), 273 (10), 271 (5), 262 (17), 255 (13), 245 (4), 231 (3), 213 (5). Acetate (**4**): $[M]^+$ at *m/z* 346.2507. $C_{22}H_{34}O_3$ requires 346.2508; ^{1}H-NMR (200 MHz): δ 0.88, 0.98 and 1.12 (each 3H, *s*), 2.07 (3H, *s*), 2.95 (1H, *d*, *J* = 6.0 Hz, H-7), 3.61 and 3.87 (each 1H, *d*, *J* = 10.8 Hz, H-18), 4.88 (1H, *dd*, *J* = 10.7 and 1.2 Hz, H-16), 4.93 (1H, *dd*, *J* = 17.5 and 1.2 Hz, H-16), 5.79 (1H, *dd*, *J* = 17.5 and 10.7 Hz, H-15); EIMS *m/z* (rel. int.): 346 $[M]^+$ (19), 331 (29), 304 (16), 286 (10), 273 (8), 271 (37), 255 (11), 245 (5), 217 (4), 199 (5).

*3.3.2. 18-Acetoxy-7-oxo-9-epi-ent-pimara-15-ene (**6**)*

IR υ_{max} cm^{-1}: 2920, 2850, 1730, 1710, 1650, 1460, 1370, 1240, 1030, 850; $[M]^+$ at *m/z* 346.2500. $C_{22}H_{34}O_3$ requires 346.2508; ^{1}H-NMR (200 MHz): δ 0.95, 0.97 and 0.99 (each 3H, *s*), 2.08 (3H, *s*), 3.69 and 3.83 (each 1H, *d*, *J* = 10.8 Hz, H-18), 4.89 (1H, *d*, *J* = 10.7 Hz, H-16), 4.97 (1H, *d*, *J* = 17.5 Hz, H-16), 5.81 (1H, *dd*, *J* = 17.5 and 10.7 Hz, H-15); EIMS *m/z* (rel. int.): 346 $[M]^+$ (17), 318 (5), 304 (9), 286 (37), 271 (51), 243 (27), 230 (14), 215 (5), 199 (5).

3.3.3. 6β,18-Dihydroxy-7α,8α-epoxy-9-epi-ent-pimara-15-ene (7)

^{1}H-NMR (200 MHz): δ 0.91, 1.09 and 1.11 (each 3H, *s*), 1.54 (1H, *d*, *J* = 9.0 Hz, H-5), 2.90 (1H, *s*, H-7), 3.11 and 3.54 (each 1H, *d*, *J* = 11.2 Hz, H-18), 4.19 (1H, *d*, *J* = 9.0 Hz, H-6), 4.90 (1H, *dd*, *J* = 10.7 and 1.2 Hz, H-16), 4.95 (1H, *dd*, *J* = 17.5 and 1.2 Hz, H-16), 5.81 (1H, *dd*, *J* = 17.5 and 10.7, H-15); EIMS *m/z* (rel. int.): 320 $[M]^+$ (2), 305 (3), 302 (6), 289 (11), 287 (10), 284 (5), 271 (34), 269 (8), 253 (17), 243 (20), 229 (11), 213 (11), 199 (9). Diacetate (**8**): $[M]^+$ at *m/z* 404.2575. $C_{24}H_{36}O_5$ requires 404.2562; ^{1}H-NMR (200 MHz): δ 0.99, 1.04 and 1.17 (each 3H, *s*), 2.08 (6H, *s*), 1.82 (1H, *d*, *J* = 10.1 Hz, H-5), 2.71 (1H, *s*, H-7), 3.53 and 3.86 (each 1H, *d*, *J* = 11.2 Hz, H-18), 4.91 (1H, *d*, *J* = 10.7 Hz, H-16), 4.95 (1H, *d*, *J* = 17.5 Hz, H-16), 5.29 (1H, *d*, *J* = 10.1 Hz, H-6), 5.80 (1H, *dd*, *J* = 17.5 and 10.1 Hz, H-15); EIMS *m/z* (rel. int.): 404 $[M]^+$ (2), 389 (2), 362 (2), 344 (13), 329 (6), 326 (8), 302 (9), 284 (18), 269 (40), 266 (10), 251 (35), 227 (15), 224 (14), 209 (18), 199 (200).

*3.3.4. 9β,18-Dihydroxy-7α,8α-epoxy-9-epi-ent-pimara-15-ene (**9**)*

^{1}H-NMR (200 MHz): δ 0.85, 1.03 and 1.17 (each 3H, *s*), 3.11 (1H, *d*, *J* = 4.0 Hz, H-7), 3.12 and 3.38 (each 1H, *d*, *J* = 10.8 Hz, H-18), 4.90 (1H, *d*, *J* = 10.7 Hz, H-16), 4.95 (1H, *d*, *J* = 17.5 Hz, H-16), 5.82 (1H, *dd*, *J* = 17.5 and 10.7 Hz, H-15); EIMS *m/z* (rel. int.): 320 $[M]^+$ (33), 305 (33), 302 (39), 289 (28), 287 (38), 284 (42), 274 (32), 272 (100), 271 (78), 253 (47). Acetate (**10**): $[M]^+$ at *m/z* 362.2462. $C_{22}H_{34}O_4$ requires 362.2457; ^{1}H-NMR (200 MHz): δ 0.94, 1.02 and 1.17 (each 3H, *s*), 2.09 (3H, *s*), 3.11 (1H, *d*, *J* = 4.0 Hz, H-7), 3.58 and 3.95 (each 1H, *d*, *J* = 10.9 Hz, H-18), 4.91 (1H, *d*, *J* = 10.7 Hz, H-16), 4.97 (1H, *d*, *J* = 17.5 Hz, H-16), 5.82 (each 1H, *dd*, *J* = 17.5 and 10.7 Hz, H-15); EIMS *m/z* (rel. int.): 362 $[M]^+$ (5), 347 (1), 344 (5), 302 (3), 289 (2), 271 (4), 253 (1), 223 (2).

*3.3.5. 6β,14α,18-Trihydroxy-9-epi-ent-pimara-7,15-diene (**11**)*

$[M]^+$ at *m/z* 320.2359. $C_{20}H_{32}O_3$ requires 320.2351; ^{1}H-NMR (200 MHz): δ 0.97, 1.01 and 1.06 (each 3H, *s*), 1.48 (1H, *d*, *J* = 9.6 Hz, H-5), 3.26 and 3.41 (each 1H, *d*, *J* = 11.2 Hz, H-18), 3.70 (1H, *s*, H-14), 4.24 (1H, *d*, *J* = 9.6, H-6), 5.14 (1H, *dd*, *J* = 17.6 and 1.2 Hz, H-16), 5.21 (1H, *dd*, *J* = 10.7 and 10.7, H-16), 5.51 (1H, *s*, H-7), 5.86 (1H, *dd*, *J* = 17.6 and 1.2 Hz, H-15); EIMS *m/z* (rel. int.): 320 $[M]^+$ (9), 305 (13), 302 (3), 289 (14), 287 (14), 271 (12), 269 (10), 253 (4), 243 (7), 221 (12), 199 (5). Acetate (**12**): $[M-AcOH]^+$ at *m/z* 386.2438. $C_{24}H_{34}O_4$ requires 386.2457; ^{1}H-NMR (200 MHz): δ 0.98, 1.00 and 1.02 (each 3H, *s*), 1.88 (1H, *d*, *J* = 10.3 Hz, H-5), 3.71 and 3.93 (each 1H, *d*, *J* = 10.8 Hz, H-18), 4.94 (1H, *s*, H-14), 4.98 (1H, *dd*, *J* = 17.6 and 1.2 Hz, H-16), 5.00 (1H, *dd*, *J* = 10.6 and 1.2 Hz, H-16), 5.42 (1H, *d*, *J* = 10.3 Hz, H-6), 5.46 (1H, *br s*, H-7), 5.78 (1H, *dd*, *J* = 17.6 and 10.6 Hz, H-15); ^{1}H-NMR (200 MHz, C_6D_6): δ 0.91, 0.93 and 0.95 (each 3H, *s*), 1.77, 1.78 and 1.94 (each 3H, *s*), 1.92 (1H, *d*, *J* = 10.2 Hz, H-5), 3.93 and 4.10 (each 1H, *d*, *J* = 10.8 Hz, H-18), 5.04 (1H, *dd*, *J* = 18.4 and 1.1 Hz, H-16), 5.06 (1H, *dd*, *J* = 10.1 and 1.1 Hz, H-16), 5.23 (1H, *s*, H-14), 5.67 (1H, *s*, H-7), 5.70 (1H, *d*, *J* = 10.2 Hz, H-6), 5.93 (1H, *dd*, *J* = 18.4 and 10.1 Hz, H-15). EIMS *m/z* (rel. int.): 386 $[M-HOAc]^+$ (1), 344 (17), 329 (1), 326 (3), 311 (3),

284 (15), 269 (13), 266 (2), 253 (9), 251 (13), 245 (13), 203 (6).

3.4. Epoxidation of **2**

Compound **2** (25 mg) in CH_2Cl_2 (4 ml) was treated with *m*-chloroperbenzoic acid (20 mg) at room temperature for 12 h, after which 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 **3** (9 mg). Further elution afforded an unseparable mixture (12 mg) of two diastereomeric epoxides at C-15, in 1:1 ratio.

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References

Bearder, J. R. (1983). In vivo diterpenoid biosynthesis in *Gibberella fujikuroi*. In A. Crozier, *The biochemistry and physiology of gibberellins*, *vol. 1* (p. 251). New York: Praeger.

Beale, M. H., Bearder, J. R., Down, G. H., Hutchison, M., MacMillan, J., & Phinney, B. O. (1982). The biosynthesis of kaurenolide diterpenoids by *Gibberella fujikuroi*. *Phytochemistry*, *21*, 1279–1287.

Cross, B. E., & Myers, P. L. (1969). The effect of plant growth retardants on the biosynthesis of diterpenes by *Gibberella fujikuroi*. *Phytochemistry*, *8*, 79–93.

Dennis, D. T., Upper, C. D., & West, C. A. (1965). An enzymic site of inhibition of gibberellin biosynthesis by AMO 1618 and other plant growth retardants. *Plant Physiology*, *40*, 948–952.

Díaz, C. E., Fraga, B. M., & Hernández, M. G. (1989). Preparation of *ent*-3α-hydroxykaur-6,16-diene and its microbiological transformation by *Gibberella fujikuroi*. *Phytochemistry*, *28*, 1053–1055.

Fraga, B.M., González, A.G., González, P., Hanson, J.R., Hernández, M.G., & Hitchcock, P.B. (1982). The formation of an *ent*-6α,7α-epoxykaurene of possible biosynthetic significance by *Gibberella fujikuroi*. *Journal of the Chemical Society, Chemical Communications*, 311–312.

Fraga, B. M., González, P., Hernández, M. G., Chamy, M. C., & Garbarino, J. A. (1998). The microbiological transformation of a 9-*epi*-*ent*-pimaradiene by *Gibberella fujikuroi*. *Phytochemistry*, *47*(2), 211–215.

Hanson, J. R., Hawker, J., & White, A. F. (1972). The sequence of oxidation on ring B in kaurene-gibberellin biosynthesis. *Journal of the Chemical Society, Perkin Transactions*, *1*, 1892–1896.

Pinto, A. C., Epifanio, R. A., Pizzolatti, M., Rezende, C. M., & Silva, B. R. (1992). Two norditerpenes with an isopimarane skeleton from *Vellozia variabilis*. *Phytochemistry*, *31*, 1679–1680.

Silva, M., Chamy, M. C., Piovano, M., & Garbarino, J. A. (1993). Diterpenoids from *Calceolaria petiolaris*. *Phytochemistry*, *34*, 449–451.