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THE BIOTRANSFORMATION OF TWO *ENT*-15β,16β-EPOXY-KAURANE DERIVATIVES BY *GIBBERELLA FUJIKUROI*

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IN HONOUR OF PROFESSOR JEFFREY HARBORNE'S SIXTY-FIFTH BIRTHDAY

Key Word Index—*Gibberella fujikuroi*; microbiological transformations; diterpenes; *ent*-15β,16β-epoxy-14α-hydroxykaurane; sideroxol.

Abstract—The microbiological transformation of the diterpene *ent*-15β,16β-epoxy-14α-hydroxykaurane into *ent*-14α,15β-dihydroxy-11α,16α-epoxykaurane, *ent*-7α,14α,15β-trihydroxy-11α,16α-epoxykaurane and *ent*-7β,14α,15β-trihydroxy-11α,16α-epoxykaurane has been carried out using the fungus *Gibberella fujikuroi*. The incubation with this fungus of sideroxol (*ent*-7α,18-dihydroxy-15β,16β-epoxykaurane) gave as main products *ent*-7α,15β,18-trihydroxy-11α,16α-epoxykaurane and *ent*-7α,13,18-trihydroxy-15α,16α-epoxykaurane. Some of these compounds were identified as their acetate derivatives. The presence of the 15α,16α-epoxy group in these two substrates inhibits transformations involving oxidation at C-19 and favours the hydroxylation at C-11(β).

INTRODUCTION

Continuing with our studies on the microbiological transformation of diterpenes with an *ent*-kaurene skeleton by the fungus *Gibberella fujikuroi*, we present here the results obtained in the incubation of two *ent*-15β,16β-epoxy-kaurane derivatives. In previous works we have shown that in diterpenes of this type, a hydroxyl group at C-15 (α) induces hydroxylation at C-11(β) and inhibits oxidation at C-19 [1–4], which is characteristic of the biosynthesis of gibberellins and kaurenolides [5]. The aim of this study is to determine the influence of a 15α,16α-epoxy group on these biotransformations and contrast the results with those obtained in the microbiological transformations of 15α-hydroxy derivatives. We therefore incubated *ent*-15β,16β-epoxy-14α-hydroxykaurane (**1**) and *ent*-7α,18-dihydroxy-15β,16β-epoxykaurane (sideroxol) (**2**) with *G. fujikuroi*.

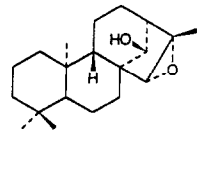
RESULTS AND DISCUSSION

The diterpene **1** was prepared by epoxidation of 14β-hydroxy-*ent*-kaur-15-ene, which had been obtained from the rearrangement of an epoxybeyerane derivative [Fraga *et al.* unpublished results]. Sideroxol (**2**) had been isolated from *Sideritis sicula* [6], and we have synthesized it in the following way: isomeration of epicandicandiol diacetate (**3**) [7, 8] gave sideridiol diacetate (**4**) [9] and the isoatisene analogue (**5**). Compound **4** was epoxidized and then hydrolysed to give **2**.

The incubation with the fungus was carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene without perturbing the post-kaurene metabolism [10, 11].

The microbiological transformation of *ent*-15β,16β-epoxy-14α-hydroxykaurane (**1**) gave *ent*-14α,15β-dihydroxy-11α,16α-epoxykaurane (**6**), *ent*-7α,14α,15β-trihydroxy-11α,16α-epoxy-kaurane (**7**) and *ent*-7β,14α,15β-trihydroxy-11α,16α-epoxy-kaurane (**8**). No metabolites were isolated from the acidic fraction.

The least polar substance isolated (**6**) had the molecular formula $C_{20}H_{32}O_3$ determined by high resolution mass spectrometry. Its ^{1}H NMR spectrum showed the presence in the molecule of four methyl groups and three protons geminal to oxygen functions. Two of these last hydrogens had the same chemical shift, δ4.21 *br s*, and the resonance of the third appeared at 3.49 *s*. To see these signals more clearly the compound was acetylated in the usual way, giving a diacetate (**9**). Its ^{1}H NMR spectrum now showed the geminal hydrogen signals at δ4.29, 4.91 and 5.36. The first of these had the same chemical shift as in the alcohol **6**, indicating that there should be a hydrogen geminal to an ether bridge. Comparison of the resonance and form of this signal (δ4.29, *t*, $J = 3$ Hz) with those given for **12** (δ4.27, *t*, $J = 3$ Hz) [12] permitted this hydrogen to be assigned to C-11, with an ether bridge between this carbon and C-16. The other two signals, at δ4.91 and 5.36, were attributed to the protons geminal to the acetates at C-15(α) and C-14(β), respectively. To



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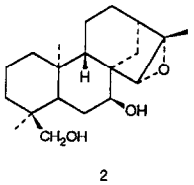

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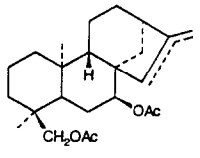
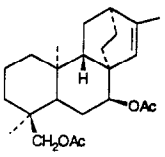


3 Δ^{16}
4 Δ^{15}



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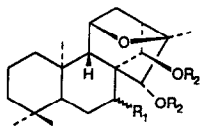
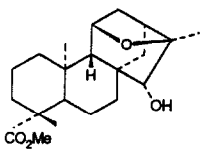


6 $R^1 = R^2 = H$
7 $R^1 = \beta$-OH $R^2 = H$
8 $R^1 = \alpha$-OH $R^2 = H$
9 $R^1 = H$ $R^2 = Ac$
10 $R^1 = \beta$-OAc $R^2 = Ac$
11 $R^1 = \alpha$-OAc $R^2 = Ac$



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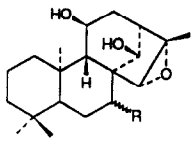


13 R = H
14 R = β-OH
15 R = α-OH

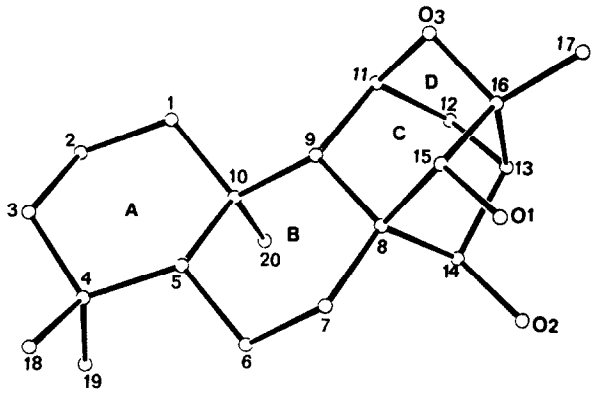


Fig. 1. ORTEP drawing of the molecule for compound **6** with the atom numbering scheme [22].

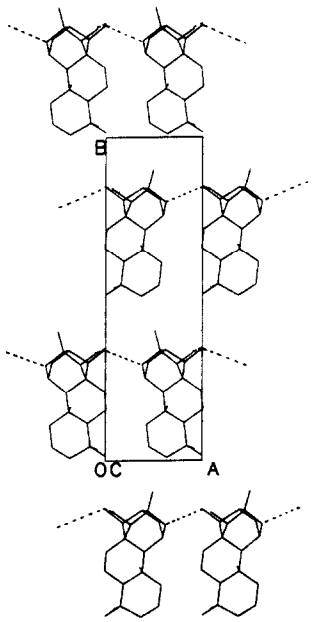


Fig. 2. Molecular packing of structure **6** by PLUTO [23], viewed down the c axis, showing the intra and intermolecular H-bonds (dashed lines).

confirm this structure we submitted the alcohol to an X-ray analysis. In this way the structure of *ent*-14α,15β-dihydroxy-11α,16α-epoxykaurane (**6**) was established for this metabolite.

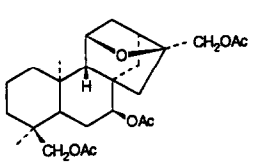
The crystal structure of **6** is shown in Fig. 1 and the packing diagram in Fig. 2. Rings A, B and C adopt a chair conformation, whilst D has an envelope one. The crystal structure is stabilized by two hydrogen bonds, one intramolecular between O_1 and O_2, and the other intermolecular between O_2 and O_3.

The substance **6** is probably formed from the product **13** by treatment of the ethyl acetate extract with aqueous acid on work-up (see Experimental) leading to opening of the oxyrane ring and neutralization of the ion formed at C-16 by the 11β-OH.

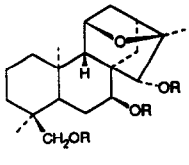
The other two metabolites obtained in the fermentation possess one oxygen atom more than the first substance and were isolated in acetate form by acetylation and chromatography of the fraction containing them. One of these was identified as *ent*-7α,14α,15β-triacetoxy-11α,16α-epoxykaurane (**10**) on the basis of its ^{1}H NMR spectrum, which, when compared with that of **9**, showed a new geminal proton to a secondary acetoxyl group at δ5.11 (*br s*). The chemical shift and the form of the signal were similar to those of the hydrogen at C-7 in **16**, previously obtained by us [3, 4].

The other acetate obtained was identified as *ent*-7β,14α,15β-triacetoxy-11α,16α-epoxykaurane (**11**) on the basis of the following considerations: its ^{1}H NMR spectrum was very similar to that of **10**, with the exception of the signal of the geminal hydrogen to the 7β-acetoxyl group, which has now been substituted by a triplet centred at δ5.26 with a coupling constant of $J = 8$ Hz, using benzene-d_6 as solvent. Thus, this acetate was assigned to the C-7(α). Although other positions for this function such as C-1(α) and C-3(α) were possible, we elected C-7(α) because a 7α-hydroxyl is normally introduced after hydroxylation at C-11(β) in the biotransformations of 15α-hydroxy derivatives by this fungus [3].

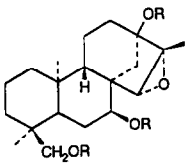
As in the case of **6**, the alcohols **7** and **8** corresponding to the triacetates **10** and **11** may be formed during the acid treatment in the isolation procedure, in which case the metabolites of **1** would be **14** and **15**, respectively.



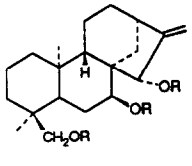
16



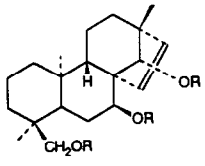
17 R = H
18 R = Ac



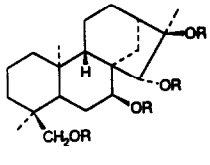
19 R = H
20 R = Ac



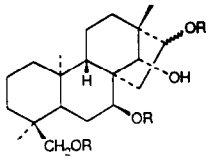
21 R = H
22 R = Ac



23 R = H
24 R = Ac



25 R = H
26 R = Ac



27 R = H
28 R = Ac

The incubation of sideroxol (**2**) gave two types of compounds, one in which a new hydroxyl group has been introduced enzymatically into the kaurenoid molecule and another mainly formed by rearrangement of the substrate. In the former, the compounds *ent*-7α,15β,18-trihydroxy-11α,16α-epoxy-kaurane (**17**) and *ent*-7α,13,18-trihydroxy-15β,16β-epoxy-kaurane (**19**) were obtained.

The first of these substances (**17**) and its triacetate **18** showed a ^{1}H NMR spectrum similar to those of **7** and **10**, respectively, except for the presence now of the AB double doublet of the hydroxymethylene group at C-4 and the absence of the hydrogen geminal to the 14β-hydroxyl group. Thus, the structure **17** was assigned to this compound. The ^{13}C NMR spectrum (Table 1) is also in accordance with this structure.

The triacetate of a second product was obtained by acetylation and chromatography from the fraction containing it, and assigned the structure **20**. Its high resolution mass spectrum is in accordance with the molecular formula $C_{26}H_{38}O_7$ corresponding to a triol $C_{20}H_{32}O_4$. Thus, a new alcohol group has been introduced during the incubation. The ^{1}H NMR of the triacetate **20** indicated that the acetate corresponding to this alcohol must be tertiary because no new geminal hydrogens to acetates were observed. This acetoxyl function must be situated at C-13, because the H-13 signal observed in the ^{1}H NMR spectrum of the substrate is absent. We must also take into consideration that one of the steps in the biosynthesis of gibberellic acid is the hydroxylation at C-13 [5]. Thus, the structure **19** was assigned to the corresponding alcohol obtained in this biotransformation.

Another four compounds were also obtained in the incubation of sideroxol (**2**). The first two products were identified with the known natural diterpenes eubotriol (**21**) [13] and pusillatriol (**23**) [14], whilst the structures of *ent*-7α,15β,16α,18-tetrahydroxy-kaurane (**25**), and *ent*-7α,14β,16,18-tetrahydroxy-beyerane (**27**) were attributed to the latter two, on the basis of NMR data (see Experimental).

These compounds may be formed during the extraction work by opening of the oxirane ring of the substrate **2** in aqueous acid medium, to give the carbonium ion at C-16 (**29**) (Scheme 1), which affords **21** and **25**, or it is rearranged to give another ion **30**, which finally leads to **23** and **27**. The facility of rearrangement of sideroxol (**2**), when compared with compound **1**, can be explained by the fact that in the latter the 14β-hydroxyl group is associated, by a hydrogen bond, with the oxygen of the oxirane ring, stabilizing it.

These biotransformations indicate that the presence of a 15α,16α epoxy group in *ent*-kaurane derivatives inhibits oxidation at C-19, and directs hydroxylation at C-11(β). These results are similar to those obtained in the microbiological transformations of *ent*-kaur-16-ene derivatives hydroxylated at C-15(α) [1–4].

Since, on the other hand, the 15β-hydroxy analogues are oxidized at C-19 and transformed into gibberellins by *G. fujikuroi* [15], the stereochemistry of the oxygen atom at C-15 is important in preventing oxidation at C-19. In this context, Hanson *et al.* [16] have shown that *ent*-kauran-16β,17-epoxide is an inhibitor of the oxidation of *ent*-kaurene at C-19 during gibberellin biosynthesis.

EXPERIMENTAL

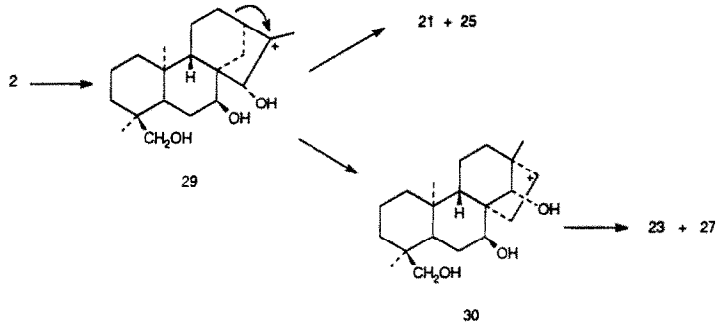
Mps: uncorr.; IR: $CHCl_3$; NMR: $CDCl_3$; MS: 70 eV (probe). CC was performed on silica gel 0.063–0.2 mm. The substances were crystallized from petrol–EtOAc except where otherwise indicated.

Preparation of **1**. *ent*-14α-Hydroxy-kaur-15-ene (230 mg) [Fraga *et al.* unpublished results] in $CHCl_3$ (15 ml) was treated with MCPA (150 mg) at room temp. for 12 hr. Usual work-up gave *ent*-15β,16β-epoxy-14α-hydroxykaurane (**1**), mp 177–179°; $[M]^+$ at *m/z* 304.2377. $C_{20}H_{32}O_2$ requires 304.2402; ^{1}H NMR (200 MHz): δ0.80, 0.88, 0.96 and 1.45 (each 3H, *s*), 2.19 (1H, *t*, *J*=3 Hz, H-13), 2.42 (1H, *m*, H-7), 2.89 (1H, *br s*, H-15), 3.04 (1H, *d*, *J* =12 Hz, H of the OH), 3.84 (1H, *br d*, *J*=12 Hz, H-14), irradiation at δ3.84 collapsed to a singlet the signal at δ3.04 [coupling H-C(14)-OH] and transformed the broad singlet at δ2.89 into a sharp singlet (W coupling between H-15 and H-14); EIMS *m/z* (rel. int.): 304 $[M]^+$

Table 1. ^{13}C NMR data (50 MHz) of compounds **1**, **6**, **9**, **18** and **26**

C	1	6	9	18	26
1	40.5	40.2	40.9	40.3	38.6
2	18.6[a]	18.9	18.8[a]	17.4	17.1
3	41.9	41.1[a]	41.2[b]	35.1	35.5
4	33.3	33.0	33.1	36.6	35.9
5	56.0	55.9	55.7	41.2	41.9
6	18.8[a]	18.9	18.6[a]	24.8	23.7[a]
7	28.0[b]	26.1	26.6	71.7	72.0
8	49.3	52.9	53.4	50.9	39.2
9	52.2	54.0	51.8	55.7	45.6
10	39.2	37.0	37.1	35.8	37.6
11	17.7	76.2	76.4	76.3	19.7
12	27.8[b]	41.7[a]	41.7[b]	40.3	22.2[a]
13	46.6	61.6	62.1	44.5	32.9
14	74.1	81.3	81.3	38.1	22.4[a]
15	70.4	91.0	88.9	83.5	78.9
16	63.7	88.1	87.6	87.3	87.0
17	15.0	18.5	18.5	21.0	18.3
18	33.6	34.0	34.0	72.2	72.2
19	21.6	21.9	22.0	17.8	17.4
20	17.3	18.7	18.6	19.0	14.4

[a, b]These values can be interchanged.



Scheme 1.

(3), 289 (7), 286 (17), 271 (10), 268 (5), 261 (5), 258 (7), 253 (6), 243 (14), 229 (4), 215 (5), 201 (5).

Preparation of sideroxol (**2**). Epicandicandiol diacetate (**3**) (1 g) [7, 8] in C_6H_6 (20 ml) was treated with *p*-TsOH (200 mg) under reflux for 1 hr. Usual work-up and chromatography of the residue on silica gel impregnated with $AgNo_3$ (15%), eluting with petrol–EtOAc (10%), afforded **5** (140 mg), $[M]^+$ at *m/z* 388.2601. $C_{24}H_{36}O_4$ requires 388.2614; ^1H NMR (200 MHz): δ0.81 and 0.99 (each 3H, *s*), 1.75 (3H, *d*, $J = 1.7$ Hz), 2.04 and 2.11 (each 3H, *s*), 2.28 (1H, *br s*, H-12), 3.70 (2H, *s*, H-18), 4.96 (1H, *br s*, H-7), 5.67 (1H, *br s*, H-15); EIMS *m/z* (rel. int.): 388 $[M]^+$ (68), 346 (11), 328 (100), 313 (58), 300 (26), 287 (20), 268 (41), 253 (57), 240 (32), 227 (41), 225 (32), 199 (26). Further elution gave starting material (290 mg) and sideridiol diacetate (**4**) (430 mg) [9].

Sideridiol diacetate (**4**) (390 mg) in $CHCl_3$ (15 ml) was epoxidized with MCPA (270 mg) at room temp. for 12 hr. Usual work-up gave the epoxide [6], which without purification was hydrolysed with aq. KOH (5%) to give sideroxol (**2**) (270 mg) [7]; ^1H NMR (200 MHz): δ0.67, 1.02 and 1.44 (each 3H, *s*), 2.16 (1H, *br s*, H-13), 2.90 and 3.51 (1H, *d*, $J = 11$ Hz, H-18), 3.08 (1H, *s*, H-15), 3.78 (1H, *br s*, H-7).

Incubation experiments. Gibberella fujikuroi (ACC 917), containing 5×10^{-5} M AMO 1618, was grown in shake culture at 25° for 2 days in 65–75 conical flasks (250 ml) each containing sterile medium (50 ml) [17]. The substrate (see below) in EtOH (13–15 ml) was distributed equally between the flasks and the incubation allowed to continue for a further 6 days. The culture broth was filtered, adjusted to pH 2 with dil. HCl, and extracted with EtOAc. The extract was sepd into acidic and neutral fractions by partitioning against $NaHCO_3$. The acidic fraction was methylated with CH_2N_2.

Incubation of *ent*-15β,16β-epoxy-14α-hydroxykaurane (**1**) (215 mg) gave, in the neutral fraction, after chromatography on silica gel eluting with petrol–EtOAc

mixts, starting material (98 mg), *ent*-14α,15β-dihydroxy-11α,16α-epoxykaurane (**6**) (29 mg) and a mixt. of compounds, which was resolved by acetylation and chromatography, eluting with petrol–EtOAc (4:1), to afford *ent*-7α,14α,15β-triacetoxy-11α,16α-epoxykaurane (**10**) (3 mg) and *ent*-7β,14α,15β-triacetoxy-11α,16α-epoxykaurane (**11**) (2 mg). No metabolites were isolated from the acidic fraction.

The neutral fraction of the incubation of sideroxol (*ent*-7α,18-dihydroxy-15β,16β-epoxykaurane) (**2**) (268 mg) was chromatographed with petrol–EtOAc (7:3), giving starting material (80 mg). Further elution with petrol–EtOAc (1:1) afforded a mixt. of compounds, *ent*-7α,15β,18-trihydroxy-11α,16α-epoxykaurane (**17**) (16 mg), compound **27**, which was characterized as *ent*-7α,14β,16,18-tetraacetoxybeyerane (**28**) (2 mg), and *ent*-7α,15β,16α,18-tetrahydroxykaurane (**25**) (10 mg). The mixt. of compounds was resolved by acetylation and chromatography, eluting with petrol–EtOAc (4:1) to give eubotriol triacetate (**22**) (6 mg) [13], pusillatriol triacetate (**24**) (3 mg) [14], and *ent*-7α,13,18-triacetoxy-15β,16β-epoxykaurane (**20**) (2 mg). No metabolites were obtained from the acidic fraction.

ent-14α,15β-Dihydroxy-11α,16α-epoxy-kaurane (**6**): Mp 253–255°; $[M]^+$ at *m/z* 320.2354. $C_{20}H_{32}O_3$ requires 320.2352; ^{1}H NMR (200 MHz): δ0.83, 0.88, 1.03 and 1.42 (each 3H, *s*), 3.49 (1H, *br s*, H-15), 4.21 (2H, *br s*, H-11 and H-14); EIMS *m/z* (rel. int.): 320 $[M]^+$ (4), 305 (5), 287 (3), 273 (100), 255 (7), 231 (8), 229 (6), 216 (21), 137 (24). Diacetate (**9**): $[M]^+$ at *m/z* 404.2616. $C_{24}H_{36}O_5$ requires 404.2563; ^{1}H NMR (200 MHz): δ0.82, 0.83, 1.15 and 1.29 (each 3H, *s*), 2.06 and 2.07 (each 3H, *s*), 4.29 (1H, *t*, *J* = 3 Hz, H-11), 4.91 (1H, *s*, H-15), 5.36 (1H, *br s*, H-14); ^{1}H NMR (200 MHz, C_6D_6): δ0.74, 0.75, 0.99 and 1.39 (each 3H, *s*), 1.74 and 1.79 (each 3H, *s*), 4.12 (1H, *t*, *J* = 3 Hz, H-11), 5.30 (1H, *br s*, H-15), 5.50 (1H, *br s*, H-14); EIMS *m/z* (rel. int.): 404 $[M]^+$ (3), 386 (9), 371 (2), 362 (6), 344 (100), 329 (5), 326 (20), 302 (25), 285 (58), 284 (50), 205 (39).

ent-7α,14α,15β-Triacetoxy-11α,16α-epoxykaurane (**10**): $[M-C_2H_2O]^+$ at *m/z* 420.2505. $C_{24}H_{36}O_6$ requires 420.2511; ^{1}H NMR (400 MHz): δ0.77, 0.81, 1.18 and 1.29 (each 3H, *s*), 1.99, 2.00 and 2.11 (each 3H, *s*), 4.32 (1H, *br s*, H-11), 5.11 (1H, *br s*, H-7), 5.17 (1H, *s*, H-15), 5.39 (1H, *s*, H-14); ^{1}H NMR (400 MHz, C_6D_6): δ0.73, 0.76, 1.04 and 1.42 (each 3H, *s*), 1.84, 1.95 and 1.96 (each 3H, *s*), 4.16 (1H, *br s*, H-11), 5.50 (1H, *br s*, H-7), 5.57 (1H, *s*, H-15), 5.63 (1H, *s*); EIMS *m/z* (rel. int.): 420 $[M-C_2H_2O]^+$ (14), 402 (27), 360 (15), 342 (60), 300 (48), 282 (39), 271 (36), 267 (15), 257 (21), 256 (10), 239 (23).

ent-7β,14α,15β-Triacetoxy-11α,16α-epoxykaurane (**11**): $[M-HOAc]^+$ at *m/z* 402.2405. $C_{24}H_{34}O_5$ requires 402.2406; ^{1}H NMR (400 MHz): δ0.85, 0.87, 1.24 and 1.31 (each 3H, *s*), 1.99, 2.03 and 2.10 (each 3H, *s*), 4.31 (1H, *br s*, H-11), 5.09 (2H, overlapped signal, H-7 and H-15), 5.75 (1H, *s*, H-14); ^{1}H NMR (400 MHz, C_6D_6): δ0.75, 0.81, 1.07 and 1.45 (each 3H, *s*), 1.69, 1.85 and 1.87 (each 3H, *s*), 4.09 (1H, *br s*, H-11), 5.26 (1H, *t*, *J* = 8 Hz, H-7), 5.52 (1H, *s*, H-15), 5.89 (1H, *s*, H-14); EIMS *m/z* (rel. int.): 462 $[M]^+$ (2), 402 (6), 360 (10), 342 (19), 324 (5), 300 (33), 282 (26), 267 (13), 256 (26), 239 (20), 226 (37), 199 (25).

ent-7α,15β,18-Trihydroxy-11α,16α-epoxykaurane (**17**): ^{1}H NMR (200 MHz): δ0.74, 1.13 and 1.37 (each 3H, *s*), 2.98 and 3.47 (each 1H, *d*, *J* = 11 Hz, H-18), 3.82 (1H, *s*, H-15), 3.89 (1H, *br s*, H-7), 4.30 (1H, *t*, *J* = 3 Hz, H-11); EIMS *m/z* (rel. int.): 336 $[M]^+$ (0.3), 318 (2), 306 (13), 288 (40), 270 (30), 255 (13), 243 (11), 214 (100), 199 (22). Triacetate (**18**): $[M]^+$ at *m/z* 462.2616. $C_{26}H_{38}O_7$ requires 462.2617; ^{1}H NMR (200 MHz): δ0.84, 1.17 and 1.24 (each 3H, *s*), 1.97, 1.99 and 2.04 (each 3H, *s*), 3.62 and 3.70 (each 1H, *d*, *J* = 11 Hz, H-18), 4.34 (1H, *t*, *J* = 3 Hz, H-11), 4.92 (1H, *br s*, H-7), 5.04 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 462 $[M]^+$ (1), 420 (23), 402 (11), 360 (43), 342 (100), 269 (100), 211 (20), 145 (15), 109 (29).

ent-7α,13,18-Triacetoxy-15β,16β-epoxykaurane (**20**): $[M]^+$ at *m/z* 462.2618. $C_{26}H_{38}O_7$ requires 462.2617; ^{1}H NMR (200 MHz): δ0.61, 1.10 and 1.44 (each 3H, *s*), 2.01, 2.07 and 2.10 (each 3H, *s*), 2.97 (1H, *s*, H-15), 3.65 and 3.73 (each 1H, *d*, *J* = 11 Hz, H-18), 4.85 (1H, *br s*, H-7); EIMS *m/z* (rel. int.): 462 $[M]^+$ (1), 447 (1), 444 (1), 420 (2), 402 (6), 389 (4), 360 (74), 342 (58), 300 (19), 285 (22), 282 (37), 269 (97), 253 (76), 241 (23), 227 (21), 199 (19).

ent-7α,15β,18-Triacetoxykaur-16-ene (eubotriol triacetate) (**22**): ^{1}H NMR (200 MHz): δ0.83 and 1.10 (each 3H, *s*), 1.98, 1.99 and 2.05 (each 3H, *s*), 2.84 (1H, *br s*, H-13), 3.60 and 2.77 (each 1H, *d*, *J* = 11 Hz, H-18), 4.97 (1H, *br s*, H-7), 5.06 and 5.21 (each 1H, *s*, H-17), 5.42 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 446 $[M]^+$ (0.2), 404 (2), 386 (5), 371 (2), 362 (5), 344 (33), 326 (34), 313 (11), 283 (13), 266 (22), 253 (55), 251 (29), 238 (7), 225 (10), 197 (13).

ent-7α,14β,18-Triacetoxy-beyer-15-ene (pusillatriol triacetate) (**24**): $[M]^+$ at *m/z* 446.2666. $C_{26}H_{38}O_6$ requires 446.2668; ^{1}H NMR (400 MHz): δ0.83, 0.84 and 0.95 (each 3H, *s*), 1.97, 1.99 and 2.05 (each 3H, *s*), 3.67 and 3.72 (1H, *d*, *J* = 11 Hz, H-18), 4.71 (1H, *s*, H-14), 5.09 (1H, *br s*, H-7), 5.52 and 5.57 (each 1H, *d*, *J* = 6 Hz, H-15 and H-16); EIMS *m/z* (rel. int.): 446 $[M]^+$ (2), 386 (7), 344 (41), 326 (67), 315 (22), 313 (10), 284 (14), 269 (16), 253 (99), 251 (27). This compound was compared with an authentic sample obtained by acetylation of pusillatriol.

ent-7α,15β,16α,18-Tetrahydroxykaurane (**25**): ^{1}H NMR (400 MHZ): δ0.70, 0.99 and 1.29 (each 3H, *s*), 2.94 and 3.49 (each 1H, *d*, *J* = 11 Hz, H-18), 3.64 (1H, *s*, H-15), 3.68 (1H, *br s*, H-7), EIMS *m/z* (rel. int.): 320 $[M-H_2O]^+$ (2), 302 (10), 290 (31), 272 (53), 246 (100), 215 (30), 199 (12). Tetraacetate (**26**): ^{1}H NMR (200 MHz): δ0.82, 1.03 and 1.44 (each 3H, *s*), 2.00 (6H, *s*), 2.04 and 2.05 (each 3H, *s*), 2.71 (1H, *br s*, H-13), 3.64 and 3.74 (1H, *d*, *J* = 11 Hz, H-18), 4.73 (1H, *br s*, H-7), 4.88 (1H, *s*, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ0.62, 0.75 and 1.62 (each 3H, *s*), 1.71 (6H, *s*), 1.90 and 1.99 (each 3H, *s*), 2.94 (1H, *br s*, H-13), 3.74 (2H, *s*, H-18), 4.99 (1H, *br s*, H-7), 5.33 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 446 $[M-HOAc]^+$ (13), 404 (17), 386 (23), 344 (64), 326 (37), 316 (24), 253 (100), 225 (16), 199 (11).

ent-7α,14β,16,18-Tetraacetoxy-beyerane (**28**): $[M-HOAc]^+$ at *m/z* 446.2671. $C_{26}H_{38}O_6$ requires 446.2668; ^{1}H NMR (200 MHz): δ0.80, 0.83 and 0.97 (each 3H, *s*), 1.98, 2.01 and 2.04 (each 3H, *s*), 2.62 (1H, *m*, H-15), 3.61 and 3.71 (each 1H, *d*, *J* = 11 Hz, H-18), 4.70 (1H, *s*, H-

14), 4.89 (1H, *m*, H-16), 5.05 (1H, *br s*, H-7); EIMS *m/z* (rel. int.): 446 $[M-HOAc]^+$ (1), 386 (14), 344 (11), 326 (52), 271 (38), 253 (100), 199 (8).

Crystal data of compound **6**. Crystals of compound **6** with approximate dimensions of $0.30 \times 0.20 \times 0.35$ mm were used for the crystal diffraction analysis. Formula $C_{20}H_{32}O$, formula weight: 320.471, Dc (g cm^{-3}) = 1.2345; $Z=2$, $\mu=5.992$ (cm^{-1}), $F(000)=352$. Cell parameters were determined from least-squares analysis of 30 high-angle reflections, range of 2θ from 24° to 74°. Symmetry monoclinic, space group $P2_1$: $a=6.9058(4)$, $b=21.013(1)$, $c=6.409(1)$ Å, $\beta=112.03(2)°$. The lattice parameters and the intensities were measured on a Philips PW 1100 diffractometer, with graphite monochromated CuKα radiation ($\lambda=1.5418$ Å) in the $\omega/2\theta$ scan mode. Number of independent reflections 1515, number of observed reflections 1400, with $I>2\sigma$ (I). The intensities of two standard reflections were monitored every 90 reflections showing no significant variation in the intensity. The data were corrected for Lorentz and polarization effects but not for absorption.

The structure was solved by direct methods [18, 19], and difference Fourier maps; the H atoms were located on DF maps and they were included in the last cycles of refinement. Weighting scheme, empirical, to prevent tendency in $\langle w\Delta^2F\rangle$ vs $\langle Fo\rangle$ and $\langle \sin\theta/\lambda\rangle$. Maximum height in final ΔF map 0.198 eA^{-3}. Scattering factors and f′ and f″ values were taken from the literature [20]. All the calculations were performed on a VAX 6410 computer, using a package of programs of the XRAY76 SYSTEM and several local programs [21].

Final atom coordinates, list of temperatures factors, hydrogen atom position, and final structure factors have been deposited at the Cambridge Crystallographic Data Centre (U.K.).

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