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THE MICROBIOLOGICAL TRANSFORMATION OF SOME *ENT*-7α,15β-DIHYDROXYKAURENE DERIVATIVES BY *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; microbiological transformations; diterpenes; *ent*-7α,15β-dihydroxy-kaur-16-ene; eubotriol.

Abstract—The microbiological transformation by the fungus *Gibberella fujikuroi* of *ent*-7α,15β-dihydroxy-kaur-16-ene gave *ent*-7α,15β,19-trihydroxy-kaur-16-ene, *ent*-7α,11α,15β-trihydroxykaur-16-ene, *ent*-7α,11α,15β,16β,17-pentahydroxy-kaurane and *ent*-7α,11α,13,15β-tetrahydroxy-kaur-16-ene. *ent*-7α,15β,18-Trihydroxy-kaur-16-ene (eubotriol) afforded *ent*-7α,11α,15β,18-tetrahydroxy-kaur-16-ene, *ent*-7α,15β,18,19-tetrahydroxy-kaur-16-ene, *ent*-7α,17,18-trihydroxy-15β,16β-epoxy-kaurane (epoxysideritriol), and *ent*-7α,17,18,19-tetrahydroxy-kaur-15-ene. The last three compounds were identified as their acetate derivatives.

INTRODUCTION

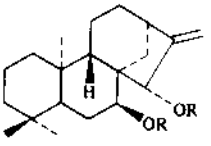
For the past few years, we have studied the microbiological transformation of *ent*-kaurene diterpenes by the fungus *Gibberella fujikuroi* [1–7]. We have shown that several 15α-hydroxy *ent*-kaurene derivatives are hydroxylated at C-11(β) by this fungus [6, 7]. At the same time, an inhibition of the oxidation at C-19 is observed. This oxidation is typical of the biosynthetic route of the gibberellins and kaurenolides [8]. We have now incubated *G. fujikuroi* with 7β,15β-dihydroxy *ent*-kaurene derivatives with the aim of seeing the influence of the 7β-hydroxyl group in the inhibition produced by the 15α-alcoholic group, taking into consideration the relative proximity between these two hydroxylic functions. The results of this study provide more information about the substrate specificity of the enzymes involved in the biosynthesis of gibberellins and kaurenolides.

RESULTS AND DISCUSSION

The two diterpenes utilized in the incubations have been previously isolated from natural sources. The *ent*-kaurene derivative **1** has been obtained from the liverwort *Plagiochila pulcherrima* [9], and Fujita *et al.* have synthesized it starting from epicandicandiol (**8**) [10]. We have now prepared it by allylic oxidation of candol A (**9**) with SeO_2 and *t*-butyl hydroperoxide. Eubotriol (**3**) has been isolated from *Sideritis euboea* [11], and we have obtained it by allylic oxidation of epicandicandiol diacetate (**10**) with SeO_2 in water, acetylation in the usual way and subsequent hydrolysis with methanolic potassium hydroxide.

The fermentations were carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene without affecting post-kaurene metabolism [12, 13], thus facilitating the study of the products formed.

The microbiological transformation of *ent*-7α,15β-dihydroxykaur-16-ene (**1**) gave *ent*-7α,15β,19-trihydroxy-kaur-16-ene (**11**), *ent*-7α,11α,15β-trihydroxy-kaur-16-ene (**13**), *ent*-7α,11α,15β,16β,17-pentahydroxy-kaurane (**15**)



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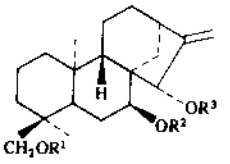
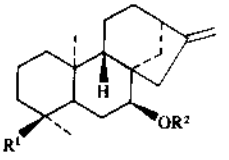
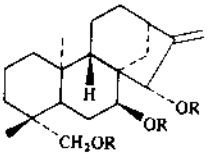


3 $R^1 = R^2 = R^3 = H$
4 $R^1 = R^2 = R^3 = Ac$
5 $R^1 = R^3 = H$, $R^2 = Ac$
6 $R^1 = R^2 = Ac$, $R^3 = H$
7 $R^1 = R^3 = Ac$, $R^2 = H$



8 $R^1 = CH_2OH$, $R^2 = H$
9 $R^1 = Me$, $R^2 = H$
10 $R^1 = CH_2OAc$, $R^2 = Ac$



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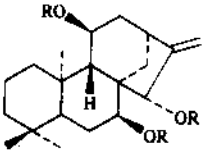

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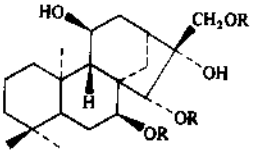


15 R = H
16 R = Ac

Table 1. ^{13}C NMR spectral data (50.3 MHz) of compounds **1**–**4**, **14**, **16**, **18**, **22** and **28**

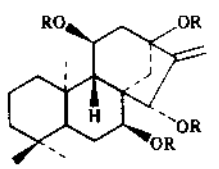
C	1	2	3	4	14	16	18	22	28
1	40.26	40.23	39.90	39.56	39.97*	40.34	39.85	39.58	39.62
2	18.71	18.62	18.11	17.78	18.55	18.43	18.50	17.49	17.52
3	42.05	41.90	35.34	35.46	41.75	41.65	41.68	30.77	30.93
4	33.20	32.65	37.24	36.21	32.80	32.55	32.80	39.58	38.90
5	49.43	49.18	38.63	41.57	46.93	47.16	46.99	42.98	43.56
6	26.91	24.12	26.31	24.20	23.96	24.92	23.94	24.70	25.45
7	73.30	75.03	73.15	74.68	74.70	77.34	74.03	74.30	79.18
8	51.66	49.90	51.68	49.79	49.29	51.33	48.29	49.69	46.78
9	46.37	47.13	49.52	48.92	56.10	55.81	55.13	49.05	51.42
10	39.38	39.53	39.14	39.43	38.50	37.05	38.43	39.28	38.90
11	17.65	17.76	18.11	17.78	68.41	71.82	69.17	17.77	17.76
12	33.20	33.37	33.24	33.32	39.93*	41.15	42.82	33.15	33.23
13	42.97	42.33	42.97	42.27	40.66	41.92	84.18	42.18	43.17
14	35.26	35.49	35.19	35.38	34.92	37.83	37.74	35.20	38.15
15	81.44	80.49	81.33	80.49	79.89	80.26	77.48	80.21	44.98
16	158.85	155.11	158.89	154.95	154.98	88.81	152.66	154.71	154.11
17	108.59	110.51	108.66	110.60	110.05	63.22	111.46	110.57	103.78
18	33.47	33.42	70.94	72.52	33.45	33.74	33.43	69.41	69.37
19	21.70	21.58	17.73	17.60	21.90	22.00	21.60	64.24	64.27
20	17.38	17.47	17.84	18.02	17.47	18.69	17.29	17.89	17.76

and *ent*-7α,11α,13,15β-tetrahydroxy-kaur-16-ene (**17**). No metabolites were obtained from the acid fraction.

The least polar compound isolated had the molecular formula $C_{20}H_{32}O_3$, and possessed one more oxygen atom than substrate **1**. Its ^1H NMR spectrum was very similar to that of **1** except that the signal of a methyl group had been replaced by that of a hydroxymethylene group. This new alcoholic group was assigned at C-19 because the hydrogen chemical shifts and the coupling constant, δ 3.47 and 3.74 ($J = 11$ Hz), are typical of this position in tetracyclic diterpenes [14]. Thus, structure **11** was assigned to this compound.

Another compound obtained in this fermentation was identified as *ent*-7α,11α,15β-trihydroxy-kaur-16-ene (**13**) on the basis of its ^1H NMR spectrum, which, when compared with that of **1**, showed a new geminal hydrogen to a secondary hydroxyl group at δ 3.93 (*d*, $J = 5$ Hz). This compound forms a triacetate (**14**). Its ^1H NMR spectrum in benzene-d_6 showed a geminal proton to one of the acetates at δ 5.19 ($J = 5$ Hz). The chemical shift and the coupling constant were similar to those of the hydrogen at C-11 in *ent*-11α,15β,18-triacetoxy-kaur-16-ene, whose structure has been well established by X-ray analysis of the corresponding alcohol [6].

The third substance isolated was a pentahydroxy derivative (**15**). Its ^1H NMR spectrum showed signals of three methyls, of one hydroxymethylene group and of three geminal protons to hydroxyl groups. Another tertiary alcoholic group was present in the molecule in accord with its M_r. The three new hydroxyls introduced in the molecule of **1** in this fermentation were placed at C-11 (β), C-16 (α) and C-17, respectively, mainly by ^{13}C NMR analysis of the triacetate **16** (Table 1). The alcoholic groups of **15** at C-16 (α) and C-11 (β) were not acetylated, because the first is a tertiary hydroxyl and the acetylation of the second is hindered by the presence of the OH-17 group. The stereochemistry at C-16 was assigned on the basis that the introduction of a new hydroxyl group in this carbon atom in an *ent*-kaur-16-ene diterpene occurs preferentially on the α-face.



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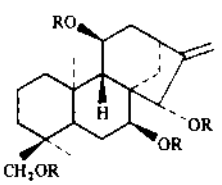
18 R = Ac



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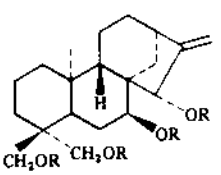

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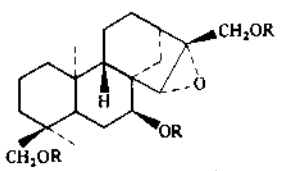

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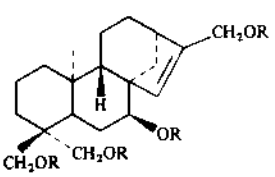
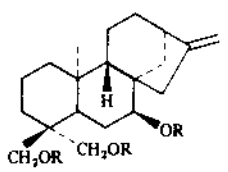


25 R = H
26 R = Ac



27 R = H
28 R = Ac

The most polar substance isolated in the incubation of **1** was the tetraol **17**. The two novel alcoholic groups introduced in the molecule were located at C-11 (β) and C-13, also in accordance with the ^1H and ^{13}C NMR data. Thus, the resonance of H-11 (α) was similar to that of

compound **13**, and the carbon chemical shift of C-13 in the tetraacetate **18** is in accord with that described in the literature for this kind of compound [15].

The microbiological transformation of eubotriol (**3**) gave *ent*-7α,11α,15β,18-tetrahydroxy-kaur-16-ene (**19**), *ent*-7α,15β,18,19-tetrahydroxy-kaur-16-ene (**21**), *ent*-7α,17,18-trihydroxy-15β,16β-epoxy-kaurane (**23**), and *ent*-7α,17,18,19-tetrahydroxy-kaur-15-ene (**25**). The last three compounds were identified as their acetate derivatives, **22**, **24** and **26**, respectively. A new alcoholic group only was introduced in the least polar compound **19** obtained in this last fermentation, this was located at C-11 by comparison of the spectroscopic data with those of other compounds described in this work.

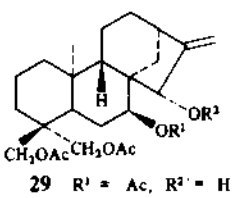
The second product obtained in the incubation of **3** was assigned the structure **21**. The ^{1}H NMR spectrum of its tetraacetate **22** was very similar to that of the triacetate of the substrate (**4**), but with additional signals characteristic of a $-CH_2OAc$ and the disappearance of a methyl group. Thus, this new acetylated alcohol group was located at C-19, by comparison with other related compounds [14], and by taking into consideration that in the biosynthesis of the gibberellins and kaurenolides a hydroxyl group is introduced in this position [8]. Finally, the structure of **21** was confirmed by synthesis of its tetraacetate **22**. Thus, compound **27**, obtained by microbiological transformation of epicandicandiol (**8**) with *G. fujikuroi* [1], was acetylated under the usual conditions to give **28**. Allylic oxidation of this triacetate with SeO_2 afforded compounds **29** and **30**, which were separately acetylated to give the same tetraacetate **22**, which was also identical with that obtained by acetylation of **21**.

The most polar compounds, **23** and **25**, obtained in the microbiological transformation of eubotriol (**3**) were two diterpenes of the *ent*-kaur-15-ene series. These two products were isolated as acetates, **24** and **26** respectively, by acetylation of the fractions that contained them. Their structures were resolved mainly by considering the chemical shifts and the form of the resonance of the epoxidic proton and the two hydrogens at C-17 in **24**, and those of the vinylic hydrogen and the three different $-CH_2OAc$ groups present in **26**. Compound **24** was identical with the triacetate obtained by acetylation of epoxysideritriol [16].

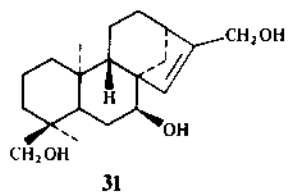
The products **23** and **25** can be formed by solvolysis of the alcohol group at C-15 in **3**, with concomitant isomerization of the double bond, and hydroxylation at C-17, to give a probable intermediate **31**. In the first case the 15(16) double bond was epoxidated by the fungus to give **23** and in the second hydroxylated at C-19 to afford **25**. Compound **23** (epoxysideritriol), the intermediate **31** (sideritriol) and the C-7 monoacetate of eubotriol (eubol) (**5**) have been isolated previously from *Sideritis sicula* [16] indicating that the biosynthetic rearrangement postulated for this incubation with the fungus may also be valid in plants.

Although further experiments are necessary to explain this 15-hydroxy solvolysis, we think that it is due to the combined influence of the OH-7β group and the increase in the polarity of eubotriol due the hydroxyl at C-18 cf. the results of the incubation with this fungus of candidiol (**32**) [6] and other non-hydroxylated diterpenes at C-7 (β) [7] with those of compound **1**.

The microbiological transformations studied here show that in substrates α-hydroxylated at C-15 there is a preference for hydroxylation at C-11 (β), as occurs with those of candidiol (**32**) and other *ent*-kaurene diterpenes of this type previously studied [6, 7]. With reference to the inhibition of the oxidation at C-19, surprisingly, compounds **11** and **21**, which are oxidated at this carbon, were isolated respectively in the two fermentations. However, no metabolites were obtained from the acid fraction, indicating that the oxidation at C-19 to the acid level was completely inhibited by the OH-15α group.



29 R^1 = Ac, R^2 = H
30 R^1 = H, R^2 = Ac



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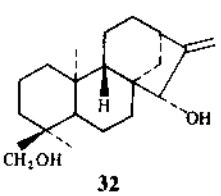

32

EXPERIMENTAL

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

Incubation experiments. Gibberella fujikuroi (ACC 917) inhibited with 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 broth was filtered, adjusted to pH 2 with dil HCl, and extracted with EtOAc. The extract was sepd into acidic and neutral frs with $NaHCO_3$. The acidic fr. was methylated with CH_2N_2, but in the chromatography of the residue no acidic kaurene derivatives were obtained.

The incubation containing *ent*-7α,15β-dihydroxy-kaur-16-ene (**1**) (320 mg) gave in the neutral fr.: starting material (80 mg), *ent*-7α,15β,19-trihydroxy-kaur-16-ene (**11**) (3 mg), *ent*-7α,11α,15β-trihydroxy-kaur-16-ene (**13**) (9 mg), *ent*-7α,11α,15β,16β,17-pentahydroxy-kaurane (**15**) (20 mg), and *ent*-7α,11α,13,15β-tetrahydroxy-kaur-16-ene (**17**) (15 mg).

The incubation containing eubotriol (**3**) (380 mg) gave in the neutral fr.: starting material (160 mg), *ent*-7α,11α,15β,18-tetrahydroxy-kaur-16-ene (**19**) (4 mg) and a mixt. of products, which was resolved by acetylation and chromatography of the mixt. of acetates to give *ent*-7α,15β,18,19-tetraacetoxy-kaur-16-ene (**22**) (10 mg), *ent*-7α,17,18-triacetoxy-15β,16β-epoxy-kaurane (**24**) (6 mg), and *ent*-7α,17,18,19-tetraacetoxy-kaur-15-ene (**26**) (4 mg).

ent-7α,15β,19-*Trihydroxy-kaur-16-ene* (**11**). Mp 196–198°; $[M]^+$ at *m/z* 320.2362. $C_{20}H_{32}O_3$ requires 320.2351; ^{1}H NMR (200 MHz): δ 0.82 and 0.89 (each 3H, *s*), 3.47 and 3.74 (each 1H, *d*, *J* = 11 Hz, H-19), 3.90 (1H, *br s*, H-7), 4.11 (1H, *br s*, H-15), 5.10 and 5.22 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 320 $[M]^+$ (1), 302 (33), 287 (24), 284 (19), 271 (100), 269 (20), 259 (7), 253 (36), 243 (7), 227 (5), 213 (6), 201 (11), 199 (7). *Triacetate* **12**, $[M]^+$ at *m/z* 446.2661. $C_{26}H_{38}O_6$ requires 446.2654; ^{1}H NMR (200 MHz):

δ0.85 and 1.07 (each 3H, *s*), 1.97, 1.99 and 2.04 (each 3H, *s*), 2.84 (1H, *br s*, H-13), 3.86 and 4.16 (each 1H, *d*, $J = 11$ Hz, H-19), 4.96 (1H, *br s*, H-7), 5.05 and 5.19 (each 1H, *s*, H-17), 5.39 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 404 $[M-H_2O]^+$ (1), 386 (5), 344 (10), 326 (15), 311 (6), 284 (21), 266 (11), 253 (16), 251 (12), 238 (3), 225 (5), 209 (4), 199 (5).

ent-7α,11α,15β-*Trihydroxy-kaur-16-ene* (**13**). $[M-H_2O]^+$ at *m/z* 302.2279. $C_{20}H_{30}O_2$ requires 302.2246; ^{1}H NMR (200 MHz): δ0.82 (3H, *s*), 0.91 (6H, *s*), 2.82 (1H, *br s*, H-13), 3.93 (1H, *d*, $J = 5$ Hz, H-11), 4.01 (1H, *br s*, H-7), 4.60 (1H, *s*, H-15), 5.25 (2H, *br s*, H-17); EIMS *m/z* (rel. int.): 320 $[M]^+$ (1), 302 (6), 287 (13), 284 (17), 269 (22), 259 (5), 251 (5), 241 (10), 228 (9), 213 (11), 199 (10). *Triacetate* **14**, $[M-HOAc]^+$ at *m/z* 386.2462. $C_{24}H_{34}O_4$ requires 386.2457; ^{1}H NMR (200 MHz): δ0.77, 0.79 and 1.00 (each 3H, *s*), 1.97 (6H, *s*), 2.00 (3H, *s*), 2.80 (1H, *br s*, H-12), 5.07 (2H, complex signal, H-7 and H-11), 5.04 and 5.12 (each 1H, *s*, H-17), 5.19 (1H, *s*, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ0.68, 0.71 and 0.80 (each 3H, *s*), 1.69, 1.86 and 1.94 (each 3H, *s*), 5.01 and 5.40 (each 1H, *s*, H-17), 5.19 (1H, *d*, $J = 5$ Hz, H-11), 5.30 (1H, *br s*, H-7), 6.17 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 386 $[M-HOAc]^+$ (5), 344 (8), 326 (24), 311 (5), 302 (3), 284 (41), 266 (26), 251 (22), 240 (10), 227 (5), 223 (5), 211 (7), 197 (13).

ent-7α,11α,15β,16β,17-*Pentahydroxy-kaurane* (**15**). Mp 240–242°; ^{1}H NMR (200 MHz): δ0.85, 0.89 and 1.11 (each 3H, *s*), 3.83 and 3.93 (each 1H, *d*, $J = 11$ Hz, H-17), 3.90 (1H, *s*, H-7), 4.11 (1H, *s*, H-15), 4.37 (1H, *t*, H-11); EIMS *m/z* (rel. int.): 336 $[M-H_2O]^+$ (1), 318 (6), 305 (2), 303 (4), 300 (6), 290 (12), 287 (12), 272 (55), 257 (14), 247 (6), 229 (72), 213 (21), 199 (4).

ent-7α,15β,17-*Triacetoxy*-11α,16β-*dihydroxy-kaurane* (**16**). Mp 145–147°; $[M-H_2O]^+$ at *m/z* 462.2628. $C_{26}H_{38}O_7$ requires 462.2617; ^{1}H NMR (200 MHz): δ0.78, 0.82 and 1.14 (each 3H, *s*), 1.96, 1.98 and 2.06 (each 3H, *s*), 3.93 and 4.52 (each 1H, *d*, $J = 11$ Hz, H-17), 4.45 (1H, *t*, H-11), 4.90 (1H, *br s*, H-7), 5.25 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 462 $[M-H_2O]^+$ (1), 402 (6), 360 (6), 342 (55), 327 (7), 324 (4), 300 (23), 282 (34), 272 (7), 269 (8), 267 (16), 257 (7), 254 (7), 249 (22), 239 (13), 228 (44), 213 (20), 207 (53), 199 (7).

ent-7α,11α,13,15β-*Tetrahydroxy-kaur*-16-*ene* (**17**). ^{1}H NMR (200 MHz): δ0.82, 0.90 and 0.92 (each 3H, *s*), 4.01 (1H, *br s*, H-7), 4.14 (1H, *d*, $J = 5$ Hz, H-11), 4.60 (1H, *s*, H-15), 5.36 (2H, *s*, H-17). *Tetraacetate* (**18**), mp 93–95° (MeOH), $[M]^+$ at *m/z* 504.2690. $C_{28}H_{40}O_8$ requires 504.2658; ^{1}H NMR (200 MHz): δ0.77, 0.79 and 1.06 (each 3H, *s*), 1.97, 1.99, 2.00 and 2.05 (each 3H, *s*), 5.05 (1H, *br s*, H-7), 5.20 (1H, *d*, $J = 5$ Hz, H-11), 5.22 and 5.30 (each 1H, *s*, H-17), 5.64 (1H, *s*, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ0.59, 0.71 and 0.77 (each 3H, *s*), 1.61, 1.67, 1.76 and 1.85 (each 3H, *s*), 5.24 and 5.49 (each 1H, *s*, H-17), 5.29 (1H, *d*, $J = 6$ Hz, H-11), 6.03 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 462 $[M-C_2H_2O]^+$ (1), 444 (8), 402 (8), 384 (17), 360 (4), 342 (27), 324 (29), 309 (6), 300 (29), 282 (79), 267 (24), 264 (20), 254 (6), 249 (13), 239 (13), 225 (11), 213 (11), 199 (14), 197 (20).

ent-7α,11α,15β,18-*Tetrahydroxy-kaur*-16-*ene* (**19**). $[M]^+$ at *m/z* 336.2282. $C_{20}H_{32}O_4$ requires 336.2266; ^{1}H NMR (200 MHz): δ0.70 and 0.94 (each 3H, *s*), 2.96 and 3.52 (each 1H, *d*, $J = 11$ Hz, H-18), 3.94 (1H, *d*, $J = 5$ Hz, H-11), 4.00 (1H, *br s*, H-7), 4.62 (1H, *s*, H-15), 5.24 and 5.25 (each 1H, *s*, H-17). EIMS *m/z* (rel. int.): 336 $[M]^+$ (1), 318 (2), 305 (2), 303 (3), 300 (3), 287 (13), 272 (4), 269 (19), 255 (16), 251 (5), 213 (17), 199 (8).

Tetraacetate (**20**). $[M]^+$ at *m/z* 504.2760. $C_{28}H_{40}O_8$ requires 504.2723; ^{1}H NMR (200 MHz): δ0.82 and 1.04 (each 3H, *s*), 1.97, 1.98, 2.00 and 2.04 (each 3H, *s*), 3.59 and 3.77 (each 1H, *d*, $J = 11$ Hz, H-18), 5.05 and 5.13 (each 1H, *s*, H-17), 5.07 (2H, complex signal, H-7 and H-11), 5.72 (1H, *s*, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ0.58 and 0.63 (each 3H, *s*), 1.66, 1.73, 1.92 and 1.94 (each 3H, *s*), 3.63 and 3.85 (each 1H, *d*, $J = 11$ Hz, H-18), 5.01 and 5.42 (each 1H, *s*, H-17), 4.17 (1H, *d*, $J = 5$ Hz, H-11), 5.27 (1H, *br s*, H-7), 6.18 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 504 $[M]^+$ (1), 462 (1), 444 (9), 420 (1), 402 (15), 384 (28), 369 (5), 342 (30), 324 (22), 309 (7), 298 (3), 283 (6), 269 (12), 264 (24), 251 (27), 249 (17), 235 (7), 223 (12), 209 (12), 197 (12).

ent-7α,15β,18,19-*Tetraacetoxy-kaur*-16-*ene* (**22**). Mp 80–82°; $[M]^+$ at *m/z* 504.2745. $C_{28}H_{40}O_8$ requires 504.2722; ^{1}H NMR (200 MHz): δ1.11 (3H, *s*), 1.96, 1.98, 2.03 and 2.04 (each 3H, *s*), 2.85 (1H, *br s*, H-13), 3.78 and 3.99 (each 2H, *d*, $J = 11$ Hz, H-18), 4.05 and 4.25 (each 2H, *d*, $J = 11$ Hz, H-19), 4.95 (1H, *br s*, H-7), 5.05 and 5.20 (each 1H, *s*, H-17), 5.39 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 504 $[M]^+$ (1), 462 (1), 444 (5), 402 (18), 384 (22), 369 (8), 342 (12), 324 (19), 309 (9), 282 (13), 264 (31), 251 (17), 249 (16), 241 (2), 235 (4), 211 (5), 197 (6).

ent-7α,17,18-*Triacetoxy*-15β,16β-*epoxy-kaurane* (**24**). Mp 137–139° (lit 138–140° [16]); $[M-C_2H_2O]^+$ at *m/z* 420.2512. $C_{24}H_{36}O_6$ requires 420.2511; ^{1}H NMR (200 MHz): δ0.81 and 1.05 (each 3H, *s*), 2.05, 2.09 and 2.10 (each 3H, *s*), 3.15 (1H, *s*, H-15), 3.64 and 3.73 (each 1H, *s*, $J = 11$ Hz, H-17), 4.08 and 4.66 (each 1H, *d*, $J = 12$ Hz, H-18), 4.83 (1H, *br s*, H-7); EIMS *m/z* (rel. int.): 420 $[M-C_2H_2O]^+$ (1), 403 (7), 385 (1), 359 (2), 342 (4), 329 (5), 327 (2), 313 (12), 283 (5), 269 (6), 253 (6), 241 (7), 239 (5), 213 (5), 199 (5).

ent-7α,17,18,19-*Tetraacetoxy-kaur*-15-*ene* (**26**). $[M]^+$ at *m/z* 504.2734. $C_{28}H_{40}O_8$ requires 504.2721; ^{1}H NMR (200 MHz): δ1.11 (3H, *s*), 2.04 (6H, *s*), 2.07 and 2.09 (each 3H, *s*), 3.83 and 3.97 (each 1H, *d*, $J = 11$ Hz, H-18), 4.04 and 4.25 (each 1H, *d*, $J = 11$ Hz, H-19), 4.57 and 4.67 (each 1H, *d*, $J = 14$ Hz, H-17), 4.74 (1H, *br s*, H-7), 5.57 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 504 $[M]^+$ (1), 462 (1), 444 (5), 402 (24), 384 (20), 369 (7), 342 (5), 324 (24), 309 (8), 264 (50), 251 (18), 236 (15), 223 (16), 221 (14), 209 (12), 195 (16).

Allylic oxidation of candol A. Compound **9** (450 mg) in CH_2Cl_2 (3 ml) was treated with SeO_2 (80 mg) and *t*-butyl hydroperoxide (0.4 ml) in CH_2Cl_2 (3 ml) under N_2 for 8 hr at room temp. [18]. The soln was poured into H_2O and extracted with EtOAc in the usual way. Chromatography of the residue and elution with petrol–EtOAc gave starting material (75 mg) and *ent*-7α,15β-dihydroxy-kaur-16-ene (**1**) (305 mg), mp 203–204° (lit 206–208° [9]), $[M-H_2O]^+$ at *m/z* 286.2292. $C_{20}H_{30}O$ requires 286.2296; ^{1}H NMR (200 MHz): δ0.81, 0.88 and 1.01 (each 3H, *s*), 3.91 (1H, *br s*, H-7), 4.11 (1H, *br s*, H-15), 5.09 and 5.21 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 286 $[M-H_2O]^+$ (26), 271 (22), 268 (5), 253 (8), 243 (4), 229 (5), 215 (4), 201 (8), 189 (8). *Diacetate* (**2**), mp 130–132°, ^{1}H NMR (200 MHz): δ0.73, 0.76 and 1.02 (each 3H, *s*), 1.94 and 1.95 (each 3H, *s*), 2.80 (1H, *br s*, H-13), 4.93 (1H, *br s*, H-7), 5.02 and 5.16 (each 1H, *s*, H-17), 5.37 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 388 $[M]^+$ (1), 346 (3), 328 (51), 313 (8), 286 (61), 268 (74), 253 (58), 240 (7), 225 (15), 213 (15), 199 (15).

Oxidation of epicandicandiol diacetate. Compound **10** (1 g) in dioxane (40 ml) was treated with SeO_2 (200 mg) and H_2O (10 ml) for 9 hr at room temp. [19]. The soln was poured into H_2O and extracted with EtOAc in the usual way. Chromatography of the residue eluting with petrol–EtOAc (10%) afforded *ent*-7α,18-diacetoxy-15α-hydroxy-kaur-16-ene (**6**) (270 mg), ^{1}H NMR (80 MHz): δ0.83 and 1.08 (each 3H, *s*), 3.70 (2H, *br s*, H-18), 4.00 (1H, *s*, H-15), 4.98 (1H, *br s*, H-7), 5.05 and 5.21 (each 1H, *s*, H-17). Further elution gave *ent*-15β,18-diacetoxy-7α-hydroxy-kaur-16-ene (**7**) (290 mg), ^{1}H NMR (80 MHz): δ0.81 and 1.06 (each 3H, *s*), 2.04 (6H, *s*), 3.50 and 4.11 (each 1H, *d*, $J = 11$ Hz, H-18), 3.85 (1H, *br s*, H-7), 5.05 and 5.19 (each 1H, *s*, H-17), 5.43 (1H, *s*, H-15).

Triacetate (**4**). Obtained by acetylation of **6** and **7** with Ac_2O and pyridine in the usual way. ^{1}H NMR (200 MHz): δ0.77 and 1.04 (each 3H, *s*), 1.92, 1.93, and 1.99 (each 3H, *s*), 2.79 (1H, *br s*, H-13), 2.54 and 3.71 (each 1H, *d*, $J = 11$ Hz, H-18), 4.91 (1H, *br s*, H-7), 5.00 and 5.16 (each 1H, *s*, H-17), 5.36 (1H, *s*, H-15).

Hydrolysis of compound **4**. Compound **4** (590 mg) in MeOH was treated with MeOH–KOH (5%) (10 ml) at room temp. for

24 hr. Usual work-up gave *ent*-7α,15β,18-trihydroxy-kaur-16-ene (*eubotriol*) (3) (390 mg), $[M-H_2O]^+$ at *m/z* 302.2235. $C_{20}H_{30}O_2$ requires 302.2246; ^{1}H NMR (200 MHz): δ0.70 and 1.04 (each 3H, *s*), 2.78 (1H, *br s*, H-13), 2.94 and 3.49 (each 1H, *d*, *J* = 11 Hz, H-18), 3.91 (1H, *br s*, H-7), 4.12 (1H, *s*, H-15), 5.09 and 5.22 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 302 $[M-H_2O]^+$ (5), 287 (4), 272 (11), 271 (11), 254 (21), 239 (10), 213 (4), 199 (4).

Oxidation of compound **28**. Compound **28** (25 mg) was treated with SeO_2 and t-butyl hydroperoxide as described above for **9**. Chromatography of the residue with petrol-EtOAc gave *ent*-7α,18,19-triacetoxy-15β-hydroxy-kaur-16-ene (**29**) (8 mg) ^{1}H NMR (200 MHz): δ1.10 (3H, s), 2.04 (6H, *s*), 2.06 (3H, *s*), 2.81 (1H, *br s*, H-13), 3.83 and 3.99 (each 1H, *d*, *J* = 11 Hz, H-18), 3.99 (1H, *s*, H-15), 4.05 and 4.26 (each 1H, *d*, *J* = 11 Hz, H-19), 4.97 (1H, *br s*, H-7), 5.06 and 5.22 (each 1H, *s*, H-17). Further elution afforded ent-15β,18,19-triacetoxy-7α-hydroxy-kaur-16-ene (**30**) (10 mg), ^{1}H NMR (200 MHz): δ1.08 (3H, *s*), 2.04 (3H, *s*), 2.06 (6H, *s*), 2.82 (1H, *br s*, H-13), 3.79 and 4.23 (each 1H, *d*, *J* = 11 Hz, H-18), 3.84 (1H, *br s*, H-7), 4.00 and 4.29 (each 1H, *d*, *J* = 11 Hz, H-19), 5.06 and 5.19 (each 1H, *s*, H-17), 5.43 (1H, *s*, H-15).

Acetylation of **29** *and* **30**. Treatment of **29** and **30** with Ac_2O and pyridine in the usual way gave the same tetraacetate **22**, identical with that obtained by acetylation of **21**.

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