

## THE MICROBIOLOGICAL TRANSFORMATION OF SOME *ENT*-15 $\beta$ -HYDROXYKAURENES BY *GIBBERELLA FUJIKUROI*

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**Key Word Index**—*Gibberella fujikuroi*; gibberellins; kaurenolides; grandifloric acid; *ent*-kaur-6,16-diene derivatives.

**Abstract**—The microbiological transformation of *ent*-15 $\beta$ ,19-dihydroxykaur-16-ene into *ent*-11 $\alpha$ ,15 $\beta$ ,19-trihydroxykaur-16-ene and *ent*-7 $\beta$ ,11 $\alpha$ ,15 $\beta$ ,19-tetrahydroxykaur-16-ene, of *ent*-15 $\beta$ ,18-dihydroxykaur-6,16-diene into *ent*-15 $\beta$ ,18-dihydroxy-6 $\alpha$ ,7 $\alpha$ -epoxykaur-16-ene, *ent*-11 $\alpha$ ,15 $\beta$ ,18-trihydroxykaur-6,16-diene and *ent*-16 $\beta$ ,17-epoxy-11 $\alpha$ ,15 $\beta$ ,18-trihydroxykaur-6-ene, and of *ent*-3 $\beta$ ,15 $\beta$ ,18-trihydroxykaur-6,16-ene into *ent*-6 $\alpha$ ,7 $\alpha$ -epoxy-3 $\beta$ ,15 $\beta$ ,18-trihydroxykaur-16-ene have been carried out using the fungus *Gibberella fujikuroi*. The presence of a 15 $\alpha$ -hydroxyl group in these substrates inhibits transformations involving oxidation at C-19.

### INTRODUCTION

Continuing with our work on the microbiological transformations of *ent*-kaurene diterpenes by the fungus *Gibberella fujikuroi* [1-6], we present here the results obtained for the incubation of some 15 $\alpha$ -hydroxykaurene derivatives with this fungus. In a previous work [6], we showed that candidiol (*ent*-15 $\beta$ ,18-dihydroxykaur-16-ene, **1**) is hydroxylated at C-11 by *G. fujikuroi*, with good yield, to give the  $\beta$ -alcohol (**2**) and not at C-19, in contrast to the normal gibberellin biosynthetic pathway after formation of *ent*-kaur-16-ene [7]. The aim of this work is to obtain more information about the inhibition of the oxidation at C-19 and the hydroxylation at C-11 produced by the presence of the 15 $\alpha$ -hydroxyl group.

### RESULTS AND DISCUSSION

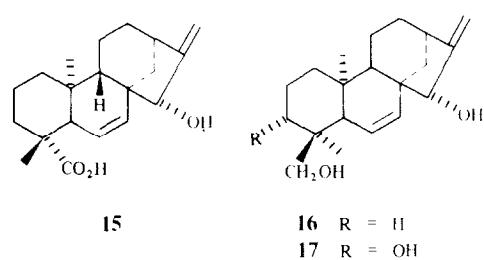
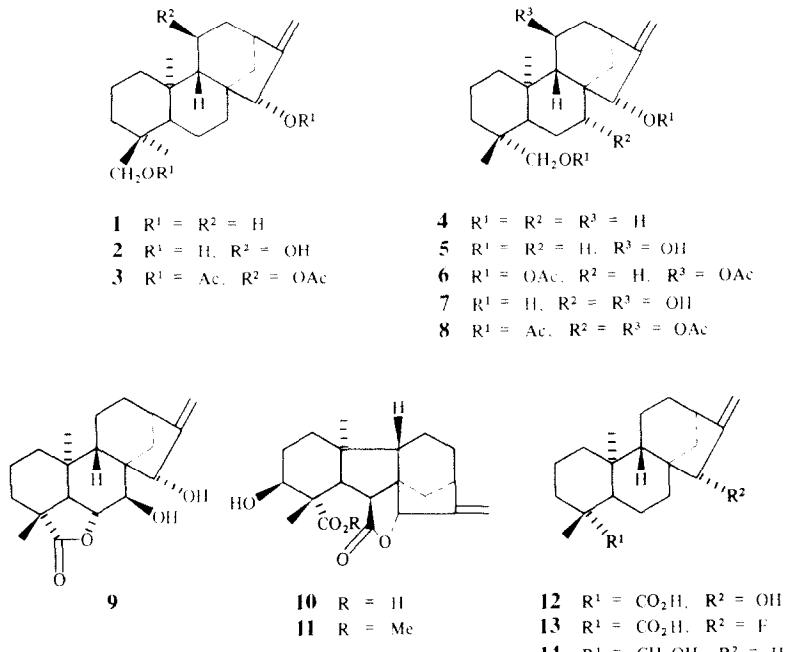
The fermentations were carried out in the presence of AMO 1618, a substance that inhibits the formation of *ent*-kaurene without perturbing the post-kaurene metabolism [8, 9], thus facilitating the analysis of the product formed.

The microbiological transformation of *ent*-15 $\beta$ ,19-dihydroxykaur-16-ene (**4**) gave *ent*-11 $\alpha$ ,15 $\beta$ ,19-trihydroxykaur-16-ene (**5**) and *ent*-7 $\beta$ ,11 $\alpha$ ,15 $\beta$ ,19-tetrahydroxykaur-16-ene (**7**). No metabolites were isolated from the acid fraction. The high resolution mass spectrum of compound **5** was in accordance with the formula  $C_{20}H_{32}O_3$ , indicating that in the fermentation a hydroxyl group has been introduced into the molecule of the substrate. Also in the  $^1H$  NMR spectrum of **5**, compared with that of **4**, a geminal proton to a new alcohol group appeared at  $\delta$  3.90. This compound formed a triacetate **6**. Its  $^1H$  NMR spectrum in  $CDCl_3$  showed a geminal proton to one of the acetates overlapped with one of the olefinic hydrogens, but in  $C_6D_6$  a doublet at  $\delta$  5.09 ( $J=5$  Hz) was observed. The chemical shift and the coupling constant were similar to those originated by the hydrogen

at C-11 in compound **3**, the triacetate of product **2** formed in the incubation of candidiol (**1**) with *G. fujikuroi* [6]. The structure of **2** has been determined by X-ray analysis [6]. Thus the structure **5** was assigned to the less polar compound of the microbiological transformation of **4**, and confirmed by assignment of its  $^{13}C$  NMR spectrum (Table 1). The more polar product (**7**) was a tetrahydroxy compound. Its  $^1H$  NMR spectrum indicated that it was very similar to **5**, but with one additional secondary hydroxyl. The hydrogen geminal to this alcohol group appeared in the spectrum as a doublet ( $J=5$  and 10 Hz) at  $\delta$  3.88, indicating an equatorial position for this hydroxyl function, allylic to a methylene group and to a tetrasubstituted carbon. Thus, this alcohol group can be at the C-1, C-3 or C-7 position. As the methyl resonances in the triol (**5**) and the tetraol (**7**) are very similar we assigned this alcohol to the C-7 position. This assumption was confirmed by the  $^{13}C$  NMR spectrum of its tetraacetate (**8**), whose carbon resonances have been determined (Table 1).

These results indicated that the 15 $\alpha$ -hydroxyl group in the substrate inhibited the oxidation of the hydroxymethylene group at C-19 to an aldehyde one. This type of oxidation occurs in the normal gibberellin biosynthetic pathway. Also, as in the incubation of candidiol (**1**) [6], the product obtained was  $\beta$ -hydroxylated at C-11.

MacMillan *et al.* [10] using GC-MS have tentatively identified  $7\beta$ ,15 $\alpha$ -dihydroxykaurenolide (**9**),  $GA_{14}$ -7,15 $\alpha$ -lactone (**10**) and a hydroxy- $GA_{14}$ -7,15 $\alpha$ -lactone as a result of the incubation of 15 $\alpha$ -hydroxykaur-16-en-19-oic acid (grandifloric acid) (**12**) [11] with a mutant of *G. fujikuroi*. In order to compare these results with those of the feeding of the alcohol **4**, we decided to repeat the incubation of grandifloric acid (**12**) with the wild strain of this fungus. In this way we isolated from the fermentation the compounds **9** and **10**, identified by the Bristol group, completing their spectral data. The  $GA_{14}$ -7,15 $\alpha$ -lactone (**10**) has also been obtained in the microbiological transformation of 15 $\alpha$ -fluorokaur-16-en-19-oic acid (**13**) with the same fungus [12, 13].

Table I.  $^{13}C$  NMR spectral data of compounds **6**, **8**, **16**, **18**, **22** and **32**

C	<b>6</b>	<b>8</b>	<b>16</b>	<b>18</b>	<b>22</b>	<b>32</b>
1	39.94	39.60	38.58	38.03	36.94	36.48
2	18.18	17.82	18.11	17.94	23.16	23.24
3	36.20	36.11	35.34	35.76	73.69	74.06
4	37.18	37.02	37.10	37.80	40.42	39.89
5	56.23	51.73	47.30	49.55	48.44	46.18
6	19.36	26.46	127.89	54.08	51.91	125.87
7	36.64	73.15	132.06	57.58	56.26	131.69
8	46.67	50.13	49.05	47.44	46.88	48.07
9	60.42	60.88	53.10	50.33	48.87	52.53
10	38.51	38.51	38.80	38.03	37.87	38.64
11	68.80	68.01	16.85	16.61	16.61	17.03
12	39.69	39.72	32.87	32.83	33.10	32.72
13	40.86	39.89	39.87	40.84	41.32	40.05
14	34.65	30.60	34.62	34.71	35.61	36.21
15	82.40	81.21	83.96	82.81	82.14	83.77
16	155.31	154.61	160.73	157.83	154.66	156.13
17	109.59	109.27	109.92	110.80	112.58	111.57
18	27.63	27.33	71.51	71.60	65.36	65.05
19	67.12	66.72	17.67	18.31	13.93	13.42
20	18.18	18.04	16.97	19.26	20.12	16.84

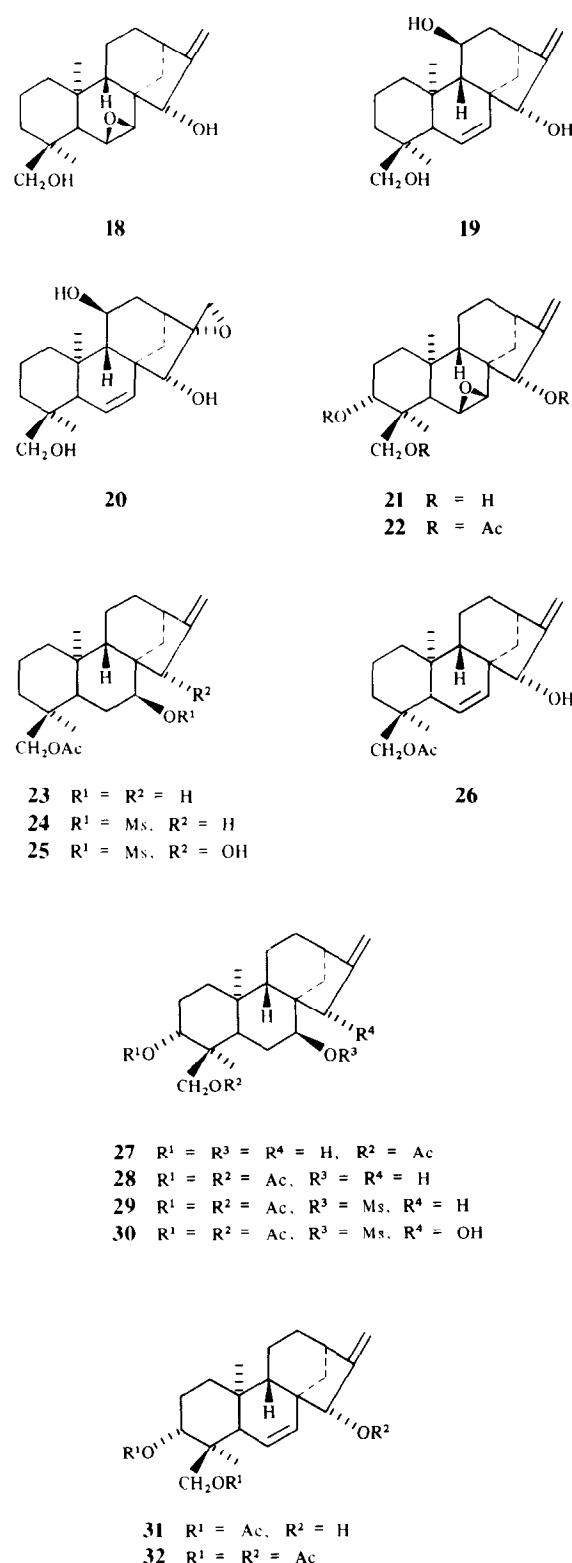
The results of the incubation of the compound **12** indicated that a  $15\alpha$ -hydroxyl group does not inhibit the  $7\beta$ -hydroxylation of **12** and the  $6\beta,7\beta$ -epoxidation of **15**, prior to and necessary for the formation of the gibberellins and kaurenolides, respectively. Compound **4** and candidiol (**1**) [6] are probably not  $\beta$ -hydroxylated at C-7 because these substances do not have a carboxyl group at C-19, as occurs in the normal gibberellin biosynthesis pathway, or two hydroxyl groups at C-3 ( $\alpha$ ) and C-18, as some of us have shown in a previous paper [3].

The kaurenolide **9** was obtained in the feeding of the  $15\alpha$ -hydroxy acid **12** as indicated above. To compare with this result and to see if in substrates hydroxylated at C- $15(\alpha)$ , and with a 6,7-double bond inhibition exists in the hydroxylation at C-19, we decided to incubate compound **16** with the fungus. The microbiological transformation of *ent*- $15\beta,18$ -dihydroxykaur-6,16-diene (**16**) gave compounds **18**, **19** and **20**. None of these three products obtained was hydroxylated at C-19, indicating that the presence in this *ent*-kaur-6,16-diene derivative of a  $15\alpha$ -alcohol group inhibits the enzymatic hydroxylation at this carbon. In two of these compounds, **19** and **20**, that conserve the 6,7-double bond after the feeding, an  $11\beta$ -hydroxylation was produced, in a similar way to that occurring in the incubation of candidiol (**1**) [6].

The structures of the three compounds obtained in the last incubation were also elucidated using spectroscopic data. Thus the chemical shifts and the coupling constants observed for the geminal protons to the oxirane ring in the  $^1\text{H}$  NMR spectrum of **18** are similar to those appearing in kaurene derivatives with this grouping [5]. The chemical shift and the form of resonance of the geminal hydrogen to the  $11\beta$ -hydroxyl group in **19** and **20** are similar to those observed in the  $^1\text{H}$  NMR spectrum of compound **5**. The  $^{13}\text{C}$  NMR spectra of compounds **18** and **22** (Table 1) are also in accordance with the proposed structures. The stereochemistry of the oxirane ring was given as  $\alpha$  in **20**, because in this type of compound the epoxidation occurs on this face.

Finally, we incubated *ent*- $3\beta,15\beta,18$ -trihydroxykaur-6,16-diene (**17**) with this fungus obtaining the epoxy-derivative **21** as the result of the fermentation. This compound was isolated in the form of its triacetate **22** by acetylation of some fractions containing it. Again, no hydroxylation at C-19 occurred, but in this case a double inhibition was probably produced, one by the  $3\alpha$ -hydroxyl group [3] and the other by the  $15\alpha$ -hydroxyl group.

The diol **4** was prepared by oxidation with selenium oxide [14] of the alcohol **14** [15]. The substrate **16** was synthesized in the following way. Treatment of epicandicandidol 18-monoacetate (**23**) [16] with mesyl chloride in pyridine gave the corresponding mesylate **24**, which was allylically oxidized with selenium oxide and *t*-butyl-hydroperoxide [17, 18] to afford compound **25** and in low yield the diene **26**. Treatment of product **25** with collidine under reflux gave further quantities of the diene **26**. This was hydrolysed with methanolic potassium hydroxide to afford the desired diol **16**. The other diene (**17**) was obtained as follows. The diterpene linearol (**27**) was partially acetylated to give the  $3,18$ -diacetate **28** [3] and then mesylated to afford compound **29**. Treatment of this with selenium oxide and *t*-butylhydroperoxide formed the allylic alcohols **30** and **31**. The former was then converted into the latter by treatment with collidine under reflux. Hydrolysis of compound **31** gave the required substrate **17**.



## EXPERIMENTAL

Mps: uncorr. IR:  $\text{CHCl}_3$ ; NMR:  $\text{CDCl}_3$ ; MS: 70 eV (probe). Column chromatography was performed on silica gel 0.063–0.2 mm. The substances were crystallized from petrol– $\text{EtOAc}$  except

where otherwise indicated.  $^{13}\text{C}$  NMR spectra were recorded at 50 MHz.

*Incubation experiments.* *Gibberella fujikuroi* (ACC 917) inhibited with  $5 \times 10^{-5}$  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) [19]. 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 separated into acidic and neutral fractions with NaHCO<sub>3</sub>. The acidic fraction was methylated with CH<sub>2</sub>N<sub>2</sub>.

The incubation of *ent*-15 $\beta$ ,19-dihydroxykaur-16-ene (**4**) (220 mg), after chromatography of the neutral fraction and elution with petrol-EtOAc (40%), gave starting material (140 mg) and *ent*-11 $\alpha$ ,15 $\beta$ ,19-trihydroxykaur-16-ene (**5**) (22 mg). Further elution with petrol-EtOAc (50%) afforded *ent*-7 $\beta$ ,11 $\alpha$ ,15 $\beta$ ,19-tetrahydroxykaur-16-ene (**7**) (6 mg).

The incubation of *ent*-15 $\beta$ -hydroxykaur-16-en-19-oic acid (grandifloric acid) (**12**) (290 mg) gave 7 $\beta$ ,15 $\alpha$ -dihydroxykaur-enolide (**9**) (8 mg), in the neutral fraction, and the 15 $\alpha$ -hydroxy-7,15-lactone of GA<sub>14</sub> methyl ester (**11**) (4 mg), in the methylated acid fraction.

The incubation of *ent*-15 $\beta$ ,18-dihydroxykaur-6,16-diene (**16**) (310 mg) gave in the neutral fraction starting material (210 mg), *ent*-15 $\beta$ ,18-dihydroxy-6 $\alpha$ ,7 $\alpha$ -epoxy-kaur-16-ene (**18**) (20 mg), *ent*-11 $\alpha$ ,15 $\beta$ ,18-trihydroxy-kaur-6,16-diene (**19**) (13 mg) and *ent*-16 $\beta$ ,17-epoxy-11 $\alpha$ ,15 $\beta$ ,18-trihydroxy-kaur-6-ene (**20**) (4 mg) by chromatography and eluting with petrol-EtOAc (20%).

The incubation of *ent*-3 $\beta$ ,15 $\beta$ ,18-trihydroxy-kaur-6,16-diene (**17**) (250 mg) gave in the neutral fraction a mixture of products, which was resolved by acetylation and chromatography of the mixture of acetates obtained. In this way the triacetate of the starting material (**32**) and *ent*-6 $\alpha$ ,7 $\alpha$ -epoxy-3 $\beta$ ,15 $\beta$ ,18-triacetoxylkaur-16-ene (**22**) (30 mg) were obtained.

*ent*-11 $\alpha$ ,15 $\beta$ ,19-Trihydroxy-kaur-16-ene (**5**). Mp 220–222°; [M]<sup>+</sup> at *m/z* 320.2325, C<sub>20</sub>H<sub>32</sub>O<sub>3</sub> requires 320.2349;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.91 and 1.00 (each 3H, s), 2.79 (1H, *br s*, H-13), 3.45 and 3.72 (each 1H, *d*, *J* = 11 Hz, H-19), 3.90 (1H, *d*, *J* = 5 Hz, H-11), 4.27 (1H, *s*, H-15), 5.24 and 5.27 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 320 [M]<sup>+</sup> (1), 302 (13), 287 (9), 284 (6), 272 (22), 271 (86), 259 (9), 253 (20), 243 (4), 213 (9), 201 (8), 199 (5), 197 (5), 189 (12), 187 (17), 177 (9), 175 (14), 173 (11), 171 (10), 163 (16), 162 (27), 161 (19), 157 (12), 153 (46), 150 (13), 148 (53). *Triacetate* (**6**).  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.94 and 0.98 (each 3H, s), 1.94, 2.03 and 2.07 (each, 3H, s), 2.76 (1H, *br s*, H-13), 3.86 and 4.15 (each 1H, *d*, *J* = 11 Hz, H-19), 5.02 and 5.09 (each 1H, *s*, H-17), 5.07 (1H, *d*, H-11), 5.63 (1H, *s*, H-15);  $^1\text{H}$  NMR (200 MHz, C<sub>6</sub>D<sub>6</sub>):  $\delta$  0.63 and 0.88 (each 3H, s), 1.70, 1.73 and 1.80 (each 3H, s), 2.48 (1H, *br s*, H-13), 3.88 and 4.22 (each 1H, *d*, *J* = 11 Hz, H-19), 5.03 and 5.24 (each 1H, *s*, H-17), 5.09 (1H, *d*, *J* = 5 Hz, H-11), 5.93 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 404 [M – C<sub>2</sub>H<sub>2</sub>O]<sup>+</sup> (1), 386 (10), 371 (3), 344 (15), 326 (18), 311 (18), 269 (6), 266 (8), 253 (25), 225 (5), 221 (6), 211 (8), 187 (10).

*ent*-7 $\beta$ ,11 $\alpha$ ,15 $\beta$ ,19-Tetrahydroxy-kaur-16-ene (**7**). [M – H<sub>2</sub>O]<sup>+</sup> at *m/z* 318.2210, C<sub>20</sub>H<sub>30</sub>O<sub>3</sub> requires 318.2192;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.91 and 1.02 (each 3H, s), 2.84 (1H, *br s*, H-13), 3.46 and 3.72 (each 1H, *d*, *J* = 11 Hz, H-19), 3.88 (1H, *dd*, *J* = 5 and 10 Hz, H-7), 3.91 (1H, *d*, H-11), 4.60 (1H, *s*, H-15), 5.19 and 5.22 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 318 [M – H<sub>2</sub>O]<sup>+</sup> (2), 300 (3), 282 (1), 269 (10), 251 (3), 241 (3), 227 (3), 213 (6), 197 (3), 185 (5). *Tetraacetate* (**8**).  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.97 and 1.01 (3H, s), 1.97, 2.00, 2.04 and 2.06 (each 3H, s), 2.80 (1H, *br s*, H-13), 3.84 and 4.13 (each 1H, *d*, *J* = 11 Hz, H-19), 4.84 (1H, *dd*, *J* = 5 and 10 Hz, H-7), 4.95 and 5.10 (each 1H, *s*, H-17), 5.13 (1H, *d*, H-11), 5.90 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 462 [M – C<sub>2</sub>H<sub>2</sub>O]<sup>+</sup> (1), 444 (3),

415 (1), 402 (6), 384 (10), 360 (2), 342 (13), 324 (8), 311 (5), 239 (5), 225 (6), 213 (6), 211 (8), 197 (8), 187 (9).

*7 $\beta$ ,15 $\alpha$ -Dihydroxy-kaurenolide* (**9**). [M]<sup>+</sup> at *m/z* 332.1970, C<sub>20</sub>H<sub>28</sub>O<sub>4</sub> requires 332.1987;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.87 and 1.32 (each 3H, *s*), 1.80 (1H, *d*, *J* = 6 Hz, H-5), 2.73 (1H, *br t*, H-13), 4.19 (1H, *br s*, H-15), 4.55 (1H, overlaped with –OH, H-7), 4.84 (1H, *dd*, *J* = 6 and 12 Hz, H-6), 5.19 and 5.24 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 332 [M]<sup>+</sup> (1), 314 (8), 299 (19), 286 (5), 271 (6), 253 (26), 197 (7), 137 (19).

*15 $\alpha$ -Hydroxy-7,15 $\alpha$ -lactone-GA<sub>14</sub> methyl ester* (**11**). [M]<sup>+</sup> at *m/z* 360.1959, C<sub>21</sub>H<sub>28</sub>O<sub>5</sub> requires 360.1936;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.70 and 1.47 (each 3H, *s*), 2.22 and 3.51 (each 1H, *d*, *J* = 12 Hz, H-5 and H-6), 2.79 (1H, *m*, H-13), 3.72 (3H, *s*), 4.20 (1H, *br s*, H-3), 4.60 (1H, *s*, H-15), 5.15 and 5.26 (each 1H, *d*, *J* = 2 Hz, H-17); EIMS *m/z* (rel. int.): 360 [M]<sup>+</sup> (1), 342 (14), 328 (5), 314 (11), 310 (6), 300 (17), 282 (13), 258 (8), 199 (11), 159 (17).

*ent*-15 $\beta$ ,18-Dihydroxy-6 $\alpha$ ,7 $\alpha$ -epoxy-kaur-16-ene (**18**). Mp 166–168°, [M]<sup>+</sup> at *m/z* 318.2191, C<sub>20</sub>H<sub>30</sub>O<sub>3</sub> requires 318.2195;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.95 and 1.01 (each 3H, *s*), 2.80 (1H, *br s*, H-13), 3.11 (1H, *d*, *J* = 4 Hz, H-7), 3.19 (1H, *q*, *J* = 2 and 4 Hz, H-6), 3.21 and 3.61 (each 1H, *d*, *J* = 11 Hz, H-18), 4.08 (1H, *s*, H-15), 5.18 and 5.29 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 318 [M]<sup>+</sup> (1), 300 (1), 287 (2), 285 (5), 283 (1), 270 (2), 269 (6), 267 (3), 257 (4), 255 (2), 243 (3), 213 (4), 159 (8).

*ent*-11 $\alpha$ ,15 $\beta$ ,18-Trihydroxy-kaur-6,16-diene (**19**). Mp 205–207°, [M – H<sub>2</sub>O]<sup>+</sup> at *m/z* 300.2098, C<sub>20</sub>H<sub>28</sub>O<sub>2</sub> requires 300.2090;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.75 and 0.92 (each 3H, *s*), 3.18 and 3.49 (each 1H, *d*, *J* = 11 Hz, H-18), 3.98 (1H, *d*, *J* = 5 Hz, H-11), 4.23 (1H, *s*, H-15), 5.31 and 5.36 (each 1H, *s*, H-17), 5.76 (2H, *br s*, H-6 and H-7); EIMS *m/z* (rel. int.): 300 [M – H<sub>2</sub>O]<sup>+</sup> (1), 285 (2), 282 (2), 269 (39), 267 (12), 255 (5), 251 (6), 242 (9), 225 (6), 213 (14), 197 (8).

*ent*-16 $\beta$ ,17-Epoxy-11 $\alpha$ ,15 $\beta$ ,18-trihydroxy-kaur-6-ene (**20**). Mp 208–210°; [M – H<sub>2</sub>O]<sup>+</sup> at *m/z* 316.2041, C<sub>20</sub>H<sub>28</sub>O<sub>3</sub> requires 316.2038;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.90 and 0.96 (each 3H, *s*), 2.85 (1H, *br s*, H-13), 3.15 and 3.74 (each 1H, *d*, *J* = 11 Hz, H-18), 3.16 and 3.21 (each 1H, *d*, *J* = 4 Hz, H-17), 3.98 (1H, *d*, *J* = 4 Hz, H-11), 4.49 (1H, *s*, H-15), 5.31 (2H, *br s*, H-6 and H-7); EIMS *m/z* (rel. int.): 316 [M – H<sub>2</sub>O]<sup>+</sup> (2), 301 (2), 286 (2), 285 (6), 284 (2), 283 (7), 270 (2), 269 (5), 268 (2), 267 (6), 255 (4), 225 (4), 213 (6), 197 (6).

*ent*-6 $\alpha$ ,7 $\alpha$ -Epoxy-3 $\beta$ ,15 $\beta$ ,18-triacetoxylkaur-16-ene (**22**). Mp 149–151°; [M]<sup>+</sup> at *m/z* 460.2415, C<sub>26</sub>H<sub>36</sub>O<sub>7</sub> requires 460.2466;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.95 and 0.97 (each 3H, *s*), 1.99, 2.03 and 2.05 (each 3H, *s*), 2.84 (1H, *br s*, H-13), 3.01 (2H, *br s*, H-6 and H-7), 3.91 (2H, *br s*, H-18), 4.76 (1H, *dd*, *J* = 11 and 5 Hz, H-3), 5.14 and 5.34 (each 1H, *s*, H-17), 5.39 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 445 [M – Me]<sup>+</sup> (0.5), 418 (1), 400 (3), 385 (2), 358 (5), 341 (4), 340 (6), 325 (8), 315 (2), 298 (6), 283 (9), 280 (43), 265 (97), 247 (11), 237 (11).

*Allylic oxidation of 14.* The alcohol **14** (650 mg) [15] in dioxane (10 ml) was treated with SeO<sub>2</sub> (260 mg) and H<sub>2</sub>O (3 ml) for 5 hr at room temp. The soln was poured into H<sub>2</sub>O and extracted with EtOAc in the usual way. Chromatography of the residue, eluting with petrol-EtOAc (20%) gave *ent*-15 $\beta$ ,19-dihydroxy-kaur-16-ene (**4**) (410 mg), mp 169–170°; [M]<sup>+</sup> at *m/z* 304.2385, C<sub>20</sub>H<sub>32</sub>O<sub>2</sub> requires 304.2400;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.96 and 1.00 (each 3H, *s*), 3.44 and 3.77 (each 1H, *d*, *J* = 11 Hz, H-19), 3.70 (1H, *s*, H-15), 5.06 and 5.19 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 304 [M]<sup>+</sup> (1), 289 (21), 273 (58), 271 (17), 255 (86), 246 (29), 229 (11), 215 (12), 213 (12), 185 (11).

*Preparation of the mesylate 24.* The compound **23** (730 mg) in dry pyridine (3 ml) was treated with mesyl chloride (0.85 ml) for 5 hr. Usual work-up gave *ent*-18-acetoxy-7 $\alpha$ -mesyloxy-kaur-16-ene (**24**) (810 mg), mp 125–127°; [M – HOMs]<sup>+</sup> at *m/z* 328.2397, C<sub>22</sub>H<sub>32</sub>O<sub>2</sub> requires 328.2400;  $^1\text{H}$  NMR (200 MHz):  $\delta$  0.83 and 1.07 (each 3H, *s*), 2.08 (3H, *s*), 2.98 (3H, *s*), 3.72 (2H, *s*, H-18), 4.61

(1H, *br s*, H-7), 4.82 (2H, *br s*, H-17); EIMS *m/z* (rel. int.): 424 [M]<sup>+</sup> (1), 328 (17), 313 (5), 269 (31), 255 (34), 253 (28), 239 (12), 225 (16), 199 (24).

**Allylic oxidation of 24.** The above mesylate **24** (890 mg) in  $\text{CH}_2\text{Cl}_2$  (15 ml) was treated with  $\text{SeO}_2$  (116 mg), and *t*-butyl hydroperoxide (0.5 ml) in  $\text{CH}_2\text{Cl}_2$  (3 ml) in inert atm. for 10 hr at room temp. The soln was poured into  $\text{H}_2\text{O}$ , extracted with EtOAc and chromatographed. Elution with petrol-EtOAc (10%) gave the diene **26** (60 mg) and *ent*-18-acetoxy-15 $\beta$ -hydroxy-7 $\alpha$ -mesyloxy-kaur-16-ene (**25**) (680 mg), mp 135–137°; <sup>1</sup>H NMR (200 MHz):  $\delta$  0.85 and 1.09 (each 3H, *s*), 2.09 (3H, *s*), 3.08 (3H, *s*), 3.74 (2H, *s*, H-18), 4.05 (1H, *s*, H-15), 4.89 (1H, *br s*, H-7), 5.07 and 5.38 (each 1H, *s*, H-17). EIMS *m/z* (rel. int.): 344 [M–HOMs]<sup>+</sup> (5), 329 (3), 326 (5), 311 (4), 284 (11), 269 (41), 253 (17), 251 (14), 241 (4), 183 (16), 143 (33).

**ent**-18-Acetoxy-15 $\beta$ -hydroxy-kaur-6,16-diene (**26**). The mesylate (**25**) (670 mg) in collidine (6 ml) was refluxed for 30 min. Usual work-up and chromatography of the residue with petrol-EtOAc (10%) gave the diene **26**: mp 71–73°; [M]<sup>+</sup> at *m/z* 344.2388,  $\text{C}_{22}\text{H}_{32}\text{O}_3$  requires 344.2349; <sup>1</sup>H NMR (200 MHz):  $\delta$  0.88 and 0.91 (each 3H, *s*), 2.04 (3H, *s*), 3.74 and 3.82 (each 1H, *d*,  $J$  = 11 Hz, H-18), 3.95 (1H, *s*, H-15), 5.12 and 5.22 (each 1H, *s*, H-17), 5.70 (1H, *dd*,  $J$  = 3 and 10 Hz, H-6), 5.71 (1H, *d*,  $J$  = 10 Hz, H-7); EIMS *m/z* (rel. int.): 344 [M]<sup>+</sup> (2), 326 (2), 311 (2), 284 (6), 269 (41), 253 (11), 227 (10), 226 (11), 197 (12), 157 (22), 105.

**ent**-15 $\beta$ ,18-Dihydroxy-kaur-6,16-diene (**16**). The monoacetate (**26**) was treated with 5% methanolic KOH (10 ml) at room temp. overnight. The soln was neutralized and the product recovered in EtOAc to afford the diol **16** (400 mg): mp 151–153°; [M]<sup>+</sup> at *m/z* 302.2250,  $\text{C}_{20}\text{H}_{30}\text{O}_2$  requires 302.2246; <sup>1</sup>H NMR (200 MHz):  $\delta$  0.80 and 0.89 (each 3H, *s*), 3.10 and 3.42 (each 1H, *d*,  $J$  = 11 Hz, H-18), 3.95 (1H, *s*, H-15), 5.11 and 5.19 (each 1H, *s*, H-17), 5.70 (1H, *dd*,  $J$  = 3 and 11 Hz, H-6), 5.71 (1H, *d*,  $J$  = 11 Hz, H-7); EIMS *m/z* (rel. int.): [M]<sup>+</sup> (6), 284 (6), 272 (10), 271 (22), 270 (5), 269 (24), 257 (5), 253 (12), 244 (5), 241 (3), 226 (7), 215 (15), 197 (15), 185 (17), 173 (19).

**ent**-3 $\beta$ ,18-Diacetoxy-7 $\alpha$ -mesyloxy-kaur-16-ene (**29**). The compound (**28**) [3] (1.1 g) was treated with mesyl chloride as above for the alcohol **23** to give the mesylate **29** (1.1 g), mp 136–138°; [M–HOMs]<sup>+</sup> at *m/z* 386.2496,  $\text{C}_{24}\text{H}_{34}\text{O}_4$  requires 386.2457; <sup>1</sup>H NMR (200 MHz):  $\delta$  0.82 and 1.09 (each 3H, *s*), 1.99 and 2.06 (each 3H, *s*), 2.95 (3H, *s*), 3.59 and 3.86 (each 1H, *d*,  $J$  = 11 Hz, H-18), 4.57 (1H, *br s*, H-7), 4.75 (1H, *m*, H-3), 4.79 and 4.82 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 386 [M–HOMs]<sup>+</sup> (2), 326 (26), 311 (2), 285 (1), 266 (38), 253 (18), 251 (24), 238 (5), 225 (10), 197 (9), 185 (24).

**ent**-3 $\beta$ ,18-Diacetoxy-15 $\beta$ -hydroxy-7 $\alpha$ -mesyloxy-kaur-16-ene (**30**). The mesylate **29** (1.1 g) was treated with  $\text{SeO}_2$  as above for the compound **24** to give, after chromatography eluting with petrol-EtOAc (20%), the diene **31** (110 mg) and the allylic alcohol **30** (420 mg): mp 152–154°, [M–HOMs]<sup>+</sup> *m/z* 402.2415,  $\text{C}_{24}\text{H}_{34}\text{O}_5$  requires 402.2409; <sup>1</sup>H NMR (200 MHz):  $\delta$  0.82 and 1.10 (each 3H, *s*), 1.99 and 2.07 (each 3H, *s*), 3.06 (3H, *s*), 3.62 and 3.83 (each 1H, *d*,  $J$  = 12 Hz, H-18), 4.05 (1H, *br s*, H-15), 4.80 (1H, *m*, H-3), 4.85 (1H, *br s*, H-7), 5.05 and 5.23 (each 1H, *s*, H-17). EIMS *m/z* (rel. int.): 402 [M–HOMs]<sup>+</sup> (1), 384 (2), 369 (1), 342 (9), 327 (5), 283 (5), 282 (18), 267 (39), 264 (11), 253 (4), 249 (14), 239 (8), 225 (8), 221 (12).

**ent**-3 $\beta$ ,18-Diacetoxy-15 $\beta$ -hydroxy-kaur-6,16-diene (**31**). The mesylate **30** (410 mg) in collidine (5 ml) was refluxed for 20 min. Usual work-up and chromatography of the residue eluting with petrol-EtOAc (20%) afforded the diene **31** (320 mg): mp 122–124°, [M–HOAc]<sup>+</sup> at *m/z* 342.2181,  $\text{C}_{22}\text{H}_{30}\text{O}_3$  requires 342.2194; <sup>1</sup>H NMR (200 MHz):  $\delta$  0.89 and 0.98 (each 3H, *s*), 2.02 and 2.04 (each 3H, *s*), 3.82 (2H, *s*, H-18), 3.98 (1H, *s*, H-15), 4.80 (1H, *dd*,  $J$  = 11 and 5 Hz, H-3), 5.14 and 5.26 (each 1H, *s*, H-17),

5.72 (1H, *dd*,  $J$  = 3 and 11 Hz, H-6), 5.71 (1H, *d*,  $J$  = 11 Hz, H-7); EIMS *m/z* (rel. int.): 402 [M]<sup>+</sup> (1), 384 (1), 369 (1), 342 (10), 327 (7), 282 (26), 267 (100), 249 (13), 241 (11), 223 (14), 209 (18), 200 (13), 195 (18).

**ent**-3 $\beta$ ,15 $\beta$ ,18-Trihydroxy-kaur-6,16-diene (**17**). The diacetate **31** was hydrolysed as above for the compound **26** to give the triol **17**: mp 208–210°, [M]<sup>+</sup> at *m/z* 318.2157,  $\text{C}_{20}\text{H}_{30}\text{O}_3$  requires 318.2195; <sup>1</sup>H NMR (200 MHz, pyridine-*d*<sub>5</sub>):  $\delta$  1.08 and 1.15 (each 3H, *s*), 3.77 and 4.18 (each 1H, *d*,  $J$  = 11 Hz, H-18), 4.24 (1H, *m*, H-3), 4.29 (1H, *br s*, H-15), 5.20 and 5.44 (each 1H, *s*, H-17), 6.00 (1H, *d*,  $J$  = 11 Hz, H-7), 6.21 (1H, *dd*,  $J$  = 3 and 11 Hz, H-6); EIMS *m/z* (rel. int.): 285 [M–H<sub>2</sub>O–Me]<sup>+</sup> (3), 269 (3), 267 (3), 255 (4), 241 (4), 239 (4), 227 (13), 225 (5), 213 (7), 209 (7), 199 (6), 197 (10), 195 (9), 185 (10), 183 (13).

**Triacetate (**32**):** mp 115–117°, <sup>1</sup>H NMR (200 MHz):  $\delta$  0.86 and 0.97 (each 3H, *s*), 2.02, 2.04 and 2.07 (each 3H, *s*), 3.75 and 3.85 (each 1H, *d*,  $J$  = 11 Hz, H-18), 4.81 (1H, *dd*,  $J$  = 11 and 5 Hz, H-3), 5.13 and 5.25 (each 1H, *s*, H-17), 5.28 (1H, *s*, H-15), 5.57 (2H, *br s*, H-6 and H-7); EIMS *m/z* (rel. int.): 384 [M–HOAc]<sup>+</sup> (4), 369 (9), 327 (2), 324 (30), 310 (100), 282 (6), 267 (10), 249 (29), 239 (6), 236 (5), 223 (13), 209 (10), 197 (14).

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## REFERENCES

1. Fraga, B. M., Hanson, J. R. and Hernandez, M. G. (1978) *Phytochemistry* **17**, 812.
2. Fraga, B. M., Hanson, J. R., Hernandez, M. G. and Sarah, F. Y. (1980) *Phytochemistry* **19**, 1087.
3. Fraga, B. M., Gonzalez, A. G., Hanson, J. R. and Hernandez, M. G. (1981) *Phytochemistry* **20**, 57.
4. Fraga, B. M., Gonzalez, A. G., Gonzalez, P., Hanson, J. R., Hernandez, M. G. and Hitchcock, P. B. (1982) *J. Chem. Soc., Chem. Commun.* 311.
5. Fraga, B. M., Gonzalez, A. G., Gonzalez, P., Hanson, J. R. and Hernandez, M. G. (1983) *Phytochemistry* **22**, 691.
6. Fraga, B. M., Gonzalez, P., Hernandez, M. G., Perales, A. and Tellado, F. G. (1986) *Phytochemistry* **25**, 1235.
7. Crozier, A. (1983) *The Biochemistry and Physiology of Gibberellins*. Vol. 1. Praeger, New York.
8. Dennis, D. T., Upper, C. D. and West, C. A. (1965) *Plant. Physiol.* **40**, 948.
9. Cross, B. E. and Myers, P. L. (1969) *Phytochemistry* **8**, 79.
10. Gaskin, P., Hutchinson, M., Lewis, N., MacMillan, J. and Phinney, B. O. (1984) *Phytochemistry* **23**, 559.
11. Martín-Panizo, F. and Rodriguez, R. (1979) *An. Quim.* **75**, 428.
12. Cross, B. E. and Erasmus, E. (1978) *J. Chem. Soc., Chem. Commun.* 1013.
13. Cross, B. E. and Erasmus, E. (1981) *J. Chem. Soc. Perkin Trans. I*, 1918.
14. Delgado, G., Alvarez, M. and Romo de Vivar, A. (1984)

*Phytochemistry* **23**, 2674.

- 15. Fraga, B. M., Hernández, M. G., Rodriguez, M. D., Díaz, C. E., González, P. and Hanson, J. R. (1987) *Phytochemistry* **26**, 1931.
- 16. Gonzalez, A. G., Fraga, B. M., Hernandez, M. G. and Luis, J. G. (1973) *Phytochemistry* **12**, 2721.
- 17. Umbreit, M. A. and Sharpless, K. B. (1977) *J. Am. Chem. Soc.* **99**, 5526.
- 18. Dolan, S. C. and MacMillan, J. (1985) *J. Chem. Soc., Perkin Trans. I* 2741.
- 19. Hanson, J. R., Hawker, J. and White, A. F. (1973) *J. Chem. Soc., Perkin Trans. I* 1892.