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TRANSFORMATION OF *ENT*-KAUR-15-ENES BY *GIBBERELLA FUJIKUROI*

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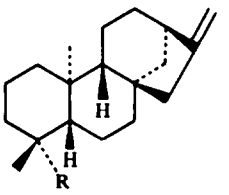
Key Word Index—*Gibberella fujikuroi*; microbiological transformation; *ent*-kaur-15-enes; gibberell-15-enes; diterpenoids.

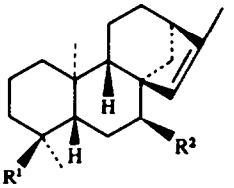
Abstract—The microbiological transformation of *ent*-kaur-15-ene and of some derivatives by *Gibberella fujikuroi* into the gibberell-15-ene analogues of GA_3, GA_7, GA_{13} and GA_{16} and into 7,18-dihydroxykaur-15-enolide and the Δ^{15}-isomer of fujenal is described.

INTRODUCTION

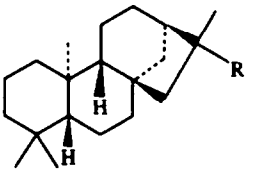
ent-Kaur-16-ene (**1**) is a key precursor of the gibberellin plant growth hormones [1]. The dwarf d_5 mutant of maize is blocked for gibberellin biosynthesis [2]. Instead of the normal gibberellin precursor *ent*-kaur-16-ene, it produces *ent*-kaur-15-ene (**5**). It has been suggested [3] that the gibberellin biosynthetic system in the dwarf maize mutant is unable to handle the double bond isomer, *ent*-kaur-15-ene. In this connection it is worth noting that whereas hydroxylated *ent*-kaur-15-enes are found quite commonly (e.g. in *Sideritis* species [4]), the corresponding gibberell-15-enes have not been isolated. Useful information concerning the structural requirements for specific biosynthetic steps in the gibberellin pathway has accrued from feeding false substrates to *Gibberella fujikuroi* [5]. It was therefore of interest to incubate *ent*-kaur-15-ene and some relatives with the fungus to see if the inability of the gibberellin pathway to handle *ent*-kaur-15-ene was a more general phenomenon. Conflicting results have been obtained in the past. It has been reported [6] that *ent*-kaur-15-ene was not metabolized to gibberellin analogues by strain GF1a but subsequently a series of gibberellin analogues were obtained [7] by the transformation of *ent*-17-hydroxykaur-15-ene.



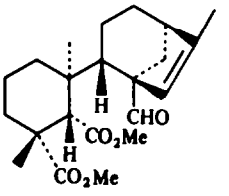
1 R = Me
2 R = CO_2H
3 R = CH_2OH
4 R = CHO



5 R^1 = Me, R^2 = H
6 R^1 = CH_2OH, R^2 = OAc
7 R^1 = CH_2Cl, R^2 = OAc
8 R^1 = Me, R^2 = OAc
9 R^1 = Me, R^2 = OH
10 R^1 = CH_2OH, R^2 = H



11 R = H
12 R = OH



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RESULTS

Preparation of substrates

ent-Kaur-15-ene (**5**) was prepared from the more readily available *ent*-kaur-16-en-19-oic acid (**2**) [8]. Reduction of the methyl ester with LAH afforded *ent*-19-hydroxykaur-16-ene (**3**) which on oxidation with PDC gave the 19-aldehyde (**4**). Wolff–Kischner reduction (Huang–Minlon conditions) brought about some double bond isomerization but the yield of *ent*-kaur-15-ene (**5**) was increased by further treatment with toluene-*p*-sulphonic acid. The hydrocarbons were then separated by chromatography on silver nitrate–silica gel.

ent-7α-Hydroxykaur-15-ene (**9**) was obtained from siderol (*ent*-7α-acetoxy-18-hydroxykaur-15-ene, **6**), by chlorination with triphenylphosphine–CCl_4 to afford **7** and subsequent reduction with tri-*n*-butyltin hydride. Hydrolysis of the resultant *ent*-7α-acetoxykaur-15-ene (**8**) afforded *ent*-7α-hydroxykaur-15-ene (**9**). *ent*-18-Hydroxykaur-15-ene (**10**) [9] was obtained by hydrolysis of isocandol B acetate.

Feeding experiments

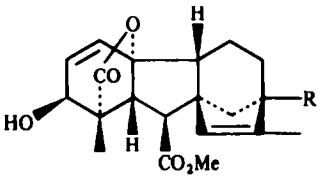
The fermentations were carried out in the presence of AMO-1618 to inhibit the formation of *ent*-kaur-16-ene [10] and consequently its endogenous metabolites. The transformations were carried out for a period of 6 days. The metabolites were then isolated and the acids were purified as their methyl esters. *ent*-Kaur-15-ene (**5**) gave a 15-isofujenal derivative (isolated as its dimethyl ester, **13**), 15-isogibberellin A_3 (**14**), 15-isogibberellin A_{16} (**16**) and 7β,18-dihydroxykaur-15-enolide (**18**). *ent*-7α-Hydroxykaur-15-ene (**9**) gave 15-isogibberellin A_7 (**15**) and 15-isogibberellin A_{13} (**16**) and *ent*-7α-hydroxy-15β,16β-epoxykaurane (**19**). *ent*-18-Hydroxykaur-15-ene (**10**) gave *ent*-7α,18-dihydroxykaur-15-en-19-oic acid (**20**) and 7β,18-dihydroxykaur-15-enolide (**18**).

The metabolites were identified by comparison of their ^{1}H NMR spectra with the spectra of their Δ^{16}-isomers. Thus 15-isogibberellin A_3 methyl ester (**14**) showed the typical H-5:H-6 doublets (δ3.15 and 2.69, J = 10 Hz) and signals characteristic of ring A (δ6.26, *d*, J = 9 Hz, H-1; 5.90, *dd*, J = 4 and 9 Hz, H-2; 4.16, *d*, J = 4 Hz, H-3). The H-15 resonance appeared as a broad singlet at 5.36 and the H-17 resonance as a doublet (1.68, J = 1.5 Hz). The corresponding carboxylic acid has been described previously [12]. 15-Isogibberellin A_7 methyl ester (**15**) showed a similar pattern of signals. 15-Isogibberellin A_{16} methyl ester (**16**) contained the signal assigned to H-1 as a quartet (J = 6 and 10 Hz) at δ4.00 (*cf.* A_{16} and A_{57}, 4.00, J = 6 and 10 Hz [13, 14]). The multiplicity of this signal and the absence of a downfield shift in the H-5 signal compared to 15-isoGA$_7$ (**15**) are in accord with the location of a hydroxyl group at C-1α. Furthermore, the ^{13}C NMR spectrum (see Table 1) closely paralleled that of gibberellin A_{16}. 15-Isogibberellin A_{13} trimethyl ester (**17**)

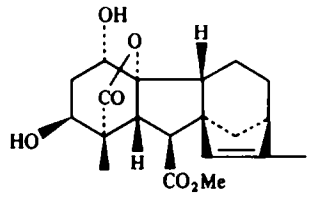
Table 1. ^{13}C NMR data for isogibberellin A_{16} and gibberellin A_{16} methyl esters* (in $CDCl_3$ at 50.32 MHz)

Carbon	IsoGA$_{16}$	GA$_{16}$	Carbon	IsoGA$_{16}$	GA$_{16}$
1	71.5	71.8	11	19.4	18.7
2	38.9	39.7	12	20.8	32.3
3	70.4	70.4	13	41.0	39.1
4	54.1	54.8	14	41.1	37.0
5	50.2	51.1	15	131.8	45.2
6	52.1	53.0	16	150.2	158.2
7	173.4	174.9	17	15.4	107.4
8	56.3	52.1	18	14.0	14.9
9	48.0	54.0	19	178.4	179.3
10	95.4	96.2			

*From ref. [11].



14 R = OH
15 R = H



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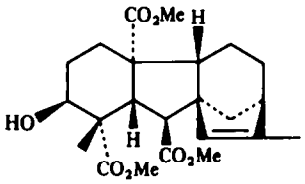

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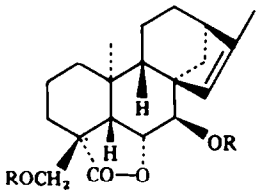
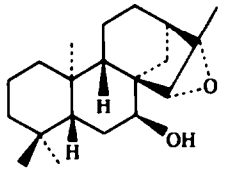


18 R = H
22 R = Ac



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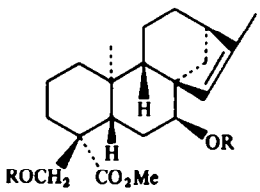

20 R = H
21 R = Ac

revealed the characteristic H-3 (δ3.70), H-5 and H-6 (2.46 and 3.53, J = 11 Hz) resonances, three methoxyl signals at 3.57, 3.60 and 3.65 together with the H-15 and H-17 signals at 5.42 and 1.60. 15-Isofujenal dimethyl ester (**13**), methyl *ent*-7α,18-dihydroxykaur-15-en-19-oate (**20**) [15] and 7β,18-dihydroxykaur-15-enolide (**18**) were identical to authentic samples. The latter were characterized as their diacetates. The epoxide, *ent*-7α-hydroxy-15β,16β-epoxykaurane (**19**) was assigned the ring D stereochemistry as shown since it was identical to material obtained by epoxidation of *ent*-7α-hydroxykaur-15-ene (**9**) with *m*-chloroperbenzoic acid. Furthermore the H-15 and H-17 proton resonances are very close in position to those of the corresponding epoxide from siderol [16].

DISCUSSION

These results, taken in conjunction with earlier experiments [7, 15] show that the gibberellin biosynthetic pathway in *Gibberella fujikuroi* is capable of accepting the 15-enes and metabolizing them, albeit less efficiently to the 15-ene isomers of the natural fungal metabolites. However, the relative proportions of 15-isogibberellin A_3 (**14**), A_7 (**15**) and A_{16} (**16**) differ from the normal series. In particular, gibberellin A_{16} is usually a minor metabolite. It is possible that 13-hydroxylation may be less efficient in the 15-ene series. The difference between the 7- and 18-hydroxykaur-15-enes in which only the latter forms a kaurenolide, parallels the difference which has been observed [15] in the 16-enes and is a reflection of the intervention of a 6-ene in kaurenolide formation [17]. Finally, the formation of a 15,16-epoxide may be a modification of the hydration at C-16 which is a probable dumping mechanism on this pathway. Alternatively, the 15-ene may be a substrate for the enzyme system which epoxidizes the 6-ene.

EXPERIMENTAL

Preparation of ent-*kaur*-15-*ene* (**5**). The methyl ester of *ent*-kaur-16-en-19-oic acid (**2**) (1.4 g) in dry THF (10 ml) was added dropwise to a suspension of LAH (450 mg) in THF (10 ml). After 2 hr reflux, EtOAc was added and the mixture was washed with dil. HCl and the product recovered in EtOAc. It was chromatographed on silica gel in petrol–EtOAc (95:5) to give *ent*-kaur-16-en-19-ol (**3**) (1.05 g); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.95 and 0.99 (each 3H, *s*), 3.42 and 3.73 (each 1H, *d*, J = 11 Hz; H-19), 4.72 and 4.78 (each 1H, *br s*, H-17). EIMS (rel. int.): 288 [M]$^+$ (22), 273 (27), 257 (100), 255 (11), 245 (14), 229 (15), 187 (15). The alcohol (**3**, 600 mg) in CH_2Cl_2 (16 ml) was treated with pyridinium dichromate (760 mg) at room temp. for 3 hr. It was then diluted with Et_2O and percolated through silica gel to afford *ent*-kaur-16-en-19-al (**4**, 530 mg); ^{1}H NMR: δ0.89 and 1.00 (3H, *s*) 4.80 (2H, *br s*, H-17), 9.73 (1H, *s*, H-19), EIMS (rel. int.): 286 (31), 271 (15), 257 (40), 243 (78), 225 (28), 199 (29), 187 (40). Hydrazine hydrate (3 ml) was added to a soln of the aldehyde (**4**, 510 mg) in diethyleneglycol (10 ml) and the mixture was then heated under reflux for 2 hr. KOH pellets (500 mg) were added and the mixture was then heated under reflux for a further 45 min. The condenser was removed and the temp. was allowed to reach 200° over 2 hr. The soln was cooled and poured into H_2O. The products were recovered in EtOAc to afford a three-component mixture of *ent*-kaurane (**11**), *ent*-kaur-16-ene (**1**) and *ent*-kaur-15-ene (**5**) as revealed by TLC. The mixture was heated with toluene-*p*-sulphonic acid (10 mg) in C_6H_6 (5 ml) under reflux for 2 hr and then chromatographed on 10% $AgNO_3$–silica gel in petrol to give *ent*-kaurane (**11**) (35 mg); ^{1}H NMR (90 MHz $CDCl_3$): δ0.80–1.02 (12H) EIMS (rel. int.): 274 [M]$^+$ (52), 259 (77), 245 (11), 231 (21), 189 (21), 177 (12). Further elution gave *ent*-kaur-16-ene (**1**, 40 mg); ^{1}H NMR (200 MHz): δ0.81, 0.85 and 1.02 (each 3H, *s*), 4.73 and 4.78 (each 1H, *br s*); EIMS (rel. int.): 272 [M]$^+$ (27), 257 (68), 229 (41), 213 (22), 201 (11), 187 (20). Further elution afforded *ent*-kaur-15-ene (**5**, 150 mg); ^{1}H NMR (80 MHz, $CDCl_3$): δ0.81, 0.85 and 1.03 (each 3H, *s*), 1.69 (3H, *d*, J = 1.5 Hz, H-17), 5.06 (1H, *br s*, H-15); EIMS (rel. int.): 272 [M]$^+$ (35), 257 (32), 244 (10), 229 (16), 213 (7), 201 (7), 187 (17). Further elution afforded *ent*-kauranol (**12**, 80 mg); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.79, 0.83 and 1.01 (each 3H, *s*), 1.35 (3H, *s*, H-17). EIMS (rel. int.): 290 [M]$^+$ (11), 275 (18), 272 (44), 257 (55), 232 (62), 229 (17), 217 (28), 201 (7), 187 (13).

ent-18-*Chloro*-7α-*acetoxykaur*-15-*ene* (**7**). Siderol (**6**), obtained from *Sideritis spinulosa*, in dry pyridine (18 ml) and CCl_4 (20 ml) was heated with triphenylphosphine (750 mg) under reflux for 4 hr. The products were recovered with EtOAc and chromatographed on silica gel in petrol–EtOAc (90:10) to afford *ent*-18-chloro-7α-acetoxykaur-15-ene, mp 109–111°. (Found: [M]$^+$ 364.215; $C_{22}H_{33}O_2Cl$ requires 364.217.) ^{1}H NMR (90 MHz, $CDCl_3$): δ0.84 and 1.08 (each 3H, *s*), 1.69, (3H, *d*, J = 2 Hz, H-17), 2.06 (3H, *s*, OAc), 3.05 and 3.41 (each 1H, *d*, J = 11 Hz, H-18) 4.69 (1H, *br s* H-7), 5.30 (1H, *br s*, H-15). EIMS (rel. int.): 364 [M]$^+$ (2), 322 (11), 304 (38), 289 (10), 276 (4), 255 (11), 199 (3), 185 (5).

Preparation of ent-7α-*acetoxykaur*-15-*ene* (**8**). The above chloro compound (**7**, 400 mg) in toluene (10 ml) was treated with tri-*n*-butyltin hydride (0.3 ml) and azobisisobutyronitrile (5 mg) under reflux for 16 hr. The solvent was evapd and the residue dissolved in Et_2O and stirred with H_2O satd with KF for 15 min. The soln was filtered, dried and the solvent evapd to give a gum which was chromatographed on silica gel. Elution with petrol–EtOAc (90:10) gave *ent*-7α-acetoxykaur-15-ene (350 mg), mp 103–105°. (Found: [M]$^+$ 330.257; $C_{22}H_{34}O_2$ requires 330.256.) ^{1}H NMR (90 MHz): δ0.79 (6H, *s*), 1.05 (3H, *s*), 1.69 (3H, *d*, J = 2 Hz, H-17), 4.75 (1H, *t*, J = 6 Hz, H-7), 5.27 (1H, *br s*, H-15). EIMS (rel. int.): 330 (3), 288 (20), 270 (93), 255 (52), 242 (13), 227 (22).

Preparation of ent-7α-*hydroxykaur*-15-*ene* (**9**). The above acetoxy derivative (**8**, 340 mg) was treated with 5% methanolic KOH (20 ml) at room temp. for 24 hr. The soln was neutralized and the product recovered in EtOAc to afford *ent*-7α-hydroxykaur-15-ene (**9**, 250 mg), mp 134–136°. (Found: [M]$^+$ 288.246; $C_{20}H_{32}O$ requires 288.245.) ^{1}H NMR (80 MHz, $CDCl_3$): δ0.81, 0.85 and 1.04 (each 3H, *s*), 1.73 (3H, *d*, J = 1.5 Hz, H-17), 3.60 (1H, *br s*, H-7), 5.50 (1H, *br s*, H-15). EIMS (rel. int.): 288 [M]$^+$ (39), 273 (9), 270 (16), 255 (16), 245 (12), 227 (7), 199 (9), 164 (55), 149 (20).

Incubation experiments. Gibberella fujikuroi (ACC 917) inhibited with 5×10^{-5} M AMO 1618, was grown in shake culture at 25° for 1–2 days in 80–100 conical flasks (250 ml) each containing sterile medium (50 ml) [15]. The substrate (see below) in EtOH (16–20 ml) was distributed equally between the flasks and the fermentation was 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 $NaHCO_3$. The acidic fraction was methylated with CH_2N_2. The fractions were then chromatographed on silica gel in petrol–EtOAc.

ent-Kaur-15-ene (**5**, 150 mg) gave the methyl ester of isofujenal (**13**, 5 mg), isogibberellin A_3 (**14**, 10 mg) and isogibberellin A_{16} (**16**, 11 mg) from the acid fraction. 7β,18-Dihydroxykaur-15-enolide (**18**, 12 mg) was obtained from the neutral fraction.

ent-7α-Hydroxykaur-15-ene (**9**, 240 mg) gave the methyl esters of isogibberellin A_7 (**15**, 20 mg) and isogibberellin A_{13} (**17**, 6 mg) and from the neutral fraction, *ent*-7α-hydroxy-15β,16β-epoxykaurane (**19**, 15 mg).

ent-18-Hydroxykaur-15-ene (**10**, 260 mg) gave the methyl ester of *ent*-7α,18-dihydroxykaur-15-en-19-oic acid (**20**, 33 mg) and from the neutral fraction, 7β,18-dihydroxykaur-15-enolide (**18**, 19 mg).

Isogibberellin A_3 methyl ester (**14**). Mp 190–193°. (Found: $[M]^+$ 360.159. $C_{20}H_{24}O_6$ requires 360.157.) ^{1}H NMR (200 MHz, $CDCl_3$): δ1.25 (3H, *s*, H-18), 1.68 (3H, *d*, *J* = 1.5 Hz, H-17), 2.69 and 3.15 (each 1H, *d*, *J* = 10 Hz, H-5 and H-6), 3.71 (3H, *s*, OMe), 4.16 (1H, *d*, *J* = 4 Hz, H-3), 5.44 (1H, *d*, *J* = 1.5 Hz, H-15), 5.90 (1H, *dd*, *J* = 4 and 9 Hz, H-2), 6.26 (1H, *d*, *J* = 9 Hz, H-1), EIMS (rel. int.): 360 $[M]^+$ (8), 342 (2), 328 (14), 301 (13), 297 (8), 237 (22), 227 (13), 209 (27).

Isogibberellin A_7 methyl ester (**15**). Mp 160–162°. (Found: $[M]^+$ 344.161; $C_{20}H_{24}O_5$ requires 344.162.) ^{1}H NMR (200 MHz, $CDCl_3$): δ1.25 (3H, *s*, H-18), 1.68 (3H, *d*, *J* = 1.5 Hz, H-17), 2.67 and 3.13 (each 1H, *d*, *J* = 10 Hz, H-5 and H-6), 3.70 (3H, *s*, OMe), 4.14 (1H, *d*, *J* = 3 Hz, H-3) 5.36 (1H, *br s*, H-15), 5.90 (1H, *dd*, *J* = 3 and 9 Hz, H-2), 6.27 (1H, *d*, *J* = 9 Hz, H-1). EIMS (rel. int.): 344 $[M]^+$ (55), 326 (1), 313 (2), 312 (6), 300 (8), 298 (4), 284 (17), 282 (53), 267 (24), 253 (16), 239 (22), 222 (45), 214 (50).

Isogibberellin A_{13} methyl ester (**17**). Gum. (Found: $[M]^+$ 420.215; $C_{23}H_{32}O_7$ requires 420.215.) ^{1}H NMR (200 MHz, $CDCl_3$): δ1.09 (3H, *s*, H-18), 3.60 (3H, *d*, *J* = 1.5 Hz, H-17), 2.46 and 3.53 (each 1H, *d*, *J* = 11 Hz), 3.57, 3.60 and 3.65 (each 3H, *s*, OMe), 3.70 (1H, *m*, H-3), 5.42 (1H, *br s*, H-15). EIMS (rel. int.): 420 $[M]^+$ (4) 388 (1), 328 (8), 300 (5), 285 (7).

Isogibberellin A_{16} methyl ester (**16**). Gum. (Found: 362.175; $C_{20}H_{26}O_6$ requires 362.173.) ^{1}H NMR (200 MHz, $CDCl_3$): δ1.14 (3H, *s*, H-18), 1.68 (3H, *d*, *J* = 1.5 Hz, H-17), 2.62 and 3.15 (each 1H, *d*, *J* = 10 Hz, H-5 and H-6), 3.68 (3H, *s*, OMe), 3.83 (1H, *br s*, H-3), 4.00 (1H, *dd*, *J* = 6 and 10 Hz, H-1), 5.34 (1H, *br s*, H-15). EIMS (rel. int.): 362 $[M]^+$ (24), 344 (13), 300 (38), 288 (32), 256 (11), 241 (21).

Isofujenal dimethyl ester (**13**). Gum. (Found: $[M-H]^+$ 375.214; $C_{22}H_{33}O_5$ requires 375.217.) ^{1}H NMR (200 MHz, $CDCl_3$): δ1.04 and 1.29(each 3H, *s*), 1.71 (3H, *d*, *J* = 1.5 Hz, H-17), 2.63 (1H, *s*, H-5), (3.61 and 3.69 (each 3H, *s*, OMe), 5.23 (1H, *br s*, H-15), 9.80 (1H, *s*, H-7). EIMS (rel. int.): 376 $[M]^+$ (7), 375 (20), 344 (13), 316 (10), 312 (6), 284 (11), 227 (15).

ent-7α,18-*Dihydroxykaur*-15-*en*-19-*oic acid methyl ester* (**20**). ^{1}H NMR (90 MHz, pyridine-d_5): δ1.06 (3H, *s*, H-20), 1.74 (3H, *d*, *J* = 2 Hz, H-17), 3.70 (3H, *s*, OMe), 3.78 and 4.41 (each 1H, *d*, *J* = 11 Hz, H-18), 3.90 (1H, *br s*, H-7), 6.00 (*br s*, H-15). The diacetate, prepared with Ac_2O in pyridine was a gum. (Found: $[M]^+$ 432.251; $C_{25}H_{36}O_6$ requires 432.251.) ^{1}H NMR (90 MHz, $CDCl_3$): δ0.91 (3H, *s*, H-20), 1.73 (3H, *br s*, H-17) 2.05 and 2.12 (each 3H, *s*, OAc), 3.70 (3H, *s*, OMe), 3.88 and 4.30 (each 1H, *d*, *J* = 11 Hz, H-18), 4.84 (1H, *t*, H-7), 5.22 (1H, *br s*, H-15), EIMS (rel. int.): 432 $[M]^+$ (1), 390 (11), 372 (6), 357 (4), 312 (28), 253 (11).

ent-7α-*Hydroxy*-15β,16β-*epoxykaurane* (**19**). Mp 193–195°. (Found: $[M]^+$ 304.242; $C_{20}H_{32}O_2$ requires 304.240.) ^{1}H NMR (200 MHz, $CDCl_3$): δ0.78, 0.85 and 0.97 (each 3H, *s*), 1.42 (3H, *s*, H-17), 3.06 (1H, *br s*, H-15), 3.78 (1H, *t*, *J* = 2 Hz, H-7). EIMS (rel. int.): 304 $[M]^+$ (3), 289 (3), 286 (7), 271 (11), 260 (8), 243 (11), 201 (12), 189 (7), 173 (8), 149 (60). The epoxide was identical to material prepared by the epoxidation of isocandol A (45 mg) with *m*-chloroperbenzoic acid (20 mg) in $CHCl_3$ (3 ml) at room temp. for 10 hr.

ent-6β,7α,18-*Trihydroxykaur*-15-*en*-19-*oic acid* 19,6β-*lactone* (**18**). ^{1}H NMR (200 MHz): δ0.91 (3H, *s*, H-20), 1.73 (3H, *s*, H-17), 1.90 (1H, *d*, *J* = 7 Hz, H-5), 3.71 (2H, *br s*, H-18), 4.12 (1H, *d*, *J* = 7 Hz, H-7), 4.79 (1H, *t*, *J* = 7 Hz, H-6), 5.50 (1H, *br s*, H-15). EIMS (rel. int.): 332 $[M]^+$ (4), 302 (4), 271 (9), 241 (8), 213 (6). The diacetate, prepared with Ac_2O-pyridine was a gum. (Found: $[M]^+$ 416.225; $C_{24}H_{32}O_6$ requires 416.220.) ^{1}H NMR (90 MHz, $CDCl_3$): δ1.02 (3H, *s*, H-20), 1.71 (3H, *d*, *J* = 2 Hz, H-17), 2.07 and 2.10 (each 3H, *s*, OAc), 4.15 (2H, *br s*, H-18), 4.27 (1H, *t*, *J* = 7 Hz, H-6), 5.50 (1H, *br s*, H-15), 5.60 (1H, *d*, *J* = 7 Hz, H-7). EIMS (rel. int.): 416 $[M]^+$ (3), 374 (12), 356 (4), 341 (9), 314 (25), 299 (10).

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