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THE MICROBIOLOGICAL TRANSFORMATION OF SOME *ENT*-3β-HYDROXYKAUR-16-ENES BY *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; tetracyclic diterpenoids; linearol; foliol.

Abstract—The preparation of *ent*-3β-hydroxykaur-16-ene from linearol and of *ent*-3β,18-dihydroxykaur-16-ene from foliol is described. The microbiological transformation of these and of foliol by *Gibberella fujikuroi* has been studied. A 3α-hydroxyl group appears to exert an inhibitory effect on transformations involving oxidation at C-19.

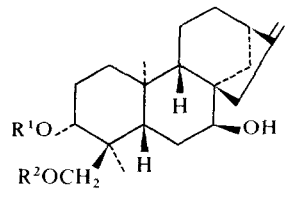
INTRODUCTION

The microbiological transformation of diterpenoids by the fungus, *Gibberella fujikuroi*, has been examined with the objective of preparing modified gibberellins and of defining the substrate requirements of various biosynthetic steps in the gibberellin pathway in *G. fujikuroi* [1–7]. In previous studies [4] with some *ent*-kaur-16-ene-7-,15- and 18-alcohols we showed that an 18-substituent apparently exerted an inhibitory effect on transformations by *G. fujikuroi* involving reaction at the C-6β position. In these molecules the 18-hydroxyl group could interfere with an active site on the enzyme surface responsible for reaction at C-6β. In this paper we report on the transformation of some *ent*-3β-hydroxylated kaurenes obtained from *Sideritis* species. In these compounds the hydroxyl group is adjacent to and on the same face of the molecule as C-19, the oxidation of which forms an important sequence in gibberellin biosynthesis. MacMillan [6] has shown that the *ent*-3α-hydroxylated analogues of the normal gibberellin intermediates, *ent*-kaur-16-en-19-ol and *ent*-kaur-16-en-19-oic acid, were efficiently converted into 3-hydroxylated gibberellins.

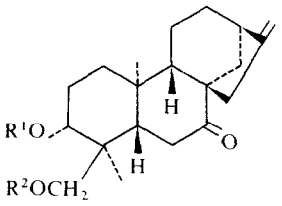
RESULTS AND DISCUSSION

The substrates were obtained in the following manner from the diterpenoids of *Sideritis* species. *Ent*-3β-hydroxykaur-16-ene (**9**) [9, 10] was prepared from linearol (**1**) [11, 12]. This was acetylated to form the *ent*-3β,18-diacetate (**2**). The diacetate was oxidized to the 7-ketone (**3**) which was then hydrolysed to afford the *ent*-3β,18-dihydroxy-7-ketone (**4**). Careful partial acetylation of the diol (CH_2OH δ 3.28 and 3.60) gave, *inter alia*, the 18-monoacetate (**5**) (CH_2OAc δ 3.62 and 4.24). This was separated from the accompanying diacetate and a small amount of the 3-mono-acetate (**6**). Under mild acid-catalysis, the acetoxyl group underwent migration to C-3 to afford the 3-monoacetate (**6**) (CH.OH δ 3.30, CH.OAc δ 4.90). The free 18-hydroxyl group was then oxidized to the 18-aldehyde (**7**) and the product immediately subjected to a Wolff–Kishner reduction. The resultant mixture of 15- and 16-enes was purified by acetylation and chromatography. The isomerization of the Δ^{16} double bond during the Wolff–Kishner reduction has been noted previously [13]. The reduction also gave a small amount of the *ent*-3β-acetoxykaurane, possibly through the generation of some di-imide in the reduction. Hydrolysis of the *ent*-3β-acetoxykaur-16-ene (**8**) gave *ent*-3β-hydroxykaur-16-ene (**9**) [9,10]. *Ent*-3β,18-dihydroxy-kaur-16-ene (**14**) was prepared from foliol (**10**) [14]. The 3- and 18-hydroxyl groups were protected as the acetonide (**11**) which was prepared with acetone and copper sulphate. The free 7-hydroxyl group was oxidized to the 7-ketone (**12**). Wolff–Kishner reduction of the keto-acetonide (**12**) afforded a separable mixture of the *ent*-3β,18-acetonide (**13**) and its Δ^{15} double bond isomer. Hydrolysis of the protecting group gave the parent diols **14** and **15**.

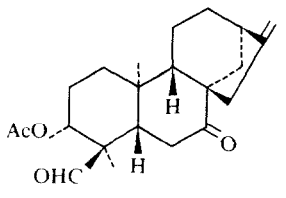
The normal diterpenoid metabolites of *G. fujikuroi* are not produced when it is grown in the presence of AMO-1618 since the latter inhibits the formation of *ent*-kaur-16-ene [15, 16]. Nevertheless the subsequent metabolism of *ent*-kaur-16-ene is not perturbed. This affords a convenient means of studying the metabolism of abnormal substrates since novel metabolites are then easily detected. Surprisingly, incubation of *ent*-3β-hydroxykaur-16-ene (**9**) on four occasions both in the presence and in the absence of the inhibitor, afforded no detectable (TLC) metabolites when compared to control fermentations. The substrate was recovered unchanged. On the other hand *ent*-3β,18-dihydroxy-kaur-16-ene (**14**) gave one major metabolite which was identified (IR and ^{1}H NMR) as *ent*-3β,7α,18-trihydroxykaur-16-ene (**10**) (foliol) [14]. When foliol (**10**) was then incubated with *G. fujikuroi*, it was metabolized to *ent*-kauran-3β,7α,16β,18-tetraol (**16**) in which hydration of the 16,17-methylene has occurred. The structure of the metabolite was proven by epoxidation of foliol triacetate with *m*-chloroperbenzoic acid and reduction of the epoxide (**17**) with lithium aluminium hydride. The epoxidation of foliol triacetate gave some additional compounds. One was identified as *ent*-15,16β-epoxy-3β,7α,18-triacetoxykauran-17-ol (**18**) by its ^{1}H NMR spectrum which contained a singlet



1 $R^1 = H$, $R^2 = Ac$
2 $R^1 = R^2 = Ac$
10 $R^1 = R^2 = H$



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



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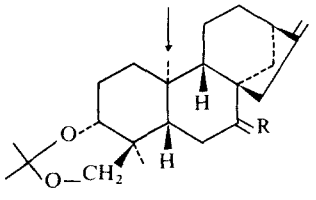
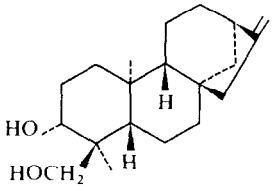
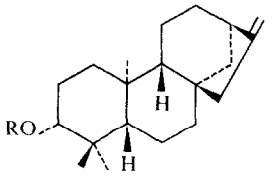


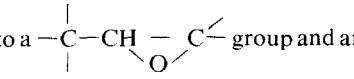
11 R = α-H, β-OH
12 R = O
13 $R = H_2$

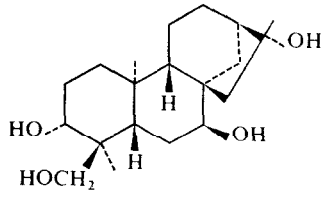


14
15 Δ^{15} isomer



8 R = Ac
9 R = H

(δ 3.21) assigned to a  group and an AB quartet (3.78 and 4.08, $J = 13$ Hz) assigned to a primary alcohol. A mixture of two further compounds was separated by acetylation and further chromatography. This afforded the tetra-acetate (**19**) and a compound which was tentatively assigned the beyerane structure (**20**). The former contained ^{1}H NMR resonances at δ 3.52 and 3.92 (each 1H, doublets, $J = 11$ Hz) and 4.63 (2H) assigned to two primary acetoxy-methyl groups and a single olefinic proton resonance (5.61). The ^{1}H NMR spectrum of the latter contained two primary acetoxy-methyl resonances (δ 3.51 and 3.97, each 1H, doublet, $J = 11$ Hz) and (4.17 and 4.40, each 1H doublet, $J = 12$ Hz) together with a disecondary epoxide (3.25 and 3.42, each 1H doublet, $J = 8$ Hz). The formation of these by-products may be interpreted in terms of the ring-opening of foliol epoxide and varying modes of discharge of the C-16 carbocation so formed.



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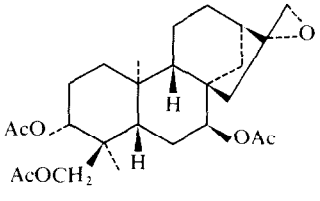

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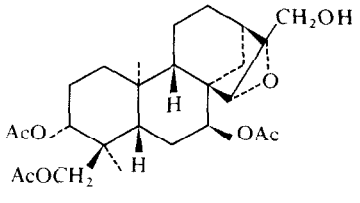

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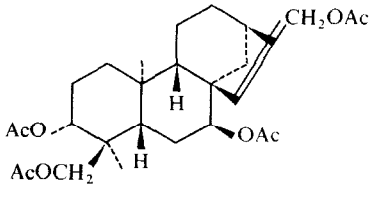

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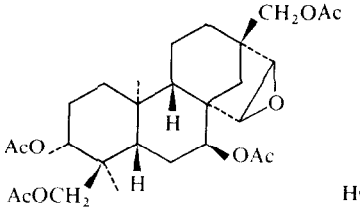

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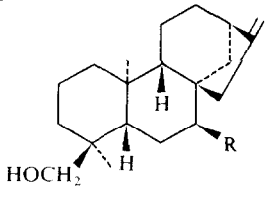

21 R = H
22 R = OH

In our previous work we showed that *ent*-18-hydroxykaur=16-ene (candol B) (**21**) [4] and *ent*-7α,18-dihydroxykaur-16-ene (epicandicandiol) (**22**) [2] gave products of oxidation at C-19 although attack at C-6β was apparently inhibited. Examination of the corresponding 3α-hydroxylated analogues shows that oxidation at C-19 and C-6α was also inhibited. In this context it should be noted that many of the successful bio-transformations with *G. fujikuroi* have utilized substrates in which C-19 is already oxidized. The 3α-hydroxyl group lies on the same face of the molecule as C-19 and it could thus interact with the enzyme system responsible for hydroxylation at this centre. The inertness of *ent*-3β-hydroxykaur-16-ene (**9**) was surprising. Since the normal order of biosynthetic events is oxidation of C-19 prior to hydroxylation at C-7, this suggests that an oxygen function at C-18 (an alcohol) or C-19 (a carboxyl) is a pre-requisite for the hydroxylation at C-7 in *G. fujikuroi*. It may be that there is a need for a polar group at C-4 to orient the substrate for hydroxylation. Hydration of the 16,17-methylene, as observed with foliol (**10**), is a common 'dumping' mechanism in *G. fujikuroi*. Such compounds (e.g. kauran-16-ol) are metabolically relatively inert (J. R. Hanson and L. F. Ball, unpublished results)

EXPERIMENTAL

General experimental details have been described previously [17].

Ent-3β,18-*diacetoxy*-7α-*hydroxykaur*-16-*ene* (**2**). Linearol (**1**) (3.5 g) in pyridine (48 ml) and Ac_2O (15 ml) was maintained at 0° for 3 hr. Recovery in the usual way gave the diacetate (**2**) (3.0 g), mp 210–213°. (Found C, 71.5; H, 9.2. $C_{24}H_{36}O_5$ requires C. 71.3; H, 9.0%). IR ν_{max} cm^{-1}: 3490, 3070, 1736, 1713, 1670, 1250: ^1H NMR ($CDCl_3$): δ 0.80 and 1.08 (each 3H, *s*, 2 C-Me), 2.00 and 2.02 (each 3H, *s*, 2 OAc), 3.56 (1H, *t*, CH.OH), 3.52 and 4.02 (each 1H, *d*, J = 11 Hz, CH_2-O), 4.80 (3H, *br. s*, $=CH_2$ and CHOAc): MS *m/e*: 404, 386, 344, 326, 284, 266. The starting material (0.8 g) was also recovered.

Ent-3β,18-*diacetoxy*-7-*oxo-kaur*-16-*ene* (**3**). The above diacetate (3.0 g) in Me_2CO was treated with a slight excess of the 8N CrO_3 reagent at room temp. for 5 min. MeOH was then added, the mixture was poured into H_2O and the product recovered in EtOAc to afford the ketone (**3**) (2.7 g) mp 119–120°. (Found: C, 71.45; H, 8.65. $C_{24}H_{34}O_5$ requires C, 71.6; H, 8.5%), IR ν_{max} cm^{-1}: 3070, 1740, 1700, 1670, 1240, 1220, 870; ^1H NMR ($CDCl_3$): δ 0.90 and 1.20 (each 3H, *s*, 2 C-Me), 2.02 (6H, *s*, OAc), 3.60 and 3.80 (each 1H, *d*, J = 11 Hz, CH_2-O) 4.88 (3H, *br. s*, $=CH_2$ and CHOAc); MS *m/e*: 402, 342, 300 and 282.

Ent-3β,18-*dihydroxy*-7-*oxo-kaur*-16-*ene* (**4**). The diacetate (**3**) (2.7 g) was treated with 5% MeOH: KOH (100 ml) at room temp. overnight. The soln was diluted with H_2O, acidified and the product recovered in EtOAc to afford the diol (**4**) (2.5 g), mp 157–158°, ^1H NMR ($CDCl_3$): δ 0.80 and 1.17 (each 3H, *s*, 2 C-Me), 3.28 and 3.60 (each 1H, *d*, J = 11 Hz, $-CH_2OH$) 4.85 (2H, *br. s*, $=CH_2$), MS *m/e*: 318, 300, 270 and 241.

Partial acetylation of the diol (**4**). Cold Ac_2O (40 ml) was added to the diol (4) (2.5 g) in pyridine (80 ml) at 0° and the mixture left at this temperature for 2 hr. The product was recovered in EtOAc and subjected to dry column chromatography on Si gel. Elution with EtOAc–petrol (30:70) gave the diacetate (**3**) (0.55 g), *ent*-18-acetoxy-3β-hydroxy-7-oxokaur-16-ene (**5**) (1.9 g) and *ent*-3β-acetoxy-18-hydroxy-7-oxo-kaur-16-ene (**6**) (0.1 g). The monoacetate (**5**) had mp 104–106°, ^1H NMR ($CDCl_3$): δ 0.81 and 1.18 (each 3H, *s*, 2 C-Me), 2.05 (3H, *s*, OAc), 3.30 (1H, *t*, CHOH), 3.62 and 4.24 (each 1H, *d*, J = 11 Hz, CH_2O), 4.88 (2H, *br. s*, $=CH_2$); MS *m/e*: 360, 342, 300, 282.

Ent-3β-*Acetoxy*-18-*hydroxy*-7-*oxo-kaur*-16-*ene* (**6**). The 18-acetate (**5**) (1.9 g) in $CHCl_3$ (150 ml) was treated with two drops of HCl and left at room temp. for 2 days. Dry column chromatography (Si gel, EtOAc–petrol, 15:85) gave the 18-acetate (**5**) (0.8 g) and the *ent*-3β-acetate (**6**) (1.1 g) mp 140–141°. (Found: C, 74.1; H, 9.44. $C_{22}H_{32}O_4$ requires C, 73.3; H, 8.95%). ^1H NMR ($CDCl_3$): δ 0.73 and 1.13 (each 3H, *s*, C-Me), 2.08 (3H, *s*, OAc), 2.60 and 3.25 (2H, *m*, CH_2OH) 4.90 (3H, *br. s*, $=CH_2$ and CHOAc); MS *m/e*: 360, 300 and 270.

Ent-3β-*acetoxy*-7,18-*dioxo-kaur*-16-*ene* (**7**). Pyridinium dichromate (1.7 g) was added to a soln of **6** (1.0 g) in CH_2Cl_2 (70 ml). The mixture was shaken at room temp. for 37 hr, diluted with Et_2O and percolated through Si gel to afford **7** (0.9 g), ^1H NMR ($CDCl_3$): δ 1.12 and 1.24 (each 3H, *s*, 2 C-Me), 1.98 (3H, *s*, OAc), 4.90 (3H, *br. s*, $=CH_2$ and CHOAc), 9.28 (1H, *s*, CHO). The product was used immediately.

Reduction of the keto-aldehyde (**7**). Hydrazine hydrate (9 ml) was added to a soln of the keto-aldehyde (**7**) (850 mg) in diethyleneglycol (50 ml) and the mixture was then heated under reflux for 2 hr. KOH pellets (1.2 g) were added and the mixture was heated under reflux for a further 45 min. The condenser was removed and the temperature was allowed to reach 200° for 2 hr. The product was recovered in EtOAc to afford a three component mixture (TLC 20% $AgNO_3$–Si gel). The mixture was acetylated (Ac_2O–pyridine and chromatographed on Si gel–20% $AgNO_3$. Elution with EtOAc–petrol (3:97) gave *ent*-3β-acetoxykaurane (40 mg) as a gum, ^1H NMR ($CDCl_3$): δ 0.82 (6H, *s*, 2 C-Me), 0.90 (3H, *s*, CH-Me), 1.03 (3H, *s*, C-Me), 2.02 (3H, *s*, OAc), 4.48 (1H, *t*, CHOAc). Further elution gave *ent*-3β-acetoxykaur-16-ene (**8**), mp 162–164° (290 mg), ^1H NMR ($CDCl_3$): δ 0.85 (6H, *s*, 2 C-Me), 1.05 (3H, *s*, C-Me), 2.04 (3H, *s*, OAc), 4.45 (1H, *t*, CHOAc), 4.78 (2H, *br. s*, $=CH_2$); MS *m/e*: 330, 287, 270, 255, 227. Further elution gave *ent*-3β-acetoxykaur-15-ene (295 mg), mp 123–125°. (Found C, 80.2; H, 10.3. $C_{22}H_{34}O_2$ requires C, 80.0; H, 10.3%). ^1H NMR ($CDCl_3$): δ 0.84 (6H, *s*, 2 C-Me), 1.06 (3H, *s*, C-Me) 1.69 (3H, *d*, J = 2 Hz, $=C-Me$), 2.03 (3H, *s*, OAc), 4.35 (1H, *t*, CHOAc) 5.08 (1H, *s*, CH=); MS *m/e*: 330, 287, 270, 255, 227.

Ent-3β-*hydroxykaur*-16-*ene* (**9**). The acetate (**8**) (290 mg) was treated with 5% methanolic KOH (20 ml) at room temp overnight. Acidification and recovery in EtOAc gave the alcohol (**9**) (240 mg), mp 176–177° (lit. [8] 172°); ^1H NMR ($CDCl_3$); δ 0.78. 0.97 and 1.02 (each 3H, *s*, C-Me), 3.18 (1H, *t*, CHOH), 4.78 (2H, *br. s*, $=CH_2$); MS *m/e*: 288, 273, 255.

Foliol acetonide (**11**). Foliol (**10**) (2.0 g) in dry Me_2CO (170 ml) and $CuSO_4$ (14 mg) was heated under reflux for 5 hr. The reaction product was filtered and the ppt. washed with Me_2CO. The solvent was evapd to afford the acetonide (**11**) (2.1 g), mp 264–266°. (Found: C, 75.2; H, 10.1. $C_{23}H_{36}O_3$ requires C, 76.6; H, 10.1%). IR ν_{max} cm^{-1}: 3460, 3075, 1660, 1390, 1370, 1070, 870; ^1H NMR ($CDCl_3$): δ 1.05 and 1.09 (each 3H, *s*, 2 C-Me), 1.43 (6H, *s*, 2 C-Me), 3.53 (4H, complex signal, CH_2O and 2CH-O), 4.70 (2H, *br. s*, $=CH_2$).

Oxidation of the hydroxy-acetonide (**11**). The acetonide (**11**) (1.6 g) diss. in hot DMF (100 ml) was cooled to 40° and treated with pyridinium dichromate (4 g) for 1 hr. The soln was dil. with H_2O and the product recovered in EtOAc to afford the keto-acetonide (**12**) (1.2 g), mp 166–168°. (Found: C, 76.7; H, 9.6. $C_{23}H_{34}O_3$ requires C, 77.05; H, 9.6%). IR ν_{max} cm^{-1}: 3080, 1700, 1660, 1390, 1370, 870; ^1H NMR ($CDCl_3$): δ 1.10 and 1.21 (each 3H, *s*, 2 C-Me), 1.42 (6H, *s*, 2 C-Me), 3.45 (2H, *s*, $-CH_2-O$), 3.54 (1H, *t*, CH−O), 4.86 (2H, $=CH_2$).

Reduction of the keto-acetonide (**12**). Hydrazine hydrate (11 ml) was added to a soln of the keto-acetonide (**12**) (1.2 g) in

diethyleneglycol (70 ml) and the mixture heated to 140° for 2 hr. KOH pellets (1 g) were added and heating was continued for 45 min. The condenser was removed and the temp was allowed to reach 200° for 2 hr. The soln was poured into water and the products recovered in EtOAc. The mixture was chromatographed on a dry column of Si gel impregnated with 20% $AgNO_3$. Elution with EtOAc–petrol (5:95) gave the acetonides of the diol (**14**) (410 mg) and (**15**) (430 mg). The acetonide of (**14**) had mp 190–192°: ^{1}H NMR ($CDCl_3$): δ 1.04 and 1.07 (each 3H, *s*, 2 C-Me), 1.42 (6H, *s*, 2 C-Me), 3.42 and 3.56 (each 1H, *d*, J = 11 Hz, $-CH_2O$) and 4.98 (2H, *br. s*, $=CH_2$); MS *m/e*: 329 (M-15), 286 and 269. The acetonide of **15** had mp 192–194° (lit., [9] 187–188°); ^{1}H NMR ($CDCl_3$): δ 1.03 and 1.08 (each 3H, *s*, 2 C-Me), 1.42 (6H, *s*, 2 C-Me), 1.68 (3H, *d*, J = 2.5 Hz, $=C-Me$), 3.41 and 3.54 (each 1H, *d*, J = 11 Hz, $-CH_2O$), 5.06 (1H, $-CH$); MS *m/e*: 344, 329 and 269.

Hydrolysis of the acetonides. The acetonide of **14** (400 mg) in EtOH (80 ml) was treated with 10% aqueous ethanolic HCl (0.5 ml) for 1 hr. The soln was poured into H_2O and the product recovered in EtOAc to afford *ent*-3β,18-dihydroxykaur-16-ene (**14**) (340 mg), mp 172–174°. (Found: C, 78.2; H, 11.2. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%). ^{1}H NMR ($CDCl_3$): δ 0.85 and 1.06 (each 3H, *s*, 2 C-Me), 3.46 and 3.70 (each 1H, *d*, J = 11 Hz, $-CH_2OH$), 3.66 (1H, *t*, CHOH) and 4.78 (2H, *br. s*, $=CH_2$); MS *m/e*: 289 (M-15), 286 (M-18), 271, 256. Under comparable conditions the acetonide of **15** gave *ent*-3β,18-dihydroxykaur-15-ene (**15**), mp 205–206°; ^{1}H NMR ($CDCl_3$): δ 0.84 and 1.06 (each 3H, *s*, 2 C-Me), 1.67 (3H, *d*, J = 2.5 Hz, $=C-Me$), 3.46 and 3.70 (each 1H, *d*, J = 11 Hz, $-CH_2OH$), 3.74 (1H, *m*, CHOH), 5.04 (1H, *m*, CH=); MS *m/e* 304 (M^+), 289, 286, 271, 256.

Incubation experiments. (i) *Ent*-3β-hydroxykaur-16-ene. *G. fujikuroi* (CMI 58289) inhibited with 10^{-4} MAMO 1618, was grown in shake culture at 25° for one day in 80 conical flasks (250 ml) each containing sterile medium [15] (50 ml). *Ent*-3β-hydroxykaur-16-ene (160 mg) in EtOH (10 ml) was distributed equally between 76 flasks and the remaining 4 flasks were retained as a control. The incubation was allowed to continue for a further 6 days. The broth was filtered, acidified with dil. HCl and extracted with EtOAc. The mycelium was washed with EtOAc. The combined extracts were separated into acidic and neutral fractions with aq. $NaHCO_3$. No transformation products were apparent in either fraction when compared (TLC EtOAc–petrol) with the control. *Ent*-3β-hydroxykaur-16-ene (120 mg) was recovered by chromatography. (ii) *Ent*-3β,18-dihydroxykaur-16-ene (**14**) (200 mg) in EtOH (20 ml) was distributed equally between 56 flasks of *G. fujikuroi* with 4 flasks as a control and incubated as above. The products were recovered in EtOAc. The neutral fraction was chromatographed on Si gel (dry column). Elution with EtOAc–petrol (1:1) gave the diol (**14**) (12 mg) and *ent*-3β,7α,18-trihydroxykaur-16-ene (**10**) (48 mg) identical (^{1}H NMR) with an authentic sample. (iii) Foliol (140 mg) in EtOH (40 ml) was distributed between 40 flasks of *G. fujikuroi* and incubated as above. The broth was extracted with EtOAc and separated into acidic and neutral fractions. The neutral fraction was chromatographed on Si gel. Elution with EtOAc gave foliol (90 mg) followed by *ent*-3β,7α,16β,18-tetrahydroxykaurane (**16**) (12 mg) which crystallized from Me_2CO–petrol as prisms, mp 226–228°. (Found: C, 67.8; H, 10.1. $C_{20}H_{34}O_4 \cdot H_2O$ requires C, 67.4; H, 10.2%). ^{1}H NMR (Py-d): δ 1.10 (6H, *s*,), 1.14 (3H, *s*) (20-H, 19-H, and 17-H), 3.66 and 4.07 (each 1H, *d*, J = 12 Hz, 18-H), 4.00 (2H, *m*, 3 and 7-H). The triacetate, prepared with Ac_2O–pyridine, was an oil, ^{1}H NMR ($CDCl_3$): δ 0.82, 1.10, 1.26 (each 3H, *s*, C-Me), 1.98 (9H, *s*, OAc), 3.46 and 3.92 (each 1H, *d*, J = 12 Hz, 18-H), 4.70 (2H, *m*, 3- and 7-H); MS *m/e*: 404 (M-AcOH), 386, 344, 326 (100%), 313, 284, 266, 253.

Epoxidation of foliol triacetate. The triacetate (900 mg) in $CHCl_3$ (25 ml) was treated with *m*-chloroperbenzoic acid (400 mg) at room temp. for 18 hr. The soln was diluted with $CHCl_3$ and thoroughly washed with aq. Na_2CO_3. The solvent was evapd and the residue chromatographed on Si gel. Elution with EtOAc–petrol (1:1) gave *ent*-3β,7α,18-triacetoxy-16β,17-epoxykaurane (**17**) (220 mg) as an oil, ^{1}H NMR ($CDCl_3$): δ 0.83 (3H, *s*, 20-H), 1.12 (3H, *s*, 19-H), 2.00 (9H, *s*, OAc), 2.82 (2H, *dd*, 17-H), 3.48 and 3.93 (each 1H, *d*, J = 12 Hz, 18-H), 4.70 (1H, *m*), 4.85 (1H, *m*,) (3 and 7-H); MS *m/e* 462, 420, 342, 318, 300, 282 (100%), 269. Further elution gave a mixture of two components which were separated by further chromatography (*vide infra*). The next fraction afforded *ent*-3β,7α,18-triacetoxy-17-hydroxy-15,16β-epoxykaurane (**18**) (200 mg) as an oil, ^{1}H NMR ($CDCl_3$): δ 0.82, 1.08, 2.02, 2.05, 2.09 (each 3H, *s*) 3.21 (1H, *s*, 15-H), 3.54 and 3.97 (each 1H, *d*, J = 11 Hz, 18-H), 3.78 and 4.08 (each 1H, *d*, J = 13 Hz, 17-H), 4.73 and 4.85 (each 1H, *m*, 3 and 7-H); MS *m/e*: 478 (M^+), 460, 418, 400, 358, 340. The tetra-acetate, prepared with Ac_2O–pyridine, had mp 166–168°. (Found: C, 64.5; H, 7.5. $C_{28}H_{40}O_9$ requires C, 64.6; H, 7.7%). ^{1}H NMR ($CDCl_3$): δ 0.82, (3H, *s*, 20-H), 1.09 (3H, *s*, 19-H), 2.02, 2.04, 2.08, 2.10 (each 3H, *s*, OAc), 3.17 (1H, *s*, 15-H), 3.52 and 3.98 (each 1H, *d*, J = 11 Hz, 18-H), 4.12 and 4.68 (each, 1H, d, J = 13 Hz, 17-H), 4.72 (1H, *m*), 4.86 (1H, *m*, 3- and 7-H). The mixture from the above chromatography was treated with Ac_2O–pyridine and chromatographed on Si gel. Elution with EtOAc–petrol (30:70) gave *ent*-3β,7α,17,18-tetra-acetoxykaur-15-ene (90 mg), mp 171–172°. (Found: C, 66.8; H, 7.8. $C_{28}H_{40}O_8$ requires C, 66.65; H, 8.0%). IR ν_{max}^{KBr} cm^{-1}: 3060, 1720, 1650, 1240, 1040, 940, 900, 830; ^{1}H NMR ($CDCl_3$): δ 0.82, 1.12, 2.02 and 2.04 (each 3H, *s*), 2.08 (6H, *s*) 3.52 and 3.92 (each 1H, *d*, J = 11 Hz, 18-H), 4.63 (2H, *br. s*, 17-H), 4.78 (2H, *m*, 3- and 7-H), 5.61 (1H, *s*, 15-H); MS *m/e* 504, 462, 444, 429, 402, 384, 369, 342, 324, 309, 283, 265 and 249. Further elution gave *ent*-3β,7α,18-tetra-acetoxy-15,16-epoxybeyerane (**20**) (70 mg), mp 155–157°. (Found: C, 64.5; H, 7.5. $C_{28}H_{40}O_9$ requires C, 64.6; H, 7.7%). ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, *s*, 20-H), 1.10 (3H, *s*, 19-H), 2.03 (9H, *s*), 2.10 (3H, *s*, 4-OAc), 3.25 and 3.42 (each 1H, *d*, J = 8 Hz, 15- and 16-H), 3.51 and 3.97 (each 1H, *d*, J = 11 Hz, 18-H), 4.17 and 4.40 (each, 1H, *d*, J = 12 Hz, 17-H), 4.72 (1H, *m*,) and 4.82 (1H, *m*, 3- and 7-H); MS *m/e*: 477 ($M^+ - 43$), 417, 357, 297.

Reduction of ent-3β,7α,18-*triacetoxy*-16β,17-*epoxykaurane.* The epoxide (220 mg) in dry THF (15 ml) was treated with $LiAlH_4$ (800 mg) under reflux for 6 hr. The excess reagent was carefully destroyed with H_2O and the product was recovered in Et_2O and chromatographed on Si gel. Elution with EtOAc–MeOH (95:5) afforded *ent*-3β,7α,16β,18-tetrahydroxykaurane (**16**) (55 mg), mp 226–228°, identical to the material described above.

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