*Phytochemistry*, 1980, Vol. 19, pp. 1087–1091. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0601-1087 $02.00/0

# THE MICROBIOLOGICAL TRANSFORMATION OF SOME *ENT*-KAUR-16-ENE 7-, 15- AND 18-ALCOHOLS BY *GIBBERELLA FUJIKUROI*

BRAULIO M. FRAGA, JAMES R. HANSON, MELCHIOR G. HERNANDEZ and FAHMI Y. SARAH

School of Molecular Sciences, University of Sussex, Brighton, Sussex, BN1 9QJ, U.K.

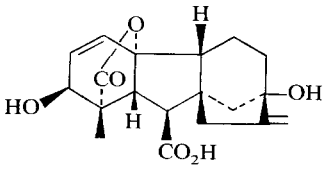
(*Revised received* 8 *October* 1979)

**Key Word Index**—*Gibberella fujikuroi*; candol A; candol B; sideridiol; 7,18-dihydroxykaurenolide.

**Abstract**—The effect of the 7-, 15- and 18-hydroxyl groups on the microbiological transformation of some diterpenoids by *Gibberella fujikuroi* has been studied. An 18-substituent appears to exert an inhibitory effect on transformations involving reaction at the 6β-position.

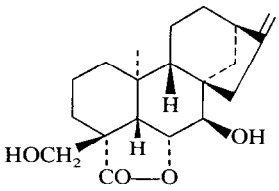
## INTRODUCTION

The fungus, *Gibberella fujikuroi*, produces a range of diterpenoid metabolites including the gibberellin plant hormones, e.g. gibberellic acid (**1**), the kaurenolide

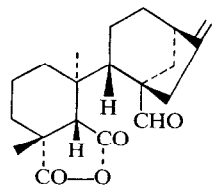


**1**

lactones, e.g. 7,18-dihydroxykaurenolide (**2**), the aldehyde–anhydride, fujenal (**3**) and a series of hydroxylated kaurenes, e.g. *ent*-7α-hydroxykaur-16-en-



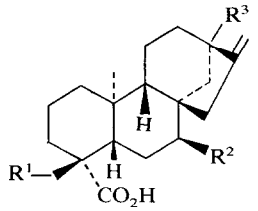
**2**



**3**

19-oic acid (**4**). These compounds are formed by a series of branching biosynthetic pathways. In the 'wild-type' fungus (strain ACC 917) certain oxidation patterns are characteristic of each branch. Thus a 13-hydroxyl group and a ring A 1,2-double bond are found as major features in the gibberellic acid branch but not commonly in the kaurenolide or in the fujenal branch. Conversely, an 18-hydroxyl group is found in the kaurenolides but not in the fungal gibberellins. It was the object of this work to incubate kaurenoid substrates with *Gibberella fujikuroi* to see whether it is possible, e.g. to induce the formation of gibberellins containing the 18-hydroxyl group characteristic of the kaurenolides or whether some of these hydroxylations have a hitherto undefined regulatory role limiting further transformation to particular pathways. We also hope to see how far the order of certain hydroxylations may be varied.

The diterpenoid pathways of *Gibberella fujikuroi* are capable of accepting a variety of artificial kaurenoid substrates. Thus steviol (**5**) which possesses an early (19-$CO_2H$) and a late (13-hydroxyl) stage in gibberellin biosynthesis is transformed along both the kaurenolide [1] and gibberellin [2, 3] pathways. The transformtaion of *ent*-kaur-2,16-dien-19-ol [4] and of some esters of *ent*-kaur-16-en-19-oic acid [5, 6] have also been studied in detail. More recently the metabolism of 2- and 3-hydroxy-kaur-16-en-19-ols and acids by *G. fujikuroi* has been studied [7].



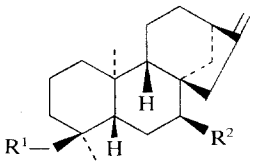
**4** $R^1 = R^3 = H$; $R^2 = OH$
**5** $R^1 = R^2 = H$; $R^3 = OH$
**7** $R^1 = R^2 = OH$; $R^3 = H$

## RESULTS AND DISCUSSION

When *G. fujikuroi* is grown in the presence of AMO 1618, the formation of *ent*-kaur-16-ene is inhibited and thus the normal diterpenoid fungal metabolites are not produced [8, 9]. However the subsequent metabolism of *ent*-kaur-16-ene is not perturbed [10, 11]. Hence the metabolism of the abnormal diterpenoid substrates can be examined in the absence of the normal diterpenoid metabolites, thus facilitating the purification and identification of novel metabolites. Without facile GC–MS facilities, our method of approach has been to compare, by TLC, the fermentation of the diterpenoids with that of a parallel control

fermentation and then to isolate any new metabolites which this assay revealed.

Candol B, *ent*-18-hydroxykaur-16-ene (**6**), isolated from *Sideritis candicans* [12], contains the last step of 7,18-dihydroxykaurenolide (**2**) biosynthesis but lacks the 6, 7 and 19-oxygen functions. It was fed to *G. fujikuroi* and two metabolites which were not present in the control, were detected and isolated. These were 7,18-dihydroxykaurenolide (**2**), identified by comparison (NMR) with authentic material [13] and *ent*-7α,18-dihydroxykaur-16-en-19-oic acid (**7**). The latter was identified by comparison of its methyl ester with known material [14].



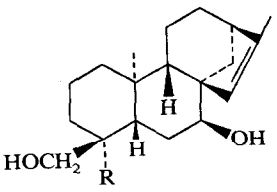

**6** $R^1 = OH, R^2 = H$
**8** $R^1 = H, R^2 = OH$
**9** $R^1 = R^2 = OH$
**14** $R^1 = R^2 = OAc$
**15** $R^1 = OH, R^2 = OAc$
**16** $R^1 = Cl, R^2 = OAc$
**17** $R^1 = Cl, R^2 = OH$

The hydrocarbon, *ent*-kaur-16-ene is the parent hydrocarbon of both the gibberellin and kaurenolide pathways. In the normal biosynthetic sequence, 7-hydroxylation follows the oxidation of *ent*-kaur-16-ene at C-19 and is the step immediately prior to the divergence between kaurenolide formation and the ring contraction to the gibberellins. The incubation of candol A, *ent*-7α-hydroxykaur-16-ene (**8**) [12] with *G. fujikuroi* was examined to see if it would be transformed along the pathway involving ring contraction without the oxidation of C-19. However, the metabolites which were obtained in amounts commensurate with their formation from the substrate, were gibberellic acid (**1**), gibberellins $A_4$ and $A_7$ [15] and fujenal (**3**) [16]. The gibberellins were identified (NMR) through the isolation of their methyl esters. They were not detected in the control fermentation. No abnormal metabolites were detected. Hence there was a substantial difference between the metabolism of 7- and 18-hydroxykaur-16-ene. Only the former was transformed along the pathway beyond the ring-contraction stage.

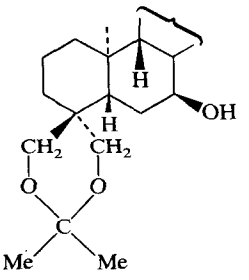
Epicandicandiol (*ent*-7α,18-dihydroxykaur-16-ene, **9**) [17, 18] and its double-bond isomer, sideridiol (*ent*-7α,18-dihydroxykaur-15-ene, **10**) [19] contain both these features within the same molecule. Epicandicandiol [14] gave *ent*-7α,18,19-trihydroxykaur-16-ene and the corresponding 19-acid on incubation with *G. fujikuroi*. When sideridiol (**10**) was fed to the fungus it gave a similar isomeric triol (**11**) which was identified by the formation of a triacetate and an 18,19-acetonide (**12**) involving the two primary alcohols. *ent*-7α,18-Dihydroxykaur-15-en-19-oic acid (**13**) was also isolated. However, no gibberellins or compounds of the fujenal type were formed in sufficient amounts to be detected. Hence the 18-hydroxyl group is apparently inhibiting reaction at the 6β-position. An 18-chloro derivative was then prepared by partial hydrolysis of *ent*-7α,18-diacetoxykaur-16-ene (**14**). The resultant 18-alcohol (**15**) [12] was treated with triphenylphosphine–carbon tetrachloride to afford *ent*-7α-acetoxy-18-chlorokaur-16-ene (**16**) which was then hydrolysed to the parent 7-alcohol (**17**). Despite several experiments no transformation products could be detected and indeed the compound appeared to be toxic to the fungus.

During the oxidative ring contraction process hydrogen atoms are lost from the 6β-position and from the 7β-hydroxyl group. A 15β-hydroxyl group possesses the same relationship to the 7β-hydroxyl hydrogen as does an 18-hydroxyl group to a 6β-hydrogen, i.e. it is on the same side of the molecule and four atoms distant from the relevant hydrogen atom. Hence the metabolism of *ent*-15α, 19-dihydroxykaur-16-ene was of interest in this context. In earlier work (Hanson, J. R. and Hawker, J., unpublished) we were unable to demonstrate any transformation of the 15β-acetoxy-acid, xylopic acid (**18**). However, more recently MacMillan [20] observed the transformation of desacetoxyxylopic acid (**19**) into gibberellin $A_{45}$ (**20**) by a mutant of *G. fujikuroi*. *ent*-15α,19-Dihydroxykaur-16-ene (**22**) was prepared by reduction of methyl xylopate (**21**) [21] with lithium aluminium hydride. Incubation of the diol with *G. fujikuroi* gave two major new metabolites which were isolated by chromatography. These were 7β,15β-dihydroxykaurenolide (**23**) which was further purified as its diacetate (**24**) ($\nu_{max}$ 1770, γ-lactone; 1740 $cm^{-1}$ OAc). The $^1H$ NMR spectrum showed that ring B contained the vicinal 6α,7β-oxygen functions characteristic of the kaurenolides (δ 5.78, *d*, $J = 6$ Hz, 7-H; 4.5, *t*, $J = 6$ Hz, 6-H; 1.96, *d*, $J = 6$ Hz, 5-H). The methyl ester of the other metabolite, *ent*-7α,15α-dihydroxykaur-16-en-19-oic acid had $^1H$ NMR signals consistent with the structure (**25**). In particular the 18-H and 7-H resonances (δ 0.85, 1.14 and 3.65) were comparable with those of methyl *ent*-7α-hydroxykaur-16-en-19-oate (0.84, 1.15 and 3.61). The MS contained an ion at *m/e* 109 associated with a non-hydroxylated ring A. Since both hydroxylation at C-7 and oxidation at C-19 had proceeded in the presence of the 15β-hydroxyl group, it was possible that further metabolism had occurred. A sample of the fermentation extract was sent to Prof. MacMillan F.R.S. for GC–MS comparison with his conversion of *ent*-15α-hydroxykaur-16-en-19-oic acid (**19**) by the mutant B1 41a. He reported the presence of gibberellin $A_{45}$ (15β-hydroxy$GA_9$), 15β-hydroxy$GA_{12}$, 15β-hydroxy$GA_{25}$, 15β-hydroxy$GA_{24}$, 15β-hydroxy$GA_{14}$, 15β-hydroxy$GA_4$, 15β-hydroxy$GA_{15}$, 15β-hydroxy$GA_{13}$, 15β-hydroxy$GA_1$ and 15β-hydroxy$GA_3$. The evidence for the structure of these compounds will be presented in his full paper.

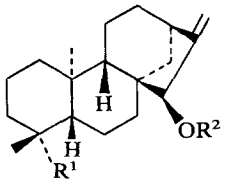
From these results we conclude that although the order of hydroxylation at C-7 and oxidation at C-19 can be varied in the substrates as in candol A, detectable quantities of 19-desoxymetabolites are not formed. The formation of 19-oxygenated gibberellins from the metabolism of *ent*-7α-hydroxykaur-16-ene suggests that oxidation of C-19 to the level of a carboxylic acid is an important prerequisite for further metabolism of ring B. Secondly an 18-substituent appears to inhibit the oxidation of the 6β-position and thus the formation of gibberellin and fujenal derivatives. However, a similar effect on the loss of the hydrogen atom from



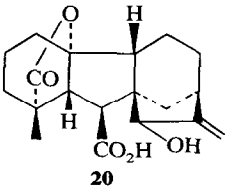
**10** R = Me
**11** R = $CH_2OH$
**13** R = $CO_2H$



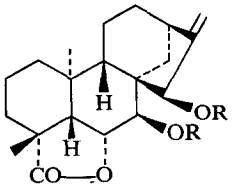
**12**



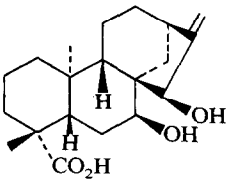
**18** $R^1 = CO_2H$, $R^2$ = Ac
**19** $R^1 = CO_2H$, $R^2$ = H
**21** $R^1 = CO_2Me$, $R^2$ = Ac
**22** $R^1 = CH_2OH$, $R^2$ = H



**20**

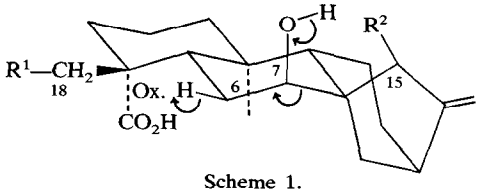


**23** R = H
**24** R = Ac



**25**

the hydroxyl group at C-7 is not shown by a 15β-hydroxyl group. In this connection it is interesting to note that 18-fluorogibberellin $A_{12}$ aldehyde was metabolized by *G. fujikuroi* into the corresponding fluorogibberellic acid and fluorogibberellin $A_9$ [22]. These features may be rationalized in terms of a mechanism for the ring contraction (Scheme 1). When



Scheme 1.

R′ = OH or Cl, the oxidative ring contraction is blocked either by tyeric effect of the substituent or by it binding too effectively to the enzyme system. This raises the interesting speculative possibility that many apparently 'irrelevant' hydroxylations in terpenoid metabolism may have a control function or code for metabolism along a particular pathway.

## EXPERIMENTAL

General experimental details have been described previously [23, 24].

*Incubation of candol B with* Gibberella fujikuroi. The fungus, inhibited with $10^{-4}$ M AMO 1618, was grown in shake culture at 25° for 1 day in 35 conical flasks (250 ml) each containing sterile medium (100 ml) [23, 24]. Candol B (280 mg) in EtOH (33 ml) was distributed equally between 33 flasks and the remaining 2 flasks were retained as a control. The incubation was allowed to continue for a further 5 days. The broth was filtered, acidified with dil HCl and extracted with EtOAc. The extract was washed with $H_2O$ and shaken first with aq. $NaHCO_3$ and then with aq. $Na_2CO_3$. These extracts were acidified with dil HCl and then extracted with EtOAc. The EtOAc extract was washed with $H_2O$, dried and the solvent evapd to afford *ent*-7α,18-dihydroxykaur-16-en-19-oic acid (10 mg), mp 145–146° (Found: MS 316.205. $C_{20}H_{30}O_4$–$H_2O$ requires: 316.203; the peaks at *m/e* 334 and 319 were too small to measure accurately). IR $\nu_{max}$ $cm^{-1}$: 3360, 1685 and 875; $^1$H NMR ($C_5H_5N$-$d_5$): δ 1.26 (3H, *s*, 20-H), 3.73 (1H, *m*, 7-H), 3.84 and 4.46 (1H, *d*, *J* = 11 Hz, 18-$H_2$), 4.84 (2H, *m*, 17-H). Irradiation at δ 3.84 caused the doublet at 4.46 to collapse to a singlet. Methylation with $CH_2N_2$ gave methyl *ent*-7α, 18-dihydroxykaur-16-en-19-oate which crystallized from EtOAc–petrol as needles, mp 229–230° (lit. [14] 228–230°) identified by its IR and $^1$H NMR spectra. The neutral fraction was chromatographed on Si gel. Elution with EtOAc–petrol (20:80) gave the starting material (50 mg). Elution with EtOAc–petrol (35:65) gave 7,18-dihydroxykaurenolide (18 mg), mp 211–213° (lit. [13] 211–214°) which was identified by its IR and $^1$H NMR spectra.

*Incubation of candol A with* Gibberella fujikuroi. Candol A (75 mg) in EtOH (20 ml) was distributed between 20 flasks of *G. fujikuroi* and incubated as above. The broth was extracted with EtOAc and separated into acidic and neutral fractions with aq. $NaHCO_3$. The acid fraction was methylated with $CH_2N_2$. TLC showed 3 new compounds compared to the control. The acid fraction was chromatographed on Si gel. Elution with EtOAc–petrol (30:70) gave dimethyl *ent*-7-oxo-6,7-seco-16-en-6,19-dioic acid 6,19-dioate (5 mg), mp 141–143°, identified by its $^1$H NMR spectrum. Further elution gave a mixture of gibberellin $A_4$ and gibberellin $A_7$ methyl esters (5 mg), identified by their $^1$H NMR spectra. Further elution with EtOAc–petrol (1:1) gave gibberellin $A_3$ methyl ester (15 mg), mp 207–210°, identified by its $^1$H NMR spectrum. Chromatography of the neutral fraction on Si gel gave the starting material (10 mg).

*Incubation of sideridiol with* Gibberella fujikuroi. Sideridiol (200 mg) in EtOH (36 ml) was distributed between 36 flasks of *G. fujikuroi* and incubated as above. The broth was extracted with EtOAc and separated into acid and neutral fractions. Elution with EtOAc gave the starting material (95 mg). Further elution with EtOAc gave *ent*-7α,18,19-trihydroxykaur-15-ene which was recrystallized from EtOAc–petrol as needles (10 mg), mp 210°. (Found: C, 75.2; H, 9.9, $C_{20}H_{32}O_3$ requires: C, 75.0; H, 10.1%), IR $\nu_{max}$ $cm^{-1}$: 3548 (sharp), 3350 (br.), 1065, 1040, 1030, 1020, 825; $^1$H NMR ($C_5H_5N$-$d_5$): δ 1.12 (3H, *s*, 20-H), 1.68 (3H, *s*, 17-H), 3.85 and 4.29 (2H each, *d*, *J* = 11 Hz, 18- and 19-H), 4.0 (1H, *m*, 7-H), 5.96 (1H, *s*, 15-H). MS *m/e:* 320, 302, 272, 271, 254, 164, 139, 121, 120, 108, 107, 105, 95, 94, (100%). The triacetate, prepared with $Ac_2O$ in Py, was an oil. [Found: MS 446.267 $C_{26}H_{38}O_6$ requires: 446.266] IR $\nu_{max}$ $cm^{-1}$: 1730 (br), 1250 (br), 1035, 1025, 980 and 822; $^1$H NMR: δ 1.09 (3H, *s*, 20-H), 1.68 (3H, *s*, 17-H), 2.0 and 2.05 (6H and 3H, *s*, 3 OAc), 3.85 (2H, *d*, *J* = 12 Hz, 19-H), 4.1 (2H, *d*, *J* = 20 Hz, 18-H), 4.7 (1H, *t*, *J* = 3 Hz, 7-H), 5.19 (1H, *s*, 15-H); MS *m/e* (rel. int.): 446 (30), 404, 386 (100), 371, 358, 344, 326, 267, 251, 238, 105. The acetonide,

prepared with $Me_2CO$ and $CuSO_4$ over 4 hr at room temp., was an oil. (Found: MS 345.2428, $C_{22}H_{33}O_3$ (M-15) requires: 345.2429). $^1$H NMR: $\delta$ 0.94 (3H, *s*, 20-H), 1.30 and 1.33 (3H each, *s*, acetonide Me), 1.71 (3H, *s*, 17-H), 3.2 and 3.55 (1H each, *d*, $J$ = 12 Hz, 19-H), 3.61 (3H, *br.m*, 18 and 7-H), 5.45 (1H, *s*, 15-H). MS *m/e* (rel. int.): 360 (10), 345, 302, 254, 239, 149 (100), 139, 121, 107, 105, 93. The acid fraction was chromatographed on Si gel. Elution with EtOAc–petrol (80:20) gave *ent*-7$\alpha$,18-dihydroxykaur-15-en-19-oic acid (8 mg) mp 115–117°. [Found: MS 334.214. $C_{20}H_{30}O_4$ requires: 334.214.] IR $\nu_{max}$ cm$^{-1}$: 3360, 1685, 1070, 1035, 805; $^1$H NMR ($C_5H_5N$-$d_5$): $\delta$ 1.31 (3H, *s*, 20-H), 1.69 (3H, *s*, 17-H), 3.85 (1H, *m*, 7-H), 3.85 and 4.52 (1H each *d*, $J$ = 11 Hz, 18-H), 5.69 (1H, *s*, 15-H); MS *m/e*: 334, 319, 316 (100%), 301, 298, 288, 286, 254, 240, 225, 197, 183.

ent-7$\alpha$-*Acetoxy*-18-*chlorokaur*-15-*ene*. Epicandicandiol 7-monoacetate (750 mg) [12] in $CCl_4$ (100 ml) and Py (10 ml) was treated with triphenylphosphine (1.1 g) under reflux for 3 hr. The solvent was evapd and the product chromatographed on Si gel. Elution with petrol afforded *ent*-7$\alpha$-acetoxy-18-chlorokaur-16-ene (720 mg), mp 123–124°. (Found: C, 71.9; H, 9.1, Cl, 10.2. $C_{22}H_{33}O_2Cl$ requires: C, 72.4; H, 9.1; Cl, 9.7%.) $^1$H NMR: $\delta$ 1.05 and 0.83 (each 3H, *s*, 19- and 20-H), 2.00 (3H, *s*, OAc), 3.42 and 3.04 (each 1H, *d*, $J$ = 11 Hz, 18-H), 4.75 (3H, *m*, H-7 and H-17). MS *m/e*: 304 (M−60) 289 (M−60−15).

ent-7$\alpha$-*Hydroxy*-18-*chlorokaur*-16-*ene*. The above chloroacetate (700 mg) was dissolved in 5% methanolic KOH (20 ml) and left at room temp. overnight. The soln was neutralized, the solvent evapd and the product recovered in EtOAc to afford *ent*-7$\alpha$-hydroxy-18-chlorokaur-16-ene (620 mg), mp 123–125°; MS *m/e*: 322 ($M^+$) 304 (M−18), 289 (M−18−15). $^1$H NMR: $\delta$ 1.04 and 0.87 (each 3H, *s*, 19- and 20-H), 3.48 and 3.17 (each 1H, *d*, $J$ = 11 Hz, 18-H), 3.58 (1H, *t*, 7-H), 4.80 (2H, *br.s*, 17-H). Despite several incubations this compound was recovered unchanged from cultures of *G. fujikuroi* which grew much less readily in its presence.

*Reduction of methyl xylopate with* $LiAlH_4$. Methyl xylopate (500 mg) in dry THF (100 ml) was heated with $LiAlH_4$ (400 mg) for 4 hr under reflux. The soln was cooled, treated with EtOAc, $H_2O$ and the product recovered in EtOAc. The product was chromatographed on Si gel. Elution with EtOAc–petrol (40:60) gave *ent*-15$\alpha$,19-dihydroxykaur-16-ene which crystallized from $Me_2CO$–petrol as prisms, mp 154–156°. (Found: C, 79.0; H, 10.4. $C_{20}H_{32}O_2$ requires: C, 78.9; H, 10.6%). IR $\nu_{max}$ cm$^{-1}$: 3410, 3290, 1660, 1110, 1025, 888; $^1$H NMR: $\delta$ 0.92 (3H, *s*, 20-H), 0.98 (3H, *s*, 18-H), 3.36 and 3.64 (1H, *d*, $J$ = 12 Hz, 19-H), 3.6 (1H, *m*, 15-H), 4.89 and 5.01H *br.d*, 17-H); MS *m/e*: 304, 289, 286, 272 (100).

*Incubation of* ent-15$\alpha$,19-*dihydroxykaur*-16-*ene with* Gibberella fujikuroi. The diol (400 mg) was dissolved in EtOH (48 ml) and distributed equally between 48 flasks of *G. fujikuroi* and incubated as above. After a further 5 days growth, the broth was extracted with EtOAc and separated into acidic and neutral fractions. The acidic fraction was methylated with $CH_2N_2$ and chromatographed on Si gel to afford methyl *ent*-7$\alpha$,15$\alpha$-dihydroxykaur-16-en-19-oate which was recrystallized from EtOAc–petrol as prisms, mp 159–160°. [Found: C, 72.4; H, 9.2. $C_{21}H_{32}O_4$ requires: C, 72.4; H, 9.3%]. IR $\nu_{max}$ cm$^{-1}$: 3480, 3440, 1730, 1150, 1075, and 895; $^1$H NMR: $\delta$ 0.85 (3H, *s*, 10-H), 1.14 (3H, *s*, 18-H), 1.9 (1H, *d*, $J$ = 4 Hz, 5-H), 3.59 (3H, *s*, OMe), 3.65 (1H, *m*, 7-H), 4.16 (1H, *m*, 15-H), 4.97 and 5.1 (2H, *dd*, $J$ = 3 Hz, 17-H). Irradiation at 4.16 caused the doublets at 4.97 and 5.1 to collapse to broad singlets. When the soln was washed with $^2H_2O$, a signal at $\delta$ 3.9 disappeared. The neutral fraction was chromatographed on Si gel. Elution with EtOAc–petrol (35:65) gave the starting material (56 mg). Further elution gave a mixture which was treated with $Ac_2O$ in Py for 1 day at room temp. The product was then chromatographed on Si gel. Elution with EtOAc–petrol (30:70) gave *ent*-7$\alpha$,15$\alpha$-diacetoxy-6$\beta$-hydroxykaur-16-en-19-oic acid 19-6$\beta$-lactone as needles (20 mg), mp 228°. (Found: C, 69.9; H, 7.7. $C_{24}H_{32}O_6$ requires: C, 69.2; H, 7.7%.) IR $\nu_{max}$ cm$^{-1}$: 1770, 1740, 1220, 1095, 1060, 1040, 975, 930, 895; $^1$H NMR: $\delta$ 0.99 (3H, *s*, 20-H), 1.25 (3H, *s*, 18-H), 1.96 (1H, *d*, $J$ = 6 Hz, 5-H), 2.1 and 2.13 (each 3H, *s*, 20Ac), 4.5 (1H, *t*, $J$ = 6 Hz, 6-H), 4.81 and 5.05 (2H, *br.d* 17-H), 5.61 (1H, *m*, 15-H), 5.78 (1H, *d*, $J$ = 6 Hz, 7-H); MS *m/e* (rel. int.): 374 (M−42, 90), 356, 314 (100), 299, 296, 285, 281, 268, 253, 137, 109.

*Acknowledgements*—We thank Mrs. N. Dransfield for growing the fermentations; B. M. F. thanks the Fundacion Juan March for financial assistance (1976) and F. S. thanks the University of Basrah for study leave (1975).

## REFERENCES

1. Hanson, J. R. and White, A. F. (1968) *Tetrahedron* **24,** 629.
2. Bearder, J. R., MacMillan, J., Frydman, V. M., Gaskin, P., Wels, C. M. and Phinney, B. O. (1976) *J. Chem. Soc. Perkin Trans.* **1,** 173.
3. Bearder, J. R., MacMillan, J., Wels, C. M. and Phinney, B. O. (1975) *Phytochemistry* **14,** 1741.
4. Bakker, H. J., Cook, I. F., Jefferies, P. R. and Knox, J. R. (1974) *Tetrahedron* **30,** 3631.
5. Jefferies, P. R., Knox, J. R. and Ratajczak, T. (1974) *Phytochemistry* **13,** 1423.
6. Croft, K. D., Ghisalberti, E. L., Jefferies, P. R., Knox, J. R., Mahoney, T. J. and Sheppard, P. N. (1974) *Tetrahedron* **30,** 3663.
7. Lunnon, M. W., MacMillan, J. and Phinney, B. O. (1977) *J. Chem. Soc. Perkin Trans.* **1,** 2308.
8. Dennis, D. T., Upper, C. D. and West, C. A. (1965) *Plant Physiol.* **40,** 948.
9. Cross, B. E. (1968) *Progr. Phytochem.* **1,** 195.
10. Cross, B. E. and Myers, P. L. (1969) *Phytochemistry* **8,** 79.
11. Light, E. N. and Lang, A. (1969) *Planta* **88,** 172.
12. Gonzalez, A. G., Fraga, B. M., Hernandez, M. G. and Luis, J. G. (1973) *Phytochemistry* **12,** 2721.
13. Cross, B. E., Galt, R. H. B. and Hanson, J. R. (1963) *J. Chem. Soc.* 3783.
14. Fraga, B. M., Hanson, J. R. and Hernandez, M. G. (1978) *Phytochemistry* **17,** 812.
15. Cross, B. E., Galt, R. H. B. and Hanson, J. R. (1962) *Tetrahedron* **18,** 451.
16. Cross, B. E., Galt, R. H. B. and Hanson, J. R. (1963) *J. Chem. Soc.* 5052.
17. Breton, J. L., Gonzalez, A. G., Rocha, J. M., Panizo, F. M., Rodriguez, B. and Valverde, S. (1969) *Tetrahedron Letters* 599.
18. Piozzi, F., Venturella, P., Bellino, A., Paternostro, M. P., Rodriguez, B. and Valverde, S. (1971) *Chem. Ind. (London)* 962.
19. Piozzi, F., Venturella, P., Bellino, A. and Mondelli, R. (1968) *Tetrahedron* **24,** 4073.

20. Bearder, J. R., Dennis, F. G., MacMillan, J., Martin, G. C. and Phinney, B. O. (1975) *Tetrahedron Letters* 669.
21. Ekong, D. E. U. and Ogan, A. U. (1968) *J. Chem. Soc. C* 311.
22. Cross, B. E. and Bateson, J. H. (1974) *J. Chem. Soc. Perkin Trans.* **1,** 1131.
23. Evans, R., Hanson, J. R. and White, A. F. (1970) *J. Chem. Soc. C* 2601.
24. Hanson, J. R., Hawker, J. and White, A. F. (1972) *J. Chem. Soc. Perkin Trans.* **1,** 1892.