Fungal Products. Part 20.[1] Transformations of 2- and 3-Hydroxylated Kaurenoids by *Gibberella fujikuroi*

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ent-3α-Hydroxy- and *ent*-2α,3α-dihydroxy-kaur-16-en-19-oic acids have been prepared from *ent*-kaur-16-ene-3β,19-diol, and their metabolites from cultures of the mutant B1-41a of *Gibberella fujikuroi* have been analysed by g.l.c.–mass spectrometry. The metabolism of *ent*-2α-hydroxy, *ent*-2β-hydroxy-, and *ent*-3α-hydroxykaur-16-en-19-ols has been similarly investigated in cultures of the parent wild-type strain, GF-1a, in which gibberellin biosynthesis was blocked by a synthetic plant growth retardant. The results show 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, are efficiently converted into 3-hydroxylated gibberellins. They also indicate that the *ent*-2β-hydroxy-analogue is converted into gibberellin A_3 by dehydration and that the conversion of *ent*-kaurenoids into gibberellins is reduced by the presence of *ent*-2α- and *ent*-2β-hydroxy-groups.

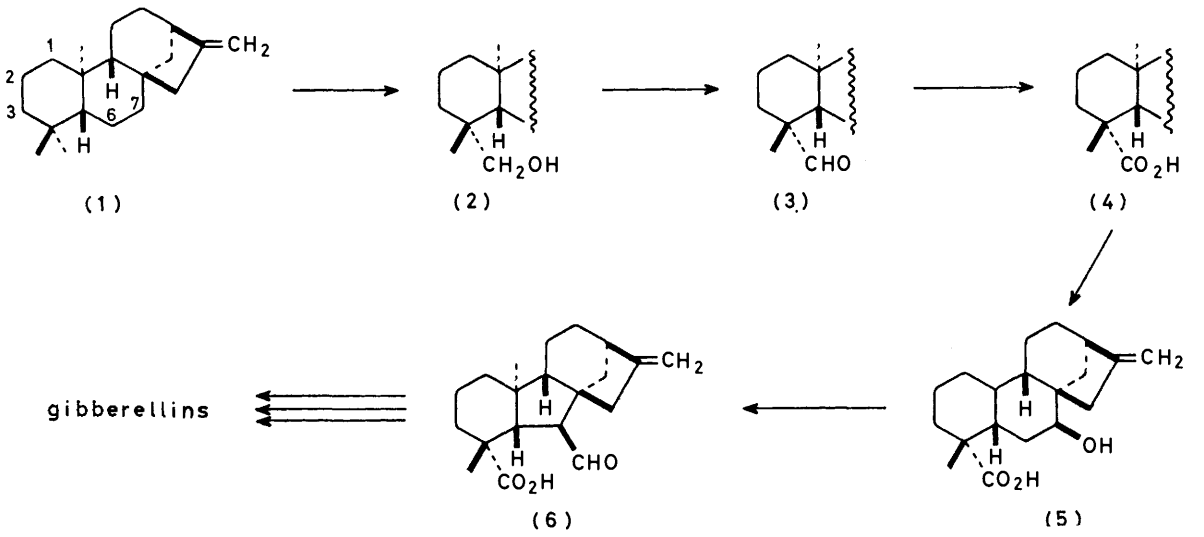
THE pathway (Scheme 1) by which gibberellins (GAs) are normally biosynthesised from *ent*-kaur-16-ene (1) by wild-type strains of *Gibberella fujikuroi* is blocked [2] in the mutant B1-41a at the step between *ent*-kaur-16-en-19-al (3) and *ent*-kaur-16-en-19-oic acid (4). This mutant converts [3] exogenously applied *ent*-kaurenoic acid (4), and later intermediates, into the GAs which are normally produced by wild-type strains such as the parent strain GF-1a [4] of the mutant. Furthermore this mutant will metabolise added analogues of *ent*-kaurenoic acid, such as steviol (7), steviol acetate (8), and isosteviol (9) into the corresponding derivatives of normal fungal GAs.[5,6]

To elucidate further this apparent lack of substrate specificity of the enzymes which catalyse the conversion of *ent*-kaurenoids into GAs, we have now examined the effect of 2- and 3-hydroxylation of the *ent*-kaurenoid intermediates. 2- and 3-Hydroxylated substrates were chosen for the following reasons. First, in the normal gibberellin biosynthetic pathway 3-hydroxylation occurs after ring B contraction [(5) ⟶ (6), Scheme 1] [7,8] and it was of interest to determine if

[1] Part 19, P. Hedden, B. O. Phinney, J. MacMillan, and V. M. Sponsel, *Phytochemistry*, in the press.
[2] J. R. Bearder, J. MacMillan, C. M. Wels, M. B. Chaffey, and B. O. Phinney, *Phytochemistry*, 1974, **13**, 911.
[3] J. R. Bearder, J. MacMillan, and B. O. Phinney, *J.C.S. Perkin I*, 1975, 721.
[4] J. MacMillan and C. M. Wels, *Phytochemistry*, 1974, **13**, 1413.
[5] J. R. Bearder, J. MacMillan, C. M. Wels, and B. O. Phinney, *Phytochemistry*, 1975, **14**, 1741.
[6] J. R. Bearder, V. M. Frydman, P. Kaskin, J. MacMillan, C. M. Wels, and B. O. Phinney, *J.C.S. Perkin I*, 1976, 173.
[7] B. E. Cross, K. Norton, and J. C. Stewart, *J. Chem. Soc.* (*C*), 1968, 1054.
[8] P. Hedden, J. MacMillan, and B. O. Phinney, *J.C.S. Perkin I*, 1974, 587.

the enzyme(s) responsible for ring contraction would accept a 3-hydroxylated substrate. Secondly, 2β-hydroxy-GAs are not normally produced by *G. fujikuroi* and 2α-hydroxylation occurs, but only to a limited extent, towards the end of the pathway.[9,10] The substrates offered a possible method of preparing otherwise scarce 2α- and 2β-hydroxy-GAs.

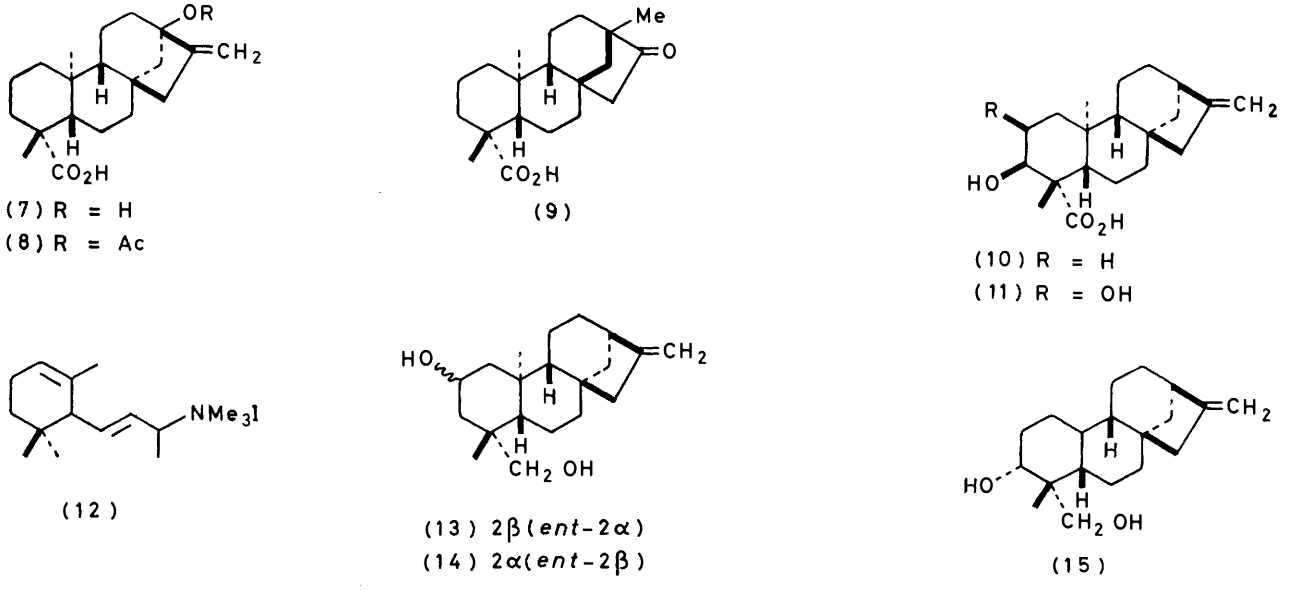
It was our original intention to examine the metabolism of 2- and 3-hydroxylated derivatives of *ent*-kaurenoic acid (4) in cultures of the mutant B1-41a and,



SCHEME 1 A portion of the GA biosynthetic pathway in *G. fujikuroi*

opposite is true of higher plants where 2α-hydroxy-GAs have not been found but 2β-hydroxylation appears to be an important deactivating process.[11] A comparison of the specificity of the fungal enzymes for 2α- and 2β-hydroxylated intermediates was therefore of interest. Thirdly the metabolism of 2α- and 2β-hydroxylated

to this end, we prepared *ent*-3α-hydroxy- and *ent*-2α,3α-dihydroxykaurenoic acids (10) and (11) by the methods described below. However, in the course of these studies we found[1] that GA biosynthesis in the parent strain GF-1a is efficiently blocked before *ent*-kaur-16-ene (1) (Scheme 1) by the synthetic plant growth retardant (12).[12] This discovery provided a method of



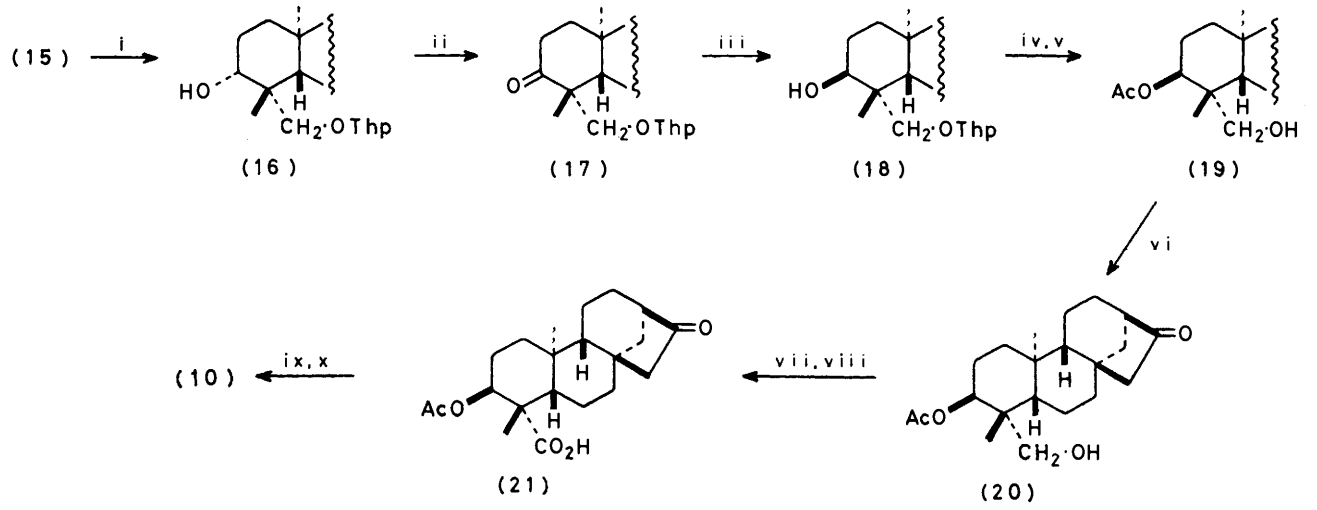
[9] J. R. Bearder, V. M. Frydman, P. Gaskin, I. K. Hatton, W. E. Harvey, J. MacMillan, and B. O. Phinney, *J.C.S. Perkin I*, 1976, 178.

[10] A. G. McInnes, D. G. Smith, R. C. Durley, R. P. Pharis, G. P. Arsenault, J. MacMillan, P. Gaskin, and L. C. Vining, *Canad. J. Biochem.*, 1977, in the press.

[11] J. MacMillan in ' Plant Growth Regulation,' ed. P. E. Pilet, Springer–Verlag, Berlin, Heidelberg, New York, 1977, p. 129.

[12] H. Haruta, H. Yagi, T. Iwata, and S. Tamura, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 417.

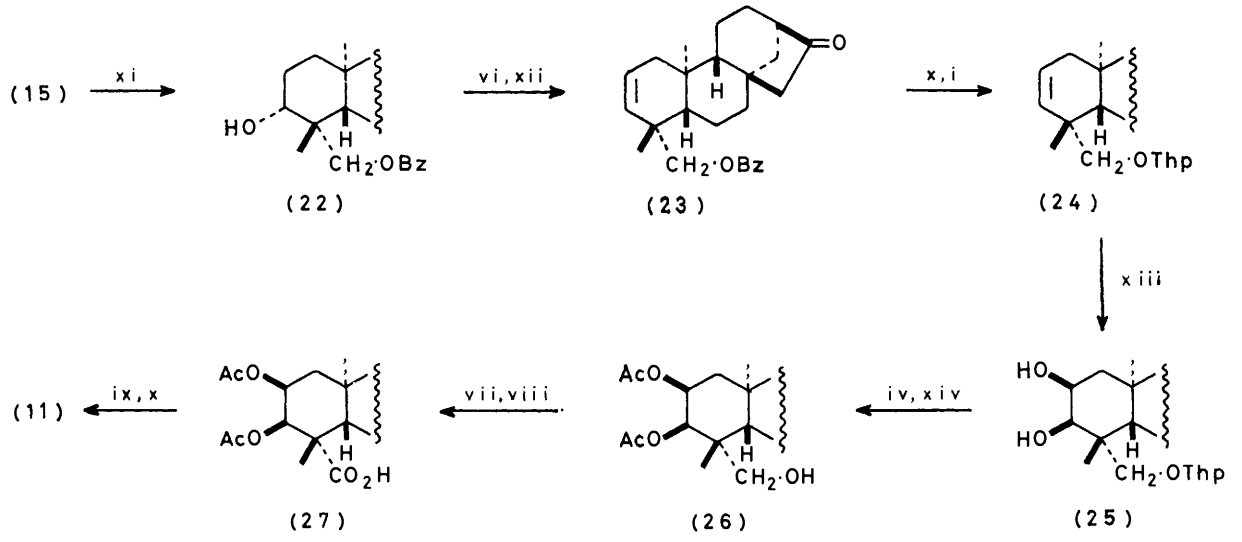
determining the metabolism of analogues of all the natural *ent*-kaurenoid intermediates in the absence of the metabolites normally produced from *ent*-kaurene (1) and allowed us to use the previously prepared [13] was necessary for the initial steps in each synthesis. For the *ent*-3α-hydroxy-acid (10) (Scheme 2) a protecting group stable under basic conditions was required and, after much experimentation, the 19-tetrahydropyranyl



Scheme 2

ent-2α- and *ent*-2β-hydroxykaurenols (13) and (14) as substrates to ascertain the effect of 2-hydroxy-substituents.

The *ent*-kaurenoic acids (10) and (11) were prepared ether (16) was obtained in 47% yield; for the *ent*-2α,3α-dihydroxy-acid (11) (Scheme 3) the acid-stable 19-benzoate (22) was prepared in 48% yield. Unexpected difficulty was experienced in oxidising the



Scheme 3

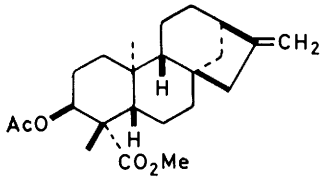
Reagents: i, dihydropyran–CH_2Cl_2–*p*-$MeC_6H_4{\cdot}SO_3H$; ii, CrO_3–C_5H_5N; iii, $Al(OPr^i)_3$–Pr^iOH; iv, $(MeCO)_2O$–C_5H_5N; v, $MeCO_2H$–MeOH; vi, OsO_4–$NaIO_4$; vii, CrO_3–Me_2CO–H^+; viii, RuO_4–CCl_4; ix, $Ph_3P{:}CH_2$; x, KOH–MeOH; xi, PhCOCl–C_5H_5N; xii, $POCl_3$–C_5H_5N; xiii, OsO_4–C_5H_5N; xiv, *p*-$MeC_6H_4{\cdot}SO_3H$

from *ent*-kaur-16-ene-3β,19-diol (15), which was obtained [14] by alkaline hydrolysis of the ether-soluble components of leaves of *Goodenia strophiolata*. The synthetic routes, shown in Schemes 2 and 3, were unexceptional except for the following points. Protection of the 19-hydroxy-group in the starting material (15) hydroxymethyl group in compound (19) to the corresponding carboxy-group. With a four-fold excess of Jones reagent, oxidation did not proceed beyond the

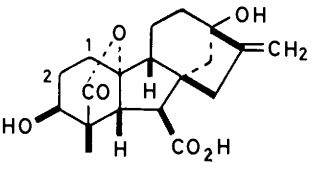
[13] M. W. Lunnon and J. MacMillan, following paper.
[14] E. J. Middleton and P. R. Jefferies, *Austral. J. Chem.*, 1968, **21**, 2349.

aldehyde level and attempts to oxidise the aldehyde with chromium trioxide in moist pyridine gave a complex mixture, shown by g.l.c.–mass spectrometry to contain the aldehyde, the carboxylic acid, and their 17-nor-ketone derivatives. This difficulty was circumvented by conversion into the nor-ketone (20), then the corresponding aldehyde, which was smoothly oxidised to the acid (21) with ruthenium tetraoxide [15] in carbon tetrachloride. The route (Scheme 3) to *ent*-2α,3α-dihydroxykaurenoic acid (11) was therefore directed *via* the nor-ketone (19). In the penultimate step of each synthesis the Wittig reactions were effected [16] in good yield using salt-free ylide and de-oxygenated solutions of the free acids in tetrahydrofuran. Initially the final step of these syntheses alkaline hydrolysis of the diacetate (27) (Scheme 3) occurred faster than that of the mono-acetate (21) (Scheme 2), presumably because of rapid hydrolysis of the 2*eq*-acetate in (27) to the 2-alkoxide ion which assists hydrolysis of the 3*ax*-acetate.

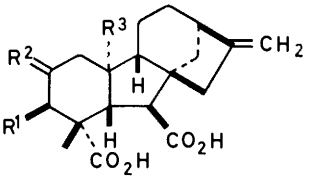
Metabolisation of the *ent*-kaurenoic acids (10) and (11) by resuspension cultures of the mutant B1-41a, and of the *ent*-kaurene diols (13) and (14) by resuspension cultures of the wild-type strain GF-1a containing the inhibitor (12) was complete after 5 days. Extracts from the mycelium contained no metabolites. Extracts from the culture filtrates were analysed by g.l.c.–mass spectrometry as the methyl ester trimethylsilyl ethers



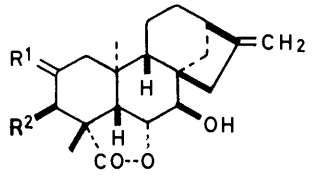
(28)



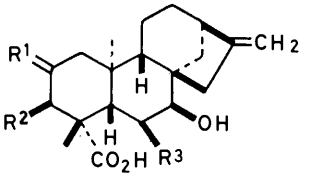
(29)
(30) $\Delta^{1\text{-ene}}$



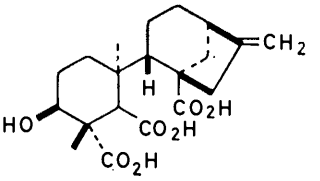
(31) $R^1 = OH, R^2 = H_2, R^3 = CO_2H$
(32) $R^1 = OH, R^2 = H, \beta\text{-}OH, R^3 = Me$
(33) $R^1 = OH, R^2 = H, \beta\text{-}OH, R^3 = CO_2H$
(34) $R^1 = OH, R^2 = H, \alpha\text{-}OH, R^3 = Me$



(35) $R^1 = H_2, R^2 = OH$
(36) $R^1 = H, \beta\text{-}OH, R^2 = OH$
(37) $R^1 = H, \beta\text{-}OH, R^2 = H$
(38) $R^1 = H, \alpha\text{-}OH, R^2 = H$



(39) $R^1 = H_2, R^2 = OH, R^3 = H$
(40) $R^1 = H, \beta\text{-}OH, R^2 = OH, R^3 = H$
(41) $R^1 = H, \beta\text{-}OH, R^2 = OH, R^3 = OH$
(42) $R^1 = H, \beta\text{-}OH, R^2 = H, R^3 = OH$
(43) $R^1 = H, \alpha\text{-}OH, R^2 = H, R^3 = OH$



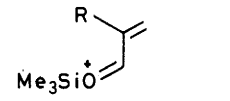
(44)

Wittig reaction was performed on the methyl ester of the acid (21) but hydrolysis of the product (28) with potassium t-butoxide in dimethyl sulphoxide also caused epimerisation at C-3 to give *ent*-3β-hydroxykaurenoic acid. Epimerisation did not occur during alkaline hydrolysis of ent-3α-acetoxykaurenoic acid in the final step of the route in Scheme 1, suggesting that in the ester (28) hydrolysis of the acetate group and epimerisation occur before hydrolysis of the ester function. Epimerisation by the retro-aldol mechanism proposed [17,18] for 3-hydroxygibberellins is possible in the methyl ester but not in the carboxylate anion. In the and in the case of the metabolites from *ent*-2α,3α-dihydroxykaurenoic acid (11), also as the trimethylsilyl ethers of the isopropylidene derivatives of the methyl esters. Representative total ion current traces, showing the identified metabolites, for identical aliquot portions of the total derivatised extracts are reproduced in the Figure. The metabolites were not isolated. Known compounds were identified by comparison of the mass spectra with reference spectra. New compounds were assigned structures from the known hydroxylation pattern in their precursors, by analogy with the normal biosynthetic intermediates, and from their mass spectra. Useful diagnostic ions in the spectra of the methyl ester trimethylsilyl derivatives were *m/e* 129 (45) indicating [19] a 3-hydroxy-group; *m/e* 147 (47) from a vicinal diol [19]

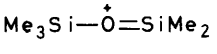
[15] J. L. Courtney and K.F. Swansborough, *Rev. Pure Appl. Chem.*, 1972, **22**, 47.
[16] J. R. Hearder, V. M. Frydman, P. Gaskin, J. MacMillan, C. M. Wels, and B. O. Phinney, *J.C.S. Perkin I*, 1976, 173.
[17] B. E. Cross, J. F. Grove, and A. Morrison, *J. Chem. Soc.*, 1961, 2498.
[18] J. MacMillan and R. J. Pryce, *J. Chem. Soc.* (*C*), 1967, 740.
[19] R. Binks, J. MacMillan, and R. J. Pryce, *Phytochemistry*, 1969, **8**, 271.

and *m/e* 217 (46) from a 2,3-diol;[20] and $M^+ - 149$ from 2,3-dihydroxy-C_{20}-gibberellins and $M^+ - 150$ from 2- or 3-monohydroxy-C_{20}-gibberellins. The isopropylidene derivatives were characterised[21,22] by intense ions at *m/e* 43 and 58. Kaurenolides unsubstituted in ring A give characteristic ions at *m/e* 137 (48) and 109 (49)[23] but with 2- or 3-hydroxylated systems the same fragmentations give[24] ions at *m/e* 135 and 107 due to the elimination of trimethylsilanol from the trimethylsilyl ethers. *ent*-6α,7α-Dihydroxykaurenoic acid shows a base peak at *m/e* 269 (50) but when 2-hydroxylated the trimethylsilyl ether gives intense ions at *m/e* 357 (51) and 267 (*m/e* 357 − Me_3SiOH).

The metabolites from *ent*-3α-hydroxykaurenoic acid (10) [Figure (A)] were the known compounds GA_1 (29), GA_3 (30), GA_{13} (31), and 3β,7β-dihydroxykaurenolide



(45) R = H
(46) R = OMe_3Si



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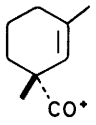
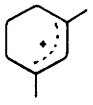


(48)



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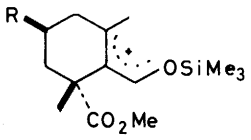
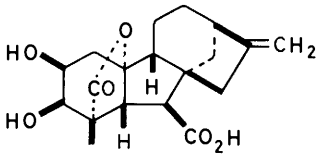


(50) R = H
(51) R = $OSiMe_3$

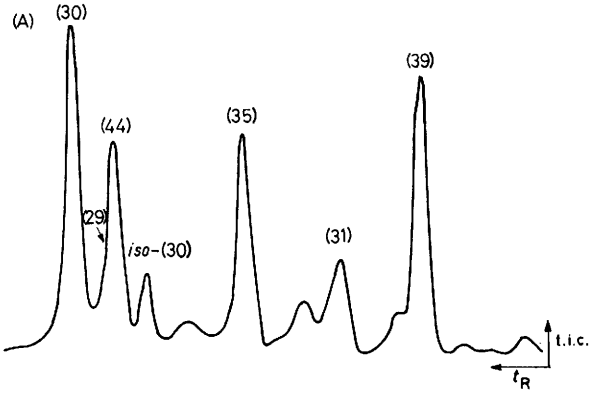


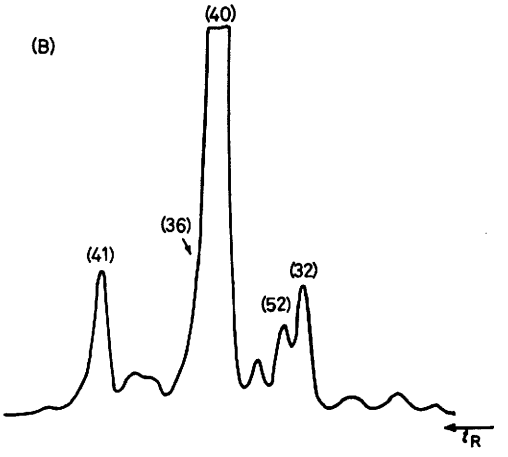
(52)

(35). Two new compounds were assigned the structures (39) and (44) from the mass spectra of their methyl ester trimethylsilyl derivatives. The same metabolites were obtained from *ent*-kaur-16-ene-3α,19-diol incubated with a culture of the wild-type strain GF-1a, grown in the presence of the inhibitor (12). The metabolites from *ent*-2α,3α-dihydroxykaurenoic acid (11) [Figures (B) and (C)] comprised the known[25] higher plant GA_{34} (52) and four new compounds tentatively assigned the structures (32), (36), (40), and (41).

GA_{34} (52) and 2β,3β-dihydroxy-GA_{12} (32) were also obtained in low yield [Figure (D)] from *ent*-kaurene-2α,19-diol (13) together with GA_{43} (33). Two other metabolites were tentatively assigned structures (37) and (42). 2β-Hydroxy-GA_{12} was also detected in trace amounts by g.l.c.–mass spectrometry on an SE-33 column. Similarly the major metabolites [Figure (E)] from *ent*-kaurene-2β,19-diol (14) were tentatively characterised as the *ent*-2β-hydroxy-analogues (34), (38), and (43) of the normal fungal metabolites. A fifth metabolite (*x*) corresponded to a 2β,3β,*x*-trihydroxy-GA_{12}. Interestingly GA_3 (30) was detected [Figure (E)] in amounts which were at least 25 times greater than in control cultures containing the inhibitor (12) but no substrate (see Figure 1b in ref. 1). Also GA_3 (30) was not detected (as a result of incomplete inhibition of GA biosynthesis) when *ent*-kaurene-2α,19-diol (13) was fed to a portion of the same, inhibitor-treated resuspension culture. It is believed therefore that GA_3 (30) is a genuine metabolite of the diol (14).

A number of conclusions may be drawn from these results. First, 2- and 3-hydroxylation of *ent*-kaur-16-en-19-ol (2) does not prevent oxidation to the corresponding *ent*-kaur-16-en-19-oic acids. Secondly, when





FIGURES (A) and (B)

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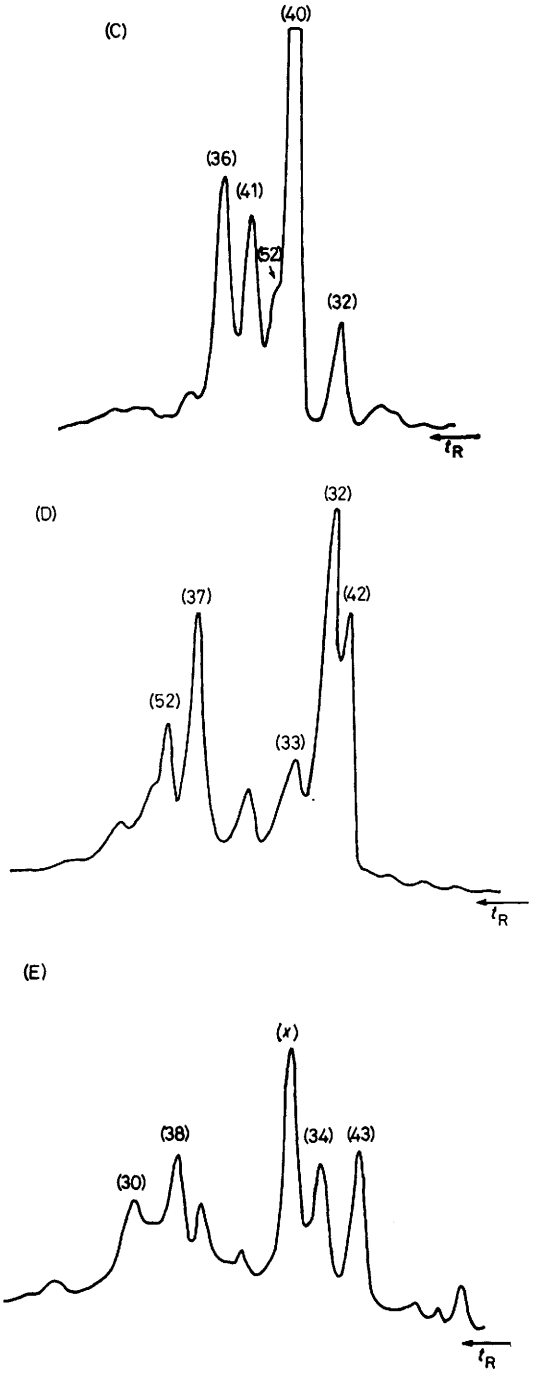
[21] D. C. De Jongh and K. Biemann, *J. Amer. Chem. Soc.*, 1964, **86**, 67.
[22] P. Gaskin and J. MacMillan, *Phytochemistry*, 1975, **14**, 1575.
[23] A. I. Kalinovsky, E. P. Serebryankov, B. M. Zolotarev, A. V. Simolin, V. K. Kucherov, and O. S. Chizhov, *Org. Mass Spectrometry*, 1971, **5**, 33.
[24] J. H. Bateson and B. E. Cross, *J.C.S. Perkin I*, 1972, 1117.
[25] N. Murofushi, T. Yokota, A. Watanabe, and N. Takahashi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1101.



G.l.c.–mass spectrometry of 1 : 200 aliquot portions of derivatised extracts from culture filtrates of *G. fujikuroi*: (A) mutant B1–41a with added acid (10), Me ester Me_3Si derivative, 2% QF–1, from 170 °C at 2° min^{-1}; (B) mutant B1–4a with added acid (11), Me ester Me_3Si derivative, 2% SE–33, from 190 °C at 2° min^{-1}; (C) as for (B), Me ester Me_3Si isopropylidene derivative; (D) GF–1a with added growth retardant (12) and diol (13), Me ester Me_3Si derivative, 2% QF–1, from 160 °C at 3° min^{-1}; and (E) GF–1a with added growth retardant (12) and diol (14), Me ester Me_3Si derivative, 2% QF–1, from 180 °C at 3° min^{-1}

2α- and 2β-hydroxy-groups are present, 3-hydroxylation still occurs and at the normal stage after ring contraction (Scheme 1). Thirdly, 2- and 3-hydroxy-groups do not prevent ring B contraction of *ent*-kaurenoids to GAs although they appear to inhibit this process to varying degrees; thus, whereas in the normal pathway (Scheme 1) the natural substrate, *ent*-7α-hydroxykaurenoic acid (5), does not accumulate, the 2- and 3-hydroxylated analogues do accumulate to varying extents [Figures (A) and (B)]. Fourthly, the presence of an *ent*-2α-hydroxy-group in a C_{20}-GA appears to prevent its conversion into a C_{19}-GA; for example, the yield of GA_{34} (52) from *ent*-2α-hydroxykaurenol (13) is very much lower than the combined yields of the GA_{12} derivatives (32) and (33). Finally an *ent*-2β-hydroxy-group may be dehydrated subsequent to ring B contraction to provide the 1,2-double bond of GA_3 (30).

The observed lack of structure specificity may be peculiar to the strain GF-1a and the mutant B1-41a derived from it, and other strains are currently being examined.

EXPERIMENTAL

For general experimental details see Part 5,[26] except for t.l.c., in which Merck Kieselgel HF was used, and for i.r. spectra, which were obtained for solutions (*ca.* 40 mg ml^{-1}) in methylene chloride (0.2 mm cells). For g.l.c.–mass spectrometry of methylated (CH_2N_2) and trimethylsilylated [hexamethyldisilazane–chlorotrimethylsilane–pyridine (2 : 1 : 1) in sealed capillary tubes] derivatives see Part 19.[1]

Reaction of ent-*Kaur*-16-*ene*-3β,19-*diol* (15) *with* 2,3-*Dihydropyran*.—The diol (15) (2.7 g), dry methylene chloride (170 ml), dihydropyran (840 μl; distilled from potassium hydroxide), and toluene-*p*-sulphonic acid (10 mg) were stirred at room temperature for 45 min. The mixture was washed with aqueous sodium hydrogen carbonate followed by water, dried, and evaporated. The resultant gum (4 g) was adsorbed on silica gel which was placed on a column of silica gel (200 g) made up in light petroleum. Fractions were eluted with increasing concentrations of ethyl acetate in light petroleum and monitored by t.l.c.

ent-3β-(*Tetrahydropyran*-2-*yloxy*)*kaur*-16-*en*-19-*ol* (16) (102 mg), eluted by 12.5—15% ethyl acetate, was crystallised from methylene chloride–light petroleum as needles, m.p. 135—140° (Found: C, 76.9; H, 10.5. $C_{25}H_{40}O_3$ requires C, 77.3; H, 10.4%); δ 0.97 (s, 20-H_3), 1.13 (s, 18-H_3), 2.63br (13-H), 3.21 and 4.20 (dd, *J* 11 Hz, 19-H_2), 4.65 (s, 2′-H), and 4.74br and 4.79br (d, $W_{\frac{1}{2}}$ 11 Hz, 17-H_2); $\nu_{max.}$ 3 625, 1 658, and 870 cm^{-1}; *m/e* (Me_3Si derivative) 390 (M^+, 0%), 375 ($M^+ - 15$, 3), and 85 (100).

Elution with 17.5—22.5% ethyl acetate gave a mixture of two components which were separated by p.l.c. on silica gel developed with ethyl acetate–light petroleum (1 : 2). Elution of the band at R_F *ca.* 0.6 gave the 3-tetrahydropyranyl ether (16) (425 mg). Elution of the band at R_F *ca.* 0.5 gave ent-19-(*tetrahydropyran*-2-*yloxy*)*kaur*-16-*en*-3β-*ol* (508 mg), which crystallised from light petroleum as prisms, m.p. 116—130° (Found: C, 77.7; H, 10.6. $C_{25}H_{40}O_3$ requires C, 77.3; H, 10.4%); $\nu_{max.}$ 3 505, 1 660, and 872 cm^{-1}; *m/e* (Me_3Si derivative) 390 (0%, M^+), 375 (7), and 85 (100); δ 1.00 (s, 20-H_3), 1.26 (s, 18-H_3), 2.63br (13-H), 3.63 and 3.84 (dd, *J* 10 Hz, 19-H_2), 4.63br (s, $W_{\frac{1}{2}}$ 5 Hz, 2′-H), and 4.74br and 4.80br (17-H_2).

[26] J. MacMillan and T. J. Simpson, *J.C.S. Perkin I*, 1973, 1487.

Fractions eluted with 22.5—27.5% ethyl acetate yielded a further 722 mg of the 19-tetrahydropyranyl ether (16). Fractions eluted with 30—32.5% ethyl acetate, after p.l.c. on silica gel with ethyl acetate–light petroleum (2 : 3), gave the 19-tetrahydropyranyl ether (16) (50 mg) and the starting diol (15) (208 mg). The latter (210 mg) was also recovered from fractions eluted with 35—40% ethyl acetate.

ent-19-(*Tetrahydropyran-2-yloxy*)*kaur*-16-*en*-3-*one* (17).—The 19-tetrahydropyranyl ether (16) (1.28 g) in pyridine (30 ml) was added to chromium trioxide (2.82 g) which had been dissolved in pyridine (90 ml) by stirring for 1 h. The solution was stirred for 24 h at room temperature, then added to an excess of water. The product, extracted in ethyl acetate, was passed through a short column of silica gel to remove coloured impurities and was recovered from the eluate to yield the required *ketone* (17) as prisms (1.2 g), m.p. 79—85° (from aqueous methanol) (Found: C, 77.1; H, 10.1. $C_{25}H_{38}O_3$ requires C, 77.7; H, 9.9%); δ 1.14 (s, 20-H_3), 1.20 (s, 18-H_3), 3.50 and 3.81 (dd, *J* 10 Hz, 19-H_2), 4.53br (s, $W_{\frac{1}{2}}$ 6 Hz, 2′-H), and 4.77br and 4.82br (17-H_2); $\nu_{max.}$ 1 705 cm^{-1}.

ent-19-(*Tetrahydropyran-2-yloxy*)*kaur*-16-*en*-3α-*ol* (18).—The ketone (17) (1.2 g) was added to aluminium isopropoxide (60 g) and propan-2-ol (600 ml) and half the solvent was distilled off over 6 h. Water and ethyl acetate were added, the mixture was filtered, and the filtrate was concentrated under vacuum. Water was added and the product (1.2 g), recovered in ethyl acetate, was fractionated by p.l.c. on silica gel with ethyl acetate–light petroleum (3 : 7). Recovery from the band centred at R_F 0.5 gave the required ent-3α-*alcohol* (18) (608 mg) which crystallised from methylene chloride–light petroleum as prisms, m.p. 119—126° (Found: C, 77.5; H, 10.9. $C_{25}H_{40}O_3$ requires C, 77.3; H, 10.4%); $\nu_{max.}$ 3 630 cm^{-1}; δ 1.05 and 1.08 (both s, 18- and 20-H_3), 2.65br (13-H), 3.39 and 3.65 (dd, *J* 10 Hz, 19-H_2), 3.92 (m, $W_{\frac{1}{2}}$ 6 Hz, 3-H), 4.53br (s, $W_{\frac{1}{2}}$ 6 Hz, 2′-H), and 4.74br and 4.78 (17-H_2); *m/e* (Me_3Si derivative) 460 (M^+, 1%), 375 (19), and 85 (100).

Extraction of the band centred at R_F 0.4 gave the *ent*-3β-alcohol (16) (340 mg).

ent-3α-*Acetoxykaur*-16-*en*-19-*ol* (19).—The *ent*-3α-alcohol (18) (608 mg) was dissolved in pyridine (130 ml) and acetic anhydride (38 ml). After 24 h at room temperature, the solution was refluxed for 1 h (t.l.c. showed the absence of starting material). The cooled mixture was added cautiously to aqueous sodium hydrogen carbonate, which was then extracted with ethyl acetate. The recovered product (800 mg) was refluxed with acetic acid–methanol (1 : 9; 150 ml) for 18 h. The usual work-up gave the ent-3α-*acetoxy*-19-*ol* (19) (500 mg), crystallising from methylene chloride–light petroleum as needles, m.p. 172—174° (Found: C, 76.4; H, 9.9. $C_{22}H_{34}O_3$ requires C, 76.3; H, 9.9%); $\nu_{max.}$ 3 640 and 1 725 cm^{-1}; δ 0.97 and 1.02 (both s, 18- and 20-H_3), 2.08 (s, MeCO), 2.65br (s, 13-H), 3.49 and 3.79 (dd, *J* 11 Hz, 19-H_2), 4.76br and 4.81br (17-H_2), and 5.07 (m, $W_{\frac{1}{2}}$ 6 Hz, 3-H).

ent-3α-*Acetoxy*-19-*hydroxy*-17-*norkauran*-16-*one* (20).—The *ent*-3α-acetoxy-19-ol (19) (475 mg) and a few crystals of osmium tetraoxide in water (28 ml) and tetrahydrofuran (28 ml) were stirred at 0 °C. Sodium periodate (760 mg) was added and the mixture was allowed to warm to room temperature. After 22 h the tetrahydrofuran was distilled off under reduced pressure. More water was added and extraction with ethyl acetate yielded a solid (550 mg), which was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (3 : 2) to give the *nor-ketone* (20) (382 mg), crystallising from methylene dichloride–light petroleum as needles, m.p. 180—182° (Found: C, 72.7; H, 9.5%; M^+, 348.230. $C_{21}H_{32}O_4$ requires C, 72.4; H, 9.3%; *M*, 348.230); $\nu_{max.}$ 3 625, 1 740, and 1 728 cm^{-1}; δ 1.01 and 1.12 (both s, 18- and 20-H_3), 2.09 (s, MeCO), 3.52 and 3.79 (dd, *J* 11 Hz, 19-H_2), and 5.07 (m, 3-H).

ent-3α-*Acetoxy*-17-*norkaur*-16-*en*-19-*oic Acid* (21).—The nor-ketone (20) (380 mg) in acetone (250 ml) was oxidised with Jones reagent (1.5 ml) at room temperature under a stream of nitrogen. The crude product, recovered in chloroform in the usual way, was dissolved in carbon tetrachloride (45 ml) and water (45 ml) together with sodium periodate (4.67 g). Ruthenium tetraoxide solution [27] (50 ml) was added and the mixture was stirred at room temperature for 20 h. The aqueous layer was adjusted to pH 3 with 2M-hydrochloric acid and extracted with ethyl acetate. This extract and the organic layer were combined and evaporated to yield a solid (520 mg), which was subjected to p.l.c. on silica gel with ethyl acetate–light petroleum–acetic acid (50 : 50 : 1). Recovery from the band at R_F 0.5 gave the 19-*oic acid* (21) (360 mg), which crystallised from acetone as needles, m.p. 220—222° (Found: C, 69.4; H, 8.6. $C_{21}H_{30}O_5$ requires C, 69.6; H, 8.3%); $\nu_{max.}$ 3 480, 3 350—2 450br, 1 742, 1 735, and 1 697 cm^{-1}; δ 1.06 (s, 20-H_3), 1.27 (s, 19-H_3), 2.10 (s, MeCO), and 5.35br ($W_{\frac{1}{2}}$ 6 Hz, 3-H).

ent-3α-*Acetoxykaur*-16-*en*-19-*oic Acid*.—Methyltriphenylphosphonium bromide (15 g; dried at 100 °C for 24 h) was dissolved in dry tetrahydrofuran (150 ml) under nitrogen, and sodium hydride (8 g of a 60% dispersion in oil, washed with dry light petroleum) was added. The suspension was stirred and warmed until the mixture appeared lime-green in colour; the mixture was stirred for a further 24 h at room temperature and then allowed to settle for 1 h. The yellow supernatant (12 ml) was then added to the nor-ketone (21) (180 mg) and the mixture was stirred for 4 h under nitrogen. The solvent was removed under vacuum and the solid residue was dissolved in water; the solution was acidified to pH 3 with 2M-hydrochloric acid. Extraction with ethyl acetate, and recovery, gave the crude product, which was purified by p.l.c. in ethyl acetate–light petroleum–acetic acid (50 : 50 : 1). Elution of the band at R_F 0.6 gave ent-3α-*acetoxykaur*-16-*en*-19-*oic acid* (143 mg), which crystallised from methylene chloride–light petroleum as plates, m.p. 202—204° (Found: C, 73.3; H, 9.2. $C_{22}H_{32}O_4$ requires C, 73.3; H, 8.95%); $\nu_{max.}$ 3 490, 3 340—2 420br, 1 732, 1 693, 1 657, and 880 cm^{-1}; δ 0.99 (s, 20-H_3), 1.25 (s, 18-H_3), 2.10 (s, $MeCO_2$), 2.66br (13-H), 4.77br and 4.82br (17-H_2), and 5.31br (s, $W_{\frac{1}{2}}$ 5 Hz, 3-H).

ent-3α-*Hydroxykaur*-16-*en*-19-*oic Acid* (10).—The acetate (21) (118 mg) was refluxed with 2% potassium hydroxide in methanol (25 ml) for 4 h. After removal of the methanol under vacuum, water was added and the solution was acidified to pH 3 (2M-HCl). The product (115 mg), recovered in methylene chloride, gave two bands upon p.l.c. in ethyl acetate–light petroleum–acetic acid (40 : 60 : 1). Extraction of the band at R_F 0.4 gave ent-3α-*hydroxykaur*-16-*en*-19-*oic acid* (10) (50 mg), which crystallised from aqueous methanol as needles, m.p. 212—214° (Found: C, 74.8; H, 9.3%; M^+, 318.220. $C_{20}H_{30}O_3$ requires C, 75.4; H, 9.5%; *M*, 318.219); $\nu_{max.}$ 3 630, 3 500, 3 350—2 420br, 1 696, and 880 cm^{-1}; δ 0.98 (s, 20-H_3), 1.35 (s, 18-H_3),

[27] H. Nakata, *Tetrahedron*, 1963, **19**, 1959.

2.67br (s, 13-H), 4.11 (m, $W_{\frac{1}{2}}$ 5 Hz, 3-H), and 4.77br and 4.82br (17-H_2); *m/e* (for Me ester Me_3Si derivative) 404 (M^+, 1%), 389 (8), 275 (73), 187 (100), and 129 (30). Starting material (50 mg) was recovered from the p.l.c. band at R_F 0.6.

ent-3β-*Hydroxykaur*-16-*en*-19-*oic Acid*.—The acid (21) in methanol was treated with ethereal diazomethane. The resulting methyl ester (74 mg) in tetrahydrofuran (1 ml) was treated in an atmosphere of nitrogen with salt-free methylenetriphenylphosphorane (4 ml; prepared as described earlier) for 4 h at room temperature. The solvent was removed under vacuum, ethyl acetate was added, and the mixture was washed with water. Evaporation of the organic layer, followed by p.l.c. in ethyl acetate–light petroleum (3 : 7) gave a solid (66 mg); δ 0.87 (s, 20-H_3), 1.18 (s, 18-H_3), 2.09 (s, $MeCO_2$), 2.66br (13-H), 3.69 (s, 19-CO_2Me_3), 4.77br and 4.82br (17-H_2), and 5.35 (m, $W_{\frac{1}{2}}$ 5 Hz, 3-H). This solid (65 mg) was dissolved in dry dimethyl sulphoxide (4 ml) and potassium t-butoxide (resublimed; 400 mg) was added. The mixture was stirred at 55 °C for 1 h under nitrogen; t.l.c then showed conversion into a more polar product. The mixture was added to water, the pH brought to 2 (1.5M-HCl), and the resulting mixture extracted with ethyl acetate. P.l.c. of the crude extract in ethyl acetate–light petroleum–acetic acid (40 : 60 : 1) and extraction of the band at R_F 0.6 gave the ent-3β-*hydroxy-acid* (30 mg), which crystallised from ethyl acetate–light petroleum as needles, m.p. 207—210° (Found: M^+, 318.219. $C_{20}H_{30}O_3$ requires M, 318.219); δ 1.00 (s, 20-H_3), 1.48 (s, 18-H_3), 2.66br (13-H), 3.14 (m, $W_{\frac{1}{2}}$ 19 Hz, 3-H), and 4.77 and 4.81br (17-H_2); $\nu_{max.}$ 3 565, 3 490, 3 300—2 400br, 1 684, 1 658, and 876 cm^{-1}; *m/e* (for Me_3Si ether Me ester) 404 (M^+, 1), 389 (8), 275 (72), 187 (100), and 129 (40).

Selective Benzoylation of ent-*Kaur*-16-*ene*-3β,19-*diol* (15) (cf. *ref.* 28).—The diol (15) (2.0 g) in pyridine (63 ml) was stirred at room temperature for 28 h with benzoyl chloride (0.81 ml). Work-up in the usual way gave an oil (3.5 g) which was adsorbed on silica and placed on a column of silica gel (200 g) made up in light petroleum. The column was eluted with increasing concentrations of ethyl acetate in light petroleum and fractions were monitored by t.l.c.

The dibenzoate (166 mg) was eluted with 20% ethyl acetate and crystallised from ethyl acetate as needles, m.p. 181—183° (lit.,[28] 162—163°) (Found: C, 79.5; H, 7.9. Calc. for $C_{34}H_{40}O_4$: C, 79.65; H, 7.9%); $\nu_{max.}$ 1 713, 1 658, and 1 603 cm^{-1}; δ 1.16 (s, 20-H_3), 1.23 (s, 18-H_3), 2.66br (13-H), 4.50 and 4.78 (dd, J 11 Hz, 19-H_2), 4.80br (17-H_2), 4.91 (m, 3-H), and 7.34 and 7.99 (10 ArH).

ent-19-Benzoyloxykaur-16-en-3β-ol (22) (937 mg) was eluted with 25—30% ethyl acetate and crystallised from ethyl acetate–light petroleum as needles, m.p. 137—139° (lit.,[28] 135—136°) (Found: M^+, 408.266. Calc. for $C_{27}H_{36}O_3$: M, 408.266); $\nu_{max.}$ 3 600, 1 715, 1 658, and 1 608 cm^{-1}; δ 1.07 (s, 20-H_3), 1.25 (s, 18-H_3), 2.66br (13-H), 3.34 (m, $W_{\frac{1}{2}}$ 18 Hz, 3-H), 4.34 and 4.62 (dd, J 12 Hz, 19-H_2), 4.74br and 4.80br (17-H_2), and 7.47 and 8.02 (5 ArH).

35—50% Ethyl acetate eluted a mixture (740 mg) which was separated by p.l.c. on silica gel with ethyl acetate–light petroleum (2 : 3) into the 19-monobenzoate (22) (280 mg; R_F 0.7) and the diol (15) (53 mg; R_F 0.3). Further elution gave a further 55 mg of the diol (15).

ent-19-*Benzoyloxy*-3β-*hydroxy*-17-*norkauran*-16-*one*.—The benzoate (22) (1.0 g) in tetrahydrofuran (50 ml) and water (50 ml) was oxidised with osmium tetraoxide (20 mg) and sodium periodate (1.35 g) in the usual way to yield the *nor-ketone*, which was purified by p.l.c. (R_F 0.5) on silica gel with ethyl acetate–light petroleum and crystallised from ethyl acetate–light petroleum as needles (714 mg), m.p. 170—172° (Found: C, 76.0; H, 8.7. $C_{26}H_{34}O_4$ requires C, 76.1; H, 8.35%); $\nu_{max.}$ 3 615, 1 737, 1 714, and 1 603 cm^{-1}; δ 1.15 (s, 20-H_3), 1.28 (s, 18-H_3), 3.36 (m, $W_{\frac{1}{2}}$ 19 Hz, 3-H), 4.40 and 4.63 (each d, J_{AB} 11 Hz, 19-H_2), and 7.49 (m), and 8.04 (dd, J 8 Hz) (5 ArH).

ent-19-*Benzoyloxy*-17-*norkaur*-2-*en*-16-*one* (23).—The foregoing nor-ketone (700 mg) in pyridine (20 ml) was treated with phosphoryl chloride (1.3 ml) at room temperature for 24 h and the solution was then refluxed for 1 h. The usual work-up gave a pink solid (756 mg) which was filtered through a column of silica gel in ethyl acetate. The olefin (23) thus obtained crystallised from ethyl acetate as feathery needles (530 mg), m.p. 182—185° (lit.,[28] 179—180°) (Found: C, 80.0; H, 8.3. Calc. for $C_{26}H_{32}O_3$: C, 79.95; H, 8.2%); for $\nu_{max.}$ and δ see ref. 28.

ent-19-*Hydroxy*-17-*norkaur*-2-*en*-16-*one*.—The benzoate (23) (530 mg) was refluxed for 1.5 h with potassium hydroxide (1.15 g) in methanol (115 ml). The usual work-up gave *ent*-19-hydroxy-17-norkaur-2-en-16-one (394 mg), needles, m.p. 157—160° (lit.,[29] 157—159°) (from aqueous methanol); for $\nu_{max.}$ and δ see ref. 29.

ent-19-(*Tetrahydropyran*-2-*yloxy*)-17-*norkaur*-2-*en*-16-*one* (24).—The foregoing alcohol (380 mg) was dissolved in methylene chloride (50 ml) and dihydropyran (1 ml), and toluene-*p*-sulphonic acid (6 mg) was added. After 3 h at room temperature t.l.c. showed the absence of starting material. The mixture was washed with water and the dried solution was evaporated. P.l.c. of the residue in ethyl acetate–light petroleum (2 : 3) with subsequent elution of the band at R_F 0.8 gave the *tetrahydropyranyl ether* (34) (425 mg), which crystallised from aqueous methanol as needles, m.p. 96—99° (Found: C, 77.7; H, 10.2. $C_{24}H_{36}O_3$ requires C, 77.4; H, 9.7%); δ 1.13 and 1.16 (both s, 18- and 20-H_3), 4.52 (m, $W_{\frac{1}{2}}$ 7 Hz, 2′-H), and 5.61 (m, $W_{\frac{1}{2}}$ 14 Hz, 2- and 3-H); $\nu_{max.}$ 1 740 and 992 cm^{-1}.

ent-2α,3α-*Dihydroxy*-19-(*tetrahydropyran*-2-*yloxy*)-17-*norkauran*-16-*one* (25).—The tetrahydropyranyl ether (24) (420 mg) was stirred in pyridine (20 ml) at 0 °C, and osmium tetraoxide (500 mg) was added. The mixture was allowed to warm to room temperature and left for 42 h; t.l.c. then showed the absence of starting material. Potassium hydroxide (5 g) and mannitol (5 g) in water (50 ml) were added and the solution was refluxed for 2 h. Isolation in ether gave a gum (390 mg), which was purified by p.l.c. in methanol–chloroform (1 : 19). Extraction of the band at R_F 0.4 gave the *diol tetrahydropyranyl ether* (25) (270 mg), which crystallised from ethyl acetate as needles, m.p. 187—193° (Found: C, 70.3; H, 9.1. $C_{24}H_{38}O_5$ requires C, 70.9; H, 9.4%); δ 1.15 and 1.17 (both s, 18- and 20-H_3) and 4.52 (m, $W_{\frac{1}{2}}$ 6 Hz, 2′-H); $\nu_{max.}$ 3 620, 3 565, and 1 740 cm^{-1}.

ent-2α,3α-*Diacetoxy*-17-*norkaur*-16-*en*-19-*ol* (26).—The diol (25) (270 mg) in pyridine (10 ml) was treated with acetic anhydride (3 ml) at room temperature for 24 h and then at 100 °C for 1 h. The cooled solution was diluted with methanol and then concentrated. An excess of water was added and the aqueous solution was extracted with ethyl acetate; the extract was then washed with aqueous sodium

[28] I. F. Cook, P. R. Jefferies, and J. R. Knox, *Tetrahedron*, 1975, **31**, 251.

[29] E. L. Ghisalberti, P. R. Jefferies, and E. J. Middleton *Austral. J. Chem.*, 1969, **22**, 455.

hydrogen carbonate followed by water. Evaporation gave an oil (400 mg). P.l.c. in ethyl acetate–light petroleum (1 : 1) and elution of the band at R_F 0.5 gave the diacetate tetrahydropyranyl ether (320 mg); δ 1.02 (s, 20-H_3), 1.25 (s, 18-H_2), 1.99 and 2.12 (both s, 2 × $MeCO_2$), 4.60 (m, $W_{\frac{1}{2}}$ 7 Hz, 2′-H), 5.28 (m, $W_{\frac{1}{2}}$ *ca.* 18 Hz, 2-H), and 5.39 (m, $W_{\frac{1}{2}}$ 5 Hz, 3-H); $\nu_{max.}$ 1 740, 1 232, and 1 035 cm^{-1}. This compound (200 mg) in acetone (6 ml) and methanol (0.6 ml) was treated with toluene-*p*-sulphonic acid (3 mg). After 8 h at room temperature t.l.c. showed the presence of starting material and a slower moving component. More methanol (0.1 ml) and toluene-*p*-sulphonic acid (one crystal) were added, and after a total of 24 h no starting material remained. The solution was concentrated under vacuum, an excess of water was added, and the solution was extracted with ethyl acetate to give an oil (173 mg). P.l.c. in ethyl acetate–light petroleum (3 : 2) and extraction of the band at R_F 0.25 gave the *diacetate alcohol* (26) (156 mg), which crystallised from ethyl acetate as prisms, m.p. 195—197° (Found: C, 67.1; H, 8.45. $C_{23}H_{34}O_6$ requires C, 67.9; H, 8.4%); δ 1.06 (s, 20-H_3), 1.26 (s, 18-H_3), 2.04 and 2.16 (both s, 2 × $MeCO_2$), 3.91 and 3.56 (AB dd, *J* 11 Hz, 19-H_2), 5.31 (m, $W_{\frac{1}{2}}$ 20 Hz, 2-H), and 5.42 (m, $W_{\frac{1}{2}}$ 5 Hz, 3-H); $\nu_{max.}$ 3 635, 1 745, 1 734, 1 232, and 1 038 cm^{-1}.

ent-2α,3α-*Diacetoxy*-16-*oxo*-17-*norkauran*-19-*oic Acid* (27). —The diacetate alcohol (26) (150 mg) in acetone (225 ml) was treated with Jones reagent (0.52 ml) under nitrogen for 2.25 h. T.l.c. of the product, isolated in the usual way, showed one component at a higher R_F value than starting material, shown to be the expected diacetate aldehyde on the basis of its n.m.r. (60 MHz) spectrum: δ 1.1 (s, 18- and 20-H_3), 2.0 and 2.2 (both s, 2 × $MeCO_2$), 5.2 (m, $W_{\frac{1}{2}}$ 21 Hz, 2-H), 5.6 (m, $W_{\frac{1}{2}}$ 5 Hz, 3-H), and 9.65 (s, 19-H). This compound was dissolved in carbon tetrachloride (19 ml) and water (19 ml), and sodium periodate (1.94 g) was added. Ruthenium tetraoxide solution (20 ml) was added to the mixture, which was then stirred at room temperature. After 24 h the organic and aqueous layers were separated. The aqueous layer was adjusted to pH 2 and extracted with ethyl acetate. This extract was added to the carbon tetrachloride layer, and the solvents were evaporated off to give a solid (216 mg). P.l.c. in ethyl acetate–light petroleum–acetic acid (50 : 50 : 1) and elution of the band at R_F 0.5 gave the acid (27) (118 mg), which crystallised from ethyl acetate–light petroleum as prisms, m.p. 238—240° (Found: M^+, 420.214. $C_{23}H_{32}O_7$ requires *M*, 420.215); δ 1.14 (s, 20-H_3), 1.29 (s, 18-H_3), 2.00 and 2.15 (both s, 2 × $MeCO_2$), 5.47 (m, $W_{\frac{1}{2}}$ 21 Hz, 2-H), and 5.60 (m, $W_{\frac{1}{2}}$ 6 Hz, 3-H); $\nu_{max.}$ 3 470, 3 350—2 410br, 1 747, 1 735, 1 228, and 1 033 cm^{-1}.

ent-2α,3α-*Dihydroxykaur*-16-*en*-19-*oic Acid* (11).—Salt-free methylenetriphenylphosphorane was prepared as described for the preparation of *ent*-3α-acetoxykaurenoic acid (same quantities). The nor-ketone (27) (60 mg) in dry tetrahydrofuran (1 ml) under nitrogen was treated with ylide supernatant (3 ml) and stirred for 3.5 h at room temperature. The tetrahydrofuran was blown off under nitrogen and the residue was dissolved in water which was acidified to pH 3 (2M-HCl) and extracted with ethyl acetate. P.l.c. of the material recovered from the ethyl acetate on silica gel with ethyl acetate–light petroleum–acetic acid (40 : 60 : 1) and recovery of the band at R_F 0.5 gave an olefinic product (45 mg); δ 1.08 (s, 20-H_3), 1.28 (s, 18-H_3), 2.00 and 2.15 (both s, 2 × $MeCO_2$), 2.68br (s, $W_{\frac{1}{2}}$ 10 Hz, 13-H), 4.78br and 4.83br (d, $W_{\frac{1}{2}}$ 10 Hz, 17-H_2), 5.48 (m, $W_{\frac{1}{2}}$ *ca.* 22 Hz, 2-H), and 5.58 (s, $W_{\frac{1}{2}}$ 5 Hz, 3-H); $\nu_{max.}$ 3 475, 3 000—2 400br, 1 744, 1 700, 1 658, 1 230, 1 034, and 880 cm^{-1}. This product (58 mg) in dry methanol (7 ml) was stirred at room temperature with 0.05M-sodium methoxide (7 ml; from sodium in dry methanol). After 18 h t.l.c. showed the presence of starting material and a slower moving spot. Further methoxide solution (5 ml) was added and after a total of 24 h no starting material remained. The methanol was removed under vacuum, water was added, and the solution was adjusted to pH 3 (1.5M-HCl). Recovery in ethyl acetate yielded the *diol* (11) (47 mg), which crystallised from methanol as microprisms, m.p. 265—269° (decomp.) (Found: C, 71.5; H, 9.05. $C_{20}H_{30}O_4$ requires C, 71.8; H, 9.0%); δ (C_5D_5N) 1.27 (s, 20-H_3), 1.78 (s, 18-H_3), 2.60br (s, $W_{\frac{1}{2}}$ 10 Hz, 13-H), 4.78 (s, $W_{\frac{1}{2}}$ 5 Hz, 3-H), *ca.* 4.86 (m, 2-H), and 4.87br (s, $W_{\frac{1}{2}}$ 10 Hz, 17-H_2); $\nu_{max.}$ 3 470, 3 250, 3 100—2 400br, 1 706, 1 676, and 868 cm^{-1}; *m/e* [for Me ester bis-(Me_3Si) ether derivative] 492 (M^+, <1%), 477 (6), 417 (35), 402 (46), 343 (100), 253 (32), 218 (26), 217 (26), 187 (33), 147 (42), 143 (44), 75 (36), and 73 (80).

Culture Conditions.—(a) *Mutant B*1-41*a of* Gibberella fujikuroi. Resuspension cultures were prepared as described in Part 9 [2] except that the concentration of potassium dihydrogen phosphate in the resuspension medium was the same (5.0 g l^{-1}) as that in the original medium. Substrates were fed and the cultures were extracted and analysed as described in Part 14.[3]

(b) *Strain GF-*1*a of* G. fujikuroi. Resuspension cultures were prepared and fed with unlabelled substrates (3 mg) in methanol (200 μl) as described in Part 19.[1] The methods of extraction and analysis by g.l.c. and g.l.c.–mass spectrometry were also as given in that paper.

Mass Spectral Data.—Data for new compounds identified from feeding studies are available as Supplementary Publication No. SUP 22102 (3 pp.).*

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* For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1976, Index issue.