Fungal Products. Part VIII.[1] New Kaurenolides from *Gibberella fujikuroi*

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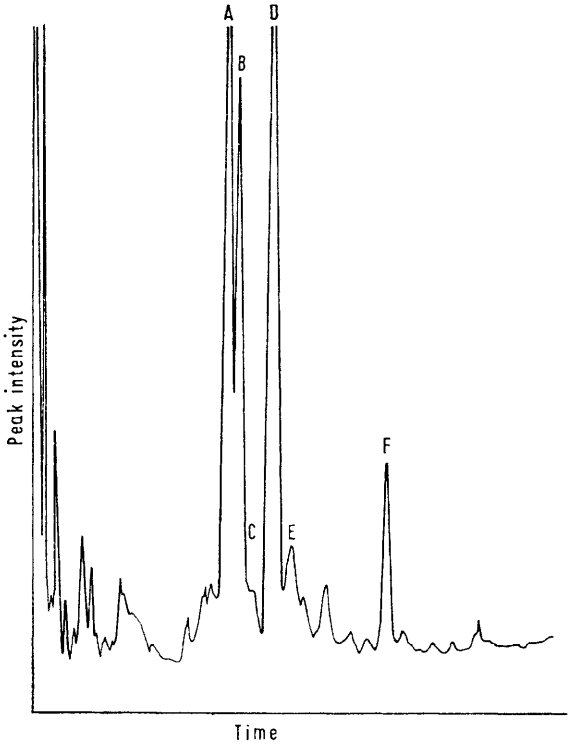
The isolation of four new kaurenolides from the culture filtrates of *Gibberella fujikuroi* strain TP70 is described. Structures (4), (6), (9), and (10), deduced for these metabolites from chemical and spectroscopic data, represent three new positions (at C-1, -3, and -11) of hydroxylation of *ent*-kaur-16-ene by this fungus. In addition 7β,11α-dihydroxy-18-norkaurenolide (11) has been isolated.

CULTURES of *Gibberella fujikuroi* produce a range of tetracyclic diterpenes. In a detailed investigation of the neutral metabolites of strain ACC917 Cross *et al.*[2] isolated three lactones subsequently shown[3,4] to be (1)—(3). A fourth lactone (5) was isolated[5] from strain PG-7 and from cultures of strain ACC917 to which steviol (7) had been added.[6] We sought a source of these and other 7β-hydroxykaurenolides (*ent*-6β,7α-dihydroxykaur-16-en-19-oic acid 19,6-lactones) † for conversion[7-9] into gibberellin A_{12}-aldehyde (8) and its derivatives which were required for biosynthetic studies of the gibberellin group of plant hormones.

After a preliminary survey of the extracts from several strains of *G. fujikuroi* by combined g.l.c.–m.s. the strain TP70 was selected for large-scale investigation of both the neutral and acidic metabolites (investigation of the acidic metabolites will be described in a later paper). G.l.c.–m.s. of the total neutral fraction from TP70, after trimethylsilylation, showed (Figure) the presence of the two known kaurenolides (1) (Peak A) and (2) (Peak D) together with four new kaurenolides which were isolated by column and thin-layer chromatography and assigned structures (4), (6), (9), and (10). A fifth new compound, the 18-norkaurenolide (11) and the previously described[10] ketone (12) were also isolated but, despite a careful search, 7β,13-dihydroxykaurenolide (5) was not detected.

3β,7β-Dihydroxykaurenolide (6), the major new metabolite (Peak B, Figure) was isolated independently by Bateson and Cross[11] and ourselves.[9,12] Our evidence for structure (6) is similar to that subsequently detailed by Bateson and Cross.[13] In addition to the data presented by the latter authors[13] evidence for a 3-hydroxy-group was provided both by the presence of an intense ion at *m/e* 129 in the mass spectrum of the bistrimethylsilyl (TMS) ether and by base-induced conversion of the derived 3,7-diketone (13) into a single epimer (presumably 4β-Me) of the 19-nor-derivative (16). The latter reaction is analogous[13] to the chromous chloride reduction of the diketone (13) to the 19-nor-derivative (17).

7β,11α-Dihydroxykaurenolide (9) was a minor metabolite (Peak E, Figure) which was separated from the 3β,7β-isomer (6) by multiple elution t.l.c. A dihydroxykaurenolide structure was indicated by combustion analysis, by the formation of a bis-TMS ether (M^+ 476),



Total ion current trace of methylated-trimethylsilylated crude neutral fraction from *G. fujikuroi* TP70 [2% QF-1 on Gas Chrom Q in a glass column (9 ft × 1/16 in); temperature programmed from 210° at 2° min^{-1}; He flow rate 6 ml min^{-1}]

by γ-lactone carbonyl absorption at 1767 cm^{-1}, and by oxidation to a diketone (14). The typical pattern for the 5-, 6-, and 7-protons of a 7β-hydroxykaurenolide was evident in the n.m.r. spectrum of the monoacetate (18) (Table) although it was obscured in the spectrum of the

† For clarity the trivial kaurenolide nomenclature is used in the Discussion section.

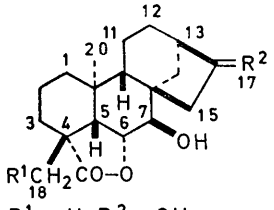
[1] Part VII, J. R. Bearder, J. MacMillan, and B. O. Phinney, *Phytochemistry*, 1973, in the press.
[2] B. E. Cross, R. H. B. Galt, J. R. Hanson, P. J. Curtis, J. F. Grove, and A. Morrison, *J. Chem. Soc.*, 1963, 2937.
[3] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1963, 2944.
[4] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1963, 3783.
[5] E. P. Serebryakov, A. V. Simolin, V. F. Kucherov, and B. V. Rosynov, *Tetrahedron*, 1970, **26**, 5215.
[6] J. R. Hanson and A. F. White, *Tetrahedron*, 1968, **24**, 6291.
[7] B. E. Cross, K. Norton, and J. C. Stewart, *J. Chem. Soc.* (*C*), 1968, 1054.
[8] J. H. Bateson and B. E. Cross, *J.C.S. Chem. Comm.*, 1972, 649.
[9] P. Hedden and J. MacMillan, *Tetrahedron Letters*, 1971, 4939.
[10] B. E. Cross and R. E. Markwell, *J. Chem. Soc.* (*C*), 1971, 2980.
[11] J. H. Bateson and B. E. Cross, *Tetrahedron Letters*, 1971, 3407.
[12] M. J. Grinsted, B.Sc. Thesis, Bristol University, 1971.
[13] J. H. Bateson and B. E. Cross, *J.C.S. Perkin I*, 1972, 1117.

N.m.r. data (τ) [a] for some protons in kaurenolides and derivatives

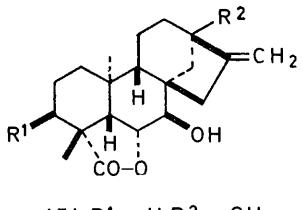
Compound	5-H [b]	6-H [c]	7-H [b]	17-H_2 [d]	18-H_3 [e]	20-H_3 [e]	Other protons
(1)	8·26	5·20	5·65	5·12, 4·99	8·72	9·18	
(6)	8·06	5·37	5·70	5·16, 5·00	8·67	9·06	*ca.* 5·62 (m, 3-H)
(9)	8·23	*ca.* 5·40	*ca.* 5·40	5·12, 5·00	8·71	8·83	5·73 (m, 11-H)
(10)	7·76	5·30	5·60	5·13, 5·00	8·72	9·25	6·42 (m, 1-H)
(20)	8·20	5·31	4·18	5·10, 4·97	8·70	9·02	
(6)-Diacetate	7·98	5·29	4·20	5·10, 4·98	8·67	8·94	
(18)	8·18	5·28	3·99	5·09, 4·99	8·71	8·74	5·74 (m, 11-H)
(10-Diacetate	7·79	5·25	4·17	5·09, 4·98	8·07	9·06	5·20 (m, 1-H)
(1)-7-Ketone	7·79	5·15 [b]		5·12, 4·91	8·69	9·30	
(13)	7·20	5·13 [b]		5·07, 4·91	8·44	9·23	
(14)	7·79	5·11 [b]		4·84, 4·76	8·74	9·11	
(15)	7·23	4·96 [b]		5·08, 4·91	8·52	9·02	

[a] For $CDCl_3$ solutions at 100 MHz. [b] Doublet, J 6—8 Hz. [c] Triplet, J *ca.* 6 Hz. [d] Multiplet. [e] Singlet.

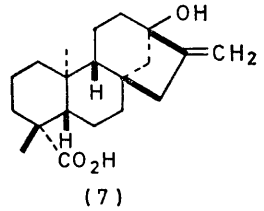
metabolite itself since the 7α-proton was deshielded and coincident with the 6β-proton signal at τ 5·40.



(1) R^1 = H, R^2 = CH_2
(2) R^1 = OH, R^2 = CH_2
(3) R^1 = OH, R^2 = α-OH,Me
(4) R^1 = OAc, R^2 = CH_2



(5) R^1 = H, R^2 = OH
(6) R^1 = OH, R^2 = H



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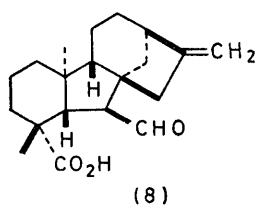

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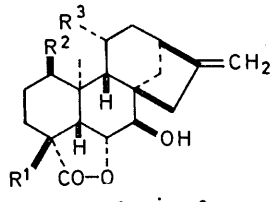
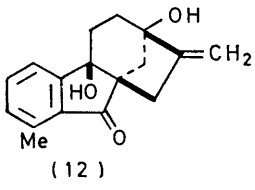


(9) R^1 = Me, R^2 = H, R^3 = OH
(10) R^1 = Me, R^2 = OH, R^3 = H
(11) R^1 = R^2 = H, R^3 = OH



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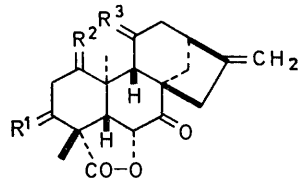
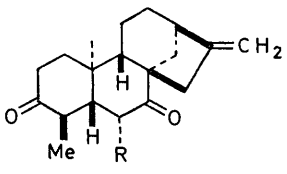
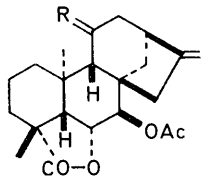


(13) R^1 = O, R^2 = R^3 = H_2
(14) R^1 = R^2 = H_2, R^3 = O
(15) R^1 = R^3 = H_2, R^2 = O

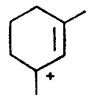


(16) R = OH
(17) R = H

The presence of a hydroxymethine proton at τ 5·75 with $W_{\frac{1}{2}}$ 10—11 Hz in the metabolite (9) and the monoacetate (18) and the oxidation of these compounds to the ketones (14) and (19) respectively established that the second hydroxy-group was secondary. This hydroxy-group was not present in ring A since the mass spectra of the metabolite (9), the bis–TMS ether, the diketone (14), and the keto-acetate (19) all showed an intense ion at *m/e* 109. This ion has been assigned [14] the structure (21) which cannot be derived from a kaurenolide substituted in ring A. Deuterium exchange by treatment of the keto-acetate (19) with deuterium oxide and sodium methoxide in tetrahydrofuran gave a dihydroxy-acid (22) which was shown to contain 58% $^2H_3$ and 42% $^2H_2$



(18) R = α-OH,H
(19) R = O
(20) R = H_2



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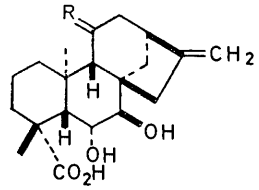
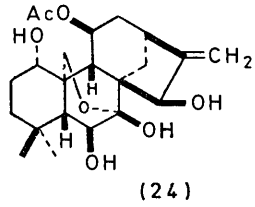


(22) R = O
(23) R = H_2



(24)

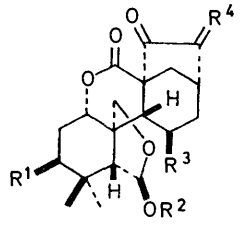
by g.l.c.–m.s. of the methyl ester. In a parallel experiment 7β-acetoxykaurenolide (20) gave the dihydroxy-acid (23) [15] containing no deuterium. These results showed the presence of an 11- or 12-hydroxy-group and of the four possibilities 11α-substitution was assigned from the following evidence. First the hydroxy-group is hindered as shown by the difficulty in preparing a di-acetate. Secondly the 20- and 7α-protons are strongly deshielded in the metabolite (9) and the monoacetate (18) compared to these protons in the other kaurenolides (Table). Thirdly the multiplicity ($W_{\frac{1}{2}}$ 10—11 Hz) of the hydroxymethine proton in the metabolite (9) and the

[14] A. J. Kalinovsky, E. P. Serebryakov, A. V. Simolin, V. F. Kucherov, and O. S. Chizhov, *Org. Mass. Spectrometry*, 1971, **3**, 33.

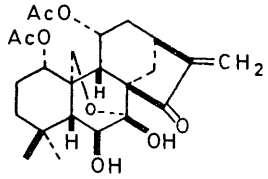
[15] J. R. Bearder, J. MacMillan, and B. O. Phinney, *Phytochemistry*, 1973, **12**, 2173.

monoacetate (18) is consistent with an 11β-proton in a boat conformation of ring C.

Several analogous 11-oxygenated kaurenoids have been isolated from *Isodon* species. Ring C in sodoponin (26) has been shown [16] to be in a boat conformation from the *J* values for the 12-, 13-, and 14-protons and a boat conformation for ring C in the compound (25), related to



(25) R^1 = OAc, R^2 = $OCOCH_2Br$, R^3 = H, R^4 = H, α-Me
(26) R^1 = R^2 = H, R^3 = OH, R^4 = CH_2



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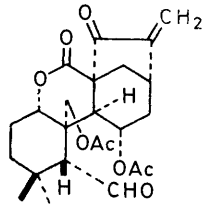
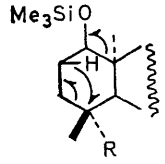


(28)

nodosin (26), has been established [17] by *X*-ray diffraction. Thus the triplet [τ 6·16 (*J* 4 Hz)] for the 11-proton in nodosin (26) [18] and the quartet [τ 4·0 (*J* 9 Hz)] for the 11-proton in sodoponin (24) [16] support the assigned structure (9) for the present metabolite with an 11α-hydroxy-group in a boat conformation of ring C. The extremely high vicinal coupling constants, reported for the 11-protons in shikokianin (27) [19] and acetylisodonal (28) [20] of 18 and 15 Hz respectively casts doubt on the interpretation of the n.m.r. of these compounds and their usefulness as model compounds.

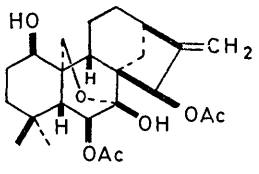
1β,7β-Dihydroxykaurenolide (10) (Peak C), obtained as a gum, was characterised as a 7β-hydroxykaurenolide containing another secondary hydroxy group by high



(29)

resolution mass spectromerty, by the formation of a diacetate, a bis-TMS ether, and a diketone (15), by γ-lactone carbonyl absorption at 1765 cm^{-1}, and by the characteristic n.m.r. data for the 5-, 6-, and 7-protons of the metabolite and its diacetate (Table). The mass spectrum of the bis-TMS ether of this new kaurenolide contained a base peak at *m/e* 129 previously considered [21] to be diagnostic of the fragmentation of a 3-TMS ether but which may also arise by an analogous fragmentation (29) of a 1-TMS ether. However, a 3-hydroxy-group was excluded by the non-identity of the derived diketone (15) with 3,7-diketokaurenolide (13). The 1β-configuration was assigned from the observed deshielding of the 5β-proton in the metabolite (10), and the diacetate (Table), and from the observed multiplicity ($W_{\frac{1}{2}}$ 7 Hz) of the 1α-proton which occurs as an incompletely resolved triplet at τ 6·42 in the metabolite (10) and at τ 5·2 in the diacetate. This multiplicity is consistent with a 1α(eq)-proton in a chair conformation of ring A. It agrees with the reported [22] triplet (*J* 2 Hz) at τ 6·42 for the 1α-proton in trichokaurin (30) and contrasts with the larger value (*J* 8 Hz) reported for the 1β-protons in oridonin (31) [23] and its 6,13-isopropylidene derivative, emmedol.[24]

18-Acetoxy-7β-hydroxykaurenolide (4) (Peak F) was obtained as a gum. The n.m.r. spectrum closely resembled that of 7,18-dihydroxykaurenolide (3) except for the additional signals of the acetate methyl and of the 18-methylene protons which occurred downfield as an AB system at τ 5·82 and 5·86. The mass spectra of



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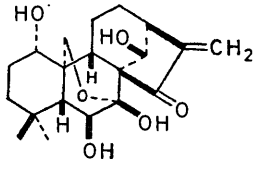


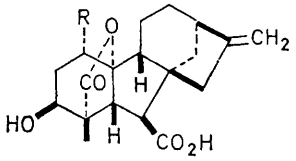
(31)

18-acetoxy- and 18-hydroxy-7β-hydroxykaurenolide were very similar apart from the loss of acetic acid in the former and water in the latter. Structure (4) was confirmed by acetylation of 7,18-dihydroxykaurenolide with an equimolar amount of acetic anhydride to give the metabolite (4) in 55% yield.

The norkaurenolide (11) was the major component of a mixture obtained by partition chromatography of the mother liquors from 3β,7β-dihydroxykaurenolide (6). The two minor components formed bis-TMS ethers with the correct molecular weight for dihydroxykaurenolides but insufficient material was available for further characterisation. The structure (11) for the major component was deduced from the molecular formula $C_{19}H_{20}O_4$ determined by high resolution mass spectrometry, and from the following spectroscopic data. The n.m.r.

[16] E. Fujita, T. Fujita, M. Taoka, H. Katayama, and M. Shibuya, *Tetrahedron Letters*, 1970, 421.
[17] M. Natsume and Y. Iitaka, *Acta Cryst.*, 1966, **20**, 197.
[18] E. Fujita, T. Fujita, and M. Shibuya, *Tetrahedron Letters*, 1966, 3153.
[19] T. Kubota and I. Kubo, *Bull. Chem. Soc. Japan*, 1969, **42**, 1778.
[20] T. Kubota and I. Kubo, *Tetrahedron Letters*, 1967, 3781.
[21] R. Binks, J. MacMillan, and R. J. Pryce, *Phytochemistry*, 1969, **8**, 271.
[22] E. Fujita, T. Fujita, M. Shibuya, and T. Shingu, *Tetrahedron*, 1969, **25**, 2517.
[23] E. Fujita, T. Fujita, H. Katayama, M. Shibuya, and T. Shingu, *J. Chem. Soc.* (*C*), 1970, 1674.
[24] S. Mori, K. Shudo, T. Ageta, T. Koizuni, and T. Okamoto, *Chem. and Pharm. Bull.*, 1970, **18**, 871.

spectrum of the norkaurenolide (11), obtained in [$^2H_5$]-pyridine, was very similar to that of 7β,11α-dihydroxykaurenolide (9) in the same solvent except for two important differences. First the spectrum of the norkaurenolide (11) contained only one tertiary methyl signal, the one at τ 8·69 in the spectrum of 7β,11α-dihydroxykaurenolide (9) being absent. This methyl signal can be assigned to the 18-protons since it is present at about the same chemical shift in 7β-hydroxy-, 1β,7β-dihydroxy-, and 7β,11α-dihydroxy-kaurenolides but occurs at lower field (Δδ *ca.* 0·5 p.p.m.) in 3β,7β-dihydroxykaurenolide. Secondly the 6β-proton in the norkaurenolide (11) occurs at higher field (0·25 p.p.m.) than in 7β,11α-dihydroxykaurenolide. A similar deshielding of the 6β-proton by the 4β-methyl group in 7β-hydroxykaurenolide is shown by the data presented by Hanson and White.[25] Further support for structure (11) for the norkaurenolide is provided by the identical fragmentation pattern in the mass spectra of the norkaurenolide and 7β,11α-dihydroxykaurenolide and of their TMS ethers.



(32) R = OH
(33) R = H

The kaurenolides (9) and (10) are the first examples of hydroxylation at C-1 and -11 in the *ent*-kaurenoid metabolites of *G. fujikuroi*. Hydroxylation at C-1 in gibberellin A_{16} (32) occurs [26] after ring contraction to the *ent*-gibberellane, gibberellin A_4 (33). The 18-norkaurenolide (11) may be an artefact formed from the corresponding 18-hydroxykaurenolide during isolation. However, if this were so, it is surprising that 7β-hydroxy-18-norkaurenolide has not been isolated along with 7β,18-dihydroxykaurenolide (2) which is a major metabolite of strains ACC917 and TP70.

EXPERIMENTAL

For general experimental details see Part V.[27]

Isolation of Neutral Metabolites.—The neutral extract from a large scale fermentation of *Gibberella fujikuroi*, strain TP70, was supplied in butyl acetate by I.C.I. Ltd. A portion (12·5 l) was reduced to *ca.* 5 l and was washed with 2N-sodium hydrogen carbonate (2 l), 2N-hydrochloric acid (1 l), and water (1 l). Recovery from the dried butyl acetate gave a viscous oil (1·05 kg) which was fractionated as follows.

(a) ent-6β,7α-*Dihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (1). The oil (1·05 kg) was adsorbed on silica gel (1·7 kg) which was placed on top of a column of Celite (3·4 kg) and silicic acid (1·7 kg), made up in light petroleum. The column was eluted in 5 l fractions of light petroleum containing increasing proportions of ethyl acetate. Fractions 12—20, eluted with 2—10% ethyl acetate contained crude *ent*-6β,7α-dihydroxykaur-16-en-19-oic acid 19,6-lactone (1) (*ca.* 78 g) m.p. 150—190°. A portion (30·6 g) was purified by further chromatography on a column of alumina (1 kg; grade II), fractions (500 ml) of light petroleum containing increasing amounts of ethyl acetate being collected. Elution with 17·5% ethyl acetate gave the lactone (1) (10·7 g), m.p. 186—188°, τ (C_5D_5N) 9·12 (s, 20-H_3), 8·74 (s, 18-H_3), 8·13 (d, *J* 6 Hz, 5-H), 5·32 (d, *J* 6 Hz, 7-H), 5·00 (t, *J* 6 Hz, 6-H), 4·97, and 4·90br (17-H_2); the TMS ether had *m/e* (g.l.c.–m.s.) 388 (M^+, 0·5%), 378(0·5), 345(5), 298(35), 283(6), 270(10), 255(7), 205(6), 163(12), 145(18), 137(100), 109(69), 93(18), and 81(18).

(b) ent-6β,7α,18-*Trihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (2). Fractions 27—32, eluted with 40—75% ethyl acetate from the silica–Celite column described in (a), gave an oil which, dissolved in the minimum of ethyl acetate, deposited the lactone (2) (20 g) m.p. 215—217°; the TMS ether had *m/e* (g.l.c.–m.s.) 476 (M^+, 1%), 461(3), 386(34), 358(8), 343(8), 296(22), 283(52), 268(100), and 103(22).

(c) ent-3α,6β,7α-*Trihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (6). A portion (130 g) of the oil recovered from the ethyl acetate mother liquors in (b) was dissolved in light petroleum–ethyl acetate (7 : 3) and chromatographed on a column of alumina (5 kg, Laporte type 0). Elution with increasing amounts of ethanol in ethyl acetate gave an oil (32 g), eluted with ethanol–ethyl acetate (1 : 9) and crystallised from ethyl acetate–light petroleum to give the lactone (6) [13] as needles, m.p. 175—176°, $[\alpha]_D^{20}$ −30° (*c* 0·43 in $CHCl_3$) (Found: C, 72·8; H, 8·6. Calc. for $C_{20}H_{28}O_4$: C, 72·3; H, 8·5%). The diacetate had m.p. 165—166° (lit.,[13] 161—162°).

(d) ent-6β,7α,11β-*Trihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (9). A portion (12 g) of the gummy residue from the mother liquors in (c) was rechromatographed on a column of alumina (500 g, Laporte type 0). The fraction (1·5 g), eluted with ethanol–ethyl acetate (1 : 20) was rechromatographed on a column of Sephadex LH20 (250 g) which had been equilibrated in the lower phase of the two-phase system, light petroleum–ethyl acetate–methanol–water (10 : 13 : 10 : 5). The column, made up in the lower phase, was eluted with the upper phase in 40 ml fractions. Fractions 27—32 gave a mixture (500 mg) of two components which were separated by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 1). Recovery of the lower R_F band gave the lactone (6) (430 mg). Recovery of the faster moving component gave the *lactone* (9), crystallised from ethyl acetate–light petroleum as needles (45 mg), m.p. 251—253°, $[\alpha]_D^{20}$ −23° (*c* 0·84 in $CHCl_3$) (Found: C, 71·9; H, 8·3. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ 3610, 3450br, 1767, 1660, and 882 cm^{-1}, τ (C_5D_5N) 8·69 (s, 18-H_3), 8·43 (s, 20-H_3), 4·90 and 4·86br (17-H_2), 4·91 (t, *J* 5 Hz, 6-H), and 4·24 (d, *J* 5 Hz, 7-H), *m/e* 332 (M^+, 1%), 317(1·5), 314(9), 299(7), 296(31), 281(20), 268(14), 256(9), 253(14), 221(6), 218(29), 137(22), 135(21), 133(12), 131(13), 109(100), 107(25), 105(25), 95(24), 93(33), 91(39), 79(32), 77(25), 67(29), 65(11), 55(30), 53(13), 43(31), and 41(42); bis-TMS ether *m/e* (g.l.c.–m.s.) 476 (M^+, 18%), 461(5), 433(8), 386(100), 371(35), 343(26), 296(54), 281(25), 257(21), 137(14), 109(43), 91(14), and 43(28). The monoacetate (18), prepared by treatment with acetic anhydride–pyridine (1 : 4) for 19 h at 20°, formed needles, m.p. 221—224° (from ethyl acetate–light petroleum), $\nu_{max.}$ 3500br, 1770, 1750, 1660, and 890 cm^{-1}.

(e) ent-6β,7α,11β-*Trihydroxy*-18-*norkaur*-16-*en*-19-*oic acid* 19,6-*lactone* (11). Fractions 36—40 from the Sephadex LH20 column described in (d) gave a solid (60 mg), shown

[25] J. R. Hanson and A. F. White, *Tetrahedron*, 1969, **25**, 2743.
[26] J. R. Bearder, Ph.D. Thesis, Bristol University, 1973.
[27] J. MacMillan and T. J. Simpson, *J.C.S. Perkin I*, 1973, 1487.

to be a mixture of three components by g.l.c. and by g.l.c.–m.s. after trimethylsilylation, which showed three peaks with M^+ 476, 476, and 462. Crystallisation of the solid from acetone–light petroleum gave the *norkaurenolide* (11) as prisms, m.p. 257—259° (Found: M^+, 318·181. $C_{19}H_{26}O_4$ requires M, 318·183), $\nu_{max.}$ 3330, 1768, 1635, and 903 cm^{-1}, τ (C_5D_5N) 8·53 (s, 20-H_3), 5·53 (m, 11-H), 5·16 (t, J 5 Hz, 6-H), 4·94 and 4·88 (m, 17-H_2), and 4·32 (d, J 5 Hz, 7-H), *m/e* 318 (M^+, 2%), 303(1), 300(37), 285(17), 282(73), 267(30), 254(17), 242(23), 239(17), 207(30), 204(39), 149(17), 147(16), 145(18), 135(46), 131(22), 123(27), 121(22), 119(20), 109(25), 107(28), 105(32), 95(100), 93(46), 91(58), 81(30), 79(51), 77(35), 67(34), 55(31), 43(20), and 41(40); the bis-TMS ether had *m/e* (g.l.c.–m.s.) 462 (M^+, 12%), 447(5), 419(1·5), 372(100), 357(35), 329(12), 282(53), 267(30), 243(24), and 95(64). An acetylation product, purified by t.l.c. on silica gel with ethyl acetate–light petroleum, gave an accumulated n.m.r. spectrum with τ 8·85 (s, 20-H_3), 8·01 and 7·91 (both s, 2 × COMe), 5·31 (t, J 7 Hz, 6-H), 5·03 and 4·96br (17-H_2), and 4·18 (d, J 7 Hz, 7-H).

(f) ent-18-*Acetoxy*-6β,7α-*dihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (4). From the alumina column described in (c), ethanol–ethyl acetate (1 : 19) eluted a gum (2·6 g), shown by g.l.c. to contain four main components. This gum was re-chromatographed on a Sephadex LH20 column (250 g) as described in (d). Fractions 13—16 contained two compounds which were separated by p.l.c. on silica gel with 4% ethanol in benzene. The faster-running compound was the lactone (1) and the slower moving one was the 18-*acetoxy-lactone* (4), obtained as a gum, $[\alpha]_D^{21}$ −21·7° (τ 2·2 in $CHCl_3$) (Found: M^+, 374·211. $C_{22}H_{30}O_5$ requires M, 374·209); $\nu_{max.}$ 3600, 3450br, 1770, 1660, and 885 cm^{-1}, τ 9·07 (s, 20-H_3), 8·06 (d, J 7 Hz, 5-H), 7·91 (s, COMe), 7·38 (m, $W_{\frac{1}{2}}$ *ca.* 8 Hz, 15-H_2), 7·23 (m, $W_{\frac{1}{2}}$ *ca.* 7 Hz, 13-H), 5·82 and 5·86 (both s, 18-H_2), 5·76 (d, J 7 Hz, 7-H), 5·35 (t, J 7 Hz, 6-H), and 5·13 and 5·00 (both m, 17-H_2), *m/e* 374 (M^+, 1·5%), 356(100), 313(61), 296(48), 268(90), 253(23), 107(39), 105(28), 93(40), 91(42), 79(42), 58(96), 55(52), 44(100), 43(100), and 41(40).

The acetate (4) was identical (optical rotation, g.l.c., i.r., n.m.r., and m.s.) with an authentic sample prepared by treatment of *ent*-6β,7α,18-trihydroxykaur-16-en-19-oic acid 19,6-lactone (3) (108 mg) with acetic anhydride (*ca.* 40 μl) in pyridine (1 ml) for 18 h at 20°. The required monoacetate (4) (53 mg) was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 3).

(g) ent-1α,6β,7α-*Trihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (10). Fractions 31—35 from the Sephadex column described in (f) gave the *lactone* (10) as a gum (180 mg), $[\alpha]_D^{20}$ −8·0° (*c* 0·62 in $CHCl_3$) (Found: M^+, 332·198, $C_{20}H_{28}O_4$ requires M, 332·199); $\nu_{max.}$ 3610, 3440br, 1765, 1660, and 885 cm^{-1}, τ (C_5D_5N) 9·18 (s, 20-H_3), 8·67 (s. 18-H_3), 7·31 (d, J 6 Hz, 5-H), 6·28 (m, 1-H), 5·24 (d, J 6 Hz, 7-H), 5·05 and 4·98br (17-H_2), and 4·84 (t, J 6 Hz, 6-H). The diacetate, prepared by treatment with acetic anhydride–pyridine (1 : 4), crystallised from acetone–light petroleum in needles, m.p. 182—185°, $\nu_{max.}$ 1772, 1735, 1660, and 888 cm^{-1}. The bis-TMS ether had *m/e* (g.l.c.–m.s.) 476 (M^+, 6%), 461(3), 386(35), 343(30), 296(100), 268(45), 252(35), 251(30), 237(40), 210(1000), 170(100), 129(83), 105(55), 98(75), 93(40), and 91(68).

(h) ent-9α,13-*Dihydroxy*-7,19,20-*norgibberella*-1,3,5(10),16-*tetraen*-6-*one* (12). From the Sephadex column described in (f), fractions 50—55 contained the known [10] ketone (12), prisms, m.p. 168—170°.

(i) ent-6β-*Hydroxy*-19-*norkaur*-16-*ene*-3,7-*dione* (16). The diketo-lactone (13) (113 mg), prepared as described by Bateson and Cross,[13] was boiled for 5·5 h in methanol (125 ml) containing potassium hydroxide (250 mg). The reaction mixture was diluted with water (100 ml) and the gummy product, which was recovered in ethyl acetate, was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 3). Recovery of the band at R_F 0·6 gave the 19-norkaurenedione (16), needles (30 μg), m.p. 128—130° (from ethyl acetate–light petroleum) (Found: M^+, 302·188. $C_{19}H_{28}O_3$ requires M, 302·188), $\nu_{max.}$ (CCl_4) 3510, 1718, and 882 cm^{-1}, τ (CCl_4) 9·04 (d, J 6 Hz, 18-H_3), 8·88 (s, 20-H_3), 5·99 (m, $W_{\frac{1}{2}}$ 7 Hz, 6-H), and 5·19 (m, $W_{\frac{1}{2}}$ 7 Hz, 17-H_2), *m/e* 302 (M^+, 100%), 274(27), 151(14), 105(10), 81(12), 79(17), 77(12), 67(12), 55(12), and 41(19).

ent-6β-*Hydroxy*-1,7-*dioxokaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (15).—The dihydroxy-lactone (10) (32 mg) in acetone (5 ml) was stirred with excess of Jones reagent for 1 h at 20°. Addition of methanol, then water, and extraction with ethyl acetate gave the *diketone* (15), needles (30 mg) m.p. 279—282° (decomp.) (from ethyl acetate–light petroleum) (Found: M^+, 328·166. $C_{20}H_{24}O_4$ requires M, 328·167), $\nu_{max.}$ 1788, 1722, 1660, and 892 cm^{-1}.

ent-6β-*Hydroxy*-7,11-*dioxokaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (14).—The dihydroxy-lactone (9) was oxidised quantitatively with Jones reagent in the usual manner to give the *diketone* (14) which crystallised from acetone–light petroleum as needles, m.p. 247—250° (Found: M^+, 328·166. $C_{20}H_{24}O_4$ requires M, 328·167), $\nu_{max.}$ 1785, 1720, 1660, and 888 cm^{-1}, *m/e* 328 (M^+, 100%), 313(3), 300(11), 165(8), 137(62), 109(40), 108(20), 107(10), 105(15), 93(18), 91(28), 79(20), 77(18), 55(15), 53(11), 43(15), and 41(30).

Oxidation of the Monoacetate (18).—The monoacetate (10 mg) was oxidised with Jones reagent in the usual way. The product was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 1) to give ent-7α-*acetoxy*-11-*oxokaur*-16-*en*-19-*oic acid* 19,6-*lactone* (19) (6 mg) (Found: M^+, 372·195. $C_{22}H_{28}O_5$ requires M, 372·194), $\nu_{max.}$ 1775, 1750, 1715, 1660, and 895 cm^{-1}, τ 8·78 (s, 20-H_3), 8·70 (s, 18-H_3), 8·26 (d, J 7 Hz, 5-H), 7·88 (s, COMe), 5·30 (t, J 7 Hz, 6-H), 4·83 (m, 17-H_2), and 4·14 (d, J 7 Hz, 7-H), *m/e* 372 (48%), 330(20), 312(100), 284(81), 256(44), 204(73), 148(100), 137(48), 109(100), 107(45), 105(48), 95(46), 93(66), 91(65), 87(36), 81(46), 79(35), 77(53), 55(69), 43(100), and 41(88).

Deuterium Exchange Experiments.—(a) *ent*-7α-Acetoxy-6β-hydroxykaur-16-en-19-oic acid 19,6-lactone (25 mg), m.p. 180—182°, tetrahydrofuran (12 ml), deuterium oxide (200 μl), and sodium methoxide (800 mg) were boiled for 16 h. The mixture was poured into 1M-potassium dihydrogen phosphate (pH 7) and the product, recovered in ethyl acetate, was purified by p.l.c. on silica gel with ethyl acetate–light petroleum–acetic acid (50 : 50 : 1) to give *ent*-6β,7α-dihydroxykaur-16-en-19-oic acid (23) (25 mg).[15] The methyl ester [28] had *m/e* (g.l.c.–m.s.) 348 (M^+, 2%), 330(8), 316(6), 315(4), 312(4), 298(22), 137(80), and 109(100).

(b) *ent*-7α-Acetoxy-6β-hydroxy-11-oxokaur-16-en-19-oic acid 19,6-lactone (19) (4 mg) was treated as in (a) with deuterium oxide (150 μl) and sodium methoxide (700 mg) in tetrahydrofuran (10 ml). The crude methylated product showed three peaks by g.l.c. The mass spectrum of the major component, obtained by g.l.c.–m.s., indicated a mixture of tri- and di-deuteriated *ent*-6β,7α-dihydroxykaur-16-en-19-oic acid (22) [Found: M^+, 365(58%) and 364(42%).

[28] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1963, 2944.

$C_{20}H_{27}D_3O_5$ requires M, 365 and $C_{20}H_{28}D_2O_5$ requires M, 364], *m/e* 365(1%), 364(1), 347(4·5), 346(3·5), 332(2·5), 331(3), 330(2·5), 315(7), 314(5), 313(2·5), 287(7), 286(5), 285(2·5), 257(6), 256(6), 257(3), 137(35), and 109(100).

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