^{13}C Nuclear Magnetic Resonance Spectra and Microbiological Hydroxylation of 7α- and 7β-Hydroxykaurenolide

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The ^{13}C n.m.r. spectra of some kaurenolides have been assigned. Changes in the spectra are used to show that microbiological hydroxylation of 7α-hydroxykaurenolide by *Rhizopus arrhizus* affords 7α,11α-, 7α,12ξ-, and 7α,13-dihydroxy- and 7α,16α,17-trihydroxykaurenolide. 7β-Hydroxykaurenolide affords 7β,11α- and 7β,13-dihydroxykaurenolide.

7α-HYDROXYKAURENOLIDE (1) is readily available from the fungal metabolite, 7β-hydroxykaurenolide (2).[1] Its derivatives can be converted into gibberellins.[2] Microbiological hydroxylation of 7α-hydroxykaurenolide was studied as part of a programme to prepare semi-synthetic gibberellins functionalized at the chemically inaccessible sites on ring C. The microbiological transformation of tetracyclic diterpenes has been reported from a number of laboratories.[3] ^{13}C N.m.r. spectroscopy has a considerable potential as a structural tool in microbiological hydroxylation since it is possible to assign each ^{13}C resonance in the substrate and metabolite and thus from the changes, to locate the site of hydroxylation. In this paper we report the assignment of the ^{13}C n.m.r. spectra of some kaurenolides and the microbiological transformation of 7α- and 7β-hydroxykaurenolide by *Rhizopus arrhizus*, a micro-organism known[4] to hydroxylate steroids at C-11.

The ^{13}C n.m.r. spectra were obtained at 25·15 MHz using a pulsed Fourier transform system with proton noise-decoupling and off-resonance decoupling. The results are tabulated. Many of the resonances were assigned with the aid of their multiplicity in the off-resonance spectra. The C-16 and C-17 olefinic and C-19 lactone carbonyl resonances at 158 (s), 107 (t), and 179 (s) p.p.m. respectively, were readily recognized. Examination of [7β-^{2}H]-7α-hydroxykaurenolide enabled the C-6 and C-7 resonances to be assigned. In this compound the C-7 resonance collapsed and showed a small deuterium isotope shift (0·5 p.p.m.). In the 7β- series, C-6 and C-7 were distinguished by the changes caused by acetylation at C-7. The C-6 resonance showed an upfield shift and the C-7 resonance a downfield shift (*cf.* ref. 5). The position of the C-6 resonance reflected variations in the nature and stereochemistry of the C-7 substituents. The C-18 and C-20 methyl groups, which gave quartets in the off-resonance spectra, could be distinguished since the 25 p.p.m. resonance was absent from the spectrum of 7β,18-diacetoxykaurenolide (4). The upfield shift of the C-20 resonance in 7-oxokaurenolide (6) reflects the influence of the shielding cone of the carbonyl group, a feature which is also seen in the proton spectrum.[6]

The singlets at 34, 42, and 45 p.p.m. were assigned to C-10, C-4, and C-8 respectively on the basis of the variation of the C-4 resonance on substitution at C-18, the effect of a C-7 carbonyl group on the C-8 resonance, and the constancy of the C-10 resonance during these variations. C-10 in steroids resonates at 36 p.p.m.[5] The doublets at 37, 51, and 56 p.p.m. were assigned to C-13, C-5, and C-9 respectively. The variation in the C-13 resonance on converting 7β,18-diacetoxykaurenolide (4) into its 16-oxo-17-nor-derivative (5) and the variation in the C-5 resonance with the introduction of a C-18 substituent [(3) ⟶ (4)] enabled these centres to be distinguished. Both the C-12 and C-14 resonances are shifted on the introduction of a C-16 carbonyl group

^{13}C N.m.r. spectra of the kaurenolides (p.p.m. from tetramethylsilane)

Compound	Carbon atom																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(1)	38·2	17·3	28·15	42·1	51·2	75·7	71·6	45·4	53·7	33·5	17·8	33·2	37·3	34·2	50·6	158·5	106·8	25·1	181·9	19·1
(2)	37·6	17·35 *	28·1	42·1	51·75	83·7	71·6	45·1	55·4	34·2	17·3 *	32·6	37·4	34·3	42·1	159·4	106·9	25·5	182·5	20·5
(3) [a]	37·3	17·35	28·15	41·2	51·75	80·15	73·2	44·3	55·5	34·1	17·1	32·5	37·3	34·2	43·2	158·2	107·7	25·7	181·4	20·3
(4) [b]	36·3	16·6	23·05	45·8	48·05	81·05	72·9	44·2	55·8	34·2	17·1	32·5	37·4	33·9	43·0	158·0	107·75	67·95	178·9	21·0 *
(5) [c]	36·0	16·4	23·0	45·8	47·9	80·05	72·8	42·8	56·5	34·3	17·7	26·2	42·8	31·7	49·8	221·3		67·6	178·4	20·9 *
(6)	34·4	16·9	28·8	41·4	52·5	76·3	208·9	53·6	57·0	35·3	17·8	31·7	37·3	31·8	47·9	156·65	108·0	26·3	179·8	15·6
(7) [d]	34·9	23·8	68·4	44·7	51·5	80·2	72·8	44·3	55·7	34·0	17·1	32·3	37·2	34·0	43·1	157·9	107·8	21·4	179·0	19·1
(8)	38·8	17·3	27·8	41·6	50·7	75·6	71·05	45·8	57·1	34·5	65·2	45·0	38·0	36·2	50·5	157·6	107·9	25·5	181·8	21·2
(9)	37·8	17·65	28·3	41·4	51·6	75·8	207·1	54·6	68·1	35·3	205·8	49·0	36·8	33·9	51·2	152·6	110·7	26·15	179·5	15·8
(16)	37·1	17·35	28·3	42·8	51·5	76·1	72·25	43·7	52·2	34·2	19·4	39·6	78·4	41·4	48·05	157·3	106·1	24·2	181·9	20·6
(21)	37·4	17·2	27·9	42·8	51·9	83·1	71·5	42·7	55·2	34·2	18·5	39·1	77·7	40·5	42·2	159·7	107·1	25·5	181·8	21·2

[a] Acetate 20·9 and 169·95. [b] Acetates 21·05 * (2), 170·0 and 170·05. [c] Acetates 20·95 * (2) and 170·3 (2). [d] Acetates 20·9 and 21·05, 169·6 and 169·8.

* Assignment may be interchanged.

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

[2] R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.*, 1965, 1565; B. E. Cross, K. Norton, and J. C. Stewart, *J. Chem. Soc.* (*C*), 1968, 1054; J. R. Hanson and J. Hawker, *Phytochemistry*, 1973, **12**, 1073; J. R. Bearder, J. MacMillan, and B. O. Phinney, *ibid.*, p. 2173.

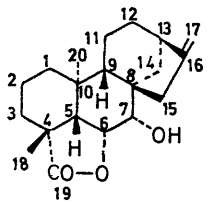
[3] J. R. Hanson and A. F. White, *Tetrahedron*, 1968, **24**, 6291; A. B. Anderson, R. McCrindle, and J. K. Turnbull, *J.C.S. Chem. Comm.*, 1973, 143; J. P. Beilby, E. L. Ghisalberti, P. R. Jefferies, M. A. Sefton, and P. N. Sheppard, *Tetrahedron Letters*, 1973, 2589; J. R. Bearder, J. MacMillan, C. M. Wels, and B. O. Phinney, *J.C.S. Chem. Comm.*, 1973, 778.

[4] D. H. C. Peterson and H. C. Murray, *J. Amer. Chem. Soc.*, 1952, **74**, 1871; P. D. Meister, D. H. Peterson, S. H. Epstein, H. C. Murray, C. M. Reineke, A. Weintraub, and H. M. L. Osborne, *ibid.*, 1954, **76**, 5679; for a recent ref. see D. S. H. Smith, N. J. Poole, and W. F. A. Jowett, *Phytochemistry*, 1973, **12**, 561.

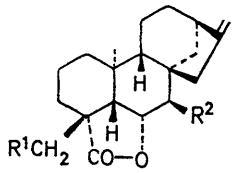
[5] H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1969, **91**, 7445; H. Eggert and C. Djerassi, *J. Org. Chem.*, 1973, **38**, 3788.

[6] J. R. Hanson and A. F. White, *Tetrahedron*, 1969, **25**, 2743.

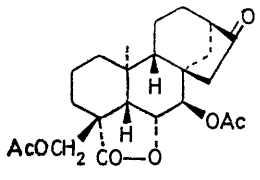
[(4) ⟶ (5)] whilst the C-5 and C-9 resonances show a shift on the introduction of a C-7 carbonyl group [(2) ⟶ (6)].



(1)



(2) R^1 = H, R^2 = OH
(3) R^1 = H, R^2 = OAc
(4) R^1 = R^2 = OAc



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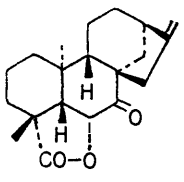

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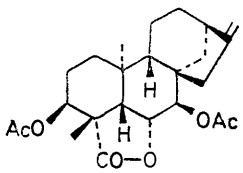
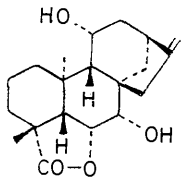


(7)

The two high field triplets at 17 p.p.m. were assigned to C-2 and C-11 as these centres are shielded by major 1,3 diaxial interactions.[7] In steroids these centres are the two highest field triplets (at 20 p.p.m.)[5] and in the pimaranes they resonate at 18—20 p.p.m.[8] The triplet at 37 p.p.m. was assigned to C-1, the adjacent axial methyl group deshielding this centre.[7] In the androstane series C-1 resonates at 38 p.p.m.[5] whilst in the pimaranes this resonance is in the range 37—40 p.p.m.[8] The triplet at 28 p.p.m. shifts to 23 p.p.m. on the introduction of a C-18 substituent [(3) ⟶ (4)] and it was therefore assigned to C-3. The lowest field triplet at 42—50 p.p.m. was assigned to C-15 which is influenced both by changes at C-16 and C-7. The resonances at 32 and 34 p.p.m. were assigned to C-12 and C-14 respectively.

The microbiological transformation of 7α-hydroxykaurenolide (1) was then examined. In order to locate the metabolites a preliminary transformation was carried out with [7β-^{3}H]-7α-hydroxykaurenolide which revealed four transformation products. The products were separated by chromatography. The first metabolite (8) (*ca.* 10% yield) analysed for $C_{20}H_{28}O_4$. The proton n.m.r. spectrum contained an additional C*H*·OH resonance at τ 5·9. The C-20 proton resonance was shifted downfield from τ 8·90 in 7α-hydroxykaurenolide to 8·58 in the metabolite. In the ^{13}C n.m.r. spectrum (see the Table) a resonance at 17 p.p.m., which had been assigned to C-11 in 7α-hydroxykaurenolide, was no longer present and a new doublet appeared at 65·2 p.p.m. Furthermore the resonances which had been assigned to C-12 and C-20 showed downfield shifts. When the metabolite was oxidized with the 8N-chromium trioxide reagent, a diketone (9) was obtained which showed only a lactone and cyclohexanone carbonyl absorption in the i.r. ($\nu_{max.}$ 1792, 1720sh, and 1715 cm^{-1}). One carbonyl group in this diketone was sterically hindered since reduction with sodium borohydride afforded a 7α-monohydroxyketone (10) [$\nu_{max.}$ 3520, 1780, and 1690 cm^{-1}, τ 5·2 (t, *J* 7 Hz, 6-H) and 5·94 (d, *J* 7 Hz, 7-H)]. The ^{13}C n.m.r. spectrum of the diketone (9), when compared to that of 7-oxokaurenolide (6), revealed a marked shift in the C-9 resonance (57·0 ⟶ 68·1 p.p.m.), the C-11 resonance (now at 205·8 p.p.m.), and the C-12 resonance (31·7 ⟶ 49·0 p.p.m.). Hence the additional oxygen function is at C-11. Further evidence for this came from a study of the mass spectrum. The hydroxylated metabolite (8), the diketone (9), and the monohydroxy-ketone (10) showed fragments at *m/e* 109, 137, and 165 which are found in the parent kaurenolides and are assigned[9] the structures (11), (12), and (13) and arise from ring A. Thus hydroxylation on ring A was excluded. The



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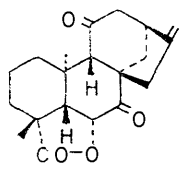

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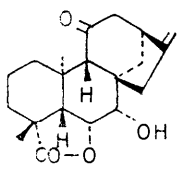

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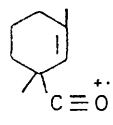

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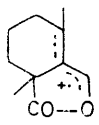

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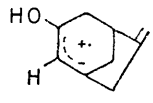

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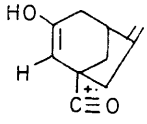


(15)

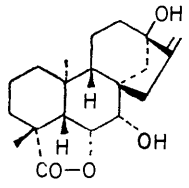
mass spectrum of the diketone contains two additional significant peaks at *m/e* 135 and 163 which are assigned

[7] G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley-Interscience, New York, p. 43.

[8] E. Wenkert and L. Buckwalter, *J. Amer. Chem. Soc.*, 1972, **94**, 4367.

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

the structures (14) and (15). Their formation requires the presence of a correctly oriented γ-C–H (*e.g.* from C-1) for the hydrogen transfer and this limits the site of oxygentation to C-11. In view of the marked effect of the additional hydroxy-group on the C-20 proton resonance, an effect which was considerably enhanced in [$^2H_5$]pyridine, Δτ 0·47 p.p.m., the C-11 hydroxy-group was assigned the 11α-configuration.



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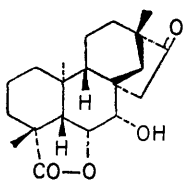

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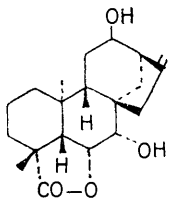

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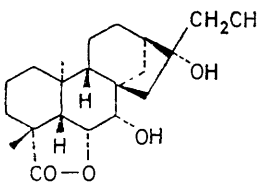
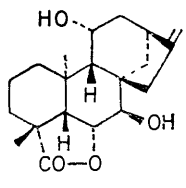


(19)

The second metabolite (16) to be isolated in 20% yield, also gave analytical data for $C_{20}H_{28}O_4$. The absence of an additional C*H*·OH resonance in the proton n.m.r. spectrum and the presence of a singlet in the ^{13}C n.m.r. spectrum at 78·4 p.p.m. replacing the C-13 doublet was clearly indicative of a tertiary alcohol located at C-13. The hydroxy-group showed the characteristic effect on the C-17 proton resonances [10] which now appeared at τ 5·09 and 4·80, an effect which was amplified in [$^2H_5$]pyridine when the resonances appeared at τ 4·90 and 4·42. In confirmation of the structure (16), the metabolite underwent a Wagner–Meerwein rearrangement [11] on treatment with acid to form a cyclopentanone ($\nu_{max.}$ 1745 cm^{-1}) (17) which possessed an additional CMe resonance in the proton n.m.r. spectrum [τ 9·25 (6H) and 8·92 (3H)].

Two minor metabolites were isolated in insufficient quantity for ^{13}C n.m.r. spectroscopy. The first of these (18), which was isomeric with the previous metabolites, showed the typical kaurenolide ring A fragments at *m/e* 109, 137, and 165 in its mass spectrum. Hence the additional oxygen function was located on ring C or D. It possessed an additional C*H*·OH resonance at τ 6·26 ($W_{\frac{1}{2}}$ 8 Hz). The corresponding diketone contained lactone and cyclohexanone absorption in the i.r. ($\nu_{max.}$ 1775, 1725, and 1710 cm^{-1}). It did not possess significant u.v. absorption. In the n.m.r. spectrum this diketone showed a doublet at τ 6·70 (*J* 6 Hz) and two more proton signals than 7-oxokaurenolide between τ 7·4 and 7·9. The C-17 protons resonated at τ 4·68 and 4·87 deshielded by an adjacent oxygen function. Reduction of a small sample of the diketone with sodium borohydride regenerated the parent dihydroxylactone [*ca.* 80% (t.l.c.)] together with an epimer (*ca.* 20%). Hence this metabolite was tentatively assigned the structure 7α,12(β)-dihydroxykaurenolide (18), although we cannot exclude a 12α-configuration for the alcohol. The second minor metabolite (19), $C_{20}H_{30}O_5$, lacked olefinic proton resonances in the n.m.r. spectrum. It contained a CH_2·OH group [τ (C_5D_5N) 5·92 (*J* 10 Hz)]. When deuterium oxide was added to the solution this resonance collapsed to a singlet. Furthermore in the mass spectrum there was a ready loss of 31 a.m.u. (CH_2OH). The metabolite was identified as 7α,16α,17-trihydroxykaurenolide (19) and was identical with a synthetic sample.[6]

Although 7β-hydroxykaurenolide (2) was not a potential substrate for chemical conversion into gibbanes, its hydroxylation was briefly examined. Two major metabolites were isolated. The first was assigned the structure (20), 7β,11α-dihydroxykaurenolide, since on oxidation it gave the diketone (9). The 11α-configuration was assigned to the hydroxy-group because of its marked deshielding effect on both the C-20 and C-7 proton resonances. After this work was completed, this compound was described [12] as a minor metabolite of *Gibberella fujikuroi*, strain TP70. The second metabolite was the known 7β,13-dihydroxykaurenolide



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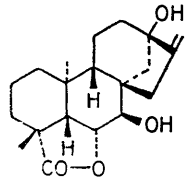

(21)

(21),[3,13] which was identified by comparison (i.r. and n.m.r.) with an authentic sample. Its ^{13}C n.m.r. spectrum (see the Table) was in accord with this structure.

We conclude that microbiological hydroxylation may provide a route to kaurenolides which are hydroxylated on ring C and are suitable for conversion into gibberellins. Furthermore the ^{13}C n.m.r. spectra of this series are a complementary structural tool to the proton n.m.r. spectra.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on a Perkin-Elmer 257 spectrometer for Nujol mulls. ^1H N.m.r. spectra were determined for solutions in [^2H]chloroform on a Varian A60A or

[10] J. R. Hanson, *J. Chem. Soc.*, 1965, 5036.
[11] E. Mosettig, U. Beglinger, F. Dolder, H. Lichti, P. Quitt, and J. A. Waters, *J. Amer. Chem. Soc.*, 1963, **85**, 2305.
[12] P. Hedden, J. MacMillan, and M. J. Grinstead, *J.C.S. Perkin I*, 1973, 2773.
[13] E. P. Serebryakov, A. V. Simolin, V. F. Kucherov, and B. V. Rosynov, *Tetrahedron*, 1970, **26**, 5215.

HA 100 spectrometer with tetramethylsilane as an internal standard. The ^{13}C n.m.r. spectra were determined on a JEOL PFT-100 Fourier transform spectrometer operating at 25·15 MHz. The spectral width was 250 p.p.m. using 8192 data points and 5—10,000 accumulations. The pulse length was 7 μs (*c.* 30°) at a pulse interval of 1·0 s. The samples (80—150 mg) were dissolved in [^{2}H]chloroform (0·5 ml), and the solvent deuterium provided the lock signal. Tetramethylsilane was used as an internal standard. The shifts are estimated to be accurate to 0·1 p.p.m. Mass spectra were determined on an A.E.I. MS9 mass spectrometer operating at 70 eV. Light petroleum refers to the fraction b.p. 60—80°.

Incubation of 7α-*Hydroxykaurenolide with* Rhizopus arrhizus.—*Rhizopus arrhizus* was grown on shake culture in 500 ml conical flasks at 25° on a medium [14] (200 ml) comprising malt extract (1 g), beef extract (1 g), bacteriological peptone (1 g), corn steep liquor (1 ml), and glucose (5 g) in water (1 l) for 6 days. 7α-Hydroxykaurenolide [1] (2·0 g) in ethanol (40 ml) was distributed between 30 flasks and the incubation was continued for a further 4 days. The mycelium was filtered and the broth saturated with sodium chloride and extracted with ethyl acetate. The extract (2·04 g) was chromatographed on silica (70 g). Elution with chloroform gave ent-6β,7β,11β-*trihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (8) (200 mg) as needles (from acetone–light petroleum), m.p. 248°, $[\alpha]_D$ −31° (*c* 0·18) (Found: C, 72·5; H, 8·1. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ 3550, 3520, 1750, 1660, and 896 cm^{-1}, τ (in $CDCl_3$) 8·78 (3H, s, 18-H_3), 8·58 (3H, s, 20-H_3), 5·90 (2H, m, 7- and 11-H), 5·2 (2H, m, 6- and 17-H), and 4·98 (1H, m, 17-H), (in C_5D_5N) 8·66 (3H, s, 18-H), 8·11 (3H, s, 20-H_3), 5·70 (1H, d, *J* 7 Hz, 7-H), 5·50br (1H, $W_{\frac{1}{2}}$ 11 Hz, 11-H), and 4·95 (3H, m, 6-H and 17-H_2). Further elution with chloroform gave ent-6β,7β,12ξ-*trihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (18) (30 mg) which crystallized from acetone–light petroleum as needles, m.p. 212—220°, $[\alpha]_D$ −15° (*c* 0·2) (Found: C, 72·3; H, 8·0. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ 3400br, 1755, 1660, and 900 cm^{-1}, τ 8·86 (3H, s, 20-H_3), 8·68 (3H, s, 18-H_3), 6·24 (1H, m $W_{\frac{1}{2}}$ 8 Hz, 12-H), 5·85 (1H, d, *J* 7 Hz, 7-H), 5·2 (2H, m, 6- and 17-H), and 4·80 (1H, m, 17-H). Further elution with chloroform gave ent-6β,7β,13-*trihydroxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (16) (400 mg) which crystallized from ethyl acetate as plates, m.p. 220—222°, $[\alpha]_D$ −131° (*c* 0·2 in pyridine) (Found: C, 72·4; H, 8·05. $C_{20}H_{28}O_4$ requires C, 72·2; H, 8·5%), $\nu_{max.}$ 3440br, 1750, 1670, and 910 cm^{-1}, τ 8·87 (3H, s, 20-H_3), 8·68 (3H, s, 18-H_3), 6·05 (1H, d, *J* 7 Hz, 7-H), 5·15 (1H, m, 6-H), 5·09br (1H, s, 17-H), and 4·80br (1H, s, 17-H), (in C_5D_5N) 8·75 (3H, s, 20-H_3), 8·63 (3H, s, 18-H_3), 5·90 (1H, d, *J* 7 Hz, 7-H), 5·0 (1H, m, 6-H), 4·90 (1H, m, 17-H), and 4·42 (1H, m, 17-H). Elution with ethyl acetate gave *ent*-6β,7β,16β,17-tetrahydroxykauran-19-oic acid 19,6-lactone which crystallized from acetone–light petroleum as prisms, m.p. 228—230° (lit.,[6] 223—226°), $[\alpha]_D$ −130° (*c* 0·2 in pyridine) (Found: C, 68·3; H, 8·5. Calc. for $C_{20}H_{30}O_5$: C, 68·5; H, 8·6%), identical (n.m.r. and i.r.) with the sample described previously.

Oxidation of 7α,11α-*Dihydroxykaurenolide* (8).—The lactone (100 mg) in acetone (10 ml) was treated with 8N-chromium trioxide reagent [15] (0·3 ml) at room temperature for 1 h. Methanol was added, and the solution concentrated, diluted with water, and the product recovered in ethyl acetate. *ent*-6β-Hydroxy-7,11-dioxokaur-16-en-19-oic acid (9) crystallized from acetone–light petroleum as needles, m.p. 260—265° (lit.,[12] 247—250°), $[\alpha]_D$ −6° (*c* 0·3) (Found: C, 73·5; H, 7·45. Calc. for $C_{20}H_{24}O_4$: C, 73·1; H, 7·4%), $\nu_{max.}$ 1790, 1720, 1715, 1660, and 890 cm^{-1}, τ 9·12 (3H, s, 20-H_3), 8·69 (3H, s, 18-H_3), 7·80 (1H, d, *J* 7 Hz, 5-H), 7·00 (1H, m, 9-H), 5·15 (1H, d, *J* 7 Hz, 6-H), and 4·86 and 4·80 (2H, m, 17-H_2).

Reduction of the Diketone (9).—The diketone (40 mg) in methanol (2 ml) was treated with sodium borohydride (50 mg) for 2 h at room temperature. Dilute hydrochloric acid was added and the product (20 mg) was recovered in chloroform. ent-6β,7β-*Dihydroxy*-11-*oxokaur*-16-*en*-19-*oic acid* 19,6-*lactone* (10) crystallized from acetone–light petroleum as needles, m.p. 190—192°, $[\alpha]_D$ −40° (*c* 0·2) (Found: C, 72·3; H, 8·3. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·9%), $\nu_{max.}$ 3510, 1780, 1690, 1650sh, and 880 cm^{-1}, τ 8·71 (3H, s, 18-H_3), 8·65 (3H, s, 20-H_3), 7·04br (1H, s, 9-H), 5·90 (1H, d, *J* 7 Hz, 7-H), 5·13 (1H, t, *J* 7 Hz, 6-H), and 4·97 and 4·91 (2H, m, 17-H_2).

Rearrangement of 7α,13-*Dihydroxykaurenolide* (16).—The kaurenolide (40 mg) was heated under reflux in aqueous ethanolic hydrochloric acid (4 ml) for 1 h. The product (30 mg) was recovered in ethyl acetate. ent-6β,7β-*Dihydroxy*-13-*methyl*-16-*oxo*-17-*nor*-13β-*kauran*-19-*oic acid* 19,6-*lactone* (17) crystallized from acetone–light petroleum as prisms, m.p. 241—243°, $[\alpha]_D$ −119° (*c* 0·1) (Found: C, 71·8; H, 8·9. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ 3540, 1778, and 1745 cm^{-1}, τ 8·98 (6H, s, 20-H_3 and 13-Me), 8·64 (3H, s, 18-H_3), 6·17 (1H, q, *J* 7 and 10 Hz, collapses to a d, *J* 7 Hz, on addition of D_2O, 7-H), and 5·08, (1H, q, *J* 4 and 7 Hz, 6-H).

Oxidation of 7α,12-*dihydroxykaurenolide* (18).—The kaurenolide (10 mg) in acetone (2 ml) was treated with 8N-chromium trioxide reagent (0·1 ml) for 1 h. Methanol was added and the solution was concentrated, poured into water, and the product recovered in ethyl acetate. ent-6β-*Hydroxy*-7,12-*dioxokaur*-16-*en*-19-*oic acid* 19,6-*lactone* crystallized from acetone–light petroleum as needles, m.p. 289—290° (Found: M^+, 328. $C_{20}H_{24}O_4$ requires *M*, 328), $\nu_{max.}$ 1775, 1725sh, 1710, 1655, and 895 cm^{-1}, τ 9·22 (3H, s, 20-H_3), 8·66 (3H, s, 18-H_3), 6·70 (1H, d, *J* 6 Hz), 5·10 (1H, d, *J* 7 Hz, 6-H), and 4·87 and 4·68 (1H each, s, 17-H_2).

Incubation of 7β-*Hydroxykaurenolide with* Rhizopus arrhizus.—The kaurenolide (2·3 g) in ethanol (40 ml) was distributed between 28 flasks of *Rhizopus arrhizus* cultured as described previously. After a further 4 days the mycelium was filtered and the broth extracted with ethyl acetate. Unchanged 7β-hydroxykaurenolide (1·4 g) was recovered from the mycelium. Chromatography of the broth extract on silica gave, in the fractions eluted with chloroform, *ent*-6β,7α,11β-trihydroxykaur-16-en-19-oic acid (75 mg) which crystallized from acetone–light petroleum as needles, m.p. 254°, $[\alpha]_D$ −34° (*c* 0·2 in pyridine) [lit.,[12] m.p. 251—253°, $[\alpha]_D$ −23° (in $CHCl_3$)] (Found: C, 72·6; H, 8·0. Calc. for $C_{20}H_{28}O_4$: C, 72·3; H, 8·5%), τ [$(CD_3)_2CO$] 8·82 (3H, s, 20-H_3), 8·75 (3H, s, 18-H_3), 5·78 (1H, m, $W_{\frac{1}{2}}$ 11 Hz, 11-H), 5·45 (2H, m, 6- and 7-H), and 5·21 and 5·08 (2H, 17-H_2). Oxidation of the kaurenolide with 8N-chromium trioxide reagent gave *ent*-6β-hydroxy-7,11-dioxokaur-16-en-19-oic acid,[12] identical (i.r. and

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[15] R. G. Curtis, E. R. H. Jones, I. M. Heilbron, and G. F. Woods, *J. Chem. Soc.*, 1953, 457.

t.l.c.) with the sample described previously. Further elution with chloroform gave *eut*-6β,7α,13-trihydroxykaur-16-en-19-oic acid 19,6-lactone (21) (100 mg) which crystallized from acetone–light petroleum as needles, m.p. 260—261°, $[\alpha]_D$ −11° (*c* 0·18 in pyridine) (lit.,[3] m.p. 261—263°, lit.,[13] m.p. 259—262°) (Found: C, 72·1; H, 8·4. Calc. for $C_{20}H_{28}O_4$: C, 72·3; H, 8·5%), $\nu_{max.}$ 3520, 1740, 1660, and 900 cm^{-1}, τ [$(CD_3)_2CO$] 9·18 (3H, s, 20-H_3), 8·75 (3H, s, 18-H_3), 5·76 (1H, d, *J* 7 Hz, 7-H), 5·40 (1H, t, *J* 7 Hz, 6-H), 5·20 (1H, s, 17-H), and 4·80 (1H, s, 17-H).

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