706. *New Metabolites of* Gibberella fujikuroi. *Part IV.*[1] *The Structures of* 7,18-*Dihydroxy- and* 7,16,18-*Trihydroxy-kaurenolide.*

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7,18-Dihydroxy- and 7,16,18-trihydroxy-kaurenolide are shown to have the structures and absolute configurations (II) and (XXVI), respectively.

The isolation of the compounds named in the title has been described in Part II.[2] Their relations to 7-hydroxykaurenolide (I) [1] and the evidence for their structures, which have been briefly reported elsewhere,[3] are set out in full in this paper.

7,18-Dihydroxykaurenolide (II), m. p. 211—214°, $[\alpha]_D^{23}$ −37°, took up 1 mol. of hydrogen on hydrogenation and gave a dihydro-compound which is presumably a mixture of 16-epimers. It contained two active hydrogen atoms (Zerewitinoff) and readily formed a diacetate which showed infrared absorption assigned to γ-lactone (1771 cm.$^{-1}$), acetate (1741 cm.$^{-1}$), and exocyclic methylene groups (1654 and 898 cm.$^{-1}$). A Kuhn–Roth determination revealed the presence of one *C*-methyl group in 7,18-dihydroxykaurenolide and this was confirmed by nuclear magnetic resonance spectroscopy which showed one methyl resonance (singlet at τ 9·07). Alkaline hydrolysis of 7,18-dihydroxykaurenolide followed by methylation yielded the trihydroxy-ester (V), which gave a diacetate * showing hydroxyl absorption in the infrared spectrum (3416 cm.$^{-1}$). 7,18-Dihydroxykaurenolide is therefore a tetracarbocyclic dihydroxy-γ-lactone.

The terminal methylene grouping was shown to be exocyclic to a 5-membered ring by ozonolysis of 7,18-dihydroxykaurenolide which gave 0·66 mol. of formaldehyde and a nor-ketone (VI), showing $\nu_{max.}$ (in Me·CN) at 1762 (γ-lactone) and 1737 cm.$^{-1}$ (cyclopentanone). Oxidation of the nor-ketone with chromic oxide–sulphuric acid in acetone, followed by careful working up of the product at room temperature, gave an amorphous acid (X) characterised as its methyl ester (XI). In hot water the acid evolved carbon dioxide and afforded a compound, $C_{18}H_{22}O_4$, shown by its infrared spectrum [1778 (γ-lactone), 1742 (cyclopentanone), and 1704 cm.$^{-1}$ (cyclohexanone)] to be a diketo-lactone and consequently assigned structure (XII). This formulation is consistent with its nuclear magnetic resonance spectrum which, like that of 7,18-dihydroxykaurenolide, showed a tertiary methyl singlet (τ 9·27). Similarly, oxidation of 7,18-dihydroxykaurenolide with an excess of chromic oxide–sulphuric acid in acetone afforded mainly acidic material as the crude product, but after purification only two neutral compounds were obtained. One of them was the diketo-lactone (XII); the other, $C_{19}H_{24}O_3$, showing infrared bands at 1784 (γ-lactone), 1712 (cyclohexanone), and 1650 and 895 cm.$^{-1}$ (exocyclic methylene), was assigned structure (XIII). These oxidations reveal the presence in 7,18-dihydroxykaurenolide of both a primary and a secondary hydroxyl group. The ready decarboxylation of the acid (X) can be explained by siting the primary hydroxyl group β to either the lactone-carbonyl or the ketone group derived from the secondary hydroxyl group. Of these alternatives the former was shown to be correct, when, on pyrolysis, 7,18-dihydroxykaurenolide underwent retroaldol fission to formaldehyde and either the hydroxy-lactone (IV) or a mixture of this and its Δ^{15}-isomer (XVIII).

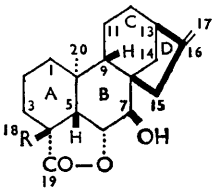
Under milder conditions the chromic oxide–sulphuric acid reagent in acetone attacks preferentially the secondary hydroxyl group in both 7,18-dihydroxykaurenolide and its nor-ketone (VI). Thus, with 6·5 equivalents of oxygen the former gave the keto-lactone

* We have shown (unpublished work) that 6,7-diols in the kaurenolide series are selectively acylated at the 7-position.

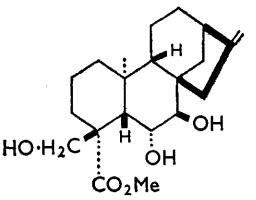
[1] Part III, *J.*, 1963, 2944.
[2] Cross, Galt, Hanson, Curtis, Grove, and Morrison, *J.*, 1963, 2937.
[3] Cross, Galt, Hanson, and Klyne, *Tetrahedron Letters*, 1962, 145.

(XIII) as the minor and a mono-alcohol, $C_{20}H_{26}O_4$, as the major product. The mono-alcohol was formulated as (XIV) on the basis of its infrared spectrum in bromoform which showed carbonyl bands at 1764 (γ-lactone) and 1718 cm.$^{-1}$ (cyclohexanone) and because on esterification with toluene-*p*-sulphonyl chloride it gave the toluene-*p*-sulphonate (XV) (see below). Confirmation of this assignment was provided when pyrolytic retroaldol fission of this mono-alcohol and of the corresponding mono-alcohol (XVI), similarly derived from the nor-ketone (VI), gave formaldehyde and the keto-lactones (XIII) and (XII), respectively.

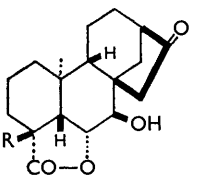
In contrast to the above oxidations, treatment of 7,18-dihydroxykaurenolide and its nor-ketone (VI) with 2·3—2·5 mol. of toluene-*p*-sulphonyl chloride in pyridine led to attack on the primary alcohol groups and gave the corresponding monotoluene-*p*-sulphonates in good yield. This was demonstrated when oxidation of the toluene-*p*-sulphonates with chromic oxide–sulphuric acid in acetone afforded the 7-keto-compounds (XV) and (XVII), respectively, without loss of carbon. Ozonolysis of the toluene-*p*-sulphonate (III) of 7,18-dihydroxykaurenolide gave the nor-ketone (VII). On the basis of the evidence presented above and of the co-occurrence of 7,18-dihydroxy- and 7-hydroxy-kaurenolide it seemed probable that the former was derived from the latter by microbiological oxidation



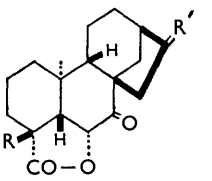
(I: R = Me)
(II: R = CH_2·OH)
(III: R = CH_2·O·SO_2·C_6H_4Me)
(IV: R = H)



(V)

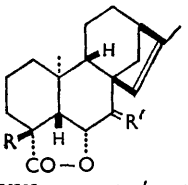


(VI: R = CH_2·OH)
(VII: R = X)
(VIII: R = CH_2I)
(IX: R = Me)

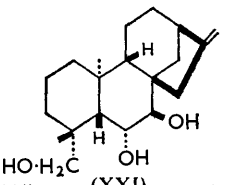


(X: R = CO_2H, R′ = O)
(XI: R = CO_2Me, R′ = O)
(XII: R = H, R′ = O)
(XIII: R = H, R′ = CH_2)
(XIV: R = CH_2·OH, R′ = CH_2)
(XV: R = X, R′ = CH_2)
(XVI: R = CH_2·OH, R′ = O)
(XVII: R = X, R′ = O)

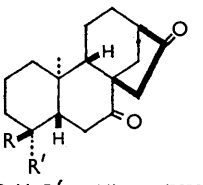
[X = CH_2·O·SO_2·C_6H_4Me]



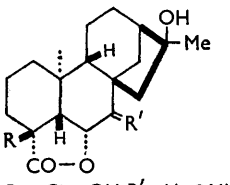
(XVIII: R = H, R′ = ···H, ═OH)
(XIX: R = CH_2·OH, R′ = ···H, ═OH)
(XX: R = CH_2·OH, R′ = O)



(XXI)



(XXII: R = CO_2H, R′ = H)
(XXIII: R = CO_2Me, R′ = CO_2H)
(XXIV: R = CO_2Me, R′ = H)
(XXV: R = H, R′ = CO_2Me)



(XXVI: R = CH_2·OH, R′ = ···H, ═OH)
(XXVII: R = H, R′ = O)
(XXVIII: R = H, R′ = ···H, ═OH)

of a methyl to a hydroxymethyl group. That these two kaurenolides are related in this manner has been established in two ways. First, reduction of the monotoluene-*p*-sulphonate (III) of 7,18-dihydroxykaurenolide with lithium aluminium hydride gave a triol (XXI), identical with the product of reduction of 7-hydroxykaurenolide (I) by lithium aluminium hydride. Secondly, treatment of the monotoluene-*p*-sulphonate (VII) with sodium iodide gave the iodo-compound (VIII), and this was reduced by zinc dust in acetic acid to a mixture of the nor-ketone [1] (IX) of 7-hydroxykaurenolide and its 7-acetate.[1] Since 7-hydroxykaurenolide has been shown [1] to have the structure and absolute stereochemistry (I), 7,18-dihydroxykaurenolide is assigned the structure and absolute stereochemistry (II).

Attempted dehydrogenations of the diketo-lactone (XII) and of the diketo-acid (XXII), prepared by hydrogenolysis of the diketo-lactone (XII) with zinc and acetic acid, were unsuccessful although the ultraviolet spectra of the intractable products indicated the presence of phenanthrenes (cf. ref. 1).

The double bond exocylic to ring D in 7,18-dihydroxykaurenolide, in contrast to that in the gibberellins but like that in phyllocladene [4] and kaurene,[5] readily migrates to the endocyclic position. Thus treatment of the dihydroxykaurenolide with 0·5% sulphuric acid in acetone at room temperature yields a mixture from which the isomer (XIX) can readily be separated by chromatography. Oxidation of the isomer with chromic oxide–sulphuric acid in acetone gave a mono-alcohol which, by analogy with the product similarly obtained from 7,18-dihydroxykaurenolide, has been formulated as the primary alcohol (XX).

Several degradation products of 7,18-dihydroxykaurenolide were made during studies on the stereochemistry at position 4 (cf. ref. 1). Hydrogenolysis of the lactone-ring in the keto-ester (XI) gave the half-ester (XXIII). The pK_{H_2O} of the latter, when compared with that of the acid-lactone (X), showed [1] that the carboxyl group in the half-ester was axial. An attempt to prepare the two 4-epimeric diketo-esters (XXIV) and (XXV) was unsuccessful, since only one could be obtained. When heated, the half-ester (XXIII) gave the 4-equatorial ester (XXIV). The latter, and not the 4-axial epimer, was also obtained by hydrogenolysis of the diketo-lactone (XII) with zinc and acetic acid followed by methylation with diazomethane, epimerisation presumably occurring during the hydrogenolysis.

The third kaurenolide, 7,16,18-trihydroxykaurenolide (XXVI), was a minor fermentation product.[2] It had m. p. 250—254°, gave analyses consistent with the formula $C_{20}H_{30}O_5$, and was inert to microhydrogenation. It showed no infrared bands attributable to double bonds, suggesting that the extra oxygen atom might have arisen by hydration of the exocyclic methylene group in 7,18-dihydroxykaurenolide. This relationship was established in the following manner. Oxidation of 7,16,18-trihydroxykaurenolide with the chromic oxide–sulphuric acid reagent, followed by decarboxylation, gave the keto-lactone (XXVII) which was also obtained by hydration of the methylene group in the keto-lactone (XIII) with boiling dilute mineral acid (cf. refs. 6 and 7). Subsequent work showed that treatment of 7,18-dihydroxykaurenolide with boiling mineral acid gave 7,16,18-trihydroxykaurenolide in about 20% yield together with a small amount of a diol, $C_{19}H_{28}O_4$, assigned the structure (XXVIII).

Experimental

For details of chromatographic materials and the determination of physical data, etc., see Part III.[1] Optical rotations were determined for ethanol solution on a E.T.L.-N.P.L. automatic polarimeter of type 143A.

7,18-*Dihydroxykaurenolide*.—7,18-Dihydroxykaurenolide [2] crystallised from aqueous methanol in felted needles, m. p. 211—214° (Found: *C*-Me, 3·9; active H, 0·57. $C_{20}H_{28}O_4$ requires 1*C*-Me, 4·5; 2 active H, 0·60%), $\nu_{max.}$ (in $CHCl_3$) ~3591, 3397 (OH), 1756 (γ-lactone), 3036, 3001, 1657, and 884 cm.$^{-1}$ (C=CH_2), τ 9·07 (>*C*-Me), 6·12 (CH_2·OH), 5·05 (C=CH_2). On microhydrogenation it took up 1·1 mol of hydrogen.

The *diacetate*, prepared with acetic anhydride in pyridine, crystallised from benzene–light petroleum in rosettes of rods, m. p. 171—172° (gas evolution), reset, and remelted at 197—202° (Found: C, 69·4; H, 7·9. $C_{24}H_{32}O_6$ requires C, 69·2; H, 7·7%), $\nu_{max.}$ 3085, 1771, 1741, 1654, 1234, and 898 cm.$^{-1}$.

Hydrogenation of 7,18-*Dihydroxykaurenolide*.—The kaurenolide (100 mg.) in ethyl acetate

[4] Brandt, *New Zealand J. Sci. Tech.*, 1952, **34***B*, 46.
[5] Briggs, Cawley, Loe, and Taylor, *J.*, 1950, 955.
[6] Grove, *J.*, 1961, 3545.
[7] Cross, Galt, and Hanson, *Tetrahedron*, 1962, **18**, 451.

(15 ml.) was reduced with hydrogen at room temperature in the presence of 25% palladised charcoal (37 mg.) until uptake ceased. The product (100 mg.) crystallised from benzene–methanol in felted needles, m. p. 211—222°, of dihydro-7,18-dihydroxykaurenolide (mixture of 16-epimers) (Found: C, 71·6; H, 9·0. Calc. for $C_{20}H_{30}O_4$: C, 71·8; H, 9·0%), $\nu_{max.}$ 3534, 3160 (br) (OH), and 1740 cm.$^{-1}$ (C=O).

Alkaline Hydrolysis of 7,18-*Dihydroxykaurenolide*.—7,18-Dihydroxykaurenolide (500 mg.) in methanol (5 ml.) was heated under reflux with 3N-sodium hydroxide (15 ml.) for 4 hr. The solution was diluted with water, cautiously acidified, and rapidly extracted with ethyl acetate. Recovery, by evaporation below 50°, gave a semi-solid which was methylated with diazomethane, dissolved in ethyl acetate, and filtered through alumina. Evaporation of the eluate and crystallisation from acetone–light petroleum gave *methyl* 6α,7β,18-*trihydroxykaur*-16-*en*-19-*oate* * (V) as needles (210 mg.), m. p. 192—194° (Found: C, 69·25; H, 8·9. $C_{21}H_{32}O_5$ requires C, 69·2; H, 8·85%), $\nu_{max.}$ 3520, 3400, 3303 (OH), 1675 (ester), 3063, 1652, and 875 cm.$^{-1}$ (C=CH_2). The *diacetate*, prepared with acetic anhydride in pyridine for 18 hr. at room temperature, crystallised from acetone–light petroleum as rods, m. p. 153—154° (Found: C, 66·7; H, 8·1. $C_{25}H_{36}O_7$ requires C, 66·9; H, 8·1%), $\nu_{max.}$ 3416 (OH), 1750, 1738 (acetates), 1698 (ester), 3070, 1668, and 885 cm.$^{-1}$ (C=CH_2).

Ozonolysis of 7,18-*Dihydroxykaurenolide*.—7,18-Dihydroxykaurenolide (66·5 mg.) in acetic acid (10 ml.) was treated with an excess of ozonised oxygen. The solution was diluted with water (10 ml.) and steam-distilled. Two 50 ml. portions of distillate were collected, treated with equal volumes of saturated dimedone solution, and kept at 0° for 48 hr. Crystals of the formaldehyde dimedone derivative (38 mg., 0·67 mol.), m. p. 187—189°, which separated, were identified by their infrared spectrum.

The non-volatile product, recovered in ethyl acetate after neutralisation of the acetic acid with sodium carbonate, was solid (64 mg.; m. p. 260—266°). It crystallised from ethanol–light petroleum in needles of 6α,7β,18-*trihydroxy*-16-*oxo*-17-*norkauran*-19-*oic acid* 19→6α-*lactone* (VI), m. p. 264—267°, $[\alpha]_D^{23}$ +40° (*c* 0·65) (Found: C, 67·9; H, 7·7. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8%), $\nu_{max.}$ (in Me·CN) 3600, 3520, 1762, and 1737 cm.$^{-1}$. The *oxime*, prepared in pyridine, crystallised from ethanol–light petroleum in felted needles, m. p. 250—254° (decomp.) (Found: C, 64·9; H, 8·0; N, 3·9. $C_{19}H_{27}NO_5$ requires C, 65·3; H, 7·8; N, 4·0%).

Oxidations of the Nor-ketone (VI).—(1) The nor-ketone (150 mg.) in acetone (15 ml.) was treated at 0° with the 8N-chromium trioxide reagent [8] (0·42 ml.) for 30 min. The mixture was poured into water (25 ml.) and extracted with ethyl acetate. The extract was evaporated to dryness *in vacuo* at 25°, giving the amorphous acid (X) (150 mg.), which became crystalline above *ca.* 200°, melted at 255—265°, and had pK_{H_2O} 2·81, $\nu_{max.}$ 1785, 1744, and 1717 cm.$^{-1}$ (C=O). The acid was covered with water (10 ml.) and heated on a water bath in a current of nitrogen for 30 min. Passage of the effluent gases through a baryta trap gave a precipitate of barium carbonate. The product was recovered in ethyl acetate and chromatographed on silica gel. Elution with light petroleum–ethyl acetate (3 : 2) gave 6α-*hydroxy*-7,16-*dioxo*-17,18-*dinorkauran*-19-*oic acid* 19→6α-*lactone* (XII), which crystallised from ethyl acetate–light petroleum in needles, m. p. 273—276° (Found: C, 71·5; H, 7·5. $C_{18}H_{22}O_4$ requires C, 71·5; H, 7·3%), $\nu_{max.}$ 1778, 1742, and 1704 cm.$^{-1}$, (in $CHCl_3$) 1789, 1749, and 1727 cm.$^{-1}$ (C=O), τ 9·27 (>C-Me).

The *methyl ester* (XI) of the above amorphous acid (74 mg.) was prepared with diazomethane and chromatographed on silica gel. It was eluted with light petroleum–ethyl acetate (7 : 3) and crystallised from acetone–light petroleum in needles (30 mg.), m. p. 265—267° (Found: C, 66·4; H, 6·75; OMe, 8·6. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7; 10Me, 8·6%), $\nu_{max.}$ (in $CHCl_3$) 1793 and 1738 cm.$^{-1}$.

(2) The nor-ketone (1 g.) in acetone (75 ml.) was treated with the 8N-chromic oxide reagent (2 ml.) at −40° for 1 hr. and then kept at 0° for 1 hr. Methanol (4 ml.) was added and the solution concentrated and diluted with water. The product, recovered by extraction with ethyl acetate and evaporation at 40°, was methylated and chromatographed on silica gel (20 × 2·5 cm.). Elution with 30—40% of ethyl acetate in light petroleum gave the above keto-ester (XI) (515 mg.) as needles, m. p. 256—261°. Further elution with ethyl acetate gave 6α,18-*dihydroxy*-7,16-*dioxo*-17-*norkauran*-19-*oic acid* 19→6α-*lactone* (XVI) (156 mg.)

* The carbocyclic nucleus of all compounds described in the Experimental section has the absolute stereochemistry of (—)-kaurene.[1]

[8] Curtis, Heilbron, Jones, and Woods, *J.*, 1953, 457.

which crystallised from acetone–light petroleum as prisms, m. p. 259—263° (decomp.) (Found: C, 68·6; H, 7·4; OMe, 0. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%), $\nu_{max.}$ 3440 (OH), 1775 (γ-lactone), 1749 (cyclopentanone), 1719 cm.$^{-1}$ (cyclohexanone), (in $CHCl_3$) 1770 and 1739 (br) cm.$^{-1}$.

Oxidations of 7,18-*Dihydroxykaurenolide*.—(1) The kaurenolide (66·4 mg.) in acetone (5 ml.) was treated with the 8N-chromium trioxide reagent (0·42 ml.) at room temperature for 4·5 hr. Methanol was added, the mixture poured into water (7 ml.) and extracted with ethyl acetate, and the extract washed with aqueous sodium hydrogen carbonate and water. Recovery gave a solid (8 mg.). Acidification of the carbonate extract and recovery in ethyl acetate gave a gum (60 mg.) which was chromatographed on silica gel (8 × 1·2 cm.). The column was eluted as follows: (i) light petroleum–ethyl acetate (9 : 1 and 85 : 15) gave gums; (ii) light petroleum–ethyl acetate (4 : 1 and 3 : 1) afforded 6α-*hydroxy*-7-*oxo*-18-*norkaur*-16-*en*-19-*oic acid* 19→6α-*lactone* (XIII) which crystallised from ethyl acetate–light petroleum in plates (4·3 mg.), m. p. 230—232° (Found: C, 75·6; H, 8·1. $C_{19}H_{24}O_3$ requires C, 76·0; H, 8·1%), $\nu_{max.}$ 1784, 1712 (C=O), 1650 and 895 cm.$^{-1}$ (C=CH_2); (iii) light petroleum–ethyl acetate (3 : 2) interband (1 mg.); (iv) light petroleum–ethyl acetate (3 : 2) gave the diketo-lactone (XII) as needles (6·2 mg.), m. p. 273—276°, identical with the sample prepared above.

(2) The kaurenolide (997 mg.) in acetone (22·5 ml.) was treated with the 8N-chromium trioxide reagent (2·45 ml.; 6·54 equiv. of oxygen) at 0° for 15 min. The product, isolated as in the preceding experiment, was suspended in water (30 ml.) and heated on a water-bath for 50 min. Filtration gave a solid (746 mg.), m. p. 205—208°, which was chromatographed on silica gel (15·5 × 2·2 cm.). Elution with light petroleum–ethyl acetate (9 : 1 and 85 : 15) afforded the keto-lactone (XIII) (266 mg.) which crystallised from ethyl acetate–light petroleum in plates, m. p. 229—232°, identical with the specimen prepared in (1). Fractions eluted with light petroleum–ethyl acetate (4 : 1 ⟶ 7 : 3) gave 6α,18-*dihydroxy*-7-*oxokaur*-16-*en*-19-*oic acid* 19→6α-*lactone* (XIV) (429 mg.) which crystallised from ethyl acetate–light petroleum as felted needles, m. p. 243—245° (Found: C, 72·6; H, 8·3. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·9%), $\nu_{max.}$ ~3100 (br) (OH), 1759 (γ-lactone), 1715 (cyclohexanone) and 1654 cm.$^{-1}$ (C=C), (in $CHBr_3$) 1764 and 1718 (C=O), 1658 and 893 cm.$^{-1}$ (C=CH_2).

The *toluene*-p-*sulphonate*, prepared with toluene-*p*-sulphonyl chloride in pyridine at room temperature, was eluted from alumina with light petroleum–ethyl acetate (3 : 1) and crystallised from ethyl methyl ketone–light petroleum in needles, m. p. 182—184° (Found: C, 66·9; H, 6·9. $C_{27}H_{32}O_6S$ requires C, 66·9; H, 6·7%), $\nu_{max.}$ 3350 (br), 1772, 1711, 1663, and 1596 cm.$^{-1}$.

Pyrolysis of 7,18-*Dihydroxykaurenolide*.—7,18-Dihydroxykaurenolide (320 mg.) was heated under a stream of nitrogen at 260—300° for 2 hr. The effluent gases were passed through aqueous dimedone which deposited formaldehyde dimethone, m. p. 189—190°. The residue was extracted with acetone, and the recovered material was chromatographed on alumina (20 × 2·5 cm.). Elution with 7 : 3 ethyl acetate–light petroleum gave 6α,7β-*dihydroxy*-18-*norkaur*-16-*en*-19-*oic acid* 19→6α-*lactone* (IV) (50 mg.) which crystallised from acetone–light petroleum as prisms, m. p. 192—193° (Found: C, 75·4; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 3501, 1750, 1655, and 884 cm.$^{-1}$. Elution with 4 : 1 ethyl acetate–light petroleum afforded a mixture of lactones (IV) and (XVIII) (60 mg.), followed by 6α,7β-*dihydroxy*-18-*norkaur*-15-*en*-19-*oic acid* 19→6α-*lactone* (XVIII) which crystallised from acetone–light petroleum in needles, m. p. 187—189° (Found: C, 75·4; H, 9·0. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%), $\nu_{max.}$ 3542, 1758, and 815 cm.$^{-1}$. On repetition this experiment sometimes gave only the isomer (IV).

Pyrolysis of 6α,18-*Dihydroxy*-7-*oxokaur*-16-*en*-19-*oic acid* 19→6α-*Lactone* (XIV). Pyrolysis of the keto-lactone as in the preceding experiment gave the keto-lactone (XIII) which crystallised from acetone–light petroleum as needles, m. p. 229—231°, identical with the sample prepared as above.

Pyrolysis of 6α,18-*Dihydroxy*-7,16-*dioxo*-17-*norkauran*-19-*oic Acid* 19→6α-*Lactone* (XVI).—Pyrolysis of the nor-ketone as in the preceding experiments but at 300° gave the diketo-lactone (XII), m. p. 274—276°, identical with the sample prepared as above.

Preparation of the Monotoluene-p-*sulphonates of* 7,18-*Dihydroxykaurenolide and the Nor-ketone* (VI).—(1) 7,18-Dihydroxykaurenolide (1·0 g.) was treated with toluene-*p*-sulphonyl chloride (1·31 g.) in pyridine (50 ml.) at room temperature for 66 hr. The mixture was poured into iced dilute sulphuric acid (250 ml.) and the product was recovered in ethyl acetate and chromatographed on alumina. Elution with light petroleum–ethyl acetate (9 : 1) gave the

toluene-p-*sulphonate* (III) which crystallised from benzene–light petroleum in felted needles (826 mg.), m. p. 185—186° (Found: C, 67·0; H, 7·2. $C_{27}H_{34}O_6S$ requires C, 66·65; H, 7·0%), $\nu_{max.}$ 3400 (br) (OH), 1782 (γ-lactone), 1650 (C=C), 1600 (aromatic), and 890 cm.$^{-1}$ (C=CH_2).

(2) The nor-ketone (100 mg.) and toluene-*p*-sulphonyl chloride (130 mg.) in pyridine (5 ml.) were left at room temperature for 92 hr. The product, isolated as in (1), crystallised from acetone–light petroleum in felted needles of the *toluene*-p-*sulphonate* (VII), m. p. 255—256° (Found: C, 63·8; H, 6·75. $C_{26}H_{32}O_7S$ requires C, 63·9; H, 6·6%).

Oxidation of the Monotoluene-p-*sulphonate* (III) *of* 7,18-*Dihydroxykaurenolide*.—(1) *With chromic oxide*. The toluene-*p*-sulphonate (99 mg.) in acetone (20 ml.) was oxidised with the 8N-chromium trioxide reagent (0·57 ml.) at 0° for 30 min. The product, isolated in the usual way, was chromatographed on alumina. Elution with light petroleum–ethyl acetate (3 : 1) followed by crystallisation from ethyl methyl ketone–light petroleum gave needles, m. p. 182—184°, identical (infrared spectrum) with the toluene-*p*-sulphonate (XV).

(2) *With ozone*. The toluene-*p*-sulphonate (76 mg.) in acetic acid (10 ml.) was treated with an excess of ozonised oxygen, diluted with water (20 ml.) and left for 2 hr. The acetic acid was neutralised with sodium carbonate solution, and the product was recovered in ethyl acetate and chromatographed on silica gel. Elution with ethyl acetate–light petroleum (1 : 1) followed by crystallisation from acetone–light petroleum gave felted needles, m. p. 255—257°, of the nor-ketone monotoluene-*p*-sulphonate (VII).

Oxidation of the Toluene-p-*sulphonate* (VII).—The ester (49 mg.) in acetone (14 ml.) was treated with the 8N-chromium trioxide reagent (0·05 ml.) at 0° for 30 min. The product was recovered in ethyl acetate and crystallised from ethyl acetate–light petroleum to give the *diketone* (XVII) as needles (37 mg.), m. p. 162—164° (Found: C, 64·4; H, 6·3. $C_{26}H_{30}O_7S$ requires C, 64·2; H, 6·2%), $\nu_{max.}$ 1797, 1750, 1716 (C=O), and 1596 cm.$^{-1}$ (aromatic).

Reductions by Lithium Aluminium Hydride.—(1) The toluene-*p*-sulphonate (III) (201 mg.) in a Soxhlet apparatus was reduced with a solution of lithium aluminium hydride (226 mg.) in dry ether (125 ml.) during 11·5 hr. Ethyl acetate followed by saturated ammonium chloride solution were added to the mixture which was then filtered. The ether layer was combined with an ether extract of the aqueous layer, washed with water, and dried. Recovery gave a gum (105 mg.) which was chromatographed on silica gel. Elution with light petroleum–ethyl acetate (1 : 3 and 3 : 7) afforded 6α,7β,19-*trihydroxykaur*-16-*ene* (XXI) (50 mg.) which crystallised from ethyl acetate–light petroleum in felted needles, m. p. 207—210°, $[\alpha]_D^{22}$ −55° (*c* 0·15) (Found: C, 74·7; H, 10·3. $C_{20}H_{32}O_3$ requires C, 75·0; H, 10·1%), $\nu_{max.}$ strong broad OH absorption, 1654 and 875 cm.$^{-1}$ (C=CH_2).

(2) 7-Hydroxykaurenolide (205 mg.) was reduced with lithium aluminium hydride (194 mg.) in ether (125 ml.) as in (1). The product crystallised from ethyl acetate–light petroleum in needles m. p. 206—210° or prisms m. p. 216—218°, $[\alpha]_D^{22}$ −58° (*c* 1·0), identical (infrared spectrum and mixed m. p.) with the triol prepared as in (1).

6α,7β-*Dihydroxy*-18-*iodo*-16-*oxo*-17-*norkauran*-19-*oic Acid* 19→6α-*Lactone* (VIII).—The nor-ketone toluene-*p*-sulphonate (VII) (1·0 g.), dry sodium iodide (2·0 g.), and dry acetone (100 ml.) were heated in a sealed tube at 140° for 31 hr. The mixture was filtered, the filtrate evaporated to dryness, and the residue washed with water, leaving crystals (878 mg.), m. p. 248—256°. Crystallisation from ethyl acetate–light petroleum gave the *iodo-compound* (VIII) as needles, m. p. 269—272° (Found: C, 51·4; H, 5·8; I, 24·8. $C_{19}H_{25}O_4I$ requires C, 51·4; H, 5·7; I, 28·5%), $\nu_{max.}$ 3420, 1772, and 1723 cm.$^{-1}$.

Reduction of the Iodo-compound (VIII).—Activated zinc dust (1·66 g.) was added to the iodo-compound (830 mg.) in acetic acid (50 ml.), and the mixture was boiled for 23 hr., then filtered and taken to dryness. Water (100 ml.) was added to the residue, and the product (607 mg.) recovered in ethyl acetate and chromatographed on alumina. Elution with light petroleum–ethyl acetate (7 : 3) gave 7β-acetoxy-6α-hydroxy-16-oxo-17-norkauran-19-oic acid 19→6α-lactone [1] (300 mg.) (identified by its infrared spectrum) which crystallised from acetone–light petroleum in needles, m. p. 244—247°. Fractions (130 mg.) eluted with light petroleum–ethyl acetate (2 : 3) crystallised from acetone–light petroleum in needles, m. p. 304—307°, identified as 6α,7β-dihydroxy-16-oxo-17-norkauran-19-oic acid 19→6α-lactone (IX) [1] by its infrared spectrum.

Attempted Dehydrogenations.—(1) The diketo-lactone (XII) (145 mg.) and selenium powder (188 mg.) were heated in a current of nitrogen at 340—350° for 22 hr. Extraction of the residue with ether and recovery gave a yellow gum (46 mg.) which was chromatographed on alumina

(8·2 × 1·4 cm.). Elution with light petroleum (b. p. 40—60°) gave a colourless oil (6 mg.) which showed $\lambda_{max.}$ 253, ~275, and 294 mμ ($E^{1\%}_{1cm.}$ 2340, 560, and 395).

(2) Dehydrogenation of the diketo-acid (XXII) (see below) (3·2 g.) with selenium powder (5·8 g.) in a current of nitrogen at 335—350° for 48 hr. gave a similar result.

Isomerisation of 7,18-*Dihydroxykaurenolide*.—The kaurenolide (5 g.) in acetone (250 ml.) was treated with concentrated sulphuric acid (1·2 ml.) and left in the dark at room temperature for 162 hr. The dark brown solution was neutralised with solid sodium carbonate, diluted with water, and extracted with ethyl acetate. The product was chromatographed on silica gel (50 × 4·8 cm.). Fractions eluted with light petroleum–ethyl acetate (4 : 1 ⟶ 7 : 3) were orange gums. Light petroleum–ethyl acetate (13 : 7) eluted starting material (2 g.). Elution with light petroleum–ethyl acetate (6 : 4 and 11 : 9) gave a solid (1·69 g.) which crystallised from ethyl acetate–light petroleum in needles, m. p. 208—210°, $[\alpha]_D^{17}$ −10° (*c* 1·0), of 6α,7β,18-*trihydroxykaur*-15-*en*-19-*oic acid* 19→6α-*lactone* (XIX) (Found: C, 72·5; H, 8·7. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%), $\nu_{max.}$ (in $CHBr_3$) 3571 and 3401 (OH), 1742 (br) (C=O), 3018, 1639, and 823 cm.$^{-1}$ (CRR′=CHR″).

Oxidation of the Isomer (XIX).—The isomer (99·7 mg.) in acetone (5 ml.) was treated with the 8N-chromium trioxide reagent at 0° for 10 min. The product, isolated in the usual way, was chromatographed on silica gel. Elution with light petroleum–ethyl acetate gave 6α,18-*dihydroxy*-7-*oxokaur*-15-*en*-19-*oic acid* 19→6α-*lactone* (XX) which crystallised from ethyl acetate–light petroleum in needles, m. p. 229—231° (Found: C, 73·3; H, 7·95. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·9%), $\nu_{max.}$ (in $CHBr_3$) 3665, 3593, 3563 (OH), 1760 (γ-lactone), and 1714 cm.$^{-1}$ (cyclohexanone).

Hydrogenolysis of the Keto-ester (XI).—The keto-ester (230 mg.) in acetic anhydride (25 ml.) was heated under reflux with freshly activated zinc dust (2 g.) for 16 hr. A further portion of zinc dust (1 g.) was added and the solution refluxed for a further 2 hr. The mixture was filtered and the filtrate evaporated *in vacuo*. The residue was taken up in ethyl acetate, and the solution was extracted with aqueous sodium hydrogen carbonate. Acidification of this extract with dilute hydrochloric acid and recovery with ethyl acetate gave 18-*methyl hydrogen* 7,16-*dioxo*-17-*norkauran*-18,19-*dioate* (150 mg.) (XXIII), m. p. 225—230° (decomp.) (Found: C, 65·85; H, 7·3. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%), $\nu_{max.}$ 3110 (carboxyl-OH), 1740 (cyclopentanone), 1725 (ester and cyclohexanone), 1706 cm.$^{-1}$ (carboxyl C=O), (in $CHBr_3$) 1738 and 1701 cm.$^{-1}$, pK_{H_2O} 4·38.

Pyrolysis of the Acid-ester (XXIII).—The acid-ester (23 mg.) was heated at 250° under a stream of nitrogen for 1 hr. The effluent gases were passed through a baryta trap which developed a precipitate. The product was recovered with acetone and crystallised from light petroleum as needles (15 mg.), m. p. 131—132°, identical with the ester (XXIV) prepared below.

Hydrogenolysis of the Keto-lactone (XII).—The keto-lactone (300 mg.) in acetic anhydride (15 ml.) was heated under reflux with zinc dust (3 g.) for 16 hr. A further portion of zinc dust (1 g.) was added and heating continued for 2 hr. The zinc dust was filtered off and the filtrate and washings were evaporated *in vacuo*. The residue was taken up in ethyl acetate, washed with water, and dried. Recovery gave a gum which crystallised from acetone–light petroleum as needles (150 mg.) of 7,16-*dioxo*-17,18-*dinorkauran*-19-*oic acid* (XXII), m. p. 225—227° (Found: C, 71·3; H, 8·1. $C_{18}H_{24}O_4$ requires C, 71·0; H, 7·95%), $\nu_{max.}$ 1750 (cyclopentanone), 1736 (cyclohexanone), 1702 cm.$^{-1}$ (carboxyl C=O), (in $CHBr_3$) 1739 and 1700 cm.$^{-1}$. The *methyl ester* (XXIV), prepared with diazomethane, crystallised from light petroleum as needles, m. p. 135—136° (Found: C, 72·1; H, 8·2. $C_{19}H_{26}O_4$ requires C, 71·7; H, 8·2%), $\nu_{max.}$ 1748, 1721, and 1711 cm.$^{-1}$. This ester (25 mg.) was recovered after treatment in methanol (1·5 ml.) with 0·5N-sodium hydroxide (0·2 ml.) at room temperature for 20 hr.

7,16,18-*Trihydroxykaurenolide*.—The trihydroxykaurenolide [2] crystallised from acetone–light petroleum in prisms, m. p. 250—255°, $\nu_{max.}$ 3400 (br), 1742 cm.$^{-1}$, $[\alpha]_D^{20}$ −40° (*c* 0·6).

Hydration of the Keto-lactone (XIII).—The keto-lactone (75 mg.) in acetone (3 ml.) was heated under reflux with dilute hydrochloric acid (10 ml.) for 2 hr. The solution was diluted to 100 ml. with water. Recovery of the product in ethyl acetate gave 6α,16-*dihydroxy*-7-*oxo*-18-*norkauran*-19-*oic acid* 19→6α-*lactone* (XXVII) (51 mg.) which crystallised from acetone–light petroleum as needles, m. p. 248—252° (Found: C, 71·2; H, 8·4. $C_{19}H_{26}O_4$ requires C, 71·7; H, 8·2%), $\nu_{max.}$ 3525 (OH), 1785 (γ-lactone), 1703 cm.$^{-1}$ (cyclohexanone).

Oxidation of 7,16,18-*Trihydroxykaurenolide*.—The kaurenolide (50 mg.) in acetone (5 ml.) was treated with the 8N-chromium trioxide reagent (0·1 ml.) for 0·5 hr. A few drops of

methanol were added and the solution diluted with water and heated on a steam-bath under nitrogen for 1 hr. Passage of the effluent gases through barium hydroxide solution gave a precipitate. The product was recovered in ethyl acetate and crystallised from acetone–light petroleum, to give the above keto-lactone (XXVII) (20 mg.) as needles, m. p. 249—251°, identical (infrared spectrum) with the specimen prepared from the keto-lactone (XIII).

Treatment of 7,18-*Dihydroxykaurenolide with Acid.*—The kaurenolide (100 mg.) in acetone (5 ml.) was refluxed with dilute hydrochloric acid (10 ml.) for 1 hr. The solution was diluted with water, and the product recovered in ethyl acetate to give prisms (20 mg.). Recrystallisation from acetone–light petroleum gave 7,16,18-trihydroxykaurenolide, m. p. 248—250°, identified by its infrared spectrum.

On repetition, the experiment gave also the *hydroxy-lactone* (XXVIII), m. p. 202—204° (Found: C, 71·45; H, 8·5. $C_{19}H_{28}O_4$ requires C, 71·2; H, 8·8%), $\nu_{max.}$ 3543, 3210 (br), and 1735 cm.$^{-1}$.

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