545. *New Metabolites of* Gibberella fujikuroi. *Part II.*[1] *The Isolation of Fourteen New Metabolites.*

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Extracts from culture filtrates of *Gibberella fujikuroi* grown under various fermentation conditions have yielded the following metabolites: 7-hydroxy-, $C_{20}H_{28}O_3$, 7,18-dihydroxy-, $C_{20}H_{28}O_4$, and 7,16,18-trihydroxy-kaurenolide, $C_{20}H_{30}O_5$; fujenal, $C_{20}H_{26}O_4$; fujenoic acid, $C_{20}H_{26}O_5$; a dicarboxylic acid (II); (−)-kaurene, (−)-kauranol, 13-epi-(−)-manoyl oxide (olearyl oxide); dehydroallogibberic acid; phthalic acid; dimethyl phthalate; phenethyl alcohol; and tyrosol. 13-Epi-(−)-manoyl oxide and dehydroallogibberic acid are shown to have structures (VIII) and (V; R = $:CH_2$), respectively.

The fungus *Gibberella fujikuroi* ACC.917 [2] is well known as a producer [2] of gibberellic acid. Other acidic metabolites which have been isolated from its culture filtrates include the fusaric acids,[3] succinic acid,[3] fumaric acid,[1] and 5-hydroxymethyl-2-furoic acid.[3] However, little attention has been paid to the neutral metabolites.

A neutral anhydride, fujenal, $C_{20}H_{26}O_4$, m. p. 169—170°, $[\alpha]_D^{26}$ −74° * (I; R = CHO), was isolated some years ago in these laboratories and an anil, $C_{26}H_{31}NO_3$, and a *p*-tolylimide, $C_{27}H_{33}NO_3$, were prepared. The infrared spectrum of fujenal in carbon tetrachloride solution showed absorption assigned to aldehyde (2702, 1723 cm.$^{-1}$), 5-ring anhydride (1855, 1783 cm.$^{-1}$) and terminal methylene (1658, 883 cm.$^{-1}$) groups. The aldehyde took up 1 mol. of hydrogen on microhydrogenation and showed only weak end-absorption in the ultraviolet region. These facts showed that fujenal was tricarbocyclic and possibly a diterpenoid connected with the biosynthesis of the fungal gibberellins.[6] The metabolites present in both the neutral (sodium hydrogen carbonate insoluble) and the acidic (sodium hydrogen carbonate soluble) fractions produced when *G. fujikuroi* is

* The *G. fujikuroi* metabolites, $C_{20}H_{26}O_4$, m. p. 165—167°, $[\alpha]_D$ −57°,[4] and gibberine, $C_{20}H_{28}O_4$, m. p. 161—162°, $[\alpha]_D$ −86°,[5] are probably identical with fujenal.

[1] Part I, Cross, Galt, and Hanson, *Tetrahedron*, 1962, **18**, 451.

[2] (*a*) Borrow, Brian, Chester, Curtis, Hemming, Henehan, Jefferys, Lloyd, Nixon, Norris, and Radley, *J. Sci. Food Agric.*, 1955, **6**, 340; (*b*) Curtis and Cross, *Chem. and Ind.*, 1954, 1066; (*c*) B.P. 783,611.

[3] Grove, Jeffs, and Mulholland, *J.*, 1958, 1236.

[4] Wierzchowski and Wierzchowska, *Naturwiss.*, 1961, **48**, 653.

[5] Sternberg, *Arch. Biochem. Biophys.*, 1962, **98**, 299.

[6] Grove, *Quart. Rev.*, 1961, **15**, 56.

grown under a wide variety of conditions have now been studied in greater detail. Some of the new products isolated have been the subject of a preliminary communication.[7]

The acidic material extracted from culture filtrates of fermentations in which the broth, after the end of the balanced phase [8] of growth, was maintained at about pH 7 has been shown to contain the gibberellins A_4, A_7,[1] and A_9.[1] Occasionally batches of crude gibberellin have afforded a new acid, $C_{20}H_{28}O_5$, m. p. 169—170°. Although this acid titrated as a monobasic acid it gave fujenal (I; R = CHO) [7,9] on treatment with acetic anhydride, and consequently it has been assigned the dicarboxylic acid structure (II).

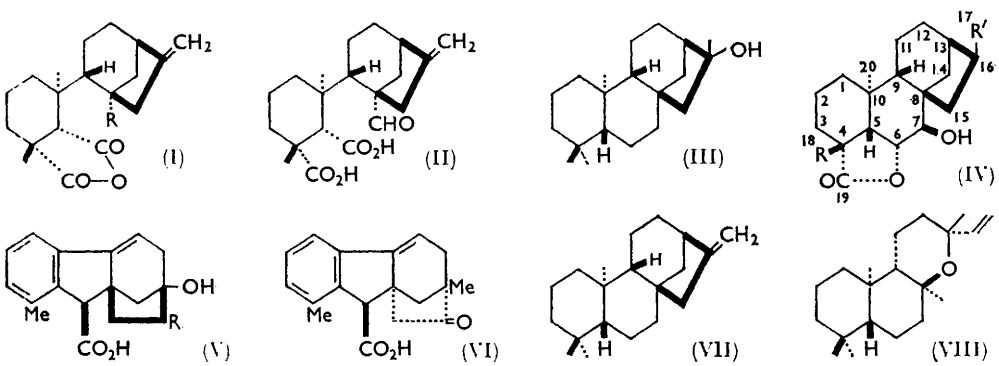
Fermentations run at 26° for 830 hr. on a medium similar to that described by Borrow *et al.*[8] but containing glycine in place of ammonium nitrate proved to be a rich source of metabolites. Early fractions from the chromatography of the combined neutral extracts (which sometimes contained small amounts of acidic substances) from three fermentations afforded (—)-kauranol (III), m. p. 214—216°, $[\alpha]_D^{22}$ —45°, identified by direct comparison with an authentic sample of (+)-kauranol (m. p. 212°,[10] $[\alpha]_D^{25}$ +48°[11]) for which we are indebted to the late Dr. J. Murray. Later fractions from the column gave fujenal, three new lactones which have been named 7-hydroxykaurenolide,[7] * $C_{20}H_{28}O_3$ (IV; R = Me, R′ = :CH_2), m. p. 187—188°, $[\alpha]_D^{22}$ —25°, 7,18-dihydroxykaurenolide,[7] * $C_{20}H_{28}O_4$ (IV; R = CH_2·OH, R′ = :CH_2), m. p. 211—214°, $[\alpha]_D^{24}$ —37°, and 7,16,18-trihydroxykaurenolide,† $C_{20}H_{30}O_5$ (IV; R = CH_2·OH, R′ = OH,Me), m. p. 250—255°, a new acid, fujenoic acid,[7] $C_{20}H_{26}O_5$ (I; R = CO_2H), m. p. 205—206° or 221—224°, $[\alpha]_D^{25}$ —59°, gibberellin A_4, and gibberellin A_9. The determination of the structures and stereochemistry of the three kaurenolides and of fujenal and fujenoic acid will be described in Parts III, IV, and V.[9] Chromatography of the combined acidic gums from these fermentations yielded succinic acid, 5-hydroxymethyl-2-furoic acid, gibberellic acid, gibberellin A_4, fujenal, fujenoic acid, and a new acid, $C_{18}H_{18}O_3$, m. p. 211—214° (decomp.), $[\alpha]_D^{20}$ —37°. The isolation of fujenal from the acid fraction is probably to be explained by its formation from the dibasic acid $C_{20}H_{28}O_5$ (II) during working-up. The infrared spectrum of the acid $C_{18}H_{18}O_3$ showed absorption attributed to hydroxyl (3330 cm.$^{-1}$), carboxyl-carbonyl (1695 cm.$^{-1}$), olefinic (1650 cm.$^{-1}$), aromatic (1580 and 775 cm.$^{-1}$), and terminal methylene (890 cm.$^{-1}$) groups. The presence of a double bond conjugated with a benzene ring was revealed by its ultraviolet spectrum ($\lambda_{max.}$ ~248, 258, 267, 288, 298 mμ, log ε 4·05, 4·15, 4·12, 3·39, 3·34, respectively) which was almost identical with that of the dehydro-derivative (V; R = H,Me) of dihydroallogibberic acid.[12] From the spectra we suspected that the acid had structure (V; R = :CH_2), *i.e.*, that of dehydroallogibberic acid, and this was confirmed by acid-catalysed rearrangement which gave dehydrogibberic acid (VI) identical with an authentic specimen.[13]

Chromatography of the neutral fraction from a fermentation run on a glucose–ammonium tartrate medium at 36° gave a hydrocarbon, $C_{20}H_{32}$, m. p. 50—50·5°, $[\alpha]_D^{20}$ —80°, and a compound, $C_{20}H_{34}O$, m. p. 98—99·5°, $[\alpha]_D^{24}$ —37°, in addition to fujenal, 7-hydroxykaurenolide, and 7,18-dihydroxykaurenolide. The acidic material from this fermentation has not been investigated. The hydrocarbon, $C_{20}H_{32}$, which was shown to contain a terminal methylene group by its infrared spectrum ($\nu_{max.}$ 1657 and 870 cm.$^{-1}$), had physical constants in close agreement with those of (—)-kaurene (VII) (lit.,[14] m. p. 51°, $[\alpha]_D^{11}$ —72°). With hydrogen chloride it gave a hydrochloride, $C_{20}H_{33}Cl$, m. p. 115—

* The nomenclature and numbering of these compounds has been agreed with the Editor.

[7] Cross, Galt, Hanson, and Klyne, *Tetrahedron Letters*, 1962, 145.
[8] Borrow, Jefferys, Kessell, Lloyd, Lloyd, and Nixon, *Canad. J. Microbiol.*, 1961, **7**, 227.
[9] Cross, Galt, and Hanson, following paper and unpublished work.
[10] McGimpsey and Murray, *J. Appl. Chem.*, 1960, **10**, 340.
[11] Murray, personal communication.
[12] Mulholland, *J.*, 1958, 2693.
[13] Cross, Grove, MacMillan, and Mulholland, *J.*, 1958, 2520.
[14] Briggs and Cawley, *J.*, 1948, 1888; Briggs, Cain, Cambie, Davis, Rutledge, and Wilmshurst, *J.*, 1963, 1345.

116°, $[\alpha]_D^{20}$ −40° {Briggs and Cawley [14] give m. p. 114—115°, $[\alpha]_D$ −40°, for (−)-kaurene hydrochloride}, and on hydrogenation it took up 1 mol. of hydrogen and gave a product, $C_{20}H_{34}$, m. p. 85—87°, $[\alpha]_D^{18}$ −33° {(−)-kaurane [α-dihydro-(−)-kaurene] has m. p. 84—85°, $[\alpha]_D^{21}$ −32° [14]}. The hydrocarbon was finally identified as (−)-kaurene when its dihydro-derivative was shown to be identical with a sample of (−)-kaurane (m. p. 85—87°) prepared from (−)-kauranol by dehydration with phosphorus oxychloride in pyridine and hydrogenation of the resultant mixture of (−)-kaurene and (−)-isokaurene.[14,15]



The compound $C_{20}H_{34}O$ must be an ether since its infrared spectrum which showed absorption attributed to olefin (3076 and 1634 cm.$^{-1}$) contained no bands due to hydroxyl or carbonyl groups. Microhydrogenation revealed the presence of one double bond; hence the compound must be tricyclic. Its nuclear magnetic resonance spectrum [16] showed five 3-proton singlets, of which three (τ 9·3, 9·2, and 9·15) were assigned to tertiary methyl groups and two (τ 8·9 and 8·8) to methyl groups of the type Me–C(–C)–O. In addition, partially resolved multiplets due to olefinic protons were centred at τ 5·27, 4·92, and 4·17.

This evidence is consistent with a view that the ether $C_{20}H_{34}O$ is a stereoisomer of manoyl oxide, possibly identical with olearyl oxide, a monounsaturated diterpenoid ether of unknown structure, for which McLean and Slater [17] give m. p. 99°, $[\alpha]_D^{15}$ −35·8°. At this point Dr. R. Hodges suggested that the ether might be the antipode of 13-epimanoyl oxide.[18,19] Subsequently comparison of the ether $C_{20}H_{34}O$ with samples of 13-epimanoyl oxide and olearyl oxide, for which the authors are indebted to Dr. G. Ohloff and Professor S. N. Slater, respectively, showed that it is identical with olearyl oxide and that the latter is the antipode of 13-epimanoyl oxide. Hence olearyl oxide, which it is proposed should now be known as 13-epi-(−)-manoyl oxide, has structure (VIII). With osmium tetroxide it gave a diol, $C_{20}H_{36}O_3$.

Fermentations run at 12°, in contrast to those run at 36°, on glucose–ammonium tartrate media gave very low yields of neutral products; consequently the neutral extracts from two fermentations on media which initially contained 0·36% and 0·92%, respectively, of ammonium tartrate were combined. Chromatography then gave a small amount of gum, believed from its infrared spectrum to be mainly (−)-kaurene, followed by fujenal, 7-hydroxykaurenolide, and 7,18-dihydroxykaurenolide. Chromatography of the acids

[15] Briggs, Cawley, Loe, and Taylor, *J.*, 1950, 955.
[16] Cf. Wenkert, Beak, and Grant, *Chem. and Ind.*, 1961, 1574.
[17] McLean and Slater, *J. Soc. Chem. Ind.*, 1945, **64**, 28.
[18] Ohloff, *Annalen*, 1958, **617**, 134.
[19] Hodges and Reed, *Tetrahedron*, 1960, **10**, 71.

from one fermentation (0·36% of ammonium tartrate) on Celite–silica (2 : 1) gave phthalic acid in a yield corresponding to ~100 mg./l. of culture filtrate.

A fermentation was carried out on a glucose–ammonium nitrate medium at 26°. It received only a limited amount of oxygen after the end of the balanced phase of growth and gave a poor yield of acidic metabolites which have not been examined. The neutral fraction was a mobile oil which yielded none of the usual metabolites on chromatography. However, it was found to contain appreciable quantities of three new fungal products, *viz.*, dimethyl phthalate, phenethyl alcohol, and tyrosol. The dimethyl phthalate and phenethyl alcohol were identified by their ultraviolet and infrared spectra and by hydrolysis of the former to phthalic acid and preparation of the dinitrobenzoate of the latter. The identity of the tyrosol was established by comparison with an authentic sample prepared by reduction of *p*-hydroxyphenylacetic acid by lithium aluminium hydride.

Experimental

The following chromatographic materials were used: silica gel M.F.C. (Hopkin and Williams), activated charcoal (B.D.H.), Celite 545 (Johns-Mandeville), and alumina (Woelm neutral alumina, grade II).

Unless otherwise stated, m. p.s were determined on a Kofler block and were corrected; infrared and ultraviolet spectra were determined for Nujol mulls and ethanol solutions, respectively, and optical rotations for ethanol solutions.

Microhydrogenations were carried out in acetic acid with a palladium black catalyst. " Light petroleum " refers to the fraction of b. p. 60—80°.

Ethyl acetate extracts were dried over anhydrous sodium sulphate.

Fujenal.—An ethyl acetate extract derived [2b] from culture filtrates of *G. fujikuroi* was extracted [2b] with phosphate buffer (pH 6·3) and then evaporated to dryness *in vacuo*. The residue crystallised from ethanol, ethyl acetate, or acetone–light petroleum in prisms of *fujenal* (I; R = CHO), m. p. 169—170°, $[\alpha]_D^{26}$ −74° (*c* 0·5 in acetone) [Found: C, 72·9, 72·5; H, 8·0, 8·0%; equiv. (potentiometric back-titration), 164; *M* (*X*-ray), 329 ± 3. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·9%; equiv. (dibasic), 165; *M*, 330], $\nu_{max.}$ (in CCl_4) 3069, 1658, and 883 (C=CH_2), 2702 and 1723 (aldehyde), 1855 and 1783 (5-ring anhydride), 918, 898, and 864 cm.$^{-1}$. The unit cell was tetragonal, space group $P4_12_12$ or $P4_32_12$ (enantiomorphous pair), a = 11·41 ± 0·04, c = 27·14 ± 0·09 Å, d 1·238. At 215 and 220 mμ ε was 810 and 480, respectively. On microhydrogenation fujenal took up 1·06 mol. of hydrogen (1·16 mol.; Adams catalyst). With concentrated sulphuric acid it gave an orange colour which became red. It was recovered after being heated with ethanol for 24 hr. and after storage for 3 hr. at 20° in methanolic ammonia. It did not form a dinitrophenylhydrazone with Brady's reagent or reduce ammoniacal silver nitrate at room temperature.

Derivatives of Fujenal.—(i) Fujenal (25 mg.) and an excess of aniline were heated for 30 min. at 100°, then cooled, and N-hydrochloric acid was added. The precipitate was collected and crystallised from ethanol, giving the *anil* as needles (11 mg.), m. p. 170—171° (Found: C, 76·8; H, 7·75; N, 3·6. $C_{26}H_{31}NO_3$ requires C, 77·0; H, 7·7; N, 3·45%), $\nu_{max.}$ 1852 and 1777 (5-ring anhydride), 1648 (C=N and C=CH_2), and 1597 cm.$^{-1}$ (aromatic ring), $\lambda_{max.}$ 271 mμ (log ε 3·62).

(ii) Fujenal (100 mg.) and *p*-toluidine (415 mg.) were heated for 65 min. at 100°. The solution was cooled, and water and 2N-hydrochloric acid were added. The p-*tolylimide*, recovered in ether, crystallised from ethanol in needles (70 mg.), m. p. 201—205° (Found: C, 77·6; H, 8·0; N, 3·4. $C_{27}H_{33}NO_3$ requires C, 77·3; H, 7·9; N, 3·3%), $\nu_{max.}$ ~2820 and 2710 (aldehyde CH), 1918, 1767, ~1713, 1701, 1655, and 1515 cm.$^{-1}$.

(iii) Fujenal (160 mg.), hydroxylamine hydrochloride (70 mg.; 2 mol.), and sodium carbonate monohydrate (70 mg.; 2 mol.) in ethanol (6 ml.) and water (2 ml.) were refluxed for 2 hr. The ethanol was distilled off *in vacuo*, water added, and the solution extracted with ethyl acetate. Recovery and crystallisation from ethyl acetate gave a *substance* as prisms, m. p. 209—210° (decomp.) (Found: C, 66·0, 65·9; H, 8·1, 8·1; N, 3·9, 4·2. $C_{20}H_{29}O_5N$ requires C, 66·1; H, 8·0; N, 3·85%), $\nu_{max.}$ 3220, 2400, 1900, 1696, ~1650, 1578 cm.$^{-1}$.

Isolation of the Dicarboxylic Acid (II).—In Part I,[1] the chromatography on a charcoal–Celite column of the crude acidic gum from a *G. fujikuroi* fermentation was described. Fraction (12) (0·69 g.) was rechromatographed on Celite–silica gel (2 : 1), elution being with light petroleum containing increasing concentrations of ethyl acetate. The fraction eluted with 20% ethyl acetate gave gibberellin A_9. In a repetition of the experiment the fraction immediately following gibberellin A_9 afforded the crystalline *dicarboxylic acid* (II), m. p. 169—170° (from acetone–light petroleum) [Found: C, 69·2; H, 8·2%; equiv. (potentiometric), 347. $C_{20}H_{28}O_5$ requires C, 68·9; H, 8·1%; *M*, 348], $\nu_{max.}$ (in $CHCl_3$) 2700 (aldehyde CH), 1717 (broad) (C=O), 1658 and 890 cm.$^{-1}$ (C=CH_2). When refluxed for 1 hr. with acetic anhydride, the acid was converted quantitatively into fujenal.

Fermentations and Isolation of Neutral and Acidic Fractions.—Gibberella fujikuroi (Saw.) Wr. strain ACC.917 [2] was grown in stirred aerated culture as described by Borrow *et al.*,[9] but on media containing different nitrogen sources. The temperature and rate of aeration of the broth were varied. When the fermentations were harvested the mycelium was filtered off and rejected. Treatment of the culture filtrates as described in Part I [1] gave ethyl acetate extracts of the neutral (insoluble in aqueous sodium hydrogen carbonate) and acidic metabolites. These extracts were washed with water, dried, and evaporated to dryness *in vacuo*. The bulk of the gibberellic acid was usually removed from the acidic fractions by crystallisation from ethyl acetate.

(i) Three fermentations were run at 26° for 830 hr. with glycine (0·188, 0·282, and 0·469%, severally) as the nitrogen source. The combined culture filtrates (75 l.) gave a neutral gum (A) (9·8 g.) and crude acids (B) (61 g.).

(ii) The culture filtrate (22·5 l.) from a fermentation run for 213 hr. at 36° with ammonium tartrate (0·36%) as the nitrogen source afforded a neutral gum (C) (2·1 g.) and acids (10·2 g.).

(iii) Fermenters 1 and 2 were run for 359 hr. at 12° with ammonium tartrate (0·92 and 0·36%, respectively) as the nitrogen source. Fermenter 1 gave 20 l. of culture filtrate from which a neutral gum (D) (0·49 g.) and acids (7·2 g.) were obtained. Fermenter 2 gave 17·5 l. of culture filtrate from which a neutral gum (E) (0·34 g.) was extracted. During concentration the acidic extract yielded crystals (F) (2·2 g.); evaporation of the mother-liquors to dryness gave a solid (G) (2 g.).

(iv) A fermentation was carried out at 26° with ammonium nitrate (0·55%) as the nitrogen source and with the usual aeration (0·5 vol. per vol. per min.). When all the inorganic nitrogen had been consumed (143 hr.) the rate of aeration was cut to 0·1 vol. and the fermentation continued for another 45 hr.

The culture filtrate (20 l.) gave a neutral mobile brown oil (H) (2·9 g.) and crude semi-solid acids (2·0 g.).

Isolation of (−)-Kauranol, Fujenal, Fujenoic Acid, and 7-Hydroxy-, 7,18-Dihydroxy-, and 7,16.18-Trihydroxy-kaurenolide.—The gum (A) was absorbed on silica gel by evaporating an acetone solution and placed on the top of a column (75 × 7 cm.) of Celite (650 g.) and silica gel (325 g.). Elution of the column with increasing concentrations of ethyl acetate in light petroleum and collection of 1 l. fractions gave the following results (% of ethyl acetate in parenthesis): Fractions (1—3) (5%) and (4—5) (7·5%) were gums (479 mg.) which, after rechromatography on alumina in 5% ethyl acetate–light petroleum, afforded (−)-kauranol (30 mg.) as felted needles (from ethyl acetate), m. p. 214—216°, $[\alpha]_D^{22}$ −45° (*c* 0·4 in $CHCl_3$) (Found: C, 82·6; H, 12·0. Calc. for $C_{20}H_{34}O$: C, 82·7; H, 11·8%). It was identified by comparison in the infrared with an authentic sample of (+)-kauranol. Fractions (6) (10%) and (7—8) (12·5%) gave fujenal (800 mg.) as prisms (from ethanol), m. p. 168°. Fractions (10) (15%) and (11) (17·5%) were crystallised from ethyl acetate–light petroleum, giving *7-hydroxykaurenolide* (IV; R = Me, R′ = :CH_2) (300 mg.) as rods, m. p. 187—188°, $[\alpha]_D^{22}$ −25° (*c* 0·5) [Found: C, 76·1; H, 9·0%; *M* (Rast), 366. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%; *M*, 316]. Fractions (12) (17·5%) and (13) (20%) gave *fujenoic acid* (I; R = CO_2H) (200 mg.) as needles (from acetone–light petroleum), m. p. 205—206° or 221—224°, $[\alpha]_D^{23}$ −59° (*c* 1·0) (Found: C, 68·7; H, 7·7%; equiv., 328. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6%; *M*, 346). Fractions (14) (20%), (15—16) (22·5%), and (17—18) (25%) were oils which were combined (1·51 g.) and rechromatographed on silica (100 g.). The fractions eluted with 35% ether in light petroleum gave gibberellin A_9 (100 mg.) as needles, m. p. 208—210°. Fractions (22) (30%), (23—24) (32·5%) and (25—26) (35%) were crystallised from ethyl acetate–light petroleum and aqueous methanol, giving *7,18-dihydroxykaurenolide* (IV; R = CH_2·OH, R′ = :CH_2) (1·2 g.) as felted needles,

m. p. 211—214°, $[\alpha]_D^{23}$ −37° (*c* 1·0) (Found: C, 72·3; H, 8·4. $C_{20}H_{28}O_4$ requires C, 72·2; H, 8·5%). Fraction (27) (35%) afforded gibberellin A_4 (25 mg.) as prisms, m. p. 214—215° (decomp.) (from acetone–light petroleum). Subsequent fractions eluted with 37·5—100% ethyl acetate remained intractable. However, elution with 10% methanol in ethyl acetate (1 l.) gave 7,16,18-*trihydroxykaurenolide* (IV; R = CH_2·OH, R′ = OH,Me) (100 mg.) as prisms, m. p. 250—255° (from acetone–light petroleum) (Found: C, 68·9; H, 8·5. $C_{20}H_{30}O_5$ requires C, 68·5; H, 8·6%).

The acidic fraction (B) (8 g.) was absorbed on silica gel from acetone and chromatographed on charcoal–Celite (charcoal 200 g.; Celite 400 g.). 32 fractions of 500 ml. each were collected by gradient elution with an increasing concentration of acetone in water over the range 0—100% acetone. Recovery of the organic material by evaporation of the acetone *in vacuo* and extraction with ethyl acetate gave the following results (fraction numbers in parentheses; crystalline fractions were identified by their infrared spectra): (1—6) were intractable; (7) gave succinic acid (40 mg.), m. p. 183°; (11) gave 5-hydroxymethyl-2-furoic acid (50 mg.), m. p. 158—162°; (15—18) gave gibberellic acid (2·5 g.), m. p. 225—228° (decomp.); (19—20) were gums (441 mg.) which were rechromatographed on silica gel (100 g.). Elution with 15% ethyl acetate in chloroform gave fujenoic acid (80 mg.) as needles, m. p. 205—206°. Elution with 35% ethyl acetate in chloroform gave gibberellin A_4 (10 mg.), m. p. 211—213° (decomp.); (24—26) gave fujenal (200 mg.) as prisms, m. p. 160°. (29—32) were gums (337 mg.) which were rechromatographed on Celite–silica gel. Elution with 10% of ethyl acetate in chloroform gave *dehydroallogibberic acid* (V; R = :CH_2) as needles (125 mg.), m. p. 211—214° (from acetone–light petroleum), $[\alpha]_D^{22}$ −37° (*c* 0·2) (Found: C, 76·65; H, 6·6%; equiv., 285. $C_{18}H_{18}O_3$ requires C, 76·6; H, 6·4%; *M*, 282), $\nu_{max.}$ 3330 (OH), 1695 (C=O), 1650 and 890 (C=CH_2), 1580 and 775 cm.$^{-1}$ (aromatic ring), $\lambda_{max.}$ ~248, 258, 267, 288, 298 mμ (log ε 4·05, 4·15, 4·12, 3·39, 3·34, respectively). It took up 1·8 mol. of hydrogen on microhydrogenation.

Acid-catalysed Rearrangement of Dehydroallogibberic Acid.—The above acid (25 mg.) in acetone (2 ml.) was refluxed with dilute hydrochloric acid (15 ml.) for 2 hr. Recovery with ethyl acetate gave a gum (18 mg.) which crystallised from ether–light petroleum as prisms, m. p. 221—222° (decomp.), identical (mixed m. p. and infrared spectrum) with dehydrogibberic acid.[13]

Isolation of (−)-*Kaurene and* 13-*Epi*-(−)-*manoyl Oxide* (VIII).—Fraction (C) was chromatographed in the usual way on Celite–silica gel (2 : 1; 300 g.). Elution with ethyl acetate–light petroleum (1 : 7→15 : 85) gave gums (407 mg.) followed, on increase in the concentration of ethyl acetate (15 : 85→35 : 65), by fujenal, 7-hydroxykaurenolide, and 7,18-dihydroxykaurenolide.

The gums (407 mg.) were chromatographed on alumina (11 × 1·5 cm.). Elution with light petroleum (b. p. 40—60°; 50 ml. fractions) gave (i) gum (211 mg.), (ii) crystals (55 mg.) and (iii) crystals (17 mg.). Elution of fraction (i) from alumina (18·5 × 1·2 cm.) with light petroleum (b. p. 40—60°) gave crystals which crystallised from methanol in needles of (−)-kaurene, m. p. 50—50·5°, $[\alpha]_D^{20}$ −80° (*c* 1·0) (Found: C, 88·5; H, 11·9. Calc. for $C_{20}H_{32}$: C, 88·2; H, 11·8%). With hydrogen chloride in ether it gave a hydrochloride which crystallised from ethyl acetate in plates, m. p. 115—116°, $[\alpha]_D^{20}$ −40° (*c* 1·3) (Found: C, 77·5; H, 10·8. Calc. for $C_{20}H_{33}Cl$: C, 77·8; H, 10·7%). On microhydrogenation, it took up 1·0 mol. of hydrogen and gave the dihydro-derivative (needles from methanol), m. p. 85—87°, $[\alpha]_D^{18}$ −33° (*c* 0·1), identical (infrared spectrum and mixed melting point) with (−)-kaurane (see below).

Fractions (ii) and (iii) crystallised from methanol in plates (60 mg.), m. p. 96—98·5°, raised to 98—99·5° by recrystallisation, of 13-epi-(−)manoyl oxide (VIII), $[\alpha]_D^{24}$ −37° (*c* 0·25) (Found: C, 82·45; H, 11·85. Calc. for $C_{20}H_{34}O$: C, 82·7; H, 11·8%). Its infrared spectrum ($\nu_{max.}$ 3076, 3055, ~1635, 1628, 1095, 1077, 1063, 961, 926, 909 and 842 cm.$^{-1}$) was identical with those of olearyl oxide [17] and 13-epi-(+)-manoyl oxide [18,19] but different from that of manoyl oxide. It depressed the m. p. of 13-epi-(+)-manoyl oxide but not that of olearyl oxide.

(−)-*Kaurane.*—(−)-Kauranol (140 mg.) in pyridine (5 ml.) was treated with phosphorus oxychloride (1 ml.) at room temperature for 12 hr. The mixture was poured into dilute hydrochloric acid and extracted with ethyl acetate, and the organic layer washed with dilute acid and water. The product crystallised from methanol in plates, m. p. 61—63°, $[\alpha]_D^{22}$ −47° (*c* 0·1 in $CHCl_3$) (Found: C, 88·4; H, 12·15. Calc. for $C_{20}H_{32}$: C, 88·2; H, 11·8%), $\nu_{max.}$ 1655, 881 (C=CH_2) and 844 (C:CH) cm.$^{-1}$. On microhydrogenation, this mixture [14,15] of (−)-kaurene and (−)-isokaurene absorbed 1 mol. of hydrogen and the major product was (−)-kaurane, m. p. 86—87°.

13-*Epi*-8,13-*epoxy*-(−)-*labdane*-14,15-*diol.*—A solution of 13-epi-(−)-manoyl oxide (125 mg.)

in dry ether (5 ml.) and pyridine (1 ml.) was treated with osmium tetroxide (250 mg.) at 0° for 2·5 days. The osmate was decomposed by refluxing it for 2 hr. with potassium hydroxide (2 g.) and mannitol (2 g.) in ethanol (10 ml.) and water (25 ml.). The solution was diluted with water and extracted with ether, and the extract washed with sodium hydroxide solution, dilute hydrochloric acid, and water and dried. Evaporation of the solvent gave a gum (95 mg.) which was chromatographed on alumina. Elution with ether gave the *diol* (75 mg.) which crystallised from acetone–light petroleum as needles, m. p. 106—107° (Found: C, 73·6; H, 11·0. $C_{20}H_{36}O_3$ requires C, 74·0; H, 11·2%), $\nu_{max.}$ 3430 (br) cm.$^{-1}$.

Isolation of Phthalic Acid.—Elution of material (F) (see above) from a Celite–silica gel (2 : 1; 500 g.) column with ethyl acetate–acetone (19 : 1 and 9 : 1) gave phthalic acid (1·31 g.) closely followed by gibberellic acid. The phthalic acid crystallised from water in prisms, m. p. 212—213°. Similarly chromatography of material (G) (see above) yielded crude phthalic acid (385 mg.).

Chromatography of the combined neutral fractions (D) and (E) gave a little gum, believed from its infrared spectrum to be impure kaurene, followed by small amounts of fujenal and 7-hydroxy- and 7,18-dihydroxy-kaurenolide.

Isolation of Dimethyl Phthalate, Phenethyl Alcohol, and Tyrosol.—Chromatography of the oil (H) (see above) on Celite–silica gel (2 : 1; 250 g.) by elution with ethyl acetate in light petroleum gave the following fractions (250 ml.; percentage of ethyl acetate in parentheses): (1—2) (5%) and (3—6) (10%) were rejected; (7—9) (15%) oil (420 mg.); (10—11) (15%) oil (206 mg.); 12 (15%), (13—15) (25%), (16—17) (50%), and (18) (100%) were rejected; (19) (100%) oil (419 mg.).

Fractions (7—9) were distilled at 50—60° (bath-temperature)/0·1 mm., giving an oil, $\lambda_{max.}$ 277 mμ ($E^{1\%}_{1\,cm.}$ 57·7) with an infrared spectrum identical with that of dimethyl phthalate. Alkaline hydrolysis gave phthalic acid.

Fractions (10) and (11) distilled at 35°/0·1 mm. as an oil (Found: C, 78·3; H, 8·3. Calc. for $C_8H_{10}O$: C, 78·65; H, 8·25%), identified as phenethyl alcohol by its infrared spectrum. It gave a dinitrobenzoate, m. p. and mixed m. p. 107·5—108°.

Fraction (19) was taken up in ether. The phenolic portion was extracted into sodium hydroxide solution and recovered in the usual manner, giving a solid, m. p. 73—87°. Sublimation at 80°/0·05—0·1 mm. followed by crystallisation from water gave prisms of tyrosol, m. p. and mixed m. p. 92—93° (Found: C, 69·05; H, 7·4. Calc. for $C_8H_{10}O_2$: C, 69·5; H, 7·3%).

Preparation of Tyrosol.—*p*-Hydroxyphenylacetic acid (1·0 g.) in dry ether (20 ml.) was added to a solution of lithium aluminium hydride (0·6 g.) in ether (50 ml.). The mixture was heated under reflux for 45 min. and left overnight. Isolation of the product in the usual way gave crude tyrosol (415 mg.), m. p. 82—92° raised by recrystallisation from water to 92—93°.

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