610. *Gibberellic Acid. Part XVII.* The Stereochemistry of Gibberic and Epigibberic Acid.*

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Gibberic acid and epigibberic acid are shown to have the absolute configurations (II) and (VI) respectively.

THE Wagner–Meerwein mechanism, proposed in earlier papers [1,2] from these laboratories, for the acid-induced isomerisation of allogibberic (I) to gibberic acid (II) has been confirmed by the determination of the absolute configuration of gibberic acid and by tracer studies which have been reported in a preliminary communication.[3] These results refute the alternative mechanism at first favoured by Birch and his collaborators,[4] in which hydration of the terminal methylene group of allogibberic acid was followed by pinacol–pinacolone rearrangement of the resulting glycol.

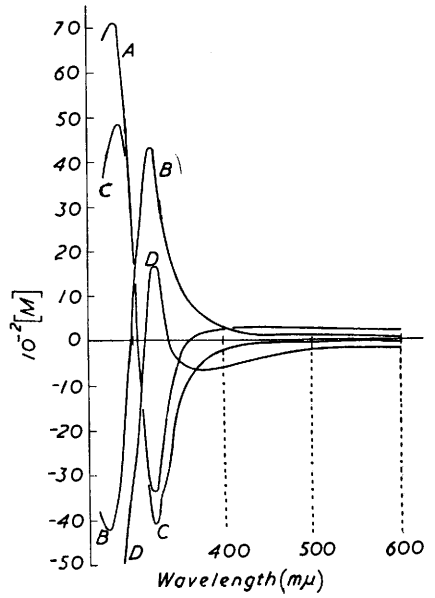
No stereochemical change at position 7 or 9a is implied in the pinacol–pinacolone mechanism; but the Wagner–Meerwein mechanism requires that the 7→9 two-carbon bridge in gibberic acid has the opposite configuration (α) to that (β) which it occupied in allogibberic acid [5] on the projection formulæ shown.

The optical rotatory dispersion curve *D* (see Figure) of the ester (XIII) derived [2] from allogibberic acid showed a positive Cotton effect and was similar to curve *B* obtained for the ketone (XIV), derived from gibberellin A_4 methyl ester [6] (XV; R = R′ = H) by

* Part XVI, preceding paper.

[1] Cross, Grove, MacMillan, and Mulholland, *Chem. and Ind.*, 1956, 954.
[2] Mulholland, *J.*, 1958, 2693.
[3] Birch, Rickards, Smith, Winter, and Turner, *Chem. and Ind.*, 1960, 401.
[4] (*a*) Birch, Rickards, and Smith, *Proc. Chem. Soc.*, 1958, 192; (*b*) Birch and Smith, Ciba Foundation Symposium on the Biosynthesis of Terpenes and Steroids, Churchill, London, 1959, p. 253; (*c*) Birch, Rickards, Smith, Harris, and Whalley, *Tetrahedron*, 1959, **7**, 241.
[5] Grove and Mulholland, *J.*, 1960, 3007.
[6] Takahashi, Seta, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 396.

ozonolysis; as expected, the peak was shifted slightly towards longer wavelengths. Thus, the sign of the Cotton effect in gibban-8-ones is not affected by a 7-hydroxyl substituent. The configuration at 4b also has little effect on the shape of the rotatory dispersion curves for such ketones: the curves for gibberic and epigibberic acids were almost superimposable after the plain positive " background " curve for deoxoepigibberic acid (X) had been subtracted from the latter. Gibberic (curve *C*) and epigibberic acid showed negative Cotton effect curves, antipodal to (*B*) and (*D*). This evidence strongly supports the absolute configuration (II) for gibberic acid; the same conclusion has been reported, briefly, by Stork and Newman.[7] Epigibberic acid, which results from the action of mineral acid on epiallogibberic acid [5] (V) (see scheme), is therefore represented by the absolute configuration (VI).



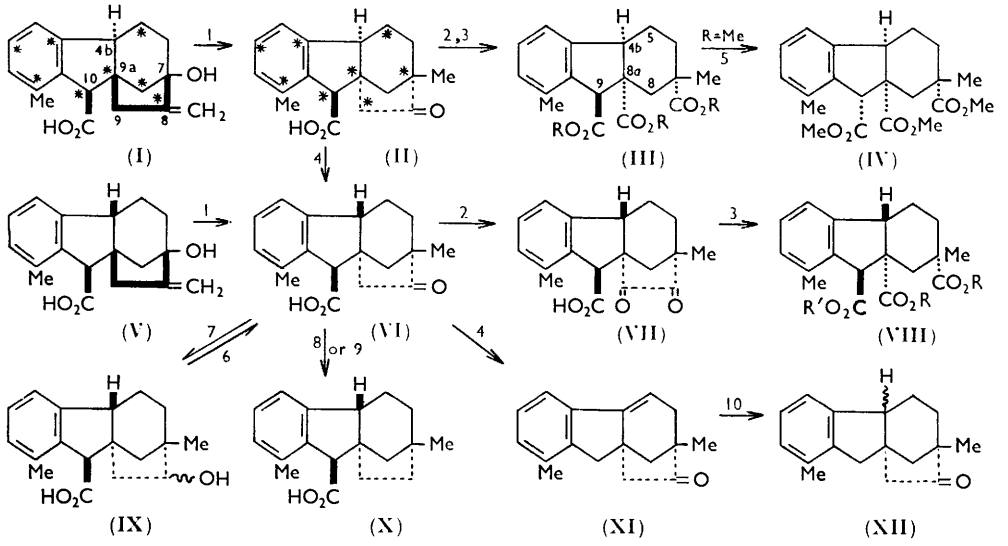
Optical rotatory dispersion curves for the ketones: (A), (XVII; R = H); (B), (XIV); (D), (XIII); and (C) gibberic acid.

The incorporation of $Me\cdot{}^{14}CO_2H$ into gibberellic acid should result in allogibberic acid having the labelling pattern shown in (I) and this was confirmed [4] by degradative experiments. If the rearrangement (I) ⟶ (II) proceeds by the Wagner–Meerwein mechanism gibberic acid should possess the labelling pattern shown in (II) and the acetic acid obtained by Kuhn–Roth oxidation of (II) should contain one-eighth of the molar activity of the gibberellic acid, and hence of the derived allogibberic acid, and should be independent of the relative yields from the two methyl groups. Birch and his collaborators reported results in which the activity of the acetic acid obtained by Kuhn–Roth oxidation varies from 0·24*C[4b] to 0·61*C per mole.[4a] We find 1·01*C per mole by Kuhn–Roth oxidation and 1·09*C per mole when the oxidation was carried out by acid permanganate.[8] The acetic acid obtained by Kuhn–Roth oxidation of dihydroallogibberic acid contained 1·13*C per mol. We conclude therefore that the Wagner–Meerwein mechanism satisfactorily accounts for the isomerisation of allogibberic to gibberic acid.

The rotatory dispersion curve (*A*) for the keto-acid (XVII; R = H), obtained by acid hydrolysis [9] of gibberellin methyl A_1 ester (XV; R = OH, R′ = H), also showed a negative Cotton effect and was antipodal to that for the hydroxy-keto-ester [9] (XVI), prepared by ozonolysis of methyl gibberellate. The two-carbon bridge in the keto-acid (XVII; R = H) therefore has the absolute configuration shown.

[7] Stork and Newman, *J. Amer. Chem. Soc.*, 1959, **81**, 3168.
[8] Cross, Grove, MacMillan, and Mulholland, *J.*, 1958, 2520.
[9] Cross, *J.*, 1960, 3022.

The chemistry of epigibberic acid is similar to that of gibberic acid [8,10] and the points of difference are explicable in terms of the stereochemistry (VI) (cf. ref. 5). Dehydrogenation with selenium at 360° gave 1,7-dimethylfluorene and gibberone (XI), and the latter compound was also obtained on dehydrogenation with 30% palladium–charcoal at 260°. Oxidation with selenium dioxide gave an orange α-diketone, epigibberdionic acid (VII), $C_{18}H_{18}O_4$, which was oxidised by alkaline hydrogen peroxide to the $C_{18}H_{20}O_6$ tricarboxylic acid (IX; R = R′ = H). Dehydrogenation of the latter with 30% palladium–charcoal gave 1,7-dimethylfluorene. Both Clemmensen and Wolff–Kishner



reduction of epigibberic acid gave deoxoepigibberic acid (X). Pondorff reduction of gibberic acid failed,[8] but epigibberic acid furnished the expected 8-epimeric alcohols (IX), which were separated by fractional crystallisation and were re-oxidised by chromic oxide to epigibberic acid.

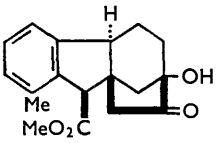
Pyrolysis of epigibberic acid on charcoal at 250° gave a complex mixture from which 1,7-dimethylfluorene (7·5%), gibberone (30%), a ketone of unknown structure but isomeric with gibberone $C_{17}H_{18}O$ (9·5%), a trace of a substance, m. p. >340°, and the decarboxylation product epidihydrogibberone (XII; 4bβ) (5·0%) were isolated. Pyrolysis of gibberic acid under the same conditions gave similar yields of 1,7-dimethylfluorene, gibberone, and the high-melting substance but only a trace of the ketone $C_{17}H_{18}O$ and no epidihydrogibberone. The last compound has, however, been isolated in small yield from the crude gibberone [8] obtained by dehydrogenation of gibberic acid with palladium–charcoal.

Under a variety of conditions [5] catalytic hydrogenation of dehydrodihydroallogibberic acid (XVIII) took place exclusively from the α-side of the molecule and regenerated the original stereochemistry at position 4b. Under the same conditions hydrogenation of dehydrogibberic acid also took place from the α-side, giving gibberic acid (cf. ref. 8) and no epigibberic acid; and methyl dehydrogibberate likewise gave methyl gibberate. It follows that the configuration of the 10-carboxyl [7] or 10-methoxycarbonyl substituent rather than that of the two-carbon bridge determines the steric course of catalytic hydrogenation in these compounds. In confirmation of this, hydrogenation of gibberone with

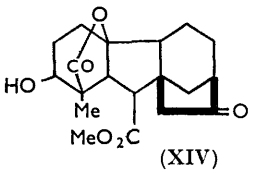
[10] Cross, *J.*, 1954, 4670.

Adams catalyst in acetic acid took place from the β-side, *trans* to the two-carbon bridge, giving epidihydrogibberone, though with a palladium–charcoal catalyst in ethyl acetate some dihydrogibberone (XII; 4bα) was also formed.

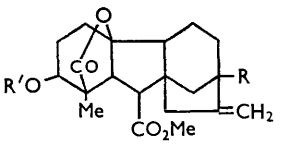
Molecular models (cf. ref. 5) show that the less-hindered configuration of the 9-carboxyl



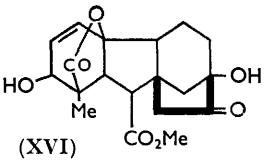
(XIII)



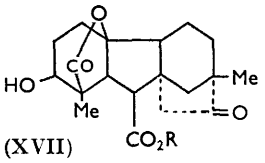
(XIV)



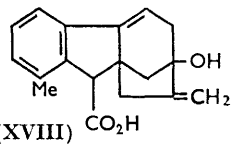
(XV)



(XVI)



(XVII)



(XVIII)

substituent is β in the B/C-*trans* 4bβ-tricarboxylic acid (VIII; R = R′ = H). In agreement with this observation alkaline hydrolysis of the ester (VIII; R = R′ = Me) proceeded without inversion of configuration at position 9, and the resulting monobasic acid (VIII; R = Me, R′ = H) regenerated the original ester (VIII; R = R′ = Me) on methylation with diazomethane. By contrast, similar treatment of the B/C-*cis*-4bα-ester (III; R = Me), derived [10] from gibberic acid, gave the 9α-ester (IV).

Although gibberic [8] and dihydroallogibberic [5] acid readily gave 4b(5)-dehydro-derivatives on permanganate oxidation, epigibberic and dihydroepiallogibberic [5] acid and the 10α-isomer of dihydroallogibberic acid, in which the 10-carboxyl substituent and the 4b-hydrogen atom are *cis*-related, were resistant to oxidation. Oxidation at position 4b in these tetracyclic compounds is therefore determined by the configuration of the 10-carboxyl group rather than by the configuration of the two-carbon bridge. The acid (III; R = H), in which the 9-carboxyl group and 4b-hydrogen atom are *trans*-related, was stable to permanganate; but in this compound access to position 4b is restricted by the adjacent bulky carboxyl substituent.

Gibberellin A_4 was first isolated [6] from the culture filtrates of certain Tokyo University strains of *G. fujikuroi*. Takahashi *et al.*[6] were unable to decide between $C_{18}H_{22}O_5$ and $C_{19}H_{24}O_5$ for the molecular formula of gibberellin A_4 although the C_{19} formula was adopted in a recent paper.[11] Gibberellin A_4 is formed in low yield from *G. fujikuroi* N.R.R.L. 2284 strain; the molecular weight of the methyl ester, determined by the *X*-ray method, and analysis of derivatives are in agreement only with the $C_{19}H_{24}O_5$ formula.

Experimental

M. p.s are corrected. Celite 545 and alumina grade II (pH 4) were used in chromatography. Light petroleum had b. p. 60—80°. Unless otherwise stated, infrared spectra were determined on Nujol " mulls " and ultraviolet spectra and specific rotations were determined for EtOH solutions. Optical rotatory dispersion curves were obtained for MeOH solutions (*c* 0·1 for plain curves: 0·01 for Cotton effect curves) at 18—22°.

Gibberellin A_4.—*Gibberella fujikuroi* N.R.R.L. 2284 strain was grown as described by Stodola *et al.*[12] but harvested by the method of Borrow *et al.*[13] The crude gibberellin (3·1 g. from 200 l. of culture filtrate) in ether (2 l.) was chromatographed [14] on Celite (200 g.) buffered

[11] Takahashi, Seta, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 405.
[12] Stodola, Raper, Fennell, Conway, Sohns, Langford, and Jackson, *Arch. Biochem.*, 1955, **54**, 240.
[13] Borrow, Brian, Chester, Curtis, Hemming, Henehan, Jefferys, Lloyd, Nixon, Norris, and Radley, *J. Sci. Food Agric.*, 1955, **6**, 340.
[14] Grove, Jeffs, and Mulholland, *J.*, 1958, 1236.

at pH 7·0, and elution was with ether (1 l. portions) saturated with water. Eluates 1—5 contained small amounts of intractable material. Eluate 6 afforded a gum (255 mg.) from which crude gibberellin A_4, m. p. 205—210° (70 mg.; 0·35 ml. per l. of culture fluid), was obtained by the addition of light petroleum to an ethyl acetate solution. Subsequent eluates furnished mixtures of gibberellin A_1 and gibberellic acid which were separated by rechromatography on a larger column of Celite.[14]

Gibberellin A_4 was dimorphic and formed rhombs (from ethyl acetate–light petroleum or benzene–methanol), m. p. 216° (decomp.) or 255° (decomp.), $[\alpha]_D^{20}$ −3° (*c* 0·4) (Found: C, 68·7; H, 7·5. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%), $\nu_{max.}$ 3500 (OH), 2700, 2615 (OH of CO_2H), 1736, 1720 (C=O), and 1657 cm.$^{-1}$ (C=C). Analytical results were erratic when gibberellin A_4 was crystallised from ethyl acetate–light petroleum (cf. Takahashi *et al.*[6]) and solvent was tenaciously retained. With concentrated sulphuric acid gibberellin A_4 gave only a straw-yellow colour. It sublimed at 190—200°/10^{-4} mm.

The *methyl ester* (XV; R = R′ = H) formed prisms (from ethyl acetate–light petroleum), m. p. 176°, $[\alpha]_D^{25}$ 0° ± 3° (Found: C, 69·4, 69·1; H, 7·6, 7·7; OMe, 9·4. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6; OMe, 9·0%), $\nu_{max.}$ 3435 (OH), 1773 (γ-lactone C=O), 1715 (ester C=O), 1655 cm.$^{-1}$ (C=C). The unit cell was orthorhombic, space group $P2_12_12_1$ or $P2_12_12$ and had a = 22·75, b = 8·56, c = 9·54 Å, and d 1·250 g. cm.$^{-3}$; M (4 molecules per unit cell), 349·8 (theor., 346·4).

The *acetyl derivative* (XV; R = H, R′ = Ac) of the ester, prepared by the action of acetic anhydride in pyridine during 24 hr., formed prisms, m. p. 130°, from methanol (Found: C, 67·7; H, 7·2; Ac, 13·2. $C_{22}H_{28}O_6$ requires C, 68·0; H, 7·3; 1 Ac, 11·1%), $\nu_{max.}$ 1776 (γ-lactone C=O), 1731 (ester C=O), 1650 cm.$^{-1}$ (C=C) (no OH absorption).

Takahashi *et al.*[6,11] give m. p. 222° (decomp.), $[\alpha]_D^{20}$ −21° or −15° for gibberellin A_4, and m. p. 175—176° and 131—133° for the methyl ester and its acetyl derivative respectively. The infrared spectra of gibberellin A_4 and its derivatives, obtained from *G. fujikuroi* N.R.R.L. 2284 strain, were identical with the spectra published by Takahashi *et al.*[6]

Ozonolysis of Gibberellin A_4 Methyl Ester.—Ozonised oxygen was passed through the ester (50 mg.) in glacial acetic acid (5 ml.) at 10° (ozone uptake: 7 mg.). After 1 hr. at room temperature water (5 ml.) was added and the mixture was steam-distilled. A saturated aqueous solution of dimedone was added to the distillate and after 48 hr. at 6° the methone derivative of formaldehyde was collected (28 mg., 0·66 mol.; m. p. and mixed m. p. 187—190°). The fraction not volatile in steam was neutralised and extracted with ethyl acetate. The gum recovered from the extract was crystallised three times from ethyl acetate–light petroleum, giving *methyl 1-carboxy-2,4a-dihydroxy-1-methyl-8-oxogibbane-10-carboxylate 1 ⟶ 4a-lactone* (XIV) (25 mg.), prisms, m. p. 205—207°, $[\alpha]_D^{25}$ +50° (*c* 0·4) (Found: C, 65·4; H, 7·9. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·9%), $\nu_{max.}$ 3525 (OH), 1761, 1744 cm.$^{-1}$ (C=O) (in $CHCl_3$, 1770 and 1740 cm.$^{-1}$). Takahashi *et al.*[11] give m. p. 203° for the ozonolysis product of gibberellin A_4 methyl ester.

Reduction of Epigibberic Acid.—(1) *Pondorff method.* Epigibberic acid[10] (284 mg.) was added to aluminium isopropoxide (1 g.) in propan-2-ol (20 ml.), and the mixture was heated under partial reflux for 2·5 hr. and then evaporated to dryness *in vacuo*. The residue was treated with 2N-hydrochloric acid (20 ml.) at 0° and the product (280 mg.) obtained by ether-extraction was separated by trituration with ether into a solid fraction (250 mg.) and an intractable gum. Fractional crystallisation of the solid from aqueous ethanol furnished a less soluble portion (*A*; 150 mg.) and a residue (*B*).

Fraction *A* recrystallised from aqueous ethanol and then from ether, giving cubes (138 mg.) of an *alcohol* (IX), m. p. 200—201°, $[\alpha]_D^{22}$ +125° (*c* 1·26) (Found: C, 75·75; H, 7·85%; equiv., 309. $C_{18}H_{22}O_3$ requires C, 75·5; H, 7·7%; M, 286), $\nu_{max.}$ 3400 (alcoholic OH), ~3100—2600 (broad; OH of CO_2H) 1691 cm.$^{-1}$ (C=O of CO_2H). Fraction *B*, together with the residues from *A*, was fractionally crystallised from ether–light petroleum (b. p. 40—60°), giving mixtures of nodules and prisms. The nodules recrystallised from ether as prisms (112 mg.) of the 8-epimeric *alcohol* (VII), m. p. 103—110° (gas evolution), $[\alpha]_D^{22}$ +134° (*c* 1·37) (Found, dried at 78°: C, 74·3; H, 8·4. $C_{18}H_{22}O_3,0{\cdot}5C_4H_{10}O$ requires C, 74·3; H, 8·4%), $\nu_{max.}$ 3430, 3340 (alcoholic OH), ~3160—2600 (broad; OH of CO_2H); 1707 cm.$^{-1}$ (C=O of CO_2H). The solvent-free alcohol, m. p. 162—164°, was obtained by drying the solvate at 100° *in vacuo* (Found: C, 75·7; H, 7·8%; equiv., 287).

(2) *Clemmensen method.* Epigibberic acid (725 mg.), zinc amalgam (1 g.), concentrated hydrochloric acid (9 ml.), water (9 ml.), and toluene (15 ml.) were heated under reflux for 96 hr.,

a further 2 ml. of concentrated hydrochloric acid being added after each 24 hr. period. The toluene layer was added to an ethereal extract of the aqueous layer, and the combined extracts were washed with dilute hydrochloric acid followed by water. The product (643 mg.; m. p. 208—215°) obtained on recovery crystallised from aqueous ethanol in plates (420 mg.), m. p. 221—222°, of *deoxoepigibberic acid* (X) (Found: C, 80·1; H, 8·3%; equiv., 279. $C_{18}H_{22}O_2$ requires C, 80·0; H, 8·2%; *M*, 270), $[\alpha]_D^{20}$ +144° (*c* 1·04), $\nu_{max.}$ 1704 cm.$^{-1}$, $\lambda_{max.}$ ~260, 265·5, 274, 288, 299 mμ (log ε 2·55. 2·59, 2·46, 1·57, 1·46 respectively).

(3) *Wolff–Kishner method.* Epigibberic acid (175 mg.), 100% hydrazine hydrate (0·40 ml.), and sodium ethoxide (0·17 g. of sodium in 3·5 ml. of ethanol) were heated in a sealed tube at 185° for 8 hr. After dilution with water, the solution was extracted with ether and then acidified with hydrochloric acid. The precipitate (76 mg.; m. p. 210—215°) was filtered off and crystallised from aqueous ethanol, giving plates (60 mg.), m. p. and mixed m. p. 220—221° with deoxoepigibberic acid prepared as described in (2) above.

Oxidation of the Alcohols (IX).—(*a*) The epimer of m. p. 200—201° (30 mg.) in acetic acid (1 ml.) was treated with chromic oxide (8 mg.) in 80% acetic acid (1 ml.) at room temperature. After 2 hr., the mixture was heated at 100° for 10 min., cooled, and diluted with water. The product (28 mg.), recovered in ethyl acetate, crystallised from ethanol in prisms of epigibberic acid, m. p. and mixed m. p. 249—251°.

(*b*) Oxidation of the alcohol of m. p. 162—164° (30 mg.) in the same way gave epigibberic acid (27 mg.).

Oxidation of Epigibberic Acid.—(1) *Selenium dioxide.* The acid (1·10 g.) in ethanol (5 ml.) was heated with selenium dioxide (2·9 g.) in a sealed tube at 140° for 4·5 hr. Extraction of the filtered and diluted mixture with ether and recovery gave an orange oil (1·2 g.) which solidified. Recrystallisation from ethyl acetate afforded *epigibberdionic acid* (VII), orange prisms (605 mg.), m. p. 288—290°, $[\alpha]_D^{24}$ +337° (*c* 0·95) (Found: C, 72·45; H, 6·1. $C_{18}H_{18}O_4$ requires C, 72·5; H, 6·1%), $\lambda_{max.}$ 258, 263, ~275, 290, 301 mμ (log ε 2·68, 2·66, 2·47, 2·07, 2·08 respectively), $\lambda_{max.}$ (in 0·1N-NaOH) 260, 265, ~275, 292, 307 mμ (log ε 2·73. 2·73, 2·58, 2·05, 2·07 respectively), $\nu_{max.}$ 3340, ~1750, 1739 cm.$^{-1}$.

In sodium hydroxide epigibberdionic acid gave a colourless solution from which it was recovered on acidification.

(2) *Attempted oxidation with potassium permanganate.* (By Dr. B. E. Cross). The acid (43 mg.) in saturated sodium hydrogen carbonate solution (0·5 ml.) and water (0·5 ml.) was treated at 25° with 1% potassium permanganate solution (2·6 ml.) during 1 hr. After 15 min. the potassium permanganate was reduced with sulphur dioxide and the solution was acidified with concentrated hydrochloric acid. The precipitate (35 mg.) was collected and crystallised from aqueous methanol, giving epigibberic acid, m. p. and mixed m. p. 250—253°.

Dehydrogenation of Epigibberic Acid.—(A) *With selenium at* 360°. The acid (48 mg.) and selenium powder (48 mg.) were heated together as described [15,8] for the dehydrogenation of gibberic acid. The acidic fraction of the product consisted of unchanged epigibberic acid (15 mg.). The neutral fraction (20 mg.) in light petroleum (b. p. 40—60°) was chromatographed on alumina (7 × 1 cm.). Elution with the same solvent gave successively 1,7-dimethylfluorene (1 mg.), m. p. 95—103°, and gibberone (XI) (12 mg.), m. p. 125—127°, identified by mixed m. p. determinations and comparison of the infrared spectra.

(B) *With palladium at* 260°. The acid (100 mg.) and 30% palladium–charcoal [8] (33 mg.) were heated together for 1·5 hr. in a stream of nitrogen. The product was separated into epigibberic acid (11 mg.) and a neutral gum (53 mg.) which was chromatographed on alumina (5 × 1 cm.) in ether. Elution of the blue fluorescent band furnished gibberone (5 mg.), m. p. and mixed m. p. 124—126°.

(C) *With charcoal at* 250°. (i) The acid (100 mg.) and charcoal (50 mg.) were heated as in (B). Carbon dioxide (0·2 mol.) was evolved (baryta trap). The neutral portion of the product afforded gibberone (25 mg.), m. p. 126—127°.

(ii) The acid (2·01 g.) and charcoal (2·04 g.) were heated for 6 hr. (0·45 mol. of carbon dioxide evolved). The neutral portion (1·08 g.) of the product in light petroleum (b. p. 40—60°) was chromatographed on alumina (16 × 1 cm.) in ultraviolet light, giving the following fractions (colour of band in parentheses): I (blue) 97 mg.; II (pale blue) 551 mg.; III (pale blue) 55 mg.; IV (inter band) 27 mg.; V (yellow) 118 mg.; VI (pale green) 205 mg.

Fractions III and IV were intractable. Rechromatography of fraction I on alumina

[15] Mulholland and Ward, *J.*, 1954, 4676.

(10 × 1 cm.) in light petroleum (b. p. 40—60°) and elution of two distinct blue fluorescent bands gave (*a*) 1,7-dimethylfluorene (64 mg.), m. p. 105—107°, and (*b*) epidihydrogibberone, m. p. 98° (see below) (17 mg.). Rechromatography of fraction II on alumina (15 × 1 cm.) and elution of four distinct blue fluorescent bands gave (*c*) 1,7-dimethylfluorene (35 mg.), m. p. 103—105°, (*d*) after further chromatography on alumina and elution with benzene, 1,7-dimethylfluorene (4 mg.), epidihydrogibberone (7 mg.), and a substance subliming without melting at 350—360° (2 mg.), (*e*) epidihydrogibberone (64 mg.), and (*f*) intractable gums (240 mg.). Fraction V was rechromatographed on alumina (10 × 1 cm.), and the following yellow bands were eluted with ether–light petroleum (b. p. 40—60°) (3 : 97), (*g*) 25 mg., intractable; (*h*) 75 mg. combined with fraction VI below; (*j*) 20 mg., intractable. Fraction VI together with fraction V (*h*) was chromatographed in benzene on alumina (20 × 1·5 cm.), giving (*k*) an intense yellow band, intractable gum (66 mg.), and (*l*) colourless bands which afforded, on sublimation at $80°/10^{-2}$ mm. and crystallisation from methanol, a *ketone* as needles (160 mg.), m. p. 124—125°, $[\alpha]_D^{24}$ +133° (*c* 1·54 in $CHCl_3$) (Found: C, 85·8; H, 7·7. $C_{17}H_{18}O$ requires C, 85·7; H, 7·6%), $\lambda_{max.}$ 229, 236, ~283, 297, ~324 mμ (log ε 4·07, 4·11, 4·31, 4·42, 4·22 respectively), $\nu_{max.}$ 1655, 1621, 1596 $cm.^{-1}$. The *dinitrophenylhydrazone* crystallised from chloroform in deep red needles, m. p. 281—282° (Found: C, 66·2; H, 5·4. $C_{23}H_{22}N_4O_4$ requires C, 66·0; H, 5·3%), $\lambda_{max.}$ (in $CHCl_3$) 269, 325, 410 mμ (log ε 4·25, 3·96, 4·56 respectively).

The ketone gave a pale yellow-green colour in concentrated sulphuric acid.

Dehydrogenation of Gibberic Acid on Charcoal at 250°.—(i) The acid (100 mg.) was treated as described for epigibberic acid (Ci, above), giving acid (60 mg.) and neutral (25 mg.) fractions consisting respectively of unchanged gibberic acid and gibberone, m. p. and mixed m. p. 126—127°.

(ii) The acid (1·0 g.) under the conditions described for epigibberic acid (C ii, above) yielded carbon dioxide (0·77 mol.), 1,7-dimethylfluorene (36 mg., 5·3%), the substance which sublimed at 350—360° (2 mg.), and the ketone, m. p. 124—125°, $[\alpha]_D^{24}$ +133° (10 mg.).

Oxidation of Epigibberdionic Acid.—The acid (122 mg.) in methanol (1·4 ml.) and 8% sodium hydroxide solution (5·83 ml.) was heated under reflux with 30% hydrogen peroxide (3·5 ml.) for 30 min. Acidification of the cooled mixture followed by ether-extraction yielded 5,6,7,8,4bβ,8a-*hexahydro*-1,7-*dimethylfluorene*-7α,9β,8aα-*tricarboxylic acid* (VIII; R = R′ = H), prisms (78 mg.), m. p. 283—284°, $[\alpha]_D$ +87° (*c* 0·97) (from acetic acid) (Found: C, 64·9; H, 6·1. $C_{18}H_{20}O_6$ requires C, 65·05; H, 6·1%), $\nu_{max.}$ ~3200—2600 (broad) 1711 $cm.^{-1}$ (OH and C=O of CO_2H).

The *methyl ester* (VIII; R = R′ = Me), prepared with diazomethane, crystallised from light petroleum in stout needles, m. p. 130—132°, $[\alpha]_D$ +84° (*c* 1·1) (Found: C, 67·15; H, 7·0; OMe, 24·4. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0; 3OMe, 24·85%), $\nu_{max.}$ 1729 $cm.^{-1}$.

Action of Alkali on the Esters (VIII; R = R′ = Me) *and* (III; R = Me).—(1) The ester (VIII; R = R′ = Me) (100 mg.) in methanol (10 ml.) was heated under reflux with 0·25N-sodium hydroxide (7 ml.) for 2 hr. Removal of the methanol *in vacuo* and ether-extraction of the residual aqueous solution gave a neutral fraction (8 mg.) which was discarded. Acidification of the aqueous mother-liquors followed by ether-extraction furnished 4bβ,5,6,7,8,8a-*hexahydro*-7α,8aα-*dimethoxycarbonyl*-1,7-*dimethylfluorene*-9β-*carboxylic acid* (VIII; R = Me, R′ = H), prisms (88 mg.), m. p. 226—228° (from benzene) (Found: C, 66·6; H, 6·8%; equiv., 350. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7%; *M*, 360), $\nu_{max.}$ ~3200—2600, 1733, 1700 $cm.^{-1}$.

Methylation of the acid with diazomethane regenerated the ester (IX; R = R′ = Me), m. p. and mixed m. p. 130—132°.

(2) The ester (III; R = Me) [10] {109 mg., $[\alpha]_D^{23}$ +5° (*c* 1·6)} was treated as described in (1). The neutral fraction (88 mg.) crystallised from light petroleum (b. p. 40—60°) in prisms, m. p. 119—120°, $[\alpha]_D^{25}$ −22° (*c* 1·26), of *trimethyl* 4bα,5,6,7,8,8a-*hexahydro*-1,7-*dimethylfluorene*-7α,9α,8aα-*tricarboxylate* (IV) (Found: C, 67·3; H, 7·1; OMe, 24·1. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0; 3OMe, 24·85%), $\nu_{max.}$ 1730 $cm.^{-1}$ (1740 $cm.^{-1}$ in CCl_4).

Attempted Oxidation of the Acid (III; R = H) (By Dr. B. E. Cross).—4bα,5,6,7,8,8a-Hexahydro-1,7-dimethylfluorene-7α,9β,8aα-tricarboxylic acid (III; R = H) (99 mg., decomp. above 158°) [10] in saturated sodium hydrogen carbonate solution (1 ml.) and water (1 ml.) at 0° was treated with 1% aqueous potassium permanganate (10 ml.) during 20 min. After 10 min. at 20° excess of permanganate was destroyed with sulphur dioxide, and the solution was acidified with concentrated hydrochloric acid and extracted with ether. Recovery afforded the acid (III; R = H) (45 mg.) identified by the infrared spectrum.

Dehydrogenation of the Acid (VIII; R = R′ = H).—The acid (100 mg.) and 30% palladium–charcoal (55 mg.) were heated as described above at 170—230° for 1 hr. and then at 260 for 2 hr. Working up in the usual way afforded gummy acid (16 mg.) and neutral fractions (30 mg.). Repeated chromatography of the neutral fraction in light petroleum (b. p. 40—60°) on alumina gave 1,7-dimethylfluorene (5 mg.), m. p. 101—103°.

Hydrogenation of Gibberone (XI).—(*a*) The compound [8] (158 mg.) in ethyl acetate (10 ml.) was shaken in hydrogen at room temperature in the presence of 10% palladium–carbon (uptake 1·0 mol. in 44 min.). The recovered product was sublimed at 120°/10^{-3} mm.; fractional crystallisation of the sublimate from methanol gave (i) prisms (61 mg.), m. p. 85—95°, (ii) needles (47 mg.), m. p. 60—80°, and (iii) mixed fractions. Fraction (i) crystallised from methanol in prisms and plates (31 mg.) of *epidihydrogibberone* (XII; 4bβ), m. p. 97—99°, $[\alpha]_D^{22}$ +30° (*c* 0·82 in $COMe_2$) (Found: C, 84·7; H, 8·5. $C_{17}H_{20}O$ requires C, 84·95; H, 8·4%), $\lambda_{max.}$ ~260, 266, 274, 291, 301 mμ (log ε 2·59, 2·66, 2·56, 1·76, 1·73 respectively), $\nu_{max.}$ (in CCl_4) 1748 cm.$^{-1}$. The prisms and plates had distinct infrared spectra in Nujol mulls. Fraction (ii) crystallised from methanol in long needles (21 mg.) of *dihydrogibberone* (XII; 4bα), m. p. 84°, $[\alpha]_D^{19}$ +6° (*c* 1·09 in $COMe_2$) (Found: C, 84·95; H, 8·4%), $\nu_{max.}$ (in CCl_4) 1744 cm.$^{-1}$.

(*b*) The compound (152 mg.) in acetic acid (10 ml.) was hydrogenated as in (*a*) in the presence of Adams catalyst (60 mg.). After 11 min. (absorption 1·05 mol.) the reaction was stopped. The recovered product sublimed *in vacuo*, giving a sticky solid (141 mg.). Crystallisation from ether and from methanol gave epidihydrogibberone (117 mg.), m. p. 90—95°, raised to 95—98° by further crystallisation.

Hydrogenation of Methyl Dehydrogibberate.—The oily *ester*, $[\alpha]_D^{25}$ +85° (*c* 1·03) (Found: OMe, 10·1. $C_{19}H_{20}O_3$ requires OMe, 10·5%), was prepared from pure dehydrogibberic acid,[8] m. p. 222 (decomp.), $[\alpha]_D$ +100°, which gave gibberic acid, $[\alpha]_D$ −3°, on hydrogenation with palladium–charcoal in ethyl acetate.

(*a*) The ester (30 mg.) in methanol (3 ml.) was shaken in hydrogen with Adams catalyst (30 mg.) in methanol (3 ml.). Recovery gave a gum (31 mg.), $[\alpha]_D^{20}$ +2° ± 3° (*c* 0·53). One crystallisation from light petroleum gave plates and prisms, m. p. 107—111°, identical (mixed m. p. and infrared spectrum) with methyl gibberate, m. p. 110—111°, $[\alpha]_D^{23}$ −4° (*c* 1·15) {methyl epigibberate, m. p. 93—94°,[10] had $[\alpha]_D^{20}$ +123° ± 3° (*c* 0·88)}.

(*b*) Hydrogenation of the ester (31 mg.) in ethyl acetate (6 ml.) with 10% palladium–charcoal (35 mg.) gave a gum (28 mg.) $[\alpha]_D^{21}$ −3° ± 3° (*c* 0·50). Crystallisation gave methyl gibberate, m. p. 109—111°, identified as in (*a*) above.

(*c*) The ester (30 mg.) was hydrogenated as in (*a*), in acetic acid (6 ml.) with Adams catalyst (30 mg.). Recovery gave a gum (22 mg.), $[\alpha]_D^{24}$ +2° (*c* 0·57). Crystallisation gave methyl gibberate, m. p. 105—110°, identified as in (*a*) (above).

Hydrogenation of Dehydrogibberic Acid (XVIII).[8]—Hydrogenation was carried out as described for the dehydro-derivative of dihydroallogibberic acid.[5] Results are tabulated.

Catalyst (mg.)	Solvent (ml.)	Starting material (mg.)	Crude product m. p.	Crude product $[\alpha]_D^{19}$ (±1°)
10% Pd–C (31)	EtOAc (6)	26	138—144°	+7° (*c* 1·05)
10% Pd–C (32)	EtOAc (6)	23	143—148	+4 (*c* 1·15) *
2% Pd–$SrCO_3$ (52)	0·1N-NaOH (6)	32	130—140	0 (*c* 1·16)
PtO_2 (31)	AcOH (7)	26	Gummy crystals	+4 (*c* 0·84)
PtO_2 (52)	EtOAc (7), conc. HCl (0·05)	32	120—135°	+1 (*c* 0·99)
PtO_2 (32)	EtOAc (9), 60% $HClO_4$ (0·002)	30	Gummy crystals	+8 (*c* 0·68)

* Recrystallisation gave gibberic acid, m. p. and mixed m. p. 148—150°, $[\alpha]_D^{20}$ +1° (*c* 0·63). Epigibberic acid [10] has m. p. 227—230° or 252—255°, $[\alpha]_D^{15}$ +131°.

Hydrogenation of the 10α-Isomer of Allogibberic Acid.—The acid [5] (804 mg.) and Adams catalyst (87 mg.) in methanol (35 ml.) were shaken in hydrogen at room temperature (absorption 1·0 mol. in 30 min.). The recovered gummy product {791 mg., $[\alpha]_D^{19}$ −108° (*c* 1·02)} consisted essentially of the 10α-*isomer* of dihydroallogibberic acid (Found: C, 75·1; H, 7·7. $C_{18}H_{22}O_3$ requires C, 75·5; H, 7·7%), $\lambda_{max.}$ at 262—263, ~267 mμ ($E_{1\,cm.}^{1\%}$ 29·9, 30·0 respectively).

Attempted Oxidation of the 10α-Isomer.—The above dihydro-derivative (200 mg.) in saturated sodium hydrogen carbonate solution (4 ml.) and water (4 ml.) was treated at 0° with ice-cold 1% potassium permanganate solution (32 ml.) during 2 min. and set aside at 0° for 7 min. Sulphur dioxide was passed through the mixture and the solution was acidified to pH 3 with

hydrochloric acid. An amorphous intractable solid (62 mg.; m. p. 105—115°) separated, and had $[\alpha]_D^{19}$ −113° (*c* 1·06), $\lambda_{max.}$ 262, 267 mμ ($E_{1\,cm.}^{1\%}$ 20·0, 19·7). It absorbed no gas on microhydrogenation. Extraction of the aqueous mother-liquor and recovery from the extract gave a gum {86 mg.; $[\alpha]_D^{19}$ −92° (*c* 1·05)}, $\lambda_{max.}$ at 261—264 mμ ($E_{1\,cm.}^{1\%}$ 34·0) (microhydrogenation uptake 0·1 mol.). The infrared spectra of the products showed that they consisted mainly of starting material.

[^{14}C]*Gibberellic Acid.*—*Gibberella fujikuroi* was grown in the usual way [13] until production of gibberellic acid had commenced (67 hr.). At this stage a solution of sodium [1-^{14}C]acetate (0·5 mc.) in distilled water (10 ml.) was added and the fermentation was continued until all the sugar had been utilized (303 hr.). A second solution of sodium [1-^{14}C]acetate (0·5 mc.) in distilled water (10 ml.) was added and the fermentation was continued for a further 24 hr. The gibberellic acid was isolated and purified in the usual way.[13,10] Incorporation of radioactivity was 0·4%.

Degradation of [^{14}C]*Gibberellic Acid.*—The gibberellic acid was degraded by procedures previously described,[10,2] and the products were assayed essentially by standard methods.[16,17] Acetic acid was assayed as *p*-bromophenacyl acetate. The results are expressed according to the method of Birch *et al.*[17]

10^{-3} *Relative molar activity.*

	(*a*) Obs.	Calc.	(*b*) Obs.	Calc.		(*b*) Obs.	Calc.
Gibberellic acid	793·8	—	415·0	—	Allogibberic acid	427·6	415·0
Gibberic acid	779·3	793·8	426·0	415·0	Dihydroallogibberic acid	414·2	415·0
Acetic acid ($KMnO_4$)	108·4	99·2	—	—	Acetic acid (Kuhn–Roth)	58·8	51·9
,, ,, (Kuhn–Roth)	52·1	51·9					

Rotatory Dispersion Curves.—Values are for [*M*]. The hydroxy-keto-ester (XVI); positive Cotton effect curve. (600 mμ) +500°; (327·5, peak) +3800°; (290, trough) −1250°; (270) +1250°.

Methyl gibberate; negative Cotton effect curve. (600 mμ) 0°; (325, peak) −4000°; (285, trough) +6600°; (275) +6000°.

Epigibberic acid; negative Cotton effect curve. (600 mμ) +350°; (400) +900°; (320, peak) −1900°; (285, trough) +9000°; (275) +6500°.

Deoxoepigibberic acid (X); plain positive curve. (600 mμ) +350°; (400) +900°; (300) +2400°.

Epidihydrogibberone; negative Cotton effect curve. (600 mμ) +100°; (320, peak) −3000°; (275) +7500°.

Dihydrogibberone; negative Cotton effect curve. (600 mμ) 0°; (325, peak) −5000°; (275) +8500°.

The keto-ester (XVII; R = Me); negative Cotton effect curve. (600 mμ) +200°. (322·5, peak) −3650°; (275, trough) +8000°; (270) +7000°.

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