Gibberellic Acid. Part I.

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Gibberellic acid, $C_{19}H_{22}O_6$, a plant-growth promoting metabolite of *Gibberella fujikuroi* (Curtis and Cross, *Chem. and Ind.*, 1954, 1066), has been shown to be a tetracyclic dihydroxy-lactonic acid. Four products have been isolated from its acid hydrolysis, one of them identical with gibberic acid and another with gibberellin B (Yabuta *et al.*, *J. Agric. Chem. Soc. Japan*, 1941, **17**, 894). The formulæ of these two products have been corrected to $C_{18}H_{20}O_3$. Some oxidations of gibberic acid are described.

The isolation of a metabolite of *Gibberella fujikuroi* (Sawada) Wollenweber, the organism which causes the Bakanae disease in rice, was reported by Yabuta and Sumiki (*J. Agric. Chem. Soc. Japan*, 1938, **14**, 1526) and Yabuta and Hayashi (*ibid.*, 1939, **15**, 257; *Chem. Abs.*, 1939, **33**, 8238). The active compound, m. p. 242—244° (decomp.), $[\alpha]_D$ +36·1°, which was first named gibberellin B and later gibberellin A, possessed remarkable growth-promoting properties and produced the elongation of the shoots of rice seedings characteristic of the Bakanae disease. Yabuta *et al.* (*J. Agric. Chem. Soc. Japan*, 1941, **17**, 721, 894, 975; 1951, **25**, 159; *Chem. Abs.*, 1950, **44**, 10,814—6; 1952, **46**, 5143), and Yatazawa and Sumiki (*J. Agric. Chem. Soc. Japan*, 1952, **25**, 503), have described the characterisation and degradation of gibberellin A for which they have proposed the formula $C_{22}H_{26}O_7$.

In these laboratories unsuccessful attempts to isolate gibberellin A led to the isolation of a new metabolite which has been named gibberellic acid (Curtis and Cross, *Chem. and Ind.*, 1954, 1066). This compound is a powerful plant-growth promoter, producing elongation of the shoots of wheat and pea seedlings (Brian, Elson, Hemming and Radley, *J. Sci. Food Agric.*, 1954, in the press) similar to that described by Yabuta *et al.*, Gibberellic acid, which decomposes at 233—235°, is a colourless crystalline optically active acid ($[\alpha]_D^{19}$ +86°; pK 3·8) giving analyses for 1·4 *C*-methyl groups but no methoxyl groups. The analysis, equivalent weight, and molecular weight (by the crystallographic method) indicate a formula $C_{19}H_{22}O_6$.*

Gibberellic acid and gibberellin A are very similar in their biological properties and have two acid hydrolysis products in common. Nevertheless they differ in optical rotation and slightly in decomposition point, and the former gives one mol. of carbon dioxide on acid hydrolysis whereas gibberellin A is reported to give none (Yabuta *et al.*, *loc. cit.*; Sumiki, personal communication). Moreover, the melting points of the methyl and *p*-bromophenacyl esters differ significantly. Finally, direct comparison showed that gibberellic acid was distinct from gibberellin A, kindly supplied by Professor Sumiki. The two compounds gave different infra-red spectra when examined as Nujol mulls and in the 10—12-μ region in dioxan solution, and, when crystallised from the same solvent mixture, gave different *X*-ray powder photographs.

In cold concentrated sulphuric acid gibberellic acid gives an intense wine-red colour with a strong blue fluorescence, but no colour with ferric chloride, and does not reduce Fehling's solution or ammoniacal silver nitrate. It is unstable to alkali and mineral acid and is rapidly oxidised by alkaline potassium permanganate. A weak positive nitrochromic acid test indicates the presence of a primary or secondary alcohol group and this has been confirmed by the infra-red spectrum (Table 1) and by the preparation of a monoacetyl derivative, $C_{21}H_{24}O_7$. Gibberellic acid yields a *p*-bromophenacyl ester and a neutral monomethyl ester, $C_{20}H_{24}O_6$. Acetylation of the latter gives methyl acetylgibberellate, $C_{22}H_{26}O_7$, also obtained by methylation of acetylgibberellic acid. The infra-red spectrum of gibberellic acid in Nujol (Table 1) shows no absorption in the region normally associated with carboxylic-OH in solid (dimeric) acids; nevertheless the band at 1736 cm.$^{-1}$ (in dioxan)

* *Added in Proof.*—Comparison of a sample of gibberellin X, from a culture filtrate of *Gibberella fujikuroi* (Stodola *et al.*, *Arch. Biochem.*, 1954, in the press), kindly supplied by Dr. Stodola, with gibberellic acid shows their identity.

is assigned to aliphatic carboxyl because (*a*) gibberellic acid forms a methyl ester without the uptake of water and (*b*) the absorption spectra exclude other acidic groupings such as a β-diketone system. Gibberellic acid and its derivatives (Table 1) all contain a high-frequency band in the C=O stretching region, showing the presence of a saturated γ-lactone, further evidence for which is provided by consumption of a second equivalent of alkali on warming the acid with a small excess of 0·1N-sodium hydroxide. The remaining oxygen atom in gibberellic acid must be an unreactive, possibly tertiary, alcohol grouping since the infra-red spectrum of methyl acetylgibberellate shows an OH band at 3510 cm.$^{-1}$.

In the ultra-violet region methyl acetylgibberellate, unlike several of the hydrolysis products of gibberellic acid (see Table 1), shows no maximum in the range 220—320 mμ and does not, therefore, contain an aromatic ring. Microhydrogenation of the acid results in the uptake of from 1·75 to 2·18 mols. of hydrogen. On the assumption of two ethylenic bonds, there must be four alicyclic rings and one lactone ring in the structure, which can be represented as $C_{17}H_{19}(OH)_2(CO{\cdot}O{\cdot}){\cdot}CO_2H$.

TABLE 1. *Infra-red absorption maxima* (*cm.*$^{-1}$).

Compound	Nujol mull		Dioxan solution	
	CO	OH	CO	OH
Gibberellic acid	1746	3305, 3390	1784, 1736	~3470
acetate	1765, 1736	3400	1786, 1740	—
Me ester	1770, 1711	3490	1777, ~1720	—
Me ester acetate	1761, 1740, 1733	3510	—	—
Gibberic acid [a]	1717, 1741	3290	1740	—
Me ester [b]	1736	—	—	—
*epi*Gibberic acid	1714, 1737	Broad	1731, 1744	—
Me ester	1735, 1745	—	—	—

[a] In CCl_4 solution, OH absorption at 3525 cm.$^{-1}$ (CO region not examined). [b] In CCl_4 solution, absorption at 1745 cm.$^{-1}$, corresponding in intensity to two CO groups.

Like gibberellin A, gibberellic acid is readily attacked by mineral acid and yields several products two of which, gibberic acid and *allo*gibberic acid, have been obtained in good yield from both precursors. Unlike gibberellin A, however, gibberellic acid rapidly evolves carbon dioxide under these conditions. Thus with dilute hydrochloric acid at 55—65° gibberellin A is reported to give gibberellin B ($C_{19}H_{22}O_3$), m. p. 194—196°, $[\alpha]_D$ −82·3°, whilst gibberellic acid yields carbon dioxide and *allo*gibberic acid ($C_{18}H_{20}O_3$), m. p. 200·5—203°, $[\alpha]_D^{20}$ −80°. Gibberellin B and *allo*gibberic acid both absorb 0·5—1·0 mol. of hydrogen during catalytic hydrogenation, giving products melting at 199—201° and 197—199·5° respectively. In each case the product does not depress the melting point of its precursor. This led Yabuta *et al.* (*loc. cit.*) to conclude that hydrogenation of gibberellin B had not occurred; however, the infra-red spectra and elementary analyses of *allo*- and dihydro*allo*-gibberic acid show quite clearly that these two hydroxy-acids are different. *allo*Gibberic acid is stable to boiling dilute alkali but is isomerised by boiling mineral acid to gibberic acid. Yabuta *et al.* found that gibberellin B was a plant-growth promoter whereas *allo*gibberic acid is not (Radley, personal communication); nevertheless it seemed possible that these two compounds were the same, and this has been proved by comparison of the infra-red spectra.*

Hydrolysis of gibberellic acid with boiling mineral acid yields one mol. of carbon dioxide and gibberic acid, m. p. 153—154° or 174—175°, $[\alpha]_D^{17\cdot5}$ −7°, as the main products; two other products *epi*gibberic acid, m. p. 252—255°, $[\alpha]_D^{15}$ +131°, and an acid, m. p. 266—269° (decomp.), have been isolated in small yield. *epi*Gibberic acid is dimorphic, sometimes crystallising in a form, m. p. 227—230°. Under similar conditions gibberellin A gives gibberic acid, m. p. 153—154°, $[\alpha]_D$ 0°, *iso*gibberic acid, m. p. 173—174°, $[\alpha]_D$ 0°, and gibberellin C, m. p. 251—252° (decomp.). Direct comparison, by infra-red spectra and mixed melting point, of gibberic acid from the two sources (the author is indebted to

* The name *allo*gibberic acid has been retained, in preference to gibberellin B, since this compound is an acid isomeric with, and closely related to, gibberic acid. The author is indebted to Professor Sumiki for a specimen of gibberellin B.

Professor Sumiki for one sample) showed them to be identical. It seems unlikely that *iso*gibberic acid and gibberic acid are different compounds since gibberic acid prepared from gibberellic acid usually has two melting points, apparently dependent on the crystal size and rate of heating. Yabuta *et al.* (*loc. cit.*) proposed the formula $C_{19}H_{22}O_3$ for gibberic acid. However, analyses and equivalent-weight determinations on gibberic acid and its derivatives conclusively establish the formula $C_{18}H_{20}O_3$ for this acid whence it follows that the hydrolysis of gibberellic acid involves the loss of carbon dioxide and water.

TABLE 2. *Ultra-violet absorption maxima* (mμ).

Compound	$\lambda_{max.}$	log ε	Compound	$\lambda_{max.}$	log ε
*allo*Gibberic acid	266, 274·5	2·50, 2·35	Methyl gibberate	265, 273, 294	2·53, 2·45, 1·61
Gibberic acid ...	265, 274, 300	2·56, 2·47, 1·49	*epi*Gibberic acid	265, 274	2·46, 2·31
Gibberic acid *...	265·5, 273·5, 292	2·63, 2·56, 1·69	Acid, m. p. 266—269° (decomp.)	293	1·52

* In 0·1N-NaOH. The other specta were measured in EtOH.

The ultra-violet absorption spectra of the hydrolysis products and their derivatives (Table 2) show that all these compounds except the acid, m. p. 266—269° (decomp.), contain one benzene ring and are probably closely related in basic structure.

Gibberic acid forms a neutral monomethyl ester, an oxime, and an ester oxime. Attempts to hydrogenate gibberic acid having been unsuccessful, it may be concluded that this acid contains no ethylenic double bond, so that the formation of gibberic acid from *allo*gibberic acid may be interpreted as the isomerisation of an unsaturated alcohol to the ketone. The infra-red spectrum of gibberic acid (Table 1) shows C=O absorption at 1717 and 1741 cm.$^{-1}$ (Nujol mull). These bands are assigned to an aliphatic carboxyl group and a five-membered ring ketone respectively. In common with several other acids described in this paper the infra-red spectrum of gibberic acid in a Nujol mull shows no OH absorption in the region 2500—2700 cm.$^{-1}$ but exhibits a high-frequency OH band (3290 cm.$^{-1}$) (cf. MacMillan, *J.*, 1953, 1697). The presence of a carboxyl group is, however, confirmed by the rise in the frequency of the C=O band from 1717 cm.$^{-1}$ in the solid to 1740 cm.$^{-1}$ in dioxan solution and by the shift of the OH band to 3525 cm.$^{-1}$ in carbon tetrachloride solution. Alcohols in carbon tetrachloride show OH absorption at frequencies greater than 3600 cm.$^{-1}$, hence the high-frequency OH band must be assigned to monomeric carboxylic OH. Consideration of these facts and the formula of gibberic acid shows that it is a tetracyclic keto-acid containing an aromatic ring. *epi*Gibberic acid closely resembles gibberic acid in its infra-red and ultra-violet absorption spectra (see Tables), in the formation of a monomethyl ester and an oxime, and in the absence of ethylenic bonds. The relation of *epi*gibberic acid to gibberic acid is being investigated and the results which have been obtained so far suggest that the acids are epimers. The other hydrolysis product, the acid of m. p. 266—269° (decomp.), has only been obtained in very small yield. Elementary analysis is in agreement with the formula $C_{19}H_{24}O_7$ corresponding to the addition of one molecule of water to gibberellic acid. The compound is presumably not a gibberellic acid hydrate because it gives only a pale greenish-yellow colour with concentrated sulphuric acid, indicating that the structure of gibberellic acid has been modified. Further, water has not been added by opening of the lactone ring since the acid in a Nujol mull shows a C=O stretching band at 1762 cm.$^{-1}$ (1778 cm.$^{-1}$ in dioxan solution).

Yatazawa and Sumiki (*loc. cit.*) have shown that oxidation of gibberic acid with selenium dioxide gives a yellow acid, m. p. 188° (gibberdionic acid), for which in agreement with their formula for gibberic acid they propose the formula $C_{19}H_{20}O_4$. By further oxidation of gibberdionic acid with alkaline hydrogen peroxide they obtained carbon dioxide and a dicarboxylic acid decomposing at 154° for which they proposed the formula $C_{17-18}H_{22-24}O_6$. This result was explained by assuming that gibberdionic acid has the partial structure $C_{16}H_{18}(CO_2H)\cdot CO\cdot CHO$. These experiments have been repeated but with somewhat different results. Selenium dioxide oxidation of gibberic acid has given gibberdionic acid, m. p. 189·5—191°, which must, however, have the formula $C_{18}H_{18}O_4$ and this is supported by analyses of the acid and its methyl ester. If the assumption that gibberic acid is a five-

membered ring ketone is correct, then gibberdionic acid must be a cyclic α-diketone. Oxidation of gibberdionic acid with alkaline hydrogen peroxide under the conditions described by Yatazawa and Sumiki yielded only a trace of carbon dioxide but gave an acid which decomposed at 158°. This acid gave analyses in fair agreement with the formula $C_{18}H_{20}O_6,\frac{1}{2}H_2O$ and was conclusively shown to be a tricarboxylic acid by analysis of its trimethyl ester for methoxyl. Gibberdionic acid would give a tricarboxylic acid on mild oxidation if it contained a cyclic α-diketone grouping but not if it had the structure suggested by Yatazawa and Sumiki. Further evidence for an α-diketone structure is provided by the infra-red spectrum of methyl gibberdionate which shows C=O bands at 1736, 1750, and 1764 cm.$^{-1}$ (Nujol mull). The band at 1736 cm.$^{-1}$ is assigned to the ester grouping and the other two C=O bands to a five-membered ring α-diketone (cf. camphorquinone which shows C=O bands at 1748 and 1769 cm.$^{-1}$; Duncanson, personal communication). The spectrum does not contain an OH or C=C band, thus showing that there is no enol present, either because there is no enolisable hydrogen atom or because structural factors prevent enolisation. The inability of gibberdionic acid to enolise is confirmed by the ultra-violet spectrum of gibberdionic acid which is almost identical in ethanol and 0·1N-sodium hydroxide and closely resembles that of gibberic acid.

Dehydrogenation of the tricarboxylic acid with selenium or palladised charcoal gives a good yield of a hydrocarbon, gibberene, $C_{15}H_{14}$, which is the main product from the dehydrogenation of gibberic acid with selenium (Mulholland and Ward, following paper).

Experimental

M. p.s are corrected. Microanalyses are by Messrs. W. Brown and A. G. Olney. Infra-red measurements were made with a Grubb–Parsons S 3A single beam spectrometer through which dry air was continuously circulated. Ultra-violet absorption was measured with a Unicam S.P. 500 Spectrophotometer. Rotations were determined in ethanol solution (1 ml.), in a 1-dm. microtube.

Gibberellic Acid.—Crude gibberellic acid (5·0 g.), extracted from the culture filtrates of *Gibberella fujikuroi* as described by Curtis and Cross (*loc. cit.*), was treated in boiling ethyl acetate (*ca.* 750 ml.) with charcoal, and the boiling filtrate diluted with light petroleum (b. p. 60—80°) (800—900 ml.) until it became faintly turbid. On cooling, clusters of white jagged lathes separated (4·0—4·5 g.), m. p. 233—235° (effervescence). For analysis this process was repeated although the melting point did not rise; the *acid* had $[\alpha]_D^{19}$ +86° (*c*, 2·12) [Found : C, 65·9, 65·85, 65·5; H, 6·6, 6·55, 6·4; *C*-Me (Kühn–Roth), 5·9%; equiv. (potentiometric), 342, 345; (phenolphthalein), 335; *M* (*X*-ray), 345. $C_{19}H_{22}O_6$ requires C, 65·9; H, 6·4; *C*-Me, 4·35%; *M*, 346]. Gibberellic acid crystallises from ethyl acetate, methanol–light petroleum, and dilute solutions in ethyl acetate–light petroleum as bipyramids. The infra-red spectrum in a Nujol mull showed bands at 1328, 1308, 1275 (shoulder at ~1280), 1263, 1250, 1233, 1212, 1191, 1172, 1156, 1128, 1117, 1105 (shoulder at ~1090), 1060, 1032, 1021, 998, 976, 942 (shoulder at ~950), 922, 889 (shoulder at ~900), 862, 847, 810, 778, 761, 744, and 691 cm.$^{-1}$ in addition to those listed in Table 1.

Gibberellic acid is only slightly soluble in water and ether but is readily soluble in methanol, ethanol, and acetone, and moderately soluble in ethyl acetate. It dissolves readily in sodium hydrogen carbonate and sodium acetate solutions. It did not give a semicarbazone or oxime or react with α-naphthyl *iso*cyanate in boiling xylene. It was warmed in 4 equivalents of 0·1N-sodium hydroxide solution on the water-bath for 1 hr.; back-titration with 0·1N-hydrochloric acid indicated consumption of 2·05 equivs. of alkali. Microhydrogenation in acetic acid resulted in the uptake of 1·75—1·88 mols. of hydrogen with a palladium catalyst and of 2·18 mols. (2 expts.) with a platinum catalyst. The *p-bromophenacyl ester* (prisms from ethanol) had m. p. 218—219° (Found : C, 57·65; H, 5·0; Br, 14·35. $C_{27}H_{27}O_7Br,H_2O$ requires C, 57·75; H, 5·2; Br, 14·2%).

Derivatives. (*a*) Gibberellic acid (207 mg.) in methanol (7 ml.) with excess of diazomethane at 0° for 5 hr. gave the *ester* as a glass which crystallised from benzene–methanol as needles (159 mg.), m. p. 208—210°, raised to 209—210° by further recrystallisation, $[\alpha]_D$ +75° (*c*, 0·5) (Found : C, 66·65; H, 6·85; OMe, 8·5. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7; OMe, 8·6%). The acid (124·7 mg.), methyl iodide (0·3 ml.), anhydrous potassium carbonate (0·7 g.), and dry acetone (7 ml.) were heated under reflux for 8 hr. The residue obtained by evaporation was

washed with water and crystallised from ethyl acetate–light petroleum and ethyl acetate–benzene, giving needles, m. p. 206—207° (Found : C, 66·1; H, 6·85%), identical with the preceding ester (mixed m. p. and infra-red spectra).

(*b*) The acid (113 mg.) in pure dry pyridine (1·8 ml.) and acetic anhydride (1·0 ml.) was left at room temperature for 45 hr. The solvents were removed on the water-bath *in vacuo* and the residue was dissolved in sodium hydrogen carbonate solution and then precipitated with hydrochloric acid. The crude *acetyl* derivative (120 mg.), m. p. 180—190° (decomp.), crystallised from ethyl acetate–light petroleum (b. p. 60—80°) as rhombs, m. p. 233—234° (decomp.), $[\alpha]_D^{17}$ +152° (*c*, 0·5) (Found : C, 65·1; H, 6·3. $C_{21}H_{24}O_7$ requires C, 64·9; H, 6·2%).

(*c*) Methyl gibberellate (79 mg.) was left in pure dry pyridine (1 ml.) and acetic anhydride (0·5 ml.) for 42 hr. The solvents were removed *in vacuo* on the water-bath, the residue was treated with sodium hydrogen carbonate solution, and the product (80 mg.) was collected by filtration. Crystallisation from benzene–light petroleum (b. p. 60—80°) gave *methyl acetylgibberellate* as prisms, m. p. 180—181°, $[\alpha]_D^{18}$ +150° (*c*, 0·4) (Found : C, 65·3; H, 6·7. $C_{22}H_{26}O_7$ requires C, 65·7; H, 6·5%). Acetylgibberellic acid (34·5 mg.) in methanol (1·25 ml.) with excess of diazomethane at 0° for 20 hr. gave a gum, which after treatment with sodium hydrogen carbonate solution had m. p. 120—140°. Crystallisation from ethyl acetate–light petroleum (b. p. 60—80°) yielded prisms, m. p. 178—180° not depressed on admixture with the preceding derivative.

Acid Hydrolysis of Gibberellic Acid.—(A) *At* 55—65°. Gibberellic acid (702 mg.), suspended in dilute hydrochloric acid (1 : 10; 10 ml.), was heated at 55—65° for 2·25 hr. (evolution of carbon dioxide). The mixture was cooled and filtered and the product (433 mg.; m. p. 172—216°) washed with water. The dried solid was extracted with boiling benzene and filtered from unchanged gibberellic acid [150 mg.; m. p. 232—234° (decomp.)]. A little ethanol was added to the hot filtrate which on cooling deposited allo*gibberic acid* as plates (245 mg.), m. p. 190—195°; recrystallisation from benzene–ethanol and finally from aqueous methanol gave colourless laths, m. p. 200·5—203°, $[\alpha]_D^{20}$ −80° (*c*, 0·7) [Found : C, 75·95, 76·05; H, 7·1, 7·3%; equiv. (potentiometric), 282. $C_{18}H_{20}O_3$ requires C, 76·0; H, 7·1%; equiv., 284 (monobasic acid)]. *allo*Gibberic acid gives no colour with cold concentrated sulphuric acid and is stable to boiling 5% potassium hydroxide solution.

Isomerisation of allo*gibberic acid to gibberic acid.* *allo*Gibberic acid (27·2 mg.) was heated under reflux with dilute hydrochloric acid (1 : 5; 4 ml.) for 1 hr. The product (22 mg.) was collected and crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving needles, m. p. 146—150°, raised to 151—153° by a second crystallisation. The product was shown to be gibberic acid (see below) by mixed m. p. and infra-red spectrum.

*Dihydro*allo*gibberic acid.* *allo*Gibberic acid (58 mg.) and 10% palladised charcoal (26 mg.) in methanol (5 ml.) absorbed hydrogen slowly (3·7 ml. at 13°/768 mm. in 3 hr.; 0·78 mol.). The recovered product crystallised from benzene–ethanol as plates (44 mg.), m. p. 197—199·5°, not raised by further crystallisation [Found : C, 75·25; H, 7·95%; equiv. (potentiometric), 289. $C_{18}H_{22}O_3$ requires C, 75·5; H, 7·7%; equiv., 286 (monobasic acid)]. *Dihydro*allo*gibberic acid* did not significantly depress the m. p. of *allo*gibberic acid.

(B) *At the boiling point.* (*a*) Gibberellic acid (4·0 g.), concentrated hydrochloric acid (120 ml.), and water (600 ml.) were heated under reflux for 1 hr. and then allowed to cool. The mixture of solidified oil and crystals was collected (3·13 g.) and crystallised from ethyl acetate–light petroleum (b. p. 60—80°) (charcoal), giving the fractions : (i) needles (2·19 g.), m. p. 152·5—154°, (ii) needles (377 mg.), m. p. 147—151°, and (iii) prisms (139 mg.), m. p. 210—220°. Continuous ether-extraction of the aqueous filtrate from the hydrolysis yielded a viscous gum which crystallised from aqueous methanol as rods (72 mg.), m. p. 215—220°, which were combined with (iii).

In one experiment, with boiling dilute sulphuric acid in a slow current of nitrogen, 1·0 mol. of carbon dioxide was evolved (barium hydroxide trap).

Fractions (i) and (ii) were combined and crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving pure *gibberic acid* as needles, m. p. 153—154° or 174—175°, $[\alpha]_D^{17\cdot5}$ −7° (*c*, 2·0), $[\alpha]_{5461}^{19\cdot5}$ −9° (*c*, 2·0) [Found : C, 76·05, 75·95; H, 7·1, 7·15; *C*-Me (Kühn–Roth), 6·5%; equiv., 278. $C_{18}H_{20}O_3$ requires C, 76·0; H, 7·1; *C*-Me, 5·3%; equiv., 284 (monobasic acid)]. Gibberic acid gives no colour with concentrated sulphuric acid and it is stable to boiling 5% potassium hydroxide solution.

The *methyl ester*, prepared with diazomethane, crystallised from light petroleum (b. p. 60—80°) in hexagonal plates, m. p. 113—115° (Found : C, 76·25; H, 7·55; OMe, 10·35. $C_{19}H_{22}O_3$ requires C, 76·5; H, 7·4; OMe, 10·4%). The *oxime*, prepared from the acid by the sodium

acetate–ethanol method, crystallised from aqueous methanol as stout rods, m. p. 250—258° (decomp.) (Found: C, 71·85; H, 7·0; N, 4·65. $C_{18}H_{21}O_3N$ requires C, 72·2; H, 7·1; N, 4·7%) [Yabuta *et al.*, *loc. cit.*, give the m. p.s of gibberic acid, its oxime, and methyl gibberate as 153—154°, 225—230° (decomp.), and 116—117° respectively]. *Methyl gibberate oxime*, prepared from the above oxime and diazomethane, crystallised from light petroleum and aqueous methanol as needles, m. p. 157—160° (Found: C, 72·9; H, 7·45; N, 4·5. $C_{19}H_{23}O_3N$ requires C, 72·8; H, 7·4; N, 4·5%).

Fraction (iii) was crystallised from methanol, giving epi*gibberic acid* as laths, m. p. either 227—230° or 252—255°, $[\alpha]_D^{15}$ +131° (*c*, 0·8) [Found: C, 75·9, 76·35; H, 7·2, 7·0%; equiv. (potentiometric), 286]. Specimens with either melting point gave the same infra-red spectrum and interchanged in melting point during crystallisation. *Methyl* epi*gibberate*, prepared with diazomethane, crystallised from aqueous methanol in needles and from light petroleum in prisms, m. p. 95—96° (Found: C, 76·45; H, 7·5%). The *oxime*, prepared as above, crystallised from aqueous methanol as hexagonal prisms or plates, m. p. 214—215·5° (decomp.) (Found: C, 72·45; H, 7·05; N, 4·7%).

(*b*) Gibberellic acid (548 mg.), concentrated hydrochloric acid (1·75 ml.), and water (10·25 ml.) were heated under reflux at a bath-temp. of 150° for 45 min. The aqueous layer was decanted from some oil whilst still hot. On cooling, the oil solidified (413 mg.; m. p. 80—110°) and the aqueous layer first deposited microcrystals (28 mg.), m. p. 140—148°, and later prisms (5 mg.), m. p. 250—258° (decomp.). Crystallisation of the solidified oil and the microcrystals from ethyl acetate–light petroleum (b. p. 60—80°) yielded gibberic acid. The prisms from this and a similar experiment were crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving prisms, m. p. 266—269° (decomp.) (Found: C, 62·3; H, 7·1. $C_{19}H_{24}O_7$ requires C, 62·6; H, 6·6%). The *compound* is soluble in sodium hydrogen carbonate solution with effervescence and gives a pale greenish-yellow colour with concentrated sulphuric acid. In the infra-red (Nujol) it showed C=O bands at 1717 and 1762 cm.$^{-1}$ and OH bands at 3480, 3420, 3300, 2750, 2690, and 2600 cm.$^{-1}$.

Gibberdionic Acid (cf. Yatazawa and Sumiki, *loc. cit.*).—Gibberic acid (352 mg.) and selenium dioxide (906 mg.) in ethanol (1·5 ml.) were heated in a sealed tube immersed in boiling xylene for 4·25 hr. The orange solution was filtered from selenium (140 mg.), and the filtrate and washings were diluted with water until just turbid and left overnight at 0°. The buff-coloured plates were collected (333 mg.) and at 100° became bright yellow with m. p. 176—184°. Crystallisation from benzene–light petroleum (b. p. 60—80°) or ethyl acetate–light petroleum (b. p. 60—80°) gave gibberdionic acid as yellow prisms, m. p. 189·5—191° (Found: C, 72·25; H, 6·15. $C_{18}H_{18}O_4$ requires C, 72·5; H, 6·1%). Yatazawa and Sumiki record the m. p. of gibberdionic acid as 188°. Gibberdionic acid crystallises from aqueous methanol in white feathery crystals, m. p. 93—96° (effervescence and turning yellow) (Found: C, 66·4; H, 7·1. $C_{18}H_{18}O_4,2CH_3{\cdot}OH$ requires C, 66·3; H, 7·2%).

Methyl Gibberdionate.—Methyl gibberate (70 mg.) and selenium dioxide (170 mg.) in methanol (0·3 ml.) were oxidised as described for gibberic acid and gave the required *ester* (70 mg.), m. p. 180—188°, which crystallised from benzene–light petroleum as pale orange plates, m. p. 193—195° (Found: C, 73·0; H, 6·55. $C_{19}H_{20}O_4$ requires C, 73·1; H, 6·45%).

Oxidation of Gibberdionic Acid (cf. Yatazawa and Sumiki, *loc. cit.*).—Gibberdionic acid (623 mg.) in methanol (30 ml.) and sodium hydroxide (8%; 30 ml.) was heated under reflux with hydrogen peroxide (30%; 18 ml.) for 30 min. After acidification of the cooled solution, the *tricarboxylic acid* was extracted with ether and crystallised once from ether–light petroleum (b. p. 60—80°), decomp. pt. 152—156° (594 mg.). A specimen for analysis was recrystallised from ether in prisms or needles, decomp. pt. above 158°, and dried *in vacuo* over phosphoric oxide at 75° (Found: C, 63·6, 63·25; H, 6·6, 6·7. $C_{18}H_{20}O_6,0{\cdot}5H_2O$ requires C, 63·3; H, 6·2%). The *trimethyl ester*, prepared with diazomethane, distilled at 90—100° (bath)/10^{-4} mm. as a colourless gum (Found: C, 67·55; H, 7·05; OMe, 25·3. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0; 3OMe, 24·85%).

Dehydrogenation. (*a*) With selenium. The tricarboxylic acid (202 mg.) and selenium powder (197 mg.) were heated in a current of nitrogen at 265°, rising to 335° in 25 min., and then at 335—345° for 2 hr. The mixture was extracted with ether, the extract washed with sodium hydroxide, and the product recovered as a yellow solid (119 mg.), which, in light petroleum (b. p. 40—60°) (6 ml.), was poured through a column of acid-washed alumina activated at 120° (12 g.). Elution of the diffuse band (fluorescing pale blue in ultra-violet light) with light petroleum (b. p. 40—60°) yielded white crystals (69 mg.), m. p. 100—103°, which crystallised from methanol in curly needles, m. p. 105·5—107° (Found: C, 92·45; H, 7·2. Calc. for $C_{15}H_{14}$: C, 92·7; H, 7·3%), identical with gibberene (mixed m. p. and infra-red spectrum)

(Mulholland and Ward, *loc. cit.*). Oxidation of gibberene (25 mg.) in acetone with potassium permanganate, as described by Mulholland and Ward, gave gibberenone (16 mg.), m. p. 74—76·5°, raised to 75·5—76·5° by crystallisation, identical with a specimen of authentic gibberenone (mixed m. p. and infra-red spectrum).

(*b*) With palladised charcoal. The tricarboxylic acid (103 mg.) and 30% palladised charcoal (57 mg.) were heated in a current of nitrogen from 170° to 230° (50 min.) and then kept at 233—237° for 1·5 hr. The product was isolated and purified as in (*a*), giving gibberene (29·5 mg.), m. p. 101—105°, raised to 105·5—107° by crystallisation from methanol.

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