606. *Gibberellic Acid. Part XIII.* The Structure of Ring* A.

By B. E. Cross.

Degradative and spectroscopic evidence is presented which shows that gibberellic acid is 2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1→4a-lactone * (I; R = H) and gibberellin A_1 is 2,4a,7-trihydroxy-1-methyl-8-methylenegibbane-1,10-dicarboxylic acid 1→4a-lactone (V; R = H) as proposed by Cross *et al.*[1] Structure (XX) advanced by Takahashi *et al.*[2] for ring A of gibberellic acid is therefore untenable.

Gibberellic acid is formulated as 2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1→4a-lactone † (I; R = H). The evidence on which this structure rests, briefly reported elsewhere,[1] is extended and described in detail in this paper. Gibberellic acid is a tetracyclic dihydroxy-acid containing a γ-lactone ring and two double bonds,[3] one of which is present as a terminal methylene group since methyl gibberellate gave 0·48 mol. of formaldehyde on ozonolysis. On mild treatment with acid, gibberellic

* Part XII, preceding paper. † For nomenclature see Part XII.

[1] Cross, Grove, MacMillan, Moffatt, Mulholland, Seaton, and Sheppard, *Proc. Chem. Soc.*, 1959, 302.
[2] Takahashi, Seta, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1958, **22**, 432.
[3] Cross, *J.*, 1954, 4670.

acid yields allogibberic acid, $C_{18}H_{20}O_3$, which under more vigorous conditions is isomerised to gibberic acid. Allogibberic and gibberic acid have been shown [4,5] to have structures (II) and (III; R = H) respectively.

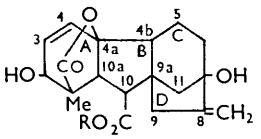
The free carboxyl group of gibberellic acid is attached to the nucleus at the same point as in gibberic and therefore in allogibberic acid since methyl gibberellate yields methyl gibberate (III; R = Me) with boiling mineral acid (cf. Kawarada *et al.*[6]). The easy, acid-catalysed aromatisation of ring A with the concomitant loss of carbon dioxide and water suggests that the lactone ring and one hydroxyl group are attached to ring A. Several attempts to open the lactone ring of gibberellic acid with hydrazine failed. When gibberellic acid was treated with hydrazine in boiling ethanol stereospecific reduction occurred, giving tetrahydrogibberellic acid * (IV; R = H), but no reaction took place when the mixture was left at room temperature for four days.[7] On the other hand, with hydrazine hydrate at 150° gibberellic acid gave gibberellenic acid, allogibberic acid (II), and its 4b-epimer epiallogibberic acid.[8] Controlled catalytic reduction of methyl gibberellate with 0·95 mol. of hydrogen gave [9] an uncharacterised acid and as the main neutral product gibberellin A_1 methyl ester (V; R = Me) which is known to retain the terminal methylene group since it yielded formaldehyde on ozonolysis.[9] Hydrogenation of methyl gibberellate until uptake ceased, in presence of Adams catalyst in methanol or palladised charcoal in ethyl acetate, gave in 30—70% yield a mixture of acids formed by hydrogenolysis of the lactone ring. The high yield of acidic products suggests that the oxygen of the γ-lactone in gibberellic acid is allylic to a double bond. The neutral product was a mixture of the 8-epimers (IV; R = Me) which was not resolved by crystallisation but was separable by chromatography on alumina into methyl tetrahydrogibberellate, m. p. 271—272°, identical with the methyl ester of the tetrahydro-acid obtained by hydrazine reduction, and the 8-epi-ester, m. p. 233—236°. Oxidation of methyl tetrahydrogibberellate (IV; R = Me) with chromic oxide–pyridine or with chromic oxide in acetic acid afforded the monoketone (VI), m. p. 161—163°. Similarly oxidation of the 8-epi-ester gave an isomeric ketone (VI), m. p. 131—133°. These ketones were most readily prepared by oxidation of the epimeric mixture of methyl tetrahydrogibberellates with chromic oxide in acetone containing sulphuric acid, the mixed ketones obtained being separated by fractional crystallisation. It follows that one of the hydroxyl groups in gibberellic acid is secondary; the other is considered to be tertiary. This conclusion is in agreement with the acetylation of gibberellic acid and its reduction products. Gibberellic acid readily yielded a monoacetyl derivative [3] and on longer acetylation a diacetate.[10] Acetylation of methyl tetrahydrogibberellate gave a mono- and a di-acetate, m. p. 213—216°. Prolonged acetylation of the 8-epi-ester gave a diacetate, m. p. 184—186°. The higher- and the lower-melting diacetate were identical with the two diacetyldihydrogibberellin A_1 methyl esters, m. p. 210—212° and m. p. 175—176° respectively, samples of which were kindly supplied by Professor Sumiki.[11]

Both gibberellic and allogibberic acid have a tertiary hydroxyl and a terminal methylene group, and carry a carboxyl group on ring B, and it was suggested in 1956 [12] that these two compounds have the same B–C–D ring structure. This conclusion is supported by the

* It is proposed to call this compound, tetrahydrogibberellic acid, and to distinguish the 8-epimer by the prefix 8-epi.

[4] Mulholland, *J.*, 1958, 2693.
[5] Cross, Grove, MacMillan, and Mulholland, *J.*, 1958, 2520.
[6] Kawarada, Kitamura, Seta, Takahashi, Takai, Tamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1955, **19**, 278.
[7] Cf. Hanus and Vorisek, *Coll. Czech. Chem. Comm.*, 1929, **1**, 223; Vorisek, *Chem. Listy*, 1934, **28**, 57.
[8] Grove and Mulholland, preceding paper.
[9] Grove, Jeffs, and Mulholland, *J.*, 1958, 1236.
[10] Cf. Moffatt and Radley, *J. Sci. Food Agric.*, in the press.
[11] Kitamura, Seta, Takahashi, Kawarada, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 71; Sumiki, *ibid.*, 1959, **23**, 408.
[12] Cross, Grove, MacMillan, and Mulholland, *Chem. and Ind.*, 1956, 954.

ozonolysis of methyl gibberellate (see below) and by the degradation of gibberellin A_1 methyl ester to 8-methylfluoren-2-ol by the sequence, (V; R = Me) ⟶ (X) ⟶ (XI; R = H) ⟶ (IX; R = H, R′ = OH).[13]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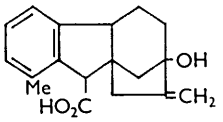
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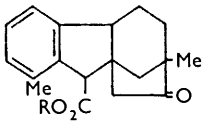
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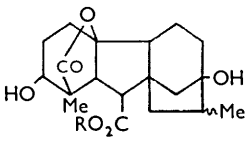
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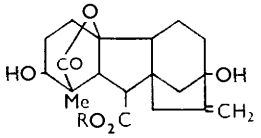
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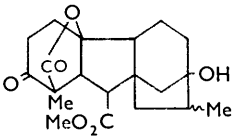
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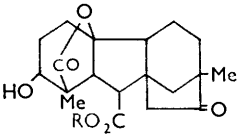
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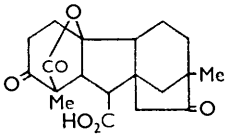
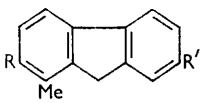

(VIII)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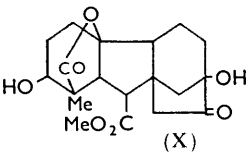
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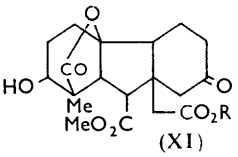
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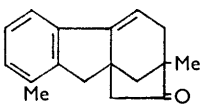

(XII)

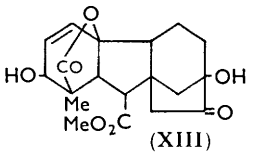
Further, the Japanese workers have reported that gibberellin A_1 [14] and its methyl ester [15] undergo the same acid-catalysed rearrangement of rings C/D as allogibberic acid does, giving as the main product gibberellin C and in very low yield an uncharacterised compound, m. p. 255—257° (decomp.). We have confirmed this result. The main product from this Wagner–Meerwein rearrangement [12] is the 5-ring keto-acid (VII; R = H) which has carbonyl absorption in dioxan at 1777 (γ-lactone), ~1742 (5-ring ketone), and 1730 cm.$^{-1}$ (CO_2H) and is identical with a specimen of gibberellin C for which the author is indebted to Professor Sumiki. The other product is an isomeric keto-acid, m. p. 268—270° (decomp.), more readily isolated as its methyl ester.

The methyl group has been shown [4,5] to be attached to ring A at position 1 in allogibberic and gibberic acid. The following evidence shows, not only that this group is also at the same position in gibberellic acid, but also that the secondary hydroxyl group is at position 2. Dehydrogenation of gibberellic acid with palladised charcoal and with selenium yielded gibberone [5] (XII) and 1,7-dimethylfluorene [16] (IX; R = H, R′ = Me) respectively. The keto-acid (VII; R = H) with chromic oxide–acetone–sulphuric acid gave in high yield the diketone (VIII) which on dehydrogenation with selenium gave 1,7-dimethylfluoren-2-ol (IX; R = OH, R′ = Me), whose structure was established by direct comparison with a specimen prepared by unambiguous synthesis [17] (cf. Seta *et al.*[18]).

Attempts to oxidise the secondary hydroxyl group in methyl gibberellate and gibberellin A_1 methyl ester with chromic oxide, and in the former by the Oppenauer reaction, gave intractable products. The difficulties with chromic oxide were probably due to oxidative attack on the terminal methylene group and consequently it was removed. Methyl gibberellate with one mol. of ozone at −70° yielded the ketol (XIII) and the keto-acid (XIV; R = H), the latter being also obtained by periodate oxidation of the former.

[13] Seta, Kitamura, Takahashi, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 73.
[14] Takahashi, Seta, Kitamura, Kawarada, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 75.
[15] Takahashi, Kitamura, Kawarada, Seta, Takai, Tamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1955, **19**, 267.
[16] Mulholland and Ward, *J.*, 1954, 4676.
[17] Cross and Melvin, following paper.
[18] Seta, Takahashi, Kitamura, Takai, Tamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1958, **22**, 61.

The spectral data for these compounds (see p. 3032) are consistent with the structures assigned. Rearrangement in ring A had not occurred during the isolation of the keto-acid (XIV; R = H) because hydrogenation of the dimethyl ester (XIV; R = Me) gave the saturated ester (XI; R = Me) identical with a specimen obtained by methylating the acidic ozonolysis product of gibberellin A_1 methyl ester (cf. ref. 18), and treatment of the dimethyl ester (XIV; R = Me) with boiling dilute mineral acid aromatised ring A and gave the keto-ester (XV) previously prepared from allogibberic acid.[4]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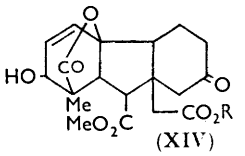
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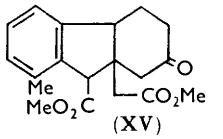
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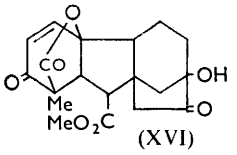
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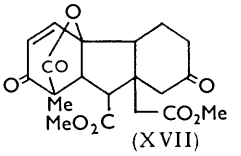
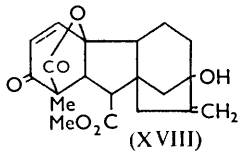

(XVII)

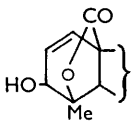

(XVIII)

With chromic oxide–acetone–sulphuric acid the ketol (XIII) gave a compound (XVI) having $\lambda_{max.}$ 229 mμ (ε 7050). This formulation is also supported by infrared carbonyl-absorption in chloroform solution at 1788 (γ-lactone), 1751 (5-ring α-ketol), 1745 (ester), and 1700 cm.$^{-1}$ (CH=CH–C=O). Similarly, although the keto-ester (XIV; R = Me) with chromic oxide in pyridine gave a gum showing no maximum in the ultraviolet spectrum, with manganese dioxide it gave the αβ-unsaturated ketone (XVII) ($\lambda_{max.}$ 229 mμ; ε 7500). Hence ring A of the ketol (XIII) and of the keto-ester (XIV; R = Me) contains an allylic hydroxyl group. This is not in agreement with an earlier failure to oxidise methyl gibberellate with manganese dioxide,[19] but it has now been found that another sample of manganese dioxide (prepared by the method of Mancera, Rosenkranz, and Sondheimer, [20]) oxidises methyl gibberellate in chloroform to the αβ-unsaturated ketone (XVIII) ($\lambda_{max.}$ 228 mμ; ε 9700) so that there can be no doubt that ring A contains the grouping CH=CH–C·OH. Hydrogenation of the unsaturated ketone (XVIII) with a palladised charcoal catalyst gave the two 8-epimeric ketones (VI), previously obtained by oxidation of methyl tetrahydrogibberellate and its 8-epimer. This showed that no rearrangement had taken place during the formation of the unsaturated ketone (XVIII). In contrast to the oxidation of the mixed tetrahydro-compounds (IV) which gave the epimeric ketones (VI) in about equal amounts, hydrogenation of the ketone (XVIII) gave about 22 parts of the ketone of m. p. 131—133° to 1 part of its isomer. Thus, under the conditions used, the hydrogenation of (XVIII), unlike that of methyl gibberellate (I; R = Me), was almost stereospecific at position 8.

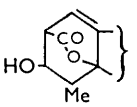
The degradative evidence leaves only two possible structures for ring A of gibberellic acid, namely, (I; R = H) and (XIX). In confirmation, the nuclear magnetic resonance spectra of methyl gibberellate and its reduction products and derivatives indicate [19,21] a tertiary methyl group. Structure (XX), advanced by the Japanese workers [2] for ring A of gibberellic acid is inconsistent with both the degradative evidence and the nuclear magnetic resonance. Structure (XIX) can be eliminated because in dilute acid solution gibberellic acid yields the heteroannular diene, gibberellenic acid (XXI).[1,22] Gibberellic acid must therefore have structure (I; R = H), and gibberellin A_1 structure (V; R = H). The exclusion of structure (XIX) for ring A is supported by further evidence. When the

[19] Cross, Grove, MacMillan, Mulholland, and Sheppard, *Proc. Chem. Soc.*, 1958, 221.
[20] Mancera, Rosenkranz, and Sondheimer, *J.*, 1953, 2189.
[21] Sheppard, *J.*, 1960, 3040.
[22] Moffatt, *J.*, 1960, 3045.

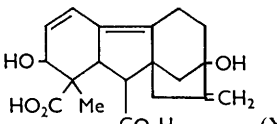
keto-ester (VII; R = Me) was boiled with methanolic hydrogen chloride the lactone ring was opened without the adition of oxygen to give a dimethyl ester (XXII; R = H, OH) which failed to take up hydrogen on attempted hydrogenation. However, the ester gave a yellow colour with tetranitromethane and a comparison of its ultraviolet absorption (ε 5300 and 2830 at 215 and 220 mμ respectively) with that of (VII; R = Me) (ε 290, 260)



(XIX)

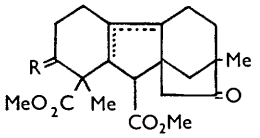


(XX)

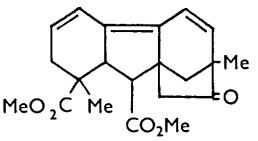


(XXI)

showed that it contains a tetrasubstituted double bond. The dimethyl ester (XXII; R = H, OH) retains a secondary hydroxyl group but is not an allylic alcohol, as would be expected for a structure derived from (XIX) for ring A of gibberellic acid, because it was not oxidised by a sample of manganese dioxide which oxidised methyl gibberellate to (XVIII) and because the derived diketone (XXII; R = O) is not an αβ-unsaturated ketone. Similarly treatment of methyl gibberellate with boiling methanolic hydrogen chloride gave, in addition to methyl gibberate and methyl epigibberate, the dimethyl ester (XXIII),[23] providing further support for structure (I; R = H) for gibberellic acid.



(XXII)



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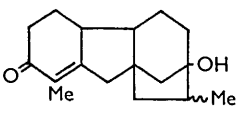


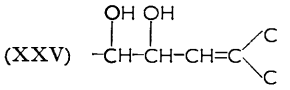
(XXIV)

In a further attempt to determine the position of the lactone carboxyl group the effect of acid on the potential β-keto-acid (VI), m. p. 161—163°, was examined. The ketone (VI) was only slowly attacked in dilute acid; in strong acid the lactone ring was eliminated but dehydration also occurred and the product was the αβ-unsaturated ketone (XXIV).[24]

Gibberellic acid consumed two mol. of dilute alkali at room temperature (cf. ref. 3) and yielded an amorphous dicarboxylic acid, $C_{19}H_{24}O_7,H_2O$, which was readily oxidised by periodate. Methylation of the dicarboxylic acid yielded a crystalline dimethyl ester, $C_{21}H_{28}O_7$, which on hydrogenation gave a tetrahydro-compound. Both the dimethyl ester, $C_{21}H_{28}O_7$, and its tetrahydro-derivative rapidly consumed about one mol. of periodate. The former was also readily oxidised by lead tetra-acetate and more slowly by sodium bismuthate. With all three oxidants the product from the dimethyl ester, $C_{21}H_{28}O_7$, was the same, a crystalline compound, $C_{21}H_{26}O_7$, which however showed neither aldehydic nor ketonic properties (negative *o*-dianisidine and Schiff's test; reduced hot Fehling's solution very slowly; in 0·01M-solution no ultraviolet maximum above 210 mμ; no carbonyl derivatives). Nevertheless, the fission by glycol-splitting reagents and consumption of one mol. of periodate suggested that the dimethyl ester is an α-glycol. Moreover it is also an allylic alcohol because on oxidation with active manganese dioxide in chloroform it yielded an αβ-unsaturated ketone, $C_{21}H_{26}O_7$. This ketone, unlike the manganese dioxide oxidation product of methyl gibberellate (XVIII), has $\lambda_{max.}$ 240 mμ (ε 17,000) and therefore carries two substituents on the double bond; in $CHCl_3$ it has carbonyl absorption at 1682 cm.$^{-1}$ (αβ-unsaturated ketone) in addition to 1728 cm.$^{-1}$ (ester C=O). Since the unsaturated ketone is derived from an α-glycol it should be an α-ketol and in agreement with this it readily formed a monoacetate, reduced cold Fehling's solution, rapidly reduced Tollens reagent, and gave a reddish-purple colour in the blue tetrazolium test. Attempts to cleave it with periodate and bismuthate failed, but it was shown that no rearrangement

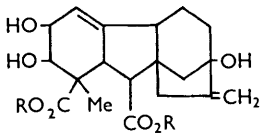
[23] Grove, unpublished work.
[24] Cf. Seta, Takahashi, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1958, **22**, 429.

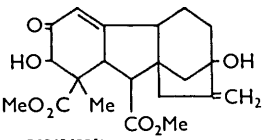
had taken place during the manganese dioxide oxidation by reducing the α-ketol with sodium borohydride back to the dimethyl ester from which it had been derived. These results establish the presence of the grouping (XXV) or (XXVI) in the dimethyl ester,

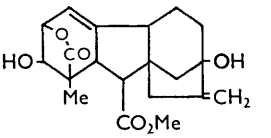

(XXV)

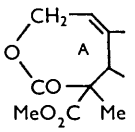

(XXVI)

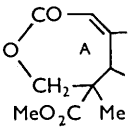
$C_{21}H_{28}O_7$, but only (XXV) can be accommodated in a 1-methoxycarbonyl-1-methyl substituted ring A. Hence the dimethyl ester, $C_{21}H_{28}O_7$, and its manganese dioxide oxidation product have structures (XXVII; R = Me) and (XXVIII) respectively, and an allylic type rearrangement must take place during the alkaline hydrolysis of gibberellic acid. The mechanism of this rearrangement is under investigation.

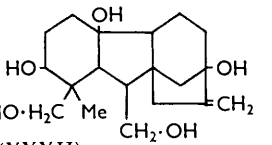

(XXVII)

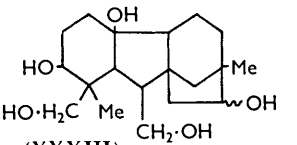

(XXVIII)


(XXIX)


(XXX)


(XXXI)


(XXXII)


(XXXIII)

We can now consider the structure of the periodate oxidation product, $C_{21}H_{26}O_7$. It was shown to contain two double bonds by microhydrogenation and by perbenzoic acid estimation. It retained only one difficultly acetylatable and presumably tertiary hydroxyl group. In boiling alkali it consumed three equivalents whilst the dimethyl ester (XXVII; R = Me) consumed only two. These results can be tentatively explained by assuming that the compound, $C_{21}H_{26}O_7$, contains a lactone ring as in (XXX) or (XXXI) formed by mutual oxidation reduction of the dialdehyde produced by glycol fission of the ester (XXVII; R = Me). The infrared spectrum of the oxidation product, $C_{21}H_{26}O_7$, in CCl_4 showed only one carbonyl band at 1738 cm.$^{-1}$ which taken in conjunction with the absence of an ultraviolet maximum between 210 and 225 mμ [25] favours formula (XXX) rather than (XXXI).

When the dicarboxylic acid (XXVII; R = H) was heated at 90° or under reflux in toluene, and the product methylated, it yielded a neutral monomethyl ester, m. p. 174°, isomeric with methyl gibberellate. This ester must be a γ-lactone (C=O absorption at 1768 and 1721 cm.$^{-1}$ in $CHCl_3$) and from its mode of formation and the fact that it was not oxidised by a sample of manganese dioxide which oxidised methyl gibberellate it has been assigned structure (XXIX), *i.e.*, the structure originally erroneously assigned to methyl gibberellate.[19] The nuclear magnetic resonance of the ester (XXIX) and its acetate are consistent with this.[21]

Sumiki and his collaborators reported [26] that reduction of gibberellin A_1 methyl ester by lithium aluminium hydride gave a pentaol, $C_{19}H_{32}O_5$, which did not react with periodate and was therefore inconsistent with the structure originally proposed for gibberellic acid.[19] In our hands vigorous reduction of gibberellin A_1 methyl ester in this way gave a poorly crystalline pentaol, $C_{19}H_{30}O_5$, which, contrary to the Japanese report, took up 0·84 mol. of hydrogen on hydrogenation and appears to be the expected reduction product (XXXII). Similar reduction of the keto-ester (VII; R = Me) gave a pentaol, $C_{19}H_{32}O_5$, assumed to be

[25] Cf. Nielsen, *J. Org. Chem.*, 1957, **22**, 1539.
[26] Takahashi, Seta, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 327.

(XXXIII). In agreement with structure (I; R = H) for gibberellic acid, neither compound (XXXII) nor (XXXIII) consumed periodate.

Experimental

M. p.s are corrected. Absorption spectra and optical rotations were determined as described previously.[3] Unless otherwise stated infrared spectra were determined on Nujol mulls and ultraviolet absorption for EtOH solutions. Alumina for chromatography was prepared as described in Part II.[16] Microhydrogenations were carried out in acetic acid with a palladium black catalyst.

Acetylation of Gibberellic Acid.—The acid (511 mg.) in dry pyridine (8 ml.) and acetic anhydride (5 ml.) was left at room temperature for 4 days. The crude product (534 mg.; m. p. 180—193°) crystallised from ethyl acetate–light petroleum (b. p. 60—80°) as (i) prisms (150 mg.), m. p. 220—226° (decomp.), and (ii) needles (295 mg.), m. p. 175—183°. Recrystallisation of fraction (i) yielded the monoacetyl derivative,[3] m. p. 233—234° (decomp.) (Found: C, 64·85; H, 6·4. Calc. for $C_{21}H_{24}O_7$: C, 64·9; H, 6·2%). Microhydrogenation resulted in the uptake of 1·81, 1·84 mol. of hydrogen.

Fraction (ii) was diacetylgibberellic acid and crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in rods,[10] m. p. 187·5—188°, $[\alpha]_D^{16}$ +176° (*c* 0·5 in EtOH) (Found: C, 64·25; H, 6·15. Calc. for $C_{23}H_{26}O_8$: C, 64·2; H, 6·1%), $\nu_{max.}$ 3170 (OH of monomeric CO_2H), 1780, 1741, and 1708 (C=O), and 1656 cm.$^{-1}$ (C=C). The methyl ester,[10] m. p. 167—169°, showed $\nu_{max.}$ 1776, 1751, and 1730 (C=O), 1664 cm.$^{-1}$ (C=C), and no OH absorption.

Action of Acid on Methyl Gibberellate.—The ester (105 mg.) was suspended in dilute hydrochloric acid (1 : 5; 18 ml.) and refluxed for 40 min. in a current of nitrogen (evolution of carbon dioxide). The gummy product (76 mg.), recovered by extraction with chloroform, crystallised from light petroleum (b. p. 60—80°) in prisms (42 mg.), m. p. 113—114° not depressed by methyl gibberate but depressed by methyl epigibberate (Found: C, 76·2; H, 7·45. Calc. for $C_{19}H_{22}O_3$: C, 76·5; H, 7·4%).

Reduction of Gibberellic Acid with Hydrazine.—(*a*) A solution of gibberellic acid (1·0 g.) and 90—95% hydrazine hydrate (2 ml.) in ethanol (20 ml.) was boiled under reflux for 14 hr. After removal of the ethanol *in vacuo* a cooled solution of the residue in a little water was acidified with concentrated hydrochloric acid, giving a precipitate [880 mg.; m. p. 285—290° (decomp.)] which crystallised from aqueous ethanol (charcoal) in prisms of *tetrahydrogibberellic acid* (IV; R = H), m. p. 300—301° (decomp.), $[\alpha]_D^{26}$ +64° (*c* 0·5 in EtOH) (Found: C, 65·2; H, 7·45. $C_{19}H_{26}O_6$ requires C, 65·1; H, 7·5%), $\nu_{max.}$ (*a*) 3520 and 3290 (OH), broad ~3200 and ~2560 (OH of CO_2H), 1744 and 1722 cm.$^{-1}$ (C=O), (*b*) in dioxan 1775 (γ-lactone) and ~1730 cm.$^{-1}$ (CO_2H). The acid took up no hydrogen on microhydrogenation and gave no colour with cold concentrated sulphuric acid. This reduction was not always reproducible; on some occasions it gave a gummy partially reduced product which was difficult to purify.

The *methyl ester* (IV; R = Me), prepared with diazomethane in ether–methanol, crystallised from aqueous methanol in prisms, m. p. 271—272°, $[\alpha]_D^{20}$ +54° (*c* 1·0 in MeOH) (Found: C, 65·7; H, 7·9; OMe, 8·65. $C_{20}H_{28}O_6$ requires C, 65·9; H, 7·7; OMe, 8·5%), $\nu_{max.}$ (*a*) 3500, 1753, and 1711 cm.$^{-1}$, (*b*) in chloroform 1767 cm.$^{-1}$ (γ-lactone) and 1735 cm.$^{-1}$ (ester).

(*b*) Gibberellic acid (100 mg.) in ethanol (2 ml.) and 90—95% hydrazine hydrate (0·1 ml.) was set aside for 4 days. After addition of water and acidification with hydrochloric acid, recovery in ethyl acetate gave gibberellic acid (94·5 mg.), m. p. 220—228° (decomp.), which crystallised from aqueous methanol in prisms (71 mg.), m. p. 234—236° (decomp.).

Acetylation of Methyl Tetrahydrogibberellate.—The ester (104 mg.) in dry pyridine (1·5 ml.) and acetic anhydride (1·0 ml.) was left for 44 hr. The solvents were then removed *in vacuo* at 35° and the residue was treated with sodium hydrogen carbonate solution, giving a solid (112 mg.), m. p. 179—209°. Crystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave fractions (i) prisms (64 mg.), m. p. 214—221°, (ii) (21 mg.), m. p. 179—199°, and (iii) rods, m. p. 189—207°. Recrystallisation of fraction (i) gave prisms of the *monoacetate*, m. p. 222—224° (Found: C, 64·9; H, 7·4; OMe, 7·8. $C_{22}H_{30}O_7$ requires C, 65·0; H, 7·4; OMe, 7·6%), $\nu_{max.}$ 3580 cm.$^{-1}$ (OH), 1779 cm.$^{-1}$ (γ-lactone), 1744 cm.$^{-1}$ (OAc) and 1728 cm.$^{-1}$ (ester). Fraction (iii) was crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving the *diacetate* as needles, m. p. 213—216° (Found: C, 64·6; H, 7·3. $C_{24}H_{32}O_8$ requires C, 64·3; H, 7·2%), $\nu_{max.}$ 1768 and 1738 cm.$^{-1}$ (C=O) (no OH absorption).

Hydrogenation of Methyl Gibberellate (With Mr. J. F. Grove).—Methyl gibberellate (4·08 g.)

and 10% palladised charcoal (1·94 g.) in ethyl acetate (380 ml.) absorbed *ca.* 2 mol. of hydrogen in 5 hr. The mixture was filtered and the filtrate and washings were shaken with aqueous sodium carbonate and water. Removal of solvent gave crystals (2·13 g.), m. p. 240—250°. Acidification of the carbonate solution and recovery in ethyl acetate yielded a gummy acid (1·26 g.).

Crystallisation of the neutral product from ethanol–light petroleum (b. p. 60—80°) and from aqueous methanol gave prisms of a mixture of methyl tetrahydrogibberellates (epimeric at position 8 and not separable by crystallisation), m. p. 246—249°, $[\alpha]_D^{23}$ +45° (*c* 1·0 in MeOH) (Found: C, 66·0; H, 7·6; OMe, 8·6. Calc. for $C_{20}H_{28}O_6$: C, 65·9; H, 7·7; OMe, 8·5%), $\nu_{max.}$ 3510 (OH), 1758, and 1713 cm.$^{-1}$ (C=O).

The gummy mixed acids crystallised from ethyl acetate in prisms (635 mg.), m. p. 215—230° (decomp.) (Found: equiv., 367), which did not take up hydrogen. They will be discussed in a later paper.

Chromatography of the crude neutral product (0·42 g.), from a similar reduction, in benzene–methanol (200 : 1; 200 ml.) on alumina (20 g.) and elution with the same solvent in 100 ml. portions gave fractions (i) and (ii) 28 mg., (iii) 125 mg., (iv) 106 mg., (v) 67 mg., and (vi) 23 mg. Fractions (iv)—(vi) crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in needles (45 mg.), m. p. 260—264°, identical (mixed m. p. and infrared spectrum) with methyl tetrahydrogibberellate.

Fraction (iii) was crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving *methyl 8-epitetrahydrogibberellate* (IV; R = Me) as needles (70 mg.), m. p. 233—236°, $[\alpha]_D^{23}$ +43° (*c* 1·0 in EtOH) (Found: C, 66·0; H, 7·8. $C_{20}H_{28}O_6$ requires C, 65·9; H, 7·7%), $\nu_{max.}$ 3520, 1759, and 1712 cm.$^{-1}$. The diacetate, prepared with acetic anhydride in pyridine for 14 days, formed needles, m. p. 184—186°, $\nu_{max.}$ 1764 and 1737 cm.$^{-1}$ (OH absent). It was identical with an authentic specimen prepared by Professor Sumiki by separation of the diacetates resulting from the acetylation of the 8-epimeric mixture of dihydrogibberellin A_1 methyl esters.[11]

Hydrogenation of methyl gibberellate (108 mg.) with Adams catalyst (25 mg.) in methanol (10 ml.) gave an acidic gum (75 mg.) and neutral material (16 mg.). The latter crystallised from aqueous methanol in prisms of the mixed methyl tetrahydrogibberellates, m. p. 244—247°.

Oxidation of Methyl Tetrahydrogibberellate (IV; R = Me).—A solution of the ester (1·0 g.) in purified pyridine (10 ml.) was shaken with the complex from chromic oxide (1·0 g.) and pyridine (10 ml.), left for 42 hr., poured into water (200 ml.), and filtered. The filtrate and washings were extracted with ethyl acetate, and the extract washed with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and water. The recovered solid (0·740 g.) was treated under reflux with Girard's reagent P (2·25 g.) in ethanol (25 ml.) and glacial acetic acid (2·5 ml.) for 1 hr. The ethanol was removed *in vacuo* and water (15 ml.) added, followed by sodium carbonate decahydrate (5·0 g.). Extraction with ethyl acetate and recovery yielded methyl tetrahydrogibberellate (269 mg.), m. p. 250—257°. The aqueous layer was left for 1 hr. after treatment with concentrated hydrochloric acid (6 ml.), then extracted with ethyl acetate, and the extract was washed with aqueous sodium hydrogen carbonate and water. Removal of the solvent gave *methyl 1-carboxy-4a,7-dihydroxy-1,8-dimethyl-2-oxogibbane-10-carboxylate 1→4a-lactone* (VI) (450 mg.), m. p. 148—155°, which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in prisms, m. p. 161—163°, $[\alpha]_D^{16}$ +144° (*c* 0·99 in MeOH) (Found: C, 66·4; H, 7·35. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%), $\lambda_{max.}$ 290 mμ (ε 52), $\nu_{max.}$ (*a*) 3480 (OH), 1792 (γ-lactone), 1728 (6-ring ketone) and 1709 cm.$^{-1}$ (ester), (*b*) in CCl_4 1793 and 1737 cm.$^{-1}$.

The ketone (VI) was also prepared in lower yield by oxidation of methyl tetrahydrogibberellate with chromic oxide in acetic acid at room temperature.

The *oxime*, prepared in pyridine, formed prisms (from methanol), m. p. 263—266° (decomp.) (Found: C, 63·9; H, 7·1; N, 3·9. $C_{20}H_{27}O_6N$ requires C, 63·6; H, 7·2; N, 3·7%), $\nu_{max.}$ 3500, 3410, 1752, 1712, and 1652 cm.$^{-1}$.

Oxidation of the Mixed Methyl Tetrahydrogibberellates.—The esters (2·13 g.) in pure acetone (80 ml.) were treated dropwise at 0° with a solution (2·0 ml.) prepared from chromic oxide (66·8 g.) in concentrated sulphuric acid (57·5 ml.) and water (100 ml.) made up to 267 ml.[27]. The whole was left for 2 hr., decanted into water (200 ml.), and extracted with ethyl acetate, and the extract was washed with aqueous sodium hydrogen carbonate and dried. Recovery gave a gum (2·12 g.) which was crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving (i) prisms (558 mg.), m. p. 142—152°, (ii) needles (449 mg.), m. p. 115—128°, and (iii) a gum.

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

Recrystallisation of fraction (i) from ethyl acetate–light petroleum (b. p. 60—80°) yielded prisms, m. p. 156—159°, identical with the above ketone (VI) (mixed m. p. and infrared spectrum). Fraction (ii) crystallised from ether–light petroleum (b. p. 40—60°) and from ethyl acetate–light petroleum (b. p. 60—80°) as rosettes of needles of the 8-*epi-ketone* (VI), m. p. 131—133°, $[\alpha]_D^{23}$ +126° (*c* 0·6 in MeOH) (Found: C, 66·25; H, 7·25; OMe, 8·05%), $\lambda_{max.}$ 293 mμ (ε 47), $\nu_{max.}$ 3350 (broad; OH), 1778 (γ-lactone), 1740 and 1728 cm.$^{-1}$; in chloroform it showed the same C=O bands as its epimer but small differences in the 7—15 μ region. Fraction (iii) yielded a further quantity of the 8-epi-ketone, m. p. 125—129°.

Oxidation of Methyl 8-Epitetrahydrogibberellate (By Mr. J. F. Grove).—The ester (19 mg.) in acetone (1 ml.) was treated with the chromic oxide solution in sulphuric acid (see above) (0·022 ml.) at −5°. Isolation of the product as in the preceding experiment gave a neutral glass (18 mg.) which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) as needles, m. p. 124—126°, identified as the 8-epi-ketone (VI) by mixed m. p. and infrared spectrum.

Action of Hydrochloric Acid on Gibberellin A_1 Methyl Ester (With Dr. T. P. C. Mulholland).—The ester (200 mg.) was boiled with dilute hydrochloric acid (1 : 5; 15 ml.) for 1·5 hr. After 18 hr. at 0°, filtration gave a solid (A) (105 mg.), m. p. 245—254° (decomp.). The filtrate was extracted with ethyl acetate, and the extract washed with sodium hydrogen carbonate solution. The neutral product (26 mg.) was intractable. Acidification of the carbonate solution and recovery of the product in ethyl acetate gave a semi-solid (B) (27 mg.).

Product (A) crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) or aqueous methanol in prisms of 2,4a-*dihydroxy*-1,7-*dimethyl*-8-*oxo*-7α-*gibbane*-1,10-*dicarboxylic acid* 1→4a-*lactone* (VII; R = H) (62 mg.), m. p. 265—267° (decomp.), $[\alpha]_D^{19}$ +50° (*c* 1·0 in EtOH) [Found: C, 62·5; H, 7·1; Active H, 1·1; H_2O (Karl–Fischer), 3·8%; equiv., 370. $C_{19}H_{24}O_6,H_2O$ requires C, 62·3; H, 7·15; 4 Active H, 1·10; H_2O, 4·9%; *M*, 366), $\lambda_{max.}$ 292·5 mμ (ε 32), $\nu_{max.}$ 3570, 3450, and 3300 (OH), ~2750—~2550 (OH of CO_2H), 1761, 1715, and 1702 (C=O), and 1646 cm.$^{-1}$ (water bending). The keto-acid took up no hydrogen on microhydrogenation. It was not dehydrated by distillation of a solution in toluene.

The *acetate*, prepared with acetic anhydride in pyridine at 20°, crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) as needles, m. p. 281—284° (decomp.) (Found: C, 64·2; H, 6·6. $C_{21}H_{26}O_7$ requires C, 64·6; H, 6·7%), $\nu_{max.}$ (*a*) 1774, 1745, and 1707 cm.$^{-1}$, (*b*) in $CHCl_3$ 1775, 1741, and 1710 (sh) cm.$^{-1}$.

The *methyl ester* (VII; R = Me), prepared with diazomethane in ether–methanol, formed needles from ethyl acetate–light petroleum (b. p. 60—80°), m. p. 226—228°, $[\alpha]_D^{19}$ +54° (*c* 1·0 in $COMe_2$) (Found: C, 65·95; H, 7·35; OMe, 8·7; Active H, 0·4. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2; OMe, 8·6; 1 Active H, 0·3%), $\lambda_{max.}$ 291 mμ (ε 32), $\nu_{max.}$ (*a*) 3500 and 1743 cm.$^{-1}$, (*b*) in $CHCl_3$ 1770 (γ-lactone), 1737 cm.$^{-1}$ (5-ring ketone and ester).

Attempts to prepare ketone derivatives failed.

Product (B) was washed with ether and crystallised from ethyl methyl ketone–ether as crystals (5 mg.) of an *acid*, m. p. 260—264° (decomp.), raise to 268—270° (decomp.) by further crystallisation (Found: C, 64·9; H, 7·0. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·9%), $\nu_{max.}$ 3430, ~3140 (broad), 1779 and 1737 cm.$^{-1}$; the spectrum was distinct from that of the isomeric acid (VII; R = H). This acid may be identical with the uncharacterised acid, m. p. 255—257° (decomp.), obtained in a similar way by Takahashi *et al.*[15]

The *methyl ester* crystallised from ethyl acetate–light petroleum (b. p. 60—80°) as prisms, m. p. 183—185°, $[\alpha]_D^{18}$ +24° (*c* 1·0 in $COMe_2$) (Found: C, 66·65; H, 7·25; OMe, 8·6. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2; OMe, 8·6%), $\lambda_{max.}$ 294 mμ (ε 37), $\nu_{max.}$ (*a*) 3535 (OH), 1755 and 1735 (C=O); (*b*) in $CHCl_3$ 1765 (γ-lactone), 1738 (5-ring ketone), and 1717 cm.$^{-1}$ (ester). No hydrogen was taken up on attempted microhydrogenation.

The keto-acid (VII; R = H) was conveniently prepared in quantity as follows: Methyl gibberellate (3·35 g.) was hydrogenated until 1 mol. of hydrogen had been absorbed [9] and the total product (neutral and acidic, 2·84 g.) was hydrolysed as in the preceding experiment. The mixture was extracted with ethyl acetate, the extract washed with aqueous sodium hydrogen carbonate and water, and the neutral product recovered as a gum (0·63 g.). Acidification of the aqueous layer and recovery in ethyl acetate afforded a gum (2·03 g.) which on crystallisation from ethyl acetate–light petroleum (b. p. 60—80°) formed (i) prisms (0·84 g.), m. p. 253—257° (decomp.), (ii) (0·16 g.), m. p. 230—247° (decomp.), and (iii) a gum. Fraction (i) crystallised from aqueous methanol in prisms of the keto-acid (VII; R = H), m. p. 259—263° (decomp.).

Fraction (ii) and other similar fractions (1·89 g.) were methylated with diazomethane. The

crude ester was crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving (*a*) ne edles (1·34 g.), m. p. 223—225° [infrared spectrum identical with that of the above keto-ester (VII; R = Me)], and (*b*) 360 mg., m. p. 219—225° with an infrared spectrum different from that of fraction (*a*). Recrystallisation of fraction (*b*) from ethyl acetate–light petroleum (b. p. 60—80°) gave prisms (270 mg.), m. p. 173—176°, raised to 183—185° by further recrystallisation and identified as the methyl ester of the acid, m. p. 268—270° (decomp.), by mixed m. p. and infrared spectrum.

Dehydrogenation of Gibberellic Acid.—(a) *With palladised charcoal.* Gibberellic acid (101 mg.) and 30% palladised charcoal (48 mg.) were heated in a current of nitrogen at 280—290° for 4 hr. Extraction with ether and recovery afforded a brown gum (49 mg.) which was chromatographed in light petroleum (b. p. 60—80°) containing a little ether on alumina (3·3 × 1·0 cm.). Elution in ultraviolet light with light petroleum (b. p. 60—80°) removed a deep blue fluorescent band. Further elution with light petroleum (b. p. 60—80°) and light petroleum (b. p. 60—80°)–ether (10 : 1) gave a blue fluorescent eluate. This yielded a solid (15 mg.), m. p. 80—105°, which crystallised from methanol as plates and needles, m. p. 121—123°, of gibberone [5] (XII) (identified by mixed m. p. and infrared spectrum).

(b) *With selenium.* The acid (218 mg.) and powdered selenium (220 mg.) were heated in a current of nitrogen from 250° to 345° in 1 hr. and kept at 345—360° for 6 hr. The brown semi-solid product (43 mg.), recovered by extraction with methanol and ether, was chromatographed in light petroleum (b. p. 40—60°) (2·5 ml.) and ether (0·5 ml.) on alumina (7·8 × 0·8 cm.). Elution in ultraviolet light with light petroleum (b. p. 40—60°) removed a violet-fluorescing band which yielded a solid (26 mg.), m. p. 77—82°. Further chromatography and crystallisation from methanol gave needles of 1,7-dimethylfluorene,[16] m. p. 104—106° (mixed m. p. and infrared spectrum).

Oxidation of the Keto-acid (VII; R = H).—The keto-acid (91·7 mg.) in pure acetone (2·5 ml.) was treated at 0° with the above chromic oxide solution (0·075 ml., 2·2 equiv.). Reaction occurred almost immediately. After 10 min. at 0° and 1 hr. at room temperature, water (10 ml.) was added. Recovery of the product in ethyl acetate gave 4a-*hydroxy*-1,7-*dimethyl*-2,8-*dioxo*-7α-*gibbane*-1,10-*dicarboxylic acid* 1→4a-*lactone* (VIII) (83·7 mg.), m. p. 279—281° (decomp.), which crystallised from aqueous methanol and from ethyl methyl ketone–light petroleum (b. p. 60—80°) in felted needles, m. p. 281—283° (decomp.), $[\alpha]_D^{16}$ +154° (*c* 1·0 in EtOH) (Found: C, 65·75; H, 6·4%; equiv., 333. $C_{19}H_{22}O_6$ requires C, 65·9; H, 6·4%; *M*, 346), $\lambda_{max.}$ 288—291 mμ (ε 88), $\nu_{max.}$ 1775, ~1746 (sh), 1728 and 1701 cm.$^{-1}$ (no OH absorption).

The *methyl ester*, prepared with diazomethane, crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) in needles, m. p. 218—219°, $[\alpha]_D^{23}$ +149° (*c* 1·0 in $COMe_2$) (Found: C, 66·5; H, 6·6; OMe, 8·75. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7; OMe, 8·6%), $\nu_{max.}$ (*a*) 1779, 1736, and 1716 cm.$^{-1}$; (*b*) in CCl_4 1792 and 1735 cm.$^{-1}$.

Dehydrogenation of 4a-*Hydroxy*-1,7-*dimethyl*-2,8-*dioxo*-7α-*gibbane*-1,10-*dicarboxylic Acid* 1→4a-*Lactone* (VIII).—The diketo-acid (345 mg.) was heated with selenium powder (368 mg.) at 335—340° for 2·5 hr. in a current of nitrogen. The product (111 mg.), collected by distillation at 15 mm., was chromatographed in light petroleum (b. p. 40—60°)–ether (2 : 1; 3 ml.) on alumina (10·5 × 1·8 cm.) and eluted in ultraviolet light. Elution of blue and bluish-white fluorescent bands with light petroleum (b. p. 40—60°)–ether (2 : 1) and with ether gave intractable oils and gums. Then ether and ether–methanol (50 : 1) gave a blue fluorescent eluate which yielded a yellow semisolid (26·2 mg.). Sublimation at 80—90°/10^{-4} mm. followed by crystallisation from benzene–light petroleum (b. p. 60—80°) gave needles, m. p. 196—198°, of 1,7-dimethylfluoren-2-ol, identical (mixed m. p., infrared and ultraviolet spectra) with an authentic specimen prepared by synthesis.[17]

Attempted Oxidation of Methyl Gibberellate.—(a) *With chromic oxide–acetone–sulphuric acid.* Oxidation of the ester (540 mg.) in acetone with the above solution of chromic oxide in sulphuric acid (0·45 ml.) and separation of the product into neutral and acidic fractions in the usual way gave an intractable acid (23 mg.). The neutral gum was separated by Girard's reagent P into an intractable ketonic gum (33 mg.) and starting material (127 mg.).

(b) *Oppenauer oxidation.* Attempts to oxidise methyl gibberellate by the Oppenauer method were abandoned when it was found that (*a*) methyl gibberellate (180 mg.), benzoquinone (540 mg.), and aluminium isopropoxide (400 mg.) in purified dioxan (5 ml.) and toluene (3 ml.), left for 8 days, gave the unchanged ester (145 mg.) and (*b*) methyl gibberellate was not recovered after being heated with aluminium isopropoxide in toluene for 2 hr.

Attempted Oxidation of Gibberellin A_1 Methyl Ester.—The ester (77 mg.) in pyridine (0·9 ml.) was added to chromic oxide (78 mg.) in pyridine (0·8 ml.) and left for 43 hr. The mixture was poured into water. Recovery in ethyl acetate gave a gum (68 mg.) which was separated by Girard's reagent P into an intractable ketonic gum (30 mg.) and starting material (38 mg.).

Ozonolysis of Methyl Gibberellate.—(*a*) (By Dr. T. P. C. MULHOLLAND). A current of ozonised oxygen (3·4 mg. of ozone per min.) was passed through a solution of methyl gibberellate (100 mg.) in acetic acid (10 ml.) at 20° for 9 min. Water (10 ml.) was added, and the solution was kept for 1 hr. and steam-distilled. Treatment of the distillate with dimedone solution (cf. ref. 4) gave the dimedone derivative of formaldehyde (39·4 mg.; 0·48 mol.), m. p. and mixed m. p. 187—189°.

(*b*) Ozonised oxygen equivalent to 1·0 mol. of ozone was passed through a solution of the ester (0·90 g.) in ethyl acetate (50 ml.) at −70° during 15 min. The ethyl acetate was removed *in vacuo* at room temperature and the resultant foam left under water (50 ml.) until it gave a negative starch–iodide test (*ca.* 70 hr.). The products from five such ozonolyses were combined and extracted thoroughly with ethyl acetate, and the extract was washed with sodium hydrogen carbonate and water. Recovery gave a neutral gum (1·59 g.). Acidification of the sodium hydrogen carbonate extract and recovery in ethyl acetate afforded an acidic gum (A) (2·12 g.).

The neutral gum, Girard's reagent P (2·98 g.), Amberlite resin IRC-50 (H) (150 mg.), and ethanol (40 ml.) were refluxed for 1 hr. and poured into water (200 ml.). Extraction with ethyl acetate afforded a solid (1·01 g.) which after crystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave methyl gibberellate, m. p. 200—201°. The aqueous layer was treated with concentrated hydrochloric acid (25 ml.) at room temperature for 1 hr., then extracted with ethyl acetate, and the extract washed with sodium carbonate and water. Removal of the solvent gave *methyl* 1-*carboxy*-2,4a,7-*trihydroxy*-1-*methyl*-8-*oxogibb*-3-*ene*-10-*carboxylate* 1→4a-*lactone* (XIII) (358 mg.), m. p. 227—231° (decomp.), which crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) in prisms, m. p. 230—232° (decomp.) (Found: C, 63·2; H, 6·45; OMe, 8·6. $C_{19}H_{22}O_7$ requires C, 63·0; H, 6·1; OMe, 8·6%), $\lambda_{max.}$ 290 mμ (ε 125), $\nu_{max.}$ (*a*) 3500 and 3470 (OH), 1756 and 1730 (C=O), (*b*) in $CHCl_3$ 1775 (γ-lactone), 1754 (C=O of 5-ring α-ketol), and 1739 cm.$^{-1}$ (ester). The ketol reduced cold Tollen's reagent but did not give a colour in the triphenyltetrazolium chloride test. It gave an intense red colour with cold concentrated sulphuric acid.

The gum (A) crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) in prisms (825 mg.) of 1-*carboxy*-1,2,4a,4b,5,6,7,8,8a,9a-*decahydro*-2,4a-*dihydroxy*-9-*methoxycarbonyl*-1-*methyl*-7-*oxofluoren*-8a-*ylacetic acid* 1→4a-*lactone* (XIV; R = H), m. p. 90—120° (gas evolution). Recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave prisms, m. p. 128—130° (gas evolution) [Found (dried at 20° *in vacuo*): C, 57·9; H, 6·2; OMe, 8·1%; equiv., 380. Found (dried at 75° *in vacuo*): C, 59·8; H, 6·1. $C_{19}H_{22}O_8,H_2O$ requires C, 57·6; H, 6·1; OMe, 7·8%; *M*, 396. $C_{19}H_{22}O_8$ requires C, 60·3; H, 5·9%]. The hydrate showed $\nu_{max.}$ 3610 (hydrate), 3500 (OH), 2730—2520 (OH of CO_2H), 1765 (γ-lactone), 1734, ~1720 (C=O), and 1619 cm.$^{-1}$ (water bending). On microhydrogenation the acid took up 1·04 mol. of hydrogen.

The *methyl ester* (XIV; R = Me), prepared with diazomethane in ether–methanol, formed needles (from benzene), m. p. 172—174°, $[\alpha]_D^{25}$ +100° (*c* 0·8 in EtOH) (Found: C, 61·3; H, 6·45; OMe, 15·45. $C_{20}H_{24}O_8$ requires C, 61·2; H, 6·2; 2OMe, 15·8%), $\lambda_{max.}$ 289 mμ (ε 27), $\nu_{max.}$ (*a*) 3430, 3255, 1776, 1763, 1736, 1720, and 1685 cm.$^{-1}$, (*b*) in $CHCl_3$ 1775 (γ-lactone), 1736 (esters), 1725 (sh) cm.$^{-1}$ (6-ring ketone). The ester took up 1·2 mol. of hydrogen on microhydrogenation.

Oxidation of the Ketol (XIII).—(a) *With periodate.* The ketol (36·4 mg.) in methanol (3 ml.) and 0·1M-sodium metaperiodate (2 ml., 2 mol.) was left at room temperature for 22 hr. The solution was diluted with water, ammonium sulphate added, and the mixture extracted with ethyl acetate. The extract was washed with sodium hydrogen carbonate and water (no neutral product). The acid fraction (37 mg.), recovered by acidification of the aqueous sodium hydrogen carbonate solution and extraction with ethyl acetate, was esterified with diazomethane, giving the ester (XIV; R = Me) which crystallised from benzene in needles (25 mg.), m. p. 171—173° (identified by mixed m. p. and infrared spectrum).

(b) *With chromic oxide.* The ketol (80·5 mg.) in pure acetone (8 ml.) was treated dropwise at 0° with the above solution of chromic oxide in sulphuric acid (0·07 ml.) and kept at 0° for 15 min. The mixture was treated with one drop of methanol, poured into water (35 ml.), and

extracted with ethyl acetate, and the extract washed with aqueous sodium hydrogen carbonate and water. Recovery gave a solid (60 mg.), m. p. 193—218°. Acidification of the alkaline extract and recovery of the product in ethyl acetate afforded an intractable gum (13 mg.).

Crystallisation of the neutral solid from ethyl methyl ketone–light petroleum (b. p. 60—80°) (1 : 1) gave *methyl* 1-*carboxy*-4a,7-*dihydroxy*-1-*methyl*-2,8-*dioxogibb*-3-*ene*-10-*carboxylate* 1→4a-*lactone* (XVI) as prisms, m. p. 215—229° (decomp.). Further crystallisation did not change the decomposition point or the ultraviolet absorption (Found: C, 63·1; H, 5·9; OMe, 8·55. $C_{19}H_{20}O_7$ requires C, 63·3; H, 5·6; OMe, 8·6%), $\lambda_{max.}$ 229 mμ (ε 7050), $\nu_{max.}$ 3580 (OH), 1784, 1751, 1739, and 1698 cm.$^{-1}$ (C=O).

Hydrogenation of the Ester (XIV; R = Me).—The ester (201 mg.) in ethyl acetate (30 ml.) with 10% palladised charcoal (75 mg.) took up 1·26 mol. of hydrogen in 45 min. The mixture was filtered and the filtrate washed with aqueous sodium hydrogen carbonate and water. Recovery gave crystals (171 mg.), m. p. 160—163°, which were recrystallised from benzene as needles, m. p. 166·5—167·5°, identical (mixed m. p. and infrared spectrum) with a specimen of the ester (XI; R = Me) prepared from gibberellin A_1 methyl ester.

Acidification of the sodium hydrogen carbonate solution and extraction with ethyl acetate yielded an intractable gum (29 mg.).

Action of Acid on the Dimethyl Ester (XIV; R = Me).—The ester (81 mg.) in methanol (2·5 ml.) and 3N-hydrochloric acid (20 ml.) was heated under reflux for 25 min. The solution was extracted with ethyl acetate, and the extract washed with aqueous sodium hydrogen carbonate and water. Recovery afforded gummy crystals (65 mg.). Acidification of the alkaline extract and recovery in ethyl acetate gave an intractable acidic gum (8 mg.). The neutral product crystallised from methanol in prisms (27 mg.), m. p. 187—197°, raised to 205—207° by recrystallisation, and identified as the keto-ester (XV) by mixed m. p. and infrared spectrum.

Oxidation of the Diester (XIV; R = Me).—(a) *With chromic oxide–pyridine.* The ester (100 mg.) in pyridine (1·0 ml.) was added to chromic oxide (100 mg.) in pyridine (1·0 ml.) and left for 46 hr., then poured into water. The intractable brown gum (43 mg.), recovered in ether, showed no ultraviolet maximum corresponding to an αβ-unsaturated ketone.

(b) *With manganese dioxide.* The ester (298 mg.) and active manganese dioxide [20] (3·08 g.) in chloroform (25 ml.) were shaken at room temperature for 96 hr. The mixture was filtered and the combined filtrate and washings were taken to dryness, giving a foam (277 mg.), $\lambda_{max.}$ 229 mμ ($E^{1\%}_{1\,cm.}$ 117), which was chromatographed on alumina (20 g.; 8·8 × 1·8 cm.) in benzene containing a little methanol. The column was eluted with benzene (300 ml.) and benzene–methanol (400 : 1; 100 ml.) (traces of gums recovered) and then with benzene–methanol (400 : 1; 100 ml.) from which a gum (A) (70 mg.), $\lambda_{max.}$ 229 mμ ($E^{1\%}_{1\,cm.}$ 170) was recovered. Further elution with benzene–methanol (200 : 1; 200 ml.) gave a solid (B) (91 mg.).

Fraction (A) was triturated with ether, giving needles, m. p. 110—118°, which on crystallisation from benzene–light petroleum (b. p. 60—80°) gave *methyl* 1-*carboxy*-1,2,4a,4b,5,6,7,8,8a,9a-*decahydro*-4a-*hydroxy*-9-*methoxycarbonyl*-1-*methyl*-2,7-*dioxofluoren*-8a-*ylacetate* 1 → 4a-*lactone* (XVII) as needles (67 mg.), m. p. 118—125°, raised to 129—131° by crystallisation from methanol (Found: C, 61·6; H, 5·8. $C_{20}H_{22}O_8$ requires C, 61·5; H, 5·7%), $\nu_{max.}$ (*a*) 1779, 1744, and 1709 cm.$^{-1}$, (*b*) in $CHCl_3$ 1792 (γ-lactone), 1738 (esters), 1727 (sh; 6-ring ketone), and 1703 cm.$^{-1}$ (–CH=CH–C=O). The compound took up 1·23 mol. of hydrogen on micro-hydrogenation.

The solid (B) crystallised from benzene in needles (57 mg.), m. p. 167—170°, of the starting material.

Oxidation of Methyl Gibberellate with Manganese Dioxide.—The ester (1·0 g.) and active manganese dioxide [20] (10 g.) in chloroform (80 ml.) were shaken for 72 hr. The mixture was filtered and the dioxide washed with chloroform. The filtrate and washings were evaporated giving a gum (700 mg.), $\lambda_{max.}$ 228 mμ ($E^{1\%}_{1\,cm.}$ 226) which could be crystallised but without increase of the $E^{1\%}_{1\,cm.}$ value. The gum was chromatographed in benzene on alumina (30 g.). Elution with benzene–methanol (400 : 1) in 100 ml. portions gave the following fractions: (1)—(3) nil, (4)—(6) (382 mg.) ($E^{1\%}_{1\,cm.}$ at 228 mμ = 268), (7) and (8) (139 mg.) ($E^{1\%}_{1\,cm.}$ at 228 mμ = 225), (9)—(12) (39 mg.). Elution with benzene–methanol (50 : 1) gave 114 mg. which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in needles of methyl gibberellate, m. p. 200—203°.

Fractions (4)—(6) crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving *methyl* 1-*carboxy*-4a,7-*dihydroxy*-1-*methyl*-8-*methylene*-2-*oxogibb*-3-*ene*-10-*carboxylate* 1→4a-*lactone* (XVIII) as plates (220 mg.), m. p. 186—187°, $[\alpha]_D^{25}$ +49° (*c* 1·0 in $COMe_2$) (Found: C, 66·9,

67·1; H, 6·6, 6·3; OMe, 8·6. $C_{20}H_{22}O_6$ requires C, 67·0; H, 6·2; OMe, 8·7%), $\lambda_{max.}$ 228 mμ (ε 9700), $\nu_{max.}$ (*a*) 3585 (OH), 1776 (γ-lactone), 1730 (ester), 1678 (CH=CH–C=O) and 1654 cm.$^{-1}$ (C=C); (*b*) in $CHCl_3$ 3615, 1787, 1739, and 1699 cm.$^{-1}$. The mother-liquors were combined with fractions (7) and (8) and crystallised, to give more of the unsaturated ketone (147 mg.), m. p. 183—185°, $\lambda_{max.}$ 228 mμ (ε 9150).

On microhydrogenation lactone (XVIII) took up 2·06 mol. of hydrogen.

Hydrogenation of the Unsaturated Ketone (XVIII).—The ketone (225·8 mg.) in ethyl acetate (30 ml.) with 10% palladised charcoal (100 mg.) took up 2·2 mol. of hydrogen in 45 min. Recovery of the products in the usual way gave an acidic gum (30 mg.) and a neutral fraction (196 mg.). The latter was separated into non-ketonic (8 mg.) and ketonic (174 mg.) gums by Girard's reagent P as in the isolation of the ketol (XIII) (see above). The ketonic gum crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in needles (158 mg.), m. p. 120—123°, almost identical in infrared absorption with the 8-epi-ketone (VI), m. p. 131—133°. The needles in benzene were chromatographed on alumina (12 g.; 7·0 × 1·5 cm.). Elution with benzene (50 ml.) and benzene–methanol (400 : 1; 50 ml.) gave intractable gums (8·8 mg.). Benzene–methanol (400 : 1) (50 ml.) and benzene–methanol (200 : 1; 50 ml.) then gave gums (76 mg.) and (62 mg.) respectively which were combined and crystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving (i) needles (75 mg.), m. p. 129—131°, (ii) needles (35 mg.), m. p. 120—123°, and (iii) nodules (9 mg.), m. p. 125—148°. Recrystallisation of fraction (i) yielded needles, m. p. 129—131°, of the 8-epi-ketone (VI) identified by mixed m. p. and infrared spectrum.

Fraction (iii) crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in prisms (5 mg.), m. p. 140—155°, raised to 153—158° by further recrystallisation and identified as the ketone (VI), m. p. 161—163°, by mixed m. p. and infrared spectrum.

Action of Methanolic Hydrogen Chloride on the Keto-ester (VII; R = Me).—A solution of the ester (305 mg.) in dry methanol (4 ml.) saturated with hydrogen chloride was heated under reflux for 4 hr. in a slow stream of hydrogen chloride. The solution was poured on ice and extracted with ether. The ether was washed with water, sodium hydrogen carbonate, and water and dried. Recovery gave a gum (A) (237 mg.) shown by its infrared absorption to contain about 20% of γ-lactone. The sodium hydrogen carbonate extract was acidified and extracted with ether to give a gum (B) (69 mg.).

The gum (A) was chromatographed in benzene on alumina (8·5 × 1·8 cm.) and eluted as follows: (i) benzene (100 ml.) gave a gum (8·5 mg.), (ii) benzene–methanol (200 : 1) (125 ml.) gave crystals (131 mg.), and (iii) benzene–methanol (200 : 1) (175 ml.) gave a yellow gum (74 mg.).

Fraction (ii) crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) in prisms of *dimethyl* 2-*hydroxy*-1,7-*dimethyl*-8-*oxo*-7α-*gibb*-4a(4b or 10a)-*ene*-1,10-*dicarboxylate* (XXII; R = H, OH) (96 mg.), m. p. 152—157°, raised to 157—158° by recrystallisation, $[\alpha]_D^{21}$ −54° (*c* 0·7 in EtOH) (Found: C, 67·25; H, 7·6; OMe, 16·4. $C_{21}H_{28}O_6$ requires C, 67·0; H, 7·5; 2OMe, 16·5%), $\nu_{max.}$ (*a*) 3495 and 1726 cm.$^{-1}$, (*b*) in CCl_4 1739 cm.$^{-1}$ (broad). It gave a very pale yellow colour with concentrated sulphuric acid and a yellow colour with tetranitromethane but took up no hydrogen on attempted microhydrogenation.

Fraction (iii) crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) in needles (41 mg.), m. p. 220—224°, of starting material.

The gum (B), methylated with diazomethane and chromatographed on alumina as for gum (A), gave the dimethyl ester (XXII; R = H, OH) (30 mg.), m. p. 155—157°, and the starting ester (9 mg.), m. p. 219—222°.

The ester (XXII; R = H, OH) was recovered after being shaken with active manganese dioxide [20] in chloroform for 71 hr.

Oxidation of the Dimethyl Ester (XXII; R = H, OH).—The ester (75 mg.) in acetone (1·5 ml.) was oxidised with the above solution of chromic oxide in sulphuric acid (0·058 ml.) at −5° and kept for 1 hr. at 20°. Water (10 ml.) was added and the product (66·5 mg.; m. p. 109—115°) recovered in ethyl acetate in the usual way. It crystallised from ethyl acetate–light petroleum (b. p. 40—60°) in needles of *dimethyl* 1,7-*dimethyl*-2,8-*dioxo*-7α-*gibb*-4a(4b or 10a)-*ene*-1,10-*dicarboxylate* (XXII; R = O) (42 mg.), m. p. 119—120° (Found: C, 67·6; H, 6·9; OMe, 16·55. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0; 2OMe, 16·6%), $\nu_{max.}$ (*a*) 1728 and 1714 cm.$^{-1}$ (no OH band), (*b*) in CCl_4 1745 (5-ring ketone and esters) and 1721 cm.$^{-1}$ (6-ring ketone). It gave a yellow colour with tetranitromethane.

Action of Acid on the Ketone (VI), *m. p.* 161—163°.—(*a*) The ketone (98 mg.) and 2N-sulphuric acid (10 ml.) were heated under reflux in a current of nitrogen for 1 hr.; very little carbon dioxide was evolved. The products, isolated in ethyl acetate, were separated into an intractable acidic glass (31 mg.) and a neutral gum (68 mg.) in the usual way. The latter crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in prisms of the starting ketone (45 mg.), m. p. 155—160°.

(*b*) The ketone (280 mg.), concentrated hydrochloric acid (15 ml.), and water (3 ml.) were heated as in (*a*) for 1·25 hr. When cold, the solution was extracted with ethyl acetate, and the extract washed with aqueous sodium hydrogen carbonate and water. Acidification of the alkaline extract and extraction with ethyl acetate yielded an intractable acidic gum (29 mg.).

The neutral product (193 mg.) was recovered and chromatographed in benzene–light petroleum (b. p. 40—60°) (1 : 1; 6 ml.) on alumina (20 g.) in ultraviolet light. Elution of a dull white fluorescent band with benzene–methanol (200 : 1) yielded a gum (92 mg.) which crystallised from chloroform–light petroleum (b. p. 60—80°) and from acetone–light petroleum (b. p. 60—80°) in prisms of 7-*hydroxy*-1,8-*dimethylgibb*-1(10a)-*en*-2-*one* (XXIV), m. p. 188—190°, $[\alpha]_D^{18}$ +26° (*c* 0·28 in EtOH) (Found: C, 78·6; H, 8·9. $C_{17}H_{24}O_2$ requires C, 78·4; H, 9·3%), $\lambda_{max.}$ 247 mμ (ε 18,000), $\nu_{max.}$ (*a*) 3540 (OH), 1645 cm.$^{-1}$ (αβ-unsaturated ketone), (*b*) in $CHCl_3$ 1652 cm.$^{-1}$.

Alkaline Hydrolysis of Gibberellic Acid (With Mr. J. F. Grove and Dr. T. P. C. Mulholland).—A solution of gibberellic acid (0·92 g.) in 0·78N-potassium hydroxide (14·0 ml., 4 equiv.) was kept for 22 hr. at room temperature and then cooled to 5° during 3 hr. Titration of the cold solution with 0·855N-hydrochloric acid showed that 2·06 equiv. of alkali had been consumed; after the addition of more 0·855N acid (total, 14·0 ml.) the solution was extracted with ice-cold ethyl acetate (6 × 35 ml.). The combined extracts were washed with a little water, dried, and evaporated *in vacuo* at 15—20°, giving 2,3,7-*trihydroxy*-1-*methyl*-8-*methylenegibb*-4-*ene*-1,10-*dicarboxylic acid* (XXVII; R = H) as an amorphous solid (0·85 g.), m. p. 145—155° (decomp.), $[\alpha]_D^{17}$ +41° (*c* 0·4 in EtOH) [Found (dried at 20°): C, 60·0; H, 6·9%; equiv., 203. Found (dried at 100°): C, 62·6; H, 6·8%; equiv., 195. $C_{19}H_{24}O_7,H_2O$ requires C, 59·7; H, 6·85%; equiv. (dibasic acid), 191; $C_{19}H_{24}O_7$ requires C, 62·6; H, 6·6%; equiv. (dibasic acid), 182]. In dioxan solution the diacid showed no infrared γ-lactone absorption. On microhydrogenation it took up 1·62, 2·02 mol. of hydrogen. It gave no colour with ferric chloride and did not react with Brady's reagent but gave a red colour with concentrated sulphuric acid. It reacted instantly with Feigl's α-glycol reagent.[28]

The *dimethyl ester* (XXVII; R = Me), prepared with diazomethane in ether–methanol, crystallised from methanol in prisms, m. p. 95—100° (gas evolution at 108°), $[\alpha]_D^{22}$ +16° (*c* 0·99 in MeOH) [Found: C, 62·45; H, 7·65; OMe, 21·9; *M* (Rast), 197. $C_{21}H_{28}O_7,CH_3{\cdot}OH$ requires C, 62·25; H, 7·6; 3OMe, 21·9%; $\frac{1}{2}M$, 212], $\nu_{max.}$ (*a*) 3410 and 3290 (OH), 1730 and 1695 (C=O), and ~1650 cm.$^{-1}$ (C=C); (*b*) in $CHCl_3$ 1729 and ~1714 cm.$^{-1}$.

On microhydrogenation it took up 1·82, 1·94 mol. of hydrogen. Drying at 90—95° *in vacuo* gave the solvent-free ester, m. p. 138—139° [Found: C, 64·9; H, 7·35; OMe, 15·5. *M* (Rast), 351. $C_{21}H_{28}O_7$ requires C, 64·3; H, 7·2; 2OMe, 15·8%; *M*, 392], $\nu_{max.}$ 1732, 1716, and 1701 cm.$^{-1}$ and OH absorption, which on crystallisation from ethyl methyl ketone–light petroleum (b. p. 60—80°) gave rods, m. p. 97—100° (gas evolution at 102°) [Found: C, 64·3; H, 7·5; OMe, 14·5; *M* (Rast), 290. $C_{21}H_{28}O_7,0{\cdot}5CH_3{\cdot}CO{\cdot}C_2H_5$ requires C, 64·5; H, 7·5; 2OMe, 14·5%; $\frac{2}{3}M$, 286].

Hydrogenation of the Dimethyl Ester (XXVII; R = Me).—The ester (102 mg.) in methanol (10 ml.) with Adams catalyst (44 mg.) took up 1·8 mol. of hydrogen. The solid product crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) in needles (33·5 mg.), m. p. softened at 210°, melted 220—235°, of the 8-epimeric tetrahydro-compounds (Found: C, 63·4 H, 8·2; OMe, 15·4. Calc. for $C_{21}H_{32}O_7$: C, 63·6; H, 8·1; 2OMe, 15·7%), $\nu_{max.}$ 3485 and 3425 (OH), 1739 and 1728 (sh) cm.$^{-1}$ (C=O).

Oxidation of the Dimethyl Ester (XXVII; R = Me).—(a) *With periodate.* The ester (127 mg.) in methanol (9 ml.) and 0·0758M-sodium metaperiodate (4·4 ml., 1·1 mol.) was left for 30 min. The methanol was removed *in vacuo* at 20°, and the residual solution diluted with a little water and extracted with chloroform. Recovery gave a gum (126 mg.) which was extracted with boiling light petroleum (b. p. 80—100°) (10 ml., then 5 ml.), leaving an intractable insoluble residue (19 mg.). Ether (*ca.* 1 ml.) was added to the combined petroleum extracts which on

[28] Feigl, "Spot Tests," Elsevier, New York, 1954, Vol. II, p. 102.

cooling deposited the *compound* as needles (75 mg.), m. p. 126—130°, raised by recrystallisation from the same solvent or from aqueous methanol to 137—139°, $[\alpha]_D^{23}$ +127° (*c* 0·6 in MeOH) (Found: C, 64·5; H, 6·85; OMe, 15·8. $C_{21}H_{26}O_7$ requires C, 64·6; H, 6·7; 2OMe, 15·9%), $\nu_{max.}$ (*a*) 3510 (OH), 1728 and 1718 (C=O), and 1641 cm.$^{-1}$ (C=C); (*b*) in CCl_4 1738 cm.$^{-1}$. The compound took up 1·67 mol. of hydrogen and consumed 2·0 mol. of perbenzoic acid in 3 days at 0°; in the same time, the ester (XXVII; R = Me) consumed 1·7 mol. of perbenzoic acid.

(b) *With sodium bismuthate.* The ester (126 mg.) in 80% acetic acid (7·5 ml.) was shaken with " AnalaR " sodium bismuthate (105 mg.) for 2·5 hr. Then 3N-sodium hydroxide (22·5 ml.) was added, the solution extracted with chloroform, and the extract washed with aqueous sodium hydrogen carbonate and water. Recovery gave a gum (120 mg.) which was treated as in (a), giving crystals (21 mg.), m. p. 123—128°, raised to 131—133° by recrystallisation and identical (mixed m. p. and infrared spectrum) with the product from (*a*).

(c) *With lead tetra-acetate.* Lead tetra-acetate (51 mg.) was added to a solution of the ester (42·4 mg.) in anhydrous benzene (3 ml.). Reaction began immediately. The mixture was left overnight, then filtered from lead acetate, and the filtrate and washings were evaporated *in vacuo*, yielding crystals (41·8 mg.), m. p. 125—131°, raised to 137—139° by recrystallisation, identical (mixed m. p. and infrared spectrum) with the compound prepared by method (a).

The compound was unstable in methanol–concentrated hydrochloric acid (100 : 1) at 20° and decomposed rapidly in hot methanolic hydrogen chloride and in hot dilute hydrochloric acid.

Treatment of this compound with acetic anhydride in pyridine for 18 days and chromatography of the gummy product in benzene on alumina gave the *acetate* which crystallised from ether–light petroleum (b. p. 40—60°) in prisms, m. p. 123—124° (Found: C, 64·0; H, 6·6; OMe, 14·25. $C_{23}H_{28}O_8$ requires C, 63·9; H, 6·5; 2OMe, 14·35%), $\nu_{max.}$ 1737 and 1642 cm.$^{-1}$ (no absorption in the OH stretching region). The compound was recovered after treatment with acetic anhydride in pyridine for 45 hr.

The compound (39·1 mg., 0·0001 mol.) and dimethyl 2,3,7-trihydroxy-1-methyl-8-methylene-gibb-4-ene-1,10-dicarboxylate (XXVII; R = Me) (42·4 mg., 0·0001 mol.) were separately heated under reflux with methanol (2 ml.) and 0·101N-sodium hydroxide (5·991 and 6·000 ml. respectively) in a current of nitrogen for 6·5 hr. After cooling, back-titration with 0·101N-hydrochloric acid showed that the two compounds had consumed 3·04 and 2·11 equivs. of base respectively.

Dimethyl 2,7-Dihydroxy-1-methyl-8-methylene-3-oxogibb-4-ene-1,10-dicarboxylate (XXVIII).—The ester (XXVII; R = Me) (613 mg.) in chloroform (30 ml.) was shaken with manganese dioxide (6 g.) for 72 hr. The mixture was filtered and the filtrate and washings were taken to dryness. The gummy product (611 mg.) ($\lambda_{max.}$ 239 mμ, $E_{1\,cm.}^{1\%}$ 175) was triturated with ethyl acetate, giving crystals (215 mg.), m. p. 95—100° (decomp.), which recrystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°) in prisms of the *ketol* (XXVIII), m. p. 105—120° (decomp.) (not altered by further recrystallisation), $[\alpha]_D^{15}$ +121° (*c* 1·0 in MeOH) [Found: C, 61·85; H, 6·8; OMe, 15·05; H_2O (Karl–Fischer), 3·8. $C_{21}H_{26}O_7,H_2O$ requires C, 61·75; H, 6·9; 2OMe, 15·2; H_2O, 4·4%], $\lambda_{max.}$ 240 mμ (ε 17,000), $\nu_{max.}$ (*a*) 3590, ~3390 (sh), 3330, 3260, 1717, and 1667 cm.$^{-1}$, (*b*) in $CHCl_3$ 1728 (esters) and 1682 cm.$^{-1}$ (cyclohexenone). It took up 2·2 mol. of hydrogen.

The *monoacetate*, prepared with acetic anhydride in pyridine and purified by chromatography on alumina in benzene–methanol (200 : 1), crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in needles, m. p. 151—153° (Found: C, 63·45; H, 6·7. $C_{23}H_{28}O_8$ requires C, 63·9; H, 6·5%), $\lambda_{max.}$ 240 mμ (ε 17,900), $\nu_{max.}$ 3520 (OH), 1759, 1746, 1721, 1681 (C=O), and 1650 cm.$^{-1}$ (C=C).

Attempts to prepare an oxime and a dinitrophenylhydrazone failed.

The ketol slowly reduced cold Fehling's solution and rapidly reduced Tollens's reagent. It gave a reddish-purple colour in the triphenyltetrazolium chloride test but did not give metallic bismuth with bismuth oxide in hot acetic acid.

The ketol (39·7 mg.) in methanol (3 ml.) was treated with 0·09M-sodium metaperiodate (1·21 ml.) for 18 hr. Recovery gave starting material (38 mg.), m. p. 100—110° (decomp.). The ketol was recovered after being shaken with sodium bismuthate in acetic acid for 19 hr.

Reduction of the Ketol (XXVIII) *by Sodium Borohydride.*—Sodium borohydride (299 mg.) in ethanol (40 ml.) was added to the ketol (259 mg.) in ethanol (10 ml.) and left for 90 min. Excess of borohydride was decomposed with acetic acid, and the ethanol removed *in vacuo* at 25°.

Water was added and the gummy product (252 mg.) recovered in ethyl acetate. The gum in benzene–ether (5 : 1; 18 ml.) was chromatographed on alumina (6 × 1·8 cm.). Elution with benzene–methanol (ratios and volumes in parentheses) gave the following gums: (i) (200 : 1; 100 ml.) 1 mg., (ii) (100 : 1; 200 ml.) 15 mg., (iii) (50 : 1; 50 ml.) 19 mg., (iv) (50 : 1; 300 ml.) 158 mg., (v) (20 : 1; 50 ml.) 15 mg. Fractions (i) and (iii) were intractable, but fractions (iv) and (v) crystallised from methanol as prisms (58·2 mg.), m. p. 88—98°. Recrystallisation from methanol gave prisms, m. p. 96—99° (gas evolution at 108°), identical (mixed m. p. and infrared spectrum) with the methanol solvate of the dimethyl ester (XXVII; R = Me). On drying at 90—95° *in vacuo* the product formed needles, m. p. 135—137°, not depressed on admixture with the solvent-free dimethyl ester (XXVII; R = Me).

Periodate Estimations.—The compounds in methanol solution were treated with excess of sodium metaperiodate solution and set aside. The periodate consumption was determined by adding sodium hydrogen carbonate solution, 0·1N-sodium arsenite, and excess of potassium iodide, and estimating the excess of arsenite with 0·05N-iodine.

Compound	Time (hr.)	Periodate consumed (mol.)
Me gibberellate	17·5	0
(XXVII; R = Me)	4	1·15
(XXVII; R = Me)	16·5	1·17
Me_2 2,3,7-trihydroxy-1,8-dimethylgibbane-1,10-dicarboxylates (8-epimers)	16·75	0·96
(XXXII) (in H_2O)	17	0·08
(XXXIII) (in H_2O)	23	0·06

Lactonisation of the Dicarboxylic Acid (XXVII; R = H).—(i) The acid, when heated *in vacuo* at 130° for 2·25 hr., frothed vigorously. The product in dioxan solution showed an infrared γ-lactone band of the same intensity as gibberellic acid.

(ii) The acid (270 mg.) was heated under reflux in dry toluene for 24 hr.; it did not dissolve. When cold the pale orange solid was collected (214 mg.) and contained 76% of γ-lactone (by infrared estimation). It was methylated with diazomethane, and the resultant gum chromatographed in benzene on alumina (30 g.) and eluted as follows: Fractions (i)—(vi) [benzene–methanol (200 : 1) (700 ml.)] and (vii) and (viii) [benzene–methanol (100 : 1) (250 ml.)] gave intractable gums (37 mg.); fractions (ix)—(xiii) [benzene–methanol (100 : 1) (500 ml.)] yielded a gum (109 mg.) which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in prisms of *methyl* 1-*carboxy*-2,3,7-*trihydroxy*-1-*methyl*-8-*methylenegibb*-4-*ene*-10-*carboxylate* 1→3-*lactone* (XXIX) (54 mg.), which after recrystallisation had m. p. 174°, $[\alpha]_D^{18}$ +122° (*c* 0·76 in EtOH) (Found: C, 66·6; H, 6·7. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7%), $\nu_{max.}$ (*a*) 3360 and 3300 (OH), 1747 and 1731 (C=O), 1679 and 1660 cm.$^{-1}$ (C=C); (*b*) in CH_2Cl_2 1772 (γ-lactone), 1725 (ester), 1672 and 1660 cm.$^{-1}$ (C=C).

Attempted Oxidation of the Ester (XXIX) *with Manganese Dioxide.*—The ester (20 mg.) and manganese dioxide (as used for the oxidation of methyl gibberellate; 200 mg.) in chloroform (1·5 ml.) were shaken for 14 days. Recovery yielded a gum which showed no ultraviolet absorption maximum between 220 and 250 mμ; it crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in prisms (8 mg.), m. p. 170—173°, of the starting ester.

Reduction of the Keto-ester (VII; R = Me) *by Lithium Aluminium Hydride.*—The ester (991 mg.) in purified dry tetrahydrofuran (50 ml.) was added dropwise to lithium aluminium hydride (1·16 g.) in boiling tetrahydrofuran (150 ml.), and the mixture heated under reflux for 24 hr. After cooling, the excess of hydride was decomposed with ethanol–ether, saturated ammonium chloride solution added, and the tetrahydrofuran removed *in vacuo* at 20°. Water was added to the residue and the suspension extracted with ten portions of ethyl acetate. The combined extracts were washed with a little water and evaporated, giving a foam (585 mg.). The product in methanol (4 ml.) and benzene (40 ml.) was chromatographed on alumina (20 g.; 9 × 1·7 cm.). Elution with benzene–methanol (50 : 1) (250 ml.) and (25 : 1) (450 ml.) gave gums (27 mg. and 235 mg. respectively). Finally elution with benzene–methanol (50 : 3) (200 ml.) yielded a gum (314 mg.) which crystallised from ethyl acetate as a mixture of a gummy amorphous powder and nodules. The latter (106 mg.), m. p. 160—165°, were separated by hand-picking and recrystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°), giving 1,10-*bishydroxymethyl*-1,7-*dimethyl*-7α-*gibbane*-2,4a,8-*triol* (XXXIII) as needles, m. p. 177—180° (Found: C, 67·2; H, 9·5. $C_{19}H_{32}O_5$ requires C, 67·0; H, 9·5%), having very strong broad OH absorption at 3330 cm.$^{-1}$ but no C=O band.

Reduction of Gibberellin A_1 Methyl Ester (V; R = Me) *by Lithium Aluminium Hydride.*—The

ester (1·0 g.) in tetrahydrofuran (40 ml.) was reduced with lithium aluminium hydride (1·0 g.) in tetrahydrofuran (125 ml.). The product (607 mg.), which showed carbonyl absorption, was isolated as in the preceding experiment. It was chromatographed in methanol (2 ml.) and benzene (30 ml.) on alumina (11 × 1·7 cm.) and eluted with benzene–methanol (ratios and volumes in parentheses), giving fractions (i) (25 : 1; 700 ml.) 297 mg., gum, (ii) (50 : 3; 200 ml.) 227 mg., gum, and (iii) (10 : 1; 220 ml.) 93 mg., gum. Fraction (i) was intractable but fractions (ii) and (iii) on dissolution in boiling ethyl acetate immediately separated as crystals (190 mg.), m. p. 190—200°, which after rechromatography on alumina gave a solid. Treatment of this with a small volume of hot ethyl acetate gave 1,10-*bishydroxymethyl*-1-*methyl*-8-*methylenegibbane*-2,4*a*,7-*triol* (XXXII) (107 mg.), m. p. 195—198° (Found: C, 67·2; H, 9·0. $C_{19}H_{30}O_5$ requires C, 67·4; H, 8·9%). In the infrared region it showed strong OH absorption but no carbonyl absorption. On microhydrogenation the pentaol took up 0·84 mol. of hydrogen.

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