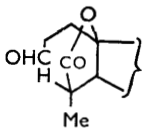
## 482. *Gibberellic Acid. Part XVIII.* Some Rearrangements of Ring* A.

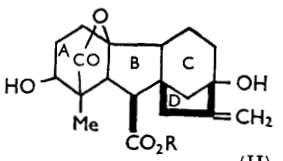
By B. E. Cross, John Frederick Grove, and A. Morrison.

Evidence is presented for the alkali-induced epimerisation of a 2-hydroxy-substituent in gibbane 1 ⟶ 4a-lactones. Under identical conditions gibb-3-ene 1 ⟶ 4a-lactones are rearranged to gibb-4-ene 1 ⟶ 3-lactones without epimerisation of a 2-hydroxyl substituent. Some degradations of the gibb-4-ene 1 ⟶ 3-lactone system are described and compared with similar degradations of the corresponding gibb-3-ene 1 ⟶ 4a-lactones.

2(*ax*)-Hydroxygibbane 1 ⟶ 4a-lactones † are epimerised [3,4] in dilute aqueous alkali to the more stable 2(*eq*)-epimers, and the evidence for the quasiaxial configuration of the 2-hydroxyl substituent in gibberellic acid has already been reported briefly.[3,4] A retro-aldol mechanism for the epimerisation *via* the intermediate (I) has been suggested.[5]




(I)

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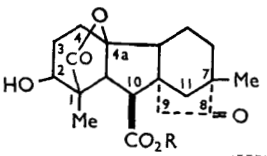


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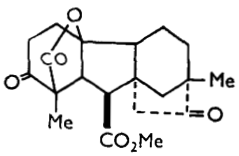


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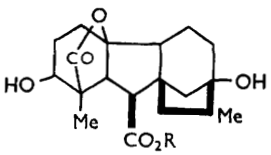


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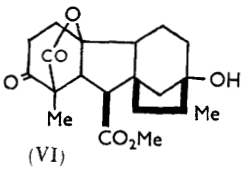



(VI)

With 0·01N-sodium hydroxide at room temperature, gibberellin $A_1$ methyl ester † (II; R = Me) gave a mixture of the 2-hydroxy-epimers, which was separated into its

* Part XVII, *J.*, 1960, 3049.

† For nomenclature see Part XII.[1] In this and subsequent publications α and β are used to indicate the absolute configuration of substituents attached to the gibbane ring system and are no longer used to differentiate stereoisomeric reduction products [2] of gibberellic acid. The name " α-dihydrogibberellic acid " [2] for (II; R = H) is withdrawn in favour of the trivial name gibberellin $A_1$.

[1] Grove and Mulholland, *J.*, 1960, 3007.
[2] Grove, Jeffs, and Mulholland, *J.*, 1958, 1236.
[3] Cross, Grove, MacMillan, Mulholland, and Sheppard, *Proc. Chem. Soc.*, 1958, 221.
[4] Cross, Grove, MacMillan, Moffatt, Mulholland, Seaton, and Sheppard, *Proc. Chem. Soc.*, 1959, 302.
[5] Cornforth, personal communication; *Chem. and Ind.*, 1959, 184.

components by chromatography on alumina. With a reaction time of 6 hr. the ratio equatorial : axial epimer recovered was approximately 2·5 : 1, but the simultaneous hydrolysis of the ester grouping rapidly reduced the total yield of neutral material and a detailed study of the course of the epimerisation was not attempted.

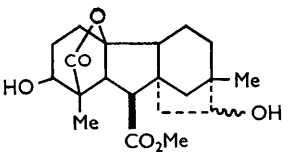
The 2(*eq*)-hydroxy-epimer (II; R = Me), m. p. 193°, underwent rearrangement of rings C/D [6] with dilute mineral acid and afforded the 2(*eq*)-hydroxy-8-keto-acid (III; R = H) and its methyl ester (III; R = Me). Oxidation of the latter by chromic oxide gave the same 2,8-diketo-ester (IV) as was obtained by oxidation of the 2(*ax*)-hydroxy-8-keto-ester (III; R = Me), derived previously [7] by acid-induced rearrangement of gibberellin $A_1$ methyl ester. The 2(*ax*)-hydroxy-8-keto-acid (III; R = H) was isomerised by 0·1N-sodium hydroxide to the 2(*eq*)-hydroxy-epimer. Similarly, with dilute alkali, methyl tetrahydrogibberellate [7] (V; R = Me) and methyl 8-epitetrahydrogibberellate [7] gave the corresponding 2(*eq*)-hydroxy-compounds, m. p. 235—239° and 166—168°, respectively. Both compounds were also obtained by catalytic reduction of the 2(*eq*)-hydroxy-epimer of gibberellin $A_1$ methyl ester followed by chromatography on alumina: separation of the 8-epimers by fractional crystallisation was not possible. Oxidation of methyl tetrahydrogibberellate and its 2(*eq*)-hydroxy-epimer by chromic oxide gave the same 2-ketone (VI), m. p. 161—163°: [7] the 8-epi-2-ketone [7] (VI), m. p. 131—133°, was obtained by oxidation of methyl 8-epitetrahydrogibberellate and its 2(*eq*)-hydroxy-epimer.

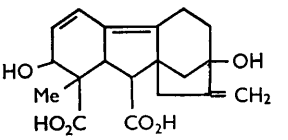
Alkaline hydrolysis of the 2(*eq*)-hydroxy-epimer (II; R = Me) gave the corresponding acid (II; R = H), identical with the pseudogibberellin $A_1$ of Takahashi *et al.*[8] since the dihydro-acid (V; R = H) (mixture of 8-epimers), obtained by catalytic reduction, was identical with an authentic specimen (for which we are indebted to Professor Y. Sumiki, University of Tokyo) of dihydropseudogibberellin $A_1$. The dihydro-acid (V; R = H) was shown to be a mixture of 8-epimers since methylation afforded a mixture of the 2(*eq*)-hydroxy-esters (V; R = Me). Isogibberellin $A_1$ obtained by Takahashi *et al.*[9] both by the action of alkali on the 2(*ax*)-hydroxy-8-keto-acid (III; R = H) (gibberellin C) and by acid treatment of pseudogibberellin $A_1$ is therefore the 2(*eq*)-hydroxy-epimer (III; R = H).

Reduction of the 2(*ax*)-hydroxy-keto-ester (III; R = Me) by sodium borohydride was stereospecific and afforded the 2(*ax*),8ξ-dihydroxy-ester (VII) which gave the 2,8-diketo-ester (IV) on oxidation by chromic oxide.

Reduction of the 2-ketone (VI), m. p. 161—163°, with sodium borohydride gave predominantly the 2(*eq*)-hydroxy-ester (V; R = Me), m. p. 235—239°, although about 10% of the 2(*ax*)-hydroxy-epimer was also formed. Some support for the retroaldol mechanism for the epimerisation of 2-hydroxygibbane **1** ⟶ 4a-lactones and for the existence of an equilibrium between the epimers in aqueous solution may be derived from the isolation of about 10% of the 2(*ax*)-hydroxy-ester (II; R = Me) when the 2(*eq*)-hydroxy-epimer, m. p. 193°, was shaken with 0·01N-sodium hydroxide.

(VII)




(VIII)

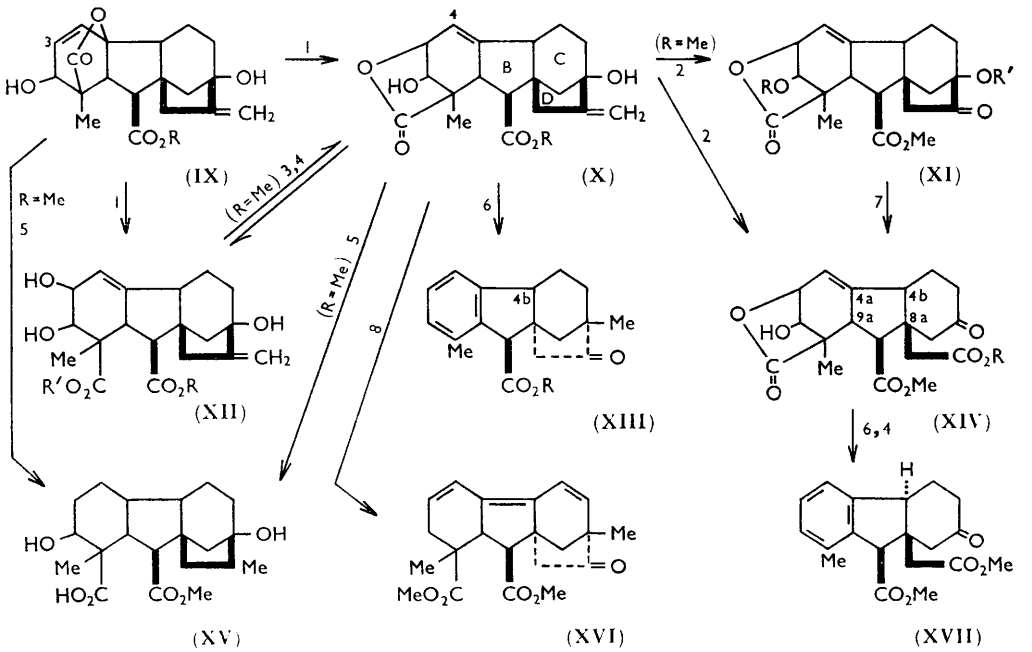
Gibberellenic acid [10] (VIII) was stable to alkali. This observation cannot be taken as indicating that the presence of a **1** ⟶ 4a-lactone bridge is essential for epimerisation since in the formation of gibberellenic acid movement of the double bond which finally appears in the 4a(4b)-position may enable the 2-hydroxy-group to become quasiequatorial.

[6] Grove, MacMillan, Mulholland, and Turner, *J.*, 1960, 3049.
[7] Cross, *J.*, 1960, 3022.
[8] Takahashi, Kitamura, Kawarada, Seta, Takai, Tamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1955, **19**, 267.
[9] Takahashi, Seta, Kitamura, Kawarada, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 75.
[10] Moffatt, *J.*, 1960, 3045.

The easy alkali-induced epimerisation of a 2-hydroxyl substituent in gibbane 1 ⟶ 4a-lactones is replaced in gibb-3-ene 1 ⟶ 4a-lactones by an allylic-type rearrangement to gibb-4-ene 1 ⟶ 3-lactones without concomitant epimerisation of the 2-hydroxyl substituent. With 0·01N-sodium hydroxide methyl gibberellate (IX; R = Me) was rapidly isomerised to the ester (X; R = Me), m. p. 174°, previously obtained [7] by lactonisation of the dibasic acid (XII; R = R′ = H) followed by methylation. That the isomerisation occurred without opening of the lactone ring was shown by solvent-extraction of the ester (X; R = Me) from the alkaline reaction medium. However, some opening of the lactone ring and hydrolysis of the ester group took place during the reaction, and the acids (X; R = H) and (XII; R = Me or H, R′ = H), isolated as their esters, were present in the acidic fraction of the products. The ester (X; R = Me) was occasionally obtained as the hydrate which readily afforded the anhydrous ester on drying *in vacuo*. Progressively more vigorous alkaline hydrolysis of the ester (X; R = Me) gave, sequentially, the half-ester (XII; R = Me, R′ = H) and the dibasic acid (XII; R = R′ = H), both isolated, after methylation, as the diester [7] (XII; R = R′ = Me).

The axial configuration of the 2-hydroxyl substituent in the ester (X; R = Me) follows from the isolation of the same acid (XV), known [11] to be in the 8-epimethyl series, after hydrogenolysis with Adams catalyst in glacial acetic acid of both methyl gibberellate and the ester (X; R = Me). In 1,3-disubstituted gibbanes an axial 2-hydroxy-substituent is in the more stable configuration.

The acid (XII; R = R′ = H) was obtained by the action of 0·1N-sodium hydroxide on gibberellic acid; [7] but with more dilute alkali, or less rapidly, in aqueous solution at room temperature, gibberellic acid undergoes rearrangement to the amorphous acid




(X; R = H), isolated as the hydrate and identified by methylation to the ester (X; R = Me).

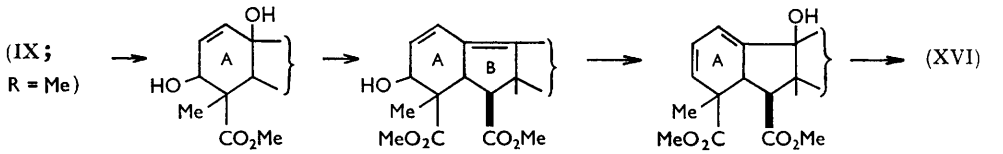
The ratio of the $R_F$'s of the acid (X; R = H) and gibberellic acid is 0·82 : 1·00 in

[11] Grove and McCloskey, unpublished work.

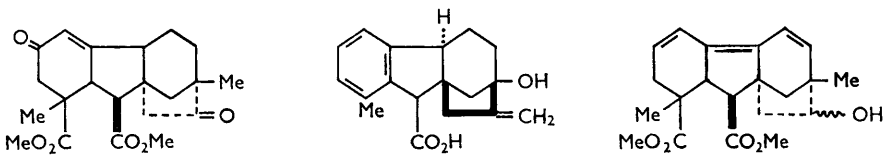
butanol–ammonia;[12] and the acid (X; R = H) is responsible for the spot at $R_F$ 0·35 detected[12] in aged aqueous solutions of gibberellic acid and sometimes when pure gibberellic acid is run in butanol–ammonia at elevated temperatures. The most convenient method of preparation of the acid (X; R = H) from gibberellic acid is to allow the isomerisation to take place at room temperature in aqueous 0·1N-ammonia.

In aqueous solution at room temperature conversion of gibberellic acid into the acid (X; R = H) and into gibberellenic acid (VIII) occurs simultaneously (by mechanisms which are probably mutually exclusive), production of the former being favoured by high pH and of the latter by low pH.

Treatment of methyl gibberellate with methanolic hydrogen chloride gave, in addition to methyl gibberate (XIII; R = Me; 4bα) and epigibberate (XIII; R = Me; 4bβ), a conjugated triene $C_{21}H_{24}O_5$ (XVI) formed by way of (XVIII)—(XX). In this




reaction sequence methanolysis of the 1 ⟶ 4a-lactone bridge and dehydration of the alcohol (XVIII) are followed by allylic rearrangement of the dienol (XIX) to the alcohol (XX) and then by a second dehydration and migration of the conjugated system of double bonds to afford the triene (XVI). Under the same conditions the ester (X; R = Me) also gave methyl gibberate, methyl epigibberate, and the triene (XVI). The initial product in the formation of the triene (XVI) from the ester (X; R = Me) is presumably the ester (XII; R = R′ = Me) which undergoes allylic rearrangement to the alcohol (XVIII), and in support of this mechanism the triene (XVI) was obtained both from the ester (XII; R = R′ = Me) and from methyl gibberellenate with boiling dilute mineral acid. In addition to the triene (XVI) much intractable ketonic material of $\lambda_{max.}$ 242 mμ was formed in all these reactions. The material of $\lambda_{max.}$ 242 mμ is unlikely to be the ketone (XXI), arising from dehydration of the α-glycol system in (XII; R = R′ = Me) since it is also formed from methyl gibberellenate.




Although the gibb-4-enes (X, R = H or Me; and XII; R = R′ = H) gave carbon dioxide and gibberic acid (or its methyl ester) with boiling dilute mineral acid, they were stable to acid at room temperature and were recovered under conditions where gibberellic acid gave gibberellenic[10] or allogibberic acid (XXII).[12] Like gibberellic acid[1] the acid (X; R = H) gave allogibberic acid with boiling water.

The structure of the triene (XVI) was deduced as follows. It contained two methoxyl and a ketone group (oxime). The infrared spectrum showed no bands due to OH absorption and only one C=O band (at 1736 cm.$^{-1}$) which was assigned to two ester groups and a five-membered ring ketone. This ketone group was not conjugated with the main chromophoric system which persisted without modification in the alcohol $C_{21}H_{26}O_5$ (XXIII) obtained by reduction of the triene ketone (XVI) with sodium borohydride. Any gross rearrangement of the carbon skeleton is unlikely to be involved in the formation of

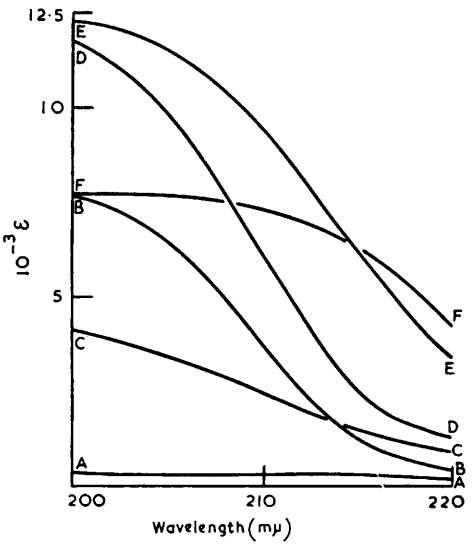
[12] Brian, Grove, Hemming, Mulholland, and Radley, *Plant Physiol.*, 1958, **33**, 329.

the triene since dehydrogenation by selenium gave 1,7-dimethylfluorene. By analogy with other ketonic C/D rearrangement products [6] the compound is assigned the 7α-gibbane stereochemistry: the optical rotatory dispersion revealed a very strong positive plain curve down to 320 mμ and the sign of the Cotton effect could not be determined.

In presence of a palladium catalyst the triene gave a tetrahydro-compound, $C_{21}H_{28}O_5$ (XXIV), which contained a tetrasubstituted ethylenic double bond exocyclic to two rings ($\lambda_{max.}$ 204 mμ, log ε 4·06).[13] The principal ultraviolet maximum, at 287 mμ, of the triene is in fair agreement with that expected for the 3,4a(4b),5-triene (XVI), both by calculation,[14] which gives 274 mμ, and by 30 mμ extension of the chromophore of gibberellenic acid (VIII) ($\lambda_{max.}$ 253 mμ); it is inconsistent with that calculated (313 mμ) for the alternative 2,4,4b-triene which contains a homoannular diene system.

The formation of the triene (XVI) provides conclusive evidence for the position of the lactone bridge in gibberellic acid (cf. ref. 7).

The increase in absorption intensity (ε 9300) at 210 mμ in the ester (X; R = Me) (see Figure) compared with that (ε 6200) of methyl gibberellate is consistent with the




*Ultraviolet absorption of* (A) *methyl tetrahydrogibberellate,* (B) *gibberellin* $A_1$ *methyl ester,* (D) *methyl gibberellate, and the esters* (E) (X; R = Me) *and* (F) (XXV; m. p. 232—235°). (C) *is the subtraction curve* (D − B).

change from a disubstituted to a trisubstituted double bond in the former compound; and evidence for this substitution pattern also comes from the infrared spectrum ($\nu_{max.}$ 820 cm.$^{-1}$) and from the nuclear magnetic resonance spectrum [15] of the acetyl derivative. But since some features of the nuclear magnetic resonance spectrum were difficult to reconcile with structure (X; R = Me) the chemistry of the ester was investigated more fully. In agreement with structure (X; R = Me) the ester was not oxidised by manganese dioxide in chloroform,[7] and hydrogenation over a palladium catalyst gave mainly acidic products resulting from hydrogenolysis of the lactone ring. The trisubstituted double bond was difficult to reduce and after uptake of 1 mol. of hydrogen the neutral portion of the product consisted essentially of a mixture of dihydro-compounds (XXV), m. p. 171—172° and m. p. 232—235°, epimeric at position 8. These compounds are known [11] to belong to the 8-methyl and 8-epimethyl series respectively.

Ozonolysis of the ester (X; R = Me) took the same course as the ozonolysis of methyl

[13] Ellington and Meakins, *J.*, 1960, 697.
[14] Fieser and Fieser, " Natural Products related to Phenanthrene," 3rd edn., Reinhold, New York, 1949, p. 187.
[15] Sheppard, *J.*, 1960, 3040.

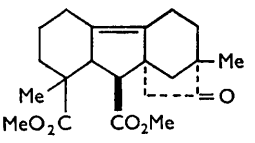
gibberellate,[7] giving formaldehyde, an α-ketol $C_{19}H_{22}O_7$ (XI; R = R′ = H), and a keto-acid $C_{19}H_{22}O_8$ (XIV; R = H) as main products. Periodate oxidation of the ketol (XI; R = R′ = H) afforded the keto-acid (XIV; R = H).

Aromatisation of the keto-acid (XIV; R = H) or its methyl ester with boiling dilute mineral acid gave (after methylation as appropriate) the keto-ester (XVII), previously obtained [1] by degradation of allogibberic acid and of known stereochemistry. It follows from this, and from the fact that the optical rotatory dispersion curve of the ketol (XI; R = R′ = H) showed a positive Cotton effect (cf. ref. 6), that the structure and stereochemistry of rings B/C/D in the ester (X; R = Me) are the same as in methyl gibberellate.

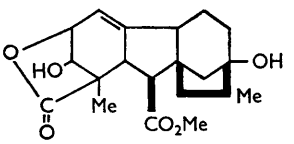
A number of by-products were isolated after the ozonolysis of the ester (X; R = Me). When the reaction was carried out in ethyl acetate at −70° a substance $C_{19}H_{22}O_7$, m. p. 206—208°, an α-ketol $C_{19}H_{22}O_8$, and the corresponding keto-acid $C_{19}H_{22}O_9$ (also obtained by periodate oxidation of the ketol and isolated as the methyl ester, m. p. 202—204°) were formed in low yield. The acetyl derivative $C_{22}H_{26}O_{10}$ of the ester, m. p. 202—204°, showed no hydroxyl absorption in the infrared spectrum, and the additional oxygen atom in the ketol $C_{19}H_{22}O_8$ and in its degradation products is considered to be in an epoxide ring (see XXVI). The absence of an ethylenic double bond in this series of compounds was confirmed by the weak ultraviolet end-absorption of the ester, m. p. 202—204°, which took up no hydrogen on attempted catalytic reduction and failed to aromatise when boiled with dilute mineral acid. Finally, oxidation of the ketol (XI; R = R′ = H) with perbenzoic acid gave the ketol $C_{19}H_{22}O_8$.

None of the above by-products was obtained when the ozonolysis was carried out in acetic acid at 20°; instead, a substance, $C_{21}H_{24}O_8$, m. p. 173—174°, was obtained in low yield; it was shown to be the 7-acetate (XI; R = H, R′ = Ac) of the ketol since it was not affected by periodate and acetylation gave the diacetate (XI; R = R′ = Ac).

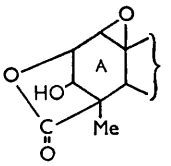
The ultraviolet end-absorption of gibberellin $A_1$ methyl ester (see Figure, curve B) was consistent with the presence of a $>C{=}CH_2$ group in this compound, and the subtraction curve (C) [= (D) − (B)] was consistent with a 1,2-disubstituted *cis*-ethylenic bond in methyl gibberellate (curve D). The trisubstituted double bond in ring A of the esters (X; R = Me) (curve E) and (XXV) (m. p. 232—235°; curve F) gave a very broad absorption band similar to that reported [13] for some $\Delta^7$-steroids.




(XXIV)




(XXV)




(XXVI)

The decomposition product gibberellenic acid is present in small amounts in gibberellic acid when this is obtained by solvent-extraction of *Gibberella fujikuroi* broths [16] followed by crystallisation from ethyl acetate–light petroleum. It is readily detected and estimated by the characteristic ultraviolet absorption at 253 mμ, but not by the infrared spectrum which (in Nujol) has few strong bands, most of which are present in the spectrum of gibberellic acid. It can be removed from the crude gibberellic acid by chromatography on " Celite " buffered at pH 7, gibberellenic acid being retained on the column, or by crystallisation from aqueous methanol. The latter method sometimes gives gibberellic acid as a hydrate which can be recognised by its characteristic infrared spectrum and readily yields the anhydrous acid *in vacuo*.

[16] Gerzon, Bird, and Woolf, *Experientia*, 1957, **13**, 487.

Methyl gibberellate tenaciously retained solvent of crystallisation, and neither chloroform nor ether was removed at 100° *in vacuo*. Benzene was removed at 130° from preparations crystallised [17] from benzene–methanol, and the infrared spectrum of the solvent-free material was identical with that of methyl gibberellate crystallised from aqueous methanol or aqueous ethanol. Crystallisation from ethyl acetate gave a different solvent-free crystalline modification, characterised by its infrared spectrum.

With an excess of ethereal diazomethane in methanol methyl gibberellate gave, in low yield, a substance $C_{21}H_{26}O_6N_2$ which no longer gave the characteristic red colour with concentrated sulphuric acid [17] but gave a positive Knorr test. From the infrared ($\nu_{max.}$ 1552 cm.$^{-1}$) and ultraviolet ($\lambda_{max.}$ 324 mμ; log ε 2·56) spectra which are consistent with the presence of the N=N grouping, it is presumably the 1-pyrazoline resulting from the addition of diazomethane to the double bond in ring A of methyl gibberellate.

## Experimental

M. p.s are corrected. Alumina of grade II and pH 4, and Celite 545, were used in chromatography. Unless otherwise stated, infrared spectra were obtained for Nujol mulls, and ultraviolet spectra and optical rotations for ethanol solutions. Optical rotatory dispersion curves were obtained for methanol solutions. Ultraviolet end-absorption data were obtained with a Unicam S.P. 500 spectrophotometer as described by Bladon *et al.*[18] Light petroleum had b. p. 60—80°. Microhydrogenations were carried out in acetic acid with a palladium-black catalyst.

*Gibberellic Acid Hydrate.*—Gibberellic acid (50 mg.) was dissolved in ethanol (25 ml.) and water (10 ml.). After removal of the ethanol and concentration *in vacuo*, the aqueous solution was set aside at 0° for 48 hr. The *hydrate* crystallised in prisms (25 mg.), m. p. (tube) 236° (decomp.) after softening at 190°, (Kofler block; rapid heating) 185—210° (Found: C, 63·0; H, 6·6. $C_{19}H_{22}O_6,H_2O$ requires C, 62·6; H, 6·6%), $\nu_{max.}$ 3413, 3240 (broad) (OH); 2660, 2584 (carboxylic OH); 1766, 1716 (C=O); ~1686 ($H_2O$?); 1653 (C=C). Between 7 and 15 μ the infrared spectrum differed considerably from that of anhydrous gibberellic acid. Anhydrous gibberellic acid (Found: C, 65·7; H, 6·6. Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·4%) was obtained on drying the hydrate *in vacuo* at 100° for 6 hr. over phosphorus pentoxide. With concentrated sulphuric acid the hydrate gave the same red colour and blue fluorescence as were given by gibberellic acid.

*Purification of Gibberellic Acid.*—(a) *By chromatography.* Crude gibberellic acid [100 mg.; m. p. 218—220° (decomp.), $E^{1\%}_{1\,cm.}$ 14 at 253 mμ], containing gibberellenic acid [10] (2·1%), in ether (300 ml.) was chromatographed on Celite (15 g.; 24 × 1·5 cm.) buffered at pH 7·0 (Sørensen M/15-phosphate buffer; 10 ml.). Elution (250 ml. fractions) was effected with ether previously saturated with water. Fractions 1—3 (10 mg.) contained intractable amorphous material. Fractions 4—15 (74 mg.) crystallised from ethyl acetate–light petroleum, giving gibberellic acid (66 mg.), m. p. 232—234° (decomp.), $[\alpha]_D^{26}$ +93° (*c* 0·4), $E^{1\%}_{1\,cm.}$ 5·0 at 253 mμ, free from gibberellenic acid (Found: C, 65·5; H, 6·5. Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·4%). Fractions 16—17 (15 mg.) furnished a powder, m. p. 215° (decomp.), $[\alpha]_D^{20}$ +75° (*c* 0·75), $\lambda_{max.}$ 255 mμ ($E^{1\%}_{1\,cm.}$ 16), consisting mainly of gibberellic acid (gibberellenic acid content, 2·3%). The infrared spectrum was indistinguishable from that of pure gibberellic acid.

*Gibberellic acid toluene-*p*-sulphonate*, prepared in pyridine during 3 days at room temperature, was obtained as an amorphous powder, m. p. 150° (decomp.), by addition of light petroleum to an ethyl acetate solution of the crude product. Recrystallisation from boiling toluene (losses) gave prisms, m. p. 152—155° (decomp.) (Found: C, 62·4; H, 5·95. $C_{26}H_{28}O_8S$ requires C, 62·4; H, 5·6%).

(b) *By crystallisation.* Water (65 ml.) was added to the crude gibberellic acid (5·0 g.) in methanol (24 ml.), and the solution was set aside for 18 hr. at 0°. Prisms (4·0 g.) of gibberellic acid, m. p. 232—233° (decomp.), $[\alpha]_D^{20}$ +89° (*c* 1·07), $E^{1\%}_{1\,cm.}$ 5·8 at 253 mμ, were obtained. The product resulting from this method of purification was frequently gibberellic acid hydrate.

*Methyl Gibberellate* (IX; R = Me).—Methyl gibberellate [17] crystallised from ethyl acetate in needles, m. p. 205—207° (Found, in sample dried at 20° *in vacuo*: C, 66·7; H, 6·8. Calc. for

[17] Cross, *J.*, 1954, 4670.
[18] Bladon, Henbest, and Wood, *J.*, 1952, 2737.

$C_{20}H_{24}O_6$: C, 66·65; H, 6·7%), $\nu_{max.}$ 3510, 3320 (OH); 1768, 1737 cm.$^{-1}$ (C=O). The infrared spectrum differed, particularly in the 7—15 μ region, from that of methyl gibberellate crystallised from benzene–methanol and dried [17] at 130° [$\nu_{max.}$ 3575, 3500 (OH); 1765, 1735w, 1710 cm.$^{-1}$ (C=O)]. The spectra of methyl gibberellate crystallised from ethyl acetate–light petroleum or from benzene–methanol but dried at 20° were identical except that the latter preparation gave a strong broad absorption at 690 cm.$^{-1}$ absent from the former. From 7 to 14 μ the spectra were essentially the same as that found for methyl gibberellate crystallised from ethyl acetate, but the C=O region showed strong bands at 1770, 1730, and 1712 cm.$^{-1}$.

Methyl gibberellate crystallised from aqueous ethanol in plates, m. p. 182—192°, $[\alpha]_D^{22}$ +80° (*c* 2·0) (Found: C, 66·9; H, 6·8%). The infrared spectrum was unaffected when the ester was dried at 100° *in vacuo* and was identical with that of methyl gibberellate crystallised from benzene–methanol and dried at 130°. Plates, m. p. 192—197°, with the same infrared spectrum were obtained from aqueous methanol.

Methyl gibberellate crystallised from ether in needles of the *ether solva e*, m. p. 170—172° (Found, in sample dried at 100° *in vacuo*: C, 66·1; H, 7·6. $C_{20}H_{24}O_6$,$C_4H_{10}O$ requires C, 66·3; H, 7·9%), $\nu_{max.}$ 3490sh, 3320, 3260 (OH): 1773, 1737 cm.$^{-1}$ (C=O). Needles of a *solvate*, m. p. 192—195°, were obtained from chloroform (Found, in sample dried at 100° *in vacuo*: C, 52·4; H, 5·3; Cl, 20·1. $C_{20}H_{24}O_6$,$CHCl_3$ requires C, 52·6; H, 5·25; Cl, 22·2%), $\nu_{max.}$ 3330 (OH); 1768, 1738 cm.$^{-1}$ (C=O).

*Reaction of Diazomethane with Methyl Gibberellate.*—Gibberellic acid (173 mg.) in methanol (2 ml.) was treated with diazomethane (105 mg., 5 mol.) in ether (10 ml.) during 48 hr. After removal of the solvents and excess of diazomethane *in vacuo*, the product, in benzene–methanol (200 : 1), was chromatographed on alumina (15 × 1·5 cm.). Elution with benzene–methanol (200 : 1; 350 ml.) and crystallisation from ethyl acetate–light petroleum afforded methyl gibberellate (117 mg.), m. p. 200—203°. Further elution of the column with benzene–methanol (50 : 1; 200 ml.) gave a gum (9 mg.) which crystallised from ethyl acetate–light petroleum in prisms, m. p. 232—234°, $[\alpha]_D^{17}$ +135° (*c* 0·4), of a *pyrazoline* (Found: C, 62·6; H, 6·6; N, 7·2. $C_{21}H_{26}O_6N_2$ requires C, 62·7; H, 6·5; N, 7·0%), $\lambda_{max.}$ 324 mμ (log ε 2·56), $\nu_{max.}$ 3515 (OH); 1772, 1719 (C=O); 1552 (N=H); 3080, 901 cm.$^{-1}$ (C=$CH_2$). It was less soluble than methyl gibberellate in ethyl acetate and gave a colourless solution in concentrated sulphuric acid. It gave a dark red colour with concentrated sulphuric acid and potassium dichromate.

*Action of Alkali on Gibberellin $A_1$ Methyl Ester* [*Methyl* 1-*Carboxy*-2(ax),4a,7-*trihydroxy*-1-*methyl*-8-*methylenegibbane*-10-*carboxylate* 1 ⟶ 4a-*Lactone*] (II; R = Me).—(*a*) The finely powdered ester (100 mg.) was shaken with 0·01N-sodium hydroxide (45·0 ml.) at room temperature until it dissolved (2·5 hr.). Back-titration with 0·1N-hydrochloric acid showed that 0·18 equiv. of alkali had been consumed. The aqueous solution (pH 7) was extracted with ether giving, on recovery, a neutral fraction (44 mg.). The aqueous layer was acidified to pH 2 with concentrated hydrochloric acid and re-extracted with ether. The extract was separated into neutral (17 mg.) and acidic (7 mg.) fractions by extraction with sodium hydrogen carbonate and recovery.

The acid fraction crystallised from ethyl acetate–light petroleum in prisms (2 mg.), m. p. 236—240° (decomp.), of gibberellin $A_1$ identified by the infrared spectrum.

The combined neutral fractions (61 mg.), in benzene–methanol (200 : 1; 100 ml.), were chromatographed on alumina (15 × 1·5 cm.) and eluted as follows: (i) benzene–methanol (200 : 1; 200 ml.) gave gibberellin $A_1$ methyl ester (34 mg.), m. p. 232—234°, (ii) benzene–methanol (200 : 1; 200 ml.) gave intractable mixtures (3 mg.), and (iii) benzene–methanol (50 : 1; 100 ml.) gave the 2(eq)-*hydroxy-epimer* (23 mg.), prisms, m. p. 193°, $[\alpha]_D^{15}$ +42° (*c* 0·5) (from ethyl acetate–light petroleum) (Found: C, 66·3; H, 7·3. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%), $\nu_{max.}$ 3470, 3430 (OH); 1773, 1707 (C=O); 1657 cm.$^{-1}$ (C=C).

Takahashi *et al.*[8] give m. p. 182—183° for pseudogibberellin $A_1$ methyl ester derived from gibberellin $A_1$ by treatment with alkali followed by methylation.

(*b*) The ester (325 mg.) and 0·01N-sodium hydroxide (100 ml.) were shaken for 6 hr. and the solution was then acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The acid fraction (106 mg., 33%), obtained as in (*a*), was chromatographed in ether (50 ml.) on Celite (20 × 1·5 cm.; pH 7·0), giving gibberellin $A_1$ (6 mg.) and intractable gums. The neutral fraction (164 mg., 51%) was separated by chromatography on alumina and fractional crystallisation into gibberellin $A_1$ methyl ester (35 mg., 11%) and the 2(*eq*)-hydroxy-epimer (80 mg., 25%).

(*c*) The ester (70 mg.) and 0·01N-sodium hydroxide (45 ml.) were shaken for 2 days and the solution was worked up as in (*b*). No neutral material was obtained. Chromatography of the acid fraction (40 mg.) afforded gibberellin $A_1$ (2 mg.) and intractable gums (27 mg.).

*Action of Alkali on the* 2(eq)-*Hydroxy-ester* (II; R = Me).—The ester (31 mg.) was shaken with 0·01N-sodium hydroxide (15 ml.) for 2 hr. Dissolution was complete in 10 min. Extraction of the acidified solution as described above gave neutral (28 mg.) and acid (3 mg.) fractions. Chromatography of the neutral fraction on alumina (7 × 1 cm.) and fractional elution with benzene–methanol (200 : 1) gave gibberellin $A_1$ methyl ester (3 mg., 10%), m. p. 231—234°, followed (complete separation of bands) by the 2(*eq*)-hydroxy-epimer (22 mg., 77%), m. p. 189—191°.

The recovered 2(*eq*)-hydroxy-ester was shaken again with 0·01N-sodium hydroxide (10 ml.) as described above, giving gibberellin $A_1$ methyl ester (2·0 mg.) and starting material (10 mg.).

*Action of Alkali on* 2(ax),4a-*Dihydroxy*-1,7-*dimethyl*-8-*oxo*-7α-*gibbane*-1,10-*dicarboxylic Acid* 1 ⟶ 4a-*Lactone*[7] (III; R = H).—The lactone (109 mg.) in 0·1N-sodium hydroxide (10 ml.) was set aside for 18 hr. at room temperature and the solution was then acidified with concentrated hydrochloric acid and extracted with ethyl acetate. Fractional crystallisation of the product from ethyl acetate gave materials, (i) m. p. 258—262° (decomp.) (47 mg.), (ii) m. p. 257—260° (decomp.) (16 mg.), (iii) m. p. 225—235° (decomp.) (14 mg.), and (iv) gums (10 mg.).

Fractions (i) and (ii) were combined and recrystallised from ethyl acetate, giving the 2(eq)-*hydroxy-lactone* (51 mg.), m. p. 262—264° (decomp.), $[\alpha]_D^{21}$ +48° (*c* 1·0) (Found: C, 65·3; H, 7·0. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·9%), $\nu_{max.}$ 3425 (OH); 1778, 1726 (broad) cm.$^{-1}$ (C=O). A mixed m. p. determination with the 2(*ax*)-hydroxy-epimer (III; R = H) showed a 25° depression. Takahashi *et al.*[9] give m. p. 260—262° for isogibberellin $A_1$ obtained by treatment of gibberellin C with alkali.

The *methyl ester*, prepared with diazomethane, crystallised from ethyl acetate–light petroleum in needles, m. p. 238°, $[\alpha]_D^{18}$ +42° (*c* 0·5 in acetone) (Found: C, 66·6; H, 7·4. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%), $\nu_{max.}$ 3530 (OH); 1762, 1736 (C=O).

*Acid Rearrangement of the* 2(eq)-*Hydroxy-ester* (II; R = Me).—The ester (17 mg.) in methanol (0·3 ml.) and 3N-hydrochloric acid (3 ml.) was heated under reflux for 1 hr. After removal of the methanol by distillation *in vacuo* the cooled solution was extracted with ethyl acetate. The extract was separated into neutral and acidic fractions by extraction with sodium hydrogen carbonate and recovery. The acid fraction (10 mg.) crystallised from ethyl acetate in prisms, m. p. 257—260° (decomp.), identified (mixed m. p. and the infrared spectrum) as the 2(*eq*)-hydroxy-acid (III; R = H). The neutral fraction (4 mg.) formed needles, m. p. 237—238° (from ethyl acetate–light petroleum), identified as the 2(*eq*)-hydroxy-ester (III; R = Me).

*Methyl* 1-(*Carboxy*-2(ax),4a,8ξ-*trihydroxy*-1,7-*dimethyl*-7α-*gibbane*-10-*carboxylate* 1 ⟶ 4a-*lactone* (VII).—To a stirred solution of the 2(*ax*)-hydroxy-ester (III; R = Me) (100 mg.) in methanol (10 ml.) at 0° was added sodium borohydride (30 mg.) in methanol (5 ml.). After 1 hr. at room temperature, the excess of borohydride was decomposed by the addition of acetic acid, and the solvent was removed *in vacuo*. After the addition of water (10 ml.), the product was recovered in ethyl acetate and crystallised from ethyl acetate–light petroleum, giving *methyl* 1-*carboxy*-2(ax),4a,8ξ-*trihydroxy*-1,7-*dimethyl*-7α-*gibbane*-10-*carboxylate* 1 ⟶ 4a-*lactone* (VII), needles (50 mg.), m. p. 171—172°, $[\alpha]_D^{16}$ +62° (*c* 1·0) (Found: C, 65·9; H, 7·7. $C_{20}H_{28}O_6$ requires C, 65·9; H, 7·7%), $\nu_{max.}$ 3495, 3370 (OH); 1761, 1730 cm.$^{-1}$ (C=O). Chromatography on alumina of the residue (44 mg.) from the mother-liquors and elution of the column with benzene–methanol (200 : 1) furnished more of the ester (VII), m. p. 170—172° (28 mg.).

*Oxidations by Chromic Oxide.*—(1) *The ester* (VII). The ester (27 mg.) in acetone (2 ml.) was treated dropwise at 0° with a solution of chromic oxide in sulphuric acid [chromic oxide (66·8 g.) in concentrated sulphuric acid (57·5 ml.) and water (100 ml.), made up to 267 ml.[19]] (0·04 ml.). After 1 hr. at room temperature, water was added and the precipitate was collected and crystallised from ethyl acetate–light petroleum, giving methyl 1-carboxy-4a-hydroxy-1,7-dimethyl-2,8-dioxo-7α-gibbane-10-carboxylate 1 ⟶ 4a-lactone[7] (IV), needles (13 mg.), m. p. 216—218°, identified by mixed m. p. and comparison of the infrared spectra. The products of the following oxidations, carried out in the same way, were identified similarly.

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

(2) *The* 2(ax)-*hydroxy-ester* (III; R = Me).[7] The ester (20 mg.) gave the diketo-ester (IV) (11 mg.), m. p. 215°.

(3) *The* 2(eq)-*hydroxy-ester* (III; R = Me). The ester (12 mg.) gave the diketo-ester (IV) (9 mg.), m. p. 216°.

(4) *Methyl* 1-*carboxy*-2(eq),4a,7-*trihydroxy*-1,8-*dimethylgibbane*-10-*carboxylate* 1 ⟶ 4a-*lactone* (V; R = Me). The ester (39 mg.) gave prisms (21 mg.), m. p. 160—162°, of methyl 1-carboxy-4a,7-dihydroxy-1,8-dimethyl-2-oxogibbane-10-carboxylate 1 ⟶ 4a-lactone [7] (VI).

(5) *Methyl* 1-*carboxy*-1(eq),4a,7-*trihydroxy*-1,8-*epi-dimethylgibbane*-10-*carboxylate* 1 ⟶ 4a-*lactone* (V; R = Me). The ester (9 mg.) gave needles (7 mg.), m. p. 131—133°, of methyl 1-carboxy-4a,7-dihydroxy-1,8-epi-dimethyl-2-oxogibbane-10-carboxylate 1 ⟶ 4a-lactone [7] (VI).

*Alkaline Hydrolysis of the* 2(eq)-*Hydroxy-ester* (II; R = Me).—(*a*) The ester (33 mg.) was heated under reflux with N-sodium hydroxide (3 ml.) for 1 hr. and the cooled solution was acidified with concentrated hydrochloric acid. The product, recovered in ethyl acetate, formed prisms (7 mg.), m. p. 227—230° (decomp.), $[\alpha]_D^{19}$ +43° (*c* 0·4) of 2(eq),4a,7-*trihydroxy*-1-*methyl*-8-*methylenegibbane*-1,10-*dicarboxylic acid* 1 ⟶ 4a-*lactone* (II; R = H) (Found: C, 65·3; H, 7·3. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·9%), $\nu_{max.}$ 3415, 3175 (broad) (OH); 1760, 1731 (C=O); 1653 cm.$^{-1}$ (C=C).

The 2(*eq*)-hydroxy-ester (II; R = Me), m. p. 188—191°, was regenerated on methylation of the acid with diazomethane.

(*b*) The ester (45 mg.) was treated as described in (*a*) but the product was crystallised from ethyl acetate–light petroleum, furnishing prisms (30 mg.) which lost solvent on drying at 100° *in vacuo*, giving the *hydrate* of the 2(*eq*)-hydroxy-acid (II; R = H) as an amorphous powder, m. p. 138—145°, resetting and remelting at 205—215° (Found: C, 62·7; H, 7·3%; equiv., 387. $C_{19}H_{24}O_6,H_2O$ requires C, 62·3; H, 7·15%; *M*, 366), $\nu_{max.}$ 3395 (broad) (OH); 1763, 1720 (C=O), 1650 (broad) cm.$^{-1}$ ($H_2O$, C=C). The anhydrous acid, m. p. 227—230° (decomp.), was obtained by drying the hydrate at 130—140° *in vacuo* and crystallising the gummy product from ethyl acetate.

Takahashi *et al.*[8] give m. p. 225—227° (decomp.), $[\alpha]_D^{28}$ +34° (*c* 3·9), for pseudogibberellin $A_1$ obtained by the action of alkali on gibberellin $A_1$.

*Action of Alkali on Methyl Tetrahydrogibberellate* (V; R = Me).—The finely powdered ester (220 mg.) was shaken with 0·01N-sodium hydroxide (150 ml.) at 24—26° for 6 hr. by which time dissolution was almost complete. The mixture was extracted with ethyl acetate, and the organic layer was washed with sodium hydrogen carbonate and evaporated, giving a solid (208 mg.). Acidification of the aqueous layer and washings followed by extraction with ethyl acetate yielded an intractable acidic gum (14 mg.). The neutral solid in methanol (3 ml.) and benzene (150 ml.) was chromatographed on alumina (13 × 1·7 cm.) and the column was eluted with 100 ml. portions of benzene–methanol (ratios in parentheses), giving the following fractions: (i)—(ii) (200 : 1) intractable gum (25 mg.), (iii)—(vi) (200 : 1) solid (78 mg., 35%), m. p. 246—266° [recrystallisation from aqueous methanol gave methyl tetrahydrogibberellate, m. p. 262—268° (75 mg., 34%)], (vii)—(ix) (100 : 1) interband (12 mg.), (x)—(xii) (50 : 1) solid (78 mg., 38%), m. p. 203—209°. Recrystallisation of the last material from aqueous methanol or ethyl acetate–light petroleum gave the 2(eq)-*hydroxy-epimer* of methyl tetrahydrogibberellate as rods or prisms (63 mg., 29%), m. p. 212—214° and 235—239°, $[\alpha]_D^{20}$ +59° (*c* 1·0) (Found: C, 65·6; H, 7·8. $C_{20}H_{28}O_6$ requires C, 65·9; H, 7·7%), $\nu_{max.}$ 3460 (OH); 1782, 1764, 1712 cm.$^{-1}$ (C=O); in $CHCl_3$, 1767 and 1732 cm.$^{-1}$.

*Action of Alkali on the Mixed Methyl Tetrahydrogibberellates* (8-*Epimers*).—The finely powdered mixture of esters (360 mg.) obtained by hydrogenation of methyl gibberellate [7] was shaken with 0·01N-sodium hydroxide (225 ml.) for 7·5 hr. The solid dissolved almost at once. After acidification the solution was extracted with ethyl acetate, and the organic layer was washed with sodium hydrogen carbonate. Acidification of the washings and recovery gave an intractable acidic gum (38 mg.). The neutral fraction (299 mg.) in methanol (4 ml.) and benzene (200 ml.) was chromatographed on alumina (16 × 2·2 cm.), and the column was eluted as follows: Fractions (i), benzene–methanol (200 : 1; 1800 ml.), and (ii) benzene–methanol (100 : 1, 600 ml.), gave mixtures (70 mg.) of methyl tetrahydrogibberellates, epimeric at $C_{(8)}$. Fraction (iii), benzene–methanol (50 : 1; 200 ml.), crystallised from ethyl acetate–light petroleum in prisms (15 mg.), m. p. 166—168°, of *methyl* 1-*carboxy*-2(eq),4a,7-*trihydroxy*-1,8-*epi-dimethylgibbane*-10-*carboxylate* 1 ⟶ 4a-*lactone* (V; R = Me) (Found: C, 66·0; H, 7·7. $C_{20}H_{28}O_6$

requires C, 65·9; H, 7·7%), $[\alpha]_D^{16}$ +46° (*c* 0·7), $[\alpha]_D^{18}$ +43° (*c* 0·5 in acetone), $\nu_{max.}$ 3475, 3270 (shoulder) (OH); 1785, 1775 (shoulder), 1711 cm.$^{-1}$ (C=O); in $CHCl_3$, 1771, 1734 cm.$^{-1}$. The ester was sometimes obtained in solvated prisms, m. p. 95° (gas evolution) and 166—168°. Solvent-free material, m. p. 166—168°, was obtained by drying *in vacuo* at 65° and the m. p. was raised on admixture with the 8-epimer of m. p. 212—214° (235—239°). Fractions (iv) and (v), benzene–methanol (50 : 1; 200 ml.), furnished prisms (79 mg.), m. p. 165—178°, and (55 mg.), m. p. 193—199°, respectively, which are presumed to be mixtures of the 8-epimeric 2(*eq*)-hydroxy-compounds (V; R = Me). Fraction (vi), benzene–methanol (50 : 1; 200 ml.), crystallised from ethyl acetate–light petroleum in blades (28 mg.), m. p. 209—214°. Recrystallisation from aqueous methanol gave prisms, m. p. 212—214° and 235—239° of the above 2(*eq*)-hydroxy-8-methyl compound (V; R = Me).

*Hydrogenation of the* 2(eq)-*Hydroxy-ester* (II; R = Me).—(*a*) The ester (36 mg.) in ethyl acetate (5 ml.) was reduced with hydrogen (2·4 ml., 1·08 mol.) at room temperature in the presence of 10% palladium–charcoal (36 mg.). The gummy product (36 mg.) crystallised from ethyl acetate–light petroleum in needles (15 mg.), m. p. 181—184°, comprising a mixture of dihydro-compounds (V; R = Me) epimeric at position 8, which could not be separated by fractional crystallisation (Found: C, 65·8; H, 7·8. Calc. for $C_{20}H_{28}O_6$: C, 65·9; H, 7·7%), $\nu_{max.}$ 3470 (OH); 1782, 1772 (sh), 1711 cm.$^{-1}$ (C=O).

(*b*) The ester (180 mg.) in ethyl acetate (40 ml.) in the presence of 10% palladium–charcoal (180 mg.) was hydrogenated as in (*a*) (uptake 1·1 mol.). The gummy product (177 mg.) in benzene (100 ml.) was chromatographed on alumina (15 × 1 cm.), and the column was eluted as follows: Fraction (i) [benzene–methanol (200 : 1; 500 ml.)] was a gum (110 mg.), which crystallised from ethyl acetate–light petroleum in prisms (40 mg.) of the 2(*eq*)-hydroxy-8-epimethyl ester (V; R = Me), m. p. 165—166°. Fractions (ii) [benzene–methanol (200 : 1, 300 ml.)], and (iii) [benzene–methanol (100 : 1; 100 ml.)] furnished mixtures (10 and 20 mg. respectively). Fraction (iv) [benzene–methanol (100 : 1; 300 ml.)], a gum (38 mg.), crystallised from ethyl acetate–light petroleum in prisms (33 mg.) of the 8-methyl epimer (V; R = Me), double m. p. 212—214° and 235—239°.

*Hydrogenation of the* 2(eq)-*Hydroxy-acid* (II; R = H).—The acid (3·02 mg.) was subjected to microhydrogenation (uptake: 0·15 ml., 0·77 mol.). The product crystallised from ethyl acetate–methanol in prisms, m. p. 280—282° (decomp.), of the dihydro-derivative. The infrared spectrum was identical with that of authentic dihydropseudogibberellin $A_1$, m. p. 290—291° (decomp.),[8] kindly provided by Professor Sumiki. The decomposition point varied with the particle size and rate of heating.

The dihydro-compound (3 mg.) in methanol with ethereal diazomethane gave prisms (2 mg.), m. p. 176—181° (from ethyl acetate–light petroleum). The infrared spectrum was identical with that of the mixture, m. p. 181—184°, of 8-epimeric dihydro-compounds obtained by hydrogenation of the 2(*eq*)-hydroxy-ester (II; R = Me).

*Reduction of the Ketone* (VI), *m. p.* 161—163°.—The ketone (100 mg.) in methanol (10 ml.) at 0° was stirred for 1 hr. during the addition of sodium borohydride (30 mg.) in methanol (5 ml.). Excess of hydride was destroyed by the addition of acetic acid, and the solution was concentrated to small bulk *in vacuo*. After the addition of water (10 ml.), the product, an amorphous powder (97 mg.), was recovered in ethyl acetate. A portion (70 mg.) in benzene–methanol (200 : 1; 50 ml.) was chromatographed on alumina (7 × 1 cm.) and the column was eluted as follows (all fractions were gums): (i) benzene–methanol (200 : 1; 200 ml.) gave 11 mg., which crystallised from ethyl acetate in prisms (7 mg., 10%), m. p. 260—263°, of methyl tetragibberellate (V; R = Me); (ii) benzene–methanol (200 : 1; 100 ml.; followed by 100 : 1, 200 ml.) gave 53 mg., which crystallised from ethyl acetate–light petroleum in prisms (36 mg., 51%), m. p. 212—216° and 235—239°, of the 2(*eq*)-hydroxy-epimer (V; R = Me) of methyl tetrahydrogibberellate.

The identities of both products were confirmed by comparison of the infrared spectra.

*Action of Sodium Hydroxide on Gibberellenic Acid*[10] (VIII).—The acid (28 mg.) in 0·1N-sodium hydroxide (6 ml.) was set aside for 48 hr. The solution was cooled to 0°, acidified to pH 3 by 2N-hydrochloric acid, and immediately extracted with ethyl acetate. Gibberellenic acid (15 mg.), m. p. 178—179° (from ethyl acetate), was recovered from the extracted material (23 mg.).

A similar recovery of gibberellenic acid was obtained (by Dr. J. S. Moffatt) after 5 hr. by using 0·02N-sodium hydroxide.

*Action of Alkali on Methyl Gibberellate.*—(*a*) Methyl gibberellate (100 mg.) and 0·01N-sodium hydroxide (45 ml.) were shaken at 24°, a clear solution being obtained after 5 min. After 2 hr. back-titration with 0·1N-hydrochloric acid showed that 0·62 equiv. of alkali had been consumed. Extraction of the solution at pH 7 with ether yielded a neutral fraction (27 mg.). The solution was then acidified to pH 2 with concentrated hydrochloric acid and re-extracted with ether. The ethereal layer was extracted with sodium hydrogen carbonate, and neutral (15 mg.) and the intractable gummy acidic (10 mg.) fractions were recovered. The combined neutral fractions in benzene–methanol (200 : 1) were chromatographed on alumina (15 × 1 cm.), and the column was eluted with nine 100-ml. portions of benzene–methanol (200 : 1). All eluates yielded methyl 1-carboxy-2(*ax*),3,7-trihydroxy-1-methyl-8-methylenegibb-4-ene-10-carboxylate 1 ⟶ 3-lactone [7] (X; R = Me), obtained, after recrystallisation from ethyl acetate–light petroleum, as prisms (20 mg.), m. p. 172—173°, and identified by mixed m. p. and comparison of the infrared spectra.

Like methyl gibberellate the ester (X; R = Me) in concentrated sulphuric acid gave a wine-red colour which developed a blue fluorescence. Microhydrogenation resulted in the uptake of 1·87 mol.

The *hydrate* crystallised from ethyl acetate–light petroleum in prisms, m. p. 102—106° (gas evolution) and 172° (Found: C, 62·9; H, 7·1. $C_{20}H_{24}O_6,H_2O$ requires C, 63·5; H, 6·9%), $\nu_{max.}$ 3495, 3410, 3305 (OH); 1765, 1743, 1715 (C=O); 1676 (C=C); 1637 (broad) cm.$^{-1}$ (C=C, $H_2O$); in acetonitrile, 1773, 1730; ~1672 cm.$^{-1}$. The infrared spectrum in acetonitrile was identical with that of the anhydrous ester in the same solvent. The hydrate lost water *in vacuo* at 80° (2 hr.) and the infrared spectrum of the product was identical with that of the anhydrous ester.

The *acetate*, prepared in pyridine with acetic anhydride during 24 hr. at room temperature, crystallised from ethyl acetate–light petroleum in prisms, m. p. 165—166° (Found: C, 65·85; H, 6·9. $C_{22}H_{26}O_7$ requires C, 65·7; H, 6·5%), $\nu_{max.}$ 3520 (OH); 1773, 1728, 1718 cm.$^{-1}$ (C=O). It was readily soluble in chloroform.[15]

The *benzoate hemihydrate* was prepared by the addition of benzoyl chloride (0·05 ml.) to the ester (100 mg.) in pyridine (0·5 ml.). After 48 hr. at room temperature the solvent was removed *in vacuo*, water was added to the semi-solid residue, and the resulting suspension was extracted with ether. The ether extract was washed with sodium hydrogen carbonate and concentrated to small volume. The solid product obtained by addition of light petroleum (b. p. 40—60°) to this solution was purified by two further precipitations from ether by light petroleum (b. p. 40—60°) and formed an amorphous powder (85 mg.), m. p. 90—95° (gas evolution) (Found: C, 68·1; H, 6·5. $C_{27}H_{28}O_7,0·5H_2O$ requires C, 68·5; H, 6·2%), $\nu_{max.}$ 3450 (very broad), (OH); 1780, 1730 (C=O); 1670 (C=C); 1650 (C=C, $H_2O$); 1600, 715 cm.$^{-1}$ (aromatic ring). The m. p. and infrared spectrum were unaltered on drying *in vacuo* at 63°.

(*b*) Methyl gibberellate (500 mg.) was shaken with 0·01N-sodium hydroxide as described in (*a*) and the product obtained by extraction of the acidified solution with ethyl acetate was separated into neutral (200 mg.) and acidic (160 mg.) fractions. The neutral fraction crystallised from ethyl acetate–light petroleum in prisms (129 mg., 26%), m. p. 174°, of the ester (X; R = Me).

A portion (30 mg.) of the amorphous acid fraction, m. p. 116—132° (decomp.), was treated with diazomethane, and the gummy product in benzene–methanol (200 : 1; 50 ml.) was chromatographed on alumina (15 × 1 cm.). The column was eluted as follows: (i) benzene–methanol (100 : 1; 150 ml.) gave the ester (X; R = Me) as prisms (5 mg.), m. p. 173—174°, from ethyl acetate–light petroleum; (ii) benzene–methanol (50 : 1; 250 ml.) gave methyl 2,3,7-trihydroxy-1-methyl-8-methylenegibb-4-ene-1,10-dicarboxylate [7] (XII; R = R′ = Me), prisms (21 mg.), m. p. 97—105° (gas evolution) (from methanol), identified by comparison of the infrared spectra.

(*c*) Methyl gibberellate (200 mg.) in 0·01N-sodium hydroxide (90 ml.) was shaken for 1 hr. and the alkaline solution was extracted with ethyl acetate, giving the ester (X; R = Me) (63 mg.), m. p. 174°.

*Acid-hydrolysis of the Ester* (X; R = Me).—(1) *At room temperature.* The ester (20 mg.) in methanol (1 ml.) and 3N-hydrochloric acid (2 ml.) was set aside for 3 days. The solution was extracted with ethyl acetate and the organic layer was washed with aqueous sodium hydrogen carbonate. The neutral fraction (18 mg.), on recovery, had no significant specific ultraviolet absorption above 240 mμ: crystallisation afforded starting material (16 mg.), m. p. 174°.

(2) *At* 100°. The ester (15 mg.) and 3N-hydrochloric acid (2 ml.) were heated under reflux for 1 hr. The neutral fraction (11 mg.) recovered in ethyl acetate crystallised from light petroleum (b. p. 40—60°) in prisms (7 mg.), m. p. 108—111°, of methyl gibberate [17] (XIII; R = Me; 4bα), identified by mixed m. p. and comparison of the infrared spectra.

*Alkaline Hydrolysis of the Ester* (X; R = Me).—(*a*) The ester (24·9 mg.) in ethanol (1·5 ml.) and 0·1N-sodium hydroxide (2·80 ml.) was set aside at room temperature for 4 hr. Back-titration with 0·1N-hydrochloric acid (2·11 ml.) showed that 1·0 equiv. of alkali had been consumed. The solution was acidified at 5° to pH 2 and after extraction with ethyl acetate the organic layer was extracted with sodium hydrogen carbonate. The amorphous acid (XII; R = Me, R′ = H) (22 mg.) obtained on recovery (ice-cold solutions [7]) showed no lactone C=O absorption at 1780 cm.$^{-1}$ in dioxan. A portion (9 mg.) was treated with diazomethane, giving the ester (XII; R = R′ = Me) as prisms (5 mg.), m. p. 100—105° (gas evolution), identified by comparison of the infrared spectra.

(*b*) The ester (100 mg.) was heated under reflux for 2 hr. with N-sodium hydroxide (3 ml.), and the cooled solution (at 0°) was acidified with concentrated hydrochloric acid and extracted with ethyl acetate (3 × 3 ml.). The foam (85 mg.) obtained on recovery was triturated with ether, giving the amorphous 2,3,7-trihydroxy-1-methyl-8-methylenegibb-4-ene-1,10-dicarboxylic acid [7] (XII; R = R′ = H), m. p. 130—150° (decomp.), identified by comparison of the infrared spectra. Methylation of a portion (60 mg.) furnished the ester (XII; R = R′ = Me) as prisms (38 mg.), m. p. 98—100° (gas evolution), identified as described above.

*Action of Alkali on Gibberellic Acid.*—(a) *Sodium hydroxide.* Gibberellic acid (35 mg.) in 0·1N-sodium hydroxide (4 ml., 4 equiv.) was kept 18 hr. at room temperature. Back-titration of the cold solution with ice-cold 0·1N-hydrochloric acid showed that 2·04 equiv. of alkali had been consumed. Extraction of the cold acidified solution with ether furnished the acid (XII; R = R′ = H), m. p. 145—155° (decomp.) (11 mg.).

(b) *Ammonia* (By G. W. Elson and Dr. T. P. C. Mulholland). A solution of gibberellic acid (3·0 g.) in 0·1N-ammonia (600 ml.) was kept at room temperature for 6 days, then acidified with hydrochloric acid. Recovery of the product in ethyl acetate gave a gum (2·44 g.). This was extracted with boiling ether (200 ml.), and the extract was concentrated and diluted with light petroleum (b. p. 40—60°), giving a precipitate [1·90 g., $[\alpha]_D^{21}$ +93° (*c* 1·17)]. Fractional precipitation from ether with light petroleum gave, first, fractions with $[\alpha]_D$ +93—100° and, secondly, the *acid* (X; R = H; 1·47 g.) as an amorphous powder, m. p. 150—160°, $[\alpha]_D^{20}$ +104° (*c* 1·12) (Found, dried at 100°: C, 63·2, 62·6; H, 6·65, 6·4. $C_{19}H_{22}O_6,H_2O$ requires C, 62·6; H, 6·6%), $\nu_{max.}$ 3415 (OH); 1755, 1713 cm.$^{-1}$ (C=O); in acetonitrile, 1773, 1739 cm.$^{-1}$. Microhydrogenation resulted in the uptake of 2·0 mol. The acid gave a yellow colour, changing to green and then violet, with concentrated sulphuric acid. The $R_F$ (Whatman No. 1 paper: descending chromatograms at 20°) for the acid (X; R = H) in 4 : 5 : 1 butanol–water–ammonia solution (*d* 0·88) was 0·255 (gibberellic acid, 0·31). This solvent system has been found to give variable results and $R_F$ values for gibberellic acid have been obtained in the range 0·22—0·43; but the relative $R_F$ values for the acid (X; R = H) and gibberellic acid were constant, *viz.*, 0·82 : 1·00.

The methyl ester, prepared with diazomethane, crystallised from ether–light petroleum (b. p. 40—60°) in prisms, m. p. 170—172°, $[\alpha]_D^{23}$ +125° (*c* 1·0), and was identified as the ester (X; R = Me) by mixed m. p. and comparison of the infrared spectra.

*Action of Water on the Acid* (X; R = H).—The acid (19 mg.) was boiled with water (10 ml.) for 40 hr. On cooling, needles (2 mg.), m. p. 185—195° (softening at 120°), separated; they were identified as allogibberic acid hydrate [12] by the infrared spectrum and mixed m. p. No crystalline products were recovered when the acid (20 mg.) in water (5 ml.) was autoclaved at 15 lb per sq. in. and 121° for 140 min.

*Action of Hydrochloric Acid on the Acid* (X; R = H).—(*a*) A solution of the acid (25 mg.) in water (16 ml.) was treated at 20° with 3N-hydrochloric acid (8 ml.). No crystals had separated after storage for 5 days at room temperature, followed by 24 hr. at 25°. The mixture was kept at 70—75° for 18 hr. On cooling, gibberic acid [17] (XIII; R = H: 4bα) (9 mg.; m. p. 150—155°) separated.

(*b*) The acid (20 mg.), concentrated hydrochloric acid (0·70 ml.), and water (3·5 ml.) were heated under reflux for 3 hr. The solid {13 mg.; m. p. 160—166°, $[\alpha]_D^{18}$ +5° (*c* 0·9)} which separated on cooling crystallised from ether–light petroleum in needles (8 mg.; m. p. 149—151°) of gibberic acid.

*Action of Hydrochloric Acid on the Acid* (XII; R = R′ = H).—(a) *At room temperature.* The acid (100 mg.) in 2N-hydrochloric acid (4 ml.) was set aside for 1 week. No precipitate of allogibberic acid was formed (cf. ref. 12) and the solution was continuously extracted with ether. Extraction of the recovered solid with a large volume of boiling toluene gave no allogibberic acid. The toluene-insoluble residue was precipitated by the addition of light petroleum to an ethyl acetate solution and formed an amorphous powder, m. p. 145—180° (decomp.). The infrared spectrum was very similar to, though not quite identical with, that of the acid (XII; R = R′ = H).

(b) *At* 100°. The acid (40 mg.) in water (5 ml.) and concentrated hydrochloric acid (1 ml.) was heated under reflux for 1 hr. Carbon dioxide was evolved and the solution rapidly became cloudy, depositing an oil which solidified. The solid (11 mg.) was collected and crystallised from ethyl acetate–light petroleum as needles (6 mg.), m. p. 145°, not depressed on admixture with gibberic acid. Identity was confirmed by comparison of the infrared spectra.

*Acid-hydrolysis of the Ester* (XII; R = R′ = Me).—(a) *At room temperature.* The ester (0·5 mg.) in ethanol (1 drop) and 0·1N-hydrochloric (2 ml.) was set aside at room temperature for 200 hr. and the ultraviolet absorption was determined at intervals. There was no significant increase in $E^{1\%}_{1\,cm.}$ at 254 or 270 mμ.

(b) *At* 100°. The ester (10 mg.) was heated under reflux for 30 min. with 3N-hydrochloric acid (1 ml.). The oil which separated during the reaction was extracted with ethyl acetate. Recovery, after washing with aqueous sodium hydrogen carbonate, gave a yellow gum (7 mg.) which crystallised from methanol in needles (1 mg.), m. p. 188—195°, of the triene (XVI) (see below). The mother-liquors showed $\lambda_{max.}$ 242 mμ in addition to bands, due to the triene (XVI), at 278, 287, and 310 mμ.

*Action of Methanolic Hydrogen Chloride on Methyl Gibberellate and on the Ester* (X; R = Me).—(*a*) Methyl gibberellate (300 mg.) in methanol (4 ml.) was saturated with dry hydrogen chloride and then heated under reflux for 1 hr. in a slow stream of hydrogen chloride. The yellow solution was cooled, then poured over crushed ice (10 g.), and the resulting mixture was immediately extracted with ether. The ether extract was washed with aqueous sodium hydrogen carbonate, and the neutral fraction, a gummy solid (221 mg.), was recovered and chromatographed on alumina (10 × 1·5 cm.) in benzene–light petroleum (1 : 1; 50 ml.). Elution of the column in ultraviolet light gave the following fractions: (i) Benzene–light petroleum (1 : 1; 800 ml.) removed a non-fluorescent band and afforded a gum (18 mg.) which crystallised from light petroleum (b. p. 40—60°) in needles (5 mg.), m. p. 96°, of methyl epigibberate [17] (XIII; R = Me; 4bβ), identified by mixed m. p. and comparison of the infrared spectra. (ii) Benzene–light petroleum (1 : 1; 400 ml.) removed a blue-fluorescent band, giving a gum (38 mg.). Crystallisation from light petroleum afforded needles (5 mg.), m. p. 109°, of methyl gibberate, identified as described above. (iii) Benzene–light petroleum (1 : 1; 400 ml.) gave, from a yellow-fluorescent band, an intractable yellow gum (29 mg.), $\lambda_{max.}$ 242 mμ. (iv) Benzene–methanol (200 : 1; 300 ml.) removed a yellow band, giving a yellow solid (92 mg.) (see below). (v) Benzene–methanol (100 : 1 and 50 : 1; 200 ml. each), from a diffuse blue-flourescent band, gave an intractable gum (31 mg.).

Fraction (iv) was recrystallised three times from light petroleum (with losses) and formed needles (62 mg.), m. p. 197—198°, $[\alpha]_D^{19}$ +762° (*c* 0·5 in acetone) of *methyl* 1,7-*dimethyl*-8-*oxo*-7α-*gibba*-3,4a(4b),5-*triene*-1,10-*dicarboxylate* (XVI) (Found: C, 70·4, 70·8; H, 6·8, 6·9; OMe, 17·4. $C_{21}H_{24}O_5$ requires C, 70·8; H, 6·8; 2OMe, 17·4%), $\lambda_{max.}$ ~275, 287, 310 mμ (log ε 4·32, 4·42, 4·27 respectively), $\nu_{max.}$ (OH absent) 1736 cm.$^{-1}$ (C=O); in chloroform 1737 cm.$^{-1}$ (broad); [*M*] (500 mμ) +2800°, (400 mμ) +11,000°, (350 mμ) +34,000°, (330 mμ) +72,000°, (320 mμ) +160,000°. Microhydrogenation led to the uptake of 2·12 mol. The triene gave an intense blue colour with concentrated sulphuric acid.

The *oxime*, prepared by heating the triene (25 mg.) under reflux with hydroxylamine hydrochloride and sodium acetate in aqueous ethanol for 18 hr., formed a microcrystalline powder (12 mg.), m. p. 146—152° (decomp.), from light petroleum (Found: N, 3·7. $C_{21}H_{25}O_5N$ requires N, 3·8%), $\lambda_{max.}$ ~280, 290, ~305 mμ (log ε 4·35, 4·50, 4·30 respectively), $\nu_{max.}$ 3560, 3440, 3250 (OH); 1728 (C=O); 1634 cm.$^{-1}$ (C=N).

No triene (XVI) was obtained when the reaction time was reduced to 0·25 hr.; and a significantly lower yield resulted after 2 hr.

(*b*) The ester (X; R = Me) (220 mg.) in methanol (5 ml.) was treated with hydrogen chloride as described in (*a*), and the neutral product (180 mg.) was chromatographed in the same manner

giving the following fractions: (i) Benzene–light petroleum (1 : 1; 100 ml.), from a non-fluorescent band gave a gum (18 mg.) which yielded methyl epigibberate (2 mg.) on crystallisation from light petroleum. (ii) Benzene–light petroleum (1 : 1; 100 ml.) (blue-fluorescent band) gave methyl gibberate (10 mg. from 24 mg. of gum). (iii) Benzene–light petroleum (1 : 1; 50 ml.), removed a blue-fluorescent band (12 mg.), (iv) benzene–light petroleum (1 : 1; 150 ml.) a yellow band (17 mg.), and (v) benzene (30 ml.) a yellow band (9 mg.), these materials being intractable. (vi) Benzene (220 ml.), from a blue-fluorescent band, gave a yellow gum (50 mg.), whence extraction with light petroleum followed by sublimation at 160°/$10^{-4}$ mm. furnished a yellow amorphous solid, m. p. <100°, $\lambda_{max.}$ 242 mμ ($E^{1\%}_{1\,cm.}$ 310), ketonic to Brady's reagent. (vii) Benzene–methanol (200 : 1; 100 ml.) gave a yellow solid (53 mg.), whence the triene (XVI) (19 mg.), m. p. 190—192°, was isolated.

*Action of Acid on Methyl Gibberellenate.*—The ester [10] (15 mg.) was heated under reflux for 20 min. with 3N-hydrochloric acid (1 ml.). The yellow gum which separated was extracted with ethyl acetate, and the extract was washed with sodium hydrogen carbonate solution. The neutral product (10 mg.) recovered was chromatographed in benzene (5 ml.) on alumina (8 × 0·5 cm.) in ultraviolet light. The following fractions were obtained: (i) A pale blue-fluorescent band gave to benzene (25 ml.) an oil (1 mg.), $\lambda_{max.}$ 257 mμ (unchanged methyl gibberellenate). (ii) Benzene–ether (5 : 1; 10 ml.), from a blue-fluorescent band, gave a yellow gum (2 mg.), $\lambda_{max.}$ 242 mμ. (iii) Benzene–ether (5 : 1; 150 ml.), from a yellow band, gave a solid (5 mg.), crystallising from methanol in needles (2 mg.), $\lambda_{max.}$ 287, 310 mμ, of the triene (XVI). The remaining fractions, eluted with benzene–methanol (200 : 1; 150 ml.), were intractable.

*Dehydrogenation of the Triene* (XVI).—The triene (116 mg.) and powdered selenium (116 mg.) were heated in a slow current of nitrogen for 6 hr. at 360°. The product (32 mg.), recovered by ether-extraction, was chromatographed on alumina (15 × 1·8 cm.) in light petroleum (b. p. 40—60°). Elution with light petroleum (b. p. 40—60°) (550 ml.) gave a semi-solid fraction (10 mg.) which furnished 1,7-dimethylfluorene,[20] identified by mixed m. p. and comparison of the infrared spectra, on recrystallisation from methanol.

*Reduction of the Triene* (XVI).—(a) *Catalytic reduction.* The triene (118 mg.) in ethyl acetate (25 ml.) in presence of 10% palladium–charcoal (118 mg.) took up 18 ml. of hydrogen (2·4 mol.). The product crystallised from ethyl acetate–light petroleum in needles (76 mg.), m. p. 127—128°, $[\alpha]_D^{17}$ +24° (*c* 0·4), of *methyl* 1,7-*dimethyl*-8-*oxo*-7α-*gibb*-4a(4b)-*ene*-1,10-*dicarboxylate* (XXIV) (Found: C, 69·8; H, 8·1. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8%), $\lambda_{max.}$ 204 mμ (log ε 4·06), $\nu_{max.}$ (OH absent) 1742 cm.$^{-1}$ (C=O). It gave no colour with concentrated sulphuric acid and was ketonic to Brady's reagent.

(b) *Reduction with sodium borohydride.* The triene (100 mg.) in methanol (20 ml.) at 10° was stirred for 1 hr. during the addition of sodium borohydride (100 mg.) in methanol (5 ml.). Excess of hydride was decomposed by the addition of acetic acid, and the solution was concentrated *in vacuo* to small bulk. After the addition of water the oily product (95 mg.) was extracted with ethyl acetate and crystallised from ethyl acetate–light petroleum in prisms (43 mg.), m. p. 149—153°, of *methyl* 8ξ-*hydroxy*-1,7-*dimethyl*-7α-*gibba*-3,4a(4b),5-*triene*-1,10-*dicarboxylate* (XXIII) (Found: C, 69·6; H, 7·6; OMe, 17·2. $C_{21}H_{26}O_5$ requires C, 70·4; H, 7·3; 2OMe, 17·3%), $\lambda_{max.}$ ~275, 286, ~296 mμ (log ε 4·38, 4·49, 4·40 respectively), $\nu_{max.}$ 3560 (OH); 1728 cm.$^{-1}$ (C=O). It gave an intense green colour with concentrated sulphuric acid.

*Hydrogenolysis of Methyl Gibberellate and of the Ester* (X; R = Me).—(*a*) (With Dr. T. P. C. Mulholland). Methyl gibberellate (500 mg.) in acetic acid (50 ml.) was added to Adams platinum oxide (200 mg.), previously reduced with hydrogen in acetic acid (20 ml.), and hydrogenated to completion (uptake *ca.* 2·9 mol. in 2 hr.). After removal of the solvent *in vacuo* the gummy product was separated into neutral (200 mg.) and acidic (305 mg.) fractions in the usual way. The neutral fraction crystallised from aqueous methanol in prisms, m. p. 242—245°, of mixed methyl tetrahydrogibberellates (8-epimers).[7] The acid fraction was recrystallised three times from ethyl acetate, giving prisms (96 mg.), m. p. 269—271° (decomp.; after previous softening) of *methyl* 1-*carboxy*-2,7-*dihydroxy*-1,8-*epi*-*dimethylgibbane*-10-*carboxylate* (XV) (Found: C, 65·2; H, 8·45. $C_{20}H_{30}O_6$ requires C, 65·55; H, 8·25%), $\nu_{max.}$ 3410, ~2650—2500 (broad) (OH); 1741, 1715 cm.$^{-1}$ (C=O); in dioxan, no band near 1780 cm.$^{-1}$ (γ-lactone C=O).

(*b*) The ester (X; R = Me) (500 mg.) in acetic acid (50 ml.) was subjected to hydrogenolysis in the presence of a reduced Adams platinum oxide catalyst (200 mg.) as described in (*a*) above.

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

The neutral fraction (27 mg.) was rejected and the acid fraction (459 mg.) recrystallised from ethyl acetate as before, giving the acid (XV) (110 mg.), m. p. 266—268° (decomp., after previous softening), identified by comparison of the infrared spectra.

*Hydrogenation of the Ester* (X; R = Me).—The ester (250 mg.) in ethyl acetate (50 ml.) was hydrogenated at room temperature in the presence of 10% palladium–charcoal (50 mg.) until 1·1 mol. had been taken up. The gummy product was separated into neutral (206 mg.) and intractable acidic (40 mg.) fractions by extraction with aqueous sodium hydrogen carbonate and recovery. The neutral fraction in benzene–methanol (200 : 1; 100 ml.) was chromatographed on alumina (14 × 1 cm.). Elution with the same solvent (volumes in parentheses) gave the following gummy fractions which crystallised from ethyl acetate–light petroleum: (i) (800 ml.; 120 mg.) prisms, m. p. 223—228°, (ii) (400 ml.; 55 mg.) intractable mixtures, (iii) (400 ml.; 28 mg.) needles, m. p. 164—166°. Fraction (i) was recrystallised three times from ethyl acetate, giving *methyl* 1-*carboxy*-2(ax),3,7-*trihydroxy*-1,8-*epi*-*dimethylgibb*-4-*ene*-10-*carboxylate* 1 ⟶ 3-*lactone* (XXV) as prisms (40 mg.), m. p. 232—235°, $[\alpha]_D^{24}$ +84° (*c* 0·5) (Found: C, 66·4; H, 7·2. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%), $\nu_{max.}$ 3485, 3360 (OH); 1741 (C=O); 1682 cm.$^{-1}$ (C=C). Fraction (iii) crystallised from ethyl acetate–light petroleum in needles or plates (16 mg.), m. p. 171—172°, $[\alpha]_D^{24}$ +112° (*c* 0·5), of *methyl* 1-*carboxy*-2(ax),3,7-*trihydroxy*-1,8-*dimethylgibb*-4-*ene*-10-*carboxylate* 1 ⟶ 3-*lactone* (XXV) (Found: C, 66·4; H, 7·5%), $\nu_{max.}$ (needle form) 3410 (broad) (OH); 1762 (broad), 1736 (C=O); 1676 cm.$^{-1}$ (C=C); (plate form) 3470, 3300; 1770, 1730; 1675 cm.$^{-1}$.

Both epimers gave intense reddish-purple colours with concentrated sulphuric acid.

Repetition of this reduction gave erratic yields of crude neutral products: on one occasion the weights of the acidic and neutral fractions were 116 mg. and 130 mg. respectively and the weights of the epimers, m. p. 232—235° and 171—172°, isolated by chromatography, were 26 and 8 mg. respectively. When the catalyst : ester ratio was increased to 1 : 1, the yield of neutral fraction was very considerably reduced and the weight of acidic material was correspondingly increased.

*Ozonolysis of the Ester* (X; R = Me).—(a) *In ethyl acetate at* −70°. Ozonised oxygen equivalent to 1·0 mol. of ozone was passed through the ester (3·6 g.) in ethyl acetate (200 ml.) at −70°. The ethyl acetate was removed *in vacuo* at 20° and the residual gum was decomposed with water (200 ml.) during 3—4 days. Needles (195 mg.), m. p. 96—115° (*A*), separated and were filtered off. The aqueous solution was extracted with ethyl acetate, and the product was separated into acidic (1·12 g. of gum) and neutral (2·19 g.) fractions as usual. The neutral fraction was separated by treatment with Girard's reagent P into a non-ketonic portion (829 mg.), which yielded unchanged ester (X; R = Me), m. p. 172°, on crystallisation from ethyl acetate–light petroleum, and a ketonic gum (667 mg.).

The latter was chromatographed on alumina (20 × 2 cm.) in light petroleum (b. p. 40—60°) containing a little ethyl acetate. A series of intractable gums (55 mg.) were eluted with various solvents. Benzene–methanol (100 : 1; 800 ml.) then eluted a solid (185 mg.) which crystallised from ethyl acetate in prisms (79 mg.), m. p. 236—241°, of *methyl* 1-*carboxy*-4,4a-*epoxy*-2(ax),3,7-*trihydroxy*-1-*methyl*-8-*oxogibbane*-10-*carboxylate* 1 ⟶ 3-*lactone* (Found: C, 60·7; 60·4; H, 5·9, 6·0. $C_{19}H_{22}O_8$ requires C, 60·3; H, 5·9%), $\nu_{max.}$ 3425 (OH); 1778, 1738, 1722 cm.$^{-1}$ (C=O); in acetonitrile, 1786, 1755, 1737 cm.$^{-1}$. It gave a yellow colour with a green fluorescence with concentrated sulphuric acid and reduced ammoniacal silver nitrate on warming.

Further elution of the column with benzene–methanol (50 : 1; 100 ml.) furnished *methyl* 1-*carboxy*-2(ax),3,7-*trihydroxy*-1-*methyl*-8-*oxogibb*-4-*ene*-10-*carboxylate* 1 ⟶ 3-*lactone* (XI; R = R′ = H), needles (32 mg.), m. p. 175—177° (Found: C, 62·8; H, 6·3. $C_{19}H_{22}O_7$ requires C, 63·0; H, 6·1%), $\nu_{max.}$ 3401 (broad) (OH); 1779, 1754, 1724 (C=O); 1678 cm.$^{-1}$ (C=C). Recrystallisation from ethyl acetate–light petroleum gave the *hemihydrate*, needles, double m. p. 118—120° (gas evolution) and 175—177° (Found: C, 61·6, 61·35; H, 6·4, 6·4. $C_{19}H_{22}O_7$,0·5$H_2O$ requires C, 61·4; H, 6·2%), $\nu_{max.}$ 3500, 3405, ~3285, ~3160 (OH); 1780, 1756, 1731 (C=O); 1678 cm.$^{-1}$ (C=C); in chloroform, 3615, 1777, 1755, 1736 cm.$^{-1}$. The anhydrous ketol (XI; R = R′ = H) was obtained by drying the hemihydrate *in vacuo* at 150°/0·005 mm.

The hemihydrate (49 mg.) was also obtained in the next eluate [benzene–methanol (50 : 1; 100 ml.)] from the column. Continued elution with the same eluant (700 ml.) gave a solid (270 mg.) which crystallised from ethyl acetate–light petroleum in needles (142 mg.), m. p. 104—106°, of the *ketol* (XI; R = R′ = H) *hydrate* (Found: C, 60·6; H, 6·45. $C_{19}H_{22}O_7$,$H_2O$ requires C, 60·0; H, 6·4%), $\nu_{max.}$ 3560, 3430, 3320 (OH); 1750 (broad), 1727 (C=O); 1677 (C=C);

1635 (broad) cm.$^{-1}$ ($H_2O$); in acetonitrile 1769, 1754, 1732, 1674 cm.$^{-1}$, [*M*], positive Cotton effect curve (330 mμ, peak) +5050°, (282, trough) −2820°. The infrared solution spectra of the ketol (XI; R = R′ = H) and its hemihydrate and hydrate were identical. The compounds gave red colours with concentrated sulphuric acid and reduced ammoniacal silver nitrate on warming.

The *diacetate* (XI; R = R′ = Ac) prepared with acetic anhydride in pyridine at room temperature during 3 days was an amorphous powder, m. p. 115—125° (Found: C, 61·7; H, 6·4. $C_{23}H_{26}O_9$ requires C, 61·9; H, 5·9%), $\nu_{max.}$ OH absent; 1767, 1734 (C=O); 1673 cm.$^{-1}$ (C=C).

The acid fraction crystallised from ethyl acetate in prisms (220 mg.), m. p. 176—178°, $[\alpha]_D^{22}$ +52° (*c* 1·0), of 1-*carboxy*-1,2,3,5,6,7,8,10,12α,13-*decahydro*-2,3-*dihydroxy*-9β-*methoxycarbonyl*-1-*methyl*-7-*oxo*-8aβ-*fluorenylacetic acid* 1 ⟶ 3-*lactone* (XIV; R = H) (Found: C, 60·7; H, 5·7; OMe, 9·0. $C_{19}H_{22}O_8$ requires C, 60·3; H, 5·9; OMe, 8·2%), $\nu_{max.}$ 3400, 3345 (OH); 1776, 1753, 1735, and 1711 (C=O); ~1680 cm.$^{-1}$ (C=C). It gave an orange colour with concentrated sulphuric acid.

The *methyl ester* (XIV; R = Me), prepared with diazomethane, was a glass which softened below 90° and distilled at 140—160° (bath)/4·7 × $10^{-3}$ mm. (Found: C, 61·0; H, 6·4. $C_{20}H_{24}O_8$ requires C, 61·2; H, 6·2%), $\nu_{max.}$ 3410 (OH); 1769, 1733 (broad) cm.$^{-1}$ (C=O). It gave a deep yellow colour with concentrated sulphuric acid.

The residue (820 mg.) from the mother-liquors, after the acid (XIV; R = H) had been filtered off, was methylated in methanol (5 ml.) with ethereal diazomethane, and the gummy product was chromatographed on alumina (30 × 2 cm.). The following fractions were eluted (all gums): (i) benzene (100 ml.) and benzene–methanol (400 : 1; 200 ml.) gave 145 mg.; (ii) benzene–methanol (200 : 1; 600 ml.) gave 66 mg.; (iii) benzene–methanol (100 : 1; 900 ml.) afforded the ester (XIV; R = Me) (563 mg.), identified after distillation at 140—160°/5 × $10^{-3}$ mm. by comparison of the infrared spectra.

Fractions (i) and (ii) crystallised from ethyl acetate–light petroleum in needles (110 mg.), m. p. 202—204°, $[\alpha]_D^{19}$ +8° (*c* 0·77 in acetone) of *methyl* 1-*carboxy*-4,11-*epoxyperhydro*-2,3-*dihydroxy*-9β-*methoxycarbonyl*-1-*methyl*-7-*oxo*-13β-*fluorenylacetate* 1 ⟶ 3-*lactone* (Found: C, 58·8; H, 6·1; OMe, 15·2. $C_{20}H_{24}O_9$ requires C, 58·8; H, 5·9; 2OMe, 15·2%), $\nu_{max.}$ 3515 (OH); 1773, 1738, 1715 cm.$^{-1}$ (C=O); in chloroform, 3455, 1786, 1737, 1722 cm.$^{-1}$. At 205, 210, 220, and 230 mμ ε was 1130, 1000, 700, and 340, respectively. There was no uptake of hydrogen on microhydrogenation. The ester gave a yellow colour with a green fluorescence with concentrated sulphuric acid.

The *acetate*, prepared with acetic anhydride in pyridine at room temperature during 20 hr., crystallised from ethyl acetate–light petroleum in needles, m. p. 228—229° (Found: C, 58·5; H, 5·9; Ac, 8·9. $C_{22}H_{26}O_{10}$ requires C, 58·7; H, 5·8; 1Ac, 9·3%), $\nu_{max.}$ (no OH absorption) 1783, 1749, 1730, 1716 cm.$^{-1}$ (C=O).

The solid (*A*) crystallised from aqueous methanol in needles (90 mg.), m. p. 206—208°, of a *substance* (Found: C, 61·1; H, 6·7. $C_{19}H_{22}O_7$,$CH_3$·OH requires C, 60·9; H, 6·6%), $\nu_{max.}$ 3555, 3495, 3410 (sh), 3320, 3125 (OH); 1788, 1710 (C=O); 1681, 1670 (sh) (C=C); in chloroform, 1796, 1732 cm.$^{-1}$. Solvent-free material was obtained on drying at 60° *in vacuo* (Found: C, 63·15; H, 6·3; OMe, 8·35. $C_{19}H_{22}O_7$ requires C, 63·0; H, 6·1; OMe, 8·6%) and had $\nu_{max.}$ 3635, 3540, 3120 (sh) (OH); 1782, 1723 (C=O); 1659 cm.$^{-1}$ (C=C). It gave an intense red colour with concentrated sulphuric acid.

(b) *In acetic acid at* 20°. Ozonised oxygen was passed through the ester (1·0 g.) in acetic acid (40 ml.) at 20° (uptake 1 mol.). After 1 hr. the mixture was steam-distilled. Treatment of the distillate with saturated aqueous dimedone gave the formaldehyde derivative (39%), m. p. and mixed m. p. 187—189°. The non-steam volatile residue was made alkaline by the addition of sodium carbonate and was extracted with ethyl acetate, giving a neutral fraction (620 mg.). The aqueous layer was then adjusted to pH 2 by addition of concentrated hydrochloric acid and was re-extracted with ethyl acetate, giving an acid fraction (350 mg.). The neutral fraction was chromatographed on alumina (40 × 2 cm.), and the column was eluted as described in (a) above. After a series of intractable gums had been eluted, benzene–methanol (100 : 1; 850 ml.) brought off a gum (120 mg.) which crystallised from ethyl acetate–light petroleum in felted needles (12 mg.) of the *ketol acetate* (XI; R = H, R′ = Ac), m. p. 173—174° (Found: C, 62·45; H, 6·1. $C_{21}H_{24}O_8$ requires C, 62·4; H, 6·0%), $\nu_{max.}$ 3430 (OH); 1759, 1738 (C=O); 1675 cm.$^{-1}$ (C=C); in acetonitrile, 1769, 1738 cm.$^{-1}$. It gave a claret colour with

concentrated sulphuric acid and was recovered after attempted oxidation with sodium metaperiodate in aqueous methanol. Acetylation gave the amorphous diacetate (XI; R = R′ = Ac), m. p. 115—125°, identified by the infrared spectrum.

Continued elution of the column with benzene–methanol (100 : 1; 800 ml.) gave a gum (157 mg.) which furnished the hydrate (26 mg.), double m. p. 102—106° and 172°, of the ester (X; R = Me). Benzene–methanol (50 : 1; 2·1 l.) then eluted a series of solid fractions (198 mg.) which gave the hemihydrate and hydrate of the ketol (XI; R = R′ = H) on recrystallisation from ethyl acetate–light petroleum.

The acid fraction was methylated and the product was chromatographed as described in (a) above. After intractable gummy fractions of 43 mg. had been eluted [benzene (400 ml.) and benzene–methanol (100 : 1; 700 ml.)], benzene–methanol (50 : 1; 600 ml.) furnished the ester (XIV; R = Me) (210 mg.), identified after distillation *in vacuo* by comparison of the infrared spectra.

*Oxidation of the Ketol* (XI; R = R′ = H).—(a) *With sodium periodate.* The ketol hydrate (27 mg.) in methanol (2 ml.) and water (1 ml.) containing sodium metaperiodate (30 mg.) was set aside at room temperature for 22 hr. The methanol was distilled off *in vacuo* and the residue was diluted with water and acidified to pH 3 with concentrated hydrochloric acid. Extraction with ethyl acetate was followed by separation into neutral (2 mg.) and acidic (22 mg.) fractions. The acid fraction was methylated with diazomethane and the product was chromatographed on alumina (7 × 1 cm.) as described above. Benzene–methanol (100 : 1) eluted a glass which after distillation at 140—160°/5 × $10^{-3}$ mm. was identified as the ester (XIV; R = Me) by the infrared spectrum.

(b) *With perbenzoic acid.* The ketol hydrate (32 mg.) in chloroform (1 ml.) was added to perbenzoic acid (14 mg., 1·2 mol.) in chloroform (1 ml.) at 0° and set aside at room temperature for 48 hr., then washed with sodium hydrogen carbonate. Evaporation gave a gum (22 mg.) which crystallised from ethyl acetate in prisms, m. p. 233—237° (10 mg.), of the ketol (m. p. 236—241°), identified by comparison of the infrared spectra.

*Acid-hydrolysis of the Acid* (XIV; R = H) *and its Ester* (XIV; R = Me).—(*a*) The acid (20 mg.) was heated under reflux for 1 hr. with 3N-hydrochloric acid (2 ml.). Extraction of the cooled solution with ethyl acetate afforded an intractable gum which was methylated with diazomethane. The product crystallised from ethyl acetate in prisms (2 mg.), m. p. 202—204°, of the ester [1] (XVII), identified by mixed m. p. and comparison of the infrared spectra.

(*b*) The ester (34 mg.) in methanol (1 ml.) and 3N-hydrochloric acid (8 ml.) was heated under reflux for 30 min. The neutral product (30 mg.), recovered in ethyl acetate, crystallised from ethyl acetate–light petroleum in prisms (10 mg.), m. p. 200—203°, of the ester (XVII), identified as before.

*Oxidation of the Ketol, m. p.* 236—241°.—The ketol (10 mg.) in methanol (1 ml.) and water (3 ml.) containing sodium metaperiodate (15 mg.) was set aside at room temperature for 20 hr. The product was worked up as described for the ketol (XI; R = R′ = H) and separated into neutral (3 mg.) and acid (7 mg.) fractions. Methylation of the acid fraction gave prisms (4 mg.) of the ester $C_{20}H_{24}O_9$, m. p. 202—204°, identified by comparison of the infrared spectra.

*Attempted Acid-hydrolysis of the Ester, m. p.* 202—204°.—The ester (30 mg.) in methanol (5 ml.) and 3N-hydrochloric acid (5 ml.) was heated under reflux for 30 min. After removal of the methanol *in vacuo* the product was recovered in ethyl acetate and separated into neutral (10 mg.) and acidic (110 mg.) fractions. The neutral fraction deposited unchanged starting material (2 mg.) on crystallisation from ethyl acetate–light petroleum. The acid fraction was an intractable gum which showed no specific absorption band near 270 mμ.

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