## 650. *Gibberellic Acid. Part XXVIII.* Some Derivatives of Gibberellins $A_4$ and $A_7$*

By D. C. Aldridge, J. R. Hanson, and T. P. C. Mulholland

Some reactions of gibberellins $A_4$ (II; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_2$) and $A_7$ (III; $R^1 = R^2 = H$) are described. The non-allylic hydroxyl group in gibberellin $A_4$ is oxidised by manganese dioxide in dichloromethane but not in dioxan. The 8-spirocyclopropane esters (X) and (XI), and the 8-ethylidene analogue (II; $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = CHMe$) have been obtained. Alkaline isomerisation of gibberellin $A_7$ methyl ester yields the 1→3-lactone (VIII; $R^1 = Me$, $R^2 = H$).

Of the naturally-occurring gibberellins $A_1$—$A_9$, gibberellins $A_2$[1] (I; $R^1 = H$, $R^2 = OH$), $A_4$[2] (II; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_2$), $A_7$[2] (III; $R^1 = R^2 = H$), and $A_9$[2] (II; $R^1 = R^2 = R^3 = H$, $R^4 = CH_2$) are distinguished by the absence of a hydroxyl group at position 7. Gibberellins $A_4$, $A_7$, and $A_9$ are much more active than gibberellic acid (III; $R^1 = H$, $R^2 = OH$) and the other gibberellins in promoting the growth of cucumber

* Part XXVII, P. J. Keay, J. S. Moffatt, and T. P. C. Mulholland, *J.*, 1965, 1605.

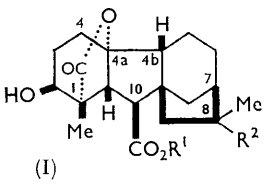
[1] J. F. Grove, *J.*, 1961, 3545.

[2] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *Tetrahedron*, 1962, **18**, 451.

seedlings. Further investigation of gibberellins $A_2$, $A_4$, and $A_7$ is described below, and of gibberellin $A_9$ in the following Paper.[3] Structure-activity relationships of many of the derivatives and transformation products have been described briefly [4] and will be fully reported elsewhere.

Gibberellin $A_4$ has been prepared from gibberellin $A_7$ [2] and converted into gibberellin $A_2$,[1] but since gibberellin $A_7$ is itself not easily freed from gibberellin $A_4$ on a preparative scale, a mixture of gibberellins $A_7$ and $A_4$ was used for some experiments.

Although attempted oxidation [5] of methyl gibberellate with chromium trioxide in acetone–sulphuric acid gave an intractable product, similar oxidation of gibberellin $A_7$ methyl ester gave a good yield of the corresponding 2-oxo-derivative (IV; $R^1 = Me$, $R^2 = CH_2$), previously obtained [2] with manganese dioxide. Ozonolysis of the 2-oxo-derivative yielded the 8-nor-ketone (IV; $R^1 = Me$, $R^2 = O$), $C_{19}H_{20}O_6$, m. p. 191—194°. Oxidation of a mixture of gibberellins $A_4$ and $A_7$ with manganese dioxide in dioxan gave



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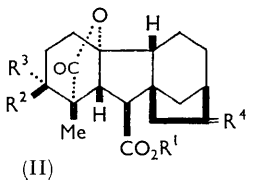

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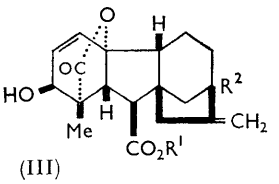

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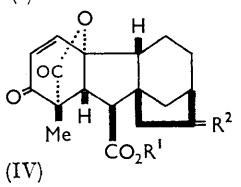

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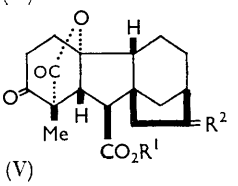

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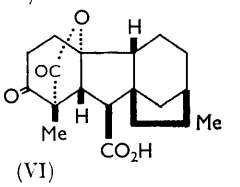


(VI)

the 2-oxo-derivative (IV; $R^1 = H$, $R^2 = CH_2$), $C_{19}H_{20}O_5$, m. p. 195—198°, of gibberellin $A_7$, and unoxidised material. However, when the oxidation was conducted in dichloromethane the gibberellin $A_4$ component was also oxidised to its 2-oxo-derivative (V; $R^1 = H$, $R^2 = CH_2$), $C_{19}H_{22}O_5$, m. p. 258—260° (decomp.). Other examples of the oxidation of non-allylic carbinols with manganese dioxide have recently been described.[6] The keto-acid, m. p. 258—260° (decomp.), and its methyl ester were also prepared from gibberellin $A_4$ and its methyl ester respectively by oxidation with the chromium trioxide reagent. Hydrogenation of the keto-acid (V; $R^1 = H$, $R^2 = CH_2$) with a palladium–carbon catalyst gave a mixture of 8-epimeric dihydro-derivatives (VI), $C_{19}H_{24}O_5$. Reduction of the keto-acid (V; $R^1 = H$, $R^2 = CH_2$) and its methyl ester, with sodium borohydride, yielded the corresponding 2α-epimers (II; $R^1 = H$ and Me, $R^2 = H$, $R^3 = OH$, $R^4 = CH_2$) of gibberellin $A_4$ and its methyl ester.

Hydrogenation of the methyl esters of gibberellin $A_4$ or $A_7$ has been reported [7,2] to give dihydrogibberellin $A_4$ methyl ester (I; $R^1 = Me$, $R^2 = H$), m. p. 148—151°, presumably a mixture of 8-epimers. When the mixed epimers were prepared by hydrogenating gibberellin $A_7$ and methylating the resulting dihydrogibberellin $A_4$ (I; $R^1 = R^2 = H$), m. p. 253—254° (decomp.), $[\alpha]_D$ +19°, the product had m. p. 149—151°, $[\alpha]_D$ +21°. The presence of unequal amounts of 8-epimers in the ester was shown by the nuclear magnetic resonance spectrum which revealed two overlapping sets of 10 : 10a quartets in the ratio of about 2 : 1 (Table).

[3] J. R. Hanson and T. P. C. Mulholland, following Paper, Part XXIX.
[4] T. P. C. Mulholland, Vth International Pesticides Congress, London, 1963.
[5] B. E. Cross, *J.*, 1960, 3022.
[6] I. T. Harrison, *Proc. Chem. Soc.*, 1964, 110.
[7] N. Takahashi, Y. Seta, H. Kitamura, and Y. Sumiki, *Bull. Agric. Chem. Soc., Japan*, 1959, **23**, 405.

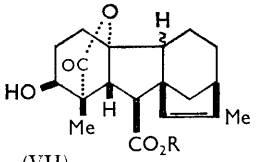
Chemical shifts ($\tau$ values) of protons, determined in $CDCl_3$ ($J$, c./sec., in parentheses)

| Compound | Position of protons: 1 | 8-Subst. | 2 | OMe | 10,10a |
|---|---|---|---|---|---|
| (I; $R^1$ = Me, $R^2$ = H) ..... | 8·87 | 9·01 (d, 7) | 6·17 | 6·30 | 6·86, 7·29 (d, 11) ~6·86, 7·39 (d, 10·5) |
| (VII; R = Me) ..... | 8·86 | 8·32 (d, 1·5) | 6·17 | 6·33 | 6·89, 7·42 (d, 9·5) |
| * (I; $R^1$ = Me, $R^2$ = OH)... | 8·55 | 8·51 | 5·92 | 6·35 | 6·21, 6·98 (d, 11) |
| * Isomer, m. p. 160—161° of (I; $R^1$ = Me, $R^2$ = OH) | 8·55 | 8·55 | 5·95 | 6·30 | 6·32, 7·08 (d, 11) |
| † (X) ..... | 8·86 | 9·55 9·43 | 6·09 | 6·24 | 6·82, 7·28 (d, 11) |
| (VIII; $R^1$ = Me, $R^2$ = H) | 8·80 | 5·21 | 5·72 | 6·23 | 7·45, 6·72 (d, 7) |

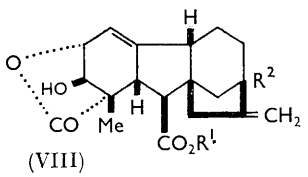
* Determined in pyridine solution. † C-3 and C-4 protons at 5·05 and 4·18 (3), respectively.

In earlier work,[2] hydrogenation of gibberellin $A_7$ over a 2% palladium–barium carbonate catalyst partially poisoned with pyridine gave gibberellin $A_4$ and hydrogenolysis products. Relactonisation of the latter fraction with hot hydrochloric acid yielded gibberellin $A_2$ and a compound, m. p. 252—254° (decomp.), considered to be dihydrogibberellin $A_4$, $C_{19}H_{26}O_5$.

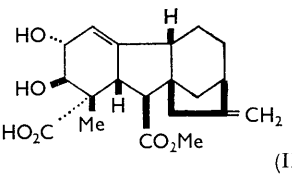
Repetition of the lactonisation on a larger scale gave a complex product. This, chromatographed on buffered Celite, yielded two fractions, m. p. 256—260° (decomp.), $[\alpha]_D$ +54°,



(VII)



(VIII)



(IX)

and m. p. 251—255° (decomp.), $[\alpha]_D$ +39°, containing monohydroxy-acids; and less easily-eluted dihydroxy-acids which were separated with difficulty into gibberellin $A_2$ and an isomer of gibberellin $A_2$.

Although the infrared spectrum of the monohydroxy-acid, $[\alpha]_D$ +54°, was almost indistinguishable from that of the mixture, $[\alpha]_D$ +21° of 8-epimeric dihydrogibberellins $A_4$, analytical data for the new acid and its methyl ester, m. p. 148—152°, $[\alpha]_D$ +59°, indicated that the acid had the formula $C_{19}H_{24}O_5$, and was isomeric with gibberellin $A_4$. The nuclear magnetic resonance spectrum of the above ester $[\alpha]_D$ +59° contained, *inter al.*, a resonance at $\tau$ 4·65 coupled with a methyl resonance at $\tau$ 8·32 ($J$ = 1·5 c./sec.) showing that the ester consisted mainly of the $\Delta^8$-olefin (VII; R = Me) and hence that the corresponding acid was (VII; R = H). Examination of the spectrum at *ca.* $\tau$ 9 showed that less than 10% of the dihydrogibberellin $A_4$ methyl esters could be present.

The monohydroxy-acid, $[\alpha]_D$ +39°, was a mixture of at least two products; the nuclear magnetic resonance spectrum revealed a 10 : 10a quartet corresponding to that shown by the above $\Delta^8$-olefin, but also a significant amount of a constituent having a 10 : 10a quartet corresponding to that ($\tau$ 6·86, 7·29) of one of the 8-epimeric dihydrogibberellin $A_4$ methyl esters.

Gibberellin $A_2$ (I; $R^1$ = H, $R^2$ = OH), $[\alpha]_D$ +13°, was obtained in the normal form,[1] m. p. 256—258° (decomp.), and in a form, m. p. 232—234°, the infrared spectrum of which in Nujol mull was distinct from that of the form, m. p. 235—237°, described by Takahashi *et al.*[8] The methyl ester, previously recorded[1] as the monohydrate, m. p. 190°, was obtained in anhydrous form, m. p. 212—214°.

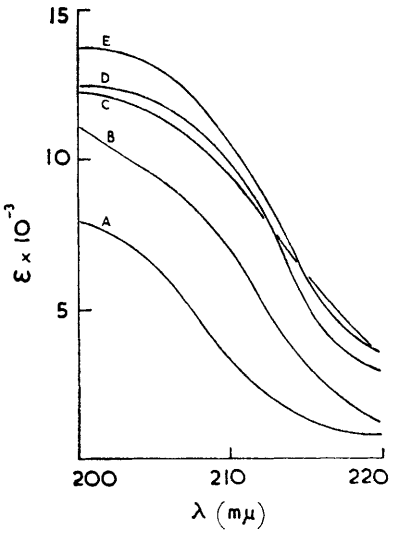
The isomer, m. p. 255—257° (decomp.), $[\alpha]_D$ +5°, of gibberellin $A_2$ yielded a methyl ester monohydrate, m. p. 160—161°. The acid was not formed in significant amount when gibberellin $A_4$ was converted into gibberellin $A_2$ with cold methanolic hydrochloric

[8] N. Takahashi, H. Kitamura, A. Kawarada, Y. Seta, M. Takai, S. Tamura, and Y. Sumiki, *Bull. Agric. Chem. Soc., Japan*, 1955, **19**, 267.

acid as described by Grove.[1] The isomer is probably epimeric with gibberellin $A_2$ at position 8, rather than position 4b (cf. ref. 9), since both compounds have the same coupling constant ($J$ = 11 c./sec.) for the 10 and 10a protons. An attempt to relate the isomer to gibberellin $A_2$ by dehydration of the methyl esters of both acids with phosphorus oxychloride in pyridine [10] did not give useful results.

Gibberellin $A_7$ is produced [2] by culturing *Gibberella fujikuroi* ACC 917 under conditions of normal pH until the inorganic nitrogen is exhausted from the medium, when the pH is adjusted to 7 with alkali. In addition to gibberellin $A_7$, an isomeric acid, $C_{19}H_{22}O_5$, m. p. 186—190°, was sometimes isolated from the fermentation.

The acid was assigned the structure (VIII; $R^1 = R^2 = H$), analogous to the compound (VIII; $R^1 = H$, $R^2 = OH$) obtained [11] by alkali-induced isomerisation of gibberellic acid. The infrared spectra of the new acid, and its methyl ester, $C_{20}H_{24}O_5$, m. p. 226—228°, showed that the acid was a monohydroxy-γ-lactonic monobasic acid. The new methyl




Ultraviolet absorption curves for (A) gibberellin $A_4$ (II; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_2$), (B) gibberellin $A_7$ (III; $R^1 = R^2 = H$), (C) the ester (VIII; $R^1$ = Me, $R^2 = OH$), (D) the acid (IX), and (E) the ester (VIII; $R^1$ = Me, $R^2 = H$)

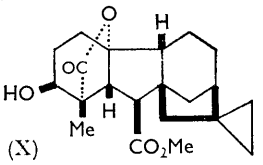
ester, unlike gibberellin $A_7$ methyl ester, showed infrared absorption (826 cm.$^{-1}$) and ultraviolet absorption (Figure, refs. 11, 12) attributed to a trisubstituted $\Delta^4$-ethylenic bond; and the change in molecular rotation (+175°) resulting from the isomerisation of methyl gibberellate to the $\Delta^4$ lactone (VIII; $R^1$ = Me, $R^2 = OH$) is similar to that (+155°) which occurs in the 7-deoxy-series. The methyl ester (VIII; $R^1$ = Me, $R^2 = H$) was also obtained directly from gibberellin $A_7$ methyl ester by the action of 0·03N-sodium hydroxide; the acid (VIII; $R^1 = R^2 = H$) is therefore probably an artifact arising during fermentation.

The nuclear magnetic resonance spectrum of the ester (VIII; $R^1$ = Me, $R^2 = H$) was consistent with the presence of the 1→3-lactone ring. In particular the spectrum showed the characteristic 10 : 10a quartet at τ 7·45 and 6·72 ($J$ = 7 c./sec.); the latter was also coupled to the C-4 proton (τ 4·18; $J$ = 3 c./sec.). The coupling constant ($J$ = 6 c./sec.) associated the C-2, C-3, and C-4 protons corresponded to an angle of about 30° between these protons and hence to an axially β-orientated 2-hydroxyl group. Alkaline isomerisation of the gibb-3-ene-1 ⟶ 4a-lactones to gibb-4-ene-1 ⟶ 3-lactones was accompanied by the appearance of strong bands in the infrared spectra at 950 cm.$^{-1}$; this was the strongest band in the spectra of the isomerised esters (VIII; $R^1$ = Me, $R^2$ = H and OH).

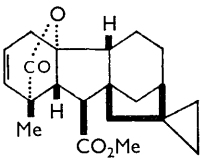
[9] D. C. Aldridge, J. F. Grove, R. N. Speake, B. K. Tidd, and W. Klyne, *J.*, 1963, 143.
[10] H. Kitmura, N. Takahashi, Y. Seta, and Y. Sumiki, *Bull. Agric. Chem. Soc., Japan*, 1958, **22**, 434.
[11] B. E. Cross, J. F. Grove, and A. Morrison, *J.*, 1961, 2498.
[12] T. P. C. Mulholland, *J.*, 1963, 2606.

With *NN'*-dicyclohexylcarbodi-imide gibberellin $A_7$ yielded a hydrated but non-crystalline anhydride, $C_{38}H_{42}O_9,3H_2O$ ($\nu_{max.}$ 1800, 1741—1736 cm.$^{-1}$), analogous to gibberellic anhydride monohydrate.

Unlike gibberellic acid,[5] gibberellin $A_7$ methyl ester with hydrazine did not yield the corresponding saturated tetrahydro-derivative, but instead yielded an unsaturated carboxylic acid, m. p. 121—122°, $C_{20}H_{26}O_6,H_2O$. The ultraviolet end absorption (Figure) and absorption at 795 cm.$^{-1}$ are consistent with the presence of an 8-methylene group and a $\Delta^4$-ethylenic bond in this acid, but the remainder of its spectrum suggests a higher degree of hydroxylation than in the ester (VIII; $R^1$ = Me, $R^2$ = H); the acid may have the structure (IX).




(X)




(XI)

Gibberellin $A_4$ methyl ester, prepared from gibberellin $A_4$ with ethereal diazomethane, was sometimes contaminated with significant amounts of the 8-spirocyclopropane derivative (X). The impure ester was not separated into its components by chromatography, but the derivative (X) was isolated after ozonolysis. Previously, ozonolysis of gibberellin $A_4$ methyl ester yielded the 8-nor-ketone (II; $R^1$ = Me, $R^2$ = OH, $R^3$ = H, $R^4$ = O), m. p. 203° [7] or 205—207°.[14] By using Girard's reagent P the ozonolysis product was separated into the pure nor-ketone, m. p. 212—213°, and a non-ketonic fraction, which yielded the spirocyclopropane derivative (X), $C_{21}H_{28}O_5$, m. p. 218—220°. The latter contained no ethylenic bond (no max. ~880 cm.$^{-1}$, low end absorption in the ultraviolet). The nuclear magnetic resonance spectrum showed no signal due to a methylene group at $\tau$ 5·1, but revealed a four proton-double peak at $\tau$ 9·43 and 9·55 ascribed to the spirocyclopropane ring. In the absence of ethylenic unsaturation, absorption at ~1070 cm.$^{-1}$ in the infrared spectrum is also assigned to this grouping.

Dehydration of the derivative (X) with phosphorus oxychloride in pyridine yielded the corresponding 2,3-dehydro-ester (XI), $C_{21}H_{26}O_4$, m. p. 252—257°, $[\alpha]_D$ −128°, containing one ethylenic bond and showing an inflection at 220 mμ in the ultraviolet spectrum. This derivative was also obtained as a by-product when the impure gibberellin $A_4$ methyl ester was dehydrated by way of the 2-methanesulphonyloxy-derivative.[3]

Ozonolysis of a mixture of gibberellins $A_4$ and $A_7$ gave the corresponding mixture of 8-nor-ketones which could not be separated by chromatography on silica. Catalytic hydrogenation of the mixed nor-ketones yielded the saturated nor-ketone (II; $R^1$ = $R^3$ = H, $R^2$ = OH, $R^4$ = O), $C_{18}H_{22}O_6,H_2O$.

Application of the Wittig reaction to this nor-ketone, methyltriphenylphosphonium iodide being used, gave a mixture of gibberellin $A_4$ and its 2-hydroxyl (equatorial) epimer (II; $R^1$ = $R^2$ = H, $R^3$ = OH, $R^4$ = $CH_2$) (see above). With ethyltriphenylphosphonium iodide the nor-ketone gave a product from which only one ethylidene derivative (II; $R^1$ = $R^2$ = H, $R^3$ = OH, $R^4$ = CHMe), $C_{20}H_{26}O_5$, m. p. 248—252°, was isolable. Oxidation of the 2-hydroxyl group of this compound yielded the 2-oxo-derivative (V; $R^1$ = H, $R^2$ = CHMe), $C_{20}H_{24}O_5$, which with sodium borohydride regenerated the parent carbinol; the latter is therefore considered to contain an equatorial 2-hydroxyl group. The nuclear magnetic resonance spectrum of the 2-oxo-derivative showed that it was probably a mixture of *cis*- and *trans*-ethylidene derivatives.

## Experimental

Melting points are corrected. Unless otherwise stated, specific rotations and ultraviolet spectra were determined for ethanol solutions and infrared spectra for Nujol mulls. Light

[13] J. MacMillan and J. S. Moffatt, *J.*, 1962, 4727.
[14] J. F. Grove, J. MacMillan, T. P. C. Mulholland, and W. B. Turner, *J.*, 1960, 3049.

petroleum had b. p. 40—60°, and solutions were dried with sodium sulphate. " Hyflo Super. Cel " Celite, and Woelm grade II acid alumina were used for chromatography. Nuclear magnetic resonance spectra were obtained with an A60 Varian Associates spectrometer (60 Mc./sec.) with tetramethylsilane as internal standard at $\tau = 10{\cdot}000$.

*Oxidation of Gibberellin $A_4$* (II; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_2$).—The acid (100 mg.) in acetone (10 ml.) was treated with an 8N-chromium trioxide–sulphuric acid reagent (0·2 ml.) [15] at room temperature. After 30 min. methanol (5 drops) was added and the solution was concentrated and diluted with water. The product was recovered in ethyl acetate, then chromatographed on a column of silica in ethyl acetate–light petroleum (b. p. 60—80°) (3 : 7) giving *4aα-hydroxy-1β-methyl-8-methylene-2-oxogibbane-1α,10β-dicarboxylic acid* 1 ⟶ *4a-lactone* (V; $R^1 = H$, $R^2 = CH_2$) (55 mg.) which formed needles, m. p. 258—260° (decomp., variable), from ethyl acetate–light petroleum (Found: C, 69·6; H, 7·0. $C_{19}H_{22}O_5$ requires C, 69·05; H, 6·7%); $\nu_{max.}$ 3145, 1752, 1734, 1661, and 883 cm.$^{-1}$.

The *methyl ester*, prepared with ethereal diazomethane, formed prisms, m. p. 120—122°, from light petroleum (b. p. 60—80°) (Found: C, 70·0; H, 7·1. $C_{20}H_{24}O_5$ requires C, 69·75; H, 7·0%); $\nu_{max.}$ 1790, 1770, 1725, 1654, and 880 cm.$^{-1}$. The ester was also obtained by oxidation of gibberellin $A_4$ methyl ester with chromium trioxide as described above.

The *oxime* of the acid (V; $R^1 = H$, $R^2 = CH_2$) formed prisms, m. p. 248—251° (decomp.), from ethyl acetate (Found: C, 66·2; H, 6·8; N, 4·1. $C_{19}H_{23}NO_5$ requires C, 66·1; H, 6·7; N, 4·1%).

*Reduction of the Keto-acid* (V; $R^1 = H$, $R^2 = CH_2$).—(*a*) *With sodium borohydride.* A solution of the keto-acid (49 mg.) in a 1 : 1 mixture (4 ml.) of methanol and tetrahydrofuran was treated with sodium borohydride (47 mg.) at room temperature for 1 hr. Drops of dilute hydrochloric acid were added, the organic solvents were evaporated, and the residue was mixed with water and more dilute hydrochloric acid. The product, recovered in ethyl acetate, was chromatographed on silica gel (15 × 1 cm.). Elution of the column with ethyl acetate–chloroform (3 : 2) gave *2α,4aα-dihydroxy-1β-methyl-8-methylenegibbane-1α,10β-dicarboxylic acid* 1 ⟶ *4a-lactone* (II; $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = CH_2$, 2-epigibberellin $A_4$) (21 mg.) which formed prisms, m. p. 215—220°, from ethyl acetate–light petroleum (b. p. 60—80°) (Found: C, 68·7; H, 7·35. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%); $\nu_{max.}$ 3490, 3120, 1758, 1718, 1654, and 872 cm.$^{-1}$.

(*b*) *Hydrogenation.* The keto-acid (201 mg.) and a 10% palladium–carbon catalyst (100 mg.) in ethyl acetate (25 ml.) were shaken in hydrogen at room temperature and pressure. The recovered product crystallised from ethyl acetate–light petroleum, giving *4aα-hydroxy-1β,8-dimethyl-2-oxogibbane-1α,10β-dicarboxylic acid* 1 ⟶ *4a-lactone* (*mixed 8-epimers*) (VI) as prisms (117 mg.), m. p. 243—246° (Found: C, 68·5; H, 7·3. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%).

*The methyl ester* formed needles, m. p. 111—113°, from light petroleum (Found: C, 69·3; H, 7·5. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6%).

The *oxime* of the acid (VI) crystallised from ethyl acetate–light petroleum in prisms, m. p. 223—226° (decomp.) (Found: C, 65·8; H, 7·3; N, 3·9. $C_{19}H_{25}NO_5$ requires C, 65·7; H, 7·25; N, 4·0%).

*Reduction of the Keto-ester* (V; $R^1 = Me$, $R^2 = CH_2$).—The ester (25 mg.) in methanol (2 ml.) was treated with sodium borohydride (50 mg.) at room temperature for 2 hr. The solution was acidified, diluted with water, and extracted with ethyl acetate. The extract was washed with sodium hydrogen carbonate solution and with water, dried, and evaporated giving *methyl 1α-carboxy-2α,4aα-dihydroxy-1β-methyl-8-methylenegibbane-10β-carboxylate* 1 ⟶ *4a-lactone* (II; $R^1 = Me$, $R^2 = H$, $R^3 = OH$, $R^4 = CH_2$) which formed prisms (15 mg.), m. p. 166—167°, from acetone–light petroleum (b. p. 60—80°) (Found: C, 69·35; H, 7·6. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6%); $\nu_{max.}$ 3508, 1770, 1720, 1660, and 884 cm.$^{-1}$.

*Oxidation of Gibberellin $A_7$ Methyl Ester* (III; $R^1 = Me$, $R^2 = H$).—The ester (50 mg.) in acetone (5 ml.) was treated with the above chromium trioxide reagent (0·1 ml.) for 30 min. at room temperature. Recovery of the product as described for the oxidation of gibberellin $A_4$ (above) gave methyl 1α-carboxy-4aα-hydroxy-1β-methyl-8-methylene-2-oxogibb-3-ene-10β-carboxylate 1 ⟶ 4*a*-lactone (IV; $R^1 = Me$, $R^2 = CH_2$) which crystallised from acetone–light petroleum (b. p. 60—80°) in prisms (35 mg.; m. p. 138—140°), identical with authentic material.[2]

*Ozonolysis of the Keto-ester* (IV; $R^1 = Me$, $R^2 = CH_2$).—Ozonised oxygen (13 mg. $O_3$/min.)

[15] R. G. Curtis, I. Heilbron, E. R. H. Jones, and G. F. Woods, *J.*, 1953, 457.

was passed through a solution of the above keto-ester (210 mg.) in acetic acid (10 ml.) for 10 min. The solution was stored for 1·5 hr., then made alkaline with sodium hydrogen carbonate solution, and the product was recovered in ethyl acetate. Crystallisation from acetone–light petroleum (b. p. 60—80°) gave *methyl* 1α-*carboxy*-4a*α*-*hydroxy*-1β-*methyl*-2,8-*dioxogibb*-3-*ene*-10β-*carboxylate* 1 ⟶ 4*a*-*lactone* (IV; $R^1$ = Me, $R^2$ = O) as prisms (139 mg.), m. p. 191—194° (Found: C, 65·7; H, 6·1. $C_{19}H_{20}O_6$ requires C, 66·3; H, 5·85%); $\nu_{max.}$ 1780, 1738, and 1685 cm.$^{-1}$.

*Oxidation of a Mixture of Gibberellins* $A_4$ *and* $A_7$.—(*a*) *With manganese dioxide.* (i) The mixed acids (250 mg.) were shaken with manganese dioxide (2·60 g., prepared as described by Attenburrow *et al.*,[16] then washed with water until the filtrates were neutral and dried for 3 hr. at 115°) in dichloromethane (50 ml.) for 24 hr.

The recovered product was chromatographed on a column of silica (18·5 × 1·8 cm.) made up in light petroleum (b. p. 60—80°)–chloroform (25 : 1). The column was eluted with portions of solvents giving fractions: (a) (25 : 1, 500 ml.), (10 : 1, 500 ml.), (5 : 1, 1·4 l.), (3 : 1, 400 ml.), (1 : 1, 200 ml.); recovery gave gums; (b) (1 : 1, 50 ml.) yielding 8 mg., m. p. 181—195°; (c) (50 ml.), 28 mg., m. p. 191—195°; (d) (200 ml.), solid products (67 mg.) with a wide range of m. p.; (e), chloroform, 300 ml.), no product; (f) (ethyl acetate, 200 ml.), giving unoxidised material (77 mg.).

Fraction (c) crystallised from ether in prisms (14 mg.), m. p. 195—198° (decomp.), of 4a*α*-*hydroxy*-1β-*methyl*-8-*methylene*-2-*oxogibb*-3-*ene*-1α,10β-*dicarboxylic acid* 1 ⟶ 4*a*-*lactone* (IV; $R^1$ = H, $R^2$ = $CH_2$) (Found: C, 69·45; H, 6·4. $C_{19}H_{20}O_5$ requires C, 69·5; H, 6·1%); $\nu_{max.}$ ~3205, 1787, 1734, 1675, and 887 cm.$^{-1}$, $\lambda_{max.}$ 226—227 mμ (log ε 3·79).

Fraction (d) crystallised from ethyl acetate–light petroleum giving prisms (13 mg.), m. p. 240—260° (decomp.), of 4a*α*-hydroxy-1β-methyl-8-methylene-2-oxogibbane-1α,10β-dicarboxylic acid 1 ⟶ 4*a*-lactone (V; $R^1$ = H, $R^2$ = $CH_2$) derived from gibberelline $A_4$, and also mixtures of both keto-acids.

(ii) The mixed gibberellins (50 mg.) were shaken with manganese dioxide (500 mg.) in dioxan (10 ml.) for 24 hr. Chromatography of the recovered product on silica as described above gave the acid (IV; $R^1$ = H, $R^2$ = $CH_2$) (19 mg.; m. p. 195—198°), and unoxidised material, but none of the acid (V; $R^1$ = H, $R^2$ = $CH_2$).

(*b*) *Ozonolysis.* A solution of the mixed acids (1·05 g.) in ethyl acetate (20 ml.) and acetic acid (5 ml.) was treated with ozone (160 mg., 1·1 mole) at −70°. The solution was allowed to warm to room temperature, then treated with zinc dust and water during 15 min. The product, recovered in ethyl acetate, was chromatographed in chloroform on silica gel (15 × 2·0 cm.). Elution of the column with chloroform–ethyl acetate (95 : 5 and 9 : 1) gave a mixture of nor-ketones (870 mg.) which was not separated by further chromatography on silica or silica–Celite.

The mixed nor-ketones (700 mg.) were shaken in hydrogen in ethyl acetate (35 ml.) with a 2% palladium–barium carbonate catalyst (200 mg.) until 1 mole of hydrogen had been absorbed (30 min.). The recovered product was chromatographed in chloroform on silica gel (15 × 2·0 cm.). Elution of the column with portions of chloroform–ethyl acetate (95 : 5 to 9 : 1) yielded a lactonic (infrared spectrum) gum (600 mg.). Further elution with ethyl acetate gave gummy hydrogenolysis products (55 mg.).

The lactonic product crystallised from ethyl acetate–light petroleum (b. p. 60—80°) containing a little water as plates (541 mg.), m. p. 120—140°, of 2β,4a*α*-*dihydroxy*-1β-*methyl*-8-*oxogibbane*-1α,10β-*dicarboxylic acid* 1 ⟶ 4*a*-*lactone* (II; $R^1$ = $R^3$ = H, $R^2$ = OH, $R^4$ = O) (Found: C, 61·1; H, 6·8. $C_{18}H_{22}O_6,H_2O$ requires C, 61·35; H, 6·9%); $\nu_{max.}$ 3510, 3440, 1769, 1750sh, 1723, and 1650 ($H_2O$) cm.$^{-1}$.

*Action of Wittig Reagents on the Nor-ketone* (II; $R^1$ = $R^3$ = H, $R^2$ = OH, $R^4$ = O).—(*a*) *Methyltriphenylphosphonium iodide.* Powdered reagent (400 mg.; *ca.* 10 equiv.) [17] was suspended in purified tetrahydrofuran (10 ml.) under nitrogen and was decomposed with an equivalent amount of butyl-lithium. A solution of the nor-ketone (24 mg.) in tetrahydrofuran (5 ml.) was added with exclusion of moisture and the mixture was boiled and stirred for 4 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in dilute hydrochloric acid (20 ml.). The solution was extracted with ethyl acetate and the extract was shaken with sodium hydrogen carbonate solution. The aqueous alkaline fraction was acidified, then extracted with ethyl acetate. Recovery from the last extract gave a gummy acid product (29

[16] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J.*, 1952, 1094.

[17] V. Schöllkopf, *Angew. Chem.*, 1959, **71**, 260.

mg.) which was chromatographed in chloroform on silica (9 × 1·0 cm.). The column was eluted with portions of solvents giving fractions: (i) (chloroform; 25 ml.) giving a trace of gum on recovery; (ii) (chloroform–ethyl acetate, 9 : 1; 50 ml.), giving 9·5 mg.; (iii) (125 ml.) giving starting material (11·1 mg.).

Fraction (ii) was rechromatographed on silica (12 × 0·6 cm.). Elution with chloroform–ethyl acetate (97·5 : 2·5, 20 ml.) yielded gibberellin $A_4$ (2·5 mg.) identified by the infrared spectrum. Further elution with a 95 : 5 mixture (20 ml.) yielded the 2-epimer (II; $R^1 = R^2 =$ H, $R^3$ = OH, $R^4 = CH_2$) of gibberellin $A_4$ (2·0 mg.) described above.

(*b*) *With ethyltriphenylphosphonium iodide.* The reagent [17] (1·25 g., 10 equiv.), suspended in tetrahydrofuran (15 ml.), was decomposed as in the preceding experiment. The nor-ketone (100 mg.) in tetrahydrofuran (10 ml.) was then added and the mixture was boiled (stirring) for 20 hr. Recovery of the products gave an acidic fraction (108 mg.) which was chromatographed in chloroform on silica (15 × 1·0 cm.). The column was washed with benzene, then eluted with chloroform–ethyl acetate (95 : 5). Recovery from the eluate gave a solid (82·5 mg.) which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in plates (72·5 mg.), m. p. 248—252°, of 8-*ethylidene*-2α,4aα-*dihydroxy*-1β-*methylgibbane*-1α,10β-*dicarboxylic acid* 1 ⟶ 4*a*-*lactone* (II; $R^1 = R^2 =$ H, $R^3$ = OH, $R^4$ = CHMe) (Found: C, 69·5; H, 7·6. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6%); $\nu_{max.}$ 3386, 1764, and 1724 cm.$^{-1}$; λ (ε) 200 (9933), 205 (8565), 210 (5754), and 215 (2877) mμ.

*Oxidation of the Ethylidene Alcohol* (II; $R^1 = R^2 =$ H, $R^3$ = OH, $R^4$ = CHMe).—The compound (88 mg.) was oxidised in acetone at 0° by portionwise addition of the chromium trioxide–sulphuric reagent during 1 hr. The gummy product, recovered in the usual way, was chromatographed in chloroform on silica (20 × 0·7 cm.) and the column was eluted portionwise with chloroform. Fractions yielding solid products (47 mg.) on recovery were crystallised twice from ethyl acetate–light petroleum (b. p. 60—80°) giving needles (35 mg.), m. p. 236—239°, of 8-*ethylidene*-4aα-*hydroxy*-1β-*methyl*-2-*oxogibbane*-1α,10β-*dicarboxylic acid* 1⟶4*a*-*lactone* (V; $R^1$ = H, $R^2$ = CHMe) (Found: C, 69·6; H, 7·1. $C_{20}H_{24}O_5$ requires C, 69·75; H, 7·0%); $\nu_{max.}$ 3170, 1753, 1738, 1729, and 1654 cm.$^{-1}$.

*Reduction of the 2-Oxoethylidene Derivative.*—The above derivative (20 mg.) in methanol (2 ml.) was treated with an excess of sodium borohydride at 0° during 45 min. The mixture was evaporated *in vacuo* at room temperature and the residue was dissolved in dilute hydrochloric acid. Crystals (10 mg., A) separated and were filtered off. The mother-liquor was extracted with ethyl acetate. Recovery from the extract gave a gum (11·5 mg., B).

Solid (A) was chromatographed on silica (12 × 0·8 cm.) in chloroform. Elution with chloroform gave a solid (8 mg.) which crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in plates (5 mg.), m. p. 245—250°, of the ethylidene alcohol (II; $R^1 = R^2 =$ H, $R^3$ = OH, $R^4$ = CHMe) described above.

The infrared spectrum of the intractable gum (B) was consistent with its being a mixture of the same product, and starting material.

*Action of Diazomethane on Gibberellin* $A_4$.—Some batches of gibberellin $A_4$ methyl ester, prepared from gibberellin $A_4$ with diazomethane, had m. p. *ca.* 180° (lit.,[14] 176°), but were impure and purification by chromatography on alumina failed.

*Ozonolysis of the Ester, m. p.* 180°.—The impure ester (351 mg.) was ozonised [14] in acetic acid and the semi-solid product (343 mg.) crystallised from ethyl acetate–light petroleum yielding prisms (169 mg.), m. p. 205—208°, and fractions with lower m. p.s.

A mixture of the material of m. p. 205—208° (201 mg.), Girard's reagent P (203 mg.), Amberlite resin IRC-50(H) (403 mg.), and ethanol (6 ml.) was boiled for 1 hr., and then filtered. The filtrate was mixed with ice–water and extracted with ethyl acetate and with ether. Recovery of the non-ketonic fraction from the extracts gave a gum (19 mg.).

The aqueous fraction was treated with concentrated hydrochloric acid (1·2 ml.), and after 2 hr. at room temperature recovery of the ketonic product in ethyl acetate gave methyl 1α-carboxy-2β,4aα-dihydroxy-1β-methyl-8-oxogibbane-10β-carboxylate 1⟶4*a*-lactone (II; $R^1$ = Me, $R^2$ = OH, $R^3$ = H, $R^4$ = O) which crystallised from ethyl acetate in plates (164 mg.), m. p. 212—213° (Found: C, 65·4; H, 6·95. Calc. for $C_{19}H_{24}O_4$: C, 65·5; H, 6·9%). The infrared spectrum was indistinguishable from that of a previously prepared [14] specimen of m. p. 205—207°.

Gummy and low-melting fractions (144 mg.) from the ozonolysis were separated into ketonic (50 mg.) and non-ketonic (70 mg.) fractions as described above. The ketonic fraction yielded

the above keto-ester (II; $R^1$ = Me, $R^2$ = OH, $R^3$ = H, $R^4$ = O) (22 mg.), m. p. 212—213°. The non-ketonic product was chromatographed on alumina (18 × 1·2 cm.). Elution of the column with benzene yielded a gum (7 mg.); further elution with benzene–methanol (200 : 1) yielded *methyl* 1α-*carboxy*-2β,4*aα*-*dihydroxy*-1β-*methylgibbane*-8-*spirocyclopropane*-10β-*carboxylate* 1 ⟶ 4*a*-*lactone* (X) (35 mg.) which crystallised from acetone–light petroleum in prisms (23 mg.), m. p. 218—220° (Found: C, 70·3; H, 7·9. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8%); $\nu_{max.}$ 3590, 3530, 3070 (cyclopropane ring), 1776, 1740, and 1716 cm.$^{-1}$; $\nu_{max.}$ in $CHCl_3$ 3620, ~3500, 3070, 1764, and 1734 cm.$^{-1}$); ε <200 at 200—230 mμ (C=C absent). The compound was also obtained in a dimorphic form, m. p. 216—220°. The infrared spectrum in chloroform was identical with that of material, m. p. 218—220°, but the spectrum in Nujol mull was distinct ($\nu_{max.}$ 3591, 3541, 3515, 3068, 1777, and 1716 cm.$^{-1}$).

*Degradation of the Spirocyclopropane* (X).—The compound (11 mg.) in pyridine (0·5 ml.) was heated with phosphorus oxychloride (0·05 ml.) at 100° for 30 min. The residue obtained by evaporation *in vacuo* was mixed with water and dilute hydrochloric acid, and the mixture was extracted with ethyl acetate. Recovery from the extract gave material (11 mg.), m. p. 247—255°, which crystallised from acetone in prisms, m. p. 252—257°, $[\alpha]_D^{23}$ −128° (*c* 0·36) in dioxan, of *methyl* 1α-*carboxy*-4*aα*-*hydroxy*-1β-*methylgibb*-2-*ene*-8-*spirocyclopropane*-10β-*carboxylate* 1 ⟶ 4*a*-*lactone* (XI) (Found: C, 73·8, 73·95; H, 7·6, 7·6; OMe, 9·9. $C_{21}H_{26}O_4$ requires C, 73·7; H, 7·7; OMe, 9·1%); $\nu_{max.}$ 3068, 3040, 1775, 1739, 1694, 1662, 1100, 938, and 893 cm.$^{-1}$; $\lambda_{infl}$ ~220 mμ (log ε 3·10); microhydrogenation with a palladium catalyst in acetic acid, uptake 1·1 mole.

This compound was also obtained as a by-product when the impure gibberellin $A_4$ methyl ester was dehydrated [3] by way of the methanesulphonyloxy-derivative.

*Hydrogenation of Gibberellin* $A_7$.—(*a*) The compound (113 mg.), 10% palladium–carbon (50 mg.), and ethyl acetate (15 ml.) were shaken in hydrogen at room temperature until uptake ceased. The recovered gummy product was deposited on Celite (1 g.) in acetone and the dried Celite was placed on a column of Celite (10 g.) buffered with a 2M-phosphate buffer (pH 6·2, 10 ml.) [18] and made up in chloroform–light petroleum (1 : 10). After the column had been washed with the solvent mixture (50 ml.), elution with a (1 : 3) mixture gave solid products (60 mg.). Further elution with chloroform–ethyl acetate (1 : 1) gave gummy hydrogenolysis products (38 mg.) which were not investigated.

The solid product crystallised from ethyl acetate–light petroleum giving an 8-epimeric mixture of 2β,4*aα*-*dihydroxy*-1β,8-*dimethylgibbane*-1α,10β-*dicarboxylic acid* 1 ⟶ 4*a*-*lactones* (I; $R^1$ = $R_2$ = H) as prisms (55 mg.), m. p. 253—254° (decomp.), $[\alpha]_D^{24}$ +19° (*c* 1·06) (Found: C, 68·6; H, 7·6. $C_{19}H_{26}O_5$ requires C, 68·2; H, 7·8%); $\nu_{max.}$ in $CHCl_3$ 3633, 3510, 1766, and 1710 cm.$^{-1}$.

The methyl ester crystallised from ether–light petroleum in prisms, m. p. 149—151°, $[\alpha]_D^{23}$ +21° (*c* 0·74) (Found: C, 68·8; H, 7·9. Calc. for $C_{20}H_{28}O_5$: C, 68·9; H, 8·1%); $\nu_{max.}$ in $CHCl_3$, 3630, 3500, 1769, and 1734 cm.$^{-1}$. The m. p. of the 8-epimeric mixture of esters obtained by hydrogenating gibberellin $A_7$ methyl ester [2] or gibberellin $A_4$ methyl ester [7] has been reported as 148—151 and 141°, respectively.

(*b*) Gibberellin $A_7$ (5·30 g.) was hydrogenated with a 2% palladium–barium carbonate catalyst in ethyl acetate containing pyridine as described previously.[2] The crude product was chromatographed on a column of Celite (200 g.), buffered with the phosphate buffer (200 ml.), and made up in chloroform–light petroleum (1 : 3). Elution with the solvent mixture (3·5 l.) gave gummy products (88 mg.). Continued elution with a 1 : 1 mixture (5·5 l.) eluted gibberellin $A_4$ (2·92 g.) which crystallised from ethyl acetate–light petroleum in prisms (2·11 g.), m. p. 217—225°.

Gibberellin $A_4$ methyl ester, prepared with ethereal diazomethane, crystallised from ethyl acetate–light petroleum in prisms, m. p. 178—180°, identical with an authentic specimen, and in prisms, m. p. 178—181°, of a dimorphic form, giving a different infrared spectrum ($\nu_{max.}$ 3533, 3070, 1743, and 1660 cm.$^{-1}$). In chloroform the spectra of the two forms were identical ($\nu_{max.}$ 1767, 1732, and 1660 cm.$^{-1}$) and the dimorph, m. p. 178—181°, was convertible into the normal form by seeded crystallisation.

*The methanesulphonyloxy-derivative* of gibberellin $A_4$ crystallised from ethyl acetate–light petroleum in prisms and needles, m. p. 199—200° (decomp.) (Found: C, 57·9; H, 6·3. $C_{20}H_{26}O_7S$ requires C, 58·5; H, 6·4%); $\nu_{max.}$ ~3125, 1748, 1359, and 1186 ($SO_2$) cm$^{-1}$.

[18] F. H. Stodola, G. E. N. Nelson, and D. J. Spence, *Arch. Biochem. Biophys.*, 1957, **66**, 438.

Further elution of the Celite column with chloroform–ethyl acetate (1 : 3; 2 l.)gave gummy hydrogenolysis products (1·74 g.).

*Lactonisation of the Hydrogenolysis Products.*—(*a*) Storage of the gum with 3N-hydrochloric acid–acetone at room temperature gave an intractable product.

(*b*) The gums (2·65 g.) from several experiments were boiled with acetone (60 ml.) and 3N-hydrochloric acid (80 ml.) for 1·25 hr. The mixture was concentrated *in vacuo* and the gummy product, recovered in ethyl acetate, was chromatographed on Celite (300 g.) buffered with the phosphate buffer (pH 6·2; 300 ml.) and made up in chloroform–light petroleum (1 : 5). Elution of the column with 500-ml. portions of solvents gave the following products on recovery (solvent and proportions in parentheses): (Fractions 1—8) (chloroform–light petroleum, 1 : 5), intractable gum (282 mg.); (9—11), trace; (12—16, 1 : 3), intractable gum (60 mg.); (17—27, 1 : 3), semi-solid product (210 mg.); (28—30), gum (30 mg.); (31—41, 1 : 3; 42—47, 1 : 1), semi-solid product (260 mg.); (48—52, 1 : 3; 53—69, chloroform–ethyl acetate, 5 : 1), intractable gum (336 mg.); (70—76, 4 : 1), semi-solid products yielding a methyl ester, m. p. *ca.* 150°; (77—79), intractable gum (211 mg.); (80—93), semi-solid products (545 mg.) giving a methyl ester, m. p. *ca.* 205—210°.

Fractions (17—27) crystallised from ethyl acetate–methanol–light petroleum in needles (129 mg.), m. p. 256—260° (decomp.), $[\alpha]_D^{23}$ +54° (*c* 1·12), of 2β,4*aα*-*dihydroxy*-1β,8-*dimethyl*-4*bξ*-*gibb*-8-*ene*-1α,10β-*dicarboxylic acid* 1 ⟶ 4*a*-*lactone* (VII; R = H) (Found: C, 68·5; H, 7·4. $C_{19}H_{24}O_5$ requires C, 68·65; H, 7·3%); $\nu_{max.}$ in $CHCl_3$ 1765 and ~1710 cm.$^{-1}$.

The methyl ester crystallised from ethyl acetate–light petroleum (b. p. 60—80°) in prisms, m. p. 148—152°, $[\alpha]_D^{25}$ +59° (*c* 0·70) (Found: C, 69·7, 69·2; H, 8·0, 7·7. $C_{20}H_{26}O_5$ requires C, 69·3; H, 7·6%); $\nu_{max.}$ in $CHCl_3$ 3620, ~3510, 1770, and 1730 cm.$^{-1}$.

Fractions (31—47) crystallised from ethyl acetate–light petroleum in needles and prisms (93 mg.), m. p. 251—255° (decomp.), $[\alpha]_D^{23}$ +39° (*c* 1·02), of a mixture probably consisting mainly of compound (VII; R = H) and dihydrogibberellin $A_4$.

The methyl ester crystallised from light petroleum (b. p. 60—80°) in prisms m. p. 149—152°, $[\alpha]_D^{25}$ +43° (*c* 0·63).

Fractions (80—93) crystallised from ethyl acetate or ethyl acetate–ether–light petroleum in prisms (317 mg.), m. p. 256—258° (decomp.), $[\alpha]_D^{20}$ +13° (*c* 0·71), of gibberellin $A_2$ (I; $R^1$ = H, $R^2$ = OH) identical (infrared spectra in Nujol mull and in $Me_2SO$) with material obtained by Grove's method [1] (see below). Gibberellin $A_2$ was also obtained in a new form which crystallised from ethyl methyl ketone–ether in needles, m. p. 232—234° (Found: C, 65·0; H, 7·6. Calc. for $C_{19}H_{26}O_6$: C, 65·1; H, 7·5%). The spectrum in Nujol mull ($\nu_{max.}$ 3490s, 3385sh, 1750s, and 1683s cm.$^{-1}$) was distinct from that of the form, m. p. 256—258°, and from that of the form, m. p. 235—237°, obtained by Takahashi *et al.*[8] Seeded crystallisation gave the normal form.

Gibberellin $A_2$ methyl ester crystallised from ethyl acetate–light petroleum in prisms m. p. 210—212° (dried *in vacuo* at 100°). The infrared spectrum in dioxan was identical with that of an authentic specimen of the anhydrous ester m. p. 212—214° (see below).

Fractions (70—76) crystallised from ethyl acetate or ethyl methyl ketone–light petroleum (b. p. 60—80°) in prisms (102 mg.), m. p. 255—257° (decomp.), $[\alpha]_D^{22}$ +5° (*c* 0·87), of an *isomer* of gibberellin $A_2$ (Found: C, 65·3; H, 7·6. $C_{19}H_{26}O_6$ requires C, 65·1; H, 7·5%); $\nu_{max.}$ ~3550sh, 3430, 2670, 1750, and 1732 cm.$^{-1}$; $\nu_{max.}$ in $Me_2SO$ ~3450, 3020, 1773, 1762, 1717, and 1710 cm.$^{-1}$.

The *methyl ester* crystallised from ethyl acetate–light petroleum in prisms, m. p. 160—161° (Found: on material dried at 100° for 3 hr. *in vacuo*: C, 62·5; H, 7·9. $C_{20}H_{28}O_6,H_2O$ requires C, 62·8; H, 7·9%); $\nu_{max.}$ 3590, 3565, 3495, 3360, 1751, and 1730 cm.$^{-1}$; $\nu_{max.}$ in $Me_2SO$ ~3460br, 1768, and 1734 cm.$^{-1}$, distinct from the spectrum of gibberellin $A_2$ methyl ester in $Me_2SO$.

*Gibberellin A*$_2$.—Gibberellin $A_4$ (200 mg.) was treated with cold methanolic hydrochloric acid as described by Grove [1] except that ten times the stated volume of methanol was needed to maintain a clear solution. The crude product was chromatographed on a column of Celite (50 g.) buffered with the phosphate buffer (pH 6·2; 50 ml.) and made up in chloroform–light petroleum (1 : 2). Elution of the column with 100-ml. portions of solvents gave fractions: (1—3) (chloroform–light petroleum, 1 : 2), giving 1 mg. on recovery; (4—6) (chloroform) giving 7 mg.; (7—16), an intractable mixed product, 36 mg.; (17—22) (chloroform–ethyl acetate, 5 : 1), no product. Further elution with chloroform–ethyl acetate (3 : 1) gave gibberellin $A_2$ (115 mg.) which formed prisms (84 mg.), m. p. 256—258° (decomp.), from ethyl methyl ketone–ethyl acetate–light petroleum (Found: C, 65·1; H, 7·5. Calc. for $C_{19}H_{26}O_6$: C, 65·1; H, 7·5%).

The infrared spectrum was identical with that of an authentic specimen.[1] Thin-layer chromatography of gibberellin $A_2$ prepared by this method revealed only traces of an impurity with an $R_F$ corresponding to that of the epimeric acid (above).

Gibberellin $A_2$ methyl ester crystallised from ethyl acetate–light petroleum in prisms, m. p. (after being dried at 100° *in vacuo*) 212—214° (Found: C, 65·9; H, 7·8. Calc. for $C_{20}H_{28}O_6$: C, 65·9; H, 7·7%); $\nu_{max.}$ 3490, 3350, 3160, 1760, 1745, and 1671 cm.$^{-1}$; $\nu_{max.}$ in dioxan 3580, 3505, 1776, 1736, and 1630 cm.$^{-1}$; the spectra were indistinguishable from those of authentic hydrated material of m. p. 190°; $\nu_{max.}$ in $Me_2CO$ ~3460, 1767, 1733, and 1660 cm.$^{-1}$.

*Isolation of the Acid* (VIII; $R^1 = R^2 = H$).—The acid fraction (19·98 g.) from a pH-adjusted fermentation (30 l.) of *Gibberella fujikuroi* 917 strain was chromatographed on Celite–charcoal (1 : 2, 81 × 5·3 cm.), and the column was eluted with increasing (stepwise) concentrations of acetone in water.[2] The gummy fraction eluted with 65% acetone in water was rechromatographed, first on silica–Celite (1 : 2; 43 × 3 cm.) in ethyl acetate–light petroleum (b. p. 60—80°) (3 : 17 ⟶ 1 : 4) and the recovered crystalline product (1·98 g.) was further chromatographed on silica gel (25 × 2·0 cm.). Elution of the column with ethyl acetate–light petroleum (1 : 3) gave gibberellin $A_7$ (468 mg.). Continued elution with a 1 : 1 mixture of the solvents yielded 2β,3α-*dihydroxy*-1β-*methyl*-8-*methylenegibb*-4-*ene*-1α,10β-*dicarboxylic acid* 1 ⟶ 3-*lactone* (VIII; $R^1 = R^2 = H$) which crystallised from acetone–light petroleum (b. p. 60—80°) in prisms (434 mg.), m. p. 186—190°, $[\alpha]_D^{20}$ +59° (*c* 0·9) (Found: C, 69·0; H, 6·7. $C_{19}H_{22}O_5$ requires C, 69·1; H, 6·7%); $\nu_{max.}$ 3460, 1774, and 1722 cm.$^{-1}$; $\nu_{max.}$ in $CHCl_3$ 1766 and 1704 cm.$^{-1}$. The acid gave a green colour with concentrated sulphuric acid; microhydrogenation (palladium–acetic acid), uptake 1·7 moles.

The *methyl ester*, prepared with diazomethane, crystallised from acetone–light petroleum (b. p. 60—80°) in prisms, m. p. 226—228°, $[\alpha]_D^{20}$ +78° (*c* 1·0) (Found: C, 69·7; H, 7·1. $C_{20}H_{24}O_5$ requires C, 69·75; H, 7·0%); $\nu_{max.}$ 3492, 1771, 1707, 1662, 1653, 868, and 826 cm.$^{-1}$. This ester could be separated from some crude preparations of gibberellin $A_7$ methyl ester by hand-picking of crystals.

*Alkaline Isomerisation of Gibberellin $A_7$ Methyl Ester.*—The ester (50 mg.) in methanol (5 ml.) was shaken with 0·05N-sodium hydroxide (10 ml.) for 3 hr. The solution was diluted with water, acidified, and extracted with ethyl acetate. The extract was washed with sodium hydrogen carbonate solution and with water, dried, and evaporated. The residual gum crystallised from acetone–light petroleum giving the above ester (VIII; $R^1$ = Me, $R^2 = H$) (35 mg.), m. p. 226—228°.

*Action of Hydrazine on Gibberellin $A_7$ Methyl Ester* (III; $R^1$ = Me, $R^2 = H$).—A solution of the ester (125 mg.) in ethanol (5 ml.) was treated with hydrazine hydrate (2 ml.) and a trace of aqueous cupric sulphate solution. The mixture was boiled for 2 hr., concentrated, diluted with water, and extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid and water, dried, and evaporated. The residue was chromatographed on silica gel (15 × 1·0 cm.). The fraction eluted with ethyl acetate–light petroleum (b. p. 60—80°) (2 : 3) yielded *methyl* 1α-*carboxy*-2β,3α-*dihydroxy*-1β-*methyl*-8-*methylenegibb*-4-*ene*-10β-*carboxylate* (IX) which crystallised from acetone–light petroleum (b. p. 60—80°) in needles (43 mg.), m. p. 121—122° (Found: C, 63·3; H, 7·8. $C_{20}H_{26}O_6,H_2O$ requires C, 63·1; H, 7·4%); $\nu_{max.}$ 3481, 3356, 3148, 1757, 1744, 1668, and 795 cm.$^{-1}$.

*Gibberellin $A_7$ Anhydride.*—Gibberellin $A_7$ (205 mg.) in warm dioxan (1·9 ml.) was treated with *NN'*-dicyclohexylcarbodi-imide (64 mg.), and the solution was allowed to cool. After 30 min. the precipitated *NN'*-dicyclohexylurea was filtered off and the filtrate was evaporated *in vacuo* giving a gum (248 mg.). Crystallisation of the gum from acetone and from acetone–light petroleum gave first the urea derivative, then prisms (24 mg.), m. p. 195—211°, showing no absorption band at *ca.* 1800 cm.$^{-1}$. The gummy residue, showing strong absorption at 1800 cm.$^{-1}$, did not crystallise, and consisted of *gibberellin $A_7$ anhydride* (Found, on material dried at 100° for 4 hr.: C, 65·6; H, 7·5. $C_{38}H_{42}O_9,3H_2O$ requires C, 65·5; H, 6·9%); $\nu_{max.}$ (film) 3480, 3400br, 1800, 1741—1736br, and 1703 cm.$^{-1}$.

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