22. *Gibberellic Acid. Part XXI.* The Stereochemistry of Rings* B, C, *and* D.

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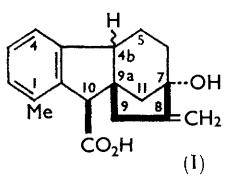
Evidence is presented that rings B, C, and D in gibberellic acid have the absolute configuration as in (III).

The absolute configuration of allo- (I; 4bα) and epiallo-gibberic acid (I; 4bβ), derived from gibberellic acid by aromatisation of ring A *via* the gibba-3,4a(4b)-diene, gibberellenic acid (II), has been determined.[1] The evidence now presented and in part briefly reported before,[2] shows that rings B/C/D of gibberellic acid (III; R = CH_2, R′ = H) have the same absolute configuration as they have in epiallogibberic acid.



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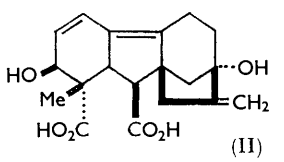

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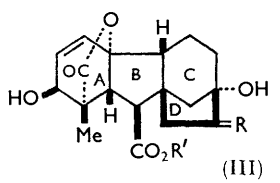

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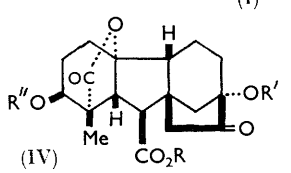

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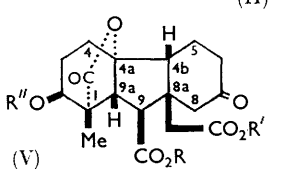

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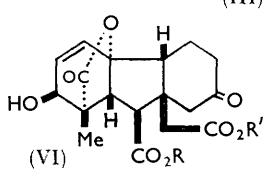


(VI)

The ozonolysis of gibberellin A_1 methyl ester is reported [3,4] to take the same course as the ozonolysis of methyl allogibberate [5] and methyl gibberellate,[6] giving (i) formaldehyde,[7] (ii) a ketol, $C_{19}H_{24}O_7$, double m. p. 135° and 170°, and (iii) a keto-acid, $C_{19}H_{24}O_8$, m. p.

* Part XX, preceding paper.

[1] Grove and Mulholland, *J.*, 1960, 3007.
[2] Cross, Grove, McCloskey, Mulholland, and Klyne, *Chem. and Ind.*, 1959, 1345.
[3] Seta, Kitamura, Takahashi, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 73.
[4] Seta, Takahashi, Kawarada, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 412.
[5] Mulholland, *J.*, 1958, 2693.
[6] Cross, *J.*, 1960, 3022.
[7] Grove, Jeffs, and Mulholland, *J.*, 1958, 1236.

98°. Our results on the ozonolysis of gibberellin A_1 methyl ester confirm those of the Japanese workers [3,4] but there are differences of detail. Thus, in our hands, the α-ketol $C_{19}H_{24}O_7$ (IV; R = Me, R′ = R″ = H), also obtained by catalytic hydrogenation of the ketol (III; R = O, R′ = Me) derived [6] from methyl gibberellate, was dimorphic, both forms having m. p. 229°, and it formed a hemihydrate, m. p. 199—201°. Contrary to the earlier reports,[3,4] the ketol (IV; R = Me, R′ = R″ = H) was oxidised by periodate to the keto-acid (V; R = Me, R′ = R″ = H), isolated as the hydrate, $C_{19}H_{24}O_8,H_2O$; m. p. 98°. The latter compound was also obtained by catalytic hydrogenation of the keto-acid (VI; R = Me, R′ = H) obtained [6] from methyl gibberellate.

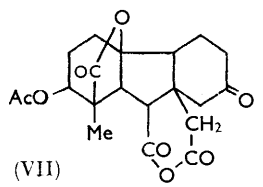
The 7-acetyl derivative (IV; R = Me, R′ = Ac, R″ = H), which gave the diacetate (IV; R = Me, R′ = R″ = Ac) on acetylation, was a minor product from the ozonolysis of gibberellin A_1 methyl ester in acetic acid at room temperature (cf. ref. 8).

Decomposition with water or hydrogen peroxide of the crude ozonide obtained from gibberellic acid with one mol. of ozone in ethyl acetate–acetic acid at −40° gave an intractable mixture, but when the ozonide was reduced with triphenylphosphine the ketol (III; R = O, R′ = H) was obtained in acceptable yield.

Hydrogenation of the last-mentioned ketol gave the dihydro-derivative (IV; R = R′ = R″ = H) which was converted into the keto-acid (V; R = R′ = R″ = H) with periodate. The latter yielded the dimethyl ester (V; R = R′ = Me, R″ = H) [3,4] with diazomethane, and an intramolecular 6-membered ring anhydride (VII) ($\nu_{max.}$ 1819, 1773 cm.$^{-1}$) [5] when heated with acetic anhydride. The anhydride (VII) was also obtained (*a*) by oxidation of the ketol (III; R = O, R′ = H) to the keto-acid (VI; R = R′ = H) [which yielded the dimethyl ester (VI; R = R′ = Me) [6]], followed by conversion of the keto-acid into the anhydride (VIII) and subsequent reduction, or (*b*) by reduction of the keto-acid (VI; R = R′ = H) to the keto-acid (V; R = R′ = R″ = H) before treatment with acetic anhydride.

With boiling methanol the anhydride (VII) yielded a mixture of the acids (V; R = Me, R′ = H, R″ = Ac) [previously obtained by acetylation of the keto-acid (V; R = Me, R′ = R″ = H)] and (V; R = H, R′ = Me, R″ = Ac), the former predominating. Hydrolysis of the anhydride with boiling water gave an acidic gum, which, with diazomethane, yielded the dimethyl ester (V; R = R′ = Me, R″ = Ac) also obtained by methylation of the acids (V; R = Me or H, R′ = H or Me, R″ = Ac) or by acetylation of the ester (V; R = R′ = Me, R″ = H).

This sequence of reactions, analogous to that obtained previously [1] with the dibasic acids (X; R = H) derived from allo- and epiallo-gibberic acid shows that intramolecular



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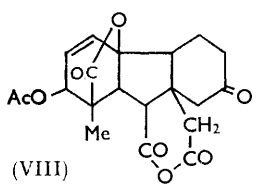

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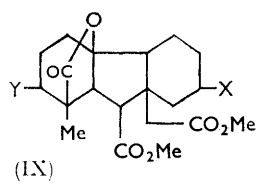

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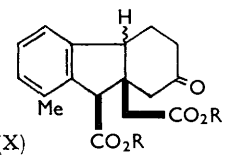

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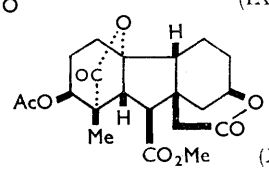


(XI)

anhydride formation in the acid (V; R = R′ = R″ = H) does not involve inversion of the 9-carboxyl substituent and hence that this substituent and the 8a-acetic acid side chain are *cis* (β) related.

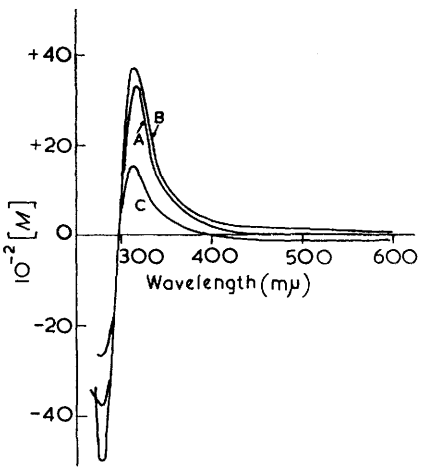
Reduction of the keto-ester (V; R = R′ = Me, R″ = H) *via* the thioketal gave the

[8] Cross, Grove, and Morrison, *J.*, 1961, 2498.

deoxo-compound (IX; X = H_2, Y = H,OH) which was oxidised by chromium trioxide to the 2-ketone (IX; X = H_2, Y = O). The deoxo-compound (IX; X = H_2, Y = H,OH) was not identical with the isomeric ester of the acid obtained [9] by Clemmensen reduction of the ester (V; R = R′ = Me, R″ = H). The only product of reduction of the acetyl derivative (V; R = Me, R′ = H, R″ = Ac) by sodium borohydride was the lactone (XI), also obtained [10] by hydrogenation over Adams catalyst in the presence of perchloric acid.

Ring D in the gibberellins, and hence the 8a-acetic acid side chain in the seco-acid (V), have been shown [11] to have the β-configuration. The 8aα-epimer of the ester (V; R = R′ = Me, R″ = H) was required for comparative studies, but attempted inversion of ring D in the ketol (IV; R = Me, R′ = R″ = H) with butyl-lithium [12] did not yield useful results.

Optical rotatory dispersion measurements on the deoxo-compound (IX; X = H_2, Y = H,OH) gave a very weak positive plain curve, unlike the steeply rising positive curve given [1] by the hydroxy-ester, alcohol C, obtained by reduction of the keto-ester (X; R = Me, 4bβ). The curve (see Figure) for the ester (V; R = R′ = Me, R″ = H)



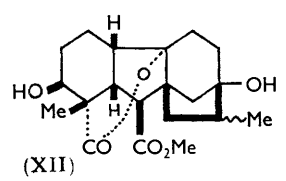
Optical rotatory dispersion curves for the ketones: (A) (V; R = R′ = Me, R″ = H); B (X; R = Me, 4bα); and (C) (X; R = Me, 4bβ); after subtraction of the curves for the corresponding dihydro- or deoxo-derivatives.

was similar in shape and amplitude ($10^{-2}a$, +70°) to that for the keto-ester (X; R = Me, 4bα), ($10^{-2}a$, +87°) derived from allogibberic acid, and unlike that for the B/C-*cis*-keto-ester (X; R = Me, 4bβ) which, after subtraction (Figure) of the plain curve for alcohol C, was of lower amplitude ($10^{-2}a$, +40°). On this basis the α-configuration was allocated to position 4b in the ester (V; R = R′ = Me, R″ = H) and in gibberellic acid.[2] Some doubt became attached to this assignment when (i) it was found that both methyl tetrahydrogibberellate and its 4b-epimer, obtained from methyl tetrahydrogibberellate by treatment with 2N-hydrochloric acid followed by chromatographic separation of the crude products, which included a δ-lactone of probable structure (XII), had almost identical weak positive plain curves, and (ii) the B/C-*cis*-fused ketone (XIII), which will be described in a later paper,[13] was found to have a Cotton effect curve with $10^{-2}a$ = +58°.

In addition, the most straightforward interpretation of the nuclear magnetic resonance data [14] for the 4b-epimeric 7α-gibbane keto-esters (XIV) [6] favours a 4bβ configuration in gibberellic acid. In methyl acetylgibberellate [15] the 4b-hydrogen resonance occurs at

[9] Seta, Takahashi, Kitamura, Takai, Tamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 499.
[10] Cross, Galt, and Hanson, *Tetrahedron*, 1962, **18**, 451.
[11] Grove, MacMillan, Mulholland, and Turner, *J.*, 1960, 3049.
[12] Djerassi, Quitt, Mosettig, Cambie, Rutledge, and Briggs, *J. Amer. Chem. Soc.*, 1961, **83**, 3721.
[13] Aldridge and Grove, in the press.
[14] Aldridge, Grove, Speake, Tidd, and Klyne, preceding paper.
[15] Sheppard, *J.*, 1960, 3040.

$\tau > 7{\cdot}7$ and some explanation, other than deshielding by the adjacent oxygen atom of the 1 ⟶ 4a-lactone bridge, must therefore be advanced for the low value, 7·2, of the chemical shift found for the 4b-hydrogen in keto-ester *A* (XIV), m. p. 226—228°, which has the same 4b-configuration as the gibberellins.[14] The shift downfield relative to the 4b-resonance at 7·61, in the epimer, keto-ester *B*, is significantly greater than the changes in the 10- and the 10a-hydrogen resonances. Molecular models of the 7α-gibbanes (XIV)



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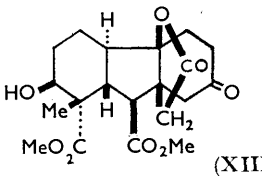

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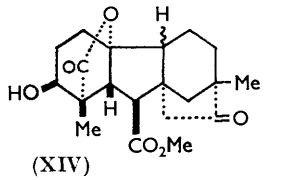


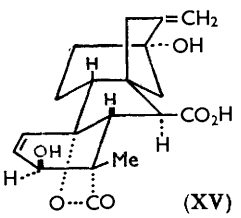
(XIV)

show that a 4bβ-hydrogen is deshielded by its proximity in the strained system to the 10-methoxy-carbonyl substituent which is steeply inclined to the plane of ring B. This deshielding does not occur with ring D in the β-configuration, as in gibberellic acid, for the 10-methoxy-carbonyl group is then more in the plane of ring B. On this basis, keto-ester *A* and the gibberellins have the 4bβ-configuration. This assignment is in accord (*a*) with the lower value (7 c./sec.: dihedral angle ~115°) of $J_{10,10a}$ for the keto-ester *A* than for the epimer (11 c./sec.) [where models show that the dihedral angle is greater (~150°)] and (*b*), by analogy with other 4b-epimers,[1,11] with the more positive $[M]_D$ value belonging to the keto-ester *A*.

Molecular models show that, although the 1,3-interaction between the 9-methylene group and the 4a-oxygen atom, prominent in the isomer (XIV; 4bβ), is largely relieved with 4bα, ring B is more strained with the latter configuration; and an equilibrium ratio [14] of 5 : 2 shows that no great difference in overall stability exists between the 4b-epimers of structure (XIV).

The 4bβ-configuration in gibberellic acid has now been established by *X*-ray crystallographic analysis.[16] That the analogy between the rotatory dispersion curves of the seco-esters (V; R = R′ = Me, R″ = H) and (X; R = Me, 4bα) is false must be due to the fact that the altered nature of ring A in (V; R = R′ = Me, R″ = H) has altered the conformation of ring B.

With sodium hydroxide, the seco-ester (V; R = R′ = Me, R″ = H), like the ester (X; R = Me, 4bβ), failed to undergo the internal Claisen condensation with inversion at position 8a which [1] remains peculiar to the ester (X; R = Me, 4bα). The asymmetric centre 9 in (V; R = R′ = Me, R″ = H) was stable to alkali, and models indicate that the more stable configuration for the 9-methoxycarbonyl substitute is β: but the 2-substituent was labile.[8] The crude product (after methylation) yielded the ester (V; R = R′ = Me, R″ = H) and, as the major component, its 2α(*eq*)-hydroxy-epimer. The epimers yielded the same diketone (IX; X = Y = O) on oxidation and had similar rotatory dispersion curves. On borohydride reduction, the behaviour of the ester (V; R = R′ = Me, R″ = H) in giving the lactone (XI) was more akin to that of the ester (X; R = Me, 4bα) than to that of the ester (X; R = Me, 4bβ), which gave only alcohols.[1]



(XV)

The allocation [11,14] to gibberellic acid of the 1*S*,2*S*,4a*R*,4b*R*,7*S*,9a*S*,10*S*,10a*R* absolute configuration (III; R = CH_2, R′ = H) with the *trans-anti-cis*-backbone (XV) present in the *Gibberella fujikuroi* metabolites related to (—)-kaurene [17] provides a satisfactory explanation for the ready formation of gibberellenic acid (II) by *trans*-elimination.[2]

[16] McCapra, Scott, Sim, and Young, *Proc. Chem. Soc.*, 1962, 185.
[17] Cross, Galt, Hanson, and Klyne, *Tetrahedron Letters*, 1962, 145.

In naming derivatives and degradation products according to the gibbane nomenclature [1] the configuration at positions 4b and 10a will be assumed to be the same (β) as in the natural product, unless stated otherwise. The compounds included in a recent review [18] are therefore correctly named; but the 4b-configurations in the structural formulæ for gibberellin relatives with a non-aromatic ring A are the inverse of those depicted.

Experimental

M. p.s are corrected. Woelm acid alumina Grade II and " Hyflo Super Cel " Celite were used in chromatography. Unless otherwise stated, infrared spectra were determined for Nujol mulls, and ultraviolet spectra and optical rotations in ethanol. Light petroleum had b. p. 60—80°. All identifications were confirmed by mixed m. p. determinations and comparison of the infrared spectra.

Ozonolysis of Gibberellin A_1 Methyl Ester.—(*a*) The non-steam-volatile product (302 mg.) (recovered in ethyl acetate) from the ozonolysis [7] of gibberellin A_1 methyl ester (286 mg.) was separated into neutral (180 mg.; A) and acidic (102 mg.; B) fractions by extraction with sodium hydrogen carbonate and recovery.

The neutral fraction (A) was crystallised three times from ethyl acetate, giving *methyl* 1α-*carboxy*-2β,4aα,7-*trihydroxy*-1β-*methyl*-8-*oxogibbane*-10β-*carboxylate* 1 ⟶ 4a-*lactone* (IV; R = Me, R′ = R″ = H) as needles (84 mg.), m. p. 226—229°, $\nu_{max.}$ 3560, 3490 (OH), 1760, 1711 cm.$^{-1}$ (C=O), or prisms, m. p. 229°, $\nu_{max.}$ 3460 (OH), 1770, 1720 cm.$^{-1}$ (C=O), $[\alpha]_D^{20}$ +50° (*c* 1·0) (Found: C, 62·5; H, 6·7; OMe, 9·0. $C_{19}H_{24}O_7$ requires C, 62·6; H, 6·6; OMe, 8·5%), $\nu_{max.}$ (both forms in $CHCl_3$) 1773 (γ-lactone), 1754 (8-ketone), 1738 cm.$^{-1}$ (ester), $\lambda_{max.}$ 294 mμ (log ε 1·64).

The *hemihydrate*, sometimes obtained after chromatography, on alumina, of the anhydrous ketol (IV; R = Me, R′ = R″ = H), formed needles, m. p. 199—201°, from ether or from ethyl acetate–light petroleum (Found: C, 60·7; H, 6·7. $C_{19}H_{24}O_7$,0·5H_2O requires C, 61·1; H, 6·75%), $\nu_{max.}$ 3440, 3330 (OH), 1745, 1700 (C=O), 1650 (H_2O) cm.$^{-1}$. The infrared spectra in chloroform and acetonitrile were identical with those of the anhydrous compound, which was obtained on drying of the hemihydrate at 100° *in vacuo*.

The ketol (IV; R = Me, R′ = R″ = H) reduced Tollens's reagent and gave a precipitate with Brady's reagent. It gave no colour with concentrated sulphuric acid.

The *diacetate* (IV; R = Me, R′ = R″ = Ac), prepared with acetic anhydride in pyridine, crystallised from ethyl acetate–light petroleum in needles, m. p. 155—157°, $\nu_{max.}$ 1772, 1739 cm.$^{-1}$, or m. p. 175°, $\nu_{max.}$ 1769, 1741 cm.$^{-1}$ (Found: C, 61·3, 62·0; H, 6·4, 6·3; Ac, 22·8. $C_{23}H_{28}O_9$ requires C, 61·6; H, 6·3; 2Ac, 19·2%). The infrared spectra of the two crystalline forms differed between 6 and 15 μ.

The acid fraction (B) crystallised from ethyl acetate–light petroleum in prisms (20 mg.), m. p. 98°, of 1α-*carboxy-perhydro*-2β,4aα-*dihydroxy*-9β-*methoxycarbonyl*-1β-*methyl*-7-*oxo*-4bβ-*fluorenyl*-8aβ-*acetic acid* 1 ⟶ 4a-*lactone hydrate* (V; R = Me, R′ = R″ = H) (Found: C, 57·3; H, 6·7%; equiv., 402. $C_{19}H_{24}O_8$,H_2O requires C, 57·3; H, 6·6%; *M*, 398), $\nu_{max.}$ 3365, 3295 (OH), 1769, 1726br cm.$^{-1}$ (C=O). The m. p. was unchanged by drying *in vacuo* at 80°.

The *acetyl derivative* (V; R = Me, R′ = H, R″ = Ac), m. p. 134—135°, prepared in acetic anhydride–pyridine, crystallised from ether. The product was usually the *hydrate*, m. p. 100—103° (decomp.), $\nu_{max.}$ 3600—2500br (OH), 1770, 1742, 1725, 1695br (C=O, H_2O) (Found: C, 57·5; H, 6·3. $C_{21}H_{26}O_9$,H_2O requires C, 57·3; H, 6·4%).

The *methyl ester* (V; R = R′ = Me, R″ = H), prepared with diazomethane, crystallised from ethyl acetate in needles, m. p. 166—168°, $[\alpha]_D^{21}$ +51° (*c* 1·04) (Found: C, 60·4; H, 6·8; OMe, 16·5. Calc. for $C_{20}H_{26}O_8$: C, 60·9; H, 6·6; 2OMe, 15·7%), $\nu_{max.}$ 3470, 3280 (OH), 1767, 1735, 1717sh, 1689 cm.$^{-1}$ (C=O), in MeCN, 1772, 1739 cm.$^{-1}$.

The acetyl derivative (V; R = R′ = Me, R″ = Ac) of the ester, obtained by boiling the alcohol with acetic anhydride or by methylation of the derivative (V; R = Me, R′ = H, R″ = Ac), formed needles, m. p. 196—198° [from ethyl acetate–light petroleum (b. p. 40—60°)], identical with material of m. p. 193—195° obtained [10] by the pyridine method and having $\nu_{max.}$ 1770, 1738, 1710, (in $CHBr_3$) 1775, 1736, 1720sh, 1704sh cm.$^{-1}$.

[18] Brian, Grove, and MacMillan, *Fortschr. Chem. org. Naturstoffe*, 1960, **18**, 350.

Seta *et al.*[3,4] give m. p. 98° for the anhydrous acid (V; R = Me, R′ = R″ = H) and m. p. 169—170°, $[\alpha]_D^{25}$ +48°, for its methyl ester.

(*b*) In one experiment the neutral fraction (292 mg.) from the ozonolysis of gibberellin A_1 methyl ester (624 mg.) was chromatographed on alumina (20 × 2 cm.) in benzene (120 ml.). Elution with benzene–methanol (200 : 1; 700 ml.) gave a gum (103 mg.) which was washed with ether and then crystallised from methanol in needles (69 mg.), m. p. 247—249°, $[\alpha]_D^{25}$ +32° (*c* 1·06), of the *ketol 7-acetate* (IV; R = Me, R′ = Ac, R″ = H) (Found: C, 62·3; H, 6·2; OMe, 8·0. $C_{21}H_{26}O_8$ requires C, 62·1; H, 6·45; OMe, 7·6%), $\nu_{max.}$ 3490 (OH), 1753, 1744 cm.$^{-1}$ (C=O), in $CHCl_3$ 1764, 1743 cm.$^{-1}$, $\lambda_{max.}$ 289 mμ (log ε 1·70). This was not oxidised by sodium periodate in aqueous methanol. Acetylation with acetic anhydride in pyridine gave the diacetyl derivative (IV; R = Me, R′ = R″ = Ac), m. p. 175°.

Further elution of the column with benzene–methanol (50 : 1, 500 ml.) gave the ketol (IV; R = Me, R′ = R″ = H) (23 mg.) and its hemihydrate (20 mg.).

Oxidation of the Ester (V; R = R′ = Me, R″ = H).—The ester (60 mg.) in acetone (2 ml.) was set aside for 1 hr. at 0° with the chromic oxide reagent [19] (0·15 ml.). The mixture was then diluted with water and extracted with ethyl acetate. The organic layer was washed with water, followed by sodium hydrogen carbonate, and the recovered neutral product (60 mg.) was distilled at 130—140° (bath temp.)/10^{-4} mm., giving *methyl* 1α-*carboxy-perhydro*-4aα-*hydroxy*-9β-*methoxycarbonyl*-1β-*methyl*-2,7-*dioxo*-4bβ-*fluorenyl*-8aβ-*acetate* 1 ⟶ 4a-*lactone* (IX; X = Y = O) as an oil (Found: C, 61·3; H, 6·4. $C_{20}H_{24}O_8$ requires C, 61·2; H, 6·2%), $\nu_{max.}$ (in $CHCl_3$) 1781, 1749, 1732 cm.$^{-1}$ (C=O).

Oxidation of the Ketol (IV; R = Me, R′ = R″ = H).—The ketol (10 mg.) in methanol (2 ml.) and water (1 ml.) containing sodium metaperiodate (15 mg.) was set aside at room temperature for 22 hr. After removal of the methanol *in vacuo* the residual aqueous solution was diluted and extracted with ethyl acetate. The extract was separated by extraction with sodium hydrogen carbonate and recovery into starting material (1 mg.) and an acidic fraction (5 mg.). The latter crystallised from ethyl acetate–light petroleum in prisms (4 mg.) of the hydrate, m. p. 96—98°, of the acid (V; R = Me, R′ = R″ = H).

Reduction of the Ketol (III; R = O, R′ = Me).—The ketol [6] (362 mg.) in ethyl acetate (100 ml.) was hydrogenated in the presence of 10% palladium–charcoal (360 mg.). After the uptake of 1·1 mol., the catalyst was filtered off and the product was separated into acid (70 mg.) and neutral (286 mg.) fractions by extraction with sodium hydrogen carbonate and recovery. The neutral fraction crystallised from ethyl acetate–light petroleum in prisms (215 mg.), m. p. 224—228°, of the ketol (IV; R = Me, R′ = R″ = H).

Attempted Reaction of the Ketol (IV; R = Me, R′ = R″ = H) *with Butyl-lithium*.—The ketol (496 mg.) in tetrahydrofuran (100 ml.) was refluxed under nitrogen with an 8% solution of butyl-lithium in hexane (12 ml.) for 3·5 hr. The neutral part of the product was oxidised with an excess of aqueous-methanolic sodium periodate but the only pure product isolated (after methylation) was the keto-ester (V; R = R′ = Me, R″ = H) as needles (5 mg.), m. p. 166—169°, $[\alpha]_D^{21}$ +51° (*c* 0·41).

Reduction of the Keto-acid (VI; R = Me, R′ = H).—The keto-acid [6] (2·62 g.) in ethyl acetate (100 ml.) was hydrogenated (uptake, 0·95 mol.) in the presence of 25% palladium–charcoal (400 mg.) previously reduced with hydrogen. After removal of the catalyst by filtration, the filtrate was concentrated and separated into acidic (2·55 g.) and neutral (0·03 g.) fractions by extraction with sodium hydrogen carbonate and recovery.

The acid fraction in chloroform–ethyl acetate (20 : 1) was chromatographed on a silica–Celite (1 : 2) column (37 × 4 cm.) by the fractional elution technique. After an intractable gum (53 mg.) had been eluted with chloroform–ethyl acetate (5 : 1; 1300 ml.), chloroform–ethyl acetate (10 : 3; 900 ml.) furnished a gum (1·28 g.) which crystallised from ethyl acetate–light petroleum in prisms (0·85 g.), m. p. 96—98°, of the hydrate of the keto-acid (V; R = Me, R′ = R″ = H). Further elution of the column with chloroform–ethyl acetate (5 : 2) and ethyl acetate gave intractable gums.

Ozonolysis of Gibberellic Acid (by Dr. R. N. Speake).—Gibberellic acid (1·0 g.) in ethyl acetate (200 ml.) at −40° was treated with ozonised oxygen (5·5 mg. of O_3/min.) for 25 min. Triphenylphosphine (900 mg.) was added and the solution was set aside at 0° for 18 hr. After removal of the solvent under reduced pressure, the residue was chromatographed on silica

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

(35 × 3·5 cm.). Triphenylphosphine oxide was first eluted with benzene–ethyl acetate (3 : 1) containing acetic acid (0·5%). Fractional elution with ether then gave (i) gibberellic acid (160 mg.), followed by (ii) prisms (448 mg.), m. p. 218—225° (decomp.), setting and remelting at 265—280°, of 2β,4aα,7-*trihydroxy*-1β-*methyl*-8-*oxogibb*-3-*ene*-1α,10β-*dicarboxylic acid* 1 ⟶ 4a-*lactone* (III; R = O, R′ = H) (Found: C, 62·1; H, 5·75. $C_{18}H_{20}O_7$ requires C, 62·1; H, 5·8%), $\nu_{max.}$ 3480, 3370, 3165br (OH); 1749, 1734 cm.$^{-1}$ (C=O). Methylation gave the ester (III; R = O, R′ = Me),[6] m. p. 230—232°.

Decomposition of the ozonide with water or with hydrogen peroxide gave intractable mixtures.

Oxidation of the Ketol (III; R = O, R′ = H).—The ketol (231 mg.) in methanol (18 ml.) and 0·1M-sodium metaperiodate (7·5 ml.) was set aside at room temperature for 20 hr. The precipitate was filtered off and washed with methanol, and the combined filtrate and washings were concentrated *in vacuo*. The residual aqueous solution was extracted with ethyl acetate, yielding, on recovery, a foam (233 mg.) which was crystallised from ethyl acetate–light petroleum (b. p. 40—60°) and then from ethyl acetate, giving prisms (212 mg.) of 1α,10β-*dicarboxy*-1,2,4a,4bβ,5,6,7,8,8a,9a-*decahydro*-2β,4aα-*dihydroxy*-1β-*methyl*-7-*oxofluoren*-8aβ-*ylacetic acid* 1 ⟶ 4a-*lactone hemihydrate* (VI; R = R′ = H), m. p. 195—199° (decomp.), $[\alpha]_D^{24}$ +64° (*c* 0·9) [Found: C, 58·3; H, 5·6%; equiv., 182. $C_{18}H_{20}O_8,0{\cdot}5H_2O$ requires C, 57·9; H, 5·6%; equiv. (dibasic), 187], $\nu_{max.}$ (in dioxan), 3408 (OH), 2760, 2708, 2589 (carboxylic acid OH), 1776, 1730, 1719sh (C = O), 1637 (C=C, H_2O).

The methyl ester, prepared with diazomethane, formed needles, m. p. 171—173°, of the ester (VI; R = R′ = Me).[6]

Reduction of the Keto-acid (VI; R = R′ = H).—The keto-acid (250 mg.) in ethyl acetate (20 ml.) was hydrogenated (uptake, 1·03 mol.) in the presence of 10% palladium–charcoal (40 mg.). Concentration of the filtered solution and recrystallisation of the solid product from ethyl acetate furnished 1α,10β-*dicarboxyperhydro*-2β,4aα-*dihydroxy*-1β-*methyl*-7-*oxo*-4bβ-*fluoren*-8aβ-*ylacetic acid* 1 ⟶ 4a-*lactone* (V; R = R′ = R″ = H), prisms (200 mg.), m. p. 247—253° (decomp.), $[\alpha]_D^{21}$ +10° (*c* 0·83) (Found: C, 58·9; H, 6·1. $C_{18}H_{22}O_8$ requires C, 59·0; H, 6·05%), $\nu_{max.}$ (in dioxan) 3565sh, 3478 (OH), 2609 (carboxylic acid-OH); 1776, 1732, 1716 cm.$^{-1}$ (C=O).

Methylation with diazomethane gave needles, m. p. 165—167°, of the ester (V; R = R′ = Me, R″ = H).

Reduction of the Ketol (III; R = O, R′ = H).—The ketol (70 mg.) in acetone (23 ml.) was shaken with previously reduced 10% palladium–carbon (28 mg.) in hydrogen at room temperature until absorption ceased (12 min.). The recovered gum crystallised from ethyl acetate–light petroleum (b. p. 40—60°), giving 2β,4aα,7-*trihydroxy*-1β-*methyl*-8-*oxogibbane*-1α.10ß-*dicarboxylic acid* 1 ⟶ 4a *lactone hemihydrate* (IV; R = R′ = R″ = H) as prisms (50 mg.), m. p. 221—227° (decomp.) (Found: C, 59·8; H, 6·45. $C_{18}H_{22}O_7,0{\cdot}5H_2O$ requires C, 60·2; H, 6·4%), $\nu_{max.}$ (in dioxan) 3585, 3505 (OH), 1778, 1754, 1734 cm.$^{-1}$ (C=O).

Methylation gave the ester (IV; R = Me, R′ = R″ = H), m. p. 225—231°.

Oxidation of the Ketol (IV; R = R′ = R″ = H).—The ketol (250 mg.) in methanol (25 ml.) was kept with 0·02M-sodium periodate (43 mg.) for 20 hr. at room temperature. Recovery of the product in ethyl acetate gave a gum (251 mg.) which crystallised from ethyl acetate in prisms (220 mg.) of the keto-acid (V; R = R′ = R″ = H), m. p. 233—240°, raised to 245—250° (decomp.) by recrystallisation.

Anhydrides of the Keto-acids (V; R = R′ = R″ = H *and* VI; R = R′ = H).—The keto-acid (100 mg.) was heated under reflux for 1 hr. with acetic anhydride (1·2 ml.). After removal of volatile material by distillation under reduced pressure, the residual brown gum was crystallised from ethyl methyl ketone.

The acid (V; R = R′ = R″ = H) furnished the *anhydride* (VII), needles or prisms (90 mg.), m. p. 280—284° (decomp.) (Found: C, 61·4; H, 5·7. $C_{20}H_{22}O_8$ requires C, 61·5; H, 5·7%), $\nu_{max.}$ (in dioxan) OH absent, 1819, 1789, 1773, 1747, 1724 cm.$^{-1}$ (C=O).

The acid (VI; R = R′ = H) furnished the *anhydride* (VIII) prisms (76 mg.), m. p. 247—256° (decomp.) (Found: C, 61·9; H, 5·3. $C_{20}H_{20}O_8$ requires C, 61·85; H, 5·2%), $\nu_{max.}$ (in dioxan) OH absent, 1820, 1789, 1774, 1749, 1715 cm.$^{-1}$ (C=O). Hydrogenation of the anhydride (VIII) in ethyl acetate in the presence of palladium–charcoal gave the anhydride (VII).

Methanolysis of the Anhydride (VII).—The anhydride (150 mg.) was boiled with methanol (2·1 ml.) for 5 hr. and the solution was evaporated *in vacuo*. The gummy product was dissolved

in ethyl acetate and the solution was extracted with sodium hydrogen carbonate solution. Acidification of the extract and recovery of the product in ethyl acetate gave an acidic gum which crystallised from ethyl methyl ketone–light petroleum, giving (i) the hydrate of the acid (V; R = Me, R′ = H, R″ = Ac) as needles (87 mg.), m. p. 96—110°, and (ii) needles (30 mg.), m. p. 160—178°. Fraction (ii) was adsorbed on Celite in acetone, and the dried Celite was placed on a column of Celite (10 g.) buffered with 2M-phosphate (pH 6·2; 10 ml.) and suspended in light petroleum (b. p. 40—60°)–chloroform (10 : 1). The column was eluted with mixtures of the same solvents, giving the following results: (*a*) 10 : 1 (300 ml.), 5 : 1 (300 ml.), 3 : 1 (500 ml.), 2 : 1 (50 ml.) gave yellow gums on recovery; (*b*) 2 : 1 (150 ml.) gave the hydrate of the acid (V; R = Me, R′ = H, R″ = Ac) (5 mg.), m. p. 99—102°; (*c*) 2 : 1 (150 ml.) gave a solid (20 mg.) which crystallised from ethyl methyl ketone in needles (12 mg.), m. p. 168—169° or 183—184° (dimorphic), of *methyl* 2β-*acetoxy*-1α,9β-*dicarboxyperhydro*-4aα-*hydroxy*-1β-*methyl*-7-*oxo*-4bβ-*fluoren*-8aβ-*ylacetate* (V; R = H, R′ = Me, R″ = Ac) (Found: C, 59·5; H, 6·4. $C_{21}H_{26}O_9$ requires C, 59·7; H, 6·2%), $\nu_{max.}$ (m. p. 168—169°) 3520, 1770, 1747, 1721, 1690 cm.$^{-1}$; (m. p. 183—184°) 3530, 1767, 1740, 1713 cm.$^{-1}$, in $CHBr_3$ (two forms identical) 3455, 1776, 1739, 1731, 1712 cm.$^{-1}$. Methylation with diazomethane gave the ester (V; R = R′ = Me, R″ = Ac), m. p. 187—190°.

Hydrolysis of the Anhydride (VII).—The anhydride (4·8 mg.) was boiled with water (1 ml.) for 2 hr. and the solution was evaporated *in vacuo*, giving an intractable acidic glass (4·3 mg.). Methylation with diazomethane gave a solid, m. p. 190—195°, which was chromatographed in benzene on alumina (10 × 0·5 cm.). Elution with benzene–methanol (100 : 1) and crystallisation of the product from ethyl acetate–light petroleum (b. p. 40—60°) gave needles (3 mg.), m. p. 196—198°, of the acetate (V; R = R′ = Me, R″ = Ac).

Reduction of the Keto-ester (V; R = R′ = Me, R″ = H).—The keto-ester (304 mg.) in chloroform (5 ml.) was treated at room temperature with boron trifluoride–ether complex (0·28 ml.), followed by ethanedithiol (0·28 ml.), and the mixture was set aside for 40 hr. The mixture was diluted with chloroform, washed with water and with saturated sodium chloride solution, and evaporated, giving a gum which was chromatographed on alumina (20 × 1·8 cm.) in 1 : 1 benzene–light petroleum (b. p. 40—60°). After the excess of ethanedithiol had been eluted with the same solvent, benzene–methanol (200 : 3) eluted the oily thioketal (324 mg.). This was heated at 100° with Raney nickel (7 g.) in dioxan (40 ml.) for 10 hr. with occasional stirring. Recovery gave a gum (249 mg.) which was chromatographed on alumina (27 × 1·8 cm.) in benzene (5 ml.). After intractable material (2 mg.) had been eluted with benzene (200 ml.) and benzene–methanol (400 : 1, 400 ml.; and 200 : 1, 250 ml.), benzene–methanol (200 : 1; 350 ml.) furnished a gum (217 mg.) which crystallised from ethyl acetate–light petroleum (b. p. 40—60°) in prisms (163 mg.) of *methyl* 1α-*carboxyperhydro*-2β,4aα-*dihydroxy*-9β-*methoxycarbonyl*-1β-*methyl*-4bβ-*fluoren*-8aβ-*ylacetate* 1 ⟶ 4a-*lactone* (IX; X = H_2, Y = H, OH), m. p. 119—120°, $[\alpha]_D^{19}$ +9° (*c* 1·0 in acetone) (Found: C, 62·9; H, 7·4. $C_{20}H_{28}O_7$ requires C, 63·1; H, 7·4%), $\nu_{max.}$ 3490 (OH), 1760sh, 1752—1723br cm.$^{-1}$ (C=O). The m. p. was depressed on admixture with the isomeric ester (m. p. 154—156°) obtained [9] by Clemmensen reduction of the keto-ester (V; R = R′ = Me, R″ = H) followed by methylation of the product.

Oxidation of the Ester (IX; X = H_2, Y = H,OH).—The ester (50 mg.) in acetone (5 ml.) at 20° was treated with the chromic oxide reagent [19] (0·07 ml.). After 2 hr., dilution with water and recovery of the product in ethyl acetate gave a gum which was chromatographed in benzene on alumina (7 × 1·3 cm.). Elution with benzene–methanol (300 : 1; 40 ml.) furnished oily *methyl* 1α-*carboxyperhydro*-4aα-*hydroxy*-9β-*methoxycarbonyl*-1β-*methyl*-2-*oxo*-4bβ-*fluoren*-8aβ-*ylacetate* 1 ⟶ 4a-*lactone* (IX; X = H_2, Y = O) (Found: C, 63·6; H, 7·05. $C_{20}H_{26}O_7$ requires C, 63·5; H, 6·9%), $\nu_{max.}$ (film) OH absent, 1777, 1743sh, 1737br cm.$^{-1}$ (C=O).

Reduction of the Acetyl Derivative (V; R = Me, R′ = H, R″ = Ac).—Sodium borohydride (50 mg.) in metnanol (1 ml.) was added to the acetyl derivative (50 mg.) in methanol (2 ml.) at 0°. After 1 hr. the excess of borohydride was decomposed by dropwise addition of acetic acid. The solvent was removed under reduced pressure and, after the addition of water, the residue was extracted with ether. Crystallisation of the product from ethyl acetate–light petroleum gave the lactone (XI), needles (21 mg.), m. p. 194—196° (Found: C, 61·8; H, 6·7. Calc. for $C_{21}H_{26}O_8$: C, 62·1; H, 6·45%). Cross *et al.*[10] give m. p. 193—194°.

Action of Alkali on the Ester (V; R = R′ = Me, R″ = H).—The ester (304 mg.) was boiled with 2N-sodium hydroxide (50 ml.) for 2 hr. The ether-washed solution was acidified with

concentrated hydrochloric acid at 0° and extracted with ethyl acetate. The gummy product (278 mg.) recovered from the extract was boiled with ethyl acetate (30 ml.) containing concentrated hydrochloric acid (0·003 ml.) for 1·5 hr. The gum recovered by evaporation of the solvent was methylated with diazomethane, and the product (280 mg.) was chromatographed on alumina (28 × 1·8 cm.). The following fractions were eluted: (i) benzene (100 ml.), 25 mg.; (ii) benzene–methanol (100 : 1; 200 ml.) 13 mg.; (iii) benzene–methanol (100 : 1; 200 ml.) 55 mg.; (iv) benzene–methanol (100 : 1; 250 ml.) 12 mg.; and (v) benzene–methanol (100 : 3; 400 ml.) 139 mg. Fractions (i), (ii), and (iv) were intractable gums. Fraction (iii) crystallised from ethyl acetate–light petroleum in needles (42 mg.), m. p. 165—167°, of the ester (V; R = R′ = Me, R″ = H). Fraction (v) crystallised from ethyl acetate–light petroleum (b. p. 40—60°; charcoal) in needles (83 mg.), m. p. 158—159°, $[\alpha]_D^{20}$ +48° (*c* 0·54), of the 2α-*hydroxy-epimer* of the ester (V; R = R′ = Me, R″ = H) (Found: C, 60·5; H, 6·7; OMe, 14·5, 17·9. $C_{20}H_{26}O_8$ requires C, 60·9; H, 6·6; 2OMe, 15·7%), $\nu_{max.}$ 3480 (OH), 1769, 1722 cm.$^{-1}$ (C=O), (in dioxan) 1772, 1729, 1713 cm.$^{-1}$. The m. p. was depressed on admixture with the ester (V; R = R′ = Me, R″ = H).

Oxidation of the ester, m. p. 158—159° (50 mg.), in acetone (5 ml.), with the chromic oxide reagent (0·07 ml.) at room temperature during 2 hr., gave, on recovery, the same oily ketone (IX; X = Y = O) as obtained (above) by oxidation of the ester (V; R = R′ = Me, R″ = H).

Action of Acid on Methyl Tetrahydrogibberellate (with P. J. Keay).—Methyl tetrahydrogibberellate [6] (100 mg.) in 2N-hydrochloric acid (26 ml.) was heated under reflux for 19 hr. The cooled solution was extracted with ethyl acetate (6 × 25 ml.), and the gummy product (96 mg.), on recovery, was methylated with diazomethane. The crude ester in benzene (100 ml.) was chromatographed on alumina (14 × 1·2 cm.). After a series of gummy fractions (15 mg.) had been eluted with 400 : 1 benzene–methanol (300 ml.), 200 : 1 benzene–methanol (200 ml.) eluted a solid (45 mg.), m. p. 130—210°. Fractional crystallisation from ethyl acetate afforded the relatively less soluble methyl tetrahydrogibberellate, m. p. 258—261° (25 mg.), and the 4bα-*epimer*, rosettes of needles, double m. p. 130—135° and 160—162° (12 mg.) (from ethyl acetate–light petroleum) (Found: C, 65·4; H, 8·0. $C_{20}H_{28}O_6$ requires C, 65·9; H, 7·7%). $[\alpha]_D^{25}$ +34° (*c* 0·25), $\nu_{max.}$ (in $CHBr_3$) 1785, 1735 cm.$^{-1}$ (C=O).

Further elution of the column with benzene–methanol (100 : 1; 100 ml.) gave a gum (8 mg.) which crystallised from ethyl acetate–light petroleum in prisms (2 mg.), m. p. 225—233°, of a δ-lactone (Found: C, 65·3; H, 8·0. $C_{20}H_{28}O_6$ requires C, 65·9; H, 7·7%), $\nu_{max.}$ 3403, 3355 (OH), 1741, 1719 cm.$^{-1}$ (C=O), (in $CHCl_3$) 1732, 1722 cm.$^{-1}$.

Rotatory Dispersion Curves.—Values for [*M*] in methanol were as follows. The keto-ester (V; R = R′ = Me, R″ = H): positive Cotton effect curve (600 mμ) +150°; (312·5, peak) +3700°; (272·5, trough) −3000°; (265) −2750°. The keto-acid (V; R = Me, R′ = R″ = H): positive Cotton effect curve (600 mμ) +100°; (310, peak) +2600°; (267·5, trough) −2300°; (260) −2100°. The ester (IX; X = H_2, Y = H,OH): plain curve (500 mμ) +100°; (290) +100°. 2-(*eq*)-Hydroxy-epimer of (V; R = R′ = Me, R″ = H): positive Cotton effect curve (600 mμ) +400°; (312·5, peak) +4000°; (267·5, trough) −2900°. The keto-ester (VI; R = R′ = Me): positive Cotton effect curve (600 mμ) +300°; (312·5, peak) +3300°; (290) −3500°. Methyl tetrahydrogibberellate: plain positive curve (600 mμ) +50°; (400) +100°; (290) +550°. 4bα-Epimer of methyl tetrahydrogibberellate: plain positive curve (600 mμ) +20°; (400) +100°; (300) +400°.

We are indebted to Miss Jane Jackson and to Messrs D. C. Aldridge, D. Gardner, and A. Morrison for technical assistance, and to Professor J. M. Robertson for a copy of a manuscript [16] before its publication.

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