2. *Gibberellic Acid. Part XIX.* The Degradation of Gibberellic Acid in Sulphuric Acid.*

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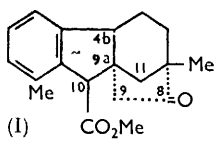
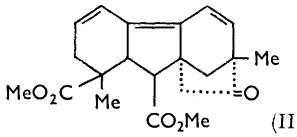
The compound believed to be responsible for the fluorescence of gibberellic acid in strong mineral acids has been isolated. In sulphuric acid the gibbane skeleton of gibberellic acid is shown to undergo rearrangement to the system (7α-isogibbane †) present in formula (VIII).

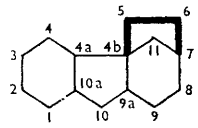
GIBBERELLIC ACID [1] (IV), in cold concentrated sulphuric acid, gives an intense wine-red colour with a strong blue fluorescence,[2] and the fluorescence produced by certain gibberellins (in particular gibberellic acid) in strong mineral acids under specified conditions is made the basis of detecting and assaying these gibberellins.[3] The chemistry underlying this reaction is described below.



(I)



(II)



(III)

When a suspension of gibberellic acid in methanol is treated with sulphuric acid at room temperature for a few minutes and the crude product is methylated with diazomethane and chromatographed on alumina, several compounds are obtained, including small quantities of methyl gibberate [4] (I; 4bα-H), methyl epigibberate [4] (I; 4bβ-H) and methyl 1,7-dimethyl-8-oxo-7α-gibba-3,4a(4b),5-triene-1,10-dicarboxylate [5] (II). The low yield of aromatic compounds contrasts with the effect of aqueous mineral acids on gibberellic acid.[2] A pale yellow compound, $C_{21}H_{24}O_5$, m. p. 173° was also obtained in 8% yield. It showed the fluorescence spectrum characteristic of gibberellic acid in alcoholic sulphuric acid (activation maxima at 280 and 418 mμ and emission maximum at 463 mμ), from which it could be recovered in high yield by dilution with water and extraction with ethyl acetate. The bulk of the product from the reaction of gibberellic acid with sulphuric acid was an intractable yellow gum from which a small quantity of amorphous yellow solid, m. p. 165—195°, was isolated. This material gave a deep magenta colour with sulphuric acid and may be responsible for the deep wine-red colour of gibberellic acid in strong acid. It was not investigated.

The compound, $C_{21}H_{24}O_5$, m. p. 173° was found to possess two methoxycarbonyl groups, alkaline hydrolysis of which gave a dicarboxylic acid, $C_{19}H_{20}O_5$, m. p. 152—154° (decomp.), which regenerated the dimethyl ester, $C_{21}H_{24}O_5$, on methylation. The fifth oxygen atom is present in a conjugated ketone system ($\lambda_{max.}$ 255 and 342 mμ). Microhydrogenation of the dimethyl ester resulted in the uptake of 3·8 mol. of gas, and reduction with sodium borohydride yielded a wax ($\lambda_{max.}$ 292 mμ). These results are consistent with the presence of a conjugated trienone system in the fluorogen, $C_{21}H_{24}O_5$, which must therefore be tetracyclic.

* Part XVIII, *J.*, 1961, 2498.

† In this and subsequent publications the name isogibbane is used for the fully saturated, tetracyclic system (III). The numbering follows that of the gibbane [6] skeleton, from which it is derived by breaking the 9a,11-bond and forming a 4b,11-bond. 7α-Isogibbane contains a 5,6-α-bridge.

[1] Cross, Grove, MacMillan, Moffatt, Mulholland, Seaton, and Sheppard, *Proc. Chem. Soc.*, 1959, 302.

[2] Cross, *J.*, 1954, 4670.

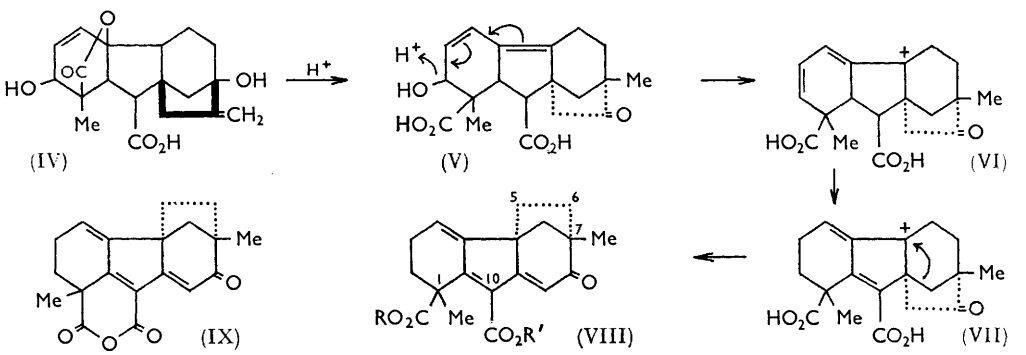
[3] Kavanagh and Kuzel, *J. Agric. Food Chem.*, 1958, **6**, 459; Theriault, Friedland, Peterson, and Sylvester, *ibid.*, 1961, **9**, 21; Elson, unpublished work.

[4] Grove, MacMillan, Mulholland, and Turner, *J.*, 1960, 3049.

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

[6] Grove and Mulholland, *J.*, 1960, 3007.

The formation of such a structure from gibberellic acid (IV) can be rationalised as (IV) ⟶ (VIII). The production of an intermediate of structure (V) is consistent with the known reactions of gibberellic acid in acid.[7,8] Under strongly acidic conditions, a compound (V) might be expected to lead to such species as (VI) and (VII), the formation of the latter being facilitated by conjugation with the carboxyl group. The conjugated trienone (VIII) can then be derived from (VII) by a Wagner–Meerwein rearrangement, the driving force for the reaction being the development of a fully conjugated system. The stereochemistry of the product is defined by the mechanism of the rearrangement, the two-carbon bridge being necessarily α. That structure (VIII; R = R′ = Me) correctly represents the constitution of the compound, m. p. 173°, obtained from gibberellic acid, is shown by the evidence below.



Treatment of methyl gibberellate with sulphuric acid led to a monomethyl ester (VIII; R = H, R′ = Me), $C_{20}H_{22}O_5$,0·5H_2O, m. p. 110—130°, which afforded the dimethyl ester (VIII; R = R′ = Me) and the dicarboxylic acid (VIII; R = R′ = H). The ultraviolet absorption spectra of the three compounds were substantially the same in neutral solution, but in 0·02N-sodium hydroxide the spectrum of the acid (VIII; R = R′ = H) was altered, although the spectra of the monomethyl and the dimethyl ester were unaffected (Table 1). The result indicates that the 10-carboxyl function is electronically associated with the trienone system, and that the 1-carboxyl group is not.

TABLE 1.

Ultraviolet absorption maxima (mμ).

Compound	In ethanol	In 0·02N-sodium hydroxide
(VIII; R = R′ = Me)	255, 342 (log ε 4·11, 4·32)	255, 344 (log ε 4·08, 4·25)
(VIII; R = H, R′ = Me)	254, 342 (log ε 4·07, 4·29)	255, 347 (log ε 4·08, 4·32)
(VIII; R = R′ = H)	252, 342 (log ε 4·00, 4·27)	244, 350 (log ε 4·05, 4·33)

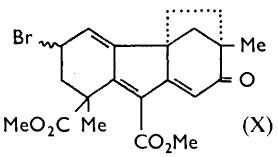
The dicarboxylic acid (VIII; R = R′ = H) was smoothly converted into the anhydride (IX) by treatment with *NN*′-dicyclohexylcarbodi-imide in 1,2-dimethoxyethane. The relative configuration of the 1-methyl and the 1-carboxyl group cannot be deduced from this reaction since molecular models show that either configuration could allow anhydride formation.

The dimethyl ester (VIII; R = R′ = Me) with *N*-bromosuccinimide in the presence of dibenzoyl peroxide gave a monobromo-derivative possessing substantially the same ultraviolet absorption spectrum ($\lambda_{max.}$ 253 and 350 mμ) as the starting material. The compound is assumed to possess structure (X) though the configuration of the bromine atom remains unknown. Attempts to dehydrobrominate it to afford a conjugated

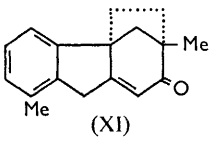
[7] Cross, Grove, MacMillan, and Mulholland, *Chem. and Ind.*, 1956, 954.
[8] Moffatt, *J.*, 1960, 3045.

tetraenone system with pyridine or quinoline failed. Reducing it with sodium borohydride gave an unstable alcohol, m. p. 176—177° ($\lambda_{max.}$ 313 mμ, $E^{1\%}_{1\,cm.}$ 420). The ultraviolet absorption spectrum of this material is to be contrasted with that of the sodium borohydride reduction product of the dimethyl ester (VIII; R = R′ = Me) ($\lambda_{max.}$ 292 mμ, $E^{1\%}_{1\,cm.}$ 70).

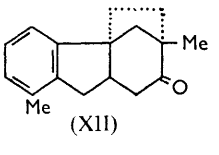
The monomethyl ester (VIII; R = H, R′ = Me) and the dicarboxylic acid (VIII; R = R′ = H) were stable to acid and showed no tendency to aromatise. Ring A of the dicarboxylic acid (VIII; R = R′ = H) was aromatised on mild dehydrogenation with selenium, a ketone, $C_{17}H_{18}O$, m. p. 138—139°, $\nu_{max.}$ (in Nujol) 1672 cm.$^{-1}$, being isolated. The ultraviolet absorption spectrum showed a gradually diminishing absorption with increasing wavelength, although inflections were observed at 215 and 250 mμ. The compound absorbed one mol. of hydrogen, to give the ketone, $C_{17}H_{20}O$, m. p. 139°, $\nu_{max.}$ (in chloroform) 1703 cm.$^{-1}$, with benzenoid ultraviolet absorption. Subtraction of the ultraviolet absorption curve of ketone $C_{17}H_{20}O$ from that of ketone $C_{17}H_{18}O$ gave a curve showing $\lambda_{max.}$ 229 mμ (log ε 4·12) and an inflection at 250 mμ (log ε 3·66). This result is consistent with the presence of an αβ-unsaturated ketone system in the compound,



(X)



(XI)



(XII)

$C_{17}H_{18}O$, which is not electronically conjugated with the benzene ring. Since the infrared spectra show that the ketonic group lies in a six-membered ring, the ketones, $C_{17}H_{18}O$ and $C_{17}H_{20}O$, are formulated as (XI) and (XII), respectively. The inflection at 250 mμ (log ε 3·66) in the ultraviolet subtraction curve may be due to homoallylic conjugation between the αβ-unsaturated ketone system and the benzene ring in ketone (XI). The dihydrocompound (XII) was identical with the neutral ketonic compound, m. p. 138—139°, previously obtained as a minor product in the dehydrogenation of gibberic acid.[9]

Vigorous dehydrogenation of the dicarboxylic acid by selenium yielded a fluorenol, m. p. 207—208°, identical with "phenol A," $C_{15}H_{14}O$, m. p. 202—205°, previously obtained [9] as a by-product in the dehydrogenation of gibberic acid, and now shown to be 1,6-dimethylfluoren-7-ol (XIII) by synthesis (cf. Morrison and Mulholland [10]).

The benzoxazine derivative of 2-amino-6-methoxy-*p*-toluic acid [11] was condensed with the Grignard derivative of *o*-tolylmagnesium bromide, giving 2-acetamido-5-methoxy-2′,4-dimethylbenzophenone. Acidic hydrolysis of the amide followed by diazotisation of the amine and ring closure furnished 6-methoxy-1,6-dimethylfluorenone. Wolff–Kishner reduction and demethylation gave 1,6-dimethylfluoren-7-ol (XIII). Direct reductive demethylation of 7-methoxy-1,6-dimethylfluorenone with red phosphorus and hydriodic acid gave 1,6-dimethylfluoren-7-ol and some 1,6-dimethylfluorene.

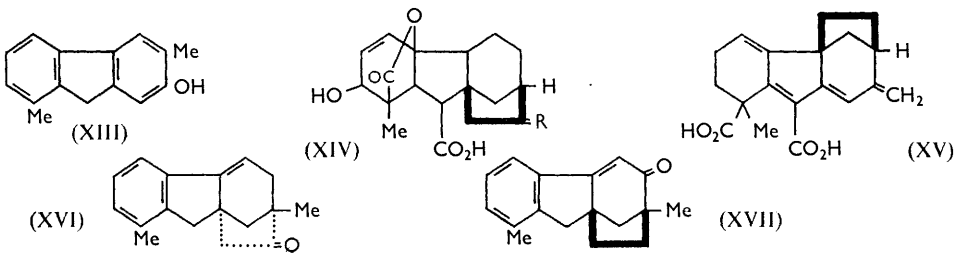
In addition to 1,6-dimethylfluoren-7-ol (XIII), vigorous dehydrogenation of the dicarboxylic acid (VIII; R = R′ = H) by selenium gave traces of ketone (XII) and 1,7-dimethylfluorene; the latter presumably arose by reverse rearrangement of the carbon skeleton of (VIII).

The nuclear magnetic resonance spectrum of the dimethyl ester (VIII; R = R′ = Me) in chloroform–carbon tetrachloride (with tetramethylsilane as internal reference) is consistent with the formulation shown. In particular, only two allylic protons are observed (τ 7·6); the two vinylic protons occur together (τ 3·9).

The molecular species responsible for the fluorescence of gibberellic acid in strong acids

[9] Cross, Grove, MacMillan, and Mulholland, *J.*, 1958, 2520.
[10] Morrison and Mulholland, *J.*, 1958, 2702.
[11] Gardner and Grove, *J.*, 1953, 3646.

has not been investigated, but since compound (VIII; R = R′ = Me) can be reversibly converted into the fluorogen in sulphuric acid, it is attributed to a protonated form of (VIII; R = R′ = H). Of the gibberellins A_1 to A_9, only gibberellin A_7 [12] (XIV; R = CH_2) shows a fluorescence comparable in intensity with that of gibberellic acid when treated



with 50% ethanolic sulphuric acid at 50° for 25 min. By analogy with gibberellic acid, the material responsible for the fluorescence of gibberellin A_7 (XIV; R = CH_2) is assumed to be a protonated form of (XV). The principal activation and emission maxima of gibberellin A_7 (XIV; R = CH_2) are at 455 and 473 mμ, respectively. Of particular significance, however, is the fluorescence spectrum of gibberellin A_7 nor-ketone [12] (XIV; R = O), which, as expected, shows the same activation and emission maxima as gibberellic acid.

In the course of the work an attempt was made to prepare the unsaturated ketone (XI) by treatment of gibberone [9] (XVI) with sulphuric acid. The product of the reaction was identified with a ketone, $C_{17}H_{18}O$, m. p. 124—125°, previously obtained [4] in the pyrolysis of epigibberic acid on charcoal at 250°. The ultraviolet absorption spectrum of the compound together with its formation from gibberone under acidic conditions suggest structure (XVII), which would be formed from gibberone by two 1,2-shifts, the rearrangement being facilitated by the formation of a fully conjugated system.

Experimental

M. p.s are corrected. Unless otherwise stated, infrared spectra were obtained for Nujol mulls, and ultraviolet spectra and optical rotations for ethanol solutions. Alumina was supplied by M. Woelm, Eschwege. Fluorescence spectra were measured, by an Aminco-Bowman spectrofluorometer, after 50% (v/v) ethanolic sulphuric acid solutions had been heated for 25 min. at 50°. Light petroleum had b. p. 60—80°.

Action of Sulphuric Acid on Gibberellic Acid.—Concentrated sulphuric acid (5 ml.) was added during 3 min. to a stirred susension of powdered gibberellic acid (0·5 g.) in methanol (1 ml.), cooled at −10°. The dark solution was stirred at room temperature for 20 min., then poured into ice and brine (300 ml.) and extracted with ethyl acetate (2 × 100 ml.). The extract was washed with small quantities of brine, dried (Na_2SO_4), and evaporated to give a yellow gum, which was treated with methanolic diazomethane. The product was chromatographed on grade I acid alumina (30 g.). From the first fractions, eluted with benzene–ether (5 : 1) and giving no colour with sulphuric acid, small quantities (5—10 mg.) of methyl gibberate and epigibberate were isolated. Ether slowly eluted a pale yellow material, which crystallised in the presence of benzene as pale yellow prisms (40 mg.), m. p. 170—173°. From the mother-liquors a trace of methyl 1,7-dimethyl-8-oxo-7α-gibba-3,4a(4b),5-triene-1,10-dicarboxylate (II) was isolated as needles, m. p. 195—196° (from light petroleum); this was identified by the infrared spectrum and formation of a deep blue colour in sulphuric acid.[5]

Further crystallisation of the pale yellow prisms from benzene–light petroleum gave almost colourless prisms, m. p. 173—174°, $[\alpha]_D^{24}$ −125° (*c* 0·25), of *methyl 1,7-dimethyl-8-oxo-7α-isogibba-4,10a,9a-triene-1,10-dicarboxylate* (VIII; R = R′ = Me) (Found: C, 70·9; H, 6·8; OMe, 18·1.

[12] Cross, Galt, and Hanson, *Tetrahedron*, 1962, **18**, 451.

$C_{21}H_{24}O_5$ requires C, 70·8; H, 6·8; 2OMe, 17·5%), $\nu_{max.}$ 1729, 1715, 1660, 1645, 1611, and 1550 cm.$^{-1}$, $\lambda_{max.}$ 255 and 342 mμ (log ε 4·11 and 4·32, respectively). The ultraviolet spectrum was unchanged in 0·02N-aqueous-ethanolic (1 : 4) sodium hydroxide. The fluorescence spectrum showed activation maxima at 280 and 418 mμ, and a single emission maximum at 463 mμ (under the same conditions gibberellic acid showed identical activation and emission maxima but with one-third of the intensity). Microhydrogenation in acetic acid solution in the presence of Adams catalyst resulted in the uptake of 3·8 mol. of gas.

Subsequent fractions from the chromatogram, eluted with ether containing methanol, were mostly deep yellow intractable gums, which did not produce the fluorescence spectrum characteristic of gibberellic acid and were not further investigated. However, from one of the fractions eluted with ether–methanol (200 : 1), yellow amorphous material was obtained (10 mg.) having m. p. 165—195°, $\lambda_{max.}$ 283 and 425 mμ, which gave an intense magenta colour with concentrated sulphuric acid.

Action of Sulphuric Acid on Methyl Gibberellate.—Methyl gibberellate (2 g.) in methanol (4 ml.) was treated with concentrated sulphuric acid (20 ml.) as described above. The crude yellow gum from the ethyl acetate extract was chromatographed on silica gel (30 × 3 cm.) (Hopkin and Williams chromatographic grade). Only those fractions giving a fluorescence with sulphuric acid were investigated. (i) Eluted with benzene–ether (6 : 1). The crude eluate (42 mg.) crystallised from benzene–light petroleum, to give the dimethyl ester (VIII; R = R′ = Me) (20 mg.), m. p. 173°. (ii) Eluted with benzene–ether (5 : 1). A pale yellow gum (405 mg.) was slowly eluted which did not crystallise. Extraction with saturated sodium hydrogen carbonate of an ethyl acetate solution and recovery furnished an acidic fraction, which was rechromatographed on silica gel as above. The fractions eluted with benzene–ether (5 : 1) (170 mg.) crystallised from ethyl acetate–light petroleum as pale yellow prisms of *10-methoxycarbonyl-1,7-dimethyl-8-oxo-7α-isogibba-4,10a,9a-triene-1-carboxylic acid hemihydrate* (VIII; R = H, R′ = Me), m. p. 110—130° (decomp.), $[\alpha]_D^{24}$ −124° (*c* 0·25) (Found: C, 68·0, 68·7; H, 6·6, 6·7; OMe, 10·1%; equiv., 343. $C_{20}H_{22}O_5,0{\cdot}5H_2O$ requires C, 68·3; H, 6·6; 1OMe, 8·8%; *M*, 351), $\nu_{max.}$ 3130 (br.), 1730, 1717, 1635, 1607, and 1550 cm.$^{-1}$, $\lambda_{max.}$ 254 and 342 mμ (log ε 4·07 and 4·29, respectively), $\lambda_{max.}$ (in 0·02N-NaOH) 255 and 347 mμ (log ε 4·08 and 4·32 respectively).

Treatment with diazomethane gave the dimethyl ester (VIII; R = R′ = Me), m. p. 173°.

Alkaline Hydrolysis of the Dimethyl Ester (VIII; R = R′ = Me).—The dimethyl ester (25 mg.) in aqueous-ethanolic (1 : 2) 2N-sodium hydroxide (4 ml.) was refluxed under nitrogen for 1 hr. The solution was acidified with dilute hydrochloric acid and extracted with ether. Crystallisation of the recovered product from ethyl acetate gave needles (16 mg.) of *1,7-dimethyl-8-oxo-7α-isogibba-4,10a,9a-triene-1,10-dicarboxylic acid* (VIII; R = R′ = H), m. p. 152—154° (decomp.), $[\alpha]_D^{24}$ −99° (*c* 0·15) (Found: C, 69·3; H, 6·7. $C_{19}H_{20}O_5$ requires C, 69·5; H, 6·1%), $\nu_{max.}$ 3240, 1743, 1692, and 1586 cm.$^{-1}$, $\lambda_{max.}$ 252 and 342 mμ (log ε 4·00 and 4·27, respectively), $\lambda_{max.}$ (in 0·02N-NaOH) 244 and 350 mμ (log ε 4·05 and 4·33, respectively).

The dicarboxylic acid (VIII; R = R′ = H) was also obtained on hydrolysis of the monoester (VIII; R = H, R′ = Me).

Methylation of the dicarboxylic acid (VIII; R = R′ = H) gave the dimethyl ester (VIII; R = R′ = Me) as prisms, m. p. 173° (from benzene–light petroleum).

Treatment of the Dimethyl Ester (VIII; R = R′ = Me) *with Sulphuric Acid.*—The dimethyl ester (49 mg.) in methanol–sulphuric acid (1 : 1, v/v) (5 ml.) was heated at 50° for 20 min., the usual fluorescence of the compound being observed. The cooled solution was poured into brine at −10° and extracted with ethyl acetate. The recovered product crystallised from ethyl acetate–light petroleum as prisms (44 mg.), m. p. 169—173°, of unchanged dimethyl ester.

Formation of the Anhydride (IX).—The dicarboxylic acid (VIII; R = R′ = H) (31 mg.) and *NN′*-dicyclohexylcarbodi-imide (25 mg.) in 1,2-dimethoxyethane (3 ml.) (purified by filtration through grade I basic alumina) were kept at 20° for 48 hr. The solvent was evaporated *in vacuo* and the residue was extracted with dry benzene. Concentration of the benzene extract and addition of light petroleum furnished almost colourless needles (25 mg.) of the *anhydride* (IX), m. p. 188—193° (Found: C, 73·7; H, 5·9. $C_{19}H_{18}O_4$ requires C, 73·5; H, 5·9%), $\nu_{max.}$ 1811, 1757, 1663, and 1640 cm.$^{-1}$, $\lambda_{max.}$ 257 and 351 mμ (log ε 4·03 and 4·23, respectively).

Reduction of the Dimethyl Ester (VIII; R = R′ = Me) *by Sodium Borohydride.*—Sodium borohydride (20 mg.) in methanol (1 ml.) was added with stirring to the dimethyl ester (20 mg.) in methanol (1·5 ml.) during 15 min. The yellow colour and pale green fluorescence disappeared

as the reaction proceeded. After 30 minutes' stirring at room temperature, the solution was acidified with acetic acid and evaporated *in vacuo* and the residue was chromatographed on grade II acid alumina (3 g.). The product (14 mg.) eluted with benzene–ether (9 : 1) did not crystallise but formed a wax, m. p. up to 115°, $[\alpha]_D^{24}$ −19° (*c* 0·25), $\nu_{max.}$ 3340 (OH), $\lambda_{max.}$ 292 mμ ($E_{1\,cm.}^{1\%}$ 70). In sulphuric acid it gave a yellow colour and green fluorescence.

Bromotrienone (X).—The dimethyl ester (VIII; R = R′ = Me) (52 mg.) and *N*-bromosuccinimide (30 mg.) in carbon tetrachloride (4 ml.) were refluxed with dibenzoyl peroxide (3 mg.) for 30 min. The solvent was evaporated *in vacuo* and the residue was chromatographed on grade I acid alumina (12 g.). Ether eluted a solid (18 mg.) which crystallised from ethyl acetate–light petroleum as pale yellow needles (11 mg.), m. p. 178—180°. Slow recrystallisation from ethyl acetate–light petroleum gave pale yellow prisms, m. p. 194—195°, $[\alpha]_D^{24}$ −30° (*c* 0·25), of a *bromotrienone* (X) (Found: C, 58·0; H, 5·5; Br, 17·5. $C_{21}H_{23}BrO_5$ requires C, 57·9; H, 5·3; Br, 18·4%), $\lambda_{max.}$ 253 and 350 mμ (log ε 3·88 and 4·38, respectively), $\nu_{max.}$ (prismatic form) 1738, 1674, and 1648 cm.$^{-1}$.

Reduction of the Bromotrienone (X).—Sodium borohydride (20 mg.) in methanol (1 ml.) was added during 5 min. to a stirred solution of the bromotrienone (X) (25 mg.) in methanol (4 ml.) at room temperature. Stirring was continued for 30 min., then the solution was acidified with acetic acid. The solvent was evaporated *in vacuo* and the residue was extracted with benzene and chromatographed on grade II acid alumina. The only material isolated from the column [eluted with benzene–ether (9 : 1)] crystallised from ethyl acetate–light petroleum as needles (8 mg.), m. p. 176—177°, $\nu_{max.}$ 3470 cm.$^{-1}$ (OH), $\lambda_{max.}$ 313 mμ ($E_{1\,cm.}^{1\%}$ 420). This product decomposed on storage at room temperature or attempted recrystallisation from ethyl acetate–light petroleum.

Attempted Dehydrobromination of the Bromotrienone (X).—(i) *With pyridine.* The bromotrienone (X) (5 mg.) in pyridine (2 ml.) was refluxed under nitrogen for 3 hr. Evaporation of the solvent and crystallisation of the residue from ethyl acetate–light petroleum gave starting material, m. p. 176—178°. The ultraviolet spectrum of the mother-liquors showed no evidence of any tetraenone. (ii) *With quinoline.* The bromotrienone (X) (4 mg.) and quinoline (12 mg.) were heated at 200—210° under nitrogen for 30 min. The deep crimson product was insoluble in ether. No crystalline material was recovered.

Mild Dehydrogenation of the Dicarboxylic Acid (VIII; R = R′ = H).—The crude dicarboxylic acid from hydrolysis of the dimethyl ester (VIII; R = R′ = Me) (60 mg.) was mixed with powdered selenium (40 mg.) in a narrow tube (25 × 0·5 cm.) and kept under nitrogen. The tube was plunged into a metal-bath at 220° and the temperature was quickly raised to and kept at 360° for 10 min. The partly crystalline sublimate which collected on the cooler parts of the tube was extracted with light petroleum and filtered through a little grade II acid alumina in light petroleum, to give a semi-solid product (28 mg.). Sublimation at 0·1 mm. gave in the 100—120° fractions a solid (19 mg.), which was crystallised from aqueous ethanol and subsequently from light petroleum (b. p. 40—60°), to yield prisms, m. p. 138—139°, of the *ketone* (XI) (Found: C, 85·4; H, 8·2. $C_{17}H_{18}O$ requires C, 85·7; H, 7·6%), $\nu_{max.}$ 1672, 1650, 850, 842, and 783 cm.$^{-1}$ (no OH absorption). The ultraviolet absorption spectrum showed inflections at 215 and 250 mμ (log ε 4·26 and 3·68, respectively).

In a microhydrogenation experiment in acetic acid with Adams catalyst, 4·115 mg. of the compound absorbed 0·60 ml. of hydrogen at S.T.P. (1 mol. = 0·40 ml.). The hydrogenated product, on crystallisation from aqueous ethanol and from light petroleum (b. p. 40—60°), furnished prisms, m. p. 139—140°, of the *ketone* (XII), $[\alpha]_D^{24}$ −62° (*c* 0·25), $\nu_{max.}$ 1707, 1603 cm.$^{-1}$, (in chloroform) 1703 cm.$^{-1}$, $\lambda_{max.}$ 266, 274, and 290 mμ (log ε 2·74, 2·67, and 1·92, respectively), identical with the neutral ketone of m. p. 138—139° obtained in the dehydrogenation of gibberic acid.[9]

Vigorous Dehydrogenation of the Dicarboxylic Acid (VIII; R = R′ = H).—Selenium powder (80 mg.) and the dicarboxylic acid (100 mg.; m. p. 152—154°) in a narrow glass tube (25 × 0·7 cm.) were heated at 360° under nitrogen for 3 hr. The mixture was cooled and then extracted with acetone and methanol, and the dark brown gummy extract distilled at 200° (bath)/0·1 mm., to give a pale yellow gum (30 mg.). The gum was chromatographed on grade II acid alumina (5 g.), and the fluorescent bands were eluted in ultraviolet light. The main fractions were: (i) Violet band, eluted with light petroleum (6 mg.). With methanol the eluted product gave crystals, which after two further crystallisations from methanol were obtained as needles (1 mg.), m. p. 105—106°. The compound was identified (infrared spectrum

and m. p.) with an authentic specimen of 1,7-dimethylfluorene.[13] (ii) Pale blue band, eluted with light petroleum–ether (20 : 1). This furnished an intractable gum (6 mg.). (iii) Pale blue band, eluted with light petroleum–ether (9 : 1). This gave a solid (3 mg.). The material was sublimed at 110/0·1 mm. and crystallised from light petroleum as needles (0·5 mg.), m. p. 136—138°. Recrystallisation from light petroleum gave prisms, m. p. 138—139°, of the ketone (XII). (iv) Violet band eluted with ether. This yielded a solid, m. p. 180—185°. Crystallisation from benzene–light petroleum gave needles (3 mg.) of 1,6-dimethylfluoren-7-ol, m. p. 205—206°, raised to m. p. 207—208° after further recrystallisations from benzene, $\nu_{max.}$ 3360 and 1580 cm.$^{-1}$, $\lambda_{max.}$ 277, 308, and 316 mμ ($E^{1\%}_{1cm.}$ 950, 420, and 440, respectively). The compound was identified with " phenol A " previously obtained as a minor product in the dehydrogenation of gibberic acid,[9] and with a synthetic specimen of 1,6-dimethylfluoren-7-ol (see below).

The acetate crystallised from methanol as plates, m. p. 153—155°, $\nu_{max.}$ 1764 cm.$^{-1}$ (no OH absorption).

2-*Amino*-5-*methoxy*-p-*toluic Acid*.—5-Methoxy-2-nitro-*p*-toluic acid [11] (526 mg.) in ethanol (20 ml.) was hydrogenated at 20° in the presence of 30% palladium–charcoal (catalyst " d ") [14] (400 mg.) until reduction ceased (2 hr.). The fluorescent solution was filtered through " Hyflo Supercel " to remove the catalyst, and the product was isolated as pale yellow crystals (399 mg.), m. p. 200—202° (decomp.), not raised by crystallisation from ethanol (lit.,[11] m. p. 202°) (Found: C, 60·2; H, 6·4. Calc. for $C_9H_{11}NO_3$: C, 59·7; H, 6·1%).

6-*Methoxy*-2,7-*dimethyl*-4-*oxo*-3,1-*benzoxazine*.—2-Amino-5-methoxy-*p*-toluic acid (2 g.) in acetic anhydride (40 ml.) was refluxed for 3 hr. under nitrogen, then cooled, and the solvent was evaporated *in vacuo*. The crude product in benzene–ethyl acetate (4 : 1) was filtered through a little grade II acid alumina, and the eluate crystallised from dry benzene–light petroleum. The *benzoxazine* was obtained as needles, m. p. 139—141° (Found: C, 63·9; H, 5·4; N, 6·0. $C_{11}H_{11}NO_3$ requires C, 64·4; H, 5·4; N, 6·8%), $\nu_{max.}$ 1753, 1656, and 1619 cm.$^{-1}$. The compound decomposed in the presence of moisture.

2-*Acetamido*-5-*methoxy*-2′,4-*dimethylbenzophenone*.—The Grignard reagent, prepared from *o*-bromotoluene (purified by distillation and filtration through grade I neutral alumina) (2·0 g.) and magnesium (260 mg.) in dry ether (20 ml.), was added dropwise during 5 min. to a stirred solution of 6-methoxy-2,7-dimethyl-4-oxo-3,1-benzoxazine (2·1 g.) in dry benzene (75 ml.) cooled at 0°. A precipitate was formed immediately. The ice-bath was removed after 10 min. and the mixture was stirred at 23° for 1 hr. and finally refluxed for 15 min. Ice and water (90 ml.) were added, followed by 3N-sulphuric acid (45 ml.). The mixture was thoroughly extracted with ether, and the combined extracts were washed with saturated sodium hydrogen carbonate solution and brine, dried (Na_2SO_4), and evaporated to a yellow gum. Chromatography of the gum on grade I neutral alumina (50 g.) gave the required product (visible on the alumina as a brown band in ultraviolet light) on elution with ether–ethyl acetate (2 : 1). Crystallisation of the solid from the light petroleum (b. p. 80—100°) yielded needles (1·106 g.), m. p. 129—133°. Recrystallisation from light petroleum (b. p. 80—100°) gave pale yellow needles of 2-*acetamido*-5-*methoxy*-2′,4-*dimethylbenzophenone*, m. p. 134—135° (Found: C, 72·9; H, 6·5; N, 4·8; OMe, 10·2. $C_{18}H_{19}NO_3$ requires C, 72·7; H, 6·4; N, 4·7; 1OMe, 10·4%), $\nu_{max.}$ 3270w, 1693, 1629, 1534 cm.$^{-1}$.

2-*Amino*-5-*methoxy*-2′,4-*dimethylbenzophenone*.—The above amide (490 mg.) in ethanol (10 ml.) and concentrated hydrochloric acid (3 ml.) was refluxed for 4 hr. The solution was neutralised with 3N-aqueous ammonia and extracted with ether–ethyl acetate (1 : 1) (3 × 30 ml.). The product was chromatographed on grade II neutral alumina, and the major fraction (360 mg.) (pale brown band on the column in ultraviolet light) was eluted with benzene–ether (9 : 1). The product was distilled at 90°/0·1 mm., to give the gummy *amine* (Found: C, 74·9; H, 6·9, 5·8; OMe, 12·1. $C_{16}H_{17}NO_2$ requires C, 75·3; H, 6·7; N, 5·5; 1OMe, 12·2%), $\nu_{max.}$ (liquid film) 3470, 3350, 1635, 1585, and 1535 cm.$^{-1}$. Acetylation of the amine (30 mg.) regenerated the amide (28 mg.), m. p. 133—135°.

Further elution of the column with ether yielded a second gum (18 mg.), which from its infrared spectrum appeared to be 2-amino-5-hydroxy-2′,4-dimethylbenzophenone, formed by demethylation.

7-*Methoxy*-1,6-*dimethylfluorenone*.—2-Amino-5-methoxy-2′,4-dimethylbenzophenone (360

[13] Cross and Melvin, *J.*, 1960, 3038.
[14] Linstead and Thomas, *J.*, 1940, 1127.

mg.) in water (7 ml.) and concentrated hydrochloric acid (25 ml.) at 0° was treated with sodium nitrite (150 mg.) in water (1 ml.) during 5 min. The solution was kept at 0° for 10 min. and then allowed to warm to room temperature during 30 min. After the solution had been heated on the steam-bath for 2 hr. and then refluxed for 50 min., the cooled solution was extracted with ether–ethyl acetate (3 : 1), and the crude extract (302 mg.) chromatographed on grade II acid alumina. Crystallisation of the major fraction from ethanol yielded orange prisms (177 mg.), m. p. 158—163°, of the *methoxyfluorenone*. Recrystallisation from ethanol raised the m. p. to 161—163° (Found: C, 80·4; H, 6·0; OMe, 12·9. $C_{16}H_{14}O_2$ requires C, 80·6; H, 5·9; 1OMe, 13·0%), $\nu_{max.}$ 1693 and 1592 cm.$^{-1}$, $\lambda_{max.}$ 270 mμ (log ε 4·89).

7-*Hydroxy*-1,6-*dimethylfluorenone*.—Crude 2-amino-5-hydroxy-2′,4-dimethylbenzophenone (29 mg.) (obtained as a minor product in the preparation of the gummy amine above) in concentrated hydrochloric acid (2·5 ml.) at 0° was treated with sodium nitrite (15 ml.) in water (1 ml.) at 0° for 10 min. The solution was then slowly heated to 100° and refluxed for 2 hr. The washed solution was extracted with ethyl acetate, to give a dark solid (29 mg.), which was filtered through a little grade II acid alumina in ether. The eluate crystallised from benzene as orange needles (9 mg.) of the *hydroxyfluorenone*, m. p. 217—220°, raised to 218—219° on recrystallisation (Found: C, 80·0; H, 5·4. $C_{15}H_{12}O_2$ requires C, 80·3; H, 5·4%), $\nu_{max.}$ 3430, 1692, and 1588 cm.$^{-1}$.

Treatment with diazomethane furnished the methoxyfluorenone as prisms, m. p. 160—163°.

7-*Methoxy*-1,6-*dimethylfluorene*.—7-Methoxy-1,6-dimethylfluorenone (39 mg.) and 64% w/w aqueous hydrazine (0·5 ml.) in diethylene glycol (5 ml.) were heated on the steam-bath for 30 min. and then at 120—130° for 20 min. Potassium hydroxide (0·5 g.) was cautiously added, and the red solution was heated at 120° for 20 min. and then at 185—190° for 4—5 hr. After cooling, the colourless solution was poured into water and extracted with ether. The organic extract was washed with water, dried (Na_2SO_4), and evaporated, giving a solid (35 mg.), which was purified by filtration through grade II acid alumina in benzene–ether (9 : 1). Crystallisation from ethanol gave needles, m. p. 159—160°, of 7-*methoxy*-1,6-*dimethylfluorene* (Found: C, 85·3; H, 7·2; OMe, 14·3. $C_{16}H_{16}O$ requires C, 85·7; H, 7·2; 1OMe, 13·8%), $\lambda_{max.}$ 277, 304, and 315 mμ (log ε 4·27, 3·91, and 3·94, respectively).

1,6-*Dimethylfluoren*-7-*ol*.—(*a*) A mixture of 7-methoxy-1,6-dimethylfluorene (25 mg.), acetic acid (2 ml.), and 48% hydrobromic acid (2 ml.; freshly distilled) was heated under reflux for 200 min. An ethereal extract of the reaction mixture was washed with water, dried (Na_2SO_4), and evaporated, to give a solid, which was chromatographed on grade II acid alumina. The band (violet in ultraviolet light) eluted with ether was collected and the product was crystallised from benzene–light petroleum as needles (10 mg.), m. p. 204—207°. Two further crystallisations gave needles, m. p. 207—209°, of 1,6-*dimethylfluoren*-7-*ol* (Found: C, 85·7; H, 7·0. $C_{15}H_{14}O$ requires C, 85·7; H, 6·7%), $\nu_{max.}$ 3345 and 1580 cm.$^{-1}$, $\lambda_{max.}$ 277, 307, and 315 mμ (log ε 4·29, 3·94, and 3·95 respectively).

The *acetate* crystallised as plates from ethanol, m. p. 155—156° (Found: C, 80·9; H, 6·5. $C_{17}H_{16}O_2$ requires C, 80·9; H, 6·4%).

(*b*) 7-Methoxy-1,6-dimethylfluorenone (51 mg.), red phosphorus (50 mg.), acetic acid (1 ml.), and hydriodic acid (0·5 ml.; *d* 1·70) were refluxed for 96 hr., then poured into water and the mixture extracted with ether. The ether solution was washed with brine, sodium thiosulphate solution, and again brine, dried (Na_2SO_4), and evaporated, to give a gummy solid (53 mg.). Chromatography on grade II acid alumina gave two main fractions: (i) eluted with light petroleum (b. p. 40—60°) (10 mg., gum), which after repeated sublimation and crystallisation from aqueous methanol gave plates (2 mg.), m. p. 115—117°, of a colourless substance, $\nu_{max.}$ 877, 805, 786, and 737 cm.$^{-1}$, assumed to be 1,6-dimethylfluorene (lit.,[15] m. p. 117—118°); and (ii) 39 mg. of solid, eluted with ether. Crystallisation of the latter fraction from benzene–light petroleum gave needles (22 mg.) of 1,6-dimethylfluoren-7-ol, m. p. 205—208°.

Action of Sulphuric Acid on Gibberone.—Gibberone [9] (72 mg.; m. p. 119—123°) in sulphuric acid–methanol (4 : 1, v/v) (1·5 ml.) was left at room temperature for 1 hr. The solution was poured into ice–water (25 ml.) saturated with sodium chloride, and the mixture extracted with ethyl acetate–ether (1 : 1) (2 × 25 ml.). The gummy product (22 mg.) was chromatographed on grade I acid alumina (4 g.). Benzene–ether (1 : 1) (30 ml.) eluted a colourless solid (12 mg.), which crystallised from light petroleum containing a little ethanol as prisms (5 mg.), m. p. 121—122°, of the *ketone* (XVII), $\nu_{max.}$ 1660, 1622, and 1596 cm.$^{-1}$, $\lambda_{max.}$ 235, 285, 296, and 320 mμ

[15] Longo and Pirona, *Gazzetta*, 1947, **77**, 127.

($E^{1\%}_{1\,cm.}$ 640, 975, 1150, and 815 respectively). It was identified with the ketone, $C_{17}H_{18}O$, m. p. 124—125°, obtained in the pyrolysis of epigibberic acid with charcoal,[4] by mixed m. p. and comparison of infrared and ultraviolet spectra.

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