609. *Gibberellic Acid. Part XVI.* The Chromophore of Gibberellenic Acid.*

By J. S. Moffatt.

Comparison of the ultraviolet light absorptions of gibberellenic acid, the conjugated dienone obtained on oxidation of methyl gibberellenate, and the monocyclic dienone, ethyl 1-methyl-6-oxocyclohexa-2,4-dienecarboxylate, whose synthesis is described, shows that in the first two compounds the conjugated double bonds are heteroannular. The above-mentioned dienone partly undergoes a dienone–phenol rearrangement in which apparently the ethoxycarbonyl group migrates.

During early studies on gibberellic acid in these laboratories and elsewhere [1,2] it was observed that aqueous solutions on storage slowly developed strong absorption at 253—257 mμ. Attempts to isolate the decomposition product, which was evidently associated with a spot at R_F 0·02 in paper chromatograms where the butanol–ammonia system was used,[3] by means of evaporation of the aqueous solutions and preparative paper chromatography of the residues with the solvent system chloroform–ethanol–water–formic acid [4]

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* Part XV, preceding paper.

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

[2] Kavanagh and Kutzel, *J. Agric. Food Chem.*, 1958, **6**, 459.

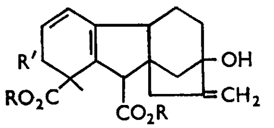
[3] Brian, Grove, Hemming, Mulholland, and Radley, *Plant Physiol.*, 1958, **33**, 329.

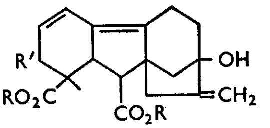
[4] Radley, *Nature*, 1956, **178**, 1070.

gave amorphous products. These were less mobile than gibberellic acid and showed strong absorption at 254 mμ but could not readily be purified. However, storage of aqueous calcium gibberellate over an excess of calcium carbonate for a prolonged period (5½ months) and working up afforded a silver salt, $C_{19}H_{20}O_6Ag_2$, $\lambda_{max.}$ 256 mμ (ε 16,750 in H_2O), of the product responsible for the strong light absorption. Thereafter, owing to the co-operation of Dr. Gerzon who kindly informed us of his results before publication,[1] we were able to isolate small quantities of the pure decomposition product gibberellenic acid by fractional crystallisation of crude gibberellic acid obtained from culture filtrates.[5] Subsequently it was obtained [6] by the action of hydrazine on gibberellic acid.

Gerzon, Bird, and Woolf [1] favoured structure (I; R = H, R′ = OH) over the alternative (II; R = H, R′ = OH) for gibberellenic acid; its light absorption, however, particularly its high intensity ($\lambda_{max.}$ 253 mμ; ε 22,400 in EtOH), seemed to be rather more in accord with the latter structure. Oxidation of methyl gibberellenate by manganese dioxide at room temperature afforded a conjugated dienone $C_{21}H_{24}O_6$, whose light absorption ($\lambda_{max.}$ 309 mμ; ε 16,500 in EtOH) decisively indicated for it the heteroannular structure (II; R = Me, R′ = O).

(I)





(II)

For comparison, the homoannular dienone, ethyl 1-methyl-2-oxocyclohexa-3,5-dienecarboxylate was synthesised. Bromination of ethyl 1-methyl-2-oxocyclohexanecarboxylate [7] in chloroform yielded the 3-bromo-ester which, contrary to statements in the literature,[8] could be readily purified by distillation. With methanolic Brady's reagent it gave, not unexpectedly,[9] the dinitrophenylhydrazone, m. p. 141—142°, of the 3-methoxy-ester; on the other hand Yanagita, Inayama, and Kitagawa [10] reported that the bromo-ketone with alcoholic 2,4-dinitrophenylhydrazine gave a tetrahydroindazolone derivative, m. p. 143—145°. Dehydrobromination of the bromo-ketone yielded ethyl 1-methyl-2-oxocyclohex-3-enecarboxylate [8,11] which, on bromination with *N*-bromosuccinimide afforded the 5-bromo-derivative. Dehydrobromination with quinoline then gave the required dienone, characterised by its formation of adducts with maleic anhydride and methyl acetylenedicarboxylate respectively and by its absorption of 1·7 mols. of hydrogen in the presence of palladium to give ethyl 1-methyl-2-oxocyclohexanecarboxylate characterised as its 2,4-dinitrophenylhydrazone. The ultraviolet light absorption of the dienone, $\lambda_{max.}$ 301 mμ (ε 3900 in EtOH) was similar to those of other cyclohexa-2,4-dienones [12] and shows that the dienone derived from gibberellenic acid has the heteroannular arrangement of double bonds as represented in (II).

For possible application to the study of gibberellic acid derivatives it was of interest to submit our synthetic dienone to the conditions of the dienone–phenol rearrangement. With warm, dilute sulphuric acid it yielded a complex mixture which was separated into *o*-cresol (about 18%), other neutral, probably dimeric products, and 3-hydroxy-2-methylbenzoic acid (about 6%). In the formation of this acid the ethoxycarbonyl group apparently migrates to an adjacent position during a dienone–phenol rearrangement. Although the previously reported [13] migration of an ethoxycarbonyl group in a somewhat

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[5] Curtis and Cross, *Chem. and Ind.*, 1954, 1066.
[6] Grove and Mulholland, *J.*, 3007.
[7] Bachmann and Raunio, *J. Amer. Chem. Soc.*, 1950, **72**, 2530.
[8] Mukherjee, *J. Indian Chem. Soc.*, 1948, **25**, 155.
[9] Cf. Ramirez and Kirby, *J. Amer. Chem. Soc.*, 1952, **74**, 4331.
[10] Yanagita, Inayama, and Kitagawa, *J. Org. Chem.*, 1956, **21**, 612.
[11] Gunstone and Tulloch, *J. Appl. Chem.*, 1954, **4**, 291.
[12] *Inter al.*, Conroy and Fireston, *J. Amer. Chem. Soc.*, 1956, **78**, 2290; Cookson and Wariyar, *J.*, 1956, 2302; Curtin and Fraser, *Chem. and Ind.*, 1957, 1358.
[13] Plieninger and Suehira, *Chem. Ber.*, 1956, **89**, 2789.

similar dienone has been interpreted [14] in terms of a 1,2-shift of this group, it seems unlikely that in our compound the ethoxycarbonyl group would migrate more readily than does the methyl group if indeed it would migrate at all. Probably, the 3-hydroxy-2-methylbenzoic acid is produced by some other, as yet unknown mechanism.

EXPERIMENTAL

M. p.s are corrected. Ultraviolet absorption was measured with a Unicam S.P. 500 spectrophotometer.

Decomposition of Gibberellic Acid in Aqueous Solution.—Solutions of gibberellic acid (100 mg.) in sterile water (100 ml.) were stored at room temperature and their absorption at 254—257 mμ measured at intervals. Maximum absorption was normally reached in about 80 days, whereafter it gradually declined. In a typical experiment a solution which had been stored for 35 days showed $\lambda_{\text{max.}}$ 254 mμ ($E^{1\%}_{1\,\text{cm.}}$ 94). It was evaporated at 25—30° under reduced pressure. Chromatography of the residue on Whatman 3 MM. paper by Radley's method [4] afforded two well-defined zones, of R_F 0·83 (gibberellic acid) and 0·61 which were revealed on spraying marker strips with 10 : 1 ethanol–sulphuric acid, drying them at 60—65°, and examining them in ultraviolet light. Elution of the slower-moving zone with 9 : 1 methanol–water afforded a gum (19 mg.) which was extracted with cold acetone. Concentration of the extract to small volume and dilution with benzene yielded amorphous, impure gibberellenic acid (7 mg.), m. p. 153—157° (decomp.), $\lambda_{\text{max.}}$ 254 mμ ($E^{1\%}_{1\,\text{cm.}}$ 239 in EtOH).

Decomposition of Calcium Gibberellate in Aqueous Solution.—A suspension of gibberellic acid (500 mg.) and powdered calcium carbonate (720 mg.) in water (25 ml.) was shaken for 24 hr. and then stored for 166 days with occasional shaking. The mixture was filtered and the residue washed with water. Evaporation of the filtrate at 25—30° under reduced pressure afforded a crude calcium salt [540 mg.; $\lambda_{\text{max.}}$ 255 mμ ($E^{1\%}_{1\,\text{cm.}}$ 197 in ethanol)]. A portion (40 mg.) of this, in water (4 ml.), was treated with 2N-silver nitrate (0·8 ml.). A trace of gelatinous material was immediately filtered off and the filtrate treated with more 2N-silver nitrate (1 ml.). Next morning the resulting precipitate of *silver gibberellenate* was filtered off and washed with small quantities of water (all with protection from light); it was microcrystalline (18 mg.) and had m. p. 154—157° (decomp.), $\lambda_{\text{max.}}$ 256 mμ (ε 16,750, in H_2O (Found: Ag, 39·4. $C_{19}H_{20}O_6Ag_2$ requires Ag, 38·6%).

Isolation of Gibberellenic Acid from Crude Gibberellic Acid.—Recrystallisation of crude gibberellic acid [5] (160 g.) from methanol–water (24 : 65 v/v, 2·5 l.) afforded a mother-liquor (containing about 50 g. of solute) which was concentrated to half volume under reduced pressure. This yielded impure gibberellic acid (22 g.), m. p. 223—225° (decomp.), $\lambda_{\text{max.}}$ 254 mμ ($E^{1\%}_{1\,\text{cm.}}$ 33). The mother-liquor was extracted with ethyl acetate (3 × 600 ml.). The extract was dried (Na_2SO_4) and fractionally concentrated; the last crystalline crop (1·6 g.), m. p. 177—180° (decomp.), $\lambda_{\text{max.}}$ 253 mμ ($E^{1\%}_{1\,\text{cm.}}$ 357 in EtOH), was refluxed with chloroform (1·6 l.), and the residue repeatedly recrystallised from ethyl acetate to give prismatic needles (210 mg.), m. p. 190—192° (decomp.), $[\alpha]_D^{25}$ $-125°$, $\lambda_{\text{max.}}$ 253 mμ (ε 22,400 in EtOH), of gibberellenic acid (Found: C, 65·8; H, 6·4%; equiv., 163. Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·4%; equiv., 173). Like gibberellic acid it gave with concentrated sulphuric acid a red colour which developed a blue fluorescence. Paper chromatography of purified gibberellenic acid on Whatman No. 3 MM. paper, by Radley's method,[4] gave a single spot of R_F 0·68. Run at the same time, gibberellic acid had R_F 0·9.

Methyl 7-*Hydroxy*-1-*methyl*-8-*methylene*-2-*oxo*-10aξ-*gibba*-3,4a(4b)-*diene*-1,10-*dicarboxylate* (II; R = Me, R′ = O).—Addition of a small excess of ethereal diazomethane, at 0°, to gibberellenic acid (209 mg.) in methanol (2 ml.) and evaporation of the resulting solution under reduced pressure afforded methyl gibberellenate (230 mg.), as a viscous gum, $\lambda_{\text{max.}}$ 253 mμ ($E^{1\%}_{1\,\text{cm.}}$ 520 in EtOH). This, in chloroform (21 ml.), was shaken with active manganese dioxide [15] (2 g.) for 23 hr. The mixture was filtered. Evaporation of the filtrate and treatment of the residual gum with ether yielded crystals (124 mg.; m. p. 122—129°) which, on repeated recrystallisation from ether, formed prisms, m. p. 133—136°, of the *dienone* (Found: C, 67·75; H, 6·6. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5%), $\lambda_{\text{max.}}$ 309 mμ (ε 16,500 in EtOH), $\nu_{\text{max.}}$ 3500, 1720, 1682, and 1662 cm.$^{-1}$.

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[14] Christol, Jacquier, and Mousseron, *Bull. Soc. chim. France*, 1959, 11.

[15] Attenburrow, Cameron, Chapman, Evans, Hems, Jansen, and Walker, *J.*, 1952, 1094.

Ethyl 3-*Bromo*-1-*methyl*-2-*oxocyclohexanecarboxylate* (cf. refs. 8, 10, 11).—Ethyl 1-methyl-2-oxocyclohexanecarboxylate [7] (21·4 g.) in dry chloroform (250 ml.) was treated dropwise, with stirring at 0°, with bromine (22·4 g.) in chloroform (100 ml.). The mixture was stirred at room temperature for 10 min., washed with 1% sodium metabisulphite solution, 5% sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and evaporated. The residue, when distilled, gave a fore-run (1·5 g.), b. p. 60—128°/2 mm., and then the *bromo-ketone* (25·2 g.), b. p. 128—136°/2 mm., n^{23} 1·4981, $\lambda_{max.}$ 269 mμ (ε 260 in EtOH) (Found: Br, 30·0. $C_{10}H_{15}O_3Br$ requires Br, 30·4%). With methanolic Brady's reagent at room temperature for 19 hr. it afforded yellow needles (from ethyl acetate), m. p. 141—142°, of *ethyl* 2-(2,4-*dinitrophenylhydrazono*)-3-*methoxy*-1-*methylcyclohexanecarboxylate* (Found: C, 51·6; H, 6·1; N, 14·1. $C_{17}H_{22}N_4O_7$ requires C, 51·8; H, 5·6; N, 14·2%).

Ethyl 1-*Methyl*-2-*oxocyclohex*-3-*enecarboxylate.*—The foregoing bromo-ketone (15·4 g.) was heated with quinoline (23 g.) at 150—160° for 30 min. The cooled mixture was treated with 5N-hydrochloric acid (74 ml.) and extracted with ether. The extract was washed with dilute hydrochloric acid and water, dried (Na_2SO_4), and evaporated. Fractional distillation gave a pale-yellow oil (7·6 g.), b. p. 114—124°/13 mm., n^{21} 1·4775, $\lambda_{max.}$ 225 mμ (ε 8370 in EtOH). This was purified by reheating it with quinoline and working up as above. The unsaturated ketone consisted of a faintly yellow oil, b. p. 120—127°/15 mm., n^{23} 1·4755, $\lambda_{max.}$ 225 mμ (ε 9140 in EtOH). Gunstone and Tulloch [11] give b. p. 113—116°/11 mm., n_D^{15} 1·4810, $\lambda_{max.}$ 226·5 mμ (ε 8910).

Ethyl 5-*Bromo*-1-*methyl*-2-*oxocyclohex*-3-*enecarboxylate.*—Ethyl 1-methyl-2-oxocyclohex-3-enecarboxylate (2·1 g.), in dry carbon tetrachloride (20 ml.), was refluxed with *N*-bromosuccinimide (2·26 g.) over a 150 w lamp for 5 hr. After being cooled, the mixture was filtered; the filtrate was evaporated and the residue distilled to give, after rejection of a small fore-run the pale-yellow oily *bromo-compound* (1·56 g.), b. p. 60—69° (bath-temp.)/0·1 mm., $\lambda_{max.}$ 220 mμ (ε 9820 in EtOH) (Found: C, 46·1; H, 5·1; Br, 29·4. $C_{10}H_{13}O_3Br$ requires C, 46·0; H, 5·0; Br, 30·6%).

Ethyl 1-*Methyl*-2-*oxocyclohexa*-3,5-*dienecarboxylate.*—The foregoing 5-bromo-compound (2·1 g.) was heated, under nitrogen, with purified quinoline (9·5 ml.) at 140° ± 2° (bath) for 30 min. After being cooled to 0°, the mixture was treated with ice-cold 5N-hydrochloric acid (53 ml.) and extracted with ether. The extract was successively washed with 5N-hydrochloric acid, water, dilute sodium hydrogen carbonate, and water. It was dried (Na_2SO_4) and then evaporated. Distillation of the residue afforded the *dienone* as a pale yellow mobile oil (0·51 g.), b. p. 60—66°/0·1 mm., $\lambda_{max.}$ 301 mμ (ε 3900), $\nu_{max.}$ 1735, 1665, 1630, and 1560 cm.$^{-1}$ (liquid film). Analysis of a number of preparations indicated that it formed a *hemihydrate* (Found: C, 63·6, 63·2, 63·7, 63·6; H, 6·9, 7·1, 6·9, 7·0. $C_{10}H_{12}O_3,\frac{1}{2}H_2O$ requires C, 63·5; H, 6·9%); passage of one preparation, in hexane, through a column of activated alumina and recovery by distillation gave the apparently anhydrous dienone (Found: C, 67·0; H, 7·1. Calc. for $C_{10}H_{12}O_3$: C, 66·6; H, 6·7%) but extinction measurements before and after this process indicated that deterioration had occurred. Likewise, storage of another preparation in the refrigerator for 3 weeks resulted in deterioration (ε 3600 $\longrightarrow$ 2740).

The freshly prepared dienone (42 mg.) in ethanol (5 ml.) absorbed hydrogen (1·7 mols.) in the presence of 20% palladium–charcoal (25 mg.), to give a liquid (26 mg.), b. p. 91—100° (bath)/15 mm., which, with methanolic Brady's reagent, afforded yellow plates (29 mg.; from methanol), m. p. 140—141°, of ethyl 2,4-dinitrophenylhydrazono-2-methylcyclohexanecarboxylate (Found: N, 15·5. Calc. for $C_{16}H_{20}N_4O_6$: N, 15·4%) whose identity was confirmed (mixed m. p. and infrared spectrum) by comparison with an authentic sample.

Storage of the dienone (99 mg.), benzene (1·5 ml.), and maleic anhydride (60 mg.) in a sealed tube for 3 days, heating at 100° for 3 hr., and then evaporation of the mixture followed by trituration of the residue with benzene afforded a *maleic anhydride adduct*, prisms, m. p. 142—145° (from benzene) (Found: C, 60·4; H, 5·2. $C_{14}H_{14}O_6$ requires C, 60·4; H, 5·1%).

Heating the dienone (100 mg.), in benzene (2 ml.), with methyl acetylenedicarboxylate (108 mg.) at 100° for 10 hr. followed by evaporation of the mixture and distillation of the residue yielded a *methyl acetylenedicarboxylate adduct monohydrate* as a pale-yellow, viscous oil (116 mg.), b. p. 130—135° (bath)/0·05 mm. (Found: C, 56·8; H, 5·9. $C_{16}H_{18}O_7,H_2O$ requires C, 56·5; H, 5·9%).

Action of Warm, Dilute Sulphuric Acid on Ethyl 1-*Methyl*-2-*oxocyclohexa*-3,5-*dienecarboxylate.*—The dienone (250 mg.) was heated at 56° (bath) with 18N-sulphuric acid (25 ml.), with frequent stirring, for 22 hr. The mixture was poured into water and extracted with ether. The extract was dried (Na_2SO_4) and then evaporated. The residual gum (143 mg.) was fractionally distilled, giving (i) a mobile liquid (27 mg.), b. p. 99—104° (bath)/13 mm., and (ii) a viscous oil (62 mg.), b. p. 185—220° (bath)/13 mm. Redistillation of fraction (i) afforded *o*-cresol (25 mg.) which with 3,5-dinitrobenzoyl chloride in pyridine yielded plates (from methanol), m. p. 137—138°, which were identical with authentic *o*-tolyl 3,5-dinitrobenzoate.

Fraction (ii) was dissolved in ether and extracted with 2N-potassium hydrogen carbonate. Recovery afforded a neutral gum (32 mg.), which, in benzene, was chromatographed on alumina (1·5 g.). Elution with 9 : 1 benzene–ether yielded a gum (22 mg.) which distilled at 142—146° (bath)/0·05 mm. as a viscous oil which crystallised. This presumably dimeric *substance* showed carbonyl absorption at 1701 and 1610 cm.$^{-1}$ (Found: C, 76·4; H, 8·3. $C_{14}H_{18}O_2$ requires C, 77·0; H, 8·3%). The potassium hydrogen carbonate extract was acidified with dilute hydrochloric acid and extracted with ether. Evaporation of the extract yielded a crystalline residue (24 mg.) which, on repeated fractional sublimation at 118—125°/0·05 mm., formed prisms (15 mg.), m. p. 141—143°, of 3-hydroxy-2-methylbenzoic acid (Found: C, 62·4; H, 5·6. Calc. for $C_8H_8O_3$: C, 63·1; H, 5·3%), whose identity was proved on comparison (mixed m. p.; infrared spectrum) with an authentic specimen prepared by Baudisch and Perkin's method.[16]

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[16] Baudisch and Perkin, *J.*, 1909, **95**, 1883.