New Intermediates in the Aqueous Decomposition of Gibberellic Acid

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The decomposition pathway of gibberellic acid (I) through gibberellenic acid (III) in aqueous solution has been investigated. Decomposition of gibberellenic acid in deuterium oxide gives [9-^{2}H]allogibberic acid (V), [9-^{2}H]-9-*epi*-allogibberic acid (VI), and unlabelled 9,11-didehydroallogibberic acid (IX). Two partially characterised hydroperoxides (XV) and (XVII) have been detected as intermediates in the oxidative transformation of gibberellic acid through gibberellenic acid to 9,11-didehydroallogibberic acid. Mechanisms are proposed on the basis of these results.

AQUEOUS solutions of gibberellic acid (I) [1] decompose to give isogibberellic acid (II), gibberellenic acid (III), allogibberic acid (V), 9-*epi*-allogibberic acid (VI), and 9,11-didehydroallogibberic acid (IX) in differing proportions depending on reaction time, temperature, and pH.[2,3] The overall pathway shown in Scheme 1 is suggested by the results of earlier work with unbuffered aqueous solutions (*ca.* pH 3) at 100 and 120°.[2] The ring A aromatic products, allogibberic acid (V) and 9-*epi*-allogibberic acid (VI), including the oxidation product 9,11-didehydroallogibberic acid (IX) were shown to arise *via* gibberellenic acid (III), and some evidence for an intermediate, unstable triene ($\lambda_{max.}$ 326 nm) of suggested structure (IV) was obtained.[2] The present paper describes experiments leading to a more detailed understanding of the decomposition pathway of gibberellenic acid, and hence gibberellic acid, in aqueous solution. Throughout this work unbuffered (*ca.* pH 3) solutions were used.

Two pathways, *a* and *b* (Scheme 1), can be postulated for the formation of the triene (IV) from gibberellenic acid. Allogibberic acid (V) and 9-*epi*-allogibberic acid (VI) could then be formed by rearrangement of the triene as shown and oxidation (see below) followed by rearrangement could lead to 9,11-didehydroallogibberic acid (IX). Pathway *b* has been tacitly assumed to be operating in the conversion of [11,11-$^3H_2$]gibberellic acid, labelled biosynthetically from (5*R*)- and (5*S*)-[5-^{3}H]-mevalonic acid, into allogibberic acid, apparently without loss of either 11-tritium.[4,5]

To distinguish between pathways *a* and *b* gibberellenic acid was decomposed in boiling deuterium oxide (>99·7% ^{2}H) under nitrogen. The product, consisting of allogibberic acid (77%) and 9-*epi*-allogibberic acid (11%) together with some inadvertently obtained oxidation product, 9,11-didehydroallogibberic acid (13%), was methylated and analysed by combined g.l.c.–mass spectrometry (g.l.c.–m.s.). Methyl 9,11-didehydroallogibberate (X) contained no deuterium, and methyl allogibberate (VII) and methyl 9-*epi*-allogibberate (VIII) each contained one deuterium atom per molecule, 92 and 69% ^{2}H respectively. Labelled methyl allogibberate and 9-*epi*-allogibberate were isolated by repetitive t.l.c. and their n.m.r. spectra, compared with the spectra of the unlabelled compounds (Table 1), showed that the deuterium atom was exclusively at C-9 in both cases. N.m.r. measurements of deuterium contents were in agreement with the g.l.c.–m.s. results. Confirmation of these deuterium labelling results was obtained as follows. Methyl [^{2}H]allogibberate obtained above was epimerised with alkali and remethylated to give methyl 6-*epi*-allogibberate (XI)[6] which from its n.m.r. spectrum (Table 1) and mass spectrum was shown to be methyl [9-^{2}H]-6-*epi*-allogibberate (94% ^{2}H).

[1] The gibberellin numbering system used here is as proposed in J. W. Rowe, 'The Common Systematic Nomenclature of Cyclic Diterpenes,' 3rd edn., October 1968, U.S. Forest Products Laboratory, Madison, Wisconsin: the proposals are currently before the I.U.P.A.C. Commission on Nomenclature. Older names of the aromatic gibberellic acid derivatives have been modified to suit this new nomenclature.

[2] R. J. Pryce, *Phytochemistry*, 1973, **12**, 507, and references therein.

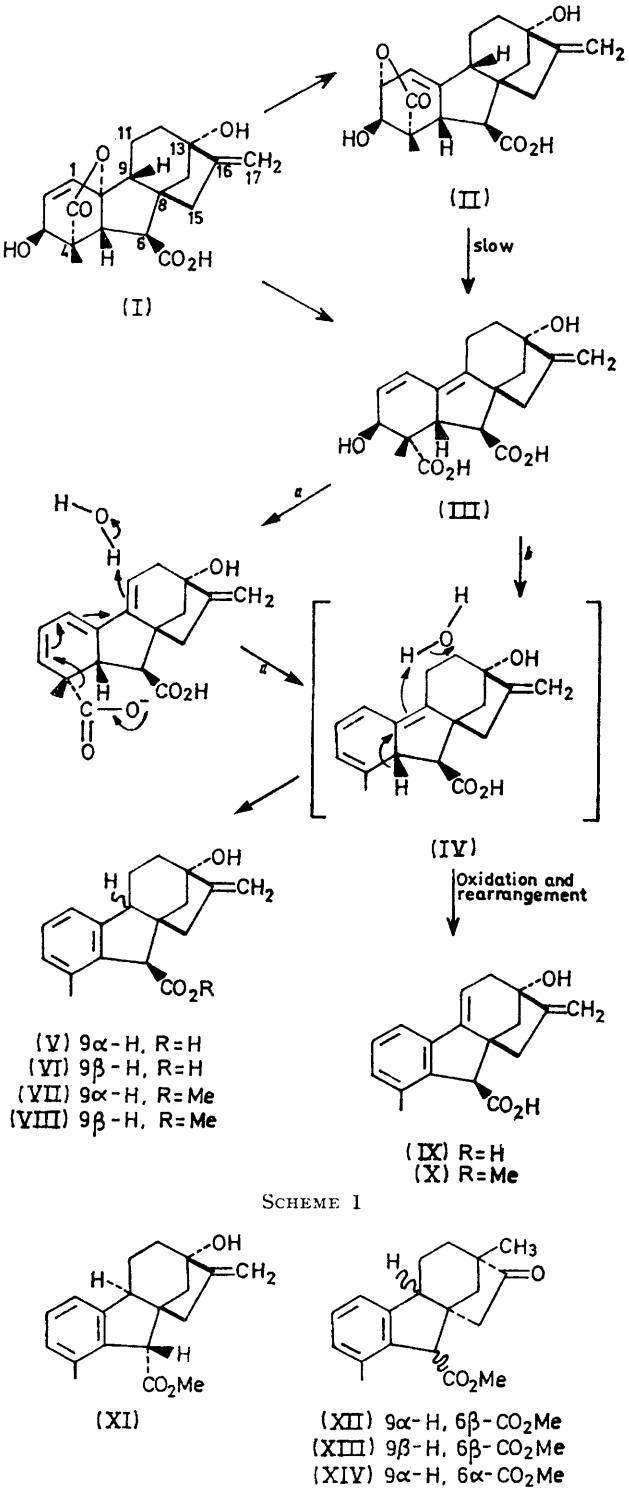
[3] R. J. Pryce, *Phytochemistry*, 1973, **12**, 1745.

[4] R. Evans, J. R. Hanson, and A. F. White, *J. Chem. Soc.* (*C*), 1970, 2601.

[5] R. Evans, J. R. Hanson, and L. J. Mulheirn, *J.C.S. Perkin I*, 1973, 753.

[6] J. F. Grove and T. P. C. Mulholland, *J. Chem. Soc.*, 1960, 3007.

Wagner–Meerwein rearrangement [7] of these three [9-^{2}H]-allogibberates, (VII), (VIII), and (XI), followed by



SCHEME 1

remethylation, provided methyl [9-^{2}H]gibberate [(XII) 92% ^{2}H], methyl [9-^{2}H]-9-*epi*-gibberate [(XIII) 61% ^{2}H], and methyl [9-^{2}H]-6-*epi*-gibberate [(XIV) 94% ^{2}H] which were analysed by combined g.l.c.–m.s.; no loss of 9-^{2}H was expected [7] and no significant change was observed. These stereospecifically labelled [9-^{2}H]allogibberates and gibberates have been used to investigate a stereochemically controlled loss of a methyl formate fragment in their mass spectra which are described in detail elsewhere.[8]

The deuterium labelling results just described exclude pathway *a* which required deuterium to be incorporated at C-9 and C-11 of allogibberic and 9-*epi*-allogibberic acids, and possibly at C-11 of 9,11-didehydroallogibberic acid. Pathway *b* satisfactorily accounts for all observed products from gibberellenic acid and the deuterium oxide decomposition results. The lower level of 9-^{2}H incorporation into methyl 9-*epi*-allogibberate (VIII) (69% ^{2}H) compared with methyl allogibberate (VII) (92% ^{2}H at C-9) could be due to some [1,3] suprafacial proton transfer of the 5β-H in the triene (IV) to the 9-position of (VIII). Similar base catalysed [1,3] suprafacial proton transfers have been observed in the indene system; [9] in the present case deuterium oxide would act as the carrier base in the intermediate contact ion-pair.

9,11-Didehydroallogibberic (IX) has been fully characterised as the major oxidised aqueous-decomposition product of gibberellic and gibberellenic acids.[2] At some pH's two uncharacterised methyl esters (W) and (Y) have been detected in methylated decomposition products and their mass spectra indicate that they are isomeric with methyl 9,11-didehydroallogibberate.[2] Time-course studies of the decomposition of gibberellic and gibberellenic acids lead to the suggestion that the acids corresponding to (W) and (Y) were decomposition products of the triene intermediate (IV) produced during work-up.[2] To further investigate the nature of (W) and (Y), aqueous gibberellenic acid was boiled under nitrogen for 5 min. The resulting solution, containing a good yield of the supposed triene (IV) as judged by u.v. spectrophotometry, was then aerated at room temperature to destroy the triene.[2] After work-up an aliquot portion of the total product (Q) was methylated and analysed by g.l.c.–m.s. where it showed the composition in Table 2. The remainder of the oxidised decomposition product (Q) was boiled again in aqueous solution under nitrogen for a period which would usually complete the decomposition of gibberellenic acid. The composition of this final decomposition product (R), determined by t.l.c. and g.l.c.–m.s., is also shown in Table 2. Complete decomposition of aqueous gibberellenic acid under nitrogen at 100° gives mainly allogibberic acid and little or no 9,11-didehydroallogibberic acid.[2] Therefore the results in Table 2 suggest that the methyl esters (W) and (Y) are either methyl esters of real intermediates or thermal decomposition products produced during g.l.c. (200°), of other intermediate oxidation products of the triene (IV) *en route* to 9,11-didehydroallogibberic acid.

[7] J. F. Grove, J. MacMillan, T. P. C. Mulholland, and W. B. Turner, *J. Chem. Soc.*, 1960, 3049.

[8] R. T. Gray and R. J. Pryce, *J.C.S. Perkin II*, in the press.

[9] J. Almy and D. J. Cram, *J. Amer. Chem. Soc.*, 1969, **91**, 4459, and references therein.

T.l.c. analysis of the product (Q) from a second experiment showed two components, (A) and (B), in addition to methyl allogibberate, methyl 9,11-didehydroallogibberate, and dimethyl gibberellenate. These two potential intermediates (A) and (B) were isolated as their methyl esters in 28 and 18% yields respectively and therefore together with the allogibberic acid and 9,11-didehydroallogibberic acid they accounted for all the gibberellenic acid decomposition products. The following results suggest that the methyl esters (A) and (B) are the methyl esters (XVI) and (XVIII) of the hydroperoxides (XV) and (XVII) respectively.

TABLE 1

100 MHz ^{1}H N.m.r. data and assignments (τ values; solutions in $CDCl_3$; J and $W_{\frac{1}{2}}$ in Hz)

Compound	6-H	9-H	17-=CH_2	6-CO_2Me	4-Me	1-, 2-, and 3-H
Methyl allogibberate (VII)	6·03 (s)	7·18 (q) J 11·5 and 4·5	5·00 (t), 5·22 (t) J 3 J 2	6·22 (s)	7·84 (s)	*ca.* 3·0 (m)
Methyl 9-*epi*-allogibberate (VIII)	6·35 (s)	6·55br (s) $W_{\frac{1}{2}}$ 12	4·83 (t), 4·96 (t) J 2·5 J 2	6·35 (s)	7·78 (s)	*ca.* 3·0 (m)
Methyl 6-*epi*-allogibberate (XI)	6·36 (s)	6·63 (q) J 12 and 5	5·00 (t), 5·25 (t) J 2·5 J 2	6·32 (s)	7·78 (s)	*ca.* 3·0 (m)
Hydroperoxide methyl esters (A) (XVI)	6·34 (s), 6·42 (s) (*ca.* 1 : 1)		4·81 (t), 4·93 (t) J 3 J 2	6·23 (s), 6·30 (s) (1 : 0·93) [a]	7·75 (s)	*ca.* 2·8 (m)
Hydroperoxide methyl esters (B) (XVIII)	5·65 (s)		4·93 (t), 5·19 (t) J 2 J 2	6·22 (s)	7·80 (s)	*ca.* 2·8 (m)

[a] Ratio of peak heights changes on addition of D_2O to (1 : 1·13) and on raising the temperature to 71° to (1 : 1·31).

TABLE 2

Esters (W) and (Y) in methylated products of partially decomposed aqueous solutions of gibberellenic acid (III)

Compound	Composition (%) [a] Methylated product (Q) [b]	Methylated product (R) [c]
Dimethyl gibberellenate (W)	27	
(Y)	31	
Methyl, 9,11-didehydroallogibberate (X)	5	
Methyl allogibberate (VII)	12	65
Others	17	25
	8	10

[a] By g.l.c.–m.s. [b] From 5 min boiling of aqueous gibberellenic acid under nitrogen followed by aeration of the product at room temperature to give product (Q). [c] From boiling the product (Q) in water under nitrogen for 1 h.

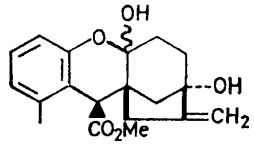
The methyl esters (A) and (B) give strong positive reactions with potassium iodide–starch reagent [10] on t.l.c. Their u.v. spectra, (A), $\lambda_{max.}$ (EtOH) 258 nm (ε 200) and (B), $\lambda_{max.}$ (EtOH) 252 nm (ε 550), suggest an aromatic chromophore and this is consistent with the observation that at room temperature air-destruction of the 326 nm chromophore of the triene (IV), present in partly decomposed gibberellic or gibberellenic acid solutions, does not produce any species with strong u.v. absorption above 240 nm. The n.m.r. spectra of (A) and (B) methyl esters and assignments are compared with the allogibberates in Table 1. The spectrum of the hydroperoxide methyl ester (B) shows a pronounced downfield shift of the 6α-H compared with (A) methyl ester whose 6α-H appears at a similar chemical shift to that of methyl 9-*epi*-allogibberate. This deshielding of the 6α-H in methyl ester (B) (XVIII) is attributed to the 9-hydroperoxide group which is *cis* to the 6α-H. The n.m.r. spectrum of methyl ester (A) (XVI) shows CO_2Me and 6α-H as doublets and this is attributed to hydrogen bonding between the 9β-hydroperoxide and the 6β-CO_2Me group; the ratio of the peak heights of the methoxycarbonyl methyl signal changes with addition of deuterium oxide and elevation of temperature (see Table 1). The spectrum of methyl ester (B) shows no such hydrogen bonding. These n.m.r. assignments and the close similarities with the spectra of the allogibberates are the chief reasons for assigning the structures and relative stereochemistries of methyl ester (A) (XVI) and methyl ester (B) (XVIII). From the n.m.r. spectra of the allogibberates in Table 1 it appears that the 17-methylene protons resonate at higher field and are more separated when the B/C-ring junction is *trans* as in methyl allogibberate (VII) and methyl 6-*epi*-allogibberate (XI) compared with the *cis*-B/C fused methyl 9-*epi*-allogibberate (VIII). This n.m.r. correlation applies very well to the proposed structures for methyl ester (A) (XVI) (*cis*-C/D-ring fusion with 17-methylene protons close to those of methyl 9-*epi*-allogibberate), and for methyl ester (B) (XVIII) (*trans*-C/D-ring fusion with 17-methylene protons close to those of methyl allogibberate). I.r. spectra of (A) and (B) methyl esters are consistent with the proposed structures.

The esters (A) and (B) were boiled in aqueous 0·001M-hydrochloric acid (pH 3) to give, after remethylation, methyl 9,11-didehydroallogibberate (X) in 50—60% yield as judged by u.v. spectrophotometry and in 73 and 85% yield respectively by g.l.c.–m.s. analysis. The ester (A) (XVI) was little changed by boiling in water alone, but the decomposition at pH 3 was closer to the situation of the aqueous decomposition of the gibberellins.

Two other products of aqueous acid treatment of methyl ester (A) were detected by g.l.c.–m.s. Both showed parent ions at *m/e* 312 (*cf.* methyl 9,11-didehydroallogibberate, P^+ 296) and could therefore be dehydration products of the anticipated cumene–phenol type rearrangement products (XIX). These latter two products (P^+ 312) were very minor components of the product derived from methyl ester (B) and were not normally detected in gibberellic and gibberellenic acid decomposition products.

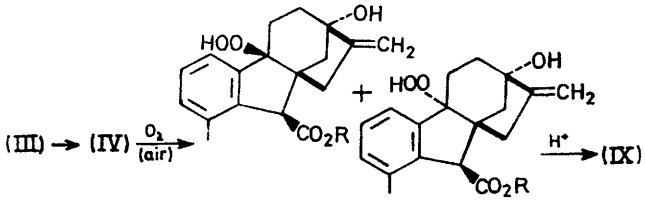
[10] D. Waldi, 'Thin-layer Chromatography,' ed. F. Stahl, Springer, Berlin, 1965, p. 494.

G.l.c. (210°) of the esters (A) and (B) apparently produced only thermal decomposition products giving peaks corresponding to (W), (Y), and methyl 9,11-didehydroallogibberate. The products of reduction of



esters (A) and (B) using sodium iodide, presumably the corresponding 9-alcohols, gave similar thermal decomposition products on g.l.c.

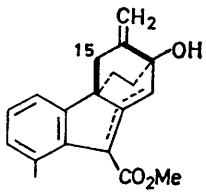
The detection of the isomers of methyl 9,11-didehydroallogibberate, (W) and (Y), by g.l.c.–m.s. in partially transformed aqueous solutions of gibberellic and gibberellenic acids [2] and the foregoing results therefore indicate that the two hydroperoxides (XV) and (XVII) are normal intermediates in the oxidative aqueous



(XV) R = H (XVII) R = H
(XVI) R = Me (XVIII) R = Me

SCHEME 2

decomposition of gibberellic and gibberellenic acids through the triene (IV) as shown in Scheme 2. Aerobic oxidation at the diallylic C-5 position of (IV) and rearrangement could lead to (A) (XV) and (B) (XVII).



(XX)

The methyl esters (W) and (Y) are thermal decomposition products of the two esters (A) (XVI) and (B) (XVIII) produced on g.l.c. and they may be rearrangement products of the type (XX). This latter proposal is supported by the occurrence of $P^+ - 28$ ions in the mass spectra of (W) and (Y), which could arise by loss of ethylene by retro-Diels–Alder fragmentation of rings C/D of (XX). The mass spectrum of methyl 9,11-didehydroallogibberate shows a similar fragmentation but of lower intensity. Compounds of the same skeleton as (XX), derived chemically from methyl allogibberate (VII) *via* species presumed to contain a 9-carbocation, have recently been described by Cross and Markwell.[11] The possibility of direct oxidation at C-9 of the allogibberic acids leading to 9,11-didehydroallogibberic acid is excluded since allogibberic and 9-*epi*-allogibberic acids remain unchanged (g.l.c. analysis) in aqueous solution even after autoclaving at 120° for 20 min.

The major decomposition product of gibberellic acid in aqueous solution, isogibberellic acid (II),[2,3] has previously been shown to be formed without incorporation of deuterium from deuterium oxide solution and therefore probably arises by intramolecular allylic rearrangement.[12] Further slow decomposition of isogibberellic acid, which is a minor pathway to (V), (VI), and (IX) at 120°,[2] could proceed *via* gibberellenic acid (Schemes 1 and 2). At room temperature, aqueous solutions of gibberellic acid decompose slowly to the same products [2,3] as at 100 or 120° and probably by the mechanism described here.

EXPERIMENTAL

Unlabelled allogibberates and gibberates were prepared by published procedures.[2] 6-*epi*-Allogibberic acid was prepared by the published procedure [6] and rearranged to 6-*epi*-gibberic acid as described below for the labelled compound. All methylations were performed by treatment of methanolic solutions of the acids with an excess of ethereal diazomethane for 5 min. T.l.c. (silica gel layers), g.l.c., and combined g.l.c.–mass spectrometry (g.l.c.–m.s.) were performed as previously described.[2] Deuterium contents were calculated from mass spectra obtained by multiple scanning of the molecular ion region. U.v. spectra were taken for ethanol solutions on a Unicam SP 800 spectrophotometer. I.r. spectra were taken for KBr discs on a Grubb–Parsons GS4 spectrophotometer. N.m.r. spectra were obtained with a Varian HA 100 spectrometer for deuteriochloroform solutions with tetramethylsilane as internal standard. Light petroleum had b.p. 60—80°.

Decomposition of Gibberellenic Acid in Deuterium Oxide.—Gibberellenic acid (III) (50 mg) was dissolved in deuterium oxide (>99·7% ^2H; 50 ml) and dry nitrogen was bubbled through the solution at room temperature for 20 min prior to boiling under nitrogen for 3 h. When cool, the reaction mixture was evaporated *in vacuo* at 30—35° and the dry product was methylated. G.l.c.–m.s. analysis of the methylated product showed it to contain methyl [^2H]allogibberate [(VII) 77%; 92% ^2H], methyl [^2H]-9-*epi*-allogibberate [(VIII) 11%; 69% ^2H], and methyl didehydroallogibberate [(X) 13%; 0% ^2H]. After isolation of [^2H]-(VII) and [^2H]-(VIII) as described below, n.m.r. spectra showed that the deuterium atom was at C-9 in both compounds [(VII) *ca.* 100% ^2H and (VIII) 63% $^2H_1$ by n.m.r. measurements]. Methyl [9-^2H]allogibberate and methyl [9-^2H]-9-*epi*-allogibberate were isolated from the methylated reaction product by preparative t.l.c. (p.l.c.): three developments in chloroform gave methyl [9-^2H]allogibberate (VII) (R_F 0·37, 22 mg, containing methyl 9,11-didehydroallogibberate, 8%, by g.l.c.) and a mixture of all three products (R_F 0·45, 8 mg); rechromatography of the latter mixture using 2 developments in ethyl acetate–light petroleum (1 : 1) gave methyl [9-^2H]-9-*epi*-allogibberate (VIII) (R_F 0·62, 2 mg, 99% pure by g.l.c.) and a mixture of all three original products (R_F 0·57, 3 mg).

Methyl [9-^2H]-6-epi-*Allogibberate* (XI).—Methyl [9-^2H]-

[11] B. E. Cross and R. E. Markwell, *J.C.S. Perkin I*, 1973, 1476.
[12] D. C. Aldridge, J. F. Grove, R. N. Speake, B. K. Tidd, and W. Klyne, *J. Chem. Soc.*, 1963, 143.

allogibberate prepared above (7·5 mg) was refluxed with 1·5N-sodium hydroxide (5 ml) under nitrogen for 2 h. The cooled product was acidified with 2N-hydrochloric acid and extracted with ethyl acetate (3 × 7 ml). The dried (Na_2SO_4) extract was evaporated to give a gum which was methylated. G.l.c.–m.s. analysis of the methylated product showed it to contain methyl [9-^{2}H]-6-*epi*-allogibberate [(XI) 92%; 94% ^{2}H)] and one unknown product (8%). N.m.r. analysis of the methylated product confirmed that the deuterium was at C-9 (*ca.* 100% ^{2}H by n.m.r.).

Methyl [9-^{2}H]*Gibberate* (XII), *Methyl* [9-^{2}H]-9-epi-*Gibberate* (XIII), *and Methyl* [9-^{2}H]-6-epi-*Gibberate* (XIV).—Labelled esters [9-^{2}H]-(VII), [9-^{2}H]-(VIII), and [9-^{2}H]-(XI) obtained above were each treated in the same manner. Each compound (*ca.* 2 mg) was boiled for 1 h in 1N-hydrochloric acid (2 ml). After cooling, the product was evaporated to dryness and remethylated prior to g.l.c.–m.s. analysis: [9-^{2}H]-(VII) gave methyl [9-^{2}H]gibberate [(XII) 92% ^{2}H], [9-^{2}H]-(VIII) gave methyl [9-^{2}H]-9-*epi*-gibberate [(XIII) 61% ^{2}H], and [9-^{2}H]-(XI) gave methyl [9-^{2}H]-6-*epi*-gibberate [(XIV) 94% ^{2}H]; each product was >90% pure by g.l.c. analysis.

The Nature of the Methyl Esters (W) *and* (Y).—Gibberellenic acid (III) (10 mg) was dissolved in water (10 ml) and nitrogen was bubbled through the solution for 20 min at room temperature before boiling the solution for 5 min under nitrogen. The solution was then rapidly cooled in an ice-bath and had a strong u.v. chromophore at 326 nm which was completely destroyed by bubbling air through the solution for 20 min at room temperature. This oxidised solution was evaporated to dryness *in vacuo* at 30—35° to give product (Q), an aliquot portion of which was methylated and analysed by g.l.c.–m.s. (200°, XE-60 column) (see Table 2). The bulk of the product (Q) (7 mg) was redissolved in water (7 ml) and after bubbling nitrogen through the solution at room temperature for 20 min, the solution was boiled for 1 h under nitrogen then cooled and evaporated to dryness as before to give product (R). An aliquot portion of the final product was methylated and analysed by g.l.c.–m.s. (Table 2). T.l.c. and u.v. analysis of the product (R) confirmed 9,11-didehydroallogibberic and allogibberic acids as the major final products.

Mass spectra of (W) and (Y) in the product (Q) above: (W), *m/e* (%) 296 (43), 281 (4), 278 (3), 268 (9), 264 (12), 254 (6), 253 (5), 249 (4), 237 (100), 221 (26), 219 (28), 209 (51), 208 (60), 202 (11), 195 (30), 194 (26), 193 (40), 179 (33), 165 (34), 155 (24), 143 (12), 128 (15), 115 (15), 95 (10), and 43 (25); (Y), 296 (60), 281 (6), 278 (4), 268 (4), 264 (13), 254 (14), 249 (8), 237 (100), 229 (6), 221 (63), 219 (36), 213 (10), 209 (62), 208 (42), 202 (26), 195 (62), 194 (75), 193 (100), 179 (70), 165 (58), 155 (27), 143 (12), 127 (11), 115 (11), 95 (6), and 43 (25); *cf.* methyl 9,11-didehydroallogibberate (X), 296 (60), 281 (4), 278 (3), 268 (2), 264 (6), 254 (3), 249 (3), 237 (63), 221 (41), 219 (22), 209 (38), 202 (18), 193 (95), 179 (81), 165 (100), 155 (20), 152 (90), 141 (25), 115 (25), 108 (21), 95 (25), and 43 (77).

The Hydroperoxide Methyl Esters (A) *and* (B).—Gibberellenic acid (10 mg) was dissolved in water (10 ml) and nitrogen was bubbled through the solution at room temperature for 20 min; the solution was then boiled for 5 min under nitrogen. After rapid cooling in an ice-bath the solution was evaporated to dryness *in vacuo* at 30—35° prior to methylation. G.l.c. analysis of the methylated product indicated the following composition, dimethyl gibberellenate (32), (W) (29), (Y) (7), methyl 9,11-didehydroallogibberate (10), methyl allogibberate (10), and others (12%). T.l.c. of the methylated product [2 elutions in ethyl acetate–light petroleum (1 : 1)] showed, apart from spots corresponding to methyl allogibberate (R_F 0·58) and dimethyl gibberellenate (R_F 0·28), two spots corresponding to methyl ester (A) (R_F 0·47) and methyl ester (B) (0·40). Methyl ester (A) (2·8 mg; gum) and methyl ester (B) (1·8 mg; gum) were isolated by p.l.c. in the system just described and on rechromatography of the isolated compounds they both showed one spot (u.v. and iodine vapour visualisation) which gave a positive peroxide response to the potassium iodide–starch spray reagent; [10] no response was given by the other reaction products. G.l.c. (210°, XE-60 column) of methyl ester (A) showed peaks corresponding to methyl 9,11-didehydroallogibberate (12), (W) (78), and others (10%) and methyl ester (B) showed peaks corresponding to methyl 9,11-didehydroallogibberate (28), (W) (40), (Y) (19), and others (17%): these compositions varied with g.l.c. conditions. The n.m.r. spectra of methyl esters (A) and (B) are shown in Table 1. Methyl ester (A) (XVI), $\lambda_{max.}$ (EtOH) 258 nm (ε 200), $\nu_{max.}$ 3400, 2930, 2850, 1710, 1650, 1203, 990, 885, 787, and 783 cm^{-1}. Methyl ester (B) (XVIII) $\lambda_{max.}$ (EtOH) 252 (ε 550), $\nu_{max.}$ 3400, 2935, 2850, 1725, 1655, 1163, 1002, 893, 787, and 776 cm^{-1}.

Methyl esters (A) and (B) were each reduced with sodium iodide as follows. The ester (0·5 mg) in methanol (0·5 ml) was added to a solution of sodium iodide [1 ml of a solution of sodium iodide (600 mg) in methanol (10 ml)]. A yellow-orange colour formed immediately and the solution was left overnight at room temperature. The product was extracted into ethyl acetate (20 ml) and after washing with sodium thiosulphate solution, the extract was dried (Na_2SO_4) and evaporated to dryness *in vacuo*. T.l.c. of the reduction products from both methyl esters (A) and (B) [one elution in ethyl acetate–light petroleum (1 : 1)] showed both products to consist of one major product and no detectable starting material (potassium iodide–starch reagent); reduced methyl ester (A) [R_F 0·23, *cf.* methyl ester (A) 0·22], reduced methyl ester (B) [R_F 0·15, *cf.* methyl ester (B) 0·18]. The reduction products from esters (A) and (B) had $\lambda_{max.}$ (EtOH) 260 and 255 nm respectively. G.l.c. (210°, XE-60 column) of the reduction products showed very similar compositions to the range of thermal decomposition products from their corresponding hydroperoxides above—small quantitative differences only could be seen.

Treatment of the Hydroperoxide Methyl Esters (A) (XVI) *and* (B) (XVIII) *with Dilute Mineral Acid.*—Both esters were treated similarly. The ester (1 mg) was boiled in 0·001N-hydrochloric acid (11 ml) for 1 h under nitrogen, then the cooled mixture was evaporated *in vacuo* at 35—40° and the dry product was methylated. The methylated product from esters (A) and (B) were analysed by u.v., t.l.c., and g.l.c.–m.s. T.l.c. showed one major component corresponding to methyl 9,11-didehydroallogibberate (X) in both products—no starting materials could be detected. As judged by their u.v. spectra [2] both products contained methyl 9,11-didehydroallogibberate (50—60%). G.l.c.–m.s. of the product from ester (A) confirmed the presence of methyl 9,11-didehydroallogibberate (73%) together with two products with higher retention time (C) (7) and (D) (13), and others (7%); (C), *m/e* (%), 312 (1), 311 (1), 310 (6), 298 (51), 296 (30), 280 (10), 265 (7), 254 (41), 252 (40), 237 (56), 227 (39), 213 (100), 209 (54), 195 (44), 148 (39), and 91 (71), (D), 312 (2), 310 (1), 298 (21), 296 (4), 280 (9), 265 (7), 254 (35), 252 (36), 237 (43), 226 (37), 213 (100),

209 (48), 195 (36), 148 (49), 105 (48), and 91 (99). G.l.c.–m.s. analysis of the product from ester (B) confirmed the presence of methyl 9,11-didehydroallogibberate (85%); other products (15%) which include $<1\%$ each of the products (C) and (D) from ester (B) above were also detected.

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