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DECOMPOSITION OF AQUEOUS SOLUTIONS OF GIBBERELLIC ACID ON AUTOCLAVING

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Key Word Index—Gibberellic acid; decomposition products; aqueous autoclaving; GLC; GLC–MS; dehydroallogibberic acid; allogibberic acid; bioassay.

Abstract—A qualitative and quantitative analysis of the decomposition of unbuffered and buffered (pH 3–8) aqueous solutions of gibberellic acid (GA_3) on autoclaving is recorded. The identified products, which vary in composition with pH, are *iso*-GA_3 (II), *iso*-GA_3 hydroxy acid (III), gibberellenic acid (IV), allogibberic acid (V), epiallogibberic acid (VI), and dehydroallogibberic acid (VII). Only 1–2% GA_3 can be detected after autoclaving in all cases. The identified products, in all cases, account for not less than 95% of the decomposition product, Dehydroallogibberic acid has not previously been recorded as an aqueous decomposition product of GA_3 and its biological activity in the lettuce hypocotyl test is recorded.

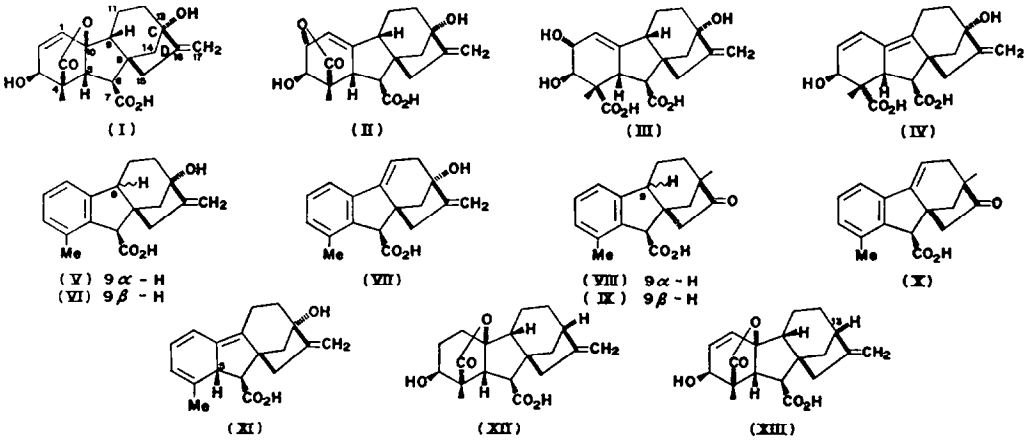
INTRODUCTION

GIBBERELLIC ACID (GA_3) (I) is well known to be unstable in aqueous solutions even at room temp. and the major decomposition products from aqueous solutions at various pH values and temperatures have been characterized.[1–3] Autoclaving aqueous solutions of GA_3 for aseptic plant culture media is known to produce a marked drop in gibberellin-like biological activity to an extent depending on the pH of the solutions.[4–7] GA_3 applied to the duckweed *Lemna perpusilla* Torr., strain 6746 increases frond multiplication, decreases frond size, and inhibits flowering; but these effects are much more marked when aqueous GA_3 solutions are autoclaved before being applied.[8] This suggested[8] production of an active substance or substances on autoclaving. As one approach to determining which substance or substances from GA_3 are responsible for the observed effects on *L. perpusilla* a detailed qualitative and quantitative analysis of decomposition of buffered and unbuffered aqueous solutions of GA_3 on autoclaving has been carried out and is reported here. The only previously attempted, essentially qualitative, analysis recorded[9] of aqueous decomposition of GA_3 at temperatures up to and including 100° at various pH values was based on UV spectroscopy and single system PC evidence only; present results show that the UV evidence was misinterpreted.

[1] B. E. CROSS, J. F. GROVE and A. MORRISON, *J. Chem. Soc.* 2498 (1961).
[2] J. F. GROVE and T. P. C. MULHOLLAND, *J. Chem. Soc.* 3007 (1960).
[3] J. S. MOFFAT, *J. Chem. Soc.* 3045 (1960).
[4] J. H. M. HENDERSON, *Nature, Lond.* **185**, 628 (1960).
[5] J. H. M. HENDERSON and H. D. GRAHAM, *Nature, Lond.* **193**, 1055 (1962).
[6] W. S. HILLMAN, *Phyton*, **14**, 49 (1960).
[7] J. VAN BRAGT and R. L. M. PIERIK, in *Effects of Sterilisation of Components in Nutrient Media* (edited by J. VAN BRAGT, D. A. A. MOSSEL, R. L. M. PIERIK and H. VELDSTRA), p. 133, Veenman, Wageningen (1971).
[8] H. K. HODSON and K. C. HAMNER, *Plant Physiol.* **47**, 726 (1971).
[9] I. KUHR, *Folia Mikrobiol.* **7**, 358 (1962).

RESULTS AND DISCUSSION

The results of analyses of autoclaved GA_3 in buffered and unbuffered aqueous solutions are shown in Table 1. Products were identified by direct comparison with authentic reference compounds in TLC, GLC and combined GLC–MS.



All the decomposition products listed in Table 1, except dehydroallogibberic acid and unknowns *W* and *Y* (see below), are previously known aqueous decomposition products of GA_3. No rearrangement[10] of the C/D-rings of GA_3 to gibberic (VIII) or epigibberic acids (IX) was observed. Very little GA_3 remains in the autoclave products and what was detected in the worked-up products might have been formed by isomerization of *iso*-GA_3, but this is unknown. The *iso*-GA_3 detected in worked-up, autoclaved GA_3 would exist mainly in the delactonised form (III) at alkaline pH.[1] Therefore the compositions recorded in Table 1 may not be the same as the actual compositions prior to work up in terms of GA_3, *iso*-GA_3 and (III). The composition of autoclaved, unbuffered aqueous solutions of GA_3 varies a little in the proportion of each product depending on the sample of distilled water used and its pH. However, results are always essentially as shown in Table 1.

The detection of dehydroallogibberic (VII) as an aqueous decomposition product of GA_3 is supported by additional evidence. The UV spectrum of autoclaved, unbuffered aq. GA_3 solutions contained a series of peaks with $\lambda_{max}^{H_2O \text{ or } EtOH}$ 258 nm and was too intense to be accounted for by the known (GLC) concentrations of allogibberic [λ_{max}^{EtOH} 264 (ϵ, 320) nm[10]] and epiallogibberic acids [λ_{max}^{EtOH} 266 (ϵ, 320 nm)[2]] which have similarly fine-structured spectra, and gibberellenic acid [λ_{max}^{EtOH} 253 (ϵ, 22 400) nm[3]]. The spectrum of this autoclaved GA_3 fitted in detail that reported for dehydroallogibberic acid [λ_{max}^{EtOH} 258 (ϵ, 14 100) nm[11]]. Estimates of the dehydroallogibberic acid content, based on UV spectra, were in close agreement with that determined by GLC.

iso-GA_3 (II) and gibberellenic acid (IV) are considered to be intermediates in the aqueous decomposition of GA_3.[1] Autoclaving unbuffered aqueous solutions of *iso*-GA_3 as above produced allogibberic, epiallogibberic and dehydroallogibberic acids in proportions similar to those found from GA_3 (Table 1), but in lower yield; *ca.* 90% of the *iso*-GA_3 remained. Gibberellenic acid, on autoclaving, was totally converted into allogibberic acid and dehydro-

[10] T. P. C. MULHOLLAND, *J. Chem. Soc.* 2693 (1958).
[11] B. E. CROSS, R. H. B. GALT, J. R. HANSON, P. J. CURTIS, J. F. GROVE and A. MORRISON, *J. Chem. Soc.* 2937 (1963).

allogibberic acid (20–40% depending on the sample of distilled water); little or no epiallogibberic acid could be detected. Boiling aq. gibberellenic acid solutions under N_2 gave no dehydroallogibberic acid and produced allogibberic acid together with some epiallogibberic acid (*ca.* 20%). These products were confirmed by GLC–MS. In a stream of air, boiling aq. gibberellenic acid solutions produced allogibberic acid (*ca.* 60%) and dehydroallo-, gibberic acid (*ca.* 40%). These observations support the identification of dehydroallogibberic acid as an oxidised decomposition product of GA_3 formed via gibberellenic acid. Surprisingly, when aqueous gibberellenic acid was boiled in a stream of O_2 instead of air a lower ratio of dehydroallogibberic to allogibberic acid and a trace of epiallogibberic acid resulted—evidently other factors affect the ratio of the products.

TABLE 1. COMPOSITION OF AUTOCLAVED AQUEOUS GIBBERELLIC ACID SOLUTIONS*

Compound	Composition (%)† Unbuffered solution‡ (initially pH 3)	Buffer (0·2 M KH_2PO_4)§ pH 3	pH 5·5	pH 8
isoGA_3 (II)	76	63	60	50
iso-GA_3 hydroxy acid (III)	1·5	0·5	0·5	0·5
GA_3 (I)	2	2	2	1
Gibberellenic acid (IV)	0·5	0·5	0·5	24
Allogibberic acid (V)	6	25	9	0·2
Epiallogibberic acid (VI)	5	6	19	19
Dehydroallogibberic acid (VII)	8	2	5	2
Unknowns W	0	0	2	0·5
Unknowns Y	0	0	0·5	0·1
Other unknowns‖	1–2	1–2	1–2	3–4

* 1 mg/ml solutions autoclaved for 20 min at 2 atmos (120°).
† Determined by GLC peak area measurements.
‡ Work-up by evaporation *in vacuo* at 30°.
§ Work-up by acidification with dil. HCl and extraction into EtOAc.
‖ Refers to sum of several unknown compounds in each product.

Further evidence for dehydroallogibberic acid as an aqueous decomposition product of gibberellenic acid was obtained by taking a product containing dehydroallogibberic acid (18%) and allogibberic acid (82%) and rearranging the mixture in boiling dilute mineral acid to a mixture of dehydrogibberic acid (X, 18%) and gibberic acid (VIII, 82%).[11,12] This rearranged mixture was catalytically hydrogenated to gibberic acid (100%).[12] Product compositions at each stage were determined by GLC and TLC, and UV spectra were as predicted by calculation from published data using GLC determined product compositions.

A time-course study of the decomposition of unbuffered autoclaved solutions of GA_3 and gibberellenic acid, and boiled gibberellenic acid solutions in air and N_2 has revealed the intermediacy of a compound having a high intensity UV chromophore at *ca.* 326 nm. This substance is very labile, being destroyed in air (but not in N_2) even at room temperature and also on concentration of unbuffered solutions or acidification of buffered aqueous solutions. This intermediate may be the unknown triene (XI) (estimated[13] λ_{max} 318 nm) which could

[12] B. E. CROSS, J. F. GROVE, J. MACMILLAN and T. P. C. MULHOLLAND, *J. Chem. Soc.* 2520 (1958).
[13] A. I. SCOTT, *Interpretation of the Ultraviolet Spectra of Natural Products*, p. 45, Pergamon Press, Oxford (1964).

undergo rearrangement to allogibberic or epiallogibberic acids or be oxidised at the diallylic C-5 position prior to further transformation into dehydroallogibberic acid. Autoclaved (20 min, 2 atmospheres and 120°) GA_3 solutions in pH 5·5 and 8 phosphate buffer (Table 1) showed the above 326 nm chromophore which was absent in pH 3 and unbuffered solutions. Time-course studies with GA_3 and gibberellenic acid suggest that the unidentified products *W* and *Y* (Table 1) are decomposition products of the intermediate 326 nm chromophore produced on work-up. GLC R_ts of *W* and *Y* methylated derivatives on an XE60 column are recorded in Table 4. When these derivatives were examined by GLC–MS they showed a very similar fragmentation pattern and the same parent ion at *m/e* 296, suggesting that, like dehydroallogibberic acid (Me ester parent ion at *m/e* 296), they could be oxidised products derived from the triene (XI).

A crystalline sample of dehydroallogibberic acid (85%) containing allogibberic acid (15%) has been obtained by making use of the rapid formation of the air-sensitive intermediate (326 nm chromophore) in boiling aqueous solutions of gibberellenic acid then oxidising it with air at room temperature followed by rearrangement at 100°. At room temp., oxidation of the intermediate is apparently faster than rearrangement to allogibberic acid.

At room temp. unbuffered aqueous solutions of GA_3 are known to form *iso*-GA_3 and gibberellenic acid.[1,3] In the present study, apart from these two products and others which are unknown, a small amount of dehydroallogibberic acid (*ca.* 2%) was detected by UV, TLC and GLC in a 17-day-old solution. A trace of allogibberic (*ca.* 0·2%) and some epiallogibberic acid (*ca.* 2%) were also detected. Present results therefore suggest that the origin of the only known pure sample of dehydroallogibberic acid, from culture filtrates of *Gibberella fujikuroi*,[11] may be from spontaneous decomposition of GA_3 and not microbial degradation of GA_3 as suggested by Hanson.[14] Dehydroallogibberic acid can be detected as an impurity in allogibberic acid prepared by the published method[15] of treating GA_3 with dil. HCl at room temp.

The presence of dehydroallogibberic acid [λ_{max}^{EtOH} 258 (ϵ, 14 100) nm[11]] in the aqueous decomposition product of GA_3 invalidates to a large extent an earlier analysis using UV and PC[9] when the UV absorbance at 253–255 nm was attributed solely to gibberellenic acid [λ_{max}^{EtOH} 253 (ϵ, 22 400) nm[3]].

TABLE 2. LETTUCE HYPOCOTYL BIOASSAY OF DEHYDROALLOGIBBERIC, ALLOGIBBERIC AND GIBBERELLIC ACIDS

Compound	Hypocotyl length (%)* Concentration (μg/ml) 0	0·001	0·01	0·03	0·1	0·3	1·0	3·0	10·0	30·0
Gibberellic acid	100	133	166	220	308	341	475	480	530	537
Allogibberic acid	100	110	114	—	141	152	202	306	327	327
Dehydroallogibberic acid	100	117	117	—	135	151	188	340	374	406

* Mean hypocotyl length of controls was 3·6 mm.

The above analysis of autoclaved aq. GA_3 solutions shows that only 1–2% of GA_3 can be detected after usual autoclaving conditions and over a range of pH values covering those

[14] J. R. HANSON, *Tetrahedron*, **22**, 701 (1966).
[15] P. W. BRIAN, J. F. GROVE, H. G. HEMMING, T. P. C. MULHOLLAND and M. RADLEY, *Plant Physiol.* **33**, 329 (1958).

commonly used for plant culture media. Therefore the residual biological activity of these autoclaved solutions or media, which has been recorded as about 10%[6,7] or 25%[4,5] in various bioassays, cannot be due entirely to residual GA_3. Some of the decomposition products of GA_3 found here, *iso*-GA_3, *iso*-GA_3 hydroxy acid (III), gibberellenic acid and allogibberic acid, have previously been shown to have some gibberellin-like activity in a number of assay systems.[16–19] epiAllogibberic acid is recorded as being inactive in a wide range of tests.[16] No data on the gibberellin-like activity of dehydroallogibberic acid have previously been recorded. Table 2 records the activity of dehydroallogibberic acid in the lettuce hypocotyl test and it is seen to have definite biological activity, lower than that of GA_3 and somewhat higher than that of allogibberic acid at the higher concentrations. The samples of dehydroallogibberic and allogibberic acids used in this test were chromatographically (TLC and GLC) pure and completely free of GA_3. The results for allogibberic acid (Table 2) are in accord with previous findings by some authors[17,18] and are opposed

TABLE 3. TLC OF GIBBERELLIC ACID AND DERIVATIVES

		R_fs (adsorbent) [solvent system†]						
		Acids			Methyl esters			
Compound	Visualization*	(SiO_2) [A]	(SiO_2) [B]	(SiO_2) [C]	(Al_2O_3) [D]	(SiO_2) [C]	(SiO_2/$AgNO_3$) [A]	(SiO_2/$AgNO_3$)$_3$ [E]
Gibberic acid (VIII)	a′,b	0·53	0·79	0·70				
Epigibberic acid (IX)	a′,b							
Epiallogibberic acid (VI)	a′,b	0·47	0·77	0·55	0·28	0·31		
Allogibberic acid (V)	a′,b	0·42	0·75	0·50	0·25	0·27	0·54	0·74
Dehydroallogibberic acid (VII)	a′.b							
iso-GA_3 (II)	a	0·29	0·65	0·17				
Gibberellenic acid (IV)	a,b	0·27	0·60	0·23				
GA_3 (I)	a	0·24	0·60	0·17				
iso-GA_3 hydroxy acid (III)	a	0·04	0·31	0·04				

* All compounds above could be visualizes as brown spots after either contact with I_2 vapour or spraying with 4% $CeSO_4$ in 10% aq. H_2SO_4 and heating at 120° for 15 min. (a) indicates a blue to blue–green fluorescence in UV light after spraying with EtOH–H_2SO_4 (19:1) and heating at 120° for 10 min; (a′) indicates a green UV fluorescence after the (a) treatment; (b) indicates an absorbing spot under UV light on SiO_2 F_{254} plates; dehydroallogibberic and gibberellenic acids produced very strongly absorbing spots.

† (A) EtOAc–$CHCl_3$–HOAc (15:5:1); (B) MeCO–light petrol. (b.p. 60–80°)–HOAc (15:5:1); (C) diisopropyl ether–HOAc (19:1); (D) benzene–EtOAc (7:3); (E) EtOAc (100%).

to those of others[15,16] who found it to be inactive in this test. There is good evidence for the gibberellin-like activity of allogibberic acid in other test systems[16–19] despite some dissent.[20,21] Allogibberic acid has also, like GA_3, been found to delay flowering in *Pisum sativum*; but unlike GA_3 allogibberic acid did not affect stem elongation.[22]

[16] P. W. BRIAN, J. F. GROVE and T. P. C. MULHOLLAND, *Phytochem.* **6**, 1475 (1967).
[17] G. SEMBDNER, G. SCHNEIDER and K. SCHREIBER, *Planta Berl.* **66**, 65 (1965).
[18] L. PALEG, D. ASPINALL, B. COOMBE and P. NICHOLLS, *Plant Physiol.* **39**, 286 (1964).
[19] F. LONA, *L'Ateneo Parmense*, **33** Suppl. **6**, 206 (1962).
[20] M. J. BUKOVAC and S. H. WITTWER, in *Plant Growth Regulation* (edited by R. N. KLEIN), p. 505, Iowa State Press, Ames (1961).
[21] A. H. HALEVY and H. M. CATHEY, *Bot. Gaz. Ital.* **122**, 63 (1960).
[22] I. MURFET and N. BARBER, *Nature, Lond.* **191**, 514 (1961).

Work is in progress to determine which, if any, of the above identified products produced on autoclaving GA_3 in unbuffered[8] aqueous solution is responsible for the observed increase in frond multiplication rate, decrease in frond size and inhibition of flowering in the short-day duckweed *Lemna perpusilla* 6746. Recent findings by Hodson and Hamner[8] suggest, as discussed above, that these effects, originally observed by Hillman,[6] are due to substance(s) produced on autoclaving. It seems also probable that similar observed effects on growth and flowering with the long-day duckweed *Lemna gibba* G3[23] may also be due to autoclave-produced products and not to GA_3 itself. Hodson and Hamner's results[8] also indicate that a substance or substances produced on autoclaving an unspecified mixture of gibberellins A_4 (XII) and A_7 (XIII), but not GA_4 and/or GA_7 themselves, also inhibited flowering in *L. perpusilla* and at lower concentrations than autoclaved GA_3 solutions. The difficulty in obtaining pure samples of GA_4 and GA_7 has precluded an analysis, similar to that above, of their autoclave-produced decomposition products. However, the known chemistry[24] of GA_7 (13-deoxygibberellin A_3) suggests that it would give products analogous to those from GA_3.

TABLE 4. GLC OF THE METHYL ESTERS AND METHYL ESTER TRIMETHYLSILYL ETHERS OF GIBBERELLIC ACID AND DERIVATIVES

	Retention time (min) Column [temp.]					
	Methyl esters				Methyl ester TMSi ethers	
Compound	1% XE60 [195°]	1% XE60 [243°]	1% OV17 [185°]	1% OV17 [215°]	1% XE60 [205°]	1% OV17 [210°]
Epigibberic acid (IX)	4·0		7·0			
Gebberic acid (VIII)	5·2		9·0			
Epiallogibberic acid (VI)	4·2	0·7	7·5	2·3	1·1	1·9
Allogibberic acid (V)	5·0	0·9	9·6	2·9	1·4	2·5
Dehydroallogibberic acid (VII)	6·3	1·1	12·2	3·5	1·7	3·1
Gibberellenic acid (IV)		3·8		11·1	2·5	5·6
GA_3 (I)		15·3		19·8	7·2	7·4
iso-GA_3 (II)		15·1		20·1	6·3	7·4
iso-GA_3 hydroxy acid (III)		15·1		20·1	2·5	6·0
Unknowns *W*	7·5					
Unknowns *Y*	11·6					

EXPERIMENTAL

Gibberellic acid (I) used in this study was purified by repetitive recrystallisation from Me_2CO-light petrol. (b.p. 60–80°) and its purity was checked by TLC and GLC. The reference compounds described were prepared from GA_3 by published procedures in the case of *iso*-GA_3 (II),[1] *iso*-GA_3 hydroxy acid (III),[1] gibberellenic acid (IV),[2] allogibberic acid (V),[15] epiallogibberic acid (VI),[2] gibberic acid (VIII),[25] epigibberic acid (IX)[2] and dehydrogibberic acid (X)[12] or were donated [dehydroallogibberic acid (VII)].

TLC. TLC was performed on Merck SiO_2 gel plates (250 μm thick) with or without fluorescent indicator (F_{254}) as appropriate. $AgNO_3$–SiO_2 plates were prepared from the above by immersing them in 5% $AgNO_3$

[23] C. F. CLELAND and W. S. BRIGGS, *Plant Physiol.* **44**, 503 (1969).
[24] B. E. CROSS, R. H. B. GALT and J. R. HANSON, *Tetrahedron* **18**, 451 (1962).
[25] B. E. CROSS, *J. Chem. Soc.* 4670 (1954).

in MeOH for 3 min and reactivating them at 120° for 1 hr. Merck Al_2O_3 plates ('E' type, 250 μm thick) were also used. Products were identified both by their R_f in various solvent systems and response to different visualization procedures (Table 3). It has not been possible to separate allogibberic (V) and dehydroallogibberic acid (VII) or their methyl esters in any solvents so far tried.

GLC. GLC was performed with a Varian aerograph 1400 instrument fitted with an FID detector. Silanized glass columns 1·5 m × 3 mm i.d. (OV 17 column) or 2 m × 3 mm i.d. (XE60 columns) were packed with stationary phases (1%) adsorbed on Gas Chrom Q (100–120 mesh). Injector and detector heaters were kept at 250° and the N_2 flow rate was 45 ml/min in all cases. Column oven temps. are recorded in Table 4. Products were identified by their R_t on the two stationary phases both as their methyl ester and as trimethylsilyl (TMSi) ethers of the methyl ester (MeTMSi) derivatives (Table 4). These derivatives were prepared as previously described.[26] Quantitative analysis was carried out by peak area measurements. Total peak areas of chromatograms derived from aliquots of autoclaved GA_3 (from 2 μg of GA_3) accounted for the peak area obtained with an equal aliquot of pure GA_3 (2 μg). Similarly GLC-observed products from *iso*-GA_3 and gibberellenic acid accounted for the total products.

Combined GLC–MS. GLC–MS was carried out with an A.E.I. MS30 mass spectrometer coupled via an all-glass silicone membrane separator[27] to a Pye 104 gas chromatograph fitted with silanized glass columns (1·5 m × 4 mm i.d.) packed with 1% OV17 or 1% XE60 adsorbed on Gas Chrom Q (100–120 mesh). The effluent from the gas chromatograph was split between an FID detector and the mass spectrometer. Gas chromatograms were recorded both as FID and total ion current traces. The He carrier gas was at 1 atmos inlet pressure and other GLC conditions were similar to those above and in Table 4. MS (10 sec/decade scans) were recorded at 70 eV with a source temp. of 300° and the separator at 200°. The identities of the major products formed during autoclaving of aq. GA_3 solutions (i.e. all in Table 1 excluding *iso*-GA_3 hydroxy acid (III) and GA_3) were confirmed by GLC–MS as their methyl esters and in the case of *iso*-GA_3 (III) also as the MeTMSi derivative. The MeTMSi derivatives of GA_3 and *iso*-GA_3 are well separated on the XE60 column (Table 4) and these derivatives, unlike their methyl esters,[28] have distinct MS. The most prominent difference between the MS of MeTMSi–GA_3 and MeTMSi-*iso*-GA_3 is in the relative intensities of the fragment ions *m/e* 208 and 207 which are considered[28] to be derived from the C and D rings of 13-hydroxy gibberellin MeTMSi derivatives. MeTMSi-*iso*-GA_3 has a small *m/e* 208 (8% of the base peak at *m/e* 73) and a larger *m/e* 207 ion (14% of base peak) while in MeTMSi–GA_3 the relative intensities are reversed, *m/e* 208 (11% of base peak at *m/e* 73) and *m/e* 207 (3% of base peak).

Autoclaving. Autoclaving was carried out in a Prestige pressure cooker for aq. GA_3 solutions (5 ml, in 25 ml Pyrex conical flasks) as described in Table 1. EtOAc extracts were dried (Na_2SO_4) before evaporation *in vacuo* at 30°. Unbuffered aqueous solutions of *iso*-GA_3 and gibberellenic acid (1 mg/ml) were autoclaved similarly and worked up by evaporation to dryness *in vacuo* at 30°.

Conversions of a mixture of dehydroallogibberic and allogibberic acids. At each stage aliquots of reaction products were taken for UV spectroscopy (0·78 mg/ml EtOH solutions) and qualitative and quantitative GLC analysis as methylated derivatives on both XE60 and OV17 columns or as indicated below. Calculated UV A values for 0·78 mg/ml solutions were based on GLC determined compositions and published[10–12] ϵ values. (a) A solution of gibberellenic acid (IV) (9·3 mg) in H_2O (9·3 ml) was autoclaved for 30 min at 2 atmos (120°). The product contained dehydroallogibberic acid (VII, 18%) and allogibberic acid (V, 82%); $A_{258\ am}$ found 1·00, calc. 1·00. (b) The product remaining from (a) above (8·4 mg) was boiled in dil. HCl (1:5, conc. HCl–H_2O; 10 ml) for 1 hr. The product was cooled and extracted into EtOAc, dried (Na_2SO_4) prior to evaporation to dryness *in vacuo*. This product contained dehydrogibberic acid (X, 18%) and gibberic acid (VIII, 82%); A_{260am} found 0·99, calc. 1·00. Methyl dehydrogibberate and methyl gibberate were not separated on the OV17 and XE60 columns. They were separated on a 3% SE30 (on Varaport, 100–120 mesh) column (stainless steel, 1·5 m × 2 mm i.d.) at 205°, N_2 flow rate 15 ml/min; Me-dehydrogibberate (R_t 7·5 min) Me-gibberate (R_t 8·0 min). (c) The product remaining from (b) above (8·0 mg) was hydrogenated over a 10% Pd–C catalyst (20 mg) in EtOAc (10 ml) at room temp. and pressure for 1 hr. The filtered product contained gibberic acid (100%); A_{265am} found 0·11, calc. 0·13.

Dehydroallogibberic acid from gibberellenic acid. The reaction sequence was monitored by UV spectroscopy of aliquots at each stage. A solution of gibberellenic acid (50 mg) in H_2O (50 ml) was flushed with N_2 at room temp. for 1 hr and was then boiled for 3 min under N_2. After rapidly cooling this solution to room temp. in an ice bath, air was bubbled through it at room temp. until its 326 nm chromophore had gone (25 min). The same cycle of N_2 flushing at room temp. boiling under N_2, cooling and decomposition of the 326 nm chromophore in air was repeated on the solution then it was boiled for 1·25 hr in air. The cooled final solution was acidified with 4 N HCl and extracted with EtOAc (5 × 10 ml) which was dried (Na_2SO_4) prior to evaporation *in vacuo* to give a gum (39·5 mg). GLC analysis XE60 and OV17 columns of a methylated aliquot of this reaction product indicated that it contained dehydroallogibberic acid (VII, 80%, allogibberic acid (V, 10%) and other unknowns (10%); the UV spectrum of the product was consistent with this amount

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of dehydroallogibberic acid. The product was partially purified by preparative TLC (SiO_2 solvent A, Table 3) to give a gum (23 mg) which was recrystallized 4× from Me_2CO–light petrol. (b.p. 60–80°). After each crystallisation the m.p. (197–204°) and composition (GLC and GLC–MS) [dehydroallogibberic acid (85%) and allogibberic acid (15%)] were unchanged.

Lettuce hypocotyl bioassay. This was a modification of the procedure of Frankland and Wareing.[29] Lettuce seeds (var. 'Arctic King', Suttons & Sons Ltd., England) were sown on wet Whatman No. 3 filter paper and incubated for 2 hr in the dark at 25°. The seeds were then exposed to natural daylight for 3 hr prior to reincubation in the dark at 25° for 24 hr. Germinating seeds with radicles *ca.* 2 mm long were then selected and placed in tubes (1·8 cm i.d.) containing a circle of Whatman No. 3 filter paper and test solutions or dist. H_2O control (0·5 ml). 2 tubes containing 6 germinating seeds each were used for each treatment and these were incubated in a glass topped humidity box for 4 days at 27° under continuous illumination from daylight fluorescent lights (4800 lx). After this incubation hypocotyls were measured and the mean values for each treatment were recorded and expressed as a percentage of the control. The results obtained (Table 2) are essentially reproducible.

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