Phytochemistry, 1973, Vol. 12, pp. 1745 to 1754. Pergamon Press. Printed in England.

ALLOGIBBERIC ACID: AN INHIBITOR OF FLOWERING IN *LEMNA PERPUSILLA*

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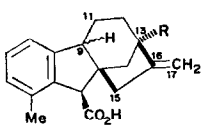
(*Received* 15 *January* 1973. *Accepted* 19 *February* 1973)

Key Word Index—*Lemna perpusilla* 6746; Lemnaceae; duckweed; gibberellic acid; autoclaving; decomposition products; allogibberic acid; 13-deoxyallogibberic acid; flowering inhibition; bioassay.

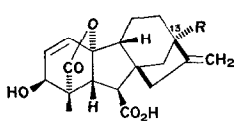
Abstract—Allogibberic acid (I) has been identified as the compound responsible for the inhibition of flowering, increase in frond multiplication rate and decrease in frond size produced in *Lemna perpusilla* 6746 by autoclaved, unbuffered aqueous solutions of gibberellic acid (VII). 13-Deoxyallogibberic acid (IV), a product of autoclaving aq. GA_7 (VIII) solutions, also inhibits flowering in *L. perpusilla* and is about 10 times more active than allogibberic acid.

INTRODUCTION

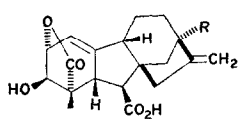
AUTOCLAVED, unbuffered aqueous solutions of gibberellic acid (GA_3) (VII) have been shown by Hodson and Hamner[1] to inhibit flowering, increase the frond multiplication rate and decrease frond size in the short-day duckweed *Lemna perpusilla* Torr., strain 6746. Non-autoclaved GA_3 had a similar but much smaller effect; this suggested production of active substance(s) on autoclaving.[1] These results confirmed a previous suggestion by Hillman[2] based on his earlier work with autoclaved aqueous GA_3 solutions alone.[3]



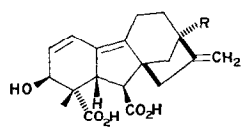
(I) 9α-H, R = OH
(II) 9β-H, R = OH
(III) $\Delta^{9,11}$, R = OH
(IV) 9α-H, R = H
(V) 9β-H, R = H
(VI) $\Delta^{9,11}$, R = H



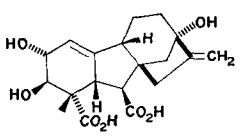
(VII) R = OH
(VIII) R = H



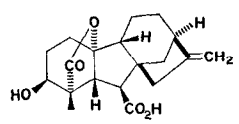
(IX) R = OH
(X) R = H



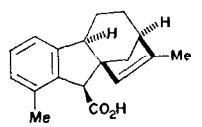
(XI) R = OH
(XII) R = H



(XIII)



(XIV)



(XV)

I have analysed the decomposition of aq. GA_3 solutions on autoclaving.[4] Each of the GA_3 decomposition products produced on autoclaving found previously[4] has now been

[1] HODSON, H. K. and HAMNER, K. C. (1971) *Plant Physiol.* **47**, 726.
[2] HILLMAN, W. S. (1969) in *The Induction of Flowering—Some Case Histories*, (EVANS, L. T., ed.) p. 186, Macmillan, Melbourne.
[3] HILLMAN, W. S. (1960) *Phyton* **14**, 49.
[4] PRYCE, R. J. (1973) *Phytochemistry* **12**, 507.

tested in bioassays with *L. perpusilla*. Allogibberic acid (I) is shown to be the GA_3 decomposition product responsible for flowering inhibition, increase in frond multiplication rate and decrease in frond size. 13-Deoxyallogibberic acid (IV) is a more active inhibitor of flowering in *L. perpusilla* than allogibberic acid and is produced by autoclaving aq. solutions of GA_7.

RESULTS AND DISCUSSION

Autoclaved, unbuffered aq. GA_3 solutions and their decomposition products were examined for their effects on vegetative growth and inhibition of flowering in *L. perpusilla* in separate groups of experiments. The results are conveniently discussed under separate headings.

TABLE 1. EFFECTS OF AUTOCLAVED GIBBERELLIC ACID AND NON-AUTOCLAVED GIBBERELLIC ACID ON VEGETATIVE GROWTH OF *Lemna perpusilla* 6746

Test	Composition of test substances* (%)		Concentration of test substances in culture medium† (μg/ml)	Mean frond multiplication rate (No. of new fronds day/frond)§	Estimated mean frond area relative to control‖
Control	—		—	0·50	100
Autoclaved GA_3‡	*iso*-GA_3 (IX)	75	38·5	0·58	25
	Allogibberic acid (I)	10			
	epiAllogibberic acid (II)	4			
	Dehydroallogibberic acid (III)	4			
	GA_3 (VII)	2			
	iso-GA_3 hydroxy acid (XIII)	2			
	Gibberellenic acid (XI)	0·5			
	Unknowns	2·5			
GA_3	Chromatographically pure		38·5	0·55	75

* TLC and GLC analysis.[4]

† *B* Medium–H_2O (24:1).

‡ Unbuffered aq. GA_3 solution (1 mg/ml) autoclaved at 2 atm. (120°) for 20 min and diluted into culture medium.

§ Calculated from the equation:
$\log (R + 1) = [(\log n_x - \log n_y)/(x - y)]$; when R = multiplication rate as above, n_x and n_y are the number of fronds on the xth and yth days respectively ($x > y$). Frond numbers were counted on the 4th and 7th days.

‖ Estimated from measurements of photographs.

Vegetative Growth

Results of experiments to determine which decomposition product(s) of autoclaved unbuffered aq. GA_3 solutions increased frond multiplication rate and decreased frond size are presented in Tables 1–3. For these bioassays plants were grown in non-inductive continuous illumination.

Initially, autoclaved unbuffered aq. GA_3 was tested against non-autoclaved GA_3 in the same experiment and the results obtained (Table 1) were in accord with those of Hodson and Hamner;[1] i.e. autoclaved GA_3 has a greater effect than non-autoclaved GA_3 on frond multiplication rate and size. The composition (Table 1) of the autoclaved, unbuffered aq. GA_3 giving rise to these results was determined as preciously described.[4] The major decomposition products were then tested individually and in the same experiment, at

concentrations approximately equivalent to those at which they were present in the test described above (Table 1), and the result, which is reproducible, is shown in Table 2. Gibberellenic acid (XI) was not tested as it is known to be produced rapidly in aq. GA_3 solutions at room temperature and achieves higher concentrations than that recorded in Table 1 in a few hours.[5] *iso*-GA_3 hydroxy acid (XIII) was tested at the same concentration as *iso*-GA_3 (IX) since it is not certain which of these two compounds is actually present, or in what proportion they occur, in aqueous solutions of GA_3 after autoclaving.[4] Of the GA_3 decomposition products tested (Table 2), only allogibberic acid (I) shows any significant and reproducible effect on frond multiplication rate and size and its effect accounts for that observed with autoclaved GA_3 (Table 1). As with autoclaved GA_3, allogibberic acid alone produced the same shortening of the axis and rounding of *L. perpusilla* fronds.

TABLE 2. EFFECTS OF PRODUCTS OF AUTOCLAVED GIBBERELLIC ACID ON VEGETATIVE GROWTH OF *Lemna perpusilla* 6746

Test	Concentration* of test substances in culture medium† (μg/ml)	Mean frond multiplication rate (No. of new fronds/day/frond)‡ (results of a replicate experiment)	Estimated mean frond area relative to control‡
Control	—	0·53 (0·51)	100
Allogibberic acid (I)	4	0·63 (0·60)	50
epiAllogibberic acid (II)	2	0·56 (0·46)	100
Dehydroallogibberic acid (III)	2	0·50 (0·54)	100
iso-GA_3 (IX)§	29	0·54 (0·45)	100
iso-GA_3 hydroxy acid (XIII)‖	29	0·54 (0·55)	100
		¶L.S.D. 10%, 0·07	
		5%, 0·09	
		2%, 0·11	

* Concentrations approx. those produced in autoclaved GA_3 tested in Table 1.
† *B* Medium–H_2O (24:1).
‡ As in Table 1.
§ Composition by TLC and GLC as in Ref. 4; *iso*-GA_3 (90%), *iso*-GA_3 hydroxy acid (4%), GA_3 (6%).
‖ Composition by TLC and GLC as in Ref. 4; *iso*-GA_3 hydroxy acid (93%), *iso*-GA_3 (7%).
¶ By analysis of variance and *t*-test.

Table 3 records the effect of allogibberic acid, autoclaved unbuffered aq. GA_3 and GA_3 each at three different concentrations and in the same experiment, on vegetative growth of *L. perpusilla*. The compositions of the autoclaved aq. GA_3 solution used, prior to its introduction to the culture medium, and the composition of the non-autoclaved GA_3 after being in the culture medium, without *L. perpusilla*, under the test conditions for the 7 day growth period are recorded in Table 3. As in water alone,[4] GA_3 in the mineral medium produced only a small amount of allogibberic acid but only 43% GA_3 remains after this time. Evidently allogibberic acid can account for the observed effects on frond multiplication rate produced by autoclaved GA_3.

[5] PRYCE, R. J. unpublished observations.

TABLE 3. EFFECTS OF ALLOGIBBERIC ACID, AUTOCLAVED GIBBERELLIC ACID AND NON-AUTOCLAVED GIBBERELLIC ACID ON VEGETATIVE GROWTH OF *Lemna perpusilla* 6746

Test	Composition of test substances* (%)	Concentration of test substances in culture medium† (μg/ml)	Mean frond multiplication rate (No. of new fronds/day/frond)‡	Estimated mean frond area relative to control‡
Control	—	—	0·50	100
Allogibberic acid	Chromatographically pure	10	0·61	25
		1	0·68	50
		0·1	0·59	50
Autoclaved GA_3	*iso*-GA_3 (IX) 68 Allogibberic acid (I) 18 epiAllogibberic acid (II) 8 Dehydroallogibberic acid (III) 2 GA_3 (VII) 2 *iso*-GA_3 hydroxy acid (XIII) 0·5 Gibberellenic acid (XI) 0·5 Unknowns 1	10	0·61	50
		1	0·58	100
		0·1	0·54	100
GA_3	*iso*-GA_3 41§ Allogibberic acid 0·5 epiAllogibberic acid 1·5 Dehydroallogibberic acid 0·5 GA_3 43 *iso*-GA_3 hydroxy acid 0·5 Gibberellenic acid 3 Unknowns 10	10	0·51	100
		1	0·55	100
		0·1	0·54	100
			‖Least significant value greater than control; 5%, 0·58 1%, 0·62	

* TLC and GLC analysis.[4] Autoclaved GA_3 as in Table 1.
† *B* Medium–H_2O (99:1).
‡ As in Table 1.
§ Composition of chromatographically pure GA_3 after being in culture medium (less *L. perpusilla*) at 10 μg/ml under culture conditions for 7 days growth period. Work-up by acidification with dil. HCl and extraction into EtOAc.
‖ Analysis of variance and *t* test.

Inhibition of Flowering

The results presented above suggest that because allogibberic acid appears to accentuate meristematic activity of *L. perpusilla* towards new frond production, i.e. vegetative reproduction, it could be an inhibitor of flowering, i.e. sexual reproduction. This prediction has been tested and shown to be correct. Tests of autoclaved unbuffered aq. GA_3 and its decomposition products on inhibition of flowering in *L. perpusilla* were carried out in a similar manner to those described above and the results are presented in Tables 4–8. For these bioassays plants were grown in inductive short-days.

The results in Table 4 show that, as previously found,[1] autoclaved aq. GA_3 is a much more effective flowering inhibitor that non-autoclaved GA_3. Gallic acid, previously found to be a naturally occurring inhibitor of flowering in *Kalanchoe blossfeldiana*,[6] was inactive against *L. perpusilla* at the single, yet high, concentration used.

TABLE 4. INHIBITION OF FLOWERING IN *Lemna perpusilla* 6746 BY AUTOCLAVED AQUEOUS GIBBERELLIC ACID SOLUTIONS

Test	Composition of test substance* (%)		Concentration of test substance in culture medium† (μg/ml)	Flowering (%)
Control	—		—	77
Autoclaved‡ GA_3	*iso*-GA_3	70	38·5	0
	Allogibberic acid (I)	16		
	epiAllogibberic acid (II)	7		
	Dehydroallogibberic acid (III)	2		
	GA_3 (VII)	2		
	iso-GA_3 hydroxy acid (XIII)	1·5		
	Gibberellenic acid (XI)	0·5		
	Unknowns	1·0		
GA_3	Chromatographically pure		38·5	78
Gallic acid	Chromatographically pure		38·5	76

* By TLC and GLC analysis.[4]
† *H* Medium–H_2O (24:1).
‡ Autoclaved as in Table 1.

Allogibberic acid alone and in combination with the combined other known GA_3 decomposition products was then tested (Table 5) at the concentrations at which they were present in the sample of autoclaved GA_3. (Table 4). The results show that allogibberic acid alone is inhibiting flowering and that the other decomposition products tested are inactive

TABLE 5. INHIBITION OF FLOWERING IN *Lemna perpusilla* 6746 BY PRODUCTS FROM AUTOCLAVED AQUEOUS GIBBERELLIC ACID SOLUTIONS

Test	Concentration* of test substances in culture medium† (μg/ml)		Flowering (%) (Result of replicate) experiment)
Control	—		77 (76)
iso-GA_3‡ +	27		71 (82)
epiAllogibberic acid +	3		
Dehydroallogibberic acid	1		
iso-GA_3 hydroxy acid†	27		71 (93)
Allogibberic acid	6		41 (67)
Allogibberic acid +	6		25 (39)
iso-GA_3† +	27		
epiAllogibberic acid +	3		
Dehydroallogibberic acid	1		

* Concentrations approx. those produced in autoclaved GA_3 tested in Table 4.
† *H* Medium–H_2O (24:1).
‡ Composition as in Table 2.

[6] PRYCE, R. J. (1972) *Phytochemistry* **11**, 1911.

but appear to enhance the inhibitory effect of allogibberic acid. Gibberellenic acid (XI) was not tested and *iso*-GA_3 hydroxy acid (XIII) was tested at the same concentrations as *iso*-GA_3 for reasons given above.

The apparent enhancement of the allogibberic acid flowering inhibition was investigated further by analysing all the decomposition products of autoclaved aq. GA_3 in all possible combinations with allogibberic acid (Table 6). It is evident from the results that no single decomposition product of autoclaved GA_3 gives any particular enhancement to the allogibberic acid flowering inhibition but all the decomposition products together give maximum enhancement. This enhancement is apparently not due to a decrease in the medium pH alone (Table 6) but pH may have some contributory effect.

TABLE 6. INHIBITION OF FLOWERING IN *Lemna perpusilla* 6746 BY ALLOGIBBERIC ACID IN COMBINATIONS WITH OTHER DEGRADATION PRODUCTS FROM AUTOCLAVED AQUEOUS GIBBERELLIC ACID SOLUTIONS

Test*	Concentration† of test substances in culture medium‡ (μg/ml)	pH of culture medium plus test substances	Flowering (%)
Control	—	6·20	87
Allogibberic acid (I)	6	6·17	63
(I) + *iso*-GA_3 hydroxy acid	6 + 27	—	73
(I) + *iso*-GA_3	6 + 27	6·02	58
(I) + epiallogibberic acid	6 + 3	6·16	64
(I) + dehydroallogibberic acid	6 + 1	6·16	51
(I) + *iso*-GA_3 + epiallogibberic acid	6 + 27 + 3	6·01	55
(I) + *iso*-GA_3 + dehydroallogibberic acid	6 + 27 + 1	6·01	40
(I) + epiallogibberic acid + dehydroallogibberic acid	6 + 3 + 1	6·15	53
(I) + *iso*-GA_3 + epiallogibberic acid + dehydroallogibberic acid	6 + 27 + 3 + 1	6·00	32

* *iso*-GA_3 and *iso*-GA_3 hydroxy acids' purity as noted in Table 2—other substances were chromatographically pure.

† Concentrations approximately those produced in autoclaved GA_3 tested in Table 4.

‡ *H* Medium–H_2O (24:1).

The flowering inhibitory activity of allogibberic acid is compared (Table 7) with that of autoclaved aq. GA_3, having the indicated composition, both over the same concentration range. These results, together with those above, confirm that allogibberic acid alone can account reasonably well for flowering inhibition of autoclaved aq. GA_3. The cut-off point in the flowering inhibition of allogibberic acid in *L. perpusilla* lies between concentrations of 1 and 10 μg/ml in the medium.

Hodson and Hamner have also shown[1] that autoclaved, unbuffered aqueous solutions of an unspecified mixture of gibberellins A_4 (XIV) and A_7 (VIII) were about 10 times more inhibitory to flowering in *L. perpusilla* than the corresponding solutions from GA_3. It seemed that GA_7 would give rise to analogous decomposition products to GA_3,[4] and examination of the decomposition products of autoclaved, unbuffered aq. GA_7 (admixed with GA_4 [15%]) by GLC and GLC–MS has confirmed the presence of 13-deoxyallogibberic acid (IV). GA_4, which lacks a 1,2-double bond, would not be expected to be as labile to decomposition on autoclaving in aqueous solution as GA_7 and GA_3. 13-Deoxyallogibberic

acid has not previously been reported in the literature but it has been obtained as a minor component (25%) of a mixture with its $\Delta^{15,16}$-isomer (XV) (75%).[7] It was prepared here (78% pure) by aqueous decomposition of 13-deoxygibberellenic acid (XII) in an analogous manner to that indicated for the preparation of allogibberic acid.[4] A sample of 13-deoxygibberellenic acid (XII) was prepared from GA_7 in the same way as for gibberellenic acid (XI) from GA_3.[8]

TABLE 7. INHIBITION OF FLOWERING IN *Lemna perpusilla* 6746 BY ALLOGIBBERIC ACID AND AUTOCLAVED AQUEOUS GIBBERELLIC ACID SOLUTIONS

Test	Composition of test substance* (%)		Concentration of test substance in culture medium† (μg/ml)	Flowering (%)
Control			—	77
Allogibberic acid	Chromatographically pure		10	36
			1	65
			0·1	81
			0·01	84
Autoclaved‡ GA_3	*iso*-GA_3 (IX)	63	10	75
	Allogibberic acid (I)	19	1	79
	epiAllogibberic acid (II)	9	0·1	80
	Dehydroallogibberic acid (III)	3	0·01	90
	GA_3 (VII)	2		
	iso-GA_3 hydroxy acid (XIII)	2		
	Gibberellenic acid (XI)	0·5		
	Unknowns	1·5		

* By TLC and GLC analysis.[4]
† *H* Medium–H_2O (99:1).
‡ Autoclaved as in Table 1.

The results in Table 8 show that 13-deoxyallogibberic acid is *ca.* 10 times more active as a flowering inhibitor in the *L. perpusilla* test than allogibberic acid. Therefore, since Hodson and Hamner[1] had shown previously that their product of autoclaving an aqueous solution of GA_7 (admixed with an unspecified amount of GA_4) was *ca.* 10 times more active in the *L. perpusilla* test than autoclaved GA_3 solutions, 13-deoxyallogibberic acid could account for the flowering inhibition produced. 13-Deoxyallogibberic has an even greater effect on reduction of frond size than allogibberic acid at equivalent concentrations. A sample of 13-deoxy *iso*-GA_3 (X) was also tested, at the maximum possible concentration with available material, and shown to be inactive (Table 8) like its GA_3-derived analogue (IX) (Table 5).

Thus, allogibberic acid (I) has been shown to be the decomposition product present in autoclaved, unbuffered aqueous solutions of GA_3 (VII) responsible for the observed inhibition of flowering, increase in frond multiplication rate and decrease in frond size of the duckweed *L. perpusilla*. Whatever systems of the duckweed respond to allogibberic acid they must have some degree of structural specificity since the 9-epimer, epiallogibberic acid (II) and $\Delta^{9,11}$-dehydroallogibberic acid (III) are inactive at the concentrations used. On

[7] CROSS, B. E., personal communication of unpublished results.
[8] GROVE, J. F. and MULHOLLAND, T. P. C. (1960) *J. Chem. Soc.* 3007.

the other hand, 13-deoxyallogibberic acid (IV), a product of autoclaving aq. GA_7 (VIII), is more active than allogibberic acid. The evidence above suggests that 13-deoxyallogibberic acid could account for all the flowering inhibitory activity found[1] to be caused by autoclaved, unbuffered aqueous solutions of a mixture of GA_7 and GA_4.

TABLE 8. INHIBITION OF FLOWERING IN *Lemna perpusilla* 6746 BY ALLOGIBBERIC ACID AND 13-DEOXYALLOGIBBERIC ACID

Test	Concentration of test substances in culture medium* (μg/ml)	Flowering (%)	Test	Concentration of test substances in culture medium* (μg/ml)	Flowering (%)
Control	—	81	13-Deoxyallogibberic acid (IV)†	10†	0
				1·0	32
Allogibberic acid (I)	10	20		0·1	71
	1·0	77			
	0·1	78	*iso*-GA_7 (X)	10	80

* *H* Medium.
† 13-Deoxyallogibberic acid (78%) by TLC and GLC analysis—see text. Concentrations tested were corrected for impurities amounting to 22%.

The results above and those previously published[4] emphasize the need for caution in interpreting the results of biological assays: the compounds tested may change during, or even before the test. In this instance, the test substance, GA_3 is very labile in the medium (water) in which it is generally applied and gives rise *inter alia* to allogibberic acid which has markedly different biological properties; only 1–2% GA_3 remains after autoclaving under usual conditions.[4] Most of the aqueous decomposition products of GA_3 have been found to have 'gibberellin-like' biological activity *per se* which is generally lower than that of GA_3 (Ref. 4 and references therein). Allogibberic acid is more active than GA_3 in the lettuce radicle elongation test[9] and while both compounds delay flowering in the pea (*Pisum sativum*) the allogibberic response does not include the stem elongation produced with GA_3.[10] GA_3 has been reported to inhibit photoperiodically-induced flowering, increase frond multiplication rate and decrease frond size in the long-day duckweed *Lemna gibba* G3.[11] While the authors of this latter report seemed aware of some possible decomposition of GA_3 on autoclaving (see Ref. 4) the possibility that the observed biological activity was due to GA_3 decomposition products was not considered. Allogibberic acid could well be the active substance affecting *L. gibba* G3 by analogy with its effects on *L. perpusilla*.

The results above, combined with the ease with which allogibberic acid is formed from GA_3 even at room temp. (Ref. 4 and references therein) suggest that allogibberic acid might be a natural product with a specific regulatory role. Allogibberic acid has not yet been isolated from, or detected in, any green plant and before it could be claimed to be a natural product an isolation would have to be devised which excluded the possibility that it is formed during work-up.

[9] PALEG, L., ASPINALL, D., COOMBE, B. and NICHOLLS, P. (1964) *Plant Physiol.* **39**, 286.
[10] MURFET, I. and BARBER, N. (1961) *Nature* **191**, 514.
[11] CLELAND, C. F. and BRIGGS, W. S. (1969) *Plant Physiol.* **44**, 503.

EXPERIMENTAL

Compounds bioassayed were obtained as previously described[4] or were donated in the case of dehydroallogibberic acid and *iso*-GA_7. 13-Deoxyallogibberic acid was prepared as described below. GA_3 and GA_7 were autoclaved as previously described.[4] GLC and GLC–MS on OV17 and XE60 columns were performed as previously described.[4]

L. perpusilla 6746 *bioassays*. Stock cultures were grown in continuous illumination from Philips 'daylight' fluorescent lights (4000 lx at plant ht) at 23–24·5° in sterile culture medium *B* or *H* (100 ml) in plugged conical flasks (250 ml). New stock cultures were started *ca.* every 7 days. Plants grew apparently non-contaminated under these conditions. *B* medium refers to an entirely mineral medium described by Bischoff and Bold[12] —'Bold's basal medium'. *H* medium is half-strength Hutner's medium containing 1% sucrose as described by Hillman[2] except that KNO_3 replaces NH_4NO_3 at the same molar concentration.[13] The plants growing on *B* medium became overgrown with microorganisms when grown on *H* medium; these stock cultures were used only for the vegetative growth bioassays. Plants growing apparently non-contaminated on *H* medium were used exclusively for the flowering bioassays. All biosssays were prepared in a similar manner. Culture media (25 ml) were sterilised by Millipore filtration and dispensed directly into sterile conical flasks (50 ml). Test substances were administered either by evaporating EtOH solutions of the substances in the test flasks with a stream of sterile-filtered N_2 prior to addition of the medium, or addition of measured vol. of autoclaved or Millipore-filtered aq. solutions to the medium. Whenever some tests in an experiment required addition of test substances in H_2O, all other tests in that experiment used culture media similarly diluted (see footnotes to Tables). Test and appropriate control cultures were started from one 3-frond colony taken from 6 to 8-day-old stock cultures.

Bioassay of vegetative growth effects (Tables 1–3). Test and control cultures (4 replicates of each) on *B* medium were grown for 7 days in continuous illumination from Philips 'daylight' fluorescent lights (4800 lx at plant ht) at 24–26°. Frond numbers were counted on the 4th and 7th days. On the 7th day cultures were tipped out into Petri dishes and photographed; measurements of enlargements of these photographs were used to estimate frond area.

Bioassay of flowering inhibition (Tables 4–8). Test and control cultures (3 replicates of each) on *H* medium were grown for 10 days under short-day photoinductive conditions; 8 hr illumination per day from Philips 'daylight' fluorescent lights (5000 lx at plant ht) at 24–26°. A sample (*ca.* 20) of fronds from each replicate of each test were then examined by microscopic dissection and each frond was scored as either flowering or vegetative—early stages of flowering were scored as flowering. Flowering percentages quoted are the percentage number of fronds flowering per total number of fronds examined per test.

13-*Deoxyallogibberic acid* (IV) *and the decomposition of unbuffered aq.* GA_7 *on autoclaving*. GA_7 (containing 15% GA_4) (100 mg) was refluxed with hydrazine hydrate (1 ml) for 30 min then the reaction mixture was poured into H_2O (10 ml). The diluted reaction mixture was cooled in ice and stirred during acidification with conc. HCl prior to extraction with EtOAc (5 × 10 ml). The EtOAc extract was dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give a gum (95 mg), UV spectrum λ_{max}^{EtOH} 255 nm [cf. gibberellenic acid (XI), λ_{max}^{EtOH} 253 nm[14]]. An aliquot of this product was methylated (CH_2N_2) and analysed by GLC [major observed product (*ca.* 90%), OV17 (190°) R_t 13·7 min, XE60 (180°) R_t 16·4 min] and GLC–MS (major observed product; P^+ at *m/e* 358). These data on the major product of the hydrazine reaction were consistent with its being 13-deoxygibberellenic acid (XII) (Me ester: MW 358). The crude hydrazine reaction product (94 mg) was dissolved in H_2O (94 ml) and the solution was flushed with N_2 at room temp. for 15 min before boiling it for 1·5 hr under N_2. After boiling, the solution was cooled in ice to room temp., adjusted to pH 3 with 4 N HCl then extracted with $CHCl_3$ (4 × 25 ml). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated *in vacuo* to a gum (60 mg) which was subjected to preparative TLC [using two, twice EtOH washed 20 × 20 cm × 250 μm Merck SiO_2 F_{254} plates, developing solvent EtOAc–$CHCl_3$–HOAc (15:5:1)—the band at R_f 0·6–0·65 was extracted with EtOAc]. An aliquot of the product, a gum (25 mg), was methylated (CH_2N_2) and analysed by GLC [major observed product (78%), OV17 (190°) R_t 2·7 min, XE60 (180°) R_t 2·2 min; two minor observed products *a* (17%) and *b* (5%) OV17 (190°) R_ts 2·3 and 3·7 min respectively, XE60 (180°) R_ts 1·8 and 3·0 min respectively] and GLC–MS (major observed product P^+ at *m/e* 282). The retention times of the major component were identical with those of the minor component of a mixture of 13-deoxyallogibberic acid (IV) (25%) and its $\Delta^{15,16}$-isomer (XV) (75%) [Me ester of (XV) GLC–OV17 (190°) R_t 2·4, XE60 (180°) R_t 1·7 min—*cf.* (IV) above] prepared[7] by refluxing GA_7 in THF with dil. HCl, and their MS were also identical. The major hydrolysis product was therefore 13-deoxyallogibberic acid (IV). The minor products were probably *a*, 13-deoxyepiallogibberic acid (V) (*a*, Me ester P^+ at *m/e* 282) and *b*, 13-deoxydehydroallogibberic acid (VI) (*b*, Me ester P^+ at *m/e* 280) by analogy with reactions of gibberellenic acid (XI).[4]

An aq. solution of GA_7 (containing GA_4 15%) (1 mg/ml) was autoclaved for 20 min at 2 atm. and 120°

[12] BISCHOFF, H. W. and BOLD, H. C. (1963) University of Texas Publication No. 6318.
[13] HILLMAN, W. S. and POSNER, H. B. (1971) *Plant Physiol.* **47**, 586.
[14] MOFFAT, J. S. (1960) *J. Chem. Soc.* 3045.

then evaporated to dryness *in vacuo* at 30°. GLC and GLC–MS analysis of an aliquot of this autoclave-product confirmed the presence of 13-deoxyallogibberic acid along with the substance *a* (probably 13-deoxyepiallogibberic acid) and *b* (probably 13-deoxydehydroallogibberic acid) above together with several unidentified products. These three products were anticipated autoclaving products from aq. GA_7 by analogy with the known GA_3 decomposition on autoclaving.[4]

Acknowledgements—I thank Dr. B. E. Cross for samples of dehgdroallogibberic acid and 13-deoxyallogibberic acid (admixed with its $\Delta^{15,16}$-isomer). *iso*-GA_7 was a gift from Dr. J. MacMillan and the samples of GA_3 and GA_7 were a gift from I.C.I. Ltd. Professor J. W. McClure kindly donated starting cultures of *Lemna perpusilla* 6746. GLC–MS was carried out by Messrs. David Green and Tony Wilkinson.