

FROM NATURAL TO SYNTHETIC PLANT GROWTH REGULATORS

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Summary Some gibberellin mimics and antagonists are described. The hydrofluorene carboxylic acids (III) to (IX) mimic allogibberic acid in their inhibition of flowering in *Lemna perpusilla*. Antagonism of gibberellic acid-induced α -amylase synthesis has been observed with a group of arylcyclo-alkyl butenolides (X).

INTRODUCTION

The difficulty of finding synthetic plant growth regulators is well known. Several approaches may be adopted to tackle the problem: random chemical synthesis and screening, synthesis of new compounds related to known synthetic plant growth regulators, or synthesis of compounds structurally related to known biologically active natural products, in particular the plant growth hormones. It is the latter approach on which the present paper concentrates. The natural products approach has been rewarding in herbicide and plant growth regulator production to date and it is a sometimes overlooked but important method of finding new synthetic plant growth regulators. A very good example of the success of this approach is the widespread use of hormonemimetics in the pharmaceutical field. Ideally, the synthetic mimics should have a similar level of biological activity to the natural growth regulators and should have advantages over the natural products such as longevity of the effect, better foliar penetration, and discrete growth regulatory effects. There is also the possibility of producing natural hormone antagonists based on the hormone mimics which could have useful plant growth regulatory effects. Apart from structural knowledge of the natural hormones, knowledge of their biosynthesis and metabolism can be useful. For example, the synthetic compounds may be mimics of biosynthesis precursors of the natural hormones which can be metabolised by the plant to active compounds or interfere with natural hormone biosynthesis, or they may be chemically blocked at a site of metabolic deactivation of the natural hormones thereby producing a longer acting hormone mimic than the natural hormone itself.

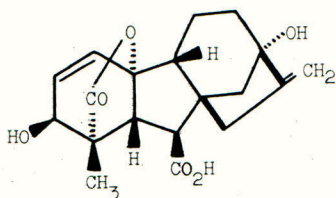
PLANT HORMONE MIMICS

In the area of herbicides and plant growth regulators some examples of the success of chemical mimicry of the plant hormones can be cited: 2,4-dichlorophenoxy acetic acid based on indole acetic acid, tetrahydropyranyl benzylaminopurine based on the natural cytokinins (e.g. zeatin), ethylene generators are a slightly different case but Ethepon is an example here. It would appear that there are no commercialised abscisic acid mimics but the recent publication¹ of the synthesis and high ABA-like activity of some aromatic mimics of abscisic acid (e.g. particularly 3-methyl-5-p-chlorophenyl- Δ^2 -trans, Δ^4 -trans-pentadienoic acid) looks promising. No doubt further work on the mimicry of these natural plant hormones could still be rewarding but there is one large and important group of plant growth regulators, the gibberellins, for which there are no highly active mimics or antagonists designed on the gibberellin structure. CCC is an example of a randomly discovered structure

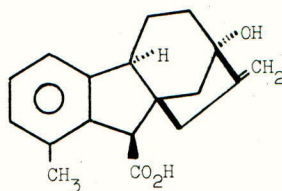
which, inter alia, inhibits gibberellin biosynthesis. This paper describes some approaches to gibberellin mimics and antagonists based on the gibberellin skeleton.

GIBBERELLIN MIMICS AND ANTAGONISTS

An important requirement for any natural hormone mimic is that it should be chemically synthesizable at a reasonable cost, and a look at the gibberellin structure (e.g. gibberellic acid (I)) shows that any mimic must be a considerably simplified chemical structure. Results of the author's work on the aqueous decomposition products of gibberellic acid in relation to inhibition of flowering *Lemna perpusilla*² showed that the decomposition product, allogibberic acid (II), was an inhibitor of flowering for this plant.



(I)



(II)

At the same time known gibberellin-like activity of allogibberic acid was further investigated. In the barley half seed α -amylase assay allogibberic acid has only about 1/1000th the activity of gibberellic acid but in the lettuce hypocotyl test it is more active with about 1/100th the gibberellic acid activity. It has now shown that, in the lettuce hypocotyl test, allogibberic acid not only produces a gibberellin-like response but also acts as a type of competitive inhibitor of the gibberellic acid response (Table 1).

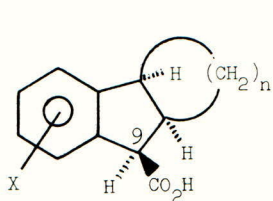
Table 1

Lettuce hypocotyl test on allogibberic acid alone and in combination with gibberellic acid

Hypocotyl lengths expressed as per cent. control (7.3mm).
Means of duplicate assays with 10 seedlings per assay.

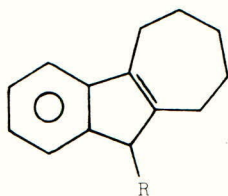
	Gibberellic acid ($\mu\text{g/ml}$)			
	0	0.01	0.1	1.0
0	100	169	227	295
Allogibberic acid 1.0	166	204	247	280
($\mu\text{g/ml}$) 10.0	203	223	232	258
100.0	203	194	191	173

This result may indicate that allogibberic acid is acting at the same "active site" as gibberellic acid and if this is so allogibberic acid offers itself as a much simplified gibberellin to try to mimic. Of the many possible simplified sub-structures of allogibberic acid, hexahydrofluorene carboxylic acids (III) and their ring C-homo analogues (IV) were investigated first.



(III; $n = 4$)

(IV; $n = 5$)

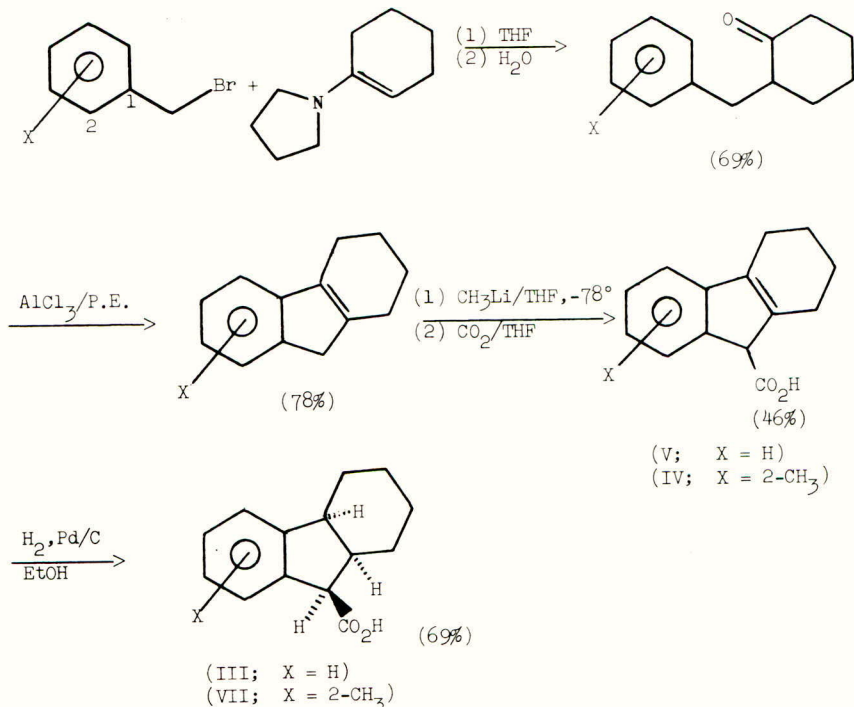


(VIII; $R = H$)

(IX; $R = CO_2H$)

In the latter compounds (IV) the 7-membered ring C is intended to mimic the shape of the gibberellin ring C/D periphery. The hexahydrofluorene-9-carboxylic acid (III, $X=H$) and its 9-epimer had previously been prepared by the author via partial saturation of fluorene-9-carboxylic acid and while both epimers were inhibitors of flowering in *L. perpusilla* the epimer (III, $X=H$) was more active and about as active as allogibberic acid itself.² In order to prepare some aromatic substituted analogues of (III) a new versatile synthesis has been developed (Scheme 1), (cf. refs. 3 and 4).

SCHEME 1 (Yields for $X = H$)



Synthesis of the new homohydrofluorene-9-carboxylic acid (IV) was achieved by carboxylation of the known hexahydrobenzazulene (VIII)⁵ via (IX); the route in Scheme 1 did not work in this case. All of these compounds (IV) - (VII) and (IX) were tested in the *L. perpusilla* flowering assay² and all were inhibitory showing a similar degree of inhibition to the hexahydrofluorene-9-carboxylic acid (III, X = H) Table 2.

Table 2
Inhibition of flowering in *Lemna perpusilla* 6746 by
hydrofluorene-9-carboxylic acid derivatives

Compound	Flowering per cent. of control (66% actually) * at test concentrations of compounds ($\mu\text{g/ml}$)		
	1	10	100
Control	----- 100 -----		
(III)	64	30	0
(IV)	92	62	36
(V)	95	26	plants dead
(VI)	64	47	plants dead
(VII)	80	41	41
(IX)	45	21	plants dead

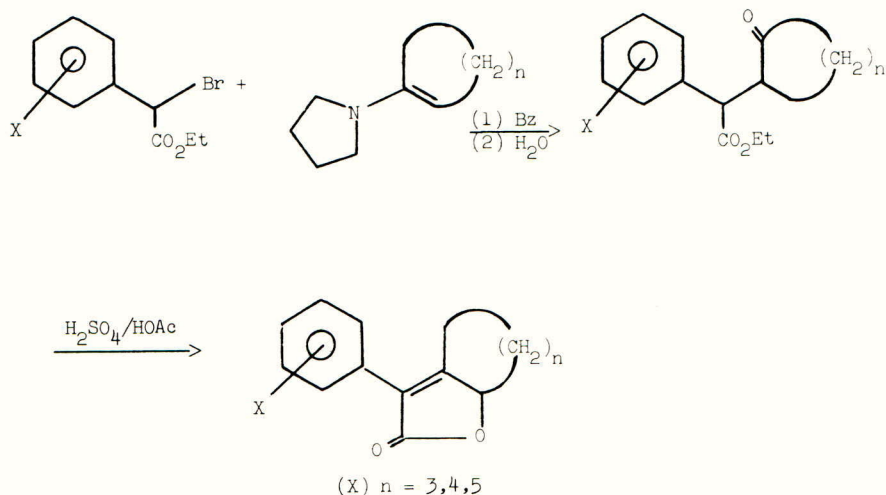
Growth inhibition or phytotoxicity was observed only at the highest dose rate (100 $\mu\text{g/ml}$) in all cases.

* Means of duplicate assays.

Only the tetrahydro ring C-homofluorene-9-carboxylic acid (IX) seemed to be somewhat more active than (III). Unfortunately none of these compounds produced any inhibition of flowering when foliarly applied to mustard or lucerne prior to induction. None of these compounds had any gibberellin-like activity in the lettuce hypocotyl or α -amylase tests and they were only very weak antagonists of the gibberellic acid-induced response in the lettuce hypocotyl test.

A more active group of gibberellin antagonists, as judged by the lettuce hypocotyl and α -amylase bioassays, was found to be the new arylcycloalkyl butenolides of general formula (X). These compounds, which still bear a structural relationship to the gibberellins and allogibberic acid, have been synthesised as shown in Scheme 2. This was in fact a failed synthetic route to hexahydrofluorene-9-carboxylic acid analogues. A number of these butenolides (X) were found to be moderately inhibitory to lettuce hypocotyl extension and they antagonised the gibberellin-induced response to this test. The most active compounds in this test were examined in the more gibberellin-specific α -amylase test where their antagonism of the gibberellic acid-induced response was confirmed, Table 3. In the few cases investigated it was found that the gibberellin antagonism of these butenolides was partially reversible by addition of more gibberellic acid; typical results are shown in Table 4.

SCHEME 2



None of these butenolides caused any inhibition of flowering in the *L. perpusilla* test but (XIV) (see Table 3) at 5 kg/ha, foliar spray, severely inhibited flowering in mustard when applied before induction; no flowering was observed in the treated plants 5 weeks after the treatment and by this time the controls were in full flower. Compound XIV did not inhibit flowering significantly in lucerne.

Acknowledgements

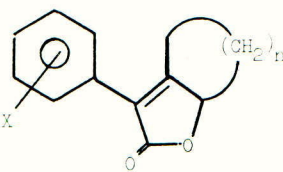
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References

1. S. Bittner, M. Gorodetsky, I. Har-Paz, Y. Mizrahi and A.E. Richmond: Phytochemistry, 1977, 16, 1143.
2. R.J. Pryce: Phytochemistry, 1974, 13, 2377 and references therein.
3. J. Colonge and J. Sibeud: Bull. Soc. Chim. 1953, 75.
4. H.O. House, T.M. Bare and W.E. Hanners: J. Org. Chem. 1969, 34, 2209.
5. W.E. Parham and D.C. Egberg, J. Org. Chem. 1972, 37, 1545.

Table 3

Inhibition of the gibberellic acid-induced α -amylase synthesis
in barley seeds by arylcycloalkyl butenolides

Compound	Per cent. inhibition of gibberellic acid 10^{-7} M response by compounds at concentrations:-*		
	2×10^{-5} M	5×10^{-5} M	2×10^{-4} M
			
(X; n = 3, X = H)		31	95
(XI; n = 3, X = 2 - Cl)	} not tested	61	99
(XII; n = 3, X = 3 - Cl)		74	94
(XIII; n = 3, X = 4 - Cl)		73	99
(XIV; n = 4, X = H)		24	91
(XV; n = 4, X = 2 - CH ₃)	37	91	
(XVI; n = 4, X = 3,4-diCl)	32	74	
(XVII; n = 5, X = H)	7	65	

* Means of triplicate assays

Table 4

Effect of arylcycloalkyl butenolides (X) and (XII)* on gibberellic acid-induced α -amylase synthesis in barley half seeds

Compound	Amylase units** at gibberellic acid concentrations			
	10^{-8}	10^{-7}	10^{-6}	10^{-5} M
Test 1 (Gibberellic acid alone	11	19	26	25
(Gibberellic acid + (X) at 10^{-4} M	0(.3)	5	13	22
Test 2 (Gibberellic acid alone	10	31	32	32
(Gibberellic acid + (XII) at 5×10^{-5} M	1	5	19	20

* See Table 3 for structures

** Means of triplicate assays. One amylase unit = 1 mg of starch hydrolysed/half seed in 15 min at 37°.

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THE INTERACTION OF GROWTH RETARDANTS AND GIBBERELLINS IN STEM

EXTENSION AND FLOWER DEVELOPMENT IN THE POT CHRYSANTHEMUM

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Summary The growth retardants chlorphonium chloride, daminozide and a new quaternary ammonium compound, piproctanyl bromide, all reduced lateral shoot length and delayed the time of flowering of the pot chrysanthemum (*C. morifolium* Ramat) cv. Bright Golden Anne. The retardants delayed flowering by reducing the rate of flower bud development and not by influencing bud initiation. The responses to a single dose of different gibberellins (GA_1 , GA_3 , GA_{4+7} , GA_5 , GA_9 and GA_{13}) given after the application of retardants varied according to the GA and the retardant supplied. Some GAs were highly active in that they overcame the retardant effects on both stem length and the time of flowering, while others were less active.

The results are consistent with a theory of retardant action in which gibberellins play the dominant role and suggest that these hormones are a major factor influencing both stem extension and the rate of flower bud development in the chrysanthemum.

INTRODUCTION

Over the last two decades the use of growth retardants has made commercially successful the large scale culture of many ornamental plants in pots. The chrysanthemum (*C. morifolium* Ramat) has become the most important and valuable of these under glass in the UK with some 20 hectares being grown in 1976, mainly on an all year round basis.

When growth retardants are used by nurserymen on pot chrysanthemums a delay in the time of flowering is associated with the decrease in lateral stem length (e.g. Bunt, 1971; Menhenett 1976 and 1977). These delays occur because growth retardants affect the rate of flower bud development, but not bud initiation. Their reduction or elimination would mean more efficient use of glasshouse space.

Different retardants vary in the extent to which they delay flowering of this crop. Foliar sprays of a new quaternary ammonium compound, 1-allyl-4-(3,7-dimethyloctyl)-piperidinium bromide (piproctanyl bromide) delayed flowering of cv. Bright Golden Anne by 2-4 days more than sprays of daminozide (the a.i. of Alar), but this was similar to the delays associated with the use of compost drenches of chlorphonium chloride, the a.i. of Phosfon formulations (Menhenett, 1977). Because piproctanyl bromide is more effective than daminozide in controlling height under summer conditions and has no adverse effects on flower colour it may be marketed for use by growers in the UK (it is already available in Switzerland, Austria and Germany).

Since there is evidence that growth retardants reduce stem extension by inhibiting the synthesis of endogenous gibberellins (see review by Dicks, 1976), and that these hormones may also be required for the rapid development of chrysanthemum flowers (Jeffcoat and Cockshull, 1972), the effects of different gibberellins (GAs)

on both lateral shoot length and flower development in the pot chrysanthemum have been studied.

METHODS AND MATERIALS

Rooted chrysanthemum cuttings cv. Bright Golden Anne were grown in G.C.R.I. peat:sand compost (3:1 by vol.) in which no retardant was incorporated prior to planting. There was either one plant in a 12 cm pot or 4 plants to each 14 cm halfpot. Experiments were carried out in glasshouses maintained at a minimum temperature of 15°C day and night, with the automatic ventilators opening at 20°C. The plants were grown from potting in short days. Daylength was artificially shortened when necessary by shading with black polythene. After "stopping" (removal of the growing point), the uppermost three lateral shoots were taken on to flowering; these shoots were disbudded. Nutrients in liquid form were supplied at every watering from about 3 weeks after planting. Times of planting, "stopping" and other relevant details are included under individual experiments.

Soil drenches of chlorphonium chloride consisted of 100 ml per pot applied to moist compost. Foliar sprays of daminozide or piproctanyl bromide were applied when the lateral shoots were about 2 cm long, to both sides of all foliage to the point of 'run-off' using a small hand sprayer (0.1% Tween 20 by vol. was added as wetting agent if none was included in the manufacturer's formulation).

GAs were applied as a single dose in 50 or 80% aqueous ethanol to the second leaf (counted from the base) on a lateral shoot using an "Agla" microsyringe. Control plants received only ethanol. Some solution ran down the stem.

'Flowering' was considered to have occurred when the inflorescence had opened to a point midway between Stages 8 and 9 according to Cockshull and Hughes (1972). Least significant differences (LSD) were calculated using a range test (for references see Menhenett, 1977).

RESULTS

In an experiment planted 29 October 1975, 40 µg of GA₃ were applied to the uppermost lateral shoot only of 'control' plants and those previously treated with piproctanyl bromide, daminozide or chlorphonium chloride. A second series of plants were given the retardants but received no GA₃. The hormone increased the rate of stem elongation of the uppermost lateral two days after it was applied. For comparable levels of retardation (considering the stem lengths at flowering of treatments not receiving GA₃) the reversal by GA₃ was more rapid and complete in the case of daminozide- and chlorphonium-treated shoots than when piproctanyl bromide was the retardant. At flowering the shoots receiving the latter chemical had not achieved the length of the "control plus GA₃" laterals (Fig. 1). The hormone increased the length of all internodes on the uppermost lateral (data presented in Menhenett, 1978). Neither the retardants nor GA₃ significantly altered leaf number (Table 1).

Fig. 1 also illustrates the relative delays in flowering brought about by different concentrations of the three retardants. Following the application of GA₃ the rate of development of the flower buds on the treated shoots increased (Table 1) so that the potential delays in the time of flowering did not materialise (see Fig. 1) even in the presence of the higher concentrations of retardants.

In view of the evidence for the basipetal and acropetal transport of GAs in plant tissues (e.g. see Jacobs, 1972 and references therein) it was surprising to find that the GA₃ applied to the uppermost lateral shoot did not change the length of the

Figure 1

Effects of growth retardants alone and growth retardants plus GA₃ (40 µg) on the length and time of flowering of the uppermost lateral shoot of *C. morifolium* cv. Bright Golden Anne (expt. 1). Figures on the graphs indicate concentrations of retardants in ppm.

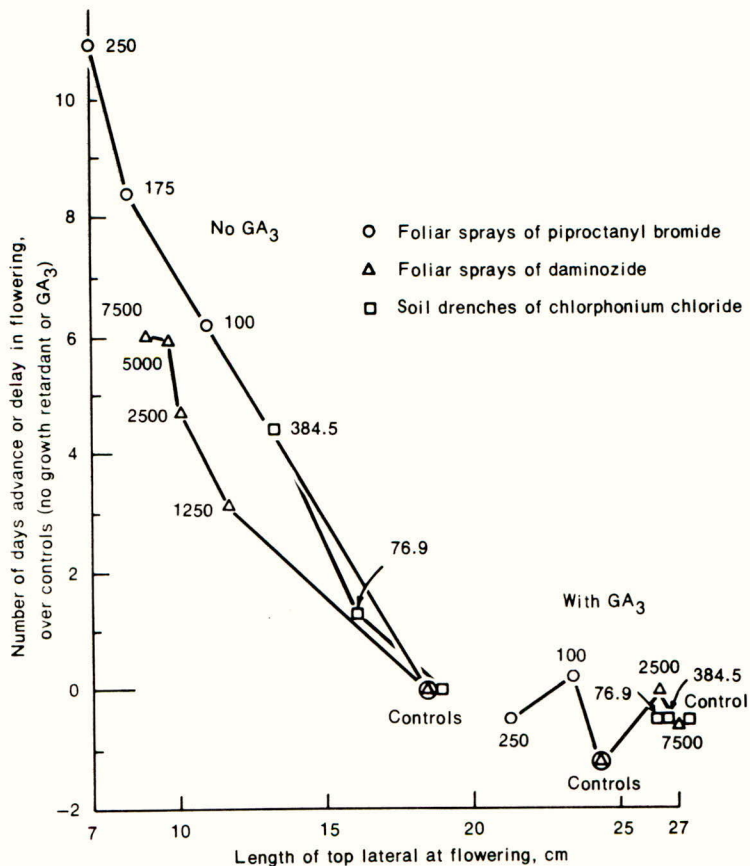


Table 1

Effects of retardants and GA₃ on flower bud development and leaf number on the uppermost lateral, and on the delay in flowering of the third lateral, of *C. morifolium* cv. Bright Golden Anne. N.B. GA₃ applied only to the uppermost lateral shoot

Retardant	Concn (ppm)	GA ₃	Uppermost lateral shoot		Leaf number	Third lateral shoot	
			Flower bud diameter, mm 6 days after GA ₃	12 days after GA ₃		Relative delay in flowering (days)*	% not flowering after 17 wks.
Control (foliar sprays)	0	No	8.7	11.5	5.4	0	0
Control	0	Yes	9.1	12.1	5.8	-0.5	0
Piproctanyl bromide	250	No	7.0	9.1	5.2	16.3	46
"	250	Yes	8.9	12.5	5.8	19.6	63
Daminozide	7500	No	7.4	9.6	5.7	12.9	30
"	7500	Yes	9.0	12.1	5.5	17.4	57
Control (soil drenches)	0	No	8.1	11.3	5.3	0	0
Control	0	Yes	8.9	12.0	5.8	0.3	5
Chlorphonium chloride	76.9	No	8.6	11.6	5.4	2.8	9
"	76.9	Yes	8.9	12.3	5.7	5.9	21
"	384.5	No	7.5	9.9	5.5	9.7	32
"	384.5	Yes	8.8	12.2	5.5	7.3	23+
LSD ($P = 0.05$)			1.4	1.6	0.8		

* The mean date of flowering for the third lateral was 14 or 16 January 1976 for the foliar spray and soil drench controls, respectively. A minus figure indicates that on average that treatment flowered before the appropriate control. For the purpose of compiling the "delay in flowering" column all shoots not bearing open flowers 17 wks. after planting (8 Feb. 1976) were considered to have flowered on that date.

+ Many laterals did not develop normally and were not counted.

second and third laterals on the same plant. However, the time of flowering of those laterals was further delayed (data for the third lateral given in Table 1). Thus, the delay in flowering of the top shoot was overcome only at the expense of greater reductions in the rate of flower bud development on the other two shoots.

The effects of other GAs, applied as single doses to all three lateral shoots on each plant, varied according to the GA and the retardant supplied (Table 2). GA₁, GA₂ and GA₄₊₇ were all highly active in reversing retardant effects on both stem length (in terms of the rate of growth and the stem length finally achieved) and the time of flowering. GA₁₃ was much less active, while GA₉ had a greater effect on the time of flowering for a given increase in stem length than the highly active GAs. An interesting feature of the results was the differential response to GA₉. This GA completely overcame the effects of piproctanyl bromide but induced a relatively small change in plants treated with daminozide. A further trial has indicated that chlorphonium chloride behaves like piproctanyl bromide in regard to its response to GA₉.

Experiments to investigate the effects of different amounts (1, 5, 10, 20 or 50 µg per shoot) of three GAs namely, GA₂, GA₉ and GA₁₃ have confirmed and extended the results. Even 1 µg of GA₂ increased stem length by 7-8 cm when applied to plants previously treated with retardants. The response to 1 µg GA₁₃ was less spectacular but, as suggested by the data of Table 2, this GA does appear to favour the rate of flower bud development rather than stem extension. The 50 µg level of any of the GAs used was not a saturating dose for stem elongation but it appeared to be at or near the optimum in most cases for inducing earlier flowering. However, for the GAs so far tested, a change in the amount of GA given has not led to any dramatic differential effect on either stem extension or flower bud development.

In a trial where 40 µg GA₂ were applied to shoots at various times after spraying with wetter alone or daminozide plus wetter (see Menhenett, 1978), the period of time during which the shoots responded to GA₂ was the same for changes in both stem length and the time of flowering. However, the duration of the period of responsiveness was prolonged following retardant application. Obviously the longer that the application of a GA is delayed then the less time available for hastening flowering. Late applications of GA₂ may delay flowering further.

DISCUSSION

The experiments described here have demonstrated that a close relationship exists between stem extension and flower bud development in C. morifolium cv. Bright Golden Anne and strongly suggest that the restrictions on stem extension and flower development consequent upon the application of retardants largely result from a common cause namely, gibberellin deficiency. Estimates of the GA content of the lateral shoots of chrysanthemum obtained by the author do indeed suggest that the above experiments, following the application of retardants, the shoots were deficient in one or more natural GAs. The results are, therefore, consistent with a gibberellin theory of retardant action, a view supported by studies indicating that compounds such as chlorphonium chloride and daminozide can block particular steps in the biochemical pathway leading to the synthesis of naturally occurring GAs, which appear necessary for the maintenance of subapical meristematic activity (Sachs and Kofranek, 1963; Ryugo and Sachs, 1969; Wylie et al., 1970; Lang, 1970).

The differential response to GA₉ indicates that retardants may not only restrict the synthesis of GA precursors but also influence the conversion of one GA to another. The degree of response to the various GAs in the absence of retardants (Table 2) may reflect the limited capacity of the lateral shoot tissues of C. morifolium cv. Bright Golden Anne to effect such interconversions. Possible

Table 2

The relationship between lateral stem length (cm) and time of flowering of *C. morifolium* cv. Bright Golden Anne following the application of 40 µg of gibberellins A₁, A₂, A₄₊₇, A₅, A₉, or A₁₃ to plants previously treated with daminozide (10,000 ppm) or piproctanyl bromide (200 ppm). GAs applied 6 days after the retardants to all three laterals.

GA	No retardant		Daminozide		Piproctanyl bromide	
	Stem length	Advance (-) or Delay (+) in Flowering*	Stem length	Advance (-) or Delay (+) in Flowering*	Stem length	Advance (-) or Delay (+) in Flowering*
None	26.5	0	16.3	5.4	17.8	5.0
A ₁	42.6	-2.9	38.1	1.6	38.3	0
A ₃	42.2	-2.6	40.8	-1.4	37.9	-2.3
A ₄₊₇	39.8	-2.4	38.7	0.6	38.3	-0.9
A ₅	32.6	-2.9	28.1	0	27.1	-2.0
A ₉	40.2	-1.6	20.7	4.1	37.3	-0.4
A ₁₃	27.3	-1.5	21.9	3.5	23.9	2.1
LSD (P = 0.05) (within and between groups)	4.1		4.1		4.1	

Planted 4 Aug. 1976, stopped 13 Aug., and retardants applied 26 Aug.

* Relative to control (no retardant or GA) which is designated day 0. A minus figure indicates that on average that treatment flowered before the control. GA₄₊₇ = 55% A₄ and 45% A₇. A₁ and A₅ were very pure; A₄₊₇ could have contained traces of A₆, A₈, and A₁₃ possibly contained traces of other GAs, while A₉ was better than 90% pure.

interference by the retardants in GA transport from the site of application to the subapical region of the shoot and to the flower bud, and/or effects on the compartmentalization, inactivation and action of GAs also merit consideration.

All these possibilities must be kept in mind in seeking to achieve the practical objective of reducing or eliminating the delays in flowering brought about by different retardants without substantially increasing plant height. It is, perhaps, worth pointing out that in attempting to devise chemical (or environmental or manipulative) treatments to maximise the number of days that flowering is advanced per unit increase in stem length, the relationship obtained must be an improvement on that which is possible merely by applying a lower concentration of retardant. The economic and commercial feasibility of any treatment is also of the utmost importance.

It is of particular relevance to consider how GAs might influence the rate of flower bud development in the pot chrysanthemum. The data presented in Fig. 1 and Table 1, showing that advances in the time of flowering of the top lateral shoots to which GA₂ was applied were associated with greater delays in the time of flowering of the untreated shoots, could have arisen because the GA₂ attracted assimilates destined for the flower buds carried on the second and third lateral stems. The fact that a considerable proportion of second and, particularly, third laterals on plants treated with the higher concentrations of retardants failed to bear open flowers (at least by a given date) could indicate that the buds on these shoots were unable to attract sufficient assimilates to develop properly. Active GAs, whether exogenously applied or naturally occurring, may therefore be important factors in determining the distribution of resources within the chrysanthemum plant.

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References

- BUNT, A.C. (1971). The use of peat-sand substrates for pot chrysanthemum culture. Acta Horticulturae, 18, 66-74.
- COCKSHULL, K.E. and HUGHES, A.P. (1972). Flower formation in Chrysanthemum morifolium: the influence of light level. Journal of Horticultural Science, 47, 113-27.
- DICKS, J.W. (1976). Chemical restriction of stem growth in ornamentals. Outlook on Agriculture, 9, 69-75.
- JACOBS, W.P. (1972). The movement of plant hormones: auxins, gibberellins and cytokinins. In Plant Growth Substances 1970. Ed. D.J. Carr. Springer-Verlag, Berlin, Heidelberg and New York. 701-709.
- JEFFCOAT, B. and COCKSHULL, K.E. (1972). Changes in the levels of endogenous growth regulators during development of the flowers of Chrysanthemum morifolium. Journal of Experimental Botany, 23, 722-32.
- LANG, A. (1970). Gibberellins: structure and metabolism. Annual Review of Plant Physiology, 21, 537-70.
- MENHENETT, R. (1976). New growth retardant for pot mums has promise. Grower, 85, 410 and 412.
- MENHENETT, R. (1977). A comparison of the effects of a new quaternary ammonium growth retardant with those of other growth retarding chemicals on the pot chrysanthemum (Chrysanthemum morifolium). Annals of Applied Biology, 87, 451-63.

- MENHENETT, R. (1978). Effects of growth retardants, gibberellic acid and indol-3-yl acetic acid on stem extension and flower development in the pot chrysanthemum (Chrysanthemum morifolium Ramat). Paper to be submitted to Annals of Botany.
- RYUGO, K. and SACHS, R.M. (1969). In vitro and in vivo studies of Alar (1,1-dimethylaminosuccinamic acid, B-995) and related substances. Journal of the American Society for Horticultural Science, 94, 529-33.
- SACHS, R.M. and KOFRANEK, A.M. (1963). Comparative cytohistological studies on inhibition and promotion of stem growth in Chrysanthemum morifolium. American Journal of Botany, 50, 772-79.
- WYLIE, A.W., RYUGO, K. and SACHS, R.M. (1970). Effects of growth retardants on biosynthesis of gibberellin precursors in root tips of peas. Journal of the American Society for Horticultural Science, 95, 627-30.

Growth Regulation (1978)

THE EFFECTS OF DIKEGULAC ON THE FLOWERING
AND GROWTH OF SOME ORNAMENTALS

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Summary Dikegulac, the a.i. of ATRINAL[®], is a new growth regulator for ornamentals. It has been found to be particularly effective as a growth retardant and pinching agent for Begonia elatior, Fuchsia hybrida, Kalanchoe blossfeldiana and Pachystachis lutea. The plants were of uniform compact shape. ATRINAL stimulated also the flower development on Cyclamen persicum and Gerbera jamesonii. The optimum concentration varies with species and environmental conditions. ATRINAL has a very low bee, fish, bird and mammalian toxicity.

Résumé Dikegulac, la substance active d'ATRINAL[®], est un nouveau régulateur de croissance pour plantes d'ornement. Elle s'est révélée particulièrement efficace comme retardateur de croissance et agent de pincement pour Begonia elatior, Fuchsia hybrida, Kalanchoe blossfeldiana et Pachystachis lutea. Les plantes présentent une forme compacte. ATRINAL a aussi stimulé le développement des fleurs du Cyclamen persicum et Gerbera jamesonii. La concentration optimale varie selon les espèces et les conditions ambiantes. ATRINAL a une toxicité très basse pour les abeilles, poissons, oiseaux et mammifères.

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INTRODUCTION

A novel plant growth regulator which has the common name dikegulac* (sodium 2,3:4,6-di-o-isopropylidene-2-keto-L-gulonate) and is the active ingredient of ATRINAL[®], was recently presented by Bocion et al (1975).

As the free acid was found to be unstable, various derivatives were examined and the sodium salt was chosen for further development. Dikegulac has an extremely low bee, fish, bird and mammalian toxicity and is not an eye or skin irritant (de Silva et al 1977).

Heursel (1975) and de Silva et al (1976) reported a loss of apical dominance and stimulation of axillary shoot production on various azalea cv. after treatment with dikegulac. Sachs et al (1975) demonstrated that dikegulac inhibited shoot elongation for more than 3 months on many shrubs and trees in California. Bocion and Walther (1976) described growth retardatory effects on hedge plants in Switzerland. In various European countries and Japan dikegulac applied to different hedge species, which were pruned in autumn and treated the following spring did not require pruning during the entire growing season. Dikegulac can also inhibit

*ISO approved common name

fruiting where this is desirable (de Silva et al 1976). Dikegulac treatment increased flower production in Gerbera jamesonii and Cyclamen persicum and axillary shoots in Begonia elatior and Fuchsia hybridum (Bocion et al 1977).

De Silva et al (1977) reported plant growth regulatory effects on many crops e.g. retardation of vegetative growth and induction of branching on olive trees, the application during early flower development of olives can entirely suppress fruit set, while inducing a large number of new shoots to develop, providing for a heavy crop the following year. On rice the number of heads and yield were increased. Storey et al (1975) and Malstrom et al (1977) demonstrated abscission activity and an increase in axillary shoot number when dikegulac was applied to pecan trees. Bocion and de Silva (1976 a) observed interactions between dikegulac and GA₃, IAA, kinetin and ethylene. Physiological studies indicated that dikegulac inhibits DNA synthesis throughout the apical meristem, but not in axillary buds (Arzee et al, 1977). Gressel (1976) showed that dikegulac depressed uridine incorporation into both plastid and cytoplasmic ribosomal RNA of axenically cultured Spirodela and therefore RNA synthesis was suppressed. Thus it was concluded that dikegulac probably acts hormonally on the apex unlike X-irradiation and other chemical pinching agents.

The aim of the present report is to summarise the research which has been done so far on this compound in respect to ornamentals and to compile our recent findings in this area.

METHODS AND MATERIALS

All experiments reported below were executed in the greenhouse using conventional growing procedures. ATRINAL, which is a water soluble concentrate containing 200 g dikegulac per liter, was diluted with the appropriate volume of water using 0.1 % nonoxynol as a wetter.

Gerbera jamesonii: Well developed 3 months old plants were transplanted into plastic containers (diam. 24 cm) containing a peat mixture (pH 5.5) to which 2 g/l of a complete fertilizer (N20 P10 K15 Mg6) were added. When the plants were well established they were fertilized once or twice weekly with 0.2 % WUXAL (N10 P10 K7). The temperature at night was kept at 18°C and during the day between 20 and 26°C depending on the radiation. A 16 h photoperiod was given during the entire season using high pressure mercury lamps (Philips HPL-N; 400 Watt/m²) when the light intensity fell below 5000 lux. ATRINAL was applied at a concn of 75 - 625 mg a.i./l with a spray vol. of 180 ml/m² at the 7 leaved stage. Between treatment (September 12, 1974) and termination of the experiment (March 5, 1975) the flowers, which were ready for sale were harvested once or twice per week. A second experiment was carried out in a commercial nursery under similar growing conditions except that no additional light was supplied. The plants were treated with a concn of 75 mg a.i./l and a spray vol. of 180 ml/m². The flowers were harvested and counted from May 31 until November 8, 1976.

Cyclamen persicum cv. Leuchtfeuer: The plants were propagated in the greenhouse in peat (pH 5.5) under practical conditions. At the beginning of June the plants were transferred outdoors. The plants were treated when the first flower buds were visible (August 13, 1976) or on September 9, 1976. ATRINAL was applied at concn of 125 - 500 mg a.i./l with a spray vol. of 250 ml/m². The number of flowers per plant were counted on December 8, 1976 and expressed as % of untreated.

Begonia elatior cv. Schwabenland: The influence of ATRINAL on the development of axillary buds was investigated on rooted cuttings. One month after transplanting into pots of 10 cm diam., when the plants were 6 - 8 cm high, they were treated with concn of 625 - 2500 mg a.i./l using a spray vol. of 250 ml/m².

Fuchsia hybrid cv. Beacon were treated when they were 5 - 10 cm high (March 3, 1975) with a concn of 1850 mg a.i./l and a spray vol. of 250 ml/m². The number of side shoots and the height were assessed on June 6, 1975. In a second experiment cv. Beacon, W. Churchill and Ortenburger Festival were sprayed with ATRINAL, when the plants were 8 - 12 cm high (May 12, 1976) at concn of 1250 - 1850 mg/l a.i. and a spray vol. of 250 ml/m². The assessment was carried out as mentioned above.

Pachystachis lutea: The plants were treated with ATRINAL at concn of 250 and 500 mg a.i./l after pinching when the primary side shoots were 4 - 8 cm long (March 24, 1975). In a additional experiment unpinched cuttings were sprayed at concn of 500 and 750 mg a.i./l. The height of the plants and the number of side shoots and inflorescences were assessed on May 5, 1975.

Kalanchoe blossfeldiana cv. Feuerzauber and cv. Feuerball: Application of ATRINAL was carried out 6 weeks (Febr. 4, 1975) after transplanting of the seedlings (1 day after pinching) at concn ranging from 625 - 2500 mg a.i./l and a spray vol. of 250 ml/m². The height of plants and the number of inflorescences was assessed on May 5, 1975.

RESULTS AND DISCUSSION

Gerbera jamesonii

Table 1

Effect of ATRINAL on flower number and petiole length
(Each value a mean of 15 plants)

ATRINAL mg a.i./l	Flowers/ plant	Length of petioles, cm
0	37	50
75	53	50
150	53	51
300	47	45
600	36	37

The best effects on flower formation and development were obtained at concn of 75 and 150 mg a.i./l ATRINAL, whereby 53 flowers were harvested compared with 36 flowers per plant on untreated plants (Table 1) from Sept. 12, 1976 to Mar. 5, 1977. The highest concn, 600 mg a.i./l, resulted in shorter flower petioles and only 37 flowers were harvested. The increase of flowers is remarkable and of commercial interest, because during the winter months the flowers command high prices. Based on the results reported above a second experiment was carried out in a commercial nursery in Switzerland where a substantial increase in flowers was also achieved (Table 2).

Table 2

Influence of ATRINAL on the flower development
of cv. Apfelblüte during the summer
(Each value a mean of 20 plants.)

Harvest period		Number of flowers		% rel. to untreated
		untreated	75 mg a.i./l	
31.V.	17.VIII.77	13.9	17.1	123
18.VIII.	- 29.IX. 77	10.5	12.1	115
30.IX.	- 8.XI. 77	6.8	7.7	113
Total		31.2	36.9	118

Usually (personal communication of Prof. Penningsfeld, Technical University Munich) the number of flowers per plant is proportional to the number of leaves. In the experiments reported above the number of leaves was not increased. From these experiments it is not possible to conclude whether ATRINAL influenced floral initiation or the further development of floral buds already initiated.

Cyclamen persicum cv. Leuchtfleur

Table 3

The effect of ATRINAL on flower production
(Each value a mean of 18 plants.)

ATRINAL mg a.i./l	Date of treatment	Number of flowers per plant	Number of flower buds per plant
0	13.VIII.76	6.8	8.8
125		10.2	11.5
250		10.0	10.5
500		10.1	9.9
125	9.IX.76	4.7	8.3
250		8.8	7.6
500		8.1	10.6

The earlier treatment on August 13, showed a better effect on flower production (Table 3) than the later application on Sept. 9. The plants were well developed and showed a compact shape.

Begonia elatior cv. Schwabenland

Table 4

Effect of ATRINAL on growth and development
(Each value a mean of 12 plants.)

ATRINAL mg a.i./l	Number of axillary shoots per plant	Number of open flowers per plant	Plant height cm
0	8.7	7.5	20
625	10.7	9.2	24
1250	12.1	6.5	16
2500	12.5	1.8	11

ATRINAL induced most side shoots if it was applied at a concn of 2500 mg a.i./l (Table 4), but the growth was severely retarded and flower development delayed. At a concn of 625 mg a.i./l 23 % more side shoots were found compared with untreated and flowering was slightly accelerated. The plants showed no phytotoxic symptoms and were of uniform shape. From experiments in commercial nurseries it is known that other varieties also respond well to ATRINAL. For practical applications 1750 mg a.i./l is recommended for unpinched and 1250 mg a.i./l for pinched plants.

Fuchsia hybrida cv. Beacon, W. Churchill
and Ortenburger Festival

Table 5

Pinching and retarding effect of ATRINAL
(Each value a mean of 15 plants.)

ATRINAL mg a.i./l	cv. Beacon		cv. W. Churchill		Ortenb. Festival	
	no. of axillary shoots	height cm	no. of axillary shoots	height cm	no. of axillary shoots	height cm
0*	10.3	28.8	9.0	31.5	5.5	19.3
1250	23.0	20.6	27.3	21.7	12.4	19.3
1850	21.3	20.9	23.3	20.6	14.7	17.2

*handpinched

On cv. Beacon and W. Churchill the best pinching effect was achieved at a concn of 1250 mg a.i./l (Table 5). On the vigorously growing cv. Ortenburger Festival the same effect was obtained at a concn of 1850 mg a.i./l. The plants showed a compact uniform shape, therefore no growth retardant application was necessary. ATRINAL can therefore be applied with success from March until May on Fuchsia. From additional experiments it is known that ATRINAL can also be used from January onwards at a concn of 1850 mg a.i./l with a spray vol. of 250 ml/m² when shoots are 5 - 10 cm long on the vigorously growing cv: Glitters, Mazda, Ortenburger Festival and Perle. A concn of 1250 mg a.i./l is sufficient for less vigorous cv.: Beacon, Berkeley, Cascade, Caramia, Cover Girl, Crescendo, Dark Secret, Dollarprinzessin, El Camino, Georgiana, Göteborgskan, Golden Glow, Hanna, Heron, Koralle, Leverkusen, Marinka, Pink Ballet Girl, Pride of Orion, Red Shadows, Red Spider, Swingtime and W. Churchill.

Kalanchoe blossfeldiana cv. Feuerzauber and Feuerball

Table 6

Effect of ATRINAL on inflorescence number and plant height
(Each value a mean of 10 plants.)

ATRINAL mg a.i./l	cv. Feuerzauber		cv. Feuerball	
	number of inflorescences	height cm	number of inflorescences	height cm
0*	6.0	19.0	9.0	17.2
625	8.5	20.5	-	-
1250	9.3	20.5	9.4	17.0
2500	9.0	18.0	8.8	13.8

*handpinched

ATRINAL treated plants of the cv. Feuerzauber developed more inflorescences compared with untreated (Table 6) at concn ranging from 625 - 2500 mg a.i./l. The best effect was achieved at a concn of 1250 mg a.i./l which gave 9.3 inflorescences compared with 6 on the untreated plants. Only small effects were recorded on plant height. On cv. Feuerball the ATRINAL treatment produced a similar number of inflorescences as handpinched but at the higher concn the growth was retarded. From unpublished results it is known that the concn must be adjusted to season and cv. ranging from 750 - 3000 mg a.i./l. With increasing light intensity and temperature higher concn of ATRINAL have to be applied to Kalanchoe blossfeldiana as was reported for azalea by Bocion and de Silva (1976 b).

Pachystachis lutea

Table 7

Effect of ATRINAL on growth and development
(Each value a mean of 8 plants.)

ATRINAL mg a.i./l	Number of axillary shoots	Number of inflores- cences	Plant height cm	Appearance
0 1)	2.3	2.0	24.7	good
500 1)	3.3	5.1	33.8	moderate
750 1)	3.1	5.0	20.1	moderate
250 2)	2.4	1.3	26.8	good
500 2)	3.8	2.6	20.7	very good

1) unpinched

2) handpinched before treatment

The best result was obtained with ATRINAL when the side shoots were 5 - 8 cm long after handpinching and if 500 mg a.i./l was applied. Application to unpinched cuttings resulted in too many inflorescences and the plants showed an irregular shape.

References

- ARZEE, T., LANGENAUER, H. and GRESSEL, J. (1977) Effects of dikegulac a new growth regulator on apical growth and development of three Compositae. Botanical Gazette, 138, 18-28.
- BOCION, P.F., HUEPPI, G.A., DE SILVA, W.H. and SZKRYBALO, W. (1975) Group of new chemicals with plant growth regulatory activity. Nature, 258, 142-144.
- BOCION, P.F. and DE SILVA, W.H. (1976 a) Some effects of dikegulac on the physiology of whole plants and tissues: interactions with plant hormones. Plant Growth Regulation, 9th International Conference on Plant Growth Substances 189-198.
- BOCION, P.F., DE SILVA, W.H. (1976 b). The interaction of dikegulac and environmental factors on apical dominance of Rhododendron simsii (Planch). Collected abstracts of the paper demonstration of the 9th International Conference on Plant Growth Substances, Lausanne Switzerland, 40 - 42.
- BOCION, P.F. and WALTHER, H.R. (1976) Atrinal als Wachstumsregler für Heckenpflanzen. Gartenwelt, 76, 179-183.
- BOCION, P.F., DE SILVA, W.H., WALTHER, H.R. and GRAF, H.R. (1977) Versuche mit Atrinal bei Gerbera, Cyclamen, Begonien und Fuchsien. Gärtnerbörse und Gartenwelt, 27, 634-636.
- GRESSEL, J., KADOURI, A., ATSMON, D. and COHEN, N. (1976) Effects of dikegulac on nucleic acid synthesis. Collected abstracts of the paper demonstrations of The 9th International Conference on Plant Growth Substances in Lausanne, August 30 to September 4, 117.
- HEURSEL, J. (1975) Results of experiments with dikegulac used on azaleas (Rhododendron simsii, Planch). Medelingen Faculteit Landbouwwetenschappen Gent, 40, 849-857.
- MALSTROM, H.L. and MC MEANS, J.L. (1977) A chemical method of pruning young pecan trees. HortScience, 12, 68-69.
- DE SILVA, W.H., BOCION, P.F. and WALTHER, H.R. (1976) Chemical pinching of Azalea with dikegulac. HortScience, 11(6), 569-570.
- DE SILVA, W.H., GRAF, H.R. and WALTHER, H.R. (1976) Dikegulac: A novel growth retardant and branching agent for hedges. Proceedings British Crop Protection Conference-Weeds, 349-356.
- DE SILVA, W.H., BOCION, P.F., OLIVER, K.W. and GUERRY, X. (1977) Dikegulac a new growth regulator and its effect on plant development. Pesticide science, (in press).
- SACHS, R.M., HIELD, H. and DE BIE, J. (1975) Dikegulac: A promising new foliar-applied growth regulator for woody species. HortScience, 10(4), 367-369.
- STOREY, J.B., SMITH, M. and HANNA, J.D. (1975) Influence of a new chemical as harvest aid on pecans. HortScience, 10, 144.