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The Biological Activity of Fluorogibberellins

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Summary. The biological activities of gibberellin A_9 (GA_9), gibberellin A_{12} (GA_{12}) and monofluoro-analogues (F-GA_9 and F-GA_{12}), substituted in the 1 β-methyl group, were compared in the barley endosperm, cucumber hypocotyl, lettuce hypocotyl, 'Meteor' dwarf pea, dwarf-5 maize and *Rumex* leaf disc assays. In most cases the fluorosubstituted compounds had a potency similar to, or less than, the relevant unmodified gibberellin but, in the lettuce assay, F-GA_9 was approximately 5 times more active than GA_9 up to a dose rate of 10^{-1} µg.

A 27–30% mixture of fluorogibberellin A_3 (F-GA_3) in GA_3 had a lower activity than 100% GA_3 in the barley endosperm, lettuce hypocotyl and dwarf maize assays. This suggested that pure F-GA_3 may be a competitive inhibitor of GA_3 action. The findings are discussed in the context of the structure/activity relationships of the gibberellins.

Introduction

Kirk and Cohen (1971) have pointed out that replacement of a hydrogen atom with fluorine produces only a small increase in size at the site of substitution but results in a considerable enhancement of electronegativity and a strong hydrogen bonding potential. It might, therefore, be expected that fluorosubstitution at significant sites in a plant or animal hormone would markedly alter the binding behaviour and, consequently, the biological activity of the molecule.

The hormonal properties of cortico-steroids can, in certain instances, be enhanced by the substitution of fluorine for a hydrogen atom or hydroxyl group; the 21-fluoro analogues of cortisone and progesterone being considerably more effective than the parent compounds when administered orally (Taylor and Kent, 1965). However, fluorination of other biochemical intermediates such as sugars, nucleotides or tricarboxylic acids leads to the production of potent inhibitors of enzyme action, as exemplified by 5-fluorouracil, p-fluorophenylalanine and fluorocitrate.

Recently, Bateson and Cross (1972) have succeeded in preparing pure samples of fluorogibberellin A_9 (F-GA_9) and fluorogibberellin A_{12} (F-GA_{12}) where the fluorosubstitution was made in the 1 β-methyl group (Fig. 1). The F-GA_9, and a 27–30% mixture of fluorogibberellin A_3 (F-GA_3) in unmodified GA_3, were isolated from *Gibberella fujikuroi* cultures fed with

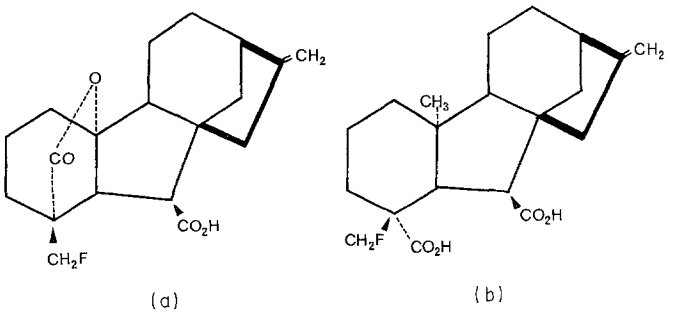

Fig. 1a and b. Structural formulae of fluorogibberellin A_9 (a) and fluorogiberellin A_{12} (b)

a F-GA_{12} aldehyde precursor during the phase of active gibberellin synthesis. As yet, it has proved impossible to prepare the F-GA_3 in a pure state. Samples of F-GA_{12} were obtained chemically by oxidation of the fluoroaldehyde.

The effects of fluorination on the biological activity of GA_9 and GA_{12} are described together with some preliminary observations on the properties of the F-GA_3/GA_3 mixture.

Materials and Methods

Gibberellin Samples

All fluorogibberellin samples were supplied by Dr. B. E. Cross, Department of Organic Chemistry, University of Leeds, U.K. The preparation and authentication of these compounds has already been described elsewhere (Bateson and Cross, 1972). A reference sample of GA_{12} was also provided by Dr. Cross. Supplies of GA_3 and GA_9 were kindly provided by Imperial Chemical Industries Ltd., Alderley Park, Macclesfield, Cheshire, U.K. It was necessary to purify the GA_9 (batch PH/FNB/753-3B) by preparative thin-layer chromatography before use, in order to remove a GA_4/GA_7 contamination of approximately 10% by weight.

All compounds were dissolved initially in absolute ethanol (1 mg in 0.25 ml) and made to a total volume of 100 ml with distilled water. No wetting agents were added.

Bioassay Methods

a) Barley, Half-Seed Assay (α-Amylase Release). Conducted according to the method of Jones and Varner (1967) except that the half grains were imbibed for 48 h on pre-sterilised pads of Whatman 3 MM filter paper. The cultivar 'Himalaya' (1966 harvest), provided by Dr. R. A. Nilan, Washington State University, Pullman, Washington, U.S.A. was used for all assays.

b) Cucumber Hypocotyl Assay. The cultivar 'Long Green Ridge' (Carters Tested Seeds Ltd, Raynes Park, Middlesex, U.K.) was used as the assay material and the procedures followed those of Brian *et al.* (1964). Responses were measured 48 h after treatment.

c) Lettuce Hypocotyl Assay. This was performed according to the method described by Frankland and Wareing (1960) using the cultivar "Arctic" (Carters Tested Seeds Ltd.) throughout. The seeds were presprouted in the light, placed upon 2.5 cm squares of Whatman No. 1 paper in 5 cm petri dishes and the test compounds were added to the substrate. Light was provided by daylight-type fluorescent tubes at an intensity of 600 lumens/sq.ft. Hypocotyls were measured 48 h after commencement of the assay.

d) Dwarf Maize Assay. The methods were essentially those described by Phinney and West (1961) and activity estimates were based upon the total growth of the second and third leaf sheaths as measured 10 days after application. Only the dwarf-5 material was used and seedlings were grown from homozygous dwarf stocks produced by intercrossing dwarf plants under glasshouse conditions. The original stocks were provided by Prof. B. O. Phinney, University of California, Los Angeles, U.S.A.

e) Dwarf Pea Assay. 'Meteor' dwarf peas (Carters Tested Seeds) were grown and utilised in the manner described by Brian *et al.* (1964). Response was assessed by measurement of the length of the third internode on the 7th day after application.

f) Rumex Leaf Disc Assay. Discs of *Rumex obtusifolius* leaf tissue, 1 cm in diameter, were prepared according to the technique of Whyte and Luckwill (1966) and placed upon 2.5 cm squares of Whatman No. 1 paper containing the test compounds. The assay units were enclosed in 5 cm petri dishes and kept in darkness until yellowing occurred in the controls. Each treatment was then extracted by grinding in 80% acetone and, after centrifugation, the chlorophyll content was estimated by measuring the optical density at 660 nm.

Cucumber, lettuce, maize and pea assays contained a 5 times replication within each test and the entire assay was repeated on several occasions. Standard errors were computed for each point on the response curves. Barley half-seed assays were based upon triplicate estimations at each dose level.

Results

The relative activities of GA_9, GA_{12} and their fluorosubstituted analogues in the various bioassay systems are detailed in Table 1.

Both GA_9 and GA_{12} have a low activity in the barley endosperm assay when compared to GA_3 and, in both instances, fluorination reduced their effectiveness still further. The cucumber hypocotyl assay is more sensitive to GA_9 than to GA_{12} and the substituted compounds again showed diminished activity although, in the case of GA_9, the difference was not significant at the 10^{-1} µg level.

In the dwarf-5 maize and 'Meteor' dwarf pea assays, the fluorosubstituted analogues produced responses which were not significantly different from those elicited by the unmodified gibberellins. However, in the *Rumex* leaf disc assay both F-GA_9 and F-GA_{12} were markedly less effective than their unsubstituted equivalents in retarding chlorophyll breakdown.

The most notable effect of fluorination was seen in the lettuce hypocotyl assay where the substituted compounds had greater activities. This effect was considerable in the case of GA_9 but barely significant with

Table 1. Biological activities of gibberellin A_9 (GA_9), gibberellin A_{12} (GA_{12}) and their fluorosubstituted analogues (F-GA_9 and F-GA_{12}) compared with gibberellin A_3 (GA_3) in 6 bioassay systems (Activities expressed as a percentage of control except where otherwise indicated)

Compound	Dose (μg)					
	Barley[a] endosperm		Cucumber hypocotyl		Lettuce hypocotyl	
	10^{-1}	10^{-2}	10^{-1}	10^{-2}	10^{-1}	10^{-2}
GA_9	70	28	302 ± 6	177 ± 4	286 ± 10	130 ± 5
F-GA_9	40	13	289 ± 11	106 ± 4	379 ± 7	291 ± 5
GA_{12}	112	36	159 ± 9	84 ± 7	156 ± 12	108 ± 7
F-GA_{12}	49	8	119 ± 12	62 ± 2	178 ± 8	125 ± 3
GA_3	249	130	380 ± 17	187 ± 8	380 ± 15	187 ± 12

Compound	Dose (μg)					
	Dwarf-5 maize		Dwarf pea		Rumex leaf disc	
	10^{-1}	10^{-2}	10^{-1}	10^{-2}	10^{-1}	10^{-2}
GA_9	326 ± 18	160 ± 9	202 ± 14	130 ± 7	237 ± 8	215 ± 12
F-GA_9	316 ± 10	145 ± 11	199 ± 3	128 ± 10	104 ± 6	96 ± 6
GA_{12}	253 ± 8	140 ± 6	143 ± 10	110 ± 2	300 ± 14	108 ± 2
F-GA_{12}	264 ± 7	180 ± 7	148 ± 9	112 ± 5	250 ± 16	92 ± 4
GA_3	283 ± 13	210 ± 4	335 ± 17	193 ± 6	560 ± 21	422 ± 13

[a] Expressed as α amylase units after subtraction of blank values. Figures are means of triplicate estimations.

GA_{12}. At the 10^{-1} μg level fluorination raised the activity of GA_9 to a level comparable with GA_3 and, at the 10^{-2} μg dose rate F-GA_9 gave a much greater response than GA_3.

In view of this result, a more detailed investigation was undertaken in oder to determine the precise dose/response characteristics of GA_9 and F-GA_9 in the lettuce hypocotyl assay. The findings from this study are presented in Fig. 2.

At all points in the concentration range, GA_9 was less effective than the fluoro-analogue but the difference at the 10^{-0} μg/dish dose rate was insignificant. Over the range of concentrations tested, GA_9 gave an essentially linear response with the threshold of sensitivity at approximately 5×10^{-3} μg. In contrast, the F-GA_9 results showed a marked saturation effect above 10^{-1} μg/dish but, at the other end of the range, a significant hypocotyl extension was noted at a dose rate of 10^{-3} μg.

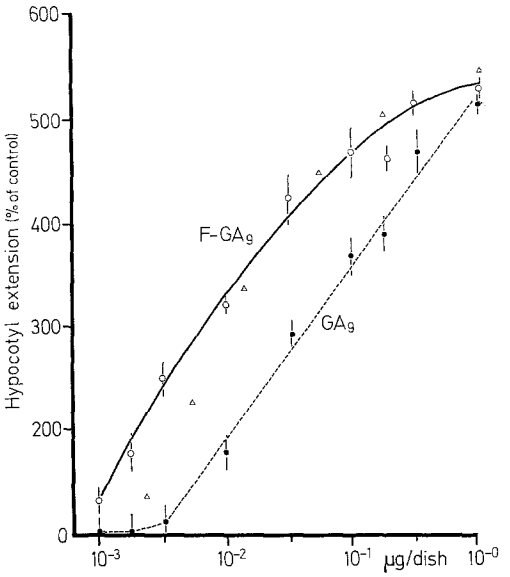

Fig. 2. Dose/response curves in the lettuce hypocotyl assay for gibberellin A_9 (GA_9) and fluorogibberellin A_9 (F-GA_9). Activities expressed as percent of control (control hypocotyl length=1.8 mm) and standard errors applicable to each point represented by vertical bars. Open triangles indicate responses to GA_3 at appropriate dose levels

Table 2. Comparative biological activities in 3 bioassays of gibberellin A_3 (GA_3) and a 27–30% mixture of fluorogibberellin A_3 (F-GA_3) in GA_3

Compound	Dose (µg)						
	Barley endosperm[a]			Lettuce Hypocotyl		Dwarf-5 maize	
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-1}	10^{-2}
GA_3	294	245	103	444±15	266±9	270±10	163±14
F-GA_3[b]	232	205	49	367± 9	221±5	214±12	129± 4

[a] Means of triplicate estimations.
[b] 27–30% mixture.
Barley assay data presented as α-amylase units. Other assays expressed as percent of control.

The data in Fig. 2 also show that at all levels up to, and including 10^{-1} µg/dish, F-GA_9 was approximately 5 times as effective as GA_9.

Although pure samples of F-GA_3 were not available it was thought desirable to make a preliminary evaluation of the biological effectiveness

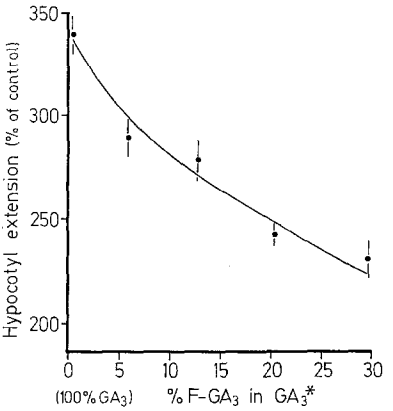

Fig. 3. Effect on lettuce hypocotyl extension of variations in the percentage of fluoro-analogue in a fluorogibberellin A_3 (F-GA_3)*/gibberellin A_3 (GA_3) mixture. Total gibberellin dose at each point=10^{-1} µg/dish. Standard errors represented by vertical bars

of the 27–30% mixture of F-GA_3 and to make a comparison with 100% GA_3. A restricted range of bioassays was used and the results are given in Table 2.

In all three bioassays and at all dose rates, the F-GA_3/GA_3 mixture gave smaller responses than GA_3 alone, suggesting that the substituted compound either exerts an inhibitory action or is biologically inactive.

This finding was confirmed by studies, using the lettuce hypocotyl system, in which the effect of variations in the proportion of F-GA_3 to GA_3 was assessed at the 10^{-1} µg/dish dose level (Fig. 3). Alterations in the percentage of fluoro-analogue being obtained by diluting the F-GA_3/GA_3 mixture with unsubstituted gibberellin.

Discussion

The preceding data demonstrate that monofluorogibberellins, substituted in the 1 β-methyl group, have biological properties which differ significantly from those of the analogous unsubstituted gibberellins. In this context, however, it is highly probable that both the degree and site of fluorination will exert large effects upon the activity of the molecule and could well result in the formation of toxic compounds. The spatial relationship between the substitution site and those structural features important in the association of the gibberellin with the receptor site is also likely to be a consideration.

* Calculations of F-GA_3 content based upon the assumption that the original mixture contained 30% fluoro-analogue.

It is, therefore, interesting to observe that fluorination at the same site in GA_3 and GA_9 results in disparate effects upon the biological activity of the molecule. A large activity increase has been demonstrated to accompany fluorination of GA_9 whereas such data as are available suggest that pure F-GA_3 could possibly be a competitive inhibitor of GA_3 action. It is probable that exocyclic features other than the A-ring methyl group are important in determining attachment of the gibberellin to a receptor site, and that the increased binding potential of the CH_2F grouping is able to effect some degree of compensation for a relative absence of such features in GA_9. Alternatively, GA_3 and GA_9 may be attached to the receptor site in different orientations.

Stimulatory effects of F-GA_9 were only observed in the lettuce hypocotyl assay, where uptake of the test compound occurred *via* the root system. There is a possibility, therefore, that this distinction may have some significance in view of the findings of Crozier and Reid (1971), who suggested that roots might modify the structure of gibberellins synthesised in other parts of the plant. The reduced activity of the F-GA_3/GA_3 mixture casts some doubt upon the probability of such a transformation but further information is required before the alternative can be dismissed.

Interest must now centre upon the possibility of an increased range of fluorosubstituted gibberellins and particularly upon the development of techniques to allow the isolation of pure F-GA_3. If this compound proves to be truly inhibitory, rather than simply inactive, it could have practical applications as a low-dosage dwarfing agent and, when combined with a radioactive label, could prove useful in the elucidation of the mode of action of GA_3 and the nature of the gibberellin receptor.

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