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BIOLOGICAL ACTIVITIES OF FLUOROGIBBERELLINS AND INTERACTIONS WITH UNSUBSTITUTED GIBBERELLINS

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Key Word Index—Gibberellins; fluorogibberellins; bioassays; barley half-seed; lettuce hypocotyl; dwarf rice; interactions.

Abstract—The activities of two mono- and two di-fluorogibberellins are compared with the equivalent unfluorinated compounds in the barley half-seed, lettuce hypocotyl and Tanginbozu dwarf rice bioassays. Interactions between the substituted and unsubstituted gibberellins are also examined. It is concluded that the effects of fluorination are dependent upon the site and degree of substitution and vary with the type of bioassay tissue involved. In the interaction studies the fluorogibberellins generally proved inhibitory, and competitive inhibition was indicated.

INTRODUCTION

Replacement of a hydrogen atom by fluorine in a carbon–hydrogen bond produces only a small increase in size, but markedly enhances electronegativity and hydrogen bonding potential [1]. It might, therefore, be expected that fluorination at critical sites in a GA molecule would significantly affect biological activity, and this suggestion is supported by the finding that some fluorinated steriods are more active then their unsubstituted equivalent [2]. Stoddart [3] also has shown that monofluorogibberellins A_9 and A_{12}, substituted at the 1_β Me group, have biological properties which differ significantly from those of the unsubstituted compounds. Recently four further fluoro GAs have become available, providing the possibility of an examination of the effects of varying degrees and sites of substitution upon the activity of various bioassays. Two difluoro GAs were tested against the appropriate unsubstituted GAs, whilst two mono-fluorodihydro GAs were compared with both the equivalent dihydro GAs of the same stereochemistry and with the unsubstituted GAs. The structures of the four fluoro compounds are shown in Fig. 1. Responses to the fluoro GAs were assessed using the barley half-seed (α-amylase), lettuce hypocotyl and Tanginbozu dwarf rice bioassays. A more detailed examination of the interactions between fluorinated and unfluorinated compounds was undertaken using only the dwarf rice bioassay.

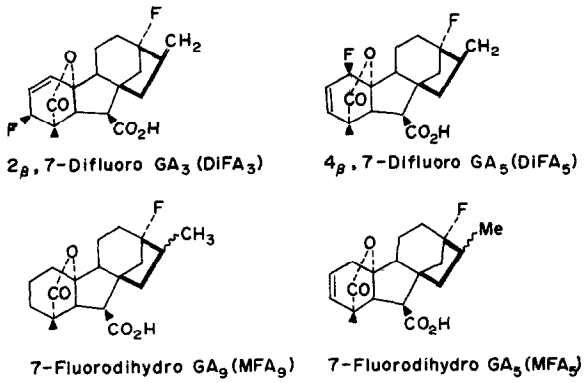

Fig. 1. Structures of the fluorinated gibberellins used in the bioassay studies.

RESULTS

The responses evoked in the barley half-seed, lettuce hypocotyl and Tanginbozu dwarf rice bioassays by GA_3, GA_5 and GA_9 and by 7, 8-dihydro GA_5 and 7, 8-dihydro GA_9, as well as by their fluorinated analogues are summarized in Tables 1, 2 and 3. 2_β, 7-Difluoro GA_3 ($DiFA_3$) gave responses significantly lower than that of the parent compound in all bioassays and at all levels of treatment. 4_β, 7-Difluoro GA_5 ($DiFA_5$) was more active than GA_5 in the barley half-seed test (Table 3) but not in the lettuce hypocotyl bioassays (Table 2). In the Tanginbozu dwarf

Table 1. Response of Tanginbozu dwarf rice seedlings to GA_3, GA_5, GA_9 and derivatives. Results expressed as length of second leaf sheath (mm) ± standard error

Compound	Concentration of test solution 10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
GA_3	12 ± 0.6	16 ± 0.8	26 ± 1.0	34 ± 1.7
$DiFA_3$	11 ± 0.4	12 ± 0.7	13 ± 0.6	24 ± 0.9
GA_5	11 ± 0.3	15 ± 0.4	20 ± 0.9	30 ± 1.3
$DiFA_5$	12 ± 0.5	15 ± 0.4	23 ± 1.0	28 ± 1.0
$DiHA_5$	12 ± 0.6	12 ± 0.7	10 ± 0.4	11 ± 0.3
MFA_5	12 ± 0.4	13 ± 0.4	18 ± 1.0	28 ± 0.9
GA_9	11 ± 0.3	13 ± 0.6	20 ± 1.0	29 ± 0.8
$DiHA_9$	12 ± 0.3	14 ± 0.5	17 ± 0.9	25 ± 0.7
MFA_9	13 ± 0.3	15 ± 0.3	16 ± 0.7	19 ± 0.6
Control	11 ± 0.3			

$DiFA_3$ = 2_β, 7-difluoro GA_3; $DiFA_5$ = 4_β, 7-difluoro GA_5; $DiHA_5$ = 7, 8-dihydro GA_5; MFA_5 = 7-fluorodihydro GA_5; $DiHA_9$ = 7,8-dihydro GA_9; MFA_9 = 7-fluorodihydro GA_9.

Table 2. Response of lettuce hypocotyls to GA_3, GA_5, GA_9 and derivatives. Results expressed as length of hypocotyl ± standard error

Compound	Concentration of test solution 10^{-8} M	10^{-7} M	5×10^{-7} M	10^{-6} M	10^{-5} M	2×10^{-5} M
GA_3	1.9 ± 0.10	2.3 ± 0.15	3.5 ± 0.19	4.1 ± 0.41	5.3 ± 0.33	5.7 ± 0.47
$DiFA_3$	1.8 ± 0.13	1.8 ± 0.13	1.8 ± 0.13	1.9 ± 0.10	2.7 ± 0.15	3.6 ± 0.34
GA_5	1.8 ± 0.13	2.2 ± 0.13	2.2 ± 0.13	2.7 ± 0.19	3.6 ± 0.22	4.9 ± 0.31
$DiFA_5$	1.8 ± 0.13	1.8 ± 0.13	2.2 ± 0.13	2.5 ± 0.16	4.0 ± 0.42	3.2 ± 0.41
$DiHA_5$	1.8 ± 0.13	2.0 ± 0.10	2.1 ± 0.10	2.6 ± 0.18	3.6 ± 0.23	4.7 ± 0.30
MFA_5	1.9 ± 0.10	2.8 ± 0.25	4.0 ± 0.30	4.2 ± 0.29	5.2 ± 0.51	2.2 ± 0.13
GA_9	1.7 ± 0.15	2.0 ± 0.0	2.1 ± 0.17	3.0 ± 0.0	5.3 ± 0.29	6.6 ± 0.27
$DiHA_9$	1.9 ± 0.10	2.8 ± 0.15	4.0 ± 0.22	4.9 ± 0.33	5.6 ± 0.40	5.8 ± 0.42
MFA_9	2.0 ± 0.15	3.5 ± 0.22	4.8 ± 0.36	5.7 ± 0.33	5.6 ± 0.48	5.1 ± 0.62
Control			1.9 ± 0.12			

Compound abbreviations as Table 1.

rice bioassay (Table 1) both fluorinated GA_5s produced responses closely similar to those of unsubstituted GA_5, whilst 7, 8-dihydro GA_5 ($DiHA_5$) was inactive at concentrations up to 10^{-3} M. Marked reductions in the response of lettuce hypocotyls were noted with applications of $DiFA_5$, MFA_5 and MFA_9 above 10^{-5} M.

Figure 2 illustrates the effects of combined applications of substituted and unsubstituted GAs upon the response of Tanginbozu dwarf rice seedlings. The results are expressed as a percentage of the response obtained using equivalent concentrations of the parent, unfluorinated compound. In general, mixtures containing a fluorinated compound gave lower responses than those obtained with an equivalent concentration of unsubstituted gibberellin. However, in combination with dihydro GA_5, 7-fluorodihydro GA_5 produced responses in excess of those obtained with the equivalent concentration of the unfluorinated compound. Overall, the inhibitory effect of a fixed amount of fluoro GA (10^{-4} M) was greater in the presence of the lower concentrations of unsubstituted GA. Similar trends were observed for interactions involving fluorinated GAs at concentrations between 10^{-3} and 10^{-6} M.

DISCUSSION

Fluorination of GAs has a demonstrable effect upon their biological properties which is dependent upon the degree of fluorination and differs with the type of bioassay tissue involved. For example, MFA_5 was generally more active than $DiFA_5$ in the lettuce hypocotyl bioassay but this was not so when the compounds were assessed in the Tanginbozu dwarf rice system. $DiFA_3$, on the other hand, was less active than GA_3 in all three bioassays, and similar results were obtained in the

Table 3. Response of barley half-seed bioassay to GA_3, GA_5 and derivatives. Results expressed as units of α-amylase released

	Concentration of test solution 10^{-8} M	10^{-7} M	10^{-6} M	2×10^{-6} M
GA_3	80.4	97.6	107.5	109.6
$DiFA_3$	2.0	21.6	69.1	88.1
GA_5	9.5	14.5	84.6	142.0
$DiFA_5$	1.8	77.8	76.5	84.2
Control		2.8		

Compound abbreviations as Table 1.

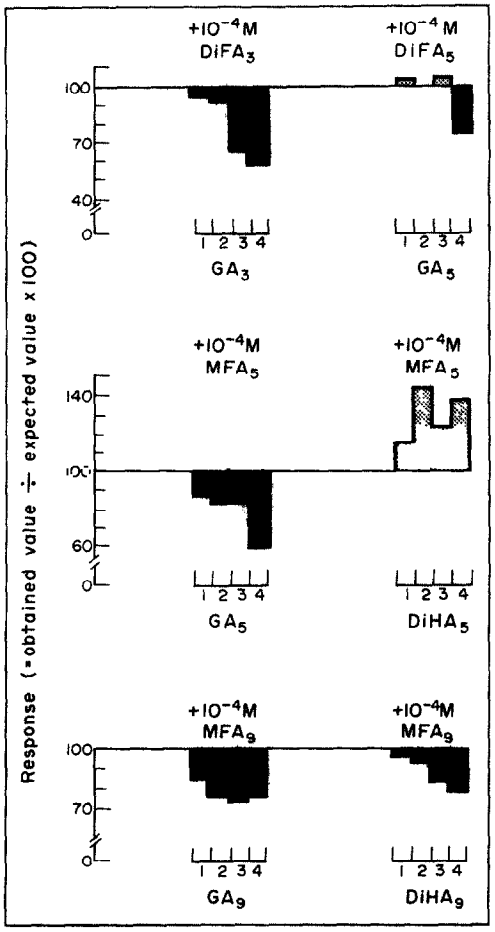

Fig. 2. The effects of combined applications of fluorinated and unsubstituted GA_3, GA_5, GA_9, dihydro GA_5 and dihydro GA_9 upon the response of Tanginbozu dwarf rice seedlings. 1 = 10^{-3} M unfluorinated gibberellin; 2 = 10^{-4} M unfluorinated gibberellin; 3 = 10^{-5} M unfluorinated gibberellin; 4 = 10^{-6} M unfluorinated gibberellin. Expected value = the response obtained from equivalent total concentration of unfluorinated gibberellin.

dwarf-5-maize test (unpublished data). The chemical nature of the substituent is also important. For example, 7-fluorodihydro GA_5 stimulated lettuce hypocotyl elongation to a greater extent than GA_5, whilst 7,8-dihydro GA_5 of the same stereochemistry did not. Furthermore $DiHA_5$ proved to be inactive in the Tanginbozu dwarf rice system, whilst the response evoked by MFA_5 was equivalent to that of GA_5. In cases where the response produced by a fluorinated compound decreased at higher levels, toxic effects were evident.

The experiments involving mixtures of fluorinated and unfluorinated compounds showed the FGAs to be generally inhibitory in this situation. The only exception involved 7-fluorodihydro GA_5, where the apparent stimulation was due to the lack of activity of the parent dihydro GA_5 in the Tanginbozu dwarf rice test. The inhibition appeared to be competitive, the depressive effect being greater the higher the proportion of fluorinated analogue present. The reduction in the response of dwarf rice seedlings was particularly marked with mixtures involving $DiFA_3$, which is consistent with previous reports that mixtures of GA_3 and 1_β-fluoro GA_3 were less active than the unsubstituted GA_3. It has also been shown that $DiFA_3$ exerts a competitive inhibition in the barley half seed bioassay, and at 10^{-6} M is capable of negating the response to an equivalent concentration of GA_3 (R. L. Jones, personal communication).

The bioassay systems employed utilize either genetically dwarfed plants or measure responses in organs which have unusually low endogenous growth rates. Further experimentation is therefore necessary in order to assess the ability of $DiFA_3$ to inhibit growth when applied to normal plant populations. The unexpected biological properties of the dihydro GAs are also of particular interest and are now being further investigated.

EXPERIMENTAL

The preparation of the fluoro GAs has been described elsewhere [4]. Each compound was initially dissolved in MeOH (1 mg per ml) and diluted to the required concentration with H_2O.

Lettuce hypocotyl bioassay. This was performed by the method of ref [5] using the cv 'Artic' throughout. Seeds were presprouted in light and placed on 3 cm squares of Whatman No. 1 in 5 cm Petri dishes together with 0.5 ml of test soln. Lighting was provided by daylight-type fluorescent tubes at an intensity of 6500 lx, and hypocotyl lengths were measured 48 hr after treatment.

Barley half-seed bioassay (α-Amylase release). The method and expression of results was as described in ref. [6], except that grains were imbibed for 48 hr on pre-sterilized pads of Whatman 3 MM filter paper. Seeds of the cv 'Himalaya' (1966 harvest) were used throughout.

Tanginbozu dwarf rice bioassay. The procedure followed was that of ref. [7] except that seeds were germinated by imbibing for 48 hr on pre-sterilized Whatman 3 MM filter paper in closed, sterile Petri dishes. Lettuce hypocotyl and dwarf rice bioassays were replicated 10 times for each test, and the assays were repeated several times. Standard errors were calculated for each point on the response curves. Barley half-seed bioassay results were based on duplicate estimations at each concentration.

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