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BIOLOGICAL ACTIVITIES OF GIBBERELLINS AND THEIR GLUCOSIDES IN *PHARBITIS NIL**

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Abstract—Growth-promoting effects of gibberellins and their glucosides isolated from immature seeds of Japanese morning glory (*Pharbitis nil*) were compared in six bioassay systems. GA_3 glucoside exhibited much less activity than GA_3 in the dwarf rice (under aseptic conditions), dwarf maize (d_1, d_2 and d_5), cucumber and dwarf pea assays. GA_8, GA_{26}, GA_{27} and GA_{29} showed low activities in all the bioassay systems, while their glucosides were even less active. Thus gibberellin glucosides do not appear to be active in growth regulation.

INTRODUCTION

IN PREVIOUS papers,[1–6] we reported the occurrence in the seeds of Japanese morning glory of GA_3, GA_5, GA_8, GA_{20}, GA_{26}, GA_{27} and the glucosides of GA_3, GA_8, GA_{26}, GA_{27}, GA_{29}, gibberellenic acid[7] and isoGA_3.[7] The biological activities of the above free gibberellins[8–10] and of GA_8 glucoside, which has also been isolated from *Phaseolus coccineus*[11,12] and *Althaea rosea*,[13] have been reported by us[2] and other workers. In this paper we report the biological activities of GA_3, GA_8, GA_{26}, GA_{27}, GA_{29},[14] gibberellenic acid[14] and their glucosides together with isoGA_3 glucoside in dwarf rice (Kotake-tamanishiki), dwarf maize (d_1, d_2, and d_5), cucumber (National Pickling) and dwarf pea (Progress No. 9) assays. In particular, we have studied the difference in activities between gibberellins and their glucosides.

* Part IV in the series "Gibberellins in Immature Seeds of *Pharbitis nil*". For Part III see ref. 17.

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TABLE 1. ACTIVITY OF GIBBERELLINS AND GIBBERELLIN GLUCOSIDES IN THE DWARF MAIZE (d_1) ASSAY

	μg/plant				Control
	50	10	1	0 1	
A_3	—	—	286	173	a
A_3 glucoside	156	127	120	—	b
A_8	132	—	—	—	c
A_8 glucoside	135	—	—	—	c
A_{26}	163	—	—	—	c
A_{26} glucoside	120	—	—	—	c
A_{27}	138	—	—	—	c
A_{27} glucoside	100	—	—	—	c
A_{29}	209	168	108	—	a
A_{29} glucoside	119	112	—	—	b
Gibberellenic acid	321	268	209	—	a
Gibberellenic acid glucoside	168	129	99	—	b
isoA_3 glucoside	—	99	96	—	b

Each value represents the mean sum of the 1st and 2nd leaf sheathl engths in % of control ($n = 4 \sim 5$)
Controls: a, 34·4; b, 35·1; c, 40·7 mm.

RESULTS

Dwarf Maize Test

The results of assays using d_1, d_2 and d_5 mutants are presented in Tables 1–3 respectively. In these assays responses which are about 30% over control were significant at $P = 0{\cdot}05$. In general, GA_8, GA_{26} and GA_{27} showed only slight activities at the level of 50 μg/plant. GA_{29} was about 10 times more active than the above three gibberellins. Gibberellenic acid[8] was

TABLE 2. ACTIVITY OF GIBBERELLINS AND GIBBERELLIN GLUCOSIDES IN THE DWARF MAIZE (d_2) ASSAY

	μg/plant				Control
	50	10	1	0·1	
A_3	—	—	270	119	a
A_3 glucoside	158	136	96	—	b
A_8	120*	—	—	—	c
A_8 glucoside	134	121	—	—	b
A_{26}	176	—	—	—	c
A_{26} glucoside	140	136	—	—	b
A_{27}	167	—	—	—	c
A_{27} glucoside	125	—	—	—	c
A_{29}	205	168	143	—	a
A_{29} glucoside	150	126	—	—	b
Gibberellenic acid	—	264	207	—	a
Gibberellenic acid glucoside	164	124	104	—	b
isoA_3 glucoside	—	113	107	—	b

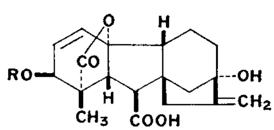
Each value represents the mean sum of the 1st and 2nd leaf sheath lengths in % of control ($n = 4 \sim 5$).
Controls: a, 45·3; b, 41·4; c, 35·0 mm
* 25 μg/plant.

fairly active, its activity being 1/10 of that of GA_3. The glucosides of GA_3, GA_8 and gibberellenic acid were slightly active in the d_1 and d_5 assays, the other glucosides being inactive. The glucosides of GA_3 and gibberellenic acid showed almost the same degree of activity which is approximately 1/500 of that of GA_3. On the other hand, in the d_2 assay, all the gibberellin glucosides except GA_{27} glucoside were active to the same degree at the dosage level of 10 μg/plant or above.

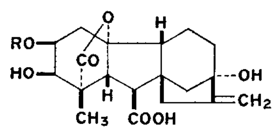
TABLE 3. ACTIVITY OF GIBBERELLINS AND GIBBERELLIN GLUCOSIDES IN THE DWARF MAIZE (d_5) ASSAY

	μg/plant				Control
	50	10	1	0·1	
A_3	—	420	326	166	a
A_3 glucoside	180	127	118	—	b
A_8	123	101	—	—	a
A_8 glucoside	127	109	—	—	a
A_{26}	169	118	—	—	a
A_{26} glucoside	109	109	—	—	a
A_{27}	102	—	—	—	a
A_{27} glucoside	119	87	—	—	a
A_{29}	240	185	124	—	a
A_{29} glucoside	114	120	—	—	b
Gibberellenic acid	336	289	217	—	a
Gibberellenic acid glucoside	207	146	117	—	b
isoA_3 glucoside	—	130	126	—	b

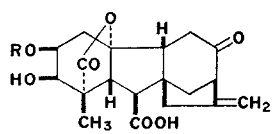
Each value represents the mean sum of the 1st and 2nd leaf sheath lengths in % of control (n = 4 ~ 5). Controls: a, 32·0; b, 29·0 mm.



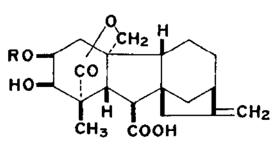
GA_3 : R = H
GA_3 glucoside : R = β-Glucosyl



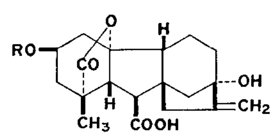
GA_8 : R = H
GA_8 glucoside : R = β-Glucosyl



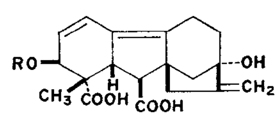
GA_{26} : R = H
GA_{26} glucoside : R = β-Glucosyl



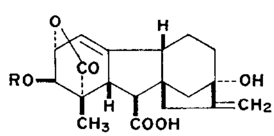
GA_{27} : R = H
GA_{27} glucoside : R = β-Glucosyl



GA_{29} : R = H
GA_{29} glucoside : R = β-Glucosyl



Gibberellenic acid . R = H
Gibberellenic acid glucoside : R = β-Glucosyl



isoGA_3 . R = H
isoGA_3 glucoside : R = β-Glucosyl

Dwarf Rice Test

(A) The results of assays under non-aseptic conditions are shown in Table 4. All the gibberellin glucosides tested were as active as their corresponding aglycones. GA_8, GA_{27},

GA_{29} and their glucosides were slightly active at 10^{-4} mol/l. GA_{26} was inactive, while its glucoside was active at 10^{-4} mol/l. This activity may be due to a trace of impurity. GA_3 glucoside showed a strong activity which is almost equivalent to that of GA_3. The glucosides of gibberellenic acid and isoGA_3 were significantly active at 10^{-4} mol/l., but much less active than GA_3.

TABLE 4. ACTIVITY OF GIBBERELLINS AND GIBBERELLIN GLUCOSIDES IN THE DWARF RICE (KOTAKE-TAMANISHIKI) ASSAY

	Mol/l.			
	10^{-4}	10^{-5}	10^{-6}	10^{-7}
A_3	—	131·3 ± 6·5	78·1 ± 2·8	29·7 ± 1·3
A_3 glucoside	—	107·8 ± 3·6	62·5 ± 2·2	27·6 ± 1·0
A_8	26·8 ± 0·7	21·8 ± 0 8	—	—
A_8 glucoside	31·4 ± 0·8	22·1 ± 0·9	—	—
A_{26}	20·5 ± 0 7	19·7 ± 0·7	—	—
A_{26} glucoside	25·3 ± 1·1	19·1 ± 1·0	—	—
A_{27}	26·1 ± 1·1	20·6 ± 1·2	—	—
A_{27} glucoside	22·5 ± 0·6	22·2 ± 0·7	—	—
A_{29}	25·4 ± 0 9	22·2 ± 0·6	—	—
A_{29} glucoside	26·3 ± 1·0	21·4 ± 0·8	—	—
Gibberellenic acid	68·3 ± 2·1	33·2 ± 1·2	23·3 ± 0·6	—
Gibberellenic acid glucoside	49·0 ± 1·9	25·9 ± 0·9	20·7 ± 0·5	—
isoA_3 glucoside	61·0 ± 2·6	26·4 ± 0·9	20·3 ± 0·7	—

Each value represents the mean length (mm) of the 2nd leaf sheath and its standard error (n = 16). Controls: 19·4 ± 1·0.

(B) The results of a test performed aseptically are shown in Table 5. It is noteworthy that the activity of GA_3 glucoside greatly decreased as compared with that under non-aseptic conditions, only slight activity being observed at 10^{-5} mol/l. The activities of the glucosides of gibberellenic acid and isoGA_3 were also very much reduced.

TABLE 5. ACTIVITY OF GIBBERELLIN GLUCOSIDES IN THE ASEPTIC DWARF RICE (KOTAKE-TAMANISHIKI) ASSAY

	Mol/l.				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Control
A_3	—	95·5 ± 3 0	70·8 ± 2·8	38·1 ± 4 0	a
A_3 glucoside	—	29·1 ± 3·5	22·4 ± 3 2	22·2 ± 2·1	a
A_8 glucoside	28·5 ± 0·7	—	—	—	b
A_{26} glucoside	21·9 ± 0·9	—	—	—	b
A_{27} glucoside	23·8 ± 1·2	—	—	—	b
A_{29} glucoside	24·0 ± 0·7	—	—	—	b
Gibberellenic acid glucoside	35·8 ± 1·1	—	—	—	b
isoA_3 glucoside	37·6 ± 1·3	—	—	—	b

Each value represents the mean length (mm) of the 2nd leaf sheath and its standard error (n = 16 ~ 33). Controls: a, 24·3 ± 0·9; b, 20·3 ± 0·6.

TABLE 6. ACTIVITY OF GIBBERELLINS AND GIBBERELLIN GLUCOSIDES IN THE CUCUMBER (NATIONAL PICKLING) ASSAY

	μg/plant			
	50	5	0·5	Control
A_3*	80·7 ± 2·4	60·6 ± 2·3	44·5 ± 1·9	a
A_3 glucoside	38·4 ± 1·4	38·3 ± 2·0	36·1 ± 1·6	a
A_8	41·7 ± 2·3	36·1 ± 2·1	36·8 ± 2·4	a
A_8 glucoside	37·8 ± 2·1	32·5 ± 1·4	33·0 ± 2·0	b
A_{26}	36·7 ± 1·7	42·4 ± 1·5	35·1 ± 1·5	a
A_{26} glucoside	36·0 ± 1·0	34·4 ± 2·9	40·7 ± 2·5	b
A_{27}	36·1 ± 1·6	39·6 ± 1·7	40·2 ± 1·5	a
A_{27} glucoside	37·3 ± 1·9	32·2 ± 1·3	36·3 ± 1·2	b
A_{29}	36·9 ± 1·7	36·4 ± 1·7	36·9 ± 2·2	a
A_{29} glucoside	41·8 ± 2·5	42·1 ± 2·3	31·0 ± 1·3	b
Gibberellenic acid	44·6 ± 1·3	39·5 ± 1·8	32·9 ± 1·7	a
Gibberellenic acid glucoside	39·8 ± 2·1	43·9 ± 2·1	38·0 ± 1·6	b

Each value represents the mean length (mm) of a hypocotyl unit and its standard error ($n = 10$).
Controls: a, 33·6 ± 2·2; b, 36·8 ± 2·1.
* 25, 2·5, 0·25 μg/plant.

Cucumber Test

The results are shown in Table 6. The tested gibberellins and their glucosides except GA_3 were almost completely inactive, up to 50 μg/plant.

Dwarf Pea Test

The results are shown in Table 7. GA_{26}, GA_{27} and their glucosides were inactive up to 5 μg/plant. GA_{29} and its glucoside were slightly active at 5 μg/plant, the degree of their

TABLE 7. ACTIVITY OF GIBBERELLINS AND GIBBERELLIN GLUCOSIDES IN THE DWARF PEA (PROGRESS NO. 9) ASSAY

	μg/plant			
	5	0·5	0·05	Control
A_3*	—	112·8 ± 9·0	78·0 ± 4·9	a
A_3 glucoside	78·8 ± 7·0	52·0 ± 4·6	51·0 ± 1·1	b
A_8	111·8 ± 9·4	83·6 ± 4·5	49·6 ± 4·4	b
A_8 glucoside	98·8 ± 10·8	63·4 ± 4·4	53·4 ± 2·5	b
A_{26}	58·8 ± 2·6	52·4 ± 0·4	55·2 ± 3·3	b
A_{26} glucoside	53·2 ± 2·8	48·2 ± 2·2	50·6 ± 1·1	a
A_{27}	45·0 ± 1·2	52·2 ± 4·4	48·8 ± 2·5	b
A_{27} glucoside	—	56·0 ± 1·5	53·8 ± 2·7	b
A_{29}	69·0 ± 5·2	59·2 ± 4·4	54·6 ± 1·8	c
A_{29} glucoside	69·5 ± 2·1	55·4 ± 2·5	58·3 ± 3·1	c
Gibberellenic acid	114·6 ± 9·4	76·2 ± 2·6	53·0 ± 1·8	b
Gibberellenic acid glucoside	62·2 ± 4·4	49·2 ± 1·5	48·0 ± 0·8	a

Each value represents the mean length (mm) of an epicotyl and its standard error ($n = 4 \sim 5$).
Controls: a, 48·4 ± 1·3; b, 53·8 ± 1·2; c, 59·4 ± 1·9.
* 0·25, 0·025 μg/plant.

activities being the same. GA_8 and its glucoside were active at 0·5 μg/plant, the latter being a little less active than the former, cf. Sembdner *et al.*[12] The activity of these two gibberellins was approximately 1/20 of that of GA_3. The activity of GA_3 glucoside was 1/200 of that of GA_3 and was less than that of GA_8 glucoside. Gibberellenic acid showed the same degree of activity as that of GA_8. However, the glucoside of the former was only slightly active at 5 μg/plant.

DISCUSSION

Generally GA_8, GA_{26}, GA_{27} and GA_{29} showed a slight or no activity in our bioassay systems. The C-3 hydroxyl or the C-2,3 glycol system in the A ring might reduce gibberellin activity, as pointed out by Crozier *et al.*[10]

In the dwarf maize test, generally all glucosides showed no or slight activities and were less active than their aglycones. Especially, the glucosides of GA_3, GA_{29}, gibberellenic acid and isoGA_3 were far less active than their corresponding aglycones. The glucosides of GA_{26} and GA_{27} tend to be less active than their corresponding aglycones. Although Sembdner *et al.*[12] observed that GA_8 glucoside was less active than GA_8, no clear difference was observed in our experiments since both showed no significant activity. It is interesting that mutant d_2 is more sensitive to gibberellin glucosides than d_1 and d_5, suggesting possibly the genotype dependence of the activity of glucosides. In the cucumber test, all glucosides were also inactive.

In the non-aseptic dwarf rice test gibberellin glucosides showed activities nearly equivalent to those of their corresponding aglycones. This contrasts with the results of the dwarf maize and cucumber tests. It can be explained by the finding that each glucoside released its aglycone in the culture media, with or without rice seedlings, when examined by TLC. To examine whether the release of aglycones is due to hydrolysis by enzymes from contaminated microorganisms or from rice seedlings, an aseptic rice test was conducted. In this test GA_3 glucoside was nearly inactive, which is in good agreement with the fact that GA_3 glucoside was almost inactive in the dwarf rice (Tangin-bozu) test by the microdrop method.[15] The glucosides of gibberellenic acid and isoGA_3[16] showed reduced activities. Aglycones could not be detected by TLC in the aseptic culture media. Thus, true activity of glucosides of GA_3, gibberellenic acid and isoGA_3 can only be determined in the aseptic test, since, under non-aseptic conditions, aglycones are released from the glucosides due to the presence of contaminating microorganisms. On the other hand, 30–100% of glucosides of GA_8, GA_{26}, GA_{27} and GA_{29} were hydrolyzed even in the aseptic rice test, indicating that these glucosides were hydrolyzed by enzymes from rice seedlings. In this connection it is interesting to note that GA_3 glucoside was only partially hydrolyzed by β-glucosidase, but glucosides of GA_8, GA_{26}, GA_{27} and GA_{29} released their aglycones very readily with this enzyme.[17]

In the dwarf pea bioassay the glucosides of GA_3 and gibberellenic acid were far less active than GA_3, whereas the glucosides of GA_8 and GA_{29} showed about the same activity as GA_8 and GA_{29}, respectively. This phenomenon, which resembles the result of the aseptic rice test, might be ascribed to selective hydrolysis of the glucosides by endogenous enzymes in dwarf pea seedlings.

It is therefore concluded that the activity of gibberellin glucosides is much less than that

[15] Private communication from Dr. Y. Murakami. We are grateful to him for these data.
[16] As for the activity of isoGA_3, see Ref. 10.
[17] T. YOKOTA, N. MUROFUSHI, N. TAKAHASHI and S. TAMURA, *Agr. Biol. Chem.* **35**, 583 (1971).

of their aglycones and the growth-promoting effects of the glucosides observed in some bioassay systems are possibly due to the aglycones liberated by hydrolysis in plant tissue. Gibberellin glucosides are probably inactive.

Ogawa[18] reported changes in the content of three gibberellin-like substances, factors I, II and III, during the development of *Pharbitis* seeds. Based on their R_fs and activities in the rice seedling test, we consider them to correspond to GA_3 (factor I), GA_3 glucoside (factor II) and gibberellenic acid glucoside (factor III). The content of factors I and II increased sharply in the early stage of seed development and attained a maximum content on 15th and 20th day after anthesis, respectively, and then sharply decreased. On the other hand, the content of factor III increased slowly until 15th day and was maintained unaltered thereafter. In mature seeds, only factors II and III were detectable. A similar result has also been reported by Murakami.[19] This suggests that gibberellin glucosides play an important role in the metabolism of gibberellins during seed development.

EXPERIMENTAL

Dwarf rice test. (A). Each test compound was dissolved in 1 ml of 1/2 strength Hoagland's solution in a tall tube (14 × 2·3 i.d. cm). Eight rice seedlings (*Oryza sativa* L., dwarf cv. Kotake-Tamanishiki), germinated in tap water for 3 days at 25°, were transplanted to each tube, which was covered with polyethylene film and incubated at 30° under continuous fluorescent light (*ca.* 3000 lx). After 7 days the length of the second leaf sheath was measured.

(B). The aseptic rice test was carried out as follows. For seed sterilization husked rice seeds were soaked in 70% EtOH for 3 min under reduced pressure and then washed with sterile water four times under atmospheric pressure and once under reduced pressure. They were further soaked in 5% chlorinated lime solution at 5° for 2 hr and washed with sterile water five times. Eight sterilized seeds and an aliquot of 5 μl of EtOH solution containing a test sample were put into a sterilized tube which contained 1 ml of the sterilized nutrient solution and stoppered with a cotton plug. The tube was covered with polyethylene film and incubated for 10 days.

Dwarf maize test. Maize seeds (*Zea mays* L., mutants d_1, d_2 and d_5) were used. The test was conducted by the procedure by Tamura *et al.*[20] with the following slight modifications. Each test compound was dissolved in 20% EtOH containing 0·1% Tween 20 and 0·1 ml aliquot of the test solution was applied on each seedling. Seedlings were grown at 25° under fluorescent light (*ca.* 2000 lx) with 10 hr illumination/day. 7 days after treatment, the lengths of the first and second leaf sheaths were measured and added together. Five seedlings were used at each dosage.

Cucumber test. The test was conducted according to the method by Katsumi *et al.*[21] using seedlings of *Cucumis sativus* L., cv. National Pickling. The length of the 'hypocotyl unit' was measured 3 days after treatment. Ten seedlings were used at each dosage.

Dwarf pea test. The test was conducted in almost the same way as that of Hayashi *et al.*[22] except that 1 μl of a test solution in 90% EtOH was applied per seedling. 7 days after treatment the length of the epicotyl was measured. Five seedlings were used at each dosage.

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