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BIOLOGICAL ACTIVITIES OF NEW GIBBERELLINS A_{30}–A_{35} AND A_{35} GLUCOSIDE

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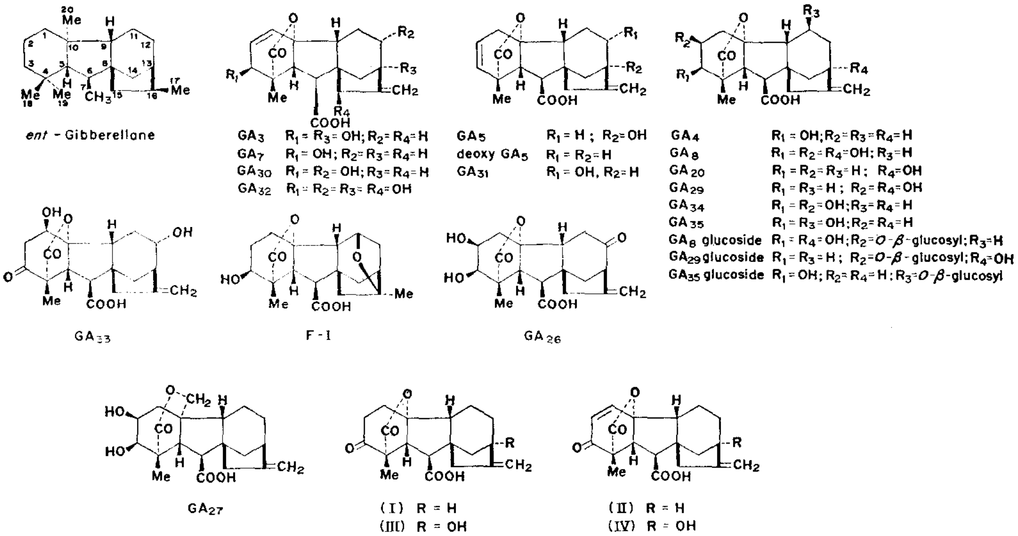
Key Word Index—Gibberellins A_{30}–A_{35}; gibberellin A_{35} glucoside; biological activity; bioassays; growth promotion.

Abstract—The plant growth-promoting activities of new gibberellins, GA_{30}, GA_{31}, GA_{32}, GA_{33}, GA_{34}, GA_{35} and GA_{35} glucoside were evaluated in seven bioassays. In general GA_{30}, GA_{31} and GA_{35} showed fairly high biological activities, whilst GA_{33}, GA_{34} and GA_{35} glucoside were almost inactive. GA_{32} was highly active, behaving similarly to GA_3. It is suggested that the C-11β and C-12α hydroxyl groups have little influence on growth-promoting activity, although the C-12α hydroxyl group reduces activity in the cucumber hypocotyl assay.

INTRODUCTION

OUR PREVIOUS papers[1–4] reported the occurrence of GA_{30}, GA_{31}, GA_{33} and GA_{34} in immature seeds of *Calonyction aculeatum*, GA_{32} in those of *Prunus persica* and GA_{35} and its glucoside in those of *Cytisus scoparius*.



THE STRUCTURES OF *ent*-GIBBERELLANE AND GIBBERELLINES ASSAYED IN THIS STUDY.

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TABLE 1. ACTIVITY OF GIBBERELLINS IN THE DWARF RICE (TAN-GINBŌZU) ASSAY

Gibberellin	ng gibberellin/seedling 0·1	1·0	10	100
A_{30}	19·80 ± 0·59	17·40 ± 0·78	25·40 ± 0·36	39·60 ± 1·37
A_{31}	17·40 ± 0·59	18·60 ± 0·96	30·20 ± 0·71	38·40 ± 0·61
A_{32}	19·20 ± 1·11	25·60 ± 0·96	31·40 ± 1·22	49·80 ± 2·36
A_{33}	18·40 ± 0·54	16·60 ± 0·61	17·80 ± 0·66	19·80 ± 0·33
A_{34}	19·00 ± 0·57	18·60 ± 0·61	17·00 ± 0·49	21·20 ± 0·33
A_{35}	21·20 ± 1·04	20·40 ± 0·92	24·60 ± 0·83	38·20 ± 1·04
F I	18·40 ± 1·46	17·00 ± 0·49	17·20 ± 1·04	28·80 ± 0·87
A_{35} glucoside	17·20 ± 0·52	17·80 ± 0·66	22·00 ± 0·80	24·80 ± 0·59
A_3	23·40 ± 1·82	28·60 ± 2·17	35·00 ± 3·17	50·40 ± 2·17
A_4	18·80 ± 1·07	21·00 ± 0·75	27·20 ± 1·73	49·20 ± 3·41
A_5	22·80 ± 1·18	26·20 ± 0·44	33·00 ± 1·20	37·80 ± 1·63
A_7	15·80 ± 0·52	21·40 ± 0·22	26·20 ± 1·63	47·60 ± 2·72
deoxy A_5	17·20 ± 0·66	22·80 ± 0·77	30·40 ± 0·78	45·60 ± 2·63
Control		17·00 ± 0·58		

Each value represents the mean length (mm) ±s.e. of the second leaf sheath (5 replicates).

We report here the plant growth-promoting activities of these new gibberellins and the compound, F-1 (a GA_{35} derivative),[4] in seven bioassays, namely, the dwarf rice (Tan-ginbōzu and Waitō-C), the dwarf maize (d_1, d_3 and d_5), the dwarf pea (Progress No. 9) and the cucumber (National Pickling) assays. Since some of these new gibberellins contain C-11β, C-12α and/or C-15β hydroxyl groups in their structures, comparisons were made between their activities and those of such known C_{19} gibberellins as GA_3, GA_4, GA_5, GA_7 and deoxy GA_5 to evaluate the effect of the hydroxyl groups at various positions in the *ent*-gibberellane[5] skeleton on gibberellin activities. On a structure-activity basis, special attention was paid to the comparisons of GA_{30} with GA_7, of GA_{31} and deoxy GA_5 with GA_5, of GA_{32} with GA_3, and of GA_{34} and GA_{35} with GA_4.

TABLE 2. ACTIVITY OF GIBBERELLINS IN THE DWARF RICE (WAITŌ-C) ASSAY

Gibberellin	ng gibberellin/seedling 0·1	1·0	10	100
A_{30}	17·00 ± 0·28	18·80 ± 0·18	22·40 ± 0·61	38·60 ± 1·15
A_{31}	18·40 ± 0·54	18·40 ± 0·22	20·80 ± 0·33	30·40 ± 0·54
A_{32}	19·80 ± 0·44	28·00 ± 0·63	44·20 ± 0·33	52·00 ± 2·17
A_{33}	16·60 ± 0·46	16·40 ± 0·22	17·60 ± 0·22	19·20 ± 0·33
A_{34}	16·20 ± 0·33	16·40 ± 0·22	18·00 ± 0·40	21·80 ± 0·77
A_{35}	18·80 ± 0·33	17·80 ± 0·33	23·20 ± 0·52	41·00 ± 2·21
F I	17·20 ± 0·18	17·80 ± 0·18	17·80 ± 0·33	22·00 ± 0·75
A_{35} glucoside	17·40 ± 0·22	17·00 ± 0·00	17·80 ± 0·18	24·60 ± 1·66
A_3	28·80 ± 0·24	31·60 ± 0·66	47·40 ± 0·67	58·20 ± 2·63
A_4	17·40 ± 0·46	19·80 ± 0·44	30·60 ± 1·78	53·80 ± 1·00
A_5	18·20 ± 0·52	18·20 ± 0·18	26·00 ± 0·75	38·80 ± 0·66
A_7	19·00 ± 0·49	21·60 ± 0·61	32·20 ± 1·56	54·20 ± 1·15
deoxy A_5	17·80 ± 0·18	20·80 ± 0·33	26·60 ± 0·61	45·20 ± 1·21
Control		15·80 ± 0·24		

Values as in Table 1.

[5] The nomenclature of *ent*-gibberellanes used in this paper is based on the proposal by J. W. ROWE, in *The common and systematic nomenclature of cyclic diterpene*. See Structures.

RESULTS

Dwarf Rice Test

The results of the assays using Tan-ginbōzu and Waitō-C varieties are shown in Tables 1 and 2, respectively. GA_{32} was most active among the new gibberellins and it was almost as active as GA_3. GA_{30}, GA_{31}, GA_{35} and deoxy GA_5 were also active. The activities of GA_{30}, GA_{31} and GA_{35} were similar and were less than that of GA_3. Deoxy GA_5 was slightly more active than these three gibberellins and was almost as active as GA_5.

TABLE 3. ACTIVITY OF GIBBERELLINS IN THE DWARF MAIZE (d_1) ASSAY

Gibberellin	μg gibberellin/seedling 0·1	1·0	10
A_{30}	61·20 ± 4·32	105·00 ± 8·74	117·00 ± 5·11
A_{31}	50·40 ± 2·44	69·40 ± 4·07	110·80 ± 1·68
A_{32}	85·20 ± 6·06	119·40 ± 6·96	122·20 ± 2·93
A_{33}	40·00 ± 1·13	48·20 ± 1·04	70·20 ± 2·46
A_{34}	35·40 ± 0·96	42·20 ± 2·49	55·40 ± 2·60
A_{35}	43·80 ± 2·66	59·40 ± 2·13	104·20 ± 4·09
FI	37·20 ± 2·03	48·40 ± 3·92	83·20 ± 3·03
A_{35} glucoside	36·60 ± 1·15	42·20 ± 1·68	50·50 ± 1·92
A_3	79·75 ± 2·56	106·80 ± 2·32	128·60 ± 7·26
A_4	66·40 ± 1·61	96·40 ± 4·84	102·60 ± 5·94
A_5	58·40 ± 2·92	86·40 ± 6·50	115·20 ± 8·23
A_7	83·20 ± 3·42	115·60 ± 1·54	133·80 ± 7·06
deoxy A_5	63·40 ± 2·48	72·60 ± 4·45	121·40 ± 3·16
Control		41·00 ± 1·72	

Each value represents the mean sum of the first and second leaf sheath lengths (mm) ±s.e. (4 or 5 replicates).

Comparison of activities between the two dwarf rice tests indicates that at 10 ng/plant, GA_{31}, GA_5 and deoxy GA_5 were less active than GA_4 and GA_7 on Waitō-C, but more active than GA_4 and GA_7 on Tan-ginbōzu. GA_{33}, GA_{34}, GA_{35} glucoside and F-I were generally inactive in both varieties, slight activity being observed at the highest dosage of 100 ng/plant.

Dwarf Maize Test

The results of the assays using d_1, d_3 and d_5 mutants are summarized in Tables 3–5 respectively. GA_{30} and GA_{32} showed almost the same degree of activity as that of GA_3 in the d_1 and d_3 assays, whilst only GA_{32} was as active as GA_3 in the d_5 assay.

The activities of GA_{30}, GA_{31}, GA_{35} and deoxy GA_5 ranged from more than 10–100% of those of GA_7, GA_5 and GA_4. However, in the d_5 assay, the activity of GA_{30} was *ca.* 10% of that of GA_7 and even lower compared with that of GA_3. GA_{33}, GA_{34}, GA_{35} glucoside and F-I were genreally inactive, although slight but significant activities were observed at the highest dosage of 10 μg per plant in some cases. In the d_3 assay F-I was as active as GA_{35}.

TABLE 4. ACTIVITY OF GIBBERELLINS IN THE DWARF MAIZE (d_3) ASSAY

Gibberellin	μg gibberellin/seedling 0·1	1·0	10
A_{30}	66·75 ± 3·15	100·04 ± 3·21	105·40 ± 5·65
A_{31}	42·25 ± 1·29	63·60 ± 2·57	97·90 ± 4·91
A_{32}	86·75 ± 3·90	117·20 ± 2·88	118·80 ± 3·32
A_{33}	35·25 ± 1·24	41·60 ± 1·78	61·60 ± 1·97
A_{34}	34·25 ± 0·54	39·20 ± 2·01	50·60 ± 3·01
A_{35}	40·75 ± 0·74	50·40 ± 1·56	86·40 ± 4·03
F I	41·50 ± 3·58	49·60 ± 2·60	73·00 ± 5·16
A_{35} glucoside	35·50 ± 1·15	37·20 ± 1·25	44·20 ± 1·31
A_3	66·60 ± 2·43	94·40 ± 4·11	120·20 ± 4·88
A_4	55·50 ± 3·91	79·80 ± 2·32	109·75 ± 6·78
A_5	52·67 ± 2·60	81·20 ± 3·04	103·80 ± 4·42
A_7	68·80 ± 3·53	109·00 ± 3·87	108·00 ± 4·66
deoxy A_5	48·00 ± 3·09	69·20 ± 5·38	99·60 ± 3·95
Control		37·20 ± 0·44	

Values as in Table 3.

Dwarf Pea Test

The results of the dwarf pea test are shown in Table 6. GA_{30}, GA_{31}, GA_{32}, GA_{35} and deoxy GA_5 were quite active. The activities of these gibberellins (except deoxy GA_5) were less than those of GA_7, GA_5, GA_3 and GA_4, respectively. However, the differences were not very large. Deoxy GA_5 was as active as GA_5. Among the new gibberellins tested, GA_{30} was the most active in this assay. GA_{34} and GA_{35} glucoside were inactive, while GA_{33} and F-I showed only slight activities at the highest dosage of 10 μg/plant.

TABLE 5. ACTIVITY OF GIBBERELLINS IN THE DWARF MAIZE (d_5) ASSAY

Gibberellin	μg gibberellin/seedling 0·1	1·0	10
A_{30}	49·25 ± 4·76	75·25 ± 7·07	95·60 ± 8·05
A_{31}	57·75 ± 4·99	68·25 ± 3·99	85·80 ± 5·16
A_{32}	82·25 ± 2·86	109·00 ± 4·60	111·25 ± 3·40
A_{33}	41·25 ± 0·96	48·75 ± 2·16	54·80 ± 2·29
A_{34}	41·50 ± 2·75	43·00 ± 3·66	61·40 ± 2·79
A_{35}	44·50 ± 4·44	57·00 ± 1·70	91·00 ± 10·36
F I	41·75 ± 3·01	43·00 ± 2·37	61·80 ± 2·25
A_{35} glucoside	44·25 ± 2·48	39·00 ± 2·60	43·80 ± 2·73
A_3	77·00 ± 2·86	114·00 ± 4·86	128·50 ± 8·28
A_4	50·75 ± 5·55	76·00 ± 7·39	101·80 ± 5·26
A_5	54·50 ± 4·55	86·50 ± 2·46	93·50 ± 5·36
A_7	77·75 ± 4·28	95·00 ± 3·26	120·50 ± 2·75
deoxy A_5	71·00 ± 6·43	88·75 ± 8·35	98·00 ± 4·81
Control		41·80 ± 2·37	

Values as in Table 3.

TABLE 6. ACTIVITY OF GIBBERELLINS IN THE DWARF PEA (PROGRESS NO. 9) ASSAY

Gibberellin	μg gibberellin/seedling 0·1	1·0	10	Control
A_{30}	78·00 ± 4·15	——	216·00 ± 18·29	*a*
A_{31}	73·60 ± 3·46	85·60 ± 8·71	107·60 ± 3·42	*c*
A_{32}	74·00 ± 8·71	82·80 ± 7·94	153·00 ± 15·49	*g*
A_{33}	63·40 ± 3·05	67·80 ± 1·56	82·20 ± 6·58	*g*
A_{34}	53·60 ± 2·54	57·00 ± 2·28	62·50 ± 1·15	*g*
A_{35}	65·60 ± 4·20	81·78 ± 3·14	106·50 ± 11·43	*g*
F I	72·40 ± 1·00	76·20 ± 7·73	97·80 ± 8·09	*c*
A_{35} glucoside	61·00 ± 3·02	64·00 ± 2·04	60·40 ± 1·22	*d*
A_3	151·00 ± 16·06	204·40 ± 29·93	248·60 ± 29·96	*f*
A_4	87·40 ± 4·96	114·60 ± 12·35	217·20 ± 32·55	*e*
A_5	78·00 ± 4·32	94·80 ± 9·40	125·00 ± 4·76	*d*
A_7	119·20 ± 7·03	166·00 ± 27·27	230·20 ± 24·67	*e*
deoxy A_5	84·40 ± 4·48	100·60 ± 11·86	125·00 ± 11·21	*b*

Controls: (*a*) 64·40 ± 2·89; (*b*) 68·00 ± 1·72; (*c*) 66·40 ± 2·17; (*d*) 56·40 ± 1·95; (*e*) 60·33 ± 2·13; (*f*) 68·60 ± 1·89; (*g*) 56·00 ± 2·32

Each value represents the mean epicotyl length (mm) ±s.e. (5 replicates).

Cucumber Test

The results of the cucumber test are shown in Table 7. GA_{32} was the most active of the new gibberellins, its activity being higher than that of GA_3. GA_{30} was much less active than GA_7; GA_{35} was almost as active as GA_4. Both GA_{31} and GA_5 were inactive; deoxy GA_5 was as active as GA_3. GA_{33}, GA_{34}, GA_{35} glucoside and F-I were all inactive.

TABLE 7. ACTIVITY OF GIBBERELLINS IN THE CUCUMBER (NATIONAL PICKLING) ASSAY

Gibberellin	μg gibberellin/seedling 0·1	1·0	10	Control
A_{30}	27·30 ± 0·53	34·30 ± 0·83	——	*a*
A_{31}	28·57 ± 1·03	29·80 ± 1·75	29·80 ± 0·44	*b*
A_{32}	32·30 ± 2·17	44·10 ± 1·92	61·80 ± 3·00	*b*
A_{33}	29·60 ± 1·66	29·50 ± 0·75	30·00 ± 1·30	*a*
A_{34}	30·70 ± 1·58	26·86 ± 1·13	28·60 ± 0·86	*c*
A_{35}	32·00 ± 1·41	42·40 ± 3·11	57·00 ± 4·03	*a*
F I	27·50 ± 1·39	29·80 ± 1·17	28·90 ± 0·77	*c*
A_{35} glucoside	23·60 ± 0·46	26·00 ± 0·72	29·00 ± 1·12	*c*
A_3	——	39·80 ± 1·29	51·00 ± 2·51	*b*
A_4	41·70 ± 2·14	48·00 ± 1·74	53·40 ± 1·77	*c*
A_5	29·10 ± 1·66	30·10 ± 1·03	29·10 ± 0·98	*c*
A_7	47·60 ± 1·01	54·50 ± 2·10	51·90 ± 2·02	*c*
deoxy A_7	32·10 ± 1·16	39·50 ± 1·18	46·70 ± 1·48	*c*

Controls: (*a*) 27·67 ± 0·94; (*b*) 29·00 ± 2·79; (*c*) 25·90 ± 1·12

Each value represents the mean length (mm) ±s.e. of a hypocotyl unit (10 replicates).

DISCUSSION

In general GA_{30}, GA_{31} and GA_{35} were quite active, while GA_{33}, GA_{34} and GA_{35} glucoside were inactive or only slightly active. GA_{32} exhibited very high activities in our bioassays and was as active as GA_3. This result is in good agreement with that of Coombe.[6] Thus, GA_{32} can be classified as one of the most active gibberellins, along with GA_3 and GA_7.

GA_{32} differs from GA_3 in possessing C-12 and C-15 hydroxyl groups. It is interesting that the addition of hydroxyl groups to the positions of C-12 and C-15 of GA_3 does not seem to affect biological activities. GA_{32} has four hydroxyl groups; GA_8 has three and yet it is generally inactive or only slightly active.[7] This implies that the degree of activity may not be determined by the number of hydroxyl groups, i.e. the hydrophilic character of the molecule, but by the position of hydroxylation. Comparison of GA_4 and GA_8 with GA_{34}, which has the same A ring structure as GA_8, indicates that the low activity or the lack of the activity in GA_8 and GA_{34} is probably due to the presence of the C-2β hydroxyl group. In fact, GA_{26}, GA_{27} and GA_{29} which have the C-2β hydroxyl group have been reported to have low activities.[8] High activity of gibberellins may be dependent upon the C-2 position being free.

GA_{31} and deoxy GA_5 have the same A ring system but the former contains the C-12α hydroxyl group. These two gibberellins generally showed a similar pattern of biological activities in our bioassay systems. The same structure-activity was also observed for GA_{30} and GA_7. This suggests that the C-12α hydroxyl group generally does not affect the growth-promoting effect.

However, in cucumber hypocotyl elongation, the effect of the C-12α hydroxyl group on activity is different. GA_{30}, containing the C-12α hydroxyl group, showed low activity, being much less active than GA_7. It should also be noted that GA_{31}, an isomer of GA_5, as well as GA_5 itself, was inactive in the cucumber bioassay, while deoxy GA_5 is quite active. It is well known[9–11] that the gibberellins lacking the C-13 hydroxyl group such as GA_4, GA_7 and GA_9 show very high activity in this assay. These results may indicate that the introduction of a hydroxyl group to either the C-12 or the C-13 position reduces the activity in the cucumber hypocotyl elongation. The inactivity of GA_{20}[7] may support this idea. It is quite interesting that GA_{32} and GA_{35} have quite high activity in this assay system. GA_{32} differs from GA_{30} in possessing two additional hydroxyl groups at C-13 and C-15, and yet GA_{32} is much more active than GA_{30}. For the explanation of this fact it is necessary to study the contribution of the C-15β hydroxyl group to gibberellin activity. The high activity of GA_{35} may suggest that the C-11β hydroxyl group does not affect activity in this assay. According to Brian *et al.*[9] the C-3 keto-compounds (I, II) derived from GA_4 and GA_7 generally show low activities, but in cucumber hypocotyl elongation these are as active as GA_4 and GA_7. On the other hand, the C-3 keto compounds (III, IV) derived from GA_1 and GA_3 are inactive in this assay. These results suggest that the C-3 keto group reduces activity in general bioassay systems (except the cucumber hypocotyl elongation), while the C-13 hydroxyl group reduces activity in the cucumber assay. The low activity of GA_{33} in the cucumber test may be due to the C-12α hydroxyl group, although the effect of the C-1β

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hydroxyl group cannot be excluded. On the other hand, low activities in other bioassays are in good agreement with the observation by Brian *et al.*[9]

Murakami[12] pointed out that GA_5, GA_9 and GA_{20}, lacking the C-3 hydroxyl group, show low activities in the Waitō-C assay, but high activities in the Tan-ginbōzu assay. It is noteworthy that GA_{31} and deoxy GA_5 showed similar activity patterns to these gibberellins in the rice seedling assays.

GA_{35} glucoside with the C-11β equatorial glucosidic bond showed no activity in the pea assay. This contrasts with the observation that GA_8 and GA_{29} glucosides having the C-2β equatorial glucosidic bond show almost the same activities as the respective aglycones in the pea assay,[8] suggesting facile hydrolysis of the glucosides in pea plants. The ease of hydrolysis of the glucosidic bond in pea plants may depend upon the position of the glucosidic bond and not solely on its conformation.

EXPERIMENTAL

Dwarf rice test. The test was carried out according to the micro-drop method by Murakami[12] and was conducted at 30° under continuous fluorescence light (*ca.* 2800 lx). Rice seeds of *Oryza sativa L.*, dwarf cv. Tan-ginbōzu and Waitō-C were used for the assay.

Dwarf maize test. The assay was conducted according to the method described by Tamura *et al.*[13] with the following modifications. Gibberellins were dissolved in 20% acetone containing 0·1% Tween 20, and a 50-μl aliquot of the test solution was applied to each seedling (*Zea mays* L., mutants d_1, d_3 and d_5). The seedlings were grown under the same conditions as those used for dwarf rice and after 7 days the length of the 1st and 2nd leaf sheaths were measured.

Dwarf pea test. The test was conducted as described by Yokota *et al.*,[8] using seeds of *Pisum sativum* L. var. Progress No. 9.

Cucumber test. According to the procedure described by Katsumi *et al.*,[14] the assay was conducted using *Cucumis sativus* L., cv. National Pickling.

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