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ACTIVITY OF SYNTHETIC GIBBERELLIN A_{15} AND (±)-GIBBERELLIN A_{15}-ISOLACTONE

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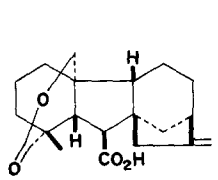
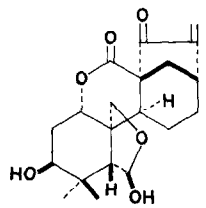
Abstract—The activities of (±)-gibberellin A_{15} ((±)-GA_{15}) and (±)-gibberellin A_{15}-isolactone ((±)-iso-GA_{15}) which were obtained by stereocontrolled total synthesis and gibberellin A_{15} (E-GA_{15}) synthesized by interconversion of enmein were assayed by the rice seedling test. As expected, (±)-GA_{15} showed half the activity of natural gibberellin A_{15} (GA_{15}). E-GA_{15} which has a natural configuration showed the same activity as natural gibberellin A_{15}, while (±)-iso-GA_{15} was almost inactive. These samples were also submitted to the cucumber hypocotyl assay. Contrary to what has already been reported, they were almost inactive.

INTRODUCTION

CROSS *et al.*[1] and Hanson[2] isolated GA_{15} from the culture medium of *Gibberella fujikuroi* and gave its structure (1a). Cross *et al.*[1] and Brian *et al.*[3] reported its biological activity as follows: GA_{15} has a very weak activity in the dwarf pea epicotyl assay, the lettuce hypocotyl assay and the dwarf maize (d_1) assay but it gave the same order of activity as gibberellin A_3 (GA_3) in the cucumber hypocotyl assay and the dwarf maize (d_3, d_4) assay. Crozier *et al.*[4] reported that GA_{15} had a high activity in the dwarf maize (d_5) assay, an intermediate activity in the lettuce hypocotyl assay, dwarf rice microdrop assay, cucumber hypocotyl assay, and dwarf maize (d_2) assay and a weak activity in the dwarf pea assay, while it was quite inactive in the barley aleurone assay and the dwarf maize (d_1, d_3) assay.



(1a), (1b)



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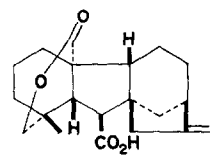

(3)

[1] CROSS, B. E., GALT, R. H. B. and HANSON, J. R. (1964) *Regulateurs Naturels de la Croissance Vegetale*, p. 265, Centre National de la Recherche Scientifique, Paris.
[2] HANSON, J. R. (1967) *Tetrahedron* **23**, 733.
[3] BRIAN, P. W., GROVE, J. F. and MULHOLLAND, T. P. C. (1967) *Phytochemistry* **6**, 1475.
[4] CROZIER, A., KUO, C. C., DURLEY, R. C. and PHARIS, R. P. (1970) *Can. J. Botany* **48**, 867.

Recently, stereocontrolled total synthesis of (±)-GA_{15} (**1b**) was completed by Nagata *et al.*[5] Their identification was made by comparing their IR, MS, NMR spectra and TLC with those of natural GA_{15}. Somei *et al.*[6] attempted to synthesize GA_{15} from enmein (**2**), which is a bitter substance isolated from *Plectranthus japonicus*, and they finally succeeded in obtaining 2 mg of pure sample of GA_{15} (**1a**), which was identified by comparing its IR and NMR spectra, optical rotatory dispersion (ORD) curve, and also by admixture with natural GA_{15}.

These samples and isomeric compounds (±)-iso-GA_{15} (**3**) have now been assayed by the rice seedling test and the cucumber hypocotyl assay, and the results will be reported in the present paper.

RESULTS AND DISCUSSIONS

The rice seedling test of GA_3, GA_{15} (**1a**), (±)-GA_{15} (**1b**), (±)-iso-GA_{15} (**3**), and GA_{15} from enmein was repeated 4 times, the results are shown in Table 1.

TABLE 1. EFFECT OF VARIOUS GIBBERELLINS ON THE SECOND LEAF SHEATH LENGTH OF RICE SEEDLINGS

Experiment	Conc. (ppm)	GA_3	GA_{15}	(±)-GA_{15}	(±)-iso-GA_{15}	GA_{15} from enmein
1	10	113·1 ± 2·2*	91·0 ± 1·3			
	5		77·8 ± 1·5			
	1	95·3 ± 1·7	46·0 ± 1·2	36·4 ± 0·8	25·2 ± 0·7	47·4 ± 1·1
	0·5		37·1 ± 0·7			
	0·1	56·4 ± 0·9	28·2 ± 0·6	24·9 ± 0·5	24·2 ± 0·3	27·9 ± 0·7
	0·05		25·2 ± 0·4			
	Control	24·8 ± 0·3				
2	1	86·6 ± 2·4	50·4 ± 1·2	40·1 ± 0·9	24·5 + 0·4	53·8 ± 1·2
	0·5		40·5 ± 0·5			
	0·1	53·8 ± 1·4	28·7 ± 0·4	24·6 ± 0·4	24·3 ± 0·3	28·6 ± 0·6
	0·05		25·3 ± 0·5			
	Control	23·7 ± 0·2				
3	1	92·8 ± 1·4	55·9 ± 1·3	38·1 ± 0·7	25·0 ± 0·2	54·9 ± 1·2
	0·5		44·8 ± 1·0			
	0·1	58·6 ± 1·1	28·0 ± 0·9	26·1 ± 0·3	23·1 ± 0·3	28·9 ± 0·4
	0·05		27·0 ± 0·4			
	Control	24·3 ± 0·2				
4	1	98·7 ± 1·4	63·7 ± 1·0	40·0 ± 0·8	25·7 ± 0·4	55·2 ± 1·0
	0·5		41·9 ± 0·4			
	0·1	68·4 ± 0·7	31·2 ± 0·4	27·0 ± 0·4	22·8 ± 0·4	28·4 ± 0·4
	0·05		30·6 ± 0·5			
	Control	24·5 ± 0·2				

Each figure means average length (mm) of 25 of second leaf sheath.
* Standard error.

As shown in this table, 1 ppm (3×10^{-6} mol) of (±)-GA_{15} gave almost the same elongation as 0·5 ppm ($1{\cdot}5 \times 10^{-6}$ mol) of GA_{15} and 0·1 ppm (3×10^{-7} mol) of (±)-GA_{15} gave almost the same elongation as 0·05 ppm ($1{\cdot}5 \times 10^{-7}$ mol) of GA_{15}. These results suggested that the antipode of natural GA_{15} is quite *inactive* in the rice seedling test. The results

[5] NAGATA, W., WAKABAYASHI, T., NARISADA, M., HAYASE, Y. and KAMATA, S. (1970) *J. Am. Chem. Soc.* **92**, 3203; (1971) *ibid.* **93**, 5740.
[6] SOMEI, M. and OKAMOTO, T. (1970) *Chem. Pharm. Bull. (Tokyo)* **18**, 2135; (1972) *Yakugaku Zasshi* **92**, 397.

also showed that (±)-iso-GA_{15} is almost inactive and GA_{15} from enmein is identical with natural GA_{15} in the bioassay. These results also reveal that GA_{15} has a specific activity of 50–60% of GA_3.

TABLE 2. EFFECT OF VARIOUS GIBBERELLINS ON THE HYPOCOTYL LENGTH OF CUCUMBER SEEDLINGS

Experiment	Dose (μg/plant)	GA_3	GA_{15}	(±)-GA_{14}	(±)-iso-GA_{15}	GA_{15} from enmein
1	10	41·6 ± 1·4*				
	1	36·0 ± 0·9	25·3 ± 0·6	25·1 ± 0·3	24·3 ± 0·5	25·3 ± 0·5
	0·5		24·8 ± 0·4			
	0·1	26·3 ± 0·3	24·8 ± 0·3	24·6 ± 0·5	23·6 ± 0·2	24·2 ± 0·3
	0·05		24·8 ± 0·3			
	Control	23·1 ± 0·2				
2	10	41·1 ± 1·1				
	1	35·6 ± 0·7	26·5 ± 0·3	25·0 ± 0·5	25·0 ± 0·4	25·3 ± 0·4
	0·5		26·5 ± 0·5			
	0·1	26·3 ± 0·3	24·3 ± 0·3	23·6 ± 0·3	23·7 ± 0·2	24·0 ± 0·3
	0·05		24·9 ± 0·2			
	Control	24·0 ± 0·2				

Each figure means average length (mm) of 20 of hypocotyl length.
* Standard error.

These samples were also tested by the cucumber hypocotyl assay. Contrary to earlier reports,[1,3,4] GA_{15}, E-GA_{15}, and also (±)-GA_{15}, (±)-iso-GA_{15} were almost inactive (Table 2).

EXPERIMENTAL

Bioassay. (1) Rice seedling test: Seeds* of dwarf variety of rice (*Oryza sativa* var. Tanginbozu) were soaked in EtOH for 10 min and rinsed 3–4 times with H_2O and then soaked in a filtered suspension of 10% $Ca(OCl)_2$ for 1 hr, and washed thoroughly. They were germinated in 300 ml of water in a Petri dish (dia., 21 cm) at 30° under nearly 4000 lx of artificial light for 2 days. The 18 ml of test solutions were mixed with 0·5% agar and heated to effect solution. When cooled, 25 germinating seeds having 1 mm of coleoptiles were selected and "planted" on the surface of the solidified agar, coleoptiles upwards. The dishes were covered with polyethylene film and kept at 30° in 4000 lx for 5 days. The length of the second leaf sheaths of seedlings were measured and mean values calculated. All the glassware and materials used were sterilized, although operations were carried out in an ordinary room.

(2) Cucumber hypocotyl assay: The procedures were almost the same as those of Katsumi *et al.*[7] Seeds of *Cucumis sativus* cv. National Pickling stock (Burpee Seeds Co.) were planted in vermiculite and grown in a growth chamber at 26° under 2000 lx of fluorescent lamps for 6–7 days. When the seedlings had reached a height of 3–4 cm and cotyledons were fully expanded, seedlings having 3 cm of hypocotyl were selected and the hypocotyl were marked with Indian ink 2 cm below the cotyledonary nodes. Test samples were dissolved in 90% EtOH and 0·01 ml of the soln was applied to the apical buds of the seedlings. Measurements were made from the Indian ink mark to the cotyledonary nodes 3 days after the treatment. Controls consisted of treatments with 90% EtOH.

Preparation of test solutions: In the case of (±)-GA_{15} and (±)-iso-GA_{15}, the amount of the samples available was so small that (220 and 300 μg) that they were dissolved in 22 and 30 ml acetone and aliquotes containing 50 and 60 μg of each were evaporated by N_2 aeration on a H_2O bath (40°). GA_{15} is sparingly soluble in H_2O and the mixture was warmed on a H_2O bath (70°) for several hours with occasional shaking until complete soln.

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* 10 g of dry seeds (about 700) are necessary for one Petri dish, which is sufficient to give 350 selected germinating seeds. The seeds were kindly provided by the Aburahi Laboratories of Shionogi & Co. Ltd., Shiga Prefecture.

[7] KATSUMI, M., PHINNEY, B. O. and PURVES, W. K. (1965) *Physiol. Plant.* **18**, 462.