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Synthetic Gibberellin Synergists in Elongation of Shoot Growth of *Oryza sativa* L.*

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Abstract. Previous studies showed that 2-ethyl-3-methoxycarbonyl-1-(*p*-tolylcarbamoyl) isourea acts as a potent GA_3-synergist in stimulating shoot growth of rice seedlings. Studies with several structurally related compounds show that the alkoxycarbonylcarbamoyl-isourea or -isothiourea skeleton is required for biological activity. Any chemical deletion from this skeleton causes complete loss of activity. From present and previous data it seems that alkoxycarbonylcarbamoyl-isourea or -isothiourea is converted by intramolecular cyclization in the rice seedlings into the corresponding triazinone that serves as the active form.

In previous communications we reported that 2-ethyl-3-methoxycarbonyl-1-(*p*-tolylcarbamoyl)isourea, **I**, promoted the growth of rice (*Oryza sativa* L.) seedlings and was a highly active synergist when tested in combination with GA_3 (Ogawa et al. 1976, 1977). These findings prompted us to study further the interactions between GA_3 and the synthetic isourea and related compounds. In particular, the physiological activities of 4-ethoxy-l-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione, **VIII**, prepared by intramolecular cyclization of **I**, have been extensively investigated using rice seedlings (Ogawa et al. 1978, 1980a, Ogawa

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and Kitamura 1980, Ogawa 1980). It is hoped that these studies might provide new insight into the role of gibberellins in plant growth and development.

We describe in this paper the synthesis of several compounds structurally related to I and their abilities to interact synergistically with GA_3 in the promotion of growth of rice seedlings.

Materials and Methods

Plant Material

Seeds of rice (*Oryza sativa* L. var. Kinmaze) were germinated by soaking them in water for 2 days at 28–30°C. Unless otherwise specified, five germinated seeds with coleoptiles of uniform length were planted on 10 ml of a 0.5% agar medium containing the test compound with or without GA_3 in a glass tube (2.6 cm in diameter × 6 cm in height). Rice seedlings were cultured for 4 days at 28–30°C under continuous fluorescent illumination of 4500 lx, and thereafter their shoot lengths were measured. Then, the abilities of each compound at three different concentrations to enhance the GA_3-induced shoot elongation of rice seedlings were calculated.

Chemicals

The compounds synthesized for this study are given in Table 1. **I**, **II**, **III**, **VIII**, **IX**, and **X** were prepared according to the methods reported by Oyamada et al. (1976) and Ogawa et al. (1976, 1977, 1978, 1980b). **IV** was prepared by the addition reaction of *p*-tolylisocyanate and ammonia. Their purity was checked by silica gel TLC, and their structures were confirmed by elemental analysis, and IR and NMR spectra. Details of the methods for preparing **V**, **VI**, and **VII** are hereinafter described.

2-Ethyl-3-(p-tolylcarbamoyl)isourea (V)

Ethyl N-(phenoxycarbonyl)thiocarbamate (Dixon 1906), 52.5 g (0.23 mol) was refluxed with 25 g (0.23 mol) of *p*-toluidine in 150 ml of EtOH for about 8 h. After cooling, the precipitated crystalline solid was separated by filtration. Recrystallization from EtOH gave 34 g (61.2% yield) of ethyl N-(*p*-tolylcarbamoyl)-thiocarbamate (**XI**), mp. 186–7°C, IR ν_{max}^{Nujol} cm^{-1}: 1690 (C=O). A mixture of 23.8 g (0.1 mol) of **XI** and 16 g of methyl iodide in 150 ml of acetone was stirred with 14 g of K_2CO_3 at room temperature for about 24 h. After removal of insoluble substances by filtration, the solvent was evaporated under reduced pressure. Recrystallization of the residue from n-hexane-benzene (1:1) gave 23.6 g (93.6% yield) of O-ethyl S-methyl N-(*p*-tolylcarbamoyl)carboimidothioate (**XII**), mp. 105–6°C, IR ν_{max}^{Nujol} cm^{-1}: 1675 (C=O). Twelve g of **XII** was dissolved in 150 ml of EtOH saturated with NH_3 and stirred at room temperature for about 24 h, and then refluxed for about 1 h. After removal of the solvent, the residue was recrystallized from EtOH to give 5.8 g (55.2% yield) of V, mp. 146–7°C, IR ν_{max}^{Nujol} cm^{-1}: 1640 (C=O).

2-Ethyl-1-methyl-3-(p-tolylcarbamoyl)isourea (VI)

A mixture of 5.0 g (0.02 mol) of **XII** and 2.4 g of monomethylamine (40% aqueous solution) in 60 ml of EtOH was stirred at room temperature for about 24 h, and then refluxed for about 30 min. After removal of the solvent, the residue was recrystallized from n-hexane to give 4.2 g (90.1% yield) of **VII**, mp. 75–6°C, IR ν_{max}^{Nujol} cm^{-1}: 1660 (C=O).

1-Methoxycarbonyl-5-p-tolylbiuret (VII)

One g of 2-ethyl-3-methoxycarbonyl-1-(*p*-tolylcarbamoyl)isourea (**I**) was refluxed with HBr (15% aqueous solution) for about 5 h. The resulting crystalline solid was separated and dried. Recrystallization from EtOH gave 0.7 g (77.8% yield) of **VII**, mp. 197–8°C, IR ν_{max}^{Nujol} cm^{-1}: 1750, 1700 (C=O).

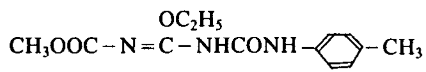
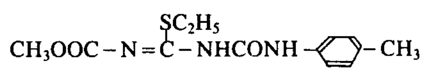
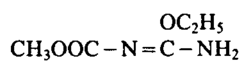
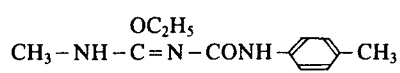
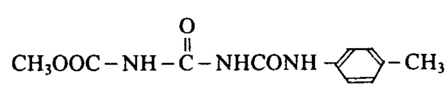
Results and Discussion

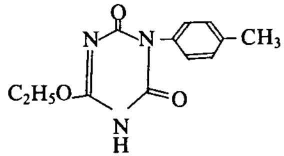
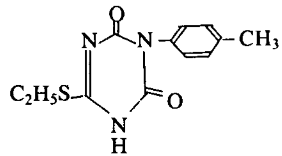
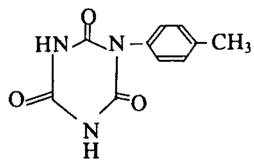
Fig. 1 shows the interaction between **I** and GA_3 in the growth of rice seedlings cultured for 5 days. **I** at 50 mg/liter induced a slight increase in shoot growth while a most marked effect was observed in the presence of 10 mg/liter of GA_3. The simultaneous application of **I** and GA_3 induced a synergistic promotion of shoot growth, even at the optimum GA_3 concentration, 10 mg/liter.

To obtain more information on structural requirements for synergistic interaction with GA_3, six analogs of I (**II–VII**) were synthesized and among them three (**I**, **II**, and **VII**) were chemically converted into their counterpart triazinones (**VIII**, **IX**, and **X**) by intramolecular cyclization. Their biological activities were examined at the concentrations of 1, 10, and 50 mg/liter in the rice seedling test in the presence or absence of 10 mg/liter GA_3. The evaluation was based on their abilities to enhance the GA_3-induced shoot elongation of rice seedlings. Table 1 summarizes the compounds tested in this study and their synergistic effects. The replacement of an oxygen atom by a sulfur atom at the position of isourea structure (**II**) maintained the biological activity, although it was slightly less than that of the corresponding isourea compound, **I**. The complete inactivity of 1-methoxycarbonyl-5-*p*-tolylbiuret (**VII**) indicates the importance of the presence of an isourea or isothiourea structure for synergistic activity. It should be noted that any chemical deletion of the alkoxycarbonylcarbamoylisourea skeleton (**III–VI**) also caused complete loss of activity. These results suggest that the total skeleton of alkoxycarbonylcarbamoyl-isourea or -isothiourea is required for biological activity.

Compounds **VIII**, **IX**, and **X**, synthesized by intramolecular cyclization of **I**, **II**, and **VII**, respectively, were also examined for biological activity. **VIII** was most active; **IX** was also active, but weaker than **VIII**, while the activity

Table 1. Synergistic activities of isourea and related compounds in the GA_3-induced shoot elongation of rice seedlings.

Compound no.	Chemical structure	Mp (°C)	Conc. (ppm)	Synergistic activity with GA_3*
I		156	1	0
			10	+ + +
			50	+ + + +
II		132–134	1	0
			10	+ +
			50	+ + +
III		56–57	1	0
			10	0
			50	0
IV		180–181**	1	0
			10	0
			50	0
V		146–147	1	0
			10	0
			50	0
VI		75–76	1	0
			10	0
			50	0
VII		197–198	1	0
			10	0
			50	0

VIII		260–262	1	+
			10	+ +
			50	+ + + +
IX		223–226	1	+
			10	+ +
			50	+ + +
X		280	1	0
			10	0
			50	0

* Synergistic activity with GA_3 was expressed as follows:

$$\frac{\text{[Elongation due to (10 mg/l } GA_3 \text{ + test compound)]}}{\text{[Elongation due to 10 mg/l } GA_3\text{] + [Elongation due to test compound]}} \times 100:$$

85–115 : 0
116–135 : +
136–155 : + +
156–175 : + + +
176 : + + + +

Control shoot length: with GA_3, 42 mm; without GA_3, 22 mm.

** Steiner A (1875) Ber. **8**, 518

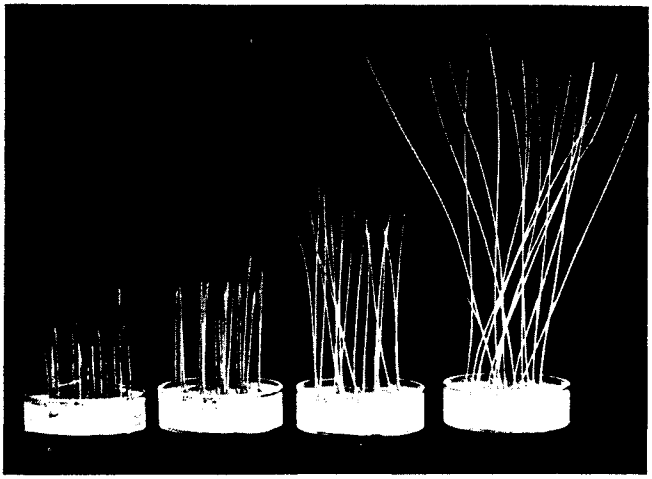


Fig. 1. Synergistic interaction between GA_3 and 2-ethyl-3-methoxycarbonyl-1-(*p*-tolylcarbamoyl)isourea, I, in the growth of rice seedlings. Twenty-five seeds of rice were planted on a 0.5% agar medium (30 ml) containing the test compound(s) in a 5.5 cm Petri dish. They were cultured for 5 days at 28–30°C under continuous illumination from fluorescent lamps of 4500 lx. From left to right: control, 50 mg/liter I, 10 mg/liter GA_3, and 50 mg/liter I plus 10 mg/liter GA_3.

of X was almost zero. These results with the three closed-ring compounds are consistent with those of our previous reports (Ogawa et al. 1978, 1980b). The magnitude of activity in these triazinones was well correlated with that of the corresponding alkoxycarbonylcarbamoyl-isourea, -isothiourea or -urea compounds. Alkoxycarbonylcarbamoylisourea compounds seem to be metabolized into the active form of triazinone compounds in rice seedlings, as suggested previously (Ogawa et al. 1977). If this is the case, it is reasonable to assume that compounds III–VI, which were subjected to chemical modification of alkoxycarbonylcarbamoylisourea skeleton, were inactive because they could not be converted into the closed-ring form.

Recently, the synergistic effects of I and VIII on the activity of various gibberellins were investigated, the results being greatly dependent on the kind of gibberellin tested (Satoh et al. 1981). In addition, the compounds were shown to be a useful tool in the biological assay for detecting minute endogenous gibberellins. Although biochemical and ultrastructural effects of VIII have been studied previously (Ogawa 1980, Ogawa et al. 1980a), its mode of action remains obscure. As far as we know, there is no report in the literature on other synthetic GA_3-synergists. We therefore think it is of value to study further the synergistic interaction of isourea derivatives and related compounds with GA_3.

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