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PLANT GROWTH-PROMOTING ACTIVITIES OF ALKOXYCARBONYLISOUREAS IN RELATION TO THEIR CHEMICAL STRUCTURES*

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Key Word Index—*Oryza sativa*; Gramineae; rice shoot elongation; gibberellic acid-synergists; isoureas.

Abstract—A number of alkoxycarbonylisourea derivatives were synthesized and their plant growth-promoting activities examined by the rice (*Oryza sativa*) seedling test. Isourea compounds with an appropriate substituent such as a halogen atom or a methyl, ethyl or methoxy group at the *para*-position on a benzene ring in 1-alkoxycarbonyl-2-alkyl-3-phenylcarbamoylisoureas promoted the growth of rice seedlings and acted as a highly active gibberellic acid-synergist when used in combination with gibberellic acid. The common structural requirements of isourea derivatives applied well for a growth promoter and a gibberellic acid-synergist.

INTRODUCTION

In a previous paper [1] we reported that synthetic 2-ethyl-1-isopropoxycarbonyl-3-(4-tolylcarbamoyl)isourea promoted the growth of rice (*Oryza sativa*) seedlings, and enhanced greatly the stimulative effect of GA on the growth of rice seedlings. This compound is the first synthetic compound known to be an effective GA-synergist. On the basis of these findings, we planned to clarify the structure–activity relationships of a series of isourea derivatives structurally related to 2-ethyl-1-isopropoxycarbonyl-3-(4-tolylcarbamoyl)isourea, on the shoot elongation of rice seedlings.

In this study, we synthesized a number of alkoxycarbonylisoureas with various substituents at the R_1, R_2 and R_3 positions in the general formula as shown in Fig. 1. These compounds were tested for their activities to promote the growth of rice seedlings and to synergize the effect of GA on rice shoot elongation.

RESULTS AND DISCUSSION

First, we prepared 13 isourea derivatives possessing a *para*-methylphenyl group as R_3 and various substituents as R_1 and R_2. They were assayed for growth pro-

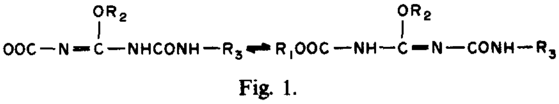

Fig. 1.

* Part 2 in the series "Plant growth-regulating activities of isourea derivatives and related compounds". For Part 1 see ref. [1].

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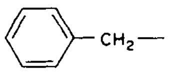
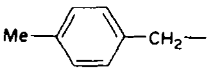
moting activity of rice seedlings in the presence or absence of 10 ppm GA. The results are summarized in Table 1. Derivatives having alkyl, alkenyl or alkynyl groups (compounds **I–1** to **I–9**) as R_1 and/or R_2, with three or less carbon atoms, were highly effective. These compounds equally promoted the growth of rice seedlings at the concentrations of 10–50 ppm. The combined application of each compound with GA showed a distinct synergistic effect on the shoot growth. The substitution of R_1 and/or R_2 with isobutyl group(s) (**I–10** and **I–11**) slightly decreased the biological activity. On the other hand, isourea derivatives with a benzyl or a substituted benzyl at R_2 (**I–12** and **I–13**) were rather biologically inactive.

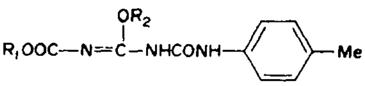
Next, we prepared 26 isourea derivatives possessing a methyl group as R_1, an ethyl group as R_2 and various substituents as R_3. They were tested in the absence of GA for a growth-promoting activity, and examined for a synergistic activity with GA in the presence of 10 ppm GA. The principal requirements for high biological activity were the same in both tests, as shown in Table 2. Methylcarbamoyl (**II–1**), cyclohexylcarbamoyl (**II–2**) or phenylcarbamoyl (**II–3**) isourea showed very low activities. If a methyl group was introduced into the *para*-position of the benzene ring in phenylcarbamoyl isourea (**II–6**), the biological activities were greatly enhanced, as previously described. However, the introduction of a methyl group into the *ortho* (**II–4**) or *meta* (**II–5**) position of a phenyl group markedly decreased the biological activity.

To discover the relationship between the length of an alkyl side chain at the *para*-position of a phenyl group and biological activity, we synthesized two additional *para*-substituted phenylcarbamoyl isoureas. *para*-Ethylphenylcarbamoyl isourea (**II–11**) was biologically as active as **II–6**. On the other hand, the biological activity completely disappeared when a *n*-propyl group (**II–12**) was introduced at the *para*-position of a phenyl group.

Table 1. Plant growth-promoting activities of 3-(*para*-methylphenylcarbamoyl)-1-alkoxycarbonylisourea derivatives

Compound no.	Compound tested* R_1	Compound tested* R_2	Concentration (ppm)	Activity: Growth-promoting activity†	Activity: Synergistic action with GA‡
I-1	Me–	Me–	1	0	+
			10	+	+ + +
			50	+ +	+ + +
I-2	Me–	C_2H_5–	1	0	0
			10	+	+ + + +
			50	+ +	+ + + + +
I-3	C_2H_5–	Me–	1	0	0
			10	+	+ + + +
			50	+ +	+ + + +
I-4	C_2H_5–	C_2H_5–	1	0	0
			10	+	+ + + +
			50	+ +	+ + + +
I-5	Me_2CH–	C_2H_5–	1	0	+
			10	+	+ +
			50	+ +	+ + + +
I-6	C_2H_5–	Me_2CH–	1	0	+
			10	+	+ + +
			50	+	+ + +
I-7	Me–	CH_2=CH–CH_2–	1	0	0
			10	+	+ + +
			50	+ +	+ + + +
I-8	CH_2=CH–CH_2–	Me–	1	0	0
			10	0	+ +
			50	+	+ + + +
I-9	CH≡C–CH_2–	C_2H_5–	1	0	+
			10	+	+ + +
			50	+	+ + + +
I-10	C_2H_5–	Me_2CH–CH_2–	1	0	0
			10	+	+ + +
			50	+	+ + +
I-11	Me_2CH–CH_2–	Me_2CH–CH_2–	1	0	0
			10	+	+
			50	+	+ + +
I-12	Me–		1	0	0
			10	0	0
			50	0	+
I-13	Me–		1	0	0
			10	0	0
			50	0	0

*



† Growth-promoting activity was measured as a percentage of control shoot length and expressed as follows:

% of control
<84 : –
85–115: 0
116–135: +
136–155: + +

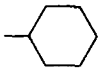
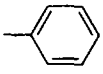
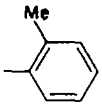
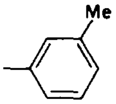
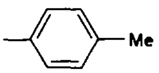
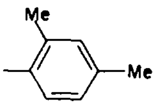
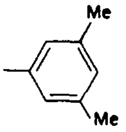
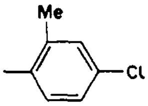
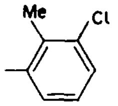
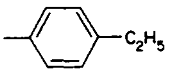
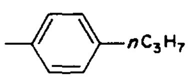
‡ Interaction with GA was expressed as follows:

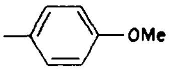
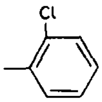
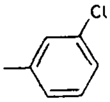
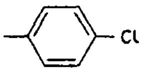
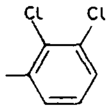
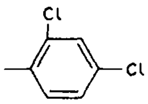
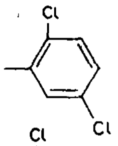
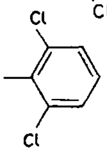
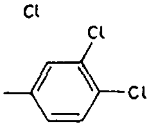
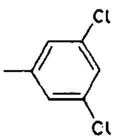
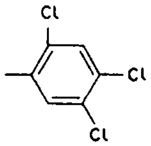
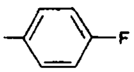
$$\frac{[\text{Elongation due to (10 ppm GA + test compound)}]}{[\text{Elongation due to 10 ppm GA}] + [\text{Elongation due to test compound}]} \times 100\,(\%)$$

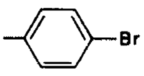
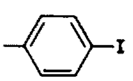
%
<84 : –
85–115: 0
116–135: +
136–155: + +
156–175: + + +
176–195: + + + +
>196 : + + + + +

Control shoot length: with GA, 42 mm; without GA, 24 mm.

Table 2. Plant growth-promoting activities of 3-phenyl(or alkyl)carbamoyl-2-ethyl-1-methoxycarbonylisoureas

Compound no.	Compound tested* R_3	Concentration (ppm)	Activity† Growth-promoting activity	Synergistic action with GA
II-1	—Me	1	0	0
		10	0	+
		50	+	++
II-2		1	0	0
		10	0	+
		50	0	+
II-3		1	0	0
		10	0	+
		50	0	++
II-4		1	0	0
		10	0	0
		50	+	+
II-5		1	0	0
		10	0	+
		50	0	++
II-6(I-2)		1	0	0
		10	+	++++
		50	++	+++++
II-7		1	0	+
		10	+	+++
		50	−	−
II-8		1	0	0
		10	0	+
		50	0	+
II-9		1	0	0
		10	+	+++
		50	++	+++
II-10		1	0	0
		10	0	0
		50	+	+
II-11		1	0	0
		10	0	+++
		50	+	++++
II-12		1	0	0
		10	0	0
		50	0	+

Compound no.	Compound tested* R₃	Concentration (ppm)	Activity† Growth-promoting activity	Activity† Synergistic action with GA
II-13		1	0	0
		10	0	+ + +
		50	+	+ + +
II-14		1	0	0
		10	0	+
		50	+	+ +
II-15		1	0	0
		10	0	0
		50	0	+
II-16		1	0	+
		10	+	+ + + +
		50	+ +	+ + + + +
II-17		1	0	0
		10	0	+
		50	+	+ +
II-18		1	0	0
		10	+ +	+ + +
		50	+ +	+ + +
II-19		1	0	0
		10	0	+
		50	0	+ +
II-20		1	0	0
		10	0	0
		50	0	+
II-21		1	0	0
		10	0	+ +
		50	0	−
II-22		1	0	0
		10	0	0
		50	0	0
II-23		1	0	0
		10	0	+
		50	0	+
II-24		1	0	0
		10	0	+ +
		50	+	+ + +

Compound no.	Compound tested* R_3	Concentration (ppm)	Activity† Growth-promoting activity	Synergistic action with GA
II-25		1	0	+
		10	0	++++
		50	+	++++
II-26		1	0	0
		10	+	++
		50	+	+++++

* $MeOOC{-}N{=}C(OC_2H_5){-}NHCONH{-}R_3$.
† For definition of symbols, see Table 1.

The introduction of a methoxy group (**II-13**) only slightly decreased the activity.

Isourea derivatives having halogen atom(s) on the benzene ring were also prepared and examined for their biological activities. If one chlorine atom was introduced into the *para*-position of a phenyl group (**II-16**), the activity was as high as that of 2-ethyl-1-methoxycarbonyl-3-(4-tolylcarbamoyl)isourea (**II-6**). On the other hand, the introduction of a chlorine atom into the *ortho* (**II-14**) or *meta* (**II-15**) position greatly decreased the biological activity. The same tendency was observed in isourea derivatives possessing an ethyl group as R_1 and a methyl group as R_2 (**III-1** to **III-4**, Table 3), and possessing a propargyl group as R_1 and an ethyl group as R_2 (**III-5** to **III-8**, Table 3). The above results show clearly that a chlorine atom, as well as a methyl or ethyl group, at the *para*-position on a benzene ring is most essential for the appearance of high biological activity.

Derivatives substituted with other halogen atoms in the *para*-position of a phenyl group (**II-24** to **II-26**) were also active, but the biological activity of phenylcarbamoyl isourea having a fluorine atom (**II-24**) was lower than that of 2-ethyl-1-methoxycarbonyl-3-(4-chlorophenylcarbamoyl)isourea (**II-16**).

As in the series of isourea derivatives possessing a *para*-methylphenyl group as R_3 (Table 1), derivatives possessing a *para*-chlorophenyl group as R_3, and various substituents as R_1 and R_2 were very active, if the number of carbon atoms in alkyl, alkenyl or alkynyl groups as R_1 and R_2 is within three (**IV-1** to **IV-9**, Table 4). The substitution of R_1 and/or R_2 with isobutyl group(s) (**IV-10** and **IV-11**) slightly decreased the biological activity. On the other hand, the biological activities of isourea derivatives with a benzyl or a substituted benzyl in the R_2 position (**IV-12** and **IV-13**) were almost zero.

As mentioned above, if only one halogen atom was introduced in the *para*-position of the phenyl group, the biological activities were very high. However, the additional introduction of halogen atom(s) resulted in the disappearance of the activity, except in the case of 2,4-dichlorophenylcarbamoyl isourea (**II-18**). Both 2,4-dichlorophenyl (**II-18**) and 2-methyl-4-chlorophenyl (**II-9**) carbamoyl isoureas showed high activity, though their biological activities were lower than that of 2-ethyl-1-methoxycarbonyl-3- (4-chlorophenylcarbamoyl)isourea (**II-16**). 2,4-Dimethylphenylcarbamoyl isourea (**II-7**) also enhanced the GA action at the concentration of 1–10 ppm, although it inhibited growth and reduced the GA action above 50 ppm.

The results described suggest that the position of substituent(s) on the benzene ring of phenylcarbamoyl isourea plays an important part in determining the biological activity. Phenylcarbamoyl isoureas with an appropriate substituent at the *para*-position of a phenyl group were found to show high activities, if the number of carbon atoms of alkyl, alkenyl or alkynyl groups in R_1 and R_2 positions was three or less.

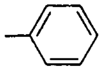
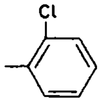
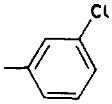
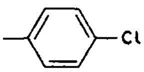
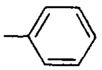
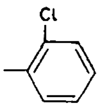
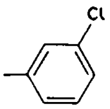
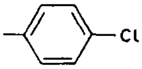
More detailed investigations on the roles of substituents on the benzene ring and on the modification of the carbamoylisourea skeleton are necessary to draw a definite conclusion about the structure–activity relationships of isourea derivatives.

EXPERIMENTAL

Preparation of the compounds. All compounds used in this work were prepared according to the method as previously reported [2]. Their purities and structures were checked by TLC, IR and NMR spectra.

Plant material and bioassay. Biological activities of the compounds on the growth of rice (*Oryza sativa* L. var. Kinmaze) seedlings were assayed according to the method as previously reported [1]. Sterilized rice seeds were soaked in H_2O for 2 days at 28–30°. Germinated seeds with uniform-sized coleoptiles were planted on 10 ml 0.5% agar medium containing test compounds in a glass vessel (2.6 cm dia. × 6 cm height), 5 seeds/vessel. For the test of activity to enhance the stimulative action of GA, a definite amount of test compound was applied in combination with 10 ppm GA. Seedlings were allowed to grow at 28–30° under continuous illumination (4500 lx of fluorescent light). Shoot length was measured after 4 days of growth.

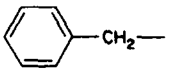
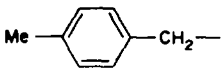
Table 3. Plant growth-promoting activities of 1-alkoxycarbonyl-2-alkyl-3-phenylcarbamoylisoureas

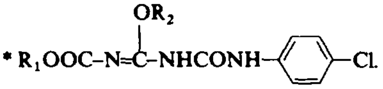
Compound no.	Compound tested* R_1	R_2	R_3	Concentration (ppm)	Activity† Growth-promoting activity	Synergistic action with GA
III-1	C_2H_5–	Me–		1 10 50	0 0 0	0 0 +
III-2	C_2H_5–	Me–		1 10 50	0 0 +	0 + ++
III-3	C_2H_5–	Me–		1 10 50	0 0 0	0 0 +
III-4	C_2H_5–	Me–		1 10 50	0 + ++	0 ++++ +++++
III-5	CH≡C–CH_2–	C_2H_5–		1 10 50	0 0 0	0 0 +
III-6	CH≡C–CH_2–	C_2H_5–		1 10 50	0 0 +	0 + ++
III-7	CH≡C–CH_2–	C_2H_5–		1 10 50	0 0 0	0 0 0
III-8	CH≡C–CH_2–	C_2H_5–		1 10 50	0 + ++	+ ++++ +++++

* $R_1OOC\text{-}N{=}C(OR_2)\text{-}NHCONH\text{-}R_3$.
† For definition of symbols, see Table 1.

Table 4. Plant growth-promoting activities of 3-(*para*-chlorophenylcarbamoyl)-1-alkoxycarbonylisourea derivatives

Compound no.	Compound tested* R_1	R_2	Concentration (ppm)	Activity† Growth-promoting activity	Synergistic action with GA
IV-1	Me–	Me–	1 10 50	0 + ++	+ ++ ++++
IV-2	Me–	C_2H_5–	1 10 50	0 + ++	+ ++++ +++++

Compound no.	Compound tested* R$_1$	Compound tested* R$_2$	Concentration (ppm)	Activity† Growth-promoting activity	Activity† Synergistic action with GA
IV-3(III-4)	C_2H_5-	Me-	1	0	0
			10	+	+ + + +
			50	+ +	+ + + + +
IV-4	C_2H_5-	C_2H_5-	1	0	0
			10	+	+ + + +
			50	+ +	+ + + +
IV-5	$(Me)_2CH$-	C_2H_5-	1	0	+
			10	+	+ + +
			50	+ +	+ + + + +
IV-6	C_2H_5-	$(Me)_2CH$-	1	0	0
			10	+	+ +
			50	+ +	+ + + +
IV-7	Me-	CH_2=CH-CH_2-	1	0	0
			10	+	+ +
			50	+	+ + + +
IV-8	CH_2=CH-CH_2-	Me-	1	0	+
			10	+	+ + + +
			50	+ +	+ + + +
IV-9(III-8)	CH≡C-CH_2-	C_2H_5-	1	0	0
			10	+	+ + +
			50	+ +	+ + + +
IV-10	C_2H_5-	$(Me)_2CH$-CH_2-	1	0	0
			10	0	+ +
			50	+	+ + + +
IV-11	$(Me)_2CH$-CH_2-	$(Me)_2CH$-CH_2-	1	0	0
			10	+	+
			50	+	+ + +
IV-12	Me-		1	+	0
			10	0	0
			50	0	0
IV-13	Me-		1	0	0
			10	0	0
			50	0	0



† For definition of symbols, see Table 1.

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