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# Plant Growth-promototing Activities of Mercaptriazinone Derivatives*

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Twenty-nine mercaptotriazinone derivatives were synthesized and their plant growth-promoting activities were examined by the rice (*Oryza sativa*) seedling test in the presence or absence of gibberellic acid ($GA_3$). For high activity in promoting the $GA_3$-induced shoot elongation, an isopropyl or an appropriately substituted phenyl group, a hydrogen atom and a lower alkyl thio group were required in the 1-, 3- and 4-positions, respectively, of the 1,3,5-triazine-2,6-dione structure. In more detailed experiments, 4-methylthio-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione, one of the most potent mercaptotriazinones, was found to synergistically promote the $GA_3$-induced elongation of the first and second leaves of rice seedlings. Several mercaptotriazinone derivatives, active or inactive, in the rice seedling test were examined by the radish (*Raphanus sativus*) leaf disk expansion test, but all of them were completely inactive. Structure-activity relationships of mercaptotriazinone derivatives are discussed in relation to those of the corresponding alkoxytriazinone derivatives.



A series of alkoxycarbonylisourea derivatives (Fig. 1A) were reported to act synergistically with gibberellic acid ($GA_3$) in the shoot elongation of rice (*Oryza sativa* L.) seedlings.[1,2] Alkoxytriazinone derivatives (Fig. 1B) chemically synthesized by intramolecular cyclization of alkoxycarbonylisoureas were also active in synergistically enhancing the effect of $GA_3$, and thought to be the active form of alkoxycarbonylisoureas in rice seedlings.[3,4] 4-Ethoxy-1-(*p*-tolyl)-*s*-triazine-2,6(1H,3H)-dione, one of the most potent alkoxytriazinones, has been investigated at the ultrastructural and biochemical levels in rice seedlings.[5,6] These studies led us to speculate that $GA_3$-synergists might be useful tools for studies on the role of $GA_3$ in plant growth and development.

In the present paper, we synthesized a number of mercaptotriazinone derivatives (Fig. 1C) and assayed them for activity in promoting the growth of rice seedlings in the presence or absence of $GA_3$. In addition some of them were assayed in the radish leaf disk expansion test. The results in the rice seedling and radish leaf tests were compared with those of alkoxytriazinone derivatives, focusing on the structure-activity relationships.

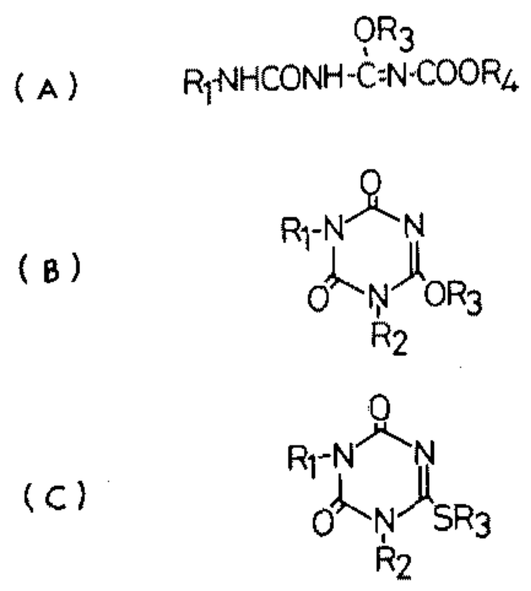


FIG. 1. Chemical Structure of Isourea and Its Cyclic Derivatives.

## MATERIALS AND METHODS

*Plant materials.* Seeds of rice (*Oryza sativa* L. var. Kinmaze) were germinated by soaking in water for 2 days at 28~30°C. Unless otherwise stated, 5 germinated seeds with uniform-sized coleoptiles were plant-

* This paper is Part 8 in the series "Plant growth-regulating activities of isourea derivatives and related compounds." For Part 7, see ref. 6.

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Abbreviation: $GA_3$, gibberellic acid.

ed on 10 ml of 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 grown at 28~30°C under continuous fluorescent illumination of 4500 lux. They were harvested at a given period of growth and their lengths of coleoptiles, first leaves or second leaves were measured.

Radish (*Raphanus sativus* L. var. *acathiformis* Makino Risodaikon) was grown in a green house under natural light conditions for 2~3 weeks. When the area of first leaves reached 0.4~0.5 $cm^2$, the seedlings were transferred to the dark room and kept for about 24 hr at room temperature to increase their sensitivity to applied chemicals. Thereafter, a disk, 5 mm in diameter, was punched out from each first leaf. The effect of compaunds on the expansion of excised leaf disks was examined by floating 15 leaf disks on 10 ml incubation solution in a Petri dish (5.5 cm in diameter). The solution contained 64 $\mu$mol $Ca(NO_3)_2 \cdot 4H_2O$, 34 $\mu$mol KCl, 10 $\mu$mol $MgSO_4 \cdot 7H_2O$, 3.7 $\mu$mol $KH_2PO_4$, 584 $\mu$mol sucrose and a test compound at appropriate concentrations. The upper surface of leaf disks was handled with care not so as to be covered with water. They were incubated at 28~30°C under fluorescent light of 4500 lux. After 18 hr the leaf disks were lightly blotted with a sheet of filter paper and its fresh weight was measured.[7]

*Chemicals.* Twenty-nine mercaptotriazinone derivatives were synthesized according to the procedure outlined in Fig. 2. Their purities were checked by silica gel TLC and their structures were confirmed by elemental analyses and if necessary IR and NMR spectra. The uncorrected melting points of each compound are shown in Fig. 3. Examples illustrating the preparation of typical mercaptotriazinones, 4-methylthio-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione derivatives are hereinafter described.

a) A mixture of 3.1 g (0.021 mol) of ethyl 3-thioallophanate[8] and 4.25 g (0.03 mol) of methyl iodide in 50 ml of acetone was stirred with 2.0 g of $K_2CO_3$ at room temperature for about 8 hr. After removal of insoluble substances by filtration, 2.66 g (0.02 mol) of *p*-tolyl isocyanate was added to the filtrate and the mixture was refluxed for 30 min. The solvent was then removed by evaporation under reduced pressure. Recrystallization of the residue from methanol gave 4.8 g (82%) of 1-ethoxycarbonyl-2-methyl-3-(*p*-tolylcarbamoyl)isothiourea (**I**) as colorless prisms, mp. 128~130°C, IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1735 (C=O), 1640 (CONH), NMR ($CDCl_3$) $\delta$ ($J$=Hz): 1.28 (3H, t, $J$=7.0), 2.30 (3H, s), 2.40 (3H, s), 4.20 (2H, g, $J$=7.0), 7.0~7.5 (4H, arom.).

b) 2.94 g of **I** in 20 ml of methanol was refluxed with 0.6 g (0.011 mol) of $CH_3ONa$ (anhydrous powder) for about 1 hr. After removal of the solvent, the residue was dissolved in 20 ml of water (insoluble substances were removed by filtration). After cooling, the solution was neutralized by adding dropwise 5 N HCl. The resulting crystalline solid was separated by filtration and dried. Recrystallization from methanol gave 1.6 g (64%) of 4-methylthio-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione (**17**) as colorless prisms, mp. 275~276°C (decomp.), IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1740, 1660 (C=O), NMR (DMSO-$d_6$) $\delta$: 2.30 (3H, s), 2.45 (3H, s), 6.9~7.2 (4H, arom.). *Anal.* Calcd. for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86; S, 12.86. Found: C, 53.15; H, 4.34; N, 16.85; S, 12.72%.

c) A mixture of 1.25 g (0.005 mol) of **17** and 1.06 g (0.0075 mol) of methyl iodide in 20 ml of acetone was stirred with 1.0 g of $K_2CO_3$ at room temperature for about 5 hr. After adding 10 ml of water, the mixture was extracted with 30 ml of chloroform. The chloroform solution was washed with water and dried over anhyd. $Na_2SO_4$. After removal of the solvent, the residue was recrystallized from methanol, giving 0.85 g (65%) of 3-methyl-4-methylthio-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione (**28**) as colorless needles, mp. 242~244°C, IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1740, 1680 (C=O), NMR ($CDCl_3$) $\delta$: 2.40 (3H, s), 2.60 (3H, s), 3.50 (3H, s), 7.1~7.4 (4H, arom.). *Anal.* Calcd. for $C_{12}H_{13}N_3O_2S$: C, 54.74; H, 4.98; N, 15.96; S, 12.18. Found: C, 54.77; H, 4.99; N, 15.89; S, 12.12%.

Alkoxytriazinone derivatives were synthesized according to the method previously reported.[3] $GA_3$ was a generous gift from Kyowa Hakko Co., Ltd. (Tokyo, Japan). Kinetin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

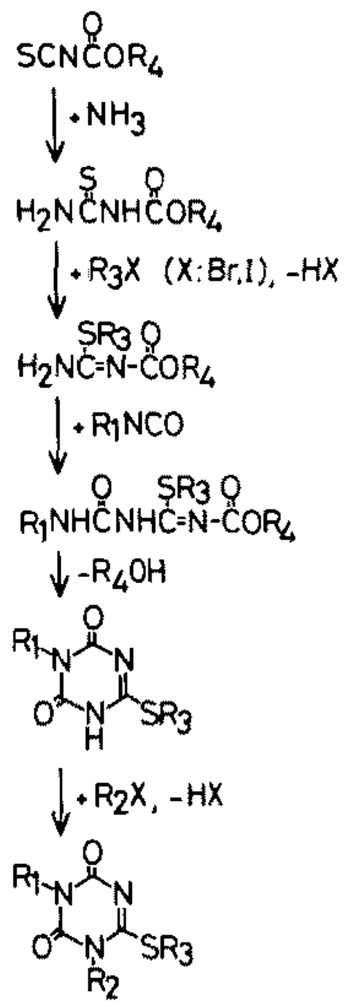



FIG. 2. Synthetic Flow Sheet of Mercaptotriazinone Derivatives.

## RESULTS AND DISCUSSION

*Structure-activity relationships*

Twenty-nine mercaptotriazinone derivatives were synthesized and their plant growth-promoting activities were examined in the concentrations of 1, 10 and 50 mg/liter by the rice seedling test in the presence or absence of 10 mg/liter $GA_3$. The evaluation was conducted by testing their abilities to promote the growth of shoots of rice seedlings and to enhance the $GA_3$-induced shoot elongation of rice seedlings. As shown in Fig. 3a ~ 3c, large variations in activity resulted from changes in the substituents $R_1$, $R_2$ and $R_3$ in the structure. The structural requirements for the growth-promoting activity in the absence of $GA_3$ and for the synergistic activity with $GA_3$ in the presence of $GA_3$ were almost the same.

As to $R_1$ in the 4-methylthio-*s*-triazine-2,6-(1H,3H)-dione series (compounds **1** ~ **21**, $R_2$ = H, $R_3$ = $CH_3$), high $GA_3$-synergist activity was obtained when $R_1$ was an isopropyl group (**4**) or an appropriately substituted phenyl group. Derivatives having a methyl (**1**), an ethyl (**2**) or a phenyl (**7**) group were weakly active, while those having a *n*-propyl (**3**), a *n*-butyl (**5**) or a cyclohexyl (**6**) group had no activity. The substitution with a chlorine atom (**10**) or a methyl group (**17**) at the 4-position of the benzene ring markedly increased the biological activity. The substitution at the 2- or 3-position of the ring with a chlorine atom (**8** and **9**) or a methyl group (**15** and **16**) caused

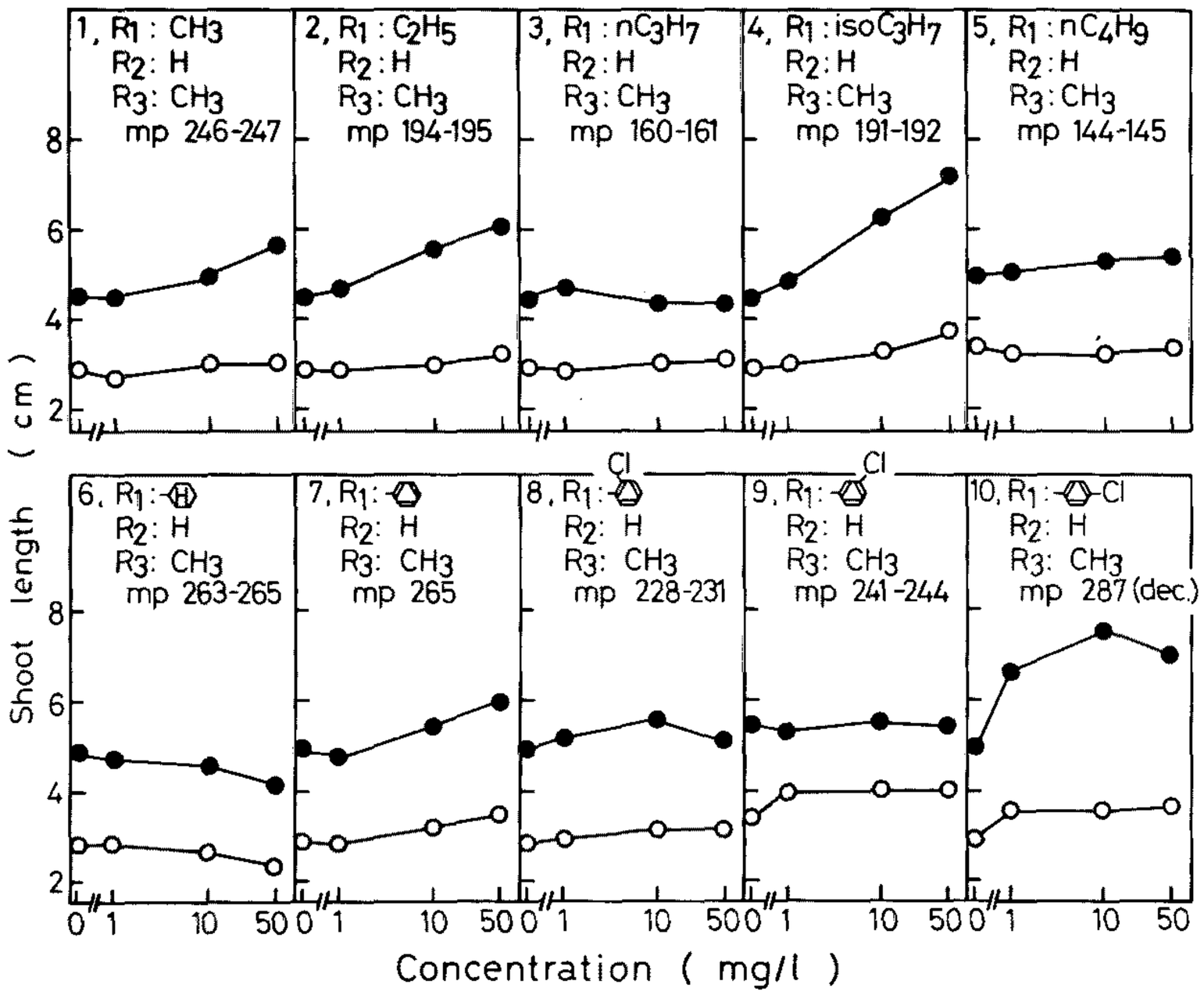
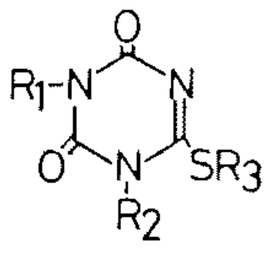



FIG. 3a

FIG. 3. Plant Growth-promoting Activities of Mercaptotriazinone Derivatives.

General structure;




○—○, without $GA_3$; ●—●, with $GA_3$ (10 mg/liter).
Each point represents the average of three to four replications; the standard errors were smaller than the symbols.

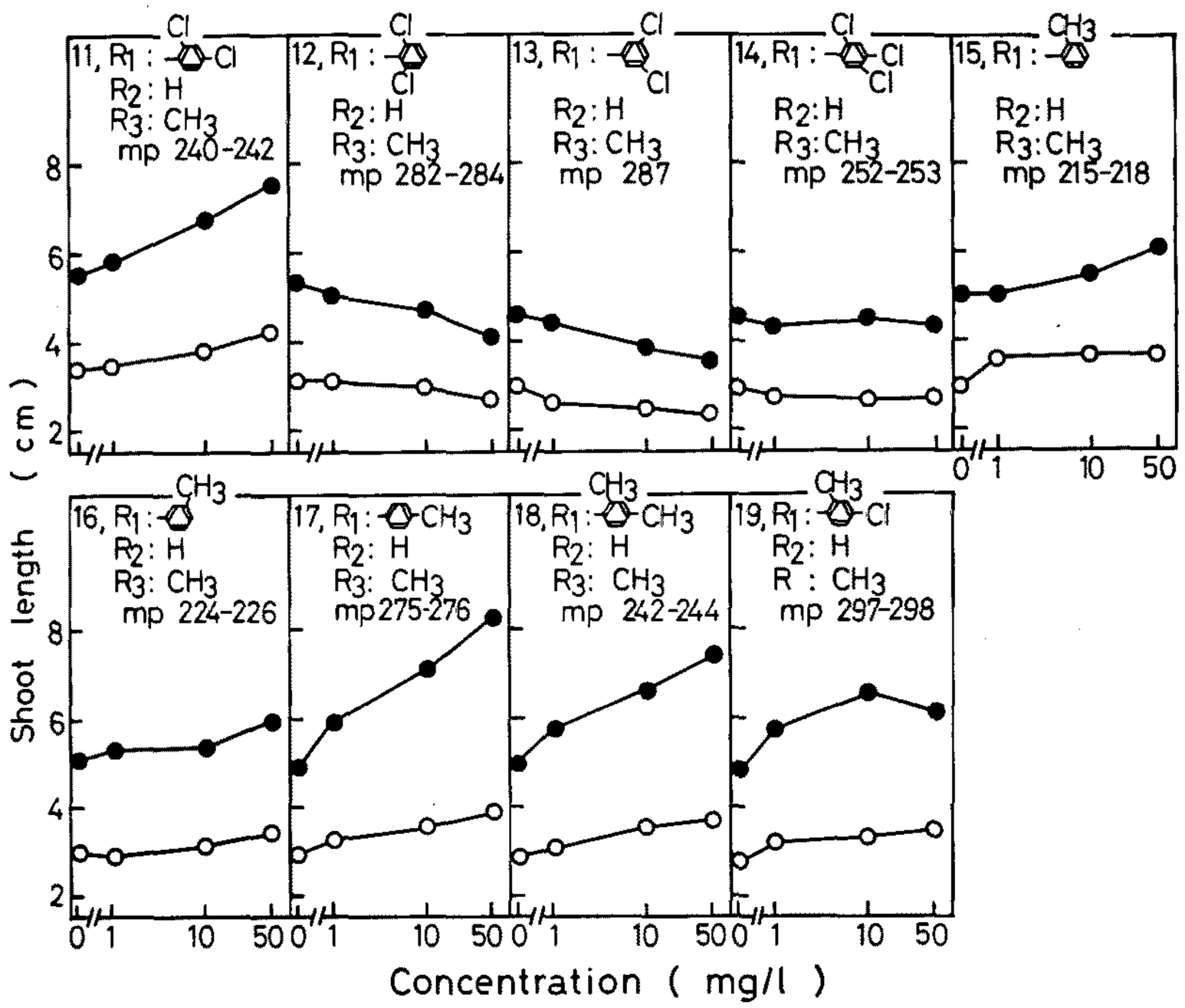



FIG. 3b

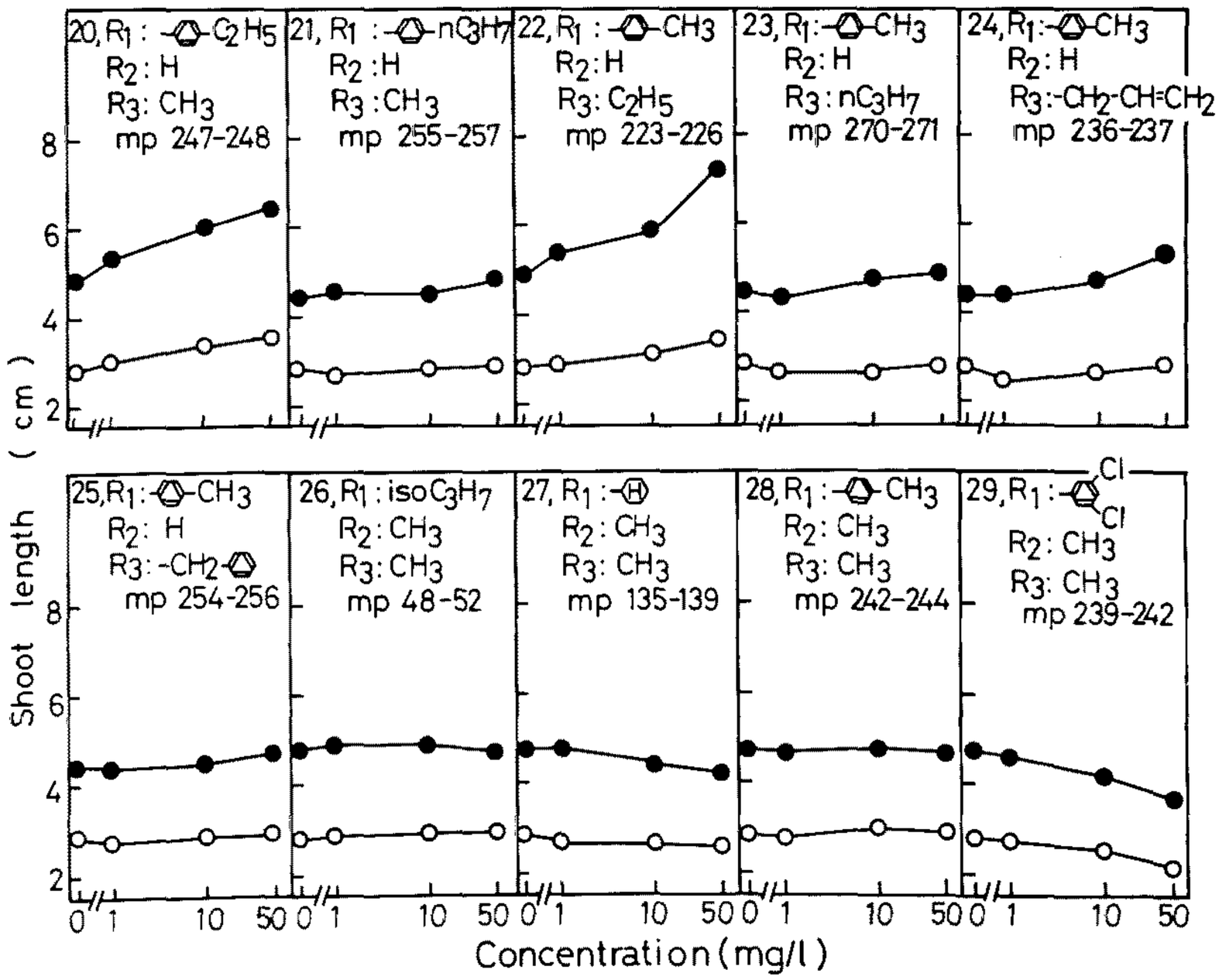



FIG. 3c

loss of activity. Of the di- or tri-substituted compounds, 2,4-dichloro (**11**), 2,4-dimethyl (**18**) and 4-chloro-2-methyl (**19**) phenyl derivatives were active, but other changes in the ring abolished the biological activity. As the number of carbon atoms in an alkyl group at the 4-position of the ring increased (**17**, **20** and **21**), the activity decreased. When a *n*-propyl group (**21**) was introduced the activity completely disappeared.

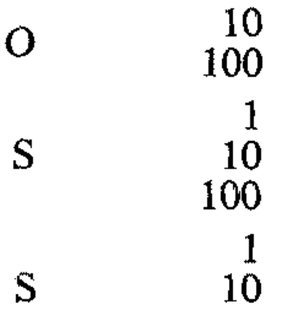
A hydrogen atom as $R_2$ appeared to be a critical requirement for the activity. The introduction of a methyl group (**26** and **28**) nearly eliminated the activity, while the unsubstituted corresponding compounds **4** and **17** demonstrated the high activity. Other two derivatives possessing a methyl group as $R_2$ (**27** and **29**) were also inactive.

As to $R_3$ the size of the substituent was critical in a series of derivatives possessing a 4-methylphenyl as $R_1$ and a hydrogen atom as $R_2$. The activity decreased with increase in chain length of $R_3$. The high activity in this series of derivatives was demonstrated by a methyl (**17**) or an ethyl (**22**) group. The former was slightly more active than the latter.

TABLE I. PLANT GROWTH-PROMOTING ACTIVITY OF ALKOXYTRIAZINONE AND MERCAPTOTRIAZINONE DERIVATIVES IN EXPANDING RADISH LEAF DISKS

| Compounds | | | | Conc. (mg/liter) | Activity | |
|---|---|---|---|---|---|---|
| $R_1$ | $R_2$ | $R_3$ | X | | Increase in fresh wt.of 15 disks (mg) | (Treated) minus (Control) (mg) |
| 4-Methylphenyl | H | Methyl | O | 1 | 35 | 1 |
| | | | | 10 | 41 | 7 |
| | | | | 100 | 48 | 14 |
| 4-Methylphenyl | H | Ethyl | O | 1 | 38 | 4 |
| | | | | 10 | 44 | 10 |
| | | | | 100 | 48 | 14 |
| 4-Methylphenyl | H | *n*-Propyl | O | 1 | 38 | 4 |
| | | | | 10 | 44 | 10 |
| | | | | 100 | 40 | 6 |
| 4-Methylphenyl | H | *n*-Butyl | O | 10 | 38 | 4 |
| | | | | 100 | 32 | −2 |
| 4-Methylphenyl | Methyl | Ethyl | O | 10 | 37 | 3 |
| | | | | 100 | 38 | 4 |
| Isopropyl | H | Methyl | S | 1 | 33 | −1 |
| | | | | 10 | 32 | −2 |
| | | | | 100 | 32 | −2 |
| 4-Methylphenyl | H | Methyl | S | 1 | 32 | −1 |
| | | | | 10 | 32 | −1 |
| | | | | 100 | 31 | −3 |
| 4-Methylphenyl | H | Ethyl | S | 1 | 31 | −3 |
| | | | | 10 | 32 | −2 |
| | | | | 100 | 25 | −9 |
| 4-Methylphenyl | H | *n*-Propyl | S | 1 | 34 | 0 |
| | | | | 10 | 30 | −4 |
| | | | | 100 | 28 | −6 |
| 4-Methylphenyl | Methyl | Methyl | S | 1 | 30 | −4 |
| | | | | 10 | 30 | −4 |
| | | | | 100 | 31 | −3 |
| $GA_3$ | | | | 1 | 51 | 17 |
| Kinetin | | | | 10 | 47 | 13 |
| Control | | | | | 34 | 0 |

General formula,




Each value represents the average of three replications. Initial fresh wt. of 15 disks before incubation was 63.0 mg.

Other three substituents, *n*-propyl (**23**), allyl (**24**) and benzyl (**25**) groups destroyed the activity. It was impossible to synthesize a compound possessing a hydrogen atom as $R_3$, although the magnitude of its activity was particularly interesting.

The results demonstrate that for high biological activity, a 4- or 2,4-substituted phenyl group or an isopropyl group as $R_1$, a hydrogen atom as $R_2$ and a methyl or an ethyl group as $R_3$ were required in the mercaptotriazinone molecule. It is clear from our present and previous results[4] that the structure-activity relationships for mercaptotriazinone derivatives are correlated well with those of alkoxytriazinones in the rice seedling test.

Several mercaptotriazinone derivatives were assayed for another growth-promoting activity using the radish leaf disk test (Table I). Five derivatives examined, however, were inactive. It is noted that among the corresponding alkoxytriazinones, three derivatives showing high activity in the rice seedling test,[4] obviously promoted the expansion of radish leaf disks in the concentrations of 10 and 100 mg/liter. $GA_3$ and kinetin were active in this test, as previously reported by Kuraishi.[7]

The alkoxytriazinone derivatives showing the promotive effect on the expansion of radish leaf disks, however, did not exhibit any synergistic activity with $GA_3$ or kinetin (data not shown).

The results suggest the response patterns of a series of alkoxytriazinone derivatives in both the rice seedling and radish leaf disk tests were the same, while mercaptotriazinone derivatives whose response pattern was applied well to that of alkoxytriazinones in the rice seedling test, were quite inactive in the radish leaf disk test. The replacement of an $-OR_3$ group by an $-SR_3$ group at the 4-position of a triazinone skeleton completely diminished the activity in promoting expansion of radish leaf disks.

*Effect of 4-methylthio-1-(p-tolyl)-s-triazine-2,6-(1H, 3H)-dione on growth of rice seedlings*

The growth-promoting activity of 4-methylthio-1-(*p*-tolyl)-*s*-triazine-2, 6(1H, 3H)-dione (**17**), which was highly active in the rice seedling test, was investigated in more detail with regard to the growth of rice seedlings. The growth for a six-day period in the presence of a mercaptotriazinone and $GA_3$ is shown in Fig. 4. A mercaptotriazinone alone slightly increased the growth of coleoptiles, first leaves and second leaves as compared to that of the control. $GA_3$ also increased the growth of first and second leaves, and the elongation of second leaves induced by $GA_3$ was significantly greater than that by a mercaptotriazinone. The combination of a mercaptotriazinone with

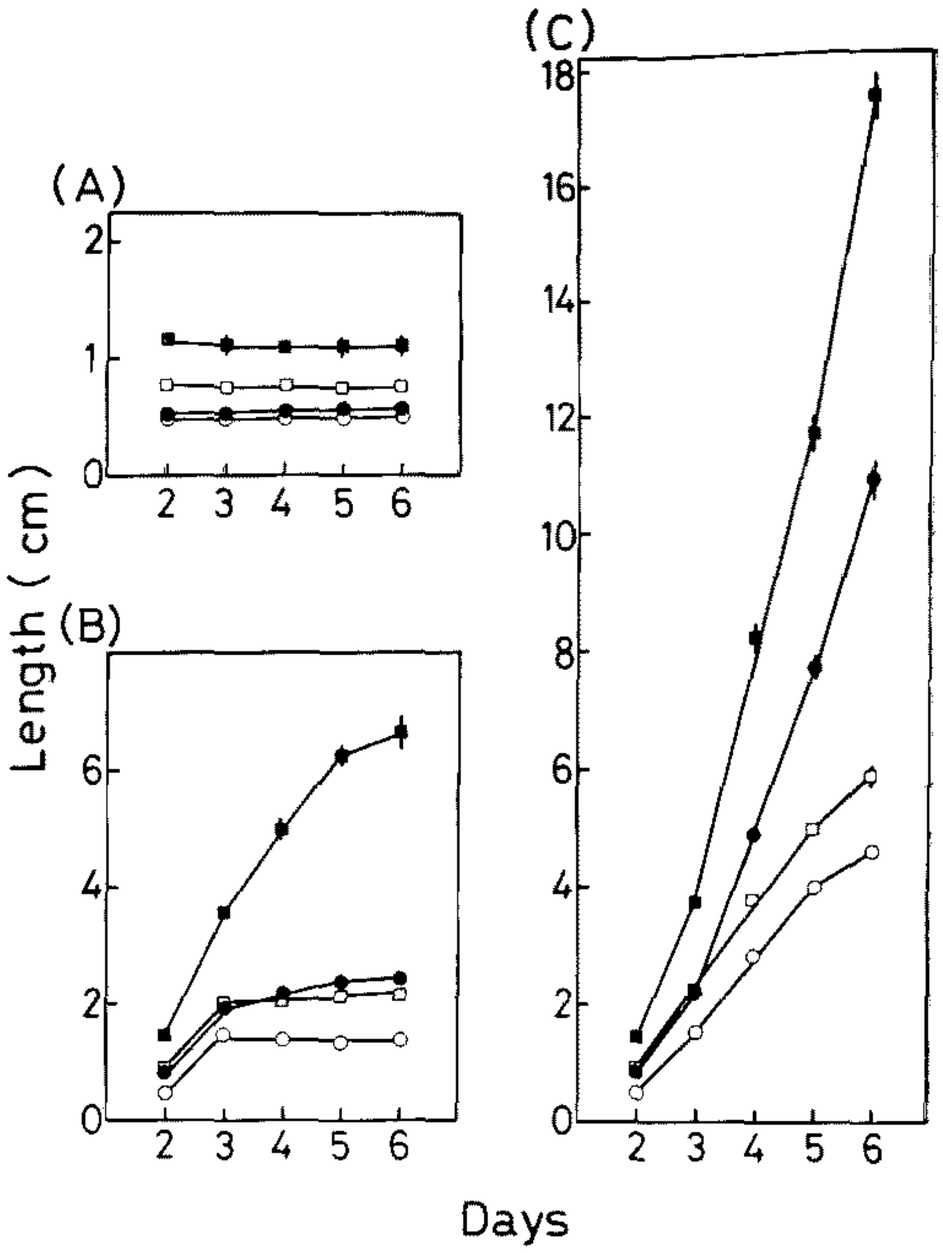



FIG. 4. Effect of 4-Methylthio-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione (MT) on Growth of Rice Seedlings in the Presence or Absence of $GA_3$.

Twenty-five germinated seeds were planted on 35 ml of 0.5% agar medium containing test compound(s) in a Petri dish (5.5 cm in diameter). Rice seedlings were incubated under the same conditions as those described in MATERIALS AND METHODS.

A, coleoptiles; B, first leaves; C, second leaves. ○—○, control; □—□, MT 50 mg/liter; ●—●, $GA_3$ 10 mg/liter; ■—■, MT 50 mg/liter plus $GA_3$ 10 mg/liter. Each point represents the average of three replications. Standard errors were given as vertical bars; where no bars appear, the standard errors were smaller than the symbols.

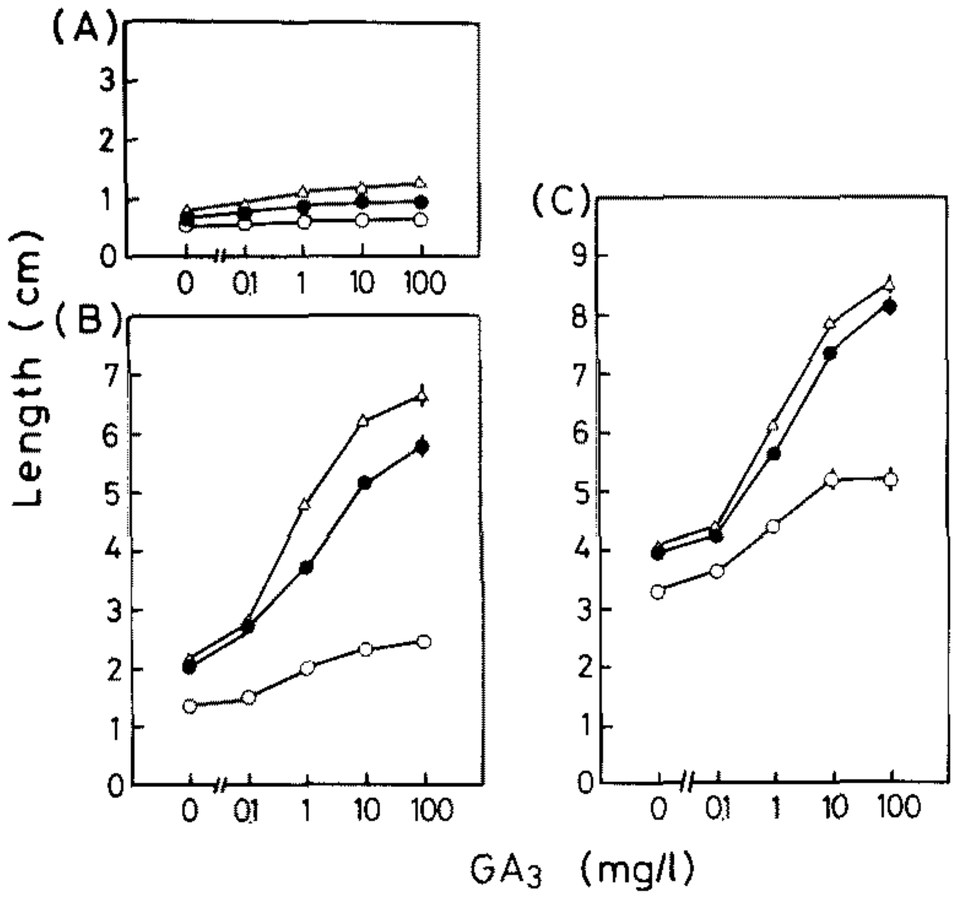



FIG. 5. Effect of 4-Methylthio-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione (MT) on $GA_3$-induced Elongation of Rice Seedlings.

A, coleoptiles; B, first leaves; C, second leaves. ○—○, control; ●—●, MT 25 mg/liter; △—△, MT 50 mg/liter.

Rice seedlings were grown for 4 days in the presence or absence of MT together with various concentrations of $GA_3$ under the same conditions as those described in the legend of Fig. 4. Each value represents the average±standard error of three replications as in Fig. 4.

$GA_3$ showed a distinct synergistic effect on each part of rice seedlings. Especially the dramatic synergism was observed in the first and second leaves from 3 to 6 days after planting.

The effects of various concentrations of $GA_3$ in the presence or absence of a mercaptotriazinone on the growth of rice seedlings are shown in Fig. 5. The $GA_3$-induced elongation of first and second leaves was synergistically increased by a mercaptotriazinone at 25 and 50 mg/liter. $GA_3$ did not induce the growth of coleoptiles, but in combination with a mercaptotriazinone slightly increased the growth.

From the present and previous works,[4] the ability of 4-methylthio-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione to synergistically enhance the action of $GA_3$ was found to be slightly less than that of the corresponding alkoxytriazinone, 4-methoxy-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione.

Further studies on the syntheses of the derivatives as well as the examination of their physiological properties will bring about new potent $GA_3$-synergists which are useful tools for practical purposes and for studies of the mechanism of $GA_3$ action.

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