Original Article

Structural Characteristics of *s*-Triazine Compounds Inducing Rhizome in *Cyperus serotinus* ROTTB.*

Hiro Yoshi OMOKAWA, Hitoshi KURAMOCHI, Yoshinori KAWATA
and Makoto KONNAI

Weed Science Center, Utsunomiya University, Mine, Utsunomiya 321, Japan

(Received May 17, 1993; Accepted October 13, 1993)

Rhizome induction activities of the *s*-triazines and synthetic cytokinins were measured on *Cyperus serotinus* ROTTB. tuber in the dark. The (*R*)-enantiomers of 2- α -methylbenzylamino-6-chloro-*s*-triazine compounds having a small alkyl group at 4-amino moiety stimulated the rhizome induction. Medium sized or long-chain alkylamino groups (butyls to decyl) resulted in decrease in activity. Their (*S*)-antipodes did not cause the rhizome induction. The racemates substituted with an ethyl or propyl group at the benzylic position of the 2-benzylamino-4-methyl (or ethyl) amino-6-chloro-*s*-triazines also exhibited a significant rhizome inducing activity. The activity of the methoxy or methylthio derivatives of corresponding chloro-*s*-triazines that was active on rhizome induction was maintained. An *s*-triazine ring system was essential for the rhizome induction. Kinetin and synthetic cytokinin compounds, forchlorfenuron [1-(2-chloro-4-pyridyl)-3-phenylurea] and thidiazuron [1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea], significantly stimulated rhizome induction similar to 6-benzylaminopurine (BA).

INTRODUCTION

Optically active 2- α -methylbenzylamino-*s*-triazines show diverse physiological properties on plants: the (*S*)-isomers are potent inhibitors against photosystem II (PS II) electron transport¹⁾ and the (*R*)-isomers are root growth inhibitors in the dark.²⁾ Racemates of them exhibit potent herbicidal activities to barnyard grass (*Echinochloa oryzicola* VASING.) in paddy conditions with double symptoms (growth inhibition and leaf burning).³⁾ *Cyperus serotinus* ROTTB. is a important weed in paddy fields. In developing seedlings, secondary rhizomes generate physiologically from the base of shoots at the 3–4 leaf stage. In the previous paper, we reported that the secondary rhizome induction was stimulated on treatment with 6-benzylaminopurine (BA), and the

stimulation to the secondary rhizome was also found by treatment with 2-(*R*)- α -methylbenzylamino-4-methyl (or ethyl) amino-6-chloro-*s*-triazine (**2**, **3**). On the other hand, their (*S*)-enantiomers was found to be active inhibitors on PS II.⁴⁾ The *s*-triazine herbicides have many kinds of physiological properties to plants. Cytokinin-like growth-promoting activities of *s*-triazine herbicides such as ametryn, atrazine and simazine have been reported on seed germination, protein synthesis, callus growth and chlorophyll retention by many researchers.^{5–7)} The objective of our experiment reported here was to elucidate the structural properties of *s*-triazine compounds and to determine the effects of kinetin and synthetic cytokinins {forchlorfenuron [1-(2-chloro-4-pyridyl)-3-phenylurea] and thidiazuron [1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea]} on secondary rhizome induction in *Cyperus serotinus* ROTTB.

* Interaction between Optical Isomerism and Plant Pharmacological Action (Part 5). For Part 4, see Ref. 15).

MATERIALS AND METHODS

1. Chemicals

The synthetic cytokinins, forchlorfenuron and thidiazuron, were donated by Tomono Nohyaku Co., Ltd. and Kyowa Hakko Kogyo Co., Ltd., respectively. Kinetin and 6-benzylaminopurine (BA) were purchased from Wako Pure Chemical Industries, Ltd. The *s*-triazine compounds were synthesized in our laboratory and their physical properties have already been communicated.^{1-3,8-10)} Kinetin and BA were dissolved in minimum quantity of 0.1 N HCl, and then diluted with 50 ppm Tween-80 solution. The test solutions of forchlorfenuron and thidiazuron were prepared from 10% wettable powder of them by dilution with 50 ppm Tween-80 solution. The test solutions of the *s*-triazines were prepared from their stock solutions (0.01 M in EtOH) by dilution with 50 ppm Tween-80 solution.

2. Assay of Rhizome Induction

The rhizome induction assay was carried out by methods similar to those described in the previous paper with some modifications.⁴⁾ Tubers collected from paddy fields in early winter were stored in a cold room (5°C) before they were used. They were selected, washed with water to remove the soil, and then sterilized with 5% sodium hypochlorite by immersing the lot for 5 min in the solution. Tubers were rinsed in running tap water to remove the excess sterilant, and pre-incubated at 30°C for 1 day. Tubers were soaked in 50 ppm Tween-80 solution with or without (control) the test compounds at 30°C for 24 hr in the dark. Five tubers each for treatment were placed in a tall Petri dish (10 cm in diameter) filled with vermiculite (80 ml), containing 50 ml of distilled water. The Petri dishes were placed in a growth chamber at 25°C in the dark. The number and length of shoots and secondary rhizomes were counted after 21 days of incubation. The rhizome induction activity of the test compounds was evaluated by assigning two numbers: secondary rhizomes per tuber and mean length of rhizomes. All experiments were replicated three times.

RESULTS

In the previous paper,⁴⁾ a specific stimulation of secondary rhizome induction in *Cyperus serotinus* ROTTB. was reported on a continuous treatment with BA among the plant hormones and with 2-(*R*)- α -methylbenzylamino-4-methyl (or ethyl) amino-6-chloro-*s*-triazine (**2**, **3**). Similar as the continuous soaking assay, typical symptoms of cytokinins (secondary rhizome induction, roots growth inhibition, a decrease of shoot growth and increase of plantlets number) were exhibited on the plantlets generating from the pre-soaking tubers in the above chemical solutions. In the control treatment (in 50 ppm Tween-80 solution), the *Cyperus* tubers germinated and grew normally, and only one long apical shoot was formed as usual. A short secondary rhizome (2.2 mm in the mean length) was introduced at the basal part of the shoots for 21 days incubation in 9.6% of the tubers in the controls. However, extended incubation periods as reported in the previous paper⁴⁾ or use of the physiologically old tubers which were stored at 5°C for more than 6 months resulted in a progressive generation of the secondary rhizome within the incubation periods.

Effect of alkyl substitution at the 4-amino moiety of the optically active 2-(*R*)- α -methylbenzylamino-6-chloro-*s*-triazine compounds was shown in Table 1. The *s*-triazine compounds having small alkylamino groups such as methyl- (**2**) and ethylamino (**3**) groups significantly stimulated the rhizome induction as reported previously,⁴⁾ the methyl derivative (**2**) showed the most potent activity. The activities of the amino (**1**) and propylamino (**4**) derivatives were weak, and the isopropylamino compound (**5**) was inactive. An introduction of sterically bulky or long-chain alkyl groups (from butyls to decyl) produced a large decrease in the activity. There was no significant difference in the growth with controls. All of the corresponding antipodes ((*S*)- α -methylbenzyl derivatives) were inactive (data not shown).

No antagonistic action of the (*S*)-enantiomers was shown in this study either as previously⁴⁾: they did not interfere the rhizome induction agitated by the antipodal (*R*)-

Table 1 Effect of the substituents at the 4-amino group of 2-(*R*)- α -methylbenzylamino-4-amino-6-chloro-*s*-triazine compounds on the rhizome induction in *Cyperus serotinus* in the dark.

Compound		Concentration (μM)	Rhizome induction activity	
R	No.		No. of rhizome per tuber	Length of rhizomes (mm) mean (max-min)
Control			0.096 (146) ^{a)}	2.2 (5-1) ^{a)}
H	1	30	1.2	6.5 (14-2)
		10	0	
		3	0	
		1	0	
CH ₃	2	30	2.7	19 (43-2)
		10	2.1	22 (64-2)
		3	2.6	18 (50-2)
		1	2.5	16 (52-1)
CH ₃ CH ₂	3	30	2.6	17 (48-2)
		10	2.3	23 (36-2)
		3	1.5	8.7 (32-2)
		1	1.2	4.8 (12-1)
CH ₃ CH ₂ CH ₂	4	30	0.8	13 (25-1)
		10	0	
		3	0	
		1	0	
(CH ₃) ₂ CH	5	30	0	
		10	0	
		3	0	
		1	0	

^{a)} A number in parenthesis indicate the sum of tubers and length of the control in this study.

The derivatives substituted with butyl, isobutyl, 1-ethylpropyl, (*R*)-*sec*-butyl, (*S*)-*sec*-butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl group at R in the structure were inactive at 30 μM as similar to control.

isomers, and the racemates (**7**, **8**) also stimulated the rhizome induction significantly (Table 2). Therefore, effect of the substituents (R_1 and R_2 in the structure shown in Table 2) at the benzylic position on the rhizome induction was demonstrated using racemates. The 2-benzylamino-4-ethylamino-*s*-triazine (**6**) was inactive on the rhizome induction. The methyl (**7**, **8**), ethyl (**9**, **10**) and propyl (**11**, **12**) derivatives exhibited the rhizome induction significantly. An introduction of a bulky alkyl group (isopropyl (**13**)) or two alkyl groups (dimethyl (**14**, **15**)), however, resulted in a drastic decrease in the activity.

On the substituents at the 4-amino moiety (R_3 in the structure shown in Table 2), the methyl derivatives (**7**, **9** and **11**) were more potent than the corresponding ethyl derivatives (**8**, **10** and **12**). This trend was consistent with that in the optically active (*R*)-enantiomers mentioned above. In a high concentration of the α -propylbenzylamino derivative (**11**), the growth of the plantlets was severely inhibited. The fact that the plants soaked in the solutions of the racemates were active on the rhizome induction also showed a typical morphogenetic response of cytokinin, but those in the inactive compounds were not

Table 2 Effect of the substituents at the benzylic position of 2-benzylamino-4-alkylamino-6-chloro-*s*-triazines compounds on the rhizome induction in *Cyperus serotinus* in the dark.

Compound				Concentration (μM)	Rhizome induction activity	
R ₁	R ₂	R ₃	No.		No. of rhizome per tuber	Length of rhizomes (mm) mean (max-min)
H	Control				0.096 (146) ^{a)}	2.2 (5-1) ^{a)}
	H	CH ₃ CH ₂	6	30	0	
				10	0	
				3	0	
				1	0	
CH ₃	H	CH ₃	7	30	2.6	22 (42-2)
				10	1.8	12 (38-2)
				3	2.3	14 (32-1)
				1	1.3	11 (42-1)
CH ₃	H	CH ₃ CH ₂	8	30	2.5	18 (50-1)
				10	2.5	14 (40-2)
				3	0.9	7.5 (35-1)
				1	0	
CH ₃ CH ₂	H	CH ₃	9	30	1.3	25 (63-2)
				10	2.9	27 (50-6)
				3	3.3	28 (75-4)
				1	1.8	19 (40-7)
CH ₃ CH ₂	H	CH ₃ CH ₂	10	30	3.3	37 (94-2)
				10	4.7	8.0 (14-2)
				3	3.0	13 (22-5)
				1	1.3	6.5 (12-1)
CH ₃ CH ₂ CH ₂	H	CH ₃	11	30	0	
				10	2.5	28 (55-7)
				3	2.8	25 (50-7)
				1	1.3	10 (20-5)
CH ₃ CH ₂ CH ₂	H	CH ₃ CH ₂	12	30	2.8	8.0 (35-1)
				10	2.5	20 (27-4)
				3	1.2	19 (30-2)
				1	0.9	4.3 (12-1)
(CH ₃) ₂ CH	H	CH ₃ CH ₂	13	30	0.7	4.5 (8-1)
				10	0.6	2.7 (7-1)
				3	0	
				1	0	
CH ₃	CH ₃	CH ₃	14	30	0	
				10	0	
				3	0	
				1	0	
CH ₃	CH ₃	CH ₃ CH ₂	15	30	0	
				10	0	
				3	0	
				1	0	

^{a)} See Table 1.

Table 3 Effect of the chloro, methoxy and methylthio groups at the 6-position of the 2-ethyl (or propyl) benzylamino-4-ethylamino-6-chloro-*s*-triazine compounds on the rhizome induction in *Cyperus serotinus* in the dark.

Compound			Concentration (μM)	Rhizome induction activity	
R	X	No.		No. of rhizome per tuber	Length of rhizomes (mm) mean (max-min)
CH_3CH_2	Control	10		0.096 (146) ^{a)}	2.2 (5-1) ^{a)}
	Cl		30	3.3	37 (94-2)
			10	4.7	8.0 (14-2)
			3	3.0	13 (22-5)
			1	1.3	6.5 (12-1)
CH_3CH_2	OCH_3	16	30	2.9	22 (36-2)
			10	2.7	15 (21-1)
			3	0.5	6.2 (11-1)
			1	0	
CH_3CH_2	SCH_3	17	30	2.9	54 (85-2)
			10	3.7	26 (67-2)
			3	2.3	9.5 (21-1)
			1	1.4	8.2 (17-1)
$\text{CH}_3\text{CH}_2\text{CH}_2$	Cl	12	30	2.8	8.0 (35-1)
			10	2.5	20 (27-4)
			3	1.2	19 (30-2)
			1	0.9	4.3 (12-1)
$\text{CH}_3\text{CH}_2\text{CH}_2$	OCH_3	18	30	0	
			10	0	
			3	0	
			1	0	
$\text{CH}_3\text{CH}_2\text{CH}_2$	SCH_3	19	30	4.2	30 (75-2)
			10	4.3	26 (53-2)
			3	1.8	15 (53-1)
			1	0.8	6.3 (10-1)

^{a)} See Table 1.

be interfered any growth of them.

The 6-methoxy and 6-methylthio analogues of the 6-chloro-*s*-triazine compounds agitating the rhizome induction were synthesized and assayed the rhizome induction activity (Table 3). The rhizome induction activity of the methylthio analogues (**17**, **19**) was higher than that of the corresponding chloro-*s*-triazine compounds (**10**, **12**), and the plants treated with them at 30 μM formed the daughter plants which generated new rhizomes. The methoxy analogue (**16**) of the α -ethylbenzylamino-*s*-

triazine compound also showed a potent rhizome induction, but the analogue (**18**) of the α -propylbenzylamino-*s*-triazine proved to be not active.

Table 4 shows the rhizome induction activity of cytokinins (forchlorfenuron, thidiazuron and kinetin) including BA and *s*-triazine compounds affecting morphogenetic responses to plants. Kinetin also showed the rhizome induction activity, but its potency was lower than BA. Increased concentrations of kinetin (30 μM) resulted in a root

Table 4 Effect of cytokinins and *s*-triazine compounds affecting morphogenetic responses of plants on rhizome induction in *Cyperus serotinus* in the dark.

Compound	Concentration (μM)	Growth condition			Rhizome induction activity	
		No. of shoots per tuber	Plant height (% of control)	Root ^{a)}	No. of rhizome per tuber	Length of rhizomes (mm) mean (max-min)
Control		1.25 (146) ^{b)}	100	3	0.096 (146) ^{b)}	2.2 (5-1) ^{b)}
BA	1	1.8	103	3	1.5	13 (29-3)
	3	2.0	90	2	2.3	12 (41-2)
	10	2.3	86	1	3.0	25 (46-2)
	30	3.4	81	1-0	2.6	15 (31-4)
Kinetin	1	2.1	102	3	0	
	3	2.3	111	3	0.6	6.0 (11-4)
	10	3.0	87	2	2.3	8.7 (20-2)
	30	3.7	20	1	1.9	4.8 (10-3)
Forchlorfenuron	0.3	1.7	103	3	0.7	6.3 (11-1)
	1	2.2	86	2	2.6	7.5 (18-3)
	3	2.5	75	2	2.5	12 (35-2)
	10	3.0	70	1	3.2	15 (45-2)
	30	2.8	30	0	0.6	6.3 (8-2)
Thidiazuron	0.1	1.7	90	2	2.4	7.2 (26-1)
	0.3	2.0	80	2	2.7	10 (42-1)
	1	2.2	75	1	2.0	11 (55-1)
	3	2.0	45	0	2.6	18 (50-1)
	10	3.5	26	0	3.4	14 (48-4)
Atrazine	30	2.7	5	0	0	
	3	1.3	92	3	0	
	10	1.5	100	3	0	
Ametryn	30	1.1	87	3	0	
	3	1.5	105	3	0	
	10	2.0	108	3	0	
CHTz ^{c)}	30	1.6	98	3	0	
	3	1.4	98	3	0	
	10	1.3	102	3	0	
	30	1.6	99	3	0	

^{a)} Growth condition of root is indicated by an index as follows: 3, similar to control, 2, slight inhibition, 1, moderate inhibition and 0, complete inhibition.

^{b)} See Table 1.

^{c)} 2-Cyclohexylamino-4-ethylamino-6-chloro-*s*-triazine.

growth inhibition, an increase in numbers of the shoots per tuber and a drastic decrease in heights of shoots, whose physiological symptoms were similar as that of BA. Optimum concentration of kinetin for the rhizome induction was 10 μM . The synthetic cytokinins, forchlorfenuron and thidiazuron, significantly stimulated the rhizome induction. A high concentration of forchlorfenuron inhibited the rhizome induction, because the growth of shoots and roots was abnormal at 30 μM concentration. Thidiazuron was the most potent

compound among cytokinins tested, showing significant stimulation of the rhizome induction even at a low concentration (0.1 μM), and the growth of the plants was completely inhibited at 30 μM concentration. Both *s*-triazine herbicides (ametryn and atrazine) and 2-cyclohexylamino-4-ethylamino-6-chloro-*s*-triazine (CHTz) interfered neither the rhizome induction nor growth inhibition (Table 4).

DISCUSSION

The rhizome induction was affected by the

compounds showing cytokinin activity (BA, Kinetin, forchlorofenuron and thidiazuron which have been found to exhibit cytokinin-like activity¹¹⁾ and induction of somatic embryogenesis¹²⁾ (Table 4), and the other plant hormones were no active.⁴⁾ The results indicated that cytokinins played some physiologically important role in controlling secondary rhizome induction of *Cyperus serotinus* plant.

The structural properties of the *s*-triazine compounds on generation of the rhizome induction activity as cytokinin action were described. The (*R*)-isomers of α -methylbenzylamino-*s*-triazine compounds exhibited a specific stimulation of the rhizome induction, but the activities of the (*S*)-antipodes drastically disappeared (Table 1). The activity was easily influenced by a slight structural change in the substituents at amino moieties and at the benzylic position (Tables 1, 2 and 4). Moreover, the activity of the methylthio-*s*-triazine compounds was higher than that of corresponding chloro-*s*-triazines, and the plantlet treated with them formed the daughter plants with new rhizomes (Table 3). The pyrimidine homologues (2- α -methylbenzylamino-4-ethylamino- and 4- α -methylbenzylamino-2-ethylamino-6-chloro-pyrimidines) of the corresponding *s*-triazine compound (**8**) active on the rhizome induction were inactive (data not shown). Ametryn and atrazine have cytokinin-like growth promoting activities. 2-Cyclohexylamino-4-ethylamino-6-chloro-*s*-triazine (CHTz) and some *s*-triazine herbicides show a potent flower induction activity in seedling of *Asparagus officinalis*,¹³⁾ and their action has been classified into anticytokinin.¹⁴⁾ However, the *s*-triazine herbicides (atrazine and ametryn) and CHTz were inactive in this assay system (Table 4). These results positively indicate not only that *R*-configuration of the α -methylbenzyl moiety is essential for the rhizome induction, but also that space of the receptor site associated with the 4-amino moiety is relatively narrow. And also, it was suggested that an *s*-triazine ring system was essential for the rhizome induction, and that the (*R*)- α -alkyl (methyl, ethyl or propyl) substituted benzylamino-*s*-triazine would be the most active cytokinin among *s*-triazine com-

pounds known hitherto.¹⁵⁾

The 2-(*R*)- α -methylbenzylamino-*s*-triazine compounds showed diverse physiological properties, cytokinin-like and root growth inhibitory activities.^{2,4)} However, some differences in the structural properties of the (*R*)-enantiomers for optimizing activity were defined between the both bioassay systems (rhizome induction of *Cyperus serotinus* and root growth inhibition of *Echinochloa crus-galli*); the 4-amino (**1**) and 4-isopropylamino (**5**) derivatives were potent inhibitor against the root growth, but not active on the rhizome induction assay, whereas the 4-methylamino (**2**) and 4-ethylamino (**3**) compounds were active on both bioassays. This result would propose that a light-independent herbicidal activity of the (*R*)-enantiomers is not necessarily correlated with cytokinin activity of them.

REFERENCES

- 1) H. Omokawa & M. Konnai: *Agric. Biol. Chem.* **54**, 2373 (1990)
- 2) H. Omokawa & M. Konnai: *Pestic. Sci.* **35**, 83 (1992)
- 3) H. Omokawa, N. Ichizen & T. Takematsu: *Agric. Biol. Chem.* **51**, 2563 (1987)
- 4) H. Omokawa, M. Takeuchi & M. Konnai: *Pestic. Sci.* **35**, 87 (1992)
- 5) L. G. Copping, D. E. Davis & C. G. P. Pillai: *Weed Sci.* **20**, 274 (1972)
- 6) C. G. P. Pillai & D. E. Davis: *Weed Sci.* **21**, 461 (1973)
- 7) H. M. Nadar, M. D. Clegg & J. W. Maranville: *Plant Physiol.* **56**, 747 (1975)
- 8) H. Omokawa, N. Ichizen & M. Konnai: *Agric. Biol. Chem.* **52**, 1047 (1988)
- 9) H. Omokawa, N. Ichizen, M. Konnai & T. Takematsu: *Agric. Biol. Chem.* **52**, 1515 (1988)
- 10) H. Omokawa, I. Kobayashi & M. Konnai: *Agric. Biol. Chem.* **53**, 2723 (1989)
- 11) M. C. Mok, D. W. S. Mok, D. J. Armstrong, K. Shudo, Y. Isogai & T. Okamoto: *Phytochemistry* **21**, 1509 (1982)
- 12) P. K. Saxena, K. A. Malik & R. Gill: *Planta* **187**, 421 (1992)
- 13) T. Abe, R. Shimizu, H. Iwamura & T. Kameya: *Physiol. Plant.* **70**, 228 (1987)
- 14) R. Shimizu, H. Iwamura, S. Matsubara & T. Fujita: *J. Agric. Food Chem.* **37**, 236 (1989)
- 15) H. Omokawa, Y. Kawata & M. Konnai: *Pestic. Sci.* **37**, 107 (1993)

要 約

ミズガヤツリの地下茎形成促進活性を示す *s*-トリアジン化合物の構造的特徴

重川弘宜, 倉持仁志, 川田好徳, 近内誠登

ミズガヤツリの塊茎を用いて, α 位にアルキル基をもつベンジルアミノ-*s*-トリアジン化合物, アトラジン, アメトリンおよび合成サイトカイニン化合物の地下茎の形成促進作用を検定した. 4-位にメチルアミノ, エチルアミノ基をもつ 2-(*R*)- α -メチルベンジルアミノ-6-クロロ-*s*-トリアジン化合物に強い促進活性が認められ, アミノ, プロピルアミノ基のものは活性が弱かった. イソプロピルアミノ基および炭素数 4 から 10 までのアルキルアミノ基をもつ誘導体には活性が認められなかつ

た. 活性を示す (*R*)-体の対掌体, (*S*)-体には活性が認められなかった. 形成促進活性を示す化合物のラセミ体でも活性は保存され, α 位のアルキル基の変化ではメチル, エチル, プロピル基に置換した化合物で活性が認められたが, イソプロピル基および無置換では活性は消失した. クロロ-*s*-トリアジン体で活性を示した化合物のメチルチオおよびメトキシ誘導体の活性は保存された. サイトカイニン様活性が報告されているアメトリンやアトラジンおよびアンチサイトカイニン作用を示すシクロヘキシル-*s*-トリアジンは活性を示さなかった. 合成サイトカイニン物質であるチアジアズロンとフォルクロフェニュロンは促進活性を示し, ベンジルアデニンより高活性であった. カイネチンの活性はベンジルアデニンより弱かった.