2′-*O*-sulfated glycoside and a 2′-*O*-linked glucuronic acid containing disaccharide, were detected in the red beet culture.

ACKNOWLEDGEMENTS

The author thanks Profs N Amrhein and W Barz for providing the red beet and soybean cell cultures, respectively.

REFERENCES

1 Mumma RO and Hamilton RH, Xenobiotic metabolism in higher plants: In-vitro tissue and cell culture techniques, in *Xenobiotic metabolism: In vitro methods*, ed by Paulson, GD, Frear DS and Marka EP, *ACS. Symp. Ser. 97.* pp 35–76 (1979).

2 Mumma RO and Davidonis GH, Plant tissue culture and pesticide metabolism, in *Progress in Pesticide Biochemistry*, ed by Hutson DH and Roberts TR, Vol 3. pp 255–278 (1983).

3 Shimabukuro RH and Walsh WC, Xenobiotic metabolism in plants: In-vitro tissue, organ and isolated cell techniques, in *Xenobiotic metabolism: In vitro methods* ed by Paulson GD, Frear DS and Marka EP, *ACS. Symp. Ser. 97.* pp 3–34 (1979).

Effect of halomethyl-1,3,5-triazines on nitrification of ammonia

Shinpei Ohki,[1]* Yuuta Kasahara,[1] Yukiharu Sato,[1] Ko Wakabayashi,[1] Yoshiko Miyamoto[2] and Johannes W Vonk[3]

[1]*Graduate School of Agricultural Science, Tamagawa University, Tamagawa Gakuen, Machida-shi, Tokyo 194-8610, Japan*

[2]*Department of Chemistry, School of Science, Kitasato University, Kitasato 1-15-1, Sagamihara-shi 228-0000, Japan*

[3]*EPP Consultancy, Homeruslaan 22, 3707 GS Zeist, The Netherlands*

Abstract: Nitrification inhibitory activity of halomethyl-1,3,5-triazines was determined by measuring the inhibitory activities on ammonia-oxidation to nitrate (NO_3^--N) in an upland soil and on ammonia-oxidation to nitrite (NO_2^--N) by *Nitrosomonas europaea* ATCC 25978 (ATCC) and *Nitrosomonas sp* TK 794 (TK).

Within the chlorinated trimethyl-1,3,5-triazines, those bearing at least one trichloromethyl group inhibited nitrification more strongly, both in soil and in cell suspension of ATCC, than other mono- or dichlorinated methyl-1,3,5-triazines. Introduction of an amino group to 2,4,6-tris(trichloromethyl)-1,3,5-triazine gave 10- and 100-fold increases of nitrification inhibitory activity in soil and ATCC cell culture, respectively. Within the trihalomethyl-1,3,5-triazines, those having tribromomethyl group(s) exhibited rather weaker nitrification inhibition in soil than the corresponding trichloromethyl-1,3,5-triazines, although they showed a strong inhibition in cell suspension.

Ammonium oxidation in ATCC was inhibited more strongly than that in TK. In QSAR studies, the optimum log *P* values were calculated as *c* 4.30. By using this value it will become possible to design highly active trichloromethyl-1,3,5-triazine nitrification inhibitors.

Keywords: halomethyl-1,3,5-triazines; trichloromethyl-1,3,5-triazines; nitrification inhibitors; *Nitrosomonas europaea* ATCC 25978; *Nitrosomonas sp* TK 794; quantitative structure–activity relationship

1 INTRODUCTION

It is known that trichloromethyl-1,3,5-triazines are highly active nitrification inhibitors in upland soil, possibly by controlling ammonia-oxidizing, but not nitrite-oxidizing, bacteria.[1–4] In a quantitative structure–activity relationship (QSAR) study, the hydrophobic parameter, log *P*, was found to be an important parameter affecting inhibitory activity by these 1,3,5-triazines.[3] A mode-of-action study of these inhibitors for ammonia-oxidizing bacteria has been presented elsewhere.[5] In this summary, the nitrification-inhibiting activity of halomethyl-1,3,5-triazines, including trichloromethyl-1,3,5-triazines, was assayed in soil and in cell cultures of ammonia-oxidizing bacteria.

2 MATERIALS AND METHODS

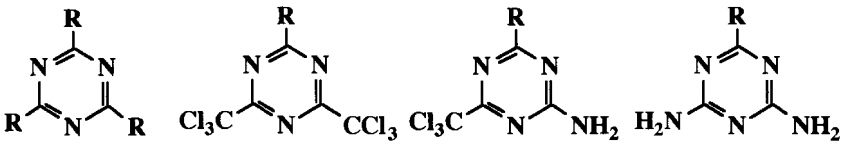
Most of the halomethyl-1,3,5-triazines were synthesized by (co-)trimerization of nitriles, condensation of *N*-(haloacetimidoyl)haloacetamidine with acid anhydride or halogenation of methyl-1,3,5-triazines.[1,6,7] 2-Amino-4-halomethyl-1,3,5-triazines were prepared by nucleophilic amination reaction of corresponding 2-trichloromethyl-4-halomethyl-1,3,5-triazines.[1–3] Nitrification inhibitory activity of the 1,3,5-triazines was determined through measurement of the inhibitory activities on ammonia-oxidation to nitrate (NO_3^--N) in an upland soil and on ammonia-oxidation to nitrite (NO_2^--N) by *Nitrosomonas europaea* Winogr. ATCC 25978 (ATCC) and *Nitrosomonas sp* TK 794 (TK). The nitrification inhibitory indices in soil, cell culture of ATCC and TK were presented as pI_{50} (soil), pI_{50} (ATCC) and pI_{50} (TK), which indicated the logarithm of the reciprocal molar concentration for 50% nitrification inhibition by the compound tested.

3 RESULTS AND DISCUSSION

Within the chlorinated trimethyl-1,3,5-triazines, those bearing at least one trichloromethyl group inhibited nitrification more strongly, both in soil and in cell suspension of ATCC, than other mono- or dichlorinated methyl-1,3,5-triazines. This fact indicated that the trichloromethyl group(s) may be essential for high activity in 1,3,5-triazine nitrification inhibitors. Introduction of an amino group to the 2,4,6-tris(trichloromethyl)-1,3,5-triazine resulted in 10- and 100-fold

* Correspondence to: Shinpei Ohki, Graduate School of Agricultural Science, Tamagawa University, Tamagawa Gakuen, Machida-shi, Tokyo 194-8610, Japan
E-mail: kwaka@agr.tamagawa.ac.jp
(*Received 29 June 1998; accepted 16 February 1999*)

Table 1. pI_{50} (soil) and pI_{50} (ATCC) values of trihalomethyl-1,3,5-triazines



Group A Group B Group C Group D

	Group A		Group B		Group C		Group D	
R	pI_{50} (*soil*)	pI_{50} (*ATCC*)	pI_{50} (*soil*)	pI_{50} (*ATCC*)	pI_{50} (*soil*)	pI_{50} (*ATCC*)	pI_{50} (*soil*)	pI_{50} (*ATCC*)
CH_3	<3.00	<3.00	4.30	6.15	5.17	5.31	<3.00	<3.00
CF_3	<3.00	<3.00	4.19	4.50	4.02	5.73	4.05	4.19
CCl_3	4.48	4.60	4.48	4.60	5.31	6.72	4.64	5.15
CBr_3	4.49	5.91	4.32	6.10	4.17	7.12	3.79	6.30

increases of nitrification inhibitory activity in soil and ATCC cell culture, respectively, (Table 1). Within the trihalomethyl-1,3,5-triazines, the 1,3,5-triazines having tribromomethyl group(s) exhibited rather weaker nitrification inhibition than the trichloromethyl analogues in soil although they showed a strong inhibition in cell suspension. This difference in activity may be related to a difference in susceptibility between ATCC and the nitrifying bacteria in soil, a different metabolic detoxification of the 1,3,5-triazine in the soil used or a different adsorption of 1,3,5-triazine compounds in soil.[1]

Ammonium oxidation of ATCC was inhibited more strongly than that of TK, as shown in eqn (1).

$$\mathrm{pI}_{50}(\mathrm{ATCC}) = 0.757(\pm 0.106)\mathrm{pI}_{50}(\mathrm{TK}) + 1.969(\pm 0.569) \quad (1)$$

$$(n=28,\ r=0.945,\ s=0.187,\ \mathrm{F}_{1,26}=215.87)$$

This higher sensitivity of ATCC to the 1,3,5-triazine inhibitors compared with TK may be explained by the thick lamellar structure of the cell membrane of TK which prevents penetration of the inhibitors into cells. Also, the susceptibility of ammonia monooxygenase (AMO), which is considered as the target enzyme of the inhibitors, may differ between the two strains of *Nitrosomonas*.

In QSAR studies, two equations (2) and (3) were obtained using pI_{50} (ATCC) and pI_{50} (TK).

$$\mathrm{pI}_{50}(\mathrm{ATCC}) = -0.071(\pm 0.015)(\log P)^2 + 0.618(\pm 0.194)\log P + 4.930(\pm 0.598) \quad (2)$$

$$(n=44,\ r=0.931,\ s=0.306,\ \mathrm{F}_{2,41}=133.10)$$

$$\mathrm{pI}_{50}(\mathrm{TK}) = -0.096(\pm 0.022)(\log P)^2 + 0.831(\pm 0.241)\log P + 3.890(\pm 0.630) \quad (3)$$

$$(n=34,\ r=0.909,\ s=0.277,\ \mathrm{F}_{2,31}=73.45)$$

Equations (2) and (3) indicate that log*P* plays a significant role in determining the nitrification activities in cell culture. The optimum log*P* values in eqns (2) and (3) were calculated as 4.35 and 4.33, respectively. By using this value it should become possible to design highly active trichloromethyl-1,3,5-trazine nitrification inhibitors. This finding also indicates that the nitrification inhibitory activity of trichloromethyl-1,3,5-triazines relates to permeability into the cell membrane or so-called hydrophobic binding of the inhibitors to the target site.

ACKNOWLEDGEMENTS

The authors are grateful to Prof Dr Tatsuaki Tokuyama and Dr Reiji Takahashi, Nihon University. The authors are also indebted to Dr Masanori Okanishi and Dr Hiroshi Kubo, both of Tamagawa University, for helpful suggestions and discussion.

REFERENCES

1 Murakami M, Tsuji A, Miyamoto Y, Yamazaki C, Ogawa H, Takeshima S and Wakabayashi K, Synthesis of trichloromethyl-1,3,5-triazines and their nitrification-inhibitory activity. *Nihon Noyaku Gakkaishi (J Pestic Sci)* **18**:147–154 (1993).

2 Okano N, Murakami M, Miyamoto Y, Koizumi K, Ogawa H and Wakabayashi K, Synthesis and structure–activity relationship of newer trichloromethyl-1,3,5-triazine nitrification inhibitors. *Nihon Noyaku Gakkaishi (J Pestic Sci)* **18**:361–368 (1993).

3 Murakami M, Takagi S, Takahashi I, Tokuyama T, Sato Y, Vonk JW and Wakabayashi K, A possible target site of 1,3,5-triazine nitrification inhibitors. *Nihon Noyaku Gakkaishi (J Pestic Sci)* **20**:471–477 (1994).

4 Takagi S, Murakami M, Sato Y, Takahashi R, Tokuyama T and Wakabayashi K, Effect of nitrification inhibitors on *Nitrosomonas* and *Nitrobacter*. *Nihon Noyaku Gakkaishi (J Pestic Sci)* **19**:19–23 (1994).

5 Kasahara Y, Takagi S, Ohki S, Vonk JW, Tokuyama T, Sato Y and Wakabayashi K, Influence of nitrification inhibitors on cell-free system of nitrosomonas. *Abstracts 9th International Congress of Pesticide Chemistry*, London (1998).

6 Tsunoda M and Omata K, (Mitsubishi Chem Ind Ltd), Jpn Kokai Tokkyo Koho 2-Substituted-4-trichloromethyl-6-trifluoromethyl-*s*-triazines, JP 52-83,577 (1977).

7 Schaefer FC and Ross JH, Chlorination and bromination of alkyl *s*-triazines. *J Org Chem* **29**:1527–1537 (1964).