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Original Article

Synthesis and Structure-Activity Relationship of Newer Trichloromethyl-1,3,5-triazine Nitrification Inhibitors

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Various trichloromethyl-1,3,5-triazines were synthesized and effects of trichloromethyl-1,3,5-triazines on nitrification in the upland soil were investigated. 2-Substituted-4,6-bis(trichloromethyl)-1,3,5-triazines were prepared by trimerization or cotrimerization using CCl_3CN in the presence of Norton-Wakabayashi complex catalyst, *e.g.* $AlBr_3$-HCl. 2-Amino-4-substituted-6-trichloromethyl-1,3,5-triazines were obtained through the haloform type of reactions of trichloromethyl-1,3,5-triazines with amines. The pI_{50} values of highly active trichloromethyl-1,3,5-triazines were 4.5–5.5. Essentially they have a CCl_3-group and an amino or alkylamino group in the three substituents of 1,3,5-triazine ring. QSAR between pI_{50} values and log P(S) parameters were investigated by multi-parameter regression analysis. Hydrophobic parameters, log P(S) estimated by semi-empirical calculation, were used for convenience instead of log P(H) determined by HPLC in this QSAR study. The optimum log P(S) was calculated as 2.91, a little more hydrophobic figure compared with the log P value (*ca.* 2.4) in our previous paper. By the use of this value, it will become possible to design highly active trichloromethyl-1,3,5-triazine nitrification inhibitors.

INTRODUCTION

Nitrification is the process of conversion of NH_4^+-N into NO_3^--N *via* NO_2^--N. The formed NO_3^--N is soluble in water and not adsorbed onto the soil as NH_4^+-N. Consequently a considerable amount of nitrogen is lost through leaching. Then the nitrification may result in inefficient crop use of nitrogen during some growth stages. Therefore control of nitrification should lead to increase in efficiency of nitrogen use by plants manifested as improvement in crop growth, yield and quality. The control can be achieved by inhibiting the nitrifying bacteria with chemicals. The ideal nitrification inhibitor should have specificity to nitrifying bacteria. The inhibitor should also be nontoxic to other soil organisms, fish, mammals, crops, and be safe in the environment. It should be effective throughout the nitrogen fertilizer-soil reaction zone on its mobility in soil, and it should be sufficiently persistent in action so that nitrification is inhibited for an adequate period of time. Furthermore, such a chemical should be a low cost additive to fertilizer. It should inhibit the conversion of NH_4^+-N to NO_2^--N *i.e.*, inhibit *Nitrosomonas* growth or activity. However, it should not inhibit *Nitrobacter* since such inhibition could cause an undesirable accumulation of nitrite.[1] It is reported that 1,3,5-triazines may have nitrification

inhibitory activity by inhibiting action of ammonia-oxidizing bacteria.[2] In the report, only limited numbers of 1,3,5-triazines with limited range of log P values were discussed concerning quantitative structure-activity relationships (QSAR). In this paper, we attempted to use wider range of log P parameters, in order to obtain more precise QSAR, and more active nitrification inhibitors.

MATERIALS AND METHODS

1. *Synthesis of 1,3,5-Triazines*

1.1 *2-(p-Chlorobenzylthio)-4,6-bis(trichloromethyl)-1,3,5-triazine* (**9**)

A mixture of CCl_3CN (31.0 g, 0.22 mol), *p*-chlorobenzyl thiocyanate (20.0 g, 0.11 mol) and $AlBr_3$ (3.0 g) were placed in a flask. Anhydrous hydrogen chloride was saturated at −20°C with stirring. The reaction mixture was kept at −10°C for 12 hr and then at room temperature for the further 12 hr. Hydrogen chloride gas was then removed from the reaction mixture by a water pump under reduced pressure. After washing with cold water, the residue was recrystallized from ethanol to give 35.7 g of 2-(*p*-chlorobenzylthio)-4,6-bis-(trichloromethyl)-1,3,5-triazine (70%). mp 91–93°C (lit.[3] 91–93°C). Anal. Found: C, 30.25; H, 1.35; N, 8.95, Calcd. for $C_{12}H_6N_3Cl_7S$: C, 30.50; H, 1.28; N, 8.89%. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 4.49 (2H, s, CH_2), 7.30 (2H, d, J=8.3 Hz, H-2 and H-6 of phenyl), 7.43 (2H, d, J=8.3 Hz, H-3 and H-5 of phenyl). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 34.84 (td, $^1J_{CH}$=143.4, $^3J_{CH}$=3.6 Hz, SCH_2), 94.37 (s, CCl_3), 128.91 (dd, $^1J_{CH}$=165.4, $^2J_{CH}$=5.6 Hz, C-3 of phenyl), 130.58 (ddt, $^1J_{CH}$=161.8, $^2J_{CH}$=3.7, $^3J_{CH}$=12.9 Hz, C-2 of phenyl), 133.89 (td, $^2J_{CH}$=3.7, $^3J_{CH}$=12.9 Hz, C-1 of phenyl), 134.12 (dd, $^2J_{CH}$=3.7, $^3J_{CH}$=11.0 Hz, C-4 of phenyl), 173.39 (s, C-4 and C-6 of triazine), 186.17 (t, $^3J_{CH}$=5.5 Hz, C-2 of triazine). IR ν_{max}^{KBr} cm^{-1}: 1540 (1,3,5-triazine ring). According to this procedure 1,3,5-triazines (**1**–**8**) were prepared.

1.2 *2-Methyl-4-propylamino-6-trichloromethyl-1,3,5-triazine* (**17**)

A solution of *n*-propylamine (0.94 g, 0.016 mol) in 5 ml of tetrahydrofuran (THF) was added dropwise at 0–5°C to a solution of 2-methyl-4,6-bis(trichloromethyl)-1,3,5-triazine (**2**) (3.3 g, 0.01 ml) in 20 ml of THF. After stirring for 3 hr at room temperature, the reaction solution was concentrated in a vacuum and the residue was recrystallized from *n*-hexane to give 1.96 g of the title compound in 73% yield. mp 77–80°C (lit.[4] 75–76°C). Anal. Found: C, 35.83; H, 4.14; N, 20.74, Calcd. for $C_8H_{11}N_4Cl_3$: C, 35.60; H, 4.14; N, 20.80%. MS *m/z*: 268 (M^+), 253 (M^+-CH_3) and so on. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 0.98 and 1.00 (3H in total, t, J=7.88 and J=7.33 Hz, together CH_3), 1.66 (2H, sextet, J=7.33 Hz, CH_2), 2.52 and 2.58 (3H in total, s, together CH_3), 3.49 and 3.50 (2H in total, m, together $NHC\underline{H}_2$), 5.94 and 6.13 (1H in total, bs, together NH). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 11.80 (qt, $^1J_{CH}$=125.0, $^3J_{CH}$=7.4 Hz, $CH_2\underline{C}H_3$), 22.44 and 22.48 (each t, $^1J_{CH}$=126.9 Hz, together $\underline{C}H_2CH_3$), 42.78 and 42.92 (each t, $^1J_{CH}$=132.9 Hz, $NHCH_2$), 95.92 and 96.18 (each s, together CCl_3), 165.76 and 166.15 (each s, together C-4 of triazine), 172.47 and 173.08 (each s, together C-6 of triazine), 177.28 and 178.44 (each q, $^2J_{CH}$=7.3 Hz, together C-2 of triazine). IR ν_{max}^{KBr} cm^{-1}: 1522, 1530 (1,3,5-triazine ring). For reference, analytical data of 2-methyl-4-methylamino-6-trichloromethyl-1,3,5-triazine (**15**) and 2-methyl-4-dimethylamino-6-trichloromethyl-1,3,5-triazine (**20**) are shown below; the triazine (**15**), Anal. Found: C, 30.25; H, 2.66; N, 22.89, Calcd. for $C_6H_7N_4Cl_3$: C, 29.90; H, 2.92; N, 23.20%. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 2.54 and 2.60 (3H in total, each d, CH_3), 3.10 and 3.12 (3H in total, d, J=4.88 Hz, $NHC\underline{H}_3$), 6.29 and 6.54 (1H in total, each bs, together NH). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 27.87 and 28.04 (q, $^1J_{CH}$=139.5 Hz, $NHCH_3$), 95.96 and 96.12 (each s, together CCl_3), 166.29 and 166.59 (each s, together C-6 of triazine), 172.42 and 173.21 (each s, together C-4 of triazine), 177.14 and 178.56 (each s, $^2J_{CH}$=7.3 Hz, together C-2 of triazine), IR ν_{max}^{KBr} cm^{-1}: 1540, 1620 (1,3,5-triazine ring), and 1,3,5-triazine (**20**), Anal. Found: C, 31.50; H, 3.62; N, 22.99, Calcd. for $C_7H_9N_4Cl_3$: C, 32.90; H, 3.55; N, 21.93%. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 2.54 (3H, s, CH_3), 3.26 and 3.27 (each 3H, each s, $N<^{CH_3}_{CH_3}$). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 26.01 (s, J=128.7 Hz, CH_3), 36.56 and 36.60 (each s, J=137.9 Hz, $N<^{CH_3}_{CH_3}$, 96.67 (s, CCl_3), 165.13 (s, J=7.3 Hz, C-4 of triazine), 172.48 (s, J=7.3 Hz, C-6 of triazine), 177.49 (s, J=7.3 Hz, C-2 of triazine),

IR ν_{max}^{KBr} cm^{-1}: 1540, 1620 (1,3,5-triazine ring). 1,3,5-Triazines (**10**–**16**, **18**–**26**) were prepared in a similar manner.

1.3 2-(p-Chlorophenylamino)-4-methylthio-6-trichloromethyl-1,3,5-triazine (***29***)

A mixture of 2-methylthio-4,6-bis(trichloromethyl)-1,3,5-triazine (3.6 g, 0.01 mol), *p*-chloroaniline (1.3 g, 0.01 mol), triethylamine (1.5 g, 0.015 mol) and 100 ml of chloroform was refluxed, with stirring, for 48 hr. After excess triethylamine and chloroform were removed by distillation, the resulting brown oil was purified by silica gel column chromatography to give 2.0 g of the title compound in 53% yield. mp 122–124°C. Anal. Found: C, 35.94; H, 2.16; N, 15.14, Calcd. for $C_{11}H_8N_4Cl_4S$: C, 35.70; H, 2.18; N, 15.14%. MS *m/z*: 368 (M^+) and so on. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 2.77 (3H, s, SCH_3), 7.32 (2H, d, J=9.0 Hz, H-2 and H-6 of phenyl), 7.60 (1H, bs, NH), 7.62 (2H, d, J=9.0 Hz, H-3 and H-5 of phenyl). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 13.67 (q, J=141.85 Hz, SCH_3), 95.56 (s, CCl_3), 121.77 (d, $^1J_{CH}$=164.94 Hz, C-2 of phenyl), 129.04 (d $^1J_{CH}$=164.14 Hz, C-3 of phenyl), 129.77 (s, C-4 of phenyl), 135.58 (s, C-1 of phenyl), 162.82 (s, C-2 of triazine), 171.70 (s, C-6 of triazine), 184.53 (s, C-4 of triazine). IR ν_{max}^{KBr} cm^{-1}: 1510, 1560 (1,3,5-triazine ring). According to this procedure, 1,3,5-triazines (**27** and **28**) were prepared.

1.4 2-Amino-4-phenylthio-6-trichloromethyl-1,3,5-triazine (***33***)

A mixture of 2-phenylthio-4,6-bis(trichloromethyl)-1,3,5-triazine (4.24 g, 0.01 mol) and 2.5 ml of concentrated ammonium hydroxide was stirred at 0°C for 1 hr. The formed soild was recrystallized from ethanol to give 2.83 g of title compound in 88% yield. mp 122–123°C (lit.[5] 122–124°C). Anal. Found: C, 37.11; H, 1.94; 17.35, Calcd. for $C_{10}H_7N_4Cl_3S$: C, 37.35; H, 2.19; N, 17.48%. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 5.95 and 6.22 (2H, each bs, NH_2), 7.41 (3H, m, phenyl), 7.58 (2H, m, phenyl). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 95.32 (s, CCl_3), 127.14 (t, $^3J_{CH}$=7.3 Hz, C-1 of phenyl), 129.15 (dd, $^1J_{CH}$=161.8, $^3J_{CH}$=11.0 Hz, C-4 of phenyl), 129.79 (dd, $^1J_{CH}$=161.8, C-3 of phenyl), 135.35 (dd, $^3J_{CH}$=163.7, C-2 of phenyl), 166.13 (s, C-2 of triazine), 172.11 (s, C-6 of triazine), 183.94 (s, C-4 of triazine). IR ν_{max}^{KBr} cm^{-1}: 1545, 1504 (triazine ring). 1,3,5-Triazines (**30**–**32**, **34**–**37**) were obtained in a similar manner described above.

1.5 2-Methoxy-4,6-bis(trichloromethyl)-1,3,5-triazine (***38***)

Fifteen gram of triethylamine was added at 0°C to the solution of 2,4,6-tris(trichloromethyl)-1,3,5-triazine (21.7 g, 0.05 mol) in 100 ml of methanol. The reaction mixture was kept between 0°C and 5°C for 5 hr. After a small amount of solid material was filtered off, the triethylamine, excess methanol and chloroform formed were removed by vacuum distillation at room temperature. The residue was purified by silica gel column chromatography to give 10.4 g of 2-methoxy-4,6-bis(trichloromethyl)-1,3,5-triazine in 60% yield. mp 45–46°C (lit.[6] 46°C). Anal. Found: C, 20.70; H, 0.95; N, 12.33, Calcd. for $C_6H_3N_3Cl_6O$: C, 20.84; H, 0.87; N, 12.15%. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 4.29 (s, 3H, OCH_3). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 57.02 (q, $^1J_{CH}$=149.0 Hz, OCH_3), 94.20 (s, CCl_3), 172.80 (s, C-4 and C-6 of triazine), 176.80 (s, C-2 of triazine). IR ν_{max}^{KBr} cm^{-1}: 1568, 1545 (1,3,5-triazine ring).

1.6 2-(p-Chlorophenoxy)-4,6-bis(trichloromethyl)-1,3,5-triazine (***39***)

The starting material, 2-chloro-4,6-bis(trichloromethyl)-1,3,5-triazine, was prepared by the chlorination reaction of 2-hydroxy-4,6-bis(trichloromethyl)-1,3,5-triazine using $POCl_3$, the former being obtained by the haloform reaction of 2,4,6-tris(trichloromethyl)-1,3,5-triazine with water in the presence of triethylamine.[7] A solution of 2-chloro-4,6-bis(trichloromethyl)-1,3,5-triazine (1.25 g, 0.005 mol) in 10 ml of THF was added dropwise at room temperature to a solution of *p*-chlorophenol (0.65 g, 0.005 mol) and triethylamine (0.5 g, 0.005 mol) in 15 ml of THF. After stirring for 2 hr, triethylamine hydrochloride formed was filtered off and the solvent was evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give 1.55 g of 2-(*p*-chlorophenoxy)-4,6-bis(trichloromethyl)-1,3,5-triazine in 70% yield. mp 85–95°C. Anal. Found: C, 30.12; H, 1.02; N, 9.25, Calcd. for $C_{11}H_4N_3Cl_7O$: C, 29.86; H, 0.91; N, 9.50%. MS *m/z*: 439 (M^+) and so on. ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm: 2.27 (d, J=8.79 Hz, H-2 and H-6 of phenyl), 7.45 (d, J=8.79 Hz, H-3

and H-5 of phenyl). ^{13}C NMR $\delta_{TMS}^{CDCl_3}$ ppm: 93.89 (s, CCl_3), 122.35 (dd, $^1J_{CH}=167.3$, $^2J_{CH}=3.7$ Hz, C-2 of phenyl), 129.92 (dd, $^1J_{CH}=169.1$, $^2J_{CH}=5.6$ Hz, C-3 of phenyl), 132.37 (dd, $^2J_{CH}=3.7$, $^3J_{CH}=11.0$ Hz, C-4 of phenyl), 149.61 (dd, $^2J_{CH}=3.7$, $^3J_{CH}=11.0$ Hz, C-1 of phenyl), 172.13 (s, C-4 of triazine), 177.34 (s, C-2 and C-6 of triazine). IR ν_{max}^{KBr} cm^{-1}: 1560 (1,3,5-triazine ring). 1,3,5-Triazines (**27**, **28**) were prepared in a similar method using corresponding 2-chloro-4-substituted-6-trichloromethyl-1,3,5-triazine and anilines.

All the reaction products were purified by recrystallization and/or column chromatography, and their structures were confirmed by IR-, NMR-, mass-spectroscopy and elementary analysis of C, H, and N, and melting points are uncorrected. IR spectra were recorded on a Shimadzu IR-420 spectrophotometer. The ^1H and ^{13}C NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solution on a JMN-EX400 spectrometer operating at 400 MHz. The mass spectra (75 eV) were recorded on a JEOL JMS D100 mass spectrometer.

2. *Evaluation of Nitrification Inhibitory Activity*

Ten gram of sterilized soil (150°C, 2 hr) and 62.5 mg of each 1,3,5-triazine were mixed to prepare 125 ppm stock treatment formulation. The upland soil was preincubated for 5 days under the same conditions as in the experiment. The properties of the soil are shown in Table 1. In each wide mouthed culture flask, 25 g of the upland soil, 16.5 mg of urea and the appropriate amount of the 1,3,5-triazine formulation were added and mixed well. Then the moisture of test soil mixture in the flask was adjusted to 50% of the field capacity by adding water and pH of the soil to 6.8 with $CaCO_3$. The flasks were sealed with cap with 5 holes, whose diameter was 2 mm, and incubated in a dark room at 28°C for 14 days. The molar I_{50} value of each 1,3,5-triazine was calculated according to the method described by Murakami *et al.*[2] To discuss the nitrification inhibitory activity of 1,3,5-triazines, pI_{50} values (negative logarithms of the molar I_{50}) were used as nitrification inhibitory indices in this paper.

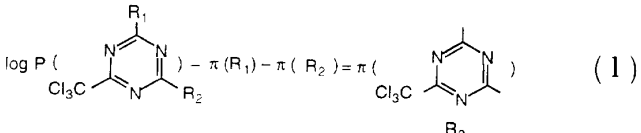
3. *Determination of Hydrophobicity Parameters; log P(H) and log P(S)*

3.1 *Log P(H) by HPLC method*

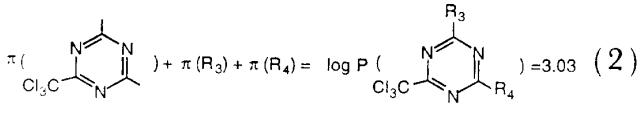
Hydrophobicity parameters, log P(H), of twelve of 1,3,5-triazines (**2**, **6**, **10**, **14**, **22**, **26**, **27**, **29**, **30**, **32**, **34**, **39**) were determined by HPLC method using ODS column (Inertsil ODS 4.6×250 mm) and methanol–water (8:2) as mobile phase.[8]

3.2 *Log P(S) by semi-empirical method*

Since it was rather hard works to determine log P(H) for all thirty-nine 1,3,5-triazines even by HPLC method, we tried to estimate log P(S) for triazines in this study, using semi-empirical method based upon the additive property of π value. In the first place, the π value (3.03) of trichloromethyl-1,3,5-triazine moiety was estimated as the mean of the π values for the twelve 1,3,5-triazines. The π values were calculated by the Eq. (1) using log P(H) values for twelve 1,3,5-triazines described in *3.1* and the substituent's π values of Rekker.[9] Then log P(S) values of all thirty-nine 1,3,5-triazines in this paper were calculated according to the Eq. (2) (see the column of log P(S) in Table 2).



(1)



(2)

For reference, see the log P(S) and log P(H) for twelve 1,3,5-triazines; Compd. **2**, 4.07 and

Table 1 Physical and chemical properties of soil tested.

pH	(H_2O)	5.67	CEC	(meq/100 g soil)	18.28
	(KCl)	5.46			
Total C	(%)	11.58	NH_4^+-N	(mg/100 g soil)	1.25
Total N	(%)	0.62	NO_3^--N	(mg/100 g soil)	1.72
C/N		18.7			

3.78; **6**, 6.17 and 6.67; **10**, 4.19 and 4.09; **14**, 4.86 and 4.93; **22**, 4.17 and 3.87; **26**, 3.26 and 3.33; **27**, 4.33 and 4.39; **29**, 5.60 and 5.71; **30**, 2.45 and 2.48; **32**, 4.01 and 3.91; **34**, 4.92 and 4.51 and **39**, 5.57 and 5.67. The estimated log P(S) values were well correlated with log P(H) as shown in the Eq. (3).

$$\log P(\mathrm{S}) = 0.884 \log P(\mathrm{H}) + 0.538 \quad (3)$$
$$(\pm 0.123)$$
$$[n=12,\ r=0.981,\ s=0.211]$$

4. Quantitative Structure-Activity Relationship (QSAR) for 1,3,5-Triazine Nitrification Inhibitor

QSAR between pI_{50} values and log P(S) parameters were investigated by multi-parameter regression analysis. Hydrophobic parameters, log P(S), were used instead of log P(H) in this QSAR study.

RESULTS AND DISCUSSION

1. Synthesis of 1,3,5-Triazines

1.1 Trimerization and cotrimerization of CCl_3CN

Trichloromethyl-1,3,5-triazines (**2**–**9**) were prepared by trimerization or cotrimerization using CCl_3CN in the presence of Norton-Wakabayashi complex catalyst, *e.g.* $AlBr_3$-HCl.[10] Although trimerization of CCl_3CN was extremely slow in the presence of HCl in contrast to aromatic nitriles trimerized readily in the atmospheric pressure, the addition of a small amount of anhydrous $AlBr_3$ with HCl in trimerization of CCl_3CN greatly activates the reaction, reducing time required for completion of the reaction to only a few hours. Also in the cotrimerization of CCl_3CN with other cyanides, the complex catalyst gave good results, and only 2-substituted-4,6-bis(trichloromethyl)-1,3,5-triazines were obtained when molar ratio, CCl_3CN/other cyanide=2/1, was used. The mechanisms of (co)trimerization are very complicated and experiments are under way to elucidate them in our laboratory.

1.2 Haloform type of reactions of trichloromethyl-1,3,5-triazines with amines or alcohols

The amino-1,3,5-triazines (**10**–**37**) were prepared by the reaction of 2-substituted-4,6-bis(trichloromethyl)-1,3,5-triazines (**1**–**9**, **30**, **38**) with appropriate amines. Although the CCl_3-group in 2-substituted-4,6-bis(trichloromethyl)-1,3,5-triazines was readily replaced by means of concentrated ammonium hydroxide or alkylamines to give 2-amino-4-substituted-6-trichloromethyl-1,3,5-triazines (**10**–**26**, **30**–**37**), 2-arylamino-4-substituted-6-trichloromethyl-1,3,5-triazines (**27**–**29**) were obtained through the reaction of trichloromethyl-1,3,5-triazines with arylamines only in the presence of trialkylamine. Since chloroform was evolved in the reactions, it was considered that CCl_3-group was replaced by amino group *via* haloform type of reactions. In the reaction of 2,4,6-tris(trichloromethyl)-1,3,5-triazine with ammonia or amines, one or two of the CCl_3-group could be replaced, but the third was resistant to attack by the amines. The replacement of the first CCl_3-group took place at 0–10°C, while the second group replaced at 20–30°C. 2-Methoxy-4,6-bis(trichloromethyl)-1,3,5-triazine was prepared by the reaction of 2,4,6-tris(trichloromethyl)-1,3,5-triazine (**1**) with methanol in the presence of triethylamine at 0°C, through the similar haloform type of reaction of the 1,3,5-triazine (**1**) with amines.

The ^{1}H NMR spectrum of 2-methyl-4-methylamino-6-trichloromethyl-1,3,5-triazine (**15**) in $CDCl_3$ showed a set of resonance for the particular protons, *i.e.*, two signals at δ 2.54 and 2.60 for the 2-methyl protons, two signals at δ 3.10 (d, J=4.88 Hz) and 3.12 (d, J=4.88 Hz) for the N-methyl protons and two signals at δ 6.29 and 6.54 for the NH proton. The ^{13}C NMR spectrum of the compound **15** also showed a similar spectral characteristic *i.e.*, two signals at δ 25.49 (q, J=128.7 Hz) and 25.90 (q, J=128.7 Hz) for the 2-methyl carbon, two signals at δ 22.87 (q, J=139.5 Hz) and 28.04 (q, J=139.7 Hz) for the N-methyl carbon, and two signals at δ 95.96 and 96.18 for the trichloromethyl carbon, two signals at δ 166.29 and 166.59 for the C-4 carbon, two signals at δ 172.42 and 173.21 for the C-6 carbon and two signals at δ 177.14 and 178.56 for the C-2 carbon. However, the dimethylamino analog (**20**) did not exhibit such sets of resonances in both ^{1}H and ^{13}C NMR spectra (see ^{1}H and ^{13}C NMR data described in MATERIALS AND METHODS *1.2*). The observed

spectral characteristic of compound **15** may be explained in terms of the non-equivalency of the observed nuclei caused by the molecular unsymmetry and the restricted rotation between the ring carbon (C-4) and methylamino nitrogen bond. The strong electron-withdrawing property of the trichloromethyl group can attract the π-electrons on the triazine ring and further the lone electron pair on the methylamino nitrogen, rendering the bond between C-4 and nitrogen a kind of partial-double bond character. Accordingly several sets of two resonances in ^{1}H and ^{13}C NMR spectra can be observed due to a sort of syn-anti isomerism of C–N bond occured in magnetic field.

1.3 Reaction of 2-chloro-4,6-bis(trichloromethyl)-1,3,5-triazine with phenol

Since the haloform reaction of 2,4,6-tris-(trichloromethyl)-1,3,5-triazine (**1**) with phenol did not take place even in the presence of triethylamine, 2-(*p*-chlorophenoxy)-4,6-bis(trichloromethyl)-1,3,5-triazine (**39**) was prepared by the reaction of 2-chloro-4,6-bis(trichloromethyl)-1,3,5-triazine with *p*-chlorophenol. By the reaction of 2-chloro-4,6-bis(trichloromethyl)-1,3,5-triazine with anilines, 1,3,5-triazines (**27**–**29**) were also prepared.

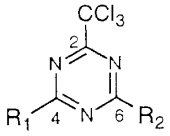
2. Relation between Structure and Nitrification Inhibitory Activity of 1,3,5-Triazines

Various trichloromethyl-1,3,5-triazines and their nitrification inhibitory activity are listed in Table 2. The pI_{50} values of highly active trichloromethyl-1,3,5-triazine were 4.5–5.5. Compounds **17** and **30** were the most potent nitrification inhibitors, compounds **2**, **3**, **26**, **31** and **38** were also active, followed by **11**, **15**, **16**, **20**, **21**, **24**, **25**, **32**, **33** and **35**. Compounds **6**, **12**, **14**, **22**, **23**, **27**, **28** and **34** were moderately active inhibitors. Less activity was observed with other compounds tested. Essentially they have a CCl_3-group and an amino or alkylamino group in the three substituents of 1,3,5-triazine ring. For example, the compound (**10**–**37**) with amino group(s) are more active than the compound (**1**–**9**, **38**, **39**) without amino group. Especially compound **10**, **17** and **26** were most potent inhibitors. Introduction of short chain (C_1-C_3) alkyl group in amino substituent improved the inhibitory activity. Introduction of aryl group in amino substituent gave less active compounds (**27**–**29**). This fact indicates that some suitable hydrophobicity is necessary for the activity. The third substituent in the active 2-amino-4-trichloromethyl-1,3,5-triazine should be methyl (**15**–**18**, **20**, **21**, **31**) or methoxyl (**24**–**26**) for high activity. It is summarized that the substituents combination consisted of a CCl_3-group, a short chain alkyl amino group and methyl or methoxyl group increase the nitrification inhibitory activity. QSAR study was also carried out to obtain the best combination of three substituents of 1,3,5-triazine ring. Electronic parameters, *e.g.* Hammett σ constants, and steric parameters, *e.g.* Verloop sterimol parameters, were omitted from QSAR study, because the inhibitory effect was not correlated with parameters mentioned above. The best correlation obtained was expressed by the Eq. (4) using $\log P(S)$.

$$pI_{50} = -0.118[\log P(S)]^2 \underset{(\pm 0.036)}{} + 0.626 \log P(S) + 4.078 \quad (4)$$
$$(\pm 0.318) \qquad (\pm 0.686)$$
$$[n=39,\ r=0.903,\ s=0.217]$$

In this equation, n stands for the number of data, r the correlation coefficient and s the standard deviation. The figures in parenthesis are the 95% confidence intervals. The Eq. (4) indicates that hydrophobic parameters, or $\log P(S)$, play an important role in determining the activity, as we predict in the quantitative consideration on the inhibitory-activity mentioned previously.[2] To confirm whether the $\log P$ relates aggregately to the permeability of the compounds in soil environment or directly to the action mechanism of the inhibitors against the nitrifying microorganisms in soil, further study has started already in our laboratory.[11,12] Here, the $\log P(H)$ values of the 1,3,5-triazines were estimated by rather laborious HPLC determination. If $\log P$ values are always available and used conveniently by synthetic chemists, the molecular design of the active 1,3,5-triazine nitrification inhibitors will proceed rapidly. Therefore we designed and estimated $\log P(S)$ values according to a semi-empirical method described in MATERIALS AND METHODS *3.2.* The optimum $\log P(S)$ in

Table 2 pI_{50} and log P values of 1,3,5-triazines.



No.	R_1	R_2	mp (°C)	pI_{50}	log P(S)
1	CCl_3	CCl_3	94–95	4.48	3.70
2	CCl_3	CH_3	96–97	5.17	4.07
3	CCl_3	CH_3S	71–72	5.12	4.21
4	CCl_3	C_6H_5	97–98	4.19	5.26
5	CCl_3	C_6H_4-Cl-*p*	158–159	3.89	6.03
6	CCl_3	S-C_6H_5	65–67	4.70	5.40
7	CCl_3	S-C_6H_4-Cl-*p*	68–69	3.67	6.17
8	CCl_3	SCH_2-C_6H_5	84–85	3.74	5.93
9	CCl_3	SCH_2-C_6H_4-Cl-*p*	91–93	3.28	6.69
10	CCl_3	NHC_2H_5	79–81	5.18	3.66
11	CCl_3	NHC_3H_7	45–47	4.88	4.19
12	CCl_3	NHC_4H_9	Liquid	4.61	4.72
13	CCl_3	NHC_5H_{11}	Liquid	4.32	5.25
14	CCl_3	NH-CH_2-C_6H_5	95–97	4.55	4.86
15	CH_3	$NHCH_3$	126–127	5.05	3.50
16	CH_3	NHC_2H_5	53–54	4.98	4.03
17	CH_3	NHC_3H_7	75–76	5.28	4.56
18	CH_3	NHC_5H_{11}	Liquid	4.49	5.61
19	CH_3	NHC_7H_{15}	46–47	3.81	6.66
20	CH_3	$N(CH_3)_2$	58–60	5.01	3.71
21	CH_3	NH-CH_2-C_6H_5	46–48	4.82	5.22
22	CH_3S	NHC_2H_5	54–56	4.73	4.17
23	CH_3S	NHC_3H_7	51–53	4.50	4.70
24	CH_3O	$NHCH_3$	134–136	4.89	3.04
25	CH_3O	NHC_2H_5	50–51	4.89	3.57
26	CH_3O	$N(CH_3)_2$	143–145	5.12	3.26
27	CCl_3	NHC_6H_5	112–114	4.68	4.33
28	CH_3	NHC_6H_5	107–108	4.73	4.70
29	CH_3S	NHC_6H_4-Cl-*p*	122–124	4.01	5.60
30	CCl_3	NH_2	154–158	5.31	2.45
31	CH_3	NH_2	160–161	5.17	2.82
32	C_6H_5	NH_2	175–176	4.79	4.01
33	C_6H_5-S	NH_2	122–123	4.82	4.15
34	*p*-Cl-C_6H_4-S	NH_2	149–150	4.68	4.92
35	C_6H_5-CH_2S	NH_2	145–147	4.86	4.68
36	*p*-Cl-C_6H_4-CH_2S	NH_2	120–122	4.39	5.45
37	NH_2	NH_2	235–236	4.64	1.21
38	CCl_3	OCH_3	45–46	5.14	3.61
39	CCl_3	O-C_6H_4-Cl-*p*	92–94	4.41	5.57

the Eq. (4) was calculated as 2.91, a little more hydrophobic compared with the log P value (*ca.* 2.4) in our previous paper.[2] Accordingly, it is considered that the activity of trichloromethyl-1,3,5-triazine nitrification inhibitors may become maximum when log P value is below 3.0. By the use of this value, it will become to possible to design highly active 1,3,5-triazine nitrification inhibitors. Design and synthetic studies of 1,3,5-triazine nitrification inhibitors using QSAR have yet to be investigated further before we make sure

how this approach using QSAR can be applied widely in the development of new nitrification inhibitors.

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要　　約

新規トリクロロメチル-1,3,5-トリアジン系硝酸化成抑制剤の合成と構造活性相関

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トリクロロメチル-1, 3, 5-トリアジン系化合物は，硝酸化成抑制剤としての効果が知られている．本研究では，新規トリクロロメチル-1, 3, 5-トリアジン系硝酸化成抑制剤を求めて，合成，硝酸化成抑制効果の測定および定量的構造活性相関を行なった．トリクロロメチル-1, 3, 5-トリアジン系化合物は，ルイス酸触媒を用いた(共)三量化反応およびトリクロロメチル-1, 3, 5-トリアジン系化合物の求核置換反応により合成した．硝酸化成抑制効果は，畑土壌を用いて硝酸菌に対する阻害効果を測定することによって求めた．高活性を示す化合物は，pI_{50} が 4.5～5.5 であり，(アルキル)アミノ基およびトリクロロメチル基を必ず一つもつものであった．また，半経験的計算法によって求めた疎水性パラメータ $\log P$(S) と，硝酸化成抑制効果 pI_{50} との間に良好な相関が見られ，最適 $\log P$(S) は 2.91 であることがわかった．また前報での最適 $\log P$ が約 2.4 であったことから，今後トリクロロメチル-1, 3, 5-トリアジン系硝酸化成抑制剤を検索するにあたっては，$\log P$(S) が 3 以下の化合物を分子設計することにより，高活性の化合物を得ることが期待できる．