Synthesis of Isothiocyanates and their Inhibitory Effects upon Soil Nitrification

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Several isothiocyanates were synthesized and their inhibitory effects upon soil nitrification were examined.

Some aryl isothiocyanates, such as 2,4- and 2,5-dichlorophenyl isothiocyanates, showed high activities.

INTRODUCTION

It is known that most of the nitrogenous fertilizers, such as urea and ammonium sulfate, are nitrified to some extent primarily by soil microorganisms and the nitrates so formed undergo leaching or reductive denitrification. If the nitrification is inhibited by chemical substances, nitrogenous fertilizers will be utilized better by plants.

Recently, the compounds effective for this purpose have been widely surveyed. J. H. Quastel and P. G. Scholefield pointed out¹⁾ that all the five active compounds, namely, methyl carbamate, ethyl N-methylcarbamate, guanidine, methylguanidine, and thiourea, have a common structure:

They reported also that other N-non-substituted or N-monosubstituted carbamates have the high activities.²⁾

We found that several N-monosubstituted

thionocarbamates as well as thioureas are effective, too.³⁾

Nishihara found that N-monosubstituted dithiocarbamates are more effective than N,N-disubstituted ones.⁴⁾ Various dithiocarbamates have long been known to be physiologically active⁵⁾ and they are widely used also in agriculture as pesticides. Most of these dithiocarbamates are, however, N-alkyl or N-alkylene substituted and not N-aryl derivatives. In a preliminary examination,³⁾ we found that several metal salts of N-phenyldithiocarbamic acid inhibit nitrification seriously; but as might be anticipated, they are unstable and the activities were gradually decreased as they decomposed.

Fungicidal dithiocarbamates are commonly classified into two groups:

- (1). N-Non- and N-monosubstituted,
- (2). N,N-Disubstituted.

H. L. Klöpping and G. J. M. van der Kerk in 1951, suggested that antifungal action of

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1) J. H. Quastel and P. G. Scholefield, *Bact. Revs.*, **15**, 1 (1951).

2) J. H. Quastel and P. G. Scholefield, *Appl. Microbiology*, **2**, 282 (1953).

3) Y. Kinoshita, A. Kage, Y. Oshima, T. Harada and T. Nishihara, unpublished data.

4) T. Nishihara, *Bull. Fac. Agr. Kagoshima Univ.*, No. 12, 107 (1962).

5) G. D. Thorn and R. A. Ludwig, "The Dithiocarbamates and Related Compounds," Elsevier, Amsterdam-New York, 1962.

the former is primarily connected with their conversion to isothiocyanates.⁶⁾

The fact that there exists a common structure,

in the nitrification inhibitors mentioned above, suggests those compounds having the general structure,

might also have the inhibitory activities. The present work was carried out to ascertain this possibility.

PREPARATION AND PROPERTIES OF MATERIALS

Following synthetic methods were adopted.

1. Isothiocyanates

2. Isocyanate

3. Carbodiimide

Some properties of synthetic compounds are shown in Table I. G. M. Dyson and H. J. George tried to prepare 2,6-dichlorophenyl isothiocyanate by the reaction of 2,6-dichloroaniline and thiophosgen, but obtained only the starting materials.¹³⁾ We obtained this sterically hindered isothiocyanate following the method of J. N. Baxter et al.,⁷⁾ involving pyrolysis of 2,6-dichlorophenyl-thiourea in chlorobenzene. 2,6-

TABLE I. PHYSICAL PROPERTIES OF SYNTHETIC COMPOUNDS

Compound Isothiocyanate $\text{R} \cdot \text{N}=\text{C}=\text{S}$ R	M. p.(B. p.)	Synthetic Method (Yield %)*	IR-Absorptions $\nu_{\text{N}=\text{C}=\text{S}}$ $\nu_{\text{N}=\text{C}=\text{X}}$ (cm^{-1})
CH_3	(45~7°/45 mm)	C(46.0)	2280, 2180
Cyclo- C_6H_{11}	(110~2°/20 mm)	C(50.7)	2240, 2160, 2100
$\text{C}_6\text{H}_5 \cdot \text{CH}_2$	(133~4°/18 mm)	C(53.7)	2250, 2150
C_6H_5	(120°/35 mm)	B(72.0)	2230, 2140
<i>p</i> - $\text{CH}_3 \cdot \text{C}_6\text{H}_4$	26~7°	B(19.0)	2240, 2160
<i>p</i> - $\text{CH}_3\text{O} \cdot \text{C}_6\text{H}_4$	(111~4°/5mm)	B(10.4)	2240, 2160
<i>p</i> - $\text{F} \cdot \text{C}_6\text{H}_4$	23~5°	A(8.4)	2220, 2140
<i>o</i> - $\text{Cl} \cdot \text{C}_6\text{H}_4$	(160°/42 mm)	A(47.3)	2230, 2090
<i>m</i> - $\text{Cl} \cdot \text{C}_6\text{H}_4$	(158°/60 mm)	D(46.0)	2250, 2150
<i>p</i> - $\text{Cl} \cdot \text{C}_6\text{H}_4$	43~5°	D(54.0)	2230, 2140
<i>p</i> - $\text{Br} \cdot \text{C}_6\text{H}_4$	56~8°	A(70.2)	2250, 2160
<i>p</i> - $\text{I} \cdot \text{C}_6\text{H}_4$	75~6°	A(55.0)	2250, 2160
2,3- $\text{Cl}_2\text{C}_6\text{H}_3$	(166°/28 mm)	A(51.2)	2290, 2090
2,4- $\text{Cl}_2\text{C}_6\text{H}_3$	41~2°	A(68.6)	2270, 2080
2,5- $\text{Cl}_2\text{C}_6\text{H}_3$	(125°/3 mm)	A(80.2)	2260, 2090
2,6- $\text{Cl}_2\text{C}_6\text{H}_3$	42~3°	A(72.2)	2270, 2080
3,4- $\text{Cl}_2\text{C}_6\text{H}_3$	(168°/33 mm)	A(16.7)	2260, 2080
3,5- $\text{Cl}_2\text{C}_6\text{H}_3$	44~6°	A(16.0)	2300, 2090, 1990
Isocyanate Carbodiimide $\text{R} \cdot \text{N}=\text{C}=\text{O}$ R			
C_6H_5	(158~65°/760 mm)	E(75.5)	2320, 2280, 2240
$\text{R} \cdot \text{N}=\text{C}=\text{N} \cdot \text{R}$ R			
Cyclo- C_6H_{11}	(142~3°/9 mm)	F(70.0)	2130

* (A): from thiourea, (B): from amine, (C): from amine (D): from amine, (E): from amine, (F): from thiourea

Dichlorophenyl-thiourea was prepared modifying the method reported by R. L. Frank and P. V. Smith.¹⁴⁾

2,6-Dichlorophenylthiourea. To a hot solution of anhydrous ammonium thiocyanate (13 g) in 300 ml of dry acetone, was added dropwise 21.2 g of benzoyl chloride. After the reaction had subsided, a solution of 2,6-dichloroaniline¹⁵⁾ (24.3 g) in 200 ml of dry acetone was gradually added. With occasional swirling the mixture was refluxed for one hour. A half of the solvent was evaporated and the residue was poured into 2 l of water. The resulting yellow precipitate was separated by filtration. The crystalline 1-benzoyl-3-(2,6-dichlorophenyl)thiourea was saponified with a boiling solution of 250 g of 10 %

6) H. L. Klöpping and G. J. M. van der Kerk, *Rec. trav. chim.*, **70**, 949 (1951); *C. A.*, **46**, 5243a (1952).

7) J. N. Baxter, J. Cymmerman-Craig, M. Moyle and R. A. White, *J. Chem. Soc.*, 1956, 659.

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11) D. A. Shirley, "Preparation of Organic Intermediates," *Jone Wiley and Sons, Inc.*, 1951, p. 255.

12) T. Hashizume, *Yukikagobutsu Goseiho*, **13**, 22 (1961).

13) G. M. Dyson and H. J. George, *J. Chem. Soc.*, **125**, 1702 (1924).

14) R. L. Frank and P. V. Smith, *Org. Synth. Coll. Vol. III*, 735 (1955).

15) Margaret K. Seikel, *ibid.*, Coll. Vol. III, 262 (1955).

FIG. 1. IR Absorption Spectrum of 2,6-Dichlorophenyl Isothiocyanate (10 % CHCl_3 Sol.)

sodium hydroxide. After cooling, the solution was acidified with concentrated hydrochloric acid to precipitate both benzoic acid and 2,6-dichlorophenylthiourea, and then made alkaline with ammonium hydroxide to dissolve benzoic acid. 2,6-Dichlorophenylthiourea was filtered and recrystallized from ethanol. Yield, 20.0 g (60.3 %). m.p. 156~158°C. *Anal.* Found: C, 38.36; H, 2.82; N, 12.73. Calcd. for $\text{C}_7\text{H}_6\text{N}_2\text{Cl}_2\text{S}$: C, 38.02; H, 2.74; N, 12.67 %.

2,6-Dichlorophenyl isothiocyanate. A solution of 2,6-dichlorophenylthiourea (18 g) in 250 ml of chlorobenzene was refluxed for ten hours. The solvent was evaporated under reduced pressure and the residue was extracted with three 100-ml portions of *n*-hexane. The combined extract was evaporated and the residue was distilled under reduced pressure. The solidified distillate was crystallized from *n*-hexane. Yield, 12.0 g (72.2%). m.p. 42~43°C. *Anal.* Found: C, 41.49; H, 1.55; N, 6.82. Calcd. for $\text{C}_7\text{H}_3\text{NCl}_2\text{S}$: C, 41.20; H, 1.48; N 6.86%. Infrared absorption spectrum of this compound is shown in Fig. 1.

Derivative. Refluxing a mixture of 2,6-dichlorophenyl isothiocyanate (3.5 g) and excess methanol for twenty hours afforded methyl N-(2,6-dichlorophenyl)thionocarbamate. Yield, 3.0 g (74.1 %). m.p. 149°C from MeOH). *Anal.* Found: C, 40.71; H, 3.07; N, 9.95. Calcd. for $\text{C}_8\text{H}_7\text{NCl}_2\text{OS}$: C, 40.69; H, 2.99; N, 5.93 %.

BIOASSAY METHOD

A soil of definite physical and chemical condi-

tions,¹⁶⁾ 25 mg of urea-nitrogen per 100 g of an air-dry soil, and an inhibitor of indicated concentration were thoroughly mixed in a jar. The jar was incubated at 30°C for two weeks. The soil was analyzed for ammonium, nitrite, and nitrate nitrogens by the previous method.¹⁶⁾ (The conversion of urea to ammonium nitrogen was not seriously prevented by any of the compounds used.)

In this paper, we showed the Inhibition Index (I) which is calculated by the following equation:

$$I = 100(A - B)/25$$

wherein *A* represents mg of $\text{NH}_4\text{-N}$ per 100 g of dry soil containing the inhibitor, *B* represents mg of $\text{NH}_4\text{-N}$ per 100 g of dry soil without the inhibitor.

RESULTS AND DISCUSSION

Table II shows the activities of six com-

TABLE II. EFFECTS OF SIX COMPOUNDS HAVING THE GENERAL STRUCTURE, $\text{R}-\text{N}=\text{C}=\text{X}$.

Compound	M. W.	(I)	
		100 p.p.m.	50 p.p.m.
$\text{CH}_3-\text{N}=\text{C}=\text{S}$	78.120		3.6
- $\text{N}=\text{C}=\text{S}$	135.191	91.6	53.6
- $\text{CH}_2-\text{N}=\text{C}=\text{S}$	149.218	44.4	28.0
- $\text{N}=\text{C}=\text{S}$	141.239	73.2	38.0
- $\text{N}=\text{C}=\text{O}$	119.125	0.0	
- $\text{N}=\text{C}=\text{N}-$	206.335	16.8	0.0

¹⁶⁾ T. Harada, Y. Oshima, T. Nishihara and Y. Kinoshita, *J. Sci. Soil and Manure, Japan*. (Nippon Dojohiryogaku Gasshi) 35, 392 (1964).

pounds having the general structure, $R-N=C=X$. Three isothiocyanates other than methyl isothiocyanate exhibited moderate inhibitory activities, whereas phenyl isocyanate and dicyclohexylcarbodiimide showed little or no activities.

It was postulated that fungicidal dithiocarbamates or their breakdown products, such as isothiocyanates, combine with the sulfhydryl groups of essential enzymes¹⁷⁾ in the fungal cell system or with those of some constituents¹⁸⁾ of the cell membranes.

Though having similar chemical properties with isothiocyanates, phenyl isocyanate and dicyclohexylcarbodiimide were practically inactive possibly because of their instability under the experimental condition.

As phenyl isothiocyanate showed the highest activity of the four isothiocyanates, we compared the activities of some aryl isothiocyanates (Table III).

TABLE III. EFFECTS OF ARYL ISOTHIOCYANATES

Compound X	M. W.	Hammett σ Constant of X ¹⁹⁾	(I)	
			50 p.p.m.	20 p.p.m.
CH ₃ O—	165.218	-0.268	63.2	33.2
CH ₃ —	149.218	-0.170	57.6	10.8
H—	135.191	0.000	53.6	8.4
F—	153.183	+0.062	80.0	28.8
Cl—	169.640	+0.227	81.6	52.4
Br—	214.099	+0.232	72.0	48.0
I—	261.093	+0.276	52.4	38.8

In considering the relationship between the chemical structure and the inhibitory activity, it must be taken into consideration that the bioassay was not conducted with same molar concentrations but with same weight concentrations.

Generally, the larger the Hammett σ constant of the group X introduced into

benzene ring is, the more electrophilic the isothiocyanate becomes.²⁰⁾ But there is no complete parallel between the inhibitory activity and the electrophilicity of the compound. (cf. ref. 20). These results are not surprising, considering that fungicidal activity of a compound may be decided not only by chemical reactivity but stability, permeability into fungal cell, steric factor of the compound and other environmental factors.

Despite of marked increase in atomic weight in the order, $F < Cl < Br < I$, the inhibitory activities of *p*-halophenyl isothiocyanates at same concentrations were not altered considerably. These data suggest that the inhibitory activities of halophenyl isothiocyanates are more or less associated with their electrophilic strength.

Of these compounds in Table III, *p*-chlorophenyl isothiocyanate showed the highest activity. We, therefore, examined the effects of the three isomers of chlorophenyl isothiocyanate. (Table IV). The activities of these compounds were too high to be graded this time.

TABLE IV. EFFECTS OF CHLOROPHENYL ISOTHIOCYANATES

Compound	(I)	
	50 p.p.m.	20 p.p.m.
2-Cl	95.2	94.8
3-Cl	97.2	96.4
4-Cl	95.2	91.6

Table V shows the activities of all the six dichlorophenyl isothiocyanates. 2,4-Dichlorophenyl and 2,5-dichlorophenyl isothiocyanates exhibited the high activities; but four other compounds showed rather low activities.

If fungicidal isothiocyanates react, as mentioned above, with nucleophiles, such as sulfhydryl groups of essential enzymes, the

17) Kaars Sijpesteijn, A. and G.J.M. van der Kerk, *Biochim. Biophys. Acta*, **13**, 545 (1954).

18) R. T. Wedding and J. B. Kendrick, Jr., *Phytopathology*, **49**, 557 (1959).

19) Robert W. Taft, Jr., in M. S. Newman, "Steric Effects in Organic Chemistry," Wiley, New York, 1956, p. 571.

20) D. W. Browne and G. M. Dyson, *J. Chem. Soc.*, **1931**, 3285.

TABLE V. EFFECTS OF DICHLOROPHENYL ISOTHIOCYANATES

Compound		(I)		
		50 p.p.m.	20 p.p.m.	
Exp. (a)	2,3-Cl ₂	71.6	18.4	
	2,4-Cl ₂	92.0*	89.6*	
	2,5-Cl ₂	90.0	84.8	
	2,6-Cl ₂	57.6	49.6	
	3,4-Cl ₂	69.6	32.0	
	3,5-Cl ₂	87.6	49.6	
* Data from Exp. (b)				
		20 p.p.m.	10 p.p.m.	5 p.p.m.
Exp. (b)	2,4-Cl ₂	89.6	83.2	40.8
	2,5-Cl ₂	90.8	88.8	54.4

approach and the subsequent combination of these reactants may be largely influenced by the steric factors. This possibility was supported by the data of Table V.

Recently, Omura found that 2,5-dichlorophenyl isothiocyanate actually inhibits the cell-free ammonia-oxidizing enzymes system of chicken liver.²¹⁾

21) H. Omura, Personal Communication.

The data of Tables III and IV apparently show that different tests sometimes give varying results because the nitrifying abilities of the soil microorganisms differ from one test to another. We, therefore, compared the activities of 4-chlorophenyl and 2,5-dichlorophenyl isothiocyanates at the same time. (Table VI).

TABLE VI. COMPARISON OF THE ACTIVITIES OF 4-CHLOROPHENYL AND 2,5-DICHLOROPHENYL ISOTHIOCYANATES

Compound	(I)			
	30p.p.m.	20p.p.m.	10p.p.m.	5p.p.m.
4-Cl	67.2	55.6	34.4	16.8
2,5-Cl ₂	88.4	87.6	86.4	47.2

2,5-Dichlorophenyl isothiocyanate was found to be the most active compound of all the isothiocyanates examined.

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