Original Article

Photolysis and Hydrolysis of the Fungicide Procymidone in Water

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The chemical photolysis and hydrolysis of systemic fungicide procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide] were studied in buffered distilled water, or natural river and sea water. Procymidone was degraded in water mainly by hydrolysis, especially in basic media, whereas sunlight had a limited effect on the breakdown of the fungicide. Hydrolysis of procymidone proceeded predominantly through neutral (pH independent) and base-catalyzed processes below pH 5 and above pH 8, respectively, while both reactions occurred between pH 5 and pH 8. Within the range of pH 6–9 normally found in natural water the half-life of procymidone by hydrolysis ranged from 0.6 to 68 days at 15°C, from 2 hr to 18 days at 30°C, and from 0.5 hr to 8 days at 45°C. Temperature dependence for the hydrolysis rate was obtained from the Arrhenius equation. The product cleaved at the cyclic imide linkage was predominant in neutral and basic water, whereas the product cleaved further at the amide linkage was predominant in acidic water.

INTRODUCTION

Procymidone [Sumilex®, *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide] is a systemic fungicide for the control of plant diseases such as gray mould of grape vine and vegetable crops, and Sclerotinia rot of kidney beans and vegetable crops.^{1–3)} The metabolism studies of procymidone in rats,⁴⁾ and cucumber and bean plants⁵⁾ have already been performed.

This paper deals with the rates and routes of transformation of procymidone by natural sunlight in three kinds of water as well as by hydrolysis in buffered distilled water at pH ranging from 2 to 10, and in natural river and sea water at 15, 30 and 45°C.

MATERIALS AND METHODS

1. Chemicals

Procymidone labeled with ¹⁴C at the carbonyl group was synthesized by Yoshitake *et al.*⁶⁾ in the Takarazuka Research Center of Sumi-

tomo Chemical Co., Ltd. The preparation had a specific activity of 4.67 mCi/mmol with more than 99.9% radiochemical purity as determined by thin layer chromatography (TLC) and liquid scintillation counting. The following unlabeled compounds were prepared for reference⁷⁾: 2-(3,5-dichlorophenylcarbamoyl)-1,2-dimethylcyclopropane-1-carboxylic acid [referred to P-NH-COOH] and 1,2-dimethylcyclopropane-1,2-dicarboxylic acid [CP-(COOH)₂].

2. Radioassay

Liquid scintillation counting (LSC) and radioautography were carried out according to the methods reported previously.⁸⁾

3. Thin Layer Chromatography (TLC)

Precoated silica gel 60F-254 chromatoplates (20×20 cm layer thickness, E. Merck) were used for analysis. The *R_f* values for procymidone and its degradation products are reported elsewhere.⁵⁾ The resolved radioactive spots were detected by radioautography and

unlabeled compounds were detected by UV light or by exposure to iodine vapor.

4. Photolysis in Water

Distilled water, and natural river and sea water sterilized by passage through a 0.1 μm Millipore filter were used in the experiment. The natural water was collected from Muko River (pH 7.4) and Nishinomiya Seaside (pH 7.8) in Hyogo Prefecture. ^{14}C -Procymidone diluted appropriately with the unlabeled compound was added to each water and stirred for 12 hr in the dark to give a concentration of 3 ppm. One liter of each solution was placed in a two-liter quartz flask, and then exposed to natural sunlight for 28 days (8 hr per day from August to September in 1979) at Takarazuka, Hyogo, Japan. During the experiment, CO_2 -free air was continuously supplied over the water surface at 100 ml/hr and the effluent air was passed serially through a polyurethane foam plug and a gas washing bottle containing 400 ml of 0.5 N NaOH solution to trap the volatile ^{14}C including $^{14}\text{CO}_2$. A dark control experiment was also carried out by covering the flask with aluminum foil.

At specified intervals, the polyurethane foam plug was eluted three times with 30 ml of acetone and the extract was radioassayed. The NaOH solution was also radioassayed using Aquasol®-2 (New England Nuclear). An aliquot of the water sample (40 ml) was extracted three times with a two-fold volume of ethyl acetate after acidification to pH 2. The aqueous and organic layers were radiocounted, and the combined ethyl acetate extracts were

concentrated and examined by TLC.

5. Hydrolysis

Buffer solutions at almost the same ionic strength (μ 0.2–0.3) were prepared by dissolving the component salts in deionized-distilled water at the concentrations listed in Table 1. The pH of the solutions was adjusted to the desired values at 15, 30 and 45°C, and checked with a pH meter model F-7AD (Horiba Co., Ltd.) calibrated with standard buffer solutions. ^{14}C -Procymidone was dissolved in the buffer solutions or filter-sterilized natural river and sea water, and stirred at 25°C for 5 min to attain a level of 1 ppm (water solubility, 4 ppm at 25°C). Each 40 ml solution in a 50 ml volumetric flask was immersed in a darkened, thermostated-water bath at 15, 30 and 45°C. At various intervals after treatment, 2 ml aliquot of the solution was extracted with ethyl acetate (5 ml $\times$ 3) immediately after acidification to pH 1 with 1 N HCl. The combined ethyl acetate layer was washed with sat. NaCl solution, concentrated and examined by TLC.

RESULTS AND DISCUSSION

1. Photolysis

Procymidone gradually degraded in distilled water with half-lives of 8 days under sunlight irradiation and 10 days in the dark (Fig. 1). By contrast, the fungicide was unstable in slightly basic media such as river and sea water, and approximately 20–45% of procymidone applied was decomposed even in the dark during the preparation of the aqueous

Table 1 Composition of buffer solutions used in the experiment.

pH	5.0 M NaCl (ml)	1.0 M Glycine + 1.0 M NaCl (ml)	2.0 M HCl (ml)	2.0 M Sodium acetate (ml)	3.5 M Acetic acid (ml)	0.5 M Na_2HPO_4 (ml)	4.0 M NaH_2PO_4 (ml)	0.2 M Boric acid + 0.2 M KCl (ml)	0.2 M Na_2CO_3 (ml)	Total (l)	Ionic strength (μ)
2.0	72	10.6	14.7							2	0.2
5.0	72			20.0	3.7					2	0.2
6.3	72					9.2	6.6			2	0.2
7.1	72					22.7	1.6			2	0.2
8.1								88.8	11.2	0.2	0.3
9.0								63.0	37.0	0.2	0.3
10.1								29.1	70.9	0.2	0.3

Fig. 1 Photodegradation of ^{14}C -procymidone in water.

○: sunlight, ●: dark.

Fig. 2 Degradation pathways for procymidone by photolysis and hydrolysis in water.

solution. The half-life of disappearance was 0.6 day and 1.0 day in sea and river water, respectively, under sunlight or dark conditions. Thus, degradation of the fungicide in water seemed to occur chiefly through hydrolysis.

Two major degradation products were formed by cleavage at the cyclic imide linkage (P-NH-COOH) followed by cleavage at the corresponding amide linkage [CP-(COOH)₂] (Fig. 2). Evidence for their identity was obtained by TLC cochromatography with the authentic standards. After 28 days P-NH-COOH and CP-(COOH)₂ amounted to 42–87% and 6–32%

of the applied ^{14}C , respectively (Table 2). Under sunlight conditions P-NH-COOH had a tendency to decrease with time in river and sea water. Furthermore, greater amounts of CP-(COOH)₂ were detected in water under sunlight than under dark conditions. It is likely therefore that sunlight accelerates the cleavage of the amide linkage of P-NH-COOH. Photomineralization of the radiocarbon to $^{14}\text{CO}_2$ occurred to some extent.

2. Hydrolysis

The psuedo-first-order rate constant (k_H) for the hydrolysis of ^{14}C -procymidone in buffer solutions were determined (Table 3). The preliminary experiments verified that the hydrolysis proceeded through first order in terms of procymidone concentration, since a satisfactory agreement was obtained among the first-order rate constants for hydrolysis at pH 7 at different concentrations. The pH depend-

Table 2 Degradation of ^{14}C -procymidone in distilled water, river and sea water under sunlight or dark conditions.

	% of the applied ^{14}C											
	Distilled water				Sea water				River water			
	Sunlight		Dark		Sunlight		Dark		Sunlight		Dark	
Days after treatment	7	28	7	28	3	28	3	28	3	28	3	28
Volatile ^{14}C	0.2	2.0	<0.1	<0.1	0.1	1.9	<0.1	<0.1	<0.1	5.4	<0.1	<0.1
Polyurethane foam	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NaOH soln.	0.2	2.0	<0.1	<0.1	0.1	1.9	<0.1	<0.1	<0.1	5.4	<0.1	<0.1
Extract ^{14}C	99.6	97.4	99.8	95.5	99.5	97.3	99.8	99.7	99.3	91.0	99.7	98.7
Procymidone	57.7	8.6	62.8	17.4	4.2	4.2	7.2	6.0	3.5	2.9	14.5	7.3
P-NH-COOH	33.4	61.9	29.5	68.8	87.0	61.5	80.9	85.8	82.6	41.9	76.2	79.2
CP-(COOH) ₂	7.0	17.0	7.5	7.0	7.1	22.0	5.5	8.0	11.6	32.0	6.1	12.1
Others	1.5	9.9	0.1	2.5	1.2	9.2	6.2	0.1	1.6	14.2	2.9	0.1
Total	99.8	99.4	99.8	99.8	99.6	99.2	99.8	99.7	99.3	96.4	99.7	98.7

Table 3 Pseudo-first-order rate constants (sec^{-1}) for hydrolysis of procymidone (k_H) and for formation of P-NH-COOH (k_a) and CP-(COOH) $_2$ (k_b) at 15, 30 and 45°C.

pH	15°C			30°C			45°C		
	$k_H \times 10^7$	$k_a \times 10^7$	$k_b \times 10^7$	$k_H \times 10^7$	$k_a \times 10^7$	$k_b \times 10^7$	$k_H \times 10^6$	$k_a \times 10^6$	$k_b \times 10^6$
2.0	0.13	0.06	0.07	0.66	<0.01	0.66	0.24	<0.01	0.24
5.0	0.27	0.15	0.12	1.27	<0.01	1.27	0.41	<0.01	0.41
6.3	1.00	0.92	0.08	3.08	2.31	0.77	0.98	0.49	0.49
7.1	2.40	2.40	<0.01	19.5	19.5	<0.1	9.38	9.38	<0.01
8.1	14.3	14.3	<0.1	121.0	121.0	<1.0	53.1	53.1	<0.1
9.0	150.0	150.0	<1.0	—	—	—	—	—	—
River (pH 7.4)	4.39	4.39	<0.01	34.1	34.1	<0.01	22.0	22.0	<0.01
Sea (pH 7.8)	8.66	8.66	<0.01	128.0	128.0	<1.0	51.2	51.2	<0.1

Table 4 Temperature dependence of rate constants in neutral (k_N) and base-catalyzed (k_B) hydrolysis of procymidone.

	Temp. (°C)	Rate constants			Arrhenius expression
		$k_H \times 10^7$ (sec^{-1})	$k_N \times 10^7$ (sec^{-1})	k_B ($\text{M}^{-1} \cdot \text{sec}^{-1}$)	
Neutral (pH 5.0)	15	0.24	0.24		$\log k_N = 5.22 - 3.68 \times 10^3/T$
	30	1.29	1.29		
	45	3.93	3.93		
Base-catalyzed (pH 9.0)	15	1.52×10^2		1.52	$\log k_B = 15.3 - 4.33 \times 10^3/T$
	30	1.03×10^3		1.03×10^4	
	45	4.07×10^3		4.07×10^4

Fig. 3 pH-Rate profile for procymidone hydrolysis.

●: observed, ○: calculated, ▲: river water, ■: sea water.

ence for hydrolysis of procymidone at 15, 30 and 45°C is expressed in Fig. 3 by a plot of $\log k_H$ as a function of pH. From this expres-

sion, it can be seen that the hydrolysis proceeds through a neutral reaction between pH 2 and pH 5 since the hydrolysis rate is almost independent upon pH at any temperature tested. By contrast, the base-catalyzed process is predominant above pH 7–8 where a slope +1 is observed, and both basic and neutral reactions occur between pH 5 and pH 8. Acid-catalyzed hydrolysis was not significant as compared with neutral and base-catalyzed hydrolysis, and therefore the pseudo-first-order rate constant (k_H) and the half-life of procymidone by hydrolysis ($T_{1/2}$) can be expressed by Eqs. (1) and (2), respectively,

$$k_H = k_N + k_B[\text{OH}^-] \quad (1)$$

$$T_{1/2} = \ln 2/k_H \quad (2)$$

where k_N and k_B are the rate constants for neutral and basic hydrolysis, respectively.

Temperature dependence of k_N and k_B was determined at pH 5 and pH 9, respectively,

Fig. 4 Hydrolytic half-life values of procymidone in buffer solutions at 15, 30 and 45°C.

△: river water, ○: sea water.

where the neutral and base-catalyzed processes were predominant, using the Arrhenius Eq. (3)

$$k = A \exp(-E/RT) \quad (3)$$

where E is the activation energy and A is the preexponential factor. Table 4 shows the Arrhenius expression for k_H and k_B , along with the rate constants of k_H , k_N and k_B at pH 5 and pH 9. The Arrhenius parameters for the neutral hydrolysis are $E_N = 16.8 \text{ kcal} \cdot \text{mol}^{-1}$ and $\log(A/\text{sec}^{-1}) = 5.2$; for the specific base-catalyzed hydrolysis, $E_B = 19.8 \text{ kcal} \cdot \text{mol}^{-1}$ and $\log(A/\text{M}^{-1} \cdot \text{sec}^{-1}) = 15.3$. As shown in Fig. 2, the $\log k_H$ calculated from Eq. (1) together with the Arrhenius expression for k_N and k_B is in good accord with the observed value in buffer solutions.

Procymidone yielded two major hydrolysis products which were identified to be P-NH-COOH and CP-(COOH)₂ by TLC cochromatography with the authentic standards. The rate constants k_a and k_b for the respective formation of P-NH-COOH and CP-(COOH)₂ were determined and the results are listed in Table 3. P-NH-COOH was predominant in neutral and alkaline solutions, whereas CP-(COOH)₂ was predominant in acidic solutions below pH 5. It appears that the cyclic imide linkage is more susceptible to neutral and base-

catalyzed hydrolysis, and the amide linkage is more susceptible to acid-catalyzed hydrolysis.

To determine if this laboratory data could be extrapolated to natural water, the rate constants for hydrolysis of procymidone and for formation of the degradation products in river and sea water were also determined (Fig. 3 and Table 3). The kinetic data from experiments in buffer solutions are in good agreement with those in natural water, indicating no significant catalytic reaction by the chemicals in natural water. Therefore, the rate constant for hydrolysis of procymidone in natural water was found to be also expressed by Eq. (1) and the Arrhenius expression for k_N and k_B , and the half-life of procymidone by hydrolysis within the ranges of pH 6–9 normally found in natural water⁹⁾ could be estimated to be 0.6–68.0 days at 15°C, 2 hr–18 days at 30°C, and 0.5 hr–8 days at 45°C (Fig. 4).

The hydrolysis products such as P-NH-COOH and CP-(COOH)₂ were biodegradable and gradually degraded to CO₂ by microorganisms in natural water.¹⁰⁾ Each product is quite low in toxicity to carp with TLm 48 values of more than 20 ppm, and further appears not to accumulate to high degrees in aquatic organisms, considering that the water solubility is more than 50 ppm. It is likely therefore that there is little possibility of environmental deterioration caused by these compounds.

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要 約

殺菌剤プロシミドンの水中における光分解と加水分解

三上信可, 今西久美子, 山田宏彦, 宮本純之
プロシミドン (スミレックス®) の緩衝液もしくは自然水中における光分解と加水分解について検討した。プロシミドンの分解は, 太陽光照射条件下においていくらか促進されたものの, おもに加水分解を経て進行した。本化合物は, pH 5 以下では水による neutral reaction,

pH 7~8 以上では塩基触媒, pH 5~8 の範囲では両者の反応を受けて分解した。自然水において通常見いだされる pH 6~9 の範囲では, プロシミドンは半減期が 0.6 日~68 日 (15°C), 2 時間~18 日 (30°C), 0.5 時間~8 日 (45°C) の速度で加水分解された。また, Arrhenius の式を用いて加水分解速度を温度の関数として表し, 計算から求めた加水分解半減期は実測値とよく一致した。中性および塩基性条件下では環状イミドの加水分解物が, 一方, 酸性条件下ではさらにアミド結合が開裂した加水分解物が多く検出された。