水田除草剤ピラゾレートの水溶液中における加水分解

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Original Article

Hydrolysis of the Rice Herbicide Pyrazolate in Aqueous Solutions

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Hydrolysis of pyrazolate, [4-(2,4-dichlorobenzoyl)-1,3-dimethylpyrazol-5-yl] *p*-toluenesulfonate, was studied in buffer solutions in a range of pH 1.0–9.0 at 25°C, and in artificial gastric and intestinal fluids (pH 1.5 and 7.5) at 37°C. The hydrolysis proceeded predominantly through base- and acid-catalyzed processes in the regions above pH 7 and below pH 3, respectively, whereas both reactions occurred between pH 3 and pH 7. The half-life (hr) of pyrazolate ranged from 4.3 (pH 9.0) to 106.0 (pH 5.0) at 25°C, and was 2.3 (pH 7.5) and 7.8 (pH 1.5) at 37°C. Hydrolysates of pyrazolate in the wide range of pH at both temperatures were *p*-toluenesulfonic acid and 4-(2,4-dichlorobenzoyl)-1,3-dimethyl-5-hydroxypyrazole; *i.e.*, destosyl pyrazolate (DTP).

INTRODUCTION

Pyrazolate, [4-(2, 4-dichlorobenzoyl)-1, 3-dimethylpyrazol-5-yl] *p*-toluenesulfonate, is a new herbicide for control of both annual and perennial weeds in paddy fields.[1)]

Pyrazolate is hardly soluble in water, whose solubility in distilled water is 56 ppb at 25°C (M. Fujimoto, unpublished observation). In water solution, pyrazolate is hydrolyzed to give *p*-toluenesulfonic acid and 4-(2, 4-dichlorobenzoyl)-1,3-dimethyl-5-hydroxypyrazole; *i.e.*, destosyl pyrazolate (DTP).[1)] The latter compound, DTP, is the actually herbicidal entity of pyrazolate.[1-4)]

A metabolic study on pyrazolate in rats, using ^{14}C-preparations labeled either at the pyrazole ring or at the chlorinated phenyl ring, has revealed that the chemical, when orally administered, was metabolized to give DTP and its further metabolites, while a certain amount of unchanged pyrazolate was excreted in feces with the ratio clearly depending on the dose[5)]; *i.e.*, unchanged pyrazolate was excreted only in feces by a ratio of 64%, 67% and 74% at a level of 10, 100 and 1000 mg/kg, respectively, within 4 days, during which the administered radiocarbon was almost completely eliminated from the body. In addition, in eleven consecutive oral administration of ^{14}C-pyrazolate at 0.63 mg/kg/day, 17% of the dose was excreted in feces unchanged, while radiocarbon was nearly completely eliminated within several days after last administration.

Therefore, hydrolyzability of pyrazolate in aqueous media is important for a better understanding of the fate of the chemical in the environment and in warm blooded animals.

This study deals with hydrolysis of pyrazolate in buffer solutions in a range of pH 1–9 at 25°C and in both artificial gastric and intestinal fluids at 37°C.

MATERIALS AND METHODS

1. *Chemicals*

Both pyrazolate and DTP labeled with ^{14}C at the 3rd position of the pyrazole ring were synthesized by Yanai.[6)] The specific activity of each preparation was 5.78 mCi/mmol with a radiochemical purity of more than 98% as determined by thin-layer chromatography, followed by autoradiography and liquid scintil-

lation counting. Radiolabeled DTP was used as the reference standard.

2. Thin-layer Chromatography (TLC)

Precoated silica gel 60 F_{254} chromatoplates (20×20 cm, 0.25 mm layer thickness, E. Merck) were used for analytical purpose. The solvent systems used were: A) *n*-hexane–ethyl acetate–formic acid (10/10/1, v/v), B) benzene–ethanol (9/1, v/v), C) *n*-butanol–acetic acid–water (8/1/1, v/v), D) ethyl acetate–methyl ethyl ketone–formic acid–water (5/3/1/1, v/v). *Rf* values for pyrazolate and DTP were 0.82 and 0.19; 0.79 and 0.17; 0.89 and 0.49; and 0.99 and 0.81 for systems A, B, C and D, respectively.

3. Radioassay

Radioactivity was counted with a liquid scintillation counter (LSC-673, Aloka). Scintillation solution was composed of 8.0 g of PPO and 0.2 g of dimethyl-POPOP in a mixture of 500 ml each of ethanol and toluene.

4. Procedure for Hydrolysis

Buffer solutions were prepared by dissolving component salts in distilled water at the ratios shown in Table 1. The ionic strength (μ) of each solution was adjusted to 0.1 with potassium chloride. The artificial gastric (pH 1.5) and intestinal (pH 7.5) fluids were prepared in accordance with the literature.[7] The pH of the solutions was measured with a pH meter model MP-6 (Horiba Co., Ltd.) at 37°C for the artificial fluids and at 25°C for the others. All glasswares were autoclaved and each buffer solution was sterilized by passing through a 0.1 μm membrane filter (Toyo Roshi Co., Ltd.). Each buffer solution (99.5 ml) was separately transferred into a 150-ml Erlenmeyer flask with a glass stopper, and the flask was preheated at each desired temperature. Reaction was initiated by adding an acetonitrile solution (0.5 ml) of ^{14}C-pyrazolate (58,400 dpm) to the flask, followed by shaking for 3 min to attain 20 ppb. Each flask was shielded from light and immersed in a thermostatic water bath at 25±0.1°C or 37±0.1°C. At specified intervals, the whole volume of the solution was shaken with three 25-ml portions of toluene for 3 min, and for samples, except one at pH 9, the combined toluene extract was washed with two 10-ml portions of 0.01 N NaOH by shaking for 1 min to remove an acidic hydrolysate, ^{14}C-DTP (p*K*a 4.15[8]). The toluene extract (**I**) was washed once with 10 ml of saturated aqueous sodium chloride solution by shaking for 1 min, and the toluene phase was radioassayed, dried over anhydrous sodium sulfate, concentrated, and subjected to TLC in solvent systems A and B. The aqueous phase and all the washings were combined and shaken with three 25-ml portions of toluene after acidifying to pH 2 with 1 N HCl, and the resulting toluene extract (**II**) was radioassayed, concentrated and subjected to TLC in solvent systems C and D. All experiments were carried out in duplicate.

Table 1 Composition of buffer solutions.

pH	0.2 M KCl (ml)	0.2 M HCl (ml)	0.2 M KH_2PO_4 (ml)	0.2 M glycine (ml)	0.2 M NaOH (ml)	Total (*l*)	Ionic strength (μ)
1.0	3	97				0.2	0.1
3.0	48	2	50			0.2	0.1
5.0	47.5		50		2.5	0.2	0.1
7.0	20.4		50		29.6	0.2	0.1
9.0	91.2			50	8.8	0.2	0.1

pH	NaCl (g)	10% HCl (ml)	Na_2HPO_4 (g)	Total (*l*)
1.5	2	24		1
7.5		6	35.8	1

RESULTS AND DISCUSSION

Thin-layer chromatography of the toluene extract (**I**) and (**II**) revealed that unreacted ^{14}C-pyrazolate and its hydrolysate, ^{14}C-DTP, were clearly partitioned into (**I**) and (**II**), respectively, and that ^{14}C-DTP was a sole radiolabeled-product in every experiment.

Profiles of the disappearance rates of pyrazolate in each buffer system at 25°C are represented in Fig. 1. A linear correlation was observed between the amount of remaining pyrazolate (expressed by log %) and reaction time in each system.

Pseudo-first-order rate constants and half-lives of pyrazolate were calculated from the data in Fig. 1 in accordance with the following rate equation:

$$-dC/dt = kC$$

where C is a percentage of remaining pyrazolate at time t and k a pseudo-first-order rate constant.

As Table 2 shows, the rate constants increased with both increasing and decreasing pH of the reaction medium in the regions of above pH 7 and below pH 3 under the constant temperature at 25°C. The half-lives (hr) of pyrazolate were 52.7 at pH 3, 17.5 at pH 1, 25.0 at pH 7 and 4.3 at pH 9. The results indicate that hydrolysis of pyrazolate in aqueous solution involves both base- and acid-catalyzed reactions initiated by hydroxide ion and hydronium ion. At pH 5 the half-life of pyrazolate was 106.0 hr at 25°C, indicating the compound is also readily hydrolyzed in slightly acidic media.

As Table 2 shows, the rate constants of pyrazolate hydrolysis in the artificial gastrointestinal fluids at 37°C were considerably large, with the half-lives (hr) of the compound being 7.8 at pH 1.5 and 2.3 at pH 7.5. Although a direct comparison of these data at 37°C with the data in the similar pH regions (pH 1 and 7) at 25°C would be irrelevant, because the components of the reaction media are different, it seems that hydrolysis of pyrazolate is significantly enhanced with rising temperature.

A plot of log k at 25°C vs. pH in Fig. 2 demonstrates that the slope of the line in the basic region is steeper than that in the acidic region, and that both slopes are less than 1. This behavior is subsequently discussed. The nonintegral features might imply that some other processes than catalyzed hydrolysis at the ester bond of pyrazolate by hydroxide ion or hydronium ion are involved in the reaction. This is, however, a very unlikely

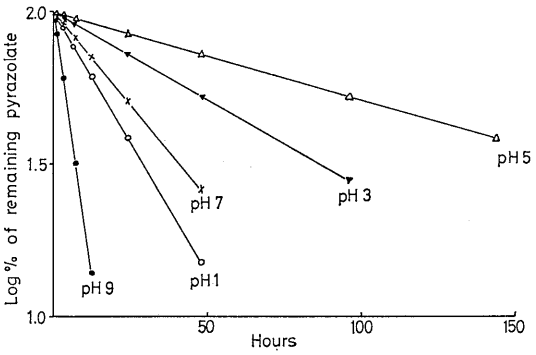

Fig. 1 Hydrolysis of pyrazolate in buffer solutions at 25°C.

Each value is the mean of duplicate samples.

Table 2 Rate constants and half-lives of hydrolysis of pyrazolate.

pH	Temp. (°C)	Rate constant (min^{-1})	Half-life (hr)
1.0	25	6.59×10^{-4}	17.5
3.0	25	2.19×10^{-4}	52.7
5.0	25	1.09×10^{-4}	106.0
7.0	25	4.62×10^{-4}	25.0
9.0	25	2.71×10^{-3}	4.3
1.5	37	1.48×10^{-3}	7.8
7.5	37	5.04×10^{-3}	2.3

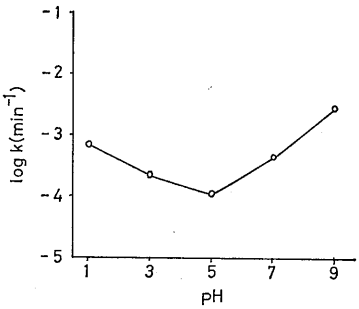

Fig. 2 pH-Rate profile for hydrolysis of pyrazolate at 25°C.

case, since DTP was a sole radiolabeled-product throughout the experiments at each reaction medium. The reason for deviation of the slopes from 1 in both acidic and basic regions remains unclarified; a real feature of pyrazolate hydrolysis may not be first-order with respect to concentrations of hydroxide ion or hydronium ion, or solubility of pyrazolate in buffered solutions may be significantly lower than that in distilled water. Pyrazolate concentration was maintained at a level of 20 ppb in the present experiments, which is less than half of the solubility of the compound in distilled water; however, even at this dilute concentration, the rate of dissolution of the applied pyrazolate to each reaction medium might have influenced the hydrolysis rates.

The present study clearly shows that pyrazolate, though it is hardly soluble in water, is readily hydrolyzed in solution to give DTP and *p*-toluenesulfonic acid irrespective of pH of the aqueous media. Its rapid hydrolysis rates suggest that the chemical hydrolysis is a predominant, if not exclusive, factor for transformation of pyrazolate in the aquatic environment over other factors, such as metabolism and sunlight photolysis. Additionally, the rapid hydrolysis of pyrazolate in the artificial gastrointestinal fluids together with the solubility of the compound in water suggests that pyrazolate orally administered to warm blooded animals is gradually dissolved and then rapidly hydrolyzed in the digestive tract, while undissolved part of the chemical remains unchanged and is excreted in feces.

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要　　約

水田除草剤ピラゾレートの水溶液中における加水分解

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ピラゾレート，[4-(2,4-ジクロロベンゾイル)-1,3-ジメチルピラゾール-5-イル] *p*-トルエンスルホナート，の緩衝液（pH 1～9, 25°C）および人工胃液と腸液（pH 1.5 と 7.5, 37°C）中における加水分解について検討した．ピラゾレートの加水分解反応速度は，pH 5 以上では反応液中の pH が高いほど，pH 5 以下では pH が低いほど，大きくなった．25°C での加水分解半減期は 4.3 時間（pH 9.0）から 106.0 時間（pH 5.0）で，37°C の人工胃液中では 7.8 時間，人工腸液中では 2.3 時間であった．いずれの条件においても，加水分解産物は *p*-トルエンスルホン酸および 4-(2,4-ジクロロベンゾイル)-1,3-ジメチル-5-ヒドロキシピラゾール（デストシルピラゾレート，DTP）であった．