pH Dependent Hydrolysis of 2-Hydroxypropyl Methanethiolsulfonate

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The hydrolysis of 2-hydroxypropyl methanethiolsulfonate (HPMTS), an industrial water treatment microbicide, was investigated at 25 °C and at three pH values (pH 5, 7, and 9). Radiolabeled (^{14}C) HPMTS at a concentration of 100 mg/L was used to determine rate constants and to identify hydrolytic degradation products. The hydrolysis at all three pHs followed *pseudo*-first-order kinetics with rate constants of 5.8×10^{-4}, 7.6×10^{-2}, and 7.3 h^{-1} at pH 5, 7, and 9, respectively. Two major radiolabeled hydrolytic degradation products were observed at all three pH values and were identified to be bis(2-hydroxyisopropyl) disulfide and bis(2-hydroxypropyl) disulfide by particle beam LC/MS. On the basis of the observed degradation products, a hydrolytic degradation pathway for HPMTS using propylene epoxide as a reactive intermediate was postulated.

Keywords: *2-Hydroxypropyl methanethiolsulfonate; HPMTS; hydrolysis; kinetics*

INTRODUCTION

Hydrolysis is one of the most important naturally occurring reactions in the environment and consequently represents one of the most important degradation pathways (Mabey and Mill, 1978; U.S. EPA, 1982). The knowledge of hydrolytic degradation pathways and kinetics over the normal pH ranges of the aquatic environment is critical for predicting the fate and transport mechanisms of a chemical. Because of its compatibility with aqueous samples, HPLC has been used extensively to monitor hydrolytic degradations of pesticides and industrial chemicals (Szeto, 1993; Smith and Aubin, 1993; Szeto *et al.*, 1989). The objective of this study was to determine the kinetics (rate constants and half-lives) of hydrolysis of HPMTS in water at three different pH values, so that the importance of hydrolysis as a transformation route could be better assessed with regard to the overall potential for persistence or elimination of HPMTS in the environment. Reversed phase HPLC and ion exclusion HPLC with radiometric detection were used to monitor the disappearance of [^{14}C]-HPMTS and the formation of radiolabeled hydrolytic products. Particle beam LC/MS was used for positive structural identification of hydrolytic products.

MATERIALS AND METHODS

Chemicals. Nonradiolabeled HPMTS, an off-white solid (CAS Registry No. 41206-16-0, molecular weight 170, chemical purity 96.5%), was obtained from Buckman Laboratories, Inc., Memphis, TN. [^{14}C]HPMTS, specific activity of 30.0 mCi/mmol and radiochemical purity of 91% (by HPLC), was obtained from Sigma Chemical Co., St. Louis, MO. Analytical grade 1-mercapto-2-propanol was obtained from Aldrich Chemical Co., Inc., Milwaukee, WI. Methyl iodide was obtained from Sigma. Hydrogen peroxide solution (3%) was obtained from Mallinckrodt Specialty Chemicals Co., Paris, KY, and used to oxidize thiol compounds. All other chemicals were of analytical or reagent grade. Water and organic solvents were of HPLC grade.

[^{14}C]Bis(2-hydroxypropyl)disulfide was synthesized by adding [^{14}C]HPMTS in dilute NaOH solution at room temperature.

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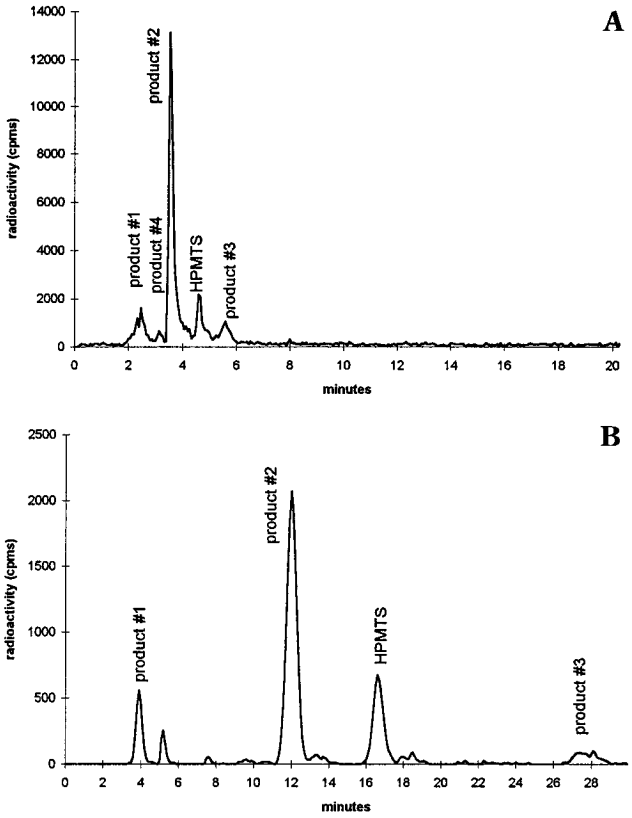

Figure 1. Representative HPLC radiochromatograms (A, reversed phase; B, ion exclusion) of a pH 9 test sample showing the separation of HPMTS and its hydrolytic degradation products.

Table 1. Rate Constants and Half-Lives of HPMTS Hydrolysis

pH	rate constant (h^{-1})	correl coeff (r^2)	half-life, $t_{1/2}$ (h)	total data points
5	5.87×10^{-4}	0.9367	1187.00	33
7	7.62×10^{-2}	0.9843	9.10	21
9	7.31	0.9897	0.10	24

The reaction mixture was partitioned with ethyl acetate. The extract was concentrated to dryness, and residues were redissolved in acetonitrile. This sample was used "as is" for

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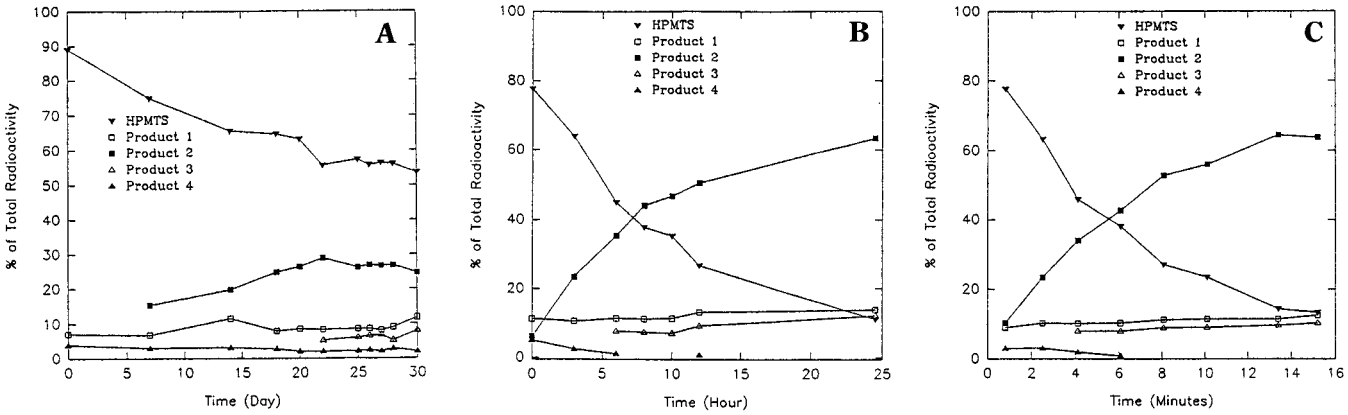

Figure 2. Formation and decline of HPMTS hydrolytic degradation products. (A) pH 5, replicate 1; (B) pH 7, replicate 1; (C) pH 9, replicate 1.

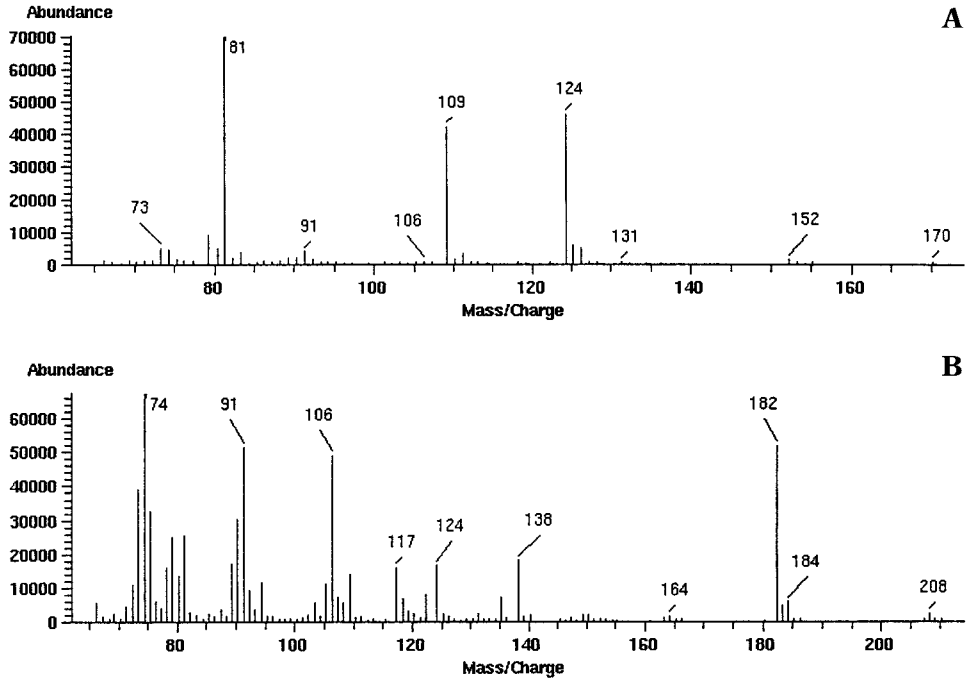

Figure 3. EI mass spectra of HPMTS (A) and bis(2-hydroxypropyl) disulfide (product 3) (B) in a pH 9 sample.

HPLC retention time determinations. The radio HPLC profile of this sample showed a single major product (>90%). The identity of this major product was confirmed by particle beam LC/MS to be [^{14}C]bis(2-hydroxypropyl) disulfide.

Test Procedures. The hydrolysis study was conducted at three pH values (pH 5, 7, and 9). pH buffers were prepared with sterilized HPLC grade water: pH 5, sodium acetate buffer (100 mM); pH 7, potassium phosphate buffer (50 mM); and pH 9, sodium borate buffer (35 mM). Triplicate test solutions were prepared at each pH in 100- or 200-mL volumetric flasks. The test concentration was 101 mg/L of HPMTS (containing 1.46% radiolabeled HPMTS). The specific activity of test solutions was calculated to be 5719.8 dpm/μg. (Nonradiolabeled HPMTS was used to increase the test concentration and subsequently enabled the positive identification of hydrolytic degradation products via mass spectrometry.) The three replicates of each HPMTS solution (pH 5, 7, and 9) were placed in a circulating water bath (Brinkman Lauda RMS-20) designed to maintain a constant temperature of 25 °C. At each sampling interval, aliquots of test solution were taken from each volumetric flask and analyzed by HPLC and liquid scintillation counting (LSC). Aseptic techniques were used during test setup and sampling for HPLC and LSC measurements.

HPLC. Two HPLC systems were developed for analysis of HPMTS and its hydrolytic degradation products: reversed phase (C_8 column) and ion exclusion (ORH-801 organic acid column) chromatography. The reversed phase system was used to quantify HPMTS and its degradation products during the study, while the ion exclusion system was used to confirm the identification of hydrolytic degradation products. HPLC instrumentation included a Waters 510 solvent pump, a Hewlett-Packard 1050 or a Waters 710B autosampler, a Beckman 171 or Radiomatic A-280 radioisotope detector with liquid scintillation cells (800 μL cell for the Beckman instrument and 1000 μL cell for the Radiomatic instrument), and a Hewlett-Packard 1047A refractive index (RI) detector.

Reversed phase HPLC separation was achieved on a Phenomenex Ultremex C_{18} (5 μm, 250 × 4.6 mm) column at ambient temperature. Isocratic mobile phase was 25/75 acetonitrile/water (pH 3 with phosphoric acid) at 1 mL/min. Ion exclusion HPLC was conducted using an Interaction ORH-801 organic acid column (300 × 6.5 mm) at 35 °C. Isocratic mobile phase was 10 mM H_2SO_4 at 0.8 mL/min. Eluted radioactivity was monitored by the flow-through radiometric detector, while nonradiolabeled reference standards were monitored by the RI detector. The flow rate of the scintillation cocktail (Beckman ReadyFlow III or Radiomatic Flo-Scint A) was set at 3 times the flow rate of HPLC eluent. [^{14}C]HPMTS and its radiolabeled degradation products were identified by comparison of their retention times with those of reference standards and quantified on the basis of integrated peak areas (dpms). To ensure that all injected radioactivity eluted off the analytical column and was measured by the detector, HPLC column recoveries were determined for each test sample. This was accomplished by comparing the total detector integrated

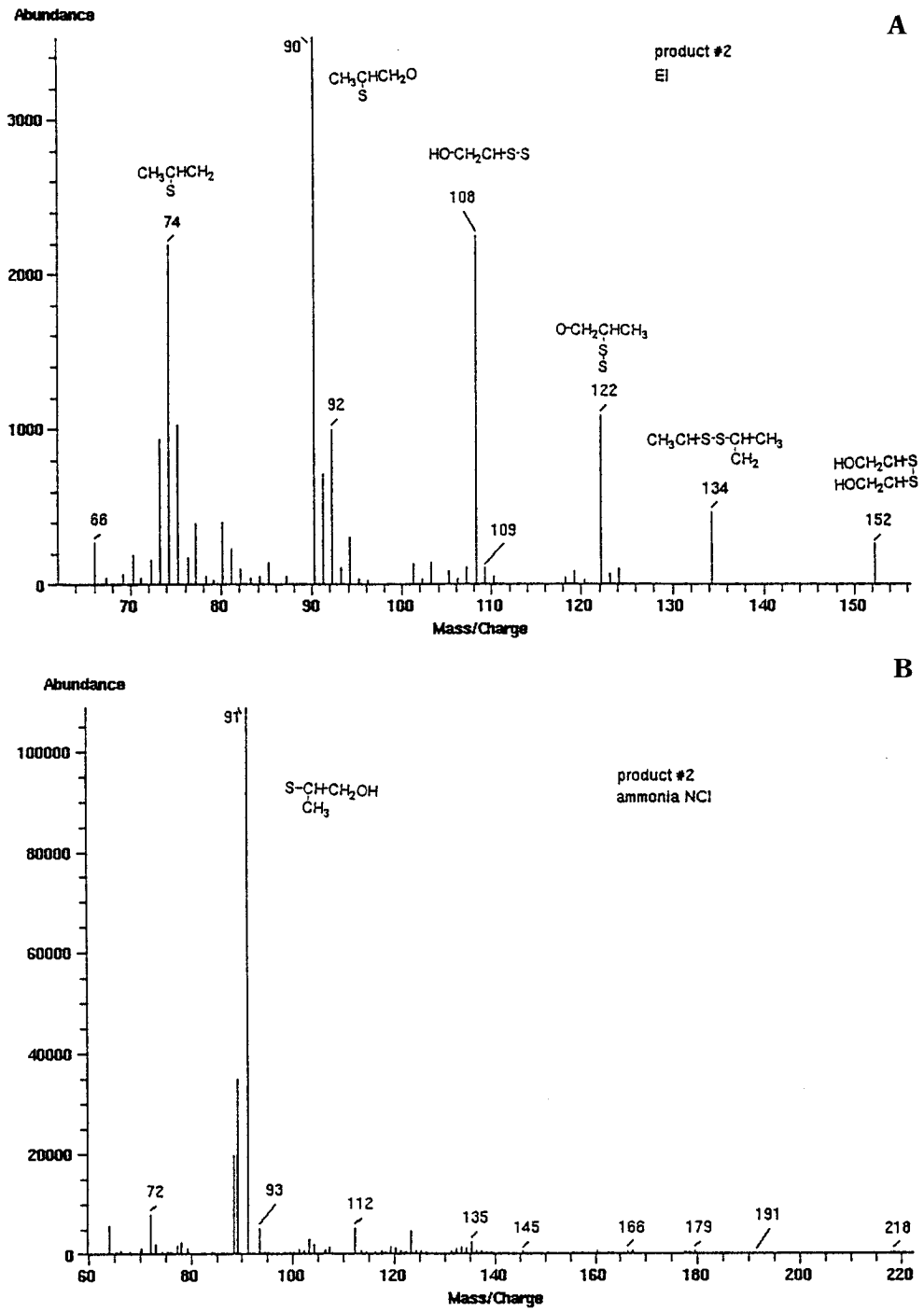

Figure 4. EI (A) and ammonia NCI (B) mass spectra of product 2.

radioactivity to that measured by LSC (Beckman LS 5000 TD or LS 1801 scintillation counter).

LC/MS. LC/MS analysis was conducted on a Hewlett-Packard Model 5989A MS engine equipped with a particle beam interface. Full scan electron impact ionization (EI) mass spectra were obtained using 70 eV of electron energy. Negative ion chemical ionization (NCI) was also used to analyze selected samples using either methane or ammonia as reagent gas. The ion source and analyzer temperatures were 250 and 125 °C for all analyses. The separation was achieved on a Nucleosil C_{18} (5 μm, 150 × 2 mm) column at ambient temperature. The mobile phase was 20/80/0.1 acetonitrile/water/acetic acid at 0.4 mL/min.

RESULTS AND DISCUSSION

Material Mass Balance. The measurement of total ^{14}C residue by LSC showed acceptable material mass balance for all test replicates during the course of the study. For pH 5 replicates, a mean mass balance of 110 ± 5% (n = 33) was obtained. For pH 7 and 9 replicates, mean mass balances of 96 ± 4% (n = 21) and 95 ± 2% (n = 25) were obtained, respectively. These adequate material mass balances suggested that, under the test conditions, HPMTS and its hydrolytic degradation products were not volatile and did not adsorb to the glass walls of test vessels.

Kinetics of HPMTS Hydrolysis. The hydrolysis of HPMTS at 25 °C was found to be pseudo-first-order and highly pH dependent. In the pH 5 test solution, the mean HPMTS concentration declined from 89.8 to 60.8 mg/L, with a mean of 54.1% of the parent compound remaining after a period of 30 days. In the pH 7 test solution, the mean HPMTS concentration declined from 78.3 to 12.2 mg/L, with a mean of 12.6% of the parent compound remaining after a period of 24.5 h. In the

pH 9 test solution, the mean HPMTS concentration declined from 72.7 to 12.6 mg/L, with a mean of 13.5% of the parent compound remaining after a period of approximately 15 min. HPLC column recoveries averaged 96 ± 5%, 98 ± 7%, and 99 ± 3% for pH 5, 7, and 9 samples, respectively. The kinetics data are summarized in Table 1.

Identification of Hydrolytic Degradation Products. The identification of hydrolytic degradation products produced in a greater than 10% yield was conducted using both chromatographic (based on retention times) and spectroscopic (LC/MS) techniques. Four degradation products were observed in test solutions at all three pH values (Figure 1). The formation and decline of these degradants are graphically presented in Figure 2. Among them, products 2 and 3 exceeded 10% of initial HPMTS concentration and were identified. Product 2 was a major product of HPMTS hydrolysis and appeared to follow the hydrolysis kinetics of HPMTS. Product 3 appeared to be a secondary reaction product rather than a product from the initial hydrolysis. Products 1 and 4 were impurities present in the radiolabeled HPMTS at the start of the study, and their concentrations increased very little with time during the experiment. The kinetics of these two products indicated that they were not hydrolytic degradation products of HPMTS. Since they were radiolabeled impurities, structural identifications were not attempted.

Identification of Product 3. To identify the two major degradants (products 2 and 3), a pH 9 sample (collected at the test termination) was extracted with ethyl acetate. The extract (containing HPMTS and products 2 and 3) was concentrated and analyzed by HPLC and LC/MS. Product 3 was identified to be bis(2-hydroxypropyl) disulfide by comparisons to the synthesized reference standard using reversed phase HPLC, ion exclusion HPLC, and particle beam LC/MS. EI mass spectra of HPMTS and product 3 are shown in Figure 3.

Identification of Product 2. EI and ammonia NCI mass spectra of product 2 are shown in Figure 4. To determine if product 2 was a thiol, a pH 9 (replicate 2) sample was reacted with hydrogen peroxide and the reaction mixture was monitored using ion exclusion chromatography. In the presence of an oxidizing agent (i.e. H_2O_2) thiols would be oxidized to disulfides and subsequently change their retention profiles. Results from this experiment were negative, indicating that product 2 was not a thiol. On the basis of the LC/MS data, the chemical structure of product 2 was proposed to be bis(2-hydroxyisopropyl) disulfide. This structure was consistent with the retention profiles of product 2 on both chromatographic systems since bis(2-hydroxyisopropyl) disulfide is a primary alcohol (diol) which was more polar than bis(2-hydroxypropyl) disulfide (a secondary alcohol).

Conclusions. The hydrolysis of HPMTS was determined to follow pseudo-first-order kinetics. The hydrolytic degradation was highly pH dependent, and kinetic half-lives were determined to be 50 days, 9 h, and 6 min at pH 5, 7, and 9, respectively. Two major and at least two minor hydrolysis products were observed. The two major degradation products were identified to be bis(2-hydroxypropyl) disulfide and bis(2-hydroxyisopropyl) disulfide. On the basis of these degradation products, a hydrolytic degradation pathway is postulated (Figure 5). HPMTS was postulated to undergo an intramolecular reaction to form a reactive intermediate—propylene epoxide. Propylene epoxide would react with HS^- to form thiols, which in turn were oxidized to disulfides. The formation of propylene epoxide and resultant thiols would be accelerated under basic conditions, which was consistent with the kinetics of hydrolysis. With propylene epoxide as the intermediate, possible degradation products could also include 1,2-propanediol and an isomer of bis(2-hydroxyisopropyl) disulfide.

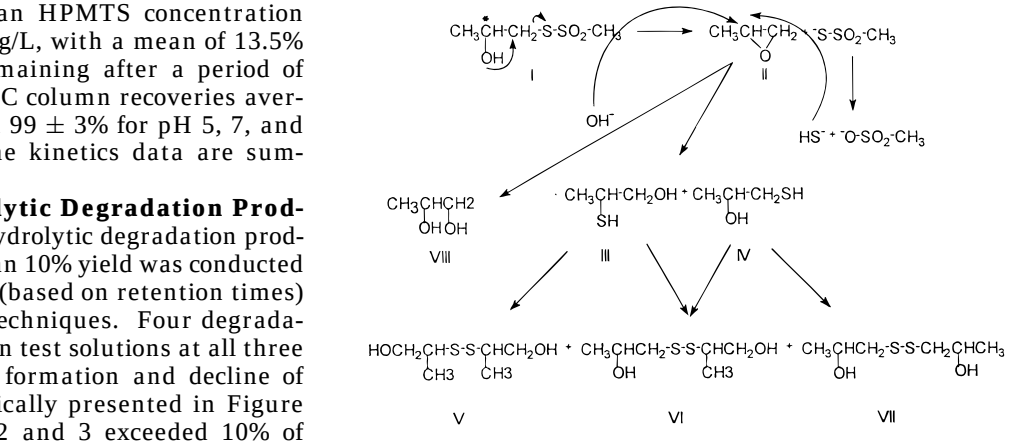

Figure 5. Proposed hydrolytic degradation pathway of HPMTS.

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