schungsgemeinschaft; the support by Professor M. A. Raftery is gratefully acknowledged.

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Dehalogenation of 5-Bromo-6-methoxy-5,6-dihydrothymine by Cysteine

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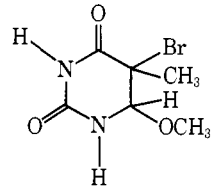
Abstract: The pseudo-first-order rate constants for the cysteine-promoted dehalogenation of 5-bromo-6-methoxy-5,6-dihydrothymine (BrMDHT) have a strict dependence on the concentration of cysteine thiol anion with a calculated second-order rate constant ($k_2^{cys\text{-}S^-}$) equal to 1652 ± 77 M^{-1} min^{-1}. The reaction which is not subject to catalysis by external buffers has thymine, Br^-, cystine, and some cysteic acid as products. Under conditions of excess cysteine, 2 mol of cysteine per mole of BrMDHT is consumed and about 88% of the BrMDHT is converted to thymine, a result which indicates the formation of 6-methoxy-5,6-dihydrothymine. Under conditions of excess BrMDHT relative to cysteine, the ratios of thymine produced to cysteine initially present are consistently above 0.50. These results argue for the participation of the E2 Hal mechanism in this reaction and are discussed in terms of the cysteine and bisulfite buffer promoted dehalogenation of the halouracils.

Halogenated pyrimidine derivatives are important antiviral agents which upon enzymatic dehalogenation lose their pharmacological activity. The pathways for enzymatic dehalogenation are not clearly elucidated; however, several hypothetical schemes have been advanced based on the abilities of bisulfite buffers[1-7] and thiols, such as cysteine and 2-mercaptoethanol, [8-10] to cause the dehalogenation, especially of 5-bromo- and 5-iodouracil. In the case of the dehalogenation of 5-iodouracil by cysteine, the intramolecular general acid catalyzed addition of cysteine to the 5,6 double bond of the pyrimidine ring system to presumably form 5-iodo-6-cysteinyl-5,6-dihydrouracil appears to control the overall rate of dehalogenation.[10] Two possible pathways, both of which have been identified in halopyrimidine dehalogenation by bisulfite buffers,[4-6] are possible for the subsequent dehalogenation of the iodocysteinyldihydrouracil intermediate. The first of these is the E2 Hal mechanism in which the thiol anion of cysteine attacks the halogen atom to initially yield a sulfenyl halide and an enolate anion of 6-cysteinyldihydrouracil. Further reaction then yields the final products: halide anion, cystine, and uracil. The second potential mechanism, proposed for the dehalogenation of 5-bromouracil by cysteine,[9] involves the SN2 attack of cysteine thiol anion on C-5 of the pyrimidine ring to yield halide anion and 5,6-dicysteinyl-5,6-dihydrouracil, which presumably further reacts with cysteine to yield uracil and cysteine. Very recent work in Pitman's laboratory, using both 5-bromo-6-methoxy-5,6-dihydrouracil and -dihydrothymine as a model for the dehalogenation of 5-bromo-5,6-dihydrouracil-6-sulfonate by SO_3^{2-},[5] has demonstrated the feasibility of the E2 Hal mechanism for similar reactions with simple thiols. The object of this report is to demonstrate the existence of the E2 Hal mechanism for the dehalogenation of 5-bromo-6-methoxy-5,6-dihydrothymine (I), a compound which can be considered a model for 5-bromo-6-cys-



I

teinyl-5,6-dihydrouracil, the proposed intermediate in the cysteine-promoted dehalogenation of 5-bromouracil.

Experimental Section

Materials. Glass distilled, deionized water stored in polyethylene bottles was used to prepare all stock solutions and reaction mixtures. Water and/or stock solutions were vigorously deaerated with water-saturated argon prior to use. Reagent grade inorganic reagents were obtained from Fisher Chemical Co. Thymine, bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bistris), ethylenediaminetetracetic acid disodium salt (EDTA), tris(hydroxymethyl)aminomethane (Tris), and 5,5′-dithiobis(2-nitrobenzoic) acid (Ellman's reagent) were obtained from Sigma Chemical Co. and used without further purification. Imidazole (Eastman Organic Chemicals) was recrystallized four times from hot benzene to remove a yellow impurity. Cysteine hydrochloride was obtained from Aldrich Chemical Co. [3-^{14}C]Cysteine hydrochloride (specific activity 15 mCi/mmol^{-1}) was obtained from Schwartz-Mann Co. 5-Bromo-6-methoxydihydrothymine was prepared by direct bromination of thymine in methanol, using a minor modification of the procedures used by Duschinsky et al.[11] for the synthesis of 5-bromo-5-fluoro-6-methoxy-5,6-dihydrouracil. The white crystalline product, after recrystallization from ethyl acetate, had the same physical properties as an authentic sample kindly provided by Dr. Ian Pitman, University of Kansas.

Kinetic Measurements. The rate of the cysteine-promoted dehalogenation of 5-bromo-6-methoxy-5,6-dihydrothymine (BrMDHT) was followed spectrophotometrically by recording the increase in the thymine absorbance at 275 nm. Measurements were obtained using a Zeiss PMQ II spectrophotometer equipped with a cell holder thermostated at 25 °C. Reactions were initiated by addition of 0.10 ml of 5×10^{-3} M freshly prepared BrMDHT solution to 2.90-ml solutions of the other reactants previously equilibrated to 25 °C in 3.00-ml capped cuvettes. Due to the sensitivity of cysteine to oxidation, all stock solutions were deaerated with water-saturated argon gas; transfers were performed using argon-purged pipettes and all reaction mixtures contained 5×10^{-3} M Na_2-EDTA. Ionic strength was maintained at 1.00 M by the addition of NaCl. Immediately upon completion of each reaction, the pH was measured using a Radiometer PHM26 pH meter equipped with a Radiometer GK 2321C combination electrode. Pseudo-first-order rate constants (k_{obsd}) were obtained from semilogarithmic plots of $A_\infty - A_t$ against time and the relationship $k_{obsd} = 0.693/t_{1/2}$.

Determination of pK_a Values. The acid dissociation constant at 25 °C and μ = 1.0 M of BrMDHT was determined spectrophotometrically at 240 nm in sodium carbonate buffers. Absorbance and pH measurements were obtained using the previously described instruments. The pK_a values for the thiol group and the protonated amine group of cysteine had been previously determined in this laboratory.[10]

Stoichiometry and Product Analysis. Thymine formation and cysteine utilization were determined under conditions of both excess cysteine and excess BrMDHT. In the former case, reaction mixtures 0.10 M in sodium phosphate buffer, pH 8.0, containing 11.70 μmol of cysteine and 23.40 μmol of cysteine, respectively, were allowed to react with 3.0 μmol of BrMDHT. Identical mixtures containing 3.0 μmol of thymine in place of BrMDHT were maintained as blanks. The reaction mixtures and accompanying blanks were incubated at 22 °C for 2.5 h to ensure completion of reaction, quenched by the addition of 0.30 ml 2.0 M HCl, and diluted ten times with H_2O. Thymine production was determined spectrophotometrically at 265 nm using $\epsilon_{thymine}^{265} = 0.77 \times 10^4$ M^{-1} cm^{-1}. Cysteine utilization was determined with Ellman's reagent.[12]

In the case of experiments conducted using an excess of BrMDHT, reaction mixtures and suitable blanks were 0.07 M in Tris buffer (pH 8.08) and 3×10^{-3} M Na_2 EDTA. BrMDHT concentration was either 5.33 or 6.40×10^{-3} M. Cysteine concentration varied from 0.26 to 1.6×10^{-3} M. After at least 2 h at room temperature (~23 °C) to ensure complete reaction, the samples and blanks were diluted tenfold with 0.10 N HCl. Absorbance at 265 nm was used to determine thymine concentration as previously described, thus allowing calculation of the ratio of thymine produced to cysteine initially present in the reaction mixtures.

To determine the utilization of cysteine to form cystine and to determine the relationship between cysteine and BrMDHT utilization under conditions of excess BrMDHT, [3-^{14}C]cysteine was employed. Reaction mixtures (16 ml) containing 0.12 mmol of BrMDHT, 0.01 mmol of cysteine hydrochloride, and 6×10^{-5} mmol of [3-^{14}C]cysteine hydrochloride adjusted with NaOH to pH 7.9 were incubated at 23 °C for 12 h sealed under argon. Controls without BrMDHT were treated in the same manner. Following incubation, the pH was adjusted to 2.0 with 1.0 N HCl. The reaction mixture and control were then quantitatively applied to duplicate Dowex 50 X4-H$^+$ columns, washed with 50 ml of H_2O, and eluted with increasing concentrations of HCl.[13] Fractions (15 ml) were collected immediately upon sample addition. Radioactivity was determined in aliquots of the column fractions using a Beckman liquid scintillation counter. Recovery of total radioactivity was 93 ± 2% for the chromatography of both the reaction mixtures and the control. Cellulose thin-layer chromatography, using 2-propanol-HCl-H_2O (65:16.6:18.4 v/v) as developing solvent followed by ninhydrin spray, was used to separate and identify the compounds present in the concentrated (rotary evaporation) radioactive fractions.

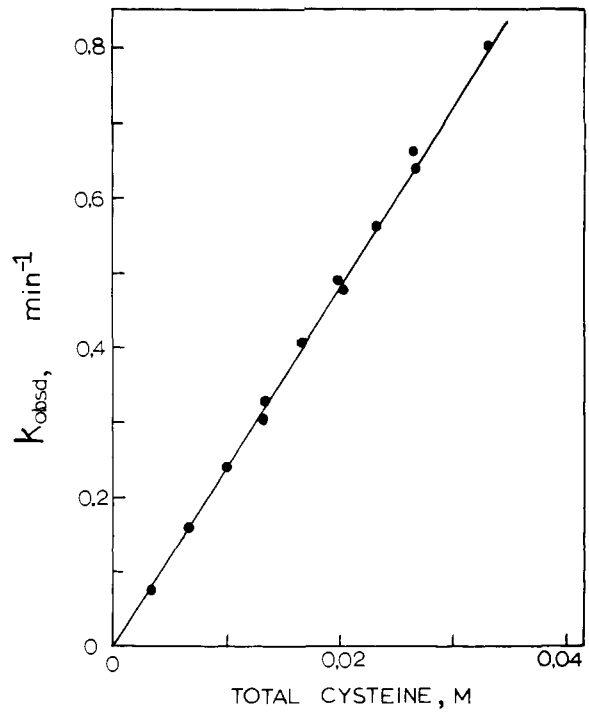


Figure 1. Linear relationship between the pseudo-first-order rate constants for the cysteine-promoted dehalogenation of BrMDHT and increasing cysteine concentration, pH 6.70, μ = 1.0 M, 25°. In addition to cysteine, reaction mixtures were 0.25 M Bistris buffer (50% base), 3.3×10^{-4} M EDTA, and 1.67×10^{-4} M BrMDHT.

Results

Kinetic Measurements. The reaction of 5-bromo-6-methoxydihydrothymine with excess cysteine followed strict first-order kinetics. Semilogarithmic plots of extent of reaction ($A_\infty - A_t$) against time were linear for at least 3 half-lives. Plots of k_{obsd} vs. increasing cysteine concentration (pH 6.73) are linear, have zero intercepts, and clearly indicate a first-order dependence on cysteine concentration (Figure 1). BrMDHT in sodium phosphate buffers of the same pH is stable for at least the same period of time.

Previous work has demonstrated that the addition of sulfur nucleophiles to halopyrimidines is subject to general acid catalysis of proton transfer.[10] Consequently, this reaction, since it is presumed to be a model for the second portion of the overall halopyrimidine dehalogenation reaction, was examined for Bistris buffer catalysis both at pH 6.10 and 6.73. Figure 2 shows that the observed pseudo-first-order rate constants for cysteine dehalogenation of

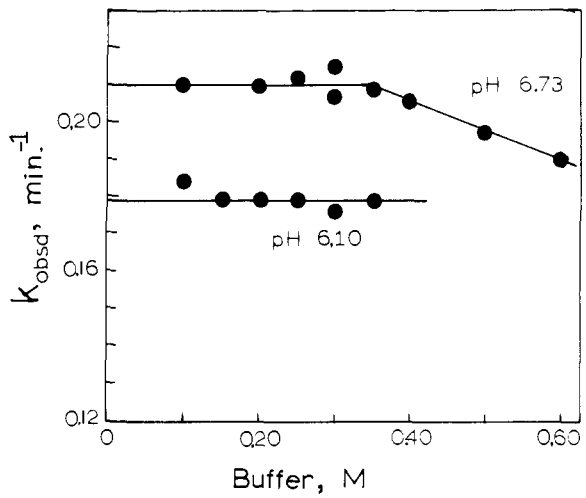


Figure 2. Effect of increasing Bistris buffer concentration on the observed first-order rate constants for the dehalogenation of 1.67×10^{-4} M BrMDHT by 0.010 M cysteine, 25°, μ = 1.0 M.

Table I. Second-Order Rate Constants for the Reaction of BrMDHT with Cysteine Thiol Anion, μ = 1.0 M, 25° [a]

Expt	Total cysteine, mM	pH	Cysteine thiol anion,[b] mM	k_{obsd}, min^{-1}	k_2, M^{-1} min^{-1}
1	8.01	8.08	1.84	2.89	1571
2	8.01	8.08	1.84	3.15	1712
3	8.01	8.09	1.84	3.01	1636
4	4.00	8.08	0.92	1.48	1609
5	4.00	8.08	0.92	1.54	1674
6	4.00	8.08	0.92	1.54	1674
7	3.61	8.09	0.83	1.46	1759
8	5.41	8.08	1.25	1.83	1464
9	7.21	8.09	1.66	2.77	1664
10	7.21	8.08	1.66	2.83	1702
11	9.02	8.08	2.08	3.52	1695
12	10.82	8.08	2.49	4.13	1655
				Av	1652 ± 77[c]

[a] Reaction mixtures were 0.25 M Tris buffer (40% base), 3.3×10^{-4} M EDTA, 8.4×10^{-5} M BrMDHT, and varying concentrations of total cysteine. [b] Calculated from the a_{H^+} and the total cysteine concentration using [cys-S$^-$] = [total cysteine]/(1 + [H$^+$]/K_a + K_c/[H$^+$] + K_b/K_a) (see ref 10). [c] Standard error of the mean.

BrMDHT are not sensitive to increasing concentrations of Bistris buffer. Indeed, at pH 6.73, buffer concentrations beyond 0.30 M cause an observable rate of depression which, based on similar decreases in k_{obsd} as a function of increasing 1,4-dioxane concentrations, can be regarded as a solvent effect. This latter conclusion is supported by similar studies using sodium phosphate buffers. With this buffer, values of k_{obsd} were invariant at lower concentrations and increased slightly at higher concentrations.

To determine the second-order rate constant for the dehalogenation of BrMDHT by cysteine, data similar to that shown in Figure 2 were obtained from pH 6.0–8.0, extrapolated to zero buffer concentration, and divided by the total concentration of cysteine present in the reaction mixtures. Semilogarithmic plots of these second-order rate constants against pH were linear with a slope equal to 1.0 (Figure 3). This result indicates the requirement for cysteine thiol anion. To confirm this result and evaluate the second-order rate constant for the reaction of cysteine thiol anion with BrMDHT, a series of 12 reactions was studied at pH 8.08 where the concentration of cysteine anion was 12- to 25-fold in molar excess relative to BrMDHT. The results of these

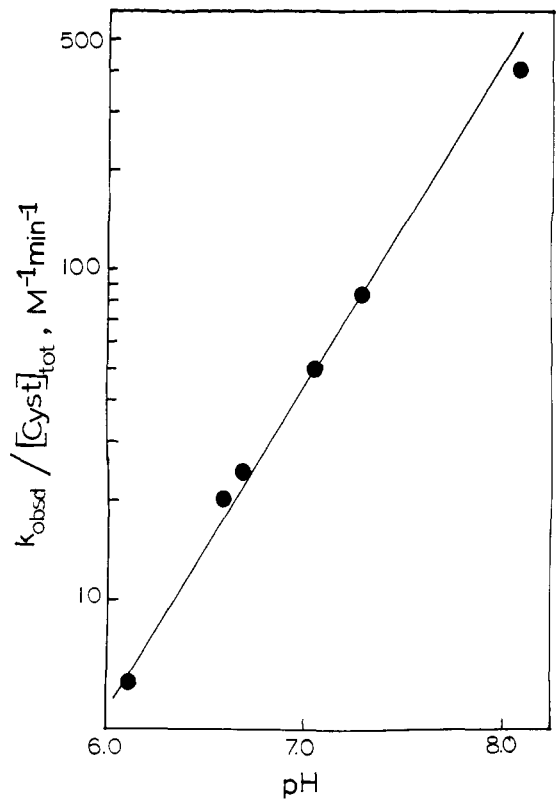


Figure 3. Semilogarithmic relationship between the apparent second-order rate constants for the dehalogenation of BrMDHT by cysteine and pH, 25°, μ = 1.0 M. Second-order rate constants were determined by dividing the zero intercepts of plots of k_{obsd} against increasing concentrations of various buffers (Tris–HCl, Bistris–HCl, and phosphate) by the total cysteine concentration present in the reaction mixtures.

studies, summarized in Table I, indicate that at 25 °C and μ = 1.0 M, $k_2^{cys\text{-}S^-}$ = 1652 ± 77 M^{-1} min^{-1}.

Acid Dissociation Constant of BrMDHT. The pK_a of BrMDHT was spectrophotometrically measured at 240 nm using previously described methods.[14] At 25 °C and μ = 1.0 M, the pK_a of BrMDHT is 9.62.

Stoichiometry and Product Analysis. The reaction products and the relationships between cysteine utilization and thymine formation were examined under conditions of both excess and limiting cysteine. In the former case, reaction mixtures initially containing 11.70 and 23.40 μmol of cysteine were incubated at 25 °C for 2.5 h with 3.0 μmol of BrMDHT in 0.10 M sodium phosphate buffer (pH 8.0). Two controls, one lacking BrMDHT and one containing 3.0 μmol of thymine in place of BrMDHT, were incubated under exactly the same conditions. Thymine and cysteine were then assayed in aliquots of these reaction mixtures and controls using uv spectrophotometry and Ellman's reagent, respectively. The results of these experiments (Table II) indicate that under these conditions only 1% of the cysteine initially present is lost via nonspecific oxidation; essentially 100% of the thymine incubated with either concentration of cysteine remains; cysteine at both concentrations converts 88% of the added BrMDHT to thymine; and, finally, 2 mol of cysteine is utilized in the reaction per mole of BrMDHT initially present.

To further examine the question of thiol utilization, similar experiments were conducted in 0.07 M Tris buffer (pH 8.08) using initial cysteine concentrations of 0.26–1.60 mM and either 5.33 or 6.40 mM BrMDHT. Thus, BrMDHT was always in molar excess relative to the initial cysteine concentration. Following incubation at 23 °C for 2.5 h, the reaction mixtures were brought to pH 2.0 by the addition of

Table II. Thymine Formation and Cysteine Utilization in the Reaction of BrMDHT with Cysteine, pH 8.0, 23° [a]

Addition	Cysteine utilized,[b] μmol	Thymine produced,[c] μmol
11.70 μmol of cysteine	0.06	
+3.0 μmol of BrMDHT	6.03	2.64
+3.0 μmol of thymine		2.94
23.40 μmol of cysteine	0.12	
+3.0 μmol of BrMDHT	5.80	2.64
+3.0 μmol of thymine		3.00

[a] Reaction mixtures and suitable controls were in 0.10 M sodium phosphate buffer (pH 8.0). Reaction mixtures were monitored spectrophotometrically for 2.5 h to ensure complete reaction, quenched by the addition of 0.30 ml of 2.0 M HCl, and ten times diluted with H_2O prior to analysis. [b] Determined using Ellman's reagent (ref 12). [c] Determined from the 265-nm absorbance of the diluted reaction mixtures using $\epsilon_{thymine}^{265} = 0.77 \times 10^4\ M^{-1}\ cm^{-1}$

Table III. Ratio of Thymine Produced to Cysteine Initially Present[a]

Expt	Cys initial, M × 10^3	BrMDHT initial, M × 10^3	Thymine produced, M × 10^3	Ratio Thy/Cys
1	0.26	6.40	0.16	0.60
2	0.52	6.40	0.31	0.60
3	0.78	6.40	0.47	0.60
4	0.27	5.33	0.17	0.65
5	0.53	5.33	0.30	0.57
6	0.80	5.33	0.44	0.55
7	1.10	5.33	0.58	0.55
8	1.30	5.33	0.69	0.53
9	1.60	5.33	0.83	0.52

[a] All reaction mixtures were 0.07 M Tris buffer, pH 8.08, 3 × 10^{-3} M EDTA.

0.1 M HCl and the thymine concentration was measured spectrophotometrically. These experiments, summarized in Table III, show that under these conditions the ratio of thymine product to initial cysteine concentration is always greater than 0.50, a result which relates to the efficacy of the E2 Hal mechanism in this reaction.

To identify cystine as a major sulfur-containing product of this reaction, duplicate reaction mixtures containing 0.04 M cysteine, 4.5 mM BrMDHT, and 0.25 M imidazole buffer (pH 7.0) were incubated at 23 °C for 15 h. Blanks containing no BrMDHT were incubated in the same manner. Cysteine assays[12] indicated a 3% cysteine loss due to nonspecific oxidation and a ratio of cysteine utilization to BrMDHT initially present of 2.2:1, thus confirming our previous observation. These experiments were conducted at pH 7.0 rather than about 8.1. At neutral pH, the solubility of cystine is considerably reduced. Consequently, a heavy white precipitate was observed in the reaction mixtures but not in the blanks which contained no BrMDHT. This precipitate was collected and shown to be cystine by a combination of cellulose thin-layer chromatographic (1-butanol–acetic acid–H_2O, 2:1:1) and spectrophotometric methods (0.01 N HCl; $A_{270}/A_{250} = 0.56$; $A_{240}/A_{250} = 1.13$; λ_{max} 243).

Participation of the E2 Hal mechanism for the dehalogenation of BrMDHT by cysteine requires the formation of a sulfenyl halide which, in addition to further reaction with cysteine to yield cystine and bromide, can also react to a limited extent with water to yield halide and mixtures of cysteic and cysteinesulfinic acid.[15] Consequently, experiments were conducted using [3-^{14}C]cysteine to identify these latter products which would be expected in low concentrations because thiol anions are more reactive toward sulfenyl halides than water and because several moles of the sulfenyl halide are required for disproportionation to yield

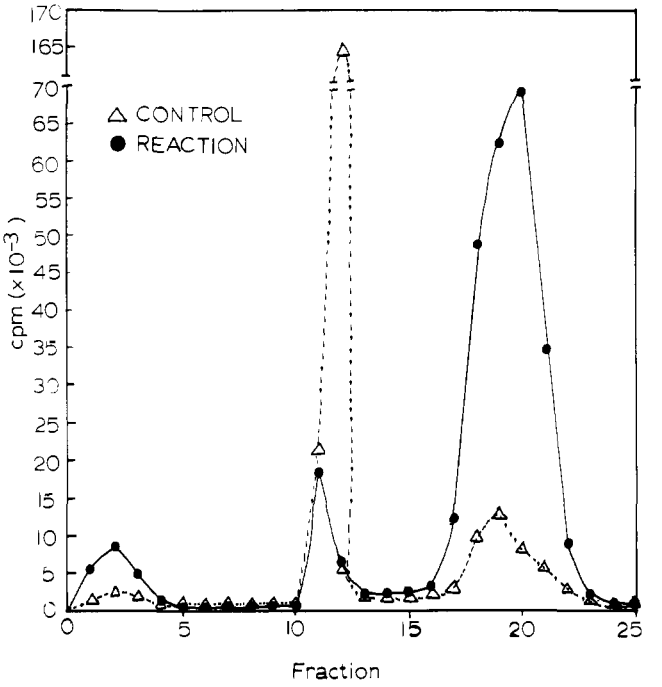


Figure 4. Dowex 50 chromatography of reaction mixtures and controls using [3-^{14}C]cysteine. Reaction mixtures contained 0.12 mmol of BrMDHT, 0.01 mmol of cysteine, and 6 × 10^{-5} mmol of [3-^{14}C]cysteine. The control contained no BrMDHT.

Table IV. Cellulose Thin-Layer Chromatography of the Peak Tubes Resulting from Dowex 50 Cation Exchange Chromatography of a Reaction Mixture Containing BrMDHT Plus Radioactive Cysteine and a Control Containing Radioactive Cysteine Alone[a]

Sample	R_f
Authentic cysteine	0.47
Authentic cystine	0.11
Authentic cysteic acid	0.49
Authentic cysteinesulfinic acid	0.34
Reaction tube 3	0.49
Reaction tube 11	0.47
Reaction tube 21	0.11
Blank tube 11	0.47
Blank tube 19	0.11

[a] After chromatography, authentic samples were visualized using ninhydrin spray. Radioactivity in the reaction and blank tubes was detected using a Packard radiochromatogram scanner.

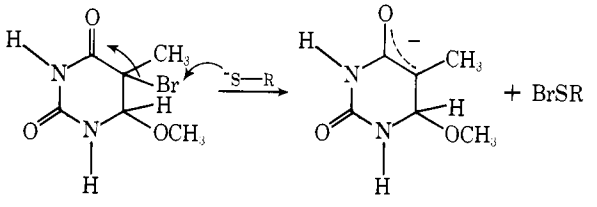
either cysteinesulfinic acid or cysteic acid.[15] A reaction mixture with limiting cysteine (0.12 mmol of BrMDHT, 0.01 mmol of cysteine, and 6 × 10^{-5} mmol of [3-^{14}C]cysteine, pH 7.9) and a control containing no BrMDHT were incubated at 23° for 12 h under argon, reduced to pH 2.0, and chromatographed on identical Dowex 50 X4-H^+ columns.[13] Under these chromatographic conditions, cysteic and cysteinesulfinic acid are not retained on the column, while cystine and cysteine are. The elution profiles obtained by column development with increasing concentrations of HCl (Figure 4) indicate three major radioactive peaks for the reaction mixture and two for the control. The small amount of radioactivity found in the void volume of the control column likely represents a minor contaminant in the [3-^{14}C]cysteine which was 98% pure material according to the supplier. Aliquots from the peak tubes of each of the radioactive peaks, along with cysteine, cystine, and cysteic acid standards, were subjected to cellulose thin-layer chromatography using 2-propanol–HCl–H_2O (65:16.6:18.4 v/v). The resulting R_f values, summarized in Table IV, indicate the presence of significant cysteic acid in the void volume of the column used for the reaction mixture.

Discussion

Both the chemical and enzymatic dehalogenation of the halogenated uracils are of interest because of the importance of this class of compounds as antiviral agents. The chemical reactivity of simple thiol and SO_3^{2-} toward the halopyrimidines may give important insights into the far more complicated enzymatic process because thiol groups are frequently found in the active site of enzymes.

In the reaction of bisulfite buffers with 5-chloro-, -bromo-, and -iodouracils, the kinetics of the dehalogenation process have been rationalized in terms of the formation of 5-halo-5,6-dihydrouracil-6-sulfonate,[4-6] an intermediate which has been clearly identified in the case of 5-chlorouracil.[6] By analogy, previous work with the cysteine-promoted dehalogenation of 5-bromo- and 5-iodouracil has been explained by the formation and subsequent dehalogenation of 5-halo-6-cysteinyl-5,6-dihydrouracil;[8-10] however, probably because of the low equilibrium constant for the addition of thiols across the 5,6 double bond of uracil and the reactivity of the intermediate in the subsequent dehalogenation reaction, this intermediate has not been clearly identified to date. The kinetics of the dehalogenation of 5-iodouracil by cysteine have been previously rationalized in terms of rate-determining addition of cysteine thiol anion to C-6 of the pyrimidine ring, a reaction in which the cysteine protonated α-amino group likely serves as an intramolecular general acid catalyst.[10] Two mechanisms have been proposed for the more rapid dehalogenation of the presumed 5-halo-6-cysteinyl-5,6-dihydrouracil, both of which are reasonable based on the analogous reaction of SO_3^{2-} with 5-bromodihydrouracil and 5-iododihydrouracil.[5] These mechanisms are shown using BrMDHT, a compound that we as well as Rork and Pitman[5] have considered as a likely model for the cysteine and the SO_3^{2-} promoted dehalogenation of either 5-bromo-6-cysteinyl-5,6-dihydrouracil and 5-bromo-5,6-dihydrouracil-6-sulfonate, respectively. The first of these mechanisms is the E2 Hal mechanism (Scheme I)

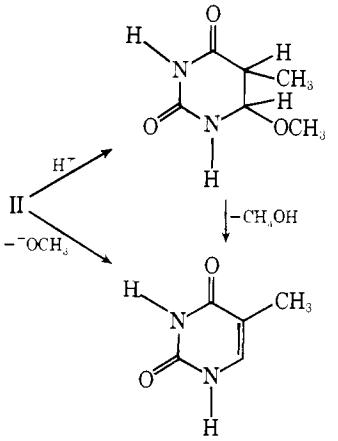
Scheme I. E2 Hal



$$R{-}S{-}Br \xrightarrow{R{-}S^-} RS{-}SR + Br^-$$

and

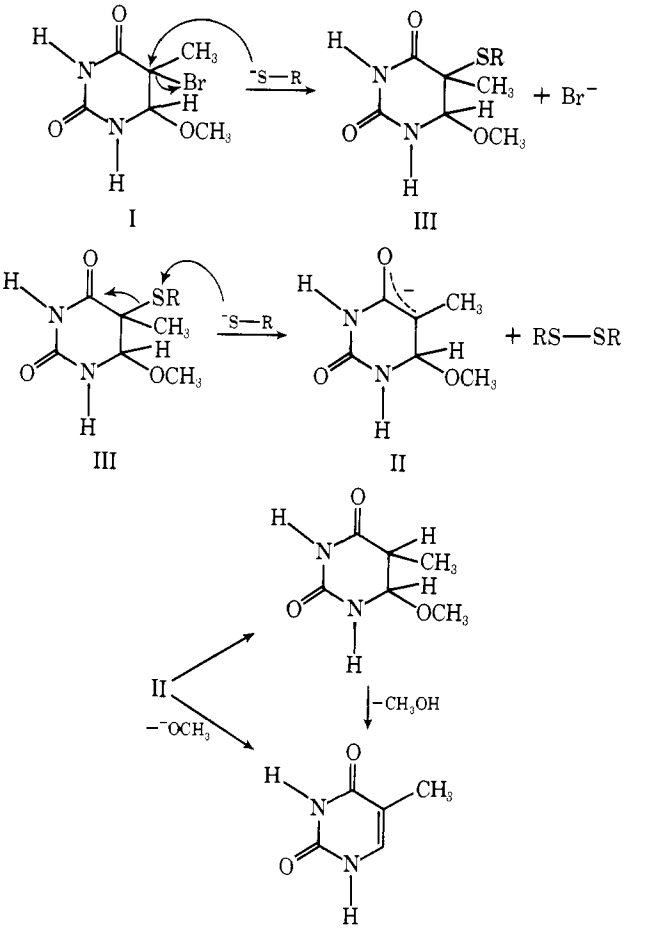
$$R{-}S{-}Br \xrightarrow{H_2O} RSO_2H + RSO_3H + RSH + Br^-$$



proposed by Ko and Parker[16] for the thiophenoxide anion debromination of 1,3-dibromocyclohexane and by us[8,10] for the dehalogenation of cysteine thiol anion adducts of 5-iodo- and 5-bromouracil. This mechanism involves the attack of thiol anion on the bromine atom to yield a sulfenyl bromide (R-S-Br) and the enolate anion of 6-methoxy-5,6-dihydrothymine (II). R-S-Br further reacts with either another mole of $R\text{-}S^-$ to yield Br^- and R-S-S-R or with H_2O to yield Br^- and $R\text{-}SO_3^-$, $R\text{-}SO_2^-$, and R-SH, while II either adds a proton to yield 6-methoxy-5,6-dihydrothymine or eliminates the 6-methoxy group to yield thymine. This mechanism can clearly be supported for the reaction of SO_3^{2-} with both 5-bromo-6-methoxydihydrouracil and -dihydrothymine.[5]

The second mechanism (Scheme II), SN2, again illus-

Scheme II. SN2



trated using BrMDHT as a model, has been proposed by Wataya et al.[9] for the cysteine-promoted dehalogenation of 5-bromo-2′-deoxyuridine (BrUdRib) and 5-bromouracil based on the fact that 5-cysteinyl-2′-deoxyuridine and 5-cysteinyluracil can be isolated as products of a side reaction which becomes more important at lower initial cysteine concentrations and higher pH values. This reaction involves the attack of $R\text{-}S^-$ on C-5 of the dihydropyrimidine ring with the formation of Br^- and 5-cysteinyl-6-methoxy-5,6-dihydrouracil (III) which, after further reaction with $R\text{-}S^-$, would yield R-S-S-R, thymine, and 6-methoxydihydrothymine as products.

The kinetic dependence of the reaction of BrMDHT with cysteine thiol anion, the fact that cystine is the major sulfur-containing product, the 2:1 relationship between cysteine and BrMDHT utilization under conditions of excess thiol, and the fact that only 88% of the BrMDHT is converted to thymine can be accommodated by both of these

mechanistic interpretations. However, under conditions where the reaction is run in limiting cysteine relative to BrMDHT concentration, the SN2 mechanism would predict that the ratio of thymine produced relative to cysteine utilized could not exceed 0.50, a restriction not placed on the E2 Hal mechanism because the second mole of $R-S^-$ required for cystine formation is involved in the reaction with a sulfenyl bromide and not with an intermediate (III) directly involved in thymine formation. Furthermore, only the E2 Hal mechanism would generate cysteic acid via the reaction of the sulfenyl bromide with H_2O. Consequently, under conditions of limiting cysteine concentration, the presence of cysteic acid as a product coupled with thymine to initial cysteine ratios consistently greater than 0.50 demonstrates that the E2 Hal mechanism is at least partially responsible for the debromination of BrMDHT. These results do not, however, eliminate the SN2 mechanism as being important in the overall dehalogenation of the halouracils by cysteine, as the E2 Hal mechanism alone cannot account for the 5-cysteinyluracil found as products in the work of Wataya et al.[9] Consequently, both mechanisms must be considered as feasible candidates for the thiol-catalyzed dehalogenation of the halogenated pyrimidines; however, their relative contribution likely varies depending upon reactant concentrations, the nature of the halogen atom, and substituents on the pyrimidine ring system.

Acknowledgments. This work was supported by U.S. Public Health Service Grant No. CA-12971 from the National Institute of Cancer. We wish to thank Dr. Ian Pitman, Department of Pharmaceutical Chemistry, University of Kansas, for generously giving us an authentic sample of 5-bromo-6-methoxy-5,6-dihydrothymine and for communicating his results on the SO_3^{2-} promoted dehalogenation of the 5-halodihydrouracils prior to publication.

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Monosodium Urate Monohydrate, the Gout Culprit

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Received September 22, 1975

Abstract: Crystals of monosodium urate monohydrate, $NaC_5H_3N_4O_3 \cdot H_2O$, are responsible for the inflammatory disease acute gouty arthritis. The crystals are triclinic needles, space group $P\bar{1}$, with $a = 10.888$ (5) Å, $b = 9.534$ (3) Å, $c = 3.567$ (1) Å, $\alpha = 95.06$ (3)°, $\beta = 99.47$ (5)°, $\gamma = 97.17$ (3)°, and $Z = 2$. The structure was solved by Patterson techniques and refined to a final R index of 0.094 for 579 observed reflections. The urate anions are hydrogen bonded together about three centers of symmetry to form sheets nearly parallel to (011). Sodium ion coordination leads to a rippling of the sheets by inducing a 7.7° tilt of the purine rings from the (011) plane and may also be partially responsible for the very intimate (3.28 Å) stacking of the urate ions. The crystal faces which interact with the lysosomal membrane during a gouty attack contain stacks of edge-on urate anions separated by stacks of sodium ions and water molecules.

The presence of crystalline monosodium urate monohydrate in the human body is prerequisite to the disease acute gouty arthritis. Electron microscopy has clearly indicated the encapsulation of the inflammatory salt crystals by white blood cells, polymorphonuclear leukocytes (PMN's), during gouty attacks.[1] The physiological function of a PMN is to phagocytize foreign materials and digest them using the digestive enzymes contained within one of its organelles, the lysosome. When crystals of monosodium urate monohydrate are phagocytized, the normal digestive process is initiated with enzymatic degradation of the protein coat adhering to the crystal. Very soon after this initial digestion, the lysosomal membrane, which separates the digestive process from the cytoplasm, lyses. The result is uncontained enzymatic activity within the entire leukocyte, leading to the rupture of the plasma membrane, and the release of the lysosomal enzymes into the serum surrounding the leukocyte. It is the release of these enzymes into the serum that precipitates the pain and inflammation associated with acute gouty arthritis.

The currently accepted mechanism for this crystal-induced membranolysis[2,3] invokes hydrogen-bond formation between donors on the crystal surface and the oxygen atoms of the phosphate head groups of the phospholipid membrane. The long-range cooperative effect of these hydrogen bonds is to bind the membrane so tightly that it becomes more rigid and tears open at the crystal edges as the membrane undulates. The single-crystal structure of monosodium urate monohydrate is of utmost importance in the verification of this mechanism or consequently in the postulation of any alternative mechanism.

Experimental Section

The colorless needles were grown by adding uric acid (Nutri-