chlorination of uracil in anhydrous CH_3OH. A suspension of 1.0 g of uracil in 15 ml of anhydrous CH_3OH was bubbled with reagent Cl_2 at 0° until the solvent remained yellow-green, due to excess Cl_2. The solid material was collected by filtration and washed with Et_2O. The white crystals decomposed with gas evolution above 150° and finally melted near 310°: NMR (DMSO-d_6) δ 10.6 (br s, 1, N_3-H), 8.8 (br s, 1, N_1-H), 5.3 (d, 1, J = 4 Hz, C_5-H), 4.6 (t, 1, C_6-H; d, 1, after adding D_2O), and 3.3 (s, 3, C_6-OCH_3). Anal. Calcd for $C_5H_7N_2O_3Cl$: C, 33.59; H, 3.92; N, 15.68. Found: C, 33.45; H, 3.90; N, 15.65. Chlorination of uracil in refluxing CH_3OH apparently resulted in formation of 5,5-dichloro-5,6-dihydro-6-methoxyuracil, which precipitated out with cooling and addition of H_2O. The white crystals were collected by filtration and washed with Et_2O: mp 227–229° (with slow loss of HCl); NMR (D_2O + NaOD) δ 5.4 (s, 1, C_6-H) and 3.3 (s, 3, C_6-OCH_3).

5-Chloro-5-methyl-5,6-dihydro-6-methoxyuracil (V) was prepared by chlorination of thymine in anhydrous CH_3OH in a similar manner to preparation of IV: mp 218–220° (with decomposition); NMR (acetone-d_6 + D_2O) δ 4.7 (s, 1, C_6-H), 3.4 (s, 3, C_6-OCH_3), and 1.8 (s, 3, C_5-CH_3). Anal. Calcd for $C_6H_9N_2O_3Cl$: C, 37.78; H, 4.67; N, 14.54. Found: C, 37.43; H, 4.62; N, 14.37.

Sodium 5,6-dihydrouracil-5-sulfonate (XIII) was isolated from the reaction of 5-bromo-5,6-dihydrouracil in aqueous pH 6 $NaHSO_3$ solution. A mixture of 0.5 g of 5-bromo-5,6-dihydrouracil was stirred in 4 ml of saturated pH 6 $NaHSO_3$ solution at room temperature for 10–12 hr. The resultant suspension of fine crystals was filtered, and the crystals were collected. They were dissolved in a minimum amount of boiling H_2O and upon cooling to room temperature, white crystals formed. The supernatant liquid was obtained by filtration and treated with several milliliters of ether. The white precipitate that formed was collected by filtration and recrystallized from a minimum volume of EtOH–H_2O. The crystals were filtered, washed with anhydrous ether, and dried: NMR (D_2O) δ 4.05 (m, 1, C_5-H) and 3.95 (m, 2, C_6-H); ir (KBr) 1725, 1710, and 1690 cm^{-1} (C=O); 1040 and 1020 cm^{-1} (S=O). Anal. Calcd for $C_4H_5N_2O_5SNa$: C, 22.21; H, 2.31; N, 12.96. Found: C, 21.98; H, 2.11; N, 12.74.

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Dehalogenation of 5-Bromouracil by Bisulfite Buffers. Kinetic Evidence for a Multistep Reaction Pathway[1]

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Abstract: Bisulfite buffers (Bis_t) of varying fraction HSO_3^- (α) dehalogenate 5-bromouracil (Br-Ura) via a multistep reaction pathway which involves SO_3^{2-} attack on C-6 of Br-Ura to yield the enolate anion of 5-bromo-5,6-dihydrouracil-6-sulfonate (Br-DHU-SO_3^-), general acid catalyzed protonation of this anion, and finally SO_3^{2-} attack on Br-DHU-SO_3^- to yield both uracil and 5,6-dihydrouracil-6-sulfonate as products. The relationship between Bis_t concentration and k_{obsd} for Br-Ura dehalogenation can best be explained by a rate equation which assumes that the concentrations of Br-DHU-SO_3^- and its enolate anion are in the steady state. At lower concentrations, the reaction has a second-order dependence on Bis_t. This dependence approaches first-order at higher Bis_t concentrations. In the concentration range where the reaction is second-order in Bis_t, the relative contributions of two different reactions to the overall dehalogenation rate are influenced by the fraction HSO_3^- (α) in the various bisulfite buffers. This is illustrated by the fact that the reaction exhibits a kinetic hydrogen–deuterium isotope effect at α = 0.20 but not at α = 0.80, that sensitivity to added general acids (Im-$^+$H) increases with decreasing α, and that relative to ClO_4^-, Cl^-, Br^-, and SO_4^{2-} enhance the reaction rate maximally at high α values. These results are briefly discussed relative to enzymatic halopyrimidine dehalogenation.

Sulfite ion is a powerful nucleophile which adds reversibly and stereoselectively to the pyrimidine ring system to yield the corresponding 5,6-dihydropyrimidine-6-sulfonate.[3-8] Following the initial formation of the dihydropyrimidine-6-sulfonate, a variety of reactions occur, depending upon the chemical nature of the parent pyrimidine base: cytosine is deaminated to yield uracil;[4,9,10] deuterium and tritium are incorporated at carbon-5 of both uridine and cytidine;[11,12] 5-iodo-, 5-bromo-, and 5-chlorouracil are dehalogenated to yield uracil;[13,14] and 5-iodo- and 5-bromocytosine are dehalogenated to yield cytosine.[15]

In the case of the bisulfite buffer promoted dehalogenation of Br-Ura, SO_3^{2-} appears to have a dual role. First, it acts to form Br-DHU-SO_3^- and then it reacts with the bromine atom of this dihydropyrimidine intermediate to yield SO_4^{2-} and Br^- as final products.[16] Another potential function for bisulfite buffers in the dehalogenation of halouracils is the use of HSO_3^- as a general acid catalyst of proton

transfer for the formation of Br-DHU-SO_3^-, a multistep reaction which, by analogy to the formation of 5-fluoro-5,6-dihydrouracil-6-sulfonate[17] and 5,6-dihydrouracil-6-sulfonate,[8] likely involves the formation of the enolate anion of Br-DHU-SO_3^- as an intermediate.

The objective of this report is to rationalize the kinetics of the reaction of Br-Ura and bisulfite buffers in terms of a multistep reaction pathway which involves SO_3^{2-} attack on Br-Ura, general acid catalyzed protonation of the resulting enolate anion, and SO_3^{2-} mediated dehalogenation of Br-DHU-SO_3^-.

Experimental Section

Materials. Reagent grade inorganic acids and salts were used without further purification. Deionized water was glass distilled and stored in polyethylene bottles prior to the preparation of solutions. Br-Ura (Sigma Chemical Co.) was used without further purification. Imidazole (Grade I, Sigma Chemical Co.) was recrystallized from hot benzene [mp 89.5–90.5° uncor (lit. 90–91°)]. Deuterium oxide (99.7%, New England Nuclear Corp.) was glass distilled before use.

Kinetic Measurements. The rates of Br-Ura disappearance in the presence of bisulfite buffers were monitored spectrophotometrically by following the decrease in 290-nm absorbance which occurs when 0.10 ml of 5.0×10^{-3} *M* Br-Ura is added to a 3-ml cuvette containing a 2.90-ml solution of the other reactants equilibrated at 25°. Ionic strength was maintained at 1.0 *M* either by the addition of $LiClO_4$ or $NaClO_4$. The halide ion dependence of the reaction was measured using mixtures of $LiClO_4$ and either LiBr or LiCl. In the experiments dealing with the SO_4^{2-} dependence of the reactions, Na^+ salts were employed. Absorbance measurements were made on either a Gilford 2000, Gilford 222, or a Zeiss PMQII spectrophotometer, equipped with cell compartments thermostated at 25°. Pseudo-first-order rate constants (k_{obsd}) were determined from linear semilogarithmic plots of the extent reaction, $A_t - A_\infty$, against time and the relationship, $k_{obsd} = 0.693/t_{1/2}$.

Immediately after each kinetic run, the pH of the reaction mixtures was measured with a Radiometer PHM-26 pH meter, equipped with a Radiometer GK2321C combination glass electrode.

Kinetic Measurements in Deuterium Oxide. Kinetic measurements were made at 25°, ionic strength 1.0 *M*, in bisulfite buffers containing either 20 or 80% HSO_3^-. All solid reagents were dissolved directly in D_2O. Perchloric acid solutions were prepared by diluting 70–72% reagent grade $HClO_4$ acid in D_2O. This resulted in final reaction mixtures which contained a negligible amount (1–2%) of water. Values of pD were calculated using pH values determined as previously described and the relationship, pD = pH + 0.40.[18]

Spectra. Ultraviolet absorption spectra were recorded on a Cary 14 recording spectrophotometer at room temperature (22–23°).

Attempts to Spectrophotometrically Identify Br-DHU-SO_3^- as a Reaction Intermediate. To evaluate the accumulation of Br-DHU-SO_3^- as an intermediate, the first 30–60 sec of the time course of Br-Ura disappearance was examined, using techniques similar to those used by Shapiro et al.,[4] Rork and Pitman,[19] and Jacobson et al.,[15] for the identification of dihydropyrimidine-6-sulfonates as intermediates in bisulfite-mediated cytosine deamination, 5-chlorouracil dehalogenation, and 5-bromocytosine dehalogenation, respectively. Experiments were conducted using bisulfite buffers of both $\alpha = 0.20$ and 0.80. Absorbance measurements were made on the Zeiss PMQII spectrophotometer at 290 nm as a function of time, using reference blanks which contained all reaction components but Br-Ura. With the more basic buffers ($\alpha = 0.20$), carefully matched 1.0-cm cuvettes were employed because the background absorbance of the reference solutions was low. In the buffers of $\alpha = 0.80$, both 1.0- and 0.2-cm cuvettes were employed because at the highest buffer concentration (0.40 *M*) employed, using 1.0-cm cuvettes, maximum dynode voltage and large slit widths (1.9 mm) were required to null the instrument. When either 1.0- or 0.2-cm cuvettes were employed, the reactions were initiated with sufficient Br-Ura to make the final concentration 1.67×10^{-4} and 8.40×10^{-4} *M*, respectively. Extreme care was taken to ensure accurate timing, since at the higher concentrations of bisulfite buffer, the reaction rates are moderately rapid ($t_{1/2}$ about 2.0 min). All measurements with 1.0-cm cuvettes were made in a cell holder thermostated at 25°. The 0.20-cm cuvettes required spacers which would not fit this cell holder. Consequently, measurements with these cells were made at ambient temperature (22–23°). Following complete reaction, A_∞^{290} values were obtained for all reaction mixtures. These values were all in the range +0.018 to −0.017 absorbance units relative to the reagent blanks. Semilogarithmic plots of $A_t^{290} - A_\infty^{290}$ vs. time were constructed and extrapolated (≤30 sec) to time zero. Differences in $A_{t=0}^{290}$ and the 290-nm absorbance of Br-Ura measured in the absence of bisulfite buffer (ΔA^{290}), but at the same pH, ionic strength, and temperature, were taken to be proportional to the equilibrium concentration of Br-DHU-SO_3^-.[20]

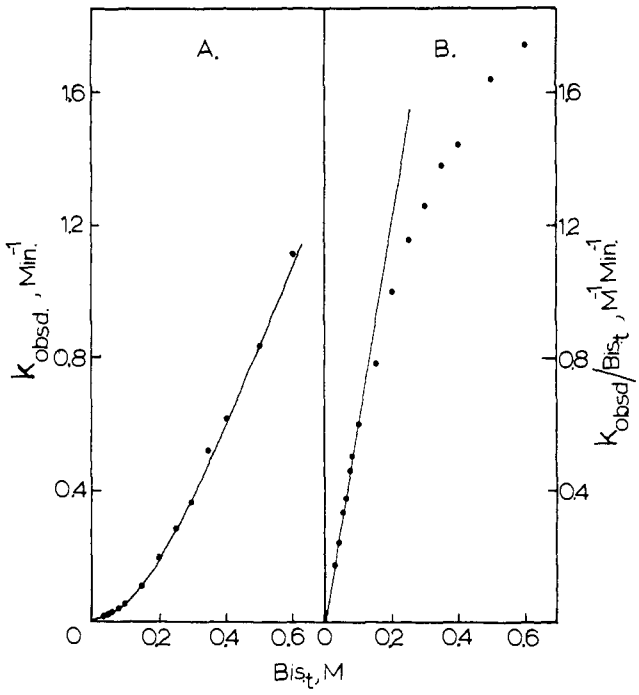


Figure 1. A. Relationship between k_{obsd} for the bisulfite buffer (Bis_t) mediated dehalogenation of 5-bromouracil and increasing Bis_t concentration: 25°, $\mu = 2.0$ *M*, $\alpha = 0.50$. The solid line was calculated using eq 7. B. Change from a second- to an almost first-order dependence on Bis_t concentration for the bisulfite mediated dehalogenation of 5-bromouracil: 25°, $\mu = 2.0$ *M*, $\alpha = 0.50$.

Results

The ultraviolet absorbance decrease which occurs when bisulfite buffers react with the 5,6 double bond of Br-Ura followed first-order kinetics in every reaction examined. Semilogarithmic plots of extent reaction vs. time were linear for at least three, and in most cases five, half-lives. The only slight deviations from linearity that were observed occurred in the first 0.5 min of reactions measured using bisulfite buffers of $\alpha = 0.80$.

Catalysis by Bisulfite Buffers. The effectiveness of bisulfite buffer systems for the dehalogenation of Br-Ura was evaluated by measuring k_{obsd} as a function of increasing buffer concentration ($\alpha = 0.20$, 0.30, 0.40, 0.50, 0.60, 0.70, and 0.80; 25°; $\mu = 1.0$ *M*). Figure 1 illustrates that the reaction changes from a second- to a first-order dependence with increasing bisulfite buffer (Bis_t) concentration. These data are typical, although the data shown in Figure 1 were measured at $\mu = 2.0$ *M*, a necessity required by the higher buffer concentrations required to show the change in kinetic order with respect to Bis_t. The slopes of plots similar to Figure 1B where the reaction was second order with respect to Bis_t were plotted as a function of α (Figure 2). These slopes, which represent the apparent third-order rate constants

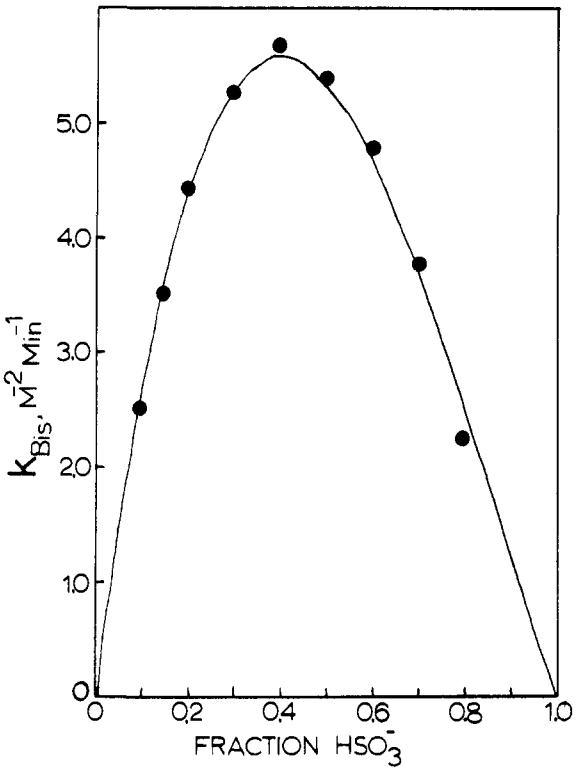


Figure 2. Relationship between the apparent third-order rate constants (k_{Bis}) for the bisulfite buffer (Bis_t) catalyzed dehalogenation of 5-bromouracil, 25° and μ = 1.0 M, and the fraction bisulfite present in the buffers. Values of k_{Bis} were evaluated from the linear portion of plots of $k_{obsd}/[Bis_t]$ against $[Bis_t]$.

(k_{Bis}) for Bis_t catalysis of the reaction increase, reach a maximum and then decrease when plotted as a function of α.

Imidazole Buffer Catalysis. Since it had been previously shown that proton transfer to dihydropyrimidine-6-sulfonate enolate anions is subject to general acid catalysis,[8,17] this reaction was examined for imidazolium ion (Im-$^+$H) catalysis using imidazole buffers of increasing fraction Im-$^+$H. A second-order dependence on Bis_t was maintained by holding Bis_t constant at 0.160 M. The slopes of linear plots of $k_{obsd}/[SO_3^{2-}]\beta$, where β equals fraction neutral Br-Ura, vs. Im-$^+$H concentration (0.22–0.52 M) are 4.80, 5.10, 3.91, 3.90, 3.66, 3.40, and 1.81 M^{-2} min^{-1} at fraction Im-$^+$H equal to 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, and 0.80, respectively.

Deuterium Isotope Effects. The results of measuring the apparent third-order rate constants (k_{Bis}) for bisulfite buffer dehalogenation of Br-Ura in both D_2O and H_2O are shown in Table I. Buffer ratios of α equal to 0.20 and 0.80 were selected because these buffer ratios are on either side of the maximum shown in Figure 2. The results indicate a small kinetic isotope effect ($k_{Bis}^{H_2O}/k_{Bis}^{D_2O}$ = 1.23) at α equal to 0.20 and no isotope effect ($k_{Bis}^{H_2O}/k_{Bis}^{D_2O}$ = 1.0) at α equal to 0.80.

Rate Enhancement by Anions. Perchlorate salts were used to maintain constant ionic strength in these studies because it had been previously reported that halide ions promoted the dehalogenation of several 5,5-dibromo-6-hydroxy-5,6-dihydrouracils.[21,22] Consequently, the effects of increasing concentrations of Cl^- on the observed rate constants for Br-Ura dehalogenation were measured in constant Bis_t concentrations of increasing α. Similar experiments for Br^- and SO_4^{2-} were performed at α = 0.50. The rate enhancements caused by Cl^-, Br^-, and SO_4^{2-} are expressed by the

Table I. Effects of Either Deuterium Oxide or Water on the Apparent Third-Order Rate Constants for Bisulfite Buffer Dehalogenation of 5-Bromouracil at 25° and Ionic Strength 1.0 M

Fraction HSO_3^-	Solvent	k_{Bis},[a] M^{-2} min^{-1}	$k_{Bis}^{H_2O}/k_{Bis}^{D_2O}$
0.20	H_2O	4.45	1.23
0.20	D_2O	3.61	
0.80	H_2O	2.25	1.00
0.80	D_2O	2.25	

[a] Evaluated from the slopes of plots of $k_{obsd}/[Bis_t]$ against $[Bis_t]$.

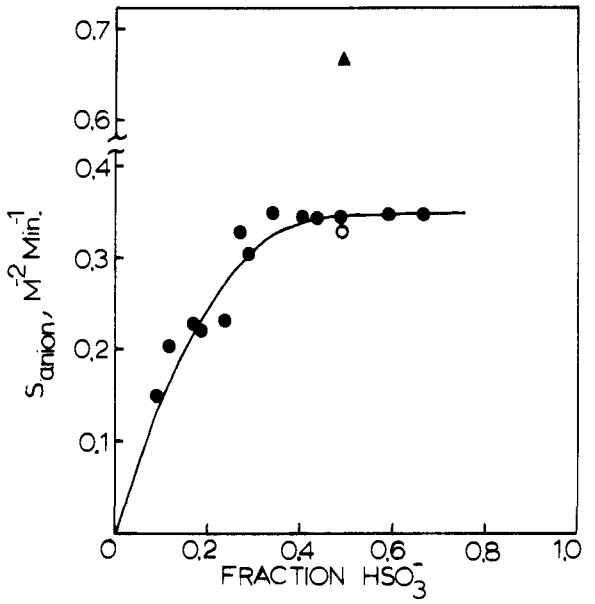


Figure 3. Relationship between fraction HSO_3^- in the bisulfite buffers and the slopes (S_{anion}) of plots of $k_{obsd}/[Bis_t]$ against either Cl^- (●), Br^- (○), or SO_4^{2-} (▲) concentration: 25°, μ = 1.0 M.

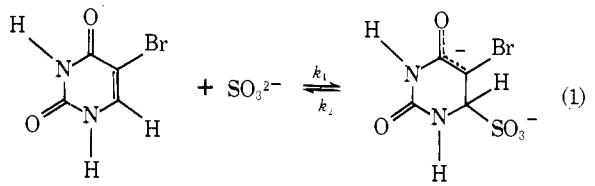
term S_{anion}, which represents the slopes of plots of $k_{obsd}/[Bis_t]$ against $[Cl^-]$ and, hence, has the same dimensions as a third-order rate constant. Figure 3 shows the results of plotting S_{anion} against α for each of the bisulfite buffers tested. This treatment of the data indicates that as α increases, the rate enhancement by Cl^- increases and reaches an invariant maximum at α equal to about 0.40. At α = 0.50, Br^- is equally as effective as Cl^-; however, under these conditions, SO_4^{2-} is about twice as effective as either of the monovalent ions.

Formation of an Intermediate. Studies designed to show the accumulation of Br-DHU-SO_3^- as an intermediate in the bisulfite-mediated dehalogenation of Br-Ura at α = 0.20 were negative. A short (~20 sec) extrapolation of semilogarithmic plots of extent reaction vs. time to time zero yields absorbance values ($A_{t=0}^{290}$) which, in all cases (0.20 and 0.35 M Bis_t in duplicate), were within experimental error equal to that obtained for the same concentration of Br-Ura initially added to the reaction mixtures. Similar experiments conducted using buffers with α = 0.80, while less conclusive, also failed to demonstrate any consistent, Bis_t concentration-dependent decrease in $A_{t=0}^{290}$ which could be attributed to the accumulation of Br-DHU-SO_3^-. These results are in accord with both the NMR data of Rork and Pitman[23] and, more recently, spectral experiments similar to these.[24]

Discussion

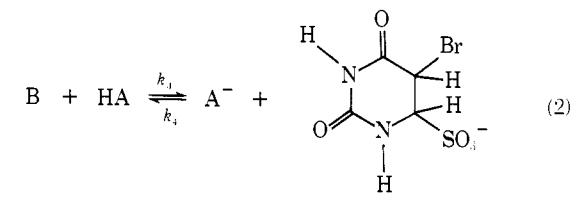
Previously, we reported that bisulfite buffers caused the dehalogenation of 5-bromo-, 5-chloro-, and 5-iodouracil.[13]

Under similar conditions, 5-fluorouracil was not dehalogenated. To explain the spectral changes of these reactions followed as a function of time, we proposed a general reaction pathway for dehalogenation which involved 5-halo-5,6-dihydrouracil-6-sulfonate as an intermediate in the formation of uracil.[13] Subsequent studies on the addition of bisulfite to 5-fluorouracil (F-Ura),[17] the elimination of SO_3^{2-} from 1,3-dimethyl-5,6-dihydrouracil-6-sulfonate,[8] and the oxidation of SO_3^{2-} to SO_4^{2-} during the dehalogenation of Br-Ura by bisulfite buffers[16] have allowed the formulation of the more-refined pathway shown in eq 1-3.



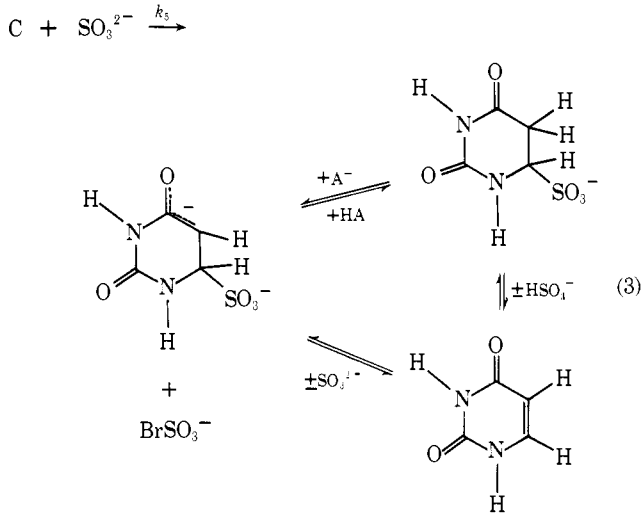
(1)

B



(2)

C



(3)

Previously, we hypothesized[13,16] that the dehalogenation of Br-DHU-SO_3^- (C) (eq 3) went via a pathway in which Ura was the initial product of the reaction and that DHU-SO_3^- was formed by the subsequent addition of bisulfite to Ura's 5,6 double bond. Rork and Pitman, studying the reaction of bisulfite buffers with both 5-bromo-6-methoxy-5,6-dihydrouracil[23] and Br-Ura,[19] have demonstrated the intermediacy of the 5,6-dihydrouracil-6-sulfonate anion shown in eq 3 by showing that, under conditions of limiting Bis_t, the percent Ura product decreased as a function of increasing phosphate buffer concentration. We have confirmed this important observation by measuring the extent of Ura formation from Br-Ura in 0.150 *M* Bis_t, pH 7.13, 25°, ionic strength 1.0 *M*, both in the presence and absence of 0.165 *M* Im-$^+$H. Our results indicate that 0.165 *M* Im-$^+$H decreases the final 270-nm absorbance, due to uracil formation, by approximately 2.5-fold.

The reaction pathway shown in eq 1-3 can be supported by the kinetics of Br-Ura disappearance in bisulfite buffers by assuming that the concentration of the enolate anion of Br-DHU-SO_3^- (B) and Br-DHU-SO_3^- (C) remains in the steady state and that there are no general acids (HA) and bases (A^-) present other than HSO_3^- and SO_3^{2-}. This would appear reasonable since no evidence can be obtained for the accumulation of Br-DHU-SO_3^-. Allowing these assumptions, the rate law for the reactions shown in eq 1-3 is given by eq 4. Substitution for the concentrations of HSO_3^- and SO_3^{2-} yields a complex expression (eq 5) which relates k_{obsd} to $[Bis_t]^2$. Inversion and rearrangement of eq 5 yields the linear relationship shown in eq 6 in which C_1 and C_2 equal $(k_2k_4 + k_2k_5)/k_1k_3k_5\alpha(1 - \alpha)$ and $(1 - \alpha)k_1$, respectively. Solution of eq 6 for k_{obsd} yields eq 7. Values of C_1 and $1/C_2$ were evaluated from the slopes and intercepts, respectively, of linear plots of $[Bis_t]/k_{obsd}$ against $1/[Bis_t]$. Substitution of these constants (C_1 = 0.145 M^2 min; C_2 = 3.125 M^{-1} min^{-1}) into eq 7 allows the calculation of the curve shown in Figure 1A which agrees well with the values of k_{obsd} measured at α = 0.50 and μ = 2.0 *M*. Similar fits were achieved with other data measured at μ = 1.0 *M*, both in this study and by Rork and Pitman who have derived a similar expression.[19]

$$\text{rate} = \frac{k_1k_3k_5[HSO_3^-][SO_3^{2-}]}{k_2k_4 + k_2k_5 + k_3k_5[HSO_3^-]}[\text{Br-Ura}] \quad (4)$$

$$k_{obsd} = \frac{k_1k_3k_5\alpha(1 - \alpha)[Bis_t]^2}{k_2k_4 + k_2k_5 + k_3k_5\alpha[Bis_t]} \quad (5)$$

$$[Bis_t]/k_{obsd} = C_1(1/[Bis_t]) + 1/C_2 \quad (6)$$

$$k_{obsd} = C_2[Bis_t]^2/(C_1C_2 + [Bis_t]) \quad (7)$$

The tendency for the reaction to change from a second- to an almost first-order dependence on Bis_t, as is shown in Figure 1, further supports the scheme shown in eq 1-3. Similar changes in kinetic order have previously been shown for the addition of bisulfite to both Ura[8] and F-Ura.[17] Consequently, at lower concentrations of Bis_t, kinetic terms which are second order in Bis_t (i.e., either the general acid catalyzed protonation of B, eq 2, or the attack of SO_3^{2-} on C, eq 3) have a greater influence on the overall reaction rate; while at higher Bis_t concentrations, the attack of SO_3^{2-} on Br-Ura, which is first order in Bis_t, exerts control on the rate of dehalogenation. As previously mentioned, at the lower Bis_t concentrations where second-order kinetics are observed, two reactions likely contribute to the overall rate of dehalogenation (eq 2 and 3); however, the degree of their individual contribution likely changes as α, the fraction HSO_3^- changes. This conclusion is supported by the following observations: a small kinetic isotope effect is observed at α = 0.20, but not at α = 0.80 (Table I); general acid catalysis by Im-$^+$H becomes more significant as both α and the fraction Im-$^+$H decrease; and, finally, salt effects by Cl^- increase and become maximum as a function of increasing α (Figure 3). Thus, when α is small, the general acid catalyzed protonation of B by HSO_3^- (eq 2) has the greater influence on the overall rate; whereas, at high α values, the dehalogenation of C by SO_3^{2-} exerts the greater control (eq 3).

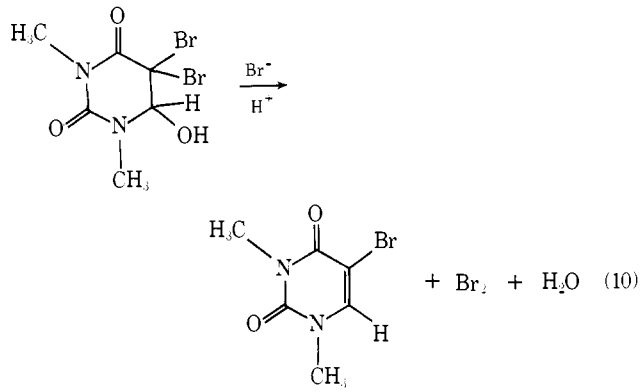
The dehalogenation of Br-Ura by Bis_t is subject to general acid catalysis by Im-$^+$H. The sensitivity to Im-$^+$H catalysis is maximum (low α values) where the reaction has least sensitivity to specific salt effects (Cl^- vs. ClO_4^-). If it can be assumed that, like similar reactions of bisulfite and other uracils,[8,17] proton transfer to the enolate anion of Br-DHU-SO_3^- (eq 2) is the reaction catalyzed, then eq 8 should describe the rate of Br-Ura utilization in imidazole buffers of varying fraction Im-$^+$H and constant concentrations of Bis_t. The apparent third-order rate constants (k_3', k_3'', and k_3''') contain the undetermined value of K_{eq} for the reaction

shown in eq 1. Thus, under pseudo-first-order conditions and after a small correction of the k_{obsd} values for the fraction of neutral Br-Ura (β) present at a particular pH, the slopes of plots of $k_{obsd}/[SO_3^{2-}]\beta$ against increasing Im-$^+$H concentration (eq 9) should have been invariant as a function of the fraction Im-$^+$H. The fact that these slopes decrease as both α and fraction Im-$^+$H increase argues that two different reactions, both of which are second-order in Bis_t, can influence the overall rate of reaction under these conditions. As fraction Im-$^+$H (α) increases, the overall reaction rate likely has a greater dependence on the dehalogenation of C by SO_3^{2-}, while proton transfer to B becomes less important as a controlling influence.

$$\text{rate} = k_3'[\text{Br-Ura}][SO_3^{2-}][H^+] + k_3''[\text{Br-Ura}][SO_3^{2-}][HSO_3^-] + k_3'''[\text{Br-Ura}][SO_3^{2-}][\text{Im-}^+\text{H}] \quad (8)$$

$$k_{obsd}/[SO_3^{2-}]\beta = k_3'[H^+] + k_3''[HSO_3^-] + k_3'''[\text{Im-}^+\text{H}] \quad (9)$$

The specific reasons for the rate enhancements of dehalogenation by Cl^-, Br^-, and SO_4^{2-} are not clear. Wang[21] and, more recently, Banerjee and Tee[22] have reported that Br^- and protons promote the debromination of several 5,5-dibromo-6-hydroxy-5,6-dihydrouracils to yield the corresponding 5-bromouracils (eq 10). This would be an attrac-



(10)

tive explanation for the rate enhancement of bisulfite-mediated Br-Ura dehalogenation caused by Cl^- and Br^-. Several lines of evidence argue against this hypothesis: compared to ClO_4^-, SO_4^{2-} is about twice as effective as either Cl^- or Br^- in bisulfite buffers of $\alpha = 0.50$; there is little sensitivity to increasing concentrations of general acids in bisulfite buffers where the rate enhancement by Cl^- is maximum; and the kinetics of the reaction, shown in eq 10, follow the acidity function H_0,[22] thus indicating the requirement for conditions of acidity far greater than those employed in this work. Rork and Pitman, studying the dehalogenation of 5-bromo-5-methyl-6-methoxy-5,6-dihydrouracil by bisulfite buffer, a reaction which can be considered a model for the dehalogenation of Br-DHU-SO_3^-, have reached the same conclusion.[23] They have shown that, in the absence of bisulfite buffer, Cl^- does not dehalogenate the model compound and that, in bisulfite buffers with ionic strength maintained at 1.0 M with KCl, plots of k_{obsd} against increasing SO_3^{2-} concentration are linear with zero intercepts. These workers ascribe these rate differences caused by Cl^-, ClO_4^{2-}, and SO_4^{2-} to differences in water structure. Thus, at least in the case of SO_3^{2-} reacting with the 5-bromo-5-methyl-6-methoxy-5,6-dihydrouracil, the competing reaction involving halide ions, shown in eq 10, is effectively eliminated as a potential mechanistic possibility. To further examine the effect of Cl^-, relative to ClO_4^-, on reactions of this type, the effects of varying the ratio of Cl^- to ClO_4^- at ionic strength 1.0 M were measured at both $\alpha = 0.20$ and 0.80 for the addition of constant concentrations of bisulfite to F-Ura, a halopyrimidine which does not dehalogenate.[17] In both buffer systems, a linear relationship in plots of $k_{obsd}/[Bis_t]$ vs. increasing Cl^- concentration was observed. The slopes of these plots (S_{anion}) were 0.024 and 0.028 M^{-2} min^{-1} at $\alpha = 0.20$ and 0.80, respectively. While Cl^- concentration affected the rate of bisulfite reacting with F-Ura in a systematic way, it should be noted that S_{anion} for the Br-Ura reaction is an order of magnitude larger when measured under the same conditions.

The halogenated uracils and their nucleotide analogs are known to have both antiviral action[25] and the ability to sensitize mammalian cells in culture to the lethal effects of radiation,[26,27] a property which has importance in radiation therapy of tumors. Both of these important biological properties of halogenated pyrimidines may be limited by the fact that, in vivo, the halogenated nucleotides are rapidly degraded to pyrimidine bases and then dehalogenated to yield uracil.[28-33] The pathways for the enzymatic dehalogenation of Br- and I-Ura are thought to involve triphosphopyridine nucleotide-linked reduction of the 5,6 double bond, followed by spontaneous elimination of either HI or HBr to yield Ura as products.[28,30,33] Preliminary kinetic data on the base-catalyzed elimination of HBr from 5-bromo-5,6-dihydrouracil indicate that at physiological pH this reaction is far too slow to account for in vivo dehalogenation.[28,34] Consequently, either the elimination of HBr from 5-bromo-5,6-dihydrouracil must be enzymatically catalyzed or mechanisms similar to the action of sulfur nucleophiles, such as sulfite and cysteine,[35] on halopyrimidine dehalogenation must be considered for the enzymatic process.

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References and Notes

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Copper(II) Complex of Sulfur-Containing Peptides. Characterization and Similarity of Electron Spin Resonance Spectrum to the Chromophore in Blue Copper Proteins

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Abstract: A green-colored α-mercaptopropionylglycine–Cu(II) complex which involves thiol, neighboring deprotonated peptide nitrogen, and terminal carboxylate groups as the coordination sites has been obtained. The liberation of peptide proton occurs at the neutral pH region and the infrared spectrum of the complex isolated directly shows the dissociation of a proton from peptide linkage by a disappearance of ν (NH) at 3300 cm^{-1}. The magnetic circular dichroism curve of the complex consists of two negative bands at 450 and 600 nm and the maximum $[\theta]$ was -0.86×10^{-3} (deg cm^2)/dmol at 600 nm under a field of 11.7 kG. The formation of the adduct complex with some heterocyclic bases such as pyridine and imidazole caused a considerable shift of the visible band to a higher frequency. Of interest is the fact that the spin Hamiltonian parameters ($g_\parallel$ = 2.259, $g_\perp$ = 2.040, and $A_\parallel$ = 82 G) and the bonding parameters (α^2 = 0.52 and $^4/_7\alpha'^2 + \kappa$ = 0.51) of the complex are very similar to those of the chromophore in blue copper proteins. The high covalency of the Cu–S bonding is indicative of a decrease in the unpaired electron density on the Cu(II) atom, namely, a small hyperfine coupling constant.

On the basis of optical and ESR properties, copper proteins are grouped into two categories: blue copper proteins and nonblue proteins. The so-called blue copper proteins have an unusually high extinction coefficient in the visible spectrum and an anomalously small copper hyperfine coupling constant ($A_\parallel$ < 100 G) in comparison with that of nonblue proteins ($A_\parallel$ > 140 G).[2a] The unique spectral properties of blue copper proteins have attracted keen interest and various model systems have been investigated.[2b] Most of the model Cu(II) complexes involve coordination to nitrogen ligands where the coordination geometry is distorted tetrahedral. Studies on the copper complexes of thiol ligands have been relatively few, despite the importance of the copper–sulfur interaction in copper enzymes as suggested by Hemmerich[3a] and Beinert.[3b] It is not even known whether the cysteinyl residue involved in a peptide chain can interact with Cu(II) ion through both the thiol and neighboring peptide amide groups.

Evidence for the presence of a thiol group as a ligand for Cu(II) in blue copper proteins has been presented by Graziani et al.[4] From the reactivity with *p*-chloromercuribenzoate and the results of the physicochemical investigations, these authors reported that the sulfur atom could be an invariant ligand for copper in plastocyanin, stellacyanin, and azurin which contain a thiol group of the cysteine residue. The resonance Raman spectra of blue copper proteins between 1700 and 200 cm^{-1} revealed the presence of the Cu–S coordinations (CuN_3S, CuN_4S, CuN_2OS, or CuN_3OS).[5] The proposal that the presence of the sulfur atom provides a logical mechanism for the intensification of the blue copper ligand field bands has been given particular stress. Furthermore, Giordano et al.[6] have implied on the basis of ESR studies that the nonlabile endogeneous axial ligand to the copper in galactose oxidase has π-bonding character and a thiol group may be the ligand involved. The Cu(III) complex of 1,1-dicarboethoxy-2,2-ethylenedithiolate has also been synthesized by Coucouvanis and his colleagues[7] as a model of copper-containing enzymes. Thus, the role of thiol group in copper binding of the proteins is being given much attention.

We have already reported that the red-violet-colored penicillamine–copper complex is isolated from an aqueous solution and has a high extinction coefficient of about 10^3 per copper at 520 nm.[8] The sulfur-containing amino acids and peptides, such as penicillamine and mercaptopropionylglycine (MPG), are useful not only as an oral treatment of Wilson's disease caused by an abnormal metabolism of copper but also as a ligand in the study of the interaction of the Cu(II) ion with the thiol group. This paper deals with ESR similarities of the chromophore between the α-MPG–Cu(II) complex and blue copper proteins. The complex is hereafter abbreviated as green complex.

Experimental Section

α-MPG and β-MPG were a gift from Santen Seiyaku Co. and were used after recrystallization from ethyl acetate. Glutathione and glycylcysteine were obtained from Sigma and Tokyo Kasei Co., respectively. The solution of cupric chloride was prepared from reagent grade material and was standardized complexometrically with EDTA. Carbonate-free potassium hydroxide solution was prepared by the procedure described by Armstrong[9] and was standardized by the titration with potassium hydrogen phthalate. Deionized water was used throughout the experiments. All other