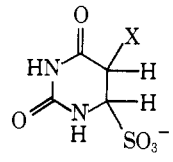
A Kinetic Study of the Dehalogenation of 5-Halo-5,6-dihydrouracils in Aqueous Solutions of Sodium Bisulfite[1]

Gerald S. Rork and Ian H. Pitman*

Contribution from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66044. *Received December 31, 1974*

Abstract: The reactions of 5-bromo- and 5-chloro-5,6-dihydro-6-methoxyuracil (II and IV), 5-bromo- and 5-chloro-5-methyl-5,6-dihydro-6-methoxyuracil (III and V), 5-bromo- and 5-iodo-5,6-dihydrouracil (VI and VII), and 5-bromo-5,6-dihydro-6-methyluracil (VIII) in aqueous solutions of sodium bisulfite with pH values between 4 and 8 have been investigated. Kinetic studies have shown that the attack of sulfite ion on the molecule is the rate-determining step in the dehalogenation process. Based on the products of the reactions, there are apparently two pathways which lead to dehalogenation. An SN2 displacement of the halogen via attack of sulfite ion on carbon-5 leads to a 5,6-dihydrouracil-5-sulfonate and halide ion as products. The other mechanism is believed to involve attack of sulfite ion on the halogen to produce a carbanion and a halosulfonic acid. The latter compound rapidly hydrolyzed to a halide ion, H^+ and SO_4^{2-}, and the carbanion was either protonated on carbon-5 to yield a 5,6-dihydrouracil or, if it contained a methoxy group at carbon-6, it eliminated a methoxy group to yield a uracil. The ratio of uracil and 5,6-dihydro-6-methoxyuracil produced in the dehalogenation of II was observed to be buffer dependent, with uracil being the predominant product at low buffer concentration.

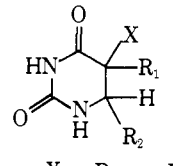
It has been established that 5-chloro-, 5-bromo-, and 5-iodouracils are dehalogenated much more rapidly in aqueous sodium bisulfite ($NaHSO_3$) solutions[2,3] than in aqueous phosphate buffers at the same pH. Such dehalogenation in $NaHSO_3$ solution has been postulated[2,3] to occur via reduction of the 5-halo-5,6-dihydrouracil-6-sulfonate (I) which is



I, X = Cl, Br, I

formed by covalent addition of HSO_3^- across the 5,6 carbon–carbon double bond of the 5-halouracil. The formation of intermediate **I** has been observed during the dehalogenation of 5-chlorouracil,[3] but the rates of the dechlorination reaction were very slow and were not investigated in detail. No evidence has been obtained, however, for any buildup of similar intermediates in the dehalogenation of 5-bromouracil and 5-iodouracil.[2,3] Consequently, it is believed that either the rates of reduction of these latter two intermediates are much faster than their formation rates and/or the values of the equilibrium constants for their formation are very small. Studies on the kinetics of reaction of these 5-halouracils in aqueous $NaHSO_3$ solutions are discussed in an accompanying paper.[3]

To find out more about the dehalogenation of intermediates such as I, studies have been made of the dehalogenation of 5-bromo-5,6-dihydro-6-methoxyuracil (II) and several other 5-halo-5,6-dihydrouracils (III–VIII) in $NaHSO_3$



	X	R_1	R_2
II	Br	H	OCH_3
III	Br	CH_3	OCH_3
IV	Cl	H	OCH_3
V	Cl	CH_3	OCH_3
VI	Br	H	H
VII	I	H	H
VIII	Br	H	CH_3

solutions in the pH range of 4–8. The compounds studied could all be isolated, and their reactions in $NaHSO_3$ solutions could be studied independently of the reactions which led to their formation from the parent uracil.

Results

Stability of II–VIII. In aqueous solutions which did not contain $NaHSO_3$, both II and IV eliminated CH_3OH to yield 5-bromouracil and 5-chlorouracil, respectively. These reactions were first order in the concentration of II or IV and had rate constants (calculated from uv measurements) of 2.2×10^{-4} sec^{-1} and 2.2×10^{-1} sec^{-1}, respectively, in 0.42 *M* phosphate buffers at pH 7.0, 25°, and $I = 1.0$ *M*. The yield of 5-bromouracil was calculated to be 100 ± 3% (from two determinations) on the basis of its molar absorptivity at 275 nm.

In the absence of $NaHSO_3$, compounds III and V–VIII showed no change in their uv spectrum in aqueous solutions in the pH range of 5–8 for at least 30 min at 25°. It has been reported that 5-halo-5,6-dihydrouracils with a hydrogen at C-6 do undergo dehydrohalogenation slowly in aqueous solution.[4] These reactions are comparatively slow, however, as the reported half-lives for VI and VIII at 37–38° are greater than 20 hr at pH 7.4. Thus, these reactions were not considered to be significant in the present study, because the dehalogenations that occurred in solutions containing $NaHSO_3$ were very much faster.

Reactions of II–V in Aqueous Sodium Bisulfite Solutions. In $NaHSO_3$ solutions with pH values in the range 4–8, IV was only observed to eliminate CH_3OH as it did in buffers which did not contain $NaHSO_3$. Thus, any direct dehalogenation of IV under these conditions appeared to be much slower than the elimination of CH_3OH.

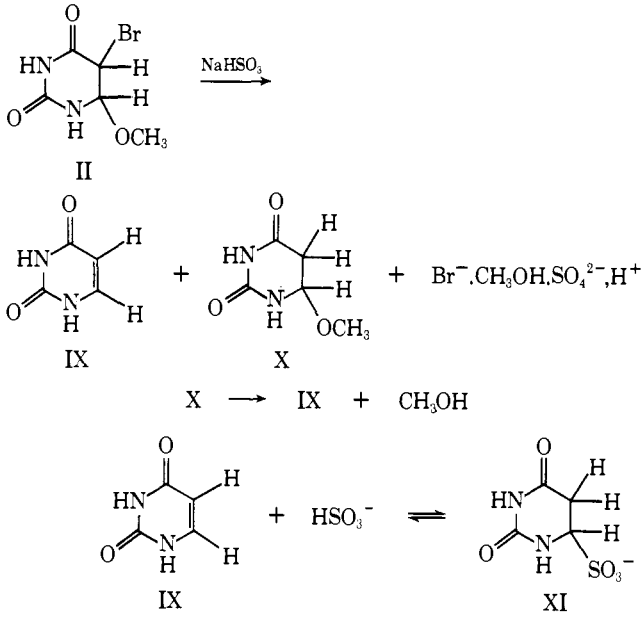
However, under the same conditions II was rapidly dehalogenated in a reaction which was very much faster than the elimination of CH_3OH. The initial products of the reaction were identified as uracil (IX) and 5,6-dihydro-6-methoxyuracil (X), along with CH_3OH, Br^-, SO_4^{2-}, and H^+. Both IX and X were observed to then undergo slow, subsequent reactions under these conditions. Compound X slowly eliminated CH_3OH to yield more IX, and IX slowly underwent covalent addition of HSO_3^- to yield 5,6-dihydrouracil-6-sulfonate (XI). These reactions are illustrated in Scheme I. Evidence for the initial formation of IX and X and for their slow, subsequent reactions was obtained from

Table I. Observed First-Order Rate Constants and Percentage Initial Yields of Uracil (IX) for Dehalogenation of II in $NaHSO_3$ Solutions at 25° and Ionic Strength 1.0 M[a]

pH	$[S_T]$,[b] M	Buffer conditions	10^3k_{obsd}, sec^{-1}	Initial % yield of IX	
				Obsd	Calcd[c]
4.50	0.01	Succinate, 0.02 M	3.92	62.6	62.4
4.50	0.01	Succinate, 0.05 M	3.44	50.2	50.6
4.50	0.01	Succinate, 0.10 M	3.56	42.5	42.4
4.50	0.01	Succinate, 0.25 M	3.31	34.6	34.8
4.50	0.01	Succinate, 0.40 M	3.10	32.6	32.4
7.00	0.016	Phosphate, 0.042 M		66.3	
7.00	0.016	Phosphate, 0.40 M		42.2	
7.00	0.016	Phosphate, 0.042 M + Morpholine hydrochloride, 0.375 M		16.6	

[a] Adjusted with KCl. [b] $[S_T] = [HSO_3^-] + [SO_3^{2-}]$. [c] Calculated using eq 4.

Scheme I



the changes in NMR and uv spectra that occurred after addition of II to aqueous $NaHSO_3$ ($NaDSO_3$) solutions.

When II was added to a D_2O solution which was 1 M in K_2SO_3, 1 M in K_3PO_4, and which had been adjusted to pD 7 (with 40% DCl in D_2O), a clear, homogeneous solution resulted within 1 min. The NMR spectrum of this solution was quickly measured, and it indicated that a mixture of IX [δ 7.65 (d, 1, J = 7 Hz, C_6-H), 5.78 (d, 1, J = 7 Hz, C_5-H)] and 5-deuterio-X [δ 4.88 (d, 1, J = 2–3 Hz, C_6-H), 2.78 (br d, 1, C_5-H), 3.40 (s, C_6-OCH_3)] had formed from the reaction of II. Subsequently under these conditions (high $KDSO_3$ concentration and pD 7), all IX underwent addition of DSO_3^- across its 5,6 carbon–carbon double bond to produce 5-deuterio-5,6-dihydrouracil-6-sulfonate (5-deuterio-XI). Evidence for this reaction was provided by the loss of the signals for IX and the appearance of the signals for 5-deuterio-XI [δ 4.64 (s, 1, C_6-H), 3.06 (br s, 1, C_5-H)][5] over a 10-min period. During this time, the signals due to X remained constant. Under these conditions, the reaction of IX to 5-deuterio-XI was much faster than the reaction of X to IX. A rapid loss of the signals due to X was observed on heating or acidifying the solutions with DCl. The above NMR assignments are consistent with those reported for a series of 5,6-dihydro-6-hydroxyuracils,[6] which would be expected to have similar NMR spectral characteristics to those of II, X, and XI.

Uv spectra of solutions of II in aqueous $NaHSO_3$ also underwent changes which were consistent with the reactions shown in Scheme I. Subsequent reactions of IX and X

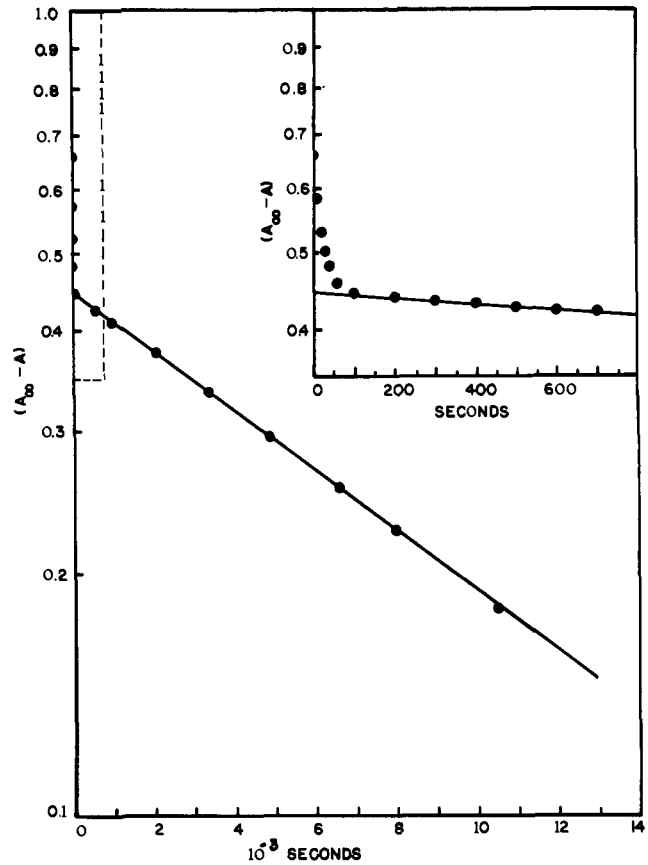


Figure 1. A semilogarithmic plot of the absorbance changes ($A_\infty - A$) vs. time that occurred at 258 nm when II was added to a 0.002 M $NaHSO_3$ solution, which was also 0.40 M in phosphate buffer, at ionic strength 1.0 M, 25°. The insert at the right is an expansion of the section of the plot enclosed by the dotted line.

under these conditions were extremely slow and were not investigated. Because of the instability of X, it was not isolated and, consequently, its uv spectral characteristics were not measured. It is expected, however, that its uv spectrum would be very similar to that of other 6-substituted 5,6-dihydrouracils such as XI, whose molar absorptivities are negligible in comparison to those of IX in the 240–280-nm region.[7] On the basis of this assumption, the initial percentage yield of IX could be calculated from its molar absorptivity (8290 cm^{-1} M^{-1}, 258 nm) and the observed absorbance at 258 nm. Table I lists the initial percentages of IX that were formed and the observed first-order rate constants for the reaction at various buffer concentrations. At pH 7.0, all reactions were somewhat faster, and the increase in absorbance was followed at λ = 258 nm in 0.42 M phosphate buffer which was also 0.002 M in $NaHSO_3$. Figure 1 shows

Table II. Observed Second-Order Rate Constants[a] for Dehalogenation at Ionic Strength 1.0 M,[b] 25°

Compd	pH	k'_{obsd}, M^{-1} sec^{-1}
II	4.00[c]	0.111
II	5.00[d]	1.23
II	6.24[e]	16.6
II	7.46[e]	40.0
III	4.50[c]	0.098
VI	7.46[e]	0.021
VIII-B	7.46[e]	0.039

[a] The slopes of k_{obsd} vs. [S_T] plots. [b] Adjusted with KCl. [c] 0.1 M succinate buffer. [d] 0.1 M acetate buffer. [e] $NaHSO_3$–Na_2SO_3 buffers.

the resultant semilogarithmic plot of the absorbance changes ($A_\infty - A$) vs. time. The terminal slope of this plot indicated a first-order rate constant of 1.0×10^{-4} sec^{-1} for the reaction of X to IX. An approximate value of the rate constant for the reaction of II to IX and X, 4.5×10^{-2} sec^{-1}, was obtained from a semilogarithmic plot of ($A_\infty - A$) vs. time, using a value of A_∞ equal to the observed absorbance at t = 100 sec. For runs at slightly higher $NaHSO_3$ concentrations, the reaction of II to IX and X became more than 450 times faster than the reaction of X to IX, and this method led to semilogarithmic plots of ($A_\infty - A$) vs. time which were strictly linear for 3–5 half-lives. Under the conditions described in the caption of Figure 1, the reaction of IX to XI was slower than the reaction of X to IX and was not investigated further.

Further evidence for the sequence of reactions shown in Scheme I came from the uv spectra of solutions of II in aqueous $NaHSO_3$ which had been subjected to a sharp increase in pH. A 0.005 M solution of II in 0.05 M $NaHSO_3$ solution at pH 6.5, which was also 0.2 M in phosphate buffer, was allowed to stand several minutes at room temperature (until dehalogenation of II was complete). When this solution was diluted 50-fold in 0.1 N NaOH solution, the uv spectrum of the resultant solution showed a maximum at 283 nm, and the yield of IX was calculated to be 100%, based on the absorbance value at 283 nm and the molar absorptivity of IX in these conditions (5950 cm^{-1} M^{-1}). Any XI that may have formed at pH 6.5 would have quickly eliminated HSO_3^- to yield IX in the 0.1 N NaOH solution.[8] When the above solution of II in $NaHSO_3$ solution at pH 6.5 was diluted 50-fold into pH 7.0 phosphate buffer, the uv spectrum of the resultant solution had a maximum absorbance at 258 nm. The yield of IX was calculated to be approximately 50% under these conditions, based on the absorbance at 258 nm and the molar absorptivity of IX (8290 cm^{-1} M^{-1}, 258 nm, pH 7.0). These data are consistent with the reaction sequence in Scheme I in which IX and X are formed in a fast dehalogenation of II and in which the rates of the subsequent reactions are markedly dependent on pH and $NaHSO_3$ concentration.

The other products of the dehalogenation of II (CH_3OH, Br^-, SO_4^{2-}, and H^+) were identified as follows. A decrease of several units in pH was observed when II was added in large amounts to a pH 7 $NaHSO_3$ solution, indicating a net production of H^+ in the reactions. Addition of $AgNO_3$ to a portion of this reaction mixture (which had been acidified with concentrated HNO_3) resulted in the formation of a yellow precipitate which was identified as AgBr. Compound II or $NaHSO_3$, alone, did not yield a precipitate in similar conditions. Likewise, the presence of SO_4^{2-} was indicated by the formation of a white precipitate upon addition of $BaCl_2$ to a portion of the reaction mixture which had been acidified with concentrated HCl. Again, $NaHSO_3$ or II did not yield a precipitate in similar conditions. The presence of CH_3OD was indicated in reaction mixtures of II in $NaDSO_3$ solutions in D_2O at pD 6 by the appearance of a singlet in the NMR spectrum 2–3 Hz upfield of the signal for the methoxy group of X.

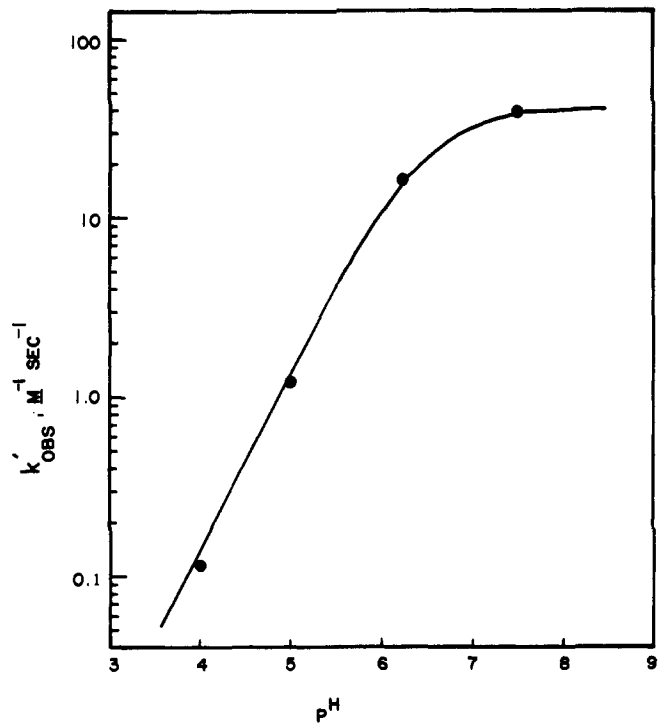


Figure 2. A semilogarithmic plot of the observed second-order rate constants, k'_{obsd}, for dehalogenation of II vs. pH at ionic strength 1.0 M (adjusted with KCl), 25°. The k'_{obsd} values shown are listed in Table II, and the solid line was calculated from eq 1 using a value of k = 42 M^{-1} sec^{-1}.

The kinetics of the formation of IX from II in $NaHSO_3$ solutions at ionic strength 1.0 M (adjusted with KCl) were investigated in the pH range of 4.0–7.5 at 25°. First-order rate constants, k_{obsd} values, were calculated for the fast increase in absorbance at 260 nm that resulted when II was added to buffers of $NaHSO_3$ and Na_2SO_3 or solutions of $NaHSO_3$ in acetate or succinate buffers. At constant pH, k_{obsd} values were linearly related to the total concentration of HSO_3^- and SO_3^{2-} in solution, [S_T], and had a value of 0 when [S_T] = 0. The observed second-order rate constants, k'_{obsd} values, were calculated from the above plots and are listed in Table II. Values of k'_{obsd} for other compounds in this study are also listed in Table II. Figure 2 shows a semilogarithmic plot of the k'_{obsd} values for II (which are listed in Table II) vs. pH. The solid line was calculated from the equation

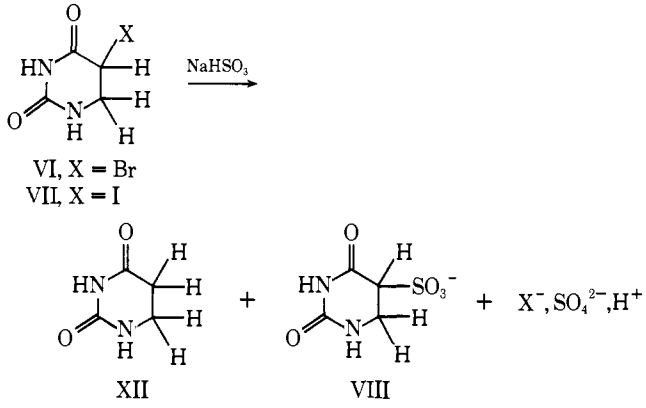
$$k'_{obsd} = 42\ M^{-1}\ \text{sec}^{-1} K_a^{HSO_3^-}/(K_a^{HSO_3^-} + [H^+])$$

where $K_a^{HSO_3^-}$ is the acid dissociation constant of HSO_3^- at ionic strength 1.0 M, 25° (pK_a = 6.50).

The effect of various salts on the dehalogenation rate of II in 0.018 M $NaHSO_3$ solutions which were 0.1 M in acetate buffer was investigated at pH 5.0, ionic strength 1.0 M. Table III lists the k_{obsd} values obtained when several different salts were used to adjust the ionic strength to 1.0 M. Because of the small changes in k_{obsd} values, a number of determinations were carried out for each salt. The values reported in Table III were generally reproducible within 5%.

5-Bromo-5-methyl-5,6-dihydro-6-methoxyuracil (III) appeared to undergo a dehalogenation reaction in aqueous $NaHSO_3$ solutions similar to that of II, although it was

Scheme II



VI, X = Br
VII, X = I

XII VIII

studied less extensively. NMR and uv spectra indicated that III was dehalogenated to thymine (5-methyluracil) in the presence of $NaHSO_3$. A linear plot of k_{obsd} vs. $[S_T]$ for the appearance of thymine was obtained in $NaHSO_3$ solutions at pH 4.50, which were also 0.1 M in succinate buffer. The k'_{obsd} value, or the slope of this plot, is listed in Table II. Since III cannot eliminate CH_3OH in a side reaction, as occurs with II, its stability was checked with respect to dehalogenation by other nucleophilic or oxidizable species present in these solutions. The uv spectrum of III remained unchanged for over 1 hr in a 0.1 M acetate buffer at pH 4.50, 25°, and in the same acetate buffer which was also 0.9 M in KCl or 0.9 M in KI.

In contrast to IV (which was only observed to eliminate CH_3OH in aqueous $NaHSO_3$ solution) 5-chloro-5-methyl-5,6-dihydro-6-methoxyuracil (V) was apparently dehalogenated to thymine in $NaDSO_3$ solution. A suspension of V in approximately 2 M $NaDSO_3$ solution in D_2O at pD 5 was heated at 60–70° for several hours. The NMR spectrum of this mixture was measured after sufficient NaOH solution in D_2O had been added to obtain a homogeneous solution. The spectrum showed a multiplet at δ 7.6 (C_6-H) and a doublet at δ 2.0 (C_5-CH_3), characteristic of thymine. One minor, unidentified peak at δ 1.5 was observed but was not investigated further. Vigorous conditions were employed to speed up the dehalogenation reaction, and this may have resulted in some decomposition of V.

Reactions of VI–VIII in Aqueous Sodium Bisulfite Solutions. Several derivatives which did not have an $-OCH_3$ group (i.e., a facile leaving group) at C-6 were also observed to undergo dehalogenation in aqueous $NaHSO_3$ solution at pH 6 to yield virtually a 1:1 mixture of 5,6-dihydrouracil (XII) and 5,6-dihydrouracil-5-sulfonate (XIII) (Scheme II). Identification of XIII was made via its isolation and characterization as described in the Experimental Section. Identification of XII was accomplished by comparison of NMR spectra of the reaction mixtures with the NMR spectrum of authentic XII and by comparison of the paper chromatographic behavior (Whatman no. 2; ascending; 85/15 EtOH–H_2O) of the reaction mixture with that of authentic XII. The product ratio was determined by carrying out the dehalogenation in a $NaDSO_3$ solution in D_2O at pD 6 and measuring the peak intensities in the NMR spectrum of the reaction mixture. (In D_2O the products were 5-deuterio-XII and 5-deuterio-XIII.) In the dehalogenation of 5-iodo-5,6-dihydrouracil (VII), both XII and XIII were again found to be products of the reaction. In this case, however, the ratio of XII to XIII formed was approximately 10:1 or higher. No XIII was observed in the NMR spectra of the reaction mixtures, but paper chromatographic analysis did indicate the presence of some XIII.

The molar absorptivities of VI and VII are larger than those of XII or XIII in the 230–250-nm region so that the dehalogenation reaction could be monitored by following changes in absorbance. At pH 7.48, the observed rate constants for dehalogenation of VI were linearly dependent on $[S_T]$, as was observed for the reactions of II. The slope of the k_{obsd} vs. $[S_T]$ plot obtained is listed in Table II. A k_{obsd} value of 0.18 sec^{-1} was obtained for dehalogenation of VII in a 0.071 M $NaHSO_3$ solution at pH 7.48. This value is approximately 120 times larger than the value obtained for VI in the same conditions.

Table III. k_{obsd} Values for Dehalogenation of II in 0.018 M $NaHSO_3$, 0.10 M Acetate Buffer at pH 5.00, Ionic Strength 1.0 M, 25°, in the Presence of Various Salts

Salt	Concn, M[a]	$10^2 k_{obsd}$, sec^{-1} [b]
LiCl	0.880	2.19 ± 0.07 (3)
NaCl	0.880	2.18 ± 0.02 (4)
Na_2SO_4	0.293	2.07 ± 0.15 (4)
KCl	0.880	1.77 ± 0.05 (5)
KBr	0.880	1.76 ± 0.04 (5)
NaCl–$NaClO_4$	0.580–0.300	2.12 ± 0.02 (2)
NaCl–$NaClO_4$	0.380–0.500	1.87 ± 0.03 (2)
$NaClO_4$	0.880	1.67 ± 0.04 (2)
KI	0.880	1.51 ± 0.04 (4)

[a] The concentration required to maintain the ionic strength of the solution at 1.00 M. [b] The average value obtained ± the average deviation for the number of determinations shown in parentheses.

The dehalogenation of 5-bromo-5,6-dihydro-6-methyluracil (VIII) in aqueous $NaHSO_3$ solution was investigated to evaluate the effect of a bulky substituent at C-6 on the reaction. As described in the Experimental Section, the two geometrical isomers of VIII, termed VIII-A and VIII-B, were isolated, but no cis–trans assignments were made. Both isomers were dehalogenated in $NaHSO_3$ solution at pH 6–7. The only pyrimidine product detectable from NMR spectra and paper chromatographic analysis (Whatman no. 2; ascending; 85/15 ethanol–H_2O) of reaction mixtures of VIII-B was 5,6-dihydro-6-methyluracil, R_f 0.60. In paper chromatographic analysis of the reaction mixture of VIII-A, a spot at R_f 0.16 was observed in addition to a spot at R_f 0.60. The spot at lower R_f suggests that 5,6-dihydro-6-methyluracil-5-sulfonate was likely formed, since it is very comparable in R_f value to that observed for compound XIII (R_f 0.11). The kinetics of dehalogenation of VIII-B were studied at pH 7.45, and the k_{obsd} values were found to be linearly dependent on $[S_T]$ values, as was observed for reactions of VI. The slope of the k_{obsd} vs. $[S_T]$ plot is listed in Table II. It was also found that VIII-B was dehalogenated six times faster than VIII-A in a 0.353 M $NaHSO_3$ solution at pH 7.45.

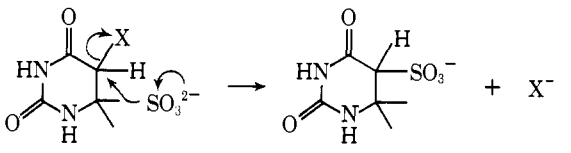
Polarographic Behavior of VI. Compound VI, 5-bromo-5,6-dihydrouracil, was found to be polarographically active at a dropping mercury electrode, with a half-wave potential of −0.3 V, vs. a saturated calomel electrode. A linear plot of diffusion current vs. the concentration of VI in solution (0.001–0.005 M) was obtained in 0.1 N KCl solution. Similar results were obtained for a 1:1 mixture of the two isomers of VIII.

Discussion

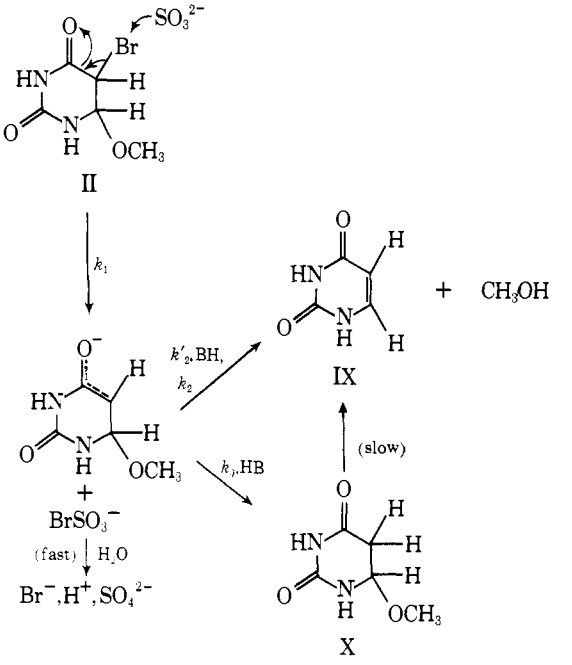
The results of this study suggest that two different mechanisms are responsible for the dehalogenation of the 5-halo-5,6-dihydrouracils in aqueous $NaHSO_3$ solutions. One process involves displacement of the halogen by SO_3^{2-} (Scheme III) and leads to products such as XIII, whereas the other process leads to products such as XII, IX, and X (Scheme IV).

The displacement reaction is almost certainly an SN2 rather than an SN1 reaction. The carbonium ion which

Scheme III



Scheme IV



would be an intermediate in the SN1 reaction would have a positive charge on a carbon atom which was adjacent to a carbonyl group and would, consequently, be an extremely high-energy species.

The mechanism of the reaction which leads to products such as XII, IX, and X is postulated to proceed via the steps shown in Scheme IV. Other possible mechanisms could involve reactions of sulfite ions with a positive halogen ion or a hypohalous acid which were in dynamic equilibrium with the dihydrouracil in aqueous solution. Both of these alternative mechanisms are, however, discounted because it would be expected that the strongly oxidizing positive halogen ion or hypohalous acid would give iodine by reaction with iodide ion. None of the compounds which were studied yielded iodine in aqueous solutions of potassium iodide at 25°.

The reaction sequence illustrated in Scheme IV involves attack of SO_3^{2-} on the halogen on C-5 to yield a carbanion and possibly a halosulfonic acid. The carbanion would rapidly pick up a proton at C-5 to form products such as XII and X or eliminate CH_3O^- to form a product such as IX. The species HB in this scheme is a general acid. The halosulfonic acid would rapidly hydrolyze to form halide ion, H^+, and SO_4^{2-}.[9]

If the attack of SO_3^{2-} is rate determining, k'_{obsd} values would be related to the calculated second-order rate constants, k'_{obsd}, by eq 1.[10] Equation 1 will apply in the pH range of 4–8, where the imino protons of II are not ionized to an appreciable extent. K_a is the acid dissociation constant for HSO_3^- ($pK_a = 6.50$). The solid line in Figure 2 was calculated using eq 1 and a value of 42 M^{-1} sec^{-1} for k_1.

$$k'_{obsd} = k_1 \left(\frac{K_a^{HSO_3^-}}{[H^+] + K_a^{HSO_3^-}} \right) \quad (1)$$

Significant changes were observed in k_{obsd} values for dehalogenation of II (listed in Table III) in the presence of various neutral salts (while maintaining the pH, $[S_T]$, and ionic strength constant). Specific kinetic salt effects in this reaction could conceivably arise from a participation of the salt anions in a redox reaction (i.e., dehalogenation of II via oxidation of I^- to I_2), from a common ion effect (Br^- and SO_4^{2-} are products of the dehalogenation of II), or from changes in the solvent structure. Since the rates were actually slowest in the presence of KI, any competition of I^- (or any other anion studied) with SO_3^{2-} as a reducing agent can be eliminated. Because the salt effects for KBr and Na_2SO_4 were no larger than for the other salts investigated, common ion effects could also be discounted. Thus, it appears that these rate differences are related to changes in the structure of water and, hence, to changes in the activity coefficients of the reactants. The data in Table III are in very good rank order correlation with activity coefficients of nonelectrolytes (i.e., benzoic acid) which were measured in solutions of these salts.[11]

The results shown in Table I indicate that the initial percentage yield of IX decreased significantly with increasing buffer concentration in the dehalogenation of II. The rate of appearance of IX, on the other hand, was essentially independent of buffer concentration at constant pH and $[S_T]$. To be consistent with these results, it was necessary to consider in Scheme IV that both protonation at C-5 of the enolate anion and elimination of CH_3OH were buffer catalyzed and that essentially only elimination of CH_3OH occurred in the absence of buffer [i.e., k'_3 (for H_2O) $\ll k_2$ in Scheme IV]. Equation 2 describes the initial percentage yield of uracil (IX) according to Scheme IV. In this equation, $[II]_0$ is the initial concentration of II in solution, and HB is the conjugate acid species of the added buffer.

$$\% IX = \frac{[IX]100}{[II]_0} = \frac{k'_2[HB] + k_2}{(k'_2 + k_3)[HB] + k_2} \times 100 \quad (2)$$

This equation is consistent with the observation that the initial percentage yield of IX decreased to a constant value with increasing buffer concentration as shown in Table I. Since the data for Table I were obtained in 0.01 M $NaHSO_3$ solutions, terms for HSO_3^- acting as a general acid must also be included in eq 2, resulting in eq 3. At constant pH and $[S_T]$, eq 3 can be arranged to eq 4 where $[B_T] = [HB] + [B^-]$; $C_1 = \alpha[k'_2/(k'_3[HSO_3^-] + k_2)]$; $C_2 = [(k''_2[HSO_3^-] + k_2)/(k'_3[HSO_3^-] + k_2)]$; and $C_3 = \alpha[(k'_2 + k_3)/(k'_3[HSO_3^-] + k_2)]$. The factor α is the fraction of the acidic species of the buffer, $[H^+]/([H^+] + K_a)$. Although succinic acid buffer was used in solutions to obtain the data shown in Table I, it has been treated in these equations as a monoprotic [i.e., $(CH_2CO_2)_2H^- \rightleftharpoons H^+ + (CH_2CO_2)_2^{2-}$] acid for the sake of simplifying the expressions. The calculated values in Table I were obtained from eq 4 using values of $C_1 = 7.271$, $C_2 = 0.8052$, and $C_3 = 26.18$.

$$\% IX = \frac{k'_2[HB] + k''_2[HSO_3^-] + k_2}{(k'_2 + k_3)[HB] + k'_3[HSO_3^-] + k_2} \times 100 \quad (3)$$

$$\% IX = \frac{C_1[B_T] + C_2}{1 + C_3[B_T]} \times 100 \quad (4)$$

The observation that H_2O was apparently not an effective proton donor to carbon-5 of the enolate ion to yield X (i.e., no term for this reaction need be included in eq 3) is consistent with similar observations made on other reactions of uracils which involve proton transfer to carbon-5.[3,8]

Evidence for both a pathway involving attack of SO_3^{2-} on halogen and the SN2 pathway shown in Scheme III was obtained with several of the compounds studied. The SN2 pathway appeared to compete most successfully when the

C-6 substituents were H. Compound VI dehalogenated to yield XIII (via the SN2 pathway) and XII (via attack of SO_3^{2-} on bromine) in virtually a 1:1 ratio. When a $-CH_3$ or $-OCH_3$ group was present at C-6, the contribution of the SN2 process was strongly reduced. The $-CH_3$ group did not significantly affect the rate of SO_3^{2-} attack on halogen but did appear to sterically hinder the attack of SO_3^{2-} on C-5. The geometrical arrangement of the $-CH_3$ group relative to the halogen was also an important factor in determining the magnitude of this effect. In the dehalogenation of the two isomers of VIII, only one isomer yielded a detectable amount of SN2 product. The $-OCH_3$ group in compound II had a similar effect in essentially blocking the SN2 reaction. The fact that II underwent attack on bromine some 2000 times faster than did VI or VIII can be attributed to the electron-withdrawing effect of the $-OCH_3$ group which would make the C-5 halogen more labile toward attack by SO_3^{2-}. The type of halogen at C-5 also had an effect on the relative significance of these two reaction pathways. On going from a bromo substituent (VI) to an iodo substituent (VII) at C-5, the SN2 pathway became less significant, and the dehalogenation rate increased some 120 times. Apparently, this effect is due to the greater ease of reduction of the iodo substituent, although other effects, such as ring conformation, may also be important.

Based on these substituent effects on the relative significance of the two dehalogenation pathways, it would be expected that the 5-halo-5,6-dihydrouracil-6-sulfonates formed from addition of HSO_3^- to 5-chloro-, 5-bromo-, and 5-iodouracil would not be likely to dehalogenate via an SN2 process. The large $-SO_3^-$ group at C-6 would be expected to strongly inhibit attack of SO_3^{2-} at C-5 by a steric effect and to enhance attack of SO_3^{2-} at the C-5 halogen by an inductive effect, similar to that observed for the $-OCH_3$ group, but smaller in magnitude. Thus, compound I, 5-bromo-5,6-dihydrouracil-6-sulfonate, is expected to dehalogenate in $NaHSO_3$ solutions in a manner very similar to that observed for compound II.

A recent paper on the polarographic activity of the 5-halouracils reported that 5-bromo-5,6-dihydrouracil was not polarographically active, whereas 5-bromouracil had a half-wave reduction potential of -1.6 V (vs. a SCE).[12] This report seems strange in light of our observed chemical reduction of VI and other similar derivatives in aqueous $NaHSO_3$ solution. A brief investigation in this study showed that VI and VIII were polarographically active with half-wave potentials of -0.3 V in aqueous 0.1 *N* KCl solution.

Experimental Section

pK_a and pH Measurement. The pK_a value of HSO_3^- was determined at ionic strength 1.0 *M*, 25°, using a potentiometric method.[13] This titration and all pH measurements were made on a Radiometer Model 26 pH meter equipped with Corning electrodes No. 476002 (calomel) and No. 476022 (glass). The pH values of solutions containing $NaClO_4$ were measured with the calomel electrode fitted with a salt bridge containing a 0.1 *M* NaCl solution.

Kinetics and Methods. NMR spectra were recorded on a Varian Associates T-60 instrument. Chemical shifts were measured relative to 3-(trimethylsilyl)propanesulfonic acid sodium salt in D_2O and relative to $Si(CH_3)_4$ in dimethyl-d_6 sulfoxide and acetone-d_6. Uv spectra were recorded on a Cary 14,15 or a Durrum-Gibson spectrophotometer equipped with thermostated sample compartments held at 25 ± 0.2°.

Polarographic measurements were made utilizing a mercury dropping electrode (4 sec/drop), an SCE, and an auxiliary carbon electrode connected with a potentiostat and x,y recorder. Solutions were also 0.1 *N* in KCl and contained gelatin to suppress maxima (they were bubbled with N_2 prior to measurements).

All solutions containing sodium bisulfite were freshly made before each experiment using N_2-bubbled water redistilled from a Pyrex apparatus. The solutions contained 1×10^{-4} *M* Na_2EDTA to help inhibit oxidation of HSO_3^-. Values of $[S_T]$ (i.e., $[HSO_3^-] + [SO_3^{2-}]$) were calculated from the $[NaHSO_3]$ in the solution as described in ref 3. All kinetic runs were carried out in solutions at ionic strength 1.0 *M* (adjusted with KCl unless otherwise stated) and at 25°. Typically, 2–3 runs were made for each solution, and the average k_{obsd} value was reported.

Materials. All commercial chemicals were reagent grade and were used without purification. Uracil and thymine were obtained from Sigma Chemical Co.

5-Bromo-5,6-dihydrouracil (VI) was prepared according to Zee-Cheng et al.,[14] and the product, mp 200° with loss of HBr (lit. mp 207–208), had an NMR spectrum consistent with that reported in the literature.[7]

5-Bromo-5,6-dihydro-6-methyluracil (VIII) was prepared by the method of Zee-Cheng et al.,[14] and the NMR spectrum of this product in DMSO-d_6 indicated that it was a 1:1 mixture of the geometrical isomers of 5-bromo-5,6-dihydro-6-methyluracil since the C_6-CH_3 peak appeared as two sets of doublets, approximately 6 Hz apart, and the N_1-H peak appeared as two broad singlets, approximately 10 Hz apart. The isomers were separated by successive fractional crystallization from EtOH–H_2O mixtures. The isomeric composition of the crystal fractions was determined by observing the ratio of the C_6-CH_3 doublets in the NMR spectra. (Since no definite assignment could be made as to which isomer was cis or trans, the isomers will be referred to as A and B.) Anal. Calcd for $C_5H_7N_2O_2Br$ (cis-trans mixture): C, 28.99; H, 3.38; N, 13.53. Found: C, 28.75; H, 3.40; N, 13.24. Isomer A: mp 190° (decomposition with loss of HBr); NMR (DMSO-d_6) δ 1.13 (d, 3, J = 6 Hz, C_6-CH_3), 3.68 (m, 1, C_6-H), 4.60 (d, 1, J < 2 Hz, C_5-H), 7.75 (br s, 1, N_1-H), and 10.35 (br s, 1, N_3-H). Isomer B: mp 205° (decomposition with loss of HBr); NMR (DMSO-d_6) δ 1.22 (d, 3, J = 6 Hz, C_6-CH_3), 3.60 (m, 1, C_6-H), 4.62 (d, 1, J = 4 Hz, C_5-H), 7.93 (s, 1, N_1-H), and 10.42 (br s, 1, N_3-H). Isomer A was observed to be slightly more soluble in 95% EtOH and less stable with respect to thermal dehydrohalogenation than isomer B.

5-Iodo-5,6-dihydrouracil (VII) was prepared from 5-bromo-5,6-dihydrouracil via the Finkelstein reaction. A solution of 20.0 g (0.13 mol) of reagent NaI in 60 ml of anhydrous acetone was prepared and 5.0 g (0.026 mol) of 5-bromo-5,6-dihydrouracil added at room temperature. The resultant solution quickly became turbid, due to precipitation of NaBr. After 25 min, the mixture was heated to reflux for 5 min and most of the acetone then removed under vacuum. After adding 100 ml of H_2O, the solution was cooled in an ice bath for several hours. The resultant crystals were collected by filtration, washed with cold H_2O and Et_2O, and recrystallized from an acetone–water mixture to yield 2.9 g of off-white needles: mp 190° (decomposition with loss of HI); NMR (F_3CCOOH) δ 7.4 (br s, 1, N_3-H), 5.5 (br s, 1, N_1-H), 3.0 (m, 1, C_5-H), and 1.7 (m, 2, C_6-H). Anal. Calcd for $C_4H_5N_2O_2I$: C, 20.00; H, 2.08; N, 11.66. Found: C, 20.25; H, 1.99; N, 11.42.

5-Bromo-5,6-dihydro-6-methoxyuracil (II) was prepared by bromination of uracil in anhydrous CH_3OH. A solution of 3.2 g (0.02 mol) of reagent Br_2 in 10 ml of anhydrous CH_3OH was slowly added to a suspension of 2.24 g (0.02 mol) of uracil in 40 ml of anhydrous CH_3OH at 0° with constant stirring. Vigorous stirring was continued for 15 min and then the product collected by filtration and thoroughly washed with Et_2O. (The crystals were not recrystallized due to the instability of the compound toward loss of CH_3OH across its 5,6 bond in solution.) The off-white crystals decomposed at 310°. The NMR spectrum of this product suggested that only one geometrical isomer was formed by this method of synthesis: NMR (DMSO-d_6) δ 8.8 (br s, 1, N_3H), 7.9 (br d, 1, J = 6 Hz, N_1-H), 4.6 (t, 1, C_6-H), 4.5 (d, 1, J = 2 Hz, C_5-H), and 3.3 (s, 3, C_6-OCH_3). Anal. Calcd for $C_5H_7N_2O_3Br$: C, 26.91; H, 2.69; N, 12.56. Found: C, 27.06; H, 2.82; N, 12.28.

5-Bromo-5-methyl-5,6-dihydro-6-methoxyuracil (III) was prepared in a similar manner by bromination of thymine in anhydrous methanol. The isolated product was recrystallized from ethyl acetate. It slowly decomposed above 150° (formation of Br_2): NMR (acetone-d_6 + D_2O) δ 4.8 (s, 1, C_6-H), 3.5 (s, 3, C_6-OCH_3), and 1.9 (s, 3, C_5-CH_3). Anal. Calcd for $C_6H_9N_2O_3Br$: C, 30.38; H, 3.80; N, 11.81. Found: C, 31.07; H, 4.01; N, 11.97.

5-Chloro-5,6-dihydro-6-methoxyuracil (IV) was prepared by

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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References and Notes

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

Frank A. Sedor, Dan G. Jacobson,[2] and Eugene G. Sander*

Contribution from the Department of Biochemistry, University of Florida, Gainesville, Florida 32610. *Received December 28, 1974*

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