A Kinetic Study of the Dehalogenation of 5-Chloro-, 5-Bromo-, and 5-Iodouracil in Aqueous Solutions of Sodium Bisulfite[1,2]

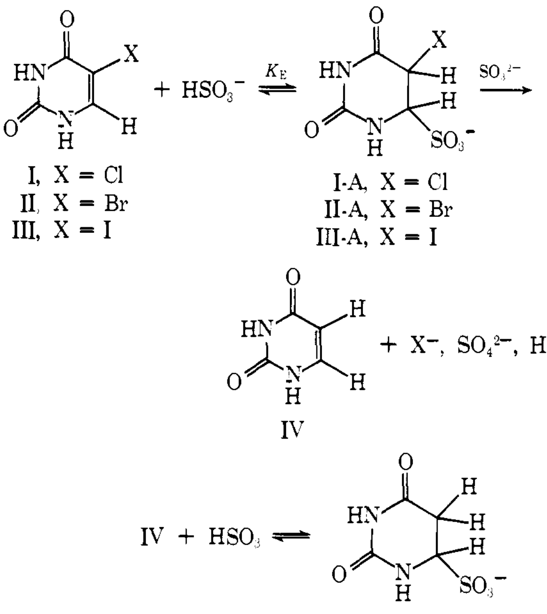
G. S. Rork and Ian H. Pitman*

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

Abstract: Kinetic studies have been made of the reactions which occurred at 25° in aqueous solutions which contained sodium bisulfite and 5-chloro-, 5-bromo-, or 5-iodouracil. Bisulfite ion added rapidly and reversibly to 5-chlorouracil to yield an approximately equimolar mixture of two isomers of 5-chloro-5,6-dihydrouracil-6-sulfonate. The equilibrium constant for this reaction was calculated to be 1.8 M^{-1} at 25° and ionic strength 1.0 M. The dependence of pseudo-first-order rate constants for the covalent addition on pH, initial concentrations of bisulfite ion, and of other general acids and bases such as phosphate ions suggested that the mechanism of addition involved (1) addition of SO_3^{2-} to C-6 of 5-chlorouracil to yield a carbanion and (2) addition of a proton to C-5 of the carbanion in both proton and general acid catalyzed reactions to yield the product. The 5-chloro-5,6-dihydrouracil-6-sulfonate reacted with sulfite ion to yield 5,6-dihydrouracil-6-sulfonate, chloride ion, and sulfate ion. The kinetics of conversion of 5-bromo- and 5-iodouracil to mixtures of uracil and 5,6-dihydrouracil-6-sulfonates suggested that the mechanisms of these reactions were similar to those involved in the dehalogenation of 5-chlorouracil. The following differences were, however, observed. (1) Appreciable amounts of 5-halo-5,6-dihydrouracil-6-sulfonates did not build up. (2) The rates of the overall reactions were not appreciably catalyzed by general acids and bases between pH 5-7, but they were proton catalyzed. (3) Both uracil and 5,6-dihydrouracil-6-sulfonate were rapidly formed following the mixing of solutions of the 5-halouracil and sodium bisulfite. The yield of the 5,6-dihydrouracil-6-sulfonate increased as the concentration of general acids in the reaction solution was increased.

Halogenated uracils are relatively stable against ring opening or dehalogenation at 25° in aqueous solutions of salts such as sodium carbonate, sodium phosphate, or sodium borate which have pH values between 5 and 10.[3] These compounds are, however, much less stable in aqueous solutions which contain the strong nucleophile and reducing agent, bisulfite ion (HSO_3^-). The quite rapid ring opening and dehalogenation of 5-fluorouracil in aqueous solutions of sodium bisulfite ($NaHSO_3$) has been described recently.[4] This manuscript deals with the kinetics and mechanism of the dehalogenation[5] of 5-chlorouracil (I), 5-bromouracil (II), and 5-iodouracil (III) in aqueous solutions of $NaHSO_3$. A brief review of the medical uses of these compounds and of possible similarities between the mechanisms of their in vivo dehalogenation and the subject of this paper is presented by Sedor et al.[2]

Scheme I



I, X = Cl
II, X = Br
III, X = I

I-A, X = Cl
II-A, X = Br
III-A, X = I

IV

V

It has been suggested[5a] that the dehalogenations of I, II, and III in aqueous solutions of $NaHSO_3$ occur via the formation and reduction of 5-halo-5,6-dihydrouracil-6-sulfonates as shown in Scheme I. A subsequent reaction of uracil (IV) with HSO_3^- yielded 5,6-dihydrouracil-6-sulfonate (V) as the final pyrimidine product of these dehalogenation reactions. No buildup of any intermediate other than uracil was, however, reported in this previous study.[5a]

We have studied reactions of the 5-halouracils in $NaHSO_3$ solutions and have also obtained results which indicate that the dehalogenations occur via reactions of 5-halo-5,6-dihydrouracil-6-sulfonates. These results differ from those of the previous study[5a] in that compound I was observed to react with HSO_3^- to form 5-chloro-5,6-dihydrouracil-6-sulfonate (I-A) in an equilibrium reaction which took place much faster than the subsequent dehalogenation of I-A. An additional finding of the present study is that both IV and V were initial products of the dehalogenation of II and III (and presumably I) in $NaHSO_3$ solutions and that the ratio of these two products was dependent on the concentration of buffer in the solutions.

Experimental Section

Materials and Equipment. Uracil, II, and III were obtained from Sigma Chemical Co. and used without further purification. 5-Chlorouracil (obtained from K and K Laboratories) was found to contain uracil as an impurity and was purified by several recrystallizations from a water-ethanol mixture. All inorganic chemicals were of reagent grade and were used as obtained from commercial sources. Distilled water was redistilled from a Pyrex apparatus and bubbled with N_2 to remove dissolved O_2.

Solutions which contained $NaHSO_3$ were made up immediately before use in the manner described previously.[6] Each solution contained 1×10^{-4} M Na_2EDTA to help inhibit metal ion catalyzed oxidation of HSO_3^-. Values of the concentration of HSO_3^- + SO_3^{2-}, $[S_T]$, were calculated from the analytical concentration of sodium bisulfite $(S_T)_A$[7] by using equations which were developed previously.[8]

Uv spectra were measured on Cary 14, 15, or 16 spectrophotometers which had thermostated cell compartments. NMR spectra were measured in D_2O on a Varian T-60 instrument and sodium 3-(trimethylsilyl)propanesulfonate was used as an internal refer-

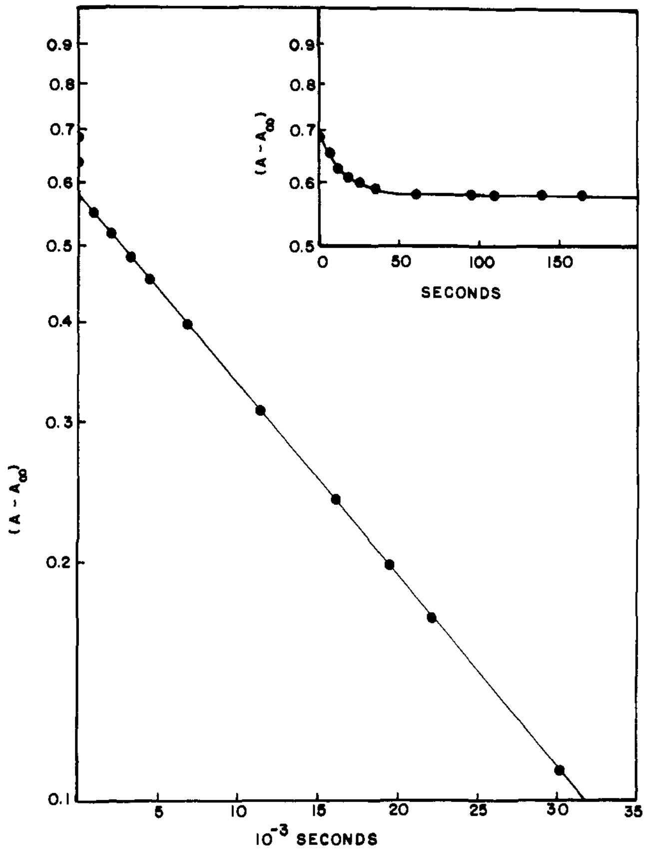


Figure 1. A semilogarithmic plot of time of the absorbance changes ($A - A_\infty$) that occurred at 295 nm after 5-chlorouracil was added to a 0.45 M $NaHSO_3$ solution at pH 6.50, ionic strength 1.00 M, 25°.

ence. Values of pH were measured on a Radiometer Model 26 pH meter. The normal calomel electrode (Corning no. 476002) was fitted with a salt bridge containing 0.1 N NaCl whenever the pH of solutions containing $NaClO_4$ was measured.

Kinetic and Equilibria Measurements. The pK_a values of compounds I and III were determined[9] spectrophotometrically at ionic strength 1.00 M, 25°.

The reactions of I-III in aqueous $NaHSO_3$ solutions at ionic strength 1.00 M, 25.0 ± 0.1°, were monitored in the kinetic studies by following the time course of absorbance changes in the 295-nm region. At high $NaHSO_3$ concentrations, 2-mm uv cells (0.8 ml capacity) were utilized to reduce the background absorbance of the $NaHSO_3$ solutions. KCl was used to adjust ionic strength unless otherwise stated.

Results

Reactions of 5-Chlorouracil in $NaHSO_3$ Solutions. Addition of a solution of compound I to solutions of $NaHSO_3$ at pH 4-7 resulted in a rapid partial decrease in the uv absorbance due to I in the 260-300-nm region. This was followed by a much slower decrease in the absorbance at these wavelengths. Figure 1 shows a semilogarithmic plot of the absorbance changes ($A - A_\infty$) vs. time which were observed at 295 nm for an aqueous solution of I and 0.45 M sodium bisulfite at pH 6.50. Under these conditions, essentially a complete loss in absorbance at 295 nm was observed after 24 hr. A value of 5.54×10^{-5} sec^{-1} was calculated for the observed first-order rate constant for the second reaction from the terminal slope of the plot in Figure 1. When the absorbance at t = 100 sec was taken as an A_∞ value for the first reaction that occurred, a semilogarithmic plot of ($A - A_\infty$) vs. time was linear for at least 3 half-lives. A value of the observed first-order rate constant for the first reaction that occurred was calculated to be 7.23×10^{-2} sec^{-1} from the slope of this plot. These data are consistent with a reaction scheme such as that illustrated in Scheme I. In this sequence of reactions, I undergoes addition of HSO_3^- to come to equilibrium with 5-chloro-5,6-dihydrouracil-6-sulfonate (I-A) and I-A is subsequently dehalogenated to yield Cl^-, H^+, SO_4^{2-}, and 5,6-dihydrouracil-6-sulfonate (V) as final products. Sander and Deyrup[5] have previously described the characterization of these reaction products and we have confirmed their observations.

The kinetics and thermodynamics of the first reaction illustrated in Scheme I were investigated in the pH range of 4.5-7.0. Based on the uv spectral data observed for the equilibrium addition of HSO_3^- to 5-fluorouracil,[4] it was expected that I-A would make no contribution to the observed absorbance in the 270-300-nm region. Uracil is the only other substance that may possibly contribute to uv absorbance between 270 and 300 nm in these solutions. However, no evidence of a buildup of uracil as an intermediate was obtained for reactions of I in $NaHSO_3$ solutions. Furthermore, the addition of HSO_3^- to uracil occurs some 10-100 times faster than the second reaction observed for I in $NaHSO_3$ solutions when measured under the same conditions.[10] Hence, any uracil that was formed as a result of reactions between SO_3^{2-} and I-A would be rapidly converted to V and would not contribute to the observed absorbance in the 270-300-nm region. Thus, it was assumed that the observed absorbance in this region is due entirely to I.

Values of equilibrium constants, K_{obsd}^{I},[11] for addition of HSO_3^- to I at pH values between 4.5 and 7.0 were calculated by using a method which has been described previously.[4] The uv absorbance at equilibrium (A_∞) was estimated to be the same as the measured absorbance when the initial fast reaction had proceeded for 7 half-lives. Values of K_{obsd}^{I} are displayed in Figure 2. The solid line in Figure 2 was calculated on the assumption that $K_{obsd}^{I} = K_E^{I}(\alpha_I \alpha_{HSO_3^-})$ (where α_I and $\alpha_{HSO_3^-}$ are the fractions of added I and sodium bisulfite that are present as the neutral molecule and HSO_3^-, respectively)[11] and that $K_E^{I} = 1.8$ M^{-1}, $pK_a^{HSO_3^-} = 6.50$, and $pK_a^{I} = 7.85$ at 25° and $I = 1.0$ M.

NMR spectral changes which were observed when I was added to a 1-2 M K_2SO_3 solution in D_2O at pD 6 also support the reaction sequence shown in Scheme I. As compound I dissolved in the solution, a singlet due to I appeared at δ 7.90 (C_6-H). Within a few minutes, additional singlets of near equal intensities appeared at δ 4.93 and 4.83. After several hours, another singlet was observed at δ 4.62; this signal is identical in chemical shift with that of 5,5-dideuterio-5,6-dihydrouracil-6-sulfonate,[12] which is the final pyrimidine product of the reaction in D_2O. The C_6-H of 5-deuterio-5-fluoro-5,6-dihydrouracil, which has been isolated and characterized, appeared at δ 4.9 in D_2O solution.[4] It is expected that the chemical shift of the C_6-H of I-A would be very similar to that of its 5-fluoro analog and that the signal would appear as a singlet. The fact that two singlets were observed near δ 4.9 suggests that both geometrical isomers of I-A form and that they are in equilibrium with I and DSO_3^-. Similar small differences (δ ~0.1) in C_6-H chemical shifts have been reported[13] for the geometrical isomers of both 1-methyl-5,6-dihydroxy-5,6-dihydrouracil and 1,3-dimethyl-5,6-dihydroxy-5,6-dihydrouracil. If this analysis of the NMR data is correct, then compound I-A in Scheme I represents both geometrical isomers of 5-chloro-5,6-dihydrouracil-6-sulfonate and K_E^{I} is a composite equilibrium constant for the formation of both isomers.

The kinetics of the addition of HSO_3^- to I in the establishment of the equilibrium between I, HSO_3^-, and I-A were studied in the pH range of 5-7. The observed first-order rate constants, k_{obsd} values, which were measured at several pH values are shown as data points in plots of k_{obsd} vs. [S_T] in Figure 3. Each data point is the average of 3-5

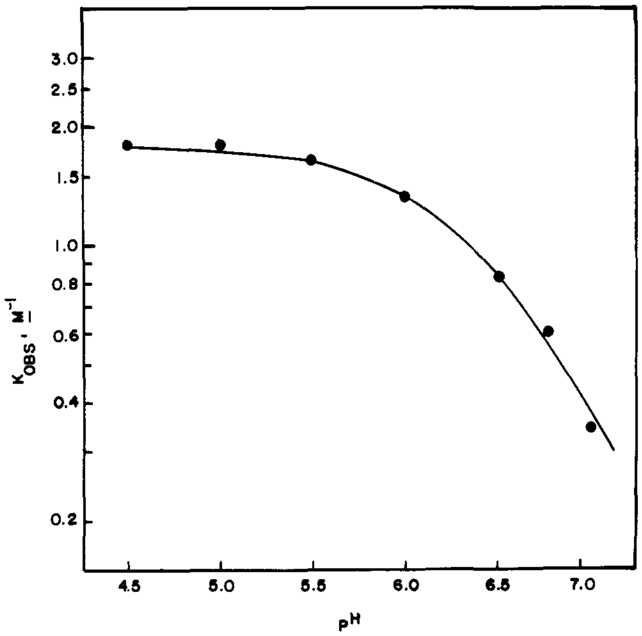


Figure 2. A semilogarithmic plot of the K_{obsd} values measured for the addition of HSO_3^- to 5-chlorouracil vs. pH.

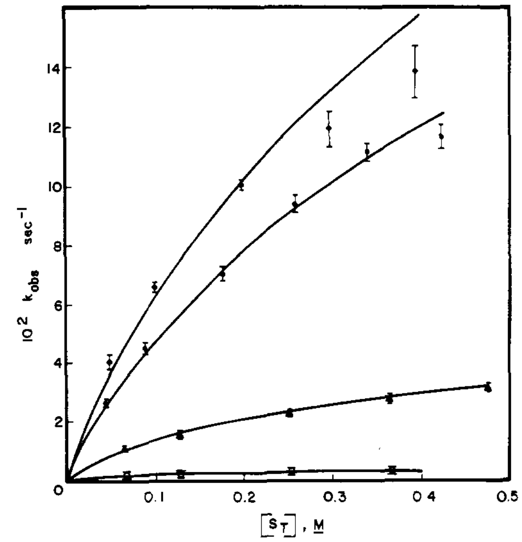


Figure 3. Plots of the observed first-order rate constants for addition of HSO_3^- to 5-chlorouracil at pH 7.00 (♦), pH 6.77 (●), pH 6.00 (▲), and pH 5.00 (○) vs. $[S_T]$. The points shown are the average of 3-5 values and the vertical bars denote the range of the values which were averaged.

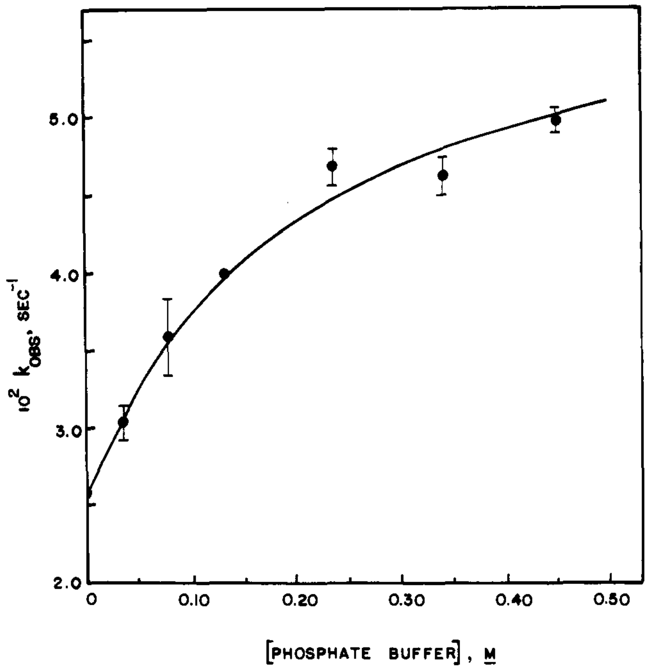


Figure 4. A plot of the observed first-order rate constants for addition of HSO_3^- to 5-chlorouracil for solutions 0.075 M in $[S_T]$ at pH 7.00 vs. the concentrations of added phosphate buffer. The points shown are the average of two values and the vertical bars denote the range of the values which were averaged. All solutions were maintained at ionic strength 1.00 M with KCl. The solid line was calculated according to eq 1 as described in the text.

values and the vertical bars denote the range of k_{obsd} values observed at each $[S_T]$. Values of k_{obsd} were measured at pH 5.00 in the presence of 0.05, 0.10, and 0.20 M acetate buffer. Within experimental error, no differences in k_{obsd} values were obtained at these different concentrations of acetate buffer. At pH 7, the k_{obsd} vs. $[S_T]$ plots exhibited a strong dependency on added phosphate buffer. Figure 4 shows a plot of k_{obsd} vs. concentration of added phosphate buffer, $[P_T]$, for solutions which were 0.075 M $[S_T]$ at pH 7.00. The solid line was calculated according to eq 1 with $C_A = 0.313\ M^{-1}\ sec^{-1}$, $C_B = 0.0251\ sec^{-1}$, and $C_c = 5.137\ M^{-1}$.

$$k_{obsd} = (C_A[P_T] + C_B)/(1 + C_c[P_T]) \qquad (1)$$

A brief study was made of the dehalogenation reaction of I in $NaHSO_3$ solutions at pH 6.50 using an initial rate technique. Since I was apparently the only absorbing species in the 270-300-nm region in these reactions, the observed first-order rate constants for the second reaction shown in Scheme I could be calculated from eq 2. A_0' is the absorbance observed at equilibrium for the addition reaction and $(A_0' - A_t)/t$ is the initial slope of the absorbance-time curve for the second reaction. Although the k_{obsd} values which were obtained by this method were somewhat variable, the results suggested a second-order dependence on $[S_T]$ with an apparent rate constant of $2.5 \times 10^{-4}\ M^{-2}\ sec^{-1}$. These reactions were not investigated further since they became very slow at lower $[S_T]$. Based on the initial rates treatment, the observed half-life of the reaction would be 62.4 hr in a 0.1 M $NaHSO_3$ solution at pH 6.5.

$$k_{obsd} = (A_0' - A_t)/A_t t \qquad (2)$$

Reactions of 5-Bromo- and 5-Iodouracil in $NaHSO_3$ Solutions. In contrast to the dehalogenation of I, in $NaHSO_3$ solutions, no 5-halo-5,6-dihydrouracil-6-sulfonate intermediate was observed in the dehalogenation of 5-bromouracil (II) and 5-iodouracil (III) under any of the conditions investigated. At wavelengths where the 5-halouracil was the only absorbing species, strict first-order losses in absorbance were observed in all reactions studied. A similar observation has previously been reported.[5] NMR spectra of solutions of II or III in 1-2 M K_2SO_3 solution at pD 6 also gave no indication of 5-halo-5,6-dihydrouracil-6-sulfonate formation.

The kinetics of the reactions of II and III in $NaHSO_3$ solutions at pH 5-8 were investigated. It was found that the observed first-order rate constant (k_{obsd}) for the loss of II and III had a second-order dependence on $[S_T]$ at low concentrations and a nearly first-order dependence on $[S_T]$ at higher sulfite buffer concentrations. The change in order of the $[S_T]$ dependence is evident when the data are plotted as $k_{obsd}/[S_T]$ vs. $[S_T]$ as shown in Figure 5. If the dependence

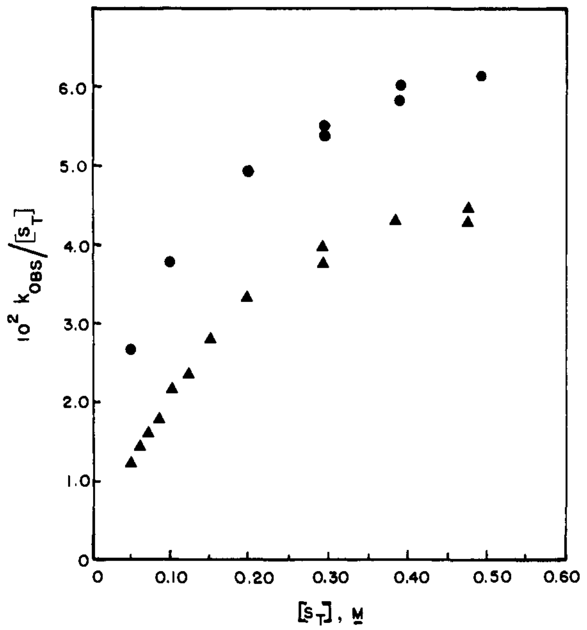


Figure 5. Plots of $10^2 k_{obsd}/[S_T]$ vs. $[S_T]$ of the reactions of 5-bromouracil (▲) and 5-iodouracil (●), in aqueous $NaHSO_3$ at pH 6.5 and ionic strength = 0.1 *M*.

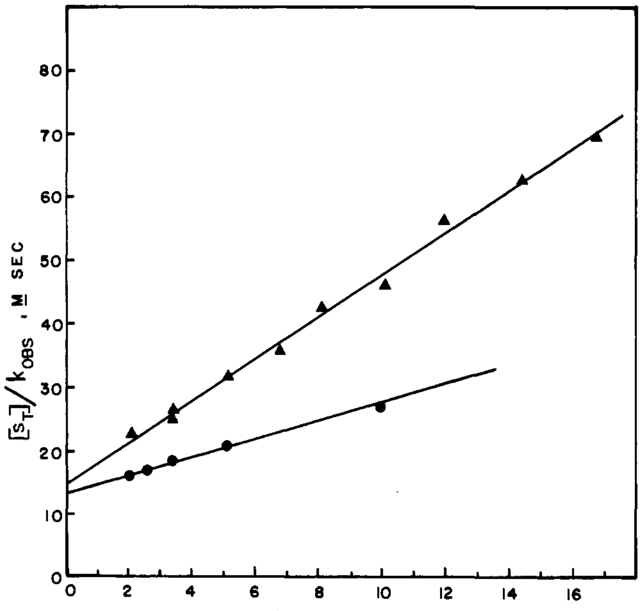


Figure 6. Plots of $[S_T]/k_{obsd}$ vs. $1/[S_T]$ of the data shown in Figure 5 for 5-bromouracil (▲) and for 5-iodouracil (●). The values of the slopes and intercepts of these plots are listed in Table I as the parameters $(1/C_1)$ and (C_1/C_2), respectively.

Table I. Values of the Parameters of Eq 4 Which Were Observed for Compounds II and III at Ionic Strength 1.00 *M*,[a] 25°

		Compound II		Compound III	
pH	Buffer	$(1/C_1)$, M^2 sec	(C_2/C_1), *M* sec	$(1/C_1)$, M^2 sec	(C_2/C_1), *M* sec
5.00	0.1 *M* succinate				208[d]
5.50	0.088 *M* phosphate	8.6	74.6		
5.78	Bisulfite–sulfite			5.2	42
5.97	Bisulfite–sulfite			2.2	27.3
6.00	Bisulfite–sulfite	6.16	20.3		
6.50	Bisulfite–sulfite	3.4	14.0	1.4	13.0
6.54[b]	Bisulfite–sulfite	4.6	13.5	1.6	13.7
6.55[c]	Bisulfite–sulfite			3.0	11.0
6.98	Bisulfite–sulfite	3.4	17.0		
7.10	Bisulfite–sulfite			1.5	13.0
7.25	Bisulfite–sulfite	6.4	24.5		
7.50	Bisulfite–sulfite	9.0	40.0	2.75	17.5

[a] Ionic strength was maintained at 1.00 *M* with KCl unless otherwise stated. [b] Ionic strength was maintained at 1.00 *M* with Na_2SO_4. [c] Ionic strength was maintained at 1.00 *M* with $NaClO_4$. [d] The reciprocal of the slope of a plot of k_{obsd} vs. $[S_T]$.

on $[S_T]$ was purely second order, the plots in Figure 5 should be linear.

Empirically, it was determined that the k_{obsd} values could be described by eq 3. This equation can be put in linear form as shown by eq 4. Figure 6 shows that a straight line was obtained when the data from Figure 5 were plotted

$$k_{obsd} = C_1[S_T]^2/(1 + C_2[S_T]) \qquad (3)$$

$$[S_T]/k_{obsd} = (1/C_1)[S_T]^{-1} + C_2/C_1 \qquad (4)$$

as $[S_T]/k_{obsd}$ vs. $[S_T]^{-1}$. The apparent linearity of the plots suggests that the dependencies of the k_{obsd} values for both II and III obey an equation of the form of eq 3. At a pH value of 5, plots of k_{obsd} vs. $[S_T]$ were almost linear. This could be occurring because the value of C_2 in eq 3 was greater than $1/[S_T]$. The $NaHSO_3$ solutions used in the latter experiment also contained succinate buffer to maintain the pH. No significant changes in the rates were observed with the variation of the succinate buffer concentration in the range of 0.10–0.40 *M* at this pH.

Table I lists the values of $1/C_1$ and C_2/C_1 for II and III which were determined from the kinetic data at several pH values. Since a linear dependence of k_{obsd} on $[S_T]$ was obtained for III at pH 5.0, the slope of this plot was taken as being equivalent to C_1/C_2.

It has previously been suggested[5] that uracil is the sole product of the dehalogenation reaction and that it is then slowly converted to 5,6-dihydrouracil-6-sulfonate. If this was the case, the absorbance at the conclusion of the dehalogenation of II or III should be the absorbance of a solution of uracil which was equivalent in concentration to the initial solution of II or III. This conclusion is reached due to the fact that the dehalogenation reactions were at least 30 times faster than addition of bisulfite to uracil (e.g., the $t_{1/2}$ value for dehalogenation of II at pH 6.5 when $[S_T]$ = 0.12 *M* was 139 sec whereas the $t_{1/2}$ value for addition of HSO_3^- to uracil under identical conditions was 4800 sec[10]). It can also be concluded that extrapolation of plots of $(A - A_\infty)$ against time for the slow addition of bisulfite to the uracil back to $t = 0$ should yield identical values of $(A_0 - A_\infty)$ if the initial concentrations of II or III were the same in each experiment. However, it was observed that values of $(A_0 - A_\infty)$ that were calculated from the results of experiments in which different concentrations of general acids and bases were employed were quite different. On the basis of what was observed to occur during the dehalogenation of 5-halo-5,6-dihydro-6-methoxyuracil under similar conditions,[14] it is concluded that both IV and V formed directly as a result of the dehalogenation reactions and that their relative yields depend on the concentrations of general acids and bases in the system. The yields of uracil that resulted directly from dehalogenation of II in several different solutions were calculated from $(A_0 - A_\infty)$ values in a manner which has been described previously[14] and they are listed in Table II.

Discussion

In contrast to what has been reported previously,[5] this study establishes clearly that significant concentrations of

5-chloro-5,6-dihydrouracil-6-sulfonate (I-A) build up during the dehalogenation of 5-chlorouracil (I) (initial concentration range 1-3 × 10^{-4} M) in aqueous sodium bisulfite solutions (initial concentration range 0.1-7 × 10^{-1} M, pH range 4.5-7.0). The equilibrium constant for covalent addition of HSO_3^- to I, $K_E{}^I$, was calculated to be 1.8 M^{-1} at 25°. This value is thus considerably smaller than the K_E value for 5-fluorouracil (560 M^{-1} at 25°).[4] It has previously been reported[15] that 5-methyluracil also has a very much smaller K_E value than that for 5-fluorouracil ($K_E < 1$). Hence, because substitution of the fluoro atom in 5-fluorouracil by either a more electropositive ($-CH_3$) or more electronegative (-Cl) substituent results in a reduction in the value of K_E, it seems highly probable that the electronic properties of the substituent play only a minor role in determining the relative values of K_E. On the other hand, both the $-CH_3$ and the -Cl substituents are larger than the -F substituent.[16] It thus seems likely that the magnitude of K_E decreases as the size of the C-5 substituent increases. Because -Br and -I substituents are larger than -Cl it seems highly likely that the K_E values for II and III would be smaller than that for I (i.e., 1.8 M^{-1}). This is consistent with the fact that no significant concentration of II-A or III-A was detected during the dehalogenation reactions of II and III. The likely importance of the size of the substituent on the stereochemistry of the reactions is further indicated by the fact that addition of HSO_3^- to I resulted in the formation of both cis and trans isomers of I-A whereas only one isomer, presumably the one with the C-5 hydrogen and the sulfonate group trans, was formed[4] in appreciable amounts when HSO_3^- added to 5-fluorouracil.

The kinetics of addition of HSO_3^- to I to form I-A can be rationalized on the basis of the reaction sequence shown in Scheme II. This sequence of reactions involves addition of SO_3^{2-} to I to yield a carbanion followed by protonation of the carbanion at C-5 in both general acid (HB) and proton-catalyzed[17] reactions. According to this scheme the desulfonation of the adduct occurs by general base (B^-) and water assisted removal of the C-5 proton followed by elimination of SO_3^{2-}. This mechanism is similar to the ones which have been proposed to account for the covalent addition of HSO_3^- to uracil[18] and 5-fluorouracil.[19] It differs slightly in that it includes both general acid and proton catalysis of the protonation of the carbanion rather than simply general acid catalysis. If it is assumed that the concentration of the carbanion remains in low steady state concentrations, the observed first-order rate constant k_{obsd} for the approach to equilibrium when $[S_T]_A$ was at least ten times the initial concentration of I is related to the rate constants in Scheme II by the identity

$$k_{obsd} = \frac{k_1\alpha'[SO_3{}^{2-}](k_3[H^+] + k_5[HB]) + k_2(k_4 + k_6[B^-])}{k_2 + k_3[H^+] + k_5[HB]} \qquad (5)$$

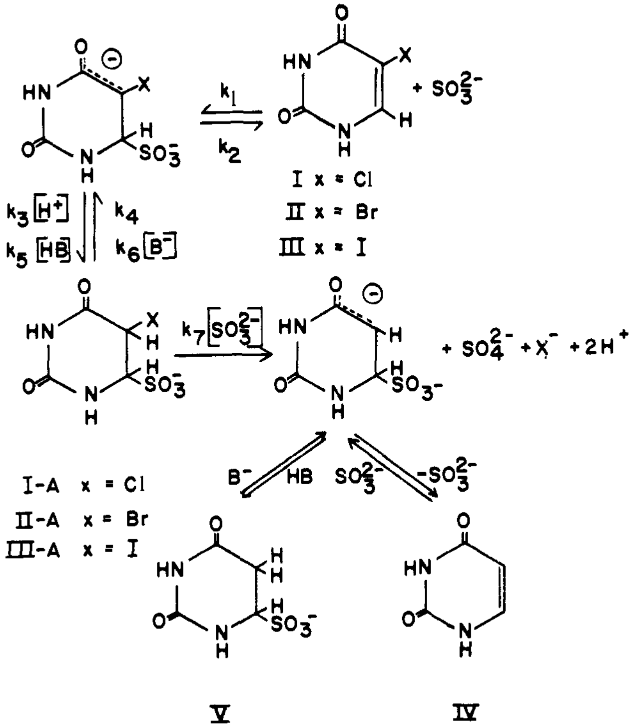
In this identity α' is the fraction of I that exists as a neutral molecule at the pH value where measurements were made. The data in Figure 3 were calculated from experiments in which HSO_3^- and SO_3^{2-} were the only general acids and bases other than water in the reaction solutions. Under these conditions $[HSO_3^-]$ and $[SO_3^{2-}]$ can be substituted for [HB] and $[B^-]$, respectively, in eq 5. A convenient rearranged form of the equation is

$$k_{obsd} = (\alpha' K_E{}^I\alpha[S_T] + 1)\frac{[k_4/k_6(1 - \alpha) + [S_T]]}{\dfrac{k_2 + k_3[H^+]}{k_2k_6(1 - \alpha)} + \dfrac{k_5(\alpha)[S_T]}{k_2k_6(1 - \alpha)}} \qquad (6)$$

Table II. Percentage Yields of Uracil at Various Buffer Concentrations for the Dehalogenation of 5-Bromouracil in $NaHSO_3$ Solutions at pH 7.00, Ionic Strength 1.00 M, 25°

$[S_T]$, M	[Phosphate buffer], M	% uracil
0.04	0.000	38.7
0.04	0.0833	26.8
0.04	0.1667	22.0
0.04	0.250	18.2
0.04	0.375	17.3
0.40	0.000	13.6

Scheme II



In eq 6, $K_E{}^I$ has been substituted for the ratio k_1k_5/k_2k_6 and $K_E{}^I/K_a{}^{HSO_3^-}$ has been substituted for the ratio k_1k_3/k_2k_4. The terms α and $(1 - \alpha)$ are the fractions of $[S_T]$ (i.e., $[SO_3^{2-}] + [HSO_3^-]$) which exist as HSO_3^- and SO_3^{2-}, respectively. Values of the ratios $k_4/k_6(1 - \alpha)$, $(k_2 + k_3[H^+]/k_2k_6(1 - \alpha)$, and $k_5\alpha/k_2k_6(1 - \alpha)$ were calculated at several pH values by nonlinear regression analysis of plots of k_{obsd} against $[S_T]$. The solid lines in Figure 3 were calculated by using eq 6 and the following values of the rate constant ratios: $k_4/k_6 = 5.6 \times 10^{-3}$ M, $(k_2 + k_3[H^+])/k_2k_6 = 1.04 + 3.66 \times 10^5[H^+]$ M sec, and $k_5/k_2k_6 = 12.1$ sec.

At pH 5, the value of k_{obsd} was unaffected by the inclusion of 0.05, 0.1, or 0.2 M sodium acetate in the reaction solution. This suggests that proton catalysis is more important than catalysis by other general acids at this pH value. At higher pH values, however, the inclusion of phosphoric acid salts in the reaction solution led to an increase in k_{obsd} values as demonstrated by the results in Figure 4. Inclusion of terms for general acid catalysis by an acid phosphate, $k'_5\alpha''P_T$ (where α'' is the fraction of the total phosphate, P_T, which is acting as a general acid), and for general base catalysis for a basic phosphate, $k'_6(1 - \alpha'')P_T$, in eq 6 and rearrangement leads to eq 7.

$$k_{obsd} = \frac{k_1[SO_3{}^{2-}][k_5[HSO_3{}^-] + k_3[H^+] + k'_5\alpha''P_T] + k_2[k_4 + k_6[SO_3{}^{2-}] + k'_6(1 - \alpha'')P_T]}{k_2 + k_3[H^+] + k_5[HSO_3{}^-] + k'_5\alpha''P_T} \qquad (7)$$

Table III. "Corrected" Values of the Slopes and Intercepts of Plots Such as Those in Figure 6[a]

	$10^7(1-\alpha)^2\alpha'[\mathrm{H^+}]/C_1$		$(1-\alpha)\alpha' C_2/C_1$	
pH	II	III	II	III
5.00				6.24
5.50	2.25		6.76	
5.78		2.20		6.69
5.97		1.25		
6.00	3.55		4.80	6.45
6.50	2.69	1.08	6.73	6.36
6.54[b]	3.62	1.23	6.76	6.98
6.55[c]		2.30		5.60
6.98	1.96		11.49	
7.10		0.70		
7.25	2.59		17.01	9.54
7.50	2.35	0.60	26.07	13.00

[a] Values of $1/C_1$ (M^2 sec) and C_2/C_1 (M sec) are in Table I. [b] Ionic strength was maintained at 1.0 M with $\mathrm{Na_2SO_4}$. [c] Ionic strength was maintained at 1.0 M with $\mathrm{NaClO_4}$.

When pH and $[\mathrm{S_T}]$ are kept constant this equation can be written as shown in eq 1 where C_A, C_B, and C_c are constants. The solid line in Figure 4 was calculated using eq 1 and values of the constants $C_A = 0.313\ M^{-1}\ \mathrm{sec}^{-1}$, $C_B = 0.0251\ \mathrm{sec}^{-1}$, and $C_c = 5.137\ M^{-1}$. Hence, again the model which is represented by the reaction sequence in Scheme II appears to be consistent with the experimental results.

Comparison of the relative values for water and sulfite ion assisted removal of a C-5 proton from I-A ($k_4/k_6 = 5.6 \times 10^{-3}\ M$) and for proton and bisulfite ion catalysis of protonation of C-5 of the carbanion ($k_3/k_5 = 3.02 \times 10^4$) suggests that the Brønsted β value for proton removal is smaller than was found for the same reaction of 5,6-dihydrouracil-6-sulfonate where β was calculated to be 0.9.[18] The reason for this difference can only be speculated on at present, but it seems likely that both the electronic and steric changes that occur when a proton is replaced by a chlorine lead to a lowering of the β value for the reaction. It will be pointed out later that the β value for the corresponding reactions of 5-bromouracil and 5-iodouracil derivatives appears to be even lower and the α values for protonation of the carbanion intermediate at C-5 appear to be higher than those for reactions of I.

A brief study of the dehalogenation of I-A to yield 5,6-dihydrouracil-6-sulfonate was carried out. This indicated that the reaction was first order in $[\mathrm{SO_3^{2-}}]$. A similar dependence has been found for dehalogenations of several other 5-halo-5,6-dihydrouracils.[14] As mentioned earlier, it is believed that I-A is dehalogenated by reactions such as those in Scheme II but no buildup of concentration of IV was observed because the rate of addition of $\mathrm{HSO_3^-}$ to IV to yield V occurs so much faster than the loss of I-A.

The reactions of 5-bromouracil (II) and 5-iodouracil (III) in aqueous sodium bisulfite solutions differed from what has just been described for I in at least two respects. In the first place no 5-halo-5,6-dihydrouracil-6-sulfonate (II-A or III-A) was observed to accumulate in the reaction solutions. Secondly, both uracil (IV) and 5,6-dihydrouracil-6-sulfonate (V) were observed to be rapidly formed from the 5-halouracils. However, because of the similarities in structure which exist between I, II, and III, it seems reasonable to assume that each of the dehalogenation reactions proceeds via a similar sequence of reactions (i.e., the ones shown in Scheme II).

By applying a steady-state treatment to the concentrations of the carbanion intermediates formed by addition of $\mathrm{SO_3^{2-}}$ to II or III and to the concentrations of II-A or III-A[20] and assuming that there are no general acids or bases present besides $\mathrm{HSO_3^-}$, $\mathrm{H^+}$, $\mathrm{SO_3^{2-}}$, and $\mathrm{H_2O}$, the following relationship[21] can be arrived at.

$$k_{\mathrm{obsd}} = \frac{k_1 k_7 \alpha'(1-\alpha)^2(k_3[\mathrm{H^+}] + k_5\alpha[\mathrm{S_T}])[\mathrm{S_T}]^2}{k_2 k_4 + k_2(k_6 + k_7)(1-\alpha)[\mathrm{S_T}] + k_7(k_3[\mathrm{H^+}] + k_5\alpha[\mathrm{S_T}])(1-\alpha)[\mathrm{S_T}]} \tag{8}$$

This complex equation has the same form as the empirical relationship (eq 3) (and thus predicts that $k_{\mathrm{obsd}} \propto [\mathrm{S_T}]^2$ at low values of $[\mathrm{S_T}]$ and $k_{\mathrm{obsd}} \propto [\mathrm{S_T}]$ at high values of $[\mathrm{S_T}]$) under two sets of conditions. These are either that $k_3[\mathrm{H^+}] \gg k_5\alpha[\mathrm{S_T}]$ and $k_4 \gg k_6[\mathrm{SO_3^{2-}}]$ or that $k_3[\mathrm{H^+}] \ll k_5\alpha[\mathrm{S_T}]$ and $k_4 \ll k_6[\mathrm{SO_3^{2-}}]$. However, only the first of these conditions is consistent with the pH dependence of values of $1/C_1$ and C_2/C_1. Under the first set of conditions, values of the term $(1/C_1)\alpha'[\mathrm{H^+}](1-\alpha)^2$ should be independent of pH and values of the term $(C_2/C_1)\alpha'(1-\alpha)$ should be independent of pH at low pH values but increase with increasing pH values when these were larger. The second set of conditions requires that values of the above terms should both be independent of pH. As shown from the "corrected" values of $1/C_1$ and C_2/C_1 in Table III the experimental results are more consistent with the condition that $k_5\alpha[\mathrm{S_T}] \ll k_3[\mathrm{H^+}]$. Another way of stating this condition is that "proton" catalyzed addition of a proton to the carbanions formed by addition of $\mathrm{SO_3^{2-}}$ to II and III predominated over protonation reaction catalyzed by the general acid $\mathrm{HSO_3^-}$. It was also found that the k_{obsd} value for dehalogenation of II in a 0.0667 M $\mathrm{NaHSO_3}$ solution at pH 7 only increased 15–20% when 0.35 M $\mathrm{NaH_2PO_4}$ was added (an 80% increase in rate was observed following a similar addition to I) and virtually no differences in k_{obsd} values were observed when 0.02–0.10 M tromethamine was added to solutions of II in aqueous $\mathrm{NaHSO_3}$ (0.01–0.25 M) at pH 8.3. Thus it appears that the α values for protonations of the carbanions in these reactions are much higher (and the β values for the deprotonations are much lower) than was found for the same reactions of uracil,[18] 1,3-dimethyluracil,[18] 5-fluorouracil,[19] and 5-chlorouracil.

The arguments that were presented above lead to the conclusion that eq 9 adequately describes the dependence of k_{obsd} values on $[\mathrm{S_T}]$ and pH.

$$k_{\mathrm{obsd}} = \frac{k_1 k_3 k_7 \alpha'(1-\alpha)^2[\mathrm{H^+}][\mathrm{S_T}]^2}{k_2 k_4 + k_7(1-\alpha)(k_2 + k_3[\mathrm{H^+}])[\mathrm{S_T}]} \tag{9}$$

This equation leads to the prediction that $k_{\mathrm{obsd}} \propto [\mathrm{S_T}]$ when the term $k_7(1-\alpha)(k_2 + k_3[\mathrm{H^+}])[\mathrm{S_T}] \gg k_2k_4$ and that $k_{\mathrm{obsd}} \propto [\mathrm{S_T}]^2$ when $k_7(1-\alpha)(k_2 + k_3[\mathrm{H^+}])[\mathrm{S_T}] \ll k_2k_4$. In the experiments reported in this paper it was observed that $k_{\mathrm{obsd}} \propto [\mathrm{S_T}]$ was approached at values of $[\mathrm{S_T}] > 0.5\ M$ when the pH value was greater than 6.0 and down to even lower values of $[\mathrm{S_T}]$ when the pH was less than 6.0. Under these conditions

$$k_{\mathrm{obsd}} \simeq \frac{k_1 k_3 \alpha'(1-\alpha)[\mathrm{H^+}][\mathrm{S_T}]}{k_2 + k_3[\mathrm{H^+}]}$$

and thus the dehalogenation step in Scheme II (with rate constant k_7) is apparently not rate determining. The rate-determining process is essentially the formation of the adducts II-A and III-A. The reactions reported in this paper approached a second-order dependence on $[\mathrm{S_T}]$ when this was <0.1 M at pH 6 or above. Equation 8 leads to the conclusion that this arises because the term $k_7(1-\alpha)(k_2 + k_3[\mathrm{H^+}])[\mathrm{S_T}]$ no longer dominates the denominator. In the extreme case where this term becomes much smaller than k_2k_4, k_{obsd} values would be given by the identity

$$k_{\text{obsd}} = \frac{k_1k_3k_7\alpha'(1-\alpha)^2[\text{H}^+][\text{S}_\text{T}]^2}{k_2k_4}$$

This identity suggests that under the above conditions both formation of the covalent adduct and its dehalogenation are rate determining. The results which are reported in the paper by Sedor et al.[2] were mainly collected from experiments in which $[\text{S}_\text{T}]$ was varied between 0.1 and 0.2 *M*. This is thus the range of values of $[\text{S}_\text{T}]$ in which a transition is expected to be occurring between rate-determining formation of the covalent adduct (II-A) and the more complex situation where both adduct formation and dehalogenation are occurring. If the k_{obsd} values in the Sedor et al. paper do have the strong second-order dependence on $[\text{S}_\text{T}]$ that was concluded by the authors it appears that the term $k_7(1-\alpha)(k_2 + k_3[\text{H}^+])[\text{S}_\text{T}]$ stayed small relative to k_2k_4 up to higher values of $[\text{S}_\text{T}]$ than was observed in the studies reported here. One difference in the experimental conditions employed by the two groups of workers was the salt that was used to maintain ionic strength at 1.0 *M*. Sedor et al.[2] used NaClO_4 whereas KCl was used in most of the experiments reported in this paper. It has previously[14] been shown that the rate of reaction of $\text{SO}_3{}^{2-}$ with 5-bromo-5,6-dihydro-6-methoxyuracil is faster by a factor of 30% in a 0.880 *M* solution of KCl than it is in a 0.880 *M* solution of NaClO_4. If a similar effect occurred during the dehalogenation of II and III the k_7 value for reactions in NaClO_4 solutions would be smaller than that in NaCl and this would cause the value of terms containing k_7 to be reduced in magnitude. Some evidence that this effect does occur comes from a comparison of $1/C_1$ and C_2/C_1 values (see Table III) for dehalogenation of III at pH values 6.54 and 6.55 in solutions that contained either Na_2SO_4 or NaClO_4. The size of C_2/C_1 relative to $1/C_1$ was greater in the example where NaClO_4 was used and it has previously[14] been established that substitution of Na_2SO_4 for KCl in reaction solutions has very little effect on dehalogenation rates. The fact that neutral salts do affect the rates of dehalogenation of II and III complicates the interpretation of the effects of general acids and bases that are ions on the reaction rates. These ions are likely to be affecting reaction rates by simply acting as salts in addition to any general acid or base catalysis that they may exert.

The fact that both uracil (IV) and 5,6-dihydrouracil-6-sulfonate (V) are formed directly in the dehalogenation of II-A and III-A emphasized the similarity of these dehalogenation reactions with the dehalogenation of 5-bromo-5,6-dihydro-6-methoxyuracil.[14] The values listed in Table II are the percent yields of IV that were obtained for reactions of II. These values had a very similar dependency on phosphate buffer concentration to what was found for the values obtained at various succinate buffer concentrations with 5-bromo-5,6-dihydro-6-methoxyuracil.

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References and Notes

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(2) A paper included in this issue by Sedor, Jacobson, and Sander describes studies that were carried out at the University of Florida at the same time that the work described in this paper was performed. We have subsequently been in close contact with Dr. Sander and his colleagues and both groups of workers feel that there are benefits in the publication of the results of these two studies at the same time. The slightly different conclusions that are drawn from each study may provide clues concerning the chemistry of an important class of compounds.

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(7) Pyrosulfite ion ($\text{S}_2\text{O}_5{}^{2-}$) is in equilibrium with $\text{HSO}_3{}^-$ in aqueous solutions and it has been assumed throughout this study that the former species is not a reactant. The equilibrium constant for the reaction $2\text{HSO}_3{}^- \rightleftharpoons \text{S}_2\text{O}_5{}^{2-}$ at 25° and $I = 1.0$ *M* was estimated to be 0.23 M^{-1}. This value was interpolated from the data in ref 8.

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(11) $K_{\text{obsd}}{}^{\text{I}} = [\text{I-A}]/[\text{I}]_\text{T}[\text{S}_\text{T}]$ where $[\text{I}]_\text{T}$ is the sum of concentrations of I and its anion $K_\text{E}{}^{\text{I}} = [\text{I-A}]/[\text{I}][\text{HSO}_3{}^-]$.

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(20) It will be argued that the failure to detect II-A or III-A arose because the rate of their dehalogenation occurred faster or at a comparable rate to their formation. It is also possible that they were rapidly formed but their K_E values were so small that their concentrations remained low under the experimental conditions. Some support for this proposal comes from the expectation that the K_E value for II-A and III-A would be smaller than $K_\text{E}{}^{\text{I}}$ (see earlier). However, if this was the situation k_{obsd} values would be proportional to $[\text{S}_\text{T}]^2$ as long as [II-A] or [III-A] remained small compared to [II] or [III], respectively. This was not observed as, although the dependence of k_{obsd} values on $[\text{S}_\text{T}]$ did change from second to first order as $[\text{S}_\text{T}]$ was increased, the values of [II-A] or [III-A] always remained small.

(21) In eq 8, α' is the fraction of II or III that exists as a neutral molecule. The pK_a value of III was calculated to be 8.15 at 25°, $I = 1.0$ *M*. The pK_a value of II was estimated to be 7.9 at $I = 1.0M$ based on a reported value of 8.05 at $I = 0.1$ *M*: R. Berens and D. Shugar, *Acta Biochim. Pol.*, **10**, 25 (1963).