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The Dehalogenation of Halocytosines by Bisulfite Buffers[1]

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Both 5-bromo- and 5-iodocytosine are rapidly dehalogenated in dilute bisulfite buffers to yield cytosine. With 5-bromocytosine, but not with 5-iodocytosine, extrapolation of semilogarithmic plots of extent reaction versus time indicates the bisulfite buffer concentration-dependent formation of an intermediate which subsequently reacts to control the rate of 5-bromocytosine dehalogenation. The disappearance of both halocytosines has a second-order dependence on bisulfite buffer concentration. Both imidazole and acetate buffers catalyze the reaction of 5-iodocytosine, but not that of 5-bromocytosine, with bisulfite. In the case of acetate buffer catalysis of the reaction of 5-iodocytosine with bisulfite, the dependence of the observed rate constants changes from first order to zero order as a function of increasing buffer concentration. The observed rate constants for 5-bromocytosine dehalogenation increase, reach a maximum at about 4.5, and then decrease as a function of pH. Iodometric titration of sulfite utilization coupled with spectrophotometric analysis of pyrimidine reactants and products indicates that 1 mole of sulfite is consumed per mole of halocytosine dehalogenated. The spectrophotometrically determined pK_a values for the conjugate acids of 5-bromo- and 5-iodocytosine at 25°C and ionic strength 1.0 *M* are 3.25 and 3.56, respectively. These results are discussed in terms of a multistep reaction pathway which is analogous to the bisulfite-catalyzed dehalogenation of the 5-halouracils.

INTRODUCTION

The halogenated pyrimidines are an important class of antiviral compounds (*1*) which, at least in the case of the 5-halouracils, are rapidly dehalogenated *in vivo* by a biochemical pathway which is not clearly delineated (*2–4*). Both 5-bromo- and 5-iodouracil are easily dehalogenated under near physiological conditions by bisulfite buffers (*5, 6*) and simple thiol compounds, such as cysteine and 2-mercaptoethanol (*7–9*). The mechanism for the reaction of 5-bromo- and 5-iodouracil with bisulfite buffers likely involves the formation of 5-halo-5,6-dihydrouracil-6-sulfonate, which is then dehalogenated to yield uracil, halide, and sulfate as products (*10*). The objective of this report is to show that 5-bromo- and 5-iodocytosine are dehalogenated by dilute bisulfite buffers under near physiological conditions where bisulfite-promoted cytosine deamination is minimal (*11, 12*).

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EXPERIMENTAL SECTION

Materials

Glass-distilled, deionized water was used to prepare all stock solutions and reaction mixtures. Reagent grade inorganic salts and standardized potassium iodate solutions were from Fisher Chemical Company. Hydroquinone and the cellulose thin-layer chromatography plates were from Eastman Organic Chemicals. Cytosine (Cyclo Chemical Corporation), 5-bromocytosine, 5-iodocytosine, uracil, and bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane (Bis-Tris) (Sigma Chemical Company) were used as received. Imidazole (Eastman Organic Chemicals) was recrystallized four times from hot benzene to remove a yellow impurity.

Ultraviolet Absorption Spectra

Stock solutions (0.50 M) of sodium sulfite, 14.8% neutralized with standardized perchloric acid, were prepared immediately before use in argon-flushed volumetric flasks. Reaction mixtures containing 0.05 M sodium sulfite, 0.05 M sodium phosphate buffer (pH 7.18), and 1.00×10^{-4} M 5-halocytosine were prepared in argon-purged, capped 3.0 ml cuvettes. Ionic strength was maintained at 1.0 M with sodium perchlorate. Reactions were initiated at room temperature (22–24°C) by the addition of the halopyrimidine. Difference spectra were recorded against blank solutions containing all components but the halopyrimidine, at various times during the course of the reaction using either a Cary 14 or a Beckman Acta C-III spectrophotometer. The pH of the reaction mixtures was measured immediately after the completion of each reaction using a Radiometer PHM-26 pH meter equipped with a Radiometer GK 2331C combination electrode.

Kinetic Measurements

The rates of 5-halocytosine disappearance in the presence of bisulfite were spectrophotometrically measured at either 294 nm (5-bromocytosine) or 297 nm (5-iodocytosine) by measuring the absorbance decrease which occurs when 0.3 μmole of 5-halocytosine is added to argon-purged 3.0 ml cuvettes containing the other reaction components preequilibrated at 25°C. Ionic strength was maintained at 1.0 M by the addition of sodium perchlorate. Absorbance measurements were made with a Zeiss PMQII spectrophotometer equipped with a cell holder thermostatted at 25°C. The pH of the individual reaction mixtures was determined following complete reaction. Pseudo first-order rate constants (k_{obsd}) were evaluated from linear semilogarithmic plots of extent reaction ($A_t - A_\infty$) against time using the relationship $k_{obsd} = 0.693/t_{1/2}$.

Product Analysis

Formation of products and utilization of reactants from 0.375 mmole cytosine, 0.375 mmole 5-bromocytosine, and 0.250 mmole 5-iodocytosine each individually reacting with either 1.0 mmole sodium sulfite (cytosine and 5-bromocytosine) or 0.50 mmole sodium sulfite (5-iodocytosine) in 0.05 M sodium phosphate buffer (pH 7.0)

containing 1×10^{-4} *M* hydroquinone were measured after incubation in argon-flushed 100 ml volumetric flasks for 3 days to insure complete reaction. Pyrimidine products were determined by ultraviolet absorption spectra and cellulose thin-layer chromatography. The utilization of sulfite was measured by iodometric titration as previously described (*10*).

Determination of pK_a Values

The pK_a of bisulfite was determined by fractional neutralization using carefully standardized solutions at 25°C and ionic strength 1.0 *M*. The pK_a values for the conjugate acids of 5-bromo- and 5-iodocytosine were spectrophotometrically determined at 310 nm, 25°C, and ionic strength 1.0 *M* in sodium acetate buffers. Absorbance and pH measurements were made using the instruments described above.

RESULTS

Ultraviolet Absorption Spectra

Sodium bisulfite buffers dehalogenate both 5-bromo- and 5-iodocytosine to yield cytosine as the pyrimidine product as evidenced by the decrease in halocytosine absorbance concomitant with the increase in cytosine absorbance which occurs when either of these halocytosines are mixed with dilute sodium sulfite buffer under conditions where bisulfite-promoted cytosine deamination is minimal (*11*, *12*). Figure 1

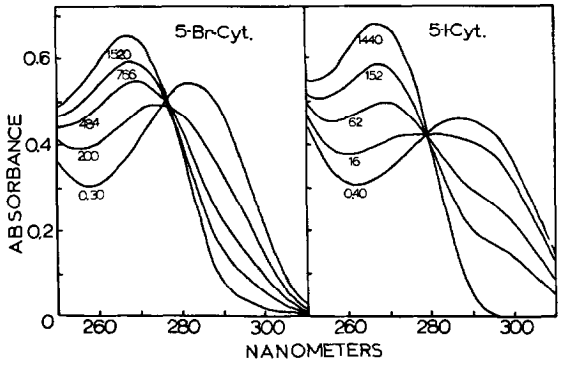


FIG. 1. Ultraviolet absorption spectra of 1.0×10^{-4} *M* 5-bromo- and 5-iodocytosine reacting with 0.05 *M* sodium bisulfite in 0.05 *M* sodium phosphate buffer, pH 7.18, ionic strength 1.0 *M*, room temperature (22–24°C). Numbers below the spectra show the time in minutes that the particular spectrum was recorded.

shows the results of a typical experiment with both 5-bromo- and 5-iodocytosine. This series of spectra measured as a function of time clearly indicates that decrease in both 5-bromocytosine ($\lambda_{max} = 283$ nm, pH 7.17) and 5-iodocytosine ($\lambda_{max} = 286$ nm, pH 7.17) absorbance with the corresponding increase in cytosine ($\lambda_{max} = 267$ nm, pH 7.17). Calculation of the ratio A_{285nm}/A_{260nm} for the spectrum of the completed reaction mixtures containing either 5-bromo- and 5-iodocytosine gave values of 0.30 and 0.31,

respectively. These values are in reasonable agreement with the similar ratio ($A_{285nm}/A_{260nm} = 0.25$) measured for cytosine in 0.05 *M* sodium phosphate buffer, pH 7.17. The small differences between the complete reaction mixtures and authentic cytosine probably reflects errors associated with the measurement of difference spectra at the lower wavelengths, where the bisulfite blanks have appreciable absorbance.

Kinetic Measurements

The reactions of both 5-bromo- and 5-iodocytosine with dilute bisulfite buffers follow strict first-order kinetics. Semilogarithmic plots of extent reaction ($A_t - A_\infty$) against time were linear for at least three and, in most cases, five half-lives. Figure 2 shows the results of measuring extent reaction as a function of time for both of the

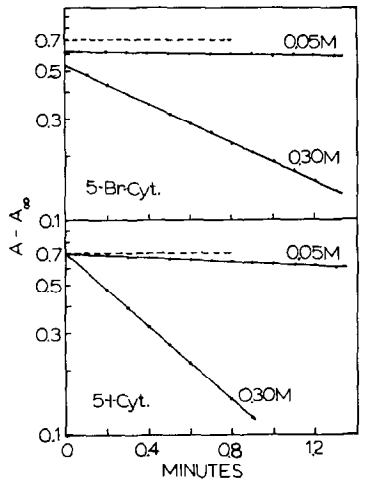


FIG. 2. Semilogarithmic relationship between extent reaction and time for the reaction of 2.0×10^{-4} *M* 5-bromocytosine (upper panel) and 2.0×10^{-4} *M* 5-iodocytosine (lower panel) with bisulfite buffers, pH 5.70, ionic strength 1.0 *M*, 25°C. Absorbance measurements were made at either 297 nm (5-I-Cyt.) or 295 nm (5-Br-Cyt.) against blanks containing all reaction components but the halopyrimidines. The dashed lines show the absorbance of 2.0×10^{-4} *M* of each of the halopyrimidines in 0.10 *M* Bis–Tris buffers of the same pH, ionic strength, and temperature as the bisulfite buffers.

halocytosines using pH 5.70 bisulfite buffers. In the case of 5-bromocytosine, extrapolation of these plots to time zero indicates a bisulfite buffer concentration-dependent decrease in the amount of 5-bromocytosine which theoretically should be present if its rate of utilization controlled the rate of the overall dehalogenation reaction. A similar, but much less extensive, time zero absorbance decrease was observed with bromocytosine using pH 7.08 bisulfite buffers—a result which might be expected based on the pH dependence of K_{eq} for the addition of bisulfite to cytosine (*12*). With 5-iodocytosine, the absorbance value obtained at time zero by extrapolation (Fig. 2) was exactly equal at both pH 5.70 and 7.06 to the absorbance of the added 5-iodocytosine. These results argue for the formation of an intermediate which subsequently reacts in a rate-determining step to control 5-bromo-, but not 5-iodocytosine, dehalogenation.

The dependencies of the pseudo first-order rate constants (k_{obsd}) for both 5-bromo- and 5-iodocytosine reacting with increasing concentrations of bisulfite buffer (pH 7.22) are complex and indicate a greater than first-power dependence on total bisulfite concentration. Consequently, plots of $k_{obsd}/[Bis_t]$ versus $[Bis_t]$ were constructed (Fig. 3). These plots clearly indicate the second-order dependence on bisulfite buffer for both 5-bromo- and 5-iodocytosine dehalogenation. Similar results have been previously observed for 5-bromouracil dehalogenation (*10*) and cytidylate deamination (*11*).

The reaction of 5-iodocytosine, but not 5-bromocytosine, with bisulfite is catalyzed by external buffer systems. The effect of increasing concentrations of imidazolium ion (Im–$^+$H) on k_{obsd} for the dehalogenation of both 5-bromo- and 5-iodocytosine is shown

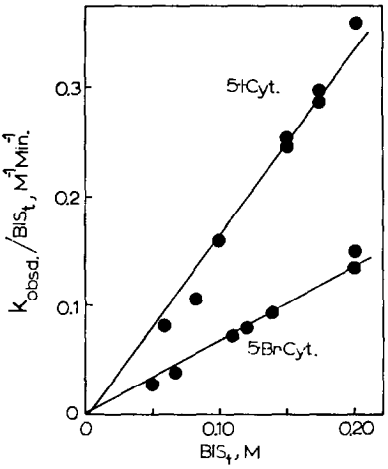


FIG. 3. Second-order relationship between the pseudo first-order rate constants for 5-iodocytosine and 5-bromocytosine dehalogenation and increasing concentration of sodium sulfite buffer (Bis_t), pH 7.22, ionic strength 1.0 *M*, 25°C.

in Fig. 4. The equality of slopes for Im–$^+$H catalysis at both pH 6.86 and 7.21 for 5-iodocytosine dehalogenation indicates that the acidic form (Im–$^+$H) of the buffer is catalytically effective, acting as a general acid catalyst of the reaction. Under similar conditions, 5-bromocytosine dehalogenation is not subject to Im–$^+$H catalysis. These results may indicate that different steps of a multistep reaction pathway control the overall rate of the reaction, depending upon the nature of the halogen at carbon 5 of the cytosine ring.

The effect of acetate buffer (pH 4.45) on the dehalogenation rate of both 5-bromo- and 5-iodocytosine is shown in Fig. 5. As in the case of imidazole buffer catalysis, k_{obsd} for 5-bromocytosine dehalogenation does not change as a function of increasing acetate buffer concentration. The results seen in Fig. 5 for 5-iodocytosine dehalogenation are strikingly different. In this case, k_{obsd} increases linearly up to about 0.20 *M* acetate, levels, and then becomes essentially invariant as a function of increasing acetate buffer concentration. These results coupled with the lack of evidence for the accumulation of an intermediate in 5-iodocytosine dehalogenation most likely illustrate the multistep nature of the formation of 5-iodo-5,6-dihydrocytosine-6-sulfonate (*13*). Similar breaks in plots of k_{obsd} against increasing general acid/base catalyst

concentration have been observed in both the general base-catalyzed elimination of sulfite from 1,3-dimethyl-5,6-dihydrouracil-6-sulfonate (*14*) and in the general acid-catalyzed addition of sulfite to 5-fluorouracil (*15*), a halopyrimidine which, under these conditions, does not dehalogenate (*5*).

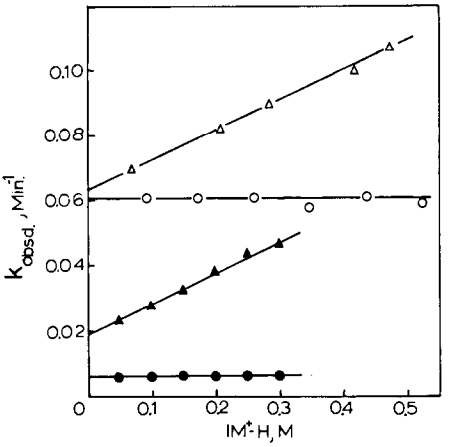


FIG. 4. Effect of increasing concentrations of imidazolium ion ($Im-^+H$) on the pseudo first-order rate constants for the reaction of bisulfite with both 5-bromo- and 5-iodocytosine, ionic strength 1.0 *M*, 25°C. In addition to imidazole buffer and halocytosine, all reaction mixtures were 0.10 *M* in total bisulfite. Open circles (○), 5-bromocytosine, pH 5.37; closed circles (●), 5-bromocytosine, pH 7.21; open triangles (△), 5-iodocytosine, pH 6.86; closed triangles (▲), 5-iodocytosine, pH 7.21.

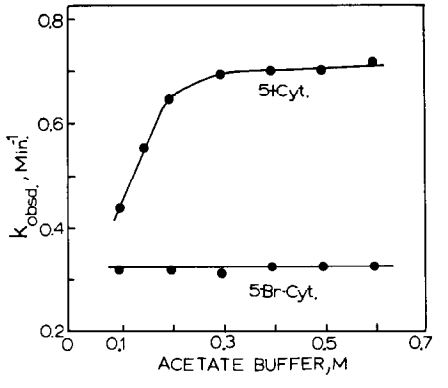


FIG. 5. Effect of increasing concentrations of acetate buffer (50% acetic acid) on the pseudo first-order rate constants for the reaction of bisulfite with both 5-bromo- and 5-iodocytosine, pH 4.45, ionic strength 1.0 *M*, 25°C. In addition to acetate buffer, reaction mixtures for 5-bromocytosine and 5-iodocytosine were 0.10 and 0.05 *M* total bisulfite, respectively.

Figure 6 shows the effect of pH on k_{obsd} for the reaction of bisulfite with 5-bromocytosine. As can be seen, k_{obsd} increases, reaches a maximum at pH 4.5, and then decreases as a function of increasing pH. From about pH 5.0 to 7.2 (data above pH 6.5 are not shown in Fig. 6), 0.10 *M* bisulfite buffers were employed. Below pH 5.0, bisulfite

($pK_a = 6.42$, ionic strength 1.0 *M*, 25°C) lacks buffering capacity; hence, 0.60 *M* acetate buffers were employed to maintain pH control. In the case of 5-bromocytosine dehalogenation, an intermediate appears to form whose subsequent reaction controls the overall rate of dehalogenation. This, coupled with the lack of observable acetate and imidazole buffer catalysis, argues for rate-determining dehalogenation of a halocytosine–SO_3^- adduct. The effect of hydrogen ion activity on the observed rate constants

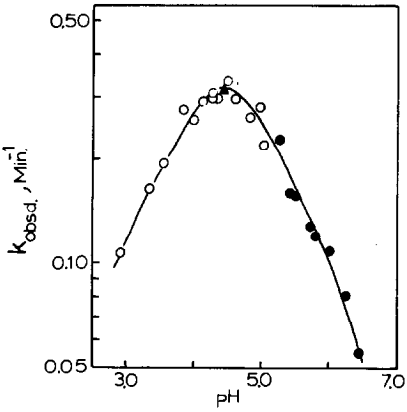


FIG. 6. Logarithmic relationship between hydrogen ion activity and the pseudo first-order rate constants for bisulfite reacting with 5-bromocytosine, ionic strength 1.0 *M*, 25°C. Open circles (○) represent reaction mixtures which were 0.10 *M* total bisulfite and 0.60 *M* acetate buffer. Closed circles (●) represent reaction mixtures which were 0.10 *M* bisulfite buffer. The closed triangle (▲) represents the zero intercept of a plot of k_{obsd} against increasing acetate buffer concentration (Fig. 5).

for this process is complex and likely involves the equilibrium concentration of 5-bromo-5,6-dihydrocytosine-6-sulfonate, its degree of protonation, and the concentration of SO_3^{2-}. Work currently in progress should help rationalize these relationships.

Sulfite Utilization

Reaction mixtures in which the molar ratio of total bisulfite to 5-halocytosine was about 2.0- to 2.5-fold were prepared along with suitable blanks and cytosine-containing controls in 0.05 *M* sodium phosphate buffer (pH 7.00–7.10). Aliquots removed immediately after reaction initiation and after 3 days incubation at room temperature were subjected to spectral analysis for pyrimidines and iodometric titration for total sulfite. Table 1 shows the results of these experiments. Blank solutions incubated for the same period of time as the reactions mixtures contained 94% of the initial concentration of sodium sulfite, which indicates that nonspecific bisulfite oxidation was minimal in these experiments. As previously indicated, conditions were chosen to minimize the deamination of the cytosine product. This was confirmed as essentially all of the cytosine initially added to these reactions mixtures was present after the 3-day incubation period. In the case of reaction mixtures containing 5-bromocytosine, 0.296 mmole of sulfite and 0.319 mmole of 5-bromocytosine were utilized during the course of the reaction. Corresponding experiments with 5-iodocytosine show the

TABLE 1

STOICHIOMETRY FOR THE DEHALOGENATION OF 5-BROMO- AND 5-IODOCYTOSINE BY BISULFITE BUFFERS[a]

Reaction component	Blank[b] (mmole)	Blank plus cytosine (mmole)	Blank plus 5-bromocytosine (mmole)	Blank plus 5-iodocytosine (mmole)
Sodium sulfite[c]				
initial	0.970	0.970	0.970	0.485
final	0.913	0.901	0.674	0.197
utilized	0.057	0.069	0.296	0.288
Cytosine[d]				
initial	—	0.375	—	—
final	—	0.374	0.290	0.250
utilized	—	0.001	—	—
5-Bromocytosine[d]				
initial	—	—	0.375	—
final	—	—	0.056	—
utilized	—	—	0.319	—
5-Iodocytosine[d]				
initial	—	—	—	0.250
final	—	—	—	0
utilized	—	—	—	0.250

[a] In addition to the indicated components all reaction mixtures were 0.05 *M* sodium phosphate buffer, pH 7.10–7.00 and 1×10^{-4} *M* hydroquinone in a final volume of 100 ml. Reactions were incubated at room temperature for 3 days.

[b] Blank solutions contained all reaction components except the pyrimidines.

[c] Determined by iodometric titration.

[d] Determined by ultraviolet absorbance measurements.

utilization of 0.288 mmole of sulfite and 0.250 mmole of 5-iodocytosine. These results are quantitatively similar to those previously observed for 5-bromouracil in bisulfite buffer (*10*) and indicate that for each mole of 5-halocytosine dehalogenated, a mole of sulfite is utilized, most likely in the formation of sulfate.

Pyrimidine Products

To confirm the ultraviolet absorption spectra which indicate that cytosine is the final pyrimidine product of these reactions, samples of completed reaction mixtures were subjected to cellulose thin-layer chromatography, using the following solvent systems: (A) H_2O; (B) *sec*-butanol:water [50:50 v/v; upper phase], and (C) isopropanol:water [80:20 v/v]. After visualization of the pyrimidine-containing spots with uv light, they were extracted with 0.01 *N* HCl and 0.01 *N* NaOH and uv spectra obtained to yield a λ_{max} (nm) and A_{285nm}/A_{260nm} ratio for each solvent system. The spectra resulting from the product of reaction of 5-iodocytosine and bisulfite exhibited $\lambda_{max}^{pH2} = 275$, $A_{285}/A_{260} = 1.16$ and $\lambda_{max}^{pH12} = 266$, $A_{285}/A_{260} = 0.29$, consistent with an authentic cytosine sample. The product of 5-bromocytosine exhibited the same values as well as values corre-

TABLE 2

R_f VALUES FOR CELLULOSE THIN-LAYER CHROMATOGRAPHY OF REACTION MIXTURES CONTAINING BISULFITE BUFFERS AND EITHER 5-BROMO- OR 5-IODOCYTOSINE[a]

Pyrimidine	R_f[b] in solvents		
	A	B	C
Uracil	0.80	0.62	0.60
Cytosine	0.76	0.25	0.44
5-Iodocytosine	0.60	0.62	0.55
5-Iodocytosine + bisulfite	0.76	0.22	0.45
5-Bromocytosine	0.65	0.60	0.54
5-Bromocytosine + bisulfite	0.65	0.58	0.54
	0.76	0.23	0.44

[a] Reaction mixtures were 0.10 M sodium phosphate, 0.005 M sodium sulfite, and 2.5×10^{-4} M pyrimidine, pH 7.22.

[b] Thin-layer plates containing a fluorescent indicator were used. Pyrimidine containing spots were visualized with uv light.

sponding to unreacted 5-bromocytosine; that is, $\lambda_{max}^{pH2} = 294$, $A_{285}/A_{260} = 0.62$ and $\lambda_{max}^{pH12} = 287$, $A_{285}/A_{260} = 0.22$. Table 2 shows the R_f values obtained for reaction mixtures containing bisulfite buffer plus either 5-bromo- or 5-iodocytosine along with R_f values obtained for authentic samples of uracil, cytosine, 5-iodocytosine, and 5-bromocytosine. These results show that the final pyrimidine product resulting from the dehalogenation of the 5-halocytosines is cytosine, thus confirming the ultraviolet spectra.

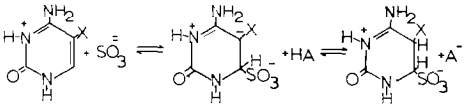
Halocytosine pK_a Values

The pK_a values for the conjugate acids of both 5-bromo- and 5-iodocytosine were determined in sodium acetate buffers, 25°C, ionic strength 1.0 M. The pK_a values were determined graphically (*16*) from the zero intercepts of linear plots with slope equal to 1.0 of $\log(A^N - A/A - A^+)$ versus pH where A^N, A^+, and A represent the absorbance at 310 nm of equimolar concentrations of the neutral, the cationic, and mixtures of these two species, respectively. Under these conditions, the pK_a values of 5-bromo- and 5-iodocytosine are 3.25 and 3.56, respectively.

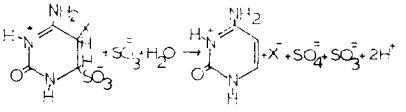
DISCUSSION

Collectively, the ultraviolet absorption spectra, the formation of an intermediate in the reaction of 5-bromocytosine, but not with 5-iodocytosine, the second-order dependence of k_{obsd} on increasing bisulfite buffer concentration, the 1:1 stoichiometry between sulfite and halocytosine disappearance, and the change in rate-determining step for 5-iodocytosine dehalogenation as a function of increasing acetate buffer

concentration all indicate that the pathway for 5-bromo- and 5-iodocytosine dehalogenation by bisulfite buffers is analogous to the similar dehalogenation of 5-bromo- and 5-iodouracil by either bisulfite (*6*, *10*, *17–19*) or simple thiol compounds such as 2-mercaptoethanol and cysteine (*7–9*). Such a reaction pathway is shown in Eqs. (1) and (2), in which –X represents either –Br or –I, HA represents a general acid, and A^-



(1)



(2)

represents a general base. As in the case of both the bisulfite-promoted dehalogenation of the 5-halouracils and the deamination of cytosine, the first step of this reaction pathway involves the formation of a 5,6-dihydropyrimidine–sulfite adduct, likely via a two-step reaction (Eq. 1) in which the attack of SO_3^{2-} on the pyrimidine ring occurs in a discrete reaction apart from general acid-catalyzed proton transfer to the resulting intermediate. The addition of bisulfite to uracil, 1,3-dimethyluracil (*14*), and 5-fluorouracil (*15*) occurs by such a pathway, and preliminary experiments on the general base-catalyzed elimination of SO_3^{2-} from 3-methyl-5,6-dihydrocytosine-6-sulfonate indicate that proton transfer from the dihydrocytosine ring may not be concerted with the elimination of SO_3^{2-} (*20*). In the case of 5-iodocytosine dehalogenation by bisulfite buffers, the two reactions shown in Eq. (1) for the formation of 5-iodo-5,6-dihydrocytosine-6-sulfonate most likely control the overall rate of dehalogenation. This conclusion is supported by the fact that an intermediate does not appear to accumulate, indicating that reactions subsequent to the formation of 5-iodo-5,6-dihydrocytosine-6-sulfonate are fast; and that there is a change from a first- to a zero-order dependence on acetate buffer concentration. The latter observation indicates that, as acetate buffer concentration increases, the rate determining step of overall reaction changes from the general acid-catalyzed protonation of the anion shown in Eq. (1) to the attack of SO_3^{2-} to 5-iodocytosine.

The second major step in the bisulfite-promoted dehalogenation of 5-bromo- and 5-iodocytosine involves the attack of sulfite, either directly or through an intervening molecule of water on the halogen atom of 5-halo-5,6-dihydrocytosine-6-sulfonate (Eq. 2) to yield cytosine and initially either a halosulfonic acid ($X–SO_3^-$) or a hypohalous acid (X–OH). Either of these halogen acids would be expected immediately to react further to yield halide ion and sulfate as final products. This step in the proposed reaction pathway is supported by the sulfite utilization data (Table 1) and its analogy to the dehalogenation of 5-bromouracil by bisulfite buffers (*5*, *9*, *17–19*). In the case of 5-bromocytosine dehalogenation, this step (Eq. 2) likely controls the overall reaction rate, as evidenced by the bisulfite buffer concentration-dependent buildup of an intermediate and lack of observable acetate and imidazole buffer catalysis. The change from rate-determining formation to dehalogenation of 5-halo-5,6-dihydrocytosine-6-

sulfonate as a function of the halogen at carbon 5 is supported by Rork and Pitman, who have shown that rates of dehalogenation of 5-halo-5,6-dihydrouracils in bisulfite solutions can be ranked iodo > bromo ≫ chloro (*18*). In the reaction of 5-bromouracil (*17, 19*) and 5-bromo-6-methoxy-5,6-dihydrouracil (*18*) with bisulfite buffers, the initial pyrimidine product formed after the removal of the halogen is a dihydrouracil-6-sulfonate anion which then either is protonated by general acids to yield 5,6-dihydrouracil-6-sulfonate or eliminates SO_3^{2-} to yield uracil. Thus, under conditions of limiting bisulfite concentration, the final product ratio, uracil/5,6-dihydrouracil-6-sulfonate, decreases with increasing concentrations of general acid. No evidence for such behavior in the case of 5-bromo- and 5-iodocytosine dehalogenation has been obtained using reaction mixtures containing 2.0×10^{-4} *M* halocytosine, 0.16 *M* total bisulfite, and either 0, 0.20, 0.40, or 0.70 *M* imidazole buffer (86% Im–$^+$H), ionic strength 1.0 *M*, 25°C. Consequently, either the elimination of SO_3^{2-} from 5-halo-5,6-dihydrocytosine (Eq. 2) is concerted with halogen removal or else SO_3^{2-} elimination from the carbanion resulting from halogen abstraction is much faster than proton transfer from Im–$^+$H.

The 5-halodeoxycytidines are believed to have anti-viral activity because they can be enzymatically deaminated to the corresponding 5-halodeoxyuridines which are then phosphorylated and incorporated into viral DNA (*21–24*). It is known that the halouracils are rapidly dehalogenated *in vivo* (*2–4*); however, to our knowledge, no evidence appears to be available concerning *in vivo* dehalogenation of the halocytosines or their derivatives. That these anti-viral compounds can be dehalogenated under near physiological conditions of temperature and pH by relatively low concentrations of nucleophilic reagents shows enzymatic 5-halocytosine dehalogenation to be chemically feasible.

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