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Reversible Addition of Bisulfite Buffer to the Cytidine Ring System

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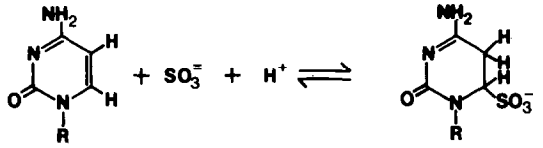
The rate constants for the reversible addition of protons and sulfite to the 5,6 double bond of cytidine and 3-methylcytidine have been spectrophotometrically measured under conditions (25°C, μ = 1.0 M) where the deamination of 5,6-dihydrocytidine-6-sulfonate is minimal. Both the addition and the elimination of sulfite from the ring system are subject to general catalysis of proton transfer. For the reaction in either direction, plots of the pseudo-first-order rate constants against increasing buffer concentration are biphasic and indicative of at least a two-step reaction pathway with both steps being subject to general acid–base catalysis. Kinetic hydrogen–deuterium isotope effects were measured for both buffer-catalyzed steps of sulfite elimination from 3-methyl-5,6-dihydrocytidine-6-sulfonate and sulfite addition to 3-methylcytidine. Both H_2O and D_2O were used as solvent. For both the addition and the elimination of SO_3^{2-} values of k_2^H/k_2^D were 6.3–7.1 and 2.3–2.6 at low and high imidazole buffer concentration, respectively. The large isotope effects values in the range of 6–7 can be attributed to rate-determining proton transfer to carbon-5 of the cytidine ring system. The smaller values are more likely caused by proton transfer to a electronegative atom such as the oxygen on carbon-2 of the cytidine ring. The equilibrium constants for bisulfite buffer addition to 3-methylcytidine and cytidine at 25°C, μ = 1.0 M, pH 7.2, are 10.2 and 1.3 M^{-1}, respectively.

INTRODUCTION

It is well established that sulfite and protons add reversibly to the 5,6 double bond of cytidine, uracil, and their derivatives, yielding a 5,6-dihydropyrimidine-6-sulfonate as product:



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Following the formation of this dihydropyrimidine–SO_3^- adduct, cytidine bases are deaminated and the 5-halopyrimidines are dehalogenated (*1–18*). These reactions also occur in "looped-out" regions of RNA and hence represent a method

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for studying both structure–function relationships (*19–26*) and protein–RNA interactions (*27–31*). Reactions of this type may also be of importance in the regulation of gene expression as the conversion of cytosine bases to dihydrouracil-6-sulfonate residues in DNA inhibits *S*-adenosylmethionine-dependent DNA methylation (*32*). It also has recently been proposed that DNA cytosine methyltransferase proceeds by a molecular mechanism that involves the formation of a Michael adduct (*33*). Other important biological and chemical reactions which may involve this type of chemistry include: the specific radiochemical labeling of uracil and cytosine residues in RNA and DNA (*34, 35*) and the proposed enzymatic mechanisms for catalysis by deoxycytidylate hydroxymethylase (*36*), deoxyuridylate hydroxymethylase (*37*), pseudouridylate (*38*), uracil-*N*-glycosidase (*38*) thymidylate synthetase (*39*), and the aminoacyl tRNA synthetase (*40*).

The purpose of this report is to present data showing that the reversible addition of SO_3^{2-} and protons to both cytidine and 3-methylcytidine proceeds via a two-step mechanism in which SO_3^{2-} adds to carbon-6 followed by proton transfer to carbon-5, resulting in the final dihydrocytidine-6-sulfonate product.

EXPERIMENTAL PROCEDURES

Materials. Cytidine and 3-methylcytidine (methosulfate salt) were from Sigma Chemical Company and used as received. All inorganic salts were of analytical grade. Deuterium oxide, 99.75%, from J.T. Baker Company, and deuterium chloride, 20% solution in 99.0% D_2O, from Aldrich Chemical Company, were used without further purification. Buffer solutions were prepared to constant composition by careful neutralization with standardized KOH and HCl. Imidazole from Sigma Chemical Company was recrystallized from hot benzene. 4-Morpholineethanesulfonic acid (MES) from Sigma Chemical Company was used as received. Glass distilled water was used in the preparation of all solutions. The dihydropyrimidine–SO_3^- adducts of cytidine and 3-methylcytidine were prepared at room temperature by adding 0.0125 g 3-methylcytidine (0.0092 g cytidine), 0.158 g K_2SO_3, 0.90 ml 1.0 N HCl in a final volume of 1.0 ml. DCl and D_2O replaced HCl and H_2O in the isotope effect studies. After 15 min, the reaction mixtures were chilled in ice to prevent significant deamination and immediately used in the kinetic studies.

Equilibrium constants. The equilibrium constant (25.0°C, μ = 1.0) for the addition of bisulfite buffer to both 3-methylcytidine and cytidine were spectrophotometrically measured at 290 nm by observing the absorbance decrease that occurs when 7.47×10^{-5} M 3-methylcytidine (2.0×10^{-4} M cytidine) is allowed to react to equilibrium with increasing concentrations (0.033–0.30 M) of K_2SO_3 buffer containing 20% $KHSO_3$ (*10*). Endpoints were measured immediately upon reaching equilibrium as spurious values were obtained if the reaction mixtures were allowed to stand, probably due to deamination of the adduct.

Kinetic measurements. The psuedo-first-order rate constants for either SO_3^{2-} elimination or addition to the cytidine ring system were spectrophotometrically measured at 290 nm using either a Zeiss PMQII or a Gilford 2000 spectrophotome-

ter, both of which were equipped with cell holders thermostated at 25 ± 0.1°C. Ionic strength was maintained at 1.0 M by the addition of KCl. All buffers were prepared by careful neutralization with standardized HCl or KOH. Reactions were initiated by the addition of either the pyrimidine (addition reaction) or the dihydropyrimidine–SO_3^- adduct (elimination reaction) to a final concentration of approximately 1.0×10^{-4} M. All reactions were followed to completion because reaction mixtures allowed to stand long periods of time gave unreliable endpoints, probably due to deamination. Following complete reaction, the pH of the reaction mixture was determined using a Radiometer PHM83 pH meter equipped with a GK-2322C combination electrode. Values of pD were determined with the same electrode using the relationship, pD = pH + 0.40 (*41*). Pseudo-first-order rate constants were determined from linear, semilogarithmic plots of extent reaction against time using the relationship, $k_{obsd} = 0.693/t_{1/2}$. Rate constants measured in this manner were reproducible to ±50%. Values of pK_a (25.0°C, μ = 1.0) for $KHSO_3$ and imidazolium ion were determined by half-neutralization in both H_2O and D_2O. They are $KHSO_3$, pK_a (H_2O) = 6.65, pK_a (D_2O) = 7.19; imidazolium ion, pK_a (H_2O) = 7.21, pK_a (D_2O) = 7.74. The pK_a of 3-methylcytidine was spectrophotometrically (290 nm) measured using phosphate, tris(hydroxymethyl) aminomethane, and ethanolamine buffers. At 25.0°C, μ = 1.0 M, its pK_a = 8.85.

RESULTS

Reaction reversibility and equilibrium constants. The overall reaction of bisulfite buffers with cytidine derivatives is complex, involving first the reversible formation of a 5,6-dihydrocytidine-6-sulfonate followed by a much slower deamination to yield 5,6-dihydrouracil-6-sulfonate as the final product. Since the objectives of this work involved only the addition reaction, it was important to determine the degree to which adduct deamination occurs under these experimental conditions. Consequently, a study was conducted in which concentrated 5,6-dihydrocytidine-6-sulfonate adducts were allowed to incubate at room temperature for various periods of time followed by reversal to cytidine by 300-fold dilution into 0.10 M potassium phosphate buffer (pH 7.0). These SO_3^{2-} elimination reactions were followed spectrophotometrically at 270 nm. The degree of reversal (cytidine formation) was determined by comparison to the 270-nm absorbance of the same concentration of authentic cytidine in 0.10 M potassium phosphate buffer. Under these conditions 95, 89, 74, 72, and 6.6% of the 5,6-dihydrocytidine-6-sulfonate could be reversed to yield cytidine after 30, 60, 120 150, and 1260 min of concentrated adduct incubation at room temperature. Furthermore, incubation of the concentrated adduct at 0°C in an ice bath for 110 min resulted in the recovery of 90% of the original cytidine. Thus, under the conditions of these experiments, the 6-sulfonate adducts used to study the kinetics of SO_3^{2-} elimination are not significantly deaminated.

Hayatsu and co-workers (*4, 6*) have reported a value for the equilibrium constant (K_{eq} = 0.77 M^{-1}) for bisulfite addition to cytidine at pH 5.8, 37°C. Consequently, equilibrium constants for the addition of bisulfite to both cytidine and 3-

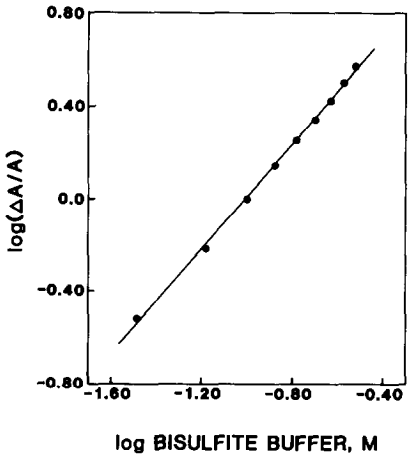


FIG. 1. Equilibrium constant for the reaction of K_2SO_3 buffer (20% $KHSO_3$) with 7.47×10^{-5} M 3-methylcytidine, 25°C, μ = 1.0 M. Data plotted according to equation 2 with K_{eq} = −log[bisulfite buffer] at $\log(\Delta A/A)_{290\,nm} = 0$.

methylcytidine were spectrophotometrically measured under the conditions of these experiments much in the same manner previously used for bisulfite addition to 1,3-dimethyluracil (*10*). Data were obtained at 25.0°C, μ = 1.0 M, by measuring the 290-nm absorbance decrease which occurs upon reaction to equilibrium of either 7.47×10^{-5} M 3-methylcytidine or 2.00×10^{-4} M cytidine with increasing concentrations (0.033–0.30 M) of K_2SO_3 buffer, 20% $KHSO_3$ (pH 7.2). Figure 1 shows the 3-methylcytidine data plotted using the equation

$$\log(\Delta A/A)_{290\,nm} = \log K_{eq} + \log[\text{bisulfite buffer}] \qquad [2]$$

in which A and ΔA are proportional to 3-methylcytidine and its 6-sulfonate, respectively. From this logarithmic plot, the apparent equilibrium constant, K_{eq} = 10.2 M^{-1}, was determined from the relationships $\log K_{eq}$ = −log[bisulfite buffer] at $\log(\Delta A/A)_{290\,nm} = 0$.

Under the same experimental conditions, an estimate of the equilibrium constant (K_{eq} = 1.3 M^{-1}) for bisulfite addition to cytidine was obtained by a short extrapolation of data similar to those seen in Fig. 1 to $\log(\Delta A/A)_{290\,nm} = 0$. This value which roughly agrees with that obtained by Hayatsu and co-workers (*4*, *6*) at another temperature and pH must be considered an approximate value because of the limitations on the range of bisulfite concentrations which could be employed at this pH below ionic strength 1.0 M.

Kinetics for the addition of bisulfite buffer to 3-methylcytidine. The kinetic behavior for the addition of SO_3^{2-} and protons to the 5,6 double bond of 3-methylcytidine in H_2O is shown in Fig. 2. Over the concentration range 0.05 to 0.25 M bisulfite buffer, values of k_{obsd} have a first-order dependence on bisulfite buffer concentration with increasing slope (k_2) directly related to decreasing fraction HSO_3^- in the buffer. Thus, in H_2O, a single step of the reaction controls the rate over the range of bisulfite buffer concentration. This step of the reaction pathway

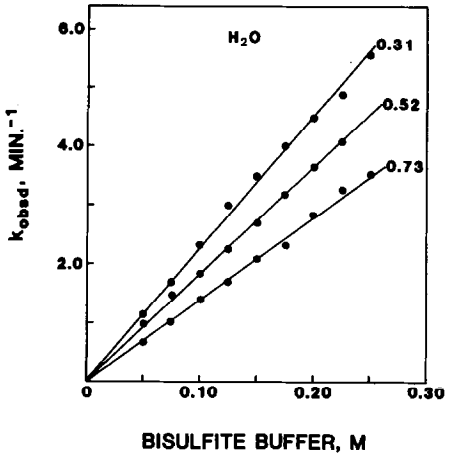


FIG. 2. First-order dependence for bisulfite buffer for addition to 1.0×10^{-4} M 3-methylcytidine in H_2O, 25°C, μ = 1.0 M. Numbers refer to the fraction $KHSO_3$ in the buffers employed.

is favored by increasing the ratio SO_3^{2-}/HSO_3^-. Since this approach is complicated by the fact that both buffer components can play a significant part in the reaction, SO_3^{2-} as the attacking nucleophile and HSO_3^- as a general acid, these reactions were repeated in D_2O at both fraction HSO_3^- equal to 0.50 and 0.80. These data, shown in Fig. 3, clearly indicate a change from second- to nearly first-order dependence on bisulfite buffer concentration and hence are indicative of a multistep reaction pathway with a change in rate-determining step as a function of increasing bisulfite buffer concentration. Estimation of the deuterium isotope effect at fraction HSO_3^- equal to 0.52 (H_2O) and 0.50 (D_2O) from the first-order parts of Figs. 2 and 3, k_2^H/k_2^D = 1.6, indicates that the kinetics shown in Fig. 2 reflect SO_3^{2-} addition to the ring rather than proton transfer to carbon-5, a conclusion in

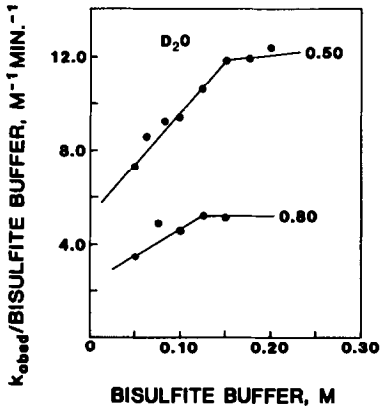


FIG. 3. Change from a second- to a first-order dependence for bisulfite buffer addition to 1.0×10^{-4} M 3-methylcytidine in D_2O, 25°C, μ = 1.0 M. Numbers refer to the fraction $KHSO_3$ in the buffers employed.

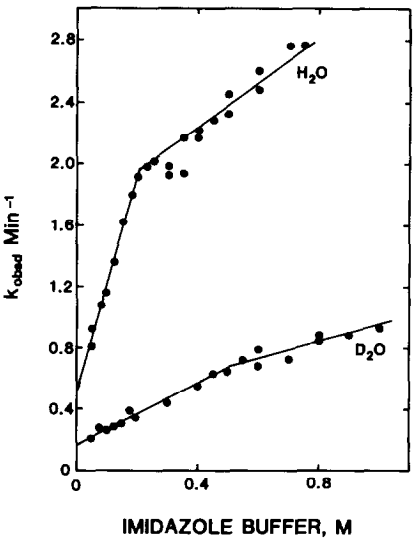


FIG. 4. Change in rate-determining step for the imidazole buffer-catalyzed addition of 0.025 M total bisulfite to 1.0×10^{-4} M 3-methylcytidine in both H_2O and D_2O, 25°C, μ = 1.0 M. Imidazole buffers were 50% imidazole. The fraction SO_3^{2-} in all of the reaction mixture is 0.78.

agreement with the observed rate enhancement caused by increasing the fraction SO_3^{2-} in the buffers.

To further elucidate this reaction pathway, values of k_{obsd} for bisulfite addition to 3-methylcytidine were measured as a function of increasing imidazole buffer concentration. These results shown in Fig. 4 indicate that imidazole buffer catalyzes two discrete steps of the overall reaction, hence showing that increasing concentrations of general catalysts of proton transfer cause changes in the rate-determining step of SO_3^{2-} addition to 3-methylcytidine. Similar results were observed using 4-morpholineethanesulfonic acid buffers in H_2O (data not shown). Also, to be discussed later, there are striking differences in the second-order rate constants (k_2) of these reactions depending on the use of either H_2O or D_2O as solvent. Similar kinetic behavior was observed using cytidine rather than 3-methylcytidine.

Kinetics of SO_3^{2-} elimination from dihydrocytidine-6-sulfonate adducts. The effects of imidazole buffer catalysis on the rate of SO_3^{2-} elimination from both 3-methyl-5,6-dihydrocytidine-6-sulfonate and 5,6-dihydrocytidine-6-sulfonate are shown in Figs. 5 and 6, respectively. As in the SO_3^{2-} addition reaction, both adducts eliminate SO_3^{2-} by a two-step pathway both steps of which are subject to buffer catalysis. Using 50% neutralized imidazole buffers in H_2O the initial and final slopes which represent the second-order rate constants (k_2) for imidazole buffer catalysis are 5.64 and 1.57 M^{-1} min^{-1}. In identical experiments using imidazole buffers containing 20% imidazole base corresponding initial and final values of k_2 are 3.15 and 1.30 M^{-1} min^{-1}, thus indicating that SO_3^{2-} elimination reaction is subject to general base catalysis by imidazole base.

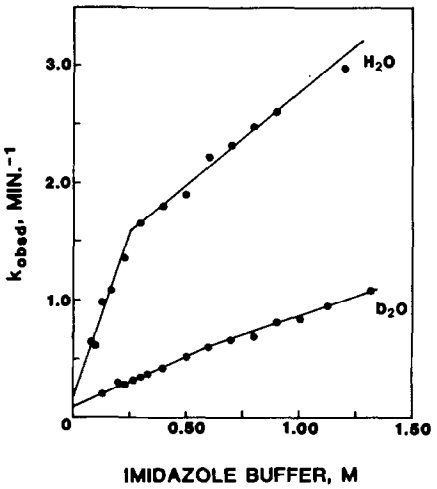


FIG. 5. Change in rate-determining step for the imidazole buffer-catalyzed elimination of SO_3^{2-} from 3-methyl-5,6-dihydrocytidine-6-sulfonate-5H and -5D in H_2O and D_2O, 25°C, μ = 1.0 M. Fraction imidazole was 0.50. Dihydrocytidine–SO_3^- adducts were prepared as described under Materials.

Hydrogen–deuterium isotope effects. To better understand the sites of proton transfer, the kinetics of the imidazole buffer-catalyzed SO_3^{2-} addition and elimination reactions with 3-methylcytidine were studied in D_2O. Representative data are shown in Fig. 4, SO_3^{2-} addition to 3-methylcytidine; Fig. 5, SO_3^{2-} elimination from 3-methyl-5,6-dihydrocytidine-6-sulfonate; and Fig. 6, SO_3^{2-} elimination from 5,6-dihydrocytidine-6-sulfonate. When studying the SO_3^{2-} addition reactions, reaction components were simply dissolved in D_2O; however, the SO_3^{2-} elimination reactions were studied using dihydrocytidine–SO_3^- adducts formed in both H_2O and D_2O followed by dilution into either H_2O or D_2O as solvent. The values of k_2 evaluated from the initial and final slopes of plots such as those illustrated in Figs.

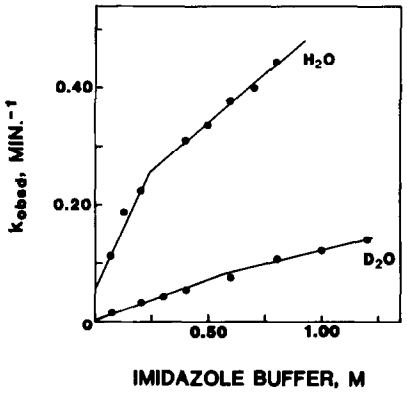


FIG. 6. Change in rate-determining step for the imidazole buffer-catalyzed elimination of SO_3^{2-} from 5,6-dihydrocytidine-6-sulfonate-5H and -5D in H_2O and D_2O, 25°C, μ = 1.0 M. Fraction imidazole was 0.50. Dihydrocytidine–SO_3^- adducts were prepared as described under Materials.

TABLE 1

DEUTERIUM ISOTOPE EFFECTS FOR THE REVERSIBLE ADDITION OF SULFITE AND PROTONS TO 3-METHYLCYTIDINE

Solvent	Atom at C-5	Initial slope k_2, M^{-1} min^{-1}	Final slope k_2, M^{-1} min^{-1}	k_2^H/k_2^D initial	k_2^H/k_2^D final
		Elimination reaction			
H_2O	-H	5.64	1.57	6.34	2.34
	-D	0.89	0.67		
D_2O	-H	5.68	1.68	6.53	2.58
	-D	0.87	0.65		
		Addition reaction			
H_2O	-H	7.20	1.42	7.13	2.54
D_2O	-H	1.01	0.56		

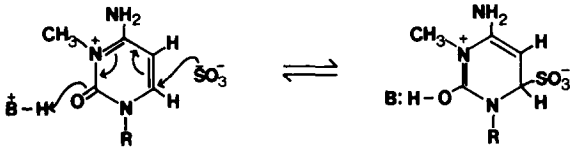
Note. All reactions were measured in 50% neutralized imidazole buffers, 25°C, μ = 1.0 M. In the elimination reactions the adduct was prepared in either D_2O or H_2O solution as described under Experimental Procedures with the rate constants being measured in either H_2O or D_2O as solvent. For the SO_3^{2-} addition reaction total bisulfite buffer was constant at 0.025 M, fraction SO_3^{2-} equal to 0.78. Values of k_2 were obtained from the slopes of plots such as those shown in Figs. 4–6.

4–6 are summarized in Table 1. In the SO_3^{2-} addition reactions, the initial step of the pathway observed at low imidazole buffer concentrations is subject to a large deuterium isotope effect, $k_2^H/k_2^D = 7.13$, with the isotope effect observed at high buffer concentrations being much smaller, $k_2^H/k_2^D = 2.54$. This result is similar in the SO_3^{2-} elimination reactions where the isotope effect at low imidazole buffer concentrations is large, $k_2^H/k_2^D = 6.34$–6.58 followed by a smaller isotope effect at high buffer concentrations ($k_2^H/k_2^D = 2.34$–2.58). These deuterium isotope effects for the imidazole buffer-catalyzed SO_3^{2-} elimination reactions are independent of using either H_2O or D_2O as solvent provided that the 3-methyl-5,6-dihydrocytidine-6-sulfonate adducts were prepared in D_2O. Thus for the elimination reaction, the large isotope effects observed at low buffer concentrations, $k_2^H/k_2^D = 6.34$–6.58, likely reflect imidazole-catalyzed abstraction of the nonexchangeable proton at carbon-5 of the dihydrocytidine ring system. The smaller isotope effect observed at high imidazole buffer concentrations, $k_2^H/k_2^D = 2.34$–2.58, likely reflects proton transfer from an electronegative atom such as the oxygen at carbon-2 of the ring system.

DISCUSSION

Based on earlier work dealing with SO_3^{2-} and proton addition to the uracils, it might be predicted that the corresponding reaction with cytidines would involve

non-buffer-catalyzed SO_3^{2-} addition to carbon-6 of the ring followed by a buffer-catalyzed proton transfer to carbon-5, thus yielding the 5,6-dihydrocytidine-6-sulfonate product (*10, 42*). This prediction is enhanced by the positively charged nitrogen available as an electron sink when SO_3^{2-} adds to 3-methylcytidine. These data indicate that this is not the case in that SO_3^{2-} and protons react with the 5,6 double bond of cytidine and 3-methylcytidine via a pathway which involves at a minimum two steps, both of which are subject to general acid–base catalysis of proton transfer. A reaction pathway consistent with the data is shown in the equations.



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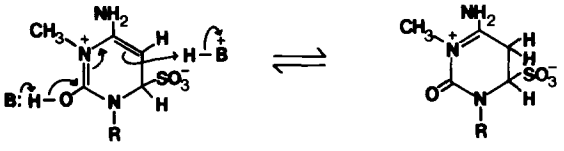
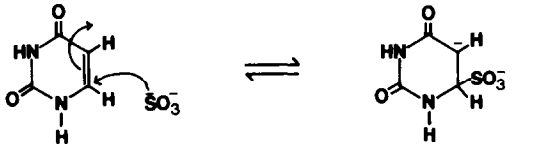


(4)

The first step in this pathway is the general acid-catalyzed addition of SO_3^{2-} to carbon-6 concomitant with proton transfer to an electronegative atom such as the oxygen on carbon-2 of the cytidine ring. This step controls the overall reaction rate at high concentrations of imidazole buffer and is subject to the smaller isotope effect, $k_2^H/k_2^D = 2.3–2.6$. With the exception of being subject to general acid catalysis of proton transfer this step of the reaction (Eq. [3]) is analogous to the attack of SO_3^{2-} on the uracils (Eq. [5]) yielding a carbanion intermediate which subsequently protonates at carbon-5 of the uracil ring (Eq. [6]) to yield 5,6-dihydrocytidine-6-sulfonate as the final product (*10, 42*):



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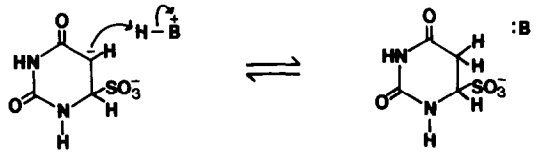

(6)

The second step in the pathway for SO_3^{2-} addition to the cytidine ring system (Eq. [4]), involves general base-catalyzed proton abstraction from the oxygen on carbon-2 concomitant with general acid-catalyzed protonation of carbon-5. This reaction controls the overall reaction rate at low imidazole buffer concentrations and

is subject to the large deuterium isotope effect, $k_2^H/k_2^D = 6.3–7.2$, which would be expected for proton transfer to carbon. This step is analogous to the proton transfer reaction involved in dihydrouracil-6-sulfonate formation (Eq. [6]) which also exhibits a relatively large deuterium isotope effect, $k_2^H/k_2^D = 4.10$, again indicating proton transfer to carbon (*10*).

Reactions of nucleophiles such as SO_3^{2-}, thiols, and NH_2OH are important models for biologically important reactions such as the enzymatically catalyzed methylation and hydroxymethylation of carbon on uracil and cytosine ring systems (*36–39*), and the aminoacylation of tRNA (*40*). While the exact details of these enzymatically catalyzed reactions are not known, a key feature, at least in thymidylate synthetase (*39*) and aminoacyl tRNA synthetase (*40*), is the formation of a covalent, dihydrouracil adduct between the uracil ring and the active site of the enzyme. In the case of deoxycytidylate hydroxymethylase, the existence of such an intermediate is widely hypothesized based on the analogies between it's catalytic features (*36*) and those of thymidylate synthetase (*39*). As pointed out by both Starzyk *et al.* (*40*) and Santi *et al.* (*33*) other enzymes such as DNA cytosine methyltransferase may also proceed by a catalytic pathway of this type. Thus, reactions of this variety may be important in a complete understanding of the control of gene expression.

ACKNOWLEDGMENTS

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REFERENCES

1. SHAPIRO, R., SERVIS, R. E., AND WELCHER, M. (1970) *J. Amer. Chem. Soc.* **92,** 422–424.
2. HAYATSU, H. (1976) *Progr. Nucl. Acids Res. Mol. Biol.* **16,** 75–124.
3. HAYATSU, H., WATAYA, Y., AND KAI, K. (1970) *J. Amer. Chem. Soc.* **92,** 724–726.
4. HAYATSU, H., WATAYA, Y., KAI, K., AND IIDA, S. (1970) *Biochemistry* **9,** 2858–2865.
5. SHAPIRO, R., DIFATE, V., AND WELCHER, M. (1974) *J. Amer. Chem. Soc.* **96,** 906–912.
6. HAYATSU, H., AND SONO, M. (1971) *Chem. Commun.,* 1178–1179.
7. SHAPIRO, R., AND SLAE, S. (1978). *J. Org. Chem.* **43,** 4197–4200.
8. SONO, M., WATAYA, Y., AND HAYATSU, H. (1973) *J. Amer. Chem. Soc.* **95,** 4745–4749.
9. SHIRAGAMI, M., AND HAYATSU, H. (1976) *Nucl. Acids Res., Spec. Publ.* **2** (*Symp. Nucl. Acids Chem.* 4th, 1976), 31–32.
10. ERIKSON, R. W., AND SANDER, E. G. (1972) *J. Amer. Chem. Soc.* **94,** 2086–2091.
11. SANDER, E. G., AND DEYRUP, C. L. (1972) *Arch. Biochem. Biophys.* **150,** 600–605.
12. FOURREY, J. (1972) *Bull. Soc. Chim. Fr.,* 4580–4581.
13. RORK, G. S., AND PITMAN, I. H. (1975) *J. Amer. Chem. Soc.* **97,** 5559–5565.
14. RORK, G. S., AND PITMAN, I. H. (1975) *J. Amer. Chem. Soc.,* 5566–5572.
15. SEDOR, F. A., JACOBSON, D. G., AND SANDER, E. G. (1975) *J. Amer. Chem. Soc.* **97,** 5572–5577.
16. SEDOR, F. A., AND SANDER, E. G. (1974) *Arch. Biochem. Biophys.* **161,** 632–637.
17. JACOBSON, D. G., SEDOR, F. A., AND SANDER, E. G. (1975) *Bioorg. Chem.* **4,** 72–83.
18. SANDER, E. G. (1978) *in* Bioorganic Chemistry: A Treatise to Supplement Bioorganic Chemistry: An International Journal (van Tamelen, E. E., ed.), Vol. 2, pp. 273–297, Academic Press, New York.

19. SINGHAL, R. P. (1974) *Biochemistry* **13,** 2924–2932.
20. FURUICHI, Y., WATAYA, Y., HAYATSU, H., AND UKITA, T. (1970) *Biophys. Biochem. Res. Commun.* **41,** 1185–1191.
21. KUCAN, Z., FREUDE, K. A., KUCAN, I., AND CHAMBERS, R. W. (1971) *Nature (London), New Biol.* **232,** 177–179.
22. GODDARD, J. P., AND SCHULMAN, L. H. (1972) *J. Biol. Chem.* **247,** 3864–3867.
23. SCHULMAN, L. H., SHAPIRO, R., LAW, D. C. F., AND LOUIS, J. B. (1974) *Nucl. Acids Res.* **1,** 1305–1316.
24. SCHULMAN, L. H., AND GODDARD, J. P. (1973) *J. Biol. Chem.* **248,** 1341–1345.
25. CHAMBERS, R. W., AOYAGI, S., FURUKAWA, Y., ZAWADZKA, H., AND BHANOT, O. S. (1973) *J. Biol. Chem.* **248,** 5549–5551.
26. SCHIMMEL, P., PUTNEY, S., AND STARZYK, R. M. (1982) *Trends Biochem. Sci.* (Pers. Ed.) **7,** 209–212.
27. TURCHINSKY, M. F., KUSOVA, K. S., AND BUDOWSKY, E. I. (1974) *FEBS Lett.* **38,** 304–307.
28. PUTNEY, S. C., AND SCHIMMEL, P. (1981) *Nature (London)* **291,** 632–635.
29. CAREY, J. LOWARY, P. T., AND UHLENBECK, O. C. (1983) *Biochemistry* **22,** 4723–4730.
30. UHLENBECK, O. C., CAREY, J., ROMANIUK, P. J., LOWARY, P. T., AND BECKETT, D. (1983) *J. Biomol. Struct. Dyn.* **1,** 539–552.
31. CAREY, J., AND UHLENBECK, O. C. (1983) *Biochemistry* **22,** 2610–2615.
32. DRAHOVSKY, D., AND MORRIS, N. R. (1972) *Biochem. Biophys. Acta* **277,** 245–250.
33. SANTI, D. V., GARETT, C. E., AND BARR, P. J. (1983) *Cell* **33,** 9–10.
34. WATAYA, Y., AND HAYATSU, H. (1972) *Biochemistry* **11,** 3583–3588.
35. IIDA, S., WATAYA, Y., KUDO, I., KAI, K., AND HAYATSU, H. (1974) *FEBS Lett.* **39,** 263–266.
36. YUN-CHI, Y., AND GREENBERG, G. R. (1967) *J. Biol. Chem.* **242,** 1307–1313.
37. KUNITANI, M. G., AND SANTI, D. V. (1980) *Biochemistry* **19,** 1271–1275.
38. SANTI, D. V., WATAYA, Y., AND MASUDA, A. (1978) *in* Proceedings, International Symposium, Substrate-Induced Irreversible Inhibition of Enzymes (Seiler, N., Jung, M. H., and Koch-Wesser, J., eds.), pp. 291–303, Elsevier, Amsterdam.
39. POGOLOTTI, A. L., JR., AND SANTI, D. V. (1977) *in* Bioorganic Chemistry: A Treatise to Supplement Bioorganic Chemistry: An International Journal (van Tamelen, E. E., ed.), Vol. 1, pp. 277–311, Academic Press, New York.
40. STARZYK, R. M., KOONTZ, S. W., AND SCHIMMEL, P. (1982) *Nature (London)* **298,** 136–140.
41. GLASCOE, P. K., AND LONG, F. A. (1960) *J. Phys. Chem.* **64,** 188–190.
42. SEDOR, F. A., JACOBSON, D. G., AND SANDER, E. G. (1974) *Bioorg. Chem.* **3,** 221–228.