Intermolecular and Intramolecular Catalysis in Deamination of Cytosine Nucleosides

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Abstract □ The rates of deamination of arabinosylcytosine (Ara-C), cytidine (Cyd) and cytosine (C) are compared in the presence and in the absence of catalytic buffers. Ratios of the rate constants in the absence of intermolecular catalysis by buffers indicate intramolecular catalysis by the 2′-hydroxyl in Ara-C. For example, the ratio of the deamination rate constants at pH 4.7, 70° is 53/1.4/1 for Ara-C/Cyd/C. In contrast to Cyd and C which show catalysis by both the acidic and basic components of the buffers, Ara-C exhibits only general-acid catalysis. These data suggest that intramolecular participation by the 2′-hydroxyl in Ara-C replaces the general-base catalytic role which the buffers perform in the deamination of C and Cyd. Mechanisms for inter- and intramolecular catalysis in the deamination of Ara-C, Cyd, and C are discussed.

Keyphrases □ Cytosine nucleosides—deamination □ Catalysis, inter- and intramolecular—cytosine nucleosides □ Kinetics—cytosine nucleosides deamination □ UV spectrophotometry—analysis

Cytosine (C), cytidine (Cyd) and 1-β-D-arabinosylcytosine (Ara-C)[1] are known to undergo hydrolytic deamination in aqueous buffered solutions to yield the corresponding uracil derivatives as shown in Scheme I.

The authors have previously reported on the kinetics of Ara-C deamination in phosphate buffers at several temperatures in the presence and absence of sodium bisulfite (1). The major catalytic species in aqueous phosphate buffers at 70°, pH 6–8, was shown to be $H_2PO_4^-$. Other workers have demonstrated that the deamination of C and Cyd is subject to buffer catalysis by carboxylate and pyridine buffers at pH < 6, 95° (2, 3). The catalytic constants for the individual buffer species could not be calculated from previous data for C and Cyd (2, 3) and the catalytic constant for $H_2PO_4^-$ was not reported (1).

In the authors' initial publication the fact was emphasized that insufficient data was available to quantitatively compare the catalytic effects of buffers on the deamination of Ara-C, Cyd, and C. The authors have now demonstrated that the kinetics of deamination of the arabinosyl nucleoside is dramatically different from either the ribosyl nucleoside or cytosine itself and that C and Cyd behave relatively similarly by comparison. This report provides the first data allowing: (*a*) comparison of the catalytic constants for several buffer components in the deamination kinetics of C, Cyd, and Ara-C and (*b*) a direct comparison of their deamination rates in the absence of buffer catalysis under a variety of experimental conditions. The results clearly indicate a significant contribution due to intramolecular participation by the 2′-hydroxyl in the hydrolytic deamination of Ara-C. There is evidence that C and Cyd are subject to both general-acid and general-base catalysis while general-acid catalysis predominates in the case of Ara-C at 70 and 80°. Under certain conditions the apparent first-order rate constants for Ara-C deamination are shown to increase rapidly as a function of acetic acid concentration and then become relatively constant and independent of the buffer concentration. This apparent change in the rate determining step is not evident in the buffer data for the deamination of C or Cyd under the conditions of this report.

[1] USAN approved name is cytarabine; common name is cytosine arabinoside.

EXPERIMENTAL

Materials—Arabinosylcytosine and arabinosyluridine (Upjohn Co., Kalamazoo, Mich.), and cytosine, uracil, cytidine, and uridine (Mann Research Laboratories, New York, N. Y. and Nutritional Biochemicals Co., Cleveland, Ohio) were used.

Analytical Methods—The wavelengths of maximum absorption as a function of pH were determined using a Cary model 15 spectrophotometer. Beer's law plots for cytosine and uracil in 0.1 *N* HCl were constructed using a Beckman DU spectrophotometer and the absorptivities, *a*, were calculated to be 1.0×10^4 (275 mμ), 5.9×10^3 (259 mμ) for C, and 3.8×10^3 (275 mμ), 8.2×10^3 (259 mμ) for U. Solution of the simultaneous equations for total absorption, *A*, from a mixture of C and U yields:

$$C = (1.37\,A_{275} - 0.625\,A_{259}) \times 10^{-4} \quad \text{(Eq. 1)}$$

$$U = (1.67\,A_{259} - 0.990\,A_{275}) \times 10^{-4} \quad \text{(Eq. 2)}$$

The concentration of each component in mixtures containing C and U were calculated from the A_{275} and A_{259} in 0.1 *N* HCl using Eqs. 1 and 2.

A similar treatment for arabinosylcytosine and arabinosyluracil results in Eqs. 3 and 4.

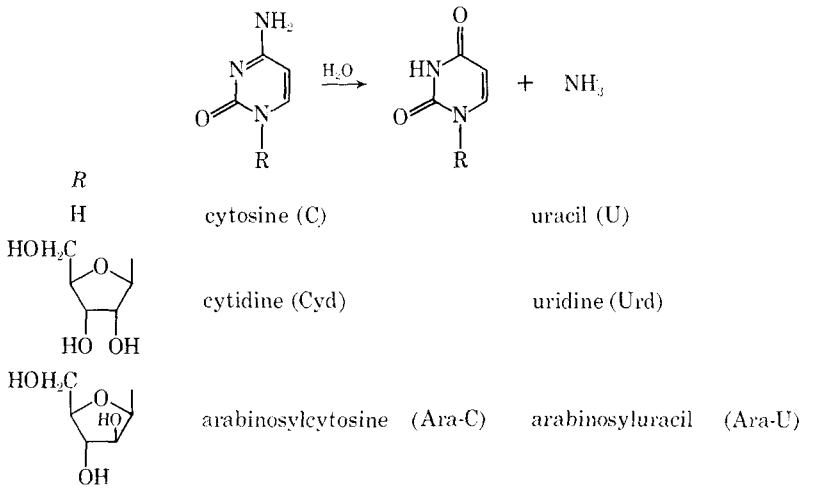
$$\text{Ara-C} = (0.927\,A_{280} - 0.389\,A_{260}) \times 10^{-4} \quad \text{(Eq. 3)}$$

$$\text{Ara-U} = (1.21\,A_{260} - 0.546\,A_{280}) \times 10^{-4} \quad \text{(Eq. 4)}$$

The concentration of Ara-C or Ara-U in a mixture was calculated from Eq. 3 or 4 using the A_{280} and A_{260} in 0.1 *N* HCl where the absorptivities were determined as 1.3×10^4 (280 mμ), 6.0×10^3 (260 mμ) for Ara-C, and 4.3×10^3 (280 mμ), 1.0×10^4 (260 mμ) for Ara-U.

The method of Loring and Ploeser (4) was employed in the analysis of cytidine and uridine mixtures.

Kinetics of Deamination—The kinetics of hydrolytic deamination of C, Cyd, and Ara-C, were determined under pseudo first-order conditions by maintaining constant pH and a sufficient excess of buffer. The temperature and ionic strength were held constant within each study. The pH was measured at the temperature of the



Scheme I

kinetic run using a digital readout pH meter (Sargent model DR) and combination electrode pH 7–14, 0–80° before and after the reactions were carried out. All reactions were followed for one to seven half-lives in the case of Cyd, for more than 40% of the reaction in the Case of C, and until complete loss of substrate in the case of Ara-C. The concentrations of the reactants and products were determined as described in the previous section. Details of the experimental conditions are given in Tables I, II, and III.

RESULTS

Kinetics of Deamination—Good first-order plots were obtained under all experimental conditions when graphed according to the equation

$$\ln X = \ln X_0 - k_1 t \qquad \text{(Eq. 5)}$$

where X is the molar concentration of unreacted C, Cyd, or Ara-C and X_0 is the corresponding initial concentration. The apparent first-order rate constants, k_1, calculated from the slopes of these plots are listed in Tables I, II, and III. The effect of buffer concentration on the apparent first-order rate constants is discussed in the following sections.

Table I—Experimental Conditions and Apparent First-Order Rate Constants for Deamination of 5.0×10^{-4} *M* Arabinosylcytosine

°C.	Observed pH	Buffer Compn. [CH_3–COOH]	Buffer Compn. [CH_3–COONa]	Buffer Compn. [NaCl]	$10^3 k_1$, hr.$^{-1}$
70	3.66 ± 0.05	3.60	0.36	0.00	53.8
		0.50	0.050	0.31	51.2
		0.30	0.030	0.33	49.1
		0.10	0.010	0.35	43.4
		0.05	0.005	0.36	38.8
	4.71 ± 0.07	0.36	0.36	0.00	25.9
		0.10	0.10	0.26	17.7
		0.050	0.050	0.31	15.3
		0.005	0.005	0.35	10.4
	5.66 ± 0.08	0.035	0.35	0.01	3.24
		0.020	0.20	0.16	2.79
		0.010	0.10	0.26	2.29
		0.005	0.05	0.31	1.84
80	3.66 ± 0.05	3.50	0.35	0.00	154
		0.50	0.050	0.30	107
		0.30	0.030	0.32	103
		0.10	0.010	0.34	93.4
	4.72 ± 0.05	0.32	0.32	0.00	49.7
		0.090	0.090	0.23	30.6
		0.045	0.045	0.27	25.2
		0.005	0.005	0.31	19.0
	5.66 ± 0.08	0.035	0.35	0.00	7.15
		0.020	0.20	0.15	5.52
		0.010	0.10	0.25	4.60
		0.005	0.05	0.30	3.95

General Acid-General Base Catalysis in Deamination of C and Cyd—Plots of the apparent first-order rate constants, k_1, *versus* total buffer concentration were linear for both C and Cyd under the conditions given in Tables II and III. The apparent first-order rate constants were shown to be defined by the equation:

$$k_1 = k_{HA}[HA] + k_{A^-}[A^-] + k_i \qquad \text{(Eq. 6)}$$

where HA is the acidic component and k_i is the rate constant in the absence of HA or A^-. At any given pH a plot of k_1 *versus* $[HA]$ will be linear with intercept, k_i, and slope defined as $S = (k_{HA} + k_{A^-}R)$ where R is the ratio $[A^-]/[HA]$. Thus a knowledge of S and R at two or more pH values will allow calculation of both catalytic constants. The calculation can also be done by a plot of k_1 *versus* $[A^-]$ where $S = (k_{HA}R + k_{A^-})$ and $R = [HA]/[A^-]$. Both of these methods were applied to the data in Tables II and III with consistent results. The catalytic constants calculated in this way are listed in Table IV. It is apparent that both the acidic and basic components of the buffers are catalytic. The effects of a number of buffer systems on the deamination of C have been established as part of another study which will examine the possibility of a Brönsted correlation of the catalytic constants for various buffers. In all cases general-acid and general-base catalysis is indicated. As evidenced in Table IV neither species appears to predominate as the catalyst.

The reaction mixtures were examined for material balance by calculating the sum of the concentrations of C and U or Cyd and Urd as a function of time. Under all conditions reported in Tables II and III there was no indication of the presence of any reaction product other than the U or Urd.

General-Acid Catalysis in Deamination of Ara-C—The method described in the previous section was applied to data for the deamination of Ara-C in phosphate buffers at pH 6.15 and 6.90 (1) by

Table II—Experimental Conditions and Apparent First-Order Rate Constants for Deamination of 5.0×10^{-4} *M* Cytidine

°C.	Observed pH	Buffer Compn. [CH_3–COOH]	Buffer Compn. [CH_3–COONa]	Buffer Compn. [NaCl]	$10^3 k_1$, hr.$^{-1}$
70	4.71 ± 0.04	0.360	0.360	0.000	0.767
		0.100	0.100	0.260	0.390
		0.050	0.050	0.310	0.328
		0.005	0.005	0.355	0.266
80	3.63 ± 0.05	1.350	0.135	0.180	4.40
		0.450	0.045	0.270	3.12
		0.270	0.027	0.288	2.93
		0.090	0.009	0.306	2.41
	4.72 ± 0.04	0.315	0.315	0.000	1.80
		0.090	0.090	0.225	0.992
		0.045	0.045	0.270	0.817
		0.0045	0.0045	0.311	0.646

Table III—Experimental Conditions and Apparent First-Order Rate Constants for Deamination of 1.8×10^{-3} *M* Cytosine at 70°

Observed pH	Buffer Compn. [CH_3COOH]	[CH_3COONa]	[NaCl][a]	$10^4 k_1$, $hr.^{-1}$
3.75 ± 0.13	0.20	0.020	0.18	3.50
	0.15	0.015	0.19	3.11
	0.082	0.008	0.19	2.77
	0.020	0.002	0.20	2.48
4.72 ± 0.02	0.20	0.20	0.00	3.59
	0.15	0.15	0.05	3.16
	0.082	0.082	0.12	2.54
	0.020	0.020	0.18	2.07
5.64 ± 0.02	0.020	0.20	0.00	1.29
	0.015	0.15	0.05	1.12
	0.0082	0.082	0.12	0.865
	[$NaH_2PO_4 \cdot H_2O$]	[Na_2HPO_4]	[NaCl][b]	
5.76 ± 0.03	0.20	0.020	0.00	1.94
	0.10	0.010	0.065	1.48
	0.05	0.005	0.13	1.18
6.70 ± 0.03	0.060	0.060	0.02	1.29
	0.040	0.040	0.10	1.10
	0.020	0.020	0.18	0.949
7.69 ± 0.04	0.0060	0.060	0.074	0.875
	0.0040	0.040	0.14	0.795
	0.0020	0.020	0.20	0.723

[a] Ionic strength adjusted with NaCl to 0.020. [b] Ionic strength adjusted with NaCl to 0.26.

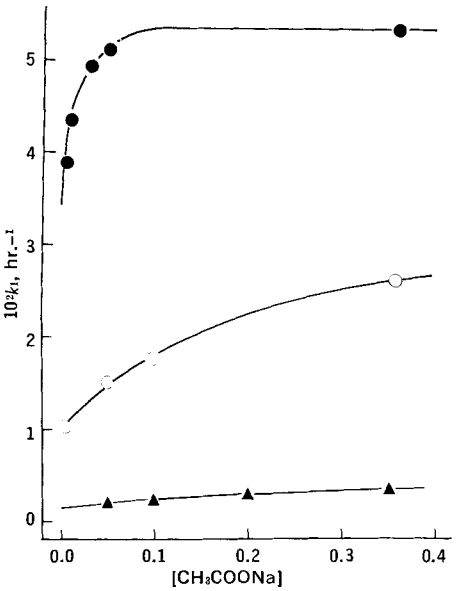


Figure 1—*Apparent first-order rate constants for deamination of Ara-C at 70° in acetate buffers at pH 3.7,* ●*; 4.7,* ○*; and 5.7,* ▲*; versus sodium acetate concentration.*

determining the slopes of the plots k_1 *versus* [$HPO_4^=$]. Solving the simultaneous equations for the catalytic constants yields a value of 2.0×10^{-2} (l./mole/hr.) for $k_{H_2PO_4^-}$ and a negligible value for $k_{HPO_4^=}$ (estimated to be 3×10^{-4} l./mole/hr.).

Catalysis in the presence of the acetic acid-acetate buffer system did not exhibit the usual linear dependency of rate constant on buffer concentration. Plots of k_1 *versus* total buffer concentration (or the concentration of either component) showed negative deviation from linearity. Typical examples are presented in Figs. 1 and 2. Figure 2 also shows a plot for the deamination of Cyd to allow a direct comparison.

The plot k_1 *versus* CH_3COONa at pH 3.7 shows the greatest degree of deviation of all cases reported in this paper. This plot shows essentially two regions. In the region of low buffer concentration there exists a sharp increase in k_1 with increasing concentration of buffer. Examination of Fig. 1 will reveal that this increase can be attributed to CH_3COOH rather than [CH_3COO^-] since the most significant buffer catalysis is seen to occur at pH 3.7 where there is primarily CH_3COOH (see Table I). As the pH is increased to 4.7 and 5.7 the slopes of the plots can be seen to decrease. Indeed the nearly linear plot of k_1 *versus* CH_3COONa at pH 5.7 represents the case where the buffer is composed primarily of the acetate with relatively little acetic acid present. This plot shows an increase in k_1 of only 1.4×10^{-3} ($hr.^{-1}$) when total buffer concentration is increased from 0.055 to 0.39 *M*. The plot at pH 3.7 shows an increase of more than 10×10^{-3} ($hr.^{-1}$) over the same range. If one assumes that all of the increase in k_1 at pH 5.7 is due to the acetate the maximum catalytic constant for CH_3COO^- would be $1.4 \times 10^{-3}/0.3$ or 4.7×10^{-3} (l./mole/hr.). (See Table I for sodium acetate and acetic acid concentrations.) This would allow calculation of the acetic acid catalytic constant at pH 3.7 from the initial slope of k_1 *versus* CH_3COOH which is 94×10^{-3} (Fig. 2) yielding a value of $k_{CH_3COOH} = 93 \times 10^{-3}$ (l./mole/hr.). Thus the maximum possible contribution due to the acetate component of the buffer at 70° is negligible in comparison to that of acetic acid.

Since the plots in Figs. 1 and 2 show decreasing buffer dependency with increasing buffer concentration, a more accurate assessment of the catalytic constants can be performed using the initial slopes. The initial slopes of k_1 *versus* CH_3COOH were obtained at pH 3.7 and 4.7 by regraphing the data in Fig. 2 on an expanded scale. Applying the technique discussed in the previous section results in the following:

$$0.094 = k_{HA} + 0.1\ k_{A^-} \qquad \text{(Eq. 7)}$$

$$0.103 = k_{HA} + k_{A^-} \qquad \text{(Eq. 8)}$$

Solving for the constants yields $k_{HA} = 9.3 \times 10^{-2}$ and $k_{A^-} = 1 \times 10^{-2}$ (l./mole/hr.). However, the initial slope of k_1 *versus* CH_3COONa at pH 5.7 is 0.0090 which would indicate a negative value for acetate since $S = k_{A^-} + 0.1\ k_{HA}$ at this pH. If it is assumed that k_{A^-} is actually zero and that the small positive value calculated from Eqs. 7 and 8 is due to the approximation in determining the initial slopes, the catalytic constant $k_{HA} = 0.093$ can be used to calculate the initial k_1 values at pH 5.7 according to Eq. 6 where k_i is 1.39×10^{-3} ($hr.^{-1}$). The calculated values for 10^3 k_1 ($hr.^{-1}$) are 2.32 at 0.01 *M* CH_3COOH and 1.84 at 0.005 *M* CH_3COOH which agree with the experimental values 2.29 and 1.84. Since the most pronounced acetate effect would be exhibited at pH 5.7 it is evident that its catalytic constant is negligible as compared to that for acetic acid.

The same generalities can be made for the acetic acid-acetate buffer at 80°. The slope of k_1 *versus* [CH_3COO^-] at pH 5.7 was again minimal in comparison to that of pH 4.7 and 3.7. Attempts to calculate the catalytic constants from the initial slopes using Eq. 6 resulted only in approximate values for k_{HA} (15×10^{-2} l./mole/hr.) and k_{A^-} (0.1×10^{-2} l./mole/hr.). However, the data are unambiguous in exhibiting the predominance of acetic acid as the catalytic species.

It is apparent that acetic acid is also responsible for the negative deviation in the k_1 *versus* buffer concentration plot since this is most pronounced at pH 3.7. When the acetic acid is increased from 0.05 to 0.10 there is a corresponding increase in k_1 of 4.6×10^{-3} for a slope value, $S_1 = 92 \times 10^{-3}$. However, when the concentration is increased from 0.5 to 3.6 *M* there is an increase of only 2.6×10^{-3} in k_1 for a value of $S_2 = 0.8 \times 10^{-3}$. Thus the ratio S_1/S_2 is 115/1. The rate constant, k_1, has become relatively independent of buffer concentration in the region of 0.5 to 3.6 *M* acetic acid, pH 3.7, 70°. This type of plot is evidence for a change in the rate determining step of the reaction (5). In the buffer region up to 0.5 *M* acetic acid the reaction was significantly catalyzed by acetic acid. At concentrations greater than 0.5 *M*, acetic acid has supplied sufficient catalysis

Table IV—Catalytic Constants for Acetate and Phosphate Buffers in Deamination of Arabinosylcytosine, Cytidine, and Cytosine

°C.	Substrate	$10^4 k$, l./mole/hr. HAc	Ac^-	$H_2PO_4^-$	$HPO_4^=$
70	C	5.43	2.99	4.75	3.45
	Ara-C	~930	~0	200	~3
80	Cyd	11.5	26.3	—	—
	Ara-C	~1500	~0	—	—

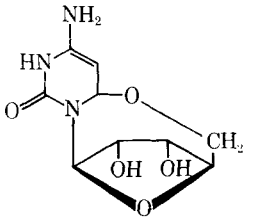
to result in a new rate determining step which is apparently independent of buffer.

The deamination of Ara-C differs from that of C and Cyd in one other aspect. Under certain experimental conditions, particularly in dilute carboxylate buffers at pH 2–4, 60–80°, material balance data provide evidence for the formation of an intermediate in the deamination of Ara-C to Ara-U. By proper control of conditions we can demonstrate formation of as much as 40% of the intermediate which subsequently reacts to form Ara-U. The isolation and identification of this intermediate is currently in progress and will be the subject of a future report.

The presence of the intermediate does not affect the validity of the rate constants reported in the present paper. It has been shown that rate constants determined from Ara-C concentration are the same as those calculated directly from the UV absorption data. In addition, the intermediate shows no effect on the spectra of the reaction mixtures from 250–300 mμ and would not therefore be expected to interfere with the spectrophotometric assays.

DISCUSSION

Intramolecular Participation in the Hydrolytic Deamination of Ara-C—The rate constants for deamination of Ara-C, Cyd, and C in the absence of buffer at a variety of pH values, 70 and 80°, are given in Table V. The data at 70° show a slight rate enhancement when the ribosyl nucleoside, Cyd, is compared to cytosine alone, C. The ratio of the rate constants is 2.2/1 at pH 4.0 and 1.4/1 at pH 4.7. This increase in deamination rate may be due to participation by the 5′-hydroxyl to form the 6,5′-anhydro cyclonucleoside intermediate, I. The formation of similar 6,5′-cyclic intermediates has



I

been proposed in the 5-H exchange of ribofuranosyl nucleosides in MeONa-MeOD (6). In the present case, however, this increase in deamination rate, which may be attributed to the ribosyl 5′-hydroxyl, is insignificant in comparison to the rate increase of the arabinosyl nucleoside which is 142 times faster than C at pH 3.7. At pH 4.7 the ratio of Ara-C/Cyd/C is 53/1.4/1. (Other ratios are listed in Table V.) It is obvious from these data that the configuration of the 2′-hydroxyl in Ara-C (Scheme I) is responsible for a 30- to 40-fold rate increase as compared to the deamination of Cyd where the 2′-hydroxyl is *trans* to the pyrimidine base. It should also be mentioned here that Fig. 2 illustrates why the uncatalyzed rate constants rather than those in buffer solutions must be compared for a true indication of the degree of intramolecular catalysis. As the buffer concentration is increased the ratio of reactivity of Ara-C to Cyd or C would be found to first increase and then decrease. Figure 1 shows the most dramatic example of this fact in that Ara-C deamination becomes practically

Table V—Comparison of First-Order Rate Constants for Hydrolytic Deamination of Arabinosylcytosine, Cytidine, and Cytosine in Absence of Buffer[a]

°C.	pH	10^4 k, hr.$^{-1}$ Ara-C	Cyd	C	Ratio Ara-C/Cyd/C
70	3.7	340	—	2.4	142/–/1
	4.0	260[b]	6.4[c]	2.9[c]	90/2.2/1
	4.7	100	2.6	1.9	53/1.4/1
	5.7	14	—	1	14/–/1
80	3.7	900	24	—	40/1
	4.7	180	6.4	—	28/1
	5.7	35	—	—	—

[a] Determined from intercepts of plots of k_1 *versus* buffer concentration. See Tables I, II, and III for experimental details. [b] Estimated from plot of k_i *versus* pH where k_1 is intercept of k_i *versus* buffer concentration for data in Table I. [c] Taken from *Reference 9*.

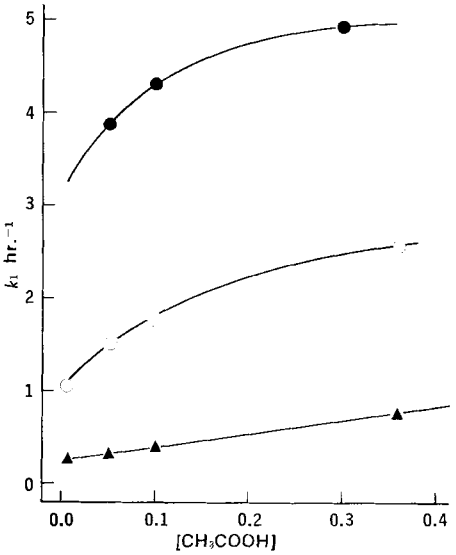


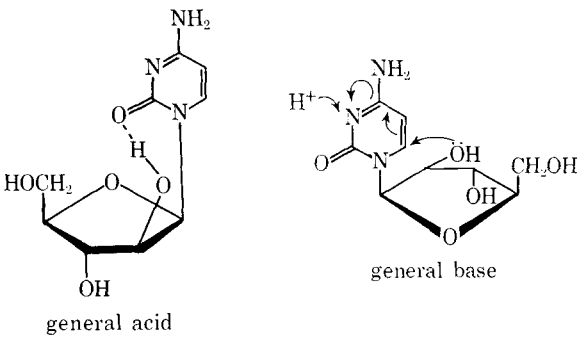
Figure 2—*Apparent first-order rate constants at 70° for deamination of Ara-C in acetate buffers at pH 3.7,* ● *and 4.7,* ○ *(10^2k$_1$ in hr.$^{-1}$) and Cyd at pH 4.7,* ▲ *(10^3k$_1$ in hr.$^{-1}$) versus acetic acid concentration.*

independent of buffer at concentrations above 0.5 *M* acetic acid. Since the rate constants in Table V do not reflect the differences in intermolecular catalysis by buffer, it is apparent that the 2′-hydroxyl in the arabinosyl nucleoside is capable of intramolecular catalysis while the 2′-hydroxyl in cytidine does not exhibit effective intramolecular participation.

Buffer Catalysis of Ara-C, Cyd, and C Deamination—Table IV summarizes the values of the catalytic constants in the acetic acid-acetate and phosphate buffer systems. It is readily apparent that catalysis of the deamination of Cyd and C differs from that of Ara-C in both magnitude and type. Cyd and C are catalyzed by both the acidic and basic components of the buffers. Thus general-acid and general-base catalysis are evident.

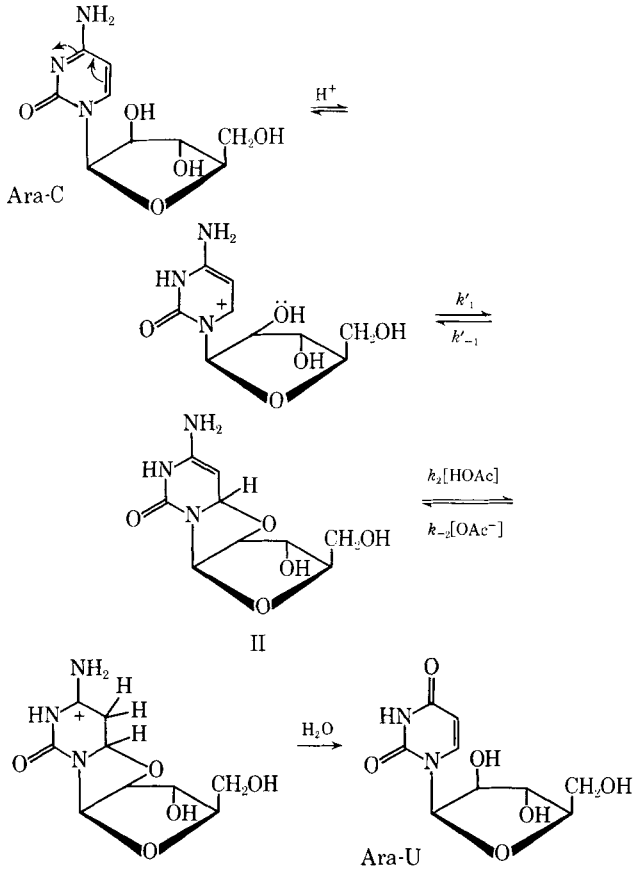
The catalytic constants for deamination of Ara-C were calculated only in dilute buffer solutions in the case of the acetic acid-acetate system because of the decreasing effect of buffer at higher concentrations (Fig. 1). It is obvious that Ara-C is subject primarily to general-acid catalysis under the conditions of this study. Furthermore, the catalytic constants for acetic acid and $H_2PO_4^-$ are significantly larger than those for the corresponding constants in C or Cyd deamination (Table IV).

In a preliminary communication we have discussed two possibilities for participation by the 2′-hydroxyl (7). One case is that of intramolecular general-acid catalysis and the other that of general-base (Scheme II).



Scheme II

Consideration of the buffer data suggests that the general base mechanism is the more likely of the two. This is evident from the fact that Ara-C deamination exhibits buffer catalysis primarily by general acids whereas C and Cyd are susceptible to catalysis by both acidic and basic buffer components. Thus the Ara-C appears to be supplying its own basic catalyst and to require only the general-acid catalyst to undergo relatively rapid deamination.



Scheme III

Intramolecular catalysis by nucleophilic attack of the 2′-oxygen on C-6 would lead to the formation of the 2′,6-cyclic intermediate, II, shown in Scheme III. This mechanism is basically similar to that previously proposed for Cyd (2) and Ara-C (1) except that the arabinosyl 2′-hydroxyl now serves as the general base.

The formation of 2′,6-cyclonucleosides has also been implicated in the 5-H exchange reactions of Ara-U and 5′-deoxy Ara-U in MeONa-MeOD (6) and Ara-U and Ara-C in aqueous acid-base (8). This mechanism accounts for the increased deamination rates of Ara-C over Cyd and C in the absence of buffer since only H_3O^+ is required for the reaction to proceed.

Scheme III is also consistent with the observed behavior of the first-order rate constants for Ara-C deamination, k_1, as a function of acetic acid concentration at pH 3.7, 70° (Figs. 1 and 2). The dependency of k_1 on acetic acid at low buffer concentrations can be explained on the basis of reversible proton addition to the C-5 of II as shown in the scheme. The maximum concentration of II would thus be a function of the rate of ring formation and the relative rate of proton addition to the cyclic intermediate. Thus at low buffer concentrations and sufficient H-ion to catalyze the 2′-hydroxyl attack, one would expect to accumulate II. This is in agreement with the authors' observation that Ara-C deamination involves formation of an intermediate under such conditions (see *Experimental*). Increasing the concentration of acetic acid would result in a more rapid loss of II which would approach a steady state in high buffer concentrations. The observed deamination rate constant in more concentrated buffer (0.5 to 3.6 *M* acetic acid) appears to approach a constant value which would represent the rate constant for the formation of II under steady state conditions at pH 3.7. This would be expected to be a constant at a given pH since it represents a process which is first-order in Ara-C protonated at N-3.

Scheme III is therefore consistent with the current data for inter- and intramolecular catalysis in Ara-C deamination. The deamination of C and Cyd presumably proceeds through a similar mechanism with the intermolecular general-acid and general-base catalysis being supplied by buffers. It is possible that the 5′-hydroxyl in Cyd participates to some extent as discussed earlier under the formation of I. This effect is minimal as compared to the 2′-hydroxyl in Ara-C under the present experimental conditions.

SUMMARY

1. At pH 3.7–4.7 70–80°, in the absence of buffers arabinosylcytosine undergoes hydrolytic deamination 30 to 40 times faster than cytidine which deaminates slightly faster than cytosine. This rate enhancement is attributed to intramolecular participation by the 2′-hydroxyl in the arabinosyl nucleoside.

2. First-order deamination rate constants, k_1, for cytidine and cytosine showed a linear dependency on buffer concentration. Catalytic constants could be calculated from slopes of appropriate plots in the usual fashion. Both compounds were susceptible to general-acid and general-base catalysis by the buffer components.

3. Deamination rate constants for arabinosylcytosine did not show the usual linear dependency on acetic acid-acetate buffer concentration. Curves were obtained with negative deviation from linearity. In the most extreme case studied, a region where k_1 is nearly independent of buffer concentration is reported.

4. Catalytic constants were estimated from the initial slopes of k_1 *versus* acetic acid concentration in the arabinosyl case. Although the values for the catalytic constants are estimates it is unequivocal that general-acid catalysis predominates in Ara-C deamination. General-acid catalysis was also shown in phosphate buffer which showed no curvature.

5. It is hypothesized that Ara-C is subject to general-base type of intramolecular catalysis by the 2′-hydroxyl group. Thus only intermolecular general-acid catalysis is observed whereas both general-acid and general-base catalysis are required in the deamination of cytosine and cytidine.

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