

off the enzyme or at least swings free of any specific binding site. This follows from the fact that phenoxylation of the free amino group of the valine of Val-tRNA^{Val} (Yaniv and Gros, 1969), or naphthoxylation of the free amino group of the isoleucine of Ile-tRNA^{Ile} (D. C. Lynch and P. R. Schimmel, unpublished data), does not prevent binding of the tRNA to its cognate enzyme.

Loftfield and Eigner (1969) have proposed that aminoacylation of tRNA proceeds through a concerted mechanism in which the adenylate is not an intermediate of the reaction. When viewed in detail, the results reported here argue strongly against this proposal. For example, when IRS·Ile~AMP is reacted with tRNA^{Ile} to produce enzyme bound Ile-tRNA^{Ile} in a "one-shot" reaction (eq 2a), the rate observed (see Table II) coincides with the initial burst of synthesis of enzyme-bound Ile-tRNA^{Ile} when starting from ATP, isoleucine, and tRNA^{Ile} (see Figure 8). Since the rates of both reactions are the same, it appears that the same rate-limiting step is being observed in both cases. This step is eq 2a, the transfer of isoleucine from Ile~AMP to tRNA^{Ile} to yield IRS-bound Ile-tRNA^{Ile}. Hence, we conclude that the adenylate intermediate is formed as a necessary intermediate in the synthesis of Ile-tRNA^{Ile}.

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Acidic Hydrolysis of Deoxycytidine and Deoxyuridine Derivatives. The General Mechanism of Deoxyribonucleoside Hydrolysis*

Robert Shapiro† and Melvyn Danzig‡

ABSTRACT: The kinetics of the acidic hydrolyses of deoxycytidine and deoxyuridine, and their 5-bromo and 5-methyl derivatives have been studied, in the range pH 6 to $H_0 = -6$. The pH-rate profiles, substituent effects, and other parameters of the reactions are consistent with a mechanism involving the formation of mono- and dications of the nucleoside, followed by rupture of the *N*-glycosyl bond. An alternative, widely accepted, mechanism involving sugar-ring opening (initial C-O

cleavage) is excluded. As the *N*-glycosyl cleavage pathway has already been established for the uncatalyzed hydrolysis of thymidine, and for the acidic hydrolysis of deoxyadenosine and deoxyguanosine, this process is general for the major naturally occurring deoxyribonucleosides. The pK_A value of 0.5 reported for uracil is incorrect. Another value reported at -3.38 has been confirmed.

Acidic hydrolysis of the *N*-glycosyl bonds of DNA has been of importance as a method for base composition analysis (Tittensor and Walker, 1967) and base sequence determination

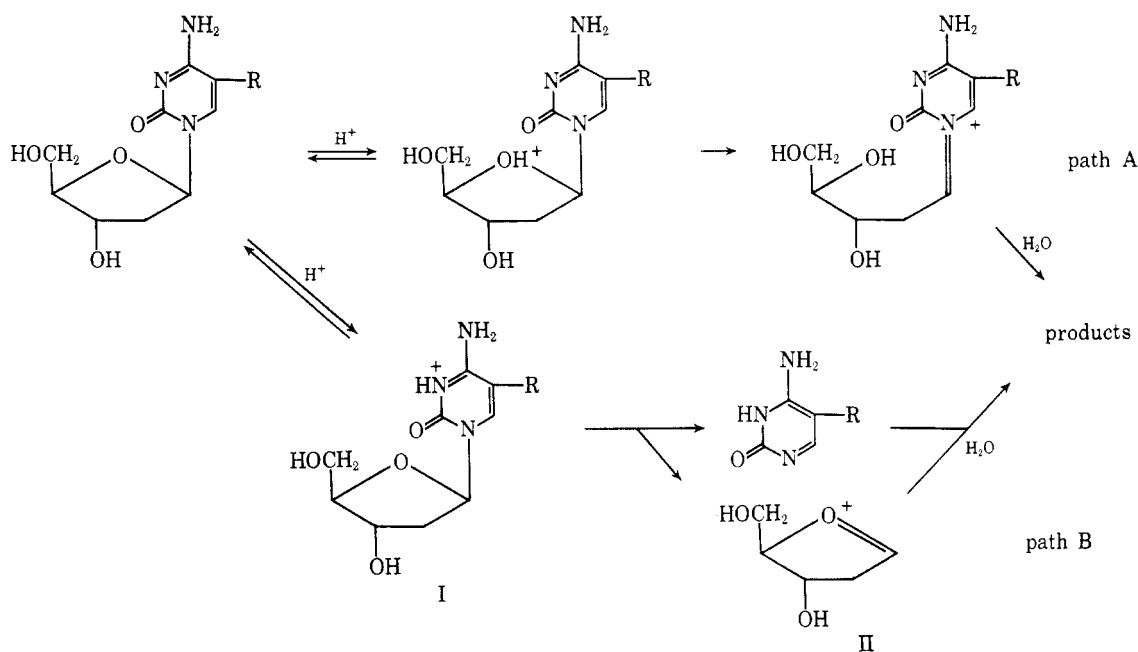
(Shapiro, 1967; Mushynski and Spencer, 1970) of DNA. The cleavage of one of these bonds *in vivo* may lead to mutations (Freese, 1963) or to inactivation of the DNA *via* chain breakage (Strauss *et al.*, 1969).

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SCHEME I



An understanding of the detailed mechanisms of these processes is clearly desirable. The most important question is the identity of the bond that is initially cleaved. Most workers in the field have favored the following pathway. The sugar ring oxygen is protonated, either directly, or by proton transfer from the nitrogen heterocycle. The glycosidic nitrogen then participates in sugar ring opening. This is followed by attack of water, and the cleavage of the heterocycle from the open-chain sugar (Kenner, 1957; Dekker, 1960; Micheel and Heesing, 1961; Michelson, 1963; Ulbricht, 1963; Pfitzner and Moffatt, 1964; Garrett *et al.*, 1966; Gin and Dekker, 1968). The process is illustrated, for the case of deoxycytidine, in Scheme I, path A. However, detailed kinetic studies of the uncatalyzed hydrolysis of deoxyuridine derivatives (Shapiro and Kang, 1969) and the acidic cleavage of deoxyadenosine and deoxyguanosine (Zoltewicz *et al.*, 1970) have indicated that the mechanism in these cases followed an alternative path—direct cleavage of the heterocycle from the protonated nucleoside (C-N cleavage). This is illustrated for deoxycytidine in Scheme I, path B. It has been suggested, however, that different nucleosides may undergo hydrolysis by different mechanisms (Capon, 1969). To complete the picture for the principal nucleoside components of DNA, we have studied the hydrolysis of deoxycytidine derivatives and the cleavage by strongly acidic solution of deoxyuridine derivatives. We find that these compounds also hydrolyze by the path involving initial C-N cleavage (path B), and that this mechanism is therefore general for the nucleoside components of DNA.

Experimental Section

Methods and Materials. Bases and nucleosides were obtained from Schwarz-Mann, Orangeburg, N. Y., Calbiochem, Los Angeles, Calif., and Cyclo Chemical Corp., Los Angeles, Calif. They were found to be homogeneous upon thin-layer chromatography in several solvent systems. Buffer solutions (pH 0–7) were prepared by dissolving the appropriate amount of buffer salt and acid in water and adding sufficient sodium chloride to bring the ionic strength to 1.0. The systems (pH

range) employed were HCl–KCl (0–1.5), H₃PO₄–KH₂PO₄ (1.5–2.5), HCO₂H–NaHCO₂ (2.5–3.5), CH₃CO₂H–CH₃CO₂Na (3.5–5.5), and KH₂PO₄–Na₂HPO₄ (5.5–7.0). Sulfuric acid solutions of known *H*₀ value were prepared by the method of Johnson *et al.* (1969). Thin-layer chromatography was run as described earlier (Shapiro and Klein, 1966). Determinations of pH (0–14) were done at the reaction temperature using a Beckman Zeromatic pH meter equipped with a Beckman combination electrode (No. 39013). Ultraviolet measurements were made using a Beckman DU spectrophotometer or Cary 15 recording spectrophotometer. Optical rotatory dispersion measurements were obtained with a Cary 60 recording spectropolarimeter. Reactions were run in ground-glass-stoppered flasks in a thermostated bath maintained at the indicated temperature $\pm 0.05^\circ$. The initial concentration of nucleoside was 5×10^{-3} M. Aliquots were withdrawn from time to time, cooled in ice, and analyzed by the procedure described below.

Hydrolysis of Deoxyuridine Derivatives. Sodium hydroxide and sodium sulfate solutions were added to the aliquots to produce a solution 0.05 M in NaOH and 0.5 M in Na₂SO₄. Recent studies (Shapiro and Kang, 1971) have shown that the ultraviolet spectra of alkaline solutions of uracil derivatives vary with the ionic strength. Therefore, care was taken to take the spectra in solutions of the same ionic strength, and to analyze them using equations derived from known standards measured at the same ionic strength. The aliquots were allowed to stand overnight in alkali for reasons discussed earlier (Shapiro and Kang, 1969). The ratio (*R*) of the absorbances in the uv region at two wavelengths was obtained. The wavelengths employed were 290:260 for deoxyuridine, 300:260 for thymidine, and 300:275 for 5-bromodeoxyuridine. The ratio was used to determine the fraction, *X*, of nucleoside hydrolyzed according to the general method described by Loring (1955). The equations used were $X = (4770 - 4390R)/(4540 + 3290R)$ for deoxyuridine, $X = (3430 - 3830R)/(3380 + 2960R)$ for thymidine, and $X = (5140 - 4380R)/(3840 + 2950R)$ for 5-bromodeoxyuridine. As a precaution, the reaction mixtures were also examined by thin-layer chromatography in 1 N NaOH–2-propanol (1:1, v/v). No ultraviolet-absorbing spots,

TABLE I: Hydrolysis of 5-Bromodeoxycytidine.

pH or H_0	Temp ($^{\circ}\text{C}$)	$10^4 K (\text{sec}^{-1})$	Method
4.90	95	0.181	ORD
4.00	95	1.50	ORD
3.70	95	1.99	ORD
0.00	65	2.81	ORD
0.00	65	2.76	Uv
0.00	55	0.700	ORD
0.00	55	0.727	Uv
-0.44	65	2.45	Uv
-0.96	65	2.83	Uv
-1.22	65	2.89	Uv
-1.46	65	3.58	Uv
-1.58	65	4.55	Uv
-1.71	65	5.08	Uv

other than the starting nucleoside and the expected base, were observed.

Hydrolysis of Deoxycytidine Derivatives. It was feared that deamination to deoxyuridine derivatives might complicate the study of the hydrolysis of deoxycytidine derivatives in weakly acidic solution (Shapiro and Klein, 1966). The following method was devised to allow hydrolysis and deamination to be followed at the same time. Aliquots were diluted into a known volume of 0.1 N HCl, and readings were taken at two wavelengths that represented the isosbestic points of the optical rotatory dispersion spectra of equimolar solutions of each nucleoside and its deamination product (λ 263.5 and 290 for deoxycytidine, λ 262.5 and 297.5 for 5-methyldeoxycytidine, and λ 271.5 and 309 for 5-bromodeoxycytidine). The magnitude of the difference between the rotations at the two wavelengths provided a direct measure of the amount of nucleoside (starting and deaminated) in the reaction mixture. The extent of deamination was followed by the use of a third wavelength in which the rotations of starting nucleoside and deamination product differed sharply (λ 277.5 for deoxycytidine, λ 280 for 5-methyldeoxycytidine, and λ 290 for 5-bromodeoxycytidine). In practice, it was found that deamination was negligible within the time periods used in this study. This was confirmed by thin-layer chromatography using 2-propanol-concentrated HCl-H₂O (65:16.7:18.3, v/v) as the developing solvent. Only the starting nucleoside and the corresponding base was observed. The above method was used to follow hydrolyses run at pH > 0.

A spectrophotometric procedure was used to follow the hydrolyses run in sulfuric acid ($H_0 < 0$). This was analogous to that described above for deoxyuridine. The solvents used for the ultraviolet measurement, wavelength ratio employed, and equation used were 1.0 N NaOH with 0.5 M Na₂SO₄, 290:270, and $X = (7190 - 6190R)/(3410 + 3800R)$ for deoxycytidine; 1.0 N NaOH with 0.5 M Na₂SO₄, 295:270, and $X = (7920 - 4360R)/(3810 + 4090R)$ for 5-methyldeoxycytidine; and 0.01 N NaOH with 0.5 M Na₂SO₄, 300:270, and $X = (6700 - 2440R)/(1960 + 2230R)$ for 5-bromodeoxycytidine. In one of the determinations, the rate constants were derived using both the ultraviolet and optical rotatory dispersion methods. The values derived from the two methods were in good agreement with each other.

Kinetic Analysis. The data from the above runs gave linear plots in the appropriate form indicating first-order reactions in

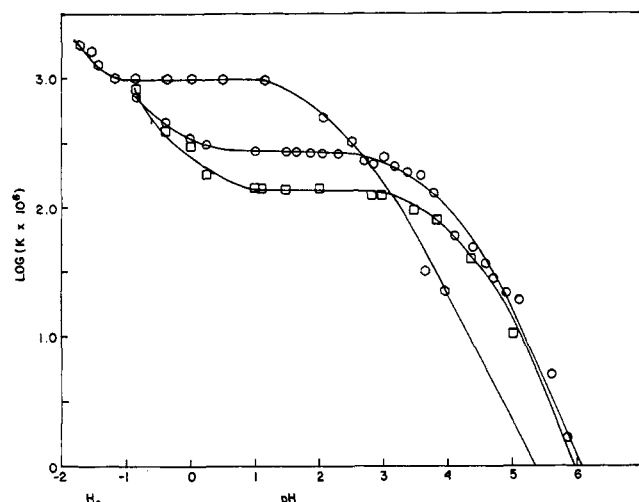


FIGURE 1: Plot of the logarithms of the rate constants (sec^{-1}) vs. pH and H_0 for the hydrolysis of deoxycytidine (○) at 95°, 5-methyldeoxycytidine (□) at 95°, and 5-bromodeoxycytidine (○) at 75°.

nucleoside. The rate constants were determined by a least-squares analysis on a Wang 700B electronic calculator. The standard errors were generally in the range 0–7%, with the slowest hydrolyses having the least accurate rate constants.

Results

Kinetics of Hydrolysis of Deoxycytidine Derivatives. The variations with pH and H_0 of the logs of K (the observed pseudo-first-order rate constant) for the hydrolysis of deoxycytidine, 5-methyldeoxycytidine, and 5-bromodeoxycytidine are given in Figure 1. For reasons of convenience, the hydrolyses of 5-bromodeoxycytidine were run at several different temperatures. The rate constants in the range pH 3.95–0 were run at 75°, and can be determined from Figure 1. Constants derived at other temperatures are summarized in Table I. These rates were also extrapolated to 75°, using the thermodynamic parameters given below, in order to display a complete pH profile at one temperature in Figure 1.

The pH-rate profiles, in the pH range 1–6, resemble titration curves with the midpoints at pH 3.70, 3.85, and 2.20 for dCyd,¹ dMeCyd, and dBrCyd, respectively. This agrees with earlier, more limited data collected for dCyd by Venner (1964, 1966). The actual pK_A values for the three nucleosides under reaction conditions could not be determined because of hydrolysis. At 55°, with an ionic strength of 0.1, they were found to be 3.92, 4.07, and 2.48 for dCyd, dMeCyd, and dBrCyd, respectively. As the pK_A values would decline with a further temperature increase, it is likely that the first set of values represents the pK_A values under reaction conditions. This indicates that a monocation form of the nucleosides is the reactive species from pH 1 to 6. The entire curve for each nucleoside in that range was generated using the equation $K = K_1 K_A [H^+] \times [Nuc]/(1 + K_A [H^+])$. In the above, K_1 represents the actual rate constant for the decomposition of the monocation, and is equal to the observed constant K , in the pH-independent (plateau) area; $[Nuc]$ represents the total concentration of

¹ Abbreviations used are: dCyd, deoxycytidine; dMeCyd, 5-methyldeoxycytidine; dBrCyd, 5-bromodeoxycytidine; dUrd, deoxyuridine; dThd, thymidine; dBrUrd, 5-bromodeoxyuridine; dNuc, deoxyribonucleoside.

TABLE II: Hydrolysis of Deoxycytidine Derivatives: Variation with Temperature.

Temp (°C)	10 ⁵ K (sec ⁻¹)		
	dCyd	dMeCyd	dBrCyd
95	27.4	14.4	
90	13.6	10.1	
85	6.16	5.82	
80	3.30	2.24	
75	1.35	1.19	96.1
65			27.6
60			12.9
55			7.00

nucleoside, protonated or nonprotonated. Activity coefficients are neglected. In this treatment, the assumption has been made that the N-3 protonated species (I in Scheme I) is the reactive species. If a tautomer were involved, then K_1 would represent the product of the actual rate constant and a tautomeric constant.

The rate constant for deoxycytidine hydrolysis (at pH 2.84, 95°, constant ionic strength of 1.0) was found not to vary in formate buffers of concentrations varying from 0.025 to 0.075 M. This excluded the possibility of a buffer addition and elimination mechanism for the hydrolysis. Such a mechanism had been observed for the hydrolytic deamination of cytosine derivatives (Shapiro and Klein, 1966). This specific acid catalysis exhibited by the hydrolysis was in accord with the results of the pH-rate profile. A moderate increase in the rate of hydrolysis in the plateau area for dCyd was observed with increasing ionic strength: $10^4 K (\text{sec}^{-1}) = 2.11$ when $\mu = 0.1$, 2.32 when $\mu = 0.4$, 2.50 when $\mu = 0.7$, and 2.74 when $\mu = 1.0$. A very similar increase in rate (32%) with a tenfold increase in ionic strength had been observed in the acidic hydrolysis of deoxyguanosine (Zoltewicz *et al.*, 1970).

In order to determine the thermodynamic parameters for the hydrolyses of the monocations, the rates were measured at several temperatures in the plateau areas. The rate constants obtained are listed in Table II, and the calculated thermodynamic parameters in Table III. The ΔS^\ddagger values were evaluated at 95° for dCyd and dMeCyd and 75° for dBrCyd. The entropies and enthalpies of activation were calculated using the absolute rate equation, $K = (kT/h)e^{-\Delta H^\ddagger/RT}e^{\Delta S^\ddagger/R}$.

TABLE III: Thermodynamic Parameters for Hydrolysis.

Compound	ΔH^\ddagger (kcal/mole) ^a	ΔS^\ddagger (eu) ^a
Deoxycytidine	33.9	16.6
5-Methyldeoxycytidine	30.7	6.6
5-Bromodeoxycytidine	29.5	12.1
Deoxyuridine	29.3	9.5
Thymidine	29.8	8.0
5-Bromodeoxyuridine	28.6	9.2

^a Evaluated in the pH-independent area for deoxycytidine derivatives and at $H_0 = -2.20$ for deoxyuridine derivatives.

TABLE IV: Hydrolysis of Deoxyuridine Derivatives.

H ₂ SO ₄ (M)	H ₀	Temp (°C)	10 ⁵ K (sec ⁻¹)
Deoxyuridine			
2.24	-0.92	75	3.24
2.74	-1.17	75	5.28
3.25	-1.41	75	7.80
3.79	-1.65	75	12.8
4.36	-1.90	75	20.7
4.94	-2.15	75	38.2
4.94	-2.26	50	1.55
5.55	-2.61	50	2.72
6.19	-2.95	50	5.43
6.86	-3.23	50	11.4
7.56	-3.67	50	29.0
8.29	-4.10	50	67.7
10.27	-5.82	25	34.6
11.14	-6.47	25	71.4
Thymidine			
2.24	-0.92	75	5.24
2.74	-1.17	75	8.15
3.52	-1.53	75	16.6
4.94	-2.15	75	54.0
4.94	-2.26	50	2.91
5.87	-2.78	50	6.25
6.52	-3.12	50	11.7
8.29	-4.10	50	72.3
10.27	-5.82	25	36.6
5-Bromodeoxyuridine			
2.24	-0.92	75	7.73
2.74	-1.17	75	12.4
3.52	-1.53	75	24.8
4.94	-2.15	75	75.5
4.94	-2.26	50	4.03
5.87	-2.78	50	8.87
6.52	-3.12	50	15.0
7.20	-3.48	50	27.2
10.27	-5.82	25	41.4

When the hydrolyses were conducted at $H_0 < 0$ for dCyd and dMeCyd, or $H_0 < -1$ for dBrCyd, the rate was seen to rise with increasing acidity (see Figure 1). This could be ascribed to the hydrolysis of a dication form of the nucleoside. The ultraviolet spectra of the compounds were taken over the range $H_0 = 0$ to -10 in sulfuric acid solutions. Changes were observed for dCyd and dMeCyd in solutions of $H_0 < -4$, and for dBrCyd in solutions of $H_0 < -5.8$. These changes continued up to $H_0 = -10$, so that no definite pK_A could be assigned to the dications.

Kinetics of Hydrolysis of Deoxyuridine Derivatives under Strongly Acidic Conditions. The hydrolysis of deoxyuridine derivatives had been studied previously over the pH ranges 0–1 (Garrett *et al.*, 1966) and 1–7 (Shapiro and Kang, 1969). We now studied the reaction in sulfuric acid solutions, $H_0 = -1$ to -6.5 . The nucleosides used were deoxyuridine, thymidine, and 5-bromodeoxyuridine. Because of the wide range of reaction rates, a number of temperatures were employed. The pseudo-first-order rate constants for hydrolysis are summarized in Table IV.

The logarithms of the rate constants (extrapolated for con-

venience to 75° were plotted against both $-H_0$ (Figure 2) and the logarithm of acid concentration. Both sets of plots were linear, with slopes of about 0.8 in the former case and 0.4 in the latter one. According to the Zucker-Hammett hypothesis (Long and Paul, 1957), a hydrolysis proceeding without the participation of water in the transition state (A-1 mechanism) will afford a linear plot of $\log K$ vs. H_0 , with a slope close to unity. As the plots vs. H_0 were closer to unity in slope than the plots vs. the logarithm of acid concentration, the nonparticipation of water in the transition state is indicated. The validity of the Zucker-Hammett hypothesis has been questioned, however (Rochester, 1970), and an alternative parameter, ω , has been proposed (Bunnett, 1961a-d). An ω value of -2.5 to 0 is supposed to indicate the nonparticipation of water in the transition state, while an ω of 1.2 – 3.3 indicates the opposite (A-2 mechanism). In the present case, we calculated $\omega = 0.86$ for dUrd, $\omega = 1.26$ for dThd, and $\omega = 1.60$ for dBrUrd, suggesting more A-2 than A-1 character for the reactions.

The pK_A values for dissociation of the cations of uracil and 5-bromouracil have been reported to be -3.38 and -7.25 , respectively (Katritzky and Waring, 1962). (There has been confusion on this point in the literature. This is discussed separately in the next section.) The pK_A values for the cations of dUrd, dThd, and dBrUrd have not been determined. As the site of protonation in uracil derivatives appears to be on O-4 (Sobell and Tomita, 1964), it is probable that the substitution of a methyl substituent on C-5 and/or a deoxyribosyl residue on N-1 would affect the pK only to a minor extent (less than 1 unit). The plots of \log rate vs. H_0 for dUrd and dThd thus pass through the region of a dissociation, with no deviation of the curves from linearity. This is strikingly dissimilar to the results in the deoxycytidine series, but analogous to those observed with deoxyadenosine and deoxyguanosine (Zoltewicz *et al.*, 1970). It was postulated in these two cases that the observed rate constant, K , was the sum of the rates of hydrolysis of the nucleoside monocation ($HNuc^+$) and the dication (H_2Nuc^{2+}) according to the equation $K = K_1 \times [HNuc^+] + K_2[H_2Nuc^{2+}]$. It was shown that a linear plot of $\log K$ vs. the activity of hydrogen ion would result if the following relationship were true: $K_1/K_{A1} = K_2/K_{A2}$, where K_{A1} and K_{A2} are dissociation constants of the nucleoside monocation and dication (Zoltewicz *et al.*, 1970).

The thermodynamic parameters for the hydrolyses were evaluated and are listed in Table III.

Our earlier report on the uncatalyzed hydrolysis of deoxyuridine (Shapiro and Kang, 1969) had not included data on the effect of ionic strength on reaction rate. This was now examined at pH 4.65, with the following results: $\mu = 0.3$, $K = 2.83 \times 10^{-6} \text{ sec}^{-1}$; $\mu = 0.7$, $K = 2.90 \times 10^{-6} \text{ sec}^{-1}$; $\mu = 1.0$, $K = 3.02 \times 10^{-6} \text{ sec}^{-1}$. This is in accord with the $SN1$ mechanism postulated for the reaction.

Dissociation Constant of Uracil Monocation. There have been two sharply disagreeing sets of values in the literature for the dissociation constant of the monocation of uracil and its *N*- and *C*-alkyl derivatives. The value of -3.38 for the pK_A of uracil was determined by spectroscopy by Katritzky and Waring (1962). The pK_A value of -4.16 has been measured for 2-thiouracil, and it has been estimated that uracil is probably slightly higher than this (Cook, 1966). On the other hand, pK_A values of 0.5 and 0.0 (derived from unpublished spectroscopic determinations) have been reported for uracil and thymine monocations, respectively (Beaven *et al.*, 1955; Cohn, 1955). These results have been republished in several biochemical handbooks and tables. Additional workers have reported pK_A

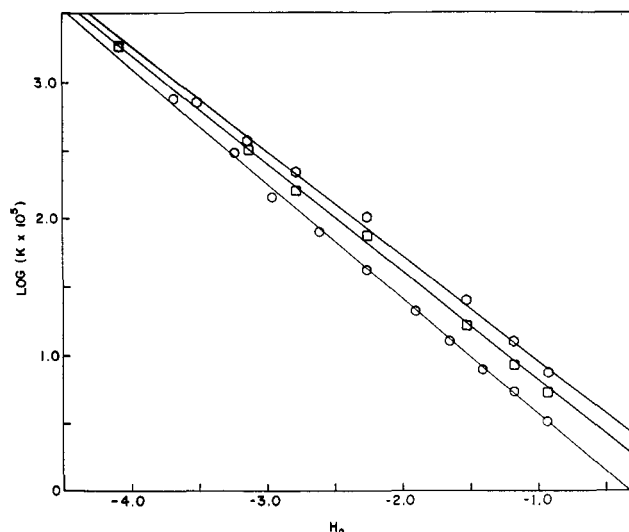


FIGURE 2: Plot of the logarithms of the rate constants (sec^{-1}), at 75°, vs. H_0 for the hydrolysis of deoxyuridine (○), thymidine (□), and 5-bromodeoxyuridine (○).

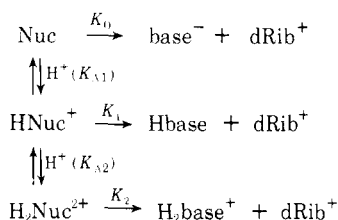
values between 0 and 1 for 1-ethyl derivatives of thymine and uracil (Parry *et al.*, 1969). The method used was a titrimetric one, with *p*-nitroaniline as indicator.

To resolve the contradiction between the sets of values, we have studied the ultraviolet, nuclear magnetic resonance, and optical rotatory dispersion spectra of uracil derivatives. The ultraviolet spectrum of uracil at pH 0.5 was found to be identical to that in neutral aqueous solution. In concentrated sulfuric acid, the shifts reported by Katritzky and Waring (1962) were confirmed. The spectrum of uracil in 6 *N* HCl ($H_0 = -2.12$) differed from that of neutral uracil, as reported by Beaven *et al.* (1955). This, however, we found to be due to partial conversion to the monocation of $pK_A = -3.38$. To guard against the possibility that an additional dissociation near pH 0.5 might have been overlooked, because it was not accompanied by a shift in the ultraviolet, the optical rotatory spectra of uridine were taken in acid. The curves at pH 1 and $H_0 = -1$ were identical: λ (peak) 282 $m\mu$ (Φ 4000); λ (crossover) 272 $m\mu$; λ (trough) 253 $m\mu$ (Φ -8800). At $H_0 = -3.3$, the values were: λ (peak) 287 $m\mu$ (Φ 3050); λ (crossover) 275 $m\mu$; λ (trough) 254 $m\mu$ (Φ -7100). The nuclear magnetic resonance spectra of uridine in H_2SO_4 were also observed, as protonation of a nucleoside has been shown to be associated with a down-field shift of the C-H protons on the base (Danyluk and Hruska, 1968). The H-5 resonance was at τ 3.95 at pH 2 and $H_0 = -0.50$, but gradually shifted to τ 3.33 as H_0 was varied from -2.95 to -7.46 . Similarly, the H-6 resonance was observed at τ 2.02 at both pH 2 and $H_0 = -2$, but shifted to τ 1.53 at $H_0 = -7.46$. It was concluded that the pK_A value reported near -3.4 was the correct one for uracil, and that the one reported near 0.5 does not exist.

Discussion

The results above, together with others already published (Shapiro and Kang, 1969; Zoltewicz *et al.*, 1970) make it clear that the four common naturally occurring deoxyribonucleosides, and a number of their simple derivatives, undergo hydrolysis by a pathway involving the initial rupture of the *N*-glycosyl bond. In the case of deoxyuridine derivatives, the neutral form can cleave; the others require mono- or dipro-

SCHEME II



nation of the nitrogenous heterocycle prior to reaction. This is illustrated for deoxycytidine in path B of Scheme I, and expressed more generally in Scheme II. In the latter scheme, Nuc represents a neutral deoxyribonucleoside, HBase a neutral base, and dRib⁺ is the deoxyribosyl cation, II.

The rates of hydrolysis of deoxyadenosine, deoxycytidine, deoxyguanosine, and thymidine are compared in Figure 3. Hydrolysis rates from this paper and earlier ones (Garrett *et al.*, 1966; Shapiro and Kang, 1969; Zoltewicz *et al.*, 1970) have been combined and extrapolated to 95° in preparing this figure, while ionic strength differences have been neglected. The level portion of the thymidine curve (pH 3–7) represents the hydrolysis of unprotonated nucleoside (K_0). At pH values below 2, both protonated and diprotonated forms of thymidine hydrolyze. The observed rate is equal to $K_1[\text{HNuc}^+] + K_2 \times [\text{H}_2\text{Nuc}^{2+}]$. The linear dependence of this upon the activity of hydrogen ion is due to the equivalency, $K_1/K_{A1} = K_2/K_{A2}$ (see the Results section). The same situation occurs in the hydrolysis of deoxyadenosine and deoxyguanosine (Zoltewicz *et al.*, 1970). Hydrolysis of both the monocation and dication take place and combine to produce a linear dependence upon hydrogen ion activity throughout the entire pH range studied. In the case of deoxycytidine, however, the above equivalency does not hold. The hydrolysis observed above pH 0 is due to the monocation. The decrease in rate observed above pH 3 is caused by the dissociation of the monocation. At $H_0 = <0$, hydrolysis of the dication contributes appreciably to the total rate, and a rate enhancement is observed. No hydrolysis of the unprotonated nucleoside has been reported for deoxyadenosine, deoxycytidine, or deoxyguanosine.

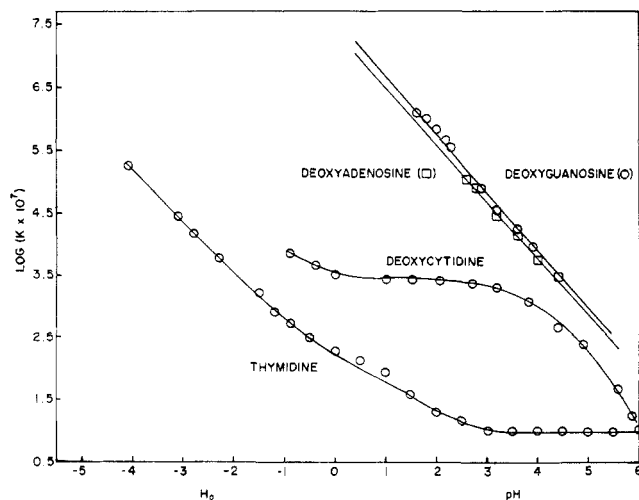
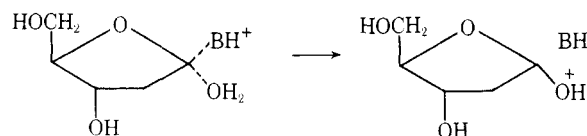


FIGURE 3: Plot of the logarithms of the rate constants (sec^{-1}), at 95°, vs. pH (above 0) and H_0 (below 0) for the hydrolysis of the four common, naturally occurring deoxyribonucleosides.

SCHEME III



The most widely accepted mechanism for the hydrolysis of deoxyribonucleosides (as illustrated by path A in Scheme I) does not fit the data and is incorrect. The detailed arguments that demonstrate this have been made elsewhere for the cases of individual nucleosides (Shapiro and Kang, 1969; Capon, 1969; Zoltewicz *et al.*, 1970) and need not be repeated again. Only a summary of the principal arguments will be presented here.

(1) The argument in favor of initial C–O cleavage (sugar ring opening) was not based upon kinetic data but on analogy to the mechanism of hydrolysis of glycosylamines (Kenner, 1957). The ring nitrogens of purines and pyrimidines are not like simple aliphatic and aromatic amines, however. In fact, the hydrolytic behavior of nucleosides most resembles that of simple *O*-glycosides. (2) The pH–rate profiles of the deoxyribonucleosides accommodate well to a pathway involving initial C–N cleavage. They do not at all resemble the profiles of the glycosylamines (C–O cleavage) which give bell-shaped curves with well-defined maxima. (3) Hydrolyses involving sugar-ring opening, such as the case of the glycosylamines, are accompanied by mutarotation due to anomerization, rearrangements, transglycosidation, and other side reactions. These have not been observed with the deoxyribonucleosides. (4) The effect of substituents upon the rate of hydrolysis can be explained by the C–N cleavage pathway, but not by the C–O cleavage pathway. This can be illustrated for the case of deoxycytidine derivatives (Scheme I). The rate of hydrolysis of dBrCyd in the plateau area (monocation hydrolysis) is approximately 100 times as rapid as those of dCyd and dMeCyd. This is consistent with path B, where an important factor is the stability of the base as a leaving group. This would be enhanced by the electron-withdrawing bromine atom. In path A, the bromine would be expected to retard the opening of the sugar ring. It is possible, of course, that the rate-determining step occurs after the opening, but this is unlikely for other reasons (see Zoltewicz *et al.*, 1970).

Another example of substituent effects is the hydrolysis of deoxyuridine derivatives in strong acid. The order of hydrolysis rates is dBrUrd > dMeUrd > dUrd. This mixed order is due to the fact that dication as well as monocation is hydrolyzing. The presence of a bromine enhances the rate of hydrolysis of the dication, but reduces the amount of dication present at a given H_0 value. A methyl substituent has the opposite effect. In path A, no such set of opposing effects would exist, and the most probable order would be dMeUrd > dUrd > dBrUrd.

A secondary mechanistic question that has been less firmly resolved is the degree of participation of water in the transition state of the hydrolysis. In Schemes I and II, it has been arbitrarily assumed that no participation by the solvent takes place. An alternative to the A-1 type cleavage, however, would be the A-2 cleavage (Scheme III). Transition states intermediate between the two extremes are also possible. The principal source of information available on this point is the value of the entropy of activation. In studies on the hydrolysis of alkyl and aryl glycosides, and $\text{S}_{\text{N}}1$ and $\text{S}_{\text{N}}2$ reactions of related systems, it has been found that the unimolecular processes were usually associated with positive entropies of activation.

The bimolecular reactions were usually accompanied by negative ΔS^\ddagger values, about 20 eu lower (Schaleger and Long, 1963; Overend *et al.*, 1962; Long *et al.*, 1957). The entropies of activation observed for the natural deoxyribonucleosides are: thymidine (hydrolysis of unprotonated nucleoside) 3.5 eu (Shapiro and Kang, 1969), thymidine (hydrolysis of monocation + dication) 8.0 eu, deoxycytidine (hydrolysis of monocation) 16.6 eu, deoxyadenosine and deoxyguanosine (hydrolysis of monocation + dication) 8.4 and 12.7 eu, respectively (Zoltewicz *et al.*, 1970). This suggests that all of these hydrolyses proceed without participation of water in the transition state. Additional information is available in the case of deoxyuridine derivatives, where the hydrolyses were studied in concentrated sulfuric acid solutions. The kinetic data were analyzed using the ω criterion of Bunnett (1961a-d). The ω values obtained indicate reactions intermediate in character between A-1 and A-2. The situation here is analogous to that existing for the acidic hydrolysis of several glycopyranosides. The entropies of activation suggest A-1, and the ω values A-2 mechanisms. It has been concluded in these cases that the ω criterion is the less valid standard, and that the reactions follow an A-1 mechanism (Rochester, 1970).

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