Kinetics and Mechanisms of Solvolysis of 5-Iodocytosine

EDWARD R. GARRETT×, TADEUSZ W. HERMANN*, and HYUK-KOO LEE‡

Abstract □ Iodocytosine is deiodinated by specific hydrogen-ion attack on the neutral species to iodouracil, which is further transformed to uracil in acid solutions. The extent of the transformation of iodouracil to uracil is dependent on its concentration and is consistent with a postulated equilibration of iodouracil and two chloride ions with uracil and the complex anion ICl_2^- and thus is a function of hydrochloric acid concentration. There are pH-independent rates of solvolysis at high acidities, indicative of negligible hydrogen-ion-catalyzed solvolysis of protonated iodocytosine. In the neutral pH region, the iodocytosine solvolysis can be rationalized by general acid–base and water catalysis to cytosine, since it was shown that phosphate and carbonate buffers served as catalysts for the deiodination although acetate buffers did not. Hydroxide ion attacked both the neutral and anionic iodocytosine and produced 6-hydroxycytosine, which further degraded on hydroxide-ion attack, probably through a fast reacting barbituric acid intermediate. Arrhenius parameters were determined.

Keyphrases □ 5-Iodocytosine—kinetics and mechanisms of solvolysis □ pH-rate profiles—5-iodocytosine solvolysis □ Solvolysis—5-iodocytosine, kinetics and mechanisms

The iodinated nucleosides have interesting antitumor and antiviral properties (1, 2). The kinetics of the solvolytic transformation of iodouridine and iodouracil were investigated (3–5) as were various pyrimidine and purine nucleosides and their derivatives (6–14). Cytosine, cytidine, and arabinocytosine undergo hydrolytic deamination to yield the corresponding uracil derivatives (12–14), and their ring system is susceptible to nucleophilic attack at the 5,6-double bond (3).

The purpose of this paper is to report the complete rate–pH profile for the solvolytic degradation of iodocytosine, its Arrhenius parameters, and the pH dependencies of the solvolytic degradative mechanisms of the compound and its initial products in acid, neutral, and alkaline solvolyses, which include iodouracil, cytosine, and 6-hydroxycytosine, where the formation of such products is pH dependent.

EXPERIMENTAL

Kinetic Measurements—Appropriate quantities of the iodocytosine[1] to produce a final concentration of 1×10^{-2} *M* were weighed into volumetric flasks and diluted to volume with nitrogen-purged distilled water. Aliquots were taken from these stock solutions and diluted with acid, alkali, or an appropriate buffer solution to produce, in general, a final nucleoside concentration of 1×10^{-4} *M*. All sample solutions were purged with nitrogen.

The fast reacting solutions in acid and alkali media were studied in 100-ml volumetric flasks. The slow reacting solutions in buffer were purged with nitrogen and sealed in 5-ml ampuls[2]. The reactions were conducted in constant-temperature oil baths controlled at selected temperatures between 70 and 90°, samples were withdrawn at suitable time intervals, and spectra were recorded[3] with time in the spectral range of 220–350 nm.

Spectrophotometric readings were also made on acidified solutions, obtained by adding 0.1–0.3 ml of concentrated hydrochloric acid to lower the pH below 1, and on alkaline solutions, obtained by adding 0.1–0.4 ml of concentrated sodium hydroxide.

pH Measurements—The pH values of the sodium hydroxide and hydrochloric acid solutions at the temperatures studied were calculated from extrapolation of the activity coefficient data in the literature (15). The pH values of buffer solutions were measured directly at the temperature of the study on a pH meter[4] equipped with a high temperature combination electrode[5], standardized with standard buffer solutions of pH 4.0, 7.0, and 10.0 at the same temperature.

TLC Studies—Solutions of iodocytosine (10^{-2} *M*) in 1 *N* HCl, distilled water, and 1 *N* NaOH were prepared and were maintained at 85° in a constant-temperature bath; aliquots were withdrawn at suitable time intervals. The acidic solutions were neutralized before spotting on TLC plates. Solutions (1×10^{-2} *M*) of cytosine, uracil, iodouracil, iodocytosine, barbituric acid, and isobarbituric acid in distilled water were prepared as standards for TLC studies.

The reaction mixtures and standard solutions were spotted on TLC plates coated with a 5-mm layer of silica gel[6] and activated at 110° overnight. The chromatograms were developed in closed tanks with a mixture of *tert*-butyl alcohol–methyl ethyl ketone–ammonium hydroxide–distilled water (4:3:2:1). The solvent front advanced 15–16 cm in approximately 4 hr. After drying, the spots were visualized under UV light. The spots of the chromatogram were extracted with 0.1 *N* HCl and 0.1 *N* NaOH, and UV spectra of the eluates were taken.

Identification of Initial Product of Alkaline Reaction, 6-Hydroxycytosine—Two grams of iodocytosine in 100 ml of 1 *N* NaOH was refluxed for 24 hr until the iodocytosine peak at λ_{max} 297 nm almost disappeared. The reacted solution was cooled and a white precipitate, which was nonchromophoric in the UV when dissolved in water, was filtered off. The filtrate was eluted with distilled water through a column of cationic resin[7] (50–100 mesh, 2.2 × 30 cm).

Aliquots of 100 ml were collected at a constant flow rate (3 ml/min). The first three fractions had no UV absorption and were discarded. Fractions 4–8 had an absorption maximum in the 256–257-nm range (9). These fractions were pale yellow and ranged in pH from 1.3 to 3.6. The fractions were combined and evaporated to dryness. The residue was washed with acetone and ether and recrystallized in methanol. The IR spectra of this compound suggested barbituric acid (16), and it is well known that 6-hydroxycytosine is readily hydrolyzed by dilute acid to this product (17).

Fractions 9 and 10, which had UV λ_{max} at 259–262 nm, were discarded. The UV absorption of fractions 10–20 had λ_{max} 264–265 nm. These were combined and evaporated to dryness, and the residue was washed with acetone and ether and recrystallized three times in water. The product exhibited UV absorption at λ_{max} (0.1 *N* HCl) 263 nm, λ_{max} (water) 265 nm, and λ_{max} (0.1 *N* NaOH) 266 nm. The UV data were in good agreement with an authentic solution of 6-hydroxycytosine and reported data (18) [λ_{max} (pH 3.0) 264 nm and λ_{max} (pH 11.0) 266 nm]. It did not melt below 310° and gave an IR spectrum identical with that of 6-hydroxycytosine. The NMR spectrum in d_6-dimethyl sulfoxide showed δ 4.4 (1, single, H-5), 6.1 (2, broad, NH_2), and 9.5 (2, broad, OH).

[1] California Corporation for Biochemical Research, Los Angeles, Calif.
[2] Kimble, neutraglass.
[3] Cary model 15 recording spectrophotometer.

* Direct-reading Beckman pH meter, Beckman Instruments Inc., South Pasadena, Calif.
[5] Corning combination electrode, Sargent-Welch Scientific Co., Birmingham, Ala.
[6] GF-254, E. Merck AG, Darmstadt, Germany.
[7] Dowex 50W-X2, J. T. Baker Chemical Co., Phillipsburg, N.J.

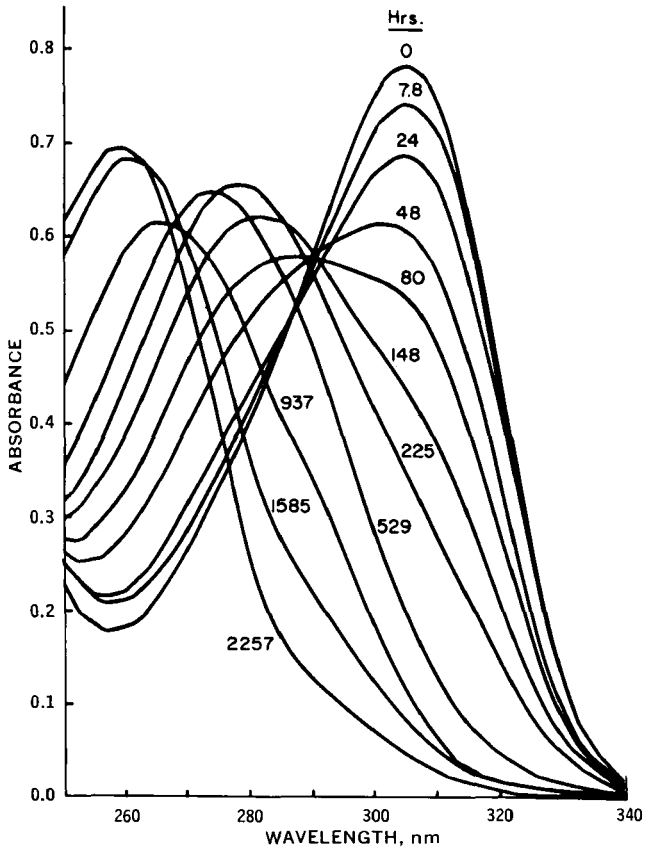


Figure 1—*Typical UV spectral changes of acidified solutions for the solvolysis of 1 × 10⁻⁴ M iodocytosine in acid and acetate buffers at 80.0°; [H₃CCOOH] = 0.1020, [H₃CCOO⁻] = 0.0090, pH 3.65. The curves are labeled as to hours after the start of reaction.*

Anal.—Calc. for $C_4H_5N_3O_2$: C, 37.8; H, 3.9; N, 33.1. Found: C, 37.41; H, 3.92; N, 32.74.

Dissociation Constant for Iodouracil—Beer's law plots for iodouracil and uracil in 1 *N* HCl solution were constructed[3] and the absorptivities were 6.08 × 10³ at 280 nm and 3.45 × 10³ at 260 nm for iodouracil and 1.88 × 10⁴ at 260 nm and 4.37 × 10² at 280 nm for uracil.

Solution of the simultaneous equations for total absorbance from a mixture of iodouracil and uracil yields expressions for the concentrations of the two compounds:

$$C_{IU} = (3.88 \times 10^{-6})A_{260} - (1.67 \times 10^{-4})A_{280} \quad \text{(Eq. 1)}$$

$$C_{U} = (3.06 \times 10^{-5})A_{280} - (5.4 \times 10^{-5})A_{260} \quad \text{(Eq. 2)}$$

Various concentrations of iodouracil in 1 *N* HCl were maintained at 80° in a constant-temperature oil bath. Spectrophotometric readings were obtained after appropriate dilution with 1 *N* HCl until final equilibrium was reached. The concentration of each component in the mixtures at equilibrium was calculated from absorbances A_{260} and A_{280} using Eqs. 1 and 2.

RESULTS AND DISCUSSION

Spectral Changes—The changes with time of typical spectra of the acidic, neutral, and alkaline samples of iodocytosine degraded in acetate buffer, phosphate buffer, and sodium hydroxide solutions, respectively, are given in Figs. 1–3.

The spectra of iodocytosine degrading in hydrochloric acid solution and in buffers up to pH 5 (after acidification) showed an initial absorbance at 305 nm, the λ_{max} of iodocytosine, which subsequently decreased with time (Fig. 1). A new band appeared at 283 nm, the λ_{max} of iodouracil, and subsequently decreased, and a new band appeared at 259 nm, λ_{max} of uracil. This information, coupled with TLC data demonstrated that iodouracil (λ_{max} 283 nm in acid, 295 nm in alkali; R_f 0.82) was the first intermediate of the acid degradation of iodocytosine and sequentially degraded to produce uracil (λ_{max} 259 nm in acid, 283 nm in alkali; R_f 0.76). The isosbestic point at 290 nm was maintained for up to 1 half-life.

The spectra of iodocytosine degrading in pH 5–9 phosphate and carbonate buffers (after acidification) showed a new band at 278 nm in 1 half-life of iodocytosine loss (Fig. 2). TLC studies on degradation in phosphate buffer at 80° showed the initial degradation product of iodocytosine to be cytosine (λ_{max} 275 nm in acid, 278 nm in alkali; R_f 0.65) and the final product to be uracil (λ_{max} 259 nm in acid, 283 nm in alkali; R_f 0.76). The isosbestic point at 285 nm was maintained for 2.5 half-lives.

The absorbance of iodocytosine in alkaline solutions above pH 9 (λ_{max} 298 nm) decreased with time, and a new band appeared with a maximum at 265 nm (λ_{max} of acidified solution 264 nm) (Fig. 3). The maintenance of an apparent isosbestic point at 275 nm was strong evidence of a one-to-one transformation. The final slow loss of absorbance at 265 nm suggested that this intermediate degrades further to a nonchromophoric product. The reaction intermediate, 6-hydroxycytosine, was the initial product of iodocytosine alkaline degradation and was separated by column chromatography and characterized by IR, NMR, and elemental analyses.

TLC Identification—The developed plates of degraded samples from acid solutions under pH 5 were visualized under UV light and indicated the presence of iodouracil (R_f 0.82) and uracil (R_f 0.76). The UV spectra of spots extracted by acid and alkali were consistent with iodouracil [λ_{max} (0.1 *N* HCl) 283 nm and λ_{max} (0.1 *N* NaOH) 295 nm] and uracil [λ_{max} (0.1 *N* HCl) 259 nm and λ_{max} (0.1 *N* NaOH) 283 nm].

TLC spots of the samples degraded in water demonstrated that the products were cytosine (R_f 0.68) and uracil (R_f 0.76). The UV

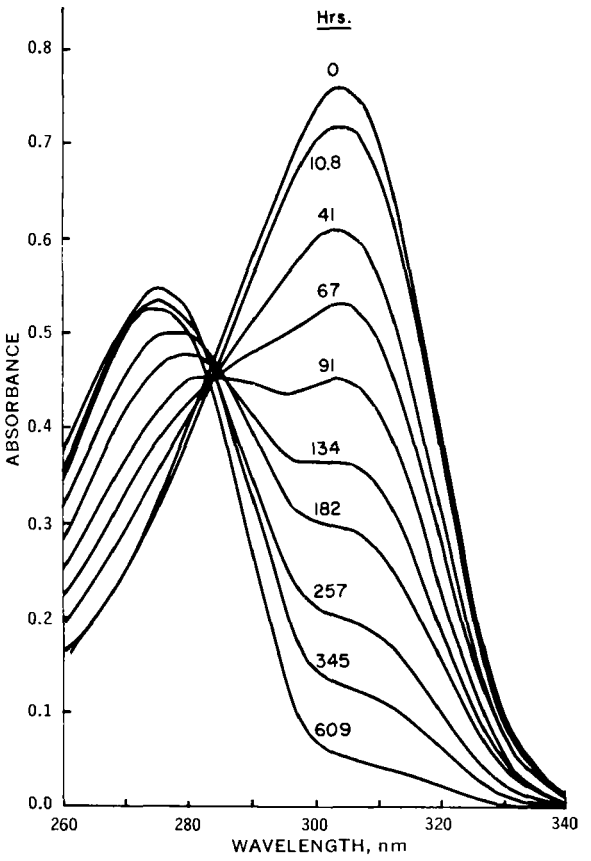


Figure 2—*Typical UV spectral changes of acidified solutions for the solvolysis of 1 × 10⁻⁴ M iodocytosine in phosphate and carbonate buffers at 80.0°; [KH₂PO₄] = 0.0314, [Na₂HPO₄] = 0.0486, pH 6.80. The curves are labeled as to hours after the start of reaction.*

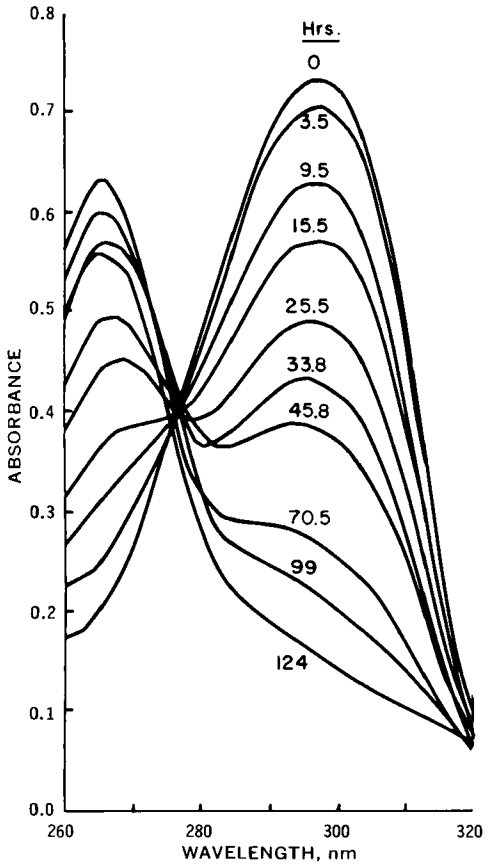


Figure 3—*Typical UV spectral changes for the solvolysis of 1 × 10⁻⁴ M iodocytosine in 0.05 N NaOH at 70°. The curves are labeled as to hours after the start of reaction.*

spectra of spots extracted by acid and alkali were in agreement with cytosine [λ_{max} (0.1 *N* HCl) 275 nm and λ_{max} (0.1 *N* NaOH) 278 nm] and uracil [λ_{max} (0.1 *N* HCl) 259 nm and λ_{max} (0.1 *N* NaOH) 283 nm]. However, the spot for the degradation intermediate in alkali (λ_{max} 263 nm in acid, 266 nm in alkali) did not correspond to any available standards of iodouracil, uracil, cytosine, and barbituric and isobarbituric acids. After isolation by column chromatography, the product was found to be 6-hydroxycytosine.

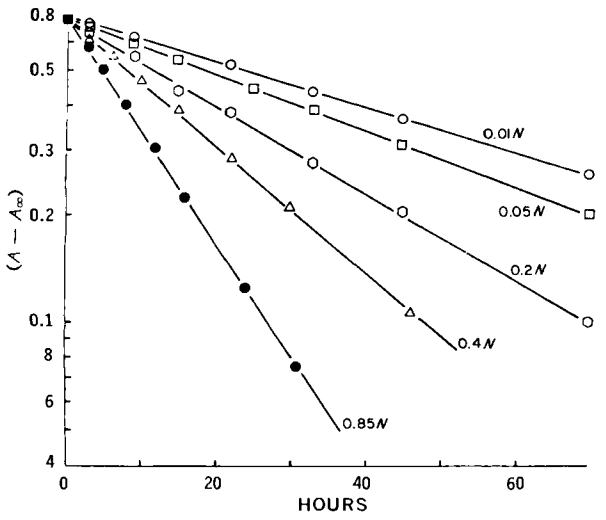


Figure 4—*Typical apparent first-order plots for the solvolysis of iodocytosine in various sodium hydroxide concentrations at 80°. The absorbance values, A, were measured at 297.5 nm, and A_∞ is the final asymptotic absorbance.*

Table I—Apparent First-Order Rate Constants, k (in Seconds^{-1}), for the Degradation of Iodocytosine at Various Temperatures and pH Values

Temperature	Buffer	pH[a]	$10^6 k$[b]
70.0°	0.85 *N* HCl	0.22	2.20
	0.05 *N* HCl	1.40	2.41
	Acetate	3.60	0.58
	Phosphate	5.85	0.14
	Carbonate	8.75	0.225
	0.05 *N* NaOH	11.4	1.93
	0.20 *N* NaOH	11.97	2.96
	0.60 *N* NaOH	12.41	5.84
	0.85 *N* NaOH	12.56	8.90
80.0°	0.85 *N* HCl	0.22	6.00
	0.40 *N* HCl	0.55	6.20
	0.10 *N* HCl	1.12	6.40
	0.05 *N* HCl	1.40	6.20
	0.01 *N* HCl	2.06	5.90
	Acetate	3.60	1.43
	Acetate	4.35	0.52
	Acetate	5.35	0.32
	Phosphate	5.85	0.44
	Phosphate	6.80	0.35
	Phosphate	8.05	0.354
	Carbonate	8.90	1.30
	Carbonate	9.50	3.40
	0.01 *N* NaOH	10.56	3.93
	0.05 *N* NaOH	11.23	4.93
	0.20 *N* NaOH	11.78	7.72
	0.40 *N* NaOH	12.05	11.3
	0.85 *N* NaOH	12.35	19.3
85.0°	0.40 *N* HCl	0.40	11.1
	Acetate	4.35	1.01
	Phosphate	6.80	0.53
	0.01 *N* NaOH	10.55	6.88
	0.10 *N* NaOH	11.41	9.63
90.0°	0.40 *N* HCl	0.40	16.0
	Acetate	5.60	8.01
	Phosphate	8.0	0.87
	0.10 *N* NaOH	11.32	16.0

[a] The pH values at various hydrochloric acid concentrations were calculated from pH = $-\log f$ [HCl] from the activity coefficient, f, data in the literature (15). The pH values of the buffer solutions were measured at the reaction temperature on the pH meter standardized at that temperature. The pH values in alkaline solutions were calculated from pKw − pOH, where pOH = $-\log f$ [NaOH]. The pKw and f values were obtained or extrapolated for the requisite temperatures from literature data (15). [b] Rate constants in the acetate, phosphate, and carbonate buffer region were determined by obtaining the intercepts from the linear plots of k *versus* buffer concentration. Ionic strengths of all buffer solutions were adjusted to 0.4 *M* with sodium chloride.

6-Hydroxycytosine, 1 × 10⁻² *M*, was heated at 80° in 0.5 *N* NaOH. The resulting degraded solution was developed on TLC plates. No spots were observed other than that of 6-hydroxycytosine, even after exposure to UV light and with the use of a spray reagent prepared with *p*-dimethylaminobenzaldehyde which visualizes barbituric acid (19).

Rate Constants—The kinetic parameters for the solvolysis of iodocytosine were determined by following the loss of absorbance, A, of the UV chromophore as a function of time to the ultimate absorbance, A_∞.

The apparent first-order rate constants (Tables I and II) for the degradation of iodocytosine at various temperatures (70, 80, 85, and 90°) were obtained from the slopes of first-order plots of absorbance at 300–320 nm for acidified solutions and at 297.5 nm for the alkaline degradations. Plots of ln $(A - A_\infty)$ *versus* time were linear for all pH values at these wavelengths. Reasonable agreement was obtained between rate constants calculated from acidified and alkaline spectra. Typical first-order plots for the degradation of iodocytosine at various alkaline concentrations are given in Fig. 4.

The apparent first-order rate constants for iodocytosine degradation determined in this manner generally represented molecular transformation to iodouracil below pH 5, to cytosine between pH 5 and 9, and to 6-hydroxycytosine above pH 9.

Kinetic studies were conducted with and without shielding from light. No significant effect on the rate constants was observed with these variations.

Table II—Apparent First-Order Rate Constants, k (in Seconds^{-1}), for the Degradation of Iodocytosine in Buffers in the 3.5–9.5 pH Region at 0.4 Ionic Strength and 80.0°

[CH_3COOH]	[CH_3COONa]	[NaCl]	pH[a]	10^6k
0.0340	0.0030	0.3960	3.55 ±	1.45
0.0426	0.0037	0.3950	0.1	1.43
0.0682	0.0059	0.3930		1.48
0.1021	0.0090	0.3900		1.48
0.0095	0.0053	0.3940	4.35	0.52
0.0017	0.0092	0.3900	5.35	0.32
[KH_2PO_4]	[Na_2HPO_4]	[NaCl]	pH[a]	10^7k
0.0175	0.0025	0.3750	5.85 ±	4.8
0.0350	0.0049	0.3500	0.05	6.4
0.0700	0.0098	0.2504		7.7
0.1230	0.017	0.2260		9.6
0.00784	0.0122	0.3560	6.80 ±	6.6
0.01568	0.0243	0.3140	0.05	9.6
0.03136	0.0486	0.2230		16.0
0.04704	0.0730	0.1340		22.6
0.00220	0.0378	0.2844	8.05 ±	7.7
0.00330	0.0567	0.2266	0.05	9.6
0.00550	0.0945	0.1100		16.0
0.00770	0.142	0.0654		19.3
[Na_2CO_3]	[$NaHCO_3$]	[NaCl]	pH[a]	10^6k
0.0032	0.0370	0.3540	8.9 ±	2.05
0.0048	0.0552	0.3300	0.1	2.45
0.0080	0.0900	0.2840		3.20
0.0112	0.1289	0.2380		3.95
0.0045	0.0055	0.3800	9.5 ±	2.62
0.0202	0.0180	0.3210	0.1	4.74
0.0404	0.0360	0.2430		5.95
0.0808	0.0720	0.0856		8.76

[a] All pH values were measured at the reaction temperature. The pH meter was standardized with standard buffers of pH 4.00, 7.00, and 10.0 at the same temperature.

Buffer Catalysis—The apparent first-order rate constant in a buffer solution is the sum of catalytic contributions from the hydrogen ion, the hydroxide ion, the acidic (HA) and basic (A^-) components of a buffer, and water.

The overall rate constant for reaction in a buffer may be expressed by (20, 21):

$$k_{app} = k_0 + k_{H^+}[H^+] + k_{HA}[HA] + k_{A^-}[A^-] + k_{OH^-}[OH^-] \quad \text{(Eq. 3)}$$

or:

$$k_{app} = k_0 + \left(k_{HA} + \frac{k_{A^-}\,Ka}{[H^+]}\right)[HA] + k_{H^+}[H^+] + k_{OH^-}[OH^-] \quad \text{(Eq. 4)}$$

or:

$$k_{app} = k_0 + k_{H^+}[H^+] + k_{OH^-}[OH^-] + \left(k_{A^-} + \frac{k_{HA}[H^+]}{Ka}\right)[A^-] \quad \text{(Eq. 5)}$$

Thus, a plot of k_{app} *versus* [HA] at a constant pH will be linear with intercept $k_0 + k_{H^+}\,[H^+] + k_{OH^-}\,[OH^-]$ and slope $S_1 = k_{HA} + k_{A^-}\,Ka\,[H^+]$. A plot of k_{app} *versus* $[A^-]$ at a constant pH will give a straight line with intercept $k_0 + k_{H^+}\,[H^+] + k_{OH^-}\,[OH^-]$ and slope $S_2 = k_{HA}\,[H^+]/Ka + k_{A^-}$. A plot of k_{app} *versus* [HA], or k_{app} *versus* $[A^-]$, at two or more pH values will permit the calculation of both catalytic constants, k_{HA} and k_{A^-}.

No significant buffer catalysis was observed in acetate buffer. However, significant buffer catalysis was observed with phosphate and carbonate buffers in the solvolysis of iodocytosine. The apparent first-order rate constants for the reactions studied at different buffer concentrations at 80.0° are given in Table II.

From the plots of k_{app} against $H_2PO_4^-$ and HPO_4^{-2} at constant pH (Fig. 5), the respective slopes of $S_1 = k_{H2PO4^-} + k_{H2PO4^-}\,Ka/[H^+]$ and $S_2 = k_{HPO4^-2} + k_{H2PO4^-2}\,[H^+]/Ka$ were obtained. The catalytic rate constants of $k_{H2PO4^-} = 1.59 \times 10^{-5}$ and $k_{HPO4^-2} = 2.53 \times 10^{-5}$ liter/mole-sec were obtained from the intercepts of S_1 against $1/[H^+]$ and S_2 against $[H^+]$ by least squares on a calculator[8], where the correlation coefficient was 0.993 in each case.

From the plots of k_{app} against $[HCO_3^-]$ and $[CO_3^{-2}]$ at constant pH (Fig. 6), the slopes of $S_1 = k_{HCO3^-} + k_{CO3^-2}Ka/[H^+]$ and $S_2 = k_{CO3^-2} + k_{HCO3^-}\,[H^+]/Ka$ were obtained. The catalytic rate constants of $k_{HCO3^-} = 2.57 \times 10^{-6}$ and $k_{CO3^-2} = 9.73 \times 10^{-6}$ liter/mole sec were obtained from the intercepts of plots of S_1 against $1/[H^+]$ and S_2 against $[H^+]$, respectively.

Log k-pH Profile—The complete log k-pH profiles for the solvolysis of iodocytosine at several temperatures are given in Fig. 7. The pH values of the highly acid and alkaline solutions were calculated from the known concentrations of hydrochloric acid and sodium hydroxide from the pKw and mean activity coefficients obtained or extrapolated from literature data (15).

The rate constants for iodocytosine are independent of pH below pH 2 where the compound is completely protonated. This implies that hydrogen-ion-catalyzed solvolysis of the protonated species is negligible. The decrease of k with increasing pH between 2 and 5 can be explained by a reduction of the fraction of the protonated species attacked by neutral solvent molecules. An alternative explanation is the kinetically equivalent hydrogen-ion attack on the neutral substrate, where the protonated species is stable with respect to deamination. The apparent first-order rate constants of iodocytosine degradation again become pH independent between 5 and 8 and indicate either water attack on the neutral species or its kinetic equivalent, hydroxide-ion attack on the protonated species. Therefore, the log k-pH profiles below pH 7 of iodocytosine were fitted (20, 21):

$$k = f_{H_2C^+}k_{H_2O} + f_{HC}k'_{H_2O} \quad \text{(Eq. 6)}$$

where k_{H2O} and k'_{H2O} are the apparent first-order rate constants (Table III) for water attack on the protonated and neutral species, respectively, and where the fraction of the protonated

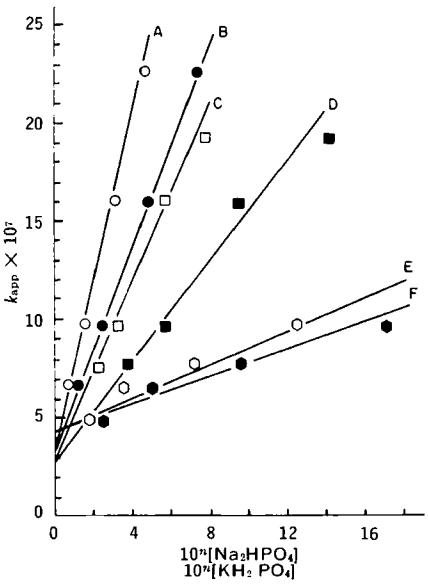


Figure 5—*Plots of apparent first-order rate constants for the solvolysis of 1 × 10^{-4} M iodocytosine at 80° in various concentrations of phosphate buffer at constant pH and 0.4 ionic strength against $[HPO_4^{-2}]$ and $[H_2PO_4^-]$. The slopes, S_1, for plots against $[H_2PO_4^-]$ were: curve A, pH 6.80, S_1 = 4.09 × 10^{-5}; curve C, pH 8.05, S_1 = 2.20 × 10^{-4}; and curve E, pH 5.85, S_1 = 4.29 × 10^{-6}. The slopes, S_2, in liters per mole seconds against $[HPO_4^{-2}]$ were: curve B, pH 6.80, S_2 = 2.64 × 10^{-5}; curve D, pH 8.05, S_2 = 1.30 × 10^{-5}; and curve F, pH 5.85, S_2 = 3.6 × 10^{-5}. The values of n were 2 except in the cases of curves C and F where they were 3.*

[8] Wang 700.

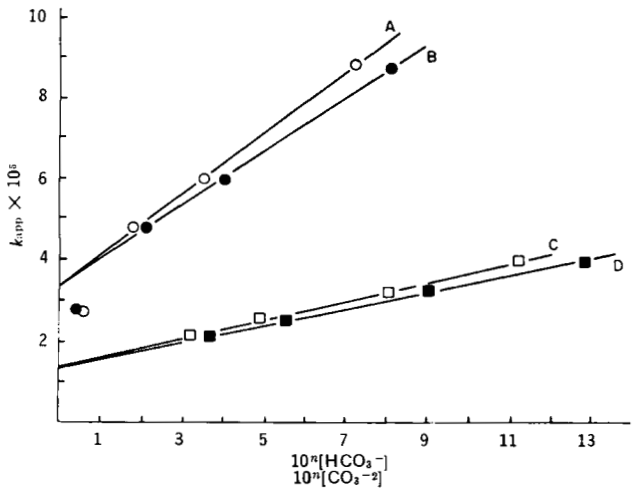


Figure 6—*Plots of apparent first-order rate constants for the solvolysis of 1 × 10⁻⁴ M iodocytosine at 80° in various concentrations of carbonate buffer at constant pH and 0.4 ionic strength against* $[HCO_3^-]$ *and* $[CO_3^{-2}]$. *The slopes,* S_1, *in liters per mole seconds for plots against* $[HCO_3^-]$ *were: curve A, pH 9.5,* $S_1 = 7.50 \times 10^{-5}$; *and curve D, pH 8.9,* $S_1 = 2.07 \times 10^{-5}$. *The slopes,* S_2, *in liters per mole seconds against* $[CO_3^{-2}]$ *were: curve B, pH 9.5,* $S_2 = 6.67 \times 10^{-5}$; *and curve C, pH 8.9,* $S_2 = 2.37 \times 10^{-4}$. *The values of* n *were 2 except in the case of curve C where it was 3.*

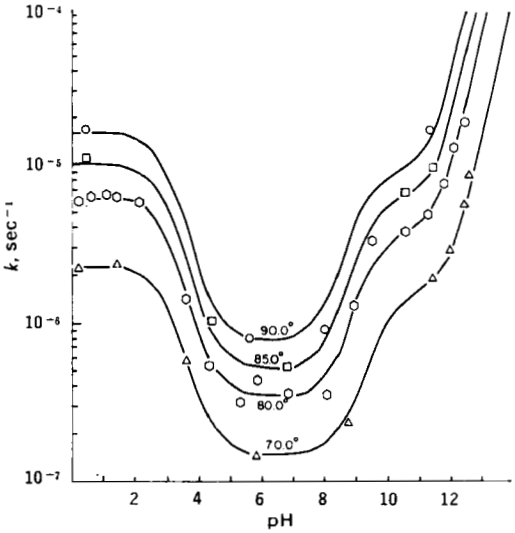


Figure 7—*Log* k–*pH profiles for the solvolysis of iodocytosine at several temperatures, where* k *is the apparent first-order rate constant for deamination to iodouracil below pH 5, for deiodination to cytosine at pH 5–9, and for hydroxylation–deiodination to 6-hydroxycytosine above pH 9.*

species is:

$$f_{H_2C^+} = \frac{[H^+]}{[H^+] + Ka_1} \qquad \text{(Eq. 7)}$$

and the fraction of the neutral species is:

$$f_{HC} = \frac{Ka}{[H^+] + Ka_1} \qquad \text{(Eq. 8)}$$

The kinetic pKa_1 values were estimated from the best fit (19) of the log k–pH curves and were also determined by the half-neutralization technique. The pKa_1 value of 3.0 was almost invariant among the temperatures studied (Table III). At room temperature, the pKa_1 value was determined spectrophotometrically to be 3.32.

The k values for iodocytosine degradation increase with pH values above 7.0. The fact that there is an inflection in the log k–pH plot (Fig. 7) in the 10–12 pH range indicates that a second acid dissociation constant, Ka_2, influences the rate of the reaction. The increase of the apparent first-order rate constant, k, with increasing pH between 8 and 10 can be explained by a hydroxide-ion attack on the neutral species or its kinetic equivalent, the attack of hydroxide ion on the monoionized molecule.

Above pH 11.5, a linear increase of log k with increasing pH to give a slope close to unity indicates specific hydroxide-ion catalysis, which can be explained by hydroxide-ion attack on the monoionized species or the kinetically equivalent water attack on the dianionic species.

The apparent first-order rate constant for iodocytosine in the alkaline region above pH 7 may be formulated (20, 21):

$$k = k_{OH^-}[OH^-]f_{HC} + k'_{OH^-}[OH^-]f_{C^-} + k'_{H_2O}f_{HC} \qquad \text{(Eq. 9)}$$

where f_{HC} is the fraction of neutral species:

$$f_{HC} = \frac{[H^+]}{[H^+] + Ka_2} \qquad \text{(Eq. 10)}$$

and f_{C^-} is the fraction of the nucleoside as the anion in the solution:

$$f_{C^-} = \frac{Ka_2}{[H^+] + Ka_2} \qquad \text{(Eq. 11)}$$

The k_{OH^-}, k'_{OH^-}, and Ka_2 values were determined from the best fit of the alkaline branch of log k–pH plots in accordance with Eq. 9, and the Ka_2 values were also determined by the half-neutralization technique (Table III). A pKa_2 value, obtained by the spectrophotometric method, was 10.59 at 25°.

Since $Kw = [H^+][OH^-]$, the components of the rate constant at any pH value from Eqs. 6–11 are:

$$k = \frac{k_{H_2O}[H^+]}{[H^+] + Ka_1} + \frac{k_{H_2O}Ka_1}{[H^+] + Ka_1} + \frac{k'_{H_2O}[H^+]}{[H^+] + Ka_2} + \frac{k_{OH^-}Kw}{[H^+] + Ka_2} + \frac{k'_{OH^-}Ka_2[OH^-]}{[H^+] + Ka_2} \qquad \text{(Eq. 12)}$$

The rate of production from iodocytosine of iodouracil is characterized by the apparent first-order rate constant given as the first term on the right-hand side of this equation. The next two terms characterize the rate of production of cytosine, and the final terms characterize the rate of production of 6-hydroxycytosine.

Dependence of Rate on Temperature—Estimates of the Arrhenius parameters for the solvolysis of iodocytosine were obtained from the slopes and intercepts of plots of the logarithm of the apparent first-order rate constants, k_{app}, *versus* the reciprocal of the absolute temperatures, T, in accordance with the expres-

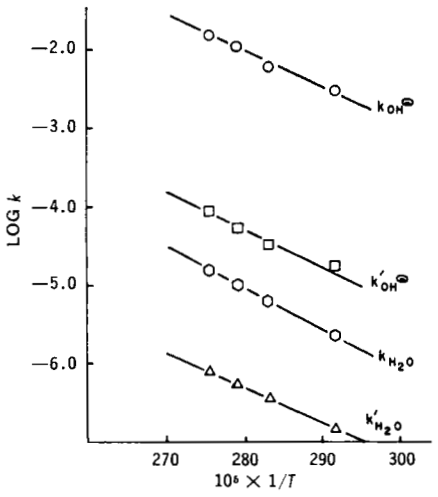


Figure 8—*Arrhenius plots for the catalytic rate constants for the solvolysis of iodocytosine.*

Table III—Microscopic Rate Constants[a] and Arrhenius Parameters for Solvolytic Degradation of Iodocytosine

Temperature	$10^6\ k_{H_2O}$	$10^7\ k'_{H_2O}$	$10^3\ k_{OH^-}$	$10^5\ k'_{OH^-}$	pKa_1[b]	pKa_2[b]
70.0°	2.20	1.40	2.98	1.77	3.0[c] 3.0[d]	9.73[c] 9.88[d]
80.0°	6.20	3.50	6.33	3.29	3.0[c] 3.0[d]	9.40[c] 9.63[d]
85.0°	10.00	5.28	11.00	5.68	3.0[c] 3.0[d]	9.24[c] 9.65[d]
90.0°	16.00	8.02	15.00	9.02	3.0[c] 3.0[d]	9.11[c] 9.55[d]
log P[e]	9.68	6.94	10.52	8.06		
ΔH_a[e], kcal/mole	24.1	21.6	20.5	20.2		
$\Delta S\ddagger$, eu[f]	−14.6	−27.1	−10.6	−22.0		

[a] Based on the kinetic expression for the overall apparent first-order rate constants, k_{app}, for the degradation of iodocytosine:

$$k_{app} = k_{H_2O}f_{H_2C^+} + (k'_{H_2O} + k_{OH^-}[OH^-])f_{HC} + k'_{OH^-}[OH^-]f_{C^-}$$

where k_{H_2O} and k'_{H_2O} are the first-order rate constants for the spontaneous decomposition of the positively and uncharged iodocytosine, respectively; k_{OH^-} is the bimolecular rate constant for the hydroxide-ion-catalyzed reaction of the neutral species; and k'_{OH^-} is for hydroxide-ion-catalyzed reaction of the monoanion of iodocytosine. The $f_{H_2C^+} = [H^+]/([H^+] + Ka_1)$ is the fraction of protonated iodocytosine; $f_{HC} = Ka_1/([H^+] + Ka_1)$ is the fraction of uncharged iodocytosine in the 0–6.5 pH range; $f_{HC} = [H^+]/(Ka_2 + [H^+])$ is the fraction of iodocytosine that is undissociated above pH 6.5; $f_{C^-} = Ka_2/([H^+] + Ka_2)$ is the fraction of iodocytosine that is monoanion at pH values above 9.5. [b] The spectrophotometrically determined values were $pKa_1 = 3.32$ and $pKa_2 = 10.6$ at 25° from the expression $pKa = pH + \log(A - A_1)/(A_2 - A)$, where A is the absorbance at any pH, and A_1 and A_2 are the absorbances of the equilibrating species. [c] Determined from the fitting of the log k–pH data. [d] Determined at the pH of half-neutralization. [e] Where $\log k_i = -\Delta H_a/2.303RT + \log P$. [f] Where $\Delta S\ddagger = [\log P - \log kT/h]\ 2.303R$ at 80°.

sion (20, 21):

$$\log k = \log P - \Delta H_a/2.303RT \qquad \text{(Eq. 13)}$$

where R is 1.987 cal deg^{-1} mole^{-1}. The energies of activation, ΔH_a, at several pH values were (kcal/mole) 24.4 (pH 2.0) and 20.8 (pH 7.0).

The Arrhenius plots for the specific rate constants are shown in Fig. 8. The ΔH_a and log P values obtained from the slope and the intercept of the Arrhenius plots for the specific rate constants are listed in Table III.

The entropies of activation, $\Delta S^{\neq}$, were calculated from the log P values using the equation:

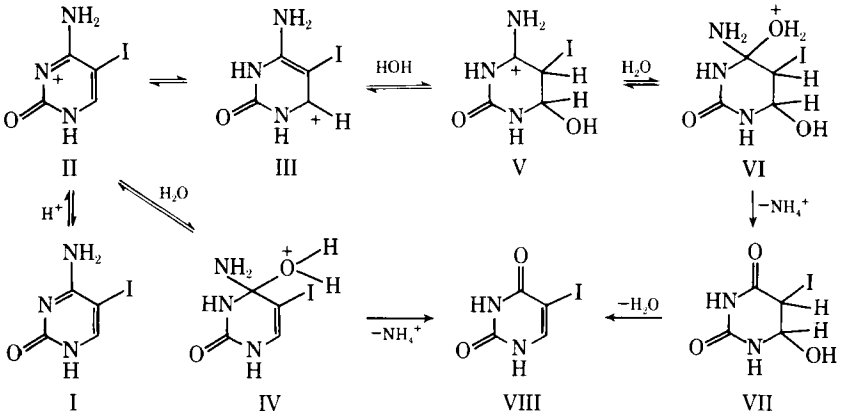
$$\Delta S^{\neq} = 2.303R\left[\log P - \log\left(\frac{kT}{h}\right)\right] \qquad \text{(Eq. 14)}$$

where k is the Boltzmann constant, 1.387×10^{-16} erg deg^{-1}, and h is the Planck constant, 6.623×10^{-27} erg sec. The calculated entropies of activation at 80° are given in Table III.

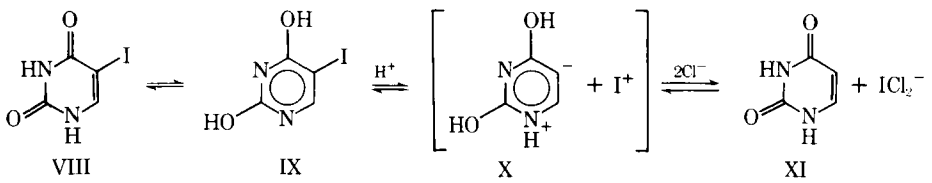
Possible Mechanisms for Solvolytic Transformations of Iodocytosine and Its Solvolytic Products—The mechanisms for the hydrolytic deamination of cytosine and its derivatives in aqueous buffer are explicable (12, 14, 22) by two kinetically equivalent processes (Scheme I). The first is analogous to the hydrolysis of an amide involving nucleophilic displacement of the amino group of the protonated cytosine, II (I → II → IV → VIII); and the second mechanism is by initial N-3 protonation of iodocytosine, I, followed by the saturation of the 5,6-double bond resulting in an electron deficiency at C-4, V, thus facilitating nucleophilic attack by water with subsequent deamination and dehydration to iodouracil (II → III → V → VI → VII → VIII). The electron-withdrawing iodo group at C-5 could help create the electron deficiency at C-4 and subsequent nucleophilic attack by water in both mechanisms. The lack of significant acetate buffer catalysis may favor the first mechanism.

In the acidic pH region, the deamination of iodocytosine occurs to give iodouracil, VIII, which subsequently produces uracil.

The deiodination mechanism of iodouracil in acidic solution was reported previously (3) and is apparently a consequence of the dissociation of iodouracil to uracil and an iodonium ion promoted by protonation of one nitrogen of the pyrimidine ring to permit the formation of an activated complex stabilized by a dipole. Facile rearrangement would give uracil (Scheme II). This is an equilibrium phenomenon, as was shown by the facile production of iodouracil from ICl and uracil in acid solution (3). The complete transformation of iodouracil to uracil occurred only in the presence of acid-degraded deoxyribose (3), which produced 5-methyl-3(2H)-furanone (23), a compound that reduced the iodon-



Scheme I



Scheme II

Table IV—Equilibration of Iodouracil with Uracil in 1 *N* HCl at 80.0°

$[IU]_0$[a]	$[IU]_e$[b]	$[U]_e$[c]	$\frac{[U]_e}{[IU]_e}$[d]	$\frac{[IU]_0 - [IU]_e}{[IU]_0} \times 100 = \%$[e]	$K_d = \frac{[U]_e^2}{[IU]_e}$[f]
4×10^{-5}	1.91×10^{-5}	2.09×10^{-5}	1.09	52.0	2.28×10^{-5}
5×10^{-5}	2.45×10^{-5}	2.58×10^{-5}	1.08	51.5	2.73×10^{-5}
1×10^{-4}	6.16×10^{-5}	3.84×10^{-5}	0.62	38.0	2.39×10^{-5}
1×10^{-3}	8.56×10^{-5}	1.44×10^{-5}	0.17	14.4	2.42×10^{-5}
				Average	2.46×10^{-5}

[a] Initial concentration of iodouracil. [b] Equilibrium concentration of iodouracil. [c] Equilibrium concentration of uracil. [d] Ratio at equilibrium of uracil to iodouracil. [e] Percent transformation of iodouracil at equilibrium. [f] Apparent dissociation constants of iodouracil may be based upon the equilibrium established in hydrochloric acid solution:

$$IU + 2Cl^- \rightleftarrows U + ICl_2^-$$

where:

$$K = [U]_e[ICl_2^-]/[IU]_e[Cl^-]^2 = [U]_e^2/[IU]_e$$

where $[ICl_2^-] = [U]_e$ and $[Cl^-] = 1.0$ in 1.0 *M* HCl.

ium ion and iodine to iodide ion and so displaced the equilibrium given in Scheme II.

These previous studies (3) were conducted at 1×10^{-3} *M* iodouracil in 1 *M* HCl at 80°, and 14.4% of iodouracil had formed the uracil product at equilibrium. In the present studies, when a 1×10^{-4} *M* concentration of iodouracil was reacted in 1 *M* HCl at 80°, 38% was transformed to uracil at equilibrium; at 5×10^{-5} *M*, it was 50%. Neither iodocytosine nor any of its degradation products, including ammonium ion, acted as a reducing agent, such as deoxyribose, to complete the transformation of iodouracil to uracil. Nor did these compounds change significantly the equilibrium between iodouracil and uracil in 1 *M* HCl solution.

However, it is apparent (Table IV) that an increase in the concentration of iodouracil in 1 *N* HCl decreases the ratio of uracil, U, to iodouracil, IU, at equilibrium. The following equilibria have been postulated (3, 24, 25) in hydrochloric acid solutions:

$$IU \rightleftarrows U + I^+$$
$$I^+ + Cl^- \rightleftarrows ICl$$
$$ICl + Cl^- \rightleftarrows ICl_2^-$$

so that the overall expression for iodouracil dissociation can be given by the sum of these equilibria:

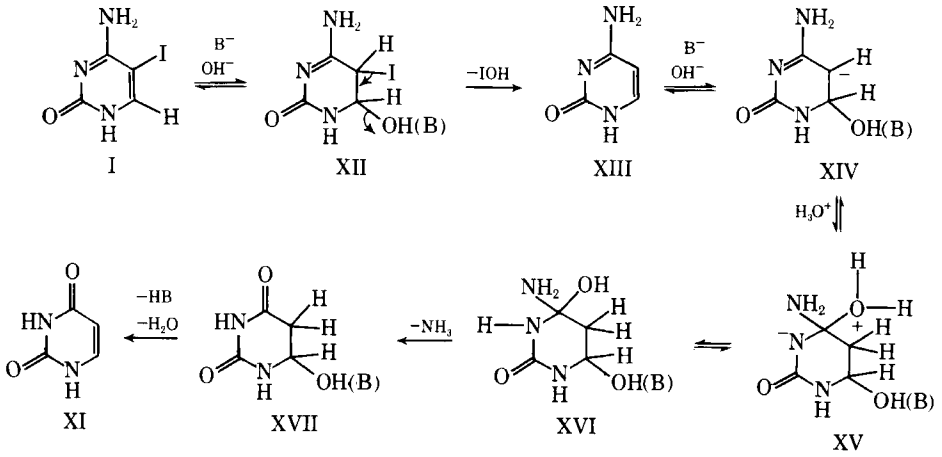
$$IU + 2Cl^- \rightleftarrows ICl_2^- + U$$

with an equilibrium constant of:

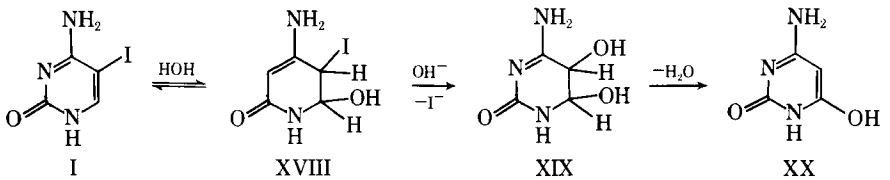
$$K = \frac{[U][ICl_2^-]}{[IU][Cl^-]^2} = \frac{[U]^2}{[IU][Cl^-]^2} \qquad \text{(Eq. 15)}$$

The premise that $[U] = [ICl_2^-]$ is based on Philbrick's (24) report that the concentration of iodine cation released in concentrated hydrochloric acid must be negligible and must be in the form of the complex anion, ICl_2^-. It also follows that, at 1.0 *M* chloride ion, the apparent dissociation constant of iodouracil is the ratio of the square of the uracil concentration to the iodouracil concentration at equilibrium. The constancy of the dissociation constant at 80.0° calculated in Table IV for various iodouracil concentrations is consistent with these relationships and the average value of K is 2.46×10^{-5} liter/mole.

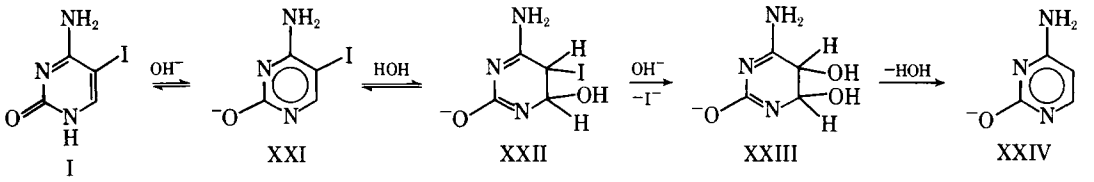
It follows from Eq. 15 that the ratio of uracil to iodouracil at equilibrium will increase at higher hydrochloric acid concentrations and decrease at higher initial concentrations of iodouracil, $[IU]_0$ (Table IV).



Scheme III



Scheme IV



Scheme V

The deiodination of iodocytosine in the 5–9 pH range produces cytosine, which then deaminates to uracil. Neutral iodocytosine could undergo a general base-catalyzed deiodination by saturation of the 5,6-double bond, XII, with subsequent regeneration of cytosine, XIII, after the elimination of hypoiodous acid, which may disproportionate to iodide ion and periodate (Scheme III). This mechanism can be rationalized with the significant phosphate and carbonate buffer catalysis observed in this pH region. The rate-determining hydroxide-ion attack on the protonated species (at N-3) is kinetically equivalent to water attack on the neutral species and can rationalize the rate constant k'_{H2O} in Eq. 6. The deamination mechanism of cytosine has been discussed previously (12, 14). This facile deamination reaction can also be explained in terms of an addition–elimination mechanism (Scheme III).

Above pH 9, the reaction intermediate isolated by column chromatographic separation was 6-hydroxycytosine. Since the product of alkaline-degraded cytosine was uracil (14), it is probable that the 6-hydroxycytosine, XX, was produced concomitantly with the deiodination process. It can be explained by saturation of the 5,6-double bond, XVIII or XXII, followed by base-catalyzed substitution of iodide ion, XIX or XXIII, with subsequent regeneration of the 5,6-double bond to give 6-hydroxycytosine, XX or XXIV (Schemes IV and V). It is possible that the final dehydration (XIX → XX, XXIII → XXIV) produced some 5-hydroxycytosine, which degraded too fast to be isolable (19).

The degradation of the 6-hydroxycytosine in alkaline range could be to barbituric acid (19) which undergoes rapid alkaline destruction of the chromophoric ring structure. This may have been indicated by the IR after concentration of degraded material separated by column chromatography, although it could have been a consequence of catalysis by H^+ (17) from the cationic-exchange resin. TLC and spectral monitoring of the reactions did not give any positive evidence. However, the difficulties inherent in the detection of barbituric acid are understandable since the rate of degradation of barbituric acid in alkali (19) is about 100–200 times faster than its probable rate of formation from 6-hydroxycytosine (Table V).

Table V—Apparent First-Order Rate Constants (in Seconds^{-1}) for the Degradation of 6-Hydroxycytosine and Barbituric Acid in Alkali at 80.0°

[NaOH]	pH	k^a Barbituric Acid	k^b 6-Hydroxycytosine
0.01	10.56	1.79×10^{-5}	6.68×10^{-8}
0.05	11.23	6.43×10^{-5}	2.59×10^{-7}
0.20	11.78	1.00×10^{-4}	6.68×10^{-7}
0.40	12.05	1.19×10^{-4}	8.02×10^{-7}

[a] Reported (19) values for apparent first-order rate constant of barbituric acid at 80°. [b] The final spectral readings, A_∞, for studies on the solvolysis of 6-hydroxycytosine in alkali were perturbed by the formation of cloudy silicate precipitates. The rate constants for 6-hydroxycytosine degradation in alkali were calculated under the assumption that the A_∞ is zero at 265 nm.

REFERENCES

(1) A. P. Mathias, G. A. Fischer, and W. H. Prusoff, *Biochim. Biophys. Acta*, **36**, 560(1959).

(2) R. Papac, E. Jacobs, C. Wong, W. Silliphant, and D. A. Wood, *Proc. Amer. Ass. Cancer Res.*, **3**, 257(1961).

(3) E. R. Garrett, T. Suzuki, and D. J. Weber, *J. Amer. Chem. Soc.*, **86**, 4460(1964).

(4) E. R. Garrett, H. J. Nestler, and A. Somodi, *J. Org. Chem.*, **33**, 3460(1968).

(5) E. R. Garrett, P. B. Chemburkar, and T. Suzuki, *Chem. Pharm. Bull.*, **13**, 1113(1965).

(6) E. R. Garrett and L. J. Hanka, *J. Amer. Pharm. Ass., Sci. Ed.*, **49**, 526(1960).

(7) E. R. Garrett, *J. Amer. Chem. Soc.*, **82**, 827(1960).

(8) E. R. Garrett, J. K. Seydel, and A. J. Sharpen, *J. Org. Chem.*, **31**, 2219(1966).

(9) E. R. Garrett and G. J. Yakatan, *J. Pharm. Sci.*, **57**, 1478(1968).

(10) E. R. Garrett and P. J. Mehta, *J. Amer. Chem. Soc.*, **94**, 8532(1972).

(11) *Ibid.*, **94**, 8542(1972).

(12) R. E. Notari, *J. Pharm. Sci.*, **56**, 804(1967).

(13) R. E. Notari, M. L. Chin, and R. Wittebort, *ibid.*, **61**, 1189(1972).

(14) E. R. Garrett and J. Tsau, *ibid.*, **61**, 1052(1972).

(15) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold, New York, N.Y., 1958.

(16) The Sadtler Research Laboratories, Philadelphia, Pa., Standard IR spectral chart #24040.

(17) A. P. Phillips and J. Mentha, *J. Amer. Chem. Soc.*, **76**, 6200(1954).

(18) G. B. Barlin and W. Pfleiderer, *J. Chem. Soc. B*, **1971**, 1425.

(19) E. R. Garrett, J. T. Bojarski, and G. J. Yakatan, *J. Pharm. Sci.*, **60**, 1145(1971).

(20) E. R. Garrett, *ibid.*, **51**, 811(1962).

(21) E. R. Garrett, in "Advances in Pharmaceutical Sciences," vol. II, Academic, New York, N.Y., 1967, pp. 1–94.

(22) R. Shapiro and R. S. Klein, *Biochemistry*, **5**, 2358(1966).

(23) J. K. Seydel, E. R. Garrett, W. Diller, and K. J. Schaper, *J. Pharm. Sci.*, **56**, 858(1967).

(24) F. A. Philbrick, *J. Amer. Chem. Soc.*, **56**, 1257(1934).

(25) J. H. Faull, Jr., *ibid.*, **56**, 522(1934).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 19, 1973, from the *College of Pharmacy, University of Florida, Gainesville, FL 32610*

Accepted for publication January 17, 1974.

* Present address: Institute of Chemistry and Analytics, Medical Academy, Poznan, Poland.

‡ Present address: Midwest Research Institute, Kansas City, Mo.

x To whom inquiries should be directed.