The Alkaline Hydrolysis of the Dihydropyrimidines[1]

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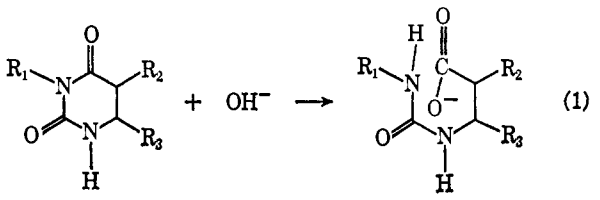
Abstract: The kinetics of the alkaline hydrolysis of the 5,6-dihydro derivatives of uracil (DHU), orotic acid (DHOA) thymine (DHT), and 3-methyluracil (3MeDHU) were studied as a function of OH^- concentration. Values of pK_a for the dissociation of the proton on the 3 position on the dihydropyrimidine ring at 25° and ionic strength 1.0 *M* are 11.66, 11.46, and 11.84 for DHU, DHOA, and DHT, respectively. The observed rate law for dihydropyrimidine (I) hydrolysis at low OH^- concentration is $d[I]/dt = k_{obsd}[I] = (k_1[OH^-] + k_2[OH^-]^2)[I]$ indicating a second-order dependence on the concentration of OH^-. With 3MeDHU, the rate changes from a second- to a first-order dependence with increasing OH^- concentration, while under the same conditions the rates of DHU, DHOA, and DHT hydrolysis become independent of OH^- concentration. The results are consistent with a multistep reaction mechanism involving the formation and decomposition of a tetrahedral addition intermediate (II) similar to that proposed for the alkaline hydrolysis of esters and amides. At lower OH^- concentration, it is proposed that the OH^- and H_2O catalyzed expulsion of the leaving group is rate determining while at higher OH^- concentration the nucleophilic attack of OH^- on the dihydropyrimidine (or H_2O attack on the dihydropyrimidine anion) becomes rate determining.

The 5,6-dihydropyrimidines are a biochemically important class of compounds as they are structural components of alanine transfer RNA[2] and are on the pathway for the biosynthesis and degradation of the pyrimidines.[3] Because of our interest in the mechanism of action of dihydroorotase (5,6-L-dihydroorotate amidohydrolase, EC 3.5.2.3), the enzyme responsible for the reversible cyclization of L-ureidosuccinic acid to L-dihydroorotic acid, we have studied the kinetics of the alkaline hydrolysis of the 5,6-dihydro derivatives of uracil (DHU), thymine (DHT), orotic acid (DHOA), and 3-methyldihydrouracil (3MeDHU) hoping to gain some insight about the more complicated enzymatic reactions.

Little is known about the alkaline hydrolysis of the dihydropyrimidines. Batt, *et al.*,[4] reported that the dihydropyrimidines were hydrolyzed to the corresponding ureido acids in 0.1 *N* NaOH (eq 1). Similar studies have been reported by Green and Cohen[5] and Janion and Shugar.[6] More recently, Pojarlieff, *et al.*,[7] have reported studies on the reversible cyclization of ureidopropionic acid to yield dihydrouracil and the alkaline hydrolysis of *cis*- and *trans*-5,6-tetramethylenedihydrouracil.



I

(1)

DHU, $R_1 = H$; $R_2 = H$; $R_3 = H$
DHT, $R_1 = H$; $R_2 = CH_3$; $R_3 = H$
DHOA, $R_1 = H$; $R_2 = H$; $R_3 = COOH$
3MeDHU, $R_1 = CH_3$; $R_2 = H$; $R_3 = H$

Experimental Section

Materials. Reagent grade inorganic salts were used without further purification. Boiled glass distilled water was used in all experiments. The 5,6-dihydro derivatives of uracil, orotic acid, and thymine were obtained from Sigma Chemical Co. and were used without further purification. The method of Hotchkiss and Johnson[8] was used to synthesize 3-methyl-5,6-dihydrouracil which after repeated recrystallization from 95% ethanol and drying under vacuum over P_2O_5 melted at 128–129° uncorrected (lit.[8] 129.5–131.0° cor). Eastman piperidine was recrystallized several times as the hydrochloride to remove impurities which absorb strongly in the ultraviolet. Tris(hydroxymethyl)aminomethane (Tris Ultra Pure) and L-arginine monohydrochloride were obtained from Mann Research Laboratories and were used without further purification. D_2O (99.7%) was obtained from New England Nuclear Corporation and was glass distilled before use.

Spectra. The ultraviolet absorption spectra of dihydrouracil, dihydroorotic acid, dihydrothymine, and 3-methyldihydrouracil were determined in both acid and base with a Cary 14 recording spectrophotometer using a 1-cm light path at room temperature.

Determinations of Acid Dissociation Constants. The acid dissociation constants of the proton on three positions of dihydrouracil, dihydroorotic acid, and dihydrothymine were determined spectrophotometrically taking advantage of the differences in the absorbance of the protonated and anionic forms at 230 mμ. The absorbance of 1×10^{-4} *M* solutions of each of these dihydropyrimidines was measured against a reagent blank with a Zeiss PMQII spectrophotometer which was equipped with a brass cell holder thermostated at 25°. Determinations of pH were made at 25° with a Radiometer PHM 4c pH meter equipped with a Sargent S-30070-10 combination electrode. Above pH 11.54 the absorbance was measured in dilute KOH. Below this pH, tris(hydroxymethyl)aminomethane and piperidine buffers were employed. Ionic strength was maintained at 1.0 *M* by the addition of KCl. At the higher pH values where hydrolysis occurs the zero time ab-

(1) Supported in part by a grant from the National Institute of Cancer (No. CA 10847) and Public Health Service general research support grant to the University of Florida College of Medicine (No. 5-SO1-FR05363-06).

(2) R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, and A. Zamir, *Science*, **147**, 1462 (1965).

(3) P. Reichard, *Advan. Enzymol.*, **21**, 263 (1959); G. W. Crosbie, "The Nucleic Acids," Vol. III, Academic Press, New York, N. Y., 1960, p 323.

(4) R. D. Batt, J. K. Martin, J. McT. Ploeser, and J. Murray, *J. Am. Chem. Soc.*, **76**, 3663 (1954).

(5) M. Green and S. S. Cohen, *J. Biol. Chem.*, **225**, 397 (1957).

(6) C. Janion and D. Shugar, *Acta Biochim. Polonica*, **7**, 294 (1960).

(7) I. G. Pojarlieff, *Tetrahedron*, **23**, 4307 (1967); I. G. Pojarlieff, R. Z. Mitova-Chernaeva, I. Blagoeva, and B. J. Kourtev, *Compt. Rend. Acad. Bulgare Sci.*, **21**, 131 (1968).

(8) R. D. Hotchkiss and T. B. Johnson, *J. Am. Chem. Soc.*, **58**, 525 (1936).

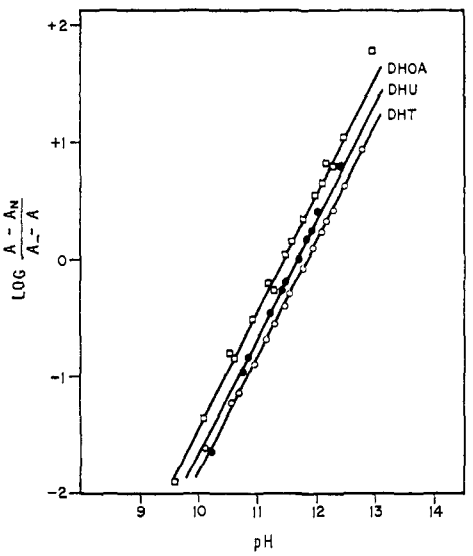


Figure 1. Results of the spectrophotometric titrations of DHU, DHOA, and DHT at 25° and ionic strength 1.0*M*.

sorbancies of the reaction mixtures were determined by extrapolating plots of log $(A_t - A_\infty)$ against time.

Determination of Rate Constants. The kinetics of dihydropyrimidine hydrolysis were studied under pseudo-first-order conditions at 25° and ionic strength 1.0 *M* by following the decrease in absorbance at 230 mμ which occurs when 3-ml reaction mixtures in stoppered cuvettes containing various concentrations of OH^- are made approximately 1×10^{-4} *M* in dihydropyrimidine. Absorbance measurements were made on either a Zeiss PMQII or a Gilford 2000 spectrophotometer both of which were equipped with cell holders thermostated at 25°. The pseudo-first-order rate constants were obtained from linear semilogarithmic plots of extent of the reaction, $A_t - A_\infty$, against time and the relationship $k_{obsd} = 0.693/t_{1/2}$. Above pH 12.0 the concentration of OH^- was taken to be the amount of standardized potassium hydroxide added. Below this pH, where buffers were employed to control pH, OH^- concentration was determined using the pH meter previously described and a semilogarithmic plot of OH^- concentration against pH. The validity of this empirical relationship was determined experimentally several times during the course of these studies by measuring at 25° the pH of carefully standardized KOH solutions maintained at ionic strength 1.0 *M* by the addition of KCl. Linear plots were obtained in all cases between pH 11.13 and 12.75. Beyond these limits, deviations from linearity were small.

Determinations of Rate Constants in D_2O. The pseudo-first-order rate constants for 3-methyldihydrouracil hydrolysis were measured in D_2O at 25° and ionic strength 1.0 *M*. All solid reagents were prepared in freshly distilled D_2O with the exception of one of the stock KOH solutions which was prepared by diluting 0.5 ml of carefully standardized 3.935 *M* KOH to 10 ml with D_2O which after equilibration resulted in a 0.197 *M* stock solution of OD^- containing 95% D_2O. Above an observed pH of 12.0 the concentration of OD^- was determined directly from the amount of stock KOD solution added to the reaction mixtures. Below this pH, OD^- concentration was determined in the same manner as was OH^- concentration using values of observed pH and a linear semilogarithmic plot of OD^- concentration against observed pH.

Results

Spectra. The ultraviolet absorption spectra of the dihydropyrimidines unsubstituted at the 3 position (DHU, DHOA, and DHT) are different when determined in either dilute acid or base. In dilute OH^- definite maxima are observed at 230 mμ. These maxima are not seen in dilute HCl. In the case of 3MeDHU, the absorption spectra in dilute acid and base are identical after small corrections for the loss in absorbance due to hydrolysis in base are made. These spectra are similar in shape to the spectra of DHU, DHOA, and DHT in acid, showing no maxima at 230 mμ. Thus the absorbance maxima at 230 mμ for the unsubstituted dihydropyrimidines in base can be explained by the dissociation of the proton at the 3 position of the dihydropyrimidine to yield the dihydropyrimidine anion. Similar conclusions were reached by Janion and Shugar[6] who also recorded the spectra of DHU and 3MeDHU in base.

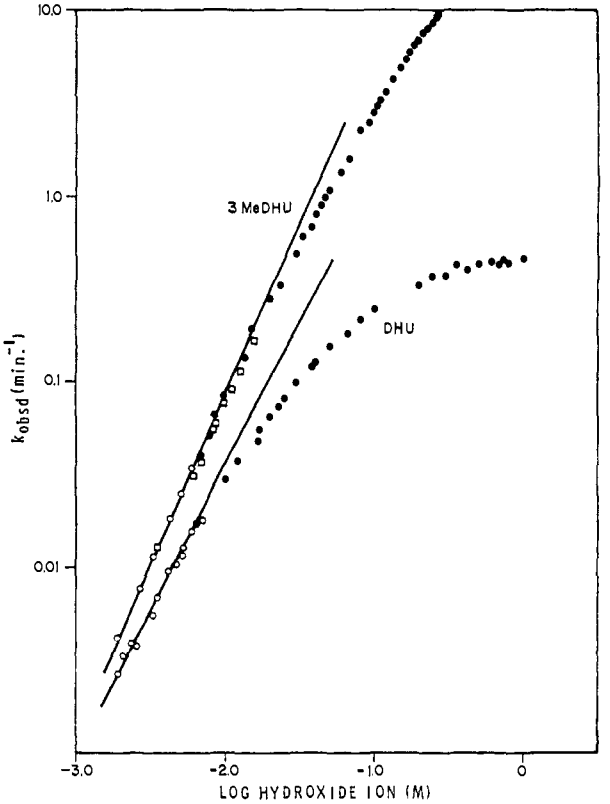


Figure 2. Logarithmic plot of k_{obsd} against OH^- concentration for DHU and 3MeDHU hydrolysis at 25°, ionic strength 1.0 *M*: ●, KOH; ○, 0.02 *M* piperidine buffers; □, 0.02 *M* arginine buffers. Solid lines calculated from eq 2 using values of k_1 and k_2 from Table I.

Acid Dissociation Constants. The results of the spectrophotometric titrations of dihydrouracil, dihydroorotic acid, and dihydrothymine are shown in Figure 1. The data, plotted according to the method of Stenström and Goldsmith,[9] were linear between 10 and 90% dihydropyrimidine anion. The pK_a values for the dissociation of the proton at the 3 position of the unsubstituted dihydropyrimidines were determined from the pH at log $[(A - A_N)/(A_- - A)]$ equal to zero where A_N, A_-, and A represent the absorbance at 230 mμ of equimolar concentrations of the neutral, the anionic, and mixtures of these two species, respectively. Values of pK_a at 25° and ionic strength 1.0 *M* were 11.66, 11.46, and 11.84 for dihydrouracil, dihydroorotic acid, and dihydrothymine, respectively. The values are in agreement with the results of Janion and Shugar[6] who estimated that the values of pK_a for several dihydrouracil derivatives were well above 11.

Rate Constants. The pseudo-first-order rate constants for dihydropyrimidine hydrolysis were determined from linear semilogarithmic plots of extent reaction, $A_t - A_\infty$, against time. These semilogarithmic

(9) W. Stenström and N. Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).

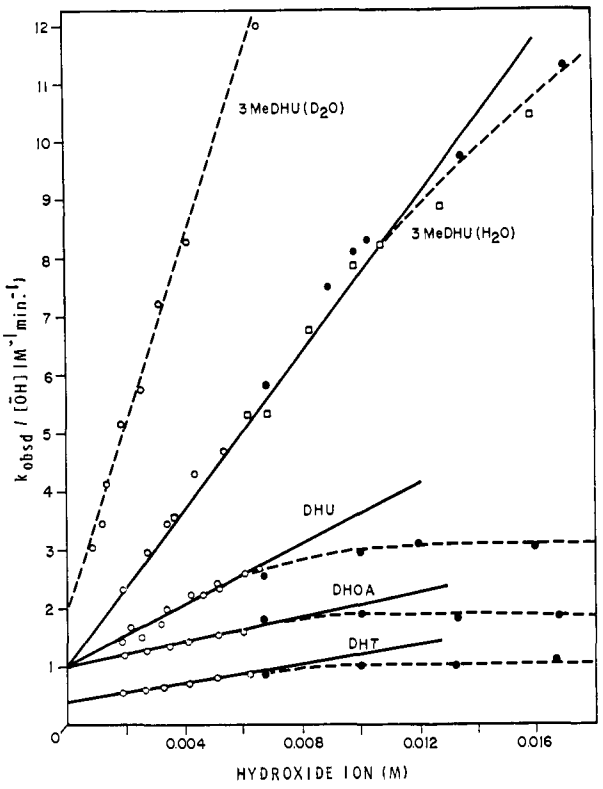


Figure 3. Dependence of the second-order rates constants for DHU, DHOA, DHT, and 3MeDHU (in H_2O and D_2O) hydrolysis on OH^- concentration at 25° and ionic strength 1.0 *M*: ●, KOH; ○, 0.02 *M* piperidine buffers; □, arginine buffers. The lines were determined by the method of least squares. Slopes and intercepts represent k_2 and k_1, respectively.

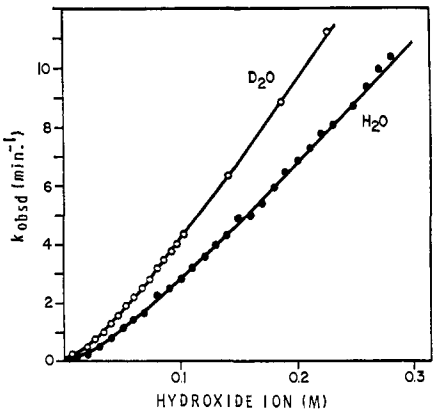


Figure 4. Dependence of the observed rate constant for 3MeDHU hydrolysis on OH^- concentration at 25°, ionic strength 1.0 *M* in both H_2O (●) and D_2O (○). Solid lines calculated from the steady-state equation (eq 4) and values of k_a, k_1, and k_2 (Table I).

plots were linear indicating that the reactions follow pseudo-first-order kinetics for at least five half-lives. In some of the slower reactions at low OH^- concentrations some small deviations from linearity were observed beyond three half-lives.

The results of plotting the logarithms of the observed pseudo-first-order rate constants against the logarithms of the OH^- concentrations for 3-methyldihydrouracil and dihydrouracil hydrolysis are shown in Figure 2. Both of these plots show a linear region at the lower OH^- concentrations; however, the slopes of these lines are greater than unity indicating a greater than first-order dependence on the concentration of OH^-. Plots of $k_{obsd}/[OH^-]$ against OH^- concentration are linear between 0.002 *M* and 0.008 *M* OH^- for 3-methyldihydrouracil both in H_2O and D_2O, dihydrouracil, dihydroorotic acid, and dihydrothymine (Figure 3). This indicates that in this region of OH^- concentration the rate law for dihydropyrimidine (I) hydrolysis can be expressed by eq 2. Similar results have been found for the alkaline hydrolysis of the N-methylanilides.[10] Table I shows the values of k_1 and k_2 obtained from the intercepts and slopes, respectively, of the least-square lines from Figure 3 for each of the dihydropyrimidines tested. The lines in Figure 2 were calculated from eq 2 using these rate constants for dihydrouracil and 3-methyldihydrouracil hydrolysis and give a good fit to the data at low OH^- concentration.

$$-\frac{d[I]}{dt} = k_{obsd}[I] = (k_1[OH^-] + k_2[OH^-]^2)[I] \quad (2)$$

(10) S. S. Biechler and R. W. Taft, Jr., *J. Am. Chem. Soc.*, 79, 4927 (1957).

Table I. Second- and Third-Order Rate Constants for the Hydrolysis of Dihydropyrimidines at 25°, Ionic Strength 1.0 *M*

Dihydropyrimidine	k_a, M^{-1} min^{-1}	k_1, M^{-1} min^{-1}	k_2, M^{-2} min^{-1}
Dihydrothymine		0.40	80
Dihydroorotic acid		1.00	106
Dihydrouracil		1.02	260
3-Methyldihydrouracil (H_2O)	43	1.06	717
3-Methyldihydrouracil (D_2O)	55	1.90	1603

At the higher concentrations of OH^-, the rates of dihydrouracil, dihydroorotic acid, and dihydrothymine hydrolysis level until k_{obsd} is independent of OH^- concentration (Figure 2). This observation was confirmed at much higher concentrations of OH^- by showing that the rate of dihydrouracil hydrolysis does not change between 0.46 and 3.0 *M* OH^- at 25°, ionic strength 3.0 *M* (Table II). Similar results have been reported for the alkaline hydrolysis of 2,2,2-trifluroacetanilide, a compound which also has a dissociable proton (pK_a 9.5) on the nitrogen atom involved in bond cleavage.[11]

Table II. Hydrolysis of Dihydrouracil in Hydroxide Ion at 25°, Ionic Strength 3.0 *M*

Hydroxide ion, *M*	k_{obsd}, min^{-1}
0.460	0.325
0.996	0.327
1.51	0.326
2.03	0.325
2.55	0.322
3.00	0.318

In the case of 3-methyldihydrouracil there is a leveling in rate at the higher concentrations of OH^-; however, it is not as pronounced as in the case of dihydropyrimidines unsubstituted at the 3 position. Instead the rate levels with increasing OH^-, until between 0.13 and

(11) P. M. Mader, *ibid.*, 87, 3191 (1965).

Table III. Effect of Buffer Concentration and Ionic Strength on the Rate of Dihydrouracil and 3-Methyldihydrouracil Hydrolysis at 25°

Solvent	pH	Ionic Strength,[a] M	k_{obsd}, min^{-1}
	Dihydrouracil		
0.20 piperidine, 92.7% free base	12.20	1.00	0.054
0.30 M	12.32	1.00	0.060
0.40 M	12.36	1.00	0.062
0.20 M piperidine, 71.6% free base	11.81	1.00	0.022
0.30 M	11.83	1.00	0.022
0.40 M	11.82	1.00	0.023
0.05 M OH^-		0.05	0.126
		1.00	0.140
		3.40	0.130
0.10 M OH^-		0.10	0.234
		1.00	0.239
		2.00	0.215
		3.00	0.206
1.02 M OH^-		1.02	0.452
		2.35	0.380
		4.02	0.302
	3-Methyldihydrouracil		
0.02 M piperidine, 70% free base	11.18	0.02	0.0056 (0.0056)[b]
	11.43	1.00	0.0175 (0.0057)[b]
	11.53	2.00	0.0205 (0.0054)[b]
0.1 M OH^-		0.10	3.04
		1.00	2.75
		2.00	2.42

[a] Varied by the addition of potassium chloride. [b] Corrected to pH 11.18 from the pH–rate profile; corrections represent small differences between larger numbers and hence are only approximate.

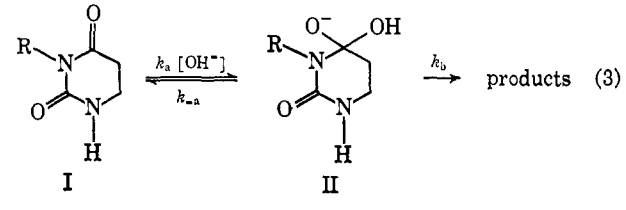
0.26 M OH^- the slope of the plot of log k_{obsd} against log $[OH^-]$ is essentially 1.0 (Figure 2), indicating that in this region the reaction has a simple first-order dependence on OH^- concentration. This behavior can be seen more readily when k_{obsd} is plotted against the first power of OH^- concentration (Figure 4). This plot shows that at the higher OH^- concentrations linearity is achieved. From the slope of the line in this linear region, k_a, the rate constant for the attack of OH^- on 3-methyldihydrouracil, was determined. Values of k_a for 3-methyldihydrouracil hydrolysis in H_2O and D_2O are reported in Table I. This change in kinetic behavior indicates that the rate of 3-methyldihydrouracil hydrolysis changes from a second- to a first-order dependence with increasing OH^- concentration.

The effects of varying ionic strength and increasing piperidine buffer concentration on the observed rate constants for dihydrouracil and 3-methyldihydrouracil hydrolysis are summarized in Table III. The rates of both dihydrouracil and 3-methyldihydrouracil hydrolysis show little or no effect of increasing salt concentration at the lower concentrations of OH^-; however, at higher concentrations of OH^- a decrease in k_{obsd} with increasing ionic strength was observed. The experiments with increasing piperidine buffer concentration at two different buffer ratios indicate that the buffers, used at the lower OH^- concentrations to control pH, do not catalyze the reaction.

Rate Constants in Deuterium Oxide. The rate behavior of 3-methyldihydrouracil hydrolysis in D_2O over a range of OD^- concentrations is shown in Figures 3 and 4. As in the case of the reaction in H_2O, the rate constants change from a second- to a first-order dependence with increasing OD^- concentration. Values of k_a, k_1, and k_2 in D_2O were determined in a manner analogous to the values found for the reaction in H_2O. These values are reported in Table I. The ratios $k_a^{H_2O}/k_a^{D_2O}$, $k_1^{H_2O}/k_1^{D_2O}$, and $k_2^{H_2O}/k_2^{D_2O}$ are 0.78, 0.56, and 0.45, respectively. The fact that $k_a^{D_2O}$ was greater than $k_a^{H_2O}$ might be expected since OD^- is a stronger base than OH^-;[12] however, the ratios involving k_1 and k_2 would indicate that there has been no loss of zero-point energy due to a proton being transferred in the transition state of the rate-determining reaction at low OH^- concentrations.

Discussion

The results show that the rate of 3-methyldihydrouracil hydrolysis changes from a second- to a first-order dependence with increasing OH^- concentration. This change in the order of the reaction with respect to OH^- cannot be explained by the dissociation of a proton on dihydropyrimidines which are substituted at the 3 position and hence indicates a multistep reaction pathway in which the rate-determining step changes with increasing OH^-. Such evidence has been previously interpreted to implicate the formation of a tetrahedral addition intermediate between a nucleophilic reagent and an acyl compound which then reacts further to expel the leaving group with the formation of products.[13] Such a reaction pathway for the alkaline hydrolysis of the dihydropyrimidines substituted at the 3 position is shown in eq 3. In this pathway k_b is equal to k_{obsd} when the decomposition of the tetra-



(12) L. Pentz and E. R. Thornton, *J. Am. Chem. Soc.*, **89**, 6931 (1967).

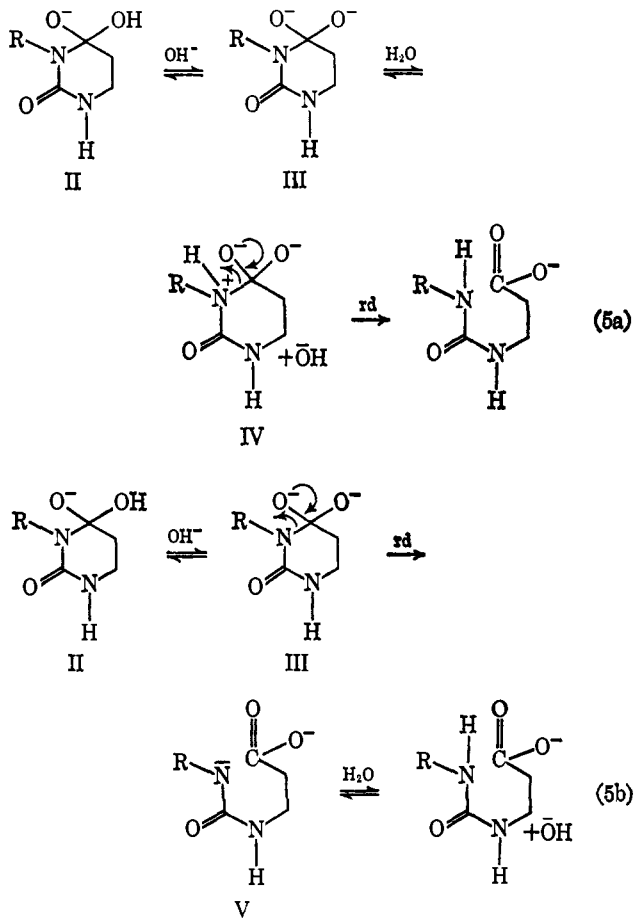
(13) W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964); T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., New York, N. Y., 1966; S. L. Johnson, *Advan. Phys. Org. Chem.*, **5**, 237 (1967).

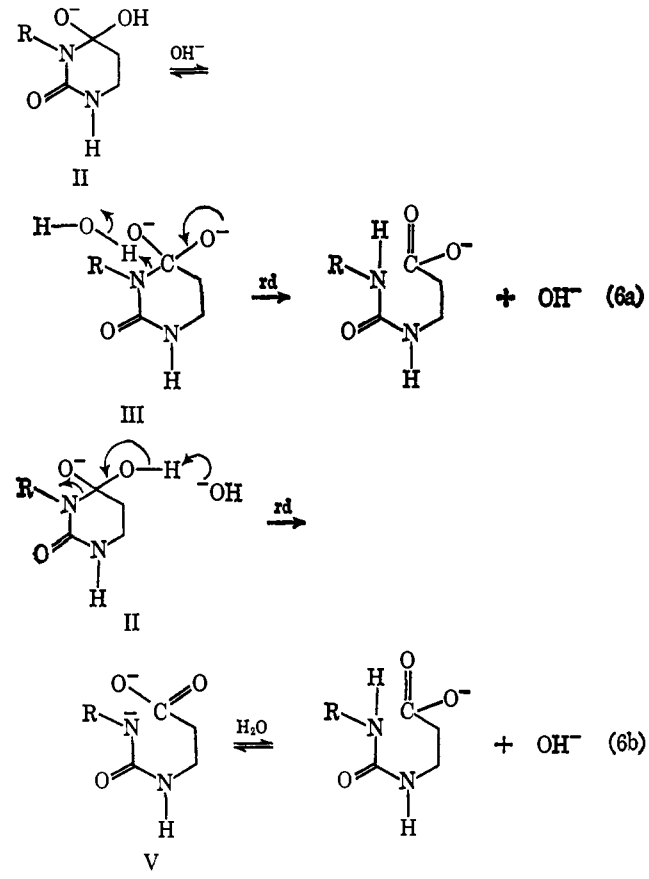
hedral addition intermediate (II) to yield products is rate determining. Under these conditions the concentration of II is in equilibrium with the concentration of I and $K_{eq} = k_a/k_{-a} = [II]/[I][OH^-]$. Thus when eq 2 is rewritten to reflect the equilibrium concentration of II, $k_b = k_1(k_{-a}/k_a) + k_2(k_{-a}/k_a)[OH^-]$. The combination of this latter relationship along with the assumption that the concentration of II is in the steady state results in eq 4. The lines in Figure 4, calculated using this steady-state rate equation and the values of k_a, k_1, and

$$-\frac{d[I]}{dt} = k_{obsd}[I] = [I]\left\{[OH^-]\frac{k_a(k_1 + k_2[OH^-])}{k_a + k_1 + k_2[OH^-]}\right\} \quad (4)$$

k_2 from Table I, show an excellent fit with the experimentally observed pseudo-first-order rate constants.

For the alkaline hydrolysis of all of the dihydropyrimidines tested, it would appear that the second step of the reaction which represents the breakdown of the tetrahedral intermediate to yield products is rate determining at the lower concentrations of OH^-. This step of the reaction is catalyzed by both OH^- and H_2O as evidenced by the fact that eq 2 describes the values of k_{obsd} in this region of OH^- concentration. This catalysis can be explained by either specific acid-base catalyzed mechanisms (eq 5a and 5b) or general acid-base catalyzed mechanisms (eq 6a and 6b). The specific acid-base catalyzed mechanisms (eq 5a and 5b) both involve the rate-determining collapse of a dianionic species preceded by either proton donation by H_2O or proton abstraction by OH^-, whereas the general acid-base catalyzed mechanisms (eq 6a and 6b) involve either rate-determining proton donation or abstraction





concominant with carbon-nitrogen bond cleavage. Biechler and Taft[10] have proposed an intermediate dianion such as III to explain their results for the alkaline hydrolysis of the N-methylanilides; however, more recently Schowen and coworkers[14] have shown that the hydrolysis of 2,2,2-trifluoro-N-methylacetanilide is subject to general base catalysis by glycine buffers and hence mechanisms such as the ones shown in eq 6a and 6b would be more applicable for N-methylacetanilide hydrolysis.

In the case of the dihydropyrimidines no clear distinction between the specific and general acid-base catalyzed mechanisms can be made; however, the specific acid-base catalyzed mechanisms might be favored in view of the fact that there is no observable increase in k_{obsd} for dihydrouracil hydrolysis with increasing piperidine buffer concentration. The inverse deuterium isotope effects observed for k_1 and k_2 in 3-methyldihydrouracil hydrolysis, which might indicate that proton transfer occurred prior to cleavage of the carbon-nitrogen bond, also support either mechanism 5a or 5b although there are several reported examples of general acid-base catalyzed reactions which exhibit either small or inverse deuterium isotope effects.[15-18]

The interpretation of the rates of dihydrouracil, dihydroorotic acid, and dihydrothymine hydrolysis at the higher concentrations of OH^- becomes more complex because of the ionization of the proton at the 3 position of the ring to yield the dihydropyrimidine

(14) R. L. Schowen and G. W. Zuorick, *J. Am. Chem. Soc.*, **88**, 1223 (1966); R. L. Schowen, H. Jayaraman, and L. Kershner, *ibid.*, **88**, 3373 (1966).
(15) B. M. Anderson and W. P. Jencks, *ibid.*, **82**, 1773 (1960).
(16) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).
(17) A. Williams and M. L. Bender, *ibid.*, **88**, 2508 (1966).
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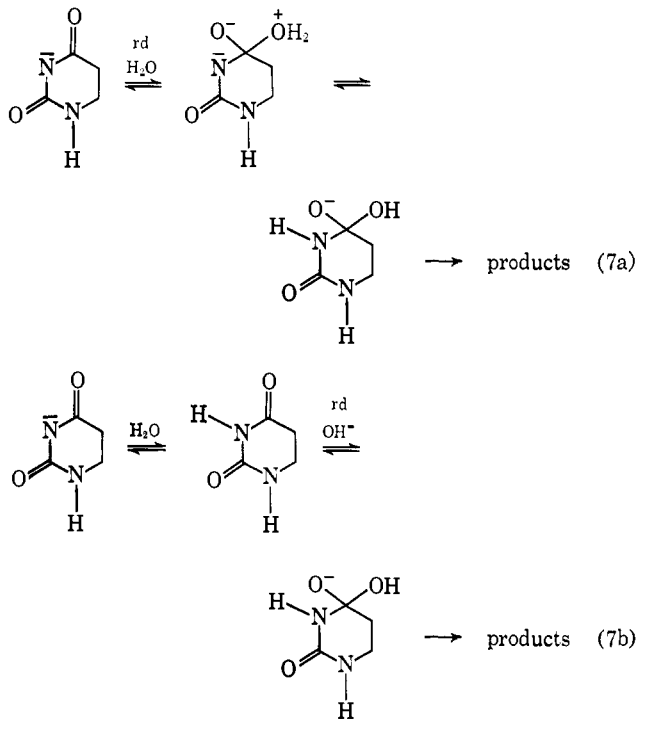

anion. For these compounds, the rates of hydrolysis change from a second- to a zero-order dependence on OH^- which like the case of 3-methyldihydrouracil indicates a change in the rate-determining step of a multistep pathway. Since at low OH^- concentration the hydrolysis of the unsubstituted dihydropyrimidines obeys a rate law which is second order in OH^- (eq 2) it can be concluded that under these conditions the rate-determining step of the reaction is the breakdown of the tetrahedral intermediate. Some of the rate measurements reported were conducted at pH values near and in several cases below the pK_a of the proton on the 3 position of the dihydropyrimidine ring. Consequently, some of the tetrahedral intermediate is likely formed by the attack of OH^- on the undissociated dihydropyrimidine as is the case with 3-methyldihydrouracil. As the OH^- concentration is increased the predominant dihydropyrimidine species becomes the anion and the rate of hydrolysis becomes invariant with OH^- concentration. This observation would be compatible with a change in the rate-determining step of the reaction from breakdown of the tetrahedral intermediate to either the attack of H_2O on the dihydropyrimidine anion (eq 7a) or the kinetically indistinguishable attack of OH^- on the protonated dihydropyrimidine preceded by proton donation to the dihydropyrimidine anion (eq 7b). Mader[11] has invoked a similar explanation for the analogous alkaline hydrolysis of 2,2,2-trifluroacetanilide.

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Isolation and Characterization of a Pyrimidine Sulfenic Acid *via* Scission of the Sulfur–Sulfur Bond in the Methyl Analog of Bis(4-thiouridine) Disulfide[1,2]

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Abstract: It has been established that bis(1-β-D-ribofuranosyl-4-thiouracil) disulfide and its methyl analog are hydrolyzed almost quantitatively in alkali to the corresponding thiones and sulfenic acids in 1:1 molar ratios. 1-Methyluracil-4-sulfenic acid was isolated and characterized as its silver salt. Uridine-4-sulfenic acid was identified by the similarity of its uv absorption spectra with those of its 1-methyl analog. Some of the properties of the sulfenic acid derivatives are reported. The cleavage of the disulfides in acid, on the other hand, gives quantitative formation of the corresponding thiones and uracil derivatives.

The recent discovery of four thiopyrimidines[3-5] and one thiopurine[6] as minor bases in tRNA has stimulated considerable interest in these compounds, particularly in 4-thiouridine, which is known to constitute a major portion of these thionucleosides in bacterial tRNA. Although the presence of bis(4-thiouridine) disulfide has not yet been demonstrated in native tRNA, it has been claimed to be artificially formed therein by iodine oxidation.[7,8] The potential significance of these thio bases in tRNA has been discussed by Irie, *et al.*[9] Since acid and alkali are very often used in degrading nucleic acids, their action on these disulfides is impor-

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