methanes (VII, R = H, R′ = COR″) are sufficiently stable to be isolated from water.[25,26]

The reaction corresponding to the k_2 term in the reverse direction is the first step of the general acid catalyzed aminolysis of an amide. It has previously been shown that the hydroxylaminolysis of amides proceeds in two steps, both of which are subject to general acid catalysis, but it was not possible to distinguish among the possible mechanisms for this catalysis.[27] The conclusion that the DPIC reaction proceeds according to mechanism 16 (or 20) suggests that the same mechanism holds generally for amide aminolysis.

The deamination of protonated cytosine, a mutagenic reaction, is subject to general base catalysis by acetate ion and proceeds according to a rate law corresponding to the k_2 term for DPIC hydrolysis.[28] It is probable that this reaction also proceeds by the mechanism of eq 16, if the deamination proceeds by a direct hydrolysis, rather than through an intermediate addition of water to the 5,6 double bond.

Acknowledgment. The authors are grateful to Miss Mary E. Grant for expert technical assistance.

(26) H. Bredereck, R. Gompper, F. Effenberger, H. Keck, and H. Heise, *Chem. Ber.*, 93, 1398 (1960); S. Hunig, *Angew. Chem. Intern. Ed. Engl.*, 3, 548 (1964).

(27) W. P. Jencks and M. Gilchrist, *J. Am. Chem. Soc.*, 86, 5616 (1964).

(28) R. Shapiro and R. S. Klein, *Biochemistry*, 5, 2358 (1966); D. M. Brown and M. J. Hewlins, manuscript in preparation.

Mechanism and Catalysis of the Hydrolysis of Methenyltetrahydrofolic Acid

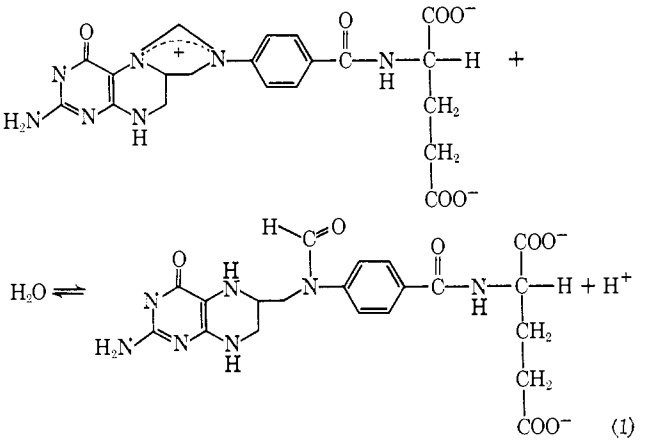
Dwight R. Robinson and William P. Jencks

Contribution[1] from the Department of Medicine, Harvard Medical School, the Medical Services (Arthritis Unit), Massachusetts General Hospital, Boston, Massachusetts, and the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received August 8, 1967*

Abstract: The hydrolysis of $N^{5,10}$-methenyltetrahydrofolate (MTF) to N^{10}-formyltetrahydrofolate has been studied over the pH range 7.2–10.0 at 25° and ionic strength 1.0 *M*. A p*K* value of 8.95 for MTF was determined spectrophotometrically and is attributed to the dissociation of the amide group in the pteridine ring. The hydrolysis of MTF at low buffer concentrations follows the rate law $v = k_2[\text{MTF}][\text{B}] + k_3[\text{MTF}][\text{B}]a_{\text{OH}^-}$, where MTF is the protonated form with respect to the p*K* of 8.95 and B is either hydroxide ion or a general base. Most of the observed reaction is accounted for by the term second order in base (k_3). Plots of the pseudo-first-order rate constants for the hydrolysis of MTF against buffer concentration at a constant pH are not linear but level off with increasing buffer concentration, providing evidence for the existence of a kinetically significant tetrahedral intermediate. At low buffer concentration, the rate-determining step is the general base catalyzed breakdown of a tetrahedral intermediate to form products. At high buffer concentrations, the formation of the tetrahedral intermediate becomes rate determining. A steady-state treatment of this mechanism accounts quantitatively for the imidazole-catalyzed reaction. The mechanism of general base catalysis in the attack step is unambiguous, and it is suggested that the same mechanism holds for general acid–base catalysis of the second step of imido ester aminolysis.

Kinetic experiments and exchange reactions provide evidence that several nucleophilic reactions of carbonyl compounds at the acyl level of oxidation proceed through the formation of unstable tetrahedral intermediates General acid–base catalysis of the formation and breakdown of these intermediates often provides facile reaction pathways for the nonenzymatic and presumably for the enzymatic reactions of these compounds. An important problem in these reactions is the resolution of the kinetic ambiguity and the determination of the detailed mechanism of catalysis.

In the accompanying paper we have reported an investigation of the hydrolysis of the amidine, N,N′-diphenylimidazolium chloride. The rate law of this reaction indicates that the rate-determining step is the general acid–base catalyzed breakdown of a tetrahedral intermediate.[2] In the present paper, we report the results of a study of the hydrolysis of $N^{5,10}$-methenyltetrahydrofolic acid (MTF) (eq 1). This reaction



(1)

(1) Publication 441 from the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, and 531 from the Graduate Department of Biochemistry, Brandeis University. Supported by grants from the U. S. Public Health Service (AM-4501 and AM-3564).

(2) D. R. Robinson and W. P. Jencks, *J. Am. Chem. Soc.*, 89, 7088 (1967).

proceeds at convenient rates nonenzymatically under mild conditions in both forward and reverse directions at alkaline and acid pH values, respectively.[3] Previous work has shown that the reaction is subject to buffer catalysis near neutral pH but the buffer effects have not been investigated in detail.[4] The rate law of the reaction at low buffer concentrations is the same as that of the hydrolysis of N,N′-diphenylimidazolinium chloride.[2] In addition, the reaction undergoes a change in rate law with increasing catalyst concentration which is interpreted as evidence for a change in rate-determining step.

Experimental Section

The calcium salt of N^5-formyltetrahydrofolic acid (calcium leucovorin) was generously supplied by the Lederle Laboratories, Pearl River, N. Y., through the kindness of Dr. Robert B. Angier of the Organic Chemical Research Section, and was routinely used without further purification. A portion of this material was recrystallized twice from aqueous ethanol containing approximately 0.01 *M* calcium chloride. The ultraviolet spectrum was unchanged by the recrystallization. The compound, MTF, was prepared by dissolving N^5-formyltetrahydrofolic acid in 0.01 *M* hydrochloric acid and allowing the solution to stand at 37° for 1.5 hr.[4b] The ultraviolet spectra of both compounds were in good agreement with published data.[3,4a]

The hydrolysis of MTF was followed by the disappearance of absorbance at 390 mμ and rate constants were measured as described in the accompanying paper. The product of this reaction is N^{10}-formyltetrahydrofolate which is susceptible to rapid oxidation by atmospheric oxygen. This product was identified by running the reaction under argon in a Thunberg tube with a 1 × 1 × 4 cm silica cuvette fused to the bottom of the tube (Pyrocell Manufacturing Co.). The product was shown to be N^{10}-formyltetrahydrofolate by its ultraviolet spectrum in the presence of each of the buffers which were used in the kinetic experiments and in which the absorbance of the buffer does not obscure the spectrum of the product. For routine measurements, the reaction could be followed in the presence of air, since neither N^{10}-formyltetrahydrofolate or its oxidation products absorb appreciably at 390 mμ. In one experiment, the kinetics were shown to be the same in the presence and absence of oxygen.

The p*K* of MTF of 8.95 was determined by measuring the absorbance of MTF at 400 mμ as a function of pH. A solution of MTF (0.2 ml) was added with a micropipet to 2.8 ml of solutions containing 0.03 *M* N-methylmorpholine or borate buffers at pH ranging from 6.94 to 9.40. The absorbance at 400 mμ was measured within 15 sec after mixing and the rate of disappearance was followed to permit extrapolation of the absorbance to the time of mixing.

Steady-State Treatment. A steady-state rate equation (eq 10) was derived for the mechanism outlined in eq 9. Initial estimates of the rate constants listed in Table II were made as described below and the final values were obtained by successive approximation to achieve the best fit to the experimental points. The initial value for k'_1 is the slope of the plot shown in Figure 6. The slopes of the plots in Figure 4 at high imidazole concentrations were similar and gave an initial value of k_2'. The values of $k_1'k_3'/k_{-1}'$ and $k_1'k_4'/k_{-1}'(K_w/K_B)$ were obtained as the slope and intercept, respectively, of a plot of the initial slopes of the lines in Figure 5 against hydroxide ion activity. The last three rate constant ratios in Table II were determined from the other values.

Spectra in Concentrated Buffers. Ultraviolet spectra were taken of N^5-formyltetrahydrofolate in duplicate tubes containing 0.68 *M* N-methylmorpholine buffer, 80% free base, and 0.33 *M* hydrazine buffer, 65% free base, and these spectra were compared to spectra taken in 0.04 *M* N-methylmorpholine buffer at the same pH of 8.4. Blank cuvettes in each case contained buffer alone. All of the spectra were identical within experimental error of 2%.

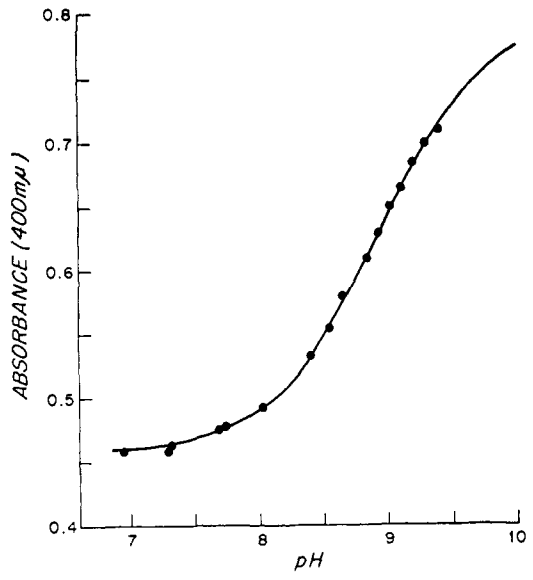


Figure 1. Spectrophotometric determination of the p*K* of MTF at 25°, ionic strength 1.0 *M*. Each point was obtained by extrapolating the absorbance to the time of mixing of MTF with buffer solutions. The line is calculated, based on a p*K* value of 8.95.

In addition, the spectrum of N^5-formyltetrahydrofolate in 0.42 *M* imidazole, 0.56 *M* N-methylmorpholine, and 0.28 *M* hydrazine and dilute KOH at pH values between 11.3 and 12.0 were identical within 3%. The percentage figures refer to extinction at 250–300 mμ. The wavelength maxima and minima were the same in all cases at the same pH, within ±0.5 mμ.

Other experimental methods were similar to those previously described.[2]

Results

p*K* of MTF. The ultraviolet absorbance of MTF at 390 mμ, extrapolated to the time of mixing with the buffer, increases with increasing pH above pH 7 in a manner consistent with the titration of a group with a p*K* of 8.95. The absorbance of MTF could not be determined above pH 9.4 because of rapid base-catalyzed hydrolysis. The p*K* determined by plotting the absorbance data according to the procedure of Hofstee[5] is 8.95. The data are shown in Figure 1 with the line calculated from the equation

$$\mathrm{pH} = \mathrm{p}K + \log \frac{(A - A_a)}{(A_b - A)} \qquad (2)$$

where A is absorbance at any pH, $A_a = 0.458$, $A_b = 0.804$, and pK = 8.95.

Hydroxide Ion Catalyzed Hydrolysis. In the pH range 8.80–9.90 the rate of hydrolysis of MTF, extrapolated to zero buffer concentration and corrected to the reactive protonated form of MTF (Figure 2) is first and second order with respect to hydroxide ion and follows the rate law of eq 3.

$$v = k_2[\mathrm{MTF}]\alpha_a a_{\mathrm{OH}^-} + k_3[\mathrm{MTF}]\alpha_a a_{\mathrm{OH}^-}{}^2 \qquad (3)$$

$$\text{fraction free acid} = \alpha_a = \frac{a_{\mathrm{H}^+}}{a_{\mathrm{H}^+} + K} \qquad (4)$$

Buffer Catalysis. The hydrolysis of MTF is catalyzed by buffers. In each case the slopes of the plots of k_{obsd} against buffer concentration decrease with increasing buffer concentration (Figures 3 and 4). The depen-

(3) M. May, T. J. Bardos, F. L. Barger, M. Lansford, J. M. Ravel, G. L. Sutherland, and W. Shive, *J. Am. Chem. Soc.*, 73, 3067 (1951).

(4) (a) J. C. Rabinowitz in "The Enzymes," Vol. 2, P. D. Boyer, H. Lardy, and K. Myrback, Ed., 2nd ed, Academic Press Inc., New York, N. Y., 1960, p 185; (b) S. C. Hartman and J. M. Buchanan, *J. Biol. Chem.*, **234**, 1812 (1959); H. Tabor and L. Wyngarden, *ibid.*, **234**, 1830 (1959).

(5) B. H. J. Hofstee, *Science*, **131**, 39 (1960).

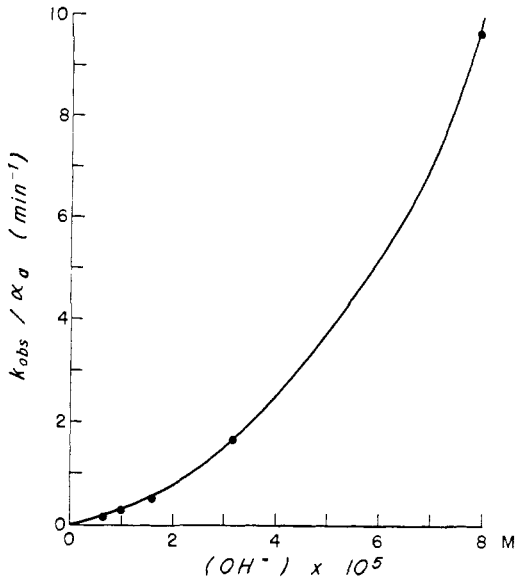


Figure 2. Hydroxide ion catalyzed hydrolysis of MTF at 25.0°, ionic strength 1.0 *M*. Each point was obtained from runs in 0.01–0.05 *M* borate buffers by extrapolating to zero buffer concentration. The line is calculated from the rate law of eq 3 and the values of k_2 and k_3 listed in Table I; α_a is defined in eq 4.

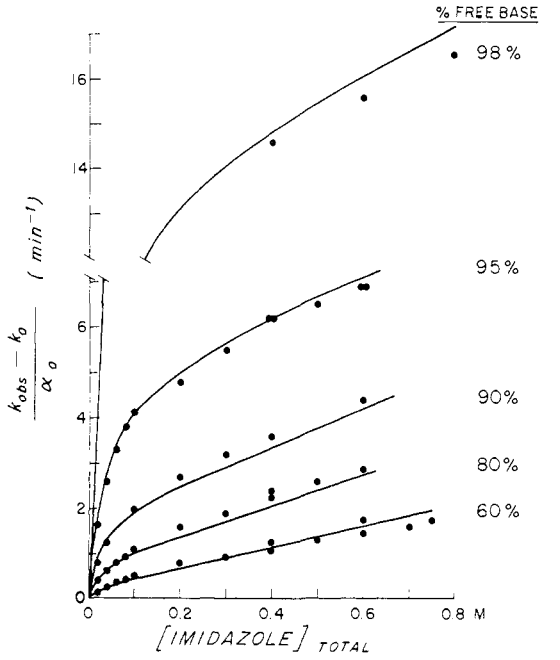


Figure 4. Hydrolysis of MTF in imidazole buffers at 25.0°, ionic strength 1.0 *M*.

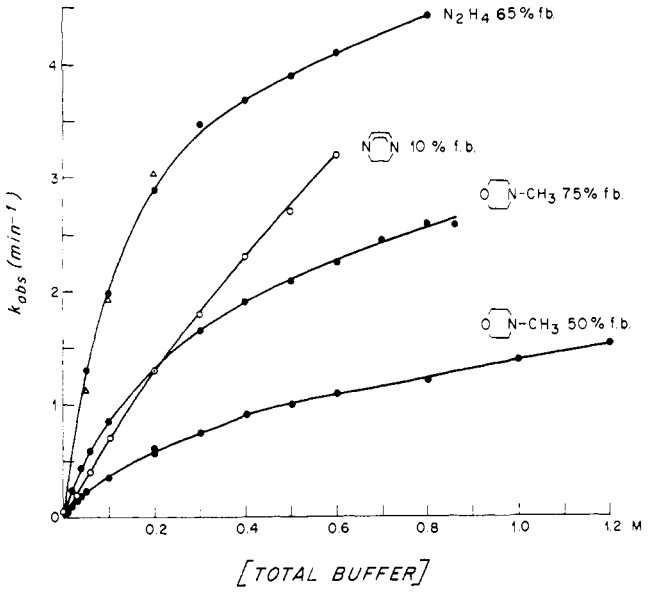


Figure 3. Buffer-catalyzed hydrolysis of MTF at 25.0°, ionic strength 1.0 *M*.

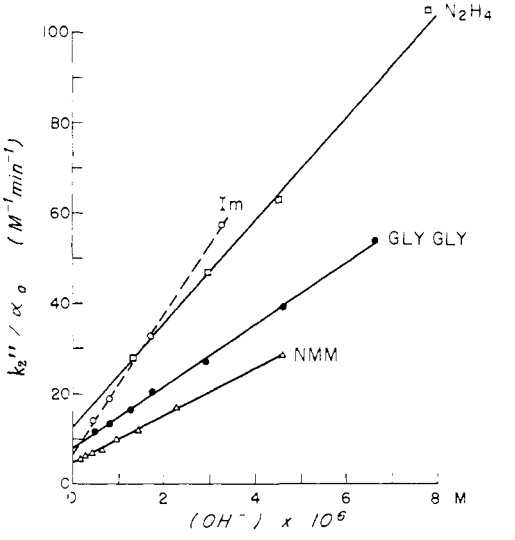


Figure 5. The pH dependence of the buffer catalysis of MTF hydrolysis at low buffer concentrations. The apparent second-order rate constant, $k_2'' = (k_{obsd} - k_0)/[B]_{fb}$.

dence of the initial slopes of these plots on pH was determined in dilute buffers (0.05 and 0.1 *M*) at a series of pH values. Some representative results are shown in Figure 5 as plots of the apparent second-order rate constants, k_2'', against hydroxide activity (eq 5).

$$k_2'' = \frac{k_{obsd} - k_0}{[B]\alpha_a} \quad (5)$$

Therefore, the hydrolysis of MTF at *low* buffer concentrations follows the rate law

$$v = k_2[MTF][\alpha_a][B] + k_3[MTF][\alpha_a][B]a_{OH^-} \quad (6)$$

where B is the concentration of buffer in the free base form. The values of k_2 and k_3 were obtained from the intercepts and slopes, respectively, of plots such as those in Figure 5 and are listed in Table I.[6]

Discussion

The hydrolysis of MTF at low buffer concentrations follows the rate law of eq 6. Most of the reactivity in the pH range 7–10 is accounted for by the term second order with respect to base. The reaction follows the same rate law as the hydrolysis of N,N′-diphenylimidazolinium chloride which is discussed in detail in

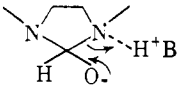
(6) Two exceptions are *t*-butylamine and borate buffers. The usual plots of k_2'' against a_{OH^-} are curved upward at high a_{OH^-}. Plots of k_2''/a_{OH^-} against a_{OH^-} are roughly linear, and the values of k_3 listed in Table I are taken from the intercepts of these plots on the k_2''/a_{OH^-} axis. A possible explanation for these results is that a reaction of the basic form of I, with respect to the p*K* of 8.95, becomes significant at high pH values.

Table I. Base Catalysis of the Hydrolysis of $N^{5,10}$-Methenyltetrahydrofolate at 25.0° (Ionic Strength = 1.0 *M*)

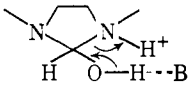
Base	pK^a	Total buffer, *M*	% free base	pH	No. of runs	k_2^b	$k_3 \times 10^{-6}$ [b]
Hydroxide	15.7			8.80–9.9	6	15,000	1200
t-Butylamine	10.9	0.03–0.05	5–15	9.45–10.16	8	*c*	3[c]
Mercaptoacetate	10.0	0.05–0.3	20	9.65	4		15[d]
Carbonate	9.8	0.05	5–20	8.68–9.21	4	260	~14
		0.01–0.04	10	8.73	3		
		0.02–0.04	10	8.84	3		
		0.05–0.3	20	9.57	4		
Ethanolamine	9.8	0.05–0.2	25	9.32	3	20	21
		0.1	10–40	8.79–9.61	5		
Acetohydroxamate	9.3	0.03	10–40	8.37–9.16	4	50	25
Triethylenediamine	9.1	0.03–0.6	10	8.20	9	12	3.2
		0.03–0.8	25	8.7	8		
		0.03	10–60	8.13–9.36	6		
Borate	9.0	0.01–0.05	40–90	8.80–9.9	27	*c*	1.7[c]
Morpholine	8.85	0.05–0.2	25	8.33	4		16[d]
Tris	8.3	0.02–0.6	75	8.84	8	5	7
		0.025–0.1	70	8.73	3		
		0.05–0.2	50	8.3	3		
		0.05–0.3	40	8.14	4		
Glycylglycine	8.25	0.05–0.2	75	8.74	3	8	6.8
		0.1	20–80	7.68–8.82	7		
Hydrazine	8.15	0.03–0.2	80	8.73	5	8	12
		0.05–0.8	70	8.6	8		
		0.05	48–84	8.12–8.89	4		
N-Methylmorpholine	7.80	0.02–1.2	50	7.87	10	4.5	5.3
		0.02–0.8	75	8.28	13		
		0.1	20–90	7.21–8.66	8		
sym-Collidine	7.7	0.01–0.05	50	7.77	5	1.0	1.8
		0.02–0.1	80	8.29	5		
Imidazole	7.2	*g*	*g*	*g*	*g*	7[f]	30[f]
Phosphate[e]	6.5	0.1–0.3	~50	6.48–6.54	3	0.33	3.8
		0.2	50–90	6.49–7.53	5		

[a] Values are from the measured pH values and the composition of the buffer solutions, except for hydroxide ion. [b] Determined at low buffer concentrations as described in the text. [c] See ref 6. [d] Approximate value, calculated assuming k_2 to be negligible under these conditions. [e] Ionic strength = 1.24 *M*. [f] This value was obtained from the slopes of the plots in Figure 4 extrapolated to zero buffer concentration. [g] See Figure 4.

the accompanying paper.[2] This indicates that the rate-determining step of MTF hydrolysis is the general acid–base catalyzed breakdown of a tetrahedral intermediate. By analogy with the hydrolysis of DPIC the k_3 term for MTF hydrolysis represents the general acid catalyzed breakdown of the anionic tetrahedral intermediate (I), and the preferred mechanism for the k_2 reaction is the general base catalyzed breakdown of the cationic intermediate (II). In both the k_3 and k_2 re-



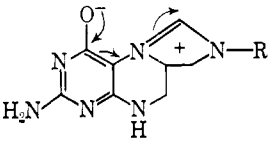
I



II

actions the two proton transfers from the neutral tetrahedral intermediate may occur either in separate steps or by a "one-encounter" mechanism in which both protons are transferred during a single encounter with the catalyst.

The pK value of 8.95 for MTF is assigned to the amide group in the pteridine ring, which has a pK of 10.5 in tetrahydrofolate under the same conditions.[7] A pK value of 8.6 for MTF determined potentiometrically has been previously reported,[3] but this value may be inaccurate because the hydrolysis of MTF becomes rapid near pH 9. The kinetics of hydrolysis of MTF indicates that its reactivity is accounted for by the protonated form under these conditions. The negative charge of the conjugate base of MTF can be delocalized to the imidazolinium ring to decrease its reactivity (III).



III

Hydrolysis of MTF at High Buffer Concentrations. The slopes of plots of k_{obsd} against buffer concentration decrease with increasing buffer concentration (Figures 3 and 4). In cases where the leveling off of these plots begins at low buffer concentrations, such as with imidazole, the slopes can be seen to approach a new constant value at high concentrations. This behavior may be explained by one of two mechanisms. Deviation of the rate of the reaction at high catalyst concentration *below* that predicted on the basis of the rate law at low catalyst concentration cannot be accounted for by addition of further terms to the rate law, but must reflect either a change in rate-determining step or the formation of a less reactive complex with the catalyst. We conclude, on the basis of the following arguments, that the hydrolysis of MTF undergoes a change in rate-determining step with changing catalyst concentration.

1. The hydrolysis of N,N′-diphenylimidazolinium chloride, a compound with structural similarity to MTF, follows a linear relationship between k_{obsd} and buffer concentrations for all the buffers examined, in-

(7) R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, **241**, 5845 (1967).

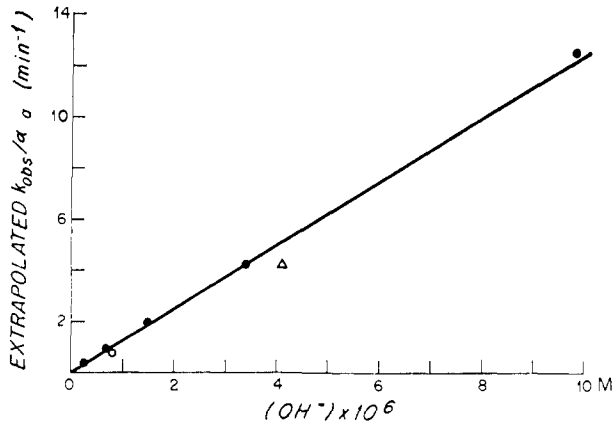


Figure 6. Hydrolysis of MTF at high buffer concentrations. The slopes of the plots of Figures 3 and 4 at high buffer concentrations were extrapolated to zero buffer concentration. The values of k_{obsd}/α_a obtained from these intercepts are plotted as a function of hydroxide ion activity: imidazole buffers, ●; N-methylmorpholine buffers, ○; hydrazine buffers, Δ.

cluding 80% free-base imidazole buffers at concentrations up to 1.0 *M*.

2. Figures 3 and 4 include plots which level off to a constant slope at high buffer concentration. In these cases, straight lines through the points at high buffer concentrations were extrapolated to zero buffer concentration. The values of k_{obsd}/α_a from these intercepts are shown as a function of pH in Figure 6, with the line based on the points from imidazole buffers. The two points obtained from hydrazine and N-methylmorpholine buffers fall near this line. The extrapolated values from three different buffers appear to be determined by the pH, which is consistent with a change in rate-determining step as discussed below. If the leveling off at high buffer concentration were due to complex formation, the break in the curves would be a function of the nature of the catalyst, not the pH, and any such relation to pH would be coincidental.

3. The leveling off of plots of k_{obsd}/α_a against buffer concentration was observed with all of the buffers listed in Table I, except borate and acetohydroxamate which were not examined at concentrations greater than 0.05 *M*. It would not be expected that complexation would occur and cause a leveling off with buffers of such different chemical nature as primary, secondary, and tertiary amines, thiol, carbonate, and phosphate.

4. The results in imidazole buffers may be accounted for by the rate law of eq 8 based on the formation of a less reactive complex according to eq 7. A reasonably

$$B + MTF \underset{}{\overset{K_c}{\rightleftharpoons}} B\cdot MTF \quad (7)$$

$$K_c = [B\cdot MTF]/[B][MTF]$$

$$k_{obsd} = k_2\left[\frac{1}{1 + K_c[B]}\right][B] + k_3\left[\frac{1}{1 + K_c[B]}\right] \times [B]a_{OH} + k_4\left[\frac{K_c[B]}{K_c[B] + 1}\right][B] \quad (8)$$

close fit to the experimental points in Figure 4 was obtained from eq 8 and the following constants: k_2 = 7 M^{-1} min^{-1}, $k_3 = 3 \times 10^7$ M^{-2} min^{-1}, k_4 = 2.4 M^{-1} min^{-1}, and K_c = 16 M^{-1}. However, the hydrolysis of DPIC and MTF at low buffer concentrations occurs principally through the k_3 term in the rate law which includes hydroxide ion activity, corresponding to the addition of hydroxide ion to the substrate before the rate-determining step. There is no corresponding hydroxide ion term in the rate law for the reaction at high buffer concentration. This rules out complexation as an explanation for the rate at high buffer concentration unless there is a change in the mechanism of the predominant reaction under these conditions.

5. The presence of high concentrations of imidazole, hydrazine, and N-methylmorpholine causes no significant change in the ultraviolet spectrum of N^5-formyltetrahydrofolate.

Change in Rate-Determining Step. The leveling off of the plots of rate against buffer concentration may be satisfactorily explained by the occurrence of a change in rate-determining step with the intermediate formation of a kinetically significant tetrahedral addition compound; the imidazole-catalyzed reaction is described in detail as an example. A kinetic scheme for the hydrolysis of MTF is given in eq 9, in which TH is

$$MTF^+ \underset{k_{-1}'}{\overset{k_2'B}{\underset{k_{-2}'BH^+}{\overset{k_1'OH^-}{\rightleftharpoons}}}} TH \underset{k_3'B}{\overset{k_4'BH^+}{\rightleftharpoons}} P \quad (9)$$

the neutral tetrahedral intermediate formed by attack of the solvent and P is N^{10}-formyltetrahydrofolate.[2] At low buffer concentration the second step, the breakdown of the tetrahedral intermediate to form N^{10}-formyltetrahydrofolate, is rate determining. With increasing buffer concentration there is a change in rate-determining step, and the formation of the tetrahedral intermediate becomes rate determining at high buffer concentrations. Other reactions which undergo a change in rate-determining step with increasing concentration of general acid–base catalysts have been described in recent years.[8] The steady-state rate equation for the mechanism of eq 9 is given in eq 10. The lines for imidazole catalysis in Figure 4 were calculated from eq 10 and the rate constants listed in

$$\frac{d[P]}{dt}\frac{1}{[MTF][\alpha_a]} = \frac{\left[k_1'[OH^-] + k_2'[B]\right]\left[1 + \dfrac{k_4'K_w}{k_3'K_B[OH^-]}\right]}{\dfrac{k_{-1}'}{k_3'[B]} + \dfrac{k_{-2}'K_w}{k_3'K_B[OH^-]} + 1 + \dfrac{k_4'K_w}{k_3'K_B[OH^-]}} \quad (10)$$

Table II. The values of the rate constants were ob-

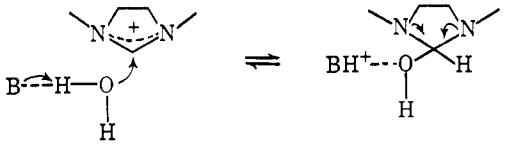
Table II. Rate Constants for the Imidazole-Catalyzed Hydrolysis of MTF at 25.0° (eq 10)

Rate constants	Values
k_1'	1.5×10^6 M^{-1} min^{-1}
k_2'	4.5 M^{-1} min^{-1}
$k_1'k_3'/k_{-1}'$	4.5×10^7 M^{-2} min^{-1}
$(k_1'k_4'/k_{-1}')(K_w/K_B)$	15 M^{-1} min^{-1}
k_4'/k_3'	3.3
k_{-1}'/k_3'	0.033 *M*
k_{-2}'/k_3'	1.0

(8) E. C. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 4319 (1962); W. P. Jencks and M. Gilchrist, *ibid.*, **86**, 5616 (1964); A. J. Kirby and W. P. Jencks, *ibid.*, **87**, 3217 (1965); R. B. Martin, R. I. Hedrick, and A. Parcell, *J. Org. Chem.*, **29**, 3197 (1964); B. A. Cunningham and G. L. Schmir, *J. Am. Chem. Soc.*, **89**, 917 (1967), and references therein.

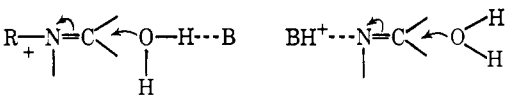
tained as described in the Experimental Section. The agreement of the calculated lines with the experimental points over a wide range of pH and buffer concentration provides support for the mechanism of eq 9.

Formation of the Tetrahedral Intermediate. The slopes of the plots in Figures 3 and 4 at high buffer concentration, corrected by α_a in each case, are 3.5 M^{-1} min^{-1} for imidazole, 3.9 M^{-1} min^{-1} for hydrazine, and 1.6 M^{-1} min^{-1} for N-methylmorpholine catalysis. We interpret this buffer catalysis as general base catalysis of the formation of the tetrahedral intermediate, and the values of the slopes listed above are equal to the k_2' terms for these bases (eq 9). The mechanism for this step is the general base catalyzed attack of water on MTF (IV) and in the reverse direction the general acid catalyzed expulsion of hydroxide ion (V). This mechanism is the same as that



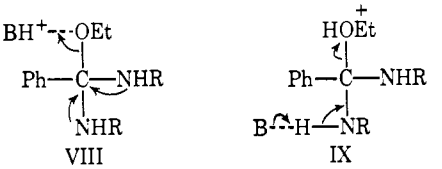
IV V

for the addition of water to several immonium compounds.[9] In the case of protonated imines mechanism VI (R = H) is kinetically indistinguishable from VII. This ambiguity is removed for several methyl-substituted immonium compounds (R = CH_3) for which mechanism VII is excluded.[10] Similarly, this ambiguity does not exist for MTF.



VI VII

Comparison of the Hydrolysis of MTF with Imido Ester Aminolysis. The aminolysis of imido esters derived from weakly basic amines proceeds through tetrahedral intermediates which break down to amidines by a general acid catalyzed pathway.[11] It was previously pointed out that this implies that the hydrolysis of amidines, by analogy, is subject to general acid–base catalysis[11] which has been demonstrated by the work reported here. Two mechanisms for general acid catalyzed imido ester aminolysis, VIII and IX, are kinetically indistinguishable. This ambiguity does



VIII IX

not exist for the attack of water on MTF (IV) because of the absence of protons on the amidinium nitrogen atoms. This suggests that mechanism VIII is correct for the corresponding step of the imido ester reactions.

Acknowledgment. The authors are grateful to Miss Mary E. Grant for expert technical assistance.

(9) W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).
(10) K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Am. Chem. Soc.*, **86**, 2413 (1964); J. E. Riemann and W. P. Jencks, *ibid.*, **88**, 3973 (1966); R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, **241**, 5851 (1966).
(11) E. S. Hand and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 3505 (1962).