nmr spectrum in $D_2O$ had a singlet at $\tau$ 7.3 assigned to the N-methyl group.

*Anal.* Calcd for $C_6H_{15}N_2O_2Cl$: C, 39.45; H, 8.28; N, 15.34. Found: C, 39.58; H, 8.43; N, 15.38.

**DL-δ-N-Methyl-$^{14}$C-ornithine monohydrochloride** was obtained by methylation of DL-α-N-benzoyl-δ-N-*p*-toluenesulfonylornithine with dimethyl sulfate-$^{14}$C as described for the α isomer.

**Chromatography of the Amino Acids.** Thin layer chromatography of the amino acids on silica gel (Eastman chromatogram sheet 6061) with methanol–triethylamine (9:1) resulted in the following $R_f$ values: α-N-methyornithine 0.28, δ-N-methylornithine 0.18, ornithine 0.24.

**Administration of the Tracers to the Plants and Isolation of the Nicotine.** The methylornithines (see Table I for amounts) were added to the nutrient solution[22] in which the roots of 5-month-old *N. tabacum* plants were growing. After 7 days the plants which had been fed the δ-N-methylornithine (residual activity in nutrient solution, 1.3%) were harvested (wet weight of plant, 856 g) and the crude alkaloids isolated as previously described.[7] After distillation the nicotine was purified by preparative thin layer chromatography on silica gel PF, eluting with a mixture of chloroform and methanol (9:1). Nicotine, having an $R_f$ of 0.5, was extracted from the silica gel with ethanol. Evaporation of the ethanol in the presence of 70% perchloric acid (0.1 ml) yielded nicotine diperchlorate (133 mg), having an activity of 5.87 × $10^3$ dpm/mg (1.25% absolute incorporation, 0.65% specific incorporation). After 13 days the plants which had been fed the α-methylornithine (residual activity in the nutrient solution, 11.1%) were harvested (wet weight of plants, 880 g) and afforded nicotine diperchlorate (192 mg), having an activity of 322 dpm/mg (0.10% absolute incorporation, 0.053% specific incorporation).

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[22] E. Leete, *J. Am. Chem. Soc.*, **78**, 3520 (1956).

# Mechanism and Catalysis of the Hydrolysis of a Formamidinium Compound[1]

**Dwight R. Robinson and William P. Jencks**

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*the Medical Services (Arthritis Unit), Massachusetts General Hospital,*
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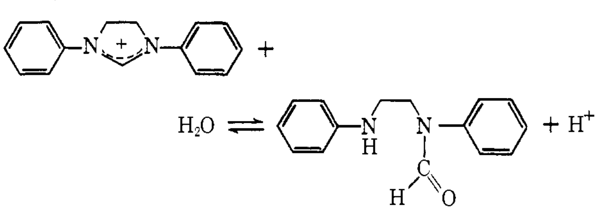
**Abstract:** The hydrolysis of the amidine, 1,3-diphenyl-2-imidazolinium chloride (DPIC), to N-(2-anilinoethyl)-formanilide (AEF) in aqueous solutions at 25°, ionic strength 1.0 *M*, follows the rate law $v = k_2[\mathrm{DPIC}][\mathrm{B}] + k_3[\mathrm{DPIC}][\mathrm{B}]a_{\mathrm{OH}^-}$, where B is a general base or hydroxide ion. Catalysis was observed by all of the 26 different bases examined, many of which have no dissociable proton, and the $k_3$ terms account for most of the observed reaction above pH 9. Nucleophilic attack of the bases on DPIC is ruled out. The presence of the term in the rate law which is second order in base is evidence for a two-step mechanism. The mechanism proposed involves addition of solvent to DPIC to form a tetrahedral addition intermediate followed by rate-determining general base catalyzed breakdown of the intermediate to form AEF. Mechanistically, the observed general base catalysis in the $k_3$ reaction is shown to involve the general acid catalyzed breakdown of an anionic tetrahedral intermediate. Structure–reactivity correlations and comparison with the mechanism of imido ester aminolysis suggest that the mechanism of the $k_2$ reaction involves general base catalyzed breakdown of the conjugate acid of the neutral tetrahedral intermediate. The increased reactivity of bicarbonate ion and the greater reactivity of phosphate dianion than methyl phosphate dianion in the $k_3$ reaction suggest that bifunctional acid–base catalysis by these compounds is slightly more efficient than monofunctional catalysis.

The hydrolysis of 1,3-diphenyl-2-imidazolinium chloride (DPIC) to N-(2-anilinoethyl)formanilide (AEF) (eq 1) has been considered as a model for the reaction of


(1)

formyl derivatives of tetrahydrofolic acid, but the mechanism of the reaction has not been previously studied in detail.[2] The hydrolysis of the related N,N′-diarylformamidines has been found by DeWolfe to occur by pH-independent and hydroxide ion catalyzed pathways.[3] It was concluded from the effects of substituents that the pH-independent hydrolysis in alkaline solutions represents the reaction of hydroxide ion with the protonated amidine or its hydrate and it was pointed out that the nonlinear Hammett plot for these reactions could indicate a change in rate-determining step. These reactions were found to be subject to buffer catalysis but the mechanism of the buffer effects was not established.[3a]

The work reported here and in the accompanying paper was carried out in order to obtain further information on the mechanism and catalysis of the hydrolysis of amidines in general and on some reactions of formyltetrahydrofolates in particular.

## Experimental Section

N-(2-Anilinoethyl)formanilide (AEF) was prepared by the method of Zienty,[4] and was recrystallized once from methanol and

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[1] Publication 440 from the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, and 530 from the Graduate Department of Biochemistry, Brandeis University. Supported by grants from the U. S. Public Health Service (AM-4501 and AM-3564).

[2] (a) 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); (b) L. Jaenicke and E. Brode, *Ann.*, **624**, 120 (1959).

[3] (a) R. H. DeWolfe, *J. Am. Chem. Soc.*, **82**, 1585 (1960); (b) R. H. DeWolfe, *ibid.*, **86**, 864 (1964).

twice further from ethanol–water, mp 62° (lit. 60–61°,[2b] 65–66°[4]). *Anal.* Calcd for $C_{15}H_{16}ON_2$: C, 74.97; H, 6.71; N, 11.66. Found: C, 74.92; H, 6.73; N, 11.65. The infrared spectrum shows strong bands at 1677, 1600, 1505, and 1495 $cm^{-1}$ ($CCl_4$).

1,3-Diphenyl-2-imidazolinium chloride (DPIC) was routinely prepared for kinetic experiments by dissolving AEF in 0.02 *N* HCl (*ca.* 0.3 mg/ml) and allowing it to stand at room temperature for 0.5 hr, which resulted in the complete conversion of AEF to DPIC as demonstrated by its ultraviolet spectrum.[2a] All organic compounds were recrystallized or redistilled before use. Inorganic salts were reagent grade materials and were used without further purification. Glass-distilled water was used throughout.

Spectra were obtained with Cary Models 14 and 15 spectrophotometers. Determinations of pH were carried out with a Radiometer TTT 1 meter equipped with a PH A630T scale expander, using G 202 C or G 222 B glass electrodes.

**Kinetic Measurements.** The reaction (eq 1) is conveniently followed spectrophotometrically using the absorbance of DPIC ($\epsilon_{312m\mu} = 1.92 \times 10^4\ M^{-1}\ cm^{-1}$) above 300 mμ, where AEF absorbance at comparable concentrations is negligible. Kinetic measurements were made with a Zeiss PMQ II spectrophotometer equipped with a hollow brass cuvette holder through which water was circulated with the temperature maintained at 25.0 ± 0.1°. Solutions were equilibrated in a water bath at 25.0° and then transferred to 1-cm path length cuvettes. The reaction was started by addition of DPIC or AEF. AEF was added in 50% ethanol–water but the final concentration of ethanol in the reaction mixture was less than 2%. Kinetics were routinely followed by the disappearance or appearance of DPIC absorbance at 320 mμ. Good pseudo-first-order kinetics were obtained in all runs except in hydrazine solutions, discussed below.

Pseudo-first-order rate constants were obtained from plots of DPIC absorbance ($A_t - A_\infty$ or $A_\infty - A_t$) against time on semilogarithmic graph paper. The half-times of the reactions were obtained from these plots and the pseudo-first-order rate constants were calculated from the relation, $k_{obsd} = 0.693/t_{1/2}$. The hydrolysis of DPIC at a given pH value follows the rate law of eq 2. Values of the second- and third-order rate constants, $k_2$ and $k_3$, for each catalyst were obtained from the intercepts and slopes, respectively, of plots of the apparent second-order rate constants, $k_2''$, against hydroxide ion activity according to eq 2, where $k_0$

$$k_2'' = (k_{obsd} - k_0)/[B] \tag{2}$$

is the solvent-catalyzed reaction and B is the free base form of the catalyst. The rate constants were routinely determined with the catalyst present in excess as a buffer and a series of runs were performed with a constant total buffer concentration and varying pH. The value of $k_2''$ was calculated for each run, using values of $k_0$ calculated from the data in Table II. In some cases, values of $k_2''$ were obtained from the slopes of plots of $k_{obsd}$ against [B] at constant pH. For some weakly basic catalysts, the $k_3$ term is insignificant at pH values in their buffering range. These compounds were added to more alkaline buffers and the contribution of the buffer was determined in separate runs in the same experiment.

Solvent-catalyzed hydrolysis (Figure 1) was determined by extrapolation of the observed rate constants to zero buffer concentration from runs in borate, triethylamine, or *n*-butylamine buffers, 0.01–0.05 *M* total concentration, at pH values below 10.6. Unbuffered solutions were used at higher pH. The two points in Figure 1 at 7.5–10 × $10^{-4}$ *M* hydroxide ion concentration each represent the average of three determinations. Since there was a fall in pH of up to 0.1 pH unit over the course of the reaction, the points are plotted at a concentration of hydroxide ion obtained from the average of the pH measurements before and after each reaction. The line through these points represents the range of variation in hydroxide ion concentration for all six runs. For the points at hydroxide concentration $>10^{-3}$ *M*, the pH drop during the course of the reaction was less than 0.05 pH unit. These reactions were started by the addition of 0.1 ml of DPIC solution to a cuvette containing 2.9 ml of buffer solution in the cuvette holder of the spectrophotometer, and rapid mixing was accomplished with a small plastic spatula. The first reading was taken 5 sec after mixing and subsequent readings at 5-sec intervals. The most rapid rates are based on four to seven points per run (Figure 1, open circles) and followed good first-order kinetics. These rate constants were reproducible within approximately 10% and slower rates were reproducible within 5%.

The hydroxide ion activity was determined from pH measurements and the autoprotolysis constant for water, $K_w = 10^{-14}$, at 25.0°. The p$K_a$ values of the buffers listed in Table II were obtained from measured pH values and the stoichiometric composition of the solutions. Statistical corrections for the Brønsted plots were applied according to eq 3, where *p* is the number of

$$k/q = G_B(p/qK_a)\beta \tag{3}$$

equivalent protons in the acid and *q* is the number of equivalent basic sites in the base.[5] The third-order rate constant, $k_3$, for water was obtained by dividing the second-order rate constant for hydroxide ion by 55.5 *M*. The second-order rate constant for water (Figure 6, filled circle) was obtained from the pH-independent hydrolysis rate of 3.4 × $10^{-3}$ $min^{-1}$ by dividing by 55.5 *M*.

The products of the reactions were identified by ultraviolet spectra of the reaction mixtures at the end of kinetic runs. Ionic strength was maintained at 1.0 *M* in all runs by the addition of potassium chloride. All solutions contained 2 × $10^{-4}$ *M* ethylenediaminetetraacetate.

**Kinetic Isotope Effect.** Deuterium oxide solutions were prepared by dissolving dried, crystalline materials in 99.7% deuterium oxide. Measurements of pD were obtained from the relation, pD = pH + 0.40, where pH refers to the reading of the pH meter in deuterium oxide solutions.[6] The activity of deuterioxide ion was calculated from the pD measurements and the autoprotolysis constant, $K_{D_2O} = 0.16 \times 10^{-14}$, at 25°.

## Results

**Equilibrium Constant.** The equilibrium constant for the reaction of eq 1, $K_{eq} = [AEF]a_{H^+}/[DPIC]$, was measured at seven different pH values using both AEF and DPIC as starting materials (Table I). The reactions were followed over a period of 24 hr and in every case two successive readings after 20 and 24 hr agreed with 1%, demonstrating that equilibrium had been reached. The average value of $K_{eq}$ from these determinations is 1.14 × $10^{-5}$ $M^{-1}$.

**Table I.** Equilibrium Constant for the Reaction
$DPIC + H_2O \rightleftharpoons AEF + H^+$ at 25.0° (Ionic Strength 1.0 *M*)

| pH[a] | Fraction[b] DPIC | $K_{eq}$[c] × $10^5$, $M^{-1}$ |
|---|---|---|
| | AEF[d] | |
| 5.58 | 0.19 | 1.12 |
| 5.23 | 0.34 | 1.15 |
| 4.99 | 0.48 | 1.11 |
| | DPIC[e] | |
| 5.48 | 0.24 | 1.05 |
| 5.40 | 0.25 | 1.20 |
| 5.16 | 0.37 | 1.17 |
| 4.92 | 0.51 | 1.15 |
| | Av | 1.14 |

[a] Solutions contained 0.12 *M* potassium acetate or 0.12 *M* potassium phosphate buffers. [b] Fraction DPIC at equilibrium = [DPIC]/[DPIC] + [AEF] = $A_{320m\mu}/(A_{320m\mu}$ in 0.02 *M* HCl). Each value is the average of two determinations which agreed within 4%. [c] $K_{eq} = [AEF]a_{H^+}/[DPIC]$. [d] Reactions started with AEF. Reactions started with DPIC.

**Hydrolysis of DPIC.** The hydroxide ion catalyzed hydrolysis of DPIC follows the rate law of eq 4. The term which is second order in hydroxide ion, $k_3$,

$$v = k_0[DPIC] + k_2[DPIC]a_{OH^-} + k_3[DPIC]a_{OH^-}{}^2 \tag{4}$$

accounts for most of the observed rate above pH 10 (Figure 1). The value of $k_2$ is difficult to determine from these data, and the value listed in Table II may

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(4) F. B. Zienty, *J. Am. Chem. Soc.*, **68**, 1388 (1946).

(5) D. M. Bishop and K. J. Laidler, *J. Chem. Phys.*, **42**, 1688 (1965); R. P. Bell and P. G. Evans, *Proc. Roy. Soc.*, (London), **A291**, 297 (1966).

(6) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

**Table II.** Base Catalysis of the Hydrolysis of DPIC at 25.0° (Ionic Strength = 1.0 *M*)

| Base | p$K_a$[a] | $p$[a] | $q$[a] | $[B]_t$,[b] $M$ | % free base | pH | No. of runs | $k_2$,[c] $M^{-1}$ min$^{-1}$ | $k_3 \times 10^{-5}$,[c] $M^{-2}$ min$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| Hydroxide | 15.7 | 2 | 1 | | | 9.22–11.20 | 17 | 500 | 90 |
| Triethylamine | 11.1 | 1 | 1 | 0.02–0.3 | 20 | 10.36 | 8 | *d* | 3 |
| | | | | 0.04–0.3 | 25 | 10.54 | 7 | | |
| *n*-Butylamine | 11.0 | 3 | 1 | 0.02–0.05 | 10 | 9.91 | 4 | *d* | 10 |
| | | | | 0.02–0.08 | 20 | 10.32 | 4 | | |
| Carbonate | 9.8 | 1 | 3 | 0.1–0.2 | 50 | 9.78 | 2 | 37 | 5.2 |
| | | | | 0.1–0.3 | 20 | 9.21 | 3 | | |
| | | | | 0.1 | 10–60 | 8.88–9.95 | 6 | | |
| Mercaptoacetate | 10.0 | 1 | 1 | 0.05–0.3 | 20 | 9.42 | 4 | <1 | 5.0 |
| ($RS^-$) | | | | 0.1 | 20–60 | 9.42–10.21 | 5 | | |
| Glycine (GLY) | 9.8 | 3 | 1 | 0.1–0.3 | 20 | 9.14 | 3 | <1 | 3.4 |
| | | | | 0.1–0.3 | 50 | 9.77 | 3 | | |
| | | | | 0.1 | 20–80 | 9.12–10.34 | 5 | | |
| Ethanolamine | 9.8 | 3 | 1 | 0.1–0.3 | 50 | 9.77 | 3 | 1.0 | 3.6 |
| (EA) | | | | 0.1 | 30–70 | 9.41–10.14 | 5 | | |
| Borate | 9.0 | 3 | 4 | 0.1 | 50–90 | 9.01–9.92 | 5 | 0.30 | 0.66 |
| 4-Aminopyridine | 9.4 | 1 | 1 | 0.1–0.3 | 30 | 9.03 | 3 | 1.0 | 5.1 |
| | | | | 0.1 | 20–80 | 8.75–9.97 | 5 | | |
| Triethylenediamine | 9.1 | 1 | 2 | 0.1–0.3 | 75 | 9.68 | 3 | 0.19 | 3.2 |
| (TED) | | | | 0.1–0.3 | 50 | 9.19 | 3 | | |
| | | | | 0.1 | 40–90 | 8.98–10.02 | 5 | | |
| Morpholine | 8.85 | 2 | 1 | 0.1–0.3 | 80 | 9.44 | 3 | 0.4 | 2.3 |
| (MOR) | | | | 0.2 | 50–90 | 8.85–9.74 | 5 | | |
| Hydrazine | 8.15 | 3 | 2 | 0.1–0.3 | 95 | 9.47 | 3 | | 1.6[e] |
| Glycine ethyl ester | 7.8 | 3 | 1 | 0.1–0.3 | 80 | 8.24 | 3 | 0.20 | 1.5 |
| (GEE) | | | | 0.2 | 60–90 | 7.99–8.62 | 4 | | |
| Triethanolamine | 8.2 | 1 | 1 | 0.050–0.40 | 90 | 9.23 | 5 | 0.06 | 2 |
| (TEA) | | | | 0.2 | 60–95 | 8.31–9.70 | 5 | | |
| *sym*-Collidine | 7.7 | 1 | 1 | 0.020–0.080 | 90 | 8.56 | 3 | 0.06 | 1.3 |
| (COLL) | | | | 0.080 | 70–95 | 8.05–8.85 | 4 | | |
| Imidazole | 7.2 | 2 | 1 | 0.1–0.3 | 90 | 8.36 | 3 | 0.19 | 3.2 |
| | | | | 0.2 | 60–95 | 7.45–8.54 | 5 | | |
| Phosphate | 6.6 | 2 | 3 | 0.2 | 75–98 | 7.07–8.27 | 6 | 0.14 | 5.2 |
| Arsenate | 6.45 | 2 | 3 | 0.2 | 70–95 | 6.81–7.75 | 6 | 0.10 | 5.8 |
| Maleate (MAL) | 5.6 | 1 | 4 | 0.10–0.25 | *f* | 9.00 | 3 | 0.015 | 0.14 |
| | | | | 0.3 | *f* | 8.39–9.42 | 3 | | |
| | | | | 0.2 | 85–95 | 6.44–7.45 | 3 | | |
| Hydroxylamine | 6.1 | 3 | 1 | 0.1–0.3 | 60 | 6.26 | 3 | 0.024 | 1.4 |
| | | | | 0.1–0.3 | 90 | 7.03 | 3 | | |
| 4-Methylpyridine | 6.0 | 1 | 1 | 0.1 | *f* | 8.31–9.56 | 4 | *d* | 1.7 |
| Trifluoroethylamine | 5.7 | 3 | 1 | 0.1–0.3 | 80 | 6.31 | 3 | 0.011 | 0.32 |
| | | | | 0.1 | 70–95 | 6.07–6.93 | 5 | | |
| | | | | 0.1 | *f* | 8.25–9.53 | 4 | | |
| Methyl phosphate | 6.0 | 1 | 3 | 0.2 | 60–95 | 6.33–7.37 | 7 | 0.07 | 0.63 |
| | | | | 0.1 | 90 | 7.05 | 1 | | |
| | | | | 0.2 | *f* | 8.26–9.53 | 5 | | |
| Pyridine | 5.2 | 1 | 1 | 0.1–0.3 | *f* | 9.00 | 3 | *d* | 0.55 |
| | | | | 0.1 | *f* | 9.25–10.01 | 4 | | |
| Acetate | 4.7 | 1 | 2 | 0.1–0.3 | *f* | 9.00 | 3 | *d* | 0.054 |
| | | | | 0.3 | *f* | 8.37–9.41 | 3 | | |
| Semicarbazide (SCZ) | 3.7 | 3 | 1 | 0.3 | *f* | 8.36–9.29 | 3 | *d* | 0.05[g] |
| $H_2O$ | −1.57 | 3 | 1 | | | | | $6.1 \times 10^{-5}$[h] | |

[a] Determined as defined in the text. [b] Total buffer concentration. [c] Defined in eq 5. Values were determined as described in text. [d] The $k_2$ term is too small to be detected under these conditions. [e] The value listed was calculated from the data at a single pH assuming the $k_2$ term to be negligible. [f] All runs were made in 0.05 *M* borate buffers. The corrections for borate buffer catalysis were determined from separate runs during the same experiments. [g] Approximate value. [h] From the data shown in Figure 2.

be in error by as much as ±50%. The rate constant of the spontaneous or water-catalyzed hydrolysis, $k_0$, was obtained from the intercepts of the plots shown in Figure 2.

The hydrolysis of DPIC is catalyzed by buffers (Figure 3, Table II). With every buffer examined there is a linear relationship between the observed pseudo-first-order rate constant and the buffer concentration. The buffer catalysis increases with increasing pH, indicating that the basic forms of the buffers are the catalytically active species. However, the catalysis increases with increasing pH more than can be accounted for by the change in the concentration of the basic forms of the buffer alone, which indicates that the rate law for the buffer-catalyzed reaction contains a term in hydroxide ion. This is illustrated for some of the buffers in Figure 4 in which the apparent second-order rate constant at each pH ($k_2'' = k_{obsd}/[B]$) is plotted against hydroxide ion activity. As is shown for these examples, and with all of the buffers examined, $k_2''$ is a linear function of ($OH^-$).

The hydrolysis of DPIC, therefore, follows the rate law of eq 5 in which B is the basic form of the buffers or hydroxide ion. All of the buffers which were

$$v = k_2[\mathrm{DPIC}][\mathrm{B}] + k_3[\mathrm{DPIC}][\mathrm{B}]a_{\mathrm{OH^-}} \qquad (5)$$

examined are catalytically active and follow the rate law of eq 5. Values for $k_2$ and $k_3$ were obtained from the

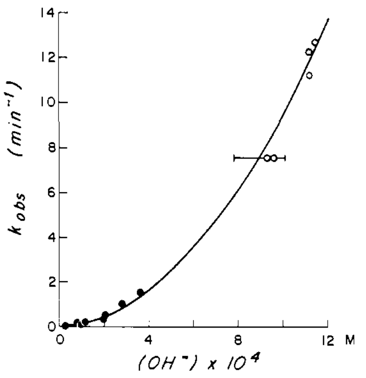

Figure 1. Hydroxide ion catalyzed hydrolysis of DPIC at 25°, ionic strength = 1.0 *M*: ●, obtained by extrapolation to zero buffer concentration; ○, unbuffered solutions. The line is calculated from eq 4 and the data in Table II.

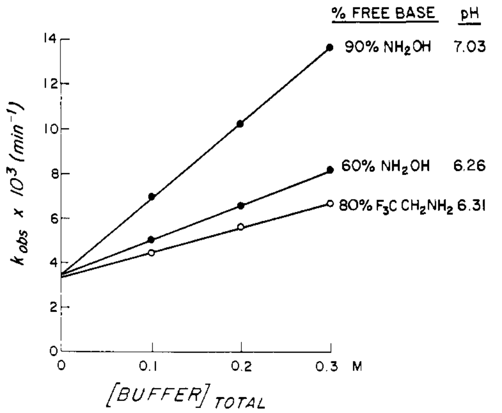

Figure 2. Hydrolysis of DPIC at low pH, 25.0°, ionic strength = 1.0 *M*.

intercepts and slopes, respectively, of plots of $k_2''$ against $a_{OH^-}$. The values of $k_2$ are difficult to determine with accuracy, especially at higher pH values, because the $k_2$ term makes only a small contribution to the observed rates under these conditions (see Figure 4).

Brønsted plots for the second- and third-order catalytic constants ($k_2$ and $k_3$) of the bases are shown in Figures 5 and 6, respectively. The line in Figure 5 provides a good fit to the points for simple primary amines and has a slope, $\beta$, of 0.26. The pyridines and imidazole fall above this line by factors of 3–5, consistent with the known greater reactivity as general acid–base catalysts of tertiary amines than primary amines of comparable basicity.[7] Points for the other tertiary amines fall below a line drawn through the points for pyridines and imidazole by factors of approximately 2–4, which may be attributed to the greater steric requirements of the former compounds. Borate ion shows a a negative deviation in at least one other reaction as a general base catalyst, which has been explained on structural grounds.[7]

The $\beta$ value for the $k_2$ terms is 0.44, from the line in Figure 6. The $k_2$ term for carbonate ion is kinetically indistinguishable from the $k_3$ term for bicarbonate, since $k_2$ is related to $k_3$ by the acid dissociation constant of bicarbonate ion, $K_{a,HCO_3^-}$, and the autoprotolysis

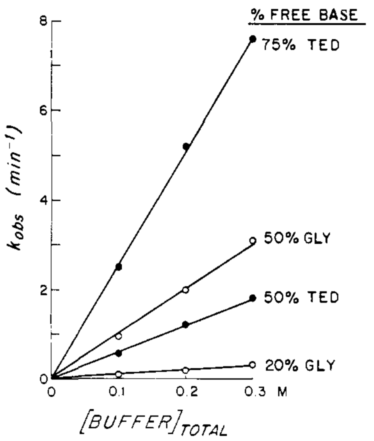

Figure 3. Hydrolysis of DPIC at 25.0°, ionic strength = 1.0 *M* in glycine (GLY) and triethylenediamine (TED) buffers.

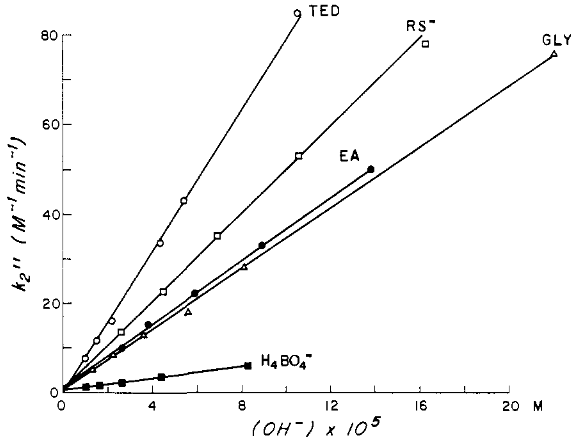

Figure 4. Dependence of the buffer catalysis of DPIC hydrolysis on hydroxide ion activity at 25.0°, ionic strength = 1.0 *M*. The apparent second-order rate constant, $k_2''$ is defined in eq 2. Abbreviations are: TED, triethylenediamine; RS⁻, mercaptoacetate; EA, ethanolamine; GLY, glycine.

constant for water, $K_{w'}$ (eq 6). Expressed either way,

$$k_2[CO_3^{2-}] = \frac{k_2K_{a,HCO_3^-}}{K_w}[HCO_3^-]a_{OH^-} = k_3[HCO_3^-]a_{OH^-} \quad (6)$$

the values fall above the lines in the Brønsted plots by factors of about 30. If the $k_3$ term for bicarbonate ion is calculated from the deviation of the $k_2$ term for carbonate ion above the line in Figure 5 instead of the total value, the value of $k_3$ for bicarbonate ion still falls above the line in Figure 6 by a factor of 10–15.

A similar ambiguity exists between the $k_3$ term for water and the $k_2$ term for hydroxide ion. Therefore, the value given for the $k_2$ term for hydroxide ion in Table II and Figure 6 is an upper limit.

**DPIC Formation.** The formation of DPIC is acid catalyzed near pH 4, but the rates level off and become nearly pH independent below pH 2. The leveling off of the rate at low pH is caused by the protonation of the aniline nitrogen of AEF. After correction for the free-base form of AEF present, the reaction follows the rate law of eq 7. The p$K_a$ value of 3.35 for AEF

$$v = k_{H^+}[AEF]\alpha_b a_{H^+} + k_a[AEF]\alpha_b[HA] \quad (7)$$

$$\text{fraction free base} = \alpha_b = \frac{K_a}{a_{H^+} + K_a}$$

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[7] R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959.

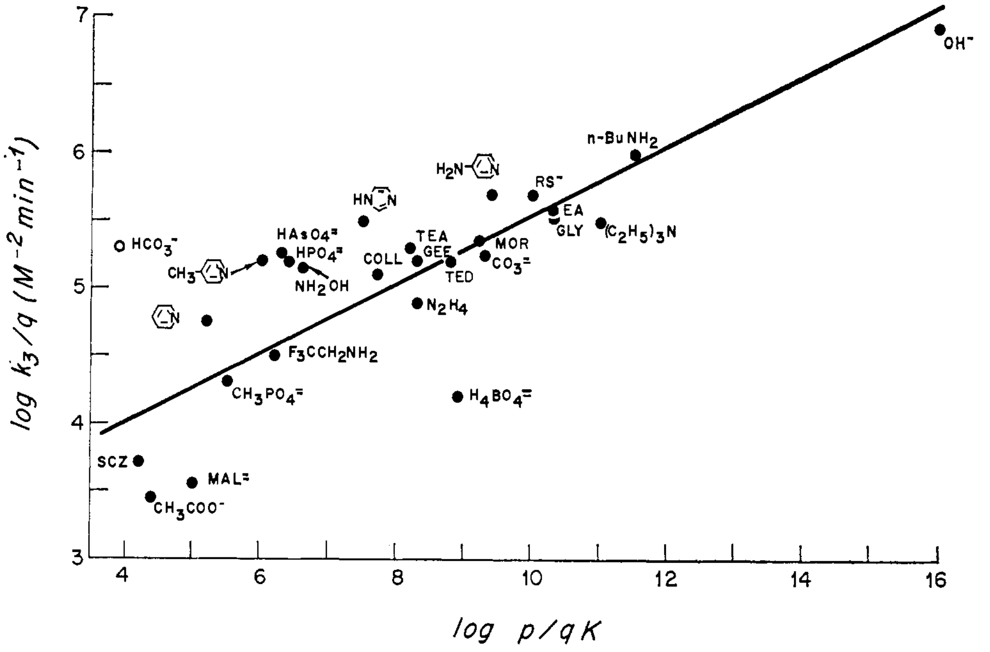


Figure 5. Brønsted plot for the hydrolysis of DPIC according to the rate law, $v = k_3[\mathrm{DPIC}][\mathrm{B}]a_{\mathrm{OH}^-}$. The abbreviations and statistical correction factors are given in Table II. The line is based on the points for primary amines and has a slope, $\beta$, of 0.26. The point for bicarbonate ion is an upper limit and is calculated as described in the Results section.

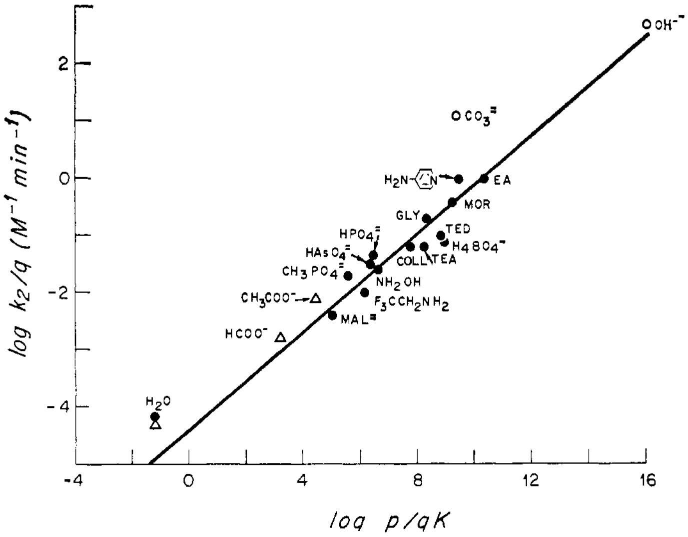


Figure 6. Brønsted plot for the hydrolysis of DPIC according to the rate law, $v = k_2[\mathrm{DPIC}][\mathrm{B}]$. The points represented by filled circles are from the data in Table II. The open triangles are calculated from the equilibrium constant and rate constants for DPIC formation as described in the Results section. The open circles ($CO_3^{2-}$ and $OH^-$) are upper limits, because the observed reactions include the $k_3$ reactions of $HCO_3^-$ and $H_2O$.

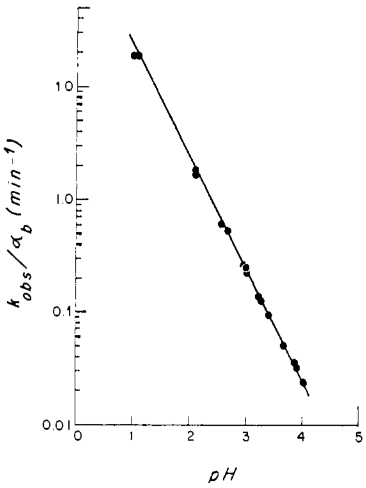


Figure 7. The rate of DPIC formation as a function of pH at 25.0°, ionic strength = 1.0 $M$. The four points at pH below 2.1 were obtained from runs in dilute hydrochloric acid. All other points are from runs in carboxylic acid buffers, extrapolated to zero buffer concentration. The line is calculated from eq 7.

was determined spectrophotometrically at 25.0°, ionic strength 1.0 $M$. The data for the specific acid catalyzed reaction are presented in Figure 7. The slope of the line gives a value of 235 $M^{-1}$ min$^{-1}$ for $k_{\mathrm{H}^+}$. All of the points above pH 2.5 were obtained by extrapolating rates determined in buffers to zero buffer concentration. The points below pH 2.5 were obtained from runs in dilute hydrochloric acid solutions. Buffer catalysis of DPIC formation is small but definite and is shown in Figure 8. The catalysis is general acid catalysis because of the increasing effectiveness of formate buffers with decreasing pH, and because the catalysis by acetic acid is less than that by formic acid. Rate constants for formic and acetic acid were calculated according to eq 7 and are listed in Table III. The average value of

Table III. DPIC Formation and Hydrolysis in Acid Solutions[a]

| Catalyst | $pK_a$ | $k_a$, $M^{-1}$ min$^{-1}$ | $K_{ab}$ | $k_{b,\mathrm{calcd}}$, $M^{-1}$ min$^{-1}$ |
|---|---|---|---|---|
| $CH_3COOH$ | 4.70 | 0.028 | 1.78 | 0.016 |
| HCOOH | 3.52 | 0.086 | 26.7 | 0.0032 |
| $H^+$ | −1.57 | 235 | $8.9 \times 10^4$ | $4.8 \times 10^{-6}$ |

[a] The terms are defined in the Results section.

$k_a$ for formic acid from the runs at three pH values is 0.086 with a range of 0.079–0.098 $M^{-1}$ min$^{-1}$.

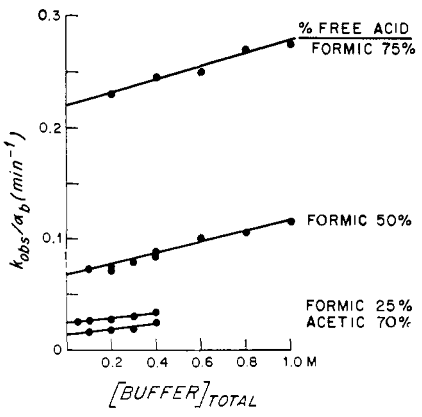


Figure 8. General acid catalysis of DPIC formation at 25.0°, ionic strength 1.0 *M*.

From these data and the equilibrium constants, the catalytic constants were calculated for the reverse reaction, DPIC hydrolysis under acidic conditions. The equilibrium constant for the general acid–base catalyzed reaction (eq 8 and 9) is $K_{ab}$ which is the product of $K_{eq}$, the equilibrium constant reported in Table I, and $K_a$, the dissociation constant of the general acid catalyst. The values of $k_b$ were calculated from the experimental values of $k_a$ and $K_{ab}$ according to eq 9. The rate constant for water-catalyzed DPIC

$$\text{AEF} + \text{HA} \underset{k_b}{\overset{k_a}{\rightleftharpoons}} \text{DPIC}^+ + \text{A}^- \qquad (8)$$

$$K_{ab} = k_a/k_b = K_{eq}K_a \qquad (9)$$

hydrolysis was similarly calculated from $K_{eq}$ (eq 1) and $k_a$ for the solvated proton and divided by 55.5 *M*. The calculated rate constants, $k_b$, for water, formate, and acetate ion catalysis of DPIC hydrolysis are plotted as open triangles in Figure 6. There is good agreement between the calculated water value and the experimental value obtained directly from the pH-independent rates of DPIC hydrolysis near neutral pH. In addition, the calculated values for formate and acetate fall close to the line determined by the data for buffer catalysis of DPIC hydrolysis at more alkaline pH. These results are in accord with the requirement that the mechanism of DPIC formation in acidic solutions must be the microscopic reverse of the mechanism of DPIC hydrolysis represented by the $k_2$ term in eq 5.

**Reaction of DPIC with Hydrazine.** The disappearance of DPIC absorbance in hydrazine buffers proceeds by two sequential first-order reactions. Some representative runs are shown in Figure 9. This behavior was seen only with hydrazine; the reaction in all other buffers followed pseudo-first-order kinetics accurately for over 90% of the reaction.

We will consider two possible explanations for the hydrazine reactions according to Scheme I. According to the first, the initial rapid reaction represents the reversible reaction of hydrazine with DPIC to form intermediates II and III, which subsequently react with water to form the product, AEF, in the slower reaction. The pathway through I in this case is negligible. In the second possible explanation, the most important pathway is through intermediate I, which is similar to the pathway proposed for catalysis by other buffers. However, a small percentage (<10%) of the starting material reacts with hydrazine to form II and III on a side pathway, and these intermediates are eventually converted to AEF *via* $k_N$ during the slow reaction.

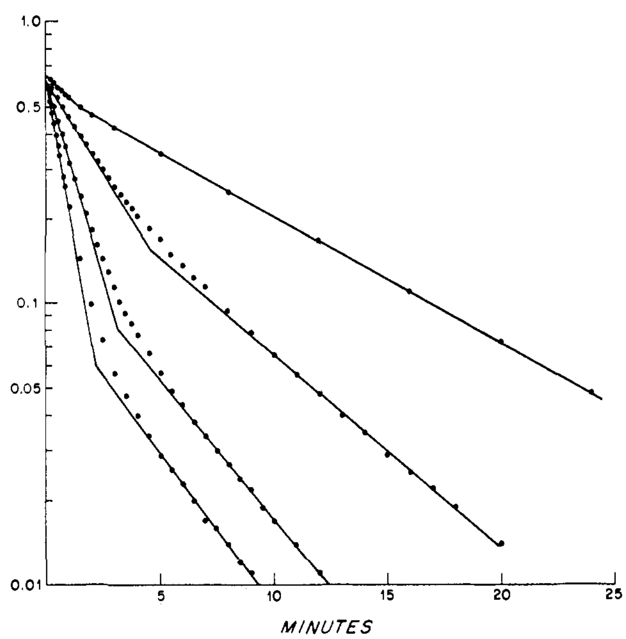
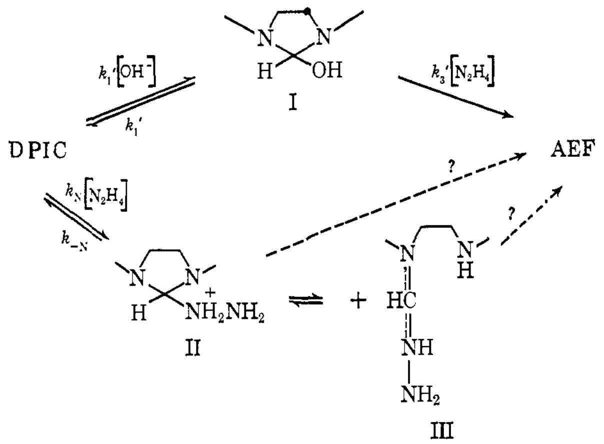


Figure 9. Hydrolysis of DPIC in hydrazine buffers at 25.0°. The conditions are: upper curve, 0.20 *M* hydrazine, pH 8.42; lower three curves 0.10, 0.20, and 0.30 *M* hydrazine (from top to bottom), all at pH 9.02. The concentrations refer to total hydrazine buffer.

**Scheme I**



The following lines of evidence indicate that no large amount of intermediates accumulates during the course of these reactions, and, therefore, the first explanation is substantially ruled out.

1. Ultraviolet spectra obtained by rapid scanning of the reaction mixtures immediately after the initial reaction showed definite but only small differences compared to spectra of solutions containing only DPIC and AEF. This is consistent with the formation of AEF during the rapid reaction as the major product. If an intermediate is accumulating, it must have a spectrum similar to AEF.
2. If the rapid reactions represent the reversible accumulation of intermediates II and III, then at pH values >8.80 (Figure 9) more than 90% of the initial amount of DPIC present should have been converted to the intermediates after the initial rapid reaction.

Rapid dilution of the hydrazine after the initial reaction and before a significant amount of the slow reaction occurs would be expected to regenerate almost all of the DPIC initially present. Five experiments were performed in 0.20 *M* hydrazine buffers at pH 8.80 and pH 9.10. The reaction mixtures were diluted either 15-fold or 21-fold with 1 *M* potassium chloride immediately after the initial rapid reaction. The absorbance of these solutions at 310 m$\mu$ was recorded at intervals after 20–30 sec following dilution. In each case, a small increase in absorbance at 310 m$\mu$ was observed followed by a slow decrease. The maximum absorbance at 310 m$\mu$ after dilution could represent regeneration of no more than 5% of the original amount of DPIC present after correction for dilution.

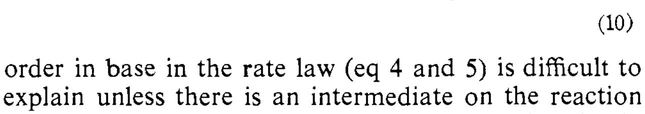
3. The experiments described below indicate that the major product of the initial rapid reactions is AEF, and that in addition, a small amount of an intermediate is also produced which can be converted back to DPIC by decreasing the pH.

The reaction of DPIC in 0.2 *M* hydrazine buffers at pH 9.0–9.5 was allowed to proceed nearly to completion ($\approx$95%). Small volumes of hydrochloric acid or acidic buffers were then added to bring the pH to values between 7.0 and 8.5. (These solutions at pH 7.0–8.5 will be referred to as solutions A.) The decrease in pH resulted in small increases in absorbance around 310 m$\mu$, consistent with the formation of *small* amounts of DPIC. The resulting spectrum agrees well with the calculated spectrum of a mixture of DPIC and AEF in proportions of 10 and 90%, respectively, with the total concentration equal to the initial concentration of DPIC present. The following experiments provide further evidence that the solutions A contain mainly AEF and small amounts of DPIC.

(a) Aliquots of solutions A were transferred to buffers at three different pH values below pH 5. The formation of DPIC from these solutions occurred with rates identical with the rates of formation of DPIC from AEF solutions under the same conditions. In addition, the yield of DPIC, based on the ultraviolet absorbance was essentially equal to the amount of DPIC originally used to prepare solutions A.

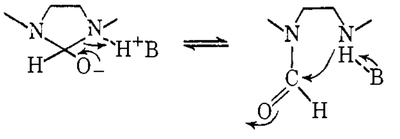
(b) To provide evidence that the solutions A contain small amounts of DPIC, aliquots of the solutions A were transferred to hydrazine buffers at pH values above 8.5 and the decrease in absorbance at 310 m$\mu$ was followed. In each case the reactions followed biphasic kinetics in good agreement with the reactions of DPIC under the same conditions.

## Discussion

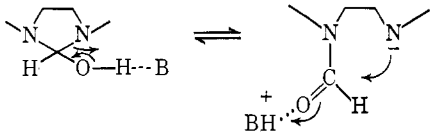


(10)

The results support a mechanism for DPIC hydrolysis in which the general acid–base catalyzed breakdown of a tetrahedral intermediate is the rate-determining step, according to eq 10. The presence of the term second order in base in the rate law (eq 4 and 5) is difficult to explain unless there is an intermediate on the reaction pathway. Other reactions which are second order in base have been interpreted similarly.[8] Nucleophilic catalysis can be ruled out for three reasons. First, the reaction shows little sensitivity to steric requirements as is shown, for example, by the reactivity of *sym*-collidine and triethanolamine. Nucleophilic reactions of carbonyl compounds show marked sensitivity to steric hindrance in the nucleophile[9] but general acid–base catalysis is much less sensitive to steric effects.[10] Second, the catalytic constants of the bases for DPIC hydrolysis do not deviate by more than a factor of ten from the lines in the Brønsted plots (with the exception of carbonate and bicarbonate ions). In contrast, the rate constants for the reactions of nucleophilic reagents of similar basicity but different structure with carbonyl compounds vary over a range of several powers of ten.[9] Third, it is difficult to explain base catalysis according to the $k_3$ term by a mechanism which involves nucleophilic catalysis, particularly in the case of tertiary amines.



(11)



(12)

The rate-determining step of the reaction according to the $k_3$ term in the rate law could be accounted for by mechanisms 11 or 12. We will now show that mechanism 12 can be excluded because the nitrogen anion which would be formed is thermodynamically so unstable that its formation as a free intermediate would require some step of the reverse reaction to occur at a greater than diffusion-controlled rate. The procedure will be given for the pyridine-catalyzed reaction, which may be written

$$\mathrm{DPIC^+ + OH^- + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AEF^- + BH^+} \qquad (13)$$

$$K' = \frac{k_1}{k_{-1}} = \frac{[\mathrm{AEF^-}][\mathrm{BH^+}]}{[\mathrm{DPIC^+}][\mathrm{OH^-}][\mathrm{B}]} \qquad (14)$$

The value of $K'$ can be calculated from the values of the dissociation constant of AEF, the equilibrium constant of reaction 1 (Table I), and the dissociation constant of the pyridinium ion. The dissociation constant of AEF to form AEF$^-$ is unknown but we will assume that it is approximately equal to that of aniline, for which a p*K* of 27 has been reported.[11] The resulting value of $K'$ is $10^{-13}$ $M^{-1}$. If $k_{-1}$ is assigned a maximum value of $10^{10}$ $M^{-1}$ sec$^{-1}$, corresponding to the rate constant for a diffusion-controlled reaction, the

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[8] L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p 350; R. G. Pearson and E. A. Mayerle, *J. Am. Chem. Soc.*, **73**, 926 (1951); S. S. Biechler and R. W. Taft, Jr., *ibid.*, **79**, 4927 (1957); P. M. Mader, *ibid.*, **87**, 3191 (1965); R. L. Schowen, H. Jayaraman, and L. Kershner, *ibid.*, **88**, 3373 (1966); M. Zanger, C. A. Vander Werf, and W. E. McEwen, *ibid.*, **81**, 3806 (1959).

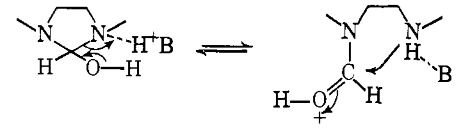
[9] W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960).

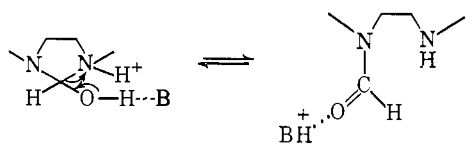
[10] R. P. Bell, M. H. Rand, and K. M. A. Wynne-Jones, *Trans. Faraday Soc.*, **52**, 1093 (1956); F. Covitz and F. H. Westheimer, *J. Am. Chem. Soc.*, **85**, 1773 (1963).

[11] D. Dolman and R. Stewart, personal communication.

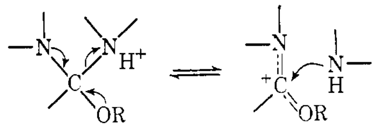
calculated value of $k_1$ is $10^{-3}$ $M^{-2}$ sec$^{-1}$. However, the observed rate constant for pyridine-catalyzed DPIC hydrolysis is $0.8 \times 10^3$ $M^{-2}$ sec$^{-1}$, which exceeds the calculated rate constant by a factor of $\sim 10^6$. Similarly, calculations for phosphate dianion and imidazole give calculated third-order rate constants which are smaller than the observed rate constants by factors of $\sim 10^5$. Therefore, mechanism 12 for the $k_3$ term requires that the reverse reaction, $k_{-1}$, exceed a diffusion-controlled rate by factors of up to $\sim 10^6$ in order to account for the observed rates. The principal assumption in this calculation is that the p$K$ for aniline is the same as the p$K$ of AEF and that this value is valid for aqueous solutions. However, in order to affect the conclusions, the p$K$ estimate would have to be falsely high by at least six pH units.

The reactions according to the $k_2$ terms could proceed by mechanisms 15 or 16. A choice between these two mechanisms may be made on the basis of a comparison of the Brønsted slopes of Figures 5 and 6. Relationships between Brønsted coefficients and other structure–reactivity relationships for nucleophilic reactions with carbonyl compounds have been described previously.[12] Equations based on the Brønsted and Hammett equations and the considerations of Hammond[13] and Leffler[14] predict that increasing reactivity of carbonyl compounds caused by electron-withdrawing substituents results in smaller $\alpha$ values for general acid catalysis; *i.e.*, less catalysis is required for a more reactive substrate. Analogous equations for general base catalysis can be derived readily. It would be expected that the value of $\beta$ for the general base catalyzed attack of an amine on a protonated amide (mechanism 15, in the reverse direction) would be smaller than for attack on the much less reactive free amide (mechanism 11). The fact that the value of $\beta$ for the $k_2$ term (0.44) is *larger* than that for the $k_3$ term (0.26) is inconsistent with the predictions of these structure–reactivity correlations according to mechanism 15 and suggests that the $k_2$ reaction follows mechanism 16.


(15)


(16)

Another method for resolving the kinetic ambiguity of mechanisms 15 and 16 is to compare this step of amidine hydrolysis to an analogous reaction in which one of the protons is replaced by an alkyl group which cannot be removed by a base catalyst. Such a comparison may be made to the first step of imido ester aminolysis, which proceeds through a cationic transition state and is identical with the breakdown of the tetrahedral amidine intermediate except that an alkyl group is substituted for hydrogen on the oxygen atom (eq 15a).[15] The reaction of eq 15a is not subject to general acid–base catalysis so that if amidine hydrolysis proceeded through the comparable mechanism 15 general acid catalysis would not be expected, contrary to what is observed. The occurrence of general acid–base catalysis in the $k_2'$ step of amidine hydrolysis may be attributed to the presence of a proton on the oxygen atom which can be removed by a general base catalyst to provide additional driving force for amine expulsion according to mechanism 16.


(15a)

For those simple carbonyl group reactions in which there is evidence favoring one or the other of the kinetically equivalent mechanisms of catalysis, it appears that the addition of weak nucleophiles to aldehydes involves proton transfer to and from the carbonyl oxygen atom, while the preferred mechanism for general base catalysis involves proton transfer to and from the nucleophile.[16] Mechanisms 11 and 16, in the direction from right to left, represent the general base and general acid catalyzed addition, respectively, of a weak nucleophile to an amide. These mechanisms are in accord with the previous generalizations for simple carbonyl addition reactions and suggest that these generalizations can be extended to a carbonyl compound at the acyl level of oxidation. In each case, the preferred mechanism of catalysis appears to be that which avoids the formation of unstable intermediates, the amine anion of mechanism 12 and the protonated carbonyl group of mechanism 15.

The Bronsted $\alpha$ value of 0.56 ($\alpha = 1 - \beta$) for the attack of the aniline nitrogen atom on an amide (eq 16 in the reverse direction) is considerably larger than the $\alpha$ value of 0.25 for the attack of aniline on *p*-chlorobenzaldehyde.[17] Presumably a larger amount of proton transfer is required for attack at an amide carbonyl group than for attack at the much more reactive aldehyde.

**One-Encounter Mechanisms.** Although it is difficult to deal with the exact timing of processes at or near the transition state, it is worthwhile to consider in more detail the relationship of the proton transfers to other parts of these reactions. In order to form the product of the reaction from the neutral tetrahedral intermediate, a proton must be removed from the hydroxyl group and donated to the aniline nitrogen leaving group. In the mechanisms discussed previously, these proton transfers occur in a stepwise manner with the formation of free and in some cases, unstable intermediates (*e.g.*, mechanism 11). Alternatively, the proton transfers may occur by two general mechanisms which avoid the formation of *free* intermediates: a one-encounter mechanism in which both proton transfers take place during the lifetime of a single encounter of the catalyst with the tetrahedral intermediate ($<10^{-10}$ sec), or a truly concerted mechanism in which

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[12] (a) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 4319 (1962); (b) G. E. Lienhard and W. P. Jencks, *ibid.*, **88**, 3982 (1966); (c) C. G. Swain and J. C. Worosz, *Tetrahedron Letters*, 3199 (1965).

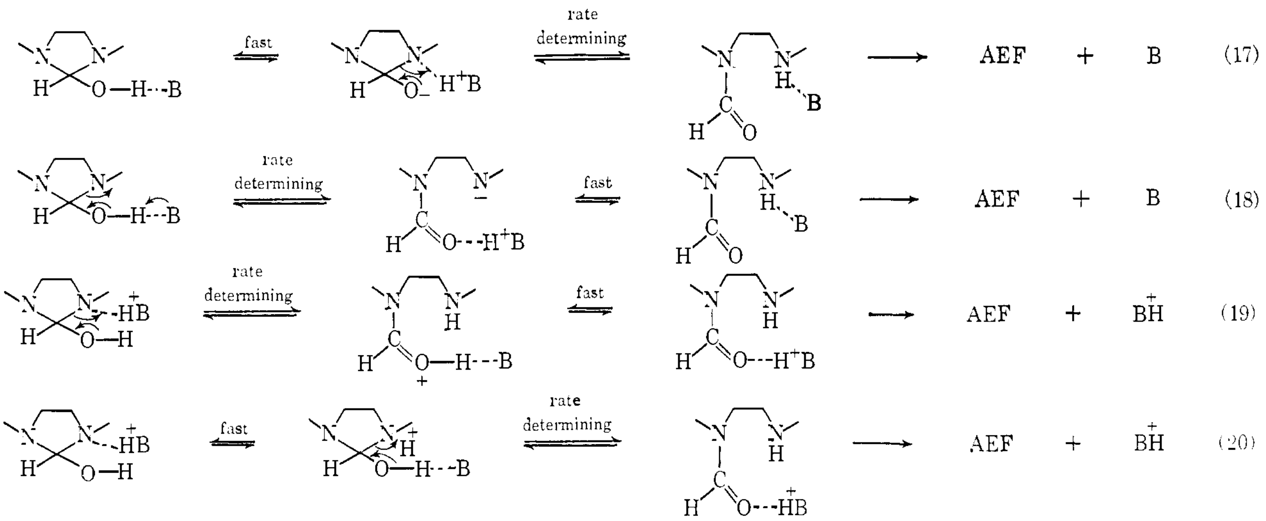
[13] G. S. Hammond, *J. Am. Chem. Soc.*, 77, 334 (1955).

[14] J. E. Leffler, *Science*, **117**, 340 (1953).

[15] E. S. Hand and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 3505 (1962).

[16] W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).

[17] E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 832 (1962).

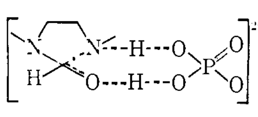


both proton transfers take place simultaneously in the transition state.[12b,18,19]

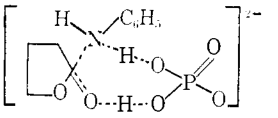
Two possible one-encounter mechanisms are shown for both the $k_3$ and the $k_2$ reactions in eq 17–20. For the $k_3$ reactions mechanism 17, which is analogous to mechanism 11, is preferred because it avoids the formation of the unstable aniline anion which is formed in 18. The formation of the aniline anion as a free intermediate (mechanism 12) has been ruled out because of its instability. In mechanism 18 the same nitrogen anion is formed as the immediate product and cannot be greatly stabilized by $BH^+$ because $BH^+$ is hydrogen bonded to the carbonyl oxygen until after the nitrogen anion is formed. By definition, there is no proton transfer from $BH^+$ to nitrogen in 18 until after the formation of the nitrogen anion.

The reactions according to the $k_2$ terms could proceed by the one-encounter mechanisms 19 or 20, which are analogous to the two-encounter mechanisms 15 and 16, respectively. Of the two one-encounter mechanisms, 20 is preferred for the same reasons that 16 is preferred to 15.

**Possible Bifunctional Acid–Base Catalysis.** In a few instances, catalysts which have both proton donor and acceptor sites react more rapidly than expected for their basicity. The $k_3$ term for phosphate dianion is greater than that for methyl phosphate dianion by a factor of eight. As discussed above, either the $k_3$ term for bicarbonate ion or the $k_2$ term for carbonate ion or both are larger than predicted by the Brønsted plots by factors of *ca.* 10–30. The increased reactivity of these compounds, and presumably arsenate also, may be explained by bifunctional acid–base catalysis (IV), in which concerted transfer of both protons takes place during the rate-determining step. This mechanism might more simply be regarded as a modification of mechanism 11 in which some extra stabilization of the transition state is achieved by hydrogen bonding of an acidic group to the anionic oxygen atom of the tetrahedral intermediate. The transition state IV is similar to that for the reaction of N-phenyliminotetrahydrofuran to form aniline and $\gamma$-butyrolactone (V), described by Cunningham and Schmir.[19] In the latter reaction bifunctional catalysts are more effective than monofunctional catalysts of comparable acidity by factors of over 200. In contrast, there is relatively little extra stabilization of the transition state for the DPIC reaction by bifunctional catalysts.

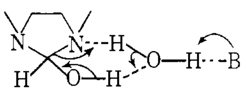


IV



V

The fact that the reactivity of tertiary amines in DPIC hydrolysis is similar to that of other amines and is only slightly smaller than that of phosphate ion means that the reaction can be catalyzed effectively by catalysts which cannot undergo direct bifunctional proton transfer. A form of bifunctional proton transfer by these and other monofunctional compounds could conceivably take place through one or more intervening water molecules (VI).[18] However, the extra reactivity seen with phosphate and bicarbonate ions is not readily explained by this mechanism and it is not clear why the base in mechanism VI should provide a driving force for concerted proton transfer in both directions so that water can act as an acid and base catalyst at the same time. It should be noted that in all of the mechanisms involving monofunctional acid–base catalysis, the proton transfers may proceed through one or more intervening water molecules.



VI

**Possible Significance of the $k_3$ Term for Water.** The maximum possible value of the $k_3$ term for water is 14 $M^{-1}$ min$^{-1}$. This value is lower than the value predicted by the line in the Bronsted plot in Figure 5 by a factor of 30. The actual negative deviation of the $k_3$ term for water must be larger than a factor of 30 since the value above contains a contribution from the $k_2$ term for hydroxide ion. A possible explanation of this negative deviation is that the reaction proceeds according to mechanism 11 and has reached the diffusion-controlled limit.

A rough calculation suggests that this explanation is not unreasonable. The reaction according to mech-

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(18) M. Eigen, *Discussions Faraday Soc.*, **39**, 7 (1965).

(19) B. A. Cunningham and G. L. Schmir, *J. Am. Chem. Soc.*, **88**, 551 (1966).

anism 11 is described by eq 21. An upper limit for the

$$\mathrm{DPIC}^+ + \mathrm{OH}^- \overset{K_1}{\rightleftharpoons} \text{intermediate} \overset{\overset{K_2}{\pm\mathrm{H}^+}}{\rightleftharpoons} \text{intermediate}^- \xrightarrow{k_x\mathrm{BH}^+} \mathrm{AEF} + \mathrm{B} \qquad (21)$$

value of $K_1$ of $10^3$ $M^{-1}$ is based on the fact that there is no spectral evidence for the accumulation of intermediates during DPIC hydrolysis at pH values up to 11.0 and the value of $K_2$ is estimated to be on the order of $10^{-12}$ $M$. The value of $k_x$ from the *observed* upper limit for the $k_3$ term of 14 $M^{-1}$ min$^{-1}$ is then *ca.* $10^{10}$ $M^{-1}$ sec$^{-1}$. The catalytic constants for mechanism 11 in the direction from left to right may be plotted based on the acidity of the acids $BH^+$ to give a Brønsted slope $\alpha$ of 0.74, instead of in the manner shown in Figure 5 to give a slope $\beta$ of 0.26. Such a plot exhibits a negative deviation of the point for $H_3O^+$ at or near the diffusion-controlled limit, corresponding to the break described by Eigen for simple proton-transfer reactions,[20] but differs from the plot for such reactions in that the slope below the diffusion-controlled limit is 0.74 instead of 1.0 over a large range of catalyst acidity. This suggests that carbon–nitrogen bond cleavage is taking place in addition to proton transfer in the transition state when the reaction proceeds at less than the diffusion-controlled rate. A similar break at the diffusion-controlled limit has been observed for hydroxide ion in the base-catalyzed decomposition of hemithioacetals.[21]

In conclusion, the most likely mechanisms (11 and 17) for the $k_3$ reaction both involve proton transfer to the leaving group in the rate-determining step. Removal of the proton from the hydroxyl group of the tetrahedral intermediate by a base B presumably occurs in a preequilibrium step either with (mechanism 11) or without (mechanism 17) diffusion of $BH^+$ away from the resulting anion before the rate-determining step. The most likely mechanisms for the $k_2$ reaction are those of eq 16 and 20. Concerted transfer of both protons apparently provides only a small advantage.

**Deuterium Isotope Effect.** The deuterium isotope effect was determined for the triethylenediamine-catalyzed hydrolysis of DPIC according to the $k_3$ term in the rate law (eq 5). The results are shown in Figure 10 in a plot based on eq 22 which is kinetically equivalent to the $k_3$ term in eq 5. The other terms in the rate law are insignificant under these conditions. The observed isotope effect, $k_{4,\mathrm{H}}/k_{4,\mathrm{D}}$ is 0.6.

$$v = k_4[\mathrm{DPIC}][\mathrm{BH}^+]a_{\mathrm{OH}^-}{}^2(\text{or } a_{\mathrm{OD}^-})^2 \qquad (22)$$

The $k_4$ term is expressed in terms of mechanism 21 in eq 23. The rate-determining step according to mechanism 21 is preceded by two equilibria, $K_1$ and $K_2$. If it is assumed that the isotope effect on $K_1$ is close to 1.0[22] and that the isotope effects on $K_2$ and $K_w$ are approximately the same,[23] then the kinetic isotope effect on the rate-determining step, $k_x^{\mathrm{H}}/k_x^{\mathrm{D}}$, is also approximately 0.6 (eq 23). This value is only a rough

$$\frac{k_4^{\mathrm{H}}}{k_4^{\mathrm{D}}} = \frac{k_x^{\mathrm{H}}K_1^{\mathrm{H}}K_2^{\mathrm{H}}K_w^{\mathrm{D}}}{k_x^{\mathrm{D}}K_1^{\mathrm{D}}K_2^{\mathrm{D}}K_w^{\mathrm{H}}} = 0.6 \qquad (23)$$

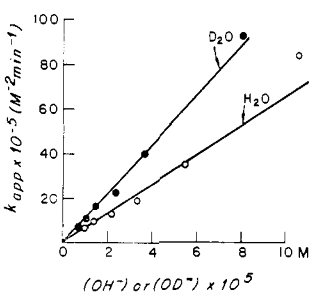
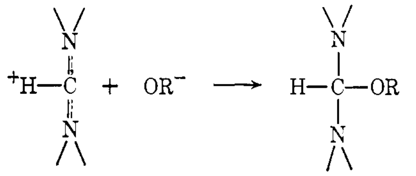


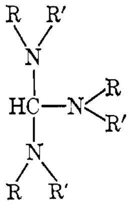
Figure 10. Deuterium isotope effect on the triethylenediamine-catalyzed hydrolysis of DPIC. The apparent third-order rate constant $k_{\mathrm{app}}$ is equal to $(k_{\mathrm{obs}} - k_0)/[\mathrm{BH}^+]a_{\mathrm{OH}^-}$ (or $a_{\mathrm{OD}^-}$).

estimate because of the assumptions involved but it indicates that the true value of $k_x^{\mathrm{H}}/k_x^{\mathrm{D}}$ is not far from one. Small or inverse deuterium isotope effects have been observed in a number of other general acid–base catalyzed reactions.[12a,24]

**Related Reactions.** The formation of a tetrahedral addition compound by the addition of hydroxide ion to the cationic amidine in a preequilibrium step should be favored by electron-withdrawing substituents on the phenyl groups, which will destabilize the starting material by withdrawing electrons from the amidinium system by both inductive and resonance effects. This is probably the principal factor which contributes to the large $\rho$ value of 3.6 for the hydrolysis of N,N′-diphenylamidinium ions substituted on both phenyl groups.[3b] The pH-independent hydrolysis of these compounds corresponds to the $k_2$ term for the hydrolysis of DPIC with B = $H_2O$.

Precedent for the addition of hydroxide ion to DPIC to form a tetrahedral intermediate is found in the addition of alkoxide ions to formamidinium ions (formed from two molecules of secondary amines) to give alkoxydiaminomethanes, which are stable in the cold in the absence of water (eq 24).[25] A number of triamino-methanes (VII) are known, isolable compounds and although they are thermodynamically unstable with respect to breakdown to formamidinium ions and amines in the presence of protons, the trisacylamino-


(24)


VII

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[20] M. Eigen, *Angew. Chem. Intern. Ed. Engl.*, **3**, 1 (1964).

[21] R. Barnett and W. P. Jencks, *J. Am. Chem. Soc.*, **89**, 5963 (1967).

[22] C. A. Bunton and V. J. Shiner, Jr., *ibid.*, **83**, 3207 (1961).

[23] P. Ballinger and F. H. Long, *ibid.*, **82**, 795 (1960).

[24] A. Williams and M. L. Bender, *ibid.*, **88**, 2508 (1966); B. M. Anderson and W. P. Jencks, *ibid.*, **82**, 1773 (1960); Y. Pocker, *Proc. Chem. Soc.*, 17, (1960); W. H. Hamill and V. K. Lamer, *J. Chem. Phys.*, **4**, 395 (1936).

[25] H. Bredereck, F. Effenberger, and G. Simchen, *Angew. Chem. Intern. Ed. Engl.*, **1**, 331 (1962); D. H. Clemens, E. Y. Shropshire, and W. D. Emmons, *J. Org. Chem.*, 27, 3664 (1962).

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.

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[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.

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# Mechanism and Catalysis of the Hydrolysis of Methenyltetrahydrofolic Acid

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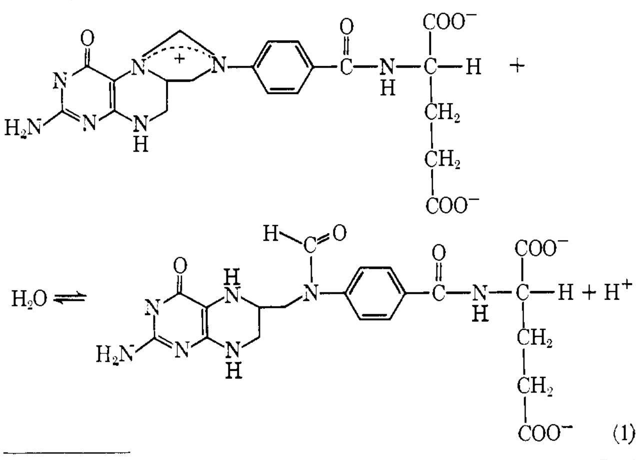
**Abstract:** The hydrolysis of N$^{5,10}$-methenyltetrahydrofolate (MTF) to N$^{10}$-formyltetrahydrofolate has been studied over the pH range 7.2–10.0 at 25° and ionic strength 1.0 *M*. A p*K* value of 8.95 for MTF was determined spectrophotometrically and is attributed to the dissociation of the amide group in the pteridine ring. The hydrolysis of MTF at low buffer concentrations follows the rate law $v = k_2[\mathrm{MTF}][\mathrm{B}] + k_3[\mathrm{MTF}][\mathrm{B}]a_{\mathrm{OH}^-}$, 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)

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