

**Role of Water in the Imidazole-Catalyzed Hydrolysis  
of *p*-Nitrotrifluoroacetanilide. A Study of  
Solvation in Acetonitrile–Water Mixtures<sup>1</sup>**John W. Henderson<sup>2</sup> and Paul Haake\**Department of Chemistry, Wesleyan University, Middletown, Connecticut 06457**Received December 27, 1976*

Solvation by water in the hydrolysis of *p*-nitrotrifluoroacetanilide has been studied through measurement of the rates of the imidazole-catalyzed hydrolysis as a function of water concentration in mixtures of acetonitrile and water. There is a complicated dependence on water with three apparently distinct regions of behavior: at  $[H_2O] < [imidazole] = 1\text{ M}$ , linear dependence of rate constant on water concentration; at  $[H_2O]$  from 1 to 10 M, linear dependence on  $[H_2O]$  with a lower slope; and at  $[H_2O] > 10\text{ M}$ , approximately fourth order in water. These results are related to probable mechanisms.

A common observation in the crystallography of enzymes has been the low concentration of water at active sites, particularly after a model substrate is bound.<sup>3</sup> This appears to be an expected result of the evolution of enzymes because of the need for strong enzyme–substrate interactions which enable substrate to be held in an oriented, even strained, conformation. Water in the active site would interfere with oriented binding if it solvated those highly polar functionalities which make significant contributions to the total binding energy between enzyme and substrate.

Understanding of enzyme function has progressed far in recent years due to structural results from crystallography, enzymology, and mechanistic results from studies of the fundamental chemistry of the reaction which is catalyzed. Yet, there are still important gaps in our knowledge of enzyme function, partly due to incompleteness of the structural information<sup>4</sup> and partly due to our lack of understanding of the catalytic and inhibitory roles that water plays in the fundamental chemistry of the reaction. We expect that the enzyme must play the catalytic roles of water in the reaction and some of the catalytic power of enzymes may be due to exclusion of inhibitory effects of water. For example, a nucleophile might be expected to be more reactive when it is stripped of hydrogen bonds.

Therefore, it appears that an important area of fundamental chemistry, necessary for total understanding of enzyme function, is a knowledge of the role of water in reactions of biological importance. Because of the importance of enzymes which catalyze the cleavage of amide bonds, we have studied the imidazole-catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide, *p*-CF<sub>3</sub>C(O)NHC<sub>6</sub>H<sub>4</sub>NO<sub>2</sub> (pNTA) in acetonitrile–water at variable water concentrations. This amide, and closely related amides, have been previously studied in aqueous solution.<sup>5–11</sup> The results that we have found appear

to be of interest both with regard to the roles of water in catalyzed hydrolysis of amides and with regard to the utility of this experimental method in studying the effects of water on chemical reactions.

**Experimental Section**

**Materials.** *p*-Nitrotrifluoroacetanilide (pNTA) was prepared from *p*-nitroaniline and trifluoroacetic anhydride,<sup>12</sup> and was recrystallized twice from ethanol–water, once from chloroform–hexane, and a final time from ethanol–water: mp 152.5–154 °C (lit.<sup>12</sup> 151.5–153 °C), IR 1745 cm<sup>-1</sup> (C=O), UV (CH<sub>3</sub>CN) 298 nm ( $\epsilon$  12 900), 219 nm ( $\epsilon$  10 700). **Imidazole** was recrystallized three times from benzene, mp 88.5–91.5 °C (lit.<sup>13</sup> 88–90). Perchloric acid (J. T. Baker “Analyzed”) was determined by titration to be 70.74% HClO<sub>4</sub> (w/w). **Water** was distilled, boiled to remove CO<sub>2</sub>, and stored under Ascarite. **1-Methylimidazole** was obtained from Aldrich Chemical Co. **Zinc perchlorate hexahydrate** was determined by EDTA titration to be 70.0% Zn(ClO<sub>4</sub>)<sub>2</sub>, i.e., Zn(ClO<sub>4</sub>)<sub>2</sub>·6.3H<sub>2</sub>O. Acetonitrile was MCB “Spectroquality” grade which we analyzed by gas chromatography and showed to contain less than 10<sup>-3</sup> M water and undetectable amounts of other impurities in chromatography on a Poropak column.<sup>14</sup>

**Kinetic Method.** Reactions were followed in acetonitrile–water mixtures on a Cary 16 spectrophotometer (thermostatted at 30.9 °C) by measuring the appearance of *p*-nitroaniline near 370 nm; the wavelength was adjusted to the  $\lambda_{max}$  which varies with the solvent. Buffers were prepared using imidazole and HClO<sub>4</sub>. Solutions were prepared with all components except pNTA in a 10-mL volumetric flask, equilibrated in the constant-temperature bath, pNTA was added, and the solution was brought to the mark with additional solvent to give 10<sup>-4</sup> M pNTA. We decided not to do these reactions at constant ionic strength because of the complications resulting from solvation of an additional solute.

**Treatment of Data.** For reactions with  $t_{1/2}$  less than ca. 2 h, the experimental infinity point obtained after ca. 10 half-lives was used for the calculation of the rate constant by the least-squares method. However, pNTA reacts so slowly under many of our conditions that it was inaccurate to use the experimental infinity point. The rate

**Table I. Dependence of the Rate Constant on Imidazole Concentration at a Constant Imidazolium Ion Concentration of 0.1 M at Different Water Concentrations**

[Imidazole], M	[Imidazole] [Imidazo- lium]	$10^6 k_{\text{obsd}}, \text{s}^{-1}$		
		[H <sub>2</sub> O] = 0.7 M	5.0 M	45 M
0.01	1:10	~0	0.13	5.7
0.033	1:3	0.1	0.47	13
0.10	1:1	0.32	1.4	51
0.30	3:1	1.1	4.5	200
1.00	10:1	4.4	16.0	520

**Table II. Dependence of the Rate Constant on the Imidazolium Ion Concentration at a Constant Imidazole Concentration of 0.1 M at Different Water Concentrations**

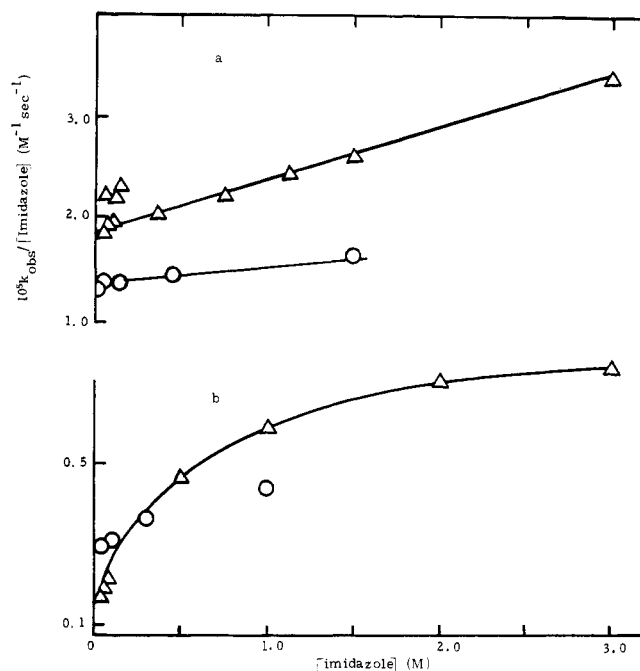
[Imidazo- lium], M	[Imidazo- lium] [Imidazole]	$10^6 k_{\text{obsd}}, \text{s}^{-1}$		
		[H <sub>2</sub> O] = 0.7 M	5.0 M	45 M
0.01	1:10	0.20	1.1	94
0.033	1:3	0.28	1.2	59
0.10	1:1	0.32	1.4	51
0.30	3:1	0.28	1.6	51
1.00	10:1	1.8	4.1	41

constants for slower reactions were calculated using the theoretical infinity point which was measured using *p*-nitroaniline (recrystallized from ethanol-water) in the reaction mixtures and with the other product, trifluoroacetic acid. Duplicate determinations of the rate constant were made for two of our slowest reactions; for  $k_{\text{obsd}} = 5.83 \times 10^{-7} \text{ s}^{-1}$  and  $k_{\text{obsd}} = 2.27 \times 10^{-6} \text{ s}^{-1}$  the rate constants agreed within 9 and 6%, respectively.

### Results

**Product Identity.** In the cases in which an infinity solution could be obtained, the UV spectrum of the solution is the same as that measured with *p*-nitroaniline, trifluoroacetic acid, and the reagents. The alternative product to trifluoroacetate, *N*-trifluoroacetylimidazole, is much more reactive than pNTA; *N*-trifluoroacetylimidazole hydrolyzed "instantaneously" at room temperature,<sup>15</sup> while pNTA hydrolyzed under similar conditions with a half-life of greater than 20 min.<sup>11</sup> While these observations do not preclude the acylimidazole as a reactive intermediate, previous results on this reaction<sup>5-11</sup> indicate water as the nucleophile in attack at the acyl carbon of pNTA. Our results (vide infra) demonstrate that at low water concentrations the reaction of pNTA requires one water molecule and, at [H<sub>2</sub>O] extrapolated to zero, the rate is indistinguishable from zero; this is strong evidence that water is the nucleophile especially since the rate of reaction of *p*-nitrophenyl acetate in similar media does not depend greatly on [H<sub>2</sub>O] at very low water concentrations and infrared spectra have demonstrated formation of an acyl imidazole.<sup>14</sup> Therefore, in the studies reported in this paper, we believe it is reasonable to conclude that water is the nucleophile and hydrolysis is the reaction.

**Dependence of the Rate Constant on the Concentrations of Imidazole, Imidazolium Ion, and Hydroxide Ion.** The dependence of the rate constant for the hydrolysis of pNTA on the concentration of imidazole at constant imidazolium concentration and its dependence on the concentration of imidazolium ion at constant imidazole concentration has been determined at 0.7, 5.0, and 45 M water (Tables I and II). The results indicate that the rate constant is dependent on the concentration of imidazole but essentially independent of the concentration of imidazolium ion in all three solvent

**Figure 1.** Plots of  $k_{\text{obsd}}/[\text{Imidazole}]$  vs.  $[\text{Imidazole}]$ : (a) 5 M water (O), 10 M water ( $\Delta$ ); (b) 0.7 M water (O), 1 M water ( $\Delta$ ).

systems, although there do appear to be salt effects (Table II). Figure 1 presents the results of the study of the dependence of the rate constant for hydrolysis of pNTA over a wide range of concentration of 10:1 imidazole/imidazolium ion in four solvent systems; in the light of Table II (no dependence on concentration of imidazolium ion), these results reflect the dependence on imidazole concentration. In 45 M water the rate constant increases linearly with the increase in imidazole concentration in agreement with the report<sup>11</sup> of the first-order imidazole dependence of the rate constant in water. However, in the four solvent systems displayed in Figure 1 the rate constants show a second-order component (eq 1). The scatter at low [imidazole] (Figure 1) is probably due to experimental error which is large because of the very slow rates (Table I).

$$k_{\text{obsd}} = k_1[\text{imidazole}] + k_2[\text{imidazole}]^2 \quad (1)$$

As shown by the linearity of the graphs (Figure 1a) of  $k_{\text{obsd}}/[\text{Im}]$  vs.  $[\text{Im}]$  in 5 M water,  $k_1 = 1.4 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$  and  $k_2 = 2.3 \times 10^{-6} \text{ M}^{-2} \text{ s}^{-1}$ . In 10 M water,  $k_1 = 1.9 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$  and  $k_2 = 7.7 \times 10^{-6} \text{ M}^{-2} \text{ s}^{-1}$ . However, the results in 0.7 and 1 M water (Figure 1b) show curvature (Figure 1); this is due to the fact that Figure 1b includes data both for water in excess of imidazole and for imidazole in excess of water as explained in the Discussion. In 45 M water,  $k_1 = 5.3 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ . All the results extrapolate to within experimental error of  $k_{\text{obsd}} = 0$  at zero concentration of imidazole, indicating little or no contribution to the rate from hydroxide in these buffered systems. These results also indicate that at 1 M imidazole, the concentration used in most of our studies, the reaction is primarily first order in imidazole.

**Dependence of the Rate Constant on the Concentration of Water.** The dependence of the rate constant for the hydrolysis of pNTA in CH<sub>3</sub>CN-H<sub>2</sub>O on the concentration of water has been studied in three systems (Table III): 1.0 M imidazole/0.1 M imidazolium; 2.0 M imidazole/0.2 M imidazolium; and 1.0 M *N*-methylimidazole/0.17 M *N*-methylimidazolium. The apparent pH of the 1.0 M imidazole/0.1 M imidazolium system varied by 0.6 pH unit over the solvent range we used, indicating considerable variation in  $\text{p}K_a$  with solvent.<sup>16</sup> Since the hydroxide-catalyzed rate is  $\ll k_1[\text{imidazole}]$ , we consider this pH variation to be insignificant in our rate

**Table III. Dependence of the Rate Constant on Water Concentration in Different Buffer Systems**

[H <sub>2</sub> O] M	10 <sup>6</sup> k <sub>obsd</sub> , s <sup>-1</sup>		
	[Imidazole] = 1.0 M [Imidazolium] = 0.1 M	2.0 M 0.2 M	[N-Methylimidazole] = 1.0 M [N-Methylimidazolium] = 0.17 M
0.12	1.0 <sup>a</sup>		
0.23	1.8		
0.50	3.5	7.5	1.6
0.70	4.4		
0.75	4.5	10.5	
1.00	5.9	13.9	2.9
1.25	6.7	16.4	3.5
1.50	7.6	19.0	4.1
2.00		22.8	
2.50	10.4		6.8
3.00		29.0	
5.00	16.0	39.7	9.8
7.50	20.7		13.0
10.0	26.2	68.0	16.7
20.0	53.5		24.2
30.0	117		88.3
36.0	210		
40.0	294		237
42.0	357		
45.0	520		
48.0	750		
50.0			588

<sup>a</sup> From extrapolation of values determined at 0.5 M imidazole-0.05 M imidazolium and 0.1 M imidazole-0.01 M imidazolium.

studies. The rate constants in Table III are not corrected for ionization of pNTA to its unreactive anion.<sup>11</sup> We observe no UV absorbance due to the anion at or below 10 M water. Above 10 M water, spectral evidence was obtained for the anion of pNTA; consequently, in analyzing the data we corrected the rate constants (see below).

The dependence of the rate constant on water concentration in the 1.0 M imidazole/0.1 M imidazolium system is plotted in Figure 2. The plot appears separable into three regions: low, medium, and high water concentrations. We use eq 2-4 as a basis for interpretation of the results.

$$k_{\text{obsd}} = k_0 + k_1[\text{H}_2\text{O}] \quad (2)$$

For [H<sub>2</sub>O] = 0 to 1 M; k<sub>0</sub> = extrapolated intercept at [H<sub>2</sub>O] = 0; k<sub>1</sub> determined by dependence of k<sub>obsd</sub> on [H<sub>2</sub>O] at [H<sub>2</sub>O] < 1.1 M.

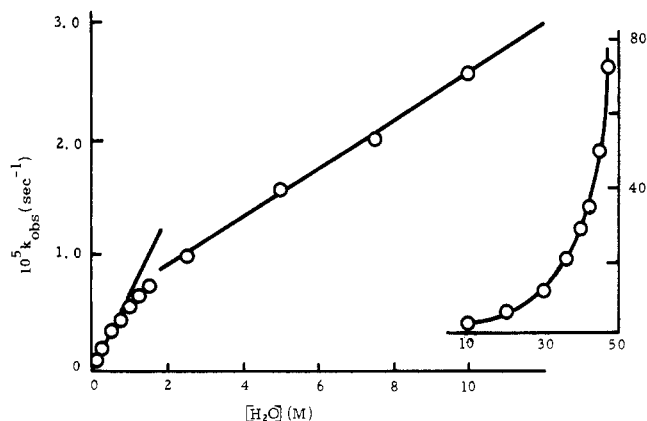
$$k_{\text{obsd}} = k_{1.1} + k_2[\text{H}_2\text{O}] \quad (3)$$

For [H<sub>2</sub>O] = 1 to 10 M; k<sub>1.1</sub> = intersection at 1.1 M H<sub>2</sub>O of lines determining k<sub>1</sub> and k<sub>2</sub>; k<sub>2</sub> determined by dependence of k<sub>obsd</sub> on [H<sub>2</sub>O] at 1.1 M < [H<sub>2</sub>O] < 10 M.

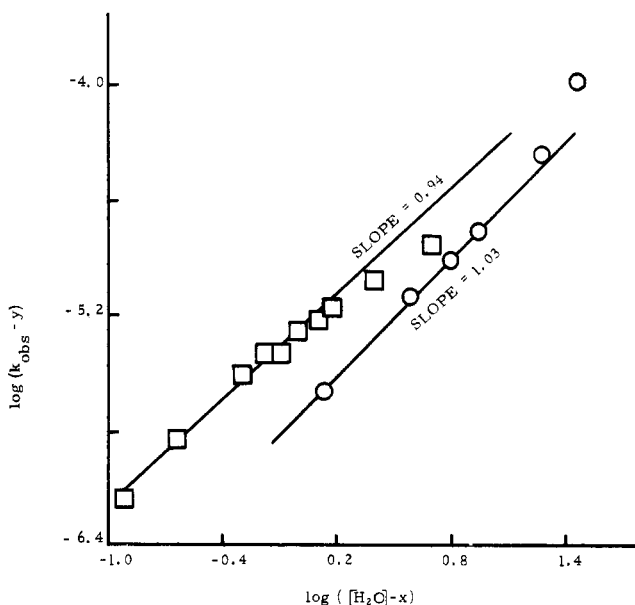
$$k_{\text{obsd}} = k_{10} + k_3[\text{H}_2\text{O}]^n \quad (4)$$

For [H<sub>2</sub>O] = 10 to 55 M; k<sub>10</sub> = intersection at 10 M H<sub>2</sub>O of lines determining k<sub>2</sub> and k<sub>3</sub>; k<sub>3</sub> and n determined by dependence of k<sub>obsd</sub> on [H<sub>2</sub>O] at [H<sub>2</sub>O] > 10 M.

In the medium water concentration, ca. 2.5 to 10 M, the rate constant is linearly dependent on the water concentration (slope = k<sub>2</sub> = 2.1 × 10<sup>-6</sup> M<sup>-1</sup> s<sup>-1</sup>), indicating that the reaction is first order in water in this region. In the low water region the rate constants at and below 0.5 M water show an initial linear dependence on water concentration (slope = k<sub>1</sub> = 6.45 × 10<sup>-6</sup> M<sup>-1</sup> s<sup>-1</sup>; intercept = k<sub>0</sub> = 2.8 × 10<sup>-7</sup> s<sup>-1</sup>). The intercept, k<sub>0</sub>, appears to be within experimental error of zero if one includes the possibility of water as an impurity in the acetonitrile or



**Figure 2.** Plot of the rate constant vs. water concentration for 1.0 M imidazole/0.1 M imidazolium ion.

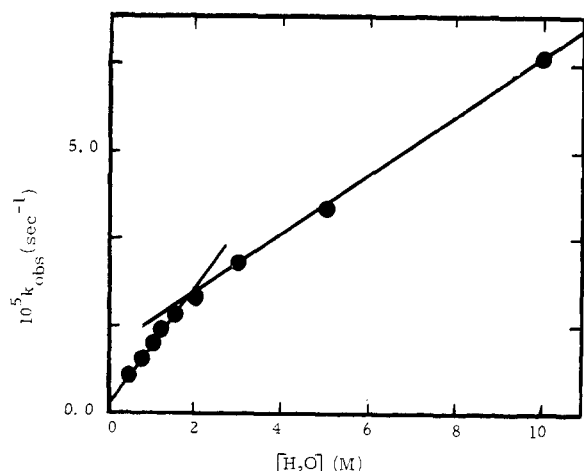


**Figure 3.** Plot of the log of the effective rate constant (1.0 M imidazole/0.1 M imidazolium) vs. the log of the effective water concentration for the low (□) and medium (○) water regions, (□) x = 0, y = 0.3 × 10<sup>-6</sup> s<sup>-1</sup>, (○) x = 1 M, y = 7.7 × 10<sup>-6</sup> s<sup>-1</sup>.

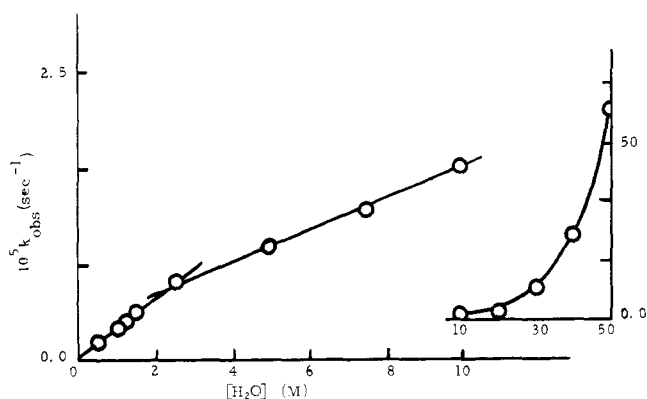
imidazole reagents. The two straight lines for low and medium [H<sub>2</sub>O] intersect at 1.1 M water and k<sub>1.1</sub> = 7.7 × 10<sup>-6</sup> s<sup>-1</sup> (eq 3 and Figure 2).

We were concerned about more than one rate term making contributions in each of these regions of water concentration; that is, one might expect that k<sub>obsd</sub> = Σk<sub>i</sub>[H<sub>2</sub>O]<sup>n<sub>i</sub></sup>. Therefore, we utilized a graphic determination of n<sub>i</sub> for each region from the slope of the plot of log(k<sub>obsd</sub> - other terms) vs. log [H<sub>2</sub>O] in that region. The upper line (□) of Figure 3 is such a plot for the low water region of Figure 2. The six points from 0.12 to 1.0 M describe a straight line with a nearly unit slope after subtraction of the intercept (using eq 2) from k<sub>obsd</sub>, implying that the reaction is first order in water in that region. The lower line (○) of Figure 3 is a similar plot for the medium water region after the values for water concentration (1.1 M) and rate constant (k<sub>1.1</sub> = 7.7 × 10<sup>-6</sup> s<sup>-1</sup>) at the intersection of the two lines in Figure 2 are subtracted from each point (eq 3). As expected from the linearity of the medium water region in Figure 2, the four points from 2.5 to 10 M water describe a straight line with unit slope, confirming the first-order dependence of the rate constant on water concentration in the medium water region.

The dependence of the rate constant on water concentration in the 2.0 M imidazole/0.2 M imidazolium system is plotted



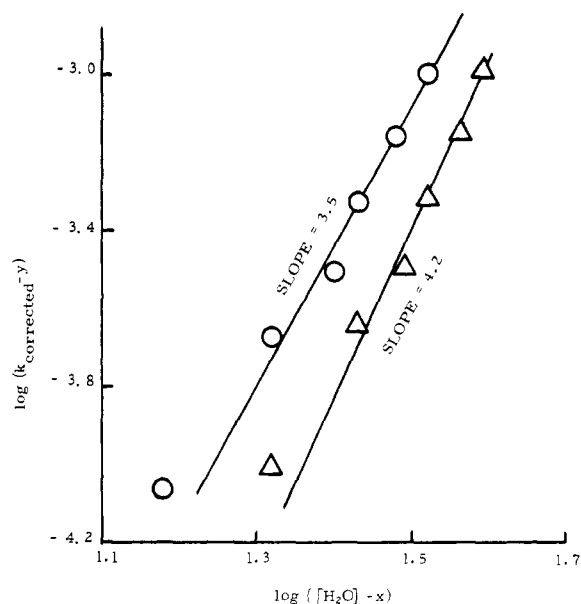
**Figure 4.** Plot of the rate constant for the 2.0 M imidazole/0.2 M imidazolium catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide vs. water concentration.



**Figure 5.** Plot of the rate constant for the 1.0 M *N*-methylimidazole/0.17 M *N*-methylimidazolium catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide vs. water concentration.

in Figure 4. Just as in the 1.0 M imidazole/0.1 M imidazolium system (Figure 2), the plot shows two water dependence regions in the range studied. In the medium water region the three rate constants from 3.0 to 10.0 M water show a linear dependence on the water concentration ( $k_2 = 5.6 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$ ). In the low water region, the five rate constants at  $[\text{H}_2\text{O}] < 2 \text{ M}$  show a linear dependence on water concentration ( $k_1 = 11.6 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$ ; intercept =  $k_0 = 1.9 \times 10^{-6} \text{ s}^{-1}$ ). The two lines intersect at 1.7 M water and  $k = 21.5 \times 10^{-6} \text{ s}^{-1}$ . The linearity of the dependence of the rate constant on water concentration in both the low and medium water regions of Figure 4 implies that the reaction is first order in water in both regions. This is confirmed by analysis of the data using graphs similar to Figure 3. In the low water region the five points from 0.5 to 1.5 M water describe a straight line with a slope of 1.01 after subtraction of the intercept from  $k_{\text{obsd}}$ . In the medium water region, after subtraction of the values for water concentration and rate constant at the intersection of the two lines in Figure 4 from each point, the three points from 3.0 to 10.0 M water describe a straight line with a slope of 1.00.

The dependence of the rate constant on water concentration in the 1.0 M *N*-methylimidazole/0.17 M *N*-methylimidazolium system is plotted in Figure 5. Just as in the 1.0 M imidazole/0.1 M imidazolium system (Figures 2 and 3), the plot shows three water dependence regions. In the medium water region the four rate constants from 2.5 to 10.0 M water show a linear dependence on water concentration ( $k_2 = 1.3 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$ ). In the low water region the four rate constants at and below 1.5 M show a linear dependence on water concentration ( $k_1 = 2.5 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$ ; intercept =  $k_0 = 3.2 \times 10^{-7}$



**Figure 6.** Plot of the rate constant (corrected for anion concentration) for the 1.0 M imidazole/0.1 M imidazolium catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide vs. the log of the effective water concentration for the region of high  $[\text{H}_2\text{O}]$ , ( $\Delta$ )  $x = 9 \text{ M}$ ,  $y = 2.4 \times 10^{-5} \text{ s}^{-1}$ , ( $\circ$ )  $x = 15 \text{ M}$ ,  $y = 3.65 \times 10^{-5} \text{ s}^{-1}$ .

$\text{s}^{-1}$ ). The two lines intersect at 2.5 M water and  $k = 6.7 \times 10^{-6} \text{ s}^{-1}$ . The linearity of the dependence of the rate constant on water concentration in both the low and medium water regions of Figure 5 implies that the reaction is first order in water in both regions. This is confirmed by log-log analysis of the data. In the low water region the four points from 0.5 to 2.5 M water describe a straight line with a slope of 1.01 after subtraction of the intercept from  $k_{\text{obsd}}$ . In the medium water region, after subtraction of the values for water concentration and rate constant at the intersection of the two straight lines in Figure 5 from each point, the three points from 5.0 to 10.0 M water describe a straight line with a slope of 1.05.

Using the values of  $k_1$ ,  $k_2$ , and  $k_{10}$  (eq 2 and 3) for 1.0 M imidazole, we have determined the order of the reaction in water at high water concentration by the method of plotting  $\log(k_{\text{obsd}} - k_{10})$  vs.  $\log[\text{H}_2\text{O}]$  (eq 4). However, a correction of  $k_{\text{obsd}}$  was required because, at concentrations of water greater than 10 M, we observed initial absorption in the electronic absorption spectra of our kinetic solutions due to the unreactive anionic form of *p*-nitrotrifluoroacetanilide. Since the anion is unreactive, we have corrected observed rate constants using eq 5, where the correction factor is calculated from the extinction coefficient of anion in pure water, the anion absorbance, and the ratio of the extinction coefficient of *p*-nitrotrifluoroacetanilide in water and the solvent. This resulted in correction factors as follows: 1.5 for the range 50 to 42 M water, 1.25 for 36 and 40 M water, 1.1 for 30 M water, and 1.02 for 20 M water. Equation 5 was applied in order to get corrected rate constants, which were used in the treatment based on eq 4 (see Figure 6).

$$k_{\text{corr}} = k_{\text{obsd}} \times \text{correction factor} \quad (5)$$

Although there is a problem in determining at what point the medium water region ends and the high water region begins and thus what values for the water concentration and rate constant should be subtracted from each point in order to apply eq 4 to the data, Figure 6 indicates, by subtracting rate constant and water concentration values which seem to be high and low limits for the transition points, that in the high water region the imidazole-catalyzed reaction is fourth order in water. This does not mean that lower and higher order

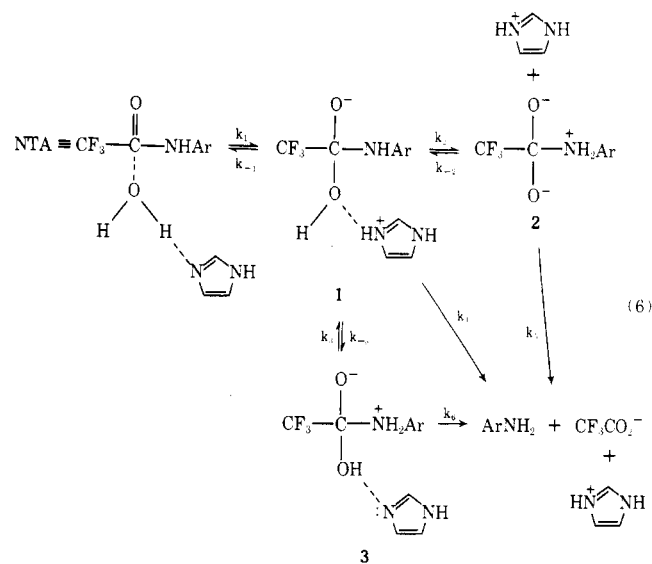
water terms are nonexistent; at the lower ends of the lines in Figure 6, the deviations of the lowest points indicate contributions from terms lower than fourth order in water. However, the excellent fit of the other points indicates that the predominant contribution to the rate is fourth order in the high region of water concentration. We find similar results when we analyze the *N*-methylimidazole data in the same way. Using the rate constants in Table III, the data from 30 to 48 M yield  $k_3 = 3.3 \times 10^{-10} \text{ M}^{-4} \text{ s}^{-1}$  for the reaction carried out at 1 M imidazole and 0.1 M imidazolium ion.

### Discussion

**General Comments.** It appears that the results are well enough defined to attempt empirical correlations leading to understanding the catalytic and inhibitory roles of water in this reaction, the hydrolysis of an amide—therefore, a reaction of biological importance. However, we emphasize at the beginning of the discussion that, although these results are encouraging, this research is only a beginning on the very difficult problem of elucidating the effects of water on a molecule along a reaction coordinate. One concern with such studies of solvation is that the activity of each component of the system should be known at each solvent composition in order to rigorously analyze observations. However, we believe that this is not necessary for progress on the problem of solvation because: (1) There has been great progress made in an empirical understanding of acid-catalyzed reactions in strongly acidic solutions.<sup>17</sup> This progress has been made largely on an empirical basis in media which change much more drastically than the mixed solvents we have employed. (2) Our cosolvent with water, acetonitrile, has major advantages: (a) It has a high dielectric constant and the dipole moment,  $\mu = 4.0$ , is even larger than that of water,  $\mu = 1.9$ , so association, ion pairing, aggregation and nonspecific solvent effects should be minimal. (b) Specific hydrogen-bonding and proton-transfer effects in these media should be entirely due to the water, not the acetonitrile, because acetonitrile has no protons which can compete in proton donation with water protons and acetonitrile appears to be  $10^8$  less basic than water ( $\text{p}K_a$  for  $\text{CH}_3\text{CNH}^+ = -10$ ).<sup>18</sup> Nevertheless, the hypotheses in this discussion must be regarded as tentative until more research is done.

We need to consider our results in terms of mechanism for three regions of water concentration which differ considerably; the low, medium, and high water regions may be characterized approximately by powers of ten differences in water concentration: 0.5, 5, and 50 M. Of particular concern is the question of the location of the transition state along the reaction coordinate in each region, since our solvation numbers indicate the extent of solvation of *transition state over ground state*. The scheme shown in eq 6 will serve as the basis for our discussion. The dependence on water and imidazole (see Results) indicates that both molecules play essential roles. Water as a nucleophile is indicated by the negligible rate at  $[\text{H}_2\text{O}] = 0$  as distinct from the imidazole-catalyzed reaction of *p*-nitrophenyl acetate which has been shown to proceed through an acylimidazole and gives a significant rate at  $[\text{H}_2\text{O}] = 0$ . The role of imidazole is demonstrated in Table I and Figure 1a,b. The complicated dependence of  $k_{\text{obsd}}$  on  $[\text{imidazole}]$  in Figure 1b is expected from the results (Table III and Figures 2, 4, and 5) which demonstrate the importance of the imidazole/water ratio; one water molecule is essential for reaction and there is a decreased dependence on  $[\text{H}_2\text{O}]$  as the imidazole/water ratio changes from greater than one to less than one (Figure 2). In Figure 1a the imidazole/water ratio is always considerably greater than one and the water concentrations are in the well-behaved, medium range of water concentration, but in Figure 1b there are data for  $[\text{H}_2\text{O}]$  both greater than and less

than  $[\text{imidazole}]$ , so the curved dependence is not surprising.



Both Figure 1a and 1b indicate some contribution to the rate by a term second order in imidazole. Although this is a minor contribution to the total rate, this indicates to us that the rate-determining step is not addition to the carbonyl group using the analogy to reactions of aryl esters.<sup>19</sup> In addition, previous research on the hydrolysis of amides leads one to expect rate-determining cleavage of the C–N bond.<sup>20</sup> The fourth order dependence on water appears to be additional evidence for this rate-determining step. Therefore, we suggest the following analysis of the roles of water in this reaction.

(1) **The Low  $[\text{H}_2\text{O}]$  Region, 0.1–1 M.** The strong, first-order dependence on  $[\text{H}_2\text{O}]$  certainly appears to be the water molecule that is the nucleophile. Although  $k_4$ ,  $k_5$ , and  $k_6$  could contribute to the formation of product, the need for a good leaving group makes  $k_5$  and  $k_6$  most likely, especially in low water concentrations where the activity of an amide anion would increase dramatically. Because the reaction is predominantly first order in imidazole, either  $k_1$  is rate determining in the  $k_6$  pathway or  $k_6$  must involve imidazole as a base, abstracting the OH proton in the transition state. Because there is a significant second-order contribution from imidazole, we suggest that the second imidazole may act as a base to remove the O–H proton as the imidazolium ion formed in  $k_1$  donates a proton to N. However, other hypotheses are possible, such as two imidazoles hydrogen bonded to one nucleophilic water with  $k_1$  rate determining.

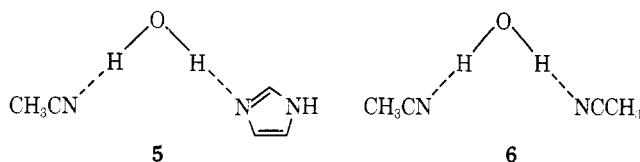
(2) **The Medium  $[\text{H}_2\text{O}]$  Region, 1–10 M.** We suggest that the second water molecule (the first being the nucleophile) is involved in the proton transfer required for  $1 \rightarrow$  products. Grunwald and co-workers<sup>21</sup> have found that one water molecule is needed for proton transfer similar to that required for  $1 \rightarrow 2$ . Even in the region of  $[\text{H}_2\text{O}] = 1$  to 10 M, it should be difficult to eliminate amide anion from 1; however, cleavage of 2 to give *p*-nitroaniline should be a facile process particularly since it also generates the resonance-stabilized  $\text{CF}_3\text{CO}_2^-$  ion with increased C–O bond strengths. The charged form of the reactive intermediate, 2, is not unprecedented.<sup>5,22</sup> Therefore, proton transfer is critical and would appear to be the most critical function for a water molecule involved in solvation.

It is possible that the increased rate of reaction in the region of  $[\text{H}_2\text{O}] = 1$  to 10 M is a bulk solvent effect, but the well-behaved first-order behavior in this region (Figures 2, 3), rather than some curved dependence, leads us to believe that there is a specific, proton-transfer role for a water molecule which leads to this preferred pathway in this region of  $[\text{H}_2\text{O}]$

concentration. We will comment on the availability of water molecules below.

(3) **The High H<sub>2</sub>O Region:** Our results demonstrate that the reaction is predominantly fourth order in water (Figure 6) in addition to the two water molecules implicated by the first-order dependence on water in the low and medium ranges of [H<sub>2</sub>O]. Since Figure 6 is based on solvation changes beyond 10 M water, it appears that the latter two water molecules are already in the ground state in the high water region. Since one water is the nucleophile, this leads to a total solvation number of 5. The solvation results indicate a highly solvated transition state which we suggest closely resembles the highly reactive intermediate **2**, which decomposes rapidly to products (*k*<sub>5</sub> very fast).<sup>5</sup> Therefore, we suggest that solvation of **2** is a suitable model for solvation of the transition state when water is readily available and that the high degree of hydration when water is readily available appears to be associated with hydrogen bonding to the O<sup>-</sup> atoms of **2**.

**In summary**, we suggest that the structure of the transition state is always near **2** but may vary in structure, solvation, and energy depending on the availability of water. It is noteworthy that three regions of water composition found experimentally to have differing solvation for the reaction are also distinguishable in the relationship of [H<sub>2</sub>O] to concentrations of other components: (1) In the low water region, [H<sub>2</sub>O] < [imidazole] so that as [H<sub>2</sub>O] increases the concentration of **5** increases. The intersection of the *k*<sub>1</sub> and *k*<sub>2</sub> lines in Figure 2 is within experimental error of the water concentration required to solvate all imidazole as in **5** and to solvate each imidazolium ion with two water molecules as proton acceptors in hydrogen bonds. (2) The medium [H<sub>2</sub>O] region, 1–10 M water, is the region in which all the water should be predominantly present as **5** or **6**. (3) In the high [H<sub>2</sub>O] region, there will be free OH groups and hydrogen bonds between water molecules.



The above discussion demonstrates ways that solvation studies such as the one reported here will be useful in our understanding of solvation, structures of transition states, and

solvent structure. As our studies of hydration in acyl transfer reactions proceed, we expect to be able to draw general conclusions which will substantiate these suggestions or will enable us to modify them and which will enable us to draw firm conclusions about the need for functional groups on enzymes to replace critical water molecules in order to lower activation barriers.

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**Registry No.**—pNTA, 404-27-3; acetonitrile, 75-05-8; imidazole, 288-32-4; water, 7732-18-5.

### References and Notes

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## On the Photochemistry of 1-Oxaspiro[2.n]alkan-5-ones

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Irradiation of an ether solution of 4,4-dimethyl-1-oxaspiro[2.4]heptan-5-one gives 4-isopropylidenepentanolide and 2-isopropylidenepentane-1,5-dial in yields of 65 and 5%, respectively. Irradiation of 4,4,7,7-tetramethyl-1-oxaspiro[2.5]octan-5-one under comparable conditions affords 3,3-dimethyl-5-isopropylidenehexanolide and 4,4-dimethyl-2-isopropylidenehexane-1,6-dial in yields of 45 and 20%, respectively. The photoproducts resulting from these reactions are readily accounted for by the general scheme we have previously advanced for the photochemistry of  $\beta,\gamma$ -epoxy cyclic ketones. These results suggest that the photochemistry previously reported for a 1-oxaspiro[2.3]-hexan-5-one, though typical of that for other cyclobutanones, is not characteristic of 1-oxaspiro[2.n]alkan-5-ones.

Recently we have suggested a general scheme to account for the photochemistry of  $\beta,\gamma$ -epoxy cyclic ketones.<sup>2</sup> It is proposed that irradiation of a  $\beta,\gamma$ -epoxy cyclic ketone **1** (Scheme I) initially leads to Norrish type I bond cleavage and

the formation of an apparent diradical species **2** which undergoes subsequent ring opening to give the acyl alkoxy diradical **3**. Unless specific substituent and/or skeletal constraints are present, product formation proceeds from **3** by