

# Metal Ion Promoted Hydroxide Ion and Water Catalyzed Hydrolysis of Amides. Effects of the Acyl Group and the Leaving Group

Thomas H. Fife\* and Ramesh Bembli

Contribution from the Department of Biochemistry, University of Southern California, Los Angeles, California 90033

Received February 2, 1993

**Abstract:** The hydrolysis of anilide derivatives of *N*-methyl-4-nitroaniline in H<sub>2</sub>O is hydroxide ion catalyzed; plots of log  $k_{\text{obsd}}$  vs pH are linear with slopes of 1.0 at pH > 8. When there is a chelating functional group in the acyl portion of the molecule (1,10-phenanthroline or 6-carboxypicolinoyl) and in the presence of saturating concentrations of Cu<sup>2+</sup>, Co<sup>2+</sup>, or Zn<sup>2+</sup>, hydroxide ion catalyzed reactions are observed at pH > 4 (rate enhancements range from 10<sup>4</sup>- to 10<sup>5</sup>-fold), and pH-independent reactions occur from pH 1 to 4. Rate constants are very nearly the same in the metal ion promoted hydrolysis reactions for the anilides with these acyl groups. Thus, the negatively charged carboxylate ligand of *N*-(6-carboxypicolinoyl)-*N*-methyl-4-nitroaniline is equally good in facilitating the metal ion promoted hydrolysis reactions as the neutral ligands of the 1,10-phenanthroline derivative. A trifluoroacetyl acyl group produces the same general effect as a chelated metal ion, i.e., a facile OH<sup>-</sup>-catalyzed reaction and a pH-independent reaction at pH < 6. The metal ions very likely exert their principal effect in a similar manner, by strong electron withdrawal from the carbonyl group. The hydroxide ion catalyzed hydrolysis of *N*-(trifluoroacetyl)-2,4-dinitroaniline at pH > 6 is favorable ( $k_{\text{OH}'} = 1.0 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  at 50 °C), even though the  $\text{p}K_{\text{a}}$  of the anilide N-H function is 5.5 and the second-order rate constant ( $k_{\text{OH}'}$ ) is that for attack of OH<sup>-</sup> on the ionized species. Amide deprotonation does not have a large effect on the rate of hydrolysis; ionization of the amide N-H function of *N*-(trifluoroacetyl)-2-carboxy-4-nitroaniline produces only a 7-fold decrease in  $k_{\text{OH}}$ . An *N*-methyl group accelerates the rate of hydrolysis of the (trifluoroacetyl)-2,4-dinitroanilide. *N*-(Trifluoroacetyl)-*N*-methyl-2,4-dinitroaniline hydrolyzes very rapidly at 50 °C,  $k_{\text{OH}} = 5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  and  $k_0 = 10^{-2} \text{ s}^{-1}$ . The pH-independent reaction governed by  $k_0$  proceeds 2.1-fold more slowly in D<sub>2</sub>O than in H<sub>2</sub>O. General base catalysis occurs, and the Bronsted coefficient  $\beta$  is 0.3.

Carboxylate esters with chelating functional groups in the molecule are highly susceptible to metal ion promotion of the hydroxide ion catalyzed hydrolysis reaction.<sup>1-6</sup> Extremely large rate enhancements (10<sup>8</sup>-10<sup>9</sup>-fold) have been observed in these hydrolysis reactions in comparison with the reactions in the absence of metal ions.<sup>7</sup> The effects of divalent metal ions on the rates of hydrolysis of amides have also been extensively studied.<sup>8-16</sup> This interest has been in part the result of the X-ray crystallo-

(1) Wells, M. A.; Rogers, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 4336. Wells, M. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 5341.  
(2) Hay, R. W.; Clark, C. R. *J. Chem. Soc., Dalton Trans.* **1977**, 1866, 1993.

(3) Fife, T. H.; Squillacote, V. L. *J. Am. Chem. Soc.* **1978**, *100*, 4787.  
(4) Fife, T. H.; Przystas, T. J.; Squillacote, V. L. *J. Am. Chem. Soc.* **1979**, *101*, 3017.

(5) (a) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 7297. (b) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 2251.

(6) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 1041.  
(7) Fife, T. H.; Pujari, M. P. *J. Am. Chem. Soc.* **1990**, *112*, 5551. The enhancement in  $k_{\text{OH}}$  due to Cu(II) in the hydrolysis of 1,10-phenanthroline-2-carbonyl phosphate of  $5 \times 10^{10}$  is the largest that has been observed in reactions of acyl derivatives.

(8) Hay, R. W. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Ed.; Pergamon: Oxford, 1987; pp 411-485 and references therein.

(9) Fife, T. H. In *Perspectives on Bioinorganic Chemistry*; Hay, R. W., Ed.; JAI Press: London, 1991, Vol. 1, pp 43-93.

(10) (a) Meriwether, L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 5119. (b) Grant, I. J.; Hay, R. W. *Aust. J. Chem.* **1965**, *18*, 1189. (c) Fife, T. H.; Squillacote, V. L. *J. Am. Chem. Soc.* **1977**, *99*, 3762.

(11) (a) Groves, J. T.; Dias, R. M. *J. Am. Chem. Soc.* **1979**, *101*, 1033. (b) Groves, J. T.; Chambers, R. R., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 630. (c) Groves, J. T.; Olson, J. R. *Inorg. Chem.* **1985**, *24*, 2715.

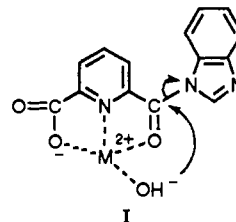
(12) (a) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145. (b) Chin, J.; Jubian, V.; Mrejen, K. *J. Chem. Soc., Chem. Commun.* **1990**, 1326. (c) Breslow, R.; Fairweather, R.; Keana, J. *J. Am. Chem. Soc.* **1967**, *89*, 2135.

(13) Large effects have been obtained with substitution inert Co(III) complexes. (a) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1970**, *92*, 6151. (b) Buckingham, D. A.; Keene, F. R.; Sargeson, A. M. *J. Am. Chem. Soc.* **1974**, *96*, 4981. (c) Schepartz, A.; Breslow, R. *J. Am. Chem. Soc.* **1987**, *109*, 1814.

(14) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1986**, *108*, 4631.

graphic studies of the Zn(II) metalloenzyme carboxypeptidase A at 2-Å resolution, which showed that the metal ion was complexed to the carbonyl oxygen of the poor peptide substrate glycylytyrosine.<sup>17</sup> However, metal ion effects in the nonenzymatic hydrolysis of amides have usually been small or absent.<sup>8-10</sup>

In contrast, metal ion promoted hydroxide ion catalysis occurs with large rate enhancements (10<sup>9</sup> with Cu(II) and 10<sup>5</sup>-10<sup>6</sup> with Ni(II), Co(II), and Zn(II)) in the hydrolysis of *N*-(6-carboxypicolinoyl)benzimidazole (I), with which nucleophilic attack at



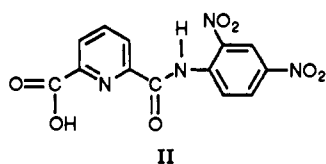
the carbonyl group by OH<sup>-</sup> is rate determining.<sup>14</sup> This reaction must involve an intramolecular attack of metal ion bound OH<sup>-</sup>, since the second-order rate constant  $k_{\text{OH}}$  calculated for bimolecular attack of an external OH<sup>-</sup> on the Cu(II) complex is  $5.4 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$ , a value exceeding that of a diffusion-controlled reaction ( $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ). Likewise, Zn<sup>2+</sup> and Cu<sup>2+</sup> give 10<sup>3</sup>-10<sup>6</sup>-fold rate enhancements in the OH<sup>-</sup>-catalyzed hydrolysis of lactams,<sup>11</sup> and a 10<sup>5</sup> enhancement occurs in the Ni<sup>2+</sup>-promoted OH<sup>-</sup>-catalyzed hydrolysis of the  $\beta$ -lactam *N*-(8-quinolyl)azetidin-2-one.<sup>15</sup> In

(15) Przystas, T. J.; Fife, T. H. *J. Chem. Soc., Perkin Trans. 2* **1990**, 393.

(16) Reddy, K. V.; Jacobson, A. R.; Kung, J. I.; Sayre, L. *Inorg. Chem.* **1991**, *30*, 3520.

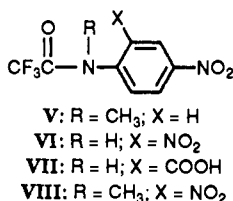
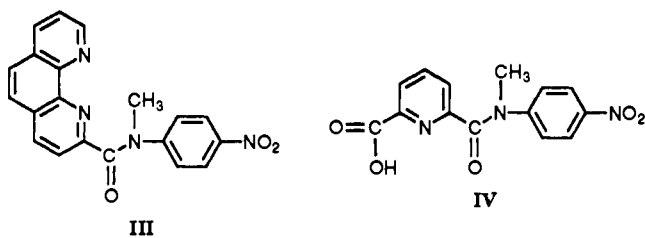
(17) Lipscomb, W. N. *Acc. Chem. Res.* **1970**, *3*, 81. Ludwig, M. L.; Lipscomb, W. N. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; American Elsevier: New York, 1973; pp 438-487.

the latter case it is again probable that nucleophilic attack by OH<sup>-</sup> is the rate-determining step. Metal ion promoted OH<sup>-</sup> reactions are not observed in the hydrolysis of *N*-(6-carboxypicolinoyl)-2,4-dinitroaniline (II) or *N*-(trifluoroacetyl)-2-car-



boxy-8-aminoquinoline at pH > 4; saturating concentrations of Ni<sup>2+</sup>, Co<sup>2+</sup>, and Zn<sup>2+</sup> give rise to pH-independent reactions from pH 4 to 7, and the rate enhancements are only moderate (Cu(II) provides an enhancement of 1000-fold in the pH-independent hydrolysis of the latter compound at pH > 3.5 but is not a catalyst in the hydrolysis of II).<sup>15</sup> The acyl groups of I and II are the same, so the differences in rate and mechanism are strictly due to the different leaving groups. The two types of mechanisms, i.e., OH<sup>-</sup>-dependent or pH-independent, could be the result of different rate-determining steps. With a poor leaving group, the C-N bond breaking reaction of a tetrahedral intermediate should become important. Oxygen-18 exchange between water and the carbonyl group occurs in the alkaline hydrolysis of such amides,<sup>18</sup> which indicates the formation of a tetrahedral species.

It is important to understand how metal ions can affect amide hydrolysis because, in addition to the intrinsic chemical interest, several peptidase enzymes are metalloenzymes, for example, carboxypeptidase A. Much of the difficulty in establishing mechanisms for the enzyme reactions resides in the lack of a conceptual basis for understanding the chemistry of metal ion promoted amide hydrolysis. Systematic studies of metal ion effects in amide hydrolysis are, therefore, required. We have in the present work investigated the hydrolysis reactions of the anilides III-VIII. These anilides are more reactive than the



peptide substrates for enzymes and may hydrolyze with different mechanisms or rate-determining steps.<sup>19</sup> The anilides are highly suitable for establishing the effect of the acyl portion of the molecule, the effect of leaving group, and the effect of a secondary or tertiary amide linkage in metal ion promoted hydrolysis reactions.

(18) (a) Bender, M. L.; Thomas, R. J. *J. Am. Chem. Soc.* **1961**, *83*, 4183. (b) Bunton, C. A.; Nayak, B.; O'Connor, C. *J. Org. Chem.* **1968**, *33*, 572. (c) Slebocka-Tilk, H.; Bennet, A. J.; Keillor, J. W.; Brown, R. S.; Guthrie, J. P.; Jodhan, A. *J. Am. Chem. Soc.* **1990**, *112*, 8507.

(19) Note that, when a peptide binds to the active site of a peptidase enzyme, the peptide becomes reactive. The rate constants in the carboxypeptidase A catalyzed hydrolysis of tripeptides are very large; the second-order rate constants  $k_{cat}/K_m$  are  $10^4$ - $10^5$  M<sup>-1</sup> s<sup>-1</sup> at 25 °C. Auld, D. S.; Vallee, B. L. *Biochemistry* **1970**, *9*, 602. Auld, D. S.; Holmquist, B. *Biochemistry* **1974**, *13*, 4355.

## Experimental Section

**Materials.** *N*-[(1,10-Phenanthrolin-2-yl)carbonyl]-*N*-methyl-4-nitroaniline (III). The acid chloride of 1,10-phenanthroline-2-carboxylic acid was prepared by the method of Sigman<sup>20</sup> and was suspended in dry ether. One equiv of *N*-methyl-4-nitroaniline and 2 equiv of triethylamine, dissolved in ether, were added dropwise. The mixture was stirred overnight and then filtered. The ether was removed by rotary evaporation, and the residue was recrystallized from a chloroform-ether mixture. The compound melted at 217 °C. Anal. Calcd for C<sub>20</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>: C, 67.04; H, 3.91; N, 15.64. Found: C, 66.93; H, 4.02; N, 15.45.

*N*-(6-Carboxypicolinoyl)-*N*-methyl-4-nitroaniline (IV). 2,6-Pyridinedicarboxylic acid was mixed with 1 equiv of trifluoroacetic anhydride. The mixture was allowed to stand for 4 h at room temperature. *N*-methyl-4-nitroaniline (1 equiv) was then added. The mixture was warmed to ensure homogeneity and was allowed to stand overnight. Chloroform was added, and the solution was washed several times with cold water. The chloroform layer was dried over sodium sulfate. The chloroform was removed by rotary evaporation, leaving a light-yellow solid. This solid was recrystallized from acetonitrile and chromatographed over silica gel. The compound melted at 205-206 °C. Anal. Calcd for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>5</sub>: C, 55.81; H, 3.65; N, 13.95. Found: C, 55.69; H, 3.63; N, 14.06. <sup>1</sup>H NMR δ 3.3 (3H) in CDCl<sub>3</sub> vs TMS.

*N*-(Trifluoroacetyl)-*N*-methyl-4-nitroaniline (V). *N*-Methyl-4-nitroaniline in dry benzene was mixed with an excess of trifluoroacetic anhydride. The mixture was allowed to stand for 4 h. The benzene was removed by rotary evaporation. The residue was taken up in hot acetonitrile and filtered. A crystalline precipitate formed upon cooling of this solution. The material was further recrystallized from a chloroform-hexane mixture and melted at 149 °C. Anal. Calcd for C<sub>9</sub>H<sub>7</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>: C, 43.54; H, 2.84; N, 11.29. Found: C, 43.58; H, 2.73; N, 11.41.

*N*-(Trifluoroacetyl)-2,4-dinitroaniline (VI) was prepared by the same method as that used for V. After recrystallization from acetonitrile, the compound melted at 85 °C. Anal. Calcd for C<sub>8</sub>H<sub>4</sub>F<sub>3</sub>N<sub>3</sub>O<sub>5</sub>: C, 34.41; H, 1.44; N, 15.06. Found: C, 34.58; H, 1.61; N, 14.92.

*N*-(Trifluoroacetyl)-2-carboxy-4-nitroaniline (VII) was prepared by the same method as that used for V but in the solvent chloroform. The residue was recrystallized from acetonitrile and then melted at 218 °C. Anal. Calcd for C<sub>9</sub>H<sub>5</sub>F<sub>3</sub>N<sub>2</sub>O<sub>5</sub>: C, 38.85; H, 1.80; N, 10.07. Found: C, 39.07; H, 2.02; N, 10.26.

*N*-(Trifluoroacetyl)-*N*-methyl-2,4-dinitroaniline (VIII) was prepared by the same method as that used for V. After recrystallization from chloroform, the compound melted at 78 °C. Anal. Calcd for C<sub>9</sub>H<sub>6</sub>F<sub>3</sub>N<sub>3</sub>O<sub>5</sub>: C, 36.86; H, 2.05; N, 14.33. Found: C, 36.96; H, 2.15; N, 14.19.

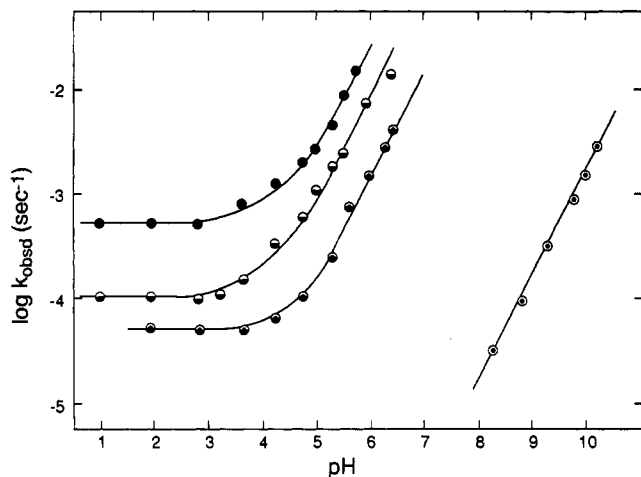
**Kinetic Methods.** The rates of hydrolysis of III-VIII were determined with a Pye-Unicam SP8-100 recording spectrophotometer by monitoring appearance of the *N*-methyl-4-nitroaniline and 4-nitroaniline at 400 nm and *N*-methyl-2,4-dinitroaniline and 2,4-dinitroaniline at 360 nm, with water as the solvent and μ = 0.1 M with KCl. In a typical experiment, 15 μL of the amide stock solution (10<sup>-2</sup> M in acetonitrile) was injected into 3 mL of reactant solution maintained at the desired temperature. The reactions followed excellent pseudo-first-order kinetics for ≥4 half-lives. The rate constants were determined by extrapolation to zero buffer concentration or in 0.02 M buffer in cases where the buffer did not have a significant effect on the rate constants. The product spectra of all the compounds matched the spectra of the appropriate nitroaniline and the carboxylic acid qualitatively and quantitatively.

The buffers employed in the metal ion studies were formate, acetate, and cacodylate. Hydrochloric acid solutions were employed at pH < 3. In the absence of added metal ions, 2 × 10<sup>-5</sup> M EDTA was added to the reaction solutions of III and IV. In the studies of the metal ion promoted reactions of III and IV, saturating concentrations of the metal ions were employed, and the ratio of the metal ion concentration to the substrate concentration was ≥100:1 to ensure formation of a 1:1 complex. The reaction solution pH was measured with a Beckman Model 3500 pH meter, and pD was determined by employing the glass electrode correction equation of Fife and Bruce.<sup>21</sup> The values of  $K_w$  were taken to be 2.04 × 10<sup>-13</sup> at 70 °C, 5.5 × 10<sup>-14</sup> at 50 °C, and 1.47 × 10<sup>-14</sup> at 30 °C. The values of  $K_{D_0}$  were estimated from  $K_{D_0}$  at 25 °C<sup>22</sup> and the assumption that  $K_{D_0}$  varies with temperature as does  $K_w$ .

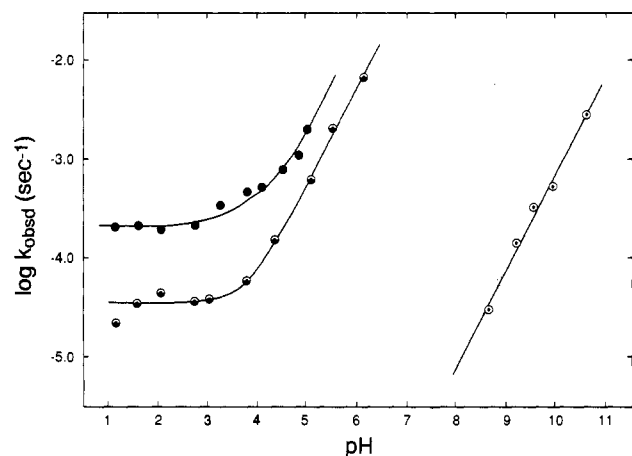
(20) Sigman, D. S.; Wahl, G. M.; Creighton, D. J. *Biochemistry* **1972**, *11*,

(21) Fife, T. H.; Bruce, T. C. *J. Phys. Chem.* **1961**, *65*, 1079.

(22) Kingerley, R. W.; LaMer, V. K. *J. Am. Chem. Soc.* **1941**, *63*, 3256.



**Figure 1.** Plots of  $\log k_{\text{obsd}}$  vs pH for the hydrolysis of *N*-[(1,10-phenanthrolin-2-yl)carbonyl]-*N*-methyl-4-nitroaniline in  $\text{H}_2\text{O}$  at  $70^\circ\text{C}$  ( $\mu = 0.1 \text{ M}$  with KCl) in the presence of  $0.01 \text{ M}$   $\text{Cu}^{2+}$  (●),  $\text{Zn}^{2+}$  (◐), and  $\text{Co}^{2+}$  (●) and in the absence of metal ions (○).



**Figure 2.** Plots of  $\log k_{\text{obsd}}$  vs pH for the hydrolysis of *N*-(6-carboxypicolinoyl)-*N*-methyl-4-nitroaniline in  $\text{H}_2\text{O}$  at  $70^\circ\text{C}$  ( $\mu = 0.1 \text{ M}$  with KCl) in the presence of  $0.01 \text{ M}$   $\text{Cu}^{2+}$  (●) and  $\text{Zn}^{2+}$  (◐) and in the absence of metal ions (○).

**$pK_a$  Determinations.** The  $pK_a$  values were determined spectrophotometrically at  $30^\circ\text{C}$ . A large series of  $0.02 \text{ M}$  buffers were prepared spanning the anticipated  $pK_a$ . The concentration of the anilide was  $1.3 \times 10^{-4} \text{ M}$ . In the initial work, complete UV-vis scans were made after addition of a constant concentration of the substrate to  $3 \text{ mL}$  of buffer in the cuvette. This allowed determination of the most favorable wavelength for measurement of the  $pK_a$  and also allowed calculation of an approximate  $pK_a$ . For more precise work, a constant concentration of the substrate was added to the buffer in the cuvette, and the absorbance was determined at the appropriate wavelength immediately after mixing.

## Results

In Figures 1 and 2 the plots are presented of  $\log k_{\text{obsd}}$  vs pH for hydrolysis of *N*-[(1,10-phenanthrolin-2-yl)carbonyl]-*N*-methyl-4-nitroaniline (III) and *N*-(6-carboxypicolinoyl)-*N*-methyl-4-nitroaniline (IV) in  $\text{H}_2\text{O}$  at  $70^\circ\text{C}$ . The reactions at  $\text{pH} > 8$  are hydroxide ion catalyzed; the slopes of the plots of  $\log k_{\text{obsd}}$  vs pH are 1.0. The second-order rate constant  $k_{\text{OH}}$  is  $0.85 \text{ M}^{-1} \text{ s}^{-1}$  for hydrolysis of III. The value of  $k_{\text{OH}}$  is similar in the hydrolysis of IV at  $70^\circ\text{C}$  ( $k_{\text{OH}} = 0.37 \text{ M}^{-1} \text{ s}^{-1}$ ).

In the presence of divalent metal ions, there is a marked enhancement of  $k_{\text{obsd}}$  with both III and IV. Saturation is achieved at low metal ion concentration ( $<0.01 \text{ M}$  with  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$  and  $<0.001 \text{ M}$  with  $\text{Cu}^{2+}$ ); plots of  $k_{\text{obsd}}$  vs  $[\text{M}^{2+}]$  are hyperbolic at constant pH. Equation 1 is followed at constant pH, where  $K_M$  is the metal ion association constant and  $k_M$  is the limiting

$$k_{\text{obsd}} = \frac{k_M K_M [\text{M}^{2+}]}{1 + K_M [\text{M}^{2+}]} \quad (1)$$

rate constant for metal ion catalysis. The experimental association constants for metal ion binding to III (uncorrected for protonation) were determined in HCl solution at pH 2.80 and are  $7 \times 10^3 \text{ M}^{-1}$  for  $\text{Cu}(\text{II})$ ,  $7 \times 10^2 \text{ M}^{-1}$  for  $\text{Zn}(\text{II})$ , and  $10^3 \text{ M}^{-1}$  for  $\text{Co}(\text{II})$ . The association constants for metal ion binding to IV are  $3.5 \times 10^3 \text{ M}^{-1}$  for  $\text{Cu}(\text{II})$  at pH 3.25 (formate buffer) and  $\text{Zn}(\text{II})$  at pH 6.1 (cacodylate buffer). At saturating concentrations of the metal ions, apparent hydroxide ion catalyzed reactions occur at  $\text{pH} > 4$  in the hydrolysis of both III and IV, as shown in Figures 1 and 2. The enhancements in  $k_{\text{OH}}$  range from  $10^4$ - to  $10^5$ -fold depending on the metal ion.<sup>23</sup> At pH less than 4, pH-independent reactions occur to at least pH 2 with all of the metal ions. Note that, with a metal ion bound to the liganding groups of III and IV, there are no ionizable groups present except metal ion bound water, which will have a  $pK_a$  greater than the highest pH studied.<sup>24</sup> Equation 2 is then followed in the metal ion promoted reaction.

$$k_{\text{obsd}} = k_0 + k_{\text{OH}} K_w / a_{\text{H}} \quad (2)$$

The lines in Figures 1 and 2 are theoretical and were constructed from eq 2 and the appropriate values of the rate constants. The rate constants for these reactions are summarized in Table I. The rate constants in Table I are best fit values.

The plot (not shown) of  $\log k_{\text{obsd}}$  vs pH for hydrolysis of *N*-(trifluoroacetyl)-*N*-methyl-4-nitroaniline (V) at  $70^\circ\text{C}$  in  $\text{H}_2\text{O}$  and  $\mu = 0.1 \text{ M}$  with KCl indicates the occurrence of an OH<sup>-</sup> reaction at  $\text{pH} > 5$  ( $k_{\text{OH}} = 1.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ ) and a pH-independent reaction at  $\text{pH} < 5$  ( $k_0 = 10^{-5} \text{ s}^{-1}$ ). Equation 2 is again followed.

Figure 3 presents a plot of  $\log k_{\text{obsd}}$  vs pH for hydrolysis of *N*-(trifluoroacetyl)-2,4-dinitroaniline (VI) at  $50^\circ\text{C}$ ,  $\mu = 0.1 \text{ M}$  with KCl. The reaction again apparently follows eq 2. However, spectrophotometric titration at  $280 \text{ nm}$  ( $30^\circ\text{C}$ ) revealed a  $pK_a$  of 5.5. Thus, the hydroxide ion catalyzed reaction at  $\text{pH} > 7$  is a reaction of the ionized species. The value of  $k_{\text{OH}'}$  is larger in  $\text{D}_2\text{O}$  than in  $\text{H}_2\text{O}$  ( $k_{\text{OH}'}/k_{\text{OH}} = 0.4$ ), whereas the pH-independent reaction is considerably slower in  $\text{D}_2\text{O}$  than in  $\text{H}_2\text{O}$  ( $k_0(\text{H}_2\text{O})/k_0(\text{D}_2\text{O}) = 2.9$ ). Similar solvent isotope effects were determined in the reactions of VII and VIII. There is less electron withdrawal in the leaving group with the 2-carboxy-4-nitro derivative VII. As seen in Figure 3, there is an inflection in the  $\log k_{\text{obsd}}$  vs pH profile near pH 6, which reflects ionization of the amide N-H. This produces a 7-fold reduction in the second-order rate constant for the OH<sup>-</sup>-catalyzed reaction. Equation 3 is then followed,

$$k_{\text{obsd}} = (k_0 + k_{\text{OH}} K_w / a_{\text{H}}) \left( \frac{a_{\text{H}}}{K_a + a_{\text{H}}} \right) + k_{\text{OH}}' K_w \left( \frac{K_a}{K_a a_{\text{H}} + a_{\text{H}}^2} \right) \quad (3)$$

where  $k_{\text{OH}'}$  is the second-order rate constant for alkaline hydrolysis of the amide anion and  $K_a$  is the dissociation constant of the neutral amide N-H group. The lines in Figure 3 are theoretical for eq 3 and the rate constants in Table II, with  $K_a = 6.3 \times 10^{-7} \text{ M}$ . Introduction of an *N*-methyl group in VIII prevents the formation of an ionized species. The shape of the  $\log k_{\text{obsd}}$  vs pH profile is the same as that of VI, but  $k_0$  and  $k_{\text{OH}}$  are enhanced

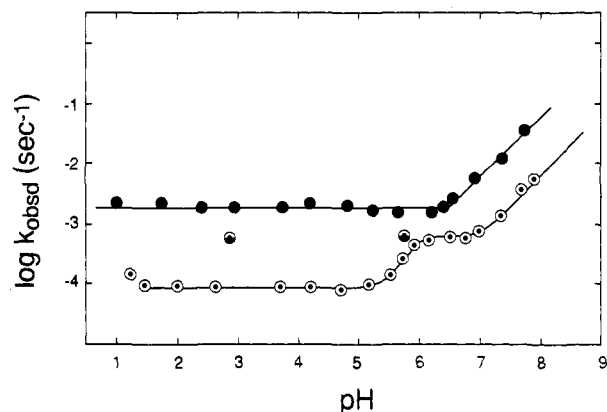
(23) Metal ion promoted OH<sup>-</sup> reactions (slope 1.0) have been reported for IV at  $40^\circ\text{C}$  with similar rate enhancements. Tang, W.; Sayre, L. M. *Abstracts*; 203 National Meeting of the American Chemical Society, San Francisco, April 5-10, 1992; INOR 679.

(24) The  $pK_a$  values at  $25^\circ\text{C}$  for ionization of metal ion bound water are 6.8, 8.8, and 8.9 for the aquo complexes of  $\text{Cu}(\text{II})$ ,  $\text{Zn}(\text{II})$ , and  $\text{Co}(\text{II})$ , respectively. Basolo, F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*, 2nd ed.; Wiley: New York, 1967; p 32.

(25) Komiyama, M.; Bender, M. L. *Bioorg. Chem.* 1978, 7, 133; 1979, 8, 141.

**Table I.** Rate Constants for Hydrolysis of Anilides in H<sub>2</sub>O at 70 °C and  $\mu = 0.1$  M with KCl

compound	metal ion	$k_o \times 10^4, s^{-1}$	$k_{OH}, M^{-1} s^{-1}$
II <sup>a</sup>	none		7.4
	Zn <sup>2+</sup>	3.5	
III	none		0.85 ± 0.1
	Cu <sup>2+</sup>	5.0 ± 0.1	1.2 ± 0.1 × 10 <sup>5</sup>
	Zn <sup>2+</sup>	1.10 ± 0.06	3.9 ± 0.5 × 10 <sup>4</sup>
	Co <sup>2+</sup>	0.48 ± 0.03	5.6 ± 0.4 × 10 <sup>3</sup>
IV	none		0.37 ± 0.02
	Cu <sup>2+</sup>	2.18 ± 0.04	1.0 ± 0.1 × 10 <sup>5</sup>
	Zn <sup>2+</sup>	0.3 ± 0.05	2.5 ± 0.2 × 10 <sup>4</sup>
	(6-carboxypicolinoyl)-4-nitroaniline <sup>a</sup>		0.044

<sup>a</sup> Reference 15.**Figure 3.** Plots of  $\log k_{\text{obsd}}$  vs pH for the hydrolysis of *N*-(trifluoroacetyl)-2,4-dinitroaniline in H<sub>2</sub>O (●) at 50 °C and in D<sub>2</sub>O (○) ( $\mu = 0.1$  M) and of *N*-(trifluoroacetyl)-2-carboxy-4-nitroaniline in H<sub>2</sub>O (○) at 70 °C ( $\mu = 0.1$  M).**Table II.** Rate Constants for Hydrolysis of (Trifluoroacetyl)anilides in H<sub>2</sub>O and D<sub>2</sub>O ( $\mu = 0.1$  M with KCl)

compound	$k_o \times 10^3, s^{-1}$	$k_{OH}, M^{-1} s^{-1}$	$k_{OH}', M^{-1} s^{-1}$	$T, ^\circ C$
V	0.01	1.2 × 10 <sup>3</sup>		70
VI	1.8		1.0 × 10 <sup>4</sup>	50
	0.614 (D <sub>2</sub> O)		2.4 × 10 <sup>4</sup>	50
VII	0.09	2.0 × 10 <sup>3</sup>	2.8 × 10 <sup>2</sup>	70
	0.041 (D <sub>2</sub> O)			70
VIII	13	5 × 10 <sup>6</sup>		50
	1.3	6.1 × 10 <sup>5</sup>		30
	0.63 (D <sub>2</sub> O)	1.5 × 10 <sup>6</sup>		30
	(trifluoroacetyl)-4-nitroaniline <sup>a</sup>	0.1	4.2 × 10 <sup>3</sup>	

<sup>a</sup> Reference 25.

7-fold and 500-fold, respectively, by the presence of the *N*-methyl group ( $k_o = 10^{-2} s^{-1}$  and  $k_{OH} = 5 \times 10^6 M^{-1} s^{-1}$  at 50 °C). Rate constants for the reactions of the trifluoroacetyl derivatives are given in Table II.

The hydrolysis of the (trifluoroacetyl)anilides is catalyzed by buffer. In view of the complications produced by the amide ionization of VI and VII, a detailed study of buffer effects was only carried out with the *N*-methyl substituted anilide VIII. Plots of  $k_{\text{obsd}}$  vs total buffer concentration at constant pH had increasing slopes as the pH was increased, which indicates that the base species of the buffer is catalytically active. Second-order rate constants for general base catalysis are given in Table III. A plot of  $\log k_B$  vs the  $pK_a$  of the conjugate acid of the base catalyst was linear with a slope  $\beta$  of 0.3.

## Discussion

The hydrolysis reactions of amides have been extensively studied in recent years to provide chemical understanding of these biologically important reactions.<sup>8-16</sup> Anilides have in particular

**Table III.** Second-Order Rate Constants for Buffer-Catalyzed Hydrolysis of *N*-(Trifluoroacetyl)-*N*-methyl-2,4-dinitroaniline (VIII) at 30 °C,  $\mu = 0.1$  M with KCl

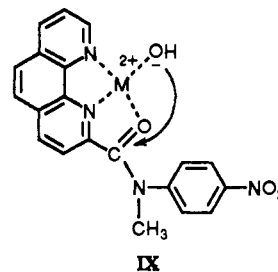
buffer	$k_B, M^{-1} s^{-1}$ <sup>a</sup>	buffer	$k_B, M^{-1} s^{-1}$ <sup>a</sup>
chloroacetate	0.009	acetate	0.044
formate	0.019	cacodylate	0.071

<sup>a</sup> Second-order rate constant for general base catalysis.

been investigated because of their absorbance characteristics and because the rates of their reactions are faster at high pH than those of amides with poorer leaving groups.<sup>15,26,27</sup> Breakdown of a tetrahedral intermediate to products is usually the rate-determining step in the alkaline hydrolysis of anilides.<sup>27</sup>

Chelated divalent metal ions significantly enhance the OH<sup>-</sup>-catalyzed hydrolysis of esters<sup>1-6</sup> and reactive amides such as *N*-acylimidazoles,<sup>14</sup>  $\beta$ -lactams,<sup>15,28</sup> and  $\epsilon$ -lactams.<sup>11</sup> Nucleophilic attack of OH<sup>-</sup> at the carbonyl group of *N*-acylimidazoles must be part of the rate-determining step, since there is little exchange of <sup>18</sup>O into the carbonyl group when the reactions are run in water enriched with <sup>18</sup>O<sup>29</sup> and there is only a small effect on the rate constants ( $k_{OH}$ ) due to substitution in the leaving group ( $\beta_{lg} = -0.28$ ).<sup>9</sup> Rate-determining nucleophilic attack probably also occurs with  $\beta$ -lactams in view of the  $\beta_{lg}$  of  $-0.4$ .<sup>28,30</sup> In contrast, chelated metal ions have a much smaller effect in the hydrolysis of substituted anilides,<sup>15,16</sup> and metal ion promoted OH<sup>-</sup>-catalyzed reactions are not invariably observed.<sup>15</sup>

The hydrolysis reactions of the *N*-methyl-4-nitroanilides III and IV are OH<sup>-</sup>-catalyzed at 70 °C. Divalent metal ions bind strongly to these amides because of the metal ion liganding functional groups. Enhancements in  $k_{\text{obsd}}$  are produced and saturation occurs at low metal ion concentrations (<0.01 M). As seen in Figures 1 and 2, metal ion promoted OH<sup>-</sup>-catalyzed reactions are observed at pH > 4 that have second-order rate constants  $k_{OH}$  (at saturating metal ion concentrations) that are 10<sup>4</sup>–10<sup>5</sup> larger than those in the absence of a metal ion. These reactions could proceed via either attack of external OH<sup>-</sup> on the metal ion complex or an intramolecular attack of metal ion bound OH<sup>-</sup>, as depicted in IX.<sup>24</sup> Cupric ion produces larger effects in



these reactions than Co(II) or Zn(II), as was observed in the hydrolysis of *N*-acylimidazoles<sup>14</sup> and esters.<sup>1-6</sup> However, in contrast with those reactions, the differences among the metal ions are not large; Zn(II) is almost as effective as Cu(II) (~5-fold difference). Thus, moderately large rate enhancements can be obtained in the hydrolysis of anilides by a series of divalent metal ions, even though the leaving group is poor (the  $pK_a$  of *N*-methyl-4-nitroaniline is 18.5).<sup>31</sup> The rate enhancements are not, however, nearly as large as those in the hydrolysis of the *N*-acylbenzimidazole (I) (10<sup>6</sup>–10<sup>9</sup>-fold),<sup>14</sup> so the leaving group clearly has a significant effect on the magnitude of the rate enhancement; note that the acyl groups of I and IV are the same.

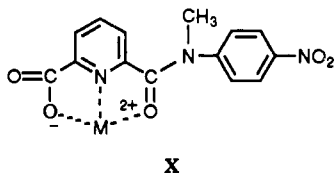
(26) (a) Mader, P. M. *J. Am. Chem. Soc.* **1965**, *87*, 3191. (b) Eriksson, S. O.; Holst, C. *Acta Chem. Scand.* **1966**, *20*, 1892.(27) (a) Kershner, L. D.; Schowen, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 2014. (b) Schowen, R. L.; Zuorick, G. W. *J. Am. Chem. Soc.* **1966**, *88*, 1223.(28) Page, M. I. *Acc. Chem. Res.* **1984**, *17*, 144.

(29) Fife, T. H.; Fee, J. A. Unpublished data.

(30) Blackburn, G. M.; Plackett, J. D. *J. Chem. Soc., Perkin Trans. 2* **1972**, 1366.(31) Dolman, D.; Stewart, R. *Can. J. Chem.* **1967**, *45*, 911.

In the presence of the divalent metal ions, pH-independent reactions are observed in the hydrolysis of **III** and **IV** at pH < 4. These pH-independent reactions extend to at least pH 2 and were found with all of the metal ions that were studied. Reactions of this type are not, however, observed in the metal ion promoted hydrolysis of *N*-acylimidazoles and are, therefore, specifically due to the anilide leaving group. It is not surprising that a pH-independent water reaction at low pH would be observed in the presence of Cu(II), because such reactions have been detected in the Cu(II)-promoted reactions of a variety of acyl derivatives.<sup>5b,7,32</sup> But pH-independent reactions are usually not seen in the reactions of Zn(II) or Co(II) complexes unless the leaving group is extraordinarily good, e.g., carboxylate or nitrophenoxide.<sup>6,33</sup> Water reactions are found in ester hydrolysis when there is electron withdrawal in the acyl group,<sup>34</sup> and this must also be an important factor in the reactions of the metal ion complexes of **II–IV**. A metal ion chelated in the acyl group will markedly increase inductive electron withdrawal from the carbonyl group, which will increase its reactivity.

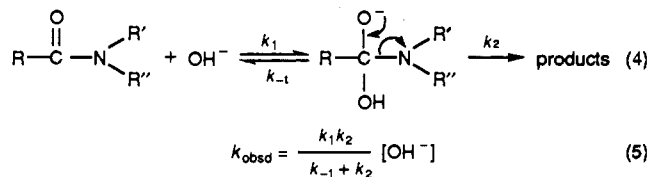
The rate constants and enhancements due to the chelated metal ions are very similar with **III** and **IV** and are, therefore, independent of the acyl group. The negatively charged carboxylate liganding group of **IV** is equally good in promoting the rate of hydrolysis as the completely neutral liganding groups of **III**. The negatively charged liganding group in **X** will reduce the effective positive



charge on the metal ion, which in turn should produce less polarization of the carbonyl group. Consequently, either the neutral nitrogen ligands of **III** are providing electrons to the metal ion to a similar extent as the ligands of **IV** or polarization effects are not of major importance in the reactions.

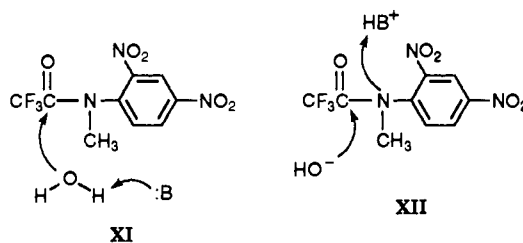
The magnitude of the metal ion effects in amide hydrolysis reactions will depend not only upon the identity of the metal ion and liganding groups but also upon whether nucleophilic attack or C–N bond breaking (decomposition of a tetrahedral intermediate) is the rate-determining step. Metal ion complexation of the carbonyl oxygen will polarize the carbonyl group and should, therefore, lead to large enhancements in the nucleophilic attack step. In addition, a metal ion can also serve to bind the attacking OH<sup>−</sup> (or H<sub>2</sub>O) so that the ensuing nucleophilic reaction is intramolecular, as in **I** and **IX**. At the same time, metal complexation of the negatively charged oxygen of the tetrahedral intermediate can retard decomposition to products (C–N bond breaking) inductively and by preventing electron release from oxygen. If a tetrahedral intermediate is stabilized by a metal ion, then breakdown of the intermediate must be retarded in the absence of further effects. Rate-enhancing effects of the metal ion could then be small or moderate if product formation is rate-determining.

If a tetrahedral intermediate is formed in amide hydrolysis (eq 4), then  $k_{\text{obsd}}$  is given by eq 5, and the rate-determining step will depend upon the ratio of  $k_2/k_{-1}$ . A second-order dependence on OH<sup>−</sup> has often been observed in anilide hydrolysis, which indicates breakdown of a tetrahedral intermediate via a dianionic species,<sup>25,27,35</sup> but with the present series of compounds, a strict first-order dependence on OH<sup>−</sup> was observed, i.e., slopes of log  $k_{\text{obsd}}$  vs pH were 1.0. In the hydrolysis of 4-nitroacetanilide, the ratio



of  $k_2/k_{-1}$  is 0.077, which indicates that breakdown of the intermediate to products is rate-determining.<sup>36</sup> However, an *N*-methyl group increases the ratio to 1.1 so that all of the rate constants influence  $k_{\text{obsd}}$ .<sup>37</sup> An increase in the  $k_2/k_{-1}$  ratio due to *N*-alkyl substitution appears to be a general effect.<sup>36,38</sup> Strong electron withdrawal in the acyl group has only a small effect on  $k_2/k_{-1}$ , and the ratio may be increased or decreased.<sup>36–42</sup> Ring-substituted (trifluoroacetyl)-*N*-methylacetanilides hydrolyze at low OH<sup>−</sup> concentration with rate-determining breakdown of a tetrahedral intermediate.<sup>27a</sup> Thus, it is probable that  $k_2$  influences  $k_{\text{obsd}}$  in the metal ion promoted reactions of **III** and **IV**,<sup>42</sup> although the values of  $k_2$  and  $k_{-1}$  may be similar. This would explain the moderate rate enhancements in the metal ion promoted reactions.

**(Trifluoroacetyl)anilides.** The (trifluoroacetyl)anilides **V–VIII** hydrolyze via a hydroxide ion reaction at pH > 6 and a pH-independent reaction at pH < 6. These reactions appear to be quite analogous to those of comparable esters. The alkaline reactions have second-order rate constants that are larger in D<sub>2</sub>O than in H<sub>2</sub>O ( $k_{\text{OH}}/k_{\text{OD}} \sim 0.4$ ), and the pH-independent reactions are slower in D<sub>2</sub>O than in H<sub>2</sub>O by factors of 2–3. The hydrolysis reactions are catalyzed by general bases with only a small dependence on the basicity of the catalyst ( $\beta = 0.3$  in the hydrolysis of **VIII**). Therefore, the reactions proceed with proton transfer in the transition state. In comparison, the general base catalyzed hydrolysis of the acyl-activated ester ethyl dichloroacetate is characterized by a  $\beta$  value of 0.47.<sup>34</sup> The mechanism can be considered to be classical general base catalysis (**XI**) or a kinetic equivalent such as **XII**.



The (trifluoroacetyl)anilides give rise to log  $k_{\text{obsd}}$  vs pH profiles that are of the same shape as those of the metal ion promoted reactions of **III** and **IV**, i.e., slopes of 1.0 and 0. In fact, the absolute values of the rate constants are similar for the *N*-methyl-4-nitroanilides **III** and **IV** in the Zn(II)-promoted reactions and in the hydrolysis reactions of **V**;  $k_{\text{OH}}$  is only 20-fold larger in the hydrolysis of the Zn(II) complex of **IV** than in the hydrolysis of **V**, and  $k_0$  is only 3-fold larger. The  $k_0$  for hydrolysis of the Zn(II) complex of the dinitroanilide **II** is 5-fold less than that of **VI**. The rapid reactions of the (trifluoroacetyl)anilides must be due primarily to the strong inductive electron withdrawal from the carbonyl group exerted by the trifluoroacetyl group. This will make nucleophilic attack at the carbonyl carbon easier but

(36) DeWolfe, R. H.; Newcomb, R. C. *J. Org. Chem.* **1971**, *36*, 3870.

(37) Pratt, R. F. Ph.D. Dissertation, University of Melbourne, Australia, 1969 (referred to in ref 36).

(38) Kijima, A.; Sekiguchi, S. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1203.

(39) Skarzewski, J.; Aoki, M.; Sekiguchi, S. *J. Org. Chem.* **1982**, *47*, 1764.

(40) Electron withdrawal in the leaving group produces a decrease in the  $k_2/k_{-1}$  ratio in the alkaline hydrolysis of substituted acetanilides. Bender, M. L.; Thomas, R. *J. Am. Chem. Soc.* **1961**, *83*, 4183. But the reverse is the case in the hydrolysis of substituted trifluoroacetanilides.<sup>41</sup>

(41) Stauffer, C. E. *J. Am. Chem. Soc.* **1972**, *94*, 7887.

(42) A pH-independent reaction might occur regardless of which step is rate-determining.

(32) Fife, T. H.; Przystas, T. J.; Pujari, M. P. *J. Am. Chem. Soc.* **1988**, *110*, 8157.

(33) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 1638.

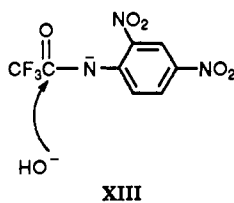
(34) Jencks, W. P.; Carriolo, J. *J. Am. Chem. Soc.* **1961**, *83*, 1743.

(35) Pollack, R. M.; Bender, M. L. *J. Am. Chem. Soc.* **1970**, *92*, 7190.

will retard C–N bond breaking, analogous to the effect of a metal ion complexed in the acyl group.<sup>43</sup>

The *N*-methyl group substituent of the anilides may alter the basicity of nitrogen somewhat, but its predominant effect should be steric. It will restrict the approach of OH<sup>-</sup> or H<sub>2</sub>O to the carbonyl group,<sup>38</sup> and it may also inhibit resonance interaction between nitrogen and the carbonyl group by restricting the ease with which the system can become coplanar. Thus, the *N*-methyl group can exert both accelerating and retarding effects on the rate of nucleophilic attack at the carbonyl group. Breakdown of a tetrahedral intermediate to products should be accelerated by relief of steric crowding in the tetrahedral intermediate.<sup>39</sup> A large retarding effect of the *N*-methyl group may, therefore, indicate that formation of the tetrahedral intermediate is rate-determining, since otherwise the effects would tend to cancel, i.e., a steric effect that retards formation of the tetrahedral intermediate would assist its breakdown to products. Note that a change in rate-limiting step from breakdown to formation of the intermediate may occur if the decomposition to products is sterically facilitated much more than that to reactants. There is only a small retardation in the rate for V as compared with the unmethylated derivative (see Table II), and the latter anilide hydrolyzes with rate-determining breakdown of the tetrahedral intermediate.<sup>25</sup> However, with the 2,4-dinitro-substituted anilide VIII, an *N*-methyl substituent greatly facilitates alkaline hydrolysis; *k*<sub>OH</sub> is enhanced 500-fold compared with that for VI. This may be due in part to a steric interaction between the *N*-CH<sub>3</sub> group and the ortho nitro substituent, which again will increase the ease of decomposition of the tetrahedral intermediate to products. The magnitude of *k*<sub>OH</sub> in the hydrolysis of VIII ( $5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  at 50 °C) is only 2000-fold less than that of a diffusion-controlled reaction.

There is no inflection in the log *k*<sub>obsd</sub> vs pH profile for hydrolysis of VI due to ionization of the anilide N–H group, even though the measured p*K*<sub>a</sub> is 5.5. The relatively facile hydroxide ion catalyzed hydrolysis of VI (*k*<sub>OH</sub>' =  $10^4 \text{ M}^{-1} \text{ s}^{-1}$  at 50 °C) is a reaction of the ionized species (XIII), which is significant because



it has been generally accepted that the ionized species of an amide is unreactive.<sup>25,26b,35,41,44</sup> Methylation of nitrogen will prevent ionization. Part of the enhancement in *k*<sub>OH</sub> for VIII as compared with VI must be due to electrostatic repulsion effects in VI, but in view of the magnitude of *k*<sub>OH</sub>' for VI ( $10^4 \text{ M}^{-1} \text{ s}^{-1}$ ), this effect is probably not large. The N–H p*K*<sub>a</sub> of *N*-(trifluoroacetyl)-2-carboxy-4-nitroaniline (VII) is higher than that of VI because of

(43) A complexed metal ion can also, of course, exert other types of rate-enhancing effects, e.g., conversion of a bimolecular reaction to intramolecular as in I and IX.

(44) Pollack, R. M.; Dumsha, T. C. *J. Am. Chem. Soc.* **1973**, *95*, 4463. Suh, J.; Klotz, I. M. *J. Am. Chem. Soc.* **1984**, *106*, 2373.

less electron withdrawal, and an inflection in the log *k*<sub>obsd</sub> vs pH profile is observed at pH 6–7. In this case NH-group ionization reduces *k*<sub>OH</sub> by a factor of 7.

It has recently been reiterated that deprotonation of an amide by a chelated Cu(II) ion might account for the small rate enhancements usually produced in amide hydrolysis<sup>45</sup> (see also refs 8 and 10). A complexed cupric ion can dramatically reduce the p*K*<sub>a</sub> of a neutral amide to 4–5 (8–10 with Ni(II) and Co(II)),<sup>8,46</sup> although there are few cases where complexed Zn(II) has produced a measurable effect.<sup>8,46,47</sup> It is clear from considering the reactions of VI and VII that simple deprotonation is not a sufficient explanation for greatly diminished reactivity. There must also be an accompanying effect, such as ligand exchange from O to N<sup>-</sup> that produces an unreactive species, to account for large effects.<sup>8,46,48,49</sup>

**Metallopeptidases.** The magnitude of metal ion effects in amide hydrolysis reactions depends markedly on the leaving group and the rate-determining step. The largest effects are obtained when the leaving group is good so that nucleophilic attack by OH<sup>-</sup> or water is clearly rate-determining.<sup>14</sup> When breakdown of a tetrahedral intermediate to products is rate-determining, the effect of a complexed metal ion will be smaller, since C–N bond breaking will be retarded by complexation of the amide carbonyl oxygen. These considerations are of importance in understanding the reactions of metallopeptidases because the leaving groups of typical substrates are very poor. A metallopeptidase will then be a more effective catalyst if it acts to maximize the rate constants for both nucleophilic attack and for C–N bond breaking, so that in the extreme case nucleophilic attack will become rate-determining. This could be accomplished in several ways, e.g., by employing the efficient metal ion bound OH<sup>-</sup> as a nucleophile rather than a functional group in the enzyme active site (the Glu270 carboxyl in the case of carboxypeptidase A) and by activating the substrate via a twisting effect. The latter effect would inhibit resonance interaction between nitrogen and the carbonyl group and would increase the ease of C–N bond breaking. Such a twisting effect is apparently required for peptides to bind in the catalytic site of carboxypeptidase A.<sup>51</sup>

**Acknowledgment.** This work was supported by a research grant from the National Science Foundation.

(45) Sayre, L. M.; Reddy, K. V.; Jacobson, A. R.; Tang, W. *Inorg. Chem.* **1992**, *31*, 935.

(46) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.

(47) Lance, E. A.; Nakon, R. *Inorg. Chim. Acta* **1981**, *55*, L1.

(48) In contrast with the anilides III and IV, the hydrolysis of the Zn(II) complex of *N*-(6-carboxypicolinoyl)-2,4-dinitroaniline (II) is pH-independent from pH 4 to 7. But at pH < 4 there is a downward bend in the plot of log *k*<sub>obsd</sub> vs pH (p*K*<sub>app</sub> = 3.8), although the metal ion still saturates. The p*K*<sub>app</sub> is considerably less than the p*K*<sub>a</sub> of the amide function, which may indicate a complex constant, or a change in rate-determining step. Fife, T. H.; Bembi, R. Unpublished results.

(49) A neutral amide nitrogen is of very low basicity, and there will be little tendency for a metal ion to bind directly. Deprotonation may occur after strong complexation of the carbonyl oxygen by Cu(II), and a ligand switch to the negatively charged nitrogen will then be favorable.<sup>46,50</sup>

(50) A system has recently been discussed in which Ni(II) binds to a ureido nitrogen, but even in this system Zn(II) binds to the carbonyl oxygen not the ureido nitrogen. Maslak, P.; Sczepanski, J. J.; Parvez, M. *J. Am. Chem. Soc.* **1991**, *113*, 1062.

(51) Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3875.