Inorg. Chem. **1993**, *32*, 5785–5791

Divalent Metal Ion Catalyzed Hydrolysis of *N*-Methyl-4′-nitro-6-carboxypicolinanilide. pH–Rate Profile Transitions and Buffer Inhibition

Wei Tang and Lawrence M. Sayre[*]

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Received August 5, 1993[®]

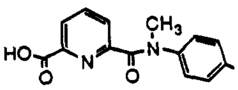
The hydrolysis of *N*-methyl-4′-nitro-6-carboxypicolinanilide (**1**) was studied in water at 40.0 °C in the absence and presence of Cu(II), Zn(II), Ni(II), and Co(II), and the results are compared to those obtained for the des-6-carboxy compound (**2**). The "spontaneous" hydrolysis exhibits a linear unit slope pH–rate profile between pH 9 and 11, indicative of a straightforward HO^--dependent reaction. Parallel, leftward-shifted lines in the pH–rate profile are seen in the presence of divalent metal ions between pH 5 and 7, implicating the dominance of a M(II)-catalyzed HO^- reaction in this pH range. The dimensionless rate enhancement factors for **1** at saturating concentrations of M(II) are about 2×10^4 for Ni(II), 4×10^4 for Co(II), 7×10^5 for Zn(II), and 1×10^6 for Cu(II). There is little difference between the kinetic behaviors of **1** and **2** except that the carboxylate ligand in **1** results in saturation at lower M(II) concentrations and permits the kinetics to be studied over a wider pH range, though the intrinsic catalytic effectiveness of the bound metal ion is actually somewhat reduced. The product pyridine-2,6-dicarboxylic acid binds M(II) more strongly than does the reactant **1**, but not by a sufficient margin to preclude the observation of true catalytic reactions (to 100% hydrolysis) using as little as 0.1 equiv of Cu(II) and Zn(II), though there is a progressive slowing in rate at high percent conversion as the amount of M(II) used decreases. Inhibition of the M(II)-catalyzed hydrolysis by so-called "biological buffers" is seen above pH 7.5, to a degree which increases with decreasing Lewis acidity of M(II). The switchover from pH-dependent to pH-independent behavior for Cu(II) catalysis at pH ~7 appears to reflect titration of the metal-bound water, producing **1**–Cu(II)–OH or a kinetically equivalent form as the species undergoing hydrolysis.

Introduction

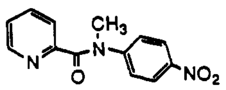
Elucidating mechanistic features of metal ion catalyzed hydrolysis of amides continues to be a subject of active research, in regard to understanding the role of metal ions in metallopeptidases and in the design of synthetic catalysts.[1] Large rate enhancements (as high as 10^7-fold) have been observed for Co(III)-promoted amide hydrolysis, and the mechanisms underlying these stoichiometric (noncatalytic) reactions have been adequately established.[2] For coordinatively labile M(II), however, it was believed for many years that only modest rate increases could be observed for amide hydrolysis realtive to the very large accelerations found for ester hydrolysis. Groves and co-workers first demonstrated that large rate enhancements for Cu(II) and Zn(II), rivaling those for Co(III), could be seen for ligand-functionalized lactams wherein the metal ion is prevented from coordinating to the amide group in the π nodal plane.[3] More recently, we showed that the limiting factor in most early model studies on amide hydrolysis was simply the formation of hydrolytically-inert amido complexes via metal-ion-coordination-induced amide NH deprotonation, which could be overcome through studies on *N*-methyl (tertiary) versions of the same amide substrates.[4] For example, whereas the Cu(II)-promoted hydrolysis of glycylglycine follows a HO^--dependent pH–rate profile only up to the pH (~4.3) where the tridentate amido chelate is formed,[5] rate enhancements of 10^7-fold are routinely seen (at pH 6.0–6.5) for Cu(II) promotion of the HO^--dependent hydrolysis of *N*-acylsarcosine derivatives.[4]

Compared to aliphatic amide hydrolysis, anilide hydrolysis may differ in terms of mechanism and has undergone more intensive structure–reactivity study.[6] We recently reported that Cu(II) promotes the HO^--dependent hydrolysis of aryl-substituted picolinanilides in the pH range 4–6, with a transition to a pH-independent process at pH ~7,[7] a behavior seen previously by Groves and co-workers for their systems.[3] Although the *N*-methylpicolinanilides bound metal ions only weakly, rate enhancement factors (REFs) up to 3×10^6 could be observed at high concentration (8 mM) of Cu(II). In contrast, Przystas and Fife observed a pH-*independent* process for the hydrolysis of 2′,4′-dinitro-6-carboxypicolinanilide in the presence of divalent metal ions at pH 4–7, indicative of a prevailing H_2O rather than HO^- catalysis in this region.[8] It could be argued that since the ancillary 6-carboxy ligand in their reactant ensured saturation of M(II) at low concentration, our observation of a pH-dependent process was an artifact of equilibria caused by weak binding of M(II) to the reactant. Thus, an unambiguous analysis of the kinetic mechanism had to await our utilization of ligand-functionalized anilides which ensured saturation at lower concentrations of metal ion.

At this time,[9] we report data on the ability of not only Cu(II) but also other coordinatively-labile divalent metal ions (zinc, cobalt, nickel) to promote the hydrolysis of the 6-carboxy derivative (**1**)of the *N*-methyl-4′-nitropicolinanilide (**2**) we reported previously.[7] Saturation is seen at accessible M(II) concentrations, permitting an in depth analysis of the pH–rate



1



2

---

[*] Abstract published in *Advance ACS Abstracts*, November 15, 1993.

(1) (a) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145. (b) Suh, J. *Acc. Chem. Res.* **1992**, *25*, 273. (c) Zhu, L.; Kostic, N. M. *J. Am. Chem. Soc.* **1993**, *115*, 4566. (d) Fife, T. H. *Metal Ion Catalyzed Ester and Amide Hydrolysis*; Perspectives on Bioinorganic Chemistry, Vol. 1; JAI Press, Ltd.: London, 1991; pp 43–93.

(2) (a) Sutton, P. A.; Buckingham, D. A. *Acc. Chem. Res.* **1987**, *20*, 357. (b) Groves, J. T.; Baron, L. A. *J. Am. Chem. Soc.* **1989**, *111*, 5442. (c) Takasaki, B. K.; Kim, J. H.; Rubin, E.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 1157.

(3) (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.

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

0020-1669/93/1332-5785$04.00/0

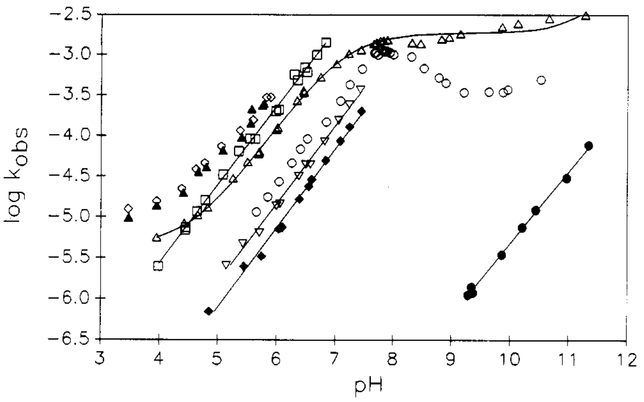


Figure 1. pH–rate profile for hydrolysis of **1** (0.2 mM) at 40.0 °C in H_2O (μ = 0.1 M) in the absence of metal ion (●) and presence of 0.2 mM Cu^{2+} (▲), 2.0 mM Cu^{2+} (◇), 4.0 mM Cu^{2+} (▲), 0.2 mM Zn^{2+} (○), 10 mM Zn^{2+} (□), 10 mM Co^{2+} (▽), and 10 mM Ni^{2+} (◆). The curve shown is a computer-generated least-squares fit to eq 1.

profile. Most importantly, the unit slope pH dependence in the pH range 5–7 is preserved, confirming the M(II)-dependent acceleration of HO^- catalysis, and the associated REFs are comparable to those we observed for the des-6-carboxy system. Improved coordination of M(II) by the 6-carboxypicolinanilide retards precipitation of M(II)–hydroxide species, and rate data up to pH 11 are obtainable. Unexpectedly large inhibitory effects of supposedly noncoordinating buffers are observed at high pH, with the degree of inhibition reflecting the identity of M(II) and the M(II):reactant:buffer stoichiometry.

Results and Discussion

Rate Enhancement Effects of M(II) on Base-Mediated Hydrolysis. Figure 1 displays plots of log k_{obs} vs pH for the hydrolysis of *N*-methyl-4′-nitro-6-carboxypicolinanilide (**1**) followed spectrophotometrically in water at 40.0 °C (ionic strength maintained at 0.1 M with KNO_3), in the presence and absence of divalent metal ions. The "spontaneous" hydrolysis at high pH is a straightforward HO^--dependent reaction, as reflected by the unit slope of the pH–rate profile. The hydrolysis is faster in the presence of M(II) to a degree indicated by the vertical displacement of the M(II) data above the "spontaneous" hydrolysis line. The reactant binds Cu(II) fairly strongly; there is only a modest increase in going from 1 to 10 equiv of Cu(II) per reactant ligand, and a further increase to 20 equiv of Cu(II) has no effect on the rate. For the more weakly binding metal ions, the rate is enhanced substantially by going from 1 to 50 equiv of M(II) per reactant ligand, as seen for Zn(II), and only the 50-equiv (10 mM) data are shown for Co(II) and Ni(II). The 10 mM concentration of M(II) has previously been shown to achieve saturation of 6-carboxypicolinyl systems.[10] From the rate data displayed in Figure 1, the order of catalytic effectiveness is seen to be Cu(II) > Zn(II) ≫ Co(II) > Ni(II). We obtained rate data over a wide pH range (4–11) in the case of both 1:1 **1**–Cu(II) and 1:1 **1**–Zn(II), and an explanation of the overall pH–rate profile in these cases requires considerable discussion (see below). Nonetheless, in the pH range 5–7, the M(II)-catalyzed hydrolysis is a straightforward HO^--dependent process, as indicated by the linear unit slope of the pH–rate profile in this region. From these lines, the M(II) rate enhancement factors (REFs) for the HO^- reaction can be determined graphically by inspection, and the values for the bimolecular HO^--dependent rate constants k_{OH} can be calculated from the autoprotolysis constant K_w (Table I).

Table I. Bimolecular Rate Constants for the Noncatalyzed and Metal Ion Catalyzed Alkaline Hydrolysis of **1** and **2** (0.2 mM) in H_2O at 40.0 °C

Reactant	M(II)	[M(II)]/[reactant]	k_{OH}, M^{-1} s^{-1} [a]	REF[b]
1	none	0[c]	1.36×10^{-2}	
1	Cu^{2+}	1	4.62×10^{3}	3.4×10^{5}
			4.56×10^{3} [d]	
			2.07×10^{4} [d,e]	
1	Cu^{2+}	(sat.)[f]	1.64×10^{4}	1.2×10^{6}
1	Zn^{2+}	1	7.67×10^{2}	5.6×10^{4}
1	Zn^{2+}	50	9.12×10^{3}	6.7×10^{5}
1	Co^{2+}	50	543	4.0×10^{4}
1	Ni^{2+}	50	243	1.8×10^{4}
2	none	0[c]	4.31×10^{-2}	
			2.28×10^{-2} [e,g]	
2	Cu^{2+}	1	684	1.6×10^{4}
			1.91×10^{3} [e,g]	8.4×10^{4}
2	Zn^{2+}	50	96.6	2.3×10^{3}
2	Zn^{2+}/bipy[h]	50	51.9	1.2×10^{3}

[a] Derived graphically from the linear slope = 1 portion of the pH–rate profile and the value of K_w at 40.0 °C. [b] Rate enhancement factor (dimensionless) on k_{OH} in the presence of M(II). [c] The reaction solution used in the absence of M(II) contained 20 μM EDTA. [d] Derived from k_2 of the computer-assisted least-squares fit of the entire pH–rate profile. [e] For EtOH–H_2O (1:2). [f] Representing data obtained using [Cu(II)]/[reactant] of 10 or 20 (unchanged). [g] From ref 7. [h] Representing a 1:1 mixture of $Zn(NO_3)_2$ and 2,2′-bipyridine.

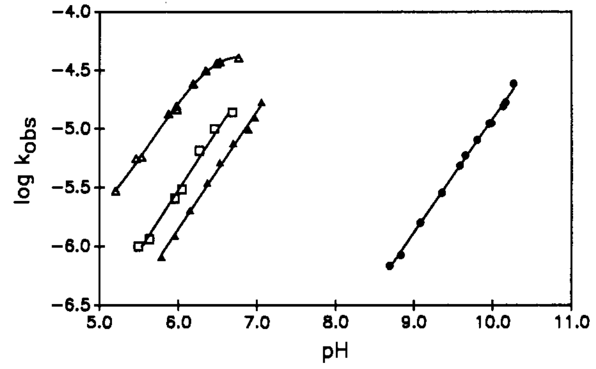


Figure 2. pH–rate profile for the hydrolysis of **2** (0.2 mM) at 40.0 °C in H_2O (μ = 0.1 M) in the absence of metal ion (●) and presence of 0.2 mM Cu^{2+} (▲), 10 mM Zn^{2+} (□), and 10 mM Zn^{2+} + 10 mM 2,2′-bipyridine (▲).

For comparison, rate data for *N*-methyl-4′-nitropicolinanilide (**2**), studied previously by us in EtOH–H_2O (1:2),[7] have now been obtained in pure water (Figure 2). Also, some hydrolysis rates for 1:1 **1**–Cu(II) were measured in our previously used EtOH–H_2O (1:2) solvent system (Figure 3). In every case, the existence of a linear unit slope region in the pH–rate profile at pH 5–7 is indicative of M(II) catalysis of alkaline hydrolysis, and the magnitude of k_{obs} simply reflects the equilibrium fraction of reactant which is tied up in a M(II) complex. This fraction is increased by use of the ancillary 6-carboxy ligand and/or through use of a large excess of M(II).

Kinetic Analysis of the pH–Rate Profiles and Comparative Rate Trends. The pH–rate profile for hydrolysis of **1** in the presence of 1 equiv of Cu(II) is sigmoidal both in water (Figure 1) and in EtOH–H_2O (1:2) (Figure 3) and additionally shows an upward curvature in the former case above pH 10. This exact behavior was observed previously by Groves and co-workers for the Cu(II)- and Zn(II)-mediated hydrolysis of ligand-functionalized lactams.[3] The sigmoidal behavior was explained in terms of dominance of a pH-independent reaction at low pH (a H_2O reaction), giving way to a HO^--dependent reaction at intermediate

---

(5) (a) Nakata, T.; Tasumi, M.; Miyazawa, T.; *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1599. (b) Grant, I. J.; Hay, R. W. *Aust. J. Chem.* **1965**, *18*, 1189.
(6) DeWolfe, R.; Newcomb, R. C. *J. Org. Chem.* **1971**, *36*, 3870.
(7) Reddy, K. V.; Jacobson, A. R.; Kung, J. I.; Sayre, L. M. *Inorg. Chem.* **1991**, *30*, 3520.
(8) Fife, T. H.; Przystas, T. J. *J. Chem. Soc., Perkin Trans. 2* **1990**, 393.
(9) A preliminary account ot this work was presented: Tang, W.; Sayre, L. M. *Abstracts of Papers*, 203rd National Meeting of the American Chemical Society, San Francisco, April 5–10, 1992; American Chemical Society: Washington, DC, 1992; INOR 679.
(10) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1986**, *108*, 4631.

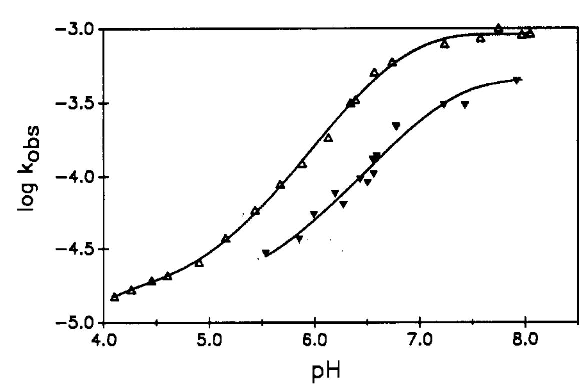
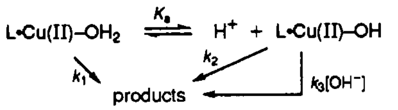


Figure 3. pH–rate profile for the hydrolysis of the 1:1 **1**–Cu^{2+} complex, at either 0.2 mM (▲) or 1.0 mM (▼), at 40.0 °C in EtOH–H_2O (1:2) ($\mu = 0.1$ M). The curves shown are computer-generated least-squares fits to eq 1.

pH with titration at pH ~7 of the responsible group, assigned as the M(II)-bound water. The upward curvature at high pH represents the emergence of a mechanism involving reaction of HO^- with the M(II)–OH form of the complex. The three-term kinetics process takes the form

$$k_{obs} = k_1\left(\frac{a_H}{K_{app} + a_H}\right) + \left(k_2 + k_3\frac{K_w}{a_H}\right)\left(\frac{K_{app}}{K_{app} + a_H}\right) \qquad (1)$$

wherein K_{app} is the apparent ionization constant required to fit the data. A mechanism consistent with this kinetic form is shown in eq 2(L = the reactant ligand). A computer-assisted least-



(2)

squares fit of the 1:1 **1**–Cu(II) data (0.2 mM) in Figure 1 to eq 1 gave $k_1 = 4.58 \times 10^{-6}$ s^{-1}, $k_2 = 1.80 \times 10^{-3}$ s^{-1}, $k_3 = 0.295$ M^{-1} s^{-1}, and $pK_{app} = 7.13$. There is excellent agreement between the two k_{OH}'s, one derived from the k_2 of this fit[11] ($k_2K_{app}/K_w = 4.56 \times 10^3$ M^{-1} s^{-1}) and another derived from the slope 1 region of the pH–rate profile (4.62×10^3 M^{-1} s^{-1}). A similar treatment of the 1:1 **1**–Cu(II) data (0.2 mM) in EtOH–H_2O (1:2) (Figure 3) yielded a value of 1.06×10^{-3} s^{-1} for k_2 with a pK_{app} of 6.75, but there were insufficient data at high pH to validate inclusion of the k_3 term, in turn causing uncertainty in the k_1 term. The value of k_2 translates to a k_{OH} of $k_2K_{app}/K_w = 2.07 \times 10^4$ M^{-1} s^{-1}.

Several comments can be made from the comparative data listed in Table I. First, the 6-carboxy substituent is seen to have a ~3-fold rate-retarding effect on the "spontaneous" hydrolysis in water, nearly identical to the effect seen by Fife and Przystas for *N*-picolinylbenzimidazole.[10] Second, in all cases of the weak-binding picolinanilide **2**, the REFs are less than those seen with the 6-carboxy compound **1**, as expected, but the discrepancy is wider for Zn(II) than for Cu(II), presumably on account of the weaker Lewis acidity of Zn(II). Third, for both compounds **1** and **2**, the k_{OH} for 0.2 mM Cu(II) (1 equiv) is 3–5 times faster in EtOH–H_2O than in H_2O alone, possibly reflecting a greater Cu(II)–ligand K_{assoc} in the mixed-solvent system. Finally, the largest REF seen in this study, a factor of 1.2×10^6 for the Cu(II)–**1** complex, is 7-fold *smaller* than the REF value reported previously by us for the Cu(II)–**2** complex obtained from extrapolation (double-reciprocal plot) of the rate data to saturating Cu(II) concentrations. Thus, if one neglects the slight difference in solvent, it is possible to conclude that the ancillary 6-carboxy substituent, although ensuring saturation at lower [M(II)], actually possesses an intrinsically *deactivating* effect on M(II)-catalyzed hydrolysis. This trend, seen by us before,[4] most likely arises from carboxylate ligation diminishing the Lewis acidity of M(II). Such effect is probably induced internally, however, because we found that external carboxylate coordination (varying [NaOAc] in the MES buffer at pH 6.4 up to 0.09 M, keeping a constant $\mu = 0.1$ M) had no effect on the rate of hydrolysis of **1** in the presence of 10 mM Zn(II) (data not shown).

M(II)-Catalyzed As Opposed to M(II)-Promoted Hydrolysis. One principal difference between the present investigation and previously studied Co(III)-promoted hydrolyses is that the latter reactions are *stoichiometric* (noncatalytic) in the case of "exchange-inert" Co(III). In the cases of the coordinatively-labile divalent metal ions examined here, however, catalysis can occur using substoichiometric quantities of M(II) as long as the product does not bind the M(II) so strongly so as to exclude binding to the reactant. In the case of **1**, the hydrolysis product pyridine-2,6-dicarboxylate (dipicolinic acid) will bind M(II) more strongly than will **1**, such that one might expect the hydrolysis reaction to slow down at 50% completion when 0.5 equiv of M(II) per **1** is used. However, dipicolinic acid forms 2:1 complexes with M(II) at higher concentrations[12] and, to whatever extent this occurs, will liberate M(II) for coordination to **1**. Thus, the greatest slowdown might occur when less than $n = 0.5$ equiv of M(II) is used, at $2n$ mole fraction of conversion, e.g., at 50% reaction using 0.25 equiv of M(II). In the case of **2**, the product (picolinic acid) will also bind M(II) more strongly than does the reactant, but *by a greater margin* than in the case of **1**. Thus, since effective acceleration of hydrolysis depends more critically for **2** than for **1** upon the presence of *excess* M(II) for optimizing the fraction of reactant present in complexation with M(II), a significant slowdown in the case of **2** is expected as soon as the concentration of M(II) falls below that of **2**.

These predictions are generally borne out by the data obtained: percent conversions at various time points chosen to allow crucial comparisons are listed in Table II for Cu(II)-catalyzed hydrolysis of **1** (at two different pH values) and **2** and for Zn(II)-catalyzed hydrolysis of **1**, all at 40.0 °C. In all three cases, all reactions eventually proceed to 100% completion. With 0.5 equiv of Cu(II) or Zn(II) at pH 7, the hydrolysis of **1** follows first-order kinetics to 50% conversion with $k_{obs} = 4.39 \times 10^{-4}$ and 1.28×10^{-4} s^{-1}, respectively. The use of 0.25 equiv of metal ion also achieved a high-yield conversion (>80%) in a remarkably short reaction period, though substantial slowing of the latter stages of hydrolysis occurred below 0.25 equiv. In contrast, for **2**, the rate was already slower using 0.50 equiv of Cu(II), and considerable further slowing occurred below this level. Nonetheless, for both compounds, when one examines the 10-day extent of hydrolysis achieved in the absence of M(II), the degree of conversion in the same time period using even 0.10 equiv of M(II) is remarkable.

Buffer Inhibition at High pH. As seen in Figure 1, whereas the pH–rate profile for 1:1 **1**–Cu(II) plateaus at pH 7–8, the curve for 1:1 **1**–Zn(II) peaks in this same pH range and then falls over the pH range 8–10. This latter range corresponds to the use of borate buffer and the high-pH end of HEPES buffer. Although these buffers are normally considered to be noncoordinating relative to such notorious buffers as Tris and phosphate, we suspected that the dip in pH–rate profile for Zn(II) might reflect buffer coordination, decreasing the concentration of the **1**–Zn(II) complex and/or the Lewis acidity of the catalytic Zn(II) in the complex. To investigate this phenomenon further, we obtained hydrolysis kinetic data for 1:1 **1**–Zn(II) (0.2 mM) using a variety

---

(11) In the range of predominance of the k_2 term, $k_{obs} = k_2K_{app}/(K_{app} + a_H)$, which, after equating to $k_{obs} = k_{OH}[HO^-]$ and considering only the pH region below the pK_{app}, yields $k_{OH} = k_2K_{app}/K_w$.

(12) (a) Faucherre, J.; Petitfaux, C.; Charlier, B. *Bull. Soc. Chim. Fr.* **1966**, 1091. (b) Anderegg, A. *Helv. Chim. Acta* **1960**, *43*, 414.

Table II. Data Demonstrating M(II) *Catalysis* of Hydrolysis of **1** and **2**[a]

	6-carboxypicolinanilide **1**				picolinanilide **2**			
M(II)	[M(II)]/[reactant]	pH	time, h	conversion, %	[M(II)]/[reactant]	pH	time, h	conversion, %
Cu^{2+}	0.50	7.00	3.3	95	0.50	7.00	50	90
Cu^{2+}	0.25	7.01	23.1	90	0.25	7.04	240	73
Cu^{2+}	0.10	7.10	240	77	0.10	7.04	240	37
Cu^{2+}	0.50	5.60	31.7	90				
Cu^{2+}	0.25	5.61	48.0	88				
Zn^{2+}	0.50	7.05	11.8	95				
Zn^{2+}	0.25	7.10	22.7	83				
Zn^{2+}	0.10	7.01	240	71				
none[b]	0	7.09	240	0.40	0	7.15	240	1.4
none[b]	0	5.67	240	0.08				

[a] The reaction was performed at 40.0 °C and μ = 0.1 M. The percent conversion was determined spectrophotometrically. [b] The reaction solution used in the absence of M(II) contained 20 μM EDTA.

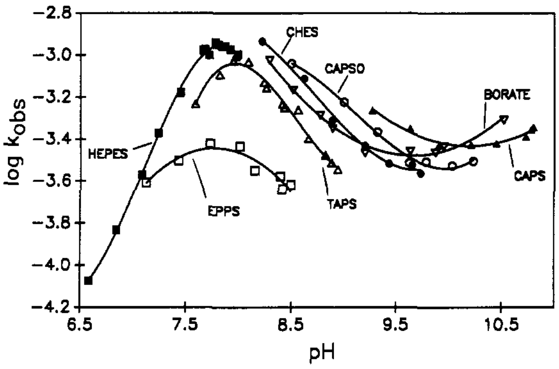


Figure 4. pH–rate profile for the hydrolysis of **1**–Zn^{2+} (1:1) (0.2 mM) at 40.0 °C in H_2O (μ = 0.1 M) using different buffers (5 mM): HEPES (■), EPPS (□), TAPS (△), CHES (●), borate (▽), CAPSO (○), and CAPS (▲).

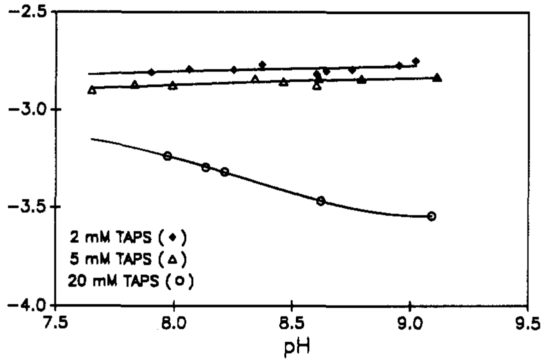


Figure 5. pH–rate profile for the hydrolysis of **1**–Cu^{2+} (1:1) (0.2 mM) at 40.0 °C in H_2O (μ = 0.1 M) in the presence of varying concentrations of TAPS buffer: 2 mM (◆), 5 mM (△), and 20 mM (○).

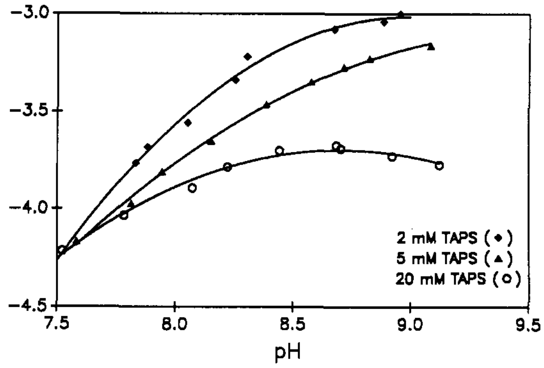


Figure 6. pH–rate profile for the hydrolysis of **1**–Co^{2+} (1:1) (0.2 mM) at 40.0 °C in H_2O (μ = 0.1 M) in the presence of varying concentrations of TAPS buffer: 2 mM (◆), 5 mM (▲), and 20 mM (○).

of other so-called "biological" buffers[13] (5 mM) with buffering action within the 7.5–11.0 pH range. Figure 4 shows that the inhibitory trend over the pH range 8–10 is general, though certain buffers appear to be more inhibitory than others. A firm conclusion regarding the structural basis of this inhibition cannot be made from our limited data set, but it appears that less inhibition is seen with borate and the secondary cyclohexylamine-based buffers (CHES, CAPSO, and CAPS), whereas more inhibition is seen (in the same pH range) with TAPS and especially the piperazine-based buffer EPPS (presumably more inhibitory than HEPES on account of its increased basicity).

In order to convince ourselves that the dip in pH–rate profile truly represented buffer inhibition, we selected one of the buffers (TAPS) to determine the effect of varying buffer concentration at constant 1:1 **1**–M(II) (0.2 mM) for various metal ions. The pertinent pH–rate profiles for Cu(II), Co(II), and Zn(II) are shown in Figures 5–7. Although the inhibitory effect is discernible in every case, the magnitude varies in the order Zn(II) ~ Co(II) > Cu(II). In the case of Cu(II) (Figure 5), the rates are the same using either 2 or 5 mM TAPS over the pH range 7.5–9.0 and nearly overlap the pH-independent rate profile in this pH range using HEPES and borate (Figure 1). Buffer inhibition is seen only at 20 mM TAPS at pH >7.5. In the case of Co(II) (Figure 6), the rate decreases significantly with increasing [TAPS] in a manner which is increasingly evident as the pH increases above 7.5 (note that the rate at pH 7.5 is invariant at $k_{obs} \sim 10^{-4.25}$). An actual dip in rate for Co(II) is seen only using 20 mM TAPS, but this is because the intrinsic rate (buffer noninhibited) is still increasing up to pH 9.0 (the switchover from pH-dependent to pH-independent hydrolysis for Co(II) appears to occur at pH ~9.5). In the case of Zn(II) (Figure 7), the dip in rate increases with increasing [TAPS] above pH 7.5 (the rate at pH 7.5 again appears to be invariant at $k_{obs} \sim 10^{-3.3}$). Interestingly, with [TAPS] kept constant at 0.5 mM, when the Zn(II) concentration is dropped from 0.2 to 0.1 mM (0.5 equiv based on [**1**]), the rate is lower, as expected from less of **1** being in the form of the **1**–Zn(II) complex, but more importantly is also nearly pH-independent between pH 8 and 9.

Figure 8 displays additional data which illustrate how buffer inhibition depends on the buffer:**1**:Zn(II) stoichiometry in the case of another commonly used buffer,[8] morpholine. It is seen that at constant [morpholine] = 0.5 mM, the rate increases with increasing [Zn(II)] only up to about 0.2 mM Zn(II) (1 equiv based on **1**), whereas at constant [morpholine] = 5.0 mM, the rate increases with increasing [Zn(II)] up to about 0.3 mM Zn(II) (1.5 equiv based on **1**). Our interpretation of these results is that there is direct competition between the buffer and the

---

(13) Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. *Biochemistry* **1966**, *5*, 467. Ferguson, W. J.; Braunschweiger, K. I.; Braunschweiger, W. R.; Smith, J. R.; McCormick, J. J.; Wasmann, C. C.; Jarvis, N. P.; Bell, D. H.; Good, N. E. *Anal. Biochem.* **1980**, *104*, 300.

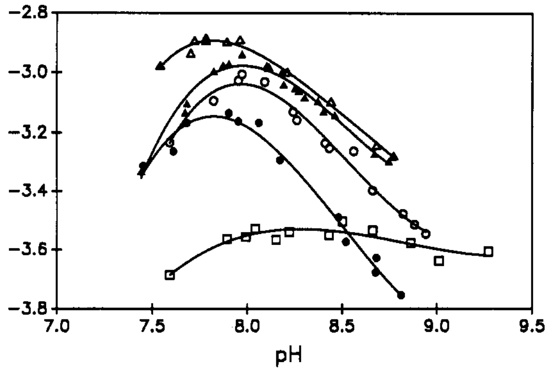


Figure 7. pH–rate profile for the hydrolysis of **1** (0.2 mM) at 40.0 °C in H_2O (μ = 0.1 M) in the presence of 0.2 mM Zn^{2+} and 0.5 mM (△), 2 mM (▲), 5 mM (○), or 20 mM (●) TAPS buffer or in the presence of 0.1 mM Zn^{2+} and 0.5 mM TAPS buffer (□).

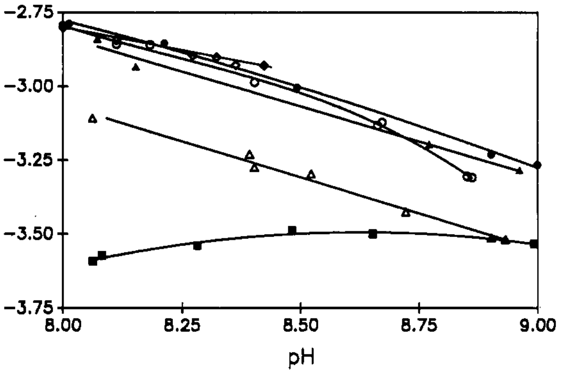


Figure 8. pH–rate profile for the hydrolysis of **1** (0.2 mM) at 40.0 °C in H_2O (μ = 0.1 M) in the presence of 5 mM morpholine and 0.2 mM (△), 0.3 mM (○), or 0.4 mM (◇) Zn^{2+} or in the presence of 0.5 mM morpholine and 0.1 mM (■), 0.2 mM (▲), or 0.3 mM (●) Zn^{2+}.

reactant ligand for binding to the Zn(II) present. However, once the reactant ligand is in excess of Zn(II), and even though the *overall* level of inhibition still increases with increasing [buffer], the ability of the buffer to exert an *increasingly greater inhibitory effect as the pH increases seems to be eliminated*, at least at lower buffer concentrations. Thus, the finding of a nearly pH-independent hydrolysis in the pH range 8–9 for 0.2 mM **1** and 0.1 mM Zn(II) in the case of both 0.5 mM TAPS ("lowest" curve in Figure 7) and 0.5 mM morpholine ("lowest" curve in Figure 8) suggests to us that this represents the intrinsic pH–rate profile behavior, in turn implying that the switchover from pH-dependent to pH-independent hydrolysis for Zn(II) occurs at pH 7.5–8.0.

Nature of the pH–Rate Profile Transition. Our above analysis of the pH–rate profile data for 1:1 **1**-Cu(II) according to a three-term kinetics equation yielded a pK_{app} of 7.13 (H_2O, 40.0 °C), which we interpret to reflect titration of the Cu(II)-bound water in the **1**–Cu(II) complex undergoing hydrolysis. From the buffer variation studies described above, the pK_{app} for the 1:1 **1**–Zn(II) system can be estimated to be 7.5–8.0, but closer to 9.5 in the case of 1:1 **1**–Co(II) (0.2 mM). The values we find for Cu(II) and Zn(II) are consistent with literature data on the acidity of water bound to cationic Cu(II) and Zn(II) chelates[14] and are close to those reported by Groves and co-workers for a family of pyridine-ligand-functionalized lactams.[3] In the latter studies, direct potentiometric titrations were performed to support the assignment of the kinetic pK_{app} as representing the M(II)-bound water. In our case, however, such attempted titration could not be performed in water owing to the occurrence of precipitation problems at concentrations amenable to direct titration. Furthermore, there is no significant chromophoric change which would allow the titration to be conducted spectrophotometrically at low concentrations.

We did find, however, that direct titration at [**1**] = [Cu(II)] = 1 mM could be performed in EtOH–H_2O (1:2) at 25.0 °C, yielding two well-behaved inflections at 1.0 and 2.0 equiv of NaOH (after which point some precipitation occurred). The two inflections were associated with pK_a values of 3.52 (representing the carboxylic acid proton) and 6.40 (representing the Cu(II)-bound water). The titrations were conducted at 25.0 °C rather than 40.0 °C in an effort to minimize the occurrence of hydrolysis during the titration. At the lower temperature, we found that the degree of hydrolysis at the second NaOH equivalence point (pH ~7) was reproducibly less than 3% (determined spectrophotometrically).

As we could not conduct the titration under the conditions of our aqueous kinetics experiments (Figure 1), it was important that we carry out kinetic studies under the mixed-solvent conditions wherein the titration could be performed. Data were obtained for [Cu(II)] = [**1**] both at 0.2 mM and at 1.0 mM (Figure 3). Excellent first-order kinetics were followed at the lower concentration, and fitting of the sigmoidal pH–rate profile data to a two- or three-term kinetics equation described above yielded a pK_{app} of 6.75. A similar inflection was observed for the data at 1 mM, which was the concentration at which the potentiometric titration was performed, though in this case first-order kinetics were followed only up to 1 half-life (the rate data plotted in Figure 3 represent initial rates obtained from the first 15% of the first-order kinetics plots). The reason for the complicated kinetics is not clear but probably is a consequence of one or more "new" equilibria which become significant at higher concentration. Considering this complication and the difference in temperature, the close agreement between the potentiometrically- and kinetically-determined pK_a values (6.40 and 6.75) suggests that the break in the pH–rate profile for Cu(II) in Figure 3, and by inference in Figure 1 as well, represents titration of the Cu(II)-bound water.

An alternative, frequently encountered mechanism that predicts a transition from slope = 1.0 to slope = 0 in the pH–rate profile is given in eq 3, where HO^- addition to a reactant S results in

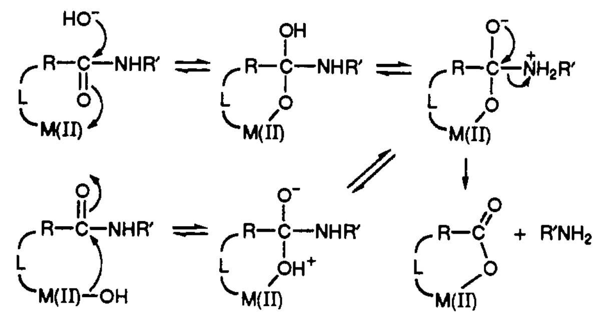
$$A + HO^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I \underset{k_2}{\overset{H^+}{\rightarrow}} \text{products} \qquad (3)$$

reversible formation of an intermediate I, which requires protonation to give products. Application of the steady-state condition to [I] yields $k_{obs} = k_1K_w/(k_{-1}/k_2 + [H^+])$. This has the same form as the rate expression for the k_2 term of eq 1; $k_{obs} = k_2K_{app}/(K_{app} + [H^+])$. In the case of eq 3, the significance of the plateauing of the pH–rate profile would be the pH at which the reaction converts from rate-limiting formation of I (pH-dependent) to rate-limiting breakdown of I (pH-independent). This is a quite reasonable scenario for amide hydrolysis, where tetrahedral-intermediate formation may be rate-limiting at low pH but C–N bond cleavage (with proton transfer to nitrogen) becomes rate-limiting at high pH. This possibility, however, can be ruled out in the present case of Cu(II) catalysis, where we have shown that deprotonation of Cu(II)-bound water does occur at the point of pH–rate profile plateau. The argument is that once the pK_a equilibrium is demonstrated independently, it must be represented in the kinetics. The observed plateau cannot represent both mechanistic explanations simultaneously. For the other two metal ions which appear to display pH–rate profile inflections, namely Zn(II) and Co(II), we did not perform potentiometric titrations, but we nonetheless prefer to interpret the kinetics also in terms of titration of M(II)-bound water.

---

(14) (a) Woolley, P. *Nature* (London) **1975**, *258*, 677. (b) Brown, R. S.; Salmon, D.; Curtis, N. J.; Kusuma, S. *J. Am. Chem. Soc.* **1982**, *104*, 3188. (c) Martell, A. E.; Chaberek, S.; Jr.; Courtney, R. C.; Westerback, S.; Hyytiainen, H. *J. Am. Chem. Soc.* **1957**, *79*, 3036. Gustafson, R. L.; Martell, A. E. *J. Am. Chem. Soc.* **1959**, *81*, 525.

Scheme I



Conclusions

One goal of the present study was to confirm that our previous findings[7] of linear unit slope behavior in the pH–rate profile for hydrolysis of *N*-methylpicolinanilides in the presence of Cu(II) were not an artifact of weak binding of the metal ion. The behavior of the 6-carboxy analog **1** has now been found to be identical to what we saw for the des-6-carboxy compound **2**, except that low concentrations of Cu(II) are sufficient to achieve saturation. The rate of the normal HO⁻-dependent reaction is enhanced ~10^6-fold by Cu(II) in both cases. A second goal of this study was to extend the phenomenon to other divalent metal ions, and although smaller rate enhancement effects are observed (~2-fold weaker for Zn(II), ~30-fold weaker for Co(II), and ~70-fold weaker for Ni(II)), it is now clear that the ability of divalent metals to catalyze the normal alkaline hydrolysis of anilides is the "norm". Thus, the pH-independent behavior observed by Przystas and Fife for the M(II)-promoted hydrolysis of 6-carboxy-2′,4′-dinitropicolinanilide[8] in the same pH range (5–7) must be the consequence of some idiosyncrasy of this reactant. Our preliminary studies suggest that the culprit responsible for the pH-independent behavior is M(II)-coordination-induced NH-deprotonation occurring below pH 5.[9]

In any event, the mechanistic implication of the unit slope pH-rate profiles for M(II)-catalyzed hydrolysis is the occurrence either of a unimolecular reaction of the **1**–M(II)–OH complex or of the kinetically equivalent mechanism of external HO⁻ attack on a **1**-M(II) complex. The usual notion of these two mechanisms involves respectively intramolecular nucleophilic attack of the M(II)-bound hydroxide on a noncoordinated amide carbonyl or external HO⁻ attack on a M(II)-coordinated amide carbonyl (i.e., electrophilic activation), as shown in Scheme I. As before,[7] although our data do not permit a choice between these two mechanisms, we have argued that the M(II)-bound tetrahedral intermediates formed from these two encounters are identical except for the position of a proton and that hydrogen bonding of the solvent would further lessen the tautomeric distinction.[15] If the two encounters (intermediates) are in rapid equilibrium during the hydrolysis reaction (i.e., rate-limiting C–N bond-breaking), the distinction between "M(II)–OH" and "carbonyl activation" mechanisms becomes a moot point. In addition, we cannot rule out the possibility of a template mechanism involving attack of the M^{2+}-bound hydroxide on the M^{2+}-bound carbonyl.

When the rate enhancement factors representing saturation of **1** with M(II) in Table I are compared, the finding of the greatest REF for Cu(II) is not unexpected. However, it is surprising how close the REF for Zn(II) comes to that of Cu(II), at the same time being very much larger than that for Co(II) and Ni(II). For **2**, as well as in other reported systems, the catalytic effectiveness of Zn(II) is substantially less than that of Cu(II) and is close to those of Ni(II) and Co(II).[3b,10] Notwithstanding the fact that Zn(II) is nearly as effective as Cu(II) in the hydrolysis of **1**, substantially higher concentrations of Zn(II) are needed for saturation, and the inhibitory effect of the buffer (competing with the ligand for binding to the metal) is substantially greater for Zn(II). Although these latter findings are expected consequences of the lower Lewis acidity of Zn(II), it appears that prediction of catalytic effectiveness cannot be made simply on this same basis and must involve more subtle influences of the coordination environment. Perhaps the fact that Zn(II) is the metal ion of choice in metallohydrolases relates somewhat to the observation made here.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded on Varian XL-200 and Gemini 300 instruments, and chemical shifts are reported relative to tetramethylsilane. High-resolution mass spectra were obtained using a Kratos MS-25 instrument. Melting points are uncorrected. The water used in the kinetic experiments was doubly distilled in an all-glass apparatus. Inorganic reagents were ACS grade, and organic chemicals were obtained from Aldrich Chemical Co. The preparation of *N*-methyl-4′-nitropicolinanilide (**2**) was described previously.[7] Reaction mixture pH values were obtained at 40.0 °C (kinetics) or 25.0 °C (titrations) as needed using a Fisher Accumet Model 810 or 910 meter and combination electrode, standardized at the respective temperature using Fisher Certified buffers (pH 4.0, 7.0, and 10.0). The autoprotolysis constants pK_w used in this study were 13.535 for H_2O (40 °C) and 14.04 for EtOH–H_2O (1:2) (40 °C).[7] The apparent pH values measured in EtOH–H_2O (1:2) were corrected to the operational pH values by subtracting 0.06 (at both 40 and 25 °C).[7]

Preparation of *N*-Methyl-4′-nitro-6-carboxypicolinanilide. According to a modification of a published method,[8] 5 mmol each of 2,6-pyridinedicarboxylic acid and trifluoroacetic anhydride were stirred together under N_2 in the absence of solvent at room temperature for 5 h, then 5 mmol of *N*-methyl-4-nitroaniline was added, and the mixture was heated with stirring at 80 °C for 5 h. After cooling to room temperature overnight, the solid mass was dissolved in a hot mixture of 100 mL of $CHCl_3$ and 50 mL of H_2O. The $CHCl_3$ layer was washed with 50 mL of water, dried (Na_2SO_4), and concentrated to give a mixture of desired monoamide, undesired diamide, and recovered aniline, from which **1** eventually precipitated. Purification was by recrystallization from $CHCl_3$–CH_3CN. Alternatively, the mixture could be chromatographed on silica gel, but in this case the desired product elutes as its sodium salt (Na^+ is the silicate counterion). Conversion of the latter to neutral **1** can be achieved by dissolving in dilute HCl (pH 3), evaporating to dryness, and selective extraction of **1** away from NaCl with cold EtOH–CH_3CN: mp 206–208 °C; ^{1}H NMR (DMSO-d_6) δ 3.51 (s, 3H), 7.43 (d, 2H, *J* = 9.2 Hz), 7.81 (app t, 1H, *J* = 4.4 Hz), 8.02 (d, 2H, *J* = 4.4 Hz), 8.10 (d, 2H, *J* = 9.2 Hz); ^{13}C NMR (CD_3CN(δ 38.42, 125.42, 125.72, 128.15, 128.85, 140.54, 146.27, 146.79, 151.09, 153.53, 164.79, 167.72. The methyl ester of **1** was prepared for the purpose of obtaining a good mass spectrum: A 0.1-mmol sample of **1** in 5 mL of CH_3CN was mixed with ethereal diazomethane, generated from Diazald with aqueous KOH.[16] Evaporation of the solvent, dissolution of the residue in $CHCl_3$, drying (Na_2SO_4), and evaporation yielded *N*-methyl-4′-nitro-6-carbomethoxypicolinanilide: mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 3H, NCH_3), 3.86 (s, 3H, OCH_3), 7.28 (d, 2H, *J* = 9.2 Hz), 7.90–8.10 (m, 3H), 8.17 (d, 2H, *J* = 9.2 Hz); EIMS (40 eV) *m/z* calcd for $C_{15}H_{13}N_3O_5$ 315.0855, found 315.0854 (17%, M^+).

Kinetics. Buffer solutions at various pH values were prepared weekly in doubly distilled water. Buffers used (respective pH ranges): hydrochloric acid (pH 1.0–3.0), formate (pH 3.5–4.7), acetate (pH 4.7–5.8), MES (2-(*N*-morpholino)ethanesulfonic acid, pH 5.5–6.7), HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid, pH 6.8–8.2), EPPS (*N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid, pH 7.3–8.7), TAPS (3-((tris(hydroxymethyl)methyl)amino)propanesulfonic acid, pH 7.7–9.1), morpholine (pH 8.0–9.0), borate (pH 8.5–12.0), CHES (2-(cyclohexylamino)ethanesulfonic acid, pH 8.6–10.0), CAPSO (3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid, pH 8.9–10.3), and CAPS (3-(cyclohexylamino)-1-propanesulfonic acid, pH 9.7–11.1). The ionic strength was maintained at 0.1 M with KNO_3, and the solution used for rate measurements in the absence of metal ions contained 2×10^{-5} M EDTA as a precaution against trace levels of transition metal ions in the buffer or salt.

Hydrolysis reactions were carried out in 1-cm quartz cells at 40.0 °C using a Perkin-Elmer Lambda 3B UV–vis spectrophotometer (with

---

[15] Sayre, L. M. *J. Am. Chem. Soc.* **1986**, *108*, 1632.

[16] Technical Information Bulletin No. AL-113, Aldrich Chemical Co.

PECSS software) equipped with a temperature-controlled six-cell changer. UV–vis spectral monitoring of the hydrolysis reactions of **1** showed a clear isosbestic point at 340 nm, with a decrease in absorbance at shorter wavelength (double λ_{max} at 275 and 295 nm) and an increase at longer wavelength (λ_{max} 405 nm) representing the aniline product. The latter absorbance increase was most conveniently followed for obtaining rate data, though the same k_{obs} values were obtained by following the absorbance decreases at 295 nm. Kinetic runs were initiated by injecting 18–30 μL of a CH_3CN solution of the amide (0.01–0.02 M) and 6–30 μL of a 1.0 M aqueous solution of the M(II) nitrate hexahydrates (hemi(pentahydrate) in the case of $Cu(NO_3)_2$) into 3 mL of the buffer solution which was temperature-equilibrated in the cell compartment. That the products of the hydrolysis were 2,6-pyridinedicarboxylic acid and *N*-methyl-4-nitroaniline was confirmed by TLC and ^1H NMR and by the fact that the UV–vis spectrum of the 1:1 mixture (containing metal ion) exactly matched the final UV–vis spectrum of the hydrolysis reaction. The reactions followed first-order kinetics, generally to greater than 3 half-lives. The linear portions of the pH–rate profiles were evaluated by linear regression. In some cases (where indicated in the figure legends) the pH–rate profile curves shown represent an analysis of the kinetic data according to eq 1, using a least-squares computer program (Lottos). In all other figures, the pH–rate curves were computer-fitted by an unspecified polynomial equation.

Potentiometric Titration. The titration of 25 mL of an $EtOH-H_2O$ (1:2) solution (0.1 M $NaNO_3$) that was 1.0 mM each in **1** and $Cu(ClO_4)_2$ was carried out in 15 min at 25.0 °C using 25 mM NaOH which was standardized against potassium hydrogen phthalate. Plots of pH, measured using a Fisher Accumet Model 910 meter, as a function of added NaOH solution exhibited clear inflections at 1.0 and 2.0 equiv of added base. The apparent pK_a values given in the text represent the average result of two titrations performed on separate weighings.

Acknowledgment. We thank Dr. F. Wang for checking some of the data, Professors J. Stuehr and F. L. Urbach for helpful suggestions, and the National Institutes of Health (Grant GM 34294) for financial support.