950 mL of the salts solution were combined in the sterile fermentor apparatus described by Vederas.[31] The buret was charged with 50% $^{18}O_2$ and was replenished as needed. The experiment was carried out twice: the first time 1.2 L was consumed in 52 h, and the second time 1.2 L was consumed in 57 h. Each was worked up separately through the Silicar CC-4 chromatography, and the combined material was purified by PLC to yield 14.2 mg of **4d**, which was analyzed by ^{13}C NMR spectroscopy.

Incubation of *S. murayamaensis* in the Presence of D_2O. *S. murayamaensis* was grown in the standard production medium (two 200-mL broths) to which 10 mL of D_2O had been added sterilely to each flask. After 48 h, the fermentations were filtered and extracted with benzene, and—after they were dried and concentrated in vacuo—the extracts were chromatographed twice by PLC. This yielded 39 mg of kinamycin D that was recrystallized to afford 9.7 mg of pure metabolite: ^2H NMR (61.4 MHz, $CDCl_3$) δ 1.09 (b), 2.14 (b), 4.55 (vb), 5.40 (vb), and 7.58 (sharp peak overlapped by broad peak).

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Registry No. Kinamycin C, 35303-08-3; kinamycin D, 35303-14-1; acetate, 64-19-7.

Divalent Metal Ion Catalysis in Amide Hydrolysis. The Hydrolysis of *N*-Acylimidazoles

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Abstract: Rate constants have been determined for hydrolysis of *N*-picolinoylimidazole, *N*-picolinoylbenzimidazole, and *N*-(6-carboxypicolinoyl)benzimidazole in water at 30 °C. Hydroxide ion, hydronium ion, and water catalyzed reactions were observed. The second-order rate constants for OH^- catalysis, k_{OH}, are similar with *N*-picolinoylimidazole and *N*-picolinoylbenzimidazole. However, the pH-independent and apparent hydronium ion catalyzed reactions of the benzimidazole derivatives are slower than those of the *N*-acylimidazole. The latter reaction is retarded because of the relatively low pK_a of the *N*-acylbenzimidazole conjugate acid ($pK_{app} < 2$). Pronounced metal-ion catalysis occurs in the hydrolysis of these compounds in the presence of Cu^{2+}, Ni^{2+}, Co^{2+}, or Zn^{2+}. With the picolinoyl derivatives the plots of k_{obsd} vs. metal ion concentration were linear even at metal ion concentrations as high as 0.01 M. At the nonsaturating metal ion concentration of 0.01 M (0.001 M with Cu^{2+}) the rate enhancements in the OH^--catalyzed reactions ranged from 4×10^2 with Co^{2+} to 4×10^5 with Cu^{2+}. A Ni^{2+}-promoted water-catalyzed reaction was also observed in the hydrolysis of *N*-picolinoylbenzimidazole. The hydrolysis of *N*-isonicotinoylbenzimidazole is not significantly catalyzed by divalent metal ions. Therefore, metal ion catalysis requires the pyridine nitrogen to be adjacent to the carbonyl. Saturation was observed at low metal ion concentrations in the hydrolysis of *N*-(6-carboxypicolinoyl)benzimidazole. Metal ion promoted OH^--catalyzed reactions then occur with large enhancements in k_{OH}, ranging from 10^5–10^6 with Zn^{2+}, Co^{2+}, and Ni^{2+} to 10^9 with Cu^{2+}. The value of k_{OH} in the presence of a saturating concentration of Cu^{2+} is 5×10^{11} M^{-1} s^{-1}. Thus, a bimolecular attack of external OH^- can be ruled out; the reaction must involve intramolecular attack of metal ion bound OH^-. At pH > 6 formation of the metal ion–*N*-(6-carboxypicolinoyl)benzimidazole complex becomes the rate-limiting step in the Ni^{2+}-catalyzed reactions, and a pH-independent reaction is observed. Large metal ion catalytic effects are found in the OH^--catalyzed reactions of the *N*-acylbenzimidazoles because the C–N bond is easily broken.

A number of proteolytic enzymes require the presence of a metal ion for activity.[2,3] Carboxypeptidase A is a Zn(II) metalloenzyme that catalyzes the hydrolysis of peptides having a free terminal carboxyl group and O-acyl derivatives of α-hydroxy carboxylic acids.[2-4] Mechanisms have been suggested for the enzymatic reactions in which the Zn(II) ion is involved.[2-4] Divalent metal ion catalyzed ester hydrolysis reactions have been extensively studied.[5-13] Metal ion promoted OH^- catalysis occurs in these reactions, and enhancements in the second-order rate constant k_{OH} as large as 10^8 have been observed.[7,9] Metal ion promoted water reactions have also been detected in the hydrolysis of phenolic esters[13] and anhydrides of picolinic acid.[14] In contrast, there have been few reports of metal ion catalysis in the hydrolysis of amides,[15-17] and in those cases the rate enhancements have been

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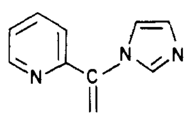
relatively small in comparison with those in ester hydrolysis. With amides having a neighboring carboxyl group that can act as a nucleophile, any metal ion catalysis in the hydrolytic reaction cannot compete with an unassisted carboxyl nucleophilic reaction.[8,18] Thus, with the present state of knowledge, it is difficult to see how a metal ion might be an effective catalyst in the enzymatic hydrolysis of peptides.

N-Acylimidazoles are amides that hydrolyze rapidly.[19-26] For example, the second-order rate constant for OH$^-$-catalyzed hydrolysis of *N*-acetylimidazole[20] at 25 °C is 316 $M^{-1}\,s^{-1}$, whereas k_{OH} for hydrolysis of the reactive ester *p*-nitrophenyl acetate[27] is 15 $M^{-1}\,s^{-1}$, even though the pK_a of the imidazole leaving group[28] is 14.5 while that of *p*-nitrophenol is 7. Thus, the C–N bond of *N*-acylimidazoles must be especially susceptible to hydrolytic cleavage. An easily broken C–O bond is an important factor leading to the metal ion promoted water reaction in the hydrolysis of phenyl esters.[13] The cutoff point in terms of the leaving group pK_a for observation of such a water reaction in the Ni^{2+}-assisted hydrolysis of esters of picolinic acid[13] lies between that of trifluoroethanol (pK_a = 12.4)[29] and ethanol (pK_a = 16.0). We thought, therefore, that metal ion catalysis might be found in the hydrolysis of *N*-acylimidazoles having a functional group in the acyl portion of the molecule capable of complexing metal ions and that both metal ion promoted OH$^-$ and H_2O reactions might be observed. As a consequence, the structural requirements for such reactions could be established in amide hydrolysis and the relationship between these reactions determined. We have, therefore, studied the hydrolysis reactions of the imidazole and benzimidazole derivatives of picolinic acid (I and II), pyridine 2,6-dicarboxylic acid (III), and isonicotinic acid (IV) (the pK_a of benzimidazole at 25 °C is 12.8).[28]



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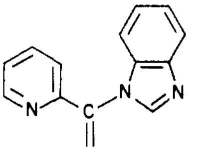

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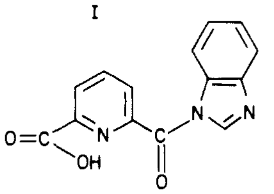

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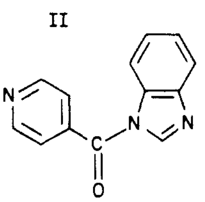

IV

Experimental Section

Materials. *N*-Picolinoylimidazole (I) and *N*-picolinoylbenzimidazole (II) were prepared by stirring 0.01 mol of picolinic acid, dicyclohexylcarbodiimide, and imidazole or benzimidazole in 125 mL of ethyl acetate for 16 h. The solution was filtered to remove the precipitated dicyclohexylurea, and the solvent was removed by rotary evaporation. *N*-Picolinoylimidazole boiled at 105 °C (0.02 mmHg), $n^{25}{}_D$ 1.5802. The *N*-picolinoylbenzimidazole was recrystallized 3 times from hexane and had mp 105–107 °C. Anal. Calcd for $C_{13}H_9N_3O$: C, 69.96; H, 4.04; N, 18.83. Found: C, 70.15; H, 4.28; N, 18.81. *N*-(6-Carboxypicolinoyl)benzimidazole (III) and *N*-isonicotinoylbenzimidazole (IV) were prepared by mixing 0.01 mol of trifluoroacetic anhydride and either 2,6-pyridinedicarboxylic acid or isonicotinic acid. After 2 h, 0.01 mol of benzimidazole was added, and the resulting mixture was allowed to stand overnight. The product was taken up in 140 mL of warm chloroform and washed with two 50-mL portions of water. The chloroform was dried with sodium sulfate, and the solvent was removed by rotary evaporation. *N*-(6-Carboxypicolinoyl)benzimidazole was recrystallized twice from acetonitrile, mp 218–220 °C dec. Anal. Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.37; N, 15.73. Found: C, 62.83; H, 3.59; N, 15.47. *N*-Isonicotinoylbenzimidazole was recrystallized twice from cyclohexane, mp 153–155 °C dec. Anal. Calcd for $C_{13}H_9N_3O$: C, 69.96; H, 4.04; N, 18.83. Found: C, 69.88; H, 4.21; N, 18.81.

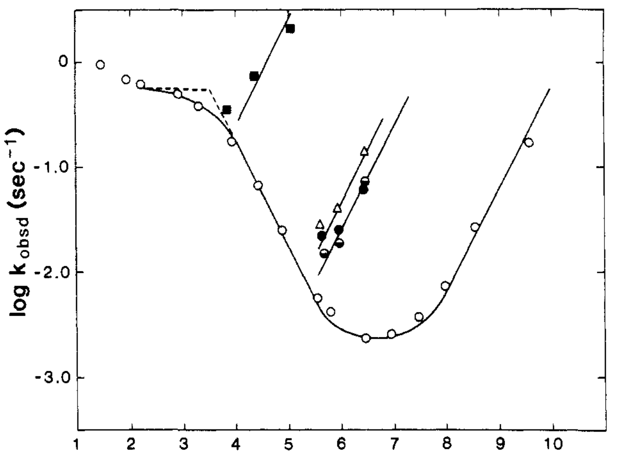

Figure 1. Plot of log k_{obsd} vs. pH for the hydrolysis of *N*-picolinoylimidazole (I) in H_2O at 30 °C with μ = 0.1 M (with KCl) in the presence of Cu^{2+} (■), Ni^{2+} (●), Co^{2+} (⊖), and Zn^{2+} (△) and in the absence of metal ions (○).

Table I. Rate Constants for Hydrolysis of I–IV in H_2O at 30 °C and μ = 0.1 M (with KCl)

compd	k_H,[a] $M^{-1}\,s^{-1}$	k_0, s^{-1}	k_{OH}, $M^{-1}\,s^{-1}$
I	1.78 × 10^3	1.6 × 10^{-3}	4.29 × 10^3
II	17.8	3.7 × 10^{-4}	2.15 × 10^3
III	31.6	6.9 × 10^{-4}	6.8 × 10^2
IV	31.6	8.0 × 10^{-4}	3.8 × 10^3

[a] k_H is equal to k_2/K_2.

Kinetic Methods. The rates of hydrolysis of I–IV were measured spectrophotometrically with a Beckman Model 25 or Durrum D110 stopped-flow spectrophotometer. In all cases, the disappearance of the reactant was monitored at 300 nm. The ionic strength was maintained at 0.1 M with KCl, and the solutions used for rate measurements in the absence of metal ion contained 2 × 10^{-5} M EDTA as a precaution against trace metal ions in the buffer or salt. The hydrolysis reactions are subject to moderate buffer catalysis. The reported rate constants were, therefore, obtained by extrapolation of k_{obsd} values obtained at several buffer concentrations to zero buffer concentration. In the pH regions where buffer catalysis was not experimentally significant, 0.01–0.02 M buffers were routinely used. No corrections were made for buffer-metal ion complexation. The buffers employed were HCl (pH 1–3), formate (pH 3.2–4.5), acetate (pH 4.5–5.5), 2,6-lutidine (pH 5.7–6.6), *N*-ethylmorpholine (pH 6.6–8.4), and carbonate (pH 8.8–10). Increasing the buffer concentration at constant pH and a constant concentration of metal ion had no effect, i.e., there is no buffer catalysis in the metal ion catalyzed reactions. Metal ion catalysis was studied in the pH range 2–8 with III and 4–7 with I and II because of precipitation at higher pH values.

Kinetic runs were initiated by injecting 15–30 μL of a stock solution of the *N*-acylimidazole (0.01 M in acetonitrile) into 3 mL of buffer solution in a 1-cm cuvette maintained at the desired temperature. It was necessary to warm the acetonitrile to completely dissolve *N*-(6-carboxypicolinoyl)benzimidazole. In rate measurements carried out with the stopped-flow apparatus, one drive syringe contained 100–150 μL of the *N*-acylimidazole stock solution in 15 mL of 0.1 M KCl, while the other

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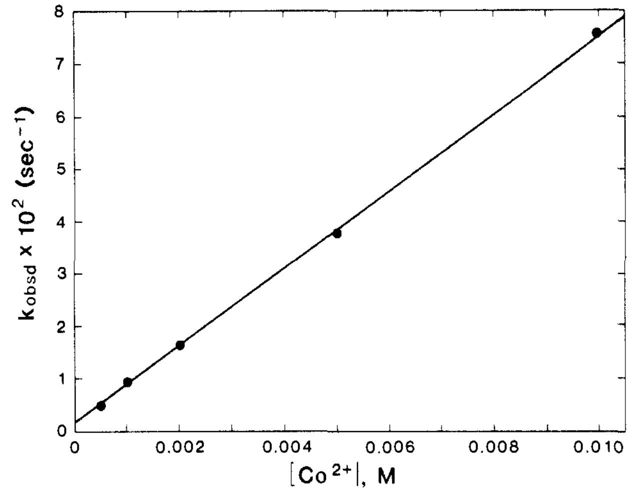


Figure 2. Plot of k_{obsd} vs. Co^{2+} concentration for the hydrolysis of *N*-picolinoylimidazole (I) in H_2O at 30 °C with $\mu = 0.1$ M (with KCl) at pH 6.47.

Table II. Second-Order Rate Constants k_{OH} (M^{-1} s^{-1}) for the Hydroxide Ion Catalyzed Hydrolysis of the Metal Ion Complexes of I, II, and III at 30 °C and $\mu = 0.1$ M

compd	Cu^{2+}	Co^{2+}	Ni^{2+}	Zn^{2+}
I[a]	1.7×10^9	1.7×10^6	1.7×10^6	3.0×10^6
II[a]	6.8×10^8	6.8×10^5	9.6×10^5 9.6×10^4 [b]	1.2×10^6
III[c]	5.4×10^{11}	4.6×10^8	7.6×10^8 2.7×10^8 [b,d]	7.6×10^8

[a] Values of the rate constants were obtained with nonsaturating concentrations of metal ions (0.001 M Cu^{2+} and 0.01 M Co^{2+}, Ni^{2+}, and Zn^{2+}). [b] 0.001 M Ni^{2+}. [c] Values of the rate constants were obtained with saturating concentrations of metal ions (0.001 M Cu^{2+} and 0.01 M Co^{2+}, Ni^{2+}, and Zn^{2+}). [d] At pH < 6.

syringe contained the buffer and the metal ion. The reactions followed pseudo-first-order kinetics for at least 4 half-lives. Reaction mixture pH values were obtained at 30 °C with a Radiometer Model 22 pH meter. Second-order rate constants for hydroxide ion catalysis were calculated by employing a K_w value of 1.47×10^{-14} at 30 °C.

Results

In Figure 1 is shown a plot of log k_{obsd} vs. pH for hydrolysis of *N*-picolinoylimidazole (I) in H_2O at 30 °C, $\mu = 0.1$ M with KCl. Hydroxide ion and apparent hydronium ion catalysis can be observed. In addition there is a pH-independent region in the profile from pH 5.5 to 7.5. The equation for k_{obsd} at $a_H < K_1$ is given in eq 1, where k_{OH} is the second-order rate constant for OH^-

$$k_{obsd} = \frac{k_2 a_H{}^2 + k_0 K_2 a_H + k_{OH} K_w K_2}{a_H{}^2 + K_2 a_H} \tag{1}$$

catalysis, k_0 is the rate constant for the pH-independent reaction, k_2 is the rate constant for reaction of the monoprotonated species, and K_1 and K_2 are the first and second acid-dissociation constants. Values of the rate constants are given in Table I. The value of pK_2 from Figure 1 is 3.5. There is then a further increase in k_{obsd} with decreasing pH at pH less than 2.5, but the presence of a positive charge in the molecule has so increased K_1 that it is not detected at pH > 1.5.

Pronounced metal ion catalysis was found in the hydrolysis of I by Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} even though metal ion binding to the acylimidazole is weak. The plot of k_{obsd} vs. Co^{2+} concentration at pH 6.47 (Figure 2) is still linear at the high metal ion concentration of 0.01 M. In Figure 1 plots are presented of log k_{obsd} vs. pH for the hydrolysis reactions in the presence of 0.001 M Cu^{2+} and 0.01 M (nonsaturating) Ni^{2+}, Co^{2+}, and Zn^{2+}. The values of k_{obsd} in the presence of the metal ions were obtained by subtracting the values for the spontaneous (zero metal ion) reaction. The plots are linear with slopes of 1.0, which shows that the reactions involve metal ion promoted OH^- catalysis. The rate constants for these reactions are given in Table II.

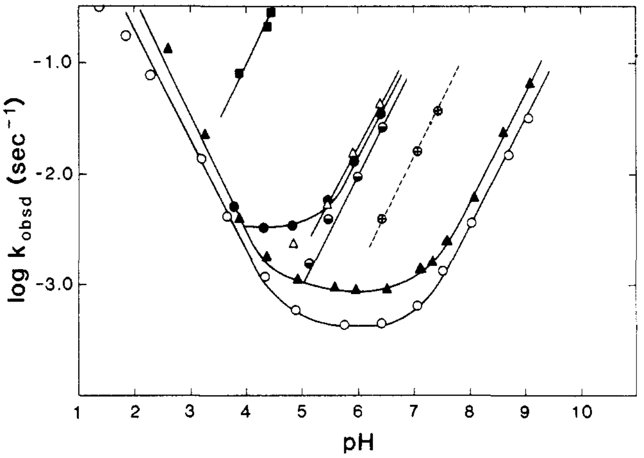


Figure 3. Plot of log k_{obsd} vs. pH for the hydrolysis of *N*-picolinoylbenzimidazole (II) in H_2O at 30 °C with $\mu = 0.1$ M (with KCl) in the presence of 0.001 M Cu^{2+} (■), 0.01 M Ni^{2+} (●), Co^{2+} (◒), and Zn^{2+} (△), 0.001 M Ni^{2+} (⊕), and in the absence of metal ions (○). Also included is the plot of log k_{obsd} vs. pH for hydrolysis of *N*-isonicotinoylbenzimidazole vs. pH (▲) at 30 °C.

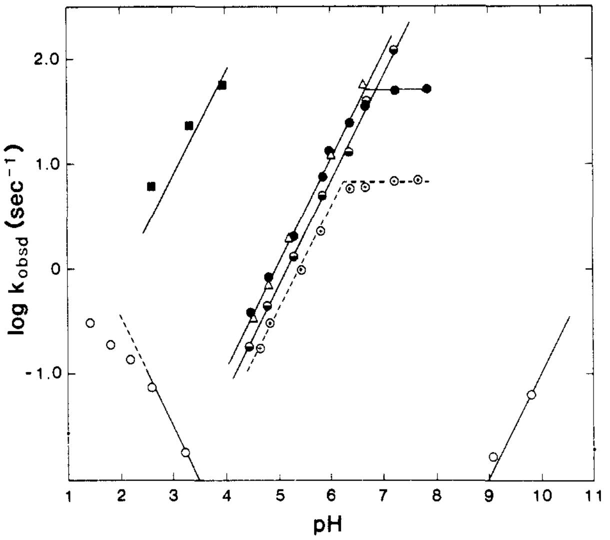


Figure 4. Plot of log k_{obsd} vs. pH for the hydrolysis of *N*-(6-carboxypicolinoyl)benzimidazole (III) in H_2O at 30 °C with $\mu = 0.1$ M (with KCl) in the presence of 0.001 M Cu^{2+} (■), 0.01 M Ni (●), Co^{2+} (◒), and Zn^{2+} (△), 0.001 M Ni^{2+} (⊕), and in the absence of metal ions (○).

The plot of log k_{obsd} vs. pH for hydrolysis of *N*-picolinoylbenzimidazole (II) in H_2O at 30 °C is shown in Figure 3. Again the data give a good fit to eq 1 except that neither K_1 nor K_2 are detectable at pH >1.5. The rate constants are given in Table I. As was the case with I, large metal catalysis was observed, and again plots of k_{obsd} vs. metal ion concentration were linear. The rate enhancements were similar in the OH^--catalyzed reaction for I and II. In addition, with 0.01 M Ni^{2+}, a metal ion promoted water reaction was detected in the hydrolysis of II with $k_0 = 3 \times 10^{-3}$ s^{-1}. Also included in Figure 3 is the plot of log k_{obsd} vs. pH for hydrolysis of *N*-isonicotinoylbenzimidazole (IV). The rate constants (Table I) are slightly greater than those of II. Significant metal ion catalysis was not observed in the hydrolysis of IV. At pH values of 4.26 and 4.84, 0.001 M Cu^{2+} increased k_{obsd} by approximately 30%, while at pH values from 6.41 to 7.51, 0.01 M Ni^{2+} increased k_{obsd} by ~40%.

The carboxyl group in the 6-position of *N*-(6-carboxypicolinoyl)benzimidazole (III) does not have great effect on the hydrolysis reactions; as seen in Figure 4 and Table I, the OH^--catalyzed reaction is 3-fold slower than that of II. There are now three ionizations that must be taken into account, i.e., the equation for k_{obsd} at $K_1 > a_H$ is that in eq 2, where k_{OH} is the second-order rate constant for OH^- catalyzed hydrolysis of the

$$k_{\text{obsd}} = \frac{k_2 a_H^3 + k_3 K_2 a_H^2 + k_4 K_2 K_3 a_H + k_{\text{OH}} K_w K_2 K_3}{a_H^3 + K_2 a_H^2 + K_2 K_3 a_H} \quad (2)$$

monoanion, k_3 and k_4 are the rate constants for water catalysis (or kinetic equivalents) of the reaction of the neutral species and the monoanion, respectively, and K_3 is the third ionization constant. An inflection in the log k_{obsd} vs. pH profile due to ionization of the 6-carboxyl group was not detected; therefore k_0 in Table I equals k_4 or k_3. The log k_{obsd} vs. pH profile is identical in shape with that of II, and the rate constants are provided in Table I. Only the k_{obsd} values at the pH extremes are shown in Figure 4 for reactions in the absence of metal ions so that the metal ion caalyzed reactions can be presented with greater clarity in the figure.

Metal ion binding to III is very strong; saturation effects are observed at low metal ion concentrations, as seen in Figure 5 where k_{obsd} is plotted vs. Co^{2+} concentration at pH 6.74. Thus, at constant pH the reactions follow eq 3, where k_M is the limiting

$$k_{\text{obsd}} = \frac{k_M K_M [M^{2+}]}{1 + K_M [M^{2+}]} \quad (3)$$

rate constant for metal ion catalysis and K_M is the metal ion association constant. At pH 6.74, k_M is 38.5 s^{-1} and K_M is 5 × 10^2 M^{-1} for Co^{2+}. The value of k_M at pH 6.00 with Zn^{2+} is 13.5 s^{-1} and K_M is 3 × 10^2 M^{-1}. At pH 5.85 with Ni^{2+} k_M is 6.7 s^{-1} and K_M is 6 × 10^2 M^{-1}. Catalysis by Cu^{2+} at pH 3.27 is characterized by $k_M = 31$ s^{-1} and $K_M = 4 \times 10^3$ M^{-1}. These values were obtained employing eq 3 and do not reflect protonation of the substrate.

At the saturating metal ion concentrations the rate enhancements in the OH^--catalyzed reactions of III are now very large (Figure 4 and Table II) ranging up to 10^9 with Cu^{2+}. The reaction in the presence of Ni^{2+} becomes pH independent at pH >6. This very likely reflects a change in rate-limiting step to rate-determining formation of the metal ion complex (see Discussion). This is supported by the fact that at pH >6 the plots of k_{obsd} vs. Ni^{2+} concentration are linear (Figure 6), in contrast with the curved saturation plots at pH <6.

Discussion

The hydrolysis of N-acylimidazoles is characterized by hydronium ion, hydroxide ion, and water catalyzed reactions[19-26] or kinetic equivalents. Neighboring carboxyl[30] and acetamido[31] group participation has also been observed. The log k_{obsd} vs. pH profile for hydrolysis of N-picolinoylimidazole (**I**) is consistent with those previously obtained.[20,31] However, the water reaction (or kinetic equivalent) in the pH range from 5.5 to 7.5 is more facile than usually encountered; k_0 is over 4-fold larger than that for hydrolysis of N-benzoylimidazole.[32] A similar pH-independent reaction from pH 5–7 is also observed in the hydrolysis of N-picolinoylbenzimidazole (II). This reaction does not involve intramolecular general base participation by the pyridine nitrogen since the corresponding isonicotinoyl derivative (IV), with which such participation is not possible, has a comparable rate constant k_0. That was also the case in the pH-independent hydrolysis of picolinic and isonicotinic cinnamic anhydride,[14] both of which hydrolyzed considerably faster than benzoic cinnamic anhydride.

The pH-independent and apparent hydronium ion catalyzed reactions of N-acylbenzimidazoles are slower than those of analogous N-acylimidazoles.[33] The effect in the latter reaction is undoubtedly a reflection of the relatively low pK_a of the N-acylbenzimidazole conjugate acid (~2).[33] This would also have an important influence in the pH-independent reaction if the reaction involved attack of OH^- on the protonated species. However, the k_0 for hydrolysis of II is only 4-fold less than that of I, whereas there is a 100-fold difference in the rate constants k_H for the apparent hydronium ion catalyzed reaction (see Table I). It is, therefore, likely that the pH-independent reactions proceed with water catalysis of the hydrolysis of the neutral species, as suggested for N-acetylimidazole.[21]

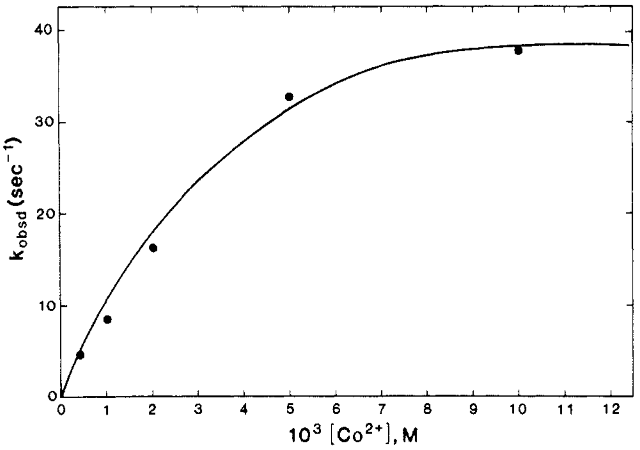


Figure 5. Plot of k_{obsd} vs. Co^{2+} concentration for the hydrolysis of N-(6-carboxypicolinoyl)benzimidazole in H_2O at 30 °C with $\mu = 0.1$ M (with KCl) and at pH 6.74.

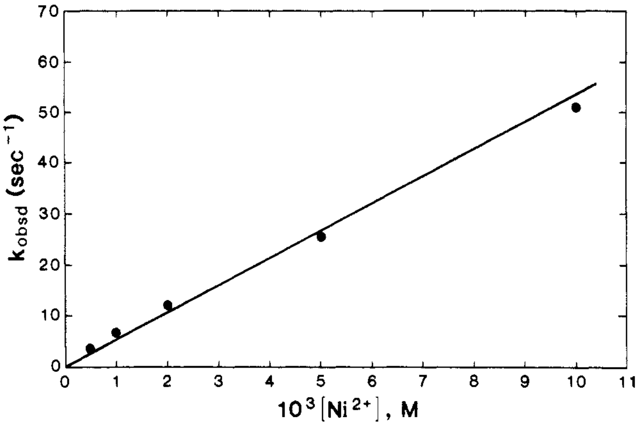
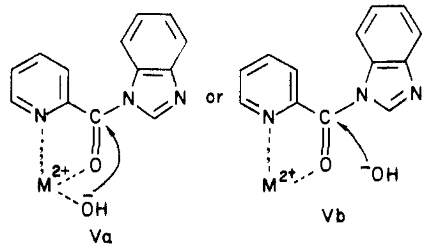


Figure 6. Plot of k_{obsd} vs. Ni^{2+} concentration for the hydrolysis of N-(6-carboxypicolinoyl)benzimidazole in H_2O at 30 °C with $\mu = 0.1$ M (with KCl) and at pH 7.20.

Metal Ion Catalysis. There is pronounced catalysis by the divalent metal ions Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} in the hydrolysis of I and II, even though metal ion binding to the reactant is weak. Plots of k_{obsd} vs. metal ion concentration were linear even at metal ion concentrations as high as 0.01 M. It can be seen in Figures 1 and 3 that at constant metal ion concentrations the catalytic effect is being exerted on the hydroxide ion catalyzed reaction. Thus, a metal ion promoted OH^- catalyzed reaction is occurring similar to that observed in the hydrolysis of esters.[5-13] Rate enhancements in this reaction increase from 4 × 10^2 with 0.01 M Co^{2+} to 4 × 10^5 with 0.001 M Cu^{2+}. Significant metal ion catalysis was not observed in hydrolysis of the analogous isonicotinoyl derivative. Therefore, a chelation effect involving the pyridine nitrogen must be important (V). Intramolecular attack of metal ion bound OH^- (Va) and attack of external OH^- on the metal ion complex (Vb) are kinetically equivalent.



Va

Vb

A pH-independent metal ion promoted water reaction takes place in the hydrolysis of anhydrides and esters with leaving groups

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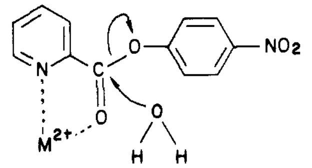
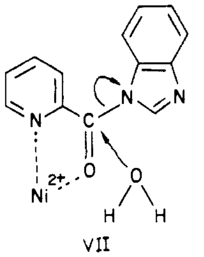
(32) Kogan, R. L., unpublished data: k_0 is 4 × 10^{-4} s^{-1} at 30 °C.

(33) Fife, T. H.; Natarajan, R.; Werner, M., unpublished data.

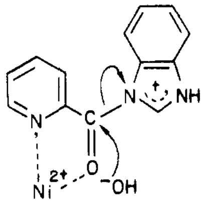
of pK_a less than 12.4, as in VI.[13] A metal ion promoted water reaction was not detected in the hydrolysis of I, but that could be due to competition from the rapid spontaneous reactions. In view of the relatively slow spontaneous hydrolysis at pH <7 and the low pK_a of the benzimidazole leaving group, a metal ion promoted water reaction or kinetic equivalent would be more readily detected in the hydrolysis of an *N*-acylbenzimidazole than the corresponding *N*-acylimidazole. As can be seen in Figure 3, that is the case for the hydrolysis of *N*-picolinoylbenzimidazole (II). In the presence of Ni^{2+} at a nonsaturating concentration of 0.01 M, both metal ion promoted OH^- and H_2O-catalyzed reactions can be observed, although with Co^{2+} and Zn^{2+} only the apparent OH^--catalyzed reaction was detected. Rate enhancements in the OH^--catalyzed reaction are comparable to those with I, and in the Ni^{2+}-catalyzed pH-independent reaction the rate enhancement is a factor of 10. While the metal ion catalyzed pH-independent reactions of anhydrides[14] and picolinate esters[13] must involve attack of a water molecule on the metal ion complex (VI), kinetic equivalents exist for the reaction of II, i.e., either attack of a water molecule could be occurring (VII) or the reaction might involve attack of OH^- on the protonated species (VIII).



VI

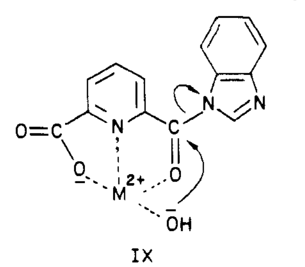


VII



VIII

Saturation effects are observed in the metal ion catalyzed hydrolysis of *N*-(6-carboxypicolinoyl)benzimidazole as seen in Figure 5. At saturating metal ion concentrations only metal ion promoted OH^- catalyzed reactions occur. The increased strength of metal ion binding has increased the efficiency of this reaction so that water catalysis is not observed. Note in Figure 4 that the plot of log k_{obsd} vs. pH in the Cu^{2+}-catalyzed reaction is linear with a slope of 1.0 at pH values as low as 3. The rate enhancements in the metal ion promoted OH^- reactions are now very large, ranging from 10^5–10^6 with Zn^{2+}, Ni^{2+}, and Co^{2+} to 10^9 with Cu^{2+}. These are the largest catalytic effects by metal ions that have been found in amide hydrolysis. Note in Table II that the second-order rate constant k_{OH} for the Cu^{2+}-catalyzed reaction is 5.4×10^{11} M^{-1} s^{-1}, a value which exceeds that of a rate constant for a diffusion-controlled reaction (10^{10} M^{-1} s^{-1}).[34] Therefore, the reaction cannot involve attack of external OH^- on the metal ion complex but must be an example of intramolecular attack of metal ion bound OH^- as in IX. This is also very likely the case with the other metal ions. The hydroxopentaamminecobalt(III) complex $(NH_3)_5CoOH^{2+}$ will act as a bimolecular nucleophile toward *N*-acetylimidazole (k_B = 14.9 M^{-1} s^{-1} at 25 °C) but will only attack the protonated species with which the leaving group pK_a is ~7.[35] On the other hand, the intramolecular nature of the metal ion promoted OH^- catalyzed reactions of III permits facile hydrolysis of the neutral species having a leaving group pK_a of 12.8.[28]



IX

An increased catalytic effect due to strong metal ion binding to the reactant has been observed previously in the hydrolysis of 8-(2-carboxyquinolyl) esters[9] and O-esters of 2-hydroxymethylpicolinic acid.[11] This would appear to be a characteristic of metal ion promoted OH^- catalysis (IX). In contrast, the strength of metal ion binding to the reactant is not a significant factor in regard to the magnitude of the rate enhancements at constant metal ion concentrations in the hydrolysis of acetals with which C–O bond breaking is rate determining.[36] In the metal ion promoted OH^- catalyzed reactions of the *N*-acylbenzimidazoles nucleophilic attack of OH^- is very likely the rate-determining step. Therefore, strong binding of the carbonyl group by a metal ion should have a large catalytic effect.

The Ni^{2+}-catalyzed reaction of III becomes pH independent at pH >6. The pK_a of the aquo complex of Ni^{2+} is 10.6;[37] therefore, the downward bend in the plot of log k_{obsd} vs. pH must be due to a change in rate-determining step rather than to an ionization (note that such a bend is not observed in Figures 1 and 3 in the hydrolysis of I and II). Replacement of a liganded water molecule in aquo complexes of Ni^{2+} is characterized by a rate constant of 3×10^4 s^{-1}, whereas with Cu^{2+}, Zn^{2+}, and Co^{2+} this rate constant is much larger (5×10^8, 3×10^7, and 4×10^5 s^{-1}, respectively).[38] It is likely that with Ni^{2+} replacement of H_2O is the rate-determining step in complex formation. Formation of the metal ion complex may, therefore, occur at a slower rate with Ni^{2+} than with the other metal ions in the series and will become rate limiting in the hydrolysis of III when the apparent OH^--catalyzed reaction becomes sufficiently rapid. For the scheme of eq 4 k_{obsd} is given by eq 5,[39] assuming that ionization of the nickel complex is fast. The rate-determining step in the

$$Ni^{2+} + S \underset{k_d}{\overset{k_f}{\rightleftharpoons}} NiS^{2+} \overset{K_{MOH}}{\rightleftharpoons} Ni(OH)S^+ + H^+ \xrightarrow{k_r} \text{products} \tag{4}$$

$$S = III$$

$$k_{obsd} = \frac{k_f k_r K_{MOH}[Ni^{2+}]}{a_H(k_d + k_f[Ni^{2+}]) + K_{MOH}(k_r + k_f[Ni^{2+}])} \tag{5}$$

Ni^{2+}-promoted OH^--catalyzed reaction will change from attack of OH^- (product formation) to complex formation at low pH when $K_{MOH}k_r/a_H$, i.e., $k_{OH}(OH^-)$, $>k_f(Ni^{2+})$ if k_d is small. In fact, however, k_d is not small enough to drop from the denominator of eq 5. If K_M for Ni^{2+} is 6×10^2 M^{-1} and $k_f = 5 \times 10^3$ M^{-1} s^{-1} (calculated from Figure 4),[40] then k_d is 8 s^{-1} since $K_M = k_f/k_d$. Therefore, the rate-determining step will change when $K_{MOH}k_r/a_H$ > ($k_d + k_f[Ni^{2+}]$). Thus, at a Ni^{2+} concentration of 0.01 M the rate-determining step should change at pH 6.7, and when Ni^{2+} is present at 0.001 M, the rate-determining step should change

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(34) Eigen, M.; De Maeyer, L. *Z. Elektrochem.* **1955**, *59*, 986.

(35) Buckingham, D. A. In "Biological Aspects of Inorganic Chemistry"; Addison, A. W., Cullen, W. R., Dolphin, D., James, B. R., Eds.; Wiley-Interscience; New York, 1977; p 191.

(36) Przystas, T. H.; Fife, T. H. *J. Am. Chem. Soc.* **1980**, *102*, 4391.

(37) The plots of log k_{obsd} vs. pH in metal ion promoted OH^- catalyzed reactions will have slopes of 1.0 at pH values below the pK_a of the metal ion coordinated water molecule. The pK_a values for ionization of the aquo complexes of metal ions at 25 °C are as follows: Cu^{2+}, 6.8; Zn^{2+}, 8.8; Co^{2+}, 8.9; Ni^{2+}, 10.6. Basolo, F.; Pearson, R. G. "Mechanisms of Inorganic Reactions", 2nd ed.; Wiley: New York, 1967; p 32.

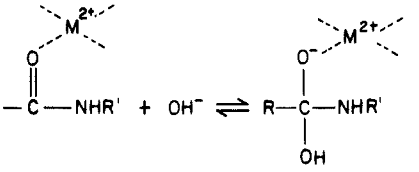
(38) Cotton, F. A.; Wilkinson, G. "Advanced Inorganic Chemistry", 3rd ed.; Wiley-Interscience: New York, 1972; p 656.

(39) Equations 4 and 5 apply to the reactions catalyzed by all of the metal ions that were studied. However, with Cu^{2+}, Co^{2+}, and Zn^{2+} $a_H(k_d + k_f[M^{2+}])$ is always greater than $K_{MOH}k_r$ at the pH values employed.

(40) The reactions of Ni^{2+} with neutral ligands have second-order rate constants of ca. 4×10^3 M^{-1} s^{-1} at 25 °C. Wilkens, R. G. *Acc. Chem. Res.* **1970**, *3*, 408.

at pH 6.1. As can be seen in Figure 4, it is near these pH values that the changes in slope occur.

The large rate enhancements due to metal ion catalysis in the hydrolysis of *N*-acylimidazoles can be contrasted with the small or negligible effects that have usually been observed in amide hydrolysis.[15,18] The very poor leaving group of an amide normally results in a very unfavorable OH^--catalyzed reaction. The rate-determining step in such reactions is very likely breakdown of a tetrahedral intermediate (C–N bond breaking). Therefore, the efficiency of metal ion promoted OH^- catalysis will be reduced or abolished, i.e., complexation of the carbonyl will enhance the nucleophilic attack step but will retard the C–N bond-breaking process by preventing electron release from oxygen (eq 6). On the other hand, the relatively low pK_a of the imidazole and benzimidazole leaving groups of *N*-acylimidazoles and *N*-acylbenzimidazoles will allow nucleophilic attack by OH^- to be rate determining (nucleophilic attack will be rate determining if the reaction is concerted or if the leaving group departs from a tetrahedral intermediate faster than the intermediate reverts to reactants). As a consequence, polarization of the carbonyl group by a metal ion can in these cases be effective in producing large catalytic effects. Thus, the key factor leading to the facile metal ion catalysis in the hydrolysis of *N*-acylimidazoles appears to be the easily broken C–N bond.



$$\mathrm{R{-}\overset{\overset{M^{2+}}{\vdots}}{\overset{O}{\overset{\|}{C}}}{-}NHR'} + OH^- \rightleftharpoons \mathrm{R{-}\underset{\underset{OH}{|}}{\overset{\overset{M^{2+}}{\vdots}}{\overset{O^-}{\overset{|}{C}}}}{-}NHR'} \qquad (6)$$

Carboxypeptidase A. An increased ease of C–N bond breaking brought about by binding of a peptide substrate to the enzyme would contribute to significant Zn(II) involvement in the carboxypeptidase A catalyzed hydrolysis reactions. Ease of bond breaking might be achieved by protonation of the leaving group nitrogen and/or strain which restricts the resonance interaction between the amide nitrogen and the carbonyl (eq 7). Protonation of nitrogen would occur most readily in an equilibrium process after formation of a tetrahedral intermediate or in a process concerted with C–N bond breaking. General acid catalysis by Tyr-248 has been considered as a mechanistic possibility,[3] but replacement of Tyr-248 by phenylalanine produced no change in k_{cat} in the hydrolysis of hippuryl-L-phenylalanine (K_m was increased about threefold).[41] Thus, the most probable proton donor would be metal ion bound water or Glu-270 (after proton abstraction from water in a general base process.[42] Nevertheless, in a concerted reaction the bond must begin to break while the proton is still at a distance, which presupposes an easily broken bond. The poor substrate glycyltyrosine binds to carboxypeptidase A with the carbonyl oxygen complexed to the metal ion, the aromatic residue associated with a hydrophobic pocket, and the carboxylate anion bound to Arg-145.[2,3] Binding of a peptide substrate in this manner requires the peptide linkage to be twisted, thereby reducing the resonance interaction.[3,43] This effect of binding could lead then to facile formation of a tetrahedral intermediate or a concerted reaction in which general acid catalysis occurs in reactions involving Glu-270, water, or OH^- attack.

$$\mathrm{R{-}\overset{\overset{O}{\|}}{C}{-}NHR'} \longleftrightarrow \mathrm{R{-}\overset{\overset{O^-}{|}}{C}{=}\overset{+}{N}HR'} \qquad (7)$$

The reactions of *N*-acylimidazoles and *N*-acylbenzimidazoles illustrate the importance of the resonance effect in amide hydrolysis. Molecular orbital calculations on *N*-acetylimidazole[44] indicate that resonance interactions of the type in eq 7, which will reduce the partial positive charge on the carbonyl carbon, are less favorable than with a usual amide. This may be due to opposed resonance, i.e., the effect of the second nitrogen in the ring (N-3) in accommodating net negative charge (−0.23 in *N*-acetylimidazole).[44] On the other hand, N-1 and the carbonyl carbon have net electronic charges of +0.475 and +0.287, respectively. Thus, the carbonyl carbon of an *N*-acylimidazole can be attacked readily by a nucleophile, the C–N bond is easily broken, and pronounced metal ion catalysis is observed.

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

Registry No. I, 100312-28-5; II, 100312-29-6; III, 100312-30-9; IV, 100312-31-0; H_3O^+, 13968-08-6; Co^{2+}, 22541-53-3; Cu^{2+}, 15158-11-9; Ni^{2+}, 14701-22-5; Zn^{2+}, 23713-49-7; hydroxide ion, 14280-30-9; water, 7732-18-5; picolinic acid, 98-98-6; imidazole, 288-32-4; benzimidazole, 51-17-2; 2,6-pyridinedicarboxylic acid, 499-83-2; isonicotinic acid, 55-22-1.

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(41) Hydrolysis of the ester substrate hippuryl-DL-phenyllactate was also not significantly affected when Tyr-248 was replaced by phenylalanine, but there was diminished activity toward O-(*p*-chloro-*trans*-cinnamoyl)-L-β-phenyllactate. Gardell, S. J.; Craik, C. S.; Urdea, M. S.; Kaiser, E. T.; Rutter, W. J. *J. Cell. Biochem. Suppl.* **9B**, **1985**, 93.

(42) Rees, D. C.; Lipscomb, W. N. *J. Mol. Biol.* **1982**, *160*, 475.

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

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