

solubility, the crude product was carried on to the next step.

**13-Nordrim-9(14)-ene (31).** Oven-dried  $\text{CuCl}_2$  (0.478 g, 3.56 mmol) was added slowly with ice-cooling to a suspension of  $\text{LiAlH}_4$  (0.270 g, 7.12 mmol) in 20 mL of THF. The resulting black heterogeneous mixture was stirred at room temperature for 45 min before a suspension of crude sulfone **30** (0.296 g, 0.89 mmol) in 5 mL of THF (plus three more THF rinses) was added. The mixture was refluxed for 8.5 h. After the solution was cooled to room temperature, 30 mL of a NaCl solution was added, followed by phase separation and three ether extractions. The combined organic layers were washed with brine and water and dried ( $\text{MgSO}_4$ ). Flash chromatography (silica gel, lbpe) afforded after vacuum drying an olefinic mixture (0.143 g of an oil, 83% based on  $\text{C}_{14}\text{H}_{24}$ ) with **31** as the major product. The details of the  $^1\text{H}$  NMR spectra (90 and 200 MHz) were as follows:  $\delta$  ( $\text{CDCl}_3$ ) 0.84, 0.86, 1.06 (3 H each,  $\text{C}_{11,12,15}$ - $3\text{CH}_3$ , three s), 4.49 (2 H, 2  $\text{H}_{14}$ , br s with fine structure,  $W \sim 3$  Hz). Most notably, the  $\delta$  4.49 signal is characteristic of the exocyclic olefinic linkage. The hydrocarbon **31** could not be resolved by HPLC, and, accordingly, the material was ozonized directly to the known ketone as described in the next section.

**5,5,8a $\beta$ -Trimethyl-3,4,4a $\alpha$ ,5,6,7,8,8a-octahydronaphthalen-1(2H)-one (32).** The crude olefinic mixture (preceding experiment; 0.143 g, 0.74 mmol based on  $\text{C}_{14}\text{H}_{24}$ ) was dissolved in 2 mL of dichloromethane and 20 mL of methanol. The ozonolysis was carried out at  $-78^\circ\text{C}$  until the appearance of the blue color. Excess ozone was flushed out with  $\text{N}_2$ , a mixture of 5 mL of 30%  $\text{H}_2\text{O}_2$  and 5 mL of acetic acid was added, and the dry ice bath was removed. After reaching room temperature the mixture was refluxed for 1 h, water was added, and the aqueous layer was extracted 3 times with  $\text{CH}_2\text{Cl}_2$ . The combined organic phases were

washed successively with water,  $\text{KI}$ ,  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $\text{NaHCO}_3$ , and  $\text{NaCl}$  solutions and dried over  $\text{MgSO}_4$ . Flash chromatography (silica gel, 12:1 lbpe ether) afforded **32** (0.071 g, 50%). The product proved pure by  $^1\text{H}$  (90 and 200 MHz) and  $^{13}\text{C}$  NMR (50.4 MHz):  $^1\text{H}$  NMR  $\delta$  ( $\text{CDCl}_3$ ) 0.89, 0.92, 1.14 (3 H each,  $\text{C}_{9,10,11}$ - $3\text{CH}_3$ , three s), 0.8-2.7 (13 H, ring proton, m);  $^{13}\text{C}$  NMR  $\delta$  ( $\text{CDCl}_3$ ) 18.1, 18.6, 20.9, 22.0, 26.3, 33.1, 34.1, 37.5, 41.6, 49.0, 53.5, 215.8. The  $^1\text{H}$  NMR spectrum (90 MHz) of this specimen was identical with the spectrum kindly provided by Dr. G. Ohloff (Firmenich, Geneva, Switzerland). The ketone **32** showed the following optical data:  $[\alpha]_D^{20} +30.8^\circ$  ( $\text{CHCl}_3$ ,  $c$  0.05) and  $[\alpha]_D^{20} +35.9^\circ$  ( $\text{CH}_3\text{OH}$ ,  $c$  0.05); Ohloff reported  $[\alpha]_D^{20} -35^\circ$  (neat).<sup>36</sup>

**Acknowledgment.** We gratefully acknowledge financial support from the National Institutes of Health (Grant EY-02452 and NCI Contract CP-05715). Gratitude is expressed to BASF (Ludwigshafen) for chemicals, the NSF Midwest Center for Mass Spectrometry for mass spectra, and the Southern California Regional NMR Facility for 500-MHz NMR spectra (NSF Grant CHE7916324). Postdoctoral fellowships to R. P. (Deutsche Forschungsgemeinschaft, West Germany) and W. R. (Fulbright) are acknowledged. Finally, we also acknowledge Beverly Scher, who under an NSF-URP grant the summer of 1980 carried out several initial experiments on this project.

**Supplementary Material Available:** Spectral ( $^1\text{H}$  and  $^{13}\text{C}$  NMR, UV, IR, and mass spectra) and analytical data (11 pages). Ordering information is given on any current masthead page.

## Divalent Metal Ion Catalysis in the Hydrolysis of Esters of Picolinic Acid. Metal Ion Promoted Hydroxide Ion and Water Catalyzed Reactions

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**Abstract:** Rate constants have been determined for hydrolysis of a series of phenolic and aliphatic esters of picolinic acid in  $\text{H}_2\text{O}$ . Hydroxide ion, hydronium ion, and water catalyzed reactions were observed in hydrolysis of the phenolic esters. Catalysis by low concentrations of  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  occurs even though binding of the metal ions is weak (saturation effects were not observed). Both metal ion promoted water and  $\text{OH}^-$  catalyzed reactions were observed with the esters having leaving groups with  $\text{pK}_a$  values of 12.4 or less. Rate enhancements produced by 0.01 M  $\text{Ni}^{2+}$  and 0.001 M  $\text{Cu}^{2+}$  range from 10- to near 200-fold in the pH-independent water reactions and from  $10^2$ - to over  $10^5$ -fold in the  $\text{OH}^-$  catalyzed reactions. Significant metal ion catalysis was not observed in the hydrolysis of 4-nitrophenyl isonicotinate or 8-(5-nitroquinolyl) isonicotinate; therefore, metal ion catalysis in the hydrolysis of the esters with the pyridine nitrogen ortho to the ester function must be associated with a chelation effect. The rate constants  $k_0$  and  $k_{\text{OH}}$  for hydrolysis of the picolinic esters in the metal ion promoted water and  $\text{OH}^-$  catalyzed reactions are little affected by the leaving group ( $\beta_{\text{lg}} \sim 0$ ) for leaving groups with  $\text{pK}_a$  values ranging from 4.1 with 2,4-dinitrophenol to 12.4 with trifluoroethanol, and ratios of  $k_{\text{OH}}/k_0$  are nearly constant. This indicates that there is little or no C-O bond breaking in the critical transition state, i.e., in both reactions the nucleophilic attack step is rate determining. When the leaving group is ethanol, then  $k_{\text{OH}}$  is markedly less than in the case of the trifluoroethyl ester, and a metal ion promoted water reaction is not detected even at pH values as low as 4. Thus, a change in rate-determining step has occurred with the change in the leaving group. Likewise only metal ion promoted  $\text{OH}^-$  catalysis is observed with ethyl 6-carboxypicolinate. Rate enhancements produced by saturating concentrations of  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  are in that case  $2.7 \times 10^4$ - and  $1.3 \times 10^5$ -fold, respectively. Intramolecular general base catalysis does not occur in the metal ion promoted water reaction of 8-quinolyl picolinate or 8-(5-nitroquinolyl) picolinate. With the nitro substituted esters of picolinic acid a metal ion promoted formate and acetate ion catalyzed reaction takes place which is quite dependent on leaving group ability. It is likely that formate and acetate ions are attacking the metal ion complexes as nucleophiles.

Carboxypeptidase A is a Zn(II) metalloenzyme that catalyzes the hydrolysis of peptides and O-acyl derivatives of  $\alpha$ -hydroxy carboxylic acids.<sup>2</sup> The metal ion presumably complexes the carbonyl oxygen of peptide substrates.<sup>2-4</sup> X-ray crystallographic

analysis at 2-Å resolution also revealed the presence of the carboxyl group of glutamic acid-270 in the active site.<sup>2-4</sup> Both nucleophilic and general base mechanisms have been suggested for the enzyme involving Glu-270<sup>3-5</sup> as well as nucleophilic attack by Zn(II)-

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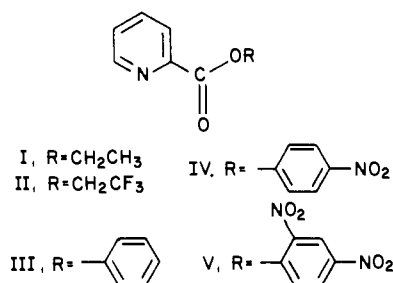
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coordinated hydroxide ion.<sup>4</sup> Experiments employing low temperature and a mixed aqueous-organic solvent system have provided kinetic evidence (biphasic kinetics) for the formation of an intermediate in the hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-*L*-β-phenyllactate<sup>6a</sup> and the corresponding *p*-dimethylamino-substituted ester<sup>6b</sup> under these conditions. It was suggested that breakdown of an anhydride intermediate is rate determining in the hydrolysis of the *p*-chlorocinnamoyl ester.<sup>7</sup> However, with other types of ester and peptide substrates the hydrolysis reactions may proceed without formation of an anhydride intermediate.<sup>8,9</sup> i.e., the reactions may involve metal ion promoted water or OH<sup>-</sup> catalysis.

Divalent metal ion catalysis by Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, and Cu<sup>2+</sup> in the hydrolysis of esters has been extensively studied.<sup>10-16</sup> In these reactions the rate constants for hydroxide ion catalysis are markedly enhanced; rate enhancements as large as 10<sup>8</sup> have been observed.<sup>14</sup> On the other hand, metal ion catalyzed attack of water on acyl derivatives has rarely been reported.<sup>17</sup> A Cu<sup>2+</sup>-promoted water reaction was detected at pH <3 in hydrolysis of 2-(6-carboxypyridyl)methyl acetate.<sup>16</sup> Also, the hydrolysis of *O*-acetyl-2-pyridinecarboxaldoxime is catalyzed by Cu<sup>2+</sup> via both OH<sup>-</sup> and H<sub>2</sub>O pathways.<sup>18</sup> At saturating concentrations of Ni<sup>2+</sup>, Co<sup>2+</sup>, and Zn<sup>2+</sup>, pH-independent reactions are clearly detectable in the hydrolysis of cinnamic 6-carboxypicolinic monoanhydride<sup>19</sup> even though the metal ion promoted OH<sup>-</sup> catalyzed reactions occur with great facility. In order to understand the chemical factors governing carboxypeptidase A catalyzed reactions it is important to determine the structural features of esters that will facilitate metal ion promoted water and OH<sup>-</sup> catalyzed reactions and to determine the relationships between these reactions. Consequently, we have studied the divalent metal ion catalyzed reactions of a series of esters of picolinic acid (I-V), in which the leaving groups

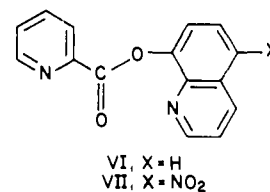


have p*K*<sub>a</sub> values between 4 and 16, to ascertain the effect of the leaving group on the OH<sup>-</sup> and water reactions. In addition we have studied the hydrolysis of the 8-quinolyl picolinate esters VI and VII. These compounds offer the possibility of intramolecular general base participation in the metal ion catalyzed reactions.

### Experimental Section

**Materials.** Ethyl picolinate (I) was prepared by refluxing overnight 5.0 g of picolinic acid in 200 mL of absolute ethanol with 5 mL of

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concentrated HCl. The solution was then neutralized with Na<sub>2</sub>CO<sub>3</sub>. The ethanol was removed by rotary evaporation, and the residual material was distilled giving a colorless liquid with bp 75 °C (0.3 mm), *n*<sub>D</sub><sup>22</sup> 1.5098 (lit.<sup>20</sup> bp 125 °C (12 mm), *n*<sub>D</sub><sup>20</sup> 1.5105). Ethyl 6-carboxypicolinate (VIII) was prepared by the method of Vasa et al.<sup>21</sup> It was a white solid with mp 120–122 °C (lit.<sup>21</sup> mp 121.5 °C).

Phenyl picolinate (III) was prepared by the method of Felton and Bruce<sup>22</sup> and had mp 81–82 °C (lit.<sup>22</sup> mp 81–82 °C). 2,2,2-Trifluoroethyl picolinate (II), 4-nitrophenyl picolinate (IV), 4-nitrophenyl isonicotinate (IX), 2,4-dinitrophenyl picolinate (V), and 8-(5-nitroquinolyl) picolinate (VII) were prepared by stirring 0.01-mol quantities of carboxylic acid, alcohol or phenol, and dicyclohexylcarbodiimide in 100 mL of chloroform for 16 h. The solution was filtered to remove the dicyclohexylurea precipitate, and the chloroform was removed by rotary evaporation under reduced pressure. The trifluoroethyl ester II was then distilled (bp 92 °C (2.5 mm), *n*<sub>D</sub><sup>21</sup> 1.4633). Anal. Calcd for C<sub>8</sub>H<sub>6</sub>N<sub>2</sub>O<sub>3</sub>: N, 6.83. Found: N, 7.10. The *p*-nitrophenyl esters were treated with decolorizing carbon in methanol and recrystallized from methanol. 4-Nitrophenyl picolinate (IV) had mp 145–147 °C dec (lit.<sup>23</sup> mp 144–146 °C). 4-Nitrophenyl isonicotinate (IX) had mp 137–139 °C (lit.<sup>24</sup> mp 134 °C). 2,4-Dinitrophenyl picolinate (V) had mp 151–153 °C dec. Anal. Calcd for C<sub>12</sub>H<sub>7</sub>N<sub>3</sub>O<sub>6</sub>: C, 49.82; H, 2.42; N, 14.52. Found: C, 49.69; H, 2.55; N, 14.50. 8-(5-Nitroquinolyl)picolinate (VII) had mp 163–165 °C dec. Anal. Calcd for C<sub>15</sub>H<sub>9</sub>N<sub>3</sub>O<sub>4</sub>: C, 61.02; H, 3.05; N, 14.24. Found: C, 61.07; H, 3.33; N, 14.16.

8-(5-Nitroquinolyl) isonicotinate (X) was synthesized by refluxing 0.01 mol of isonicotinic acid in 50 mL of thionyl chloride until the suspension completely dissolved. The excess thionyl chloride was removed by distillation, and the remaining liquid was added to 100 mL of dry THF containing 0.01 mol of 8-hydroxy-5-nitroquinoline and triethylamine. After being stirred for 2 h the solution was filtered, and the solvent was removed under reduced pressure. The solid product was treated with decolorizing carbon in chloroform and recrystallized from chloroform-hexane. The compound melted at 150–152 °C. Anal. Calcd for C<sub>15</sub>H<sub>9</sub>N<sub>3</sub>O<sub>4</sub>: C, 61.02; H, 3.05; N, 14.24. Found: C, 61.22; H, 3.43; N, 13.77.

8-Quinolyl picolinate (VI) was prepared by mixing 0.01-mol quantities of picolinic acid and trifluoroacetic anhydride. The resulting liquid was allowed to stand for 1 h, and 0.01 mol of 8-hydroxyquinoline was slowly added. The reaction mixture became hot and turned reddish in color. After standing overnight, the mixture was dissolved in 150 mL of chloroform and extracted with several 50-mL portions of aqueous sodium bicarbonate. The chloroform layer was dried with sodium sulfate, and the solvent was removed under reduced pressure. The ester product was treated with decolorizing carbon in chloroform and recrystallized from methanol. The compound melted at 140–142 °C. Anal. Calcd for C<sub>15</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>: C, 72.00; H, 4.00; N, 11.20. Found: C, 72.02; H, 4.16; N, 11.10.

**Kinetic Methods.** The rates of hydrolysis of the esters were measured spectrophotometrically with a Beckman Model 25 or Durrum D110 stopped-flow spectrophotometer. The hydrolysis of ethyl picolinate (I) and ethyl 6-carboxypicolinate (VIII) was followed by monitoring the absorbance decrease at 280 and 285 nm, respectively, while that of 2,2,2-trifluoroethyl picolinate (II) was followed at 275 nm. In the hydrolysis of 4-nitrophenyl picolinate (IV), reactant disappearance was followed at 280 nm (pH 1–6) and product appearance at 330 nm (pH 4–6) and 400 nm (pH 7–8). The hydrolysis of 4-nitrophenyl isonicotinate (IX) was followed by monitoring product appearance at 400 nm (pH 6–8) or 330 nm (pH 1–6). With 2,4-dinitrophenyl picolinate (V) product appearance was followed at 310 nm (pH 1–4) or 400 nm (pH 4–8). In the hydrolysis of 8-(5-nitroquinolyl) picolinate (VII) or isonicotinate (X) the appearance of product was followed at 380 nm (pH 1–6) or 450 nm (pH 6–9); product appearance in the metal ion assisted reactions was followed at 450 nm. With 8-quinolyl picolinate (VI) reactant disappearance was followed at 290 nm. In the cases where different wave-

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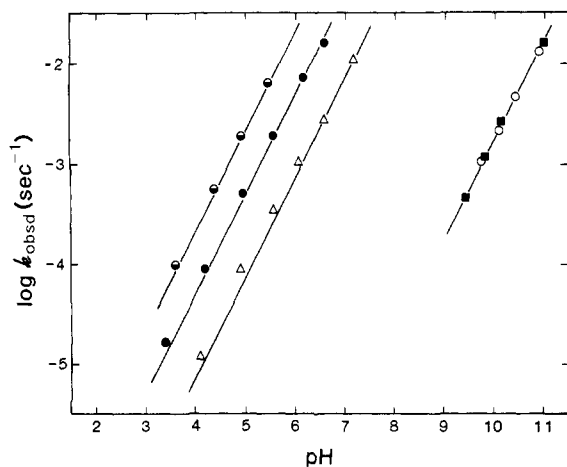


Figure 1. Plots of  $\log k_{\text{obsd}}$  vs. pH for hydrolysis of ethyl picolinate (I) in  $\text{H}_2\text{O}$  at  $50^\circ\text{C}$  ( $\mu = 0.1 \text{ M}$ ) in the presence of  $0.01 \text{ M Ni}^{2+}$  ( $\Delta$ ) and in the absence of metal ion ( $\blacksquare$ ). Also shown is the plot of  $\log k_{\text{obsd}}$  vs. pH for hydrolysis of ethyl 6-carboxypicolinate (VIII) in  $\text{H}_2\text{O}$  at  $50^\circ\text{C}$  ( $\mu = 0.1 \text{ M}$ ) in the presence of  $0.01 \text{ M Ni}^{2+}$  ( $\bullet$ ) or  $0.001 \text{ M Cu}^{2+}$  ( $\circ$ ) and in the absence of metal ion ( $\circ$ ).

lengths were employed, the rate measurements in overlapping pH regions showed no wavelength dependence. The ionic strength was  $0.1 \text{ M}$ , maintained with  $\text{KCl}$ , and the solutions used for rate measurements in the absence of metal ions contained  $2 \times 10^{-5} \text{ M EDTA}$  as a precaution against trace metal ion in the buffer or salt. With the esters IV–VII and IX and X the rate constants at zero buffer concentration were obtained by extrapolation in both the metal ion studies and those in the absence of metal ion. The values of  $k_{\text{obsd}}$  in the hydrolysis of I–III and VIII, with which buffer catalysis was not significant, were obtained in  $0.02 \text{ M}$  total buffer. No corrections were made for buffer–metal ion complexation. The buffers used were  $\text{HCl}$  (pH 1–3), formate (pH 3.2–4.2), acetate (pH 4.2–5.2), 2,6-lutidine (pH 5.6–6.6), *N*-ethylmorpholine (pH 6.6–8.0), and carbonate (pH 8.8–10.5).

Kinetic runs were initiated by injecting  $15\text{--}30 \mu\text{L}$  of ester stock solution ( $0.003\text{--}0.01 \text{ M}$  in  $\text{THF}$ ) into  $3 \text{ mL}$  of the buffer solution maintained at the desired temperature. In rate measurements carried out with the stopped-flow apparatus, one  $15\text{-mL}$  drive syringe contained  $60\text{--}90 \mu\text{L}$  of ester stock solution in  $0.1 \text{ M KCl}$ , while the other syringe contained the metal ion and buffer. The reactions followed pseudo-first-order kinetics for at least 4 half-lives, and the kinetic parameters were evaluated by using a non-linear least-squares program. Reaction mixture pH values were obtained at  $30^\circ\text{C}$  with a Radiometer Model 22 and at  $50^\circ\text{C}$  with a Beckman Model 35 pH meter. Second-order rate constants for hydroxide ion catalysis were calculated by using  $K_w$  values of  $1.47 \times 10^{-14}$  at  $30^\circ\text{C}$  and  $5.50 \times 10^{-14}$  at  $50^\circ\text{C}$ .

## Results

In Figure 1 is shown a plot of  $\log k_{\text{obsd}}$  vs. pH for hydrolysis of ethyl picolinate (I) in  $\text{H}_2\text{O}$  at  $50^\circ\text{C}$  and  $\mu = 0.1 \text{ M}$ . The plot has a slope of 1.0 which indicates  $\text{OH}^-$  catalysis ( $k_{\text{OH}} = 3.4 \text{ M}^{-1} \text{ s}^{-1}$ ). Hydrolysis of the ester is markedly enhanced by  $0.01 \text{ M Ni}^{2+}$  ion (a nonsaturating concentration). As seen in Figure 1, only  $\text{OH}^-$  catalysis is observed in the presence of  $\text{Ni}^{2+}$  even at pH values as low as 4.09. The value of  $k_{\text{OH}}$ , the second-order rate constant for  $\text{OH}^-$  catalysis, is then  $1.4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ , i.e., 4100-fold larger than in the absence of metal ion. Inclusion of a second chelating functional group in the molecule with ethyl 6-carboxypicolinate (VIII) does not lead to any change in the value of  $k_{\text{OH}}$  in the absence of metal ions. However, strong metal ion binding to the ester then occurs. Saturation effects are observed at low metal ion concentration as shown in Figure 2. The plot of Figure 1 shows that only  $\text{OH}^-$  catalysis is observed in the hydrolysis of VIII at saturating concentrations of  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  at pH values above 3.5. The values of  $k_{\text{OH}}$  are  $4.6 \times 10^5$  and  $9.1 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  with  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$ , respectively, in comparison with  $3.4 \text{ M}^{-1} \text{ s}^{-1}$  in the absence of metal ion. Saturation occurs with  $\text{Cu}^{2+}$  ion at concentrations less than  $0.001 \text{ M}$  and with  $\text{Ni}^{2+}$  ion at concentrations less than  $0.01 \text{ M}$ . In the latter case the metal ion association constant is  $10^3 \text{ M}^{-1}$ .

The hydrolysis of esters IV and V at  $50^\circ\text{C}$  and  $\mu = 0.1 \text{ M}$  is characterized by plots of  $\log k_{\text{obsd}}$  (obtained by extrapolation to

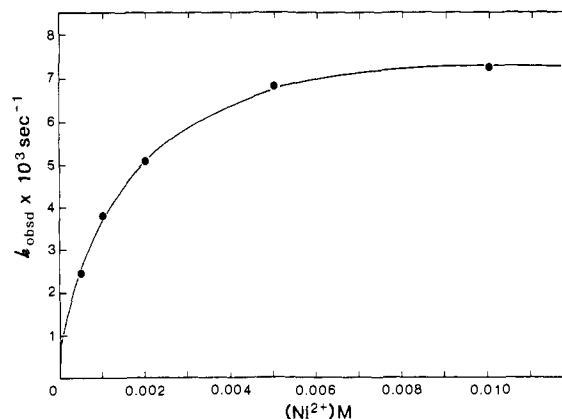


Figure 2. Plot of  $k_{\text{obsd}}$  vs.  $\text{Ni}^{2+}$  concentration for hydrolysis of ethyl 6-carboxypicolinate (VIII) in  $\text{H}_2\text{O}$  at  $50^\circ\text{C}$  ( $\mu = 0.1 \text{ M}$ ) at pH 6.18.

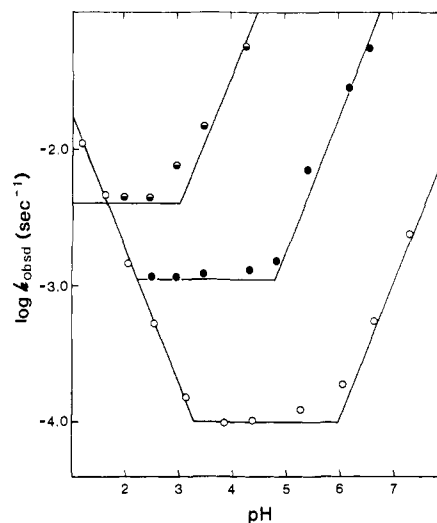


Figure 3. Plot of  $\log k_{\text{obsd}}$  vs. pH for hydrolysis of 2,4-dinitrophenyl picolinate (V) in  $\text{H}_2\text{O}$  at  $50^\circ\text{C}$  ( $\mu = 0.1 \text{ M}$ ) in the presence of  $0.01 \text{ M Ni}^{2+}$  ( $\bullet$ ) or  $0.001 \text{ M Cu}^{2+}$  ( $\circ$ ) and in the absence of metal ion ( $\circ$ ).

Table I. Rate Constants for the Hydrolysis of Esters of Picolinic Acid at  $30^\circ$  or  $50^\circ\text{C}$ ,  $\mu = 0.1 \text{ M}$

compd	$\text{pK}_a$ (leaving group)	$k_H \times 10^2$ , $\text{M}^{-1} \text{ s}^{-1}$	$k_0 \times 10^5$ , $\text{s}^{-1}$	$k_{\text{OH}} \times 10^{-2}$ , $\text{M}^{-1} \text{ s}^{-1}$
I <sup>a</sup>	16.0 <sup>b</sup>			0.034
II <sup>a</sup>	12.4 <sup>b</sup>			0.82
III <sup>a</sup>	9.9 <sup>c</sup>			0.82
IV <sup>d</sup>	7.1 <sup>c</sup>	8.8	3.16	7.95
V <sup>d</sup>	4.1 <sup>e</sup>	16.1	8.5	16.4
VI <sup>f</sup>	9.8 <sup>g</sup>		21.8	0.13

<sup>a</sup> At  $50^\circ\text{C}$  in  $0.02 \text{ M}$  total buffer. <sup>b</sup> Reference 28. <sup>c</sup> Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622. <sup>d</sup> At  $50^\circ\text{C}$  with the values of  $k_{\text{obsd}}$  obtained by extrapolation to zero buffer concentration. <sup>e</sup> Reference 29. <sup>f</sup> At  $30^\circ\text{C}$ , with the  $k_{\text{obsd}}$  values obtained by extrapolation to zero buffer concentration. <sup>g</sup> Sillen, L. G.; Martell, A. E. "Stability Constants of Metal Ion Complexes", The Chemical Society: London, 1964.

zero buffer concentration) vs. pH that indicate facile hydroxide ion and hydronium ion catalysis. There is also a large pH-independent region near neutrality. The plot of  $\log k_{\text{obsd}}$  vs. pH for hydrolysis of 2,4-dinitrophenyl picolinate (V) is presented in Figure 3. The expression for  $k_{\text{obsd}}$  at zero buffer concentration is given in eq 1

$$k_{\text{obsd}} = k_1 \left[ \frac{a_{\text{H}}}{K_a + a_{\text{H}}} \right] + \left[ k_0 + \frac{k_{\text{OH}} K_w}{a_{\text{H}}} \right] \left[ \frac{K_a}{K_a + a_{\text{H}}} \right] \quad (1)$$

where  $k_1$  is the rate constant for the reaction of the protonated species,  $k_0$  is the rate constant for the pH-independent reaction, and  $K_a$  is the dissociation constant of the protonated species. The

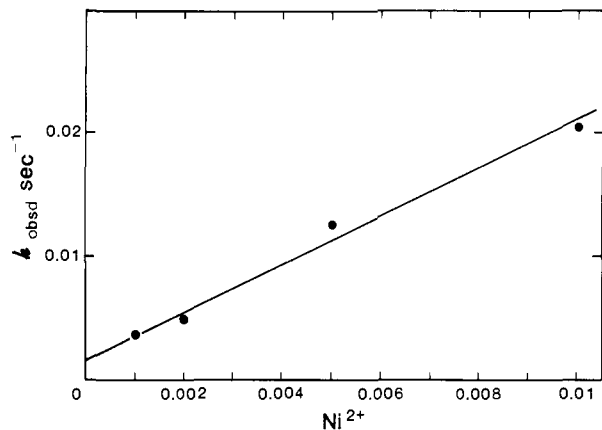


Figure 4. Plot of  $k_{\text{obsd}}$  vs.  $\text{Ni}^{2+}$  concentration for hydrolysis of 2,4-dinitrophenyl picolinate (V) in  $\text{H}_2\text{O}$  at  $50^\circ\text{C}$  ( $\mu = 0.1\text{ M}$ ) and pH 6.23.

Table II. Rate Constants for Hydrolysis of Esters of Picolinic Acid at 30 or  $50^\circ\text{C}$  in the Presence of  $0.01\text{ M Ni}^{2+}$  or  $0.001\text{ M Cu}^{2+}$ ,  $\mu = 0.1\text{ M}$

compd	$\text{pK}_a$ (leaving group)	metal ion	$k_0 \times 10^3$ , $\text{s}^{-1}$	$k_{\text{OH}} \times 10^{-5}$ , $\text{M}^{-1}\text{ s}^{-1}$
I <sup>a</sup>	16.0 <sup>b</sup>	$\text{Ni}^{2+}$		0.14
II <sup>a</sup>	12.4 <sup>b</sup>	$\text{Ni}^{2+}$	0.25	1.81
III <sup>a</sup>	9.9 <sup>c</sup>	$\text{Ni}^{2+}$	0.24	2.54
IV <sup>d</sup>	7.1 <sup>c</sup>	$\text{Ni}^{2+}$	1.05	7.06
		$\text{Cu}^{2+}$	5.96	460
V <sup>d</sup>	4.1 <sup>e</sup>	$\text{Ni}^{2+}$	1.0	2.54
		$\text{Cu}^{2+}$	3.65	489
VI <sup>f</sup>	9.8 <sup>g</sup>	$\text{Ni}^{2+}$	5.5	67.5
VII <sup>f</sup>	6.0 <sup>h</sup>	$\text{Ni}^{2+}$	4.22	35.4

<sup>a</sup> At  $50^\circ\text{C}$  in  $0.02\text{ M}$  total buffer. <sup>b</sup> Reference 28. <sup>c</sup> Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622. <sup>d</sup> At  $50^\circ\text{C}$  with the values of  $k_{\text{obsd}}$  obtained in HCl solution or by extrapolation to zero buffer concentration. <sup>e</sup> Reference 29. <sup>f</sup> At  $30^\circ\text{C}$  with the values of  $k_{\text{obsd}}$  obtained by extrapolation to zero buffer concentration. <sup>g</sup> Sillen, L. G.; Martell, A. E. "Stability Constants of Metal Ion Complexes"; The Chemical Society: London, 1964. <sup>h</sup> The value was obtained by extrapolation of measurements in dioxane-water solutions to zero dioxane concentrations. Steger, H. F.; Corsini, A. *J. Inorg. Nucl. Chem.* **1973**, *35*, 1637.

values of the rate constants are given in Table I ( $k_{\text{H}} = k_1/K_a$ ). 4-Nitrophenyl isonicotinate (IX) had  $k_{\text{OH}} = 3.4 \times 10^3\text{ M}^{-1}\text{ s}^{-1}$ ,  $k_0 = 7.3 \times 10^{-5}\text{ s}^{-1}$ ,  $k_1 = 1.2 \times 10^{-3}\text{ s}^{-1}$ , and  $\text{pK}_a = 2.8$  at  $50^\circ\text{C}$ . The rate constants for the hydrolysis of 8-(5-nitroquinolyl) isonicotinate (X) at  $30^\circ\text{C}$  are the following:  $k_{\text{OH}} = 6.8 \times 10^2\text{ M}^{-1}\text{ s}^{-1}$ ,  $k_0 = 2.3 \times 10^{-4}\text{ s}^{-1}$ , and  $k_{\text{H}} = 4.6\text{ M}^{-1}\text{ s}^{-1}$ .

Pronounced metal ion catalysis takes place in the hydrolysis of II-V. A typical plot of  $k_{\text{obsd}}$  vs. the concentration of  $\text{Ni}^{2+}$  at constant pH is shown in Figure 4. The plot is linear even at high metal ion concentrations ( $0.01\text{ M}$ ), which shows that saturation is not occurring. Both metal ion promoted  $\text{OH}^-$  and water catalyzed reactions are observed in the presence of  $0.01\text{ M Ni}^{2+}$  or  $0.001\text{ M Cu}^{2+}$  ion, which are nonsaturating concentrations. Values of these rate constants are given in Table II. Addition of  $0.01\text{ M Ni}^{2+}$  to 4-nitrophenyl isonicotinate (IX) increased its rate of hydrolysis at pH < 7 by only approximately 25%. That was also the case with 8-(5-nitroquinolyl) isonicotinate (X) at pH < 7.3.

The hydrolysis of 8-quinolyl picolinate (VI) proceeds with  $\text{OH}^-$  catalysis and a pH-independent reaction from pH 4-9 (Figure 5). Rate constants are given in Table I. The pH-independent reaction is slower in  $\text{D}_2\text{O}$  than in  $\text{H}_2\text{O}$  ( $k_0^{\text{H}_2\text{O}}/k_0^{\text{D}_2\text{O}} = 3.75$ ). Nickel ion markedly catalyzes the hydrolysis of VI even though metal ion binding to the ester is weak. Figure 6 shows that saturating effects are not observed with  $0.01\text{ M Ni}^{2+}$ . Nevertheless, the  $\text{OH}^-$  catalyzed reaction is enhanced by  $5 \times 10^5$ . A reaction that is nearly pH independent was also observed at pH less than 4.5 (see Figure 5). A reaction that is clearly pH independent was observed in the hydrolysis of 8-(5-nitroquinolyl) picolinate (VII) at pH < 5 in the presence of  $0.01\text{ M Ni}^{2+}$ . As

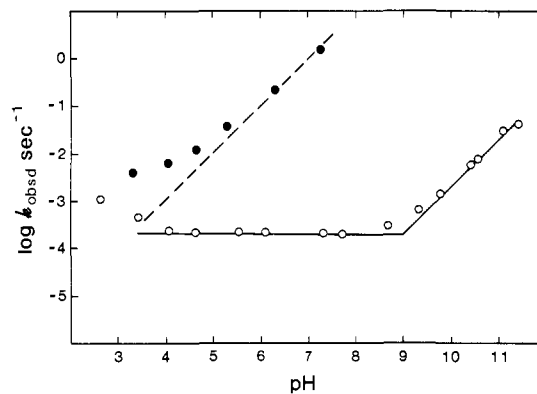


Figure 5. Plot of  $\log k_{\text{obsd}}$  vs. pH for hydrolysis of 8-quinolyl picolinate (VI) in  $\text{H}_2\text{O}$  at  $30^\circ\text{C}$  ( $\mu = 0.1\text{ M}$ ) in the presence of  $0.01\text{ M Ni}^{2+}$  (●) and in the absence of metal ion (○).

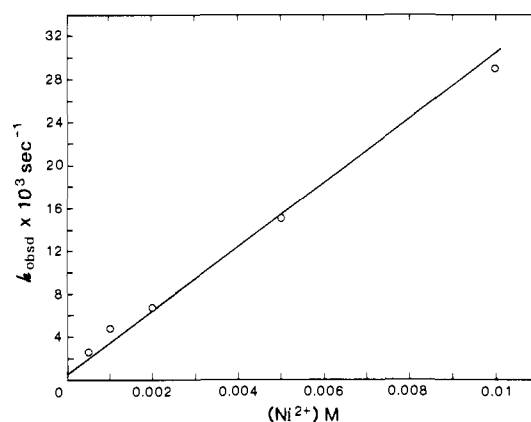


Figure 6. Plot of  $k_{\text{obsd}}$  vs.  $\text{Ni}^{2+}$  concentration for hydrolysis of 8-quinolyl picolinate (VI) in  $\text{H}_2\text{O}$  at  $30^\circ\text{C}$  ( $\mu = 0.1\text{ M}$ ) and pH 5.27.

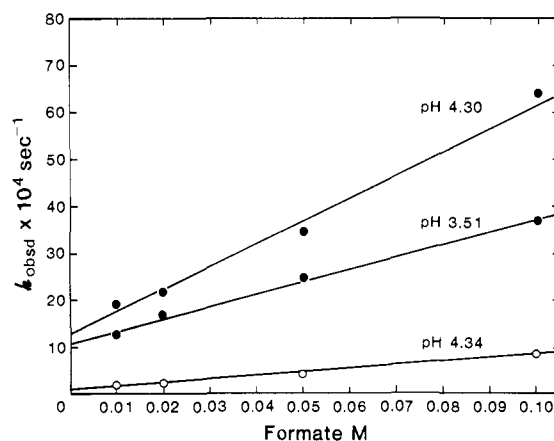


Figure 7. Plot of  $k_{\text{obsd}}$  vs. total formate buffer concentration for hydrolysis of 2,4-dinitrophenyl picolinate (V) in  $\text{H}_2\text{O}$  at  $50^\circ\text{C}$  ( $\mu = 0.1\text{ M}$ ) in the presence of  $0.01\text{ M Ni}^{2+}$  (●) at pH 4.30 and 3.51 and in the absence of metal ion (○) at pH 4.34.

was the case with esters II-V, the metal ion promoted water reactions of VI and VII occur with similar rate constants (Table II).

The hydrolysis reactions of the nitro-substituted esters, IV, V, and VII are catalyzed by formate and acetate ions. This catalysis is markedly enhanced in the presence of  $0.01\text{ M Ni}^{2+}$  as shown in Figure 7 for the hydrolysis of V. Rate constants for these reactions are given in Table III. In the presence of  $0.001\text{ M Cu}^{2+}$ , increasing buffer concentrations produced a small rate decrease. In contrast with formate and acetate, increasing concentrations of lutidine produced no effect on the  $\text{Ni}^{2+}$ -promoted reactions of IV, V, or VII. Buffer catalysis by acetate was not observed in the metal ion catalyzed reactions of III or VI. Measurement of

**Table III.** Second-Order Rate Constants for Catalysis of the Hydrolysis of 4-Nitrophenyl Picolinate (IV), 2,4-Dinitrophenyl Picolinate (V), and 8-(5-Nitroquinolyl) Picolinate (VII) by Formate and Acetate Buffer

compd	catalyst	temp., °C	$k_B \times 10^3,^a$ M <sup>-1</sup> s <sup>-1</sup>	$k'_B \times 10^3,^b$ M <sup>-1</sup> s <sup>-1</sup>
IV	formate	50	0.44	4.75 <sup>c</sup>
	acetate	50	0.16	12 <sup>d</sup>
V	formate	50	8.5	55.5 <sup>e</sup>
	acetate	50	8.4	100.0 <sup>d</sup>
VII	formate	30		220 <sup>f</sup>
	acetate	30		2200 <sup>g</sup>

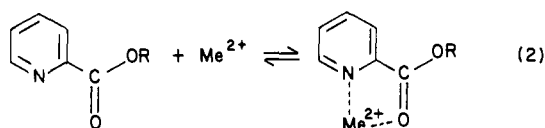
<sup>a</sup> Second-order rate constants for buffer base catalysis. <sup>b</sup> Second-order rate constants for buffer base catalysis in the presence of 0.01 M Ni<sup>2+</sup>. <sup>c</sup> Determined at pH 3.92 and 4.14. <sup>d</sup> Determined at pH 4.83. <sup>e</sup> Determined at pH 3.51 and 4.34. <sup>f</sup> Determined at pH 3.48 and 4.04. <sup>g</sup> Determined at pH 4.89 and 5.30.

$k'_B$ , the second-order rate constant for buffer base catalysis in the presence of 0.01 M Ni<sup>2+</sup> in D<sub>2</sub>O (formate buffer at pD 3.98 and 4.48), gave a D<sub>2</sub>O solvent isotope effect ( $k'_{B,H_2O}/k'_{B,D_2O}$ ) of 0.8 for the hydrolysis of V.

### Discussion

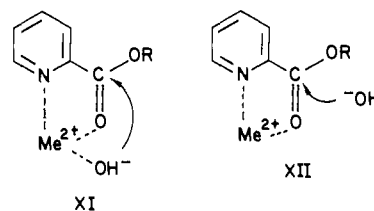
The plots of log  $k_{obsd}$  vs. pH for hydrolysis of IV, V, IX, and X (shown for V in Figure 3) indicate catalysis by hydronium ion, hydroxide ion, and water or a kinetic equivalent. The relatively facile hydronium ion catalyzed reactions at low pH very likely reflect protonation of the pyridine nitrogen, i.e., the reaction involves attack of H<sub>2</sub>O on the conjugate acid species. The reaction of 4-nitrophenyl isonicotinate (IX) again becomes pH independent near pH 2.8, which must correspond with the pK<sub>a</sub> of the pyridine conjugate acid. The pH-independent regions in the profiles near neutrality could be due to attack of H<sub>2</sub>O on the neutral species or kinetically equivalent possibilities, e.g., attack of OH<sup>-</sup> on the protonated species. General base catalysis by the pyridine nitrogen is not occurring since 4-nitrophenyl isonicotinate (IX), with which the pyridine nitrogen is para to the ester function, has rate constants that are closely similar to those of the ortho derivative 4-nitrophenyl picolinate (IV). The extensive pH-independent region in the profile for hydrolysis of the 8-hydroxyquinoline derivative VI (Figure 5) may however represent general base catalysis by the quinoline nitrogen, and the reaction is slower in D<sub>2</sub>O than in H<sub>2</sub>O. Intramolecular general base participation has previously been observed in the hydrolysis reactions of 8-acetoxyquinoline and substituted phenyl quinoline 8-carboxylate esters.<sup>22,25</sup>

Significant metal ion catalysis by Ni<sup>2+</sup> and Cu<sup>2+</sup> was observed in both the OH<sup>-</sup> and H<sub>2</sub>O catalyzed reactions of II–VI. Large rate enhancements of 10<sup>2</sup>–10<sup>5</sup> are produced in the OH<sup>-</sup> catalyzed reactions by 0.01 M Ni<sup>2+</sup>, a nonsaturating concentration (plots of  $k_{obsd}$  at constant pH vs. Ni<sup>2+</sup> concentration were linear). Likewise 0.001 M Cu<sup>2+</sup> produces rate enhancements of 10<sup>4</sup>–10<sup>5</sup> (5.8 × 10<sup>4</sup> with IV). These minimum rate enhancements are similar to those which have previously been observed in metal ion promoted OH<sup>-</sup> catalyzed reactions of other types of esters.<sup>10–16</sup> The pH-independent reactions of II–VI are enhanced by factors of 10–200 in the presence of the metal ion. The pyridine nitrogen is clearly important in coordination of the metal ions. Binding must involve a chelation effect (eq 2) since 4-nitrophenyl isonicotinate (IX), with which such a chelation effect is not possible, does not show significant metal ion catalysis in the hydrolysis reactions.



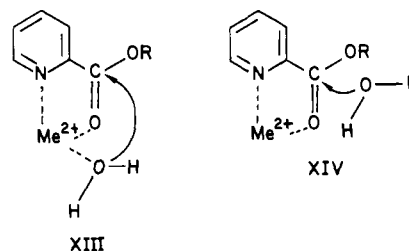
(25) Bruce, P. Y.; Bruce, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 5523.

The metal ion promoted OH<sup>-</sup> catalyzed reactions could involve nucleophilic attack of metal ion coordinated hydroxide ion at the carbonyl of the ester (XI) or attack by external OH<sup>-</sup> on the metal ion complex (XII).<sup>26</sup> In view of the good leaving groups of II–VII,



it is likely that nucleophilic attack is the rate-determining step rather than breakdown of a tetrahedral intermediate to products. There is little effect of the leaving group on  $k_{OH}$  ( $\beta_{lg} \approx 0$ )<sup>27</sup> in the presence of metal ions for the esters II–V with which the leaving group pK<sub>a</sub> changes from 12.4<sup>28</sup> to 4.1.<sup>29</sup> As the leaving group is made poorer than trifluoroethanol the rate constant does decline sharply ( $k_{OH}$  for the ethyl ester I is 13 times less than that of II). This effect is similar to that in the OH<sup>-</sup> catalyzed reactions in the absence of metal ions ( $\beta_{lg} = 0.2$ ).

In a corresponding manner, the metal ion promoted water reactions might involve nucleophilic attack of a metal ion coordinated water molecule (XIII) or attack of external water (XIV).



Since the pyridine nitrogen is a site of metal ion binding, the pH-independent reactions must reflect water reactions, not the kinetic equivalent that is possible in the reactions without metal ions (attack of OH<sup>-</sup> on a protonated species). As in the case of the OH<sup>-</sup> catalyzed reactions, there is very little effect of the leaving group on the rate constants for the water reactions. It can be seen in Table II that  $k_0$  changes by only a factor of 4 upon going from a dinitrophenol leaving group of pK<sub>a</sub> 4.1 to trifluoroethanol (pK<sub>a</sub> = 12.4), i.e.,  $\beta_{lg} \sim 0$ . Thus, there is little or no C–O bond breaking in the critical transition state, and electron withdrawal in the leaving group has little influence upon the ease of nucleophilic attack. In contrast, changes in the structure of the acyl group bring about large changes in the rate constants; the Ni<sup>2+</sup> and Cu<sup>2+</sup> promoted water reactions of cinnamic picolinic acid anhydride<sup>19</sup> are at least 100-fold faster than those of the dinitrophenyl ester V even though the leaving groups in both cases have pK<sub>a</sub> values near 4.

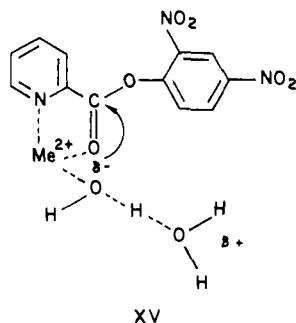
Water is weakly basic and is therefore a poor nucleophile. The lack of a significant effect of electron withdrawal in the leaving group on the rate constants, which indicates an early transition state in the nucleophilic attack step, must reflect considerable proton transfer from water in the transition state (XV), i.e., the transition state of the metal ion promoted water reaction must resemble that of the metal ion promoted OH<sup>-</sup> catalyzed reaction

(26) The plots of log  $k_{obsd}$  vs. pH in metal ion promoted OH<sup>-</sup> catalyzed reactions will have slopes of 1.0 at pH values below the pK<sub>a</sub> of the metal ion coordinated water molecule. The pK<sub>a</sub> values for ionization of the aquo complexes of Ni<sup>2+</sup> and Cu<sup>2+</sup> are 10.6 and 6.8, respectively. Basolo, F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*, 2nd ed.; Wiley: New York, 1967; p 32.

(27) The rate constants, of course, reflect both the preequilibrium metal ion binding and the OH<sup>-</sup> catalyzed reaction, i.e.,  $k_{OH} = K_{Me}k'_{OH}$  where  $K_{Me}$  is the metal ion association constant. Since the effect of the leaving group is the same in the presence or absence of metal ions it is clear that the effect of the leaving group on  $K_{Me}$  is small.

(28) Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 795.

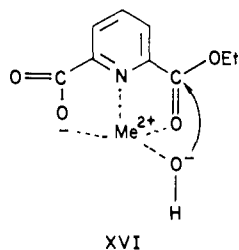
(29) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415.



in terms of the extent of bond making and breaking. That the effect of the leaving group is nearly the same in the metal ion promoted  $\text{OH}^-$  and  $\text{H}_2\text{O}$  catalyzed reaction is clearly shown by the fact that the ratios of  $k_{\text{OH}}/k_0$  are nearly constant; with  $\text{Ni}^{2+}$  as the catalyst these ratios vary from  $2.5 \times 10^8$  to only  $1.2 \times 10^9 \text{ M}^{-1}$ . The  $k_{\text{OH}}/k_0$  ratio for  $\text{Cu}^{2+}$  in the reactions of IV and V are considerably larger,  $7.7 \times 10^9$  and  $1.3 \times 10^{10} \text{ M}^{-1}$ , respectively, because of the more favorable  $\text{OH}^-$  catalyzed reactions. In these ratios,  $K_{\text{Me}}$ , the metal ion association constant, cancels out.

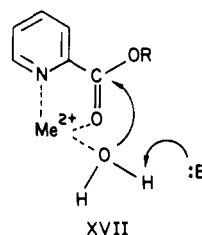
Breslow and McAllister<sup>30</sup> have previously studied the hydrolysis of phenyl 6-carboxypicolinate in the presence of 0.2 M  $\text{Ni}^{2+}$ . A small pH-independent region was observed in the log  $k_{\text{obsd}}$  vs. pH profile from pH 3-4 that is very likely due to a water reaction. The rate constant for this reaction at 25 °C is  $4.2 \times 10^{-4} \text{ s}^{-1}$ , which is similar to that for phenyl picolinate (III) ( $2.4 \times 10^{-4} \text{ s}^{-1}$  at 50 °C) at a  $\text{Ni}^{2+}$  concentration of 0.01 M. Thus, the increased strength of metal ion binding brought about by the additional functional group does not produce a large effect on the rate constant of the water reaction.

There is no detectable pH-independent reaction in the hydrolysis of ethyl picolinate (I) in the presence of  $\text{Ni}^{2+}$  or  $\text{Cu}^{2+}$  even at pH values as low as 4 (see Figure 1).<sup>31</sup> This is not a consequence of the weakness of metal ion binding because again there is no detectable pH-independent reaction in the metal ion catalyzed hydrolysis of ethyl 6-carboxypicolinate with which metal ion binding is very strong. Saturation effects are observed at low metal ion concentrations in that case. As a result, the metal ion promoted  $\text{OH}^-$  catalyzed reaction (XVI) is facilitated by factors of  $2.7 \times$

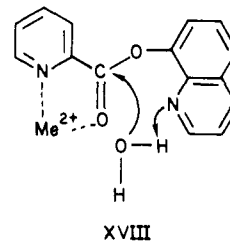


$10^4$  and  $1.3 \times 10^5$  with  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  and is the only reaction observed even at low pH. Thus it is clear that a change in rate-limiting step has taken place in the water reaction with the ethyl ester. If in fact a water reaction occurs it must be at least 100-fold slower than that with the trifluoroethyl ester. Breakdown of a tetrahedral intermediate to products is very likely rate determining with esters having leaving groups that are poorer than trifluoroethanol.

As noted, a water molecule coordinated to a metal ion should be a very poor nucleophile. However, the possibility exists for general base catalysis in which a proton is partially abstracted from the water molecule in the transition state (XV), thereby greatly increasing its nucleophilicity. This reaction might be catalyzed by various general bases, as in XVII. Such a possibility has been considered to be a plausible mechanism in ester hydrolysis reactions<sup>18</sup> and also in reactions catalyzed by carboxypeptidase A.<sup>32</sup> The 8-quinolyl esters (VI and VII) provide an opportunity

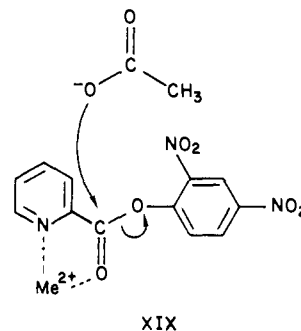


for intramolecular general base catalysis in the metal ion promoted reactions (XVIII). Again the pyridine nitrogen must be a site



of metal ion chelation because the corresponding isonicotinic acid derivative (X) does not display metal ion catalysis in its reactions. Since metal ion binding to VI and VII is weak it is probable that the quinoline nitrogen is not chelated in a 1:1 complex. Nevertheless, the ratio  $k_{\text{OH}}/k_0$  is not appreciably different in the presence of added 0.01 M  $\text{Ni}^{2+}$  with VI and VII than in the hydrolysis of the corresponding phenolic esters III and IV which lack a second functional group that might act as an intramolecular general base. Thus, significant intramolecular catalysis does not occur in the metal ion promoted water reactions of VI and VII. The quinoline nitrogen evidently cannot compete efficiently as a general base catalyst with the solvent water, i.e., its effective molarity is less than 55.5 M.

The metal ion promoted reactions of the picolinate esters having good leaving groups are indeed base catalyzed by formate and acetate ion as shown in Figure 7. As can be seen, the magnitude of the buffer catalysis is dependent upon the presence of the metal ion. This catalysis is also highly dependent upon the leaving group; the acetate ion general base catalyzed reactions occur with the dinitro and nitro phenyl esters where the leaving group  $\text{p}K_{\text{a}}$  values are 4 and 7 but not with the phenyl esters III and VI with which the leaving group  $\text{p}K_{\text{a}}$  is  $\sim 10$ . Thus, it is likely that the catalysis by formate and acetate is not general base catalysis of the type shown in XVII but rather is due to nucleophilic attack at the carbonyl of the ester as in XIX or a kinetic equivalent.<sup>33</sup> A



nucleophilic reaction would be expected to have a much greater dependence on the leaving group than a general base catalyzed reaction.<sup>34</sup> This is substantiated by the fact that lutidine (2,6-dimethylpyridine) is not a catalyst in the metal ion promoted

(30) Breslow, R.; McAllister, C. *J. Am. Chem. Soc.* **1971**, *93*, 7096.

(31) There is a significant water reaction in the hydrolysis of the palladium complex of ethyl picolinate. See ref 17.

(32) Geoghegan, K. F.; Holmquist, B.; Spilburg, C. A.; Vallee, B. L. *Biochemistry* **1983**, *22*, 1847.

(33) A kinetic equivalent possibility would have acetate ion complexed to the metal ion analogous to XI and XIII.

(34) Kirsch, J. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 833, 837. Bruice, T. C.; Benkovic, S. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966; p 60.

reactions. It has been shown that 2,6-lutidine does not act as a nucleophile toward *p*-nitrophenyl esters.<sup>35</sup>

**Carboxypeptidase A.** The present work shows that divalent metal ion promoted water catalyzed reactions will occur in the hydrolysis reactions of esters by a mechanism that is similar to that previously found in the hydrolysis of anhydrides.<sup>19</sup> These reactions show little dependence on the  $pK_a$  of the leaving group for esters with leaving groups of  $pK_a$  less than 12.4, but there is a pronounced decrease in the efficiency of this reaction with esters having poorer leaving groups. There are pH-independent regions in the log  $k_{cat}$  vs. pH profiles for carboxypeptidase A catalyzed hydrolysis of various ester substrates.<sup>36,37</sup> These pH-independent reactions could reflect water reactions of either anhydride intermediates or the substrates themselves. The  $pK_a$  of the alcohol groups of  $\beta$ -phenyllactate (ionized species) and mandelate, estimated by the method of Fox and Jencks,<sup>38</sup> are 14.7 and 14.2, respectively. Thus, a metal ion catalyzed water reaction of ester substrates of these alcohols can be considered unlikely on the basis of leaving group ability. Also, the rate constants for the metal ion promoted water reactions of II-VII are at least  $10^4$  less than in the enzymatic hydrolysis of *O*-(*trans*-cinnamoyl)-L- $\beta$ -phenyllactate<sup>39,40</sup> at pH 6.5-9.0. A water reaction of the enzyme substrate would appear to be chemically unable to achieve the experimentally determined rate constants in the enzyme reactions even though general base catalysis by other functional groups in the active site could occur in a water reaction. Such reactions are not observed in the metal ion catalyzed reactions of VI and VII nor is intramolecular general base catalysis by a neighboring carboxyl group observed in the metal ion catalyzed hydrolysis of salicyl phenanthroline-2-carboxylate.<sup>14</sup>

It may also be noted that while the rate constants for metal ion catalyzed hydrolysis of mixed cinnamic acid anhydrides<sup>19</sup> are

(35) de Jersey, J.; Willadsen, P.; Zerner, B. *Biochemistry* **1969**, *8*, 1959.

(36) Hall, P. L.; Kaiser, B. L.; Kaiser, E. T. *J. Am. Chem. Soc.* **1969**, *91*, 485.

(37) Bunting, J. W.; Murphy, J.; Myers, C. D.; Cross, G. G. *Can. J. Chem.* **1974**, *52*, 2648.

(38) Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436.

(39) The values of  $k_{cat}$  for enzyme catalyzed hydrolysis of *O*-(*trans*-cinnamoyl)-L- $\beta$ -phenyllactate are pH independent from pH 6.5 to 9 ( $k_{cat} = 200 \text{ s}^{-1}$  at 30 °C),<sup>40</sup> while  $k_{cat}$  for hydrolysis of hippuryl-L- $\beta$ -phenyllactate is pH independent from pH 5 to 10 ( $k_{cat} = 580 \text{ s}^{-1}$  at 30 °C).<sup>37</sup>

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reasonably similar to the  $k_{cat}$  values for carboxypeptidase A catalyzed hydrolysis of *O*-(*trans*-cinnamoyl)-L- $\beta$ -phenyllactate,<sup>40</sup> nevertheless the rate constants for metal ion promoted intramolecular nucleophilic carboxyl group participation in the hydrolysis of esters with aliphatic alcohol leaving groups are many orders of magnitude less, even with phthalate monoesters where the steric fit is excellent.<sup>15,16</sup> Therefore, on the basis of present knowledge it is difficult to explain how nucleophilic attack by Glu-270 on an ester substrate could lead to the experimental rate constants in the enzyme reactions. The only mechanism that presently might account for these rate constants would involve initial nucleophilic attack by metal ion coordinated  $\text{OH}^-$ , similar in type to mechanism XVI.<sup>41</sup> If such a species is present in the ES complex, it would be the most effective nucleophile in the active site. This is an important consideration in view of the poor leaving groups of the ester substrates. Over a wide range of structural type in esters metal ion promoted  $\text{OH}^-$  catalysis has been found to be the favored mechanism in hydrolytic reactions at pH > 6.<sup>10-19</sup> A drawback to such a mechanism is, of course, the kinetic evidence for a stable intermediate at low temperatures in the hydrolysis of para-substituted cinnamate esters of L- $\beta$ -phenyllactic acid.<sup>6a,b,42</sup> However, there is no evidence that an anhydride is a general intermediate in carboxypeptidase A catalyzed reactions,<sup>43</sup> and in some cases it appears that an anhydride is not formed.<sup>9</sup>

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

**Registry No.** I, 2524-52-9; II, 93111-30-9; III, 26838-86-8; IV, 74104-89-5; V, 76519-50-1; VI, 93111-31-0; VII, 93111-32-1; VIII, 21855-16-3; IX, 2882-35-1; X, 93111-33-2;  $\text{Ni}^{2+}$ , 14701-22-5;  $\text{Cu}^{2+}$ , 15158-11-9;  $\text{HCO}_2^-$ , 71-47-6;  $\text{CH}_3\text{CO}_2^-$ , 71-50-1;  $\text{HO}^-$ , 14280-30-9;  $\text{H}_2\text{O}$ , 7732-18-5; EtOH, 64-17-5;  $\text{CF}_3\text{C}(\text{O})\text{OC}(\text{O})\text{CF}_3$ , 407-25-0; picolinic acid, 98-98-6; isonicotinic acid, 55-22-1; 8-hydroxy-5-nitroquinoline, 4008-48-4; 8-hydroxyquinoline, 148-24-3.

(41) An attack of external  $\text{OH}^-$  on the enzyme-bound ester *O*-(*trans*-cinnamoyl)-L- $\beta$ -phenyllactate can be ruled out at pH values less than 7; a second-order rate constant greater than that for a diffusion-controlled reaction would be required.

(42) There appears to be no spectral evidence for the accumulation of an anhydride intermediate at low temperature.<sup>6b</sup>

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