

constant given as $Ne^2/2$ and $f(r_{\pm})$ is a function of the radius of both ions in solution.³⁰ As applied to the solvation energy of R_4Sn^+ relative to Me_4Sn^+ important in this study, the anionic contribution cancels out, and the relative solvation energy is given by eq 27. Since ΔG_r^\ddagger in acetonitrile

$$\Delta G_r^\ddagger = -\alpha(1 - 1/\epsilon)\Delta f(r_+) \quad (27)$$

is available from a previous study,¹⁷ the value of ΔG_r^\ddagger in other solvents

(30) $f(r_{\pm})$ is given as $[(r_+ + r_+')^{-1} + (r_- + r_-')^{-1}]$, where r_+ (or r_-) and r_+' (or r_-') correspond to the crystal radius of the cation (or anion) and their correction factor applied to solution, respectively. In the present case, the usage of eq 28 does not rely on these values, since they cancel out.

can be evaluated as in eq 28, where $\Delta G_r^{\ddagger 0}$ is the solvation energy in

$$\Delta G_r^\ddagger = \beta \Delta G_r^{\ddagger 0} \quad (28)$$

acetonitrile as the reference solvent and $\beta = (1 - \epsilon^{-1})/(1 - \epsilon_0^{-1})$. From the known values of the dielectric constants,⁶ β is obtained as 1.0 for MeOH, 0.91 for CH_2Cl_2 , and 0.57 for CCl_4 . The values of ΔG_r^\ddagger for various alkyltin compounds evaluated by eq 28 are listed in Table IV. [Note: the radial function $\Delta f(r_+)$ in eq 27 is cancelled out in the conversion to eq 28.]

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Transition-State Effects in the Divalent Metal Ion Catalyzed Hydrolysis of Esters. Hydrolysis of 2-Pyridylmethyl Hydrogen Phthalate

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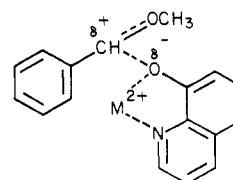
Abstract: Rate constants have been obtained for hydrolysis of 2-pyridylmethyl hydrogen phthalate, an ester with a poor leaving group, at 90 °C in the pH range 6–11. The pH–rate constant profile shows hydroxide ion catalysis at high pH and a plateau due to nucleophilic carboxyl group participation. Divalent metal ions (Ni^{2+} , Co^{2+} , Zn^{2+}) exert a large catalytic effect, although binding to the substrate is weak. Saturation effects were not observed even at 0.01 M metal ion (>100-fold excess over ester). Catalysis is dependent upon the presence of the pyridine nitrogen in the 2-position; metal ions are without effect in hydrolysis of the 4-pyridyl analogue. The metal ion catalyzed reactions are pH independent (pH 4.74–7.15 at 50 °C). Therefore, metal ion catalysis in reactions of the phthalate ester is associated with carboxyl group participation. Since breakdown of a tetrahedral intermediate must be rate determining in the carboxyl nucleophilic reactions of esters with poor leaving groups, the metal ions must exert their effect in the transition state by stabilization of the leaving group. In contrast, the metal ion effects observed in hydrolysis of 2-pyridylmethyl benzoate represent metal ion promoted OH^- catalysis.

Carboxypeptidase A is a Zn(II)-requiring enzyme, catalyzing the hydrolysis of ester and peptide substrates.² X-ray crystallographic analysis at 2-Å resolution has shown the zinc ion to be chelated to the carbonyl oxygen of poor peptide substrates.^{2–4} The carboxyl group of glutamic acid-270 has also been implicated in the catalytic process, and mechanisms have been suggested involving nucleophilic attack and classical general base catalysis (proton transfer in the transition state from a water molecule).^{3,4} The role of the complexed metal ion in these mechanisms has been to provide polarization of the carbonyl group. However, this should not be a catalytic factor in the case of a carboxyl nucleophilic reaction because breakdown of a tetrahedral intermediate would be rate determining when the pK_a of the leaving group greatly exceeds that of the nucleophile. A tetrahedral intermediate might be greatly stabilized by metal ion binding. An alternative proposal is that Zn(II) exerts a transition-state effect in which the leaving group is stabilized,⁵ but there have not been any assessments of the efficiency or chemical feasibility of the latter mechanism.

Chelated divalent metal ions have not been found to enhance the intramolecular carboxyl nucleophilic reactions of esters^{6,7} or amides.^{6,8} The reactions of amides can in fact be strongly inhibited by metal ion chelation to the carbonyl oxygen.⁸ There are no metal

ion effects in the carboxyl group nucleophilic reactions of phenolic esters, even though rate enhancements of up to 10^9 can be obtained in the metal ion promoted OH^- -catalyzed reaction.^{6,7} When the metal ion is strongly chelated to the substrate, nucleophilic attack by a carboxylate anion cannot compete with the metal ion promoted OH^- reaction.⁷

We have recently found that divalent metal ions will markedly enhance the rates of hydrolysis of benzaldehyde methyl 8-quinolyl acetals by binding to the leaving group in the transition state.⁹ In those reactions the catalytic effectiveness does not depend upon strength of binding to the reactant; inclusion of an additional functional group which leads to strong chelation of the metal ion to the acetal does not lead to an increased rate enhancement in comparison with acetals where binding to the reactant is weak at metal ion concentrations greater than 0.01 M. Catalysis occurs because of chelation of the metal ion to the quinoline nitrogen and oxygen of the leaving group in the transition state as the C–O bond breaks.

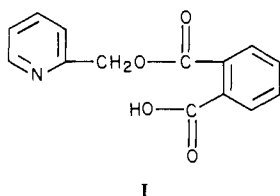


This is quite analogous to the manner in which a metal ion might function in assisting breakdown of a tetrahedral intermediate in ester hydrolysis reactions. Thus, the best opportunity for demonstration of such a transition-state effect in the intramolecular

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nucleophilic carboxyl group catalyzed reactions of esters would be with a substrate combining the following structural features: (1) a poor leaving group, so that bond breaking will be difficult, although not as difficult as with amides, which require protonation before bond breaking can occur; (2) a functional group sterically located to chelate a metal ion to the leaving group oxygen but not to the carboxyl group nucleophile, thereby allowing strong binding in the transition state; (3) the absence of other metal ion chelating functional groups, so that metal ion binding to the reactant will be weak. Strong binding to the reactant would very likely result in only a metal ion promoted OH^- reaction.⁷ We have therefore studied metal ion effects in the hydrolysis of 2-pyridylmethyl hydrogen phthalate (I) in which these structural features occur.



Experimental Section

Materials. 2-Pyridylmethyl hydrogen phthalate (I) was prepared by refluxing equivalent amounts of phthalic anhydride (Mallinckrodt) and 2-pyridinemethanol (Aldrich) in dry benzene for 48 h. The benzene was removed by rotary evaporation. Recrystallization of the solid residue from ethyl acetate-acetone yielded white crystals, mp 148–149 °C. Anal. Calcd for $\text{C}_{14}\text{H}_{11}\text{O}_4\text{N}$: C, 65.37; H, 4.28; N, 5.45. Found: C, 65.55; H, 4.48; N, 5.39.

4-Pyridylmethyl hydrogen phthalate (II) was prepared by refluxing equivalent amounts of phthalic anhydride and 4-pyridinemethanol (Aldrich) in dry benzene overnight. The resulting suspension was cooled, and ether was added. The white solid recovered upon filtration was recrystallized from methanol, mp 176–177 °C dec. Anal. Calcd for $\text{C}_{14}\text{H}_{11}\text{O}_4\text{N}$: C, 65.37; H, 4.28; N, 5.45. Found: C, 65.46; H, 4.41; N, 5.38.

2-Pyridylmethyl benzoate (III) was synthesized by slowly adding an equivalent of benzoyl chloride (Mallinckrodt) to an ether solution of 2-pyridinemethanol and trimethylamine. The solid trimethylamine hydrochloride was filtered off, and the ether was removed by rotary evaporation. Vacuum distillation of the residue yielded the benzoate ester: bp 102 °C (0.03 mm); n_D^{25} 1.5691 [lit.¹⁰ bp 93 °C (0.04 mm); n_D^{25} 1.5711].

Kinetic Measurements. The rates of hydrolysis of the esters were measured by following the disappearance of the reactant at 287 nm on a Beckman Model 25 (50 °C) or Pye-Unicam SP8-100 (90 °C) recording spectrophotometer. The ionic strength was maintained at 0.1 M with KCl, and solutions used for nonmetal ion assisted reactions contained 2×10^{-5} M EDTA as a precaution against trace metal ions in buffer or salt. The buffer concentration was 0.02 M, and no correction was made for any possible metal ion–buffer complexation.

The kinetic run was initiated by introducing 15–30 μL of ester stock solution into 2–3 mL of the buffer solution maintained at the desired temperature. The stock solutions were $(2\text{--}5) \times 10^{-3}$ M ester in acetonitrile. The reactions followed pseudo-first-order kinetics for at least 3 half-lives. Rate constants and subsequent kinetic parameters were evaluated by a nonlinear least-squares program. Reaction mixture pH values were measured at 50 and 90 °C with a Beckman Model 3500 digital pH meter.

The pK_a values of the esters were determined at 50 °C, $\mu = 0.1$ M (with KCl), using a Radiometer Type SBR2c/TTTlc autotitrator.¹¹ Ester I has pK_a values of 4.2 and 2.7, while ester II has pK_a 's of 4.8 and 2.8. 2-Pyridylmethyl benzoate could not be titrated in this manner because of low water solubility. Second-order rate constants for hydroxide ion catalysis (k_{OH}) were calculated by using K_w values of 5.5×10^{-14} at 50 °C and 3.55×10^{-13} at 90 °C.¹²

Results

Figure 1 shows a plot of $\log k_{\text{obsd}}$ vs. pH for hydrolysis of 2-pyridylmethyl hydrogen phthalate at 90 °C in H_2O ($\mu = 0.1$ M with KCl). Hydroxide ion catalysis is observed with the

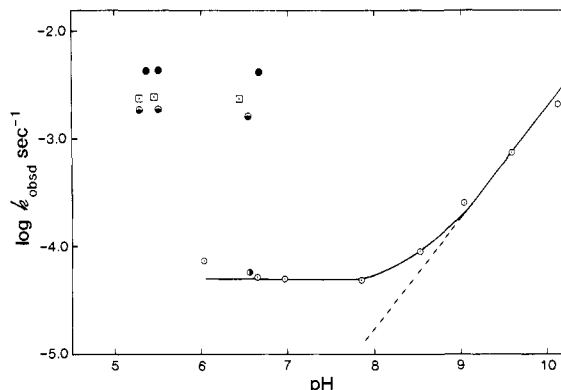


Figure 1. Plots of $\log k_{\text{obsd}}$ (s^{-1}) vs. pH for hydrolysis of 2-pyridylmethyl hydrogen phthalate in H_2O (O) and D_2O (●) at 90 °C ($\mu = 0.1$ M with KCl) in the absence of metal ions and in the presence of 0.001 M Ni^{2+} (●), Zn^{2+} (□), and Co^{2+} (●).

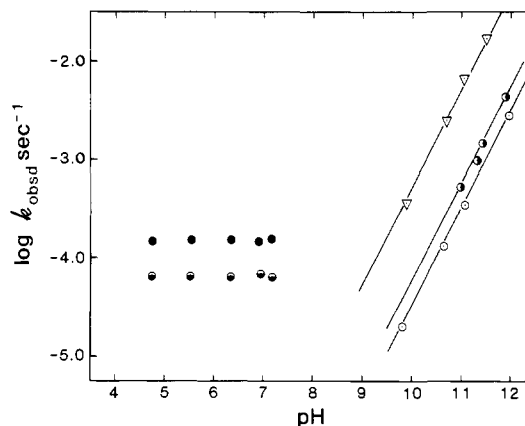


Figure 2. Plots of $\log k_{\text{obsd}}$ (s^{-1}) vs. pH in H_2O at 50 °C ($\mu = 0.1$ M with KCl) for hydrolysis of 2-pyridylmethyl hydrogen phthalate in the absence of metal ion (O) and in the presence of 0.005 M Ni^{2+} (●) and Co^{2+} (●) and hydrolysis of II (●) and III (▲) in the absence of metal ions.

Table I. Second-Order Rate Constants for Metal-Ion Catalysis of the Hydrolysis of 2-Pyridylmethyl Hydrogen Phthalate at 50 °C, $\mu = 0.1$ M (with KCl)

metal ion	$10^2 k_M$, $\text{M}^{-1} \text{s}^{-1}$	metal ion	$10^2 k_M$, $\text{M}^{-1} \text{s}^{-1}$
Ni^{2+}	3.1 ^a	Zn^{2+}	1.7 ^b
Co^{2+}	1.4 ^a		

^a pH 6.90, 0.02 M *N*-ethylmorpholine buffer. ^b pH 5.50, 0.02 M cacodylate buffer.

second-order rate constant $k_{\text{OH}} = 0.51 \text{ M}^{-1} \text{ s}^{-1}$. At pH values less than 9 the reactions become pH independent ($k_0 = 4.80 \times 10^{-5} \text{ s}^{-1}$). Rate constants could not be obtained at pH values less than 5 because of insufficient absorbance changes. Hydroxide ion catalysis was also observed at 90 °C in hydrolysis of 4-pyridylmethyl hydrogen phthalate ($k_{\text{OH}} = 0.69 \text{ M}^{-1} \text{ s}^{-1}$) and 2-pyridylmethyl benzoate ($k_{\text{OH}} = 8.8 \text{ M}^{-1} \text{ s}^{-1}$). At 50 °C values for k_{OH} ($\text{M}^{-1} \text{ s}^{-1}$) are as follows: I, 0.057; II, 0.094; III, 0.89. In Figure 2 $\log k_{\text{obsd}}$ at 50 °C for hydrolysis of these esters is plotted vs. pH.

The divalent metal ions Ni^{2+} , Co^{2+} , and Zn^{2+} exert a marked catalytic effect on the rate of hydrolysis of 2-pyridylmethyl hydrogen phthalate, as seen in Figure 1, where $\log k_{\text{obsd}}$ at metal ion concentrations of 0.001 M are plotted vs. pH. Saturation effects of the metal ions could not be observed even at concentrations as large as 0.01 M. Plots of k_{obsd} vs. metal ion concentration were linear, as illustrated in Figure 3 where such a plot is presented for Ni^{2+} at pH 6.90 (0.02 M *N*-ethylmorpholine buffer) at 50 °C. From the slopes of these plots, second-order rate constants (k_M) were calculated. These rate constants are given in Table I.

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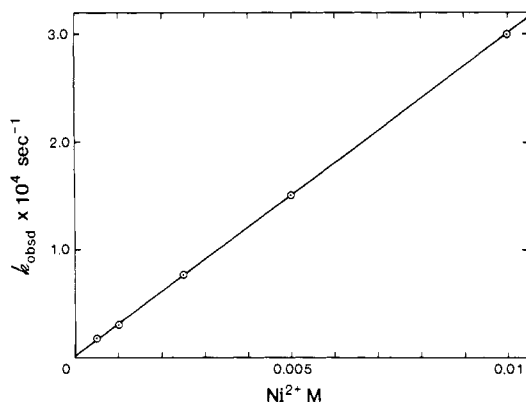


Figure 3. Plot of k_{obsd} (s^{-1}) vs. Ni^{2+} concentration in hydrolysis of 2-pyridylmethyl hydrogen phthalate in H_2O at 50°C ($\mu = 0.1 \text{ M}$ with KCl) at pH 6.90 (0.02 M *N*-ethylmorpholine buffer).

Table II. Rate Constants (k_{obsd} , s^{-1}) for Hydrolysis of 2-Pyridylmethyl Hydrogen Phthalate at 50°C , $\mu = 0.1 \text{ M}$, in the Presence of 0.005 M Co^{2+} and 0.005 M Ni^{2+}

pH	Ni^{2+}	Co^{2+}
4.74	1.53×10^{-4}	6.40×10^{-5}
5.54	1.56×10^{-4}	6.60×10^{-5}
6.35	1.58×10^{-4}	6.58×10^{-5}
6.93	1.51×10^{-4}	6.99×10^{-5}
7.15	1.65×10^{-4}	6.93×10^{-5}

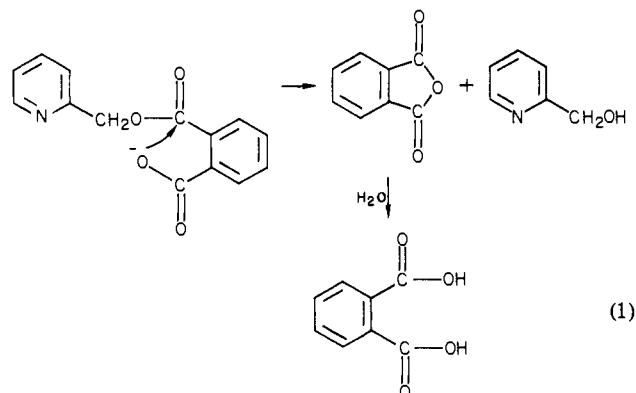
At constant metal ion concentration, values of k_{obsd} for hydrolysis of I are pH independent, as seen in Figures 1 (90°C) and 2 (50°C). A wider pH range could be studied at 50°C , and it was found that k_{obsd} was pH independent in the pH range 4.74–7.15. It was not possible to obtain rate constants for the metal ion catalyzed reactions outside this pH range. At higher pH values precipitation occurred, while at lower pH absorbance changes became too small for accurate determination of the rate constants. Rate constants are given in Table II.

Metal ion catalysis was not detected in the hydrolysis of 4-pyridylmethyl hydrogen phthalate. For example, at pH 6.05 k_{obsd} (90°C) in the absence of metal ion is $8.09 \times 10^{-5} \text{ s}^{-1}$, whereas in the presence of 0.005 M Ni^{2+} k_{obsd} is $8.31 \times 10^{-5} \text{ s}^{-1}$. Metal ion catalysis was detected in the hydrolysis of 2-pyridylmethyl benzoate at 90°C . A plot of k_{obsd} at pH 6.19 (cacodylate buffer) vs. the concentration of Ni^{2+} had a slope k_{M} of $1.3 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$. Variation of the pH at a constant Ni^{2+} concentration of 0.01 M showed that k_{obsd} is linearly related to pH with a slope of 1.0 ($k_{\text{OH}} = 2.8 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$).

Discussion

Intramolecular carboxyl group participation in ester hydrolysis has been extensively investigated.^{13–17} Large rate enhancements are observed in the intramolecular nucleophilic reactions of phenolic monoesters of succinic or glutaric acid.^{14,15} The neighboring carboxyl group of phthalate monoesters also participates as a nucleophile.¹⁶ In that case, when the leaving group is phenol, the carboxylate anion is the active species, but with the methyl ester the pH–rate constant profile indicates that the neutral species is maximally reactive. This probably reflects a reaction of a zwitterionic species, i.e., carboxylate anion and protonated leaving group. When the leaving-group pK_a is intermediate between that of phenol and methanol, the observed rate constants are pH independent, which can be interpreted as due to involvement of both types of mechanisms.¹⁶ In the present study, the pH–rate constant profile for hydrolysis of the monophthalate ester of 2-(hydroxymethyl)pyridine (Figure 1) shows OH^- catalysis at high pH and a plateau at pH <9 which is undoubtedly due to carboxyl

group participation (eq 1). Phthalic anhydride has been indirectly shown to be an intermediate in the hydrolysis of phthalate monoesters.¹⁶ It hydrolyzes at 30°C in the pH range 1.6–5.7 in a pH-independent reaction with a rate constant of 0.012 s^{-1} , which is much greater than the observed rate constants for the reactions of I and II.



The pK_a of the hydroxyl group of 2-(hydroxymethyl)pyridine would be expected to be somewhat less than that of methanol (15.5),¹⁸ and it has been reported to be 13.9.¹⁹ Thanassi and Bruice¹⁶ found that the rate of hydrolysis of propargyl hydrogen phthalate was invariant with pH in the range of the carboxyl pK_a (pH 2–4.3). Propargyl alcohol has a pK_a of 13.55, and it was considered that near this leaving-group pK_a , COO^- and COOH participation would be equally effective. Therefore, the plateau in the pH–log k_{obsd} profile for hydrolysis of I (pH 6–8) must represent carboxyl group participation by the anionic species. A nucleophilic reaction is supported by a D_2O solvent isotope effect near unity. The value of the rate constant for pH-independent hydrolysis of the propargyl monoester is $6.9 \times 10^{-5} \text{ s}^{-1}$ (78.5°C), which is comparable to k_0 in hydrolysis of I ($4.8 \times 10^{-5} \text{ s}^{-1}$ at 90°C). Consequently, 2-pyridylmethyl hydrogen phthalate exhibits hydrolytic behavior expected for phthalate monoesters with a leaving-group pK_a of 13.9. It may also be noted in Figure 2 that I undergoes OH^- -catalyzed hydrolysis at a rate which is similar to that of 4-pyridylmethyl hydrogen phthalate and 15-fold less than that of 2-pyridylmethyl benzoate. Since the electronic effect of an ionized carboxyl group is negligible ($\sigma \approx 0$), this may indicate an unfavorable electrostatic effect on approach of OH^- .

Divalent metal ions (Ni^{2+} , Co^{2+} , Zn^{2+}) produce a large enhancement in the rate of hydrolysis of I, even though binding of metal ion to the ester is quite weak. Saturation effects by the metal ions could not be observed, even at relatively high concentrations (0.01 M). Nevertheless, the enhancement in k_{obsd} (90°C) brought about by a 0.001 M concentration of Ni^{2+} at pH 6.68 is a factor of 90. This enhancement could, of course, be made much larger by employing higher metal ion concentrations, if that were experimentally feasible. Since metal ion binding is so weak, it is clear that the neighboring carboxyl group is not being chelated. Such chelation would be unlikely in a 1:1 complex on steric grounds (10-membered ring). It is necessary that the pyridine nitrogen be in the 2-position for the metal ions to exert a catalytic effect; metal ions are without effect in hydrolysis of the analogous 4-pyridyl ester (II).

Values of k_{obsd} for hydrolysis of I are pH independent at constant metal ion concentration. An interpretation that the pH-independent metal ion reactions represent a transition from a metal ion promoted OH^- catalyzed reaction of the anionic species to a similar reaction of a neutral species is highly unlikely in view of the pK_a values of I (4.2 and 2.7) and the length of the plateau that could be determined at 50°C . Since k_{OH} values for the metal ion– OH^- catalyzed reactions of I and III should be roughly similar, it can be estimated that the plateau for the Ni^{2+} -catalyzed reaction

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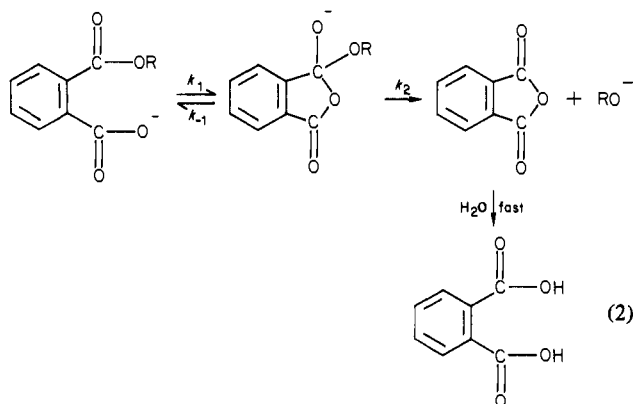
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of I would not intersect the profile for the metal ion-OH⁻ reaction until pH 8-9. Therefore, if the pH-independent reactions of I were to be explained in that manner, then the difference in the second-order rate constants for the metal ion promoted OH⁻ reactions of the two species at constant metal ion concentration would necessarily be at least 10⁴-10⁵, which is much too large to explain on the basis of electronic effects due to carboxyl group ionization. Addition of a proton to the pyridine nitrogen would, of course, necessarily reduce the observed rate by preventing binding of metal ion. Only a small plateau (~1 pH unit) was observed in the pH-log k_{obsd} profiles for metal ion promoted OH⁻-catalyzed reactions of 8-(2-carboxyquinolyl) hydrogen glutarate due to ionization of the carboxyl group.⁷ Metal ion catalysis in the hydrolytic reactions of I is clearly associated with the neighboring carboxyl group nucleophilic reaction.

A pH-independent metal ion catalyzed reaction of the type observed is quite unusual in ester hydrolysis reactions. Typically, metal ion promoted OH⁻-catalyzed reactions have been observed in ester hydrolysis reactions when there is a functional group present in the ester which will allow metal ion chelation.^{6,7,20-24} Such reactions have pH-log (rate constant) profiles with slopes of 1.0 at pH values below the metal ion-bound water pK_a and are only pH independent at pH values above the pK_a.²⁰ The pH range in which metal ion catalysis was studied in the present case is clearly below the pK_a values.²⁵ Marked metal ion effects were observed in hydrolysis of 2-pyridylmethyl benzoate (III), and, as expected, the reactions involve metal ion promoted OH⁻ catalysis. Thus, the neighboring carboxyl group of I is necessary for occurrence of the pH-independent metal ion catalyzed reactions.

If, in carboxyl group nucleophilic reactions (eq 2), a tetrahedral



intermediate is formed, then its breakdown must be rate determining. The expression for k_{obsd} is given in eq 3. Since the

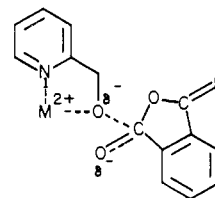
$$k_{\text{obsd}} = k_1 k_2 / (k_{-1} + k_2) \quad (3)$$

leaving-group pK_a is much greater than that of the carboxyl group nucleophile, $k_{-1} > k_2$ and, therefore,

$$k_{\text{obsd}} = k_1 k_2 / k_{-1} \quad (4)$$

i.e., C-O bond breaking must be part of the rate-determining step. If the metal binds to the carbonyl oxygen, then the carbonyl group will be polarized, increasing k_1 , but the tetrahedral intermediate will be greatly stabilized; i.e., both k_{-1} and k_2 will be decreased. It might be expected that k_2 would be decreased to a greater extent

than k_{-1} , in view of the relatively poor leaving group. Consequently, carbonyl chelation would very likely lead to a metal ion promoted OH⁻-catalyzed reaction in which attack is rate limiting. Metal ion chelation of the carbonyl oxygen of I would require formation of a seven-membered ring and is unlikely. The metal ion catalysis observed in hydrolysis of I can therefore be considered to involve preequilibrium metal ion binding followed by stabilization of the transition state for C-O bond breaking (IV). When the leaving



IV

group is good, as with phenolic esters, C-O bond breaking occurs readily, and chelated metal ions are without effect in the carboxylate anion nucleophilic reactions.^{6,7} In those cases the transition state must be attained before metal ion binding to the leaving group becomes catalytically effective. However, in view of the relative high pK_a of the leaving-group alcohol of I, C-O bond breaking would normally be difficult, and considerable bond breaking would be required to reach the transition state. Metal ion binding to the leaving group would therefore greatly facilitate the reaction. While metal ion binding to the reactant is weak, negative charge will be generated on oxygen as the C-O bond breaks, thereby allowing strong binding in the transition state²⁶ (IV) in which a five-membered chelate ring is formed.

In the metal ion catalyzed hydrolysis of benzaldehyde methyl 8-quinolyl acetals,⁹ catalysis is also achieved by binding to the leaving-group oxygen in the transition state. In those reactions, the important factor is ease of binding in the transition state rather than to the reactant, and, as in reactions of I, the metal ion catalyzed reactions are pH independent. Thus, the similarities in these reactions are striking. It can be concluded that metal ions may enhance a variety of bond-breaking reactions through transition-state effects if the structural features in the substrate are favorable.

Ester substrates for carboxypeptidase A (esters of phenylactic and mandelic acids)² have poor leaving groups. Consequently, transition-state effects of the type observed with I could also be important in the enzymatic reactions, if the substrate binds to the enzyme so that Zn(II) can chelate the leaving-group oxygen. The pH- k_{cat} profiles of the specific ester substrates *O*-(*trans*-cinnamoyl)-*L*-β-phenyllactate and its chloro derivative^{27,28} can be considered to represent OH⁻ catalysis at high pH superimposed on a bell-shaped region.²⁹ Suh and Kaiser²⁷ suggested that the profiles for the cinnamoyl phenyllactate esters might reflect nucleophilic participation by Glu-270 and attack of OH⁻ on an intermediate anhydride at high pH.³⁰ Recent evidence³² supports a nucleophilic mechanism with ester substrates, probably involving Glu-270. The present work shows that transition-state effects in which the leaving group is stabilized by metal ion binding are capable of generating sizable rate enhancements in carboxyl nucleophilic reactions and could be of importance in carboxypeptidase A reactions with the types of ester substrates commonly employed.

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