# Metal Ion Catalysis in the Hydrolysis of Esters of 2-Hydroxy-1, 10-phenanthroline: The Effects of Metal Ions on Intramolecular Carboxyl Group Participation

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Rate constants have been determined for hydrolysis of the acetate, glutarate, and phthalate monoesters of 2-hydroxy-1,10-phenanthroline in water at 30°C and  $\mu = 0.1$  M with KCl. The hydrolysis reactions of the esters are hydroxide ion catalyzed at pH > 9. The phthalate and glutarate monoesters have in addition pH-independent reactions from pH 5.5 to 9 that involve intramolecular participation by the neighboring carboxylate anion. The pH-independent reaction of the glutarate monoester is  $\sim$ 5-fold faster than that of the phthalate monoester. The plots of  $\log k_{obsd}$  vs pH for hydrolysis of the carboxyl substituted esters are bell shaped at pH < 5, which indicates a rapid reaction of the zwitterionic species (carboxyl anion and protonated phenanthroline nitrogen). The divalent metal ions,  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Zn^{2+}$ , and  $Co^{2+}$ , complex strongly with the esters; saturation occurs at metal ion concentrations less than 0.01 M. The 1:1 metal ion complexes have greatly enhanced rates of hydrolysis; the second-order rate constants for the  $OH^-$  reactions are increased by factors of  $10^5$  to  $10^8$  by the metal ion. The pH-rate constant profiles for the phthalate and glutarate ester metal ion complexes have a sigmoidal region below pH 6 that can be attributed to a metal ion-promoted carboxylate anion nucleophilic reaction. The carboxyl group reactions are enhanced  $10^2$ - to  $10^3$ -fold by the metal ions, which allows the neighboring group reaction to be competitive with the favorable metal ion-promoted  $OH^-$  reaction at pH < 6, but not at pH > 6. The half-lives of the pH-independent neighboring carboxyl group reactions of the Cu(II) complexes at 30°C are  $\sim 2$  s. The other metal ion complexes are only slightly less reactive (half-lives vary from 2.5 to 40 s). These are the most rapid neighboring carboxyl group reactions that have been observed in ester hydrolysis. © 2000 Academic Press

## INTRODUCTION

A carboxyl group has been implicated in the catalytic action of the esterase and peptidase Zn(II) metalloenzyme carboxypeptidase A (1-5). A nucleophilic mechanism involving the  $\gamma$ -carboxyl group of Glu-270 has been proposed for the enzyme-catalyzed hydrolysis of ester substrates (3,6,7), although trapping experiments designed to demonstrate the presence of an anhydride intermediate have not been successful (8). In addition to the intrinsic chemical interest, knowledge of the mechanisms and the magnitude of the rate constants for coordinated metal ion and carboxyl group participation in the hydrolysis of acyl derivatives is necessary for the meaningful assessment of proposed enzyme mechanisms.



A neighboring carboxyl group can participate in the hydrolysis of esters as an intramolecular nucleophile without assistance from metal ions (9-15). However, the rate constants in the nucleophilic reactions are considerably less than those in carboxypeptidase A esterase reactions (16, 17). The intramolecular carboxyl group reactions of esters continue to be of great interest as examples of "steric fit" in nucleophilic reactions (15).

The demonstration of bifunctional mechanisms in hydrolytic reactions is important because many enzymatic reactions give bell-shaped pH-rate constant profiles that may be due to such mechanisms. Esters with two substituent carboxyl groups can give  $k_{obsd}$  vs pH profiles that are bell shaped (13,14). The profile for the reaction of salicyl succinate was interpreted in terms of the concerted bifunctional mechanisms I and II where a carboxylate anion acts as a nucleophile and an un-ionized carboxyl functions as a general acid with proton donation to oxygen (Scheme 1). However, Maugh and Bruice (14) considered that the reactions of salicyl succinate involve nucleophilic attack by a carboxylate anion and a changing inductive effect as the second carboxyl group ionizes, or a "series nucleophilic" reaction in which there are two stepwise nucleophilic processes. The same general conclusions were drawn in regard to other proposed bifunctional mechanisms. A properly located chelated metal ion might function with great effect analogous to a general acid in a bifunctional reaction, and there would be no kinetic ambiguity (Eq. [1]).<sup>1</sup>



Determination of the effects of metal ions in intramolecular nucleophilic reactions is difficult: first, because of the necessity of the metal ion binding to the substrate in a position that is remote from the nucleophile, and secondly, because of the extremely favorable metal ion-promoted OH<sup>-</sup> catalysis that is always observed in the hydrolysis of esters having metal ion liganding groups (19-26) The facile OH<sup>-</sup> reaction restricts competition from other mechanistic pathways (23). Rate enhancements in metal ion-promoted OH<sup>-</sup> reactions of acyl derivatives are very large (19,20,23,25-31), ranging up to  $10^8$ -fold with Cu<sup>2+</sup> ( $10^{10}$ -fold with an acyl phosphate) (31). In contrast, a significant effect ( $10^2$ - to  $10^4$ -fold) has only been observed in metal ion-promoted carboxyl nucleophilic reactions in cases where the leaving group is an aliphatic alcohol and the metal ion can complex the leaving group oxygen in the transition state (24,25). Complexed divalent metal ions were previously found to have little or no

<sup>&</sup>lt;sup>1</sup> For an analogy of general acid catalysis and metal ion catalysis in acetal hydrolysis see Ref. (18).



SCHEME 1

effect in carboxyl group nucleophilic reactions of esters of 8-hydroxyquinoline and 2-carboxy-8-hydroxyquinoline (22,23). However, there have been no previous studies of the effects of a metal ion strongly complexed to the carbonyl oxygen of an ester with a carboxyl group substituent capable of functioning as a nucleophile.<sup>2,3</sup> If a carboxyl nucleophilic mechanism is to be considered chemically feasible for an esterase metalloenzyme, then such a mechanism must be capable of generating much larger rate constants and rate enhancements than have hitherto been observed in chemical reactions. It is therefore important to investigate systems that are maximally suited to provide large rate constants.

The 1,10-phenanthrolyl ligand has advantages for the study of metal ion effects, among which is the very strong complexing ability of the phenanthroline nitrogens (23,31-34). The position of the metal ion in a 1:1 complex can often be specified. Furthermore, the neutral liganding groups will minimize the charge reduction on the metal ion that results with negatively charged ligands. In esters of 2-hydroxy-1,10-phenanthroline a metal ion will be complexed by the phenanthroline nitrogens and can also complex with the ester carbonyl oxygen in a stable 6-membered chelate ring. A phthalate monoester would allow intramolecular carboxyl group attack via a kinetically favored 5-membered ring transition state in which the strength of metal ion binding should increase as negative charge is generated on oxygen. Therefore, we have determined the effects of the divalent metal ions  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Zn^{2+}$ , and  $Co^{2+}$  in the reactions of 2-(1,10-phenanthrolyl) hydrogen phthalate (**III**) and for comparison, the corresponding monoglutarate (**IV**) and acetate (**V**) esters (Scheme 2). The carboxyl group substituent of **III** and **IV** will not be bound to the metal ion in a 1:1 complex

 $<sup>^{2}</sup>$  The metal ion complexation of the carbonyl oxygen of salicyl 1,10-phenanthroline-2-carboxylate could be strong, but nucleophilic attack by the carboxylate anion would only result in a reversible rearrangement and is therefore not detected (23).

<sup>&</sup>lt;sup>3</sup> Metal ion complexation of the carbonyl oxygen of amides results in rate retardation in the neighboring carboxyl group reaction (*32*).

R or Ar



**SCHEME 2** 

## **EXPERIMENTAL**

Materials. 2-Hydroxy-1,10-phenanthroline was prepared by the synthetic scheme previously outlined (35) and was recrystallized from a benzene-ethanol mixture, mp 159–160 °C; lit. (35), mp 159–160 °C. The synthesis of the esters III and IV was carried out by dissolving freshly prepared 2-hydroxy-1,10-phenanthroline in tetrahydrofuran that had been distilled and dried. An equivalent amount of the appropriate anhydride was added and the solution was refluxed for at least 48 h. The solvent was then removed by rotary evaporation. The oil residue was then extracted several times with dry benzene. The solid that was obtained was recrystallized from dry chloroform or benzene. The acetate ester V was prepared similarly except that excess acetic anhydride was employed. 2-(1,10-Phenanthrolyl) hydrogen phthalate (III) had mp 142°C after recrystallization. Anal. Calcd for C<sub>20</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>: C, 69.76; H, 3.51; N, 8.12. Found: C, 69.94; H, 3.49; N, 8.12. 2-(1,10-Phenanthrolyl) hydrogen glutarate (IV) had mp 148 °C after recrystallization. Anal. Calcd for  $C_{17}H_{14}N_2O_4$ : C, 65.80; H, 4.55; N, 9.03. Found: C, 65.69; H, 4.53; N, 9.13. 2-(1,10-Phenanthrolyl) acetate (V) had mp 152 °C after recrystallization. Anal. Calcd for C<sub>14</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.55; H, 4.30; N, 11.78. Spectra were consistent with the expected structures. The esters are sensitive to air, but can be stored in a desiccator in the dark at low temperature.

Acetonitrile was Eastman Kodak Spectrograde. Amine buffer components were recrystallized or distilled prior to use.

*Kinetic measurements.* The rates of hydrolysis of **III–V** were measured with a Beckman DU-7500 or a Pye Unicam SP8-100 spectrophotometer by following the absorbance increase at 300–320 nm. The buffers employed were formate, acetate, cacodylate, 2,6-lutidine, *N*-ethylmorpholine, Tris, morpholine, and carbonate. All buffer solutions were maintained at a constant ionic strength of 0.1 M with KCl. The buffer concentration was 0.02 M; buffer catalysis is negligible at that concentration. A typical kinetic run was initiated by injecting 20–30  $\mu$ L of a 0.004 M stock solution

of the ester in acetonitrile into 3 mL of buffer maintained at 30 °C. Product spectra at the conclusion of the reactions matched those of identical concentrations of 2-hydroxy-1,10-phenanthroline and the appropriate carboxylic acid. The solutions employed for kinetic measurements in the absence of metal ions included  $2 \times 10^{-5}$  M EDTA.

Reactions that were too fast to be measured with a conventional spectrophotometer were monitored with a Durrum D-110 stopped-flow spectrophotometer equipped with a Hewlitt-Packard Model 1207B storage oscilloscope. The substrate was dissolved at the desired concentration in  $10^{-2}-10^{-3}$  M HCl,  $\mu = 0.1$  M with KCl. This solution was introduced into one of two identical drive syringes. The second syringe contained the appropriate buffer maintained at the same ionic strength and the chosen metal ion (Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, and Co<sup>2+</sup>). With each buffer three to four reactions were tabulated. The observed reactions followed good first-order kinetics, and infinity points were stable. The hydrolysis of phthalic anhydride or glutaric anhydride has no effect on the observed kinetics in the reactions of **III** and **IV** monitored at 320 nm. Pseudo-first-order rate constants ( $k_{obsd}$ ) were computer-calculated.

Reaction mixture pH values were measured with a Radiometer-type PHM 22A pH meter equipped with a combination electrode. In calculating second-order rate constants for hydroxide ion catalysis ( $k_{\text{OH}}$ ) the ion product of water,  $K_{w}$ , was taken to be  $1.47 \times 10^{-14}$  at 30°C.

## RESULTS

In Fig. 1 a plot is shown of log  $k_{obsd}$  vs pH for hydrolysis of 2-(1,10-phenanthrolyl)hydrogen phthalate (III). The plot has a slope of 1.0 at high pH, which indicates a



**FIG. 1.** Plot of log  $k_{obsd}$  vs pH for the hydrolysis of 2-(1,10-phenanthrolyl) hydrogen phthalate in H<sub>2</sub>O at 30°C,  $\mu = 0.1$  M with KCl, in the presence of 0.002 M Cu<sup>2+</sup> ( $\bigcirc$ ) and 0.004 M Zn<sup>2+</sup> ( $\bigcirc$ ), and in the absence of metal ion ( $\odot$ ).

hydroxide ion-catalyzed reaction. At pH 6-9 there is a pH-independent reaction, and at pH < 6 the plot of Fig. 1 is bell shaped. The reaction scheme is that of Eq. [2].



The equation for  $k_{obsd}$  is then

$$k_{\text{obsd}} = \frac{k_1 K_1 a_{\text{H}} + k_2 K_1 K_2 + k_{\text{OH}} K_1 K_2 \text{ [OH}^{-}\text{]}}{{a_{\text{H}}}^2 + K_1 a_{\text{H}} + K_1 K_2},$$
[3]

where  $K_1$  and  $K_2$  are the first and second dissociation constants. Thus, the pHindependent region of Fig. 1 represents a reaction of the anionic species, while the bell shaped portion of the profile is due to a reaction of the neutral species, or more likely a kinetic equivalent zwitterion (ionized carboxyl group and protonated nitrogen). Values of the rate constants are given in Table 1. The apparent  $pK_a$  values of 4.0 and 5.0 provide a good fit to the experimental data employing Eq. [3]. There is, however, some positive deviation at low pH (< 3) which indicates the incursion of a protonated species reaction. The solid line in Fig. 1 is theoretical for Eq. [3] and the constants in Table 1.

A similar plot of log  $k_{obsd}$  vs pH is also produced in the hydrolysis of 2-(1,10phenanthrolyl) hydrogen glutarate (**IV**), as seen in Fig. 2, and again Eq. [3] provides a good fit for the experimental data with  $pK_{app}$  3.5 and 4.5. The rate constants for **IV** in Table 1 are several-fold larger than those of **III**.

**IV** in Table 1 are several-fold larger than those of **III**. The divalent metal ions  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$ , and  $Zn^{2+}$  bind strongly to **III–V**. Figure 3 is a plot of  $k_{obsd}$  vs the concentration of  $Zn^{2+}$  in the hydrolysis of **IV** at pH 3.2. Saturation occurs at  $Zn^{2+}$  concentrations less than 0.005 M. The equation for  $k_{obsd}$  at constant pH is

$$k_{\rm obsd} = \frac{k_{\rm M} K_{\rm M} \, [{\rm M}^{2+}]}{(1 + K_{\rm M} \, [{\rm M}^{2+}])}, \qquad [4]$$

where  $K_{\rm M}$  is the metal ion association constant and  $k_{\rm M}$  is the rate constant at the

#### TABLE 1

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Compound	Metal ion	$k_1 (s^{-1})$	$k_2 (s^{-1})$	$k_{\rm OH} \ ({ m M}^{-1} \ { m s}^{-1})$	$pK_{app}$
Ш	None	$2 \times 10^{-3}$	$1.3 \times 10^{-4}$	2.5	4.0, 5.0
	$Cu^{2+}$		$4.2 \times 10^{-1}$	$1.1 \times 10^{8}$	4.3
	Ni <sup>2+</sup>		$1.8 imes10^{-1}$	$9.0 \times 10^{7}$	4.5
	$Zn^{2+}$		$4.5  imes 10^{-2}$	$2.1 \times 10^{6}$	5.0
	$\mathrm{Co}^{2+}$		$1.6  imes 10^{-2}$	$1.0  imes 10^{6}$	5.1
IV	None	$1 \times 10^{-2}$	$7.0 imes10^{-4}$	12.2	3.5, 4.5
	$Cu^{2+}$		$2.5 \times 10^{-1}$	$6.8  imes 10^{7}$	4.4
	Ni <sup>2+</sup>		$1.3  imes 10^{-1}$	$2.7 \times 10^{7}$	4.15
	$Zn^{2+}$		$1.3 \times 10^{-1}$	$4.3 \times 10^{7}$	4.15
	$\mathrm{Co}^{2+}$		$6.7  imes 10^{-2}$	$4.0  imes 10^{6}$	4.2
V	None			10.2	
	$Cu^{2+}$			$1.2 \times 10^{6}$	
	Ni <sup>2+</sup>			$2.7 \times 10^{5}$	
	$Zn^{2+}$			$9.0  imes 10^4$	
	$\mathrm{Co}^{2+}$			$8.8 imes10^4$	

Values of the Rate Constants for Hydrolysis of 2-(1,10-Phenanthrolyl) hydrogen Phthalate (III), Glutarate (IV), and Acetate (V) in Water at 30°C and  $\mu = 0.1$  M with KCl

specified pH for hydrolysis at a saturating concentration of the metal ion. Values of the association constants are given in Table 2.

Plots of log  $k_{obsd}$  vs pH are shown in Figs. 1 and 2 at saturating concentrations of Cu<sup>2+</sup>, Zn<sup>2+</sup>, and Co<sup>2+</sup>. Similar plots were also obtained with Ni<sup>2+</sup>, but were omitted



**FIG. 2.** Plot of log  $k_{obsd}$  vs pH for the hydrolysis of 2-(1,10-phenanthrolyl) hydrogen glutarate in H<sub>2</sub>O at 30°C,  $\mu = 0.1$  M with KCl, in the presence of 0.003 M Zn<sup>2+</sup> ( $\bullet$ ) and 0.003 M Co<sup>2+</sup> ( $\bullet$ ), and in the absence of metal ion ( $\odot$ ).



**FIG. 3.** Plot of  $k_{obsd}$  vs the concentration of Zn<sup>2+</sup> at pH 3.20 in the hydrolysis of 2-(1,10-phenanthrolyl) hydrogen glutarate in H<sub>2</sub>O at 30°C,  $\mu = 0.1$  M with KCl.

from the figures for clarity. The plots have a slope of 1.0 at pH > 6, and a pH-independent region from pH 4 to 6, which is followed by a downward bend to again give a line of slope 1.0 at lower pH. The reaction scheme for hydrolysis of the 1:1 metal ion complexes **VI–VIII** is given in Eq. [5].

TABLE 2

Compound	Metal ion	$K_{ m M}~{ m M}^{-1}$
III	$Cu^{2+}$	$2 imes 10^{4a}$
	Ni <sup>2+</sup>	$7  imes 10^{3a}$
	$Zn^{2+}$	$2.5 imes 10^{4b}$
	$\mathrm{Co}^{2+}$	$7  imes 10^{3b}$
IV	$Cu^{2+}$	$1 \times 10^{3c}$
	Ni <sup>2+</sup>	$3.5  imes 10^{3a}$
	$Zn^{2+}$	$5  imes 10^{3a}$
	$\mathrm{Co}^{2+}$	$4 \times 10^{3a}$
V	$Cu^{2+}$	$10^{5d}$
	Ni <sup>2+</sup>	$3  imes 10^{4d}$
	$Zn^{2+}$	$3  imes 10^{4d}$
	$\mathrm{Co}^{2+}$	$1  imes 10^{4d}$

Values of the Association Constants for Binding of Divalent Metal Ions with 2-(1,10-Phenanthrolyl) hydrogen Phthalate (**III**), Glutarate (**IV**), and Acetate (**V**) in Water at 30°C and  $\mu = 0.1$  M with KCl

<sup>a</sup> At pH 3.20.

<sup>b</sup> At pH 4.28.

<sup>c</sup> At pH 2.80.

<sup>d</sup> At pH 6.20.



In these reactions the ratio of metal ion to reactant is > 100:1 to ensure a 1:1 complex.

The equation for  $k_{obsd}$  as a function of pH at saturating metal ion concentration is then

$$k_{\rm obsd} = \frac{k_2' K_{\rm a} a_{\rm H} + k_{\rm OH}' K_{\rm a} K_{\rm a}'}{a_{\rm H}^2 + K_{\rm a} a_{\rm H} + K_{\rm a} K_{\rm a}'}.$$
 [6]

The slopes of 1.0 at pH > 6 show that pK<sub>a</sub>' has not been approached at the highest pH employed, that is,  $a_H > K_a'$  at all experimental pH values.<sup>4</sup> Equation [6] then reduces to Eq. [7] at  $a_H > K_a'$ . At  $K_a > a_H$  Eq. [8] is then obtained.

$$k_{\rm obsd} = \frac{k_2' K_{\rm a} + k_{\rm OH}' K_{\rm a} K_{\rm a}' / a_{\rm H}}{a_{\rm H} + K_{\rm a}}$$
[7]

$$k_{\rm obsd} = k_2' + \frac{k_{\rm OH}' K_a'}{a_{\rm H}}.$$
 [8]

The lower arm of the profile will have  $k_{obsd}$  given by

$$k_{\rm obsd} = \frac{k_2' K_{\rm a}}{a_{\rm H}}.$$
[9]

<sup>4</sup> The p $K_a$  values for acid ionization of the aquo complexes of the metal ions at 25°C are Cu<sup>2+</sup>, 6.8; Zn<sup>2+</sup>, 8.8; Co<sup>2+</sup>, 8.9; and Ni<sup>2+</sup>, 10.6 (36).

These equations assume a mechanism involving intramolecular attack of metal ion bound  $OH^-$  on the carbonyl carbon at high pH. However, at pH values below the  $pK_a$  of metal ion bound water, a kinetically equivalent reaction would be attack of external hydroxide ion on the metal ion complex **VII**. Equations [10] and [11] would then follow, so that at high pH Eq. [12] results.

$$k_{\rm obsd} = \frac{k_2' K_{\rm a} a_{\rm H} + k_{\rm OH} K_{\rm a} K_{\rm w}}{{a_{\rm H}}^2 + K_{\rm a} a_{\rm H}}$$
[10]

$$k_{\rm obsd} = \frac{k_2' K_{\rm a} + k_{\rm OH} K_{\rm a} [\rm OH^-]}{a_{\rm H} + K_{\rm a}}$$
[11]

$$k_{\rm obsd} = k_{\rm OH} \ [\rm OH^-].$$
 [12]

The values of  $k_{OH}$ , the second-order rate constant for hydroxide ion catalysis of the hydrolysis of the metal ion complex, are reported in Table 1. The lines in Figs. 1 and 2 for the metal ion-promoted reactions are theoretical for Eq. [11] and the values of the constants in Table 1.

Hydroxide ion catalysis also occurs in the hydrolysis of 2-(1,10-phenanthrolyl) acetate (V) as seen in Fig. 4. The value of  $k_{OH}$  at 30°C is 10.2 M<sup>-1</sup> sec<sup>-1</sup>, which is only slightly less than the  $k_{OH}$  found for **IV**. However, in contrast with **IV**, the reaction of **V** becomes slow at low pH. The divalent metal ions again bind strongly to the phenanthroline nitrogens of **V**. In Fig. 4 the plot of log  $k_{obsd}$  vs pH for hydrolysis of



**FIG. 4.** Plot of log  $k_{obsd}$  vs pH for the hydrolysis of 2-(1,10-phenanthrolyl) acetate in H<sub>2</sub>O at 30°C,  $\mu = 0.1$  M with KCl, in the presence of 0.003 M Cu<sup>2+</sup> ( $\bigcirc$ ) and 0.003 M Co<sup>2+</sup> ( $\bigcirc$ ) and in the absence of metal ion ( $\odot$ ).

the Cu(II) and Co(II) complexes of **V** is shown. Only metal ion-promoted OH<sup>-</sup> reactions are observed. The plots are linear with slopes of 1.0. The plot for hydrolysis of the Cu(II) complex is linear at pH values from 3 to 6.5 with  $k_{\rm OH} = 1.2 \times 10^6$  M<sup>-1</sup> s<sup>-1</sup>.

## DISCUSSION

Intramolecular nucleophilic carboxyl group participation occurs in the hydrolysis of several types of esters, including glutarate and phthalate monoesters (9-15,37). The "steric fit" of the carboxyl group to the carbonyl carbon is highly important in the nucleophilic reactions (10,15); nucleophilic attack via a 5-membered ring transition state is 230-fold more favorable than through a 6-membered ring (10). This difference is most likely due to the increased restriction of unfavorable rotational states in the ester reacting via a 5-membered ring (10,15).

The neighboring carboxylate anions of **III** and **IV** are sterically able to participate in the hydrolysis reactions as efficient nucleophiles. The large rate constants in the pH-independent reactions (pH 6–9) show convincingly that carboxyl participation is occurring. The anhydrides produced in these reactions hydrolyze rapidly (10,12).<sup>5</sup> Phthalic anhydride has been shown to be an intermediate in the hydrolysis of phthalate monoesters (*12*). The ease with which nucleophilic attack can take place might be expected to be greater with **III** than **IV** since a 5-membered ring transition state can be formed in the former case. The carboxyl group of III is rigidly held adjacent to the carbonyl group. Nevertheless, the rate constant  $k_2$  for the nucleophilic reaction of the anionic species of **III** (Eq. [3]) is 5-fold less than with **IV**. This is most likely a reflection of the more deactivated carbonyl group of the phthalate ester. Note that the  $k_{OH}$  values for **III** and **IV** are in the same ratio as the  $k_2$  values, while  $k_{OH}$  for **IV** and the acetate ester **V** are nearly the same.

The bell-shaped  $k_{obsd}$  vs pH profiles at low pH indicate that the neutral species of **III** and **IV** also undergo a rapid reaction. It is likely that the kinetically equivalent zwitterion is the reactive species (ionized carboxyl group and protonated phenanthroline nitrogen) since the carboxyl anion would be a better nucleophile than the neutral species and the protonated phenanthroline would provide a better leaving group (**IX**) (Scheme 3). Again,  $k_1$  is larger in the case of the glutarate ester than with **III**.

*Metal ion catalysis.* Metal ion effects in the intramolecular nucleophilic reactions of esters with good leaving groups have previously been undetectable; the metal ion-promoted OH<sup>-</sup> reaction is the favored pathway even at low pH in the hydrolysis of phenolic esters (23). When the leaving group is an aliphatic alcohol, a complexed metal ion can provide assistance for breakdown of a tetrahedral intermediate to products (24,25). With a poor leaving group the OH<sup>-</sup> pathway is relatively less favorable, which then allows other mechanisms to be observable. In none of the previously studied nucleophilic reactions has it been sterically possible for the metal ion to complex favorably with the carbonyl oxygen. Such complexation should further increase the efficiency of the metal ion-promoted OH<sup>-</sup> pathway but might also greatly

 $<sup>^5</sup>$  The rate constant for pH-independent hydrolysis of phthalic anhydride in H<sub>2</sub>O at 30°C has been reported as 1.2  $\times$  10<sup>-2</sup> s<sup>-1</sup> (12).



**SCHEME 3** 

increase the efficiency of the carboxyl nucleophilic reaction. The present study shows that this is indeed the case.

The divalent metal ions,  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Zn^{2+}$ ,  $Co^{2+}$ , bind very strongly to **III–V**. A stable 6-membered chelate ring (**VI–VIII**) can be formed with complexation of the carbonyl oxygen. Enhancements of 10<sup>5</sup>- to 10<sup>8</sup>-fold are obtained for  $k_{OH}$  in the apparent OH<sup>-</sup> reactions of the 1:1 complexes of **III** and **IV** as compared with the reactions in the absence of metal ions. The reactions could involve either an internal attack of metal ion bound OH<sup>-</sup> (**X**) or the attack of external OH<sup>-</sup> (**XI**) on the metal ion complex (Scheme 4). The hydrolysis of the metal ion complexes of *N*-acylimidazoles must involve a reaction of metal ion bound OH<sup>-</sup> because the alternative external attack analogous to **XI** demands a second-order rate constant greater than that of a diffusion-controlled reaction (*30*). Cupric ion is the most effective promoter of the hydrolysis reactions of **III–V**, while Co<sup>2+</sup> is least effective in the series of metal ions, although Co<sup>2+</sup> is still capable of producing a sizable rate enhancement.

At pH < 7 the metal ion-promoted reactions of **III** and **IV** become pH independent. Subsequently, the profiles of Figs. 1 and 2 break downward to give slopes of 1.0. These reactions are clearly dependent on the neighboring carboxyl group; at lower pH (< 6) the only proton that can dissociate from the metal ion complex is that of



the carboxyl group. Note that the Cu<sup>2+</sup>-promoted hydrolysis of the acetate ester V has a log  $k_{obsd}$  vs pH profile that is linear with a slope of 1.0 at pH values as low as 3.5. The  $pK_{app}$  values in the reactions of **III** and **IV** are well below the  $pK_a$  of metal ion-bound water (*36*). At higher pH the plots of log  $k_{obsd}$  vs pH are linear to pH values above 7; the water  $pK_a$  values must then be still higher. Since the metal ion saturation is maintained at low pH values, as shown by the plots of  $k_{obsd}$  vs  $[M^{2+}]$ , the pH-independent reaction and subsequent downward bend of the log  $k_{obsd}$  vs pH profile are due to the ionization of the neighboring carboxyl group.

The observed metal ion-promoted reactions in Figs. 1 and 2 could be influenced by the changing inductive effect of the carboxyl group produced by ionization. An un-ionized carboxyl group is more electron withdrawing than a carboxylate anion, which would lead to greater reactivity for the un-ionized species in a OH<sup>-</sup> reaction. Therefore, the reactions might be considered as OH<sup>-</sup> reactions of the ionized and un-ionized species with the near pH-independent regions of the profiles representing a transition between these reactions. However, with the glutarate monoester **IV** the carboxyl inductive effect must be transmitted through three saturated carbon atoms and would therefore be small. On that basis it would be predicted that there would be a wider separation of the rate constants  $k_{OH}$  for the reactions of the two species with **III** than **IV**; but the opposite is the case. It can be seen in Fig. 2 that the near pH-independent reaction of the Co(II) complex of **IV** extends over 2 pH units, which is clearly more than can be accounted for by a small change in the inductive effect. Thus, the metal ion-promoted reactions at pH < 6 involve participation by the carboxylate anion.

A nucleophilic reaction (**XII**) is analogous to the reactions in the absence of metal ions (Scheme 5). The enhancements in  $k_2'$  in the carboxyl nucleophilic reaction provided by the complexed metal ions range from  $10^2$ - to  $10^3$ -fold. A metal ionpromoted carboxyl group reaction can therefore be competitive with the metal ionpromoted OH<sup>-</sup> reaction at pH < 6. The rate constants  $k_2'$  for reactions of the Cu(II) and Ni(II) complexes of **III** are sufficiently large that they are comparable to the rate constant for the pH-independent hydrolysis of phthalic anhydride (12).<sup>5</sup>

The apparent  $pK_a$  values of 4.2–4.4 in the hydrolysis of the metal ion complexes



**SCHEME 5** 

of the glutarate ester are in accord with the expected  $pK_a$  of the  $\gamma$ -carboxyl group. However, the apparent  $pK_a$  values found in the hydrolysis of the metal ion complexes of the phthalate ester (4.3–5.1) are higher than expected and show some dependence on the identity of the metal ion. The measured  $pK_a$  of monomethyl phthalate (30 °C) is 3.32 (12), and that of phthalic acid monoamide is 3.75 (38). Any direct interaction of the carboxyl group with the metal ion should stabilize the carboxylate anion and therefore reduce the  $pK_a$ . Likewise, a very favorable equilibrium prior to the ratelimiting step would reduce the apparent  $pK_a$  (39). The high  $pK_{app}$  values for **III** could reflect either a possible hydrogen bonding interaction of the carboxyl group with metal ion-bound water that stabilizes the proton on the carboxyl oxygen, or proximity of the carboxyl to the negative charge of the partially ionized metal ion bound water. On the other hand, in the stable extended conformation of the glutarate monoester, the carboxyl group can point out into the solvent (15).

A change in rate-determining step can also give rise to a downward bend in a pH rate constant profile but is unlikely in the reactions of **III** and **IV**. Breakdown of a tetrahedral intermediate should be rate determining in the scheme of Eq. [13] at all pH in view of the greater ease of the reverse step than expulsion of the leaving group to give products. The steady-state equation for  $k_{obsd}$  in the neighboring carboxyl group reaction at metal ion saturation is Eq. [14],



$$k_{\rm obsd} = \frac{k_{\rm f} k_{\rm p}}{k_{\rm r} + k_{\rm p}} \left[ \frac{K_{\rm a}}{K_{\rm a} + a_{\rm H}} \right],$$
[14]

where  $k_{\rm f}$ ,  $k_{\rm r}$ , and  $k_{\rm p}$  are the rate constants for the formation of a tetrahedral intermediate and the decomposition of the intermediate to reactants and products, respectively. Release of a carboxylate anion from the tetrahedral intermediate to regenerate the reactant should be relatively favorable. Therefore, if as expected, the rate constant  $k_{\rm r} > k_{\rm p}$ , then  $k_{\rm p}$  is the rate constant of the rate-determining step. Breakdown to products would be more difficult than is usually the case because it would involve breaking a stable 6-membered chelate ring. A high  $pK_{\rm app}$  is then best explained by an environmentally perturbed  $pK_{\rm a}$  for the carboxyl group of **III** in the metal ion complex.

A metal ion complexed to the carbonyl oxygen will greatly facilitate the nucleophilic attack step but will inhibit breakdown of a tetrahedral intermediate to products by restricting electron release from oxygen (Eq. [13]). Large rate enhancements are

achieved by metal ion complexation despite this necessary compromise of effects. The opposition of metal ion effects on the two steps of the reaction explain in part the large difference in catalytic efficiency for a chelated metal ion in the  $OH^-$  and carboxyl group-promoted reactions; the  $OH^-$  reaction should proceed with rate-determining nucleophilic attack, whereas the carboxyl group reaction should have rate-determining breakdown of the tetrahedral intermediate to products.

The metal ion-promoted carboxyl nucleophilic reactions of **III** and **IV** are the most favorable intramolecular carboxyl group reactions that have been observed. The reactions are general examples of metal ion promotion in intramolecular reactions where the metal ion can favorably complex the carbonyl group oxygen. The log rate constant pH profiles of Figs. 1 and 2 show that both of the possible mechanisms, metal ion-promoted OH<sup>-</sup> catalysis and metal ion-promoted carboxyl group attack, are represented on a single profile, and this is because of the increased favorability of the carboxyl group reaction. The former mechanism is predominant at pH > 6, and the latter at pH < 6.

Carboxypeptidase A. The pH-rate constant profiles for the hydrolysis of the metal ion complexes of **III** and **IV** are similar in shape to the plots of  $k_{cat}$  vs pH obtained in the carboxypeptidase A (CPA)-catalyzed hydrolysis of cinnamoyl-L,  $\beta$ -phenyllactate (16,17). The ascending arm of those plots occurs at pH > 8.5, i.e.,  $\sim 2$  pH units higher than with **III** and **IV**. The sigmoidal portion of the  $k_{cat}$  vs pH plot at lower pH has a  $pK_{app}$  of 6.2, which may be the  $pK_a$  of the  $\gamma$ -carboxyl group of Glu-270. The pH-independent value of  $k_{cat}$  in the enzymatic reaction (195 s<sup>-1</sup> at 30 °C) (16) is only ~500-fold larger than  $k_2'$  with III and IV. However, in contrast with the excellent leaving group of **III** and **IV**, the leaving group alcohol of the  $\beta$ -phenyllactate ester is of much higher  $pK_a$ ; that  $pK_a$  was calculated to be 14.7 (40). Therefore, the enzymatic reaction is considerably more favorable than the metal ion-promoted carboxyl nucleophilic reactions of III and IV.<sup>6</sup> The steric fit of the carboxyl group to the reaction center of the phthalate ester III would be difficult to improve upon. The rate constants  $k_2'$  for III and IV are then close to maximal for metal ion promoted carboxyl group participation in the reactions of esters having a phenanthrolyl liganding group. A simple Glu-270 nucleophilic mechanism in the CPA-catalyzed hydrolysis of esters can then be considered doubtful on chemical grounds. Metal ion-promoted  $OH^-$  catalysis, as with III and IV at pH > 6, is the only mechanism found to date that is capable of generating rate constants of the magnitude obtained in the CPA reactions. Thus, a reasonable mechanism for the enzyme reaction would be XIII (8,41) (Scheme 6). Mechanism XIII is kinetically equivalent to a mechanism in which the Glu-270 anion partially abstracts a proton from a metal ion bound water molecule in the transition state, but XIII employs the better nucleophile. The kinetically equivalent carboxylate anion general base mechanism is not likely since there should be a significant concentration of Zn(II)-OH<sup>-</sup> at pH values from 6 to 9. Even if the concentration was 1% at pH 7 (assuming a pKa of metal ion bound water of 9), the reaction should go 100% via the ionized species because of the great nucleophilicity.

<sup>&</sup>lt;sup>6</sup> When the leaving group is a phenol with a carbonate diester substrate for CPA, the values of  $k_{cat}$  are reduced (40).



## **SCHEME 6**

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