# Metal Ion Effects on Intramolecular General Base and Nucleophilic Carboxyl Group Participation in Ester Hydrolysis. Hydrolysis of Salicyl Phenanthroline-2-carboxylate and 8-(2-Carboxyquinolyl) Hydrogen Glutarate

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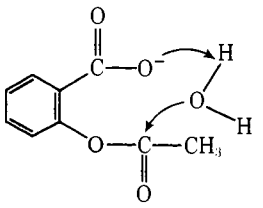
**Abstract:** A plot of log $k_{obsd}$ vs. pH for hydrolysis of salicyl phenanthroline-2-carboxylate at 50 °C has a pH-independent region from pH 5 to 7, indicating participation by the carboxylate anion or a kinetically equivalent reaction ($k_{H_2O}/k_{D_2O}$ = 1.9). Plots of log $k_{obsd}$ vs. pH for hydrolysis in the presence of divalent metal ions ($Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, and $Cu^{2+}$) in the pH range 2–7 are linear with slopes of 1.0. Saturating concentrations ($5 \times 10^{-3}$ M) of the divalent metal ions produce enhancements in $k_{OH}$, the second-order rate constant for hydroxide ion catalysis, ranging from $3 \times 10^3$ with $Zn^{2+}$ to $8 \times 10^4$ with $Cu^{2+}$. The order of hydrolytic reactivity of the metal ion complexes is Cu(II) > Ni(II) > Co(II) > Zn(II), which is also the order of the equilibrium constants for binding of metal ion to 1,10-phenanthroline. The reactions involving metal ion promoted attack of hydroxide ion on the ester carbonyl proceed with such facility that mechanisms utilizing carboxyl group general base participation cannot compete. The pH-log (rate constant) profile for hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate at 30 °C has a bell-shaped region at pH 2–7 due to intramolecular nucleophilic attack by the glutarate carboxyl of the zwitterionic species. From pH 7 to 9 there is a plateau due to a nucleophilic reaction of the dianionic species, and at pH greater than 9 hydroxide ion catalysis occurs. In the presence of 0.01 M $Co^{2+}$ and $Ni^{2+}$ (nonsaturating) and 0.01 M $Zn^{2+}$ and 0.001 M $Cu^{2+}$ (saturating) the pH-log (rate constant) profiles show hydroxide ion catalysis with enhancements in $k_{OH}$ ranging from $2 \times 10^6$ ($Ni^{2+}$) to $4 \times 10^7$ ($Cu^{2+}$). The order of reactivity of the metal ion complexes is $Cu^{2+} > Zn^{2+} > Co^{2+} \sim Ni^{2+}$, again paralleling the order of the equilibrium constants for metal ion binding, although differences in reactivity within the series are small. Upper limits for rate enhancements due to metal ion catalysis of the carboxylate anion nucleophilic reaction are $10^2$ with $Ni^{2+}$ and $Co^{2+}$ and $10^3$ with $Cu^{2+}$ and $Zn^{2+}$. These enhancements could arise from a transition-state effect in which the leaving group is stabilized. However, it is likely that the observed reactions only involve metal ion facilitated $^-OH$ catalysis at all pH values. Thus, an intramolecular nucleophilic reaction can also be overcome by a metal ion promoted $^-OH$ reaction when the nucleophilic reaction does not proceed with maximum efficiency because of the presence of degrees of freedom for rotation of the carboxyl away from the carbonyl. For the occurrence of significant intramolecular nucleophilic attack by a neighboring carboxyl group in systems in which a metal ion is strongly chelated, the steric fit of the carboxyl and the carbonyl must be excellent. The mechanistic implications of these results for carboxypeptidase A catalyzed hydrolysis of esters are discussed.

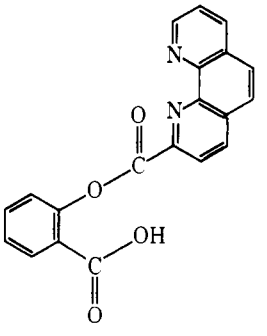
Carboxypeptidase A has Zn(II) chelated at the active site.[1-3] The metal ion presumably complexes the carbonyl of ester and amide substrates. It was suggested that Zn(II) might polarize the substrate carbonyl thereby increasing its susceptibility to nucleophilic attack. General base and nucleophilic mechanisms involving Glu-270 have been proposed as well as nucleophilic attack by Zn(II)-coordinated hydroxide ion. Consequently, studies of metal ion effects in the hydrolysis of esters and amides having neighboring carboxyl groups capable of participating in the reaction are of great relevance to an understanding of the mechanism of action of the enzyme.

The carboxyl group has perhaps been more extensively studied as an intramolecular participant in reactions of esters and amides than any other functional group.[4-20] Fersht and Kirby have presented convincing evidence that the mechanism of hydrolysis of aspirin involves classical general base catalysis by the neighboring carboxyl group.[17,18] A suitably located

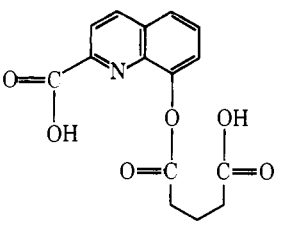


carboxyl group can also act as an intramolecular nucleophile, and large rate enhancements have been observed due to such participation in ester hydrolysis reactions[4-8] including those of glutarate monoesters.[19,20]

Numerous studies have been made of metal ion effects in hydrolysis reactions of esters.[21-23] However, there have been no previous reports of intramolecular systems where participation by a neighboring carboxyl group is possible in cases where the metal ion is strongly bound and a correlation can be drawn between stability of the metal ion complexes and resultant catalytic effects. In order to ascertain the effect of metal ions on intramolecular carboxyl group catalyzed ester hydrolysis we have studied the hydrolysis of salicyl phenanthroline-2-carboxylate (I) and 8-(2-carboxyquinolyl) hydrogen glutarate (II). It would be expected that carboxyl group participation would occur in hydrolysis of I by a general base mechanism analogous to that for aspirin, and in hydrolysis of II by a nucleophilic mechanism via an anhydride intermediate. In both cases a metal ion can be strongly chelated close to the ester carbonyl but in a position in which chelation of the participating carboxyl group would be sterically difficult in a 1:1



I



II

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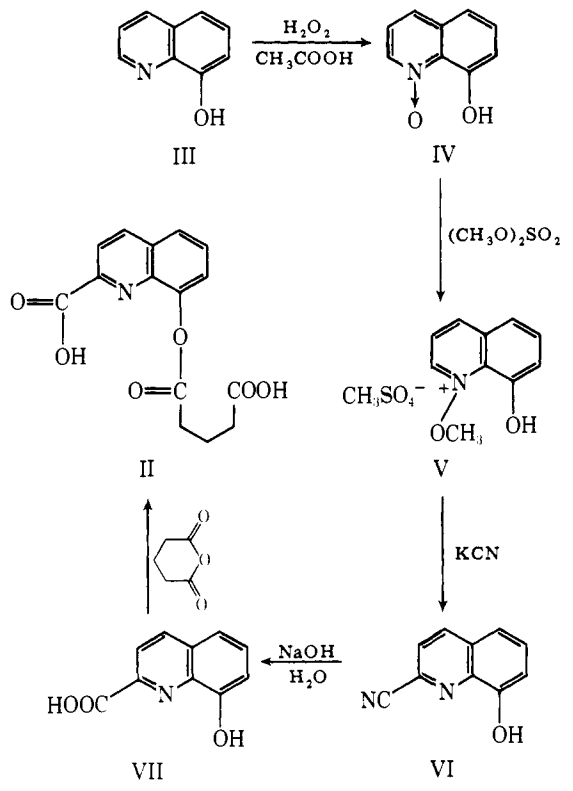
Scheme I




complex. The relative efficiencies of carboxyl group participation and metal ion facilitated $^-OH$ catalysis can thereby be determined with esters where metal ion binding is strong. Divalent metal ions give rise to large enhancements in the second-order rate constant for hydroxide ion catalyzed hydrolysis of 8-quinolyl hydrogen glutarate.[23] The carboxyl group in the 2 position of II is sterically not capable of direct participation in the reaction but serves as an additional chelating group for a metal ion so that the equilibrium constant for metal ion binding is much larger than in the case of the unsubstituted ester.

## Experimental Section

**Materials. Salicyl Phenanthroline-2-carboxylate (I).** One equivalent of 2-phenanthrolinecarboxylic acid chloride hydrochloride, which was prepared using a previously reported reaction sequence,[24,25] was suspended in 50 mL of ether with stirring. An ether solution containing 1 equiv of salicylic acid (Mallinckrodt) and 2 equiv of freshly distilled triethylamine (Matheson) was added dropwise over a 30-min period. The reaction vessel was fitted with a drying tube, and the mixture was allowed to stir overnight at room temperature. The resulting suspension was suction filtered, and the solid was resuspended in chloroform. The undissolved white solid was recovered, recrystallized from ethanol, and vacuum dried in an Abderhalden apparatus over calcium sulfate. The compound had mp 146–147 °C. Anal. Calcd for $C_{20}H_{12}O_4N_2 \cdot H_2O$: C, 66.30; H, 3.89; N, 7.73. Found: C, 66.47; H, 4.02; N, 7.34.

**8-(2-Carboxyquinolyl) hydrogen glutarate (II)** was prepared as outlined in Scheme I. 8-Hydroxyquinoline (III) was converted to the *N*-oxide (IV) by the procedure of Ramaiah and Srinivasan[26] (mp 138–139 °C, lit.[26] 139 °C) with the following modification. The acetic acid–peroxide–quinoline mixture was not concentrated in vacuo but was cooled in ice and neutralized by the slow addition of a saturated solution of ice-cold potassium carbonate. The 8-hydroxyquinoline *N*-oxide was converted to the methyl sulfate salt (V) according to the method of Krasavin et al. [27] 2-Cyano-8-hydroxyquinoline (VI) was synthesized from the methyl sulfate salt by the method of Clark and Hay[28] (mp 133 °C, lit.[28] 135 °C), and the cyano derivative was hydrolyzed to 8-hydroxyquinoline-2-carboxylic acid following the procedure of Hay and Clark[29] (mp 214–215 °C, lit.[29] 217–218 °C). 8-(2-Carboxyquinolyl) hydrogen glutarate (II) was synthesized by refluxing an equimolar mixture of glutaric anhydride (recrystallized from ether) and 8-hydroxyquinoline-2-carboxylic acid overnight in tetrahydropyran which had been dried over lithium aluminum hydride and freshly distilled prior to use. The solvent was removed by rotary evaporation, and the solid residue was extracted with dry benzene. The benzene was removed by rotary evaporation, and the residue was dissolved in chloroform. Hexane was added until the solution became slightly cloudy, and pale yellow crystals were recovered after standing in the freezer overnight, mp 96 °C dec. The ester is hygroscopic and decomposes upon standing in air. Anal. Calcd for $C_{15}H_{13}NO_6 \cdot H_2O$: C, 56.08; H, 4.67; N, 4.36. Found: C, 56.23; H, 4.50; N, 4.58. The infrared spectrum (KBr pellet) had strong absorbance at 1750 (ester C=O), 1710 and 1685 (acid C=O), 1210, and 1135 $cm^{-1}$.

**Kinetic Methods.** Stock solutions of I ($4 \times 10^{-3}$ M) were made up in methanol. In studies employing a Beckman Model 25 spectrophotometer, 25 $\mu$L of the substrate stock solution was injected into a reaction cuvette containing 2 mL of buffer, and the reaction was monitored at 285 nm after stirring. The spectrum of the solution upon completion of the reaction was invariably both qualitatively and quantitatively that of equivalent concentrations of the appropriate acid and alcohol. Temperature was controlled at 50 ± 0.1 °C, and the ionic strength of the buffers was kept constant at 0.5 M with either KCl or $LiClO_4$. The rates of hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate were measured at 30 °C, $\mu$ = 0.1 (maintained with $LiClO_4$). In the nonmetal ion studies, the reaction was monitored by following appearance of phenol at 254 (pH 1–9) and 268 nm (pH 9–12). The metal ion catalyzed reactions were followed at 254 nm with a few determinations at 268 nm with $Ni^{2+}$ and $Co^{2+}$. Noncomplexing buffers were employed whenever possible ($HClO_4$, cacodylate, and *N*-methylmorpholine). At pH values where it was necessary to employ carboxylic acid buffers no corrections were made for metal ion binding to buffer. Low concentrations of total buffer were routinely utilized at pH >3 (0.05 and 0.02 M with I and II, respectively). In reactions of II 3–5 $\mu$L of a $10^{-2}$ M acetonitrile stock solution (kept at −10 °C when not in use) was injected into 2 mL of reactant solution maintained at 30 °C. A spectrum of the solution taken after the reaction was complete was always identical with that of the product phenol or metal ion–phenol complex.

Reactions that were too rapid to be monitored with a conventional spectrophotometer (metal ion complexation of I and metal ion catalyzed hydrolysis of I at 50 °C and II at 30 °C) were followed using a Durrum-Gibson stopped-flow spectrophotometer (Model D110). The substrate (I) was dissolved at the desired concentration in $10^{-2}$ M $HClO_4$, $\mu$ = 0.5 ($LiClO_4$). This solution was introduced into one of two identical drive syringes. The other syringe contained the appropriate buffer, maintained at the same ionic strength with $LiClO_4$, and the chosen metal ion ($Co^{2+}$, $Cu^{2+}$, $Ni^{2+}$, or $Zn^{2+}$ perchlorate). In stopped-flow studies of II, one drive syringe of the stopped-flow apparatus contained 20 $\mu$L of the ester stock solution diluted to 20 mL with 0.10 M lithium perchlorate and 0.001 M Tris buffer (pH about 8.5). The other drive syringe contained the metal ion and reaction buffer. The drive syringes, mixing chamber, and cuvette were suspended in a water trough whose temperature was maintained at 30 ± 0.1 or 50 ± 0.1 °C. Optical density changes after mixing were recorded on a Hewlett-Packard storage oscilloscope (Model 1207B). With each buffer, three to four reactions were tabulated. Pseudo-first-order rate constants were calculated with an IBM 360 computer.

To avoid trace metal ion contamination in buffers the following precautions were taken. Deionized water was used throughout. Plastic labware was used whenever possible. Buffer solutions were extracted with a 0.001 M solution of dithizone in carbon tetrachloride[30] to remove metal ion from the buffer itself and salts used to maintain ionic strength. Spot checks of $k_{obsd}$ values were made using the same buffer plus $10^{-5}$ M EDTA, and values of the rate constants were identical in the presence and absence of EDTA.

Good first-order kinetics were obtained for at least 3 half-lives in all cases, and the first-order rate constants and subsequent kinetic parameters were evaluated using a nonlinear least-squares program. Reaction pH values were obtained using a Radiometer Model 22 pH meter or a Beckman Model 3500 digital pH meter.

**Spectrophotometric Determination of p$K_a$ Values.** Ester I was not sufficiently soluble in $H_2O$ for accurate titrimetric determination of the p$K_a$ values.[31] However, it was found that changes in absorbance occurred with changes in pH so that a p$K_a$ value could be determined

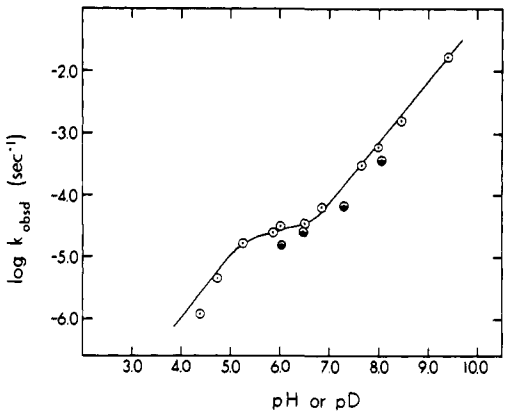



**Figure 1.** Plots of log $k_{obsd}$ vs. pH or pD for hydrolysis of salicyl phenanthroline-2-carboxylate at 50 °C and $\mu$ = 0.5 (with KCl) in $H_2O$ (⊙) and $D_2O$ (◒).

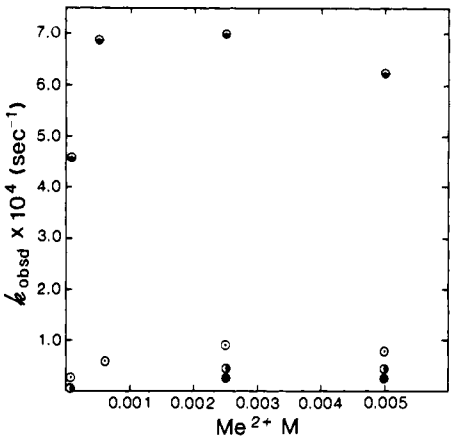



**Figure 2.** Plot of $k_{obsd}$ for hydrolysis of salicyl phenanthroline-2-carboxylate at 50 °C and pH 2.95 ($HClO_4$, $\mu$ = 0.5 with $LiClO_4$) vs. the concentration of metal ion: $Cu^{2+}$, ◒; $Ni^{2+}$, ⊙; $Co^{2+}$, ◐; $Zn^{2+}$, ●.

**Table I.** Second-Order Rate Constants for Hydroxide Ion Catalyzed Hydrolysis of Metal Ion Complexes of Salicyl Phenanthroline-2-carboxylate[a] at 50 °C ($\mu$ = 0.5 M with $LiClO_4$)

| metal ion | $k_{OH} \times 10^{-5}$ M$^{-1}$ s$^{-1}$ [b] | $k_{rel}$ | $k_{OD} \times 10^{-5}$ M$^{-1}$ s$^{-1}$ [b,c] |
|---|---|---|---|
| none | 0.001 | 1.0 | 0.005 |
| $Zn^{2+}$ | 3.10 | 3 100 | |
| $Co^{2+}$ | 6.83 | 6 800 | |
| $Ni^{2+}$ | 15.8 | 15 800 | 44.7 |
| $Cu^{2+}$ | 79.4 | 79 000 | |

[a] Ester concentration was $5 \times 10^{-5}$ M. Metal ion concentration was $5 \times 10^{-3}$ M. [b] In calculating second-order rate constants at 50 °C the ion product of water $K_w$ and $K_{D_2O}$ were taken to be $5.50 \times 10^{-14}$ and $7.94 \times 10^{-15}$, respectively: R. C. Weast, Ed., "Handbook of Chemistry and Physics", 54th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1973, p D131. [c] In calculating pD at 50 °C, the glass electrode correction formula of Fife and Bruice was employed: T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961).

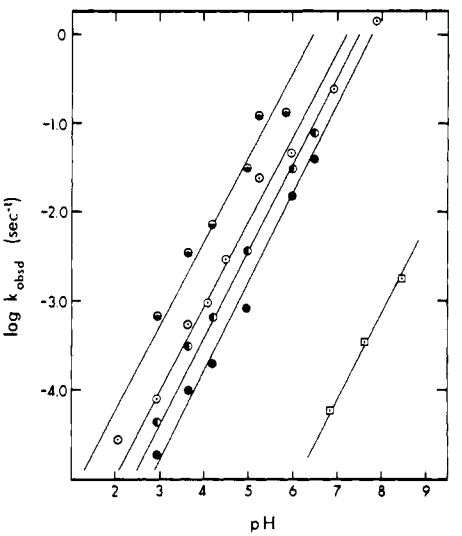



**Figure 3.** Plot of log $k_{obsd}$ vs. pH for hydrolysis of salicyl phenanthroline-2-carboxylate at 50 °C and $\mu$ = 0.5 (with $LiClO_4$) in the presence of $5 \times 10^{-3}$ M metal ion: $Cu^{2+}$, ◒; $Ni^{2+}$, ⊙; $Co^{2+}$, ◐; $Zn^{2+}$, ●; in the absence of added metal ion, ⊡.

spectrophotometrically. A series of buffer solutions was prepared to cover a pH range of 0–12, and the ionic strength was kept constant at 0.5 M with KCl. A $4 \times 10^{-3}$ M stock solution (25 $\mu$L) was injected into a cuvette containing 2 mL of buffer which had been thermally equilibrated at 50 ± 0.1 °C. After mixing, the absorption spectrum was taken using a Beckman Model 25 spectrophotometer. A plot of absorbance at the appropriate wavelength vs. pH was made. Ester I has one discernible p$K_a$ of 4.2 at 50 °C measured at 283 nm. Employing 50% dioxane–$H_2O$ (v/v) as the solvent, the p$K_a$ is 3.8. Thus, the value in $H_2O$ of 4.2 must be the p$K_a$ of the phenanthroline conjugate acid; the p$K_a$ of a carboxyl group should be 1.0–1.5 p$K_a$ units greater in 50% dioxane–$H_2O$ than in $H_2O$.[32] A p$K_a$ of 4.2 is quite reasonable for the phenanthroline conjugate acid; the p$K_a$ of the protonated species of 1,10-phenanthroline is 4.4 at 50 °C.[33]

## Results

Figure 1 presents a plot of log $k_{obsd}$ vs. pH or pD for hydrolysis of salicyl phenanthroline-2-carboxylate at 50 °C. The plot shows hydroxide ion catalysis ($k_{OH}$ = 100 M$^{-1}$ s$^{-1}$), a plateau at pH values less than 7, and decreasing rate constants as pH is decreased below pH 5. The data give a satisfactory fit to eq 1 in the pH range 4–10

$$k_{obsd} = \frac{K_a K_a'[k_2 + k_{OH}(OH^-)]}{a_H^2 + K_a a_H + K_a K_a'} \quad (1)$$

where $K_a'$ is the dissociation constant of the neutral species, $K_a$ is the dissociation constant of the conjugate acid, and $k_2$ is the rate constant for participation by the carboxylate anion when the nitrogen is unprotonated. The measured p$K_a$ of 4.2 must correspond with that of the phenanthroline conjugate acid. Assuming a p$K_a'$ of 5.3 to give the best fit to the experimental data, the rate constant $k_2$ for maximum participation by the carboxyl group is $3 \times 10^{-5}$ s$^{-1}$ in $H_2O$ and $1.6 \times 10^{-5}$ s$^{-1}$ in $D_2O$ ($k_2^{H_2O}/k_2^{D_2O}$ = 1.9 and $k_{OH}/k_{OD}$ is 0.2). Significant buffer catalysis was not observed in hydrolysis of I at the low total buffer concentration generally employed, but there was evidence that $k_{obsd}$ is increased at high buffer concentrations. For example, in cacodylate buffers at pH 6.05 $k_{obsd}$ is increased 55% in 0.2 M total buffer as compared with 0.05 M buffer. The second-order rate constant for general base catalysis by cacodylate is $3.8 \times 10^{-4}$ M$^{-1}$ s$^{-1}$.

Divalent metal ions ($Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$) produce a very large catalysis of the rate of hydrolysis of I at low concentrations of metal ion. A saturating effect of metal ion on the rate of hydrolysis is observed at metal ion concentrations close to $5 \times 10^{-4}$ M when the ester is at a concentration of $5 \times 10^{-5}$ M (Figure 2). In Figure 3 a plot is presented of log $k_{obsd}$ vs. pH for hydrolysis of I in the presence of $5 \times 10^{-3}$ M metal ion (100-fold excess). The plots are linear for the various metal ions, and the lines are drawn with slopes of 1.0. Thus, the reactions involve apparent hydroxide ion catalysis in the pH range 2–6.5. The second-order rate constants $k_{OH}$ are given in Table I. The enhancements in $k_{OH}$ produced by the metal ions range from $3 \times 10^3$ with $Zn^{2+}$ to $8 \times 10^4$ for $Cu^{2+}$. Metal ion catalyzed reactions could not be studied at higher pH values because of precipitation problems.

**Table II.** Rate Constants for Complexation of Metal Ions with Salicyl Phenanthroline-2-carboxylate[a] at 30 °C

| metal ion | metal ion concn, M × 10⁴ | pH | $k_{obsd}$, $s^{-1}$ | $k_d$,[b] $s^{-1}$ | $k_f \times 10^{-6}$,[c] $M^{-1} s^{-1}$ | $K_1 \times 10^6$,[c] M | lit.[d] $k_f \times 10^{-6}$ $M^{-1} s^{-1}$ | lit.[d] $k_d$, $s^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| $Ni^{2+}$ | 1.0 | 2.95 | 0.045 | 0.031 | 0.0009 | 34.4 | 0.0031 | 0.000 010 |
| | 5.0 | 2.95 | 0.041 | | | | | |
| | 15.0 | 2.95 | 0.117 | | | | | |
| | 25.0 | 2.95 | 0.155 | | | | | |
| | 37.5 | 2.95 | 0.21 | | | | | |
| $Cu^{2+}$ | 1.0 | 2.95 | 0.028 | 0.027[e] | | | 10.0 | 0.04 |
| | 5.0 | 2.95 | 0.038 | | | | | |
| | 15.0 | 2.95 | 0.038 | | | | | |

[a] Ester concentration was $5 \times 10^{-5}$ M, $\mu$ = 0.5 M with $LiClO_4$. [b] Intercept of a plot of $k_{obsd}$ vs. metal ion concentration: Me $phen^{2+} \rightarrow Me^{2+}$ + phen ($k_d$); $Me^{2+}$ + phen → Me $Phen^{2+}$ ($k_f$). [c] The $k_f$ value was calculated from the slope of a plot of $k_{obsd}$ vs. metal ion concentration employing the equation of R. S. Bell and N. Sutin, *Inorg. Chem.*, **1**, 359 (1962):

$$k_{obsd} = k_d(1 + K_a'(Me^{2+}))/K_1(H^+)$$

where $K_a'$ is the acid dissociation constant and $K_1$ is $k_d/k_f$. [d] Rate constants for 1,10-phenanthroline were calculated from the data of ref 41. [e] $Cu^{2+}$ rate constants indicate saturation at a very low metal ion to ester concentration ratio, so $k_d$ determination is not as accurate as values found for $Ni^{2+}$.

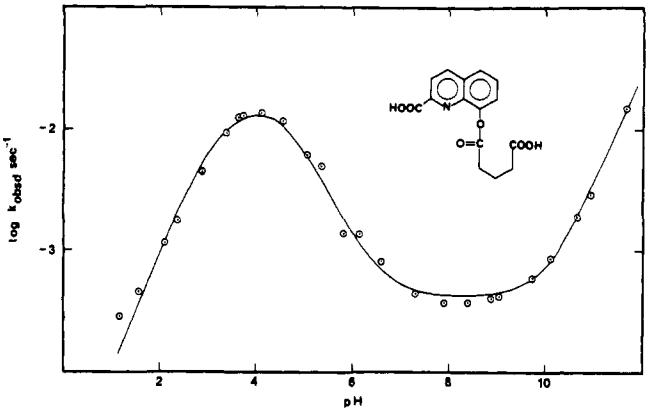



**Figure 4.** Plot of log $k_{obsd}$ vs. pH for hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate at 30 °C and $\mu$ = 0.1 (with $LiClO_4$) in $H_2O$.

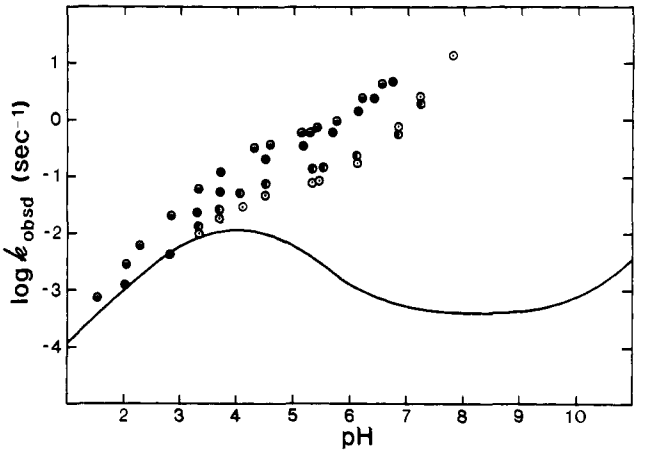



**Figure 5.** Plots of log $k_{obsd}$ vs. pH for hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate at 30 °C and $\mu$ = 0.1 (with $LiClO_4$) in the presence of 0.001 M $Cu^{2+}$ (⊖) (0.000 25 M at pH >5.40) and 0.01 M $Zn^{2+}$ (●), $Co^{2+}$ (⊙), or $Ni^{2+}$ (◐). The solid line is the theoretical profile for hydrolysis in the absence of metal ions.

The rates of complexation of $Ni^{2+}$ and $Cu^{2+}$ with I were measured spectrophotometrically at 270 nm at pH 2.95 (30 °C). Rate constants for these reactions are given in Table II. From the linear plot of $k_{obsd}$ vs. $Ni^{2+}$ concentration values of the rate constants for the formation ($k_f$) and dissociation ($k_d$) of the metal ion complex were obtained. The value of the equilibrium constant for binding of $Ni^{2+}$ ($k_f/k_d$) is $2.9 \times 10^4$ $M^{-1}$.

Figure 4 presents a plot of log $k_{obsd}$ vs. pH for hydrolysis of II at 30 °C in $H_2O$. In the equation

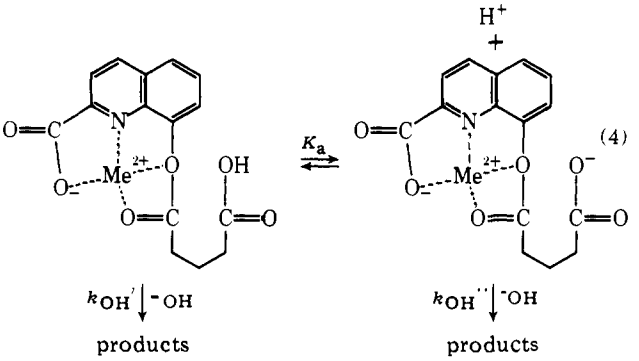
$$k_{obsd} = \frac{k_1K_1K_2a_H^2 + k_2K_1K_2K_3a_H + k_{OH}K_wK_1K_2K_3}{a_H^4 + K_1a_H^3 + K_1K_2a_H^2 + K_1K_2K_3a_H} \quad (2)$$

$K_1$, $K_2$, and $K_3$ are dissociation constants for the conjugate acid, neutral, and monoanionic species, $k_1$ is the rate constant associated with the monoanionic species, $k_2$ is the rate constant associated with the dianionic species, and $k_{OH}$ is the second-order rate constant for hydroxide ion catalyzed hydrolysis of the dianionic ester. When $a_H < K_1$ the equation simplifies to

$$k_{obsd} = \frac{k_1K_2a_H^2 + k_2K_2K_3a_H + k_{OH}K_wK_2K_3}{a_H^3 + K_2a_H^2 + K_2K_3a_H} \quad (3)$$

to which the data give a good fit. From the data $k_{OH}$ was determined to be 2.21 $M^{-1} s^{-1}$. The theoretical line in Figure 4 was calculated using this $k_{OH}$ value plus the best fit values $k_1$ ($1.73 \times 10^{-2} s^{-1}$), $k_2$ ($4.16 \times 10^{-4} s^{-1}$), $K_2$ ($5.89 \times 10^{-4}$ M), and $K_3$ ($1.69 \times 10^{-5}$ M).

Figure 5 is a plot of log $k_{obsd}$ vs. pH for hydrolysis of II in the presence of 0.01 M $Zn^{2+}$, $Ni^{2+}$, or $Co^{2+}$, or 0.001 M $Cu^{2+}$ ($2.5 \times 10^{-4}$ M at pH values greater than 5.40). The plots indicate hydroxide ion catalysis at both high (5.5-7.5) and low (2-4) pH with a small inflection in between due most likely to ionization of the glutaryl carboxyl group, i.e., the scheme of eq 4 is being followed. This scheme leads to




(4)

$$k_{obsd} = \frac{k_{OH}'K_wa_H + k_{OH}''K_wK_a}{a_H^2 + K_aa_H} \quad (5)$$

Values of the rate and equilibrium constants are given in Table

**Table III.** Rate and Equilibrium Constants Associated with Divalent Metal Ion Catalysis of the Hydrolysis of 8-(2-Carboxyquinolyl) Hydrogen Glutarate in $H_2O$ at 30 °C, $\mu$ = 0.1

| metal | $k_{OH'} \times 10^{-8}$, $M^{-1}\ s^{-1}$ | $k_{OH''} \times 10^{-8}$, $M^{-1}\ s^{-1}$ | $K_a \times 10^5$, M | $k_{rel}$[a] |
|---|---|---|---|---|
| none | | $2.21 \times 10^{-8}$ | | 1.0 |
| $Ni^{2+}$ [b] | 2.62 | 0.056 (0.096)[c] | 2.59 | $2.5 \times 10^6$ ($4.3 \times 10^6$)[c] |
| $Co^{2+}$ [b] | 2.01 | 0.087 (0.180)[c] | 5.08 | $3.9 \times 10^6$ ($8.1 \times 10^6$)[c] |
| $Zn^{2+}$ [c] | 6.00 | 0.551 | 2.51 | $2.5 \times 10^7$ |
| $Cu^{2+}$ [c] | 24.2 | 0.821 | 7.34 | $3.7 \times 10^7$ |

[a] $k_{OH''}$ in the presence of metal ion divided by $k_{OH}$ in the absence of metal ion. In calculating second-order rate constants at 30 °C, the ion product of water $K_w$ was taken to be $1.47 \times 10^{-14}$: R. C. Weast, Ed., "Handbook of Chemistry and Physics", 54th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1973, p D131. [b] At a nonsaturating concentration of metal ion (0.01 M). [c] At a saturating concentration of metal ion. Calculated in the case of $Ni^{2+}$ and $Co^{2+}$ employing the value of $K_{Me}$.

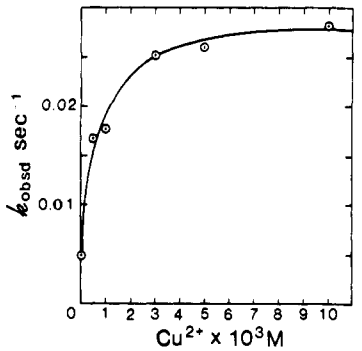



**Figure 6.** Plot of $k_{obsd}$ for hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate at 30 °C and pH 2.79 ($\mu$ = 0.1 with $LiClO_4$) vs. the concentration of $Cu^{2+}$.

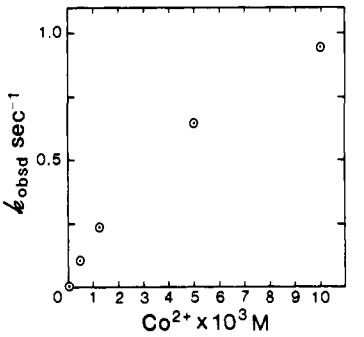



**Figure 7.** Plot of $k_{obsd}$ for hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate at 30 °C and pH 6.93 ($\mu$ = 0.1 with $LiClO_4$) vs. the concentration of $Co^{2+}$.

III. The enhancements in $k_{OH}$ produced by the metal ions range from $2.5 \times 10^6$ with $Ni^{2+}$ (0.01 M, nonsaturating) to $3.7 \times 10^7$ with $Cu^{2+}$ (0.001 M, saturating). Calculation of $k_{OH''}$ values for saturating concentrations of $Ni^{2+}$ and $Co^{2+}$ gives enhancements of $4.3 \times 10^6$ and $8.1 \times 10^6$, respectively. The concentrations employed of $Cu^{2+}$ and $Zn^{2+}$ were saturating. Figure 6 shows the dependence of the rate of hydrolysis on $Cu^{2+}$ concentration at pH 2.79 in 0.02 M chloroacetate buffer. Similar dilutions were carried out at pH 2.04 ($HClO_4$) and 5.29 (0.02 M cacodylate buffer). In the latter case a $2.5 \times 10^{-4}$ M concentration of $Cu^{2+}$ was saturating. The equilibrium constants for metal ion binding ($K_{Me}$) were calculated from eq 6, which holds in the pH range 2-4.

$$k_{obsd} = \frac{k_1 K_2 a_H + k_{OH'} K_w K_{Me} K_2 [Me^{2+}]}{a_H^2 + K_2 a_H (1 + K_{Me}[Me^{2+}])} \quad (6)$$

With $Ni^{2+}$ and $Co^{2+}$ at pH > 6, a 0.01 M concentration of metal ion is not saturating, as shown in Figure 7. Values of the equilibrium constants for metal ion binding were then calculated from eq 7 and are given in Table IV.

$$k_{obsd} = \frac{k_{Me} K_{Me} [Me^{2+}]}{(1 + K_{Me}[Me^{2+}])} \quad (7)$$

The limiting rate constant $k_{Me}$ at a saturating concentration of metal ion at a given pH is equal to $k_{OH} K_w / a_H$.

## Discussion

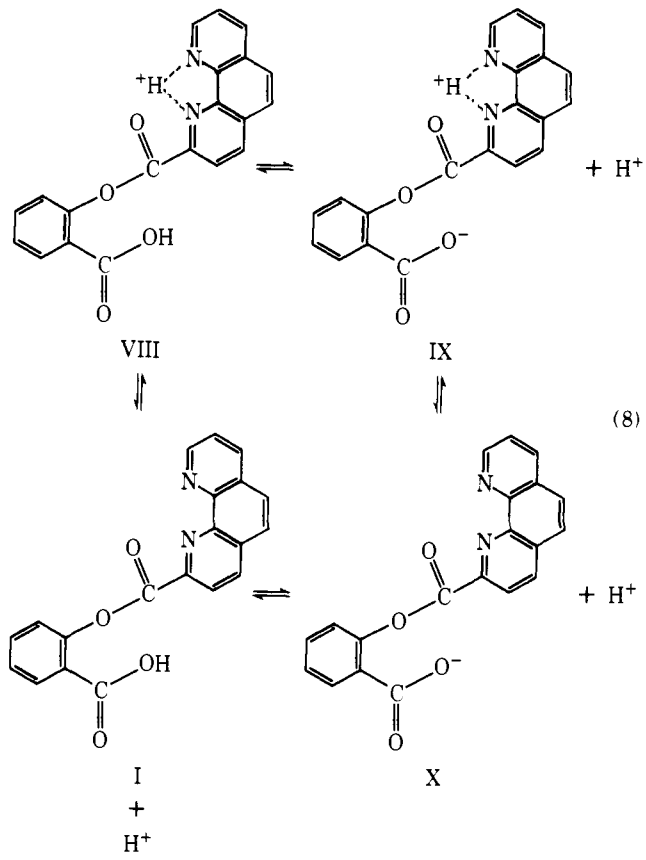
**Hydrolysis of Salicyl Phenanthroline-2-carboxylate (I).** There are four species that must be considered in the hydrolysis of salicyl phenanthroline-2-carboxylate. These are depicted in eq 8. Species I and IX are, of course, kinetically equivalent. At pH values greater than 7, hydroxide ion catalysis occurs ($k_{OH}$ = 100 $M^{-1}\ s^{-1}$ at 50 °C) in the hydrolytic reactions which must reflect reaction of the anionic species (X). The plateau in the pH-rate constant profile of Figure 1 from pH 7 to 5 could represent carboxyl group participation in the hydrolysis of X or a kinetically equivalent hydroxide ion catalyzed reaction of IX.

**Table IV.** Values of the Equilibrium Constants for Binding of Divalent Metal Ions to 8-(2-Carboxyquinolyl) Hydrogen Glutarate in $H_2O$ at 30 °C, $\mu$ = 0.1

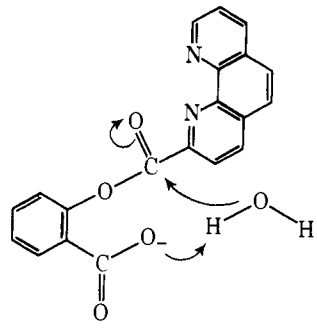
| metal ion | pH | $k_{Me}$, $s^{-1}$ | $K_{Me}$, $M^{-1}$ [a] |
|---|---|---|---|
| $Cu^{2+}$ | 2.05 | | $9.79 \times 10^3$ |
| | 2.79 | | $6.00 \times 10^3$ |
| $Zn^{2+}$ | 3.68 | | $8.93 \times 10^1$ |
| | 6.16 | 1.61 | $6.23 \times 10^2$ |
| $Ni^{2+}$ | 6.58 | 0.643 | $1.40 \times 10^2$ |
| $Co^{2+}$ | 6.93 | 1.98 | $9.68 \times 10^1$ |
| | 7.78 | 31.5 | $9.34 \times 10^1$ |

[a] Values of $K_{Me}$ at low pH refer to binding of metal ion to the monoanionic species of the substrate whereas values at pH >6 refer to binding to the dianion.

Fersht and Kirby[17] have argued persuasively that the plateau in the pH-rate constant profile for hydrolysis of acetylsalicylic acid represents general base catalysis by the carboxyl group rather than kinetically equivalent possibilities. A variety of experimental approaches were brought to bear on this problem.[17,18] It is reasonable that salicyl phenanthroline-2-carboxylate would hydrolyze through a similar mechanistic pathway. The magnitude of $k_{obsd}$ for the near pH-independent reaction at 50 °C ($3 \times 10^{-5}\ s^{-1}$) is similar to that for aspirin at 39 °C. Also the $D_2O$ solvent isotope effect ($k_{H_2O}/k_{D_2O}$ = 1.9) is consistent with a general base mechanism. As in the case of aspirin hydrolysis,[17] buffer anions have a small rate-accelerating effect. With cacodylate at pH 6.05, 0.2 M total buffer increases the rate 55%. In aspirin hydrolysis[17] the effect of acetate was very small (1 M buffer increased the rate only 12%), but the effect of phosphate was much greater. In view of the high $pK_a$ of the salicylate leaving group[34] it is probable that the weakly basic anions are acting as bimolecular general bases in these reactions. Even with such a reactive ester as *p*-nitrophenyl acetate, acetate ion functions as a general base.[35]




If buffer anions participate in the hydrolysis of aspirin and I as general bases, then the carboxyl substituent group should participate in an entropically favored intramolecular general-base-catalyzed reaction (XI). Involvement of a kinetically



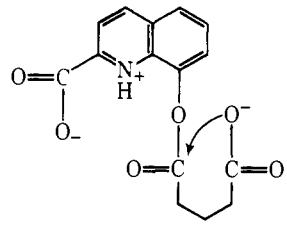
XI

equivalent mechanism (attack of $^{-}OH$ on IX) would, of course, be due to the fact that carboxyl group general base catalysis is not normally a highly favored mechanism in that it does not give rise to large rate enhancements in comparison with alternative pathways.[4-8]

Intramolecular general base catalysis by the quinoline ring nitrogen takes place in the hydrolysis of 8-acetoxyquinoline and phenolic esters of quinoline-8-carboxylic acid.[36,37] It is very unlikely that a similar reaction is contributing to the rate constants for hydrolysis of I. The apparent $pK_a$ of 5.3 is higher than the $pK_a$ of the phenanthroline conjugate acid (4.2). Secondly, the rate constants for ring nitrogen general base catalysis in the quinoline derivatives are small. The rate constant for pH-independent hydrolysis of *p*-nitrophenyl quinoline-8-carboxylate[36] is comparable to $k_2$ for I even though the leaving group of the quinoline ester has a $pK_a$ which is perhaps 3 $pK_a$ units less than that of the salicyl leaving group of I.[34] Furthermore, intramolecular general base catalysis is not observed in hydrolysis of phenyl picolinate,[37] which is sterically of greater similarity to I than the quinoline 8-carboxylate esters.

There are two significant differences in the pH-rate constant profiles for hydrolysis of salicyl phenanthroline-2-carboxylate (50 °C) and aspirin (39 °C). Hydroxide ion catalysis is approximately 100 times more favorable with the phenanthroline derivative. This has the effect of reducing the length of the plateau in the pH-rate constant profile by approximately 2 pH units in comparison with aspirin.[14] It is unlikely that trace metal ion contamination in the buffers is contributing to the observed rate constants in view of the precautions taken to avoid such contamination (see Experimental Section). A second difference is that, in contrast with aspirin, a hydronium ion catalyzed reaction is not observed in hydrolysis of salicyl phenanthroline-2-carboxylate. Even in 2 M HCl rates are too slow to accurately measure. This is very likely the result of a protonated phenanthroline nitrogen inhibiting protonation of the ester carbonyl.[38]

**8-(2-Carboxyquinolyl) Hydrogen Glutarate.** The pH-log (rate constant) profile for hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate (II) shown in Figure 4 is very similar both qualitatively and quantitatively to that for hydrolysis of 8-quinolyl hydrogen glutarate in the absence of metal ions.[23,39] This shows that the 2-carboxy substituent is exerting only a very small electronic effect on the hydrolysis reaction. The bell-shaped profile at pH 2-7 is very likely due to a nucleophilic reaction of a zwitterionic species where there is a proton on the quinoline nitrogen and the glutaryl carboxyl is ionized (XII).



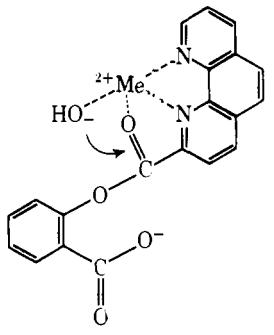
XII

A plateau in the profile from pH 7 to 9 is due to a nucleophilic reaction of the dianionic species (unprotonated quinoline nitrogen). At higher pH values hydroxide ion catalyzed hydrolysis occurs with a second-order rate constant $k_{OH}$ that is identical with that of 8-quinolyl hydrogen glutarate, showing that a carboxylate anion in the 2 position does not electrostatically inhibit approach of hydroxide ion. The large rate constants for hydrolysis of 8-quinolyl hydrogen glutarate and II show convincingly that nucleophilic participation is occurring. In comparison with hydrolysis of 8-acetoxyquinoline[39] or 2-carboxy-8-acetoxyquinoline[29] the rate enhancement is $10^2$-$10^3$.

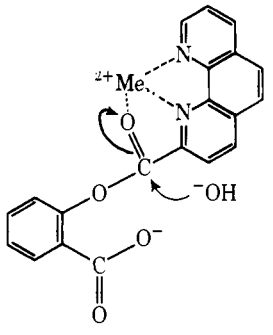
**Metal Ion Effects.** Divalent metal ions bind rapidly to salicyl phenanthroline-2-carboxylate. The rate constant $k_f$ for binding of $Ni^{2+}$ is similar to that for binding to 1,10-phenanthroline.[40,41] Thus, the ring nitrogens are undoubtedly chelating the metal ion. A rate constant for dissociation of the metal ion complex ($k_d$) is larger than for 1,10-phenanthroline and the equilibrium constant for complex formation ($k_f/k_d$) is smaller with the salicyl derivative showing that the metal ion complex is relatively less stable. In general, substituent groups in the 2 position of 1,10-phenanthroline appear to influence metal ion binding by affecting $k_d$ rather than $k_f$.[41] It would be expected that chelation of an additional group ($COO^-$) would confer added stability on the complex. Therefore, either the salicyl carboxyl group is not chelated or else it dissociates rapidly from the complex prior to the rate-determining step. Ring size (eight membered) would be very unfavorable for chelation of the carboxyl group.

There is pronounced catalysis of the hydrolysis of salicyl phenanthroline-2-carboxylate by low concentrations of divalent

metal ions ($Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$). The pH-rate constant profiles in the presence of metal ion are linear in the pH range 2-7 with slopes of 1.0, indicating hydroxide ion catalysis. At a saturating concentration of $5 \times 10^{-3}$ M metal ion (100-fold excess over ester), enhancements in the second-order rate constants $k_{OH}$ range from $3 \times 10^3$ with $Zn^{2+}$ to $8 \times 10^4$ with $Cu^{2+}$. Kinetically equivalent possibilities exist for metal ion catalysis (XIII and XIV).



XIII



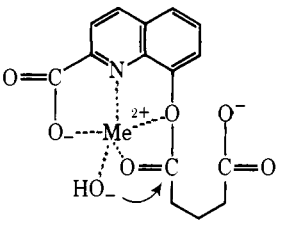
XIV

The order of reactivity of the metal ion complexes of I is Cu(II) > Ni(II) > Co(II) > Zn(II), which is also the order of the equilibrium constants for binding to 1,10-phenanthroline.[41] However, the difference in reactivity of the metal ion complexes is not large (25-fold), and even $Zn^{2+}$, which has a stability constant for binding to 1,10-phenanthroline[41] that is almost $10^3$ less than that for $Ni^{2+}$, gives a rate enhancement which is only fivefold less than that for $Ni^{2+}$. The rate enhancements of $10^3$-$10^5$ produced by saturating concentrations of divalent metal ions in hydrolysis of I are similar in magnitude to those obtained with nonsaturating concentrations (0.01 M, 100-fold excess over ester) in hydrolysis of 8-quinolyl hydrogen glutarate.[23] This may imply that the steric fit of metal-bound water (hydroxide ion) and the ester carbonyl of I is not optimal.

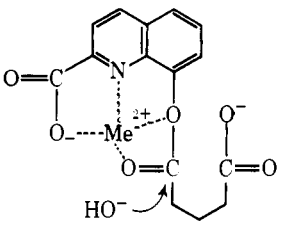
Evidence has been obtained previously that intramolecular attack of metal-bound hydroxide ion is capable of giving rise to large rate enhancements in the hydrolysis of chelated esters,[22] anhydrides,[42] and stable Co(III) complexes of esters and amides.[43] The rate of hydroxide ion catalyzed hydrolysis of the salicylic acid ester of pyridine-2,6-dicarboxylic acid is catalyzed $10^3$-fold by 0.2 M $Ni^{2+}$, but the stability constant of the metal ion complex was not ascertained.[44] A pH-independent reaction was observed in the presence of $Ni^{2+}$ as was also the case with the phenyl ester.[44]

It will be noted in Figure 3 that the pH-rate constant profiles for hydrolysis of I in the presence of metal ion are strictly linear in the pH range in which the neighboring carboxyl group is ionizing. Thus, the reaction rate is not dependent on the ionization state of the carboxyl group, i.e., the carboxyl group is not participating in the metal ion catalyzed reaction. Intramolecular general base catalysis of ester hydrolysis by a neighboring carboxyl group is therefore not capable of competing with metal ion promoted attack of hydroxide ion. If an ester with metal chelating groups can hydrolyze with both hydroxide ion catalysis and neighboring carboxyl group participation in the absence of metal ion, then whether or not carboxyl participation can be detected in the presence of metal ion will depend on the relative rate enhancements produced by metal ion in the two reactions. In view of the 3100-fold enhancement in $k_{OH}$ and the linear pH-log (rate constant) profile produced by $Zn^{2+}$, an upper limit of $\sim 10^2$ can be placed on any possible rate enhancement in the general base reaction. The actual enhancement might, of course, be much smaller. If the metal ion catalyzed reaction proceeds as in XIII there should be a large $\Delta S^{\ddagger}$ advantage compared to a general base reaction in which a water molecule attacks the metal ion complex. Nucleophilic attack by a carboxylate anion which does not suffer from such an entropic disadvantage, and for which rate enhancements are generally much larger than in general-base-catalyzed reactions, would be able to compete more effectively with metal ion promoted hydroxide ion catalysis.

Divalent metal ions $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$ bind strongly to II as shown by the equilibrium constants in Table IV.[45] This leads to saturation at low metal ion concentrations (0.01 M). It is unlikely that the glutaryl carboxyl is complexed to the metal ions in the 1:1 complexes in view of the similar equilibrium constants for metal ion binding to II and to 2-carboxy-8-acetoxyquinoline.[29] Likewise, in the metal ion complexes of the analogous ester 8-quinolyl hydrogen glutarate the carboxyl group cannot be chelated since the intramolecular nucleophilic reaction of the anionic species is unaffected by metal ions.[23] The pH-log (rate constant) profiles for hydrolysis of II in the presence of saturating concentrations of these metal ions show $^-OH$ catalysis. The slopes are 1.0 at high and low pH (as low as 2) with a small plateau in between. The rate enhancements at high pH in the hydroxide ion catalyzed reaction (Table III) are extremely large, ranging from $2 \times 10^6$ with $Ni^{2+}$ to $4 \times 10^7$ with $Cu^{2+}$. In the presence of a metal ion the mechanism of the hydrolysis reaction must involve intra- or intermolecular attack of hydroxide ion on the ester carbonyl group (XV or XVI).



XV



XVI

As was the case with I there is only a small dependence of $k_{OH}$ in the metal ion catalyzed reactions of II on the identity of the metal ion. The $pK_a$ values for a metal ion bound water molecule should differ considerably in this series.[46] Therefore, ease of ionization of the metal ion bound water molecule and the nucleophilic properties of the Me-OH species may nearly compensate in their effects on the rate constants. Likewise, metal ion $^-OH$-catalyzed hydrolysis of propionic anhydride is characterized by a Brønsted $\beta$ of 0.2.[47] The order of reactivity of the metal ion complexes of II is Cu(II) > Zn(II) > Co(II) ~ Ni(II) whereas that of I is Cu(II) > Ni(II) > Co(II) > Zn(II). In both cases the order is that of the respective binding constants. The rate constants would vary in this order if metal ion polarization of the ester carbonyl group, promoted by delocalization of d electrons into the phenanthroline or quinoline rings, were an important factor. The order of reactivity based on the $pK_a$ of metal ion bound water would be Ni(II) > Co(II) > Zn(II) > Cu(II). Thus, if both factors were contributing to the rate constants, then the observed orders of reactivity and the fairly small differences within the series could result. With the exception of Cu(II) the order for II is the reverse of that obtained with I, which must reflect N-O vs. N-N binding and different geometrical requirements.

The small inflections in the pH-log (rate constant) profiles of II are consistent with a changing inductive effect in the metal ion promoted $^-OH$ reaction due to ionization of the glutaryl carboxyl. However, metal ion enhancements in the intramolecular carboxyl group reaction (dianionic species) of $10^2$ with $Ni^{2+}$ and $Co^{2+}$ and $10^3$ with $Cu^{2+}$ and $Zn^{2+}$ would also lead to the same observed rate constants. These values then represent upper limits on metal ion catalysis in the nucleophilic re-

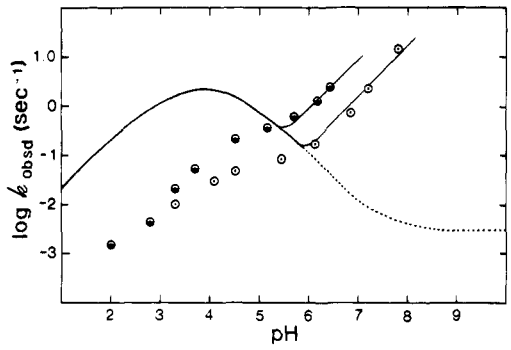



**Figure 8.** Plot of log $k_{obsd}$ vs. pH for hydrolysis of 8-quinolyl hydrogen succinate at 30 °C (data of ref 39) with the values of $k_{obsd}$ for hydrolysis of II in the presence of 0.01 M $Zn^{2+}$ (●) and $Co^{2+}$ (⊙) superimposed. The solid line is the resultant profile in the presence of metal ion. The dashed line represents the profile in the absence of metal ion.

action and might arise from a transition-state effect in which the leaving group is stabilized. Hay and Clark[29] found a rate enhancement of $2 \times 10^8$ in $Cu^{2+}$-promoted attack of $^-OH$ on 2-carboxy-8-acetoxyquinoline. If the $Cu^{2+}$-catalyzed reaction of II at low pH is considered to be an $^-OH$-catalyzed reaction the rate enhancement is only slightly larger ($10^9$), as would be expected with the different acyl group. Thus, any participation by the carboxyl group is contributing little to the observed rate constants.

It was ascertained in the hydrolysis of 8-quinolyl hydrogen glutarate that the carboxyl nucleophilic reaction is not significantly affected by the metal ion.[23] The upper limit for $Zn^{2+}$ catalysis in that reaction was only a factor of 20. The lack of metal ion catalysis of the nucleophilic reaction reflects the fact that the attack step is not rate limiting when the nucleophile (carboxylate anion) is much less basic than the leaving group. Polarization of the carbonyl group will then not be a significant factor since breakdown of a tetrahedral intermediate to products will be rate determining. The metal ions bind much more strongly to the product 8-hydroxyquinoline and presumably to the transition state than to the reactant, but marked transition-state effects are absent. A catalytic effect of metal ion through stabilization of the leaving group, of course, depends on the structure of the transition state, i.e., the amount of bond breaking. In the absence of such a transition state effect it is clear that chelated metal ions cannot effectively catalyze nucleophilic attack by a carboxylate anion in the hydrolysis of esters. On the other hand, hydroxide ion catalysis of the hydrolysis of phenolic esters involves rate-determining nucleophilic attack.[4] This is also very likely the case in the metal ion promoted $^-OH$ reactions of Zn(II), Co(II), Ni(II), and perhaps Cu(II), although with Cu(II) the $pK_a$ of metal-bound $H_2O$ should be 2-3 $pK_a$ units less than that of the leaving group. As a consequence, polarization of the carbonyl group by a chelated metal ion should lead to large rate enhancements as observed.

It is probable that carboxyl group participation in hydrolysis of II is not appreciably enhanced by divalent metal ions so that the nucleophilic reaction cannot compete with the metal ion promoted $^-OH$-catalyzed reaction. Although large rate enhancements are obtained in the intramolecular nucleophilic reactions of glutarate monoesters,[19,20] the steric fit of the carboxyl group and the carbonyl is far from maximal. Degrees of freedom exist, and the preferred conformation has the carboxyl pointing out into the solvent.[4,19] Removal of degrees of freedom, i.e., restricting the carboxyl in closer proximity to the carbonyl, would allow the intramolecular nucleophilic reaction to compete more effectively even if it was not catalyzed by metal ions. However, such restriction would have to be severe. Maugh and Bruice[39] determined the pH-rate constant profile for 8-quinolyl hydrogen succinate. Substitution of a carboxyl group into the 2 position of the succinate ester should have little effect on the pH-log (rate constant) profile as was the case for II in comparison with 8-quinolyl hydrogen glutarate. Reasonably assuming that values of $k_{OH}$ would be nearly the same in the presence of saturating concentrations of metal ions for II and the corresponding succinate ester, the pH-log (rate constant) profile for the $Zn^{2+}$-catalyzed reaction (Figure 8) is qualitatively very similar to that obtained in hydrolysis of 8-quinolyl hydrogen glutarate and to the plot of $k_{cat}$ vs. pH for carboxypeptidase A catalyzed hydrolysis of *O*-(*trans*-cinnamoyl)-L-β-phenyllactate and its chloro derivative.[48,49] Removal of a methylene group from II would produce only a small change in the inductive effect exerted by the carboxyl but would decrease the ease of rotation of the carboxyl away from the carbonyl and lead to a nucleophilic reaction 200-fold more favorable,[4,19] thereby allowing the nucleophilic attack by carboxyl to be predominant at pH <6. Thus, for intramolecular nucleophilic attack by a neighboring carboxyl group to occur in systems in which a metal ion can be chelated, either metal ion binding to the reactant must not lead to a rapid metal ion promoted $^-OH$ reaction (because of weak binding or steric factors) or else steric fit of the carboxyl and the carbonyl must be excellent.

**Carboxypeptidase A.** Various metal ions can be substituted for Zn(II) in carboxypeptidase A. Ester substrates apparently require the presence of metal ion for binding, whereas peptides do not, but metal ion is required for hydrolysis of both.[52,53] The Zn(II), Co(II), and Ni(II) enzymes are comparably active,[54] but the Cu(II) enzyme is inactive in both the esterase and peptidase reactions.[52,53] This is of interest since $Cu^{2+}$ gives a $10^5$-$10^9$-fold rate enhancement in reactions of I and II and is a superior catalyst in comparison with the other metal ions. X-ray crystallographic studies have shown that carboxypeptidase A bound Zn(II) has a distorted tetrahedral geometry.[1] However, the coordination geometries of Co(II), Ni(II), and Cu(II) carboxypeptidase A are five-coordinate (or possibly distorted tetrahedral), octahedral, and distorted tetrahedral, respectively.[55-57] It was suggested that the Cu(II) enzyme may be inactive because $Cu^{2+}$ binding induces a change in the active site through a shift of mechanistically important groups.[57] From the ability of $Cu^{2+}$ to catalyze ester hydrolysis through a metal ion promoted attack of $^-OH$ it is clear that the Cu(II) enzyme must bind esters so that the ester carbonyl and metal ion bound water or $^-OH$ are not in close proximity. It has been shown that the Cu(II) enzyme is able to bind substrates.[58,59] Ester substrates may, however, bind to the metal ion in the active site through the free carboxyl group.[60]

There may be pronounced differences in mechanism in carboxypeptidase A catalyzed ester and peptide hydrolysis.[60] This is supported by the striking differences in metal ion effects observed in the hydrolysis of carboxyl substituted esters and amides. In contrast with the catalytic effects of metal ions in the hydrolysis of I and II, divalent metal ions produce significant rate retardations in the hydrolysis of *N*-(2-phenanthrolyl)phthalamic acid.[25] This is most likely due to metal ion inhibition of protonation of the leaving group, a requirement in amide hydrolysis. Metal ion promoted $^-OH$ catalysis does not take place in hydrolysis of *N*-(2-phenanthrolyl)phthalamic acid[25] or *N*-(8-quinolyl)phthalamic acid[23] at accessible pH values.

Both nucleophilic and general base mechanisms have been suggested for carboxypeptidase A involving the carboxyl group of glutamic acid-270.[1-3] Evidence has been presented for the formation of an anhydride intermediate from an ester substrate at very low temperature.[61] However, experiments designed to detect the presence of an intermediate in peptide hydrolysis at normal temperatures failed to do so.[62] Detection of an anhydride intermediate under normal conditions of temperature

and solvent would conclusively demonstrate a nucleophilic mechanism, but in the absence of direct evidence other mechanisms must be considered. The present data showing that intramolecular general base catalysis cannot effectively compete with metal ion promoted $^-OH$ attack indicates that the former possibility is not likely in enzyme-catalyzed ester hydrolysis, if indeed the zinc ion of carboxypeptidase A is coordinated in close proximity to the carbonyl of an ester substrate in the enzyme-substrate complex. Metal ion promoted attack of $^-OH$ would then be expected at high pH values. Metal ion promoted $^-OH$ catalysis can also completely overcome carboxyl group nucleophilic catalysis when steric fit of the carboxyl and the carbonyl is not maximal. However, because of the very large rate enhancements associated with nucleophilic catalysis, when steric fit is good the carboxyl nucleophilic reaction will be predominant at pH <6-7 even though the metal ion promoted $^-OH$ reaction is very facile. Thus, in the enzyme reaction, if the steric fit of Glu-270 and the substrate carbonyl is good, then nucleophilic attack will be the preferred pathway at pH below neutrality even if Zn(II) does not catalyze the reaction. Whether the carboxyl group of Glu-270 is sterically capable of efficient participation as an intracomplex nucleophile is the principal remaining question in regard to the mechanism of action of the enzyme. If that could be determined, the enzyme mechanism and a quantitative explanation of the observed rate enhancements would follow from the chemical studies.

## Conclusions

In summary, the following conclusions can be drawn from the present work.

1. Divalent metal ion promoted attack of hydroxide ion at saturating concentrations of metal ion will occur in the hydrolysis of esters with rate enhancements of $10^3$-$10^9$ in cases where the metal ion is tightly chelated.

2. Reactivity in ester hydrolysis is dependent upon the ability of a metal ion to bind to the reactant. However, differences in rate enhancements are small in comparison with differences in the stability constants. The metal ion complexes of II react in the order Cu(II) > Zn(II) > Co(II) ~ Ni(II), but there is only a tenfold difference within the series.

3. Intramolecular general base catalysis by a neighboring carboxylate anion is not capable of sufficient rate enhancements in the presence of a chelated metal ion to compete with metal ion promoted attack of hydroxide ion. Metal ion promoted $^-OH$ catalysis can also overcome intramolecular nucleophilic catalysis by a carboxylate anion when the metal ion is tightly chelated and the carboxyl is not rigidly held in proximity to the carbonyl as in the case of 8-(2-carboxyquinolyl) hydrogen glutarate. However, removal of degrees of freedom for rotation of the carboxyl away from the carbonyl will allow the nucleophilic mechanism to be predominant at pH <6.

4. Intramolecular general base catalysis by Glu-270 is very unlikely in the carboxypeptidase A catalyzed hydrolysis of esters if Zn(II) is mechanistically important. It is highly probable that the enzyme mechanism involves either nucleophilic attack by Glu-270 or metal ion promoted attack of $^-OH$, with the added possibility that both mechanisms occur as a function of pH.

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# Concurrent General Acid and General Base Catalysis in the Hydrolysis of an Imidate Ester. 2. Bifunctional Catalysis[1a]

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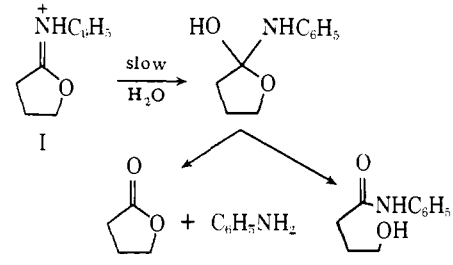
**Abstract:** Bifunctional buffers efficiently catalyze the expulsion of amine from the tetrahedral intermediate generated by hydration of imidate ester III. Rate enhancements of up to 300-fold have been calculated from a comparison of the effects of bifunctional catalysts to those of a series of monofunctional general acid and general base catalysts. No single factor ($pK_a$ of the acidic or basic groups, catalyst geometry, ring size of a cyclic transition state) has been identified as primarily responsible for high catalytic activity. In the region of catalyst $pK > 7$, monofunctional oxy acids are about eight times better general acid catalysts than monofunctional nitrogen acids.

In 1952, Swain and Brown reported that the mutarotation of tetramethylglucose in benzene was efficiently catalyzed by 2-pyridone, and proposed a mechanism involving bifunctional acid-base catalysis via a cyclic transition state.[1b] Their suggestion that polyfunctional catalysis may account, at least in part, for the high catalytic activity of enzymes has received continued attention, though few unequivocal examples of such catalysis in model systems have been described.[2-6] Bifunctional catalysis has been proposed to occur in a number of reactions carried out in *aprotic solvents*. These include the reaction of amines with 2,4-dinitrofluorobenzene,[7] the aminolysis of *p*-nitrophenyl acetate,[8] the addition of amines to isocyanates,[9] and the isomerization of cholestenone,[10] as well as additional studies on the mutarotation of tetramethylglucose.[8,11,12] Among the catalysts for which special effectiveness has been claimed have been carboxylic acids, pyrazole, 1,2,4-triazole, ureas, amides, and various phosphorus oxy acids.

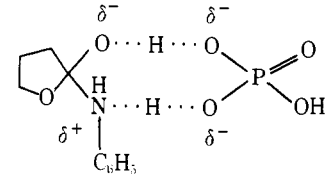
*In water*, 2-pyridone loses its unusual catalytic properties, and is no better a catalyst for the mutarotation of glucose than other general bases of similar $pK$.[12] Presumably, the ability of water to act as a proton donor and acceptor suppresses the need for special catalytic pathways for proton transfer in this reaction. Nevertheless, there are known several reactions carried out in aqueous medium in which the enhanced reactivity of some general acid-base catalysts (usually phosphate, arsenate, or bicarbonate ions) has been ascribed to bifunctional catalysis. Among these are the hydrolysis of amides,[13] imidate esters,[14] and amidines,[15] the aminolysis of esters[16] and thiol esters,[17] the decomposition of the adduct of hydrogen peroxide and aldehydes,[18] the addition of urea to formaldehyde,[19] the hydration of pteridine,[20] and a transimination reaction.[21]

The experiments described in this paper were undertaken to define more closely the relationship between the structure of an acid-base catalyst and its ability to function effectively as a bifunctional catalyst in aqueous solution. The reaction chosen for this purpose was the hydrolysis of an imidate ester, earlier studies having shown that the nature of the hydrolysis products is markedly affected by the presence of general acid-base catalysts.[14,16a,22] Thus, the yield of aniline obtained on hydrolysis of the cyclic imidate I is increased by increasing concentrations of phosphate or imidazole buffers at constant pH (eq 1a). Despite the fact that the $H_2PO_4^-$-$HPO_4^{2-}$ and the imidazole-imidazolium buffers have approximately the same $pK_a$, phosphate buffer is at least 200 times as effective as imidazole buffer in catalyzing the expulsion of aniline from the intermediate shown in eq 1a. The unusual reactivity of



(1a)

phosphate ions has been ascribed to their bifunctional character, which enables them to participate in more or less simultaneous proton transfers, as in transition state II.[14] It



II

should be noted that catalytic effects on the breakdown of the intermediate are seen as a change in the product distribution, with little or no change in the rate of the overall reaction. Under

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