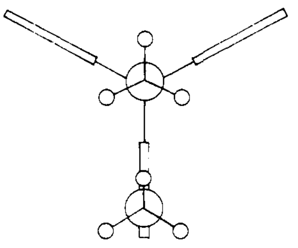

Figure 6. A schematical illustration of the transition state conformation for peri methyl rotation in **3**.

Table VI. Intra- and Intergroup Energy Differences between the Transition and the Ground States for the Peri Methyl Rotation Calculated by the CNDO/FK Method[a]

	1	4	3	6
peri Me	0.60	0.72	1.18	1.67
bh C-H, C-Me	0.96	0.99	2.09	2.47
peri Me···bh C-H, C-Me	−0.45	−0.43	−0.45	−0.33
peri Me···others	0.53	0.36	0.64	0.29
others	−0.26	−0.22	−0.18	−0.03
total	1.38	1.42	3.28	4.07
obsd	1.62[b]	1.65	2.26	2.24
	1.17[c]			

[a] Energies are given in units of kcal/mol. [b] Value above 147 K. [c] Value below 147 K.

MMI Calculation for Peri Methyl Barriers. For the MMI calculations of the rotational transition states, Wiberg–Boyd's one-bond driving technique is also applied to rotation of peri methyls. The results of MMI calculations are listed in Table V. They are in satisfactory agreement with experiment suggesting the absence of electronic effects such as the nonbonded attractive interaction for bh methyls. The calculated transition-state conformation for **3** is sketched in Figure 6, with the methyl groups taking a meshed-gear conformation. The angle bending and the van der Waals terms make a dominant contribution to the rotational barrier. The increase in the steric energy by the bh methyl substitution comes mainly from the increase in the angle bending term.

CNDO/FK Calculation for Peri Methyl Barriers. The CNDO/FK rotational barriers presented in Table VI are in substantial agreement with the experimental as well as the MMI barriers. A nonbonded attractive interaction between the peri methyl and the bh substituent negligibly negligible small, about 0.4 kcal/mol for all the compounds in Table VI. Therefore, the MMI calculations gave results in good agreement with experimental results. The major origin of the peri methyl barrier is the destabilization of peri methyl and bh substituents.

To conclude, the observed appreciable increment of barriers to rotation of bh methyls by methyl substitution at peri positions is not compatible with the gear effect. Furthermore, both MMI and CNDO/FK calculations indicate the two adjacent methyl groups prefer a clashed-gear conformation in the ground state and bh and peri methyls need not rotate synchronously. Thus, the idea of gearlike rotation may be needless for two adjacent methyl groups.

Acknowledgment. We wish to express our thanks to Professor Eiji Ōsawa of Hokkaido University for his kind offer of the modified MMI program source. The efficient service of the Data Processing Center at Kyoto University and the Computer Center at the Institute for Molecular Science is gratefully acknowledged. This work was supported by a grant for Fundamental Research in Chemistry from the Japan Society for the Promotion of Science.

Registry No. 1, 20711-97-1; **2**, 793-39-5; **3**, 75979-95-2; **4**, 17417-17-3; **5**, 15254-35-0; **6**, 33671-75-9.

Effects of Divalent Metal Ions on Hydrolysis of Esters of 2-(Hydroxymethyl)picolinic Acid. Metal Ion Catalysis of the Carboxyl, Hydroxide Ion, and Water Reactions

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Contribution from the Department of Biochemistry, University of Southern California, Los Angeles, California. *Received May 18, 1981*

Abstract: The effects of divalent metal ions (Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}) on the hydrolysis reactions of phthalate, succinate, and acetate esters of 2-(hydroxymethyl)picolinic acid have been determined at 50 °C. With these esters the metal ion saturates at low metal ion concentration (<0.01 M). Large rate enhancements are observed in the hydroxide ion catalyzed reactions at saturating concentrations of metal ion, ranging from 10^4 with Ni^{2+} to 10^6 with Cu^{2+}. A pH-independent reaction also occurs in the case of the phthalate monoester which is associated with the neighboring carboxyl nucleophilic reaction. Rate enhancements of 10^2–10^4 are obtained in this reaction at saturating concentrations of Ni^{2+}, Co^{2+}, and Cu^{2+}. Rate constants in the Ni^{2+} and Co^{2+} catalyzed reaction are closely similar to those for 2-pyridylmethyl hydrogen phthalate with which metal ion binding is weak. Thus, metal ion catalysis of the nucleophilic reaction, which occurs through leaving group stabilization in the transition state, is not appreciably enhanced by increased strength of binding to the reactant. Such a reaction does not occur with Cu^{2+} in hydrolysis of the succinate monoester, showing that when the leaving group is poor, steric fit of the nucleophile and the carbonyl must be excellent for a nucleophilic mechanism to occur even though the leaving group can be greatly stabilized by a metal ion, i.e., there cannot be degrees of freedom for rotation of the nucleophile away from the carbonyl. At pH <3 k_{obsd} is pH independent in the Cu^{2+}-catalyzed hydrolysis of the succinate and acetate esters due to a metal ion promoted water catalyzed reaction. Thus, three different mechanisms for metal ion catalysis can be observed in hydrolysis of esters of 2-(hydroxymethyl)picolinic acid: (1) catalysis of a neighboring carboxyl nucleophilic reaction, (2) metal ion promoted OH^- catalysis, and (3) metal ion promoted water catalysis. The first of these mechanisms is dependent upon the strength of binding of the metal ion to the leaving group oxygen in the transition state, whereas the latter two mechanisms are facilitated by strength of binding to the reactant.

Carboxypeptidase A is a Zn(II)-requiring enzyme, catalyzing the hydrolysis of ester and peptide substrates.[2] X-ray crystallographic analysis at 2-Å resolution has shown the zinc ion to be chelated to the carbonyl oxygen of poor peptide substrates.[2-4] The

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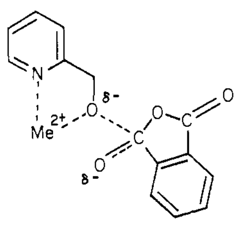
carboxyl group of glutamic acid-270 has also been implicated in the catalytic process, and mechanisms have been suggested involving nucleophilic attack and classical general base catalysis (proton transfer in the transition state from a water molecule).[3,4] The role of the complexed metal ion in these mechanisms has been to provide polarization of the carbonyl group. However, this should not be a catalytic factor in the case of a carboxyl nucleophilic reaction because breakdown of a tetrahedral intermediate would be rate determining when the pK_a of the leaving group greatly exceeds that of the nucleophile. A tetrahedral intermediate might be stabilized by metal ion binding. An alternative possibility is that Zn(II) exerts a transition-state effect in which the leaving group is stabilized.[5]

Divalent metal ions have little effect on the intramolecular nucleophilic carboxyl group reactions of phenolic esters even though rate enhancements of 10^4–10^9 are observed in the metal ion promoted OH^- reactions.[6,7] However, we have found divalent metal ion catalysis of the neighboring carboxyl nucleophilic reaction of 2-pyridylmethyl hydrogen phthalate in which the leaving group is stabilized in the transition state for C–O bond breaking (I).[8] Such an effect should be important with esters having poor leaving groups. Metal ion binding to the reactant is weak, but as the C–O bond breaks, partial negative charge will be generated on oxygen thereby allowing formation of a strong five-membered chelate ring with metal ion in the transition state. This mechanism might only be observed when metal ion binding to the reactant is weak, since strong binding should enhance the metal ion promoted OH^- reaction.[7] On the other hand, strong binding of metal ion to the leaving group oxygen of the reactant might also enhance the neighboring carboxyl nucleophilic reaction through stabilization of the leaving group, thereby allowing that reaction to be competitive. To determine the relative importance of these effects we have studied the metal ion catalyzed reactions of esters of 2-(hydroxymethyl)picolinic acid (II–IV) with which the metal ions are chelated strongly to the reactant. 2-(6-Carboxypyridyl)methyl hydrogen phthalate provides a direct comparison with 2-pyridylmethyl hydrogen phthalate. With such esters the leaving group oxygen can be chelated, but the carbonyl oxygen or the carboxyl nucleophile should not be chelated in a 1:1 complex because of the unfavorable steric situation. An important question also concerns the importance of steric fit of the nucleophile to the carbonyl in these reactions with a poor leaving group, i.e., will a chelated metal ion which stabilizes the leaving group permit a nucleophilic mechanism even if there are degrees of freedom for rotation of the carboxyl group away from the carbonyl? This question has been approached with the corresponding succinate ester (III).



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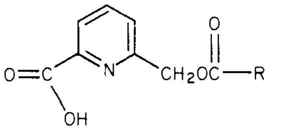
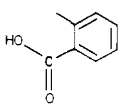
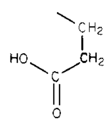


II–IV

II, R =  ; III, R =  ; IV, R = $-CH_3$

Experimental Section

Materials. 2-(Hydroxymethyl)picolinic Acid. 2,6-Pyridinedicarboxylic acid (Aldrich) was refluxed with thionyl chloride. After removal of the thionyl chloride by distillation and rotary evaporation with dry benzene, the remaining liquid was added dropwise to a cooled stirred solution of dry ethanol. The ethanol solution was made neutral to pH paper by the addition of sodium carbonate, and the ethanol was removed to yield the diethyl ester of 2,6-pyridinedicarboxylic acid (bp 115 °C at 0.02 mmHg). The diethyl ester (20 g) was refluxed with 3.4 g (0.6 equiv) of sodium borohydride in 200 mL of dry ethanol for 2 h.[9] After being cooled, the solution was concentrated to a volume of 50 mL, and 50 mL of water was added. The solution was further concentrated to a final volume of 50 mL and extracted with several 50-mL portions of chloroform. The combined chloroform extracts were dried with sodium sulfate, and the chloroform was removed by rotary evaporation, yielding about 8 g of ethyl 2-(hydroxymethyl)picolinate (mp 95–97 °C). The ester was hydrolyzed by dissolving it in a minimum volume of 1 M OH^- and allowing 2 h for hydrolysis. The pH was then adjusted to 3 by the addition of HCl, and the solvent was removed. The remaining residue was extracted and recrystallized from hot acetone to yield the 2-(hydroxymethyl)picolinic acid, mp 136–137 °C (lit.[10] mp 136 °C).

2-(6-Carboxypyridyl)methyl hydrogen phthalate (II) was prepared by refluxing equivalent amounts (0.003 mol) of 2-(hydroxymethyl)picolinic acid and phthalic anhydride in 75 mL of dry benzene overnight. The solution was cooled and filtered, and the white solid was recrystallized from acetonitrile in approximately 50% yield, mp 172–174 °C dec. Anal. Calcd for $C_{15}H_{11}NO_6$: C, 59.80; H, 3.65; N, 4.56. Found: C, 59.82; H, 3.89; N, 4.88.

2-(6-Carboxypyridyl)methyl hydrogen succinate (III) was prepared in the same manner and with the same yield as the phthalate monoester, mp 118–120 °C. Anal. Calcd for $C_{11}H_{11}NO_6$: C, 52.17; H, 4.35. Found: C, 52.11; H, 4.31.

2-(6-Carboxypyridyl)methyl acetate (IV) was synthesized by refluxing equivalent amounts of 2-(hydroxymethyl)picolinic acid and acetic anhydride overnight in dry benzene. Removal of the solvent yielded a white solid, mp 113–115 °C dec. The material was recrystallized from benzene (20% yield).

Kinetic Measurements. The rates of hydrolysis of esters II–IV were measured on a Beckman Model 25 or Pye-Unicam SP8-100 recording spectrophotometer at 280 nm by following appearance of product (III and IV) or disappearance of reactant (II). In the metal ion catalyzed reactions disappearance of reactant was followed at 280 nm with all the esters. The ionic strength was maintained at 0.1 M with KCl, and solutions used for nonmetal ion assisted reactions contained 2×10^{-5} M EDTA as a precaution against trace metal ions in the buffer or salt. A few buffer solutions were prepared which did not contain EDTA but which had been extracted with 0.001 M dithizone in CCl_4, and these yielded the same results as the EDTA-containing buffers. The buffer concentration was 0.02 M, and no correction was made for any metal ion–buffer complexation. Buffers employed were HCl (pH 1–3), chloroacetate (pH 3.2–3.8), acetate (pH 4.2–5.3), cacodylate (pH 5.5–6.5), *N*-ethylmorpholine (pH 6.7–7.8), morpholine (pH 8.0–8.6), and carbonate (pH 9–11).

Kinetic runs were initiated by injecting 15–30 μL of ester stock solution (0.005–0.02 M in acetonitrile) into 2–3 mL of the buffer solution maintained at the desired temperature. The reactions followed pseudo-first-order kinetics for at least 4 half-lives. Kinetic parameters were evaluated with a nonlinear least-squares computer program. Reaction mixture pH values were measured at 50 and 90 °C with a Beckman Model 3500 digital pH meter. Second-order rate constants for hydroxide

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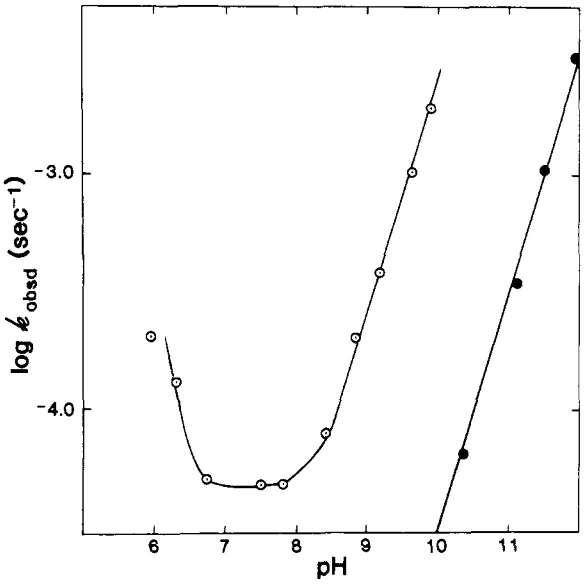

Figure 1. Plots of log k_{obsd} vs. pH for hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen phthalate at 90 °C (○) and 50 °C (●) with $\mu = 0.1$ M in H_2O.

Table I. Values of k_{obsd} for Hydrolysis of 2-(6-Carboxypyridyl)methyl Hydrogen Phthalate at pH 6.83 (50 °C and $\mu = 0.1$ M) in the Presence of Varying Concentrations of Metal Ions

metal ion	concn × 10^3, M	k_{obsd} × 10^3, s^{-1}
Co^{2+}	0.2	1.34
	0.5	1.39
	1.0	1.32
	2.0	1.28
	5.0	1.31
	10.0	1.33
Ni^{2+}	0.2	1.46
	0.5	1.38
	1.0	1.30
	2.0	1.28
	5.0	1.28
	10.0	1.28

ion catalyzed hydrolysis of the esters were calculated by using K_w values[11] of 5.5×10^{-14} at 50 °C and 3.35×10^{-13} at 90 °C.

Results

In Figure 1 is shown a plot of log k_{obsd} vs. pH for hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen phthalate (II) at 90 °C. There is OH^- catalysis and at pH less than 8 a pH-independent region. Three functional groups are present in II whose ionization state must be considered. With the assumption that in the pH range 6–10 $a_H \ll K_1$, values of k_{obsd} are given by

$$k_{obsd} = \frac{k_1K_2a_H^2 + k_0K_2K_3a_H + k_{OH}K_2K_3K_w}{a_H^3 + K_2a_H^2 + K_2K_3a_H} \quad (1)$$

where k_{OH} is the second-order rate constant for hydroxide ion catalysis, k_0 is the rate constant for the carboxyl group reaction involving the dianionic species, k_1 is the rate constant for the corresponding reaction of the monoanionic species, K_2 and K_3 are the second and third dissociation constants of the molecule, and K_w is the ion product of water. Values of k_{OH} and k_0 are 0.665 M^{-1} s^{-1} and 3.51×10^{-5} s^{-1}, respectively. At pH less than 6.5 k_{obsd} increases with decreasing pH, showing that neighboring group participation is more favorable via the monoanionic species. The lack of sufficient absorbance change at low pH precluded following the reaction at pH values near pK_2 so that k_1 could not be determined. Also included in Figure 1 are values of k_{obsd} for hydrolysis of II obtained at 50 °C, $k_{OH} = 0.051$ M^{-1} s^{-1}.

Divalent metal ions (Cu^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+}) exert large catalytic effects in the hydrolysis of II. Metal ion binding to the reactant is very strong. Saturation effects were observed at metal ion concentrations less than 0.01 M as seen in Table I; increasing Co^{2+} or Ni^{2+} concentration in the range 0.0002–0.01 M at constant pH (6.83) has no effect on k_{obsd}. In Figure 2 plots are presented of log k_{obsd} vs. pH at 50 °C for hydrolysis of II at saturating concentrations of the metal ions. With Ni^{2+} and Co^{2+} an OH^- catalyzed reaction is observed along with a pH-independent reaction at pH <6. Best-fit values of the rate constants are given in Table II. With Cu^{2+}, however, only the pH-independent reaction is observed (pH 4–6.2) with a rate considerably greater than displayed by the other metal ions ($k_0 = 6.78 \times 10^{-3}$ s^{-1}).

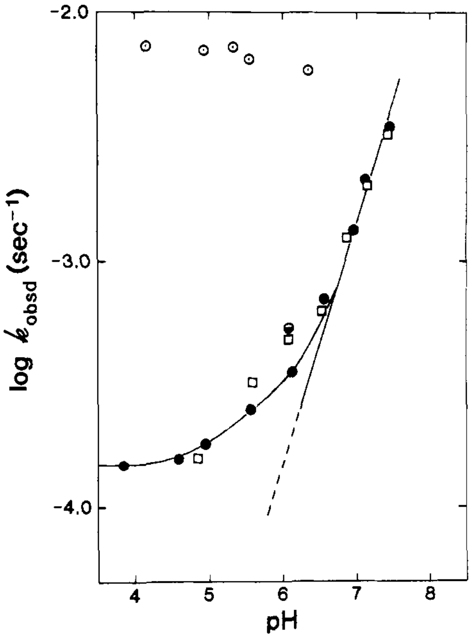

Figure 2. Plots of log k_{obsd} vs. pH for hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen phthalate at 50 °C in the presence of saturating concentrations of Cu^{2+} (○), Zn (⊖), Ni^{2+} (●), and Co^{2+} (□), with $\mu = 0.1$ M in H_2O.

Table II. Rate Constants for Hydrolysis of 2-(6-Carboxypyridyl)methyl Hydrogen Phthalate in H_2O at 50 °C, $\mu = 0.1$ M with KCl

metal ion[a]	k_0 × 10^3, s^{-1}	k_{OH}, M^{-1} s^{-1}
none		0.0510
Cu^{2+}	6.78	
Ni^{2+}	0.172	2500.0
Co^{2+}	0.181	2560.0

[a] At saturating concentrations of metal ion.

Figure 3 presents plots of log k_{obsd} vs. pH for hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen succinate (III) at 50°C in the presence of saturating concentrations of the divalent metal ions. The effect of metal ion dilution on k_{obsd} was ascertained. At low pH higher concentrations were required for saturation than at pH >5, but in all cases concentrations of 0.01 M or less could be employed. Metal ion promoted OH^- catalyzed reactions are observed at both high and low pH values with a small inflection in the profile near pH 4. This probably corresponds to addition of a proton to the molecule with metal ion promoted OH^- catalyzed reactions of both the dianionic and anionic species. Thus, at saturating metal ion concentrations k_{obsd} is given by eq 2, where

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

k_{OH} and k_{OH}' are the second-order rate constants for metal ion enhanced OH^- catalysis of hydrolysis of the dianionic and anionic species, respectively, and K_a is the apparent dissociation constant. Values of the constants are given in Table III. As seen in Figure 3, in the case of the Cu^{2+} catalyzed reaction, the pH-independent

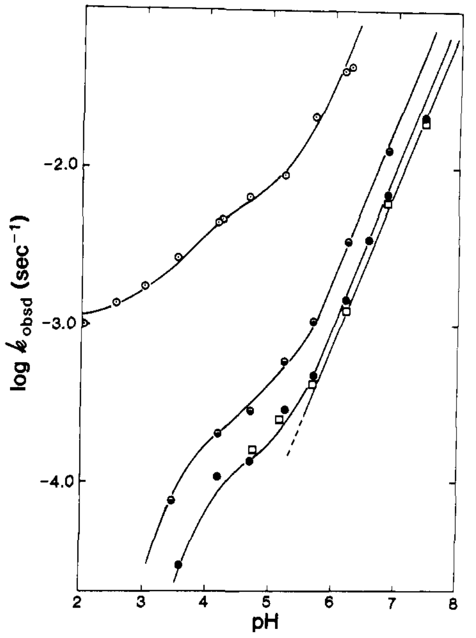

Figure 3. Plots of log k_{obsd} vs. pH for hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen succinate at 50 °C in the presence of saturating concentrations of Cu^{2+} (○), Zn^{2+} (◓), Ni^{2+} (●), and Co^{2+} (□) with μ = 0.1 M in H_2O.

Table III. Rate Constants for Hydrolysis of 2-(6-Carboxypyridyl)methyl Hydrogen Succinate and 2-(6-Carboxypyridyl)methyl Acetate at 50 °C, μ = 0.1 M

ester	metal ion^a	$k_0' \times 10^3$, s^{-1}	$K_a \times 10^4$, M	k_{OH}', M^{-1} s^{-1}	k_{OH}, M^{-1} s^{-1}
acetate	none				1.85
	Cu^{2+}	1.05			6.80×10^5
	Zn^{2+}				8.17×10^4
	Ni^{2+}				3.38×10^4
	Co^{2+}				6.20×10^4
succinate	none				0.551
	Cu^{2+}	1.1	1.26	1.3×10^7	5.5×10^5
	Zn^{2+}		1.26	6.65×10^5	3.25×10^4
	Ni^{2+}		0.64	1.75×10^5	1.48×10^4
	Co^{2+}				1.38×10^4

^{a} At saturating concentrations of metal ion.

reaction at pH <3 necessitates an additional term (k_0') in the equation for k_{obsd} (eq 3) ($k_0' = 1.1 \times 10^{-3}$ s^{-1}).

$$k_{obsd} = \frac{k_{OH}K_wK_a + k_{OH}'K_wa_H + k_0'a_H^2}{a_H^2 + K_aa_H} \quad (3)$$

Figure 4 presents plots of log k_{obsd} vs. pH for hydrolysis of 2-(6-carboxypyridyl)methyl acetate (IV) at 50 °C in the presence of saturating concentrations of the divalent metal ions. As with III metal ion concentrations of 0.01 M or less were saturating even at pH <5. In contrast with III, metal ion promoted OH^- catalyzed reactions are observed without a significant inflection in the log k_{obsd}–pH profile. Values of k_{OH} for IV are given in Table III. With Cu^{2+} as the catalyst a pH-independent region is again observed in the profile below pH 3 ($k_0' = 10^{-3}$ s^{-1}).

Discussion

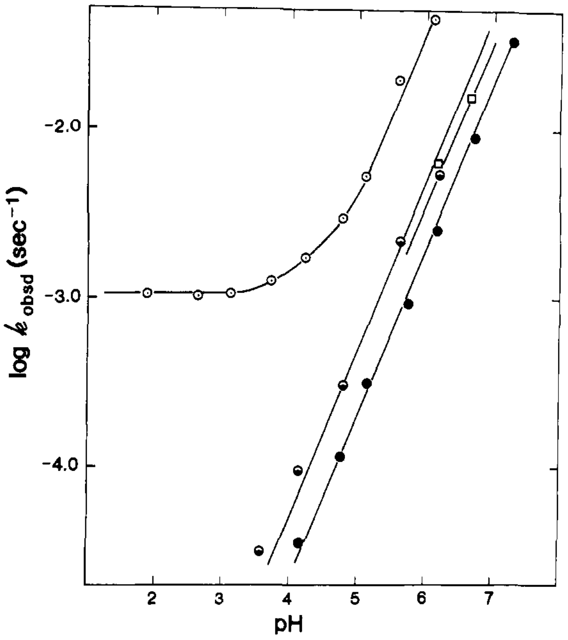

Figure 4. Plots of log k_{obsd} vs. pH for hydrolysis of 2-(6-carboxypyridyl)methyl acetate at 50 °C in the presence of saturating concentrations of Cu^{2+} (○), Zn^{2+} (◓), Ni^{2+} (●), and Co^{2+} (□) with μ = 0.1 M in H_2O.

Intramolecular carboxyl group participation in ester hydrolysis has been extensively investigated.[12-16] Large rate enhancements are observed in the intramolecular nucleophilic reactions of phenolic monoesters of succinic, glutaric, or phthalic acid.[13-15] When the leaving group of phthalate monoesters is phenol, the carboxylate anion is the active species, but with the methyl ester the pH–rate constant profile indicates that the neutral species is maximally reactive.[15] This possibly reflects a reaction of a zwitterionic species, i.e., carboxylate anion and protonated leaving group. When the leaving group pK_a is intermediate between that of phenol and methanol, the observed rate constants are pH independent in the range of the carboxyl pK_a, indicating involvement of both types of mechanisms.[15] The pH–log rate constant profile for hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen phthalate (II) (Figure 1) shows OH^- catalysis at high pH and a plateau at pH <8.5 which is undoubtedly due to carboxyl group participation (eq 4). Phthalic anhydride has been shown to be an intermediate in the hydrolysis of phthalate monoesters.[15] It hydrolyzes at 30 °C in the pH range 1.6–5.7 in a pH-independent reaction with a rate constant of 0.012 s^{-1}, which is much greater than the observed rate constants for reaction of II.

The log k_{obsd}–pH profile for hydrolysis of II is quite analogous to that for 2-pyridylmethyl hydrogen phthalate,[8] and values of the rate constants are closely similar except that an apparent hydronium ion catalyzed reaction is observed below pH 6.5 with II. The pK_a of the hydroxyl group of 2-(hydroxymethyl)pyridine has been reported to be 13.9,[17] and the pK_a of the 6-carboxyl-substituted derivative should be similar. Therefore, the plateau in the pH–log k_{obsd} profile for hydrolysis of II (pH 6.5–8) must represent carboxyl group participation by the anionic species as in the case of 2-pyridylmethyl hydrogen phthalate.[8] The value of the rate constant for pH-independent hydrolysis of propargyl hydrogen phthalate (pK_a of the leaving group alcohol = 13.55) is 6.9×10^{-5} s^{-1} at 78.5 °C, comparable to k_0 in hydrolysis of II (3.51×10^{-5} s^{-1} at 90 °C). In view of the similarity of the rate constants for hydrolysis of these esters, II is exhibiting hydrolytic behavior expected for phthalate monoesters with a leaving group pK_a near 13.9.

Binding of metal ions to II is quite strong as might be expected from the known binding constants of metal ions to the alcohol product (Cu^{2+}, 5×10^6 M^{-1}; Ni^{2+}, 2×10^5 M^{-1}; Co^{2+}, 2×10^4

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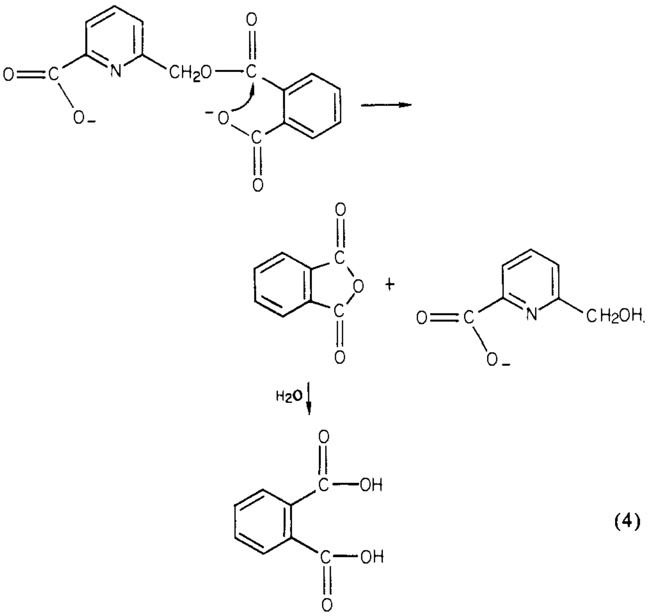


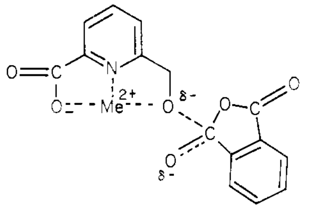
(4)

M^{-1}; Zn^{2+} 2 × 10^4 M^{-1})[10] and the fact that esterification does not modify the major chelating groups. Divalent metal ions (Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}) produce a large enhancement in the rate of hydrolysis of II. Saturation effects by the metal ions were observed at relatively low concentrations (<0.01 M). It can be seen in Figure 2 that at saturating metal ion concentrations both metal ion promoted OH^- catalyzed reactions and nearly pH-independent reactions are observed with Ni^{2+} and Co^{2+}, although the pH range of the latter reaction is limited. However, with Cu^{2+}, which is the best catalyst, the reaction is completely pH independent in the pH range 4.2–6.2, analogous to the more extensive pH-independent metal ion catalyzed carboxyl nucleophilic reactions of 2-pyridylmethyl hydrogen phthalate.[8]

The metal ion catalyzed hydrolysis of esters which have metal ion chelating functional groups is characterized by promotion of the OH^- catalyzed reaction.[6,7,18-22] Such reactions have log k_{obsd}–pH profiles with slopes of 1.0 at pH values below the pK_a of metal ion bound water, and are only pH independent at pH values above the pK_a.[18,23] Metal ion promoted OH^- catalyzed reactions are enhanced by increased strength of binding of metal ion to the reactant.[7] As a consequence, substitution of a carboxyl group into the 6 position of 2-pyridylmethyl hydrogen phthalate to give II should allow metal ion saturation at low concentrations and enhance the metal ion promoted OH^- reactions. This will shift the lines of slope 1.0 for OH^- catalysis on the log k_{obsd}–pH profiles to the left and thereby restrict the length of the plateau due to the carboxyl nucleophilic reaction unless that reaction is also enhanced by increased strength of metal ion binding. In the divalent metal ion catalyzed carboxylate nucleophilic reaction of 2-pyridylmethyl hydrogen phthalate saturation effects could not be observed even at high metal ion concentrations (>0.01 M).[8] The profiles in Figure 2 and ref 8 show that indeed the metal ion assisted OH^- reaction is facilitated by the carboxyl group in the 6 position. However, the pH-independent reactions of II with Ni^{2+} and Co^{2+} at saturating concentrations occur at approximately the same rate as with 2-pyridylmethyl hydrogen phthalate at the nonsaturating metal ion concentration of 0.005 M (1.56×10^{-4} s^{-1} and 6.70×10^{-5} s^{-1}, respectively, at 50 °C). Consequently, the pH-independent reactions of II are restricted in regard to their pH range by the more facile metal ion promoted OH^- reactions, although it will be noted in Figure 2 that definite plateaus in the profiles are observed, and with Cu^{2+} the reaction is completely pH independent over 2 pH units with no indication of OH^- catalysis.

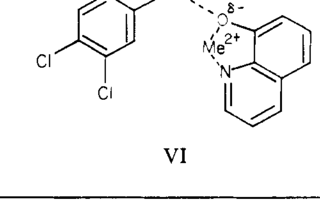
A transition from a metal ion promoted OH^- catalyzed reaction of the dianionic species to a faster OH^- catalyzed reaction of a monoanion should not give an extensive well-defined plateau since a large difference in these rate constants would not be expected.[7,8] A difference of at least 10^4 would be required in the Cu^{2+} catalyzed reactions. The profiles in Figure 3 for hydrolysis of the monosuccinate ester (III) where such a transition does occur show only small inflections near pH 5. Therefore, the pH-independent reactions of II must be associated with the neighboring carboxyl nucleophilic reaction. From the k_0 value at 90 °C for the neighboring carboxyl reaction (eq 1) and employing the ΔH^* measured for the carboxyl catalyzed reaction of methyl hydrogen phthalate,[15] a rough estimate of k_0 at 50 °C is 10^{-6} s^{-1}. Thus, saturating concentrations of Ni^{2+} and Cu^{2+} provide rate enhancements of ~10^2 and 10^4, respectively, in the carboxyl nucleophilic reaction.

If a tetrahedral intermediate is formed in carboxyl group nucleophilic reactions of esters with poor leaving groups, then its breakdown must be rate determining since the leaving group pK_a is much greater than that of the nucleophile, i.e., C–O bond breaking must be part of the rate-determining step. In reactions of the 2-pyridylmethyl esters a metal ion would be in position to bind strongly to the leaving group oxygen in the transition state via a five-membered chelate ring. Thus, metal ion catalysis of the carboxyl reaction of II must occur through a transition state effect (V) which is not enhanced by strong binding of metal ion to the reactant. Coordination of the metal ion to a carboxylate anion will reduce the effective charge on the metal ion, which could affect the rate constant for the bond-breaking step. Strong binding of a metal ion will also stabilize the reactants. Consequently, a rate enhancement will only be obtained if the transition state is stabilized to an even greater extent. The additional stabilization of the transition state in the metal ion catalyzed reaction provided by the carboxyl group in the 6 position of II is evidently not sufficient to significantly offset the increased metal ion stabilization of the reactants in comparison with 2-pyridylmethyl hydrogen phthalate. The maximum effect in reactions of this type will be achieved when binding in the transition state is very strong but binding to reactant is weak.



V

As the C–O bond of 2-pyridylmethyl phthalate monoesters breaks in the transition state (I and V) the partial negative charge that is generated on oxygen permits strong metal ion chelation to the leaving group. This type of transition state effect was also observed in hydrolysis of substituted benzaldehyde methyl 8-quinolyl acetals (VI).[24] Again, metal ion binding to the reactant



VI

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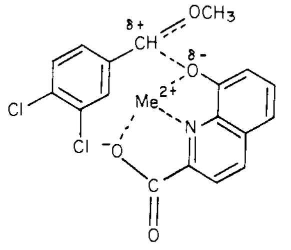
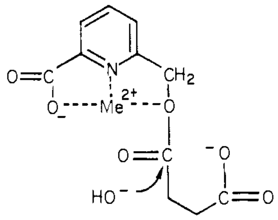
is weak, but stabilization of the leaving group in the transition state provides large rate enhancements (>10^5). At constant metal ion concentration these reactions are pH independent. Substitution of a carboxyl group into the 2 position of the quinoline ring (VII)



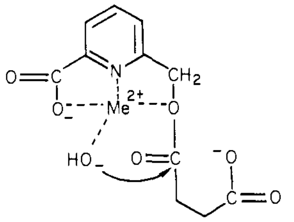
VII

allows strong metal ion binding to the reactant (saturation effects are observed at low metal ion concentrations), but this does not facilitate the reaction rate in comparison with VI at metal ion concentrations >0.01 M. Thus, the acetal hydrolysis reactions, in which C–O bond breaking occurs in the transition state, are quite analogous to the intramolecular carboxyl catalyzed reactions of the esters 2-pyridylmethyl hydrogen phthalate and II. In both cases the metal ion effects are produced by binding in the transition state to stabilize the leaving group.

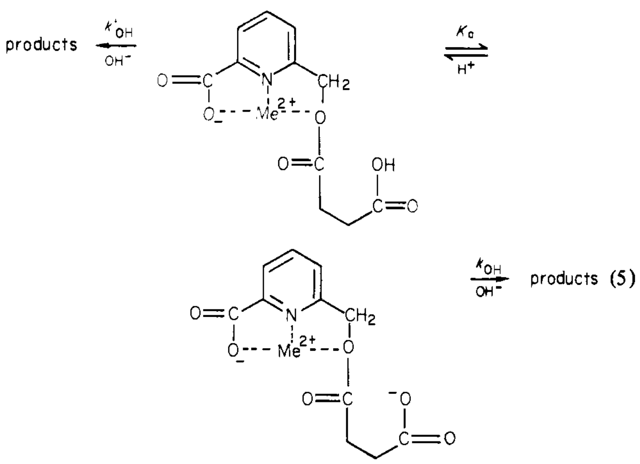
Degrees of freedom exist for rotation of the carboxylate nucleophile away from the ester carbonyl of glutarate and succinate monoesters, which will decrease the ease of nucleophilic attack.[13,14] Thus, while nucleophilic participation will occur with phthalate monoesters when the leaving group is poor (an aliphatic alcohol),[15] it is doubtful whether such a reaction would be detectable with a glutarate or succinate ester when the pK_a of the leaving group greatly exceeds that of the nucleophile. However, if a chelated metal ion can stabilize the leaving group sufficiently such a mechanism might occur with facility. In hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen succinate (III) extremely large rate enhancements are observed in the metal ion promoted OH^- catalyzed reactions of the dianion species ranging up to 10^6 at a saturating concentration of Cu^{2+} (6×10^4 with Zn^{2+} and 3×10^4 with Ni^{2+}).[25] This reaction must occur either through catalysis of the attack of external OH^- (VIII) or via an intramolecular attack of metal bound OH^- (IX). Rate constants for this reaction are 10-fold larger than with the phthalate monoester II. This is undoubtedly due to the more deactivated carbonyl of the phthalate ester. There is a small inflection in the log k_{obsd}–pH profile at constant metal ion concentration near pH 4 which probably corresponds to addition of a proton to the molecule. Thus, the scheme of eq 5 is being followed with k_{obsd} given by eq 2. A similar small inflection in the log k_{obsd} vs. pH profile was also observed in the divalent metal ion catalyzed hydrolysis of 8-(2-carboxyquinolyl) hydrogen glutarate.[7] To further establish that indeed the inflection in the profile for hydrolysis of III near pH 4 (Ni^{2+} and Co^{2+}) is due to protonation of the carboxyl group, the log k_{obsd}–pH profile for the corresponding acetate ester IV was obtained. It will be noted in Figure 4 that there is no inflection at pH <5. Thus, there is no evidence that the succinate carboxyl group of III can participate in the hydrolysis reaction at saturating concentrations of metal ions even though the leaving group can be greatly stabilized. Nucleophilic participation by a neighboring carboxyl group must require an excellent steric fit of nucleophile and carbonyl when the leaving group is poor (as with II) for that mechanism to be competitive with the metal ion promoted OH^- and H_2O catalyzed reactions.



VIII



IX

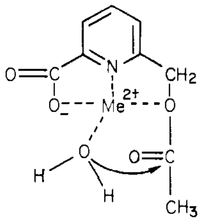


(5)

The scheme of eq 5 provides an explanation of the profiles for hydrolysis of III in the presence of Ni^{2+} and Co^{2+} at pH >3.5, but the experimental values of k_{obsd} at saturating concentrations of Cu^{2+} do not give a good fit to eq 2 below pH 3.5. It is clear that a pH-independent reaction is occurring at low pH which requires an additional term in eq 2. Assuming that a water-catalyzed reaction governed by k_0' is occurring at low pH and employing eq 3, a good fit to the experimental data (Figure 3) can be achieved with appropriate values of the constants ($k_0' = 1.1 \times 10^{-3}$ s^{-1} and pK_{app} = 3.90).

Reactions of the acetate ester IV in the presence of saturating concentrations of Cu^{2+} could be followed to low pH values and, as seen in Figure 4, at pH <3 a definite plateau is observed in the profile for the Cu^{2+} catalyzed reaction. Corresponding pH-independent reactions are not observed with Zn^{2+}, Ni^{2+}, or Co^{2+}. Therefore, the Cu^{2+} catalyzed reaction must be at least 100 times more favorable. The rate constant for the Cu^{2+} catalyzed water reaction of IV is nearly identical with that calculated for III (10^{-3} s^{-1}), but is much less than the rate constant for the Cu^{2+} catalyzed pH-independent reaction of II (7×10^{-3} s^{-1}) at higher pH in spite of the much more deactivated carbonyl of II. The latter reaction is clearly different and must correspond to Cu^{2+} catalysis of the carboxylate nucleophilic reaction.

The pH-independent Cu^{2+} catalyzed reactions of III and IV could correspond to attack of metal bound water on the ester (X) or a kinetic equivalent. The kinetically equivalent OH^- catalyzed hydrolysis of the neutral species can be ruled out since (1) metal ion binding to the neutral species should be quite weak, yet Cu^{2+} still saturates at low concentrations below pH 3, and (2) the k_{OH}



X

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(25) The enhancements in k_{OH} in the OH^- catalyzed reaction for saturating concentrations of Ni^{2+} with five esters having poor leaving groups (II-IV and previously published studies)[18,26] are closely similar, ranging from 1.2×10^4 to 4.9×10^4 (av 2.5×10^4). All of these compounds allow the divalent metal ion to bind strongly to the reactant and in excellent steric position to promote OH^- catalysis. Thus, the influence of structural features in the ester, e.g., leaving group, etc., is similar in the metal ion promoted OH^- reactions and the reactions uncatalyzed by metal ion. Larger rate enhancements can be achieved in the reactions of phenolic esters.[27] When the leaving group is a phenol with a leaving group pK_a of 10 or less it is probable that nucleophilic attack at the carbonyl by OH^- is the rate-determining step, whereas with esters having aliphatic alcohol leaving groups of pK_a 14–16 breakdown of a tetrahedral intermediate may be rate determining. This might then result in similar rate enhancements in the Ni (II) promoted OH^- catalyzed reactions for esters of similar leaving group pK_a.

value required would be considerably greater than 2×10^8 M^{-1} s^{-1}. Such a reaction would be approaching diffusion control, and considering the poor leaving group there is no basis for expecting a rate constant of that magnitude.[7,8,18,19] A water reaction has not previously been unambiguously observed in metal ion catalyzed ester hydrolysis.[28] In the absence of a metal ion, water catalysis occurs in the hydrolysis of acyl activated esters[29–32] but apparently not in the hydrolysis of aliphatic esters with poor leaving groups. A metal ion chelated strongly to the leaving group would, of course, provide electron withdrawal, and the complex would be equivalent to a protonated ester intermediate in hydronium ion catalyzed ester hydrolysis, which occurs with attack of water on the ester conjugate acid.[32] A chelated metal ion could also greatly stabilize the leaving group in the transition state for C–O bond breaking in the same manner as in the neighboring carboxyl group reaction of II, but for a metal ion promoted H_2O reaction to occur, it is very likely necessary that the metal ion be strongly chelated to the reactant. This type of reaction has not been observed previously in ester hydrolysis reactions presumably because of the facility of the other types of catalyzed reactions, i.e., metal ion promoted OH^- catalysis and metal ion enhancement of the neighboring carboxyl group reaction. A water reaction might only be detected when a metal ion is chelated to a poor leaving group. It is not seen with phenolic esters[6,7,18–21] because of the reduced need for stabilization of the leaving group and the favorable OH^- catalyzed reaction. Metal ion binding to III and IV is so favorable that saturating concentrations can be achieved even at pH values below the pK_a of the chelating groups. The hydrolysis reaction could therefore be followed at very low pH values so that this mechanism could be demonstrated.

Conclusions

Metal ion catalysis of intramolecular carboxyl nucleophilic reactions occurs via stabilization of the leaving group in the transition state and is not enhanced by strong chelation. In contrast, divalent metal ion promoted OH^- catalysis is facilitated by increases in the strength of chelation to the reactant. Consequently, when an additional chelating functional group is included in the molecule as with II, only the most effective metal ion (Cu^{2+}) catalyzes the carboxyl group reaction sufficiently so that it is clearly competitive with OH^- catalysis at pH >5. For the above reasons, an intramolecular carboxyl group nucleophilic reaction is not detected with an ester (III) having a poor leaving group when the nucleophilic reaction is not of maximum efficiency due to degrees of freedom for rotation of the nucleophile away from the carbonyl, even though a metal ion is strongly chelated to the leaving group oxygen. At low pH (<3) the pH-independent metal ion promoted water catalyzed reaction detected in hydrolysis of the acetate ester (IV) is quite analogous to hydronium ion catalyzed ester hydrolysis and very likely depends upon very strong chelation of metal ion to the leaving group oxygen of the reactant. Thus, three different types of mechanisms for metal ion catalysis can be observed in hydrolysis of esters of 2-(hydroxymethyl)picolinic acid: (1) catalysis of a neighboring carboxyl nucleophilic reaction, (2) metal ion promoted OH^- catalysis, and (3) metal ion promoted water catalysis.

These results are of considerable interest in regard to the mechanism of action of the hydrolytic enzyme carboxypeptidase A because ester substrates for the enzyme have poor leaving groups (e.g., esters of phenyllactic or mandelic acid).[2] Carboxypeptidase A requires the presence of metal ion for binding of esters.[2,3] If an ester is bound in the active site so that a Zn (II) complex can be formed these mechanisms could be operative. However, there are quantitative factors that have not yet been accounted for. Evidence has been presented supporting a nucleophilic mechanism in the enzyme catalyzed hydrolysis of ester substrates.[33] The rate constant $k_{\text{cat.}}$ for hydrolysis of *O*-(*trans*-cinnamoyl)-L-*β*-phenyllactic acid is pH independent in the pH range of 6.4–9[34] and is at least 10^8 larger than k_0 for the phthalate ester II[35] and 10^6 larger in comparison with k_0 for the Ni^{2+} and Co^{2+} catalyzed reactions. Therefore, other factors must be involved. Nevertheless, the present results show that mechanisms involving both metal ion and Glu-270 participation are chemically reasonable.

Acknowledgment. This work was supported by research grants from the National Institutes of Health.

Registry No. II, 80540-77-8; III, 80540-78-9; IV, 80540-79-0; 2-(hydroxymethyl)picolinic acid, 1197-10-0; 2,6-pyridinedicarboxylic acid, 499-83-2; diethyl 2,6-pyridinedicarboxylate, 15658-60-3; Co^{2+}, 22541-53-3; Ni^{2+}, 14701-22-5; Cu^{2+}, 15158-11-9; Zn^{2+}, 23713-49-7.

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(27) 8-(2-Carboxyquinolyl) hydrogen glutarate, an ester with a phenolic leaving group, gives a rate enhancement in the Ni (II) promoted OH^- catalyzed reaction of 4.3×10^6.[7]

(28) It has been previously reported that in the hydrolysis of some amino acid esters coordinated to a complex of nitrilotriacetic acid with Cu (II) that the equation for k_{obsd} contains a unimolecular term. However, kinetic equivalents exist for this reaction. Angelici, R. J.; Hopgood, D. *J. Am. Chem. Soc.* **1968**, *90*, 2514.

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(35) The leaving group pK_a of phenyllactic acid ester substrates for the enzyme would be expected to be in the range 13–14, similar to that of II.