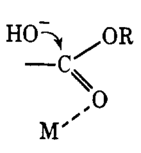
Intramolecular Catalysis of Ester Hydrolysis by Metal Complexed Hydroxide Ion. Acyl Oxygen Bond Scission in Co^{2+} and Ni^{2+} Carboxylic Acid Complexes

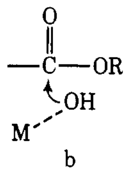
Michael A. Wells and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106. *Received December 10, 1976*

Abstract: A study of metal ion complexation and the catalysis of hydrolysis of the methyl (Ia), β-chloroethyl (Ib), propargyl (Ic), 2′,2′,2′-trifluoroethyl (Id), and *p*-chlorophenyl (Ie) esters of 2-(2′-hydroxyphenyl)-4(5)-methyl-5(4)-(2″,2″-dimethylacetate)imidazole (II) has been carried out. In addition metal ion complexation to II has been studied (30 °C, H_2O, 0.2 M $NaClO_4$). The metal ions employed have been Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+}. Of these, complexation of ester with Ca^{2+}, Mg^{2+}, and Mn^{2+} could not be observed; Zn^{2+} was found to form dimeric complexes (i.e., $(ligand)_2Zn$) which were not particularly labile to hydrolysis, and Cd^{2+}, though forming a 1:1 complex with esters, did not exhibit a significant catalytic effect upon their hydrolysis. On the other hand, very large rate enhancements over hydroxide ion mediated hydrolysis (10^3–10^5) were obtained on formation of the 1:1 complexes of esters with the hydroxo species of Ni^{2+} and Co^{2+}. For the 1:1 M^{2+} complexes of esters Ia–d, as well as the parent carboxylic acid (II), the metal is bound to the unprotonated imidazolyl moiety and the dissociated phenolic hydroxyl group. On the basis of the inability to establish the spectral characteristics of four- or five-coordinate Co^{2+}, it is assumed that M^{2+} is octahedral in the 1:1 complex of esters as well as with II. All experimental results (titrimetric as well as kinetic) for metal ion mediated ester hydrolysis may be explained by the sequence of reactions shown in Scheme I. The important features of Scheme I include: *preequilibrium formation of a tetrahedral intermediate accompanying nucleophilic addition of metal bound HO^-; the formation of an intermediate metal carboxylate capable of acting as an acylating agent ("carboxyl-metal anhydride"); and acid catalysis of the hydrolysis of the "carboxyl-metal anhydride" intermediate.*

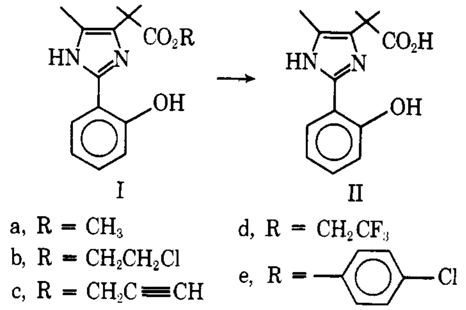
The catalytic role of metal ions in metallohydrolases, such as carboxypeptidase, can be explained in two alternative ways: (a) direct coordination of the ester or amide carbonyl group to the metal ion with the consequent activation to nucleophilic attack either by solvent HO^- or a nucleophile on the enzyme; and (b) nucleophile attack by metal coordinated HO^-. Analysis of model systems involving divalent cations such as Co^{2+}, Ni^{2+}, and Zn^{2+} is usually complicated by the ligand lability of complexes of these ions. Such complications generally make it impossible to distinguish kinetically between mechanisms a and b,[1] although distinctions can sometimes be made on the basis of the effects of added nucleophiles.[2] In the case of stable complexes of Co^{3+}, the distinction between mechanism a and b can be made on the basis of ^{18}O experiments.[3] Studies by Buckingham et al.[4,5] have shown that both mechanisms a and b can occur. The clearest example of carbonyl activation is the base hydrolysis of dimethylformamide pentaamine Co^{3+} in which case a $\sim 10^4$ rate enhancement over hydrolysis of the uncomplexed species was observed. In the case of glycinamide hydrolysis, the rate constant associated with path b was estimated to be 10^7–10^{11} greater than that for the uncomplexed amide, whereas the rate constant associated with path a was $\sim 10^4$ greater than that for hydrolysis of the uncomplexed species. For dipeptide hydrolysis, the b path was also found to be considerably more facile than the a path. In addition to the above reports comparing rates of reactions through paths a and b in Co^{3+}-mediated hydrolytic reactions, other studies involving Co^{3+} complexes demonstrate increased nucleophilicity of metal-bound nucleophiles in halogen elimination,[6a] aminolysis,[6b] and transesterification reactions.[6c]


a


b

The stability of Co^{3+} complexes is due to their inner-sphere nature. This chemistry is not shared by metal ions of biochemical importance which possess the oxidation state of M^{2+}. The retention of esterase activity in Co^{3+} carboxypeptidase[7] has been used to suggest that the metal ion cannot participate directly in the catalytic process. As we show in this paper, the study of labile complexes leads to mechanisms for the breakdown of metal-product complexes which can account for these data and still retain a direct catalytic role for the metal ion in carboxypeptidase.

We report herein studies of the M^{2+} complexes of esters of 2-(2′-hydroxyphenol)-4(5)-methyl-5(4)-(2″,2″-dimethylacetate)imidazole (I).[8] These studies provide thermodynamic data on the metal–I and metal–II complexes, as well as kinetic data on the hydrolyses of esters (Ia–e).


(1)

I

II

a, $R = CH_3$

b, $R = CH_2CH_2Cl$

c, $R = CH_2C{\equiv}CH$

d, $R = CH_2CF_3$

e, R = –C₆H₄–Cl

Experimental Section

Materials. Reagent grade perchlorate salts were obtained from ROC/RIC (Sun Valley, Calif.) and stored under vacuum. $NaClO_4$ solutions were extracted with 0.01% dithizone in CCl_4 to remove any heavy metal contamination.[9] All water used was deionized, double glass distilled, and extracted with dithizone solution.

2-(2′-Hydroxyphenyl)-4(5)-methyl-5(4)-(2″,2″-dimethylacetate)imidazole (II) and the acid chloride were prepared by the method of Rogers and Bruice.[8] Esters Ia–d were prepared as the hydrochlorides by heating the acid chloride from 0.1 g of acid at 50 °C under anhydrous conditions with 5 mL of anhydrous alcohol for 1 h. After removal of solvent in vacuo, the ester was crystallized from a solvent mixture of acetonitrile and the alcohol used to make the ester. Ester Ie was prepared by refluxing the acid chloride with a twofold excess of freshly sublimed *p*-chlorophenol in anhydrous chloroform. The phenyl ester crystallized from the reaction mixture after 1 h, and the

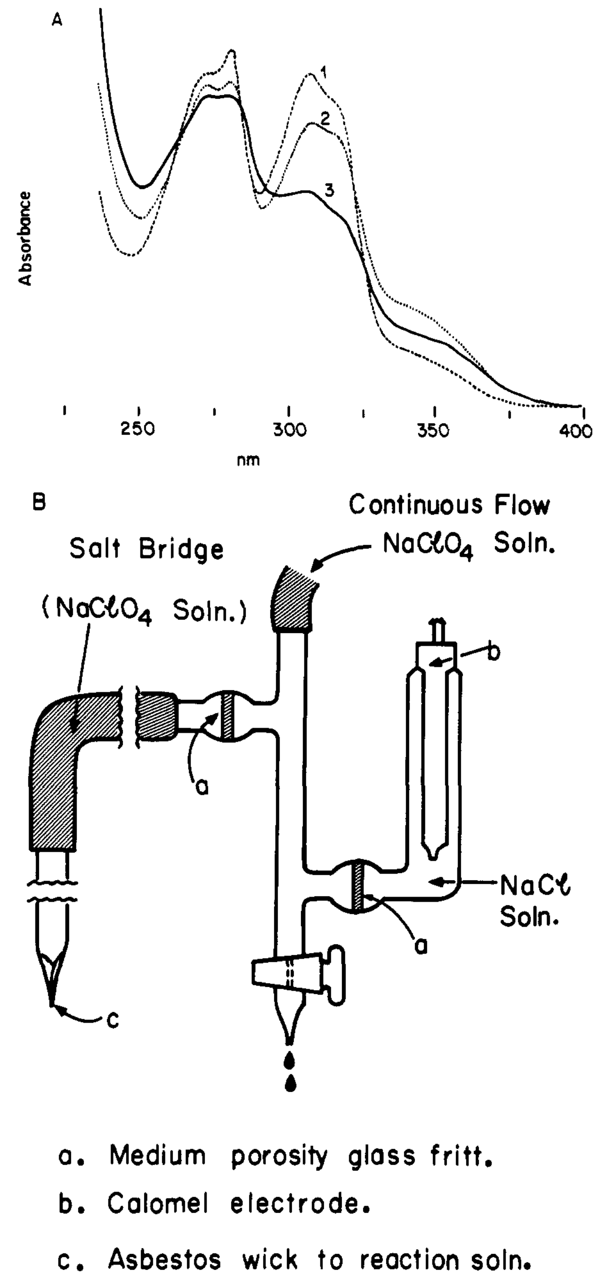


Figure 1. (A) Spectra in aqueous solution (0.2 M $NaClO_4$) of (1) trifluoroethyl ester (Id); (2) hydroxamate of II; and (3) *p*-chlorophenyl ester (Ie) at pH 7.75. (B) Flowing liquid junction.

crystals were removed and washed extensively with ether. Yields varied from 40 to 80%, mp Ia 195–197 °C, Ib 175–177 °C, Ic 164–166 °C, Id 180–182 °C, Ie 205–207 °C. All esters melted without decomposition, as opposed to the acid hydrochloride which melts with decomposition at 215–217 °C.

Esters were characterized as follows: IR (KBr) showed the presence of a single C=O stretch at 1725 cm^{-1}, whereas the acid hydrochloride has a doublet at 1690, 1725 cm^{-1}, and a band at 1640 cm^{-1} (ImH^+); $pK(ImH^+) = 6.05$–6.15 with no change in the UV spectra in the pH range 3–5 as seen for II; quantitative conversion to II after alkaline hydrolysis with $pK_{COOH} = 3.15$–3.30, $pK_{ImH^+} = 6.85$–6.95, and $pK_{PhOH} = 9.75$–9.85. The spectra of esters Ia–d as well as II produced by hydrolysis are identical with the spectra of Ia and II reported by Rogers and Bruice.[8] Although the spectrum of Ie was different (Figure 1A) than those of the alkyl esters, its IR spectrum, $pK(ImH^+)$, and conversion by OH^- to II and *p*-chlorophenol showed it to be the correct ester.

Apparatus. Spectral characterization of esters and products and kinetic and metal binding studies were carried out in the Cary 15 pH stat apparatus of Bruice and Maley[10] under N_2 at 30 ± 0.1 °C. pH was measured with a Radiometer Model 26 pH meter. In order to prevent leakage of Cl^- from the calomel electrode into the reaction vessel a flowing liquid junction was used, as shown in Figure 1B. $NaClO_4$ (0.2 M) was passed through the left arm at 100 mL/h. The calomel electrode was filled with saturated NaCl (in order to avoid precipitation of $KClO_4$ in the electrode) and immersed in saturated NaCl. The junction between the $NaClO_4$-filled salt bridge and the reaction vessel was made through a 0.1-mm diameter asbestos tip. Flow through this arm was less than 0.1 mL/h. Under these conditions no Cl^- could be detected in the reaction vessel even after 48 h. pH was measured with a Radiometer type G-202C glass electrode and maintained with a Radiometer Autoburette AUB 12 which added 0.01 M NaOH through a fine capillary (drop size ~5 μL) which was placed just above the level of the liquid in the cell. This arrangement prevented precipitation of metal hydroxides on the tip of the capillary and maintained pH at ±0.02 for several hours. A KNO_3/agar bridge between the calomel electrode and the reaction vessel was not nearly as effective as the arrangement pictured above in preventing Cl^- leakage and diffusion of NO_3^- from the bridge into the reaction solution which caused an increase in absorbance in the UV which made spectral studies impossible ($a_m{}^{300}$ nm of twice crystallized $KNO_3 \approx 10$).

pK_a Determinations. As shown by Rogers and Bruice,[8] the pK_a associated with each ionization of Ia and II can be determined by spectrophotometric titration. The same can be done for esters Ib–e. The wavelengths used follow: Ia–e, pK_{ImH^+} at 255.7 nm, pK_{PhOH} at 240.5 nm; II, pK_{CO_2H} at 283.5 nm, pK_{ImH^+} at 351.6 nm, pK_{PhOH} at 247.8 nm. The pK_as of metal aquo complexes, $M(H_2O)_6 \rightleftarrows M(H_2O)_5OH + H^+$, were determined by titrating 20 mL of 2×10^{-4} M metal perchlorate in 0.2 M $NaClO_4$ in the apparatus pictured and described above. At each pH, after correcting for the water blank, α = equivalents of OH^-/equivalent of metal ion was calculated, and the pK_a obtained according to

$$pK_a = \log \frac{\alpha}{(1-\alpha)} - \mathrm{pH} \qquad (2)$$

Ten to fifteen values of the pK_a were determined in the first 15–20% of the titration curve (for Co^{2+} and Ni^{2+} pH 7.8–8.8, for Zn^{2+} pH 7.0–8.0) and those values were found to vary by but ±0.1 pK_a. At higher pHs precipitation of metal hydroxide occurred giving erratic values for pK_a.

Metal Binding Equilibria. The pH dependence of the metal ion-substrate dissociation constants for esters Ia–e and II were determined spectrophotometrically. Stock solutions of ester or acid, 1.25×10^{-5} M in 0.2 M $NaClO_4$, were used. Metal ion binding was evidenced by a lowering of the pK_a for the phenolic group (see Figure 2). For esters the binding was followed by measuring the decrease in absorbance at 292 nm (Δ molar extinction coefficient = $\Delta a_m = -2.8 \times 10^4$, path length of reaction vessel 3.3 cm). For the acid the increase in absorbance at 335 nm ($\Delta a_m = 3.3 \times 10^4$) was measured.

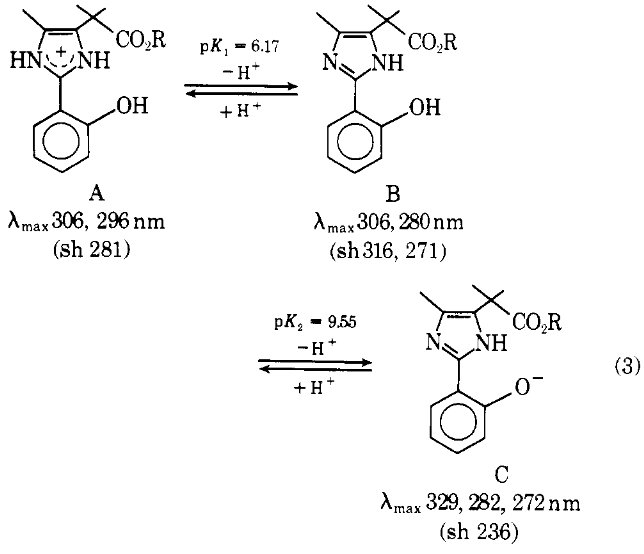
Metal-substrate dissociation constants were calculated via equations provided in the Appendix. The fraction of substrate present as the complex, as determined from phenolic absorbance, was measured: (1) at constant pH as a function of addition of small increments of metal ion (see Appendix A-8); and (2) at constant metal ion concentration as a function of pH with correction for pH-dependent phenol ionization in the absence of metal ion (see A-11). Both methods were employed with the ester substrates below pH 8.5 and provided excellent agreement. Above pH 8.5 only method 1 was used in order to avoid complications due to hydrolysis of the ester, and a fresh aliquot of the ester stock solution was used for each determination. By method 1 and with the ester and metal ion concentrations employed it was possible to collect data up to pH ~9.5 without apparent metal hydroxide precipitation. Using method 1, data were collected in less than 10 s. The experimental data points were fit to eq A-8 and A-11 (Appendix) by use of a Hewlett-Packard 9820A computer and programs written in this laboratory.

Kinetics. All kinetic measurements were made in 0.2 M $NaClO_4$ using 5×10^{-6} M ester and metal concentrations of 5×10^{-4} to 1×10^{-2} M depending on pH. The pH was maintained by employing the assembly used for titration in the pH-stat mode. Formation of II was followed at 335 nm and disappearance of Ia–e was followed at 258.5 nm. Both methods were used interchangeably. Reactions were followed to completion and the pseudo-first-order rate constants (k_{app}) were calculated from plots of ln ($OD_\infty - OD_t$) vs. time using 2–3 half-lives of the reaction and a Hewlett-Packard 9820A computer and programs written in this laboratory. At each pH the rate (k_{obsd}) at infinite metal ion concentration ($[M_T]$) was determined by extrapolation of plots of $1/k_{app}$ vs. $1/[M_T]$ to $[M_T] = \infty$. At least four metal concentrations were used and the computation was carried out using a linear least-squares regression analysis. The kinetically determined metal-substrate dissociation constant was determined from the ratio of the slope/intercept of these plots.

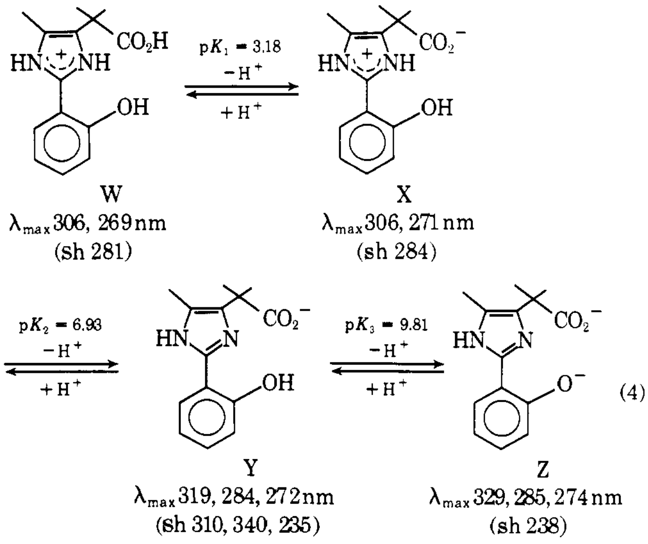
Hydroxylammonium perchlorate was prepared by treating NH_2OH-HCl with an equivalent amount of $AgClO_4$ and removing AgCl. Treatment of ester Id with 0.05 M NH_2OH-$HClO_4$ at pH 7.75 produced the hydroxamate of II as suggested by the following evidence. The hydroxamate showed an increased absorbance between 330 and 360 nm and decreased absorbance between 290-320 and 260-282 nm and isosbestic points with Id at 322, 284, and 260 nm (see Figure 1a). The spectrum was distinctly different from that of II at this pH. Rapid titration showed the absence of spectral changes between pH 5 and 3, indicating the absence of a COOH group. After 30 min at pH 3 significant quantities of II could be detected by spectral titration. Addition of Co^{2+} to the hydroxamate at pH 7.75 gave a spectrum identical with that of the Co^{2+}-II complex. At pH 7.75 the hydroxamate was stable for at least 2-3 h in the absence of added metal ion.

Results

As thoroughly discussed by Rogers and Bruice[8] the various ionic forms of II and its methyl ester (Ia) have distinct spectra. This distinctiveness of spectra allowed the determination of the ionic species present at any pH and the ionic species to which metal ion binds. For esters Ia-e ionic species A-C (eq 3) need to be considered whereas for II ionic species W-Z (eq 4) can be present. In addition the spectral changes, brought about by metal ion binding (Figure 2), were employed in the determination of metal ion-ester dissociation constants. Metal ion titrations were carried out at several pHs in order to determine which ionic form(s) of ester (A, B, or C) bind metal ions and the stoichiometry of complexation. The results of such titrations with Ni^{2+} and ester Ia are shown in Figure 3 as plots of $1/F$ vs. $1/[M_T]$, where F represents the fraction of ester in form C at the given metal ion concentration and $[M_T]$ the concentration of metal ion. These results establish that complexation occurs only between metal ion and ionic form C; otherwise the value of F at $[M_T] = \infty$ would have been pH dependent (see eq A-7, Appendix). Since the plots of $1/F$ vs.



(3)



(4)

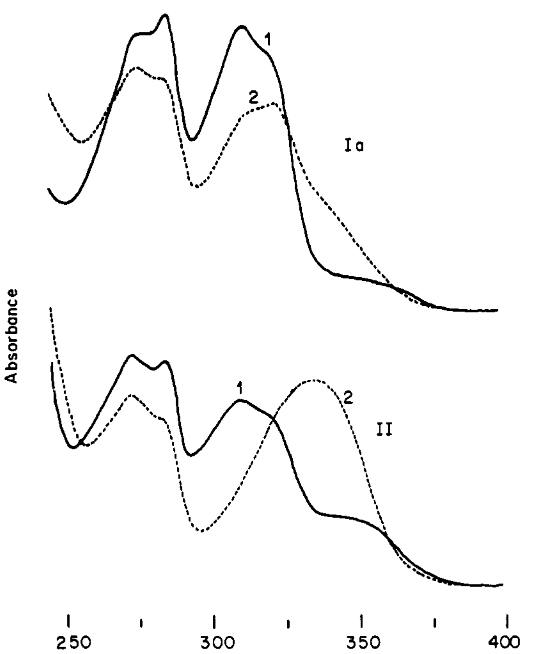


Figure 2. Effect of metal ion on the spectra of Ia and II. Upper panel: (1) ester Ia at pH 7.5 without metal ion; (2) ester Ia at pH 7.5 and 5×10^{-3} M Co^{2+}. Lower panel: (1) II at pH 7.5 without metal ion; (2) II at pH 7.5 and 5×10^{-3} M Co^{2+}.

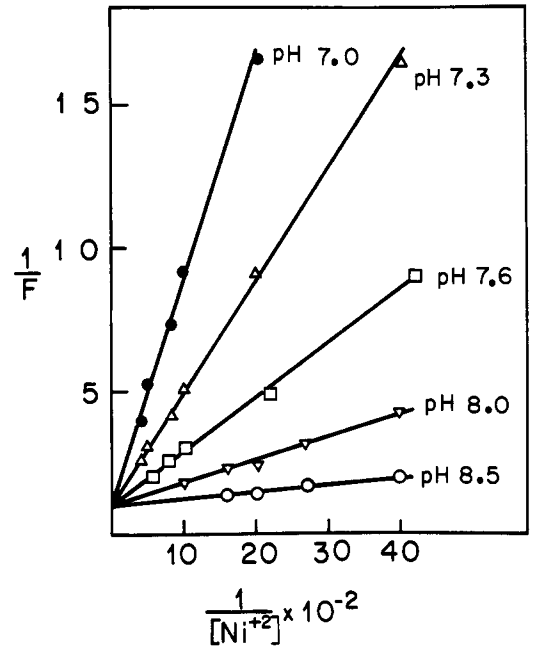


Figure 3. Effect of pH and $[Ni^{2+}]$ on the fraction of ester Ia as the metal complex (c)—c = III + C + D (see Appendix).

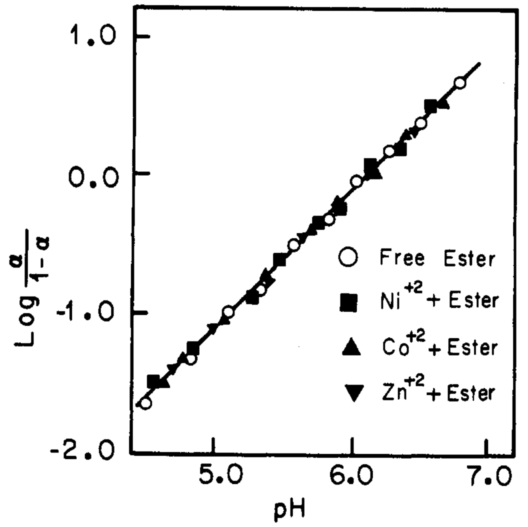


Figure 4. Effect of metal ions on the pK_1 (see eq A-14).

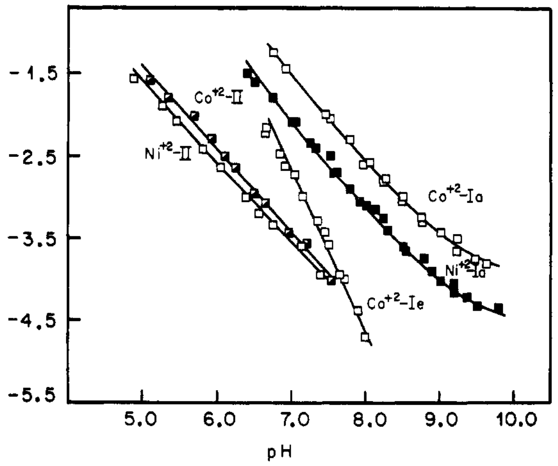


Figure 5. pH dependence of the metal-substrate dissociation constant (K_{pH}) of Co^{2+}-Ia, Co^{2+}-Ie, Co^{2+}-II, Ni^{2+}-Ia, and Ni^{2+}-II complexes. Curves were calculated as detailed in the Appendix (i.e., eq A-10 and A-17).

Table I. Values of K_m, K_6, and K_4 Obtained by Iterative Fitting of the pH Dependence of the Metal Substrate Dissociation Constants (K_{pH}) to Equations A-10, A-17, and A-21

A. Binding to Esters[a]

Metal[b]	Eq	Ester	Log K_m	pK_6
Ni^{2+}	A-10	Ia	4.85	9.6
Co^{2+}	A-10	Ia	5.42	9.6
Co^{2+}	A-10	Ie	5.10	6.25
Cd^{2+}	A-10	Ia	5.62	>10
Zn^{2+}	A-21	Ia	6.075	

B. Binding to Carboxylic Acid (Eq A-17)[c]

Metal	Log K_m	pK_4
Ni^{2+}	3.40	6.9
Co^{2+}	3.58	6.8
Zn^{2+}	5.05	4.0
Cd^{2+}	4.12	6.95

[a] For esters pK_1 = 6.1, pK_2 = 9.6. [b] pK_x, Ni^{2+} = 9.4, Co^{2+} = 9.4, Zn^{2+} = 8.5, Cd^{2+} = 9.2. [c] For carboxylic acid pK_1 = 3.2, pK_2 = 6.8.

Table II. pH-Independent Metal–Substrate Dissociation Constants

A. Esters			B. Carboxylic Acid (II)	
Metal	Ester	Log K_{dis}	Metal	Log K_{dis}
Ni^{2+}	Ia	−4.75[a]	Ni^{2+}	−3.40[c]
Co^{2+}	Ia	−4.18[a]	Co^{2+}	−3.22[c]
Co^{2+}	Ie	−4.50[a]	Zn^{2+}	−1.75[c]
Cd^{2+}	Ia	−3.98[a]	Cd^{2+}	−2.69[c]
Zn^{2+}	Ia	−13.43[b]		

[a] Equation 7. [b] Equation 8. [c] Equation 9.

$1/[M_T]$ are linear at all pHs, a 1:1 complex of ester and metal is indicated. For example, a 2:1 complex would only give linear plots if $1/F$ were plotted vs. $1/[M_T]^{1/2}$, etc.

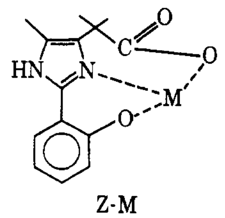
In Figure 4 the left-hand side of eq 5

$$\log \frac{\alpha}{1-\alpha} = \log K_1 + \text{pH} \qquad (5)$$

(see Appendix A-14 for derivation) is plotted as a function of pH. In this equation α = fraction of ester in form A and $1 - \alpha$ = fraction of ester in form B. In Figure 4, pK_1 = pH at that point where $\log(\alpha/(1-\alpha)) = 0$ (eq 5). Since the presence of the various metal ions does not affect the fit to the line of Figure 4, it is obvious that metal ions have no effect on pK_1. This provides further evidence that metal ion complexation of A and B cannot be important (see eq A-14, Appendix).

In the case of the carboxylic acid (II), metal titration data show that (1) pK_1 is not affected by metal ions indicating that complexation does not occur to W or X; (2) the spectrum of the metal complex is identical with that of Z; and (3) both pK_2 and pK_3 (eq 4) are lowered by the presence of the metal ion. These data suggest metal complexation to Y with loss of the proton from the phenolic hydroxyl to give the metal complex of Z.

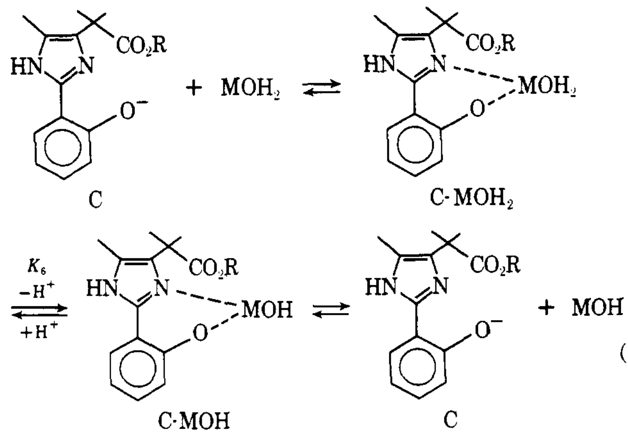
Plots of the pH-dependent metal-substrate dissociation constant (K_{pH}) vs. pH for the Ni^{2+}-II, Co^{2+}-Ia, Co^{2+}-Ie, and Co^{2+}–II complexes are shown in Figure 5. Data were collected either by titration with metal ion at constant pH (see eq A-9, Appendix) or by pH titration at constant metal ion concentration (see eq A-11, Appendix). The points for the metal-ester complexes were fit to eq A-10 (Appendix) and the points for metal-II complexes were fit to eq A-17 (Appendix) using the constants given in Table I. These data (Figure 5) for metal-II complexes are also consistent with the binding of the metal ion to ionic form Y of the carboxylic acid (eq 4) with simultaneous or stepwise loss of a proton from the phenolic hydroxyl group to form Z-M. It is assumed that the metal ion is coordinated to all three ionized groups; however, the carboxylic acid (II) is not sufficiently soluble in water to allow unequivocal spectral identification of the metal coordination number in Z-M. Nevertheless, it is assumed that octahedral complexation is present, because a 1×10^{-3} M II-Co^{2+} solution failed to show spectral characteristics of four- or five-coordinate Co^{2+}. Based on published extinction coefficients (Table II of ref 11) four and probably five coordination should have been detectable.



Z-M

In order to properly analyze the pH-dependent metal-ester dissociation constant (K_{pH}), account must be taken of the hydrolysis of the metal aquo complex

$$M(H_2O)_6 \overset{pK_x}{\rightleftharpoons} M(H_2O)_5OH + H^+$$



(6)

and the equilibria of eq 6. Determinations of pK_x are provided in the Experimental Section. Binding of only MOH to the ester gives an equation which will not fit the data because the slope obtained when log K_{pH} (calculated according to eq A-23) is plotted vs. pH is found to be ~2 instead of ~1 as required by the experimental data (Figure 5). The important point is that both the aquo (C-MOH$_2$) and hydroxo (C-MOH) metal ion complexes of the ester (eq 6) must be taken into account in order to arrive at eq A-10. It has been previously established that metal ion complexation to imidazole occurs at the pyridine-like nitrogen and does not involve the loss of a proton.[12] Though the second pK_a of imidazole (to form imidazole anion) is depressed from 14.45 to 10.34 on complexing to metal ion (to the Fe^{2+} in myoglobin)[13] cognizance of this feature need not be taken in the pH range employed.

In eq A-10, K_m (= [B][MOH$_2$]/[C-MOH$_2$]a_H) controls the overall fit of the curve to the data points whereas pK_6 controls the fit above pH 8.5. For the Ni^{2+} and Co^{2+} complexes of Ia, pK_6 = 9.6, whereas for the Co^{2+} complex of Ie, pK_6 = 6.25. The presence of the ester phenyl ring near the metal (π complex?) may account for this change in pK_6. In the case of the Cd^{2+}-Ia complex pK_6 was greater than 10, but its value could not be accurately assessed. Based on spectral evidence similar to that described above for the II-Co^{2+} complex, it may be assumed that the metal ion is also octahedrally coordinated in the ester complex.

For the complexation equilibria described above for Co^{2+} and Ni^{2+}, K_{pH} was independent of ester concentration. For Zn^{2+} complexation different results were obtained as shown in Figure 6. The data of Figure 6 were fit to eq A-21, derived for an (ester)$_2$M complex, by employing the parameters listed in Table I. The pH dependence of K_m for the Zn^{2+}-(II)$_2$ complex is similar to that of the Co^{2+}-II and Ni^{2+}-II complexes, with the exception that the ionization of the phenolic group (pK_5) of the aquo complex (eq A-17) was lower (see Table I for the parameters). Zinc has been noted to show an increasing affinity for imidazole[12,14] and other nitrogen ligands[15] as the degree of complex formation increases.

Equations A-10, A-17, and A-21 are convenient for iterative fitting; however, the value of K_m has little physical meaning. For purposes of comparison to other metal-ligand systems, values of the metal-substrate dissociation constants for the following equilibria are presented in Table II.

Esters plus all metals except Zn^{2+}

$$\text{C-MOH}_2 \rightleftharpoons \text{C} + \text{MOH}_2 \qquad K_{dis} = \frac{[\text{C}][\text{MOH}_2]}{[\text{C-MOH}_2]} = K_m K_2 \tag{7}$$

Esters plus Zn^{2+}

$$(\text{C})_2\text{-M} \rightleftharpoons 2\text{C} + \text{MOH}_2$$

$$K_{dis} = \frac{[\text{C}]^2[\text{MOH}_2]}{[(\text{C})_2\text{-M}]} = \frac{1}{2}K_m(K_2)^2 \tag{8}$$

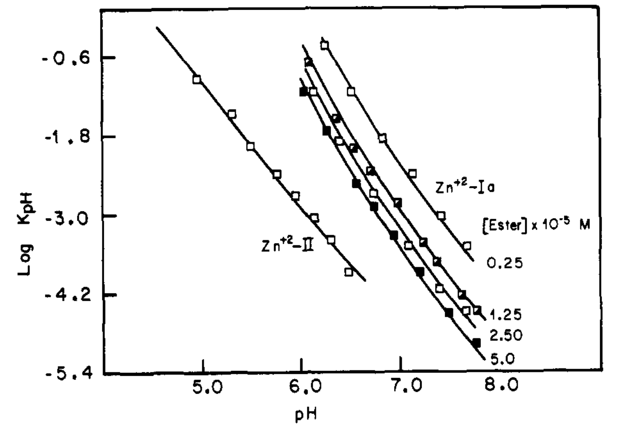


Figure 6. pH dependence of the dissociation constant (K_{pH}) for Zn^{2+}-Ia as a function of ester concentration and for Zn^{2+}-II. (Curves were calculated according to eq A-17 and A-21.)

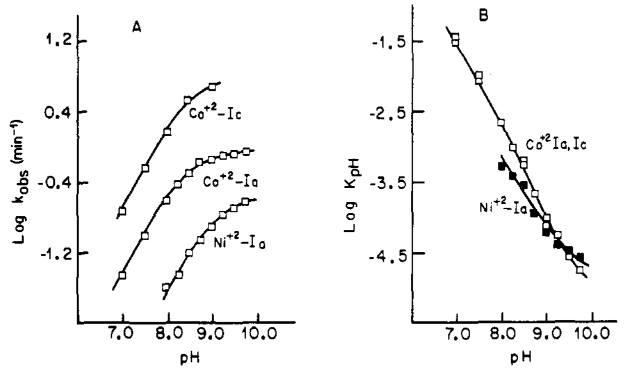


Figure 7. (A) pH-rate profiles for hydrolysis of Co^{2+}-Ic, Co^{2+}-Ia, and Ni^{2+}-Ia. (B) pH dependence of the kinetically determined metal ester dissociation constant (K_{pH}) for Co^{2+}-Ic, Co^{2+}-Ia, Ni^{2+}-Ia.

Carboxylic acid with all metals

$$\text{Y-MOH}_2 \rightleftharpoons \text{Y} + \text{MOH}_2 \qquad K_{dis} = \frac{[\text{Y}][\text{MOH}_2]}{[\text{Y-MOH}_2]} = K_m K_2 \tag{9}$$

Kinetic Studies. The apparent first-order rate constant (k_{app}) for hydrolysis of esters at any pH increases with increasing metal ion concentration until saturation of the ester with metal ion occurs. Plots of 1/k_{app} vs. 1/[M$_T$] were linear at all pHs studied. The value of 1/k_{app} at 1/[M$_T$] = 0 gives 1/k_{obsd}, where k_{obsd} is the first-order rate constant for hydrolysis of the ester-metal complex. The kinetically determined and pH-dependent metal-ester dissociation constant (K_{pH}) is given by the ratio of the slope of the 1/k_{app} vs. 1/[M$_T$] plot to the intercept at 1/[M$_T$] = 0. Figure 7A contains plots of log k_{obsd} vs. pH for Ni^{2+}-Ia, Co^{2+}-Ia, and Co^{2+}-Ic complexes. The experimental data were fit to

$$k_{obsd} = k\frac{K_a}{K_a + a_H} \tag{10}$$

using the following constants: Ni^{2+}-Ia, k = 0.29 min^{-1}, pK_a = 9.1; Co^{2+}-Ia, k = 0.92 min^{-1}, pK_a = 8.4; Co^{2+}-Ic, k = 6.45 min^{-1}, pK_a = 8.5. In Figure 7B the determined values of log K_{pH} are plotted vs. pH. The data were fit to eq A-10 using the same parameters given in Table I except that the value of the kinetically apparent pK_a for ionization of the ester metal aquo complex (pK_6) was 9.2 for the Ni^{2+}-Ia complex and 8.5 for the Co^{2+}-Ia and Co^{2+}-Ic complexes. The fact that pK_6 determined kinetically is significantly less than that determined titrimetrically is of particular importance.

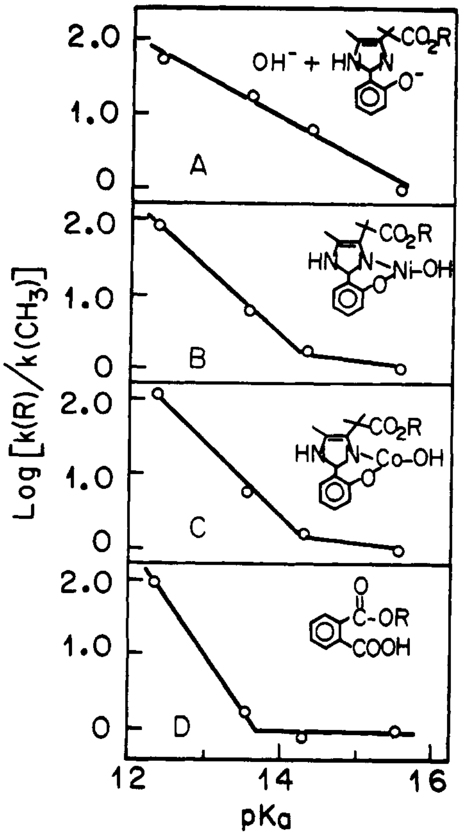


Figure 8. Effect of leaving group pK_a on the rate constants for hydrolysis of esters Ia-d. (A) OH^--catalyzed reaction. (B) Ni^{2+}-catalyzed reaction (pH 8.0). (C) Co^{2+}-catalyzed reaction (pH 7.0). (D) Hydrolysis of monophthalate esters (Thanassi and Bruice, ref 16) with neighboring COO^- and COOH participation.

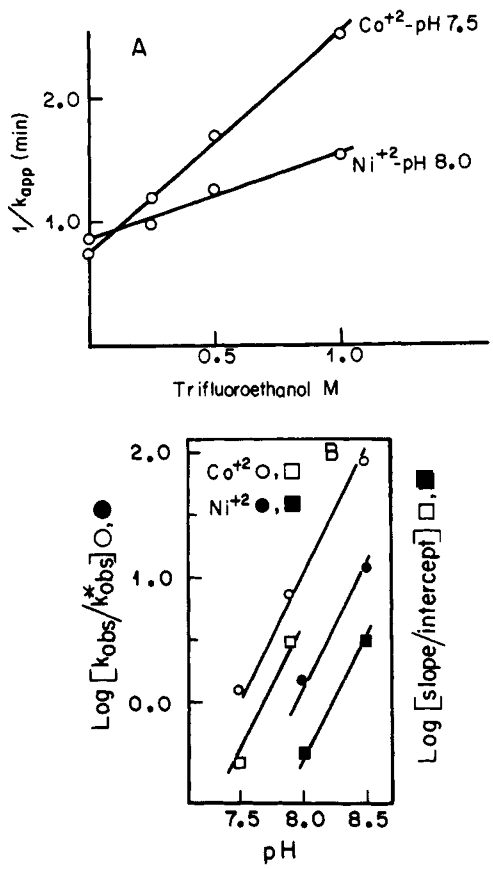


Figure 9. (A) Effect of trifluoroethanol concentration on the metal ion catalyzed hydrolysis of Id-Co^{2+} (1.25×10^{-3} M) catalyzed reaction at pH 7.5, Ni^{2+} (5×10^{-4} M) catalyzed reaction at pH 8.0. (B) (□) Log [slope/intercepts] from plots of $1/k_{app}$ vs. [ROH] Co^{2+} and Ni^{2+} catalyzed hydrolysis of ester Id in the presence of CF_3CH_2OH. (○) Log ratio k_{obsd} (in the absence of alcohol)/k^*_{obsd} (in the presence of alcohol) for Co^{2+} and Ni^{2+} catalyzed hydrolysis of ester Id ([CF_3CH_2OH] = 0.25 M).

In that portion of the log k_{obsd} vs. pH profile (Figure 7A) which has a slope of +1 the metal-catalyzed hydrolysis of esters Ia-d exceeds that for HO^- catalysis by 10^3-10^5 depending on the ester and the metal ion. The observation that the rate enhancement for catalysis by a particular metal ion depends on the ester studied results from the fact that the logarithm of the rate constants associated with metal catalysis does not exhibit a linear dependence on the pK_a of the leaving alcohol, whereas the logarithms of the rate constants for HO^- catalysis do. These data are shown in Figure 8 where plots of log [k(R)/k(CH_3)] (where k(R) = rate for various esters and k(CH_3) = rate for the methyl ester) vs. the pK_a of the alcohols from which esters Ia-d were prepared are compared for the HO^--catalyzed reaction, the Ni^{2+}-catalyzed reaction at pH 8.0, and the Co^{2+}-catalyzed reaction at pH 7.0. Also included in Figure 8 for comparison are data for neighboring carboxyl-catalyzed hydrolysis of the phthalate half-esters of the same alcohols.[16]

The rates of hydrolysis of the Co^{2+} complex of Ie and Zn^{2+} complexes of esters Ia-e in general were found to be very slow. Thus, although the rate constant for HO^- hydrolysis of Ie is large ((k_{OH^-} = 820 vs. 1.63 (Ia); 1.98 (Ib); 26.7 (Ic), and 82.9 M^{-1} min^{-1} (Id)), k_{obsd} for hydrolysis of the Co^{2+} complex of Ie at pH 8.0 was but 9×10^{-3} min^{-1}. This amounts to only a ~40-fold rate enhancement over the HO^--mediated reaction at this pH. Based on the data in Figure 6, the rate constants for Co^{2+}-Ie hydrolysis at pH 8.0 would be predicted to be ~3×10^4 min^{-1}. Thus the rate of hydrolysis of Co^{2+}-Ie is ca. 10^6 slower than anticipated from the data of Figure 8. No detectable hydrolysis of the Zn^{2+}-Ia dimer occurred. However, with Zn^{2+}-$(Id)_2$, k_{obsd} at pH 7.5 was 1.7×10^{-2} min^{-1} compared to 11.4 min^{-1} for Co^{2+}-Id and 0.65 min^{-1} for Ni^{2+}-Id. Since this was the highest pH at which Zn^{2+} could be studied without precipitation of $Zn(OH)_2$ further studies were not conducted on the $Zn^{2+}(ester)_2$ complexes. At pH 8.5 the rate of hydrolysis of Cd-Ia complex was too slow to measure; however, the Cd-Id complex gave k_{obsd} = 0.193 min^{-1}. No detectable hydrolysis of Ia with 1×10^{-3} M Ca^{2+}, Mg^{2+}, or Mn^{2+} at pH 9.5 was observed.

Transesterification Reactions. The metal ion catalyzed hydrolysis of the trifluoroethyl ester (Id) was markedly inhibited by the addition of trifluoroethanol. This is illustrated as a plot of $1/k_{app}$ vs. [trifluoroethanol] in Figure 9 (upper panel) for the Co^{2+}-Id reaction at pH 7.5 (1.25×10^{-3} M Co^{2+}) and the Ni^{2+}-Id reaction at pH 8.0 (5×10^{-4} M Ni^{2+}). At constant pH and trifluoroethanol concentration, plots of $1/k_{app}$ vs. $1/[M_T]$ were linear as is the case in the absence of added alcohol. The ratio of the slope/intercept of these plots gives the kinetically determined pH-dependent metal-ion ester dissociation constant (K_{pH}), which, as shown in Table III, is not affected by the presence of trifluoroethanol. The intercept at $1/[M_T] = 0$ provides $1/k_{obsd}$ which is markedly affected by the alcohol. These latter data are presented in Figure 9 (lower panel) as plots of log (k_{obsd}/k^*_{obsd}) vs. pH where k_{obsd} = rate constant observed in the absence of alcohol and k^*_{obsd} = rate constant observed in the presence of alcohol (eq A-41). The slope of this plot ≈ 2. Also shown in Figure 9 (lower panel) is a plot of the logarithm of the ratio of the slope/intercept of plots of $1/k_{app}$ vs. [trifluoroethanol] at constant metal ion concentration vs pH. This plot has a slope of 2.

Inhibition by trifluoroethanol could result from competition between the ester and the alcohol for metal ion. That this is not

Table III. Kinetically Determined Co^{2+}–Substrate Dissociation Constants (K_{pH})

	Log K_{pH}			Id + CF_3CH_2OH at given concn		
pH	Ia	Ic	Id	Log K_{pH}	M	Log K_{pH} required if inhibition is due to competition of CF_3CH_2OH with Id for Co^{2+} (alcohol at 0.25 M)
7.0	−1.50	−1.45	−1.46			
7.5	−2.00	−1.96	*a*	−2.00	1.0, 0.25	−1.47
8.0	−2.65	−2.84	*a*	−2.65	0.5, 0.25	−1.00
8.5	−3.25	−3.41	*a*	−3.42	0.25, 0.1	−0.10

[a] Reactions too rapid to follow at these pH values.

Table IV. Characterization of the Products Formed during the Co^{2+}-Catalyzed Hydrolysis of Esters Ia and Id in the Presence of *p*-Chlorophenol

Ester and incubation conditions	k_{OH}, M^{-1} min^{-1}, of ester remaining after incubation	% ester[a] remaining	Identity of[b] ester remaining
Trifluoroethyl ester (Id)[c]			
(a) pH 8.5, no additions	78.1	>90	Id
(b) pH 8.5, 5×10^{-4} M Co^{2+}		0	Free acid
(c) pH 8.5, 1×10^{-4} M *p*-chlorophenol	83.9	>90	Id
(d) pH 8.5, 5×10^{-4} M Co^{2+}, 1×10^{-4} M *p*-chlorophenol	830	10	Ie
Methyl ester (Ia)[d]			
(a) pH 8.5, no additions	1.58	>90	Ia
(b) pH 8.5, 5×10^{-4} M Co^{2+}			Free acid
(c) pH 8.5, 1×10^{-4} M *p*-chlorophenol	1.65	>90	Ia
(d) pH 8.5, 5×10^{-4} M Co^{2+}, 1×10^{-4} M *p*-chlorophenol	800	12	Ie
p-Chlorophenyl ester (Ie)[d]			
(a) pH 8.5, no additions	825	>90	Ie
(b) pH 8.5, 5×10^{-4} M Co^{2+}	805	>90	Ie

[a] Based on $\Delta_{am}{}^{335} = 5 \times 10^3$ for Ia,d and 4.9×10^4 for Ie. [b] Based on k_{OH}. [c] Reaction time 2 min. [d] Reaction time 10 min. Reaction stopped by addition of $HClO_4$ and EDTA. See text for details.

the case was shown by the fact that the pH-dependent metal ion–ester dissociation constant (K_{pH}), determined kinetically, was not affected by the presence of the alcohol. The data for the Co^{2+} reactions, in the presence of trifluoroethanol, along with kinetic K_{pH} data for Co^{2+}–Ia, Co^{2+}–Ic, and Co^{2+}–Id in the absence of trifluoroethanol are provided in Table III. Also shown in Table III are the calculated values of log K_{pH} which would be necessary in order to explain the inhibition data if complexation of metal ion to trifluoroethanol were in effect. These data clearly show that competition between ester and alcohol for metal ion are unimportant. In addition it was also shown that 0.5 M trifluoroethanol at pH 7.5 caused no detectable inhibition of the Co^{2+}-catalyzed hydrolysis of Ic. The apparent rate constant for hydrolysis of Id was decreased by a factor of 2 under these conditions. This experiment also eliminates alcohol–ester competition for metal ion as a source of alcohol inhibition of ester metal complex hydrolysis. If trifluoroethanol transesterification of Ic to yield Id occurred, it would not have been detected since hydrolysis of Id is more rapid than hydrolysis of Ic.

The data in Figure 9 suggest that trifluoroethanol inhibition of hydrolysis is due to the resultant re-formation of M^{2+}–Id by trapping of a reactive intermediate by $CF_3CH_2O^-$. Attempts to trap the presumed intermediate, formed during Co^{2+}–Id hydrolysis, by using propargyl alcohol in the hopes of obtaining the propargyl ester (Ic) were not completely successful. When the hydrolysis of Co^{2+}–Id was carried out in the presence of propargyl alcohol (0.5 M) the initial rate of disappearance of Id was observed to be the same as in the absence of propargyl alcohol (0.82 vs. 0.89 min^{-1}). The detection of the presence of Co^{2+}–Ic through determination of the rate constant for the second slower reaction was obscured by a slow precipitation of Co^{2+} in the presence of propargyl alcohol.

Definitive evidence for the formation of an intermediate, upon hydrolysis of metal ion complexed ester, which is capable of undergoing trans acylation was obtained using *p*-chlorophenol as a trapping agent. For these experiments, esters Ia and Id were employed. This experiment depends on the following: (1) ester Ie shows minimal Co^{2+}-catalyzed hydrolysis so that if it is formed by reaction with a reactive intermediate present during the Co^{2+}-catalyzed hydrolysis of other esters, it will not be significantly hydrolyzed; (2) k_{HO} for Ie in the absence of metal is large and since Δa_m for hydrolysis of Ie at 335 nm = 4.9×10^4, as compared to 5×10^3 for the aliphatic esters, the presence of Ie is easily detected by spectrophotometric monitoring of its hydrolysis (after removal of metal ion). The following experiments were carried out at pH 8.5 (0.2 M $NaClO_4$) using 20 mL of 2.5×10^{-5} M ester: (a) ester alone; (b) ester + 5×10^{-4} M Co^{2+}; (c) ester + 1×10^{-4} M *p*-chlorophenol; (d) ester + 5×10^{-4} M Co^{2+} + 1×10^{-4} M *p*-chlorophenol. For ester Ia incubation the reaction time was 10 min and for Id 2 min. These times correspond to ~95% hydrolysis of these esters under condition (b) above. In addition, ester Ie was incubated for 10 min under conditions (a) and (b). At the end of the reaction period the hydrolytic reactions were quenched by adjusting to pH ~4 with $HClO_4$ and made 5×10^{-3} M in EDTA. Excess *p*-chlorophenol was removed by extracting four times with hexane and the aqueous phase was freed of hexane by bubbling N_2 through the solution for 1 h. The HO^--catalyzed hydrolysis of any esters present was monitored spectrophotometrically after diluting 5 mL of the extracted solution to 20 mL with 0.2 M $NaClO_4$ and adjusting the pH with concentrated NaOH. In those cases where no ester hydrolysis was observed, titration of another aliquot of the original reaction mixture confirmed the presence of the acid (II). The results of these experiments are shown in Table IV.

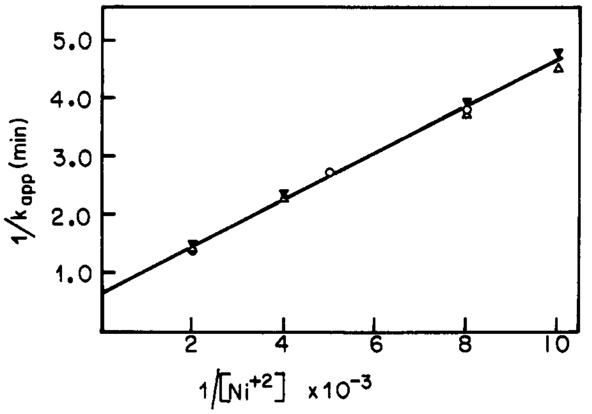


Figure 10. Effect of hydroxylamine on the Ni^{2+}-catalyzed hydrolysis of Id: (○) reaction in the absence of NH_2OH; (△) reaction in the presence of NH_2OH not corrected for NH_2OH attack on uncomplexed ester; (▼) reaction in the presence of NH_2OH, corrected for NH_2OH attack on uncomplexed ester.

In all cases where ester hydrolysis was detectable a single first-order reaction was observed whose associated rate constant was identical with that of authentic Ie or unreacted starting material. These data clearly show that in the presence of *p*-chlorophenol and Co^{2+} both esters Ia and Id are converted in about 10% yield to the *p*-chlorophenyl ester. In the absence of Co^{2+} there was no detectable production of Ie. Any mechanism which requires the phenolate ion to be bound to Co^{2+}-Ia,d is made highly unlikely by the observation that at pH 8.5 a 100-fold excess of Co^{2+} did not significantly alter the spectrum of *p*-chlorophenol (at 1×10^{-4} M *p*-chlorophenol). Nucleophilic attack of *p*-chlorophenol on Id in the absence of Co^{2+} did not occur to any measurable extent. Based on Δa_{335} for hydrolysis of Ie, the presence of 1% Ie in the presence of Id could easily have been detected.

Metal ion catalysis of ester hydrolysis can occur by either mechanism a or b (introductory section). If metal ion activation of the ester to HO^- attack occurs then activation of ester to nucleophilic attack by NH_2OH should also be seen. It is not to be expected that hydroxylamine would favorably compete, as a ligand, with H_2O and HO^- for ester complexed metal ion.[2b] In order to achieve a reasonable rate of ester hydroxylaminolysis at a pH where metal ion catalyzed hydrolysis could also be observed, the trifluoroethyl ester (Id) was used. At pH 7.75 and 0.05 M NH_2OH-$HClO_4$, $k_{NH_2OH} = 1.09 \times 10^{-2}$ min^{-1}. This is 2.3×10^2 greater than $k_{HO}[HO^-]$ at this pH. If metal ion is complexed to the ester carbonyl group then the kinetics for hydroxylaminolysis and HO^--mediated hydrolysis are given by

$$-\frac{d[Id]}{dt} = k_N'[NH_2OH][E] + \{k_N^2[NH_2OH] + k_{HO}[OH^-]\}(M\text{-}E) \quad (11)$$

where k_N' = rate constant for attack of NH_2OH on the uncomplexed ester, k_N^2 = rate constant for attack of NH_2OH on the complexed ester, and k_{HO} = rate constant for attack of HO^- on the complexed ester. In the event that metal ion catalysis is due to intramolecular attack of metal-bound HO^- the rate of disappearance of Id is given by

$$-\frac{d[Id]}{dt} = k_N'[NH_2OH][E] + k^*[E\text{-}MOH] \quad (12)$$

where k^* = rate constant for the intramolecular reaction. Under the conditions of this experiment $k_N'[NH_2OH]$ was determined to be 1.09×10^{-2} min^{-1} and assuming $k_{HO}[HO^-]$ is 1.84 min^{-1} then $k_N^2[NH_2OH]$ would be predicted to be $\sim 4.2 \times 10^2$ min^{-1} based on the difference between $k_N'[NH_2OH]$ and $k_{HO}[HO^-]$ for uncomplexed ester. Thus, if metal ion complexes the ester carbonyl oxygen it is to be expected that hydroxylaminolysis would occur in the stopped-flow time range. In Figure 10 is shown a comparison of the Ni^{2+}-catalyzed hydrolysis of ester Id in the presence and absence of 0.05 M NH_2OH-$HClO_4$. For the reactions carried out in the presence of NH_2OH both $1/k_{app}$ and $1/(k_{app} - k_N'[NH_2OH])$ are plotted vs. $1/[Ni^{2+}]$, whereas in the absence of NH_2OH, $1/k_{app}$ vs. $1/[Ni^{2+}]$ is plotted. All the data points may be seen to fit a single line. Since neither the slope nor intercept of the plot of Figure 10 is changed on addition of NH_2OH we may conclude that NH_2OH does not influence the binding of metal ion to ester nor the rate of its hydrolysis. Correction of the data for $k_N'[NH_2OH]$ does not significantly alter the results and it can be seen that the presence of NH_2OH does not significantly increase the rate of ester disappearance. In the absence of NH_2OH, k_{obsd} at $[M_T] = \infty$ was 1.84 min^{-1} and $\log K_{pH} = -3.18$; in the presence of NH_2OH, without correction for $k_N'(NH_2OH)$, $k_{obsd} = 1.85$ min^{-1}, $\log K_{pH} = -3.09$, and with correction for $k_N'(NH_2OH)$, $k_{obsd} = 1.81$ min^{-1}, $\log K_{pH} = 3.10$. Further, the change in absorbance at 335 nm during the course of reaction either in the presence or absence of NH_2OH was found to be the same within ±10%. This eliminates the possibility that the bulk of product was formed very rapidly by the k_N^2 pathway. These data establish that the mechanism of metal ion catalyzed hydrolysis is by intramolecular attack of metal-bound hydroxide. In addition, the lack of metal ion promoted hydroxylaminolysis makes it highly unlikely that the *p*-chlorophenol trapping experiments could be interpreted as being due to metal ion promoted transesterification by *p*-chlorophenol. The hydroxamic acid of II is rapidly hydrolyzed in the presence of metal ion.

Discussion

A complete description of the metal ion catalyzed hydrolysis of esters Ia-d must account for the following: (1) at saturation in metal ion the $\log k_{obsd}$ vs. pH rate profiles follow a titration curve which is interpretable in terms of the hydrolyzing species being the hydroxo-metal-ester complex formed by ionization of the aquo-metal-ester complex; (2) the observation that the kinetically determined pK_a is smaller than the titrimetrically determined pK_a of the aquo-metal ester complex; (3) that the kinetically determined metal-ester dissociation constant is significantly different from that determined thermodynamically; (4) the nonlinear dependence of $\log k_{obsd}$ upon the pK_a of the leaving alcohol; (5) the inhibition of hydrolysis of ester Id by the alcohol from which it was prepared; (6) the trapping of a reactive intermediate by *p*-chlorophenol in the Co^{2+}-catalyzed hydrolysis of esters Ia and Id; and (7) the lack of catalysis of hydroxylaminolysis of ester by metal ion.

From the pH dependence of metal-binding equilibria it may be concluded that two metal-ester species exist in solution over the pH range where kinetic data were collected. These are the aquo (C-MOH_2) and hydroxo (C-MOH) complexes (eq 6). The lack of effect of hydroxylamine on the metal ion catalyzed reaction shows that hydrolysis proceeds not by metal ion complexation of ester carbonyl (introduction, mechanism a), but by intramolecular attack of the metal-bound HO^- in C-MOH (mechanism b). The simplest mechanism would be

$$\text{C-MOH}_2 \underset{+H^+}{\overset{K_6 \atop -H^+}{\rightleftarrows}} \text{C-MOH} \xrightarrow{k_r} \text{hydrolytic products} \quad (13)$$

(C-MOH_2 refers to aquo complex and C-MOH to the hydroxo complex) for which

$$k_{obsd} = k_r \left[\frac{K_6}{K_6 + a_H}\right] \quad (14)$$

provides the correct shape of the log k_{obsd} vs. pH-rate profile but predicts that the kinetic and titrimetric values of pK_6 should be identical. Also, this mechanism predicts that the pH-dependent ester-metal ion dissociation constant (K_{pH}) determined kinetically would be the same as that determined titrimetrically (see Appendix, eq A-28 and A-29).

Bruice and Schmir[17] have shown that kinetically determined pK_as can differ from the thermodynamic values if either partner of the acid-base pair (C-MOH$_2$ and C-MOH) is involved in an additional equilibrium process before the rate-determining step. In general terms this equilibrium can involve the following sets of equations. For simplicity, we assume for this analysis that all reactions are carried out at infinite metal ion such that all the ester is present as the metal complex (eq 15). It is necessary that reaction occur with only one of the

$$\begin{tikzcd}
 & \text{C}'\text{—MOH}_2 \arrow[r, shift left, "K_e'"] \arrow[d, shift right, "K_6'\ -\text{H}^+"'] & \text{C—MOH}_2 \arrow[l, shift left] \arrow[d, shift right, "K_6-\text{H}^+"'] \\
\text{prod} & \text{C}'\text{—MOH} \arrow[l, "k_r"'] \arrow[u, shift right, "+\text{H}^+"'] \arrow[r, shift left, "K_e"] & \text{C—MOH} \arrow[l, shift left] \arrow[u, shift right, "+\text{H}^+"']
\end{tikzcd} \tag{15}$$

hydroxo complexes, for example, C′-MOH. Assuming pre-equilibrium in C-MOH$_2$, C′-MOH$_2$, C-MOH, and C′MOH and noting that $K_6K_e = K_6'K_e'$, the observed rate constant is given by

$$k_{\text{obsd}} = \frac{k_rK_6K_e}{a_H(1+K_e')+K_6(1+K_e)} = \frac{k_rK_e}{1+K_e}\,\frac{K_6\dfrac{(1+K_e)}{(1+K_e')}}{a_H+K_6\dfrac{(1+K_e)}{(1+K_e')}} \tag{16}$$

Three cases can be considered: (1) $K_e = K_e'$, which means $K_6 = K_6'$ and there will be no difference between the kinetic and thermodynamic pK_a; (2) $K_e' > K_e$, which means that the reaction path is C-MOH$_2$ → C′-MOH$_2$ → C′-MOH → products. In this case eq 16 becomes

$$k_{\text{obsd}} = \frac{k_rK_e}{1+K_e}\,\frac{\dfrac{K_6}{1+K_e'}}{a_H+\dfrac{K_6}{1+K_e'}} \tag{17}$$

which predicts that the K_6 determined kinetically, $K_6[K_6/(1+K_e)]$, is smaller than that determined thermodynamically. This is not in accord with the data; (3) $K_e > K_e'$, which means that the reaction path is C-MOH$_2$ → C-MOH → C′-MOH → products. In this case eq 13 becomes

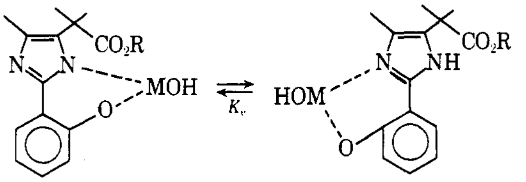
$$k_{\text{obsd}} = \frac{k_rK_e}{1+K_e}\,\frac{K_6(1+K_e)}{K_6(1+K_e)+a_H} \tag{18}$$

which predicts that K_6 determined kinetically ($K_6(1+K_e)$) is larger than that determined thermodynamically. This is in accord with the experimental findings. Furthermore, the kinetically determined metal-substrate dissociation constant, K_{pH}, which is given by

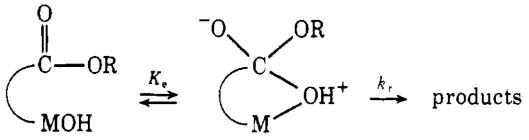
$$K_{\text{pH}} = \frac{K_m a_H\left(1+\dfrac{a_H}{K_1}+\dfrac{K_2}{a_H}\right)\left(1+\dfrac{K_x}{a_H}\right)}{\left[1+\dfrac{K_6(1+K_e)}{a_H}\right]} \tag{19}$$

(see Appendix, eq A-34, A-35) predicts that the kinetic apparent K_6 of the ester-MOH$_2$ ⇄ ester-MOH (apparent $K_6 = K_6(1+K_e)$) will increase. This result is also in accord with the experimental data. Thus, among the equilibria considered, the only explanation for the observation that the pK_a of ester-MOH$_2$ is lower in the kinetic experiments than in the thermodynamic experiments is that ester-MOH is involved in an equilibrium following formation from ester-MOH$_2$ but before the rate-determining step. Equation 18 can be used to fit the pH-rate profiles of Figure 7A using the thermodynamic pK_a for ester-MOH$_2$ (pK_6 = 9.6) with $k_r = 1.05$ min^{-1}, $K_e = 14.5$ for Co^{2+}-Ia; $k_r = 7.0$ min^{-1}, $K_e = 11.6$ for Co^{2+}-Ic; $k_r = 0.6$ min^{-1}, $K_e = 2.5$ for Ni^{2+}-Ia. Equation 19 can be used to fit the kinetic K_{pH} data of Figure 7B using the thermodynamic value for pK_6 and $K_e = 13.5$ for the Co^{2+} reactions and 1.5 for the Ni^{2+} reactions. Thus, we assign a value of $K_e \sim 14$ for Co^{2+}-Ia and Co^{2+}-Ic and $K_e \approx 2$ for Ni^{2+}-Ia.

Three possibilities for the equilibrium process of C-MOH ⇄ C′MOH (eq 15) may be considered. (1) Rotation about the imidazole–phenyl bond (eq 20). This possibility may be ruled out since the requirement that $K_e > K_e'$ is tantamount to the assumption that the hydroxo–metal complex exists predominantly in the C-MOH isomeric form whereas the aquo metal complex exists predominantly in form C′-MOH$_2$. (2) Change in metal coordination, e.g., six to five coordination. Again, it does not seem reasonable that the hydroxo complex should favor a coordination number change in comparison to the aquo complex. (3) Formation of a tetrahedral intermediate (eq 21).



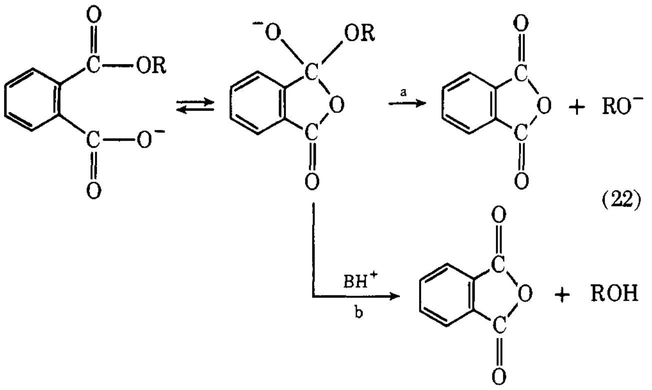
C′-MOH C-MOH (20)



C-MOH C′-MOH (21)

In this case only the hydroxo complex can be involved. This mechanism is tenable if the equilibrium between the hydroxo complex and the tetrahedral intermediate is slowly established with respect to the time required for titrimetric determination of K_{pH}, but rapidly established with respect to hydrolysis of the ester. These conditions are satisfied in the case of the methyl ester (Ia), the only ester for which it was possible to collect both kinetic and thermodynamic data.

In Figure 8 there is plotted the logarithm of the observed rate constants (pH 7.0 for Co^{2+}, pH 8.0 for Ni^{2+}) for hydrolysis of the metal complexes of esters Ia-d vs. the pK_a of the alcohols from which the esters were prepared. Examination of Figure 8 reveals that a sharp break in slope (from 0.15 to 1.07 for Co^{2+}; from 0.18 to 0.97 for Ni^{2+}) occurs near the point for ester Ic. Thanassi and Bruice,[16] in an examination of the kinetics of hydrolysis of monophthalate esters prepared from the same alcohols employed in the synthesis of Ia-d, noted a similar break in slope (from 0.11 to 1.47) in their plots of log k_{rate} vs. pK_a of the leaving alcohol moiety (Figure 8D). For the monophthalate esters prepared from alcohols of lower pK_a the rate of hydrolysis was dependent upon the mole fraction of ester possessing a neighboring COO$^-$ group, while for the esters prepared from alcohols of higher pK_a the rate of hydrolysis was dependent upon the mole fraction of ester possessing a neighboring COOH group. These results find interpretation[18] through the mechanism of eq 22 where the rate-determining steps involve departure of the leaving group to yield phthalic anhydride.[16] The expulsion of alkoxide ions of lower basicity occurs spontaneously (path a) while the rate-determining step for expulsion of the more strongly basic alkoxide ions requires

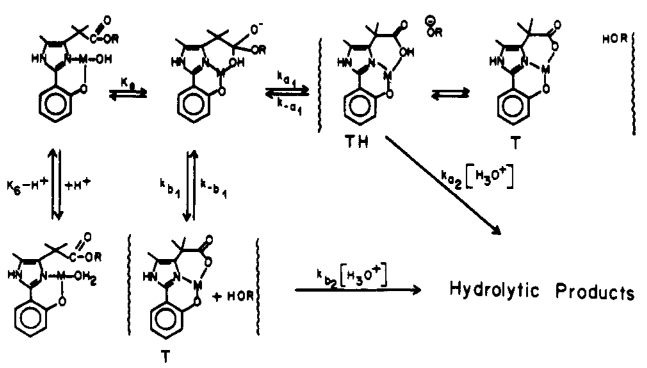


(22)

general acid catalysis (path b). A similar mechanism can be applied to the breakdown of the tetrahedral intermediate (eq 21) formed during metal–OH catalyzed hydrolysis of esters Ia–d.

The following observations establish that the metal ion complexed esters yield, via M–OH group participation, an intermediate on the way to hydrolytic products which possesses a carboxyl function susceptible to nucleophilic attack. By analogy with the anhydride intermediate in eq 22 we term this intermediate the *"metal-carboxyl anhydride"*. First, trifluoroethanol decreases the rate of hydrolysis of the trifluoroethyl ester (Id). Secondly, hydrolysis of the metal ion complexes of the most (Id) and least (Ia) reactive esters in the presence of *p*-chlorophenol yields the *p*-chlorophenyl ester. Neither trifluoroethanol nor *p*-chlorophenol react with the metal salts of the carboxylic acid (II). That direct reaction of *p*-chlorophenol with metal ion activated ester is not involved is established by the fact that metal ion complexation does not activate the esters to attack by hydroxylamine, and hydroxylamine is a better nucleophile than *p*-chlorophenol. These and all other experimental observations may be accounted for in the mechanism of Scheme I where path a pertains to hydrolysis of esters with moderately basic leaving groups and path b to hydrolysis of esters with strongly basic leaving groups. For path a, assumption of equilibrium formation of tetrahedral intermediate and steady state in TH and T leads to

Scheme I



$$k_{app} = \frac{k_{a_1}k_{a_2}a_H}{k_{-a_1}\dfrac{K_{ROH}}{a_H}[ROH] + k_{a_2}a_H} \times \left[\frac{\dfrac{[M_T]K_6K_e}{K_m\left(1+\dfrac{K_x}{a_H}\right){a_H}^2}}{\left(1+\dfrac{a_H}{K_1}+\dfrac{K_2}{a_H}\right)+\dfrac{[M_T]}{K_m\left(1+\dfrac{K_x}{a_H}\right)}\left[1+\dfrac{K_6}{a_H}(1+K_e)\right]\dfrac{1}{a_H}}\right] \quad (23)$$

Equation 23 relates the observed first-order rate constant to the pH, alcohol concentration, and metal ion concentration. In eq 23 K_{ROH} refers to the pK_a of the alcohol and other constants are defined as before (Scheme I and Appendix, eq A-37 and A-44). At constant concentration of alcohol, $1/k_{app}$ is related to $1/[M_T]$ by

$$1/k_{app} = \left(\frac{\left(1+\dfrac{a_H}{K_1}+\dfrac{K_2}{a_H}\right)K_m a_H\left(1+\dfrac{K_x}{a_H}\right)}{\dfrac{k_{a_1}K_6K_e}{a_H}} \times \left[1+\frac{k_{-a_1}}{k_{a_2}}\frac{K_{ROH}}{(a_H)^2}[ROH]\right]\right)\frac{1}{[M_T]} + \frac{a_H+K_6(1+K_e)}{k_{a_1}K_6K_e}\left[1+\frac{k_{-a_1}}{k_{a_2}}\frac{K_{ROH}}{(a_H)^2}[ROH]\right] \quad (24)$$

The pH-dependent metal–substrate dissociation constant (K_{pH}) given by the ratio of slope/intercept of such plots is given by

$$K_{pH} = \frac{\left(1+\dfrac{a_H}{K_1}+\dfrac{K_2}{a_H}\right)K_m a_H\left(1+\dfrac{K_x}{a_H}\right)}{1+\dfrac{K_6(1+K_e)}{a_H}} \quad (25)$$

which is the same as eq 19 and which predicts that the presence of alcohol does not affect K_{pH} as is experimentally observed. Ethanol (to 18% (v/v)) has previously been shown to have no effect on the association constants of imidazole metal ion complexes.[12] The intercept of eq 24 at $[M_T] = \infty$ (i.e., $1/k_{obsd}$) is given by

$$\frac{1}{k_{obsd}} = \frac{a_H+K_6(1+K_e)}{k_{a_1}K_6K_e}\left[1+\frac{k_{-a_1}}{k_{a_2}}\frac{K_{ROH}}{(a_H{}^2)}[ROH]\right] \quad (26)$$

which at [ROH] = 0 is identical with eq 18, which in turn has been established to correctly provide a fit to the plots of log k_{obsd} vs. pH. Thus, at [ROH] = 0 the rate-determining step in path a (Scheme I) is k_{a_1} while at increasing values of [ROH], k_{a_2} becomes rate limiting. For path b, k_{b_1} is rate limiting. The ratio of $1/k_{obsd}$ in the absence of added alcohol (k_{obsd}) to that (Figure 7) found in the presence of alcohol (k^*_{obsd}) is given by

$$\frac{k_{obsd}}{k^*_{obsd}} = 1 + \frac{k_{-a_1}}{k_{a_2}}\frac{K_{ROH}}{(a_H{}^2)}[ROH] \quad (27)$$

Inspection of eq 27 reveals that a plot of log $[k_{obsd}/k^*_{obsd}]$ vs. pH at constant [ROH] should have a slope of 2. Such is the case (Figure 9). Further, plots of $1/k_{app}$ vs. [ROH] at constant pH and metal ion concentration will be linear as shown by

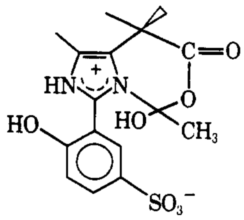
$$1/k_{app} = \left[\frac{\left(1+\dfrac{a_H}{K_1}+\dfrac{K_2}{a_H}\right)K_m a_H\left(1+\dfrac{K_x}{a_H}\right)}{[M_T]\dfrac{K_6K_e}{a_H}} + \frac{a_H+K_6(1+K_e)}{\dfrac{K_6K_e}{a_H}}\right]\frac{1}{k_{a_1}} + \left[\frac{\left(1+\dfrac{a_H}{K_1}+\dfrac{K_2}{a_H}\right)K_m a_H\left(1+\dfrac{K_x}{a_H}\right)}{[M_T]\dfrac{K_6K_e}{a_H}} + \frac{a_H+K_6(1+K_e)}{\dfrac{K_6K_e}{a_H}}\right]\frac{k_{-a_1}}{k_{a_1}k_{a_2}}\frac{K_{ROH}}{(a_H)^2}(ROH) \quad (28)$$

This may also be observed in Figure 9. This feature of saturation in the inhibition of k_{obsd} rules out any explanation for this phenomenon based upon a medium effect. Finally, the ratio of the slope to intercept of these plots is given by

$$\text{slope/intercept} = \frac{k_{-a_1}}{k_{a_2}} \frac{K_{\text{ROH}}}{(a_{\text{H}})^2} \qquad (29)$$

so that plots of logarithm of the ratio (slope/intercept) vs. pH should have a slope of 2 as was also observed (Figure 9). The rate of reaction of $CF_3CH_2O^-$ with *p*-nitrophenyl acetate is known to be 4.7×10^3 M^{-1} min^{-1} in water at 30 °C.[19] The constant k_{-a_1} is not likely to exceed this value since the pK_a of the conjugate acid of the leaving group (i.e., II-Co^{2+}-OH_2) exceeds the pK_a for *p*-nitrophenol by 2.5 units. At 0.25 M CF_3CH_2OH (pK_a = 12.36)[20] and assuming a value for k_{-a_1} of 10^2 M^{-1} min^{-1} it can be calculated that $k_{a_2} = 10^5$ M^{-1} min^{-1}. This value of k_{a_2} would be in the range anticipated for ligand exchange on protonation of the phenolic oxygen of TH.

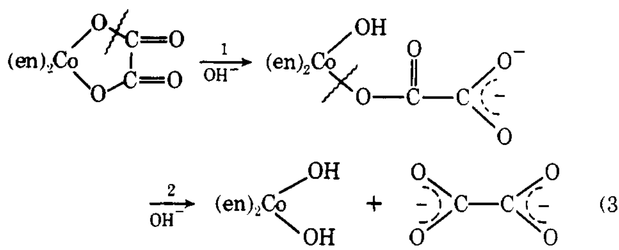
The *"metal-carboxyl"* anhydride intermediates (T and TH) of Scheme I must owe their existence in time to the trialkyl lock[21] (i.e., the *gem*-dimethyl groups and the 4(5)-methyl group). As shown in other instances[22] the trialkyl lock provides a great degree of rigidity to lactonelike ring systems and also a degree of compression within the ring systems. These two factors (rigidity and compression) should profoundly reduce the rate constant for carboxyl ligand exchange with H_2O and apparently provides a degree of covalent character (inner sphere) to the RCO_2-M bond. It should be noted that the trialkyl lock system as present in II has allowed the isolation[23] and study[24] of the tetrahedral intermediate III whose structure resembles broadly those of TH and T of Scheme I. The presence of the trialkyl lock does not confer unusual reactivity in the HO^--catalyzed hydrolysis of Ia. Based on the dependence of k_{HO} on the pK_a of the carboxylic acid of a series of methyl esters,[25] and assuming that the steric effects in Ia would be similar to that found in pivalate esters,[25] it is estimated that k_{OH} for Ia should be approximately 20 min^{-1} (at 25 °C), as compared to the measured value of 1.63 min^{-1} (at 30 °C). Thus, the stabilization of the "metal-carboxyl anhydride" and the tetrahedral intermediate depends on the presence of the trialkyl lock.



III

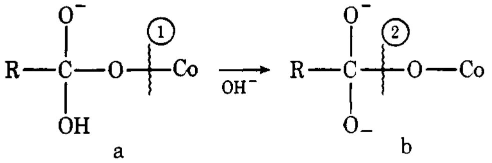
Implicit in the existence of the "metal-carboxyl anhydride" acylation reactions (k_{-a_1} and *p*-chlorophenol trapping) is the necessity that the anhydride have the potential to open by C-O rather than metal-O bond cleavage. Although C-O bond cleavage in the hydrolysis of metal-carboxylate complexes is a rather rare event, there are two cases which are of interest in the present context. In HO^--catalyzed hydrolysis of a Co^{3+} complex of oxalate (eq 30),[26] the first bond is broken by C-O bond cleavage whereas the second occurs by M-O bond cleavage. The second example involves the base-catalyzed hydrolysis of the pentaamino Co^{3+} complex of trifluoroacetate which follows the rate law[27]

$$\text{rate} = \{k_1[\text{HO}^-] + k_2[\text{HO}^-]^2\}\,[\text{complex}] \qquad (31)$$



(30)

It has been proposed that the k_2 pathway, which gives carbon-oxygen bond cleavage, follows conversion of a to b and C-O bond breakage (eq 32).



(32)

The existence of C-O bond cleavage in these two Co^{3+} complexes, as well as in the k_{-a_1} pathway and *p*-chlorophenol trapping of the "metal-carboxyl anhydride" of this study can be understood in terms of the stereoelectronic control of cleavage of tetrahedral intermediates as described by Deslongchamps.[28] According to this theory, the nature of the products formed depends on the conformation of the tetrahedral intermediate and specifically the breakdown of a particular conformer depends on the orientation of the lone pair orbitals of the heteroatoms, specific cleavage of a carbon-oxygen bond being allowed only if the other two heteroatoms in the tetrahedral intermediate each have an orbital oriented antiperiplanar to the leaving group. Supplemental to this hypothesis is the postulate that an O-H bond is equivalent to a lone pair orbital. Application of this theory to these three metal carboxyl systems is provided in Schemes II, III, and IV. In the case of the $(en)_2Co$-OX complex (Scheme II), the conformation of the tetrahedral intermediate is such that lone pair orbitals on two oxygens are oriented antiperiplanar to the C-O metal bond, thus predicting C-O bond cleavage. A more striking example is obtained in the HO^--catalyzed hydrolysis of $(NH_3)_5Co$-$OCOCF_3$. In this case (see Scheme III) the tetrahedral intermediate (a) formed by initial attack of OH^- cannot give rise to a conformer oriented properly to expel the metal-O group because steric hindrance by the M-$(NH_3)_5$ group forces the H of the attacking OH to be antiperiplanar to the leaving metal-O group; thus there are not two lone pair orbitals correctly positioned. When this H^+ is removed at high [HO^-] to provide b, the orbital generated is now situated in the correct antiperiplanar position to act with the orbital on the other oxygen to expel the metal-O group. In this context it would not appear as though the O-H bond is equivalent to the lone pair orbital generated by proton removed.

In the case of ester Ia-d metal complexes (Scheme IV) the orientation of the attacking OH group generates a tetrahedral intermediate which can readily break down to form the "metal-carboxyl anhydride" intermediate by expulsion of OR. The re-formation of the ester can occur since by the principle of microscopic reversibility the attack of RO^- must generate the same tetrahedral intermediate (b → a) which can break down to give either ester or the "metal-carboxyl anhydride".

The nature of the "metal-carboxyl anhydride" can most clearly be appreciated from structure b in Scheme IV. Owing to the presence of the trialkyl lock, and the fact that the metal-OH attacks from below the carboxyl group of the ester, the "anhydride" is forced to remain in the lactone conformation. The more stable ester conformation can only be achieved by breaking the chelate ring and allowing re-formation of the complex in the ester conformation. It is possible that attack of H_2O on structure b of Scheme IV, then protonation of the M-O-C oxygen, could lead to breaking of the chelate ring. It seems more probable that the chelate ring is broken by pro-

Table V. Comparison of the Rate Constants for Metal Ion Catalyzed Hydrolysis of Esters Ia-d and k_{cat} for Carboxypeptidase Catalyzed Hydrolysis of Ester Substrates

A. Metal Ion Catalyzed Hydrolysis of Esters

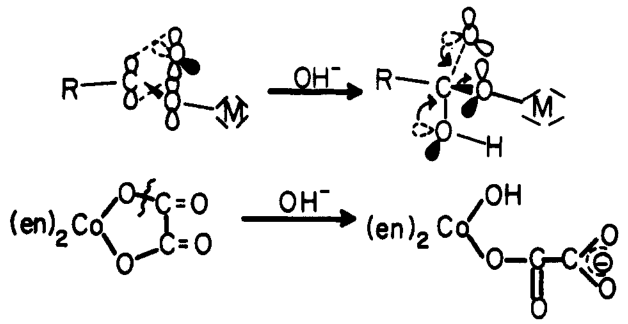
Ester	Co^{2+} [a] k_r, min^{-1}	Co^{2+} [a] k_rK_{eq}, min^{-1}	Ni^{2+} [b] k_r, min^{-1}	Ni^{2+} [b] k_rK_{eq}, min^{-1}
Ia	1.05	14.7	0.6	1.2
Ib	1.6	22.4	1.0	2.0
Ic	7.0	98	6.3	12.6
Id	115	1610	50	100

B. Carboxypeptidase[c]

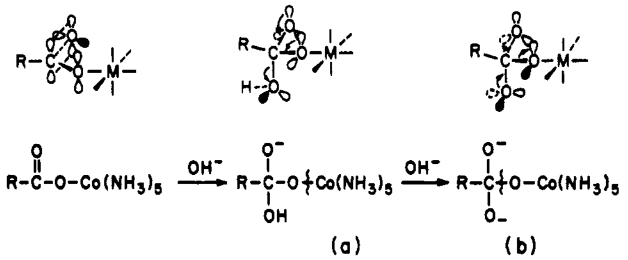
Substrate	k_{cat}, min^{-1}
p-Nitrocinnamoyl L-β-phenyl lactate	13 800
Cinnamoyl L-β-phenyl lactate	4000
p-Nitrocinnamoyl L-mandelate	307
Acetyl L-mandelate	29

[a] Calculated from eq 18 using K_{eq} = 14. [b] Calculated from eq 18 using K_{eq} = 2. [c] At pH = 7.5 (ref 29).

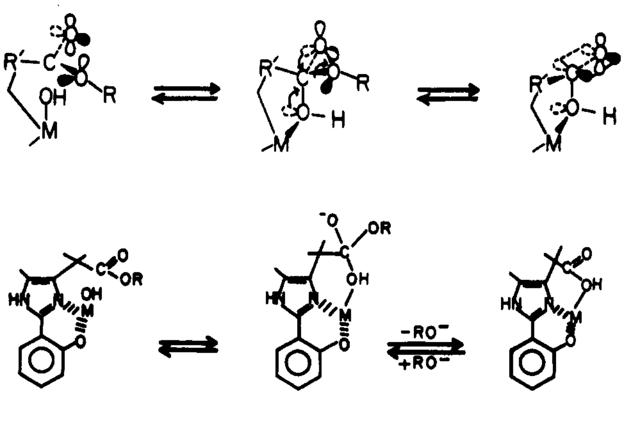
Scheme II



Scheme III



Scheme IV



tonation of the oxygen of the phenol group. Thus, the ligand possessing an ionized imidazole, but a un-ionized phenolic function, does not complex to metal ion. Model building shows that breaking any of the three ligand bonds in the "metal-carboxyl anhydride" will allow assumption of the more stable ester conformation.

The data presented in this paper on labile metal-ester complexes, as well as previous studies on stable metal-substrate complexes,[5] show that a metal bound HO^- is a very effective nucleophile in intramolecular catalysis of ester hydrolysis. In Table V are compared the rate constants for hydrolysis of esters Ia-d and k_{cat} for the carboxypeptidase catalyzed hydrolysis of some ester substrates.[29] The values of k_r and K_{eq} were derived from eq 18, which assumes that all ester hydrolysis with a given metal ion (Co^{2+} or Ni^{2+}) follows the same rate law. This assumption is probably valid since both esters Ia and Ic did show the same shape log k_{obsd} vs. pH profile in the presence of Co^{2+} (Figure 7). It can be seen from Table V that the values of k_rK_{eq} fall roughly in the range of k_{cat} for carboxypeptidase hydrolysis of L-mandelate esters, but are smaller than k_{cat} for L-β-phenyllactate esters. It has been concluded[30] that the latter esters bind more effectively to the enzyme through hydrophobic interactions.[30] Thus, it appears that metal-OH attack could be rapid enough to account for the catalysis of metallohydrolases such as carboxypeptidase, assuming that additional rate enhancements arise in the enzyme-catalyzed reaction through substrate binding.

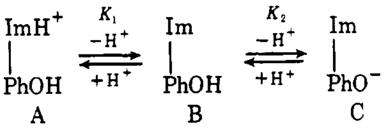
The "metal-carboxyl anhydride" of Scheme I may also be of relevance to recent attempts to identify an acyl-enzyme intermediate in the carboxypeptidase reaction.[31,32] Kinetically the "metal-carboxyl anhydride" would be indistinguishable from an anhydride formed at Glu 270.[30,31] The inability to trap an anhydride intermediate of carboxypeptidase[32] with methanol might find an explanation in the lack of reactivity of the metal anhydride intermediate of this study with methanol.[32] Finally, the potential for the "metal-carboxyl anhydride" to break down to hydrolysis products by C-O bond cleavage rather than M-O bond cleavage may explain the apparently anomalous activity of Co^{3+} carboxypeptidase.[7]

Acknowledgment. This work was supported by a grant from the National Institutes of Health to Thomas C. Bruice. Michael A. Wells was a Macy Faculty Scholar (1975-1976).

Appendix

I. Analysis of Equilibrium Formation of Metal Ion Complexes. A. Ester. The following equilibria are considered:

uncomplexed ester

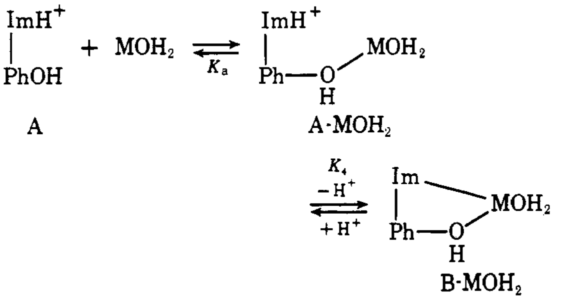


$$\begin{array}{c}\mathrm{ImH^+}\\ |\\ \mathrm{PhOH}\\ \mathrm{A}\end{array} \underset{+H^+}{\overset{K_1,\ -H^+}{\rightleftharpoons}} \begin{array}{c}\mathrm{Im}\\ |\\ \mathrm{PhOH}\\ \mathrm{B}\end{array} \underset{+H^+}{\overset{K_2,\ -H^+}{\rightleftharpoons}} \begin{array}{c}\mathrm{Im}\\ |\\ \mathrm{PhO^-}\\ \mathrm{C}\end{array} \qquad \text{(A-1)}$$

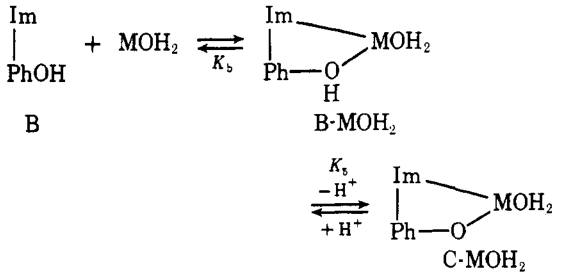
metal ion

$$\mathrm{MOH_2} \overset{K_x}{\rightleftharpoons} \mathrm{MOH} \qquad \text{(A-2)}$$

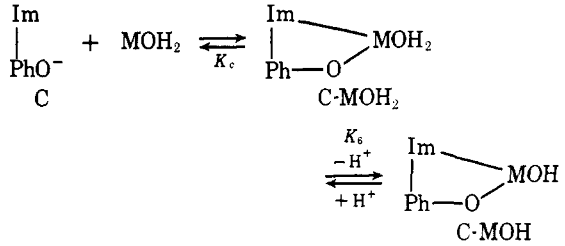
ester–metal complexes



$$\begin{array}{c}\mathrm{ImH^+}\\ |\\ \mathrm{PhOH}\\ \mathrm{A}\end{array} + \mathrm{MOH_2} \underset{K_a}{\rightleftharpoons} \begin{array}{c}\mathrm{ImH^+}\\ |\quad\ \ \mathrm{MOH_2}\\ \mathrm{Ph{-}O}\\ \mathrm{H}\\ \mathrm{A{\cdot}MOH_2}\end{array} \underset{+H^+}{\overset{K_4,\ -H^+}{\rightleftharpoons}} \begin{array}{c}\mathrm{Im}\qquad\\ |\quad\ \ \mathrm{MOH_2}\\ \mathrm{Ph{-}O}\\ \mathrm{H}\\ \mathrm{B{\cdot}MOH_2}\end{array} \qquad \text{(A-3)}$$



(A-4)



(A-5)

Assuming that metal complexation to a particular species of organic ligand does not alter the spectra of this species, we may define three spectral species: a = A + A-MOH$_2$, b = B + B-MOH$_2$; c = C-MOH$_2$ + C-MOH + C. At most pHs studied, C was quite small, so c $\sim$ C-MOH$_2$ + C-MOH.

Titration with metal ion at constant pH was carried out spectrally by observation of the conversion of b to c. Assuming that M_T (total metal ion) is greater than ester, then the fraction of total ester as C-MOH$_2$ + C-MOH = F is given by

$$F = \frac{\frac{K_2}{a_H}\left[\frac{[M_T]}{K_c\left(1+\frac{K_x}{a_H}\right)}\left(1+\frac{K_6}{a_H}\right)\right]}{\left(1+\frac{a_H}{K_1}+\frac{K_2}{a_H}\right)+\frac{[M_T]}{1+\frac{K_x}{a_H}}\left(\frac{a_H}{K_1K_a}+\frac{1}{K_6}\right)+\frac{K_2}{a_H}\left[\frac{[M_T]}{K_c\left(1+\frac{K_x}{a_H}\right)}\left(1+\frac{K_6}{a_H}\right)\right]} \qquad \text{(A-6)}$$

Noting $K_c/K_2K_b = 1/K_5$, $K_c/K_2K_1K_a = 1/K_4K_5$, $1/F$ becomes

$$\frac{1}{F} = 1 + \frac{\frac{a_H}{K_2}\left[1+\frac{a_H}{K_1}+\frac{K_2}{a_H}\right]K_c\left(1+\frac{K_x}{a_H}\right)}{\left(1+\frac{K_6}{a_H}\right)[M_T]} + \frac{\frac{a_H}{K_5}\left[1+\frac{a_H}{K_4}\right]}{1+\frac{K_6}{a_H}} \qquad \text{(A-7)}$$

A plot of $1/F$ vs. $1/[M_T]$ should be linear with an intercept at $1/[M_T] = 0$ of

$$1 + \frac{a_H}{K_5}\left[1+\frac{a_H}{K_4}\right]\Big/\left(1+\frac{K_6}{a_H}\right)$$

Since the experimental intercept (Figure 3) is 1.0 this means that

$$\frac{a_H}{K_5}\left(1+\frac{a_H}{K_4}\right) = \frac{K_c(a_H)^2}{K_2(a_H+K_6)}\left(\frac{1}{K_b}+\frac{a_H}{K_1K_a}\right) = 0$$

and, therefore, K_a and K_b must be large and can be ignored (i.e., the equilibria of eq A-3,4 can be ignored). Defining $K_c/K_2 = K_{m_1}$ (i.e., $K_{m_1} = ([B][MOH_2])/([C\text{-}MOH_2]\,a_H)$, eq A-7 may be reduced to

$$\frac{1}{F} = 1 + \frac{a_H\left[1+\frac{a_H}{K_1}+\frac{K_2}{a_H}\right]K_{m_1}\left(1+\frac{K_x}{a_H}\right)}{\left(1+\frac{K_6}{a_H}\right)M_T} \qquad \text{(A-8)}$$

The pH-dependent ligand metal ion dissociation constant (K_{pH}) is provided by the slope of a plot of $1/F$ vs. $1/[M_T]$. Thus

$$K_{pH} = \frac{a_H\left[1+\frac{a_H}{K_1}+\frac{K_2}{a_H}\right]K_{m_1}\left(1+\frac{K_x}{a_H}\right)}{\left(1+\frac{K_6}{a_H}\right)} \qquad \text{(A-9)}$$

or

$$\log K_{pH} = \log K_{m_1} - \text{pH} + \log\left(1+\frac{a_H}{K_1}+\frac{K_2}{a_H}\right) + \log\left(1+\frac{K_x}{a_H}\right) - \log\left(1+\frac{K_6}{a_H}\right) \qquad \text{(A-10)}$$

For pH titration at constant metal ion concentration, define α = mole fraction of complexed ester = C-MOH$_2$ + C-MOH and $(1-\alpha)$ = the mole fraction of uncomplexed ester = A + B + C (assuming metal ion does not complex to A or B).

$$\frac{\alpha}{1-\alpha} = \frac{\frac{K_2}{a_H}\left[\frac{M_T}{K_c\left(1+\frac{K_x}{a_H}\right)}\right]\left(1+\frac{K_6}{a_H}\right)}{1+\frac{a_H}{K_1}+\frac{K_2}{a_H}} \qquad \text{(A-11)}$$

Define

$$\log K_{pH} = \log [M_T] - \log\frac{\alpha}{1-\alpha} \qquad \text{(A-12)}$$

and $K_c/K_2 = K_{m_1}$, then

$$\log K_{pH} = \log K_{m_1} - \text{pH} + \log\left(1+\frac{a_H}{K_1}+\frac{K_2}{a_H}\right) + \log\left(1+\frac{K_x}{a_H}\right) - \log\left(1+\frac{K_6}{a_H}\right) \qquad \text{(A-13)}$$

Equation A-13 may be seen to be equal to eq A-10.

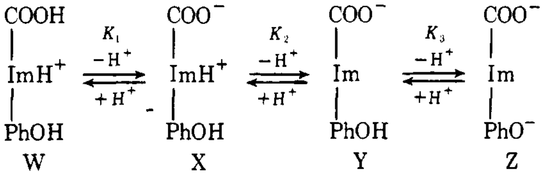
Conversion of a to b in the Presence of Metal Ion. Defining $\alpha = a/(a+b)$ and $1-\alpha = b/(a+b)$, and ignoring c since pH $\ll$ pK_3, one obtains

$$\log\frac{\alpha}{1-\alpha} = \log K_1 + \text{pH} + \log\left[1+\frac{M_T}{K_b\left(1+\frac{K_x}{a_H}\right)}\right] - \log\left[1+\frac{M_T}{K_a\left(1+\frac{K_x}{a_H}\right)}\right] \qquad \text{(A-14)}$$

The lack of effect of $[M_T]$ upon pK_1 (conversion of a to b) requires that K_b and K_a must be large compared to $[M_T]$ and thus they may be ignored.

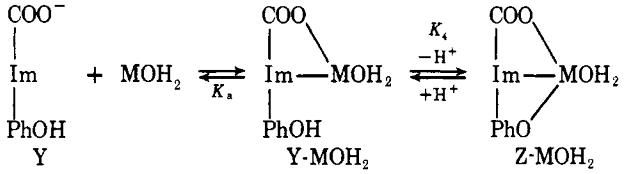
B. Binding to Carboxylic Acid. The value of pK_1 is not

uncomplexed acid



(A-15)

changed by the presence of the metal so binding to W and X is not important. Both pK_2 and pK_3 are changed on metal binding so that complexing of metal ion occurs to Y. Since pK_3 > pH below pH 8, Z may be ignored, providing

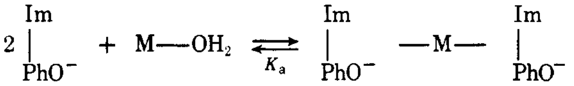


(A-16)

Z-MOH$_2$ is the spectral species measured. Defining $K_a/K_2 = K_{m_2}$ = [X][MOH$_2$]/[Y-MOH$_2$]a_H and proceeding as above for eq A-10

$$\log K_{pH} = \log K_{m_2} - pH + \log\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right) + \log\left(1 + \frac{K_x}{a_H}\right) - \log\left(1 + \frac{K_4}{a_H}\right) \quad (A\text{-}17)$$

C. Metal-Induced Dimerization of Ester. Equilibria A-1 and A-2 plus



$$2\,\begin{matrix}\text{Im}\\|\\\text{PhO}^-\end{matrix} + \text{M—OH}_2 \underset{K_a}{\rightleftharpoons} \begin{matrix}\text{Im}\\|\\\text{PhO}^-\end{matrix}\text{—M—}\begin{matrix}\text{Im}\\|\\\text{PhO}^-\end{matrix} \quad (A\text{-}18)$$

Proceeding as above as for eq A-10, eq A-19 is derived which contains the concentration of B.

$$K_{pH} = \frac{\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right) K_a \left(1 + \frac{K_x}{a_H}\right)\left(\frac{a_H}{K_2}\right)^2}{[B]} \quad (A\text{-}19)$$

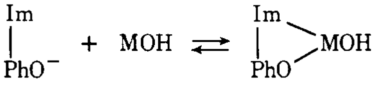
Since K_{pH} refers to the metal ion concentration where ½ of ester is complexed B under these conditions is given by

$$B = \frac{[T.E.]}{2\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right)} \quad (A\text{-}20)$$

where T.E. = total ester. Define $K_m = 2K_a/(K_2)^2$. Thus, eq A-19 becomes

$$\log K_{pH} = \log K_m - 2pH + 2\log\left[1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right] + \log\left(1 + \frac{K_x}{a_H}\right) - \log(T.E.) \quad (A\text{-}21)$$

D. Binding of Hydroxo Metal Ion to Ester. Equilibria A-1, A-2, and A-22 provide

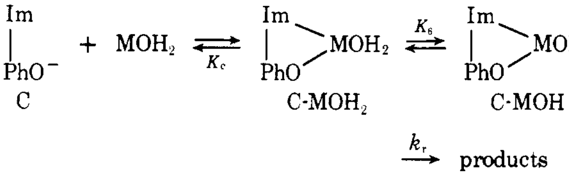


(A-22)

This gives

$$\log K_{pH} = \log K_{m_1} - pH + \log\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right) + \log\left(1 + \frac{a_H}{K_x}\right) \quad (A\text{-}23)$$

II. Kinetic Equations. Reactions of Hydroxo Complex. A. Without Equilibrium.



(A-24)

$$v = k_r[\text{C-MOH}]$$

By mass balance

$$\nu = \frac{k_r \frac{[M_T]}{K_c\left(1 + \frac{K_x}{a_H}\right)} \frac{K_2}{a_H}\frac{K_6}{a_H}}{\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right) + \frac{[M_T]}{K_c\left(1 + \frac{K_x}{a_H}\right)}\left(1 + \frac{K_6}{a_H}\right)\frac{K_2}{a_H}[\text{ester}]} \quad (A\text{-}25)$$

Noting $K_{m_1} = K_c/K_2$, then the equation for plots of $1/k_{app}$ vs. $1/[M_T]$ are of the form

$$1/k_{app} = 1/k_r\left[\frac{K_m a_H\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right)\left(1 + \frac{K_x}{a_H}\right)}{[M_T]\frac{K_6}{a_H}} + \frac{1 + \frac{K_6}{a_H}}{\frac{K_6}{a_H}}\right] \quad (A\text{-}26)$$

At $[M_T] = \infty$, $1/k_{app} = 1/k_{obsd}$ and

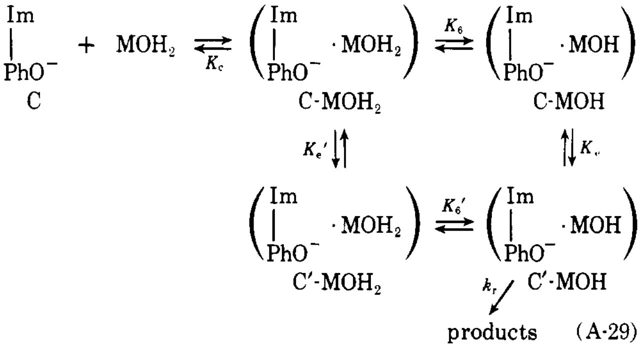
$$k_{obsd} = k_r\frac{K_6}{a_H + K_6} \quad (A\text{-}27)$$

The kinetically determined and pH-dependent metal–substrate dissociation constant, K_{pH}

$$K_{pH} = \frac{K_m a_H\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right)\left(1 + \frac{K_x}{a_H}\right)}{\left(1 + \frac{K_6}{a_H}\right)} \quad (A\text{-}28)$$

is given by the ratio of the slope/intercept of a plot of $1/k_{app}$ vs. $1/[M_T]$ (eq A-26). Equation A-28 may be compared to eq A-9.

B. Equilibria Involving the Metal Ester Complexes.



(A-29)

$$\upsilon = k_r[\text{C}'\text{-MOH}]$$

By mass balance

$$\nu = \frac{k_r \frac{[M_T]}{K_c\left(1 + \frac{K_x}{a_H}\right)}\frac{K_2}{a_H}\frac{K_6}{a_H}K_e[\text{ester}]}{\left(1 + \frac{a_H}{K_1} + \frac{K_2}{a_H}\right) + \frac{K_2}{a_H}\frac{[M_T]}{K_c\left(1 + \frac{K_x}{a_H}\right)} \times \left[1 + K_e' + \frac{K_6}{a_H}(1 + K_e)\right]} \quad (A\text{-}30)$$

Noting $K_m = K_c/K_2$ then plots of $1/k_{app}$ vs. $1/[M_T]$ are given by

$$1/k_{\mathrm{app}} = 1/k_{\mathrm{r}}\left[\frac{K_{\mathrm{m}}a_{\mathrm{H}}\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right)\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}{[\mathrm{M_T}]\frac{K_6}{a_{\mathrm{H}}}K_{\mathrm{e}}} + \frac{1+K_{\mathrm{e}}'+\frac{K_6}{a_{\mathrm{H}}}(1+K_{\mathrm{e}})}{\frac{K_6}{a_{\mathrm{H}}}K_{\mathrm{e}}}\right] \quad \text{(A-31)}$$

At $[\mathrm{M_T}] = \infty$, $1/k_{\mathrm{app}} = 1/k_{\mathrm{obsd}}$ and

$$k_{\mathrm{obsd}} = \frac{k_{\mathrm{r}}K_6K_{\mathrm{e}}'}{K_{\mathrm{a}}(1+K_{\mathrm{e}}) + a_{\mathrm{H}}(1+K_{\mathrm{e}}')}$$

$$= \frac{k_{\mathrm{r}}K_{\mathrm{e}}}{1+K_{\mathrm{e}}}\left\{\frac{K_6\left(\frac{1+K_{\mathrm{e}}}{1+K_{\mathrm{e}}'}\right)}{K_6\left(\frac{1+K_{\mathrm{e}}}{1+K_{\mathrm{e}}'}\right)+a_{\mathrm{H}}}\right\} \quad \text{(A-32)}$$

The kinetically determined and pH-dependent metal-substrate dissociation constant, K_{pH}, is given by the ratio of slope/intercept of a plot of $1/k_{\mathrm{app}}$ vs. $1/[\mathrm{M_T}]$ (eq A-31).

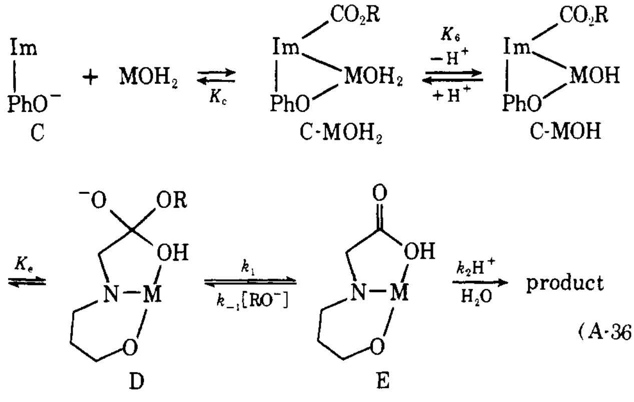
$$K_{\mathrm{pH}} = \frac{\frac{K_{\mathrm{m}}}{(1+K_{\mathrm{e}}')}a_{\mathrm{H}}\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right)\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}{\left[1+\frac{K_6}{a_{\mathrm{H}}}\frac{(1+K_{\mathrm{e}})}{(1+K_{\mathrm{e}}')}\right]} \quad \text{(A-33)}$$

If K_{e}' is small then eq A-32 becomes eq A-34 and eq A-31 becomes eq A-35 (compare eq A-27 to eq A-28).

$$k_{\mathrm{obsd}} = \left(\frac{k_{\mathrm{r}}K_{\mathrm{e}}}{1+K_{\mathrm{e}}}\right)\frac{K_6(1+K_{\mathrm{e}})}{a_{\mathrm{H}}+K_6(1+K_{\mathrm{e}})} \quad \text{(A-34)}$$

$$K_{\mathrm{pH}} = \frac{K_{\mathrm{m}}a_{\mathrm{H}}\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right)\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}{\left[1+\frac{K_6(1+K_{\mathrm{e}})}{a_{\mathrm{H}}}\right]} \quad \text{(A-35)}$$

C. Inhibition by Trifluoroethanol.



(A-36)

$$v = k_2a_{\mathrm{H}}[\mathrm{E}]$$

Assumption of steady state in E and mass balance in C + C-MOH$_2$ + C-MOH + D provides

$$v = \frac{k_1k_2a_{\mathrm{H}}\frac{[\mathrm{M_T}]}{K_{\mathrm{c}}\left[1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right]}\frac{K_6}{a_{\mathrm{H}}}\frac{K_2}{a_{\mathrm{H}}}K_{\mathrm{e}}\,[\text{total ester}]}{\left[k_{-1}\frac{K_{\mathrm{ROH}}}{a_{\mathrm{H}}}(\mathrm{ROH})+k_2a_{\mathrm{H}}\right]\left\{\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right) + \frac{[\mathrm{M_T}]}{K_{\mathrm{c}}\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}\frac{K_2}{a_{\mathrm{H}}}\left[1+\frac{K_6}{a_{\mathrm{H}}}(1+K_{\mathrm{e}})\right]\right\}} \quad \text{(A-37)}$$

Noting $K_{\mathrm{m}} = K_{\mathrm{c}}/K_2$ and $\mathrm{p}K_{\mathrm{ROH}} = \mathrm{p}K_{\mathrm{a}}$ for ROH

$$k_{\mathrm{app}} = \frac{k_1k_2a_{\mathrm{H}}}{\left[k_{-1}\left(\frac{K_{\mathrm{ROH}}}{a_{\mathrm{H}}}\right)(\mathrm{ROH})+k_2a_{\mathrm{H}}\right]} \times \left\{\frac{\frac{[\mathrm{M_T}]}{K_{\mathrm{m}}\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}\frac{K_6K_{\mathrm{e}}}{(a_{\mathrm{H}})^2}}{\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right)+\frac{[\mathrm{M_T}]}{K_{\mathrm{m}}\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}\frac{a_{\mathrm{H}}+K_6(1+K_{\mathrm{e}})}{(a_{\mathrm{H}})^2}}\right\} \quad \text{(A-38)}$$

For experiments at constant [ROH]

$$1/\mathrm{k}_{\mathrm{app}} = \left\{\frac{\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right)K_{\mathrm{m}}a_{\mathrm{H}}\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}{\frac{K_6K_{\mathrm{e}}}{a_{\mathrm{H}}}} \times \left(\frac{1}{k_{-1}}+\frac{k_{-1}K_{\mathrm{ROH}}}{k_1k_2(a_{\mathrm{H}})^2}[\mathrm{ROH}]\right)\right\}\frac{1}{[\mathrm{M_T}]} + \frac{a_{\mathrm{H}}+K_6(1+K_{\mathrm{e}})}{K_6K_{\mathrm{e}}}\left[\frac{1}{k_1}+\frac{k_{-1}}{k_1k_2}\frac{K_{\mathrm{ROH}}}{(a_{\mathrm{H}})^2}(\mathrm{ROH})\right] \quad \text{(A-39)}$$

At $[\mathrm{M_T}] = \infty$, $1/k_{\mathrm{app}} = 1/k_{\mathrm{obsd}}$.

$$1/k_{\mathrm{obsd}} = \frac{K_6(1+K_{\mathrm{e}})+a_{\mathrm{H}}}{K_6K_{\mathrm{e}}}\left(\frac{1}{k_1}+\frac{k_{-1}}{k_1k_2}\frac{K_{\mathrm{ROH}}}{(a_{\mathrm{H}})^2}[\mathrm{ROH}]\right) \quad \text{(A-40)}$$

The ratio of k_{obsd} in the absence of added alcohol to k_{obsd} in the presence of alcohol (k^*_{obsd}) is given by

$$\frac{k_{\mathrm{obsd}}}{k^*_{\mathrm{obsd}}} = 1+\frac{k_{-1}}{k_2}\frac{K_{\mathrm{ROH}}}{(a_{\mathrm{H}})^2}[\mathrm{ROH}] \quad \text{(A-41)}$$

For experiments at constant metal and changing [ROH] plots of $1/k_{\mathrm{app}}$ vs. [ROH] are given by

$$1/k_{\mathrm{app}} = \left[\frac{\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right)K_{\mathrm{m}}a_{\mathrm{H}}\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}{\frac{K_6K_{\mathrm{e}}}{a_{\mathrm{H}}}} + \frac{1+\frac{K_6}{a_{\mathrm{H}}}(1+K_{\mathrm{e}})}{\frac{K_6K_{\mathrm{e}}}{a_{\mathrm{H}}}}\right]\frac{1}{k_1[\mathrm{M_T}]} + \left[\frac{\left(1+\frac{a_{\mathrm{H}}}{K_1}+\frac{K_2}{a_{\mathrm{H}}}\right)K_{\mathrm{m}}a_{\mathrm{H}}\left(1+\frac{K_{\mathrm{x}}}{a_{\mathrm{H}}}\right)}{[\mathrm{M_T}]\frac{K_6K_{\mathrm{e}}}{a_{\mathrm{H}}}} + \frac{1+\frac{K_6}{a_{\mathrm{H}}}(1+K_{\mathrm{e}})}{\frac{K_6K_{\mathrm{e}}}{a_{\mathrm{H}}}}\right] \times \frac{k_{-1}}{k_1k_2}\frac{K_{\mathrm{ROH}}}{(a_{\mathrm{H}})^2}[\mathrm{ROH}] \quad \text{(A-42)}$$

The ratio of slope/intercept of a plot of $1/k_{\mathrm{app}}$ vs. $1/[\mathrm{M_T}]$ for eq A-42 is given by

$$\text{slope/intercept} = \frac{k_{-1}}{k_2}\frac{K_{\text{ROH}}}{(a_{\text{H}})^2} \qquad \text{(A-43)}$$

References and Notes

(1) R. Breslow, R. Fairweather, and L. J. Keana, *J. Am. Chem. Soc.*, **89,** 2135 (1967).
(2) (a) R. J. Angelici and B. E. Leach, *J. Am. Chem. Soc.*, **90,** 2499 (1968); (b) R. Breslow, D. E. McClure, R. S. Brown, and J. Eisenach, *ibid.*, **97,** 194 (1975).
(3) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.*, **91,** 4102 (1969).
(4) (a) D. A. Buckingham, C. E. Davis, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.*, **92,** 5571 (1970); (b) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *ibid.*, **96,** 1726 (1974).
(5) (a) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.*, **92,** 6151 (1970); (b) D. A. Buckingham, F. R. Keene, and A. M. Sargeson, *ibid.*, **96,** 4981 (1974).
(6) (a) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.*, **91,** 3451 (1969); (b) D. A. Buckingham, C. E. Davis, and A. M. Sargeson, *ibid.*, **92,** 6159 (1970); (c) D. S. Sigman and C. T. Jorgesen, *ibid.*, **94,** 1724 (1972).
(7) E. P. Kang, C. B. Storn, and F. W. Carson, *J. Am. Chem. Soc.*, **97,** 6723 (1975).
(8) G. A. Rogers and T. C. Bruice, *J. Am. Chem. Soc.*, **96,** 2473 (1974).
(9) R. E. Thiers, *Methods Biochem. Anal.*, **5,** 273 (1957).
(10) T. C. Bruice and J. R. Maley, *Anal. Biochem.*, **34,** 275 (1970).
(11) R. C. Rosenberg, C. A. Root, R. H. Wang, M. Cerdonio, and H. B. Gray, *Proc. Natl. Acad. Sci. U.S.A.*, **70,** 161 (1973).
(12) N. C. Li, J. M. White, and E. Doody, *J. Am. Chem. Soc.*, **76,** 6219 (1954).
(13) P. George, J. H. Hanania, D. H. Irvine, and I. Au-Jssa, *J. Chem. Soc., Suppl. 1,* 5689 (1964).
(14) Y. Yasuhiko, F. R. N. Gurd, R. F. Chen, and J. T. Edsell, *J. Am. Chem. Soc.*, **79,** 2123 (1957).
(15) F. R. N. Gurd and P. E. Wilcox, *Adv. Protein Chem.*, **11,** 311 (1956).
(16) J. W. Thanassi and T. C. Bruice, *J. Am. Chem. Soc.*, **88,** 747 (1966).
(17) T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **81,** 4552 (1959).
(18) M. F. Aldersley, A. J. Kirby, and P. W. Lancaster, *J. Chem. Soc., Perkin Trans. 2,* 1504 (1974).
(19) T. C. Bruice, T. H. Fife, J. T. Bruno, and N. E. Brandon, *Biochemistry,* **1,** 7 (1962).
(20) P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **81,** 1050 (1959).
(21) (a) S. Milstein and L. A. Cohen, *J. Am. Chem. Soc.*, **94,** 9158 (1972); (b) R. T. Borchardt and L. A. Cohen, *ibid.*, **94,** 9166, 9175 (1972); (c) *ibid.*, **95,** 8308, 8313, 8319 (1973).
(22) J. M. Karle and I. I. Karle, *J. Am. Chem. Soc.*, **94,** 9182 (1972).
(23) G. A. Rogers and T. C. Bruice, *J. Am. Chem. Soc.*, **95,** 4452 (1973).
(24) G. A. Rogers and T. C. Bruice, *J. Am. Chem. Soc.*, **96,** 2481 (1974).
(25) L. P. Hammett, "Physical Organic Chemistry", 1st ed, McGraw-Hill, New York, N.Y., 1940, p 211.
(26) C. Andrade and H. Taube, *J. Am. Chem. Soc.*, **86,** 1328 (1964).
(27) R. B. Jordan and H. Taube, *J. Am. Chem. Soc.*, **88,** 4406 (1966).
(28) (a) P. Deslongchamps, S. Dube, C. Lebreux, D. R. Patterson, and R. J. Taillefer, *Can. J. Chem.*, **53,** 2791 (1975); (b) P. Deslongchamps, *Tetrahedron,* **31,** 2463 (1976).
(29) G. Tomalin, B. L. Kaiser, and E. T. Kaiser, *J. Am. Chem. Soc.*, **92,** 6046 (1970).
(30) E. T. Kaiser and B. L. Kaiser, *Acc. Chem. Res.*, **5,** 219 (1972).
(31) J. Suh and E. T. Kaiser, *J. Am. Chem. Soc.*, **98,** 1940 (1976).
(32) R. Breslow and D. Wernich, *J. Am. Chem. Soc.*, **98,** 259 (1976).

Dialkoxycarbonium Ions as Ambident Electrophiles in Their Hydrolysis in Aqueous Sulfuric Acid Solutions

Robert A. McClelland* and Mahmood Ahmad

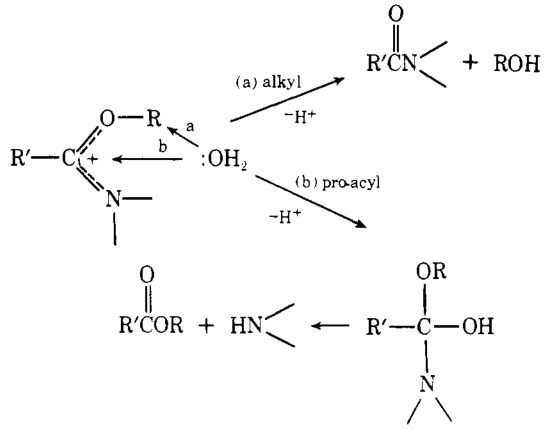
Contribution from the Department of Chemistry, University of Toronto, Scarborough College, West Hill, Ontario, Canada M1C 1A4.

Received December 6, 1976

Abstract: The dimethoxy-2,4,6-trimethylphenylcarbonium ion (II) hydrolyzes with cleavage of both the methyl carbon-oxygen bond and the pro-acyl carbon-oxygen bond, the amount of former increasing with increasing sulfuric acid concentration. The dimethoxyphenylcarbonium ion (I) cleaves only the pro-acyl carbon-oxygen bond at all acidities. These results are compared with those obtained for analogous imidatonium ions, where alkyl carbon-oxygen bond cleavage is more dominant. It is concluded that dealkylation of alkoxycarbonium ions is more important for a less reactive (or more stable) cation. The possibility of observing $A_{Al}2$ carboxylate ester hydrolysis is considered.

Recently we have demonstrated[1,2] that there are two routes for the acid-catalyzed hydrolysis of imidate esters, differentiated by the position of nucleophilic attack of a water molecule on the imidatonium ion (Scheme I).

Scheme I



In this paper will be reported an evaluation of the importance of the dealkylation reaction in the hydrolysis of methylated and protonated carboxylate esters, in other words, an evaluation of the importance of $A_{Al}2$ carboxylate ester hydrolysis as compared to the normal $A_{Ac}2$ mechanism.[3] According to the imidate results,[2] the type of ester for which the former reaction is most likely to occur, if at all, is the methyl ester of a sterically congested carboxylic acid, for example, a 2,6-dimethylbenzoate. Such esters, however, appear not to undergo bimolecular hydrolyses in acid solutions, preferring a unimolecular route via the acylium ion.[4]

We have turned therefore to what might be considered as models for carbonyl protonated esters, dialkoxycarbonium ions $R'C^+(OR)_2$.[5] These can be conveniently prepared from the ortho ester ($R'C(OR)_3$) either as a salt with a weakly nucleophilic counterion,[6] or on solution in strong acid media.[7] A number of studies[5,7,8] of the NMR spectra of these species, along with various other properties, have now appeared, and their hydrolysis does give the expected products of ester and alcohol.[6] For our purposes these are ideally suited since there is little possibility for unimolecular reactions providing that the alkoxy group consists of a primary alkyl group. The ap-