Intramolecular Nucleophilic Attack of Carboxyl on Amides. The Metal Ion Inhibited Hydrolysis of *N*-(2-Pyridyl)phthalamic Acid and *N*-(2-Phenanthrolyl)phthalamic Acid

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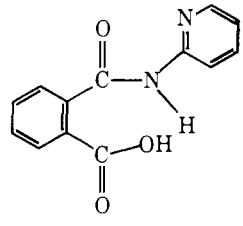
Abstract: Rate constants for hydrolysis of *N*-(2-pyridyl)phthalamic acid have been measured in H_2O at 50 °C. A plot of log k_{obsd} vs. pH is bell shaped with pK_{app} values of 3.2 and 3.9. Carboxyl group participation in this system is marked by a large facilitation in the rate of hydrolysis. At pH 3.5 there is a 10^4-fold enhancement in k_{obsd} in comparison with *N*-(2-pyridyl)benzamide even with a temperature difference of 40 °C. A small metal ion inhibition of the rate of hydrolysis of *N*-(2-pyridyl) phthalamic acid (~ twofold at 0.002 M metal ion) is observed with Ni^{2+} and Co^{2+} at all pH values. *N*-(2-Phenanthrolyl) phthalamic acid at 50 °C hydrolyzes with a pH–rate constant profile indicating participation by one group in the acid form. Divalent metal ions (Ni^{2+}, Co^{2+}, Zn^{2+}, and Cu^{2+}) bind rapidly to this compound with rate constants comparable to those for complexing with 1,10-phenanthroline, suggesting that binding involves the phenanthroline nitrogens. There is a large metal ion inhibition of the rate of the hydrolysis reaction. At pH 2.75, k_{obsd} is 20–40-fold less when the ratio of metal ion (Cu^{2+}, Co^{2+}, or Zn^{2+}) to substrate is 2/1, and in the presence of Ni^{2+} hydrolysis is not observed. At pH 5.35 the compound is so stable in the presence of these metal ions that in all cases no significant hydrolysis was observed over a period of 7 days. These observations indicate that either complexation involves the carboxyl group or that a metal ion chelated carbonyl will not permit facile proton transfer to the leaving group, which is a requirement in the hydrolysis of amides. The former explanation is unlikely in view of the small metal ion effects in the hydrolysis of *N*-(2-pyridyl)phthalamic acid.

Enzyme reactions, proceeding through an enzyme substrate complex in which the substrate is held in close proximity to functional groups in the enzyme active site, bear a striking resemblance to chemical intramolecular reactions.[1-3] The study of intramolecular catalysis has therefore been of great importance in attempts to understand enzymatic catalysis.

The carboxyl group has perhaps been more extensively studied as an intramolecular participant in reactions of esters and amides than any other functional group.[1-5] Phthalic anhydride is formed as an intermediate in hydrolysis of phthalic acid monoesters,[6] phthalamic acid,[7] and phthalanilic acid,[8] indicating that nucleophilic attack takes place. Large rate enhancements have been obtained due to carboxyl group participation in hydrolysis of phthalamic acid (10^5 in comparison with hydrolysis of benzamide) and maleic acid monoamides.[9,10]

Investigation of carboxyl group participation in reactions of amides is of relevance to the mechanism of action of carboxypeptidase A since the carboxyl group of glutamic acid-270 has been shown to be present at the active site and has been implicated as a participant in the reaction.[11-13] Both general base and nucleophilic mechanisms have been recognized as possibilities. Carboxypeptidase A has Zn^{2+} liganded at the active site, and the metal ion presumably complexes the carbonyl oxygen of substrate esters and amides in the binding process.[11-13] Although numerous studies have been made of metal ion effects in hydrolysis reactions of esters and amides,[14] there have been no previous reports of metal ion effects on carboxyl group participation. One problem has been to design a simple system which will chelate a metal ion to the carbonyl group but where the carboxyl group is uncomplexed and where nucleophilic attack is sterically possible. We have, therefore, investigated the hydrolysis reactions of *N*-(2-pyridyl)-phthalamic acid (I), *N*-(2-pyridyl)benzamide (II), and *N*-(2-phenanthrolyl)phthalamic acid (III). Chelation of the carbonyl oxygen and heterocyclic nitrogen will produce a stable six-membered ring in the case of I and III. A Stuart–Brieglieb space-filling model of III indicates that if a metal ion binds to the phenanthroline nitrogens and the carbonyl oxygen, then chelation of carboxyl oxygen would be sterically difficult. This



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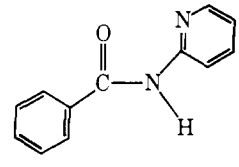

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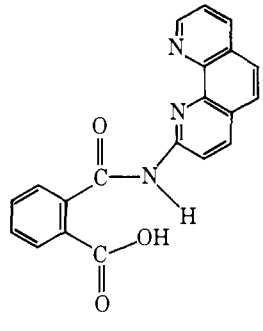


III

is the case because of the planarity of the phenanthroline ring system, the planarity of the amide function, and the unfavorable ring size required for carboxyl group chelation. Since metal ion chelation to the nitrogens would be expected to be more favorable than simple complexing of the carboxyl group, III represents an appropriate system in which to determine the effect of a metal ion on carboxyl group participation in amide hydrolysis.

Experimental Section

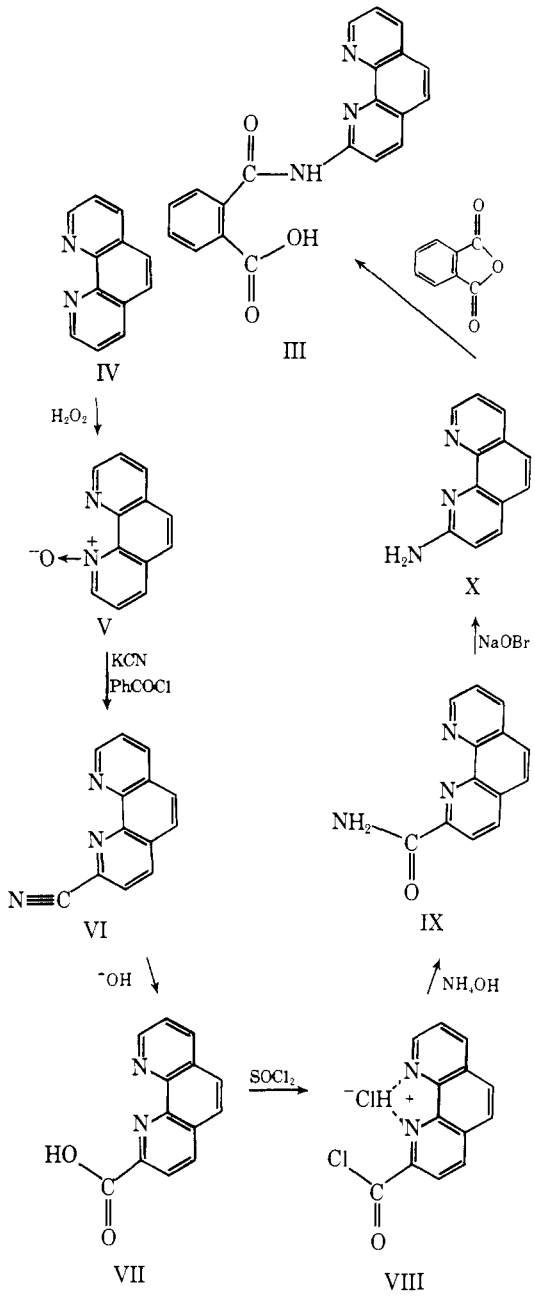
Materials. *N*-(2-Pyridyl)phthalamic Acid (I). Equivalent amounts of phthalic anhydride (Mallinckrodt) and 2-aminopyridine (Aldrich) were refluxed for 2 h in dry benzene. The resulting suspension was suction filtered. The white solid was recrystallized from ethanol to constant melting point after treatment with decolorizing carbon and was vacuum dried in an Abderhalden apparatus over calcium sulfate.

The compound had mp 243–243.5 °C. Anal. Calcd for $C_{13}H_{10}O_3N_2$: C, 64.45; H, 4.16; N, 11.56. Found: C, 64.44; H, 4.30; N, 11.49.

***N*-(2-Pyridyl)benzamide (II)** was prepared by the method of Huntress and Walter.[15] The product had mp 82–83 °C (lit.[15] 82–83 °C).

***N*-(2-Phenanthrolyl)phthalamic acid (III)** was prepared according to the reaction sequence outlined in Scheme I. The procedure of Corey

Scheme I



et al.[16] was used to effect the conversion of 1,10-phenanthroline (IV) (Aldrich) to 2-carboxy-1,10-phenanthroline (VII) (mp 209–210 °C, lit.[16] 209–210 °C) via 1,10-phenanthroline *N*-oxide (V) (mp 179–179.5 °C, lit.[16] 180–181 °C) and 2-cyano-1,10-phenanthroline (VI) (mp 235–235.5 °C, lit.[16] 237–238 °C). The 2-carboxy derivative (VII) yielded the acid chloride (VIII) upon treatment with thionyl chloride, as reported by Sigman et al.[17]

1,10-Phenanthroline-2-carbonamide (IX) was synthesized by adding the solid acid chloride (VIII) in increments (a spatula-tip full) directly to a flask containing 50 mL of cold, concentrated (28%) aqueous ammonia, which was being rapidly stirred. The flask was immersed in an ice bath to ensure that the temperature of the reaction mixture did not exceed 15 °C. Stirring was continued for 1 h after the addition of the acid chloride was completed. The solution containing the precipitated carbonamide was suction filtered, and the white solid was treated with decolorizing carbon, recrystallized from ethanol, and vacuum dried over calcium sulfate, mp 304–304.5 °C (lit.[18] 304.5–305.5 °C).

2-Amino-1,10-phenanthroline (X) was formed via a Hofmann rearrangement reaction. A solution containing 2.55 g of potassium hydroxide and 2.70 g of bromine in 25 mL of water was poured into a flask containing 3.14 g of phenanthroline-2-carbonamide (IX). This mixture was poured into another flask containing 3.60 g of potassium hydroxide in 10 mL of water. The flask was immersed in a water bath maintained at 70–75 °C, and the suspension was stirred for 45 min, resulting in the formation of a dark brown oil. The aqueous phase was decanted, and 50 mL of acetone was added to the oil, yielding a suspension of a light tan solid. The acetone was decanted and another 50 mL was added. This step was repeated three times. The amine was maintained as a suspension in acetone, as it proved to be hygroscopic and was not stable to air.

Amide III was prepared by rapidly adding 2-amino-1,10-phenanthroline (X) suspended in a minimal amount of acetone to a solution of dry benzene containing an equivalent amount of phthalic anhydride. The mixture was refluxed for 2 h, and the resulting cream-colored solid was recovered by suction filtration. Treatment with decolorizing carbon was followed by two recrystallizations from ethanol–water. The compound was then vacuum dried over calcium sulfate, mp 297–298 °C. Anal. Calcd for $C_{20}H_{13}O_3N_3 \cdot 1H_2O$: C, 66.47; H, 4.18; N, 11.62. Found: C, 66.18; H, 3.95; N, 11.27. The identity and purity of the compounds I–III is indicated by the sharp melting points, spectral data (IR, UV, and NMR), thin layer chromatography (single spot), and quantitative hydroxamate tests for amide.

Kinetic Methods. Stock solutions of substrate (10^{-2} M) were made up in methanol or water. In studies employing a Zeiss PMQII, Gilford Model 2000, or Beckman Model 25 spectrophotometer, 30 μL of the substrate stock solution was injected into the reaction cuvette containing 3 mL of buffer, and the reaction was monitored at 295 nm after stirring. The spectrum of the solution upon completion of the reaction was invariably both qualitatively and quantitatively that of equivalent concentrations of the appropriate amine and phthalic acid. Thus, imide formation is not competing with the hydrolytic reaction in aqueous solution.[19] Temperature was controlled at 50 ± 0.1 °C, and the ionic strength of the buffers was kept constant at 0.5 M with either KCl or $LiClO_4$. Each reaction was measured either in duplicate or triplicate.

Reactions conducted at 90 °C were monitored in the following manner. Large screw-capped test tubes were filled with 50 mL of the appropriate buffer, thermally equilibrated in a Haake Ultra-Thermostat circulating bath containing a 1:1 mixture of water–ethylene glycol, and maintained at 90 ± 0.1 °C. The tubes were removed from the bath, injected with 0.5 mL of 10^{-2} M substrate stock solution, and quickly inverted ten times. They were immediately replaced in the bath. Aliquots (3 mL) were withdrawn at regular intervals and placed in smaller test tubes immersed in an ice bath. The aliquots were then placed in cuvettes and allowed to equilibrate to 30 ± 0.1 °C, and the absorbance was directly read on a Zeiss PMQ II spectrophotometer.

Complexation reactions between various metal ions and III that were too rapid to be monitored with a conventional spectrophotometer were followed using a Durrum-Gibson stopped flow spectrophotometer (Model D110). The substrate was dissolved at the desired concentration in 0.5 M $LiClO_4$. This solution was introduced into one of two identical drive syringes. The other syringe contained the appropriate buffer ($HClO_4$ or cacodylate) maintained at the same ionic strength with $LiClO_4$, and the chosen metal ion (Co^{2+}, Cu^{2+}, Ni^{2+}, or Zn^{2+} perchlorate). The drive syringes, mixing chamber, and cuvette were suspended in a water trough whose temperature was maintained at 30 ± 0.1 °C. Optical density changes (270 nm) after mixing were recorded on a Hewlett-Packard storage oscilloscope (Model 1207B). With each buffer, three to four reactions were tabulated.

Reaction solution pH values were measured with a Radiometer Model 22 pH meter or a Beckman Model 3500 digital pH meter standardized with Mallinckrodt standard buffer solutions.

Spectrophotometric Determination of pK_a Values. The amides I–III were not sufficiently soluble in H_2O for accurate titrimetric determination of the pK_a values.[20] However, it was found that large changes in absorbance at appropriate wavelengths occurred with changes in pH so that pK_a values could be determined spectrophotometrically. A series of buffer solutions were prepared to cover a pH range of 0–12.

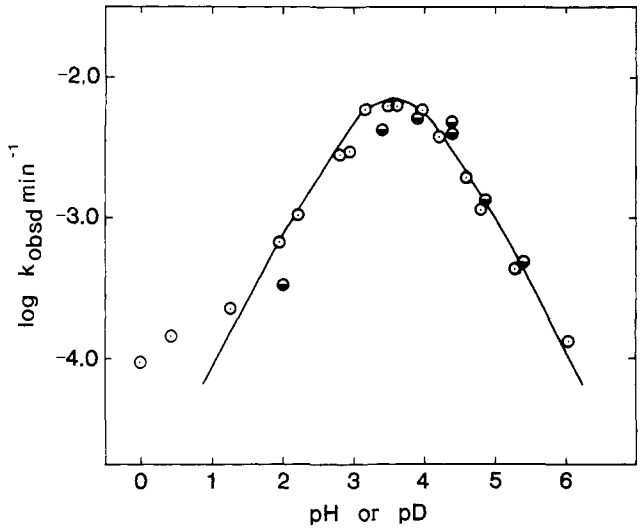


Figure 1. Plot of log k_{obsd} vs. pH or pD for hydrolysis of *N*-(2-pyridyl)-phthalamic acid at 50 °C and μ = 0.5 with KCl in H_2O (⊙) and D_2O (◒). The line was calculated from eq 1 using appropriate values of the constants.

and the ionic strength was kept constant at 0.5 M with KCl. A 10^{-2} M stock solution (30 μL) was injected into a cuvette containing 3 mL of buffer which had been thermally equilibrated at 30 ± 0.1 °C or 50 ± 0.1 °C. After mixing, the absorption spectrum was taken using a Beckman Model 25 spectrophotometer. A plot of absorbance at the appropriate wavelength vs. pH was made. Amide I has one discernible pK_a of 3.90 at 30 °C and 3.65 at 50 °C measured at 295 nm, and 4.10 measured at 275 nm. Amide III has pK_a values of 4.55 and 5.50 at 30 °C (measured at 295 and 275 nm, respectively). These values at 50 °C are 4.53 and 5.10. Since the lower value is relatively unchanged with temperature it must correspond with the carboxyl group pK_a.

Hydroxamic Acid Assays. Adaptations of the procedures of Hestrin[21] and Bruice and Marquardt[22] were employed in assessing the purity of the amide starting material. Benzamide was employed as the concentration standard. Compounds I and III were in that manner shown to be 99 and 98% amide, respectively, i.e., the tests were quantitative within the accuracy of the determination.

Results

Values of k_{obsd} at 50 °C in H_2O or D_2O for hydrolysis of *N*-(2-pyridyl)phthalamic acid (I) and *N*-(2-phenanthrolyl)-phthalamic acid (III) are given in Table I. In Figure 1 is shown a plot of log k_{obsd} vs. pH or pD for reaction of I. The plot shows an apparent dependence of k_{obsd} on the ionization of two groups with pK_a values of 3.22 and 3.95. The value of k_{obsd} at the maximum in the profile is 6.51×10^{-3} min^{-1}. This value is only slightly less when D_2O is the solvent (5.25×10^{-3} min^{-1}). The neutral species or the kinetically equivalent zwitterionic species must provide maximum reactivity. The data give a satisfactory fit to eq 1

$$k_{obsd} = \frac{k_r K_a a_H}{a_H^2 + K_a a_H + K_a K_a'} \quad (1)$$

where K_a and K_a' are the apparent dissociation constants and k_r is the rate constant assumed to give the best fit of the data ($k_r = 1.31 \times 10^{-2}$ min^{-1}). The ratio $k_r^{H_2O}/k_r^{D_2O}$ is 1.4.

In Figure 2 a plot is presented of log k_{obsd} vs. pH for hydrolysis of III. The plot shows dependence of k_{obsd} on one group in the acid form. The rate constant for maximum participation by this group (k_1) is 3.42×10^{-3} min^{-1} in H_2O and 3.10×10^{-3} min^{-1} in D_2O (k_{H_2O}/k_{D_2O} = 1.10). Assuming that both the neutral and protonated species are reacting, the equation for k_{obsd} is that given in eq 2

$$k_{obsd} = \frac{(k_H a_H^2 + k_1 a_H + k_2 K_a) a_H}{K_a K_a' + K_a a_H + a_H^2} \quad (2)$$

Table I. Rate Constants for Hydrolysis of *N*-(2-Pyridyl)phthalamic Acid at 50 °C and *N*-(2-Phenanthrolyl)phthalamic Acid at 50 °C (μ = 0.5)

Compd	pH	pD[a]	$k_{obsd} \times 10^3$, min^{-1}
N-(2-Pyridyl)phthal-amic acid	0[b]		0.1
	0.5		0.158
	1.25		0.223
	1.95		0.663
	2.20		1.05
	2.80		2.87
	2.90		3.77
	3.15		5.95
	3.50		6.29
	3.60		6.51
	3.98		5.95
	4.20		3.83
	4.60		1.95
	4.80		1.18
	5.30		0.432
	6.03		0.136
		1.99	0.338
		3.39	4.23
		3.89	5.26
		4.36	4.79
		4.39	4.106
		4.84	1.35
		5.39	0.475
N-(2-Phenanthrolyl)-phthalamic acid	0[b]		37.03
	0.50		16.17
	1.25		6.03
	1.95		4.40
	2.05		3.99
	2.35		3.53
	2.50		3.50
	2.60		3.14
	2.75		3.33
	3.10		3.33
	3.55		2.08
	4.17		1.21
	4.60		0.772
	4.98		0.408
	5.30		0.295
		1.05	5.14
		2.00	3.10
		3.40	1.64
		4.84	1.02

[a] In calculating pD at 50 °C, the glass electrode correction formula of Fife and Bruice was employed: T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961). [b] 1.0 M HCl.

where K_a is the apparent dissociation constant for the carboxyl group (10^{-4}), K_a' is the dissociation constant of the phenanthroline conjugate acid (pK_a = 5.10), k_1 is the rate constant for participation by the carboxyl group (protonated nitrogen), and k_2 is the rate constant for participation by the carboxyl group when the compound is neutral (4.30×10^{-4} min^{-1}). The apparent value of pK_a (4.0) was chosen to give the best fit of the theoretical line in Figure 2 and is slightly less than the experimentally determined thermodynamic pK_a of 4.5.

Hydronium ion catalysis is observed in reactions of II and III, but not in the case of I at HCl concentrations as high as 1.0 M. The points in Figure 1 for hydrolysis of I that deviate positively from the theoretical line at low pH probably represent a transition to hydronium ion catalysis at higher acid concentrations, but at 1.0 M HCl the rate constants are still declining with decreasing pH. Rate constants for hydronium ion catalysis are given in Table II. Plots of log k_{obsd} vs. log HCl concentration are linear with II and III with slopes of 1.10 and 1.36, respectively. Significant buffer catalysis was not observed

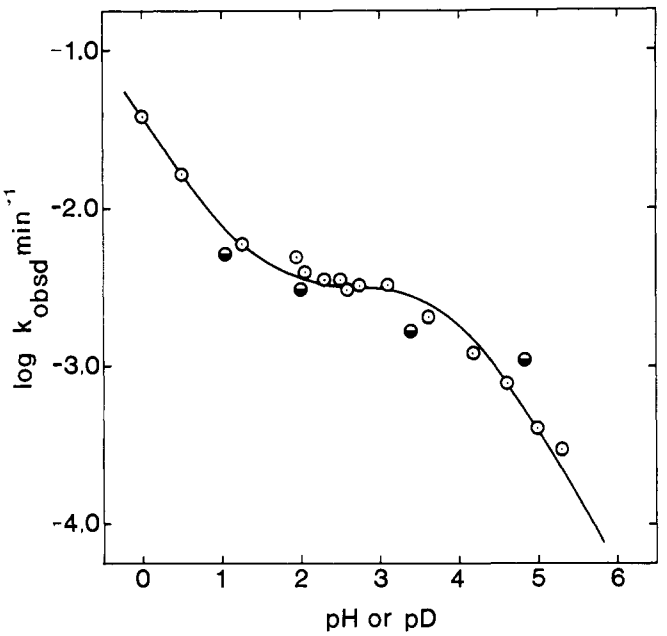


Figure 2. Plot of log k_{obsd} vs. pH or pD for hydrolysis of *N*-(2-phenanthrolyl)phthalamic acid at 50 °C and μ = 0.5 with KCl in H_2O (⊙) and D_2O (◒). The line was calculated from eq 2 using appropriate values of the constants.

Table II. Rate Constants for Hydronium Ion Catalyzed Hydrolysis of *N*-(2-Pyridyl)benzamide (II) at 90 °C and *N*-(2-Phenanthrolyl)phthalamic Acid (III) at 50 °C

	$k_{obsd} \times 10^2$, min^{-1}	
HCl, M	II	III
0.5	0.128	1.62
1.0	0.272	3.70
2.0	0.571	7.70
3.0	0.925	16.44
4.0	1.30	26.82
5.0		40.03

in hydrolysis of I or III at total buffer concentrations less than 0.1 M, which was the concentration generally employed. However, there was evidence that k_{obsd} is increased slightly at very high buffer concentrations. Increasing total chloroacetate buffer concentration in the range 0.05–0.35 M at pH 2.75 produced a 13% increase in k_{obsd} for I, and acetate buffer (0.05–0.5 M) at pH 4.50 increased k_{obsd} by 17%.

Divalent metal ions (Co^{2+} and Ni^{2+}) produce a small rate-retarding effect on the hydrolysis of I in the pH range 1–6. Rate constants for these reactions in the presence of 0.002 M metal ion are given in Table III. Retardation of the rate increases as the metal ion concentration is increased at pH 3.75 as illustrated in Figure 3. This effect is greater when the anion is ClO_4^- rather than Cl^-.

Divalent metal ions complex very rapidly with III. The rates of complexation were measured spectrophotometrically at 270 nm at pH 2.75 and 5.35 (30 °C). Observed rate constants for these reactions at 30 °C are listed in Table IV. Plots of k_{obsd} vs. metal ion concentration for complexation of Co^{2+} are shown in Figure 4. Similar plots for Ni^{2+}, Cu^{2+}, and Zn^{2+} were obtained. Pronounced metal ion inhibition of the rate of reaction of III is observed. Rate constants at pH 2.75 in the presence of metal ions are presented in Table V. The metal ion complexes of III are so stable at pH 5.35 that absorbance changes were not observed over a period of 7 days.

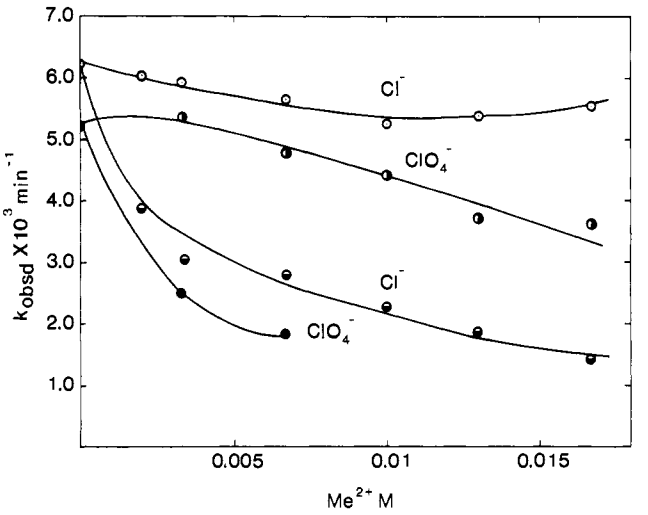


Figure 3. Plot of k_{obsd} vs. metal ion concentration (Co^{2+} ⊙, ◑ or Ni^{2+} ◒, ●) for hydrolysis of *N*-(2-pyridyl)phthalamic acid at 50 °C and pH 3.75 (μ = 0.5 M) with KCl or $LiClO_4$.

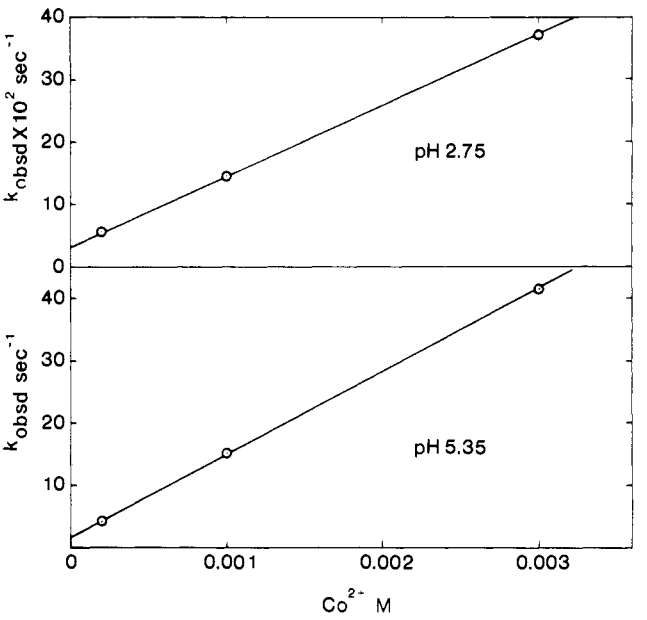


Figure 4. Plots of k_{obsd} vs. Co^{2+} concentration for complexation to *N*-(2-phenanthrolyl)phthalamic acid at 30 °C and pH 2.75 or 5.35.

Table III. Effect of Metal Ions (0.002 M) on the Hydrolysis of *N*-(2-Pyridyl)phthalamic Acid[a] at 50 °C (μ = 0.5)

Metal ion	pH	$k_{obsd} \times 10^3$, min^{-1}
Co^{2+}	1.25	0.185
	2.60	1.45
	3.10	2.92
	3.60	4.96
	3.98	4.21
	4.98	1.01
Ni^{2+}	2.60	0.964
	3.10	2.76
	3.60	4.02
	3.98	1.91
	4.98	0.733
	6.03	0.057

[a] Amide concentration was 10^{-4} M.

Table IV. Rate Constants for Complexation of Metal Ions with *N*-(2-Phenanthrolyl)phthalamic Acid[a] at 30 °C (μ = 0.5 M with $LiClO_4$)

Metal ion	Concn, M × 10^4	pH	k_{obsd}, s^{-1}	k_d[b], s^{-1}	k_f[b] × 10^{-6}, s^{-1}	Lit.[c] k_f × 10^{-6}, s^{-1}	Lit.[c] k_d, s^{-1}
Co^{2+}		5.35		1.46			
	2.0	5.35	4.19				
	10.0	5.35	15.21				
	30.0	5.35	41.55				
		2.75		0.0325	0.064	0.31	0.0159
	2.0	2.75	0.0562				
	10.0	2.75	0.145				
	30.0	2.75	0.372				
Ni^{2+}	2.0	5.35	0.0665				
	10.0	5.35	0.324				
	30.0	5.35	1.094				
		2.75		0.00035	0.0022	0.0031	0.00001
	2.0	2.75	0.00101				
	10.0	2.75	0.00431				
	30.0	2.75	0.0122				
Zn^{2+}	2.0	5.35	127.0				
		2.75		0.384	1.76	2.0	4.0
	2.0	2.75	0.947				
	10.0	2.75	3.747				
	30.0	2.75	9.742				
Cu^{2+}	2.0	5.35	230.				
		2.75		0.923	11.8	10.0	0.04
	2.0	2.75	3.535				
	10.0	2.75	25.18				
	30.0	2.75	64.22				

[a] Amide concentration was 10^{-4} M. [b] Intercept of a plot of k_{obsd} vs. metal ion concentration (Me phen^{2+} (k_d) $\rightleftharpoons$ Me^{2+} + phen (k_f)). The k_f values were calculated from the slopes of plots of k_{obsd} vs. metal ion concentration employing the equation of R. S. Bell and N. Sutin, *Inorg. Chem.*, **1**, 359 (1962), where K_a' is the acid dissociation constant and K_1 is k_d/k_f: $k_{obsd} = k_d(1 + K_a'(Me^{2+})/K_1(H^+))$. [c] Rate constants for 1,10-phenanthroline calculated from the data of R. H. Holyer, C. D. Hubbard, S. F. A. Kettle, and R. G. Wilkins, *Inorg. Chem.*, **3**, 929 (1965).

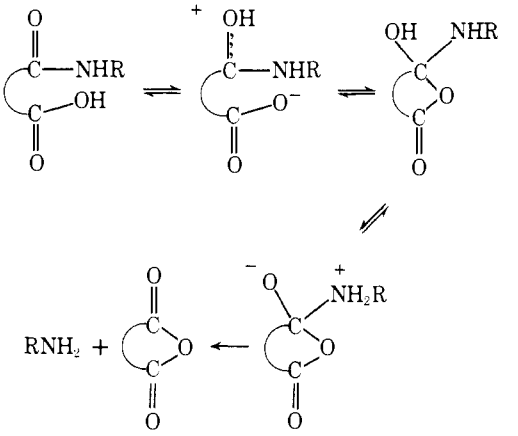
Table V. Divalent Metal Ion Inhibition of the Hydrolysis of *N*-(2-Phenanthrolyl)phthalamic Acid[a] at 50 °C (μ = 0.5 with $LiClO_4$)

Metal ion	pH[b]	Concn., M	k_{obsd} × 10^5, min^{-1}	$k^0_{obsd}/k^{Me^{2+}}_{obsd}$
None	2.75		333.0	
	5.35		29.46	
Co^{2+}	2.75	2×10^{-4}	8.87	37.5
	2.75	1×10^{-3}	11.6	
	3.00	2×10^{-4}	6.63	
	3.25	2×10^{-4}	3.71	
	5.35	2×10^{-4}	0[c]	
Cu^{2+}	2.75	2×10^{-4}	12.96	25.7
	2.75	1×10^{-3}	14.9	
Ni^{2+}	2.75	2×10^{-4}	0[d]	
Zn^{2+}	2.75	2×10^{-4}	15.63	21.3
	2.75	1×10^{-3}	19.4	
	5.35	2×10^{-4}	0[b]	

[a] 1×10^{-4} M. [b] pH 2.75, 3.00, and 3.25 were maintained with $HClO_4$ and pH 5.35 was maintained with 0.1 M cacodylate buffer. [c] No observable absorbance change over a period of 7 days. [d] No observable absorbance change over a period of 10 days.

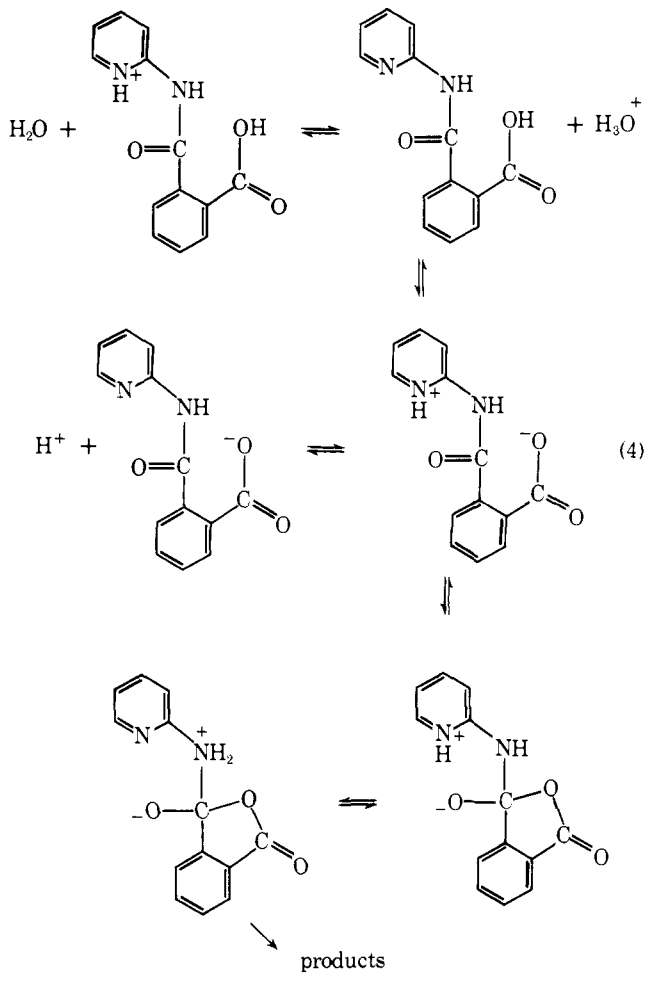
Discussion

The previously determined pH–rate constant profiles for intramolecular carboxyl group attack on amides have invariably shown a dependence of the observed rate constants on an un-ionized carboxyl group,[7-10] although the hydrolysis of *N*-(2-carboxyphenyl)phthalamic acid[8a] and diisopropylmaleamic acids derived from β-amino acids[23] have pH–rate constant profiles that are bell shaped. Since the nucleophilic reaction is most likely proceeding with attack by the anionic species, it is reasonable to assume that the amide function is protonated to assist the nucleophilic attack and/or to aid departure of the leaving group. A characteristic of nucleophilic reactions of amides appears to be a requirement for protonation of the leaving group to avoid expulsion of a highly unstable amine anion from the tetrahedral intermediate.[2,24,25] In the intramolecular aminolysis of the amide 2-aminomethylbenzamide to phthalimidine, proton transfer and C–N bond breaking are concerted.[25] General acid catalysis is observed in cyclization of substituted maleamic acids to anhydride with $\alpha = 0$ at $pK_a < 4$, implying that in those cases proton transfer is diffusion controlled and rate limiting.[10] Thus, a generalized scheme for intramolecular carboxyl group attack on amides can be formulated as in eq 3 in which proton transfer to the



(3)

leaving group may be a preequilibrium process with C–N bond breaking rate determining, or in which proton transfer is concerted with bond breaking or wholly rate limiting. It is a reasonable expectation that breakdown of the tetrahedral intermediate would normally be rate limiting in view of the low basicity of a carboxylate anion. Hawkins[8b] has presented evi-



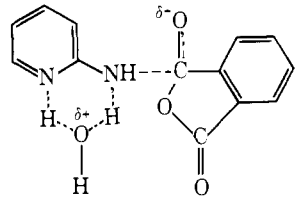
(4)

dence supporting rate-limiting tetrahedral intermediate breakdown in hydrolysis of phthalanilic acid in 0.1 M HCl. Nucleophilic attack can only be rate determining if departure of the amine leaving group is more favorable than breakdown to starting materials.

The extremely rapid rate of hydrolysis of I and III in comparison with II and with simple amides such as benzamide[7] shows convincingly that the neighboring carboxyl group is participating in the reactions. The D_2O solvent isotope effects close to unity indicate that the carboxyl group is functioning as a nucleophile. A general base or general acid mechanism would be expected to proceed much more slowly in D_2O than in H_2O. If phthalic anhydride is formed as an intermediate in a nucleophilic reaction it would hydrolyze rapidly to phthalic acid in a fast step. Phthalic anhydride has been indirectly demonstrated to be an intermediate in the hydrolysis of phthalamic acid[7] and phthalate monoesters.[6] It hydrolyzes in the pH range 1.6–5.7 in a pH-independent reaction with a rate constant of 0.739 min^{-1} at 30 °C,[6b] which is several orders of magnitude greater than the k_{obsd} values for I–III in that pH range at higher temperatures. *N*-Methylphthalimide is formed in addition to hydrolysis products in reactions of *N*-methylphthalamic acid,[19] but in the present study of I and III there was no evidence for imide formation. Likewise, Hawkins[8b] found no evidence for imide formation during hydrolysis of phthalanilic acids in aqueous solution.

The pH–rate constant profile for hydrolysis of *N*-(2-pyridyl)phthalamic acid (I) (Figure 1) is quite different than those profiles obtained previously. The bell-shaped profile suggests that the ionization state of two functional groups is important with kinetic pK_{app} values of 3.2 and 3.9. Only one pK_a could be detected in a spectrophotometric titration at 50 °C in the pH range 0–12 at 295 nm (3.65), which is very likely the result of the dissociation constants for the two groups being closely similar. This is supported by the fact that at 275 nm the experimental value is slightly higher (4.10). The bell-shaped curve can be interpreted as indicating that maximum reactivity is displayed by the neutral form of the compound or the kinetically equivalent zwitterionic species. Therefore, a reasonable reaction scheme is that given in eq 4.

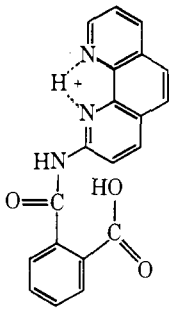
At pH 3.5, k_{obsd} for hydrolysis of I (50 °C) is 10^4 larger than k_{obsd} for II (90 °C) even with a temperature difference of 40 °C. A large rate advantage is also associated with the pyridyl nitrogen in the ortho position; *N*-(4-pyridyl)phthalamic acid hydrolyzes at pH 3.5 20-fold more slowly at 90 °C than I at 50 °C.[26] These structural features and the bell-shaped pH–rate constant profile of I might suggest a concerted bifunctional reaction, but a stepwise reaction with a tetrahedral intermediate, as in eq 4, is chemically more reasonable.[26] The relatively rapid rate of hydrolysis of I could be due to a favorable equilibrium constant for ring closure or to the ease of proton transfer to the leaving group in breakdown of the tetrahedral intermediate (XI). The small D_2O solvent isotope effect ($k_r^{H_2O}/k_r^{D_2O}$ = 1.4) indicates that proton transfer is either nearly complete in the critical transition state or has not progressed to a significant extent. Transition state XI could be

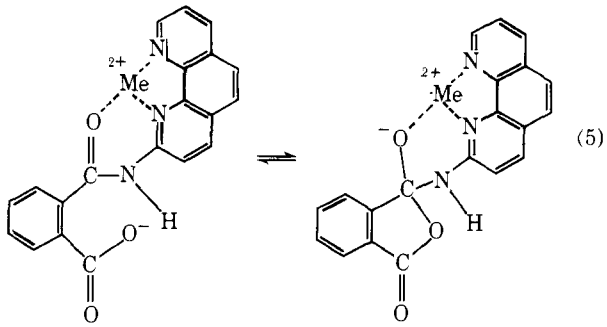


XI

important if the reaction proceeds through the zwitterionic species. The lack of buffer catalysis provides a measure of support for XI since it might reasonably be expected that buffer catalysis would be observed in hydrolysis of I in the absence of competing internal catalysis. Buffer catalysis has been found in the hydrolysis of aryl-substituted maleanilinic acids and has a small Brönsted coefficient, indicating a concerted reaction.[27] The bell-shaped profile could also be the result of a favorable reaction of the neutral species with inhibition by the pyridine nitrogen conjugate acid. Protonation of the leaving group by hydronium ion or a general acid would be difficult with a protonated pyridine nitrogen in close proximity. This is very likely the explanation for the absence of hydronium ion catalysis in hydrolysis of I at low pH.

The pH–rate constant profile for hydrolysis of the phenanthroline derivative III is quite different than that for I. The apparent dependence of the rate on the un-ionized carboxyl group is similar to what has been observed previously in amide reactions involving participation by a carboxyl group.[7-10] The maximum in the pH–rate constant profile must correspond to reaction of the protonated species. A striking difference between I and III is the absence of hydronium ion catalysis at low pH in the cyclization of I. Whereas the values for k_{obsd} are approximately threefold greater for I in the pH range 3.5–6, III hydrolyzes much faster than I at low pH. At pH 0 (1 M HCl) the difference in k_{obsd} is a factor of more than 400, which probably reflects the difficulty in protonating the amide function of the conjugate acid of I. Thus, the steric situation in monoprotonated I and III must be different. The ortho nitrogen of III has a similar steric relationship with the amide function as the pyridine nitrogen of I, but the proton in the monoprotonated species must partially or wholly reside on N-10. The much more favorable hydronium ion catalysis with III very likely results because positive charge is further from the amide group and provides less electrostatic repulsion to approach of hydronium ion to the amide. The differently





(5)

shaped pH–rate constant profiles for I and III can be explained in terms of a sterically less favorable internal proton transfer to the leaving group with III than I and/or to the lack of reactivity of the protonated species of I.

Metal Ion Effects. The divalent metal ions Ni^{2+} and Co^{2+} produce a small inhibitory effect on the hydrolysis of I. In the presence of 0.002 M metal ion, the pH–rate constant profile is still bell shaped but the values of k_{obsd} are reduced by approximately twofold. This small inhibitory effect could arise from partial complexing to the carboxylate ion, which would inhibit nucleophilic attack, or it could be due to complexing of the pyridine nitrogen which would make protonation of the leaving group more difficult.

In contrast with I, divalent metal ions have a large inhibitory effect on hydrolysis of III. At pH 2.75, where the hydrolysis reaction is pH independent and where protons strongly compete with metal ion for binding to the substrate, k_{obsd} is 20–40-fold less when the ratio of metal ion (Cu^{2+}, Co^{2+}, or Zn^{2+}) to substrate is 2/1. In the presence of Ni^{2+} a hydrolysis reaction is not observed. At pH 5.35, III is so stable when the metal ion to substrate ratio is 2/1 with all the metal ions that no significant absorbance changes were detected over a period of 7 days. Coordination of the carboxyl group would not explain complete loss of reactivity, especially since only small metal ion effects are observed in the cyclization of I where an equal opportunity for carboxyl group complexing exists. The rates of complexing of metal ions with III were measured spectrophotometrically with a Durrum stopped-flow spectrophotometer, and the rate constants are similar to those for chelation of 1,10-phenanthroline[28] (Table IV). Thus the metal ions must be binding the phenanthroline nitrogens of III. It would be expected that metal ions would bind much more readily to III than I because of the possibility of chelation with a second nitrogen.

The pronounced metal ion inhibition most likely results from inhibition of protonation of the amine leaving group. Even though nucleophilic attack of the carboxylate ion might be greatly enhanced by chelation of a metal ion to the carbonyl oxygen, the overall kinetic effect would be inhibition because the attack step is not rate limiting and breakdown of the tetrahedral intermediate to products would be retarded unless the metal ion can duplicate exactly the ability of a proton to facilitate departure of the leaving group. This is not necessarily the case, especially when binding of metal ion to the leaving group nitrogen might be sterically unfavorable as with III (four-membered ring).[29] With a metal ion bound to III in the manner of eq 5, breakdown of the tetrahedral intermediate to products should be strongly retarded. Lone pair orbital orientation may be important in the partitioning of a tetrahedral intermediate in amide hydrolysis reactions,[30] specific cleavage of a carbon–nitrogen bond being allowed only if the oxygens of the tetrahedral intermediate each have an orbital oriented antiperiplanar to the leaving group. Thus, part of the large rate inhibition could also be due to this factor, if indeed metal ion chelation has an unfavorable influence on the conformation of the tetrahedral intermediate resulting from carboxyl group attack. Metal ions can exert a catalytic effect on the hydrolysis of amino acid amides[31] and peptides,[14,32] but these effects are small.

Conclusions

The following conclusions can be drawn from the present study.

(1) Nucleophilic attack on amides by a neighboring carboxyl group will give a bell-shaped pH–rate constant profile and an enhancement in the rate of the reaction at the rate maximum in the presence of a second ionizable group that can transfer a proton to the leaving group.

(2) Protonation of the leaving group is very likely a requirement in nucleophilic attack by carboxyl on amides, as is the case with other intramolecular nucleophiles.

(3) Divalent metal ions strongly inhibit intramolecular nucleophilic reactions of III, an amide capable of chelating the metal ion.

(4) It seems clear that a nucleophilic role for glutamic acid-270 in reactions of carboxypeptidase A could lead to large rate enhancements. This is especially true considering the possibilities for facile proton transfer from other functional groups in the active site. Evidence has been presented for the existence of an anhydride intermediate at low temperature in the reaction of carboxypeptidase A with an ester substrate.[33] A nucleophilic mechanism is also to be preferred at the present time over a general base role[34] for glutamic acid-270 in the hydrolysis of amides since, when possible, nucleophilic attack is normally the more favorable process and is capable of generating much larger rate enhancements. However, whatever the role of Glu-270, protonation or comparable stabilization of the leaving group must occur.

Possibly a catalytic role for Zn^{2+} in carboxypeptidase A catalyzed hydrolysis of amide and peptide substrates[11-13,35] should be reconsidered. It is difficult to see how a metal ion chelated to a tetrahedral intermediate could have a large facilitating effect on the bond-breaking process. It is possible that the effect of Zn^{2+} is to maintain proper positioning of the substrate carbonyl group during formation of the enzyme substrate complex. Internal attack of metal bound hydroxide ion has been shown to lead to rapid hydrolysis of esters,[36] amides,[37] and anhydrides,[38] and so might also be considered as a possibility for carboxypeptidase A catalyzed hydrolysis reactions.

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References and Notes

(1) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", W. A. Benjamin, New York, N.Y., 1966.
(2) W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969.
(3) T. C. Bruice in "The Enzymes", Vol. II, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1970, Chapter 4.
(4) A. J. Kirby and A. Fersht, "Progress in Bioorganic Chemistry", Vol. I, E. T. Kaiser and F. J. Kezdy, Ed., Wiley-Interscience, New York, N.Y.,

1971.
(5) T. H. Fife, *Adv. Phys. Org. Chem.*, 11, 1 (1975).
(6) (a) M. L. Bender, F. Chloupek, and M. C. Neveu, *J. Am. Chem. Soc.*, **80**, 5384 (1958); (b) J. W. Thanassi and T. C. Bruice, *ibid.*, **88**, 747 (1966).
(7) M. L. Bender, *J. Am. Chem. Soc.*, **79**, 1258 (1957); M. L. Bender, Y. Chow, and F. Chloupek, *ibid.*, **80**, 5380 (1958).
(8) (a) H. Morawetz and J. Shafer, *J. Am. Chem. Soc.*, **84**, 3783 (1962); (b) M. D. Hawkins, *J. Chem. Soc., Perkin Trans. 2*, 642 (1976).
(9) A. J. Kirby and P. W. Lancaster, *J. Chem. Soc., Perkin Trans. 2*, 1206 (1972).
(10) M. F. Aldersley, A. J. Kirby, P. W. Lancaster, R. S. McDonald, and C. R. Smith, *J. Chem. Soc., Perkin Trans. 2*, 1487 (1974).
(11) W. N. Lipscomb, *Acc. Chem. Res.*, **3**, 81 (1970).
(12) W. N. Lipscomb, G. N. Reeke, Jr., F. A. Quiocho, and P. H. Bethge, *Philos. Trans. R. Soc. London, Ser. B*, **251**, 177 (1970).
(13) E. T. Kaiser and B. L. Kaiser, *Acc. Chem. Res.*, **5**, 219 (1972).
(14) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins", Wiley-Interscience, New York, N.Y., 1971.
(15) E. H. Huntress and H. C. Walter, *J. Org. Chem.*, **13**, 735 (1948).
(16) E. J. Corey, A. L. Borror, and T. Foglia, *J. Org. Chem.*, **30**, 288 (1965).
(17) D. S. Sigman, G. M. Wahl, and D. J. Creighton, *Biochemistry*, 11, 2236 (1972).
(18) R. Breslow, R. Fairweather, and J. Keana, *J. Am. Chem. Soc.*, **89**, 2135 (1967).
(19) J. Brown, S. C. K. Su, and J. A. Shafer, *J. Am. Chem. Soc.*, **88**, 4468 (1966).
(20) The low solubility of *III* also precluded a thorough spectrophotometric (UV–visible) study of the coordination geometry and the nature of the ligands of the metal ion complexes.
(21) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).
(22) T. C. Bruice and F. H. Marquardt, *J. Am. Chem. Soc.*, **84**, 365 (1962).
(23) A. J. Kirby, R. S. McDonald, and C. R. Smith, *J. Chem. Soc., Perkin Trans. 2*, 1495 (1974).
(24) D. G. Oakenfull and W. P. Jencks, *J. Am. Chem. Soc.*, **93**, 178 (1971); D. G. Oakenfull, K. Salvesen, and W. P. Jencks, *ibid.*, **93**, 188 (1971).
(25) T. H. Fife and B. R. DeMark, *J. Am. Chem. Soc.*, in press.
(26) Hydrolysis at 90 °C of *N*-(4-pyridyl)phthalamic acid, with which a bifunctional mechanism is not possible, also has a bell-shaped pH–rate constant profile. The conjugate acid of this compound has a much higher pK_a than I thereby making direct comparisons of the rate constants difficult.
(27) R. Kluger and C. H. Lam, *J. Am. Chem. Soc.*, **97**, 5536 (1975).
(28) R. S. Bell and N. Sutin, *Inorg. Chem.*, **1**, 359 (1962); R. H. Holyer, C. D. Hubbard, S. F. A. Kettle, and R. G. Wilkins, *ibid.*, **4**, 929 (1965).
(29) In the case of *N*-(8-quinolyl)phthalamic acid it is sterically possible for a metal ion to be chelated to the amine leaving group, and metal ion effects on the rate of the hydrolysis reaction are small: T. H. Fife and V. L. Squillacote, unpublished work.
(30) P. Deslongchamps, C. Lebreux, and R. Taillefer, *Can. J. Chem.*, **51**, 1665 (1973); P. Deslongchamps, *Tetrahedron*, **31**, 2463 (1975).
(31) L. Meriwether and F. H. Westheimer, *J. Am. Chem. Soc.*, **78**, 5119 (1956).
(32) J. R. Cronin, D. A. Long, and T. G. Truscott, *Trans. Faraday Soc.*, **67**, 2096 (1971).
(33) M. W. Makinen, K. Yamamura, and E. T. Kaiser, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3882 (1976).
(34) R. Breslow and D. Wernick, *J. Am. Chem. Soc.*, **98**, 259 (1976).
(35) M. L. Ludwig and W. N. Lipscomb in "Inorganic Biochemistry", Vol. I, G. L. Eichhorn, Ed., American Elsevier, New York, N.Y., 1973, pp 438–487.
(36) M. A. Wells, G. A. Rogers, and T. C. Bruice, *J. Am. Chem. Soc.*, **98**, 4336 (1976).
(37) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.*, **92**, 6151 (1970); D. A. Buckingham, F. R. Keene, and A. M. Sargeson, *ibid.*, **96**, 4981 (1974).
(38) R. Breslow, D. E. McClure, R. S. Brown, and J. Eisenach, *J. Am. Chem. Soc.*, **97**, 194 (1975).

Solvolysis of Haloallenes. The Effect of Added Salts on the Polarimetric and Titrimetric Rates of Solvolysis of 1-Bromo-3-methyl-1,2-pentadiene

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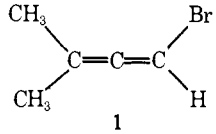
Abstract: The effect of added LiCl, LiBr, $LiClO_4$, and NaN_3 and the effect of solvent nucleophilicity on the polarimetric and titrimetric rates of solvolysis of (*R*)-(−)-1-bromo-3-methyl-1,2-pentadiene and its racemic modification are reported. The data are reconciled with a mechanism of solvolysis involving nucleophilic attack on a tight ion pair. Marked increases in k_{rac} with added Br^- in 97:3 (w/w) 2,2,2-trifluoroethanol–water and with added N_3^- in 60:40 (v/v) ethanol–water and $k_{rac} \neq 0$ in the absence of added nucleophile are key elements in this interpretation. Internal return with rearrangement is also discussed.

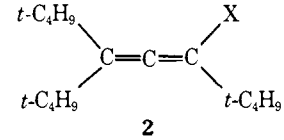
The previous paper in this series[2] reported the effect of changing solvent nucleophilicity on the rate of solvolysis of a variety of di- and trisubstituted haloallenes. In general these compounds exhibited behavior typical of saturated halides undergoing solvolysis by a limiting mechanism but with notable exceptions.[3] Typical of these exceptions is the behavior of **1**



1

which yields $m = 0.88$ vs. Y in aqueous ethanol, $(k_H/k_D)_\alpha = 1.20$ in 50E,[4] 1.23 in 70T,[4] and 1.28 in 97T,[4] and $(k_H/k_{CD_3})_\beta = 1.33$ in 50E, 60E,[4] 70T, and 97T. Interestingly, however, the $(T/E)_Y$[5] rate ratio exhibited by **1** is 0.63. We concluded that while the former data might imply solvolysis via a limiting mechanism, the lower rate of solvolysis in the nonnucleophilic solvent at constant Y was more consistent with the behavior of saturated substrates undergoing solvolysis with considerable assistance by solvent attack on neutral substrate or ion pair.[3b,6]

Nor is the phenomenon unique to **1**. In fact, the 70T/50E rate ratio diminishes along the series $(t\text{-}C_4H_9)_2C{=}C{=}C(H)Br > t\text{-}C_4H_9(CH_3)C{=}C{=}C(H)Br > (CH_3)_2C{=}C{=}C(H)Br$, being 8.2, 3.7, and 1.8 respectively.[2] In addition **2** (X = Cl)



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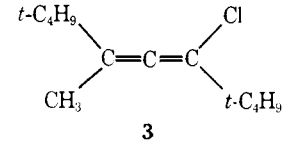

3

exhibits a 97T/60E rate ratio of 900 while **3** reacts only 12 times as rapidly in this nonnucleophilic solvent.[2] These data thus imply the possibility of an increasingly important component of nucleophilic solvent assistance as steric hindrance is removed. Indeed the relatively low CH_3/H ratio, **1**/**4** = $10^{4.3}$ in 80E at 25 °C, provides some support for this contention, particularly when one notes that the $t\text{-}C_4H_9/H$ ratio **2** (X = Br)/**5** = $10^{3.1}$. It might be expected that the greater electron-releasing effect of the *tert*-butyl group would result in a larger ratio than is observed for methyl substitution. Impor-