Aminolysis of Esters. IX. The Nature of the Transition States in the Aminolysis of Phenyl Acetates

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Abstract: The rate constants for the HO^- catalyzed hydrolysis and aminolysis (by six amines) of nine α-substituted phenyl acetates and four β-substituted phenyl propionates have been determined. Of the esters all but the α-cyanoacetate and β-nitropropionate followed the rate law $v = k_{OH}[HO^-][ester]$ for alkaline hydrolysis. The pH–log k_{obsd} profiles for the α-cyanoacetate and β-nitropropionate esters establish the formation of carbanions which undergo alkaline hydrolysis at rates appreciably slower than the undissociated esters. The decreased rates of carbanion hydrolysis find most reasonable explanation through resonance and inductive stabilization of the ground state. From the aminolysis data, the sensitivity to electronic effects of the various pathways to amide were deduced. By (1) comparing the sensitivity to electronic effects in both acyl and leaving phenol for aminolysis paths to the like sensitivity for hydroxide ion catalyzed hydrolysis; (2) consideration of the sensitivity of rate terms to the base strength of amine and catalyst; (3) considering electrostatic effects; and (4) from a reasonable knowledge of the transition state structure for hydroxide ion catalyzed hydrolysis, transition state structures for the various aminolysis paths are arrived at (see Conclusion).

The aminolysis of phenyl esters by primary and secondary amines has been shown to occur through both general base and general acid catalyzed nucleophilic attack of amine at the ester bond.[1-8] In aqueous solution when amine and its conjugate acid serve as buffer, the following kinetic terms are discerned: k_n(amine)(H_2O)[7]; k_{gb}(amine)[2]; k_{ga}(amine)(amineH^+); k_{OH}'(amine)(HO^-).

In this study the sensitivity of k_n, k_{gb}, and k_{ga} to the electronic nature of α and β substituents on phenyl acetate and phenyl propionates is described. Based on the ρ values for substitution of the acyl functions, and previously found ρ values for substitution of the leaving group,[3,4] Brønsted β values was related to base strength of amine,[8] electrostatic effects,[9] and deuterium solvent isotope effects[2,3] possible transition state structures for the processes related to k_n, k_{gb}, and k_{ga} are considered.

Experimental Section

Materials. Potassium chloride, potassium hydroxide, potassium phosphate monobasic, and potassium bicarbonate were reagent grade and used without further purification. Morpholine, bp 125–126°, was distilled through a 1-ft Vigreux column. Glycine (Fisher, reagent grade) was used directly and glycylglycine (Aldrich) was recrystallized from aqueous ethanol. Hydrazine monohydrochloride (Matheson Coleman and Bell) was twice recrystallized from aqueous ethanol and had mp 94°. Methoxyamine, mp 149–150°, was recrystallized from ethanol–ether.

Phenyl Dichloroacetate (Method A). To 7.2 g of dichloroacetic anhydride (0.03 mol) was added 2.82 g of phenol (0.03 mol) and pyridine (0.5 ml). The mixture was refluxed for 2 hr and then distilled *in vacuo*. The fraction with bp 60–61° (0.4 mm) was retained; this solidified on standing and after several crystallizations from petroleum ether (30–60°) had mp 48–48.5° (lit.[10] mp 48–49°).

Phenyl Cyanoacetate (Method B). Cyanoacetic acid (10 g) was heated at 110° for 25 min with 11 g of phenol (0.116 mol) and 4 ml of phosphorus oxychloride. The mixture on cooling was neutralized with aqueous potassium carbonate and extracted twice with ether. On evaporation of the dried ($MgSO_4$) ether extracts, the residual oil was distilled *in vacuo*. The distillate, bp 113° (0.4 mm), solidified slowly to give a white solid, mp 40–42°. On crystallization from aqueous ethanol the ester has mp 43° (lit.[11] 41°).

Phenyl Ethylthioacetate (Method C). Dicyclohexylcarbodiimide (DCC), 6.8 g (0.03 mol), was dissolved in 30 ml of tetrahydrofuran (THF) with 2.82 g (0.03 mol) of phenol. The solution was maintained at 0° for 1 hr and then added to 3.60 g of ethylthioacetic acid (0.03 mol), also dissolved in 30 ml of cooled THF. The mixture was allowed to stand at 0° overnight; the precipitated urea was filtered off and the THF evaporated *in vacuo*. Distillation of the residual oil gave the ester, bp 145° (1.7 mm), $n^{30}D$ 1.5309. *Anal.* Calcd for $C_9H_{12}O_2S$: C, 61.20; H, 6.16; S, 16.33. Found: C, 61.01; H, 6.31; S, 15.65.

Phenyl β-Bromopropionate (Method D). To 12.60 g of trifluoroacetic anhydride (0.06 mol) was added an equimolar amount of β-bromopropionic acid (8.18 g). A solution was obtained after *ca.* 10 min; after a further hour at room temperature, 5.64 g of phenol (0.06 mol) was added. The solution was allowed to stand for 18 hr, concentrated *in vacuo*, and distilled twice. The ester had bp 87° (0.9 mm), $n^{23}D$ 1.5292. *Anal.* Calcd for $C_9H_9BrO_2$: C, 47.18; H, 3.96. Found: C, 47.35; H, 4.03.

The other esters used in this study were synthesized using one of the four general methods above (the actual method that was used in each case is noted in parentheses) and had the following physical data: **phenyl ethoxyacetate** (C) bp 76–78° (0.8 mm), $n^{22}D$ 1.4999 [lit.[12] bp 75–77° (0.7 mm), $n^{20}D$ 1.4040]; **phenyl β-nitropropionate** (D) bp 108–110°, $n^{23}D$ 1.5136. *Anal.* Calcd for $C_9H_9NO_4$: C, 55.43; H, 4.64. Found: C, 55.68; H, 4.76; **phenyl methoxyacetate** (D) bp 69–70° (1.1 mm), $n^{23}D$ 1.4990 (lit.[10] bp 127–128°, $n^{25}D$ 1.5000); **phenyl β-ethoxypropionate** (D) bp 73° (0.6 mm), $n^{23}D$ 1.4920. *Anal.* Calcd for $C_{11}H_{14}O_3$: C, 68.22; H, 7.26 Found: C, 68.22; H, 6.38; **phenyl chloroacetate** (C) mp 41–41.5° (lit.[10] 40–41°); **phenyl phenylacetate** (C) mp 39.5–40° (lit.[10] 39.5–40.5°); **phenyl acetate** (A) bp 76° (9 mm), $n^{27}D$ 1.5018 (lit.[13] $n^{25}D$

(1) J. F. Bunnett and G. T. Davis, *J. Amer. Chem. Soc.*, **82**, 665 (1960).
(2) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).
(3) T. C. Bruice and M. F. Mayahi, *ibid.*, **82**, 3067 (1960).
(4) T. C. Bruice and S. J. Benkovic, *ibid.*, **86**, 418 (1964).
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(6) W. P. Jencks and M. Gilchrist, *ibid.*, **88**, 104 (1966).
(7) L. do Amaral, K. Koehler, D. Bartenbach, T. Pletcher, and E. H. Cordes, *ibid.*, **89**, 3537 (1967).
(8) T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *ibid.*, **89**, 2106 (1967).
(9) B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 2875 (1969).
(10) A. S. A. S. Shawali and S. S. Biechler, *J. Amer. Chem. Soc.*, **89**, 3020 (1967).
(11) E. Ziegler, G. Wildtgrube, and H. Junek, *Monatsh. Chem.*, **88** 166 (1957).
(12) H. Baganz and J. Pflug, *Chem. Ber.*, **90**, 386 (1967).

1.5012); **phenyl phenoxyacetate** (C) bp 162–164° (2 mm), mp 56.5–57.5° (lit.[14] mp 56–57°); **phenyl bromoacetate** (C) mp 31.5–32° (lit.[15] mp 32°); **phenyl propionate** (C) bp 43–45° (1.2 mm), $n^{25}D$ 1.4965 [lit.[10] bp 82–83° (2 mm), $n^{25}D$ 1.4952]; **phenyl β-chloropropionate** (D) bp 95° (1.5 mm), $n^{25}D$ 1.5168 [lit.[16] bp 132–135° (12 mm)].

Kinetic Measurements. The aminolysis reactions were studied spectrophotometrically (unless otherwise stated) by following the appearance of phenolphenoxide ion at the isosbestic point, 275 mμ. For the slower reactions a Gilford Model 2000 or a Zeiss PMQ II spectrophotometer was used; a Durrum-Gibson Model 13001 stopped-flow spectrophotometer equipped with an all Kel-F valve and cuvette assembly, being used for the more rapid reactions. In both cases the reaction solutions were thermostated at 30°. Some hydrolyses reactions were followed simultaneously, both spectrophotometrically and autotitrimetrically, using the following apparatus. A thermostated polyethylene cell with quartz windows was placed in the sample compartment of a Cary 15 ultraviolet spectrophotometer so that the contents of the cell could be stirred magnetically. The pH of the reaction solution in the cell was monitored using a Metrohm EA 125 U universal glass electrode and Radiometer Type PHM 26 pH meter. The pH was maintained constant by the addition of 0.05 *N* potassium hydroxide using Radiometer Titrator 11 Type TTT 11b, Titrigraph Type SBR 2c, and Auto-Burette Type ABU 1c. The hydrolytic rate constants obtained by both methods (followed by the appearance of phenol or by the addition of hydroxide) were in agreement; in general, however, better rate profiles were obtained by the spectral method in the faster reactions.

The amines were used both as nucleophiles and as buffers, to maintain constant pH. Studies with a given amine were therefore in the region (±1 pH unit) of its pK_a. The amine–amine hydrochloride buffers were prepared just prior to the kinetic run by the addition of standardized hydrochloric acid or potassium hydroxide to the solutions of the free amine or amine hydrochloride, ionic strength being maintained at 1.0 by the addition of potassium chloride. All solutions were prepared in fresh doubly distilled (from an all glass apparatus) water. The pH's of the kinetic solutions were determined both prior to and after a kinetic experiment using a Radiometer pH meter Type PHM 22 equipped with a PHA 630 scale expander, thermostated at 30°. Any run showing a pH drift greater than 0.03 unit was discarded.

The esters were dissolved (0.01–0.03 *M*) in spectral quality dioxane, and the reactions were initiated by the addition of one drop of this solution to the 2-ml cuvette containing the appropriate buffer. In experiments using the stopped-flow apparatus the same final concentration, *i.e.*, 2% of dioxane, was used. To obviate problems caused by the development of peroxides in the stock dioxane solutions of esters, these were kept frozen and reprepared frequently.

The values of the pseudo-first-order rate constants were calculated using Olivetti-Underwood Programma 101 computer with either a weighted least-squares analysis in determining the slopes of plots of $\log [(OD_\infty - OD_0)/(OD_\infty - OD_t)]$ *vs.* time (t) or by the method of Guggenheim.[17]

Results

The rates of hydroxide-catalyzed hydrolyses (k_{OH}) of fifteen phenyl acetates and phenyl propionates (α and β substituted in the acyl portion) have been determined at 30°, μ = 1.0 (with KCl) using a combination of experimental techniques. In the pH range 6–11 either an external buffer was employed and the appropriate hydroxide-catalyzed rate constant obtained by extrapolation of the serially diluted buffer to zero buffer concentration, or the pH was maintained constant by use of a pH-stat. At higher pH's, buffer capacity was maintained by hydroxide ion itself. In general, good plots of log k_{OH} *vs.* pH with unit slope were obtained in all cases and the resultant k_{OH} values obtained from these,

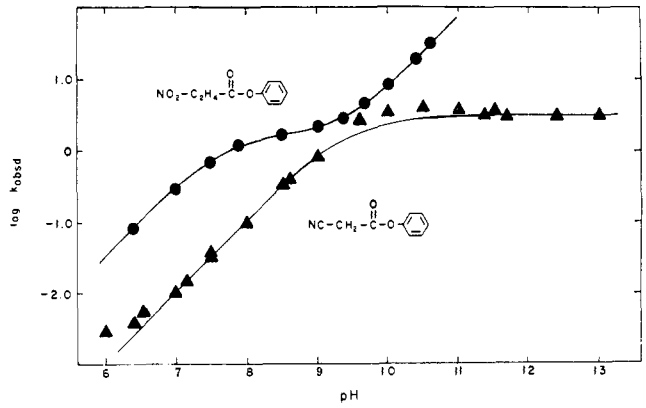


Figure 1. Plot of the observed pseudo-first-order rate constant (min^{-1}) *vs.* pH for the hydrolysis of phenyl β-nitropropionate and phenyl α-cyanoacetate.

together with the experimental conditions used for the individual ester, are listed in Table I. In two instances, for the ester phenyl β-nitropropionate and phenyl α-cyanoacetate, the rate *vs.* pH profile was studied in more detail. For the α-cyano ester the rate of hydrolysis is

Table I. Second-Order Rate Constants (k_{OH}) for HO^--Catalyzed Hydrolysis of Acyl-Substituted Esters ($XC(=O)OC_6H_5$)

Substituent (where X =)	Method[a]	pH Range (no. of values)	k_{OH}, M^{-1} min^{-1} [b]
CH_3	A		2.23×10^2 [c]
C_2H_5	A, C	9.39–10.33 (5)	1.51×10^2
CH_3OCH_2	B	11.30–12.00 (3)	4.95×10^3
$C_2H_5OCH_2$	B	11.30–12.00 (3)	4.76×10^3
$ClCH_2$	A	7.13–10.33 (7)	5.94×10^4
Cl_2CH	B, C	6.51–11.70 (5)	1.70×10^6
$BrCH_2$	B	6.51–8.60 (5)	9.50×10^4
$C_2H_5SCH_2$	A	8.60–10.33 (4)	1.65×10^3
$CNCH_2$	A,B,C	6.51–8.60 (5)	7.08×10^4
$C_6H_5CH_2$	A	8.60–10.33 (4)	4.47×10^2
$C_6H_5OCH_2$	A	8.01–10.33 (5)	5.25×10^4
$BrCH_2CH_2$	B	11.30–12.30 (4)	3.40×10^2
$ClCH_2CH_2$	B	11.30–12.30 (4)	3.41×10^2
$C_2H_5OCH_2CH_2$	B	11.30–12.30 (4)	3.80×10^2
$NO_2CH_2CH_2$	B,C	8.50–12.59 (12)	1.70×10^4

[a] A, external buffer dilution, pH = 6.5–7.5, phosphate; 8.0–9.0, borate; 9.5–10.5, carbonate; in each case five buffer concentrations of 1.0–0.05 *M* were used; B, buffered by hydroxide ion at high pH; C, autotitrator and spectrophotometric reactions monitored simultaneously. [b] μ = 1.0 with KCl, 30°. [c] T. C. Bruice and S. J. Benkovic, *J. Amer. Chem. Soc.*, **85**, 1 (1963).

first order in hydroxide ion at low pH and independent of hydroxide ion concentration at high pH, while for the β-nitropropionate ester the log k_{obsd} *vs.* pH profile exhibits regions of +1 slope separated by a plateau region (Figure 1). The k_{OH} values quoted in Table I for these esters are for the reaction of hydroxide ion with the neutral ester (see Discussion), and are therefore directly comparable with the other values listed in that table.

The rates of aminolysis of these acyl-substituted esters have also been studied under the same conditions (30°, μ = 1.0). The amines used in this study were hydrazine, ammonia, morpholine, glycine, glycylglycine, and methoxyamine. The most comprehensive data are available for the reactions with hydrazine; the experimental conditions used with this ester are listed

(13) T. C. Bruice and G. L. Schmir, *J. Amer. Chem. Soc.*, **79**, 1663 (1957).
(14) C. M. Hill, M. B. Towns, and G. Senter, *ibid.*, **71**, 257 (1949).
(15) S. M. McElvain and B. Fajardo-Pinzon, *ibid.*, **67**, 690 (1945).
(16) F. Mayer and L. von Zütphen, *Chem. Ber.*, **57**, 200 (1924).
(17) E. A. Guggenheim, *Phil. Mag.*, **2**, 538 1926).

Table II. Rate Constants and Experimental Conditions for the Hydrazinolysis of the Esters ($XC(=O)OC_6H_5$)[a]

Substituent (where X =)	pH range (no. of values)	Concn range (M)[b]	No. of k_{obsd}	k_n, l. mol^{-1} min^{-1}	k_{gb} l.^{2} mol^{-2} min^{-1}	k_{ga}	$k_{OH'}$
CH_3	7.59–9.02 (5)	0.5–0.05	30	0.49	14.1	4.90	2.6×10^4
C_2H_5	7.58–9.21 (9)	0.2–0.01	46	0.40	5.3	0.48	7.0×10^5
CH_3OCH_2	7.53–9.10 (6)	1.0–0.02	29	0.56	250	90	2.2×10^4
$C_2H_5OCH_2$	7.68–9.10 (6)	1.0–0.02	36	0.60	300	28	4.4×10^5
$ClCH_2$	7.51–9.10 (3)	1.0–0.10	15	12.0	2850	180	1.7×10^7
Cl_2CH	7.55–9.10 (3)	1.0–0.2	16	390	90000	4800	7.1×10^7
$BrCH_2$	7.50–9.20 (3)	1.0–0.05	12	13.2	2400	*c*	2.6×10^6
$C_2H_5SCH_2$	7.70–9.10 (3)	0.5–0.02	18	1.1	32	8.0	2.4×10^5
$CNCH_2$	7.52–9.10 (3)	1.0–0.05	12	21	2000	120	1.2×10^5
$BrCH_2CH_2$	7.53–9.64 (5)	1.0–0.05	29	0.42	8.5	0.36	1.6×10^4
$ClCH_2CH_2$	7.69–9.07 (6)	1.0–0.20	40	0.25	7.7	0.50	2.2×10^4
$C_2H_5OCH_2CH_2$	7.50–9.61 (6)	1.0–0.02	32	0.09	5.1	0.90	1.0×10^4
$NO_2CH_2CH_2$	7.50–9.50 (6)	1.0–0.02	43	1.98	13.2	0.60	8.4×10^4

[a] At 30°; μ = 1.0 (KCl). [b] Total concentration of amine (N_T). [c] Experimentally not detectable.

in detail in Table II. The rates of aminolysis were studied under pseudo-first-order conditions by maintaining the amine concentration in a large excess (relative to the ester) and in all cases the observed rate constants (k_{obsd}) were found to follow the general relationship

$$k_{obsd} = k_{OH}[HO^-] + k_n[N] + k_{gb}[N]^2 + k_{ga}[N][NH^+] + k_{OH'}[N][HO^-] \quad (1)$$

where [N] refers to the free (unprotonated) amine concentration (*i.e.*, $K_a = [N][H^+]/[NH^+]$ and the total amine concentration $[N_T] = [N] + [NH^+]$). The individual rate constants have the following designations: k_{OH}, hydroxide ion catalysis of ester hydrolysis; k_n, unassisted (or water assisted) amine nucleophilic attack; k_{gb}, nucleophilic attack assisted by neutral amine (general base); k_{ga}, nucleophilic attack assisted by the conjugate acid of the amine (general acid); and $k_{OH'}$, hydroxide ion catalysis of nucleophilic displacement by the amine.

Equation 1 can be rearranged to eq 2, separating the terms which are first and second order in amine. The

$$(k_{obsd} - k_{OH}[HO^-])/[N] = (k_n + k_{OH'}K_w/a_H) + (k_{gb} + k_{ga}a_H/K_a)[N] \quad (2)$$

relative contributions of the various terms in the kinetic equation to the overall observed rate were determined as follows. Each ester was studied at several pH's with a given amine; at each pH a minimum of five serial dilutions of the amine (which also acted as a buffer) were used. Plots of $(k_{obsd} - k_{OH}[HO^-])/[N]$ *vs.* [N] at a single pH gave $(k_n + k_{OH'}K_w/a_H)$ as intercept (I) and $(k_{gb} + k_{ga}a_H/K_a)$ as slope (S). Secondary plots of these slopes (S) *vs.* a_H/K_a (obtained at various pH values) gave the third-order constants k_{gb} and k_{ga} (as intercept and slope, respectively). Likewise the intercepts (I) were plotted against K_w/a_H to give the constants dependent on the first power of the amine concentration, k_n and $k_{OH'}$. The values of the constants obtained by this plotting technique are listed in Tables II and III. Although the values of k_n and k_{gb} could be obtained accurately, the slopes of the secondary plots were often close to zero [or, in the case of plots of S *vs.* $(k_{gb} + k_{ga}a_H/K_a)$, had slight curvature at low pH] so that the values of k_{ga} and $k_{OH'}$ have a lower confidence limit. This is particularly true for $k_{OH'}$ which was often almost an insignificant contributor to disappearance of ester (this being particularly true for the amines with lower pK_a). For hydrazine, this point is illustrated in Table IV where the per cent contributions of the various species to k_{obsd} (calculated using data from Table III)

Table III. Rate Constants for the Aminolysis of Substituted-Acyl Phenyl Esters[a]

Substituent	Amine	k_n, l. mol^{-1} min^{-1}	k_{gb}, l.^{2} mol^{-2} min^{-1}	k_{ga}, l.^{2} mol^{-2} min^{-1}
CH_3	NH_3	0.15	0.90	0.055
C_2H_5		0.14	0.50	0.028
$C_6H_5CH_2$		0.18	0.43	0.036
$CNCH_2$		7.6	56	48
$ClCH_2$		20	200	
$BrCH_2$		7.6	300	
$C_2H_5SCH_2$		0.52	0.56	
CH_3		0.025		
C_2H_5		0.0068		
$C_6H_5CH_2$		0.012		
$C_6H_5OCH_2$		0.95		
$ClCH_2$		3.5		
$C_2H_5SCH_2$		0.074		
CH_3	Glycine	0.50	1.10	
C_2H_5		0.19	0.35	0.28
$C_6H_5CH_2$		0.10	0.63	0.35
$C_6H_5OCH_2$		1.8	0.55	25
$CNCH_2$		0.85	140	56
$ClCH_2$		8.9	450	
$C_2H_5SCH_2$		1.5		
CH_3	Glycylglycine	0.009	0.045	
C_2H_5		0.011	0.014	
$C_6H_5CH_2$		0.071	0.25	
$C_6H_5OCH_2$		0.13		
$CNCH_2$		0.70	1.5	
$ClCH_2$		1.20	1.9	
$C_2H_5SCH_2$		0.050	0.19	
CH_3	Methoxyamine	0.0018	0.005	0.064
$C_6H_5CH_2$		0.0040		0.024
$CNCH_2$		0.095	1.30	0.068
$ClCH_2$		0.085	1.05	0.090
$C_2H_5SCH_2$		0.0034	0.014	0.060

[a] μ = 1.0, 30°.

Table IV. Ratio of Acylhydrazide formed by the Various Reaction Paths of Hydrazine with Phenyl Acetate[a]

	pH 7.11	pH 8.11	pH 9.11
k_n, %	41	33	18
k_{gb}, %	11	47	47
k_{ga}, %	47	16.5	16.5
$k_{OH'}$, %	0.4	3.5	18.5

[a] N_T = 0.1 M; μ = 1.0, 30°.

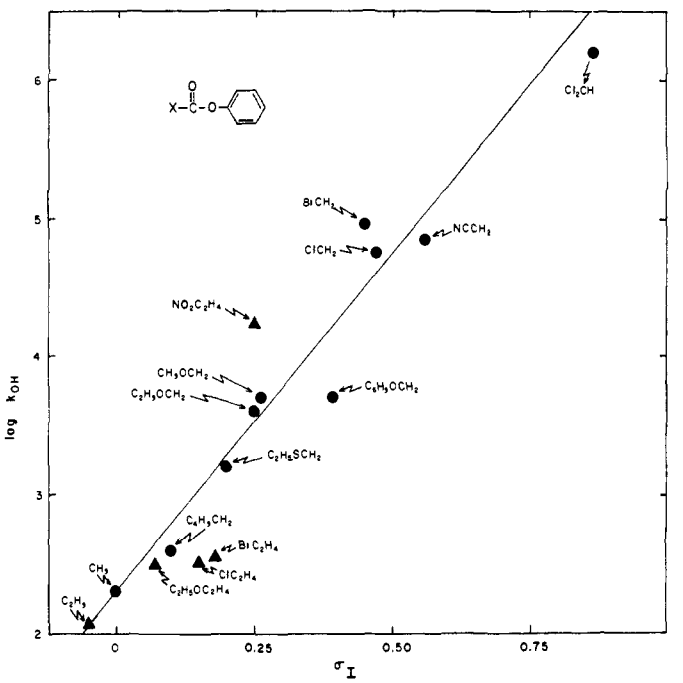


Figure 2. Plot of the logarithm of the second-order rate constant (l. mol^{-1} min^{-1}) for hydroxide ion catalyzed hydrolysis of phenyl propionates (▲) and acetates (●) *vs.* σ_I.

are listed; consequently, although it was obvious that in some cases there was a contribution of k_{obsd} attributable to $k_{OH'}$, these values have not been listed because of their uncertainty.

Discussion

Hydroxide Ion Catalyzed Hydrolyses. The rates of hydroxide-catalyzed hydrolysis of a series of eleven phenyl esters with various α substituents in the acyl position are listed in Table I. The rate data are correlated ($\rho = +4.58$) with some precision (see Figure 2) with the reduced form of the Taft equation (ignoring steric correction parameters) using the σ_I substituent constants listed by Charton.[18] Any attempt to include E_s corrective values resulted in a less precise correlation. But clearly this relationship (which is essentially between log k_{OH} and the pK_a's of the corresponding aliphatic acids) shows important deviations—in particular by the β-substituted phenylpropionates, three of which lie below and one (phenyl β-nitropropionate) above the line. To largely eliminate the common factors causing such deviations, the log k_{OH} values are taken subsequently as standards (rather than σ_I's) in the treatment of the aminolysis rate data. The slopes of such plots then indicate whether a given mechanistic pathway is more (slope greater than unity) or less (slope less than unity) sensitive to acyl substituent variation than is the standard reaction with HO^- as nucleophile. Multiplication of the slopes of these plots by ρ_I for k_{OH} (+4.58) then gives the ρ_I values for the other pathways.

Data for two compounds, phenyl α-cyanoacetate and phenyl β-nitropropionate, which deviated from the normal pattern of linear plots with unit slope of log k_{OH} *vs.* pH over the pH range 8–12, are given in Figure 1. Two kinetically indistinguishable schemes account for the observed log k_{obsd} *vs.* pH–rate profile for phenyl α-cyanoacetate.

(18) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).

Scheme I

$$E \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} E^- \xrightarrow{k_r} \text{product}$$

$$v = k_r \frac{K_a}{K_a + a_H}[E + E^-]$$

a

$$E^- \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} E \xrightarrow{k_r[HO^-]} \text{product}$$

$$v = k_r \frac{K_w}{K_a} \frac{K_a}{K_a + a_H}[E + E^-]$$

b

Scheme Ia, which describes a rate-determining decomposition of the ester carbanion to form a ketene intermediate (NC—CH=C=O), which is rapidly hydrated to give products), has recently been shown[19] to occur in selected cases. Scheme Ib involves the nonproductive formation of the anion; only the un-ionized ester leads to products in a rate-determining step involving hydroxide ion. One of the possible unambiguous methods to distinguish between these two possibilities is that pathway Ia would constitute an abnormal hydrolytic mechanism and consequently, if the ester were reacting entirely by this pathway, data for it should deviate from linear free-energy relationships embracing the other α-substituted esters. Clearly from Figure 2 this is not the case. In fact, the point for phenyl α-cyanoacetate (calculated from the equation in Scheme Ib, with apparent $pK_a = 9.50$) actually lies a little *below* the line. We conclude, therefore, that unlike *o*-nitrophenyl α-cyanoacetate (which on a similar plot shows a 40-fold rate enhancement), the phenyl ester hydrolyzes normally (Scheme Ib), presumably because the carbanion mechanism (Scheme Ia) is more sensitive to the nature of the leaving group ($C_6H_5O^-$ *vs.* o-$NO_2C_6H_4O^-$).

(19) B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 2993 (1969).

Phenyl β-nitropropionate (**1**) behaves differently, although carbanion formation is also being observed

$$\underset{\text{1}}{NO_2CH_2CH_2COC_6H_4} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} \underset{\text{2}}{NO_2\overset{-}{C}HCH_2COC_6H_5}$$

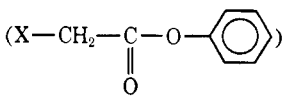
(Figure 1). But since the carbanion is in this case β to the carbonyl group (**2**), the ketene formation mechanism (in Scheme Ia) is not possible. Moreover, the negative charge is one further carbon removed from the reaction center (relative to phenyl α-cyanoacetate) and is, therefore, not able to bring about as great a diminution in reactivity toward hydroxide ion. The pH profile for **1** is, therefore, again linear with unit slope beyond pH 12 (hydroxide ion catalyzed hydrolysis of the anion **2**). The k_{OH} constant calculated for the anion **2** is 5.64×10^2 l. mol^{-1} min^{-1}, *i.e.*, the anion is *ca.* 30 times less susceptible to hydroxide ion attack than is the neutral ester. Assuming that the insertion of a methylene group has a damping effect on the transmission of electronic effects of 2.8,[20] it can be calculated that a carbanion α (rather than β) to the carbonyl group would diminish its reactivity (in normal hydrolysis, relative to the unionized ester) *ca.* 20,000-fold. This factor would probably be even higher if the negative charge were concentrated mainly on the β-carbon. It is probable in the present case that it is largely concentrated on the oxygen atoms of the nitro substituent. This is consistent with the observation (Figure 1), that the measured rate constant for phenyl α-cyanoacetate does not vary over a change of three powers of 10 in the hydroxide ion concentration. The pK_a calculated for the ester **1** which best fits the kinetic data is 9.87, comparable to the values of 10.2 for nitromethane and 8.6 for nitroethane at 25°.[21]

Aminolysis. The literature covering the aminolysis of phenyl acetates has been extensively reviewed recently.[7,8] Our objective herein is to employ the present data to evaluate the variously proposed transition state structures for the several pathways to amide. The approach employed involves, in part, the comparison of the sensitivity of the various aminolysis rate constants to electronic effects using the sensitivity of the rate constant for hydroxide ion catalyzed hydrolysis as standard. The procedure obviates the necessity of comparing ρ_I and Hammett ρ values.

Sensitivity of k_n to Electronic Effects. It is clear from the data listed in the second column of Table V (derived from the results listed in Tables II and III) that the uncatalyzed aminolysis reactions are *less* sensitive to the nature of the acyl substituent than is the corresponding hydroxide ion catalyzed hydrolysis reaction. The average sensitivity constant is, in fact, 0.78 ± 0.07 times that of k_{OH} for the six amines studied. Only with the secondary amine, morpholine, are the ρ_I values similar. These extensive data are a confirmation of earlier observations. Cordes and coworkers,[22] comparing the reactivities of a number of neutral amines and negatively charged nucleophiles (*e.g.*, HO$^-$, F$^-$) with phenyl acetate and with phenyl chloroacetate, found that rate differences between these two esters were *less* for the neutral nucleophiles. More detailed recent studies have confirmed this general finding. For nucleophilic displacement on substituted *o*-nitrophenyl acetates, a number of oxygen bases were found to exhibit an average ρ_I value of 5.2 ± 0.4 while for a number of amines the average ρ_I value was 3.2 ± 0.4.[9] Like results have been obtained for the aminolysis of substituted phenyl benzoates.[23]

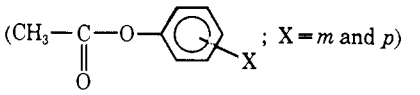
Though ρ values for sensitivity to acyl substitution are less for amines than for HO$^-$ the opposite is true for substitution of the leaving phenoxy group (Table VI).

Table V. Slopes of Plots of Log k_n, Log k_{gb}, and Log k_{ga} *vs.* Log k_{OH} for α-Substituted Acyl Phenyl Esters



Amine	k_n	k_{gb}	k_{ga}
$(NH_2)_2$	0.83	1.00	1.03
NH_3	0.73	1.13	1.22
Morpholine	1.05		
Glycine	0.66	1.06	0.96
Glycylglycine	0.83	0.65	
NH_2OCH_3	0.70	1.00	

Table VI. Slopes of Plots of Log k_n, Log k_{gb}, and Log k_{ga} *vs.* Log k_{OH} for Aryl Acetates

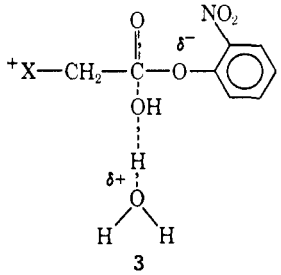


Nucleophile	k_n	k_{gb}	k_{ga}	Ref
HO$^-$	[1.00]			
$(CH_3)_3N$	2.4			Footnote *c* (Table I)
NH_3	1.9	0.50		3
Piperidine	1.9			7
Morpholine	2.4			7
$(NH_2)_2$	2.7	0.50	0.60	4
Imidazole	1.6	0.45		4
CH_3ONH_2	1.5	0.90	0.40	7

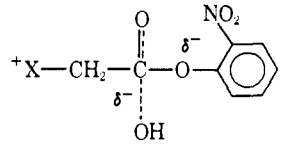
It has been suggested that this distinct difference is due to the presence of ion–dipole interaction between the incoming amine (which is developing a positive charge), and the polarizable α substituents on the acyl moiety.[22] This is not likely the case since *para*-substituted benzoate esters of phenol exhibit the same sensitivity pattern as do α-substituted acetate esters of phenol.[23] Jencks and Gilchrist[24] have recently provided evidence that covalent bond formation between amine and carbonyl carbon is concerted with a certain amount of C–OC$_6$H$_5$ bond loosening. It would appear that the most logical transition state structure associated with k_n that could explain the sensitivity to electronic effects would be one in which these sensitivities are related to the extent of bond making and breaking. Since all values in Tables V and VI are relative to HO$^-$ as standard, a knowledge of the transition state for attack by HO$^-$ allows an approximation of the transition state structure for the

(20) J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 99.
(21) R. G. Pearson and R. L. Dillon, *J. Amer. Chem. Soc.*, **75**, 2439 (1953).
(22) K. Koehler, R. Skora, and E. H. Cordes, *ibid.*, **88**, 3577 (1966).
(23) J. F. Kirsch and A. Kline, *ibid.*, **91**, 1841 (1969).
(24) W. P. Jencks and M. Gilchrist, *ibid.*, **90**, 2622 (1968).

aminolysis mechanism associated with k_n. Previous studies have indicated that the transition states for attack of the nucleophiles H_2O and HO^- are not subject to electrostatic effects by formally charged substituents in the α position of α-substituted *o*-nitrophenyl acetates.[9,25] In contrast, the transition states for oxyanions of medium pK_a are *subject to electrostatic* effects. These results have been rationalized through the assumption that the transition state for attack of H_2O (which is general base catalyzed by H_2O) resembles **3**, while that for attack of HO^- resembles **4**.[26] In **3** the

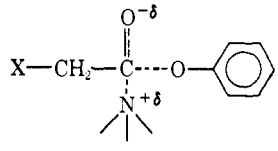


3



4

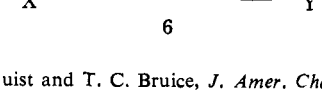
partial positive charge is far removed from the attacking oxygen which has a short partial bond to carbon. In **4** the partial oxygen carbon bond has only scarcely begun to form in the transition state and is quite long. Structures **3** and **4** not only explain the lack of electrostatic effects but are reasonable on consideration of the very low basicity of H_2O and great basicity of HO^- in terms of the Hammond postulate.[27] In the absence of contrary evidence, as nonlinear free-energy plots, it is fair to assume that structures **3** and **4** pertain regardless of the nature of the substituent groups of this study. For the case of the aminolysis pathway associated with k_n the transition state must resemble that of **3** much closer than **4** (*i.e.*, **5**). The order $\rho^{amine} > \rho^{HO^-}$ (change



5

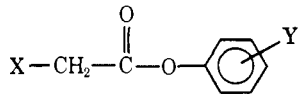
in leaving group) is explained by the greater amount of bond breaking, while $\rho_I^{HO^-} > \rho_I^{amine}$ though not nearly so marked, finds explanation in the compensating effects of more bond making and dipole charge repulsion between the δ^+ charge on the incoming nitrogen and the carbonyl carbon. The explanation for the sensitivity to substitution on the acyl moiety is similar if not identical with that provided by Jencks and Gilchrist.[24]

Sensitivity of k_{gb} and k_{ga} to Electronic Effects. For phenyl benzoates (**6**), $\rho_X = 2.0$ and $\rho_Y = 1.24$ for hydroxide-catalyzed hydrolysis.[28] Since ρ_X and ρ_Y for this system are comparable (both are Hammett ρ's), this allows us to relate the hydroxide-catalyzed rates used as standards for the acetates listed in Tables V and VI to a common scale, *i.e.*, the slopes in Table V are *ca.* twice those listed in Table VI on an absolute scale. The averaged values of Table V are provided in Table VII.



6

Table VII. Relative Sensitivities to the Electronic Nature of Substituents and the Basicity of Amine



	$(\rho/\rho_{HO^-})_X$	$(\rho/\rho_{HO^-})_Y$
k_{OH}	1.0	0.5
k_n (β = 1.05)	0.8 ± 0.07	1.0 ± 0.2
k_{gb} (β = 1.08)	1.0 ± 0.08	0.3 ± 0.1
k_{ga} (β = 0.74)	1.0 ± 0.08	0.3 ± 0.1

Included also in Table VII are the averaged values of Table VI divided by two. The values of Table VII are, therefore, relative sensitivities of rate expressions to those for HO^- nucleophilic attack when ρ_X for HO^- is taken as 1.0 and ρ_Y as 0.5. Although there are some minor deviations (*i.e.*, for CH_3ONH_2 with substitution on the leaving group and k_n for morpholine with substitution in the α position) all values have been averaged and are felt to be meaningful. Included in Table VII are the Brønsted β values from a previous study[8] which provides an index to the sensitivities of the various rate terms to amine base strength where phenyl acetate is substrate.

Inspection of Table VII reveals that the sensitivity to electronic effects in both the acyl and phenoxy portions of phenyl acetates is essentially identical for k_{gb} and k_{ga}. Furthermore, the terms k_{gb} and k_{ga} differ markedly in sensitivity to electronic effects from k_n but resemble rather closely the sensitivity of the rate constant for hydroxide ion catalyzed hydrolysis. The accumulated data of Tables V and VI, which are summarized in Table VII, provide the first opportunity to compare the sensitivity of k_n, k_{gb}, and k_{ga} to electronic effects for both acyl and leaving group in aqueous solution. Previous to this study the sensitivity of k_{ga} to electronic effects in the acyl portion of phenyl esters was not available. Shawali and Biechler[10] determined the sensitivity to electronic effects of k_n and k_{gb} for the *n*-butylaminolysis of α-substituted phenyl acetates. In this study anhydrous dioxane was employed as solvent and as in the present study the ρ^* value for k_{gb} (3.03) exceeded that for k_n (2.14). However, for the studies in dioxane a steric correction of considerable magnitude was required. Kirsch and Kline[23] obtained an even greater ρ value ratio (1.72) for k_{gb} *vs.* k_n while studying the ammonolysis of *p*-chlorophenyl benzoates in 33% aqueous acetonitrile.

Two possible structures involving simultaneous amine attack and phenol departure which offer a reasonable rational for the difference in the sensitivity of k_{gb} and k_n to electronic effects may be considered for the transition state associated with k_{gb} (*i.e.*, **7** and **8**).[29]

(25) B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 2982 (1969).

(26) In structures **3** and **4**, it is assumed that the formation of a covalent bond between nucleophile and carbonyl carbon is not concerted with departure of the leaving group (*i.e.*, formation of a tetrahedral addition intermediate). Alternatively, structures involving considerable and very little concerted bond breaking in **3** and **4**, respectively, would also suffice to explain the experimental observations (no tetrahedral intermediate). The features of importance in **3** and **4** are the extent of bond making.

(27) G. S. Hammond, *J. Amer. Chem. Soc.*, 77, 334 (1955).

(28) J. F. Kirsh, W. Clevell, and A. Simon, *J. Org. Chem.*, 33, 127 (1968).

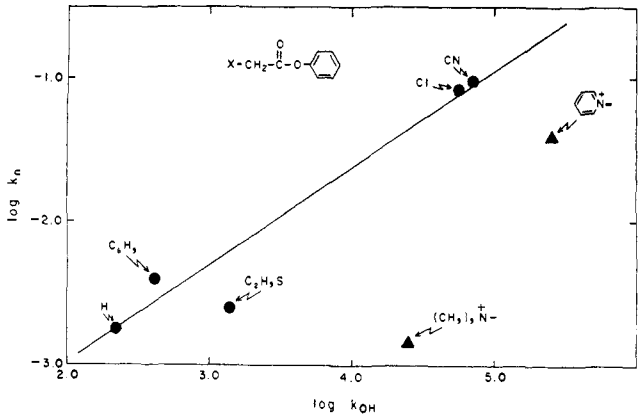
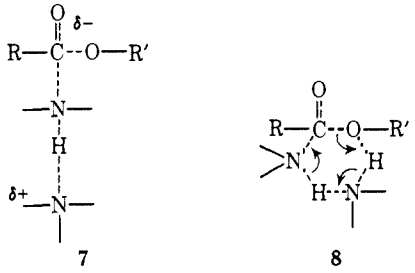


Figure 3. Plot of the logarithm of the second-order rate constant for methoxylaminolysis (k_n, l. mol^{-1} min^{-1}) *vs.* the logarithm of the second-order rate constant for alkaline hydrolysis (k_{OH}, l. mol^{-1} min^{-1}) for selected α-substituted phenyl acetates.

Both **7** and **8** involve the partial removal of a proton from the attacking amine by the second amine molecule. Since the pK_a's of amines are in the region of 30–35,[30] partial breaking of the N–H bond should create



7 **8**

a nucleophile as basic or more than HO$^-$ so that in **7** and **8** N–C bond formation is less than in **5**. Structure **8** differs from **7** in that the catalytic species acts as not only a general base but a general acid to transfer a proton in a cyclic process. Apart from the absence of formal negative charge and the presence of catalyst, structure **7** shares the characteristics of the transition state for hydroxide ion attack (as **4**)[29]—long C–N and short C–O bonds—and is consistent with the relative sensitivities listed in Table VII. A further observation supports this. While the hydroxide-catalyzed rate is not subject to electrostatic effects by positively charged α substituents (because of the long carbonyl–nucleophile bond in the transition state, *cf.* structure **4**), the aminolysis rate constants k_n generally are.[9] Figure 3 illustrates that the rates of nucleophilic attack (log k_n) by methoxyamine on the positively charged phenyl esters of 1-(carboxymethyl)trimethylammonium chloride and 1-(carboxymethyl)pyridinium bromide[31] lie well below the line for the other neutral esters when plotted against log k_{OH}. In contrast, k_{gb} values for these compounds do not deviate seriously in a similar plot (Figure 4), showing that the interaction with the

(29) On the basis of the experimental data of Jencks and Gilchrist (see ref 24), that amine attack is accompanied by loosening of C–OC$_6$H$_5$ bond for the unassisted or water-assisted aminolysis pathway (k_n), it is most reasonable to consider initially transition states for the k_{gb} and k_{ga} mechanisms which possess this feature.

(30) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 87.

(31) The rate constants for these compounds were calculated from data given in Table IX of ref 9 for the corresponding *o*-nitrophenyl esters by employing the ρ values of this study.

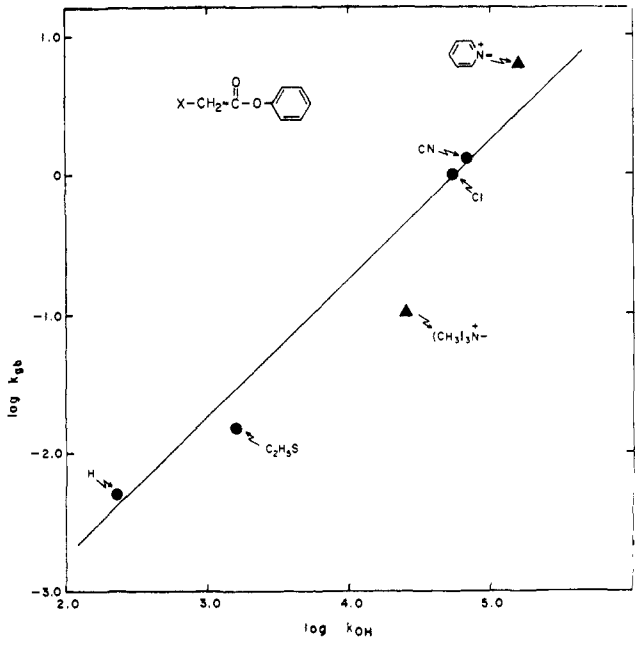
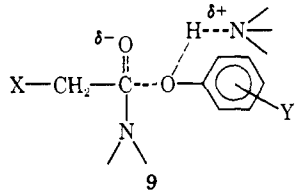


Figure 4. Plot of the logarithm of the third-order rate constant (k_{gb}, l.^{2} mol^{-2} min^{-1}) for the methoxylaminolysis *vs.* the logarithm of the second-order rate constant for alkaline hydrolysis (k_{OH}, l. mol^{-1} min^{-1}) of selected α-substituted phenyl acetates.

nucleophile is about the same as in the hydroxide rate. This is consistent with structure **7** since the C–N bond is long in the transition state minimizing interactions (as in the attack of hydroxide ion, **4**) and much of the charge has been funneled away to the amine molecule.

The cyclic mechanism (**8**) was originally proposed by Bruice and Mayahi[3] to account for the low sensitivity of the k_{gb} term to leaving group tendency and has since been favored by many investigators using both organic–aqueous mixed solvent[23] and nonaqueous[10,32,33] solvent. Such a mechanism is not subject to the criticism of Jencks and coworkers[2,34] of noncyclic pathways (**9**) involving protonation of the leaving group.



9

Employing acetonitrile as solvent, Satchell and Secemski[32] were unable to detect any general base catalysis of the *n*-butyl aminolysis of two *p*-nitrophenyl esters by triethylamine. On the basis that only primary and secondary amines could act as catalysts for the cyclic mechanism of **8**, this transition state structure was favored for k_{gb}. Though general base catalysis has never been noted in aqueous solution with tertiary amines it should be noted that the pathway to amide through k_{gb} exhibits much more steric hindrance than that through k_n. Thus, the catalytic terms with secondary amines (*e.g.*, morpholine, piperidine) are of considerably less importance than with primary amines[8] and consequently rarely observed (see Tables V and VI).

(32) D. P. N. Satchell and I. I. Secemski, *J. Chem. Soc., C*, 130 (1969).

(33) F. M. Menger, *J. Amer. Chem. Soc.*, **88**, 3081 (1966).

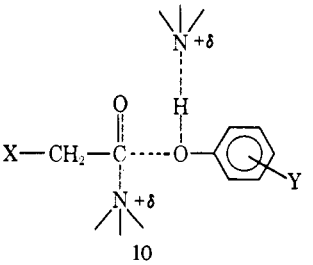
(34) G. M. Blackburn and W. P. Jencks, *ibid.*, **90**, 2638 (1968).

A similar conclusion follows from the diminution in the ratio of k_{gb}/k_n for the amines RNH_2 with increasing size of the R group.[5,32] It therefore seems possible that the failure to observe triethylamine-catalyzed *n*-butyl aminolysis[32] is a manifestation of steric hindrance. Triethylamine catalysis of the attack of *n*-butylamine upon phenyl dichloroacetate has been recorded.[10]

The enumerated considerations make it difficult to decide whether **7** or **8** should be favored as transition state structures for k_{gb}. Whether either is correct depends upon the correctness of the assumption that N–C bond formation and C–OPh bond breaking are synchronous.[26] Any choice of transition state structures must provide a rational for the marked similarity of the sensitivity of the k_{gb} and k_{ga} terms to electronic effects and the fact that these terms resemble k_{OH} in sensitivity more closely than k_n. For k_{ga} a cyclic transition state involving proton transfer cannot be drawn.

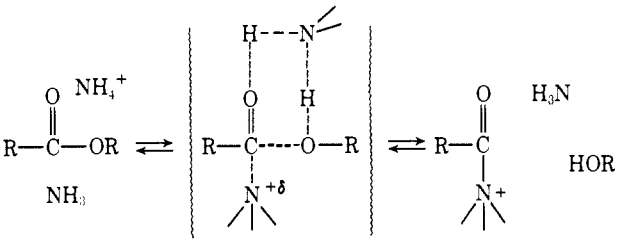
Sensitivity of k_{gb} and k_{ga} to Amine Base Strength. The Brønsted β values[8] associated with k_n, k_{gb}, and k_{ga} are included in Table VII. The β value for k_{gb} takes into account the sensitivity of the term to both the basicity of the attacking amine and amine catalyst (both are identical). For maximum proton transfer, β = 1.0 and for complete formation of the N–C bond β has been estimated to be 1.7,[24] therefore, the maximum value of β for k_{gb} may be taken as 2.7. The summed fraction of N–C bond formation and proton dissociation is then 40% assuming **7** to be correct. For k_n the maximum value of β should be 1.7[24] so that N–C bond formation may be taken to be 60% complete which is in accord with **5**. Less bond formation in k_{gb} than in k_n is in accord with the Hammond postulate[26] (and structures **5** and **7**) since the incipient amine anion species formed by general base catalysis is a much stronger base than neutral amine.

The value of β for k_{ga} includes a term for N–C bond formation (1.7 max) and α for proton donation from the amine conjugate acid (−1.0 max). The experimental value of β (0.74) suggests that there is almost complete N–C bond formation and proton transfer in the transition state. The transition state structure of **10** for the pathway to amide associated with k_{ga} possesses these aspects. The retrograde mechanism for



10

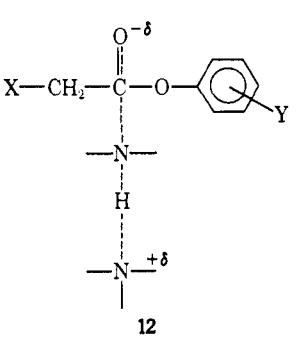
k_{ga}, based on **10**, would involve amine general base catalyzed attack of phenol upon protonated amide. The reasonableness of **10** may be judged by examining the retrograde process. When simple nucleophilic attack of amine upon phenyl ester is competitive with the general acid catalyzed mechanism (Table IV) then the retrograde mechanisms must also be competitive. This is so since the ground-state free energies are determined only by the composition of the reaction solution prior to and after completion of aminolysis. If one compares **10** and **5**, the conclusion is reached that under the experimental conditions in which the pathways to amide for k_n and k_{ga} are competitive, **10** would appear unreasonable since for the retrograde process general base catalyzed attack of phenol to eliminate amine could not be competitive with attack of phenolate ion to eliminate amine. To provide a mechanism for the retrograde of the pathway associated with k_{ga} that might be competitive with the retrograde of k_n under the conditions in which the forward reactions are competitive, **11** might be considered. In the phenolysis of



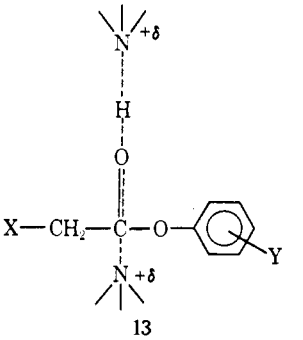
11

protonated amide *via* **11**, hydrogen bonding of catalyst increases the positive nature of the carbonyl carbon and thus its susceptibility to nucleophilic attack. But on the basis that the solvent water should hydrogen bond to the carbonyl carbon as well as the incipient amine catalytic species, **11** appears dubious for reaction in water.

The transition state structures of **5**, **7**, **8**, **10**, and **11** all involve a concerted attack of amine accompanied with departure of the leaving group.[26] The concerted mechanisms have been based on the postulation that the mechanism of k_n is concerted.[24] If tetrahedral intermediates are involved in the pathways associated with k_{gb} and k_{ga} then the rate-determining step would be formation of the tetrahedral intermediate. It is most difficult to conceive of a suitable transition state structure for the k_{ga} process which has the features of concerted attack of nucleophile and departure of leaving group. Thus, it is reasonable to assume that for the k_{ga} process the rate-determining step is the formation of a tetrahedral intermediate. There being no evidence to the contrary the mechanism associated with the k_{gb} term may also be nonsynchronous. The structures of **12** and **13** have been previously postulated for the



12



13

transition states for k_{gb} and k_{ga} by Bruice and coworkers as those which were most in accord with the experimental evidence of Nov 1966 (see ref 8 for discussion). With the addition of the data presented herein these structures still appear to be the least encumbered.

Conclusion

For direct nucleophilic attack of amines upon phenyl esters the transition state most reasonably resembles

5. For amine general base catalyzed attack of amine the transition state structures of **7**, **8**, and **12** are in accord with out present knowledge. Structures **7** and **12** differ only as to the delocalization of partial negative charge to carbonyl or phenol oxygens. Structure **8** involves a cyclic proton transfer. For the amine conjugate acid general acid catalyzed attack of amine, reasonably concerted or cyclic structures involving proton transfer are not apparent and the lone transition state structure of **13** is suggested.

These considerations apply to water as a solvent. In a poor ion supporting solvent it is to be anticipated that the necessity of internal stabilization of charges will result in cyclic transition state structures. The process associated with k_{ga} has not been seen in such solvents.[10,32,33,35]

Acknowledgments. This work was supported by a grant from the National Institutes of Health.

(35) H. Anderson, Chi-Wu Su, and J. W. Watson, *J. Amer. Chem. Soc.*, **91**, 482 (1969).

Studies of the Chymotrypsinogen Family of Proteins. IX. Steady-State Kinetics of the Chymotryptic Hydrolysis of N-Acetyl-L-tryptophan Ethyl Ester at pH 8.0[1a]

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Abstract: Thermodynamic changes for significant steps in the reaction sequence of the chymotrypsin-catalyzed hydrolysis of N-acetyl-L-tryptophan ethyl ester have been determined at pH 8.0 as a function of temperature and ethanol concentration. The reaction follows simple catenary chain kinetics up to 1.5 *M* ethanol. The reaction profile is symmetric about the acyl enzyme at all temperatures. There is no unique rate-limiting step. For ethanol and water standard states of 1 *M* and unit activity, respectively, the phenomenological rate parameters α_0 and β_0 (alcohol independent) and α_p and β_p (alcohol dependent) have the following values at 35° and the following activation enthalpies and entropies in order (α_0, 4.7×10^5 M^{-1} sec^{-1}) (β_0, 78 sec^{-1}) (α_p, 5.0×10^5 M^{-1} sec^{-1}) (β_p, 250 sec^{-1}). There are three "metastable" intermediates which can be distinguished: two Michaelis–Menten complexes ES and EP_2H and an acylenzyme EA with the following standard enthalpies and entropies of formation (ES, −9.9 kcal, −17 eu) (EP_2H, −8.6 kcal, −14 eu) (EA, −12.3 kcal, −26 eu). A cooperative protein transition centered at pH 8 and 25° is detectable in α_0 and α_p and the two substates have different catalytic parameters. EA as well as ES and EP_2H appear to belong to the class of enzyme–inhibitor complexes which manifest a common pattern of linear compensation of enthalpy change by entropy change. Judging by the number of other cases in which the same pattern is found, these results imply that water plays a direct role in the catalytic process.

As a foundation for a study of the transient-phase kinetics of chymotryptic catalysis, a grid of the responses of the steady-state parameters for the variation of a minimum set of independent variables is necessary. No comprehensive study of the steady-state kinetics has been reported thus far. In particular there has been little study of the temperature dependence of the kinetic parameters for chymotryptic catalysis. In addition, the finding by Kim and Lumry[2a,b] that α-chymotrypsin near pH 8.0 and 25° exists in roughly equal proportions of two substates, A_b and A_f, necessitates a reexamination of the catalysis kinetics in this pH and temperature region in order to determine if the transition between these two substates manifests itself in any of the rate parameters. A reexamination of the hydrolysis kinetics of N-acetyl-L-tryptophan ethyl ester has also become necessary to eliminate uncertainties associated with a group of contaminants frequently found in commercial preparations of α-chymotrypsin[3] not treated by the simple purification procedures of Yapel, *et al.*[3] It is important to emphasize the finding of these authors[3] that strong acylating agents[4] used as the "active site" reagents displace the contaminants and thus give spurious assurances of purity. More reliable tests for such purity are provided by Yapel, *et al.*,[3] but the simplest test still appears to be the determination of reaction velocity constants under a fixed set of "standard conditions." We report here a study of the rates of chymotryptic hydrolysis of N-acetyl-L-tryptophan ethyl ester as a function of temperature and ethanol concentration at a fixed pH of 8.0. At this pH, the rate parameters are nearly pH independent thus facilitating their analysis in terms of elementary rate constants or simple combinations of these. The tentative analysis of the rate parameters is based on the work of Bender[5–7] and Wilson[8,9] and

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