1490, 1445, 995, 785, 748, 701 cm^{-1}; nmr δ 6.95–7.80 (m, 14 H), 1.0–2.8 (m, 16 H); mass spectrum, molecular ion, *m/e* 418.[31]

Anal. Calcd for $C_{31}H_{30}O$: C, 88.95; H, 7.22. Found: C, 89.19; H, 7.18.

Pyrolysis of 1 with Furan. The amine oxide (0.30 g, 1.3 mmol) and furan (0.70 g, 10 mmol) were sealed in a glass tube and immersed in an oil bath preheated to 140°. The reaction mixture was kept at this temperature for 1 hr, and then was allowed to cool. The tube was broken and the resultant brown solution was analyzed by glpc on columns II and IV. Results indicate formation of homoadamantane (**5**; 23%), *N,N*-dimethyl-*O*-(3-homoadamantyl)hydroxylamine (**4**; 9%), 3-hydroxyhomoadamantane (**6**; 3%), 2-(3-homoadamantyl)furan (**12**; 30%), *N,N*-dimethyl-3-aminohomoadamantane (**7**; 18%), and a minor amount of unidentified material (~8%). No dimers (**2**) or 4-homoadamantene (**3**) were detected.

Spectral data for compound **12** include: ir[32] (neat) 2900, 1580, 1505, 1450, 1150, 1020, 904, 887, 790, 726 cm^{-1}; nmr[32] δ 7.2 (broad s, 1 H), 6.1 (m, 1 H), 5.8 (m, 1 H), 1.5–2.2 (m, 17 H); mass spectrum, molecular ion, *m/e* 218.

Anal. Calcd for $C_{15}H_{20}O$: C, 83.28; H, 9.32. Found: C, 82.98; H, 9.04.

HI Cleavage of 4. A mixture of the basic material from Cope elimination [0.50 g containing **4** (0.12 g, 0.57 mol)] and constant boiling HI (10 ml) was refluxed for 8 hr. The solution was poured over ice and extracted with ether. After the ether solution was washed in succession with 0.1 *N* $Na_2S_2O_3$, 5% $NaHCO_3$, and water, the dried solution was evaporated, leaving 80 mg of neutral material. Analysis on column II indicated a mixture of iodomethyladamantane (75%), identified by comparison of ir and nmr spectra and glpc retention time to authentic material, and a product (25%) which has an ir spectrum consistent with the 3-iodohomoadamantane structure.

1-Iodomethyladamantane (14). 3-Hydroxyhomoadamantane (**6**), or the isomeric 1-hydroxymethyladamantane (1.0 g, 6.0 mmol) was heated at reflux in constant boiling HI (15 ml) for 1 hr. The reaction mixture was poured over ice and extracted with ether. The ether solution was washed in succession with 0.1 *N* $Na_2S_2O_3$, 5% $NaHCO_3$, and water. Evaporation of the dried solution gave 1.3 g (78%) of the iodide, mp 51–53°. Recrystallization from ethanol gave white plates, mp 52–53°; ir (CCl_4) 2800, 1435, 1335, 1190, 1095, 970, 915 cm^{-1}; nmr δ 3.0 (s, 2 H), 1.5–2.4 (m, 15 H); mass spectrum, molecular ion, *m/e* 276.

Anal. Calcd for $C_{11}H_{17}I$: C, 47.84; H, 6.20. Found: C, 47.53; H, 5.80.

(31) The aromatic region of the nmr spectrum was essentially identical with that of DPIBF adducts of bicyclo[3.2.2]non-1-ene and bicyclo[3.2.1]oct-1-ene; J. A. Chong Ph.D. Thesis, University of Michigan, 1971.

(32) The spectral data are similar to those reported for 2-substituted furans; J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 2, Pergamon Press, New York, N.Y., 1966, p 782; C. J. Pouchert, "The Aldrich Library of Infrared Spectra," Aldrich Chemical Co., Milwaukee, Wis., 1970, p 882.

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The Mechanism of the Aminolysis of Acetate Esters[1]

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Abstract: The reactions of hydrazine with ethyl acetate, methoxyethyl acetate, and probably 2-chloroethyl acetate exhibit a change in rate-determining step with decreasing pH similar to that with methyl formate; no such change is observed with trifluoroethyl or phenyl acetates. Structure-reactivity correlations suggest identical transition state structures and rate-determining steps for the general base-catalyzed reactions of alkyl and phenyl esters, but for the uncatalyzed reactions there is a leveling of the rate with changing leaving group for the alkyl esters that requires a different mechanism from that of the phenyl esters. This change in mechanism and a requirement for proton transfer in the reactions of primary and secondary amines are also evident in the slow reactions of tertiary amines with alkyl esters. Bifunctional cyclic mechanisms are excluded by the effectiveness of tertiary amines as general base catalysts for hydrazinolysis. A mechanism of aminolysis is proposed that is consistent with these and other data. With most esters, the attack of amine to form the labile intermediate $T^{\pm}$ is rapid and reversible, and the rate-determining step at high pH is the trapping of this intermediate by proton removal with a general base, a proton switch through water (alkyl esters), or the direct breakdown of $T^{\pm}$ to products (phenyl esters). For very fast reactions, there is a change to rate-determining formation of $T^{\pm}$ accompanied by a sharp decrease in sensitivity to substituent effects. The application of these considerations to the mechanism of chymotrypsin catalysis is described.

The aminolysis of alkyl esters in aqueous solution proceeds through a tetrahedral addition intermediate, as shown by the existence of a change in rate-determining step with changing pH.[2,3] The assignment of the rate-determining step in the different pH regions has been made by determining the preferred direction of breakdown of the tetrahedral intermediate formed in the hydrolysis of the corresponding imidate as a function of pH.[3,4] For the aminolysis of methyl formate, it was shown that the formation of an anionic addition compound, T^{-}, is rate determining at high pH, and

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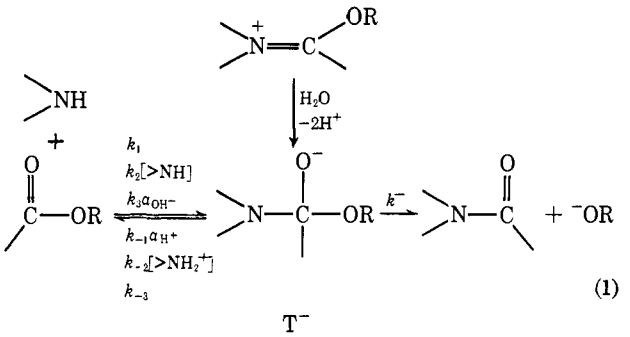
(2) B. Hansen, *Acta Chem. Scand.*, 17, 1307 (1963); B. A. Cunningham and G. L. Schmir, *J. Amer. Chem. Soc.*, **89**, 917 (1967).

(3) G. M. Blackburn and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 2638 (1968).

(4) (a) G. L. Schmir and B. A. Cunningham, *J. Amer. Chem. Soc.*, **87**, 5692 (1965); B. A. Cunningham and G. L. Schmir, *ibid.*, **88**, 551 (1966); R. B. Martin, S. Lowey, E. L. Elson, and J. T. Edsall, *ibid.*, **81**, 5089 (1959); (b) T. C. Pletcher, S. Koehler, and E. H. Cordes, *ibid.*, **90**, 7072 (1968); M. Kandel and E. H. Cordes, *J. Org. Chem.*, **32**, 3061 (1967).

that the breakdown of this intermediate to products becomes rate determining as the pH is lowered (eq 1);[3]



(1)

this conclusion has recently been confirmed by measurements of the pH dependence of the heavy-atom isotope effect in the hydrazinolysis of methyl formate-*methoxyl*-^{18}O.[5] Based on the assumption that proton-transfer steps are fast, the rate-determining step at high pH was assigned to attack of amine on the ester.[3] Similarly, the rate-determining step in the aminolysis of phenyl acetates was assigned to amine attack, based on the fact that the intermediates formed in the hydrolysis of phenyl imidates break down predominantly (but not exclusively) with phenol expulsion to give amides.[4b,6] Although the main outline of the mechanism of ester aminolysis appeared to be established by these results, several puzzling problems remained.

(1) Structure–reactivity correlations show that the (class II) transition state for the reactions of primary, secondary, and tertiary amines with phenyl acetates maintains an almost constant-structure, intermediate between reactants and products with a large amount of N–C bond formation and C–O bond cleavage, over a range of 10^8 in reaction rates; inclusion of the aminolysis of acetylpyridinium ions extends this range to 10^{13} (for N–C bond formation). This is followed by a rather sudden change to a (class I) transition state with little bond formation or cleavage for very fast reactions with strong nucleophiles and good leaving groups.[6,7] This structure–reactivity behavior is surprising for a reaction involving rate-determining attack, even if the intermediate has little or no lifetime. Furthermore, if amine attack is rate determining for class II reactions, no entirely satisfactory explanation is available for the mechanism of class I reactions.

(2) The addition intermediate formed during the hydrolysis of the imidate corresponding to phenyl acetate and methylamine breaks down with a significant amount of amine expulsion to give ester.[4b,6] It follows that with better amine leaving groups, such as (protonated) trifluoroethylamine (pK = 5.8) and nicotinamide (pK = 3.6), amine expulsion should become predominant, and the aminolysis reaction should undergo a change to rate-determining breakdown. There is no evidence for such a change nor for any change in transition-state structure for the reactions of phenyl acetate with these amines.[6] The assignment to rate-determining attack requires further that the addition intermediate expels phenolate ion (pK = 10) faster than nicotinamide (pK = 3.6; the pK_a's refer to the conjugate acid of the leaving group); this requirement becomes even more disturbing with the recent demonstration that amines are expelled some 10^5 times faster than oxyanion leaving groups of comparable pK from a tetrahedral addition compound formed from a phthalimidium ion.[8]

(3) Fersht has pointed out that the linear correlation of the rate constants for the reactions of oxyanions, including *p*-nitrophenoxide and acetate, with AMPP and *N*-acetyl-4-methylpyridinium ions suggests that attack of the anion is the rate-determining step in the reaction with the acylpyridinium ion, as it certainly is with AMPP. It follows from the principle of microscopic reversibility that in the reverse reactions of pyridines with PNPA and acetic anhydride, for example, *breakdown* of the addition compound T± must be the rate-determining step.[7]

In this paper, we describe a mechanism for ester aminolysis that resolves these problems and appears to be consistent with the available and some new experimental data. According to this mechanism, the rate-determining step for the uncatalyzed aminolysis of all but the most reactive phenyl esters is phenolate expulsion, and the rate-determining step for the formation of T$^-$ from alkyl acetates is a proton transfer through water, rather than the attack of amine on the ester. In the following paper, we show that different intermediates that are not in protonic equilibrium are generated in imidate hydrolysis and in the aminolysis of the corresponding phenyl ester, so that the products formed from imidate hydrolysis do not necessarily show which step is rate determining in ester aminolysis.[9] It is concluded that proton-transfer steps are critical for both rate and product determination in these reactions. Preliminary reports of portions of this work have appeared.[10–12]

Experimental Section

Materials. Organic reagents, including tetramethylammonium chloride, were recrystallized or redistilled before use. Phenyl acetate,[6] 2,2,2-trifluoroethyl acetate,[13] *p*-chlorophenyl acetate,[14] and *m*-nitrophenyl acetate[14] were prepared and purified by published procedures. Hydrazine dihydrochloride was recrystallized from ethanol–water. *Exercising great caution*, hydrazine monohydrochloride (mp 91–93°), a highly toxic compound, was prepared by slowly adding 0.95 equiv of cold concentrated hydrochloric acid dropwise to commercial liquid hydrazine in a large flask in an ice bath in an efficient hood, removing most of the water by rotary evaporation and crystallizing from the remaining water. The composition of the product was confirmed by titration. All experiments were carried out in glass-distilled water.

(5) C. B. Sawyer and J. F. Kirsch, *J. Amer. Chem. Soc.*, **95**, 7375 (1973).
(6) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968).
(7) A. R. Fersht and W. P. Jencks, *J. Amer. Chem. Soc.*, **92**, 5442 (1970).
(8) N. Gravitz and W. P. Jencks, *J. Amer. Chem. Soc.*, **96**, 499 (1974).
(9) A. C. Satterthwait and W. P. Jencks, *J. Amer. Chem. Soc.*, **96**, 7031 (1974), following paper.
(10) J. P. Fox, M. I. Page, A. Satterthwait, and W. P. Jencks, *J. Amer. Chem. Soc.*, **94**, 4729 (1972).
(11) W. P. Jencks, *J. Amer. Chem. Soc.*, **94**, 4731 (1972). In applying this rule, it should be kept in mind that the pK of a group in a metastable intermediate may be different from that of a corresponding group at equilibrium in solution, if solvation has not kept up with the rate of formation of the group. Although we do not have information to assess this point critically at this time, the effect is not expected to be large unless the solvation energy of the immediate product of the proton-transfer reaction is much smaller than that of the group in question. The problem of estimating pK values is, of course, more difficult in enzymic reactions, in which microsolvent and other local environmental effects may perturb ionization equilibria.
(12) W. P. Jencks, *Chem. Rev.*, 72, 705 (1972).
(13) T. C. Bruice, T. H. Fife, J. J. Bruno, and N. E. Brandon, *Biochemistry*, 1, 7 (1962).
(14) F. D. Chattaway, *J. Chem. Soc.*, 2495 (1931).

Table I. Experimental Conditions for the Determination of Rate Constants for the Hydrazinolysis of Alkyl[a] and Phenyl[b] Acetates at 25°

Ester	[Hydrazine], M	pH	No. of runs	Intercept,[c] M^{-1} min^{-1}	k_{cat},[c] M^{-2} min^{-1}
m-Nitrophenyl acetate	0.2–1.0	6.94	5	56.7	13.5–18.5
	0.4–0.8	8.12	12[d]	56.0	74.5
	0.1–0.6	8.24	6	57.5	79.0
	0.22–0.5	9.20	20[d]	56.5	131
p-Chlorophenyl acetate	0.1–0.5	7.80	5	2.0	14.5
	0.05–0.3	8.25	6	1.75	21.0
	0.07–0.27	8.75	4	2.0	29.0
	0.10–0.45	12.50	6	[e]	
Trifluoroethyl acetate	0.6–1.2	7.6	2	$\leq$3.0	1.6
	0.2–0.4	8.3	2	$\leq$1.0	4.0
	0.06–0.61	9.0	10	$\leq$1.0	6.3
	0.1–0.5	11.2[f]	6	9.5	7.8
Propargyl acetate	0.15–0.75	10.5[g]	9	$\leq$0.1	1.3
2-Chloroethyl acetate	0.16–0.50	8.60	2	$\leq$0.015	0.21
	0.05–0.25	9.07	5	4×10^{-3}–1.5×10^{-2}	0.29
	0.4	10.9–12.8[h]	5	[e]	
Methoxyethyl acetate	0.30–0.75	11.0[h]	7	$\leq$0.02	0.17
	0.4	11.7–12.6[i]	6	[e]	
Ethyl acetate	0.30–1.5	10.95[h,j]	11	0.010–0.017	0.047
	1.5	10.5–13.0[i]	12	[e]	

[a] Ionic strength maintained at 2.25 with 1.5 M KCl + 0.75 M (tetramethylammonium chloride + amine hydrochloride buffers), except where noted. [b] Ionic strength maintained at 1.0 with KCl. [c] k_{cat} is equal to the slope of a plot of $(k_{obsd} - k_0 a_{OH^-})/[NH_2NH_2]_{total}$ against the concentration of total hydrazine; the intercept is the sum of terms first order in hydrazine. [d] Some or all of these rate constants were determined with the stopped-flow apparatus. [e] For the determination of k_3. [f] In 0.12 M *tert*-butylamine buffer. [g] In 0.18 M *tert*-butylamine buffer. [h] In 0.30 M diisopropylamine buffer. [i] In 0.15 M diisopropylamine buffer. [j] Ionic strength 2.55 M with 1.5 M KCl + 0.75 M TMA-Cl + 0.30 M diisopropylamine buffer.

Kinetics. The hydrazinolysis of alkyl acetates was followed by measuring the rate of ester disappearance by conversion to the hydroxamic acid.[3] Liquid ester was added to 10–20 ml of a reaction mixture that had been brought to 25 ± 0.1°, to give a final concentration in the range 10^{-2}–10^{-3} M; the solution was thoroughly mixed. The tubes were capped with serum stoppers to prevent evaporation of the ester. Aliquots of 0.25–1.0 ml were withdrawn into a syringe and added to 1.0 ml of alkaline hydroxylamine (4 M hydroxylamine hydrochloride–3.5 M sodium hydroxide, 1:1.5; prepared within 2 hr of use). After 2 min, 4 ml of ferric chloride solution (10% $FeCl_3 \cdot 6H_2O$ in 2.5 M hydrochloric acid) was added, and after a further 20 min and a thorough shaking, the absorbance was read at 540 nm.

The hydrazinolysis of phenyl acetates was followed spectrophotometrically.[6] Pseudo-first-order reactions were carried out with 10^{-3}–10^{-4} M ester. Initial rate measurements at 275 nm with phenyl acetate at pH <7.0 were carried out with 5×10^{-3} M ester. The final absorbance was determined from a hydrolyzed reaction mixture and was corrected for any initial absorbance. Pseudo-first-order rate constants were then obtained by dividing the initial rate of change of absorbance by the corrected final absorbance. Interference from an impurity that caused an initial rapid change in absorbance at pH <5 was greatly reduced by passing argon through the solutions for 5 min before initiating the reaction. It was shown that no chloride ion is released during the reactions of 2-chloroethyl acetate by testing with silver nitrate.

End points for pseudo-first-order reactions were obtained after 10 or more halftimes or (for the hydroxamic acid method) from a reaction blank. Reactions were generally followed for 3–5 halftimes with 10–20 points.

Some experiments with *p*-chlorophenyl acetate (280 nm) and *m*-nitrophenyl acetate (335 nm) at high pH and hydrazine concentration were carried out by mixing equal volumes of ester and hydrazine solutions, both brought to ionic strength 1.0 with potassium chloride, in a stopped-flow apparatus. Some reactions of *p*-chlorophenyl acetate that were initiated by adding a small volume of ester in ethanol gave rate constants that were independent of hydrazine concentration, as a consequence of rate-determining mixing and dissolving of the ester in the aqueous reaction mixture.

The ionic strength of the reactions of alkyl esters was maintained constant by adding 1.5 M potassium chloride, hydrazine buffers, and sufficient tetramethylammonium chloride to bring the ionic strength to 2.25. This procedure served to minimize specific salt effects by maintaining a constant total concentration of ammonium ions. For the reactions of phenyl acetates, the reaction mixtures were brought to an ionic strength of 1.0 with potassium chloride, in order to permit comparison with earlier data. The pH of reaction mixtures was determined at 25° before and after each experiment.

The experimental conditions for the hydrazinolysis experiments are summarized in Table I. The observed pseudo-first-order rate constants for hydrolysis of the phenyl esters are known,[6,15] and those for alkaline hydrolysis of the alkyl esters were measured under the conditions of the hydrazinolysis experiments in the pH range 10–12 in diisopropylamine or *tert*-butylamine buffers, using the hydroxamic acid method for determining ester disappearance and five to ten runs for each ester. The buffers were shown not to affect the rate significantly.

After correction for hydrolysis, the observed pseudo-first-order rate constants were divided by the concentration of free hydrazine to give observed second-order rate constants, k'_{obsd}, which are described by eq 2. The values of k'_{obsd} were plotted against total

$$k'_{obsd} = \frac{k_{obsd} - k_0 a_{OH^-}}{[NH_2NH_2]} = k_1 + k_3 a_{OH^-} + k_5[\text{buffer}] + k_2[NH_2NH_2] + k_4[NH_2NH_3^+] \quad (2)$$

hydrazine concentration to give an intercept containing the terms first order in hydrazine (k_1, k_3, and k_5) and a slope which is the sum of the terms second order in hydrazine, k_2 and k_4 (Table I). For reactions in which the k_4 term is significant, the slopes were plotted against the fraction of hydrazine as the free base, and the values of k_4 and k_2 were obtained from the intercepts at 0 and 100% free base, respectively. The rate constant k_3 for the hydroxide ion catalyzed hydrazinolysis of *p*-chlorophenyl acetate was obtained by determining rate constants at a series of hydrazine concentrations in the range 0.05–0.45 M at pH 12.5, where the k_3 term is predominant, and correcting for the contribution of the k_1 and k_2 terms, which were determined at lower pH.

The rate constants for the overall reaction, under conditions in which the change in rate-determining step occurs, may be described in the same way as previously by combining the rate constants for the steps involved in the formation and breakdown of the anionic addition intermediate, T^-, as shown in eq 1 and 3.[3,12] The data for

$$\frac{\text{rate}}{[NH_2NH_2][\text{ester}]} = \frac{k_1 + k_2[NH_2NH_2] + k_3[OH^-]}{1 + \dfrac{k_{-1}a_{H^+} + k_{-2}[NH_2NH_3^+] + k_{-3}}{k^-}} \quad (3)$$

the ethyl acetate reaction over a range of pH values were treated using this rate equation, after determination of the individual values at the extremes of the pH–rate profiles.

(15) J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 837 (1964).

Table II. Rate Constants for the Alkaline Hydrolysis and Hydrazinolysis of Alkyl Acetates at 25° and Ionic Strength 2.25[a]

Ester	pK_a,[b] resident alcohol	k_0,[c] M^{-1} min^{-1}	k_1, M^{-1} min^{-1}	k_2, M^{-2} min^{-1}	k_3,[c] M^{-2} min^{-1}	k_4, M^{-2} min^{-1}	k_{-1}/k^-, M^{-1}	k_{-2}/k^-, M^{-1}
Ethyl acetate (EA)	16.0	4.5	0.005–0.012[d]	0.047[d]	3.8		9×10^8	15.0
Methyl formate[e]	15.5	2000	5.0	170			5.5×10^8	9.0
Methoxyethyl acetate (MEA)	14.8	8.9	≤0.02	0.18	15			
2-Chloroethyl acetate (CEA)	14.3	17.0	0.004–0.015	0.35	32			
Propargyl acetate (PRA)	13.5	28.0	≤0.10	1.3				
Trifluoroethyl acetate (TFEA)	12.4	102.0	≤0.15	7.5		~0.5		

[a] Ionic strength maintained at 2.25 with 1.5 M KCl + 0.75 M (tetramethylammonium chloride + amine hydrochloride buffers), except where noted. [b] W. P. Jencks and J. Regenstein, "Handbook of Biochemistry," 2nd ed, H. A. Sober, Ed., Chemical Rubber Co., Cleveland, Ohio, 1970, pp J-150–189. [c] Based on the activity of hydroxide ion as measured by pH and $K_w = 10^{-14}$. [d] Ionic strength 2.55 with 1.5 M KCl + 0.75 M tetramethylammonium chloride + 0.30 M diisopropylamine buffer. [e] Reference 3.

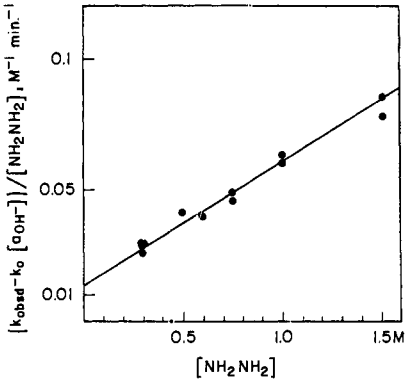


Figure 1. Observed second-order rate constants for the hydrazinolysis of ethyl acetate as a function of hydrazine concentration at pH 10.95, 25°, and ionic strength 2.55 in 0.3 M diisopropylamine buffer.

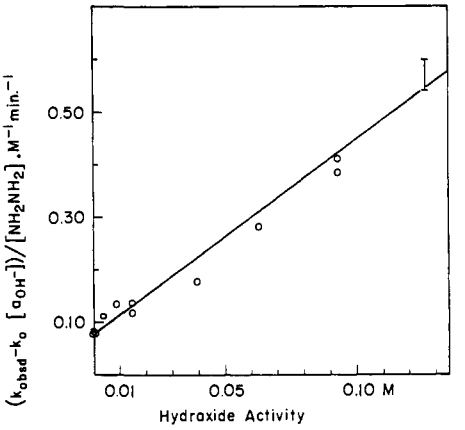


Figure 2. Observed second-order rate constants for the reaction of 1.5 M hydrazine free base with ethyl acetate as a function of hydroxide ion activity at 25° and ionic strength 2.25. The reactions were buffered with 0.2 M diisopropylamine buffers from a_{OH^-} = 0.0016 to 0.016 M.

Results

The reactions of alkyl and phenyl acetates with hydrazine follow the rate law of eq 4 at pH values

$$k_{obsd} - k_0 a_{OH^-} = k_1[N_2H_4] + k_2[N_2H_4]^2 + k_3[N_2H_4]a_{OH^-} + k_4[N_2H_4][N_2H_5^+] + k_5[N_2H_4][\text{Buffer}] \quad (4)$$

sufficiently high that the change in rate-determining step is not significant. We are interested mainly in the terms for the uncatalyzed and hydrazine catalyzed reactions described by k_1 and k_2, respectively. The experimental data were treated as described in the Experimental Section, as illustrated for the ethyl acetate reaction in Figures 1 and 2. The slope of Figure 1 gives the k_2 term, and the intercept gives the sum of the contributions of the k_1, k_3, and k_5 terms. The k_1 term for the alkyl acetates is difficult to determine accurately because of the large contribution of the k_2 term, and definite evidence for its existence was obtained only for the reactions of ethyl acetate and 2-chloroethyl acetate, although intercepts that are probably significant were observed also for 2-methoxyethyl acetate and trifluoroethyl acetate. The rate constant k_3 for hydroxide ion catalysis of hydrazinolysis was obtained from the slope of Figure 2. In a separate experiment it was shown that the third-order rate constant k_5 for catalysis by diisopropylamine buffer at pH 10.9 is <0.005 M^{-2} min^{-1}. The corrected value of k_1 for ethyl acetate is 0.005–0.012 M^{-1} min^{-1}. The observed rate constants and limiting values for the constants which were not demonstrated unequivocally are summarized in Tables II and III. The rate constants are given in units of min^{-1} rather than sec^{-1} to facilitate comparison with previous results; earlier data for methyl formate[3] are included in Table II.

The hydrazinolysis of 2-chloroethyl acetate and *p*-chlorophenyl acetate is catalyzed by tertiary amines, as illustrated for catalysis of the latter reaction by triethylenediamine in Figure 3. Small corrections have been made for alkaline hydrolysis and the direct reaction of triethylenediamine with the ester. Division of the slopes by the fraction of buffer as the free base, indicates that only the free base form of triethylenediamine has significant catalytic activity. Accurate determination of the rate constants for catalysis by 3-quinuclidinol and quinuclidine buffers was not possible because of curvature in plots of k_{obsd} against buffer concentration, presumably caused by complexation of ester with the amine. Approximate values for these catalytic constants, estimated from the initial slopes of the plots, and for the corresponding catalytic constants of the 2-chloroethyl acetate reaction are summarized in Table IV.

pH–rate profiles for the reactions of a series of esters with hydrazine at a constant concentration of free base are shown in Figure 4. As observed previously for methyl formate,[3] the reactions of ethyl acetate and

Table III. Rate Constants for the Hydrazinolysis and Alkaline Hydrolysis of Phenyl Acetates at 25° and Ionic Strength 1.0 (KCl)

Ester	pK_a,[a] resident alcohol	k_0,[b,c] M^{-1} min^{-1}	k_1, M^{-1} min^{-1}	k_2, M^{-2} min^{-1}	k_3, M^{-2} min^{-1}	k_4, M^{-2} min^{-1}
p-Methoxyphenyl acetate (PMPA)	10.2	94	0.24[d]	13.8[e]		2.6[e]
p-Methylphenyl acetate (PMePA)	10.2	88	0.34[e]	11.8[e]		2.6[e]
Phenyl acetate (PA)	9.95	113	0.56[e]	16.3[e]		3.0[e]
p-Chlorophenyl acetate (PCPA)	9.38	197	2.0	35.5	1.8×10^{3}[c]	6.5
m-Nitrophenyl acetate (MNPA)	8.35	615	56.5	145.0		7–12
p-Nitrophenyl acetate (PNPA)	7.14	850	425[e]			
2,4-Dinitrophenyl acetate (DNPA)	4.02	4.8×10^{3}	1.8×10^{4} [f]			
1-Acetoxy-4-methoxypyridinium perchlorate (AMPP)	2.0	8.5×10^{5} [f]	4.9×10^{5} [f]			

[a] W. P. Jencks and J. Regenstein, "Handbook of Biochemistry," 2nd ed, H. A. Sober, Ed., Chemical Rubber Co., Cleveland, Ohio, 1970, pp J-150–189. [b] k_0 values taken from ref 15 except where noted. [c] Based on the activity of hydroxide ion as measured by pH and $K_w = 10^{-14}$. [d] T. C. Bruice and S. J. Benkovic, *J. Amer. Chem. Soc.*, **86**, 418 (1964), from data at 25°. [e] Calculated for 25° from the reported enthalpies and entropies of activation.[d] [f] Reference 6.

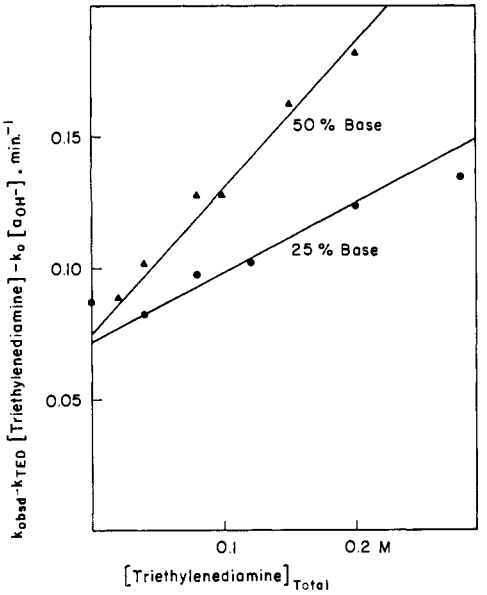


Figure 3. Catalysis by triethylenediamine buffers of the reaction of 0.025 *M* hydrazine (free base) with *p*-chlorophenyl acetate at 25° and ionic strength 1.0 (KCl).

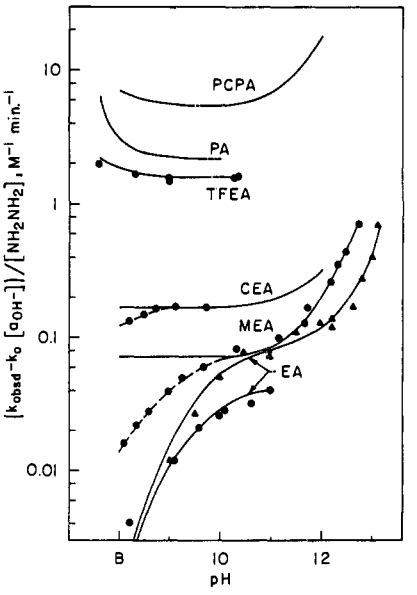


Figure 4. Dependence on pH of the observed second-order rate constants for the reactions of esters with a constant concentration of hydrazine free base at 25°. The hydrazine free base concentrations are 0.5 and 1.0 *M* for ethyl acetate (EA), 0.4 *M* for methoxyethyl acetate (MEA), 0.5 *M* for 2-chloroethyl acetate (CEA), 0.2 *M* for trifluoroethyl acetate (TFEA), and 0.1 *M* for phenyl acetate (PA) and *p*-chlorophenyl acetate (PCPA). Ionic strength 2.25 (alkyl acetates) or 1.0 (phenyl acetates). The solid lines were calculated from the rate constants in Tables II and III. The solid calculated lines for ethyl acetate are based on the complete rate law (eq 3), whereas those for the other esters show the behavior expected if there were no change in rate-determining step. The absence of a break for the phenyl acetates is based on the observed adherence to the simple rate law of eq 4 (see text). Added buffers were 0.05–0.10 *M* dimethylglycine (pH 9.6–10.5), 0.2 *M* *tert*-butylamine (pH 10), 0.2 *M* diisopropylamine (pH 10.5–12.2), and hydroxide ion (pH > 12.2) for ethyl acetate; 3-quinuclidinol (k_{obsd} extrapolated to zero buffer concentration; pH 9.7 and 10.35) and 0.15 *M* diisopropylamine (pH 11.2–12.6) for methoxyethyl acetate.

methoxyethyl acetate exhibit a negative deviation of the rate with decreasing pH that is evidence for a change in rate-determining step. The deviations for 2-chloroethyl acetate are small but probably significant; negative deviations between 20 and 40% were observed in a series of experiments at 0.1, 0.2, 0.3, and 0.5 *M* hydrazine free base. The solid lines for the phenyl acetates were calculated from the rate constants in Table III, which were shown to describe the observed rate in the pH range shown in Figure 4. No negative deviation of the rate at low pH occurs with the phenyl acetates or trifluoroethyl acetate. The positive deviations in these curves at low pH are caused by catalysis by the hydrazinium ion.

The solid lines for ethyl acetate were calculated from the rate law described by eq 1 and 3, in the same manner as for the methyl formate reaction[3] and show satisfactory agreement with the data. The absence of a break for trifluoroethyl acetate, even at low pH values, was confirmed by a series of experiments carried out at pH values in the range 5–7, which gave observed rate constants that agree well with those calculated from the data at high pH (Table V). A similar experiment with phenyl acetate, in the presence of 0.09–0.45 *M* total hydrazine at pH 4.86, also gave satisfactory agreement with the rate constants obtained at higher pH values (Table III). Thus, no change in rate-determining step with changing pH occurs in the hydrazinolysis of esters with relatively acidic leaving groups.

Table IV. General Base Catalysis of the Hydrazinolysis of 2-Chloroethyl Acetate and *p*-Chlorophenyl Acetate by Tertiary Amines at 25° [a]

Ester	Tertiary amine	pK_a, amine	No. of runs	Concn of catalyst, *M*	% base	k_{cat},[b] M^{-2} min^{-1}	k_b,[c] M^{-2} min^{-1}
2-Chloroethyl acetate	3-Quinuclidinol	10.30[d]	5	0.0–0.4[e]	50	0.44	0.88
	Quinuclidine	11.6[d]	5	0.0–0.5[e]	20	0.42	2.1
p-Chlorophenyl acetate	Triethylenediamine	9.20[f]	6	0.0–0.28[g]	25	10.6	
			6	0.02–0.20[g]	50	22.2	43
	3-Quinuclidinol	10.1[f]	4	0.05–0.2[g]	50	26	
			6	0.05–0.50[g]	80	70	$\lesssim 200$
	Quinuclidine	11.5[f]	2	0.2–0.7[h]	10	12	$\lesssim 200$

[a] Ionic strength maintained at 2.25 with 1.5 *M* KCl + 0.75 *M* (tetramethylammonium chloride + amine hydrochloride buffer) for 2-chloroethyl acetate. Ionic strength maintained at 1.00 (KCl) for *p*-chlorophenyl acetate. [b] For total buffer concentration. [c] Based on the free base form of the catalyst. [d] Determined from the pH of reaction mixture (ionic strength 2.25). [e] Hydrazine free base = 0.3 *M*. [f] Reference 6; J. M. Sayer and W. P. Jencks, *J. Amer. Chem. Soc.*, **91**, 6353 (1969). [g] Hydrazine free base = 0.025 *M*. [h] Hydrazine free base = 0.10 *M*.

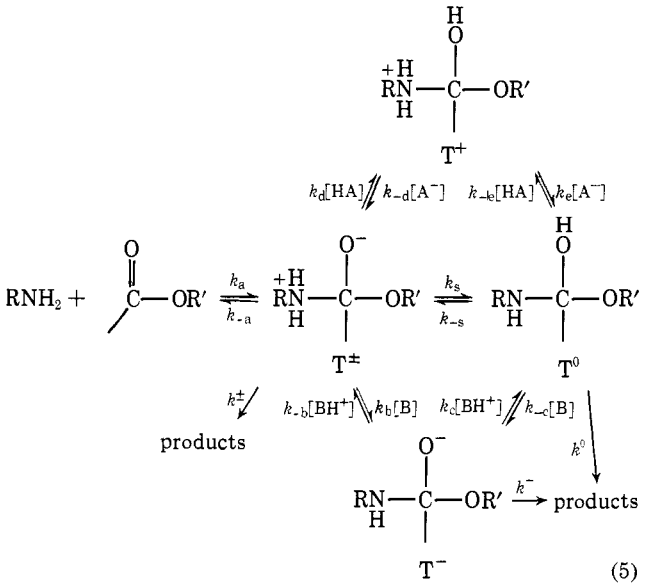
Table V. Hydrazinolysis of Trifluoroethyl Acetate between pH 5.0 and pH 7.0 at 25°

pH	k_{obsd}, min^{-1}	k_{calcd},[a] min^{-1}
4.96	0.0023[b]	0.0021
5.96	0.023[c]	0.022
6.93	0.35[d]	0.28
6.98	0.38[d]	0.34
7.00	0.35[d]	0.36

[a] Calculated rates are based on the rate constants in Table II, eq 4, and a pK_a for hydrazinium ion of 8.33 at 25°, ionic strength 2.25. [b] Run in 3 *M* hydrazine monohydrochloride and buffered with 0.17 *M* acetate. [c] Run in 3 *M* hydrazine monohydrochloride and buffered with 0.17 *M* phosphate. [d] Run in 3 *M* hydrazine monohydrochloride and buffered with 0.10 *M* phosphate.

Discussion

A mechanism for ester aminolysis is described in eq 5.



(5)

According to this mechanism, the formation of the dipolar addition intermediate $T^{\pm}$ (k_a) is rapid and reversible, for the alkyl and moderately reactive phenyl esters examined in this work, but becomes rate determining for fast reactions of esters that exhibit a small sensitivity to substituent effects. The pH- and buffer-independent breakdown of this intermediate represents rate-determining expulsion of phenolate ion for phenyl acetates ($k^{\pm}$). As the leaving group becomes poorer (alkyl acetates) a proton switch through water (k_s) that converts $T^{\pm}$ to T^0 becomes the rate-determining step; this mechanism provides a lower energy pathway and a positive deviation from structure–reactivity correlations for the reactions of primary and secondary amines. The general-base-catalyzed reaction represents rate-determining proton removal from $T^{\pm}$ (k_b[B]) that serves to trap this unstable intermediate and prevents it from reverting rapidly to starting materials, for both classes of esters. Similarly, the general-acid-catalyzed reaction involves trapping of $T^{\pm}$ by rate-determining proton donation to give T^+, which proceeds to amide through T^0. Alkyl esters generally undergo a change to rate-determining breakdown of T^- (k^-) with decreasing pH and at still lower pH values may exhibit rate-determining breakdown of the addition intermediate through pH-independent and buffer-catalyzed pathways.[3]

The following points are consistent with the proposed mechanism but not with previously proposed mechanisms.

(1) The change in rate-determining step with decreasing pH (Figure 4) shows that there is a kinetically significant *intermediate* in the reactions of acetate esters with poor leaving groups, as well as with methyl formate. This change involves a shift from rate-determining formation of the intermediate T^- at high pH to its rate-determining breakdown at lower pH, as described previously.[3] The proposed mechanism (eq 5) differs from the earlier mechanism in that a proton-transfer step, rather than amine attack, is rate determining for the formation of T^-. The experimental rate constant k_1 for the formation of T^- (eq 1) includes both the amine attack and subsequent proton-transfer steps; it was assumed previously that the proton transfers were fast, and that amine attack was rate determining. It will be shown in the following paper that this assumption is not justified, because the various ionic forms of the addition intermediates formed in ester aminolysis and in imidate hydrolysis are not in protonic equilibrium.[9]

(2) Structure–reactivity correlations show that the structure of the transition state for the *general base-catalyzed reactions* of alkyl and phenyl esters is the same and is consistent with that expected for rate-determining removal of a proton from $T^{\pm}$ by a second molecule of amine. An addition intermediate has been demonstrated in the reactions of the alkyl esters, and this result suggests that the same intermediate must be sufficiently stable to have a finite existence in the reactions of phenyl acetates; *i.e.*, neither group of reactions is "concerted." The structure–reactivity rela-

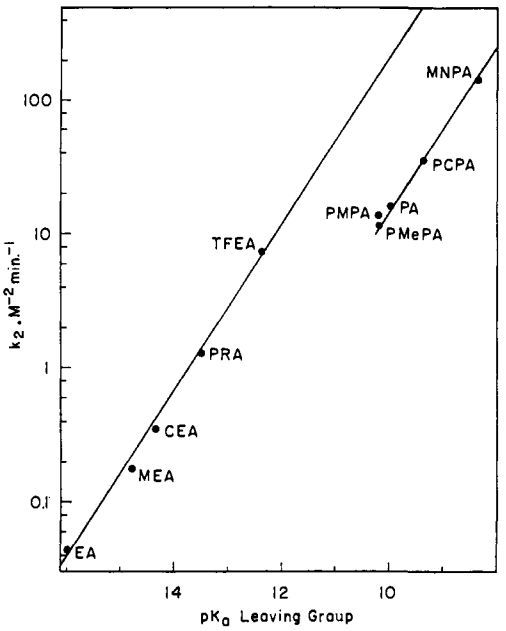


Figure 5. Plot of log k_2 for the hydrazine-catalyzed hydrazinolysis of alkyl and phenyl acetates at 25° as a function of the pK_a of the leaving alcohol.

tionships, expressed as the slopes, β, of plots of log k against the pK_a of the conjugate acid of the attacking amine or the leaving group, are summarized in Table VI.

Table VI. Summary of Structure-Reactivity Correlations for Ester Aminolysis[a]

Brønsted-type slopes	β_{nuc}	$-\beta_{lg}$
Class I: acidic alcohol, basic amines		
k_1 (primary, secondary, tertiary amines)	0.2 ± 0.2	0.2 ± 0.2
Class II: intermediate reactivity, most phenyl esters and amines		
k_1 (primary, secondary, tertiary amines)	0.9 ± 0.1	1.0 ± 0.1
k_2 (primary, secondary amines)	0.9 ± 0.1	0.6 ± 0.1
Class III: low reactivity, aliphatic esters		
k_1 (primary, secondary amines)	0.8 ± 0.1	0.2 ± 0.2
k_1 (tertiary amines)	1.4 ± 0.2	1.4 ± 0.2
k_2 (primary, secondary amines)	0.9 ± 0.1	0.6 ± 0.1

[a] k_1 refers to the buffer-independent reaction; k_2 refers to the amine-catalyzed reaction; see text for references.

(a) The sensitivity of the reactions of alkyl acetates and phenyl acetates to the pK of the leaving group is the same; the slope of the lines in Figure 5, $-\beta_{lg}$, is 0.62. The line for the phenyl acetates falls an order of magnitude below that for the alkyl acetates, presumably because of a steric effect. A similar difference is observed for the reaction of hydroxide ion.[15] A comparison of the rate constant for the hydrazine-catalyzed hydrazinolysis of an *O*-formylserine ester[16] with the corresponding rate constant for methyl formate[3] gives a value of $-\beta_{lg} = 0.57$, in close agreement with the result for the acetate series.

According to the proposed mechanism (eq 5), the observed rate constant for the base-catalyzed reaction is given by eq 6, where $K_{T^\pm}$ is the equilibrium constant

$$k_{2(obsd)} = k_b \frac{k_a}{k_{-a}} = k_b K_{T^\pm} \quad (6)$$

for the formation of $T^\pm$. Since the simple proton-transfer step, k_b, is expected to have little or no dependence on substituents in the leaving group, the substituent dependence of the observed rate reflects principally the effect on $K_{T^\pm}$. A value of $-\beta_{lg} = 0.6$ is expected for the conversion of an ester to an addition intermediate $T^\pm$ from equilibrium measurements which show that the hydrolysis of an ester exhibits a value of $-\beta_{eq} = 0.6$ that is caused by the loss of positive charge on the alcohol oxygen atom of the ester, **1**, upon cleavage to the alcohol.[17]

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(b) Plots of log k_2 against the pK of the nucleophile have slopes $\beta_{nuc} = 0.9 \pm 0.1$ for the reactions of amines with methyl formate[3] and with phenyl acetate.[18] This means that the sensitivity of these reactions to substituents on the amine is essentially the same as for protonation of the amine; *i.e.*, the reactions behave as if there is a development of a shared charge of approximately +1 on the attacking and catalyzing amine molecules in the transition state. This is consistent with a rate-determining reaction of the intermediate $T^\pm$ with a base in the transition state, as it is also in the aminolysis reactions of acetylimidazole and acetyltriazole. Brønsted plots for general base catalysis of the hydrazinolysis of acetylimidazole and the methoxyaminolysis of acetyltriazole exhibit β values approaching zero for strong base catalysts that suggest a diffusion-controlled encounter of $T^\pm$ with the base in the rate-determining step.[10,19,20]

Proton transfer from $T^\pm$ to the strong base hydroxide ion will be thermodynamically favorable, so that the value of k_b is taken as 10^{10} M^{-1} sec^{-1} for hydroxide ion.[21] The ratios of the rate constants for base catalysis by hydroxide ion and by hydrazine, k_3/k_2, are equal to 81, 83, 91, and 51 for the reactions of ethyl, methoxyethyl, chloroethyl, and *p*-chlorophenyl acetates, respectively (Tables II and III). Since $K_{T^\pm}$ is the same for both catalysts, the average value of k_b for hydrazine is then 1.3×10^8 M^{-1} sec^{-1}. The approximate ionization constants for the various forms of the addition intermediate formed from ethyl acetate were estimated, as described elsewhere,[9,20] to be pK_b = 9.6, pK_c = 12.6, pK_d = 7.8, and pK_e = 4.8 (the subscripts refer to the ionizations in eq 5). Since proton transfer from $T^\pm$ to hydrazine is slightly unfavorable thermodynamically (by some 1.3 pK units), it is reasonable that k_b should be smaller for hydrazine than for hydroxide ion. The smaller value of k_3/k_2 for *p*-chlorophenyl acetate suggests a larger value of k_b for hydrazine in this reaction, which is in accord with the slightly greater acidity of

(16) G. M. Blackburn and H. L. H. Dodds, *J. Chem. Soc. B*, 826 (1971).

(17) J. Gerstein and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 4655 (1964).

(18) T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *J. Amer. Chem. Soc.*, **89**, 2106 (1967).

(19) M. I. Page and W. P. Jencks, *J. Amer. Chem. Soc.*, **94**, 8828 (1972).

(20) J. P. Fox and W. P. Jencks, *J. Amer. Chem. Soc.*, **96**, 1436 (1974).

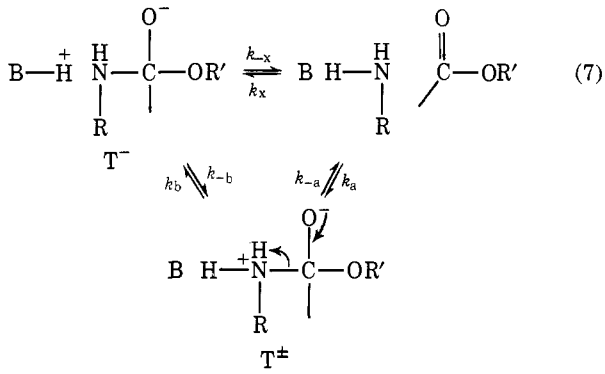
(21) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964).

$T^{\pm}$ that is expected as a consequence of the electron-withdrawing phenyl group.

Neglecting the relatively small variation in k_b for hydrazine with varying leaving group, the values of the equilibrium constant $K_{T^{\pm}}$ may be estimated from eq 6 to be on the order of 6×10^{-12}, 1×10^{-9}, 2×10^{-9}, and 2×10^{-8} M^{-1} for ethyl, trifluoroethyl, phenyl, and *m*-nitrophenyl acetates, respectively; the value of $-\beta_{lg}$ for $K_{T^{\pm}}$ is 0.6. Since there is no indication of a change in rate-determining step with increasing hydrazine concentration up to over 1 *M* (Figure 1), the value of k_{-a} must be $> k_b[N_2H_4]$ or $> 10^8$ sec^{-1}.

(3) The observation of general base catalysis requires that the base be present in the transition state. Since proton transfer from the free amine and from $T^{\pm}$ to hydrazine is thermodynamically unfavorable, it will also be thermodynamically unfavorable in the transition state so that a concerted mechanism of general base catalysis is not possible if the addition intermediate has a finite lifetime, *i.e.*, as long as it may be called an intermediate.[11,12] The general-base-catalyzed reaction must therefore proceed through a stepwise mechanism with rate-determining proton transfer.

This point may be explained more fully as follows. In any encounter of the nitrogen atom of T^- with an acid BH^+ that is sufficiently strong and in the correct position so that proton transfer is favorable, the proton will jump to nitrogen (k_{-b}, eq 7) faster than a concerted



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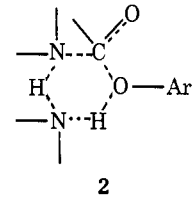
proton transfer and C–N bond cleavage (k_{-x}) can take place, as long as there is a significant energy barrier for the breakdown of the fully protonated intermediate $T^{\pm}$. The direct expulsion of a fully protonated amine (k_{-a}) will be more rapid than the expulsion of a partially protonated amine (k_{-x}). The breakdown of T^- to ester and amine will then proceed by the energetically more favorable stepwise pathway (k_{-b} and k_{-a}) rather than through the concerted pathway (k_{-x}). Since the stepwise mechanism is thermodynamically favored for the breakdown reaction, it must also be the lowest energy path for T^- formation.

The stepwise will be more favorable than the concerted mechanism of general base catalysis, unless the free energy of the proton transfer from $T^{\pm}$ to the base and the instability of the intermediate provide a sufficient free-energy advantage to overcome the entropic and enthalpic requirements for coupled multiatom transfers between three properly located molecules in the transition state for the concerted mechanism.[12,22] Although there does not appear to be strong evidence for a concerted mechanism in any known ester aminolysis reaction, concerted catalysis might become the favored reaction path for catalysis by strong bases, such as hydroxide ion, of reactions of weakly basic amines that give an intermediate $T^{\pm}$ with a low pK_a, or of esters with such a good leaving group that T^- becomes too unstable to exist.

The change in rate-determining step in the reactions of the alkyl acetates shows that a metastable intermediate is formed. If general base catalysis involves proton transfer from $T^{\pm}$ to the base in these reactions, it should also in the phenyl acetate reactions, since the structure–reactivity correlations indicate a closely similar transition-state structure and no evidence for a change in rate-determining step for the two classes of compounds. The addition intermediates formed during the hydrolysis of phenyl imidates must have a finite lifetime because the rates and product ratios of these reactions may be varied independently;[4b,6] further evidence for the existence of $T^{\pm}$ is given in the following paper.[9]

(4) The limited data that were obtained for catalysis by bases of differing strength are consistent with a stepwise mechanism. The catalytic constants (Tables II, III, and IV) show a small increase with increasing pK ($\beta \leq 0.25$) as expected for a simple proton-transfer reaction in the region near and above $\Delta pK = 0$.[21] The rate constants for hydroxide ion are only about tenfold larger than those for much less basic tertiary-amine catalysts, and some of this increase may be ascribed to an electrostatic effect and more facile diffusion of hydroxide ion; a similar increase has been observed in 2-methylthiosemicarbazone formation for which there are more complete data supporting a β value approaching zero for catalysis by strong bases.[23] The previously mentioned variation of less than twofold in the ratio of the catalytic constants for hydroxide ion and for hydrazine, k_3/k_2, in the reactions with ethyl, methoxyethyl, chloroethyl, and *p*-chlorophenyl acetates, over a range of leaving group basicity of $10^{6.6}$, provides further evidence that these base-catalyzed reactions proceed by a common mechanism.

The fact that tertiary amines, which have no transferable proton, are even more active than hydrazine as general base catalysts serves to rule out cyclic mechanisms, such as **2**, that have been proposed for catalysis of ester aminolysis in water.[18,24-26]



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(5) The *pH- and buffer-independent reactions* that are first order in amine (k_1) proceed through three different rate-determining transition states, depending on the pK of the leaving group. Since the three transition states have different structures, structure–reactivity correlations exhibit breaks that correspond to the

(22) J. Hine, *J. Amer. Chem. Soc.*, 94, 5766 (1972).

(23) J. Sayer and W. P. Jencks, *J. Amer. Chem. Soc.*, 95, 5637 (1973).

(24) T. C. Bruice and M. F. Mayahi, *J. Amer. Chem. Soc.*, 82, 3067 (1960).

(25) J. F. Kirsch and A. Kline, *J. Amer. Chem. Soc.*, 91, 1841 (1969).

(26) T. C. Bruice, A. F. Hegarty, S. M. Felton, A. Donzel, and N. G. Kundu, *J. Amer. Chem. Soc.*, 92, 1370 (1970).

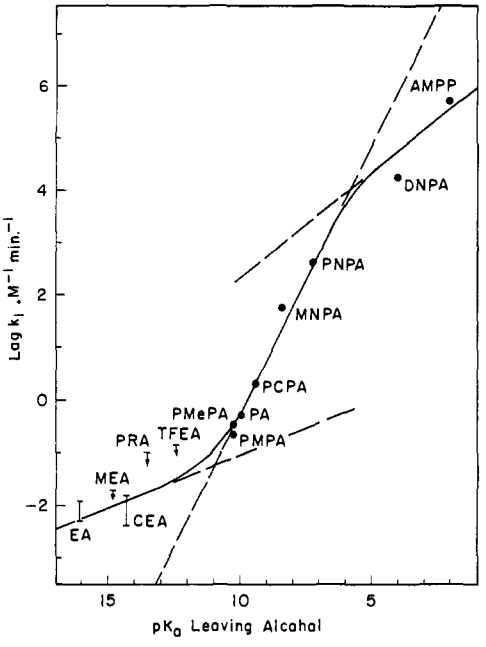
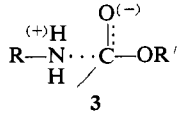


Figure 6. Plot of log k_1 (uncatalyzed or water-catalyzed reaction) for the hydrazinolysis of alkyl and phenyl acetates as a function of the pK_a of the alcohol leaving group at 25°. Upper limits are indicated for several of the alkyl acetates. The dashed lines are drawn with slopes $-\beta_{lg} = 0.2$, 1.0, and 0.4.

changes in the rate-determining step. This results in a sigmoid curve for the correlation of log k_1 with the pK of the leaving group, in which the three regions correspond to the three different transition states (Figure 6). The break between the class I and class II regions is more clearly evident in previously reported correlations of log k_1 for the reactions of phenyl esters with a series of amine nucleophiles of increasing pK.[6,7]

(a) Class I reactions of basic amines and acyl compounds with very good leaving groups, such as 1-acetoxy-4-methoxypyridinium (AMPP) and acetylpyridinium ions, are assigned to rate-determining amine attack (k_a, eq 5). These reactions exhibit only a small sensitivity to changes in the structure of the nucleophile and leaving group, as expected for an early transition state (**3**), and show a negative deviation from structure–



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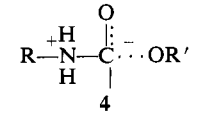
reactivity correlations for class II reactions.[6,7] In the hydrazine series this class is rather poorly represented by the points for AMPP and probably dinitrophenyl acetate (DNPA) in Figure 6.

The break in the structure-reactivity correlation for the aminolysis of AMPP appears[6] with amines of pK approximately 8, 6 units above the pK of the leaving group of **2**. This is consistent with the approximately 10^5 times better leaving ability of amines than of alkoxide ions of comparable pK from the corresponding addition compounds formed from a phthalimide.[8]

The intermediate $T^{\pm}$ formed in the reaction with AMPP probably has a short but significant lifetime. The rate constant k_{-a} for breakdown to reactants should not be larger than that for the ethyl acetate adduct, because the basic oxygen atom of ethanol should provide more driving force than the corresponding oxygen atom of the AMPP addition compound to assist amine expulsion. The value of $k^{\pm}$ may be estimated from structure–reactivity data for less reactive esters for which $k^{\pm}$ is rate determining, so that $k_1 = k^{\pm}K_{T^{\pm}}$, and the value of $-\beta_{lg}$ for k_1 is 1.0 (see below). Since $-\beta_{lg}$ for $K_{T^{\pm}}$ is 0.60, the value of $-\beta_{lg}$ for $k^{\pm}$ is $1.0 - 0.6 = 0.4$. Based on the value of $k^{\pm} = k_1/K_{T^{\pm}} = 5 \times 10^6$ sec^{-1} for the phenyl acetate reaction, this gives $k^{\pm} = 8 \times 10^9$ sec^{-1} for the AMPP addition compound. An analogous calculation for the methylamine reaction with AMPP gives $k^{\pm} = 3 \times 10^{10}$ sec^{-1}. The extrapolated value of $K_{T^{\pm}}$ for the AMPP–hydrazine reaction is 1.3×10^{-4} M^{-1}, and assuming that $k_1 = k_a$ for this reaction, the value of k_{-a} is 6×10^7 sec^{-1}. This is smaller than $k^{\pm}$, as expected for rate-determining amine attack.

(b) Class II reactions of most phenyl esters with amines are assigned to rate-determining breakdown of $T^{\pm}$ through $k^{\pm}$ (eq 5), so that $k_1 = K_{T^{\pm}}k^{\pm}$. Reactions in this class characteristically exhibit values of $\beta_{nuc} = 0.9 \pm 0.1$ and of $-\beta_{lg} = 1.0 \pm 0.1$ and constitute the central region of Figure 6.[6,18,27] The comparable reactivities of primary, secondary, and tertiary amines means that no significant loss of a proton from the amine has occurred in the transition state.[6] The value of β_{nuc} is similar to that for the equilibrium formation of $T^{\pm}$ and suggests a transition state at a similar or later point along the reaction coordinate than $T^{\pm}$. The value of $-\beta_{lg}$ is significantly larger than that for the formation of $T^{\pm}$ and suggests a significant amount of C–O bond cleavage in the transition state, consistent with rate-determining breakdown of $T^{\pm}$ (**4**). Menger



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and coworkers have concluded from the large effect of substituents in the phenol (ρ = 4–6) that the rate-determining step in the aminolysis of phenyl esters in aprotic solvents is the breakdown of $T^{\pm}$ and have suggested that base catalysts act by abstracting a proton from $T^{\pm}$.[28]

These assignments provide an explanation, other than a cyclic mechanism of general base catalysis, for the unexpected finding that substituents on the acyl group have a larger effect on the general base catalyzed (ρ = 1.8) than on the uncatalyzed (ρ = 1.1) ammonolysis of *p*-chlorophenyl benzoates[25] and for a similar relationship for acyl-substituted phenyl acetates.[26] These reactions involve rate-determining proton removal (k_b) and rate-determining phenolate ion expulsion ($k^{\pm}$), respectively, from the common intermediate $T^{\pm}$. Electron-withdrawing substituents favor the formation of $T^{\pm}$ to the same extent in both reactions. The difference in the substituent effects is expected from a small increase in k_b, caused by an increase in the acidity of $T^{\pm}$, and a decrease in $k^{\pm}$, caused by a decrease in the driving force for phenolate expulsion from $T^{\pm}$ in the presence of electron-withdrawing substituents.

(27) T. C. Bruice and S. J. Benkovic, *J. Amer. Chem. Soc.*, **86**, 418 (1964).
(28) F. M. Menger and J. H. Smith, *J. Amer. Chem. Soc.*, **94**, 3824 (1972); F. M. Menger and A. C. Vitale, *ibid.*, **95**, 4931 (1973).

(c) The aminolysis of esters with poor leaving groups is sharply divided into two groups: typical class III reactions of tertiary amines, with no possibility of proton removal from the amine and rate-determining leaving group expulsion ($k^{\pm}$, eq 5), and the much faster reactions of primary and secondary amines, which are assigned to a rate-determining proton switch at high pH (k_s) followed by the rapid loss of a proton from oxygen (k_{-c}) and the expulsion of alcoholate ion from the anionic intermediate T^- (k^-), which becomes rate determining at lower pH.

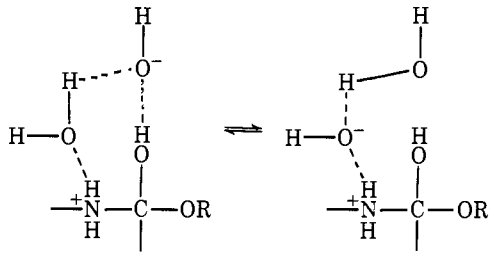
Although the data are inadequate to provide a value of β_{lg}, the rate constants and limits for the hydrazinolysis of the alkyl acetates in Figure 6 show that this reaction proceeds much more rapidly than the extrapolated line for esters with better leaving groups and exhibits little if any sensitivity to electron-withdrawing substituents on the leaving alcohol. The data are consistent with a value of $-\beta_{lg}$ in the range 0–0.3, and the lower dashed line in Figure 6 is arbitrarily drawn with a slope of 0.2. The insensitivity to substituent effects is confirmed in the formate series by the identical rate constants for the hydrazinolysis of methyl (pK = 15.5) and substituted serine (pK = 13.6) formate esters.[3,16]

The requirement for proton transfer is also evident in the absence of an observed nucleophilic reaction of alkyl esters with tertiary amines. The rate constants for these reactions have been calculated from the equilibrium constants and the rate constants for the reverse reactions and are some 10^5 times smaller than those for primary amines of comparable pK.[7]

The value of β_{nuc} = 0.7 for the aminolysis of methyl formate indicates considerable charge development on the attacking nitrogen atom in the transition state but is slightly smaller than that for the equilibrium formation of $T^{\pm}$. It is much smaller than the value of β_{nuc} = 1.5 for the reactions of tertiary amines and alkyl esters, with rate-determining breakdown of $T^{\pm}$ in a late transition state.[7]

The positive deviation from the structure–reactivity correlation of Figure 6 for the pH-independent reactions of alkyl acetates and their diminished sensitivity to substituents in the leaving group stand in marked contrast to the similar behavior of alkyl and phenyl acetates in general-base-catalyzed reactions (Figure 5). This means that there is a change to a different mechanism and transition state structure for the pH-independent but not the base-catalyzed reactions of alkyl acetates.

The data are consistent with a rate-determining proton switch mechanism through one or two water molecules in which proton donation to the oxygen anion has proceeded further than proton abstraction from the nitrogen atom of $T^{\pm}$. Although a concerted transfer is not excluded, the results are most easily explained by a stepwise mechanism[22,29] with the most facile proton transfer proceeding first to form a cationic intermediate (*e.g.*, **5**) followed by rapid proton abstraction from nitrogen to give T^0. The structure–reactivity data are best considered starting with the intermediate $T^{\pm}$. The small observed effect of substituents in the leaving group ($-\beta_{lg}$ = 0–0.3) means that the proton switch is aided by electron-donating substituents since the value of



5a **5b**

$-\beta_{lg}$ for the equilibrium formation of $T^{\pm}$ from the ester is approximately 0.6. It is expected that the thermodynamically unfavorable abstraction of a proton from water by the oxyanion of $T^{\pm}$ to form **5** will be aided by electron-donating substituents. The smaller observed value of β_{nuc} (0.7) for this reaction than for the equilibrium formation of $T^{\pm}$ (β_{nuc} = 0.9) suggests that the proton-switch step is facilitated to a small extent by electron-withdrawing substituents on nitrogen, consistent with hydrogen bonding or a small amount of proton transfer from the nitrogen atom in the transition state. The rate of the analogous intermolecular proton-transfer reaction to an amine from an ammonium ion through water increases with increasing basicity of the amine, consistent with a predominant role of proton abstraction from water in the transition state, and in the intramolecular proton switch of carboxylic acids the rate increases with increasing acidity of the acid (α = 0.5), suggesting an initial protonation of the solvent.[22,29] Both of these results support a predominant role of the thermodynamically more favorable proton transfer in the transition state.

The values of k_1 are generally 10^1–10^2 times smaller than those for the base catalyzed reaction, k_2 (Table II and ref 1). If the value of k_b is 1.3×10^8 sec^{-1}, k_s is then in the range 10^6–10^7 sec^{-1}, which is not unreasonable for a proton switch.[29,30] It is noteworthy that the values of k_2/k_1 differ by less than a factor of 3 for the reactions of methyl formate with morpholine, glycinamide, methoxyethylamine, ethylenediamine, hydrazine, and glycylglycine, although the absolute values of the rate constants vary over a range of 250-fold.[3] This is in accord with the proposed mechanisms, for which the ratio $k_2/k_1 = k_b/k_s$ should be approximately constant for amines of similar pK, as is also the increase in the ratio by a factor of 5 for the *n*-propylamine reaction,[3] since the proton switch should be relatively slower with this more basic amine.

The subsequent proton abstraction from T^0 to give T^- will be fast and not kinetically significant as long as the pH is sufficiently high that the diffusion-controlled reaction with hydroxide ion ($k_{-c} \sim 10^{10}$ M^{-1} sec^{-1}) or proton abstraction by other bases in the solution is faster than the reversion of T^0 to $T^{\pm}$.

The change to rate-determining breakdown of T^- is not observed for trifluoroethyl and phenyl esters (Figure 4), because breakdown of $T^{\pm}$ and T^- through $k^{\pm}$ and k^- becomes fast relative to the rate of the proton switch step as the leaving group becomes better.

The pH-independent reaction cannot represent simple proton abstraction by water acting as a general base for two reasons. First, the rate constant for this reaction is only one or two magnitudes slower than the

(29) E. Grunwald, P. J. Karabatsos, R. A. Kromhont, and E. L. Purlee, *J. Chem. Phys.*, **33**, 556 (1960); E. Grunwald and S. Meiboom, *J. Amer. Chem. Soc.*, **85**, 2047 (1963); D. D. Eley, A. S. Fawcett, and M. J. Hey, *J. Chem. Soc., Faraday Trans. 1*, 399 (1973).

(30) E. Grunwald, C. F. Jumper, and S. Meiboom, *J. Amer. Chem. Soc.*, **85**, 522 (1963); Z. Luz and S. Meiboom *ibid.*, **85**, 3923 (1963).

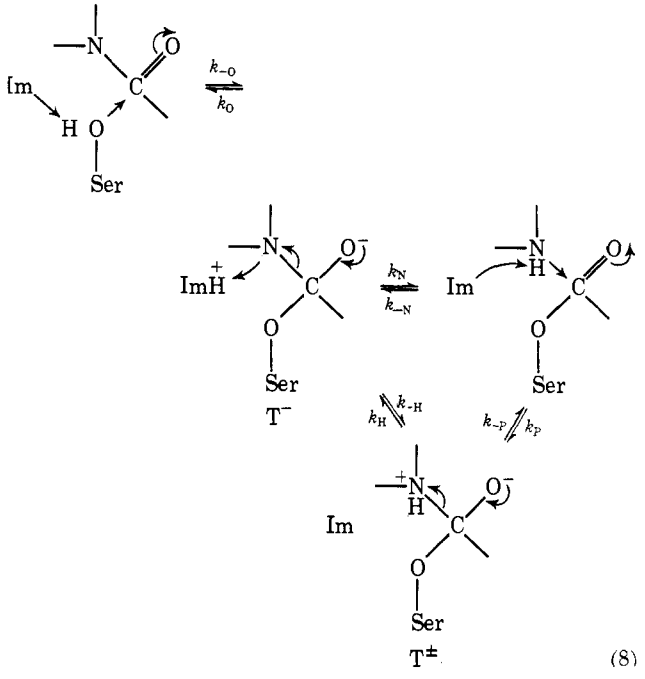
general base reaction, whereas the rate constant for the thermodynamically unfavorable removal of a proton from $T^{\pm}$ by water is many orders of magnitude slower.[21] Second, the rate-determining step of such a thermodynamically unfavorable proton-transfer reaction is the separation of T^- from H^+ (solvated). The transition state for such a reaction would have no charge on the nitrogen atom and would not be stabilized by electron-donating substituents, which is inconsistent with the observed β_{nuc} of 0.7.

(6) The available data for *general acid catalysis of ester aminolysis* are uncertain for several experimental reasons, which include the predominance of other terms and large specific salt and solvent effects, so that only a few comments are warranted at this time. The rate constants for general acid catalysis by the conjugate acid of the amine of hydrazinolysis (Tables I and II) and of essentially all other aminolysis reactions involving moderately basic amines are smaller than those for general base catalysis by a second molecule of the amine, usually by a factor on the order of tenfold.[18,26] The pK_a of the hydroxyl group of the intermediate T^+ formed from phenyl acetate and methylamine has been estimated[9] to be about 7.0, and the corresponding pK_a for the hydrazine addition compound will be slightly lower. The pK_a for $T^{\pm}$ formed from methylamine and phenyl acetate is approximately 10.8, almost the same as that of the parent amine.[9] Since the substituent added to the amine is the same when a series of amines reacts with phenyl acetate to form the corresponding addition compounds $T^{\pm}$, the ΔpK will remain approximately zero for the entire series. Thus, according to the mechanism of eq 5, the k_4 term for general acid catalysis by the conjugate acid of moderately basic amines represents a thermodynamically unfavorable proton transfer, k_d, to the oxygen atom of $T^{\pm}$, whereas the k_2 term for general base catalysis represents a faster proton transfer, k_b, with a $\Delta pK \sim 0$ for reactions with phenyl acetate, in at least qualitative agreement with the observed magnitudes of k_2 and k_4.

On the other hand, in the methoxyaminolysis of phenyl acetate, the term for general acid catalysis is 13 times larger than that for general base catalysis; with electron-withdrawing substituents on the phenol, the ratio decreases progressively, to 2.5 for *p*-nitrophenyl acetate.[31] Allowing a fall-off factor of 0.4 per atom for the transmission of substituent effects from the amine,[32] the pK_a of the hydroxyl group of the intermediate T^+ formed from methoxyamine will be approximately $7.0 - (0.4 \times 0.4)(10.6 - 4.6) = 6.0$. Thus, proton transfer from methoxyammonium ion ($pK = 4.6$) to $T^{\pm}$ will be thermodynamically favorable and faster than that for general base catalysis, for which $\Delta pK \sim 0$, in accord with the experimental result. Electron-withdrawing substituents on the phenol are expected to reduce the basicity and increase the acidity of $T^{\pm}$ so that the ratio $k_d/k_b = k_4/k_2$ should decrease, as observed. This analysis should be regarded as tentative, however, because it is possible that $T^{\pm}$ is too unstable to exist as an intermediate in the methoxyamine reaction.

Chymotrypsin. It is widely believed that catalysis of proton transfer between electronegative atoms provides an important contribution to the rate accelerations brought about by enzymes, but there has been little experimental evidence or detailed evaluation of the mechanism and driving force for this catalysis.[33-35] We describe here the application of the considerations developed above for nonenzymic aminolysis reactions to the mechanism of action of chymotrypsin, in order to illustrate some of the points that deserve consideration in enzymic reactions of this kind.

A typical mechanism for the initial steps of the hydrolysis of an amide catalyzed by chymotrypsin is shown in the k_{-O} and k_N steps of eq 8. According to



(8)

this mechanism, imidazole acts as a general base to assist the attack of the serine hydroxyl group (k_{-O}), and imidazolium ion acts as a general acid to facilitate amine expulsion from the addition intermediate (k_N). In the reverse reaction, the aminolysis of the acyl-enzyme, imidazole acts as a general base to facilitate amine attack (k_{-N}) and as a general acid to assist serine expulsion (k_O). The essential aspects of the mechanism are unchanged if there is a further proton transfer to or from the carboxyl group of aspartate-102, and in the following, we will take imidazole (Im) to represent the aspartate–imidazole pair.

This mechanism is untenable in its simplest form for the reason shown in eq 8.[11,12] The basicity of the nitrogen atom in the tetrahedral intermediate T^- is expected to be similar to that of the parent amine.[9] Proton transfer from imidazolium ion to this nitrogen atom will therefore be thermodynamically favorable for most amide substrates so that the proton will jump to the nitrogen atom (k_{-H}) more rapidly than the intermediate can break down, and the expulsion of the fully

(31) L. do Amaral, K. Koehler, D. Bartenbach, T. Pletcher, and E. H. Cordes, *J. Amer. Chem. Soc.*, **89**, 3537 (1967).

(32) P. R. Wells, "Linear Free Energy Relationships," Academic Press, New York, N. Y., 1968, p 39.

(33) C. G. Swain and J. F. Brown, Jr., *J. Amer. Chem. Soc.*, **74**, 2538 (1952); B. M. Anderson, E. H. Cordes, and W. P. Jencks, *J. Biol. Chem.*, **236**, 455 (1961); J. H. Wang and L. Parker, *Proc. Nat. Acad. Sci., U. S.*, **58**, 2451 (1967); W. P. Jencks, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 1 (1971).

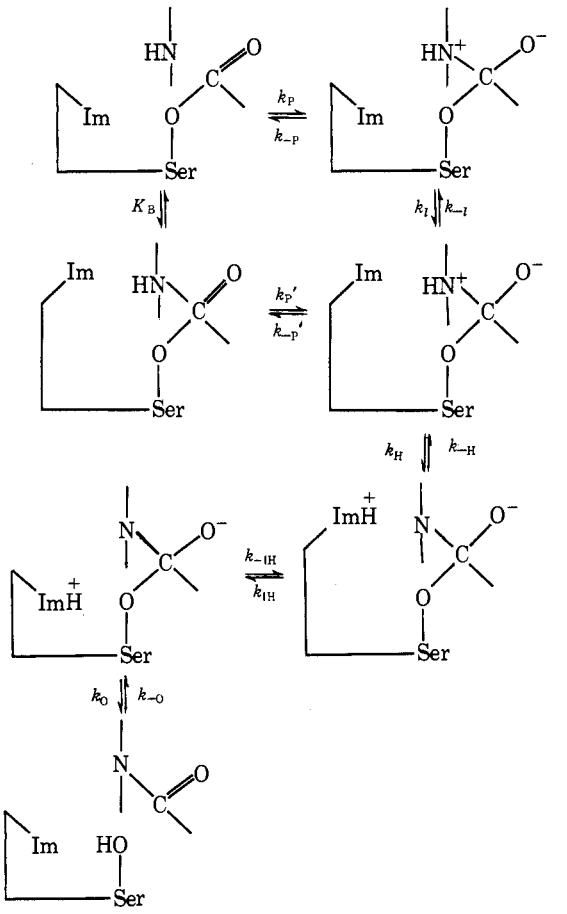
(34) L. Parker and J. H. Wang, *J. Biol. Chem.*, **243**, 3729 (1968); T. Inagami, A. Patchornik, and S. S. York, *J. Biochem. (Tokyo)*, **65**, 809 (1969).

(35) P. W. Inward and W. P. Jencks, *J. Biol. Chem.*, **240**, 1986 (1965).

protonated amine will occur through a stepwise (k_{-H} and k_{-P}, eq 8) rather than a concerted (k_N) mechanism. In the reverse, aminolysis reaction, microscopic reversibility requires that the same stepwise path must be followed, with proton transfer to imidazole (k_H) occurring *after* the formation of the addition intermediate (k_P) as in nonenzymic ester aminolysis. Although the mechanism must be stepwise rather than concerted, the catalytic role of imidazole in transferring a proton to or from nitrogen is essential in order to avoid the formation of an amine anion $>N^-$ (in the forward direction) or an N-protonated amide $-CONH_2R^+$ (in the reverse direction) as the immediate product; both of these species are too unstable to be formed as intermediates in the course of the reaction.

The aminolysis of the acyl-enzyme intermediate by aliphatic amines occurs with very little charge development on the attacking nitrogen atom in the transition state, as shown by a value of $\beta_{nuc} \sim 0.1$ for the aminolysis of furoylchymotrypsin;[35] the absence of a significant substituent effect for aliphatic amines has recently been confirmed for the specific acyl-enzyme intermediate *N*-acetyl-L-tyrosylchymotrypsin.[36] Earlier indications of a favorable effect of electron-donating substituents on the maximum velocities for the hydrolysis of substituted anilides[34] now appear to have been due to the examination of too small a series of compounds and may reflect nonproductive binding of the less reactive substrates; with a larger series of anilides or after a correction for nonproductive binding, there is little or no effect of polar substituents on the rate.[37] The small amount of charge development on the attacking amine was previously interpreted in terms of concerted general base catalysis in the transition state,[35] an interpretation which we now see is untenable in its simplest form. The experimental fact remains, and we can consider the following possible explanations for it.

(1) The mechanism of the enzymic reaction is similar to that of the analogous mechanism for general base catalysis of aminolysis in solution, except that the diffusion process in solution is replaced by movement of the imidazole group relative to the substrate at the active site of the enzyme. According to this mechanism (eq 9), the proton is transferred rapidly back and forth between the nitrogen atom of the tetrahedral intermediate and imidazole (k_H and k_{-H}) but will be located mainly on the nitrogen atom of basic amines. The rate-determining step is then the movement of the protonated imidazole away from the amine nitrogen (k_{IH}), presumably to a position at which the proton is directed toward the leaving serine oxygen atom in order to permit the reaction to proceed further. Since the proton is on the imidazole in the rate-determining step, the nitrogen atom will be uncharged, in agreement with the observed absence of a significant effect of polar substituents on the rate. Although this mechanism represents a rate-determining "conformation change," the change is only a small shift in the relative positions of atoms on the substrate and catalyst so that proton transfer may occur. It is reasonable to expect that such small changes will be rate determining for some enzymic reactions, and X-ray structural analysis of the trypsin–



(9)

trypsin inhibitor complex suggests that the preferred conformation of the imidazole group is near the serine oxygen atom and different from the optimum position for proton transfer to or from the nitrogen atom of the addition intermediate.[38]

For the relatively simple case in which the addition intermediates are present at low concentration and the isomerization steps are rate determining, the observed initial rate is described by eq 10, in which the subscripts

$$k = \left(\frac{1}{1 + K_B}\right)\frac{k_I k_{IH} K_P K_H}{k_{-I} + k_{IH} K_H} \qquad (10)$$

refer to the steps identified in eq 9, $K_P = k_P/k_{-P}$, and $K_H = k_H/k_{-H}$. Equation 10 is identical with that for a simple proton-transfer reaction, except that the k_I and k_{IH} steps refer to the intramolecular movement of the imidazole or imidazolium ion between two positions relative to the substrate, instead of to the formation or separation of encounter pairs in solution.[21] If the rate of movement of the imidazole group is independent of its state of protonation so that $k_{IH} = k_{-I}$, k_I will become rate determining when K_H becomes >1.0, *i.e.*, when the attacking base is weak and the pK_a of $T^{\pm}$ is less than that of imidazolium ion. With amines of progressively decreasing basicity, this will result in a break in a plot of log k against pK close to the position at which $\Delta pK = 0$, just as in the case of proton-transfer reactions in solution.[21] If the pK of the imidazolium ion is 7.0, and the pK_a of $T^{\pm}$ formed from serine is 1 unit above that of the parent amine, the break should occur with amines of $pK \sim 6$.

(36) B. Zeeberg and M. Caplow, *J. Biol. Chem.*, 248, 5887 (1973).
(37) J. Fastrez and A. R. Fersht, *Biochemistry*, 12, 1067 (1973); M. Philipp, R. M. Pollack, and M. L. Bender, *Proc. Nat. Acad. Sci. U. S.*, 70, 517 (1973).
(38) A. Rühlmann, D. Kukla, P. Schwager, K. Bartels, and R. Huber, *J. Mol. Biol.*, 77, 417 (1973).

It is probable, however, that the breakdown of the addition intermediate $T^{\pm}$ with amine expulsion will be faster than the isomerization step so that $k'_{-P} > k_{-I}$. When this is the case, the reaction will proceed preferentially through the $k'_P - k'_{-P}$ pathway, and the rate is described by eq 11. The break will then occur

$$k = \left(\frac{K_B}{1 + K_B}\right)\frac{k'_P k_{IH} K_H}{k'_{-P} + k_{IH} K_H} \qquad (11)$$

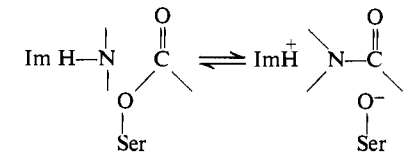
when $k'_{-P} = k_{IH}K_H$, *i.e.*, when $K_H = k'_{-P}/k_{IH}$. The reaction is then asymmetrical, and if $k'_{-P} = 10^2 k_{IH}$, for example, the break will occur 2 pK units below the point at which $\Delta pK = 0$. The rate-determining step with weak bases will then be the attack of amine on the acyl-serine, k'_P. This situation is analogous to a "pre-association mechanism" and has been described in more detail elsewhere.[19]

If the proton-transfer step itself ($k_H - k_{-H}$) becomes kinetically significant, it will result in a decrease in the sharpness of the break, as in the case of simple proton transfer reactions in solution.[21] However, this is less likely in the enzymic reaction, because the isomerization steps are probably slower than the rate of separation of encounter pairs in solution (*ca.* 10^{11} sec^{-1}) so that proton transfer in the favorable direction is more likely to be fast and kinetically insignificant.

Although there is strong evidence[35,36] that the rate of reactions of amines with acyl-chymotrypsins is essentially independent of amine basicity for amines of p$K >$ 6, the experimental data for less basic amines are inconclusive. Two studies[36,39] agree that the rates of reaction of substituted anilines increase with increasing basicity, with $\beta_{nuc} = 0.4$–0.5. If the rate of reaction of substituted anilides with chymotrypsin, in the reverse direction, is actually independent of substituents,[37] this corresponds to a value of $\beta_{nuc} = 0.6$ in the forward direction since the equilibrium constant for anilide formation increases with increasing aniline basicity[40] with $\beta_{eq} = 0.6$. A value of $\beta_{nuc} = 0.5$ is probably reasonable for rate-determining attack on a serine ester, k'_P.

(2) Concerted general acid catalysis by imidazolium ion of the cleavage of the C–O bond to serine (k_O) or, in the other direction, concerted base catalysis of serine attack on the amide (k_{-O}) is rate determining. In contrast to the situation with amines, concerted general acid–base catalysis of oxygen expulsion and attack is permitted, because a thermodynamically unfavorable proton transfer (ImH^+ to Ser–O–C) becomes favorable when the C–O bond is cleaved (ImH^+ to Ser–O^-).[11] This mechanism does not require a large development of positive charge on the nitrogen atom in the transition state. The driving force for the catalysis is the avoidance of the formation of an O-protonated serine ester or serine alkoxide ion that would have to be formed as an intermediate in the absence of concerted catalysis. This assignment is essentially the same as that for the non-enzymic aminolysis of most aliphatic esters near neutrality, in which the expulsion of alcohol from the addition intermediate is rate determining.[41] This expulsion is known to be subject to general acid catalysis.[3] It is possible that an earlier step may become rate determining with weakly basic amines.

(3) The overall reaction occurs by a fully concerted mechanism without the formation of a tetrahedral-addition intermediate so that concerted general base catalysis to avoid the formation of an N-protonated amide product is permitted (eq 12). This requires that



(12)

the addition "intermediate" be so unstable that it has no significant lifetime ($<10^{-13}$ sec). There is evidence that a stepwise mechanism is converted to a concerted mechanism of catalysis in an analogous solution reaction, when the intermediate is destabilized.[19] However, the addition intermediates in ester aminolysis have a significant lifetime in solution reactions and may be stabilized further on the enzyme, if the active site is complementary to this structure[38] so that this mechanism appears unlikely. The mechanism has the additional disadvantage that it requires the formation of the serine alkoxide ion as an intermediate.

(4) The proton is effectively in a single-potential well so that there is no significant free energy of activation for proton transfer; *i.e.*, the proton transfers denoted by k_H and k_{-H} take place so rapidly that the two species $Im \cdot T^{\pm}$ and $ImH^+ \cdot T^-$ have no separate existence. The geometry of the active site may cause compression of the $[Im \cdot H \cdot N]^+$ system so that the hydrogen-bond distance is shortened, and the barrier for proton transfer is reduced. There is evidence that the lowest energy transition state for proton transfer between nitrogen atoms in solution involves motion of the heavy atoms toward each other to decrease the barrier for transfer,[42] and it is reasonable that enzymes should facilitate proton transfers by this mechanism. The decrease in the vibration frequency of the bond to the proton in a broad single-potential well will result in a loss of zero-point energy and a deuterium isotope effect on the observed rate.

In the chymotrypsin reaction, it is not likely that there is much transfer of the proton or charge in the transition state for this mechanism. If the attacking amine undergoes a change in acidity from pK 30 to 10 upon forming $T^{\pm}$, it will be only weakly acidic in the transition state so that there will be little driving force for proton transfer to imidazole (pK 7), and the proton will remain close to the amine nitrogen atom. To the extent that it may be significant, this mechanism will be facilitated by the "charge-relay system."[43]

(5) The rate-determining step involves the attack of amine on the ester. This mechanism would require

(39) A. R. Fersht, D. M. Blow, and J. Fastrez, *Biochemistry*, 12, 2035 (1973).

(40) W. P. Jencks, B. Schaffahusen, K. Tornheim, and H. White, *J. Amer. Chem. Soc.*, **93**, 3917 (1971).

(41) Alcohol expulsion is rate determining near neutrality for methyl formate,[3] ethyl acetate, methoxyethyl acetate, and probably 2-chloroethyl acetate. It has been shown by Blackburn and Dodds that, for a substituted serine ester of formate, the k_b step (eq 5) is rate determining at physiological pH,[16] but in the enzymic reaction it is possible that the proton-transfer reaction to a properly located imidazole group is more rapid than to a base in dilute solution so that a later step may be rate determining.

(42) J. J. Delpeuch, G. Serratrice, A. Strich, and A. Veillard, *J. Chem. Soc., Chem. Commun.*, 817 (1972); P. Merlet, S. D. Peyerimhoff, and R. J. Buenker, *J. Amer. Chem. Soc.*, **94**, 8301 (1972).

(43) D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature* (*London*), 221, 337 (1969).

an early transition state with little development of positive charge on nitrogen. It involves a different rate-determining step than the reaction in solution, which proceeds through a rapid and reversible addition of the amine.

(6) There is a rate-determining conformation change of the acyl-enzyme with a molecule of amine bound at the active site. It is difficult to explain the large differences in the reaction rates of specific and nonspecific substrates by this mechanism.

The formation and aminolysis of the acyl-enzyme intermediate exhibit a solvent deuterium isotope effect k_{H_2O}/k_{D_2O} of about 2–3 in the reactions of amines with *N*-acetyl-L-tyrosylchymotrypsin and in the hydrolysis of anilides.[34,36] A pH-dependent isotope effect has also been reported for the hydrolysis of *N*-acetyl-L-tryptophanamide.[44] If these isotope effects represent loss of the zero-point energy of the N–H proton in the transition state rather than a secondary isotope effect (*e.g.*, on the structure and catalytic activity of the protein), they serve to exclude Mechanisms 1, 5, and 6. A small, pH-dependent nitrogen isotope effect ($^{14}N/^{15}N$ = 1.006–1.010) has been observed for the hydrolysis of *N*-acetyltryptophanamide.[45] This result may be interpreted as evidence for rate-determining C–N bond cleavage in the transition state[45] or for a decrease in the C–N bond order upon conversion of the amide to the addition intermediate. A significant heavy-atom isotope effect has been observed upon formation of the addition intermediate in three reactions of methyl formate-*methoxyl*-^{18}O in which expulsion of alcohol is not occurring in the rate-determining step,[5] and the hydrolysis of *N*-benzoyl-L-argininamide by papain, in which C–N bond cleavage is presumably fully rate determining, exhibits a larger nitrogen isotope effect ($^{14}N/^{15}N$ = 1.022) and a small solvent deuterium isotope effect (k_{H_2O}/k_{D_2O} = 1.35).[46]

In our opinion the available data are insufficient to distinguish among Mechanisms 1–6 or other possible mechanisms for chymotrypsin at the present time. However, enough is now known about the mechanisms of nonenzymic reactions to indicate that mechanisms of this kind and their experimental implications deserve serious consideration with respect to the mechanism of catalysis by chymotrypsin and by other enzymes. We venture to express a slight preference for mechanisms 1 and 2 at this time.

The absence of an effect of polar substituents on the rate of reaction of alcohols with furoylchymotrypsin is consistent with a mechanism involving concerted general base catalysis of alcohol attack, as suggested previously.[35] The result is inconsistent or difficult to explain with mechanisms for alcohol attack analogous to 1, 5, and 6, but is not inconsistent with Mechanisms 2, 3 and 4.

Acknowledgment. We are grateful to Alan Fersht for helpful discussions.

(44) M. L. Bender, G. E. Clement, F. J. Kézdy, and H. D'A. Heck, *J. Amer. Chem. Soc.*, **86**, 3680 (1964).

(45) M. H. O'Leary and M. D. Kluetz, *J. Amer. Chem. Soc.*, 92, 6089 (1970); M. H. O'Leary and M. D. Kluetz, *ibid.*, **94**, 3585 (1972).

(46) M. H. O'Leary and M. D. Kluetz, *J. Amer. Chem. Soc.*, **94**, 665 (1972); J. R. Whitaker and M. L. Bender, *ibid.*, **87**, 2728 (1965).

The Mechanism of Partitioning of the Intermediates Formed in the Hydrolysis of Phenyl Imidates[1]

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Abstract: The observed rate constants for the reactions of methylamine and dimethylamine with *p*-tolyl acetate remain constant over a range of pH in which a partial change in rate-determining step may be predicted from the observed change of the % ester/% amide product ratio in the hydrolysis of the corresponding *p*-tolyl imidates. Brønsted plots for general acid catalysis of ester formation from these imidates are curved, consistent with rate-determining proton transfer in the product-determining step. These results show that different, nonequilibrated ionic forms of the addition intermediate are formed in the aminolysis of the ester and in the hydrolysis of the corresponding imidate. Three predictions required by a proposed mechanism for imidate hydrolysis are supported experimentally: (1) the pH- and buffer-independent product ratio remains constant upon substitution of electron-withdrawing substituents into the phenyl group; (2) the decrease in ester yield with increasing pH is correlated with the fraction of the imidate that reacts with hydroxide ion; (3) the observed rate constants for general acid catalysis of ester formation remain almost constant as the buffer-independent ester yield decreases with increasing pH.

As discussed in the previous paper[2] and elsewhere,[3–6] an understanding of the mechanism of breakdown to ester and to amide of the tetrahedral intermediate that is formed during the hydrolysis of imidates is essential for an understanding of the mechanism of ester aminolysis. The mechanism we have proposed for ester aminolysis requires that under certain conditions

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(2) A. C. Satterthwait and W. P. Jencks, *J. Amer. Chem. Soc.*, **96**, 7018 (1974).

(3) R. B. Martin, R. I. Hedrick, and A. Parcell, *J. Org. Chem.*, **29**, 3197 (1964); G. L. Schmir, *J. Amer. Chem. Soc.*, **90**, 3478 (1968); A. C. Satterthwait, Ph.D. Thesis, Brandeis University, 1973.

(4) M. Kandel and E. H. Cordes, *J. Org. Chem.*, **32**, 3061 (1967).

(5) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968).

(6) G. M. Blackburn and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 2638 (1968).