

[CONTRIBUTION NO. 87 FROM THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

General Base Catalysis of Ester Hydrolysis<sup>1</sup>

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RECEIVED OCTOBER 24, 1960

The hydrolysis of esters activated in the acyl portion, including the ethyl chloroacetates, ethyl difluoroacetate, ethyl oxamide and glycine ethyl ester hydrochloride, is catalyzed by general bases. This catalysis follows the Brønsted catalysis law with a slope of 0.47, for ethyl dichloroacetate, is decreased two- to threefold in deuterium oxide solution, is not a function of the nucleophilic reactivity of the catalyzing base and, in the case of aniline, does not result in acylation of the catalyzing base. In contrast to esters activated in the alcohol portion, which are subject to nucleophilic catalysis by bases, it therefore appears that esters activated in the acyl portion are subject to classical general base-catalyzed hydrolysis. The rate of neutral, "uncatalyzed" hydrolysis is also two- to threefold decreased in deuterium oxide solution and water falls on the same Brønsted plot as other general bases, suggesting that the neutral hydrolysis of these esters involves water as a proton transferring agent as well as a nucleophilic reagent.

It has not been clear whether ester hydrolysis is subject to classical general acid-base catalysis. Early studies, particularly those of Dawson and co-workers, suggested the existence of such catalysis in ethyl acetate hydrolysis<sup>2-4</sup> and Hinshelwood reported general acid catalysis of the esterification of a variety of acids in alcohol solution.<sup>5</sup> It has been difficult, however, to differentiate this type of catalysis from salt effects<sup>6</sup> and in at least one case, that of bisulfate ion, it has been demonstrated that the observed catalysis can be entirely accounted for by hydrogen ions.<sup>7</sup> More recently, it has been shown that the hydrolysis of esters with a good leaving group, such as the phenyl and thiol acetates, is catalyzed by bases through nucleophilic catalysis, with the intermediate formation of the acylated base.<sup>8-10</sup> Unactivated esters are not detectably subject to such catalysis and esters activated in the acyl portion have been little studied, although Brouwer, *et al.*, have reported imidazole catalysis of the hydrolysis of methyl oxalate, which was interpreted as an example of nucleophilic catalysis.<sup>10</sup>

It has been shown recently that the aminolysis of esters<sup>11,12</sup> and a number of acyl transfer reactions of acetylimidazole, including hydrolysis,<sup>13</sup> are subject to classical general base catalysis, in which the catalyst functions as a proton transferring agent in the activated complex. The hydrolysis of acetic anhydride is subject to general base catalysis by acetate ion<sup>14</sup> and De Wolfe recently has reported a small increase in the rate of N,N'-diphenylformamidine hydrolysis in the presence of aniline,

which may represent general base catalysis.<sup>15</sup> Imidazole catalysis of the hydrolysis of the ester linkage of N,O-diacetylserinamide, a model for the acylated active site of chymotrypsin, is decreased twofold in deuterium oxide solution, which suggests that, in the case of this relatively unreactive ester, imidazole is acting as a classical general base catalyst.<sup>16</sup>

Esters which are activated by electron-withdrawing substituents in the acyl rather than in the alcohol portion undergo neutral ("water") and alkaline hydrolysis at readily measurable rates in neutral or slightly acidic solutions at room temperature.<sup>17</sup> Since it is unlikely that a water molecule could displace an alkoxide ion, it appeared probable that proton transfer occurs in the course of neutral hydrolysis and that these reactions might be aided by catalysis of proton transfer. A study of the hydrolysis of such esters in the presence of increasing buffer concentrations at constant pH is reported here.

## Experimental

**Materials.**—Ethyl chloroacetate, b.p. 143.5–144°,  $n_D^{20}$  1.4222; ethyl dichloroacetate, b.p. 61–61.5° at 19 mm.,  $n_D^{20}$  1.4386; ethyl trichloroacetate, b.p. 65–65.5° at 16 mm.,  $n_D^{20}$  1.4504; ethyl difluoroacetate, b.p. 98°,  $n_D^{20}$  1.3474; pyridine, b.p. 115°,  $n_D^{20}$  1.5094; *sym*-collidine, b.p. 169°,  $n_D^{20}$  1.4984; aniline, b.p. 182–183°; 2,6-lutidine, b.p. 143°,  $n_D^{20}$  1.4973; and 4-picoline, b.p. 143°,  $n_D^{20}$  1.5048, were fractionally distilled from commercial products. Deuterium oxide, 99.8%, was obtained from the Atomic Energy Commission through the courtesy of the Department of Chemistry, Harvard University. Water and deuterium oxide were glass distilled before use. In some experiments, the addition of small amounts of reagents which had not been pre-equilibrated caused slight dilution, but in all experiments carried out in deuterium oxide solution, the final concentration of deuterium oxide was over 99%.

**Rate Measurements.**—The kinetic procedures employed were essentially the same as those previously reported.<sup>12</sup> Reactions were generally carried out in a 5–10-ml. volume in glass-stoppered tubes with a small air space. Controls were run frequently in sealed tubes to ensure that ester was not being lost by evaporation during the removal of aliquots. Reactions were started by adding a small amount of a concentrated solution of ester in water or alcohol to give a 0.01–0.02 M solution and were followed by 6–10 analyses of residual ester, determined by incubation of aliquots with hydroxylamine and measurement at 540 m $\mu$  of the red color formed on the addition of FeCl<sub>3</sub>.<sup>15</sup> For runs in formate buf-

(1) Supported by grants from the National Science Foundation and the National Cancer Institute of the National Institutes of Health (C-3975).

(2) (a) H. M. Dawson and W. Lowson, *J. Chem. Soc.*, 393 (1929);

(b) H. M. Dawson and J. E. Smith, *J. Chem. Soc.*, 79 (1930).

(3) H. S. Taylor, *J. Am. Chem. Soc.*, **37**, 551 (1915).

(4) H. Goldschmidt, *Trans. Faraday Soc.*, **24**, 662 (1928).

(5) (a) A. C. Rolfe and C. N. Hinshelwood, *ibid.*, **30**, 935 (1934);

(b) C. N. Hinshelwood and A. R. Legard, *J. Chem. Soc.*, 587 (1935).

(6) R. P. Bell, "Acid-Base Catalysis," Oxford University Press, London, 1941.

(7) D. W. Barnum and G. Gorin, *J. Phys. Chem.*, **58**, 1169 (1954).

(8) (a) M. L. Bender and B. W. Turnquest, *J. Am. Chem. Soc.*, **79**, 1652, 1656 (1957); (b) M. L. Bender and W. A. Glasson, *ibid.*, **81**, 1590 (1959).

(9) T. C. Bruice and G. L. Schmir, *ibid.*, **79**, 1663 (1957).

(10) D. M. Brouwer, M. J. v.d. Vlugt and E. Havinga, *Koninkl. Nederl. Akad.*, **B60**, 275 (1957).

(11) J. F. Bunnett and G. T. Davis, *J. Am. Chem. Soc.*, **82**, 665 (1960).

(12) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).

(13) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).

(14) M. Kilpatrick, Jr., *J. Am. Chem. Soc.*, **50**, 2891 (1928).

(15) R. H. DeWolfe, *ibid.*, **82**, 1585 (1960).

(16) B. Anderson, E. Cordes and W. P. Jencks, *J. Biol. Chem.*, **236**, Feb. (1961).

(17) R. Skrabal, *Monatsh.*, **71**, 298 (1938).

(18) (a) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945); (b) S. Hestrin, *ibid.*, **180**, 249 (1949).

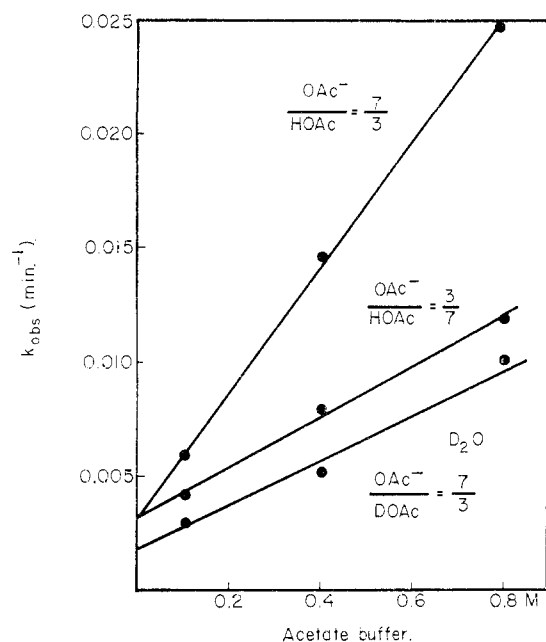


Fig. 1.—Effect of acetate buffer concentration on the rate of hydrolysis of ethyl difluoroacetate in water and in deuterium oxide solution.

fers the ester was added in acetonitrile instead of ethanol, in order to avoid the formation of ethyl formate. Constant ionic strength was maintained by the addition of potassium or sodium chloride.

Because of variations in the amount and stability of color obtained with the hydroxylamine- $\text{FeCl}_3$  assay with different esters, it was necessary to modify and standardize the procedure carefully for each ester. In each case, the sample was added to 1 ml. of freshly prepared hydroxylamine mix, incubated, water was added to 2.0 ml. and 4 ml. of  $\text{FeCl}_3$  solution was added. The tubes were read at 540  $m\mu$  at a constant time, usually about 5 minutes, after addition of  $\text{FeCl}_3$ . For ethyl chloroacetate and glycine ethyl ester the mix consisted of 5 vol. 3.5  $M$   $\text{NaOH}$ , 4 vol. 4.0  $M$   $\text{NH}_2\text{OH}\cdot\text{HCl}$  and 1 vol. water; incubation was carried out for 2 min., and 10%  $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$  in 0.3  $M$   $\text{HCl}$  was added. For ethyl dichloroacetate and ethyl oxamide the mix contained 1.1 vol. 3.5  $M$   $\text{NaOH}$ , 1.0 vol. 4.0  $M$   $\text{NH}_2\text{OH}\cdot\text{HCl}$  and 0.4 vol. 2  $M$  tris-(hydroxymethyl)-amino-methane (final  $\text{pH}$  about 9.0); incubation was carried out for 5 min., and 20%  $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$  in 0.3  $M$   $\text{HCl}$  was added. For ethyl trichloroacetate and ethyl difluoroacetate the mix contained equal volumes of 3.5  $M$   $\text{NaOH}$  and 4.0  $M$   $\text{NH}_2\text{OH}\cdot\text{HCl}$ ; incubation was carried out for 20 and 10 minutes, respectively, and 20%  $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$  in 0.3  $M$   $\text{HCl}$  was added. For phenyl acetate in the presence of concentrated phenol, 0.2 ml. of sample was added to 0.5 ml. of a mix containing equal parts of 3.5  $M$   $\text{NaOH}$  and 4.0  $M$   $\text{NH}_2\text{OH}\cdot\text{HCl}$ ; incubation was carried out for 10 minutes, the volume was made up to 2.5 ml. and 1.0 ml. of 20%  $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$  in 2.5  $M$   $\text{HCl}$  was added.

Second-order rate constants were obtained from the slopes of plots of observed pseudo first-order rate constants against the concentration of the second reactant or catalyst; and the rates of uncatalyzed (or solvent-catalyzed) hydrolysis were obtained from the intercepts. The individual rate constants are accurate to within approximately  $\pm 5\%$ , while derived rate constants are subject to a correspondingly greater error. A representative example is given in Fig. 1. The rates of hydroxide ion hydrolysis were estimated from the intercepts of plots of rate constants against buffer concentration from experiments carried out in buffers near  $\text{pH}$  7; at this  $\text{pH}$  the neutral (water) reaction makes only a small contribution to the rate. The values for hydroxide ion hydrolysis, while probably at least as accurate as previously reported values, can be regarded as no more than approximations, because of uncertainties in the extrapolation and interpretation of highly  $\text{pH}$ -dependent rates to zero buffer concentration.

**Analysis of Products.**—The solvolyses of 0.2  $M$  ester solutions were carried out in 0.5  $M$  imidazole or aniline buffers in deuterium oxide solution (ethyl trichloroacetate was run in 0.2  $M$  sodium acetate in 40% dioxane-deuterium oxide). The products were analyzed by infrared spectroscopy in calcium fluoride cells with a Perkin-Elmer model 21 double beam spectrophotometer. The asymmetric and symmetric carboxylate absorption bands of the products, given in Table I, agree with those of authentic carboxylate salts in deuterium oxide solution to within 3  $\text{cm.}^{-1}$ .

TABLE I  
INFRARED SPECTRA IN  $\text{D}_2\text{O}$  OF REACTION PRODUCTS FROM BUFFER-CATALYZED ESTER HYDROLYSIS

Ethyl ester, 0.2 $M$	Buffer	Assym. $\text{COO}^-$ , $\text{cm.}^{-1}$	Sym. $\text{COO}^-$ , $\text{cm.}^{-1}$
Chloroacetate	Imidazole	1600	1394
Dichloroacetate	Imidazole	1630	1373
	Aniline	1633	1377
Trichloroacetate <sup>a</sup>	Acetate	1676	1329
Difluoroacetate	Imidazole	1640	1322
Oxamide	Imidazole	1630	1342
		Amide bands: 1660, 1440	

<sup>a</sup> 40% dioxane in  $\text{D}_2\text{O}$ ; also acetate peak at 1560  $\text{cm.}^{-1}$ .

Aliquots of reaction mixtures in which 0.02  $M$  solutions of ethyl difluoroacetate and ethyl dichloroacetate in acetate buffer and 0.01  $M$  ethyl difluoroacetate in imidazole buffer had undergone solvolysis were analyzed for the presence of free fluoride and chloride ions by the addition of calcium chloride and silver nitrate, respectively. No indication of the presence of these ions was found under conditions in which 0.001  $M$  fluoride or chloride was readily detectable.

Aliquots of the reaction mixtures from the reaction of ethyl dichloroacetate with aniline were analyzed for dichloroacetanilide by the neutral hydroxylamine method of Katz, Lieberman and Barker,<sup>19</sup> with the time of heating extended to 60 minutes. No dichloroacetanilide was found as a reaction product, although added dichloroacetanilide was recovered quantitatively from reaction mixtures and was shown to be stable under the conditions of the experiment. A similar assay, but with only 10 minutes, heating, of the reaction mixtures from the reaction of ethyl chloroacetate with ammonia indicated that approximately 50% of the increased rate of ester disappearance in the presence of ammonia could be accounted for as dichloroacetamide. Similarly, assay of the reaction mixtures from the reaction of ethyl dichloroacetate with tris-(hydroxymethyl)-amino-methane, using the 5:4:1 hydroxylamine mix described above, indicated that the principal product of this reaction is the acylated base, rather than dichloroacetate ion.

Bunton and Hadwick have shown with experiments in  $\text{H}_2\text{O}^{18}$  enriched water that the neutral hydrolysis of ethyl trifluoroacetate involves acyl-oxygen fission.<sup>20</sup>

## Results

The rates of hydrolysis of ethyl difluoroacetate at  $\text{pH}$  4.25 and 4.93 in a series of acetate buffers of constant buffer ratio and increasing buffer concentration are shown in Fig. 1. The rates increase with increasing buffer concentration and increase more rapidly at the higher  $\text{pH}$ , indicating that the hydrolysis is catalyzed by acetate ion. The rate of uncatalyzed hydrolysis in this  $\text{pH}$  range, obtained by extrapolation to zero buffer concentration, is independent of  $\text{pH}$  and is therefore a neutral ("water") reaction. In deuterium oxide solution both the acetate-catalyzed hydrolysis, from the slope of the line, and the neutral hydrolysis, from the intercept, are decreased approximately two-fold.

(19) J. Katz, I. Lieberman and H. A. Barker, *J. Biol. Chem.*, **200**, 417 (1953).

(20) C. A. Bunton and T. Hadwick, *J. Chem. Soc.*, 3248 (1958).

TABLE II  
 RATES OF ESTER HYDROLYSIS AT VARYING BUFFER CONCENTRATIONS AT IONIC STRENGTH 1.0 AND 25°

Buffer, <i>M</i>	Buffer ratio A <sup>-</sup> /HA	pH	No. detns.	$k_0$ , <sup>a</sup> min. <sup>-1</sup>	$k_2$ , <sup>b</sup> l. mole <sup>-1</sup> min. <sup>-1</sup>
Ethyl difluoroacetate					
Aniline, <sup>c,d</sup> 0.2-0.8	3/7	4.22	4	0.0032	0.0027
Same, 0.1-0.4	6/4	4.78	4	.0032	.0056
K acetate, <sup>e</sup> 0.1-0.8	3/7	4.25	3	.0034	.011
Same	7/3	4.93	3	.0034	.027
Same, in D <sub>2</sub> O	7/3		3	.0016	.010
Imidazole, <sup>e</sup> 0.1-0.4	4/6	7.00	4	.033	.246
Same	1/1	7.26	4	.038	.303
Same	7/3	7.52	4	.080	.438
Same, in D <sub>2</sub> O	1/1		4	.020	.11
Ethyl dichloroacetate					
Na formate, <sup>f</sup> 0.2-0.8	1/1	3.28	4	0.00034	0.00094
Same	8/2	3.96	4	.00034	.00159
Aniline, <sup>g</sup> 0.2-0.8	3/7	4.29	4	.00030	.00025
Same, 0.1-0.2	6/4	4.82	2	.00030	.00066
K acetate, <sup>g</sup> 0.1-0.8	3/7	4.26	3	.00027	.00089
Same	7/3	4.95	3	.00030	.0022
Same, in D <sub>2</sub> O	7/3		3	~ .00006	.00096
Pyridine, <sup>g</sup> 0.2-0.8	1/1	5.37	4	.0004	.0058
Na succinate, <sup>h</sup> 0.1-0.4	4/6	5.00	4	.00030	.0061
Same	7/3	5.42	4	.00030	.0084
4-Picoline, <sup>e</sup> 0.2-0.8	4/6	5.9	4	.002	.0069
Same	7/3	6.5	4	.003	.0123
K phosphate, <sup>i,j</sup> 0.2-0.8	4/6	6.3	3	...	.015
Same	7/3	7.0	3	...	.033
Same <sup>g,k</sup>	4/6	6.4	4	.006	.17
Same	7/3	7.1	4	.009	.32
Imidazole, <sup>e</sup> 0.2-0.8	4/6	6.99	3	.0057	.033
Same, in D <sub>2</sub> O	4/6		3	.0032	.011
Tris-(hydroxymethyl)-aminomethane, <sup>e</sup> 0.2-0.8	3/7	8.00	4	.035	.189
Same	6/4	8.52	4	.095	.475
Ethyl chloroacetate					
K acetate, <sup>e</sup> 0.2-0.8	3/7	4.28	4	$7 \times 10^{-6}$	$1.4 \times 10^{-6}$
Same	7/3	4.95	4	$6.5 \times 10^{-6}$	$3.1 \times 10^{-6}$
Imidazole, <sup>e</sup> 0.2-0.8	4/6	7.00	4	0.00025	0.00098
Same	7/3	7.50	4	.00087	.00150
Same, in D <sub>2</sub> O	7/3		4	.00053	.00049
Ammonia, <sup>e</sup> 0.2-0.8	1/3	8.8	4	.014	.037
Same	1/1	9.4	4	.048	.107
Ethyl trichloroacetate <sup>l</sup>					
K acetate, 0.1-0.7	3/7	4.9	4	0.0016	0.0036
Same	7/3	5.7	4	0.0018	0.0090
Ethyl oxamide					
Imidazole, 0.2-0.8	1/1	7.2	4	0.0035	0.0073
Glycine ethyl ester hydrochloride					
Imidazole, 0.2-0.8	1/1	7.2	4	0.00016 <sup>m</sup>	0.00019 <sup>m</sup>

<sup>a</sup> Rate extrapolated to zero buffer concentration. <sup>b</sup>  $(k_{obs} - k_0)/[\text{total buffer}]$ . <sup>c</sup> In 1% ethanol. <sup>d</sup> Ionic strength 0.4. <sup>e</sup> In 2% ethanol. <sup>f</sup> In 1% acetonitrile. <sup>g</sup> In 4% ethanol. <sup>h</sup> Ionic strength 2.2. <sup>i</sup> In 5% ethanol. <sup>j</sup> Ionic strength 2.0. <sup>k</sup> No added salt. <sup>l</sup> In 40% ethanol; pH values uncorrected. <sup>m</sup> Observed values  $\times 1.26$  to correct for dissociation of the hydrochloride ( $pK_a$  7.75).

The results of similar experiments with ethyl difluoroacetate in imidazole and aniline buffers and with ethyl dichloroacetate, ethyl chloroacetate, ethyl trichloroacetate (in 40% ethanol), ethyl oxamide and glycine ethyl ester hydrochloride in the presence of one or more catalysts are given in Table II. The first-order rate constants for neutral hydrolysis, the second-order rate constants for catalysis by the catalytically active ionic species

of the buffers and the ratios of the rate constants in water and deuterium oxide are summarized in Table III. Catalysis by buffer was found in every case, and in all instances in which the pH dependence of the catalysis was investigated only the basic form of the catalyst was found to be active. The rates of neutral and base-catalyzed hydrolysis are two- to threefold lower in deuterium oxide solution.

TABLE III  
 SUMMARY OF RATE CONSTANTS FOR CATALYSIS OF ESTER HYDROLYSIS AT 25° AND IONIC STRENGTH 1.0

Ester	Reactant	$k_0$ , min. <sup>-1</sup>	$k_2^a$ , l. mole <sup>-1</sup> min. <sup>-1</sup>	$k_{H_2O}/k_{D_2O}$
Ethyl difluoroacetate	Water	0.0034	$6.1 \times 10^{-5}$	2.1
	D <sub>2</sub> O	0.0016	$2.9 \times 10^{-5}$	
	Aniline		0.0092	
	K acetate		.037	2.7
	In D <sub>2</sub> O		.014	
	Imidazole		.62	2.8
	In D <sub>2</sub> O		.22	
Ethyl dichloroacetate	OH <sup>-</sup>		$2.7 \times 10^{5b}$	
	Water	0.0030	$5.3 \times 10^{-6}$	5 <sup>b</sup>
	D <sub>2</sub> O	0.0006 <sup>c</sup>	$1.1 \times 10^{-6}$	
	Na formate		0.0019	
	Aniline		.0010	
	K acetate		.0030	2.2
	In D <sub>2</sub> O	.0014		
	Pyridine		.012	
	Na succinate <sup>e</sup>		.014	
	4-Picoline		.017	
	K phosphate <sup>d</sup>		.044	
	Imidazole		.082	3.0
	In D <sub>2</sub> O		.027	
Tris-(hydroxymethyl)-aminomethane <sup>f</sup>		.71		
Ethyl chloroacetate	OH <sup>-</sup>		$5.3 \times 10^{4b}$	
	Water	$6.5 \times 10^{-6}$	$1.2 \times 10^{-7}$	
	K acetate		$4.4 \times 10^{-6}$	
	Imidazole		0.0022	3.0
	In D <sub>2</sub> O		.0007	
	Ammonia <sup>f</sup>		.18	
Ethyl trichloroacetate <sup>e</sup>	OH <sup>-</sup>		$2.2 \times 10^{3b}$	
	Solvent <sup>e</sup>	0.0017		
Ethyl oxamide	K acetate		0.013	
	Imidazole		0.015	
Glycine ethyl ester HCl	OH <sup>-</sup>		$2.5 \times 10^{4b}$	
	Imidazole		0.00038	
	OH <sup>-</sup>		1200 <sup>b</sup>	

<sup>a</sup> For the reactive ionic species of the reactant. <sup>b</sup> Approximate value. <sup>c</sup> Ionic strength 2.2. <sup>d</sup> No added salt. In 40% alcohol. <sup>e</sup> Rate constant for ester disappearance, which represents largely a nucleophilic reaction (see text).

The effects of high concentrations of salts and of urea on the rate of the neutral hydrolysis of ethyl difluoroacetate are given in Table IV. So-

 TABLE IV  
 EFFECTS OF SALTS AND UREA ON THE NEUTRAL HYDROLYSIS OF ETHYL DIFLUOROACETATE AT 25°<sup>a</sup>

	$k \times 10^3$ , min. <sup>-1</sup>		$k \times 10^3$ , min. <sup>-1</sup>
Control	4.4	KCl	4.4
NaClO <sub>4</sub>	0.8 M		3.7
	3.2 M		2.5
	7.2 M		
NaCl	1.0 M	Imidazolium	
	2.1 M	Cl	4.4
	4.7 M		4.5
			3.1
Urea	9.0 M	Guanidinium	
		Cl	1.8

<sup>a</sup> In 0.08 M potassium acetate buffer, pH 4.2, 2% ethanol.

dium perchlorate, 7.2 M, causes an 18-fold decrease in the rate; sodium chloride, potassium chloride and guanidinium chloride cause smaller decreases, while imidazolium chloride and urea cause still smaller decreases. The decreases in rate caused by salt concentrations of the order used in the buffer catalysis experiments are considerably smaller, and

are in the opposite direction, compared to the rate differences due to base catalysis, so that specific salt effects, while certainly present, probably do not cause a large error in the reported catalytic rate constants. The rate of neutral hydrolysis of ethyl difluoroacetate shows a considerable decrease in the presence of increasing concentrations of alcohol (Table V).

 TABLE V  
 EFFECT OF ETHANOL ON THE NEUTRAL HYDROLYSIS OF ETHYL DIFLUOROACETATE AT 25°<sup>a</sup>

Ethanol, <sup>b</sup> %	$k \times 10^3$ , min. <sup>-1</sup>	Ethanol, <sup>b</sup> %	$k \times 10^3$ , min. <sup>-1</sup>
3	4.6	32	1.3
7	4.2	52	0.53
12	3.6	72	0.28
22	1.8		

<sup>a</sup> In 0.08 M potassium acetate buffer, pH 4.2 in water. Vol. EtOH  $\times 100$  / [vol. EtOH + vol. H<sub>2</sub>O].

An attempt was made to demonstrate catalysis of phenyl acetate hydrolysis by increasing concentrations of phenol buffers. Although increases from 0.2 to 0.8 M of the concentrations of 30% and 60% neutralized phenol buffers caused approximately 50% increases in rate, there was also an increase in the apparent pH of the more con-

centrated buffer solutions and the ratio  $k_{\text{obs}}/[\text{OH}^-]$  showed no trend with increasing buffer concentration.

No catalysis by *sym*-collidine or 2,6-lutidine buffers could be detected. While this might be due to the decreased effectiveness of these buffers as general bases because of steric hindrance,<sup>21</sup> it may also reflect complex formation in the buffers, since the *pH* values of increasing concentrations of these buffers at constant ionic strength were not constant. Catalysis of acetyl phenyl phosphate hydrolysis by these compounds showed a leveling off at high buffer concentrations, also suggestive of complex formation.<sup>22</sup>

The rate constant for the neutral hydrolysis of ethyl dichloroacetate,  $0.00030 \text{ min.}^{-1}$ , is in reasonable agreement with the value of  $0.00033 \text{ min.}^{-1}$  reported by Salmi and Suonpää.<sup>23</sup> The corresponding methyl ester has a fourfold faster neutral hydrolysis, and several other rate constants reported here for ethyl esters may be compared to two to fourfold larger values previously reported for the corresponding methyl esters.<sup>17</sup>

No catalysis of the hydrolysis of ethyl acetate by imidazole could be detected, in confirmation of previous reports.<sup>8</sup> This may be related to the very slow neutral hydrolysis of this compound, which, if it occurs at all, has a half-time of some 89 years.<sup>24</sup> No evidence was obtained for general acid catalysis of the hydrolysis of ethyl haloacetates.

#### Discussion

**Mechanism.**—The observed general base catalysis of acyl-activated ester hydrolysis appears to represent classical general base catalysis rather than nucleophilic catalysis, because: (1) The catalyzed reaction is decreased two- to threefold in deuterium oxide solution. While the absence of such a deuterium isotope effect does not rule out general base catalysis,<sup>12,25</sup> its presence strongly suggests that a bond to hydrogen is stretched in the transition state of the catalyzed reaction and that the catalysis involves proton transfer. Nucleophilic catalysis of the hydrolysis of phenyl acetate and *p*-nitrophenyl acetate by imidazole<sup>16</sup> and of acetic anhydride by formate ion<sup>26</sup> is not significantly decreased in deuterium oxide solution. The pyridine-catalyzed hydrolysis of acetic anhydride is decreased in deuterium oxide, although it almost certainly proceeds by nucleophilic catalysis, but it is probable that the attack of pyridine is not the rate-limiting step under the experimental conditions used.<sup>26</sup> The general base-catalyzed hydrolyses of acetylimidazole,<sup>13</sup> acetic anhydride<sup>26</sup> and *N,O*-diacetylserinamide,<sup>16</sup> as well as several imidazole-catalyzed acyl transfer reactions from acetylimidazole<sup>13</sup> and the general base-catalyzed solvolysis of tetrabenzyl pyrophosphate in pro-

(21) R. P. Bell, M. H. Rand and K. M. A. Wynne-Jones, *Trans. Faraday Soc.*, **52**, 1093 (1956).

(22) Unpublished experiments. Half-maximal rate increases were observed with solutions  $0.03 \text{ M}$  and  $0.06 \text{ M}$  in respect to the free base, for collidine and lutidine, respectively.

(23) E. J. Salmi and T. Suonpää, *Ber.*, **73B**, 1126 (1940).

(24) (a) A. Skrabal and Z. Zahorka, *Monatsh.*, **53**, 562 (1929); (b) W. Poethke, *Ber.*, **68B**, 1031 (1935).

(25) S. D. Ross, M. Finkelstein and R. C. Petersen, *J. Am. Chem. Soc.*, **81**, 5336 (1959).

(26) A. R. Butler and V. Gold, *Proc. Chem. Soc.*, 15 (1960).

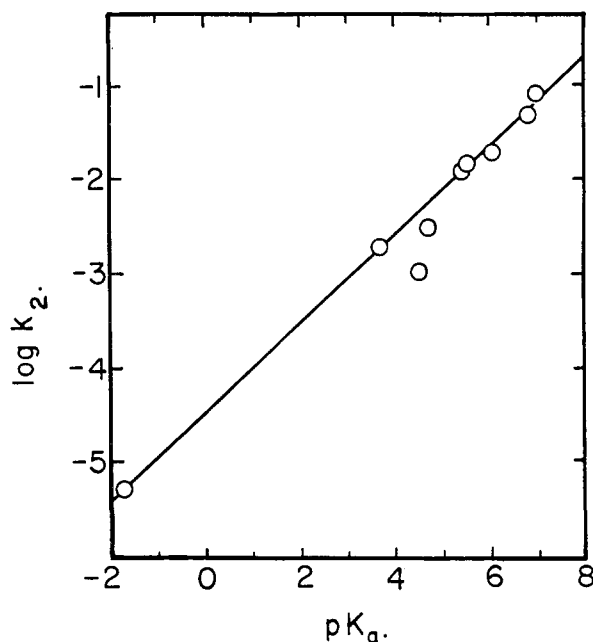


Fig. 2.—Brønsted plot of the catalytic constants for general base-catalyzed hydrolysis of ethyl dichloroacetate. No statistical corrections have been made. The bases shown are listed in Table III.

panol,<sup>27</sup> are one-half to one-third as fast in deuterium oxide.

(2) Imidazole and the phosphate dianion, which are of almost equal basicity, are almost equally effective as catalysts for the hydrolysis of ethyl dichloroacetate. In contrast, imidazole is some 4,000 times more reactive than the phosphate dianion as a nucleophilic catalyst for the hydrolysis of *p*-nitrophenyl acetate.<sup>28,29</sup> This suggests that basicity, which largely determines the efficiency of classical general base catalysts, rather than nucleophilic reactivity, determines the effectiveness of catalysts in the former reaction.

In previously reported examples of classical general base-catalyzed reactions of acyl groups, it was not possible to study the effect of the structure of the catalyzing base on the reaction rate, because the special conditions required for detection of general base catalysis allowed study of catalysis by only a single base for a given reaction.<sup>11-13</sup> The general base-catalyzed hydrolysis of acyl-activated esters is not subject to this limitation. The Brønsted plot of the catalytic rate constants for the hydrolysis of ethyl dichloroacetate has a slope of 0.47 (Fig. 2). The experimental values have been plotted without correction for statistical effects, abnormal basicity in water, etc., because of the uncertainties involved in such corrections.<sup>6</sup> Corrections would slightly improve or worsen the fit to the line, depending upon which corrections were chosen. The most marked deviation from the line is aniline which, in contrast to its faster rate in nucleophilic reactions,<sup>28</sup> is less reactive than carboxylate catalysts; this may reflect the known low

(27) G. O. Dudek and F. H. Westheimer, *J. Am. Chem. Soc.*, **81**, 2641 (1959).

(28) W. P. Jencks and J. Carriolo, *ibid.*, **82**, 1778 (1960).

(29) T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958).

efficiency of primary amines as general base catalysts.<sup>30</sup> The relationship between basicity and nucleophilic reactivity toward esters may be linear for a given class of compounds, but shows very marked deviations for different types of compounds.<sup>28,29,31</sup> In view of the widely varying structure and charge type of the bases represented in Fig. 2, which include imidazole, phosphate and succinate dianions, pyridines, aniline, acetate and formate, the fit to the line is reasonably good and provides further support for the conclusion that basicity, rather than nucleophilic reactivity, determines the effectiveness of this series of catalysts.

(3) The reaction of aniline with ethyl dichloroacetate does not give dichloroacetanilide as a product (see Experimental). The reaction with aniline, therefore, appears to represent catalysis of hydrolysis, rather than a nucleophilic reaction.

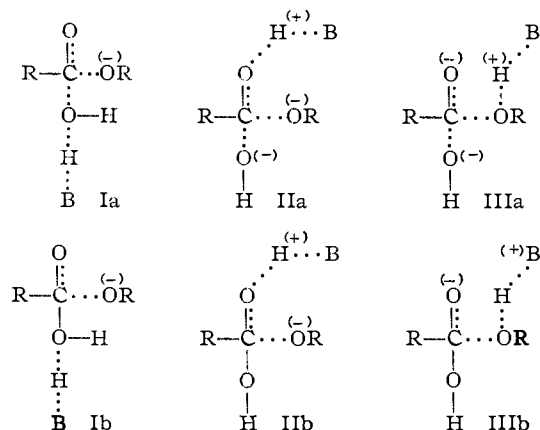
The stronger bases, ammonia and tris-(hydroxymethyl)-aminomethane, react more rapidly with ethyl chloroacetate and ethyl dichloroacetate, respectively, than would be expected from Brönsted plots based on less basic catalysts. It was found that in both of these reactions, the acyl group was transferred, at least in part, to the attacking molecule to form a stable product (see Experimental), so that these reactions represent nucleophilic reactions rather than catalysis of hydrolysis. The hydroxide ion reactions also show positive deviations from the Brönsted plots, suggesting that these also represent nucleophilic reactions, and not hydroxide ion-catalyzed attack of water.

It therefore appears that for the compounds under consideration here, there is a shift from general base catalysis to nucleophilic attack at a  $pK$  value near 7–8. The slopes of Brönsted plots for nucleophilic reactions of reagents of a given chemical class are typically in the range 0.7–0.8,<sup>12,28,29,31</sup> which is greater than that for general base-catalyzed hydrolysis. The region of  $pK$  7–8 thus appears to represent an intersection of the Brönsted lines for general base catalysis, by weaker bases, and for nucleophilic reaction, by stronger bases. Imidazole is a considerably weaker base, but is a stronger nucleophilic reagent than either ammonia or tris-(hydroxymethyl)-aminomethane toward  $p$ -nitrophenyl acetate. The importance of basicity near this region of intersection is emphasized by the fact that the weakly basic imidazole molecule reacts only relatively slowly and as a general base catalyst, while the two stronger bases react more rapidly and as nucleophilic reagents with ethyl haloacetates.

General base-catalyzed ester hydrolysis may proceed through one of the following kinetically indistinguishable transition states, which may be preceded by one or more pre-equilibrium steps. Mechanisms involving addition of the catalyst to the ester to form a tetrahedral intermediate, followed by displacement, appear to be less likely, because of the generally low reactivity of saturated, compared to carbonyl, carbon atoms in displacement reactions.

(30) R. P. Bell and A. F. Trotman-Dickenson, *J. Chem. Soc.*, 1288 (1949).

(31) M. L. Bender, *Chem. Revs.*, **60**, 53 (1960).



Structure I involves removal of a proton from the attacking water molecule by the general base and III the addition of a proton to the leaving alcohol molecule, which must follow a previous proton removal from water to preserve the correct stoichiometric composition of the transition state and  $pH$  dependence of the rate. Structure II involves proton transfer to and from the carbonyl oxygen atom to aid hydroxide addition to the ester or alkoxide expulsion from an addition intermediate. Series a and b vary only in whether a more or less fully formed bond exists between carbon and the attacking water molecule, *i.e.*, in whether or not formation of an intermediate tetrahedral addition compound<sup>32</sup> precedes the transition state. It should be noted that the reaction is symmetrical, since the entering and leaving groups are oxygen atoms of similar nature; if alcohol instead of water were the attacking reagent, the reaction would be completely symmetrical. It is, therefore, likely that catalysis aids the entering and leaving groups in a similar manner, although the failure to observe a term second order in respect to catalyst suggests that only a single type of catalysis is occurring in the transition state. The symmetry of the reaction also demands that a catalyst which is removing a proton when the reaction is going in one direction must donate a proton in the reverse direction; *i.e.*, there is no reason *a priori* to suggest that the observed general base catalysis actually represents general base catalysis rather than general acid (accompanied by a previous proton removal) catalysis.

**The Neutral ("Water") Hydrolysis.**—Extension of the Brönsted plot for general base-catalyzed ester hydrolysis (Fig. 2) to include water, based on the rate of neutral hydrolysis, reveals that water falls on the same line as other general base catalysts; *i.e.*, the neutral hydrolysis of ethyl dichloroacetate appears to be a water-catalyzed attack by water. Parallel Brönsted lines, based on the more limited available data, lead to the same conclusion for the other esters examined. This is relatively direct evidence that at least two molecules of water are required for neutral hydrolysis, one of which acts as a proton transferring agent. Such a proton transfer in the transition state offers a reasonable explanation for the two- to threefold decrease in deuterium oxide solution of the rates

(32) M. L. Bender, *J. Am. Chem. Soc.*, **73**, 1626 (1951).

of the reactions with water of these esters and of a number of other acyl compounds, including acetyl-imidazole,<sup>13</sup> acetyl-imidazolium,<sup>13</sup> acetic anhydride,<sup>26</sup> acetyl phenyl phosphate<sup>33</sup> and possibly acetylpyrazole<sup>34a</sup> and ethyl benzoate.<sup>34b,35</sup> The ratio  $k_{H_2O}/k_{D_2O}$  is 0.85 for the conjugate acid of acetamide in aqueous acid, but this may reflect a special property of strong aqueous acids, since the corresponding rate of solvolysis in methanol is decreased twofold in deuteriomethanol.<sup>36</sup> Considerably smaller solvent isotope effects are found in the solvolysis of saturated carbon compounds.<sup>37</sup>

Certain other features appear to be characteristic of the neutral ("water") hydrolysis of activated acyl compounds. The entropy of activation for the water hydrolysis of alkyl trifluoroacetates (ca. -50 e.u.),<sup>38</sup> ethyl trichloroacetate (-43 e.u.),<sup>39</sup> acetyl phenyl phosphate (-27 e.u.),<sup>33</sup> acetyl-imidazolium (-30 e.u.)<sup>13</sup> and acetic anhydride (-40 e.u.)<sup>40</sup> are unusually large and negative. The rates of neutral hydrolysis of ethyl trifluoroacetate,<sup>38</sup> ethyl difluoroacetate (Table IV) and acetic anhydride<sup>41</sup> are markedly decreased by the addition of relatively small amounts of alcohol or acetone, and Koskikallio has interpreted this in the case of acetic anhydride as evidence for participation of more than a single water molecule.<sup>41</sup> Concentrated salt solutions cause marked decreases in the rate of water hydrolysis of ethyl difluoroacetate (Table V), ethyl trifluoroacetate,<sup>20</sup> acetyl-imidazolium<sup>13</sup> and acetyl phenyl phosphate.<sup>33</sup> This is analogous to the behavior of aqueous solutions of strong acids, and may be at least partly accounted for by a decrease in the availability of free water in the presence of strong electrolytes; the  $H^0$  values of aqueous acids are known to become more negative in the presence of concentrated salts.<sup>42-44</sup> It is difficult to account for these results in terms of simple electrostatic or solvent effects, but they may be at least partially accounted for in terms of a requirement for several moles of available water, which must be properly oriented in the polar transition state.

**Effects of Ester Structure.**—Acyl compounds with a good *leaving group* are subject to nucleophilic attack, while compounds with a poor leaving group tend to be subject to general base catalysis. If the product of nucleophilic attack in the former

(33) G. DiSabato and W. P. Jencks, unpublished experiments.

(34) (a) R. Hüttel and J. Kratzer, *Ber.*, **92**, 2014 (1959); (b) A. Kailan and F. Ebeneder, *Z. physik. Chem.*, **180A**, 157 (1937); it does not appear certain that only a *pH*-independent reaction was studied in these cases.

(35) C. A. Bunton, N. Fuller, S. G. Perry and V. J. Shiner, Jr., *Chemistry & Industry*, 1130 (1960), have recently added several more acyl compounds, including ethyl trifluoroacetate ( $k_{H_2O}/k_{D_2O} = 1.8$ ), to this group.

(36) O. Reitz, *Z. physik. Chem.*, **183A**, 371 (1938).

(37) (a) R. E. Robertson and P. M. Laughton, *Can. J. Chem.*, **35**, 1319 (1957); (b) P. M. Laughton and R. E. Robertson, *ibid.*, **37**, 1491 (1959); (c) J. G. Pritchard and F. A. Long, *J. Am. Chem. Soc.*, **78**, 6008 (1956).

(38) A. Moffat and H. Hunt, *ibid.*, **81**, 2082 (1959).

(39) From the data of M. H. Palomaa, E. J. Salmi and R. Korte, *Ber.*, **72**, 790 (1939).

(40) J. Koskikallio, D. Pouli and E. Whalley, *Can. J. Chem.*, **37**, 1360 (1959).

(41) J. Koskikallio, *Acta Chem. Scand.*, **13**, 665 (1959).

(42) M. A. Paul and F. A. Long, *Chem. Revs.*, **57**, 1 (1957).

(43) J. A. Leisten, *Chemistry & Industry*, 397 (1959).

(44) J. F. Bunnett, *J. Am. Chem. Soc.*, **82**, 499 (1960).

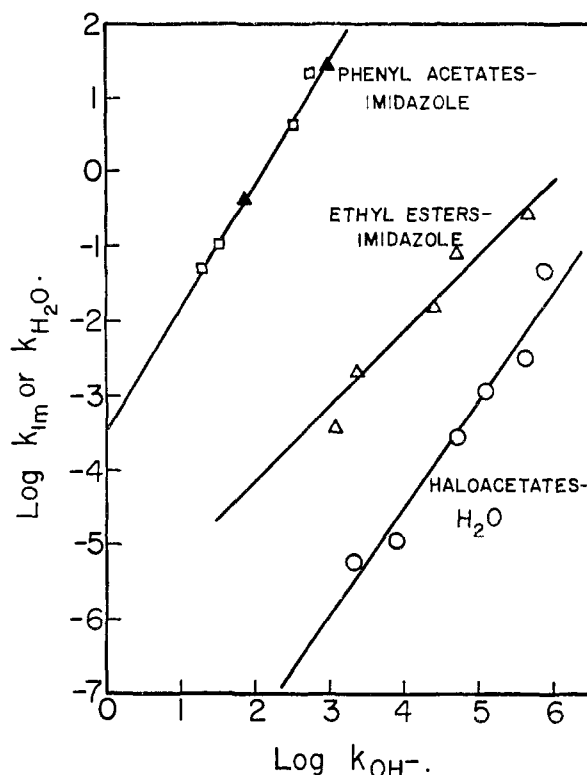


Fig. 3.—Rates of neutral or imidazole-catalyzed ester hydrolysis as a function of the rate of base hydrolysis: methyl<sup>17</sup> and ethyl haloacetate neutral hydrolysis, O; imidazole-catalyzed hydrolysis of acyl-activated ethyl esters,  $\Delta$ ; imidazole-catalyzed hydrolysis of substituted phenyl acetates in water,<sup>12</sup>  $\blacktriangle$ ; or mixed solvents,<sup>9</sup>  $\square$ .

case is unstable, the result is nucleophilic catalysis of hydrolysis, as in the case of *p*-nitrophenyl acetate and imidazole.

Electron-withdrawing substituents in the *acyl* portion of the molecule cause parallel increases in the rates of hydroxide ion,<sup>17</sup> water,<sup>17,23</sup> and general base-catalyzed hydrolysis (Fig. 3). The reaction of hydroxide ion with glycine ethyl ester hydrochloride shows a small positive deviation, presumably because of electrostatic attraction between these two oppositely charged ions. The rates of water and general base-catalyzed hydrolysis of acyl-activated esters show a closer correlation with the rates of hydroxide ion hydrolysis than with the acidity of the parent acid or  $\sigma^*$ . This presumably reflects similar steric requirements for the different hydrolysis reactions, which are not reflected in acidity or  $\sigma^*$ , and suggests the use of the saponification rate as a standard index for comparisons of the effects of polar substituents on these reactions.

In addition to the difference in mechanism, the reaction of imidazole with alcohol-activated esters, such as the phenyl acetates, differs from that with acyl-activated esters in that: (i) nucleophilic reactions with phenyl acetates are several orders of magnitude faster than the general base catalyzed reactions and (ii) the nucleophilic reactions show a greater sensitivity to structure than the general base-catalyzed reactions (Fig. 3). Bruice and Schmir have interpreted this high sensitivity to

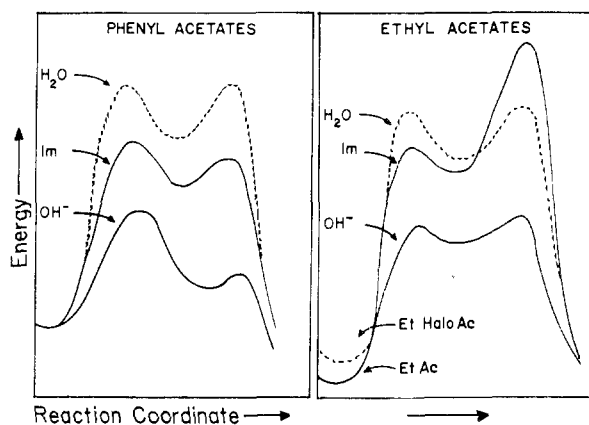


Fig. 4.—Transition state diagrams for reactions of esters with a good leaving group, such as the phenyl acetates, and with a relatively poor leaving group, such as the ethyl haloacetates. The energy barriers for the water reactions involve proton transfer as well as the making and breaking of bonds to carbon.

structure in terms of a progressive increase in the difficulty of leaving group expulsion by the weakly basic imidazole molecule.<sup>9</sup> Qualitatively, the absence of a nucleophilic reaction of imidazole with esters containing a poor leaving group may be regarded as due to the breakdown of a tetrahedral intermediate<sup>37</sup> always to expel imidazole to give starting materials, rather than  $\text{RO}^-$  to give products,<sup>45</sup> or simply to the inability of a weak base to displace a stronger base.

Quantitatively, the relationships shown in Fig. 3 require further explanation. No nucleophilic reaction of imidazole with ethyl acetate has been detected, and the rate of such a reaction, if it occurs at all, must fall far below that predicted by the line for nucleophilic reactions of imidazole shown in Fig. 3; *i.e.*, there is a sharp break in this line as the leaving group becomes worse than a substituted phenolate ion. It is of interest that this break occurs at a point at which the leaving group is considerably more basic than the attacking imidazole molecule. The results are consistent with the hypothesis that the energy barrier to the nucleo-

philic reactions reflects primarily the energy barrier to nucleophilic attack on the carbonyl group, which is altered by electron-withdrawing substituents on the leaving group, while with a sufficiently basic leaving group the energy barrier to its expulsion is larger and becomes rate controlling. Since substituents on the leaving group will affect the leaving step more than the attacking step, there will be a break in the curve and a greater sensitivity to structure when the leaving step becomes rate controlling; the phenyl acetates presumably fall near this transition point. For nucleophilic reactions of imidazole with ethyl esters, ethoxide expulsion is rate controlling and the energy barrier to this step is so large that no reaction occurs; in this case the otherwise undetectable general base catalysis by imidazole is the only reaction observed. These points are illustrated by the transition state diagrams shown in Fig. 4. The shift from general base catalysis to nucleophilic reaction as the basicity of the attacking group increases, discussed above, represents an analogous change in reaction path which results from variation of the attacking group rather than the leaving group.

**Conclusion.**—The change in mechanism and rate-limiting step of catalysis of ester hydrolysis in passing from alcohol-activated to acyl-activated esters must reflect a major difference in mechanism for hydrolysis and nucleophilic attack on the acyl group in the two series of compounds. Although we do not wish to speculate on the lifetime of any possible addition intermediate, the above considerations suggest that in the case of esters with a good leaving group relative to the attacking group, the rate-limiting step reflects principally nucleophilic attack on the carbonyl group and is sensitive to the nucleophilic reactivity of the attacking reagent, while with a relatively poor leaving group the transition state also reflects the expulsion of this group from a species which resembles a tetrahedral addition compound. In the latter case nucleophilic reactivity, as reflected in the rate of nucleophilic attack, becomes of secondary importance, while proton transfer, to aid expulsion of the leaving group and prevent expulsion of the attacking group, becomes an important, or even a necessary, part of the reaction.

(45) K. B. Wiberg, *J. Am. Chem. Soc.*, **77**, 2519 (1955).