The Question of the Importance of Electrostatic Catalysis. I. Comparison of the Reactivity of *o*-Nitrophenyl Hydrogen Oxalate and Ethyl *o*-Nitrophenyl Oxalate toward Nucleophiles

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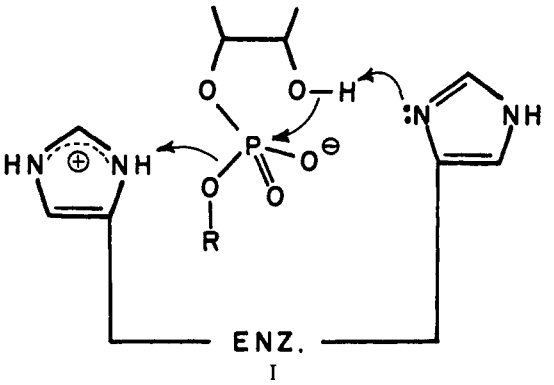
Abstract: The pH–log k_{obsd} profiles for the hydrolysis of *o*-nitrophenyl hydrogen oxalate (*o*-NPHO $\rightleftarrows$ *o*-nitrophenyl oxalate (*o*-NPO^-) + H^+) and ethyl *o*-nitrophenyl oxalate (*o*-NPEO) have been determined in water at 30° between pH 9 and 8 *M* hydrochloric acid. In the pH range of 1 to 9 the profiles are quantitatively expressed by spontaneous general base catalyzed hydrolysis of *o*-NPHO, *o*-NPO^-, and *o*-NPEO (supported by deuterium solvent kinetic isotope effects and salt effects) and hydroxide ion catalyzed hydrolysis of *o*-NPO^- and *o*-NPEO. In the acidity range of 0 to 8 *M* hydrochloric acid, the rates of hydrolysis of *o*-NPHO and *o*-NPEO decrease with increase in acidity. This decrease has been established to be primarily due to a depression in the spontaneous rates of hydrolysis brought about by a decrease in a_{H_2O}. The second-order rate constants for the reaction of 18 nucleophiles with *o*-NPEO and 16 nucleophiles with *o*-NPO^- have been determined. A plot of the log $k_{o\text{-NPEO}}$ *vs.* log $k_{o\text{-NPO}^-}$ was found to be linear of slope 0.9. The fact that the log k_{rate} points for nucleophiles possessing formally negative or positive charges fit well to the line for nucleophiles without charge shows that electrostatic facilitation of nucleophilic displacement on *o*-NPO^- is unimportant. Suggested cases of electrostatic facilitation in other bimolecular reactions in water are discussed.

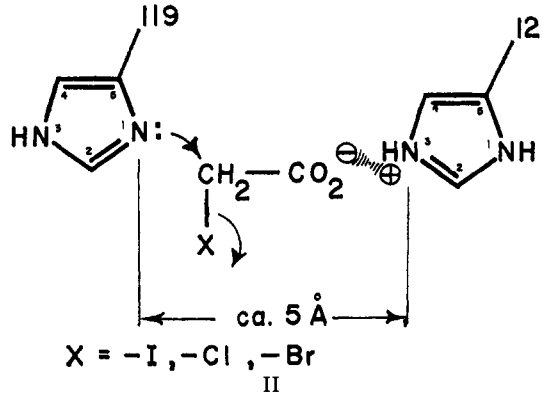
It is likely that the catalytic mechanisms available to the hydrolytic enzymes are limited to those established in physical organic studies (general base, general acid, and nucleophilic). The efficiency of these processes in the case of enzymatic reactions presumably is due to the lowering of $\Delta F^{\pm}$ by steric alignment of the substrate at the active site in a media favoring the formation of the transition state. From these most reasonable assumptions one is led to postulate that enzymes, on the whole, should not be specific to the making or breaking of one particular type of bond. This supposition is supported by studies in recent years among which may be mentioned the heightened activity of the serine hydroxyl group of the serine esterases toward alkylating agents[2] and the esteratic activity of carbonic anhydrase[3] and 3-phosphoglyceraldehyde dehydrogenase.[4]

An enzyme whose sequential structure is known, whose tertiary structure is expected to be known soon, and whose specificity is still believed to be restricted is ribonuclease. The only known substrates for ribonuclease are RNA and the cyclic phosphate esters produced as intermediates in RNA hydrolysis. A postulated mechanism for ribonuclease action involves the imidazolyl groups of histidine-12 and histidine-119 (I).[5] Supporting evidence for the involvement of His_{12} and His_{119} has been reviewed recently.[6] This evidence includes the enhanced reactivity of these two functional groups to alkylation by haloacetates and the



I

alkylation of either histidine moiety being dependent on the intact and unmodified nature of the other. These experimental findings have led to the suggestion that the alkylation reaction is electrostatically facilitated as in II.[7] A very large electrostatic enhancement of



II

nucleophilic catalysis has been reported for the reaction of the anion (*o*-NPO^-) of *o*-nitrophenyl hydrogen oxalate (*o*-NPHO) with the conjugate acid of 2-amino-

(1) Predoctoral Fellow of the National Institutes of Health. Work to be submitted by B. H. as partial fulfillment for the Ph.D. degree in chemistry.

(2) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., New York, N. Y., 1966, Chapter 2.

(3) Y. Pocker and J. T. Stone, *J. Am. Chem. Soc.*, 87, 5497 (1965).

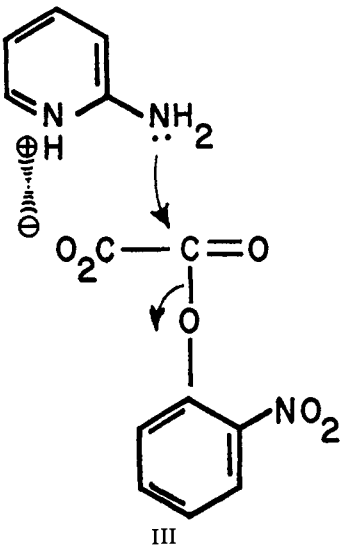
(4) J. H. Park, B. P. Meriwether, P. Clodfelder, and L. W. Cunningham, *J. Biol. Chem.*, 236, 136 (1961).

(5) D. Findlay, D. G. Herries, A. P. Mathias, B. R. Rabin, and C. A. Ross, *Biochem. J.*, 85, 152 (1962). For a modified version, see A. Deavin, A. P. Mathias, and B. R. Rabin, *Nature*, 211, 252 (1966).

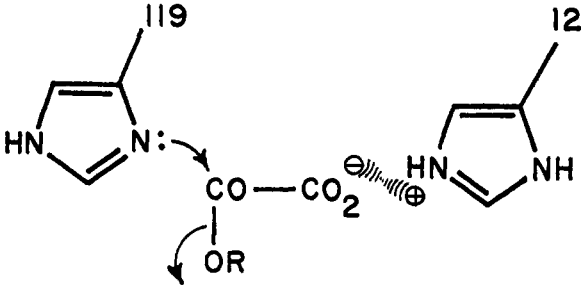
(6) J. P. Hummel and G. Kalnitsky, *Ann. Rev. Biochem.*, 33, 15 (1964).

(7) A. M. Crestfield, W. H. Stein, and S. Moore, *J. Biol. Chem.*, 238, 2421 (1963).

pyridine (III).[8] Comparison of II and III suggested to us that bovine pancreatic ribonuclease-A might catalyze the hydrolysis of *o*-NPHO *via* IV.

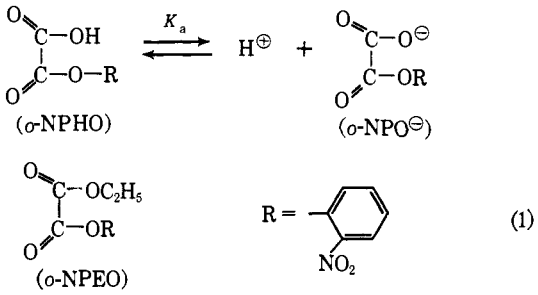


III



IV

Preliminary accounts of our studies of the reaction of RNase with *o*-NPHO and its inhibition by known inhibitors of RNase action[9] are reported in an accompanying communication. In the present paper we report the pH–rate profiles and the comparative reactivity of *o*-NPHO and its ethyl ester (*o*-NPEO) with a series of nucleophiles (eq 1). From the comparative



(1)

reactivities of *o*-NPHO, *o*-NPO$^-$, and *o*-NPEO, particularly in regard to negatively charged, neutral, and positively charged nucleophiles, a more correct evaluation of the importance of electrostatic facilitation and hindrance to nucleophilic catalysis has been determined.

Experimental Section

Materials. Potassium carbonate, potassium chloride, hydroxylamine, lithium chloride, potassium phosphate monobasic, and potassium phosphate dibasic were reagent grade (Baker) and used without further purification. Formic acid was CP grade (98%, Baker); glacial acetic acid of reagent grade (Baker) was redistilled. 2,2,2-Trifluoroethanol (Aldrich, bp 74°) and propargyl alcohol (Aldrich, bp 113°, n^{28}D 1.4318) were redistilled on a Nester and Faust spinning-band column. Aniline (Baker reagent) was distilled from zinc dust while pyridine (Baker Reagent) was dried over potassium hydroxide and distilled from barium oxide. Imidazole (Eastman White Label) was recrystallized from acetone–petroleum ether (bp 30–60°) and exhibited mp 88–89°. Methoxylamine (Eastman White Label) was recrystallized from ethanol–diethyl ether (mp 149–150°). Glycine (Calbiochem A Grade), ethylenediamine dihydrochloride (Eastman White Label), *o*-phenylenediamine (Eastman practical, mp (after recrystallization) 102–103°), and hydrazine hydrochloride (Eastman White Label) were recrystallized from ethanol–water mixtures. Ethyl oxalyl chloride and oxalyl chloride were obtained from Eastman. 2-Aminopyridine (Matheson Coleman and Bell) was recrystallized from chloroform–petroleum ether, mp 59–60°. All crystalline solids were dried and stored over P_2O_5 prior to use. Deionized freshly double glass-distilled and degassed water was employed to prepare all solutions. Deuterium oxide (Bio-Rad, Lot 3888) was 99.84 mole %. Moisture was excluded from experiments involving deuterium solvent kinetic isotope effects with the exception of potassium deuterioxide solutions which were prepared with potassium hydroxide pellets containing some potassium hydroxide hydrate. Deuteriochloric acid was prepared by bubbling dry HCl through deuterium oxide under anhydrous conditions.

***o*-Nitrophenyl hydrogen oxalate** (*o*-NPHO) was prepared by partial hydrolysis of the corresponding diester. Modifications of synthetic procedures of Adams and Gillman[10] for the diester and Bender and Chow[8] for the conversion of the diester to the monoester were employed.

To 25 ml of pyridine at 0° was added dropwise 5 g (0.0395 mole) of oxalyl chloride. To the yellow suspension still at 0° was then added 27.8 g (0.2 mole) of *o*-nitrophenol dissolved in 25 ml of pyridine. After allowing the reaction mixture to stand at 0° for an additional 2 hr, it was poured into a suspension of several hundred grams of ice in 300 ml of concentrated hydrochloric acid. The yellow precipitate was collected by filtration, the filter cake washed with ethanol, and the product washed with ether by suspension in this solvent and recollecting by filtration. The diester was then dried *in vacuo* (mp 185–187°; lit.[10] mp 185°) and employed without further purification.

A solution of 2 g (0.015 mole) of sodium acetate trihydrate in 300 ml of H_2O was added to 4 g (0.012 mole) of the diester dissolved in 500 ml of acetone at 0°. After stirring for 15 min, 5 ml of 5.8 *N* hydrochloric acid (0.029 mole) was added, the resultant solution was evaporated to one-third its initial volume on a rotary evaporator, and 200 ml ether was added. After standing at room temperature for 12 hr, the ether and aqueous layers were separated and the aqueous layer was extracted with 50 ml of ether. The combined ether extracts were dried over anhydrous magnesium sulfate, decanted from the drying agent, and evaporated to dryness under vacuum. Residual *o*-nitrophenol was removed from the resulting solid crude monoester by sublimation at 40° for 8 hr. The solid remaining was then dissolved in ether, the ether removed under vacuum, and the product recrystallized from a minimum of hot chloroform by addition of petroleum ether (bp 30–60°) until a slight cloudiness appeared. After recrystallization was complete the ester was collected by filtration and recrystallized three times from chloroform–petroleum ether. In this manner there was obtained 0.152 g (6% yield) of *o*-NPHO, mp 123–124° (lit.[8] 123–125°).

Anal. Calcd for $C_8H_5O_6N$: C, 45.51; H, 2.38; N, 6.64. Found: C, 45.55; H, 2.37; N, 6.64.

Ethyl *o*-Nitrophenyl Oxalate (*o*-NPEO). Ethyl oxalyl chloride (0.136 g, 0.001 mole) was added under anhydrous conditions at 0° to 0.53 g (0.003 mole) of potassium *o*-nitrophenolate.[11] When reaction was complete the suspension was brought to room temperature and extracted with anhydrous diethyl ether. The extract was then dried over anhydrous magnesium sulfate and flash evaporated to yield a yellow viscous oil. Purification was achieved by horizontal zone sublimation at a temperature differential of 30 to 200° under vacuum which provided the ester as the

(8) M. L. Bender and Y. L. Chow, *J. Am. Chem. Soc.*, **81**, 3929 (1959).

(9) T. C. Bruice, B. Holmquist, and T. P. Stein, *ibid.*, **89**, 4221 (1967).

(10) R. Adams and H. Gilman, *ibid.*, **37**, 2716 (1915).

(11) J. Cason and H. Rapoport, "Laboratory Text in Organic Chemistry," 2nd ed, Prentice-Hall Inc., Englewood Cliffs, N. J., 1962, p 148.

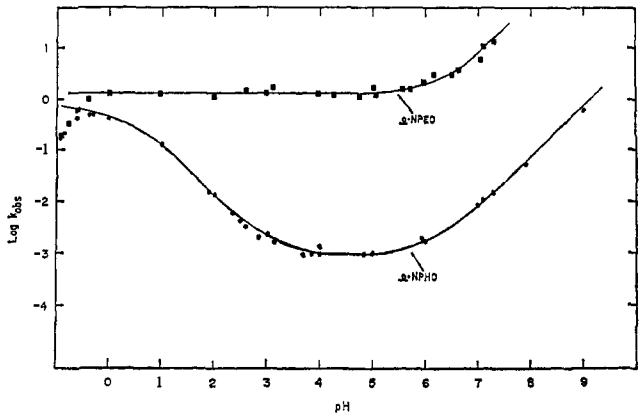


Figure 1. Plots of the log of the pseudo-first-order rate constants (k_{obsd} in units of min^{-1}) *vs.* pH for the hydrolysis of *o*-NPHO and *o*-NPEO. Points are experimental and the lines calculated from eq 2 for *o*-NPHO and eq 3 for *o*-NPEO.

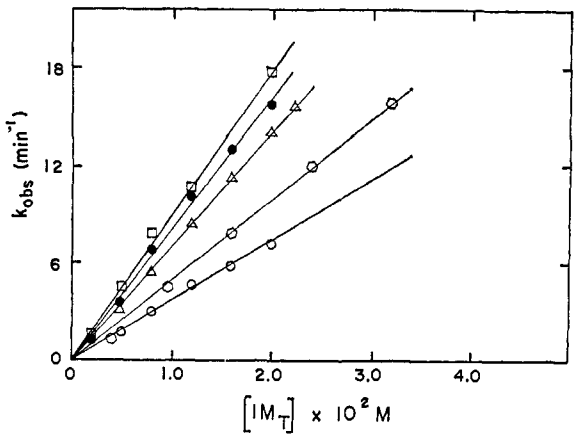


Figure 2. Plots of the pseudo-first-order rate constants for the hydrolysis of *o*-NPHO *vs.* total imidazole buffer concentration at constant pH values: pH 7.54, □; pH 7.33, ●; pH 7.14, Δ; pH 6.96, ○; and pH 6.73, O.

last liquid band. The portion of the sublimation tube containing the ester fraction was separated by cutting, and the ester was taken up in absolute diethyl ether which was then evaporated to yield the product as a yellow oil, $n^{25}D$ 1.5195.

Anal. Calcd for $C_{10}H_9NO_6$: C, 50.21; H, 3.79; N, 5.88. Found: C, 49.94; H, 3.90; N, 6.02.

Apparatus. Ultraviolet and visible spectra were measured on a Perkin-Elmer 350 recording spectrophotometer. Infrared spectra were recorded by use of a Perkin-Elmer 137 sodium chloride spectrophotometer. All kinetic measurements were made on a Gilford 2000 spectrophotometer equipped with four thermospacers through which was circulated water at 30 ± 0.1° or on a 13001 Durrum-Gibson stopped-flow spectrophotometer equipped with a Kel-F cell and valve block through which water was circulated at 30 ± 0.2°. All pH measurements were taken at 30 ± 0.1° with a Radiometer Model 22 pH meter equipped with a Model PHA 630 scale expander and combined glass calomel electrode (Radiometer GK 2021C).

Kinetics. All kinetic measurements were carried out in water at μ = 1.0 (with KCl), unless otherwise noted, and at a temperature of 30°. The appearance of *o*-nitrophenol with time was followed spectrophotometrically by recording the increase in optical density at 372.5 mμ, the isosbestic point of *o*-nitrophenol and *o*-nitrophenolate ion. In a typical run one drop of an ethanolic solution of the ester was added to a 2-ml glass-stoppered cuvet containing a solution of the nucleophile previously equilibrated at 30°. The cuvet was quickly shaken and returned to the thermostated cell housing of the spectrophotometer for recording of the change of absorbance. The concentration of ester was such that the absorbance of the solution at completion of the reaction did not exceed 0.4, corresponding to a concentration of *o*-nitrophenol less than 2×10^{-4} *M*. In all kinetic experiments the concentration of the nucleophile and its conjugate acid was in great excess over that of the ester so that pseudo-first-order kinetics were obtained in all cases. Reaction rates were followed to a minimum of three half-lives. The pH of the reaction solution was determined before and after each run to ensure constancy. Kinetic runs exhibiting pH drifts in excess of 0.03 pH unit were discarded. The concentration of nucleophilic base species was corrected for those instances in which a pH drift of over 0.03 unit accompanied serial dilution. The values of the pseudo-first-order rate constants (k_{obsd}) were calculated from the slopes of plots of log $OD_\infty/(OD_\infty - OD_t)$ *vs.* *t*. The nucleophile and its conjugate acid served as buffer except where noted in Tables I and II.

The rate constants for reactions whose half-lives were less than 2.5 sec were determined from reactions carried out on the stopped-flow spectrophotometer. For these experiments a 1 *M* KCl solution containing an appropriate concentration of ester was mixed with the solution of nucleophile previously adjusted to μ = 1.0 (with KCl) and the correct pH.

The pD was determined from pH meter readings by the equation of Fife and Bruice.[12] The autoprotolysis constants used for water and deuterium oxide were 1.47×10^{-14} and 2.24×10^{-15}, respectively.[13]

(12) T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961).

Results and Discussion

pH–Log k_{obsd} Profiles. The pH dependency for the hydrolysis of *o*-NPHO and *o*-NPEO between pH 0 and 9 are depicted in Figure 1. The points of Figure 1 are experimental, having been obtained by determining the dependence of the pseudo-first-order rate constants (k_{obsd}) on buffer concentration at constant pH. Since the reaction of all nucleophiles investigated with the ester were found to be first order in nucleophile and ester, extrapolation of plots of [buffer] *vs.* k_{obsd} to zero concentration provided the values of k_{obsd} dependent upon the concentration of lyate species. A sampling of the buffer dilution plots for *o*-NPHO is shown in Figure 2 wherein k_{obsd} is plotted *vs.* the concentration of total imidazole buffer at five pH values from pH 6.73 to 7.54. The profiles of Figure 1 are theoretical, having been derived from eq 2 for *o*-NPHO + *o*-NPO$^-$ and eq 3 for *o*-NPEO. The values of k_0, k_0', and k_{OH} employed are tabulated in Table I.

$$k_{hydr} = k_0' \frac{a_H}{K_a + a_H} + \frac{k_0 K_a}{K_a + a_H} + k_{OH}[OH^-] \quad (2)$$

$$k_{hydr} = k_0 + k_{OH}[OH^-] \quad (3)$$

Table I. Rate Constants for the Hydrolysis of *o*-Nitrophenyl Hydrogen Oxalate (*o*-NPHO), Its Anion (*o*-NPO$^-$), and Ethyl *o*-Nitrophenyl Oxalate (*o*-NPEO)

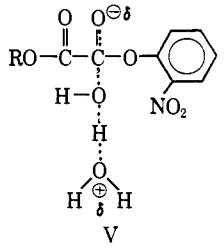
	o-NPHO[a]	*o*-NPO[a]	*o*-NPEO[b]
k_0, min^{-1}	...	9.5×10^{-4}	1.34
k_0', min^{-1}	5.79×10^{-1}	...	...
k_{OH}, l. $mole^{-1}$ min^{-1}	...	4.84×10^4	5.5×10^6
K_a (*M*) = 0.416[c]			

[a] 30°, μ = 1.0 with KCl; solvent, water. [b] 30°, μ = 1.0 with KCl; solvent, 2% EtOH–H_2O (v/v). [c] Obtained by fitting the experimental points of the pH–k_{obsd} profile to theoretical titration curves between pH 0 and 9.

The broad plateau associated with the hydrolysis of *o*-NPEO between pH 0 and 5 is best described as a water-catalyzed hydrolysis. This is supported by the value of the deuterium solvent kinetic isotope effect

(13) See T. C. Bruice and J. J. Bruno, *J. Am. Chem. Soc.*, **83**, 3494 (1961).

$(k_0^{H_2O}/k_0^{D_2O})$ of 3.25.[14] The plateau in the pH–rate profile for *o*-NPO⁻ is with assurance also the result of a water-catalyzed hydrolysis ($k_0^{H_2O}/k_0^{D_2O}$ = 1.49[14]). The much greater rate constant obtained in water as compared to D_2O is in accord with proton transfer in the rate-determining step (V). The constant k_0' is then



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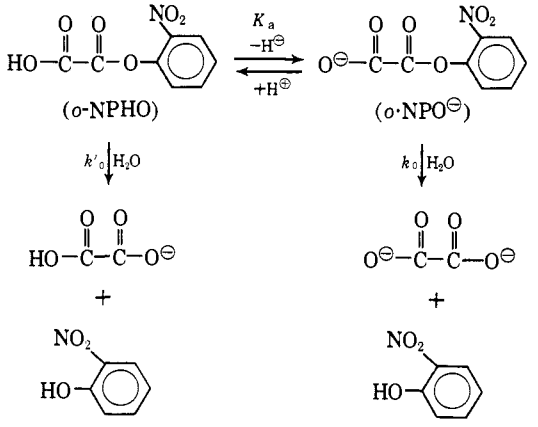
most assuredly associated with a spontaneous water catalysis of the hydrolysis of *o*-NPHO (eq 4). The



(4)

constant k_{OH} pertains to hydroxide ion catalyzed hydrolysis.

It may be noted from Table I that k_0 for *o*-NPEO and k_0' for *o*-NPHO are of the same order of magnitude, which is anticipated on the basis of the similarity of σ_I for the -COOEt (0.34) and -COOH (0.39) groups.[15] The decrease in k_{obsd} for both esters in the acid range of 1 to 8 *M* resembles in some respects the decrease in the plateau rate constants between pH 1 and 1 *M* acid in the hydrolysis of ethyl trifluorothiolacetate as reported by Fedor and Bruice.[16] However, for the oxalate esters this can be attributed to the decrease in activity of water while for the thiol ester the correct explanation is one involving acid-catalyzed partitioning of a tetrahedral intermediate to starting ester and spontaneous partitioning to product. Thus, the thiol ester hydrolysis is not affected by LiCl concentrations equivalent to those of a_H which bring about a decrease in its spontaneous rate of hydrolysis,[17] and the proposed mechanism has been verified through O^{18}-exchange experiments by Bender and Heck.[18]

To ascertain the cause of the rate depression obtained in concentrated acid (Figure 1), a depression similar to that previously found by Bender and Chow[8] with *o*-NPHO, the effect of high salt concentrations (LiCl) on the observed rates of both *o*-NPHO and *o*-NPEO

(14) Determined in acetate buffer by extrapolation to zero buffer concentration.
(15) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).
(16) L. R. Fedor and T. C. Bruice, *J. Am. Chem. Soc.*, **87**, 4138 (1965).
(17) M. Gregory and T. C. Bruice, *ibid.*, **89**, 2121 (1967).
(18) M. L. Bender and H. d'A. Heck, *ibid.*, **89**, 1211 (1967).

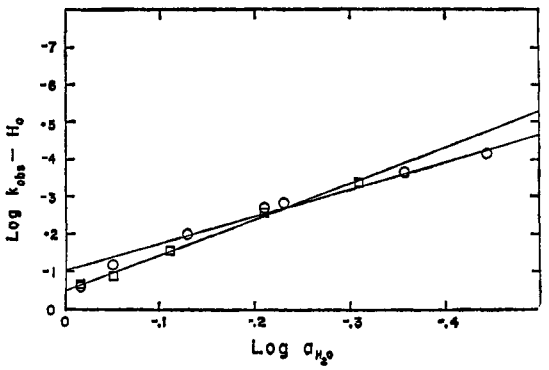


Figure 3. Bunnett plot for the hydrolysis of *o*-NPHO (○) and *o*-NPEO (□) in hydrochloric acid. The values of log a_{H_2O} were taken from M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957), and are for 25°. The H_0 values for aqueous solutions of HCl at 25° were taken from J. F. Bunnett, *J. Am. Chem. Soc.*, **83**, 4956 (1961). Units for k_{obsd} are min^{-1}. Deviations from linearity at low acid concentrations in the case of *o*-NPHO can be accounted for by assuming incomplete protonation of *o*-NPO⁻.

hydrolyses at a constant pH of 2 was measured (Table II). The rate depression of approximately tenfold over an ionic strength change of 1 to 6 at constant acidity is very similar to the change seen on increasing the concentration of hydrochloric acid, showing that the ionic strength in decreasing the activity of water is alone responsible for the rate depression. This is further supported by the results obtained for the hydrolysis of both esters at constant activity of water and increasing hydronium ion concentrations. In solutions of varying proportions of LiCl and HCl, prepared so that the total concentration of chloride ion is 8.0 *M*, a rate increase is noted with increase in hydronium ion concentration (Figure 3). Since the activity of water is nearly constant over the entire range of hydronium ion concentration,[19] the small and nonproportional increase in rate with increasing hydronium ion concentration may be best ascribed to a not completely equal effect of H^+ and Li^+ on the activities of both water and the ester.

Table II. Effect of LiCl Concentration on the Hydrolysis of *o*-NPHO and *o*-NPEO at pH 2.0

LiCl, *M*[a]	k_{obsd}, min^{-1}	
	o-NPHO[b]	*o*-NPEO[c]
1	9.2×10^{-3}	1.11
2	5.96×10^{-3}	0.784
3	4.25×10^{-3}	0.404
4	1.58×10^{-3}	...
5	1.25×10^{-3}	...
6	0.856×10^{-3}	0.107

[a] LiCl concentrations were determined by titration of Cl^- with standard $AgNO_3$ using dichlorofluorescein indicator as described by W. C. Pierce, D. T. Sawyer, and E. L. Haenisch, "Quantitative Analysis," John Wiley and Sons, Inc., New York, N. Y., 1958, p 320. [b] 30 ± 0.1°; solvent, water. [c] 30 ± 0.1°; solvent, 2% C_2H_5OH in H_2O (v/v).

It is interesting to note the results when a Bunnett[20] plot is applied to the rate constants in strong acid solu-

(19) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworth & Co. (Publishers) Ltd., London, 1959, p 483.
(20) J. F. Bunnett, *J. Am. Chem. Soc.*, **83**, 4956, 4968, 4973, 4978 (1961).

Table III. The Reaction of Nucleophiles with *o*-Nitrophenyl Hydrogen Oxalate (Solvent, H_2O; 30 ± 0.1°; μ = 1.0 with KCl)

	Nucleophile	pH range investigated	No. of pH values	Concn range, *M*	No. of k_{obsd} values detd
1	*o*-Phenylenediamine	4.76–5.21	2	0.2–0.02	12
2	Imidazole (H_2O)	6.73–7.54	6	0.002–0.032	33
	(D_2O)	7.20	1	0.004–0.02	5
3	Ethylenediamine[c]	6.45–7.78	4	0.004–0.2	25
4	Propargyl alcohol[c]	8.93–9.45	2	0.061–1.0	14
5	2,2,2-Trifluoroethanol[c]	8.14–9.07	3	0.04–1.0	18
6	2-Aminopyridine[f]	6.17–7.34	4	0.03–0.3	27
7	Methoxylamine	4.37–4.82	3	0.05–1.0	18
8	Glycine	1.94–2.60	3	0.05–1.0	18
9	Aniline	4.31–5.09	5	0.03–0.3	36
10	Pyridine	4.94–6.03	5	0.05–0.5	30
11	Acetate (H_2O)	3.96–4.81	4	0.02–0.5	23
	(D_2O)	4.69	1	0.01–0.5	6
12	Formate	2.87–3.70	4	0.10–1.0	23
13	Phosphate	5.97–7.90	5	0.01–0.30	27
14	Hydroxylamine	6.06	1	0.12–1.0	4
15	Hydrazine	7.75	1	0.12–1.0	4
16	Hydroxide ion[b,e]	7.10–9.04	3		
17	Water	−1.0–9.0	24	*Ca.* 53.4[a]	24[b]
18	Deuterium oxide	4.69	1	*Ca.* 53.6[d]	

[a] Approximate molarity of water 1.0 *M* in KCl, density 1.04. [b] Number of intercept values obtained by extrapolating plot of pseudo-first-order rate constants *vs.* concentration to zero buffer concentration. [c] Carried out in 0.02 *M* carbonate buffer. [d] Approximate molarity of deuterium oxide 1.0 *M* in KCl, density 1.149. [e] Carried out in phosphate or carbonate buffers. [f] No reaction of 2-aminopyridinium ion could be detected at pH 3.3 (formate buffers) or at pH 3.03 (HCl buffer at μ = 1.0 and 0.02 at concentrations in nucleophile as high as 0.2 and 0.019 *M*, respectively) and, therefore, the observations of ref 8 could not be substantiated. That reaction does not involve unprotonated 2-aminopyridine and protonated ester is indicated by the fact that the value of the second-order rate constant for this process would be *ca.* 10^4 greater than that determined for the reaction of pyridine with *o*-NPEO.

tion (Figure 4). The slopes, ω, obtained from a plot of log $k_{obsd} - H_0$ *vs.* a^{H_2O} for *o*-NPHO and *o*-NPEO are +7.4 and +9.7, respectively. Large positive values of ω (*i.e.*, above +3.3) are ascribed to water playing the role of a proton-transfer agent in the rate-determining step, in accord with the proposed mechanism of V.[21]

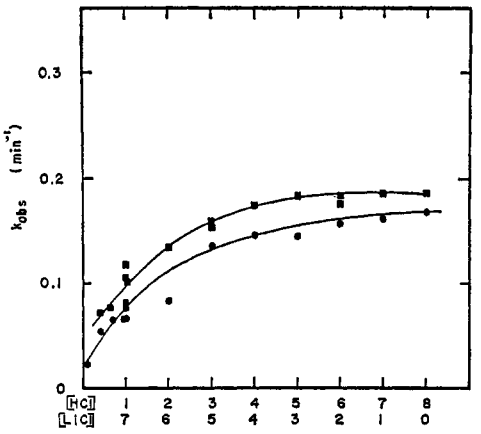


Figure 4. Plot of the pseudo-first-order rate constant for the hydrolysis of *o*-NPHO (●) and *o*-NPEO (■) *vs.* the molar concentration of HCl–LiCl at constant chloride ion concentration.

Reaction with Nucleophiles. Under the conditions of nucleophile plus nucleophile conjugate acid in great excess over ester, constant pH, and kinetics first order in nucleophile and ester, eq 5 pertains. k_{obsd} is the first-

$$k_{obsd} - k_{hydr} = k_n[N_T]\{K_a/(K_a + a_H)\} \quad (5)$$

order rate constant for appearance of *o*-nitrophenol plus *o*-nitrophenolate, k_n the second-order rate constant for reaction of nucleophile with ester, $[N_T] = [N_f] + [NH]$, where $[N_f]$ represents the concentration of nucleophile and [NH] that of its conjugate acid, and k_{hydr} is the first-order hydrolytic constant provided by (2) and (3). A summary of experimental conditions for the reaction of 18 nucleophiles with *o*-NPO^- and 16 nucleophiles with *o*-NPEO are provided in Tables III and IV, respectively. The calculated k_n constants for both esters are recorded in Table V. With the exception of a few nucleophiles the concentrations of $[N_f]$ and [NH] were sufficient to provide needed buffer capacity. For the alcohols 2,2,2-trifluoroethanol and propargyl alcohol, reactions were studied in carbonate or acetate buffers. In the pH range investigated $a_H \gg K_a$, so that (5) reduces to

$$k_{obsd} - k_{hydr} - k_{buffer}[buffer] = k_nK_a[N_T]/a_H = k_nK_a[OH^-][N_T]/K_w \quad (6)$$

From (6) it follows that a plot of $[N_T]$ *vs.* k_{obsd} at constant a_H and constant buffer concentration provides as slope $k_nK_a[OH^-]/K_w$ and as intercept the constant $k_{hydr} + k_{buffer}[buffer]$. With a knowledge of the values of K_a, K_w, and $[OH^-]$ the value of k_n may be derived from the determined specific base constant k_nK_a/K_w by carrying out experiments at various pH values (Tables III and IV). For the case of imidazole and ethylenediamine reacting with *o*-NPEO a similar procedure was employed because the value of k_n was of such a magnitude that it was not possible to work near the pK_a value. Stopped-flow kinetics could not be employed with *o*-NPEO since the spontaneous rate of

(21) The considerations of Bunnett[20] and R. B. Martin [*J. Am. Chem. Soc.*, 84, 4130 (1962)] on the applicability of the Bunnett treatment to the hydrolysis of *o*-NPHO must be viewed in the light of the present findings that there is likely no acid catalysis of the hydrolysis of either *o*-NPHO or *o*-NPEO in the acidity range studied and also the fact that the data employed[8] by these workers do not agree quantitatively with the data of this study.

Table IV. The Reaction of Nucleophiles with Ethyl *o*-Nitrophenyl Oxalate [Solvent, 2% C_2H_5OH in H_2O (v/v); 30 ± 0.1°; μ = 1.0 with KCl]

	Nucleophile	pH range investigated	No. of pH values	Concn range, *M*	No. of k_{obsd} values detd
1	*o*-Phenylenediamine	4.74–5.24	2	0.2–0.02	14
2	Imidazole[a]	4.63–5.12	2	0.004–0.0008	12
3	Ethylenediamine[a]	4.41–5.48	3	0.002–0.12	28
4	Propargyl alcohol[a]	4.93–5.32	3	0.10–1.0	25
5	2,2,2-Trifluoroethanol[a]	5.00–5.43	2	0.07–1.0	17
6	2-Aminopyridine	6.15–7.65	5	0.003–0.03	20
7	Methoxylamine	4.37–4.70	2	0.005–0.012	9
8	Glycine	2.34–2.60	2	0.05–1.0	12
9	Aniline	4.31–5.09	5	0.03–0.3	34
10	Pyridine	4.94–6.03	5	0.05–0.5	30
11	Acetate (H_2O)	3.96–4.82	3	0.05–0.5	23
	(D_2O)	4.69	1	0.01–0.5	6
12	Formate	3.70	2	0.10–1.0	12
13	Phosphate	6.56–7.21	3	0.01–0.066	17
14	Hydroxide ion[b,c]	6.15–7.65	5		
15	Water	−1.0–9.0	21	*Ca.* 53.4[d]	21
16	Deuterium oxide	4.69	1	*Ca.* 53.6[e]	6

[a] Carried out in 0.02 *M* acetate buffer. [b] Number of intercept values obtained by extrapolating plots of pseudo-first-order rate constants *vs.* concentration to zero buffer concentration. [c] Carried out in 2-aminopyridine buffer. [d] Approximate molarity of water in 1 *M* KCl solution, density 1.04. [e] Approximate molarity of deuterium oxide in 1 *M* KCl solution, density 1.149.

Table V. Second-Order Rate Constants (k_n) for the Reaction of a Series of Nucleophiles with Ethyl *o*-Nitrophenyl Oxalate (*o*-NPEO) and with *o*-Nitrophenyl Oxalate (*o*-NPO^-)

	Nucleophile	pK_a'	k_n, l. mole^{-1} min^{-1} *o*-NPHO[a]	k_n, l. mole^{-1} min^{-1} *o*-NPEO[i]
1	*o*-Phenylenediamine[j]	1.3, 4.74[h,d]	2.90	1.59×10^2
2	Imidazole (H_2O)	7.15[d]	1.36×10^3	4.5×10^5
	(D_2O)	7.60[d]	1.23×10^3	
3	Ethylenediamine[j]	7.53, 10.18	1.24×10^3	1.08×10^5
4	Propargyl alcohol[k]	13.55[c]	3.04×10^5	4.32×10^8
5	2,2,2-Trifluoroethanol[k]	12.36[b]	6.03×10^4	1.39×10^8
6	2-Aminopyridine[j]	6.86[d,h]	2.08	2.04×10^2
7	Methoxylamine	4.68[d]	3.0×10^1	7.86×10^2
8	Glycine[j,k]	2.34[f]	1.25×10^{-2}	1.79
9	Aniline	4.71[d]	2.61	1.66×10^2
10	Pyridine	5.45[e]	9.30	2.7
11	Acetate (H_2O)	4.61[d]	1.57×10^{-2}	3.1
	(D_2O)	5.12[d]	1.44×10^{-2}	4.0
12	Formate	3.70	1.73×10^{-2}	9.6
13	Phosphate	7.10	2.0×10^{-1}	5.40×10^2
14	Hydroxylamine	5.98	5.46×10^3	
15	Hydrazine	8.10[g]	4.6×10^4	
16	Hydroxide	15.74	4.56×10^{-4}	4.77×10^7
17	Water	−1.74	1.96×10^{-5}	2.55×10^{-2}
18	Deuterium oxide		1.40×10^{-5}	7.80×10^{-3}

[a] 30 ± 0.1°; solvent, water; μ = 1.0 with KCl. [b] P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **81**, 1050 (1959). [c] P. Ballinger and F. A. Long, *ibid.*, **82**, 795 (1960). [d] Determined by half-neutralization. [e] T. C. Bruice and R. G. Willis, *J. Am. Chem. Soc.*, **87**, 531 (1965). [f] R. M. C. Dawson, *et al.*, "Data for Biochemical Research," Oxford Claredon Press, London, 1959, p 13. [g] T. C. Bruice, J. J. Bruno, and W. Chow, *J. Am. Chem. Soc.*, **85**, 1659 (1963). [h] Determined by spectrophotometric titration. [i] 30 ± 0.1°; solvent, 2% C_2H_5OH in H_2O (v/v); μ = 1.0 with KCl. [j] As monocation. [k] As monoanion.

hydrolysis of the ester precluded its incorporation into aqueous stock solutions. Owing to the magnitude of the rate constants, hydrazine and hydroxylamine could not be studied with *o*-NPEO. Inspection of a Brønsted plot (not shown) of the data of Table V for *o*-NPO^- reveals that both hydrazine and hydroxylamine possess undue reactivity which may be ascribed to the α effect.[22]

Product analyses have not been generally carried out in this study because no suitable analytical technique could be found to determine quantitatively the concentration of oxalyl nucleophiles at less than 10^{-4} *M* in the presence of high salt concentrations. For the reaction of *o*-NPO^- with imidazole, however, an intermediate absorbing in the region 240–260 mμ was observed. The rate of disappearance (257 mμ) of the intermediate from solution was found to be much slower than the rate of disappearance of ester as measured by the rate of nitrophenol production, the ratio of rate constants extrapolated to zero buffer concentration being 29.2 at pH 7.14. The hydrolysis of the intermediate was found to be catalyzed by imidazole (k_2 = 4.62 l. mole^{-1} min^{-1}), and its spontaneous rate of hydrolysis exhibits a deuterium solvent kinetic isotope effect of 2.02. The fact that N-acetylimidazole is subject to imidazole general base catalyzed hydrolysis and exhibits a deuterium solvent kinetic isotope effect of 2.5 and a λ_{max} at 245 mμ supports the contention that the intermediate is

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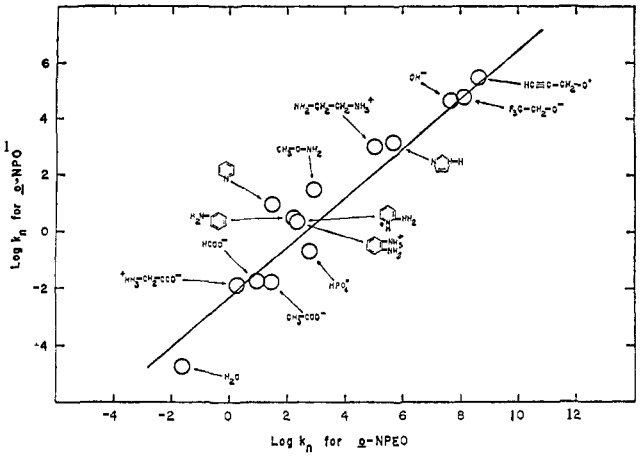
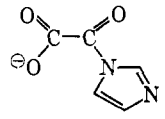


Figure 5. Plot of the log of the second-order rate constants (l. mole^{-1} min^{-1}) for the reaction of a series of nucleophiles with *o*-NPO$^-$ *vs.* the log of the second-order rate constants for the reaction of the same nucleophiles with *o*-NPEO.

N-oxalylimidazole (VI).[23] Thus, imidazole is involved in a direct nucleophilic attack on the ester carbonyl of *o*-NPO$^-$. Under a variety of experimental conditions no intermediate could be detected in the imidazole-catalyzed hydrolysis of *o*-NPEO. This result is best interpreted on the basis that if an intermediate were formed its rate of hydrolysis would be much greater than that of VI; compare values of k_0 for *o*-NPO$^-$ and *o*-NPEO (Table I).



VI

Electrostatic Catalysis. Figure 5 is a plot of the values of log k_n for *o*-NPO$^-$ (log $k_{o\text{-NPO}^-}$) *vs.* the corresponding values for *o*-NPEO. The line of Figure 5 has been drawn from eq 7. The intercept value of

$$\log k_{o\text{-NPO}} = +0.90 \log k_{o\text{-NPEO}} - 2.20 \qquad (7)$$

-2.20 is most assuredly related to the difference in σ_I values for the $-COO^-$ (-0.17) and $-COOEt$ ($+0.34$) groups[15] while the factor 0.90 indicates the near identity of the slope of the Brønsted plots for various types of nucleophiles reacting with both esters. An inspection of Figure 5 reveals that there is no discrimination between the charge type of the nucleophile. Thus, neutral and negatively and positively charged nucleophiles are equally correlated by eq 7. Therefore, there is no justification for electrostatic facilitation in the reaction of nucleophiles with *o*-NPO$^-$. In particular, since the protonated 2-aminopyridine shows no appreciable deviation from the best line for Figure 5, there is no justification for III.

The importance of electrostatic facilitation (defined in this study as rate enhancement due to attraction of formal positive and negative charges) is not well understood, as shown by examination of the pertinent literature. Though negatively charged nucleophiles are more reactive with the positively charged acetylimidazolium ion than are neutral nucleophiles,[24] the same preference is exhibited by the neutral esters, *p*-nitrophenyl chloroacetate and phenyl dichloroacetate.[25] For the latter substrates the enhanced reactivity has been "*tentatively* ascribed to electrostatic stabilization of the transition state resulting from ion–dipole or dipole–dipole interactions between acyl-activated substrate and anionic nucleophilic reagent." Cationic nucleophiles are reported to possess abnormal reactivity toward the *p*-nitrophenylphosphate dianion[26] and isopropyl methylphosphonofluoridate;[27] however, it should be noted that negatively charged anions do not exhibit enhanced reactivity with 1-(N,N-dimethylcarbamoyl)pyridinium chloride.[28] It is possible that for this substrate as well as *o*-NPO$^-$ the lack of an electrostatic facilitation is due to dispersion of the negative charge on the pyridinium and carboxylate moieties, but dispersion of charge should be more important with N-acetylimidazolium ion than with the dimethylcarbamoylpyridinium ion. Bell[29] has considered the question of electrostatic facilitation in esters of the type X–CH_2COOEt by comparing their rate constants for alkaline hydrolysis to that for ethyl acetate. It should be noted that the question of electrostatic catalysis is not completely clarified by these studies since by employing the σ_I values of Charton[15] it can be shown that substituents X in X–CH_2COOEt fit with excellence eq 8, regardless of the charge or lack

$$\log \frac{k_{OH}}{k_0} = 3.89\sigma_I \qquad (8)$$

of charge for the substituent X. Interpretation of this result as showing no electrostatic facilitation is beclouded by the fact that σ_I is determined by use of the pK_a values of XCH_2COOH which are, of course, not only an index of the induction effect of X but of its electrostatic influence.[30-33]

Means of establishing the importance of increase or decrease of collision frequency on nucleophilic attack of esters of type $X(CH_2)_nCOOR$ are now under investigation in this laboratory. Regardless of the end result of these studies it can be assuredly stated that electrostatic facilitation is of no significance in the reactivity of *o*-nitrophenyl oxalate ion with negatively or positively charged or neutral nucleophiles.

We wish to emphasize that all arguments presented in this paper pertain to the reaction of small molecules in aqueous solutions.

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

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