shows that this species is susceptible to nucleophilic attack. A preferred reaction with this species is not unreasonable, in view of the negative charge of the fluoride ion. However, a slow reaction of concentrated fluoride with phosphoramidate monoanion is detectable at neutral or slightly alkaline pH, and an analogous reaction must account for the formation of phosphorofluoridate from the intermediates in the reactions of phosphoramidate with pyridine and triethylenediamine.

The Reactivity of Nucleophilic Reagents toward the *p*-Nitrophenyl Phosphate Dianion[1]

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The nucleophilic reactivity of a series of amines toward the p-nitrophenyl phosphate dianion displays only a very small sensitivity to the basicity of the amine. Cationic substituents on the amine cause an enhanced reactivity, which is ascribed to an electrostatic effect. Substituents which introduce steric hindrance decrease nucleophilic reactivity. The rate constant for the hydrolysis of p-nitrophenyl phosphate dianion falls on the same line as those of benzoyl phosphate dianions in a logarithmic plot against the pK of the leaving group. It is concluded that the principal driving force for the bimolecular reactions of phosphoramidate monoanion, acetyl phosphate dianion, and p-nitrophenyl phosphate dianion arises from electron donation from the oxygen atoms and electron withdrawal by the leaving group, with little bond formation by the nucleophilic agent.

As a continuation of the experiments described in the previous paper of this series, the reactivity of a number of nucleophilic reagents toward the dianion of *p*-nitrophenyl phosphate has been determined. This substrate has the advantage that its reaction rate may easily be followed spectrophotometrically and its reaction with basic amines may be studied in alkaline solution.

Experimental

Disodium *p*-nitrophenyl phosphate was obtained from the Aldrich Chemical Co., and proved to be a hydrated mixture of the salt with inorganic phosphate. Each lot was assayed by spectrophotometric measurement of *p*-nitrophenol release on complete acid hydrolysis[2] and was found to contain from 65 to 70% of *p*-nitrophenyl phosphate, and less than 0.1% of *p*-nitrophenol. This salt, which is similar to that used in other kinetic investigations,[3] was used without further purification. Amines and their hydrochlorides were redistilled or recrystallized before use. Water and deuterium oxide were glass distilled. Reagent grade inorganic salts were used without further purification.

Initial rates of *p*-nitrophenolate release were followed spectrophotometrically at 400 mμ using a Zeiss PMQ II spectrophotometer equipped with a thermostated cell compartment. Solutions were brought to 39.0° in a water bath before mixing. Reactions were started by the addition of 0.5 ml. of a freshly prepared solution, 0.03–0.06 *M* in *p*-nitrophenyl phosphate, to 2.5 ml. of reaction mixture. The ionic strength was maintained at 1.0 with potassium chloride.

The pH of the reaction mixture was measured at room temperature at the end of each run. With the exception of certain experiments with ethylenediamine cation, the pH of the solutions was always more than one pH unit above the pK_a of *p*-nitrophenol (7.1). However, for all reactions in which the final pH was below 10, the degree of ionization of the product was determined by measuring the absorbance at 400 mμ of 1.0 ml. of a standard solution of *p*-nitrophenol added to a further 5.0 ml. of reaction mixture, and the result was used to calculate the amount of *p*-nitrophenol formation from the spectrophotometric measurements of the rate experiments.

First-order rate constants were calculated from the slopes of the linear plots of optical density against time, by converting to concentration units and dividing by the initial concentration of phosphate ester. (This was calculated using ϵ 18,320 for *p*-nitrophenolate ion at 400 mμ,[4] but the conversion from optical density to concentration units also involves this constant, and the rate constants obtained do not depend directly on a particular value for the extinction coefficient.)

The observed first-order rate constants were corrected for the rates of uncatalyzed hydrolysis, which were obtained from the intercepts of plots of rate against amine concentration or from independent measurements of the rate of hydrolysis in the absence of amine (Figure 1). No precautions were normally taken to exclude light[5] or metal ions, but it was shown that the hydrolysis rates are identical in the dark, in the presence and absence of 10^{-4} *M* ethylenediamine-

(1) Supported by grants from the National Science Foundation and the National Institute of Child Health and Human Development of the Public Health Service (HD-01247), and by a Public Health Service Training Grant from the National Institute of General Medical Sciences (5T1-GM-212-05).

(2) O. A. Bessey and R. H. Love, *J. Biol. Chem.*, **196**, 175 (1952).

(3) J. D. Chanley and E. Feageson, *J. Am. Chem. Soc.*, **85**, 1181 (1963).

(4) F. Kezdy and M. L. Bender, *Biochemistry*, 1, 1097 (1962).

(5) (a) R. L. Letsinger and O. B. Ramsay, *J. Am. Chem. Soc.*, **86**, 1447 (1964); (b) E. Havinga, R. O. De Jongh, and W. Dorst, *Rec. trav. chim.*, **75**, 378 (1956).

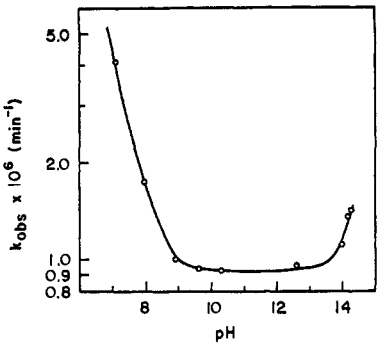


Figure 1. Plot of rate of hydrolysis of *p*-nitrophenyl phosphate at 39° and ionic strength 1.0 against pH. Conditions are given in Table II.

of free base or at constant total amine concentration. Deviations from linearity were observed with concentrated solutions of several amines. Piperidine and morpholine show a decrease and an increase, respectively, in the second-order constants at concentrations approaching 2 *M* in free base. Since the solution contains some 10% of amine at these concentrations, solvent effects would not be unexpected, and it was shown that changes of a similar magnitude were induced by the addition of up to 1 *M* dioxane to 1.2 *M* amine under the same conditions (see Table III, and Table II of ref. 6).

The heterocyclic bases showed larger deviations, with a decrease in the second-order rate constants at higher base concentrations (Figure 2). The implications of this

Table I. Rate Constants for the Reactions of Amines with the Phosphoryl Group of *p*-Nitrophenyl Phosphate at 39° and Ionic Strength 1.0

	pK_a	Concn. range,[a] *M*	No. of runs	Fraction[b] P–O	$k_2 \times 10^5$,[c] M^{-1} min.$^{-1}$
Nicotinamide	3.4	0.07–1.2[d]	9		0.80[e]
Aniline	4.6	0.3[f]	1		—[g]
Methoxylamine	4.7	0.13–0.75[f]	6		—[g]
Pyridine	5.2	0.2–1.2[d]	6		1.5[e]
Hydroxylamine	6.0	0.2–0.96[h]	5	1.00	2.0
4-Picoline	6.0	0.2–0.8[d]	5		2.0[e]
2,6-Lutidine	6.8	0.17–0.33[f]	2		—[g]
Imidazole	7.0	0.2–1.0	3	0.99	0.33
Tris	8.1	0.1–1.0[i]	3		—[g]
Hydrazine	8.1	0.05–0.45[i,k]	6	0.97	6.3
Morpholine	8.4	0.4–2.1[l]	6	0.87	1.8[m]
Ethanolamine	9.5	0.3–0.8[i,k]	6	0.86	1.5
Glycine anion	9.6	0.2–0.8[i,k]	6	0.83	1.3
Trimethylamine	9.8	0.5–1.0[n]	4		0.72
n-Butylamine	10.6	0.3–0.8[i,k]	6	0.73	2.0
Methylamine	10.6	0.17–0.67[o]	5	0.72	5.1
Dimethylamine[p]	10.6	0.05–0.5[q,k]	9	*p*	4
Triethylamine	10.65	0.2–0.3[i,k]	2		<0.2
Diethylamine	11.0	0.2–2.0[r]	6		<0.5
2-Methylpiperidine	11.0	0.2–0.5[i]	3		—[g]
Piperidine[p]	11.2	0.1–1.6[k,s]	12	*p*	4.6
HO^-[t]	15.7	0.17–1.0	6		0.05[t]
		Diamino compounds[u]			
Triethylenediamine	8.8	0.2–2.0[i,v]	12		6.2
Monocation	3.6	0.2–0.8[i,v]	12		2.5
N,N,N′,N′-Tetramethylethylenediamine	9.1	0.1–0.9[i,w]	12		0.43
Monocation	5.7	0.1–0.9[i,w]	12		1.9
Ethylenediamine	10.0	0.2–1.0[i,x]	12	0.83	3.0
Monocation	7.5	0.2–1.0[i,x]	12	0.95	1.8
1,3-Diaminopropane	10.6	0.4–1.0[i]	5	0.69	3.2
Monocation	8.6	0.2–1.0	5	0.82	1.4
1,4-Diaminobutane	10.4	0.2–0.8[i]	3	0.62	3.5
Monocation	9.3	0.2–0.8[i]	3	0.69	1.5

[a] Concentration of ionic form specified. [b] Fraction of total color increase at 400 mμ from *p*-nitrophenolate formation; this color disappears on acidification to pH 3–4. [c] For P–O bond cleavage. [d] Measured in 0.2 *M* K_2CO_3 buffer, pH 9.9. [e] k_2 decreases with increasing base concentration; see text. [f] Measured in 0.2 *M* Tris buffer, pH 8.3. [g] No reaction detectable within the concentration range specified. [h] As 96% free base, pH 7.6. [i] As 50% free base. [j] Measured by varying pH with total amine concentration constant at 1.0 *M*. [k] The conjugate acid gives no detectable reaction. [l] As 86% free base, pH 9.6. [m] Small increase in k_2 at high base concentrations; see text. [n] As 80% free base. [o] As 67% free base, pH 11.2. [p] Some C–O fission occurs; the figure for P–O fission depends on amine concentration and [OH^-] and is derived as described in ref. 6. [q] As 50% free base, pH 11.0. [r] As 67% free base, pH 11.6. [s] As 40% free base, pH 11.3, and 50% free base, pH 11.4. [t] This reaction probably represents C–O bond fission; see text. [u] No correction was made for overlap of pK_a' values in calculations of the fraction of free base in buffer solutions made by adding HCl to the free diamine. [v] Also as 20% free base, pH 8.5. [w] Also as 17% free base, pH 8.7. [x] Also as 50% free base, pH 10.2.

tetraacetic acid (EDTA), and at three different concentrations of both carbonate and tris(hydroxymethyl)-aminomethane (Tris) buffers (Tables I and II).

Second-order rate constants were obtained from the slopes of plots of first-order rate constants against the concentration of free amine, either at constant fraction

observation are discussed below. The rate constants were obtained from the initial slopes of these curves. Measurements of the rate of reaction of heterocyclic bases with *p*-nitrophenyl acetate at 39° were carried out

(6) A. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, 87, 3217 (1965).

Table II. Hydrolysis of *p*-Nitrophenyl Phosphate at 39° and Ionic Strength 1.0

Conditions	pH[a]	$k_{obsd} \times 10^6$, min.$^{-1}$
0.2 *M* Tris buffer[b]	7.4	4.2
0.2 *M* Tris buffer[b]	8.3	1.75
0.2 *M* Tris buffer[b]	9.3	1.01
0.07 K_2CO_3 buffer[c]	10.2	0.93
0.13 *M* K_2CO_3 buffer[c]	10.2	0.93
0.27 *M* K_2CO_3 buffer[c]	10.2	0.93
0.1 *M* K_2CO_3 buffer, no KCl added	10.0	0.78
0.2 *M* Na_2CO_3 buffer	9.6	0.94
0.2 *M* Na_2CO_3 buffer, in D_2O	*d*	0.84
Hydrolysis in		
0.17 *M* NaOH[e]		0.96
0.33 *M* NaOH[e]		1.04
0.50 *M* NaOH[e]		1.12
0.67 *M* NaOH[e]		1.27
0.83 *M* NaOH[e]		1.36
1.0 *M* NaOH[e]		1.41
0.83 *M* NaF[e]		0.93
0.83 *M* KBr[e]		0.93
0.83 *M* KI[e]		0.94
0.83 *M* KNO_3[e]		0.90
0.83 *M* KNCS[e]		0.93
0.28 *M* K_2SO_3[e]		1.02
0.28 *M* $Na_2S_2O_3$[e]		0.84
0.28 *M* K_2HPO_4[e]		0.92
0.28 *M* potassium oxalate[e]		0.98
0.28 *M* potassium citrate[e]		1.03
0.83 *M* potassium acetate[e]		1.05
Hydrolysis at 58°, K_2CO_3 buffer[f]	10.2	15.6
Hydrolysis at 75°, K_2CO_3 buffer[f]	10.2	168.0
ΔS^*_{av}, +3.5 e.u.[g]		
ΔH^*_{av}, 30.6 kcal./mole.[g]		

[a] Measured at room temperature, 25 ± 1°. [b] Tris base does not catalyze the reaction; see Table I. Rates identical in the presence of 10^{-4} *M* ethylenediaminetetraacetic acid. [c] Rates identical in the dark. [d] pH 9.6 in water. [e] 0.1 *M* K_2CO_3 buffer, pH 9.8 ± 0.1. [f] Rates identical at 0.07 and 0.13 *M* buffer. [g] Calculated from the relations $\Delta H^* = E_a - RT$, $\Delta S^* = (\Delta H^* - \Delta F^*)/T$; $\Delta F^* = -RT \ln kh/K_BT$.

Table III. Effect of Morpholine and of Dioxane on the Second-Order Rate Constants for the Reaction of *p*-Nitrophenyl Phosphate with Morpholine at 39°, Ionic Strength 1.0

Morpholine concn.,[a] *M*	$k_2 \times 10^5$, M^{-1} min.$^{-1}$	Dioxane added 0.5 *M*	Dioxane added 1.0 *M*
0.63	2.41		
1.27	2.68	2.81	3.05
1.70	2.94		
2.12	3.22		

[a] 84% free base, pH 9.6.

at 400 mμ, as previously described,[7] in 0.05 *M* Tris buffer at pH 9.3. The observed first-order rate constants were corrected for hydrolysis, measured independently under the same conditions in the absence of heterocyclic base.

Products. The two possible aromatic products from the reaction of *p*-nitrophenyl phosphate with a primary or secondary amine are *p*-nitrophenolate ion and a *p*-nitroaniline. Both absorb strongly at 400 mμ, but the phenol would be protonated, and therefore colorless, at pH 3–4. Attack of a tertiary amine at the aromatic ring would give a cationic product with no free electron pair on nitrogen; it would not be expected to absorb at 400 mμ. The products obtained in the reactions of primary amines were determined by adding aliquots of reaction mixtures to acetic acid solutions to give a pH of 3 to 4 and comparing the color yield with that obtained with aliquots added to alkaline solutions.[6] With the primary amines, *p*-nitrophenol is the primary product and the rate constants were corrected for any aniline formation. With secondary amines a larger amount of aniline is formed under certain conditions, and the rate constants for these amines were obtained as described in the accompanying paper.[6]

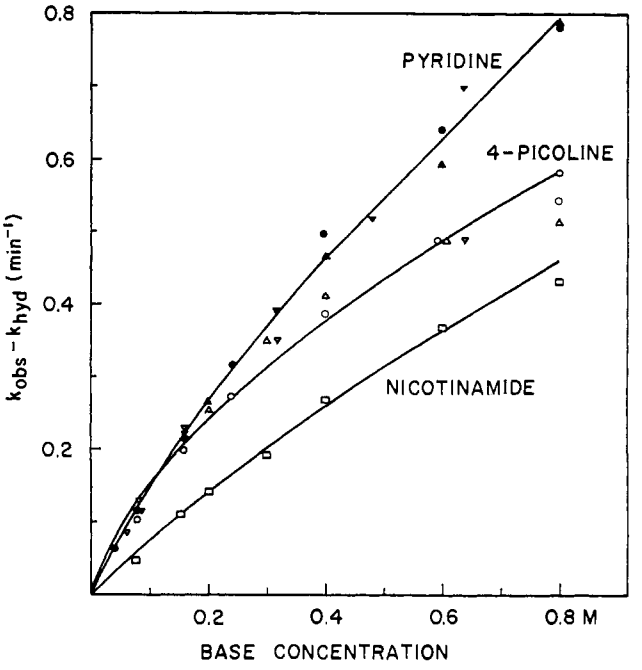


Figure 2. Rates of hydrolysis reactions as a function of the concentrations of heterocyclic bases at 39°, ionic strength 1.0. ▲, △, □: hydrolysis of *p*-nitrophenyl phosphate, $k \times 10^5$; ●, ○: hydrolysis of *p*-nitrophenyl acetate (0.05 *M* Tris buffer, pH 9.3), $k \times 3.3$ for pyridine, $k \times 0.42$ for 4-picoline; ▼, ▽: hydrolysis of phosphoramidate in buffers of 80% free base, pH 6.0, $k \times 1.9$ for pyridine, pH 6.8, $k \times 1.8$ for 4-picoline. The curves are calculated from the equation, rate = k_2B (see text).

The rate of formation of the phosphoramidate by attack on phosphorus was generally slower than its expected rate of hydrolysis, so that the formation of a substituted phosphoramidate was difficult to demonstrate in most reactions. The reaction of *p*-nitrophenyl phosphate with 1 *M* methylamine and 1 *M* hydrazine at pH 11.9 and 39° for 18,540 min. gave yields of 29 and 34% inorganic phosphate and 44 and 61% substituted phosphoramidate, respectively, measured by the Fiske and Subbarow and ethylene glycol methods.[8] The reaction of 1 *M* pyridine at pH 10.54 for 18,540 min. gave only 18% inorganic phosphate. *p*-Nitrophenol was identified by its absorption spectrum at several pH values.

In order to determine the position of bond cleavage, the solvolysis of the *p*-nitrophenyl phosphate dianion was followed at 99 ± 1° in water and in 99% ethylene glycol, in the presence of 0.01 *M* potassium hydroxide. Solvolysis proceeded with rate constants of 3.6 ×

(7) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, 82, 675 (1960).

(8) W. P. Jencks and M. Gilchrist, *ibid.*, 87, 3199 (1965).

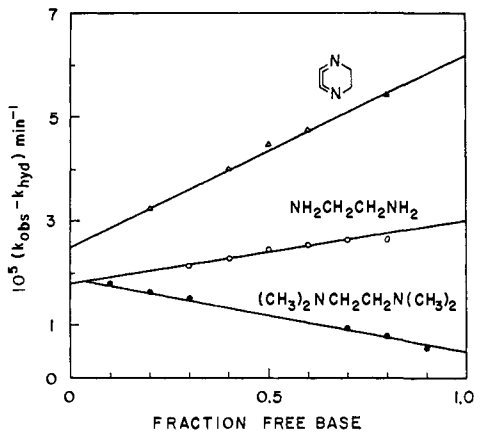


Figure 3. Rates of reaction of *p*-nitrophenyl phosphate with three diamino compounds, at 39°, ionic strength 1.0, and total amine concentration 1.0 *M*; as a function of per cent of free base.

10^{-3} and 3.3×10^{-3} min.$^{-1}$, respectively, in the two solvents. Equal amounts of *p*-nitrophenol were formed in the two solvents, but the yield of inorganic phosphate in the ethylene glycol solution was only 4% of that in water, which shows that solvolysis proceeds with at least 96% P–O cleavage to give the phosphate ester.

Results

Nucleophilic Attack. The rate constants for the reactions of 30 amines with *p*-nitrophenyl phosphate dianion to give *p*-nitrophenolate ion, in aqueous solution at 39° and ionic strength 1.0, determined from measurements of initial rates as a function of amine concentration, are given in Table I. The rate constants have been corrected, if necessary, for substituted aniline formation caused by attack at the aromatic ring, as described in Experimental. The reactions are slow, with half-lives of weeks in the presence of 1 *M* amine. No reaction was detected with five amines (for details see Table I): tris(hydroxymethyl)aminomethane (Tris), 2,6-lutidine, 2-methylpiperidine, aniline, and methoxylamine.

The dependence on pH of the reactions with diamino compounds, with the exception of hydrazine and imidazole, shows that both the free base and the monocation are active nucleophilic reagents toward *p*-nitrophenyl phosphate dianion (Figure 3). The reactions with the monocations almost certainly do not represent the kinetically indistinguishable reactions of the amine–free base with the *p*-nitrophenyl phosphate monoanion, because no reaction of a number of other amines with *p*-nitrophenyl phosphate monoanion can be detected.[3,9] In most cases the reaction of the free base is faster than that of the monocation, but in the case of N,N,N′,N′-tetramethylethylenediamine the monocation is more reactive than the free base.

At high amine concentrations, the rates of reaction of *p*-nitrophenyl phosphate with pyridine and substituted pyridines do not increase linearly with increasing pyridine concentration (Figure 2). This does not appear to be a specific interaction of pyridines with *p*-nitrophenyl phosphate because a similar nonlinearity is found for the reactions of the same amines with phosphoramidate monoanion and with *p*-nitrophenyl acetate (Figure 2). The decrease in the apparent second-order rate constant with increasing pyridine concentration, therefore, represents a self-interaction effect of the substituted pyridines, which is most marked for 4-methylpyridine and least for nicotinamide. The results may be described with moderate success by assuming that the pyridines undergo self-association in aqueous solution to an unreactive dimer, with an association constant *K*. The concentration of reactive monomer, B, is then given in terms of total amine concentration, B_T, by eq. 1. The solid lines

$$[B]^2 + [B]/2K - [B_T]/2K = 0 \qquad (1)$$

shown in Figure 2, which were calculated using values of *K* of 0.35, 0.5, and 3.0 M^{-1} for nicotinamide, pyridine, and 4-methylpyridine, respectively, show reasonably good agreement with the experimental data for the reactions with both phosphate compounds and with *p*-nitrophenyl acetate. It is probable that the situation is more complex than indicated by this treatment and, in fact, a somewhat better fit is obtained if the data are calculated assuming multiple equilibria for pyridine self-association; however, the data are not of sufficient accuracy to justify a more complete treatment. A similar, but more marked leveling off of the rate with increasing amine concentration was observed in the reaction of acetyl phenyl phosphate with collidine and lutidine.[10] It is probable that this represents a more powerful manifestation of the same effect. It is known that the activity coefficient of pyridine in dilute aqueous solution is much larger than in pure pyridine and decreases rapidly as the pyridine concentration is increased; the same is true to an even greater extent for methyl-substituted pyridines.[11] This behavior of substituted pyridines would seem to represent a "hydrophobic" interaction.[12]

Hydrolysis. The dependence on pH of the rate of uncatalyzed hydrolysis of *p*-nitrophenyl phosphate at 39° is shown in Figure 1 and Table II. In addition to a reaction of the monoanion at low pH values, there is a pH-independent hydrolysis of the dianionic species between pH 9 and 13 and a reaction of the dianion with hydroxide ion in strong alkali. Desjobert has shown previously[13] that the rate of hydrolysis of *p*-nitrophenyl phosphate is independent of pH between pH 9 and 13 at 100°, and Bender and Lawlor[14] have reported a rate constant for hydrolysis of the dianion at pH 9.99. An Arrhenius plot of the data of Table II for the hydrolysis of the dianion at three temperatures and also of a rate constant for hydrolysis at 100°, from previously reported data,[13,14] is shown in Figure 4; values of ΔH^* and ΔS^* of 30.6 kcal./mole and +3.5 e.u., respectively, are obtained from these data. The rate of dianion hydrolysis is only slightly de-

(9) See footnote *k*, Table I. A reaction of an amine with *p*-nitrophenyl phosphate monoanion would be expected to follow the rate law

$$\text{rate} = k[RNH_2][PNPP^-] = k'[RNH_3^+][PNPP^{2-}]$$

No reaction corresponding to this rate law was found for a number of monoamines which were examined over a range of pH values.

(10) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, **83**, 4396 (1961), Table I.

(11) (a) R. J. L. Andon, J. D. Cox, and E. F. G. Herington, *J. Chem. Soc.*, 3188 (1954); (b) N. Ibl, G. Dändliker, and G. Trümpler, *Helv. Chim. Acta*, **37**, 1661 (1954).

(12) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

(13) A. Desjobert, *Bull. soc. chim. France*, 683 (1963).

(14) M. L. Bender and J. M. Lawlor, *J. Am. Chem. Soc.*, **85**, 3010 (1963).

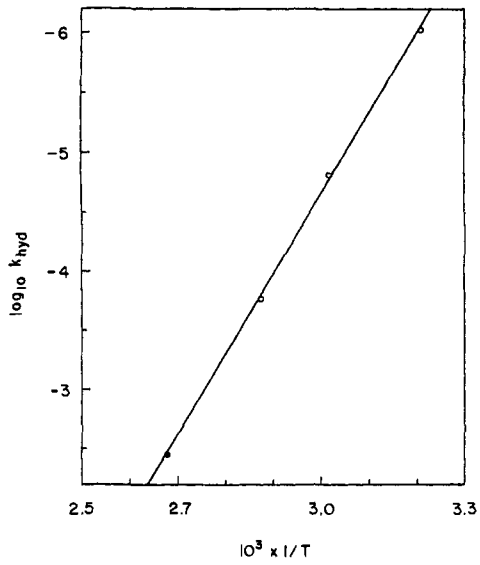


Figure 4. Arrhenius plot for the hydrolysis of *p*-nitrophenyl phosphate at pH 10.2 and ionic strength 1.0, for 39, 58, and 75°. The closed circle is from previously reported results at pH 10 and 100°.

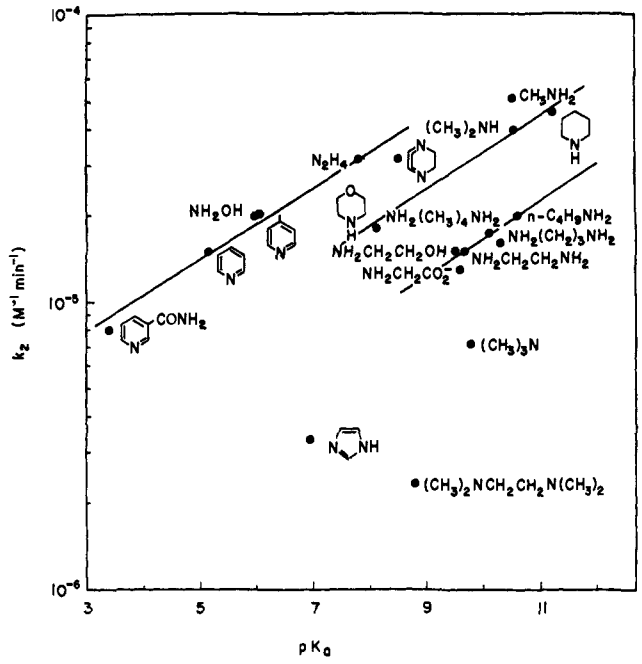


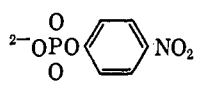
Figure 5. Second-order rate constants for reactions of amines with the *p*-nitrophenyl phosphate dianion at 39° and ionic strength 1.0, plotted against the pK_a of the attacking amine. Values for diamines are corrected for statistical factors by halving the given values for K_a and k.

creased in deuterium oxide solution. The rate of hydrolysis shows a small increase with increasing salt concentration but is very little affected by a number of different salts, some of which are effective nucleophilic agents toward other compounds (Table II). Solvolysis of the dianion at 99 ± 1° in ethylene glycol proceeds at almost the same rate (3.3×10^{-3} min.$^{-1}$) as in water (3.6×10^{-3} min.$^{-1}$) and gives nitrophenol and the phosphate ester as products, which shows that the reaction proceeds with P–O bond cleavage.

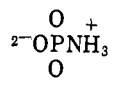
In view of the well-known resistance of phosphate ester dianions to attack by hydroxide ion, it is probable that the slow reaction of *p*-nitrophenyl phosphate with hydroxide ion represents attack at the aromatic ring with C–O bond cleavage.[6] This interpretation is supported by the fact that the rate constant for this reaction, 4.9×10^{-7} M^{-1} min.$^{-1}$, is similar to that for the reaction of hydroxide ion with 1-chloro-4-nitrobenzene in 16.7% dioxane, 1.34×10^{-6} M^{-1} min.$^{-1}$; it is probable that attack on the aromatic ring is rate determining in both cases.[6]

Discussion

Nucleophilic Reactivity. The order of nucleophilic reactivity toward the dianion of *p*-nitrophenyl phosphate (I) is similar to that toward the monoanion of phosphoramidate (II),[8] and the reactions with both compounds will be discussed here. In both cases the



I



II

phosphorus atom is bound to three unsubstituted oxygen atoms, which carry two negative charges; the difference in the net charge of the two compounds is the result of the positive charge on the leaving group of phosphoramidate. The rate constants for the reactions with *p*-nitrophenyl phosphate dianion are plotted logarithmically against the basicity of the amine in Figure 5. It is probable that the results are representative of those which may be expected for nucleophilic reactions with other monosubstituted phosphates, including those of biological importance. The order of nucleophilic reactivity is the same as that toward the phosphorus atom of the acetyl phosphate dianion for those compounds which have been examined with this substrate.[15] As might be expected, the two negative charges provide a considerable electrostatic barrier toward reactions with nucleophilic reagents which have a negative charge on the attacking atom. However, this barrier is not absolute, as shown by the reactions of fluoride ion with the monoanions of phosphoramidate and acetyl phosphate,[15] and a negative charge several atoms removed from the reaction center, as in glycine, does not have a large effect on the rate with either phosphoramidate or *p*-nitrophenyl phosphate.

The reactivity of nucleophilic reagents toward ionized phosphates shows moderate sensitivity to steric effects. The presence of an α-methyl group in a substituted pyridine eliminates detectable nucleophilic reactivity. Methylamine and piperidine are highly reactive, while 2-methylpiperidine and tris(hydroxymethyl)aminomethane are unreactive. Furthermore, the order of reactivity is dimethylamine ~ piperidine >> diethylamine, and triethylenediamine > trimethylamine > triethylamine, a series in which basicity actually increases. Steric hindrance is not of overriding importance, however, in view of the fact that *n*-butylamine reacts at an appreciable rate, and the order of reactivity of amines is generally tertiary > secondary > primary. This order of reactivity is the same as that found in a number of nucleophilic reactions and probably reflects an abnormally high basicity rather than an abnormally low reactivity of primary and secondary

(15) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, 83, 4393, 4400 (1961).

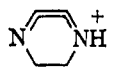
amines; the differences in the reactivities of primary, secondary, and tertiary amines largely disappear if the rate constants are plotted against σ^*[16] rather than against the pK of the amine. Hydroxylamine and hydrazine, compounds which exhibit the "α-effect,"[17] exhibit a reactivity which falls well above the line for other primary amines. It is not known whether it is the oxygen or the nitrogen atom of hydroxylamine that reacts with *p*-nitrophenyl phosphate.

It is of interest that imidazole is less reactive than pyridine toward phosphoramidate and *p*-nitrophenyl phosphate. Since the order of reactivity is opposite for acyl groups, this provides an explanation for the fact that imidazole reacts with the acyl group whereas pyridine reacts with the phosphoryl group of acetyl phosphate.[15] However, the reason for this difference in reactivity is not apparent. Imidazole is more reactive than pyridine in reactions with fully substituted dialkyl halogenophosphates[18] and in general base catalyzed hydrolysis of methyl ethylene phosphate,[19] presumably because of its greater basicity.

The most striking characteristic of the reactions of nucleophilic reagents with these ionized phosphates is the very small sensitivity of the rates of the reactions to the basicity of the attacking nucleophilic reagent. Such a low sensitivity was suggested previously by the relatively small difference between the rates of reaction of pyridine and of 4-methylpyridine with phosphoramidate[3] and acetyl phosphate.[15] The Brønsted slope, β, of a logarithmic plot of rate constant against basicity for the reaction of substituted pyridines with phosphoramidate is 0.22 (ref. 8, Figure 1), and the rates of reaction with phosphoramidate of the other amines which were examined show only a small dependence on the basicity of the amine.[8] The more extensive data obtained with *p*-nitrophenyl phosphate dianion (Figure 5) show a tendency for the reactivities of primary, secondary, and tertiary amines to fall into distinct groups, and there is an increase in reaction rate with increasing basicity within each group. However, as with phosphoramidate, the sensitivity to basicity is small and the points fall near lines of slope 0.13. There is no indication of an accompanying high sensitivity to polarizability; a number of effective, highly polarizable nucleophilic reagents toward carbon do not react with ionized phosphates, and fluoride ion, which has a low polarizability, has a high reactivity toward phosphate.

This small sensitivity to basicity is in marked contrast to the results of previous studies of nucleophilic reactivity toward fully substituted phosphate compounds which, like acyl groups, are highly sensitive to the basicity of the nucleophilic reagent.[20-23] The Brønsted slopes, β, for the reactions of isopropyl methylphosphonofluoridate with hydroxamic acids,[20] oximes,[21] and phenols,[22] are all greater than 0.5, and in most cases are close to 0.80. The Brønsted slopes for the reactions of pyridines with acetic anhydride[24] and *p*-nitrophenyl acetate[25] are 0.8–0.9, and even the reaction of allyl bromide with substituted pyridines[26] exhibits a Brønsted slope of 0.37. The corresponding slope for the reaction of pyridines with ethyl methanesulfonate is 0.11, and it has been suggested that this decrease is correlated with increasing monomolecular character of the reaction compared to the values of 0.2–0.32 for other displacements by pyridine.[27] The low sensitivity to basicity of the reactions with the ionized phosphates suggests that, in contrast to the reactions of substituted phosphates, only a small amount of bond formation has taken place in the transition state. A similar low sensitivity to basicity has been observed in nucleophilic reactions with N,N,N′,N′-tetramethylphosphorodiamidic chloride.[28] Although it has been suggested that the solvolysis of this compound occurs by a monomolecular mechanism with the intermediate formation of the metaphosphate-like ion, $((CH_3)_2N^{\cdots})_2{}^+PO$, the existence of nucleophilic reactions suggests that its reactions may be described according to a bimolecular mechanism in which little bond formation to the nucleophilic reagent has occurred in the transition state.[28]

The rates of the reactions of monocations of diamines with the dianion of *p*-nitrophenyl phosphate are abnormally rapid, and the rapid rate is even more striking if allowance is made for the statistical effect introduced by the presence of two nitrogen atoms in the corresponding free diamine bases. In the case of tetramethylethylenediamine, the monocation (pK = 5.7) reacts even more rapidly than the free base (pK = 9.1). At first sight, this high reactivity of the monocation might be attributed to hydrogen bonding between the protonated amine and the substrate, but this explanation appears to be untenable, in view of the high reactivity of the conjugate acid of triethylenediamine

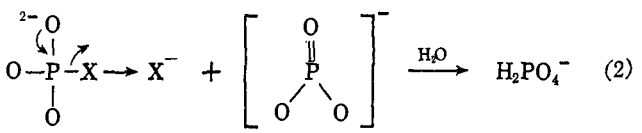


which is sterically prevented from taking part in such hydrogen bonding. It appears, therefore, that the enhanced reactivity of these monocations results from electrostatic attraction to the two negative charges of the phosphate ester. In view of the low sensitivity of the reaction to the basicity of the nucleophile and the fact that the rate constant for reaction of the monocation may be even higher than that of the corresponding free base, the rate enhancement cannot be ascribed to an effect of the positive charge on the basicity of the amine. Epstein, *et al.*, have reported that certain phenols which carry a positively charged substituent

(16) H. K. Hall, Jr., *J. Am. Chem. Soc.*, **79**, 5441 (1957).
(17) J. O. Edwards and R. G. Pearson, *ibid.*, **84**, 16 (1962).
(18) T. Wagner-Jauregg and B. E. Hackley, Jr., *ibid.*, **75**, 2125 (1953).
(19) F. H. Westheimer, *ibid.*, **85**, 1773 (1963).
(20) (a) G. F. Endres and J. Epstein, *J. Org. Chem.*, **24**, 1497 (1959); (b) R. W. Swidler, R. E. Plapinger, and G. M. Steinberg, *J. Am. Chem. Soc.*, **81**, 3271 (1959).
(21) A. L. Green, G. L. Sainsbury, B. Saville, and M. Stansfield, *J. Chem. Soc.*, 1583 (1958).
(22) (a) J. Epstein, D. H. Rosenblatt, and M. M. Demek, *J. Am. Chem. Soc.*, **78**, 341 (1956); (b) J. Epstein, R. E. Plapinger, H. O. Michel, J. R. Cable, R. A. Stephani, R. J. Hester, C. Billington, Jr., and G. R. List, *ibid.*, **86**, 3075 (1964); (c) J. Epstein, H. O. Michel, D. H. Rosenblatt, R. E. Plapinger, R. A. Stephani, and E. Cook, *ibid.*, **86**, 4959 (1964).
(23) B. Miller, *ibid.*, **84**, 403 (1962).
(24) V. Gold and E. G. Jefferson, *J. Chem. Soc.*, 1409 (1953).
(25) T. C. Bruice and R. Lapinski, *J. Am. Chem. Soc.*, **80**, 2265 (1958).
(26) K. Clarke and K. Rothwell, *J. Chem. Soc.*, 1885 (1960).
(27) R. F. Hudson and R. J. Withey, *ibid.*, 3513 (1964).
(28) (a) H. K. Hall, Jr., *J. Org. Chem.*, **21**, 248 (1956); (b) D. Samuel and F. H. Westheimer, *Chem. Ind.* (London), 51 (1959); (c) E. W. Crunden and R. F. Hudson, *J. Chem. Soc.*, 3591 (1962); (d) H. K. Hall, Jr., *J. Org. Chem.*, **28**, 2818 (1963); (e) P. S. Traylor and F. H. Westheimer, *J. Am. Chem. Soc.*, 87, 553 (1965).

exhibit an unusually large reactivity toward isopropyl methylphosphonofluoridate.[22]

Hydrolysis. The hydrolysis of the dianion of *p*-nitrophenyl phosphate, while considerably slower than that of the monoanion, still proceeds at a significant rate at 39° (Figure 1). There is evidence which suggests that the solvolysis of the monoanions of phosphate esters,[29] acyl phosphates,[15] and related compounds[30] proceeds by a mechanism which involves proton transfer to the leaving group and has many of the characteristics of a monomolecular reaction; because of the requirement for proton transfer, the rates of such reactions display little sensitivity to the nature of the leaving group.[15,31] It has been suggested that the solvolysis of the dianions of acyl phosphates proceeds by a similar monomolecular elimination mechanism to give a monomeric metaphosphate intermediate, which is rapidly hydrated to inorganic phosphate (eq. 2).[15] These dianion reactions exhibit



(2)

a large sensitivity to the nature of the leaving group because there is no requirement for proton transfer.[15] The dianion of *p*-nitrophenyl phosphate might be expected to undergo solvolysis by a similar mechanism because the *p*-nitrophenolate ion is only a few powers of ten more basic than benzoate. Indeed, the rate of hydrolysis of *p*-nitrophenyl phosphate dianion falls on the same line as the rates of hydrolysis of the dianions of substituted benzoyl phosphates in a plot of log k against the pK of the leaving group (Figure 6[32]). The large slope of this line, -1.2, is more than twice that for the reaction with hydroxide ion of a series of fully substituted phosphates with different leaving groups, 0.43.[33] The reactions are also similar in that there is a small or negligible solvent deuterium isotope effect, an insensitivity to the nature of the solvent, and a small, positive entropy of activation in both cases. The incomplete evidence which is available indicates that the rates of reactions of phosphoramidates also increase rapidly with increasing electrophilic character of the leaving group; the rate increase is particularly marked if the leaving group has two proton acceptor sites, so that two positive charges may be formed on the leaving group.[8,34]

The mechanism of other phosphate dianion solvolyses in which there is a good leaving group may be interpreted in a similar way. A particularly striking example is unsymmetrical diethyl pyrophosphate, which undergoes solvolysis at a rate which is too fast to measure.[35] Similarly, phosphorochloridate ($^{2-}O_3PCl$)

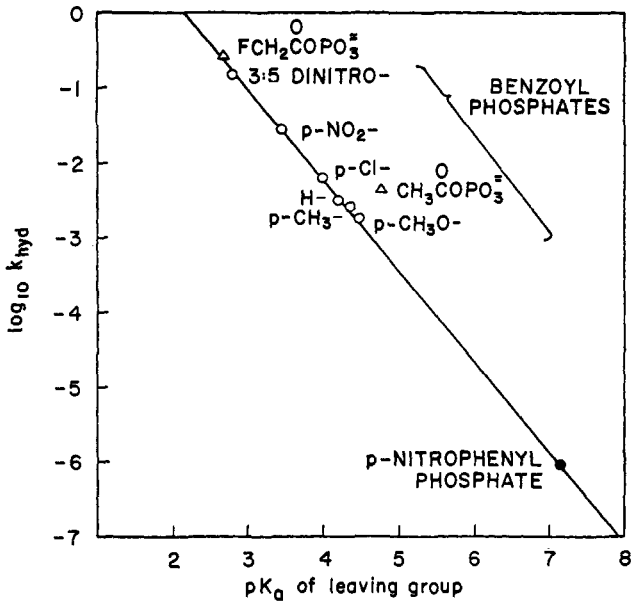
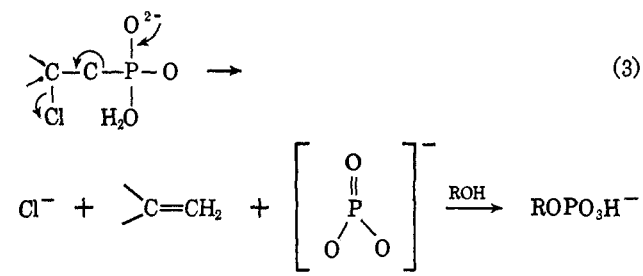


Figure 6. Logarithmic plot of hydrolysis rates at 39° for substituted acetyl (△) and benzoyl (○) phosphate dianions and for *p*-nitrophenyl phosphate dianion (●). The point for fluoroacetyl phosphate is based on the ratio of 63 for its rate of hydrolysis to that of acetyl phosphate, both measured at 24°.[32]

undergoes extremely rapid hydrolysis at a rate which is faster than that of phosphorodichloridate ($^{-}O_2PCl_2$).[36] A mechanism of this kind may contribute to the rapid hydrolysis of 2,4-dinitrophenyl phosphate, a suggested intermediate in uncoupled oxidative phosphorylation, which undergoes hydrolysis too rapidly to permit its isolation.[37] 2-Chloroalkylphosphonic acids undergo a very rapid solvolysis as the dianion, although the monoesters are comparatively stable, and an elimination mechanism provides an attractive mechanism for this reaction[38] (eq. 3). Maynard and Swan have argued that this reaction proceeds by a bimolecular



(3)

mechanism because solvolysis of 2-chloroalkylphosphonates in the presence of alcohol gives phosphate esters, whereas a metaphosphate intermediate would not be expected to be an effective phosphorylating agent for alcohols, and solvolysis does not proceed by this route in aprotic solvents in the presence of base.[38] We do not find these arguments convincing, because the great instability of anions in aprotic solvents[39] would be

(29) W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, 77, 2420 (1955); C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3574 (1958).
(30) (a) R. F. Hudson, *Advan. Inorg. Chem. Radiochem.*, 5, 347 (1963); (b) J. R. Cox, Jr., and O. B. Ramsay, *Chem. Rev.*, 64, 317 (1964).
(31) C. A. Vernon, Special Publication No. 8, The Chemical Society, London, England, 1957, p. 17.
(32) A. Marcus and W. B. Elliott, *J. Am. Chem. Soc.*, 80, 4287 (1958).
(33) D. F. Heath, "Organophosphorus Poisons," Pergamon Press, New York, N. Y., 1961, p. 79.
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(35) D. M. Brown and N. K. Hamer, *J. Chem. Soc.*, 1155 (1960).
(36) R. F. Hudson and G. Moss, *ibid.*, 3599 (1962).
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expected to inhibit the formation of the reactive dianion in such solvents and there does not seem to be good reason to believe that metaphosphate would not be an effective phosphorylating agent for alcohols; a monomolecular mechanism (eq. 3) may, therefore, warrant further consideration as a possible mechanism for this reaction.

On the other hand, Chanley and Feageson have shown conclusively by analysis of the products of the reaction in aqueous alcohol that the solvolysis of phosphoramidate monoanion, which might be expected to proceed through a similar elimination mechanism (eq. 2), does not give a free metaphosphate anion as a metastable intermediate.[3] The same method has ruled out such a free intermediate in the formaldehyde- and hypochlorite-assisted solvolyses of phosphoramidate, although the latter reactions show one characteristic of monomolecular reactions in that they are insensitive to nucleophilic reagents which react with phosphoramidate itself.[34] Furthermore, the fact that phosphoramidate monoanion[3,8,40] and the acetyl phosphate[15] and *p*-nitrophenyl phosphate dianions undergo reactions with nucleophilic reagents suggests that the solvolyses of these compounds may also involve attack by water.

This somewhat unsatisfactory state of affairs is, in fact, closely similar to that which exists in carbon chemistry. Although there are many reactions of carbon compounds which are clearly bimolecular and some which have many properties ascribed to monomolecular, carbonium ion reactions, carbonium ion reactions do not occur readily in the gas phase, and in many reactions the "carbonium ion" is so closely associated with the leaving group or the replacing group that the reaction is of mixed character and displays only some of the characteristics of a monomolecular reaction.[41] In phosphate chemistry, substitution reactions at fully substituted phosphate or phosphonate esters display a large sensitivity to the nucleophilic reactivity of the attacking reagent and are clearly bimolecular while, at the other extreme, most phosphate monoester monoanions and the formaldehyde- and hypochlorite-assisted solvolyses of phosphoramidate display little or no selectivity or kinetic sensitivity to the nature of the nucleophilic reagent. The dianion reactions, like the solvolysis of N,N,N′,N′-tetramethylphosphorodiamidic chloride,[28] are assisted by the presence of a nucleophilic reagent but display only a very small sensitivity to its nucleophilic reactivity. These reactions, then, may be regarded as intermediate in character and derive their driving force principally by electron donation from the atoms attached to phosphorus and electron withdrawal by the leaving group.

This conclusion suggests that enhancement of the reactivity of nucleophilic groups at the active site of an enzyme, either by special properties of these groups or by general base catalysis, will not, in itself, make a large contribution to the rate enhancements brought about in enzymatic catalysis of reactions of phosphate dianions. More effective catalysis might be brought about by the enzyme by enhancing the leaving ability of the leaving group, by reduction of the activation energy through distortion or compression of the substrate(s), or by bringing about a change in the mechanism of the reaction, perhaps by metal ion catalysis. Binding of a metal ion to a phosphate would be expected to make the compound more sensitive to nucleophilic attack, like fully substituted phosphates, by neutralizing the anionic centers.

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