Mechanism of Hydrolysis of Phosphorylethanolamine Diesters. Intramolecular Nucleophilic Amine Participation

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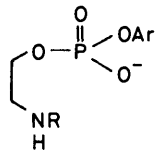
Intramolecular displacement reactions at phosphorus have been examined in a series of *N*-alkyl-*O*-arylphosphorylethanolamines in water at 35 °C. The examination of the pH–rate profiles and the direct observation by ^{31}P n.m.r. of the reaction products implicate a nucleophilic role for the amine. A rate enhancement of 10^6—10^7 is observed. Structure–reactivity correlations derived by changing the pK_a of the amine and leaving group yield values for $\beta_{nuc} \simeq 0.7$ and $\beta_{lg} \simeq -1.25$ and support an uncoupled concerted mechanism. A discussion of the mechanisms of nucleophilic reactions involving amines and oxyanions with inter- and intra-molecular phosphate di- and tri-esters is presented.

THE reactions of phosphate diesters represent an important process in biological systems and yet their uncatalysed hydrolysis [1] and bimolecular nucleophilic reactions [2] are quite slow.[3,†] Previous investigations involving neighbouring group participation have demonstrated intramolecular nucleophilic attack by carboxy or carboxylate,[4] hydroxy,[5] and pyridine-like amines.[6]

We have been interested in exploring the catalytic effect of an intramolecular amino-function on phosphate ester hydrolysis. Three different mechanisms, nucleophilic, general base, and electrostatic catalysis, have recently been demonstrated in amine-catalysed triester hydrolysis.[7] The results presented in this paper deal with the hydrolysis of a series of arylphosphorylethanolamines (1)—(3).



(1) R = Et

(2) R = $CH_2CH_2OCH_3$

(3) R = CH_2CF_3

a; Ar = C_6Cl_5

b; Ar = 2-Cl-4-$NO_2C_6H_3$

c; Ar = 2,4,6-$Cl_3C_6H_2$

d; Ar = 4-$NO_2C_6H_4$

e; Ar = 4-CNC_6H_4

f; Ar = 3-$NO_2C_6H_4$

g; Ar = C_6H_5

The structure–reactivity correlations derived by changing amine and leaving group acidities support an uncoupled nucleophilic mechanism and permit a tentative assignment of the effective charges in the transition state. A comparison of the catalytic effects of the amino-group in the di- and tri-ester leads to a much greater effect in the diester, to the extent that the phosphate diester reacts faster than the corresponding triester.

† For example, the second-order rate constant for the spontaneous hydrolysis of bis-*p*-nitrophenyl phosphate at 100 °C is only 6.81×10^{-8} l mol^{-1} min^{-1}.[1a]

EXPERIMENTAL

M.p.s were taken on either a Fisher–Johns or a Thomas Hoover apparatus and are uncorrected. Microanalyses were performed by MHW Laboratories, Phoenix, Arizona. I.r. spectra were obtained on a Perkin-Elmer model 267 instrument and were calibrated with polystyrene film. ^1H N.m.r. spectra were recorded on a Varian Associates A-60 instrument and chemical shifts (δ) are reported relative to internal tetramethylsilane. ^{31}P N.m.r. measurements were taken on a JEOL PS-100-FT spectrometer at 40.29 MHz and chemical shifts (δ) are reported in p.p.m. from external 85% H_3PO_4, downfield shifts being designated as positive. Mass spectra (70 eV) were measured on an MS-902 A.E.I. spectrometer.

Materials.—Boiled, doubly distilled, deionized water was used throughout. All chemicals and solvents were commercially available reagent grade materials and were generally either distilled or recrystallized. Inorganic salts and acetic acid were analytical grade and used without further purification. The preparation of *p*-nitrophenyl phenyl phosphate has been previously described.[7]

Preparation of 2-Alkylaminoethanols.—2-Ethylaminoethanol was purchased from Aldrich. 2-(2,2,2-Trifluoroethylamino)ethanol was prepared as described previously.[7] 2-(2-Methoxyethylamino)ethanol was prepared by slowly condensing ethylene oxide (10 ml, 0.2 mol) into a solution of 2-methoxyethylamine (19.5 g, 0.26 mol; Aldrich) in methanol (25 ml) at −5 °C over 1.5 h. After stirring at room temperature overnight, the methanol was removed by rotary evaporation and the product distilled at 98—100 °C at 6 mmHg to give a clear liquid (9.7 g, 41%), $\delta_H(CDCl_2)$ 2.75 (4 H, m, CH_2NCH_2), 3.37 (3 H, s, OCH_3), 3.51 (2 H, t, *J* 6 Hz, CH_2OMe), 3.65 (2 H, t, *J* 6 Hz, CH_2OH), and 3.8br (2 H, s, NH, OH); *m/e* 119, 88, and 74.

Preparation of Aryl Phosphorodichloridates.—These compounds were prepared by the method of Katyshkina and Kraft.[8] Typically a mixture of the pure phenol (0.1 mol), freshly distilled $POCl_3$ (0.5 mol), and dry NaCl (0.1 g) were refluxed for *ca.* 6 h. The excess of $POCl_3$ was removed under reduced pressure and the product distilled using a short path condenser under high vacuum. The products ob-

tained were: 2-chloro-4-nitrophenyl phosphorodichloridate (63%), b.p. 150 °C at 0.90 mmHg; *p*-nitrophenyl phosphorodichloridate (54%), b.p. 140 °C at 0.55 mmHg, which solidified to give crystals, m.p. 42—43 °C; *m*-nitrophenyl phosphorodichloridate (63%), 126 °C at 0.07 mmHg; *p*-cyanophenyl phosphorodichloridate (53%), 241 °C at 0.25 mmHg, which solidified to give crystals, m.p. 69—70 °C; 2,4,6-trichlorophenyl phosphorodichloridate (40%), 130 °C at 0.5 mmHg, which solidified to give crystals, m.p. 76—77 °C; and pentachlorophenyl phosphorodichloridate (37%) as crystals from heptane, m.p. 91—92 °C. Phenyl phosphorodichloridate was purchased from Aldrich. ^{1}H N.m.r., i.r., or mass spectra confirmed the structures.

Preparation of 3-Alkyl-2-aryloxy-2-oxo-1,3,2-oxazaphospholidines.—These compounds were prepared as described previously.[9] The structures of the isolated oils were confirmed by mass spectra containing a correct parent ion with a consistent fragmentation pattern. ^{1}H N.m.r. of the cyclic phosphoramidates contained resonances due to the particular phenol as well as the following ones in the aliphatic region: δ *ca.* 1.25 (3 H, t, *J* 7.5 Hz, CH_3), 2.9—3.7 (4 H, m, CH_2NCH_2), and 4.1—4.6 (2 H, m, CH_2O) for 2-ethylaminoethanol compounds; δ 3.3—4.1 (4 H, m, CH_2NCH_2) and 4.2—4.7 (2 H, m, CH_2O) for 2-(2,2,2-trifluoroethylamino)ethanol compounds; and δ 3.35 (3 H, s, OCH_3), 3.1—3.8 (4 H, m, CH_2NCH_2), and 4.2—4.7 (2 H, m, CH_2O) for 2-(2-methoxyethylamino)ethanol compounds.

The oil isolated for 3-ethyl-2-oxo-2-pentachlorophenoxy-1,3,2-oxazaphospholidine crystallized to give crystals, m.p. 178—179 °C. The synthesis of 3-ethyl-2-oxo-2-phenoxy-1,3,2-oxazaphospholidine has been previously reported.[7] Generally the crude product was employed without further purification in the following step.

Preparation of N-Alkyl-O-arylphosphorylethanolamine Hydrochlorides.—The desired esters were prepared by hydrolysis of a solution of the crude 3-alkyl-2-aryloxy-2-oxo-1,3,2-oxazaphospholidine (*ca.* 3 mmol) in CH_3CN (3 ml) and H_2O (3 mmol). If no crystals appeared after 1 h, the solution was acidified with anhydrous HCl and Et_2O added to precipitate the crude hydrochloride salt which was then recrystallized from either CH_3CN or EtOH–Et_2O. I.r. (KBr) spectra contained absorptions at *ca.* 2 980w (CH), 2 820w, 2 700m, and 2 480w (NH_2^+), 1 590m and 1 490s (Ar), 1 260s (P=O), 1 100s, 1 040s, 900s (P–O), 1 520s, and 1 350s (NO_2) for nitro-compounds and 2 230w (CN) cm^{-1} for cyano-compounds. Data for these compounds are presented in Table 1.

TABLE 1

Data for *N*-alkyl-*O*-arylphosphorylethanolamine hydrochlorides

Compound [a]	Yield (%) [b]	M.p. (°C)	Found (%)				Calc. (%)			
			C	H	N	Cl	C	H	N	Cl
(1a)	45	192—194	26.65	2.4	2.9	47.0	26.45	2.65	3.1	46.85
(1b) [c]	29	179—180	36.9	4.4	8.4		37.0	4.35	8.65	
(1c) [c]	56	219	34.7	3.75	3.75	29.85	34.45	3.75	4.0	30.5
(1d)	11	108—111	37.0	4.95	8.35		36.75	4.95	8.55	
(1e)	25	129—131	42.95	5.35	9.05		43.1	5.25	9.15	
(1f)	52	128—129	36.85	4.95	8.7		36.75	4.95	8.55	
(1g) [c]	48	197—199	48.75	6.5	5.5		49.0	6.6	5.7	
(2b)	44	127—128	34.0	4.4	7.4		33.75	4.4	7.15	
(3b)	20	193	28.9	2.8	6.85		28.95	2.65	6.75	
(3e)	75	147—148	36.25	3.7	7.5		36.65	3.65	7.75	

[a] Compound (2e) was prepared in *ca.* 5% yield, m.p. 70—80 °C which did not improve upon recrystallization. The hydrolysis was carried out on the crude salt. [b] No attempt was made to maximize the yield. Yields were based on the amount of crude oxazaphospholidine. [c] Isolated as the internal zwitterion.

Methods.—pH Measurements were taken with a Radiometer model 22 pH meter equipped with a model PHA 630 Pa scale expander and a GK2302C glass electrode at 35 °C. pK_a Determinations were performed on a Radiometer TTT1C autotitrator at an ionic strength of 1.0 (KCl) at 35 °C. A Gilford model 2000, 220, or 240 spectrophotometer equipped with a thermostatted cuvette holder (±0.1 °C) was used for kinetic measurements. Proton decoupled pulsed Fourier transform ^{31}P n.m.r. measurements were obtained on a solution (*ca.* 40mM) of the phosphodiester in 0 4M-buffer (2 ml) in a 10 mm n.m.r. tube. Buffers employed were acetate, fluoride, dimethylarsinate, ethanolamine, imidazole, n-butylamine, and triethylamine. The pH of the solution was measured before and after the hydrolysis. Spectra were recorded at ±30 p.p.m. of 85% H_3PO_4 using a 45° pulse and a repetition rate of 2.2 s. Good signal-to-noise ratios could be obtained within 20 s. A capillary tube filled with D_2O was inserted and used as the heteronuclear lock.

Kinetics.—Hydrolysis of the esters was carried out at 35 °C and μ 1.0 (KCl). Stock solutions (10^{-3}—10^{-2}M) were prepared in either 0.05M-acetate (pH 4.7) or methanol and stored frozen between experiments without decomposition. Reactions were initiated by addition of stock solution (*ca.* 0.02 ml) of the ester to buffer (2 ml) in a cuvette which had been equilibrated for 15 min in the cell holder. The hydrolysis of phosphates (1)—(3) was monitored for phenol release at either 400 (2-Cl-4-NO_2, *p*-NO_2), 392 (*m*-NO_2), 274 (*p*-CN), 289 (*p*-H), 322 (Cl_5), or 311 nm (2,4,6-Cl_3). Reactions were generally followed for at least three half-lives and infinity readings taken after ten, except for very slow reactions, which were followed by the method of initial rates. The pH of the medium was measured before and after each kinetic run at 35 °C; those runs exhibiting pH drift of greater than ±0.03 were discarded.

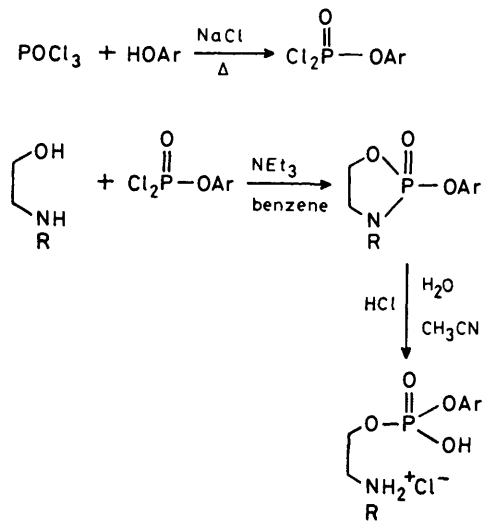
The observed pseudo-first-order rate constants were calculated from the slopes of linear plots of ln ($OD_\infty - OD_t$) against time. Buffers employed were n-butylamine, ethanolamine, Tris, imidazole, and acetate. Buffer concentrations ranged from 0.05 to 1.0M; however no buffer catalysis was detected. Experiments to examine Zn^{2+} catalysis were run at 0.05M-acetate and 0.05M-$Zn(ClO_4)_2$ at pH 5.5 with μ 0.20 ($NaClO_4$).

The intermolecular reactions of amines with *p*-nitrophenyl phenyl phosphate at 35 °C and μ 1.0 (KCl) were followed by the method of initial rates at 400 nm. Initial concentrations of the ester were *ca.* 5mM. The amine (1M)

was 50% in its free base form. A portion of the hydrolysis products was acidified to pH 3 and corrected for C–O bond cleavage due to aniline formation as described by Kirby and Younas.[2a]

RESULTS

Synthesis.—The general procedure for the synthesis of the desired arylphosphorylethanolamines (1)—(3) is shown in Scheme 1. The success of this synthesis is derived from



SCHEME 1

the lability of the P–N bond upon acidic hydrolysis to form the protonated β-amino phosphodiester which is relatively resistant to further hydrolysis.

Kinetics.—The hydrolyses of phosphate diesters (1)—(3) were investigated under pseudo-first-order conditions over the pH range 5—12 at 35 °C (μ 1.0, KCl). The reactions proceed with the stoicheiometric release of the phenol indicating complete P–O bond cleavage.

TABLE 2

Dissociation and rate constants for the hydrolysis of phosphate diesters (1)—(3) at 35 °C and μ 1.0 (KCl)

Compound	pK_a [a] amine	pK_a [b] XArOH	k_0 [c]/min^{-1}
(1a)	9.40	4.80	1.90
(1b)	9.40	5.45	3.10
(1c)	9.40	6.10	0.048
(1d)	9.40	7.15	0.16
(1e)	9.40	7.95	0.015
(1f)	9.40	8.35	0.0047
(1g)	9.40	9.99	6.1×10^{-6}
(2b)	8.37	5.45	0.63
(2e)	8.37	7.95	0.002 3
(3b)	4.60	5.45	0.002 3
(3e)	4.60	7.95	5.9×10^{-6}

[a] Determined by potentiometric titration. Values are accurate to ±0.04 pK_a units. [b] Values taken from A. J. Kirby and A. G. Varvoglis, *J. Amer. Chem. Soc.*, 1967, **89**, 415, and A. Albert and E. P. Seargent, 'The Determination of Ionization Constants,' Chapman and Hall, London, 1971. [c] Determined from plateau region of pH–rate profile.

The phosphodiesters all exhibit a sigmoidal pH dependence which is described by equation (1) where k_0 is the rate coefficient for amine assisted P–O bond cleavage and K_a is the dissociation constant for the amine in (1)—(3). The kinetically determined pK_a was equal to the thermodynamically measured pK_a within experimental error (±0.1). No buffer or Zn^{2+} catalysis was detected. The The pH–rate profile for (1b) is shown in Figure 1. Values for k_0 are listed in Table 2.

$$k_{obs} = k_0 \left(\frac{K_a}{K_a + a_H} \right) \quad (1)$$

The second-order rate constants for the reactions of

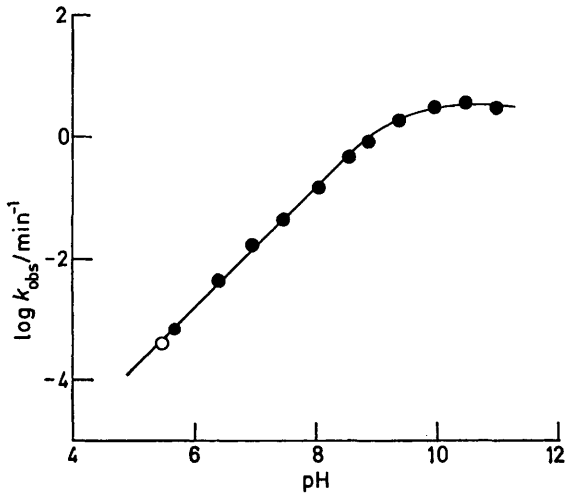
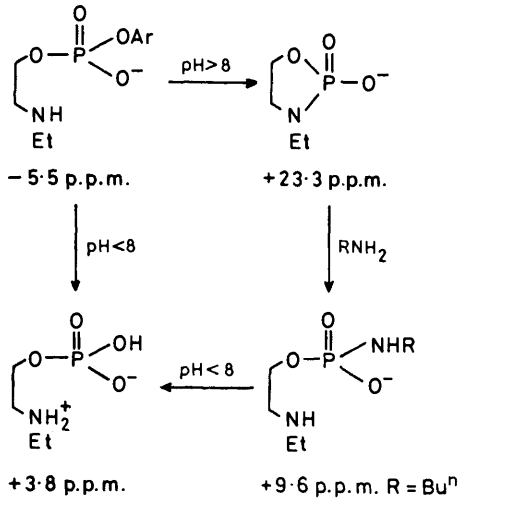


FIGURE 1 Dependence on pH of the observed pseudo-first-order rate constants for the hydrolysis of (1b) at 35 °C, μ 1.0 (KCl). The open circle represents the rate of hydrolysis in 0.05M-acetate and 0.05M-$Zn(ClO_4)_2 \cdot 6H_2O$ at pH 5.46

p-nitrophenyl phenyl phosphate with n-butylamine, ethanolamine, 2-ethylaminoethanol, and diethanolamine at 35 °C and μ 1.0 (KCl) give values of 5.4×10^{-5}, 1.35×10^{-5}, 1.6×10^{-6}, and 6.0×10^{-7} l mol^{-1} min^{-1}, respectively, for the loss of *p*-nitrophenol *via* P–O bond cleavage.

Products.—The products arising from hydrolysis of the phosphodiesters were analysed using proton decoupled ^{31}P



SCHEME 2

pulsed Fourier transform n.m.r. spectroscopy. A general scheme for the hydrolysis of (1) is shown in Scheme 2. In basic media (pH >8) the initial absorption at δ −5.5 p.p.m. for the diester is replaced by a resonance at +23.3 p.p.m.

corresponding to the cyclic phosphoramidate monoanion.[9] When the buffers employed were n-butylamine and ethanolamine, the signal at δ +23.3 p.p.m. is replaced by one at δ +9.6 and +9.2 p.p.m., respectively, corresponding to the acyclic phosphoramidate. The resonance at δ +23.3 p.p.m. is seen immediately when Ar = 2-chloro-4-nitrophenyl, however as the leaving group becomes poorer (Ar = *m*-nitrophenyl, phenyl) its rate of loss becomes slow relative to attack by the amine on the phosphoroamidate monoanion and only the acyclic phosphoramidate is observed. As the pH is lowered both the acyclic and the cyclic phosphoramidate are hydrolysed to the phosphate monoester (δ +3.8 p.p.m.).* When triethylamine was used as a buffer only signals at δ +23.3 and +3.8 p.p.m. could be detected since NEt_3 cannot form a stable phosphoramidate.

Other buffers (acetate, imidazole, dimethylarsinate, and fluoride) were used to study the hydrolysis at pH <8. The reactions were much slower (see Figure 1) but only resonances corresponding to the initial diester (δ5.5 p.p.m.) and the phosphate monoester (δ +3.8 p.p.m.) were detected since the decomposition of the cyclic phosphoramidate becomes faster than its formation with decreasing pH. No evidence for the formation of a phosphorofluoridate due to nucleophilic attack by fluoride, an exceptionally good nucleophile toward phosphorus, was detected in the ^{31}P n.m.r.

DISCUSSION

The pH–rate profiles for the hydrolyses of the aryl-ethanolamine phosphate diesters implicate the free base form of the amino-group and the phosphate monoanion or their kinetic equivalents as the reactive species. The direct observation by ^{31}P n.m.r. of formation of the cyclic phosphoramidate monoanion demonstrates that the amine is acting as a nucleophile and therefore removes the possibility of general base, specific base–general acid, or electrostatic catalysis.

The rate acceleration due to the amine group can be estimated in two ways. The final product of hydrolysis (pH <8) is the phosphate monoester so that the amino-group is acting as a true catalyst. A comparison of the rates of hydrolysis for (1b) and 2-chloro-4-nitrophenyl methyl phosphate or (1d) and methyl *p*-nitrophenyl phosphate gives values of *ca.* 4.1×10^6 and 1.2×10^7 respectively.† A more meaningful number is obtained upon comparison of an intramolecular *versus* an intermolecular reaction involving nucleophilic amine attack. An effective molarity of *ca.* 1.6×10^5M is calculated from a comparison of the rates of hydrolysis for (1d) and for *p*-nitrophenyl phenyl phosphate catalysed by a secondary aliphatic amine of the same pK_a.‡ Although the amine-catalysed hydrolysis of methyl *p*-nitrophenyl phosphate proceeds predominantly with C–O bond cleavage *via* substitution at the aromatic carbon, the measured rates for P–O bond cleavage at 39 °C with dimethylamine and piperidine are reported as 3.5×10^{-4} and 2.5×10^{-4} l mol^{-1} min^{-1}, respectively.[10] Thus a similar effective molarity of 1.5×10^5M is calculated from a comparison of the dimethylamine-catalysed hydrolysis of methyl *p*-nitrophenyl phosphate and the hydrolysis of the *p*-nitrophenyl ester of a phosphoryl-ethanolamine of the same pK_a.§ Either comparison is about a factor of 20-fold larger than the effective molarity of *ca.* 7 000 obtained for the hydrolysis of *p*-nitrophenyl quinolin-8-yl phosphate.[6]

A semiquantitative picture of the effective charges in the transition state for this reaction can be deduced from the respective Brönsted coefficients.[7] Brönsted plots of log k_0 *versus* $pK_{a\ amine}$ (β_{nuc}) and of log k_0 *versus* $pK_{a\ phenol}$ (β_{1g}) are shown in Figures 2 and 3 and yield

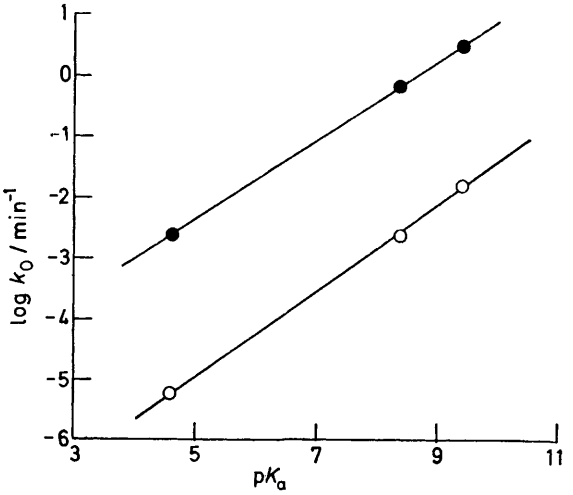


FIGURE 2 Brönsted plot for the rate constants for the hydrolysis of (1b)—(3b) (●) and (1e)—(3e) (○) *versus* the pK_a of the amine. The data were taken from Table 2 and fit the equations log $k_0 = 0.65pK_a - 5.62$ (●) and log $k_0 = 0.70pK_a - 8.48$ (○)

values for $\beta_{nuc} \simeq 0.70$ and $\beta_{1g} \simeq -1.25$, respectively. The values of β_{nuc} [0.65 for (1b)—(3b); 0.70 for (1e)—(3e)] are almost totally independent of the leaving group which is in accord with an uncoupled transition state; *i.e.* the degree of bond formation is not directly proportional to bond cleavage. Using an estimated value of $\beta_{eq} \simeq 1.2$ for complete phosphoryl transfer,[7] the changes in effective charge in the transition state for the intramolecular reaction are drawn in equation (2) and imply a very late transition state with respect to the leaving group but an earlier one with respect to the

* Phosphorylcholine has a resonance at δ +3.1 p.p.m.

† The calculation of the hydrolysis rates for 2-chloro-4-nitrophenyl methyl phosphate as well as methyl *p*-nitrophenyl phosphate was based on the rate of hydrolysis of 2,4-dinitrophenyl methyl phosphate at 39 °C [2a] assuming that $\beta_{1g} = -0.97$ from the rates of hydrolyses for a series of diaryl phosphate anions *versus* the pK_a of the phenol at 100°.[1a] There is only a three-fold change in reactivity between the aryl methyl and diaryl phosphate esters.[2a] An alternative comparison with bis-2-chloro-4-nitrophenyl phosphate or bis-*p*-nitrophenyl phosphate can be made assuming a temperature dependence for the rates of hydrolysis similar to bis-2,4-dinitrophenyl phosphate.[1a] This gives a rate acceleration of 2.9×10^6 and 7.7×10^6, respectively.

‡ This rate was derived from interpolation of the Brönsted plot (log $k_{a\ amine}$ *versus* $pK_{a\ amine}$) obtained from the reaction of *p*-nitrophenyl phenyl phosphate with 2-ethylaminoethanol and diethanolamine at 35 °C.

§ This was calculated based on a pK_a of 10.7 for dimethylamine and extrapolation of the rate of hydrolysis for (1d) to that pK_a assuming $\beta_{nuc} = 0.7$.

amine. The fact that the change in the effective charge on the amine is less than on the leaving group suggests that if a stable pentacovalent intermediate exists, its breakdown to product must be rate determining. This system is quite different from both the intermolecular reaction for primary amine attack on 2,4-dinitrophenyl methyl phosphate (β_{nuc} 0.47) [2a] or amines of varying classes on *p*-nitrophenyl methylphosphonate (β_{nuc} 0.35) [11a] and from the intramolecular attack by an amino-group

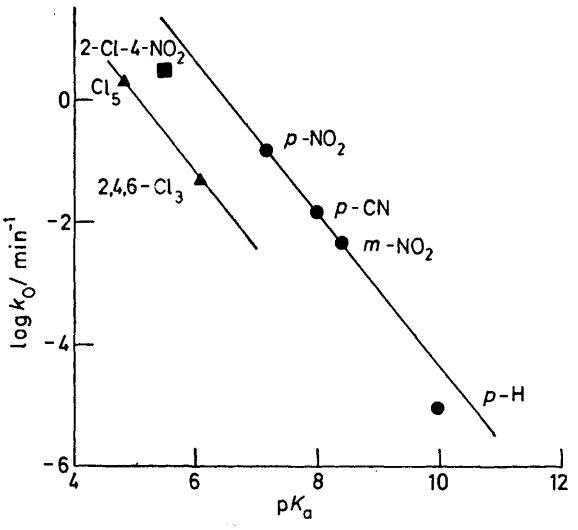
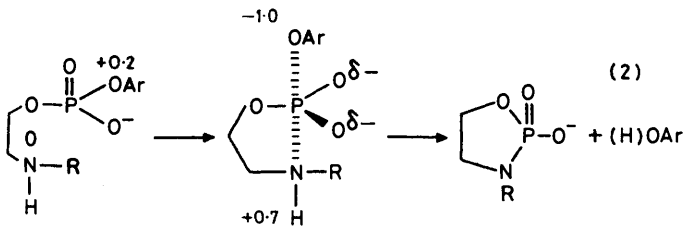


FIGURE 3 Brönsted plot for the rate constants for the hydrolysis of (1) *versus* the pK_a of the phenol; ●, no *ortho*-substituents; ■, one *ortho*-chloro-substituent; ▲, two *ortho*-chloro-substituents. The data were taken from Table 2 and fit the equation $\log k_0 = -1.29pK_a + 8.46$ (●) and $\log k_0 = -1.23$ pK_a + 6.19 (▲)

on a phosphotriester [7] where $\beta_{lg} \simeq -0.6$ and $\beta_{nuc} \simeq +0.3$. The latter occurs with concomitant external buffer catalysis to remove the amino-proton (β_{gb} +0.8). Apparently the two equatorial oxyanions in the transition state of the diester furnish sufficient driving force for leaving group expulsion so that the need for removal of the amine proton *via* external catalysis vanishes. They also act to uncouple the bond forming and breaking process; an extreme example is the attack of amines on substituted phenyl phosphate dianions where $\beta_{nuc} \simeq 0$ and $\beta_{lg} \simeq -1.0$. In this case the two oxyanions are



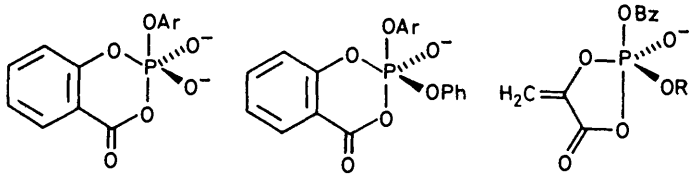
already present in the initial ground state for the reaction.[11b] In their absence as in the case of phenoxide attack on sultones, the values of $\beta_{nuc} \simeq \beta_{lg}$ in accord with a coupled concerted process.[11c] Although in the present case the amino-proton could be in equilibrium with the solvent, thus precluding any general base catalysis, or transferred to a neighbouring phosphoryl oxyanion the relatively high value of β_{nuc} indicates that the amine probably remains protonated in the transition state. The appearance of general base catalysis in the intramolecular triester system and its absence in the diester system is consistent with a postulate based on stereoelectronic control,[12] *i.e.* two lone pairs of electrons antiperiplanar to the leaving group are required for its expulsion.

A point of some importance is the effect of *ortho*-substitution on the departing phenol. The value of β_{lg} −1.23 for the pentachlorophenyl and 2,4,6-trichlorophenyl phosphates is within experimental error identical to β_{lg} −1.29 for the *para*- and *meta*-substituted compounds, indicative of the same general mechanism. However the rate of hydrolysis of the *o*-Cl_2 compounds (1a and c) is depressed by *ca.* 90-fold and the 2-Cl-4NO_2 compound (1b) by *ca.* 9-fold. Therefore the steric hindrance due to the introduction of an *ortho*-Cl results in a 9-fold decrease in rate and a second *o*-Cl depresses the rate by another factor of 9. Smaller steric effects due to *o*-Cl substituents have been encountered in phosphate monoester hydrolysis [13] which may represent a small degree of S_N2 character in the transition state of the reaction. Recently inhibitory effects have been observed for *ortho*-substitution for the rate-determining attack of amines on substituted diphenyl carbonates [14a] and for the nucleophilic attack by hydroxide and imidazole on substituted aryl trimethylacetates.[14b] This steric effect at the *ortho*-position demonstrates the necessity for structurally similar compounds in the construction of structure–reactivity correlations (*i.e.* Brönsted plots).

The data presented here and elsewhere permit a comparison of the effects of amine *versus* oxygen nucleophiles in both the inter- and intra-molecular reactions of phosphate di- and tri-esters. The rate accelerations due to intramolecular nucleophilic carboxylate attack in the reactions of aryl 2-carboxyphenyl phosphate diesters are *ca.* 10^7—10^8, *ca.* 10 times more effective than the present case.[4] There is considerable evidence for the involvement of pentacovalent species for intramolecular carboxylate attack in both the di- and tri-esters.[15] The observation of both endocyclic and exocyclic P–O bond cleavage products has been explained on the basis of pseudorotation from the pentacovalent intermediate (4b),[16] (5a), and (5b).[4a] The exclusive exocyclic displacement observed for (4a) has been attributed to the fact that in this case the respective intermediate cannot freely pseudorotate owing to the presence of two oxyanions in the equatorial position.[4b] Values of β_{lg} of −1.26 and −1.44 for (4a and b) likewise imply a late transition state with respect to the departing phenol, consistent with rate-determining breakdown of the pentacovalent intermediate. The values of β_{lg} ($\simeq -1.0$) for intermolecular reactions of oxyanions and phosphate di- [2a] and tri-esters,[17] although not as large as in the intramolecular systems are still greater than the values

of β_{nuc} ($\simeq$ 0.3) and suggestive of a late transition state relative to phenolate expulsion.

The hydrolysis of lactic acid *O*-phenyl phosphate as well as (4a) is subject to divalent metal ion catalysis.[18] The catalytic effect of the divalent metal ion has been ascribed to the stabilization of the pentacovalent intermediate (4a) and the concomitant facilitation of its breakdown. The value of $\beta_{lg} = -0.7$ for the Zn^{2+} catalysed reaction is considerably less than the spontaneous reaction. Collectively one can conclude that metal ion catalysis serves to lower the free energy of (4a) as well as move the associated transition state in accord with Hammond behaviour [19] to an earlier one along the reaction co-ordinate.

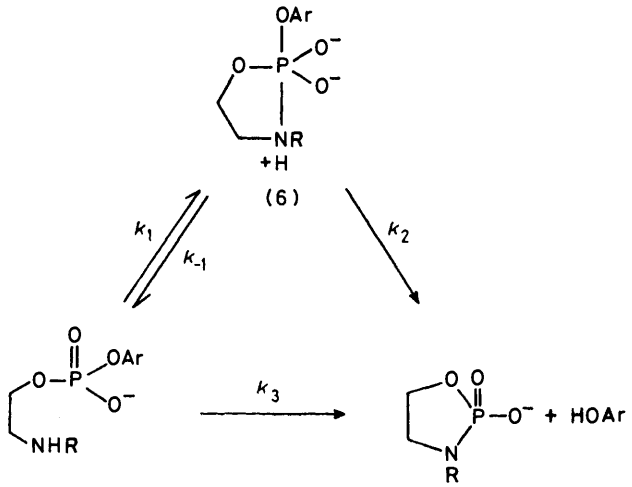


(4a) (4b) (5)

a; R = H

b; R = Bz

The question that arises in the intramolecular amine catalysed reactions of the diester is whether a stable pentacovalent intermediate (6) exists, *i.e.* is the reaction



SCHEME 3

stepwise or concerted (Scheme 3)? An estimate of 10^{12} s^{-1} for k_{-1} has been made for the corresponding intermediate formed from the phosphate triester.[7] In the case of the diester the presence of a second oxyanion in (6) should increase k_{-1} so that (6) probably does not exist as a stable intermediate. The absence of divalent metal ion catalysis may reflect the fact that (6) does not have a sufficient lifetime to be stabilized as for intramolecular cases with oxyanions. The metal ion catalysis observed in the hydrolysis of *O*-(*p*-nitrophenyl)-2-pyridylphosphonate [20] has been attributed to chelation.

The inhibitory effects observed for *ortho*-substitution also are in accord with the concerted pathway (k_3). If breakdown of (6) were rate limiting (k_2) *ortho*-substitution on the phenol should either have a slight accelerating effect on the hydrolysis due to the decrease in steric crowding in that transition state or a negligible one if the former is merely compensated for by an unfavourable equilibrium for the formation of (6). The observed decrease in reactivity would be consistent with a mechanism involving an increase in steric crowding in the transition state which would involve rate-determining amine attack (k_1 or k_3). However, the large values of k_{-1} and for β_{lg} are inconsistent with k_1 being rate determining. Thus in summary the most reasonable mechanism is the concerted one (k_3). This implies that displacement reactions by amines on phosphate diesters as with the triesters [7] occur *via* an in-line mechanism which would restrict the stereochemical course of the reaction to inversion.

The interpretation of the observed breaks in the structure–reactivity correlations in the aminolysis of phenyl acetates [21] and diaryl carbonates [14a] by Jencks and his co-workers involves the partitioning of a tetrahedral intermediate formed *via* nucleophilic attack; *i.e.* a change in the rate-determining step from rate-determining attack by the more basic amines (β_{nuc} 0.3, β_{lg} −0.2) to rate-determining breakdown to products with less basic amines (β_{nuc} 1.0, β_{lg} −1.3). The change in the rate-determining step occurs when the attacking amine is 4—5 pK units more basic than the departing phenolate. The authors concluded that the greater leaving ability from the tetrahedral intermediate of amines than of aryl oxide leaving groups of a given pK reflects the greater stability of the ester compared with the cationic amide that is the immediate reaction product.

Kinetic studies of the intermolecular aminolysis of phosphate di- and tri-esters and their corresponding intramolecular reactions give rise to linear structure correlations. The experimentally achieved ΔpK range between the amine nucleophile and the phenolate leaving group is *ca.* 4—5 units which may not be sufficient to observe the partitioning of a pentacovalent intermediate. Since the thermodynamic strength of a P–N bond (*ca.* 70 kcal mol^{-1}) is weaker than a P–O bond (*ca.* 91 kcal mol^{-1}) [22] the expected break in the Brönsted plot may occur where $\Delta pK > 5$ units. When both

attacking and leaving groups are oxyanions then $\Delta pK \simeq 0$ for a break in the structure–reactivity correlation based on symmetry arguments. Nevertheless the rates of reactions of phosphate triesters containing a six-membered ring with oxyanions [17] correlate linearly against the pK_a of the departing phenolate which is inconsistent with a stable pentacovalent intermediate in the intermolecular process.* This difference between the inter- and intra-molecular oxyanion reactions may reside in the anticipated greater stability of the pentacovalent intermediate in the latter owing to the removal of nonbonded repulsions arising between equatorial and axial ligands by incorporation into either a five- or a *constrained* six-membered ring. It has recently been suggested based on theoretical calculations that stereoelectronic catalysis may be responsible for the extra 6 kcal mol^{-1} stabilization of the five-membered cyclic *versus* the acyclic phosphate diester transition states.[24]

An unexpected observation is the rate enhancement of the amino-group in the diester relative to the triester. The rate of hydrolysis of (1b) is 3.1 min^{-1} whereas the rate of hydrolysis of the corresponding triester,[7] *O*-(2-chloro-4-nitrophenylphenylphosphoryl)-*N*-ethylethanolamine is 0.36 min^{-1}. Therefore the presence of the amino-group catalyses the rate of hydrolysis such that the diester is *ca.* 10 times more reactive than the triester. Typically phosphate triesters are much more reactive toward either intermolecular or intramolecular nucleophilic oxyanion attack than diesters. For example the values of the spontaneous hydrolysis rate constant for 2-carboxyphenyl 4-nitrophenyl phosphate,[4b] and 2,3-dicarboxyphenyl 4-nitrophenyl phenyl phosphate [10] are 0.60 and 60 min^{-1}, respectively, representing a 100-fold increase in the triester. In both cases the conversion of a di- to a tri-ester involves the introduction of a phenyl group. Therefore by changing the catalyst from a carboxy-group to an amino-group the diester has become more reactive than the triester by 1 000-fold. This phenomenon may reflect increased electrostatic stabilization between the protonated amino-group and the negatively charged phosphoryl oxygens in the di- *versus* tri-ester cases.

The results presented in this paper have two important biochemical implications. First, phosphoryl transfer reactions involving amino-groups present either in the substrate or at the active site of the enzyme should proceed stereochemically through an inversion process. Secondly, amine attack on a phosphodiester apparently requires no general base or metal ion catalysis at the phosphorus centre undergoing reaction.

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* The rates of reactions of the acyclic and six-membered ring triesters are similar in magnitude whereas the five-membered ring triesters are much faster. See ref. 23.

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