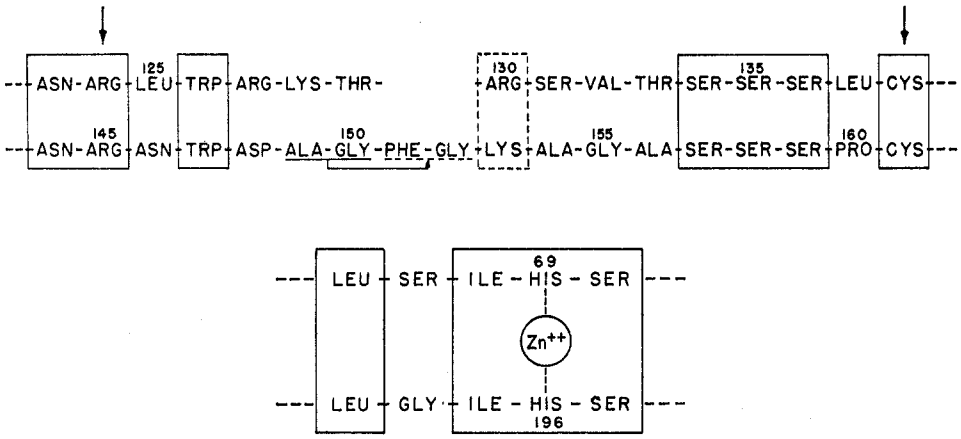

Figure 8. Comparison of the amino acid sequences surrounding the half-cystinyl residues in bovine carboxypeptidase A (upper) and the zinc-binding histidyl residues in bovine carboxypeptidase A (lower).

crevice containing the active-site residues, thus converting the endopeptidase enzyme into one with exopeptidase activity.

The second observation deals with the proposed binding site of the enzyme as deduced by chemical and X-ray analyses. As described above, residues 69 and 196 have been shown to be the histidyl residues binding the zinc. An examination of each of the sequences containing these histidyl residues, as shown in the lower part of Figure 8, shows pronounced similarity in that four of the five residues are identical. Thus it may be proposed that an extremely early genetic event may have resulted in partial or even entire gene duplication of the histidyl peptide sequence, resulting in a protein with two histidines of proper environment to bind the zinc metal. The preservation of this short sequence may be indicative of the requirements creating the appropriate orientation for the metal binding site. It should be noted that no apparent homology has been detected in the segments of polypeptide chain extending beyond that shown.

The observations described above give information about the evolutionary scheme proposed in Figure 6 only with regard to the appearance of endopeptidase activity going to exopeptidase activity and to the appearance of the metalloprotein from the protein–metal complex. The tenuous nature of the second observation is such that no detailed conclusions can be drawn regarding the early steps of the scheme. Further substantiation must be obtained by examination of proteins which would fit into the proposed scheme. For example, examination of other zinc metalloenzymes (such as carbonic anhydrase or thermolysin) would add substantial proof to the proposed scheme if they were found to contain similar structures. In this regard, it is significant that a histidine residue may be a zinc ligand in the carbonic anhydrase molecule, as judged by chemical modification experiments that indicate that one or possibly two histidines play an important role in the active site of this enzyme.[63,64] Solution of the primary and three-dimensional structures of these and other zinc metalloenzymes should supply the evidence needed to test the proposed hypotheses for the evolution of exopeptidase function.

(63) P. L. Whitney, G. Fölsch, P. O. Nyman, and B. G. Malmström, *J. Biol. Chem.*, **242**, 4206 (1967).
(64) S. L. Bradbury, *ibid.*, **244**, 2002 (1969).

Hydrolysis of Orthophosphate Esters

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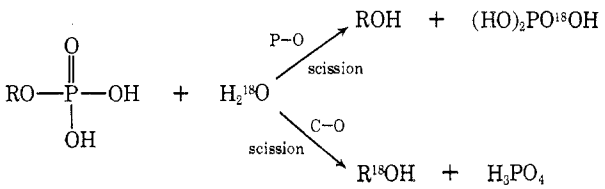
In many biological systems phosphate esters, *e.g.*, nucleotides, nucleic acids, and sugar phosphates, are important intermediates whose *in vivo* reactions are of considerable interest to biochemists; an understanding of the mechanisms of phosphate ester hydrolysis is of obvious biochemical significance. These mechanisms have intrinsic interest to the physical organic chemist. Orthophosphoric acid is tribasic, and its esterification can give not only trisubstituted esters but di- and monosubstituted esters whose acidic dissociation is governed by the pH of the medium (for mono- and disubstituted phosphates, $ROPO(OH)_2$ and

$(RO)_2PO(OH)$, pK_1 is generally about 1, and for disubstituted phosphates pK_2 is generally about 6.5). The degree of esterification and the pH of the reaction solution are therefore important mechanistic variables, and in addition structural variations in the alkyl or aryl groups can have important kinetic and mechanistic consequences.

In this Account, attention is restricted to the hydrolysis of acyclic orthophosphate esters derived from alcohols and phenols. Some important aspects of the subject are excluded, among them being metal ion and intramolecular catalysis, the hydrolysis of cyclic phosphates, and oxidative phosphorylations and hydrolyses. Much pioneering work was done by Bailly[1] and Desjobert,[2] who noted, for example, the importance of the pH of the reaction medium upon the rate of hydrolysis of monoalkyl phosphates. It is a pleasure to acknowledge their contributions as well as those of many other workers over the past two decades. Two recent reviews by Cox and Ramsay[3] and Bruice and Benkovic[4] are noteworthy; the latter authors in particular emphasize the biological significance of phosphate ester hydrolysis.

This Account first discusses the mechanisms of hydrolysis in terms of the extent of alkyl or aryl substitution into orthophosphoric acid and considers possible high-energy intermediates, *e.g.*, carbonium and metaphosphate ions, and the influence of pH on reaction mechanism, and then discusses the hydrolysis of aryl phosphates in moderately concentrated strong acids.

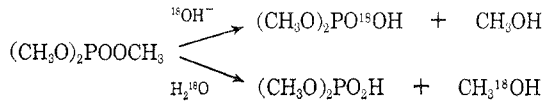
Tools of Investigation. The position of bond scission is of key importance, but it can in general be determined by using oxygen-18 as an isotopic tracer, *e.g.*



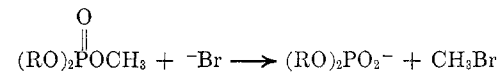
The nature and relative reactivities of reacting species, differing in their acidic dissociation, can be determined by examination of pH–rate profiles, and structural effects on reaction rate give evidence on steric and electronic influences on the various reaction mechanisms. Kinetic salt and solvent effects and variations of reaction rate with concentration of strong acid can also provide useful mechanistic information. Finally, trapping experiments can provide evidence, from reaction products, for high-energy intermediates.

Trisubstituted Esters

Reactions of trialkyl phosphates are simple. A nucleophile can attack either at phosphorus or at carbon, and the results fit the predictions of Pearson's theory of hard and soft reagents.[5] For example, hydroxide ion (a hard nucleophile) rapidly attacks the phosphoryl group of trimethyl phosphate, but water (a softer nucleophile) attacks the methyl group.[6,7]



Fluoride ion is very reactive toward phosphoryl phosphorus,[8-10] but the other halide ions attack the methyl group; for example, bromide or iodide ion in aprotic solvents are particularly effective reagents for removing one primary alkyl group from a trisubstituted phosphate, because the negative charge on the product effectively inhibits further nucleophilic attack.[11,12] The reaction can also be carried out using halide salts of amines under conditions in which the amine salt of the organic phosphate crystallizes out.[13]



Acids do not catalyze the hydrolysis of trimethyl phosphate in water, but if the attack of water upon the alkyl group is hindered, for example, by adding an organic solvent, an acid-catalyzed reaction which probably involves bimolecular attack upon the conjugate acid of the ester can be observed,[14] although the position of bond fission is not determined.

The attack of hydroxide ion upon the phosphoryl group could be a concerted SN2-like reaction or an addition followed by elimination. The low activation enthalpy, 9.6 kcal mol^{-1}, and the negative activation entropy of −38 eu for the reaction of triphenyl phosphate suggest an addition mechanism, but there is no oxygen exchange between phosphoryl oxygen and water during the reaction,[7] and there is a strong evidence for inversion of configuration in nucleophilic attack upon phosphoryl phosphorus.[15,16] These apparent inconsistencies are explained by the preference rules put forward to explain the decomposition of a pentavalent intermediate formed by nucleophilic addition to phosphoryl groups.[16] (This subject has recently been reviewed, especially with regard to reactions of cyclic phosphates.[16]) In addition phenoxide

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(4) T. C. Bruice and S. J. Benkovic, "Bio-organic Mechanisms," W. A. Benjamin, New York, N. Y., 1966, Chapters 5–7.
(5) R. G. Pearson and J. Songstad, *J. Amer. Chem. Soc.*, **89**, 1827 (1967).
(6) P. W. C. Barnard, C. A. Bunton, D. R. Llewellyn, K. G. Oldham, B. Silver, and C. A. Vernon, *Chem. Ind. (London)*, 760 (1955); C. A. Vernon, *Chem. Soc., Spec. Publ.*, **No. 8**, 17 (1957).
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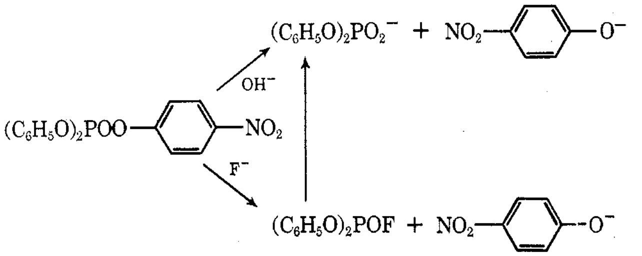
is a much better leaving group than hydroxide, for example, in carboxylic ester hydrolysis.[17]

In the absence of electron-attracting substituents, aryl groups are insensitive to nucleophilic attack[18] and triaryl phosphates react very readily with hard nucleophiles, *e.g.*, hydroxide or fluoride ions, with attack on phosphorus,[3,4,7,10,19] but water molecules are rela-



tively unreactive toward triaryl phosphates with $k_{OH^-}/k_{H_2O} \approx >2 \times 10^4$, based on the calculated second-order rate constants for *p*-nitrophenyl diphenyl phosphate.[19] (The acid hydrolysis of aryl phosphates is discussed in a later section.)

Disubstituted Esters

Except at low pH, disubstituted phosphate esters exist as anions in water, p$K \approx 1.5$,[3,20] and except for five-membered cyclic phosphates,[21] and diaryl phosphates which contain strongly electron-attracting substituents,[22] they are relatively unreactive toward anionic nucleophiles.[20,23]

The behavior of dimethyl phosphate is typical of that of simple dialkyl phosphates.[20] The monoanion is unreactive and reacts slowly with hydroxide ion, but undissociated dimethyl phosphate reacts with water at 100° with a first-order rate constant of 4.2×10^{-6} sec^{-1} and the major reaction is bimolecular attack on the methyl group. Hydrogen ions catalyze the hydrolysis, and the predominant acid-catalyzed reaction involves attack of water on the methyl group.

$$H_2{}^{18}O + CH_3 \vdots O\text{-}PO_3CH_3 \longrightarrow H^{18}OCH_3 + HO\text{-}PO_2OCH_3$$

$$H^+ + (CH_3O)_2PO_2H \rightleftharpoons (CH_3O)_2PO_2{}^+H_2$$

$$H_2{}^{18}O + CH_3 \vdots O\text{-}\underset{\displaystyle OCH_3}{\underset{|}{P}}O_2{}^+H_2 \longrightarrow H^{18}OCH_3 + (CH_3O)_2PO_2H$$

The kinetic behavior of diphenyl phosphate is generally similar to that of dimethyl phosphate[24] except that there is a slight increase of rate with increasing pH, probably due to a reaction with hydroxide ion, and in acid solution the rates reach a maximum at 4 *M* perchloric acid and then decrease. This behavior in acid is often found with aryl phosphates and is discussed in more detail later in the article. The acid, and probably the other, hydrolyses involve attack upon phosphoryl phosphorus.[24]

Diaryl phosphates are reactive toward nucleophiles provided that strongly electron-attracting groups are present; for example, at pH 4, where the phosphates are present wholly as monoanions, the first-order rate constant for hydrolysis of diphenyl phosphate is 3×10^{-9} sec^{-1} at 100°,[24] whereas that of bis(2,4-dinitrophenyl) phosphate is 1.8×10^{-5} sec^{-1} at 74.6°,[22] and the reactivity difference, calculated at 100°, is 4×10^4.

At high pH bis(2,4-dinitrophenyl) phosphate reacts readily with hydroxide ion (Figure 1), which attacks the phosphate monoanion; at pH <3 the undissociated phosphate ester is attacked by water; and there is an acid-catalyzed hydrolysis at low pH.[22] Only phosphorus–oxygen fission was observed in the reaction with water, and it predominated for the attack of hydroxide ion, showing that nucleophilic attack could occur readily upon phosphorus despite the negative charge on this acyclic diaryl phosphate. Based on the calculated second-order rate constants for attack of hydroxide ion and water, hydroxide ion is *ca.* 10^6 times as reactive as water despite the negative charge on the phosphate ester.[22]

Monosubstituted Phosphates

Hydrolysis of the Monoanion. For simple monoalkyl and aryl phosphates p$K_1 \approx 1$ and p$K_2 \approx 6.5$ in water, so that three possible reactive species have to be considered.[3,4,6,25,26] Most monosubstituted phos-

$$\underset{\text{I}}{ROPO_3H_2} \rightleftharpoons ROP\bar{O}_3H \rightleftharpoons ROPO_3{}^{2-}$$

phates derived from primary alcohols or phenols show a very simple kinetic relationship between rate constant, k_ψ, and pH. Figure 2 shows this relationship for the hydrolyses of methyl and neopentyl phosphates at 100° and shows that for pH 2–7 the value of k_ψ calculated from the relative concentration of phosphate monoanion (broken line) agrees with the experimental value.[26] Acid-catalyzed and spontaneous hydrolyses of the undissociated ester become important at low pH.

Hydrolysis of the monoanion involves phosphorus–oxygen bond fission in all the reactions studied.[25,26] The reaction of the monoanion is slightly assisted by electron-attracting substituents, and plots of log k_ψ against $-$pK_a of the acid or phenol are linear with slope ≈ 0.3 (Figure 3).[27,28] Bulky *ortho* substituents

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(19) C. A. Bunton, S. J. Farber, and E. J. Fendler, *J. Org. Chem.*, **33**, 29 (1968).
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(26) (a) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3574 (1958); (b) C. A. Bunton, D. Kellerman, K. G. Oldham, and C. A. Vernon, *ibid.*, *B*, 292 (1966).
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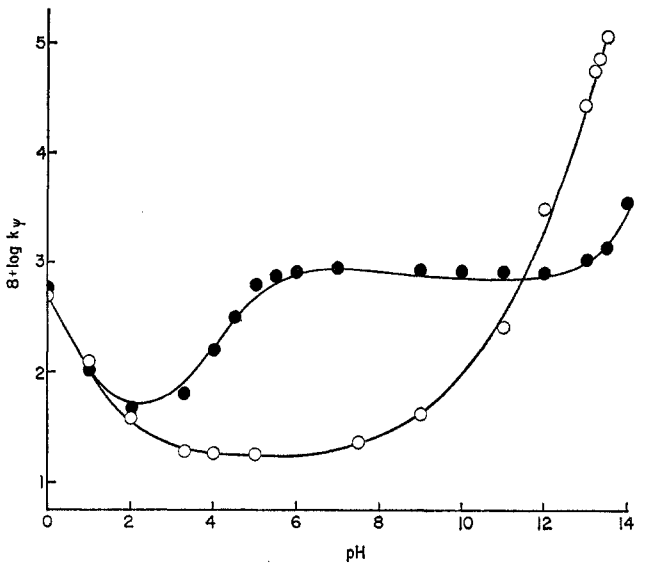


Figure 1. Variation of k_ψ with pH at 45.0° for the hydrolysis of bis(2,4-dinitrophenyl) phosphate (○) and 2,4-dinitrophenyl phosphate (●).

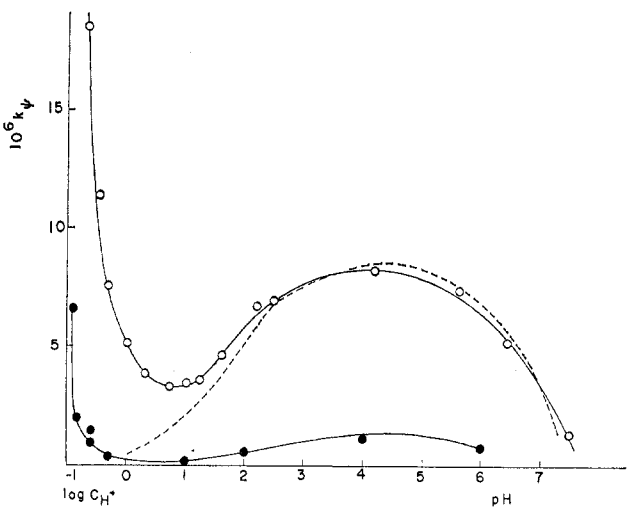
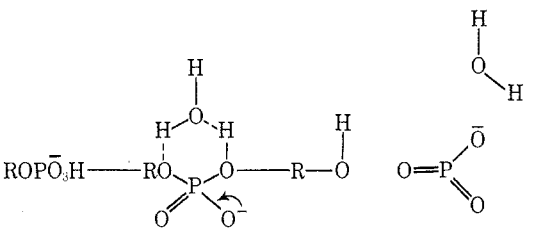


Figure 2. Hydrolyses of methyl and neopentyl phosphate in water at 100°. The broken line is that calculated for hydrolysis of the monoanion; methyl phosphate (○); neopentyl phosphate (●).

decrease k_ψ only slightly,[28] and neopentyl is only about three times less reactive than methyl phosphate;[26b] these results, together with the low reactivity of the undissociated ester I, exclude bimolecular attack on phosphorus in the rate-limiting step. Despite some unresolved questions, there is general agreement on the mechanism,[3,4] which is believed to involve loss of a (hypothetical) metaphosphate ion, probably concerted with proton transfer to the alkoxy or aryloxy group. The proton could be transferred directly, or through water molecules, but the rate is insensitive to added organic solvents,[29] suggesting that the actual arrangement of water molecules around the transition state is not particularly critical with regard to the rate of breaking of the P–O bond. However, as will be noted later, the fate of the metaphosphate ion is critically dependent upon solvation of the anionic



phosphate ester. The relatively small steric effects upon the spontaneous heterolysis of the monoanion (Figure 3) could be caused by steric hindrance to proton transfer in the transition state.

A problem with this mechanism is that there is no large deuterium solvent isotope effect, as might be expected if a proton were being transferred in the transition state. One way of avoiding this problem is to assume that the proton is transferred in a pre-equilibrium step[27] and the reaction rate–structure rela-

$$RO—PO_3H^- \rightleftharpoons RO^+(H)—PO_3^{2-} \longrightarrow ROH + PO_3^- \quad (1)$$

tionship is consistent with this suggestion (*cf.* ref 3). However, small deuterium isotope effects are often found for reactions which involve proton transfers between electronegative atoms,[30] and at present it does not seem to be possible to distinguish between these mechanistic variants, in part because they both involve a transition state in which the leaving alkoxy or aryloxy group bears very little charge. The electronic effects suggest that this leaving group bears a slight negative charge, which might not be expected in terms of reaction 1.

Hydrolysis of the Dianion. In the hydrolysis of these phosphate monoanions the driving force for reaction is provided by the negative charge on phosphate and proton transfer to the alkoxy or aryloxy group. However, if breaking of the P–O bond can generate a stable anion the dianion of the phosphate ester becomes a reactive species. The first examples of this behavior were found in hydrolyses of acyl phosphates[31] (eq 2),

$$RCOOPO_3^{2-} \longrightarrow RCO_2^- + PO_3^- \quad (2)$$

but subsequently this mechanism was also found in hydrolyses of dinitrophenyl phosphates[27,32] (eq 3). For

$$(NO_2)_2C_6H_3OPO_3^{2-} \longrightarrow (NO_2)_2C_6H_3O^- + PO_3^- \quad (3)$$

the hydrolysis of 2,4- and 2,6-dinitrophenyl phosphates the rate of hydrolysis increases smoothly from pH 2 to a plateau from pH 6 to 12; a plot of k_ψ against pH follows the relative concentration of the dianion (Figure 1), and the hydrolysis involves phosphorus–oxygen fission.[33] This dianion reaction is much more sensitive to electronic effects than that of the mono-

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(33) C. A. Bunton and J. M. Hellyer, *J. Org. Chem.*, **34**, 2798 (1969).

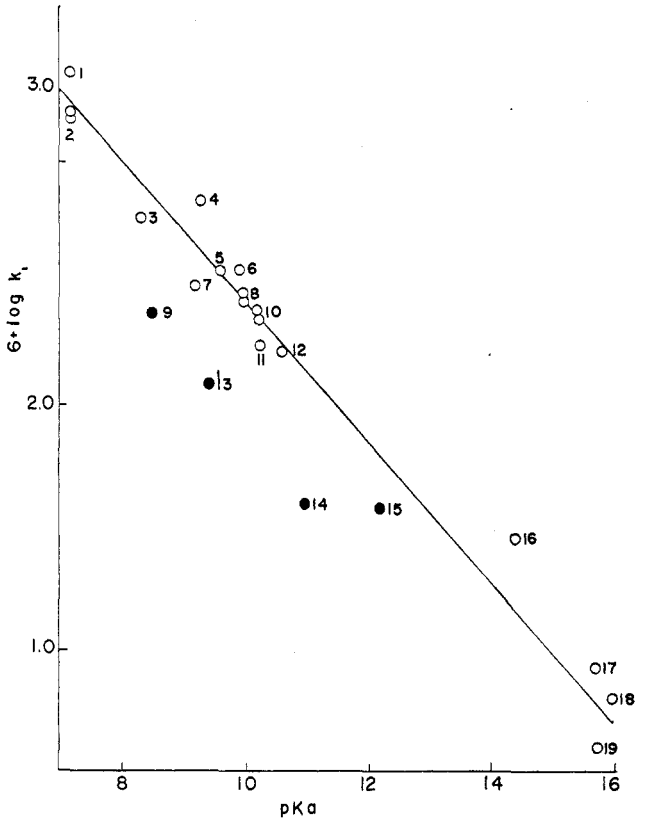

Figure 3. Plot of log k_M against pK_a of the phenol or alcohol. The shaded points refer to aryl phosphates having bulkyl alkyl groups adjacent to the phosphate residue. The following phosphates were used: (1) *o*-nitrophenyl; (2) *p*-nitrophenyl; (3) *m*-nitrophenyl; (4) *p*-carboxyphenyl; (5) 2-naphthyl; (6) *m*-carboxyphenyl; (7) *p*-chlorophenyl; (8) phenyl; (9) *o*-chlorophenyl; (10) *p*-tolyl; (11) *p*-*t*-butylphenyl; (12) 4,6-dimethylphenyl; (13) 1-naphthyl; (14) *o*-*t*-butylphenyl; (15) 2,6-di-*t*-butyl-4-methylphenyl; (16) 2-glyceryl; (17) methyl; (18) ethyl; (19) free acid (oxygen exchange).

anion: for monoalkyl and -aryl phosphates $\rho \approx 0.3$, but for dinitrophenyl phosphates it is considerably higher, although there are insufficient data to establish a good value. Solvent effects upon the reaction of the dianion (but not the monoanion) are large, and the rates of hydrolysis of 2,4- and 2,6-dinitrophenyl phosphate dianions increase on addition of organic solvents to water.[27,32] The dianion, with its negative charge on the phosphate group, should have a much higher hydration energy than the transition state in which the negative charges are delocalized over a nitrophenoxide ion and a metaphosphate ion.

With 2,5-dinitrophenyl phosphate the pH–rate profile depends upon temperature. At 25° there is a rate maximum at pH ~4, which is due to a monoanion hydrolysis, but at 45° both mono- and dianions have similar reactivities and k_ψ is only slightly dependent on pH, and at 73° the dianion is the more reactive species, showing that for a given compound the activation enthalpy is higher for the dianion than for the monoanion hydrolysis.[32] For the hydrolysis of 2,4-dinitrophenyl phosphate dianion $\Delta H^* = 25$ kcal mol^{-1} and $\Delta S^* = +2.5$ eu, and the enthalpy of activation is generally in the range 27–30 kcal mol^{-1} for monoanion hydrolysis. Somewhat similarly the mono- and dianions of 2-nitro-4-chlorophenyl phosphate have similar reactivities at 39°.[27]

Figure 1 shows that at high pH there is attack of hydroxide ion upon the phosphate dianion, and at pH 2 there is a rate minimum with a rate increase at higher acidities. The hydrolyses of dinitrophenyl phosphates are light sensitive,[34] and 3,5-dinitrophenyl phosphate dianion, although not particularly reactive in the dark, is readily hydrolyzed under strong irradiation.[35] Under these conditions a nitrophenyl group can be used as a blocking group, and the reaction is ascribed to formation of a highly reactive triplet state.[36]

The Metaphosphate Ion as an Intermediate. The spontaneous reactions of the phosphate mono- and dianions are assumed to generate a metaphosphate ion which can be trapped either by water or an alcohol in an aqueous alcoholic solvent. For many reactions the metaphosphate ion is captured by water and methanol indiscriminately on a molar basis, although ethanol is often less effective (Table I). Lack of selectivity is understandable for such a reactive intermediate as the metaphosphate ion, but, even for monophosphates, this lack of selectivity depends upon the fortuitous choice of temperature and solvent composition, and metaphosphate ions generated from aryl phosphate dianions and phosphoramidate ion react preferentially with methanol rather than with water. In one particular case, the hydrolysis of inorganic pyrophosphate, which at pH 4 probably involves elimination of metaphosphate ion from $H_3P_2O_4^-$ and $H_2P_2O_4^{2-}$, no methyl phosphate was detected when the reaction was carried out in aqueous methanol, but the rate was very similar in water and aqueous methanol.[37] These differences do not necessarily mean that metaphosphate ions are not generated in these hydrolyses, because they may have a very short life so that their capture by solvent is probably governed by the composition of the solvation layer around the substrate and therefore upon the structure of the substrate. For example, a pyrophosphate ion should be solvated by water rather than by methanol, and the metaphosphate ion may never become sufficiently free to be captured indiscriminately by solvent molecules. On the other hand a phosphate ester should be solvated to some extent by alcohols, and striking differences in product can be observed with minor changes in substrate or solvent.[27,37]

Nucleophilic Attack. In general anions do not readily attack anions of monosubstituted phosphates, but with 0.1–1.0 *M* hydroxide ion there is some nucleophilic attack upon phosphorus[33] (Figure 1) and with alkoxide ion in methanol or ethanol one can observe alkoxide attack upon both aryl and phosphoryl groups,

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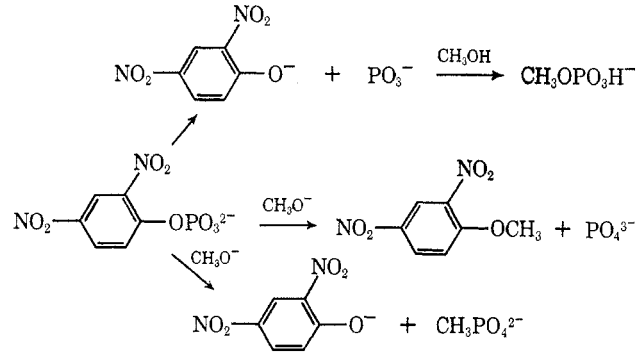
Table I
Capture of Metaphosphate Ion by Water and Alcohols

Substrate	Medium	[H₂O]/[ROH]	[H₃PO₄]/[ROPO₃H₂]	Selectivity (H₂O:ROH)
$(CH_2OH)_2CHOPO_3H^-$	Aq C_2H_5OH, 100°	2.14	3.13[a]	1.4
		0.36	0.55[a]	1.5
$C_6H_5OPO_3H^-$	Aq CH_3OH, 100°	5.45	3.55[b]	0.65
		2.42	2.23[b]	0.92
	Aq C_2H_5OH, 100°	3.46	6.12[b]	1.8
		1.48	3.0[b]	2.0
p-$NO_2C_6H_4OPO_3H^-$	Aq CH_3OH, 100°	5.45	5.25[b]	0.96
	37°	5.45	3.55[b]	0.65
	100°	2.42	2.45[b]	1.0
	37°	2.42	1.64[b]	0.67
$CH_2{=}C(C\bar{O}_2)OPO_3H^-$	Aq CH_3OH, 55°	2.24	2.13[c]	0.95
2,4-$(NO_2)_2C_6H_3OPO_3^{2-}$	Aq CH_3OH, 25°	5.45	0.92[d]	0.17
	25°	2.43	0.49[d]	0.20
	Aq C_2H_5OH, 25°	3.45	1.70[d]	0.49
2,6-$(NO_2)_2C_6H_3OPO_3^{2-}$	Aq CH_3OH, 25°	2.43	0.16[d]	0.07
$H_3P_2O_7^-$	Aq CH_3OH, 100°	1.49	∝[e]	∝
$H_2NPO_3H^-$	Aq CH_3OH, 37°	5.45	0.82[b]	0.15
	37°	2.42	0.38[b]	0.16

[a] Reference 29a. [b] Reference 29b. [c] S. J. Benkovic and K. J. Schoray, *Biochemistry*, **7**, 4090 (1968). [d] Reference 32. [e] Reference 37.

accompanying the spontaneous solvolysis of the dianion.[32]



Amines attack p-nitrophenyl phosphate, and there is then considerable attack on the aryl group.[38]

Carbonium Ion Reactions. At low pH, *e.g.*, 1, the monosubstituted phosphate esters exist as undissociated acids (I) which, except in special cases, are not particularly reactive in hydrolysis. A major exception to this generalization is provided by phosphate esters whose alkyl groups readily ionize as carbonium or oxocarbonium ions, for example α-glucopyranose and α-ribofuranose 1-phosphates and t-butyl phosphate.[39-41] For all these compounds the phosphate ester (I) is so reactive that hydrolysis of the monoanion makes only a slight contribution to the overall reaction, even at pH 4 (Figure 4). This SN1 reaction of the undissociated alkyl phosphate is very dependent upon the nature of the alkyl group, whereas hydrolysis of the monoanion is not; therefore, although we find hydrolyses of the monoanions of t-butyl phosphate[41] and α-glucopyranose 1-phosphate,[39] none could be detected in the hydrolysis

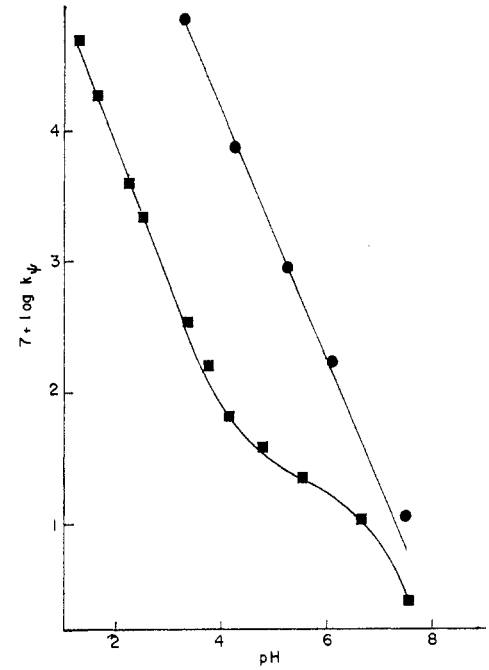


Figure 4. Relation between log k_ψ and pH for the hydrolysis of ribose 1-phosphate (●) and glucose 1-phosphate (■) at 84.0°.

of the more reactive α-ribofuranose 1-phosphate.[40] The kinetic behavior of these undissociated phosphate esters is typical of SN1 reactions leading to carbonium ion intermediates, *e.g.*, α-ribofuranose 1-phosphate is 200–400 times more reactive than α-glucopyranose 1-phosphate. This reactivity difference is readily understandable in view of the ease with which the furanose ring can take up a planar conformation which allows delocalization of the forming positive charge in the carbonium-like transition state.[40]

Acid Hydrolysis. The hydrolyses of monoalkyl phosphates are catalyzed by strong acids, and the mechanisms depend upon the alkyl group and the nature of the strong acid. Compounds such as α-glucopyranose 1- and α-ribofuranose 1-phosphates and t-butyl phos-

(38) A. J. Kirby and W. P. Jencks, *J. Amer. Chem. Soc.*, **87**, 3209, 3217 (1965).
(39) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3588 (1958).
(40) C. A. Bunton and E. Humeres, *J. Org. Chem.*, **34**, 572 (1969).
(41) A. Lapidot, D. Samuel, and M. W. Broday, *J. Chem. Soc.*, 637 (1964).

Table II

Acid Hydrolyses of Monosubstituted Phosphates

Substituent	ΔS^*, eu	ΔH^*, kcal mol^{-1}	k_{H_2O}/k_{D_2O}
CH_3[a]	−16.3	24.6	0.83
C_2H_5[a]	−6.0	28.7	0.76
			0.91[c]
i-C_3H_7[a]	+8.2	30.7	0.69
			0.64[c]
t-C_4H_9[a]			0.56
Glucose-1-[b]	+14.9	27.9	0.57
Ribose-1-[b]	+7.4	22	

[a] Reference 45. [b] Reference 40. [c] Values in $HClO_4$; the other values of k_{H_2O}/k_{D_2O} were obtained in H_2SO_4.

phate follow an A1 mechanism in which the conjugate acid of the phosphate gives a carbonium or oxocarbonium ion in the rate-limiting step.[39-41] The rates of hydrolysis increase sharply with increasing acidity, and approximately follow Hammett's acidity function, H_0.[42] Consistently the w values relating rate at a given acidity to water activity are negative,[42b] as is typical of A1 reactions, and the deuterium solvent isotope effects, k_{H_2O}/k_{D_2O}, are low, 0.5–0.6 (Table II), as expected.[43]

For many A1 hydrolyses values of the entropies of activation, ΔS^*, are positive,[44] as is found for the acid hydrolyses of t-butyl phosphate and α-glucofuranose 1- and α-ribofuranose 1-phosphates (Table II). The lower value of ΔS^* for the acid hydrolysis of α-ribofuranose 1-phosphate, as compared with α-glucopyranose 1-phosphate, can be explained in terms of the entropy decrease which is occasioned by the requirement that the sugar residue adopt a rigid conformation which permits delocalization of the positive charge to the ring oxygen atom in the transition state.[40] Despite the more negative entropy of activation, α-ribofuranose 1-phosphate is considerably more reactive than α-glucopyranose 1-phosphate in the acid as well as the spontaneous hydrolysis.

The acid hydrolyses of primary monoalkyl phosphates follow A2 mechanisms with nucleophilic attack on either the alkyl or phosphoryl group of the conjugate acid.[21a,26,45] The deuterium solvent isotope effect, k_{H_2O}/k_{D_2O}, is 0.76–0.9 for the hydrolyses of methyl and ethyl phosphates,[45] and the values of the entropy of activation ΔS^* (Table II) are as expected for A2 hydrolyses. The values of k_ψ increase more rapidly than C_{H^+} but the kinetic forms are generally similar to those found for A2 hydrolyses.[42] The hydrolysis of isopropyl phosphate appears to follow an A1 mechanism based on the activation parameters (Table II) and the relation between rate and Hammett's acidity function.[45] (The values of k_{H_2O}/k_{D_2O} for methyl, ethyl, and isopropyl phosphates (Table II) were obtained at 100° and the higher temperature brings them closer to unity.)

(42) (a) F. A. Long and M. A. Paul, *Chem. Rev.*, **57**, 935 (1957); (b) J. F. Bunnett, *J. Amer. Chem. Soc.*, **83**, 4956 (1961); (c) J. F. Bunnett and F. R. Olsen, *Can. J. Chem.*, **44**, 1899, 1917 (1967).

(43) J. G. Pritchard and F. A. Long, *J. Amer. Chem Soc.*, **78**, 6008 (1956); **80**, 4162 (1958); C. A. Bunton and V. J. Shiner, *ibid.*, **83**, 3207 (1961).

(44) L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963).

(45) L. Kugel and M. Halmann, *J. Org. Chem.*, **32**, 642 (1967).

Table III

Deuterium Solvent Isotope Effects for Acid Hydrolysis of Nitrophenyl Phosphates[a]

Phosphate	Reagent	k_{H_2O}/k_{D_2O}
p-Nitrophenyl	2.4 M H_2SO_4	0.73
p-Nitrophenyl	4.96 M H_2SO_4	0.73
2,4-Dinitrophenyl	3 M H_2SO_4	0.67
2,4-Dinitrophenyl	8 M H_2SO_4	0.67
Bis-2,4-dinitrophenyl	3 M H_2SO_4	1.18
Bis-2,4-dinitrophenyl	8 M H_2SO_4	0.91
p-Nitrophenyl diphenyl	1–4 M H_2SO_4	≈1.0

[a] Data from reference 49.

The position of bond fission has only been determined in a few systems, and corrections have to be made for oxygen exchange of the reactants and products under the reaction conditions.

In the acid hydrolysis of methyl phosphate at 100° and 5 M $HClO_4$, there is *ca.* 73% carbon–oxygen fission,[26a] but the phosphoryl oxygens exchange during hydrolysis, either by addition of water to the conjugate acid to give an intermediate which loses either water or methanol[21a,26a] (eq 4) or by a direct displacement on

$$CH_3O\text{-}PO_3^+H_3 \underset{H_2{}^{18}O}{\rightleftharpoons} CH_3O\overset{{}^{18}\dot{O}H_2}{P}(OH)_3 \rightleftharpoons CH_3OP^{18}OO_2^+H_3 \quad (4)$$
$$\downarrow$$
$$CH_3OH + H_3PO_4$$

phosphorus (eq 5). For the corresponding hydrolysis

$$H_2{}^{18}O + CH_3O\overset{O}{\overset{\|}{P}}(OH)\text{—}\overset{+}{O}H_2 \rightleftharpoons H_2{}^{18}O^+\text{—}\overset{O}{\overset{\|}{P}}(OH)\text{—}OCH_3 + H_2O \quad (5)$$

of ethyl phosphate in 4 M $HClO_4$ there is 52% carbon–oxygen fission, but little exchange of the unhydrolyzed ethyl phosphate.[45]

The strong halogen acids are very good catalysts for the decomposition of primary alkyl phosphates. The reaction is not hydrolysis, but SN2 attack of halide ion on the alkyl group, *e.g.*, eq 6 and 7, and the hydrolysis

$$CH_3OPO_3H_2 + H^+ \rightleftharpoons CH_3OPO_3^+H_3 \quad (6)$$
$$\bar{X} + CH_3OPO_3^+H_3 \longrightarrow X\text{-}CH_3 + H_3PO_4 \quad (7)$$
$$X = Cl, Br, I$$

of methyl phosphate in acid solutions containing halide ions follows eq 8 (where P = $CH_3OPO_3H_2$) showing that

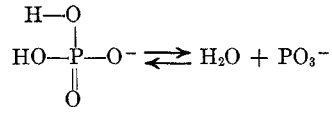
$$v = k_1[P] + k_2[H^+][P] + k_3[P][Hal^-] + k_4[H^+][P][Hal^-] \quad (8)$$

the halide ion can attack both undissociated methyl phosphate and its conjugate acid.[26b] As is generally found for reactions in hydroxylic solvents the nucleophilicity increases in going from chloride to iodide ion. For the acid hydrolysis of neopentyl phosphate nucleophilic attack upon carbon is sterically hindered, the attack upon phosphorus is slower than the overall reaction of methyl phosphate, and halide ions are not catalysts.[26]

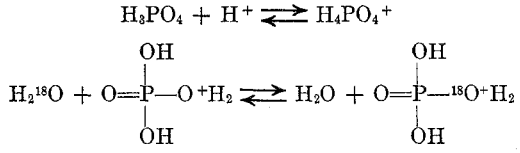
Oxygen Exchange of Inorganic Phosphate. Oxygen exchange between water and inorganic phosphate

follows the mechanisms which have been outlined for hydrolyses of primary alkyl phosphates,[46] *e.g.*

Exchange of the monoanion



Acid-catalyzed exchange



(This exchange is written as a bimolecular substitution, but it could equally well be an addition–elimination.) After statistical correction the rate constants for oxygen exchange are very similar to those found for the corresponding hydrolysis of methyl phosphate.

Acid-Catalyzed Hydrolysis of Aryl Phosphates

The absence of acid catalysis in the hydrolysis of phenyl phosphate and its alkyl- or halo-substituted derivatives is easily explained in terms of the low basicity of aryl as compared with alkyl compounds.[24,28] It was not so easy to explain why the hydrolyses of nitrophenyl phosphates are acid catalyzed and why the rates increase to maxima at 5–7 *M* acid (Figure 5). This behavior appears to be general for aryl phosphates which contain strongly electron-attracting groups, *e.g.*, NO_2 or CH_3CO, and for di- and triaryl phosphates, including the unsubstituted derivatives.[19,22,24,28,32] These rate maxima have also been found in acid hydrolyses of nitrophenyl phosphinates.[47]

These reactions show characteristic and sometimes unexpected kinetic features (*e.g.*, the entropies of activation are always large and negative), and the rate is very sensitive to water activity (*e.g.*, it is decreased by addition of solvents such as dioxane), and w values[42b] are large and positive, although plots of log $k_\psi + H_0$ against log a_w are not always linear and w depends upon the nature of the acid. Electron-attracting substituents increase the reaction rate, whereas electronic effects are generally small for A2 hydrolyses and electron-donating groups assist A1 hydrolyses. Rather unexpectedly nitrophenyl phosphates are more reactive than the primary alkyl phosphates in acid hydrolysis. Deuterium solvent isotope effects k_{H_2O}/k_{D_2O} are in the range 0.7–1.2 (Table III) and tend to be closer to unity than those generally found for reactions in which a conjugate acid is in equilibrium with reactants and decomposes slowly to products.[43] They are the same on both sides of the rate maxima, showing that these rate maxima are not related to complete protonation of the substrate, as in amide hydrolysis.[48] These conclusions are supported by pK_a measurements, *e.g.*, for triphenyl phosphate p$K_a \approx -5$.[49] Moreover, the kinetic evidence suggests that the transition states are strongly hydrated and the phosphorus–oxygen bond is broken, but the phosphoryl oxygen atoms do not exchange with water.[22,24,49] In all the systems studied the reaction rate is greater in HCl and H_2SO_4 than in $HClO_4$, but this difference reflects not the differing nucleophilicities of the anions of the acids, as is found for alkyl phosphate hydrolysis, but, at least in part, a "salting in" of the initial state by a large anion such as perchlorate.[19,24] The rate maxima disappear if the ionic strength is kept constant, and k_ψ generally increases linearly with C_{H^+} for mixtures of HCl and LiCl of constant ionic strength (Figure 5).

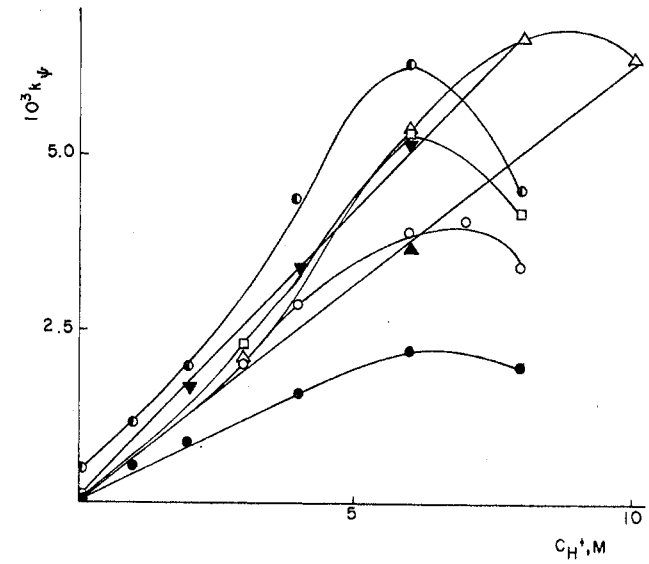
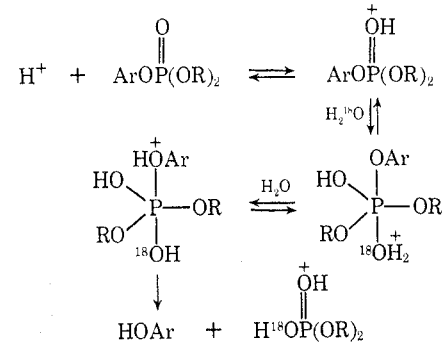


Figure 5. Acid hydrolyses at 73.0°: (●) 2,5-dinitrophenyl phosphate in $HClO_4$; (◐) 2,6-dinitrophenyl phosphate in $HClO_4$; (○) 2,4-dinitrophenyl phosphate in $HClO_4$; (□) 2,4-dinitrophenyl phosphate in H_2SO_4; (△) 2,4-dinitrophenyl phosphate in HCl; (▼) 2,4-dinitrophenyl phosphate in HCl + LiCl, I = 8.0; (▲) 2,4-dinitrophenyl phosphate in HCl + LiCl, I = 10.0

The kinetic evidence is consistent with mechanisms in which proton transfer is involved in the rate-limiting step. Mechanisms of this type have been invoked for acid-catalyzed reactions of organic sulfides with weakly basic sulfinyl sulfones.[50]

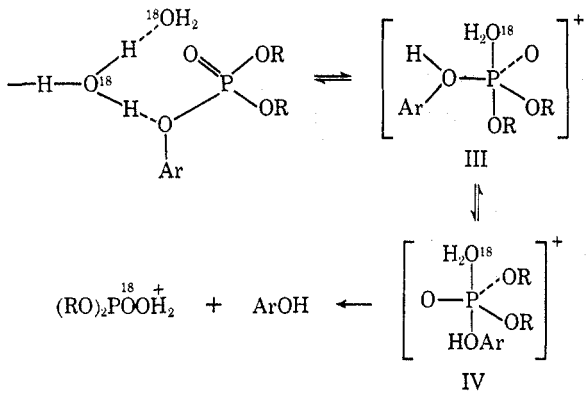
Possible reactions are shown in Schemes I and II.

Scheme I



(46) C. A. Bunton, D. R. Llewellyn, C. A. Vernon, and V. A. Welch, *J. Chem. Soc.*, 1636 (1961).
(47) P. Haake and G. Hurst, *J. Amer. Chem. Soc.*, **88**, 2544 (1966).
(48) J. T. Edward and S. C. R. Meacock, *ibid.*, **79**, 2000 (1957).
(49) C. A. Bunton and S. J. Farber, *J. Org. Chem.*, **34**, 3402 (1969).
(50) J. Kice and G. Guaraldi, *J. Amer. Chem. Soc.*, **89**, 4113 (1967).

Scheme II



In Scheme I it is assumed that addition of a proton and water precedes a slow proton transfer to the leaving group, in which water molecules are involved, and the rate of this transfer should decrease with decreasing water activity. The absence of oxygen exchange during hydrolysis is understandable if pseudorotation is sufficiently slow for the phosphoryl oxygen never to move into an apical position.

In the alternative, Scheme II, the attacking water molecule is close to the proton, and therefore the chance of concerted attack by both reagents will depend upon the number of water molecules which are closely hydrating the proton. It is generally assumed that in dilute acid each proton is closely associated with four water molecules,[51] but this number should decrease with increasing acid concentration;[52] thus as long as $H_9O_4^+$, for example, is the bulk acid species the rate should increase with increasing acid concentration, but when less hydrated species begin to appear the rate should fall. The situation is therefore completely different from that for acid reactions in which the substrate is in equilibrium with its conjugate acid.

Although Scheme II includes pentavalent intermediates, we can explain the absence of oxygen exchange during acid hydrolysis of triphenyl phosphate on the assumption that intermediates III and IV rapidly interconvert by several pseudorotations[16] so that the labeled oxygen and the leaving group with its electron-attracting group rapidly take up apical positions, to give IV which rapidly decomposes to the products. Schemes I and II involve completely different assumptions about pseudorotation rates.

(51) K. W. Bascombe and R. P. Bell, *Discuss. Faraday Soc.*, **24**, 158 (1957).

(52) E. B. Robertson and H. B. Dunford, *J. Amer. Chem. Soc.*, **86**, 5081 (1964).

Westheimer and his coworkers have shown that pseudorotation can be sufficiently slow to determine the relative amounts of ring opened and exocyclic products in the hydrolysis of methyl ethylene phosphate.[53] However the five-membered cyclic phosphates are very reactive compounds, and it is quite possible that the intermediate steps in the hydrolysis of the relatively unreactive aryl phosphates could be slower than pseudorotation in acyclic phosphorus compounds.[54]

These two mechanisms differ considerably, but they have certain common features in that electron-attracting substituents should hinder protonation but assist nucleophilic attack by water, and all these phosphate esters which show rate maxima not associated with extensive substrate protonation contain strongly electron-attracting groups. In addition these mechanisms explain why a compound which contains such groups but also has a structural feature which allows it to generate a carbonium ion in an A1 hydrolysis does not show a rate maximum in acid.[56] In a normal A2 hydrolysis nucleophilic attack and loss of the leaving group follow complete proton transfer, but in Schemes I and II it is assumed that they can occur after partial proton transfer, and therefore a weakly basic aryl phosphate should then be more reactive than methyl phosphate, as is observed; in other words we interpret the rate maximum in terms of effects (*e.g.*, partial protonation) which assist, not hinder, formation of the transition state.

Solvent deuterium isotope effects upon the acid hydrolysis of these aryl phosphates are not large (Table III) even though slow proton transfers may be involved. There is, however, precedent for this behavior in other reactions.[30,57]

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(53) R. Kluger, F. Covitz, E. Dennis, L. D. Williams, and F. H. Westheimer, *ibid.*, **91**, 6066 (1969).

(54) The rate of acid hydrolysis of a bicyclic phosphinate ester reaches a maximum in dilute acid,[55] which cannot be ascribed to a decrease in water activity, and in the hydrolysis of this relatively rigid bicyclic ester it is reasonable to assume that pseudorotation is rate limiting.

(55) R. Kluger and F. H. Westheimer, *J. Amer. Chem. Soc.*, **91**, 4143 (1969).

(56) C. A. Bunton and T. Hadwick, *J. Chem. Soc.*, 3248 (1958); 943 (1961).

(57) C. A. Bunton and R. M. DeWolfe, *J. Org. Chem.*, **30**, 1371 (1965); A. J. Kresge and R. J. Prieto, *J. Amer. Chem. Soc.*, **87**, 4593 (1965).