Pure & Appl. Chem., Vol.52, pp.1021-1045.
Pergamon Press Ltd. 1980. Printed in Great Britain.

0033-4545/80/0401-1021$02.00/0

PHOSPHORYL TRANSFER FROM PHOSPHOMONOESTERS IN APROTIC AND PROTIC SOLVENTS

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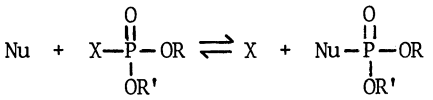
Abstract - The "addition-elimination" mechanism of nucleophilic displacements on phosphotriesters and phosphodiesters via oxyphosphorane intermediates is reviewed. Recent data on nucleophilic catalysis of this mechanism in aprotic and protic solvents is summarized. The implications of positional exchange of ligands in the phosphorane intermediate with respect to the stereochemistry of the product are emphasized. The "elimination-addition" mechanism of displacements on phosphomonoesters via a monomeric metaphosphate anion intermediate is considered, first from earlier data obtained in aqueous solutions, and then from the results of the present work in both aprotic and protic solvents. Evidence is provided for the operation of the addition-elimination and the elimination-addition mechanisms in nonenzymatic phosphoryl transfer from aryl phosphomonoesters, $ArOPO_3H_2$, as a function of the structure of the nucleofugic group, ArOH, and of the state of ionization of the ester, i.e., neutral acid, monoanion, $ArOPO_3H^-$, or dianion, $ArOPO_3^{2-}$. The medium is very important in controlling the acidity of the phosphomonoester and, hence, its state of ionization, and in determining relative solvation of the more polar ground states vs the less polar transition state in both mechanisms. Only monoanions are susceptible to nucleophilic catalysis in displacement reactions of phosphomonoesters, and the catalysis is exerted via oxyphosphorane intermediates. Dianions are not susceptible to nucleophilic catalysis due to their relatively low phosphorus electrophilicity. A very slight rate acceleration of dianion displacements observed in the presence of most types of amines is attributed to medium effects not associated with nucleophilic catalysis. The first direct observation of the monomeric metaphosphate anion and metaphosphoric acid in the gas phase is described. Monomeric alkyl metaphosphates are also generated by purely thermal reactions in the gas phase at temperatures as low as 200°C. It is concluded that the characteristics of the elimination-addition mechanism of phosphoryl-transfer are such that this mechanism is unlikely to be involved in enzymatic reactions of phosphorus compounds with two ionizable hydrogens on the same phosphate group, e.g. phosphomonoesters, acyl phosphates and the terminal (P_γ) group of ATP. These enzymatic reactions probably involve the monoprotonated species, XPO_3H^-, and proceed via oxyphosphorane intermediates, without or with assistance of nucleophilic catalysis. The essential role of Mg^{2+} ions in most of the enzymatic reactions is probably associated with the simultaneous binding of the metal ion to the phosphate and to amino acid residues of the enzyme, which reinforces the direct binding of the phosphate to enzyme at the active site pocket. Magnesium ions do not increase significantly the electrophilicity of the phosphorus in nonenzymatic phosphoryl transfer.

INTRODUCTION

Nucleophilic displacements on phosphoric acid derivatives lead to a variety of "phosphoryl transfer" reactions of considerable synthetic value and biochemical significance:



The number of acidic protons in the electrophilic phosphorus is an important parameter in any discussion of these reactions. The phosphates, pyrophosphates and phosphoramidates found in biological systems have one or two ionizable hydrogens per phosphate group, which imparts water solubility to the molecule. Different states of ionization of a phosphorylating reagent have significantly different phosphorus-electrophilicity, and can undergo nucleophilic displacements by fundamentally different mechanisms. This is one of the main questions to be examined in this paper.

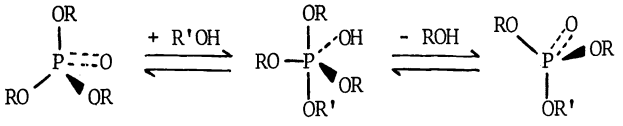
In studies of enzymatic phosphoryl transfer mechanisms, much use has been made of model phosphate esters, e.g. 2,4-dinitrophenyl, 4-nitrophenyl, and simple alkyl phosphates. This raises two questions. (1) Does the phosphorylation mechanism type change as a function of the nucleofugicity of group-X in phosphates, pyrophosphates, etc. (2) What is the effect of the structure of group-X on the acidity of the phosphomonoesters? This question is quite pertinent if, as was suggested above, different ionization states of the same phosphate may be capable of undergoing displacements by different types of mechanisms. Moreover, the effect of medium and of other variables, e.g., the presence of divalent metal ions, on the acidity of the phosphates must also be considered in terms of possible changes of mechanism types. It is known that the magnesium ion, which is required in many biophosphoryl transfers, increases significantly the acidity of the protons in nucleotide-5' polyphosphates. Thus, the complexes MgADPH and $MgATPH^-$ have pKa values of 5.3 and 5.4 respectively, which represent an increase in acidity of about 1.5 pK units relative to the values observed for the sodium or tetramethylammonium salts, all in aqueous medium [1,2].

In the past, studies on phosphoryl transfer mechanisms involving phospho- and pyrophosphomonoesters have been carried out in aqueous solutions. Initially, we undertook the study of nucleophilic displacements on phosphotriesters in aprotic solvents in order to explore the effect of the medium on activation energy parameters. We found significant increases in the rates of reaction of alcohol and water with the triesters in changing from water to acetonitrile and dichloromethane as the solvent. We attributed this effect mainly to a destabilization of the phosphotriester ground state in the aprotic vs the protic solvent as a result of the significantly lower degree of solvation of the highly polar 4-coordinate phosphate function by the aprotic solvent. This differential solvation effect in the relatively less polar transition state which generates the 5-coordinate oxyphosphorane intermediate should not be as important as the ground state solvation effect. In the present study of phosphoryl transfers from phosphomonoesters we have also included rate measurements in aprotic and in mixed aprotic/protic solvents for two reasons: (a) to detect possible effects on ground state-solvation analogous to those observed among the phosphotriesters; (b) to detect effects which could result from changes in the acidity of the phosphomonoesters in aprotic vs protic solvents. Such changes might enhance the separation between the first and the second pKa values of a phosphomonoester, which should facilitate an examination of possible differences in mechanism types in displacements at the mono- and dianion forms of the phosphates. In this respect, it is conceivable that the medium which prevails in the partially hydrophobic enzymatic active site pocket may be significantly different from that represented by water.

ADDITION-ELIMINATION MECHANISM OF PHOSPHORYL TRANSFER

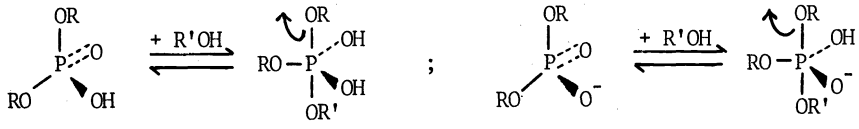
Nucleophilic displacements on phosphotriesters are satisfactorily described by an addition-elimination mechanism which involves an oxyphosphorane intermediate containing five ligands covalently bonded to the phosphorus [3-5]; Scheme 1. Most of these studies have been carried out in aqueous media [6-18]; however, recent work from this Laboratory has explored the behavior of triesters in aprotic solvents [19-22].

Scheme 1



Displacements on phosphodiesters in their nonionized state are also adequately explained in terms of oxyphosphorane intermediates; Scheme 2. Again, most of the pertinent investigations have been carried out in aqueous solution [23-29], but recent work has been done in aprotic solvents [30]. The phosphodiester anion is much less reactive than its conjugate acid, which is a reasonable corollary of the oxyphosphorane mechanism, since the oxyanion ligand should decrease the stability of the phosphorane and of the transition state leading to it. There is, at present, no evidence that phosphates with only one ionizable proton per phosphate group undergo displacements via a monomeric metaphosphate ester intermediate (see below).

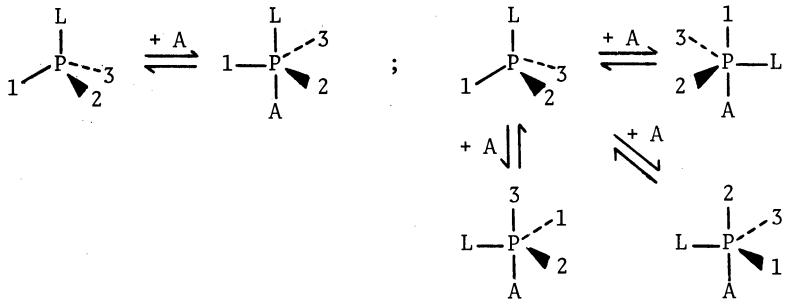
Scheme 2



In general, four stereoisomeric phosphoranes can be derived by addition of a nucleophile to tetrahedral phosphorus; Scheme 3. A = attacking group, L = leaving group. These are among the twenty possible stereoisomers (ten pairs of enantiomers) of a phosphorane with five different and symmetrical ligands [31]. The nucleophile, A, can add to the tetrahedron in two ways [4,5,32]: "trans or in-line" and "cis or adjacent":

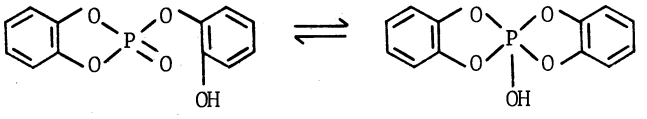
Scheme 3

Trans or In-line Attack: Cis or Adjacent Attack:

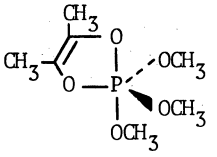


An oxyphosphorane intermediate that provides no direct or indirect information of its existence is operationally indistinguishable from a transition state analogous to that postulated for S_N2 displacements at tetrahedral carbon, e.g. in Walden-inversion. Therefore, there is no assurance that all displacements at phosphorus by the addition-elimination mechanism must proceed via an oxyphosphorane intermediate. However, there is a growing body of work which supports the formation of this type of intermediate as a general phenomenon in phosphorus chemistry. Hydroxyphosphoranes have, in fact, been observed in solution, in equilibrium with phosphotriesters which contain tetrahedral phosphorus in the crystalline state [33,34]; Scheme 4. There are further examples of detection of such hydroxyphosphoranes among acyl phosphotriesters and phosphinate esters [35-39].

Scheme 4



The formation of oxyphosphorane intermediates in displacement reactions can be inferred from stereochemical arguments, and from the incorporation into the product of more than one isotopically labeled atom from the reagent. This is a corollary of the phenomenon of permutational isomerization, i.e., of ligand positional exchange, in trigonal bipyramidal phosphorus [40]. Permutational isomerization of phosphoranes can take place by intramolecular deformation of bonds ("regular isomerization"), and by much slower processes which require the rupture and the reformation of bonds at the phosphorane stage ("irregular isomerization") [32,41]. Regular isomerizations of phosphoranes, in particular when the five ligands are oxygen atoms, can be very fast, e.g. > 10^5 sec^{-1} in some monocyclic pentaoxyphosphoranes:



Two mechanisms have been suggested for the regular permutational isomerization: pseudorotation [42] and turnstile rotation (TR) [43]. The similarities and differences between these two concepts have been described elsewhere [40].

The net result of the "single TR" is to exchange the relative positions of a pair of ligands on the bipyramidal skeleton, while imposing upon a trio of ligands the type of motion which is usually associated with a "turnstile". The "double TR^2" causes no change in the positioning of the pair of ligands, but causes the turnstile motion of the trio of ligands. The "triple TR^3" exchanges the relative positions of the pair of ligands, but does not affect the positioning of the trio-ligands [40].

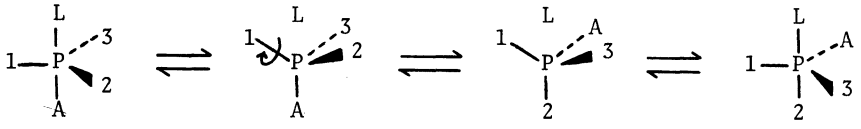
It can be shown [32,40] that all possible single TR processes of a given initial isomer, regardless of the choice of pair-trio combinations, and of the direction of the trio rotation, will result in the movement of both apical ligands to equatorial positions. Moreover, the choice of certain pair-trio combinations and directions of trio-rotation, in conjunction with the single TR will move a given equatorial ligand to an apical position, if this is desirable. The selection of certain pair-trio combinations and directions of trio-rotation, in conjunction with the TR^2 and TR^3 processes, can leave a given apical ligand in the apical position, or can move an equatorial ligand to an apical position. These characteristics of regular permutational isomerizations of phosphoranes, in conjunction with the addition-elimination mechanism of phosphoryl transfer, permit a general correlation between

the relative configurations of the tetrahedral reactant and product, on the one hand, and the mode of attack of the nucleophile on the 4-coordinate phosphorus [32]. Thus, it can be shown that if apical entrance-departure [4] of groups A and L is observed in these displacements, a trans or in-line attack which is not followed by permutational isomerization in the phosphorane intermediate must result in inversion of configuration of the tetrahedral product relative to the reactant. Trans attack followed by a single TR (or by one pseudorotation) prevents displacement, since in all cases, the leaving group is moved from the apical to an equatorial position. However, a cis or adjacent attack which is not followed by permutational isomerization in the phosphorane intermediate prevents displacement, since by definition the cis attack places the leaving group in an equatorial position. Cis attack followed by a single TR (or by one pseudorotation) can result in retention of configuration of the tetrahedral product relative to the reactant, provided that the appropriate pair-trio combination in TR (or pivot in pseudorotation) is chosen. By "appropriate choice" is meant that the leaving group L must move from an equatorial to an apical position as a result of that particular isomerization.

These stereochemical differences associated with the trans vs the cis modes of attack no longer exist if the phosphorane intermediate has a sufficiently long life-time to allow the occurrence of TR^2 or TR^3 (or of sequences of two or three successive pseudorotations), assuming of course that the nature of the ligands in the phosphorane is such that these types of regular isomerizations are energetically and structurally possible. The trans or the cis attack followed by TR^2 has the same effect, namely inversion of product configuration vs reactant configuration. The trans or the cis attack followed by TR^3 also has the same effect but this time there is retention of configuration.

As previously stated, the regular permutational isomerization is not the only way in which ligand positional exchange can take place in trigonal bipyramidal phosphorus. Irregular permutational isomerizations [41], although usually much slower than the regular isomerizations, must also be considered in displacements at tetrahedral phosphorus. There are three ways of performing irregular isomerizations. In one type of irregular process, 5-coordinate phosphorus becomes 4-coordinate phosphorus in an intermediate; Scheme 5. In the two other types of irregular process, not considered further here, 5-coordinate phosphorus becomes 6-coordinate phosphorus in an intermediate. The dissociative irregular isomerization of Scheme 5 has the same stereochemical consequences as the TR^2 regular isomerization.

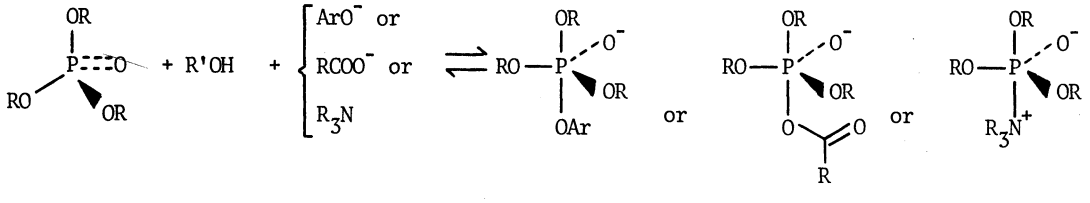
Scheme 5



In summary, inversion of configuration at a chiral phosphate may be the result of: (a) regular isomerizations by the in-line attack followed by no TR or by TR^2; (b) regular isomerization by the adjacent attack followed by TR^2; (c) irregular isomerization after either in-line or adjacent attacks. Retention of configuration may be the result of (a) regular isomerizations by the adjacent attack followed by TR or TR^3; (b) regular isomerization by the in-line attack followed by TR^3. If no special structural features interfere with the appropriate multiple-TR processes, i.e. TR^2 or TR^3, the observation of certain stereochemical result (inversion or retention), or of a certain degree of isotope incorporation from nucleophile into product, cannot be interpreted exclusively in terms of the regular isomerization process (at the exclusion of the irregular isomerization), or in terms of the single TR, or a single pseudorotation (at the exclusion of TR^2, TR^3 or the respective succession of pseudorotations).

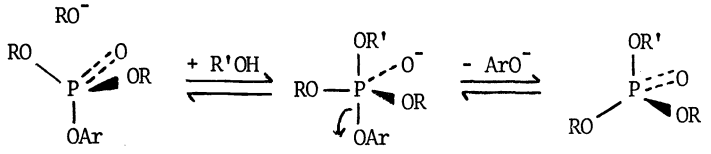
A significant question in phosphoryl transfer mechanisms of phosphotriesters is the possibility of nucleophilic catalysis. This phenomenon has been studied mainly in aqueous solutions of phosphotriesters and phosphodiesters, although there is a report of imidazole catalysis in the solvolysis of tetrabenzyl pyrophosphate in 1-propanol [7]. We have detected nucleophilic catalysis in displacements on phosphotriesters by imidazole, tertiary amines, phenoxide ion, and acetate ion in aprotic solvents, e.g. acetonitrile and chloroform [19-22]. The catalysis is adequately explained by formation of the oxyphosphorane intermediates depicted in Scheme 6.

Scheme 6



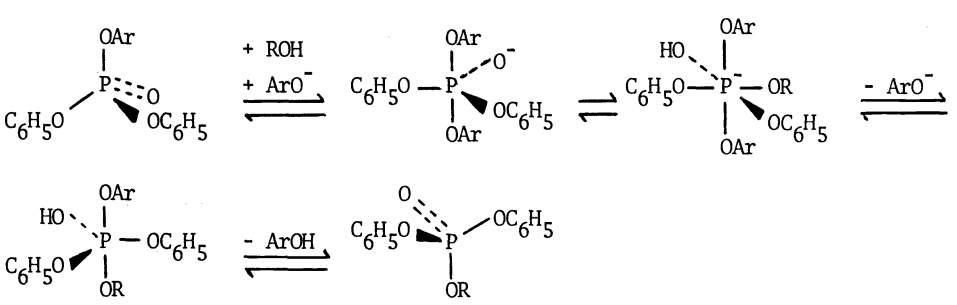
The second step in nucleophilic catalysis via an oxyphosphorane intermediate can be formulated as the collapse of the phosphorane to a new intermediate, this time with 4-coordinate phosphorus; Scheme 7. This type of "phosphorylated-catalyst", e.g. an aryl or acyl phosphate, or N-phosphorylimidazole, has a relatively high energy content and should accept the reagent, R'OH, to yield a second oxyphosphorane intermediate. The reaction is completed by ejection of the catalyst from the second phosphorane. With or without the actual participation of the phosphorane intermediates in the overall reaction sequence, the phosphorylated-catalyst hypothesis of nucleophilic catalysis in phosphoryl transfer has been widely accepted [9-18,23,24,44].

Scheme 7



The operation of the phosphorylated-catalyst mechanism in reactions of phosphomonoesters is supported by the actual observation of the intermediate in aprotic media, as described in another Section of this paper. It is probable that this mechanism also operates in reactions of phosphodiesters and in some reactions of phosphotriesters. However, there appears to be another pathway by which certain nucleophiles can increase the rate of reaction of phosphotriesters with alcohols, at least in aprotic media. This alternate pathway is illustrated in Scheme 8. p-Nitrophenyldiphenyl phosphate does not react appreciably with one molequivalent of methanol within 14 days in a 0.2 M acetonitrile solution at 25°C. However, the addition of one mol equiv of tetra-n-butylammonium p-nitrophenoxide has a marked catalytic effect, and methyldiphenyl phosphate is produced with a half-life of about 45 min, under comparable conditions. Evidently, the phosphorylated-catalyst mechanism does not explain this observation, since in this case the phosphorylated-catalyst is identical with the reactant itself. A possible interpretation involves the formation of an intermediate with 6-coordinate phosphorus (a phosphoride), for which there is adequate precedent in the literature [45,46]. The phosphoride can generate the observed product by loss of catalyst, followed by loss of leaving-group from the new oxyphosphorane intermediate.

Scheme 8

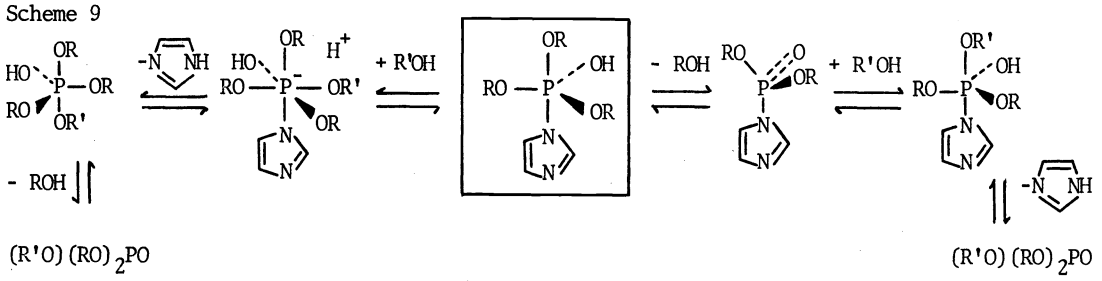


The "phosphoride-intermediate" mechanism of nucleophilic catalysis helps to explain several other observations made among certain phosphotriesters, when the catalyst is aroxide ion, acetate ion or a tertiary amine [19-22].

Imidazole is one of the most powerful, and because of its relevance to histidine catalysis in enzymatic phosphoryl transfer, one of the most important nucleophilic catalysts. Scheme 9 contrasts the phosphorylated-catalyst and the phosphoride-intermediate mechanistic alternatives for this catalysis.

Acid catalysis provides a relatively modest rate acceleration of displacements on phosphotriesters by the oxyphosphorane intermediate mechanism, but these effects are not as dramatic as those provided by nucleophilic catalysis.

Scheme 9



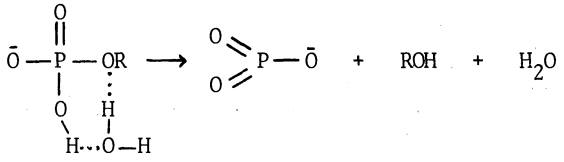
ELIMINATION-ADDITION MECHANISM OF PHOSPHORYL TRANSFER

The elimination-addition mechanism with formation of an intermediate monomeric metaphosphate anion, PO_3^-, was proposed to account for the observation that maximum rate of hydrolysis of alkyl phosphomonoesters in aqueous solution occurs at the pH which corresponds to a maximum concentration of monoanion [47-52]

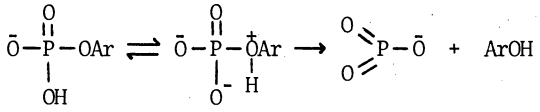
$$ROPO_3H^- + H_2O \xrightarrow{H_2O} PO_3^- + ROH \longrightarrow H_2PO_4^-$$

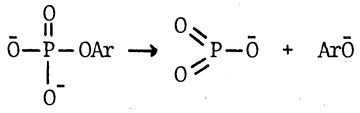
Subsequently, it was found [53-58] that aryl phosphates derived from phenols with pKa values above 5.5 in water have pH-rate profiles analogous to those of alkyl phosphates in water, e.g. 4-nitrophenyl phosphate exhibits maximum hydrolysis rate at the pH which corresponds to a maximum concentration of monoanion. On the other hand, aryl phosphates derived from phenols with pKa values below 5.5 in water, e.g. 2,4-dinitrophenyl phosphate, show maximum hydrolysis rate at the pH which corresponds to a maximum concentration of dianion [59-61].

The formation of metaphosphate from the alkyl phosphate monoanion was pictured as follows [47,48]:



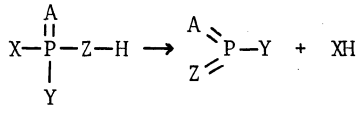
The hydrolysis of aryl phosphates, at the stages of monoanion and of dianion, was also assumed to occur via the metaphosphate anion intermediate, and the following mechanisms were suggested for these reactions [59,60]:





In this hypothesis, it is necessary to postulate that metaphosphate is generated at a faster rate from a monoanion than from a dianion if the nucleofugic phenol has a pKa > 5.5, while the same metaphosphate species is generated at a faster rate from a dianion than from a monoanion if the phenol has pKa < 5.5, since these are the results obtained from the hydrolysis pH-rate profiles of 4-nitrophenyl and 2,4-dinitrophenyl phosphates, respectively. These differences have been attributed to differences in driving force associated with the ejection of a neutral species (ArOH) vs a negatively charged species (ArO^-) of a weakly acidic vs a strongly acidic phenol. It should be noted that the formation of metaphosphate from monoanion via a proton-shift, and via a bimolecular complex involving water, are kinetically indistinguishable.

The formation of the PO_3^- intermediate has been invoked [62-64] to interpret nucleophilic displacements on acyl phosphates, pyrophosphates [65], and phosphoramidates, and this hypothesis has played an important role in the theory of biophosphorus reactions [66]. It is important to draw a distinction between the generation of PO_3^- anion and the generation of other species with diunsaturated three-coordinate phosphorus, where the ligands to phosphorus are elements other than oxygen:



Several examples of such structures, sometimes generated by pyrolysis reactions, are known or inferred [67-73]. Differences in phosphorus bonding to carbon, nitrogen and sulfur, on the one hand, and oxygen, on the other hand, are such as to preclude extrapolations from one system to the other, in matters concerning the possible participation of structures P(A)(Z)Y vs PO_3^- in nucleophilic displacements of the respective tetrahedral precursors in solution.

Several recent papers have reexamined the question of the participation of monomeric metaphosphate in displacement reactions of phosphomonoesters in solution, both from the experimental [17,74,75] and the theoretical [76,77] points of view. Although the hypothesis has received considerable support from these and previous studies, the subject remains controversial [78].

Concerning the possible involvement of nucleophilic catalysis in reactions of phosphomonoesters in solutions, a number of studies have been carried out in aqueous solution, in the presence of a large excess of various types of amines [56,59,60]. It has been concluded that nucleophilic amines catalyze hydrolyses according to a bimolecular mechanism in which the amine participates in the rate limiting step. This nucleophilic catalysis is said to occur in both the 4-nitrophenyl and 2,4-dinitrophenyl phosphate reactions, and in both forms of the phosphate, namely, monoanion and dianion.

In the investigation summarized in the next section, we turned to aprotic and mixed aprotic-protic solvents as the medium in which to study phosphoryl transfer of phosphomonoesters. The choice of solvent system rests on the ability of the aprotic medium, e.g. acetonitrile, to separate the first and second ionization constants of the phosphomonoesters. We selected the aryl phosphates: 2,4-dinitrophenyl-, 4-nitrophenyl- and phenyl phosphates as substrates on account of their relatively high reactivity. We followed the approximate rates of appearance of the phenol by ^{1}H NMR, and of the phosphorus-containing products by ^{31}P NMR. In view of the large number of reactions required to observe trends related to changes in phosphate structure and on experimental conditions, and in view of the difficulties associated with accurate kinetic studies of ester displacements in aprotic solvents, we relied on measurements of half-times of reaction. The results are, therefore, of a qualitative nature, and the times given are accurate to about 25%. Only relatively large differences in rates, i.e. differences by factors of 3 or more, are taken into consideration.

By means of these techniques we sought to obtain further information on several questions related to phosphoryl transfer from phosphomonoesters:
(1) Why is monomeric metaphosphate formed faster from the monoanion of 4-nitrophenyl phosphate, and from the dianion of 2,4-dinitrophenyl phosphate?
(2) What is the nature of the "bimolecular mechanism" by which sterically unhindered amines increase the rate of phosphoryl transfer via the PO_3^- intermediate; i.e. what is the exact nature of the participation of the nucleophilic amine in the transition state of the decomposition of a phosphate mono- or dianion into PO_3^-?
(3) Can the same phosphomonoester react via oxyphosphorane and metaphosphate intermediates, with the contribution of the two mechanisms being affected by experimental conditions, and can this contribution change among different phosphates under comparable experimental conditions?
(4) If the rate-limiting step in the addition-elimination mechanism is the addition of nucleophile to phosphate, while the rate-limiting step in the elimination-addition mechanism is the elimination of PO_3^- from the phosphate, can one find experimental conditions to distinguish unequivocally between the two mechanisms in terms of reaction rates and product structures using certain nucleophiles, e.g. the sterically hindered tert-butyl alcohol?

REACTIONS OF ARYL PHOSPHATES IN APROTIC SOLVENTS

This type of study requires the preparation of anhydrous samples of the acids, $ArOPO_3H_2$, and of quaternary ammonium salts of suitable solubility in aprotic and protic solvents, $ArOPO_3H^-R_4N^+$, $ArOPO_3^{2-}\ 2(R_4N^+)$. Solutions in acetonitrile-rich media were obtained with the tetra-n-butylammonium salts; solutions in water-rich media were obtained with tetramethylammonium salts. Solutions of the phosphates in the desired medium (1.0 M) were studied at 35° or 70°C by means of ^{31}P and ^{1}H NMR spectroscopy, and approximate values of the reaction half-times (τ) were estimated ($\pm$ 25%) from the times at which reactant signal intensity became equal to product signal intensity.

Prior to a discussion of the results it is necessary to consider certain aspects of the acidity of Brönsted acids in solvents other than water.

Acid-base equilibria of aryl phosphates in acetonitrile solutions.
Acids become progressively weaker when water is replaced by alcohols and by aprotic organic liquids as solvent [79-82]. Among carboxylic acids and phenols, this effect is enormous when water is replaced by acetonitrile, as illustrated in Table 1. The decrease in the acidity of aminium cations, R_3NH^+, is not nearly as large. In the carboxylic acids, the maximum decrease in acidity is observed among the weakest acids of the series, and a similar effect is observed in the phenols. Note that the value of ΔpKa ($H_2O \rightarrow CH_3CN$) is ca. 17 units for acetic acid and phenol, and 14 units and 12 units for 3,5-dinitrobenzoic acid and 2,4-dinitrophenol, respectively.

From these data, and on the assumption that the effect of the aprotic solvent on the acidity of phosphoric and carboxylic acids should be of comparable order of magnitude, we have drawn the following inferences. The aryl phosphates have a relatively weak and a relatively strong

acidic function. The weakly acidic function should be affected to a greater extent than the strongly acidic function in going from water to acetonitrile. Thus, using the approximate values of ΔpKa $\sim$ 17 and 13 for the decrease in acidity of the second and the first dissociation constants of the aryl phosphates, respectively, 2,4-dinitrophenyl phosphate becomes an acid with pKa2 $\sim$ 22 and pKa1 $\sim$ 14, in acetonitrile (a separation of $\sim$ 8 pKa units), instead of pKa2 = 4.6 and pKa1 = 1.0, in water (a separation of only 3.6 pKa units). The corresponding figures for 4-nitrophenyl phosphate are: pKa2 $\sim$ 23 and pKa1 $\sim$ 14, in acetonitrile (a separation of $\sim$ 9 pKa units), instead of pKa2 = 5.5 and pKa1 = 1.2, in water (a separation of only 4.3 pKa units). In other words, a study of the aryl phosphates in acetonitrile as solvent should facilitate the task of elucidating the mechanism types that may operate in phosphoryl transfer from the monoanion and the dianion of a given aryl phosphate, since it is easier to "isolate" for study these two forms in acetonitrile than in water.

Two additional considerations enter into this picture. An acetonitrile solution containing the aryl phosphate dianion salt and several mole equivalents of an alcohol or water, should not contain appreciable concentrations of the monoanion salt and alkoxide (or hydroxide) ion:

$$ArOPO_3^{2-}2M^+ + ROH \rightleftharpoons ArOPO_3H^- + RO^- + 2M^+ \quad ; \quad [M^+ = (n\text{-}C_4H_9)_4N^+]$$

Data on alcohol and water acidity in acetonitrile as solvent are not available; however, from the corresponding data for phenol it is not unreasonable to expect decreases in acidity of the order of 16 pKa units for Brönsted acids of the type ROH. Therefore, the decrease in acidity of $ArOPO_3H^-$ is compensated by a decrease in acidity of ROH, when both are present in acetonitrile; or stated differently, ROH is too weak an acid in acetonitrile to protonate even the strongly basic dianion, $ArOPO_3^{2-}$. The same can be said for an acetonitrile solution of the aryl phosphate monoanion salt containing some alcohol or water:

$$ArOPO_3H^-M^+ + ROH \rightleftharpoons ArOPO_3H_2 + R\bar{O} + M^+$$

The second consideration is that an acetonitrile solution containing the aryl phosphate monoanion salt and one mole equivalent of an amine such as diisopropylethylamine or quinuclidine, should contain a relatively low concentration of the phosphate dianion and the aminium cation:

$$ArOPO_3H^-M^+ + R_3N \rightleftharpoons ArOPO_3^{2-} + R_3NH^+ + M^+$$

Table 1 shows that the replacement of water by acetonitrile decreases the acidity of the phosphate monoanion to a much greater extent than it decreases the acidity of the aminium cation; or conversely, R_3N is too weak a base in acetonitrile to shift the equilibrium from the monoanion to the dianion to a relatively large extent because the monoanion is such a weak acid in this solvent. On the other hand, an acetonitrile solution containing the aryl phosphate neutral acid and one mole equivalent of the amine should contain significant concentrations of the phosphate monoanion and aminium cation, since the first acidic function of the phosphate is so strong in water that it probably remains stronger than the aminium cation in acetonitrile:

$$ArOPO_3H_2 + R_3N \rightleftharpoons ArOPO_3H^- + R_3NH^+$$

A final consideration is that the displacement reactions of aryl phosphates produce a phenol and an alkyl phosphate, or inorganic phosphate, when the nucleophile is alcohol or water, respectively:

$$ArOPO_3H^- + ROH \rightleftharpoons ROPO_3H^- + ArOH \quad ; \quad ArOPO_3^{2-} + ROH \rightleftharpoons ROPO_3^{2-} + ArOH$$

The replacement of water by acetonitrile results in a decrease in acidity of both phosphates and phenols. The magnitude of this decrease will vary somewhat in both series, but the value of ΔpKa should be quite large in both cases, as shown in Table 1. Hence, the equilibrium involving the alkyl phosphate and the phenol products must be taken into account in connection with the acid strength of the phenol.

$$ROPO_3^{2-} + ArOH \rightleftharpoons ROPO_3H^- + ArO^-$$

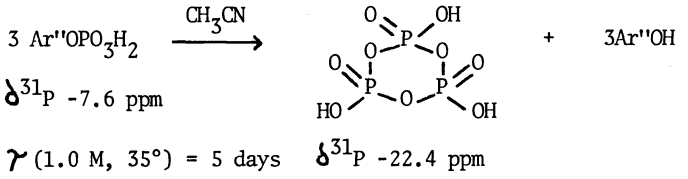
The corresponding equilibrium is probably irrelevant, in both solvents, for displacements of the aryl phosphate monoanion; now, the resulting alkyl phosphate species is the monoanion, which is a relatively weak base:

$$ROPO_3H^- + ArOH \rightleftharpoons ROPO_3H_2 + ArO^-$$

TABLE 1. Acid dissociation constants of Brönsted acids in water and in acetonitrile (data from Ref. 82).

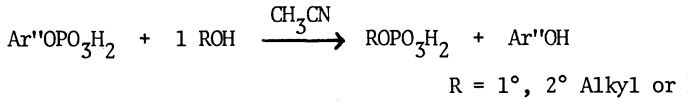
Acid	pKa Water	pKa Acetonitrile	ΔpKa
Acetic	4.8	22.3	17.5
Benzoic	4.2	20.7	16.5
4-Nitrobenzoic	3.4	18.7	15.3
3,5-Dinitrobenzoic	2.8	16.9	14.1
Phenol	10.0	26.6	16.6
4-Nitrophenol	7.2	20.7	13.5
2,4-Dinitrophenol	4.1	16.0	11.9
Pyrrolidine	11.3	19.6	8.3
Piperidine	11.2	18.9	7.7
Diethylamine	11.0	18.7	7.7
Triethylamine	10.7	18.5	7.8
Ethylamine	10.6	18.4	7.7
Ammonia	9.2	16.5	7.3
Pyridine	5.2	12.3	7.1

Reactions of 2,4-dinitrophenyl phosphate [83,84], $Ar''OPO_3H_2$; Ar'' = 2,4-$(NO_2)_2.C_6H_3$. The acid is reasonably stable in the crystalline state, but it is slowly transformed into cyclic trimetaphosphoric acid in acetonitrile solution (Eq. 1).



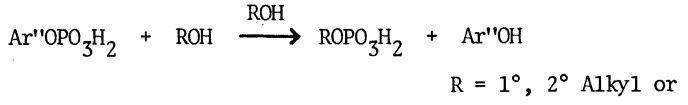
(Eq. 1)

Phosphoryl transfer from the acid to alcohols or water is observed in acetonitrile, but the reaction is quite sensitive to the structure of the alcohol and does not take place with tert-butyl alcohol (Eq. 2).



(Eq. 2)

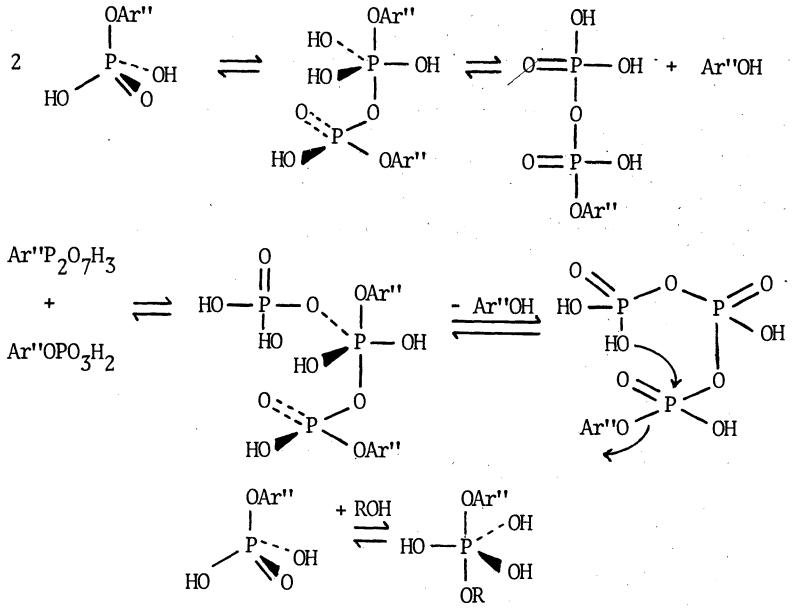
The solvolysis of the acid is not significantly faster in pure alcohol or water relative to the reaction with limited amounts of nucleophile in acetonitrile, in spite of the large increase in the alcohol/acid ratio (Eq. 3).



(Eq. 3)

A reasonable interpretation of the data is that the acid reacts via oxyphosphorane intermediates (Scheme 10). There is significant retardation of the reactions that produce cyclic trimetaphosphoric acid and alkyl phosphate as the medium is changed from acetonitrile to protic solvents. This effect is quite general (see below), and we conclude that there is more solvation of the more polar phosphate ground state than of the less polar transition state which generates the oxyphosphorane. The effect, which can cause rate depression [85], should increase as the solvating power of the medium increases.

Scheme 10



The monoanion salt decomposes into cyclic trimetaphosphate in acetonitrile at a rate which is similar to that observed with the acid (Eq. 4).

$$Ar''OPO_3H^-R'_4N^+ \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''OH \qquad \text{(Eq. 4)}$$

γ(1.0 M, 35°) = 2 days

When one mol equiv of alcohol or water is introduced into the above solution, there is still exclusive formation of trimetaphosphate (Eq. 5).

$$Ar''OPO_3H^-R'_4N^+ + 1\ ROH \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''OH \qquad \text{(Eq. 5)}$$

R = 1°, 2°, 3° Alkyl or H

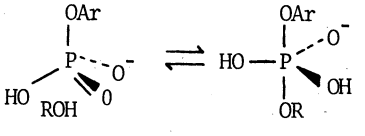
As more alcohol is introduced into the solution, alkyl phosphate appears as a component of the products (Eq. 6). The phosphoryl transfer is subject to steric hindrance in the alcohol; phosphorylation of ROH is not competitive with cyclization in mixtures of ROH and acetonitrile, when the alcohol is larger than methanol. Primary and secondary alcohols, but not tert-butyl alcohol, are phosphorylated in the pure alcohol at the appropriate concentration.

$$Ar''OPO_3H^-R'_4N^+ + ROH \xrightarrow{ROH} 1/3\ (P_3O_9)^{3-} + ROPO_3H^- + Ar''OH \qquad \text{(Eq. 6)}$$

R = 1°, 2° Alkyl or H

A significant rate depression is observed as acetonitrile is being replaced by alcohols or water, in both the formation of trimetaphosphate and alkyl phosphate or inorganic phosphate. Evidently, any increase in alcohol/phosphate ratio inevitably alters the medium; i.e. while the alcohol concentration increases, the rate-depression due to preferential ground-state solvation also increases. In general, the data supports the operation of an oxyphosphorane mechanism in these reactions of the dinitrophenyl phosphate monoanion, as shown in Scheme 11.

Scheme 11



The conclusion that the dinitro-ester monoanion reacts via oxyphosphorane intermediates contradicts previous interpretations of the data on the hydrolysis of this compound in aqueous solution [56,59,60]. It is conceivable that the ester-oxygen atom of an aryl phosphate derived from a phenol as acidic as 2,4-dinitrophenol (pKa 4.1 in water) is not sufficiently basic to allow the proton shift to the reactive form: $Ar''OP(O)(OH)O^- \rightleftharpoons Ar''O^+(H)PO_3^{2-} \dashrightarrow Ar''OH + PO_3^{2-}$. The ester-oxygen should become less basic for the same

electronic reasons that result in lower basicity of the 2,4-dinitrophenoxide anion, $Ar''O^-$. In this interpretation, the formation of the dipolar structure $X^+(H)PO_3^{2-}$ of a given phosphate monoanion, $XP(O)(OH)O^-$, is a prerequisite for the generation of PO_3^- from the monoanion. Presumably, the ejection of PO_3^- from a phosphate is a permissible process in solution, in contrast to the generation of the neutral species, $HOPO_2$ or $ROPO_2$. The possibility of symmetrical charge-delocalization in PO_3^- provides a justification for these differences.

There are no significant differences in reaction rates or in product composition when unhindered or hindered amines are added to the acid in equimolar amounts, in acetonitrile solution (Eq. 7).

$$Ar''OPO_3H_2 + \left\{\begin{array}{l}1\ (i\text{-}C_3H_7)_2C_2H_5N \\ \quad\text{or} \\ 1\ CH(CH_2CH_2)_3N\end{array}\right. \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''OH \qquad \text{(Eq. 7)}$$

γ(1.0 M, 35°) = 6 or 3 days

The similarities between the above types of reactions persist in the presence of limited amounts of alcohols or water (Eq. 8).

$$Ar''OPO_3H_2 + 1\ ROH + \left\{\begin{array}{l}1\ (i\text{-}C_3H_7)_2C_2H_5N \\ \quad\text{or} \\ 1\ CH(CH_2CH_2)_3N\end{array}\right. \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''OH \qquad \text{(Eq. 8)}$$

R = 1°, 2°, 3° Alkyl or H

The two types of amines display analogous behavior when added to the acid in equimolar amounts in the pure alcohols or water (Eq. 9). All of these reactions are analogous to those of the monoanion salt, $Ar''OPO_3H^-R_4N^+$, under comparable conditions. We conclude that the role of both types of amines in the reactions of the relatively acidic 2,4-dinitrophenyl phosphate is to act simply as proton acceptors. When one mol equiv of the amine is added to the acid in the aprotic solvent, practically all the acid is converted into monoanion and there is no free amine present in the solution.

$$Ar''OPO_3H_2 + ROH + \left\{\begin{array}{l}(i\text{-}C_3H_7)_2C_2H_5N \\ \quad\text{or} \\ CH(CH_2CH_2)_3N\end{array}\right. \xrightarrow{ROH} 1/3\ (P_3O_9)^{3-} + ROPO_3H^- + Ar''OH \qquad \text{(Eq. 9)}$$

R = 1°, 2° Alkyl or H

Bis-quaternary ammonium salts of 2,4-dinitrophenyl phosphate dianion cannot be isolated due to their extreme instability. Therefore, we studied the system which results when one mol equiv of amine is added to solutions of the monoanion salt (Eq. 10). The hindered amine causes an extraordinarily rapid formation of cyclic trimetaphosphate in acetonitrile solution. It is probable that this trimetaphosphate stems from whatever amount of dianion, $Ar''OPO_3^{2-}$ is present in the solution in equilibrium with monoanion, $Ar''OPO_3H^-$, since the cyclization promoted by diisopropylethylamine is much faster than the cyclization from the monoanion salt, $Ar''OPO_3H^-R_4N^+$.

$$Ar''OPO_3H^-R'_4N^+ + 1\ (i\text{-}C_3H_7)_2C_2H_5N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''O^- \qquad \text{(Eq. 10)}$$

γ(1.0 M, 35°) = 1 min

The unhindered amine also promotes a very fast formation of trimetaphosphate when added to the monoanion salt in acetonitrile solution (Eq. 11).

$$Ar''OPO_3H^-R'_4N^+ + 1\ CH(CH_2CH_2)_3N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''O^- \qquad \text{(Eq. 11)}$$

γ(1.0 M, 35°) < 1 min

The most reasonable interpretation of these results is that the amines generate a small but finite concentration of dianion from the monoanion salt, and that the dianion decomposes into the monomeric metaphosphate anion in a rate-limiting step which corresponds to a reaction with an extraordinarily large rate constant: $Ar''OPO_3^{2-} \rightarrow Ar''O^- + PO_3^-$. The PO_3^- is so electrophilic [86] that it attacks the aryl phosphate mono- or dianion to form alkyl polyphosphates, e.g.: $Ar''OPO_3H^- + PO_3^- \rightarrow Ar''P_2O_7H^{2-}$; $Ar''P_2O_7H^{2-} + PO_3^- \rightarrow Ar''P_3O_{10}H^{3-}$.

The polyphosphate then undergoes cyclization to the trimetaphosphate. An alternative route to cyclic trimetaphosphate is the trimerization of PO_3^-, which, however, requires a build-up in the solution of this very reactive species.

This picture is consistent with the fact that addition of hindered amine to the monoanion salt in acetonitrile and in the presence of any type of alcohol, or of water, produces the alkyl phosphate, or inorganic phosphate, at a rate which is independent of the structure of the alcohol (Eq. 12) or the water.

$$Ar''OPO_3H^-R'_4N^+ + 1\ ROH + 1\ (i\text{-}C_3H_7)_2C_2H_5N \xrightarrow{CH_3CN} ROPO_3H^- + Ar''O^- \qquad \text{(Eq. 12)}$$

R = 1°, 2°, 3° Alkyl or H

These observations would be difficult to understand unless one postulates reaction via a metaphosphate intermediate: $PO_3^- + ROH \longrightarrow ROPO_3H^-$.

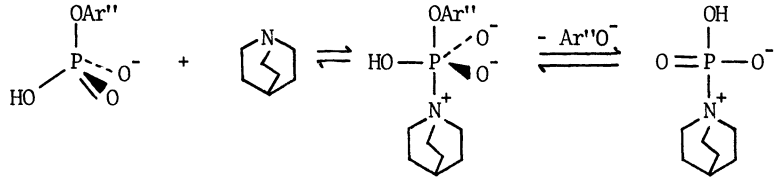
The comparable reaction with quinuclidine also gives alkyl phosphate, but now some cyclic trimetaphosphate is also formed with all alcohols or water (Eq. 13).

$$Ar''OPO_3H^-R'_4N^+ + 1\ ROH + CH(CH_2CH_2)_3N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + ROPO_3H^- + Ar''O^- \qquad \text{(Eq. 13)}$$

R = 1°, 2°, 3° Alkyl or H

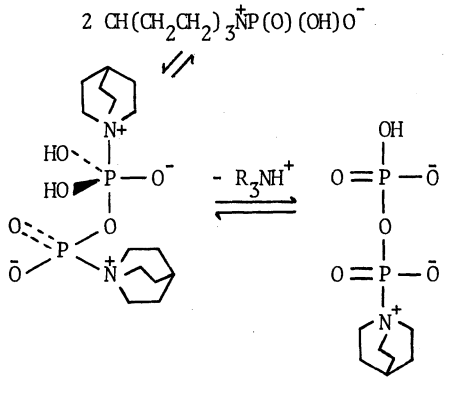
The cyclic trimetaphosphate results from that portion of the phosphate which exists in the solution as the monoanion in equilibrium with the dianion. The monoanion is sufficiently electrophilic to react with the unhindered amine to form an oxyphosphorane; Scheme 12. Collapse of the phosphorane produces the phosphorylated catalyst.

Scheme 12



The dipolar phosphoramide dimerizes as shown in Scheme 13. The final product can result from the pyrophosphoramide and the phosphoramide in an analogous step, and it should be emphasized that the tripolyphosphoramide (not shown) still contains an electrophilic center capable of undergoing cyclization to trimetaphosphate.

Scheme 13



As expected, the differences between the hindered and unhindered amines vanish when they are introduced into solutions of the monoanion salt in pure alcohols or water (Eq. 14). As the alcohol becomes more polar, e.g. methanol, the equilibrium shifts in the direction of dianion and this deprives the solution of monoanion and of nucleophilic amine. This effect is probably complete in aqueous solution. Now, the product, alkyl phosphate or HPO_4^{2-} results by way of decomposition of the dianion into PO_3^-. It should be noted that in the pure alcohols and water, there is rate depression resulting from the preferential solvation of the phosphate ground state relative to the less polar transition state that generates PO_3^-.

$$Ar''OPO_3H^-R'_4N^+ + ROH + \begin{cases} 1\ (i\text{-}C_3H_7)_2C_2H_5N \\ \text{or} \\ 1\ CH(CH_2CH_2)_3N \end{cases} \xrightarrow{ROH} ROPO_3H^- + Ar''O^- \qquad \text{(Eq. 14)}$$

R = 1°, 2°, 3° Alkyl or H

These phenomena are more clearly observed when the amines are introduced, to the extent of two mol equiv, into solutions of the acid, $Ar''OPO_3H_2$. The results observed in pure acetonitrile are given in Eq. 15 and Eq. 16.

$$Ar''OPO_3H_2 + 2\ (i\text{-}C_3H_7)_2C_2H_5N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''O^- \qquad \text{(Eq. 15)}$$

γ(1.0 M, 35°) = 40 min

$$Ar''OPO_3H_2 + 2\ CH(CH_2CH_2)_3N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''O^- \qquad \text{(Eq. 16)}$$

γ(1.0 M, 35°) = 1 min

The results of adding different amines to acid in the presence of alcohols or water in acetonitrile solution are dramatically different; Eq. 17 and Eq. 18. The hindered amine produces alkyl phosphate exclusively, regardless of the structure of the alcohol. Only the elimination-addition mechanism from dianion appears to be operative. Quinuclidine produces cyclic trimetaphosphate exclusively, from all types of alcohol or water. Now, the prevailing mechanism is nucleophilic catalysis by amine on monoanion. The solutions made from acid and quinuclidine in acetonitrile, apparently contain more monoanion and less dianion than the corresponding solutions made from monoanion salt and quinuclidine.

$$Ar''OPO_3H_2 + 1\ ROH + 2\ (i\text{-}C_3H_7)_2C_2H_5N \xrightarrow{CH_3CN} ROPO_3H^- + Ar''O^- \qquad \text{(Eq. 17)}$$

R = 1°, 2°, 3° Alkyl or H

$$Ar''OPO_3H_2 + 1\ ROH + 2\ CH(CH_2CH_2)_3N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar''O^- \qquad \text{(Eq. 18)}$$

R = 1°, 2°, 3° Alkyl or H

Superimposed on the problem of the relative concentrations of monoanion vs dianion in solutions of the phosphate in different solvents in the presence of amines, is the rate depression that occurs when the medium becomes poorer in acetonitrile and richer in alcohols and water. The rate depression is more important for reactions that stem from dianion (formation of alkyl phosphate via PO_3^-), than for reactions which stem from monoanion (formation of trimetaphosphate via oxyphosphorane under nucleophilic catalysis). These effects are apparent in the rates and product compositions for the reactions of acids in the presence of amines in pure alcohol or water. The results with the hindered amine are summarized in Eq. 19. Only alkyl phosphate is detectable in the products with all alcohol types.

$$Ar''OPO_3H_2 + ROH + 2\ (i\text{-}C_3H_7)_2C_2H_5 \xrightarrow{ROH} ROPO_3H^- + Ar''O^- \qquad \text{(Eq. 19)}$$

R = 1°, 2°, 3° Alkyl or H

The results with quinuclidine are given in Eqs. 20-22. With methanol or water, only alkyl phosphate is observed.

$$Ar''OPO_3H_2 + ROH + 2\ CH(CH_2CH_2)_3N \xrightarrow{ROH} ROPO_3H^- + Ar''O^- \qquad \text{(Eq. 20)}$$

R = CH_3 or H

With secondary alcohols, both trimetaphosphate and alkyl phosphate are detected.

$$Ar''OPO_3H_2 + ROH + 2\ CH(CH_2CH_2)_3N \xrightarrow{ROH} 1/3\ (P_3O_9)^{3-} + ROPO_3H^- + Ar''O^- \quad \text{(Eq. 21)}$$

R = 2° Alkyl

With tertiary alcohols, only trimetaphosphate is detected.

$$Ar''OPO_3H_2 + ROH + 2\ CH(CH_2CH_2)_3N \xrightarrow{ROH} 1/3\ (P_3O_9)^{3-} + Ar''O^- \quad \text{(Eq. 22)}$$

R = 3° Alkyl

The ^{31}P NMR spectrum of a 1.0 M acetonitrile solution of 2,4-dinitrophenyl phosphate, $Ar''OPO_3H_2$, shows the appearance of a new signal at +10.2 ppm (to low-field of the reference H_3PO_4 = 0) two minutes after the addition of two mol equiv of quinuclidine. This signal decreases in intensity as the signal due to the cyclic trimetaphosphate ($\delta^{31}P$ = -20.5 ppm) becomes more intense. It is quite probable that the +10.2 ppm signal is due to the dipolar phosphoramide intermediate, $CH(CH_2CH_2)_3N^+P(O)(OH)O^-$. The transformation of the +10.2 ppm signal into the -20.5 ppm signal is observed in solutions in which no signal due to the original aryl phosphate can be detected. The inference is that cyclic trimetaphosphate can be derived by trimerization of the dipolar intermediate as well as by reaction of the intermediate with aryl phosphate. Intermediates analogous to this phosphoramide have been previously suggested on kinetic grounds [24].

We found no evidence of nucleophilic catalysis in any of the reactions of the dinitrophenyl phosphate dianion. Evidence for such catalysis had been described in previous work in aqueous solutions [56,59,60]. Therefore, we examined the effect of adding one mol equiv of various amines to the following system:

$Ar''OPO_3H_2 + 2\ (CH_3)_4N^+OH^- \xrightarrow{H_2O} Ar''OPO_3^{2-}\ 2\ (CH_3)_4N^+ + 2\ H_2O$. Within the limited sensitivity of our measurements there was no significant effect in the rate of formation of inorganic phosphate plus 2,4-dinitrophenol upon addition of either triethylamine or quinuclidine. Pyridine had a reproducible but slight rate acceleration (by a factor of 4). Analogous observations were made on the system:

$Ar''OPO_3H_2 + 2[(n\text{-}C_4H_9)_4N]^+OH^- \xrightarrow{CH_3OH} Ar''OPO_3^{2-}2[(n\text{-}C_4H_9)_4N]^+ + 2\ H_2O$. Neither quinuclidine nor pyridine had a significant effect on the rate of formation of methyl phosphate. It seems that any relatively small rate acceleration by amines on reactions of the dianion are due to unknown medium effects and are not the result of the addition of the amine to the phosphate phosphorus.

In summary, 2,4-dinitrophenyl phosphate, in its diprotonated form or as its monoanion, undergoes nucleophilic displacements by the addition-elimination mechanism, and as its dianion, by the elimination-addition mechanism. Sterically unhindered amines add to the monoanion, but not to the dianion. The oxyphosphorane generated by addition of quinuclidine to the dinitrophenyl phosphate monoanion decomposes into a dipolar phosphoramide, $CH(CH_2CH_2)_3N^+P(O)(OH)O^-$, which can be detected as a transient intermediate by ^{31}P NMR spectroscopy. The monoprotonated dipolar phosphoramide reacts with itself or with the initial monoanion, $Ar''OPO_3H^-$, to yield cyclic trimetaphosphate, $P_3O_9^{3-}$ as the final product, even when alcohols are present. The occurrence of phosphoryl transfer from monoanion *via* oxyphosphorane, in the absence of nucleophilic catalysis, is recognized by the occurrence of relatively slow reaction rates, by a significant decrease in the rate of formation of alkyl phosphates with an increase in steric hindrance in the alcohol, and by the absence of any tert-butyl phosphate when the reaction is carried out in the presence of tert-butyl alcohol. The occurrence of phosphoryl transfer from dianion *via* monomeric metaphosphate anion is recognized by relatively fast reaction rates, by insensitivity of rates of formation of alkyl phosphates to changes in alcohol structure, and by the formation of tert-butyl phosphate when the reaction is carried out in the presence of tert-butyl alcohol. Medium effects are very important and operate at two levels: (1) aprotic solvents decrease acid strength relative to protic solvents, hence the medium controls the state of ionization of the phosphate and, thus, the relative concentrations of monoanion and dianion in the solution. (2) Protic solvents participate in solvation of ground state monoanion and dianion to a much greater extent than they participate in solvation of the less polar transition states which result in formation of oxyphosphorane or monomeric metaphosphate anion intermediates. This preferential ground-state solvation results in a significant rate decrease when the aprotic solvent, *e.g.* acetonitrile, is replaced by protic solvents, *e.g.* methanol or water. This effect is greater in the dianion than in the monoanion due to the higher dianion charge density.

TABLE 2. Approximate Half-time[a] of Reactions of Aryl Phosphates in 1.0 M Solutions.

Solvent	T,°C	Reagent (mol equiv.)	τ	Results[b]
$2,4\text{-}(NO_2)_2.C_6H_3OPO_3H_2$				
CD_3CN	35	CH_3OH(1)	15 h	$ROPO_3H_2$
CD_3CN	35	$(CH_3)_2CHOH$(1)	6 d	$ROPO_3H_2$
CD_3CN	35	$(CH_3)_3COH$(1)	...	$P_3O_9H_3$
CH_3OH	35	CH_3OH(25)	7 h	$ROPO_3H_2$
$(CH_3)_2CHOH$	35	$(CH_3)_2CHOH$(13)	7 d	$ROPO_3H_2$
CD_3CN	35	H_2O(1)	10 h	H_3PO_4
H_2O	35	H_2O(55)	10 h	H_3PO_4
$2,4\text{-}(NO_2)_2C_6H_3OPO_3H^-(n\text{-}C_4H_9)_4N^+$				
CD_3CN	35	CH_3OH(1)	3 d	$P_3O_9^{3-}$
CD_3CN/CH_3OH[c] 60 40	35	CH_3OH(10)	6 d	$P_3O_9^{3-}$ + $ROPO_3H^-$; 1:1
CH_3OH	35	CH_3OH(25)	14 d	$ROPO_3H^-$
CD_3CN	35	H_2O(1)	2 d	$P_3O_9^{3-}$
CD_3CN/H_2O 60 40	35	H_2O(22)	6 d	$P_3O_9^{3-}$ + $H_2PO_4^-$; 1:4
H_2O	35	H_2O(55)	6 d	$H_2PO_4^-$
$2,4\text{-}(NO_2)_2C_6H_3OPO_3H_2$ + $(i\text{-}C_3H_7)_2C_2H_5N$				
CD_3CN	35	CH_3OH(1)	7 d	$P_3O_9^{3-}$
CD_3CN/CH_3OH 60 40	35	CH_3OH(10)	10 d	$P_3O_9^{3-}$ + $ROPO_3H$; 1:1
CD_3CN/H_2O 60 40	35	H_2O(22)	8 d	$P_3O_9^{3-}$ + H_2PO_4; 1:4
$2,4\text{-}(NO_2)_2C_6H_3OPO_3H_2$ + $CH(CH_2CH_2)_3N$				
CD_3CN	35	CH_3OH(1)	2 d	$P_3O_9^{3-}$
CD_3CN/CH_3OH 60 40	35	CH_3OH(10)	4 d	$P_3O_9^{3-}$ + $ROPO_3H^-$; 1:1
CD_3CN/H_2O 60 40	35	H_2O(22)	8 d	$P_3O_9^{3-}$ + $H_2PO_4^-$; 1:4

[a] τ corresponds to [phosphate reactant]=[phosphate products], from measurements of ^{31}P NMR signal intensity; the values are accurate to within 25% of the indicated time.

[b] Products observed at t_∞.

[c] All mixed solvents are v/v.

Solvent	T,°C	Reagent (mol equiv.)	λ	Results
$2,4\text{-}(NO_2)_2C_6H_3OPO_3H^-(n\text{-}C_4H_9)_4N^+ + (i\text{-}C_3H_7)_2C_2H_5N$				
CD_3CN	35	CH_3OH(1)	1 m	$ROPO_3H^-$
CD_3CN	35	$(CH_3)_3COH$(1)	1 m	$ROPO_3H^-$
CD_3CN	35	H_2O(1)	1 m	$H_2PO_4^-$
$CD_3CN/(CH_3)_2CHOH$ 10 90	35	$(CH_3)_2CHOH$(12)	12 m	$ROPO_3H^-$
CH_3OH	35	CH_3OH(25)	1 h	$ROPO_3H^-$
H_2O	35	H_2O(55)	25 h	$H_2PO_4^-$
$2,4\text{-}(NO_2)_2C_6H_3OPO_3H^-(n\text{-}C_4H_9)_4N^+ + CH(CH_2CH_2)_3N$				
CD_3CN	35	CH_3OH(1)	<1 m	$P_3O_9^{3-} + ROPO_3H^-$; 1:4
CD_3CN	35	$(CH_3)_3COH$(1)	<1 m	$P_3O_9^{3-} + ROPO_3H^-$; 1:4
CD_3CN	35	H_2O(1)	<1 m	$P_3O_9^{3-} + H_2PO_4$; 1:5
$CD_3CN/(CH_3)_2CHOH$ 10 90	35	$(CH_3)_2CHOH$(12)	10 m	$ROPO_3H^-$
CH_3OH	35	CH_3OH(25)	1 h	$ROPO_3H^-$
H_2O	35	H_2O(55)	2.5 h	$H_2PO_4^-$
$2,4\text{-}(NO_2)_2C_6H_3OPO_3H_2 + 2(i\text{-}C_3H_7)_2C_2H_5N$				
CD_3CN	35	CH_3OH(1)	30 m	$ROPO_3H^-$
CD_3CN	35	$(CH_3)_3COH$(1)	35 m	$ROPO_3H^-$
CD_3CN	35	H_2O(1)	20 m	$H_2PO_4^-$
$CD_3CN/(CH_3)_2CHOH$ 10 90	35	$(CH_3)_2CHOH$(12)	2 h	$ROPO_3H^-$
CH_3OH	35	CH_3OH(25)	3.5 h	$ROPO_3H^-$
H_2O	35	H_2O(55)	2.5 h	$H_2PO_4^-$
$2,4\text{-}(NO_2)_2C_6H_3OPO_3H_2 + 2CH(CH_2CH_2)_3N$				
CD_3CN	35	CH_3OH(1)	1 m	$P_3O_9^{3-}$
CD_3CN	35	$(CH_3)_3COH$(1)	1 m	$P_3O_9^{3-}$
CD_3CN	35	H_2O(1)	1 m	$P_3O_9^{3-}$
$CD_3CN/(CH_3)_2CHOH$ 10 90	35	$(CH_3)_2CHOH$(12)	10 m	$P_3O_9^{3-} + ROPO_3H^-$; 5:1
CH_3OH	35	CH_3OH(25)	3 h	$ROPO_3H^-$
$CD_3CN/(CH_3)_3COH$ 10 90	35	$(CH_3)_3COH$(10)	10 m	$P_3O_9^{3-}$
H_2O	35	H_2O(55)	2.5 h	$H_2PO_4^-$

Solvent	T,°C	Reagent (mol equiv.)	λ	Results
$4\text{-}NO_2.C_6H_4OPO_3H_2$				
Dioxane	70	CH_3OH(1)	6 d	$ROPO_3H_2$
CH_3OH	70	CH_3OH(25)	2 d	$ROPO_3H_2$
Dioxane	70	H_2O(1)	3.5 d	H_3PO_4
H_2O	70	H_2O(55)	6 h	H_3PO_4
$4\text{-}NO_2.C_6H_4OPO_3H^-(n\text{-}C_4H_9)_4N^+$				
CD_3CN	70	CH_3OH(1)	8 h	$ROPO_3H^- + RP_2O_7H^{2-} + P_3O_9^{3-}$; 6:3:1
CD_3CN	70	$(CH_3)_3COH$(1)	7 h	$ROPO_3H^- + RP_2O_7H^{2-} + P_3O_9^{3-}$; 4:2:1
CD_3CN	70	H_2O(1)	7 h	$H_2PO_4^- + H_2P_2O_7^{2-}$; 4:3
CH_3OH	70	CH_3OH(25)	30 h	$ROPO_3H^-$
$CD_3CN/(CH_3)_3COH$ 10 90	70	$(CH_3)_3COH$(10)	30 h	$ROPO_3H^-$
H_2O	70	H_2O(55)	6 h	$H_2PO_4^-$
$4\text{-}NO_2.C_6H_4OPO_3^{2-}2[(n\text{-}C_4H_9)_4N^+]$				
CD_3CN	35	CH_3OH(2)[d]	5 m	$ROPO_3^{2-} + HPO_4^{2-}$; 1:1
CD_3CN	35	$(CH_3)_3COH$(2)[d]	4 m	$ROPO_3^{2-} + HPO_4^{2-}$; 1:1
CD_3CN	35	H_2O(3)	5 m	HPO_4^{2-}
CH_3OH	35	CH_3OH(25)	...	No reaction after 12 days
CH_3OH	70	CH_3OH(25)	6 h	$ROPO_3^{2-}$
$(CH_3)_3COH$	35	$(CH_3)_3COH$(10)	5 h	$ROPO_3^{2-}$
$(CH_3)_3COH$	70	$(CH_3)_3COH$(10)	1 m	$ROPO_3^{2-}$
H_2O	35	H_2O(55)	...	No reaction after 12 days
H_2O	70	H_2O(55)	3.5 d	HPO_4^{2-}
$4\text{-}NO_2.C_6H_4OPO_3H^-(n\text{-}C_4H_9)_4N^+ + (i\text{-}C_3H_7)_2C_2H_5N$				
CD_3CN	70	CH_3OH(1)	7 h	$ROPO_3^{2-} + RP_2O_7^{3-} + P_3O_9^{3-}$; 15:4:1
CD_3CN	70	$(CH_3)_3COH$(1)	6 h	$ROPO_3^{2-} + RP_2O_7^{3-} + P_3O_9^{3-}$; 6:3:1
CD_3CN	70	H_2O(1)	5 h	$HPO_4^{2-} + HP_2O_7^{3-}$; 3:2
H_2O	70	H_2O(55)	3 d	HPO_4^{2-}
$4\text{-}NO_2.C_6H_4OPO_3H^-(n\text{-}C_4H_9)_4N^+ + CH(CH_2CH_2)_3N$				
CD_3CN	70	CH_3OH(1)	45 m	$P_3O_9^{3-} + ROPO_3^{2-}$; 1:3
CD_3CN	70	$(CH_3)_3COH$(1)	45 m	$P_3O_9^{3-} + ROPO_3^{2-}$; 1:1
CD_3CN	70	H_2O(1)	30 m	$HPO_4^{2-} + HP_2O_7^{3-}$; 3:2
H_2O	70	H_2O(55)	3 d	HPO_4^{2-}

[d] Plus one mol equiv of water of crystallization in the salt.

Reactions of 4-nitrophenyl phosphate [87], $Ar'OPO_3H_2$; Ar' = 4-NO_2.C_6H_4.
This acid is quite stable in aprotic solvents, e.g. no reaction is observed after 7 days at 70°C in 1.0 M dioxane solution, although phosphoryl transfer to methanol or water takes place slowly in the same solvent (Eq. 23).

$$Ar'OPO_3H_2 + 1\ ROH \xrightarrow{\text{Dioxane}} ROPO_3H_2 + Ar'OH \qquad \text{(Eq. 23)}$$

R = CH_3, τ(1.0 M, 70°) = 6 days;

R = H, τ(1.0 M, 70°) = 4 days

The solvolysis of the acid is only slightly faster in pure alcohol or water (Eq. 24; unless otherwise specified, the reactions of 4-nitrophenyl phosphate were studied at 70°C). There is little doubt that these displacement reactions occur via oxyphosphorane intermediates.

$$Ar'OPO_3H_2 + ROH \xrightarrow{ROH} ROPO_3H_2 + Ar'OH \qquad \text{(Eq. 24)}$$

R = CH_3 or H

In contrast to the dinitro-ester monoanion salt, the mononitro-ester monoanion salt decomposes into cyclic trimetaphosphate at a much faster rate than the neutral acid, $Ar'OPO_3H_2$, in acetonitrile as solvent (Eq. 25).

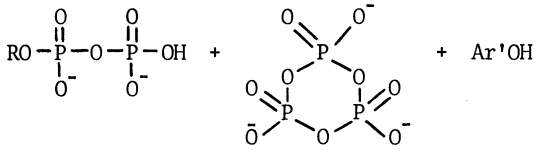
$$Ar'OPO_3H^-R'_4N^+ \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar'OH \qquad \text{(Eq. 25)}$$

τ(1.0 M, 70°) = 5 hr

This striking difference in behavior in the two types of esters is understandable if the mononitro-ester monoanion undergoes displacements via monomeric metaphosphate: $Ar'OPO_3H^- \rightleftharpoons Ar'O^+(H)PO_3^{2-} \rightleftharpoons Ar'OH + PO_3^-$. Apparently, the more basic ester oxygen of mononitrophenyl phosphate is able to accept a proton, and thus can generate the intermediate which seems to be required for the generation of metaphosphate anion. As PO_3^- is formed, it reacts with aryl phosphate to give arylpyrophosphate, which can be detected by ^{31}P NMR: $PO_3^- + Ar'OPO_3H^- \longrightarrow Ar'P_2O_7H^{2-}$. Eventually cyclic trimetaphosphate is produced, as described above, from pyrophosphate and more PO_3^-.

The transfer of the phosphoryl group from mononitro-ester monoanion salt to alcohols or water in acetonitrile solution occurs at a relatively rapid rate (Eq. 26). This reaction occurs at about the same rate (τ = 8 hr, 1.0 M at 70°) as the decomposition of the monoanion in the absence of alcohol or water. In fact, the alkyl phosphate is produced at about the same rate with all types of alcohol, including tert-butyl alcohol. Water reacts at about the same rate as alcohols. Significant amounts of alkyl pyrophosphates, and traces of cyclic trimetaphosphate are also detected among the products of these reactions.

$$Ar'OPO_3H^-R'_4N^+ + ROH \xrightarrow{CH_3CN} RO-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-O^- + \qquad \text{(Eq. 26)}$$



(Traces) R = 1°, 2°, 3° Alkyl or H

All of these observations are strongly suggestive of the formation of PO_3^- in the rate-limiting step, followed by rapid addition of nucleophile to PO_3^-: $PO_3^- + ROH \longrightarrow ROPO_3H^-$ $PO_3^- + ROPO_3H^- \longrightarrow RP_2O_7H^{2-}$.

In pure alcohols or water, the rate of phosphorylation decreases significantly, and all the phosphorus appears as alkyl phosphate (Eq. 27). We see here another manifestation of rate-depression resulting apparently from preferential ground state solvation relative to transition state solvation.

$$Ar'OPO_3H^-R'_4N^+ + ROH \xrightarrow{ROH} ROPO_3H^- + Ar'OH \qquad \text{(Eq. 27)}$$

R = 1°, 2°, 3° Alkyl or H

The mononitro-ester dianion salt is remarkably unstable in acetonitrile solution. The dianion transfers its phosphoryl group to alcohols or water in this solvent at a very fast rate (Eq. 28). Note that these reactions had to be carried out at 35°C, since they proved to be too fast for measurements at 70°C. Again, the rates were approximately the same regardless of the structure of the alcohol, and the reaction has all the characteristics associated with the elimination-addition mechanism, in particular the formation of tert-butyl phosphate from tert-butyl alcohol.

$$Ar'OPO_3^{2-}2R'_4N^+ + ROH \xrightarrow{CH_3CN} ROPO_3^{2-} + Ar'OH \qquad \text{(Eq. 28)}$$

R = 1°, 2°, 3° Alkyl or H

γ(1.0 M, 35°) = 5 min

It is significant that the phosphorylation of methanol from the mononitro-ester dianion is much slower in pure methanol than in acetonitrile as solvent, even though methanol is present only to the extent of one mol equiv in the aprotic medium. However, even in methanol solution, phosphorylation is still faster from dianion than from monoanion. On the other hand, phosphorylation becomes faster from monoanion than from dianion (by a factor of 15) when the solvent is water. It seems that the dianion is more susceptible to solvation than the monoanion. Therefore, as the solvent is changed from acetonitrile to methanol and to water, the rate depression due to preferential solvation of ground state vs transition state increases and eventually leads to reversal in the rate of formation of $\overline{PO_3^-}$ from the mono- and dianion.

$$Ar'OPO_3^{2-}2R'_4N^+ + ROH \xrightarrow{ROH} ROPO_3^{2-} + Ar'OH \qquad \text{(Eq. 29)}$$

R = 1°, 2°, 3° Alkyl or H

An acetonitrile solution of 4-nitrophenyl phosphate monoanion salt and one mol equiv of diisopropylethylamine in acetonitrile behaves like the solution of the same salt in the absence of the hindered amine; compare Eqs. 30 and 25. We take this to mean that, in the aprotic solvent, most of the monoanion remains protonated under these conditions.

$$Ar'OPO_3H^-R'_4N^+ + (i\text{-}C_3H_7)_2C_2H_5N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar'O^- \qquad \text{(Eq. 30)}$$

γ(1.0 M, 70°) = 5 hr

Quinuclidine and diisopropylethylamine have about the same basicity and, therefore, it is interesting that the acetonitrile solution of the monoanion salt plus quinuclidine no longer behaves like the monoanion in the absence of the amine (Eq. 31). Note the increase in the rate of formation of trimetaphosphate caused by the unhindered amine. In line with the discussion on the effect of quinuclidine on the dinitro-ester monoanion, we assume that this increase in the rate of trimetaphosphate formation from mononitro-ester is due to nucleophilic catalysis, i.e. formation of oxyphosphorane and dipolar phosphoramide intermediates, $\overline{CH(CH_2CH_2)_3N^+P(O)(OH)O^-}$.

$$Ar'OPO_3H^-R'_4N^+ + 1\ CH(CH_2CH_2)_3N \xrightarrow{CH_3CN} 1/3\ (P_3O_9)^{3-} + Ar'O^- \qquad \text{(Eq. 31)}$$

γ(1.0 M, 70°) = 30 min

When the hindered amine is added to the monoanion salt in acetonitrile solution containing one mol equiv of alcohols or water, the results are very similar to those obtained in the absence of the amine, as would be expected if little or no deprotection of monoanion takes place under those conditions (Eq. 32).

$$Ar'OPO_3H^-R'_4N^+ + ROH + (i\text{-}C_3H_7)_2C_2H_5N \xrightarrow{CH_3CN} ROPO_3^{2-}, RP_2O_7^{3-} + 1/3\ (P_3O_9)^{3-} + Ar'OH \text{ (Traces)} \qquad \text{(Eq. 32)}$$

R = 1°, 2°, 3° Alkyl or H

If the unhindered amine is added to the monoanion salt in acetonitrile solution containing limited amounts of alcohols and water, the results differ from those obtained in the absence of amine. Rates of reaction increase, and product composition changes significantly. Thus without amine (or with diisopropylethylamine) ratios of alkyl phosphate to trimetaphosphate are of the order of 5/1, and there are significant amounts of alkyl pyrophosphate (about one part for every two of alkyl phosphate). In the presence of quinuclidine, the ratios of alkyl phosphate to trimetaphosphate are 3/1 with methanol, and 1/1 with tert-butyl alcohol, and there is no detectable alkyl pyrophosphate.

$$Ar'OPO_3H^-R'_4N^+ + 1\ ROH + CH(CH_2CH_2)_3N \xrightarrow{CH_3CN} ROPO_3^{2-} + 1/3\ (P_3O_9)^{3-} + Ar'OH \qquad \text{(Eq. 33)}$$

R = 1°, 2°, 3° Alkyl or H

If one accepts the view that the protonated phosphoramide intermediate $CH(CH_2CH_2)_3\overset{+}{N}P(O)(OH)\bar{O}$ should produce only trimetaphosphate and not alkyl phosphate, as was the case in the analogous reaction of 2,4-dinitrophenyl phosphate monoanion with quinuclidine, one is forced to assume that a new process participates in the quinuclidine catalysis of 4-nitrophenyl phosphate monoanion. This process could be the deprotonation of the phosphoramide by the relatively strong base 4-nitrophenoxide. The aroxide is formed from the monoanion and quinuclidine: $Ar'OPO_3H^- + CH(CH_2CH_2)_3N \rightleftharpoons$ oxyphosphorane $\rightleftharpoons Ar'O^- + CH(CH_2CH_2)_3\overset{+}{N}P(O)(OH)O^- \rightleftharpoons Ar'OH + CH(CH_2CH_2)_3N^+PO_3^{2-} \longrightarrow CH(CH_2CH_2)_3N + PO_3^-$. Thus, although the rate of reaction increases as a result of the amine catalysis, the composition of the product is determined by fast reaction of PO_3^- with the alcohol, or with aryl phosphate, or with additional PO_3^- species. In the dinitro-ester, the base present would be the weakly basic 2,4-dinitrophenoxide which may not be able to deprotonate the phosphoramide to any significant extent.

It is not surprising to find that, in aqueous solution, the mixtures of mononitro-ester and the hindered and unhindered amines behave very much alike. Now, nearly complete shift of the equilibrium to the dianion form occurs, and there is no longer free amine or monoanion; hence no nucleophilic catalysis is observed. The product is formed from the dianion via PO_3^-.

$$Ar'OPO_3H^-R'_4N^+ + H_2O + \begin{cases} 1\ (i\text{-}C_3H_7)_2C_2H_5N \\ \text{or} \\ 1\ CH(CH_2CH_2)_3N \end{cases} \xrightarrow{H_2O} H_2PO_4^- + Ar'O^- \qquad \text{(Eq. 34)}$$

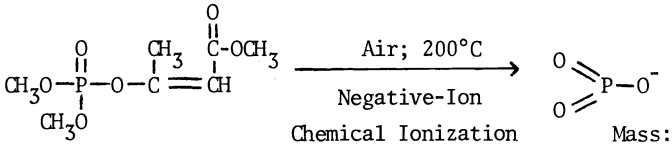
In summary, 4-nitrophenyl phosphate, in its diprotonated form, undergoes nucleophilic displacements by the addition-elimination mechanism, and as its monoanion and dianion, by the elimination-addition mechanism. The formation of monomeric metaphosphate anion intermediate is much faster from the dianion than from the monoanion in solution of 4-nitrophenyl phosphate in aprotic solvents and in alcohols. However, the metaphosphate anion is generated at a much faster rate from monoanion than from dianion in solutions of 4-nitrophenyl phosphate in water. This is attributed to greater water-solvation of the dianion ground state than of the monoanion ground state relative to the respective transition states which generate monomeric metaphosphate; the preferential ground-state vs transition state solvation by alcohols is not as strong as that of water, and cannot compensate for the greater tendency of dianion to generate metaphosphate when compared to monoanion. The operation of the elimination-addition mechanism in the 4-nitrophenyl phosphate monoanion, but not in the 2,4-dinitrophenyl phosphate monoanion, is attributed to the occurrence of a proton shift in the mononitro-case: $4\text{-}NO_2.C_6H_4OP(O)(OH)O^- \rightleftharpoons 4\text{-}NO_2.C_6H_4O^+(H)P(O)O^{2-} \longrightarrow 4\text{-}NO_2.C_6H_4OH + PO_3^-$. This proton-shift does not seem to take place to any appreciable extent in the dinitro-case, presumably, because the basicity of its aroxide-oxygen is too low. Basicity of the aroxide-oxygen in an aryl phosphate decreases as the acidity of the corresponding phenol, ArOH, increases. The factors which increase aroxide stability, ArO^-, and hence promote the dissociation $ArOH \rightleftharpoons ArO^- + H^+$, are the same factors which operate to lower oxygen basicity in the phosphate, $ArOP(O)(OH)O^-$. Sterically unhindered amines add to the 4-nitrophenyl phosphate monoanion, but not to its dianion, just as in the 2,4-dinitrophenyl phosphate case. However, there are significant differences in the stability of the dipolar phosphoramide that is produced from the intermediate oxyphosphorane in reactions of these two aryl phosphates. In reactions of 4-nitrophenyl phosphate catalyzed by quinuclidine, the protonated dipolar phosphoramide appears to be in equilibrium with its deprotonated form as a result of the relatively strong basicity of 4-nitrophenoxide ion. The deprotonated phosphoramide is unstable and decomposes to the amine and monomeric metaphosphate anion. In this case, quinuclidine catalyzes the disappearance of monoanion, but the final products of the reaction are those derived from reactions of metaphosphate anion. The occurrence of phosphoryl transfer from 4-nitrophenyl phosphate monoanion and dianion via monomeric metaphosphate anion (in the absence of nucleophilic catalysis by sterically unhindered amines) is recognized by the same criteria as in the dinitrophenyl-case, namely, rapid rates, insensitivity

of rates of alkyl phosphate formation to alcohol size, and formation of tert-butyl phosphate when tert-butyl alcohol is present. Medium effects in 4-nitrophenyl phosphate reactions operate also by the mechanisms discussed in the dinitrophenyl-case, i.e. by controlling the state of ionization of the phosphate, and by preferential solvation of phosphate ground state relative to the transition state leading to monomeric metaphosphate anion. In addition, aprotic solvents disfavor the proton-shift in the monoanion: $ArOP(O)(OH)O^- \rightleftharpoons ArO^+(H)PO_3^{2-} \longrightarrow ArOH + PO_3^{2-}$, which seems to be a prerequisite for generation of metaphosphate from monoanion.

Reactions of phenyl phosphate, $C_6H_5OPO_3H_2$.
This acid is, as expected, even less reactive than 4-nitrophenyl phosphate by the addition-elimination mechanism. The reactions of the monoanion salt $C_6H_5OPO_3H^-R_4N^+$ and the dianion salt, $C_6H_5OPO_3^{2-}2R_4N^+$, occur by the elimination-addition mechanism and show the lower reaction rates expected from the weaker nucleofugic groups, phenol and phenoxide.

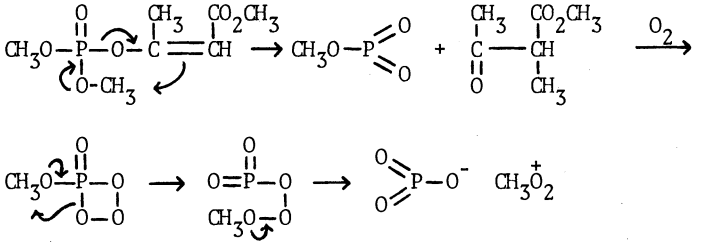
DIRECT OBSERVATION OF MONOMERIC METAPHOSPHATE ANION, ACID AND ESTERS IN THE GAS PHASE

The monomeric metaphosphate anion, PO_3^-, has been observed [88] in the gas phase by the technique of negative chemical ionization mass spectrometry [89]. The spectrum of dimethyl phosphoenolacetoacetate (Eq. 35), obtained at a source temperature of 200°C, in the presence of air as the reagent gas or moderator, contains a peak at mass 78.9590. The species responsible for this peak, which constitutes 99.9% of the total ion current at M/z 79 is the monomeric metaphosphate anion, PO_3^-, with a calculated mass of 78.9585. The phosphinate $CH_4PO_2^-$ has a calculated mass of 78.9949, and therefore it is possible to make a definitive structural assignment on the basis of high-resolution mass measurement.



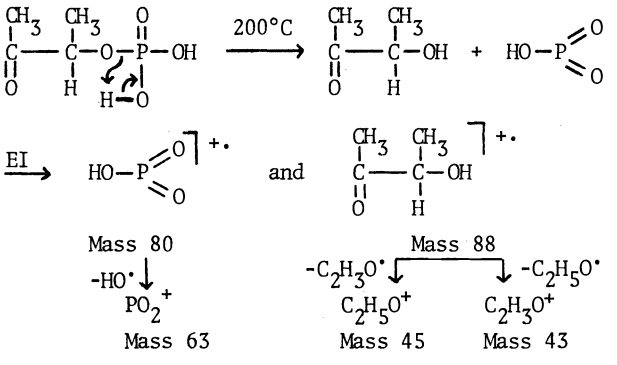
(Eq. 35)

The phenomena that take place in negative chemical ionization are complex; however, one can rationalize these, and analogous observations made on several related organophosphorus pesticides, by the hypothetical mechanisms depicted in Eq. 36.



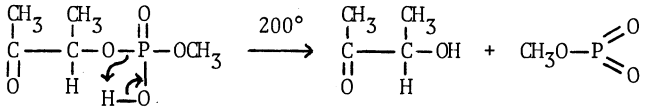
(Eq. 36)

Monomeric metaphosphoric acid, PO_3H, has also been observed [90] in the gas phase by electron impact (EI) mass spectrometry. Phosphoacetoin undergoes thermal decomposition into metaphosphoric acid and acetoin at a relatively low temperature (ca. 200°C); Eq. 37. Subsequent electron-ionization generates the corresponding molecular ions, and their expected fragment ions, all of which have been observed.



(Eq. 37)

Finally, we have been able to show [91] that, at about 200°C, methylphosphoacetoin undergoes thermal decomposition into monomeric methyl metaphosphate and acetoin; Eq. 38. An examination of the earlier literature on mass spectrometry of organophosphorus compounds reveals that the loss of alkyl metaphosphate and of other species of type $X\text{-}PO_2$ is, indeed, a rather general phenomenon in the gas phase.



(Eq. 38)

These results complement the earlier work of Westheimer and his coworkers [92,93], who carried out the pyrolysis of a six-membered cyclic unsaturated phosphonate methyl ester at 600°C. These authors were able to detect products reasonably ascribed to the intermediacy of CH_3OPO_2 in the gas phase.

CONCLUSIONS

There is compelling evidence for the existance of two types of mechanisms for nonenzymatic transfer of phosphoryl groups. In phosphotriesters and phosphodiesters, the only detectable mechanism is addition-elimination via an oxyphosphorane intermediate. In phosphomonoesters, and in other derivatives of phosphoric acid with two ionizable protons per phosphate group, the two mechanisms, addition-elimination and elimination-addition, can become operative depending on the structure of the phosphate, the state of ionization of the molecule, and the presence or absence of nucleophilic catalysts. The neutral diprotonated form of the phosphate reacts via oxyphosphoranes in all cases. The monoanion can react via oxyphosphorane or via metaphosphate in the absence of nucleophilic catalyst, depending on the structure of the phosphate. The monoanion reacts via an oxyphosphorane and a phosphorylated-catalyst intermediate, in the presence of nucleophilic catalyst. The dianion reacts only via metaphosphate regardless of the structure of the phosphate and of the amine.

There is no need to assume that some phosphates react by addition-elimination via a pentacoordinate phosphorus transition state, while other phosphates react via an oxyphosphorane intermediate.

Oxyphosphoranes have been directly observed in solutions in dynamic equilibrium with phosphate esters. However, monomeric metaphosphate anions, acids or esters have not been directly observed in solution. The species, PO_3^-, has been generated in the gas phase by pyrolytic reactions at temperatures as low as 200°C. The anion is observed directly by negative-ion chemical ionization mass spectrometry. The monomeric metaphosphoric acid, $HOPO_2$, has been generated in the gas phase by pyrolysis of certain phosphomonoesters at about 200°C. The acid, obtained in a purely thermal process, is observed directly in the form of its molecular ion, $HPO_3^{+\cdot}$, by electron-impact mass spectrometry. Monomeric alkyl metaphosphates $ROPO_2$ are easily produced by thermal decomposition of alkyl phosphodiesters in the gas phase at about 200°C, and by other pyrolytic reactions of phosphorus compounds.

Indirect evidence supports the involvement of the metaphosphate anion as an intermediate in certain reactions in solution. There is no analogous evidence with respect to the acid, $HOPO_2$, or the ester, $ROPO_2$. Possibly, this reflects a preference for the formation of oxyphosphoranes under the conditions that would be necessary to generate the metaphosphate acid or ester and/or, the difficulty associated with the formation of the acid or ester relative to the anion.

There is no conclusive evidence for or against the formation of monomeric metaphosphate in enzymatic phosphoryl transfer. The existence of regular and irregular permutational isomerizations in the intermediate of the alternate mechanism, namely the oxyphosphorane, and the possibility of multiple regular isomerizations of the phosphorane, increase the difficulties of any attempt to distinguish between addition-elimination and elimination-addition mechanisms in these reactions [94]. Some of the properties of enzymatic phosphoryl transfer are difficult to reconcile with the formation of metaphosphate in the reaction pathway. For example, it is known that there is an extensive intermediate oxygen exchange in muscle actomyosin MgATPase, i.e. three or four oxygen atoms in the water of the medium appear in the inorganic phosphate that is eventually released to the medium [95]. In view of the extraordinary reactivity of PO_3^-, it is necessary to include a number of ad hoc assumptions to explain the incorporation of so much medium water into released phosphate. The oxyphosphorane hypothesis, however, provides a reasonable interpretation for the currently available experimental data [95].

The effect of magnesium ion on phosphoryl transfer appears to be limited to enzymatic reactions [96-98]. Magnesium bound to oxyanions of phosphate or pyrophosphates does not significantly increase the electrophilicity of the phosphorus atom in nonenzymatic reactions [99]. In a recently advanced hypothesis [95], the main role of Mg^{2+} ions in biophos-

phoryl transfer is attributed to: (1) enhancement of the binding of the phosphorus reactant to the enzyme; (2) maintenance of the integrity of the "protein-product complex". With respect to the first point, Mg^{2+} ions bind strongly to both the phosphorus reactant and the protein. Histidine and N^{π}-methylhistidine appear to be the relevant amino acid residues for direct ligation to Mg^{2+} ions [100,101], just as histidine ligates to zinc ions in alcohol dehydrogenase [102], carboxypeptidase [103] and carbonic anhydrase [104]. In addition, other suitable amino acid residues can hydrogen-bond to water directly bound to the magnesium. These interactions supplement others which occur between the phosphorus reactant and the protein, e.g. nucleoside-protein binding. With respect to the second point, Mg^{2+} ions bind pairs of phosphorus oxyanions very strongly [105], $>P(O)OMgOP(O)<$. The protein-product complex in ATPases contains ADP strongly bound to inorganic phosphate through magnesium, and the whole complex remains bound to the protein [95]. The energy formerly in ATP has been transferred, as conformational energy, to a relatively small peptide segment in the protein-product complex. Further transformations depend on the biological role of the energy transduction associated with the phosphoryl transfer.

In the above hypothesis, the interactions between enzymes, certain metal ions and the phosphorus reactants modify significantly the mechanistic possibilities inherent in the process of phosphoryl transfer from derivatives of phosphoric acid to oxygen- and nitrogen-containing nucleophiles.

Acknowledgment - This work has been supported by grants from the National Institutes of Health (GM 20672) and the National Science Foundation (CHE76-16785).

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