U.D.C. 547.953.1

Reactions of Phosphoramidic Acids

N.N.Preobrazhenskaya

The mechanisms of the hydrolysis and solvolysis of phosphoramidic acids have been examined. The concepts of phosphoramidates as donors of the phosphate residue in phosphorylation reactions of acids, alcohols, and amines are discussed. The use of phosphoramidates as phosphorylating agents in synthetic chemistry and also their possible involvement in biological phosphorylation processes have been demonstrated.
The bibliography includes 142 references.

CONTENTS

I.	Introduction	54
II.	The structure of phosphoramidates	54
III.	Hydrolysis and solvolysis of phosphoramidates	56
IV,	Interaction of phosphoramidates with nucleophilic reagents	58
V.	General intramolecular acid catalysis of the reactions of phosphoramidates	60
VI.	Imidazole derivatives of phosphoric acid	61
VII.	Interaction of phosphoramidates with electrophilic reagents	62
VIII.	Phosphoramidates as intermediates in biological phosphorylation reactions	62

I. INTRODUCTION

During the last decades derivatives of orthophosphoric acid have been continually attracting the attention of many organic chemists and biochemists in connection with the outstanding advances in the study of nucleic acids, protein, biosynthesis and the investigation of bioenergetics, the structure of complex phospholipids, phosphoproteins, etc. Many studies have been made on phosphoric acid esters and anhydrides, in particular pyrophosphates, triphosphates, and acyl phosphates. Nowadays, yet another type of phosphoric acid derivatives—phosphoramidates—has aroused considerable interest.

Phosphoramidates include phosphonocreatine, phosphonotaurocyamine, phosphonoarginine, and other phosphonoguanidines isolated from the muscles of various animals and involved in the formation of ATP and ADP in muscular contraction, (see, for example, Straub[1]). The study of biological phosphorylation involving these compounds promoted the use of phosphoramidates as starting materials in the synthesis of pyrophosphates and triphosphates, including nucleoside derivatives (nicotinamide-adenine dinucleotide, coenzyme A, ADP, ATP, etc.). On the other hand, the study of biological phosphorylation showed that it involves labile intermediate phosphoramidates, namely enzymes phosphorylated at the imidazole ring of the histidine residue.

The determination of the role of phosphonoimidazoles in enzymatic processes is closely related to the study of the effect of organophosphorus poisons, pesticides, insecticides, etc. Thus there are data showing that di-isopropyl phosphonofluoridate—an effective inhibitor of many enzymes with a powerful toxic activity—phosphorylates the imidazole residue of the enzyme (see, for example [O'Brien][2]). Many powerful poisons, insecticides, and pesticides are phosphoramidates (Tabun, Schradan, etc). The synthesis of many phosphoramidates with carcinostatic activity has been described (see, for example, Stock[3] and Burstone[4]). (P–N)-aminoacid derivatives of nucleotides constitute an interesting variety of phosphoramidates. There are data suggesting that DNA- and RNA-peptides with a P–N bond between the nucleotide and the amino-group of the peptide are involved in the formation of the structure and in the functioning of nucleic acids (see, for example, Shabarova and Prokofiev[6]). One of the approaches employed to establish the structure and role of phosphoramidates is the study of model compounds—synthetic nucleotidoamides. Many interesting facts were observed in these studies, significantly supplementing the available knowledge of the reactivity of phosphoramidates, particularly about the intramolecular catalysis of their reactions (see the reviews of Preobrazhenskaya and Shabarova[5] and Shabarova and Prokofiev[6]).

The amides of nucleotides have found a novel application for synthetic purposes: the use of the amide derivative of the nucleotide for the blocking of the phosphate group in the synthesis of the dinucleoside phosphate has been described[7]; a method has been proposed whereby the nucleotide can be fixed on a polymeric carrier in the synthesis of oligonucleotides via the formation of a phosphoramidic bond between the phosphate residues of the nucleotides and the amino-group of the polymeric carrier[8].

Yet another cause which has stimulated interest in recent years in phosphoramidates is the discovery of enzymes cleaving these compounds—the so called phosphoamidases.

Despite the important role of phosphoramidates and the large number of studies on their chemistry and biochemistry, there are virtually no reviews of such investigations. Phosphoramidates are briefly mentioned in a discussion of phosphoesters[9–14] are dealt with only in a single review by Peacock[15] and in a chapter in the book by Bruice and Benkovic[16].

II. THE STRUCTURE OF PHOSPHORAMIDATES†

Phosphoramidates, like other derivatives of orthophosphoric acid, have a tetrahedral structure in the ground

† For a more detailed treatment see Refs. 9, 13 and 14.

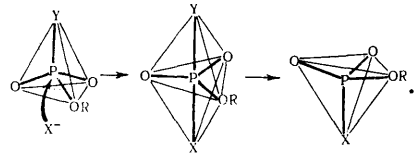
state having the phosphorus atom in the centre of the tetrahedron, and the four bonds directed to the vertides. The vacant 3*d* orbitals of the phosphorus atom may be involved in the formation of additional π bonds with the unshared electron pairs of nitrogen and oxygen (the so called $d_\pi-p_\pi$ conjugation). Thus the P–N and P–O bonds in phosphoramidates have some double-bond character[22]. This hinders attack by nucleophilic reagents and is shown particularly in a considerable resistance of phosphoramidates (in contrast to carboxylic acid amides) to the action of alkalis[17-21].

A necessary condition for nucleophilic substitution reactions at the phosphorus atom is the breakdown of $d_\pi-p_\pi$ conjugation in order to liberate the 3*d* orbitals of phosphorus and make them available for interaction with the *p* electrons of the nucleophilic reagent in the transition state.

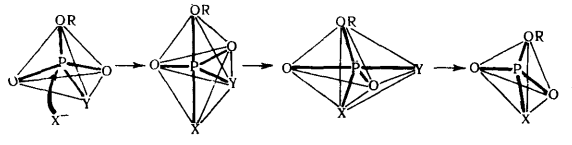
The most probable structure of the transition state in nucleophilic substitution at the phosphorus atom is a trigonal bipyramid[12-14,22]. Hence the entering and leaving groups can be located at the vertices of the bipyramid (a transition state of S_N2) type and the reaction takes place with inversion of configuration at the phosphorus atom. Such a transition state has been assumed for the majority of reactions of phosphoramidates on the basis of a study of nucleophilic substitution of optically active amido-derivatives of phosphoric acids (methyl *N*-cyclohexylphosphoramidothionate)[23] and also of intermolecular substitution reactions involving phosphoramidate derivatives with an additional amino-group[24].

However, these data do not fully exclude transition states of other types. The attack by nucleophilic reagents on the phosphorus atom can be facilitated if it is not directed along the P–N axis, since the unfavourable charge distribution then has a smaller effect[15]. This type of reaction is accompanied by so-called "pseudorotation", which is well known for phosphoesters (see, for example Refs. 25–28). As a result of pseudorotation, the nucleophilic reagent (X^-) attacking the phosphorus atom at an angle to the P–N axis passes from the plane of the base of the bipyramid to its vertex. Unfortunately, the stereochemistry of nucleophilic substitution at the phosphorus atom in phosphoramidates has been hardly investigated and therefore one can only speak of the possibility of "pseudorotation" in principle.

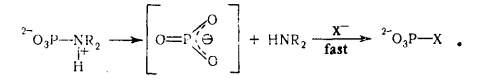
"Linear" substitution:



Substitution with "pseudorotation":



An extreme case of a trigonal bipyramid with an axial disposition of the entering and leaving groups is a transition state in which a labile intermediate metaphosphate is eliminated[24]. Its formation has been postulated for the reactions of many acyl phosphates, phosphoesters, and phosphoramidates, the common feature of which is the presence of a powerful electron-accepting substituent at the oxygen or nitrogen atom undergoing elimination and its marked dependence on the pK_a of the group eliminated[29]. Metaphosphate has a planar structure and therefore reactions involving it should take place with racemisation. However, the process has not been investigated for phosphoramidates. In reactions involving the intermediate metaphosphate, the rate-determining stage is unimolecular decomposition of the protonated phosphoramidate:

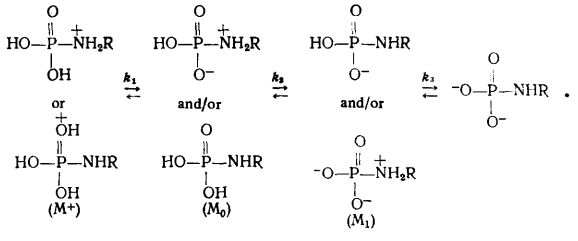


There are also many reactions in which a transition state, intermediate between the trigonal bipyramid and the planar metaphosphate[15,30-32] and characterised by a long P–N bond and a short bond between the phosphorus atom and the nucleophilic reagent being eliminated, has been postulated[30]. Such reactions exhibit the features of both bimolecular (in particular, the selectivity with respect to the nucleophilic reagent) and unimolecular reactions.

Table 1. Dissociation constants of phosphoramidic acids [and their esters]

Compound	pK_1	pK_2	References
Phosphoramidic acid	3.00	8.15	37
	4.6	7.7	17
	3.3	8.28	38
	2.83	8.03	39
	2.8	8.2	40
	2.72		15
	2.92	7.68	40
N-(*p*-Chlorophenyl)phosphoramidic acid	1.6	6.8	37
N-(*o*-Carboxyphenyl)phosphoramidic acid		7.25	32
N-(*p*-Carboxyphenyl)phosphoramidic acid		6.99	32
N-Acetylphosphoramidic acid	1.3	6.0	16
N-Benzoylphosphoramidic acid	1.99	6.42	41
	2.67	5.67	42
N-(*p*-Methylbenzoyl)phosphoramidic acid	2.04	6.20	41
N-(*p*-Nitrobenzoyl)phosphoramidic acid	1.97	6.24	41
Methyl *N*-cyclohexylphosphoramidate	3.1		43
Methyl *N*-cyclohexylphosphoramidothionate	3.1		18
Ethyl *NN*-diethylphosphoramidate		7.2	42
Phenyl *N*-benzoylphosphoramidate	1.9	13.4	41
N-Phosphorourethane	2.4	6.3	44

The reactions of phosphoramidates depend significantly on their degree of ionisation. Depending on the conditions and structure, phosphoramidic acids can exist in the form of the conjugate acid species (M^+), the neutral molecular (M_0), the monoanion (M_1), and the dianion (M_2). For example



Phosphoramidic acid may be protonated both at the nitrogen and at the phosphoryl oxygen. Its conjugate acid (M^+) is probably an equilibrium mixture of two forms: protonated at the oxygen and nitrogen atoms[33,34]. The neutral species (M_0) and the monoanion (M_1) can probably

exist in a zwitterionic form[35,36], and it is most likely that the derivatives of aromatic amines (for which $pK_a < pK_2$ for orthophosphoric acid) are protonated at the oxygen atom of the phosphate residue, while those of aliphatic amines (for which $pK_a > pK_2$ for orthophosphoric acid) exist mostly in a zwitterionic form[32] (Table 1).

III. HYDROLYSIS AND SOLVOLYSIS OF PHOSPHORAMIDATES

1. Hydrolysis of Phosphoramidates

The study of the hydrolysis of unsubstituted phosphoramidic acid as a function of pH showed[37] the presence of a plateau in the pH range 3.5–6.5; at higher pH the hydrolysis is retarded and at pH ≥ 10 phosphoramidate is virtually stable; at pH < 3 the rate of hydrolysis increases. These data show that the phosphoramidate dianion (M_2, $NH_2PO_3^{2-}$) is resistant to the action of water. The pH range 4.5–6.5 corresponds to the monoionic form (M_1), at lower pH the neutral form (M_0) makes a significant contribution, and at pH < 2 the cationic form (M^+) appears and the hydrolysis is acid catalysed.

When aromatic substituents are introduced at the phosphoramidate nitrogen, the profile of the curve relating the rate of hydrolysis of the phosphoramidate to pH hardly changes[45]. Only at pH < 3 is the hydrolysis of *N*-arylphosphoramidates somewhat slower than that of unsubstituted phosphoramidate. The explanation is probably that the electrons of the nitrogen atom are involved in conjugation with the aromatic ring, as a result of which the concentration of the active protonated form of the *N*-arylphosphoramidate (M^+) diminishes.

When powerful electron-accepting substituents (benzoyl[42], diphenylphosphoryl[42], benzenesulphonyl[46], and ethoxycarbonyl[44,46]) are introduced at the phosphoramidate nitrogen, the variation of the rate of hydrolysis with pH changes very significantly: the corresponding curves have a maximum at a pH of about 4 and at higher and lower pH the rate of hydrolysis falls sharply. Acid catalysis is as a rule not observed for these compounds[46,47] (*N*-benzoylphosphoramidate occupies an intermediate position: the acid catalysis of its hydrolysis occurs only in a very acidic medium[42]).

Probably acyl substituents, which are very powerful electron acceptors, hinder the protonation of the phosphoramidate and decrease the fraction of the neutral (M_0) and cationic (M^+) forms, which determine the rates of hydrolysis of the phosphoramidate at pH < 3.

There is no unanimous view at present concerning the possible reaction mechanisms. Therefore we present in Table 2 the suggested mechanisms of the solvolysis of various ionic forms of phosphoramidates according to different investigators and shall attempt to consider the motives which led them to give preference to a particular mechanism.

Phosphoramidic acid. The reactive ionic species are M_1, M_0, and M^+. Chanley and Feageson[37] attribute the activity of M_0 and M_1 to their ability to exist in a zwitterionic form. Conclusions concerning the solvolysis mechanism have been arrived at on the basis of a study of the composition of the solvolysis products in methanol–water mixtures: since for the monoionic form (M_1) the alkyl phosphate : phosphoric acid ratio is appreciably higher than the alcohol : water ratio in the reaction mixture, a bimolecular S_N2 (P) mechanism was assumed. This is consistent also with the catalysis of the solvolysis by pyridine and nicotine but not by the sterically hindered 2-methylpyridine.

However, Oney and Caplow[49] suggest a unimolecular solvolysis of the M_1 form of phosphoramidate, since it reacts at a rate close to that of the neutral form (M_0). They believe that with a bimolecular mechanism one should expect greater differences between the rates of solvolysis of the two forms owing to the more intense electrostatic repulsion of the nucleophilic reagent by the first form compared with the second.

Table 2. Suggested mechanisms of the solvolysis of phosphoramidates.

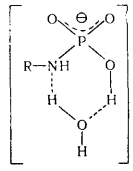
Compound	Ionic forms hydrolysed			Refs
	M^+	M_0	M_1	
Phosphoramidic acid	unimolecular bimolecular bimolecular *	unimolecular bimolecular	intermediate unimolecular bimolecular intermediate	15 48 37 11
N-Arylphosphoramidic acids	unimolecular * bimolecular	unimolecular bimolecular	unimolecular intermediate	37 11 32
N-Acylphosphoramidic acid			unimolecular * *	44 47 42

*The authors adduce data supporting both the unimolecular and bimolecular mechanisms without giving preference to either.

Cox and Ramsay[11] adduce evidence in favour of both the bimolecular and unimolecular mechanisms for the M_1 form of phosphoramidate. They suggest that, in the case of a direct attack by water on the monoanion (S_N2 mechanism), one should expect that phosphoramidate would react much more slowly than its aromatic derivatives. However, in fact phosphoramidate is hydrolysed approximately 10^3 times faster. On the other hand, if the solvolysis in aqueous alcohol proceeded via metaphosphate, the same ratio of alkyl phosphate and phosphoric acid should have been obtained as in the solvolysis of *p*-nitrophenyl phosphate under the same conditions, for which a unimolecular mechanism has been generally assumed. In fact phosphoramidate gives rise to approximately twice as much alkyl phosphate as does *p*-nitrophenyl phosphate[45]. Thus it is most likely that the solvolysis of the M_1 form proceeds via a transition state characterised by features of both the bimolecular and unimolecular mechanism. Peacock[15] arrives at this conclusion by considering the entropy of activation for the solvolysis of phosphoramidate.

A bimolecular solvolysis mechanism has been proposed[11,37,49] for the cationic form (M^+) of phosphoramidic acid in view of the lower yield of alkyl phosphate in solvolysis in aqueous alcohol compared with M_0 and M_1 (8, 44, and 72% respectively in 50% methanol[37,49]). However, the rate of hydrolysis of phosphoramidic acid is proportional to the Hammett acidity function H_0 and the corresponding plot has a slope of unity[17], which is characteristic of hydrolysis with elimination of metaphosphate[11]. Peacock is also inclined to favour the unimolecular mechanism[15].

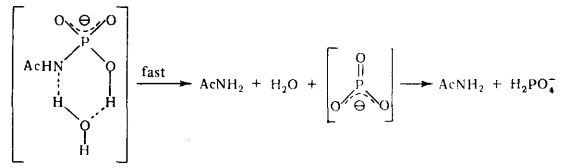
N-Arylphosphoramidates. A unimolecular solvolysis mechanism has been proposed for the monoionic (M_1) and neutral (M_0) forms of *N*-arylphosphoramidates, and it is suggested that M_0 is hydrolysed faster owing to the extremely high reactivity of its zwitter-ion[37]. However, in solvolysis in aqueous alcoholic mixtures with different alcohols different selectivities are observed compared with water[32]. This is inconsistent with the formation of free metaphosphate in the course of these reactions, since it cannot exhibit selectivity in relation to the nucleophilic reagent. On the other hand, the composition of the products in the methanol–water mixture is independent of the presence of an adjacent carboxy-group near the phosphoramidate group, which can be explained by a unimolecular mechanism[32]. In accordance with these data, it is suggested that the solvolysis of the monoanions of *N*-arylphosphoramidates includes a transition state with both unimolecular and bimolecular characteristics[32]. Possibly the hydrolysis of the monoanions involves the formation of a cyclic intermediate in which water participates[11,45], by analogy with that proposed for the hydrolysis of the monoanions of phosphate monoesters[50].



The neutral form (M_0) of *N*-arylphosphoramidic acids is selective with respect to the nycleophilic reagent in solvolysis in aqueous alcohol[32,37]. In addition, it is very sensitive to steric factors[32]. This suggests a bimolecular mechanism for the solvolysis of the M_0 form of *N*-arylphosphoramidic acids.

Examination of the solvolysis products in aqueous alcohol shows that the protonated form (M^+) is hydrolysed unimolecularly[37] (the alkyl phosphate : phosphoric acid ratio is close to the alcohol : water ratio in the solution). However, Benkovic and Benkovic[32] preferred a bimolecular pathway, including attack by the solvent on the protonated phosphoramidic acid. According to Cox and Ramsay[11], the linear relation between the rate of hydrolysis and the molarity of the acid which they observed[45] suggests that the hydrolysis of the M^+ form includes bimolecular attack by water, but the ratio of the products suggests that the reaction is unimolecular.

N-Acylphosphoramidates and *N*-phosphourethane. The reactive form of *N*-acylphosphoramidates and also of *N*-phosphourethane is the monoanion (M_1). The decrease of its proportion as both the dianion and the neutral form are formed retards the hydrolysis fairly markedly[42,44,47], which resembles somewhat the situation in the hydrolysis of monoalkyl phosphates[10]. The absence of an effect of nucleophilic (NaF, di-n-butylamine, butanethiol, aniline) and electrophilic (Ag^+, Cu^+) reagents on the hydrolysis of *N*-phosphonourethane[47] and the absence of exchange of the phosphoryl oxygen in the hydrolysis of *N*-phosphonourethane in $H_2^{18}O$ are inconsistent[47] with both unimolecular and bimolecular reactions. One of the possible mechanisms for the hydrolysis of *N*-acylphosphoramidates and *N*-phosphonourethane[41,42,47] is the cleavage of a hydrated cyclic intermediate, as in the hydrolysis of *N*-arylphosphoramidates examined above:



2. Hydrolysis of Phosphoramidate Monoesters

Phosphoramidate monoesters are cleaved at the P–N bond only in an acid medium[6,7,48,51–58]. The linear relation between the hydrolysis rate constant and the concentration of H^+ ions is evidence that the hydrolysis is acid catalysed[48].

The neutral forms of methyl phosphoramidate and unsubstituted phosphoramidic acid are both hydrolysed and solvolysed in 50% aqueous methanol at similar rates[48]. This finding indicates a bimolecular reaction mechanism for both compounds; in a unimolecular reaction involving an intermediate metaphosphate one would expect lower rates for the methyl ester because the resonance stabilisation of metaphosphate esters is more difficult than that of the metaphosphate ion. The bimolecular hydrolysis and solvolysis of phosphoramidate esters was demonstrated also for methyl *N*-cyclohexylphosphoramidate[43]: the lower yield of ethyl methyl phosphate (10%) in the solvolysis of this compound in 50% ethanol contradicts the formation of a highly reactive intermediate metaphosphate, which should not exhibit selectivity with respect to one of the nucleophilic reagents. However, in the catalysis of the solvolysis of methyl *N*-cyclohexylphosphoramidate by pyridine, for which the formation of active *O*-methylphosphonopyridinium has been suggested, the yield of ethyl methyl phosphate virtually corresponds to the content of ethanol in the reaction mixture, so that in this case one cannot rule out the possibility of the formation of an intermediate metaphosphate[43]. Thus it is extremely likely that the mechanism of the reaction of phosphoramidate monoesters depends on the type of groups eliminated, in other words on the substituents at the amide nitrogen.

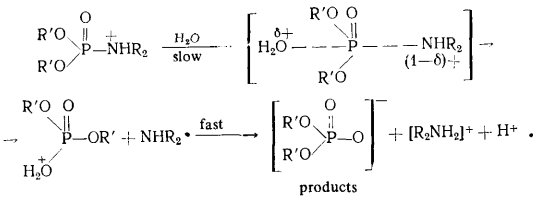
The view that the reactivity of phosphoramidates increases with the basicity of the initial amine[42,43,51], based on a study of the hydrolysis of three compounds (the piperidide, morpholide, and *p*-anisidide of adenosine 5′-monophosphoric acid)[51], has been very widely expressed in the literature. However, this conclusion contradicts the results obtained in the study of the hydrolysis of many other alkyl phosphoramidates[34,51,59,60], for which a decrease of reactivity with increasing basicity of the initial amine can be clearly traced.

In this connection a comparative study of the hydrolysis of many aliphatic and aromatic phosphoramidates derived from uridine 5′-monophosphate[61] is of interest. Comparison of the rates of hydrolysis of these compounds in 0.05 *N* HCl (37 °C) shows that the rate of hydrolysis of derivatives of weak amines (aromatic amines and 2,2,2-trifluoroethylamine, pK_a = 1–5.7) increases with the basicity of the initial amine, while the rates of hydrolysis of derivatives of strongly aliphatic amines (pK_a = 9.34 to 10.64) falls as the basicity of the initial amine rises. These results are consistent with the hypothesis of the dual effect of the substituent at the nitrogen atom on the hydrolysis of phosphoramidates[34]: on the one hand, electron-donating substituents promote the protonation of the

phosphoramidate, stabilising the positive charge generated, and, on the other hand, electron-accepting substituents facilitate the formation of the transition state, reducing the electron density at the phosphorus atom and promoting thereby nucleophilic substitution at this atom. Possibly[61] in the case of derivatives of weak amines the first effect predominates and therefore the rate of hydrolysis of the P–N bond increases as the electron-donating properties of the substituent become more pronounced. On the other hand, in the case of derivatives of strong amines the formation of the transition state is decisive and accordingly the rate of hydrolysis falls for more powerful electron-donating substituents.

3. Hydrolysis of Phosphoramidate Diesters

Phosphoramidate diesters are also hydrolysed at the P–N bond in an acid medium and are stable in an alkaline medium (see, for example, Sokolova et al.[62]). The study of the kinetics of the acid hydrolysis of phosphoramidate diesters, for 2,4-dichlorophenyl methyl *N*-alkylphosphoramidates, showed that the reaction is bimolecular and most probably involves direct attack on the phosphorus atom by the water molecule with simultaneous elimination of the amine[34]:



The results of a study of the hydrolysis of dimethyl phosphoramidate in D_2O and its solvolysis in 50% aqueous methanol[49] and also the results obtained in a study of the hydrolysis of dimethyl *NN*-dialkylphosphoramidates[60,63] are consistent with this mechanism.

Phosphoramidate diesters are much more stable in acid solutions than the corresponding phosphoramidic acids and phopshate monoesters[62,64–67]. In some cases, as a result of the stabilisation of the P–N bond in phosphoramidate diesters, the phosphate ester and not the phosphoramide bond is the first to be cleaved in an acid medium[34,64].

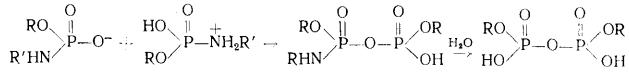
The reason for this change in the stabilities of the P–N and P–O bonds may involve[64] a change in the d_π–p_π – conjugation of the lone electron pairs of the nitrogen and oxygen atoms linked to the reaction centre and in the population of the 3*d* orbitals of the phosphorus atom in these compounds compared with phosphoramidic acids and phosphate monoesters. Conjugation of the electrons of the oxygen atom with the phosphorus atom in the P–OH and particularly the $P–O^-$ bond is known[22] to be much more pronounced than for the P–OR bond. This should have two consequences: firstly, a decrease of the stability of the P–O bond in phosphate diesters and secondly a decrease of the population of the 3*d* orbitals of the phosphorus atom in such diesters and consequently their greater accessibility to the electrons of the nitrogen atom, which should entail an increase in the stability of the P–N bond. The appreciably higher stability of diphenyl phosphoramidates compared with di-n-butyl phosphoramidates is consistent with these concepts[59]. It is also possible that the hydrolysis of phosphoramidate diesters is slower than that of the monoesters because, owing to the absence of a hydroxyl capable of dissociation in the phosphate residue of the diesters, their monomolecular hydrolysis via an intermediate metaphosphate is precluded.

IV. INTERACTION OF PHOSPHORAMIDATES WITH NUCLEOPHILIC REAGENTS

When phosphoramidates react with nucleophiles such as phosphoric acid and its esters, pyrophosphoric acid and its esters, carboxylic acids, amines, alcohols, etc., they can behave as donors of the phosphate group.

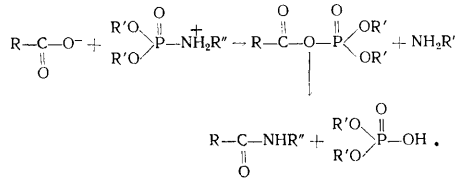
In the transfer of the phosphate residue from phosphoramidates to phosphoric acid or its esters, pyrophosphates are formed; if the nucleophilic agent is pyrophosphoric acid or its esters, triphosphates are formed. The formation of pyrophosphates from [unsubstituted] phosphoramidate was already observed in the previous century[68]. Later it was established that other phosphoramidates are readily converted into symmetrical pyrophosphates[51,67,69,70].

Kinetic studies showed that pyrophosphates are formed as a result of the attack by the phosphoramidate anion on the protonated molecule with elimination of one mole of the base[23,36,43,71]:



This reaction and also the known capacity of biological systems to employ phosphoramidates the so-called phosphagens, for example; phosphonocreatine, phosphonotaurocyamine, phosphonoglycocyamine, and phosphonolombricine (see, for example, Refs. 1, 72, and 73)—for the synthesis of pyrophosphates have served as a basis of preparative methods for the synthesis of pyrophosphates and triphosphates, including nucleotide coenzymes[69,74–77]. The most convenient amides of nucleotides for the synthesis of pyrophosphates are morpholides[51]. Many nucleoside polyphosphates have been synthesised from them. This problem has been considered in detail in monographs[78,79]. Mention should be made of those later studies[80] where the effect of reaction conditions on the yield of nucleoside diphosphate derivatives was investigated.

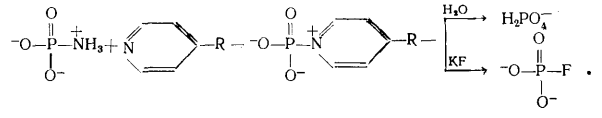
When the phosphate residue is transferred from phosphoramidate to carboxylic acids, acyl phosphates are formed[43,67,83]. Then the amine present in the reaction mixture (this may be a specially added amine[43,67,70] or the amine liberated as a result of the reaction between phosphoramidate and carboxylic acid[81]) can react with the acyl phosphate to form a carboxylic acid amide. When carboxylic acids are heated with phosphoramidate diesters and particularly with phosphoric triamide $PO(NH_2)_3$, the corresponding amides are formed in yields of 40–80%:[82]



The phosphate group in phosphoramidates can be transferred to another amine. The free phosphoramidic acids $(HO)_2PONR_2$ (R = H, Alk, or Ar) are least effective as phosphate donors in the phosphorylation of primary amines[19,39], probably because under conditions where

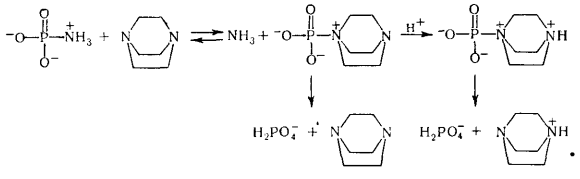
phosphoramidates are active the majority of primary amines are protonated and therefore cannot function as nucleophilic reagents. Nevertheless, the preparation of *N*-phosphonoaminoacids and *N*-phosphonopeptides using phosphoramidate as the phosphorylating agent has been described recently[84]. The phosphorylation of hydrazine in its reaction with phosphoramidate has also been described[39]

Phosphoramidate monoesters and particularly diesters are much more active as donors of the phosphate group, probably because of their higher p*K* values. However, the phosphate group is transferred only to weak amines. Thus methyl *N*-cyclohexylphosphoramidate and its thioanalogue enter into transamination reactions with 1-naphthylamine, 2,2,2-trifluoroethylamine, and *O*-methylhydroxylamine[23,43]. Among bases, the most effective acceptors of the phosphate group are tertiary bases[18], particularly pyridine and its homologues. Synthetic reactions are frequently carried out in pyridine (see the synthesis of pyrophosphates mentioned above), which is well known to catalyse the transfer of the phosphate group. The formation of an intermediate labile phosphonopyridinium ion has been frequently postulated in these reactions[18,37,85-90]. Its formation has been demonstrated in the reaction of phosphoramidic acid; in the presence of pyridine its hydrolysis is accelerated, and when fluoride ions are added, phosphorofluoridate is also formed[39]:



Pyridine derivatives catalyse also the hydrolysis of diisopropyl phosphorofluoridate and dimethyl phosphorofluoridate, 3- and 4-methylpyridines being more effective and 1-methylpyridine equally as effective as unsubstituted pyridine[85].

Other tertiary bases behave similarly, for example triethylenediamine is a still more effective catalyst (probably because of steric strain and the ready protonation of the second nitrogen atom, a more readily eliminated group is formed)[39]:



Phosphorylated tertiary amines can be useful phosphorylating agents in syntheses owing to their high reactivity and particularly because they remain reactive in an alkaline solution—under conditions where other phosphoramidates lose a proton and become inactive[39]. Examples of this kind are diphosphonoimidazole (see Section VI) and 4-methyl-*N*-phosphonopyridinium, which reacts completely with 0.5 M solution of methylamine at pH 11.7 in less than one minute[39].

An important problem in the chemistry of phosphoramidic acids is the transfer of the phosphate residue to the hydroxy-group of alcohols. The achievement of this reaction on a preparative scale would provide a method for the phosphorylation of alcohols which would be convenient for the following reasons: (a) the relative simplicity of the synthesis of the phosphorylating agent (phosphoramidate); (b) the possibility of storing it for a fairly long time; (c) the possibility of carrying out the reaction in an aqueous medium, this being particularly important in the phosphorylation of nucleosides and nucleotides, which are sparingly soluble in organic solvents. In this connection, a number of studies have been made on the solvolysis of phosphoramidates in water–alcohol mixtures. However, despite the fact that phosphoramidates effectively phosphorylate phosphates, carboxylic acids, and certain amines, in the vast majority of cases they proved to be unsuitable for the phosphorylation of alcohols[43,67]. This is because alcohols as a rule cannot compete as nucleophilic agents with water (hydrolysis) or with a second molecule of phosphoramidate (the formation of pyrophosphates). Thus, although in very dilute aqueous ethanol methyl *N*-cyclohexylphosphoramidate yields methyl phosphate (hydrolysis product) and ethyl methyl phosphate (ethanol phosphorylation product), in more concentrated aqueous ethanol considerable amounts of pyrophosphoramide are formed, the product being gradually hydrolysed, so that ultimately more than 90% of phosphorus is detected as methyl phosphate[43].

However, it is noteworthy that the ratio of the hydrolysis and phosphorolysis products depends considerably on the structure of the phosphoramidate, namely on the presence and nature of ester substituents at the phosphorus atom and of substituents at the nitrogen atom: the yield of methanolysis products depends on the introduction of a methyl group into the phosphate residue and amounts to 8, 21, and 12% for unsubstituted phosphoramidate, monomethyl phosphoramidate, and dimethyl phosphoramidate respectively[49].

The yield of phosphorylation products depends significantly on the reaction conditions, probably due to the specific mechanism of the solvolysis of phosphoramidate. But, in the solvolysis of the monoanionic form of phosphoramidate (bimolecular reaction), the ratio of the alkyl phosphate and phosphoric acid formed greatly exceeds the ratio of the alcohol to water in the reaction mixture[37]. This may be due to the greater nucleophilicity of alcohols compared with water. On passing to a unimolecular mechanism (for example acid-catalysed solvolysis), the effect of the nucleophilicity of the reactant diminishes and there is a corresponding decrease of the percentage of phosphorolysis. Until recently, the formation of alkyl phosphate and phosphoric acid in the solvolysis of phosphoramidates in water–alcohol mixtures in proportions close to those of the alcohol and water was regarded as evidence of a unimolecular mechanism involving the formation of an intermediate metaphosphate[37]. However, it was shown recently that the product ratio depends to a considerable degree on the nature of the group eliminated even when the mechanisms are known to be identical[29,91] and on the nature of the alcohol[29]. The latter finding had been explained previously by steric factors, but this explanation is invalid, since in the phosphorylation of amines significant differences as a function of the structure of the group are not observed. It has been suggested that the difference between the reactivities of alcohols can be due to a non-static distribution of solvent molecules in the solvation shell around the phosphate group[29], the degree of solvation in the transition state being apparently important[49]. This suggests that phosphoramidates are in principle unsuitable for the phosphorylation of alcohols with a bulky alkyl group.

However, the role of the alcohol alkyl group is abolished in the phosphorylation by phosphoramidates derived from tertiary amines. Above it was already mentioned that

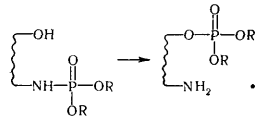
pyridine derivatives catalyse the reactions of phosphoramidates with phosphates and amines by forming active phosphorylating compounds. This is also observed in the reaction of phosphoramidates with alcohols. Thus in the solvolysis of methyl *N*-cyclohexylphosphoramidate in aqueous ethanol (50 mole %) in the presence of pyridine, the yield of ethyl methyl phosphate increases to 40% compared with 8% in the absence of pyridine[43].

N-Benzoylphosphoramidate proved to be an effective phosphorylating agent for alcohols on a preparative scale. It was used successfully in the phosphorylation of ethyl and benzyl alcohols, cyclohexanol and phenol (yield 40–80%); ethanolamine was phosphorylated to 2-aminoethylphosphoric acid. The reaction was carried out in the presence of triethylamine; the highest yields were obtained when it was used in an equimolar amount in relation to the phosphoramidate[41].

Thus apart from the phosphonoimidazoles, which will be discussed separately in Section VI, only phosphoramidates with powerful electron-accepting substituents at the nitrogen atom are of preparative value as phosphorylating agents for alcohols.

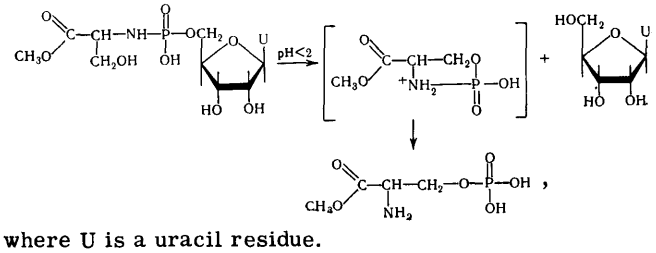
The situation is different when the phosphoramide group and the alcoholic hydroxyl are near one another in space: the intramolecular phosphorylation of hydroxy-groups is achieved under a wide variety of conditions and with high yields. It is possible to distinguish two types of reactions.

1. The amino-group of a hydroxylated compound is phorphorylated. In this case intramolecular phosphorylation results in N → O migration of the phosphate residue:



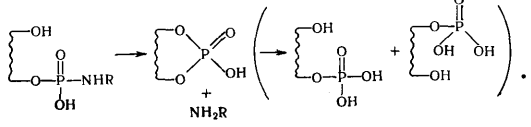
These reactions take place as a rule in an acid medium (see, for example Plapinger and Wagner-Jauregg[92]).

The finding that intramolecular phosphorylation of hydroxy-groups by phosphoramidates takes place only with esterified phosphono-derivatives is striking[18]. This phenomenon is attributed to the necessity for a cyclic intermediate, which in the case of monoesters and diesters can be formed fairly readily as a result of transesterification whereas in the case of a non-esterified phosphate group its formation is difficult[18]. N → O migration of this kind with transesterification in the acid hydrolysis of (P–N)-serine derivatives of nucleotides has been described[93]:

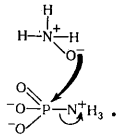


where U is a uracil residue.

2. The hydroxy-compound contains a second OH group esterified by the phosphoramidate residue. In this case intramolecular phosphorylation leads to the elimination of the amine and the formation of a cyclicphosphate, which under sufficiently severe conditions may be hydrolysed further to the monophosphate[7,94–98]:



Among other nucleophilic reagents capable of reacting with phosphoramidates, hydroxylamine should be mentioned. A study of its reaction with unsubstituted phosphoramidate at pH 7 established that it attacks the phosphorus atom via its oxygen atom rather than the nitrogen[39]:



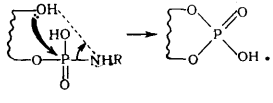
The reaction of hydroxylamine with phosphoramidates has been suggested for the specific cleavage of nucleotido-(P–N)-peptides[99,100]. When they are treated with hydroxylamine at pH 5, hydroxylamine derivatives of nucleotides are formed together with peptides, and the latter can be determined quantitatively by conversion into dinitrophenyl derivatives[99]. *N*-Methylhydroxylamine proved to be more reactive and *O*-methylhydroxylamines less reactive than unsubstituted hydroxylamine[100], in agreement with the mechanism described above.

Finally, phosphoramidates react with hydrogen halides. Hydrogen fluoride is the most reactive in this process. It reacts with any phosphoramidate, in contrast to hydrogen chloride and hydrogen bromide, which reacts only with *NN*-disubstituted phosphoramidates[101,102].

In aqueous solutions phosphoramidates react with fluoride ions to form phosphorofluoridates, rapidly in a weakly acid medium[17,103] and slowly in neutral and alkaline media[39]. Phosphorofluoridate is formed from phosphoramidate and potassium fluoride in the presence of pyridine or triethylenediamine, which are responsible for the nucleophilic catalysis of the transfer of the phosphate residue[39] (see pp. 58, 59).

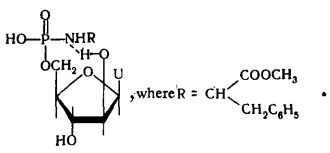
V. GENERAL INTRAMOLECULAR ACID CATALYSIS OF THE REACTIONS OF PHOSPHORAMIDATES

The transfer of the phosphate residue from the amino-group to the hydroxy-group with formation of cyclicphosphates, described in the preceding sections, probably constitutes a special case of general intramolecular acid catalysis in which the hydroxy-group behaves as a proton donor for the amide nitrogen, disrupting the $d\pi$–$p\pi$ conjugation between nitrogen and phosphorus and increasing the electrophilicity of the latter[93,98]:



In the majority of the examples quoted above, general acid catalysis involving the hydroxyl-group leads to a sharp change in the properties of the phosphoramidates: the P–N bond becomes labile not only in acid but also in neutral and alkaline media. In other cases, catalysis by the hydroxyl group can lead only to an increase of the reactivity of phosphoramidates in an acid medium. Thus the methyl ester of 1-B-D-arabofuranosyluracil 5′-phospho-(P–N)-phenylalanine is hydrolysed faster at pH 1–5 than the corresponding ribose derivative, probably

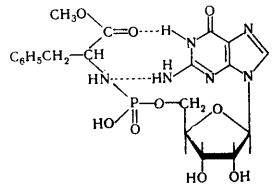
because in the former the 2'-OH group is in the *cis*-position relative to the 5'-P–N bond and may protonate its nitrogen atom[96]:



Apart from the hydroxy-group, the undissociated carboxy-group can function as a proton donor in the general intramolecular acid catalysis of reactions of phosphoramidates. Thus *o*-carboxylphenyl phosphoramidate is hydrolysed faster (in the monoanionic and neutral forms) than the corresponding *p*-carboxyl-derivative[32]. A similar effect of the carboxyl group is observed in the hydrolysis of phosphonocreatine[104].

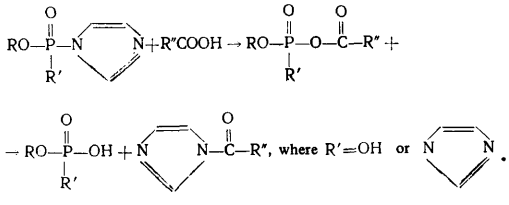
The increase of the reactivity of the P–N bond as a result of the catalytic effect of the amino-group as the proton donor is observed in the hydrolysis of phosphonohistidines. Histidine derivatives are less stable than the corresponding imidazole derivatives both in hydrolysis and in the transfer of the phosphate residue to imidazole derivatives, the P–N bond in the 1-position of the imidazole ring being the most labile. The protonated α-amino-group is responsible for the increase in the lability of phosphonohistidines[84,105].

An intramolecular acceleration of the hydrolysis of the phosphoramide bond by the amino-group has been observed also for 5'-guanylyl-(P–N)-phenylalanine[57]:



VI. IMIDAZOLE DERIVATIVES OF PHOSPHORIC ACID

Imidazolides occupy a special place among phosphoramidates. They are distinguished by high reactivity towards nucleophilic reagents of various types. Thus phosphonoimidazole monoesters and diesters give quantitative yields of pyrophosphates in reactions with phosphoric acids and its esters[108-109]. In the reaction with carboxylic acids, phosphonoimidazole monoesters and also the esters of phosphoric acid di-imidazolide yield acyl phosphates, which react further with the liberated imidazole, forming acylimidazole and a phosphate monoester[107,108]:



Among phosphoramidates, phosphonoimidazoles are the most active donors of the phosphate residue towards amines. Phosphonoimidazole and its esters phosphorylate aromatic and aliphatic amines, the latter reacting more readily[107,108,110,111]. Diphosphonoimidazole is particularly effective in the phosphorylation of amines. It phosphorylates primary aliphatic amines at alkaline pH. Diphosphonoimidazole can be used to prepare phosphoramidic acid (75%) and to phosphorylate aminoacids[40,112], ethanolamine, aromatic amines, *p*-aminobenzoic acid, and sulphanilic acid[18].

Phosphonoimidazoles are even more effective phosphorylating agents towards alcohols, phosphonoimidazole esters being the most reactive[107,108,111].

Evidently (see Sections VI and V) the intramolecular phosphorylation of an alcoholic hydroxyl by phosphonoimidazoles would be most effective. In this connection, the synthesis of dinucleoside phosphates on a polynucleotide matrix by the reaction of the nucleotide imidazolide with the nucleoside is exceptionally interesting[113-115]. The complementary interaction of polyuridylic acid with a mixture of *N*-(5'-adenylyl)-imidazole and adenosine ensures relative positions of the phosphoramide group and the alcoholic hydroxyl resembling those in the intramolecular reaction, as a result of which the phosphate residue is transferred from imidazole to the hydroxy-group of the nucleoside, i.e. a dinucleoside phosphate ApA is formed (yield 41%).

Thus phosphonoimidazoles are the most active donors of the phosphate residue in the phosphorylation of phosphates, carboxylic acids, amines, and alcohols. On the other hand, imidazole is the most effective acceptor of the phosphate group from phosphoramidates[18,39,116]. This has served as a basis for a preparative method of phosphorylating imidazole by phosphoramidic acid[84,105].

The imidazole residue can be phosphorylated not only by phosphoramidates but also by phosphonoimidazoles. Examples of this are the reaction between diphosphonoimidazole and histidine with formation of phosphonohistidine and the hydrolysis of 1-phosphonoimidazole, where one molecule probably phosphorylates another[18] and also the conversions of 1-phosphonohistidine into the corresponding 3-derivatives[84], for example: 1-phosphonohistidine + histidine → histidine + 3-phosphonohistidine; 1-phosphonohistidine + α-*N*-acetylhistidine → histidine + α-*N*-acetyl-3-phosphonohistidine.

The unique capacity of phosphonoimidazoles to function as effective donors and acceptors of the phosphate residue probably constitutes the basis of their catalytic effect in the reactions of many phosphoric acid derivatives. Thus imidazole catalyses the hydrolysis of phosphoramidate[18,39,116], *p*-nitrophenyl phosphate[31], dialkyl phosphorofluoridates[35], and ethylene methyl phosphate[117]. It has been suggested[85] that the catalytic properties of imidazole can be accounted for by the intermediate formation of phosphorylated imidazole. Indeed, the exceptional lability of di-isopropylphosphonoimidazole—the potential intermediate in the hydrolysis of di-isopropyl phosphorofluoridate—has been demonstrated. In a bicarbonate buffer at pH 7.6 at 30°C it hydrolyses to the extent of 50% in 2.7 h and 100% in 13 h.[85]

The catalytic activity of imidazole in reactions *in vitro* suggests that phosphonoimidazoles may be intermediates in the reactions of phosphorylated compounds *in vivo*. Imidazole in the form of the histidine residue enters into the active centres of transphosphorylation enzymes. Phosphonohistidine has been isolated in many cases. The involvement of histidine is particularly likely, since its *N*-1-phosphono-derivative is much more labile than *N*-1-phosphonoimidazole[84]. As stated above (see section V), this is associated with the effects of the histidine side chain, namely its protonated amino-group, which is

close to the *N*-1-position. In protein the histidine amino-group is of course most likely to be combined in a peptide chain, but the labilisation of the P–N bond of phosphono-imidazole may be induced by the amino-group of another aminoacid located in its vicinity owing to the specific conformation of the enzyme. Evidently, in principle, there is then a possibility of the activation of both phosphor-amide groups, in the 1- and 3-positions of imidazole, and the specific result will be determined by steric factors.

It is interesting to note that phosphonoimidazole itself is not more reactive than phosphoramidic acid but its esters are much more reactive towards nucleophilic attack. One may suppose therefore that phosphonohistidine partici-pates in enzymatic processes associated with the transfer of the phosphate ester residue (for further details, see Section VIII).

VII. INTERACTION OF PHOSPHORAMIDATES WITH ELECTROPHILIC REAGENTS

General and specific acid catalysis (see Sections V and III) and catalysis by metal ions are special cases of elec-trophilic catalysis. Catalysis by metal ions (Zn^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, and Cu^{2+}) has been described for phospho-guanidines—natural phosphagens[118]. It is known that *in vitro* they are much more stable than simple phos-phoramidates. This is because of the delocalisation of the positive charge at the amide nitrogen. The catalytic effect of the metal ion consists in preventing the delocalisation by the formation of a phosphoramidate–metal cation com-plex[72,73,118].

Electrophilic catalysis has been investigated in detail in the hydrolysis of phosphoramidates in the presence of formaldehyde, pyridoxal, and hypochlorite[30], nitrous acid, and isopentyl nitrite[119]. The role of such catalysts is probably the conversion of the amino-group into a more readily eliminated group. For example, the following mechanism has been suggested[30] for the hydrolysis of phos-phoramidate in the presence of formaldehyde:

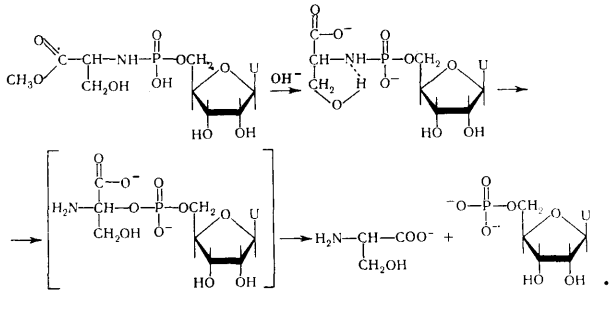
$$^{-2}O_3P\overset{+}{N}H_3 + HCHO \overset{\text{fast}}{\rightleftharpoons} {}^{-2}O_3P\overset{+}{N}H_2CH_2OH\,,$$

$$^{-2}O_3P\overset{+}{N}H_2CH_2OH + HCHO \overset{\text{fast}}{\rightleftharpoons} {}^{-2}O_3P\overset{+}{N}H(CH_2OH)_2\,,$$

$$^{-2}O_3P\overset{+}{N}H_2CH_2OH \rightarrow H_2PO_4^- + NH_2CH_2OH\,,$$

$$^{-2}O_3P\overset{+}{N}H(CH_2OH)_2 \rightarrow H_2PO_4^- + NH(CH_2OH)_2\,.$$

Electrophilic catalysis combined with nucleophilic catalysis, particularly when they take place intramole-cularly, is most effective in relation to phosphoramidates. For example, in the case of 5′-uridilyl-(P–N)-serine[93] and other nucleotidoamines containing the OH group capable of approaching the amide nitrogen[97], combined electro-philic and nucleophilic catalysis results in the lability of the P–N bond in an alkaline medium:



VIII. PHOSPHORAMIDATES AS INTERMEDIATES IN BIOLOGICAL PHOSPHORYLATION REACTIONS

The exceptional sensitivity of phosphoramidates to nucleophilic and electrophilic catalysis and in particular to combined intramolecular nucleophilic and electrophilic catalysis suggests that they can play the role of active intermediates in biological phosphorylation processes.

A number of enzymes cleaving phosphoramidates, the so called phosphoamidases (here we are not considering phosphagens), have been described. However, hitherto in all cases the phosphoamidase activity has been merely supplementary to the main activity of the enzyme—usually phosphoesterase of phosphotransferase activity[120–127]. Only ribonucleoside 5′-phosphoamidase from mammalian tissue extract has not so far shown any additional activity[128]. Phosphoamidase activity is also shown by certain proteo-lytic enzymes[127].

The phosphoesterase and phosphotransferase activity of phosphoamidases and the fact that free phosphoramidates have not been detected in biological systems, synthetic compounds being employed as substrates in enzymatic reactions, suggest that the phosphoamidase activity of the enzymatic preparations described is a kind of artefact reflecting certain details of the mechanism of enzyme action.

Possibly, in phosphoesterase and other reactions, intermediate enzyme–substrate compounds of the phos-phoramidate type are formed and this is why the enzymes are capable of cleaving phosphoramidates when these are supplied in a finished form.

The phosphorylation of the enzyme protein during the reaction has been described for a number of enzymes[129–142]. For certain enzymes involved in nucleic acid metabolism, it has been suggested[110,127,138–140] that the substrate can interact initially with the active centre of the enzyme, forming intermediate active nucleoside 5′-phospho-enzyme derivatives. In many cases it has been suggested that phosphate adds to the imidazole moiety of the histidine residue[105,129–135,142]. Phosphonohistidine was also isolated from mitochondria and from a preparation of succinate thiokinase and was identified by reference to a synthetic specimen of 3-phosphonohistidine[105]. However, subse-quently the exceptional case of the transfer of the phosphate residue from the 1-position of histidine to various acceptors, including the *N*-3-position in histidine, was demonstrated. Thus the problem of the site where histi-dine is phosphorylated in the enzyme remains open[84].

In connection with the possibility of the formation of enzyme–substrate complexes with a P–N bond between the fragments, synthetic phosphoramidates can be regarded as models of such complexes. The differences between them and natural phosphoramidates consists in the fact that the enzyme has been replaced by an amine residue as a donor of amide nitrogen. If the phosphoramidate can approach the active centre of the enzyme without affecting sifnifi-cantly its structure, there is a possibility of nucleophilic-electrophilic activation of the phosphoramidate site with participation of the functional groups of the enzyme, which under "normal" conditions acts on the enzyme's own phosphonoimidazole system. Ultimately, when a phos-phate acceptor is present, transphosphorylation takes place, while in the absence of a specific acceptor the phos-phoramidate is hydrolysed. This hypothesis has been discussed in detail for ribonucleoside 5′-phosphoamidase and it was suggested that, like other phosphoamidases, this enzyme has no independent importance and its activity is based on the formation of intermediate phosphoramidate structures in the synthesis of hydrolysis of internucleotide bonds of RNA.[61]

REFERENCES

1. F. B. Straub, "Biochemie" (Translated into Russian), Published by the Hungarian Academy of Sciences, Budapest, 1965, p. 613.
2. R. D. O'Brien, "Toxic Phosphate Esters, Chemistry, Metabolism, and Biological Effects" [Acad. Press, New York, 1960] (Translated into Russian), Izd. Mir, Moscow, 1964, pp. 102, 132.
3. J. A. Stock, Chem. in Britain., 6, 11 (1970).
4. M. S. Burstone, "Enzyme Histochemistry and Its Applications in the Study of Neoplasms" [Acad. Press, New York, 1962] (Translated into Russian), Izd. Mir, Moscow, 1965, pp. 421–424.
5. N. N. Preobrazhenskaya and Z. A. Shabarova, Uspekhi Khim., 38, 222 (1969) [Russ. Chem. Rev., No. 2 (1969)].
6. Z. A. Shabarova and M. A. Prokofiev, Progress in Nucleic Acid Research and Mol. Biol., 10, 145 (1970).
7. E. Ohtsuka, K. Murao, M. Ubasawa, and M. Ikehara, J. Amer. Chem. Soc., 91, 1537 (1969); 93, 3441 (1970).
8. G. M. Blackburn, M. J. Borwn, and M. R. Harris, J. Chem. Soc. (C), 2438 (1967).
9. R. S. Hudson, "Organic Chemistry" (Translated into Russian, Vol. 6, "Structure and Mechanism in Organophosphorus Chemistry" (Translated into Russian), Izd. Mir, Moscow, 1967.
10. E. M. Kosower, "Molecular Biochemistry" (Translated into Russian), Izd. Mir, Moscow, 1964.
11. J. R. Cox and O. B. Ramsay, Chem. Rev., 64, 317 (1964).
12. N. A. Loshadkin, "Mekhanizm Nukleofil'nogo Zameshcheniya u Tetraedricheskogo Atoma Fosfora" (Mechanism of Nucleophilic Substitution at a Tetrahedral Phosphorus Atom) (Appendix to the Russian translation of Ref. 2).
13. A. J. Kirby and S. G. Warren, "The Organic Chemistry of Phosphorus, Elsevier Publ. Comp., Amsterdam, 1967.
14. J. R. Van Wazer, "Phosphorus and Its Compounds" (Translated into Russian), Inostr. Lit., Moscow, 1962.
15. C. J. Peacock, Z. Naturforsch., 24b, 391 (1969).
16. T. C. Bruice and S. J. Benkovic, "Bio-organic Mechanisms", W. A. Benjamin, Inc., New York–Amsterdam, 1966, Vol. 2.
17. M. Halmann, A. Lapidot, and D. Samuel, J. Chem. Soc., 1299 (1963).
18. T. Rathlev and T. Rosenberg, Arch. Biochem. Biophys., 65, 319 (1956).
19. J. F. Morrison, A. H. Ennor, and D. E. Griffiths, Biochem. J., 68, 447 (1958).
20. E. W. Crunden and R. F. Hudson, J. Chem. Soc., 3591 (1962).
21. S. E. Severin, Uspekhi Biol. Khim., 2, 355 (1954).
22. D. W. Cruickshank, J. Chem. Soc., 5486 (1961).
23. N. K. Hamer, J. Chem. Soc., 2731 (1965).
24. N. K. Hamer, J. Chem, Soc., (C), 404 (1966).
25. P. C. Haake and F. H. Westheimer, J. Amer. Chem. Soc., 83, 1102 (1961).
26. D. A. Usher, Proc. Nat. Acad. Sci. U. S. A, 62, 661 (1969).
27. M. A. Harris, D. A. Usher, H. P. Albrecht, G. H. Jones, and J. G. Moffatt, Proc. Nat. Acad. Sci. U. S. A, 63, 246 (1969).
28. S. J. Benkovic and K. J. Schray, J. Amer. Chem. Soc., 91, 5653 (1969).
29. A. J. Kirby and A. G. Varvoglis, J. Amer. Chem. Soc., 89, 415 (1967).
30. W. P. Jencks and M. Gilchrist, J. Amer. Chem. Soc., 86, 1410 (1964).
31. A. J. Kirby and W. P. Jencks, J. Amer. Chem. Soc., 87, 3209 (1965).
32. S. J. Benkovic and P. A. Benkovic, J. Amer. Chem. Soc., 89, 4714 (1967).
33. P. S. Traylor and F. H. Westheimer, J. Amer. Chem. Soc., 87, 553 (1965).
34. A. W. Garrison and C. E. Boozer, J. Amer. Chem. Soc., 90, 3486 (1968).
35. E. Hobbs, D. C. C. Corbridge, and B. Raistrick, Acta Cryst., 6, 621 (1953).
36. V. M. Clark and S. G. Warren, J. Chem. Soc., 5509 (1965).
37. J. D. Chanley and E. Feageson, J. Amer. Chem. Soc., 85, 1181 (1963).
38. R. R. Irani and C. F. Callis, J. Phys. Chem., 65, 934 (1961).
39. W. P. Jencks and M. Gilchrist, J. Amer. Chem. Soc., 87, 3199 (1965).
40. O. Meyerhof and K. Lohman, Biochem. Z., 196, 22 (1928).
41. C. Zioudrou, Tetrahedron, 18, 197 (1962).
42. M. Halmann, A. Lapidot, and D. Samuel, J. Chem. Soc., 4672 (1960).
43. N. K. Hamer, J. Chem. Soc., 46 (1965).
44. A. Lapidot and M. Halmann, J. Chem. Soc., 1713 (1958).
45. J. D. Chanley and E. Feageson, J. Amer. Chem. Soc., 80, 2686 (1958).
46. M. Halmann, A. Lapidot, and D. Samuel, J. Chem. Soc., 3158 (1961).
47. M. Halmann and A. Lapidot, J. Chem. Soc., 419 (1960).
48. D. F. Heath, J. Chem. Soc., 3796 (1956).
49. J. Öney and M. Caplow, J. Amer. Chem. Soc., 89, 6972 (1967).
50. W. W. Butcher and F. H. Westheimer, J. Amer. Chem. Soc., 77, 2420 (1955).
51. J. G. Moffatt and H. G. Khorana, J. Amer. Chem. Soc., 83, 649 (1961).
52. T. S. Ryabova, Z. A. Shabarova, and M. A. Prokof'ev, Biokhimiya, 30, 235 (1960).
53. E. P. Savel'ev, T. S. Ryabova, I. P. Beletskaya, and Z. A. Shabarova, Dokl. Akad. Nauk SSSR, 155, 1457 (1964).
54. O. E. Vorob'ev, Z. A. Shabarova, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, 158, 143 (1964).
55. T. S. Ryabova and Z. A. Shabarova, Vestnik Moskov. Gos. Univ., Ser. Khim., No. 1, 114 (1966).
56. V. A. Bakanova, Z. A. Shabarova, and M. A. Prokof'ev, Khim. Prirodn. Soed., 35 (1966).
57. N. I. Sokolova, Z. Stumbravichute, P. P. Purygin, Z. A. Shabarova, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, 174, 722 (1967).
58. L. G. Gatinskaya, N. I. Sokolova, Z. A. Shabarova, and M. A. Prokof'ev, Zhur. Vses. Khim. Obshch. im Mendeleeva, 14, 583 (1969).
59. J. A. Stock, W. J. Hopwood, and P. D. Regan, J. Chem. Soc., (C), 637 (1966).
60. M. M. Sélim and T. N. Thanh, Compt. rend., 250, 2377 (1960).
61. R. K. Ledneva, N. N. Preobrazhenskaya, Z. A. Shabarova, and M. A. Prokof'ev, Mol. Biol. 5, 264 (1971).

62. N. I. Sokolova, V. I. Mel'nikova, Z. A. Shabarova, and M. A. Prokof'ev, Vestnik Moskov. Gos. Univ., Ser. Khim., No. 5, 119 (1966).
63. T. N. Thanh and M. Sélim, Compt. rend., 250, 2724 (1960).
64. N. N. Preobrazhenskaya, Z. A. Shabarova, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, 174, 100 (1967).
65. Yu. P. Shvachkin, M. T. Azarova, and M. A. Prokof'ev, Zhur. Obshch. Khim., 31, 2107 (1961).
66. R. Rätz, J. Org. Chem., 22, 371 (1957).
67. A. R. Todd, Proc. Chem. Soc., 199 (1962).
68. H. N. Stokes, Amer. Chem. J., 15, 198 (1893).
69. V. M. Clark, G. W. Kirby, and A. R. Todd, J. Chem. Soc., 1497 (1957).
70. V. M. Clark, A. R. Macrae, F. P. Richter, and A. R. Todd, Tetrahedron, 1966, Suppl. 7, 307.
71. V. M. Clark and S. G. Warren, Proc. Chem. Soc., 178 (1963).
72. V. M. Clark and S. G. Warren, Nature, 199, 657 (1963).
73. V. M. Clark, Proc. Chem, Soc., 129 (1964).
74. R. W. Chambers and H. G. Khorana, J. Amer. Chem. Soc., 80, 3749 (1958).
75. J. G. Moffatt and H. G. Khorana, J. Amer. Chem. Soc., 80, 3756 (1958).
76. R. W. Chambers and H. G. Khorana, Chem. Ind (London), 1022 (1956).
77. J. Machleidt, E. Cohen, and R. Tschesche, Ann. Chem., 672, 215 (1964).
78. H. G. Khorana, "New Trends in the Chemistry of Biologically Important Phosphate Esters" (Translated into Russian), Izd. Mir, Moscow, 1964, p. 97.
79. A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides" (Translated into Russian), Izd. Mir, Moscow, 1966, p. 258.
80. N. K. Kochetkov, E. I. Budovskii, V. N. Shibaev, and K. S. Lebedeva, Izv. Akad. Nauk SSSR, Ser. Khim., 897 (1969).
81. Z. A. Shabarova, L. G. Andronova, M. Bezdek, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, 130, 346 (1960).
82. Z. Skrowaczewska and P. Mastalerz, Roczniki Chem., 31, 531 (1957).
83. D. M. Brown, in Symposium "Uspekhi Organicheskoi Khimii" (Advances in Organic Chemistry) (Translated into Russian), Izd. Mir, Moscow, 1966, Vol. 3.
84. D. E. Hultquist, Biochim. Biophys. Acta, 153, 329 (1968).
85. T. Wagner-Jauregg and B. E. Hackley, J. Amer. Chem. Soc., 75, 2125 (1953).
86. J. H. Park and D. E. Koshland, J. Biol. Chem., 233, 986 (1958).
87. H. C. Khorana and J. P. Vizsolyi, J. Amer. Chem. Soc., 81, 4660 (1959).
88. G. DiSabato and W. P. Jencks, J. Amer. Chem. Soc., 83, 4393 (1961).
89. G. DiSabato and W. P. Jencks, J. Amer. Chem. Soc., 83, 4400 (1961).
90. R. Letters and A. M. Michelson, J. Chem. Soc., 71 (1962).
91. C. A. Bunton, E. J. Fendler and J. H. Fendler, J. Amer. Chem. Soc., 89, 1221 (1967).
92. P. E. Plapinger and T. Wagner-Jauregg, J. Amer. Chem. Soc., 75, 5757 (1953).
93. E. P. Savel'ev, E. A. Yuodka, Z. A. Shabarova, and M. A. Prokof'ev, Vestnik Moskov Gos. Univ., Ser. Khim., No. 5, 128 (1967).
94. C. A. Dekker and H. G. Khorana, J. Amer. Chem. Soc., 76, 3522 (1954).
95. T. Ueda, Chem. Pharm. Bull., 8, 459 (1960).
96. N. N. Preobrazhenskaya, N. G. Shinskii, M. G. Ivanovskaya, Z. A. Shabarova, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, 192, 1060 (1970).
97. N. G. Shinskii, N. N. Preobrazhenskaya, Z. A. Shabarova, and M. A. Prokof'ev, Zhur. Obshch. Khim., 40, 1122 (1970).
98. O. E. Vorob'ev, Z. A. Shabarova, and M. A. Prokof'ev, Vestnik Moskov. Gos. Univ., Ser. Khim., No. 6, 66 (1964).
99. T. S. Ryabova, Z. A. Shabarova, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, 162, 1068 (1965).
100. B. A. Yuodka, V. K. Nedbai, Z. A. Shabarova, and M. A. Prokof'ev, Biokhimiya, 34, 849 (1969).
101. Z. Skrowaczewska and P. Mastalerz, Roczniki Chem., 29, 415 (1955).
102. R. Greenhalgh and J. R. Blanchfield, Canad. J. Chem., 44, 501 (1966).
103. W. P. Jencks, Brookhaven Symp. Biol., 15, 134 (1962).
104. A. Lapidot and D. Samuel, Biochim. Biophys. Acta, 111, 537 (1965).
105. D. E. Hultquist, R. W. Moyer, and P. D. Boyer, Biochemistry, 5, 322 (1966).
106. A. H. Staab, H. Schaller, and F. Cramer, Angew. Chem., 71, 736 (1959).
107. H. Schaller, H. A. Staab, and F. Cramer, Chem. Ber., 94, 1621 (1961).
108. F. Cramer and H. Schaller, Chem. Ber., 94, 1634 (1961).
109. I. Goldman, J. W. Marsico, and G. Anderson, J. Amer. Chem. Soc., 82, 2969 (1960).
110. J. Baddiley, J. G. Buchanan, and R. Letters, J. Chem. Soc., 2812 (1956).
111. L. N. Nikolenko and E. V. Degterev, Zhur. Obshch. Khim., 37, 1350 (1967).
112. T. Rosenberg, Arch. Biochem. Biophys., 105, 315 (1964).
113. B. J. Weimann, R. Lohrmann, L. E. Orgel, H. Schneider-Bernloehr, and J. E. Sulston, Science, 161, 387 (1968).
114. H. Schneider-Bernloehr, R. Lohrmann, L. E. Orgel, J. Sulston, and B. J. Weimann, Science, 162, 809 (1968).
115. H. Schneider-Bernloehr, R. Lohrmann, J. Sulston, L. E. Orgel, and H. T. Miles, J. Mol. Biol., 47, 257 (1970).
116. T. Müller, T. Rathlev, and T. Rosenberg, Biochim. Biophys. Acta, 19, 563 (1956).
117. F. H. Westheimer, J. Amer. Chem. Soc., 85, 1773 (1963).
118. V. M. Clark, A. R. Todd, and S. G. Warren, Biochem. Z., 338, 591 (1963).
119. M. Ikehara, S. Hesugi, and T. Fukui, Chem. Pharm. Bull., 15, 440 (1967).
120. K. M. Moller, Biochim. Biophys. Acta, 16, 162 (1955).
121. N. Katunuma, Arch. Biochem. Biophys, 76, 547 (1958).
122. R. A. Smith and D. J. Burrow, Biochim. Biophys. Acta, 34, 274 (1959).
123. M. F. Singer and J. S. Fruton, J. Biol. Chem., 229, 111 (1957).
124. A. Fujimoto and R. A. Smith, Biochim. Biophys. Acta, 56, 501 (1962).

125. M. E. Holzer, K. D. Johnson, and R. A. Smith, Biochim. Biophys. Acta, **122**, 232 (1966).
126. R. Parvin and R. A. Smith, Biochemistry, **8**, 1748 (1969).
127. M. S. Burstone, "Enzyme Histochemistry and Its Applications in the Study of Neoplasms" [Acad. Press, New York, 1962], (Translated into Russian), Izd. Mir, Moscow, 1965, pp. 180–183.
128. R. K. Ledneva, Z. A. Shabarova, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, **172**, 977 (1967).
129. R. A. Mitchell, L. G. Butler, and P. D. Boyer, Biochem. Biophys. Res. Comm., **16**, 545 (1964).
130. G. Kreil and P. D. Boyer, Biochem. Biophys. Res. Comm., **16**, 551 (1964).
131. J. S. Nishimura, Biochemistry, **6**, 1094 (1967).
132. A. W. Norman, R. T. Wedding, and M. K. Black, Biochim. Biophys. Res. Comm., **20**, 703 (1965).
133. O. Walinder, J. Biol. Chem., **243**, 3947 (1968).
134. R. D. Simoni, M. Levinthal, F. D. Kundig, W. Kundig, B. Anderson, P. E. Hartman, and S. Roseman, Proc. Nat. Acad. Sci. U. S. A., **58**, 1963 (1967).
135. B. Weiss, A. Thompson, and C. C. Richardson, J. Biol. Chem., **243**, 4556 (1968).
136. Y. W. Little, S. B. Zimmerman, C. K. Oshinsky, and M. Gellert, Proc. Nat. Acad. Sci. U. S. A., **58**, 2004 (1967).
137. Y. Nishizuka, K. Ueda, K. Yoshihara, H. Yamamura, M. Takeda, and O. Hayaishi, Cold. Spring Harbor Symp., **34**, 781 (1969).
138. Z. W. Hall and I. R. Lehmann, J. Biol. Chem., **244**, 43 (1969).
139. P. L. Pedersen, J. Biol. Chem., **243**, 4305 (1968).
140. T. Y. Shen, Angew. Chem., **82**, 729 (1970).
141. M. Dixon and E. Webb, "Enzymes" (Translated into Russian), Izd. Mir, Moscow, 1966, pp. 266–269.
142. M. DeLuca, K. E. Ebner, D. E. Hultquist, G. Kreil, J. B. Peter, R. W. Moyer, and P. D. Boyer, Biochem. Z., **338**, 512 (1963).

Moscow State University