DEUTERIUM ISOTOPE EFFECTS AS CRITERIA OF MECHANISM IN THE REACTIONS OF ORGANOPHOSPHORUS COMPOUNDS

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We have been using deuterium isotope effects in the elucidation of the mechanisms of reaction of organophosphorus compounds for some years. In addition to their considerable biological interest, derivatives of phosphoric acid are interesting examples of various types of reaction mechanism. Even the simplest mono-alkyl esters and anhydrides of phosphoric acid can react in a very large number of ways depending on the precise conditions prevailing in the solution.

As a result of this interest, an enormous volume of experimental detail has accumulated from the work of bio- and physical-chemists. However, in spite of very thorough kinetic analyses, many important details of the mechanism of reaction are difficult to determine by classical methods. Since proton transfer and proton removal are often critical steps in determining the course of these reactions, substitution of hydrogen by deuterium in either substrate or solvent can often provide very significant information.

The reactions of phosphorus compounds can, on the other hand, be used to obtain more experimental data on isotope effects of various kinds. They are particularly suited for this purpose for the following reasons: (*a*) the variety of reactions possible and the attention that has been devoted to mechanism in phosphorus chemistry; (*b*) the fact that most mono-substituted phosphates are soluble in water which eliminates the need for using mixed solvents and the errors involved thereby; (*c*) the accuracy with which the release of inorganic phosphate can be determined using various colorimetric methods.

We shall discuss here the effect of substitution of deuterium in various positions of the organic part of the esters, anhydrides and amides of phosphoric acid. The effect of deuteration on phosphorus acid and its derivatives will also be described briefly, and finally the more uncertain field of deuterium solvent effects in the solvolysis of organophosphorus compounds will be discussed.

Kinetic and tracer studies using oxygen-18 have shown[1] that a very large number of monoalkyl phosphates are hydrolysed by a standard and extensively documented mechanism. In order to discuss the effect of isotopic substitution more clearly, I shall first summarize very briefly what is known about the hydrolysis of phosphates. In basic solution these compounds are very stable, probably due to electrostatic repulsion. As the pH decreases, the rate of breakdown increases, reaching a maximum at about pH 4 where

the predominant species is the monoanion. Experiments with an ^{18}O-labelled medium show that hydrolysis at this point occurs with P–O bond fission. On increasing the acidity of the solution the rate decreases to a minimum at about pH 0. Finally, in strongly acid solution the hydrolysis of some phosphates is acid-catalysed and of others is not—this point will be discussed later.

Although this pattern is widespread, a number of alkyl phosphates are nevertheless found to be alkali labile, liberating phosphate in basic solution with C–O bond fission. Many of these labile compounds are of biological interest, although the rôle of the dephosphorylation reaction is not yet clear. It was suggested[2] some time ago that in glucose-3-phosphate, for instance, a β-elimination reaction occurs with phosphate as a leaving group. A similar β-elimination has been suggested to account for the facile breakage of bonds to phosphorus in nucleic acids[3] and in casein[4]. The phosphorus in most phosphoproteins is bound to the hydroxyl of the amino-acid serine and hence we have examined the dephosphorylation reactions of serine phosphate[5] itself using kinetic and isotopic methods. The pH profile and the products in the acid range are quite normal, but in basic solution another reaction occurs, as is shown by the fact that

(*a*) the products are pyruvic acid, ammonia and phosphate—all formed at the same rate;

(*b*) the rate of dephosphorylation increases with OH^- concentration, in accordance with a H—function which has been discussed elsewhere[6];

(*c*) ^{18}O studies show that on dephosphorylation, the bond from carbon to oxygen is cleaved, *i.e.*, 100 per cent C–O bond fission occurs.

Confirmation of a β-elimination reaction in which proton removal is rate determining was obtained by substitution of the α-hydrogen by deuterium. DL-[α-D]-serine phosphate was prepared by an unequivocal synthesis and the rate of dephosphorylation of this compound compared to that of the normal one.

The results are shown as the ratio of the two rate constants in *Table 1*.

Table 1. Deuterium isotope effect on the elimination of serine phosphate at 100°

Medium	6N $HClO_4$	pH 4·0	pH 9·6	pH 9·65	2N NaOH	6N NaOH
k_H/k_D	(1·0†)	(1·0†); 1·85	1·40	1·50	1·90	1·83

† Hydrolysis reaction.

It is seen that in basic solution $k_H/k_D = 1{\cdot}4$ to 1·9 over a wide range of alkalinity (pH 9 to 6N NaOH). This is a rather low value for a primary isotope effect, even accounting for the fact that experiments were conducted at 100°. The theoretical maximum at this temperature is 4·7 and other well-known examples of the primary isotope effect in β-elimination reactions are included in *Table 2* for comparison.

Small k_H/k_D primary isotope effects have recently been discussed[11].

It was suggested that one reason for small k_H/k_D ratios in some cases

is that the proton is not removed in the rate-determining step, *i.e.* that nucleophilic substitution or some equilibrium reaction competes with the direct abstraction of hydrogen. That this is not the case here, is shown by both products and bond fission using ^{18}O experiments. However, there remains some chance that the removal of the proton and loss of phosphate

Table 2. Primary deuterium isotope effects in β-elimination reactions

Compound	*Temperature*	*Solvent*	k_H/k_D	*Reference*
C–H (theoretical)	0	—	8·3	7
	25	—	6·9	7
	100	—	4·7	7
CD_3 \ CHBr / CD_3	25	Ethanol	7	8
$PhCD_2CH_2Br$	50	Ethanol	6	9
DL-[α-D]-serine phosphate	100	Water	1·4–1·9	5
$PhCD_2CH_2N^+Me_3Br$	50	Ethanol	3	9
$CD_3CH_2{\cdot}N^+Me_3OH$	137	Glycol	3·9	10

are not entirely synchronous, since some lability of the α-hydrogen of an amino-acid in basic solution has been demonstrated[12] and may equally apply to serine phosphate. Another possibility is that the transition state may not be linear. Under the basic conditions used here, the carboxyl group is fully ionized and obviously electrostatic repulsion can occur between incoming hydroxyl and substrate carboxyl. Indeed the part played by charged groups on elimination reactions is not at all clear. Attempts[13] to investigate the effect of either a carboxyl or a charged amino-group in a simpler system were foiled by the fact that both β-hydroxy propionic acid phosphate and ethanolamine phosphate are resolutely stable in basic solution. Attempts[13] at removing the effect of the charge by using esters or amides of serine phosphate were also not successful since the blocking groups are removed more rapidly than the phosphate. We have however made some headway in this problem by examining[14] the deuterium isotope effect in the base-catalysed β-elimination of β-cyanoethyl phosphate. This is an important[15] phosphorylating agent, in which the organic residue is readily removed under mild basic conditions. The products, ^{18}O-studies on bond fission and the direct proportionality of rate of hydrolysis to base concentration all indicate a β-elimination reaction[16], with phosphate as a leaving group.

[β-D_2]-cyanoethyl phosphate was synthesized and the position and deuterium content determined by proton n.m.r. The rate of hydrolysis of the deutero and normal phosphate at various base concentrations at 50° are given in *Table 3.*

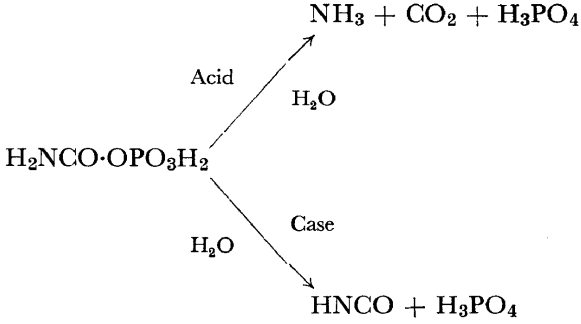
It is seen that at low base concentrations, the primary effect $k_H/k_D = 3{\cdot}0$ which approaches the theoretical one when allowance is made for an 80 per cent deuterium content. As the concentration of base increases the k_H/k_D ratio falls. Here is an indication of a transition in mechanism from

E2, in which proton removal is rate determining, to ElcB in which a conjugate base is formed in a rapid pre-equilibrium, followed by a rate-determining dephosphorylation. This change in mechanism has been confirmed[14] by following the rate of exchange of deuterium with solvent as the OH^- concentration increases, again using n.m.r. as an analytical tool. It should

Table 3. Primary deuterium isotope effect in the base-catalysed hydrolysis of β-cyanoethyl phosphate at 50·1°

N NaOH	0·1	0·2	0·3	0·5	1·0
k_H/k_D	3·0	2·38	1·98	1·43	1·0

be mentioned in passing that the ElcB mechanism is fully operative[17] in the breakdown of carbamoyl phosphate in basic solution. In acid solution this compound reacts normally giving phosphate and the elements of carbamic acid which immediately form ammonia and carbon dioxide. In neutral and basic solution the products are isocyanate and phosphate and base-catalysis occurs with C–O bond fission indicating an elimination reaction

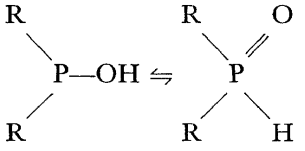


However, after partial reaction in D_2O solvent at pH 7, the remaining carbamoyl phosphate can be isolated, purified and analysed for its deuterium content. The enrichment in the amino group amounted to at least 81 per cent, indicating that proton exchange with solvent is far more rapid than elimination, *i.e.* an ElcB reaction occurs.

$$D_2O + H_2NCO{\cdot}OPO_3^{2-} \rightleftharpoons H_2O + D_2N{\cdot}CO{\cdot}O{\cdot}PO_3^{2-}$$

Further support for this is shown by the fact that when both β-hydrogen atoms are replaced by alkyl groups an entirely different type of reaction[14] takes place. At the other extreme, when the hydrogen atoms in the methyl group of acetyl phosphate are replaced by deuterium, the rate of hydrolysis[16] is virtually not affected, $k_H/k_D = 1{\cdot}17$ at 23° in 0·056N NaOH. This confirms evidence from other sources that the mechanism of hydrolysis of acetyl phosphate in basic solution is nucleophilic displacement on the carbonyl group, *i.e.* an S_N2 reaction.

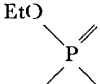
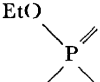
Substitution of deuterium for hydrogen in organophosphorus compounds in other positions in the molecule has also been used as a mechanistic criterion. Thus, phosphorus acid and its derivatives have been assumed to exist in two tautomeric forms



(where R = alkyl, aryl, alkoxyl or hydroxyl)

This tautomerism is implied by many chemical reactions, although physical evidence[18] indicates that the phosphonate form is by far the more predominant. By replacing the hydrogen bonded to phosphorus by deuterium some evidence has been found to indicate which structure participates in the rate-determining step. These studies are summarized in the following table.

Table 4. Deuterium isotope effects in reactions of phosphorous acid and its derivatives

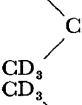
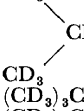
Compound	*Reactant*	*Solvent*	*Temperature* (°C)	k_H/k_D	*Reference*
 (Theoretical)	—	—	22	5·3	19
	I_2	aq. HCl	22	4	19
	I_2	pH 8·6 (borate)	22	3·6	20
	I_2	0·1M acetate	20	1·07	21
	NOCl	Cyclohexane or dibutylether	23	1	22

The oxidation reactions with a primary isotope effect very close to the theoretical, (calculated from the P–H and P–D vibrations in liquid diethyl phosphonate) indicate that a proton is removed in the rate-determining

step. In the acetate-catalysed reaction, in which a very small difference in rate is observed on isotopic substitution, it is suggested[21] that the transition state is non-linear, similar to reactions involving hydride shifts. In the reaction with nitrosyl chloride, in which tetraethylpyrophosphate is formed after a complex series of reactions[22], the absence of an isotope effect is probably due also to a non-linear reaction, possibly a four-centre transition state as in many reactions of the oxides of nitrogen. This is supported by the insensitivity of the reaction to solvent polarity.

Deuterium substitution on carbon not involved in the rate-determining step has been used to confirm the mechanism[23] of hydrolysis of t-butyl phosphate. Here an unusual mechanism operates in that, although no reaction occurs in basic solution, the rate increases steadily with increasing acidity of the solvent, and there is no inflection in the pH profile at pH 4. Hydrolysis occurs with complete C–O bond fission, and no olefins could be detected in the products indicating that no elimination occurs. The rate of hydrolysis of t-butyl phosphate fully deuterated in all three methyl groups is slower than the normal compound for a factor of $k_H/k_D = 2{\cdot}8$ at both 27° and 74°. This is a rather large secondary β-deuterium isotope effect when compared with those found for other alkyl compounds as shown in *Table 5.*

Table 5. Secondary β-deuterium isotope effects in solvolysis of alkyl compounds

Compound	*Solvent*	*Temperature* (°C)	k_H/k_D	*Reference*
$(CH_3)_2CCl{\cdot}CD_2CH_3$	80% EtOH	25	1·40	24
$(CD_3)_2CClCH_2CH_3$	80% EtOH	25	1·77	24
$(CD_3)_2CClCD_2CH_3$	80% EtOH	25	2·35	24
CD_3CH_2Br	Water	60	1·03	25
CD_3CH_2OTs	Water	60	1·02	25
	Water	60	1·37	25
	Water	30	1·55	25
$(CD_3)_3CCl$	Water	2	2·56	25
$(CD_3)_3COPO_3H_2$	0·6 N $HClO_4$	27	2·8	23
	pH 4	74	2·75	23

It has been stated[25] that the secondary isotope effects increase with the tendency to an ionic mechanism. The k_H/k_D ratio is increased by the polarity of the solvent, by an increasingly ionic leaving group and by increased branching at the reaction centre. A combination of all these factors probably accounts for the very large β-secondary effect ($k_H/k_D = 2{\cdot}8$) in the hydrolysis of t-butyl phosphate.

In addition to deuterium substitution in the substrate, changing the solvent from H_2O to D_2O has also been of value in the elucidation of

mechanisms of hydrolysis of organophosphorus compounds. Below pH 0, as mentioned above, the hydrolysis of many monoalkyl and amido-phosphates is acid catalysed. The dependence on acid concentration can be either proportional to the stoichiometric acidity or to one of the various forms of acidity function. The form of the relationship to acid strength is to some extent also a criterion of mechanism, the limitations of which, however, will not be discussed here. Whatever the form of acid catalysis, quite significant effects on the rate have been observed on substituting D_2O for H_2O in the acid hydrolysis of many organophosphorus compounds. These are summarized in *Table 6*.

Table 6. Solvent deuterium isotope effects in hydrolysis of organophosphorus compounds in acid solution

Compound	*Acidity*	*Acid catalysis*	*Temperature* (°C)	k_D/k_H	*Reference*
$CH_3PO_3H_2$	4M $HClO_4$	Yes	100	1·1	26
$C_6H_{11}O_6PO_3H_2$†	3M $HClO_4$	Yes§	25	1·5	27
$(CH_3)_3COPO_3H_2$	2M $HClO_4$	Yes§	10	1·55	23
$NH_2CO{\cdot}OPO_3H_2$	0·75 and 2 M $HClO_4$	Yes	37	1·0	17
$EtO{\cdot}CO{\cdot}NH{\cdot}PO_3H_2$	0·1M HCl	No	37	1·0	28
$NH_2PO_3H_2$	0·4 and 0·9 M $HClO_4$	Yes	10	1·4	29
$PhCONHPO_3H_2$	1·7 and 7·6 M $HClO_4$	Yes	37	1·2–1·35	30
$(PhO)_2PONHPO_3H_2$	0·1N H_2SO_4	No	61	1·0	30
$PhSO_2NHPO_3H_2$	0·1M H_2SO_3	No	60	1·0	31
$Et_2N{\cdot}CO{\cdot}OPO_3H_2$	2·74M $HClO_4$	Yes	50	1·4	14

† α-D-Glucose-1-phosphate.
§ C–O bond fission.

When these phosphates are hydrolysed with acid catalysis (those marked "yes" in the table), the bridging oxygen (or nitrogen) is protonated in a pre-equilibrium.

$$ROPO_3H_2 + H_3O^+ \leftrightharpoons R\overset{+}{O}HPO_3H_2 + H_2O$$

$$RNHPO_3H_2 + H_3O^+ \leftrightharpoons R\overset{+}{N}HPO_3H_2 + H_2O$$

As has been shown in many other systems[32] of this type the difference in acidity of D_2O and H_2O will cause a displacement of this equilibrium to the left and hence slow down the measured rate of hydrolysis in H_2O. However, in certain cases, the tendency of protonation on the bridging atom is reduced by substitution (by diphenoxyphosphoryl or benzenesulphonyl groups) or by competition with the amino group of carbamoyl and hence neither acid catalysis or solvent isotope effect is observed.

A more significant area of investigation is the hydrolysis of the monoanion at pH 4. Here, it has been suggested[1] that a cyclic mechanism occurs, the proton required for the organic leaving group being transferred, either directly or *via* a chain of water molecules from the phosphate group. This leaves an unstable metaphosphate intermediate, which is immediately hydrated to give phosphoric acid. Numerous attempts to trap or identify this intermediate have not been entirely successful although a great deal of

circumstantial evidence has accumulated in favour of this concept. Deuterium solvent isotope effects have also been brought to bear on this point and the rates of hydrolysis of the monoanion (at or about pH 4) in H_2O and in D_2O solvents are summarized in *Table 7*.

Table 7. Solvent deuterium isotope effects in solvolysis of organophosphorus compounds at pH 4

Compound	*Temperature* (°C)	k_H/k_D	*Reference*
$CH_3OPO_3H^-$	100	1.1	26
$(CH_3)_3COPO_3H^-$	74	1·66	23
$CH_3CO{\cdot}OPO_3H^-$	39	1·0	33
$NH_2COOPO_3H^-$	37	1·34	16
$Et_2NCO{\cdot}OPO_3H^-$	50	1·35	14
$EtO{\cdot}CO{\cdot}NHPO_3H^-$	37	1·0	28
$NH_2PO_3H^-$	37	1·0	29
$PhCONHPO_3H^-$	37	1·2	30
$(PhO)_2PONHPO_3H^-$	61	1·0	30
$PhSO_2NHPO_3H^-$	50	1·0	31

Here no equilibrium is concerned since the reactions are buffered. It is evident that most of the compounds are insensitive to a change of solvent from D_2O to H_2O. This would indicate no important rôle for water molecules in the rate-determining step, the reactions being essentially unimolecular. This can be considered further evidence for the cyclic mechanism. Three compounds are however affected by a change of solvent, t-butylphosphate[23], which undergoes a different type of hydrolysis, unimolecular heterolysis. Here the solvent deuterium isotope effect is of a similar magnitude to these found[34] for the hydrolysis of t-butyl chloride in water, the effect being considered due to the changes in solution of the substrate on activation. A similar mechanism may also operate in carbamoyl phosphate[14] and its *N,N*-diethyl derivative[14]. Alternatively, the isotope effect may indicate a bimolecular reaction with water as has been suggested by Jencks[33] for the hydrolysis of phenyl acetylphosphate. At the present moment, however, there is insufficient evidence on this point to draw any definite conclusions.

We are now synthesizing a number of organophosphorus compounds specifically labelled in a given position with deuterium in order to test some of the suggestions outlined above, and extending our studies to a more rigorous examination of deuterium solvent isotope effects in the hydrolysis of alkyl phosphates.

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