

P-O splitting with the acetyl phosphate monoanion. Pyridine does not form an unreactive complex with acetyl phosphate, since the rate of reaction of 0.22 *M* aniline with acetyl phosphate at pH 5.2 was found to be unchanged on the addition

of 0.5 *M* pyridine. No reaction of acetyl phosphate with acetate, arsenate, tris-(hydroxymethyl)-amino-methane or phosphate could be detected.³⁰

(30) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, **83**, 4400 (1961).

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Mechanism and Catalysis of Reactions of Acyl Phosphates. II. Hydrolysis¹

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The following evidence supports the hypothesis that the hydrolysis of simple acyl phosphates near neutrality proceeds through monomeric metaphosphate ion, the dianion (AcP^{2-}) by direct elimination of carboxylate ion and the monoanion (AcP^-) by proton transfer and elimination of carboxylic acid, while the hydrolysis of the acetyl phenyl phosphate monoanion (AcPhP^-) proceeds through a nucleophilic reaction of water with the acyl group: (1) Values of ΔS^\ddagger for hydrolysis are 3.8 e.u. for AcP^{2-} , -3.6 e.u. for AcP^- and -28.8 e.u. for AcPhP^- . (2) There is no significant solvent deuterium isotope effect on the hydrolysis of AcP^{2-} and AcP^- , while the hydrolysis of AcPhP^- is decreased 2.5-fold in deuterium oxide. (3) The hydrolysis of simple acyl phosphates is much faster than that of other phosphate esters and AcPhP^- . (4) The rate of hydrolysis of substituted benzoyl phosphate dianions is increased by electron-withdrawing substituents ($\rho = 1.2$), while hydrolysis of the corresponding monoanions is much less sensitive to structure. (5) Solvolysis of AcP^{2-} and AcP^- is known to proceed with P-O bond cleavage, while the solvolysis of AcPhP^- in 90% methanol proceeds with C-O cleavage. (6) The rates of hydrolysis of AcP^{2-} and AcP^- are slightly increased by concentrated salt solutions, while that of AcPhP^- is decreased. (7) The addition of acetonitrile to the reaction medium has little or no effect on the rates of hydrolysis of AcP^{2-} and AcP^- , while it considerably decreases that of AcPhP^- . (8) Values of ΔV^\ddagger for hydrolysis are -1.0 cm.³/mole for AcP^{2-} , -0.6 cm.³/mole for AcP^- and -19 cm.³/mole for AcPhP^- .² (9) Solvolysis of AcP^{2-} in 7 *M* NaClO_4 gives pyrophosphate.

There is a considerable body of evidence which is consistent with the hypothesis that the solvolysis of phosphate monoester monoanions and related compounds proceeds through protonation and elimination of the leaving group to give the unstable monomeric metaphosphate anion as the initial reaction product.³⁻¹⁰ The experiments reported here provide support for such a mechanism in the solvolysis of simple acyl phosphate monoanions and suggest that the corresponding dianions eliminate carboxylate ion to give metaphosphate ion directly; substituted acyl phosphates, such as acetyl phenyl phosphate, undergo normal acyl-oxygen cleavage.

Experimental

Rate determinations were carried out as described in the preceding paper.¹¹ Substituted benzoyl phosphates were prepared by the procedure of Avison.¹² The desired anhydride was prepared from the acid and acid chloride in dry pyridine and added, without isolation, to 0.5 to 1.0 molar

equivalent of K_2HPO_4 in water at 0°, to give a final pyridine concentration of 30-40%. The mixture was stirred at 0° for 30 minutes (at room temperature for the anisoyl compound) and extracted three times with ether. The benzoyl and toluyl compounds were prepared directly from the anhydrides. The monoacyl phosphates were separated from contaminating diacyl phosphate by precipitation as the barium salts at pH 7.2-7.4, and were redissolved by stirring with Na_2SO_4 just before use. The compounds were obtained in approximately 50% yield and were used without further purification. Reactions of the substituted benzoyl phosphates were followed by analyzing aliquots of the reaction mixtures for remaining acyl phosphate with the alkaline hydroxylamine method of Hestrin,¹³ except for 3,5-dinitrobenzoyl phosphate, which was assayed by the neutral hydroxylamine method.

Results

The rates of acetyl phosphate monoanion (AcP^-) and dianion (AcP^{2-}) hydrolysis are summarized in Table I; the rates in 0.6 *M* KCl at 39° agree satisfactorily with those previously obtained by Lynen¹⁴ and by Koshland.^{15,16} The rates of monoanion and dianion hydrolysis are not significantly altered in solvent deuterium oxide and are not changed or are slightly increased by concentrated KCl, NaClO_4 and NaCl. The rates are also insensitive to the concentration of tris-(hydroxymethyl)-amino-methane (TRIS) buffer and do not show a significant reaction or common ion effect with concentrated phosphate, acetate or arsenate buffers. In 30 and 50% acetonitrile the rate of AcP^{2-} hydrolysis is unchanged and that of AcP^- is slightly decreased. The rates of hydrolysis at 0°, 25° and 50° and the derived ΔH^\ddagger and ΔS^\ddagger values also are recorded.

The rate of hydrolysis of acetyl phenyl phosphate increases with increasing concentrations of phosphate buffer in H_2O and D_2O (Fig. 1). The de-

(1) Supported in part by grants from the National Cancer Institute of the National Institutes of Health (C-3975 and CRT-5033) and the National Science Foundation. For a preliminary report, see Abstracts, 137th Meeting, American Chemical Society, Cleveland, Ohio, 1960, p. 77-O.

(2) G. Di Sabato, W. P. Jencks and E. Whalley, unpublished experiments.

(3) (a) W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, **77**, 2420 (1955); (b) J. Kumamoto and F. H. Westheimer, *ibid.*, **77**, 2515 (1955); (c) D. Samuel and F. H. Westheimer, *Chemistry & Industry*, 51 (1959).

(4) C. A. Vernon, *Chem. Soc. (London) Spec. Publ.*, **48**, 17 (1957).

(5) C. A. Bunton, D. R. Llewellyn, K. G. Oldham and C. A. Vernon, *J. Chem. Soc.*, 3574 (1958).

(6) J. D. Chanley and E. Feageson, *J. Am. Chem. Soc.*, **80**, 2686 (1958).

(7) E. W. Crunden and R. F. Hudson, *Chem. & Ind. (London)*, 1478 (1958).

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(9) M. Hulmann, A. Lapidot and D. Samuel, *ibid.*, 4672 (1960).

(10) D. M. Brown and N. K. Hamer, *ibid.*, 1155 (1960).

(11) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, **83**, 4308 (1961).

(12) A. W. D. Avison, *J. Chem. Soc.*, 732 (1955).

(13) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).

(14) F. Lynen, *Ber.*, **73**, 367 (1940).

(15) D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **73**, 4103 (1951).

(16) D. E. Koshland, Jr., *ibid.*, **74**, 2286 (1952).

TABLE I
HYDROLYSIS OF 0.004 M ACETYL PHOSPHATE AT 39° AND
IONIC STRENGTH 0.6

Conditions	pH	$-k_{obs} \times 10^3, \text{min.}^{-1}$	
		AcP ^a	AcP ^b
Uncatalyzed		13	4.3
In denterium oxide		12.2 ^c	4.5 ^d
		11.9 ^{e,d}	4.5 ^{e,d}
Added, to ionic strength			
KCl, 1.0		13	4.3
KCl, 2.0		13	4.7
KCl, 3.0		13	5.5
NaClO ₄ , 0.6		11 ^f	4.3 ^g
NaClO ₄ , 2.0		11 ^f	4.1 ^g
NaClO ₄ , 3.0		11 ^f	4.1 ^g
NaClO ₄ , 5.0		10 ^f	4.3 ^g
NaCl, 0.6 ^g	9.3 ^h		4.2
NaCl, 3.2 ^g	9.2 ^h		5.0
In sodium arsenate, 0.1 M	7.9 ⁱ		4.4
In sodium arsenate, 0.5 M	9.2 ^j		4.5
In TRIS buffer, 0.1 M	7.2		4.4
In TRIS buffer, 0.2 M	7.2		4.4
In 0.05 M potassium acetate buffer	4.1	10	
In 0.8 M potassium acetate buffer	4.1	10	
In 0.1 M potassium acetate buffer	5.9		4.6
In 0.6 M potassium acetate buffer	6.1		4.2
In 0.05 M potassium phosphate buffer	6.4		4.3
In 0.9 M potassium phosphate buffer	6.4 ^k		5.0
In 0.1 M potassium phosphate	3.5	11 ^l	
In 0.8 M potassium phosphate	3.5	11 ^l	
In water		10.5 ^m	4.3
In 30% acetonitrile		9.7 ^m	4.4 ⁿ
In 50% acetonitrile		8.6 ^m	4.4 ⁿ
Hydrolysis at 0°		0.05 ^o	0.012 ^p
Hydrolysis at 25°		1.95 ^o	0.56 ^p
Hydrolysis at 50°		38.4 ^o	18.2 ^p
$\Delta H^\ddagger, ^\circ 0-25^\circ$, kcal./mole		22.9	24.2
$\Delta H^\ddagger, ^\circ 25-39^\circ$, kcal./mole		22.1	26.6
$\Delta H^\ddagger, ^\circ 39-50^\circ$, kcal./mole		22.5	25.3
$\Delta H^\ddagger, ^\circ$ av., kcal./mole		22.5	25.4
$\Delta S^\ddagger, ^\circ 39^\circ$, e.u.		-3.6	3.7

^a In potassium formate buffer 0.1 M, pH 3.0, unless otherwise indicated. ^b In potassium phosphate buffer 0.1 M, pH 6.9, unless otherwise indicated. ^c In sodium formate buffer 0.25 M, pH 3.2 in water. ^d Control in water. ^e In potassium phosphate buffer 0.1 M, pH 6.0 and 6.6 in water. ^f In sodium formate buffer 0.1 M, pH 3.5. ^g In sodium phosphate buffer 0.1 M, pH 6.5. ^h In sodium carbonate buffer 0.1 M. ⁱ In tris-(hydroxymethyl)-aminomethane HCl buffer 0.1 M. ^j In sodium carbonate buffer 0.1 M, ionic strength 3.2. ^k Ionic strength 1.4. ^l In potassium formate buffer, 0.1 M. ^m In 0.1 M potassium formate buffer, pH 3.5 in water. ⁿ In 0.1 M potassium phosphate buffer, pH 7.1 in water. ^o In potassium formate buffer 0.1 M, pH 3.2. ^p In potassium phosphate buffer 0.1 M, pH 6.8. ^q Calculated from the relations: $\Delta H^\ddagger = E_a - RT$; $\Delta S^\ddagger = (\Delta H^\ddagger - \Delta F^\ddagger)/T$; $\Delta F^\ddagger = -RT \ln kh/k_B T$; where E_a is the Arrhenius energy of activation, h is the Planck constant and k_B is the Boltzmann constant.

pendence of the rate on the concentration of buffer is the same in H₂O and D₂O, but the hydrolysis rate extrapolated to zero buffer concentration is 2.5-fold greater in H₂O than in D₂O. A similar increase in rate with increasing buffer concentration was found for all buffers examined, with the exception of phenyl phosphate, indicating that the hydrolysis is subject to nucleophilic catalysis. The rates of hydrolysis, extrapolated to zero buffer concentration, and the data from which they were derived are shown as a function of pH in Fig. 2. Acid- and base-catalyzed reactions are important at the extremes of pH, but between pH 2.7 and 7.5 hydrolysis occurs by a neutral, pH-independent, "water" reaction. The rate constants for the uncatalyzed and catalyzed hydrolyses and for the reactions with buffer salts are summarized in Table

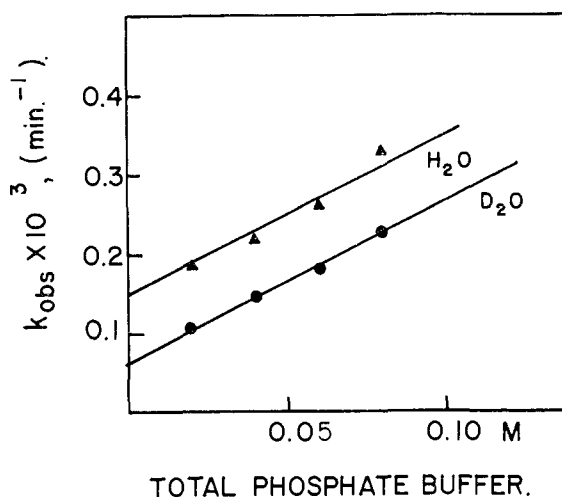


Fig. 1.—Rate constants for the solvolysis of acetyl phenyl phosphate in H₂O and D₂O in potassium phosphate buffer, pH 5.90 in water, 39°, ionic strength, 0.6.

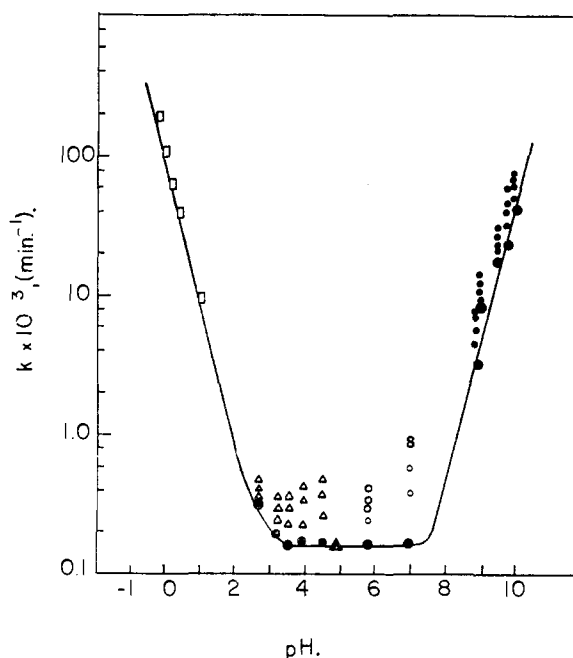


Fig. 2.—Hydrolysis of acetyl phenyl phosphate at 39°: □, HClO₄ 0.1, 0.4, 0.6, 1.0 and 1.5 M; △, potassium formate buffer 0.1, 0.2 and 0.3 M for each pH; ▲, sodium phenyl phosphate buffer 0.05 and 0.2 M; ○, potassium phosphate buffer 0.05, 0.10, 0.15 and 0.20 M at each pH; ●, potassium carbonate-bicarbonate buffer 0.05, 0.10, 0.15 and 0.20 M at each pH; ●, rates extrapolated to zero buffer concentration; all data at ionic strength 0.6, except in perchloric acid.

II. The rate of the neutral hydrolysis is decreased approximately twofold in 30 and 50% acetonitrile. It is decreased from 1.5×10^{-4} to 1.0×10^{-4} min.⁻¹ by 3.0 M KCl and by 5.0 M NaClO₄, but is only slightly decreased by NaCl. The rates of hydrolysis at 50°, 60° and 73° and the derived thermodynamic parameters also are recorded.

The rates of hydrolysis of *p*-methoxybenzoyl phosphate, *p*-nitrobenzoyl phosphate and 3,5-dinitrobenzoyl phosphate as a function of pH are

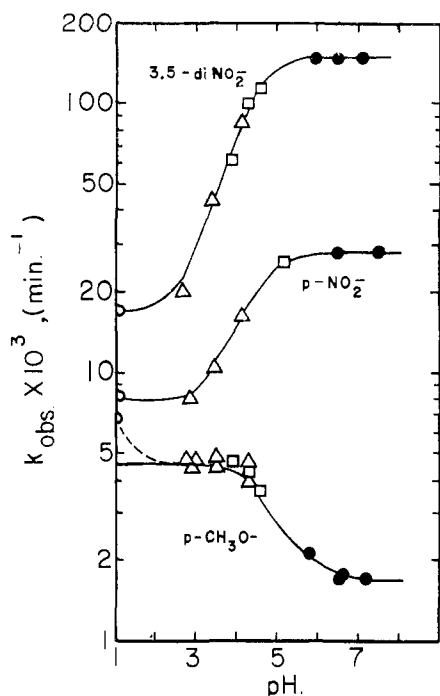


Fig. 3.—Hydrolysis of 3,5-dinitrobenzoyl phosphate, *p*-nitrobenzoyl phosphate and *p*-methoxybenzoyl phosphate as a function of *pH* at 39°. Solid lines are theoretical curves calculated from the rate constants in Table III. Buffers: ●, potassium phosphate, 0.1 *M*; □, potassium acetate, 0.1 *M*; △, potassium formate, 0.1 *M*; ○, HCl, 0.1 *M*.

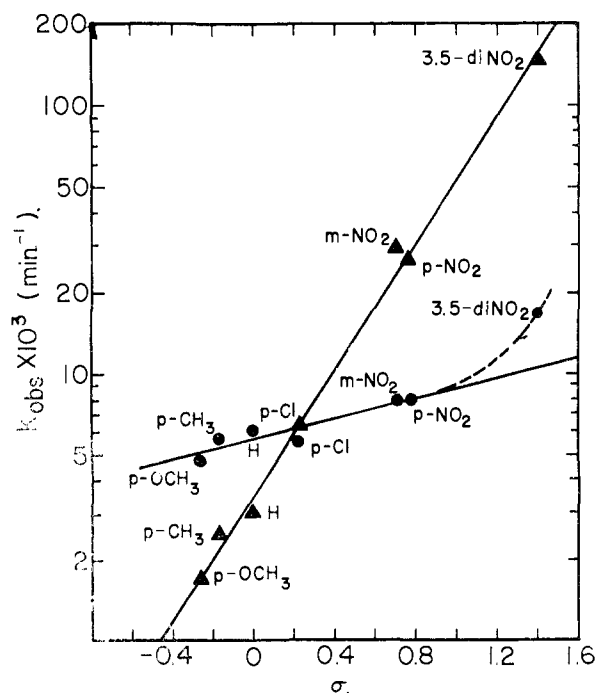


Fig. 4.—Rates of hydrolysis of substituted benzoyl phosphates as a function of Hammett's substituent constants, σ : ●, monoanion reactions; ▲, dianion reactions.

shown in Fig. 3. The rate of solvolysis of the *p*-methoxybenzoyl phosphate dianion near neutrality, as in the case of acetyl phosphate, is slower than

TABLE II
HYDROLYSIS OF 0.004 *M* ACETYL PHENYL PHOSPHATE AT 39° AND IONIC STRENGTH 0.6

	<i>k</i> , min. ⁻¹
<i>k</i> _{neut.} ^a	1.5 × 10 ⁻⁴
<i>k</i> _{H⁺} ^b , 1. mole ⁻¹ min. ⁻¹	0.10
<i>k</i> _{OH⁻} ^{c,d} , 1. mole ⁻¹ min. ⁻¹	500
In deuterium oxide	0.6 × 10 ⁻⁴ , ^d 1.5 × 10 ⁻⁴ , ^{d,f}
Added, to ionic strength	
KCl, 3.0 ^g	1.0 × 10 ⁻⁴
NaClO ₃ , 0.6 ^h	1.7 × 10 ⁻⁴
NaClO ₃ , 3.0 ^h	1.2 × 10 ⁻⁴
NaClO ₃ , 5.0 ^h	1.0 × 10 ⁻⁴
NaCl, 0.6 ^h	1.7 × 10 ⁻⁴
NaCl, 3.0 ^h	1.5 × 10 ⁻⁴
NaCl, 4.0 ^h	1.4 × 10 ⁻⁴
<i>k</i> _{HCOOH} ⁱ , 1. mole ⁻¹ min. ⁻¹	3.2 × 10 ⁻⁴
<i>k</i> _{HCOO⁻} ⁱ , 1. mole ⁻¹ min. ⁻¹	0.0011
<i>k</i> _{HPO₃²⁻} ^j , 1. mole ⁻¹ min. ⁻¹	.0072
<i>k</i> _{CO₃²⁻} ^k , 1. mole ⁻¹ min. ⁻¹	.26
In 30% acetonitrile ^l	.75 × 10 ⁻⁴
In 50% acetonitrile ^l	.63 × 10 ⁻⁴
<i>k</i> _{neut.} at 50° ^{k,d}	4.35 × 10 ⁻⁴
<i>k</i> _{neut.} at 60° ^h	1.01 × 10 ⁻³
<i>k</i> _{neut.} at 73° ^h	2.72 × 10 ⁻³
Δ <i>H</i> [‡] , ^l 39–50°, kcal./mole	17.6
Δ <i>H</i> [‡] , ^l 50–60°, kcal./mole	17.3
Δ <i>H</i> [‡] , ^l 60–73°, kcal./mole	16.7
Δ <i>H</i> [‡] , ^l av., kcal./mole	17.2
Δ <i>S</i> [‡] , ^l 39°, e.u.	–28.8

^a In sodium phenyl phosphate-buffer 0.05 and 0.2 *M*, *pH* 4.9. ^b In 0.1–1.5 *M* HClO₄. ^c In potassium carbonate-bicarbonate buffer 0.05, 0.10, 0.15 and 0.20 *M*; *pH* 8.9, 9.5, 9.8 and 10.0. ^d Values extrapolated to zero buffer concentration. ^e Control in water. ^f In potassium phosphate buffer, 0.02, 0.04, 0.06 and 0.08 *M*; *pH* 5.9 in water. ^g In potassium phosphate buffer 0.025, 0.050, 0.075 and 0.10 *M*; *pH* 5.8. ^h In sodium phenyl phosphate buffer 0.1 *M*, 50% neutralized. ⁱ In potassium formate buffer 0.1, 0.2, and 0.3 *M*; *pH* 2.7, 3.2, 3.5, 3.9 and 4.4. ^j In potassium phosphate buffer 0.05, 0.10, 0.15 and 0.20 *M*; *pH* 5.8 and 7.0. ^k In potassium phosphate buffer 0.015, 0.025 and 0.050 *M*; *pH* 6.2. ^l Calculated according to footnote *q* of Table I.

that of the monoanion at *pH* 3, while for the compounds with electron-withdrawing substituents on the benzoyl group, the rate of dianion solvolysis is greater than that of the corresponding monoanion.

The *pK_a*' values for the dissociation of the monoanions of 3,5-dinitrobenzoyl phosphate, *p*-nitrobenzoyl phosphate and *p*-methoxybenzoyl phosphate may be estimated from the kinetic data to be 4.0, 4.3 and 4.8, respectively. The rates of hydrolysis of the mono- and dianions of a larger series of substituted benzoyl phosphates are given in Table III and are plotted as a function of Hammett's substituent constants¹⁷ in Fig. 4.

The solvolysis of concentrated acetyl phosphate in 87% acetonitrile and 87% dioxane in the presence of half-neutralized formate and tris-(hydroxymethyl)-aminomethane buffers gave a 10 to 14% conversion of the phosphate to pyrophosphate, measured as acid-labile phosphate after precipitation as the manganese salt.¹⁸ In 93% pyridine, 51% of the phosphate was present as pyrophos-

(17) H. H. Jaffé, *Chem. Revs.*, **53**, 191 (1953).

(18) A. Kornberg, *J. Biol. Chem.*, **182**, 779 (1950).

TABLE III
HYDROLYSIS OF SUBSTITUTED BENZOYL PHOSPHATES AT 39°

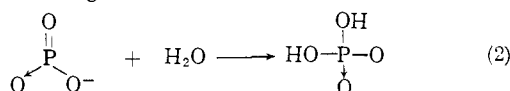
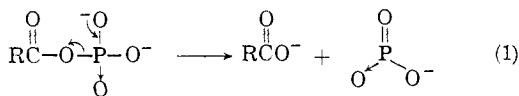
Substituent	$k \times 10^3, \text{min.}^{-1}$	
	Monoanion ^a	Dianion ^b
<i>p</i> -CH ₃ O-	4.8	1.8
<i>p</i> -CH ₃ -	5.7	2.6
H	6.1	3.1
<i>p</i> -Cl-	5.6	6.5
<i>m</i> -NO ₂ -	8.2	30
<i>p</i> -NO ₂ -	8.1	28
3,5-diNO ₂ -	17.0	150

^a In potassium formate buffer pH 2.7–3.1, except 3,5-diNO₂- in 0.1 M HCl. ^b In potassium phosphate buffer, pH 6.9–7.5.

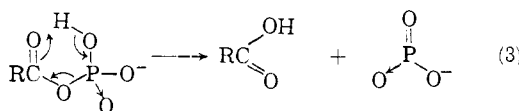
phate after solvolysis. The presence of pyrophosphate was confirmed by paper electrophoresis in acetate buffer, pH 4.7; no more than traces of trimetaphosphate could be detected. Solvolysis of 0.4 M solutions of AcP⁻ and AcP⁼ in water gave only traces (1–3%) of pyrophosphate. However, if the activity of water was decreased by carrying out the reaction in 7.3 M NaClO₄, a larger formation of pyrophosphate was observed and was confirmed by paper electrophoresis. In one experiment, carried out without added buffers, the pH was maintained at about 4.0, 5.6 and 9.7 during the reaction and a 9, 15 and 33% conversion of 0.22 M AcP to pyrophosphate, respectively, was observed. Similar results were obtained with disodium acetyl phosphate, instead of the dilithium salt.

Discussion

The results are discussed in terms of the hypotheses that: (i) the hydrolysis of acyl phosphate dianions proceeds by a monomolecular elimination of carboxylate ion to form the monomeric metaphosphate ion (eq. 1) which reacts rapidly with water to give phosphate (eq. 2)

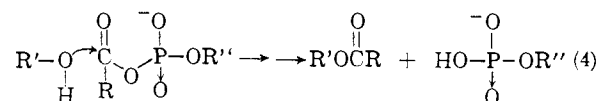


(ii) The hydrolysis of acyl phosphate dianions proceeds by a similar mechanism (eq. 3 and 2), possibly facilitated by a concerted proton transfer from



phosphate to the leaving acetic acid molecule. (iii) The solvolysis of acetyl phenyl phosphate proceeds by a bimolecular reaction involving solvent and the carbonyl group, probably aided by other solvent molecules (eq. 4). As discussed in detail elsewhere, there are several kinetically indistinguishable and equally probable alternatives to this mechanism, which involve the same stoichiometric composition of the transition state and differ only in the position of protons and the degree of bond making and breaking; it is also likely that

several molecules of solvent are involved, as proton transfer agents, in the transition state.¹⁹



(1).—The entropies of activation for the neutral hydrolysis of AcP⁼, AcP⁻ and AcPhP⁻ are 3.7, –3.6 and –28.8 entropy units, respectively.²⁰ The hydrolyses of other phosphate monoester and phosphoramidate monoanions also exhibit ΔS[‡] values which are positive or near zero.^{4,8,9} The “water” hydrolyses of acetylimidazolium,²² carboxylic acid esters,²³ acetic anhydride²⁴ and diphenylformamide hydrochlorides,²⁵ which presumably involve one or several molecules of solvent, exhibit ΔS[‡] values of –20 to –50 e.u. For reactions which have a similar charge in the starting materials and transition state, it may be expected that a bimolecular attack of solvent will result in a negative value of ΔS[‡], because of the requirement for orientation of the attacking solvent molecule(s), while a monomolecular reaction will exhibit a ΔS[‡] value near zero.²⁶ There appear to be exceptions to this generalization in the case of certain acid-catalyzed reactions, because of the entropy of the preliminary proton transfer,²⁷ but this is not a problem in the case of the dianion, and probably not in the monoanion reactions considered here. It is possible that the slightly more negative ΔS[‡] for AcP⁻ than for AcP⁼ solvolysis reflects a restriction to rotation in the transition state due to intramolecular proton transfer (eq. 3).

(2).—The rates of neutral hydrolysis of AcP⁼ and AcP⁻ are not significantly changed, while that of AcPhP⁻ is decreased 2.5-fold in deuterium oxide solution. The rates of hydrolysis of methyl phosphate, phosphourethan, N-benzoylphosphoramidate and N-dihydroxyphosphinylphosphoramidate monoanions also are not decreased in deuterium oxide.^{5,8,9} The pH-independent solvolysis of activated acyl groups by attack of water is quite generally decreased 2- to 3- fold in deuterium oxide solution^{19,22,28,29}; a decrease may be superimposed on the expected increase in acid-catalyzed solvolyses

(19) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **83**, 1743 (1961).

(20) The ΔS[‡] values reported here are more positive than those recently reported by Kurz and Gutsche in 50% dioxane²¹ for the neutral solvolysis of AcP⁻. This may partly reflect the difference in solvent, but mainly results from the assumption of these authors that the reaction is bimolecular and their calculation of ΔS[‡] from bimolecular rate constants, obtained by dividing the observed rate constants by the concentration of water.

(21) J. L. Kurz and C. D. Gutsche, *J. Am. Chem. Soc.*, **82**, 2175 (1960).

(22) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).

(23) (a) A. Moffat and H. Hunt, *J. Am. Chem. Soc.*, **81**, 2082 (1959); (b) M. H. Palomaa, E. J. Salmi and R. Korte, *Ber.*, **72**, 790 (1939).

(24) J. Koskikallio, D. Pouli and E. Whalley, *Can. J. Chem.*, **37**, 1360 (1959).

(25) R. H. DeWolfe, *J. Am. Chem. Soc.*, **82**, 1585 (1960).

(26) F. A. Long, J. G. Pritchard and F. E. Stafford, *ibid.*, **79**, 2362 (1957), and references therein.

(27) E. Whalley, *Trans. Faraday Soc.*, **55**, 798 (1959).

(28) A. R. Butler and V. Gold, *Proc. Chem. Soc.*, 15 (1960).

(29) C. A. Bunton, N. Fuller, S. G. Perry and V. J. Shiner, Jr., *Chem. & Ind. (London)*, 1130 (1960).

in deuterium oxide.³⁰ The neutral hydrolysis of catechol sulfite is 5.95-fold faster in 60% dioxane-water than in the corresponding deuterated solvent.³¹ The solvolyses of saturated carbon compounds and of (*i*-PrO)₂POCl and (*i*-PrO)₂POF, which involve a variable amount of participation by solvent, show smaller solvent deuterium isotope effects, but nearly always are significantly slower in deuterium oxide solution.^{29,32,33} The effects of deuterium oxide on the solvolyses of AcP⁼ and AcPhP⁻ thus support monomolecular and bimolecular mechanisms, respectively, for the two reactions. The conclusion is less clear in the case of AcP⁻ and other monosubstituted phosphate monoanions because of possible pre-equilibrium or concerted proton transfers in these reactions, but in view of the unknown effects of deuterium substitution on the pertinent equilibria and the apparent absence of a deuterium isotope effect in certain reactions which almost certainly involve a proton transfer as part of the rate-determining step,³⁴⁻³⁶ the absence of a deuterium isotope effect is not evidence against a monomolecular mechanism in these reactions also.

(3).—The volumes of activation for the neutral hydrolysis of AcP⁼, AcP⁻ and AcPhP⁻ are -1.0 ± 1.0 , -0.6 ± 1.0 and -19 ± 2 cm.³/mole, respectively.² Values of ΔV^\ddagger , which generally parallel ΔS^\ddagger values, may be expected to be negative for reactions which involve bimolecular attack of solvent, because of the smaller volume of the transition state than the starting materials, and near zero for monomolecular reactions, provided that there is a similar charge on the starting materials and the transition state.^{24,27,37-39}

(4).—The rates of solvolysis of AcP⁼ and AcP⁻ are unchanged or slightly increased, while that of AcPhP⁻ is considerably decreased by concentrated salt solutions. The rate of solvolysis of methyl phosphate monoanion⁵ is also insensitive to 0.95 *M* NaClO₄. The *p*H-independent solvolyses of activated acyl groups and of catechol sulfite, a group of reactions of varying charge type which appear to proceed through attack of water, are markedly decreased by high concentrations of salt.^{22,31,40,41} In addition to effects on the activity coefficients of the substrates and transition states, this may reflect a decrease in the availability of water in concentrated salt and acid solutions.⁴²

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The decrease in the rate of AcPhP⁻ solvolysis in concentrated salt solutions is less striking than for other bimolecular reactions, but is still in marked contrast to the results with AcP⁼ and AcP⁻. The rate decrease in the former case may be partially counteracted by a specific rate-accelerating effect due to binding of sodium ion; this is suggested by the larger decrease in rate observed with KCl compared to NaCl and even compared to NaClO₄.

(5).—The rate of hydrolysis of AcP⁼ is unchanged, that of AcP⁻ is only slightly decreased and that of AcPhP⁻ is considerably decreased by addition of 30 to 50% acetonitrile to the solvent. The solvolysis of AcP⁼ has been shown to be faster in 50% dioxane than in water²¹ and that of glycerol phosphate monoanion is only slightly affected by increases in dioxane and ethanol concentration to 87–90%.⁴³ The *p*H-independent hydrolysis of activated acyl groups is generally considerably decreased by the addition of nonpolar solvents to water,^{41,44} because of activity coefficient effects and the decrease in the amount of water available for nucleophilic attack.

(6).—The postulated mechanism of solvolysis of monoanions of phosphate monoesters and phosphoramidates involves the elimination of an uncharged alcohol or amine, formed by proton transfer either before or during the reaction.^{3-6,9} Such reactions show only rather small effects of substituents in the leaving group, presumably because electronic effects which aid proton transfer hinder leaving group expulsion, and *vice versa*. It might be expected that if the leaving group could form a sufficiently stable anion it could leave directly, without proton transfer. This would result in a reaction of the ester dianion and is the mechanism here postulated for the solvolysis of acyl phosphate dianions. Since proton transfer is not involved in such a reaction, the rate should be increased by electron-withdrawing substituents which aid leaving group expulsion. This is observed in the hydrolyses of substituted benzoyl phosphate dianions (Figs. 3 and 4), which follow a Hammett σ - ρ relationship with a ρ -value of 1.2.

The hydrolysis of acyl phosphate monoanions occurs readily at 39°, while other phosphate monoanion hydrolyses are conveniently studied at 100°; the calculated rate of AcP⁻ hydrolysis at 100° is 3.9 min.⁻¹, compared to 5×10^{-4} min.⁻¹ for methyl phosphate mono-anion.⁵ In the case of methyl phosphate, proton transfer must occur either before the reaction, to give a very unstable zwitterion, or during the reaction, through a four-membered ring or an intermediate water molecule.⁴ The rapid rate for acyl phosphate mono-anions may reflect the possibility of a facile direct proton transfer to the carbonyl group through a six-membered ring during the reaction, with expulsion of uncharged carboxylic acid (eq. 3). In any case, the requirement for proton transfer should make these reactions, like other monoanion solvolyses, relatively insensitive to substituent effects, as is observed

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(Figs. 3 and 4). An analogous situation is found with substituted acetyl phosphates: acetyl phosphate and fluoroacetyl phosphate monoanions undergo solvolysis at similar rates, while the fluoroacetyl phosphate dianion undergoes solvolysis 63 times faster than the acetyl phosphate dianion⁴⁵; similarly, the hydrolysis of the β -aspartyl phosphate dianion (pK_2 of aspartic acid = 3.9) at 30° is three times as fast as acetyl phosphate at 39°.⁴⁶

The rate of neutral hydrolysis of AcPhP⁻ at 39° is 70 times slower than that of AcP⁻. The hydrolysis of dibenzoyl phosphate is some tenfold slower than that of monobenzoyl phosphate and that of benzoyl phenyl phosphate is still slower.⁴⁷ The difference in the rates of P-O bond cleavage is considerably greater than this, at least in the case of acetyl phenyl phosphate, since the observed reaction involves C-O cleavage.¹¹ This requirement that the phosphate moiety be unsubstituted, except for the leaving group, is found also in the solvolysis of phosphate ester monoanions³⁻⁵ and is consistent with a mechanism requiring the formation of an unsubstituted, resonance-stabilized, monomeric metaphosphate anion.

(7).—The hydrolysis of AcP⁻ and AcP⁼ has been shown^{48,49} to occur with P-O bond cleavage by studies in H₂¹⁸O. The solvolysis of acetyl phenyl phosphate in 90% methanol occurs with C-O cleavage.¹¹ The alkaline hydrolysis of benzoyl adenylate, a related compound, also occurs with C-O cleavage.⁵⁰

(8).—The formation of pyrophosphate during acetyl phosphate solvolysis in largely non-aqueous solvents and in water in the presence of concentrated NaClO₄ is reminiscent of the formation of condensed phosphates which accompanies other phosphate solvolyses.^{10,51} This type of reaction may be interpreted as: (a) nucleophilic reaction of phosphate or a second mole of substrate with the activated phosphate of the substrate; (b) reaction of phosphate or substrate with a reactive intermediate produced during solvolysis, such as metaphos-

phate ion; or (c) attack of solvent on the substrate to form a reactive intermediate, which can react with phosphate or a second mole of substrate. Mechanism c probably accounts for the high yield of pyrophosphate found in aqueous pyridine and for the related reaction observed by Khorana and Vizsolyi.⁵² In aqueous solution at alkaline pH, mechanism a is unlikely, since it would require a reaction between two doubly charged negative ions, and mechanism c is improbable on chemical grounds. Pyrophosphate formation under these conditions is thus further evidence favoring the intermediate formation of metaphosphate ion.

The solvolyses of related compounds display certain similarities. The solvolysis of the carbamyl phosphate dianion at neutrality occurs approximately 20% by P-O bond cleavage and the rate constant for P-O cleavage may be estimated to be $3 \times 10^{-3} \text{ min.}^{-1}$ at 37° from the data of Jones and Lipmann⁵³; it is likely that the mechanism of this reaction is the same as that for AcP⁼ solvolysis. The solvolysis of acetyl, carbamyl and glucosyl arsenates proceeds very rapidly with As-O bond cleavage,⁵⁴ arsenate itself undergoes rapid exchange with H₂¹⁸O at neutrality⁵⁵ and there is a rapid interconversion between arsenate and pyroarsenate in aqueous solution⁵⁶; these reactions may proceed through a facile formation of the meta-arsenate anion. The solvolysis of sulfamic acid proceeds with a k_{D_2O}/k_{H_2O} ratio of 0.95 and a ΔS^\ddagger of 3.2 e.u. and may proceed by an elimination reaction to give SO₃ as the immediate product.⁵⁷

Conclusion.—While no single one of these considerations should be taken alone as conclusive proof of the monomolecular or bimolecular nature of a reaction, taken together they constitute strong evidence that the neutral hydrolyses of acyl phosphates occur by the mechanisms shown in eqs. 1-4.

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