The Reactivity of Phosphate Esters. Monoester Hydrolysis

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Abstract: Linear free-energy relationships between the rate constants for hydrolysis and the pK_a of the leaving group are obtained for both mono- and dianions of phosphate esters, $ROPO(OH)_2$. The reactivity of the monoanions depends only weakly on the leaving group, but that of the dianions is nine times more sensitive to the basicity of RO^-. Both ionic species are considered to be hydrolyzed by unimolecular mechanisms, involving the elimination of the leaving group from $ROPO_3^{2-}$ or $RO^+HPO_3^{2-}$, and the formation of monomeric metaphosphate, although nonselective phosphorylation of mixed alcohol–water solvents is not in general observed. The hydrolysis of the dianion of 2,4-dinitrophenyl phosphate is accelerated many hundredfold in the presence of dipolar aprotic solvents.

Any meaningful discussion of the kinetics and mechanism of enzymic or intramolecular catalysis must be based on a sound knowledge of the type of reaction concerned in the absence of catalysis. The rates of simple reactions can often be predicted with some accuracy if a linear free-energy relationship can be found to describe the variation of reactivity with the structure of the reactants. Preliminary to a study of more complex reactions, we are engaged in an examination of simple substitution processes at the phosphorus center of esters of phosphoric acid.

We have shown previously[1] that the nucleophilic reactivity of a series of aliphatic amines toward the dianion of a single ester, *p*-nitrophenyl phosphate, can be described with some success by a linear free-energy relationship, namely, a plot of the logarithms of the second-order rate constants for the displacement reaction, against the pK_a's of the conjugate acids of the amines. The present paper is concerned with the variation of reactivity with the structure of the leaving group in phosphate monoester hydrolysis and with the mechanism of this reaction.

Experimental Section

Materials. Inorganic salts were of analytical grade and were used without further purification. All liquid reagents were purified by distillation; distilled water was further glass-distilled twice before use. Disodium *p*-nitrophenylphosphate, obtained from the Aldrich Chemical Co., was used in a few product analysis experiments in mixed solvents. It was similar to samples used in previous investigations,[1,2] containing negligible amounts of free *p*-nitrophenol, but 6.4% of inorganic phosphate.

Commercially available phenols were recrystallized. 3,5-Dinitrophenol, mp 126° (anhydrous) from dilute HCl, was prepared by demethylation of 3,5-dinitroanisole[3] with $AlCl_3$.[4] 2-Nitro-4-acetylphenol[5] had mp 131–132° (from EtOH); 2-nitro-4-chlorophenol[6] had mp 87° (from EtOH); and 2-nitro-4-carbomethoxyphenol[7] had mp 74° (from aqueous HOAc).

Aryl phosphorodichloridates were prepared either from the solution of the phenol in refluxing $POCl_3$, in the presence of KCl,[8] or by stirring the sodium salt of the phenol with $POCl_3$ at room temperature.[9] After removal of inorganic chlorides by filtration, the solutions were evaporated *in vacuo*, and the residues were purified by distillation or sublimation.

Dibenzyl aryl phosphates were prepared from the phenol and dibenzylphosphorochloridate[10] in the presence of 1 mole of 2,6-lutidine in refluxing ether, or, alternatively, by stirring a suspension of the potassium salt[11] of the phenol in a solution of the phosphorochloridate in dry benzene.[12] Removal of the precipitated ionic chloride by filtration, and of the solvent by evaporation, gave the crude triester as an oil. This was used without further purification, with the exception of dibenzyl 2,4-dinitrophenyl[13] and dibenzyl pentachlorophenyl phosphates, which crystallized on standing and were recrystallized from ethanol (mp 65–66 and 69°, respectively).

Phosphate Monoesters. The usual methods of debenzylation fail with dibenzyl 2,4-dinitrophenyl phosphate,[14] but dry HBr[15] in inert solvents gives excellent results with dibenzyl aryl esters and even with some aliphatic esters. The triester was dissolved or suspended in dry ether or dioxane, and dry HBr passed in until it was no longer absorbed. Both benzyl groups were usually removed by this procedure, but monodebenzylation occurred with triesters derived from *m*-nitro and 3,5-dinitrophenols, and from 2-chloro-4-nitro- and 2-nitro-4-chlorophenols. These esters could be completely debenzylated by passing HBr through the solution in gently refluxing chloroform for 2 hr, and these are the most generally useful conditions. The esters from 2,4,6-trichloro- and pentachlorophenols were debenzylated by hydrogenation over 5% palladized charcoal in dry methanol.

After debenzylation, the solvent and excess of HBr were removed *in vacuo*, and the mixture of arylphosphoric acid and benzyl bromide was dissolved in ether (a large volume may be needed). 2,6-Lutidine or cyclohexylamine was added with stirring, and the amine salt was precipitated immediately. For phenol phosphates the amine was added until the yellow color of the phenolate anion was observed otherwise a large excess of amine was used. This procedure gave salts containing 2 equiv of amine, except in the case of 2,4-dinitrophenyl phosphate, where the monoanion salt was obtained.[13]

The salts were recrystallized from water or ethanol–water mixtures. They could readily be converted into the salts of the monoanions by treatment of a concentrated aqueous solution with the acid form of Amberlite IR (120) ion-exchange resin. The filtrate was brought back to pH 3.5 by addition of the amine, the water evaporated off, and the salt recrystallized again from water or aqueous ethanol. Yields were 30–40% over-all, based on phenol.

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(1) A. J. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, **87**, 3209 (1965).

(2) J. D. Chanley and E. Feageson, *ibid.*, **85**, 1181 (1963).

(3) L. de Braun, *Rec. Trav. Chim.*, **9**, 209 (1890).

(4) N. V. Sidgwick and T. W. J. Taylor, *J. Chem. Soc.*, 1853 (1922).

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(6) A. Faust and E. Saame, *Ann. Chem., Suppl.*, 7, 191 (1870).

(7) G. W. K. Cavill, *J. Soc. Chem. Ind.* (London), **84**, 212 (1945).

(8) M. Y. Kraft and V. V. Katyshina, *Dokl. Akad. Nauk SSSR*, **86**, 725 (1952); *Zh. Obshch. Khim.*, **26**, 3060 (1956).

(9) A. F. Turner and H. G. Khorana, *J. Am. Chem. Soc.*, **81**, 4651 (1959).

(10) G. W. Kenner, A. R. Todd, and F. J. Weymouth, *J. Chem. Soc.*, 3675 (1952). Dibenzyl phosphorochloridate prepared in this way from a good sample of dibenzyl phosphite can be stored at 0° for at least a week without serious decomposition.

(11) *Caution!* Alkali metal salts of nitrated phenols are potentially explosive, and when dry may be detonated by friction at temperatures well below the explosion point.

(12) J. Baddiley, V. M. Clark, J. J. Michalski, and A. R. Todd, *J. Chem. Soc.*, 815 (1949).

(13) A. J. Kirby and A. G. Varvoglis, *J. Am. Chem. Soc.*, **88**, 1823 (1966).

(14) V. H. Parker, *Biochem. J.*, **69**, 306 (1958); R. Azerad, D. Gautheron, and M. Vilkas, *Bull. Soc. Chim. France*, 2078 (1963).

(15) A. Cosmatos, I. Photaki, and L. Zervas, *Chem. Ber.*, **94**, 2644 (1961).

2,6-Lutidinium 2,4-dinitrophenyl hydrogen phosphate had mp 142° from ethanol.[16] *Anal.* Calcd for $C_{13}H_{14}N_3O_8P$: C, 42.1; H, 3.77; N, 11.3; P, 8.35. Found: C, 41.7; H, 4.0; N, 11.5; P, 8.29.

Cyclohexylammonium 2-nitrophenyl hydrogen phosphate gave crystals from 95% ethanol. *Anal.* Calcd for $C_{12}H_{19}N_2O_6P$: C, 45.3; H, 6.0; N, 8.8; P, 9.7. Found: C, 45.2; H, 5.4; N, 8.4; P, 9.5.

Cyclohexylammonium 4-nitrophenyl hydrogen phosphate gave crystals from water. *Anal.* Calcd for $C_{12}H_{19}N_2O_6P$: C, 45.3; H, 6.0; N, 8.8; P, 9.7. Found: C, 45.5; H, 5.3; N, 8.7; P, 9.6.

Cyclohexylammonium 2,2,2-trifluoroethyl hydrogen phosphate gave crystals from ethanol. *Anal.* Calcd for $C_8H_{17}F_3NO_4P$: C, 34.4; H, 5.9; N, 5.0; P, 11.1. Found: C, 34.6; H, 5.9; N, 5.2; P, 8.3. We suspect that fluoride may interfere with the phosphorus determination by forming phosphorofluoridic acid in the acid-hydrolysis stage.

Cyclohexylammonium 3,5-dinitrophenyl hydrogen phosphate gave crystals from aqueous ethanol. *Anal.* Calcd for $C_{12}H_{18}N_3PO_8 \cdot H_2O$: C, 37.1; 5.29; N, 11.1; P, 8.15. Found: C, 36.9; H, 4.69; N, 11.6; P, 8.4.

Dicyclohexylammonium 3-nitrophenyl phosphate gave crystals from aqueous ethanol. *Anal.* Calcd for $C_{18}H_{32}N_3O_6P \cdot H_2O$: C, 49.6; H, 8.02; N, 9.65; P, 7.1. Found: C, 49.6; H, 8.25; N, 9.6; P, 7.3.

Dicyclohexylammonium 2-chloro-4-nitrophenyl phosphate gave crystals from aqueous ethanol. *Anal.* Calcd for $C_{18}H_{31}ClN_3O_6P \cdot H_2O$: C, 46.1; H, 7.09; N, 8.95; P, 6.60. Found: C, 46.6; H, 6.43; N, 9.1; P, 6.8.

Dicyclohexylammonium 2-nitro-4-chlorophenyl phosphate gave crystals from aqueous ethanol. *Anal.* Calcd for $C_{18}H_{31}ClN_3O_6P \cdot H_2O$: C, 46.1; H, 7.09; N, 8.95; P, 6.60. Found: C, 46.0; H, 7.04; N, 8.95; P, 7.06.

Dicyclohexylammonium pentachlorophenyl phosphate gave crystals from aqueous ethanol. *Anal.* Calcd for $C_{18}H_{28}Cl_5N_2O_4P$: C, 39.7; H, 5.15; Cl, 32.6; P, 5.7. Found: C, 40.2; H, 5.6; Cl, 32.9; P, 5.4.

Cyclohexylammonium 2-nitro-4-acetylphenyl hydrogen phosphate gave crystals from methanol–ether, then from ethanol. *Anal.* Calcd for $C_{14}H_{21}N_2O_7P \cdot 0.5H_2O$: C, 45.5; H, 6.00; N, 7.59; P, 8.39. Found: C, 45.9; H, 6.00; N, 7.99; P, 8.8.

Dicyclohexylammonium 2,4,6-trichlorophenyl phosphate gave crystals from aqueous ethanol. *Anal.* Calcd for $C_{18}H_{30}Cl_3O_4N_2P \cdot 0.5H_2O$: C, 44.6; H, 6.43; Cl, 22.0; N, 5.78; P, 6.39. Found: C, 44.5; H, 6.78; Cl, 22.3; N, 6.4; P, 6.35.

Kinetic Methods. When this work was begun, no satisfactory procedure was available for the preparation of many of the phosphate monoesters required. Their rates of hydrolysis were therefore obtained by using as substrates the aryl phosphorodichloridates, $ArOPOCl_2$. The hydrolysis of both P–Cl bonds was expected to be very much faster than that of the phosphorus–oxygen bond: the slowest stage in the hydrolysis of $POCl_3$ is that of the dichloro acid, $HOPOCl_2$, with a half-life of about 4 min at 25°.[17] The release of phenol was always considerably slower than this, so the rate of release of phenol from $ArOPOCl_2$ was taken as a measure of the rate of hydrolysis of the monoester. When pure salts of the monoesters became easily accessible, these rates were measured again, and good agreement was found. The results in this paper refer to experiments with the pure ester anions, but the dichloridate may sometimes be the more convenient substrate.

The very slow hydrolysis of monoesters at 39° was measured by following initial rates of release of the phenols. A solution (1 ml) of known concentration of the phosphate ester in water was made up to 25 ml in a volumetric flask containing the buffer constituents and sufficient KCl to maintain a final ionic strength of 1.0. The solutions were incubated in the dark[18] in a water bath at 39°. Aliquots (1 ml) were removed at intervals and diluted to 5 ml with water and 1 ml of 1 *N* NaOH; the optical density was measured at the wavelength of the absorption maximum of the phenolate ion concerned (Table I), using a Zeiss PMQII spectrophotometer. The pH of the reaction mixture was measured at room temperature at the end of each run. First-order rate constants were calculated as described previously,[1] using the extinction coefficients listed in Table I. The first 1-5% of reaction was followed.

The faster hydrolysis of 2,4-dinitrophenyl phosphate at 39° and all reactions at 100° were usually followed to three half-lives and final readings taken after at least ten. The release of 2,4-dinitrophenolate at 39° from the ester dianion, above pH 4.5, was followed in the thermostated cell compartment of the spectrophotometer. For the hydrolysis of the monoanion at lower pH values, aliquots were taken as before, and for experiments at 100° aliquots were sealed in separate Pyrex tubes. All these reactions gave satisfactory first-order kinetics.

The rate constants for the hydrolysis of the separate ionic species were obtained from the pH–rate profiles. For each ester the rate of hydrolysis was measured at least six different pH values in the range 2–12 (see Figure 1). The rate for the dianion is that observed in the pH-independent region above pH 7. The rate for the monoanion was taken as that at the maximum at pH 3–4,[19] except where the dianion was hydrolyzed faster. Then the rate in the small pH-independent region near pH 2 was used.[20] These last rates might be expected to contain a contribution from the hydrolysis of the neutral species; but, since they are actually somewhat slower than predicted from the linear free-energy relationship discussed below, this contribution is unlikely to be large. In any case our conclusions will not be affected. Inorganic phosphate was estimated by the method of Allen.[21]

The buffer solutions used were $HClO_4$–NaCl, HCl–KCl, phthalate–HCl, acetate–HCl, formate–HCl, phosphate, Tris–HCl, and carbonate–bicarbonate, generally at 0.05 *M*.[22] The ionic strength for all reactions in water was maintained at 1.0 with KCl, except where inorganic phosphate was to be determined, when NaCl was used, to avoid precipitation of $KClO_4$.

Reactions in Mixed Solvents. Rates were followed in mixed aqueous solvents at ionic strength 0.2, as described above, by following the release of phenol, except that the reactions were started by dissolving a weighed amount of the crystalline ester in the reaction mixture. In a few cases the appearance of inorganic phosphate was also followed. The rates obtained in this way agreed with those based on the release of phenol but were less precise, particularly for faster reactions. The ratio of alkyl phosphate to inorganic phosphate produced in alcohol-water mixtures was constant throughout a given reaction.

This ratio was obtained by measuring the concentrations both of inorganic phosphate formed and of phenol released, usually after complete solvolysis, but more conveniently after 1–3 half-lives for slow reactions. The difference in these two concentrations gave the amount of alkyl phosphate formed. The pure alkyl phosphates were shown not to be hydrolyzed under the conditions of the reactions or of the estimation. A small correction was made in one case, where the solvolysis of the monoanion of 2,4-dinitrophenyl phosphate was allowed to proceed for 1 week at 39° and pH 2.4 in aqueous isopropyl alcohol. Under these conditions isopropyl phosphate gave 3.6% of the theoretical amount of inorganic phosphate.

The monoanions of monoalkyl[23] and some monoaryl[19] phosphates are known to be hydrolyzed with fission of the phosphorus–oxygen bond. This has been demonstrated also for the dianion of *p*-nitrophenyl phosphate.[1] Solvolysis of 2,4-dinitrophenyl phosphate in ethylene glycol containing NaOH (0.01 *M*) was four times faster than the hydrolysis of the dianion in water at the same ionic strength ($k_{EG} = 2.2 \times 10^{-2}$ min^{-1} at 39°) and gave 2% of the calculated amount of inorganic phosphate. In this case also, therefore, the phosphorus–oxygen bond is broken.

Results

Hydrolysis. Two distinct types of pH–rate profile for hydrolysis are observed for phosphate esters. One has the familiar rate maximum near pH 4, as exemplified by the curves for *p*-nitrophenyl phosphate[19,24] and

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[16] Melting points are not reported for other salts, because they were not sharp. They occur with decomposition and are not considered reliable either for identification or as a criterion of purity.

[17] R. F. Hudson and G. E. Moss, *J. Chem. Soc.*, 3599 (1962).

[18] A number of the esters were light sensitive (Table I); *cf.* E. Havinga, R. O. De Jongh, and W. Dorst, *Rec. Trav. Chim.*, 75, 378 (1956).

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

(21) R. J. L. Allen, *Biochem. J.*, 34, 848 (1940).

(22) All rates observed were independent of the concentration of buffer constituents at a given pH, so these are not specified, except in the hydrolysis of 2,4-DNPP below pH 1, where halide ion catalysis is observed (Table II).

(23) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3574, 3588 (1958).

Table I. Hydrolysis of Monoaryl Phosphates at 39°, Ionic Strength 1.0

Phenol	pK_a	Spectrum of ArO$^-$ λ_{max}, mμ	Spectrum of ArO$^-$ ϵ_{max}	Rate constant for hydrolysis, min^{-1} × 10^5 Monoanion	Rate constant for hydrolysis, min^{-1} × 10^5 Dianion
Phenol	9.99	289.4	4,780	0.573	*a*
2-Nitro	7.23	416	4,800[b]	5.37	0.404
4-Nitro	7.14	400	18,320[c]	6.42	0.093[d]
3,5-Dinitro[e]	6.68[f]	397	2,200[g]	5.95	0.17
2-Nitro-4-chloro[e]	6.36[h]				
	6.46[i]	425	4,380[g]	3.68	1.97
2,4,6-Trichloro	6.1[j]	311	4,900[k]	2.20	0.27
2-Chloro-4-nitro[e]	5.45[i]	400	24,320[g]	1.46	1.36
2-Nitro-4-COMe	5.09[h]	399	4,560[g]	13.8	61.7
Pentachloro	4.8[j]	322	4,800[g]	*l*	11.4
2,4-Dinitro	4.07	360	14,700[g]	30.4	1050

[a] Too slow to measure. [b] L. Doub and J. M. Vandenbelt, *J. Am. Chem. Soc.*, **71**, 2414 (1949). [c] F. Kezdy and M. L. Bender, *Biochemistry*, **1**, 1097 (1962). [d] Reference 1. [e] Light sensitive. [f] D. C. Martin and J. A. V. Butler, *J. Chem. Soc.*, 1366 (1939). [g] Measured using a Cary Model 14 recording spectrophotometer. [h] M. Rapoport, C. K. Hancock, and E. A. Meyers, *J. Am. Chem. Soc.*, **83**, 3489 (1961). [i] V. E. Bower and R. A. Robinson, *J. Phys. Chem.*, **64**, 1078 (1960). [j] G. E. Blackman, M. H. Parke, and G. Garton, *Arch. Biochem. Biophys.*, **54**, 55 (1955). [k] W. R. Jondorf, D. V. Parke, and R. T. Williams, *Biochem. J.*, **61**, 512 (1955). [l] Phenol was precipitated at pH lower than 5.

Table II. Hydrolysis of 2,4-Dinitrophenyl Phosphate at 39°, Ionic Strength 1.0

Conditions	k_{hyd} × 10^4 min^{-1}
3 *N* HCl	86.1
1 *N* HCl + 2 *M* KCl	51.3
1 *N* HCl + 2 *M* KClO$_4$	43.0
1 *N* HCl	38.5
1 *N* HClO$_4$	37.1
pH 1.95[a]	3.12
2.30	3.12
2.65	5.78
3.60	22.0
4.65	67.3
5.10	85.6
5.55	107.3
7.1	108.3
8.4	101.2
10.4	105.1
11.6	102.0

Conditions	Monoanion	Dianion
At ionic strength		
1.0, KCl	3.12[b]	104.0[c,d]
1.0, LiCl	...	82.1
0.2, KCl	1.78	64.7
In D$_2$O	2.16	102.5
At 27.0°	...	18.1
At 50.0°	...	407
At 60.0°	34.1	...
At 100°	1850	...
$\Delta H_{av}^{\ddagger}$ (kcal/mole)	24.2	25.7
$\Delta S^{\ddagger}$ at 39° (eu)	−6.0	+6.6

[a] See footnote 22. [b] At pH 1.8 in water. [c] Average of 27 values in the pH-independent region, 6–13. [d] Rate unchanged with NaCl.

Table III. Hydrolysis of Monoanions ROP(OH)O$_2^-$ at 100°, Ionic Strength 1.0

Ester	pK_a of ROH	k_{hyd}, min^{-1}
2,4-Dinitrophenyl phosphate	4.07	1.85 × 10^{-1}
3,5-Dinitrophenyl phosphate	6.68	7.83 × 10^{-2}
4-Nitrophenyl phosphate[a]	7.14	6.83 × 10^{-2}
3-Nitrophenyl phosphate	8.35	2.95 × 10^{-2}
2,2,2-Trifluoroethyl phosphate	12.37[b]	3.42 × 10^{-3}
Propargyl phosphate[c]	13.55[b]	1.54 × 10^{-3}

[a] Data from ref 2. [b] P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **82**, 795 (1960). [c] Data from E. Cherbuliez, G. Weber, and J. Rabinowitz, *Helv. Chim. Acta*, **46**, 2464 (1963).

monoalkyl phosphates.[23,25] The second type, of which the curve for 2,4-dinitrophenyl phosphate is an example,[13] is obtained for esters of phenols with pK_a less than about 5.5, where hydrolysis of the dianion is faster than that of the monoanion. For 2-chloro-4-nitrophenyl phosphate (pK_a of phenol = 5.45), the monoanion and the dianion are hydrolyzed at almost identical rates at 39° (Figure 1 and Table I).

Data for ten substituted monoaryl phosphates at 39° are summarized in Table I. Three representative pH–rate profiles are shown in Figure 1. The rates of hydrolysis of both ionic species increase with decreasing pK_a of the phenol, but the values for the dianion cover a much wider range. Detailed data for the most reactive ester studied, 2,4-dinitrophenyl phosphate, appear in Table II. The rate of hydrolysis of this ester in 1 *M* acid at ionic strength 3.0 increases linearly with the con-

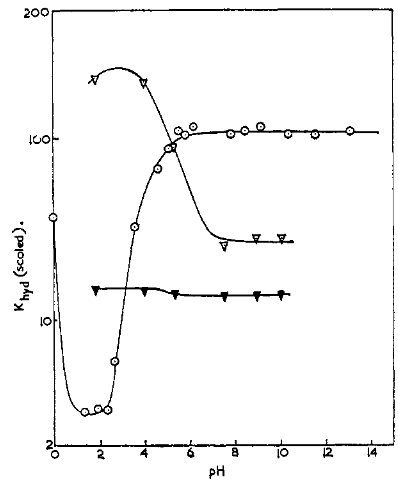

Figure 1. pH-rate profiles for three representative esters at 39°, ionic strength 1.0, on adjusted scales: ▲, k_{hyd} × 10^7 min^{-1}, for 2,4,6-trichlorophenyl phosphate; ▼, k_{hyd} × 10^6 min^{-1} for 2-chloro-4-nitrophenyl phosphate; ○, k_{hyd} × 10^4 min^{-1}, for 2,4-dinitrophenyl phosphate.

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(24) A. Désjobert, *Bull. Soc. Chim. France*, 683 (1963).

(25) M. C. Bailly, *ibid.*, **9**, 340, 405 (1942); A. Désjobert, *ibid.*, **14**, 809 (1947).

Table IV. Per Cent of Alkyl Phosphate Formed on Solvolysis of Phosphate Monoesters in Alcohol-Water Mixtures at 100°, Ionic Strength 0.2

Solvent mixture	Mole % of ROH	*p*-Nitrophenyl phosphate Monoanion	*p*-Nitrophenyl phosphate Dianion	*o*-Nitrophenyl phosphate dianion	2,4-Dinitrophenyl phosphate Monoanion	2,4-Dinitrophenyl phosphate Dianion
30% methanol	15.5	16[a]	16.9	23.1	14.3	29.5[b] 31.8[c]
50% methanol	29.2	29[a]	29.0	36.8	...	...
70% methanol	48.0	47[a]	46.8	63.7	...	...
30% 2-propanol		0[d]	0[d]			
50% 2-propanol		0[d]	0[d]			
70% 2-propanol		...	0[d]			

[a] Data from ref 2. [b] At pH 5.7. [c] At pH 9.2. [d] 100 ± 2% of the calculated amount of inorganic phosphate formed.

Table V. Per cent of Alkyl Phosphate Formed on Solvolysis of 2,4-Dinitrophenyl Phosphate in Alcohol-Water Mixtures at 39°, Ionic Strength 0.2

	Alcohol											
	Methanol				Ethanol			Trifluoroethanol		2-Propanol		
% v/v	20	30	50	70	30	50	70	50	70	30	50	70
Mole % ROH	10.2	15.5	29.2	48.0	11.3	22.4	40.4	22.0	36.8	9.4	19.1	35.5
Monanion[a]	...	32.0	38.9	52.1	...	...	...	...	...	0[c,d]	0[c,d]	0[c,d]
Dianion[b]	28.3	44.8	62.7[e]	79.1	20.5	35.3	58.0	8.3	14.5	0[c]	0[c]	0[c]

[a] Measured at pH 1.8 in water. [b] Measured at pH 9.2 in water. [c] Found: 100 ± 2% of the theoretical amount of inorganic phosphate. [d] Isopropyl phosphate was 3.6% hydrolyzed under the conditions of these experiments. [e] Falls to 39% in a solvent composed of 15% methanol, 15% water, and 70% dimethyl sulfoxide.

Table VI. Rates of Solvolysis of 2,4-Dinitrophenyl Phosphate in Mixed Aqueous Solvents at 39°, Ionic Strength 0.2 ($\text{min}^{-1} \times 10^2$)

Solvent	%, v/v: 0	30	50	70	80
		Monoanion			
Methanol	0.0178	0.0136	0.0186	0.0152	...
		Dianion			
Methanol	0.647	1.43	3.22	7.30	...
2-Propanol		1.75	2.95	6.30	17.3
Acetonitrile		1.69	2.77	...	...
Acetone		2.10	4.33	12.6	...
Dioxane		3.56	9.91	19.8	...
Formamide		3.96	8.40	21.3	...
Dimethylformamide		3.15	15.4	138	...
Dimethyl sulfoxide		2.83	21.7	346	...

centration of chloride or bromide ion as the anion present is changed from ClO_4^-. This is most likely a specific salt effect, although nucleophilic catalysis by halide ion of the hydrolysis of the protonated acid would also account for the results.

Rates of hydrolysis at 100° were measured for several esters; these results appear in Table III. Finally, data concerning solvolysis in mixed aqueous solvents are given in Tables IV–VI.

Discussion

Dianion Hydrolysis. We have shown previously[1] that the rate of hydrolysis of the dianion of *p*-nitrophenyl phosphate is that predicted from a logarithmic plot of the rate constants for hydrolysis of a series of substituted benzoyl phosphates[20] against the pK_a of the leaving group. Data for the dianions of a further 11 esters are plotted on the same graph in Figure 2 and show that the original agreement was not fortuitous.

The rates of hydrolysis are too slow to measure at 39° for derivatives of phenols with pK_a above about 7, so that only esters of strongly acidic phenols are represented. Many of these have *ortho* substituents, which are presumably responsible for the small but systematic deviations from the original straight line. Compounds with *o*-nitro groups, for instance, are hydrolyzed just twice as fast as predicted and give a straight line parallel to the original. This may be a steric effect: it is consistent with a decrease in nonbonded interaction between the phosphoryl group and the *ortho* substituent in the transition state, due to the extension of the phosphorus–oxygen bond. A constant, passive, role for the *o*-nitro group is supported by the good Hammett plot shown in Figure 3. Here the hydrolysis data for 2-nitro-4-substituted phenyl phosphate dianions are plotted against σ^- for the 4 substituent, giving a value for $\rho = 2.6$ for the hydrolysis reaction.

Compounds with *o*-chloro substituents are hydrolyzed more slowly than expected. The displacement from the line in this case is by a factor of about 6 in rate, or 0.6 in pK_a. We are unable to account for this observation.

The equation of the straight line of Figure 2 is

$$\log k_{\text{hyd}} = 2.64 - 1.23\text{p}K_a \qquad \text{(i)}$$

in min^{-1} at 39°. The slope agrees with the value of $\rho = 2.6$ obtained from Figure 3, taking ρ for the

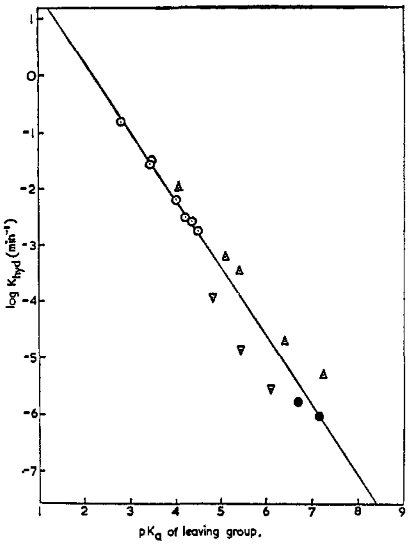
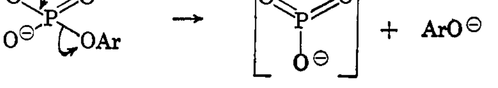


Figure 2. Hydrolysis rates for the dianions of phosphate monoesters at 39°, as a function of the pK_a of the conjugate acid of the leaving group: ⊙, benzoyl phosphates (data from ref 20); ●, monoaryl phosphates with no *ortho* substituents; △, monoaryl phosphates with *o*-nitro groups; ▽, monoaryl phosphates with *o*-chloro groups. Data from Table I.

ionization of phenols as 2.11.[26] This equation may be expected to apply generally to the hydrolysis at 39° of derivatives $ROPO_3^{2-}$, such as mixed anhydrides, in which a phosphorus–oxygen bond is cleaved.[27]

The high sensitivity of the rate of hydrolysis of the dianions to the pK_a of the leaving group, as measured by the slope of −1.23 for the plot of Figure 2, indicates that bond breaking is well advanced in the transition state. We have suggested[1] that this is evidence for a unimolecular elimination mechanism (1), in which the anion of monomeric metaphosphate is formed. Strong evidence for this mechanism is available for the dianions of acyl phosphates,[20] and the esters listed in Table I are almost certainly hydrolyzed by the same route, since data for both types of compound fall on the same curve of Figure 2. Both also show a small, positive entropy of activation for hydrolysis and insignificant deuterium isotope effects.



(1)

The magnitude of the slopes of the straight line of Figure 2 is considerably greater than seems likely for a reaction involving bimolecular displacement by a water molecule, although there are not sufficient data available to establish this point definitely. The slope of the corresponding plot for the alkaline hydrolysis of a series of diethyl aryl phosphates is 0.49;[28] and a similar figure, of 0.56, has been found by Brown and Usher[30] for a series of diester anions. Both of these studies, however, involved alkaline hydrolysis, catalyzed in the latter instance by a 2-hydroxy group, and the corresponding figures for neutral hydrolysis might be expected to be higher. The data of Aldridge and Davison[29] for the "neutral" hydrolysis of diethyl aryl phosphates in phosphate buffer at pH 7.6 (which, however, may well involve nucleophilic catalysis by phosphate dianion) do not in fact suggest a markedly greater sensitivity to the pK_a of the leaving group than those in alkali.[28] But again, the data are not sufficiently extensive for an accurate comparison.

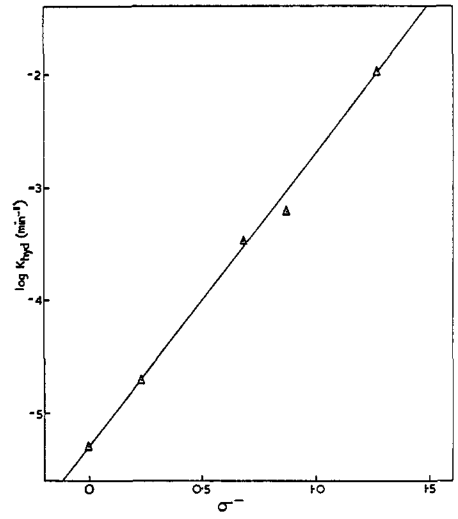


Figure 3. Hammett plot for the hydrolysis of the dianions of 2-nitro-4-substituted monoaryl phosphates at 39°, using σ^- for the *para* substituent. Data from Table I.

Unlike the hydrolysis of the dianion of acetyl phosphate, which preceeds at the same rate in water and in 30 and 50% acetonitrile,[20] the hydrolysis of aryl phosphate dianions is accelerated, often markedly, by the addition of organic solvents to the medium. The data in Table VI refer to 2,4-dinitrophenyl phosphate, but *p*-nitrophenyl phosphate behaves similarly. We can find no systematic relationship between the increase in rate caused by a given solvent and its dielectric constant or Grunwald–Winstein *Y* value,[31] but dipolar aprotic solvents have the greatest effect.

The accelerating effect of all organic solvents tested (Table VI) is predicted by the qualitative theory of Hughes and Ingold[32] for the unimolecular mechanism (1), which involves dispersion of the negative charge in the transition state. The special effect of dipolar aprotic solvents may be due to weaker solvation of the $-PO_3^{2-}$ group as the amount of water in the solvent falls sufficiently for the organic solvent to take part (see below).[33] This special effect is only observed at fairly high concentrations of the dipolar aprotic solvent; 2,4-dinitrophenyl phosphate dianion is actually hydrolyzed more slowly in 30% dimethyl sulfoxide than in 30% dioxane, although 17 times faster in water containing 70% of the organic solvent.[34]

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[26] H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).

[27] It does not apply to the cleavage of bonds to phosphorus from elements other than oxygen.

[28] Data taken from T. R. Fukuto and R. L. Metcalf, *J. Agr. Food Chem.*, **4**, 930 (1956); corrected for neutral hydrolysis using data from ref 29.

[29] W. N. Aldridge and A. N. Davison, *Biochem. J.*, **52**, 663 (1952).

[30] D. M. Brown and D. A. Usher, *J. Chem. Soc.*, 6558 (1965).

[31] E. Grunwald and S. Winstein, *J. Am. Chem. Soc.*, **70**, 846 (1948). There is a linear relationship between log k_{hyd} and *Y* for several solvents at lower concentrations, usually below about 50% v/v.

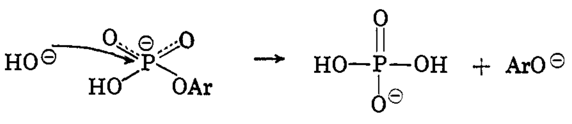
[32] C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p 346.

[33] A. J. Parker, *Quart. Rev.* (London), **16**, 163 (1962).

[34] We have data at lower ionic strengths which show that the rate of hydrolysis goes on increasing as the concentration of water in the solvent is decreased below 30% v/v. This probably accounts for the difficulty of earlier workers, who tried to synthesize 2,4-dinitrophenyl phosphate in

Finally, it is significant that the dianion of 2,4-dinitrophenyl phosphate is hydrolyzed faster than the monoanion. It is difficult to see how the removal of a proton from the monoanion could make the phosphorus center in $ROPO_3^{2-}$ *more* electrophilic,[36] so that bimolecular attack, particularly by a hard[38] nucleophile like water, would be expected to occur more readily on the monoanion. Since nucleophilic attack by water is available to both ionic species, *and yet the dianion is hydrolyzed faster*, we consider that the rate-determining step in the hydrolysis of the dianion cannot be bimolecular attack by water.

The remaining alternative to the unimolecular elimination (1) is the kinetically equivalent attack of hydroxide ion on the monoanion (2). Hydroxide ion reacts only very slowly with the anions even of activated

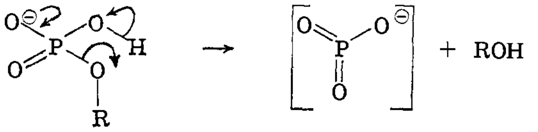

(2)

diesters,[39] and it seems unlikely that mechanism 2 can account for the observed rate of the reaction of the dianion, which in the case of 2,4-dinitrophenyl phosphate would require a second-order rate constant for the attack of hydroxide ion of at least 10^7 M^{-1} min^{-1}[40] at 39°.

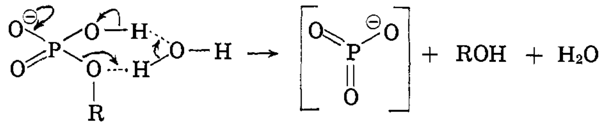
We consider therefore that the hydrolysis of the dianions of both acyl and monoaryl phosphates involves the unimolecular elimination process (1). A number of other cases where this mechanism is likely are discussed in ref 1, and by Miller and Westheimer.[41] More detailed features of the mechanism, and in particular the fate of the metaphosphate ion in mixed solvents containing both water and an alcohol, are discussed below.

Monoanion Hydrolysis. It is now generally accepted that the hydrolysis of the monoanions of phosphate monoesters does not involve simple bimolecular displacement by a molecule of water.[42] A special mechanism is necessary to account for the rate maximum observed near pH 4, where the concentration of the monoanion is a maximum, and the fact that this maximum rate is much greater than that for the hydrolysis of the corresponding diesters at the same pH.[23]

It is not likely that an alkoxide anion is displaced in aqueous solution at pH 4, and, since anad dition–elimination mechanism is ruled out by isotopic evidence,[23] it seems inescapable that at least partial protonation of the oxygen atom of the leaving group must have occurred by the time the transition state is reached. Two similar mechanisms have been proposed, involving intramolecular general acid catalysis by the undissociated hydroxyl group of the monoanion. The proton may be transferred to the leaving group either directly,[43] as in (3) or by way of a six-membered cyclic


(3)

transition state, involving a molecule of water[44] (4).


(4)

This latter formulation avoids the four-membered cyclic transition state and might therefore be favored energetically. But this factor would be offset to some extent by a less favorable entropy of activation.

Activation parameters for the hydrolysis of a number of phosphate monoester monoanions are collected in Table VII. The entropies of activation are all close to zero, in the range observed for many solvolysis reactions thought not to involve a molecule of solvent in the transition state.[45] Bimolecular solvolysis reactions, such as the hydrolysis of carboxylic esters, commonly have entropies of activation some 20 entropy units more negative.[46a] The near-zero values for the hydrolysis of the monoanions of phosphate monoesters are therefore consistent with a mechanism which does not

Table VII. Activation Parameters for the Hydrolysis of Phosphate Monoester Monoanions

Ester	k_{hyd}, min^{-1}, at 100°	$\Delta H^{\ddagger}$, kcal/mole[a]	$\Delta S^{\ddagger}$, eu[a]
Methyl phosphate[b]	4.94×10^{-4}	29.9	−2.2
Glycerol-1-phosphate[c]	8.1×10^{-4}	29.2	−3.1
$H_3N^+ CH_2CH_2OPO_3H^-$[c]	1.84×10^{-4}	28.9	−3.2
Phenyl phosphate[d]	1.67×10^{-3}	27.9	−0.6
4-Nitrophenyl phosphate[e]	6.83×10^{-2}	25.4	−4.5
2,4-Dinitrophenyl phosphate[f]	1.85×10^{-1}	24.2	−6.0

[a] Calculated from data in references cited. [b] Reference 23. [c] P. A. T. Swoboda and E. M. Crook, *Biochem. J.*, **52**, xxiv (1952). [d] Data from Table I: J. D. Chanley and E. Feageson, *J. Am. Chem. Soc.*, **77**, 4002 (1955); from ref 2; and from ref 19. [e] Data from Table I; from ref 2; and from K. A. Holbrook and L. Ouellet, *Can. J. Chem.*, **36**, 686 (1958). [f] Data from Table II.

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organic solvents, such as acetonitrile.[35] Even in 70% dimethyl sulfoxide the half-life of the dianion is reduced to 12 sec.

(35) R. Azerad, D. Gautheron, and M. Vilkas, *Bull. Soc. Chim. France*, 2078 (1963).

(36) But not impossible. It is conceivable, for instance, that the P–O bond broken in the hydrolysis of $ArOPO_3^{2-}$ is very much weaker than that of $ArO(HO)PO_2^-$, because of a sharp reduction in pπ–dπ conjugation. This would presumably be reflected in a marked change in the length of the phosphorus–oxygen bond concerned; and we are at present examining the length of this bond in a representative series of phosphate monoesters by X-ray crystallography.[37]

(37) With Drs. O. Kennard, D. G. Watson, J. K. Fawcett, and K. A. Kerr, of this department.

(38) R. G. Pearson, *J. Am. Chem. Soc.*, **85**, 3533 (1963).

(39) J. A. A. Ketelaar and H. R. Gersmann, *Rec. Trav. Chim.*, 77, 973 (1958).

(40) Calculated from the relation $k_2 = k_1K_2/K_w$, where k_2 is the second-order rate constant concerned, K_2 the second dissociation constant for 2,4-dinitrophenylphosphoric acid, and k_1 is the observed first-order rate constant for the hydrolysis of the dianion. At 39° k_1 is near 10^{-2} min^{-1} and pK_2 is about 4.6 (rough value by titration).

(41) D. L. Miller and F. H. Westheimer, *J. Am. Chem. Soc.*, **88**, 1511 (1966).

(42) J. R. Cox and O. B. Ramsay, *Chem. Rev.*, **64**, 317 (1964).

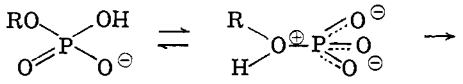
(43) C. A. Vernon, Special Publication No. 8, The Chemical Society, London, 1957, p 17.

(44) W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, 77, 2420 (1955).

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

(46) (a) Although these correlations have been obtained mainly with neutral substrates, the presumably bimolecular hydrolysis of the anion of acetyl phenyl phosphate, and the probably unimolecular hydrolysis of both monoanion and dianion of acetyl phosphate, have entropies of activation which fall in the appropriate ranges.[20] (b)This mechanism has been suggested as a possibility by: W. P. Jencks, *Brookhaven Symp. Biol.*, **No. 15**, 134 (1962); M. L. Bender and J. M. Lawlor, *J. Am. Chem. Soc.*, **85**, 3010 (1963); and no doubt by other authors.

involve a molecule of water in the transition state, such as (3). However, a simpler mechanism consistent with the facts is the specific acid-catalyzed hydrolysis of the dianion (5).[46b]



I

$ROH + [PO_3^{\ominus}]$ (5)

We believe that this mechanism accounts quantitatively for the observed rate of the reaction. Consider the hydrolysis of methyl phosphate. The first-order rate constant for the hydrolysis of the monoanion, at pH 4.17, is 8.23×10^{-6} sec^{-1} at 100°.[23] The pK_2 for methylphosphoric acid at 100° is 6.85,[23] and the pK_a of I is probably not far different from that of ROH_2^+, since the pK_a of the phosphoramidate monoanion, H_3N^+-PO_3^{2-}, is almost the same as that of NH_4^+.[2] The pK_a of $MeOH_2^+$ is about −2.5,[47] but since pK_a for Me_2OH^+ is lower, at −3.8,[47] a similar effect, probably due to solvation differences, could lower the pK_a of I also. We use therefore an estimated value of −4 for the pK_a of I. Allowing finally for the three protonation sites on the dianion giving the monoanion, compared with only one leading to I, the difference in basicity between the ester oxygen and the anionic oxygen atoms of $MeOPO_3^{2-}$ is approximately $6.85 - (-4) + 0.5 = 11.35$ powers of ten. If the observed hydrolysis at pH 4 goes entirely through I, therefore, the first-order rate constant for the breakdown of I must be near $8.23 \times 10^{11.35-6}$, *i.e.*, about 2×10^6 sec^{-1} at 100°.

The value of this rate constant at 39° can be calculated from eq i. Using pK_a = −2.5 for $MeOH_2^+$, the calculated value is 8.7×10^3 sec^{-1}, which is equivalent to 2.7×10^7 sec^{-1} at 100° (E_a = 30.6 kcal/mole[23]). An important assumption involved in this calculation is that eq i can be applied to a species with a neutral leaving group. Otherwise the approximations are not large, and this result, giving an estimated rate constant for the hydrolysis of I one order of magnitude larger than is necessary to account for the observed rate, shows that mechanism 5 can account satisfactorily for the rate of the hydrolysis. A similar calculation[48] for phenyl phosphate, for which we have measured the rate of hydrolysis at 39°, gives a similar result.

A logarithmic plot of the rates of hydrolysis at 100°, for a series of monoanions, against the pK_a of the leaving group, shows a good linear relationship over a wide range of phosphate esters (Figure 4). The equation of this line is

$$\log k_{\text{hyd}} = 0.91 - 0.27\text{p}K_a \qquad \text{(ii)}$$

in min^{-1} at 100°. The first deviations from the line are negative and occur for aromatic phosphates with leaving groups less basic than phenol.

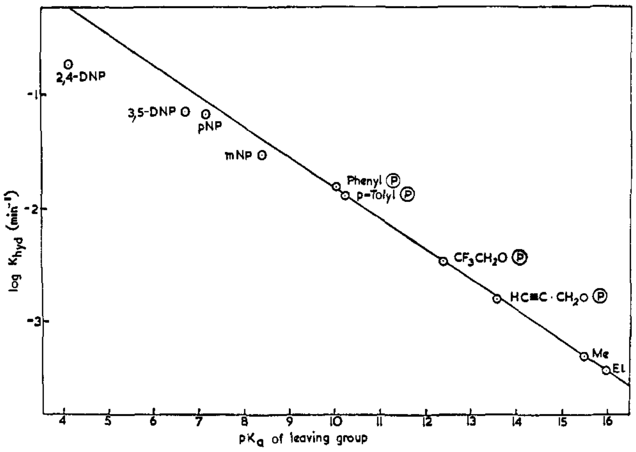
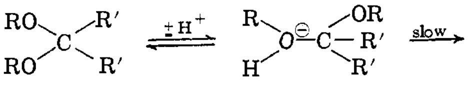


Figure 4. Hydrolysis rates for the monoanions of phosphate esters at 100°, as a function of the pK_a of the conjugate acid of the leaving group. D, p, and mNP are di-, *p*-, and *m*-nitrophenyl phosphates. Data from Tables III and VII.

We interpret the evidence as follows. The hydrolyses of the monoanions of methyl and phenyl phosphates, and of esters with leaving groups of intermediate basicity, occur by mechanism 5, with rapid preequilibrium formation of the zwitterion I, followed by phosphorus–oxygen bond fission in the rate-determining step. As the pK_a of the leaving group falls, however, the very large rate constants predicted for the hydrolysis of the zwitterions of type I rapidly approach the value at which the rate must become limited by diffusion control of their formation, by proton transfer, from the monoanion. An arbitrary upper limit for this process of 10^{10} sec^{-1} corresponds (eq i) to a leaving group of pK_a = −7.4, a little less basic than phenol. We suggest therefore that for those esters with very good leaving groups, the proton transfer becomes partially rate-determining, as in mechanisms 3 and 4 above. Consistent with this picture, we find k_H/k_D = 1.45 for the hydrolysis of the monoanion of 2,4-dinitrophenyl phosphate at 39° (Table II); the figure for methyl phosphate is 0.87 at 100°.[23]

Mechanism 5 is closely analogous to the A1 mechanism 6, believed to account for the hydrolysis of acetals.[51] The PO_3^{2-} group probably reduces the basicity of the alkoxyl oxygen less than does the $CR_2'OR$



III

$(RO^{\oplus}{=}CR_2') + ROH$ (6)

group in an acetal (the pK_a of III, R = R′ = CH_3, has been estimated to be −5.4[52]). The analogy extends also to the compounds with less basic leaving groups. Bunton and DeWolfe have argued[52] that the basicity of orthoesters is too low to account for the rate of their acid-catalyzed hydrolysis by the A1 mechanism. They and others[53] consider that the slow step involves protonation concerted with cleavage of the carbon–oxygen bond.

The catalysis of the hydrolysis of the dianion of salicyl phosphate by the *o*-carboxyl group has been attributed to stabilization of the zwitterion IV by the adjacent anionic group.[50] A similar effect may account

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(47) N. C. Deno and J. O. Turner, *J. Org. Chem.*, **31**, 1969 (1966).

(48) Using pK_a = −6.74 for $PhOH_2$,$^{+49}$ −6.5 for $PhOH^+PO_3^{2-}$ (II), and 6.0 for $PhOPO_3H^-$ (pK_2 for salicyl phosphate is 6.03[50]). The rate constant calculated for the hydrolysis of II at 39° is 7×10^8 min^{-1}; the value necessary to account for the observed rate of the reaction is 1.8×10^7 min^{-1}.

(49) E. M. Arnett and C. Y. Wu, *J. Am. Chem. Soc.*, **82**, 5660 (1960).

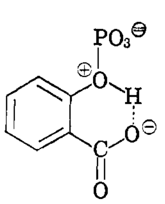
(50) Bender and Lawlor, ref 46b.

(51) D. McIntyre and F. A. Long, *J. Am. Chem. Soc.*, **76**, 3240 (1954).

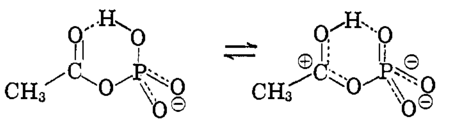
(52) C. A. Bunton and R. H. DeWolfe, *J. Org. Chem.*, **30**, 1371 (1965).

(53) A. J. Kresge and R. J. Preto, *J. Am. Chem. Soc.*, **87**, 5493 (1965).

for the high reactivities of acyl phosphate monoanions,[20] which are hydrolyzed at 39° about 20 times faster than esters with comparable leaving groups.



IV



Both cases of enhanced reactivity could also, however, be explained by exceptionally fast proton transfers, possible between basic centers linked by a hydrogen bond,[54] if the proton transfer were partially rate determining.

Product Composition in Mixed Solvents. A valuable test for the presence of a highly reactive electrophilic species is to generate it in the presence of competing nucleophiles.[55] In a mixed solvent consisting of two nucleophilic components of sufficiently similar solvent properties, such an electrophile may be expected to react nonselectively, to form products in a molar ratio equal to that of the solvent mixture. Of mixed aqueous solvents, methanol–water meets the criteria most closely. When the *t*-butyl carbonium ion is generated in methanol–water mixtures at 25°, the molar ratio of ether to alcohol formed is close to the mole ratio in the solvent.[56] Similarly, the ratio of methyl phosphate to inorganic phosphate formed on solvolyis of the monoanions of phenyl and *p*-nitrophenyl phosphates at 100° is equal to the molar ratio of the methanol–water solvent mixture.[2] This is strong evidence for a mechanism for hydrolysis involving monomeric metaphosphate as an intermediate, since methanol is generally a more effective nucleophile than water toward phosphoryl phosphorus.

The solvolysis of the dianion of *p*-nitrophenyl phosphate in methanol–water mixtures at 100° also leads to nonselective phosphorylation of the solvent (Table IV). This result completes the evidence for the unimolecular elimination mechanism for the hydrolysis of phosphate monoester dianions, and establishes that metaphosphate can act as a nonselective phosphorylating agent at relatively high pH (9.2 in water).

More extensive data (Tables IV and V) for mixed solvolysis reactions show that such agreement is unusual. Phosphorylation by the monoanion of 2,4-dinitrophenyl phosphate in 30% methanol is nonselective at 100°, but methanol is more reactive than water toward the dianion at 100°, and toward both ionic species at 39°.

Chanley and Feageson[2] found that methanol is seven times more reactive than water toward the monoanion of phosphoramidic acid. They were able to account quantitatively for both product composition and the modest increase in rate as the methanol concentration was increased, using a two-term, second-order rate expression. They concluded therefore that bimolecular attack by solvent was involved.

Toward the dianion of 2,4-dinitrophenyl phosphate, methanol is up to four times as reactive as water, but the rate increase with increasing methanol concentration here is too rapid to be due to the increase nucleophilicity of the solvent. Moreover, alcohols increase the solvolysis rate less than such solvents as dioxane and acetone, which have negligible nucleophilic reactivity.

Ethanol competes less effectively than methanol in the solvolysis of the dianion of 2,4-dinitrophenyl phosphate, being no more than twice as reactive as water. 2-Propanol appears not to react as a nucleophile at all.[57] We considered first the possibility that methanol might be more effective than ethanol because of its slightly greater acidity, which might facilitate general base catalysis by solvent. The much more strongly acidic 2,2,2-trifluoroethanol, however, gives markedly less alkyl phosphate even than ethanol (Table V), and is actually less reactive than water.

The simplest explanation of the relative reactivities of the alcohols is that the introduction of substituents at the α-carbon atom of the alcohol leads to steric hindrance of attack by 2-propanol on phosphorus. This explanation would apply only for a bimolecular mechanism, and in the absence of an acceptable alternative must throw considerable doubt on the unimolecular mechanism. The following evidence, however, suggests that the explanation is not valid.

The bimolecular attack of amine nucleophiles on the dianion of *p*-nitrophenyl phosphate is unlikely to have very different steric requirements than the similar attack of alcohols. If 2-propanol is so much less reactive than ethanol because of steric hindrance, there should be a marked difference in reactivity also between isopropylamine and straight-chain primary amines. We therefore compared the reactions of *p*-nitrophenyl phosphate with normal and isopropylamine. (Conditions were as previously described, except that, because of solubility problems, we used 50% aqueous dimethyl sulfoxide as solvent.) The kinetics are complicated slightly by a third-order term,[58] but the second-order rate constants for nucleophilic attack on the phosphorus atom of *p*-nitrophenyl phosphate dianion at 39° are 5.1×10^{-5} M^{-1} min^{-1} for *n*-propylamine, and 3.1×10^{-5} M^{-1} min^{-1} for isopropylamine. Clearly there is no serious steric hindrance to attack on phosphorus associated with the introduction of the α substituent in this case, and it seems unlikely that steric hindrance can be the explanation of the relative reactivities of the alcohols.

This result shows that the total lack of phosphorylation of 2-propanol cannot readily be explained in terms of a bimolecular mechanism. In fact the same result is observed in 2-propanol even in cases where nonselective phosphorylation is observed in methanol–water and ethanol–water[2] mixtures, for example, the solvolysis of the monoanion of *p*-nitrophenyl phosphate at 100°. It seems certain therefore that it is possible to generate metaphosphate in the presence even of an excess of 2-propanol, without producing detectable amounts of

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(54) M. Eigen, *Angew. Chem. Intern. Ed. Engl.*, 3, 1 (1964).

(55) Reference 32, p 350.

(56) L. C. Bateman, E. D. Hughes, and C. K. Ingold, *J. Chem. Soc.*, 881 (1938).

(57) This is observed also with phosphoramidate monoanion.[2] In a solvent containing 94% of 2-propanol (ionic strength 0.01, Tris buffer, pH 9.2 in water) solvolysis of the dianion of 2,4-dinitrophenyl phosphate gave 99.6% of the calculated amount of inorganic phosphate.

(58) We have not examined this reaction in detail as yet, but it is most interesting that the third-order term appears to be associated with attack on *phosphorus* rather than on the aromatic ring.[59] The third-order constant is small for isopropylamine, but for *n*-propylamine the rate equation for attack on phosphorus is $v/[p\text{-NPP}^{2-}] = k_{\text{hyd}} + 5.1 \times 10^{-5}[n\text{-PrNH}_2] + 4.5 \times 10^{-5}[n\text{-PrNH}_2]^2$.

(59) A. J. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, 87, 3217 (1965).

isopropyl phosphate. Since phosphorylation by metaphosphate can be nonselective under the right conditions, its failure to phosphorylate 2-propanol is not likely to be due to an abnormal proportion of unproductive collisions in solution, but rather to an abnormally low chance of collision.

These considerations lead us to propose that the differences in reactivity observed between different alcohols may be due to nonstatistical distribution of solvent molecules in the solvation shell about the phosphoryl group. This *selective solvation* would be most pronounced for 2-propanol, the least effective ionizing solvent, which in consequence is totally excluded from the solvation shell of the phosphoryl group of both monoanions and dianions of phosphate monoesters. The differences will be less pronounced for the lower alcohols, and would be expected to be less for the monoanion than the dianion. Like other forms of selectivity, it will be expected to be reduced also at higher temperatures.

This picture accounts qualitatively for the results observed in mixed alcoholic solvents, if the reactivity of the metaphosphate intermediate is such that it does not survive long enough to penetrate its solvation shell. Clearly product distribution can no longer be regarded as a simple measure of metaphosphate formation.[60] We now regard nonselective phosphorylation in mixed alcohol–water solvents as sufficient, *but not necessary*, evidence for a metaphosphate intermediate.

Implications for Phosphate Transfer *in Vivo*. The transfer of the free phosphoryl group is a common and important process in living systems. We believe that our results indicate very clearly that the most effective way a simple phosphate derivative, XPO_3^{2-}, can be activated to transfer its phosphoryl group to another nucleophilic center

$$XPO_3^{2-} + Y \longrightarrow YPO_3^{2-} + X$$

is by modification of X, to convert it to a more effective leaving group. This would consist of either electrophilic or oxidative attack on X.[61] The process can be considerably further facilitated if solvation of the PO_3^{2-} group by hydrogen-bonding solvent molecules is reduced.

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[60] Dr. J. D. Chanley (personal communication) informs us that he has reached a similar conclusion in a study of the solvolysis of acetyl phosphate.

[61] V. M. Clark, D. W. Hutchinson, A. J. Kirby, and S. G. Warren, *Angew. Chem. Intern. Ed. Engl.*, **3**, 678 (1964).

Comparison of Mercaptide and Alkoxide Ions as Catalysts for Ketone Isomerization[1]

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Abstract: In methanol solution, thioethoxide ion is from one-fifth to one-fifteenth as effective as methoxide ion in catalyzing the isomerization of (−)-menthone to (+)-isomenthone and of Δ^5- to Δ^4-cholesten-3-one. An improved method for dissecting catalysis by solutions of basic nucleophiles into contributions by nucleophile and lyate ions has been developed. The catalytic activity of thioethoxide ion is much greater than one would have anticipated from its basicity and the Brønsted β values for carboxylate ion catalysis of enolate ion (or enol) formation from acetone. In the functioning of enzymes, thiol groups require consideration as possible sites for proton donation or acceptance.

It is well known that many basic reagents have the capacity to remove an α-hydrogen atom from ketones, forming reactive intermediates which are enolate ions or enols. The rate of formation of these intermediates can be estimated from kinetic information on halogenation, hydrogen isotope exchange, or isomerization reactions. Many kinetic studies involving hydroxide ions, alkoxide ions, carboxylate ions, amines, and other types of bases have been made, but the reactivity of mercaptide ions in this type of reaction seems not to have been measured.

This paper reports the kinetics of the (−)-menthone (I)[3,4] $\rightleftarrows$ (+)-isomenthone (II) equilibration and of the isomerization of cholest-5-en-3-one (III) to cholest-4-en-3-one (IV), as catalyzed by methoxide and thioethoxide ions in methanol. Our results enable a comparison of the catalytic effectiveness of representative alkoxide and mercaptide ions.

The kinetics of acid-catalyzed menthone isomerization have been studied by many workers.[5b,6] Rates of isomerization as catalyzed by ethanolic ethoxide

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[3] The official *Chemical Abstracts* name of menthone is *p*-menthan-3-one.

[4] For a discussion of conformations, see C. Djerassi, P. A. Hart, and C. Beard, *J. Am. Chem. Soc.*, **86**, 85 (1964).

[5] (a) C. Tubandt, *Ann.*, **339**, 41 (1905); (b) *ibid.*, **354**, 259 (1907); (c) C. Tubandt, K. Mohs, W. Tubandt, and H. Weinhausen, *ibid.*, **377**, 284 (1910).

[6] P. D. Bartlett and J. R. Vincent, *J. Am. Chem. Soc.*, **55**, 4992 (1933); R. P. Bell and E. F. Caldin, *J. Chem. Soc.*, 382 (1938); A. Weissberger, *J. Am. Chem. Soc.*, **65**, 102, 245, 402 (1943); **67**, 1622 (1945); F. Covitz and F. H. Westheimer, *ibid.*, **85**, 1773 (1963).