carbanion, and it would appear that the N–Mg bond in the Grignard reagent in ether must have substantial covalent character.[33] THF, being a stronger base than ether, would coordinate more strongly with magnesium and increase the ionic character of the N–Mg bond.[34,35] Similarly, coordination of several molecules of water

about each magnesium would likewise increase the ionic character of this N–Mg bond until a point is reached where its behavior is identical with that of the alkali metal derivatives of indoles and N-protonation occurs. Our results seem to exclude a highly ionic Grignard reagent in ether, since its behavior would then follow that of alkali metal derivatives of indole. Therefore, we conclude that the N–Mg bond of the Grignard reagent has considerable covalent nature in ether.[36]

(33) An N–Mg bond has 55% ionic character and an N–Na bond 67% ionic character calculated on the basis of electronegativity differences: L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960, Chapter 3.
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(35) L. I. Zakharkin, O. Y. Okhlobystin, and K. A. Bilevitch, *Tetrahedron*, **21**, 881 (1965), report that the more solvating (complexing) the medium, the higher the nucleophilic activity of organomagnesium compounds.

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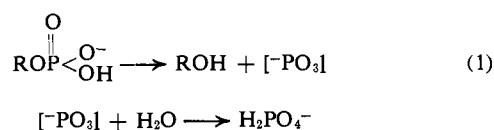
The Hydrolysis of S-Aryl Phosphorothioates

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Abstract: The rates of hydrolysis of the *p*-H, *p*-Cl, and *p*-NO$_2$ S-phenyl phosphorothioates have been measured in water at 35° as a function of pH. The typical maximum in rate for the monoanionic species was found for the *p*-H and *p*-Cl derivatives, although the dianion rate of hydrolysis was of much greater magnitude for *p*-Cl than for *p*-H. No rate maximum for the monoanionic species was found for S-(4-nitrophenyl) phosphorothioate, the dianion rate being 16.2 times more rapid than the monoanion rate. Deuterium oxide has little effect on the rates of the dianion reactions, but for monoanion hydrolysis k_{H_2O}/k_{D_2O} is 1.78 for *p*-NO$_2$ and 1.4 for *p*-H. These values may indicate that the mechanism for monoanion hydrolysis involves a proton-transfer step that is partially rate determining. The monoanion rates are nearly insensitive to electronic effects, decreasing slightly as the leaving group becomes better. The values of ΔS^* are −1.9 eu for hydrolysis of S-(4-nitrophenyl) phosphorothioate monoanion and +7.9 for hydrolysis of the dianion, consistent with decomposition to metaphosphate ion for both species. Acid catalysis is not observed with any of the compounds, the rates being nearly invariant at concentrations of HCl from 1.0 to 6.12 M. Electron withdrawal in the leaving group facilitates to only a small extent hydrolysis of the neutral species ($k_{NO_2}/k_H = 1.7$). The neutral species of the *p*-NO$_2$ derivative hydrolyzes much more slowly in D$_2$O than in H$_2$O ($k_{H_2O}/k_{D_2O} = 1.83$) and has a ΔS^* of −13.2 eu. Thus the mechanism of neutral species hydrolysis probably involves nucleophilic attack by water in concert with a proton-transfer step. The amount of methyl phosphate formed from solvolysis reactions in aqueous methanol solutions is two to three times greater for both monoanion and dianion reactions of the *p*-NO$_2$ derivative than would be expected from a nonselective phosphorylation of the solvent components, but the *p*-Cl and *p*-H compounds give ratios of inorganic phosphate to methyl phosphate similar to the solvent ratios of H$_2$O/CH$_3$OH.

The hydrolysis reactions of various types of phosphate esters have been actively studied.[2] The pH–rate profiles for hydrolysis of monoalkyl phosphates generally show a maximum around pH 4.[2] To account mechanistically for this relatively rapid monoester monoanion reaction has been one of the intriguing problems of phosphate ester chemistry. It has been postulated that this reaction involves the formation of metaphosphate as a reactive intermediate[2,3] (eq 1). The hydrolysis of the dianionic species

$$\text{RO}\overset{\displaystyle O}{\underset{\displaystyle}{\overset{\|}{P}}}\!\!<\!\!\begin{array}{c}O^-\\OH\end{array} \longrightarrow \text{ROH} + [^-\text{PO}_3] \qquad (1)$$

$$[^-\text{PO}_3] + \text{H}_2\text{O} \longrightarrow \text{H}_2\text{PO}_4{}^-$$

of acetyl phosphate[4] and 2,4-dinitrophenyl phosphate[3,5] very likely also involves metaphosphate formation. Kirby and Varvoglis[3b] have recently suggested that hydrolysis of phosphate monoester monoanions proceeds with preequilibrium zwitterion formation in which the leaving group is protonated, but when the pK_a of the leaving group is low the protonation step may become partially rate determining.

The same general pH dependence has been found for hydrolysis of thiolphosphates as for the oxygen esters of orthophosphoric acid,[6–10] with P–S cleavage oc-

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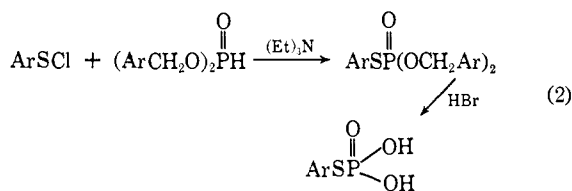(8) S. Akerfeldt, *Acta Chem. Scand.*, **15**, 575 (1961).
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curring exclusively. Since the bond energy of the P–S bond (45–50 kcal/mole) is considerably less than P–O bond energy (95–100 kcal/mole),[2] P–S bond cleavage is much more facile than P–O cleavage, as is reflected in a lower energy of activation for thiolphosphate ester hydrolysis.[7] In view of the increased difficulty of protonating sulfur rather than oxygen, if protonation is indeed occurring then it might be partially rate determining. A convincing demonstration of this would be of great significance in the elucidation of the mechanism of hydrolysis of phosphate esters, since if internal protonation is important in the hydrolysis of thiolphosphate esters then it is reasonable that it would be important in the hydrolysis of the more difficultly cleaved oxygen ester monoanions, for which the evidence concerning the occurrence of internal protonation has been mainly suggestive. The mechanism of hydrolysis of thiolphosphate esters is of interest not only because of the insight which might be afforded in regard to the mechanism of hydrolysis of the corresponding oxygen esters, both chemical and enzymic, but also because of the possible biochemical significance of compounds of this type. Several phosphate-transferring enzymes are dependent for activity on the presence of sulfhydryl groups,[11] and S-phosphoryl CoA is a possible intermediate in acetate activation and in the formation of adenosine triphosphate from adenosine diphosphate,[12] although evidence is lacking. In this investigation the rates of hydrolysis of several S-monoaryl thiolphosphate esters have been measured so that by variation of the *para* substituent the pK_a of the leaving group may be systematically varied. S-Monoaryl phosphorothioates have not been prepared previously.

Experimental Section

Materials. Conventional methods for the preparation of phosphate monoesters and S-alkyl phosphorothioates were found generally not to be applicable to the synthesis of S-aryl phosphorothioates. The reaction of phosphorus oxychloride with thiophenols by the method of Tener[13] in ether or benzene yielded only di- and triesters even with a tenfold excess of phosphorus oxychloride. Attempts to prepare S-phenyl phosphorothioate by treating dibenzyl phosphorochloridate[14] with thiophenol yielded little product upon debenzylation. Morrison[15] has prepared triesters of thiophosphoric acid by treating alkyl- and arylsulfenyl chlorides with trialkyl phosphites. We have prepared the S-monoaryl phosphorothioates by treating arylsulfenyl chlorides with dibenzyl phosphite in the presence of triethylamine and then debenzylating as shown in eq 2. The synthetic method, as described below, should be general

$$ArSCl + (ArCH_2O)_2PH \xrightarrow{(Et)_3N} ArSP(OCH_2Ar)_2$$

$$\downarrow HBr \qquad (2)$$

$$ArSP \overset{\displaystyle O}{\underset{\displaystyle \diagdown OH}{\diagup OH}}$$

for the synthesis of most thiolphosphate monoesters and offers the advantages that the reagents are fairly stable and the yields are high (although optimum conditions were not determined).

Sulfenyl Chlorides. Sulfenyl chlorides were prepared by the method of Almasi and Hantz[16] by treating the appropriate thiophenol in 50 ml of dry carbon tetrachloride dropwise with 100 ml of dry carbon tetrachloride containing an equivalent amount of chlorine at room temperature. The mixture was stirred for an additional hour. The solvent was removed *in vacuo* and the residue distilled (1 mm) except in the case of the *p*-nitrobenzenesulfenyl chloride which was crystallized from anhydrous ether. Benzenesulfenyl chloride boiled at 44.5–45.1° (1.1 mm) [lit.[17] bp 44–47.5° (1 mm)]. 4-Chlorobenzenesulfenyl chloride boiled at 65–67° (1.1 mm) [lit.[18] bp 94° (6 mm)]. 4-Nitrobenzenesulfenyl chloride melted at 52° (lit.[18] mp 52°).

Triesters. The O,O-dibenzyl thiolphosphate triesters were prepared by adding dropwise, with stirring, a mixture of 1 equiv of dibenzyl phosphite[14] plus 1 equiv of redistilled triethylamine in 100 ml of dry ether at room temperature to a solution of 1 equiv of the appropriate benzenesulfenyl chloride in 300 ml of dry ether. The precipitation of triethylammonium hydrochloride commenced immediately. Stirring was continued for an additional 4 hr at room temperature. The solution was then filtered and the triester concentrated to an oil *in vacuo*. In the cases of the benzenethiol and 4-nitrobenzenethiol triesters, crystallization took place when the oils were dissolved in a small amount of ether and placed in the freezer. The triesters were recrystallized from ether giving melting points of 74–76 and 47–49° (uncorrected), respectively. Examination of the infrared spectra of crystalline O,O-dibenzyl S-phenyl phosphorothioate showed that the band due to P–H in the starting material at 4.1 μ was absent.

S-Aryl Phosphorothioates. Debenzylation of the triesters and crystallization as the dicyclohexylammonium salts was accomplished by the procedure described by Kirby and Varvoglis.[3b] Analyses were performed by Elek Microanalytical Laboratories, Los Angeles, Calif., and Micro-Tech Labs, Skokie, Ill.: dicyclohexylammonium S-phenyl phosphorothioate, *Anal.* Calcd for $C_{18}H_{33}N_2O_3PS \cdot 0.5H_2O$: C, 54.38; H, 8.62; N, 7.04; P, 7.78. Found: C, 54.26; H, 8.88; N, 7.19; P, 7.44; dicyclohexylammonium S-(4-chlorophenyl) phosphorothioate, *Anal.* Calcd for $C_{18}H_{32}ClN_2O_3PS \cdot 0.5H_2O$: C, 50.10; H, 7.70; N, 6.48; P, 7.17. Found: C, 50.60; H, 7.75; N, 6.40; P, 6.47; dicyclohexylammonium S-(4-nitrophenyl) phosphorothioate (gave a quantitative *p*-nitrothiophenol release at pH 8.90 and a quantitative release of inorganic phosphate), *Anal.* Calcd for $C_{18}H_{32}N_3O_5PS \cdot 2.5H_2O$: C, 45.20; H, 7.74; N, 8.78; P, 6.48. Found: C, 45.15; H, 7.20; N, 8.65; P, 6.79.

Benzenethiol and *p*-chlorobenzenethiol were obtained from Matheson Coleman and Bell. *p*-Nitrobenzenethiol was obtained from Aldrich Chemical Co. as a moist powder and was recrystalized twice from 95% ethanol and dried at room temperature *in vacuo* (mp 75°).

Buffers were prepared from commercial, reagent grade materials using deionized, distilled water. All buffers contained 10^{-5} M EDTA to eliminate heavy metal catalyzed oxidation of the thiol compounds and were generally 0.05 M in buffer components.

Kinetic Measurements. The rates were measured spectrophotometrically by following the appearance of thiophenol or thiophenoxide ion at the appropriate wavelength (245 and 270 mμ for *p*-Cl and *p*-H; 330 and 410 mμ for *p*-NO$_2$) with a Beckman DU spectrophotometer equipped with a Gilford Model 2000 recording attachment.

Since thiophenol is relatively insoluble in aqueous solutions, all rate measurements were made at approximately 10^{-4} M substrate concentration in 1% acetonitrile. Stable stock solutions of the thiolphosphate monoesters were prepared by dissolving the dicyclohexylammonium salts in acetonitrile with the aid of Dow 50-X8 (200–400 mesh, H$^+$ form) ion-exchange resin. The stock solutions were stable for several days when stored at 0°.

The reactions were started by adding a known amount of the stock solution to the thermostated buffer solution which had been previously deoxygenated by bubbling in nitrogen for several minutes. Several rates were run by adding small amounts of the salt directly to the cuvettes and yielded almost identical rate constants. The contents of the cuvette were then stirred with a fine capillary tube through which nitrogen was passed, and the cuvette was stoppered tightly with a Teflon stopper. The rates were generally followed to at least 75% of the reaction, and infinity points were

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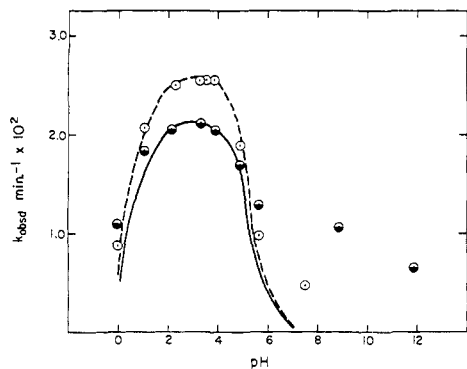(18) F. Montanari, *Gazz. Chim. Ital.*, **86**, 406 (1956).

Figure 1. Plot of k_{obsd} vs. pH for the hydrolysis of S-phenyl phosphorothioate, ○, and S-(4-chlorophenyl) phosphorothioate, ●, at 35°, μ = 1.0 M. The lines are theoretical assuming only a monoanion reaction. The constants for S-phenyl phosphorothioate were taken to be: $k_{monoanion}$, 2.55 × 10^{-2} min^{-1}; K_2, 4.0 × 10^{-6}; and K_1, 3.1 × 10^{-1}. The constants for S-(4-chlorophenyl) phosphorothioate were taken to be: $k_{monoanion}$, 2.12 × 10^{-2} min^{-1}; K_2, 4.0 × 10^{-6}; and K_1, 3.1 × 10^{-1}.

taken at ten half-lives. Pseudo-first-order rate constants were calculated by an Olivetti–Underwood Programma 101 using a computer program designed to calculate a least-squares evaluation of the slope and the intercept of a plot of ln $((OD_\infty - OD_0)/(OD_\infty - OD_t))$ vs. time. The correlation coefficient varied from 0.9990 to 0.9999. Buffer catalysis was not observed with any of the buffer solutions employed. The ionic strength was maintained at 1.0 M by the addition of KCl. Constant temperature was maintained at ±0.1° during the kinetic runs by circulating water from a Precision Scientific Co. Temptrol Model 154 circulating bath around the cell compartment. All temperatures were measured with the temperature probe supplied with the Gilford instrument and were monitored continuously. The pH of each solution was measured on a Model 22 Radiometer pH meter. In work utilizing 99.8% D$_2$O as the solvent, the glass electrode correction formula of Fife and Bruice[19] was employed in the determination of pD.

Determination of Ionization Constants. The ionization constants of the free thiols were determined spectrophotometrically by the method of Flexser.[20] The pK_a's of the thiophenols at 35° (μ = 1.0) are: unsubstituted, 6.43; 4-chloro, 5.70; 4-nitro, 4.42.

The dissociation constants of the phosphorothioates were measured with a Radiometer TTT1 Autotitrator and Radiometer Titrigraph utilizing a Metrohm EA 115 X electrode. Constant temperature was maintained by circulating water from a Haake Model F constant-temperature bath through the water jacket around the microtitration cell. pK_2 was estimated from the inflection point of the titration curve. The cyclohexylammonium salts were titrated by dissolving approximately 25 mg in 2 ml of 1 M KCl in the microtitration cell, quickly acidifying with HCl, and titrating with 0.075 M NaOH (μ = 1.0). Hydrolysis was too rapid at 35° for accurate titrations and even at 25° reproducibility was poor. Therefore, the free acid of S-phenyl phosphorothioate was titrated at 15 and 25° by dissolving the compound in acetonitrile in the presence of an excess of dry Dow 50-X8 (H$^+$) at a concentration such that a 2.5% (v/v) solution of acetonitrile in 1 M KCl could be titrated. S-(4-Nitrophenyl) phosphorothioate hydrolyzed too rapidly to titrate at 15°. The pK_2 of S-phenyl phosphorothioate was found to be 5.21 ± 0.05 for the dicyclohexylammonium salt and 5.26 ± 0.05 for the free acid at 15°. The pK_2 value for the free acid increased to 5.42 ± 0.05 at 25°. pK_2 for the dicyclohexylammonium salt of S-(4-chlorophenyl) phosphorothioate was found to be 5.26 ± 0.05 at 15°. The kinetically determined apparent pK_2 values obtained from the midpoints of the pH–rate profiles at 35° have the values: 4-H, 5.4; 4-chloro, 5.2; and 4-nitro, 4.4.

Product Composition in Mixed Solvents. Hydrolysis of the thiolphosphate esters was allowed to proceed to completion at 35° in aqueous and alcohol–water buffer solutions. The inorganic phosphate was then measured by King's method,[21] and the difference
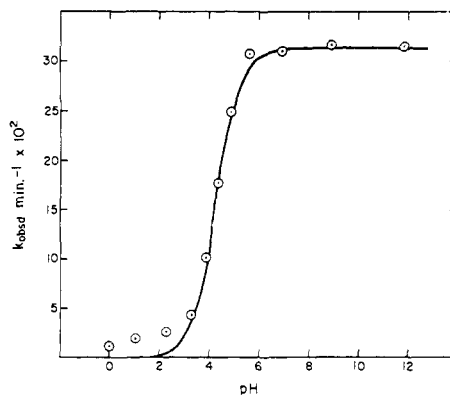

Figure 2. Plot of k_{obsd} vs. pH for the hydrolysis of S-(4-nitrophenyl) phosphorothioate at 35°, μ = 1.0 M. The line is theoretical assuming only a dianion reaction taking the constants to be: $k_{dianion}$, 3.13 × 10^{-1} min^{-1}; K_2, 4 × 10^{-5}; and K_1, 7 × 10^{-1} (estimated from the pH–rate profile).

between the amount of inorganic phosphate measured and the theoretical amount was taken to be the amount of alkyl phosphate. Buffers were prepared with NaCl to avoid precipitation of potassium perchlorate. The dianion of S-(4-nitrophenyl) phosphorothioate was studied at pH 8.83 in water. The apparent pH decreased to 8.70 in 40% methanol. The monoanions of p-NO$_2$, p-Cl, and p-H were hydrolyzed at pH 2.63 in water. The apparent pH increased to 3.14 in 40% (v/v) methanol. These variations should have little effect on product composition as they are on "plateaus" of pH–rate profiles. Methyl phosphate is not hydrolyzed under these conditions of assay. There was no detectable phosphorylation of isopropyl alcohol.

Results

In Table I are given rate constants for the hydrolysis of S-(4-substituted phenyl) phosphorothioates at 35° in H$_2$O, μ = 1.0 M, at various pH values. Plots of k_{obsd} vs. pH are shown in Figures 1 and 2. It can be seen that a maximum in rate occurs at approximately pH 3–4 for the p-H and p-Cl derivatives, but in the case of the p-NO$_2$ derivative the rate at higher pH values where the dianion is the predominant species is 16.2 times faster than at low pH values where the compound exists as the monoanion. Also included in Table I are the rate constants determined in D$_2$O at several pD values for hydrolysis of S-(4-nitrophenyl) and S-phenyl phosphorothioate. The rates are much slower in D$_2$O except at high pH values (dianion rate) where there is only a small effect.

The rate constants for hydrolysis of the thiolphosphate esters in moderately concentrated HCl solutions (1.0–6.12 M) are presented in Table II. The rates of hydrolysis decrease very slightly as acid concentration increases, very likely due to salt effects. Thus no acid catalysis can be observed at acid concentrations up to 6.12 M, the reaction being hydrolysis of the neutral species. Electron withdrawal facilitates to only a small extent the hydrolysis of the neutral species (k_{NO_2}/k_H = 1.7; ρ = +0.29).

In Table III are given rate constants for the hydrolysis of S-(4-nitrophenyl) phosphorothioate as a function of temperature at several pH values. Plots of log k_{obsd} vs. $1/T$ are shown in Figure 3. Activation parameters are reported in Table IV. Values of ΔS^* are quite similar to those found for monoaryl phosphate

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Table I. Rate Constants (k_{obsd} min^{-1} × 10²) for Hydrolysis of S-(4-Substituted Phenyl) Phosphorothioates at Various pH or pD Values, 35°, and μ = 1.0 M

pH	pD	Buffer[a]	4-Substituent H	Cl	NO₂
0		1 M HCl	0.88	1.10	1.11
1.03		0.1 M HCl	2.12	1.84	1.95
2.14		0.01 M HCl			2.06
2.30		Formate	2.39		2.39
3.30		Acetate	2.55	2.18	4.40
3.55		Formate	2.54		
3.87		Acetate	2.56	2.04	10.1
4.38		Acetate			17.7
4.87		Acetate	1.90	1.70	24.9
5.04		Citrate		1.73	
5.65		Acetate	0.99	1.29	30.8
6.95		Phosphate			31.0
7.50		Phosphate	0.48		30.4
8.90		Tris		1.06	31.7
11.85		0.1 M NaOH		0.65	31.5
	1.04	DCl			1.09
	3.55	Formate	1.83		
	4.44	Acetate			9.03
	9.60	Tris			26.1

[a] All buffers were 0.05 M.

Table II. Rate Constants (k_{obsd} min^{-1} × 10²) for Hydrolysis of S-(4-Substituted Phenyl) Phosphorothioates in Moderately Concentrated HCl at 35°
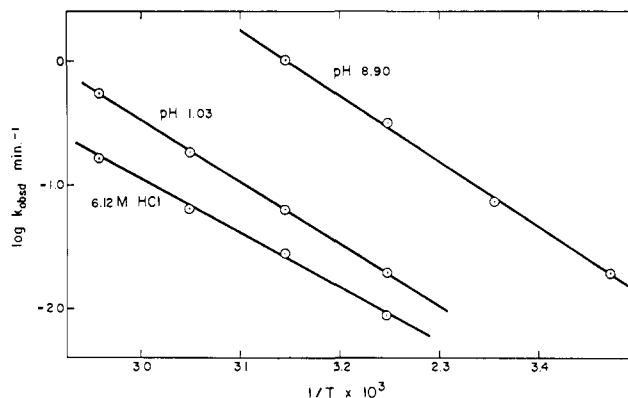
HCl concn, M	4-Substituent H	Cl	NO₂
1.00	0.88	1.10	1.11
1.80			1.00
3.06	0.55	0.67[a]	0.945
4.20			0.915
6.12	0.52	0.60[b]	0.879
6.0[c]			0.480

[a] 2.95 M HCl. [b] 5.90 M HCl. [c] DCl in D₂O.

Table III. Rate Constants (k_{obsd} min^{-1} × 10²) for Hydrolysis of S-(4-Nitrophenyl) Phosphorothioate at Various Temperatures and μ = 1.0 M

Temp, °C	6.12 M HCl	pH 1.03	pH 6.95	pH 8.90
15			1.77	1.91
25			7.49	7.43
35	0.88	1.95	31.0	31.7
45	2.80	6.29	98.3	101.8
55	6.50	18.6		
65	16.6	55.1		

Table IV. Activation Parameters for Hydrolysis of S-(4-Nitrophenyl) Phosphorothioate

Species	pH	ΔH*, kcal/mole	ΔS*,[a] eu
Neutral	6.12 M HCl	19.3 ± 0.4[b]	−13.2 ± 1.2
Monoanion	1.03	22.3 ± 0.1	−1.9 ± 0.4
Dianion	6.95	23.8 ± 0.3	+8.4 ± 1.0
Dianion	8.90	23.6 ± 0.3	+7.9 ± 1.1

[a] Calculated at 35° with the rate constants having the units sec^{-1}. [b] Reported errors in ΔH* were calculated from the standard error of a plot of ln k_{obsd} vs. 1/T.

Figure 3. Plots of log k_{obsd} vs. 1/T for the hydrolysis of S-(4-nitrophenyl) phosphorothioate.

In Table V are rate constants for hydrolysis of S-(4-nitrophenyl) phosphorothioate in various aqueous–organic solvent mixtures. Organic solvents have an accelerating effect on the rate of dianion hydrolysis and a retarding effect at low apparent pH values where the monoanion is in high concentration.

Table V. Rate Constants (k_{obsd} min^{-1} × 10²) for Hydrolysis of S-(4-Nitrophenyl) Phosphorothioate in Various Aqueous, Organic Solvent Mixtures, μ = 1.0 M

Solvent	Apparent pH[a]	Monoanion	Apparent pH[b]	Dianion
H₂O	1.03	1.95	8.90	31.7
10% methanol			8.90	36.9
20% methanol	1.06	1.22	8.90	43.0
40% methanol	1.16	0.819	8.89	54.2
60% methanol			8.95	64.6
20% dioxane	1.07	1.11		
40% dioxane	1.10	0.546		
20% acetonitrile		1.18		

[a] 0.1 M HCl. [b] 0.05 M Tris buffer.

At the conclusion of S-(4-nitrophenyl) phosphorothioate hydrolysis reactions in aqueous methanol solvents, the mixture was analyzed for inorganic phosphate. Table VI shows the results of these experiments. It

Table VI. The Effect of Methanol on the Product Composition (Moles of Inorganic Phosphate/Mole of Methyl Phosphate) in the Hydrolysis of S-(4-Substituted Phenyl) Phosphorothioates at 35°

4-Substituent	(10[a]) 20.2[b]	(20[a]) 9.04[b]	(40[a]) 3.38[b]
NO₂ dianion[c]	6.0	2.54	1.14
NO₂ monoanion[d]	9.3	3.95	1.59
Cl monoanion[d]	12.3	7.95	
H monoanion[d]	15.7	6.05	2.67

[a] Per cent CH₃OH v/v. [b] Moles of H₂O/mole of CH₃OH in the solvent. [c] 0.05 M Tris buffer, μ = 0.25 M with NaCl, pH 8.83 in H₂O (1% acetonitrile); pH at 10, 20, and 40% CH₃OH is 8.78, 8.75, and 8.70. [d] 0.025 M phthalate buffer, μ = 0.25 M with NaCl, pH 2.63 in H₂O (1% acetonitrile); pH at 10, 20, and 40% CH₃OH is 2.73, 2.86, and 3.14.

esters,[3b,5] being +7.9 eu at pH 8.90 where the dianion is the predominant species and −1.9 eu at pH 1.03 where the monoanion is predominant.

can be seen that in each case the mole ratio of inorganic phosphate to methyl phosphate is less than the mole ratio of H₂O to CH₃OH in the solvent. Thus alcohol is being selectively phosphorylated, but the selectivity

factor is only 2–3. With the p-H and p-Cl derivatives the mole ratio of inorganic phosphate to methyl phosphate is more nearly in accord with the solvent mole ratio of H_2O/CH_3OH.

Discussion

From the pH–rate profiles shown in Figures 1 and 2 it can be seen that variation of the *para* substituent in the S-monoaryl phosphorothioates from H to NO_2 produces a striking change in the relative ease of hydrolysis of the mono and dianionic species. In the case of the unsubstituted derivative the typical bell-shaped profile is obtained with a maximum at pH 3–4, the dianion having an extremely slow rate of hydrolysis. The p-Cl derivative also shows a rate maximum at pH 3–4, but the dianion has become considerably more reactive as evidenced by the tail on the curve at pH values above 5. The dianionic species of S-(4-nitrophenyl) phosphorothioate, however, hydrolyzes 16.2 times faster than its monoanion. With only few exceptions,[2–5] the dianions of monoalkyl and monoaryl phosphates are quite unreactive. p-Nitrophenyl phosphate dianion hydrolyzes quite slowly,[22] but introduction of a second nitro group into the aromatic ring in the *ortho* position gives rise to a dianionic species hydrolyzing faster than the monoanion.[3,5] Bunton, Fendler, and Fendler[5] have discussed reasons for believing that the reaction from pH 6 to 12 involves the dianionic species and not a hydroxide ion catalyzed monoanion reaction. It is reasonable that this is also the case for S-(4-nitrophenyl) phosphorothioate. A single nitro group in the *para* position of an S-monoaryl thiolphosphate, therefore, has the same effect as two nitro groups in the oxygen ester in regard to the induction of lability into the dianionic species. This is undoubtedly a reflection of the pK_a difference between p-nitrothiophenol and p-nitrophenol, the pK_a of the thiophenol being considerably lower, 4.4, compared to 7.14 for p-nitrophenol.[23] Thus p-nitrothiophenoxide ion is a relatively good leaving group and can depart from phosphorus without a proton being donated to it, as is also the case for carboxylate ion[4] and 2,4-dinitrophenoxide ion.[3,5] More basic alkoxy and aryloxy groups cannot depart readily from phosphate ester dianions. The reactivity of the p-nitrophenyl thiolphosphate dianion, therefore, provides striking confirmation for the importance of pK_a of the leaving group in dianion reactions.

The most probable mechanism for the rapid dianion reactions of acetyl phosphate[4] and 2,4-dinitrophenyl phosphate,[3,5] as shown by several pieces of evidence, involves a unimolecular decomposition to metaphosphate ion. It is very likely that the same type of mechanism is operative in the hydrolysis of S-(4-nitrophenyl) phosphorothioate dianion. Thus, (a) the value of ΔS^* is $+7.9$ eu, typical of a unimolecular reaction where ground state and transition state have the same charge;[24] (b) there is essentially no D_2O solvent isotope effect ($k_{H_2O}/k_{D_2O} = 1.2$); and (c) organic solvents produce a slight accelerating effect in accord with the Hughes

and Ingold theory of solvent effects,[25] which predicts small rate increases with decreases in the solvent dielectric constant for a reaction involving charge dispersal in the transition state. Similar values of ΔS^* and k_{H_2O}/k_{D_2O} and similar solvent effects have been found for 2,4-dinitrophenyl phosphate dianion.[3,5] The rate of hydrolysis of acetyl phosphate dianion, however, is not affected by addition of acetonitrile,[4] and the rate of hydrolysis of 2,6-dinitrophenyl phosphate dianion decreases at methanol concentrations greater than 15 mole %.[5] A difference in product composition is encountered when the effects of methanol are compared for 2,4-dinitrophenyl phosphate and S-(4-nitrophenyl) phosphorothioate dianions. The amounts of methyl phosphate formed during the hydrolysis of the thiolphosphate in aqueous methanol solutions are in each case more than would be expected from the solvent composition on the basis of a nonselective reaction of alcohol and water with metaphosphate ion: methanol is being phosphorylated about two to three times as readily. The amounts of methyl phosphate formed from the p-Cl and unsubstituted derivatives are considerably less than with p-NO₂. The ratio of inorganic phosphate to methyl phosphate is more nearly in accord with the ratio of water to methanol in the solvent. The ease of phosphorylation of alcohol is thus dependent upon the thiolphosphate. This might indicate that in the hydrolysis of the S-(4-nitrophenyl) phosphorothioate dianion, metaphosphate ion is not completely free when captured by alcohol or water. The phosphorylation of alcohol by various dinitrophenyl phosphates is also dependent upon the phosphate ester,[5] but with these compounds methanol is phosphorylated with much greater ease than water: for 2,4-dinitrophenyl phosphate the reactivities toward metaphosphate are CH_3OH, 6, H_2O, 1.[5] A different ease of phosphorylation of methanol than water is also found in the solvolysis of pyrophosphate anions,[26] phosphoroamidate,[27] and monoaryl phosphoroamidates.[28] In contrast, in the solvolysis of the monoanions of several monophosphate esters, including p-nitrophenyl phosphate, water and alcohol have similar reactivities.[29] The product composition is therefore greatly dependent upon the compound being solvolyzed, even though with some of these reactions in which methanol is phosphorylated with widely varying ease there is considerable evidence favoring a metaphosphate intermediate. It is thus evident that product composition studies cannot be employed with any confidence to distinguish a unimolecular reaction from a bimolecular reaction. It would seem probable that product composition is governed by factors such as transition-state solvation.

The D_2O solvent isotope effect measured for hydrolysis of the p-NO₂ monoanionic species at pH 1 ($k_{H_2O}/k_{D_2O} = 1.78$) may indicate that a proton is being transferred in the critical transition state. For purposes of comparison the D_2O solvent isotope effects of several phosphate esters are listed in Table VII. The value

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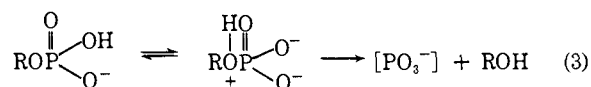(28) S. J. Benkovic, personal communication.
(29) P. A. T. Swoboda, Special Publication No. 8, The Chemical Society, London, 1957, p 41; J. D. Chanley and E. Feageson, *J. Am. Chem. Soc.*, 85, 1181 (1963).

Table VII. D$_2$O Solvent Isotope Effects in Hydrolysis Reactions of Phosphate Monoesters

Compound	k_{H_2O}/k_{D_2O}	Ref
Methyl phosphate monoanion	0.87	a
Salicyl phosphate dianion	0.96	b
Acetyl phosphate monoanion	1.1	4
2,4-Dinitrophenyl phosphate monoanion	1.45	3b
S-Phenyl phosphorothioate monoanion	1.4	c
S-(4-Nitrophenyl) phosphorothioate monoanion	1.78	c
S-(4-Nitrophenyl) phosphorothioate dianion	1.20	c
S-(4-Nitrophenyl) phosphorothioate neutral species	1.83	c

[a] C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3574, 3588 (1958). [b] M. L. Bender and J. M. Lawlor, *J. Am. Chem. Soc.*, **85**, 3010 (1963). [c] This study.

k_{H_2O}/k_{D_2O} = 1.45 at 39° for hydrolysis of 2,4-dinitrophenyl phosphate was considered by Kirby and Varvoglis[3b] as support for a mechanism involving protonation of the leaving group, either internally or through a solvent molecule, that is partially rate determining. For hydrolysis of methyl phosphate monoanion at 100°, however, the D$_2$O solvent isotope effect of less than unity possibly indicates preequilibrium zwitterion formation (eq 3). The value of the D$_2$O solvent isotope

$$\underset{\text{O}^-}{\overset{\text{O}}{\text{ROP}}}\text{--OH} \rightleftharpoons \underset{\text{O}^-}{\overset{\text{HO}}{\text{ROP}}}\text{--O}^- \longrightarrow [\text{PO}_3{}^-] + \text{ROH} \quad (3)$$

effect should be at a maximum when the system O···H···X is symmetrical.[30] When the proton is either not transferred or is completely transferred in the transition state, the isotope effect will be much smaller. The isotope effect of 1.4 for the monoanion reaction of S-phenyl phosphorothioate may indicate that proton transfer in the transition state takes place even when the pK_a of the leaving group is considerably higher than that of the *p*-NO$_2$ derivative. The ratio k_{H_2O}/k_{D_2O} is, however, small (about equal to that for 2,4-dinitrophenyl phosphate monoanion), and differences in the stretching and bending frequencies of H–S and H–O bonds might influence the observed ratios.[31] Mechanisms for the hydrolysis of substituted S-phenyl phosphorothioate monoanions involving partially rate-determining protonation are shown in I and II. The
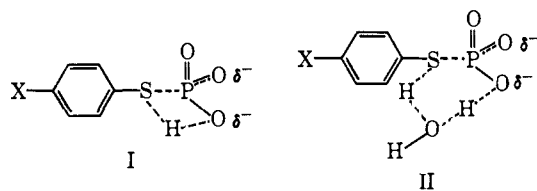
isotope effects found for the monoanions can be contrasted with the small solvent isotope effect (k_{H_2O}/k_{D_2O} = 1.2) for the dianion reaction of S-(4-nitrophenyl) phosphorothioate. Additional support for a mechanism involving a protonation step for the monoanions is provided by the near-insensitivity of rate to changes in the *para* substituent, electron withdrawal having compensating effects, facilitating cleavage and hinder-

(30) F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).
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ing protonation of the leaving group. The dianion rate is by contrast very susceptible to electronic effects.

The ΔS^* of -1.9 eu for the monoanion hydrolysis of S-(4-nitrophenyl) phosphorothioate is approximately 10 eu more negative than for the dianion reaction. This difference is possibly expressing the additional restriction of a water molecule in the monoanion transition state II. A ΔS^* difference of 10 eu is similar to that observed in the hydrolysis of other phosphate esters.[3b]

An additional similarity to the monoanion reactions of other phosphate esters is the rate retardation produced by the addition of organic solvents,[3] in contrast to the acceleration observed for the dianions. The product composition in water–methanol solutions is, however, nearly the same for solvolysis of both monoanions and dianions, which indicates that phosphorylation probably takes place by the same mechanism in the two cases.

Kirby and Varvoglis[3b] presented a plot of log k_{hyd} at 100° for a series of phosphate monoester monoanions *vs.* the pK_a of the leaving group. A good linear correlation was obtained over a wide range with the equation

$$\log k_{hyd} = 0.91 - 0.27 \, pK_a$$

The points for the nitro-substituted phenyl phosphates deviated slightly in a negative manner from the line. This was interpreted as indicating that the rate was beginning to be limited by proton transfer with these compounds so that the predicted rate for a zwitterion species could not be attained. It can be seen in Table VIII, where rate constants for several thiolphosphate
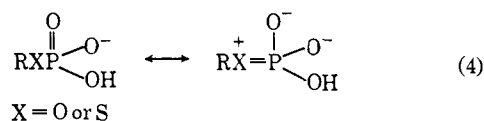
Table VIII. Rate Constants for Hydrolysis of S-(4-Substituted Phenyl) Phosphorothioates at 35°, μ = 1.0 M, and Monoaryl Phosphates at 39°, μ = 1.0 M

Thiophenol	Phenol	pK_a	Neutral, 6.12 M HCl	Rate constant for hydrolysis, min^{-1} × 10^2 Monoanion	Dianion
4-NO$_2$		4.42	0.879	1.95[a]	31.3[b]
4-Cl		5.70		2.12[c]	
4-H		6.43	0.52	2.55[c]	
S-*n*-Butyl phosphorothioate[d]				7.80	
	2,4-Di-NO$_2$[e]	4.07		0.03	1.05
	4-NO$_2$[e]	7.14		0.0064	0.000093
	4-H[e]	9.99		0.00057	

[a] The observed rate constant at pH 1.03. [b] The observed rate constant in the pH-independent region above pH 7. [c] The observed rate constant at the pH–rate maximum at pH 3–4. [d] Data from ref 7 at 37.1°. [e] Data from ref 3b.
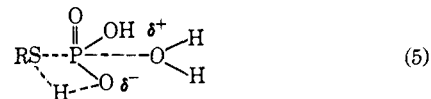
and oxygen phosphate esters are assembled, that a large sensitivity of rate to the pK_a of the leaving group still exists for the nitrophenyl phosphates. In contrast, the rate constants for the thiolphosphate monoanions are fairly insensitive to the pK_a of the leaving group but with the rates actually showing a slight decrease as the leaving group becomes better. The most reasonable explanation for this behavior is that protonation of the leaving group is more important than bond breaking in the transition state. The great facility with which thiolphosphate monoanions hydrolyze is thus not dependent on leaving group pK_a. The cause of the rela-

tively facile hydrolysis of these compounds may reside in a smaller resonance interaction between sulfur and phosphorus than between oxygen and phosphorus in the ground state.

$$RXP\overset{\displaystyle O}{\underset{\displaystyle OH}{{<}}}{-}O^{-} \quad\longleftrightarrow\quad RX\overset{+}{=}P\overset{\displaystyle O^{-}}{\underset{\displaystyle OH}{{<}}}{-}O^{-} \tag{4}$$

X = O or S

Neutral Species. There is no observable acid catalysis of the hydrolysis of any of the thiolphosphate esters at HCl concentrations up to 6.12 M. A similar observation was made for the alkyl ester S-n-butyl phosphorothioate.[6] Dittmer, Ramsay, and Spalding[7] found that the rate of hydrolysis of that compound decreased as acid concentration was increased. The value of k_{H_2O}/k_{D_2O} for p-NO$_2$ in 6 M HCl and DCl is 1.83. Thus hydrolysis of the neutral species also probably involves a slow proton-transfer step not involving hydronium ion. Electron withdrawal in the leaving group does not facilitate the reaction greatly ($k_{NO_2}/k_H = 1.7$). A unimolecular decomposition to metaphosphate with internal protonation in the rate-determining step should proceed more slowly in D$_2$O than in H$_2$O and would be expected to be insensitive to electronic effects, as in the monoanion reaction. The observed ΔS^* is, however,

-13.2 eu, 21 eu more negative than that for the dianion reaction and 11 eu more negative than for hydrolysis of the monoanion. Thus it seems probable that solvent is involved in the hydrolysis of the neutral species, and that attack by water is taking place. An internal protonation should markedly lower the sensitivity of rate to electron withdrawal in R. For a reaction in-

$$RS{\cdots}\overset{\displaystyle O}{\underset{\displaystyle \underset{H}{{\backslash}}{\cdots}O\,\delta^{-}}{\overset{\displaystyle {\nearrow}OH\ \delta^{+}}{{-}}P{<}}}{\cdots}{-}O\overset{H}{\underset{H}{{<}}} \tag{5}$$

volving water it might be expected that increasing the ionic strength up to 6 M would retard the rate much more strongly than observed as the activity of water is decreased. This objection is, however, not necessarily valid. The rate of hydrolysis of N-(3,3-dimethylbutyryl)-imidazolium ion, which certainly involves water in the transition state, is unaffected by increasing HCl concentration to 6 M at 30°.[32]

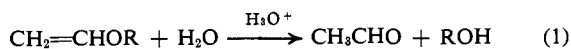(32) J. A. Fee and T. H. Fife, *J. Phys. Chem.*, 70, 3268 (1966).

Structure and Reactivity of α,β-Unsaturated Ethers. The Acid-Catalyzed Hydrolysis of Alkenyl Alkyl Ethers[1]

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Abstract: The rates of the acid-catalyzed hydrolysis of various α,β-unsaturated ethers, R$_1$R$_2$C=CHOR$_3$, in 80% aqueous dioxane have been measured at varying temperatures. The reaction is first order with respect to both ether and acid. The second-order rate constants of ethyl ethers have been found to decrease in the order: vinyl > β-monoalkylvinyl > β,β-dialkylvinyl. Neither *cis*- nor *trans*-β-monoalkylvinyl alkyl ethers suffer geometrical isomerization during the course of hydrolysis. *cis*-β-Monoalkylvinyl alkyl ethers have proved to be several times more reactive than the corresponding *trans* isomers. The heats of activation for the *trans* isomers are greater than those of the *cis* isomers by 1–3 kcal/mole. The activation entropies are positive in sign in the majority of cases. All these results are best interpreted in terms of the rate-determining protonation of the unsaturated bond of the ethers, in which the transition state possesses substantial carbonium ion character.

Alkyl vinyl ethers are known to be readily hydrolyzed in dilute aqueous acids to give acetaldehyde and alcohols[3-7] (eq 1). The course of the reaction must

$$CH_2{=}CHOR + H_2O \xrightarrow{H_3O^+} CH_3CHO + ROH \tag{1}$$

apparently be different from that of the hydrolysis of saturated ethers.

The mechanism of the unsaturated ether hydrolysis is currently being unraveled by several workers. Kiprianova and Rekasheva[8] first noted that the alkyl–oxygen bond in vinyl ether is not broken during the acid-catalyzed hydrolysis, and thereby established that the unsaturated carbon–carbon bond constitutes a center vulnerable to the primary attack of the hydronium ion. Jones and Wood[9] investigated the hydrolysis of various alkyl vinyl ethers kinetically and suggested on the basis of the

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