Reactivity of Thiophosphates. II. Hydrolysis of S-*n*-Butylphosphorothioate and S-(2-Aminoethyl)phosphorothioate[1-3]

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Received November 21, 1962

The hydrolysis of S-*n*-butylphosphorothioate ($n\text{-}C_4H_9SPO_3H_2$) in aqueous solution at constant ionic strength has a rate maximum at pH 3.25. As the acidity is increased to 10 *M* perchloric acid and to 8 *M* hydrochloric acid, the rate decreases. The rate decrease is interpreted as a salt effect on the hydrolysis of the neutral acid. At *ca.* pH 3.50, variation of the ionic strength or kind of buffer had very little effect on the rate. At pH 3.25 and 1 *M* ionic strength, addition of Mn(II), Ni(II), Mg(II), and Zn(II) ions had little effect on the rate. Decreasing the ionic strength or dielectric constant of the solvent at pH 8 increases the rate. Added magnesium chloride decreased the rate at pH 8. These effects are interpreted as being caused primarily by variation of the second ionization constant and by complexing of the dianion with magnesium ion. S-(2-Aminoethyl)phosphorothioate is less reactive than the butyl ester from pH 1–7. It has a rate maximum between pH 2 and 3 and appears to have a second maximum between pH 8 and 9. It protects mice against the effects of radiation.

Monoesters of thiols and phosphoric acid have been suggested as models for intermediates in enzymic phosphate transfers.[5] The activity of several phosphate-transferring enzymes is dependent on the presence of sulfhydryl groups[6a] and S-phosphoryl derivatives have been suggested as intermediates in the formation of acetylcholine from acetate and adenosine triphosphate from adenosine diphosphate,[6b] in succinate activation,[6c] and in the enzymic reduction of amino acids.[6d]

Wieland and Lambert have shown that S-*n*-butylphosphorothioate is capable of transferring phosphate to acetic acid (yielding acetyl phosphate), to alcohols and to phenol,[7] and it has been shown more recently that S-*n*-butylphosphorothioate can yield pyrophosphate when treated with phosphoric acid.[8]

Walsh has found that S-*n*-propylphosphorothioate was hydrolyzed completely in seven minutes at 100° in 1 *N* hydrochloric acid.[5] The hydrolysis of S-cysteinylphosphorothioate is reported to yield cysteine and phosphate in 1 *N* sodium hydroxide and hydrogen sulfide, phosphoric acid, and ammonia in 1 *N* perchloric acid.[9] The corresponding ester of coenzyme A was reported to be unstable in acidic and alkaline solutions.[10] After the work in this paper was completed, data on the hydrolysis of S-(2-aminoethyl)phosphorothioate were reported.[11] The single rate maximum was reported at pH 3.

While the work reported in this paper was in progress, Koshland and Herr reported briefly on the rate of hydrolysis of S-*n*-butylphosphorothioate.[12] They reported a rate maximum for hydrolysis between pH 2 and 4 and a leveling off of the rate between 1 and 4 *N* acid.

This paper reports the effects of various changes in the medium on the rate of hydrolysis of thiophosphate esters.

Kinetic data cannot be used to distinguish between a reaction of water with a monoanion of a phosphorothioate or of hydroxide ion with the neutral acid, nor can a reaction of water with a dianion be distinguished kinetically from a reaction of hydroxide ion with the monoanion. It may be noted, however, that hydroxide ion shows much greater reactivity than water toward phosphorus in triphenyl phosphate and trimethyl phosphate.[13] For the sake of organization, the discussion is arranged according to the major ionic species present in each pH range.

pH-Rate Profiles.—The profile for S-*n*-butylphosphorothioate shown in Fig. 1 agrees essentially with that of Koshland and Herr.[12] A profile calculated from values of the first and second ionization constants (10^{-1} and 3.16×10^{-6} at *ca.* 25°) of the S-*n*-butyl ester and values of the rate constants for hydrolysis of the neutral acid ($k_N = 2.92 \times 10^{-4}$ sec.$^{-1}$) and the monoanion ($k_M = 13.1 \times 10^{-4}$ sec.$^{-1}$) agrees well with the observed profile. The second ionization constant of the ester at 1 *M* ionic strength was determined experimentally, but the first ionization constant was assumed in order to give the best fit of the calculated curve to the experimental curve. The rate constants, k_N and k_M, were determined from experimental data by the method of least squares from the relationship,

$$k_{\text{obs}} = k_N \frac{C_N}{C_P} + k_M \frac{C_M}{C_P}$$

where C_N = concentration of neutral acid, C_M = concentration of monoanion, $C_P = C_N + C_M + C_D$, and C_D = concentration of dianion.

The pH-rate profile for S-(2-aminoethyl)phosphorothioate is shown in Fig. 2. Determinations of the rate constants for this ester were less precise than for the *n*-butyl ester because of difficulty in determination

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[1] We are grateful to the National Institutes of Health (grant A-1023) and the Walter Reed Army Institute of Research for support of this work.

[2] Taken in part from O. B. Ramsay, Ph.D. thesis, University of Pennsylvania, 1960.

[3] Reported in part at the 133rd National Meeting of the American Chemical Society, San Francisco, Calif., April, 1958, and at the 139th National Meeting of the American Chemical Society, St. Louis, Mo., March, 1961.

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[5] E. O'F. Walsh, *Nature*, **169**, 546 (1952).

[6] (a) E. S. G. Barron, *Advan. Enzymol.*, **11**, 243 (1951); L. Noda, *J. Biol. Chem.*, **232**, 237 (1958); (b) G. Feuer and M. Wollemann, *Acta Physiol. Acad. Sci. Hung.*, **7**, 343 (1955); *Chem. Abstr.*, **50**, 1097 (1956); (c) R. A. Smith, I. F. Franck, and I. C. Gunsalus, *Federation Proc.*, **16**, 251 (1957); (d) T. Stadtman, P. Elliott, and L. Tiemann, *J. Biol. Chem.*, **231**, 972 (1958).

[7] T. Wieland and R. Lambert, *Chem. Ber.*, **89**, 2476 (1956).

[8] D. C. Dittmer and V. B. Opshelor, *J. Org. Chem.*, **26**, 4706 (1961).

[9] F. Binkley, *J. Biol. Chem.*, **195**, 283 (1952).

[10] G. Feuer and M. Wollemann, *Acta Physiol. Acad. Sci. Hung.*, **10**, 1 (1956); *Chem. Abstr.*, **51**, 513 (1957).

[11] S. Akerfeldt, *Acta Chem. Scand.*, **14**, 1980 (1960).

[12] D. E. Koshland and E. B. Herr, Jr., *Biochim. Biophys. Acta*, **25**, 219 (1957); E. B. Herr, Jr., and D. E. Koshland, Abstracts of Papers, 131st National Meeting of the American Chemical Society, Miami, Fla. April, 1957, p. 44C.

[13] R. F. Hudson and D. C. Harper, *J. Chem. Soc.*, 1356 (1958); P. W. C. Barnard, C. A. Bunton, D. R. Llewellyn, C. A. Vernon, and V. A. Welch, *ibid.*, 2670 (1961).

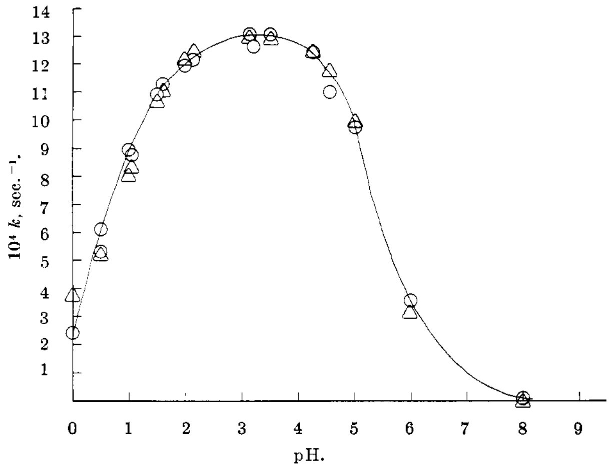

Fig. 1.—pH-Rate profile for the hydrolysis of S-*n*-butylphosphorothioate at 37.1°, 1 *M* ionic strength: ○, observed; △, calculated.

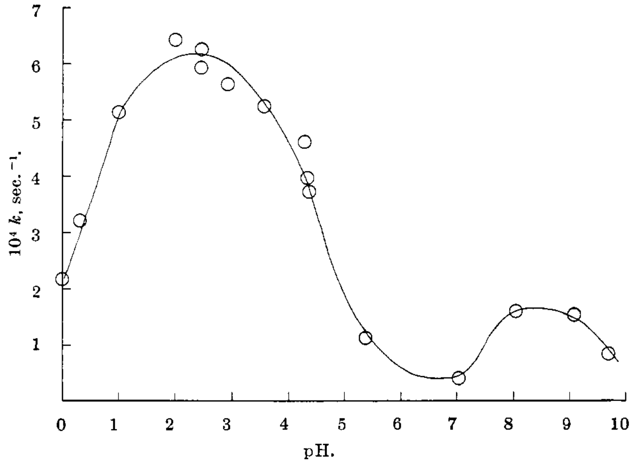

Fig. 2.—pH-Rate profile for hydrolysis of S-(2-aminoethyl)-phosphorothioate at 37°, 1 *M* ionic strength. All points except the ones at pH 0 and pH 9.70 were obtained by use of an arbitrary infinity point.

TABLE I

RATES OF HYDROLYSIS OF 1.64 × 10^{-2} *M* S-*n*-BUTYLPHOSPHOROTHIOATE AT 37.1° AND 1 *M* IONIC STRENGTH (KCl)

pH	Buffer	$10^4 k_{\text{obsd}}$, sec.$^{-1}$
0.50	0.1 *M* Phosphate	6.13
0.51	.1 *M* Phosphate	5.33
1.00	.1 *M* Phosphate	8.94
1.05	.1 *M* Phosphate	8.77
1.50	.1 *M* Phosphate	10.91
1.59	.1 *M* Phosphate	11.26
2.00	.1 *M* Phosphate	11.94
2.15	.1 *M* Phosphate	12.15
3.15	.1 *M* Phthalate	13.07
3.20	.1 *M* Phthalate	12.60
3.50	.1 *M* Phosphate	13.08
4.25	.1 *M* Phthalate	12.40
4.55	.1 *M* Phthalate	11.00
5.00	.1 *M* Phthalate	9.75
6.00[a]	.1 *M* Phosphate	3.55
8.00[b]	.05 *M* Tris[c]	0.0933

[a] Ionic strength = 0.95 *W*. [b] $BuSPO_3Na_2$ = 1.27 × 10^{-3} *M*. [c] Tris(hydroxymethyl)aminomethane.

TABLE II

RATES OF HYDROLYSIS OF S-(2-AMINOETHYL)PHOSPHOROTHIOATE AT 37° AND 1 *M* IONIC STRENGTH (KCl)

pH	Substrate concn., 10^2 *M*	Buffer	$10^4 k$, sec.$^{-1}$
0 (1 *M* HCl)	1.25	...	2.18
0.30 (0.5 *M* HCl)	1.25	...	2.82 (3.21)[a]
1.03	1.25	0.01 *M* Phthalate	4.62 (5.14)
2.02	1.27	.01 *M* Phthalate	5.85 (6.42)
2.46	1.20	.01 *M* Phthalate	4.72 (5.93)
2.47	1.20	.01 *M* Phthalate	4.62 (6.25)
2.92	1.20	.01 *M* Phthalate	4.62 (5.63)
2.93	1.20	.01 *M* Phthalate	4.81 (5.63)
3.58	1.13	.05 *M* Phthalate	4.62 (5.25)
3.58	1.19	.05 *M* Phthalate	3.85 (5.02)
3.58	1.19	.05 *M* Phthalate	4.62 (5.25)
4.28	1.13	.05 *M* Phthalate	3.98 (4.62)
4.33	0.486	.05 *M* Phthalate	3.50 (3.98)
4.36	0.972	.05 *M* Phthalate	3.39 (3.73)
5.33	0.620	.05 *M* Phthalate	1.02
5.37	1.24	.05 *M* Phthalate	0.95
5.38	1.24	.05 *M* Phthalate	.96 (1.13)
7.02	1.20	.05 *M* Tris[b]	.38
7.03	1.20	.05 *M* Phthalate	.35 (0.42)
8.06	1.20	.05 *M* Tris[b]	1.31 (1.60)
9.08	1.20	.05 *M* Tris[b]	0.99 (1.54)
9.70	1.20	.05 *M* Borate	0.86[c]

[a] Values in parenthesis were obtained by using an arbitrary infinity point so that a plot of log $\frac{D - D_t}{D - D_0}$ against time gave the best straight line. Values not in parenthesis were from data obtained in the first 50 or 60% of reaction. [b] Tris(hydroxymethyl)aminomethane. [c] Based on first 25% of reaction. Rate plot deviated from first-order rate law.

of the infinity point, but it appears that the aminoethyl ester is less reactive than the *n*-butyl ester over much of the pH range. A second maximum appears at pH 8–9.

The general shapes of the curves in Fig. 1 and Fig. 2 are similar to those observed for other phosphate esters for which the monoanion is considered the most reactive species.[14a,b,15]

Rate data from pH 0.50 to pH 8.0 for S-*n*-butylphosphorothioate and from pH 0 to pH 9.7 for S-(2-aminoethyl)phosphorothioate are given in Tables I and II.

Hydrolysis in the Neutral Acid Region.—The apparent first ionization constant (10^{-1}) at *ca.* 25° of S-*n*-butylphosphorothioate which gives the best fit of calculated to experimental curve in Fig. 1 is somewhat greater than K_1 for the corresponding oxygen ester (1.3 × 10^{-2})[16] although the latter value is not at 1 *M* ionic strength. The first ionization constant was difficult to determine experimentally, but the second ionization constant (3.16 × 10^{-6}) which was determined is greater than the second ionization constant (1.45 × 10^{-7}) of the oxygen ester[16] which lends some support to the assumption of a greater acidity for the neutral thioester. This greater acidity may be the result of a decreased electron density on phosphorus in the thioester. Phosphorus n.m.r. spectra of phosphate

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(14) (a) A. Desjobert, *Bull. soc. chim. France*, 809 (1947); *Compt. rend.* **224**, 575 (1947); (b) C. A. Bunton, D. R. Llewellyn, K. O. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3574, 3588 (1958).

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

(16) W. D. Kumler and J. J. Eiler, *ibid.*, **65**, 2355 (1943).

esters have indicated that replacement of oxygen by sulfur decreases the shielding at phosphorus.[17]

In contrast to the oxygen esters of phosphoric acid,[14b,18] there is no evidence of C–S cleavage in the hydrolysis of the thioester. In the hydrolysis in 3.6

$$RSPO_3H_2 + H_2O \longrightarrow RSH + H_3PO_4$$

M sulfuric acid or in 6 *M* hydrochloric acid, no hydrogen sulfide was produced as would be the case if phosphorothioic acid were formed.[19] In the sulfuric acid solution a 96% yield of *n*-butanethiol was isolated as its mercury derivative. Only sulfur–phosphorus cleavage has been reported for hydrolyses of tri(S-ethyl)phosphorotrithioate[20] and of di(S-isopropyl)methylphosphonodithioate.[21] Hydrolysis of S - (2 - aminoethyl)-phosphorothioate in 1 *M* perchloric acid results in only sulfur–phosphorus cleavage.[22]

Resonance involving the electrons of the ether oxygen may predispose the oxygen ester to carbon–oxygen cleavage, whereas such resonance is absent in the sulfur derivatives.[23]

$$ROPO_3H_2 \longleftrightarrow R\overset{+}{O}{=}\overset{-}{P}O_3H_2$$

The rate of hydrolysis of S-*n*-butylphosphorothioate continues to decrease as the acidity increases, which contrasts with the behavior of oxygen esters[13–15] and with previous reported data on the hydrolysis of S-*n*-butylphosphorothioate which indicated a leveling-off of the observed rate in acidic solutions.[12] The rate data which are reported here in perchloric and in hydrochloric acids were obtained by the use of two different analytical methods (for inorganic phosphate and for unchanged ester) for following the reaction. The results obtained by each method were quite similar and are given in Table III. The analytical method for inorganic phosphate is sensitive to the acidity at which the determination is carried out and the acid concentration must be adjusted to ensure reproducible results.

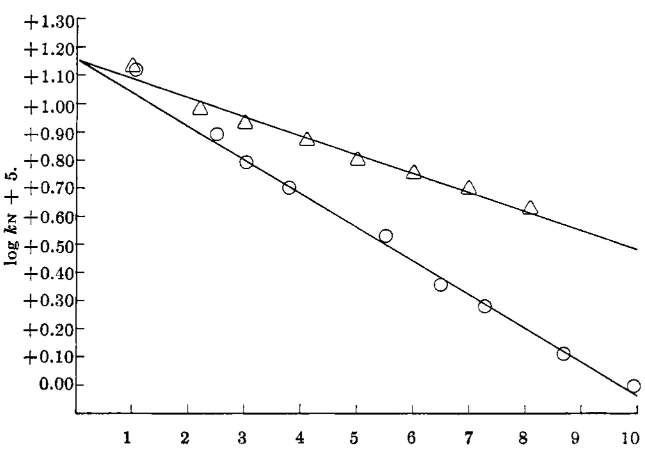

Fig. 3.—Relationship of log k_N for S-*n*-butylphosphorothioate to ionic strength: ○, $HClO_4$; △, HCl.

TABLE III

RATES OF HYDROLYSIS OF S-*n*-BUTYLPHOSPHOROTHIOATE IN ACID AT 37.1°

Acid, *M*	Substrate, 10^3 *M*	Ionic strength, *M*	10^4k_{obsd}, sec.$^{-1}$
1.00 (HCl)	4.97	1	2.40
1.00 (HCl)	4.97	11 (LiCl)	1.99
2.20 (HCl)	16.4	2.20	1.44
2.80 (HCl)	1.27	5 (KCl)	1.31[a]
3.00 (HCl)	4.97	3	1.19
3.00 (HCl)	4.97	11 (LiCl)	0.939
4.11 (HCl)	16.4	4.11	.979
4.20 (HCl)	1.27	5 (KCl)	.979[a]
5.00 (HCl)	4.97	5	.808
5.00 (HCl)	4.97	11 (LiCl)	.656
6.03 (HCl)	16.4	6.03	.700
7.00 (HCl)	1.27	7	.592[a]
7.00 (HCl)	4.97	7	.607
7.00 (HCl)	4.97	11 (LiCl)	.502
8.10 (HCl)	16.4	8.10	.510
1.06 ($HClO_4$)	16.4	1.06	2.31
2.50 ($HClO_4$)	1.27	2.50	1.19[a]
2.51 ($HClO_4$)	16.4	2.51	1.18
3.03 ($HClO_4$)	16.4	3.03	0.940
3.81 ($HClO_4$)	16.4	3.81	.739
3.82 ($HClO_4$)	1.27	3.82	.750[a]
5.52 ($HClO_4$)	16.4	5.52	.449
6.50 ($HClO_4$)	16.4	6.50	.310
7.29 ($HClO_4$)	16.4	7.29	.238
8.00 ($HClO_4$)	1.27	8.00	.210[a]
8.69 ($HClO_4$)	16.4	8.69	.154
9.94 ($HClO_4$)	16.4	9.94	.105

[a] Rates followed by colorimetric analysis of inorganic phosphate. All other rates were followed by iodometric analysis of unchanged thioester.

The decreasing rate of hydrolysis of S-*n*-butylphosphorothioate with increasing acidity can be rationalized as being caused by salt effects of hydrochloric and perchloric acids.[24] Plots of the logarithm of the rate constant (k_N) of the neutral acid[25] against the concentration of hydrochloric or perchloric acid are linear from 2-10 *M* acid concentration as shown in Fig. 3. The linearity is poorer at low acidities if the rate constant is not corrected for the amount of reaction proceeding through the monoanion. All data in Table III from 3–10 *M* acid can be correlated by

$$\log k_{obsd} = -3.648 - 0.083[HCl] - 0.017[LiCl] - 0.132[HClO_4]$$

Plots of log k_N or log k_{obsd} against $-H_0$ have small slopes (*ca.* −0.2). Bunnett plots are not linear.[26]

The effects of ionic strength on the rate of reaction of neutral molecules has been discussed.[27]

In contrast to the decrease in rate for hydrolysis of S-*n*-butylphosphorothioate with increasing acidity, the

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(17) J. R. Van Wazer, C. F. Callis, J. N. Shoolery, and R. C. Jones, *J. Am. Chem. Soc.*, **78**, 5715 (1956). This could be caused also by a neighbor-anisotropy effect. [J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 176.]

(18) C. A. Vernon in "Phosphoric Esters and Related Compounds," Special Publication no. 8, The Chemical Society, London, 1957, p. 17.

(19) D. C. Dittmer and O. B. Ramsay, *J. Org. Chem.*, **28**, 1268 (1963).

(20) E. Thain, *J. Chem. Soc.*, 4694 (1957).

(21) R. F. Hudson and L. Keay, *ibid.*, 3269 (1956).

(22) S. Akerfeldt, *Acta Chem. Scand.*, **13**, 1479 (1959).

(23) Delocalization of the electrons on the ether oxygen in phenylphosphate and in phosphonate esters has been discussed by H. R. Gersmann and J. A. A. Ketelaar, *Rec. trav. chim.*, **77**, 1018 (1958), and briefly by R. F. Hudson and L. Keay, *J. Chem. Soc.*, 2463 (1956).

(24) Salt–non-electrolyte interaction coefficients for sodium chloride and sodium perchlorate are considerably different [E. Grunwald and A. F. Butler, *J. Am. Chem. Soc.*, **82**, 5647 (1960)]. The differences in rates between hydrochloric and perchloric acids may be due in part to specific anion effects on the molar activity coefficients of the reacting species.

(25) A correction has been applied for the amount of reaction proceeding through the monoanion.

(26) J. F. Bunnett, *J. Am. Chem. Soc.*, **82**, 499 (1960).

(27) F. A. Long and W. F. McDevit, *Chem. Rev.*, **51**, 119 (1952); F. A. Long, W. F. McDevit, and F. B. Dunkle, *J. Phys. Colloid Chem.*, **55**, 813 (1951); F. A. Long, F. B. Dunkle, and W. F. McDevit, *ibid.*, **55**, 829 (1951).

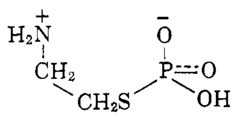
rate of hydrolysis of phosphorothioic acid is increased.[19] This reversal of behavior could be caused by differences in the magnitudes and signs of the salt effects on the initial state, transition state or on both states of the neutral acids.

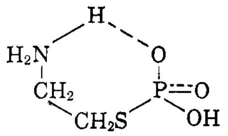
The lack of acid catalysis which distinguishes this sulfur ester from the oxygen esters is probably the result of sulfur being less basic to protons than the ether oxygen. The lack of C–S cleavage lends some support to this explanation.

Intervention of the conjugate acid of the substrate has been considered but an unusually low ionization constant (4.5×10^{-2}) for it is required to fit the data to such an assumption.

Hydrolysis in Monoanion Region.—In the monoanion region, S-*n*-butylphosphorothioate and S-(2-aminoethyl)phosphorothioate are more reactive than the oxygen esters which in part may be because the P–S bond is weaker than a P–O bond. The mechanisms of hydrolysis of oxygen esters have been discussed[28] and there is no reason to propose any unique new mechanisms for the hydrolysis of thiophosphate esters. Possible mechanisms are discussed elsewhere.[2,19]

The somewhat lower reactivity of S-(2-aminoethyl)-phosphorothioate as compared with S-*n*-butylphosphorothioate may be caused by an electrostatic effect of the β-ammonium group or by inter- or intramolecular hydrogen bonding.





Effect of Metal Ions.—The effects of Mg(II), Ni(II), and Mn(II) ions on the rate of hydrolysis of S-*n*-butylphosphorothioate at pH 3.5 and 1 *M* ionic strength was negligible. Zn(II) ions caused an 18% decrease in the rate; a complex of zinc ions with the sulfur and the negative oxygen might decrease the rate by hindering proton transfer from water. Complexing of the ions with the buffer might decrease their catalytic effect.

At pH 8, Mg(II) ions significantly decrease the rate of hydrolysis of S-*n*-butylphosphorothioate with the decrease apparently proportional to the concentration of magnesium ions. The effect of Mg(II) ions seems likely to be an effect on the second ionization constant, since it is known that the apparent pK_2 of phosphoric acid is decreased by Mg(II) ions.[29] Magnesium and other divalent ions complex with the dianions of phosphoric acid and its esters.[30] Such complexing would decrease the concentration of the reactive monoanion (or neutral acid).

$$RSPO_3H^- \rightleftharpoons RSPO_3^= + H^+$$

$$RSPO_3^= + Mg^{++} \rightleftharpoons RSPO_3Mg$$

The data on metal-ion effects are given in Table IV.

The effect of metal ions on the hydrolysis of S-(2-aminoethyl)phosphorothioate, which should be interesting, has not yet been investigated extensively but Hg(II) ions are reported to catalyze the hydrolysis.[22] Complexing of an amino group and a β-sulfur atom with a Cu(II) ion is effective in promoting an elimination reaction in S-alkyl cysteine derivatives.[31]

TABLE IV

RATES OF HYDROLYSIS OF S-*n*-BUTYLPHOSPHOROTHIOATE AT 37.6° IN THE PRESENCE OF METAL IONS

pH	Sub-strate, 10^3 *M*	Buffer, *M*	Ionic strength, *M*	Metal ion, *M*	10^4 k_{obsd}, sec.$^{-1}$
3.25	16.4	0.01 Phthalate	1.0 (KCl)	0.05 $MnCl_2$	12.0
3.25	16.4	.01 Phthalate	1.0 (KCl)	.05 $NiCl_2$	12.1
3.25	16.4	.01 Phthalate	1.0 (KCl)	.05 $MgCl_2$	12.2
3.25	16.4	.01 Phthalate	1.0 (KCl)	.05 $ZnCl_2$	10.0
8.00	1.27	.05 Tris[a]	0.50 (KCl)	...	0.126[b]
8.00	1.27	.05 Tris[a]	.50 (KCl)	.0196 $MgCl_2$	.114[b]
8.00	1.27	.05 Tris[a]	.50 (KCl)	.0492 $MgCl_2$	$.088_2$[b]
8.00	1.27	.05 Tris[a]	.50 (KCl)	.0980 $MgCl_2$	$.058_1$[b]

[a] Tris(hydroxymethyl)aminomethane. [b] Rates followed by analysis for inorganic phosphate. In the acid molybdate solution used in the analysis, magnesium acid phosphate is largely dissociated and magnesium ion does not interfere in the analysis. (Private communication from Dr. Robert Rutman, University of Pennsylvania.)

Effect of Ionic Strength and Buffer Concentration.—Increasing the ionic strength with potassium chloride at pH 8 decreased the rate of hydrolysis of S-*n*-butylphosphorothioate. The data are given in Table V.

TABLE V

EFFECT OF IONIC STRENGTH AND BUFFERS ON THE HYDROLYSIS OF S-*n*-BUTYLPHOSPHOROTHIOATE AT 37.1°

pH	Substrate, 10^3 *M*	Ionic strength, *M*[c]	Buffer, *M*	10^4 k_{obsd}, sec.$^{-1}$
8.00	1.27	0.03	0.05 Tris[a]	0.206[b]
8.00	1.27	.10	.05 Tris[a]	.186[b]
8.00	1.27	.20	.05 Tris[a]	.151[b]
8.00	1.27	.30	.05 Tris[a]	.135[b]
8.00	1.27	.50	.05 Tris[a]	.127[b]
8.00	1.27	.75	.05 Tris[a]	.110[b]
8.00	1.27	1.00	.05 Tris[a]	.0934[b]
8.00	1.27	1.00 (Na_2SO_4)	.05 Tris[a]	.0961[b]
3.50	16.4	0.19	.20 Citrate	12.1
3.50	16.4	1.00	.20 Citrate	12.8
3.15	16.4	1.00	.10 Phthalate	13.1
3.50	16.4	1.00	.10 Phosphate	13.1
3.25	16.4	0.13	.10 Phthalate	12.5
3.28	16.4	.13	.10 Phthalate 5 ml. dioxane per 50 ml.	12.6
4.00	16.4	1.00	0.05 Phthalate	12.6
3.50	1.27	1.00	.05 Phthalate	13.1[b]
6.00	16.4	0.95	.05 Phosphate	3.73
6.00	16.4	.95	.10 Phosphate	3.55
6.00	16.4	.20	.10 Phosphate	3.19

[a] Tris(hydroxymethyl)aminomethane. [b] Rates followed by analysis for inorganic phosphate. [c] Potassium chloride added when necessary.

Increasing the ionic strength, besides affecting the rate constants, probably increases the concentration of

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(28) "Phosphoric Esters and Related Compounds," Special Publication no. 8, The Chemical Society, London, 1957.

(29) I. Greenwald, J. Redish, and A. C. Kibrick, *J. Biol. Chem.*, **135**, 65 (1940).

(30) H. Tabor and A. B. Hastings, *ibid.*, **148**, 627 (1943); J. R. Van Wazer and C. F. Callis, *Chem. Rev.*, **58**, 1011 (1958).

(31) D. C. Dittmer and J. R. Schaeffer, *J. Am. Chem. Soc.*, **83**, 2475 (1961).

the dianion and decreases that of the monoanion.[32] The first and second ionization constants of phosphoric acid are increased as the ionic strength is increased.[33]

Variation of the buffer and ionic strength at pH 3.2–3.7 had little effect on the rate of hydrolysis of S-*n*-butylphosphorothioate. The data are given in Table V.

Earlier studies had indicated that pyridine and 2,6-lutidine were catalysts of the hydrolysis of S-*n*-butylphosphorothioate at pH 8, the stronger base being more efficient,[34] and such results might be interpreted as indicating proton removal in the transition state as was found for the solvolysis of tetrabenzylpyrophosphate.[35] However, experiments with imidazole, a stronger base than pyridine and 2,6-lutidine, indicated it was not the base strength of the latter two compounds that was important in determining the rates. Although the kinetic data for imidazole were not as precise as most of the other data, this base definitely showed no catalytic effect. The rate acceleration with pyridine and 2,6-lutidine could have been caused by a medium effect on a rate constant (perhaps the monoanion is destabilized relative to the transition state) or the decrease in the dielectric constant of the medium could have altered the relative concentrations of the mono- and the dianion in favor of the monoanion. Dioxane caused a rate acceleration analogous to pyridine. Plots of log k_{obsd} against $1/D$, where D is the dielectric constant of the medium, are linear for pyridine[36] and dioxane but not for methanol. The latter, of course, can react with the ester.[7] The data are given in Table VI.

TABLE VI

EFFECT OF DIOXANE, PYRIDINE, METHANOL, AND IMIDAZOLE ON THE RATES OF HYDROLYSIS OF 1.27×10^{-3} *M*. S-*n*-BUTYLPHOSPHOROTHIOATE AT "pH 8.0," IONIC STRENGTH 0.10 *M*, AT 37.1° IN 0.05 *M* TRIS BUFFER

Solute, vol. %	$10^4 k_{\mathrm{obsd}}$, sec.$^{-1}$[a]
1.96 Dioxane	0.197
3.29 Dioxane	.234
9.80 Dioxane	.325
1.96 Pyridine	.195
3.92 Pyridine	.229
9.80 Pyridine	.312
4.90 Methanol	.259
9.80 Methanol	.297
0.178[b] Imidazole	.0837
0.888[b] Imidazole	.0828

[a] Rates followed by analysis for inorganic phosphate. [b] Molar. These molar quantities are approximately equal to 1 and 5 vol. % calculated by assuming the density of imidazole was 1.1.

Reactivity in the Dianion Region.—Data are insufficient to determine if the dianion of S-*n*-butylphosphorothioate has any reactivity at all but it is reported stable in alkali for four hours at 37°.[12] The slight increase in rate around pH 8–9 for S-(2-aminoethyl)phosphorothioate may be caused by a nucleophilic attack of the free amino group on phosphorus as has been suggested for the hydrolysis of diphenyl 2-aminoethyl phosphate,[37] or by an elimination reaction. The decrease in rate observed at pH 10 may not be real. There was difficulty in obtaining an experimental point at the pH of 9.70, and the rate constant was calculated on the basis of only 25% of reaction.

Activation Energies and Entropies.—The energies and entropies of activation for S-*n*-butylphosphorothioate at pH 3.2 and in 2.5 *M* perchloric acid are given in Table VII. The decrease in rate on going from pH 3.2 to 2.5 *M* acid is the result of a decrease in entropy of activation. In 2.5 *M* perchloric acid, approximately 96% of the reaction proceeds through the neutral acid; and at pH 3.2, nearly 99% of the reaction proceeds through the monoanion (or the neutral acid and hydroxide ion).

TABLE VII

ACTIVATION DATA FOR THE HYDROLYSIS OF S-*n*-BUTYLPHOSPHOROTHIOATE

pH	Buffer, *M*	Ionic strength	*T*, °C.	10^5 k_{obsd}, sec.$^{-1}$	E_a kcal./-mole	Δ*S*, e.u.
3.35	0.10 Phthalate	1.0 (KCl)	14.9	6.60[a]	23.85	...
3.20	.10 Phthalate	1.0	25.2	27.3[a]	23.85	+3.08
3.20	.10 Phthalate	1.0	37.1	131[a]	23.85	...
2.50 *M* $HClO_4$	...	2.50	25.2	3.14[b]	21.64	−8.62
2.50 *M* $HClO_4$	...	2.50	37.1	11.9[b]	21.64	...
2.50 *M* $HClO_4$	...	2.50	46.8	36.4[b]	21.64	...

[a] 1.64×10^{-2} *M* substrate. [b] 1.27×10^{-3} *M* substrate.

The initial state of the monoanion[38] probably is strongly hydrogen bonded (water plus monoanion) and as these hydrogen bonds are broken or stretched in the transition state, the entropy increases. The initial state of the neutral acid may be less strongly hydrogen bonded and the transition state may be more polar and more highly solvated relative to the initial state. One might suppose that the energy to break up the hydrogen-bonded initial state of the monoanion is reflected in the higher energy of activation.

Antiradiation Results.[39]—Monosodium S-(2-aminoethyl)phosphorothioate gave "good" protection to mice when administered thirty minutes before irradiation. One hundred percent of the mice survived (30 days) a dose of 825 r. after administration of 400 mg. of the thiophosphate per kg. of mouse. Smaller doses of the compound gave much less protection, and larger doses were toxic. Disodium S-*n*-butylphosphorothioate gave only "slight" protection at levels of 1000. mg./kg.

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(32) One might speculate that, if esters of thiols and phosphoric acid are intermediates in phosphorylations in living systems, the permeability of the cell walls to ions and the transport of ions may play an important part in the regulation of phosphorylation reactions. Specific effects, such as observed for Mg(II) ions, may be especially important. It has been reported that the phosphorylation of adenosine diphosphate in rat brain mitochondria occurs in a hypotonic medium but not in an isotonic one [A. Fonyo and J. Somogy, *Acta Physiol. Acad. Sci. Hung.*, **18**, 191 (1960); *Chem. Abstr.*, **55**, 13492 (1961)]. Calcium ions inhibit oxidative phosphorylation [F. Aiello and R. Maggio, *Experientia*, **17**, 390 (1961)].

(33) J. W. H. Lugg, *Trans. Faraday Soc.*, **27**, 297 (1931); *J. Am. Chem. Soc.*, **53**, 2554 (1931).

(34) D. C. Dittmer and O. B. Ramsay, Abstracts of Papers, 133rd National Meeting of the American Chemical Society, San Francisco, Calif., April, 1958, p. 28 N.

(35) G. O. Dudek and F. H. Westheimer, *J. Am. Chem. Soc.*, **81**, 2641 (1959).

(36) A linear relationship of dielectric constant to weight per cent of pyridine was assumed.

(37) G. J. Durant, J. H. Turnbull, and W. Wilson, *Chem. Ind.* (London) 157 (1958).

(38) It is assumed that the reactive species is the monoanion.

(39) The testing was done at the Walter Reed Army Institute of Research, Washington 12, D. C., and these results were kindly communicated by Dr. David P. Jacobus.

Experimental

Dioxane was purified by treatment with hydrochloric acid, potassium hydroxide, and sodium.[40] Pyridine, A.C.S. Analytical Reagent Grade, was distilled from calcium hydride, b.p. 114.5–115°. Other reagents were reagent grade and were used without further purification.

Barium S-*n*-Butylphosphorothioate.—The salt was prepared by the method of Wieland and Lambert.[7]

Anal. Calcd. for $C_4H_9O_3PSBa.C_2H_5OH$: S, 1.752 mmoles. Found: S, 1.755 mmoles (iodometric analysis).

Disodium S-*n*-Butylphosphorothioate.—The disodium salt was prepared by trituration of the barium salt with an equivalent amount of sodium sulfate. The barium sulfate was removed by filtration and a large excess of isopropyl alcohol (*ca.* 20 vol.) was added to the filtrate. The gelatinous disodium salt was filtered and dried *in vacuo*. Several recrystallizations may be required to give a pure sample.

Anal. Calcd. for $C_4H_9O_3SPNa_2$: C, 22.43; H, 4.24; S, 14.97. Found: C, 22.17; H, 4.56; S, 14.76.

The disodium salt could be prepared also by passage of a solution of the barium salt through an ion-exchange column of Amberlite IR-120 (sodium form).

Sodium S-(2-Aminoethyl)phosphorothioate.—The monosodium salt was prepared by the same method as that used by Akerfeldt by reaction of trisodium phosphorothioate with 2-bromoethylammonium bromide in aqueous dimethylformamide.[22]

Determination of Products of Acid Hydrolysis of S-*n*-Butylphosphorothioate.—To 50 ml. of 3.6 *M* sulfuric acid was added 1.059 g. (0.00494 mole) of disodium S-*n*-butylphosphorothioate, and the mixture was heated on the steam bath for 3.5 hr. The butanethiol was trapped in 5% solution of mercuric cyanide. No mercuric sulfide was observed. The yield of the air-dried mercury salt of butanethiol was 0.9002 g. (96%), m.p. 80–82°. The derivative was recrystallized from ethanol, m.p. 84.5–85° (cor.). No depression of the melting point was observed with an authentic sample of the mercury salt.[41] The sulfuric acid solution was adjusted to pH 4 and refluxed overnight. No further precipitate was observed in the mercuric cyanide trap and the reaction solution gave a negligible titer with iodine.

No mercuric sulfide was observed when the hyrolysis was carried out in 6 *M* hydrochloric acid. A considerable amount of C_4H_9SHgCl was produced along with $(C_4H_9S)_2Hg$.

Determination of Dissociation Constants of S-*n*-Butylphosphorothioate.—The disodium salt was titrated at *ca.* 25° with 0.60 *N* hydrochloric acid and the pH was read from a Beckman Model H-2 pH meter. A plot of pH *vs.* ml. of acid gave a curve with two sharp inflection points. From this curve, $pK_1 = 2.21$ and $pK_2 = 5.93$. The determination of the ionization constants by titration is subject to error because of the hydrolysis which occurs during the time for titration. It is also necessary to use inconveniently large samples for the determination of the K_1 of strong acids. Glass and calomel electrodes were used.

When 10 ml. of 0.10 *N* hydrochloric acid was added to a solution of 0.143 g. (6.67×10^{-4} mole) of $C_4H_9SPO_3Na_2$ and 2.021 g. (0.0271 mole) of potassium chloride in 15 ml. of water, the pH was 1.90. When 5.0 ml. of 0.10 *N* hydrochloric acid was added to a solution of 0.214 g. (0.001 mole) of $C_4H_9SPO_3Na_2$ and 1.722 g. (0.0228 mole) of potassium chloride in 20 ml. of water, the pH was 5.50. Since these pH values are of half-neutralized acid and monoanion, they are equal to pK_1 and pK_2, respectively, but the determination of the pK_1 is subject to the limitations mentioned in the preceding paragraph.

Kinetic Procedure.—The rates of hydrolysis of S-*n*-butylphosphorothioate were followed either by determination of inorganic phosphate as described in the preceding paper[19] or by determination of unchanged ester. In the latter case, 5-ml. aliquots are removed from the reaction flask and drained into *ca.* 25 ml. of phosphate buffer at pH 8.5 to quench the reaction. The *n*-butanethiol is extracted with carbon tetrachloride (extraction is complete at this pH). For hydrolyses in strong acid solutions, it is necessary to add a calculated amount of sodium hydroxide solution to the buffer in order to neutralize the excess acid. After two extractions with carbon tetrachloride, the aqueous buffer solution was acidified to pH 1–2 with 6 *N* hydrochloric acid. The unchanged ester was determined by addition of 1.00 ml. of 0.050 *N* iodine solution and back titration with 0.050 *N* sodium thiosulfate.

Pyridine has a pronounced inhibitory effect upon the development of the phosphomolybdenum blue which is used to determine inorganic phosphate. Pyridine also causes the phosphomolybdate complex to become colloidal. These difficulties may be overcome by a change in the order of addition of reagents. After addition of 1 ml. of the reaction solution to the molybdate solution, 10 ml. of isobutyl alcohol is added and the mixture shaken in a separatory funnel for 10 sec. Then, 1 ml. of the reducing agent is added, and the mixture shaken for 10 sec. more. The usual procedure is then followed.[19] Optical densities resulting from this procedure are higher than expected; this is also the case for runs in dioxane, but the rate constants from runs in which the "normal" procedure was followed are not different from the rate constants from runs followed by the modified procedure.

The measurement of "pH" of pyridine solutions was best done by means of a sleeve-type calomel electrode (Beckman 4970–71); a fiber-type calomel electrode gave erratic results.

The hydrolysis of S-(2-aminoethyl)phosphorothioate was followed by analysis of inorganic phosphate since the thiol formed could not be extracted from the reaction mixture. Some difficulty was experienced in determination of some of the infinity points. Two sets of rate constants could be determined depending upon whether one uses half-lives determined from a plot of $\log (D_\infty - D_t)/(D_\infty - D_0)$ *vs.* time for the first 50–60% of reaction or a more arbitrary infinity point chosen to give a straight line through all of the observed points.

Calculation of Rate Constants.—Values of k_{obsd} for the hydrolyses followed by the iodometric procedure were obtained by plotting log 10 $(1 - z)$, where z is the fraction reacted, *vs.* time, whence

$$k_{\text{obsd}} = \frac{0.693}{t_{1/2}}$$

where $t_{1/2}$ is the half-life. Runs in which phosphate was determined colorimetrically were treated as previously described.[19]

Acknowledgment.—We wish to thank Dr. Daniel E. Koshland, Jr., for a helpful discussion and for communication of his results.

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[40] L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed., D. C. Heath and Co., Boston, 1955, p. 285.

[41] E. Wertheim, *J. Am. Chem. Soc.*, **51**, 3661 (1929).