

Further Studies on S-Substituted Phosphorothioic Acids

II *. Synthesis and Certain Properties of some Potential Antiradiation Drugs

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1. 14 new compounds containing the $-\text{SPO}_3^{2-}$ group have been synthesized. The synthetic methods consisted of: (a) reaction of a substituted alkyl halide with the phosphorothioate ion; (b) reaction of an amino alkyl halide with the phosphorothioate ion followed by guanylation of the amino group by S-ethyl isothiuronium bromide; (c) reaction of dibromopropanols with the phosphorothioate ion. Reaction (c) gave compounds containing two phosphorylated thiol groups per molecule.

2. The reaction between cysteamine S-phosphate and bromine was investigated. The results favour the conception that the S-P bond is broken at an early stage of the oxidation, which most likely proceeds *via* sulfenylbromides.

3. Attempts to demonstrate intramolecular phosphate group transfer from S to O in some of the prepared compounds failed.

As a result of a search for new antiradiation drugs at this Institute it was recently reported by Hansen and Sörbo¹ that one of the S-substituted phosphorothioic acids earlier synthesized by Åkerfeldt, namely cysteamine S-phosphate, is a potent radioprotective agent when given to mice. This fact has stimulated the synthesis of a series of related compounds and their properties as antiradiation drugs are now being evaluated. The results of these studies will be reported elsewhere.

In this communication the synthesis and some properties of 12 new S-substituted phosphorothioic acids and of two S-phosphorylated dithiols is reported.

RESULTS AND DISCUSSION

Synthetic methods. In the method earlier developed by the author for the preparation of S-substituted phosphorothioic acids²⁻⁵ a compound containing

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a reactive halogen is allowed to react at room temperature with trisodium phosphorothioate in aqueous medium with or without the use of DMF* as a catalyst. In all cases studied so far a quantitative reaction of the phosphorothioate ion has been obtained after a few hours of reaction.

It was earlier found advantageous to isolate the S-substituted phosphorothioic acids as mixed lithium sodium salts, since small amounts of orthophosphate formed could be conveniently removed as trilithium phosphate⁵.

To simplify the analysis of the isolated products most of the compounds described in this paper were prepared from trilithium phosphorothioate. By the use of this compound only one metallic cation was (usually) present in the isolated products.

Trilithium phosphorothioate is easily obtained from PSCl_3 and lithium hydroxide. For its preparation a procedure similar to that worked out for the synthesis of trisodium phosphorothioate⁴ was followed with the exception that water of crystallization was not removed by methanol. This step was omitted since trilithium phosphorothioate forms a gel-like suspension in this alcohol and isolation of the dehydrated product therefore becomes difficult.

The phosphorus content of trilithium phosphorothioate was determined by the bromine method⁵. The sulfur content can be determined by potentiometric iodine titration in aqueous medium as described by Yasuda and Lambert⁶ for trisodium phosphorothioate. Visible detection of the endpoint is not feasible because of lack in sharpness. However, it was found during this investigation that it is possible to get a perfectly sharp endpoint for visible titration if the sample is dissolved in glacial acetic acid and titrated with iodine in methanol. This method also works well for trisodium phosphorothioate.

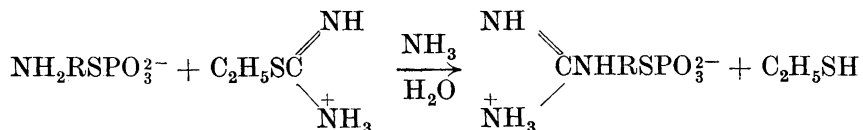
The S-substituted phosphorothioic acids described in this communication were prepared as follows:

(a) trilithium phosphorothioate (in a few cases Na_3SPO_3) was allowed to react with compounds containing one reactive halogen atom (usually bromine) in the presence of DMF:



In this way 10 compounds were prepared. In a few cases it was found to be more convenient to isolate the substances as their barium salts and in one case the sodium salt was isolated.

(b) Trilithium phosphorothioate was first allowed to react with an amino-alkyl bromide as described under (a). The amino group was then guanylated by S-ethyl isothiuronium bromide in the presence of ammonia without isolation of the intermediary amino compound:



* Abbreviations: DMF = N,N-dimethyl formamide; EDTA = (ethylenedinitrilo) tetraacetic acid; CMNP = 2-chloromercuri-6-nitrophenol.

In this way two compounds were prepared, namely S-(2-guanidinoethyl) phosphorothioic acid and S-(3-guanidinopropyl) phosphorothioic acid.

Pant and Dubey⁸ have recently shown that 2-aminoethanol O-phosphate can be N-guanylated by S-methyl isothiuronium bromide.

(c) Trilithium phosphorothioate was allowed to react with compounds containing two reactive bromine atoms in the presence of DMF. In this way the lithium salts of 1,3-dimercapto-2-propanol S₁,S₃-diphosphate (I) and 2,3-dimercapto-1-propanol S₂,S₃-diphosphate (II) were prepared.



Compound I has the same spacing between the sulfur atoms as lipoic acid. It is of interest in this connection that Stadtman *et al.*⁷ have shown 1,3-dimercapto-2-propanol to serve as a cofactor in the enzymic reduction of glycine in *E. sticklandii*. As an intermediate in this reaction an S-phosphorylated compound similar to I was suggested. Compound II is S-phosphorylated British Anti Lewisite (BAL). Compounds I and II represent a new class of compounds containing two phosphorylated thiol groups in the same molecule.

Structural investigations. As was pointed out in Part I of this series, and in earlier papers, there exists the theoretical possibility that the substituted alkyl halides used in the preparation of the S-substituted phosphorothioic acids by the outlined methods could also react with an oxygen atom of the phosphorothioate ion and thus form Q-substituted phosphorothioic acids (ROPSO₂⁻) as contaminants of the major reaction products. In no case, however, could the presence of such contaminants be demonstrated in earlier synthesized compounds. All compounds described in this paper were tested for the presence of O-substituted phosphorothioic acids. It was not possible to demonstrate their presence in any isolated product.

The testing methods used included: (1) acid hydrolysis (5 min at 90–100° in 1 M HCl) of the compound and determination of liberated thiol (usually) by iodine titration. A subquantitative yield of thiol could indicate the presence of contaminants, but exact knowledge of the behavior of the corresponding O-substituted phosphorothioic acids towards oxidation by iodine is lacking. However, in no case tested was the amount of thiol released below the expected amount (within experimental error). The quantity of thiol obtained always corresponded very well with the phosphorus content of the compounds as determined by the bromine method [see (2)], a circumstance that would not be expected in the presence of O-substituted phosphorothioic acids. (S-Ethyl phosphorothioate could not be tested in this way because of liberation of volatile ethanethiol during hydrolysis). (2) Phosphate determination according to the bromine method⁵. Only the S–P bond would be expected to be broken by this mild method. The more stable O–P bond is usually not attacked at all by bromine treatment. A subquantitative yield of orthophosphate, as deter-

mined by the bromine method, would thus indicate the presence of contaminants. Within experimental error the phosphate recovery was quantitative for all compounds.

A possible decomposition of S-[2-(trimethylammonium)ethyl] phosphorothioate (thiocholine S-phosphate) was tested for by reacting the compound in alkaline solution with 2-chloromercuri-6-nitrophenol¹⁷, CMNP. This treatment breaks down the S—P bond and MNP-thiols¹⁷ are formed². Paperchromatographic analysis of the resulting solutions according to Ref.¹⁷ in the solvent systems 1, 2 and 3 gave only one thiol spot (R_F 's 0.64, 0.76, and 0.70, respectively), much lower in position than the thiol spot obtained from, e.g., S-(2-dimethylaminoethyl) phosphorothioate (R_F 's 0.90, 0.90, and 0.95, respectively). Acid hydrolysis of the compound followed by coupling to CMNP gave the same results. The compound thus appears to be homogeneous.

Guanylation of the two S-(aminoalkyl) phosphorothioates by S-ethyl isothiuronium bromide could possibly give contaminants that were guanylated on the phosphate residue. Acid hydrolysis of such contaminants should result in the formation of urea. Acid hydrolysates of S-(2-guanidinoethyl)- and of S-(3-guanidinopropyl) phosphorothioate were therefore subjected to paperchromatography in ethanol: pyridine: water, 2:1:1, in butanol:pyridine:water, 1:1:1 and in butanol: acetic acid:water, 4:1:1. After spraying with *p*-dimethylamino benzaldehyde urea could not be detected on the chromatograms.

A nonquantitative guanylation of the amino group would result in the presence of S-(aminoalkyl) phosphorothioates in the isolated products. Coupling to CMNP, as described above, followed by paper chromatographic analysis according to Ref.¹⁷ showed the presence of only one thiol spot for each substance. No trace of MNP-2-aminoethanethiol or MNP-3-aminopropanethiol was detected in this way.

The analysis of 1,3-dimercapto-2-propanol S_1, S_3 -diphosphate (I) and its isomer (II) presented certain difficulties. After acid hydrolysis sulfur in the liberated thiol could not be determined by iodine titration in the usual manner since the oxidation product is of unknown structure (see e.g. Ref.²⁷). Instead a method depending on spectrophotometric measurements of the reduction of 2,6-dichlorophenol indophenol was used*. The S-substituted phosphorothioic acids themselves are not oxidized by this reagent. The developed procedure is a modification of the one described by Basford and Huennekens⁹. The method was standardized by the use of thiols obtained by acid hydrolysis of three of the prepared compounds with known composition (S-(carbamylmethyl-, S-[2-(trimethylammonium)ethyl]-, and S-(2-hydroxyethyl)-phosphorothioates). These three thiols gave the following absorbance values (per μ mole in 3 ml of solution) at 600 $m\mu$ (1 cm light path): 3.73, 3.71, and 3.68, respectively. (Thiols containing reactive amino groups gave different values). An absorbance of 3.71 was considered representative. When the SH-content of the thiols obtained by hydrolysis at pH 4 for 5 min at 70° of compounds I and II was determined by this method a value of 2.04 ± 0.02 SH groups per

* The earlier developed thiol reagent, CMNP¹⁷, was not useful for these SH determinations since the reaction products obtained with this reagent were soluble in toluene or in 1,2-dichloroethane. The nitroprussiate method²⁵ gave a very low color yield with the liberated dithiols.

molecule was obtained. Acid hydrolysis was thus considered to give a quantitative yield of thiol also in these two cases.

Paper chromatographic analysis according to Ref.¹⁷ of the reaction product obtained from CMNP and the thiol released by acid hydrolysis of compound II gave identical results with commercial 2,3-dimercapto-1-propanol treated the same way.

Reaction with bromine. Phosphorus in the prepared compounds was determined as orthophosphate by the bromine method⁵. This method was preferred over the mercury method² since mercury (II) ions sometimes form insoluble mercaptides with the thiols released from S-substituted phosphorothioic acids. It was demonstrated in an earlier paper⁵ that bromine liberates orthophosphate quantitatively from these compounds. No investigation, however, was performed at that time to establish what became of the thiol part of the compounds during this treatment. For an investigation of this problem cysteamine S-phosphate was chosen primarily because its oxidation products could be easily demonstrated by paperchromatographic analysis using ninhydrin as a spot detector*, secondly because oxidation products of cysteamine are easily available.

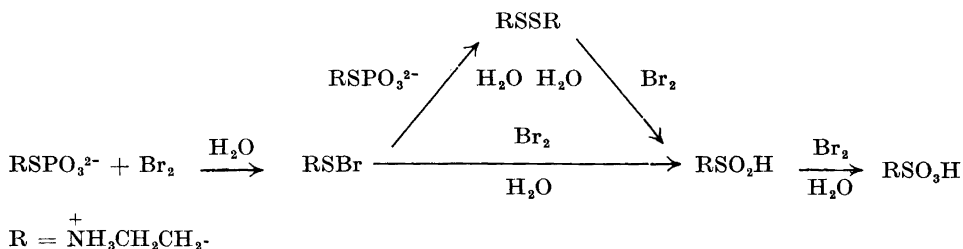
Saturated bromine water was added to cysteamine S-phosphate of pH < 7 until the bromine color persisted. Paperchromatographic analysis was performed of the resulting solution after 24 h in butanol:pyridine:water, 1:1:1, in ethanol:water:pyridine, 2:1:1 (saturated with solid NaCl); and in butanol:acetic acid:water, 4:1:1. Subsequent development with ninhydrin gave only one spot in all three solvents identical in position and in ninhydrin color with 2-aminoethane sulfonic acid (taurin). However, if chromatograms were run immediately after bromine treatment of cysteamine S-phosphate three major spots were obtained. The spots were identical in position to taurin, 2-aminoethane sulfinic acid (hypotaurin)** and 2,2'-dithiobisethylamine (cystamine) and gave identical ninhydrin colors to these. The spot corresponding to cystamine gave a positive cyanide-nitroprussiate reaction and reduced hexaiodoplatinate. The spot corresponding to hypotaurin gave a negative cyanide-nitroprussiate reaction but reduced hexaiodoplatinate. None of the ninhydrin positive spots gave orthophosphate on hydrolysis at 100° by acid molybdate spray. (In this way orthophosphate and a trace of unreacted cysteamine S-phosphate was found).

It was further noticed that bromine oxidation of cystamine gave exactly the same chromatograms as bromine oxidation of cysteamine S-phosphate. This fact and the actual demonstration of the presence of cystamine (the amount of cystamine in the cysteamine S-phosphate used was negligible) indicates that the oxidation of cysteamine S-phosphate to taurin by bromine at least partially proceeds *via* cystamine. This compound may be formed by a reaction similar to the one proposed for the reaction between cysteamine S-phosphate and iodine⁴. Other possible routes for the formation of interme-

* Preliminary chromatographic experiments had shown that the amino group of the molecule is little affected by bromine treatment. Any reaction taking place at this end of the molecule lay outside the scope of this investigation.

** 2-Aminoethyl 2-aminoethanethiol sulfonate could be separated from hypotaurin on the chromatograms. Small amounts of this substance were possibly present. It was prepared according to Field *et al.*²⁸

diates *via* sulfenylhalides have been discussed by Stirling¹⁰ for S-benzyl O,O-diethyl phosphorothioate.



The present investigation does not give support to a scheme similar to the one suggested by Lordy and Epstein¹¹ for the oxidation of O,O,S-triethyl phosphorothioate by chlorine, where all the oxidation of sulfur to sulfonic acid should occur while sulfur was still bound to phosphorus. Obviously a disulfide would then not result, nor would a sulfinic acid be formed. One would then also expect to find traces of these phosphorus containing intermediates on the chromatograms.

Does intramolecular phosphate group migration occur in certain S-substituted phosphorothioic acids containing a hydroxyl group? Acetyl group migration from sulfur to oxygen in the same molecule has been demonstrated to occur under certain circumstances (*cf.*, *e.g.* Ref.¹²). The free energy of thiol ester hydrolysis is high¹² and so is the free energy of hydrolysis of the S—P bond in S-substituted phosphorothioic acids^{13,14}.

The question of intramolecular phosphate group migration from sulfur to oxygen was investigated for three compounds: S-(2-hydroxyethyl), S-(3-hydroxypropyl) phosphorothioate and 1,3-dimercapto-2-propanol S₁S₃-diphosphate. A phosphate group migration taking place in the fashion mentioned would result in the formation of (a) free thiol groups, and (b) O—P bonds relatively resistant to hydrolysis. Since thiol groups are also being liberated by hydrolysis of the S—P bond the method chosen for the investigation was based on the formation of the relatively stable O—P bond. The bromine method for phosphate determination⁵ was suitable since it would only be expected to break the S—P bond. The formation of O—P bonds would thus result in the disappearance of orthophosphate.

Samples of the three compounds were incubated at 35° in buffers of different pH for various lengths of time and orthophosphate was determined by the bromine method. Within experimental error no decrease of the orthophosphate amount was detected in any case. An intramolecular phosphate group transfer could thus not be demonstrated.

General remarks. The formulas as well as found and calculated analytical values for the 14 isolated compounds are tabulated in Table 1.

Some of the isolated compounds contained ethanol of crystallization (all compounds had been dried for at least 20 h at 8–10 mm Hg over fresh silica gel at + 4° before analysis was made). A strong affinity for methanol or ethanol was also earlier reported for several S-substituted phosphorothioic acids^{5,15}.

Table 1. Formulas, found and calculated analytical values of the isolated compounds.

Com- pound No.	Formula of isolated compound	Mol. weight	ANALYSIS											
			C		H		P		S		OTHER			
			calc.	found	calc.	found	calc.	found	calc.	found	calc.	found		
1.	$C_2H_5SPO_3Li$	154.0	15.4	3.3	3.4	20.1	19.9	20.8	20.3 ^a	—	—	—	—	
2.	$H_2OCH_2CH_2SPO_3Li_2$	170.0	14.0	3.0	3.0	18.2	18.4	18.9	18.7	—	—	—	—	
3.	$H_2OCH_2CH_2CH_2SPO_3Ba, 2.5H_2O$	352.5	10.2	3.4	3.4	8.80	8.80	9.10	9.20	Ba	39.0	38.8	—	
4.	$H_3NCOCH_2SPO_3Li_2$	183.0	13.2	2.2	2.0	16.9	16.9	17.5	17.6	N	7.7	7.7	—	
5.	$H_3NCH_2CH_2CH_2SPO_3Li, 0.5H_2O$	186.1	19.4	5.4	5.2	16.6	16.7	17.2	17.1	—	—	—	—	
6.	$CH_3NHCH_2CH_2SPO_3Ba(C_2H_5OH)_{0.15}$ 1.5 H ₂ O	340.4	11.6	3.5	3.4	9.10	9.13	9.41	9.56	Ba	40.4	40.9	0.15	
7.	$(CH_3)_2NCH_2CH_2SPO_3Ba, 2H_2O$	356.6	13.5	3.9	4.1	8.70	8.68	8.99	8.90	Ba	38.6	38.4	—	
8.	$(CH_3)_2NCH_2CH_2SPO_3Na, 8H_2O$ ^d	365.3	16.4	8.0	7.7	8.47	8.51	8.77	8.80	N	3.8	3.8	—	
9.	$(e-C_3H_7)_2NCH_2CH_2SPO_3Ba, 2.5H_2O$	421.7	22.8	5.5	5.4	7.34	7.45	7.6	7.2 ^c	Ba	32.5	31.4	—	
10.	$(CH_3)_2NCH_2CH(OH)SPO_3Li, 2H_2O$ HN	247.1	24.3	6.5	6.2	12.5	12.7	13.0	12.9	—	—	—	—	
11.	$C-NHCH_2CH_2SPO_3^-(NH_4)_{0.2}Li_{0.8}, 1.5$ H_3N^+ H ₂ O HN	234.4	15.4	5.5	5.3	13.2	13.3	13.7	13.5	N	19.1	19.0	0.82	
12.	$C-NHCH_2CH_2SPO_3^-(NH_4)_{0.9}Li_{0.3}$ H_3N^+ H ₂ O	246.0	19.5	6.6	6.9	12.6	12.5	13.0	13.0	N	21.6	21.4	0.18	
13.	$Li_2PO_3SCH_2CH(OH)CH_2SPO_3Li_2$ $(C_2H_5OH)_{0.9}, 3H_2O$ ^b	403.4	14.3	4.3	4.3	15.4	15.5	15.9	16.3	C_2H_5OH/P	0.9	0.90	—	
14.	$Li_2PO_3SCH_2CH(SPO_3Li_2)CH_2OH$ $(C_2H_5OH)_{0.9}, 2H_2O$ ^b	385.4	15.0	4.0	3.7	16.1	16.1	16.6	16.9	C_2H_5OH/P	0.9	0.85	—	

^a) Determined as sulfate after combustion of the substance.

^b) Ethanol was not removed by further drying at 10 mm Hg.

^c) Determined by 2,6-dichlorophenolindophenol reduction after acid hydrolysis of a 6 months old prepn. Iodine gives a colored adduct with the corresponding disulfide and iodine titration is therefore not suitable for S-detn.

^d) The result of acid titration of the compound excluded the structure $(CH_3)_2N(OH)CH_2CH_2SPO_3^{2-}$.

Several of the compounds contained water of crystallization. No method was found that was entirely satisfactory for its quantitative determination. The amount of water of crystallization in the compounds was calculated from (a) the molecular weight as calculated from C, S, and P content; (b) from H determinations. For all compounds methods (a) and (b) were in close agreement.

Due to different reaction rates, different solubilities of the isolated salts and various experimental difficulties that arose during the preparation of the compounds it was found most suitable to present the synthesis of each individual compound *per se*.

DMF was found to catalyze the formation of cysteamine S-phosphate². In other instances the reaction between the phosphorothioate ion and the substituted alkyl bromide proceeds at a convenient rate without the catalyst (*e.g.* in Ref.⁵). In the present investigation the reaction rate without DMF was not determined, but it was routinely added in all cases.

EXPERIMENTAL

General methods

Phosphate was determined colorimetrically by the bromine method⁵. Sulfur was determined as liberated thiol by iodine titration after hydrolysis at 90–100° of about 100 mg of substance in 25 ml of 1 M HCl for 5 min (unless otherwise stated). Ethanol was determined using the colorimetric method by Reid and Salmon¹⁶ after passage of the sample through Dowex 50 W × 8 (H⁺) and through Dowex 21 K (OH⁻). Barium was determined by EDTA titration according to Anderegg *et al.*²³ Lithium was determined by the colorimetric method of Thomason²⁶ after passage of the sample through Dowex 21 K (OH⁻).

Melting points were determined on the Kofler Heiz Bank.

Determination of thiols with 2,6-dichlorophenol indophenol. The following was added to a 4 ml cuvette (1 cm light path): 1.00 ml of a 1 M phosphate buffer pH 6.8; 0.10 ml of 2,6-dichlorophenol indophenol (50 mg in 250 ml of water) and 0.10 ml of thiol. The contents of the cuvette were mixed and allowed to react for 30 min at room temperature whereafter 1.80 ml of water were added. The absorbance was read at 600 μ immediately. Water served as a blank. At the most individual readings of samples with the same composition varied 3–4 % of the mean, but were usually within 1–2 % of the mean.

Comments. To prevent a rapid autooxidation of reduced dye a pH slightly below 7 was chosen. To obtain a complete oxidation of the thiol by the dye an excess of dye must be present. The absorbance after completed reaction should therefore not be considerably below 0.200. Regardless of the nature of the thiol Beer's law was followed for the reduction of the dye up to absorbance differences well over 0.500 (provided an excess of dye was present). For the measurements a Beckman Model B Spectrophotometer was used.

Trilithium phosphorothioate

183 g (4.05 mole) of lithium hydroxide hydrate (containing 53 % LiOH, Merck and Co) were stirred up in 1000 ml of water and 70 ml (0.675 mole) of freshly distilled PSCl₃ were added. The temperature of the reaction mixture was kept at 80–85° under constant rapid stirring. After about 2 h all PSCl₃ had dissolved. The content of the reaction flask was cooled to room temperature under tap water and any precipitate was filtered off. 1500 ml of ethanol was added to the clear filtrate under rapid stirring and the precipitated trilithium phosphorothioate was filtered off and washed thoroughly with ethanol (500 ml). After drying first in the open air and then in vacuum about 136 g (88 %) of hydrated trilithium phosphorothioate was obtained. (Found: P⁵ 13.5; S 13.7; H 5.0. Calc for Li₃SPO₃, 5.5 H₂O (231.0): P 13.4; S 13.9; H 4.8).

Determination of sulfur in trilithium phosphorothioate. About 200 mg of substance were dissolved in 30 ml of glacial acetic acid and titrated to persistent iodine color with iodine in methanol (50 mequiv/l). Anhydrous trisodium phosphorothioate⁴ titrated in this fashion also gave the quantitative amount of iodine reduction (*i.e.* one equivalent of iodine reacts with one mole of phosphorothioate).

Synthesis of intermediates

The substituted alkyl halides used in this investigation were partly synthesized in this laboratory, partly commercial products.

2-Bromoethyl dimethylammonium bromide was prepared by a modified Leuckart methylation of 2-bromoethyl ammoniumbromide according to Bobranski *et al.*¹⁸, m.p. 188–189° (ethanol). Ref.¹⁸ gives m.p. 186–187°.

2-Bromoethyl trimethylammoniumbromide was prepared from trimethylamine and 1,2-dibromoethane according to Renshaw¹⁹.

2-Bromoethyl methylammonium bromide was prepared from 2-methylaminoethanol and HBr according to Knorr and Meyer²⁰, m.p. 82° (ethanol).

1,3-Dibromo-2-propanol (Eastman Kodak Co) was redistilled and the fraction boiling at 103° at 16–17 mm Hg was used. $n_D^{25} = 1.5496$.

2,3-Dibromo-1-propanol was prepared by the addition of bromine to allyl alcohol²¹, b.p. 102–105° at 11.5 mm Hg, $n_D^{25} = 1.5492$.

3-Bromopropylammonium bromide was prepared in principle according to Schöberl *et al.*²² from 3-amino-1-propanol and HBr. Washing the product with ether and recrystallizing it from isopropanol gave a much more pure product than earlier described²². M.p. 171°. Yield: 67%. (Ref.²² gives m.p. 162°). (Found: C 16.5; H 4.1. Calc for $\text{Br}^-\text{NH}_3^+\text{CH}_2\text{CH}_2\text{CH}_2\text{Br}$ (218.9): C 16.4; H 4.2).

Synthesis of S-substituted phosphorothioic acids

Silver ion test for unreacted phosphorothioate. The test used to follow the rate of reaction between the alkyl halides and the phosphorothioate ion was the following. To two drops of test solution were added three drops of 5 M HNO_3 followed by five drops of 3% AgNO_3 . At the start of the reaction, when much unreacted phosphorothioate was still present, the Ag^+ -test gave a black precipitate. It grew progressively lighter in color as the reaction proceeded. A light yellowish color of the precipitate indicated the endpoint of the reaction. In no case was the reaction stopped before all phosphorothioate had reacted as judged by this test.

Notes on the experimental technique. All substances prepared were precipitated by ethanol from aqueous solution, filtered and washed thoroughly with ethanol. They were dried in vacuum (about 8–10 mm Hg) at 2–4° in a refrigerator. *Due to the lability of the S–P bond all compounds were stored at –15° at all times when not in use.*

1. *Dilithium S-(ethyl) phosphorothioate.* 11.5 g of $\text{Li}_3\text{SPO}_3 \cdot 5.5 \text{ H}_2\text{O}$ (50 mmole) were dissolved in 75 ml of water and 4.2 ml of bromoethane were added followed by 25 ml of DMF. The solution was stirred for 45 min after which time 1.0 ml of bromoethane (totally 69 mmole) was again added. After another hour of stirring the reaction product started to separate. Addition of 200 ml of ethanol precipitated most of the substance. Yield*: 6.7 g (87%).

2. *Dilithium S-(2-hydroxyethyl) phosphorothioate.* 11.5 g of $\text{Li}_3\text{SPO}_3 \cdot 5.5 \text{ H}_2\text{O}$ (50 mmole) were dissolved in 100 ml of water. 8.75 g of 2-bromoethanol (70 mmole) and 25 ml of DMF were added. After stirring for 1.5 h the substance was precipitated by the addition of 250 ml of ethanol. Yield: 7.0 g (59%).

3. *Barium S-(3-hydroxypropyl) phosphorothioate.* 9.0 g of Na_3SPO_3 (50 mmole) were dissolved in 125 ml of water (the reaction product is only sparingly soluble in water). 7.1 ml of 3-bromo-1-propanol (80 mmole) and 25 ml of DMF were added (the reaction is very slow with less bromopropanol). The solution was stirred for 1.5 h when 20.5 g of barium acetate monohydrate (75 mmole) dissolved in 50 ml of water were added under

* All yields are calculated on the amount of phosphorothioate used.

vigorous stirring. A microcrystalline precipitate was formed, which was completely precipitated by the addition of 100 ml of ethanol. The precipitate was stirred up in 300 ml of water for 20 min, filtered and pure material was precipitated from the clear filtrate by the addition of 200 ml of ethanol. Yield: 6.7 g (38 %).

4. *Dilithium S-(carbamylmethyl) phosphorothioate*. 11.5 g of $\text{Li}_3\text{SPO}_3 \cdot 5.5 \text{H}_2\text{O}$ (50 mmole) were dissolved in 100 ml of water. 5.2 g of chloroacetamide (55 mmole) and 25 ml of DMF were added. After 30 min of stirring 200 ml of ethanol were added. The crystalline precipitate was filtered off. Yield: 8.4 g (84 %).

5. *Lithiumhydrogen S-(3-aminopropyl) phosphorothioate*. This substance was prepared as No. 4 except that 12.0 g of 3-bromopropylammonium bromide (55 mmole) were added. Reaction time: 4.5 h. The addition of 300 ml of ethanol precipitated 6.7 g (72 %) of substance.

6. *Barium S-[2-(methylamino)ethyl] phosphorothioate*. 9.0 g of Na_3SPO_3 (50 mmole) were dissolved in 75 ml of water and 12.0 g of 2-bromoethylmethylammonium bromide (55 mmole) and 25 ml of DMF were added. The solution was stirred for 30 min. 20.5 g of barium acetate monohydrate (75 mmole) were added and a small precipitate was filtered off. 3.0 g of NaOH (75 mmole) in 25 ml of water were added followed by 400 ml of ethanol. Yield: 11.9 g (70 %).

7. *Barium S-[2-(dimethylamino)ethyl] phosphorothioate*. 9.0 g of Na_3SPO_3 (50 mmole) were dissolved in 75 ml of water and 12.8 g of 2-bromoethyl dimethylammonium bromide (55 mmole) and 25 ml of DMF were added. After 30 min of stirring 20.5 g of barium acetate monohydrate (75 mmole) were added in 50 ml of water. A small precipitate was filtered off. 3.0 g of NaOH (75 mmole) in 25 ml of water were added followed by 200 ml of ethanol. Yield: 12.3 g (69 %).

8. *Sodium S-[2-(trimethylammonium)ethyl] phosphorothioate*. 9.0 g of Na_3SPO_3 (50 mmole) were dissolved in 175 ml of water. 13.6 g of 2-bromoethyltrimethylammonium bromide (55 mmole) and 30 ml of DMF were added. After 3 h of stirring the reaction mixture was slowly poured into 1500 ml of ethanol of 10° under rapid stirring. A microcrystalline precipitate was filtered off. Yield: 11.3 g (61 %). (Attempts to isolate the compound by precipitation of its barium or lithium salt from aqueous solution by ethanol failed).

9. *Barium S-[2-(diisopropylamino)ethyl] phosphorothioate*. 9.0 g of Na_3SPO_3 (50 mmole) were dissolved in 75 ml of water and 15.3 g of 2-bromoethyl-diisopropylammonium bromide (53 mmole) and 25 ml of DMF were added. The solution was stirred for 30 min. 12.2 g of barium chloride dihydrate (50 mmole) and 2.0 g of NaOH (50 mmole) were added dissolved in 75 ml of water under vigorous stirring. 200 ml of ethanol were then added under continuous stirring and the microcrystalline precipitate was filtered off. 14.2 g (67 %) of dry substance were obtained.

10. *Dilithium S-[(2-dimethylamino-1-methyl)ethyl] phosphorothioate*. 11.5 g of $\text{Li}_3\text{SPO}_3 \cdot 5.5 \text{H}_2\text{O}$ (50 mmole) were dissolved in 100 ml of water. 8.7 g of 2-chloropropyl dimethylammonium chloride (55 mmole) and 25 ml of DMF were added. After stirring for 45 min 2.5 g of lithium hydroxide hydrate (containing 53 % LiOH, 55 mmole) were added in 30 ml of water. A small precipitate was filtered off and the substance was precipitated by the addition of 900 ml of ethanol. The fine precipitate was centrifuged down and stirred up in another 300 ml of ethanol. After renewed centrifugation the substance was dried in the open air and then in vacuum at 4°. Yield: 6.4 g (49 %).

11. *Ammonium lithium S-(2-guanidinoethyl) phosphorothioate*. 11.5 g of $\text{Li}_3\text{SPO}_3 \cdot 5.5 \text{H}_2\text{O}$ (50 mmole) were dissolved in 100 ml of water. 10.9 g of 2-bromoethylammonium bromide (53 mmole) and 25 ml of DMF were added. The mixture was stirred for 1 h. The precipitated crystals were dissolved by the addition of 100 ml of ammonia (29 %) and 50 ml of water. 21.3 g of S-ethylisothiuronium bromide²⁴ (115 mmole) were added under stirring in small portions during 15 min. The reaction mixture was left at room temperature over night in a well ventilated hood (large amounts of ethanethiol are evolved). A small precipitate was filtered off and the reaction mixture was poured dropwise into 1000 ml of ethanol under rapid stirring. A crystalline precipitate containing a small amount of coprecipitated unreacted S-ethylisothiuronium bromide was obtained. The substance was purified by dissolving it in 50 ml of water containing 2.3 g of lithium hydroxide hydrate (LiOH content 53 %, 50 mmole). 200 ml of ethanol were slowly added under rapid stirring. The precipitated crystals were filtered off. Yield: 6.5 g (56 %).

12. *Ammonium lithium S-(3-guanidinopropyl) phosphorothioate*. 11.5 g of Li_3SPO_3 , 5.5 H_2O (50 mmole) were dissolved in 150 ml of water. 12.0 g of 3-bromopropylammonium bromide (55 mmole) and 25 ml of DMF were added. After stirring for 2.5 h 50 ml of ammonia (29 %) were added. The reaction mixture was then treated with 13.9 g of S-ethylisothiuroniumbromide (75 mmole) as described under No. 11. The guanylated derivative was precipitated by the addition of 500 ml of ethanol. No further purification was needed in this case. Yield: 9.5 g (77 %).

13. *Tetralithium 1,3-dimercapto-2-propanol S_1, S_3 -diphosphate*. 11.5 g of Li_3SPO_3 , 5.5 H_2O (50 mmole) were dissolved in 100 ml of water. 3.0 ml of 1,3-dibromo-2-propanol (28 mmole) and 25 ml of DMF were added. The solution was stirred for 1.5 h and a precipitate was filtered off and discarded (mostly consisting of Li_3PO_4). 400 ml of ethanol were added to the clear filtrate and the crystalline precipitate was filtered off and washed with ethanol. It was first dried in the open air and then in vacuum at 4° (repeated drying in vacuum did not remove the ethanol of crystallization). Yield: 6.3 g (31 %).

14. *Tetralithium 2,3-dimercapto-1-propanol S_2, S_3 -diphosphate*. 11.5 g of Li_3SPO_3 , 5.5 H_2O (50 mmole) were dissolved in 100 ml of water. 2.95 ml of 2,3-dibromo-1-propanol (28 mmole) and 25 ml of DMF were added. The reaction mixture was set aside for 23 h at room temperature (this long reaction time was found necessary for quantitative reaction). A precipitate was filtered off and discarded. 400 ml of ethanol were added to the clear filtrate and the precipitated substance was filtered off. (Repeated drying in vacuum did not remove the ethanol of crystallization). Yield: 4.7 g (25 %).

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