# The Reaction Between the Phosphorothioate Ion and Substituted Alkyl Bromides
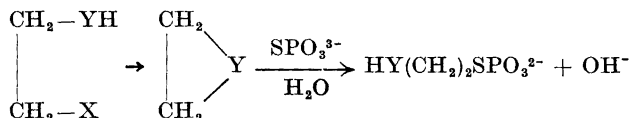
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The mechanism of reaction for the formation of S-substituted phosphorothioic acids from substituted alkyl bromides and the phosphorothioate ion has been investigated. Two types of mechanisms have been demonstrated to occur: (*a*) a direct reaction between the SPO_3^{3-} ion and the alkyl bromide:

$$SPO_3^{3-} + RBr \rightarrow RSPO_3^{2-} + Br^-$$
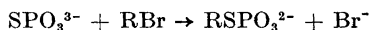
(*b*) cyclization of the substituted alkyl bromide followed by an addition of the phosphorothioate ion:

$$\begin{array}{c} CH_2-YH \\ | \\ CH_2-X \end{array} \rightarrow \begin{array}{c} CH_2 \\ | \quad \diagdown \\ | \quad \diagup Y \\ CH_2 \end{array} \xrightarrow[H_2O]{SPO_3^{3-}} HY(CH_2)_2SPO_3^{2-} + OH^-$$
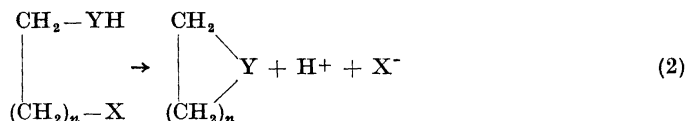
where YH represents a hydroxyl or an (substituted) aminogroup and X is a halogen. When YH represents an (substituted) aminogroup the immonium ion formed is the reactive ionic species.

It was shown in several preceding papers [1-4] that the phosphorothioate ion reacts with substituted alkyl bromides with the formation of S-substituted phosphorothioic acids according the over all formula:

$$SPO_3^{3-} + RBr \rightarrow RSPO_3^{2-} + Br^- \tag{1}$$

The present paper deals with an investigation of the mechanism(s) of reaction of (1).

It is well known [5,6] that alkyl halides containing a hydroxyl group or an (substituted) amino group (YH) under favorable conditions may form cyclic compounds according to scheme (2):
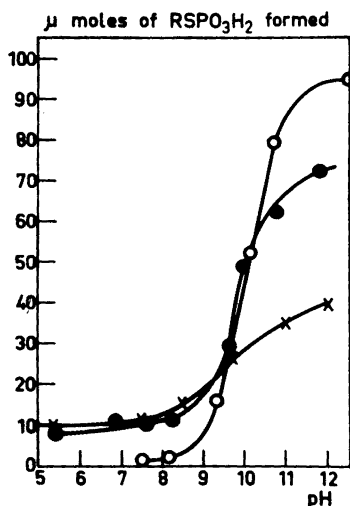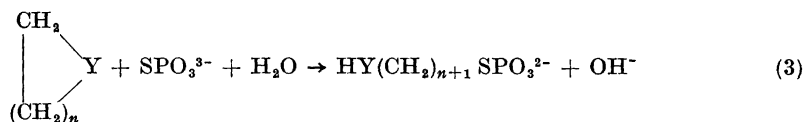
$$\begin{array}{c} CH_2-YH \\ | \\ (CH_2)_n-X \end{array} \rightarrow \begin{array}{c} CH_2 \\ | \quad \diagdown \\ | \quad \diagup Y \\ (CH_2)_n \end{array} + H^+ + X^- \tag{2}$$

μ moles of RSPO₃H₂ formed

Fig. 1. Rate of formation of S-substituted phosphorothioic acids from the phosphorothioate ion (25 mM) and 2-bromoethanol (50 mM; open circles), 3-bromo-1-propanol (50 mM; filled circles) and 2-bromoethyl trimethylammonium bromide (50 mM; crosses) as a function of pH. Incubation 30 min, 35°, at $I = 0.3$. Total volume 5 ml.

where X is a halogen. It is possible that reaction (1) can proceed *via* such cyclic intermediates (3):

$$\begin{array}{c} CH_2 \\ | \quad \diagdown \\ \quad \quad \diagup Y + SPO_3{}^{3-} + H_2O \rightarrow HY(CH_2)_{n+1} SPO_3{}^{2-} + OH^- \\ | \quad \diagup \\ (CH_2)_n \end{array} \qquad (3)$$

On the other hand, scheme (3) can not be the sole mechanism of reaction (1), since also compounds unable to form cyclic intermediates (like 2-bromoethyl trimethylammonium bromide) do react with the phosphorothioate ion [4].

METHODS AND RESULTS

The effect of pH on the rate of reaction between the phosphorothioate ion and the alkyl bromides was first investigated. The amount of S-substituted phosphorothioic acid formed was plotted as a function of pH of the reaction medium.

Two principally different types of curves were obtained. One type of curves was obtained when 2-bromoethanol, 3-bromo-1-propanol and 2-bromoethyl trimethylammonium bromide reacted with phosphorothioate (Fig. 1). These curves are all similar to titration curves and have an inflection point close to pH 9.8 *i.e.* at pK' of the $HSPO_3{}^{2-}$ ion under the experimental conditions *. If follows that the most reactive ionic species of the phosphorothioate ion in these cases is the $SPO_3{}^{3-}$ ion.

The other type of curves was obtained when 2-bromoethyl ammonium-bromide, 2-bromoethyl methylammonium bromide and 2-bromoethyl dime-

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* At 35° and $I = 0.3$ pK_3' was found to be 9.79 and pK_2' 5.19 (*cf.* Ref. [7]). The titration method used was the one described previously [12].

μ moles of RSPO$_3$H$_2$ formed

Fig. 2. Rate of formation of S-substituted phosphorothioic acids from the phosphorothioate ion (25 mM) and 2-bromoethylammonium bromide (50 mM; open circles; incubation time 30 min), 2-bromoethyl methylammonium bromide (50 mM; filled circles, incubation time 30 min) and 2-bromoethyl dimethylammonium bromide (50 mM; crosses; incubation time 5 min). Temperature: 35°. I = 0.3. Total volume 5 ml.
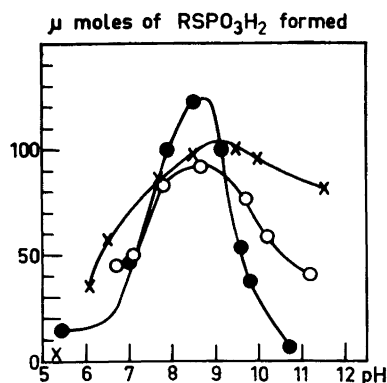
thylammonium bromide reacted with the phosphorothioate ion (Fig. 2). A maximal rate of formation of S-substituted phosphorothioic acid was observed near pH 8—9. Common to all alkyl bromides giving the last mentioned type of curves is that they (*a*) carry a nitrogen atom in the substituent; and (*b*) are able to form cyclic compounds according to scheme (2).

The curves illustrated in Fig. 2 could therefore be explained by presuming a reaction between the phosphorothioate ion and positively charged ions such as bromoalkyl ammonium or alkylimmonium ions.

To investigate the reaction mechanism(s) further it was desirable to compare the rates of reaction of (1) with the rate of liberation of bromide from the alkyl bromides in the absence of phosphorothioate but otherwise under conditions identical with the synthesis reaction. If the rate of liberation of bromide was found to be much slower than the rate of (1) the cyclization reaction (2) could in all likelyhood be excluded as an intermediary step of reaction (1).

Table 1. Half lives of alkyl bromides (12.5 mM) at 35° in buffers with ionic strength close to 0.3.

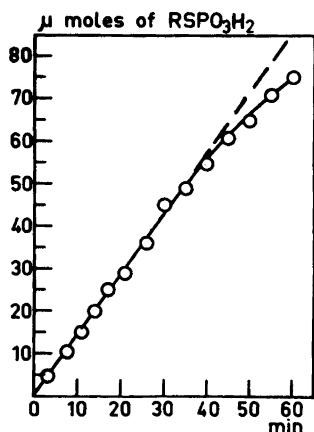| Alkyl bromide	pH	half life, min
HOCH$_2$CH$_2$Br	12	0.009 *
HOCH$_2$CH$_2$CH$_2$Br	12	3000
NH$_2$CH$_2$CH$_2$Br	8.0	31
NH$_2$CH$_2$CH$_2$Br + DMF (25 %)	8.0	14
CH$_3$NHCH$_2$CH$_2$Br	8.0	8.5
CH$_3$NHCH$_2$CH$_2$Br + DMF (25 %)	8.0	3.4
(CH$_3$)$_2$NCH$_2$CH$_2$Br	8.0	1.2
(CH$_3$)$_2$NCH$_2$CH$_2$Br + DMF (25 %)	8.0	0.3
(CH$_3$)$_3$$\overset{+}{N}CH_2CH_2$Br	12	840

* Estimated from data in Ref. [6].

Fig. 3. Demonstration of linearity of the rate of formation of cysteamine S-phosphoric acid (RSPO$_3$H$_2$) with time during the early part of the reaction between 2-bromoethyl ammonium bromide (12.5 mM) and Na$_3$SPO$_3$ (12.5 mM). Temperature: 35° and pH 8.0. Total volume 10 ml.

Table 1 summarizes the results of measurements of the alkyl bromides. Their half lives were measured at a pH where the over all reaction (1) proceeded with or close to maximal rate.

It is evident that the half lives of the bromides under the experimental conditions are short for all compounds with the exception of 3-bromo-1-propanol and 2-bromoethyl trimethylammonium bromide. The two last mentioned bromides are therefore likely to react with the phosphorothioate ion according to (1) *. This is especially true for 2-bromoethyl trimethylammonium bromide, which is unable to form a cyclic intermediate.

2-bromoethanol, on the other hand, is practically momentarily transferred to ethylene oxide [6] at pH 12, and must therefore react with the phosphorothioate ion exclusively according to (3) at this pH.

When determining rate of reaction constants for scheme (1), several side reactions must be taken into consideration, namely (*a*) Na$_3$SPO$_3$ is slowly hydrolyzed to sulfide and orthophosphate (at 53° and pH 8 the half life of Na$_3$SPO$_3$ is about 290 min[17]); (*b*) the alkyl bromides and/or their corresponding cyclic forms are decomposed by hydrolysis or by condensation reactions [16,18]. These factors resulted in maximum yields of S-substituted phosphorothioic acids varying from 50 to 95 %. An expression of the *initial* rate of reaction (1) could be obtained, however, by approximating the initial rate of reaction by a straight line (Fig. 3).

In Table 2 the initial rates of reaction are tabulated for different concentrations of alkyl bromide and phosphorothioate. The initial rate of formation of S-substituted phosphorothioic acid is approximately proportional to the concentration of alkyl bromide as well as to the concentration of phosphorothioate.

For the bromoalkylammonium bromides with a short half life there exists the possibility of a mixed reaction mechanism according to both (1) and (3).

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* However, there exists the remote possibility that the phosphorothioate ion catalyzes the cyclization reaction (2).

Table 2. Initial rates of formation of S-substituted phosphorothioic acids (v) as a function of alkyl bromide and phosphorothioate ion concentrations. Temperature 35°. Volume: 10 ml.

Reactants (mM)		pH	v μmoles min^{-1}
HOCH$_2$CH$_2$Br	Na$_3$SPO$_3$		
12.5	12.5	12	7.5
12.5	6.25	12	4.9
25.0	12.5	12	12
HOCH$_2$CH$_2$CH$_2$Br	Na$_3$SPO$_3$		
12.5	12.5	12	1.07
12.5	6.25	12	0.66
25.0	12.5	12	2.50
NH$_2$CH$_2$CH$_2$Br	Na$_3$SPO$_3$		
12.5	12.5	8.0	1.53
12.5	6.25	8.0	0.74
25.0	12.5	8.0	2.48
12.5 + DMF (25 %)	12.5	8.0	2.60
CH$_3$NHCH$_2$CH$_2$Br	Na$_3$SPO$_3$		
12.5	12.5	8.0	1.52
12.5	12.5	8.0	1.52 *
12.5	6.25	8.0	1.10
25.0	12.0	8.0	3.35
12.5 + DMF (25 %)	12.5	8.0	2.02
(CH$_3$)$_2$NCH$_2$CH$_2$Br	Na$_3$SPO$_3$		
12.5	12.5	8.0	10.1
12.5	12.5	8.0	10.6 *
12.5	6.25	8.0	5.70
25.0	12.5	8.0	14.0
12.5 + DMF (25 %)	12.5	8.0	16.2
(CH$_3$)$_3$$\overset{+}{N}CH_2CH_2$Br	Na$_3$SPO$_3$		
12.5	12.5	12	0.86
12.5	6.25	12	0.38
25.0	12.5	12	1.75
16.5	12.5	12	1.30
16.5 + DMF (25 %)	12.5	12	1.37

* The alkyl bromide was first converted to imino compound (by incubating for 6 half lives at pH 8.0 and 35°) and then allowed to react with Na$_3$SPO$_3$.

To investigate this question 2-bromoethyl methylammonium bromide and 2-bromoethyl dimethylammonium bromide were first incubated at pH 8 until they were calculated to be practically completely * converted into the cyclic forms. Na$_3$SPO$_3$ was then added and the initial rate of formation of S-substituted phosphorothioic acid was determined (Table 2). The experiment clearly shows that the cyclic forms do react with phosphorothioate at a rapid rate.

* That is under the assumption that the cyclic form did not decompose. 2-Bromoethyl ammonium bromide was not investigated due to its long half life.

It is, however, surprizing that the initial rates of formation of S-substituted phosphorothioic acids from the two cyclic imines and phosphorothioate are not much different from those obtained when the corresponding alkylbromides are reacted with phosphorothioate. This fact could be due to (*a*) the alkylbromides and the imines reacted with about the same rate with the phosphorothioate ion, or (*b*) much of the imines had decomposed during the incubation at pH 8 so that their concentrations were considerably lower than those of the initially added alkyl bromides.
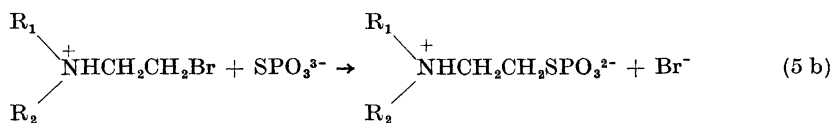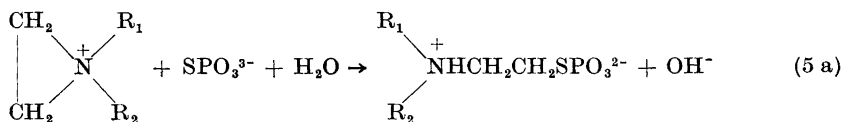
To get information about the rate of decomposition of the cyclic imines, 2-bromoethyl methylammonium and 2-bromoethyl dimethylammonium bromides (13 mM) were incubated at pH 8 and 35° until it was calculated that 98 % of the compounds should have been converted into cyclic forms. After another hour of incubation an excess of Na_3SPO_3 (molar ratio 1:1.5) was added and the formation of S-substituted phosphorothioic acids was followed until the reaction was terminated. The N,N-dimethyl ethyleneimmonium ion required 40 min for completion of the reaction and gave a yield of 63 % of S-substituted phosphorothioic acid. N-Methyl ethyleneimine required 2.5 h and gave a yield of S-substituted phosphorothioic acid of 49 %.

This experiment shows that the cyclic imines are decomposed at pH 8, but not at an extremely rapid rate.

It would thus appear that the rate measurements discussed above are best explained by presuming that the alkyl bromides and the cyclic imines are roughly equally reactive towards the phosphorothioate ion at pH 8. It can be calculated from the half lives of the aminoalkyl bromides (Table 1) that the rate of cyclization is greater than the initial rate of reaction (1). Reaction (3) will therefore rapidly become the dominating one.
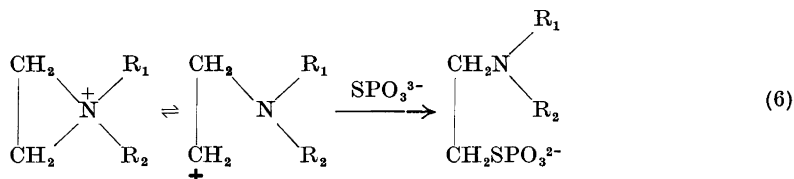
Since the results indicated the involvment of cyclic imines in the reactions of Fig. 2, the interpretation of the effect of pH becomes more complex. Only the uncharged forms of the aminoalkyl halogenides are converted to cyclic imines (see, *e.g.*, Ref.[5]). A change of pH of the reaction medium will therefore affect both the rate of cyclization and the rate of formation of S-substituted phosphorothioic acid.

The pH dependence of the rate of reaction between 2-bromoethyl ammonium, 2-bromoethyl methylammonium and 2-bromoethyl dimethylammonium bromides and the phosphorothioate ion (Fig. 2) can, however, be explained by the following reactions:

$$\begin{array}{c}CH_2 \\ | \quad \overset{R_1}{\diagdown} \\ | \quad \overset{+}{N} \\ | \quad \diagup \\ CH_2 \quad R_2\end{array} + SPO_3{}^{3-} + H_2O \rightarrow \begin{array}{c}R_1 \\ \diagdown \overset{+}{} \\ NHCH_2CH_2SPO_3{}^{2-} + OH^- \\ \diagup \\ R_2\end{array} \qquad (5\ a)$$

$$\begin{array}{c}R_1 \\ \diagdown \overset{+}{} \\ NHCH_2CH_2Br \\ \diagup \\ R_2\end{array} + SPO_3{}^{3-} \rightarrow \begin{array}{c}R_1 \\ \diagdown \overset{+}{} \\ NHCH_2CH_2SPO_3{}^{2-} + Br^- \\ \diagup \\ R_2\end{array} \qquad (5\ b)$$

(5 a) and (5 b) should give rate maxima at approximately pH $= 1/2$ $(pK'_1 + pK'_2)$ * where K'_1 is the dissociation constant of the ammonium or immonium ion and K'_2 is the dissociation constant of the $HSPO_3^{2-}$ ion.

Scheme (5 a) is supported by the results of Earley et al.[14] who found that several different nucleophiles add to the *immonium* ion of substituted ethylene imines. The function of the positive charge of the immonium ion is likely to be represented by (6) as suggested for similar cases [15,16]:

$$
\begin{array}{ccc}
\underset{\underset{+}{\overset{|}{CH_2}}}{\overset{CH_2}{|}}\diagdown\underset{N}{\overset{R_1}{\diagup}}\diagdown R_2 & \underset{\underset{+}{\overset{|}{CH_2}}}{\overset{CH_2}{|}}\diagdown\underset{N}{\overset{R_1}{\diagup}}\diagdown R_2 & \underset{CH_2SPO_3^{2-}}{\overset{CH_2N}{|}}\diagup\overset{R_1}{\diagdown}R_2
\end{array} \qquad (6)
$$

The pK' values for ethyleneimine is 7.8 (35°) [8], for N-methyl ethyleneimine 7.9 (25°)[8], for 2-bromoethylamine 8.5 (24°) [13], and for 2-bromoethyl dimethylamine 8.0 (estimated) [19].

Rate of reaction maxima for (5 a) and (5 b) should thus be expected to be found around pH 9. The position of the theoretical rate of reaction maximum agrees well with the experimental values (Fig. 2).

A reaction between uncharged amines and imines and the $HSPO_3^{2-}$ ion could also explain the pH optimum obtained in Fig. 2. However, since the ion (7), which is unable to form an uncharged imino

$$
\underset{\underset{+}{\overset{|}{CH_2}}}{\overset{CH_2}{|}}\diagdown\underset{N}{\overset{CH_3}{\diagup}}\diagdown CH_3 \qquad (7)
$$

form, reacts rapidly with phosphorothioate, (5 a) is the only possible mechanism. By analogy (5) should also be the major mechanism of reaction for the other immonium and ammonium compounds studied here. As was shown to be the case for the compounds in Fig. 1, the SPO_3^{3-} ion then becomes the reactive ionic species of phosphorothioate.

Since 2-bromoethyl dimethylamine is cyclisized at a very high rate, the ion (7) will rapidly become the main reactive species. The reaction between this ion and phosphorothioate will not be expected to decrease at high pH values since a dissociable proton is lacking. The observed decrease in reaction rate above pH 9 (Fig. 2) is therefore most likely due to destruction of (7) by hydroxyl ion attack [16,18].

The effect of N,N-dimethyl formamide. It was earlier shown [1] that N,N-dimethyl formamide (DMF) catalyzes the over all reaction (1) when RBr is 2-bromoethylamine. As is seen from Table 2 the presence of 25 % of DMF (by volume) approximately doubles the initial reaction rate. In the case of 2-bromoethyl trimethylammonium bromide, however, hardly any catalytic

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* This expression can easily be derived from the equation: rate of reaction $=$ constant $[SPO_3^{3-}]$ [immonium ion]. The concentration of the species $H_2SPO_3^-$ and H_3SPO_3 were neglected in the derivation.

effect of DMF was observed. The presence of DMF about doubles the rate of cyclization of 2-bromoethyl amine (Table 1). A likely explanation of these results is therefore that reaction (1) *per se* is not catalyzed by DMF, but that the cyclic immonium ion, being the more reactive species in this case, is formed at a faster rate in the presence of DMF.

The rate of reaction between 2-bromoethyl methylamine and phosphorothioate is only slightly catalyzed by DMF (Table 2) in spite of the fact that the cyclization of this amine is strongly catalyzed by DMF (Table 1). This result strongly supports the earlier conclusion that the 2-bromoethyl methylammonium and the N-methylethylene immonium ions are roughly equally reactive towards phosphorothioate. The same conclusion applies to 2-bromoethyl dimethylammonium bromide, although the difference in reactivities is somewhat greater in this case.

EXPERIMENTAL

The alkyl bromides used were prepared as described previously [4]. *Trisodium phosphorothioate* was prepared by an earlier reported procedure [3]. The preparation used was 99.7 % pure as judged by phosphate and sulfur determinations [9].

Determination of reaction rates

1. Effect of pH. 125 μmoles of Na_3SPO_3 and 250 μmoles of alkyl bromide were dissolved in 5.0 ml of buffers prewarmed to 35° and having an ionic strength close to 0.3. The solutions were incubated at 35.0 $\pm$ 0.2°, usually for 30 min.

The amounts of S-substituted phosphorothioic acids formed were determined in the following way: two samples (0.1 ml) were withdrawn from the reaction mixture. 1 M HCl (0.9 ml) was added to both samples, which were then heated in a boiling water bath for 2 min. This accomplished a quantitative hydrolysis of the S-substituted phosphorothioic acids. The liberated thiols were determined colorimetrically according to Grunert and Phillips [10]. The color yield of the various thiols had earlier been determined by hydrolysis of the corresponding pure S-substituted phosphorothioic acids, prepared according to Ref [4]. A correction was made for traces of H_2S liberated by acid hydrolysis of unreacted Na_3SPO_3.

The pH of the reaction mixture was determined before and after the reaction. The readings maximally differed $\pm$ 0.1 pH unit from the average value.

2. Determination of initial reaction rates. The alkyl bromide and Na_3SPO_3 (for amounts taken see Table 2) were dissolved in 10 ml of prewarmed buffers (ionic strength close to 0.3). After incubation of the reaction mixture at 35.0 $\pm$ 0.2° for various lengths of time samples were withdrawn and analyzed for S-substituted phosphorothioic acids as described under 1 above. 15 to 20 samples were withdrawn for each run.

Determination of the rate of liberation of bromide from the alkyl bromides. 1.25 mmoles of alkyl bromide were dissolved in 100 ml of buffer ($I = 0.3$) of 35°. After incubation at 35.0 $\pm$ 0.2 for various lengths of time 10 ml samples were withdrawn. Excess acetic acid was immediately added and bromide was determined titrimetrically [11]. 9 samples were analyzed for each run.

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