Synthesis of Potential Radioprotective Compounds

1. Studies on the Reaction between 2-Methylaziridine, 1-Amino-3-chloro-2-propanol, 3-Amino-2-bromo-1-propanol and the Phosphorothioate Ion Including Structural Investigations of the Compounds Formed

STIG ÅKERFELDT

Pharmaceutical Chemistry Section, Research Institute of National Defence, Dept 1, Sundbyberg 4, Sweden

1. 2-Methylaziridine was shown to react with the phosphorothioate ion in such a manner that the primary carbon-nitrogen bond is broken yielding S-(2-aminopropyl) phosphorothioate.
2. It was shown that 1-amino-3-chloro-2-propanol and 3-amino-2-bromo-1-propanol are both easily cyclisized to 2-aminomethyl oxirane. This compound reacts with the phosphorothioate ion in such a manner that the primary carbon-oxygen bond is broken yielding S-(3-amino-2-hydroxypropyl) phosphorothioate.
3. The isolated reaction products showed considerable radioprotective properties in mice.

In some respects compounds containing a phosphorylated thiol group appear to be superior to the corresponding thiols when used as radioprotectors. For instance, Airapetyan and Zherebchenko [1] have found that cysteamine S-phosphate is an appreciably more potent protector when given orally to mice than is cysteamine. Secondly, cysteamine S-phosphate when injected subcutaneously or intramuscularly, does not, like sometimes cysteamine, cause a local inflammatory-necrotic reaction in the injected animals.[1]

The radioprotective effects of S-phosphorylated thiols are, as expected, much dependent on the substituents present in the alkyl group.[2] Thus, it was shown earlier that in a series of compounds of the general formula $R(CH_2)_n$-SPO_3^{2-} ($n = 2$ or 3), those where R is an unsubstituted amino group or a guanidino group showed radioprotective effects. When R is hydrogen, an alkylsubstituted amino group, a hydroxyl, or a carbamoyl group, no protective effects were observed.[2]

The object of the present investigation was to obtain information of the effect of certain substituents on the radioprotective properties of S-aminopropyl phosphorothioic acid.

Synthesis of dilithium S-(2-aminopropyl) phosphorothioate

Usually aminoalkyl halides react rapidly with the SPO_3^{3-} ion with the formation of S-substituted phosphorothioic acids. With 1-amino-2-chloropropane, however, this is not the case. This aminoalkyl chloride reacts extremely slowly, if at all.

In earlier studies [3] it was shown that the aziridine derivatives, formed by cyclisation of aminoalkyl halides, are often more reactive towards the SPO_3^{3-} ion, than the original aminoalkyl halides. 2-Chloropropyl amine was therefore first converted to 2-methylaziridine by heating in strongly basic solution. Since it is only the aziridinium form of the compound that would be expected to react with the SPO_3^{3-} ion,[3] the pH of the solution must after the cyclisation be brought to a value where both the SPO_3^{3-} and the aziridinium ions exist. The theoretical pH-value for optimal reaction is defined by pH $= 1/2$ $[pK(HSPO_3^{2-}) + pK(aziridinium)]$. Since $pK(HSPO_3^{2-}) = 9.8$ and pK(aziridinium) ≈ 8.0 (estimated) the optimal pH for reaction between the two ionic species shold be at pH 8.5—9.
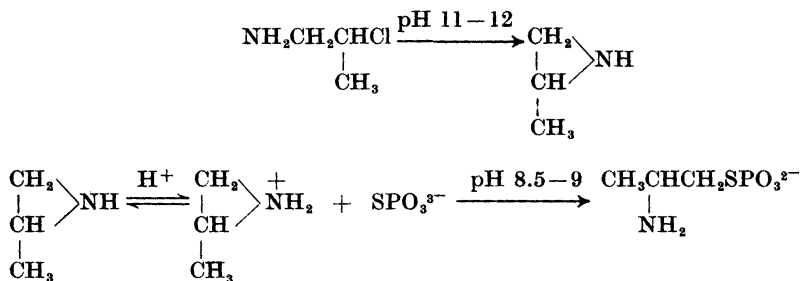
The above reaction scheme has been shown to work excellently, and a quantitative reaction of the SPO_3^{3-} ion was obtained. As in earlier studied reactions, it was found advantageous to isolate the synthesized compounds as the lithium salts, since small amounts of orthophosphate formed are easily removed as sparingly soluble Li_3PO_4.

However, since nucleophilic attack by the SPO_3^{3-} ion on the aziridinium ion can occur in two ways, yielding two isomeric compounds, the structure of the product formed had to be established.

This was accomplished by hydrolyzing the compound to orthophosphate and a thiol followed by sulfur removal using Raney nickel catalyst. The resulting propylamine was coupled to 2,4-dinitrofluorobenzene and the derivative thus obtained was subjected to paper chromatographic analysis using dimethylformamide as the stationary phase.

With the solvent used, the 2,4-dinitrophenyl derivatives of 1-propylamine and 2-propylamine show $R_F = 0.23$ and 0.39, respectively. Only the presence of 2-propylamine could be demonstrated in this way. The synthesized compound was thus identified as the lithium salt of S-(2-aminopropyl)phosphorothioic acid.

Recently Klayman et al.[4] have shown by NMR studies that the 2-methyl-aziridinium ion reacts with the thiosulfate ion in an analogous manner to the phosphorothioate ion giving exclusively S-(2-aminopropyl)thiosulfate.

The reaction between phosphorothioate, 1-amino-3-chloro-2-propanol and 3-amino-2-bromo-1-propanol

In the presence of dimethylformamide (DMF) 1-amino-3-chloro-2-propanol and 3-amino-2-bromo-1-propanol react rapidly and quantitatively with the phosphorothioate ion yielding aminomercaptopropanol S-phosphates. From a study of the mechanism of this reaction (cf. Ref. 3) it became apparent that the two aminohalopropanols used in the synthesis rapidly form cyclic products containing oxiran and/or aziridine rings. In addition to the aminohalopropanols the new cyclic intermediates react with the phosphorothioate ion rapidly.

At pH 11 and in the presence of DMF the formation of cyclic intermediates is a very fast process (Table 1), whereas the rate of formation of S-phosphoryl-

Table 1. Half lives of various substituted alkylhalogenides (0.05 M solutions) with and without dimethylformamide (DMF).

Compound	Half life at 23° and pH 11; min
$ClCH_2CH(OH)CH_2NH_2$ + 25 % DMF	9.0 7.0
$HOCH_2CHBrCH_2NH_2$ + 25 % DMF	8.0 1.7
$BrCH_2CH_2CH_2NH_2$ + 25 % DMF	210
$NH_2CH_2CH_2Br$ + 25 % DMF	10.5
$BrCH_2CH_2OH$ + 25 % DMF	6.3
$CH_3CHClCH_2NH_2$ + 25 % DMF	600 550

ated thiols is not as rapid. In consequence, the cyclic intermediates will soon become the dominant species reacting with phosphorothioate. The reaction products might therefore consist of mixtures of several possible isomers or indeed be identical in structure (Fig 1). An investigation of the actual relative positions of the three substituents in the compounds therefore became necessary.
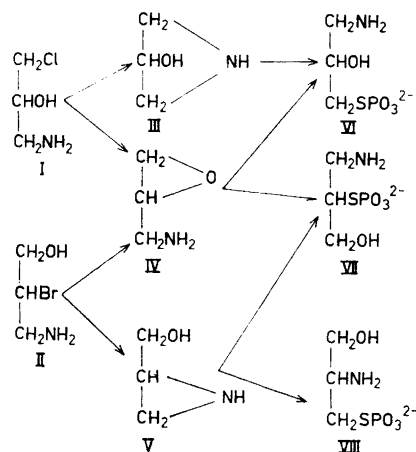
$$
\begin{array}{ccccc}
& & \text{CH}_2 & & \text{CH}_2\text{NH}_2 \\
\text{CH}_2\text{Cl} & & \text{CHOH} \quad \text{NH} \longrightarrow & \text{CHOH} \\
\text{CHOH} & & \text{CH}_2 & & \text{CH}_2\text{SPO}_3{}^{2-} \\
\text{CH}_2\text{NH}_2 & & \text{III} & & \text{VI} \\
\text{I} & & \text{CH}_2 & & \text{CH}_2\text{NH}_2 \\
& & \text{CH} \quad \text{O} & & \text{CHSPO}_3{}^{2-} \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{NH}_2 & & \text{CH}_2\text{OH} \\
\text{CHBr} & & \text{IV} & & \text{VII} \\
\text{CH}_2\text{NH}_2 & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
\text{II} & & \text{CH} & & \text{CHNH}_2 \\
& & \text{CH}_2 \quad \text{NH} & & \text{CH}_2\text{SPO}_3{}^{2-} \\
& & \text{V} & & \text{VIII}
\end{array}
$$

Fig. 1. Possible routes for the formation of S-substituted phosphorothioic acids from cyclic intermediates.

Structural investigations. The first problem to be investigated was the homogeneity of the two preparations. The phosphorothioates obtained from 1-amino-3-chloro-2-propanol (I) and from 3-amino-2-bromo-1-propanol (II) were converted to the corresponding thiols (by acid hydrolysis) and coupled to 2-chloromercuri-6-nitrophenol [5] and chromatographed using several solvent systems described in Ref. 5. In each case only one thiol spot was observed and the thiols obtained from I and II, respectively, showed identical positions on all chromatograms.

The two preparations were then converted to the corresponding disulfides (by iodine oxidation) and these were reacted with naphthylisocyanate in DMF. Only one derivative could be isolated in each case having identical melting points (m.p. 267°, decomp.) undepressed by admixture with each other.

IR studies of the phosphorothioates, their corresponding thiols and disulfides gave further indications that the two phosphorothioates obtained from I and II were homogeneous and indeed identical.

NMR-Spectra in D_2O and in dimethylsulfoxide-d_6 of the two thiols were identical. The shift * of the hydrogen linked to the secondary carbon (in dimethylsulfoxide-d_6) was τ *ca.* 6.3 ppm and thus very different from $>$C*H*NH$_2$, which usually is found around $\tau = 7.2$ ppm in similar propane derivatives. On the other hand $>$C*H*SH and $>$C*H*OH is usually found between 6.0−6.8 ppm. These NMR studies thus indicated the product to be either VI or VII (Fig. 1).

The final structural proof was obtained by removing the sulfur of the thiol by heating with Raney nickel followed by paper chromatographic analysis of the aminopropanol formed. In this way only 1-amino-2-propanol was detected, thus showing that the phosphorothioates obtained from I and II are identical and have the structure VI.

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* Relative TMS as external reference.

DISCUSSION

The fact that identical products are obtained shows that the bromide II does not react with the phosphorothioate ion to any appreciable extent. No trace of 3-amino-1-propanol could be detected after Raney nickel treatment of the corresponding thiol. II is rapidly cyclisized (Table 1) evidently giving IV, which is opened (by SPO_3^{3-}-ion attack) between the primary carbon and the oxygen. Thus the oxiran ring is opened in the same manner as is the aziridine ring in 2-methylaziridine.

The chloride I could principally react in three ways with phosphorothioate 1) by direct displacement of chloride; 2) through cyclic product III; and 3) through cyclic product IV. To investigate this question further, solutions of I and II were treated with DMF at pH 11 and incubated until more than 95 % of the substances should have been converted to cyclic forms. After paper chromatography the sheets were sprayed with a weak solution of sodium phosphorothioate. After some minutes, spraying with silver ions revealed the reacted areas as white spots on a black background.

I gave only one spot identical in position to the major spot of II. II also contained a minor spot. From this experiment it would seem likely that I is cyclisized only via IV. The bromide II appears possibly to give rise to a small amount of V (the minor spot). If so, its reaction products with the phosphorothioate ion seem to have been removed by the purification process during isolation of the substance.

The above assumptions regarding the nature of the cyclic intermediates are supported by the data in Table 1. Thus, the half life of 1-amino-3-bromopropane is much longer than those of 2-bromoethanol and 2-bromoethylamine. This fact makes the intermediary formation of III from I less likely. Similarly, oxiran ring formation is favoured from the bromide II, since 2-bromoethanol is more rapidly cyclisized than 2-bromoethylamine.

Radioprotective properties. When tested in mice (50—60 μmole per animal) the prepared compounds showed good radioprotective properties. S-(2-Aminopropyl) phosphorothioate was about as effective as cysteamine giving dose reduction factors of 1.86 and 1.84, respectively. S-(3-Amino-2-hydroxypropyl) phosphorothioate was more effective giving a dose reduction factor of 2.16. A full report of the radioprotective properties of these and similar compounds will appear elsewhere.

EXPERIMENTAL

IR-spectra were recorded on a Perkin Elmer Model 225 instrument. For the NMR spectra a Varian Model A60A high resolution instrument was used.

2-Chloropropylammonium chloride. 75 g (1 mole) of 1-amino-2-propanol, dissolved in 150 ml of chloroform, were treated with a slow stream of hydrogen chloride for 2.5 h. 60 g (0.5 mole) of thionyl chloride were added dropwise under stirring during 1 h whereafter the mixture was heated to reflux for a few minutes. Another portion of thionyl chloride (72 g, 0.6 mole) was then added under stirring and refluxing. Heating was continued until the mixture become too thick to be stirred. Excess thionyl chloride was removed in a rotating evaporator. The product was recrystallized from 400 ml of acetone and 40 ml of ethanol. Yield 50 g (39 %), m.p. 182—185°. (Found: C 27.6; H 6.7; N 11.1. Calc. for $CH_3CHClCH_2NH_3Cl$ (130): C 27.6; H 6.9; N 10.8).

1-Amino-3-chloro-2-propanol hydrochloride was prepared according to Gabriel and Ohle,[6] m.p. 107—109° (Ref. 6 gives m.p. 103—104°).

3-Amino-2-bromo-1-propanol hydrobromide was prepared according to Ball et al.[7] m.p. 107-108°.

Synthesis of dilithium S-(2-aminopropyl) phosphorothioate. 13.0 g (0.1 mole) of 2-chloropropylammonium chloride and 10.4 g (0.2 mole) of lithiumhydroxide monohydrate were dissolved in 100 ml of water and heated to boiling under reflux for 5 min. After cooling to 20° 17.1 g (0.08 mole) of hydrated trilithium phosphorothioate[8] and 50 ml of water were added. Glacial acetic acid was added to pH 8.5 (glass electrode). This pH-value was maintained throughout the reaction. The solution was stirred for 1 h at 20—23°, after which time the silver ion test[8] indicated that all the phosphorothioate had reacted. Solid lithium hydroxide monohydrate was then added to pH 11. A small amount of precipitated trilithium phosphate was filtered off and 600 ml of methanol was added to the clear filtrate. To facilitate filtration the mixture was chilled in an ice box for 1 h and then filtered. The precipitate was washed with methanol and dried in vacuum. Yield 8.2 g (45 %). (Found: C 19.8; H 4.4; N 7.68; S 17.8; P 16.5. Calc. for $CH_3CH(NH_2)CH_2SPO_3Li_2$ (183.0): C 19.7; H 4.4; N 7.64; S 17.5; P 16.9.)

Synthesis of dilithium S-(3-amino-2-hydroxypropyl)phosphorothioate. 1. From 1-amino-3--chloro-2-propanol. 12.7 g (50 mmole) of hydrated trilithium phosphorothioate were dissolved in 100 ml of water. 8.05 g (55 mmole) of 1-amino-3-chloro-2-propanol hydrochloride and 25 ml of dimethylformamide were added. The solution was adjusted to pH 11 with a saturated solution of lithium hydroxide monohydrate and maintained at this pH during the reaction by successive additions of lithium hydroxide solution. After 1.5 h of stirring the silver ion test[8] for unreacted phosphorothioate was negative. 700 ml of ethanol were then added and the precipitate filtered off and washed with ethanol. The substance was redissolved in 100 ml of water and reprecipitated by the addition of 500 ml ethanol. After drying in vacuum the yield was 7.3 g (73 %).
(Found: C 18.2; H 4.2; N 7.1; S 16.2; P 15.2; Li 7.1. Calc. for $NH_2CH_2CH(OH)CH_2SPO_3Li_2$ (199): C 18.1; H 4.1; N 7.0; S 16;1; P 15.6; Li 7.0.)

2. From 3-amino-2-bromo-1-propanol. 8.52 g (40 mmole) of hydrated trilithium phosphorothioate were dissolved in 80 ml of water. 10.4 g (44 mmole) of 3-amino-2-bromo-1-propanol hydrobromide and 20 ml of dimethylformamide were added. The solution was adjusted to pH 11—11.5 with saturated solution of lithium hydroxide monohydrate and maintained at this pH during the reaction by successive additions of lithium hydroxide solution. After 1 h of stirring the silver ion test[8] for unreacted phosphorothioate was negative. 400 ml of ethanol were added and the precipitate was purified as under 1 above. Yield: 4.8 g (60 %). (Found: C 18.2; H 4.2; N 7.2; S 16.1; P 15.4; Li 7.1).

Desulfurisation of the compounds with Raney nickel. About 500 mg of the S-substituted phosphorothioic acid salt were dissolved in 30 ml of water and the solution was adjusted to pH 4—5 (1 M HCl) and refluxed for 10 min. This accomplished a quantitative hydrolysis of the S—P bond in the compounds. The solution was then neutralized to pH 7 (2 M NaOH) and about 1—2 g of Raney nickel[9] was added. The mixture was refluxed for 6 h. The resulting alkylamines were either chromatographed as such or as their 2,4-dinitrophenyl derivatives (see below).

Paper chromatography of 2,4-dinitrophenyl propylamines. The amine solution obtained by Raney nickel reduction of the thiol from the reaction product between 2-methylaziridine and phosphorothioate was made basic with an excess of triethylamine. An equivalent amount of 2,4-dinitrofluorobenzone was added. After 10 min of stirring the reaction product was extracted into ethyl acetate and chromatographed on dimethylformamide impregnated paper (Whatman No. 1) using hexane as the solvent. (For R_F-values see p. 1784).

Paper chromatography of propanolamines was carried out on Whatman No. 1 paper impregnated with 0.1 M sodium borate-HCl buffer of pH 8.7. Solvent system: butanol:ethanol:water, 8:2:1. After development the sheet was sprayed with ninhydrin (0.1 %) in ethanol containing acetic acid (10 %). With this method it is possible to separate 3-amino-1-propanol ($R_F = 0.16$) from 1-amino-2-propanol ($R_F = 0.30$). 2-Amino-1--propanol ($R_F = 0.16$) is separated from the other isomers ($R_F = 0.42$) by the same solvent, but using unimpregnated paper.

Paper chromatography of the cyclic products obtained from the aminoalkylhalides were carried out with the solvent butanol:pyridine:water, 1:1:1.

Half lives of the aminoalkylhalides were determined by titration of the liberated hydrogen ions at constant pH and also by the method previously described.[3]

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