

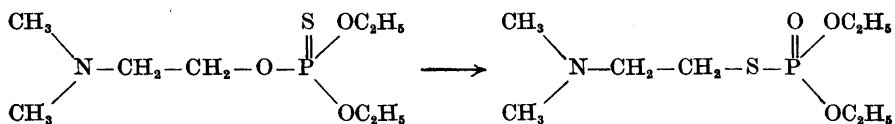
## Isomerisation of $\omega$ -Dimethylaminoethyl-diethyl-thionophosphate

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The structures of  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate and the thio isomer  $S,\omega$ -dimethylaminoethyl-diethyl-thiolophosphate, have been studied by infrared spectra. Some spectra of related compounds are given. Isomerisation of the thiono compound has been shown by means of infrared spectrophotometry and paper chromatography to yield  $S,\omega$ -dimethylaminoethyl-diethyl-thiolophosphate. The reaction rate at 100°C of the isomerisation has been studied. Two tentative reaction mechanisms are given for the isomerisation.

Organophosphorus compounds containing an  $\omega$ -dimethylaminoethylthio group as, e.g.,  $S,\omega$ -dimethylaminoethyl-diethyl-thiolophosphate have been described as potent insecticides and cholinesterase inhibitors<sup>1, 2</sup>. Reports have been given about their synthesis<sup>1-3</sup> and among the methods is isomerisation at elevated temperature of  $\omega$ -dialkylaminoethyl-diethyl-thionophosphate<sup>3</sup>.



Few details, however, have been given about the rearrangement from thiono to thio ester and no evidence has been given for the position of the sulfur atom. Since this reaction seems interesting both in connection with the syntheses of compounds of this type and with the confirmation of their structures, it was decided to study the reaction rate semiquantitatively with spectrophotometry and to investigate the reaction products qualitatively with paper chromatography. Infrared spectrophotometry seemed to be an applicable method for the kinetic studies as well as for a confirmation of the structures in general. It was thus decided to record spectra of  $S,\omega$ -dimethylaminoethyl-diethyl-thiolophosphate and related compounds.

## METHODS

## Synthesis of dimethylaminoethyl-diethyl-thionophosphate

One mole of diethoxy-thionophosphorylchloride and one mole of sodium dimethylaminoethanolate are mixed in benzene at room temperature. The sodium chloride formed is filtered off, and the filtrate is fractionated under reduced pressure by distillation. Yield 60 %. B.p.  $90^{\circ}/0.1$  mm  $n_D^{20} = 1.4590$ .

The compound was purified as the oxalate. One mole of dimethylaminoethyl-diethyl-thionophosphate (25 % solution in methanol) and 1.05 moles of oxalic acid (10 % solution in methanol) were mixed and the methanol evaporated. Recrystallisation from acetone gives colorless crystals M.p.  $132^{\circ}\text{C}$  (Kofler Heizbank). (Found: C 36.1; H 6.6. Calc. for  $\text{C}_{16}\text{H}_{22}\text{NO}_7\text{PS}$  (331.3): C 36.3; H 6.7).

The oxalate of  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate was titrated in 0.10 M KCl with sodium hydroxide as described for analogous compounds in an earlier paper <sup>2</sup>. The mixed dissociation constant, as  $\text{p}K'_a$ , was determined by the curve fitting method <sup>4</sup>. The calculated amount of sodium hydroxide was found to be consumed. The thermodynamic dissociation constant, was estimated as in the earlier paper <sup>2</sup>.

## Infrared spectra and photometry

Spectra of  $\omega$ -dimethylaminoethyl-diethylthionophosphate, S- $\omega$ -dimethylaminoethyl-diethyl-thiophosphate and related compounds were determined with a Perkin and Elmer Mod. 21 spectrophotometer. The spectra were obtained using the following solvents: carbon tetrachloride between 2 and 12  $\mu$ , benzene between 12 and 14  $\mu$  and carbon tetrachloride between 14 and 15  $\mu$ . The solutions were 0.3 M.

In order to study the rearrangement of  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate, samples were taken at different lengths of time after the preparation of the pure compound, which was stored at  $100^{\circ}\text{C}$ . These samples were studied spectrophotometrically between 6 and 9  $\mu$ . Transmittance was determined at 7.95  $\mu$ . The refractive index of each sample was determined by an Abbe refractometer. Standard curves for the correlation of concentrations to I.R. transmittance ( $T_{7.95}$ ) and to refractive index ( $n_D^{20}$ ) were determined by mixing S- $\omega$ -dimethyl-aminoethyl-diethyl-thiophosphate <sup>2</sup> with  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate in different proportions. The spectrophotometer was used with a sodium chloride prism, resolution = 927, response = 2 : 1170, gain = 5, speed = 1  $\mu/\text{min}$ , suppression = 3 and scale 5  $\text{cm}/\mu$ . The thickness of the cell was 0.116 mm.

## Paper chromatography of the thio and thiono ester

*Solvent* <sup>5</sup>: *n*-butanol 8, ethanol 2, acetic acid 2 and water 3 parts by volume.

*Reagent* <sup>5</sup>: 0.2 g of dipicrylamine is dissolved in 50 ml of acetone and 50 ml of water (Reacts with the amino group).

*Paper*: Whatman 1. Height 35 cm.

*Procedure*: Spots of 10  $\mu\text{l}$  of about 1 % solution of the samples were dried with hot air. The paper was equilibrated with air in equilibrium with the solvent for 1 h. An ascending technique was used at  $25^{\circ}\text{C}$  for 17 h. After drying with hot air the paper was sprayed with the reagent.

## Paper chromatography of hydrolysis products

An approximately 10 % solution of S- $\omega$ -dimethylaminoethyl-diethyl-thiophosphate was hydrolyzed at  $50^{\circ}\text{C}$  overnight in 0.1 M sodium hydroxide solution. The same experiment was done with the isomerized thiono compound. The hydrolysis products together with  $\omega$ -dimethylaminoethanthiol were investigated as follows.

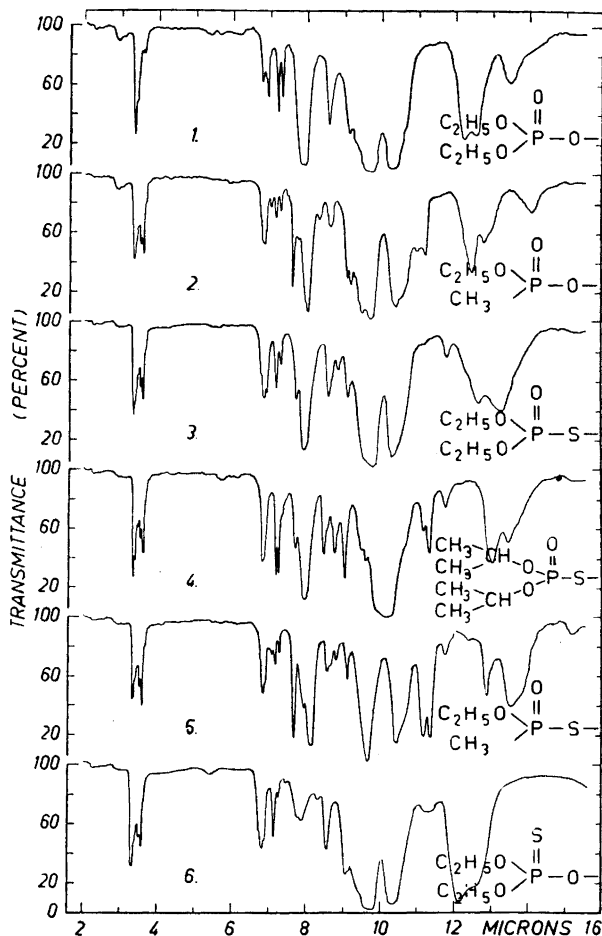


Fig. 1. Infrared spectra of  $\omega$ -dimethylaminoethyl esters of organophosphorus acids.

*Solvent:* *n*-butanol 8, ethanol 2, acetic acid 1 and water 3 parts by volume.

*Reagent:* Grotes reagent<sup>9</sup> (SH-reagent).

*Paper:* Whatman 1. Height 35 cm.

*Procedure:* Spots of 10  $\mu\text{l}$  of about 10 % solution of the three samples were dried with hot air. The paper was equilibrated with air in equilibrium with the solvent for 1 h. An ascending technique was used at 25°C for 17 h. After drying with hot air the paper was sprayed with the reagent. The papers were kept at 100°C for 5 min. Parallel tests were performed and developed by the dipicrylamine reagent<sup>5</sup> which colors amino groups.

#### Determination of the cholinesterase inhibiting effect ( $pI_{50}$ )

The cholinesterase inhibiting effect of  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate was determined by means of an electrometric method. All conditions were identical with

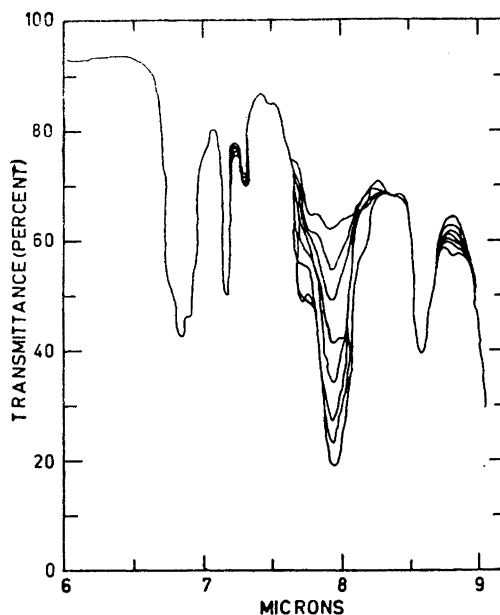


Fig. 2. Infrared spectra showing the isomerisation at 100°C of  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate. Samples taken at different times show increasing phosphoryl concentration by the decreased transmittance at 7.95 microns.

those in experiments with the thiolester<sup>2</sup>. Human erythrocytes and plasma were used as enzyme sources and acetylcholine as substrate. The results are expressed as the negative logarithm of the inhibitor concentration which decreases enzyme activity to 50 % ( $pI_{50}$ ).

## RESULTS

The infrared spectra obtained (Fig. 1) are in good agreement with those obtained from related compounds in earlier literature<sup>6,7</sup>. They thus need few comments. However, at 11.76  $\mu$  the thiole compounds show a weak absorption which seems characteristic. It has not been possible to correlate this band with some special part of the molecular structure. The phosphoryl group, as seen in Fig. 1, gives a strong absorption near 8  $\mu$ <sup>6-8</sup> which is not found in the thiono compounds, although a weak absorption in this region indicates contamination with thiole isomer presumably formed during the distillation. Spectrophotometric measurements made in this region during the reaction were used for the study of the rearrangement. The result of the kinetic investigation is shown in Fig. 2 where the increasing concentration of a phosphoryl group is demonstrated and in Fig. 3 which shows the reaction rate. The specific change in the infrared spectra shown in Fig. 2 and the relatively good agreement of the results from different methods shown in E (Fig. 3) demonstrate that the reaction product is rather homogeneous. If the process is allowed to proceed for more than 5 h at 100°C, side reactions become obvious. The

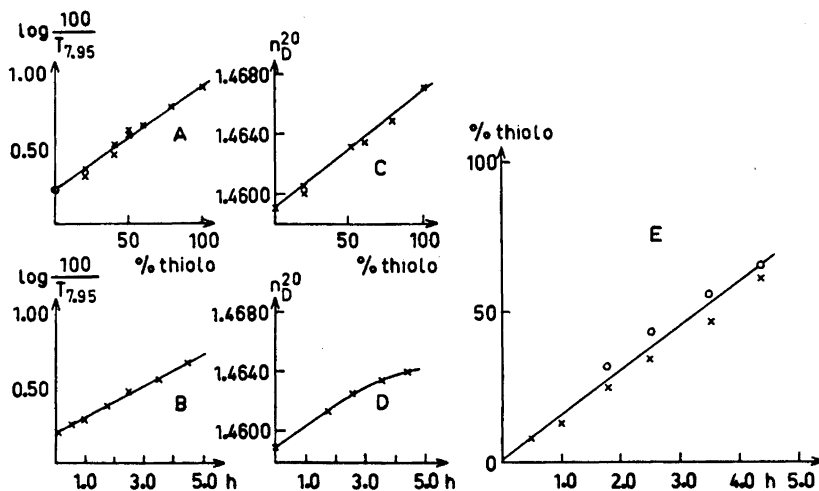


Fig. 3. The diagrams show the time dependence of the isomerisation of  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate to S, $\omega$ -dimethylaminoethyl-diethyl-thiolophosphate at 100°C. A and C are standard curves showing transmittance at 7.95 microns ( $T_{7.95}$ ) and refractive index ( $n_D^{20}$ ) as functions of the composition of the isomeric mixture. B and D show the primary results. E shows the velocity of the isomerisation at 100°C. The points  $\circ$  are from refractive index measurements and  $\times$  from I.R. transmittance.

refractive index/time diagram (D, Fig. 3) bends against the time axis. A precipitate of white crystals with salt character is formed in the reaction vessel, and an unidentified absorption band at about  $5.5 \mu$  is found in the infrared spectrum. The precipitate has approximately the same percent composition as the original compound (C 34.9; H 8.3) but has not been investigated further. Tests with isomerisation at 70°C gave a slower reaction, but side reactions were not avoided.

Infrared spectra of S, $\omega$ -dimethylaminoethyl-diethyl-thiolophosphate prepared by direct synthesis and of the isomerized product show, on the whole, identical patterns, which seems to justify the conclusion that the main product of isomerisation is identical with the reference compound. However, at  $8 \mu$  and in the region between 12 and  $15 \mu$  the transmittance differs at the coinciding absorption maxima. This is probably caused by a residue of thiono component in the isomerized compound but might also be due to the other components in the reaction mixture.

The paper chromatogram obtained from a sample of dimethylaminoethyl-diethyl-thionophosphate which had been incubated at 100°C for 4 h gave only two tailless spots with  $R_F = 0.76$  and  $R_F = 0.87$ . A mixture of oxalates of the thiono and thio<sup>2</sup> compounds which had been synthesized with direct methods gave two spots with  $R_F = 0.74$  and  $R_F = 0.87$ . Separate experiments with thio compound as free amine gives  $R_F = 0.75$  and thiono compound as oxalate (see syntheses) gives  $R_F = 0.86$ . The paper chromatography in order to study the hydrolysis products shows three identical spots ( $R_F = 0.37$ ) corresponding to  $\omega$ -dimethylaminoethanthiol, hydrolysates of S, $\omega$ -dimethyl-

aminoethyldiethyl-thiolphosphate and isomerized thionophosphate, respectively. The dimethylaminoethanthiol spot was obtained with both reagents <sup>5,9</sup>. Three other spots were obtained. Two of them were developed by both reagents and could be identified by their  $R_F$  values as unhydrolyzed thiono and thiolo compounds. The third spot,  $R_F = 0.5$ , was only developed by the sulfhydryl reagent <sup>9</sup> and might be caused by O,O-diethyl-thiophosphate. This result shows that  $\omega$ -dimethylaminoethanthiol is among the main products of hydrolysis of the isomerized compound as well as of the S, $\omega$ -dimethylaminoethyl-diethyl-thiolphosphate. *These experiments together with the infrared spectrophotometric studies show that S, $\omega$ -dimethylaminoethyl-diethyl-thiolphosphate is the main product of isomerisation of  $\omega$ -dimethylaminoethyl-diethyl-thionophosphate and exclude considerable formation of an ethyl thiolo isomer.*

The  $pK_a$  and  $pI_{50}$  values of the thio isomers and a related oxygen compound <sup>2</sup> are given in Table 1. The low  $pI_{50}$  value of the thiono ester compared to the thiolo ester emphasizes the specific role of a P—S—C structure in cholinesterase inhibitors.

Table 1.

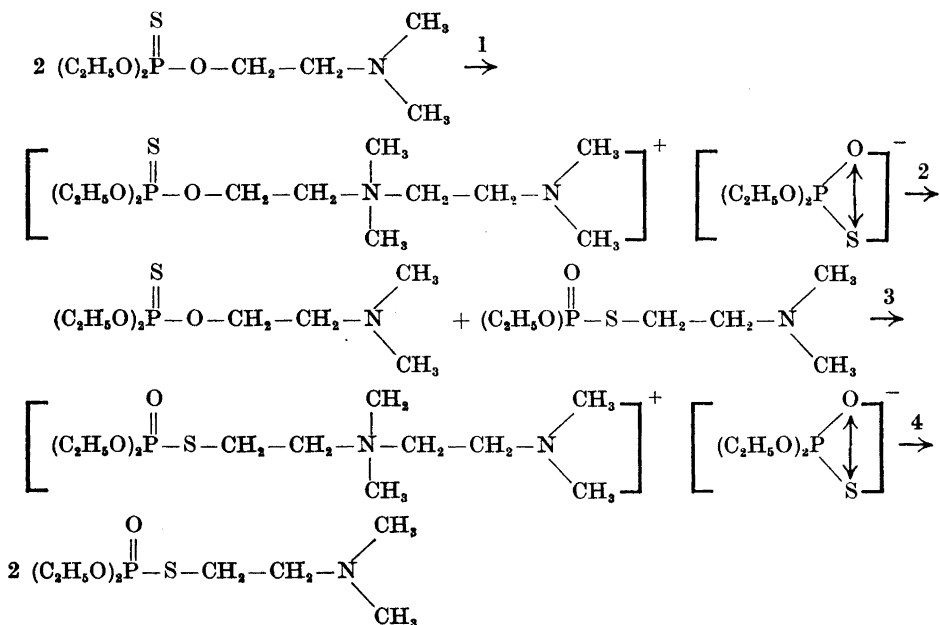
	X = —CH <sub>2</sub> —CH <sub>2</sub> —N(CH <sub>3</sub> ) <sub>2</sub>		
	$(EtO)_2\overset{\overset{O}{\parallel}}{P}-S-X$	$(EtO)_2\overset{\overset{S}{\parallel}}{P}-O-X$	$(EtO)_2\overset{\overset{O}{\parallel}}{P}-O-X$
$pK_a$	7.73	8.12	8.03
$pI_{50}$ erythrocytes	7.9	4.4	4.6
plasma	8.0	5.5	4

## DISCUSSION

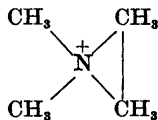
The mechanism of the rearrangement from thiono to thiolo isomer is not elucidated by the present experiments. However, some conclusions can be drawn from the results which may permit the postulation of a tentative reaction scheme.

The rate of the isomerisation is unexpectedly independent of the time, giving a picture (Fig. 3) which seems characteristic of a zero order reaction. During prolonged heating, an impurity with approximately the same composition as the starting compound but of salt character, which might be a quaternary ammonium compound, precipitates in considerable amounts. The isomerisation yields only one of the two possible thiolo isomers, the S, $\omega$ -dimethylaminoethyl-phosphoryl compound. These facts may be explained by the following reaction scheme.

If steps 2 and 4 are rate determining (with saturated solutions of the salts as an added condition) the zero order reaction is explained by the scheme, which also excludes formation of other isomers than the S, $\omega$ -dimethylaminoethyl-diethyl-thiolphosphate. The quaternary ammonium compounds should be



the precipitate observed upon prolonged heating. Other types of possible quaternary ammonium ions than the one proposed above might be formed, e.g.



but formation of the dialkoxy-thiophosphate ion seems very probable.

*Addendum:* While this paper was in press a paper by Fukuto and Stafford<sup>10</sup> has appeared which gives strong experimental support to the idea that an immonium ion is formed during the process of isomerisation.

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