[CONTRIBUTION FROM THE DEPARTMENT OF ENTOMOLOGY OF THE UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION]

The Isomerization of *O,O*-Diethyl *O*-2-Diethylaminoethyl Phosphorothionate[1]

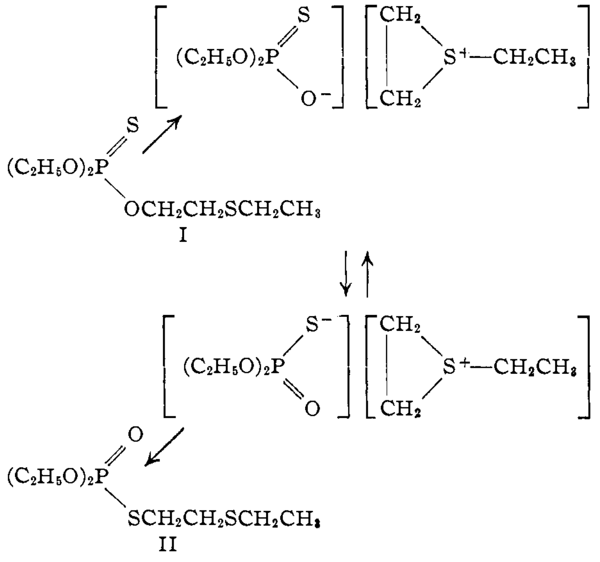
BY T. R. FUKUTO AND E. M. STAFFORD

RECEIVED JUNE 10, 1957

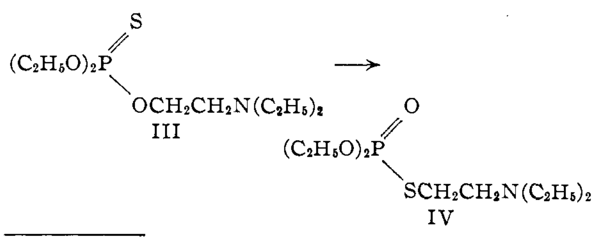
O,O-Diethyl *O*-2-diethylaminoethyl phosphorothionate rearranges with heat to its isomer *O,O*-diethyl S-2-diethylaminoethyl phosphorothiolate. This rearrangement has been shown to approximate a first-order reaction. The kinetics of the reaction of both isomers with thiosulfate ion and hydroxide ion were investigated. The results show that the phosphorothionate readily forms an immonium ion; it is postulated that the rearrangement, also, proceeds through this immonium ion.

A previous paper[2] reported studies concerning the rearrangement of *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (I) to the corresponding phosphorothiolate II. It was shown that the rearrangement followed first-order reaction kinetics[3] and that polar solvents increased the isomerization rate.

In view of these findings it was suggested that the rearrangement proceeded through a sulfonium ion intermediate, as



O,O-Diethyl *O*-2-diethylaminoethyl phosphorothionate (III) has recently been reported[4] to isomerize to *O,O*-diethyl *S*-2-diethylaminoethyl phosphorothiolate (IV).



In the form of its crystalline oxalate salt and under the code designation I. C. I.[5] R-6199, IV is now undergoing extensive evaluation as an insecticide and acaricide. The phosphorothiolate IV is an extremely potent inhibitor of the enzyme acetylcholinesterase; the concentration required for 50% inhibition of fly-brain acetylcholinesterase (I_{50}) has been determined and found to 5×10^{-9} *M*.[6] The I_{50} for the thiono isomer III, however, is 3×10^{-6} *M*, and it is relatively inactive as an insecticide.

In connection with our investigations regarding the behavior of IV in certain biological systems, we prepared P^{32}-labeled samples of both III and IV. The phosphorothionate III was obtained by condensing diethyl phosphorothionochloridate with the sodium salt of 2-diethylaminoethanol. When heated above room temperature, III was rapidly converted to IV. The rate of isomerization of III to IV was determined at 73.8°. The isomerization was followed over a 7-hr. period, at the end of which time 74% of III had been converted to IV. Over the first 4.25 hr. (43% conversion) the rearrangement approximated that of a first-order reaction, and the calculated rate constant was 4.5×10^{-5} sec.$^{-1}$. Therefore, at 73.8° the rearrangement of III to IV is approximately 10 times faster than the rearrangement of I to II (3.7×10^{-6} sec.$^{-1}$ at 78.2°).

The similarity in structure of III and IV to the 2-chloroethylamines[7] strongly suggests that the rearrangement proceeds through a three-membered immonium ion. Direct evidence for the rearrangement of III to IV through the immonium ion was demonstrated by the reaction of III with thiosulfate ion.[7,8] Thiosulfate ion has been shown to react exclusively with immonium ions formed from nitrogen mustards. Kinetic data have shown that the reaction between mustard and thiosulfate ion, and hydroxide ion, proceed at the same rate and is first order with respect to mustard. Esters of dialkylaminoethanols also have been shown to react with thiosulfate ion through the immonium ion; *e.g.*, the benzenesulfonate, sulfate and tribromobenzoate of 2-dibenzylaminoethanol all react with thiosulfate ion with first-order velocity constants.[9]

The curves in Fig. 1 show the reactivity of III and IV toward thiosulfate and hydroxide ion under

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[1] Paper No. 964, University of California Citrus Experiment Station, Riverside, California.

[2] T. R. Fukuto and R. L. Metcalf, THIS JOURNAL, **76**, 5103 (1954).

[3] That the rearrangement of I to II proceeds by first-order kinetics has also been demonstrated by (a) A. Henglein and G. Schrader, *Z. Naturforsch.*, **10b**, 12 (1955), who followed the reaction by infrared spectroscopic methods. More recently, the kinetics of this rearrangement have been studied by means of nuclear magnetic resonance spectroscopy; *cf.* (b) N. Muller and J. Goldenson, THIS JOURNAL, **78**, 5182 (1956). and the authors state that the rate is not first order but intermediate between zero and first.

[4] R. Ghosh and J. F. Newman, *Chemistry & Industry*, 118 (1955).

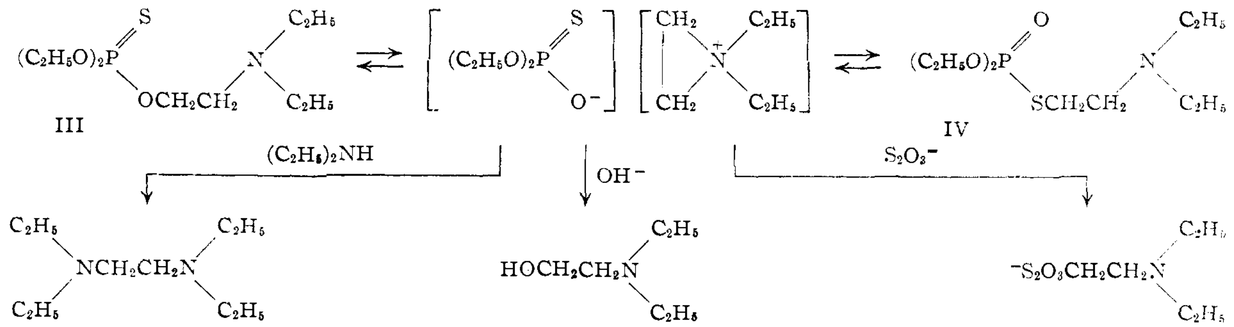
[5] Imperial Chemical Industries, Limited, England.

[6] R. L. Metcalf, E. M. Stafford. T. R. Fukuto and R. B. March, *J. Econ. Entomol.*, in press.

[7] P. D. Bartlett, S. D. Ross and C. G. Swain, THIS JOURNAL. **69**, 2971 (1947).

[8] C. Golumbic, J. S. Fruton and M. Bergman, *J. Org. Chem.*, **11**, 518 (1946).

[9] T. A. Geissman, H. Hochman and T. R. Fukuto. THIS JOURNAL **74**, 3313 (1952).



comparable conditions. Plotted is the fraction of III or IV unreacted against time. Figure 1 shows that III reacts with thiosulfate and hydroxide ions with the same velocity. Analysis of the kinetic data showed that both reactions proceed with comparable first-order velocities, indicating that the unimolecular formation of the immonium ion is the rate-determining step in both reactions as depicted below. The calculated first-order velocity constants for thiosulfate and hydroxide ions were 2.0×10^{-4} and 2.5×10^{-4} sec.$^{-1}$, respectively.

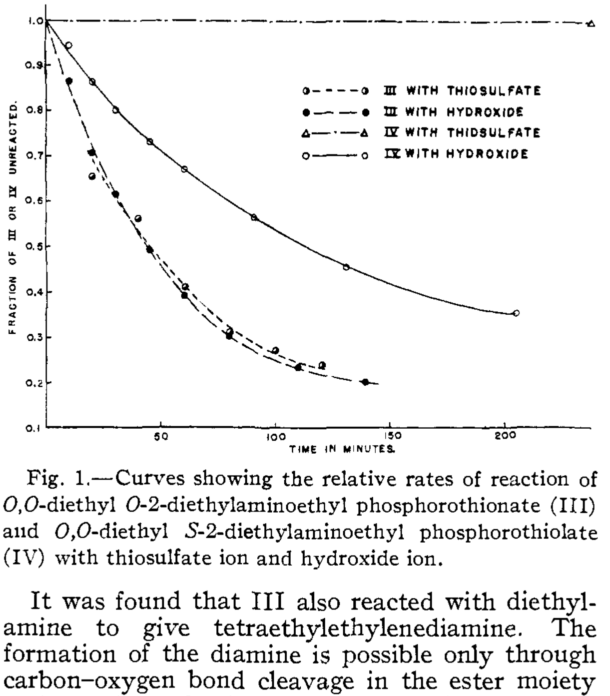
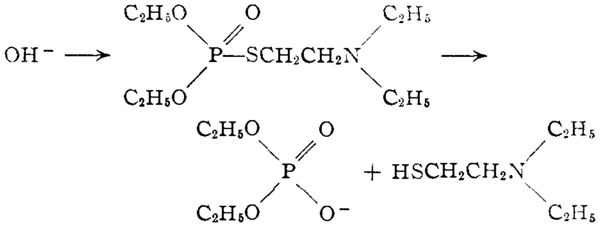


Fig. 1.—Curves showing the relative rates of reaction of *O,O*-diethyl *O*-2-diethylaminoethyl phosphorothionate (III) and *O,O*-diethyl *S*-2-diethylaminoethyl phosphorothiolate (IV) with thiosulfate ion and hydroxide ion.

It was found that III also reacted with diethylamine to give tetraethylethylenediamine. The formation of the diamine is possible only through carbon–oxygen bond cleavage in the ester moiety and stands as further evidence for the formation of the immonium ion. However, IV did not show any reaction with diethylamine under the same reaction conditions.

It was found that IV also reacted with thiosulfate ion but at a much slower rate than did III. Under the same conditions the rate approximated a first-order reaction (up to about 25% reaction after 150 hours) with a velocity constant of 2.7×10^{-7} sec.$^{-1}$. The consumption of thiosulfate ion indicates that IV also forms an immonium ion but at a rate approximately 1/1000 as fast as that of III.

The reaction rate between IV and hydroxide ion was studied by two different means: (a) by solvent partition analyses of P^{32}-labeled IV and (b) by iodometric estimation of the diethylaminoethanethiol liberated. Both methods were in good agreement and showed that IV reacted with hydroxide ion at a slower rate than did III. The reaction differed from that of III, however, in that it followed second-order kinetics. The second-order constants by P^{32} and iodometric analysis were 9.3×10^{-3} and 7.1×10^{-3} l./mole sec.$^{-1}$, respectively. The value obtained iodometrically is probably a little low because of oxidative coupling of the ethanethiol in solution, since no attempt was made to exclude air from the reaction mixture. These results indicate that the reaction between IV and hydroxide ion occurs by displacement of the diethylaminoethyl mercaptide moiety, as



$$OH^- \longrightarrow (C_2H_5O)_2P(O)SCH_2CH_2N(C_2H_5)_2 \longrightarrow (C_2H_5O)_2P(O)O^- + HSCH_2CH_2N(C_2H_5)_2$$

The phosphorothiolate IV is relatively stable and can be kept indefinitely under refrigeration. However, the phosphorothionate III isomerizes readily to IV with heat and, in addition, dimerizes to the tetraethylpiperazinium derivative even under refrigeration, a behavior similar to that of the nitrogen mustards, which dimerize to piperazinium compounds through the immonium ion.

As further evidence of the immonium ion intermediate in the rearrangement of III to IV, it was found that the oxalate salt of III, which cannot form an immonium ion, showed no isomerization even when heated for 3 hr. at 100°. In this connection the sulfoxide of I, *O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphorothionate (VI), which cannot form the sulfonium ion, is also quite stable to heat and showed no rearrangement to its corresponding phosphorothiolate even after 4 hr. at 120°. The three-membered ion intermediates appear to be the most reasonable explanation for the unusually rapid isomerization of I and III to their thiolate isomers.

Experimental Part

***O,O*-Diethyl *O*-2-Diethylaminoethyl Phosphorothionate Oxalate.**—To a mixture of 4.1 g. of sodium hydride and 100 ml. of anhydrous benzene was added dropwise 20 g. of 2-diethylaminoethanol. The mixture was stirred and heated at reflux until the sodium hydride disappeared. The flask was then cooled in ice and 32.2 g. of *O,O*-diethyl phosphorothionochloridate added dropwise. The mixture was stirred for 2 hr., washed with water and dried over anhydrous sodium sulfate. The benzene solution was treated with 20 g.

of oxalic acid in ethyl alcohol, giving crystals which melted at 85–86° after three recrystallizations from ethyl acetate. The yield of recrystallized product was 22 g.

Anal. Calcd. for $C_{12}H_{26}NO_7PS$: C, 40.09; H, 7.29. Found: C, 40.55; H, 7.64.

O,O-Diethyl *O*-2-diethylaminoethyl phosphorothionate (III) was liberated from its oxalic acid salt by treatment with two equivalents of aqueous potassium hydroxide. The free amine was taken up in ether, dried over sodium sulfate and distilled in a falling-film molecular still at 100° (0.1 mm.), n^{25}D 1.4594. The infrared spectra of the distilled and undistilled product showed that very little isomerization to the phosphorothiolate had occurred during distillation.

O,O-Diethyl *S*-2-diethylaminoethyl phosphorothiolate oxalate was obtained from Imperial Chemical Industries, England (code no. R-6199), and was recrystallized from isopropyl alcohol and ether, m.p. 98–99°. The amine IV was liberated from the oxalate by treatment with aqueous potassium hydroxide, taken up in ether, dried over sodium sulfate and distilled, b.p. 110° (0.2 mm.), n^{27}D 1.4655.

Kinetic Methods.—The rate of isomerization of III to IV was determined by the infrared spectrophotometric technique described by Henglein, *et al.*,[3a] by measuring the change in P=O absorption (1260 cm.$^{-1}$). The 1490 cm.$^{-1}$ band was used as an internal standard. Small samples of III (about 200 mg.) were sealed under nitrogen and immersed in a constant-temperature oil-bath maintained at 73.8°. At measured intervals samples were removed and made up as a 10% solution in carbon tetrachloride; absorption at 1260 and 1490 cm.$^{-1}$ was measured in a Perkin–Elmer model 21 recording spectrophotometer fitted with sodium chloride optics. The areas of the 1260 and 1490 cm.$^{-1}$ bands were measured with a planimeter and their ratios compared to that of a standard curve. The standard curve was obtained by plotting the mole fraction of IV against the 1260/1490 ratio obtained from spectra of known quantities of III and IV.

The kinetics of the reaction of III and IV with thiosulfate ion were carried out in 25% ethyl alcohol at 37.1°. The reaction mixture was made up 0.01 *M* in compound and 0.02 *M* in sodium thiosulfate. At measured intervals, 10-ml. aliquots were removed, cooled, acidified and titrated with standard iodate solution (0.05 *M*). Blank determination showed that the titer of the thiosulfate solution remained constant at 37.1° over the period of time covered by the rate runs.

The rates of hydrolysis were carried out on P^{32}-labeled samples of III and IV at 37.1°. Both III and IV were found to partition in favor of chloroform to water in the ratio greater than 125 to 1. The P^{32}-labeled hydrolysis products diethylphosphoric acid and *O,O*-diethylphosphorothioic acid, however, partition essentially completely in favor of water over chloroform. It is possible, therefore, to determine the degree of hydrolysis by partitioning the reaction mixture between the two solvents and measuring the radioactivity in each solvent. The reaction mixture was made up 0.005 *M* in compound and 0.01 *M* sodium hydroxide in 25% ethyl alcohol. At measured intervals, small samples were removed, added directly to equal volumes of chloroform and water in a separatory funnel, shaken vigorously and the two phases separated. Appropriate aliquots were taken from each phase, and the radioactivity was determined by a thin-window Geiger tube. The following data in Table I are typical of those obtained.

The liberation of 2-diethylaminoethanethiol from IV by hydroxide ion was followed iodometrically by the procedure described by Kimball, *et al.*[10] Aliquots of a solution made up of 0.005 *M* in III and 0.01 *M* in sodium hydroxide in 25% ethyl alcohol were removed at measured intervals and added directly to a solution containing 10 ml. of 0.01 *N* iodine and 10 ml. of 1 *N* sulfuric acid. The excess iodine was estimated iodometrically with standard thiosulfate solution. 2-Diethylaminoethanethiol (obtained through the courtesy of Imperial Chemical Industries, Ltd., England) reacted quantitatively with iodine under the same titration conditions. There was a slow decrease in the titer of the ethanethiol solution upon standing (about 10% in 3 hr.), but no attempt was made to correct for this spontaneous change, since the rate runs were not carried on beyond 2 hr.

TABLE I

HYDROLYSIS OF *O,O*-DIETHYL *S*-2-DIETHYLAMINOETHYL PHOSPHOROTHIOLATE (IV) BY HYDROXIDE ION

Time, min.	Counts/sec. Chloroform	Counts/sec. Water	Fraction IV hydrolyzed	$k \times 10^{-3}$, l./mole sec.$^{-1}$
0	155.0	1.2	. . .	
10	100.5	8.6	0.037	7.6
20	98.0	16.9	.135	9.5
30	92.3	24.9	.200	9.8
45	89.6	34.9	.270	9.6
60	83.0	42.6	.329	9.2
90	46.6	38.1	.441	9.2
130	38.3	48.2	.550	9.1

P^{32}-Labeled III and IV were prepared, beginning from neutron-irradiated red phosphorus. The synthesis of P^{32}-*O,O*-diethyl phosphorothionochloridate from phosphorus-32 already has been described.[11]

P^{32}-III was prepared from the above *O,O*-diethyl phosphorothionochloridate and 2-diethylaminoethanol in the same manner as described for the non-labeled material. The phosphorothiolate IV was obtained by heating III at 130° for 4 hr. and distilling, b.p. 110° (0.2 mm.). Samples used for rate studies were carefully purified by the procedures described above for the non-radioactive materials.

Tetraethylpiperazinium Di-*p*-toluenesulfonate.—Freshly prepared III was allowed to stand at 5° in the refrigerator for two months. Ether was added and an oil separated which upon standing crystallized slowly to a hygroscopic solid. The solid was treated with excess *p*-toluenesulfonic acid in ethyl alcohol, giving crystals which after recrystallization from ethyl alcohol melted at 285–286° (uncor.). A mixed melting point with an authentic sample of tetraethylpiperazinium di-*p*-toluenesulfonate, m.p. 286–287°, prepared according to Gough and King,[12] gave no depression. The diiodide was unmelted at 356°.

Reaction between III and Diethylamine.—A mixture of 5.0 g. of III and 3.0 g. of diethylamine was heated on a steam-bath for 3 hr. The excess diethylamine was removed under vacuum and the residual oil was taken up in chloroform. The chloroform solution was washed with 6 *M* sodium hydroxide and dried over anhydrous potassium carbonate; the product was then distilled. About 1 g. of tetraethylethylenediamine distilling at 72° (7 mm.) was collected. It was characterized further by its dihydrochloride, m.p. 186–187°, and its dipicrate, m.p. 242–243°.

The Action of Heat on the Oxalate of III and on *O,O*-Diethyl *O*-Ethyl-2-sulfinylethyl Phosphorothionate (V).—The oxalate of III (1.3 g.) was sealed in glass, heated at 100° for 3 hr.; III was liberated into 10 ml. of carbon tetrachloride by treatment with aqueous potassium hydroxide. The infrared spectrum showed that no rearrangement to IV had occurred.

O,O-Diethyl *O*-ethyl-2-sulfinylethyl phosphorothionate (V) was also sealed in glass, heated at 120° for 4 hr. The infrared spectrum showed no rearrangement to the corresponding phosphorothiolate isomer. The synthesis of V has been described elsewhere.[13]

RIVERSIDE, CALIFORNIA

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[10] J. W. Kimball, R. L. Kramer and E. E. Reid, THIS JOURNAL, **43**, 1199 (1921).

[11] T. R. Fukuto, J. P. Wolf, III, R. L. Metcalf and R. B. March, *J. Econ. Entomol.*, **49**, 147 (1956).

[12] G. A. C. Gough and H. King, *J. Chem. Soc.*, 2437 (1928).

[13] T. R. Fukuto, R. L. Metcalf, R. B. March and M. G. Maxon, *J. Econ. Entomol.*, **48**, 347 (1955).