

SYNTHESIS, CHEMICAL AND BIOLOGICAL PROPERTIES OF A  
SERIES OF O,O-DIETHYL S-ARYL PHOSPHOROTHIOLATES

by

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B. A., DePauw University, 1964

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A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1966

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L.D  
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TABLE OF CONTENTS

INTRODUCTION . . . . .	1
REVIEW OF LITERATURE . . . . .	2
METHODS AND MATERIALS . . . . .	10
Synthesis . . . . .	10
Phosphorochloridate Coupling Method . . . . .	10
Free Radical Method . . . . .	11
Purification and Structure Determination . . . . .	12
Column Chromatography . . . . .	12
Thin Layer Chromatography . . . . .	13
Infrared Spectroscopy . . . . .	14
Elemental Analysis . . . . .	15
Hydrolytic Rate Determination . . . . .	15
Direct Color Formation Method . . . . .	16
Triester Method . . . . .	16
Comparative Toxicity to the House Fly . . . . .	18
Rearing and Maintenance of House Fly Cultures . . . . .	18
Treatment and Holding of Flies . . . . .	18
Comparative Inhibition of House Fly Head Cholinesterase . . . . .	19
RESULTS AND DISCUSSION . . . . .	21
Phosphorochloridate Coupling Synthesis . . . . .	21
Products of the Free Radical Method . . . . .	24
Properties and Verification of Structures . . . . .	24
Infrared Spectra . . . . .	24
Hydrolytic Rates . . . . .	32
Rates from Direct Color Formation Method . . . . .	32
Rates from Triester Method . . . . .	37

Comparison of Methods . . . . .	40
Comparative Toxicity to the House Fly . . . . .	40
Comparative Cholinesterase Inhibition . . . . .	41
Structure-Activity Relationships . . . . .	43
SUMMARY AND CONCLUSIONS . . . . .	53
ACKNOWLEDGMENTS . . . . .	56
LITERATURE CITED . . . . .	57
APPENDIX . . . . .	60

## INTRODUCTION

The history of man's struggle against insect pests is surely as old as civilization itself. The struggle continues but with the development of modern insecticides a great battle, though not the war, has been won.

Organophosphorus insecticides having high insect toxicity while presenting but a small residue hazard have become one of the primary weapons used against economic insect pests in recent years. There are several types of organophosphorus compounds in practical use as insecticides; two major groups of these are the dialkyl aryl phosphorothionates and their oxidation products, the dialkyl aryl phosphates. Parathion and ronnel, paraoxon and ronnoxon are examples of each class. A third closely related class that has never been systematically examined is the dialkyl S-aryl phosphorothiolates. What little work that has been done with this type of compound indicates that members of the class might have some unusual properties such as extremely high cholinesterase inhibitory activity but low toxicity to insects; high mammalian toxicity but low insect toxicity; high rates of aqueous hydrolysis and other unusual properties. Further study of this class of compound was prevented by the absence of a simple, convenient preparative method.

This study was undertaken to elucidate some of the properties of a series of diethyl S-aryl phosphorothiolates. First, a simple, general synthetic route to a series of these compounds had to be found whereby experimental quantities could be conveniently prepared. Next a study of the stability of the compounds was undertaken in which it was necessary to develop a method to determine hydrolytic rates. Thirdly, two biological properties were examined; comparative mortality to the housefly, Musca domestica L.; and comparative inhibition of the cholinesterase enzyme.

By these various studies it was hoped (1) to clarify any relationships between chemical structure and biological activity and (2) to uncover any unusual properties of this class of compounds.

#### REVIEW OF LITERATURE

The discovery of the excellent insecticidal properties of parathion (O,O-diethyl O-[4-nitrophenyl] phosphorothionate) initiated a search for related compounds which might have valuable insecticidal activity. Among the first new compounds examined were the parathion S-phenyl isomer (O,O-diethyl S-[4-nitrophenyl] phosphorothiolate) and the S-ethyl isomer (O,S-diethyl O-[4-nitrophenyl] phosphorothiolate). The S-phenyl isomer was reported to be a solid, m.p. 42.3° C, a 0.005% spray solution of which killed 80% of aphids feeding on treated leaves. The subcutaneous lethal dose to the white mouse was given as 50-100 mg/kg. The S-ethyl isomer was reported as a non-distillable liquid, unstable to base. A 0.005% spray solution killed 90% of aphids feeding on treated leaves. The subcutaneous lethal dose to the white mouse was 50 mg/kg. It was decided that although the parathion isomers had the requisite toxic properties, the rapidity with which they were hydrolyzed precluded any potential value as commercial insecticides (Schrader, 1952).

The striking success of parathion as an insecticide aroused interest in understanding the nature of the pharmacological activity of this compound. To this end, Hecht and Wirth (1950) investigated a series of isomers of parathion and methyl parathion that were synthesized for them by G. Schrader and W. Lorentz. The S-phenyl parathion isomer was reported as light yellow crystals having m.p. 40-41° C. The average lethal dose to the frog, administered in the lymph sac in "losungsmittel M," was 10 mg/kg.

To the mouse, administered subcutaneously in oil, the lethal dose was 2.5-5 mg/kg. To the mouse, administered in water and "losungsmittel M" subcutaneously, the average lethal dose was 1.25 mg/kg. The average oral lethal dose to the rat, given with water and wetting agent, was 2.5-5 mg/kg. The molar concentration necessary for 50% inhibition ( $I_{50}$ ) of horse serum cholinesterase was  $1.43 \times 10^{-9}$  M. The S-ethyl isomer of parathion was reported to be a non-distillable, reddish yellow oil,  $D_{40}^{20} = 1.21$ . The comparable toxicity values were as follows: frog 50-100 mg/kg; mouse, in oil, 25-60 mg/kg; mouse, in water with wetting agent, 20 mg/kg; rat, 50-100 mg/kg;  $I_{50} = 0.86 \times 10^{-6}$  M.

Methyl parathion S-phenyl isomer (O,O-dimethyl S-[4-nitrophenyl] phosphorothiolate) was reported as light yellow prisms, m.p. 58-59° C. Its lethal dose to the frog was 50-75 mg/kg; to the mouse, in oil, 20-50 mg/kg; to the mouse in water and "losungsmittel M," 7.5-10 mg/kg; oral dose to the rat 45 mg/kg,  $I_{50} = 5.9 \times 10^{-8}$  M. The S-methyl isomer of methyl parathion (O,S-dimethyl O-[4-nitrophenyl] phosphorothiolate) was a bright yellow oil, not distillable,  $D_{40}^{20} = 1.36$ . This compound was toxic to the frog at 70-80 mg/kg; to the mouse, in oil, 90-100 mg/kg; to the mouse, in water and "losungsmittel M," 35 mg/kg; orally to the rat, 200 mg/kg. The  $I_{50}$  was  $1.35 \times 10^{-7}$  M.

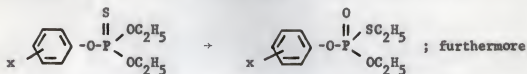
Practically all early work with organophosphorus compounds was plagued by impurities present in compounds under study. Comparable compounds administered in the same way had widely divergent toxicities. The parathion S-phenyl isomer tested by Hecht and Wirth was ten times as toxic to white mice as the same compound tested by Schrader. The higher melting point of the batch of compounds reported by Schrader may be an indication of greater purity. No comparable differences in the

toxicity of the o-ethyl compounds was evident. Nevertheless, certain tentative generalizations concerning the relative toxicities were indicated: (1) the mammalian toxicities of o-phenyl compounds were greater than o-ethyl compounds; (2) the ethyl homologs were considerably more toxic to mammals than the methyl compounds; (3) the ethyl homologs were better cholinesterase inhibitors than methyl compounds; (4) the o-phenyl compounds were better cholinesterase inhibitors than the o-ethyl isomers; (5) the order of strength of inhibition of the compounds tested by Hecht and Wirth was: ethyl o-phenyl >paraoxon >methyl o-phenyl >methyl paraoxon.

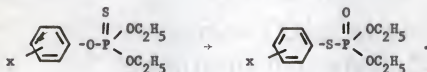
Martin (1950) studied the toxic properties of parathion and its isomers. The following values are for the subcutaneously administered lethal dose to the white rat: parathion, 50 mg/kg; methyl parathion, 45 mg/kg; o-ethyl isomer of parathion, 5 mg/kg; o-phenyl parathion isomer, 1 mg/kg. When D. oleracea was fed these compounds parathion was found most toxic, followed by the o-ethyl isomer, with the o-phenyl isomer being least toxic. The same relationship held when the compounds were administered to E. granaria by contact. Thus, the interesting result was gained; to rats parathion o-phenyl isomer was 50 times as toxic as parathion, while by contrast, to E. granaria parathion was 20 times as toxic as the o-phenyl isomer. The isomers of parathion exhibited a property optimally opposite to the differential insect toxicity desired. Martin (1956) reported the activity of parathion and its isomers against 4 insect species and found the order of toxicity to be parathion >o-ethyl >o-phenyl isomer. In considering the previous work it was indicated that studies of why this differential toxicity occurs might shed light on the problem of sculpturing insect poisons to order and on the nature of toxic action in general.

Variations in toxicities of different batches of commercial parathion

initiated a search for the cause of the variation. It was known that thionophosphate compounds could undergo a molecular isomerization:



the possibility of the formation of the S-phenyl isomer had not been ruled out:



The high mammalian toxicity of these compounds coupled with a reasonable scheme whereby they might be formed indicated that the presence of these isomers was indeed responsible for variations in parathion toxicity.

Diggie and Gage (1951) investigated the inhibitory activity of a series of samples of parathion and its isomers. Their work revealed the following trend of relative inhibitory activity: paraoxon >S-phenyl isomer >S-ethyl isomer >chromatographically pure parathion. On the basis of analytical procedures the authors concluded that high inhibitory activity of impure samples of parathion was due to the presence of the S-ethyl isomer.

Woodcock and Stringer (1951) tested the toxicity of parathion, paraoxon, the S-phenyl isomer and the S-ethyl isomer to grain weevils (Calandra granaria L.) by tarsal contact to filter papers impregnated with the toxicant. Paraoxon and the parathion isomers were found less toxic than parathion itself.

Callaway, et al. (1952), investigated a "pure" sample of parathion, an impure sample, the S-phenyl isomer and the S-ethyl isomer as to the comparative toxicity of their water solutions to the fresh water shrimp



Gammarus pulex. The order of toxicity was found to be S-phenyl > impure parathion > S-ethyl isomer > pure parathion. These results did nothing to deny the hypothesis that one or both of the isomers were responsible for batch toxicity variations although they did nothing to confirm it as well.

In seeking to establish that the inhibition of cholinesterase by a series of compounds increased as the stability to hydrolysis decreased, Aldridge and Davidson (1952) confirmed that this relationship held for parathion and its two isomers. The S-ethyl isomer had a rate constant of hydrolysis, pH 7.6 in phosphate buffer, of  $1.1 \times 10^{-3} \text{ min}^{-1}$ ; the corresponding value for the S-phenyl isomer being  $5.3 \times 10^{-4} \text{ min}^{-1}$ ; for parathion  $9.6 \times 10^{-7} \text{ min}^{-1}$ ; and for paraoxon  $3.3 \times 10^{-5} \text{ min}^{-1}$ .  $I_{50}$ 's for these compounds corresponded well with the rate of hydrolysis, the exception being the S-ethyl compound which had such a very rapid rate of hydrolysis that the extent of inhibition was lowered due to hydrolysis in the medium before cholinesterase was encountered.

Aldridge and Barnes (1952) compared the toxicity to rats with the ability to inhibit red blood cell cholinesterase using (I) parathion, (II) paraoxon, (III) S-phenyl isomer, and (IV) S-ethyl isomer. The  $I_{50}$ 's (30 min incubation at 37° C, sheep erythrocytes) were (I)  $1.7 \times 10^{-4} \text{ M}$ , (II)  $2.0 \times 10^{-8} \text{ M}$ , (III)  $2.8 \times 10^{-8} \text{ M}$ , (IV)  $2.5 \times 10^{-8} \text{ M}$ . The corresponding approximate lethal dosages, administered in alcoholic solution to the male rat, were (I) 3 mg/kg, (II) 0.4 mg/kg, (III) 0.5 mg/kg, (IV) 1.2 mg/kg. The order of relative toxicity to the rat is the same as was found in other researches with mammals, S-phenyl > S-ethyl > parathion.

Heath (1956) examined the hydrolytic rates of parathion isomers and attempted to explain their rapid hydrolytic rates on the basis of electro-meric and steric effects. The hydrolytic rate constants  $\left(\frac{[\text{OH}^-]}{\text{min}}\right)$  were:

parathion,  $5.7 \times 10^{-2}$  (25° C); paraoxon,  $5.2 \times 10^{-1}$  (25° C); S-ethyl isomer, 800 (37° C), S-phenyl isomer, 150 (37° C). The rapid hydrolysis of compounds containing the P-S-C bond was attributed to the ease of polarizability of the sulfur atom which facilitates the formation of an energized transition complex. The S-phenyl isomer hydrolyzed more slowly than the S-ethyl compound because the formation of the polarized complex increased the strength of the P-S-C bond.

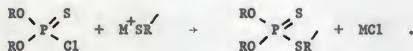
Fukuto, et al. (1959), prepared and studied a series of alkylphosphonic acid esters. The S-alkyl phosphonothionates, e.g., S-ethyl, S-n-propyl and S-isopropyl 4-nitrophenyl ethylphosphonothionates were approximately one-sixth as toxic to house flies as their oxygen analogs. Unlike the phosphonothionates, these compounds were excellent cholinesterase inhibitors. The large drop in toxicity was probably due to their relatively high susceptibility to aqueous hydrolysis. The S-ethyl and S-isopropyl compounds were 32 and 50 times less stable in phosphate buffer than their respective oxygen analogs. Ethyl S-(4-nitrophenyl) ethylphosphonothiolate was less effective than the S-ethyl isomer against house flies, even though similar activity against cholinesterase was exhibited. The reason for this difference was not apparent. From its rate of inhibition and rate of hydrolysis it was expected that the insecticidal activity of the S-phenyl isomer should parallel the phosphonate and be better than the S-ethyl isomer.

Menn and Szabo (1965) investigated a series of Q-alkyl S-aryl alkylphosphonodithioates. Q-ethyl S-aryl phosphonodithioates were found to have considerably less acute oral toxicity to the rat than corresponding phosphonothiolates, though the toxicities of the two classes of compounds to the house fly were approximately equivalent. Phosphonodithionate

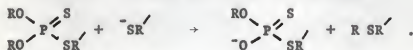
esters were found to be considerably better insecticides than corresponding phosphorodithioate compounds. The enhanced toxicity may be due to the asymmetry of the phosphonate molecule. As a group, the phosphonodithioates were poor inhibitors of cholinesterase but two related compounds, O-ethyl S-phenyl ethylphosphonothiolate and O-ethyl S-[p-methylphenyl] ethyl phosphonothiolate were very powerful inhibitors of cholinesterase, even though the phenyl ring was not deactivated or was even somewhat activated by an electron-donating methyl group.

The toxicity of parathion and its isomers and paraoxon to 2 day old female houseflies by topical application was determined by Abdallah (1963). The LD<sub>50</sub> values (micrograms/fly) found were: parathion, 0.033; paraoxon, 0.07; S-phenyl isomer, 0.28; S-ethyl isomer, 0.49.

The first appearance in the literature of a method for the synthesis of organophosphorus compounds containing the S-aryl moiety utilized the reaction of dialkyl phosphorochloridithioates with thiol salts (Schrader, 1953):



The practical utility of this method for the synthesis of a series of S-aryl compounds was in question because the highly nucleophilic thiol anion could react with the product of the reaction:



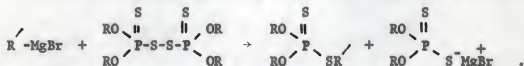
It was found that a maximum yield of 32% could be gained by this method (Miller, 1960).

Bianchetti (1957) used the reaction of aryl diazonium salts with salts of O,O-dialkyldithiophosphoric acids:



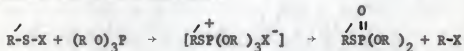
The utility of this method was severely limited by the difficulties inherent in preparing and handling diazonium salts.

Miller (1964) utilized the reaction of organometallic reagents upon the -S-S- bond of phosphorothionyl disulfides as a method of synthesis:



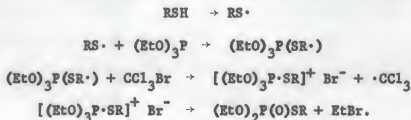
Lithium reagents as well as Grignard reagents gave smooth reactions and excellent yields. The limiting factor in the use of this scheme was the difficulty in preparing many of the organometallic reagents.

Pilgrim and Korte (1965) summarized the methods employing trialkyl phosphites and thiol derivatives:



where X = Cl, R', R'', CN, O<sub>2</sub>R.

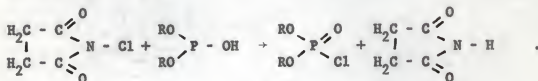
Use of the reaction between CCl<sub>3</sub>Br, trialkyl phosphite and butanethiol was made by Bunyan and Cadogan (1962) to synthesize diethyl S-butyl phosphorothiolate. The synthesis proceeded via a chain transfer mechanism:



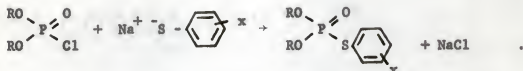
## METHODS AND MATERIALS

## Synthesis

Phosphorochloridate Coupling Method. Synthesis of dialkyl  $\underline{S}$ -aryl phosphorothiolates was first approached by reacting dialkyl phosphorochloridate with the sodium salt of the thiophenol at a low temperature. The first step in the reaction was preparation of the phosphorochloridate using N-chlorosuccinamide:



The phosphorochloridate was isolated by vacuum distillation and then reacted with the sodium thiophenolate:



Recommending the method was its successful application to the synthesis of dialkyl aryl phosphates (Blair, personal communication) and its use in the synthesis of dialkyl aryl phosphorodithioates (Schrader, 1953).

Synthesis of O,O-dimethyl  $\underline{S}$ -(2,4,5-trichlorophenyl) phosphorothiolate was attempted by the coupling method described above. Dimethyl phosphorochloridate was prepared according to the method of Goldwhite and Saunders (1955) and purified by vacuum distillation. Sodium 2,4,5-trichlorothiophenolate was prepared by dissolving 2,4,5-trichlorophenol (Dow Chemical Co., Midland, Mich.) in acetone and adding an equimolar amount of NaOH, then evaporating.

To a 50 ml round bottom flask containing 10 ml of dimethyl formamide

(DMF) was added 0.70 gm (0.0051 mole) of O,O-dimethyl phosphorochloridate. To an additional 10 ml of DMF in a 25 ml beaker was added 1.2 gm (0.0051 mole) of sodium 2,4,5-trichlorothiophenolate. The reaction flask was placed in an ice bath and the thiol salt solution added in 1/2 ml increments so that the temperature of the reaction mixture did not rise above 5° C. When all the salt solution had been added the reaction mixture was washed with three 10 ml portions of Shellysolve B. These washes were combined and rewashed with 10 ml of distilled water, then 10 ml of 10% NaHCO<sub>3</sub>, then again with 10 ml of distilled water. The aqueous washes were added back to the DMF, which was then cooled to 0° C. Crystals produced were collected on a buchner funnel.

Free Radical Method. This synthetic method employed the reaction between bromotrichloromethane, triethyl phosphite and the aryl thiol. The method had been successfully employed to synthesize O,O-diethyl S-butyl phosphorothiolate (Bunyan and Cadogan, 1962), but had not been employed to synthesize aryl phosphorothiolates.

The appropriate amounts of triethyl phosphite, bromotrichloromethane and the thiol in the respective molar ratio 1:2:4 to give a 1 gm theoretical yield were mixed in a 20 x 2.5 cm test tube. After an induction period of 2 to 3 minutes the reaction mixture turned red and became hot. The reaction mixture was allowed to cool back to room temperature; then 50 ml of distilled Shellysolve B was added. The test tube was then stoppered and placed in a refrigerator until purification.

Triethyl phosphite and bromotrichloromethane were Eastman practical grade, used without purification; 4-bromobenzenethiol, Matheson, Coleman and Bell; 2-chloro-, 4-nitro-, and benzenethiol, K & K Laboratories, New York; 4-chlorobenzenethiol, Evans Chemetics, Inc., New York; 2,4,5-

trichlorobenzenethiol was furnished by Dr. Ectyl Blair, Dow Chemical Co., Midland, Michigan. Shellysolve B was glass distilled before use.

#### Purification and Structure Determination

Column Chromatography. Many different procedures have been used to purify experimental quantities of potential pesticides. These include molecular distillation, recrystallization, countercurrent extraction, absorption and partition chromatography. Each of these techniques have limitations of yield, purity of product and convenience. The technique employed in the purification of the S-aryl phosphorothiolates was multi-molecular absorption chromatography which had been shown to give products of >99% purity with a series of similar compounds (Patchett and Batchelder, 1961).

The column used was 45 cm long x 2.8 cm outside diameter fitted with a  $\frac{3}{8}$  29/45 chromatographic receptacle containing a medium porosity fritted disc. Florisil (Floridin Co., Tallahassee, Fla.), 200-400 mesh, was heated overnight at 120° C to remove free moisture and stored in a sealed jar until used. All solvents were distilled before use.

A slurry of 50 grams of Florisil in 150 ml of Shellysolve B was prepared and poured into the column; the column was tapped to expel air bubbles and ca. 3 lb of pressure was applied at the top of the column to expel excess hexane. The synthetic reaction mixture was added to the column in 50 ml of hexane and air pressure applied to drive the sample into the packing, the hexane layer being driven down to one-half inch above the packing. Elution was done using 75 ml each of a series of solutions of hexane, chloroform and acetone of increasing polarity. A slight air pressure was applied at the top of the column so that a fraction was collected

in a tared 250 ml beaker every 5-7 minutes. When the eluting solvent reached a level ca. one-half inch above the packing bed the air pressure was removed, the beakers changed and another solvent mixture of higher polarity added. This procedure was repeated until a total of fifteen fractions had been collected. After a sample had been taken for TLC, fractions were evaporated in an air stream produced by drawing a hood window down close to the top of a row of beakers placed along the front of a hood. Total evaporation usually took somewhat less than an hour. The tared beakers were then reweighed and the weight change noted.

Thin Layer Chromatography. Thin layer chromatographic techniques developed by El-Refai and Hopkins (1965) were used to monitor column chromatographic fractions and to determine  $R_f$  values of the compounds under study.

Only the first three compounds purified were monitored using these techniques because initial results indicated that different compounds of the class would be collected in the same fractions. As soon as the last column fraction was collected a microliter spot from each of the fifteen fractions was placed 3 cm from the base of a thin-layer plate. The thin layer plate was an 8 x 8-inch glass plate coated with MN cellulose 300 G (Desaga-Brinkman, Westbury, N.Y.). A stationary phase of N,N-dimethyl formamide was applied by dipping the plate in a 20% DMF-acetone solution. Immediately after the acetone evaporated the plate was placed base-first into a trough containing 25 ml of 2,2,4-trimethyl pentane; the trough itself was inside a chromatographic tank saturated with 2,2,4-trimethyl pentane vapor. The tank was immediately sealed with masking tape and the solvent front allowed to ascend approximately 10 cm. The plate was then removed from the tank, mobile solvent allowed to evaporate, and heated



for a few minutes in an oven at  $110^{\circ}\text{C}$  to drive off DMF.

Spots were developed by spraying the plate with an alkaline silver nitrate solution consisting of 1 part 10% aqueous silver nitrate stock solution, 1 part concentrated  $\text{NH}_4\text{OH}$  and 8 parts methanol. Exposing to short wavelength ultraviolet light developed the spots.

In order to determine the relative polarities of the various compounds and to establish a basis for any qualitative analytical techniques, spots of approximately 5  $\mu\text{g}$  of each compound were applied to thin-layer plates in 1  $\mu\text{l}$  of acetone. The plates were run with the normal (non-aqueous) system described earlier and spots detected using alkaline  $\text{AgNO}_3$ . Three replicate runs were made to establish an average  $R_f$  value for each compound.

In addition, a single  $R_f$  determination by the cholinesterase technique for the detection of cholinesterase inhibiting compounds was made. The cholinesterase technique consisted of spraying the prepared plates with a cholinesterase enzyme-basic bromthymol blue solution, allowing the plate to dry and then spraying with a 2% aqueous acetylcholine bromide solution. Spots where inhibition had occurred were blue while other areas of the plate were yellow--reflecting a change of indicator color resulting from the liberation of acetic acid by the enzyme.

Infrared Spectroscopy. For the purposes of structure and purity verification an infrared spectrum of each compound was prepared. The instrument used was a Beckman IR 10 Infrared Spectrophotometer operated at a scanning speed of 42 minutes over a spectral range of  $4000$  to  $300\text{ cm}^{-1}$ . Spectra were prepared from ca. 25%  $\text{CCl}_4$  and  $\text{CS}_2$  solutions. Carbon tetrachloride solutions were used from  $4000$  to ca.  $1300\text{ cm}^{-1}$ . Carbon disulfide solutions were used from ca.  $1300\text{ cm}^{-1}$  to  $300\text{ cm}^{-1}$ . Both  $\text{CCl}_4$  and  $\text{CS}_2$  were spectrograde products of Fisher Scientific Co., St. Louis, Mo.

Spectra were made in a sealed IR liquid cell (Limit Research Corporation, Darien, Conn.). The cell used was a Limit M-08 amalgamated sealed cell with KBr windows, 0.1 mm path length. After the spectrogram had been prepared, the  $1601.1 \text{ cm}^{-1}$  peak of polystyrene was traced on the spectrum to serve as a point of wavelength reference. Spectra of analytically pure (>99%) ethyl ronnel and ethyl ronnoxon (Dow Chemical Co., Midland, Mich.) and parathion and paraoxon (American Cyanamid, Princeton, N.J.) were prepared as standards to serve as a basis for comparison. Initial spectra of the S-phenyl, S-(4-chlorophenyl) and S-(2,4,5-trichlorophenyl) phosphorothiolates were prepared and interpreted by Dr. R. A. Nyquist of Dow Chemical Co., Midland, Mich.

Elemental Analysis. To serve as further verification of structures and purity, elemental analyses of the purified compounds were made by Galbraith Laboratories, Inc., Knoxville, Tenn. One compound, O,O-diethyl S-(4-chlorophenyl) phosphorothiolate, was analyzed for all elements except oxygen. The remaining compounds were analyzed only for percent hydrogen and carbon since proportions of these two elements would adequately reflect the molecular content of other elements and determinations for only these two elements reduced costs per analysis considerably.

#### Hydrolytic Rate Determination

Hydrolytic rates were determined in 0.01 N aqueous sodium hydroxide in which the hydroxyl ion concentration was greatly in excess. The course of the reaction followed pseudo first order kinetics; hydroxyl ion concentration not being effectively changed during the course of the reaction. Two different methods were used in those cases where both methods were applicable.

Direct Color Formation Method. This method could be utilized only for those compounds containing a nitrophenyl group. These gave highly colored nitrophenoxide or nitrothiophenoxide ions upon hydrolysis. Color formed was measured against a reagent blank in a Beckman Model DB Spectrophotometer. The spectral wavelength of maximum absorption was determined by scanning wavelength versus per cent transmission. All readings were made at this wavelength. Optical density values were converted to concentrations by the use of a standard curve prepared by dissolving a weighed quantity of nitrophenol or nitrothiophenol in 0.01 N base and diluting to a series of concentrations which gave a linear optical density vs. concentration curve.

Kinetic runs were made by pipetting 0.5 ml of acetone stock solution of the ester into 50 ml of base, swirling to mix, and taking samples at measured time intervals. Samples were transferred to a Beckman Class AA silica cell, a glass stopper inserted and the color measured on the spectrophotometer. The temperature was measured by a thermometer kept beside the hydrolyzing solution.

Triester Method. This method was utilized for all compounds studied. The principle of this method was the extraction of unhydrolyzed triester from the incubating medium with chloroform and analysis by the triester method of Getz (1962).

A stock solution of a compound (Stock I) was prepared by weighing out 10 mg of the compound, transferring to a 10 ml volumetric flask and diluting to the mark with acetone. One milliliter of this solution was diluted to 50 ml with acetone to give a solution containing 20 g/ml (Stock II). To prepare a standard curve 0.1, 0.25, 0.5, 0.75 and 1 ml of Stock II were transferred to a series of six Getz tubes, the acetone

evaporated and the residue analyzed by the triester method. The procedure was repeated and the average used to plot a standard curve of optical density versus micrograms.

Kinetic runs were made by adding to each of six 35 ml centrifuge tubes having T 19 standard taper receptacles 2 ml of 0.01 N NaOH solution. Sodium hydroxide solution was standardized against potassium acid phthalate. At intervals of one-half minute, 15  $\mu$ l (15  $\mu$ g) of the acetone stock solution was injected into the alkali using a 50  $\mu$ l Hamilton microliter syringe. An appropriate series of incubation times was determined from the results of a preliminary run. After incubation, 2 ml of 0.01 N HCl was added to the tube from a buret and the tube shaken. When all the tubes had received the HCl, 3 ml of distilled chloroform was added to each of the tubes and shaken. The chloroform layer was then drawn from the centrifuge tube using a 5 ml syringe and transferred to a Getz tube. The aqueous layer was washed twice more with 3 ml of chloroform, individual extracts combined, evaporated in a stream of nitrogen and analyzed by the triester method. Rates of disappearance were determined by taking an average of three replicate runs for each of the incubation times. Runs were made at ambient temperature, temperature being recorded by means of a thermometer kept near the reacting solutions.

The hydrolytic rate constants were calculated as follows: If the reaction is first order or is being made to follow first order kinetics then the rate of reaction is proportional to the amount present at any time:

$$\frac{dx}{dt} = k(a - x) \quad ,$$

where  $a$  is the amount of triester in the base at zero time,  $t$  is the time of hydrolysis,  $x$  units of the triester have hydrolyzed, and  $k$  is the rate constant.

Solving this differential equation:

$$\ln \frac{a}{a-x} = kt$$

A plot was made of  $\ln \frac{a}{a-x}$  versus time and the slope of the estimated best straight line determined algebraically. The slope was equal to the hydrolytic rate constant.

#### Comparative Toxicity to the House Fly (Musca domestica L.)

Rearing and Maintenance of House Fly Cultures. The strain of flies used in the toxicity determinations, the "B" strain, was procured from the USDA laboratory at Beltsville, Maryland, and has been maintained in this department for several years. The strain of flies is "susceptible" in that it has never been pressured by any insecticide.

Gravid adult female house flies were stimulated to oviposit into a cotton wad in a beaker containing fermenting larval medium and dilute ammonium carbonate. A quantity of eggs was measured out by means of a pipette and transferred to a crock containing the larval medium. The larval medium was composed of CSMA mixture (Ralston-Purina Company, St. Louis, Mo.), diamsalt and water. After pupation the upper layer of the medium was removed, the pupa separated and placed in a souffle cup in a wire cage. When the flies had emerged they were given water and fed a mixture consisting of 1 part sucrose and 1 part dried milk.

Treatment and Holding of Flies. The toxicity of each compound to four day old adult female house flies was determined by topical application of acetone solutions to individual insects. Groups of flies were treated with a series of concentrations initially to determine the approximate range of toxicity. From the mortality data of these treatments it was possible to prepare a narrower range of five concentrations which

gave stepwise-increasing mortalities; the lowest mortality per cent being preferably 10-20% and the highest value preferably 85-90%, with three graded increments between these limits.

Flies were treated by applying a one microliter drop of an acetone solution of the toxicant to the notum of the fly by means of a micrometer-driven microdrop applicator acting on a 1/4 cc calibrated Yale tuberculin syringe. The syringe was calibrated by delivering 20 drops of mercury into a tared beaker and then weighing, and calculating the volume actually delivered with the appropriate temperature-corrected mercury density obtained from the Handbook of Chemistry and Physics (Chemical Rubber Co., 40th ed., 1959).

Treatments were made to batches of 25 female flies anesthetized by CO<sub>2</sub>. Flies were held in pint mason jars having screen lids. The jars contained a cotton wad saturated with 10% sucrose solution. Mortality counts were made after 24 hours. A prostrate fly was adjudged dead that exhibited no movement whatsoever during a short period of observation.

The toxicity was expressed as LD<sub>50</sub>; the lethal dose to 50% of a population treated under identical conditions. The LD<sub>50</sub> was found by inspection of a log concentration-probit mortality curve which was plotted with results from at least 3 replicate treatments of the 5 doses to groups of 25 flies.

#### Comparative Inhibition of House Fly Head Cholinesterase

At present, the best hypothesis of the mode of action of organophosphorus insecticides is that they inhibit the cholinesterase enzyme system of the insect (Winteringham and Lewis, 1959). Death results after many integrated physiological systems are disrupted. For this reason, the

cholinesterase inhibiting power of this series of compounds was determined. It is expressed as  $I_{50}$ ; the molar concentration of inhibitor necessary to produce 50% inhibition of a preparation of fly brain cholinesterase.

The strain of flies used was the KUN strain, a susceptible strain of flies which has been cultured in this department for several years. Flies used were of both sexes, 4 to 5 days old. The method used for assay of cholinesterase activity was that of Hestrin (1949), as modified by Robbins et al. (1957) and Monroe and Robbins (1959). Groups of flies were anesthetized with  $CO_2$ , decapitated by shaking with dry ice chips, the heads separated by a 20 mesh wire sieve, and ground in a Potter-Elvehjem homogenizer using 25 heads at a time with 5 ml of 0.134 M phosphate buffer. Grinding was performed in an ice bath to prevent denaturation of the enzyme. Thimersol, .075 ml of a 0.01% solution, was used to prevent bacterial decomposition and homogenates were frozen until use.

A standard curve was prepared by adding 0.2, 0.4, 0.6, 0.8, 1.2, 1.6 and 2 ml of a 5 micromole/ml solution of acetylcholine bromide to test tubes, corresponding to 1, 2, 3, 4, 6, 8 and 10 micromoles of substrate per tube. Increments of pH 7.2 phosphate buffer were added to make the final volume to 2 ml. Four ml of 2M alkaline hydroxylamine was added to each tube with vigorous mixing, followed two minutes later by 2 ml of HCl solution (1 part concentrated HCl diluted with 2 parts water) and then after mixing by 2 ml of 0.37 M  $FeCl_3$  in 0.1 M HCl. The solutions were filtered through Whatman No. 40 filter paper, and after 10 minutes the optical density of each solution was determined in a Beckman DB Spectrophotometer against a reagent blank prepared by reversing the order of addition of the alkaline hydroxylamine and HCl solutions. The average of three readings was taken and a plot of optical density versus micromoles

substrate prepared.

Before each inhibition run the activity of the enzyme brei was ascertained. A non-enzymatic control was prepared by adding 1.0 ml of phosphate buffer and 0.1 ml of acetone to a tube and incubating in a water bath at 37° C. After 15 minutes 0.2 ml of AchBr (10 micromoles) was added and the solution incubated another 15 minutes, then color developed as previously described and read against a reagent blank. An enzymic control was prepared by adding 1.0 ml of  $\text{PO}_4^{=}$  buffer, 0.1 ml acetone and 0.5 ml brei to a test tube, then incubating 15 minutes and adding 0.2 ml AchBr (10 micromoles), incubating 15 more minutes, developing the color and determining the optical density. Micromoles substrate hydrolyzed was found by subtracting micromoles remaining in the enzymic control from micromoles found in the non-enzymatic control. Acceptable levels of enzymic activity were about 3.5 micromoles AchBr hydrolyzed during the time period.

To determine the activity of the inhibitors, inhibitor solutions were added in 0.1 ml of acetone. Five inhibitory concentrations were found which gave a linear range of inhibition when per cent inhibition was plotted against log molar concentration. Each run was made in duplicate and replicated at least three times so that one point on the plot represented the average of six determinations. The  $I_{50}$  was determined as the concentration at which the plotted line crossed the 50% inhibition line.

## RESULTS AND DISCUSSION

### Phosphorochloridate Coupling Synthesis

The product of the phosphorochloridate coupling reaction was a white crystalline material which softened at 58-64° C and melted at 64-5° C.



In order to determine whether this was the desired product an elemental analysis was performed. It was considered that three possible products might be recovered from the reaction: 2,4,5-trichlorothiophenol recovered unchanged; a side product, methyl 2,4,5-trichlorophenyl sulfide; the desired product, O,O-dimethyl S-(2,4,5-trichlorophenyl) phosphorothiolate. Table 1 compares the elemental analysis results with the theoretical per cent of each element for the three possible compounds.

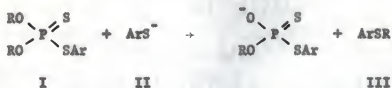
TABLE 1. Elemental per cent found and theoretical elemental per cent for possible products of phosphorochloridate coupling synthesis.

Element	% found	% calculated		
		2,4,5-trichlorothiophenol	methyl 2,4,5-trichlorophenyl sulfide	<u>O,O</u> -dimethyl <u>S</u> -(2,4,5-trichlorophenyl) phosphorothiolate
C	36.12	33.72	36.92	29.86
H	1.49	1.45	2.20	2.49
Cl	46.43	49.88	46.81	33.13
S	14.04	14.95	14.07	9.95

Evidently the major component of the product was methyl 2,4,5-trichlorophenyl sulfide. There was also a probability that 2,4,5-trichlorothiophenol was part of the mixture. The less than sharp melting characteristics of the product were consistent with the assertion that the product was a mixture and Nyquist (personal communication) found spectral evidence for the presence of both unreacted thiol and the methyl aryl sulfide in the product.

A similar side-product has been found after attempted synthesis of phosphorodithioates by a coupling procedure (Miller, 1964). The yield of side product was rationalized by postulating the following side reaction

to have occurred:



I was formed initially by coupling dialkyl phosphorothionylchloridate and the aryl sulfide ion. Once this product was formed it was attacked at an oxygen-adjacent carbon in an alkyl group by the thiophenolate anion (II), resulting in an alkyl aryl sulfide (III) being formed. This scheme was used to explain low (30%) yields of product resulting from using the coupling scheme.

Apparently the same type of reaction occurred in attempts to couple dimethyl phosphorochloridate and 2,4,5-trichlorothiophenolate. However, the side reaction occurred more extensively in this case because essentially no desired product was isolated. The reaction which occurred may have involved dealkylation at an earlier stage:



with no significant amounts of the desired product being produced.

Reactions involving displacements by nucleophilic thiophenolate ions are accelerated when the negative sulfide group is polarized. Miller (1962) points out that phosphates are insensitive to anion polarizability. However, carbons adjacent to electrophilic oxygen, as in the methoxy group of dimethyl phosphorochloridate, are quite susceptible and are therefore the preferred point of attack by the nucleophilic 2,4,5-trichlorothiophenolate ion.

## Products of the Free-Radical Method

Properties and Verifications of Structures. The free-radical method gave products of high purity after column purification and in relatively high yields of 60-70%. The S-aryl phosphorothiolates were generally colorless or very slightly yellowish, slightly viscous liquids at room temperature. The 4-nitrophenyl phosphorothiolate was a yellow crystalline solid, m.p. 37.5-39<sup>o</sup> C (uncorrected); properties which agree well with those reported by Schrader (1952) and Hecht and Wirth (1950).

$R_f$  values, elemental analysis data and physical properties of compounds synthesized are given in Table 2. The  $R_f$  values listed are those of the principal spot detected by the  $AgNO_3$  reagent. Faint spots indicating minor impurities were also detected in some cases. S-aryl phosphorothiolates gave negative spots to the  $AgNO_3$  spray, i.e., areas of the thin layer plate were darker than the spot. Usually a compound gives a spot darker than the background with this reagent. Elemental analyses substantiate the purity of the compounds and indicate that they contain the requisite proportions of constituent atoms since the actual elemental analysis agrees closely with that expected from theoretical calculations. The exception is the 4-nitrophenyl compound which may contain impurities, but other properties such as the infrared peaks, toxicity data and hydrolytic rates of this product indicate substantial purity.

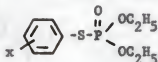
Infrared Spectra. Organophosphorus esters have several characteristic absorption peaks in the IR region. Bellamy (1958) lists the principal group-frequency correlations useful in the interpretation of these spectra: P=O stretching vibration, 1260-1250  $cm^{-1}$ ; P-O-ethyl, 1170-1150  $cm^{-1}$ ; P-S-phenyl, not firmly known but suggested to occur 575-510  $cm^{-1}$ . The IR spectra of these compounds also contain peaks due to other functional

TABLE 2. Properties of  $O_2O$ -diethyl  $S$ -aryl phosphorothiolates.

No.	Chemical name	Structure	% yield	% elemental analysis Calculated Found	TLC $R_f$ values	Physical properties	
I	$O_2O$ -diethyl $S$ -phenyl phosphorothiolate		69	C - 48.77 H - 6.14	C - 49.00 H - 6.24	0.25	Slightly yellowish liquid
II	$O_2O$ -diethyl $S$ -(4-chlorophenyl) phosphorothiolate		68	C - 42.78 H - 5.03 Cl - 12.63 S - 11.42 P - 11.03	C - 43.04 H - 5.20 Cl - 12.53 S - 11.28 P - 11.08	0.33	Clear liquid
III	$O_2O$ -diethyl $S$ -(2-chlorophenyl) phosphorothiolate		66	C - 42.78 H - 5.03	C - 42.59 H - 5.19	0.20	Slightly yellowish liquid
IV	$O_2O$ -diethyl $S$ -(2,4,5-trichlorophenyl) phosphorothiolate		60	C - 34.36 H - 3.46	C - 34.58 H - 3.48	0.55	Clear liquid
V	$O_2O$ -diethyl $S$ -(4-bromophenyl) phosphorothiolate		65	C - 36.94 H - 4.34	C - 36.70 H - 4.50	0.22	Clear liquid
VI	$O_2O$ -diethyl $S$ -(40-nitrophenyl) phosphorothiolate		--	C - 41.23 H - 4.81	C - 38.46 H - 5.38	0.63	Yellow crystals m.p. 37.5-39°C (uncorrected)

groups in the molecule. Some of the correlations of value are: aromatic-H,  $3030\text{ cm}^{-1}$ ; C-H stretch of the ethyl group,  $3000\text{-}2800\text{ cm}^{-1}$ ; C-C stretch,  $980\text{ cm}^{-1}$ ; C-O stretch  $1045$  and  $1020\text{ cm}^{-1}$ ; P-O single bond stretch (Nyquist, personal communication),  $795$  and  $760\text{ cm}^{-1}$ . Photocopies of the six spectra are found on Plate I. Table 3 lists the wavenumbers of principal peaks of each spectrum that coincide with the above correlations.

The relative constancy of the most characteristic bands taken together with the functional group peaks support the general structure of the compounds:



In conjunction with elemental analysis results and TLC the structural identity and purity of this series of compounds was well established. Other properties such as hydrolytic rates, toxicities and cholinesterase inhibition constants had values which might be expected with a series of compounds of this structure.

Heretofore, the spectral positions of P-S-aryl stretching frequencies have not been studied sufficiently to establish a correlation. Bellamy (1958) states that the frequency is probably variable. McIvor, et al. (1956), suggest that it may occur beyond the rock salt range,  $575$  to  $510\text{ cm}^{-1}$ . Early work was limited by the lower spectral range of spectrophotometers being only about  $600\text{ cm}^{-1}$ . In the last few years commercial instruments have become available which scan well into the CsBr region, down to  $300$  or  $250\text{ cm}^{-1}$ . The spectrophotometer used in this study scanned effectively to about  $450\text{ cm}^{-1}$ , being restricted by the IR limit of the KBr cell used. Interpretation of spectral peaks in the region  $600$  to  $450\text{ cm}^{-1}$  was necessary, especially since the phosphorothiolates may have both P-S-(C)

EXPLANATION OF PLATE I

Infrared spectra of some O,O-diethyl S-aryl phosphorothiolates.

## PLATE I

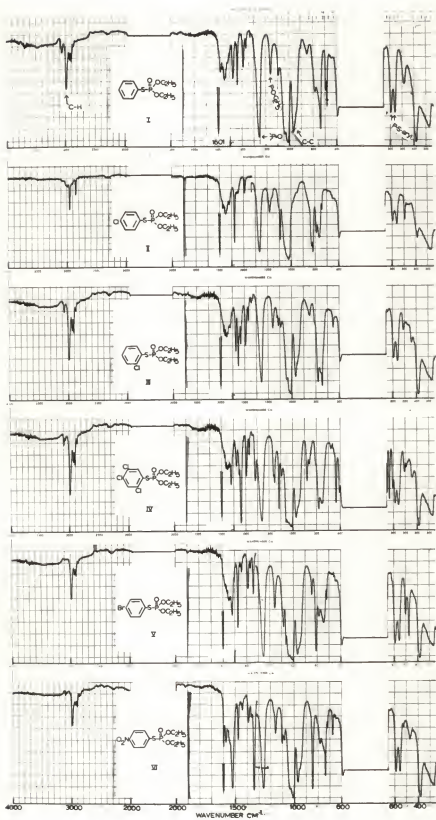


TABLE 3. Infrared spectral characteristics of  $\Omega,\Omega$ -dialkyl  $\xi$ -aryl phosphorothiolates.

Correlation	I	II	III	IV	V	VI
P=O	1255	1258	1259	1261	1256	1261
P-O-ethyl	1160	1157	1160	1152	1158	1160
P-S-aryl*	595 560	592 559	593 560	585 558	590 558	590 557
P-O	739 785	740 780	745 780	753 780	745 782	739 783
aromatic-H	3060, Shoulder 3080	--	3082	3065 3095	--	3100
C-H of C <sub>2</sub> H <sub>5</sub>	2990 2925 2900 2865	2985 2930 2905 2865	2985 2935 2905 2865	2990 2935 2910 2870	2985 2930 2900 2865	2980 2930 2900 2865
C-C	968	968	970	970	965	970
C-O	1015 1042	1010 1040	1015 1045	broad band 1010 to 1060	1005 1040	1010 1040
others	Five adjacent ring H-740 v.s. 695-684 doublet	2 adjacent ring H, 815 (v.s.)	aryl-Cl-650 <sup>†</sup> 4 adjacent H vs, 745	one free ring H; 880,860: aryl-Cl-645	aryl-Br-503 2 adjacent rings H 815	aryl-NO <sub>2</sub> , 1523 and 1342, ring 2 adjacent H 847

\* These are peaks which may be due to this unit.

<sup>†</sup> White (1964) gives aryl-Cl solution spectra correlation = 650 cm<sup>-1</sup>.



and C-S-(P) frequencies in this range.

It is necessary and expedient to realize that correlations suggested on the basis of a limited number of spectra without a corresponding Raman study must be necessarily tentative, and temptations to generalize are unwise. Nevertheless, some limited, speculative interpretations seem worthwhile. Literature citations of the aromatic-S frequency have the limits 702-673  $\text{cm}^{-1}$  (Bellamy, 1958). However, the Beckman Far IR Vibrational Frequency Chart gives correlations for aromatic-S- units conjugated with a strongly electrophilic group:  $\text{R-S-C}^{\text{O}}\text{-S-aryl}$ , 580-545  $\text{cm}^{-1}$ ;  $\text{aryl-S-C}^{\text{O}}\text{-S-aryl}$ , 585-560;  $\text{Cl-C}^{\text{O}}\text{-S-aryl}$ , two peaks, 655-685  $\text{cm}^{-1}$  and 675-650  $\text{cm}^{-1}$ . Structurally and electronically the phosphorothiolates very closely resemble these compounds:  $\text{RO}^{\text{O}}\text{P}^{\text{O}}\text{-S-aryl}$ , an  $\underline{\text{S}}$ -aryl group conjugated with an electrophilic unit. By analogy, two inferences can be made. First, the aryl-S-stretching frequency may be found in the area 600  $\text{cm}^{-1}$  to 550  $\text{cm}^{-1}$ . Secondly, the compounds above most closely resembling the aryl phosphorothiolate structure are the  $\underline{\text{S}}$ -aryl-carbonyl chloride compounds, which have two peaks in this region. It seems reasonable that the double set of peaks found in this region with the phosphorothiolates may be rationalized on this basis.

The restrictions which apply to the above correlation also apply to proposing a (P)-S-aryl frequency. Fukuto and Metcalf (1956) have shown that a graph of the Hammett Sigma value vs the P-O-aryl frequency for a group of dialkyl,  $\underline{\text{O}}$ -aryl phosphates is approximately linear. Figure 1 shows that a similar plot for  $\underline{\text{S}}$ -aryl phosphorothiolates gives roughly a linear curve. Of course the conclusion must be very tentative, but the P-S- stretching peaks are apparently weak to medium intensity bands from 525 to 475  $\text{cm}^{-1}$ . P-S-aryl compounds having a deactivated ring

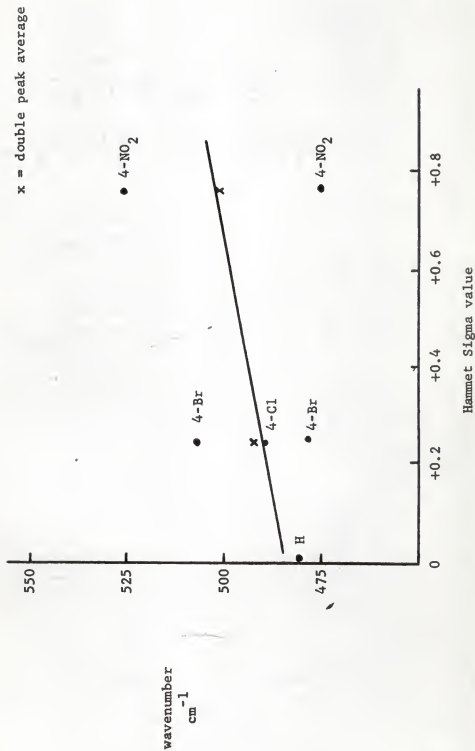


Figure 1. 550-450  $\text{cm}^{-1}$  peaks versus Hammett Sigma value for some O,O-diethyl S-aryl phosphorothiolates.

increase the bond constant and absorb at the high frequency end of the range, while the unactivated ring peak is found at the lower end of the range.

### Hydrolytic Rates

Rates from Direct Color Formation Method. Maximum spectral absorption of sodium 4-nitrophenolate and 4-nitrothiophenolate were found at 400 m and 410 m respectively. Standard curve data for each compound are given in Table i. Results from kinetic runs with paraoxon are listed in Table ii and are plotted in Figure 2. The hydrolysis of paraoxon in strong alkali follows a first order rate equation throughout the range studied, at three different temperatures. The basic hydrolysis of the S-(4-nitrophenyl) phosphorothiolate also follows a first order rate equation but only approximately (Figures 3 and 4). Data are given in Table iii. Some of the rate curves show some deviation from linearity, while those at other temperatures do not. The hydrolytic rate constant was calculated for the "best" straight line drawn through these points. Figure 5 is an Arrhenius plot for paraoxon and O,O-diethyl S-(4-nitrophenyl) phosphorothiolate. Paraoxon has an activation energy of 11.7 Kcal/mole, a value which agrees fairly well with that reported by Heath (1956) of 12.5 Kcal/mole. The Arrhenius plot for the phosphorothiolate shows some scatter from strict linearity. The activation energy for this compound is 9.7 Kcal/mole, explaining why it is hydrolyzed much more rapidly than paraoxon under these conditions. Why the phosphorothiolate hydrolysis is slightly curvilinear while paraoxon hydrolysis is quite linear is not clear. It may be accounted for by deviations from Beer's Law and experimental variability.

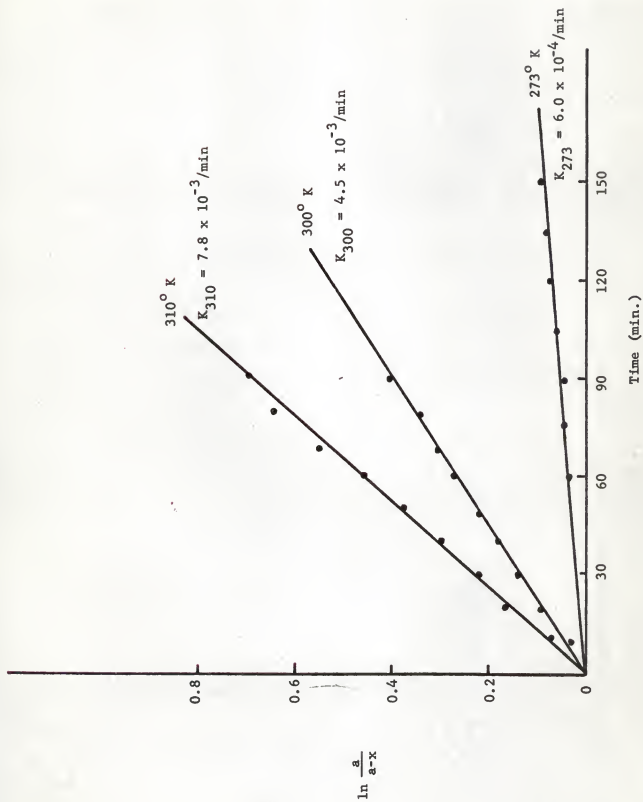


Figure 2. First order hydrolytic rate curves for paraoxon at three temperatures.

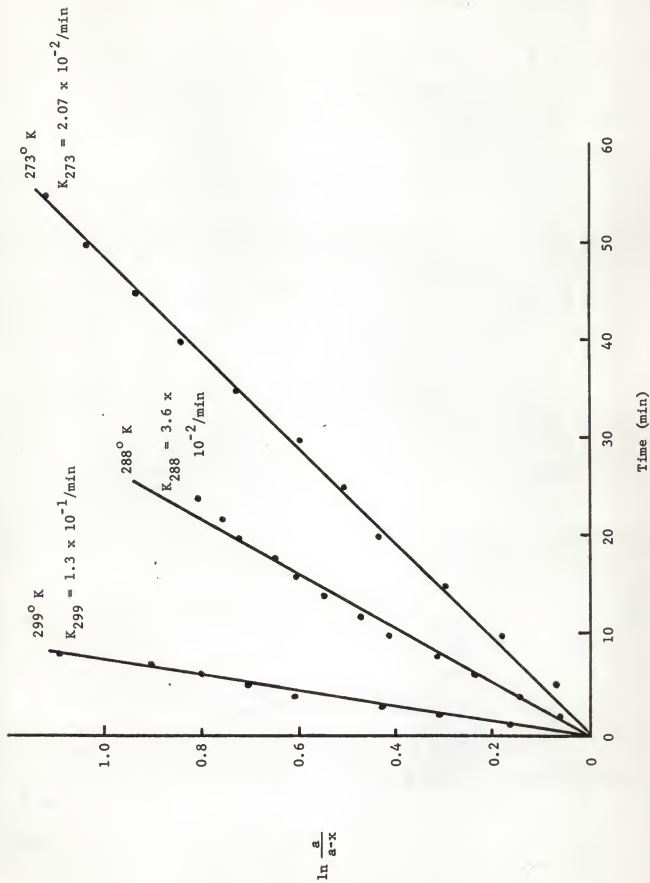


Figure 3. First order hydrolytic rate curves for O,O-diethyl S-(4-nitrophenyl) phosphorothiolate.

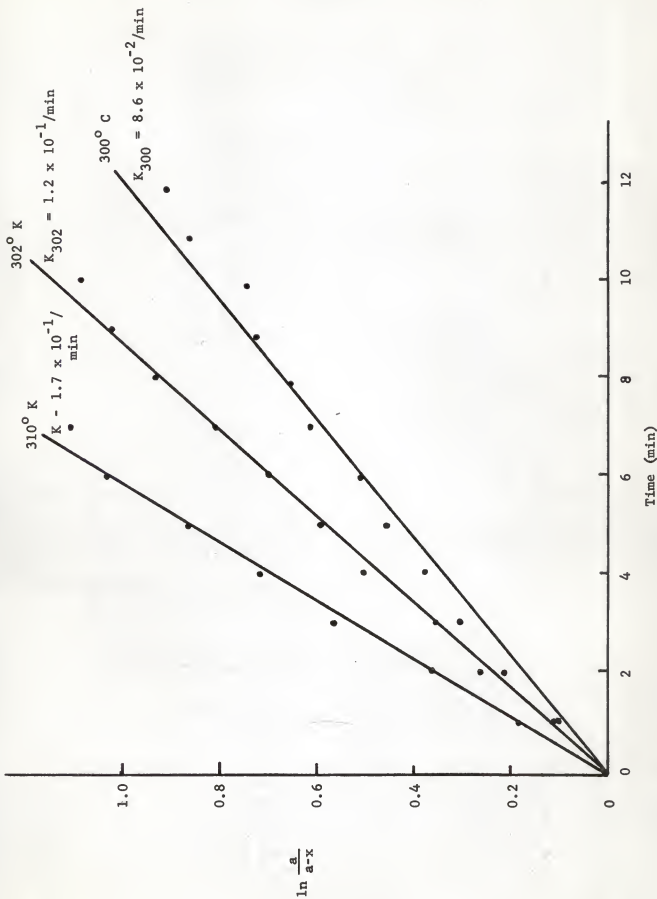


Figure 4. First order hydrolytic rate curves for O,O-diethyl S-(4-nitrophenyl) phosphorothiolate.

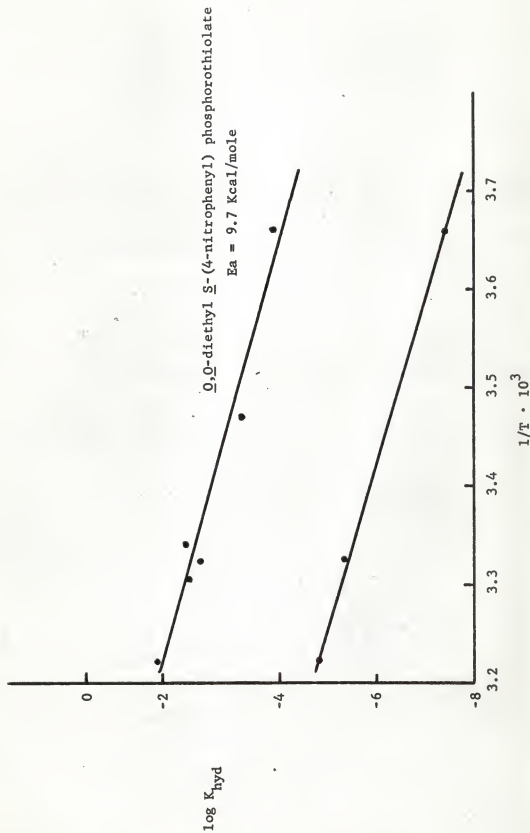


Figure 5. Arrhenius plot for the hydrolysis of paraoxon and  $\underline{O}, \underline{O}$ -diethyl  $\underline{S}$ -(4-nitrophenyl) phosphorothiolate.

Rates from Triester Method. Data for standard curves are collected in Table iv. Each compound is listed under a Roman numeral designation as in Table 2. Figure 6 is a plot of standard curves for the phosphorothiolates and phosphates using moles versus O.D. The plot gives an idea of the relative intensity of red color produced when the various triester complexes with 4-nitrobenzyl pyridine are formed (Getz, 1964):

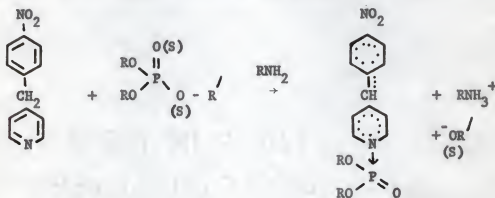
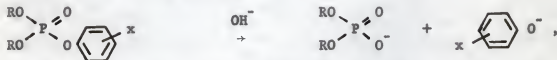


Table v contains the data from the triester hydrolysis determinations. These results are plotted on Figure 7. The first order hydrolytic rate constant is noted beside the curve for each compound.

The hydrolytic rate of organophosphate esters in base is dependent upon the acidity of the phenolic leaving group (Heath, 1956; O'Brien, 1960). With dialkyl substituted-phenyl phosphates the primary route of hydrolysis is cleavage of the phenoxy group:



(Fukuto and Metcalf, 1956). Within a group of structurally similar compounds, the more acidic the leaving group the more rapidly does hydrolysis occur, i.e., diethyl 4-nitrophenyl phosphate hydrolyzes more rapidly than diethyl phenyl phosphate. The hydrolysis diethyl S-aryl phosphorothiolates follows the same pattern.



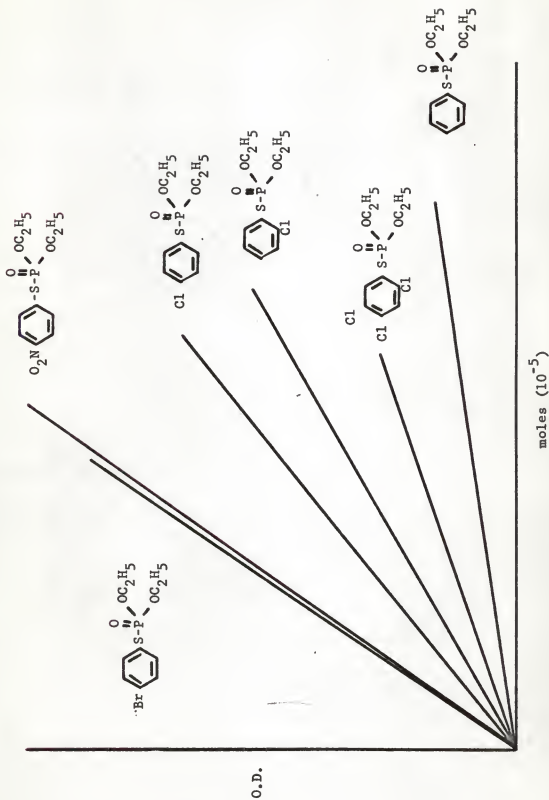


Figure 6. Standard curves of six phosphorothioates showing relative intensity of color formed from the triester method.

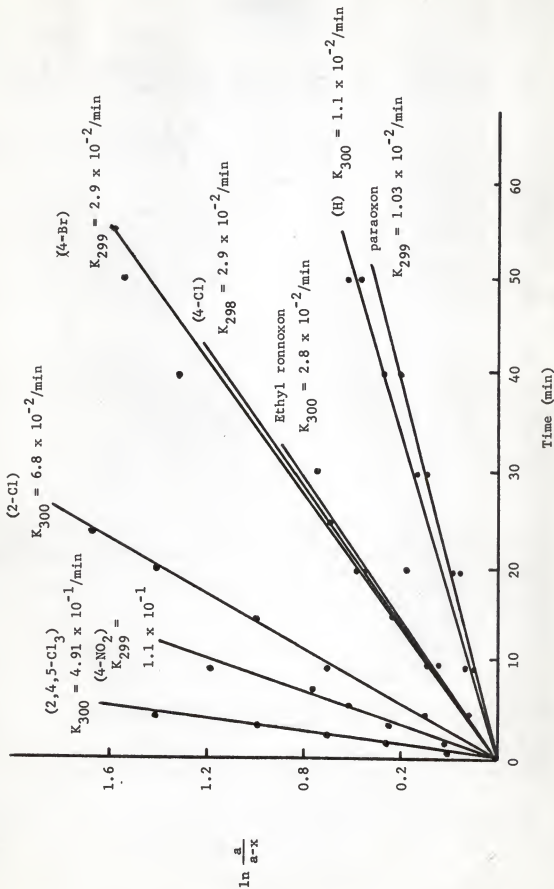
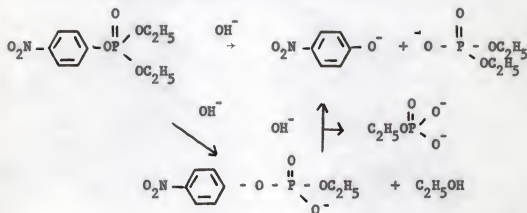


Figure 7. First order plot of the hydrolysis of six phosphorothiolates and two phosphates

Comparison of Methods. The basic hydrolysis of a compound of the type  $(RO)_2POX$ , where X is an acidic substituent, i.e., Cl, Br, phenoxide, etc., is usually thought to occur by the displacement of the X group by hydroxyl ion (O'Brien, 1960). Since the direct color formation method had as its basis the formation of the highly colored 4-nitrophenoxide ion there was evidence that paraoxon hydrolysis was proceeding by another mode. Paraoxon hydrolysis occurred more than twice as fast when determined by the triester method as when determined directly. The triester method was sensitive to hydrolytic cleavage of any of the three ester groups, whereas the direct method measured only hydrolysis of the 4-nitrophenolate moiety. A series of reactions which may have occurred are:



Alkyloxy hydrolysis has been shown to occur with similar compounds (Plapp and Casida, 1958). This result throws into doubt hydrolytic rates quoted by Aldridge and Davidson (1952), Heath (1956), Fukuto and Metcalf (1956) and Fukuto, et al. (1959), since these authors followed the hydrolysis of phosphorus triesters by analytical techniques measuring only the production of phenoxide ion.

#### Comparative Toxicity to the House Fly

The six diethyl phosphorothiolates, two phosphorothionates, parathion

and ethyl ronnel, and two phosphates, paraoxon and ethyl ronnoxon, were tested for activity against the house fly. Dosage and mortality data are found in Table vi. Figure 8 is a plot of log dosage versus probit mortality for each of the ten compounds tested. The only phosphorothiolate discussed with any frequency in the literature was the 4-nitro derivative. Compared with the LD<sub>50</sub> value found in this work, Abdallah (1963) reported an LD<sub>50</sub> of 0.28 µg/fly, which compares fairly well considering differences in experimental conditions. Abdallah also reported an LD<sub>50</sub> of 0.033 µg/fly for parathion and 0.075 µg/fly for paraoxon, values which also agree fairly well with those found in this study. The order of phosphorothiolate toxicity was 4-chlorophenyl >4-bromophenyl >4-nitrophenyl >phenyl >2,4,5-trichlorophenyl >2-chlorophenyl.

It was expected that those phosphorothiolates containing the most highly deactivating substituents, i.e., 4-NO<sub>2</sub> and 2,4,5-trichloro, would be the most toxic since this has been found with the corresponding phosphates (Fukuto and Metcalf, 1956), but this was not found to be true. Evidently permeability through the cuticle was not a very important factor affecting the toxicity of the class since the 4-NO<sub>2</sub>, 2,4,5-trichloro, and 2-chloro compounds would be considered the most polar and hence would penetrate through the house fly cuticle most rapidly (Olsen and O'Brien, 1958). Other factors such as detoxication within the organism, penetration to site of action, etc., may have accounted for the order of toxicity found.

#### Comparative Cholinesterase Inhibition

Inhibition of the cholinesterase enzyme system gives a measure of the relative ability of members of a series of compounds to phosphorylate a free hydroxyl group (O'Brien, 1960). Thionates are generally poor ChE

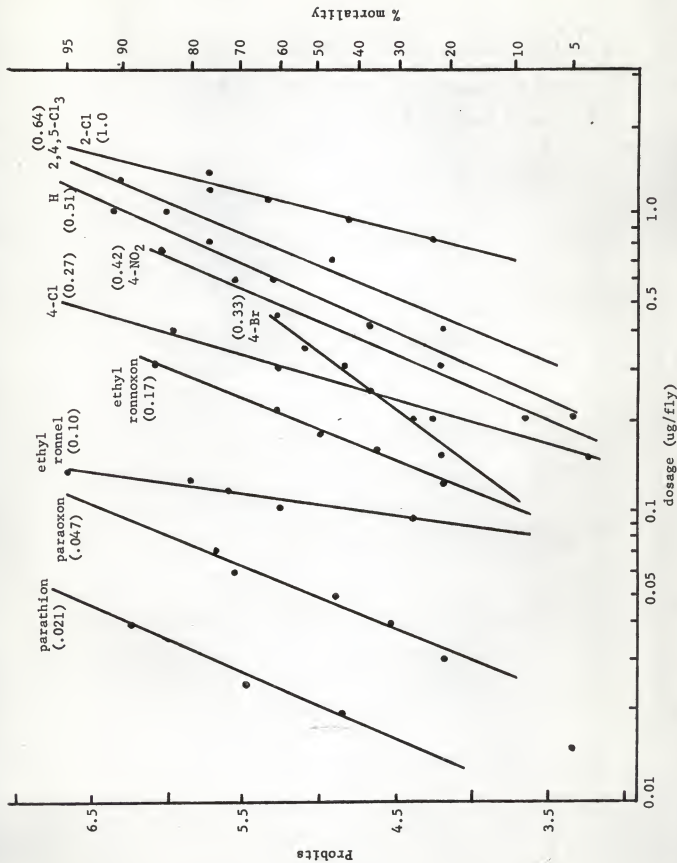


Figure 8. Log dosage - probit mortality curves for related phosphorothionates, phosphorothiolates and phosphates.

inhibitors because the thionyl sulfur reduces the relative positive charge at the phosphorus atom (Heath, 1956). Experimental determinations of  $I_{50}$ 's for phosphorothionates have shown that significant inhibitory activity of these compounds is due to impurities (Diggle and Gage, 1951). For this reason no experimental determinations of  $I_{50}$ 's were made for parathion and ethyl ronnel.  $I_{50}$ 's were determined for the six phosphorothiolates, paraoxon, and ethyl ronnoxon. Concentration-percent inhibition data are found in Table vii.

Figure 9 is a plot of concentration versus log percent inhibition for the 8 compounds studied. The  $I_{50}$  for each inhibitor is noted beside its curve.

The phosphorothiolates were found to be excellent inhibitors of cholinesterase, being in general more active than their corresponding phosphates. Those compounds containing deactivating ring substituents, i.e., 4-Cl, 4-Br, 4-NO<sub>2</sub> and 2,4,5-trichlorophenyl, were the best inhibitors. This inhibitory ability arises primarily from the deactivating influence because the 2-chloro compound has a lower magnitude of activity than the 2,4,5-trichloro compound, which also contains a 2-chloro group; therefore steric hindrance at the enzyme surface is probably of no great importance with these compounds. This assertion is also supported by the low inhibition by the phenyl compound, which has no deactivating substituent.

#### Structure-Activity Relationships

The diethyl S-aryl phosphorothiolates as a group were found to be moderately toxic to insects, being less toxic to the house fly than either corresponding phosphates and phosphorothionates. The order of increasing toxicity of the 4-nitrophenyl analog was S-(4-nitrophenyl) <paraoxon

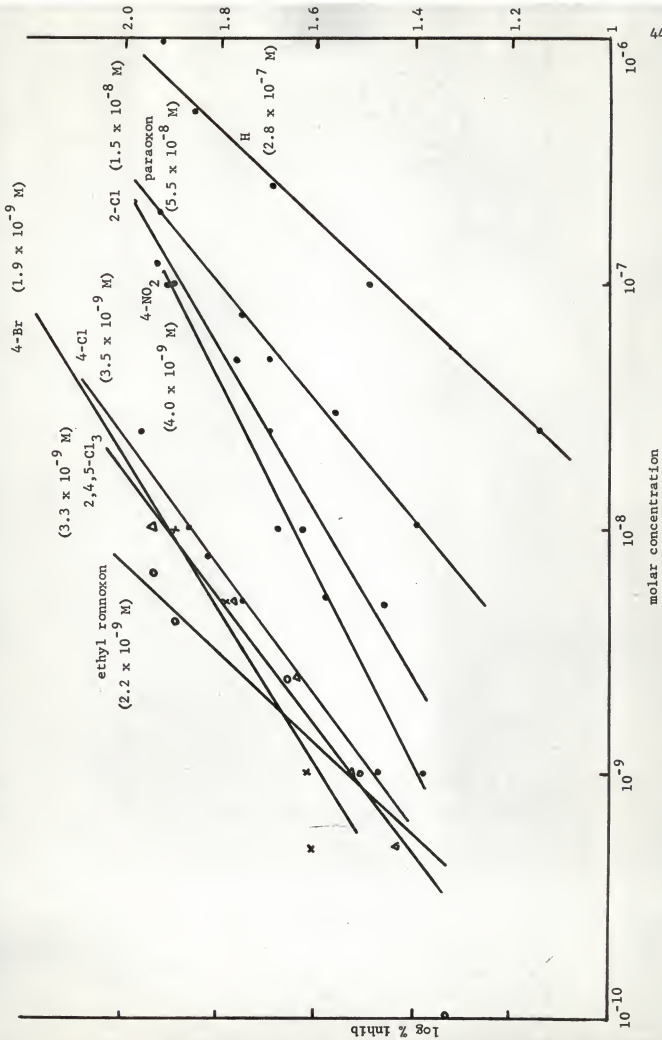


Figure 9. Inhibition of house fly head cholinesterase by six phosphorothiolates and two phosphates.

<parathion. A similar comparison for the 2,4,5-trichlorophenyl compounds gave the same order:  $\underline{S}$ -(2,4,5-trichlorophenyl) <ethyl ronnoxon <ethyl ronnel. Abdallah (1963) found the same order for the 4-nitro analogs with topically treated house flies. Decreased toxicity of the  $\underline{S}$ -(4-nitrophenyl) isomer in comparison to the oxygen analog and the thionate analog has also been found by Schrader (1952) with "leaf lice" fed on sprayed leaves; by Martin (1950) in feeding experiments with *D. oleracea* and by contact to *E. granaria*; and by Woodcock and Stringer (1951) using residual films against "grain weevils."

Table 4 lists the various properties of the phosphorothiolates studied and forms the basis for the preparation of graphs to establish trends or correlations between the properties.

Figure 10 is a plot of  $\log LD_{50}$  vs  $\log K_{hyd}$  for the six phosphorothiolates. There is no apparent correlation between hydrolytic rates and toxicity. Though the hydrolytic rates are not strictly comparable due to the fact that they were taken at slightly different temperatures, one would expect a trend to be found if it existed.

Figure 11 is a plot of  $\log I_{50}$  versus  $\log K_{hyd}$ . No close correlation is found between hydrolytic rates and inhibitory activity. There is a trend toward stronger inhibition by more rapidly hydrolyzed compounds. Fukuto and Metcalf (1956) found that a similar plot for several diethyl substituted-phenyl phosphorothiolates was approximately linear;  $I_{50}$  increasing with increasing ease of hydrolysis.

Figure 12 is a plot of  $\log LD_{50}$  versus  $\log I_{50}$ . There is a trend toward higher toxicity with increasing anti-cholinesterase activity of the phosphorothiolates. Fukuto and Metcalf (1956) found a parallel result in their work with phosphates, but there was no close correlation found.



TABLE 4. Properties of O,O-diethyl S-aryl phosphorothiolates.

Compound	Aryl substituent	LD <sub>50</sub> mole/fly	I <sub>50</sub> (M)	K <sub>hyd</sub>	P-S IR band(s) 530-450 cm <sup>-1</sup>	log LD <sub>50</sub>	log I <sub>50</sub>	log K <sub>hyd</sub>	log K <sub>hyd</sub>
I	--	2.1 x 10 <sup>-3</sup>	2.8 x 10 <sup>-7</sup>	1.1 x 10 <sup>-2</sup> @ 299° K	485	-2.68	-6.55	-1.96	0
II	4-Cl	1.0 x 10 <sup>-3</sup>	3.5 x 10 <sup>-9</sup>	2.9 x 10 <sup>-2</sup> @ 298° K	489	-3.00	-8.46	-1.54	0.23
III	2-Cl	3.5 x 10 <sup>-3</sup>	1.5 x 10 <sup>-8</sup>	6.8 x 10 <sup>-2</sup> @ 299° K	510	-2.46	-7.82	-1.17	--
IV	2,4,5-Cl <sub>3</sub>	2.0 x 10 <sup>-3</sup>	3.3 x 10 <sup>-9</sup>	4.9 x 10 <sup>-1</sup> @ 300° K	494,507	-2.70	-8.48	-0.31	--
V	4-Br	1.0 x 10 <sup>-3</sup>	1.9 x 10 <sup>-9</sup>	2.9 x 10 <sup>-2</sup> @ 299° K	474,502	-3.00	-8.72	-1.54	0.23
VI	4-NO <sub>2</sub>	1.5 x 10 <sup>-3</sup>	9.0 x 10 <sup>-9</sup>	1.3 x 10 <sup>-1</sup> @ 300° K	470,527	-2.82	-8.05	-0.89	0.78

\* Taken from Gould (1959).

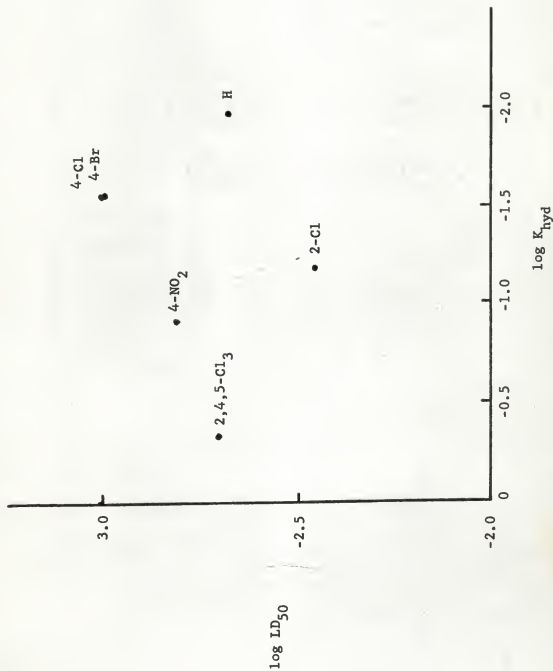


Figure 10.  $\log LD_{50}$  versus  $\log K_{hyd}$  for six phosphorothiolates.

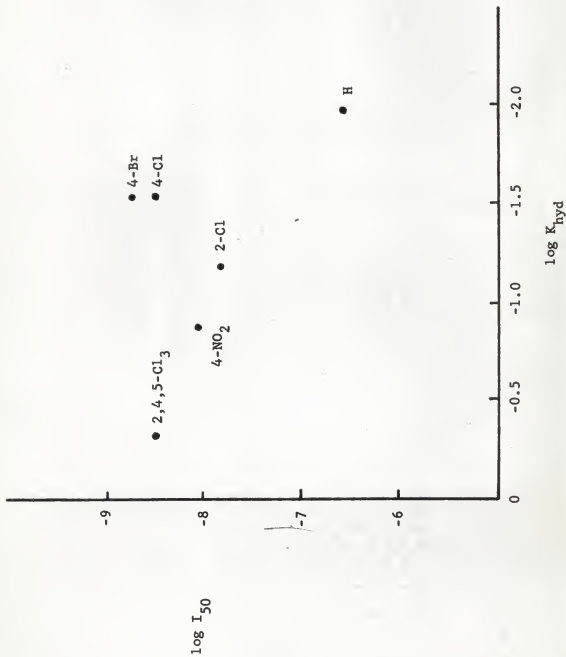


Figure 11. Plot of  $I_{50}$  versus  $\log K_{\text{hyd}}$  for six phosphorothiolates.

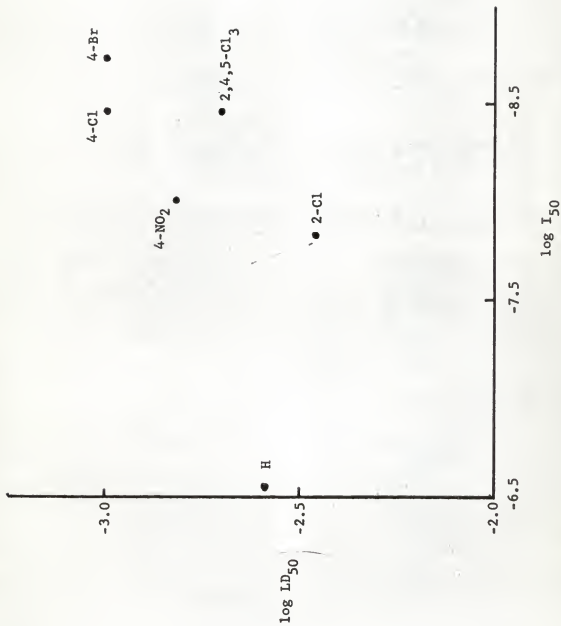


Figure 12. Log LD<sub>50</sub> versus log I<sub>50</sub> for six phosphorothiolates.

Other factors besides cholinesterase inhibition are affecting the toxicity.

Figure 13 is a plot of  $\log K_{\text{hyd}}$  versus the Hammett Sigma value for each compound for which this treatment applies. If x is a substituent on an aryl ring adjacent to a reacting center,

$$\log \frac{K_x}{K_0} = \sigma \rho$$

where  $K_x$  is the reaction rate for a derivative of a parent compound (where x = H), which itself has a reaction rate,  $K_0$ ,  $\rho$  is a constant characteristic of the reaction and  $\sigma$  is a constant which is characteristic of the substituent's electronic character and position. The treatment only applies to para and meta substituents; ortho substituents cause unpredictable electronic and steric effects (Gould, 1959). The plot of phosphorothiolate hydrolytic rates against the Hammett Sigma value is linear. This is further indication, taken with the demonstrations of Fukuto and Metcalf (1956) and Heath (1956), that certain rules derived in classical organic chemistry apply also to reactions at phosphorus centers.

Figure 14 is a plot of  $\log K_{\text{hyd}}$  versus IR peaks in the  $530\text{-}450\text{ cm}^{-1}$  region. Several of the phosphorothiolates have two peaks in this region. If the peaks are plotted and the mean taken for double peaks (x), the resulting plot is nearly linear. The 2-chloro compound is an exception, but this may be rationalized on the basis of an ortho effect. Evidently a correlation exists. The hydrolysis of the phosphorothiolates is believed to proceed by cleavage of the P-S bond (Heath, 1956). If peaks in the 530-450 region are due to some vibration of the P-S-aryl bonds, then one would expect this correlation to exist; i.e., more strained bond  $\rightarrow$  more energetic absorption frequency  $\rightarrow$  more rapid hydrolytic rates. This correlation hinges upon the assumption that the mean of two peaks in

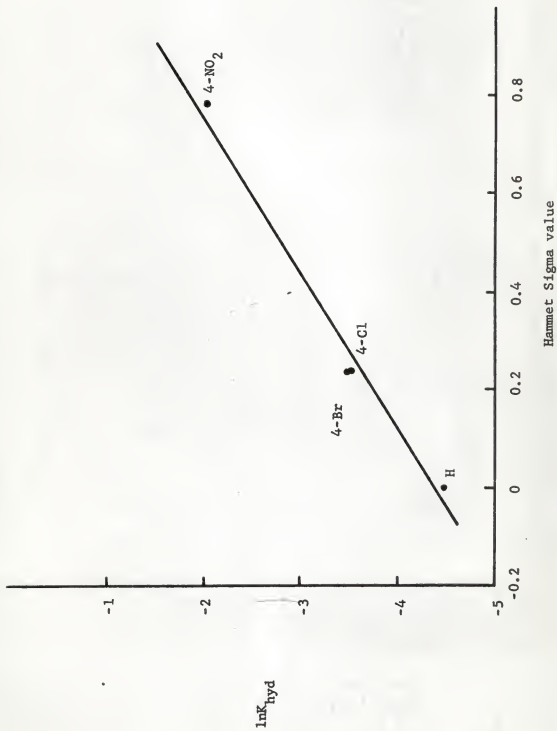


Figure 13.  $\ln K_{\text{hyd}}$  versus Hammett Sigma value for four diethyl para substituted phenyl phosphorothiolates.

x = double peak average

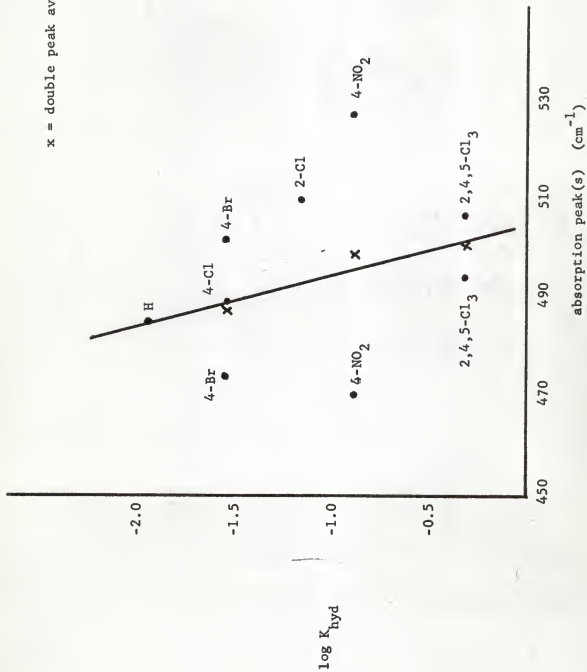


Figure 14.  $\log K_{\text{hyd}}$  versus IR absorption peaks in the 530-450  $\text{cm}^{-1}$  region for six phosphorothiolates.

this region is somehow related to modes of IR vibration or rotation. The correlation could, however, be fortuitous.

#### SUMMARY AND CONCLUSIONS

In the experiments described, a simple one-step synthetic method for O,O-diethyl S-aryl phosphorothiolates is described. The aryl group in compounds I-VI was phenyl, 4-chlorophenyl, 2-chlorophenyl, 2,4,5-trichlorophenyl, 4-bromophenyl and 4-nitrophenyl, respectively.

The method utilized a free-radical reaction between triethyl phosphite, an aryl thiol and bromotrichloromethane. Products were gained in fairly high yield and after column purification, in high purity, as validated by thin layer chromatography, IR spectra and elemental analysis. Diethyl S-aryl phosphorothiolates were found to be colorless or slightly yellowish, odorless, rather viscous oils; one exception being the S-(4-nitrophenyl) compound, which was a yellow, crystalline solid at room temperature. The attempted synthesis of a member of this class of compounds by another route, i.e., the phosphorochloridate coupling technique, was unsuccessful, and the side-product gained was rationalized on the basis of a nucleophilic displacement of an oxygen-adjacent carbon by thiolate anion.

Hydrolysis in alkali was studied using two different techniques. The direct method utilized formation of highly-colored 4-nitrophenolate or 4-nitrothiophenolate ions with optical density measurement in a colorimeter. Hydrolytic rates for paraoxon and O,O-diethyl S-(4-nitrophenyl) phosphorothiolate were measured at several temperatures and their Arrhenius activation energies determined. In general, paraoxon was hydrolyzed more slowly than its analogous phosphorothiolate. Paraoxon, as would be expected, had a higher activation energy than its analog. These rates



were also measured using triester analysis for all six phosphorothiolates and two phosphates, ethyl ronnoxon and paraoxon. The thiolates were hydrolyzed more rapidly than corresponding phosphates in the two cases studied. Phosphorothiolates containing strongly deactivated aryl rings were hydrolyzed more rapidly than those not having strongly deactivating substituents. Comparison of the two methods indicated that paraoxon hydrolysis was occurring by at least two modes because hydrolytic rates determined by the triester method were considerably more rapid than those by the direct method, which measured only one mode of hydrolysis.

To the house fly, the phosphorothiolates were less toxic than corresponding phosphorothionates. However, among the six phosphorothiolates prepared, two of the least toxic contained strong ring deactivating groups, i.e., 4-nitro and 2,4,5-trichlorophenyl. In corresponding phosphates and phosphorothionates this type of substituent generally leads to greater toxicity so the explanation of the aberrant behavior of these phosphorothiolates is not clear.

The phosphorothiolates were excellent inhibitors of house fly head cholinesterase, being in general as powerful inhibitors as corresponding phosphates. The best inhibitors were those phosphorothiolates containing strong deactivating groups, the unsubstituted phenyl compound and the 2-chlorophenyl compound being least active of those examined.

The infrared spectra of the phosphorothiolates contained the peaks expected from organophosphorus compounds of this type. In addition, spectral peaks in the  $600-450\text{ cm}^{-1}$  region were rationalized on the basis of C-S-(P) and P-S-(C) vibrations. A possible correlation between spectral peaks in the  $530-475\text{ cm}^{-1}$  region and hydrolytic rates was found.

Structure activity relationships were examined. There was an

indication that the stronger anti-cholinesterases were more toxic than weaker inhibitors, but no close correlation was found. Hydrolytic rates were found to fit the Hammet equation. No correlation was found between hydrolytic rates and inhibitory strength but there was an indication that more rapidly hydrolyzed phosphorothiolates were better cholinesterase inhibitors.

## ACKNOWLEDGMENT

I wish to express my gratitude to Dr. Theodore L. Hopkins, whose enthusiasm, patience, occasional cajolery and flattery, suggestions, and kindness have provided both stimulus and example so necessary for the completion of this work. To my wife, Susie, I am indebted for her incredible patience in renouncing so much so that this task could be done. I am indebted to Dr. Herbert Knutson for making the assistantship available without which this research could not have been performed. To Emilita Demeterio and Phyllis Lofgren I express my appreciation for their assistance in the determination of inhibition constants, hydrolysis constants and in the synthetic work. To Jon Thomas I am indebted for help with the synthetic work and with thin layer chromatography. To Magdalena Briones I acknowledge valuable assistance in the determination of LD<sub>50</sub> values. I wish also to thank Lois Williams for translating my veritable scrawl into clearly typed English. I am also indebted to Dr. R. A. Nyquist of the Dow Chemical Co. for preparing and aiding in interpretation of IR spectra and to Dr. Ectyl Blair, also of Dow, for advice concerning the synthetic work.

## LITERATURE CITED

- Abdallah, M. D.  
Interaction of some organophosphorus compounds in susceptible and resistant houseflies (Musca domestica L.). Ph.D. dissertation, University of Wageningen, The Netherlands. 1963.
- Aldridge, W. N., and J. M. Barnes.  
Some problems in assessing the toxicity of the organophosphorus towards mammals. *Nature*. 169:345-347. 1952.
- Aldridge, W. N., and A. N. Davidson.  
The inhibition of erythrocyte cholinesterase by triesters of phosphoric acid. 2. Diethyl p-nitrophenyl thionophosphate (E605) and analogues. *Biochem. J.* 52:663-71. 1952.
- Bellamy, L. J.  
The infra-red spectra of complex molecules. 2nd ed. New York: John Wiley and Sons. 1958.
- Bennet, S. H., H. Martin, A. Stringer, and D. Woodcock.  
The qualitative examination of insecticidal properties. *Ann. Rept. Long Ashton, Res. Sta. Bristol*, 138-152. 1948.
- Bianchetti, G.  
Action of aromatic diazo compounds on the salts of  $O,O$ -dialkylmonothiosphoric and  $O,O$ -dialkyldithiosphoric acids. *Rend. Ist. lombardo sci. Pt I*, 91:68-75. 1957. *Chem. Abstr.* 52:11769b. 1958.
- Bunyan, P. J., and J. T. G. Cadogan.  
The reactivity of organophosphorus compounds. Part XIII. Radical-chain transfer reactions of triethyl phosphite: a new phosphorothiolate synthesis. *J. Chem. Soc.* 2953-2958. 1962.
- Callaway, S., P. Dunhuber, and K. M. Wilson.  
A new method for the bioassay of cholinesterase inhibitors. *Nature*. 170:843-844. 1952.
- Diggle, W. M., and J. C. Gage.  
Cholinesterase inhibition in vitro by  $O,O$ -diethyl  $O$ -p-nitrophenyl thiophosphate (parathion, E 605). *Biochem. J.* 49:491-94. 1951.
- El-Refai, A., and T. L. Hopkins.  
Thin-layer chromatography and cholinesterase detection of several phosphorothiono insecticides and their oxygen analogs. *Ag. and Food Chem.* 13:477-481. 1965.
- Fukuto, T. R., and R. L. Metcalf.  
Structure and insecticidal activity of some diethyl substituted phenyl phosphates. *J. Ag. and Food Chem.* 4:930-935. 1956.

- Fukuto, T. R., R. L. Metcalf, and M. Winton.  
Alkylphosphoric acid esters as insecticides. *J. Econ. Entomol.* 52:1121-27. 1959.
- Getz, M. E., and R. R. Watts.  
Application of 4-(p-nitrobenzyl) pyridine as a rapid quantitative reagent for organophosphorus pesticides. *J.A.O.A.C.* 47:1094-1096. 1964.
- Goldwhite, H., and B. C. Saunders.  
Esters containing phosphorus. Part XIII. Dialkyl phosphorobromidates. *J. Chem. Soc.* 3564. 1955.
- Gould, E. S.  
Mechanism and structure in organic chemistry. New York: Holt, Rinehart and Winston. 1959.
- Heath, D. F.  
The effects of substituents on the rates of hydrolysis of some organophosphorus compounds. I. Rates in alkaline solution. *J. Chem. Soc.* 3:3796-804. 1956.
- Hecht, G., and W. Wirth.  
Zur pharmakologie der phosphorsäureester. Derivate der thiophosphorsäure. *Arch. Exper. Path. v. Pharmakol.* 211:264-277. 1950.
- Hestrin, S.  
The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. *J. Biol. Chem.* 180:249-261. 1949.
- Martin, H.  
Advances in chemical methods of crop protection. *J. Sci. Food Agric.* 1:163-167. 1950.
- \_\_\_\_\_.  
The chemistry of insecticides. *Ann. Rev. Entomol.* 1:149-166. 1956.
- McIvor, R. A., G. A. Grant, and C. E. Hubley.  
Infrared studies of sulfur-containing organic derivatives of phosphorus pyroacids. *Can. J. Chem.* 34:1611-1640. 1956.
- Menn, J. J., and K. Szabo.  
The synthesis and biological properties of new O-alkyl S-aryl alkylphosphonodithionates. *J. Econ. Entomol.* 58:734-739. 1965.
- Miller, B.  
Phosphorothioates. II. The effect of the nucleophilicity of the attacking anion upon rates of displacement on O,O-diphenyl phosphorochloridothioate. *J. Am. Chem. Soc.* 84:403-409. 1962.
- \_\_\_\_\_.  
Phosphorothioates. III. Reaction of phosphorothionyl disulfides with organometallic reagents. *Tetrahedron.* 20:2069-2078. 1964.

- Monroe, E. R., and W. E. Robbins.  
Studies on the mode of action of synergized Bayer 21/199 and its corresponding phosphate in the housefly. *J. Econ. Entomol.* 52: 643-647. 1959.
- Obrien, R. D.  
Toxic phosphorus esters. New York, London: Academic Press. 1960.
- Olson, W. P., and R. D. Obrien.  
The relation between physical properties and penetration of solutes into the cockroach cuticle. *J. Ins. Physiol.* 9:777-786. 1963.
- Patchett, G. G., and G. H. Batchelder.  
Multimolecular adsorption chromatography for purification of gram quantities of pesticides. *Ag. Food Chem.* 9:395-396. 1961.
- Pilgrim, K., and F. Korte.  
Zur reaction aromatisches disulfide mit trialkylphosphiten. *Tetrahedron.* 21:203-210. 1965.
- Plapp, F. W., Jr., and J. E. Casida.  
Hydrolysis of the alkyl-phosphate bond in certain dialkyl aryl phosphorothionate insecticides by rats, cockroaches, and alkali. *J. Econ. Entomol.* 51:800-803. 1958.
- Robbins, W. E., T. L. Hopkins, and A. R. Roth.  
Application of the colorimetric whole blood method to the measurement of bovine red-blood-cell cholinesterase activity. *J. Econ. Entomol.* 51:326-329. 1958.
- Schrader, G.  
Monographien zu "Angewandte Chemie" und "Chemie-Ingenieur-Technik, No. 62. Die Entwicklung neuer insektizide auf grundlage organischer fluor-und phosphor-verbindungen. Inded Weinheim: Verlag Chemie. 1952.
- 
- German patent 855,176. 1953.
- White, R. G.  
Handbook of industrial infrared analysis. New York: Plenum Press. 1964.
- Winteringham, F. P. W., and S. E. Lewis. On the mode of action of insecticides. *Ann. Rev. Entomol.* 4:303-318. 1959.
- Woodcock, D., and A. Stringer.  
The insecticidal activity of parathion, its isomers and some related compounds. *Ann. Appl. Biol.* 38:111-120. 1951.

## APPENDIX

TABLE I. Standard curve data for 4-nitrophenolate and 4-nitrothiophenolate ions.

moles 4-nitrophenolate ion	0	$1.29 \times 10^{-5}$	$2.58 \times 10^{-5}$	$3.87 \times 10^{-5}$	$5.16 \times 10^{-5}$
optical density	0	0.115	0.223	0.345	0.442
moles 4-nitrothiophenolate ion	0	$0.69 \times 10^{-5}$	$1.38 \times 10^{-5}$	$2.07 \times 10^{-5}$	$2.76 \times 10^{-5}$
optical density	0	0.100	0.222	0.345	0.442

TABLE II. Kinetic data for hydrolysis of paraoxon at three different temperatures.

Temperature; 273° K a - $3.64 \times 10^{-5}$ M			300° K a = $3.64 \times 10^{-5}$ M			310° K a = $3.64 \times 10^{-5}$ M		
Time (min)	Average O.D.*	$\ln \frac{a}{a-x}$	Time (min)	Average O.D.	$\ln \frac{a}{a-x}$	Time (min)	Average O.D.	$\ln \frac{a}{a-x}$
60	0.065	0.0284	10	0.058	0.0268	10	0.088	0.069
75	0.073	0.0436	20	0.106	0.0888	20	0.156	0.165
90	0.073	0.0436	30	0.143	0.140	30	0.201	0.223
105	0.086	0.0618	40	0.178	0.182	40	0.252	0.300
120	0.094	0.0736	50	0.202	0.215	50	0.301	0.378
135	0.101	0.0862	60	0.234	0.270	60	0.340	0.451
150	0.107	0.0910	70	0.253	0.300	70	0.389	0.548
			80	0.275	0.336	80	0.431	0.642
			90	0.309	0.399	90	0.452	0.693

\* Average optical density of three runs.



TABLE III. Kinetic data for hydrolysis of *O,O*-diethyl *S*-(4-nitrophenyl) phosphorothiolate at six different temperatures.

Temperature; 273° K $a = 3.44 \times 10^{-5}$ M			288° K $a = 3.44 \times 10^{-5}$ M			299° K $a = 3.44 \times 10^{-5}$ M		
Time (min)	Average O.D.	$\ln \frac{a}{a-x}$	Time (min)	Average O.D.	$\ln \frac{a}{a-x}$	Time (min)	Average O.D.	$\ln \frac{a}{a-x}$
			2	0.033	0.056	1	0.091	0.174
5	0.039	0.0652	4	0.076	0.140	2	0.147	0.300
10	0.094	0.182	6	0.121	0.239	3	0.193	0.425
15	0.146	0.300	8	0.153	0.315	4	0.252	0.604
20	0.194	0.432	10	0.188	0.412	5	0.278	0.693
25	0.221	0.501	12	0.208	0.463	6	0.308	0.802
30	0.250	0.593	14	0.232	0.542	7	0.330	0.900
35	0.285	0.718	16	0.253	0.604	8	0.364	1.085
40	0.318	0.842	18	0.265	0.647	9	0.377	1.197
45	0.337	0.936	20	0.283	0.713	10	0.402	1.297
50	0.357	1.029	22	0.293	0.746			
55	0.375	1.121	24	0.307	0.802			
60	0.406	1.316						

Temperature; 300° K $a = 3.44 \times 10^{-5}$ M			302° K $a = 3.44 \times 10^{-5}$ M			310° K $a = 3.44 \times 10^{-5}$ M		
Time (min)	Average O.D.	$\ln \frac{a}{a-x}$	Time (min)	Average O.D.	$\ln \frac{a}{a-x}$	Time (min)	Average O.D.	$\ln \frac{a}{a-x}$
1	0.061	0.113	1	0.057	0.104	1	0.093	0.182
2	0.107	0.207	2	0.135	0.262	2	0.168	0.357
3	0.149	0.307	3	0.164	0.350	3	0.238	0.559
4	0.173	0.370	4	0.214	0.501	4	0.285	0.717
5	0.203	0.451	5	0.250	0.593	5	0.321	0.863
6	0.221	0.507	6	0.277	0.693	6	0.357	1.029
7	0.256	0.615	7	0.307	0.802	7	0.376	1.105
8	0.267	0.652	8	0.333	0.928	8	0.394	1.235
9	0.284	0.718	9	0.355	1.018			
10	0.292	0.742	10	0.366	1.078			
11	0.320	0.859	11	0.383	1.178			
12	0.330	0.904						

TABLE iv. Standard curve data found with the triester method.

Compound	II		III		IV		V		VI		ethyl rounoxon		
	$\mu\text{g}$	O.D.*	$\mu\text{g}$	O.D.	$\mu\text{g}$	O.D.	$\mu\text{g}$	O.D.	$\mu\text{g}$	O.D.	$\mu\text{g}$	O.D.	
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2	0.028	2	0.044	2	0.035	2	0.020	2	0.043	2	0.080	2	0.070
5	0.093	5	0.110	5	0.100	5	0.040	5	0.083	5	0.150	5	0.0140
10	0.141	10	0.295	10	0.185	10	0.110	10	0.208	10	0.340	10	0.305
15	0.220	15	0.375	15	0.310	15	0.160	15	0.297	15	0.415	15	0.427

\* Average optical density of two determinations.

TABLE V. Results of triester hydrolysis technique.

Compound I; T = 300° K			II, T = 299° K			III, T = 290° K			IV, T = 300° K		
Time (min)	O.D.*	$\ln \frac{a}{a-x}$	Time (min)	O.D.*	$\ln \frac{a}{a-x}$	Time (min)	O.D.*	$\ln \frac{a}{a-x}$	Time (min)	O.D.*	$\ln \frac{a}{a-x}$
10	0.206	0	5	0.278	0	5	0.177	0	0.5	0.133	0
20	0.189	0.081	10	0.239	0.140	10	0.128	0.322	1.0	0.107	0.223
30	0.178	0.148	15	0.206	0.254	15	0.084	0.727	1.5	0.083	0.476
40	0.146	0.343	20	0.182	0.438	20	0.063	1.01	2.0	0.066	0.708
50	0.129	0.457	25	0.154	0.593	25	0.043	1.42	2.5	0.048	1.02
60	0.108	0.593	30	0.139	0.698	30	0.033	1.69	3.0	0.035	1.34

V; T = 299° K			VI; T = 299° K			Paraconon, T = 299° K			ethyl renonon; T = 27° C		
Time (min)	O.D.*	$\ln \frac{a}{a-x}$	Time (min)	O.D.*	$\ln \frac{a}{a-x}$	Time (min)	O.D.*	$\ln \frac{a}{a-x}$	Time (min)	O.D.*	$\ln \frac{a}{a-x}$
10	0.202	0	2	0.247	0	10	0.386	0	5	0.140	0
20	0.178	0.131	4	0.195	0.239	20	0.348	0.104	10	0.118	0.140
30	0.138	0.385	6	0.163	0.451	30	0.322	0.182	15	0.098	0.285
40	0.091	0.751	8	0.137	0.620	40	0.286	0.300	20	0.92	0.438
50	0.053	1.33	10	0.113	0.770	50	0.261	0.398	25	0.080	0.560
60	0.043	1.56	12	0.073	1.20	60	0.217	0.590			

\* Average of three runs.

TABLE vi. The toxicity of related phosphorothionates, phosphorothiolates and phosphates to the house fly.

Compound	dosage (ug/fly) mortality (%)				
I	0.20	0.40	0.60	0.80	1.00
	5	37	62	76	91
II	0.15	0.20	0.25	0.30	0.40
	4	23	37	61	83
III	0.80	0.95	1.10	1.20	1.40
	23	43	63	76	76
IV	0.40	0.70	1.00	1.30	1.60
	21	47	84	90	100
V	0.15	0.20	0.25	0.30	0.35
	21	27	38	44	54
VI	0.20	0.30	0.45	0.60	0.75
	9	21	61	71	85
parathion	0.01	0.015	0.020	0.025	0.030
	1.3	5.3	44	68	89
ethyl ronnel	0.09	0.10	0.115	0.125	0.135
	27	60	72	80	95
paraoxon	0.03	0.04	0.05	0.06	0.07
	21	32	45	71	75
ethyl ronnoxon	0.12	0.14	0.16	0.18	0.21
	21	31	35	50	69

TABLE vii. Inhibitory activity of phosphorothiolates and phosphates.

Compound	molar concentration				
	% enzyme inhibition				
I	$10^{-6}$	$5 \times 10^{-7}$	$2.5 \times 10^{-7}$	$10^{-7}$	$2.5 \times 10^{-8}$
	83	71	49	31	14
II	$2.5 \times 10^{-8}$	$10^{-8}$	$7.5 \times 10^{-9}$	$5 \times 10^{-9}$	$10^{-9}$
	92	72	63	56	29
III	$1.5 \times 10^{-7}$	$10^{-7}$	$2.5 \times 10^{-8}$	$10^{-8}$	$5 \times 10^{-9}$
	86	81	50	43	31
IV	$10^{-8}$	$5 \times 10^{-9}$	$2.5 \times 10^{-9}$	$10^{-9}$	$5 \times 10^{-10}$
	86	57	42	35	26
V	$2.5 \times 10^{-8}$	$10^{-8}$	$5 \times 10^{-9}$	$10^{-9}$	$5 \times 10^{-10}$
	100	78	60	42	41
VI	$10^{-7}$	$5 \times 10^{-8}$	$10^{-8}$	$5 \times 10^{-9}$	$10^{-9}$
	81	57	51	40	25
paraoxon	$10^{-7}$	$7.5 \times 10^{-8}$	$5 \times 10^{-8}$	$3 \times 10^{-8}$	$10^{-8}$
	82	57	50	36	24
ethyl ronnoxon	$8 \times 10^{-9}$	$5 \times 10^{-9}$	$2.5 \times 10^{-9}$	$10^{-9}$	$10^{-10}$
	83	77	43	29	22

SYNTHESIS, CHEMICAL AND BIOLOGICAL PROPERTIES OF A  
SERIES OF O,O-DIETHYL S-ARYL PHOSPHOROTHIOLATES

by

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B. A., DePauw University, 1964

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1966

Initial attempts to synthesize O,O-dimethyl S-(2,4,5-trichlorophenyl) phosphorothiolate by coupling of dimethyl phosphorochloridate and the sodium salt of 2,4,5-trichlorothiophenol failed due to the occurrence of a side reaction which produced methyl 2,4,5-trichlorophenyl sulfide as a main product.

Six diethyl S-aryl phosphorothiolates were synthesized by a technique involving a free radical reaction between triethyl phosphite, bromotrichloromethane and an aryl thiol. The aryl groups in compounds I through VI were: phenyl, 4-chlorophenyl, 2-chlorophenyl, 2,4,5-trichlorophenyl, 4-bromophenyl and 4-nitrophenyl, respectively. Structures and purity were verified by thin layer chromatography, elemental analysis and infrared spectroscopy. The infrared spectrum of each compound contained all peaks expected for phosphorus triesters of this structure as well as three or more peaks in the  $600-450\text{ cm}^{-1}$  region which were interpreted as arising from P-S(C) and (P)-S-C vibrations.

Stability in basic solution of the six phosphorothiolates, paraoxon and ethyl ronnoxon was determined using two techniques, where both were applicable. The first technique involved direct measurement of color produced when paraoxon and the S-(4-nitrophenyl) phosphorothiolate were hydrolyzed. Hydrolytic rate constants were ascertained at several temperatures for each compound and Arrhenius activation energies calculated. Paraoxon had an activation energy of hydrolysis of 11.7 Kcal/mole while the S-(4-nitrophenyl) phosphorothiolate had a value of 9.7 Kcal/mole. The second technique involved following the course of hydrolysis by analysis of unhydrolyzed triester and was applicable to all eight compounds. The order of hydrolytic rates found was 2,4,5-trichlorophenyl > 4-nitrophenyl > 2-chlorophenyl > 4-bromophenyl = 4-chlorophenyl > ethyl ronnoxon > phenyl

>paraoxon. The S-(4-nitrophenyl) phosphorothiolate was hydrolyzed at almost the same rate when studied by both methods, but paraoxon underwent more rapid hydrolysis with measurement by the triester method as compared to the direct method. This result indicated that paraoxon hydrolysis was occurring by two modes, i.e., phenoxy hydrolysis and alkoxy cleavage.

The toxicity of the six phosphorothiolates, paraoxon and parathion, ethyl ronnoxon and ethyl ronnel to the house fly Musca domestica L. was determined. The order of toxicity found was parathion >paraoxon >ethyl ronnel >ethyl ronnoxon >4-chlorophenyl >4-bromophenyl >4-nitrophenyl >phenyl >2,4,5-trichlorophenyl >2-chlorophenyl phosphorothiolate.

Activity against house fly head cholinesterase was measured for the six phosphorothiolates, paraoxon and ethyl ronnoxon. The phosphorothiolates were found to be excellent inhibitors, fully as good as corresponding phosphates where comparisons could be made. The best inhibitors contained deactivating ring substituents, activity falling off with the phenyl phosphorothiolate. The relative order of inhibitory activity found was 4-bromophenyl >ethyl ronnoxon >2,4,5-trichlorophenyl >4-chlorophenyl >4-nitrophenyl >2-chlorophenyl >paraoxon >phenyl phosphorothiolate.

Structure-activity relationships were examined. No correlation between  $LD_{50}$  and hydrolytic susceptibility was evident. A trend toward greater inhibition by more rapidly hydrolyzed compounds was indicated and it appeared that better cholinesterase inhibitors had enhanced toxicity to the house fly. A possible correlation between infrared absorption peaks in the  $530-450\text{ cm}^{-1}$  region and hydrolytic rates was found, but the validity of this correlation itself hinged upon the assumption that the average of double peaks for certain of the compounds had structural significance.



O,O-diethyl S-aryl phosphorothiolates were slightly viscous, colorless or very light yellow oils, the one exception among the compounds examined being the 4-nitrophenyl phosphorothiolate which was a low melting yellow crystalline solid. In general they were hydrolyzed more rapidly in base than corresponding phosphates. To the housefly they were less toxic than analogous phosphorothionates and phosphates, but they inhibited the cholinesterase enzyme as well or better than corresponding phosphates.