Synthesis and Biological Properties of Insecticidal *N*-(Mercaptomethyl)phthalimide-*S*-(*O*-alkyl)-alkylphosphonodithioates and Thiolates

Karoly Szabo[1] and Julius J. Menn[2]

A number of *N*-(mercaptomethyl)phthalimide-*S*-(*O*-alkyl)-alkylphosphonodithioates and thiolates were synthesized and examined for their anticholinesterase activity, toxicity to rats, and insecticidal and miticidal properties. The enzymatic inhibition data suggest that the thiolo esters were only moderately more inhibitory than the corresponding dithio esters. The dithio compounds showed greater specificity for insect enzymes. Unusually high anticholinesterase activity was demonstrated by the isopropyl and isoamyl esters. Generally, the ethyl esters showed superior insecticidal and miticidal activity. Considering mammalian toxicity and insecticidal and miticidal activity, the isobutyl ester (X) is the preferred member in the series.

Although, among the numerous organophosphorus insecticides and acaricides which are commercially available, only three are phosphonates [EPN, trichlorofon, and Dyfonate (Stauffer Chemical Co.)], many publications have described the excellent insecticidal properties of various phosphonate esters (Fukuto and Metcalf, 1959; Fukuto *et al.*, 1959; Hollingworth *et al.*, 1967; Menn and Szabo, 1965; Metcalf and Fukuto, 1960).

Systematic examination of structure-activity relationships in selected groups of alkylphosphonate insecticides have revealed that insecticidal activity and cholinesterase inhibition within a series are less dependent on variations in the alkyl and alkoxy groups than in the corresponding phosphate series having two alkoxy groups. In some instances the phosphonate analogs of corresponding phosphate esters proved superior insecticides (Menn and Szabo, 1965).

In the course of chemical synthesis of heterocyclic phosphorus insecticides, a highly active class based on phthalimide was prepared. These compounds are represented by the generic formula illustrated in Figure 1. Analogous dithiophosphates were previously reported as active and useful pesticides. Notable among these is the insecticide Imidan [*N*-(mercaptomethyl) phthalimide-*S*-(*O*,*O*-dimethyl-phosphorodithioate)] and the fungicidal phthalimidophosphorothionates described by Tolkmith *et al.* (1967).

We report here the synthesis, insecticidal and miticidal activity, anticholinesterase activity, and structure-activity relationship of a new series of *O*-alkyl-*S*-phthalimidomethyl-alkylphosphonodithioates and certain thiolate analogs.

MATERIALS AND METHODS

Ethyl- and butylphosphonodichloridothioates were prepared by reaction of the appropriate trialkyl aluminum with PCl_3 and S:

$$AlR_3 + 3\ PCl_3 \xrightarrow[\text{Heptane}]{30°-50°\ C.} 3\ RPCl_2 \cdot (AlCl_3) \qquad (I)$$

$$RPCl_2 \cdot (AlCl_3)\ 1/3 + S \xrightarrow{40°\ C.} RPCl_2 \cdot (AlCl_3)\ 1/3$$

$$RPCl_2 \cdot (AlCl_3)\ 1/3 \xrightarrow[H_2O]{0°-10°\ C.} RPCl_2$$

(70 to 80% over-all yields)

Methylphosphonodichloridothioate was prepared by the $AlCl_3$-catalyzed addition of S to CH_3PCl_2 according to Hoffman *et al.* (1958). The dichloridothioates were purified by fractional distillation using a 2-foot Vigreux column.

The *O*-alkyl-alkylphosphonodithioic acids were prepared by a modified method of Malatesta (1945). NaHS was prepared under anhydrous conditions from Na alkoxides and dry H_2S:

$$R'OH \rightarrow R'ONa \xrightarrow[R'OH]{H_2S} R'OH + NaHS$$

Using three equivalents of NaHS and an excess of the appropriate alcohol as solvent, the *O*-alkyl-alkylphosphonodithioic acids were obtained in one step and yields of from 70 to 85% according to the equation:

$$RPSCl_2 + 3\ NaHS + R'OH \rightarrow \begin{matrix} R & S \\ \diagdown & \diagup\!\!\diagdown \\ & P \\ \diagup & \diagdown \\ R'O & SNa \end{matrix} + 2\ NaCl + 2\ H_2S \qquad (II)$$

On acidification, the free acids are obtained and purified by fractional distillation.

O-Ethyl-ethylphosphonodithioic Acid. Twenty-three grams of sodium was dissolved in 400 ml. of absolute alcohol and 35 grams of dry H_2S was gradually introduced. The reaction was exothermic and completion was clearly indicated by a drop in temperature. The mixture was cooled to 0° C., when some of the NaHS precipitated in the form of a yellow solid.

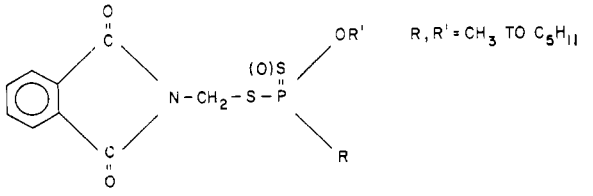
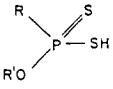


Figure 1. Heterocyclic phosphorus insecticides based on phthalimide

[1] Life Sciences Division, Syracuse University Research Corp., Syracuse, N. Y. 13210
[2] Agricultural Research Center, Stauffer Chemical Co., Mountain View, Calif. 94040

Table I. Physical Constants of *O*-Alkyl-Alkylphosphonodithioic Acid Intermediates



R	R′	B.P., ° C.	*P*, Mm.	n_D^{25}
Me	Me	66–7	1.2	1.5637
Me	Et	74–76	1.25	1.5381
Et	Me	60	0.6	1.5546
Et	Et	70	1.0	1.5398
Et	i-Pr	80–90	0.4	1.5164
Et	*n*-Pr	78–79	1.0	1.5275
Et	i-Bu	108	1.4	1.5124
Et	i-Am	Not isolated		
n-Bu	Et	97–100	0.6–1.4	1.5220
Me	i-Bu	Not isolated		

$EtPSCl_2$ (54.3 grams) was then added dropwise with vigorous agitation while the temperature was maintained at 0° C. When the addition was complete, the mixture was allowed to warm gradually to ambient temperature. During this period, the color changed from yellow to white, indicating that all NaHS had been used up. The reaction mixture was poured into 800 ml. of water, acidified with concentrated HCl [use of H_2SO_4 led to the formation of the *O*-ethyl-ethylphosphonothiolic acid, (EtO) EtP (O) SH], and the precipitated oily product was separated. The aqueous phase was extracted twice with benzene. Fractionation of the organic phase in vacuum yielded 86% of *O*-ethyl-ethylphosphonodithioic acid (b_1 = 70° C., n_D^{25} = 1.5398). Generally, the *O*-alkyl-alkylphosphonodithioic acids were obtained in nearly quantitative yields and high purity. A few were successfully used without isolation and characterization for the preparation of the appropriate *O*-alkyl-*S*-(phthalimidomethyl)-alkylphosphonodithioates.

The physical properties of the *O*-alkyl-alkylphosphonodithioic acids prepared are given in Table I.

O-Alkyl-S-(phthalimidomethyl)-alkylphosphonodithioates. The methods of Fancher (1956) and Lorenz (1955) described for the preparation of *O*,*O*-dialkyl-*S*-(phthalimidomethyl)-phosphorodithioates were applied by reacting *O*-alkyl-alkylphosphonodithioic acids with *N*-halomethylphthalimide in the presence of a base to yield the *O*-alkyl-*S*-(phthalimidomethyl)-alkylphosphonodithioate (Szabo, 1964).

O-Methyl-S-(phthalimidomethyl)-ethylphosphonodithioate (III). Twenty-four grams of *N*-bromomethylphthalimide was suspended in a mixture of 15.6 grams of *O*-methyl-ethylphosphonodithioic acid and 100 ml. of benzene. A 10.3-gram portion of triethylamine was gradually added while the mixture was being stirred. Spontaneous reaction took place with concomitant precipitation of the amine-hydrobromide. During the addition, the temperature rose to 53° C. The mixture was subsequently refluxed for 1 hour. The hydrobromide, obtained in quantitative amount, was separated by filtration. The filtrate was washed with 50 ml. of 3% NaOH solution and water. After removing the solvent at reduced pressure, 19.5 grams of an off-white solid product was obtained, which melted at 87° C. after repeated recrystallizations from methanol (III).

Infrared analysis gave an absorbance pattern practically identical to that of Imidan used as a standard. Strong absorption bands were observed at 1770, 1700, 1070, and 1045 cm^{-1}. The oxo analog was essentially identical except for an additional moderately strong band at 1270 cm^{-1}.

GC (Versamide, T = 185° C., DC – 200) showed no detectable impurity in III. However, good resolution was achieved, with respective retention times of 8.2 minutes (IV) and 11.8 minutes (III) when III and its oxo analog (IV) were cochromatographed. After establishing the lower detection limit for IV (weaker response), increasing amounts of III were injected until the maximum practical limit was reached. Thus, it was demonstrated that IV could not be present in III as an impurity in concentrations higher than 0.1%.

TLC on precoated silica gel S plates (Brinkman Instruments, Westbury, N. Y.) was successfully used to resolve the thiono and oxo compounds when eluted with a 9 to 1 ethyl acetate–benzene mixture. The respective R_f values were 0.22 (IV) and 0.54 (III). The use of 2,6-dibromo-*N*-chloro-*p*-benzoquinoneimine solution as colorimetric detector (Menn *et al.*, 1957) did not permit greater sensitivity than that obtained by GC.

A further attempt was made to exact more information relevant to the extent of the presence of oxo contaminants in the purified thiono compounds by using the method of Winterlin, Walker, and Frank (1968). This is based on detection of the anticholinesterase activity of the resolved components on bee-brain cholinesterase with subsequent incubation with indophenyl acetate. After incubation the indophenyl acetate provides a blue background with distinct white spots left in the inhibited area. The detectable limits were found to be in the range of 0.1 μg. for the P = S compounds and around 0.1 nanogram for the P = O compounds. Thus, the P = S compounds were oxidized with bromine vapors on the plates to enhance the sensitivity of the method. By this method, utilizing cochromatographic techniques, it was estimated that less than 0.05% of IV was present in III.

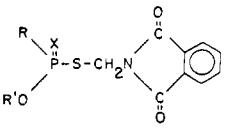
^{31}P NMR was used on selected compounds for proving the structures as well as for establishing purities. The chemical shifts were calculated in the form of parts per million from 85% aqueous phosphoric acid, which is the generally accepted primary standard.

The chemical shifts obtained were in good agreement with literature data available for related structures—for example, the chemical shift for compound IV was −58.5 p.p.m. All other P = O compounds had chemical shifts in the same range. P = S containing dithio compounds demonstrated chemical shifts in the −110 p.p.m. region—for example, the chemical shift of X was −109.6 p.p.m. No detectable amount of P = O compound was found in the P = S compounds scanned under the actual conditions of the measurements.

O-Alkyl-S-(phthalimidomethyl)-alkylphosphonothiolates. The oxo analogs were similarly made from *N*-(halomethyl)-phthalimide and *O*-alkylalkylphosphonothiolic acids, the latter compounds having been prepared *in situ* by Schrader's method (1959) from *O*-alkylphosphonochloridothioates and alkali hydroxides in aqueous alcohols, schematically shown in Figure 2.

The physical constants and the elemental analysis data of the phosphonodithioates and phosphonothiolates are presented in Table II. Since relatively minute amounts of the oxo analog can substantially alter the antienzyme activity of the thiono compounds, particular attention was given to the purification of the thiono compounds. The solids were recrystallized as many times as was needed to reach stable melting points. When needed, methanol, ethanol, and ether were alternately used as solvents for the recrystallizations. Excellent recovery was secured at temperatures as low as −20° C. in these recrystallizations. The liquids were used as technical products after instrumental and analytical identification. Structures

Table II. Physical Constants of *N*-(Mercaptomethyl) phthalimide-*S*-(*O*-alkyl)-alkylphosphonodithioates and Alkylphosphonothiolates



Code	R	R′	X	M.P., ° C. (or n_D^{25})	Analysis, % Theory	Found
I	Me	Me	S	89	C = 43.84; H = 4.01 N = 4.65; P = 10.28 S = 21.28	C = 43.68; H = 3.87 N = 4.80; P = 10.31 S = 21.31
II	Me	i-Bu	S	49	C = 48.97; H = 5.28 N = 4.07; P = 9.01	C = 49.68; H = 5.44 N = 3.97; P = 10.01
III	Et	Me	S	87	C = 45.71; H = 4.47 N = 4.44; S = 20.33	C = 45.90; H = 4.39 N = 4.57; S = 20.18
IV	Et	Me	O	76	C = 48.16; H = 4.71 N = 4.67; P = 10.34 S = 10.72	C = 48.55; H = 4.70 N = 4.46; P = 10.20 S = 10.94
V	Et	Et	S	56	C = 47.41; H = 4.89 N = 4.25; S = 19.47	C = 47.69; H = 4.66 N = 4.35; S = 19.72
VI	Et	Et	O	71	C = 49.83; H = 5.15 N = 4.47; P = 9.89 S = 10.24	C = 50.24; H = 4.97 N = 4.66; P = 9.70 S = 10.32
VII	Et	*n*-Pr	S	(1.5898)	C = 48.97; H = 5.28 N = 4.07; S = 18.68	C = 49.15; H = 5.35 N = 4.14; S = 18.55
VIII	Et	*n*-Pr	O	71	C = 51.37; H = 5.54 N = 4.27; P = 9.46 S = 9.80	C = 51.46; H = 5.59 N = 4.26; P = 9.27 S = 10.10
IX	Et	i-Pr	S	39	C = 48.97; H = 5.28 N = 4.08; P = 9.01 S = 18.68	C = 49.31; H = 5.35 N = 4.33; P = 8.84 S = 18.93
X	Et	i-Bu	S	63	C = 50.40; H = 5.64 N = 3.92; P = 8.66	C = 50.39; H = 5.67 N = 4.06; P = 8.45
XI	Et	i-Am	S	42	N = 3.77; P = 8.34 S = 17.27	N = 3.90; P = 8.50 S = 17.42
XII	*n*-Bu	Et	S	(1.5850)	C = 50.40; H = 5.64 N = 3.92; P = 8.66	C = 50.40; H = 5.79 N = 3.78; P = 8.66

were confirmed by infrared spectroscopy and purities established by elemental analyses, gas chromatography, ^{31}P NMR, and TLC.

Insecticide Testing. Toxicity tests to houseflies, *Musca domestica* (L.), were conducted with a susceptible strain (S, Stauffer) and a chlorothion-resistant strain (R) obtained from R. B. March, University of California, Riverside, Calif. A film contact bioassay was used, and LD_{50} values were determined from log concentration-probit lines by estimation or where data fitted by the method of Litchfield and Wilcoxon (1949). Fourth nymphal instar of the large milkweed bug, *Oncopeltus fasciatus* (Dallas), and fifth nymphal instar of the American cockroach, *Periplaneta americana* (L.), were sprayed with aqueous suspensions containing the candidate compounds. Third instar larvae of the salt-marsh caterpillar, *Estigmene acrea* (Drury), were exposed to leaves of sour dock, *Rumex crispus* (L.), which had been dipped in aqueous suspensions of the test compounds. Mortality counts were made 72 hours after treatment.

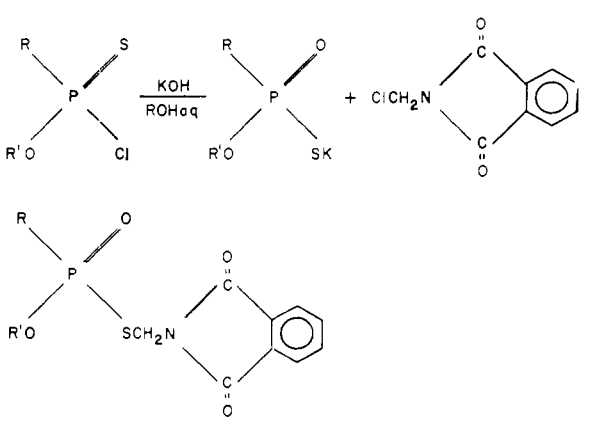
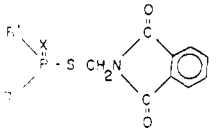


Figure 2. Oxo analogs

To determine the miticidal properties, pinto bean plants in the primary leaf stage were infested with several hundred two-spotted spider mites, *Tetranychus urticae* (Koch). Dispersions of test compounds were prepared by dissolving them in acetone and further diluting with water containing 0.175% (v./v.) of Sponto 221 emulsifier (Retzloff Chemical Co., Houston, Tex.). The test suspensions were sprayed on the infested plants with a spray gun (DeVilbiss, Type RGA, Ser. 502, with No. 390 nozzle) at 10 p.s.i. Treated plants were held in the greenhouse, and ovicidal and miticidal action was determined after 7 days.

Nasturtium plants, *Tropaeolum sp.*, were infested with 50 to 75 black bean aphids, *Aphis fabae* (Scop.). Twenty-four hours later the plants were sprayed, to the point of runoff, with

Table III. Toxicity of *N*-(Mercaptomethyl)phthalimide-*S*-(*O*-alkyl)-alkylphosphonodithioates and Alkylphosphonothiolates to Insects and Mites[a]



Code	R	R′	X	LD_{50}, μg./25 HF	LC_{50}, % AR	MWB	SMC	BA	2 SM PE	2 SM Eggs
I	CH_3	CH_3O	S	10	0.01	0.1	>0.1	0.005	0.001	0.005
II	CH_3	i-C_4H_9O	S	8		>0.1	0.1	0.001	0.001	0.008
III	C_2H_5	CH_3O	S	3	0.03	0.03	0.1	0.0008	0.001	0.005
IV	C_2H_5	CH_3O	O	1	0.03	0.03	0.05	0.0005	0.003	0.05
V	C_2H_5	C_2H_5O	S	8	0.01	0.01	0.05	0.0008	0.001	0.001
VI	C_2H_5	C_2H_5O	O	5	0.03	0.1	0.08	0.0008	0.03	0.05
VII	C_2H_5	*n*-C_3H_7O	S	3		0.01		0.0005	0.001	0.003
VIII	C_2H_5	*n*-C_3H_7O	O	3	0.007	>0.01	0.1	0.003	0.001	0.05
IX	C_2H_5	i-C_3H_7O	S	5	0.008	0.01	>0.1	0.0005	0.001	0.005
X	C_2H_5	i-C_4H_9O	S	8	0.008	0.1	0.03	0.005	0.001	0.01
XI	C_2H_5	i-$C_5H_{11}O$	S	10		0.1	>0.1	0.003	0.001	0.003
XII	*n*-C_4H_9	C_2H_5O	S	8	0.1	0.1	>0.1	0.005	0.003	0.003
XIII	CH_3O	CH_3O	S	4	0.06	0.12	>0.1	0.005	0.01	0.03
XIV	C_2H_5O	C_2H_5O	S	5	0.03	0.5	>0.1		0.01	0.01
XV	C_2H_5O	C_2H_5O	O	5	0.03	0.5	>0.1		0.01	0.03
XVI	i-C_3H_7O	i-C_3H_7O	S	40	0.05	>0.5	>0.5		0.01	0.01
XVII	i-C_4H_9O	C_2H_5O	S	9		0.1	>0.1	0.01	0.003	0.008
XVIII	i-C_4H_9O	i-C_4H_9O	S	80						

[a] HF = *Musca domestic* (*Linn.*), housefly; AR = *Periplaneta americana* (Linn.), American cockroach; MWB = *Oncopeltus fasciatus* (Dallas), spotted milkweed bug; SMC = *Estigmene acrea* (Drury.), salt-marsh caterpillar; 2SM = *Tetranychus urticae* (Linn.), two-spotted spider mite; BA = *Aphis fabae*, bean aphid; PE = postembryonic forms.

aqueous suspensions of the toxicant. Mortality was recorded after 48 hours and the LC_{50} values were expressed as per cent active ingredient in the aqueous suspensions.

Mammalian Toxicity. Single doses were administered in an aqueous mixture containing 1% Tragacanth and 0.05% Tween-20 by stomach tube to 200- to 250-gram male Sprague-Dawley rats. All animals were fasted overnight prior to dosage. The observation period was 14 days.

Cholinesterase Inhibition (I_{50}). I_{50} values were determined on three types of cholinesterase: crystalline bovine erythrocyte (Sigma Chemical Co., St. Louis, Mo.) and bee-head and fly-head cholinesterase by the direct colorimetric method of Archer and Zweig (1959) using indophenyl acetate as the substrate.

One-milliliter portions of the inhibitor at three concentrations were placed in test tubes and 1 ml. of distilled water in a fourth for control. Four milliliters of the purified enzyme solution or brei were added to all four test tubes and incubated at 37° C. for 30 minutes. Indophenyl acetate solution (150 μl. of $3.3 \times 10^{-3}M$ in absolute ethanol) was added to each sample to give a final concentration of $10^{-4}M$ indophenyl acetate and the samples were transferred to 1-cm. cuvettes. A Gilford apparatus was used to record the resultant color at 625 mμ. Four readings were made at 90-second intervals for 30 seconds at a time. Compression studies at three additional concentrations were made for the determination of the exact I_{50} values.

RESULTS AND DISCUSSION

Toxicity to Insects and Mites. The comparative toxicity of phthalimidomethyl-phosphonodithioates and -thiolates and of several corresponding phosphorodithioates and -thiolates to insects and one mite species is given in Table III.

Housefly toxicity data indicate that IV is the most toxic compound, being three times more toxic than the corresponding dithio analog (III). Compounds VI and IX are intermediate in fly toxicity, followed by II, V, X, and XII. Least active are compounds I and XI. High toxicity is apparently dependent on R being ethyl. Increasing the length of R′ reduces toxicity, in the order: III > IX > X > XI. The lower activity of the methyl ester (I) in comparison with the ethyl analog (III) may be due in part to inadequate lipophilicity and to the greater reactivity of the methyl group, which may lead to more rapid detoxication in vivo. In contrast to a previous report (Menn and Szabo, 1965), the phosphorodithioates (XIV and XIII) were more toxic to flies than their corresponding phosphonodithioates (V and I).

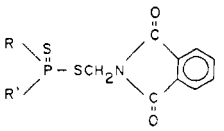
Previously, Fukuto *et al.* (1959) and Menn and Szabo (1965) showed that *S*-aryl and substituted aryl phosphonothiolates and dithioates were highly toxic to phosphate-resistant houseflies. Cross resistances of phosphate-resistant *S*-chlorothion flies to a selected phosphonate (X) and to its corresponding phosphorodithioate analogs (XVII) are compared in Table IV.

The resistant flies showed least cross resistance to the phosphonodithioate (X), followed closely by the corresponding asymmetrical phosphorodithioate (XVII). The level of resistance to the symmetrical analog (XIV) is low. In contrast, a very high level of resistance is shown toward the symmetrical diisobutoxy analog (XVIII).

As noted with the housefly toxicity data, when R is ethyl we have obtained better, over-all insecticidal activity than where R is methyl (I and II) or *n*-butyl (XII). Fukuto *et al.* (1959) reported that insecticidal activity was reduced when the size of the alkyl group attached to phosphorus was increased in a series of phosphonates based on paraoxon. A similar trend was also observed in several other phosphonate series reported by Metcalf and Fukuto (1960). Although the over-all insecticidal activity of compound X is only moderate, it combines a high degree of activity against a lepidopterous species (SMC) and mites (2SM).

Varying R from ethyl to methyl or butyl decreases insecticidal activity. There is remarkably little change in over-all activity when the alkoxy group (R′) is changed from C_1 to C_5

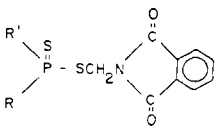
Table IV. Comparative Toxicity of Certain *N*-(Mercaptomethyl)phthalimide-*S*-(*O*-alkyl)-alkylphosphonodithioates to Susceptible (S) and *S*-Chlorothion-Resistant (R) Houseflies



Code	R	R′	LD_{50}, μg./25 Flies: Susceptible (S)	Resistant (R)	R/S[a]
X	C_2H_5	i-C_4H_9O	7	50	7
XVII	C_2H_5O	i-C_4H_9O	8	90	11
XIV	C_2H_5O	C_2H_5O	4	65	16
XVIII	i-BuO	i-BuO	80	>10,000	>125
	Parathion		1	70	70

[a] Resistance ratio = LD_{50} resistant strain/LD_{50} susceptible strain.

Table V. Toxicity to Rats and Selectivity Factor of *N*-(Mercaptomethyl)phthalimide-*S*-(*O*-alkyl)-alkylphosphonodithioates



Code	R	R′	LD_{50}/for Rats, Mg./Kg.	Selectivity Factor, LD_{50} Rats/LD_{50} Flies (S)[a]
I	CH_3	CH_3O	5.8	0.58
III	C_2H_5	CH_3O	7.1	2.38
V	C_2H_5	C_2H_5O	14.1	1.77
VII	C_2H_5	*n*-C_3H_7O	20	6.66
IX	C_2H_5	i-C_3H_7O	10	2.0
X	C_2H_5	i-C_4H_9O	75	9.40
II	CH_3	i-C_4H_9O	37	4.35

[a] Housefly LD_{50} values taken from Table III.

and R = ethyl. This is in contrast to structure activity relationships reported for phosphate series by Metcalf and March (1949), Toy (1948, 1950), and others. The data on the phosphorodithioate compounds (XIII, XIV, and XVI) corroborate the previous findings.

While the difference in activity between the dimethoxy (XIII) and diethoxy (XIV) compounds is negligible, a further increase in the size of the alkoxy group (XVI) results in a significant loss of insecticidal activity. Compound XVI is severalfold less active on insects than XIII and XIV. This trend does not hold for miticidal activity; here, increase in chain length of the alkoxy group slightly enhances the activity.

Although the activity of the symmetrical phosphorodithioates (XIII, XIV, and XVI) is not outstanding *cis-a-cis* the phosphonodithioates, it appears from other data that activity can be enhanced by substituting two unequal alkoxy groups in the phthalimidomethyl-phosphorodithioate molecule (Fancher and Hallett, 1964; Sherman *et al.*, 1967).

The thiolo analogs (IV, VI, and VIII) have insecticidal properties comparable to their thiono counterparts (III, V, and VII, respectively), but inferior ovicidal action. This appears to be also the case in the phosphate series (XIV and XV).

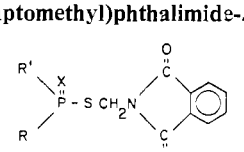
The phosphonodithioates in this series show superior miticidal activity (10×) in comparison to the phosphorodithioates reported in Table III. The exceptionally good activity of compounds IX and X against two citrus rust mite species was reported by Reed *et al.* (1967) and Johnson (1968).

Mammalian Toxicity. Unlike the insecticidal activity, the mammalian toxicity is substantially altered by variations in the alkyl and alkoxy groups (Table V).

When the appropriate methyl and ethyl phosphonates (I *vs.* III and II *vs.* X) are compared, the methylphosphonates are at a disadvantage in respect to mammalian toxicity and the selectivity factor (LD_{50} rats per LD_{50} flies). Increasing the size of the alkoxy group leads to reduced mammalian toxicity, with the exception of the isopropoxy compound (IX) which is approximately equally toxic to the methyl ester (III). The selectivity factor for compound X appears most favorable. It is least toxic to rats and in over-all insecticidal and miticidal activity ranks among the most active members of the series (Table III).

Anticholinesterase Activity. On examining the I_{50} values (Table VI) obtained with bovine erythrocyte and bee- and fly-head cholinesterase, there are several points to consider. Most of the thiono compounds show some degree of specificity for the insect enzymes as compared to the enzyme from bovine erythrocytes. Notable exceptions are I and XI. The *O*-methylmethylphosphonate (I) is a more active inhibitor of bovine erythrocyte cholinesterase than of the fly-head enzyme, in good agreement with the respective LD_{50} and "selectivity factor" values. Similarly, the correlation between the same constants is noteworthy for compound X, which is 1/8.4

Table VI. Anticholinesterase Activity of *N*-(Mercaptomethyl)phthalimide-*S*-(*O*-alkyl)-alkylphosphonodithioates and Thiolates



Code	R	R′	X	I_{50} (Molar Concentration): Bovine	Fly	Bee
I	CH_3	CH_3O	S	5.0×10^{-5}	10^{-4}	3.0×10^{-7}
II	CH_3	i-C_4H_9O	S	5.0×10^{-6}	1.6×10^{-6}	3.1×10^{-7}
III	C_2H_5	CH_3O	S	1.4×10^{-6}	3.3×10^{-7}	1.4×10^{-7}
IV	C_2H_5	CH_3O	O	1.6×10^{-8}	1.6×10^{-8}	5.3×10^{-10}
V	C_2H_5	C_2H_5O	S	3.5×10^{-6}	7.0×10^{-6}	2.2×10^{-7}
VI	C_2H_5	C_2H_5O	O	6.7×10^{-8}	1.4×10^{-9}	2.5×10^{-10}
VII	C_2H_5	*n*-C_3H_7O	S	6.8×10^{-6}	3.5×10^{-6}	6.8×10^{-8}
VIII	C_2H_5	*n*-C_3H_7O	O	4.5×10^{-8}	1.9×10^{-9}	4.4×10^{-10}
IX	C_2H_5	i-C_3H_7O	S	3.9×10^{-7}	2.1×10^{-7}	2.4×10^{-8}
X	C_2H_5	i-C_4H_9O	S	8.4×10^{-5}	10^{-5}	5.6×10^{-6}
XI	C_2H_5	i-$C_5H_{11}O$	S	2.3×10^{-8}	3.5×10^{-8}	1.4×10^{-8}

as active against the bovine enzyme than the fly enzyme and has a "selectivity factor" of 9.4 (Table V). The agreement holds equally well for compounds II and IX. This strict relationship does not hold for III and VII, although in none of these cases a reversal in the general trend of the corresponding constant ratios was observed. Thus it can generally be stated that in this group the ratio of the I_{50} values for the bovine erythrocyte and fly-head enzyme is a useful indicator for predicting selectivity. It is also apparent that the phosphonodithioates and thiolates are more inhibitory toward bee-head cholinesterase than to bovine and flyhead cholinesterase.

With few exceptions the thiono compounds are potent inhibitors of cholinesterase (Table VI). Since it is widely accepted (O'Brien, 1967) that enzyme inhibition results from conversion of the thiono esters ("indirect inhibitors") to their thiolo analogs ("direct inhibitors"), the high inhibition level of the thiono *cis-à-vis* the thiolo esters (IV, VI, and VIII) is somewhat surprising. As a possible explanation, it may be assumed that the thiono compounds are spontaneously converted to the corresponding thiolo esters in the incubation process. Indeed McBain and Menn (1964) reported that certain alkylphosphonodithioate insecticides underwent more rapid conversion to their thiolo analogs than the corresponding phosphorodithioates when exposed to light and air.

Considering the results of insect and mammalian toxicity, and anticholinesterase activity, it appears that *N*-(mercaptomethyl) phthalimide-*S*-(*O*-isobutyl)-ethylphosphonodithioate (X) is the most promising candidate insecticide and miticide in this series. The performance of X as an economic insecticide and miticide was described recently by van den Brink *et al.* (1967).

ACKNOWLEDGMENT

Cholinesterase determinations were performed by H. Willcox, III, Syracuse University Research Corp., and nontoxicity assays by G. D. Meyoing, Stauffer Chemical Co.

LITERATURE CITED

Archer, T. E., Zweig, G., J. AGR. FOOD CHEM. **7,** 178 (1959).
Fancher, L. W. (to Stauffer Chemical Co.), U. S. Patent **2,767,194** (1956).
Fancher, L. W., Hallett, J. T. (to Stauffer Chemical Co.), Australian Patent **272,185** (1964).
Fukuto, T. R., Metcalf, R. L., *J. Am. Chem. Soc.* **81,** 372 (1959).
Fukuto, T. R., Metcalf, R. L., Winton, M., *J. Econ. Entomol.* **52,** 1121 (1959).
Hoffman, F. W., Wadsworth, D. H., Weiss, H. D., *J. Am. Chem. Soc.* **80,** 2945 (1958).
Hollingworth, R. M., Fukuto, T. R., Metcalf, R. L., J. AGR. FOOD CHEM. **15,** 235 (1967).
Johnson, R. B., *Proc. Fla. State Hort. Soc. 1967,* **80,** 73 (1968).
Litchfield, J. T., Wilcoxon, F., *J. Pharmacol. Exptl. Therap.* **96,** 99 (1949).
Lorenz, W. (to Farbenfabriken Bayer), Ger. Patent **930,446** (1955).
McBain, J. B., Menn, J. J., Abstract, Entomological Society of America, National Meeting, Philadelphia, Pa., Nov. 30–Dec. 3, 1964.
Malatesta, L., Pizzotti, R., *Chim. Ind.* **27,** 6 (1945); *CA* **40,** 7939.
Menn, J. J., Erwin, W. R., Gordon, H. T., J. AGR. FOOD CHEM. **8,** 601 (1957).
Menn, J. J., Szabo, K., *J. Econ. Entomol.* **58,** 734 (1965).
Metcalf, R. L., Fukuto, T. R., *J. Econ. Entomol.* **53,** 127 (1960).
Metcalf, R. L., March, R. B., *J. Econ. Entomol.* **42,** 721 (1949).
O'Brien, R. D., "Insecticides, Action and Metabolism," Academic Press, New York, 1967.
Reed, D. K., Crittenden, C. R., Lyon, D. J., *J. Econ. Entomol.* **60,** 668 (1967).
Schrader, G. (to Farbenfabriken Bayer), Ger. Patent **1,064,512** (1959).
Sherman, M., Herrick, R. B., Chang, M. T. Y., Menn, J. J., *J. Med. Entomol.* **4,** 451 (1967).
Szabo, K. (to Stauffer Chemical Co.), Brit. Patent **954,271** (1964).
Tolkmith, H., Senkbeil, H. O., Mussell, D. R., *Science* **155,** 85 (1967).
Toy, A. D. F., *J. Am. Chem. Soc.* **70,** 3882 (1948).
Toy, A. D. F., *J. Am. Chem. Soc.* **72,** 2065 (1950).
Van Den Brink, B. J., Antognini, J., Menn, J. J., Fourth British Insecticide and Fungicide Conference, Proceedings, Vol. **I,** Brighton, England, p. 406, 1967.
Winterlin, W., Walker, G., Frank, H., J. AGR. FOOD CHEM. **16,** 808 (1968).

Received for review July 29, 1968. Accepted April 18, 1969. Research supported in part by NIH Grant GM-14800 to Karoly Szabo