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SYNTHESIS AND ACTIVITY

Synthesis and Insecticidal Activity of O-Alkyl O-2,4,5-Trichlorophenyl Phosphoramidothioates

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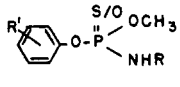
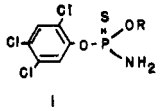
The synthesis, physical properties, and insect toxicities of a series of O-alkyl O-2,4,5-trichlorophenyl phosphoramidothioates are described. The amidothiophosphate esters were prepared from O-2,4,5-trichlorophenyl phosphoramidochloridothioates and from O-alkyl O-2,4,5-trichlorophenyl phosphorochloridothioates. The latter intermediates were prepared by partial esterification of O-2,4,5-trichlorophenyl phosphorodichloridothioate with an alcohol and by treating an O-alkyl phosphorodichloridothioate with sodium 2,4,5-trichlorophenate. While the amidothiophosphates have a wide spectrum of insecticidal activity, their use on plant insects is limited by phytoxicity. They are active against a variety of household insects and grain pests and may be useful in such applications.

RECENT reports from our laboratories have been concerned with the synthesis of aryl methyl phosphoramidates (*4*, *9*) and *O*-aryl *O*-methyl phosphoramidothioates (*2*) and the relationship among chemical structure, anticholinesterase properties, and insecticidal activities.

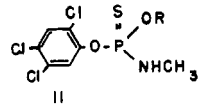


In an early study (*2*), it was demonstrated that insecticidally active compounds could be obtained by replacing a methoxy group of Ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate) (*7*) with an amide (*5*) or a substituted amide group (*8*). In many instances, these compounds, especially *O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate, were more active toward certain insects and mites than the parent triester.

In the early studies the methyl ester was kept constant and the aromatic and amide portions of the molecules were changed. The purpose of the present investigation was to change only the aliphatic ester groups of *O*-alkyl *O*-2,4,5-trichlorophenyl phosphoramidothioates I and II and relate the change in structure to insecticidal activities.



I

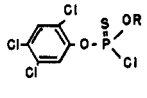


II

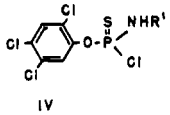
R = CH_3, C_2H_5, *n*-C_3H_7, i-C_3H_7, *n*-C_4H_9, i-C_4H_9, *sec*-C_4H_9

Chemical Studies

O-Alkyl *O*-2,4,5-trichlorophenyl phosphoramidothioates (I) and *O*-alkyl *O*-2,4,5-trichlorophenyl methylphosphoramidothioates (II) were prepared by the amidation of *O*-alkyl *O*-2,4,5-trichlorophenyl phosphorochloridothioates (III) with ammonia or methylamine and by the reaction of *O*-2,4,5-trichlorophenyl phosphoramidochloridothioates (IV) with sodium alkoxides.



III



IV

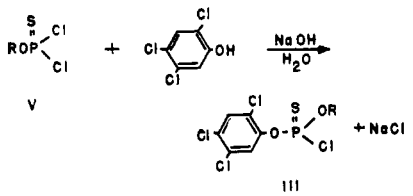
R = R in I and II R′ = H, CH_3

O-Alkyl *O*-aryl phosphorochloridothioates (type III intermediates) have been prepared by treating *O*-aryl phosphorodichloridothioates with excess alcohol in the absence of an acid acceptor (*2*) and by the esterification of *O*-alkyl phosphorodichloridothioates with phenols in the presence of hydrogen chloride acceptors (*6*).

O-2,4,5-Trichlorophenyl phosphorodichloridothioate and an alcohol (2- to 3-mole excess) dissolved in methylene dichloride were heated at 30° to 45° C. (*14*). When the evolution of hydrogen chloride was complete, the acid chloride (III) was washed with water and/or aqueous sodium hydroxide to remove dissolved HCl, unreacted alcohol, and hydrolysis products. Phosphoramidothioates I and II were obtained on treatment with aqueous (*1*) or anhydrous ammonia or methylamine.

This method was particularly suited to the synthesis of the methyl, ethyl, and propyl esters of III. With secondary and higher molecular weight alcohols esterification did not proceed to completion and secondary reactions occurred. The latter intermediates on treatment with ammonia or methylamine gave I or II contaminated with considerable quantities of *O*-2,4,5-trichlorophenyl phosphorodiamidothioates. These particular diamides were insoluble in hydrocarbon solvent and were removed from I and II by recrystallization.

O-Alkyl *O*-2,4,5-trichlorophenyl phosphorochloridothioates (III) were also prepared by the addition of aqueous sodium 2,4,5-trichlorophenate to *O*-alkyl phosphorodichloridothioates (*12*).



This method of synthesis was adaptable to a large number of different *O*-alkyl phosphorodichloridothioates and to a variety of phenols. To facilitate the formation of III and to ensure the complete removal of V, it was desirable to use about 10% excess sodium hydroxide and trichlorophenol. If dichloride (V) was not eliminated, it appeared as an impurity in III and was converted to a diamide on treatment with ammonia or methylamine. The diamides prepared from *O*-methyl and *O*-ethyl phosphorodichloridothioate were partially soluble in water and were removed by washing. However, the diamides from the propyl and butyl phosphorodichloridothioates were insoluble in water and remained in I and II. The use of excess sodium 2,4,5-trichlorophenate eliminated the diamides but produced small amounts of *O*-alkyl *O*,*O*-bis-2,4,5-trichlorophenyl phosphorothioates as impurities in products I and II. The latter impurities, however, were easily removed by distillation and crystallization techniques.

O-Aryl phosphoramidochloridothioates such as IV, readily prepared from *O*-aryl phosphorodichloridothioates and amines (*3*), are less useful intermediates for the preparation of I and II. The addition of a sodium alkoxide solution to IV dissolved in a suitable solvent gave I in about 25% yields and II in about 50% yields. These low yields may be the result of further attack of alkoxide ion on products I and II.

All phosphoramidothioates of type I were crystalline solids with well-defined melting points. Many of the crude products were obtained in a crystalline state by removing the solvent and allowing them to stand. Products of very high purity were readily obtained by recrystallization. All but two of the phosphoramidothioates of type II were crystalline solids and were easily purified by crystallization. The liquid phosphoramidothioates (II) from *n*-propyl alcohol and *n*-butyl alcohol were purified by low-pressure distillations. The phosphoramidothioates were soluble in most organic solvents and insoluble in water. They appeared to be relatively stable to base hydrolysis but were hydrolyzed by strong inorganic acids. The yields, physical properties, and analytical data of the *O*-alkyl *O*-2,4,5-trichlorophenyl phosphoramidothioates are listed in Table I.

The *O*-alkyl phosphorodichloridothioates were prepared by the esterification of of thiophosphoryl chloride with the appropriate alcohol (*10, 11*). *O*-2,4,5-Trichlorophenyl phosphorodichloridothioate (*13*) and *O*-2,4,5-trichlorophenyl phosphoramidochloridothioates (*3*) have been reported elsewhere. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. The synthesis procedures for the preparation of the various phosphoramidothioates are illustrated by the following examples. The yields, physical properties, and analytical data are given in Table I.

Synthesis from *O*-2,4,5-Trichlorophenyl Phosphorodichloridothioate. *O*-Propyl *O*-2,4,5-Trichlorophenyl Methylphosphoramidothioate. Two moles (165 grams) of propyl alcohol was added rapidly to a stirred solution of 165 grams (0.5 mole) of *O*-2,4,5-trichlorophenyl phosphorodichloridothioate in 200 ml. of methylene dichloride. The temperature increased to 43° C. and held at this point for 4 hours with external heat. The solution was then cooled to room temperature and poured into 200 ml. of cold water. The organic phase was separated and washed twice with 150-ml. portions of water. To 50% of the solution diluted with an additional 150 ml. of methylene dichloride was added 25 grams of gaseous methylamine. External cooling was required to keep the reaction mixture at 25° C. Following the addition of the amine the reaction mixture was stirred for 1 hour, then poured into 200 ml. of water. The methylene dichloride solution was separated and washed twice with water. On distilling the solvent, 61 grams (70% of theory) of an oily product was obtained (d_D^{25} 1.3760, n_D^{25} 1.5612).

Analysis. Calculated for $C_{10}H_{13}Cl_2NO_2PS$: Cl, 30.51; N, 4.02. Found: Cl, 30.38; N, 3.98.

This material was 99.5% pure by vapor phase chromatography. The infrared spectrum was identical to an analytical sample prepared by distilling 7 grams through a 1-foot Claisen head still at 0.9 micron. The 3.5 grams of colorless oil distilled at 94–98° C. The physical properties and analytical data for the distilled product are recorded in

Table I. Physical and Analytical Data of O-Alkyl O-2,4,5-Trichlorophenyl Phosphoramidothioates and Methylphosphoramidothioates

Alkyl	Yield, %[a]	M.P., °C.[b]	Calcd., % N	Cl	P	S	Found, % N	Cl	P	S
O-Alkyl *O*-2,4,5-Trichlorophenyl Phosphoramidothioates (I)										
—CH_3	80	66–67	4.57	34.69	10.10	10.46	4.41	34.82	10.16	10.50
—C_2H_5	78	61–62	4.37	33.17	9.66	10.00	4.43	33.08	9.94	10.22
—C_3H_7	73	42–43	4.18	31.78	9.25	9.58	3.99	31.79		
—$CH(CH_3)_2$	80	62–63	4.18	31.78	9.25	9.58	4.18	31.50	9.36	9.86
—C_4H_9	69	43–44[c]	4.01	30.50	8.88	9.19	4.01	30.44		
—$CH(CH_3)C_2H_5$	45	38–39	4.01	30.50	8.88	9.19	4.11	30.38	9.15	9.59
—$CH_2CH(CH_3)_2$	80	71–72[d]	4.01	30.50	8.88	9.19	4.03	30.59		
O-Alkyl *O*-2,4,5-Trichlorophenyl Methylphosphoramidothioates (II)										
—CH_3	69	42–43[c]	4.37	33.17	9.66	10.00	4.29	33.22	9.80	10.14
—C_2H_5	80	30–31	4.18	31.78	9.25	9.58	3.97	32.00	9.43	9.77
—C_3H_7	70	[e]	4.01	30.50	8.88	9.19	4.03	30.51	8.78	9.18
—$CH(CH_3)_2$	70	54–55	4.01	30.50	8.88	9.19	4.00	30.21		
—C_4H_9	52	[f]	3.86	29.32	8.54	8.84	3.81	29.33	8.50	9.07
—$CH(CH_3)_2C_2H_5$	56	33–34	3.86	29.32	8.54	8.84	3.93	29.26		
—$CH_2CH(CH_3)_2$	63	41–42	3.86	29.32	8.54	8.84	3.81	29.51		

[a] Yield of purified material. [b] Crystallized from petroleum ether (b.p. 30–60° C.). [c] Recrystallized from 2-propanol. [d] Final crystallization from methanol. [e] B.p. 94–98° (0.9μ), d_4^{25} 1.3491, n_D^{25} 1.5611. [f] B.p. 95–100° (0.6 μ), d_4^{25} 1.3182, n_D^{25} 1.5550.

Table I. Compounds I [R = CH_3, C_2H_5, $CH(CH_3)_2$, $CH(CH_3)C_2H_5$] and II [R = $CH(CH_3)C_2H_5$] were prepared similarly.

Synthesis from *O*-Alkyl Phosphorodichloridothioates. *O*-ISOPROPYL *O*-2,4,5-TRICHLOROPHENYL PHOSPHORAMIDOTHIOATE. A solution of 246 grams (1.25 moles) of 2,4,5-trichlorophenol and 54 grams (1.35 moles) of sodium hydroxide in 250 ml. of water was added with stirring in 10 minutes to 207 grams (1.0 mole) of *O*-isopropyl phosphorodichloridothioate. The temperature was maintained at 25° to 30° C. during the addition. After stirring at 30° C. for 4 hours the organic phase was separated and washed twice with water and twice with a 2% sodium hydroxide solution. The organic phase was taken up in 200 ml. of methylene dichloride and divided into two equal volumes. To one portion was added an excess of a 25% ammonium hydroxide solution. The mixture was stirred at 30° to 35° C. for 1.5 hours and kept alkaline with 25% ammonium hydroxide solution. The organic layer was separated, washed a number of times with water, and dried. After removal of solvent, the solid was recrystallized from petroleum ether [b.p. 30° to 60° C., yield 80% (138 grams), m.p. 62–63° C.]. Compounds I [R = CH_3 C_2H_5, C_3H_7, C_4H_9, $CH_2CH(CH_3)_2$] were prepared similarly.

O-ISOBUTYL *O*-2,4,5-TRICHLOROPHENYL METHYLPHOSPHORAMIDOTHIOATE. To 310 grams (1.5 moles) of *O*-isobutyl phosphorodichloridothioate was added a solution of 400 grams (2.0 moles) of 2,4,5-trichlorophenol and 90 grams (2.25 moles) of sodium hydroxide in 400 ml. of water. The addition was carried out with agitation at 20° to 30° C. After stirring at 33° to 37° C. for 4 hours, 1 liter of water was added. The oil phase was separated, washed twice with 1 liter of 2% sodium hydroxide solution, and taken up with 300 ml. of methylene chloride.

To 25% of the methylene chloride solution (0.375 mole of III) was added 31 grams (1.0 mole) of gaseous methylamine in 0.5 hour. Cooling was necessary to keep the temperature at 20° to 35° C. After the reaction mixture was stirred for 1.5 hours at 22° to 30° C., 1 liter of water was added to dissolve methylamine hydrochloride. The organic phase was separated and washed two additional times with water. After distilling the solvent, 120 grams of liquid product remained. This was dissolved in 500 ml. of 30° to 60° C. petroleum ether and cooled in a dry ice–acetone bath. The crystalline product was collected and recrystallized from 200 ml. of methanol and from 500 ml. of 30° to 60° C. petroleum ether. The amide was filtered and dried [m.p. 41–42° C., yield 63% (86 grams)]. Compounds II [R = CH_3, C_2H_5, $CH(CH_3)_2$, C_4H_9, $CH_2CH(CH_3)_2$] were prepared similarly.

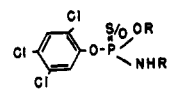
Synthesis from *O*-2,4,5-Trichlorophenyl Phosphoramidochloridothioates. *O*-*sec*-BUTYL *O*-2,4,5-TRICHLOROPHENYL METHYLPHOSPHORAMIDOTHIOATE. A sodium *sec*-butylate solution from 7.7 grams (0.33 mole) of sodium and 300 ml. of *sec*-butyl alcohol was added to 108 grams (0.33 mole) of *O*-2,4,5-trichlorophenyl methylphosphoramidochloridothioate dissolved in 100 ml. of *sec*-butyl alcohol. The addition was carried out at 15° to 25° C. and was complete in 15 minutes. The reaction mixture was stirred at 25° C. for 2 hours, then poured into 1 liter of water. The organic phase was separated and washed twice with 1-liter portions of water. After removal of excess alcohol under reduced pressure, the liquid product (106 grams) was taken up in 300 ml. of hot petroleum ether (b.p. 30° to 60° C.) and cooled in an ice bath. The crystalline product was collected and dried (66 grams, yield 56%, m.p. 34–35° C.). After two additional recrystallizations from low-boiling petroleum ether the amide melted at 33–34° C. Compounds II (R = C_4H_9) and I (R = CH_3) were prepared similarly.

Insecticide Testing Procedure and Results

The test methods used for screening and topical evaluations were as reported previously (*2*). The LD_{50} and LD_{95} are given for each compound on eight species. The insecticidal data on the *O*-2,4,5-trichlorophenyl phosphoramidothioates and the *O*-alkyl *O*-2,4,5-trichlorophenyl methylphosphoramidothioates are given in Table II. Both groups of compounds show a rather wide spectrum of insecticidal activity.

In Table II, changes in order of toxicity to the housefly are due to differences in the screening and topical test methods. In the topical test the housefly is brought in contact dorsally with the toxicant in an acetone solution, but is not exposed to a treated surface and is held for a 24-hour mortality count. In the screening test the housefly is immersed in an acetone-water emulsion containing the toxicant and allowed to walk and feed on a treated surface for 72 hours before making mortality counts.

The phosphoramidates and phosphoramidothioates discussed in previous papers (*2*, *4*) and in the present paper may be represented by the following structure.



The molecular weight of the 30 compounds varies from 291 to 363, with no apparent correlation with insect specificity. Likewise the total number of carbon atoms combined from the OR and NHR groups varies from 1 to 5 without a consistent pattern of insect toxicity. Apparently the differences in the spectrum of activity and in the degree of toxicity to species depend upon an interplay of a number of chemical and physical factors such as molecular shape, vapor pressure and molecular weight, hydrolytic stability, and solubilities in insect tissues.

While these compounds have a wide

Table II. Insecticidal Activity of *O*-Alkyl *O*-2,4,5-Trichlorophenyl Phosphoramidothioates and Methylphosphoramidothioates

Alkyl	Screening Tests Insect Species,[a] P.P.M. LD	2SSM	MBB	PC	SAW	BA	AR	HF	CFB	Topical Tests, μg./Female Housefly
O-Alkyl *O*-2,4,5-Trichlorophenyl Phosphoramidothioates (I)										
—CH_3	50	47	130	26	270	30	90	2.8	P[b]	0.062
	95	120	220	36	360	40	210	4.5	P	0.082
—C_2H_5	50	9	450	60	P	P	350	18	P	0.05
	95	15	P	100	P	P	P	25	P	0.13
—C_3H_7	50	70	25	80	500	P	220	55	P	0.14
	95	170	50	180	P	P	330	150	P	0.35
—$CH(CH_3)_2$	50	3	11	45	220	16	14	8	13	0.18
	95	4.2	14	80	320	20	18	11	16	0.35
—C_4H_9	50	500	16	45	P	380	400	50	P	0.22
	95	P	21	65	P	500	P	130	P	0.44
—$CH(CH_3)C_2H_5$	50	55	15	500	90	220	150	150	P	0.076
	95	75	19	P	190	320	500	250	P	0.12
—$CH_2CH(CH_3)_2$	50	300	18	P	P	P	200	180	P	0.036
	95	P	37	P	P	P	P	450	P	0.05
O-Alkyl *O*-2,4,5-Trichlorophenyl Methylphosphoramidothioates (II)										
—CH_3	50	27	80	28	270	40	100	9	330	0.12
	95	85	300	33	P[b]	95	380	52	P	0.21
—C_2H_5	50	3.7	3.9	52	200	230	95	34	350	0.096
	95	5.6	4.7	70	P	320	170	56	P	0.20
—C_3H_7	50	35	5.5	150	62	370	500	35	P	0.16
	95	105	8.0	250	150	450	P	80	P	0.30
—$CH(CH_3)_2$	50	15	17	110	110	P	500	115	P	0.25
	95	25	20	210	210	P	P	215	P	0.36
—C_4H_9	50	70	12	220	190	200	300	125	P	0.35
	95	110	20	320	280	320	P	185	P	0.44
—$CH(CH_3)C_2H_5$	50	20	9	P	220	P	400	300	P	0.62
	95	80	14	P	320	P	P	480	P	1.44
—$CH_2CH(CH_3)_2$	50	70	7.5	P	180	220	450	125	P	0.30
	95	80	11	P	280	320	P	210	P	0.47

[a] 2SSM = two spotted spider mite, MBB = Mexican bean beetle, PC = plum curculio, SAW = Southern army worm, BA = bean aphid, AR = American cockroach, HF = housefly, CFB = confused flour beetle. [b] P = greater than 500 p.p.m.

spectrum of insecticidal activity, their use on plant insects is limited by phytotoxicity. They are active against a variety of household and grain pests and may be useful in such applications.

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CHEMOSTERILANTS

Structure-Activity Relationships in Apholate Analogs

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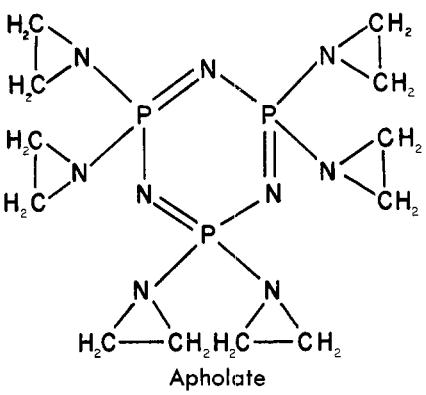
Twenty-five analogs of apholate [2,2,4,4,6,6-hexakis (1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine] were evaluated for chemosterilant activity. A minimum of two aziridinyl substitutions were required in the dimethylamino analogs for effective sterilization of houseflies. As aziridinyl substitutions were increased from two to five, more highly active chemosterilants were obtained. A minimum of four aziridinyl groups were required, however, in the chloro analogs for effective housefly sterilization. This difference between the two series may be related to water solubility. All of the aziridinyl substituted dimethylamino analogs were water-soluble, whereas water solubility in the chloro analogs did not occur until at least four aziridinyl groups were present. Monosubstituents in the apholate molecule other than chlorine or dimethylamine did not alter activity. Substitutions on the aziridinyl groups of apholate reduced chemosterilant activity. The tetrameric analog of apholate containing eight aziridinyl groups, instead of the six in apholate, did not improve activity.

APHOLATE, 2,2,4,4,6,6-hexakis (1-aziridinyl) - 2,2,4,4,6,6 - hexahydro-1,3,5,2,4,6-triazatriphosphorine is one



Apholate

of the more promising chemosterilants containing the aziridinyl group. Although first synthesized by Rätz and Grundmann in 1954 and patented in 1958 (*8*), it was the discovery of its chemosterilant activity by LaBrecque of the U. S. Department of Agriculture in 1960 (*3, 4*) that stimulated interest in compounds of this type and led to the synthesis of several analogs (*5, 6, 9*). The biological evaluation of these analogs is reported in this paper.

Methods and Materials

Chemosterilant activity has been assessed in the authors' laboratory by two different methods. In the first, newly emerged houseflies were fed a granular sugar diet containing 0.5% of the chemosterilant. Eggs subsequently laid in a milk-food cup were removed from the cellucotton with tweezers and floated in a Syracuse watch glass. Approximately 200 eggs were distributed with an eye dropper onto two green blotter tabs. These paired tabs were placed in covered Petri plates and were incubated at 78° F. for 24 hours (Figure 1). The eggs were then observed with a microscope, and the unhatched eggs were expressed as per cent nonviable or sterile (Figure 2).

A second rapid in vitro screening test employed was an existing Squibb Institute cytotoxicity test (*7*) which utilized mouse fibroblast cells grown in tissue culture. Although results from the cytotoxicity method demonstrate that active chemosterilants were not missed, these data also show that the cells are not sufficiently sensitive to separate the highly active chemosterilants from each other.

Results and Discussion

Because apholate is usually made by the substitution of aziridinyl groups for chlorine atoms in trimeric phosphonitrilic chloride, one of the first chemical series studied biologically was the chloro-substituted analogs. Significant housefly sterility or cytotoxicity was not attained in this series until four aziridinyl

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