Structure Activity Analysis of Some *O,O*-Dialkyl (*p*-Methylthio, *p*-Methylsulfonyl) Phenyl Phosphates and Phosphorothioates Prepared for Their Insecticidal Activity

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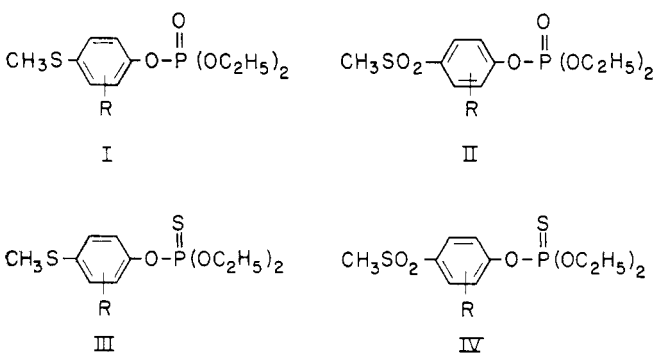
Multiple regression analysis was used to separate the contribution that the thiomethyl and sulfonylmethyl group as well as the P=O and P=S groups made on the insecticidal activity in a series of organophosphates. The analysis indicated the possibility that thiomethyl and the P=S group had to undergo transformation to a more reactive species. The biological activity of the series of phosphates represented by the sulfonylmethyl and the P=O correlated quite well with the partition coefficient and the rate of alkaline hydrolysis. One surprising observation is the indication that the thiomethyl phosphorothioate which is most stable to nucleophilic attack should be the most active as an insecticide.

This is a report of the structure activity features present in the four groups of phosphates shown below (I–IV).



I II III IV

R = H, 2Cl, 3Cl, 2,6-diCl, $2CH_3$, $3CH_3$, and $2NO_2$

The purpose of the investigation was to determine, if possible, the nature of the contribution that the thiomethyl and the sulfonylmethyl groups gave to the insecticidal activity of the phosphate. In addition, the role of the P=O and P=S was also examined. This is a continuation of our studies in the area of structure activity relationships (Neely, 1965; Neely *et al.*, 1964; 1968).

Metcalf *et al.* (1964) and Reynolds *et al.* (1966) pointed out that the conversion of P=S → P=O and CH_3S → CH_3SO_2 provide reactions which result in a change in the lipophilicity of the parent compound. They studied the influence of these changes on the systemic action of various phosphates in cotton plants. Our investigation is an attempt to separate the contributions that such conversions make on the lipophilicity as well as on the reactivity of the insecticide. For this purpose, the structure activity model as proposed by Hansch *et al.* (1965) is used. Briefly, the model assumes that the biological response (B.R.) is a function of the partition coefficient (π) and the reactivity index (k_x). By simple algebraic manipulation, a linear combination of π and k_x may be derived (Equation 1) and the subsequent use of multiple regression analysis allows an assessment of the contribution that both π and k_x make to the biological response.

$$\log \text{B.R.} = a\pi^2 + b\pi + c \log k_x + d \qquad (1)$$

where a, b, c, and d are constants

What is necessary is the accumulation of sufficient data on the series of phosphates indicated in I–IV, followed by an analysis of these data. The following data were necessary for this study:

Biological Response, in this case the lethal action on insects was the response measured and LD_{50} values on flies were obtained by topical application procedures.

Partition Coefficient or π, which is a measure of the amount of drug found at the receptor site.

Reactivity Index, once the drug reaches the receptor site, the inherent activity of the molecule takes over and caused the critical reaction which results in the biological response. In the present study, those phosphates which are most susceptible to nucleophilic attack are assumed to be the best insecticides. Consequently, the rate of hydrolysis in base was used as a measure of reactivity.

MATERIALS AND METHODS

Insecticidal Test Methods. The compounds to be tested were dissolved in acetone. A portion of the acetone solution was then put into a special dispenser and the required number of microliters was applied dropwise to the thorax of the housefly. The treated flies were placed in insect cages and observed for mortality three days post-treatment.

Synthesis of Chemicals. The (methylthio)phenols were prepared by treating the corresponding thiocyanophenols with either sodium methoxide (Method A) (Reifschneider and Kelyman 1966) or with methyl iodide and alkali hydroxide (Method B) (Reifschneider and Kelyman, 1967). The (methylthio)phenols were converted to the methylsulfonylphenols by oxidation with hydrogen peroxide in glacial acetic acid.

The synthetic procedures for the preparation of the phenols and of the various phosphates and phosphorothioates are illustrated in the following samples. The physical properties of the phenols and phosphates are given in Tables I through VI.

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Table I. Physical and Analytical Data of Substituted *p*-Methylthiophenols

R	Method	Yield	M.P. ° C, or B.P. ° C/mm.	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found
H[a]	A	63	84–85	59.96	59.74	5.75	5.62
2-Cl[b]	B	72	83–87/0.6	48.14	48.15	4.04	4.03
3-Cl[a]	B	68	66.5–68	48.14	48.10	4.04	4.11
2,6-Cl_2[c,d]	B	94	65–67	40.21	40.38	2.89	2.78
2-CH_3[a]	A	70	121–122/3	62.30	62.30	6.54	6.50
	B	82	35–36				
3-CH_3[a]	B	97	56–57	62.30	62.32	6.54	6.51
2-NO_2[a]	B	94	60.5–61.5	45.39	45.56	3.81	3.98

[a] Delfs and Wedemeyer (1961); [b] Chien and Chow (1939); [c] Reifschneider and Kelyman (1966); [d] Reifschneider and Kelyman (1967).

Table II. Physical and Analytical Data of Substituted *p*-Methylsulfonylphenols

R	Yield, %	M.P., ° C	Recrystallization Solvent	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found
H[a]	59	93–95	Benzene	48.62	48.74	4.68	4.70
2-Cl	74	168–170	Ethanol	40.68	40.98	3.41	3.22
2,6-Cl_2	84	219–221	Ethanol	34.87	34.88	2.51	2.29
2-CH_3[b]	72	123–124	Benzene	51.59	51.80	5.41	5.50
3-CH_3[c]	65	102–104	Benzene	51.59	51.70	5.41	5.32
2-NO_2[d]	93	166–168	Ethanol	38.71	38.80	3.25	3.24

[a] Zincke and Ebel (1914); [b] Wessely *et al.* (1960); [c] Corral *et al.* (1966); [d] Lavrishchev *et al.* (1960).

Table III. Physical and Analytical Data of *O,O*-Diethyl *O*-[*p*-(Methylthio)phenyl]Phosphates, Structure I

R	Yield,[a] %	B.P. ° C/mm.	$n^{25}D$	$d^{25}4$	Phosphorus, % Calcd	Phosphorus, % Found	Sulfur, % Calcd	Sulfur, % Found	Chlorine, % Calcd	Chlorine, % Found
H[b]	97	83–86/0.0002	1.5151	1.1929	11.21	11.50	11.61	11.69		
2-Cl	98	80–90/0.0002	1.5204	1.2724			10.32	10.31	11.41	11.53
3-Cl	98	84–93/0.0002	1.5197	1.2647	9.97	10.38	10.32	10.10	11.41	11.12
2,6-Cl_2	100	91–115/0.00005	1.5246	1.3264	8.97	9.62			20.54	20.77
2-CH_3[c]	81	91/0.0001	1.5118	1.1741	10.67	11.60	11.05	10.81	Nitrogen, % Calcd	Nitrogen, % Found
3-CH_3	74	83–88/0.0001	1.5180	1.1750	10.67	10.72	11.05	11.22		
2-NO_2	55		1.5277	1.2954	9.64	10.64	9.40	9.98	4.36	4.21

[a] Yield of technical grade material; [b] Benjamini *et al.* (1959); [c] Schrader (1964).

Table IV. Physical and Analytical Data of *O,O*-Diethyl *O*-(*p*-Methylsulfonylphenyl)Phosphates, Structure II

R	Yield,[a] %	M.P., ° C	Phosphorus, % Calcd	Phosphorus, % Found	Sulfur, % Calcd	Sulfur, % Found	Chlorine, % Calcd	Chlorine, % Found
H[b]	50	42–44	10.05	10.25	10.40	10.06		
2-Cl	60	86–88	9.04	9.13	9.36	9.46	10.35	10.21
2,6-Cl_2	90	92–93	8.21	8.37	8.50	8.75	18.80	18.90
2-CH_3	84	72–73	9.61	9.52	9.95	9.89	Nitrogen, % Calcd	Nitrogen, % Found
3-CH_3[c]	50	[d]			9.95	9.75		
2-NO_2	70	34–36	8.77	8.96	9.08	8.98	3.97	3.90

[a] Yield of technical grade material; [b] Benjamini *et al.* (1959); [c] Schrader (1964); [d] This compound had a n^{25}/D of 1.5009 and $d^{25}/4$ of 1.2641.

Table V. Physical and Analytical Data of *O,O*-Diethyl *O*-[*p*(Methylthio)phenyl]Phosphorothioates, Structure III

R	Yield,[a] %	M.P. ° C, or B.P. ° C/mm.	n^{25}/D	d^{25}/D	Phosphorus, % Calcd	Phosphorus, % Found	Sulfur, % Calcd	Sulfur, % Found	Chlorine, % Calcd	Chlorine, % Found
H[b]	72		1.5462	1.1947	10.59	10.50	21.94	22.40		
2-Cl	71	92/0.0001	1.5597	1.2798	9.48	9.37	19.62	19.76	10.85	11.20
3-Cl[c]	83	98/0.0001	1.5604	1.2716	9.48	9.49	19.62	19.55	10.85	10.81
2,6-Cl_2	61	45–47			8.57	8.40	17.75	17.40	19.63	19.83
2-CH_3	47	92/0.0001	1.5487	1.1780	10.11	9.95	20.93	20.93	Nitrogen, % Calcd	Nitrogen, % Found
3-CH_3[c]	61	88–90/0.0002	1.5499	1.1782	10.11	10.13	20.93	20.95		
2-NO_2	71		1.5627	1.2888	9.18	9.22	19.01	18.63	4.15	3.93

[a] Yield of technical grade material; [b] Benjamini *et al.* (1959); [c] Schegk and Schrader (1962).

Table VI. Physical and Analytical Data of *O,O*-Diethyl *O*-(*p*-Methylsulfonylphenyl)Phosphorothioates, Structure IV

R	Yield,[a] %	M.P., ° C	n^{25}/D	$d^{25}/4$	Phosphorus, % Calcd	Phosphorus, % Found	Sulfur, % Calcd	Sulfur, % Found	Chlorine, % Calcd	Chlorine, % Found
H[b]	59	43–44			9.55	9.75				
2-Cl[c]	60	66–67			8.63	8.70	17.87	18.20	9.88	9.90
2,6-Cl_2	80	95–97			7.88	7.88	16.31	16.91	18.03	18.00
2-CH_3[d]	68		1.5313	1.2600	9.15	9.13	18.95	18.48	Nitrogen, % Calcd	Nitrogen, % Found
3-CH_3	66		1.5348	1.2628	9.15	9.27	18.95	19.32		
2-NO_2	78	65–66			8.39	8.18	17.36	17.47	3.79	3.59

[a] Yield of technical grade material; [b] Benjamini *et al.* (1959); [c] Schegk and Schrader (1962); [d] Metivier (1957).

p-(METHYLTHIO)PHENOL (METHOD A). To a solution of 0.2 mole of sodium methoxide in 200 ml. of methanol (prepared by the addition of 4.6 grams (0.2 mole) of sodium to 200 ml. of methanol), a solution of 20.2 grams (0.2 mole) of *p*-thiocyanophenol in 150 ml. of methanol was added in a slow stream. After the addition was completed, the reaction mixture was heated under reflux for 4 hours. The methanol was then removed by evaporation, and the residue was poured into a mixture of ice and excess hydrochloric acid. The solid which separated on standing was collected by filtration, dried, and recrystallized from methylcyclohexane. Seventeen and one-half grams (63%) of white crystals, m.p. 84–85° C. were obtained.

2,6-DICHLORO-4(METHYLTHIO)PHENOL (METHOD B). To a solution of 55.0 grams (0.25 mole) of 2,6-dichloro-4-thiocyanophenol and 35.5 grams (0.25 mole) of methyl iodide in 300 ml. of methanol, a solution of 50.0 grams (0.75 mole) of 85% potassium hydroxide in 150 ml. of water was added in a slow stream. The addition was completed and the mixture was allowed to stir at room temperature for 2 hours. The methanol was removed by distillation under reduced pressure, and the residue was poured into a mixture of ice and excess hydrochloric acid. The solid which separated on standing was collected by filtration, dried, and recrystallized from methanol-water. Forty-nine grams (94%) of white crystals, m.p. 54–56° C. were obtained.

2,6-DICHLORO-4-METHYLSULFONYLPHENOL. A mixture of 105.55 grams (0.5 mole) of 2,6-dichloro-4-(methylthio)phenol, 500 ml. of glacial acetic acid, and 150 grams (1.3 moles) of 30% hydrogen peroxide was heated under gentle reflux for 2 hours. The solution was then concentrated in vacuum, and the solid residue was recrystallized from ethanol. One hundred and two grams (84%) of white crystals, m.p. 219–221° C. were obtained.

O,O-DIETHYL *O*-(4-METHYLTHIO-*m*-TOLYL) PHOSPHATE. A solution of 20.0 grams (0.13 mole) of 4-methylthio-*m*-cresol, 29.0 grams (0.17 mole) of *O,O*-diethyl phosphorochloridate and 15.0 grams (0.15 mole) of triethylamine in 100 ml. of chloroform (amylene inhibited) was heated to 75° C. for 2.5 hours. The reaction mixture was cooled to 25° C. and washed once with a 500-ml. portion of 0.5*N* aqueous sodium hydroxide solution and then twice with 500-ml. portions of deionized water. The chloroform solution was dried over anhydrous calcium sulfate and filtered. The solvent was removed by distillation at 60° C./1 mm., and the remaining amber colored oil (36 grams) was distilled in a molecular still. A center cut of 19.7 grams of a colorless oil was collected between 83–88° C. at 0.0001 mm.

O,O-DIETHYL *O*-(2-CHLORO-4-METHYLSULFONYLPHENYL) PHOSPHATE. A solution of 20.0 grams (0.1 mole) of 2-chloro-4-methylsulfonylphenol, 26.0 grams (0.15 mole) of *O,O*-diethyl phosphorochloridate and 15.0 grams (0.15 mole) of triethylamine in 100 ml. of chloroform (amylene inhibited) was heated to 75° C. for 2 hours. The reaction mixture was cooled to 30° C. and washed once with a 500-ml. portion of 0.5*N* aqueous sodium hydroxide solution and then twice with 500-ml. portions of deionized water. The chloroform solution was dried over anhydrous calcium sulfate and filtered. The solvent was removed by distillation at 60° C./1 mm., and the remaining yellow oil was extracted three times with 300-ml. portions of boiling methylcyclohexane. The methylcyclohexane extract was decanted each time and cooled in an ice bath. The product crystallized and was collected by filtration. The compound was recrystallized first from methylcyclohexane and then from isopropanol. Nineteen grams (36%) of white crystals, m.p. 66–67° C. were obtained.

Table VII. π Values for Phosphates Indicated by Structures I–IV

Compound	π[a]
$C_6H_5OP(OC_2H_5)_2$ (O)	1.64
$C_6H_5OP(OC_2H_5)_2$ (S)	3.46
p-CH_3S—$C_6H_4OP(OEt)_2$ (O)	2.24
p-CH_3SO_2—$C_6H_4OP(OEt)_2$ (O)	0
Group	
2-Cl	0.68[b] (0.59)[d]
3-Cl	1.22[c] (1.20)
2,6-diCl	0.58[c] (0.52)
2-CH_3	0.49[b] (0.52)
3-CH_3	−0.23[b] (−0.23)
2-NO_2	−1.64
p-CH_3SO_2	0.60 (0.62)
p-CH_3S	

[a] Determined experimentally from the procedure described by Hansch *et al.* (1963).
[b] Based on parent compound of *p*-$CH_3SC_6H_4OP(OC_2H_5)_2$.
[c] Based on parent compound of *p*-$CH_3SO_2C_6H_4OP(OC_2H_5)_2$.
[d] Values in parenthesis were determined by Hansch *et al.* (1963).

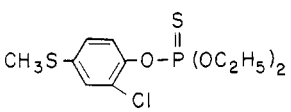
Partition Coefficients. These were determined using octanol and water as described by Hansch *et al.* (1963). In the case of the various substituents, the experimental π was very similar to the value reported by Hansch. The partition coefficient and the π values for the groups in question are shown in Table VII. The π values for structures I–IV are

Table VIII. Compilation of Data for Various Phosphates (I–IV)

R	LD_{50}[a]	π[b]	$k \times 10^3$[c]
Group I.	***O,O*-Diethyl *O*-(*p*-Methylthiophenol)Phosphates**		
H	0.01	2.24 (2.24)	50
2-Cl	0.04	2.84	151
3-Cl	0.03	3.00 (2.94)	98.5
2,6-diCl	0.12	3.44 (3.46)	195
2-CH_3	0.13	2.76 (2.82)	13.6
3-CH_3	0.04	2.76 (2.73)	20.2
2-NO_2	0.15	2.01 (2.01)	730
Group II.	***O,O*-Diethyl *O*-(*p*-Methylsulfonylphenyl)Phosphates**		
H	0.05 (0.050)[d]	0.0	182
2-Cl	0.04 (0.041)	0.59	485
2.6-diCl	0.22 (0.219)	1.20	625
2-CH_3	0.09 (0.095)	0.52 (0.58)	107
3-CH_3	0.08 (0.074)	0.52	152
	Group III.		
	***O,O*-Diethyl *O*-(*p*-Methylthiophenyl)Phosphorothioates**		
H	0.04	4.06	2.05[e]
2-Cl	0.03	4.65	6.9
3-Cl	0.02	4.82	0.95
2-CH_3	0.07	4.58	1.84
3-CH_3	0.10	4.58	2.50
2-NO_2	0.37	3.83	75
	Group IV		
	***O,O*-Diethyl *O*-(*p*-Methylsulfonylphenyl)Phosphorothioates**		
H	0.05	1.82	26.8
2-Cl	0.04	2.41	101
2-CH_3	0.07	2.34	11.6
3-CH_3	0.09	2.34	19.5

[a] These values represent μg toxicant/fly. [b] CH_3SO was assigned a π value of 0. (The values in parentheses were determined experimentally.) [c] k is the rate constant in min.$^{-1}$ determined in 0.1N NaOH at 50° C. [d] Calculated by means of Equation 4. [e] These rate constants were determined at pH 10 in 0.1M HCO_3^- buffer at 50° C.

given in Table VIII along with the other data on these phosphates. Using the additivity nature of π, the value for the phosphates may be calculated from the data in Table VII. As an example, the π value for V may be determined as follows:



V

The parent compound for this series is II where R = H, this is given a π value of 0. Removing the CH_3SO_2 group adds a π value of 1.64. Adding SCH_3 will add a π value of 0.60. The 2 Cl has a π of 0.59. π for structure V is the addition of these values, *i.e.*, 2.83.

All of the π values in Table VIII were determined in a similar manner. The values in parenthesis were determined experimentally and there is excellent agreement between the two numbers.

Reactivity. The hydrolysis constants were obtained by measuring the appearance of phenol when the organophosphate was treated with 0.1N NaOH at 50° C. A Gilford attachment for the Beckman DU spectrophotometer was used for automatically measuring the hydrolysis rate. A reaction mechanism based on pseudo first order kinetics was assumed and the rate constant was determined by Equation 2. At 50° C., the reactions were sufficiently fast to obtain final equilibrium values; there was no detectable pH shift during the reaction.

$$k = \frac{2.303 \log 2}{t_{1/2}\ (\text{min.})} \qquad (2)$$

The data are collected in Table VIII.

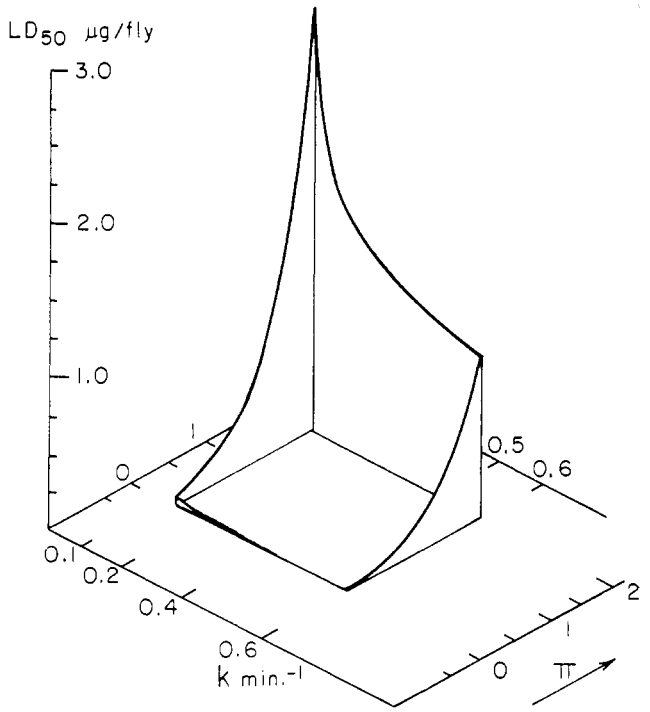


Figure 1. Structure activity surface generated by Equation 4 for the series of phosphates in Group II

RESULTS AND DISCUSSION

The results of fitting the data of the Group II compounds to Equation 1 generated the following two equations:

$$\log LD_{50} = 0.528\pi - 1.399 \qquad (3)$$
$$r^2 = 0.735,\ s = 0.152$$

$$\log LD_{50} = 0.84\pi^2 - 0.194\pi - 0.626 \log k - 1.76 \qquad (4)$$
$$r^2 = 0.98,\ s = 0.011$$

In these equations, r^2 is the square of the correlation coefficient and s is the standard deviation of regression. An F test indicated that the inclusion of the three terms in Equation 4 was significant at the 95% level.

As may be noted from Equation 3, there is a strong dependence of the observed activity on π. In fact, 74% of the variation may be "explained" on the basis of partition coefficients. Assuming an optimum π, then increasing the susceptibility of the chemical to nucleophilic attack results in increased activity. This pattern is similar to that of many other organophosphates (O'Brien, 1967). The results of Equation 4 are seen graphically in Figure 1. Hansch and Deutsch (1966) found a correlation with a series of phosphoramidates using an electronic factor, no dependence on π was noted. This difference as discussed in an earlier paper Neely *et al.* (1968), may be explained on the basis that the present work was performed with in vivo data while Hansch and Deutsch used in vitro data.

The data for compounds in Group I could not be correlated with the Hansch equation. This would imply a mechanism of action other than phosphorylation of an enzyme.

One possible explanation could be based on the thiomethyl to sulfonylmethyl conversion as discussed by Metcalf *et al.* (1964) and Reynolds *et al.* (1966). If such a transformation is rate limiting, a correlation of Group I chemicals might be achieved if the rate constant for such a conversion was used in Equation 1 instead of the one listed in Table VIII.

The compounds in Group III were interesting in that the results were fitted by Equation 5.

$$\log LD_{50} = 0.524 \log k + 0.045 \qquad (5)$$
$$r^2 = 0.83,\ s = 0.08$$

As F test indicated that this equation was statistically significant at the 95% level. Additional terms in π and π^2 were not significant. This equation is rather surprising in that con trary to experience it predicts that the compound which is most stable to base hydrolysis will have the highest activity. Such a situation demonstrates the inability to make predictions of biological activity based on model systems. Before a defini e statement can be made regarding the activity, the structure must be tested in vivo.

There were not enough compounds in Group IV to analyze with the multiple regression technique. However, since the parameters π and k are common to both series, it is possible to combine Groups II and IV and perform the analysis. This was done and visual examination of the data indicated no correlation. This was checked with the multiple regression program and no significant correlation of LD_{50} with π and k could be found within the framework of Equation 1. This would indicate that the chemicals in Group IV act differently than the chemicals in Group II. For example, the high π value for Group IV might cause the molecule to partition into a site where phosphorylation would take place. The end result would be a comparable LD_{50} to the analogous compounds in Group II.

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Received for review June 18, 1969. Accepted September 2, 1969.