Physical and Chemical Basis for Systemic Movement of Organophosphorus Esters in the Cotton Plant

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The relationship between the octanol-water partition coefficients, solubilities in distilled water, steric substituent effects, and systemic movement of a series of analogs of Monitor (*O,S*-dimethyl phosphoramidothioate) in the cotton leaf petiole was investigated by means of multiple parameter approach. For the *O,S*-dimethyl *N*-*n*-alkylphosphoramidothioates, regression analysis showed the existence of a parabolic relationship between systemic movement and π or log *P*. However, in the case of the branched *N*-alkyl substituent, steric effects reduced systemic movement even when π was near optimal.

Although systemic insecticides have been used for the control of plant feeding insects for more than two decades, relatively little information is available on the relationship between chemical structure and the ability of the compound to be translocated in a plant. Since a wide variety of organic insecticides of different physical and chemical properties, *e.g.*, water solubility and solvent partitioning, have been demonstrated to move systemically in plants, the question, what are the critical factors which govern systemic movement, has often been raised. Past investigations have indicated that high lipophilicity is required by chemicals for facile penetration through the plant cuticle (Metcalf *et al.*, 1964) but high water solubility is needed for movement within the plant (Metcalf, 1967). Collander (1954) reported that the permeability of a variety of organic chemicals into the Nitella cell increased with an increase in their ether–water partition coefficients and the more nonpolar compounds moved through the protoplasm at a faster rate. Subsequent reports (Hansch *et al.*, 1963; Hansch and Fujita, 1964) have indicated that movement of organic chemicals in plants is parabolically related to oil–water partition and there is an optimum value where maximum movement is attained. Thus, it appears that the movement of chemicals in plant tissues is strongly dependent on its partitioning properties between lipophilic and hydrophilic phases in the plant although the size and shape of the molecule also appear to be contributing factors (Collander, 1954; Miller, 1971).

This paper presents results of a systematic analysis of the relation between systemic movement in the cotton plant of a series of phosphoramidothioate esters and their octanol–water partitioning properties. These compounds were selected because of the known systemic activity and stability in plants of *O,S*-dimethyl phosphoramidothioate (Leary, 1971), the parent compound of the series.

MATERIALS AND METHODS

Synthesis of Experimental Compounds. The *O,S*-dialkyl phosphoramidothioates prepared by Quistad *et al.* (1970) were available for this study. The *O,O*-dimethyl *N*-alkylphosphoramidothioates were prepared according to conventional methods (Mel'nikov and Zen'kevich, 1955) by reaction of the desired amine with dimethyl phosphorochloridothionate in anhydrous ether. Liquid products were purified by distillation at reduced pressure and solid products by recrystallization from *n*-hexane. The *O,O*-dimethyl *N*-alkylphosphoramidothioates were isomerized to *O,S*-dimethyl *N*-alkylphosphoramidothioates by heating the thionate in a several-fold excess of methyl iodide according to Burn and Cadogen (1961).

Elemental analyses and physical properties are given in Table I. Several compounds gave unsatisfactory analyses but were characterized by pmr spectrometry. All pmr spectra were obtained with a Varian Model T-60 spectrometer in deuteriochloroform using Me_4Si as a standard. Microanalyses were done by C. F. Geiger, Ontario, Calif.

Octanol–Water Partition Coefficients. Octanol–water partition coefficients of the compounds were determined according to Fujita *et al.* (1964). Equal volumes of 1-octanol and distilled water were shaken for 15 min in a separatory funnel and the mixture was allowed to stand for 0.5 hr at room temperature. The two phases were separated and each centrifuged for 15 min at 2480 rpm to remove globules of the opposite phase. Octanol or water phases prepared in this manner were used to determine partition coefficients. As a general procedure, a 1-mg/ml solution of the compound was made up separately in 30 ml of octanol and 30 ml of water. Each solution was separated into three 10-ml portions. One 10-ml portion of each of the octanol and water solutions was used in making a series of dilutions with the same solvent for the purpose of establishing a standard analysis line. Each of the other two 10-ml portions was shaken vigorously with an equal volume of the opposite phase for 15 min in a separatory funnel and then allowed to stand for 0.5 hr. The two phases were separated and each phase was centrifuged at 2480 rpm. In this way, four replicates of each phase were obtained. Analysis of each phase was done by gas–liquid chromatography (glc) or uv spectrophotometry. For glc determinations, the octanol phase was diluted with distilled acetone prior to injection to reduce the viscosity of solution and analyzed with a Hewlett Packard Model 402 gas–liquid chromatograph equipped with a phosphorus-sensitive detector. The procedure used for glc analysis is described in a following section. The water phase was analyzed by means of a Unicam Model SP 800A ultraviolet spectrophotometer.

Solubility in Distilled Water. The solubilities of the various *O,S*-dimethyl *N*-alkylphosphoramidothioates in distilled water were estimated by adding an excess of each compound to 10 ml of distilled water in a 50-ml flask. The contents of each flask were stirred with a magnetic stirrer for 15 min, transferred to a Potter-Elvejhem homogenizer, homogenized for a period of 5 min to disperse completely the material in water, and then transferred to a 20-ml test tube. The test tubes were mechanically shaken in a constant temperature water bath at 25° for a period of 2.5 hr. The contents of the test tubes were filtered and each filtrate transferred to a 10-ml plastic centrifuge tube. The tubes were tightly capped and centrifuged at 20,000g for 2 hr in a Beckman Model L2-50 ultracentrifuge at 25°

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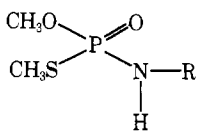
Table I. Physical Constants of *O,S*-Dimethyl *N*-Alkylphosphoramidothioates



No.	R	Mp, °C	Bp, °C (mm)	n^{25}D, deg	Anal. Calcd C	Anal. Calcd H	Anal. Found C	Anal. Found H
I	CH_3		99–100 (0.4)	1.4945				
II	C_2H_5		114–115 (2.0)	1.4903	28.40	7.10	29.63	6.69
III	*n*-C_3H_7		120 (2.0)	1.4865	32.80	7.65	33.43	8.00
IV	*n*-C_4H_9		130 (0.9)	1.4836	36.50	8.00	36.23	8.23
V	*s*-C_4H_9		106–108 (0.15)	1.4840	36.50	8.00	37.80	8.40
VI	*i*-C_4H_9		116–117 (0.3)	1.4825	36.50	8.00	37.77	8.34
VII	*t*-C_4H_9	76.5			36.50	8.00	38.19	8.89
VIII	*n*-C_6H_{13}		136 (0.25)	1.4787	42.67	8.90	42.78	8.61
IX	*n*-C_8H_{17}		160–162 (0.7)	1.4770	47.40	9.50	44.60	9.27

using a 50 Ti type rotor. The top 1 ml of water solution was pipetted out from each centrifuge tube and diluted to 10 ml with distilled water. Since injection of water solution into glc would adversely affect the column, this final dilution was shaken with an equal volume of 1-octanol (previously saturated with distilled water). The octanol phase was separated and further diluted with distilled acetone to reduce the viscosity of the solution. Injections of the acetone dilution (5 μl) were made in glc and the unknown amount originally dissolved in water was calculated from the partition coefficients.

Free-Energy Parameters. π values for the different *N*-alkyl substituents were calculated from octanol–water partition values according to Fujita *et al.* (1964), *i.e.*, $\pi = \log P_X - \log P_H$, where P_X is the octanol–water partition coefficient of the *N*-alkyl derivative and P_H is that of the basic molecule, in this case *O,S*-dimethyl phosphoramidothioate. E_s values used in this study are from the literature (Taft, 1956) after normalizing them to the same scale as those of other free-energy parameters, *i.e.*, to a scale relative to H (E_s for H is zero). This was accomplished simply by subtracting the value 1.24 (E_s for H relative to methyl) from all E_s values (Fahmy *et al.*, 1973).

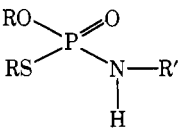
Rates of Systemic Movement (k_{tr}) in Cotton Leaf Petiole. "Delta pine smooth leaf" cotton plants were grown from seed in sterilized soil in the greenhouse at 80 ± 5°F. After the plants were 7–8 weeks old, leaves at the fifth–eighth nodes from the soil level were removed at the axils and transferred into 20-ml vials containing ordinary tap water, placing the excised end into the water. Each vial contained one leaf. Vials were then transferred to a Percival Plant Growth Lab, Model PGC-78, equipped with control for regulating photoperiod, temperature, and humidity. The growth cabinet was programmed to provide 14 hr of light from 7 a.m. until 9 p.m. and 10 hr of darkness. The temperature was set at 74–76°F and the humidity, which was not regulated, fluctuated between 45 and 65%. After a conditioning period of 24 hr, leaves which had transpired an equivalent amount of water from the containing vial were selected for treatment, thus providing relatively homogenous leaves with respect to the rate of water transpiration. Treatments were made at the fixed time of 8:30 a.m. For treatment, a 5-μl droplet of 20-mg/ml stock acetone solution of the test compound was deposited externally on the surface of the leaf petiole 2 in. below the leaf blade with a 10-μl syringe to deliver 100 μg of the chemical. The treated part of the petiole remained outside the vial out of contact with water. The amount of material moved from petiole surface into the leaf blade was analyzed 2, 4, 8, and 12 hr after treatment. For each analysis, leaf blades were cut off from the petiole and four blades were pooled together and macerated in 200 ml of distilled acetone. The extract was centrifuged at 3500 rpm and the residue was resuspended and extracted three additional times with 60-ml portions of acetone. The combined extracts were concentrated on a rotary evaporator under reduced pressure at a temperature of 40° or less. Acetone (5–10 ml) was added to the residue and the solution was stored in the freezer until analysis.

For analysis the extract was injected directly into the glc. A capillary glass tube flared at one end was inserted into the injection port end of the glc column to contain the burned out residue from the plant material. A series of dilutions was made from a 20-mg/ml stock acetone solution of the compound and 5 μl of each was injected into the glc. A glass column packed with a suitable stationary phase on Gas Chromosorb Q 80–100 mesh was used. The stationary phases used were 4% DEGS or 5% Carbowax 20M for phosphoramidothioates, 5% Apiezon L for paraoxon and methyl paraoxon, 2.5% OV-3 or 5% 200DC for demetonthiol isomer, 5% OV-17 for naled, and 5% 200DC for dimethoate. The flow rate of the carrier gas (N_2) varied between 20 and 35 ml/min. Peak heights were plotted against the amount injected and a standard line was plotted. The amount of the test compound in the extract was calculated from this line. Since the sensitivity of the detector gradually decreased with increasing number of injections, the injections of the extracts and each of the dilutions for the standard line were alternated. For each replication, the order of injections was reversed; thus variation due to detector response was reduced or virtually eliminated. Analyses were replicated up to eight times for each compound and the average values for the fraction (per cent) of the applied amount which moved into the leaf blade 2, 4, 8, and 12 hr after treatment were plotted against time. The slopes of the lines through the points were calculated by least-squares analysis using an Olivetti Programma 101 desk computer and provide a measure of the initial rates (k_{tr}) of systemic movement of the compounds in the cotton leaf petiole. Regression analyses between k_{tr} and the parameters π, E_s, and log P were conducted by means of an IBM 360/50 computer.

RESULTS

Relevant data concerning octanol–water partitioning properties of the various phosphoramidothioate esters and miscellaneous organophosphorus insecticides are presented in Table II. For the *O,S*-dimethyl *N*-alkylphosphoramidothioate analogs (I–IX) a gradual increase in the octanol–water partition coefficient (P) was observed as the *N*-

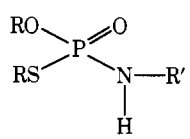
Table II. Physical and Chemical Properties of *O,S*-Dialkyl *N*-Alkylphosphoramidothioates and Other Organophosphorus Insecticides



No.	R	R′	% distribution: Octanol phase	% distribution: Water phase	Part. coeff (*P*)	Log *P*	π	Water solubility, mg/ml, 25°	E_s
I	CH_3	CH_3	46.22	53.78	0.86	−0.07	0.59	>1000.00	−1.24
II	CH_3	C_2H_5	54.21	45.79	1.18	0.07	0.73	>1000.00	−1.31
III	CH_3	*n*-C_3H_7	81.75	18.25	4.48	0.65	1.31	176.10	−1.60
IV	CH_3	*n*-C_4H_9	89.71	10.29	8.71	0.94	1.60	58.61	−1.63
V	CH_3	*s*-C_4H_9	89.85	10.15	8.85	0.95	1.61	67.78	−2.37
VI	CH_3	*i*-C_4H_9	90.37	9.63	9.39	0.97	1.63		−2.17
VII	CH_3	*t*-C_4H_9	90.02	9.98	9.03	0.96	1.62	78.88	−2.78
VIII	CH_3	*n*-C_6H_{13}	92.79	7.21	12.87	1.11	1.77	4.92	−1.60
IX	CH_3	*n*-C_8H_{17}	97.00	3.00	32.33	1.51	2.17	0.36	−1.57
X	CH_3	H	18.00	82.00	0.22	−0.66	0.00	>1000.00	
XI	C_2H_5	H	58.50	41.50	1.41	0.15		>200.00	
XII	*n*-C_3H_7	H	94.50	5.50	17.20	1.23			
XIII	Demetonthiol		98.85	1.15	85.96	1.93		2.00[a]	
XIV	Dimethoate		76.25	23.75	3.21	0.50		25.00[a]	
XV	Methyl paraoxon		95.00	5.00	19.00	1.28		6.43	
XVI	Paraoxon		97.50	2.50	38.84	1.59		2.4[a]	
XVII	Naled		96.00	4.00	24.00	1.38			

[a] Literature values of Gunther *et al.*, 1968.

Table III. Translocation Data for the Various *O,S*-Dialkyl *N*-Alkylphosphoramidothioates and Other Organophosphorus Insecticides



No.	R	R′	% applied amt moved at time, hr: 2	4	8	12	Rate of movement in petiole (k_{tr})	r^2 for slope
I	CH_3	CH_3	3.31	5.59	15.97	21.35	1.97	0.967
II	CH_3	C_2H_5	3.29	8.63	21.37	29.23	2.57	0.981
III	CH_3	*n*-C_3H_7	3.12	11.13	25.70	40.81	3.71	0.999
IV	CH_3	*n*-C_4H_9	7.04	14.00	30.90	39.90	3.24	0.970
V	CH_3	*s*-C_4H_9	3.21	10.21	22.60	27.30	2.13	0.937
VI	CH_3	*i*-C_4H_9	1.80	7.50	13.85	15.10	0.95	0.869
VII	CH_3	*t*-C_4H_9	0.12	0.28	0.67	0.68	0.05	0.768
VIII	CH_3	*n*-C_6H_{13}	0.22	7.26	15.30	23.28	2.00	0.999
IX	CH_3	*n*-C_8H_{17}	0.68	0.91	3.18	4.80	0.48	0.991
X	CH_3	H	3.85	9.47	22.00	37.13	3.45	0.998
XI	C_2H_5	H	2.44	12.90	25.90	59.10	5.77	0.940
XII	*n*-C_3H_7	H	2.25	10.86	33.15	42.55	3.92	0.944
XIII	Demetonthiol		11.45	18.12	21.82	16.83	2.61	1.000[a]
XIV	Dimethoate		0.00	3.00	9.50	27.35	3.04	0.932
XV	Methyl paraoxon		0.30	0.84	3.02	9.39	1.07	0.926
XVI	Paraoxon		2.39	8.25	20.86	34.90	3.33	0.999
XVII	Naled						1.77[b]	

[a] Regression analysis for three points only. [b] Rate of movement for first 4 hr only.

alkyl chain size was increased, although there appeared to be an abrupt increase between the *N*-ethyl and *N*-propyl derivatives (compare II and III). Branching in the alkyl group of the *N*-butyl analogs (IV and VII) did not result in any significant change in partition coefficients. The partition coefficients for the limited number of *O,S*-dialkyl phosphoramidothioates (X–XII), as expected, also increased with lengthening of the alkyl chain on oxygen and sulfur but the relative increase appeared to be slightly greater than with the *N*-alkyl derivatives (compare X with II and X with XI).

Water solubility, in the absence of significant inductive or polar effects, should be inversely related to octanol–water partition coefficients. As expected, the water solubilities of the analogous series of *O,S*-dimethyl *N*-alkylphosphoramidothioates decreased as the length of the alkyl chain was increased and the *n*-octyl analog (IX) was found to be the least soluble compound in the series.

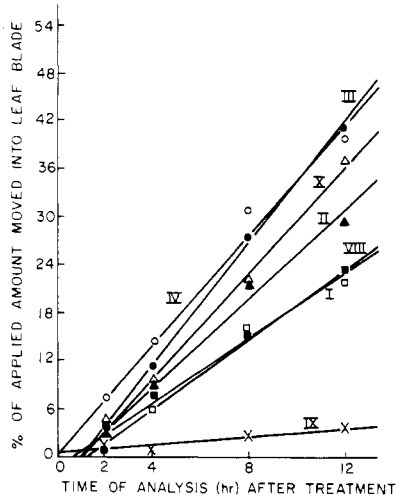


Figure 1. Rate of systemic movement of representative *O,S*-dimethyl *N*-alkylphosphoramidothioates in the cotton leaf petiole.

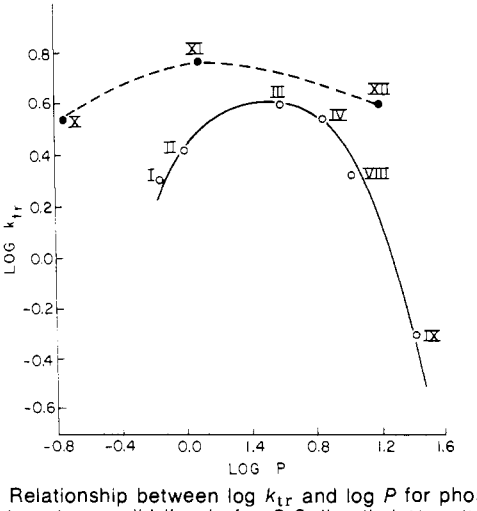


Figure 2. Relationship between log k_{tr} and log P for phosphoramidothioate esters; solid line is for *O,S*-dimethyl *N*-*n*-alkylphosphoramidothioates and dotted line is for *O,S*-dialkyl phosphoramidothioates.

The octanol-water partition coefficients of the other organophosphorus esters were in the order: demetonthiol > paraoxon > naled > methyl paraoxon > dimethoate. The literature values for the water solubilities of these compounds were found to be in the reverse order, except for naled for which a water solubility value was not available.

Data for the movement of the various organophosphorus esters from the leaf petiole surface into the leaf blade are given in Table III. From the relative amounts translocated at the indicated time intervals, the values for the rate of translocation (k_{tr}) were calculated. Typical plots showing the rate of movement are presented in Figure 1. Examination of the data for the *O,S*-dimethyl *N*-*n*-alkylphosphoramidothioate series (I–IV, VIII, IX) reveals the existence of a parabolic relationship between the rate of translocation and octanol-water partition coefficients and optimum rate was found with the *N*-propyl derivative (III). The parabolic relationship which is obtained is shown graphically in Figure 2 where log k_{tr} is plotted against log P. A similar plot was obtained (*cf.* Figure 3) when log k_{tr} was plotted against π. This is to be expected since for an analogous series of compounds π is directly proportional to log P.

The values of k_{tr} and π for the *O,S*-dimethyl *N*-*n*-alkylphosphoramidothioates were subjected to multiple regression analysis. By linearly combining π and π^2 terms the following regression equation 1 was obtained (r = correlation coefficient (0.999), n = number of compounds (6)). The excellent value for the correlation coefficient (r) indicates good fit of the data to the equation.

$$\log k_{tr} = -0.753 + 2.318\pi - 0.974\pi^2 \qquad (1)$$

For the limited number of *O,S*-dialkyl phosphoramidothioates examined (X, XI, and XII), a similar parabolic relationship, as with the *N*-*n*-alkyl derivatives, was observed between k_{tr} and π (or log P). The replacement of the methyl groups in X with ethyl (XI) resulted in increased lipophilicity as well as in systemic movement. However, substitution with propyl evidently resulted in excessive lipophilicity and k_{tr} for the di-*n*-propyl derivative (XII) was substantially less than that for XI.

Although the *O,S*-dialkyl phosphoramidothioates and *O,S*-dimethyl *N*-*n*-alkylphosphoramidothioates are structurally closely related to each other, it appears that the two series of compounds follow a separate parabolic relationship. This is evident from the dotted curve in Figure 2 and from regression analysis when data for XI and XII were included with those of the *N*-*n*-alkylphosphoramidothioates. In this case, regression equation 2 with a

$$\log k_{tr} = -0.736 + 2.401\pi - 0.992\pi^2 \qquad (2)$$

correlation coefficient of 0.844 and n = 8 was obtained. The significantly poorer correlation coefficient obtained compared to the *O,S*-dimethyl *N*-*n*-alkylphosphoramidothioate series (*cf.* eq 1) indicates that the *N*-*n*-alkyl and unsubstituted phosphoramidothioates should be treated separately. Evidently, alkyl substitution on the amido nitrogen atom results in compounds with reduced rates of systemic movement in the cotton plant, even though individual N-substituted and unsubstituted phosphoramidothioates may have similar octanol-water partitioning properties.

Profound changes in systemic movement were observed when branching was introduced into the *N*-alkyl chain. For the various isomeric *N*-butyl derivatives (IV, V, VI, and VII), marked reduction in k_{tr} values occurred with an increase in branching and translocation was minimal with the *tert*-butyl derivative (VII). In spite of rather similar values of π for the four different butyl isomers (1.60–1.63), there was approximately a 65-fold difference in k_{tr} between the *n*-butyl and *tert*-butyl derivatives with the values of the other two isomers ranging in between. The relation between k_{tr} and π for the four isomeric butyl derivatives is shown graphically in Figure 3 by the solid line. These results indicate that within the series of nine *O,S*-

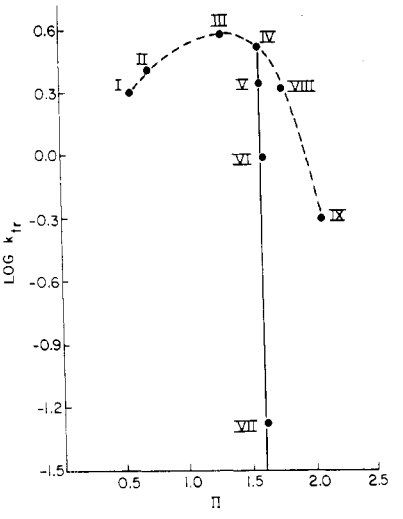


Figure 3. Relationship between log k_{tr} and π for *O,S*-dimethyl *N*-alkylphosphoramidothioates; dotted line is for *n*-alkyl esters and solid line is for branched butyl esters.

dimethyl *N*-alkylphosphoramidothioates, solvent partitioning properties alone, *i.e.*, π or log *P*, do not totally account for systemic movement.

The reduction in systemic movement with increase in branching in the *N*-butyl chain suggests that steric effects also should be considered in seeking correlation of the systemic data. For the *O,S*-dimethyl *N*-alkylphosphoramidothioates (I-IX), including the branched butyl derivatives, the following regression equation (3) relating k_{tr} and π

$$\log k_{tr} = 0.307 + 0.273\pi - 0.267\pi^2 \quad (3)$$

was obtained, with $r = 0.372$, $n = 9$, s (standard error) = 0.636, and F (F statistics) = 0.483. Use of Taft's steric substituent constant (E_s) alone in the analysis resulted in eq 4, where $r = 0.680$, $n = 9$, $s = 0.465$, and $F = 6.033$.

$$\log k_{tr} = 1.498 + 0.782E_s \quad (4)$$

From the magnitude of the correlation coefficients for eq 3 and 4, evidently neither E_s alone nor the combination of π and π^2 alone is able to provide significant correlation with the translocation data. On the other hand, linear combination of π, π^2, and E_s resulted in substantial improvement in correlation, in accordance with eq 5, where r =

$$\log k_{tr} = -0.196 + 4.187\pi - 1.551\pi^2 + 1.209E_s \quad (5)$$

0.863, $n = 9$, $s = 0.379$, and $F = 4.865$. The F statistics show significant correlation at the 10% level. These results provide quantitative evidence that steric effects, in addition to solvent partitioning properties, are important in predicting systemic movement.

In addition to octanol-water partitioning properties, water solubilities of several representative phosphoramidothioates were determined (Table II). The results indicate that, within the limits of the compounds examined, water solubility is not a critical factor in regulating the degree of systemic movement. For example, compounds I and II with solubilities of >1000 mg/ml were decidedly poorer in their ability to penetrate and move through the petiole than was IV, a compound of much lower water solubility. In contrast, the highly water-soluble *O,S*-dimethyl phosphoramidothioate (X) translocated rapidly and the poorly soluble *n*-octyl derivative IX quite slowly. Similarly, the systemic movement of the other organophosphorus esters included in this study (XIII-XVII) evidently was not related to their water solubilities.

DISCUSSION

The foregoing results clearly indicate that even within a relatively restricted series of compounds, systemic movement in the cotton plant is not directly correlated with any single chemical or physical parameter. This is not at all surprising since penetration and translocation of foreign materials in a plant are doubtlessly complex processes and a number of different factors must be involved (Collander, 1954; Edgington, 1972; Shephard, 1972; Wain and Carter, 1967; Frey-Wyssling, 1948).

The development of π as a useful parameter originated from an attempt to correlate plant growth promoting properties with the structure of a series of substituted phenoxyacetic acids (Hansch *et al.*, 1963). In this classic study π was developed to account for substituent effects on the rate of penetration of an externally applied acid to the site of action. Penetration to the site of action was visualized as a random walk process involving multiple partitions between lipophilic and hydrophilic phases in the plant and the addition of π to the regression equations provided highly significant improvement to the correlation. Therefore, the failure of π (or log *P*) to correlate satisfactorily the rate of systemic movement of the phosphoramidothioates (compounds I-XII) was unexpected. Only in the limited case of the *N*-*n*-alkyl derivatives was satisfactory correlation obtained with π and π^2 alone.

There is little doubt that steric effects play a significant

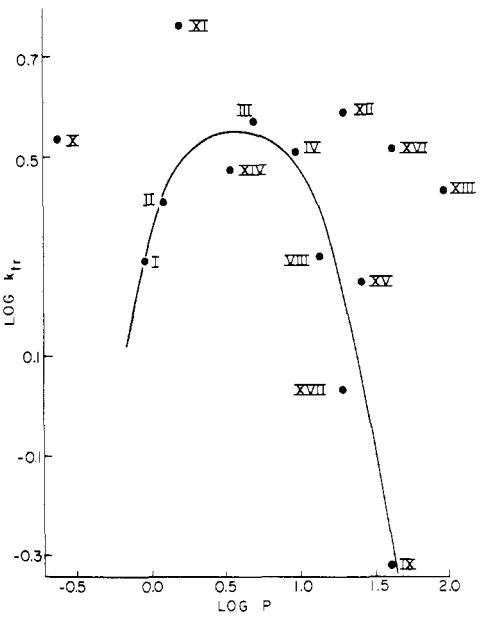


Figure 4. Plot of log *P* *vs.* log k_{tr} for phosphoramidothioate and other organophosphorus esters.

role in determining the rate in which the *N*-alkylphosphoramidothioates penetrate and move in the cotton leaf petiole. Collander (1954) in his studies noticed a similar effect of side chain branching of nonelectrolytes permeating Nitella cells. The poor ability of the isobutyl (VI) or *tert*-butyl (VII) derivatives to move systemically may be explained purely on the basis of the bulkiness of the branched butyl moieties which may impede the movement of the molecule through plant cuticle and membranes. Another possible explanation resides in hydration effects associated with the amido moiety. The effect of alkyl substituents in limiting the extent to which water is solvated to amines is well known (Hall, 1957) and the possibility remains that hydration of the amido moiety, or its absence owing to steric effects, may drastically affect rates of systemic movement. For example, in the case of VIII, the bulky *tert*-butyl group prevents solvation by water from occurring, thus rendering the molecule more hydrophobic and increasing the possibility of retention by lipophilic phases in the plant. On the other hand, compounds without alkyl substituents on the amido proton probably are highly solvated by water and hydrophobic interaction with lipophilic plant constituents which reduces penetration and systemic movement is minimized. The greater than expected systemic movement of the *O,S*-dialkyl phosphoramidothioates (X, XI, and XII) is consistent with this kind of interpretation.

It is, therefore, not surprising that the systemic movement of several of the other phosphate esters (XIII-XVII) in Table III did not fit into any systematic pattern in relation to their solvent partitioning properties (Figure 4). It should be pointed out, however, that the stability of these compounds in the plant during the duration of the translocation experiments is not known and it is possible that some of them, particularly methyl paraoxon and naled, are rapidly degraded to hydrolytic products during the translocation process. Rate constants (k_{tr}) in these cases must be considered as minimum values. Demetonthiol isomer and dimethoate, of course, are subject to rapid oxidative metabolism and undoubtedly are also translocated in the form of their metabolites. No attempt was made to analyze for movement for the metabolites, however.

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Uptake and Metabolism of Dichlobenil by Emersed Aquatic Plants

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[^{14}C]Nitrile-labeled 2,6-dichlorobenzonitrile (dichlobenil) was readily absorbed by the roots of the emersed aquatic plants, alligator weed (*Alternanthera philoxeroides*) and parrot feather (*Myriophyllum brasiliense*) and was translocated to the shoots. The concentration of the ^{14}C was greater in parrot feather than in alligator weed 24 hr after treatment. The roots of the alligator weed contained a considerably greater amount of ^{14}C than the shoots, whereas in parrot feather, a major portion of the absorbed ^{14}C was present in the shoot. [^{14}C]Dichlobenil leaked from the roots of alligator weed but not from those of parrot feather. Alligator weed was not able to metabolize dichlobenil; on the contrary, parrot feather transformed the herbicide into several metabolites. The major metabolite resulting from the transformation was 3-hydroxy-2,6-dichlorobenzonitrile; small amounts of 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid were also present. A significant amount of radioactivity (14–18% of ^{14}C in the shoot) was present as highly polar, unknown metabolites. The tolerance of parrot feather to dichlobenil appears to be related to its ability to metabolize the herbicide.

The herbicide dichlobenil (2,6-dichlorobenzonitrile) is recommended for the control of many aquatic weeds. The utility of this herbicide for killing a variety of aquatic plants was demonstrated by a number of investigators (Frank *et al.*, 1963; Lawrence *et al.*, 1963; Walker, 1964). The metabolic fate of dichlobenil in the aquatic environment is of obvious concern since the herbicide metabolites may be more persistent and more toxic than the parent molecule to the aquatic fauna and flora. Metabolism by plants is one of the important factors which determine the fate of an herbicide in the aquatic environment.

Presently, the information available on the fate of dichlobenil in aquatic plants is very limited; however, a few workers have studied the uptake and metabolism of this herbicide in terrestrial plants. Massini (1961) reported that dichlobenil was readily absorbed by roots of bean (*Phaseolus vulgaris*) seedlings and was translocated throughout the plant *via* the xylem, but more slowly than water due to affinity of the herbicide for plant tissue. Pate and Funderburk (1966) and Verloop and Nimmo (1969) reported that dichlobenil was absorbed only slightly by bean and alligator weed (*Alternanthera philoxeroides*) when applied as a foliar spray but it was readily absorbed by the root. Verloop and Nimmo (1969, 1970) found that bean, wheat (*Tritium vulgare*), and rice (*Oryza sativa*) plants absorbed the herbicide through the roots and translocated it fairly rapidly to the shoots. They observed that an appreciable amount of dichlobenil was lost from the leaves due to evaporation. Price and Putnam (1969) found that dichlobenil readily entered the corn roots (*Zea mays*), but was not actively held within the cells. About 71% of the absorbed herbicide was excreted from the roots into the nutrient solution in 24 hr.

Pate and Funderburk (1966) described 2,6-dichlorobenzoic acid as a metabolite of dichlobenil in bean and alligator weed. However, according to the results of Verloop and Nimmo (1969, 1970), hydrolysis of dichlobenil is a very minor metabolic process in bean and wheat seedlings, since very low amounts of 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid were found. They reported that hydroxylation, followed by conjugation, was the main metabolic process by which dichlobenil was converted in the plants.

The present investigation was conducted to study the fate of [^{14}C]dichlobenil in two species of emersed aquatic plants, parrot feather (*Myriophyllum brasiliense*) and alligator weed, which differ markedly in their tolerance to the herbicide.

MATERIALS AND METHODS

Chemicals. [^{14}C]Dichlobenil labeled in the nitrile carbon, with a specific activity of 28.8 μCi/mg, was supplied by N. V. Philips-Duphar, Weesp, The Netherlands. Nonradioactive samples of dichlobenil, 3-hydroxy-2,6-dichlorobenzonitrile, 4-hydroxy-2,6-dichlorobenzonitrile, and 3-hydroxy-2,6-dichlorobenzamide were supplied by Thomp-

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