Statistical Analysis of Insecticidal Activity in a Series of Phosphoramidates

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A structure activity analysis of the in vitro and in vivo insecticidal data of a series of phosphoramidates has been made. Correlation studies indicate that in certain insects the insecticidal activity is more related to the lipophilicity of the toxicant than any other parameter, while in other species the biological activity is more dependent on the chemical reactivity. A further observation is the difficulty of comparing in vivo with in vitro data. A distinct possibility arises that the in vitro cholinesterase inhibition by these agents may not be the critical step in their in vivo activity.

This investigation, a continuation of studies conducted in this laboratory in the area of structure activity relationships (Neely, 1965; Neely *et al.*, 1964), deals with an analysis of the insecticidal data on a series of trichlorophenyl *O*-methyl methylphosphoramidates (Blair *et al.*, 1965), as well as with some new data on the aryl *O*-methyl methylphosphoramidates. The biological results include both in vivo and in vitro data.

The problem of correlating chemical structures with biological activities is complex. The complexity is readily appreciated when consideration is given to all the biological parameters that are involved in a rigid structure activity relationship. A simple model is required for a starting point. If the model works—i.e., can predict the data—it may give new insights into the mechanism of action. Conversely, if it fails, the reasons for the failure must be examined; this in itself could provide new concepts for investigation.

The starting point for this analysis is the model developed by Hansch *et al.* (1965) and used extensively in a number of different biological applications (Hansch *et al.*, 1963; Hansch and Fujita, 1964). In effect, the model assumes that the amount of externally applied drug found at the internal receptor site is related to a single substituent constant, π, which is derived from octanol-water partition coefficient measurements. The reaction of the chemical at the receptor site is related to an electronic parameter. The model is illustrated by Equation 1.

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

where

B.R. = biological response
π = $\log (P_x/P_H)$
P_x. P_H = partition coefficient of substituted compound P_x and parent compound P_H
k_x = rate constant
a, b, c, d = equation constants

In the present case, the rate constant is measured by the rate at which the compound hydrolyzes in sodium hydroxide. This mechanism is based on the hypothesis that if phosphoramidates are to be cholinesterase inhibitors, they must be susceptible to attack by nucleophilic reagents (Neely, 1965; O'Brien, 1960; Spencer and Sturtevant, 1959). Furthermore, a direct relation existing between cholinesterase inhibition and insecticidal activity is assumed.

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MATERIALS AND METHODS

Chemicals. The preparation of the phosphoramidates has been described (Blair *et al.*, 1965; Neely *et al.*, 1964). The chemicals were used without any further treatment except dilution for bioassay.

Biological Data. The insecticidal data for the 2,4,5-trichlorophenyl *O*-methyl phosphoramidates, reproduced in Table I, were taken from the paper by Blair *et al.* (1965). The insecticidal data for the substituted phenyl *O*-methyl methylphosphoramidates were obtained by the topical method of Blair *et al.* (1963) and are reported in Table II. The cholinesterase data for this series were previously reported (Neely *et al.*, 1964) and are reproduced in Table II.

Partition Coefficients. The π values cited in Tables I and II were either obtained from Fujita *et al.* (1964) or determined experimentally (Hansch *et al.*, 1963). The parent compound for both series was the unsubstituted phenyl *O*-methylphosphoramidate and was given a π value of 0.

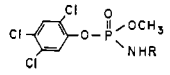
Reactivity. The hydrolysis constants were obtained by measuring the appearance of phenol when the phosphoramidate was treated with 0.1*N* NaOH at 50° C. A Gilford attachment for the Beckman DU spectrophotometer was used for automatically measuring the hydrolysis rate. The technique of using half life was employed, since this eliminated the necessity of knowing the exact concentration (approximately $10^{-5}M$) of phosphate. At 50° C., the reactions were sufficiently fast to obtain final equilibrium values; there was no detectable pH shift during the reaction. The rate constants, k, were then evaluated by Equation 2.

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

RESULTS

The correlations were made with the aid of the multiple regression analysis program at the Dow Computation Research Laboratory using a Burroughs B5500 computer. The data in Table I were fitted to Equation 1, and the

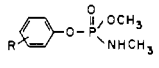
Table I. Chemical and Biological Activity of 2,4,5-Trichlorophenyl *O*-Methyl Phosphoramidates



R	Screening Test, P.P.M. (LD_{50}) MBB[c]	PC	SAW	AR	HF	CFB	HF Topical, μg./Fly LD_{50}	LD_{95}	π[a]	k,[b] Min.$^{-1}$
H	220	70	P[d]	100	70	P	0.084	0.20	2.04	60.5
Methyl	7	65	300	90	50	300	0.076	0.12	2.56	3.45
Ethyl	70	50	400	125	17	400	0.05	0.076	3.07	2.24
N-Propyl	35	35	P	110	20	P	0.067	0.104	3.60	2.10
Isopropyl	40	12	450	12	12	330	0.036	0.094	3.42	1.47
N-Butyl	52	70	P	P	45	P	0.166	0.27	4.04	1.96
tert-Butyl	65	6	170	11	17	14	0.35	0.86	3.72	0.10
s-Butyl	50	45	70	7	10	35	0.152	0.230	3.86	1.43
Isobutyl	26	20	P	38	7	70	0.18	0.29	3.99	1.72

[a] See text for description of π values.
[b] Rate of hydrolysis in 0.1*N* NaOH at 50° C.
[c] MBB, Mexican bean beetle (*Epilachna varivestis*); PC, plum curculio (*Conotrachelus nenuphar*); SAW, southern army worm (*Prodenia eridania*); AR, American cockroach (*Periplaneta americana*); HF, housefly (*Musca domestica*); CFB, confused flour beetle (*Tribolium confusium*).
[d] *P* = >500 p.p.m.

Table II. Chemical and Biological Data for Substituted Phenyl *O*-Methyl Methylphosphoramidates



R	Topical, μg./Fly, LD_{50}	K[a]	π[b]	k,[c] Min.$^{-1}$
4-OCH_3	>10	4.28×10^{-2}	0.56	0.12
4-*tert*-butyl	>10	7.83×10^{-3}	2.22	0.078
3-*tert*-butyl	>10	7.11×10^{-3}	2.14	0.12
4-Cl	0.26	6.02×10^{-3}	1.28	0.36
2,4-di-Cl	0.1	1.07×10^{-5}	1.86	1.87
2-Cl	3.0	7.40×10^{-3}	1.11	1.00
2-Cl-4-*tert*-butyl	0.48	1.10×10^{-6}	2.87	0.608
H	>10	6.94×10^{-2}	0.52	0.12
2,4,5-tri-Cl	0.076	1.07×10^{-6}	2.56	3.45

[a] Bimolecular rate constant for inhibition of fly-head cholinesterase (Neeley *et al.*, 1964).
[b] See text.
[c] Rate of hydrolysis in 0.1*N* NaOH at 50° C.

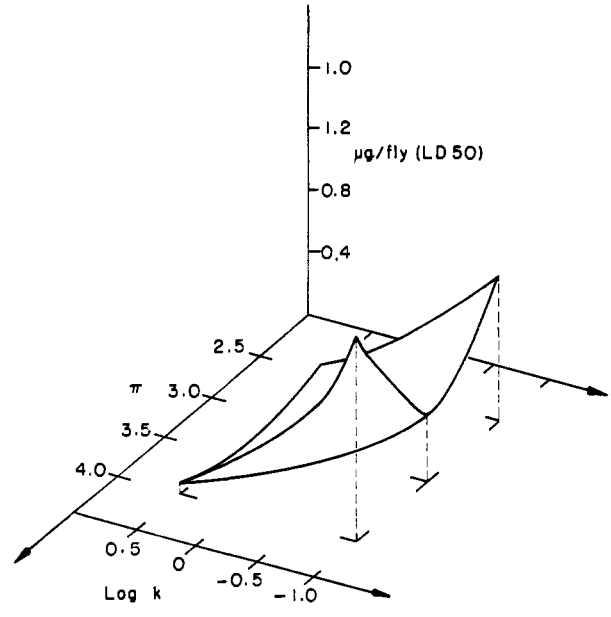


Figure 1. Topical data (micrograms per fly) from Table I plotted against partition coefficient, π, and hydrolysis constant, k, for a series of trichlorophenyl phosphoramidates

results are shown in Table III. The analysis using the topical data on flies (*Musca domestica*) was most striking, and Figure 1 is an attempt to illustrate the influence of the two parameters π (derived from partition coefficients) and k (hydrolysis constants) on the insecticidal activity of the substituted trichlorophenyl *O*-methyl phosphoramidates.

The in vitro fly-head cholinesterase inhibition (K values) and the in vivo housefly (*Musca domestica*) topical mortality data (LD_{50}'s) of the substituted phenyl *O*-methyl methylphosphoramidates (Table II) were fitted to Equation 1. These results are best described by Equations 3 to 6,

	r^2	n	s	
$\log K = 1.17 \log K + 4.75$	0.40	9	0.846	(3)
$\log K = 0.905\pi + 2.93$	0.38	9	0.858	(4)
$\log K = 0.905\pi + 1.17 \log K + 3.21$	0.92	9	0.315	(5)
$\log (LD_{50})$ = no correlation				(6)

where r^2 is the square of the correlation coefficient, n is the number of data points used in the regression, and s is the standard deviation of regression. An F test indicated that the inclusion of both π and k in Equation 5 was significant at the 1% level. In Equation 6, the LD_{50} values from Table II which were greater than 10 p.p.m. were not used in the attempted regression.

The LD_{50} values from Table I on the housefly were regressed against π and k, using Equation 1. The results are shown in Equation 7.

$$\log (LD_{50}) = 0.904\pi^2 - 6.03\pi - 0.509 \log k + 8.44 \quad (7)$$

where the number of points regressed = 9, r^2 = 0.88, and the standard deviation = 0.116

This equation is suitable for predicting LD_{50} values in a series of phosphoramidates. The only criteria that must be met are that all the parameters have a common basis. The substituted phenyl *O*-methyl methylphosphoramidates in Table II meet this requirement. Consequently, it is possible to use Equation 7 to calculate an LD_{50} value for each member of the series. This was done and the results indicated a complete lack of agreement between the experimental and the calculated LD_{50}—e.g., the 4-Cl derivative in Table II has an experimental LD_{50} of 0.26; the calculated value using Equation 7 was 330.

DISCUSSION

The general screening data for the various insect species in Table I are approximate figures and should not be weighted too heavily. The topical data on the flies in Tables I and II are more precise than the screening data; hence, they may be used with greater confidence. Different application methods were used on the various species; therefore, comparable correlations between the screening data and the chemical and physical data for all compounds and species should not be expected. The correlation that exists for the screening data in Table III with the various species is not strong but does indicate some interesting trends. For example, if an agent to control Mexican bean beetle (*Epilachna varivestis*) is desired, more attention should be paid to optimizing the π values which, in turn, might maximize the concentration of toxicant at the receptor site. The reactivity of the material, as far as this insect species and this chemical series are concerned, appears to be a relatively unimportant factor under the conditions of this test method. Conversely, in the case of the plum curculio (*Conotrachelus nenuphar*), cockroach (*Periplaneta americana*), and confused flour beetle (*Tribolium confusium*), the reactivity appears to be important. This indicates an apparent difference among the species as to mode of penetration or mechanism of action. Furthermore trends like this should be of help in the design of more selective insecticides.

The correlation of the housefly topical data with the chemical and physical parameters from Table I, and shown in Table III, is excellent and demonstrates the greater reliability that may be placed on this type of biological information. The results of this analysis indicate the strong dependence of the practical insecticidal activity on both π and reactivity index. Figure 1 emphasizes this dependence. The analysis is especially interesting when compared with the recent work of Hansch and Deutsch (1966) on a similar series of phosphates. These authors correlated the ability of the phosphoramidates to inhibit fly-head cholinesterase with π and an electronic factor. Their results indicated a total dependence on the electronic factor.

A possible explanation for the different conclusions could be based on the following: The 2,4,5-trichlorophenyl substituent is constant in the series listed in Table I. Consequently, in the in vitro system, all the compounds will have similar opportunities for association with the active site of the enzyme; thus, the correlation will be dependent on the ability of the toxicant to phosphorylate the esteratic site. On the other hand, in the in vivo systems described in this report, the chemical must penetrate the insect integument and move among the many potential binding sites that are available. Movement of a toxicant through such a path will be strongly dependent on π, which will help dictate the final concentration of agent at the active site. This hypothesis is partially substantiated, since varying the substituents on the phenyl group in Table II causes the in vitro correlation to be a function of both π and reactivity index (Equations 3 and 5). In this case, the association of the chemical with the enzyme will vary with the groups attached to the phenyl ring and thus will be related to π.

Finally, with the phosphoramidates listed in Table II, the complete lack of correlation between the topical data and the various parameters is surprising (Equation 6). One explanation for no correlation is that some of the observed insecticidal activity must be operating by a mechanism other than inhibiting the cholinesterase enzyme system. This is reflected again in the use of Equation 7, which fails to predict the observed in vivo biological activity. The model for insecticidal activity of these toxicants may be incomplete. An investigation should be undertaken to discover if there is an alternative mechanism for the toxic action of these phosphoramidates in insects.

Table III. Results of Fitting the Data in Table I to the Equation

$$\log \text{B. R.} = a\pi^2 + b\pi + c \log k + d$$

Species	Equation Constants				% Correlation
	a	*b*	*c*	*d*	
MBB[a]	−0.866	6.12		−9.08	58[b]
PC			0.654	1.38	60
SAW		No correlation			
AR			0.711	1.57	15
HR		No correlation			
CFB, LD_{50}, μg./fly	0.941	−6.03	−0.509	8.44	88
HF,[c] LD_{95}, μg./fly	0.827	−5.30	−0.640	7.58	99

[a] See Table I for explanation of code.
[b] % correlation is $r^2 \times 100$ where r is correlation coefficient obtained from regression analysis.
[c] Represents topical data on housefly from Table I.

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