Structure and Insecticidal Activity of Some Diethyl Substituted Phenyl Phosphates

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Twenty-four diethyl substituted phenyl phosphates were quantitatively examined for toxicity to insects and inhibition of insect cholinesterase, and these properties were compared with the lability of the phosphorus-oxygen-aromatic bond as measured by hydrolytic stability, infrared stretching frequencies, and Hammett's sigma values. The correlation between insect cholinesterase inhibition and the lability of the phosphorus-oxygen-aromatic bond was excellent, although steric factors appear to operate in some cases. There was some correlation between insect toxicity and insect cholinesterase inhibition. Insect metabolism, steric factors, and ionic charges all appear to influence the insecticidal activity. Several new compounds predicated on high sigma values are of outstanding insecticidal effectiveness, notably, diethyl *p*-cyanophenyl phosphate, and diethyl *p*-methylmercaptophenyl phosphate and its sulfoxide and sulfone oxidation products, thus demonstrating the practical value of the physicochemical approach.

DETAILED MECHANISMS OF ORGANOPHOSPHORUS INSECTICIDE toxicity to insects and mammals depend largely on the biochemical processes of the animal and the physicochemical properties of the phosphorus compound. Fundamentally, however, the toxic symptoms produced in animals by these organic phosphates are manifestations of the inhibition of certain enzyme systems. It is generally agreed that the toxicity to mammals is associated with the inhibition of the cholinesterase enzymes, although other enzymes such as liver esterase, chymotrypsin, and trypsin are also inhibited (*16*); and from recent work (*4*, *9*, *21*, *23*) it appears that the toxicity to insects is clearly associated with the cholinesterase enzyme system.

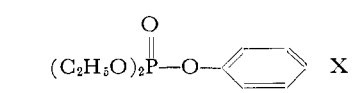
The reaction mechanisms between these organophosphorus compounds and various esterases have been studied in much detail. It is generally concluded that the inhibition of these enzymes results from irreversible phosphorylation of the enzyme at some active site by the phosphorus compound. Thus the inhibition of chymotrypsin by diethyl *p*-nitrophenyl phosphate (paraoxon) and diisopropyl fluorophosphate (DFP) results from equimolar reaction of the phosphate with the enzyme (*15*, *16*), producing inactive phosphorylated chymotrypsin. Aldridge and Davison (*1*) have shown that the inhibition of erythrocyte cholinesterase by paraoxon and some of its analogs follows first-order kinetics and is bimolecular. The bimolecular rate constants for this inhibition were determined for the various paraoxon analogs and these values were usually parallel to the rates of hydrolysis of these phosphates in water.

A mechanism proposed for the inhibition of these enzymes by the organophosphorus compounds may be depicted as follows (*1*, *13*):

$$EH + (RO)_2P(O)—X \overset{K_e}{\rightleftharpoons} EH\cdot(RO)_2P(O)—X \xrightarrow{k_i} E—P(O)(OR)_2 + HX \qquad (1)$$

where EH is the enzyme, R is any short-chain alkyl group, and X is any displaceable group—e.g., halogen, alkoxy, or aryloxy. On the basis of the mechanism proposed it is apparent that the enzyme-inhibiting ability of an organophosphorus compound $(RO)_2P(O)—X$ is directly related to the lability of the P—X bond. Therefore, the inhibition of the enzyme may be thought of as a simple bimolecular reaction between enzyme and organic phosphate, and it becomes possible to correlate structure and reactivity on this basis.

To explore further the relationships between structure and activity, it was decided to investigate the reaction between the cholinesterase enzyme and a series of diethyl substituted phenyl phosphates. This series was selected because of the direct relationship between the substituent X (or substituents) on the benzene nucleus and the reactivity of the phosphorus-oxygen bond shown in the structure below. The phosphates were preferred to the corresponding thionophosphates (parathion) series because of ease of purification resulting from the absence of isomerides, and the simplicity of action resulting from freedom from oxidative metabolism (*21*, *22*).



For meta and para substituents a quantitative relationship is furnished by Hammett's equation (*12*)

$$\log k/k_o = \rho\sigma$$

k_o and k being rate constants for the unsubstituted reactant and for the substituted reactant, respectively, for any reaction series. The substituent constant, σ, determines the nature of the substituent X and is independent of the reaction. The reaction constant, ρ, is

a constant for all substituents in a given reaction series.

A number of diethyl substituted phenyl phosphates containing substituents over a wide range of σ values were synthesized, and their inhibition to fly-brain cholinesterase and toxicity to insects and mites was determined.

Experimental Procedure and Materials

The anticholinesterase activity of the diethyl phenyl phosphates was measured by the standard Warburg manometric method (*20*), using a brei of three homogenized fly brains per milliliters of 0.025*M* sodium bicarbonate, 0.15*M* sodium chloride, 0.04*M* magnesium chloride, and 0.01*M* acetylcholine bromide. The inhibitors were added as standard molar concentrations in 0.1-ml. aliquots in acetone solution, and after flushing with 95% nitrogen–5% carbon dioxide, and 15-minute equilibration, the volume of carbon dioxide produced at 37.5° C. in 30 minutes was measured and compared with that of a standard without inhibitor. The values obtained were plotted as log molar concentration against percentage inhibition, and the 50% inhibition values were determined by inspection of the resulting straightline plots.

It has been shown by Aldridge and Davison (*1*) that a number of diethyl substituted phenyl phosphates contain small amounts of a powerful cholinesterase inhibitor which can be destroyed in alkaline media without affecting the phosphate. In this work the diethyl phenyl phosphates were allowed to stand 24 hours in 0.1*M* diethyl barbituric acid buffer (pH 9.5) just prior to the inhibition measurements. Further standing in buffer had no effect on the degree of inhibition.

Diethyl Hydrogen Phosphite. Diethyl hydrogen phosphite was prepared according to the method of McCombie, Saunders, and Stacey (*18*), using 207 grams of absolute ethyl alcohol and 206 grams of phosphorus trichloride in 375 ml. of carbon tetrachloride. The product distilled at 83° C. (20 mm.). The yield was 168 grams.

Diethyl Chlorophosphate. This compound also was prepared according to the method of McCombie, Saunders, and Stacey (*18*), by passing chlorine gas into diethyl hydrogen phosphite. The product distilled at 59.5–61° C. (2.0 mm.).

***m-tert*-Butylphenol.** This compound was prepared according to the six-step procedure of Carpenter, Easter, and Wood (*7*). Forty grams of *m-tert*-butylphenol (melting point 39–41° C.) was obtained from 138 grams of acetanilide.

***p*-Cyanophenol.** This compound was prepared according to the method of Ashley, and coworkers (*3*) by the Sandemeyer reaction on *p*-aminophenol. The product distilled at 132° C. (0.3 mm.) and solidified upon standing (melting point 110° C.).

***m*-Dimethylaminophenol.** *m*-Dimethylaminophenol methiodide was prepared according to Stedman (*26*) from *m*-aminophenol and methyl iodide (melting point 183° C., decomposes). The methiodide was heated to 180 to 190° C. under water-aspirator vacuum until complete decomposition occurred. The product was then distilled (boiling point 137° C. at 7 mm.), and crystallized from *n*-hexane. The *m*-dimethylaminophenol melted at 83° C.

***p*-Methylmercaptophenol.** This compound was prepared according to the method of Miller and Read (*24*). From 110 grams of *p*-aminophenol, 95 grams of thiohydroquinone was obtained (boiling point 92 to 96° C. at 0.1 mm.). The thiohydroquinone was then converted to *p*-methylmercaptophenol by treating it with methyliodide and sodium carbonate in ethyl alcohol. The product distilled at 96 to 100° C. (0.3 mm.) and solidified upon standing (melting point 81° C.).

***p*-Methylsulfinylphenol.** *p*-Methylmercaptophenol was converted to *p*-methylmercaptophenyl acetate (melting point 42° C.), by the method of Chattaway (*8*). The acetate was then oxidized and hydrolyzed to *p*-methylsulfinylphenol by the procedure of Zincke and Ebel (*27*). The product was recrystallized from benzene (melting point 90–1° C.).

***p*-Methylsulfonylphenol.** This compound was prepared by the oxidative procedure of Zincke and Ebel (*27*) from *p*-methylmercaptophenyl acetate. The *p*-methylsulfonylphenol was recrystallized from benzene (melting point 94° C.).

***m*-Methoxyphenol.** Resorcylic acid was prepared according to the method described by Blatt (*6*). To 80 grams of resorcylic acid and 150 grams of dimethyl sulfate in 150 ml. of methanol was added 84 grams of potassium hydroxide dissolved in 200 ml. of water. The mixture was stirred and heated for 1 hour, and another 60 grams of potassium hydroxide was then added. The mixture was heated for another hour and acidified with concentrated hydrochloric acid, and the *p*-methoxyresorcylic acid which separated was collected and recrystallized from ethyl alcohol and water (melting point 154–6° C.). The *p*-methoxyresorcylic acid was decarboxylated to the phenol by refluxing a water solution for 24 hours. The *m*-methoxyphenol was then extracted from the aqueous mixture with ether, dried over anhydrous sodium sulfate, and distilled (boiling point 80° C. at 0.05 mm.). The yield was 30 grams.

The phenols *p*-methoxyphenol and *p*-hydroxybenzaldehyde were Eastman white label grade and were used without further purification.

Diethyl Phenyl Phosphates. The diethyl substituted phenyl phosphates described in Table I were prepared by the condensation of diethyl chlorophosphate and the sodium salts of the phenols. The following procedure for the synthesis of diethyl *p*-methoxyphenyl phosphate is typical. Sodium (2 grams) was dissolved in 20 ml. of absolute ethyl alcohol. Anhydrous benzene and 10.4 grams of *p*-methoxyphenol were added, and the ethyl alcohol was removed by azeotropic distillation with benzene until the temperature of the distillate reached 79° C. The sodium salt of *p*-methoxyphenol precipitated out of solution at this point. The temperature of the mixture was lowered to 70° to 80° C., and 14.4 grams of diethyl chlorophosphate was added with stirring. The mixture was stirred for 2 hours and filtered; the benzene solution was washed, first with dilute sodium carbonate and then several times with water, and was dried over anhydrous sodium sulfate. The benzene was removed and the product was distilled under reduced pressure (boiling point 114° C. at 0.05 mm.).

Diethyl *m*-dimethylaminophenyl phosphate compound (X, Table I) has been reported by Morrison and Atherton (*25*) (boiling point 116-20° C. at 0.003 mm.; n_D^{22} 1.5112). X was further characterized by converting it to diethyl *m*-dimethylaminophenyl phosphate methio-

Table I. Physical Constants of Diethyl Substituted Phenyl Phosphates

Diethyl Substituted Phenyl Phosphate	Boiling Point, °C. (0.05 mm.)	n_D^{25}	Analysis: Empirical formula	Calc. P	Found P
I. *m*-Nitro	140 (0.1 mm.)[a]	1.4972	$C_{10}H_{14}NO_6P$	11.3	11.0
II. *p*-Cyano	103	1.4920	$C_{11}H_{14}NO_4P$	12.2	12.7
III. *m-tert*-Butyl	110	1.4770	$C_{14}H_{23}O_4P$	10.8	10.7
IV. *m*-Methoxy	114–118	1.4842	$C_{11}H_{17}O_5P$	11.9	11.6
V. *p*-Methoxy	114	1.4861	$C_{11}H_{17}O_5P$	11.9	12.1
VI. *p*-Methylmercapto	131–133	1.5254	$C_{11}H_{17}O_4PS$	11.2	11.1
VII. *p*-Methylsulfinyl	165	1.5146	$C_{11}H_{17}O_5PS$	10.6	10.2
VIII. *p*-Methylsulfonyl	185	1.5028	$C_{11}H_{17}O_6PS$	10.1	9.8
IX. *p*-Formyl	130	1.5002	$C_{11}H_{15}O_5P$	12.0	12.4
X. *m*-Dimethylamino	135	1.5100			

[a] Distilled in falling-film molecular still.

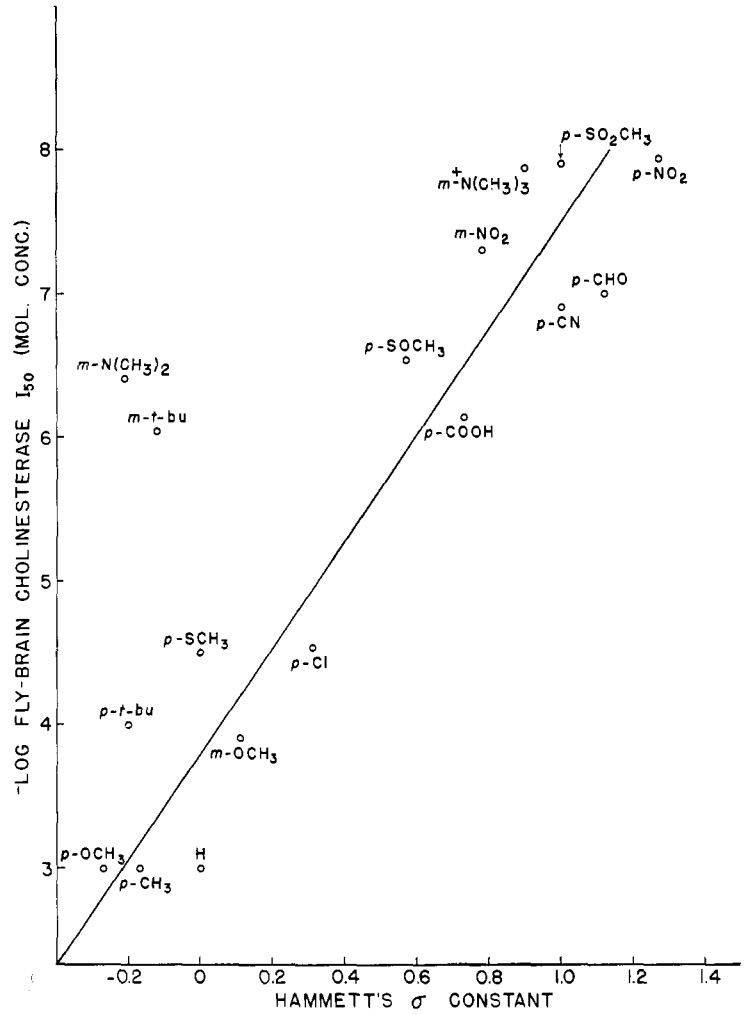


Figure 1. Relation of $-\log$ fly-brain cholinesterase I_{50} to Hammett's constants for meta- and para-substituted phenyl diethyl phosphates

Table II. Biological Activity of Diethyl Substituted Phenyl Phosphates

Diethyl Substituted Phenyl Phosphates	I_{50} Fly-brain ChE, Mol. Concn.	LD_{50}[a] Housefly, γ/gram	Thrips, % concn.	Mites, % concn.
XIX. 2,4-di-NO_2	3.0×10^{-9}	155	0.01	0.01
XVI. *p*-NO_2	2.6×10^{-8}	0.5	0.0001	0.001
XVII. *o*-NO_2	5.0×10^{-8}	7.0	0.001	0.003
I. *m*-NO_2	5.0×10^{-8}	9.8	0.005	0.03
XXII. 2,4,5-tri-Cl	6.0×10^{-9}	8.0	0.03	0.0003
XXIII. 2,4,6-tri-Cl	3.3×10^{-6}	175	0.03	0.03
XXIV. 2,4-di-Cl	5.0×10^{-7}	15.0	0.003	0.1
XXI. *o*-Cl	2.0×10^{-5}	250	0.01	>0.1
XX. *p*-Cl	3.0×10^{-5}	150	0.01	>0.1
XII. *p*-$\dot{S}(CH_3)_2 . CH_3SO_4^-$	3.4×10^{-9}	17.5	0.0002	0.001
VIII. *p*-SO_2CH_3	2.5×10^{-7}	2.5	0.0001	0.001
VII. *p*-$SOCH_3$	3.1×10^{-6}	1.5	0.0001	0.0008
VI. *p*-SCH_3	3.3×10^{-5}	2.0	0.0001	0.0002
XI. *m*-$\dot{N}(CH_3)_3I^-$	3.0×10^{-8}	>500	>0.1	>0.1
X. *m*-$N(CH_3)_2$	4.0×10^{-7}	25	0.1	0.1
II. *p*-CN	1.3×10^{-7}	3.5	0.00002	0.002
IV. *m*-OCH_3	1.3×10^{-4}	>500	0.02	0.3
V. *p*-OCH_3	$>1.0 \times 10^{-3}$	>500	1.0	1.0
III. *m*-*tert*-butyl	9.0×10^{-7}	500	0.003	>0.1
XVIII. *p*-*tert*-butyl	1.0×10^{-4}	>500	0.1	>0.1
XV. *p*-CH_3	$>1.0 \times 10^{-3}$	>500	>0.1	>0.1
XIV. H	$>1.0 \times 10^{-3}$	>500	>0.1	>0.1
IX. *p*-CHO	1.5×10^{-7}	>500	0.05	0.05
XIII. *p*-COOH	8.5×10^{-7}	150	0.005	0.01

[a] The standard deviation of 5 replicate LD_{50} determinations by the methods employed was ±30%.

dide (XI), a hygroscopic solid. Analysis. Calculated for $C_{13}H_{23}O_4NPI$: I, 30.60; found: I, 31.20.

Diethyl *p*-methylmercaptophenyl phosphate (VI) was converted to the methosulfate by heating it with an equivalent amount of dimethyl sulfate on the steam bath for several hours. The product, diethyl methylmercaptophenyl phosphate methosulfate (XII), was a thick, viscous oil that could not be induced to crystallize. Analysis. Calculated for $C_{13}H_{23}O_8PS_2$: P, 7.7; found: P, 7.5.

Diethyl *p* - formylphenyl phosphate (IX) was oxidized to diethyl *p*-carboxyphenyl phosphate (XIII) by treatment with alkaline potassium permanganate. The product was not distilled, n_D^{25} 1.4923. Analysis. Calculated for $C_{11}H_{15}O_6P$: P, 11.3; found: P, 11.4.

The techniques for determination of the contact toxicity of the phenyl phosphates to the citrus red mite, *Metatetranychus citri* (McG.), the greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché), and the common house fly, *Musca domestica* L., have been described (*20*, *22*).

The rate of hydrolysis of a number of the diethyl substituted phenyl phosphates was determined colorimetrically at 37.1° C. in *0.1M* diethyl barbituric acid buffer (pH 9.5) by colorimetric measurement of free phenol by reaction with 4-aminoantipyrine and alkaline potassium ferricyanide (*17*). The hydrolysis of diethyl *p*-nitrophenyl, *m*-nitrophenyl, and 2,4-dinitrophenyl phosphates was determined colorimetrically by measuring the amount of liberated phenate ion (*17*).

The infrared spectra were determined in a Perkin-Elmer Model 21 self-recording infrared spectrophotometer, with sodium chloride optics and carbon tetrachloride as a solvent.

Discussion of Results

On the hypothesis that the diethyl phenyl phosphates inhibit the cholinesterase enzyme by reacting with it to form the phosphorylated inactivated enzyme, it is of interest to compare the effect of variation in substituents of the benzene nucleus on the degree of enzyme inhibition. The molar concentrations of these compounds for 50% inhibition (I_{50}) of fly-brain cholinesterase (ChE) and for toxicity (LD_{50}) to the common housefly, the greenhouse thrips, and the citrus red mite are shown in Table II. Examination of the I_{50} data shows that the degree of inhibition is a direct function of the electron-withdrawing capacities of the substituents on the benzene nucleus. The relationship is graphically presented in Figure 1, which shows a plot of $-\log I_{50}$ values against Hammett's sigma (σ) constants for the substituents. Since the I_{50} value is related to the bimolecular

rate constant, k, for the reaction between the cholinesterase enzyme and the diethyl phenyl phosphate according to Equation 2 (*1*),

$$k = \frac{2.3}{tI} \log \frac{100}{b} \qquad (2)$$

where t = time, I = molar inhibitor concentration, and b = percentage residual activity at time t, it follows that a plot of $-\log I_{50}$ *vs.* σ will differ from a plot of $\log k$ *vs.* σ only by the slope of the line.

Examination of Figure 1 shows that most of the points are dispersed along a straight line, with $-\log I_{50}$ increasing with increasing values of σ. Since Hammett's equation applied only to meta and para substituents, 2,4-dinitrophenyl (XIX), *o*-nitrophenyl (XVII), 2,4,5-trichlorophenyl (XXII), 2,4,6-trichlorophenyl (XXIII), 2,4-dichlorophenyl (XXIV), and *o*-chlorophenyl diethyl phosphates (XXI) were not included in Figure 1. The I_{50} values for these compounds show that the effect of two or more substituents is largely additive. Of interest is the large difference in inhibition between XXII and XXIII. The relatively low activity of XXIII is probably due to steric hindrance by the chlorine atoms in the 2 and 6 positions, which slows down the reaction rate between the phosphate and enzyme.

Ingraham and coworkers (*14*) found that the shifts in oxygen-hydrogen stretching frequency produced by substitution in phenols and catechols were a direct function of Hammett's σ values. Variations in the phosphorus-oxygen-carbon (aromatic) stretching frequencies have been reported by Bellamy (*5*), and these values have been found to range from 1190 to 1240 cm.$^{-1}$, with those of the para-substituted nitrophenyl phosphates nearest 1240 cm.$^{-1}$. As a corollary to Hammett's treatment the infrared spectra of a number of these substituted phenyl diethyl phosphates were determined, and the shifts in the phosphorus-oxygen-carbon (aromatic) stretching frequency examined critically. The frequencies of this bond given are listed in order of increasing wave number in Table III. The data show that the wave number is a function of the electron-donating or attracting property of the substituent, and is a measure of the reactivity of the phosphorus-oxygen bond. The plot of $-\log$ fly-brain cholinesterase I_{50} against frequency (Figure 2) was similar to that obtained with σ values. Diethyl 2,4-dinitrophenyl and 2,4,5-trichlorophenyl phosphate were not included in Figure 2 because they possess ortho substituents, but the large shifts in frequency produced by these multiple substituents are in line with their high cholinesterase inhibitory activity.

The first-order rate constants for hydrolysis ($K_{hyd.}$) in 0.1*M* diethyl barbituric acid buffers (pH 9.5) were calculated, and the results are given in Table IV. The colorimetric method used was applicable only to meta-substituted phosphate and para-substituted chlorophenyl phosphate. A plot of $\log K_{hyd.}$ *vs.* σ shows that these monosubstituted phenyl phosphates conform to Hammett's equation, as shown by Figure 3. From the slope of the line, σ was calculated and found to be 1.3.

The data presented show that cholinesterase inhibition, hydrolysis constants,

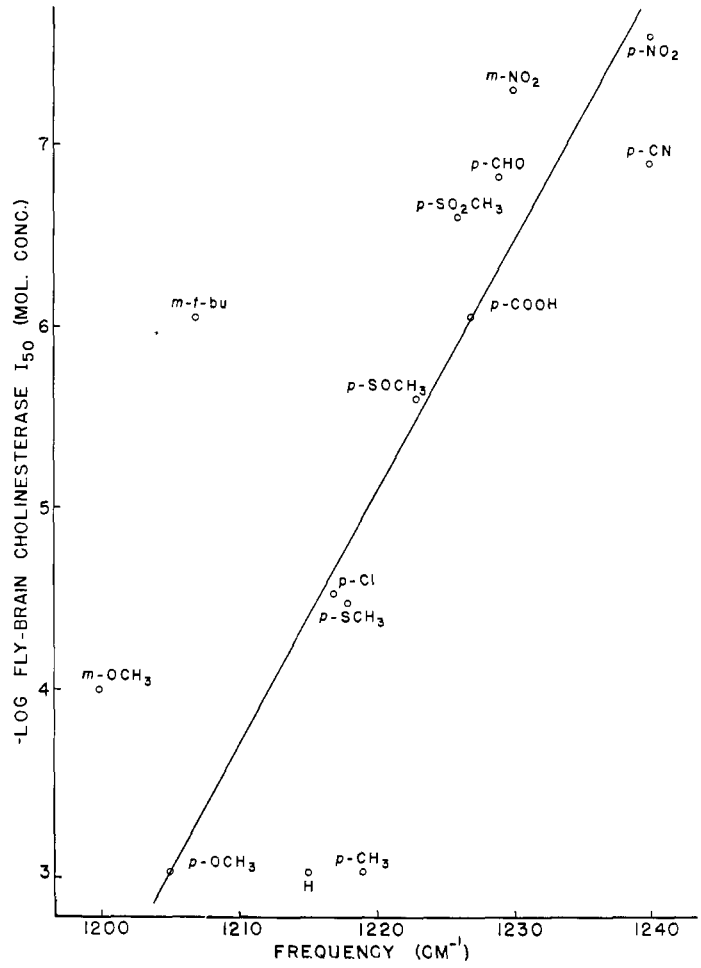


Figure 2. Relation of $-\log$ fly-brain cholinesterase I_{50} to frequency (wave number) of phosphorus-oxygen-aromatic stretching vibration for meta- and para-substituted phenyl diethyl phosphates

Table III. Frequency (Cm.$^{-1}$) of Phosphorus-Oxygen-Aromatic Stretching Vibrations in Various Diethyl Substituted Phenyl Phosphates

Diethyl Substituted Phosphate	*Wave N., Cm.$^{-1}$*
m-Methoxyphenyl	1200
p-Methoxyphenyl	1205
m-tert-Butylphenyl	1207
Phenyl	1215
p-Chlorophenyl	1217
p-Methylmercaptophenyl	1218
p-Methylphenyl	1219
p-Methylsulfinylphenyl	1223
p-Methylsulfonylphenyl	1226
p-Carboxyphenyl	1227
p-Formylphenyl	1229
m-Nitrophenyl	1230
p-Nitrophenyl	1240
p-Cyanophenyl	1241
2,4,5-Trichlorophenyl	1253
2,4-Dinitrophenyl	1265

Table IV. First-Order Hydrolysis Constants of Some Diethyl Substituted Phenyl Phosphates in 0.1M Diethyl Barbituric Acid (pH 9.5)

Diethyl Substituted Phosphate	$K_{hyd.}$ *Min.*$^{-1}$
Phenyl	9.2×10^{-6}
m-tert-Butylphenyl	8.6×10^{-6}
m-Dimethylaminophenyl	1.9×10^{-6}
p-Chlorophenyl	3.2×10^{-5}
o-Chlorophenyl	5.1×10^{-5}
2,4,5-Trichlorophenyl	7.9×10^{-5}
2,4-Dichlorophenyl	4.8×10^{-5}
m-Methoxyphenyl	8.9×10^{-6}
p-Nitrophenyl	2.7×10^{-4}
m-Nitrophenyl	9.8×10^{-5}
2,4-Dinitrophenyl	5.7×10^{-3}

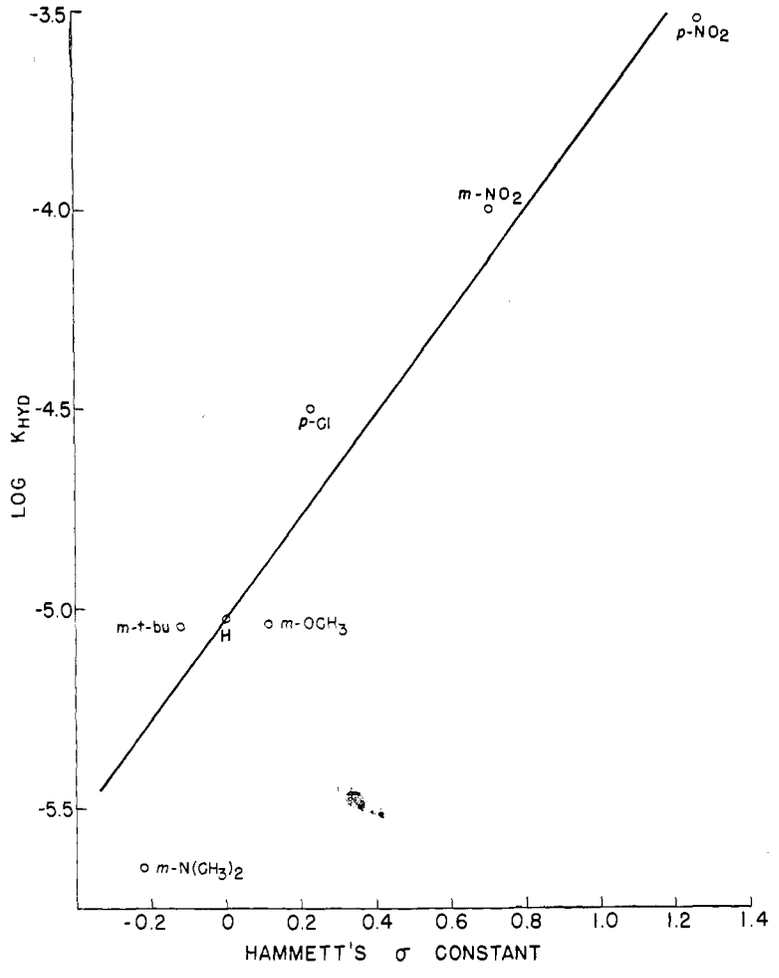


Figure 3. Relation of log $K_{hyd.}$ and Hammett's σ constants for some meta- and para-substituted phenyl diethyl phosphates

Hammett's sigma constants, and phosphorus-oxygen-aromatic stretching frequencies are interrelated. It is, therefore, obvious that sigma values, which are readily obtainable from the literature, and infrared measurements, which are easily determined, are of great value in predicting cholinesterase inhibition by compounds of this type.

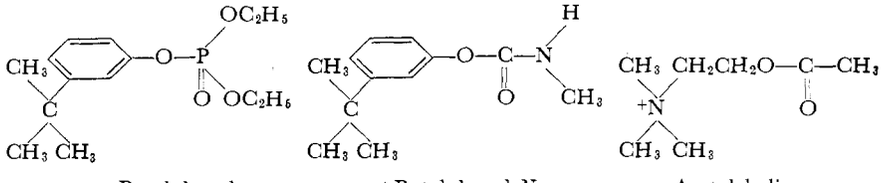
The *m*-dimethylaminophenyl (X) and *m-tert*-butylphenyl diethyl phosphates (III) are exceptions in that they are far more inhibitory than Hammett's treatment would predict for them. Their rates of hydrolysis, however, are in line with Hammett's equation, as shown in Table IV and Figure 3. The remarkable influence on activity as shown by the *m*-dimethylamino and *m-tert*-butyl groups has also been observed with the substituted phenyl *N*-methylcarbamates: *m*-dimethylaminophenyl *N*-methylcarbamate and *m-tert*-butylphenyl *N*-methylcarbamate (*17*). In view of the relative stability of III and X to aqueous hydrolysis (see Table IV) and their structural similarities to the corresponding carbamates and with acetylcholine, it would appear that these two compounds inhibit cholinesterase by a competitive type mechanism and not by irreversible phosphorylation.

housefly against $-\log$ cholinesterase I_{50} shows the dispersal of a large proportion of the points around a common straight line (see Figure 4). A number of points somewhat removed from the line require explanation, however, because of their unusual toxicities.

The relatively low toxicity of diethyl *m* - dimethylaminophenyl phosphate methiodide (XI) and diethyl *p*-methylmercaptophenyl phosphate methosulfate (XII) suggests that penetration through the insect cuticle or lipoid nerve sheaths is an important factor, and that polar compounds (ions) are poor in this respect. Moderate stability to aqueous hydrolysis is another factor which must be considered, and the low toxicity of diethyl 2,4-dinitrophenyl phosphate (XIX) is probably due to hydrolytic degradation before it reaches the site of action.

Another important consideration is the metabolic or chemical change which may occur in or on the insect. The unusually high activities of *p*-methylmercaptophenyl (VI) and *p*-methylsulfinylphenyl diethyl phosphate (VII) are probably due to oxidative conversion of these compounds in the insect to the sulfone (VIII), which shows about the same toxicity but is a far more potent inhibitor of cholinesterase. In this connection March and coworkers (*19*) have shown that *O,O*-diethyl *S*-2-ethylmercaptoethyl phosphorothiolate and *O,O*-diethyl *O*-2-ethylmercaptoethyl phosphorothionate are metabolized to the respective sulfoxides and sulfones in the white mouse and American cockroach.

The lack of toxicity of *p*-formylphenyl diethyl phosphate (IX) is difficult to



m-tert-Butylphenyl diethyl phosphate | *m-tert*-Butylphenyl *N*-methylcarbamate | Acetylcholine

This concept of the inhibition of cholinesterase by an organic phosphate through a competitive mechanism is of interest because it helps to explain the unusually high inhibitory activity of a number of phosphates such as *O,O*-diethyl *S*-2-diethylaminoethyl phosphorothiolate (*11*), 3-(diethoxyphosphinyloxy)-*N*-methylquinolinium methyl sulfate (*2*), and others.

Kolbezen, Metcalf, and Fukuto (*17*) have shown that with the substituted phenyl *N*-methylcarbamates a direct relationship exists between $-\log$ cholinesterase I_{50} and log median lethal concentration to the greenhouse thrips. A similar plot with the diethyl phenyl phosphates of log LD_{50} (γ per gram) explain. It is well known that benzaldehyde is unstable in air and readily oxidizes to the acid, and oxidative metabolism of this nature might be suspected. Oxidation of IX gives the *p*-carboxyphenyl phosphate (XIII), which as shown in Table II, is less potent as an inhibitor corresponding to its σ. XIII, however, showed slightly greater toxicity than IX. As discussed earlier, because of its structural similarity to acetylcholine and its relative stability to hydrolysis, compound III appears to inhibit cholinesterase by a competitive type mechanism. In view of the poor insecticidal activity of III it appears that competitive inhibition of cholinesterase without phosphorylation by an organic

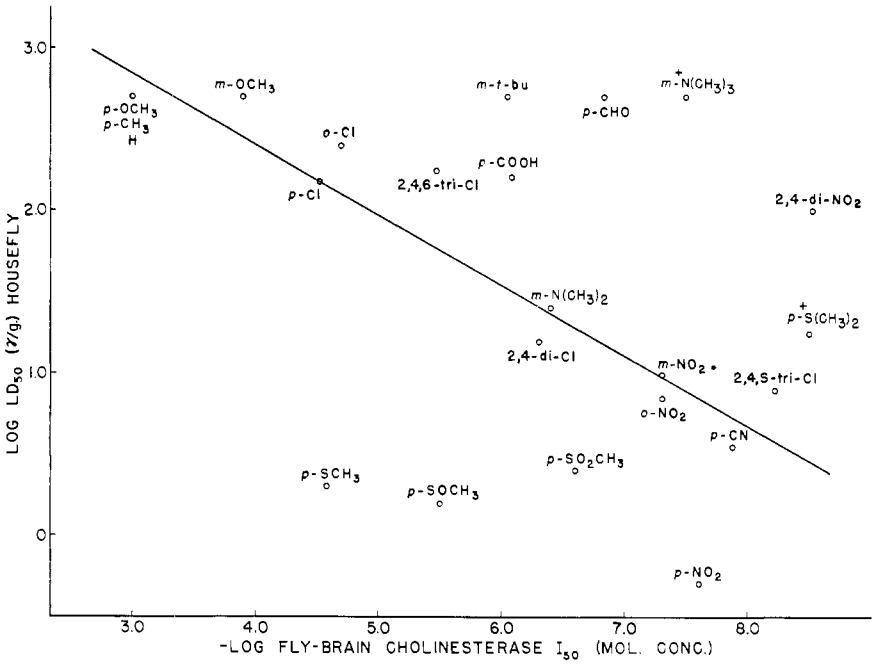


Figure 4. Relation of −log fly-brain cholinesterase I_{50} to log median lethal dosage (LD_{50} housefly) for substituted phenyl diethyl phosphates

phosphate is a relatively inefficient process in insect poisoning. The moderately high toxicity of X suggests that transformation to an amine salt takes place in vivo, and results in a compound which acts as an irreversible phosphorylating agent. The σ value for the amine salt predicts the development of a potent irreversible-type enzyme inhibitor from this conversion.

The insect and mite toxicity data showed that a number of compounds were active enough to warrant further investigation. Diethyl *p*-cyanophenyl phosphate (II) was extremely potent against thrips (0.00002% concentration). Compounds VI, VII, VIII, and XII were all highly toxic to the insects and mites tested. The data show that only those compounds containing substituents with favorable σ values were active, with the lone exception of VI, which is probably metabolized to the sulfoxide or sulfone. This investigation supports the value of the use of certain fundamental chemical concepts for the solution of practical problems of this nature.

The data and discussion presented are in agreement with the general mechanism of inhibition proposed by Aldridge and Davison (*1*) and, later, by Hartley and Kilby (*13*), as shown in Equation 1. In the case of irreversible phosphorylation, the enzyme inhibitor complex can be thought of as the transition state in the nucleophilic displacement of the X moiety on the phosphorus atom. The data indicate, however, that reversible inhibition can also occur with certain phosphates. Presumably, both mechanisms operate in certain cases, and the degree of inhibition depends on the values of K_e and k_i. Compounds *O,O*-diethyl *S*-2-diethylaminoethyl phosphorothiolate oxalate (I_{50} 3×10^{-9} *M*), 3-(diethoxyphosphinyloxy)-*N*-methylquinolinium methyl sulfate (I_{50} 1.5×10^{-10} *M*), and *O,O*-diethyl *S*-2-ethylmercaptoethyl phosphorothiolate methosulfate (I_{50} 3.3×10^{-8} *M*) (*10*) are a few of those compounds which closely resemble acetylcholine spatially and possesses relatively labile P-X bonds. The K_e values for these compounds should favor complex formation and should, therefore, increase the over-all rate of inhibition. *m*-Dimethylaminophenyl and *m-tert*-butylphenyl diethyl phosphates are examples in which K_e is near optimum and k_i is very small.

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Diethyl phenyl phosphate (XIV), diethyl *p*-tolyl phosphate (XV), diethyl *p*-nitrophenyl phosphate (XVI), diethyl *o*-nitrophenyl phosphate (XVII), diethyl *p-tert*-butylphenyl phosphate (XVIII), diethyl 2,4-dinitrophenyl phosphate (XIX), diethyl *p*-chlorophenyl phosphate (XX), and *o*-chlorophenyl phosphate (XXI) were obtained through the cooperation of Arthur Toy, Victor Chemical Works, Chicago Heights, Ill. Diethyl 2,4,5-(XXII) and 2,4,6-trichlorophenyl phosphates (XXIII) were obtained from the Dow Chemical Co., Midland, Mich. Diethyl 2,4-dichlorophenyl phosphate (XXIV) was obtained from the Chemical-Biological Coordination Center, National Research Council, Washington 25, D.C.

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