# Selectivity of Sumithion Compared with Methyl Parathion. Influence of Structure on Anticholinesterase Activity

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Methyl parathion, Sumithion, and a number of closely related analogs were examined for toxicity to susceptible and organophosphorus insecticide-resistant houseflies, the German cockroach, and the white mouse. In general, the compounds were uniformly toxic to the susceptible flies and cockroach, but there was wide variation in their toxicity to the mouse. The selectivity of these compounds is due in part to differences in inhibition of mammalian cholinesterases by different organophosphorus esters. Detailed analysis of the inhibition process by methyl paraoxon and its meta-alkyl substituted analogs provided evidence that the difference in inhibition is due to variation in the distance between the anionic and esteratic sites in enzyme from insect and mammalian sources.

Since its discovery by Schrader, methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate) has become one of the most widely used organophosphorus insecticides despite its relatively high mammalian toxicity. Introduction of a chlorine atom into the three-position of the phenyl ring gives Chlorthion, which is considerably safer to mammals but often only one tenth as toxic to insects, and shows an unfortunately reduced spectrum of insecticidal activity. Strangely, the development of Chlorthion in 1952 did not immediately lead to the examination of its isostere, *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate, which will be referred to as Sumithion (fenitrothion). This compound was first described and its remarkable mammalian selectivity noted by Drabek and Pelikan (*4*) in Czechoslovakia and later named by them as Metation. This work apparently went unnoticed elsewhere since Sumithion was rediscovered and patented by Farbenfabriken Bayer in Germany as Folithion (Bayer 41831) (*26*) and by Sumitomo in Japan as Sumithion (*23*).

By oral dosage Sumithion is from 0.02 to 0.05 times as toxic as methyl parathion to most mammals yet retains a comparable toxicity and range of activity against insects. Consequently, it has had wide application in many parts of the world and is used in large quantities for the control of such insects as the rice stem borer, *Chilo supressalis*. This compound is one of the most promising new insecticides (OMS-43) for the control of insects of public health importance and is currently undergoing large scale evaluation in malaria eradication programs.

The development and widespread usage of Sumithion represents a remarkable advance in the sophistication of pesticide design when compared to its hazardous antecedents. The biochemical basis for this outstanding selectivity provides a toxicological puzzle whose solution would be beneficial in the devising of other selective pesticides, hence the present investigation. The problem is made more fascinating by the apparently insignificant difference in structure between Sumithion and methyl parathion.

This paper, the first of three parts, is concerned with the relationship between the toxicity of Sumithion and its analogs to insects and mammals and the ability of these compounds to inhibit the target enzyme, cholinesterase. Particular attention is paid to two groups of analogs, the phosphonates and phosphinates and compounds in which the *m*-methyl group is replaced by other alkyl moieties.

## *Materials and Methods*

3-Alkyl-4-nitrophenols were prepared by nitrosation of 3-alkylphenols (*9*) followed by molybdate-catalyzed oxidation of the nitrosophenol with hydrogen peroxide. The following procedure for the synthesis of 3-methyl-4-nitrophenol is typical.

A mixture of 46 grams of 3-methyl-4-nitrosophenol, 2 grams of ammonium molybdate, and 400 ml. of glacial acetic acid in a 1000-ml. flask was heated on a steam bath and 53 ml. of 30% hydrogen peroxide were added in approximately 10 equal portions with vigorous shaking. An exothermic reaction began after most of the peroxide had been added and maintained itself on removal from the steam bath. After completion of the reaction the mixture was diluted with water, and the product was extracted into ether and dried over anhydrous sodium sulfate. Removal of the ether gave the crystalline product which, after recrystallization from water, melted at 125–25.5° C. with 62% yield. The 3-ethyl- and 3-isopropyl-4-nitrophenols were prepared similarly except these compounds were distilled prior to recrystallization from *n*-hexane–benzene. 3-Ethyl-4-nitrophenol, b.p. 149–151° C. (1.3 mm.), m.p. 71–3° C. was obtained in 74% yield; 3-isopropyl-4-nitrophenol, b.p. 147–48° C. (1.3 mm.), m.p. 80–1° C. was ob-

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tained in 86% yield. This method for the conversion of nitrosophenols to nitrophenols proved to be superior to the use of nitric acid described by Hodgson and Crouch (*11*) and hydrogen peroxide in alkaline media described by Travagli (*28*).

*O,O*-Dimethyl phosphorochloridothioate, b.p. 65–69° C. (16 mm.), $n_D^{25}$ 1.4788, was prepared from *O,O*-dimethyl phosphorodithioic acid according to Fletcher *et al.* (*5*). Dimethyl phosphorochloridate, b.p. 56–57° C. (3.5 mm.), $n_D^{25}$ 1.4100, was prepared from trimethyl phosphite by the action of chlorine (*3*). Methyl methylphosphonochloridothionate was prepared from methylphosphonothioic dichloride (*13*) by treatment with equivalent amounts of methanol and triethylamine in ether or with sodium methoxide in benzene. The yield varied from 48 to 63%. Both methods gave products which were contaminated with varying amounts of dimethyl methylphosphonothionate with range of refractive index $n_D^{25}$ 1.5010–1.5054, b.p. 61–65° C. (22 mm.). Kabachnik *et al.* (*14*) report b.p. 54–55° C. (21 mm.), $n_D^{20}$ 1.5085 for methyl methylphosphonochloridothionate. Dimethylphosphinothioic chloride was prepared from tetramethyldiphosphine disulfide (*15*) in 60% yield by treatment with chlorine according to Schrader (*27*), b.p. 65–66° C. (8 mm.). Dimethylphosphinic chloride, b.p. 88–93° C. (10 mm.), was prepared from the same tetramethyldiphosphine disulfide, according to Pollart and Harwood (*25*), using thionyl chloride.

**O,O-Dimethyl O-(3-alkyl-4-nitrophenyl) Phosphorothioates.** 3-Methyl-, 3-ethyl-, 3-isopropyl- and unsubstituted 4-nitrophenyl *O,O*-dimethyl phosphorothionates were prepared in the usual manner by reaction between the sodium salt of the appropriate 3-alkyl-4-nitrophenol and *O,O*-dimethyl phosphorochloridothioate and acetone or methyl ethyl ketone under reflux for 4 to 6 hours. In each case the product was taken up in benzene, washed twice with 5% sodium hydroxide and twice with water, and dried over sodium sulfate.

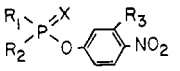
*O,O*-Dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate (Sumithion) was purified by column chromatography as follows. To a column (3 × 25 cm.) prepared from Woelm acid alumina—anionotropic, activity grade 1, 3% $H_2O$ added—which had been previously activated at 200° C. for several hours, was added 5 grams of impure Sumithion in benzene. Continuous elution with benzene gave a Sumithion fraction after about 250 ml. of benzene had passed through. Removal of the benzene gave a yellow oil, $n_D^{25}$ 1.5532, which solidified upon cooling to −10° C. and melted rapidly at room temperature. Elemental analysis of this compound is given in Table I along with the other phosphorothioate esters prepared similarly but not purified by column chromatography.

**O-4-Nitrophenyl and O-(3-Methyl-4-nitrophenyl) O-Methyl Methylphosphonothioates.** These phosphonothionates were prepared from *O*-methyl methylphosphonochloridothioate and the appropriate phenol as described for the phosphorothionates. Both compounds were recrystallized for *n*-hexane. Physical properties are given in Table I.

**O-4-Nitrophenyl and O-(3-Methyl-4-nitrophenyl) Dimethylphosphinothioates.** These compounds were prepared in the same manner described above and were purified by repeated crystallizations from *n*-hexane containing a little benzene.

**3-Alkyl-4-nitrophenyl Dimethyl Phosphates and Methyl Methylphosphonates.** The phosphate and phosphonate esters listed in Table I were prepared also in a similar manner from the appropriate phenol and chloridate. The products were liquids and were purified by distillation. The phosphonate esters X and XI were difficult to purify, and satisfactory analyses could not be obtained owing to the presence of small amounts of

**Table I. Physical Properties of Phosphate, Phosphonate, and Phosphinate Esters of 3-Alkyl-4-nitrophenols**



| | | | | | | | Analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Calcd. | | Found | |
| | $R_1$ | $R_2$ | X | $R_3$ | B.P., ° C. | $n_D^{25}$ | C | H | C | H |
| I | $CH_3O$ | $CH_3O$ | S | H | 35–35.5[a] | | | | | |
| II | $CH_3O$ | $CH_3O$ | S | $CH_3$ | ... | 1.5532 | 38.99 | 4.36 | 39.31 | 4.23 |
| III | $CH_3O$ | $CH_3O$ | S | $C_2H_5$ | ... | 1.5418 | 41.24 | 4.85 | 41.55 | 4.97 |
| IV | $CH_3O$ | $CH_3O$ | S | *i*-$C_3H_7$ | ... | 1.5313 | 43.28 | 5.28 | 43.30 | 5.27 |
| V | $CH_3O$ | $CH_3O$ | O | H | 143–144 (0.2 mm.) | 1.5199 | | | | |
| VI | $CH_3O$ | $CH_3O$ | O | $CH_3$ | 146.5–148 (0.7 mm.) | 1.5168 | 41.39 | 4.63 | 41.10 | 4.75 |
| VII | $CH_3O$ | $CH_3O$ | O | *i*-$C_3H_7$ | 155 (0.3 mm.) | 1.5062 | 45.68 | 5.58 | 46.45 | 5.74 |
| VIII | $CH_3$ | $CH_3O$ | S | H | 51–52[a] | ... | | | | |
| IX | $CH_3$ | $CH_3O$ | S | $CH_3$ | 36–39[a] | ... | 41.38 | 4.63 | 42.13 | 4.84 |
| X | $CH_3$ | $CH_3O$ | O | H | 160–163 (0.3 mm.) | 1.5381 | | | | |
| XI | $CH_3$ | $CH_3O$ | O | $CH_3$ | 153–155 (0.3 mm.) | 1.5365 | | | | |
| XII | $CH_3$ | $CH_3$ | S | H | 142.5–144[a] | ... | | | | |
| XIII | $CH_3$ | $CH_3$ | S | $CH_3$ | 64.5–65[a] | ... | | | | |

[a] Melting point.

nitrophenol. They were purified by TLC by applying 40 to 50 mg. of the compound to a 750-micron layer of Adsorbosil 1 silica gel with 10% calcium sulfate binder and using benzene–ethyl acetate (9 to 1) as the developing solvent. The $R_f$ values for the phosphonates were 0.15–0.18. The products were eluted with absolute ethanol and removal of the solvent gave light yellow oils which were homogeneous according to thin layer and paper chromatography and infrared spectra.

Compounds in Table I for which elemental analysis is not given have been reported previously: I (*10*), V (*3*), VIII (*14*), X (*2*), XII (*27*), XIII (*17*).

Several attempts were made to synthesize 4-nitrophenyl and 3-methyl-4-nitrophenyl dimethylphosphinates by the condensation of the sodium salt of the phenol and dimethylphosphinic chloride. In every case only the free phenol was recovered although the reaction appeared to have taken place.

Methods for the determination of toxicity to houseflies have been described previously (*21*). Two strains of houseflies were examined, a susceptible strain $S_{NAIDM}$ and a phosphate resistant strain $R_{SC}$ developed in this laboratory by selection with Chlorthion (*19*, *20*). Toxicity to the German cockroach, *Blattella germanica*, was determined by application of the compound to the ventral surface between the hind legs. Mortality was estimated 24 hours after dosage. Mammalian toxicity was determined orally on 3- to 6-month old male Swiss white mice (Curd's Caviary, La Puente, Calif.). The compounds were dissolved in olive oil and administered to the mice by means of a 0.25-ml. syringe fitted with a 22-gage hypodermic needle. The tip of the needle was filed off and enclosed in a ball of solder to prevent injury to the mice. The volume of solvent was 0.25 ml. After dosage the mice were placed in cages and mortality determined after 3 days at 80° C. Five to 10 mice were used at each dosage level.

The techniques for the preparation of fly head cholinesterase and the determination of anticholinesterase activity ($I_{50}$ and $k_2$ values) have been described previously (*7*, *8*). $I_{50}$ values were determined after 20 minutes' incubation with the cholinesterase. Purified bovine erythrocyte acetylcholinesterase was obtained from the Sigma Chemical Co. Mouse brain cholinesterase was obtained by killing the animals with chloroform and immediately dissecting out the brain. The brains were washed with Warburg buffer and homogenized at 3 brains per 10 ml. of buffer. The crude homogenate was diluted with buffer to a final concentration of 1 brain per 10 ml. and used immediately.

Affinity constants ($K_i$) and phosphorylation rate constants ($k_p$) were determined for the cholinesterases and dimethyl 3-alkyl-4-nitrophenyl phosphates according to Main (*18*). Fly head or bovine erythrocyte cholinesterase solutions were prepared in pH 7.6, 4m*M* phosphate buffer. The enzyme concentration was adjusted until an initial rate of about 1.0 μmole of AChBr hydrolyzed per minute under the test conditions. Enzymic activity was followed by a pH-stat method with pH at 7.6, temperature at 37.5° C., and with $CO_2$-free technique throughout. The titrator (International Instruments, Canyon, Calif.) simultaneously delivered 0.1*N* NaOH and AChBr solutions into a 10-ml. cell. For each inhibition run, 1.5 ml. of the enzyme preparation was incubated with 0.5 ml. of the appropriate concentration of an aqueous solution of the inhibitor in a 10-ml. tube at 37.5° C. for intervals from 0.5 to 3.0 minutes. The incubate was then quickly added to 8.0 ml. of AChBr solution in the cell—final concentration of AChBr, 10m*M*—and the rate of enzymic hydrolysis calculated after correction for solvolysis of the substrate. For each of seven inhibitor concentrations within a given enzyme-inhibitor series, plots were made of log per cent activity against incubation time and invariably gave first-order results. Replotting of this data as described by Main (*18*) then gave the required constants (Figure 2).

First-order rate constants ($k'$) for hydrolysis of the phosphates in 0.067*M* Sorensen's phosphate buffer at pH 8.5 and 37.5° C. were determined according to previously described procedures (*8*). Phosphorothionate hydrolysis was determined similarly in 0.01 to 0.03*M* sodium hydroxide to give the second-order rate constant ($k_{OH}$).

*Results and Discussion*

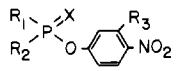
**Toxicity to Insects and the White Mouse.** The toxicity of methyl parathion, Sumithion, and their analogs to susceptible and resistant houseflies, the German cockroach, and the white mouse is given in Table II. Inhibition of fly head, bovine erythrocyte, and mouse brain cholinesterases by the phosphate and phosphonate esters is given, also. Anticholinesterase activities of the thionate esters are not given because of their inherently low activity.

There are several points of interest regarding the toxicity data which deserve comment. Generally, there is little variation in the toxicity of the compounds to susceptible houseflies and the cockroach. Sumithion (II) is 2 to 4 times less toxic than methyl parathion (I), and the toxicity decreases further as the size of the 3-alkyl group is increased to ethyl and isopropyl. Phosphate esters (V, VI) are slightly less toxic to the housefly than the corresponding phosphorothioate, but toxicity is reversed with the German cockroach. Methyl paraoxon (V) is considerably more toxic to resistant flies than methyl parathion (I), while Sumithion (II) is more toxic than Sumioxon (VI).

The levels of resistance of the Chlorthion-resistant strain ($R_{SC}$) against the phosphorothionate esters are generally very high with resistance ratios ($LD_{50}$ $R_{SC}$/$LD_{50}$ $S_{NAIDM}$) ranging from 30 to 90. In contrast, the analogous phosphonothionates (VIII and IX) are quite toxic to resistant flies, with resistance ratios between 5 and 7. Further, these compounds are somewhat more toxic to susceptible flies and cockroaches than their phosphorothionate counterparts. The phosphinate analogs of methyl parathion and Sumithion (XII and XIII) also are effective against resistant flies, with resistance ratios of 4 to 5, although they are two- to fivefold less toxic to susceptible flies.

The oral $LD_{50}$ data against the mouse clearly shows the extraordinary safety of Sumithion compared with

Table II. Toxicity and Anticholinesterase Activity of Methyl Parathion, Sumithion, and Their Analogs



| | $R_1$ | $R_2$ | X | $R_3$ | Topical $LD_{50}$, μg./G., *Musca domestica* Females $S_{NAIDM}$ | Topical $LD_{50}$, μg./G., *Musca domestica* Females $R_{SC}$ | *Blatella germanica* | White Mouse Oral $LD_{50}$, Mg./Kg. | Anticholinesterase Activity ($I_{50}$) ($M$) Fly head $S_{NAIDM}$ | Mouse brain | Bovine erythrocyte |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | $CH_3O$ | $CH_3O$ | S | H | 1.2 | 89 | 0.99 | 23 | ... | ... | ... |
| II | $CH_3O$ | $CH_3O$ | S | $CH_3$ | 3.1 | 126 | 4.2 | 1250 | ... | ... | ... |
| III | $CH_3O$ | $CH_3O$ | S | $C_2H_5$ | 4.5 | 131 | 6.1 | 2200 | ... | ... | ... |
| IV | $CH_3O$ | $CH_3O$ | S | $i$-$C_3H_7$ | 6.3 | 568 | 12.0 | 880 | ... | ... | ... |
| V | $CH_3O$ | $CH_3O$ | O | H | 2.5 | 25 | 0.55 | 21 | $1.0 \times 10^{-7}$ | $3.3 \times 10^{-7}$ | $1.7 \times 10^{-6}$ |
| VI | $CH_3O$ | $CH_3O$ | O | $CH_3$ | 4.3 | 141 | 1.5 | 120 | $5.6 \times 10^{-8}$ | $1.9 \times 10^{-6}$ | $7.8 \times 10^{-6}$ |
| VII | $CH_3O$ | $CH_3O$ | O | $i$-$C_3H_7$ | 6.5 | >500 | ... | >500 | $1.6 \times 10^{-8}$ | $1.2 \times 10^{-5}$ | $3.4 \times 10^{-5}$ |
| VIII | $CH_3$ | $CH_3O$ | S | H | 0.73 | 3.7 | 0.63 | 3.7 | ... | ... | ... |
| IX | $CH_3$ | $CH_3O$ | S | $CH_3$ | 1.9 | 13.0 | ... | 14.0 | ... | ... | ... |
| X | $CH_3$ | $CH_3O$ | O | H | 1.5 | 6.3 | ... | ... | $9.5 \times 10^{-8}$ | $9.5 \times 10^{-8}$ | ... |
| XI | $CH_3$ | $CH_3O$ | O | $CH_3$ | 2.2 | 50 | ... | ... | $3.8 \times 10^{-8}$ | $3.4 \times 10^{-7}$ | ... |
| XII | $CH_3$ | $CH_3$ | S | H | 6.4 | 29 | 1.2 | 51 | ... | ... | ... |
| XIII | $CH_3$ | $CH_3$ | S | $CH_3$ | 6.3 | 22 | ... | 370 | ... | ... | ... |

methyl parathion. Further, *O*,*O*-dimethyl *O*-(3-ethyl-4-nitrophenyl) phosphorothioate (III) is even less toxic to the mouse than Sumithion. The selectivity factor ($LD_{50}$ 3-alkyl analog/$LD_{50}$ methyl parathion) for Sumithion (II), ethyl (III), and isopropyl analogs (IV) are 54, 96, and 34, respectively. Methyl paraoxon and methyl parathion are about equal in toxicity to the mouse, while Sumioxon is greater than tenfold more toxic than Sumithion. The phosphonothionates (VIII and IX) showed high toxicity to the mouse, and insertion of a 3-methyl group had little effect in reducing toxicity as occurs with Sumithion. The selectivity factor between VIII and IX is 3.8. There was slightly greater selectivity between the methyl-substituted and unsubstituted phosphinothioate esters (XII and XIII) compared with the corresponding phosphonothioates, with a selectivity factor of 7.2.

**Anticholinesterase Activity.** Since the toxic action of thionate phosphorus esters occurs through their intermediate phosphate esters, the anticholinesterase activity of the latter compounds was determined, and the data are given in Table II as $I_{50}$ values for $S_{NAIDM}$ fly head, bovine erythrocyte, and mouse brain cholinesterases. Inhibition of $R_{SC}$ cholinesterase, although unreported here, was determined also and was almost identical with $S_{NAIDM}$ (Figure 1). The inhibition of bovine erythrocyte cholinesterase followed a pattern similar to that of mouse brain, but the bovine enzyme was always more resistant to inhibition.

One of the most important aspects of these findings is that with both phosphates and phosphonates, introduction of alkyl groups in the three-position renders the compound more inhibitory to fly head cholinesterase, with inhibition increasing in the order H < $CH_3$ < $i$-$C_3H_7$. Just the opposite effect is observed for the mammalian cholinesterases, with inhibition decreasing with 3-alkyl substitutions. The results are presented graphically in Figure 1 in which $pI_{50}$ is plotted against the number of carbon atoms in the 3-alkyl moiety. Determinations of $k_e$ for Sumioxon and methyl paraoxon showed an

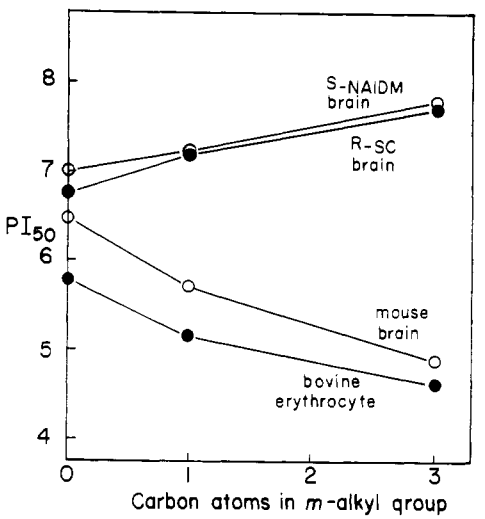



Figure 1. Inhibitory potency of *m*-alkyl-substituted dimethyl *p*-nitrophenyl phosphates against insect and mammalian cholinesterase

almost identical influence of the *m*-methyl group on the rates of inhibition of the different cholinesterases when compared with the $I_{50}$ values.

The inhibition of fly head and mammalian cholinesterase was examined in greater detail to arrive at a basis for the disparity in cholinesterase activities. In this case, $S_{NAIDM}$ and bovine erythrocyte ChE were used, the latter because of its more homogeneous nature compared with the crude mouse brain ChE. The kinetic analysis proposed by Main (*18*) was used to determine the equilibrium constant for reversible association between enzyme and inhibitor ($K_i$) and the rate constant for the subsequent phosphorylation reaction ($k_p$) as shown in the equation below:

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow{k_p} EI' + P$$

$$k_e$$

$K_i$ is given by $k_{-1}/k_1$, $E$ and $I$ are the enzyme and inhibitor, $EI$ is the association complex between enzyme and inhibitor, $EI'$ is the phosphorylated enzyme, and $k_e$ is Aldridge's bimolecular inhibition constant. The equivalent constant as calculated by Main's method is designated as $k_e'$ and is equal to $k_p/K_i$.

Figure 2 gives a typical plot showing the relationship between $1/[I]$ and $\Delta t/2.3\ \Delta \log v$ for Sumioxon and fly head ChE from which values for $K_i$, $k_p$, and $k_e'$ were calculated. The line of best fit for such plots did not pass through the origin but gave significant intercepts and thus significant values for $K_i$ and $k_p$. Table III gives these values along with $k_e$ values determined by Aldridge's method for comparison.

Several interesting conclusions arise from these results. Comparison of the $k_p$ values with rate constants for hydrolysis of the same phosphates in pH 8.5 buffer (Table IV) shows that the phosphorylating ability of these compounds falls off, with increase in size of the *m*-alkyl group similarly in both reactions. This relationship is shown clearly in Figure 3, and gives excellent confirmation that phosphorylation of the enzyme depends on the same factors which also determined purely chemical hydrolytic rates, a conclusion which has been

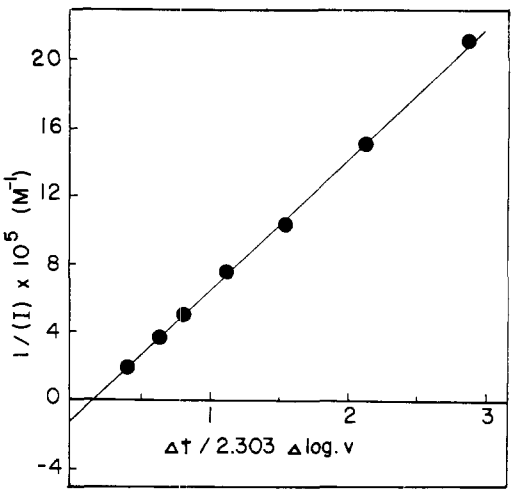



Figure 2. Plot of relationship between $1/[I]$ and $\Delta t/2.303\Delta$ log $v$ for Sumioxon and fly head cholinesterase to obtain $K_a$, $k_p$, and $k_e'$

**Table III. Bimolecular, Phosphorylation, and Affinity Constants for the Inhibition of Fly Brain and Bovine Erythrocyte AChE by Various *m*-Alkyl-Substituted Dimethyl *p*-Nitrophenyl Phosphates**

General Structure: $(CH_3O)_2P(O)O$-(*m*-R)-$C_6H_3$-*p*-$NO_2$

| R | $k_e' \times 10^{-5}$, $M^{-1}$ Min.$^{-1}$ | $K_i \times 10^5$, $M$ | $k_p$, Min.$^{-1}$ | $k_e \times 10^{-5}$,[a] $M^{-1}$ Min.$^{-1}$ |
|---|---|---|---|---|
| | | S$_{NAIDM}$ Brain | | |
| H | 2.9 | 3.7 | 10.6 | 2.6 |
| $CH_3$ | 7.6 | 1.1 | 8.3 | 7.9 |
| *i*-$C_3H_7$ | 22.6 | 0.33 | 7.5 | 21.6[b] |
| | | Bovine Erythrocyte | | |
| H | 5.2 | 1.3 | 6.6 | ... |
| $CH_3$ | 0.73 | 6.7 | 5.0 | .. |
| *i*-$C_3H_7$ | 0.22 | 15.8 | 3.5 | ... |

[a] $k_e$ as determined by Aldridge's method (*18*).
[b] Calculated from $I_{50}$.

**Table IV. Hydrolysis Rate Constants for Various *m*-Alkyl-Substituted Dimethyl *p*-Nitrophenyl Phosphates and Phosphorothionates**

General Structure: $(CH_3O)_2P(X)O$-(*m*-R)-$C_6H_3$-*p*-$NO_2$

| R | X=O $k' \times 10^{-4}$, Min.$^{-1}$ | X=S $k_{OH}$, $M^{-1}$ Min.$^{-1}$ | $\Sigma\sigma$[a] | $\sigma_{obsd.}$[b] |
|---|---|---|---|---|
| H | 5.25 | 0.606 | 1.27 | 1.27 |
| $CH_3$ | 3.50 | 0.444 | 1.20 | 1.17 |
| $C_2H_5$ | ... | 0.436 | 1.20 | 1.15 |
| *i*-$C_3H_7$ | 2.89 | 0.352 | 1.20 | 1.08 |

[a] $\Sigma\sigma$ = Sum of Hammett $\sigma$ values for the ring substituents; *p*-$NO_2$ = 1.27, *m*-alkyl = −0.07 [Charton (*1*)].
[b] $\sigma_{obsd}$ = Observed $\sigma$ value [$\rho$ = 1.3 (*7*)].

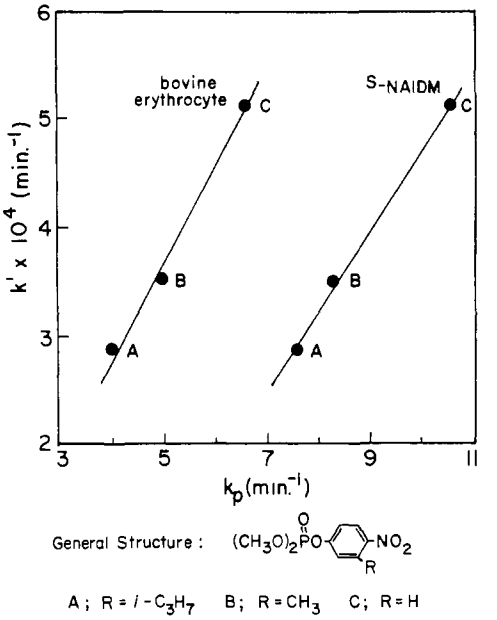



Figure 3. Relationship between rate of hydrolysis ($k'$) and rate of phosphorylation ($k_p$) for cholinesterase in *m*-alkyl substituted dimethyl *p*-nitrophenyl phosphates

suggested frequently before but, until now, not shown directly.

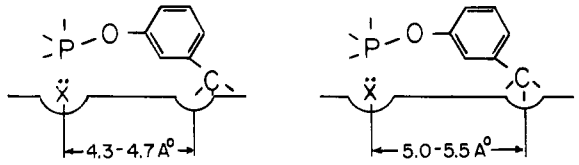
The effect of the *m*-alkyl groups in decreasing reactivity toward such nucleophiles can be attributed partly to the inductive effect of these groups and partly to their interference with the mesomerism of the nitro group with the ring. This latter effect would be expected to depend on the bulkiness of the *m*-alkyl group and such is the case, as the data in Table IV show. The difference between $\Sigma\sigma$ and $\sigma_{obsd}$ presumably is a measure of the steric interaction with the nitro group, and this difference increases with size of the *m*-alkyl moiety.

A second conclusion from the $k_p$ values is that there is little difference between the two enzymes in the rate of phosphorylation or in the response of this parameter to changes in the *m*-alkyl group. The very large differences in inhibitory potency of these compounds against the two types of enzyme must be a result of differences in steric and binding effects. Such a conclusion is confirmed by the data for $K_i$. Since this constant measures the tendency for the enzyme-inhibitor complex to dissociate, the lower the $K_i$, the more effective the binding.

In the housefly enzyme, increasing the size of the *m*-alkyl substituent greatly increases the stability of the enzyme-inhibitor complex—i.e., the change from H to *m*-$CH_3$ increases stability 3.4-fold and the change from H to *m*-isopropyl increases it 11-fold. The $k_e'$ value is, however, not raised proportionally owing to the concomitant decrease in phosphorylation rate. Thus, the $k_e'$ values increase by only 2.7-fold and 7.9-fold, respectively.

This situation is reversed with the bovine enzyme since the change from hydrogen to methyl gives a 5.4-fold decrease in stability of the complex and from hydrogen to isopropyl a 13-fold decrease. These differences are accentuated by the influence of the *m*-alkyl groups on phosphorylation rates, and thus the $k_e'$ values are decreased 7.2 times for Sumioxon and 25 times for the *m*-isopropyl analog.

These data show that a significant difference must exist between the structures of insect and mammalian enzymes and these differences may explain in part the selectivity of Sumithion. The difference in structure may be due to either difference in shape and size of the anionic site, or difference in distances between the anionic site and the vital center in the esteratic site. There is considerable evidence (*6*, *16*, *22*, *24*, *29*) suggesting that the distance between the anionic and esteratic sites differs between insect and mammalian cholinesterase. On the basis of this evidence, the distance between anionic site and the electron donor in the esteratic site in bovine erythrocyte ChE lies in the region of 4.3 to 4.7 A., and in the housefly enzyme, this distance is probably 5.0–5.5 A. The interatomic distance between the phosphorus atom and the carbon atom attached to the ring in the three-position from molecular models shows a value of about 5.2–6.5 A. depending upon the rotation of the bonds involved. From this, the 3-alkyl group should fit well on the anionic site of the fly enzyme, but will fit poorly on




Bovine erythrocyte ChE  Housefly ChE

bovine enzyme, even at a minimum P-C distance, as illustrated above.

On this basis, initial binding with fly head ChE as estimated by $K_i$ values will be aided by 3-alkyl groups of appropriate dimensions owing to van der Waals or hydrophobic interaction of the 3-alkyl group with anionic site. In the same light, 3-alkyl groups in the ring will interfere with bovine ChE–phosphate binding and this interference will be accentuated with increasing size of the alkyl moiety.

**Cholinesterase Inhibition and Toxicity.** Examination of the insect toxicity and anticholinesterase data in Table II for the phosphate and phosphorothionate esters (I through VII) shows that the toxicity of both P=O and P=S compounds is not consistent with the relative anticholinesterase activity of the P=O esters. For example, methyl parathion (I) is five times more toxic to $S_{NAIDM}$ houseflies than the corresponding 3-isopropyl analog (IV), yet methyl paraoxon (V) is approximately one sixth as inhibitory against $S_{NAIDM}$ fly head ChE as dimethyl 3-isopropyl-4-nitrophenyl phosphate (VII). The same relationship holds true for the phosphonothioate and phosphonate esters (VIII through XI). The lack of correlation between anticholinesterase activity of the P=O esters and insect toxicity of the corresponding P=S esters and also the P=O esters themselves is not surprising in view of the many variables involved in intoxication—e.g., penetration, activation of P=S to P=O, translocation to site of action, detoxication, and enzyme inhibition. The fact that insect toxicity decreases and anticholinesterase activity increases with alkyl substitution suggests that the change in toxicity is influenced by variation in rates involving the first four factors. The effect of these variables on the toxicity of Sumithion and related phosphorothionates to the mouse is even more pronounced (*12*). Upon analysis of mouse toxicity, although methyl parathion (I) and methyl paraoxon (V) are about equitoxic to the mouse, Sumioxon (VI) is considerably more toxic than Sumithion (II). Thus, according to Table II, the selectivity factor between methyl parathion and Sumithion is 54, while that for the corresponding phosphates is only 5.7. Significantly, Sumioxon is 5.7 times less effective than methyl paraoxon as an inhibitor of mouse brain ChE. Although there is a probability of oversimplification, that the selectivity between the phosphate esters is due solely to their different potencies against mouse brain ChE may be suggested. The difference in anticholinesterase activity must play a considerable part in this selectivity and also in that between the phosphorothionates. However, in view of the very high level of selectivity between methyl parathion and Sumithion, other differences in behavior must exist between these compounds.

In the complex series of events which control toxicity, there are many factors which may explain why Sumithion is 54 times less toxic than methyl parathion and not 5 times less, as would be predicted from anticholinesterase results. These other factors which may contribute to the amazing selectivity of Sumithion have been considered (*12*).

Turning to the phosphonothioate esters, the Sumithion analog (IX) is 3.8 times less toxic to the mouse than the methyl parathion analog (VIII). The relative toxicities in this case are consistent with the approximately fourfold lower anticholinesterase activity of the Sumioxon analog (XI) compared to the methyl paraoxon analog (X). The relatively high toxicity of both these compounds to mice may be due to suppression of one of the detoxication mechanisms which plays an important role in the selective action of Sumithion, namely removal of one of the methoxy methyl groups (*12*).

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