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# Phosphonofluoridate Inhibition of Acetylcholinesterase: an Exceptionally Rapid Reactant

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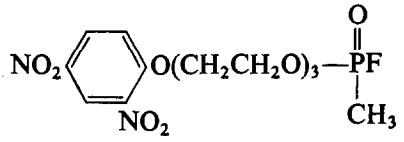
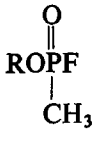
Both isomers of [3,6-dioxa-(8-(2,4-dinitrophenoxy))octyl] methylphosphonofluoridate (I) react rapidly with eel acetylcholinesterase. At pH 6.61 and 25°C, the reaction rate constants for the fast and slow isomers are $3 \times 10^9$ and $5 \times 10^7$ $M^{-1}$ $min^{-1}$. Despite the very high rate of reaction with acetylcholinesterase, which for the fast isomer is approximately 40 times that of sarin, the toxicity of I is only $\frac{1}{8}$ that of sarin. Enzyme inhibited by I is resistant to reactivation by TMB-4.

## INTRODUCTION

To prepare antibodies that may be specific for acetylcholinesterase (AChE)[1] (acetylcholine acetylhydrolase, EC 3.1.1.7), a reagent was desired which would attach the haptenic grouping, dinitrophenyl, to the active site of the enzyme. Several factors were important. The reagent must react rapidly to provide selectivity and specificity for the active site. The dinitrophenyl group should not be positioned too closely so as to be masked by the protein. Reactivatability of the inhibited enzyme, although not essential, would be highly desirable. To this end, compound I was designed and prepared. This paper describes its preparation and the kinetics of its reaction with eel AChE.



I



II

As a class, the alkyl methylphosphonofluoridates (II) are rapid, highly specific reactants for AChE (*1–3*). They display considerable enantiomeric kinetic specificity, particularly at the phosphorus atom, so that only one of the isomers is generally considered to be reactive (*2–4*). In prior reports, the longest chain length of the group R,

[1] Abbreviations: sarin, isopropyl methylphosphonofluoridate; TMB-4, 1,1′-trimethylenebis(4-formylpyridinium bromide oxime); AChE, acetylcholinesterase; bicine, *N,N*-bis(2-hydroxyethyl)-glycine; MES, 2-(*N*-morpholino)ethane sulfonic acid.

compound II, is 7 atoms. However, as Kabachnik and co-workers (*5*) have pointed out, the active site is surrounded by hydrophobic areas. It was our hope that the hydrophobic areas would extend sufficiently to accommodate the very long chain in compound I. If this were so, binding to enzyme would be enhanced, and we might expect particularly high reactivity in the active enantiomer, and also considerable activity in the slow or "unreactive" one. This, in fact, turned out to be the case.

The rate of reaction of AChE with phosphonofluoridates (II) varies with reaction conditions. Therefore, isopropyl methylphosphonofluoridate (sarin) was used as a reference standard for the kinetic measurements. It also served as a titrant for determination of the active site molarity of the AChE preparation.

## EXPERIMENTAL METHODS

Sarin was obtained locally. Its purity was reported to be in excess of 95%. Except where otherwise indicated, all chemicals were obtained commercially and were of CP grade. AChE was Worthington electric eel, purified, code: ECHP.

### *Preparation of [3,6-dioxa-(8-(2,4-dinitrophenoxy))octyl] methylphosphonofluoridate, I*

*a. 3,6-Dioxa-[8-(2,4-dinitrophenoxy)] octan-1-ol, III.* To gently warmed triethylene glycol (150 g, 1.0 mole) under nitrogen there was slowly added 2.7 g (0.12 mole) of sodium. Final solution temperature, 96°C. The solution was cooled to 65°C, 20.3 g (0.1 mole) of 1-chloro-2,4-dinitrobenzene were added and the mixture was stirred and heated for 0.5 hr at 167°C. Upon cooling, there was added 300 ml of aqueous sodium carbonate (10.6 g) and the reaction mixture was extracted with three 200 ml portions of chloroform. The chloroform extract was washed progressively with 200 ml portions of water (2×) 2 *M* hydrochloric acid (2×) and water, dried over Drierite, decolorized with 2 g Darco K-B activated charcoal and stripped under vacuum. The residue which was freed of solvent traces by pumping under an efficient oil pump, gave 27.0 g of crude product. This was dissolved in 200 ml benzene, washed progressively with 100 ml portions of aqueous sodium carbonate (10.6 g), water (2×), 2 *M* hydrochloric acid and water (3×). The benzene layer was dried over sodium sulfate, treated with 2 g Darco K-B, filtered and the solvent was distilled. The residue was dried on a high vacuum oil pump over Drierite for 18 hr. There was obtained 11.6 g of viscous pale yellow-amber syrup.

*Anal.* Calcd for $C_{12}H_{16}N_2O_8$: C, 45.57; H, 5.10; N, 8.84; O, 40.47. Found: C, 45.4; H, 5.7; N, 8.5; O, 39.7.

*b. Compound I.* To a gently refluxing solution of 10 ml methylene chloride, 2.1 g (0.016 mole) methylphosphonodichloridate and 1.6 g (0.016 mole) methylphosphonodifluoridate contained in a round bottomed flask fitted with Teflon covered stirring bar, vertical condenser and Drierite tube, there was added a solution of compound III (5.0 g, 0.0158 mole) in 5 ml methylene chloride followed by a rinse of 10 ml methylene chloride. After reflux for 1 hr, the solution was cooled in ice and 35 ml of ice water were added slowly. The organic layer was extracted twice with 25 ml ice water, dried over sodium sulfate, filtered and the solvent was distilled. The residue was dried overnight over

Drierite under an efficient oil pump. There was obtained 5.8 g of I, as a pale amber viscous syrup.

*Anal.* Calcd for $C_{13}H_{18}FN_2O_9P$: C, 39.40; H, 4.57; F, 4.79; N, 7.07; P, 7.82. Found: C, 39.4; H, 4.7; F, 4.5; N, 6.9, P, 7.8; Cl, 0.0.

*Phosphonofluoridate purity.* Fluoride was determined potentiometrically (*6*) using a Beckman Research Model pH meter, Orion fluoride electrode (Model 94-09) with calomel reference. Measurements were made at 25°C (pH 8.02) $8 \times 10^{-3}$ *M* bicine. Standardization with three concentrations of potassium fluoride over the range $3 \times 10^{-4}$ to $1.3 \times 10^{-3}$ *M* gave a good straight-line relationship on linear log paper corresponding to the equation, $E = E_0 + 59\log[F^-]$. Equilibration of the electrodes with the bath solution often took several min. Equilibrium was assumed when three sequential 30 sec readings were identical.

In phosphonofluoridate determination, an appropriate sample was prepared in acetonitrile, a small aliquot was added to 50 ml distilled water and an "initial" fluoride measurement was made. For hydrolysis of the phosphonofluoridate, one pellet of solid sodium hydroxide was added to the solution and it was allowed to stand at room temperature for 10 min. There was added 2 ml of 0.2 *M* bicine (pH 8.02) and 2 ml of 4 *N* hydrochloric acid to reduce pH to near neutrality and the "final" fluoride ion concentration was measured. Sample concentration was computed from the difference between the final and initial fluoride values. Purity determinations for each compound were made in duplicate.

*Titration of AChE.*[2] A standard concentration of enzyme ($E_w$) was prepared by dissolving 10,000 units of the Worthington product in 1.6 ml of 0.225 *M* potassium chloride (pH 7) containing 0.02% sodium azide (to prevent growth of foreign organisms) and $\frac{1}{4}$% gelatin (KCl/gel). $E_w$ was appropriately diluted as required with KCl/gel. The various enzyme solutions were stored at 5°C and appeared to be stable indefinitely. Reactions and assays were all performed at 25°C, in a standard medium, 0.1 *M* MES, pH 6.734. Assay of enzymatic activity was measured by the rate of hydrolysis of phenyl acetate, $8.23 \times 10^{-3}$ *M* in MES, spectrophotometrically at 270.0 or 272.5 nm, 25°C, using a Cary Model 14 recording spectrophotometer fitted with a thermostated cell holder. For reaction, 25 $\mu$l of $E_w/5$ ($E_w$ diluted 1:5 with KCl/gel) was mixed with 500 $\mu$l MES. To 150 $\mu$l of this solution there was added 200 $\mu$l of phosphonofluoridate, approximately $1.2 \times 10^{-7}$ *M* (serially diluted first in acetonitrile, then twice in distilled water) and reaction was allowed to proceed for 30–60 min. With sarin, assuming reaction with one enantiomer, $E_w = 1.8 \pm 0.05 \times 10^{-5}$ *M*; with I, assuming reaction with *both* enantiomers, $E_w = 2.06 \times 10^{-5}$ *M*.

### *Rate of Reaction with AChE*

#### *a. Method A. Inhibitor in Excess; First Order Kinetics*

*1. Sarin.* To a thermally equilibrated cuvette mounted in the spectrophotometer containing 2.0 ml of $1.04 \times 10^{-3}$ *M* phenyl acetate in MES, there was added a precisely measured volume (25–75 $\mu$l) of $E_w/5000$. In a control run, the record of absorbance increase at 270.0 nm was linear over the full recorder range (0.1 absorbance units). For

[2] This procedure is based upon an unreported method of H. O. Michel. A similar procedure has been reported recently by Schoene (*7*) using soman.

rate measurement, there was added approximately 0.5 min after the enzyme sample (long enough to obtain a measure of initial velocity) 50 $\mu$l of $1.28 \times 10^{-6}$ *M* sarin. A typical record is shown in Fig. 1.

2. *Compound I.* Reaction rate was measured in a similar fashion using 50 $\mu$l $E_w/5000$, $1.125 \times 10^{-3}$ phenyl acetate and $4.6 \times 10^{-8}$ *M* I, 25–55 $\mu$l.

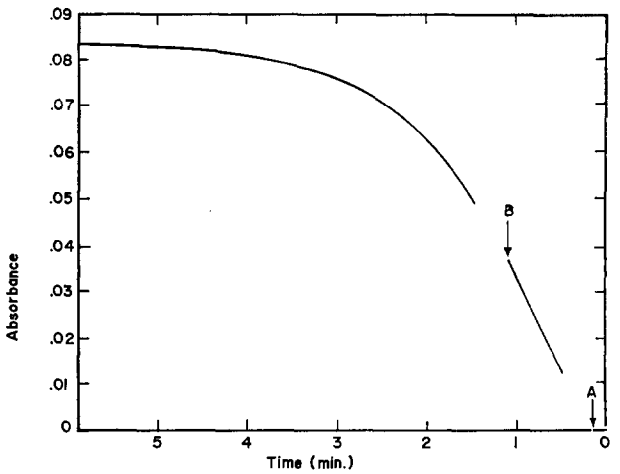



FIG. 1. Progress Curve. Method A. Reaction between AChE ($1.4 \times 10^{-10}$ *M*) and sarin (active isomer, $1.50 \times 10^{-8}$ *M*) (pH 6.734) in the presence of phenyl acetate, $1.04 \times 10^{-3}$ *M*. $\lambda = 270.0$ nm. (A) enzyme added. (B) sarin added. The blank areas after each addition represent mixing time.

3. *Calculations.* These were performed by a modification of the procedure of Main and Dauterman (*8*), Eq. (1).

$$k_i = \frac{1}{(t_2 - t_1)(1 - \alpha)\mathrm{P}} \ln \frac{v_1}{v_2}, \tag{1}$$

where $k_i$ is the second-order reaction rate constant, P = concentration of the phosphonylating compound, $\alpha = \mathrm{S}/(K_m + \mathrm{S})$, where S and $K_m$ are, respectively, the substrate concentration and its $K_m$, and $v_1$ and $v_2$ are, respectively, the slopes of the progress curve at times $t_1$ and $t_2$. Setting $t_1 = 0$ and $v_2 = v_t$, we can rearrange Eq. (1) to

$$-\ln v_t = k_i(1 - \alpha)\mathrm{P}t - \ln v_0. \tag{2}$$

A plot of $(-\ln v_t)$ vs $t$ should be linear with slope $= [k_i(1 - \alpha)\mathrm{P}]$ and intercept $(-\ln v_0)$. $v_t$ was estimated from the relationship:

$$v_t = (\mathrm{Abs}_{t+\beta} - \mathrm{Abs}_{t-\beta})/\Delta t, \tag{3}$$

where $\mathrm{Abs}_{t+\beta}$ and $\mathrm{Abs}_{t-\beta}$ represent the absorbance at times $t + \beta$ and $t - \beta$, respectively, and $\Delta t = (t + \beta) - (t - \beta) = 2\beta$. Equation (3) is without error only if the curve represents a perfect circle, or when the value of $\beta$ approaches zero. The choice of the value of $\beta$ is made by trial and error and is usually quite simple. When $\beta$ is too small, the plot of Eq. (2) contains too much scatter. As $\beta$ is increased progressively, the plots become

satisfactory and the values of slope and intercept remain substantially constant. Finally, as $\beta$ becomes large, the values of slope and intercept begin to change significantly. In this work, the value of $\beta$ was maintained at the lowest practical level. Typical plots are given in Fig. 2.

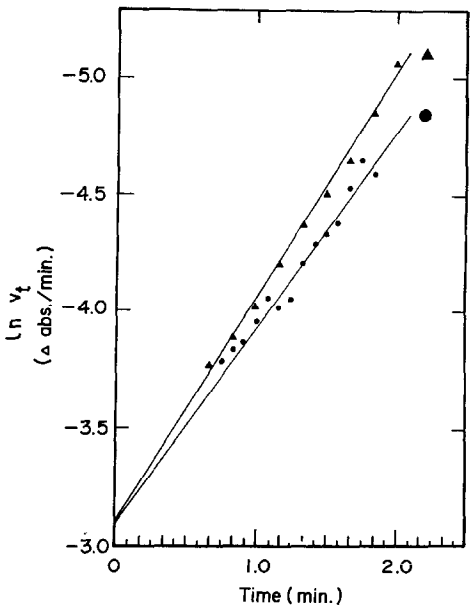



FIG. 2. Method A. (▲) Plot of data from Fig. 1 according to Eq. (2); $\Delta t = 2\beta = \frac{1}{3}$ min. (●) Reaction between AChE ($9 \times 10^{-11}$ *M*) and I ($1.2 \times 10^{-9}$ *M*) (pH 6.734) in presence of phenyl acetate ($1.25 \times 10^{-3}$ *M*); $\Delta t = \frac{1}{8}$ min.

*b. Enzyme in Excess; Second Order Kinetics, Compound I.*

*1. Method B; substrate present. Fast isomer.* Reaction conditions and record of the progress curve were the same as described above. The substrate was phenyl *i*-butyrate, $1.27 \times 10^{-3}$ *M*; reaction progress was followed at 270.0 nm. AChE concentration was varied over the range $2\text{–}4 \times 10^{-10}$ *M*. The concentration of I was adjusted to 120–180% that of AChE, so that reaction of the fast isomer could be followed to its completion (60–90% inhibition).

*2. Method C; substrate absent. Fast isomer.* For reaction, there was added 25 $\mu$l, $1.7 \times 10^{-8}$ *M* compound I to 40 ml, $1.47 \times 10^{-11}$ *M* enzyme, both solutions previously equilibrated and the mixture maintained at 25°C. Zero readings were obtained prior to addition of I. For assay, 5 ml aliquots of reaction solution were mixed with 5 ml phenyl acetate, $6.7 \times 10^{-3}$ *M* in MES, and the rate of hydrolysis measured at 25°C, 270.0 nm, Zeiss spectrophotometer PMQ II fitted with thermostated cell compartment, 5 cm cells.

*Slow isomer.* For the spectrophotometric assay 50 $\mu$l aliquots of reaction mixture were added to 2 ml of phenyl acetate, $8.0 \times 10^{-3}$ *M* in MES and the hydrolysis rate measured on the Cary 14. For reaction, there was added to 0.975 ml of $3.50 \times 10^{-9}$ *M* enzyme in

MES approximately 0.7 equivalents of I. Under these conditions it is estimated that 95% of the fast isomer will react within 0.5 min, see Fig. 3.

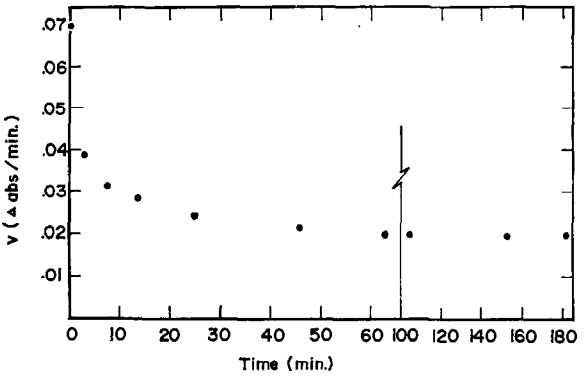



FIG. 3. Method C. Reaction of I with excess enzyme; $[E_0]$, $2.19 \times 10^{-9}$ *M*.

*3. Calculations.* Calculations were performed using the conventional second-order rate relationship (*9*) for the reaction $E + P \xrightarrow{k_i}$ products, Eq. (4).

$$k_i t = \frac{1}{E_0 - P_0} \ln \frac{P_0 E_t}{E_0 P_t}, \tag{4}$$

where E and P represent concentration of enzyme and phosphonylating compound, respectively, and the subscripts 0 and $t$ represent times zero and $t$. Enzyme concentration, $E_t$, is proportional to $v_t$, the slope of the progress curve at time $t$; $v_t = AE_t$.

$$P_0 = E_0 - E_\infty = (v_0 - v_\infty)/A, \tag{5}$$

$$P_t = P_0 \left( \frac{v_t - v_\infty}{v_0 - v_\infty} \right) = (v_t - v_\infty)/A. \tag{6}$$

Substitution of Eqs. (5) and (6) into Eq. (4) and rearrangement of terms yields:

$$\ln \frac{v_t(v_0 - v_\infty)}{v_0(v_t - v_\infty)} = \frac{k_i v_\infty E_0}{v_0} t. \tag{7}$$

The plot of $\ln\{[v_t(v_0 - v_\infty)]/[v_0(v_t - v_\infty)\}$ vs $t$ is linear with slope $= k_i \times v_\infty \times E_0/v_0$, so that $k_i = (\text{slope} \times v_0)/(v_\infty \times E_0)$, see Figs. 4 and 5.

*Attempted reactivation of I.* Samples of AChE inhibited, respectively, by I and sarin were prepared as follows: To 500 $\mu$l water there was added 5 $\mu$l carbonate–bicarbonate (0.05 *M*, pH 10), 20 $\mu$l $E_w/50$ and finally 50 $\mu$l of phosphonylating compound ($4–6 \times 10^{-6}$ *M*). After 30 min incubation it was established that all enzymatic activity had disappeared and that no free phosphonylating compound remained (addition of aliquot to fresh enzyme substrate mix). Assays were performed titrimetrically at 25°C using a Radiometer TTT1 fitted with jacketed reaction vessel and SBR2C recorder. To 3 ml KCl (0.225 *M*) plus 100 $\mu$l acetylcholine (0.1359 *M*), at pH 7.0, there was added a 10 $\mu$l aliquot of test solution. Full scale (100 units) = 0.25 ml of barium hydroxide, 0.00545 *N*.

In the absence of enzyme, blank hydrolysis rate was 0.5 units/min. Addition of 10 $\mu$l $E_w/1000$ (approx $2 \times 10^{-8}$ $M$) gave hydrolysis rate = 9 units/min.

a. To the assay solution containing 10 $\mu$l sarin-inhibited AChE, there was added 100 $\mu$l, $10^{-3}$ $M$ TMB-4. Slope of the hydrolysis curve increased progressively from the blank value (0.5 units/min) to approximately 2.5 units/min in 4 min. Addition of 500 $\mu$l of TMB-4 gave complete reactivation to 3 units/min. Final concentration of TMB-4,

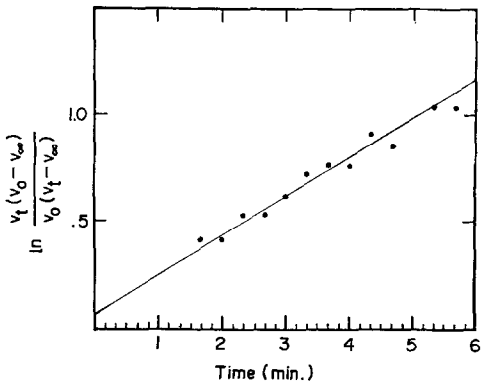



FIG. 4. Method B. Plot of data according to Eq. (7). $E_0$, $2.24 \times 10^{-10}$ $M$; [I] (fast isomer), $1.4 \times 10^{-10}$ $M$; phenyl $i$-butyrate, $1.39 \times 10^{-3}$ $M$; $v_0 = 7.98 \times 10^{-3}$ and $v_\infty = 2.69 \times 10^{-3}$ $\Delta$Abs/min; $\Delta t = \frac{4}{3}$ min.

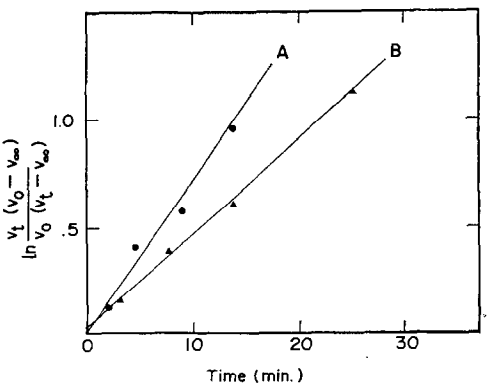



FIG. 5. Method C. Compound I. Plot of data according to Eq. (7). Fast isomer, (A) $E_0$, $1.47 \times 10^{-11}$ $M$, $v_0 = 25.34 \times 10^{-3}$ and $v_\infty = 16.2 \times 10^{-3}$ $\Delta$ Abs/min. Slow isomer (B) data from Fig. 3.

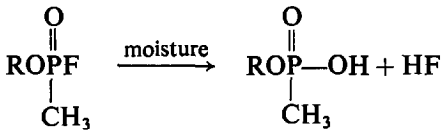
$1.7 \times 10^{-4}$ $M$. At this concentration, TMB-4 is known to inhibit AChE reversibly to a considerable extent. In the corresponding treatment of AChE inhibited by I, there was no observable increase in hydrolysis rate over a period of 21 min.

b. To 500 $\mu$l of each of the pH 10 inhibition solutions, there was added 50 $\mu$l, 1 $M$ TMB-4 (final concentration 0.08 $M$). After 10 min, assay of 10 $\mu$l gave 3.7 units/min for the sarin-inactivated enzyme, but only the blank value for enzyme inhibition by I.

It is estimated that a clearly discernible difference in slope = 0.05 units/min.

## RESULTS AND DISCUSSION

The samples of sarin and I were pure when orignally prepared. On storage, phosphonofluoridates decompose to enzymatically unreactive products[3] [Eqs. (8) and (9)] (*10*). Decomposition is autocatalytic, its rate depending upon structure and also upon

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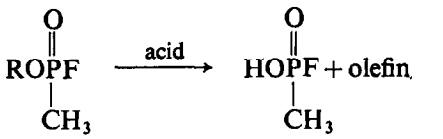


(9)

initial purity and conditions of storage. At the time of the kinetic study the purity of I had fallen to 73.5%, that of sarin remained in excess of 98%.

The stoichiometry of the titration of AChE solution $E_w$ by the two phosphonofluoridates, 1.8 and $2.06 \times 10^{-5}$ *M*, respectively, are identical within the error of the measurement procedure. The observation that I reacts with twice as much AChE as does sarin confirms the earlier reports that only one isomer of sarin is a fast reactant and also indicates that with I, both isomers react rapidly. In further confirmation, stoichiometry of the reaction between AChE and I, computed from reaction end points, method B, in each run was consistent with the above values.

The method of Main and Dauterman requires suitable adjustment of the concentrations of enzyme, phosphonylating agent, and to a lesser extent substrate in order to obtain the value of $v_0$ and the record of the full progress curve over the absorbance range of the recorder. To maintain first order kinetic conditions, the phosphonylating compound should be present in considerable excess.[4] With too little enzyme one can achieve a full record, but the curve is compressed. With too much enzyme, recorder runs off scale before much of the inhibition has occurred.

Although reaction between AChE and sarin or I in excess is extremely rapid, conditions could be found to satisfy these requirements. Kinetics of the reaction of I with AChE was also examined under conditions of excess enzyme, which gives more scatter, but permits the establishment of stoichiometry. For second-order reaction under these conditions, phenyl acetate was hydrolyzed too rapidly and it was replaced by the slower reacting substrate phenyl *i*-butyryate.[5]

The kinetic results are presented in Table 1. The reaction rate for sarin is in reasonable accord with that reported by Michel (*13*) for eel AChE and by Boter and Van Dijk (*2*)

[3] Fluoride is an inhibitor of AChE (*11*), but not at the very low concentrations used for phosphonylation rate studies.

[4] Corbett (*12*) has recently suggested a modified calculation which permits the use of as little as a two fold excess.

[5] Under the conditions of this study, for phenyl acetate, $k_m = 1.3 \times 10^{-3}$ *M*, $k_{cat} = 8.1 \times 10^5$ min$^{-1}$; phenyl *i*-butyrate, $K_m = 7.0 \times 10^{-3}$ *M*, $k_{cat} = 1.7 \times 10^5$ min$^{-1}$. The value of $\Delta\varepsilon$ at 270.0 nm for both substrates is 1340 (G. M. Steinberg and E. G. Letscher, unpublished data).

TABLE 1

REACTION RATES WITH AChE

| Compound | Method | Rate constant, $k_i \times 10^{-7}$ ($M^{-1}$ min$^{-1}$) | |
|---|---|---|---|
| | | Fast isomer | Slow isomer |
| Sarin | A | 8.2 | — |
| I | A | 260 | — |
| I | B | 330, 259, 268, 348, 274, 360 | — |
| I | C | 755 | — |
| I | C | — | 4.7 |

with bovine RBC AChE. The rate of reaction of Compound I is approximately 40 times greater than that of sarin, and it is, to our knowledge, the most rapidly reacting organophosphonate reported. The three methods for determination of the reaction rate constant for I—progress curve in the presence of substrate with excess phosphonate, progress curve in the presence of substrate with excess enzyme, and reaction in the absence of substrate with periodic assay—are in general agreement. The latter value is somewhat higher than those calculated from the substrate present data. Similar differences have been reported (*14*). These were ascribed, quite reasonably, to extra interactions between the phosphonylating compound and enzyme or enzyme–substrate complexes. Important to this work is its demonstration that the very rapid reaction of I is not an artifact due to some unexpected substrate connected phenomenon.

It is also quite striking that the slow isomer of I reacts nearly as rapidly as the fast isomer of sarin. Also, the rate ratio of the two isomers of I lies in the range 60–80, whereas with other phosphonofluoridates the values are much higher (*2*, *3*). With an analogous (to sarin) enantiomeric pair of phosphonothiolates, DeJong and Van Dijk (*4*) found that the fast/slow rate ratio of 3000 was due principally to differences in binding, reaction (first-order) rate ratio being only 6. We suggest a similar situation here. With small alkyl groups (R), as in sarin, binding to the near active site is principally dictated by the fit at the phosphorus atom, where the (–) isomer is more favorably accepted. With the additional binding at the far active site resulting from the long hydrophobic chain in I, fit at the near active site becomes relatively less important. If, as observed with the phosphonothiolates, reaction rate itself is relatively independent of conformation, we might expect that the isomer rate ratio would decrease with increasing chain length—as is observed here.

Despite its high rate of reaction with AChE, I is appreciably less toxic than sarin.[6] We may speculate either that AChE does not play the crucial role in sarin lethality, as is generally believed, or alternatively that I fails in large part to reach the critical stores of AChE. We favor the latter hypothesis. I is a viscous liquid of low water solubility. It may well be retained in the circulating blood (as microdroplets, fat or protein bound,

[6] I: $LD_{50}$ mouse (IV), 0.9 mg/kg; J. Lennox, private communication. Sarin: $LD_{50}$ mouse (IV), 0.1 mg/kg (*16*).

etc.) where it can be detoxified instead of being discharged rapidly into the vital organs.

The fast isomer of compound I is quite resistant to reactivation by the oxime, TMB-4. In this study no evidence of reactivation was observed.[7] This result is consistent with the observation of Agabekova, Rozengart and Sitkevitch (*15*) that increase in bulk in group R of the phosphonylating inhibitor, II, leads to resistance to reactivation.

*ADDENDUM. Significance in prophylaxis and therapy of G type anticholinesterase poisons.* From the viewpoint of defense against G type poisons, the phosphonofluoridate anticholinesterases, this work emphasizes three important factors relating chemical structure to properties which are physiologically important:

1. By extension and increasing the bulk of the alkoxy chain on a G-type compound, II, one can appreciably increase the rate of reaction with acetylcholinesterase.

2. The highest rate of reaction with acetylcholinesterase does not necessarily result in the greatest toxicity. This may reflect differences in the mechanism for toxicity or alternatively differences in rate or extent of distribution of the agent as a result of changes in physical properties, i.e., lipid solubility, oil–water distribution, nonspecific protein binding. While it is generally accepted that AChE is the critical locus of the toxic action of the organophosphorus anticholinesterases, this conclusion is not absolutely certain. Compound I may provide a useful tool for examination of this very important point.

3. The extended alkoxy chain interferes extensively with oxime reactivation, and animals poisoned by such compounds will be unresponsive to oxime treatment. Should agents exist which couple extended alkoxy chains with high toxicity, such compounds will fall into the refractory class along with soman.

[7] If reactivation does in fact occur, it would take place at least 1000 times more slowly than the reactivation of sarin inhibited enzyme. Note that AChE had been inhibited by an excess of I. Hence, all reaction must have been with the fast isomer.

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