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## Abstract

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## Chemistry

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# Anticholinesterase Properties of Some O-Ethyl-S-Alkylmethylthiophosphinates

According to current ideas, the mechanism of action of organophosphorus inhibitors (OPI) of cholinesterase (ChE), containing $\beta$-dialkylaminoethylthio or $\beta$-alkylthioethylthio groupings: $= P(O)SCH_2CH_2NR_2$ or $= P(O)SCH_2CH_2SR$, consists of two stages. In the first stage (reversible), sorption of the OPI molecule occurs on the surface of ChE at a close and quite definite distance from the reactive center of the enzyme. In the second stage, phosphorylation of the reactive center takes place. The reaction of ChE with its natural substrate—acetylcholine—proceeds analogously, with the sole difference that the acetylated reactive center of the enzyme is readily regenerated as a result of hydrolysis, whereas the phosphorylated one is regenerated only very slowly (reactivation of the enzyme). In accordance with the foregoing, the ChE molecule is considered to contain a so-called anionic center, at which

schematic diagram of the "anionic" center and "esterase" center of cholinesterase interacting with acetylcholine

sorption of acetylcholine occurs through electrostatic bonding of its ammonium nitrogen atom with the "anionic" group of the enzyme, and an "esterase" center (an appropriately framed hydroxyl group of serine), which enters into a transesterification reaction with acetylcholine. It is believed that analogous processes also occur with the OPI molecule, in which the sulfur or nitrogen atom located in the $\beta$-position of the thioether radical imitates the trimethylammonium group of acetylcholine. It is assumed here that the nitrogen or sulfur atom first acquires, in one way or another, a positive (integral or fractional) charge. Thus, sulfide sulfur is converted into sulfoxide and then into sulfonium sulfur ([1]), while amine nitrogen is converted into ammonium nitrogen through addition of a proton ([2]), etc.

This assumption has certain grounds and is attractive in its simplicity. However, it has no direct experimental evidence, and it is possible that both the structure of the anionic center of ChE and the mechanism of sorption of OPI on ChE are in fact much more complex.

The anticholinesterase activity of OPI of the type under consideration, as a rule, increases sharply upon alkylation of the heteroatom located in the $\beta$-position in the thioether radical ([3–9]); alkylation is naturally accompanied by transfer of

this atom into the onium state and by the appearance of a positive ionic charge: $= P(O)SCH_2CH_2\overset{+}{N}R_3]X'$, $= P(O)SCH_2CH_2\overset{+}{S}R_2]X'$.

In this effect of increased activity upon alkylation, the principal role is usually ascribed precisely to the action of the positive charge ([4,5]);

which promotes the formation of a stronger bond with the "anionic" center, increases the lifetime of the OP inhibitor molecule in the adsorbed state, and thereby raises the probability of reaction with the "esterase"center of the enzyme.

At the same time, the appearance of a positive charge exerts a powerful inductive influence on the P–S bond, increasing both the specific reactivity toward the "esterase" center of ChE and the nonspecific reactivity of OP inhibitors toward nucleophilic reagents, for example, toward $OH^-$ ions (aqueous-alkaline hydrolysis).

In the preceding work [9] we showed that, in parallel with the increase in anticholinesterase activity upon alkylation of the OP-inhibitor molecule, an increase in the rate of noncatalytic hydrolysis is also observed. These two effects proved experimentally difficult to distinguish. There is, however, a third aspect that should be borne in mind when studying the increase in anticholinesterase activity of OP inhibitors upon alkylation. This is the change in the spatial structure of the thioether radical, with its approximation to the configuration of acetylcholine. The role of the steric factor in the activity of ChE was vividly demonstrated by comparing the action of ChE on acetylcholine and on 3,3-dimethylbutyl acetate [10]:

$$\overline{X}\,[(CH_3)_3\overset{+}{N}CH_2CH_2OCOCH_3] \qquad (CH_3)_3CCH_2CH_2OCOCH_3$$

acetylcholine 3,3-dimethylbutyl acetate

Unlike acetylcholine, 3,3-dimethylbutyl acetate does not contain a positively charged atom. Instead of an ammonium nitrogen atom, it contains a quaternary carbon atom. Nevertheless, this substance is readily cleaved by ChE, and the rate of its enzymatic hydrolysis is only slightly lower than the rate of hydrolysis of acetylcholine [10]. There are indications of high anticholinesterase activity of the 3,3-dimethylbutyl derivative of phosphoric acid [11]. Undoubtedly, the steric similarity of the molecules of these substances to acetylcholine plays the principal role here, in a definite way facilitating fixation of the substrate at the sorption center. This prompted us to undertake a systematic study of the dependence of the anticholinesterase action of OP inhibitors that do not contain heteroatoms in the thioether radicals on the geometrical structure of the thioether radicals. We synthesized a series of O-ethyl-S-alkylmethylthiophosphinates: $CH_3(C_2H_5O)P(O)SAlk$, containing alkyl radicals with different numbers of carbon atoms and different degrees of branching, and studied the rate of ChE inhibition by these compounds.

**Synthesis of O-ethyl-S-alkylmethylthiophosphinates** was carried out by the interaction of sodium O-ethyl methylthiophosphinate with the corresponding alkyl bromides* according to the method described earlier [12]:

$$\begin{matrix}CH_3\\C_2H_5O\end{matrix}\Big\rangle P\Big(\begin{matrix}S\\ONa\end{matrix}\Big) + AlkBr \rightarrow \begin{matrix}CH_3\\C_2H_5O\end{matrix}\Big\rangle P\Big(\begin{matrix}O\\SAlk\end{matrix}\Big) + NaBr$$

$$Alk = C_2H_5;\ n\text{-}C_3H_7,\ n\text{-}C_4H_9,\ iso\text{-}C_5H_{11},$$

$$n\text{-}C_6H_{13},\ CH_2CH_2C(CH_3)_3$$

The constants, yields, and analytical data of the compounds obtained are given in Table 1.

**Determination of anticholinesterase activity** was carried out using a preparation of purified ChE from horse blood serum, produced by the Kashintsy Biofactory. A solution of the enzyme (4 mg protein/ml) was incubated with a solution of the inhibitor ($10^{-3}$—$10^{-7}$ mol/l) at the given temperature, and after specified time intervals the residual ChE activity was determined by potentiometric titration [13,14].

* In the reaction of sodium O-ethyl methylthiophosphinate with tert-butyl bromide, instead of the expected O-ethyl-S-tert-butylmethylthiophosphinate, O-ethyl methylthiophosphonate was isolated almost quantitatively.

**Table 1**

**O-Ethyl-S-alkyl methylthiophosphinates:** $CH_3PO(OC_2H_5)(SR)$

| Laboratory no. | R | b.p., °C | Pressure, mm | $n_D^{20}$ | $d_4^{20}$ | $MR_D$ found | $MR_D$ calc. | Yield, % | C, found | C, calc. | H, found | H, calc. | P, found | P, calc. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K-9 | $C_2H_5$ * | 62–64 | 1 | 1,4760 | 1,0969 | 43,20 | 43,35 | 77 | — | — | — | — | — | — |
| -57 | *n*-$C_3H_7$ | 85–86 | 2,5 | 1,4820 | 1,0712 | 48,44 | 47,97 | 85 | 39,93 | 39,6 | 8,58 | 8,2 | 16,71 | 17,0 |
| -58 | *n*-$C_4H_9$ | 100–102 | 2 | 1,4810 | 1,0530 | 52,97 | 52,59 | 61 | 42,54 | 42,8 | 8,58 | 8,7 | 16,21 | 15,8 |
| -63 | *n*-$C_6H_{13}$ | 105–106 | 1 | 1,4712 | 1,0178 | 61,54 | 61,81 | 87 | 47,74 | 47,2 | 9,69 | 9,4 | 14,01 | 13,8 |
| -62 | $CH_2CH_2CH(CH_3)_2$ | 73–74 | 1 | 1,4710 | 1,0394 | 56,50 | 57,20 | 59 | — | — | — | — | 15,01 | 14,8 |
| -56 | $CH_2CH_2C(CH_3)_3$ | 76–77 | 1 | 1,4746 | 1,0126 | 62,44 | 61,83 | 32 | 47,94 | 47,2 | 9,49 | 9,4 | 13,71 | 13,8 |

* Literature data (15): b.p. 87–88° (7 mm), $d_4^{20}$ 1,0951; $n_D^{20}$ 1,4768.

The rate constants of the reaction of OPIs with ChE were calculated by the formula for a pseudomonomolecular reaction:

$$k = \frac{1}{t[I]} \ln \frac{A_0}{A_t},$$

since the concentration of inhibitor considerably exceeded the concentration of the active centers of ChE.

Here $t$ is the duration of contact of the OPI with ChE in minutes, $[I]$ is the molar concentration of the OPI, $A_0$ is the initial activity of ChE, and $A_t$ is the activity of ChE at time $t$. The results were subjected to statistical treatment. Each value of $k$ is the mean of 15–20 determinations. From the constants $k$, measured at different temperatures, the activation energies ($E$) and the preexponential factor of the Arrhenius equation ($PZ$) were calculated in the usual manner. The results obtained are given in Table 2.

**Table 2**

**Kinetic parameters of the reaction of ChE with O-ethyl-S-alkyl methylthiophosphinates**

| Laboratory no. | R | $k \cdot 10^3$ L·mol/min, 10° | $k \cdot 10^3$ L·mol/min, 25° | $k \cdot 10^3$ L·mol/min, 33° | $k \cdot 10^3$ L·mol/min, 40° | $E$, kcal/mol | $PZ \cdot 10^{10}$ |
|---|---|---|---|---|---|---|---|
| K-9 | $C_2H_5$ | 0,023±0,001 | 0,064±0,001 | 0,12±0,01 | 0,15±0,01 | 12,1 | 4 |
| -57 | *n*-$C_3H_7$ | 0,038±0,001 | 0,13±0,01 | 0,23±0,01 | 0,36±0,01 | 12,2 | 10 |
| -58 | *n*-$C_4H_9$ | 0,20±0,01* | 0,82±0,01 | 1,1±0,01 | 1,7±0,1 | 12,3 | 60 |
| -63 | *n*-$C_6H_{13}$ | 12±1,1 | 38±1,2 | 55±1,7 | 83±1,2 | 12,3 | 3200 |
| -62 | $CH_2CH_2CH(CH_3)_2$ | 0,77±0,01 | 2,3±0,1 | 3,9±0,1 | 5,4±0,1 | 12,0 | 130 |
| -56 | $CH_2CH_2C(CH_3)_3$ | 1,6±0,1** | 4,7±0,13*** | 8,1±0,12 | 10±0,11 | 12,3 | 350 |

* At 9°.
** At 14°.
*** At 26°.

**Results.** From the data in Table 2 it is evident that the reactions of the compounds studied with ChE are characterized by practically identical activation energies, while the values of the preexponential factors differ sharply. This may be explained by the different lifetime of the complex of the OPI molecule and ChE.

The different anticholinesterase activity of the OPIs studied by us, which have the same phosphoryl part, is undoubtedly determined by the different configuration of the thiophosphoryl radical of the OPI.

Among the compounds studied it is of interest to single out a series of substances of the following type: $CH_3(C_2H_5O)P(O)SCH_2CH_2CR'R''R'''$, where $R'$, $R''$, and $R'''$ may be hydrogen atoms or methyl groups. The thioether radicals of these compounds are, to varying degrees, close in configuration to the choline residue: $—CH_2CH_2\overset{+}{N}(CH_3)_3$. In this series all possible variants of the above structure are represented: $R' = R'' = R''' = H$ (LG-57); $R' = R'' = H$; $R''' = CH_3$ (LG-58); $R' = H$; $R'' = R''' = CH_3$ (LG-62); $R' = R'' = R''' = CH_3$ (LG-56). Compound LG-56, where all the radicals $R$ are methyl groups, completely imitates the spatial structure of the choline residue of acetylcholine. Adams and Wittaker[^10] studied alkyl acetates as substrates of ChE and showed that, as their structure approaches that of acetylcholine, the rate of their enzymatic hydrolysis increases: $n$-propyl < $n$-butyl < isoamyl < 3,3-dimethylbutyl. It could be assumed that the corresponding change in the structure of the organophosphorus inhibitors would likewise lead to an increase in the affinity of such compounds for the enzyme.

Our experimental data confirmed this assumption. Thus, anticholinesterase activity increased distinctly in the series: LG-57 < LG-58 < LG-62 < LG-56. An increase in anticholinesterase activity is observed not only with $\beta$-branching of the radicals, but also with lengthening of the main hydrocarbon chain (compounds K-9, LG-57, LG-58, and LG-63). This increase is particularly distinct in the case of compound LG-63, where an $n$-hexyl radical is present. Compound LG-63 proved to be 560 times more active than preparation K-9, which contains an S-ethyl radical, and even almost 10 times more active than the previously studied preparation GD-7[^9], which contains a sulfur atom in the $\beta$-position*: $CH_3(C_2H_5O)P(O)SCH_2CH_2SC_2H_5$.

Apparently, the primary sorption of organophosphorus inhibitors on the ChE surface is a more complex phenomenon than was previously assumed. It is possible that van der Waals interactions, complicated by the requirements of steric correspondence, play an important role here. We do not, of course, deny the important role of the positive charge in acetylcholine or in the structures of the organophosphorus inhibitors considered above, but there is no doubt that the determining role belongs not only to the charge.

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* These results may be compared with the data obtained by Adams and Wittaker[^10], who found that, in a series of alkyl acetates—substrates of ChE—lengthening of hydrocarbon radicals of normal structure is accompanied by an increase in the rate of hydrolysis. However, this is observed only up to the butyl derivative; *n*-amyl acetate is hydrolyzed more slowly, and *n*-hexyl acetate very slowly indeed.

*Note: Figure translations are in progress. See original paper for figures.*