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Abstract

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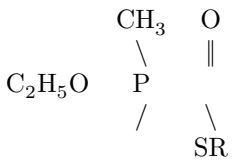
ANTICHOLINESTERASE PROPERTIES OF O-ETHYL-S-ALKYLMETHYLTHIOPHOSPHINATES

KINETICS OF INHIBITION AND THE STRUCTURE OF THE ACTIVE SURFACE OF CHOLINESTERASES

The anticholinesterase activity of organophosphorus compounds (OPCs) depends substantially on their structure. This dependence is now known in the form of a number of particular regularities linking structural elements of OPCs with their action.

In our article we consider the question of the influence of the structure of the alkyl radical R in O-ethyl-S-alkylmethylthiophosphinates:



on the rate of inhibition of cholinesterase (ChE). The solution of this particular question makes it possible, as will be seen from the further exposition, to answer a number of general questions concerning the mechanism of cholinesterase inhibition by inhibitors.

Earlier we showed* that the anticholinesterase properties of O-ethyl-S-alkylmethylthiophosphinates change substantially depending on the size and branching of the hydrocarbon radical R ([1]). Thus, in the series of O-ethyl-S-*n*-alkylmethylthiophosphinates, the rate constant of ChE inhibition increases markedly with an increase in the number of carbon atoms in the radical R from C_2 to C_6. For the hexyl derivative the rate constant of inhibition is almost 600 times greater than for the ethyl derivative. With a further increase in

the number of carbon atoms in the radical R, the anticholinesterase activity practically does not change.

It was also shown that the anticholinesterase activity of the compounds under consideration depends strongly on the degree of branching of the radical; however, this question had not been studied sufficiently fully, and therefore it seemed of interest to investigate this dependence in greater detail. We studied O-ethyl-S-alkylmethylthiophosphinates of the following structure: $CH_3(C_2H_5O)P(O)S(CH_2)_{nR}$, where R was either a tert-butyl group located at various distances from the phosphorus atom ($n = 1$—6), or an isopropyl group ($n = 1, 2, 4$).

Most of these compounds were synthesized by the action of the corresponding alkyl bromides on sodium O-ethyl methylthiophosphinate by the method described earlier ([2]):

$$CH_3(C_2H_5)P(S)ONa + Br(CH_2)_{nR} \rightarrow CH_3(C_2H_5O)P(O)S(CH_2)_{nR} + NaBr.$$

The exception was O-ethyl-S-neopentyl-methylthiophosphinate, which was obtained by interaction of O-ethyl-methylchlorothiophosphinate with sodium neopentyl mercaptide**

$$CH_3(C_2H_5O)P(O)Cl + NaSCH_2C(CH_3)_3 \rightarrow CH_3(C_2H_5O)P(O)SCH_2C(CH_3)_3 + NaCl.$$

The constants, yields, and analytical data for the substances obtained are given in Table 1.

* The work was submitted to the journal *Izvestiya AN SSSR*, Chemical Series.
** The starting neopentyl mercaptan was synthesized by Bardwell' s method ([3]); the mercaptan had the following constants: b.p. 100—102°; n_D^{20}1.4330; d_4^{20}0.8375.

Found, %: C 57.9; 57.6; H 11.5; 11.5
$C_5H_{12}S$. Calculated, %: C 57.6; H 11.5

Table 1

O-ethyl-S-alkyl methylthiophosphinates: $CH_3(C_2H_5O)P(O)SR$

Code	R	b.p., °C	P, mm	n_D^{20}	d_4^{20}	MR_D found	MR_D calculated	Yield, %	C, % found	C, % calculated	H, % found	H, % calculated	P, % found	P, % calculated
- 60	$CH_2CH(CH_3)_2$	78 – 80	2	1.4640	1.0262	56.47	57.20	45	45.3; 45.3	44.7	9.4; 9.3	9.2	15.0; 15.0	14.8
- 69	$(CH_2)_3C(CH_3)_3$	84 – 85	3	1.4710	1.0015	66.41	66.45	71	50.1; 50.0	50.4	9.7; 9.7	9.7	12.8; 13.0	13.0
- 67	$(CH_2)_4C(CH_3)_3$	124 – 126	4	1.4700	0.9896	71.21	71.07	77	52.1; 52.2	52.4	10.0; 10.0	9.9	10.0; 9.9	9.9
- 78	$(CH_2)_5C(CH_3)_3$	126 – 128	5	1.4670	0.9817	75.64	75.52	68	53.6; 53.6	54.1	10.3; 10.3	10.1	11.2; 11.4	11.6
- 87	$(CH_2)_6C(CH_3)_3$	134 – 136	6	1.4700	0.9723	81.34	80.14	69	55.2; 55.6	55.7	10.6; 10.4	10.5	10.9; 10.8	11.1
- 79	$CH_2CH(CH_3)_2$	120 – 121	2	1.4770	1.0615	52.20	52.58	46	42.5; 42.7	42.8	8.6; 8.6	8.7	16.1; 16.1	15.8
- 73	$(CH_2)_4CH(CH_3)_2$	126 – 127	4	1.4698	1.0016	66.31	66.40	71	50.4; 50.3	50.4	10.0; 9.8	9.7	12.8; 12.8	13.0

The anticholinesterase activity of O-ethyl-S-alkyl methylthiophosphinates was evaluated by the value of k_2, the rate constant for their interaction with ChE of horse blood serum. The determinations were carried out at different temperatures, which made it possible to find the activation energy of ChE inhibition (E) and, from the Arrhenius equation, to calculate the preexponential factors (pz). In addition, the constants of nonenzymatic water-alkaline hydrolysis of these compounds (k_{hydr}) were determined. The results of the study of the anticholinesterase activity of O-ethyl-S-alkyl methylthiophosphinates are given in Table 2; in the same table, for comparison, are also given kinetic parameters of ChE inhibition and constants of water-alkaline hydrolysis for some previously studied ChE inhibitors.

Table 2

Anticholinesterase activity of organophosphorus compounds of the type $CH_3(C_2H_5O)P(O)S(CH_2)_{nC}(R_1R_2R_3)$

Code	n	R_1	R_2	R_3	k_2 (10^3), l/mol · min	E, kcal/mol	$pz \cdot (10^{10})$	k_{hydr}, l/mol · min
K-9*	1	H	H	H	0.064± 0.0014	12.1	4	0.138± 0.013
- 57*	1	H	CH_3	H	0.137± 0.008	12.2	10	0.131± 0.002
-79	1	H	CH_3	CH_3	0.671± 0.031	–	–	0.136± 0.004
-60	1	CH_3	CH_3	CH_3	21.0 ± 0.9	12.5	3550	0.099± 0.003
- 57*	2	H	H	H	0.137± 0.008	12.2	10	0.131± 0.002
- 58*	2	H	CH_3	H	0.825± 0.003	12.3	60	0.145± 0.005
- 62*	2	H	CH_3	CH_3	2.31 ± 0.08	12.0	130	0.156± 0.014
- 56*	2	CH_3	CH_3	CH_3	4.70 ± 0.13	12.3	350	0.161± 0.013
-69	3	CH_3	CH_3	CH_3	18.2 ± 0.6	12.2	1660	0.134± 0.003
- 64*	4	H	H	H	2.57 ± 0.05	12.0	130	0.132± 0.004
- 63*	4	H	CH_3	H	37.9 ± 1.2	12.3	3200	0.122± 0.001
-73	4	H	CH_3	CH_3	59.2 ± 1.7	–	–	0.162± 0.004
-67	4	CH_3	CH_3	CH_3	90.5 ± 1.7	12.0	6750	0.148± 0.005
-78	5	CH_3	CH_3	CH_3	88.8 ± 3.0	–	–	0.123± 0.006
-87	6	CH_3	CH_3	CH_3	98.7 ± 2.7	–	–	0.146± 0.017

* These compounds were studied by us previously.

From the data given in Table 2 it is evident that the reactions of the compounds studied with ChE are characterized by practically identical activation energies, with sharply differing values of the preexponential factors. The values of the constants of water-alkaline hydrolysis, which characterize the electrophilic reactivity of the $P—S$ bond in the compounds studied, differed little from one another. Undoubtedly, the considera-

inhibitors react with the esterase center of ChE as electrophilic reagents.

Fig. 1. Dependence of k_2 on the size and structure of R. 1–$CH_3(C_2H_5O)P(O)S(CH_2)_{nC}2H_5$, 2–$CH_3(C_2H_5O)P(O)S(CH_2)_{nC}(CH_3)_3$, $m = n + 2$.

Figure 1: Fig. 1. Dependence of k_2 on the size and structure of R. 1–$CH_3(C_2H_5O)P(O)S(CH_2)_{nC}2H_5$, 2–$CH_3(C_2H_5O)P(O)S(CH_2)_{nC}(CH_3)_3$, $m = n + 2$.

Therefore, it should be assumed that the constancy of the rate constants of nonenzymatic hydrolysis and the constancy of the activation energies for inhibition of ChE indicate that the anticholinesterase activity of O-ethyl-S-alkylmethylthiophosphinates is determined not by the rate of their direct interaction with the esterase center, but by the rate of some preceding process that depends on the size and spatial configuration of the alkyl radical R.

This is especially clearly seen in the examples of compounds containing tert-butyl groups located at different distances from the phosphorus atom (see Fig. 1). Thus, on going from compound LG-60 ($n = 1$) to compound YaG-56 ($n = 2$), the value of the inhibition rate constant (k_2) decreases by approximately 4.5-fold; then, as n is increased, it again increases and, for LG-67 ($n = 4$), reaches a maximum value exceeding k_2 for YaG-56 by almost 20-fold. With further removal of the tert-butyl group from the phosphorus atom, k_2 practically does not change.

Fig. 1. Dependence of k_2 on the size and structure of R.
1–$CH_3(C_2H_5O)P(O)S(CH_2)_{nC}2H_5$,
2–$CH_3(C_2H_5O)P(O)S(CH_2)_{nC}(CH_3)_3$,
$m = n + 2$.

The dependence of the anticholinesterase activity of O-ethyl-S-alkylmethylthiophosphinates containing a tert-butyl group on the distance between this group and the phosphorus atom is presented in Fig. 1. For comparison, the same figure gives the dependence of the anticholinesterase action of O-ethyl-S-*n*-alkylmethylthiophosphinates on the length of the alkyl-radical chain. It is of interest to note that the value of k_2 for LG-57 is almost 1400 times greater than the k_2 of compound K-9 ($R = C_2H_5$). To explain these experimental facts we propose the following hypothesis.

It is known that on the effective surface of ChE there are two active sites—an anionic center and an esterase center. At the first, specific sorption of the substrate or inhibitor takes place, after which the sorbed molecule acylates (acetylates in the case of the normal substrate—acetylcholine—or phosphorylates in the case of an organophosphorus inhibitor) the esterase center. Both the anionic and esterase centers are hydrophilic regions of the polypeptide chain of the enzyme (the serine –OH group in the esterase center and the $–COO^-$ group in the anionic center). At the same time, in any polypeptide chain of a protein there are hydrocarbon radicals—the side chains of amino acids such as valine,

leucine, isoleucine, phenylalanine, and others. These radicals, naturally, under conditions of microheterogeneity of the system, should group together, forming hydrophobic regions—peculiar "islets" on the protein surface ([4],[5]). If a foreign molecule consisting of a polar group and a nonpolar radical is sorbed on the surface of the enzyme, then, naturally, the polar group should be sorbed on the polar (hydrophilic) centers, while the nonpolar radical should be "pushed out" onto the hydrophobic islets; this displacement should lead to a decrease in the free energy of the system, obeying the same laws as the sorption of molecules of this type at the interface of polar and nonpolar immiscible phases. It follows from this that the nonpolar hydrocarbon radicals of the substrate and inhibitor molecule should "adhere" to the hydrophobic islets on the enzyme surface. It may be thought that, in the course of evolution, ChE has become adapted to the presence of methyl groups around the onium nitrogen atom of choline and, consequently, it may be expected that the anionic center is surrounded by hydrophobic islets.

In compound L-60 ($n = 1$), the distance between phosphorus and the quaternary carbon is 4.4 Å, i.e., close to the distance between the carbonyl carbon and the ammonium nitrogen in acetylcholine (4.7 Å), and, consequently,

commensurate with the distance between the anionic and esteratic centers of ChE. The methyl groups of the neopentyl radical of LG-60 can interact with the hydrophobic environment of the anionic center in the same way as the methyl groups of trimethylammonium in acetylcholine interact with it. It is characteristic that a decrease in the number of methyl groups at the quaternary carbon causes a sharp decrease in k_2. Thus, in going from LG-60 to compound K-9, the value of k_2 decreases by a factor of 330.

When the $C(CH_3)_3$ group is moved one carbon atom farther from the phosphorus atom (LG-56, $n = 2$), the value of k_2 decreases. Probably, with such an arrangement the $C(CH_3)_3$ group (the distance from the phosphorus atom is already 5.3 Å) does not provide maximal interaction of the alkyl radical with the hydrophobic regions of the anionic center of ChE. In this case, a decrease in the number of methyl groups at the quaternary carbon does not cause such a sharp decrease in k_2: in going from LG-56 to LG-57, the value of k_2 decreases only by a factor of 35.

Further removal of the $C(CH_3)_3$ group from phosphorus (LG-69, $n = 3$, and LG-57, $n = 4$) leads to a substantial increase in k_2. Evidently, behind the anionic center there is a hydrophobic region on which hydrocarbon radicals are readily sorbed. Compound LG-67, in its anticholinesterase action, approaches methylsulfomethylate of O-ethyl-S-β-ethylmercaptoethyl methylthiophosphinate (Gd-42), which has in its molecule a positively charged sulfonium grouping and, owing to this, is a powerful inhibitor of ChE ($k_2 = 1.8 \cdot 10^6$ l/mol $\cdot$ min). A decrease in the number of methyl groups at the quaternary carbon in LV-17 also leads to a decrease in the value of k_2, although by fewer times than in comparison with LG-60.

It should be especially noted that LG-78 ($n = 5$) and LG-87 ($n = 6$) have practically the same value of k_2 as LG-67 ($n = 4$). An analogous dependence was also observed for O-ethyl-S-normal alkyl methylthiophosphinates (see Fig. 1). This is clear evidence of the limited dimensions of the hydrophobic region behind the anionic center.

These facts give grounds to suppose that on the active surface of ChE there are at least two hydrophobic regions that play a major role in the interaction of OPC with the enzyme, one of them being located in the region of the anionic center and the other behind it.

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