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Abstract

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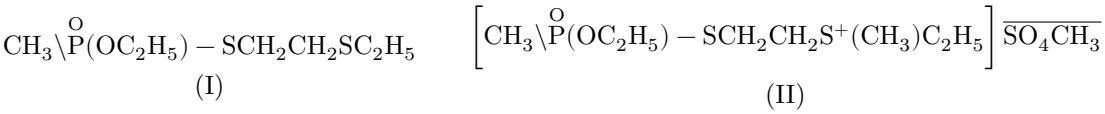
Anticholinesterase Properties of O,O-Diethyl-S-[(β-arylmethylamino)-ethyl]-thiophosphates and Their Methylsulfomethylates

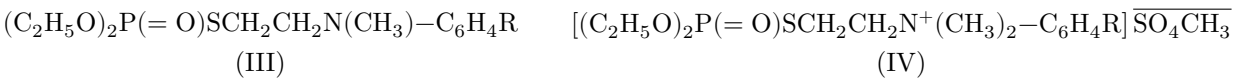
One of the most effective ways of increasing the reactivity of organophosphorus inhibitors (OPI) toward cholinesterase (ChE) is the introduction into the OPI molecule of a positively charged onium group at such a distance from the phosphoryl group as corresponds to the distance between the carbonyl carbon and the quaternary nitrogen atom in the acetylcholine molecule ([1]).

It was established earlier ([2]) that the sharp enhancement of the anticholinesterase activity of OPI on going from sulfide compounds (I) to sulfonium compounds (II)



(I) (II)

apparently occurs not so much because of the inductive effect of the positive charge as because of the formation of an ionic bond with the anionic center of cholinesterase. This is indicated by the sharp increase in the probability factor (PZ) and the absence of changes in the activation energies in the reaction with cholinesterase when sulfide sulfur in the inhibitor molecule is replaced by sulfonium sulfur.



(III) (IV)

In this connection, it is of great interest to study the influence of the magnitude of the effective positive charge in compounds of type II on the anticholinesterase activity of OPI.

As objects of study for this purpose we used O,O-diethyl-S-[(β-arylmethylamino)-ethyl]-thiophosphates (III) and their methylsulfomethylates (IV), having

substituents (R) of different electron-withdrawing ability in the para- or meta-positions of the phenyl radical, such as CH_3, Cl, OCH_3 (Table 1). The polar influence of substituents R was characterized by Hammett σ-constants ([3]). The anticholinesterase action of OPI was judged from the values of the rate constants of their interaction with serum cholinesterase (acylcholine acylhydrolase, EC 3.1.1.3),

Table 1

O,O-Diethyl-S-((β-arylmethylamino)-ethyl)-thiophosphates (III), their methylsulfomethylates (IV), compounds Gd-80 and Gd-83

Laboratory No.	Compound type	R	B.p., °C/mm	n_D^{20}	d_4^{20}
GT-23	III	H	132–133/10^{-3}	1.5424	1.1500
GT-32	III	m-CH_3	140–142/10^{-3}	1.5400	1.1322
GT-33	III	n-CH_3	135–137/10^{-3}	1.5385	1.1235
GT-39	III	m-Cl	133–140/10^{-4}	1.5517	1.2167
GT-42	III	n-Cl	140–142/10^{-4}	1.5508	1.2177
GT-52	III	n-OCH_3	162/10^{-4}	1.5407	1.1674
Gd-80*			105–107/1	1.4740	–
GT-45	IV	H	–	1.5215	–
GT-47	IV	m-CH_2	–	1.5205	–
GT-48	IV	n-CH_3	–	1.5206	–
GT-59	IV	m-Cl	–	1.5237	–
GT-60	IV	n-Cl	m.p. 70–71°		
Gd-83**			m.p. 74–76°		

* Literature data (8): b.p. 97° at 0.2 mm; n_D^{21} 1.4732.

** Compound Gd-83 was obtained by the action of dimethyl sulfate on O,O-diethyl-S-(β-diethylaminoethyl)-thiophosphate by the usual procedure, m.p. 74–76°.

Found, %: C 36.4, 36.3; H 7.8, 7.8; P 7.2, 7.3; S 16.7, 16.8
Calculated, %: C 36.4; H 7.6; P 7.8; S 16.2

in $M/50$ phosphate buffer with pH 7.5 at 25° by the method described previously (4, 5). These constants were calculated by the formula for pseudomonomolecular

reactions
$k_2 = (2.3)/t[y]_0 \lg(A_0/A_t)$, or by the formula for bimolecular reactions

$$k_{II} = \frac{2.3}{([y]_0 - [E]_0)t} \lg \frac{[E]_0([y]_0 - x)}{[y]_0([E]_0 - x)},$$

if the inhibitor was very strong and it was necessary to work with such concentrations of it that were close to the enzyme concentrations and therefore changed appreciably during the course of inhibition.

Table 2

Rate constants for the interaction of organophosphorus inhibitors with ChE (k_2 and k_{II}) and rate constants of aqueous-alkaline hydrolysis (k_{hydr})

Laboratory No.	k_2, L/mol · min	k_{II}, L/mol · min	k_{hydr}, L/mol · min
GT-23	$(3.5 \pm 0.17) \cdot 10^5$	–	0.14 ± 0.01
GT-32	$(3.8 \pm 0.15) \cdot 10^5$	–	0.16 ± 0.01
GT-33	$(5.5 \pm 0.25) \cdot 10^5$	–	0.14 ± 0.01
GT-39	$(5.8 \pm 0.15) \cdot 10^5$	–	0.18 ± 0.04
GT-42	$(7.2 \pm 0.38) \cdot 10^5$	–	0.17 ± 0.01
GT-52	$(3.0 \pm 0.12) \cdot 10^5$	–	0.18 ± 0.01
GT-45	–	$(6.8 \pm 0.22) \cdot 10^8$	44.5 ± 1.43
GT-47	–	$(6.4 \pm 0.22) \cdot 10^8$	47.1 ± 0.73
GT-48	–	$(6.1 \pm 0.23) \cdot 10^8$	49.9 ± 2.21
GT-59	–	$(7.4 \pm 0.24) \cdot 10^8$	46.7 ± 1.65
GT-60	–	$(7.0 \pm 0.20) \cdot 10^8$	51.3 ± 2.61
Gd-80	–	$(1.2 \pm 0.10) \cdot 10^7$	–
Gd-83	–	$(2.3 \pm 0.17) \cdot 10^7$	–

$[y]_0$ is the initial molar concentration of the inhibitor, A_0 is the initial activity of the enzyme in 1 ml of titrated alkali solution, A_t is the residual activity of the enzyme, t is the time of incubation of the enzyme with the inhibitor, $[E]_0$ is the initial molar concentration of the active centers of the enzyme, which was determined separately by the described method (5), and x is the decrease in active centers of the enzyme or inhibitor at time t.

The data obtained are presented in Table 2. For comparison, the rate constants of inhibition of ChE for O,O-diethyl-S-(β-diethylaminoethyl) thiophosphate (Gd-80) and its methylsulfomethylate (Gd-83) are also given there. For the organophosphorus inhibitors studied, the constants of aqueous-alkaline hydrolysis, k_{hydr}, were also determined; these too are presented in Table 2.

Fig. 1. Dependence of the rate constants of cholinesterase inhibition (k_2) on Hammett' s σ constants: 1 –GT-23; 2 –GT-32; 3 –GT-33; 4 –GT-39; 5 –GT-42; 6 –GT-52; 7 –GT-45; 8 –GT-47; 9 –GT-48; 10 –GT-59; 11 –GT-60

Figure 1: Fig. 1. Dependence of the rate constants of cholinesterase inhibition (k_2) on Hammett' s σ constants: 1 –GT-23; 2 –GT-32; 3 –GT-33; 4 –GT-39; 5 –GT-42; 6 –GT-52; 7 –GT-45; 8 –GT-47; 9 –GT-48; 10 –GT-59; 11 –GT-60

As is seen from the data of Table 2, compounds containing a tertiary nitrogen atom (GT-23; GT-32; GT-33; GT-39; GT-42; GT-52) exceed in their anticholinesterase activity such a strong inhibitor as "armin" ([4]). However, they are almost 100 times less active than O,O-diethyl-S-(β-diethylaminoethyl) thiophosphate (Gd-80). This is a consequence of the reduced ability of compounds having an aryl radical substituted at the nitrogen atom to form ammonium cations in aqueous medium; compounds containing alkyl radicals at the nitrogen atoms are stronger bases (pK_b 4 or 5). They are strongly ionized in aqueous solution at pH 7.5, which promotes an increase in their anticholinesterase activity.

Fig. 1. Dependence of the rate constants of cholinesterase inhibition (k_2) on Hammett' s σ constants:
1 –GT-23; **2** –GT-32; **3** –GT-33; **4** –GT-39; **5** –GT-42; **6** –GT-52; **7** –GT-45; **8** –GT-47; **9** –GT-48; **10** –GT-59; **11** –GT-60.

Organophosphorus inhibitors containing a quaternary nitrogen atom (GT-45, GT-47, GT-48, GT-59, GT-60) proved to be extremely strong inhibitors of ChE. The rate constants of ChE inhibition (k_{II}) for them were 100 times greater than for compound Gd-42 (II) and almost 30 times higher than for the methylsulfomethylate of O,O-diethyl-S-(β-diethylaminoethyl) thiophosphate (Gd-83). In comparison with phosphorilthiocholine, for which, according to Tammelin ([6]), the value $k_{\text{II}} = 2 \cdot 10^4$ liter/mole $\cdot$ sec or $k_{\text{II}} = 1.2 \cdot 10^6$ liter/mole $\cdot$ min, the quaternary compound GT-45 is more than 500 times more active, although it differs from phosphorilthiocholine only in that one of the methyl groups at the nitrogen atom is replaced by a phenyl group.

Such a strong anticholinesterase action of GT-type compounds with a quaternary nitrogen atom can apparently be explained by the presence in these compounds of hydrophobic aryl radicals, which, together with the positive charge, facilitate sorption of the organophosphorus-inhibitor molecule on the enzyme surface ([7]).

The rate constant of interaction of the organophosphorus inhibitors with ChE (k_2) depends noticeably on the nature and position of substituent R in the phenyl ring. In the coordinates $\log k_2$ and σ, a linear Hammett dependence is observed, although not very clearly (Fig. 1).

The rather large scatter of the points, in particular the elevated value of k_2 for compound GT-33, which has a methyl group in the para position, is probably a consequence of steric factors associated with a greater or lesser complementarity

of the substituted radicals to the hydrophobic regions surrounding the anionic site of the active center of ChE. It is noteworthy that the rate constants of nonenzymatic hydrolysis for these substances are practically identical.

The influence of the nature of substituent R on the anticholinesterase activity of organophosphorus inhibitors having a quaternary nitrogen atom is weaker than for the corresponding organophosphorus inhibitors with a tertiary nitrogen atom. It may be thought that the rate of interaction of these compounds with ChE is determined mainly by the presence of a positive charge, whereas the polar influence of substituent R plays a subordinate role. At the same time, a much better linear correlation is observed here (Fig. 1). Apparently, the steric influence here also recedes into the background.

Thus, the anticholinesterase activity of the organophosphorus compounds we studied depends strongly on the ability of these compounds to become fixed at the anionic site of the enzyme, which is ensured by the presence in them of a positive charge at the nitrogen atom, as well as of an aryl hydrophobic radical. The polar influence of substituents has a strong effect on amine compounds (change in the basicity of nitrogen), but here it is complicated by steric factors.

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