SYNTHESIS AND REACTION OF O,O-DIBUTYL S-ALKYL THIOPHOSPHATES WITH CHOLINESTERASE

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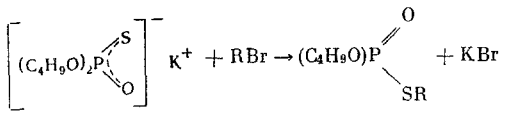
When studying the anticholinesterase activity of some S-alkyl diphenylthiophosphinates and O-ethyl S-alkyl phenylthiophosphonates it was shown that they exhibit a combined inhibition of the catalytic activity of the enzyme [1-3]. This effect is apparently associated with the presence of either one or two bulky hydrophobic phenyl groups, which facilitates the formation of an enzyme–inhibitor complex that is incapable of being converted to the phosphorylated enzyme.

To study the character of the inhibition of cholinesterase by thiolphosphates that contain hydrophobic butoxyl groups in place of the phenyl groups we synthesized a number of O,O-dibutyl S-alkyl thiophosphates and studied their inhibiting activities toward acetylcholinesterase (ACE) and butyrylcholinesterase (BuCE).

The O,O-dibutyl S-alkyl thiophosphates were obtained by the following scheme:



$$\left[(C_4H_9O)_2P\begin{matrix}S\\O\end{matrix}\right]^- K^+ + RBr \rightarrow (C_4H_9O)P(O)SR + KBr$$

R = CH_3—C_6H_{13}

EXPERIMENTAL METHOD

The K salt of dibutylthiophosphoric acid was obtained as described in [4]. The purity of the esters was checked by TLC [5], using KSK silica gel, a 2:1 hexane–acetone mixture as the eluant, and a solution of $KMnO_4$ in H_2SO_4 for detection. The constants, yields, and analysis data for the obtained compounds are given in Table 1.

As BuCE we used the purified preparation of horse blood serum (acylhydrolase of acylcholines, EC 3.1.1.8) produced by the I. I. Mechnikov Institute of Vaccines and Serums, while as ACE we used the partially purified preparation from human blood erythrocytes (acetylcholine acetylhydrolase, EC 3.1.1.7). Analytical grade acetylcholine chloride served as the substrate.

TABLE 1. Properties of O,O-Dibutyl Thiophosphates

R	Yield, %	bp, °C (p, mm of Hg)	d_4^{20}	n_D^{20}	MR		Found, %			Empirical formula	Calculated, %		
					found	calc.	C	H	P		C	H	P
CH_3	79,3	81–82 (1,5)	1,0506	1,4591	62,50	62,96	44,7	8,8	12,9	$C_9H_{21}O_3PS$	45,0	8,7	12,9
C_2H_5	75	75–76 (1)	1,0344	1,4586	67,17	67,57	46,6	9,0	11,9	$C_{10}H_{23}O_3PS$	47,3	9,0	12,2
C_3H_7	74	98–99 (1)	1,0222	1,4583	71,68	72,19	48,9	9,4	11,5	$C_{11}H_{25}O_3PS$	49,2	9,3	11,5
C_4H_9	76	109 (1)	1,0098	1,4571	76,26	76,81	51,2	9,7	10,9	$C_{12}H_{27}O_3PS$	51,0	9,6	11,0
C_5H_{11}	80	119 (1)	1,0001	1,4581	80,89	81,43	52,4	9,9	10,6	$C_{13}H_{29}O_3PS$	52,7	9,8	10,5
C_6H_{13}	73	129 (1)	1,9932	1,4588	85,43	85,05	53,9	10,0	9,8	$C_{14}H_{31}O_3PS$	54,2	10,0	10,0

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TABLE 2. Kinetic Parameters for Inhibition of Cholinesterase by O,O-Dibutyl Thiophosphates $(C_4H_9O)_2P(O)SR$

R	ACE			BuCE		
	k_a, mole^{-1}·liter·min^{-1}	$K_{i,r}$, mole/liter	$K_{i,s}$, mole/liter	k_a, mole^{-1}·liter·min^{-1}	$K_{i,r}$, mole/liter	$K_{i,s}$, mole/liter
CH_3	$7{,}46\cdot10^{3}$	$3{,}35\cdot10^{-5}$	$1{,}08\cdot10^{-5}$	$1{,}22\cdot10^{4}$	$2{,}75\cdot10^{-6}$	$1{,}13\cdot10^{-6}$
C_2H_5	$1{,}2\cdot10^{4}$	$1{,}9\cdot10^{-5}$	$3{,}05\cdot10^{-5}$	$1{,}28\cdot10^{4}$	$1{,}56\cdot10^{-6}$	$3{,}05\cdot10^{-6}$
C_3H_7	$8{,}5\cdot10^{3}$	$3{,}1\cdot10^{-5}$	$2{,}37\cdot10^{-5}$	$8{,}62\cdot10^{4}$	$3{,}27\cdot10^{-6}$	$1{,}44\cdot10^{-6}$
C_4H_9	$1{,}16\cdot10^{4}$	$4{,}5\cdot10^{-6}$	$9{,}9\cdot10^{-6}$	$3{,}22\cdot10^{5}$	$7{,}1\cdot10^{-7}$	$5{,}5\cdot10^{-7}$
C_5H_{11}	$2{,}1\cdot10^{4}$	$1{,}7\cdot10^{-5}$	$4{,}4\cdot10^{-6}$	$7{,}7\cdot10^{5}$	$1{,}95\cdot10^{-7}$	$1{,}88\cdot10^{-7}$
C_6H_{13}	$2{,}22\cdot10^{4}$	$7{,}5\cdot10^{-6}$	$3{,}38\cdot10^{-6}$	$6{,}14\cdot10^{6}$	$1\cdot10^{-8}$	$2{,}6\cdot10^{-8}$

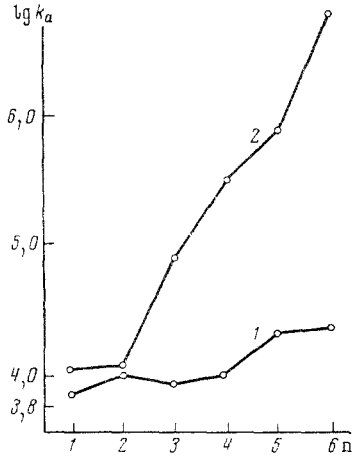

Fig. 1. Constants for the irreversible inhibition of ACE (1) and BuCE (2) by O,O-dibutyl thiophosphates $(C_4H_9O)_2P(O)SR$ as a function of number of carbon atoms in alkylthiol radical.

The enzymatic hydrolysis rate of acetylcholine was determined by the method of measuring the initial rate of change in the pH [6] at 25°C and the optimum pH values and substrate concentrations (pH 7.55 and $[S] = 5\cdot10^{-3}$ M in the case of ACE, and pH 7.8 and $[S] = 5\cdot10^{-2}$ M in the case of BuCE).

To calculate the constants of the combined type of inhibition k_a, $K_{i,r}$, and $K_{i,s}$ (Table 2) we used the equations given in [7].

DISCUSSION OF RESULTS

The O,O-dibutyl thiophosphates are inhibitors of the combined type. This is evidenced by the fact that the k_2 values, obtained for various inhibitor concentrations, are not constant, and by the presence of an intercept on the log (v_0/v_t) axis when plotted in the coordinates log (v_0/v_t) vs incubation time (t) of enzyme with inhibitor [7].

As can be seen from Table 2, the constants for the irreversible inhibition of both enzymes by dibutyl thiophosphates steadily increase with increase in the length of the alkylthiol radical R and reach a maximum value for the hexyl derivative (Fig. 1). This increase is slight in the case of ACE, and the k_a values when going to the hexyl derivative increase a total of three times (see Fig. 1). The equilibrium constants $K_{i,r}$ and $K_{i,s}$, which characterize the reversible inhibition, also decrease slightly (Fig. 2).

In the inhibition of BuCE an increase in the length of the R radical materially affects the change in the values of the irreversible inhibition rate constant k_a (see Fig. 1 and Table 2). The transition from the methyl to the ethyl derivative is accompanied by a slight increase in k_a, and then a sharp increase is observed in the irreversible inhibition rate. On the whole the k_a constants increase by 500 times, i.e., the different dibutyl thiophosphates exhibit a selective action toward BuCE. These data testify to the greater tendency of BuCE to undergo hydrophobic interactions when compared with ACE.

The change in the equilibrium constants $K_{i,r}$ and $K_{i,s}$ has a somewhat different character (Fig. 3). Thus, for the first three members of the series these constants remain practically constant, and then their sharp decrease is observed. The similarity of the change in the k_a, $K_{i,r}$, and $K_{i,s}$ values gives reason to assume that the "effective sorption" of the inhibitor on the active surface of the enzyme, which leads to irreversible inhibition, and the "nonproductive sorption", both take place due to the hydrophobic interactions of the acyl radicals on the same hydrophobic sections of the active surface of the enzyme. However, in the first case the inhibitor molecule is fixed in such manner relative to the esterase center that the subsequent phosphorylation of the esterase center takes place. In the case of "nonproductive sorption" the inhibitor molecule is apparently fixed so that the next step of the process is either not realized or else it is strongly hindered.

CONCLUSIONS

1. A number of O,O-dibutyl S-alkyl thiophosphates were synthesized and their anticholinesterase activities were studied.

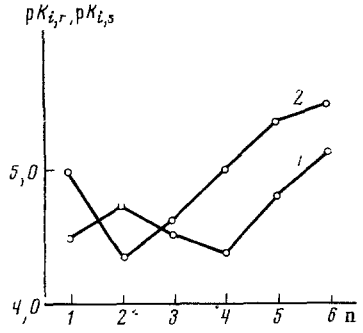

Fig. 2

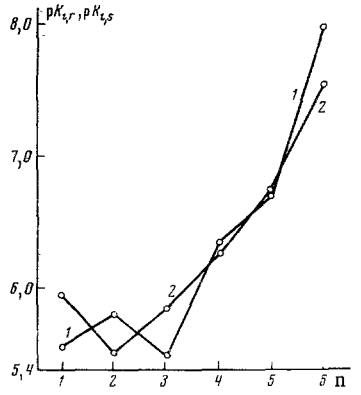

Fig. 3

Fig. 2. Constants for the reversible inhibition $K_{i,r}$ (1) and $K_{i,s}$ (2) of acetylcholinesterase by O,O-dibutyl S-alkyl thiophosphates as a function of number of carbon atoms in alkylthiol radical.

Fig. 3. Constants for the reversible inhibition $K_{i,r}$ (1) and $K_{i,s}$ (2) of butyrylcholinesterase by O,O-dibutyl S-alkyl thiophosphates as a function of number of carbon atoms in alkylthiol radical.

2. The presence of two butoxyl radicals in the uncleavable portion of the inhibitor leads to the combined type of enzyme inhibition.

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