SYNTHESIS AND ANTICHOLINESTERASE PROPERTIES OF O-ETHYL-S-(β-ALKYLSULFOXYETHYL)METHYLTHIOPHOSPHONATES

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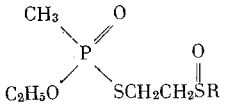
It is known that the transition from the O-ethyl-S-(β-alkylmercaptoethyl)methylthiophosphonates to the sulfonium derivatives is accompanied by a sharp increase in the anticholinesterase activity toward butyrylcholinesterase (BuCE) and acetylcholinesterase (ACE) [1]. One of the main reasons for this effect is an improvement in the conditions for sorption of the inhibitor on the active surface of the enzyme due to the coulomb interaction of the onium group with the anionic center. It was interesting to ascertain the anticholinesterase activity of analogous compounds that contain the sulfoxide grouping, which is a dipole, in the β-position of the alkyl radicals.

The available data on the inhibition of cholinesterase by the O,O-dimethyl- and O,O-diethyl-S-(β-ethylsulfoxyethyl)thiophosphates [2, 3] are contradictory and do not permit ascertaining the rules for the effect of the sulfoxide group on the anticholinesterase activity. In the present paper we report the synthesis of some O-ethyl-S-(β-alkylsulfoxyethyl)methylthiophosphonates (I) and their anticholinesterase activity



(I)

The (I) compounds were obtaindd by the oxidation of the corresponding O-ethyl-S-(β-alkylmercaptoethyl)methylthiophosphonates with H_2O_2 as described in [4]. The anticholinesterase activity of the (I) compounds was judged by the bimolecular rate constants when they are reacted with cholinesterases.

EXPERIMENTAL METHOD

The starting O-ethyl-S-(β-alkylmercaptoethyl)methylthiophosphonates were obtained by the alkylation of sodium O-ethyl methylthiophosphonate with the appropriate β-chloroethyl alkyl sulfides [5].

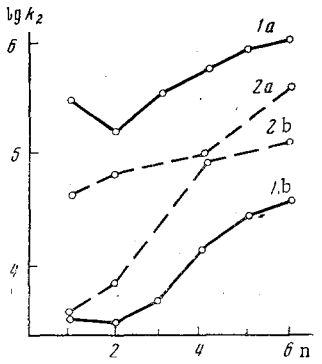


Fig. 1. Irreversible inhibition constant (k_2) for acetylcholinesterase (a) and butyrylcholinesterase (b) as a function of the length of the alkyl radical of the compounds: 1) CH_3-$(C_2H_5O)P(O)SCH_2CH_2SC_nH_{2n+1}$; 2) $CH_3(C_2H_5O)P(O)$-$SCH_2CH_2SC_nH_{2n+1}$.

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TABLE 1. O-Ethyl-S-(β-alkylsulfoxyethyl)methylthiophosphonates $CH_3(C_2H_5O)P(O)SCH_2CH_2SR$ (‖ O)

R	Yield, %	d_4^{20}	n_D^{20}	Found, %			Empirical formula	Calculated, %		
				C	H	P		C	H	P
CH_3 *	65	1,2506	1,5261	31,46	6,61	13,22	$C_6H_{15}PO_3S_2$	31,31	6,56	13,21
C_2H_5	73	1,2012	1,5242	35,08	7,21	12,44	$C_7H_{17}PO_3S_2$	34,42	7,01	12,68
C_3H_7	67	1,1830	1,5164	36,63	7,42	11,67	$C_8H_{19}PO_3S_2$	37,20	7,41	12,00
C_4H_9	86	1,1651	1,5155	39,53	7,99	10,87	$C_9H_{21}PO_3S_2$	39,70	7,77	11,40
C_5H_{11}	67	1,1431	1,5116	42,00	8,30	10,80	$C_{10}H_{23}PO_3S_2$	41,90	8,10	10,70
C_6H_{13}	70	1,1169	1,5090	43,70	8,60	9,80	$C_{11}H_{25}PO_3S_2$	44,00	8,40	10,30

* Bp 165-167° C(0.01 mm).

TABLE 2. Rate Constants for Inhibition of Cholinesterase by O-Ethyl-S-(β-alkylsulfoxyethyl)-methylthiophosphonates $CH_3(C_2H_5O)P(O)SCH_2CH_2SR$ (‖ O)

R	k_2, $mole^{-1} \cdot min^{-1}$	
	ACE · 10^5	BuCE · 10^5
CH_3	(2,86±0,15)	(2,95±0,10)
C_2H_5	(1,51±0,12)	(2,97±0,10)
C_3H_7	(3,20±0,20)	(4,35±0,13)
C_4H_9	(4,81±0,10)	(13,0±0,15)
C_5H_{11}	(7,24±0,16)	(27,5±0,11)
C_6H_{13}	(9,13±0,14)	(34,3±0,10)

Synthesis of O-Ethyl-S-(β-alkylsulfoxyethyl)methylthiophosphonates. With stirring, to a solution of 0.015 mole of the appropriate O-ethyl-S-(β-alkylmercaptoethyl)methylthiophosphonate in 8 ml of 50% aqueous MeOH at 40-45°C was added in drops 1.68 ml of 34% H_2O_2 solution. A drop of 50% H_2SO_4 solution was added as the catalyst. The mixture was stirred at 40° for 2 h, $NaHSO_3$ was added, the pH was adjusted to 7.5, K_2CO_3 was added, and the mixture was extracted with CH_2Cl_2. After removal of the solvent the residue was dried by distilling off the water with benzene and then kept in the vacuum of an oil pump for 1 h at 60-80°.

The purity of the obtained compounds was checked by TLC on SiO_2 in the system: 1:1 hexane–alcohol, with detection of the compounds employing iodine vapors. The results are given in Table 1.

Determination of Anticholinesterase Activity. As BuCE we used the purified preparation of horse blood serum (acylhydrolase of acylcholines, KF 3.1.1.8) manufactured by the I. I. Mechnikov Institute of Vaccines and Serums, and the partially purified ACE preparation of human blood erythrocytes (acetylhydrolase of acetylcholine KF 3.1.1.7).

As the substrate we used analytical grade acetylcholine iodide. The rate of the enzymatic hydrolysis of acetylcholine was determined by the method of measuring the initial rate of change in the pH [6] at 25° and at the optimum values of the pH and substrate concentrations (pH 7.55 and $[S] = 5 \cdot 10^{-4}$ M in the case of ACE and pH 7.8 and $[S] = 1 \cdot 10^{-2}$ M in the case of BuCE). All of the experiments were run 3-4 times.

The values of the bimolecular constants (k_2) were calculated using the equation:

$$k_2 = \frac{1}{t\,[\text{Ing}]} \ln \frac{v_0}{v_t}$$

where [Ing] is the inhibitor concentration; t is the incubation time; v_0 is the rate of the enzymatic hydrolysis of the substrate in the absence of inhibitor; and v_t is the same after t minutes of incubating the enzyme with the inhibitor. The obtained data are given in Table 2.

DISCUSSION OF RESULTS

The O-ethyl-S-(β-alkylsulfoxyethyl)methylthiophosphonates (I) are irreversible cholinesterase inhibitors. In all cases a certain decrease in the calculated k_2 values is observed with increase in the concentration [Ing], but the observed changes are extremely small and for this reason the components of the reversible inhibition [7] cannot be determined with sufficient reliability.

From Table 2 it can be seen that the anticholinesterase activity of the studied compounds depends on the length of the alkyl radical that is attached to the sulfoxide sulfur atom, in which connection they are much more active toward ACE than toward BuCE (Fig. 1). In the case of BuCE the quantity k_2 practically does not change when going from the methyl to the ethyl derivative, while in the case of ACE a certain decrease in it is even observed. For both enzymes an increase in the anticholinesterase activity is observed

with further increase in the length of the alkyl radical attached to the sulfoxide sulfur atom. This increase is expressed more sharply for BuCE than for ACE. Thus, for the first enzyme the increase in k_2 is 12 times when going from the ethyl to the hexyl derivative, and a total of 3.5 times for the second enzyme.

This difference in the effect of the chain length of the hydrophobic alkyl radical attached to the sulfoxide sulfur atom on the anticholinesterase activity toward ACE and BuCE is apparently associated both with a different topography of the hydrophobic sections in the vicinity of the anionic center and with a different relative role of these sections in the formation of the enzyme—inhibitor complex.

The (I) inhibitors, which contain a polar sulfoxide group, are apparently more specific toward ACE, since in ACE the anionic center is expressed quite distinctly and the hydrophobic sections have a smaller effect on the sorption of the inhibitor than in BuCE.

In both enzymes the values of k_2 for the O-ethyl-S-(β-alkylmercaptoethyl)methylthiophosphonates increase more sharply than for the (I) compounds with increase in the chain length of the alkyl radical (see Fig. 1). This indicates that the hydrophobic radicals play a more important role in the reaction of the inhibitor with the enzyme for compounds that do not contain a dipole grouping.

As was to be expected, the anticholinesterase activity of the (I) inhibitors, which have a polar sulfoxide grouping, toward ACE is considerably greater than that of the corresponding O-ethyl-S-(β-alkylmercaptoethyl)methylthiophosphonates. However, the opposite relation is observed toward BuCE. Only for the methyl derivatives of both series of inhibitors are the k_2 values nearly the same. For the higher homologs the k_2 values are noticeably higher for the sulfide type of compounds. The (I) inhibitors, which contain a polar sulfoxide group, are apparently sorbed more easily on the active surface of ACE than that of BuCE, since for the first enzyme the anionic point is expressed more distinctly. It is possible to assume that in ACE the dipole interaction of the sulfoxy group with the anionic point leads to an improvement of the conditions for sorption of the inhibitor on the active surface of the enzyme. It is probable that in BuCE, for which stronger hydrophobic interactions are characteristic, the polar sulfoxide groupings prevent to a certain degree the hydrophobic sorption of the inhibitor on the active surface of the enzyme. In view of this the sulfoxy derivatives are even less active than the sulfide inhibitors corresponding to them.

CONCLUSIONS

1. A number of O-ethyl-S-(β-alkylsulfoxyethyl)methylthiophosphonates was synthesized.

2. The synthesized compounds are more active inhibitors than the corresponding O-ethyl-S-(β-alkylmercaptoethyl)methylthiophosphonates for acetylcholinesterase, and less active for butyrylcholinesterase.

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