ANTICHOLINESTERASE PROPERTIES OF O-ETHYL-S-[β-DIALKOXYPHOSPHINYL]ETHYL]-METHYLTHIOPHOSPHONATES

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In an investigation of the anticholinesterase properties of O,O-diethyl-S-[β-(acrylmethylamino)ethyl]-thiophosphates (I) [1-4] and O-ethyl-S-[β-(aryloxyethyl)methylthiophosphonates] (II),

i.e., esters of thio phosphoric and thio phosphonic acids, containing a substituted aniline or phenol residue in the β-position of the thioester radical, forming dipole groups, which interact with the anionic center of the enzyme, differences were found within each series of compounds in the inhibiting ability with respect to butyrylcholinesterase (BuCE) and acetylcholinesterase (ACE). These differences are apparently determined by two factors: the polar influence of the substituent R and the sorbability of the aryl group on the hydrophobic portion situated behind the anionic center of the active surface of the enzyme. The ratio of these factors for the indicated enzymes proved to differ. The influence of the substituent R on hydrophobic sorption of the inhibitor molecule for BuCE was more significant than for ACE; the polar influence of the substituent on the ion–dipole interaction with the anionic center of the enzyme, on the other hand, played a deciding role for ACE and had less of an effect for BuCE.

It was of interest to determine whether the detected patterns would be preserved in the presence of a dipole group of a different type, for example, phosphoryl, in the β-position of the thioester residue of the inhibitor.

As the object of investigation we selected O-ethyl-S-[β-(dialkoxyphosphinyl)ethyl]methylthiophosphonates (III) [5]:

where R represents alkyl radicals of normal structure. For these compounds the rate constants of the interaction with butyrylcholinesterase ($k_{2BuCE}$) and acetylcholinesterase ($k_{2ACE}$) and the rate constants of nonenzymatic alkaline hydrolysis ($k_h$) were measured. The data obtained are cited in Table 1. To characterize the polar influences of the OR groups we used the constants $\sigma_0$ [7-10]: the influence of the OR groups on the sorbability was characterized by the values of $R_m$, found by the method of thin-layer chromatography 11, 12.

EXPERIMENTAL

To determine the rate constants of inhibition we used the following enzyme preparations; butyrylcholinesterase – partially purified preparation from coarse blood plasma produced by the Kashintsev Biological Institute of Heteroorganic Compounds, Academy of Sciences of the USSR; I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 4, pp. 814-817, April, 1969. Original article submitted May 8, 1968.
Fig. 1. Dependence of the rate constants of inhibition of ACE (a) and BuCE (b) by O-ethyl-S-[β-(dialkoxyphosphinyl)ethyl]-methylthiophosphonates on the value of $R_m$ of these compounds. The numeration of the points here and in Figs. 2 and 3 corresponds to the numeration of the substituents in Table 1.

Fig. 2. Dependence of the rate constants of inhibition of ACE (a) and BuCE (b) by O-ethyl-S-[β-(dialkoxyphosphinyl)ethyl]-methylthiophosphonates (III) on $\Sigma \sigma_\phi$ of the substituents OR.

Fig. 3. Ratio between the values of $R_m$ of O-ethyl-S-[β-(dialkoxyphosphinyl)ethyl]methylthiophosphonates (III) and $\Sigma \sigma_\phi$ of the substituents OR.

**TABLE 1. Dependence of the Anticholinesterase Properties of O-ethyl-S-[β-(dialkoxyphosphinyl)ethyl]methyl-**

<table>
<thead>
<tr>
<th>n</th>
<th>$k_{m1}$ liter/mole-min</th>
<th>$k_{m2}$ liter/mole-min</th>
<th>$k_{m3}$ liter/mole-min</th>
<th>$k_{m4}$ liter/mole-min</th>
<th>$k_{m5}$ liter/mole-min</th>
<th>$k_{m6}$ liter/mole-min</th>
<th>$R_f$</th>
<th>$\Sigma \sigma_\phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.64±0.04</td>
<td>3.4±0.4·10^{-4}</td>
<td>2.0±0.1·10^{-4}</td>
<td>0.33</td>
<td>0.33</td>
<td>-0.428</td>
<td>1</td>
<td>-0.428</td>
</tr>
<tr>
<td>2</td>
<td>0.49±0.16</td>
<td>1.2±0.2·10^{-4}</td>
<td>2.1±0.1·10^{-4}</td>
<td>0.39</td>
<td>0.39</td>
<td>-0.633</td>
<td>2</td>
<td>-0.633</td>
</tr>
<tr>
<td>3</td>
<td>0.42±0.02</td>
<td>6.4±0.4·10^{-4}</td>
<td>7.0±0.2·10^{-4}</td>
<td>0.46</td>
<td>0.46</td>
<td>-0.822</td>
<td>3</td>
<td>-0.822</td>
</tr>
<tr>
<td>4</td>
<td>0.31±0.03</td>
<td>1.2±0.2·10^{-4}</td>
<td>6.0±0.1·10^{-4}</td>
<td>0.55</td>
<td>0.55</td>
<td>-0.822*</td>
<td>4</td>
<td>-0.822*</td>
</tr>
</tbody>
</table>

* $\sigma_\phi$ of the $\text{OC}_2\text{H}_n$ group was calculated on the basis of the data obtained in [8].

Factory (acetylcholine acylhydrolase EC 3.1.1.8) acetylcholinesterase (acetylcholine acetylhydrolase EC 3.1.1.7) in the form of bovine erythrocyte stroma (30-fold purified preparation). The rate constants of the inhibition of these enzymes were measured in M/1500 phosphate buffer solution (for BuCE) and in a 0.01 N solution of KCl (for ACE) at pH 7.5 and 25°C according to the procedure described in [13, 14]. The rate constants of alkaline hydrolysis were determined by the usual method [15]. $R_f$ of the investigated compounds were determined by the method of thin-layer chromatography [11]; the carrier was KSK silica gel, the eluent - acetone. The values of $R_m$

$$R_m = \lg \left( \frac{1}{R_f} - 1 \right)$$

which are cited in Table 1, were calculated according to the $R_f$ values found.