Methylene chloride and methylene chloride—ethanol in mixtures of increasing polarity were used as eluents. The ratio of adsorbent to the mixture to be separated was 150:1. The three glycosides so obtained were crystallized from acetone, and the aglycon from ethanol. As a result, the following were obtained in the individual state: digitoxigenin, mp 235-239/250-256°C; digitoxin, mp 263-273°C; digitoxigenin bisdigitoxoside, mp 226-230°C, $[\alpha]_D^{21}$ +7.1 ± 2° (c 0.70; methanol), found, %: C 66.01, H 8.59 ($C_{35}H_{54}O_{10}$); and digitoxigenin monodigitoxoside, mp 195-200°C, $[\alpha]_D^{20}$ −5.6 ± 2° (c 0.65; methanol), found, %: C 68.86, H 8.72 ($C_{29}H_{44}O_7$).

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SYNTHESIS AND ANTICHOLINESTERASE PROPERTIES OF NEW DERIVATIVES OF LUPININE AND ELILUPININE

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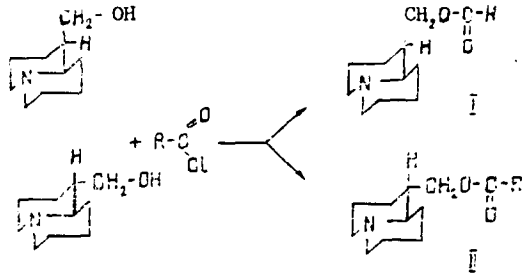
Esters of lupinine and epilupinine have been obtained by their acylation with isovaleroyl, cinnamoyl, trichloroacetyl, and trimethylacetyl chlorides. The methiodide derivatives of these esters have been investigated in reactions with the blood cholinesterases of warm-blooded animals. The interaction has a reversible nature, and the inhibition of the activity of the enzyme is both quantitative and qualitative in dependence on the structure of the acid taken and the conformational properties of the lupinine epimers.

Continuing investigations begun earlier [1], we have synthesized some new esters of lupinine (I) and epilupinine (II) and have studied the kinetics of the interaction of the compounds obtained with such blood enzymes of warm-blooded animals as acetylcholinesterase (ACE) and butyrylcholinesterase (BuCE). The scheme of the synthesis is given below:



where R = $-C(CH_3)_2$ (Ia, IIa); $-CH_2CH(CH_3)_2$ (Ib, IIb); $-CH=CH-C_6H_5$ (Ic, IIc); $-CCl_3$ (Id, IId)

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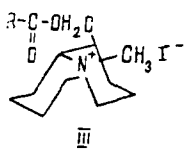
TABLE 1. Some Physicochemical Constants and Cholinergic Activities of the New Esters of Lupinine (I) and of Epilupinine (II)

Compound	n_D^{20}	R_f	Yield, %	Methiodide, mp, °C	$+[\alpha]_D^{20}$ (ethanol)	pK_i ACE	Ti	BuCE	Ti
Ia	1,4715	0,73	92	180—182	4,0	4,24	M	4,61	N
Ib	1,4677	0,79	79	149—151	5,33	4,31	M	4,84	U
Ic	1,2585	0,82	47	184—186	48,0	5,02	N	4,88	M
Id	1,3610	0,61	78	133—135	46,6	5,07	N	5,74	M
IIa	1,4755	0,78	81	156—158	16	4,65	M	4,84	M
IIb	1,4701	0,85	75	130—141	19,3	5,26	N	5,72	M
IIc	1,3550	0,75	54	152—154	56,0	5,82	M	5,18	M
IId	1,3553	0,69	70	Hygr.	84,0	3,68	M	3,89	M

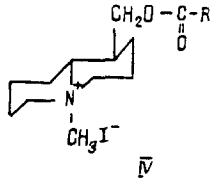
The letters indicate the types of inhibition (Ti): M) mixed; U) uncompetitive; N) noncompetitive.

To obtain the esters of (I) and (II) we used the chlorides of the following carboxylic acids: trimethylacetic, isovaleric, cinnamic, and trichloroacetic, which were obtained by the method of [2]. The reaction products were then converted into the corresponding methiodides. The physicochemical properties of the compounds obtained are characterized in Table 1.

Investigation of spatial models of lupinine and epilupinine methiodides [3] have shown that (III) has the cis- and (IV) the trans-linkage:



III



IV

These differences in structure, and also the optical activities of the compounds may exert an influence on the hydrolytic properties of the cholinesterases, which, as is known [4], exhibit a higher stereospecificity than other hydrolases. Peculiarities of the two types of cholinesterases have been shown previously with the aid of ephedrine and pseudoephedrine derivatives [5]. Table 1 shows the cholinergic activities of the lupinine and epilupinine esters (I and II) obtained. The sensitivity of the enzymes investigated increased with a change in R from (Ia) to (Id). This characteristic is shown particularly clearly in the case of ACE, the most effective in this series of compounds being (Id). It must be mentioned that with an increase in $[\alpha]_D$ the anticholinesterase activities of the lupinine derivatives increased. When the epilupinine derivatives are considered it is impossible to see the clear tendency to a rise in the anticholinesterase activities of the compounds (from (IIa) to (IId)) that was traced in the case of the lupinine esters. The highest activities for ACE and BuCE were possessed by substances (IIb) and (IIc). In the one case $[\alpha]_D$ was 19, and in the other it was 56. In contrast to the lupinine derivatives there was no regular rise of the activity of the substance with an increase in $[\alpha]_D$ here. It must be noted that substance (IId) with the highest $[\alpha]_D$ had the lowest anticholinesterase activity, this activity being characteristic for both hydrolases.

Thus, it may be concluded that in the case of lupinine the anticholinesterase activity depends on the structure of R and the values of the angles of rotation. Although the epilupinine derivatives were somewhat more active than the lupinine derivatives, here no regular rise in anticholinesterase activity with an increase in the optical density [sic] of the compounds synthesized was revealed. The results obtained indicate that in the structures of the active centers of ACE and BuCE there are identical binding sections responsible for interactions with derivatives both of lupinine and epilupinine.

EXPERIMENTAL

For general observations, see [1].

Lupinine Ester of Trimethylacetic Acid (Ia). With stirring and cooling (0-2°C), 2.4 g (0.02 mole) of trimethylacetyl chloride dissolved in 50 ml of absolute benzene was added dropwise over 1.5 h to a mixture of 2.4 g (0.02 mole) of lupinine and 2.02 g (0.02 mole) of dry triethylamine in 100 ml of absolute benzene.

The reaction mixture was stirred at 60-70°C for 3 h. The precipitate of triethylamine hydrochloride that had deposited was filtered off, the benzene was distilled off, and the residue was purified by column chromatography.

IR spectrum (ν, cm^{-1}): 1730 (—OCO—), 1280 (—COC—), 2840 (trans-quinolizidine system). The other esters were obtained similarly.

Compound (Ib). IR spectrum (ν, cm^{-1}): 1735 (—OCO—), 1170 (—COC—), 2670 (trans-quinolizidine system). PMR system (δ, ppm): 1.6-2.2 (14H, m, CH_2), 2.3-2.5 (1H, m, CH, J = 6.7 Hz), 2.71-2.73 (2H, d, $H_{2\bar{e}}$ and $H_{10\bar{e}}$, J = 10.6 Hz), 3.94-4.2 (2H, m, OCH_2).

Compound (Ic). IR spectrum (ν, cm^{-1}): 1740 (—OCO—), 1280 (—COC—), 2850 (trans-quinolizidine system). PMR spectrum (δ, ppm): 7.50 (1H, d, =CH—Ar, J = 17.0 Hz). 7.1-7.4 (5H, m, Ar—H), 6.28 (1H, d, —CO—CH, J = 17.0 Hz), 4.05-4.50 (2H, m, OCH_2), 2.79 (2H, d, $H_{2\bar{e}}$ and $H_{10\bar{e}}$, J = 10.6 Hz), 1.0-2.2 (14H, m, CH_2).

Compound (Id). IR spectrum (ν, cm^{-1}): 1750 (—OCO—), 1140 (—COC—), 2840 (trans-quinolizidine system) 980 (C—Cl).

Compound (IIa). IR spectrum (ν, cm^{-1}): 1735 (—OCO—), 1175 (—COC—).

Compound (IIb). IR spectrum (ν, cm^{-1}): 1735 (—OCO—), 1180 (—COC—). PMR spectrum (δ, ppm): 1.0-2.1 (14H, m, CH_2), 2.2-2.4 (1H, m, CH, J = 6.7 Hz), 2.71-2.72 (2H, d, $H_{2\bar{e}}$ and $H_{10\bar{e}}$, J = 10.6 Hz), 3.7-3.9 (2H, m, OCH_2).

Compound (IIc). IR spectrum (ν, cm^{-1}): 1730 (—OCO—), 1320 (—COC—). PMR spectrum, (δ, ppm): 7.48-7.51 (1H, d, =CH—Ar, J = 17.0 Hz), 7.2-7.34 (5H, m, Ar—H), 6.20-6.25 (1H, d, —CO—CH, J = 17.0 Hz), 3.5-3.8 (2H, m, OCH_2), 2.75-2.77 (2H, d, $H_{2\bar{e}}$ and $H_{10\bar{e}}$, J = 10.6 Hz), 1.1-2.0 (14H, m, CH_2).

Compound (IId). IR spectrum (ν, cm^{-1}): 1745 (—OCO—), 1140 (—COC—), 990 (C—Cl).

As the enzyme preparations we used human blood erythrocyte ACE (E.C. 3.1.1.7) with a specific activity of 2.7 U/mg and horse blood serum BuCE (E.C. 3.1.1.8) with a specific activity of 29 U/mg produced by the Perm Scientific-Research Institute of Vaccines and Sera. The efficacy of the compounds used was judged from the magnitude of the reciprocal inhibitory constant ($\bar{K}_i$) calculated graphically and expressed in the form of $p\bar{K}_i = -\log \bar{K}_i$. The type of inhibition of the enzymes was determined as described in [6].

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