SYNTHESIS OF SOME BIS(2-MORPHOLINO-1-METHYLETHYL) ESTERS OF CARBOXYLIC ACIDS AND STUDY OF THEIR INTERACTION WITH THE CHOLINESTERASES OF WARM-BLOODED ANIMALS

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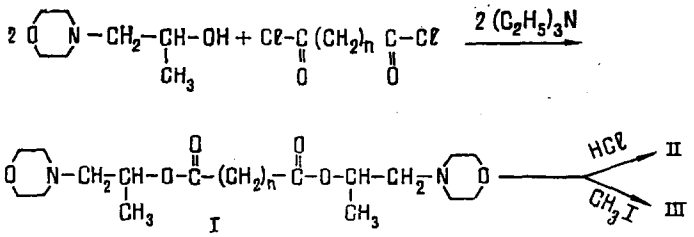
The bis(2-morpholino-1-methylethyl) esters of glutaric, adipic, azelaic, and sebacic acids have been synthesized, and their dihydrochlorides and dimethiodides have been obtained. The structures of the compounds obtained have been shown by their IR and PMR spectra. The interaction of the compounds synthesized with the acetylcholineresterase (ACE) of human blood erythrocytes and the butyrylcholinesterase (BuCE) of horse blood serum have been investigated. All the bis-esters are reversible competitive inhibitors of ACE and BuCE, while the dimethiodides exhibit specificity of their action with respect to ACE.

Depending on the structure of the cationic head and the length of the polymethylene chain, many bischoline esters of carboxylic acids exhibit various types of action on the nicotinic and muscarinic receptors, and therefore some of these compounds have found use in medical practice [1]. Dicholine esters of carboxylic acids are inhibitors of cholinesterases [2], although in the majority of cases they are hydrolyzed under the action of butyrylcholinesterase [3-5].

The present work begins systematic investigations of the dependence of the structure on the functions of analogs of bis-β-methylcholine esters of dicarboxylic acids with the aim of finding highly selective acetylcholinesterase inhibitors. Here we give information on the synthesis of the bis-2-morpholino-1-methylethyl) esters of dicarboxylic acids – glutaric (n = 3), azelaic (n = 7), adipic (n = 4), and sebacic (n = 8) – and their dimethiodides and dihydrochlorides, and also the results of the influence of the structure of these compounds on the enzymatic activity of human blood erythrocyte acetylcholinesterase (ACE) and horse blood serum butyrylcholinesterase (BuCE).

The substances were synthesized by the following scheme.



The physicochemical constants of compounds (I) and (II) are given in Table 1. The structures of the substances obtained were confirmed by their IR and PMR spectra. The IR spectra revealed absorption bands at 1045, 1130, and 1175 cm^{-1}, which are characteristic for the stretching vibrations of C–O–C bonds, while at 1725 cm^{-1} an intense band of the absorption of C=O groups was observed.

The PMR spectra of the bis derivatives of the corresponding acids are clearly in harmony with the proposed structures. The spectrum of compound (I) contained a multiplet at 4.98 ppm relating to two methine protons at ester groups. Its splitting was due to spin-spin coupling with neighboring methylene and methyl groups. A doublet of the corresponding groups

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TABLE 1. Physicochemical Properties of the Bis(2-morpholino-1-methylethyl) Esters of Dicarboxylic Acid and of Their Dihydrochlorides (II) and Dimethiodides (III)

n	I								II	III
	yield, %	bp. °C/mm Hg	d_4^{20}	n_D^{20}	MR_D found	MR_D calc.	empirical formula	R_f^{**}	R_f^{***}	mp, °C
3	53	190/7	1,0691	1,4762	101,87	101,4	$C_{19}H_{34}O_6N_2$	0,71	0,33	Hygroscope
4	36	198/7 mp 35–37°	—	—	—	—	$C_{20}H_{36}O_6N_2$	0,73	0,36	142
7	61	220/7	1,0451	1,4750	119,87	119,28	$C_{23}H_{42}O_6N_2$	0,75	0,41	170
8*	42		1,0330	1,4732	123,90	124,49	$C_{24}H_{44}O_6N_2$	0,78	0,44	172

*Purified chromatographically on a column of Al_2O_3 (activity grade II).
**Chromatographic mobilities determined on Al_2O_3 plates with the benzene–ether–ethanol (10:5:2) system. Revealing agent: iodine vapor.
***Paper chromatography. Benzene–acetic acid–water (100:15:27) system; Leningrad rapid paper.

TABLE 2. Anticholinesterase Efficiencies of the Dihydrochlorides (II) and Dimethiodides (III) of Bis(2-morpholino-1-methylethyl) Esters of Dicarboxylic Acids

n	II		III	
	K_i, (M)			
	ACE	BuCE	ACE	BuCE
3	—	—	$(8,6\pm0,01)\cdot10^{-4}$	$(3,2\pm0,07)\cdot10^{-4}$
4	$(4,3\pm0,07)\cdot10^{-3}$	$(8,2\pm0,04)\cdot10^{-4}$	$(1,6\pm0,01)\cdot10^{-5}$	$(1,2\pm0,12)\cdot10^{-4}$
7	$(4,3\pm0,07)\cdot10^{-4}$	$(1,8\pm0,16)\cdot10^{-4}$	$(4,6\pm0,6)\cdot10^{-6}$	$(2,6\pm0,03)\cdot10^{-4}$
8	$(1,2\pm0,09)\cdot10^{-5}$	$(8,2\pm0,04)\cdot10^{-5}$	$(6,4\pm0,18)\cdot10^{-6}$	$(7,9\pm0,03)\cdot10^{-5}$

(J = 7.0 Hz) was located at 1.14 ppm. An intense triplet (8 H) at 3.51 ppm belonged to the OCH_2 protons of morpholine rings. All the $N-CH_2$ protons resonated in the 2.1-2.6 ppm region. The absence from this region of a sharp triplet analogous to the signal of the OCH_2 protons observed in the spectrum of morpholine itself and its derivative indicated the nonequivalence of the $N-CH_2$ protons of the morpholine ring. In its turn, the sharp doublet theoretically possible for the $N-CH_2$ protons of the substituent was absent, which indicated the nonequivalence of these protons, as well. Apparently, the nonequivalence was due to the presence of a neighboring asymmetric center. The signal of the methylene protons at the carbonyls of the ester groups was located at 2.22 ppm in the form of a triplet because of spin-spin coupling with the neighboring methylene group resonating at 1.95 ppm.

In the spectra of the other bis-derivatives of the acids there was an additional comparatively narrow signal at 1.3 ppm the intensity of which was proportional to the number of methylene groups betwen the carbonyl carbons. The signal of the $\rangle\overset{+}{N}-CH_3$ protons at 3.16 ppm were characteristic for the methiodides.

In view of the fact that the carboxylic acid bis(2-morpholino-1-methylether) esters that had been synthesized proved to be sparingly soluble in water, while the investigation of the catalytic properties of ACE and BuCE is carried out in an aqueous medium, the bis-esters obtained were converted into the corresponding dihydrochlorides and dimethiodides. An investiation of the influence of these substances on the hydrolyzing properties of ACE and BuCE (Table 2) showed that they were all reversible competitive inhibitors of both types of esterases, the hydrochlorides being less active than the corresponding methiodides.

It must be mentioned that the smaller the value of K_i the more pronounced was the anti-enzyme activity of the substance, i.e., the stronger the reversible enzyme-inhibitor complex that was formed.

With an increase in the number of methylene groups in the dihydrochlorides from n = 4 to n = 8, K_i fell by a factor of 84.2 for ACE. This correlation of the change in K_i with the length of the polymethylene chain also applied, although in less pronounced form to the case of their interaction with BuCE. No appreciable specificity of the action of these compounds in relation to BuCE was detected.

For the methiodides, the relationship between the length of the polymethylene chain and the anticholinesterase efficiency had a somewhat different nature. Quaternization of the nitrogen atoms present in the morpholine rings of the bis-esters led to a sharp fall in K_i for the reactions with ACE: for the adipate, 52-fold, for the azelate, 100-fold, and for the sebacate 2-fold in comparison with the corresponding dihydrochloride. A change in the number of methylene groups in the dimethiodides also affected their antienzyme, which differed somewhat from the dihydrochlorides of the bis-esters. While in the case of the dihydrochlorides a lengthening of the polymethylene chain increased the antiacetylcholinesterase activity uniformly on passing from the adipate to the sebacate, the results of the action of the dimethiodide had a jump-like nature; the passage from the adipate to the azelate was accompanied by a 3.5-fold rise in K_i. On comparing these results with the analogous results for the dihydrochlorides it can be observed that the dependence of K_i on n for the dimethiodides was less pronounced.

The nature of the interaction of the dimethiodides with BuCE was almost comparable with that of the dihydrochlorides.

Here it must be mentioned that no particular specificity with respect to ACE or BuCE was observed for the dihydrochlorides, while the dimethiodides of the bis-esters of azelaic, adipic, and sebacic acids exhibited a selectivity of their action in relation to ACE.

Thus, the regular rise in the anticholinesterase activity with a lengthening of the polymethylene chain in the dihydrochlorides of the dicarboxylic acid bis(2-morpholino-1-methylethyl) esters for ACE and BuCE can be explained by an enhancement of the hydrophobic interaction between the polymethylene groups and the corresponding sections of the active surface of these enzymes. The observed specificity of the action of the hydrochlorides in relation to BuCE is connected with the fact that a hydrophobic interaction is characteristic for this cholinesterase.

The high reversible inhibiting activity of the dimethiodides with respect to ACE is completely explicable, since ion-ion interaction is characteristic for ACE [6]. Apparently, the appearance of a stable positive charge on the two morpholine nitrogen atoms sharply increases the affinity of the methiodides for ACE, in which, as is known [7], the anionic sections on the active surface are strongly expressed. The fact that these compounds were not hydrolyzed under the action of BuCE agrees well with the known facts that acetyl-β-methylcholine inhibits the activity of these enzymes [8], while all the bis-esters of the series synthesized are structural analogs of acetyl-β-methylcholine.

EXPERIMENTAL

Synthesis of Bis(2-morpholino-1-methylethyl) Adipate. A three-necked flask was charged with 7.34 g (0.04 mole) of adipoyl dichloride [9], and this was dissolved in 200 ml of absolute ether. With cooling, a mixture of 11.44 g (0.08 mole) of N-β-hydroxypropylmorpholine and 8.08 g (0.08 mole) of dry triethylamine dissolved in 50 ml of absolute ether was added slowly, dropwise. The precipitate of triethylamine hydrochloride that deposited was filtered off, the ether was distilled off, and the residue was purified by column chromatography on alumina (Al_2O_3, activity grade II). The eluent was benzene–ether–ethanol (100:20:5). The corresponding dimethiodide and dihydrochloride were obtained by the action of 0.4 g (0.0034 mole) of methyl iodide on a methanolic solution of 0.4 g of the bis(2-morpholino-1-methylethyl) adipate, and by its treatment with hydrogen chloride, respectively. The other bischoline esters were obtained similarly.

Methods of Investigation. PMR spectra were taken on a Varian XL 2200 NMR spectrometer with a working frequency of 200 MHz. The samples were 10% solutions of the compounds under investigation in carbon tetrachloride. HMDS was used as internal standard.

IR spectra were taken on a specord 71-IR instrument, with CCl_4 as solvent.

The Catalytic Activities of ACE and BuCE were determined by Ellman's method [10] from the rate of hydrolysis of thiocholine substrates (acetylthiocholine and butyrylthiocholine,

respectively). The rate of hydrolysis of the substrates was determined with the aid of a KFK-2 UKhL 4,2 photoelectric colorimeter at a wavelength of 400 nm. Enzymatic activity was determined in phosphate buffer, pH 7.5, at 25°C.

The type of reversible inhibition and the inhibition constants (K_i) were determined by the Lineweaver-Burk method [11].

The enzymes used were purified preparations of ACE (activity 3.5 units/mg) and BuCE (activity 9.6 units/mg) produced by the Perm Scientific Institute of Vaccines and Sera. The acetylthiocholine and butyrylthiocholine, and also the 5,5-dithiobis(nitrobenzoic acid) were commercial preparations.

SUMMARY

The bis(2-morpholino-1-methylethyl) esters of glutaric, adipic, azelaic, and sebacic acid have been synthesized and their dimethiodides and hydrochlorides have been obtained. It has been shown that they are all reversible competitive inhibitors of ACE and BuCE. The dimethiodides of the bis-esters possess selectivity of their action in relation to ACE.

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