SYNTHESIS AND ANTIACETYLCHOLINESTERASE PROPERTIES OF CARBAMATES OF MONO- AND BICYCLIC ALCOHOLS OF PYRROLIDINE SERIES

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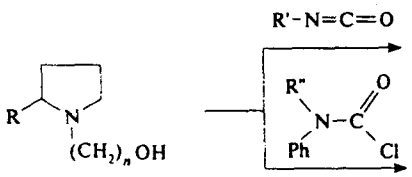
By now, a large body of experimental data has been accumulated on the structure of the active surface of acetylcholinesterases and the mechanisms of their inhibition (mostly by organophosphorus compounds) [1 – 3]. However, compounds of the pyrrolidine group have been insufficiently studied with respect to their antiacetylcholinesterase activity. As is known, the pyrrolidine and dihydropyrimidine fragments enter into the composition of highly-active natural inhibitors of cholinesterases, such as physostigmine and desoxypeganin [4].

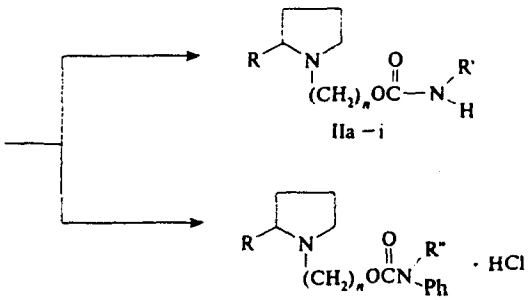
We expected that introducing alkyl subsituents at the second carbon atom of pyrrolidine ring would produce some increase in the basicity and anticholinesterase activity of carbamates of pyrrolidine alcohols. It was also of interest to study analogous derivatives including a bicyclic fragment with a hexahydropyrimidine ring annelated to the pyrrolidine ring, because these structures contain two nitrogen atoms capable of protonation under the intact organism conditions.

In this paper, we present the results on the synthesis and the *in vitro* anticholinesterase activity of carbamates of 2-alkyl(phenyl)-N-(ω-hydroxyalkyl)pyrrolidines (IIa – i; IIIa – c) and 9-alkyl-5-(2-hydroxyethyl)-1,5-diazabicyclo[4.3.0]-nonanes.

The initial ω-(2-alkyl-N-pyrrolidino)alkanols Ia – g were synthesized as described in [5]. The carbamates of compounds Ia – g, IIa – i, and IIIa – c were obtained by two methods: (i) acylation of Ia – g by butyl-, phenyl-, benzyl-, and 2,4-dichlorophenylisocyanates and (ii) reaction of Ia – g with methyl(ethyl)phenylcarbamoyl chlorides.



Ia – g



Ia: R = C_3H_7, $n = 2$;
b: R = C_3H_7, $n = 3$;
c: R = C_4H_9, $n = 2$;
d: R = C_5H_{11}, $n = 2$;
e: C_5H_{11}, $n = 3$;
f: R = C_6H_{13}, $n = 2$;
g: R = Ph, $n = 2$.

IIa: R = C_3H_7, R' = Ph, $n = 2$;
b: R = C_3H_7, R' = $C_6H_5CH_2$, $n = 2$;
c: R = C_3H_7, R' = $C_6H_5CH_2$, $n = 3$;
d: R = C_4H_9, R' = Ph, $n = 3$;
e: R = C_5H_{11}, R' = C_4H_9, $n = 2$;
f: R = C_5H_{11}, R' = $C_6H_3Cl_2$, $n = 2$;
g: R = C_5H_{11}, R' = $C_6H_5CH_2$, $n = 3$,
h: R = C_6H_{13}, R' = $C_6H_5CH_2$, $n = 2$;
i: R = C_6H_5, R' = $C_6H_5CH_2$, $n = 2$.

IIIa: R = C_3H_7, R" = C_2H_5, $n = 3$;
b: R = C_3H_7, R" = CH_3, $n = 4$;
c: R = C_4H_9, R" = CH_3, $n = 2$.

5-Hydroxyethyl-1,5-diazabicyclo[4.3.0]nonanes (Va and b) were synthesized by interaction of 9-alkyl-1,5-diazabicyclo[4.3.0]nonanes (IVa and b) with excess of ethylene oxide at 100 – 120°C. Acylation of these compounds by methylphenylcarbamoyl chloride yielded carbamates VIa and b. The initial diazabicyclononanes IVa and b were obtained by the method described elsewhere [6].

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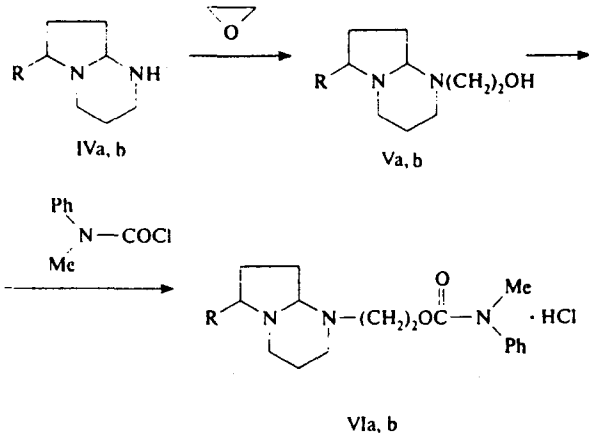


IVa, b

Va, b

VIa, b

IVa – VIa: R = C_3H_7; IVb – VIb: R = C_7H_{15}.

Spectroscopic characteristics of synthesized compounds corresponded to the proposed structures. The IR spectra of compounds Va and Vb exhibited intensive absorption bands at 3400 – 3200 cm^{-1} attributed to the stretching vibrations of associated OH groups. In the spectra of carbamates II, III, VI, the most pronounced bands are those of carbonyl group. In the N-monosubstituted compounds (II), these bands are observed in the region of 1736 – 1725 cm^{-1}, and in the disubstituted compounds (III and VI) at 1715 – 1710 cm^{-1}. The ^{13}C NMR spectrum contains signals due to carbon atoms of the pyrrolidine ring and substituents at the N-1 and C-2 positions. For example, the spectra of IIa and IIb show the following signals (ppm): 57.17 (C-2), 29.95 (C-3), 21.82 (C-4), 53.72 (C-5), 40.49 (C-6), and 64.49 (C-7). The signals of alkyl substituents in the aza heterocycle correspond to a strong field of 20.30 – 14.07 ppm.

EXPERIMENTAL CHEMICAL PART

The IR absorption spectra were recorded on UR-20 and IKS-29 spectrophotometers using samples prepared as suspensions in vaseline oil and hexachlorobutadiene. The ^{13}C NMR spectra were measured on a Varian FT-80A spectrometer using $CDCl_3$ as solvent and HDMS as the internal standard.

The individuality of synthesized compounds was checked by thin-layer chromatography on Silufol UV-254 plates using the ethanol – ethyl ether – chloroform system (5 : 1 : 1).

The properties of synthesized compounds are listed in Table 1. The results of elemental (C, H, N) analyses of compounds IIa – i, IIIa – c, Va – c, VIa, and b agreed with calculations.

9-Alkyl-5-(2-hydroxyethyl)-1,5-diazabicyclo[4.3.0]nonanes (Va, b). A mixture of 2 mmole IVa or IVb and 3 mmole of ethylene oxide is placed in an autoclave and heated at 100 – 120°C for 1.5 – 2 h. After cooling, the viscous reaction mass is dissolved in ethyl ether, the ether is evaporated, and the residue is distilled in vacuum to obtain Va or Vb.

N-butyl-, phenyl-, benzyl-, and 2,4-dichlorophenylcarbamates of 2-alkyl(phenyl)-N-(hydroxyalkyl)pyrrolidines (IIa – i). To a mixture of 15 mmole of aminoalcohol I in 30 ml of absolutized toluene is added with stirring and heating 15 mmole of butyl-, phenyl-, or 2,4-dichlorophenylisocyanate. The mixture is heated at 70 – 75°C for 2 h, cooled, and extracted with 20% aqueous HCl. The aqueous solution is neutralized with an alkali to pH 7, extracted with $CHCl_3$, and dried over Na_2SO_4. Finally, the solvent is evaporated and the residue is distilled in a nitrogen flow under vacuum to obtain compounds IIa – i.

N,N-Methyl(ethyl)phenylcarbamates of 2-alkyl-N-(2-hydroxyalkyl)pyrrolidines and 9-alkyl-5-(2-hydroxyethyl)-1,5-diazabicyclo[4.3.0]nonanes (IIIa – c, VIa, b). To a mixture of 10 mmole of aminoalcohol I, Va, or Vb is added 15 mmole methyl(ethyl)phenylcarbamoyl chloride. The mixture is heated at 120 – 125°C for 2.5 – 3 h. Upon cooling, the solid mass is poured over with ethyl ether and allowed to stand at 5 – 8°C for 2 – 4 days. The crystalline product is purified by repeated washing with an ethanol – chloroform (1 : 1) and ethanol – ethyl ether – chloroform (1 : 1 : 1) mixtures to obtain compounds IIIa – c, VIa and b.

EXPERIMENTAL BIOLOGICAL PART

The inhibiting activity of the synthesized compounds (at concentrations from 10^{-3} to 10^{-6} M) with respect to enzymatic hydrolysis of acetylcholine, induced by acetylcholinesterase extracted from human blood erythrocytes (KF 3.1.1.7), was studied by the method of potentiometric titration [7] performed in a phosphate buffer of 6 mM at pH 7.0 and a temperature of 25°C in the presence of 0.1 M KCl.

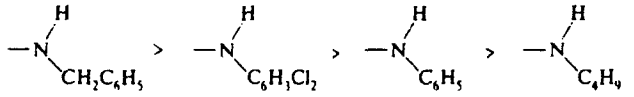
The anticholinesterase activity was evaluated as pI_{50}, which is a negative logarithm of the inhibitor concentration

TABLE 1. Characteristics of Synthesized Compounds

Compound	Yield, %	T_b, °C / Torr	Empirical formula	pI_{50}
IIa	70	238 – 240/2	$C_{16}H_{24}N_2O_2$	2.6
IIb	70	249 – 251/2	$C_{17}H_{26}N_2O_2$	3.0
IIc	73	201 – 204/1	$C_{18}H_{28}N_2O_2$	2.8
IId	69	238 – 240/2	$C_{17}H_{26}N_2O_2$	3.0
IIe	71	211 – 214/2	$C_{16}H_{32}N_2O_2$	2.9
IIf	73	219 – 222/1	$C_{18}H_{26}N_2O_2Cl_2$	3.6
IIg	69	218 – 221/1	$C_{20}H_{32}N_2O_2$	2.9
IIh	69	225 – 228/1	$C_{21}H_{34}N_2O_2$	2.1
IIi	70	189 – 200/1	$C_{20}H_{24}N_2O_2$	3.8
IIIa	74	93 – 95	$C_{19}H_{31}N_2O_2Cl$	3.6
IIIb	73	94 – 96	$C_{19}H_{31}N_2O_2Cl$	3.4
IIIc	70	89 – 91	$C_{18}H_{29}N_2O_2Cl$	4.0
Va	61	147 – 149/3	$C_{12}H_{24}N_2O$	–
Vb	63	178 – 180/3	$C_{16}H_{32}N_2O$	–
VIa	68	78 – 81	$C_{20}H_{32}N_3O_2Cl$	4.5
VIb	70	87 – 89	$C_{24}H_{40}N_3O_2Cl$	4.7

reducing the catalytic activity of the enzyme to half the initial value.

The anticholinesterase activities of the synthesized compounds and their structural formulas are listed in Table 1. Among the carbamates of pyrrolidylalkanols (IIa – i), containing butyl-, phenyl-, benzyl-, and dichlorophenyl substituents at the carbamate nitrogen, the most efficient inhibitors are represented by compounds with a benzyl radical (IIi, pI_{50} 3.8). The anticholinesterase activity of compounds II decreases in the following sequence:



The maximum inhibiting activity (pI_{50} 3.4 – 4.0) was observed for compounds IIIa – c, disubstituted at the carbamate nitrogen.

Increasing the number of methylene groups between the cationic and estrase fragments of the inhibitor from 2 to 4 in compounds IIa – i and IIIa – c produces a decrease in the anticholinesterase activity. Apparently, the structure of inhibitor with $n = 2$ most adequately models the structure of acetylcholine and favors its immobilization on the active surface of the enzyme. Carbamates of diazabicyclononanes VIa and VIb have a structure of the carbamide group analogous to that of carbamates of pyrrolidine alcohols IIIa – c, but differing from these by the structure of cationic center. The former compounds exhibit a ten times higher inhibition activity: pI_{50} 4.5 – 4.7 for compounds VI against 3.4 – 4.0 for compounds III.

We have studied correlations between the main properties of pyrrolidine alcohols, 5-(hydroxyethyl)-diazabicyclononanes, and their carbamates, on the one hand, and the anticholinesterase activity, on the other. The basicity of compounds was determined by potentiometric titration in anhydrous acetic acid. We have also calculated the ionization constants of compounds I, II, V, and VI. No clear correlation was found between the anticholinesterase effect and the basicity of the compounds studied. Alcohols and their carbamates, markedly differing in their anticholinesterase activities, exhibit close pK_A values (9.63 – 10.35) [8]. The basicity is determined primarily by the character of nitrogen-containing part of the compound and by the length of the carbon chain between nitrogen atoms and the hydroxy or carbamate group: these fragments are identical in the compounds studied.

The anticholinesterase activity is apparently mostly determined by the character of the acidic fragment in the molecule, the nature of substituents at the carbamate nitrogen, the spatial orientation of the substituents in the hydrophobic part of the enzyme, and some others. The search for anticholinesterase substances in the series of compounds disubstituted at the carbamate nitrogen atom seems to give most promising results in derivatives of 9-alkyl-5-hydroxyethyl-1,5-diazabicyclo[4.3.0]nonanes.

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