ANABASINE AND CYTISINE DERIVATIVES AS REVERSIBLE CHOLINESTERASE INHIBITORS

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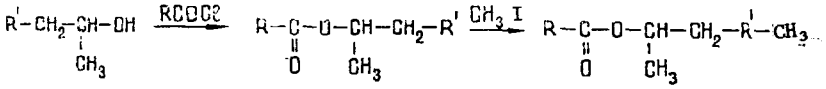
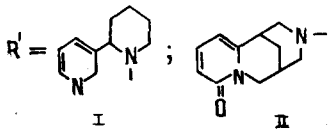
A number of analogs of acetyl-β-methylcholine, containing residues of the alkaloids anabasine and cytisine as cyclic ammonium groupings, have been synthesized. The structures of the substances obtained have been confirmed by their IR and PMR spectra and the result of elementary analysis. The kientic parameters of the interaction of the compounds synthesized with acetylcholinesterase (ACE) of human blood erythrocytes and with butylcholinesterase (BuCE) of horse blood serum have been investigated. All the substances synthesized proved to be reversible inhibitors of ACE and BuCE.

A number of acyl analogs of acetyl-β-methylcholine containing piperidine residues have, as we have shown [1], proved in the main to be reversible cholinesterase inhibitors. Continuing the search for specific substrates and inhibitors, we have synthesized derivatives of acyl-β-methylquinolines containing as cyclic ammonium groupings residues of the alkaloids anabasine (I) and cytisine (II) and their methiodides by the following scheme:



Where R = a) CH_3-, b) C_2H_5-, c) C_3H_7-, d) C_4H_9-, e) iso-C_4H_9-;



I II

The carboxylic acid chlorides, the N-(β-hydroxypropyl)anabasine and -cytisine and their methiodides were obtained by known methods [2, 3]. The compounds synthesized were characterized by their IR and PMR spectra. The characteristics of compounds (I) and (II) are given in Table 1.

We studied the interaction of compounds (I) and (II) with human blood serum acetylcholinesterase (ACE) and with horse blood serum butyrylcholinesterase (BuCE). The results of the experiments on the interaction of the enzymes with compound (I) and (II) are given in Table 2.

All the anabasine and cytisine derivatives proved to be reversible inhibitors of ACE and BuCE. It was impossible to detect the hydrolysis of the compounds synthesized even at concentrations of ACE and BuCE more than ten times greater than those that are used in the hydrolysis of acetylcholine and butyrylcholine. The anabasine acylates suppressed ACE by the noncompetitive type of action, while the inhibition of BuCE bore a competitive nature.

The anabasine dimethiodide derivatives possessed a more pronounced inhibiting action than the bases. The latter inhibited the two enzymes at almost the same rate, and their antienzymatic activity depended little on the number of carbon atoms in the acyl moiety of the ester grouping. The methiodides suppressed the enzymatic activity of ACE and BuCE almost identically, but here a definite dependence of the activity of the inhibitors on the structures of the acyl radical was shown: with an increase in the length of R up to C_3H_7 the reactivity of the compounds rose, and then it fell for C_4H_9. In the case of ACE, the acetyl

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TABLE 1. Physicochemical Constants of N-(β-Acyloxypropyl)anabasines (I) and N-β-(Acyloxypropyl)-cytisines (II)

Compound*	Yield, %	n_D^{20}	R_f^{**}
Ia	75	1,5162	0,82
Ib	67	1,5185	0,87
Ic	72	1,5197	0,85
Id	69	1,5208	0,89
Ie	70	1,5173	0,87
IIa	78	1,5282	0,86
IIb	72	1,5330	0,91
IIc	67	1,5350	0,96
IId	70	1,5362	0,90
IIe	69	1,5386	0,89

*Elementary compositions of all the substances obtained corresponded to the calculated figures.
**Benzene–ether–methanol (10:5:2) system.

TABLE 2. Reversible Anticholinesterase Activities (K_i) of N-(β-Acyloxypropyl)anabasines (I) and Their Methiodides and of N-(β-Acyloxypropyl)cytisines (II) and Their Methiodides

Compound	K_i (M) 10^{-4}			
	ACE	BuCE	ACE	BuCE
Ia	75,0±0,04	58,0±0,01	4,1±0,08	0,11±0,08
Ib	80,0±0,02	1,6±0,09	0,061±0,02	2,4±0,06
Ic	2,3±0,01	3,8±0,06	7,8±0,04	3,7±0,02
Id	37,0±0,07	4,5±0,01	50,0±0,08	5,1±0,04
Ie	91,0±0,03	9,5±0,06	35,0±0,06	0,30±0,04
IIa	2,1±0,2	4,5±0,01	0,084±0,1	0,063±0,08
IIb	3,8±0,01	5,3±0,03	8,1±0,04	0,043±0,02
IIc	3,0±0,05	6,8±0,08	0,051±0,01	0,078±0,06
IId	73,0±0,09	30,0±0,01	6,3±0,06	6,0±0,08
IIe	95,0±0,07	4,5±0,06	2,8±0,05	5,7±0,01

*Action of the corresponding methiodides.

derivative exhibited a pronounced activity, while in the case of BuCE the propionyl derivative did so. The butyryl derivative was ten times more active in relation to ACE and BuCE than the acetic acid ester. The β-methylcholine analogs of anabasine that were synthesized had less pronounced inhibiting properties than the choline analogs described previously [4] among which there were specific inhibitors of BuCE.

As follows from the figures of Table 2, the acyl derivatives of cytisine (II) were also reversible inhibitors of ACE and BuCE, but their efficiency was far lower than that of the anabasine derivatives. The absence of hydrolysis of the bases is in complete agreement with the incapacity of cholinesterase for hydrolyzing cyclic amino alcohol bases that has been observed previously [5].

On the whole, all the cytidine derivatives were more active inhibitors of BuCE than of ACE.

The inhibition of ACE by the cytisine and anabasine derivatives synthesized can be explained by the fact that, according to PMR spectroscopy, these compounds are present in the form of two stereoisomers, which leads to an inhibition of the catalytic activity of the enzyme [6]. This was not observed in a case [7] where cytisine contained fragments of acetylcholine in its molecule.

EXPERIMENTAL

IR spectra were taken on a Specord 71-IR instrument (GDR) in paraffin oil, and PMR spectra on a Varian XL-200 instrument (USA) in CCl_4 with HMDS as standard.

Synthesis of N-(β-Acyloxypropyl)anabasines. With cooling and stirring, 0.02 mole of the chloride of the appropriate carboxylic acid was added slowly to a mixture of 4.4 g (0.02 mole) of N-β-hydroxypropylanabasine and 2.02 g (0.02 mole) of dry triethylamine dissolved in 100 ml of absolute benzene. The mixture was stirred at room temperature for 2 h; the resulting precipitate of triethylamine hydrochloride was filtered off, the benzene was distilled off, and the residue was purified on a column of Al_2O_3 (activity grade II) with dry ether as eluent. The bases obtained were converted by the action of freshly distilled CH_3I into the corresponding methiodides. All the cytosine derivatives were synthesized in the same way.

Methods of Investigation. The catalytic activity of ACE (EC 3.1.1.7) and of BuCE (EC 3.1.1.8) were determined by Ellman's method [8] from the rate of hydrolysis of acetylthiocholine (ATC) for CE and of butyrylthiocholine (BTC) for BuCE with the aid of a KFK-2 UKhL-42 photoelectric colorimeter at a wavelength of 400 nm. Esterase activity was determined at pH 7.5 and a temperature of 25°C. The anticholinesterase activities of the anabasine and cytosine derivatives were evaluated from the magnitude of the inhibition constant K_i, found by the Lineweaver–Burk method [9].

Enzymes. Purified preparations of ACE (activity 3.5 units/mg) and BuCE (activity 9.5 units/mg) produced by the Perm Scientific-Research Institute of Vaccines and Sera were used.

Substrates. The ATC, BTC, and 5,5-dithiobis-(2-nitrobenzoic acid) were commercial preparations.

The IR spectrum of N-(β-acetoxypropyl)anabasine contained the following absorption bands (ν, cm^{-1}): 300 (C–H); 1760 (C=O); 1510 (C–N); 1460 (CH_2); 1020 (C–O–C). Similar absorption bands appeared in the spectra of the other N-(β-acyloxypropyl)anabasines.

The IR spectrum of N-(β-hydroxypropyl)cytosine was characterized by the following features (ν, cm^{-1}): 2910-2865 (trans-quinolizidine); 1820 (C=O); 1515 (C–N); 1060 (C–O–C).

The PMR spectra of N-(β-acetoxypropyl)anabasine and N-(β-acetoxypropyl)cytosine were characterized by a double set of signals, which indicated the presence in solution of two stereoisomers of the substances under investigation with unequal concentrations and, therefore, below we give the ranges of chemical shifts of the signals. PMR spectrum of N-(β-acetoxypropyl)anabasine, δ, ppm: 8.3-8.5 (H_α and H_α:2H); 7.45-7.6 (H_γ); 7.1-7.2 (H_β); 4.75-5.04 (H_f); 3.2-3.4 (H-6e); 2.9-3.05 (H-2a), 1.96 and 1.84 (C(=O)–CH_3, 3H); 1.01 and 0.94 (–C–CH_3, 3H).

The PMR spectrum of N-(β-acetoxypropyl)cytosine had the following signals of protons, δ, ppm: 7.1 (1H dd, H-4), 6.14 (1H, d, J = 10 Hz, H-3), 5.5 (1H, d, J = 7.6 Hz, H-5); 4.83 (H, m), 3.86 (1H, d, J = 19 Hz, H-10); 3.6 (1H, dd, J = 19 Hz, J = 6 Hz, H-10a), 1.72, 3H-S, C(=O)–CH_3; 0.9, 3H-α, J = 6.5 Hz, C–CH_3.

CONCLUSIONS

Derivatives of acetyl-β-methylcholine have been synthesized from the alkaloids anabasine and cytosine. It has been shown that the anabasine and cytosine derivatives obtained are reversible inhibitors of ACE and BuCE. A change in the structure of the acyl radical does not fundamentally affect the inhibition of ACE and BuCE.

LITERATURE CITED

1. A. G. Dobren'kov, Z. Tilyabaev, D. N. Dalmov, and A. A. Abduvakhabov, Khim. Prir. Soedin., 763 (1986).
2. A. A. Abduvakhabov, A. A. Sadykov, D. N. Dalimov, and Kh. A. Aslanov, Alkaloids and Their Derivatives as Tools for Studying the Cholinergic System [in Russian], FAN, Tashkent (1984), p. 288.
3. A. A. Abduvakhabov, Kh. A. Aslanov, and A. S. Sadykov, Dokl. Akad. Nauk UzSSR, No. 4, 40 (1976).

4. Yu. R. Khakimov, E. B. Maizel', E. V. Rozengart, A. A. Abduvakhabov, A. A. Sadykov, and Kh. A. Aslanov, Dokl. Akad. Nauk UzSSR, No. 1, 45 (1978).
5. B. S. Zhorov, E. V. Rozengart, V. A. Govyrin, A. K. Kasymov, A. A. Abduvakhabov, Sh. K. Kasymov, Kh. A. Aslanov, and A. S. Sadykov, Dokl. Akad. Nauk SSSR, 228, No. 6, 1460 (1976).
6. E. B. Maizel', E. V. Rozengart, Yu. R. Khazimov, A. A. Abduvakhabov, and Kh. A. Aslanov, Biokhimiya, 43, No. 7, 1150 (1978).
7. A. S. Sadykov, E. V. Rozengart, A. A. Abduvakhabov, and Kh. A. Aslanov, Cholinesterases: The Active Site and the Mechanism of Their Action [in Russian], FAN, Tashkent (1976).
8. G. L. Ellman, R. D. Ceuntey, V. Anders, and K. M. Reather, Biochem. Pharm., 7, 88 (1961).
9. V. A. Yakovlev, The Kinetics of Enzymatic Catalysis [in Russian], Moscow (1965).

^{13}C NMR SPECTROSCOPY OF THE RING-CHAIN TAUTOMERISM OF THE PRODUCT OF REDUCTION OF DUBINIDINONE

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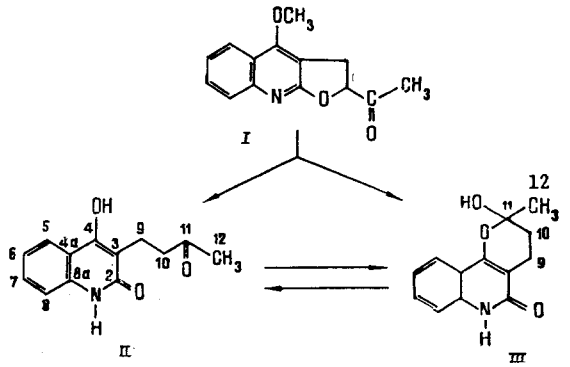
The existence of ring-chain tautomerism between a cyclic semiketal form and an open-chain δ-ketol form of the product of the reduction of dubinidinone has been shown unambiguously by ^{13}C NMR spectroscopy, and this has been confirmed by the results of PMR spectroscopy. It has been shown that in the solution in deuteropyridine and deuterodimethyl sulfoxide the tautomeric equilibrium is shifted in the direction of the formation of the cyclic semiketal, while in trifluoroacetic acid it is shifted in the direction of the open-chain δ-ketol. Analysis of the IR spectrum has shown that the product of the reduction of dubinidinone exists predominantly in the cyclic semiketal form in the crystalline state.

Earlier [1], for a compound obtained on the Clemensen reduction of dubinidinone (I), structure (II) was proposed [2] on the basis of the change in the absorption curve of the UV spectrum in an alkaline medium, the results of a study of the mass spectrum of (II), and its d_7-deutero analog obtained by the method of [3], and an analysis of the IR, PMR, and mass spectra of the O-acetyl derivative of product (II).



It is known, however, that when the molecule of a substance contains two groups capable of intramolecular interaction (in particular, CO and OH), the formation of a cyclic tautomer is possible, and if, in this process, a six-membered ring is produced, the cyclic tautomer is fairly stable [4]. In compound (II), the phenolic hydroxyl is present in the δ-position to the carbonyl group and, therefore, this substance may exist in tautomeric equilibrium with the six-membered semiketal (III).

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