LITERATURE CITED

1. R. Adams, T. A. Geismann, and I. D. Edwards, Chem. Rev., 60, 555 (1960).
2. A. L. Markman and V. P. Rzhekhin, Gossypol and Its Derivatives [in Russian], Pishchevaya, Prom-st', (1976), p. 44.
3. L. Biktimirov, N. I. Baram, A. I. Ismailov, F. G. Kamaev, and V. B. Leont'ev, Khim. Prir. Soedin., 286 (1975).
4. I. M. Dechary and L. E. Brown, J. Am. Chem. Soc., 33, 86 (1956).
5. B. Ya. Simkin, V. A. Bren', and V. I. Minkin, Zh. Org. Khim., 13, 1710 (1977).
6. V. P. Rybalkin, A. Ya. Bushkov, and V. A. Bren', Zh. Org. Khim., 22, 555 (1986).
7. V. I. Minkin, O. A. Osipov, and V. N. Sheinker, Zh. Fiz. Khim., 11, 23 (1970).
8. Zh. V. Bren', V. A. Bren', B. Ya. Simkin, and V. I. Minkin, Zh. Org. Khim., 13, 1723 (1977).
9. V. N. Sheinker, V. I. Minkin, and O. A. Osipov, Zh. Fiz. Khim., 11, 2438 (1970).
10. V. A. Bren' and V. I. Minkin, Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol., 25, No. 6, 663 (1982).
11. D. K. Zheng, Y. K. Si, J. K. Meng, J. Zhon, and L. Huang, J. Chem. Soc. Chem. Commun., 168 (1985).
12. S. A. Talipov, B. T. Ibragimov, G. B. Nazarov, T. F. Aripov, and A. I. Ismailov, Khim. Prir. Soedin., 663 (1984).

INTERACTION WITH CHOLINESTERASES AND CONFORMATIONAL ANALYSIS OF ANALOGS OF ACETYLCHOLINE CONTAINING THE ALKALOIDS EPHEDRINE AND PSEUDOEPHEDRINE

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Acetyl-β-methylcholine analogs of ephedrine and pseudoephedrine have been synthesized. The kinetics of their interaction with cholinesterases have been studied. To explain the differences in the sensitivity of the enzymes to conformers, a conformational analysis of the compounds synthesized has been made by the NMR method.

Continuing work in the field of the synthesis of alkaloid derivatives containing the structural elements of acetylcholine, we have performed the synthesis of diacetyl-N-(β-hydroxypropylephedrine and of diacetyl-N-β-hydroxypropylpseudoephedrine. Using these compounds as examples, it appeared of interest to elucidate the influence of conformational states on the catalytic activity of cholinesterases. As has been shown previously [1], substances with analogous structures may be present in solution in structurally fluctuating states, thanks to which they are capable of forming complexes with the acetylcholine receptor and with cholinesterases [2-4].

TABLE 1. Anticholinesterase Efficacy of O-Diacetyl-N-(β-acetoxypropyl)ephedrine (I, II), -pseudoephedrine (III, IV), and Their Methiodides

Compound	K_i (M)		K_i BuCE/K_i ACE
	ACE	BuCE	
I	$7{,}4 \cdot 10^{-4}$	$2{,}0 \cdot 10^{-3}$	2,7
II	$5{,}5 \cdot 10^{-5}$	$3{,}6 \cdot 10^{-4}$	6,5
III	$2{,}3 \cdot 10^{-4}$	$1{,}6 \cdot 10^{-3}$	7 0
IV	$8{,}5 \cdot 10^{-4}$	$4{,}9 \cdot 10^{-3}$	5,7

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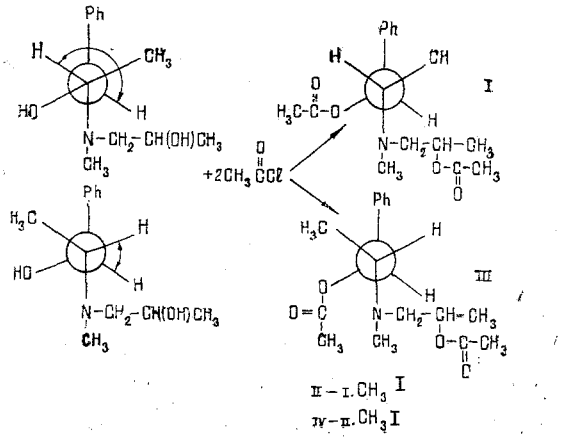
As has been shown previously [1], substances with analogous structures may be present in solution in structurally fluctuating states, thanks to which they are capable of forming complexes with the acetylcholine receptor and with cholinesterases [2-4].

The substances were synthesized by the scheme given below.

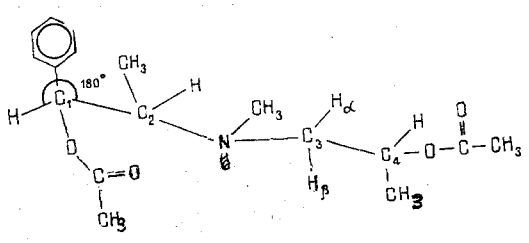


The products of the reaction of acetyl chloride with N-(β-hydroxypropyl)ephedrine and -pseudoephedrine, provided that the stereochemical configurations have been retained, can be distinguished by the position of the ester grouping relative to Ph and C–H at C-1: erythro in the case of ephedrine, and threo in the case of pseudoephedrine. On the basis of the structural similarity of the substances obtained to acetylcholine, it may be assumed that they will prove to be substrates and will be hydrolyzed under the action of cholinesterases. No hydrolysis of the ephedrine and pseudoephedrine derivatives under the action of acetylcholinesterase, ACE, and butyrylcholinesterase, BuCE, was detected even with a tenfold increase in the concentration of the enzymes. In the presence of these compounds, the reaction of acetylcholine with ACE and BuCE bore a competitive nature, and they reversibly inhibited the catalytic activity of both esterases. Results on the inhibiting activity of the ephedrine and pseudoephedrine derivatives are given Table 1.

All the substances inhibited the enzymatic capacity of ACE more effectively than that of BuCE. The inhibiting efficacy of the ephedrine derivative was more than ten times greater in the case of ACE than in the case of BuCE. This fact shows that the anionic center present in the enzymes investigated is more exposed in the case of ACE. Although the bases did inhibit the activity of cholinesterases their inhibiting efficacy was lower than that of the iodomethylated analogs. The presence of erythro and threo forms nevertheless had an effect on the sensitivity of the enzymes: for ACE and BuCE interaction with the erythro form of the inhibitor was preferred.

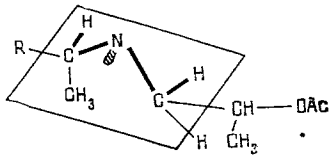
To explain the differences in the sensitivity of the enzymes to the compound synthesized, we investigated their conformational states in solutions by the ^1H NMR method.

Ephedrine and pseudoephedrine, and also their derivatives, in solutions in concentrated hydrochloric acid revealed several conformational forms due to the protonation of the nitrogen atom and the stabilization under the conditions of limited proton exchange of isomers the nonequivalence of the structures of which was due to different orientations of the chemical bonds about the nitrogen atom. This means that, in solvents of different natures (water, alcohol, CCl_4, etc.) dynamic interconversions take place between conformers the rate of which is too great for the scale of the NMR method. In these cases, the number of signals usually corresponds to only one nature.



In the spectrum of the O-acetyl-N-(β-acetoxypropyl)ephedrine (I) synthesized (see Table 1) the following signals were detected: H-1, d, J ≅ 10 Hz, δ = 5.55 ppm. The value of the vicinal constant showed that the magnitude of the dihedral angle between the directions of the H-1 and H-2 bonds was ∿180°C. The proton at C-4 formed a multiplet at 4.8 ppm, shifted downfield due to the influence of the π-electrons of the carbonyl group of the acetyl residue, and showed that H-4 was located almost in the nodal plane of the π-orbitals of C=O; and H-2 formed a multiplet at δ = 3 ppm, this position of the signal apparently indicating a noncoplanar orientation of C-2 and the unshared pair of electrons of the N–Me nitrogen atom. The two protons at C-3 formed a multiplet at δ = 2.5 ppm, the nature of the multiplet showing some nonequivalence of the positions of these atoms in the molecule. The signals of the resonance of the methyl groups were located at 2.3 ppm for N–Me, 1.92 ppm for the methyls of the acetyl groups, 1.15 ppm for C-4–Me, and 0.7 ppm for C-2–Me.

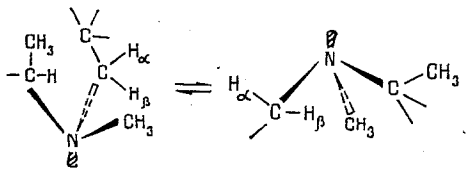
The change in configuration led to a number of effects in the spectrum of O-acetyl-N-(β-acetoxypropyl)pseudoephedrine (III) in comparison with (I); namely: the C-1–H signal was located at 5.75 ppm with a vicinal constant of ∿4-5 Hz, which showed a value of the dihedral angle between C-1–H and C-2–H of approximately 30°. C-4–H gave a multiplet at 4.68 ppm and C-2–H one at 2.95 ppm; correspondingly, the nonequivalent C-3-H-2 protons gave two individual signals at δ 2.6 and 2.2 ppm; the high-field signal apparently corresponding to the C-3–H proton located in the anticoplanar position to the unshared pair of electrons of the nitrogen atom.



The most interesting effect was the nonequivalence of the Me protons of the acetyl groups of (III), which resonated at 1.95 and 1.78 ppm, while, as can be seen from the spectra, the signals of one of them had a chemical shift almost the same as in (I).

The change in the value of the chemical shift in the upfield direction can apparently be explained on the basis of the structure under consideration only by a change in the orientation of C–Me relative to the plane of the phenyl radical at C-1, the methyl group in (III) being located above the Ph plane. The signals of the other methyl groups had the following chemical shifts: 2.22 ppm for N–Me; 0.8 ppm for C-2–Me; and 1 ppm for C-4–Me. Thus, the NMR results show structural changes in the systems of interconverting forms, depending on the type of diastereoisomers.

Labile active centers are the oxygen atoms forming ester bonds and the trivalent nitrogen atom, the unshared pair of electrons of which inverts with E_{act} ∿ 5-7 kcal/mole. It is obvious that the considerable increase in the degree of nonequivalence of the protons at C-3–H can be explained by the fact that in (III) the difference in the microenvironment of these protons in the process of inversion is considerably greater:



These dynamic differences in the structures are clearly shown in the interaction of these compounds with cholinesterases.

EXPERIMENTAL

PMR spectrum were taken on a Varian XL-200 instrument in CCl_4 and concentrated HCl solutions. N-(β-Hydroxypropyl)ephedrine and -pseudoephedrine were obtained by a known method [5].

O-Acetyl-N-(β-acetoxypropyl)ephedrine. With stirring and cooling (0-2°C), 1.57 g (0.02 mole) of acetyl chloride dissolved in 50 ml of absolute benzene was added dropwise over 1.5 h to a mixture of 2.22 g (0.01 mole) of N-(β-hydroxypropyl)ephedrine and 2.02 g (0.02 mole) of dry triethylamine in 100 ml of absolute benzene. Then the mixture was stirred at 60-70°C for 3 h. The precipitate of triethylamine hydrochloride that had deposited was filtered off

and the benzene was distilled off, and the residue (a viscous transparent oily product) was purified by column chromatography on the support Al_2O_3 (activity grade II) with ether as the eluent; R_f 0.84 (benzene–ether–ethanol (10:5:2) system); yield 82%.

O-Acetyl-N-(β-acetoxypropyl)pseudoephedrine was synthesized similarly; R_f 0.93; yield 85%. The results of the analysis of the compounds synthesized corresponded to the calculated figures.

The methiodides of O-acetyl-N-(β-acetoxypropyl)ephedrine and -pseudoephedrine were synthesized by the action of a slight excess of methyl iodide. However, a crystalline product could not be obtained because of pronounced hygroscopicity, and both products proved to be oily substances.

Methods of Determining the Catalytic Activities of Enzymes. The catalytic activities of ACE (EC 3.1.1.7) and BuCE (EC 3.1.1.8) were determined by Ellman's method [6] from the rate of hydrolysis of acetylthiocholine (for ACE) and butyrylthiocholine (for BuCE). Esterase activities were determined at pH 7.5 and a temperature of 25°C.

The acetylcholinesterase activities of the ephedrine and pseudoephedrine derivatives were evaluated from the magnitude of the reciprocal inhibition constant K_i found by the Lineweaver–Burk method [7].

Enzymes. The enzymes used were samples of ACE (3.5 U/mg) and BuCE (9.6 U/mg) produced by the Perm Scientific-Research Institute of Vaccines and Sera.

Substrates. Acetylthiocholine bromide and butyrylthiocholine bromide were Chemapol preparations, and 5,5'-dithiobis-2-nitrobenzoic acid was a commercial preparation from Koch-Light.

SUMMARY

1. It has been shown that the reaction of cholinesterases with O-acetyl-N-(β-acetoxypropyl)ephedrine and -pseudoephedrine has a reversible-competitive nature.

2. The higher sensitivity of cholinesterases to O-acetyl-N-(β-acetoxypropyl)ephedrine methiodide is due to the stabilization of the structure by the introduction of methyl groups at the nitrogen atom.

LITERATURE CITED

1. A. G. Dobren'kov, V. B. Leont'ev, D. N. Dalimov, A. A. Abduvakhabov, and A. S. Sadykov, Dokl. Akad. Nauk. SSSR, 290, No. 1, 124 (1986).
2. A. G. Dobren'kov, Z. Tilyabaev, D. N. Dalimov, and A. A. Abduvakhabov, Khim. Prir. Soedin., 763 (1986).
3. A. G. Dobren'kov, A. A. Abduvakhabov, D. N. Dalimov, and M. K. Karimov, Dokl. Akad. Nauk. UzSSR, No. 5, 36 (1985).
4. D. N. Dalimov, G. M. Vaizburg, L. K. Abdullaeva, A. A. Abduvakhabov, and A. S. Sadykov, Dokl. Akad. Nauk. SSSR, 289, No. 1, 227 (1986).
5. A. S. Sadykov, E. V. Rozengart, A. A. Abduvakhabov, and Kh. A. Aslanov, Cholinesterases: Active Center and Action Mechanism [in Russian], Fan, Tashkent (1976).
6. G. L. Ellman, K. D. Courtney, V. Anders, and R. M. Featherstone, Biochem. Pharmacol., 7, 88 (1961).
7. A. Cornish-Bowden, Principles of Enzyme Kinetics, Butterworths, London (1976).