SYNTHESIS, STRUCTURE, AND ANTICHOLINESTERASE ACTIVITY OF SOME ORGANOPHOSPHORUS DERIVATIVES OF ANABASINE

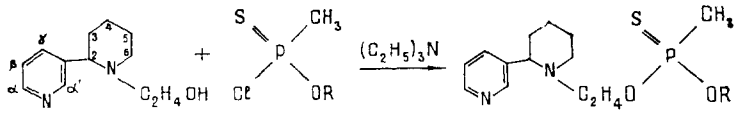
D. N. Dalimov, M. B. Gafurov,
F. G. Kamaev, and A. A. Abduvakhabov

UDC 577.153

Anabasine derivatives based on methylphosphonothoic acid have been synthesized. It has been shown by PMR spectroscopy that in solution O-alkyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioates exist in two stereomeric forms, which is due to the influence of the optically active anabasine residue. It has been shown that these compounds are competitive reversible inhibitors of acetylcholinesterase and of butyrylcholinesterase.

The reactivity of organophosphorus compounds (OPCs) on interaction with cholinesterases depends on their structure and stereochemistry [1]. It is also known that diastereomeric anisochromicity appears in NMR spectra with a doubling of the signals of the corresponding nuclei, the most characteristic signal in the case of OPCs being the signal of the protons of a methyl group attached to a phosphorus atom [2].

In the present paper we consider features of the structure and anticholinesterase activity of compounds obtained on the interaction of O-alkyl methylphosphonochloridothioates with optically active N-(β-hydroxyethyl)anabasine with the aim of finding the influence of the latter on the diastereomeric anisochromicity of O-alkyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioates. These compounds were synthesized by the following scheme:



R = $-C_2H_5$, $-C_3H_7$, $-C_4H_9$, $-C_5H_{11}$, $i-C_4H_9$

Methyl phosphonothioic acid dichloride and O-alkyl methylphosphonochloridothioates were obtained as described in [3-5]. The final products, the O-alkyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioates, were obtained by the action of N-(β-hydroxyethyl)anabasine on the O-alkyl methylphosphonochloridothioates in the presence on an HCl acceptor – dry triethylamine. These compounds were purified by column chromatography, since partial isomerization may take place during distillation.

The final products obtained were characterized by their physicochemical constants (Table 1). The structures of these compounds were confirmed by their IR and PMR spectrum. Thus, the IR spectrum of O-ethyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioate has the following characteristic absorption bands (ν, cm^{-1}): 2940 – CH vibrations of a methyl group; 1570 – aromatic ring of pyridine; 1035 – P–O–C_2H_5; 800-750 – P–C group; 605 – P=S.

Figure 1 gives the PMR spectrum of O-ethyl O-[β-(anabasin-1-yl)] methylphosphonothioate in CCl_4 solution. The signals of the α', α, β, and γ protons of the pyridine ring are located at 8.36, 8.32, 7.11, and 7.56 ppm. The signals of the protons of the piperidine ring have the following distribution: the H_{2a} and H_{6e} protons resonate in the 3.04-3.25 ppm region, the H_{6a} signal is located at 2.15 ppm, and the signals of the other protons of the piperidine ring in the 1.2-1.8 ppm region.

The values of the chemical shifts and the multiplicities of the signals are typical for N-substituted anabasines [6]. A four-proton multicomponent signal in the 3.7-4.2 ppm region belongs to two hydroxymethylene groups. By the double-resonance method (with saturation of

Institute of Bioorganic Chemistry of the Uzbek SSR Academy of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 561-565, July-August, 1987. Original article submitted January 6, 1987.

0009-3130/87/2304-0469$12.50

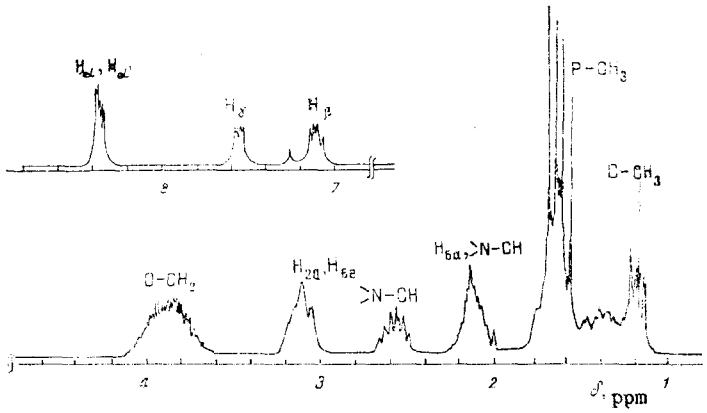
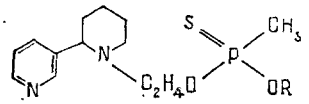


Fig. 1. PMR spectrum of O-ethyl O[β-(anabasin-1-yl)] methylphosphonothioate.

TABLE 1. Physicochemical Constants of O-Alkyl O-[β-(Anabasin-1-yl)ethyl] Methylphosphonothioates



	R	n_D^{20}	d_4^{20}	MR$_D$ found	MR$_D$ calc.	Yield, %	Found, % C	Found, % H	Found, % P	Calculated, % C	Calculated, % H	Calculated, % P
1	C_2H_5	1,5176	1,0890	91,99	91,93	37,3	54,51	7,33	9,70	54,87	7,62	9,45
2	C_3H_7	1,5216	1,0958	95,63	96,54	35,3	56,44	7,67	8,95	56,14	7,59	9,06
3	C_4H_9	1,5229	1,0891	100,20	101,14	35,5	58,02	8,40	8,42	57,86	8,15	8,70
4	C_5H_{11}	1,5232	1,0180	104,56	105,76	29,6	59,34	8,65	8,17	58,92	8,38	8,38
5	i-C_4H_9	1,5030	1,0478	100,59	101,14	32,1	57,42	8,74	9,07	57,86	8,15	8,70

the OCH_2 protons). It was established that the N—CH_2 protons of the substituent are nonequivalent and resonate at 2.10 and 2.58 ppm. A distinguishing feature of the spectrum is the distinct splitting of the signals of the P—CH_3 methyl group and of the methyl group of the ethyl radical. The differences in the chemical shifts of the corresponding signals amount to 0.08 and 0.01 ppm. Furthermore, a splitting of the signals of the α'-proton and a broadened nature of the signals of the other protons of the piperidine ring are observed. The dual nature is characteristic of the PMR spectra of the other derivatives, and the parameters of the spectra scarcely change with a variation in the temperature.

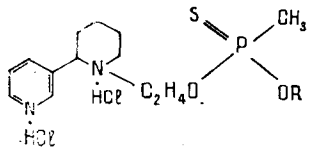
It is known that compounds with two (and more) asymmetric centers can exist in several diastereomeric forms the existence of which can be recorded by the methods of NMR spectroscopy [1, 7]; in particular, ^1H NMR and ^{31}P spectroscopy have shown the existence of diastereomers of substituted O-ethyl methylphosphonothioates with two asymmetric centers [2]. It was therefore natural to assume the existence of O-ethyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioate in diastereomeric forms. The similar intensities of the signals of the two forms indicate their approximate equal concentrations in the solution. It must be mentioned that in the hydrochloride of this compound one of the forms clearly predominates. It must also be mentioned that a determination of $[\alpha]_D$ for anabasine gave an angle of rotation of −81° while for O-[N-(β-hydroxyethyl)anabasine $[\alpha]_D$ is −23.7° and for O-ethyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioate $[\alpha]_D$ is −35.1°, and a lengthening of the O-alkyl radical does not lead to an appreciable change in the value of $[\alpha]_D$.

The anticholinesterase properties of the compounds synthesized were studied on acetylcholinesterase (ACE) of human blood erythrocytes and on butylcholinesterase (BuCE) of horse blood serum.

Anticholinesterase activity was determined by Ellman's method [8]. The type of inhibition of the catalytic activity of the enzyme was established by the Lineweaver—Burk method [9].

All the compounds studied proved to be reversible competitive inhibitors of ACE and BuCE.

TABLE 2. Reversible Inhibition Constants (K_i) of the Dihydrochlorides of the O-Alkyl O-[β-(Anabasin-1-yl)ethyl] Methylphosphonothionates



R	K_i (M)		K_i ACE / K_i BuCE
	ACE	BuCE	
C_2H_5	$1{,}9\cdot10^{-5}$	$2{,}68\cdot10^{-5}$	0,7
C_3H_7	$1{,}04\cdot10^{-5}$	$6{,}5\cdot10^{-6}$	1,6
C_4H_9	$2{,}1\cdot10^{-5}$	$6{,}2\cdot10^{-7}$	33,9
C_5H_{11}	$I_{50}=1\cdot10^{-3}$	$5{,}54\cdot10^{-6}$	—
i-C_4H_9	$2{,}56\cdot10^{-5}$	$5\ 26\cdot10^{-6}$	4,9

It can be seen from Table 2 that in the case of ACE a lengthening of the O-alkyl radical from ethyl to amyl had no appreciable influence on the anticholinesterase activity of the O-alkyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioates. Apparently, in the case of interaction with ACE the anabasine part of the inhibitor molecule is less complementary to the anionic point of the catalytic surface of the enzyme. Because of this, the phosphoryl group is poorly sorbed on the corresponding hydrophobic sections of the active surface of the ACE. A lengthening of the O-alkyl radical therefore has practically no effect on the magnitude of K_i.

In the case of the interaction of these substances with BuCE, a similar lengthening of the alkyl radical from ethyl to butyl led to a 43-fold increase in anticholinesterase activity. The further passage from the butyl to the amyl and isobutyl derivatives lowered the inhibiting activity by an order of magnitude. Such a dependence of the value of K_i on the length of the O-alkyl radical agrees with ideas according to which hydrophobic interaction is characteristic for this enzyme [10].

The specificity of the action with respect to BuCE was evaluated by the selectivity coefficient determined from the formula: (K_i ACE)/(K_i BuCE).

It can be seen from the figures given in Table 2 that, apart from O-ethyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioate all the compounds were selective in relation to BuCE. The selectivity of their action rose with an increase in the size of the O-alkyl radical in the inhibitor molecule.

EXPERIMENTAL

IR spectra were taken on a Specord IR-71 instrument (GDR) in paraffin oil and in KBr tablets. PMR spectra were taken on a Varian XL-200 instrument with a working frequency of 200 MHz. Angles of rotation $[\alpha]_D$ were determined on a Polamat instrument in ethanol at a 3% concentration of the substance.

Column chromatography was performed on Al_2O_3 (activity grade II) with absolute ether as eluent. The solvent system for TLC on Al_2O_3 (activity grade II) was benzene–ether–ethanol (10:5:2).

O-Ethyl O-[β-(Anabasin-1-yl)ethyl] Methylphosphonothioate. With cooling, 0.01 mole (1.58 g) of O-ethyl methylphosphonochloridothioate was added to 0.01 mole (2.06 g) of N-(β-hydroxyethyl)anabasine and 0.01 mole (1.01 g) of triethylamine in 50 ml of absolute ether. After this, the reaction mixture was heated in the water bath at 30-35°C for 2 h and was left for 12 h, and then the precipitate of triethylamine hydrochloride that had deposited was filtered off and the ether was distilled off. The course of the reaction was monitored by TLC. The final product was purified on a column of Al_2O_3 (activity grade II).

The anticholinesterase activity of the substance synthesized was determined on a SF-26 spectrophotometer at 412 nm [8]. The enzyme preparations used were human blood erythrocyte acetylcholinesterase (EC 1.1.7) with a specific activity of 2.7 U/mg and horse serum butyrylcholinesterase (EC 1.1.8) with a specific activity of 29 U/mg produced by the Perm Scientific Research Institute of Vaccines and Sera. The experiment was carried out at 30°C at the pH of the reaction mixture of 7.5. Samples for determining enzyme activity contained: 1.8 ml of 0.1 M phosphate buffer (pH 8.01), 0.2 ml of a solution of ACE or BuCE prepared in water, 0.4 ml of ATC in a concentration of $2 \cdot 10^{-3}$ M (or, in the case of BuCE, BTC in a concentration of 10^{-3} M), and 0.2 ml of the Ellman reagent (concentration $1 \cdot 10^{-3}$ M) prepared in 0.1 M phosphate buffer (pH 7.0).

To determine the influence of the inhibitors on the activity of the enzyme, part of the water was replaced by a solution of the necessary concentration. The reversible inhibition constant K_i was found from a double-reciprocal graph [9].

CONCLUSION

1. It has been shown by PMR spectroscopy that O-alkyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioates exist in two diastereomeric forms in solution.

2. O-Alkyl O-[β-(anabasin-1-yl)ethyl] methylphosphonothioates have proved to be reversible competitive inhibitors of acetylcholinesterase and butylcholinesterase, the anticholinesterase activity depending substantially on the structure of the inhibitor molecule.

LITERATURE CITED

1. M. Mikolaichik and M. Laitloff, Usp. Khim., 15, 1419 (1975).
2. T. A. Mastryukova, A. É. Chipov, M. S. Vaisberg, P. V. Petrovskii, and M. I. Kabachnik, Izv. Akad. Nauk SSSR, Ser. Khim, 1841 (1971).
3. M. I. Kabachnik and N. N. Godovikov, Dokl. AN SSSR, 110, 210 (1956).
4. I. V. Komkov, K. V. Karavanov, and S. Z. Ivin, Zh. Obshch. Khim., 28, 2960 (1958).
5. A. A. Abduvakhabov, N. N. Godovikov, and M. I. Kabachnik, The Chemistry of Organic Compounds of Phosphorus [in Russian], Nauka, Leningrad (1967), p. 3.
6. M. Karimov, A. S. Sadykov, Kh. A. Aslanov, V. B. Leont'ev, F. G. Kamaev, and T. K. Yunusov, Khim. Prir. Soedin., 207 (1972).
7. T. H. Siddall and W. E. Stewart, Progr. NMR Spectrosc., 5, 33 (1969).
8. G. L. Ellman, D. R. Cennetey, V. Anders, and K. M. Reather, Biochem. Pharmacol., 7, 88 (1961).
9. J. L. Webb, Enzyme and Metabolic Inhibitors, Academic Press, New York (1963-1966) [Russian translation, Mir (1976, p. 163].
10. 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).