Chemistry of Natural Compounds, Vol. 30, No. 1, 1994

METHYLPHOSPHONOTHIONATES OF N-(β-HYDROXYPROPYL)ANABASINE — SYNTHESIS, STRUCTURE, AND SENSITIVITY OF CHOLINESTERASES TO THEM

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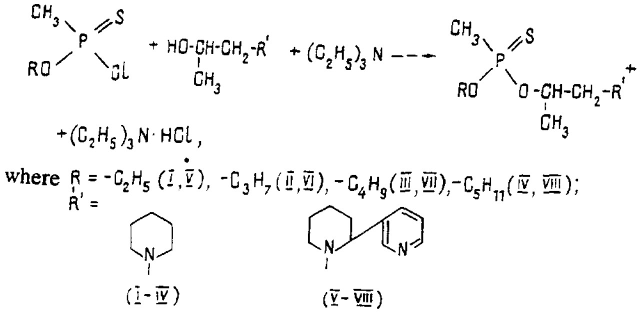
UDC 547.341:547.94:577.151.042

The synthesis has been effected of alkyl anabasinoprop-2-yl and alkyl piperidinoprop-2-yl methylphosphonothionates. It has been shown by ^1H, ^{13}C and ^{31}P NMR spectroscopies that the anabasine derivatives exist in solution as four diastereomeric forms. The kinetics of the interaction of the compounds synthesized with two types of cholinesterases has been studied.

It is known that compounds with two (and more) asymmetric centers can exist in several diastereomeric forms, the presence of which can be determined by the methods of NMR spectroscopy [1, 2]. In particular, the existence of diastereomers of O-ethyl methylphosphonothioates of amino acids with two asymmetric centers, one of them being optically active, has been shown by ^1H and ^{31}P spectroscopies [3]. In view of this, continuing investigations begun earlier on determining the effects of diastereomeric anisochronicity, it was of interest to study the ^1H, ^{13}C, and ^{31}P NMR spectra of alkyl anabasinoprop-2-yl methylphosphonothionates and, for comparison, alkyl piperidinoprop-2-yl methylphosphonothionates.

These compounds were synthesized by the following scheme:



Methylphosphonothionic dichloride and alkyl methylphosphonochloridothionates were obtained by the methods of [4, 5], and N-(β-hydroxypropyl)piperidine and N-(β-hydroxypropyl)anabasine as in [6]. The final products — the alkyl piperidinoprop-2-yl methylphosphonothionates (I-IV) and alkyl anabasinoprop-2-yl methylphosphonothionates (V-VIII) were synthesized by the action of the appropriate amino alcohol on the corresponding O-alkyl methylphosphonochloridothionates in the presence of an HCl acceptor — dry triethylamine. These compounds were purified by column chromatography.

The physicochemical characteristics of the compounds obtained are given in Table 1. Their structures were confirmed by their IR, PMR, and ^{13}C and ^{31}P NMR spectra. Thus, the IR spectra of (V) contained the following characteristic absorption bands (ν, cm^{-1}: 2935 — CH vibrations of a methyl group; 1570 — the aromatic ring of pyridine; 1050 — $P-O-C_2H_5$; 800—750 — $P-C$ group; 605 —$P=S$. The PMR spectrum of (I) confirmed its structure, containing the following signals: doublet (J = 6.6 Hz at 1.15 ppm, relating to the methyl of an isopropyl group; triplet (J = 6.5 Hz) at 1.25 ppm corresponding to the methyl of an oxyethyl group; a doublet (J = 12.0 Hz) of a methyl at a phosphorus atom resonating at 1.73 ppm; multiplets in the 2.2-2.52 ppm region belonging to the six $N-CH_2$ protons; and multiplets in the 3.72-4.1 ppm region and at 4.67 ppm relating to $O-CH_2$ and $O-CH$ protons. The other protons of the piperidine ring resonated in the 1.2-1.6 ppm region.

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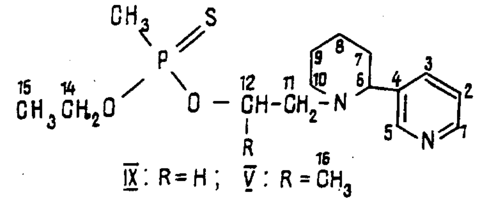
TABLE 1. Physicochemical Characteristics and Anticholinesterase Activities of Compounds (I-VIII)

Compound	n_D^{20}	d_4^{20}	Yield, %	MR_D		pK_I	
				found	calc.	ACE	BuCE
I	1.4938	1.0491	58	73.52	73.09	4.22	4.29
II	1.4880	1.0378	48	77.45	77.71	4.91	4.67
III	1.4813	1.0169	41	82.04	82.33	5.66	5.41
IV	1.4778	1.0045	47	86.48	86.95	5.97	4.47
V	1.5209	1.0703	31	97.28	96.54	3.42	4.62
VI	1.5121	1.0496	34	101.75	101.14	5.49	5.99
VII	1.5109	1.0448	30	106.06	105.76	5.09	4.11
VIII	1.5091	1.0355	34	110.96	110.38	5.39	5.46

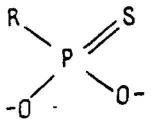
We have shown previously [7] that, according to PMR spectroscopy, O-alkyl O-(β-anabasinoethyl) methylphosphonothionates exist as two diastereomeric forms, the appearance of which is due to the existence of the optically active molecule of natural anabasine. The PMR spectrum of (V) also showed the existence of this compound in several diasteromeric forms. In the 1.56-1.86 ppm regions were located four doublets characteristic for $P-CH_3$ groups and corresponding to four diastereomeric forms. The O-ethyl O-(β-anabasinoethyl) methylphosphonothioate (IX) synthesized previously and compound (V) were studied by ^{13}C NMR spectroscopy:



The assignment of the lines in the ^{13}C spectra of (V) was made with the aid of the off-resonance method and by comparison with the spectra of model compounds. It must be mentioned that the optically active anabasine molecule causes the appearance of two groups of signals of several carbon atoms due to the presence of two diastereomeric forms of the (IX) molecule in solution, as has also been observed in the PMR spectrum [7]. The assignment of the lines is given in Table 2. The presence of another asymmetric center in the molecule of (V), as compared with (IX) causes an additional splitting of the signals of the majority of the carbon atoms (Table 2).

It must be mentioned that, in contrast to the ^{13}C NMR spectrum of (IX), in which the intensities of the signals of the optical isomers are practically identical, in the ^{13}C NMR spectrum of (V) the corresponding signals were of different intensities. This indicates a different quantitative ratio of the concentrations of the diastereomers in the solution of the sample.

The signals of the phosphorus resonance of (IX) and (X) were located in the region of 93-96 ppm, which is characteristic for the fragment



In the spectrum of (IX), the existence of two diastereomeric forms was shown by the presence of two peaks of approximately equal intensity [96. 9 (51%) and 96.5 ppm (49%)]. In the ^{31}P NMR spectrum of (V) the phosphorus signals for four optical isomers were observed [96.4 (17%); 95.7 (43%); 95.4 (30%), and 93.5 ppm (10%)]. As in the ^{13}C NMR spectra, their intensities differed considerably, which showed the predominance of certain optical forms during the reaction.

TABLE 2. Chemical Shifts of the Carbon Atoms of Compounds (V) and (IX)

No. of the carbon atom	Chemical shift, ppm		No. of the carbon atom	Chemical shift, ppm	
	IX	V		IX	V
5	149.1	149.1 (2)*	11	54.5	61.4—60.6 (gr.)
1	148.3	148.1 (2)		54.4	
4	140.0 (2 sign.)	140.0 (2)	10	53.8	54.3—53.7 (gr.)
3	134.8	135.1 (2)	7	36.6	36.8—36.3 (gr.)
2	123.3	123.1			
14	65.7 65.6	66.0	9 8	25.6 24.5	25.5 24.5
12	63.2 63.0	71.9—70.6 (gr.)	13	22.4 20.2	23.5—20.9 (gr.)
6	62.0 61.9 61.7	60.3 60.1		$J_{(C-H)}$= 115.3 Hz	$J_{(C-H)}$=118.0 Hz
			15	16.0	15.8 (gr.)
			16	–	19.7 (gr.)

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*The number of signals is shown in parentheses.

Thus, in solution, compound (V) is present in four diastereomeric forms, and therefore in an investigation of its biological activities it must be borne in mind that different forms of diastereomers interact with enzyme systems, and the kinetic parameters found are their total effect.

In a study of the anticholinesterase activities of the derivatives of N-(β-hydroxypropyl)anabasine that had been synthesized (V-VIII), just as in the case of their N-(β-hydroxyethyl) analogues [7], a definite inhibiting effect in relation to ACE and BuCE appeared. To determine the contribution of the pyridyl fragment forming a component of the anabasine molecule, Table 1 gives information on the inhibiting activity of analogous phosphorylated derivatives based on piperidine (I-IV).

It can be seen from Table 1 that a change in the structure of R caused almost the same effect for the inhibition of ACE and BuCE, while the best compound in this series is (VI), the difference in the inhibition of the two enzymes by it being insignificant. Among the piperidine derivatives, substances (IV) (for ACE) and (III) (for BuCE) were active. The dependence of the activities of these compounds on R largely resembles the dependence of the activity of the N-(β-hydroxyethyl)anabasine derivatives [7]. The introduction of a methyl group into the ethylene bridge of the OPC molecule leads to a rise in the efficiency of the compounds for ACE (by 1-2 orders of magnitude) in comparison with the N-(β-hydroxyethyl) analogues. The introduction of a methyl group affects the sensitivity of BuCE, its effect proving to be the opposite to that in the case of ACE. The experimental facts obtained are in complete agreement with results on the substrate specificity of ACE, since it is known that the introduction of methyl group into the β-positions of various choline esters leads to a rise in their affinity for ACE.

Thus, on the basis of the NMR spectra on ^1H, ^{12}C, and ^{31}P nuclei it has been shown that alkyl anabasinylprop-2-yl methylphosphonothionates exist in solution as four diastereomeric forms in different concentrations By modifying the structure of the alkaloid anabasine it is possible to achieve an increase in its inhibition of CE, which is most probably connected with a greater complementarity of the substances obtained to the structure of the active center of BuCE.

EXPERIMENTAL

The IR spectra of the compounds synthezed were taken on a Specord IR-71 instrument in KBr tablets, and the PMR and ^{13}C and ^{31}P NMR spectra on a Varian XL-200 instrument, the solvents being CCl_4 and $CDCl_3$.

For column chromatography we used Al_2O_3 (activity grade II), wit' absolute ether as the eluent. The system for TLC was benzene–ether–ethanol (10:5:2).

Ethyl Anabasinoprop-2-yl Methylphosphonothionate. With cooling and stirring, 1.58 g (0.01 mole) of ethyl methylphosphonochloridothionate in 30 ml of absolute ether was added to 2.2 g (0.01 mole) of N-(β-hydroxypropyl)anabasine and

1.01 g (0.01 mole) of triethylamine in 50 ml of absolute ether. The reaction mixture was heated in the water bath at 30-35°C for 2 h and was left overnight. The precipitate of triethylamine hydrochloride that deposited was filtered off. After the ether had been distilled off, the final product was purified on a column of Al_2O_3.

The other compounds were obtained similarly.

The anticholinesterasse activities of the organophosphorus derivatives of anabasine and piperidine were determined by Ellman's method [8] in a SF-26 spectrophotometer at a wavelength of 412 nm. The enzyme preparations used were human blood erythrocyte acetylcholinesterase (specific activity 2.7 U/mg) and horse blood serum butyrylcholinesterase (26 U/mg). Both enzymes were products of the Perm Scientific-Research Institute of Vaccines and Sera. The activities of the enzymes were investigated at 30°C in a reaction medium with a pH of 7.5. Samples for determining of activity of the CEs contained 1.8 ml of 0.1 M phosphate buffer, 0.2 ml of a solution of the enzyme, 0.4 ml of the substrate (acetylthiocholine) in a concentration of $2 \cdot 10^{-3}$ M, and 0.2 ml of the Ellman reagent (Sigma) prepared in 0.1 M phosphate buffer, pH 7.0. The inhibition constants were found from a graph of double reciprocal magnitudes according to [9] and was expressed in the form $pK_i - \log K_i$.

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