SYNTHESIS, STRUCTURE, AND ANTICHOLINESTERASE ACTIVITIES OF METHYLPHOSPHONOTHIONATES OF N-β-HYDROXYETHYLMORPHOLINE AND N-β-HYDROXYPROPYLMORPHOLINE

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

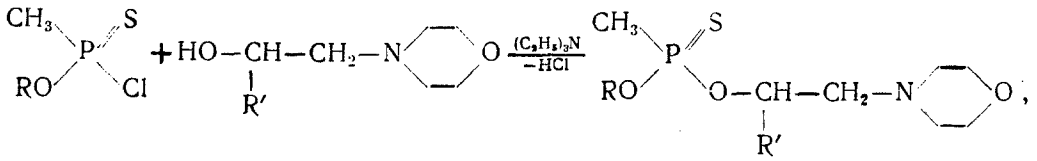
UDC 577.151.042

The work was based on the interrelationship of the diastereomeric anisochromicity on the introduction of an asymmetric center outside the ring of the amino alcohol and the anticholinesterase activity of the compounds including a morpholine heterocycle. A method is described for obtaining the compounds presented. Physicochemical constants and a method of determining anticholinesterase activity are given. In the series of O-ethyl derivatives of these compounds significant activity with respect to acetylcholinesterase can be distinguished. The efficiency of the series tested is comparable with that of tetraalkylammonium salts. It has been shown that alkyl 1-methyl-2-morpholinoethyl methylphosphonothionates are present in solution in two optically isomeric forms in a ratio of 1:3.

Esters of methylphosphonothionic acid obtained from N-β-hydroxyethylpiperidine and also from the alkaloids lupidine and epilupinine are fairly strong reversible inhibitors of cholinesterases [1, 2]. On the other hand, the combination of alkyl methylphosphonochloridothionates with optically active alcohols such as N-β-hydroxyethylanabasine has led to the appearance of diastereomeric anisochromicity, observed in the form of a doublet of the signals of the protons of the methyl groups at the phosphorus atom [3].

The present work was based on the problem of determining such diastereomeric anisochromicity in the case of the removal of an isometric center beyond the framework of the ring of a cyclic amino alcohol. In addition to this, an estimate has been given of the anticholinesterase activities of compounds including a morpholine ring, since the morpholine ring includes a hydrophilic oxygen atom, the presence of which may have a substantial effect on the anticholinesterase activity of these substances.

Methylphosphonothionic dichloride was obtained by the action of phosphorus pentasulfide on methylphosphonic acid [4]. Methylphosphonochloridothionates were obtained by the method of [5]. N-β-hydroxypropylmorpholine and N-β-hydroxyethylmorpholine were obtained by condensing morpholine with propylene oxide and with ethylene oxide in ethanol, respectively. The final products alkyl 1-methyl-2-morpholinoethyl methylphosphonothionates and alkyl 2-morpholinoethyl methylphosphonothionates were obtained by the action of N-β-hydroxypropylmorpholine and N-β-hydroxyethylmorpholine on the corresponding alkyl methylphosphonochloridothionates in the presence of an HCl acceptor – dry triethylamine. The compounds obtained were purified by column chromatography. Their synthesis was carried out by the following scheme:



where R = $-C_2H_5$, $-C_3H_7$, $-C_4H_9$, $-C_5H_{11}$, i-C_4H_9; R′ = –H, $-CH_3$.

A. S. Sadykov Institute of Bioorganic Chemistry, Uzbek SSR Academy of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 543-546, July-August, 1989. Original article submitted October 10, 1988; revision submitted December 30, 1988.

0009-3130/89/2504-0465$12.50

The compounds obtained were characterized by their physicochemical constants (Table 1). Their structures were confirmed by IR and PMR spectra. The IR spectrum of ethyl 1-methyl-2-morpholinoethyl methylphosphonothionate had the following characteristic absorption bands (ν, cm^{-1}): 2820 – CH stretching vibrations of a methyl group; 2700 – C–N (in CH_2–N–); 1040 – P–O–C_2H_5; 1120 – C–O–C of a ring; 605 – P=S.

The PMR spectrum of ethyl 1-methyl-2-morpholinoethyl methylphosphonothionate was characterized by a double set of signals of the protons of the main functional groups, which indicates the existence of this molecule in the diasteromeric form due to the presence of an asymmetric center in it [6]. The integral intensities of the signals indicated a concentration ratio of these forms of 1:3. The arrangement of the signals was as follows: a multiplet at 4.70 ppm belonged to the O–CH proton of the substituent; the signals of the O–CH_2 protons of the ethyl radical were located in the 3.8-4.2 ppm region, and the triplets of the O–CH_2 protons of the morpholine ring to the two diastereomeric forms at 3.56 and 3.54 ppm; the N–CH_2 protons resonated in a broad region of 2.1-2.6 ppm; and two doublets at 1.75 and 1.54 ppm belonged to the P–CH_3 group (J_{P-H} = 16.6 Hz). Triplets at 1.27-1.26 ppm belonged to the methyl group of the ethyl radical, and a doublet at 1.20 and 1.18 ppm to the methyl group at the asymmetric center. The double-resonance method was used to determine accurate values of the chemical shifts of the N-CH_2 protons of the substituent – 2.45 and 2.15 ppm (J = 11.4 Hz) – and of the N–CH_2 protons of the morpholine ring – 2.88 and 2.44 ppm (J = 10.8 Hz). The nonequivalence of the N–CH_2 proton of the morpholine ring could indicate a retardation of the conversion [sic; ? inversion ?] of the ring of the NMR scale, but this hypothesis was not confirmed, since the O–CH_2 protons of the ring were equivalent and, moreover, the N–CH_2 protons are nonequivalent even in the initial amino alcohol. This nonequivalence is apparently due to the asymmetric center of the substituent [6, 9]. It must be mentioned that no doubling of the signal corresponding to the diastereomeric form was observed in the PMR spectrum of ethyl 2-morpholinoethyl methylphosphonothionate.

The anticholinesterase properties of the compounds synthesized were studied on acetylcholinesterase (ACE) from human blood erythrocytes and butyrylcholinesterase (BuCE) from horse blood serum. Anticholinesterase activity was determined by Ellman's method. The constants of reversible inhibition of catalytic activity were determined by Cornish-Bowden's method using equations derived from a model of the ratio of the competitive and noncompetitive action of the inhibitor [7, 8].

All the compounds studied proved to be reversible inhibitors of the mixed type for both cholinesterases. The model of interaction that was being analyzed permitted the calculation of the competitive, K_i, noncompetitive, K_i', and generalized, $\bar{K}_i$, inhibition constants (see Table 1).

The figures in Table 1 give an idea of the interrelationship of the structural changes of the inhibitor molecules and their anticholinesterase activities. A lengthening of the alkyl radical in alkyl 2-morpholinoethyl methylphosphonothionates did not substantially change the anticholinesterase activity so far as a consideration of $\bar{K}_i$ is concerned. However, propyl 2-morpholinoethyl methylphosphonothionate was approximately an order of magnitude more effective than the other methylphosphonothionates. The butyl derivative differed from the other compounds of the series tested by the fact that it was an effective noncompetitive reversible inhibitor. The mixed type of action of the other inhibitors was initiated predominantly through the competitive action.

In the alkyl 1-methyl-2-morpholinoethyl methylphosphonothionate series the generalized constant of reversible inhibition $\bar{K}_i$ showed a lower efficacy in comparison with inhibitors of the preceding series. However, with respect to noncompetitive action the butyl derivative was stronger than the analogous compound – butyl 2-morpholinoethyl methylphosphonothionate. Characteristic for this series of compounds was the predominantly noncompetitive component of anticholinesterase efficiency.

The presence of two optically active forms in solution for representatives of the latter series may partially explain its lower anticholinesterase activity. It is possible, on the one hand, that one of the optical isomers possesses a lower specific sorbing efficiency. On the other hand, the hydrophilicity of the morpholine ring due to the presence of an oxygen atom has a substantial effect on the orientation of the inhibitor molecule and the ionic site of the enzyme, thereby lowering the specific sorption and leading to rapid breakdown of the enzyme-inhibitor complex.

TABLE 1. Physicochemical Constants and Reversible Anticholinesterase Activities of Alkyl 1-Methyl-2-morpholinoethyl Methylphosphonothionates and Alkyl 2-Morpholinoethyl Methylphosphonothionates

R	R'	n_D^{20}	d_4^{20}	MR_D found	MR_D calculated	Yield, %	Constants of the reversible inhibition of the hydrochlorides: ACE $\overline{K_i}$	ACE K_i	ACE K_i'	BuCE $\overline{K_i}$	BuCE K_i	BuCE K_i'
							$(M)\cdot10^{-5}$					
C_2H_5	H	1,4960	1,1160	66,21	65,50	45	6,83	12,4	14,8	17,0	28,7	40,0
C_3H_7	H	1,4927	1,0953	70,81	70,12	42	0,591	1,65	0,919	0,268	1,93	0,236
C_4H_9	H	1,4909	1,0823	75,18	74,74	38	2,18	—	2,18	13,9	138	15,4
i-C_4H_9	H	1,4893	1,0797	75,16	74,74	42	6,70	13,9	10,8	7,20	31,1	9,37
C_5H_{11}	H	1,4812	1,0539	79,68	79,35	35,6	7,76	22,3	9,68	—	—	—
C_2H_5	CH_3	1,4931	1,0955	70,84	70,12	42,3	19,8	39,8	30,6	21,8	162	25,2
C_3H_7	CH_3	1,4828	1,0655	75,29	74,74	40,4	16,7	23,5	56,9	70,0	175	116
C_4H_9	CH_3	1,4840	1,0554	79,97	79,35	36,5	0,524	—	0,492	0,863	—	0,863
C_5H_{11}	CH_3	1,4810	1,0514	83,63	83,97	37	10,4	1240	10,6	4,45	61,5	4,80
i-C_4H_9	CH_3	1,4850	1,0586	79,85	79,35	34,4	3,89	6,61	9,52	9,49	14,4	28,1

Note. The elementary compositions found for all the substances corresponded to the calculated figures.

The compounds considered possessed no specific activity in relation to BuCE. In the ethyl derivatives a high activity in relation to ACE was distinguishable. The efficiency of the series tested was comparable with that of tetraalkylammonium salts. It is obvious that the investigation of the anticholinesterase activity of the separated optical isomers will permit a definitive answer to the question of the level of true activity of the methylphosphonothionates as reversible cholinesterase inhibitors.

EXPERIMENTAL

The IR spectra of the compounds tested were recorded on a Specord 71-IR instrument in CCl_4 and paraffin oil, and the PMR spectra on a Varian XL-200 instrument.

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

Ethyl 1-Methyl-2-morpholinoethyl Methylphosphonothionate. With cooling, 0.01 mole (1.585 g) of ethyl methylphosphonochloridothionate was added to 0.01 mole (1.45 g) of N-β-hydroxypropylmorpholine and 0.01 mole (0.01 g) of triethylamine in 50 mg of absolute ether. After this, the reaction mixture was heated in the water bath at 30-35°C for 2 h. The course of reaction was monitored by TLC. Then the reaction mixture was left overnight. The triethylamine hydrochloride that had deposited was filtered off, and the ether was distilled off. The final product was purified by column chromatography.

The anticholinesterase activities of the compounds synthesized were determined by Ellman's method [7] on a Specol-221 spectrometer at 412 nm. The enzyme preparations used were human blood erythrocyte acetylcholinesterase (EC 3.1.1.7) with a specific activity of 3.5 U/mg and horse blood serum butylcholinesterase (EC 3.1.1.8) with a specific activity of 28 U/mg produced by the Perm Scientific-Research Institute of Vaccines and Sera.

The optical densities of the colored samples of reaction mixture were measured at 30°C and pH 7.5. The samples to determine enzymatic activity contained 0.4 ml of a 0.001 M solution of Ellman's reagent in 0.1 M phosphate buffer (pH 7.0), 0.7 ml of 0.1 M phosphate buffer (pH 8.0), 0.4 ml of water, and 0.3 ml of an aqueous solution of enzyme calculated to contain 0.1 activity unit per ml of reaction mixture. In place of the 0.4 ml of water, the "experiment" sample contained 0.2 ml of water and 0.2 ml of an aqueous solution of inhibitor, and, finally, 0.2 ml of ATC was added in a concentration within a range approximating to the Michaelis constant.

SUMMARY

1. A series of alkyl 2-morpholinoethyl methylphosphonothionates and alkyl 1-methyl-2-morpholinoethyl methylphosphonothionates have been synthesized and they have been shown to be reversible inhibitors of acetylcholinesterase and butyrylcholinesterase.

2. It has been shown that the alkyl 1-methyl-2-morpholinoethyl methylphosphonothionates exist in solution in the form of two optical isomers in a ratio of 1:3.

LITERATURE CITED

1. M. B. Gafurov, A. A. Abduvakhabov, G. M. Vaizburg, D. N. Dalimov, K. M. Zuparova, E. K. Balashova, and N. N. Godovikov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 647 (1987).
2. D. N. Dalimov, M. B. Gafurov, G. M. Vaizburg, A. A. Abduvakhabov, and N. N. Godovikov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 650 (1987).
3. D. N. Dalimov, M. B. Gafurov, F. G. Kamaev, and A. A. Abduvakhabov, Khim. Prir. Soedin., No. 4, 561 (1987).
4. M. I. Kabachnik and N. N. Godovikov, Dokl. Akad. Nauk SSSR, 110, 210 (1956).
5. A. A. Abduvakhabov, N. N. Godovikov, M. I. Kabachnik, The Chemistry of Organic Compounds of Phosphorus [in Russian], Nauka, Leningrad (1967), p. 3.
6. M. A. Mastryukova, A. E. Shchipov, M. S. Vaisberg, P. V. Petrovskii, and M. I. Kabachnik, Izv. Akad. Nauk SSSR, Ser. Khim., 1841 (1971).
7. G. L. Ellman, R. D. Courtney, V. Anders, and K. M. Featherstone, Biochem. Pharmacol., 7, 88 (1961).
8. G. M. Vaizburg, D. N. Dalimov, and A. A. Abduvakhabov, Dokl. Akad. Nauk UzSSR, No. 10, 32 (1985).
9. T. H. Siddall and W. E. Stewart, Prog. NMR Spectrosc., 5, 33 (1969).

SYNTHESIS OF ENKEPHALINS BY THE METHOD OF POLYMERIC ACTIVATED ESTERS BASED ON 4-HYDROXY-3-NITROBENZOPHENONE

E. I. Grigor'ev and S. V. Chernova

UDC 547.96

Leucine- and methionine-enkephalins have been synthesized by the successive growth of the peptide chain from the C-end by the method of polymeric activated esters based on 4-hydroxy-3-nitrobenzophenone with yields of 90 and 70%, respectively, calculated on the initial C-terminal amino acid. Polystyrene with 2% of divinylbenzene was used as the polymeric matrix. Using the synthesis of methionine-enkephalin as an example, the possibility has been shown of using polymeric activated esters for the synthesis of peptides with a free carboxy group.

In spite of the comparative simplicity of the chemical structure of the enkephalins, their synthesis give low yields of the desired products and require additional purifications of both the intermediate and the final substances, which, in our opinion, makes their economic efficiency problematical. In a review [1] giving some information on investigations in this field up to 1982, it was correctly observed that so far the syntheses of even short peptides "in their absolute majority is far from perfect." In actual fact, in classical methods of synthesizing the enkephalins in solution, variants of fragment condensation are most frequently used. However, in these cases the absence of a statement of the overall yield from the initial compounds and the fact that the yields given relate only to the stages of obtaining protected pentapeptides from two fragments and subsequent, frequently multistage, purification are indicative. Thus, the yields of leucine-enkephalin in the last stage have amounted to 40-70%, and those of methionine-enkephalin to 55-77% (see, for example, [2, 3]).

Leningrad State University. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 547-553, July-August, 1989. Original article submitted February 23, 1988; revision submitted February 14, 1989.

 0009-3130/89/2504-0468$12.50