20(S),24(R)-Epoxydammarane-3α,12β,17α,25-tetraol 3-O-Acetate 12,25-Di-O-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranoside) (8). IR spectrum (ν, cm^{-1}): 1756, 3376, 3536. ^1H spectrum (δ, ppm): 0.85 (s, 3H), 0.90 (s, 6H), 1.00 (s, 3H), 1.14 (s, 3H), 1.18 (s, 3H), 1.20 (s, 6H), 2.00-2.11 (s, 27H, 9 × OAc), 3.11 (s, 1H, OH), 3.70 (m, 3H, 2H-5', H_a-12), 3.99 (m, 1H, H-24), 4.12-4.24 (m, 4H, 4H-6'), 4.56 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1' at C-12), 4.66 (t, 1H, J = 1.7 Hz, H_e-3), 4.95 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1' at C-25), 5.01-5.23 (m, 6H, 2H-2', 2H-3', 2H-4').

CONCLUSIONS

1. The condensation of 20(S),24(R)-epoxydammarane-3α,12β,17α,25-tetraol(betulafolienetetraol oxide), isolated from birch leaves, with α-acetobromoglucose in the presence of mercury cyanide and of insoluble silver compounds has been studied.

2. Betulafolienetetraol oxide 3- and 12-mono-, 3,12- and 12,25-di-O- and 3,12,25-tri-β-D-glucopyranosides have been obtained for the first time.

LITERATURE CITED

1. T. Namba, K. Matsushige, T. Morita, and O. Tanaka, Chem. Pharm. Bull., 34, No. 2, 730 (1986).
2. O. Tanaka and S. Yahara, Phytochemistry, 17, 1353 (1978).
3. N. I. Uvarova, G. I. Oshitok, N. F. Samoshina, and G. B. Elyakov, Khim. Prir. Soedin., 460 (1974).
4. N. K. Kochetkov, A. Ya. Khorlin, and A. F. Bochkov, Tetrahedron, 23, 693 (1967).
5. L. N. Atopkina and N. I. Uvarova, Khim. Prir. Soedin., 451 (1986); 329 (1981).
6. H. Paulsen and O. Lockhoff, Chem. Ber., 114, 3102 (1981).
7. N. I. Uvarova, G. V. Malinovskaya, Yu. N. El'kin, V. V. Isakov, A. K. Dzizenko, and G. B. Elyakov, Khim. Prir. Soedin., 757 (1976).

SYNTHESIS OF A NUMBER OF DERIVATIVES OF ALKALOIDS AND OF NITROGEN-CONTAINING HETEROCYCLES AND THEIR ANTICHOLINESTERASE ACTIVITIES

D. N. Dalimov, D. T. Karimov, G. N. Vaizburg, A. A. Abduvakhabov, L. K. Abdullaeva, and F. G. Kamaev

UDC 547.94:547.1.118

N-Methyl- and N-phenylcarbamates based on a number of alkaloids and nitrogen-containing heterocycles have been synthesized, and they have proved to be weak irreversible inhibitors of acetylcholinesterase and butyrylcholinesterase. It has been shown that the choline fragments of the above-mentioned carbamates and their β-methylcholine analogs are reversible inhibitors of both cholinesterases and make a substantial contribution to the anticholinesterase activity. Selective inhibitors of ACE and BuCE have been found among the compounds synthesized.

Many organophosphorus compounds having onium nitrogen in a ring exhibit high anticholinesterase activities [1-3]. A study of the influence of carbamates containing a nitrogen atom in a ring may lead to the creation of highly specific and selective cholinesterase effectors, since it is known [4, 5] that carbamates, like organophosphorus inhibitors, interact chemically with the active sites of the cholinesterases. Furthermore, by studying the laws of the antienzyme activity of carbamates with systematic variations in their structure it will be possible to obtain additional information of the structure and topography of the catalytic site of the cholinesterases.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent.

Translated from Khimiya Prirodynkh Soedinenii, No. 6, pp. 825-831, November-December, 1988. Original article submitted December 9, 1987.

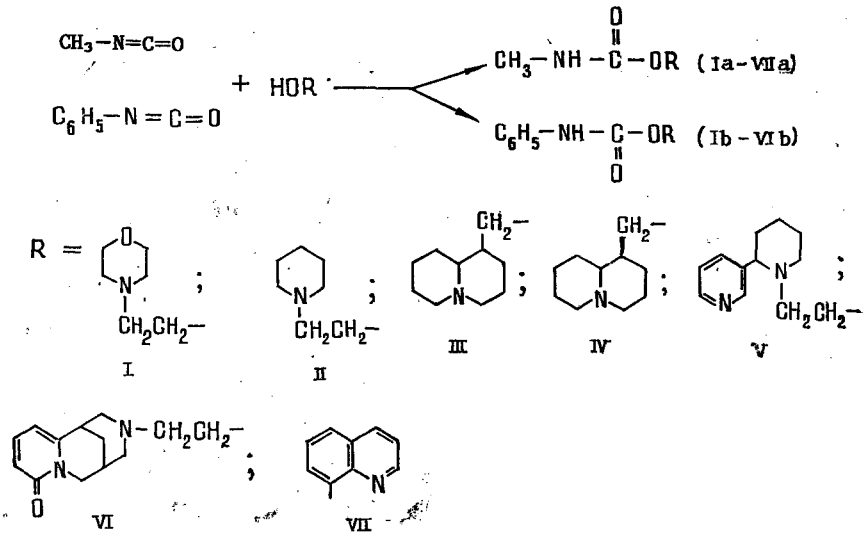
0009-3130/88/2406-0702$12.50

TABLE 1. Anticholinesterase Activities of N-Methylcarbamates (a) and N-Phenylcarbamates (b)

Compound	k_2, liter/mole·min		k_2 ACE / k_2 BuCE	Compound	k_2, liter/mole·min		k_2 ACE / k_2 BuCE
	ACE	BuCE			ACE	BuCE	
Ia	$4.0 \cdot 10^1$	$5.4 \cdot 10^1$	0,74	Ib	$1.7 \cdot 10^1$	$5.1 \cdot 10^1$	0,3
IIa	$4.0 \cdot 10^1$	$4.8 \cdot 10^1$	0,83	IIb	$4.3 \cdot 10^1$	$3.10 \cdot 10^2$	0,14
IIIa	$9.8 \cdot 10^1$	$5 \cdot 10^2$	0,20	IIIb	$3.70 \cdot 10^2$	$1.9 \cdot 10^4$	0,019
IVa	$1.7 \cdot 10^1$	$1.2 \cdot 10^1$	1,42	IVb	$4.9 \cdot 10^1$	$2.4 \cdot 10^3$	0,021
Va	$3.0 \cdot 10^1$	$9.4 \cdot 10^1$	0,32	Vb	$5.4 \cdot 10^1$	$2.80 \cdot 10^2$	0,2
VIIa	$1.3 \cdot 10^3$	$1.0 \cdot 10^1$	100	VIb	$6.0 \cdot 10^2$	$5.7 \cdot 10^3$	0,1

We have synthesized N-methyl- and N-phenylcarbamates including within their molecules residues of morpholine (I), piperidine (II), lupinine (III), epilupinine (IV), anabasine (V), cytisine (VI), and 8-hydroxyquinoline (VII) and have studied their anticholinesterase properties.

The N-methyl- and N-phenylcarbamates had been synthesized previously by methods described in [6, 7] using the following schemes:



The structures of the substances obtained were confirmed by the IR and PMR spectra.

The anticholinesterase activities of the carbamates synthesized were investigated in relation to human blood erythrocyte acetylcholinesterase (ACE) and horse blood serum butyrylcholinesterase (BuCE).

It follows from the results, which are given in Table 1, that all the carbamates synthesized are irreversible inhibitors of ACE and of BuCE. In the case of the N-methylcarbamates, the active center of ACE actually "feels" the difference between the morpholine (Ia), piperidine (IIa), and anabasine (Va) derivatives. Passage to the lupinine derivative (IIIa) was accompanied by a doubling of the irreversible inhibiting activity. The conformational differences between the lupinine (IIIa) and the epilupinine (IVa) analogs had a substantial difference on the value of k_2 of the corresponding substances. The presence in the structure of an inhibitor of the rigidly fixed 8-hydroquinoline system (VIIIa) led to a 32-fold increase in k_2. This compound proved to be the strongest inhibitor in this series of compounds.

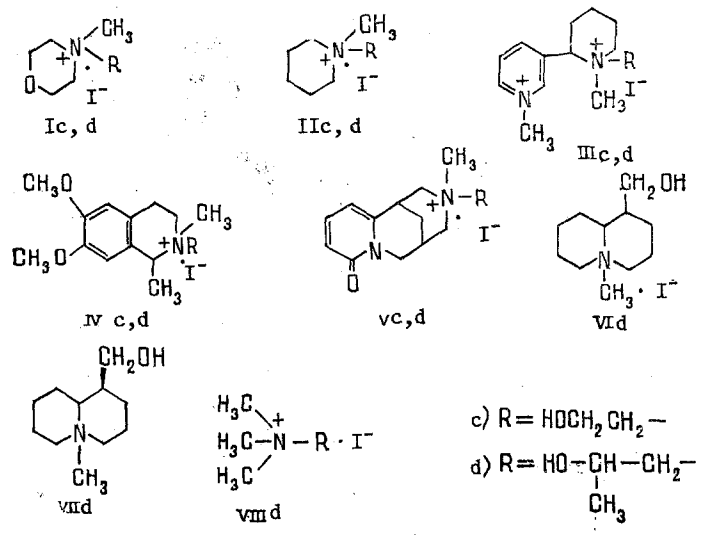
The interaction of the above-mentioned substances with BuCE differed somewhat from the interaction with ACE. While the morpholine and piperidine derivatives interacted similarly with the active site of the enzyme, passage to the anabasine derivative increased the irreversible inhibitory activity twofold, and passage to the lupinine derivative tenfold. Here, also, differences existed in the values of k_2 between the lupinine and the epilupinine derivatives. The latter substance (IVa) proved to be more than 40 times less active than the former (IIIa). It is a surprising fact that the 8-hydroxyquinoline derivative (VIa) was, on an average, 5 times less active than the morpholine and piperidine derivatives. The pronounced hydrophobic environment of the nitrogen atom in the molecule, apparently, should lead to an increase in the affinity of the inhibitor molecule for the active surface of BuCE

in which, as has been shown [8], the anionic point is surrounded by a broad hydrophobic region. However, this compound proved to be the weakest inhibitor of BuCE.

In the interaction of the N-phenylcarbamates with ACE, passage from the morpholine (Ib) to the lupinine (IIIb) derivative led to a 20-fold increase in k_2. Here, also, differences were observed in the inhibiting activities of substances containing lupinine (IIIb) and epilupinine (IVb) residues. A comparison of these facts with those for the corresponding methylcarbamates showed that the phenylcarbamates were three times more active.

The phenylcarbamates were more active in relation to BuCE than to ACE. Lupinine N-phenylcarbamate was the most active. In this series of inhibitors the epilupinine derivative (IVb) was eight times weaker than the lupinine derivative.

On the basis of the results of the analysis of the structures of the phosphorylated derivatives of the above-mentioned alkaloids and heterocycles, which are irreversible cholinesterase inhibitors [9], we expected a greater antienzymatic activity from the carbamates that we had synthesized, but they proved to be weak inhibitors of ACE and BuCE. In this connection, it was important to determine the influence of the steric factors of the choline-like fragments of the inhibitor molecule on its anticholinesterase activity. For this purpose we synthesized the corresponding methiodides of N-β-hydroxyethylmorpholine (Ic), -piperidine (IIc), -anabasine (IIIc), -salsolidine (IVc), and -cytosine (Vc) and of N-β-hydroxypropylmorpholine (Id), -piperidine (IId), -anabasine (IIId), -salsolidine (IVd), -cytosine (Vd), -lupinine (VId), and -epilupinine (VIId).



All the compounds studied proved to be reversible inhibitors with the competitive and mixed types of action in relation to both cholinesterases (Table 2). It can be seen from Table 2 that complication of the piperidine ring and of the ethylene link (with the introduction of a methyl group into the β-position) led to a rise in the value of K_i.

With respect to ACE, N-β-hydroxymorpholine methiodide (Ic) had an inhibiting activity 1.5 times weaker than that of the corresponding β-hydroxypropyl derivative (Id). An appreciable influence of a methyl group in the β-position of the ethylene link was observed on passing from N-β-hydroxyethylpiperidine methiodide (Ic) to N-β-hydroxypropylpiperidine methiodide (IId). The inhibiting activity of (IId) was 23 times as great as that of (IIc), 44 times that of N-β-hydroxyethylmorpholine methiodide (Ic), and 2.5 times that of (VIIId). It follows from this that the introduction of a methyl group into the β-position of the ethylene link substantially intensifies the inhibiting activity with respect to ACE. The presence of an oxygen atom in the morpholine ring has an adverse effect on the adsorption of the cationic part of the molecule on the ionic section of the active surface of the enzyme in comparison with a piperidine ring. The further complication of the piperidine ring, including passage to the anabasine analog, led to an increase in K_i, i.e., to decrease in the reversible inhibiting activity. Thus, the N-β-hydroxyethyl derivative of anabasine (IIIc) was only half as effective as the piperidine analog (IIc) and 15.5 times less effective than (VIIId). However, the introduction of a methyl group into the ethylene link intensified the anticho-

TABLE 2. Anticholinesterase Efficiencies of a Number of Choline Analogs Based on Nitrogen-Containing Heterocycles and Alkaloids

Compound	R_f	R_m	K_i (M)		K_i ACE / K_i BuCE
			ACE	BuCE	
Ic*	0,24	1,31	$8,3 \cdot 10^{-3}$	$1,3 \cdot 10^{-2}$	0,63
IIc**	0,41	1,69	$4,25 \cdot 10^{-3}$	$2,3 \cdot 10^{-3}$	1,84
IIIc**	0,08	1,08	$7,0 \cdot 10^{-3}$	$3,7 \cdot 10^{-3}$	1,9
IVc**	0,53	2,12	$1,6 \cdot 10^{-3}$	$4,9 \cdot 10^{-4}$	3
Vc**	0,19	1,23	$4,3 \cdot 10^{-3}$	$7,8 \cdot 10^{-3}$	0,06
Id**	0,35	1,53	$5,6 \cdot 10^{-3}$	$3,1 \cdot 10^{-3}$	1,86
IId**	0,53	2,12	$1,85 \cdot 10^{-4}$	$4,8 \cdot 10^{-4}$	0,38
IIId*	0,18	1,21	$1,19 \cdot 10^{-4}$	$6,5 \cdot 10^{-5}$	2,5
IVd*	0,75	4,0	$7,03 \cdot 10^{-3}$	$1,57 \cdot 10^{-4}$	44,9
Vd**	0,24	1,31	$5,3 \cdot 10^{-4}$	$4,3 \cdot 10^{-3}$	0,13
VId***	—	—	$1,73 \cdot 10^{-4}$	$3,4 \cdot 10^{-3}$	
VIId**	—	—	$2,1 \cdot 10^{-3}$	$2,5 \cdot 10^{-4}$	
VIIId**	—	—	$4,5 \cdot 10^{-4}$	—	—

*Possesses the competitive type of inhibition of ACE and BuCE.
**Possesses the mixed type of inhibition of ACE and BuCE.
***Taken from the literature [15].

linesterase activity by a factor of 36 (IIId) and it was 30 times greater than that of the similar morpholine methiodide (Id) and was 2.5 times more active than choline.

The salsolidine derivatives were characterized by a highly pronounced hydrophobicity. Thus, for N-β-hydroxyethylsalsolidine methiodide (IVc) R_m = 2.12 and for (IVd) R_m = 4.0. Therefore, the introduction of a methyl group into the ethylene link of (IV) leads to a 4.3-fold decrease in the reversible inhibiting activity with respect to ACE. This decrease in the reversible inhibiting activity of the salsolidine derivative can be explained by the fact that hydrophobic interactions with inhibitors are less characteristic for ACE [10].

In interaction with ACE, N-β-hydroxypropylcytidine methiodide (Vd) was 8 times more active than (Vc) and 3.7 times more active than (VIIId). In the case of the interaction of the same substances with BuCE, a complication of the piperidine ring for the N-β-hydroxyethyl derivatives led to only a slight change in anticholinesterase activity. An exception from this series of substances is the salsolidine derivative, the K_i value of which was 5 times smaller than that of the corresponding piperidine analog.

In all cases, the introduction of a methyl group into the ethylene link enhanced anticholinesterase activity. It can be seen from Table 2 that the majority of substances that we investigated exhibited a specificity of the reaction with respect to BuCE. The most specific in relation to BuCE was N-β-hydroxypropylsalsolidine methiodide, and with respect to ACE the most specific substances were N-β-hydroxypropylpiperidine and -cytosine methiodides.

As we see, in the case of the interaction of the choline analogs obtained with ACE and BuCE, a complication of the piperidine ring had little effect on the reversible inhibiting activity. The introduction of a methyl group into the β position of the ethylene group improved the complementarity of these compounds with the active sites of the two enzymes. This enhancement was particularly pronounced in the case of the interaction with ACE.

Thus, the choline fragments of the molecules of the irreversible cholinesterase inhibitors that we are discussing make a definite contribution to their anticholinesterase activity. An investigation of the dependence of the activity of a substance on its structure showed that the β-methylcholine analogs were more specific in relation to ACE than the choline analogs. The low activity of the carbamates can be explained by the assumption that in the region of the esterase point of ACE there is a strictly specific section that is adapted to the sorption of a CH_3 group. The binding of a CH_3 group with a N—C—O bond through an NH bridge apparently leads to a disturbance of the complementarity of the corresponding

section of the active site of the enzyme. In the case of BuCE, the phenyl group is apparently sorbed better than the corresponding hydrophobic section of the active site of the enzyme, but the influence of an NH group is also shown here.

An analysis of the interrelationship between the structures of the carbamate analogs of choline and β-methylcholine that had been synthesized with their anticholinesterase activities shows us possible routes to the synthesis of more active carbamates, which can apparently be obtained by modeling the methylcarbamate part of the inhibitor in the direction of greater hydrophobicity and replacing the choline fragment by β-methylcholine analogs, since these compounds exhibited greater complementarity to the active site of ACE.

EXPERIMENTAL

IR spectra in the 400-3500 cm^{-1} interval were recorded on a Specord 75-IR instrument in paraffin oil.

IR spectrum, ν_{max}, cm^{-1} (IIa): 3280-3240 (NH), 1710 (C=O), 1200 (C–O–C). Analogous absorption bands were present in the IR spectra of the other methylcarbamates. For the phenylcarbamates bands at (cm^{-1}) 3090 (CH) and at 1590, 1480, and 1320 (C=C) were characteristic. PMR spectra were recorded on a Varian XL-200 spectrometer (200 MHz, CCl_4). The samples used were 5% solutions of the substances under investigation, with HMDS as internal standard.

Phosgene, N,N-diphenyl-N-methylurea,* methyl isocyanate, and phenyl isocyanate were obtained by methods described in the literature [6, 7].

Morpholinoethyl N-Methylcarbamate (Ia). With stirring, 11.7 g (0.09 mole) of N-β-hydroxyethylmorpholine was added dropwise to a solution of 5 g (0.09 mole) of methyl isocyanate in 20 ml of pyridine. The reaction mixture was heated at 70-80°C for 7-8 h. The course of the reaction was monitored by thin-layer chromatography [in the benzene–ether–ethanol (10:5:2) system on the support Al_2O_3 (Brockman activity grade II)], nonfixed layer, with iodine as the revealing agent. Then the solvent was distilled off and the residue was purified by column chromatography on a column of Al_2O_3 with absolute ether as the eluate. Compound (Ia), oil, R_f 0.93, yield 91%; hydrochloride with mp 185-187°C. PMR of (Ia): 5.1 (NH), 4.0 (OCH_3, J = 6 Hz), 3.5 (4H), 2.3-2.6 (6H), 2.64 (CH_3, J = 4.3 Hz). The other methylcarbamates were obtained similarly. Compound (IIa), oil, R_f 0.92, yield 89%; hydrochloride with mp 131-132°C; (IIIa), mp 92-94°C, R_f 0.94, yield 92%; PMR of (IIIa): 4.6 (NH), 4.1 (m, 2H), 2.64 (d, 3H), 2.5-2.7 (H_{2e}-H_{10e}), 1.0-2.0 (H of the quinolizidine nucleus). Compound (IVa), oil, R_f 0.91, yield 90%. Compound (Va), oil, R_f 0.93, yield 94%; hydrochloride with mp 192-194°C. PMR spectrum of (Va): 8.38 (d, Hα), 8.30 (dd, Hα), 7.52 (dt, H_γ), 7.08 (dd, H_β), 5.3 (NH), 2.64 (d, 3H), 3.85 (2H), 2.08 (2H). Compound (VIa), mp 164-165°C, R_f 0.95, yield 93%; hydrochloride with mp 147-148°C.

Morpholinoethyl N-Phenylcarbamate (Ib). At the boiling point of toluene, 2.58 g (0.02 mole) of N-β-hydroxyethylmorpholine was added dropwise to a solution of 2.38 g (0.02 mole) of phenyl isocyanate in 25 ml of dry toluene. The reaction mixture was heated for 6-8 h. The course of the reaction was monitored by thin-layer chromatography [support – Al_2O_3; solvent system – chloroform–ethanol (24:1); revealing agent – iodine vapor]. Then the solvent was distilled off, and the residue was purified on a column of Al_2O_3 with absolute ether as the eluent. The final product obtained was dried in vacuum. Compound (Ib), mp 73-74°C, R_f 0.92, yield 91%; hydrochloride with mp 210-212°C.

The other phenylcarbamates were obtained similarly. Compound (IIb), oil, R_f 0.97, yield 92%; hydrochloride with mp 184-185°C. Compound (IIIb), mp 75-76°C, R_f 0.95, yield 87%; hydrochloride with mp 200-201°C. Compound (IVb), mp 43-44°C, R_f 0.91, yield 84%; hydrochloride with mp 212-214°C. Compound (Vb), mp 69-71°C, R_f 0.92, yield 90%; hydrochloride with mp 150-152°C. Compound (VIb), oil, R_f 0.93, yield 86%; hydrochloride with mp 188-189°C.

Characteristic for the PMR spectra of the corresponding phenylcarbamates was the presence in the weak field at 6.86-1.14 ppm of the signals of a benzene ring and a single signal of the NH proton in the same region.

*As in Russian original; one of the N's should be N' – Publisher.

The synthesis of the methiodides of the N-β-hydroxyethyl- and N-β-hydroxypropylamines containing morpholine, piperidine, anabasine, salsolidine, and cytosine residues was carried out as described in [3]. The values of R_f* were determined by paper chromatography on "Leningradskaya bistraya [fast]" paper in the butanol–hydrochloric acid–water (100:15:27) system. The R_f values, which characterize the hydrophobicities of inhibitor molecules, were determined from the formula $1/(1 - R_f^*)$.

Anticholinesterase activities were determined by Ellman's method [11] and were studied on enzyme preparations produced by the Perm Scientific-Research Institute of Vaccines and Sera: ACE (Ec 1.1.7) with a specific activity of 8 U/mg, and BuCE (EC 1.1.8) with a specific activity of 29 U/mg. The values of k_2 characterizing the irreversible inhibiting activity of a carbamate were calculated from the following formula:

$$k_2 = \frac{1}{[I]t} \ln\left(\frac{V_0}{V_t}\right),$$

where V_0 and V_t are the rates of enzymatic hydrolysis of the substrate in the absence of the inhibitor and after incubation with the inhibitor for t minutes, respectively; and [I] is the concentration of inhibitor.

Reversible inhibiting activities were determined by the graphical methods of Lineweaver and Burk [12] and of Eisenthal and Cornish-Bowden [13, 14], and were evaluated by the magnitude of the equilibrium inhibition constant K_i. Experiments were performed at 30°C with a reaction mixture having a pH of 7.5. The samples for the determination of the activity of the enzymes contained 1.8 ml of 0.1 M phosphate buffer (pH 8.01), 0.2 ml of a solution of ACE or BuCE in water, 0.4 ml of a solution of ATC in a concentration of $2 \cdot 10^{-3}$ M (in the case of BuCE, the concentration of BTC was $1 \cdot 10^{-3}$ M); and 0.2 ml of Ellman's 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 activities of the enzymes, part of the water was replaced by a solution of inhibitor with the necessary concentration. The specificity of the action with respect to ACE or BuCE was determined by the coefficient of action selectivity, found from the ratio K_iACE/K_iBuCE.

CONCLUSIONS

1. N-Methyl- and -phenylcarbamates based on a number of alkaloids and nitrogen-containing heterocycles have been synthesized and have been found to be weak irreversible inhibitors of acetylcholinesterase and butyrylcholinesterase.

2. It has been shown that the choline fragments of these carbamates and their β-methylcholine analogs are reversible inhibitors of both cholinesterases and make a substantial contribution to the cholinesterase activity.

3. Selective inhibitors of ACE and BuCE have been found among the compounds synthesized.

LITERATURE CITED

1. M. I. Kabashnik and N. N. Golovnikov, Dokl. Akad. Nauk SSSR, 196, 348 (1971).
2. A. P. Brestkin, I. L. Brik, L. M. Ginetsinskaya, N. N. Godovikov, and M. I. Kabashnik, Izv. Akad. Nauk SSSR, Ser. Khim., 4, 883 (1975).
3. A. S. Sadykov, E. V. Rozengart, A. A. Abduvakhobov, and Kh. A. Aslanov, Cholinesterases: Active Sites and Action Mechanism [in Russian], Fan, Tashkent (1976), p. 205.
4. R. L. Metcalf, "Relationship between the structure of carbamates and their insecticidal activity," WHO Bull., 44, 44 (1972).
5. W. N. Aldridge, "Mechanism of the action of organophosphorus compounds and carbamates with esterases," WHO Bull., 44, 27 (1972).
6. W. Siefken, Ann. Chem., 562, 75 (1949).
7. J. N. Saunders and R. J. Slocombe, Chem. Rev., 43, No. 2, 203 (1948).
8. M. I. Kabashnik, A. A. Abduvakhabov, I. I. Agabekova, A. P. Brestkin, R. I. Volkova, N. N. Godovikov, E. N. Godyna, S. S. Mikhailov, M. Ya. Mikhel'son, V. I. Rozengart, and R. V. Sitkevich, Usp. Khim., 39, 1050 (1970).
9. A. S. Sadykov, D. N. Dalimov, N. N. Godovikov, "Organophosphorus derivatives of alkaloids and nitrogen-containing heterocycles as cholinesterase inhibitors," Usp. Khim., 52, No. 10, 1602 (1983).
10. V. I. Rozengart and O. E. Sherstobitov, The Selective Toxicity of Organophosphorus Insectoacaricides [in Russian], Nauka, Leningrad (1978).

11. G. L. Ellman, K. D. Courtney, V. Anders, and K. M. Featherstone, Biochem. Pharmacol., 7, 88 (1961).
12. J. L. Webb, Enzyme and Metabolic Inhibitors, Academic Press, New York, Vol. I (1963) [Russian translation: Mir, Moscow (1967), p. 163].
13. R. Eisenthal and A. Cornish-Bowden, Biochem. J., 159, 715 (1974).
14. List of Biochemical Procedures Used in the Member Countries of Comecon on Subject 11-8.11.2. Mechanism of the Action of Insecticides and Acaricides [in Russian], CÉV/VNIIKhSZR [Comecon/All-Union Scientific-Research Institute of Chemical Agents for Plant Protection] Coordination Center, Moscow–Poznan (1978).
15. G. M. Vaizburg, D. N. Dalimov, and A. A. Abduvakhabov, Dokl. Akad. Nauk UzSSR, No. 10, 32 (1986).

MULTIMERIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PLANT STEROLS USING UV DETECTION

Yu. F. Krylov, B. S. Krynetskii, B. S. Prokhorov, and T. S. Oretskaya

UDC 543.544:615.357

A method for the multimeric high-performance liquid chromatography of plant sterols is proposed which permits the separation of compounds close in structure with similar chromatographic properties. The first stage includes the chemical modification of the sterols with the aid of a bis(p-nitrophenyl) phosphate group. The phosphotriesters formed as the result of the reaction are separated by normal-phase HPLC. The compounds isolated are treated with ammonia, as a result of which the sterol phosphotriesters are converted into the corresponding phosphodiesters. These phosphodiesters are then subjected to reversed-phase HPLC. The chromatographic separation of UV-absorbing sterol derivatives using several variants of HPLC substantially increases the resolving power of the method.

Plant sterols, or phytosterols, form an important field of investigation, since they play a significant role in biochemical processes in plants and are also an initial raw material in the industrial synthesis of hormone preparations. Phytosterols compose a group of structurally related compounds the chromatographic properties of which are very close. This complicates the analysis and preparative processing of individual compounds of this class [1, 2]. Numerous schemes for isolating sterols have been proposed, but the problem still cannot be regarded as having been completely solved [3]. The use for these purposes of such a powerful method of separation as high-performance gas-liquid chromatography (HPLC) is associated with certain difficulties. These are due to the absence of a convenient method of detection, since the majority of sterols contain no chromophoric groups, and also to their low solubility in aqueous organic phases [4]. To circumvent the first of these difficulties it has been proposed to introduce UV-absorbing modifying groups into the sterol molecule. Such a modification is possible, for example, by esterifying the 3-hydroxy groups of sterols [5].

In the present paper we propose a method for the chemical modification of plant sterols with the aid of the bis(p-nitrophenyl) phosphate group which facilitates the detection of these compounds in the process of chromatographic analysis and permits different variants of HPLC to be used for their separation. The triester scheme of synthesis widely known in oligonucleotide chemistry [6] has been used for the introduction of a bis(p-nitrophenyl) phosphate grouping at the 3-hydroxy group of a sterol:

State Scientific-Research Institute on the Standardization and Control of Drugs, USSR Ministry of Health, Moscow. L. V. Lomonosov Moscow State University. Translated from Khimiya Prirodynkh Soedinenii, No. 6, pp. 831-836, November-December, 1988. Original article submitted January 11, 1988; revision submitted April 12, 1988.

 0009-3130/88/2406-0708$12.50