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RELATIONSHIP BETWEEN THE STRUCTURE AND CHOLINERGIC EFFICIENCY OF VARIOUS DERIVATIVES OF THE ALKALOID ANABASINE

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Results on the study over the last 20 years of the relationship between the structure of derivatives of the alkaloid anabasine and their cholinotropic activity are generalized.

The school created by Academician A. S. Sadykov has made a substantial contribution to the development of the chemistry of the alkaloids of Uzbekistan. A considerable position here is occupied by investigations on the chemical modification of alkaloids and the study of their physiological activity [1-15].

Anabasine, having in its molecule a piperidine fragment with a secondary amino group and a pyridine residue with aromatic delocalization of the electron density, is characterized by conformational lability. These properties impart considerable synthetic possibilities to anabasine and determine its reactivity in complex-formation, alkylation, and acylation and electrophilic exchange processes.

By analogy with nicotine, which possesses a similar physiological action, it is considered that the mechanism of the action of anabasine is based on its interaction with the cholinergic structures of living organisms [11]. The aim of the present review is to generalize and analyze the results of investigations carried out in the last 20 years of the cholinotropic activity of anabasine derivatives.

Modification of the structure of anabasine has been achieved mainly by replacing the hydrogen atom at the nitrogen of the piperidine ring [2-9, 12-16, 321-35, 42-58]. These reactions are shown schematically in Figs 1 and 2.

Esters of Anabasine Derivatives and Carboxylic Acids. Structurally, esters based on anabasine (see Fig. 1, compounds (I) and (II)) are analogues of acetylcholine [12-15] and acetyl-β-methylcholine [16], which has permitted their investigation as substrates of cholinesterases (CEs). A detailed kinetic analysis of the interaction of esters of N-β-hydroxyethyl- and N-β-hydroxypropylanabasine with CEs has shown that they do not undergo enzymatic hydrolysis even at a CE concentration 10 times exceeding those at which the hydrolysis of acetylchloine is usually studied. These results have served as a basis for the study of the esters obtained as inhibitors of CEs from various biological materials [12-21, 24].

Acylates of the N-β-hydroxyethyl and N-β-hydroxypropyl derivatives of anabasine and the methiodides of these acylates (Table 1) reversibly suppressed the hydrolysis of acetylcholine under the action of acetylcholinesterase (ACE) from human blood erythrocytes and that of butyrylcholinesterase (BuCE) from horse blood serum. The inhibition of the activity of BuCE has a competitive nature, and that of ACE — noncompetitive. As a rule, the dimethiodides are more effective enzyme inhibitors than the bases. Compounds of this series have proved to be specific inhibitors of BuCE, particularly the compound with R = C_2H_5, the efficiency of which for BuCE is 65 times higher than for ACE. The absence of a linear dependence of the activity of the esters on the structure of the acyl moiety for the bases and the dimethiodides is due to the different cationic structures of their molecules: the presence of two unsymmetrically arranged cationic heads in each dimethiodide and of only one protonated grouping in each base. The greater affinity of these inhibitors for BuCE is connected with their possible interaction with the peripheral anionic grouping of the active center of the enzyme.

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TABLE 1. Anticholinesterase Activities, pK_i of Esters of N-(β-Hydroxyethyl)- and N-(β-Hydroxypropyl)anabasines (I) and (II) [12-16]

Compound No.	R	Bases (I)		Methiodides of (I)				Bases (II)		Methiodides of (II)	
		ACE	BuCE	ACE	BuCE	Frog CE	Squid CE	ACE	BuCE	ACE	BuCE
1	CH_3	3.38	4.68	4.22	4.96	4.20	3.85	2.12	2.23	3.38	4.96
2	C_2H_5	3.30	4.55	4.37	6.18	4.11	3.68	2.09	3.79	5.21	3.62
3	C_3H_7	3.21	4.47	4.47	5.49	4.16	4.02	3.60	3.42	3.10	3.43
4	C_4H_9	2.92	4.07	4.07	5.09	3.75	3.48	2.43	3.35	2.30	3.29
5	i-C_4H_9	3.36	4.54	4.27	6.01	4.80	—	2.04	3.02	2.45	4.52

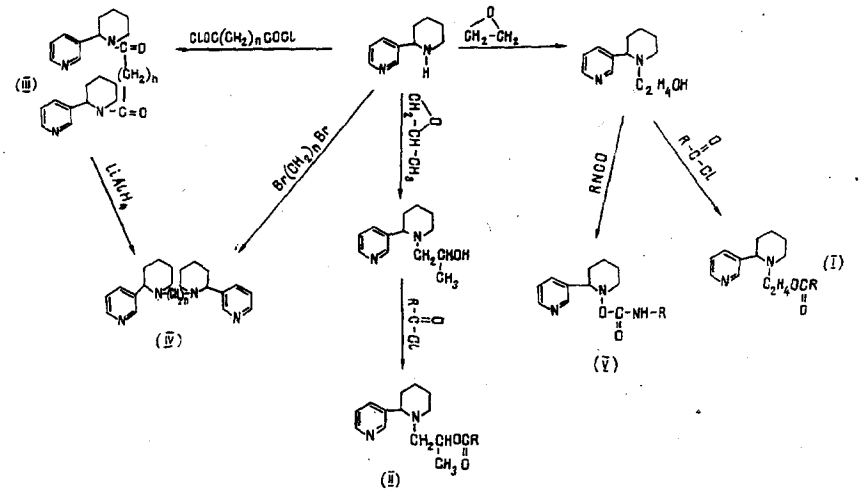

Fig. 1. Scheme of synthesis of anabasine derivatives including carboxylic acid residues.

These substances are also reversible inhibitors for the CEs of the frog and of the Pacific Ocean and Komandorskii squids, i.e., their efficacies do not depend on the time of incubation with the enzymes and the degree of inhibition falls when the reaction medium is diluted [17-20].

The efficacy of anabasine esters in relation to the CEs of these animals scarcely depended on the length of the acyl radical, apparently as a result of a disturbance to the complementarity of binding because of the cumbrousness of the bicationic head. In the reactions of anabasine derivatives with the CE from the Pacific Ocean squid [18] the competitive type of inhibition proved to be more pronounced, and for frog CE the mixed-competitive type.

It is known that the second very important component of the cholinergic system is the cholinoreceptor (CR), which, like the CEs, acts as a target of the action of acetylcholine and ammonium comounds analogous to it. A. F. Danilov et al. [21] have studied the interaction of esters of N-(β-hydroxyethyl)anabasine with the *rectus abdominis* muscle of the common frog *Lana temporaria* and the isolated *retractor infundibula* muscle of the Pacific Ocean squid *Todarodes pacificus*. In relation to the CR of the frog the esters of N-β-hydroxyethylanabasine proved to be amost total antagonists (Table 2). The nature and strength of the action of these substances on the CEs and thebCR of one and the same animal differ in terms of the structure–activity relationship. The cumbrousness of the head did not prevent the complementary binding of the compounds with the active centers of the CRs but prevented it in the case of the CEs. The sensitivity of the CRs to the valerate (R = C_4H_9) was the highest.

All the N-β-hydroxypropylanabasine esters, just like their N-β-hydroxyethyl analogues, possess the properties of reversibly inhibiting ACE and BuCE [16] (see Table 1). The corresponding dimethiodides possess a more pronounced inhibiting effect than the bases. The latter inhibit the two enzymes to almost the same extent, and their antienzyme activity depends little on the number of carbon atoms in the acyl part of the este- grouping. The methiodides suppress the catalytic activities of ACE and BuCE almost identically. A definite dependence of activity on the structure of the alkyl radical is shown (Table 1): with a lengthening of R to C_3H_7 the inhibiting effect rises, and it then falls at C_4H_9. In the case of ACE the acetyl derivative possesses a pronounced action, and in relation to BuCE the propionyl derivative does so. The butyl derivative is 10

TABLE 2. Interaction of Esters of N-(β-Hydroxyethyl)anabasine (I) with the Cholinoreceptors (CRs) of the Frog and the Squid [18]

Compound No.	R	Frog CR			Squid CR		
		ME	Pec_{50}	pA_2	ME	Pec_{50}	pA2
1	CH_3	0,90	3.40	—	—	—	2.47
2	C_2H_5	0,83	3.65	—	—	—	2.93
3	C_3H_7	0,93	3.70	—	—	—	2.72
4	C_4H_9	—	—	—	—	—	3.84
5	i-C_4H_9	—	—	—	—	—	—

Notes. ME) maximum efficiency of the substance; pEC_{50}) negative logarithm of the concentration of agonist causing 50% of the maximum possible contraction of the muscle; pA_2) negative logarithm of the concentration of antagonist in the presence of which the sensitivity of the muscle to acetylcholine is halved.

times more active for ACE and BuCE than the acetate. Characteristic for the N-β-hydroxypropyl analogues is a less pronounced inhibitory activity than in the case of the N-β-hydroxyethyl derivatives, among which there are specific inhibitors of BuCE [13].

The technical preparation anabasine sulfate is highly toxic for many insects (thrips, bugs, caterpillars, beetle larvae, etc.) but it is most highly toxic for various species of aphids and is considered one of the best aphicides [22, 23]. In view of this, for a better understanding of the mechanism of the insecticidal action of anabasine, and also for the purposes of finding new effective insecticides among anabasine derivatives, we have investigated the toxicity for the greenbug and the cholinotropic activity of anabasine itself and a number of its derivatives [24].

It can be seen from Table 3 that all the substances investigated effectively suppress greenbug and human ACEs. Anabasine and its derivatives inhibit human ACE by the noncompetitive mechanism; i.e., at any concentration of the substrate K_i is equal to 1. A direct comparison of the anticholinesterase activities of the compounds in relation to the aphid and the human ACEs is therefore possible. Erythrocyte ACE is 2-14 times more sensitive to anabasine and its derivatives than aphid ACE. The ability of the compounds investigated to inhibit aphid ACE shows that in the evaluation of the toxicity of anabasine and some of its derivatives it is impossible to ignore their anticholinesterase action. The mechanism of their action on animals is, apparently, complex and includes both interaction with the cholinoreceptor and the suppression of ACE. This complex action can explain the high toxicity of anabasine [25].

The dimethiodides of the N-β-hydroxyethylanabasine esters (compounds 8-11) proved to be reversible inhibitors of aphid and human ACEs. Their activity rose on passing from R = C_3H_7 to R = C_4H_9 and then to C_5H_{11}. This tendency was followed similarly for both human and aphid ACEs. As is known [4], in the hydrolysis of choline esters under the action of ACE a different situation exists: esters with R = CH_3 are hydrolyzed most rapidly, those with R = C_3H_7 more feebly, and those with R = C_4H_9 extremely poorly. The difference in the actions of the choline and anabasine esters is obviously connected with the orientation of these two types of compounds on the active surface of the enzyme. Apparently, esters based on anabasine are sorbed on ACE through Coulomb and hydrophobic interactions in such a way that the ester grouping is oriented "incorrectly" in relation to the esterase point of the enzyme, and these compounds are, therefore, not substrates but inhibitors, and their efficiency rises with an extension of their acyl moiety, wnich leads to an increase in the hydrophobicity of the compound.

The importance of a hydrophobic interaction in the anticholinesterase activity of anabasine derivatives is also shown by the fact that the inhibiting capacity of anabasamine (cpd 5) is several times higher than that of anabasine (cpd 1) for both aphid and human ACEs. However, the introduction of charged quaternary nitrogen atoms into the inhibitor molecules has different effects on their anticholinesterase activities in relation to the two ACEs. This shows differences in the active surfaces of the aphid and human ACEs.

A different pattern is observed on the analysis of the insecticidal properties of anabasine. It is known that the more hydrophobic substances penetrate more readily through the insect cuticle and therefore prove to be more toxic. At the same time an appreciable increase in the weight of the molecule may have an unfavorable efect on the rate of its penetration. It can seen from toxicity results (see Table 3) that anabasine itself and its salts are good aphicides with LC_{50} values of of the order of 0.01% and lower. On passing to N-methylanabasine the toxicity for aphids falls 10-fold, and to anabasamine as much as

TABLE 3. Anticholinesterase Activity of Anabasine and some of its Derivatives and their Toxicity for the Greenbug [24]

Formula	LC_{50} for the greenbug, %	I_{50} for bug ACE (M)	K_i for human ACE (M)
1. Anabasine base	0.0048+0.0007	4.5×10^{-3}	1.04×10^{-3}
2. Anabasine dimethiodide	No effect 1.3	5.3×10^{-3}	5.2×10^{-4}
3. Anabsine sulfate	0.0016+0.0003	1.3×10^{-3}	3.2×10^{-4}
4. Anabasine hydrochloride	0.0125+0.0015	2.5×10^{-3}	3.29×10^{-4}
5. Anabasamine base	0.1+0.015	5.4×10^{-4}	3.18×10^{-4}
6. Anabasamine trimethiodide	No effect 1.0 No effect	3.5×10^{-4}	5.1×10^{-5}
7. R=CH_3	0.5	7.35×10^{-4}	6.0×10^{-5}
8. R=C_2H_5	0.5	4.72×10^{-4}	4.4×10^{-5}
9. R=C_3H_7	1.0	1.65×10^{-4}	3.4×10^{-5}
10. R=C_4H_9	0.5	7.3×10^{-4}	8.5×10^{-5}
11. R=i-C_4H_9	1.0	5.82×10^{-4}	5.4×10^{-5}

20-fold. None of the compounds containing a quaternary nitrogen possessed appreciable insecticidal activity. This is apparently due to the impossibility of their penetration into the insect organism. On the other hand, it is known that, in comparison with other insects, the cuticle of aphids exhibits a high permeability for certain substances bearing a charged sulfur atom [26, 27]. Each of the iodomethylated derivatives of anabasine and anabasamine contains two or three quaternary nitrogen atoms in its molecule. To all appearance, this is what acts as an obstacle to their penetration through the aphid integument. The difference in the toxicities of anabasine and some of its salts shows the important role of the anion in the realization of insecticidal properties, and this must be taken into account in the search for new insecticides.

Thus, the facts considered in this section lead to the conclusion that human and greenbug ACEs, and also frog and squid CEs, are more sensitive enzymes than BuCE to a change in the the structure of the acid moieties of anabasine-containing esters. Characteristic for ACE and BuCE is an increase in their activity on the introduction of a CH_3 group (in the β- position) into the polymethylene bridge connecting the anabasine fragment with the acid moiety of the inhibitor.

Bisanabasine Derivatives of Dicarboxylic Acids. Some effective ganglion-blockers (hexamethonium, tubocurarine, etc.) belong to the bis-nitrogen compounds, while such bis-nitrogen substances as suberoylbischoline are strong ganglion-stimulators [28, 29]. On the other hand, it is known that anabasine possesses a pronounced ganglion-stimulating action. This fact has served as a basis for the synthesis [4, 30-38] of substances containing anabasine fragments as as the bis-nitrogen moieties (see Fig. 1, compounds (III) and (IV)).

On the basis of the structures of the compounds obtained, it may be assumed that they will be CE inhibitors, since among bisammonium derivatives the most effective inhibitors are those with two onium heads [31-33]. In actual fact, characteristic for the whole series of bisanabasine derivatives of dicarboxylic acids (Fig. 1, compounds (III)) is reversible inhibiting activity in relation to ACE and BuCE (Table 4) and a pronounced dependence of efficiency on structure [33-35]. Among the dimethiodides, stronger inhibitors of ACE and BuCE were compounds with n = 7 and n = 8, respectively, proving to be 65 and 10 times more effective than the weakest compound in this series, with n=3.

For comparison, we studied anabasine derivatives not bearing an onium charge. In relation to ACE, the compounds with n = 3 and n = 4 were equally effective, but in the case of BuCE the compound with n = 4 was 10 times more effective than its charged homologue. Among the uncharged derivatives, the substances with n = 7 and n = 8 proved to be stronger inhibitors of both ACE and BuCE. The compound with n = 3 was 40 times more effective in relation to to ACE than to BuCE.

In a comparison of the compounds with n = 3 (charged and uncharged) and with n = 4 (charged and uncharged), the effect of the quaternization of the nitrogen atoms in the bisanabasides of dicarboxylic acids was expressed in a 40-fold enhancement towards ACE. In the case of BuCE, however, for the pair with n = 4 the efficiency was increased by a factor

TABLE 4. Anticholinesterase Efficiencies (pK_i) of Bisanabasine Derivatives of Dicarboxylic Acids (III) [33-35]

Compound No.	n	Methiodides		Bases	
		ACE	BuCE	ACE	BuCE
1	3	5.18	6.22	3.58	3.75
2	4	5.33	5.92	3.69	4.68
3	7	7.0	6.85	4.86	5.28
4	8	6.26	7.26	5.08	5.37

TABLE 5. Ganglion-stimulating and Ganglion-blocking Activities of the Anabasine Derivatives (III) and (IV) [34]

n	Stimulating effect		Blocking effect	
	ED_{50}	Equieffective molecular ratios for anabasine = 1*	ED_{50}	Equieffective molecular ratios for hexamethonium = 1**
III				
2	0.45	5	—	—
3	0.04	0.5	—	—
4	0.1	1	1—5	50—70
7	0	0	5	100
Dimethiodides				
2	0	0	1	50—100
3	1—2.5	100	2.5	100
4	0	0	2.5	100
5	0	0	0.4—0.6	50
6	0	0	0.25—0.6	30
7	0	0	0.25—0.6	25
8	0	0	0.25—0.6	25
IV				
1	0.04	0.5	—	—
Methiodide	0	0	—	—
2	—	—	—	—
Methiodide	—	—	5	100

*Efficiency of anabasine taken as unity.

**Efficiency of hexamethonium taken as unity.

of almost 300. This agrees with literature statements that the quaternization of nitrogen enhances anticholinesterase action through ion-ion interaction with the anionic centers of CEs.

The results of an investigation of the interaction of some bisanabasinylalkanes with nicotinic receptors are shown in Table 5. Their ganglion-blocking activities were comparable with that of hexamethonium.

The ganglion-stimulating action of anabasine itself is almost 4 times less than that of such a strong stimulator of ganglions as nicotine. The methylation of anabasine is also accompanied by a fall in mimetic activity. These results are in harmony with literature information obtained on the methylation of other cholinomimetic amines — nicotine and cytisine [37]. The weakening of activity is apparently connected with the steric hindrance arising on the methylation of the piperidine nitrogen, which disturbs its interaction with the cholinoreceptors of the ganglionic neurons.

Almost all bisanabasine compounds possess a pronounced stimulating activity, apparently because the functionally important groups interacting with the anionic points of the CRs are the tertiary nitrogens of the pyridine rings. The compounds

TABLE 6. Anticholinesterase Efficiencies of N-Methyl- and N-Phenylcarbamates based on Anabasine (compounds 1 and 2) and on Piperidine (compounds 3 and 4) [42-46]

Compound No.	R	K_2, M^{-1}, min^{-1}	
		ACE	BuCE
1	CH_3	3.1×10^1	9.4×10^1
2	C_6H_5	5.4×10^1	2.7×102
3	CH_3	4.0×10^1	4.8×101
4	C_6H_5	4.3×10^1	3.1×101

with n = 3 and n = 4, having a distance of 13 or 14 atoms between the nitrogens of the pyridine rings proved to be the most active. As in a number of diacylcholines [38], this distance is the optimum for interaction with the nicotinic cholinoreceptors of warm-blooded animals. Among the bisanabasinylalkanes, compound (4) proved to be active, while its analogues with quaternary piperidine nitrogens were free from mimetic effects. Only for compound (5) (n = 3) were weak stimulating properties detected, but they were 200 times more feeble than for its tertiary analogue.

Methylation of the piperidine nitrogens had an adverse effect on the capacity of these substances for interacting with CRs. It must be mentioned that the methylation of the alkaloid anabasine itself leads to a weak ganglion-blocking action. This effect begins to increase with a rise in the number of methylene groups between the two anabasine heads. The higher homologues of the series of anabasine derivaties are 25 times weaker than hexamethonium. The weakening or even complete disappearane of a stimulating action on ganglia when the piperidine nitrogens are methylated in bisanabasine compounds is very interesting, and, in the future, analysis of this fact may assist in the investigation of the mechanism of the interaction of cholinomimetic amines with the cholinoreceptors of vegetative ganglia.

N-Methyl- and N-Phenylcarbamates Based on Anabasine. In plant-protection practice, derivatives of carbamic acids, which, like anabasine, block the functioning of cholinergic structures of the organism, are used as insecticides [22, 39-43]. In view of the possibility of creating insecticides with a directed action, and also with the aim of intensifying the biological properties of anabasine by its modification, we have synthesized anabasinyl N-methyl- and N-phenylcarbamates (see Fig. 1, compounds (V)).

It follows from Table 6 that that carbamoylated anabasine derivatives (compounds 1 and 2) exhibit weak blocking properties in relation to the enzymatic activities of ACE and BuCE. The sensitivity of BuCE to carbamates is higher than that of ACE. For comparison we give the anticholinesterase efficiencies of the piperidine homologues: methyl- and phenylcarbamates (compounds 3 and 4). Judging from these results, in the case of ACE, the passage from N-phenylcarbamoylpiperidine to the corresponding anabasine derivative is accompanied by a 1.25-fold increase in activity. At the same time, in the N-methylcarbamate series there is a 1.29-fold decrease.

Among the carbamates of the anabasine series the most active is anabasine N-phenylcarbamate, the efficiency of which in relation to BuCE is 5 time higher than for ACE. The anabasine derivative is 8.75 times more active than the piperidine analogue, which confirms literature statements of the greater tendency of BuCE to take part in hydrophobic interaction than ACE.

The relatively small amount of factual material gives no grounds for conclusions relative to the relationship between the structure and cholinotropic activity of carbamates including an anabasine molecule.

Phosphate Esters Based on Anabasine. Among organophosphorus inhibitors of cholinesterases, great interest is presented by phosphorylated derivatives of anabasine. The molecular structure of such anabasine derivatives is characterized by the presence of various functional groups at the phosphorus atom and also between the phosphorus atom and the nitrogen in piperidine fragment of the anabasine molecule [44-57]. The synthesis of these compounds is shown schematically in Fig. 2.

N-{2-[Methyl(ω-alkoxyalkoxy)phosphinyloxy]propyl}anabasines (see Fig. 2, compounds I, Table 7) are reversible inhibitors of CE of the competitive type [4, 9] A lengthening of the alkyl radical in RO leads to a small (30-fold) increase in inhibiting activity both for ACE and for BuCE. The methiodide analogues of these compounds are more active than the bases (see Table 7). The inhibiting effect is most pronounced in the case of BuCE and is only slight in relation to ACE. An increase in the total length of R causes a rise in the inhibiting effect which is very small for BuCE but appears appreciably in the case of ACE.

TABLE 7. Anticholinesterase Activities (pK_i) of Compounds (I) [4-9]

Compound No.	R	n	Bases		Methiodides	
			ACE	BuCE	ACE	BuCE
1	CH_3	2	3.02	3.73	3.19	4.53
2	CH_3	3	3.27	3.99	3.44	4.51
3	CH_3	4	3.43	4.14	3.51	4.34
4	CH_3	5	3.54	4.27	3.26	4.53
5	C_2H_5	2	3.12	3.9	3.37	4.91

TABLE 8. Anticholinesterase Activities (pK_i) of Compounds (II)

Compound No.	R	A	ACE	BuCE
1	n-C_5H_{11}	$-CH_2-C\equiv C-CH_2-$	3.75	5.71
2	i-C_5H_{11}	$-CH_2-C\equiv C-CH_2-$	3.91	6.3
3	n-C_6H_{13}	$-CH_2-C\equiv C-CH_2-$	4.24	6.49
4	n-C_5H_{11}	$-CH(CH_3)-CH_2-$	3.89	6.36
5	i-C_5H_{11}	$-CH(CH_3)-CH_2-$	3.06	5.68
6	n-C_6H_{13}	$-CH(CH_3)-CH_2-$	5.07	6.71

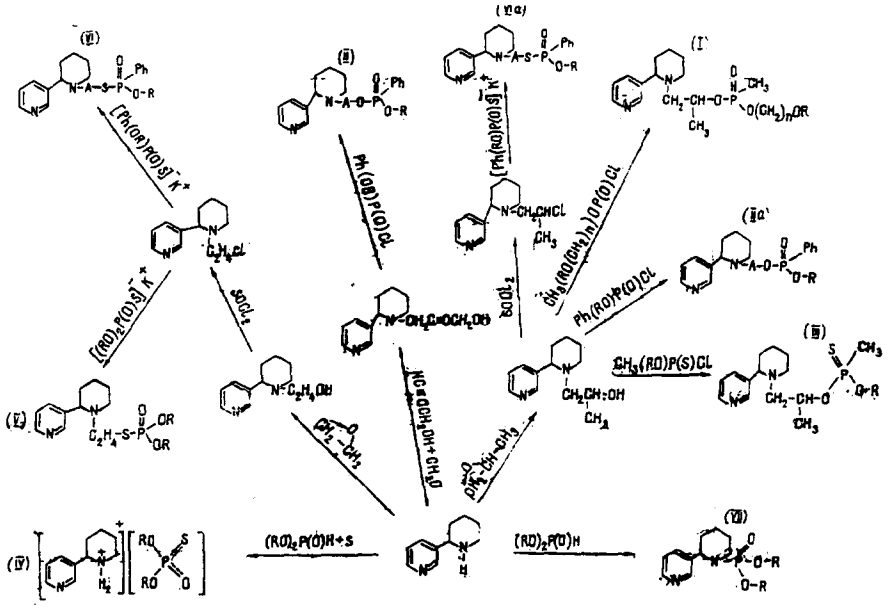

Fig. 2. Scheme of the synthesis of anabasine derivatives based on phosphoric acid

The compounds shown in Table 8 (see Fig. 2, compounds (II) and (IIa)) also caused reversible inhibition of the competitive type of the activities of CEs [51]. The first three compounds differ from the last three by the structure of the bridge linking the phosphorus atom with the anabasine. The phosphorylating activities of compounds 1-3 as functions of the nature of R rose for both types of CEs. The anticholinesterase effect of substances 4-6 differed somewhat from that of compounds 1-3. Replacement of a normal pentyl radical by its iso analogue causes a fall in the activity of compound 5 both for ACE and for BuCE. A considerable jump in activity is observed for compound 6. Characteristic for this series of compounds is a higher sensitivity of BuCE, which is most probably caused by their greater complementarity to the structure of its active center.

It follows from Table 9 (see Fig. 2, compounds (III)) that a lengthening of the O-alkyl radical from ethyl to amyl had no substantial inhibiting effect on the activity of ACE [49]. Apparently, the anabasine part of the molecules of the inhibitors is less complementary to the anionic point of the catalytic surface of ACE, which affects the value of pK_i.

On the interaction of these substances with BuCE a lengthening of R from C_2H_5 to C_4H_9 leads to a 43-fold increase in antibutyrylcholinesterase activity. The presence of amyl and isobutyl radicals causes a fall in activity by an order of magni-

TABLE 9. Anticholinesterase Activities ($K_i \times 10^{-5}$ M) of Compounds (III) [49]

Compound No.	R	ACE	BuCE
1	C_2H_5	1.9	2.68
2	C_3H_7	1.64	0.65
3	C_4H_9	2.1	0.062
4	C_5H_{11}	$I_{50}=1\times10^{-3}$	0.55
5	i-C_4H_9	2.6	0.53

TABLE 10. Anticholinesterase Efficiencies (K_2, $M^{-1}\cdot min^{-1}$) of Compounds (V) [47]

Compound No.	R	Bases		Methiodides	
		BuCE$\times10^5$	BuCE $\times10^6$	ACE$\times10^6$	BuCE$\times10^7$
1	C_2H_5	1.3	0.80	0.39	0.15
2	n-C_3H_7	7.4	2.4	3.6	0.53
3	n-C_4H_9	35	7.1	14	2.5
4	n-C_5H_{11}	10	7.7	15	4.9
5	i-C_3H_7	0.53	4.9	0.50	1.3
6	i-C_4H_9	18	10	33.0	6.2
7	i-C_5H_{11}	47	90	50	11

tude. This rule of the change in the value of pK_i is in agreement with ideas on the structure of the hydrophobic sections of the active center of this enzyme.

To determine the influence of the structures of the fragments on the biological activities of OPCs based on anabasine, complex anabasine phosphorothioates have been obtained (see Fig. 2, compound (IV), R = C_2H_5, i-C_3H_7). These compounds proved to be typical reversible CE inhibitors. An increase in the number of methylene groups in the O-alkyl radical was accompanied by an increase in their inhibiting efficiency. It must be mentioned that in the nature of their action on CEs they have a similarity with alkylammonium compounds, which are capable of entering into interaction with the anionic sections of the protein molecules of ACE and BuCE.

Particular interest is presented by organophosphorus derivatives of anabasine, which irreversibly suppress the activity of CEs, since in many cases such compounds exhibit a high toxicity, and substances of this type can be uased as agents for the fight against agricultural pests [51, 53].

The irreversible anticholinesterase activity of compounds (V) (see Fig. 2, compounds (V), and Table 10, bases) in relation to ACE has a tendency to rise with a lengthening of R [45-50]. An increase in inhibiting activity is also observed for compounds containing radicals with an iso structure. Here attention must be directed to the low activity of the diisopropoxy derivative, the constant k_2 of which is considerably lower than for the other substances studied. This is apparently connected with inadequate complementarity of he inhibitor to the active surface of the enzyme, which can be explained by the difficulties of the sorption of two branched isopropyl groups on the corresponding hydrophobic section of the active surface of acetylcholinesterase located in the region of the esterase center.

The irreversible inhibiting activity of the N-[β-(dialkoxyphosphinylthio)ethyl]anabasines (Table 11, Fig. 2, compounds (V), bases) is higher for BuCE than for ACE. It is important to note that these differences depend to a considerable extent on the length and degree of branching of the alkoxy groups. The smallest differences are observed for the dibutyl ester — 2-fold, and the largest for the diisopropyl ester — 93-fold.

Thus, in the case of BuCE the appearance of a second nitrogen-containing group in the thioester radical split out from the OPI exerts a great influence on its anticholinesterase activity. This influence is largely due to the nature of the hydrophobic alkyl radicals of the alkoxy groups, which are sorbed on the hydrophobic sections of the active surface of the enzyme present in the region of the esterase center. It may be concluded that the presence in the leaving part of the OPI molecule of two nitrogen-containing groupings that, under the conditions of the experiment (pH 7.5), exist mainly in the protonated form worsens the conditions for the hydrophobic sorption of the inhibitor on the active surface of BuCE. In the case of ACE, this situation is shown less clearly because of the lower tendency of acetylcholinesterase to participate in hydrophobic interactions.

The dimethiodides of the N-[β-(dialkoxyphosphinylthio)ethyl]anabasines are more active inhibitors of cholinesterases than their uncharged analogues (see Table 11). In the case of ACE the increase in irreversible anticholinesterase activity is

TABLE 11. Anticholinesterase Activities (K_2, $M^{-1}\cdot min^{-1} \times 10^4$) of Compounds (VI)

Compound No.	R	A	ACE of the turnip moth	Human ACE	BuCE
		VI			
1	n-C_5H_{11}	$-C_2H_4-$	5.14	0.493	58.5
2	*i*-C_5H_{11}	$-C_2H_4-$	4.74	0.574	55.5
3	n-C_6H_{13}	$-C_2H_4-$	10.4	0.693	2.86
		VIa			
4	*i*-C_5H_{11}	$-CH(CH_3)CH_2-$	10	0.344	169
5	n-C_6H_{13}	$-CH(CH_3)CH_2-$	11	0.478	196

TABLE 12. Anticholinesterase Activities (K_2, $M^{-1}\cdot min^{-1} \times 10^5$ of Compounds (VII) [52-54]

Compound No.	R	R	ACe	BuCe
1	*i*-C_3H_7	Piperidine	0.031	13.3
2	n-C_4H_9	Piperidine	0.056	1.99
3	*i*-C_3H_7	Morpholine	0.081	1.2
4	*i*-C_3H_7	*i*-C_3H_7	0.35	45.05

slight for compounds the phosphorus atom of which bears two ethoxy, propoxy or butoxy groups. The irreversible inhibiting activity of the other compounds is approximately an order of magnitude higher.

Under the experimental conditions, unmethylated OPIs already exist in the protonated form to a considerable degree, and, as in the case of the mononitrogen compounds, their methylation should lead only to a small increase in anticholinesterase activity. This is observed for compounds containing short and unbranched radicals in the alkoxy groups. A rise in anticholinesterase activity for compounds with long and branched radicals can be explained by the concerted influence of hydrophobic alkyl radicals and two nitrogen-containing groupings having positive charges.

In the case of butyrylcholinesterase, the appearance of two positive charges in the OPI molecule shows up differently. Here the anticholinesterase activity increases 2- to 6-fold, while in the case of the isoamyl derivative a decrease is actually observed. In the last example, apparently, there is a well defined absorption of the OPI on the active surface of the enzyme, where the hydrophobic alkoxy and onium groupings exert a nonconcerted influence,which leads to a decrease in inhibiting activity.

The compounds of the onium type that have been studied show only very slight differences in the inhibition of ACE and BuCE; the activities of the majority of them are similar for the two enzymes, with the exception of the diisopropyl derivative, which shows a greater tendency to inhibit ACE (see Table 11). In this connection it is interesting to note that the toxicity of N-[β-(diethoxyphosphinylthio)ethyl]anabasine in its action on the greenbug aproximates to that of the standard — Rogor (dimethoate) [4].

The N-β-hydroxyethyl- and N-β-hydroxypropylanabasine compounds (see Table 11 and Fig. 2, compounds (VI) and (VIa)) lower the catalytic activities of all the three CEs that were studied. The sensitivity of the ACE of the turnip moth is two orders of magnitude higher than that of erythrocyte ACE. Human ACE is less sensitive than the moth ACE to a change in the nature of R in the series of N-β-hydroxypropyl derivatives of anabasine. For this series of substances it is possible to detect elements of similarity of their inhibiting effect in relation to the two ACEs: the replacement of an O-pentyl by an O-hexyl radical is accompanied by an increase in the inhibiting effect of the latter. A tendency to a rise in the values of k_2 for the human enzyme is observed in the cases of both the N-β-hydroxyethyl- and the N-β-hydroxypropylanabasine esters. In relation to the moth ACE, such a change in the sensitivity of the enzyme is characteristic only of N-β-hydroxyethylanabasine compounds. This series of compounds shows the highest activity for serum BuCE, and, among them, substances with O-pentyl radicals. This behavior of BuCE can be explained by the presence of extensive hydrophobic regions in the active center of this enzyme. The presence of a hydroxypropyl fragment (Table 11, compounds 4 and 5) lowers the efficacy of the latter in relation to serum CE more than 4-fold, which agrees with information on the substrate specificity of this enzyme. Thes facts clearly show elements of difference and similarity in the sensitivities of the three CEs that are most probably caused by differences in the catalytic centers of these serine hydrolases.

The synthesis of the dialkyl N-anabasinophosphonates (see Fig. 2, compounds (VII)) was achieved by the classical Todd-Atherton reaction [52-54], and their influence on the catalytic properties of CE has been studied (Table 12). It is appropriate to mention that OPCs with P–N bonds were first studied as CE inhibitors. These compounds (Table 12), like many other OPCs, cause irreversible inhibition of the activities of ACE and BuCE. The sensitivity of ACE is 2-3 orders of magnitude lower than that of BuCE. The activity of pyridine-containing compounds is shown differently with a change in the structure of the O-alkyl radical: while for the erythrocyte enzyme the replacement of an isopropyl radical by a *n*-butyl fragment raises the inhibiting effect of the latter, in relation to blood serum CE such replacement is accompanied by a fall in the activity of the compound, which may be caused by a dissociation of the concerted sorption of the inhibitors on the active surface of BuCE. The presence of a morpholine fragment in the structure has a favorable effect on the activity of this substance towards ACE and is undesirable in the inhibition of the catalytic characteristics of BuCE, which is connected with the different sensitivities of CE to the hydrophilic morpholine residue. In its toxic action on the housefly, diisopropyl anabasinylphosphonate (see Table 12, cpd 1) proved to be equal to chlorofos (trichlorfon), while its eficiency in relation to the rice weevil exceeded that of chlorofos by a factor of 1.7. At the same time, its aphicidal activity was 15 times greater than that of carbophos (malathion).

Analysis of the literature and of the results of our investigations show that, in spite of the presence of an ester grouping, anabasine derivatives based on carboxylic acids cause the reversible inhibition of all the CEs tested. The type of inhibition and the strength of the inhibiting action depend on the degree of complementarity of the effector molecule to the active surface of the enzyme and the harmony of all the types of interaction in the regions both of the anionic and the esterase points of the CEs. An investigation of the dependence of anticholinesterase activity on the structure of esters based on anabasine has revealed elements of similarity and dissimilarity in the catalytic centers of ACEs and BuCEs from various sources.

All types of CEs undergo the inhibiting influence of a large number of the organophosphorus derivatives of anabasine that have been synthesized, which, unlike its carboxylic acid esters, cause both reversible and irreversible inhibition of the hydrolysis of substrates under the action of CE. The difference in the specificities of these enzymes is shown above all in their sensitivity to a change in the structure of the acid moiety of the inhibitor molecule: the more hydrophobic compounds are effective blockers of BuCEs and CRs,and the less hydrophobic one block human and insect ACEs.

The investigation of the biological activities of some new phosphorylated derivaties of anabsine has shown that these compounds, besides having an action on the cholinergic structures of the organism, may exert a substantial influence on the functions of glutathione transferase and the microsomal oxidases of mammals and arthropods [58-61], which opens up possibilitied for revealing the mechanism of pest resistance.

The study of the physiological activity of anbsine derivatives containing residues of various acids is also of interest for public health and for agriculture.

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