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NEW PHOSPHORYLATED DERIVATIVES OF ANABASINE AND OF LUPININE, AND THEIR ANTICHOLINESTERASE PROPERTIES

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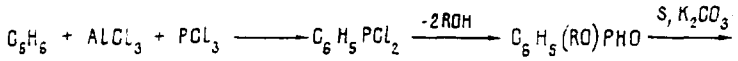
A series of new phosphorylated esters of anabasine, lupinine, and piperidine differing by the nature of the alkyl radicals attached to the phosphorus atom has been synthesized. The results of investigations of the antiesterase properties of the compounds in relation to the acetylcholinesterase of human blood erythrocytes and of the turnip moth and the carboxylesterase of porcine kidney have shown a selectivity of their action in relation to the insect enzyme and a substantially lower activity towards esterases of warm-blooded animals.

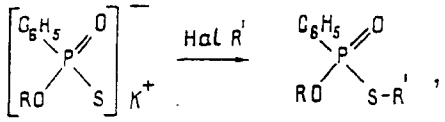
An investigation of the properties of cholinesterase (CE) plays a large role in the purposeful synthesis of new plant-protecting agents, since the toxicity of insectoacaricides of organophosphorus and of carbamate nature is connected with the capacity for the irreversible inhibition of the acetylcholinesterase (ACE) of the insect nervous system. In addition to ACE, such compounds irreversibly inhibit carboxylesterase (CBE) in the organism, which weakens their toxicity because of a decrease in their concentration on interaction with the CBE. In addition to everything else, CBE may take part in the detoxification of organophosphorus insecticides of the malathion type

These objective circumstances have led to the situation that the sensitivity of CE and CBE to compounds irreversibly phosphorylating esterases has become the object of intensive investigations [2-7]. By using this approach it is possible to create effective agents for protecting the cotton plant from harmful insects.

Considering the possibility [8, 9] of creating insecticides with a directed action by modifying alkaloids, we have synthesized O-alkyl S-(β-anabasinoethyl) phenylphosphonothioates (Ia, Ib, Ic), O-alkyl S-(β-anabasinoisopropyl) phenylphosphonothioates (IIb, IIc), and O-alkyl S-lupinanyl phenylphosphonothioates (IIIa, IIIb) and also O-hexyl S-(β-piperidinoisopropyl) phenylphosphonothioate (IVc) and have investigated their antienzyme properties in relation to the ACEs of human blood erythrocytes and of the heads of the turnip moth *Agrotis segetum* Schiff and also to porcine kidney CBE.

The substances mentioned were synthesized by the scheme given below.





where R = n-C_5H_{11} (a), i-C_5H_{11} (b), n-C_6H_{13} (c);

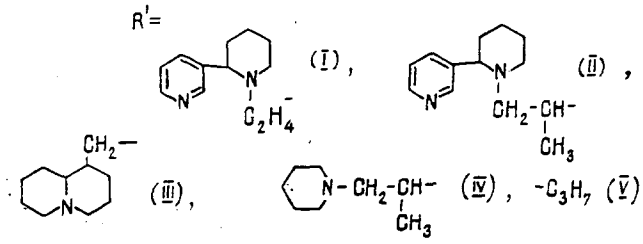


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TABLE 1

Compound	Yield, %	n_D^{20}	K_2, $M^{-1}min^{-1} \times 10^4$		
			ACE*	ACE**	CBE***
Ia	52	1.5226	0.493	5.14	0.41
Ib	57	1.5342	0.579	4.74	0.48
Ic	51	1.5411	0.693	10.4	0.63
IIb	56	1.5421	0.344	10	0.0445
IIc	59	1.5305	0.478	11	0.0331
IIIa	61	1.5017	4.71	43.3	0.0084
IIIb	53	1.5032	2.8	39.6	0.472
IVc	62	0.504	0.438	5.34	7.6
IVa	66	1.5452	0.323	0.433	33.6
Vb	63	1.5201	0.138	3.94	2.99
Vc	59	1.5300	0.46	4.34	12.2

*Human blood erythrocytes.

**Heads of the turnip moth.

***Porcine kidneys.

Phenyldichlorophospine was synthesized by Gefter's method [10]. Potassium O-alkyl phenylphosphonothioates were obtained by heating alkyl phenylphosphonites, sulfur, and potassium carbonate in absolute ethanol. The final products — O-alkyl phenylphosphonothioates — were obtained by the interaction of the potassium O-alkyl phenylphosphonothioates with the appropiate alkyl halides.

The structures of the compounds were confirmed by their IR and PMR spectra.

The IR spectrum of (Ia) showed the following characteristic absorption bands (cm^{-1}): 680-700 (−P−S−C−), 1160 (−P−O−C), 1250 (−P=O), 1450 (−P−C_6H_5), 1370 (−C−CH_3), 1540 (−N−C in a ring).

The PMR spectrum of (IIIa) gave the signals of the protons of a −S−CH_2 group in the form of doublet at 2. 9 ppm. Signals in the 7.19-7.31 ppm region corresponded to the protons of a phenyl group. A broad doublet at 2.65 ppm belonged to equatorial protons at C_2 and C_{10} of the quinolizidine radical. An intense signal in the form of a triplet at 0.89 ppm belonged to the protons of a CH_3 group. The other methylene protons of the pentyl and quinolizidine fragments produced a signal in the 1.1-2.0 ppm region.

In the PMR spectrum of O-pentyl S-(β-anabasinoethyl) phenylphosphonothioate the signals of the α,α′, β, and γ-protons of the pyridine ring were located at 8. 34, 8.30, 7.12, and 7.55 ppm. The signals of the piperidine ring were located in the following way: the H_{2a} and H_{6a} protons resonated in the 3.0-2.25 ppm region, and the signals of H_{6a} were located at 2.15 ppm [sic].

Table 1 gives the physicochemical constants and anticholinesterase activities of the compounds synthesized.

The N-β-oxyethylanabasine derivatives (Ia, Ib, and Ic) and N-β-oxypropylanabasine derivatives (IIb and IIc) and also the lupinine derivatives (IIIa and IIIb) lowered the enzymatic activity of the ACE of turnip moth heads more effectively than the ACE of human blood erythrocytes. The activities of (Ic), (IIb), and (IIc) in relation to the ACE of the moth heads was two orders of magnitude higher than to the ACE of human blood erythrocytes.

The lupinine compounds were also effective in relation to the moth CE but four times weaker than compounds (Ic), (IIb), and (IIc). Human ACE was less sensitive to a change in the structure of the O-alkyl radical in the series of N-β-oxyethyl derivatives of anabasine than the moth ACE. For this series of compounds it was possible to observe elements of similarity of their inhibiting activity in relation to both ACEs: passage from the O-pentyl radical to the O-hexyl radical was accompanied by a rise in the inhibiting effect in relation to the ACEs both of Man and of the insects.

The enzyme of the blood erythrocytes exhibited a definite regularity in relation to a change in the length of the O-alkyl radical in the case of the N-β-hydroxyethyl and N-β-hydroxypropyl analogues of anabasine which consisted in an increase in the inhibiting effect of compounds (Ic) and (IIc) as compared with (Ib) and (IIb). In relation to the turnip moth ACE, an analogous change in sensitivity was observed only for the N-(β-hydroxyethyl)anabasine derivatives.

Table 1 gives for comparison the K_2 values for compound (IVc), which differs from the anabasine analogues by the absence of a pyridyl radical. If we compare its efficiency with that of substance (IIc), it is not difficult to observe that the absence of the pyridyl radical has no effect on the inhibiting efficiency for the erythrocyte ACE, while the moth enzyme is sensitive to such a rearrangement of the structure of the inhibitor — the inhibiting activity of the substances towards the turnip moth ACE decreases. These results indicate differences in the structure of the active surface of the enzyme where the sorption of the heterocyclic fragment of the inhibitor takes place.

CBE was the least sensitive to the whole series of anabasine and lupinine derivatives, particularly to compounds containing a nitrogen atom in the heterocycle. Compounds (Va), (Vb) and (Vc) are given for comparison; they exhibit a high inhibiting efficiency in relation to CBE. Apparently, as compared with CE, the structure of the active surface of CBE is more adapted to the sorption of simple hydrocarbon radicals. In this connection, it must be mentioned that good CBE substrates are aliphatic esters formed by low-molecular-mass alcohols and short-chain acids, for example: methyl butyrate, ($C_3H_7C(O)OCH_3$), and ethyl butyrate ($C_3H_7C(O))C_2H_5$) [11].

Thus, an investigation of the kinetics of the irreversible inhibition of the enzymatic activity of CE and CBE by organophosphorus derivatives of anabasine and lupinine has shown their dissimilar sensitivity to the structures of these compounds, which is most probably connected with differences in the structures of the active sections of the catalytic surfaces of the esterases studied. The high inhibiting efficiency of organophosphorus derivatives of anabasine and lupinine to the turnip moth ACE gives grounds for the assumption that they will exhibit a high toxicity in relation to this pest.

EXPERIMENTAL

The IR spectra of the compounds synthesized were taken on a Specord IR-71 instrument in KBr tablets, and the PMR spectra on Varian XL-200 instrument with a frequency of 200 MHz using CCl_4 as solvent. Column chromatography was conducted on Al_2O_3 (activity grade II) with absolute ether as the eluent.

Synthesis of O-Pentyl S-Lupinanyl Phenylphosphonothioate (IIIa). A round-bottomed flask fitted with a reflux condenser was charged with 5.64 g (0.02 mole) of potassium O-pentyl phenylphosphonothioate and 4.62 g (0.02 mole) of bromolupinine in 100 ml of absolute ethanol. The reaction mixture was boiled for 4-6 h. The resulting precipitate of KBr was filtered off. After the solvent had been distilled off, the final product was purified by column chromatography. The other compounds were synthesized similarly.

We used commercial preparations of the enzymes: human blood erythrocyte ACE with a specific activity of 2. 1 U/mg (Perm Scientific Institute of Vaccines and Sera) and porcine kidney CBE with a specific activity of 260 U/mg (Sigma). As the turnip moth ACE we used a homogenate of the heads of fifth–sixth instar caterpillars of the pest. The homogenate was prepared in 0.05 M phosphate buffer at a ratio of 1 g of tissue to 9 ml of buffer (pH 7.4) and was subjected to centrifugation in a Beckman centrifuge at 10,000 rpm at +4°C for 20 min. The supernatant part of the homogenate was used as the moth ACE. The catalytic activities of the CE and CBE were determined by Ellman's thiocholine method [12/ from the rate of hydrolysis of acetylthiocholine (for ACE) and of ethyl thiobutyrate (for CBE) [13, 14]. In the results of the determination we introduced corrections for the nonenzymatic hydrolysis of the substrates. The interaction of the enzymes with the inhibitors was evaluated from the bimolecular constants (K_2) of the rate of their interaction. For this purpose, the enzymes were incubated with the inhibitors for 10, 15, and 25 min. The values for K_2 were calculated by the method of [15/ , starting from the slope of the straight line on a graph in the coordinates log (V_0N_t) versus the time t. The activities of the enzymes and the values of T_2 were determined at 25°C in a reaction medium with a pH of 7.4. The inhibitor solutions were prepared in ethyl alcohol. Before an experiment, these solutions were diluted with water to such an extent that the concentrations of inhibitor obtained caused 50% inhibition of the activities of the enzymes on incubation for 5 minutes. Table 1 gives the mean values of K_2.

REFERENCES

1. V. I. Rozengart and O. E. Sherstobitov, Selective Toxicity of Organophosphorus Insectoacaricides [in Russian], Nauka, Leningrad (1978).
2. Z. Tilyabaev, A. A. Abduvakhabov, and D. N. Dalimov, "Harmful insects of the cotton plant and an investigation of their cholinesterases and carboxylases," in: Problems and Prospects of the Development of the Chemistry of Natural and Physiologically Active Substances [in Russian], FAN, Tashkent (1988), p. 212.
3. O. B. Charyeva, A. M. Kuliev, S. N. Moralev , and V. I. Rozengart, Izv. Akad. Nauk Turkmenistana. Ser. Biol. Nauk, No. 3, 78 (1991)
4. Luc P. Belzunces and M. E. Golin, Neuroreport, No. 5 265 (1991).
5. N. Prabhu Saxena and S. C. Saxena, Proc. Natl. Acad. Sci. India, **B61**, No. 1, 1 (1991).
6. Y. T. Suh and J. H. Shim, Kor. J. Apicult., **3**, No. 1, 48 (1988).
7. S. A. Roslavtseva, O. E. Eremina, and Yu. V. Lopatina, Zh. Évol. Biokhim. Fiziol., **27**, No. 6, 712 (1991).
8. A. A. Abduvakhabov, A. A. Sadykov, D. N. Dalimov, and Kh. A. Aslanov, Alkaloids and their Derivatives as Tools for the Study of Cholinergic Systems [in Russian], Fan, Tashkent (1984), p. 153.
9. A. M. Gazaliev, M. Zh. Zhurinov, Z. Tilyabaev, D. N. Dalimov, K. D. Mukanova, and S. A. Dyusembaev, Khim. Prir. Soedin., No. 4, 584 (1989).
10. E. P. Gefter, Zh. Obshch. Khim., **28**, No. 5, 1338 (1958).
11. K. Krisch, "Carboxylic ester hydrolases," in: The Enzymes (ed. P. B. Boyer, Academic Press, New York, 3rd. edn., Vol. 5 (1971), p. 43.
12. G. L. Ellman, K. B. Courtney, U. Anders, and R. M. Featherstone, Biochem. Pharm., **7**, 89 (1981).
13. Z. Tilyabaev, L. A. Srybnaya, and V. N. Babaev, "Investigations of porcine liver carboxylesterase with the aid of thiosubstrates and organophosphorus compounds," in: Abstracts of Lectures at the 5th Conference of Biochemists of Central Asia and Kazakhstan [in Russian], Tashkent (1991), p. 103.
14. L. A. Srybnaya, E. Tilyabaev, and M. B. Gafurov, Dokl. Akad. Nauk RUz., Nos 6-7, 42 (1992).
15. A. P. Brestkin and I. L. Brik, Dokl. Akad. Nauk SSSR, **299**, No. 1, 103 (1971).