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SYNTHESIS AND ANTICHOLINESTERASE ACTIVITIES OF QUININYL DIALKYL PHOSPHATES

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The results are given of the synthesis of dialkyl phosphate derivatives of the alkaloid quinine and the study of their inhibitory activity in relation to the cholinesterases of various animal species.

The search for new highly selective insectoacaricides is connected with the study of the relationship between the structures of the compounds investigated and their anticholinesterase activities [1, 2]. In view of this, the main interest is presented by compounds with large hydrophobic groupings that are capable of exerting selective activities in relation to butyrylcholinesterase [3]. In this connection, it appeared desirable to synthesize organophosphorus compounds containing in an ester radical a residue the alkaloid quinine – the quininyl dialkyl phosphates **(1-4)** – and to study their anticholinesterase activities. The synthesis of these compounds was achieved by the interaction of quinine with dialkyl phosphites under the conditions of phase-transfer catalysis in the presence of various crown ethers – 18-crown-6, dibenzo-18-crown-6, 15-crown-5. It was established that, under the classical Todd–Atherton conditions, the desired products were formed in low yields (35-40%),which is apparently connected with the existence of intramolecular hydrogen bonds in the quinine molecule.

We studied the dependence of the yield of the desired products on the nature of the crown ethers and the reaction conditions. The highest yields of the desired products (**1-4**) were obtained in the presence of 18-crown-6.

The compounds (**1-4**) obtained consisted of white crystalline substances.

Their anticholinesterase properties were studied on human blood erythrocyte acetylcholinesterase (HEAcChE), equine blood serum butyrylcholinesterase (EBuChE) and the cholinesterases of an aphid, the greenbug *Schizaphis gramina* Rond. The study of differences in the sensitivity to inhibitors of the cholinesterases of various animal species is the basis of the search for insecticides with a selective action.

The anticholinesterase activities of compounds **(1-4)** were evaluated from the magnitude of the inhibition constant $\bar{K}_i$, which, with the mixed type of inhibition is connected with the competitive (K_i) and noncompetitive (K_i') components of the relation $1/K_i = 1/K_i - 1/K_i'$. The inhibition constants were determined graphically and were expressed in the form $p\bar{K}_i = -\log\bar{K}$. The type of inihibitionb was determined from the ratio of pK_i and K_i': competitive ($pK_i \to 0$), mixed ($pK_i > K_i'$), or noncompetitive ($pK_i = K_i'$) [4].

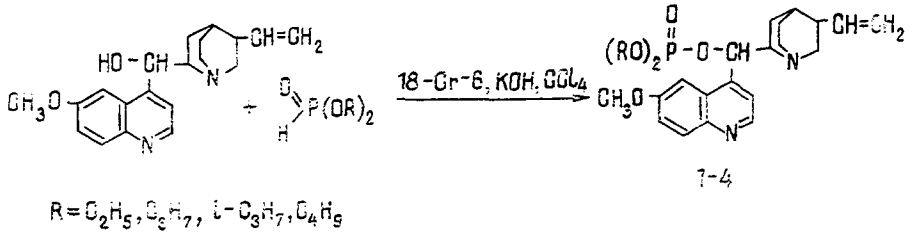

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TABLE 1. Anticholinesterase Activities of Derivatives of the Alkaloid Quinine

Compound	R	HEAChE				BuChE			
		T.i.*	pK_i	pK_i'	$p\bar{K}_i$	T.i.	pK_i	pK_i'	$p\bar{K}_i$
1	C_2H_5	m	3.43	2.28	3.53	c/m	5.12	3.67	5.14
2	C_3H_7	m	3.10	3.53	3.66	c/m	5.03	4.13	5.07
3	i-C_3H_7	c/m	4.04	3.02	4.08	m	4.94	4.49	5.07
4	C_4H_9	m	3.54	2.84	3.62	m	5.20	4.96	5.40

*Type of inhibition: m – mixed; c – competitive; n – noncompetitive

TABLE 2. Anticholinesterase Activities of Quinine Derivatives for the Greenbug

Compound	R	Aphid AcChE				Aphid BuChE			
		T.i.	pK_i	pK_i'	$p\bar{K}_i$	T.i.	pK_i	pK_i'	$p\bar{K}_i$
1	C_2H_5	m	3.90	3.27	4.00	c	3.70	-	3.70
2	C_3H_7	m/n	3.33	3.25	3.59	c	4.35	-	4.35
3	i-C_3H_7	m/n	3.44	3.42	3.72	c	5.64	-	5.64
4	C_4H_9	c/n	3.66	2.38	3.68	c	5.70	-	5.70

Tables 1 and 2 give the results of measurement of the enzymatic activities of the dialkyl phosphate derivatives of the alkaloid quinine (**1-4**), from which it follows that these groups of compounds are specific inhibitors of BuChE. The compounds proved to be equally effective in relation to AcChE. It may be assumed that an important factor in the realization of the inhibition reaction is the quinuclidine fragment, since an increase in the length of the alkyl group in the phosphorus-containing moiety did not affct the anticholinesterase activity of the compounds.

The results of a study of the interaction of the quinine derivatives with the AcChE of an aphid, the greenbug (Table 2), also permitted the assumption that the anticholinesterase activities of these compounds (**1-4**) were due only to the sorption of the quinine residue on the active surface of the enzyme, since practically all the quinine derivatives (**1-4**) had equally effective inhibition constants, in the range of $p\bar{K}_i$ = 3.20–3.60. The slight changes in inhibitory activity (see Tables 1 and 2) can very probably be explained merely by a fine balance of all the factors determining the stereochemistry of the reaction (angle and direction of attack by the molecule of the organophosphosphorus inhibitor on the active centers of the cholinesterases, the conformational state of the inhibitor molecule, etc.).

A comparison of the results of experiments on the influence of the inhibitors studied on aphid BuChE (see Table 2) with those obtained on mammalian BuChE (see Table 1) showed that equine BuChE was 1-2 orders more sensitive to them. However, while in relation to equine BuChE a lengthening of the alkyl radical from ethyl (**1**) to butyl (**4**) had practically no influence on the inhibitory capacity of the compounds, in relation to the aphid BuChE, it led to a rise in their inhibitory capacity (see Table 2). It is quite likely that the reason for this is the more favorable orientation of compound (**4**) for hydrophobic sorption on the active surface of the enzyme, since it is known [5] that aphid BuChE differs from the majority of known cholinesterases by a low sensitivity to organophosphorus compounds. It must be mentioned that for the aphid BuChE all the quinine derivatives studied proved to be competitive reversible cholinesterase inhibitors.

EXPERIMENTAL

Quininyl Diethyl Phosphate (1). A mixture of 0.018 mole of quinine, 0.027 mole of solid potassium hydroxide, 0.036 mole of carbon tetrachloride, and a catalytic amount of dibenzo-18-crown-6 in 30 ml of absolute tetrahydrofuran was treated dropwise with 0.02 mole of diethyl phosphite. To complete the phosphorylation reaction, the reaction mixture was stirred at room temperature for 5 h, and then the potassium hydroxide was filtered off and the solvent was distilled off. The product was purified by recrystallization from benzene. Yield 74.2%, mp 161°C. PMR spectrum (δ, ppm) 1.25 t (3H, CH_3), 3.97 m (2H, CH_2), 3.91 s (3H, OCH_3). ^{31}PNMR spectrum, δ, ppm: 2.0. Found %: C 62.26; H 7.48; P 6.27. $C_{24}H_{33}N_2O_5P$. Calculated %: C 62.60; H 7.22; P 6.74.

Compounds (**2-4**) were obtained under analogous conditions.

Quininyl Dipropyl Phosphate (2). Yield 73.1%, mp 132°C. PMR spectrum (δ, ppm): 0.91 s (3H, CH_3), 3.80 m (2H, CH_2), 3.98 s (3H, OCH_3). ^{31}P NMR spectrum (δ, ppm): 2.0. Found %: C 63.63; H 7.75; P 6.20. $C_{26}H_{37}N_2O_5P$. Calculated % C 63.93; H 7.58; P 6.35.

Quininyl Diisopropyl Phosphate (3). Yield 68.3%, mp 144°C. PMR spectrum (δ, ppm): 1.00 t (3H, CH_3), 4.23 m (2H, CH_2), 3.67 s (3H, OCH_3). ^{31}P NMR spectrum (δ, ppm): 1.8. Found %: C 64.09; H 7.40; P 6.55. $C_{26}H_{37}N_2O_5P$. Calculated %: C 63.93; H 7.58; P 6.35.

Quininyl Dibutyl Phosphate (4). Yield 64.4%, mp 104°C. PMR spectrum (δ, ppm): 0.90 t (3H, CH_3), 3.86 m (2H, CH_2), 3.92 s (3H, OCH_3). ^{31}P NMR spectrum (δ, ppm): −1.0. Found %: C 65:30; H 7.70; P 5.88. $C_{28}H_{41}N_2O_5P$. Calculated %: C 65.11; H 7.94; P 6.00.

The PMR spectra of compounds (**1-4**) were recorded on a Tesla BS-467, 250.13 MHz, instrument in $CDCl_3$ solution with TMS as internal standard. ^{31}P NMR spectra were taken on an instrument constructed in Kazan' State Univesity with a working frequency of 8 MHz.

The anticholinesterase activities of the substances were investigated in the laboratory of invertebrate biochemistry of the I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences. We made use of the common greenbug *Schizaphis gramina* Rond (fam. Aphididae) and purified preparations of AcChE and of BuChE (with specific activities of 2.2 and 9.6 units/mg, respectively) produced by the Perm Scientific-Research Institute of Vaccines and Sera. The catalytic activities of the AcChE and BuChE were determined by Ellman's method from the rates of hydrolysis of acetylcholine and butyrylcholine with the aid of a KFK-2MP photoelectric colorimeter at a wavelength of 412 nm. The coefficient of molar extinction calculated from a calibration curve for a sample with an optical path length of 1 cm was 13,600 cm^{-1}. Esterase activities were determined at pH 7.5 for the HEAcChE, EBuChE, and the aphid BuChE, and at pH 7.0 for the aphid AcChE.

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