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SYNTHESIS AND ANTIENZYME ACTIVITY OF 2-OXO-2*SR*-4-METHYL-1,3,2-DIOXAPHOSPHORINANES CONTAINING SALSOLINE, SALSOLIDINE, CYTISINE, AND DECAHYDROQUINOLINE RESIDUES

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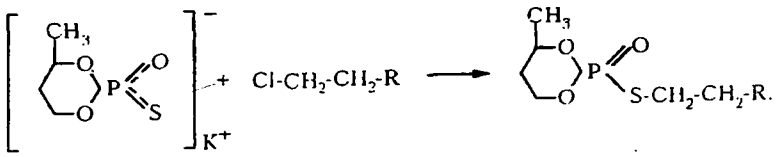
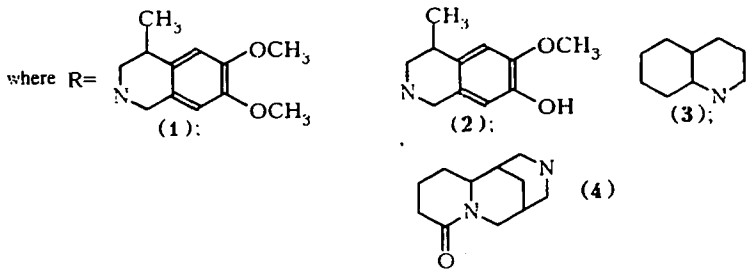
New derivatives of 2-oxo-4-methyl-1,3,2-dioxaphosphorinane containing residues of salsoline, salsolidine, cytisine, and decahydroquinoline have been synthesized, and their antienzyme activities have been investigated. The new compounds are irreversible inhibitors of the AChE, BuChE, and CBE of warm-blooded animals and of insects: the antienzyme activities of the compounds depend substantially on the structure of the alkaloid component of the inhibitor molecule.

It has been shown previously [1-3] that the thiophosphates of decahydroquinoline, cytisine, and other alkaloids, which contain a N-(β-dialkoxyphosphinyl)thiooethyl group, possess high anticholinesterase activities and exhibit a higher tendency to interact with butyrylcholinesterase (BuChE) than with acetylcholinesterase (AChE). The selectivity with respect to enzymes depends not only on the structure of the phosphoryl part but also on the volume and conformational features of the separable part of the molecule. However, cyclic phosphates the molecules of which contain the residues of the alkaloid series have not been studied as effectors of metabolic enzymes.

In view of this, we have synthesized new 2-oxo-2*SR*-4-methyl-1,3,2-dioxaphosphorinanes containing residues of salsolidine (**1**), salsoline (**2**), decahydroquinoline (**3**) and cytisine (**4**). The substances were obtained by the interaction of the potassium salt of 2-oxo-4-methyl-1,3,2-dioxaphosphorinane [4-8] with an alkyl halide derivatives of the appropriate amine in absolute alcohol:





The required N-β-chloroethylamines were obtained by a published method.

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TABLE 1. Physicochemical Constants and Constants ($k_2 \times 10^5$ M^{-1}) of the Irreversible Inhibition of Cholinesterases and of Carboxyesterase by 2-Oxo-2*SR*-4-methyl-1,3,2-dioxaphosphorinanes

Compound	Yield, %	n_D^{20}	d_4^{20}	MR_d		AchE	BuChE	$\frac{k_{AChE}}{k_{BuChE}}$	AChE	BuChE	CBE porcine liver
				found	calculated	man	horse		turnip moth		$I_{50} \times 10^{-3}$
1	65.15	1.5422	1.2611	99.10	98.91	26.8	17.1	0.64			
2	74.12	1.5323	1.2310	93.49	92.85	1.6	6.53	4.09	0.078	0.468	7.0
3	45.45	1.4890	1.0810	88.60	88.02	0.799	1.73	2.16			3.0
4	68.10	1.5083	1.2211	92.26	91.50	0.612	1.32	2.15	0.302	0.501	3.5

The compounds obtained were characterized by their physicochemical constants and the results of elementary analysis. Their structures were confirmed by their IR and PMR spectra.

The study of the influence of compounds (**1-4**) on cholinesterases showed (Table 1) that they all irreversibly inhibited human blood erythrocyte AChE and horse blood serum BuChE. The study of the anticholinesterase activities of the new 2-oxo-4-methyl-1,3,2-dioxaphosphorinane derivatives showed that they all exhibited the irreversible type of inhibition of the catalytic activity of AChE and BuChE.

The highest activity in relation to AChE was exhibited by 2-oxo-2*S*-(salsolidinoethylthio)-4-methyl-1,3,2-dioxaphosphorinane. The salsoline derivative (compound **2**) was an order of magnitude less active. Passage to the decahydroquinoline derivative and then to a more complex derivative — the cytisine derivative — led to a fall in the irreversible inhibiting activity. The interaction of these compounds with BuChE showed that they were more active irreversible inhibitors of this enzyme than of AChE. In all probability, this is connected with the hydrophobic sorption of the alkaloid residues on the corresponding hydrophobic section of the anionic point of the active surface of the enzyme.

The study of the antienzyme activities of compounds (**2**) and (**4**) in relation to the AChE and BuChE of the turnip moth showed that these substances were weaker inhibitors of these enzymes than of the corresponding enzymes of warm-blooded animals. The investigation of compounds (**2**) and (**4**) as effectors of porcine liver carboxyesterase (CBE) showed that they were weak inhibitors of CBE. The dependence on the structure of the alkaloid residue is demonstrated by the fact that when salsoline was replaced by cytisine in the structure of the organophosphorus compound (OPC) the efficiency of the latter was twice as high, which is possibly connected with the greater hydrophobicity of the cytisine residue.

Thus, the new 2-oxo-4-methyl-1,3,2-dioxaphosphorinane derivatives that we synthesized have proved to be irreversible inhibitors of the AChE, BuChE, and CBE of warm-blooded animals and insects. The antienzyme activities of these compounds depend substantially on the structures of the alkaloid residues included in the OPC molecule.

EXPERIMENTAL

IR spectra were taken on a Specord-71 instrument (in paraffin oil). PMR spectra were recorded on a XL-100 Varian spectrometer (USA) in CCl_4 solution.

Commercial enzyme preparations were used: human blood erythrocyte AChE, horse blood serum BuChE (produced by the Perm Scientific Research Institute of Vaccines and Sera) and porcine liver CBE (Sigma) with specific activities of 2.1, 610, and 260 U/mg. As the turnip moth ChEs we used homogenates of the head and thoracic parts of 2nd-3rd instar caterpillars [9, 10].

Synthesis of 2-Oxo-2*S*-(salsolidinoethylthio)-4-methyl-1,3,2-dioxaphosphorinane. A mixture of 0.04 mole of the potassium salt of 2-oxo-2-thioxo-4-methyl-1,3,2-dioxaphosphorinane and 0.04 mole of chloroethylsalsolidine in 100 ml of abs. alcohol was boiled for 5 h and left for 12 h. The precipitate of KCl was filtered off, and the residue was purified on a column of Al_2O_3 (activity grade II). Yield 74.12%.

The IR spectra of compounds (**1**) and (**3**) contained absorption bands of the following functional groups (ν, cm^{-1}): 1495 and 1510 (C−C), 1360 and 1370 (C−N), 1220 and 1210 (P═O), 1070 and 1065 (P═O), 870 and 885 (P−S).

In the PMR spectrum of compound (**1**) the following signals were observed (ppm): 6.40 (2H, br.s, Ar−H), 3.9-4.6 (3H, m, OCH, OCH_2), 3.71, 3.73 (3H, s, OCH_3), 2.2-3.1 (8H, m, N−CH_2, S−CH_2), 1.4-2.1 (4H, m, C−CH_2), 1.23 (3H, d, C−CH_3, J = 6.5 (Hz).

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

The catalytic activities of ChE and CBE were determined by Ellman's thiocholine method [11] from the rates of hydrolysis of acetylcholine (for ChE) and of ethyl thiobutyrate (for CBE) [12].

The interaction of the enzymes with the inhibitors was evaluated from the bimolecular rate constant (k_2). The enzymes were incubated with the inhibitors for 10, 15, and 25 min and the interaction was stopped by the addition of the substrate. The k_2 values were calculated from the slope of the straight line of a graph in the coordinates log (ν/ν_t) as a function of the time t. Activities of the enzymes and values of k_2 were determined at 25°C in a medium having pH 7.5.

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