Chemistry of Natural Compounds, Vol. 30, No. 3, 1994

SYNTHESIS AND ANTICHOLINESTERASE ACTIVITIES OF O-PHOSPHORYLATED DERIVATIVES OF *d*-ALLYLPSEUDOEPHEDRINE

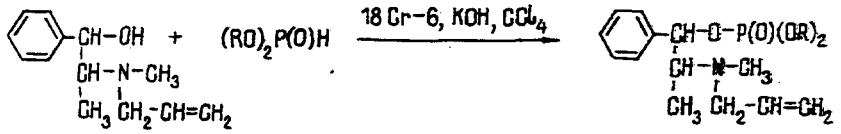
S. D. Fazylov, A. M. Gazaliev, K. D. Mukanova, E. B. Maizel', A. E. Khovanskikh, and A. B. Tateeva

UDC 547.94:577.153

The synthesis of O-phosphorylated derivatives of d-allylpseudoephedrine under the conditions of phase-transfer catalysis is described. The results of a study of their anticholinesterase properties are given.

In recent years, the arsenal of substrates and reversible inhibitors of cholinesterase has been considerably enlarged through derivatives of several classes of compounds [1, 2]. More than 40 acyl analogs of acetyl-β-methylcholine containing ephedrine alkaloids [2, 3] have, in the main, proved to be reversible inhibitors of cholinesterases. Continuing a search for specific substrates and inhibitors, we have synthesized previously undescribed O-phosphorylated derivatives of d-allylpseudoephedrine and have studied their anticholinesterase activities. We have previously reported on the anticholinesterase properties of phosphoramidates of *l*-ephedrine and of *d*-pseudoephedrine.

The performance of the phosphorylation of *d*-allylpseudoephedrine by known methods using dialkyl phosphorochloridites [5] is associated with certain experimental difficulties. For the synthesis of the allylpseudoephedrinyl dialkyl phosphates we therefore used the method of phase-transfer catalysis in the presence of catalytic amounts of benzo-18-crown-6, by the following scheme:



R=C_2H_5(1), C_3H_7(2), i-C_3H_7(3), C_4H_9(4)

1-4

The structures of the compounds synthesized (**1-4**) were characterized by IR and PMR spectroscopies. The physicochemical characteristics of compounds (**1-4**) are given in Table 1. The elemental analyses of all the compounds corresponded to the calculated figures.

The anticholinesterase properties of the compounds synthesized (**1-4**) were studied on human blood erythrocyte acetylcholinesterase (HEAcChE), equine blood serum butyrylcholinesterase (EBuChE), and the cholinesterases of an aphid, the greenbug *Schizaphis gramina* Rond. Differences in the sensitivity of the cholinesterases of insect pests and of warm-blooded animals to inhibitors form one of the bases for a directed search for specific insecticides.

As the results of the investigations showed, the allylpseudoephedrinyl dialkyl phosphates (**1-4**) proved to be practically equally effective reversible inhibitors of HEAcChE, but exhibited stronger inhibiting properties in relation to equine BuChE (Table 2). The antibutyrylcholinesterase efficiency of compounds (**1-4**) depended on the structure of the radical, and the compounds reached their maximum inhibition constant on passing from ethyl (**1**) to isopropyl (**3**). The strength of inhibi-

Institute of Organic Synthesis and Coal Chemistry, National Academy of Sciences, Republic of Kazakhstan, Karaganda. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 408-411, May-June, 1994. Original article submitted July 6, 1993.

0009-3130/94/3003-0373$12.50

TABLE 1. Physicochemical Characteristics of the Dialkyl Allylpseudoephedrinyl Phosphates (1-4)

Compound	R	Yield, %	IR spectra, ν, cm^{-1}			PMR spectra, δ, ppm			
			P=O	P—O—C	C=C	C—CH_3	N—CH_3	P—O—R	Ph
1	C_2H_5	72	1265	1040	1680	0.90d	2.50 s	3.64m	7.30m
2	C_3H_7	69	1256	1060	1680	0.91 d	2.52 s	3.78m	7.25 m
3	*i*-C_3H_7	73	1260	1055	1670	0.92d	2.53 s	3.75 m	7.24 m
4	C_4H_9	66	1255	1076	1680	0.92d	2.51 s	3.77 m	7.20m

TABLE 2. Anticholinesterase Effectivenesses of the Dialkyl Allylpseudoephedrinyl Phosphates (1-4)

Compound	R	Aphid AcChE				Aphid BuChE				Aphid AcChE				Aphid BuChE			
		T.i.*	pKi	pK_i'	$p\bar{K}_i$	T.i.	pKi	pK_i'	$p\bar{K}_i$	T.i.	pKi	pK_i'	$p\bar{K}_i$	T.i.	pKi	pK_i'	$p\bar{K}_i$
1	C_2H_5	c	3.40	-	3.40	c	3.80	-	3.80	n	3.92	3.93	4.23	-	2		
2	C_3H_7	c	2.97	1.80	3.00	m/n	4.27	4.25	4.50	m	3.89	4.36	4.50		2		
3	*i*-C_3H_7	m/m	3.20	3.14	3.48	m	5.06	4.76	5.25	m	4.53	3.90	4.62	n/m	3.04	3.20	3.44
4	C_4H_9	m	3.40	3.55	3.46	m/n	4.18	4.05	4.36	n/m	3.60	3.83	4.04	m	2.18	2.80	2.90

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

tion of butyrylcholinesterase activity was 28 times greater for compound (**3**) than for compound (**1**), with an ethyl radical. However, compounds (**1**) and (**2**) did not exhibit antibutyrylcholinesterase activity for the greenbug, and the rate of reaction of the other two derivatives (**3** and **4**) was also low (see Table 2). Only compound (**1**) exhibited a true noncompetitive type of action ($pK_i > pK_i'$) in relation to both HEAcChE and EBuChE.

Thus, the phosphorus derivatives of allylpseudoephedrine that have been studied are moderately strong reversible inhibitors of cholinesterases and have proved more specific for AcChE in the case of the greenbug and for BuChE in the case of mammals. The inhibition constants obtained give grounds for assuming that the hydrophobic regions on the surface of equine BuChE are more extensive than in the case of the aphid BuChE, and the compound with an isopropyl radical proves to be the most complementary to the BuChE surface.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in tablets with KBr, and PMR spectra on a Tesla BS-467 instrument at a frequency of 80 MHz, with HMDS as internal standard. The anticholinesterase activities of the preparations were investigated in the laboratory of invertebrate biochemistry of the I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences. We investigated a common aphid, the greenbug *Schizaphis gramina* Rond (fam. Aphididia), and purified preparations of AcChE and BuChE (with specific activities of 2.2 and 9.6 units/mg of protein, respectively) produced by the Perm Scientific Research Institute of Vaccines and Sera.

Synthesis of Allylpseudoephedrinyl Diethyl Phosphate (1). With stirring, 4.0 g (0.03 mole) of diethyl phosphite was added slowly, dropwise, to a mixture of 4.57 g (0.02 mole) of *d*-allylpseudoephedrine, 1.63 g (0.03 mole) of KOH, 12.0 g (0.08 mole) of carbon tetrachloride, and catalytic amounts of benzo-1-crown-6 in tetrahydrofuran. The mixture was stirred at room temperature for 5 h, the precipitate of potassium chloride that had deposited was filtered off, the solvent was distilled off, and the residue was purified on a column of Al_2O_3 (activity grade II) with benzene as the eluent. The reaction product was a viscous oily substance with a yellow tinge, yield 72%.

Compounds (**2-4**) were obtained analogously.

Methods of Investigation. The catalytic activities of AcChE and BuChE were determined by Ellman's method [6] from the rate 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 aphid BuChE, and at pH 7.0 for the aphid AChE.

REFERENCES

1. A. S. Sadykov, D. N. Dalimov, and N. N. Godovikov, Usp. Khim., **52**, No. 10 1602 (1983).
2. A. A. Abduvakhabov, D. I. Sadykov, D. N. Dalimov, and Kh. A. Aslanov, Alkaloids and Their Derivatives as Tools for the Study of the Cholinergic System [in Russian], Fan, Tashkent (1984).
3. A. A. Abduvakhabov, Yu. R. Khakimov, E. B. Maizel', E. V. Rozengart, A. A. Sadykov, Kh. A. Aslanov, and A. S. Sadykov, Dokl. Akad. Nauk SSSR, **24**, No. 1, 227 (1978).
4. M. Zh. Zhurinov, A. M. Gazaliev, and S. D. Fazylov, The Chemistry of the Ephedrine Alkaloids [in Russian], Nauka, Alma-Ata (1990).
5. A. A. Abduvakhabov, Uzb. Khim. Zh., No. 4, 45 (1989).
6. G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, Biochem. Pharmacol., No. 7, 88 (1961).