SYNTHESIS AND ANTICHOLINESTERASE ACTIVITIES OF A NUMBER OF DERIVATIVES OF THE ALKALOID LUPININE

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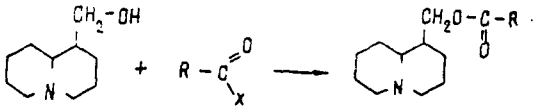
A series of new lupinine derivatives and their methiodides has been synthesized. It has been shown that they are powerful reversible inhibitors of acetylcholinesterase and butyrylcholinesterase.

Cholinesterases are some of the most important components of the cholinergic system. The creation of effective reversible inhibitors of these enzymes provides the possibility of determining the form of their action on the transmission of a nerve impulse [1].

In order to find effective reversible cholinesterase inhibitors we have performed the synthesis of a number of derivatives of the alkaloid lupinine and have studied their anticholinesterase activities. A scheme of the synthesis is given below:



where X = Cl, OCH_3, OC_2H_5; R = $-CH(OH)-CH_3$ (I); $-CH_2-Cl$ (II);

$-(CH_2)_{16}-CH_3$ (III); $-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$ (IV); $-CH_2-NH-C(=O)-C_6H_5$ (V)

The new esters (II) and (IV) were obtained by the interaction of lupinine with the corresponding acid chlorides in the presence of triethylamine in an absolute solvent (benzene or ether), and the esters (I), (III), and (IV) by the transesterification of the methyl (ethyl) esters of the corresponding acids with lupinine in the presence of a catalytic amount of metallic sodium in chlorobenzene solution. The structures of the substances obtained were confirmed by IR and PMR spectroscopy. The corresponding hydrochlorides and methiodides of all the compounds synthesized were obtained.

The anticholinesterase properties of the compounds synthesized (I-V) were studied on human blood erythrocyte acetylcholinesterase (ACE) and horse blood serum butyrylcholinesterase (BuCE). All the new methiodides of lupinine derivatives (I-V) proved to be competitive reversible inhibitors of both types of cholinesterase. Information on inhibitory activity in relation to ACE and BuCE and on the specificity of the action is given in Table 1. On the interaction of substances (I-V) with ACE, the anticholinesterase activity depended on the structure of the radical of the acid moiety of the methiodide molecule. The most active in relation to ACE proved to be the methiodides of (I) and (II). A further complication of the acyl moiety of the inhibitor molecule led to an impairment of its sorption in the active center of the ACE and to a corresponding fall in anticholinesterase activity.

The fairly high anticholinesterase activity of compounds (IV) and (V) on their interaction with BuCE must be mentioned, this being connected with the well-defined hydrophobicity of these compounds, since hydrophobic interactions are characteristic for BuCE.

On comparing the values of the magnitudes $\overline{K}_i$ on their interaction with ACE and BuCE, $(\overline{K}_i$ ACE$)/(\overline{K}_i$ BuCE), we obtained values for the specificity of the action of these substances

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TABLE 1. Constants of the Reversible Inhibition of ACE and by the Methiodides of the New Lupinine Derivatives (I-V)

Compound	ACE			Buce			$\frac{\overline{K}_i ACE}{\overline{K}_i Buce}$
	$K_i \cdot 10^{-5}$	$K'_i \cdot 10^{-5}$	$\overline{K}_i \cdot 10^{-5}$	$K_i \cdot 10^{-5}$	$K'_i \cdot 10^{-5}$	$\overline{K}_i \cdot 10^{-5}$	
I	83,1	0,55	0,55	56,9	15,2	12,9	0,045
II	1,61	1,49	0,77	—	0,78	0,79	0,974
III	2,56	1,19	0,81	14,3	12,2	6,62	0,122
IV	12,9	7,66	4,86	40,3	1,58	1,53	3,176
V	4,04	5,28	2,28	—	0,10	0,10	22,8

on ACE and BuCE. As can be seen from Table 1, compounds (I) and (III) are selective inhibitors of ACE, while (IV) and (V) are specific in relation to BuCE.

Thus, by varying the acyl moiety of the inhibitor molecule it is possible to achieve different specifications in relation to the two types of cholinesterase.

EXPERIMENTAL

The TLC of the products was performed on Silufol UV-254 plates using the chloroform–ethanol (2:1) system, with revelation by means of iodine vapor.

IR spectra were taken on a Specord-71 IR instrument (GDR), and PMR spectra on a Varian XL-200 instrument (USA) in CCl_4 solution with HMDS as standard.

Anticholinesterase efficiency was determined by Ellman's method [2] on a Specol-221 instrument (GDR) in the regime of the KIN program.

Lupinine Ester of Chloroacetic Acid (II). With stirring and cooling to 0°C, 2.48 g (0.022 mole) of chloroacetyl chloride in 30 ml of absolute ether was added dropwise over 30 min to a mixture of 3.4 g (0.02 mole) of lupinine and 2.02 g (0.02 mole) of triethylamine in 80 ml of absolute ether.

The reaction mixture was stirred for 3 h and was heated to 30-40°C and then cooled. The triethylamine hydrochloride that deposited was filtered off and was washed several times with ether. The unchanged lupinine was eliminated by treatment with a 5% solution of boric acid. The solvent was distilled off, and the residue was passed through a column filled with Al_2O_3 (activity grade II). The product, obtained with a yield of 3.65 g (74%) was an oil, R_f 0.76, n_D^{20} 1.4700. Hydrochloride with mp 94-96°C; methiodide with mp 208-210°C.

IR spectrum (ν, cm^{-1}): 2720 (trans-quinolizidine), 1730 (–OCO–), 1130 (–COC–), 750 (C–Cl). PMR spectrum (δ, ppm): 4.1-4.45 (2H, m, OCH_2), 3.89 (2H, s, $-CH_2Cl$), 2.69 (2H, d, H_{2e} and H_{10e}, J = 10.6 Hz), 1.0-1.9 (14H, m, CH_2).

Compound (V) was synthesized analogously. Yield 5.15 g (78%), R_f 0.60, n_D^{20} 1.4420.

IR spectrum (ν, cm^{-1}): 3150 (N–H), 2800 (trans-quinolizidine), 1725 (–OCO–), 1590 (Ar), 1550 (–C=C–), 1200 (–COC–). PMR spectrum (δ, ppm): 7.5-7.8 (5H, m, Ar–H), 6.72 (1H, t, NH, J = 5 Hz), 3.8-4.4 (2H, m, OCH_2), 4.07 (2H, d, $-CO-CH_2$, J = 5 Hz), 2.69 (2H, d, H_{2e} and H_{10e}, J = 10.6 Hz), 1.0-2.0 (14H, m, CH_2).

Lupinine Ester of Lactic Acid (I). A mixture of 3.4 g (0.02 mole) of lupinine, 2.36 g (0.020 mole) of ethyl lactate, and 0.04 g (0.0017 mole) of metallic sodium in 30 ml of chlorobenzene was heated in an oil bath at a temperature of 120-130°C under water-pump vacuum. The ethanol formed during the reaction and the chlorobenzene were distilled off to dryness. The residue was treated with a mixture of 50 ml of water and 10 ml of concentrated HCl. The liberated base was extracted with benzene, and the extract was dried with Na_2SO_4. The final product was purified by column chromatography on Al_2O_3 (activity grade II), with elution by ether.

Yield 4.5 g (93%), R_f 0.64, n_D^{20} 1.4976. Hydrochloride with mp 185-187°C; methiodide with mp 220-222°C.

IR spectrum (ν, cm^{-1}): 3320 (O–H), 2820 (trans-quinolizidine), 1730 (–OCO–), 1210-1250 (–COC–). PMR spectrum (δ, ppm): 4.05-4.4 (2H, m, OCH_2), 4.04 (1H, q, OCH, J = 6.3 Hz), 2.69 (2H, d, H_{2e} and H_{10e}, J = 10.6 Hz), 1.28 (3H, d, CH_3, J = 6.3 Hz), 1.0-2.0 (14H, m, CH_2).

Compounds (III) and (IV) were synthesized analogously. The yield of (III) was 5.75 g (66%), R_f 0.53. Hydrochloride with mp 66-68°C; methiodide with mp 106-108°C. IR spectrum (ν, cm^{-1}): 2830 (trans-quinolizidine), 1735 (—OCO—), 1210 (—COC—). PMR spectrum (δ, ppm): 3.8-4.2 (2H, m, OCH_2), 2.84 (2H, d, $H_{2\bar{e}}$ and $H_{10\bar{e}}$, J = 10.6 Hz), 1.2-2.3 (46H, m, CH_2), 0.84 (3H, t, CH_3).

The yield of (IV) was 4.68 g (54%); R_f 0.68, n_D^{20} 1.3544; methiodide with mp 239-242°C.

IR spectrum (ν, cm^{-1}): 2810 (trans-quinolizidine), 1750 (—OCO—), 1220 (—COC—). PMR spectrum (δ, ppm): 5.22 (2H, t, CH=CH), 3.95-4.3 (2H, m, OCH_2), 2.76 (2H, d, $H_{2\bar{e}}$ and $H_{10\bar{e}}$, J = 10.6 Hz), 1.0-2.4 (42H, m, CH_2), 0.83 (3H, t, CH_3).

The samples for determining the activities of the enzymes contained 0.4 ml of a 0.001 M solution of Ellman's reagent, 0.7 ml of 0.1 M phosphate buffer with pH 8.0, 0.3 ml of an aqueous solution of ACE or BuCE (prepared to give 0.2 activity unit per 1 ml), 0.2 ml of a solution of the substrate acetylcholine (ATC) (the concentration in the sample varied from $1.25 \cdot 10^{-4}$ M to $7.5 \cdot 10^{-5}$ M), and 0.2 ml of a 0.2 M solution of the substance under investigation.

As control we used a sample in which the 0.2 ml of the solution of the substance under investigation had been replaced by an equivalent amount of water. To exclude the possibility of irreversible inhibition of the substance under investigation and to show the reversible nature of the inhibition of the enzyme, before the inhibition of the substrate the sample was incubated with the inhibitors under investigation at 30°C for ~20 min in the reaction mixture, and then the ATC was added and hydrolysis was carried out. The constant of reversible inhibition was found from a graph of double reciprocal values of $\overline{K}_i$ according to [3].

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PREPARATION OF CONJUGATES OF PEPTIDES IMITATING PART OF THE ANTIGEN-DETERMINANT SECTION OF PROTEIN VP1 OF FOOT-AND-MOUTH DISEASE VIRUS A_{12} WITH VARIOUS SUPPORTS

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The preparation of conjugates of peptides 143-148, 153-159, 149-159, 146-159, and 143-159, imitating a section of a protein of the foot-and-mouth disease (FMD) virus of type A_{12}, with bovine serum albumin, with a copolymer of N-vinylpyrrolidone with acrylic acid, and with a copolymer of N-vinylpyrrolidone with maleic anhydride is described. The dependence of the degree of conjugation on various factors is discussed.

At the present time there are two approaches to investigations of the creation of synthetic vaccines. The first, proposed by Sela [1] consists in obtaining the determinant groups of the antigens synthetically and their subsequent conjugation with synthetic poly(amino acid)s or with native proteins. This approach has been used for those viruses in

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