SYNTHESIS AND MEMBRANOTROPIC ACTIVITY OF N-ADAMANTANOYLAMINO AND N-ADAMANTYLACETYLAMINO ACIDS

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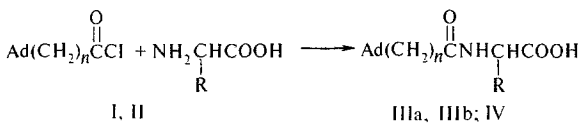
Amines and amides of the adamantane series exhibit a broad spectrum of pharmacological activity, including analgesic [1], antiinflammatory [2], anticonvulsive [3], and antiviral [4, 5]. Adamantane and its derivatives are capable of changing the hexagonal packing of methylene groups in the alkyl chains of phospholipids, thus affecting the functional properties of membrane proteins. Data obtained on models of erythrocytic membranes and liposomes showed that remantadine may incorporate into the lipid membranes, modifying their structure and imparting to them a more "liquid-phase" character [6]. In the context of an investigation of the membrane activity of adamantane derivatives and the role of this factor in the antiviral and cytotoxic properties of these compounds, of special interest are amides containing, on the one hand, the adamantyl radical and, on the other hand, an amino acid residue.

The purpose of this work was to synthesize some new adamantane-containing amino acids (IIIa, IIIb, and IV) and to study their effects upon the state of hemoglobin and membranes in human blood erythrocytes.

The adamantane-containing amino acids IIIa, IIIb, and IV were synthesized by interaction of 1-adamantanecarboxylic (I) and (1-adamantyl)acetic (II) acids with glycine, *D,L*-alanine, and *L*-valine.



I, II IIIa, IIIb; IV

Ad = 1-adamantyl; n = 0 (I, IIIa, IIIb), 1 (II, IV); R = H (IIIa), *iso*-Pr (IIIb), Me (IV).

EXPERIMENTAL CHEMICAL PART

The IR absorption spectra were measured on an IKS-29 spectrophotometer (LOMO, Russia) using samples pelletized with KBr. The ^{1}H NMR spectra were recorded on a Bruker HX-90 spectrometer using CD_3OD as the solvent and HMDS as the internal standard. The TLC analyses were conducted on Silufol UV-254 plates. The data of elemental analyses agree with the results of analytical calculations.

N-Adamantanoylglycine (IIIa). To a solution of 2.25 g (30 mmole) of glycine in 7.5 ml of water were successively added 7.5 ml (30 mmole) of a 4 N aqueous NaOH solution, 7 ml of 1,4-dioxane, and eventually, by portions with stirring, a solution of 6 g (30 mmole) of adamantanoyl chloride in 20 ml of absolute 1,4-dioxane. As the reaction proceeded, 4 N aqueous NaOH solution was added to maintain the initial level of pH 9 – 11. The chloroanhydride solution was added in five portions over a time period of approximately 45 min. The stirring was continued for 30 min at 20°C. Then the reaction mixture was doubly diluted with water and acidified to pH 2 – 3 with HCl. The white clotted precipitate was filtrated, washed with water until neutral pH, dried, and recrystallized from acetone to obtain 6.4 g (90.0 %) of compound IIIa; m.p., 191 – 192°C; R_f, 0.35 (DMF – hexane – chloroform, 5 : 1 : 1); IR spectrum (δ, cm^{-1}): 1740 (C=O amide), 1750 (C=O), 2860, 2910 (CH_2 adamantane), 3340 (NH); ^{1}H NMR spectrum in CD_3OD (δ, ppm): 1.9200 (m, 15H, adamantane), 3.9531 (d, 2H, CH_2), 7.7867 (m, 1H, NH); $C_{13}H_{19}NO_3$.

Aides IIIa and IV were obtained by similar procedures.

N-Adamantanoyl-*L*-valine (IIIb). Yield, 77.2%; m.p., 194 – 195°C; R_f, 0.57 (DMF – hexane – chloroform, 5 : 1 : 1); IR spectrum (δ, cm^{-1}): 1700 (C=O amide), 1770 (C=O), 2840, 2890 (CH_2 adamantane), 3360 (NH); ^{1}H NMR spectrum in CD_3OD (δ, ppm): 1.4974 (d, 3H, CH_3), 1.9865 (m, 15H, adamantane), 4.4683 (m, 1H, CH), 7.5143 (d, 1H, NH); $C_{16}H_{25}NO_3$.

N-Adamantylacetyl-*D,L*-alanine (IV). Yield, 82.4%; m.p., 183 – 185°C; IR spectrum (δ, cm^{-1}): 1700 (C=O amide), 1750 (C=O), 2850, 2900 (CH_2 adamantane), 3350 (NH); ^{1}H NMR spectrum in CD_3OD (δ, ppm): 1.08064 (dd, 3H, CH_3), 1.8616 (m, 15H, adamantane), 2.1272 (s, 2H, CH_2),

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2.2554 (m, 1H, $CH(CH_3)_2$), 4.3828 (m, 1H, CH), 7.7 (d, 1H, NH); $C_{15}H_{23}NO_3$.

EXPERIMENTAL BIOLOGICAL PART

The whole donor blood was incubated with compounds IIIa, IIIb, or IV at a concentration of 20 μg/ml for 20 min at 37°C. The control sample was incubated with the solvent (ethyl alcohol). In order to maintain the hematocrit level and avoid ethanol damage, the solvent volume added amounted to 0.01 of the total sample volume. Upon incubation, the samples were characterized with respect to the rates of enzymatic [7] and chemical [8] ferricyanide reduction by erythrocyte hemolysates, the contents of methemoglobin and oxyhemoglobin [9], and the osmotic resistance of erythrocytes [10].

It was established that, among the substances studied, a maximum increase in the rate of ferricyanide reduction in the presence of NADH was induced by compound IIIb (Table 1). Taking into account that the ability of hemolysates to produce the ferricyanide reduction (enzymatic) can be partly controlled by the rate of membrane enzyme conversion from latent to active form [11] and by the activity of (NADH-dependent) methemoglobin reductase with respect to one-electron oxidizers [7], we may suggest that compound IIIb exhibits maximum membranotropic activity.

In addition, the more pronounced membranotropic activity of compound IIIb leads to the accelerated chemical oxidation of hemoglobin by ferricyanide (Table 2), which is usually explained by a decrease in pH leading to protonation of a distant histidine and by an increase in the access of external oxidizers to the heme iron ion [12]. The oxidation process is accelerated via oxyhemoglobin stabilization by organic phosphates (adenosine triphosphate or 2,3-dihydrophosphate) [12].

Note that the somewhat stressed metabolism caused by amides III (especially IIIb) and IV does not lead to any increase in the rate of hemoglobin autooxidation and, hence, the amount of methemoglobin remains unchanged (Table 2). All the compounds studied favored an increase in the osmotic resistance of erythrocytes.

Thus, the synthesized adamantane derivatives do not affect the process of methemoglobin formation and increase the osmotic resistance of erythrocytes. The action of compound IIIb leads to some stress in the metabolism of erythrocytes, as manifested in an increase in the activity of the membrane enzyme reducing lipid hydroperoxides and in the rate of hemoglobin oxidation by ferricyanide. Therefore, compound IIIb exhibits the most pronounced action upon the erythrocytic membrane.

TABLE 1. Effect of Compounds IIIa, IIIb, and IV upon the Rate of Chemical Reduction (in Arbitrary Units) of Ferricyanide by Erythrocytic Hemolysates

Compound	Time of incubation with potassium ferricyanide, sec				
	30	90	150	180	300
Control	0.840 ± 0.093	1.761 ± 0.136	2.527 ± 0.189	2.874 ± 0.199	4.122 ± 0.243
IV	1.144 ± 0.201	2.447 ± 0.333	3.639 ± 0.498*	4.162 ± 0.587*	5.818 ± 0.760*
IIIa	0.799 ± 0.098	2.129 ± 0.247	3.330 ± 0.354*	3.802 ± 0.401*	5.419 ± 0.544*
IIIb	1.089 ± 0.138	2.800 ± 0.315*	4.062 ± 0.431*	4.728 ± 0.514*	6.955 ± 0.744*

* $p < 0.05$.

TABLE 2. Effect of Compounds IIIa, IIIb, and IV upon the Parameters of State of Hemoglobin and Erythrocytic Membrane

Compound	NADH-ferricyanide reductase activity (n = 16), (μM gHb)/sec	Content (n = 14)		Osmotic resistance, % (n = 12)
		HbO_2, μM/gHb	MetHb, μM/gHb	
Control	6.346 ± 0.993	11.49 ± 0.38	3.33 ± 0.46	92.84 ± 1.00**
IV	7.249 ± 1.075	12.77 ± 0.61	2.72 ± 0.57	84.91 ± 2.49**
IIIa	7.196 ± 0.714	11.02 ± 0.82	4.73 ± 0.42	78.72 ± 2.88*
IIIb	9.689 ± 0.569*	14.29 ± 0.72*	2.66 ± 0.45	85.52 ± 1.32**

* $p < 0.01$.
** $p < 0.05$.

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