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SYNTHESIS OF N_4-PROPYLAJMALINE BROMIDE AND A STUDY OF ITS ANTIARRHYTHMIC ACTIVITY

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It has been shown in recent years that the quaternary derivatives of some antiarrhythmic agents have a significantly greater antiarrhythmic action and of longer duration than their precursors. This relationship has been established for Lidocaine [14], Trimecaine [5, 6], and Anaprylin [10], and has already been successfully applied in medical practice to Ajmaline from which an effective new generation of antiarrhythmic preparations called Neo-Gilurythmal has been manufactured [12, 13, 16].

A study of the relationship between chemical structure and the pharmacological activity of the quaternary derivatives of ajmaline has established that N_4-propylajmaline bromide (N-PAB) exhibits the greatest activity [15]. N-PAB was synthesized at the I. F. Makarevich Kharkov Scientific Research Institute of Pharmaceutical Chemistry, and its pharmacological properties were investigated by É. I. Gendenshtein [2, 3]. N-PAB was clinically tested successfully in 1974 and was approved by the Pharmacology Committee of the Ministry of Health of the USSR for use in medical practice as an antiarrhythmic drug. However, because of the lack of domestic raw materials, this preparation was never manufactured.

The Leningrad Pharmaceutical Chemistry Institute devised a means of cultivating isolated cells of Rauwolfia serpentina [1] which provided a promising source for the production of ajmaline alkaloids [8] from which N-PAB was synthesized.

The present work offers a modified method of obtaining N-PAB and cites the results of a study of its antiarrhythmic activity as tested on experimental models of cardiac arrhythmia.

EXPERIMENTAL CHEMICAL

The starting material for the production of N-PAB was ajmaline which was isolated from the biomass of a Rauwolfia serpentina tissue culture and which corresponded to the requirements of the All-Union Pharmacology Committee 42-1876-82. N-PAB was synthesized by method [15] as modified by us. In the process of synthesizing the derivative, the toxic and expensive solvent acetonitrile was replaced by rectified ethyl alcohol. We established the optimal time and temperature conditions of the reaction as well as the consumption rate of the alkylating agent which enabled us to increase the yield by 16%. N-PAB was obtained in the following manner: a 1-g portion of ajmaline was dissolved in 8 ml of rectified ethyl alcohol which was mixed upon heating with 0.7 g (0.5 ml) of n-propyl bromide. The reaction proceeded at 70°C for 9.5 h. The resultant residue was filtered and recrystallized from methanol, followed by precipitation by diethyl ether. The yield of the pharmacopeia-standard product was 78%. The resultant product was a white crystalline powder, mp 281-283°C. Found, %: C 61.12, H 7.56, Br 17.18, N 5.97. $C_{23}H_{33}Br_2O_2$. Calculated, %: C 61.17, H 7.40, Br 17.78, N 6.23. UV spectrum, alcohol, γ_{max}, nm (log ε): 245 (3.81), 290 (3.33). IR spectrum, γ_{max}, cm^{-1}: 760 (C—H), 1200 (C—O), 1300 (C—N), 1610 (aromatic ring), 3300 (OH), TLC (Silufol UV-254, Czechoslovakia). R_f 0.66 ± 0.02 ($CHCl_3$—acetone—diethylamine, 5:4:1).

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TABLE 1. Antiarrhythmic Activity of N-PAB and Ajmaline

Test	Dose, mg/kg		Index discrepancy ratio
	N-PAB	Ajmaline	
Ventricular extrasystole in dogs in the late stage of experimental myocardial infarct (IV injection)	1,3±0,5 (10)	5,7±1,4 (7)	4,4
Strophanthin arrhythmia in dogs	0,5 (6)	2,5±0,5 (6)	5,0
Aconitine arrhythmia in rats, protective action (ED_{50})	0,33±0,068 (32)	3,3±0,3 (24)	10,0
Atrial flutter in dogs induced by electric shock	0,44±0,05 (8)	3,0±0,24 (6)	6,8
Aconitine-induced atrial fibrillation in dogs	0,32±0,04 (5)	2,6±0,18 (6)	8,0
LD_{50} for mice in ip injection	19,0±1,3 (100)	150,6±6,8 (80)	7,9
LD_{50} for mice in enteral administration	31,0±1,5 (60)	220,0±15,5 (60)	7,1
LD_{50} for rats in ip injection	12,2±0,83 (30)	94,0±5,0 (40)	7,7

Note. Number of observations in parentheses.

EXPERIMENTAL PHARMACOLOGICAL

The antiarrhythmic activity of N-PAB was studied on 5 experimental models of arrhythmia. Ventricular arrhythmia was induced in 31 dogs by ligating the descending branch of the left coronary artery [2, 4] and by strophanthin intoxication (90-110 µg/kg) of 18 dogs. Atrial-ventricular arrhythmias were simulated in 81 rats by the IV injection of aconitine nitrate (40-50 µg/kg). Atrial arrhythmias in 24 dogs were produced by electric pulse stimulation of the atria [17] and the application of a 0.05% solution of aconitine nitrate [2].

Our study of the antiarrhythmic activity of N-PAB during ventricular arrhythmia in the late stage of an experimental myocardial infarct showed that an IV dose of 0.5 mg/kg suppressed ventricular extrasystole in 8-12 min and restored sinus rhythm in three out of ten experiments. Moreover, this effect was maintained for 115-250 min. A significant ectopic pulse attenuation (by more than 40%) was observed in the remaining tests.

N-PAB was administered repeatedly at 30 min intervals at a dose of 0.5 mg/kg to the animals in which sinus rhythm was not completely restored, and at a subsequent dose of 1 mg/kg if a complete effect was not obtained at the previous doses.

A second dose at 0.5 mg/kg resulted in the return of a normal rhythm in three out of seven tests, while a dose of 1 mg/kg eliminated arrhythmia in all cases and restored sinus rhythm.

Consequently, the back-titered ED for N-PAB which produced antiarrhythmic activity upon IV injection in all 10 experiments was found to be 1.3 ± 0.5 mg/kg. This effect was retained for 219 ± 18 min. The antiarrhythmic action of N-PAB was not accompanied by any side effects and did not adversely affect the general condition of the unanesthetized experimental animals.

For the sake of comparison we should note that the back-titered ED for ajmaline under similar experimental conditions was found to be 5.7 ± 1.4 mg/kg and that effect lasted 52 ± 8 min.

When seven unanesthetized dogs with ventricular arrythmia in the late stage of experimental myocardial infarct were given N-PAB in the form of 20-mg tablets through a gastric tube, we found that a 2 mg/kg dose of the preparation merely lessened (by 27%) the intensity of ventricular extrasystole. In another series of experiments on seven animals, a 4 mg/kg dose of N-PAB completely restored sinus rhythm in four experiments and reduced the number of extrasystoles by 70-80% in the remaining tests. When N-PAB was administered internally, the antiarrhythmic effect occurred after 43 ± 5 min and lasted for 395 ± 56 min.

The antiarrhythmic effect of N-PAB during strophanthin-induced arrhythmia was studied in 12 dogs in two series of experiments. A 0.1 mg/kg dose of N-PAB completely eliminated

arrhythmia in only two out of six tests whereas a 0.5 mg/kg dose restored proper sinus rhythm in all cases.

Our study of the aconitine model of arrhythmia in rats showed that the ED_{50} for N-PAB (protective action) was 0.33 ± 0.68 mg/kg as calculated by the Miller and Tayntner method.

N-PAB was observed to have a reliable cut-off effect in advanced aconitine arrhythmia in experiments on 25 rats given a dose of 0.5 mg/kg.

N-PAB was found to be equally effective in experimental atrial arrhythmias in dogs where an IV dose of 0.44 ± 0.05 mg/kg arrested atrial flutter induced by electrical pulse stimulation of the heart (n = 8). At a dose of 0.32 ± 0.04 mg/kg N-PAB suppressed aconitine fibrillation of the atria (n = 5).

DISCUSSION AND RESULTS

As can be seen from the data given in the Table, N-PAB is several times stronger than ajmaline in antiarrhythmic activity both in ventricular and atrial experimental arrhythmias. Moreover, the antiarrhythmic index established for N-PAB in the case of aconitine arrhythmia in rats was found to be markedly greater (36.9) than that of ajmaline (28.4). A characteristic feature of N-PAB which conveniently distinguishes it from ajmaline is its considerably longer antiarrhythmic effect in ventricular arrhythmia in the late stage of experimental myocardial infarct. This is apparently due to the presence of a conjugated quaternary nitrogen atom in the N-PAB molecule. Consequently, this preparation blocks the internal openings of the sodium channels in the myocardium cellular membranes in which the preparation remains for an extensive period [7].

The high level of N-PAB effectiveness in cardiac arrhythmias induced by aconitine and a disturbance of myocardial blood supply whose genesis is associated with the activation of the sodium canals of the myocardial cell membranes [11] allows us to hypothesize that N-PAB's antiarrhythmic activity is apparently dependent upon its ability to inhibit afferent sodium and efferent potassium transmembrane flow, i.e., its ability to exhibit quinidine-like action.

The results of our pharmacological study of N-PAB obtained from ajmaline isolated from Rauwolfia cultivated in a tissue culture indicate that this preparation exhibits a high degree of antiarrhythmic activity with a broad spectrum of action and effect of long duration. These results allow us to view N-PAB as a very promising antiarrhythmic agent which is more economical to manufacture than ajmaline.

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ANTIFIBRINOLYTIC ACTIVITY OF NICOTINIC ACID CONJUGATES WITH 6-AMINOHEXANOIC, p-AMINOMETHYLBENZOIC, AND trans-4-AMINOMETHYLCYCLOHEXANECARBOXYLIC ACIDS

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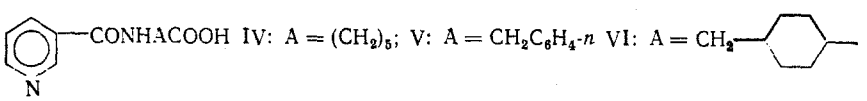
Studies of the antifibrinolytic activity of 6-aminohexanoic acid (I), p-aminomethylbenzoic acid (II), trans-4-aminomethylcyclohexanocarboxylic acid (III), and their analogs have been covered in a wide range of literature that offers data on biological action—structure relationships and a qualitative analyses of the contribution made by some functional groups to overall inhibitory activity [6]. It has been shown in particular that the presence of free amino and carboxyl groups separated by a distance of 0.7 nm is an important condition for the high level of antiproteolytic activity of this group of substances [9]. For the purpose of obtaining potential medicinal agents from compounds I-III, we undertook the synthesis of a number of their vitamin conjugates. Because of the vitamin presence in their molecular composition, we believed such compounds would be less toxic, exhibit a high degree of organotropy, as well as concomitant vitamin activity, and might also have better pharmacokinetic characteristics. That supposition has been recently justified by the production of vitamin conjugates with γ-aminobutyric acid [3, 7]. The present work cites results of studies on the antifibrinolytic activity of N-amide derivatives of nicotinic acid with substances of compounds I-III as compared to I. N-nicotinoyl-6-aminohexanoic acid (IV), p-aminomethylbenzoic acid (V), and trans-4-aminomethylcyclohexanecarboxylic acid (VI) were synthesized by the condensation of nicotinic acid chloroanhydride with salts of the corresponding acids in a water medium with a relatively low yield. This was apparently due to the partial hydrolysis of the chloroanhydride in the Schotten—Baumann reaction:



The structure of the substances obtained was confirmed by IR and UV spectra. The UV spectra of compounds IV-VI have maximum absorption bands at 259.5 nm that are due to the presence of a β-pyridylcarbonyl residue whereas the spectrum of compound V has a supplemental absorption band at 233 nm because of the disubstituted phenyl residue. The presence of intense absorption bands was observed in the IR spectrum of substances IV-VI in the region of 3300 (NH), 1710 (COOH), 1630-1640 (amide I), 1540-1550 (amide II), and 1590-1595 cm^{-1} (pyridine ring). Compounds IV-VI appear as crystalline substances that are slightly soluble in water and alcohol. The easily water-soluble sodium and potassium salts were obtained for our pharmacological study.

EXPERIMENTAL CHEMICAL

The IR spectra of compounds IV-VI were recorded in petroleum jelly on a Carl Zeiss UR-10 spectrophotometer (GDR). Compound purity was controlled by TLC on silufol UV-254

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