SYNTHESIS AND ANTIARRHYTHMIC ACTIVITY OF (1,3-DIMETHYL-5-NITRO-5-HEXAHYDROPYRIMIDINYL)-PROPIONIC ACID METHYL ESTER

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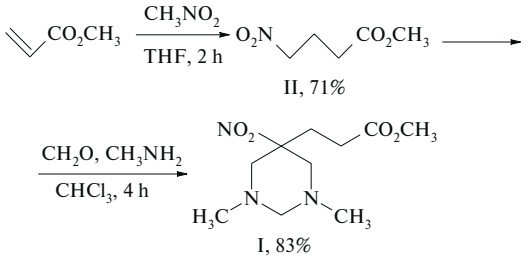
(1,3-Dimethyl-5-nitro-5-hexahydropyrimidinyl)propionic acid methyl ester (I) was obtained with a 83% yield using a Mannich type reaction of 4-nitrobutanoic acid methyl ester with excess formalin and methylamine. It was found that compound I possesses low toxicity and shows antiarrhythmic activity on models of arrhythmia induced by intravenous injections of calcium chloride and aconitine in rats.

Pyrimidine derivatives are known to exhibit high biological activity and are widely used in medicine as antimicrobial, antiviral, antitumor, and other purposes [1, 2]. Unfortunately, there are practically no data on the antiarrhythmic properties of these compounds, although some nitrous heterocycles with close chemical properties and structures — such as pyrazines, 3-aza-, and 3,7-diazabicyclo[3.3.1]nonanes — were reported to possess antiarrhythmic properties [1, 3, 4]. These data, as well as the fact that the nitro group is an important structural element in some drugs [1], allowed us to assume that nitrohexahydropyrimidines can also possess antiarrhythmic properties.

In this study, we have synthesized (1,3-dimethyl-5-nitro-5-hexahydropyrimidinyl)propionic acid methyl ester (I) and characterized it with respect to the antiarrhythmic activity. Compound I was obtained with a yield of 83% using the Mannich reaction of 4-nitrobutanoic acid methyl ester (II) with methylamine and formalin in a molar ratio of 1 : 5 : 10. The reaction proceeded in chloroform and took about 4 h at a temperature of 20°C. The initial ester II was synthesized via the Mannich reaction between methylacrylate and nitromethane in tetrahydrofuran (THF) in the presence of KF/Al_2O_3 [5].



The proposed structure of hexahydropyrimidine I was confirmed by the data of ^1H and ^{13}C NMR, IR spectroscopy, and elemental analyses. Indeed, the one-dimensional ^{13}C NMR spectrum measured in the regime of J-modulated CH parameters unambiguously identify carbon atoms in the functional groups $\underline{C}H_3N$, $CO_2\underline{C}H_3$, $\underline{C}O_2$, and $\underline{C}NO_2$ with the characteristic chemical shifts $\delta = 42.25$, 51.76, 171.99, and 86.48 ppm, respectively, and the characteristic signal from carbon in the $N\underline{C}H_2N$ moiety is manifested at 78.13 ppm. In the ^1H NMR spectrum (measured at 50°C), it is also possible to identify with sufficient precision the chemical shifts of axial and equatorial protons in the hexahydropyrimidine ring. In particular, the signals from equatorial protons at the carbon atoms in $2NC\underline{H}_2$ and $NC\underline{H}_2N$ groups are observed in higher fields ($\delta = 2.44$ and 2.79 ppm) than the signals from axial protons (3.20 and 3.26 ppm). The singlet signals at 2.29 and 3.66 ppm are due to protons of the methyl groups at nitrogen and the carboxy groups, respectively.

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EXERIMENTAL CHEMICAL PART

The ^1H and ^{13}C NMR spectra of the synthesized compounds were measured on a Bruker AM-300 spectrometer (Germany) operating at 300.13 MHz (^1H mode) and 75.47 MHz (^{13}C mode). The samples dissolved in $CDCl_3$ and the chemical shifts were determined using tetramethylsilane (Me_4Si) as the internal standard. The IR spectra were recorded with a Specord M-80 (Carl Zeiss Jena, Germany) spectrophotometer using samples prepared as thin films or Vaseline oil suspensions. The mass spectra were obtained using an MKh-1300 spectrometer (Russia) operating at an injector temperature of 100°C and an electron-impact ionization energy of 12 and 70 eV. The gas chromatography measurements were carried out on a Khrom-5 (Russia) chromatograph equipped with a plasma-ionization detector and a 1200 × 5 mm stainless-steel column filled with 5% SE-30 on Inerton N-AW DMCS (0.125 – 0.160). The carrier gas was helium. TLC analyses were performed on Silufol UV-254 plates (Czech Republic).

4-Nitrobutanoic acid methyl ester (II) [6]. To a solution of 11.9 g (138 mmole) of methylacrylate and 34.18 g (560 mmole) nitromethane in 450 ml of anhydrous THF was added 20 g of KF/Al_2O_3 (base) and the reaction mass was stirred for 2 h at 20°C. Upon cooling and filtration, the residue was washed with ethyl acetate (2 × 50 ml). The solvent was removed at a reduced pressure, and the residue was distilled in a 10-cm-long Vigreaux column to obtain 15.19 g (75%) of compound II; b.p., 90 – 92°C/2 Torr. The physicochemical properties of the product coincide with the published data [6].

1,3-Dimethyl-5-nitro-5-hexahydropyrimidinyl)propionic acid methyl ester (I). To a solution of 0.32 g (2.17 mmole) of 4-nitrobutanoic acid methyl ester (II) in 15 ml $CHCl_3$ at 0 – 10°C was added with stirring a mixture of 2.17 g (21.7 mmole) of a 30% aqueous formalin solution and 1.40 g (10.85 mmole) of 24% aqueous methylamine solution. The mixture was stirred for 4 h at room temperature, washed with water (3 × 5 ml), and dried over Na_2SO_4. Then, the solvent was distilled off at reduced pressure and the residue was chromatographed on a column filled with silica gel and eluted with petroleum ether – chloroform (9 : 1) mixture. Finally, the solvent was distilled off to obtain compound I in the form of white crystals; yield, 0.44 g (83%); $C_{10}H_{19}N_3O_4$; mass spectrum (*m/z*): 245 $[M]^+$; ^1H NMR spectrum in $CDCl_3$ at $T = 50$°C (δ, ppm; J, Hz): 2.20 – 2.36 (m, 4H, 2H($C\underline{H}_2C\underline{H}_2CO_2$)), 2.26 (s, 6H, $2C\underline{H}_3N$), 2.44 (d, 2H, J 12.2, He ($2C\underline{H}_2N$)), 2.79 (d, 1H, J 8.6, He ($NC\underline{H}_2N$)), 3.20 (d, 1H, J 8.6, Ha ($NC\underline{H}_2N$)), 3.26 (d, 2H, J 12.2, Ha ($2C\underline{H}_2N$)), 3.65 (s, 3H, $CO_2C\underline{H}_3$); ^{13}C NMR spectrum in $CDCl_3$ (δ, ppm): 27.71 (t, $\underline{C}H_2CH_2CO_2$), 31.29 (t, $\underline{C}H_2CO_2$), 42.25 (q, $2\underline{C}H_3N$), 51.75 (q, $CO_2\underline{C}H_3$), 58.95 (t, $2\underline{C}H_2N$), 78.13 (t, $N\underline{C}H_2N$), 86.48 (s, CNO_2), 171.99 (s, CO_2).

TABLE 1. Effect of Compound I on the Arrhythmia Induced by Aconitine in Rats ($n = 6$)

Treatment	Dose (i.v.), mg/kg	Percentage arrhythmia incidence	Arrhythmia duration, min
Control	—	100	120.6 ± 1.02 $p_2 < 0.001$
I	0.5	50	59.3 ± 3.40 $p_1 < 0.001$ $p_2 < 0.001$
	0.3	50	65.8 ± 3.48 $p_1 < 0.001$ $p_2 < 0.001$
	0.2	67	73.3 ± 14.11 $p_1 < 0.01$ $p_2 < 0.01$
	0.15	83	91.5 ± 8.67 $p_1 < 0.01$ $p_2 < 0.001$
Allapinine	0.15	33	26.5 ± 1.49 $p_1 < 0.001$
	0.05	50	28.3 ± 1.66 $p_1 < 0.001$
	0.04	83	36.9 ± 0.95 $p_1 < 0.001$

Notes: p_1 and p_2 are confidence intervals for the difference from control and reference (allapinine), respectively.

EXPERIMENTAL PHARMACOLOGICAL PART

The antiarrhythmic activity of the synthesized compound I was studied on urethane-narcotized mongrel rats weighing 160 – 200 g with model arrhythmia caused by intravenous injections of aconitine (50 μg/kg) or calcium chloride (250 mg/kg, 10% aqueous solution). Compound I and the reference drug allapinine were introduced into the tail vein by single injections 1 – 2 min before introduction of the arrhythmogenic agents, as recommended in [7]. The antiarrhythmic activity was evaluated in terms of the de-

TABLE 2. Effect of Compound I on the Arrhythmia Induced by Calcium Chloride in Rats ($n = 6$)

Treatment	Dose (i.v.), mg/kg	Percentage arrhythmia incidence	Percentage ventricular fibrillation incidence	Percentage loss of animals
Control	—	100	100	100
I	0.20	67	33	17
	0.15	83	50	33
	0.10	100	67	50
Allapinine	0.35	17	17	0
	0.30	50	17	0
	0.25	83	17	0

crease in the arrhythmia onset rate and duration and in the loss of animals.

As can be seen from the data presented in Tables 1 and 2, compound I produce an antiarrhythmic action on both models, although the effect is less pronounced as compared to that of the reference drug.

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