Synthesis and Antiarrhythmic Activity of New Lappaconitine Derivatives

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Abstract

Novel lappaconitine derivatives modified in the aromatic and heterocyclic fragments of the molecule were obtained. Antiarrhythmic activity of those derivatives has been investigated.

INTRODUCTION

The diterpene alkaloid lappaconitine **1** being a basic agent for an antiarrhythmic medicinal preparation (Allapinin) [1, 2] for the last decade has been a subject of detailed researches [3–10]. An important role in the increase of interest to the chemistry and pharmacology of the substance is played by a wide prevalence of different plant of *Aconitum* genus producing this metabolite. The resources of these aconites and the rate of their restoration over the territories of South Ural, West Siberia and Altai are so abundant that an environmentally sustainable lappaconitine production with respect to plant raw material could be organized in Russia and, hence, a release of medicinal preparations could be realized on this basis [11–14].

The disadvantages of lappaconitine as well as of the Allapinine pharmaceutical consist in their high toxicity and cumulating properties. In this connection, the investigation of synthetic transformations aimed at the search of novel lappaconitine derivatives those exhibit simultaneously both a higher antiarrhythmic activity and a lower toxicity, is of currently central value.

In this paper we describe the modification of alkaloid lappaconitine in aromatic and heterocyclic moieties of the molecule, as well as the antiarrhythmic activity of the new compounds.

RESULTS AND DISCUSSION

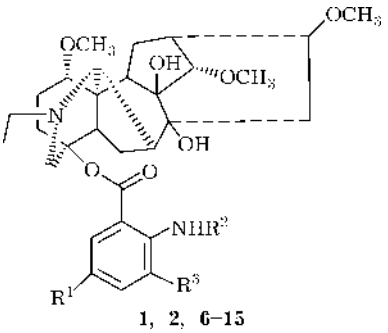
We have recently proposed a method for obtaining 5'-bromolappaconitine **2**, and demonstrated that 5'-bromolappaconitine hydrobromide **2** · HBr almost an order of magnitude surpasses lappconitine hydrobromide (**1** · HBr, Allapinine) with respect to antiarrhythmic activity in the model of calcium chloride and adrenaline arrhythmias [15]. A series of repetitive experiments presented in this communication has lent support to the validity of these results (Table 1). It is significant that high activity was exhibited by a salt **2** · HBr formed *via* 5'-bromo derivative **2** binding with hydrobromic acid, though the base **2** itself demonstrated no activity within the range of dozes from 0.033 to 3.5 mg/kg.

We have obtained a series of novel derivatives of 5'-bromide **2** with a changed structure of the heterocyclyc fragment. The oxidation with perbenzoic acid resulted in the formation of N-oxide **3** that can exhibit readily splitting out of ethylene molecule to produce hydroxylamine **4**. As the latter oxidized under the action of $K_3Fe(CN)_6$ nitrone **5** is formed. From Table 1 one can notice that only a base such as **4** at a doze of 3.5 mg/kg can exhibit antiarrhythmic activity comparable to a reference compound (**1** · HBr, lappaconitine hydrobromide). Thus, the removal of an alkyl substituent at the nitrogen atom in the heterocyclic fragment of compound **2** and even not so sig-

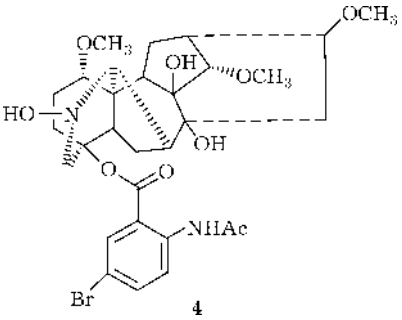
TABLE 1

Antiarrhythmic activity of lappaconitine derivatives. Arrhythmogens: $CaCl_2$ (250 mg/kg), adrenaline (0.3 mg/kg); intravenous injection

Agent	Doze, mg/kg	Percentage of surviving rats, %			
		Injection protocol			
		agent + arrhythmogen		arrhythmogen + agent	
		$CaCl_2$	Adrenaline	$CaCl_2$	Adrenaline
Lappaconitine hydrobromide (allapinine) **1** · HBr	0.290	50	50	0	0
5'-Bromolappaconitine **2**	3.5–0.035	0	0	0	0
5′-Bromolappaconitine hydrobromide **2** · HBr	0.035	100	100	100	100
5′-Bromolappaconitine-N-oxide **3**	3.5–0.35	0	0	0	0
N-Deethyl-5′-bromolappaconitine hydroxylamine **4**	3.5	50	0	0	0
Hydroxylamine hydrobromide **4** · HBr	3.5	0	0	0	0
N-Deethyl-5′-bromo-lappaconitine nitrone **5**	3.5–0.35	0	0	0	0
Deacetyl-5′-bromo-lappaconitine dihydrochloride **6** · 2HCl	0.035	50	0	50	0
Deacetyllappaconitine dihydrochloride **7** · 2HCl	0.035	50	0	50	0
Deacetyl-5′-iodolappaconitine dihydrochloride **8** · 2HCl	0.035	30	0	–	–
Deacetyl-5′-iodolappaconitine dihydrobromide **8** · 2HBr	3.5–0.00035	0	0	0	0
5′-Iodolappaconitine hydrobromide **9** · HBr	0.00035	80	0	–	–
3′-Nitrolappaconitine hydrobromide **10** · HBr	0.35	50	0	–	–
	0.035	30			
5′-Nitrolappaconitine hydrobromide **11** · HBr	3.5	0	0	0	0
3′-Nitro-5′-bromolappaconitine hydrobromide **12** · HBr	3.5	100	0	100	0
	0.175	100		100	
5′-[2-(6-Methyl-pyrid-3-yl)-1-(E)-etheno]lappaconitine **13**	0.035	50	0	50	0
5′-(3-Hydroxypropinyl)-lappaconitine **14**	3.5–0.00035	0	0	0	0
5′-(3-Methyl-3-hydroxybutine-1-yl) lappaconitine **15**	3.5–0.35	0	0	0	0



1, 2, 6–15



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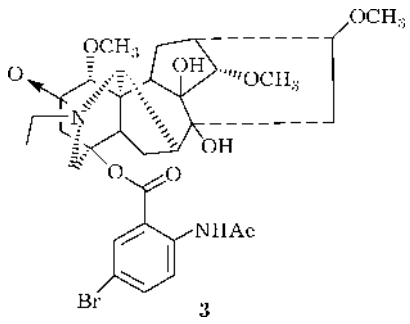

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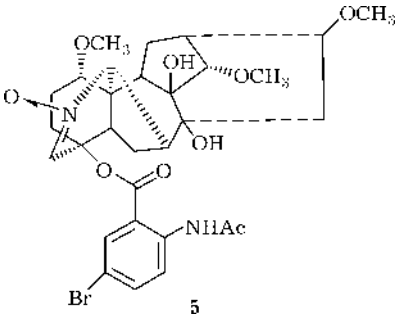

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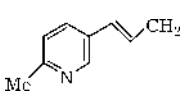
Compound	R^1	R^2	R^3
1	H	Ac	H
2	Br	Ac	H
6	Br	H	H
7	H	H	H
8	I	H	H
9	I	Ac	H
10	H	Ac	NO_2
11	NO_2	Ac	H
12	Br	Ac	NO_2
13		Ac	H
14	$-C{\equiv}CH_2OH$	Ac	H
15	$-C{\equiv}C(CH_3)_2OH$	Ac	H

nificant changes in the structure as N-oxidation could result in a sharp decrease (down to full disappearance) of antiarrythmic activity.

According to [16], *in vivo* a process of lappaconitine N-deacetylation occurs, which allows one to assume that N-deacetyl derivatives exhibit a pronounced antiarrhythmic activity. In our experiments, deacetyl derivatives dihydrochlorides such as **6** · 2HCl and **7** · 2HCl at a doze of 0.035 mg/kg in the model of calcium chloride arrhythmia exhibit the activity equal to that value for Allapinine at a doze of 0.290 mg/kg (ED_{50}).

We succeeded in the introduction of iodine atom into the lappaconitine molecule *via* iodine chloride action on N-deacetyllappaconitine **7**.

As a result, 5'-Iodo derivative **8** with the yield of 74 % was obtained. 5'-iodo-N-deacetyllappaconitine dihydrochloride **8** · 2HCl at a doze of 0.035 mg/kg in the model of calcium chloride arrhythmia blocked its development to an insignificant extent. Dihydrobromude **8** · 2HBr at a doze ranging from 0.00035 mg/kg to 3.5 mg/kg does not exhibit any activity. 5'-iodolappaconitine hydrobromide **9** · 2HBr obtained *via* acetylation of base **8** demonstrated rather high activity in the model of calcium chloride arrhythmia at a doze of 0.00035 mg/kg, having prevented the death of 80 % of animals.

In order to obtain nitro derivatives we investigated lappaconitine **1** nitration process under various conditions. It was established, that the action of KNO_3 in the solution of trifluoroacetic acid according to the method presented in [17] resulted in the formation of a mixture of 3'-nitro and 5'-nitrolappaconitine derivatives such as **10**, **11** with the yield amounting to 80 % in the ratio of 1 : 1.25, respectively. The isomers were separated using a chromatography technique. At dozes of 0.35 and 0.035 mg/kg, a weak activity with respect to calcium chloride arrhythmia was exhibited by 3'-nitrolappaconitine hydrobromide **10** · HBr, whereas hydrobromide of its 5'-isomer **11** · HBr

in the same model demonstrated the absence of any activity at all. The nitration of 5'-bromolappaconitine **2** under the conditions described allowed us to obtain 3'-nitro-5'-bromo derivative **12** whose hydrobromide **12** · HBr was revealed to exhibit a high activity at dozes of 3.5 and 0.175 mg/kg in the model of calcium chloride arrhythmia, resulting in a complete blocking the development of the latter.

Using the introduction of substituents with more complicated structure into the aromatic fragment resulted in the obtaining of compounds such as **13–15**. The Heck reaction [18] between 5'-iodolappaconitine and 2-methyl-5-vinylpyridine resulted in the formation of pyridylstyrene derivative **13** (with the yield of 86 %) that exhibited an activity at a doze of 0.035 mg/kg in the model of calcium chloride arrhythmia. A cross-coupling reaction of 5'-iodolappaconitine with 2-propine-1-ol allowed us to obtain compound **14** (with the yield of 72 %). Within the range of dozes amounting from 0.0003 to 3.5 mg/kg this lappaconitine derivative exhibited no expected antiarrhythmic activity. Acethylene derivative **15** obtained according to the above mentioned method also exhibited no antiarrhythmic activity at a doze of 0.3 mg/kg.

From the data obtained it may be concluded that the most active antiarrhythmic agents are presented by lappaconitine derivatives containing bromine atom in the aromatic fragment of a molecule (compounds **2** · HBr and **12** · HBr, see Table 1). In this connection it should be noted that 5'-bromolappaconitine possesses a rather attractive property such as lowered toxicity (LD_{50} for compound **2** · HBr amounting to 28.7 mg/kg, for initial 5'-bromolappaconitine **2** LD_{50} = 14.0 mg/kg, whereas the LD_{50} value for a reference pharmacopoeial drug substance such as lappaconitine hydrobromide (**1** · HBr, Allapinine) amounts to 6.0 mg/kg [19]).

EXPERIMENTAL

Chemistry

NMR spectra of the solutions of compounds in $CDC1_3$, CD_3OD or D_2O (for salts) were obtained using Bruker AV-300 [with operation frequencies of 300.13 (^{1}H) and 75.47 MHz (^{13}C)], AM-400 [with operation frequencies of 400.13 (^{1}H) and 100.78 MHz (^{13}C)] and Bruker DRX-500 [with operation frequencies of 500.13 (^{1}H) and 125.76 MHz (^{13}C)] NMR spectrometers. Infrared spectra were record on a Specord M-80 spectrometer (KBr); mass spectra were registered with the use of a Finnigan MAT 8200 mass spectrometer (electron impact ionisation energy amounting to 70 eV). UV absorption spectra were obtained on an HP 8453 UV-Vis spectrometer as solution in ethanol (concentration of 10^{-4} mol/L). The values of specific optical rotation were measured using a Polar 3005 polarimeter.

The conversion level with respect to source reagents was checked with the use of a TLC technique. A Merck silica gel (70–230 nm) was used in order to isolate compounds. Compounds **14**, **15** were isolated by means of a preparative TLC technique with unfixed $A1_2O_3$ layer.

The synthesis of 5′-bromolappaconitine **2** is described in [15, 20], the syntheses of deacetyl-5′- bromolappaconitine **6**, 5′-iodolappaconitine **9**, deacetyl-5′-iodolappaconitine **8** and 4β-{2-(acetylamino)-5-[2-(6-methylpyridine-3-yl)-(1E)-ethenyl]}benzoyloxy-1α,14α,16β-trimethoxy-20-ethylaconitane-8,9-diol **13** are presented in [20]. N-Deacetyllappaconitine **7** was obtained through acid hydrolysis of lappaconitine **1** using a technique described in [21].

5'-Bromolappaconitine hydrobromide 2 · HBr. To a solution of 1.11 g (1.67 mmol) of base **2** in 2.5 mL of CH_2C1_2 was added a solution of 0.295 g (1.70 mmol) of 46 % HBr in 5.0 mL of 95 % EtOH, dropwise under stirring. The solvent was distilled off in vacuum at 50 °C. A resinous residue obtained after grinding with about 5 mL of diethyl ether formed a crystalline colorless powder. The suspension in diethyl ether was kept during 15 h. A salt precipitate obtained was filtered, washed out with diethyl ether, and then it was dried in air. The yield amounted to 1.25 g (100 %) of 5'-bromolappaconitine hydrobromide; m.p. being at 208–210 °C (with decomp.). $[\alpha]_{578}^{20}$ +30 (c 5.6, H_2O). Found, %: Br (bromometric titration) 11.04. $C_{32}H_{43}BrN_2O_8 \cdot HBr$. Calculated, %: Br (ionic) 10.74. ^{1}H NMR spectrum (D_2O, 400.13 MHz, δ, ppm, *J*, Hz): 1.53 (t, 3H, C(22)Me, *J* = 7); 2.37 (s, 3 H, $NHCOC\underline{H}_3$); 3.51, 3.53 and 3.54 (all s, 3H, 1-, 16-, *и* 14-OMe); 7.74 (dd, 1H, H (4′), *J* = 9, *J*

= 2); 7.82 (d, 1H, H (3′), J = 9) and 8.03 (d 1H, H (6′), J = 2). IR spectrum (KBr), ν, cm^{-1}: 965, 1039, 1084, 1130, 1134, 1256, 1287, 1307, 1394, 1464, 1505, 1579, 1599, 1636, 1689 and 1702 (C=O), 2823, 2878, 2934 *и* 3015. UV spectrum (EtOH), λ_{max}, nm (log ε): 226 (4.33), 258 (4.06), 324 (3.52).

Following to the present technique, hydrobromides of the bases **4**, **8–12** were obtained.

5'-Bromolappaconitine-N-oxide, {4β-[2-acetylamino-5-bromobenzoyloxy]-1α,14α,16β-trimethoxy-20-ethylaconitane-8,9-diol-20-oxide} (3). To a solution of 2.65 g (4 mmol) of 5'-bromolappaconitine **2** in 25 mL of chloroform was rapidly added a solution of 2.07 g (12 mmol) of perbenzoic acids in 25 mL of chloroform. The mixture was stirred at 20 °C during 12 h, washed out with saturated $NaHCO_3$ solution (2 times × 40 mL); the organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was distilled off at the temperature below 40 °C, the reaction product was chromatographed on silica gel (chloroform: ethanol mixture in the ratio of 20 : 1, then 1 : 1 being used as eluents). The yield of N-oxide amounted to 32.12 g (80 %), m.p. being at 190–192 °C (with decomp.). ^{1}H NMR spectrum ($CDCl_3$; δ, ppm, J, Hz): 1.51 (т, 3H, C(22)H_3, J = 6.5), 2.16 (s, 3H, NHCOC$\underline{H}_3$); 3.24, 3.29, 3.36 (all s, for 3H OC(1)H_3, OC(14)H_3, OC(16)H_3); 7.55 (dd, 1H, J_1 = 9 and J_2 = 2); 7.89 (d, 1H, J = 2); 8.56 (d, 1H, J = 9); 10.78 (s, 1H, N$\underline{H}$COCH$_3$). ^{13}C NMR spectrum ($CDCl_3$; δ, ppm): 7.7 C(22), 24.0 C(6), 23.0 C(2), 25.3 (NHCO$\underline{C}$H$_3$), 26.9 C(12), 30.9 C(3), 35.5 C(13), 43.5 C(15), 47.0 C(5), 50.8 C(10), 51.5 C(11), 52.5 C(7), 55.9 (OC(16)H_3), 57.0 (OC(1)H_3), 57.8 (OC(14)H_3), 66.5 C(21), 73.9 C(19), 74.7 C(8), 77.0 C(17), 77.8 C(9), 81.7 C(16), 85.5 C(1), 85.8 C(4), 89.0 C(14), 114.4 C(5′), 116.2 C(1′), 121.9 C(3′), 132.8 C(6′), 137.4 C(4′), 140.7 C(2′), 165.9 ($\underline{C}$OO), 168.8 (NH$\underline{C}$OCH$_3$). IR spectrum (ν, cm^{-1}): 529, 670, 734, 790, 834, 899, 965, 1022, 1038, 1086, 1119, 1151, 1232, 1257, 1289, 1310, 1368, 1394, 1466, 1508, 1581, 1598, 1688, 2824, 2931, 3327, 3402. Found, %: C 55.25, H 6.23, N 3.60, Br 12.44. $C_{32}H_{43}BrN_2O_9$. Calculated, %: C 55.04, H 6.38, N 4.12, Br 11.76.

N-Deethyl-5'-bromolappaconitinehydroxylamine, {4-β-[2-acetylamino-5-bromobenzoyloxy]-1α,14α,16β-trimethoxyaconitane-8,9,20-triol} (4). A sample of 1.8 g (2.7 mmol) N-oxide **3** was heated during 3 h at the temperature of 95–100 °C under reduced pressure (5 Torr). The resinous residue was dissolved in chloroform; compound **4** was isolated by a column chromatography on SiO_2 (chloroform: ethanol in the ratio of 20 : 1, then 4 : 1 being used as eluents). The yield of hydroxylamine **4** amounted to 75 %, m.p. being at 184–186 °C (from acetone). ^{1}H NMR spectrum ($CDCl_3$; δ, ppm, J, Hz): 2.17 (s, 3H, NHCOC$\underline{H}_3$); 2.75 (dd, 1H, J_1 = 15 and J_2 = 8); 3.05 (d, 1H, J = 11); 3.26, 3.29, 3.37 (all s, for 3H OC(1)H_3, OC(14)H_3, OC(16)H_3); 3.41 (d, 1H, J = 5); 3.87 (d, 1H, J = 11); 7.55 (dd, 1H, J_1 = 9 and J_2 = 2); 7.93. (d, 1H, J = 2); 8.57 (d, 1H, J = 9); 10.91 (s, 1H, N$\underline{H}$COCH$_3$). ^{13}C NMR spectrum ($CDCl_3$; δ, ppm): 23.8 C(6), 25.2 (NHCO$\underline{C}$H$_3$), 25.8 C(2), 26.6 C(12), 30.7 C(3), 36.4 C(13), 44.1 C(15), 46.5 C(7), 46.9 C(5), 49.8 C(10), 50.3 C(11), 55.9 (OC(16)H_3), 56.3 (OC(1)H_3), 57.8 (OC(14)H_3), 59.9 C(19), 66.3 C(17), 75.2 C(8), 78.4 C(9), 82.6 C(16), 82.7 C(1), 85.6 C(4), 89.7 C(14), 114.5 C(5′), 116.8 C(1′), 121.8 C(3′), 133.0 C(6′), 137.1 C(4′), 140.8 C(2′), 165.9 ($\underline{C}$OO), 168.8 (NH$\underline{C}$OCH$_3$). IR spectrum (ν, cm^{-1}):529, 671, 734, 790, 834, 898, 965, 1038, 1086, 1120, 1151, 1232, 1257, 1289, 1311, 1368, 1394, 1467, 1509, 1581, 1598, 1689, 1707, 2823, 2933, 3339, 3402. Found, %: C 54.75, H 6.00, N 4.28, Br 11.85. $C_{30}H_{39}BrN_2O_9$. Calculated, %: C 55.30, H 6.05, N 4.29, Br 12.26. Hydroxylamine hydrobromide **4** · HBr, m. p. at 218–220 °C (with decomp.). Found, %: Br (bromometric titration) 11.55. $C_{30}H_{39}BrN_2O_9$ · HBr. Calculated, %: Br (ionic) 10.92.

N-Deethyl-5′-bromolappaconitine nitrone, {4β-[2-acetylamino-5-bromobenzoyloxy]-1α,14α,16β-trimethoxyaconit-19-ene-8,9-diol-20-oxide} (5). Hydroxylamine **4** (0.65 g, 1 mmol) was dissolved in 20 mL of chloroform and mixed with a solution of 10 mmol (3.29 g) of $K_3[Fe(CN)_6]$ and a solution of 10 mmol (0.84 g) Na_2CO_3 in 30 mL of water; then the mixture was vigorously stirred during 12 h. An organic layer was separated; an aqueous layer was extracted with chloroform. Organic phases were joined together and dried over $MgSO_4$, the solvent was distilled off. Nitrone **5** was isolated by means of a column chromatography technique on SiO_2 (chloroform with a gradient of ethanol being used as an eluent). The yield amounted

to 0.48 g (74 %), m.p. being at 204–206 °C. ^{1}H NMR spectrum ($CDCl_3$; δ, ppm, *J*, Hz): 2.17 (s, 3H, NHCOC<u>H</u>$_3$); 3.23, 3.27, 3.37 (all s, for 3H OC(1)H$_3$, OC(14)H$_3$, OC(16)H$_3$); 3.78 (d, 1H, *J* = 11); 7.26 (s, 1H); 7.55 (dd, 1H, J_1 = 9 and J_2 = 2); 7.92 (d, 1H, *J* = 2); 8.58 (d, 1H, *J* = 9); 10.78 (s, 1H, N<u>H</u>COCH$_3$). ^{13}C NMR spectrum ($CDCl_3$; δ, ppm): 21.5 C(2), 23.8 C(6), 25.2 (NHCO<u>C</u>H$_3$), 26.7 C(12), 29.1 C(3), 36.3 C(13), 41.2 C(5), 43.4 C(15), 48.8 C(7), 52.6 C(10), 52.8 C(11), 56.0 (OC(16)H$_3$, 56.6 (OC(1)H$_3$), 57.7 (OC(14)H$_3$), 74.2 C(8), 75.3 C(17), 76.2 C(9), 79.6 C(16), 81.6 C(1), 85.4 C(4), 89.0 C(14), 114.5 C(5′), 116.0 C(1′), 121.9 C(3′), 132.8 C(6′), 132.9 C(19), 137.4 C(4′), 140.7 C(2′), 165.8 (<u>C</u>OO), 168.8 (NH<u>C</u>OCH$_3$). IR spectrum (ν, cm^{-1}): 529, 669, 755, 789, 805, 832, 895, 973, 993, 1039, 1086, 1126, 1151, 1232, 1254, 1289, 1308, 1394, 1465, 1509, 1581, 1598, 1691, 2824, 2932, 3322, 3417, 3469. Found, %: C 53.30, H 5.81, N 4.28, Br 12.43. $C_{30}H_{37}BrN_2O_9$. Calculated, %: C 55.47, H 5.74, N 4.31, Br 12.38.

Dihydrochlorides such as **7** · 2HCl, **8** · 2HCl were obtained *via* the addition of a calculated amount of concentrated HCl to alcoholic solutions of deacetyl derivatives such as **7** and **8**, respectively, subsequent vacuum stripping, washings with ether and drying. For dihydrochloride **7** · 2HCl it was found, %: C 58.28, H 6.91, Cl 12.05, N 5.20. Calculated for $C_{30}H_{44}N_2Cl_2O_7$, %: C 58.53, H 7.15, Cl 11.54, N 4.55. For dihydrochloride **8** · 2HCl it was found, %: C 48.09, H 6.18, Cl 10.20, I 16.41, N 4.26. $C_{30}H_{43}Cl_2IN_2O_7$. Calculated, %: C 48.58, H 5.80, Cl 9.58, I 17.14, N 3.78.

4β-[2-Acetylamino-5-nitrobenzoyloxy]-1α,14α,16β-trimethoxy-20-ethylaconitane-8,9-diol (10) and 4β-[2-acetylamino-3-nitrobenzoyloxy]-1α,14α,16β-trimethoxy-20-ethylaconitane-8,9-diol (11). Lappaconitine (0.5 mmol, 292 mg) was dissolved in 3 mL of fluoroacetic acid. Then 1 mmol (101 mg) of KNO_3 was added, and the solution was stirred at room temperature during 85 h. The reaction mixture was alkalified with 25 % NH_4OH solution upon cooling by ice and extracted with chloroform. The extract was dried over $MgSO_4$; the solvent was distilled off by means of a rotary evaporator. The mixture of compounds **10** and **11** obtained was separated with the use of a column chromatography technique on SiO_2. For compound **10** diethyl ether was used as an eluent, whereas for the elution of compound **11** chloroform/ethanol mixture (9 : 1) was used. The yield of nitro derivative **10** amounted to 140 mg (46 %), m.p. being at 200–202 °C (with decomp.). ^{1}H NMR spectrum ($CDCl_3$; δ, ppm, *J*, Hz): 1.56 (t, 3H, C(22)H$_3$, *J* = 6.5) 2.26 (s, 3H, NHCOC<u>H</u>$_3$); 3.27, 3.29, 3.38 (all s, for 3H OC(1)H$_3$, OC(14)H$_3$, OC(16)H$_3$); 8.28–8.32 (dd, 1H, J_1 = 9.3 and J_2 = 2.6); 8.72. (d, 1H, *J* = 2.6); 8.87 (d, 1H, *J* = 9.3); 11.4 (s, 1H, N<u>H</u>COCH$_3$). ^{13}C NMR spectrum ($CDCl_3$; δ, ppm): 13.0 C(22), 23.6 C(6), 25.2 (NHCO<u>C</u>H$_3$), 25.6 C(2), 26.3 C(12), 31.3 C(3), 35.8 C(13), 44.4 C(15), 47.2 C(7), 47.4 C(5), 48.6 C(21), 50.5 C(10), 50.7 C(11), 55.0 C(19), 55.7 (OC(16)H$_3$), 56.0 (OC(1)H$_3$), 57.4 (OC(14)H$_3$), 61.0 C(17), 75.1 C(8), 78.0 C(9), 82.4 C(16), 83.5 C(1), 86.2 C(4), 89.6 C(14), 115.1 C(1′), 119.8 C(3′), 126.3 C(4′), 128.7 C(6′), 141.1 C(2′), 146.2 C(5′), 165.4 (<u>C</u>OO), 169.0 (NH<u>C</u>OCH$_3$). IR spectrum (ν, cm^{-1}): 457, 517, 552, 647, 676, 735, 749, 791, 851, 875, 891, 923, 944, 968, 1021, 1086, 1116, 1144, 1222, 1264, 1340, 1448, 1509, 1540, 1587, 1617, 1719, 2820, 2926, 3300, 3484. Found: 629.29482 $[M]^+$. $C_{32}H_{43}N_3O_{10}$. Calculated: *M* 629.29484. 5′-Nitrolappaconitine hydrobromide **10** · HBr, m.p. being at 225–227 °C (with decomp.). Found, %: Br (bromometric titration) 12.15. $C_{32}H_{43}BrN_3O^{10}$. Calculated, %: Br (ionic) 11.31.

The yield of 3′-nitrolappaconitine **11** amounted to 111 mg (35 %), m.p. being at 148–150 °C (with decomp.). ^{1}H NMR spectrum (CD_3OD; δ, ppm, *J*, Hz): 1.43 (t, 3H, C(22)H$_3$, *J* = 6.5), 1.76 (s, 3H, NHCOC<u>H</u>$_3$); 2.81, 2.83, 2.94 (all s, for 3H OC(1)H$_3$, OC(14)H$_3$, OC(16)H$_3$); 720 (t, 1H, J_1 = 7.8 and J_2 = 7.8); 8.01 (d, 1H, *J* = 7.8); 8.18 (d, 1H, *J* = 7.8). ^{13}C NMR spectrum ($CDCl_3$; δ, ppm): 13.0 C(22), 23.7 C(6), 23.7 (NHCO<u>C</u>H$_3$), 25.7 C(2), 26.3 C(12), 31.3 C(3), 35.8 C(13), 44.4 C(15), 47.1 C(7), 47.1 C(5), 47.2 C(21), 49.3 C(10), 50.6 C(11), 54.9 C(19), 56.1 (OC(16)H$_3$), 56.7 (OC(1)H$_3$), 57.5 (OC(14)H$_3$), 61.0 C(17), 75.1 C(8), 78.1 C(9), 82.4 C(16), 83.5 C(1′), 85.6 C(4), 89.6 C(14), 127.6 C(1′), 127.8 C(5′), 128.9 C(6′), 131.8 C(3′), 134.6 C(4′), 143.9 C(2′), 164.7 (<u>C</u>OO), 168.2 (NH<u>C</u>OCH$_3$). IR spectrum (ν, cm^{-1}): 524, 711, 749, 875, 890, 944, 968, 994, 1035, 1088, 1125, 1236, 1276, 1363, 1453, 1498, 1540, 1586, 1606, 1717, 2820, 2982, 3404, 3518. Analysis: Found: 598.27643

$[M\text{-}CH_3O]^+$. $C_{31}H_{40}N_3O_9$. Calculated: *M* 598.27645. Found, %: C 57.25, H 6.41, N 5.24. $C_{32}H_{43}N_3O_{10}$. Calculated, %: C 61.04, H 6.88, N 6.67. 3′–Nitrolappaconitine hydrobromide **11** · HBr, m. p. being at 223–225 °C (with decomp.). Found, %: Br (bromometric titration) 12.98. $C_{32}H_{43}N_3O_{10}$. Calculated, %: Br (ionic) 11.31.

4β-[2-Acetylamino-3-nitro-5-bromobenzoyloxy]-1α,14α,16β-trimethoxy-20-ethylaconitane-8,9-diol (12). To a solution of 331 mg (0.5 mmol) of 5′-bromolappaconitine in 3 mL of trifluoroacetic acid was added 101 mg (1 mmol) of KNO_3 and a mixture was then stirred at a room temperature during 85 h. The reaction mixture under ice cooling was alkalified with 25 % NH_4OH solution and then extracted with chloroform. After a usual treatment, compound 12 was isolated by means of a column chromatography on SiO_2 (chloroform being used as an eluent). The yield amounted to 270 mg (76 %), m.p. being at 234–236 °C (with decomp.). ^1H NMR spectrum ($CDCl_3$; δ, ppm, *J*, Hz): 1.43 (t, 3H, C(22)H_3, *J* = 6.5), 2.19 (s, 3H, NHCOC$\underline{H}_3$); 3.28, 3.29, 3.42 (all s, for 3H (OC(1)H_3), (OC(14)H_3), (OC(16)H_3); 8.12, 8.16 (both d, H(4′), H(6′), *J* = 1.8); 11.65 (s, 1H, N$\underline{H}$COCH$_3$). ^{13}C NMR spectrum ($CDCl_3$; δ, ppm): 13.4 C(22), 23.9 C(6), 24.1 (NHCO$\underline{C}$H$_3$), 26.2 C(2), 26.7 C(12), 31.8 C(3), 36.3 C(13), 44.8 C(15), 47.5 C(7), 48.2 C(5), 48.8 C(21), 49.7 C(10), 51.1 C(11), 55.2 C(19), 56.0 (OC(16)H_3), 56.4 (OC(1)H_3), 57.8 (OC(14)H_3), 61.3 C(17), 75.5 C(8), 78.4 C(9), 82.7 C(16), 83.8 C(1), 86.7 C(4), 90.0 C(14), 115.6 C(1′), 124.6 C(4′), 129.0 C(6′), 131.9 C(3′), 137.3 C(5′), 141.1 C(2′), 163.9 ($\underline{C}$OO), 169.3 (NH$\underline{C}$OCH$_3$). IR spectrum (ν, cm^{-1}): 534, 669, 739, 791, 855, 876, 892, 944, 967, 1035, 1088, 1146, 1236, 1269, 1344, 1469, 1543, 1585, 1617, 1698, 1721, 2821, 2928, 3451. Found, %: C 55.91, H 6.40, N 5.64, Br 10.94. Calculated for $C_{32}H_{42}BrN_3O_{10}$, %: C 54.24, H 5.97, N 5.93, Br 11.28. 3′-Nitro-5′-bromolappaconitine hydrobromide **12** · HBr, m.p. being at 226–228 °C (with decomp.). Found, %: Br (bromometric titration) 10.87. Calculated for $C_{32}H_{42}BrN_3O_{10}$, %: Br (ionic) 10.14.

4β-[2-(acetylamino)-5-(3-hydroxyprop-1-inyl)benzoyloxy]-1α,14α,16β-trimethoxy-20-ethylaconitane-8,9-diol (14). A reaction vessel was charged with 355 mg (0.500 mmol) of 5′-iodolappaconitine **9**, 2 mg (0.01 mmol, 2 mol. %) of CuI, 7 mg (0.01 mmol, 2 mol. %) of $Pd(PPh_3)_2Cl_2$ and 7 mg (0.03 mmol, 0.6 mol. %) PPh_3. Using a flow of argon (1 mL/min) above the mixture, air was displaced from the reactionary vessel. Then 2 mL of benzene were introduced through a backflow condenser. Upon stirring, were introduced successivelly 0.4 mL (290 mg, 2.9 mmol) of triethylamine and a solution of 0.1 mL (97 mg, 1.7 mmol) of 2-propine-1-ol in 2 mL of benzene. Further the mixture was heated in a flow of argon at 60–65 °C during 4 h, was cooled then down to 25 °C and the argon flow was cut off. Volatile components of the reaction mixture were removed under reduced pressure. A residue was dried at 60 °C (3 Torr). Upon stirring, 10 mL of chloroform and 5 mL of water were successively added. An organic layer was separated. An aqueous layer was extracted with chloroform (2 times × 10 mL). Organic extracts joined together were extracted with 10 % solution of H_2SO_4 (3 times × 7 mL). To an acid extract was added a 25 % solution of ammonia to gain pH 8, upon stirring. A precipitate formed was extracted three times with 15 mL of chloroform. The extract was dried over anhydrous $MgSO_4$; then it was concentrated to give volume of 3 mL and subjected to a preparative TLC on an unfixed layer of Al_2O_3 containing 1 mass % of a K-35 luminophore, the thickness of the adsorbent layer being 2 mm, the length of the starting band amounting to 60 cm, Et_2O being used as an eluent. A zone of the adsorbent was gathered that fluoresced with blue light under UV irradiation. The product was eluted from the adsorbent with the use of methanol. After removal of methanol, the residue was triturated with about 5 mL of diethyl ether. The yield of crystalline compound **14** amounted to 230 mg (72 %), m.p. being at 178–180 °C. Found, %: C 65.42, H 7.38, N 4.09. $C_{35}H_{46}N_2O_9$. Calculated, %: C 65.81, H 7.26, N 4.39. ^1H NMR spectrum ($CDCl_3$, 400.13 MHz; δ, ppm, *J*, Hz): 1.05 (t, 3 H, C(22)Me, *J* = 7); 1.48 (dd, 1 H, H_b(6), *J* = 15, *J* = 8); 1.76 (m, 1 H, H_b(3)); 2.15 (s, 3H, $COCH_3$); 2.93 (s, 1H, H(17)); 3.09 (dd, 1H, H(1), *J* = 10, *J* = 7); 3.21, 3.24 *и* 3.34 (all s, for 3H, 1-, 16-, and 14-OMe, respectively); 3.37 (d, lH, H(14), *J* = 5); 3.46 (d, lH, H_a(19), *J* = 11); 4.41 (s, 2H, C$\underline{H}_2$OH); 7.43 (dd, 1H, H (4′), *J* = 9, *J* = 2); 7.87 (d, 1H, H(6′), *J* = 2); 8.55

(d, 1H, H(3′), J = 9) and 11.20 (s, 1H, NH). ^{13}C NMR spectrum (100.61 MHz, δ, ppm): 83.8 (C1), 26.6 (C2), 31.6 (C3), 85.1 (C4), 48.1 (C5), 23.9 (C6), 47.5 (C7), 75.3 (C8), 78.4 (C9), 49.7 (C10), 50.8 (C11), 26.0 (C12), 36.1 (C13), 89.9 (C14), 44.3 (C15), 82.8 (C16), 61.3 (C17), 55.2 (C19), 48.7 (C21), 13.3 (C22), 56.2 (1-OCH_3), 57.7 (14-OCH_3), 55.9 (16-OCH_3), 115.5 (C1′), 141.2 (C2′), 119.9 (C3′), 137.1 (C4′), 116.6 (C5′), 134.0 (C6′), 166.5 (COO), 25.3 ($\underline{C}H_3CO$), 168.9 ($CH_3\underline{C}O$), 51.0 (C3″, 83.9 (C2″ and 87.4 (C1″)). IR spectrum (ν, cm^{-1}): 793, 945, 966, 993, 1035, 1088, 1116, 1146, 1232, 1293, 1323, 1382, 1448, 1510, 1587; 1685 and 1705 (C=O); 2230 (C≡C); 2816, 2928; 3317 and 3464 (NH и OH). UV spectrum (EtOH), λ_{max}, nm (log ε): 232 (4.35), 273 (4.20), 281 (4.20), and 326 (3.60).

4β-[2-(Acetylamino)-5-(3-methyl-3-hydroxybut-1-inyl)benzoyloxy]-1α,14α,16β-trimethoxy-20-ethylaconitane-8,9-diol (15) was obtained following the procedure described for compound **14**, from 711 mg (1 mmol) of 5′-iodolappaconitine **9**, 0.20 mL (168 mg, 2 mmol) of 2-methyl-3-butine-2-ol, 2 mg of CuI, 4 mg of $Pd(PPh_3)_2Cl_2$, 4 mg of PPh_3, 1.0 mL of triethylamine and 8.0 mL of benzene. The mixture was heated in a flow of argon at 60–65 °C during 4 h under control. A precipitate was filtered, washed out with of benzene (3 times × 2 mL), and dried. The yield of triethylamine hydroiodide amounted to 220 mg (96 %), m.p. being at 180–181 °C. The main solution was joined together with benzene washings, and then volatile components were removed from the mixture in vacuum. A residue was dried at 60 °C (3 Torr) and further it was treated in the same fashion as this was made in the case of the isolation of compound **14**. The yield of crystalline compound **15** amounted to 509 mg (76 %), m.p. being at 172–174 °C (from Et_2O). Found, %: C 66.80, H 7.30, N 4.14. Calculated for $C_{37}H_{50}N_2O_9$, %: C 66.64, H 7.56, N 4.20. ^{1}H NMR spectrum ($CDCl_3$, 400.13 MHz; δ, ppm, J, Hz): 1.10 (t, 3H, C(22)Me, J = 7); 1.54 (dd, 1H, H_b(6), J = 15, J = 8); 1.59 (s, 6H, $(CH_3)_2C$); 1.83 (m, 1H, H_b(3)); 2.18 (s, 3H, $COCH_3$); 2.69 (dd, 1H, H_a(6), J = 15, J = 7); 2.99 (s, 1H, H(17)); 3.17 (dd, 1H, H(1), J = 10, J = 7); 3.26, 3.28 and 3.38 (all s, for 3H, 1-, 16-, and 14-OMe, respectively); 3.41 (d, lH, H(14), J = 5); 3.53 (d, lH, H_a(19), J = 11); 7.48 (dd, 1H, H (4′), J = 9, J = 2); 7.88 (d, 1H, H(6′), J = 2); 8.60 (d, 1H, H(3′), J = 9) and 11.05 (s, 1H, NH). ^{13}C NMR spectrum (75.47 MHz, δ, ppm): 83.9 (C1), 26.6 (C2), 31.6 (C3), 85.1 (C4), 48.1 (C5), 24.0 (C6), 47.5 (C7), 75.4 (C8), 78.4 (C9), 49.7 (C10), 50.9 (C11), 26.1 (C12), 36.2 (C13), 89.9 (C14), 44.6 (C15), 82.7 (C16), 61.3 (C17), 55.4 (C19), 48.8 (C21), 13.3 (C22), 56.3 (1-OCH_3), 57.8 (14-OCH_3), 55.9 (16-OCH_3), 115.5 (C1′), 141.2 (C2′), 120.0 (C3′), 137.2 (C4′), 116.7 (C5′), 133.7 (C6′), 166.7 (COO), 25.4 ($\underline{C}H_3CO$), 168.9 ($CH_3\underline{C}O$), 80.8, and 93.8 (C1″, C2″), 65.3 (C3″), and 31.3 ($(\underline{C}H_3)_2C$). IR spectrum (KBr), ν, cm^{-1}: 792, 943, 966, 1021, 1147, 1255, 1294, 1321, 1368, 1452, 1511, 1586; 1683, and 1693 (C=O); 2233 (C≡C); 2819, 2929, 2975; 3400 (NH and OH). UV spectrum (EtOH), λ_{max}, nm (log ε): 232 (4.45), 273 (4.31), 281 (4.31), and 327 (3.68).

Biology

The experiments were performed with mature male rats 190–220 g in mass anaesthetized using sodium thiopental at a doze of 30 mg/kg injected intraperitoneally. (Animals were obtained from the Laboratory of Experimental Animals of the Institute of Cytology and Genetics, SB RAS (Novosibirsk); all the manipulations were carried out according to the rules and principles of humane handling of animals.) All the experiments were carried out at the same time of day between 9 a.m. and noon. For the studies, animals were grouped; each experimental group comprised ten animals.

The determination of antiarrhythmic activity of agents was carried out using an intravenous injection. The agents under investigation were preliminary dissolved in physiological salt solution with the addition of Twin 80; the injection form obtained was injected into a femoral vein of an animal through a catheter at doze values specified in Table 1. Arrhythmia was initiated by a single injection of either 10 % $CaCl_2$ solution into the same vein at a doze amounting to 250 mg/kg or 10 % adrenaline hydrochloride (AH) solution at a doze of 0.3 mg/kg. Such $CaCl_2$ and AH dozes were lethal ones in 100 % of cases [22]. We used two protocols in the experiments: the agents were injected either 1 min prior to the injection of an arrhythmogen or 10 s after the injection.

In the capacity of a basic reference drug substance applied in medical practice as an antiarrhythmic agent, Allapinine was used that was injected once intravenously at a doze of 0.29 mg/kg.

The electrocardiogram was registered using second standard (bipolar extremity) lead within 10 min. All parameters were recorded by means of a device from Coulbourn Instruments (the USA). The analysis of electrocardiograms was carried out with the help of LabVIEW 5.1 National Instruments software. Statistica 6.0 software was used for statistical treatment of the data. A mean error value was taken as average deviation; the results were processed using Student's t test.

Under the development of calcium chloride arrythmia in the case of a lethal termination there was an atrial flutter observed passing into comprehensive auricular conduction disorder as well as into a sinoatrial node block. Then atrioventricular bradycardia occurred with a subsequent development of vigorous ventricular tachycardia and cardiac arrest.

As adrenaline arrhythmia developed, there were bradycardia and insignificant auricular conduction disorder observed in the beginning indicated by the reduction of P wave amplitude. It was also established that there was a reduction of R wave amplitude observed which the reduction appeared in 1–2 min and disappeared in several minutes.

Further a recovery occurred, both of cardiac beat frequency and of R and P wave amplitudes. In the case of lethal termination, the ventricular premature beats arose against a background of a sharp bradycardia with an atrioventricular heart block of various degrees, which in 2–3 min passed into a polytopic extrasystole and ventricular tachycardia. The bradycardia was of reflex nature as a response to an abrupt increase in arterial pressure caused by adrenaline.

CONCLUSION

Thus, structural modification of lappaconitine consisting in the introduction of bromine atom into an aromatic fragment of the molecule could be rather promising from the viewpoint of the creation of pharmaceuticals with antiarrhythmic effect to be exhibited in much lower dozes as compared to the pharmacopoeial drug product such as Allapinine.

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