Chemistry of Natural Compounds, Vol. 47, No. 2, May, 2011 [*Russian original No. 2, March-April, 2011*]

CRYSTAL STRUCTURES OF ATISINE 15-ACETOXYAZOMETHINE AND 15-HYDROXYAZOMETHINE AND THEIR BIOLOGICAL ACTIVITIES

B. T. Salimov, B. Tashkhodzhaev,* M. Sh. Kodirova, F. M. Tursunkhodzhaeva, and F. N. Dzhakhangirov

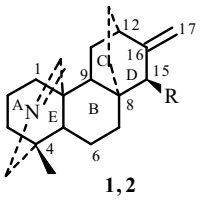
UDC 547.944/945+548.737

The 15-acetoxyazomethine derivative of atisine was prepared from atisine base. Its structure was confirmed by converting it to atisine 15-hydroxyazomethine and proved by an XSA. Atisine 15-acetoxyazomethine exhibited antiarrhythmic activity in rat cardiac arrhythmia models induced by $CaCl_2$ and aconitine. Atisine 15-acetoxyazomethine, in contrast with atisine 15-hydroxyazomethine, exhibited local anesthetic activity.

Keywords: atisine, atisine 15-acetoxyazomethine and 15-hydroxyazomethine, XSA, curare-like, antiarrhythmic and local anesthetic activity.

We showed previously that antiarrhythmic activity instead of curare-like activity appears upon converting atisine to dihydroatisine [1]. It seemed interesting to explain the change of pharmacological activity on going from atisine to atisine 15-acetyxoazomethine (**1**), which was prepared from atisine base using a simplified method based on modification of the literature procedures for preparing it from atisine chloride [2, 3]. The structure of **1** was confirmed by converting it to atisine 15-hydroxyazomethine (**2**) [4] and proved by an x-ray structure analysis (XSA).

The XSA molecular structures of **1** and **2** in approximately the same projection are shown in Fig. 1 (one of two molecules in the asymmetric unit is shown for **2**). The molecules of **1** and **2** contain a carbon skeleton with the least substitution and form a three-dimensional framework of five six-membered rings, four of which are cyclohexane A (C1-5, C10), B (C5-10), C (C8, C9, C11-14), and D (C8, C9, C11, C12, C15, C16), and heterocycle E (C4, C5, C10, C20, C19, N). The functional groups are acetyl in **1** and hydroxyl in **2** in the 15-position with the β-orientation.



1, 2
1: R = OAc
2: R = OH

Six-membered rings A and B in the crystal of **1** adopt the chair conformation; C and D, the boat conformation with slight deviations from the ideal shape. Heterocycle E adopts a conformation close to a half-chair with C5 deviating by 0.686 Å from the plane (determined to an accuracy of ±0.042 Å) of the other five heterocycle atoms because of the presence of the C20=N double bond [1.257(4) Å].

The XSA of **2** showed that the crystal asymmetric unit contained two molecules. As expected, the same canonical rings were found in them as in **1** (Fig. 1). The stability of the C framework can be noted by comparing the geometries of **1** and **2** with those observed for dihydro- [5] and tetrahydroatisine [6], i.e., the introduction of new functional groups in the carbon skeleton practically did not change the conformation of the carbon framework. However, the conformation of ring E changed from a chair (as observed in dihydroatisine [5] and tetrahydroatisine [6]) to a half-chair because of the C20=N double bond.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences, Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: tashkhodjaev@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 238–241, March–April, 2011. Original article submitted June 14, 2010.

TABLE 1. Toxicity and Antiarrhythmic Activity of Test Compounds in $CaCl_2$ and Aconitine Rat Arrhythmia Models

Compound	LD_{50}, i.v., mg/kg	$CaCl_2$, i.v., 250 mg/kg		Aconitine, i.v., 15 μg/kg	
		ED_{50}, mg/kg	AAI, LD_{50}/ED_{50}	ED_{50}, mg/kg	AAI, LD_{50}/ED_{50}
Atisine 15-acetoxyazomethine (**1**)	20.0	0.7	28.6	0.5	40.0
Atisine 15-hydroxyazomethine (**2**)	50.0	5.0	10.0	1–3 (–)*	–
Quinidine	66.9	16.0	4.2	15.4	4.3
Novocainamide	138.0	24.0	5.8	40.7	3.4

*(–), no effect.

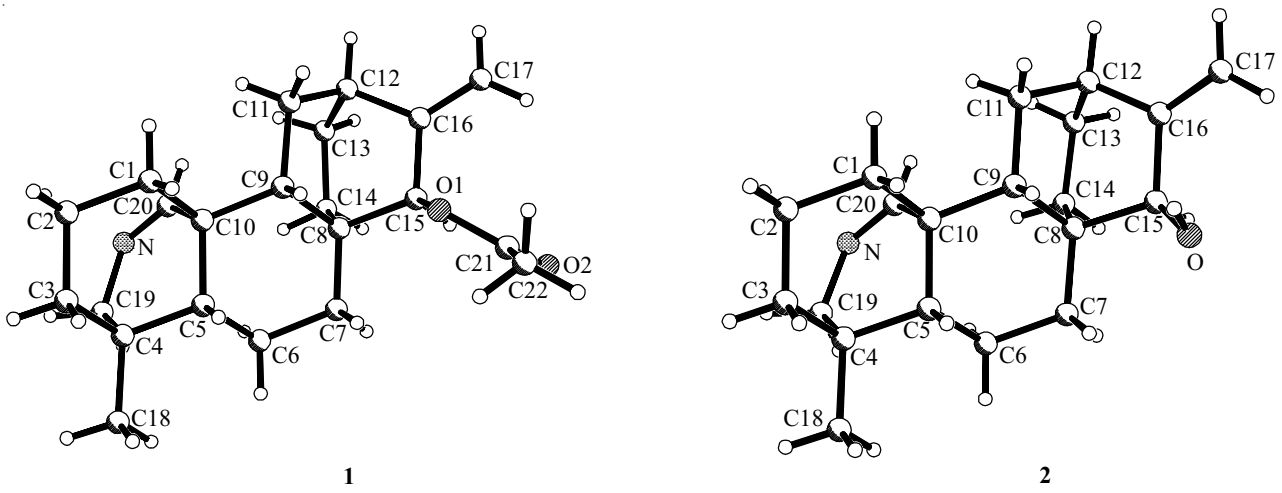


Fig. 1. Molecular structure and atomic numbering in **1** and **2**.

The alkaloid molecules in the crystal of **1** are situated at van-der-Waals distances. Anomalously short intermolecular contacts were not observed. Both molecules of the asymmetric unit in the crystal of **2** are involved in intermolecular O–H…N H-bonds between identical molecules. H-bonds were formed between the C15 hydroxyl of the initial molecule and the N atom of the translated molecule by one unit along the diagonal of the *a* and *b* axes. The parameters of these H-bonds are O…N 2.828 Å, H…N 2.07 Å, O–H…N angle 154° and O′…N′ 2.869, H…N 2.11, O′–H …N′ 153°.

Pharmacological Studies. Tests in mice with i.v. administration of **1** at doses of 10–30 mg/kg caused motor excitation, tactile and audigenic hyperreflexia, tremors, and clonic-tonic contractions. Animals died from toxic doses beginning within the first 60 min after administration with cardiovascular and respiratory insufficiency symptoms. The LD_{50} was 20.0 mg/kg. The toxicity profile of **2** differed from that of **1**. Intravenous administration to mice of **2** at doses of 25–80 mg/kg caused respiratory suppression, skeletal muscle weakening, and clonic contractions. The LD_{50} for **2** was 50.0 mg/kg. The acute toxicity for i.v. administration to mice of **2** was 2.5 times less than that of **1** and differed in the nature of the resorptive activity.

The R–R interval in the ECG increased by 20–30% whereas the duration of the PQ, QRS, and QT intervals did not change reliably in tests on anesthetized rats with i.v. administration of **2** at doses of 5–10 mg/kg.

Both compounds exhibited pronounced antiarrhythmic and antitoxic activity that exceeded those of quinidine and novocainamide in the rat heart arrhythmia model caused by $CaCl_2$ (Table 1).

Compound **1** was seven times more active and had an antiarrhythmia index (AAI) 2.9 times greater than that of **2**. In contrast with **2**, **1** showed high antiarrhythmic activity in tests of the rat heart arrhythmia model caused by aconitine. The antiarrthythmic activity and AAI of **1** were greater than those of quinidine by 30.8 and 9.3 times, respectively; of novocainamide, by 81.4 and 11.9 times, respectively.

Compound **1**, in contrast with **2**, exhibited pronounced local anesthetic activity in tests on conscious rabbits. Administration of solutions of **1** (0.2 mL, 0.25 and 0.5%) to the eye conjunctival sac of rabbits caused local anesthesia of the cornea that lasted 36 and 184 min, respectively. Compound **2** at these same concentrations did not exhibit local anesthetic activity.

TABLE 2. Principal Crystallographic Parameters and Characteristics of the X-ray Structure Analysis

Parameters	**1**	**2**	Parameters	**1**	**2**
Molecular formula	$C_{22}H_{31}NO_2$	$C_{20}H_{29}NO$	ρ, g/cm^3	1.190	1.197
MW, g/mol	341.48	299.44	Crystal size, mm	0.80 × 0.50 × 0.35	0.30 × 0.20 × 0.04
Space group	P 2_1	P 2_1	Scan range, 2θ°	3.88 ≤ θ ≤ 70.90	4.85 ≤ θ ≤ 59.58
Z	2	4	μ_{exp}, cm^{-1}	0.583	0.552
a, Å	11.3674 (8)	7.131 (4)	Number of reflections	2420	2494
b, Å	7.3623 (5)	36.48 (2)	Number of refl. with $I > 2\sigma$ (I)	1705	2240
c, Å	11.5953 (8)	7.197 (4)	R_1 ($I > 2\sigma$ (I) and total)	0.0457 (0.0645)	0.0420 (0.0510)
α, °	90	90	wR_2	0.1130 (0.1225)	0.0960 (0.1041)
β, °	100.884 (6)	117.49 (4)	COOF	0.932	1.140
γ, °	90	90	ED difference peaks, *e* Å^{-3}	0.14 and –0.12	0.13 and –0.14
V, Å^3	953.0 (1)	1661 (2)			

Thus, introduction of an acetoxy substituent at the C-15 position (C_{15}-OAc) of atisine 15-acetoxyazomethine increased the toxicity, redirected the pharmacological properties, and caused the appearance in the compound of pronounced anti-arrhythmic and local anesthetic activity.

EXPERIMENTAL

The purity of the compounds was checked on Al_2O_3 chromatography plates using benzene:EtOH (9:1) and Et_2O:hexane (1:1 and 3:1). Deactivated Al_2O_3 of the same grade was used for column chromatography.

Conversion of Atisine Base to Atisine 15-Acetoxyazomethine (1). A mixture of atisine, which was obtained from atisine chloride (10.0 g), acetic anhydride (20 mL), and Py (10 mL) was left at room temperature for 4 d. The mixture was condensed in vacuo. Traces of Py were removed by adding EtOH with subsequent evaporation of the resulting solution. The residue was dissolved in $CHCl_3$ (150 mL) and refluxed for 45 min. The solvent was distilled off. The residue was chromatographed over a column of Al_2O_3 (130 g) with elution by hexane, hexane:Et_2O (1:1), and $CHCl_3$:MeOH (10:1). The hexane:Et_2O eluates afforded **1** (1.2 g), mp 153–154.5°C (hexane).

The residue obtained from the $CHCl_3$:MeOH eluates was treated with KOH solution (40%) and $CHCl_3$ (100 mL). The $CHCl_3$ solution was dried over Na_2SO_4 and refluxed for 45 min. The solvent was distilled off. The residue was chromatographed over a column of Al_2O_3 (130 g). Compounds were eluted by hexane, hexane:Et_2O (1:1), and $CHCl_3$:MeOH (10:1). The hexane:Et_2O eluates afforded **1** (2.5 g).

Conversion of 1 to 2. A solution of **1** (0.89 g) in methanolic KOH (5%, 20 mL) was refluxed for 1 h. The usual work up afforded **2** (0.78 g).

X-ray Structure Analysis (XSA). Crystals of **1** were grown from hexane solution. The unit-cell constants were determined and refined on an Xcalibur Ruby diffractometer (Oxford Diffraction) (293 K, graphite monochromator). Table 2 lists the principal parameters of the XSA and calculations for **1**. A three-dimensional dataset of reflections for **1** was obtained on the same diffractometer using CuK_α-radiation. Absorption corrections were made using the SADABS method [7].

Crystals of **2** (mp 180–182°C) were grown from EtOH. Unit-cell constants of crystals of **2** were determined and refined on a Stoe Stadi-4 diffractometer (293 K, graphite monochromator). A three-dimensional dataset of reflections was obtained on the same diffractometer using ω/2θ-scanning and CuK_α-radiation. Absorption corrections were not applied. Table 2 lists the principal parameters of the XSA and the calculations for the crystal of **2**.

The structures were solved by direct methods using the SHELXTL PLUS 5.0 program set. All nonhydrogen atoms were refined by full-matrix anisotropic least-squares methods (over F^2). Positions of H atoms were found geometrically by a rider method and refined with fixed isotropic thermal parameters $U_{iso} = nU_{eq}$, where n = 1.5 for methyls and 1.2 for others and U_{eq} is the equivalent isotropic thermal parameter of the corresponding C, N, or O atoms. The hydroxyl H atom in **2** was found experimentally from a difference electron-density (ED) synthesis and refined isotropically while bound to the corresponding O atom.

Results of the XSA were deposited as CIF files in the Cambridge Crystallographic Data Centre (CCDC).

Pharmacological Tests. The biological activity of **1** and **2** was studied in tests on white mongrel mice (22–24 g) and white rats (240–260 g). The nature of the resorptive activity and acute toxicity was determined in conscious mice. The drug was injected into a tail vein. The effect of i.v. administration of the drugs on cardiac bioelectric activity was studied in tests on rats. ECGs were recorded in II standard leads using a SUPRODIL cardiograph.

Antiarrhythmic activity of the drugs was studied in heart arrhythmia models induced by $CaCl_2$ (250 mg/kg) [8] and aconitine (15 μg/kg) [9]. Control tests with i.v. injection of $CaCl_2$ at this dose caused in 100% of cases development of irreversible cardiac fibrillation and death of animals within the first 55–90 sec. Control tests with aconitine arrhythmia models showed allorhythmic and extrasystolic arrhythmia and disrupted conductivity for greater than 60 min. The antiarrhythmic activity of the drugs was assessed from their ability to prevent the arrthymogenic action of $CaCl_2$ and aconitine. The drug at each dose was tests in 5–6 animals.

The toxicity and antiarrhythmic activity of **1** and **2** were compared to each other and to quinidine and novocainamide. The mean lethal dose (LD_{50}) was determined by the Litchfield–Wilcoxon method [10]; mean effective anti-arrhythmic dose, graphically. The spectrum of antiarrhythmic activity was assessed from the LD_{50} to ED_{50} ratio, which was termed the anti-arrhythmic index (AAI).

Local anesthetic activity of **1** and **2** was studied using a rabbit eye cornea surface anesthesia model by determining the cornea reflex after injection into the conjunctival sac of solutions (0.2 mL; 0.1, 0.25, and 0.5%).

REFERENCES

1. B. T. Salimov, Zh. Kh. Kuzibaeva, and F. N. Dzhakhangirov, *Khim. Prir. Soedin.*, 384 (1996).
2. D. Dvornik and O. E. Edwards, *Can. J. Chem.*, **35**, 860 (1957).
3. S. W. Pelletier and P. C. Parthasarathy, *J. Am. Chem. Soc.*, **87**, 777 (1965).
4. B. T. Salimov, *Khim. Prir. Soedin.*, 84 (1993).
5. S. W. Pelletier, W. H. De Camp, and N. V. Mody, *J. Am. Chem. Soc.*, **100**, 7976 (1978).
6. S. W. Pelletier, N. V. Mody, H. K. Desai, J. Finer-Moore, J. Nowacki, and B. S. Joshi, *J. Org. Chem.*, **48**, 1787 (1983).
7. G. M. Sheldrick, *Program for Empirical Absorption Correction of Area Detector Data*, University of Goettingen, Goettingen, 1996.
8. M. R. Malinow, F. F. Batlle, and B. Malamud, *Am. J. Physiol.*, **175**, 8 (1953).
9. F. N. Dzhakhangirov, M. N. Sultankhodzhaev, B. Tashkhodzhaev, and B. T. Salimov, *Khim. Prir. Soedin.*, 257 (1997).
10. M. L. Belen′kii, *Elements of Quantitative Assessment of the Pharmacological Effect* [in Russian], Medgiz, Leningrad, 1963.