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# ARENOBUFAGIN AND GAMABUFOTALIN FROM THE VENOM OF THE CENTRAL ASIAN TOAD *Bufo viridis*

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From ancient times, toad venom has been widely used in folk medicine. The main property of the whole venom is the stimulation of the respiration and of cardiac activity [1]. The toxicity of toad venom is low in comparison with the venoms of other amphibia; nevertheless, cases of fatal poisonings of men and animals by this venom have been described in the literature. Among the physiologically active component of the venom, the main role with respect to toxicity is played by the cardiotonic steroids [2, 3].

The isolation of the physiologically active components of the venom of *Bufo viridis* and the study of their structures and biological activities are of considerable interest. This venom has scarcely been studied, as compared with the venoms of toads living in other regions [1, 2].

By a combinationof the methods of gel-permeation chromatography on a column of Sephadex LH-20 (column 2. 6 × 100 cm, buffer 0.05 M $NH_4HCO_3$, pH 8.2, containing 60% of ethanol, rate of elution 45 ml/h), hydrophobic chromatography on a column with the support Polikhrom P-1 (column 1 × 10 cm, stepwise concentration gradient of ethanol: 10, 20, 30, 40, 50, and 60%), and reversed-phase HPLC on an Octadecyl Si-100 column (column 0.46 × 25 cm, buffer A: 0.1% TFAA, pH 2.0; buffer B: acetonitrile, rate of flow 1 ml/min), we have isolated two individual compounds toxic for insects with yields of 0.01 and 0.02% on the initial dry mass of the whole venom.

The paralytic activity of the toxins was determined on cockroaches *Periplaneta american* weighing about 500 mg. The injection of the first toxin in a dose of 5 μg/individual caused paralysis (for 2-3 min), followed by death. The injection of the second toxin in a dose of 5 μg/individual led to persistent paralysis which remained for a considerable time.

Preliminary results of NMR spectroscopy showed that both toxins had structures of steroid nature with a bufadienolide skeleton. Satisfactory single crystals for x-ray structural analysis were obtaied from ethanolic solution. The first compound was arenobufagin, and the second was gamabufotalin. They were identical with the compounds isolated from the venom of the North American toad *Bufo arenarum* [4, 5], but differed from the named bufadienolides by some crystallographic parameters. The crystallographic parameters were determined on a Syntex $P2_1$ x-ray diffractometer: a = 12.539 (6) Å, b = 10.579 (4) Å, c = 15.792 (5) Å, v = 2095.0 (1.5)$A^3$, space group $P2_12_12_1$; (II): a = 10.166 (3) Å, b = 13.360 (5) Å, c = 17.523 (19) Å, V = 2390 (2) $A^3$, space group $P2_12_12_1$.

Thus, the x-ray structural analysis showed that compound (I) was a polymorphic modification of arenobufagin (3β,11α,14-trihydroxy-12-oxo-5β,14β-bufa-20,22-dienolide) and compound (II) was an adduct of gamabufatalin (3β,11α,14-trihydroxy-5β,14β-bufa-20,22-dienolide) with ethanol [6]. Detailed results on isolation and structure determination will be published separately.

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