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BIOTRANSFORMATION OF BENZOYLHETERATISINE IN THE ANIMAL ORGANISM AND BIOLOGICAL ACTIVITY OF ITS MAIN METABOLITE

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The metabolism of the alkaloid benzoylheteratisine in the animal organism has been studied and it has been shown that its main metabolic product is heteratisine. In experiments on various models of arrhythmia the antiarrhythmic activity of heteratisine proved to be lower than that of benzoylheteratisine.

In IKhRV AN RUz [Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan] a targeted search for drugs with a cardiovascular action among various classes of substances of plant origin has been carried on for many years. Earlier investigations have shown the high antiarrhythmic activity of a diterpene alkaloid, benzoylheteratisine (**1**), isolated from *Aconitum zeravschanicum* [1].

Many antiarrhythmic agents are subjected in the organism to a metabolism the study of which is expanding our ideas on the mechanism of their action, is creating certain prerequisites for developing rational schemes, and can be used for the directed synthesis of new drugs.

An investigation of the total metabolites isolated from rat urine has been carried out with the aid of electron-impact (EI) mass spectrometry and of secondary-ion mass spectrometry using liquid matrices (LSIMS) [2]. Multipeak monitoring [3, 4] and measurement of the elementary composition of key ions have been used for the qualitative characterization of the total metabolites in the EI regime. Mass-spectrometric analysis of the total metabolites isolated from the urine of rats that had received (**1**) in doses of 5 and 10 mg/kg showed that, regardless of the dose of drug administered, the main metabolite was heteratisine (**2**), while a small fraction of the (**1**) was recovered in unchanged form. This followed from the EI spectrum, which contained the peaks of ions with *m/z* 495, 480, and 464 (relating to (**1**)) and 391, 376, 374, and 360 (relating to (**2**)). This conclusion was confirmed by the LSIMS spectra of samples isolated from the urine of rats that had received (**1**) in doses of 5 and 10 mg/kg, which contained peaks of ions with *m/z* 496 $(M + H)^+$ and 484 $(M - H)^+$, belonging to (**1**), and with *m/z* 395 $(M + H)^+$ and 374 $(M - OH)^+$, characterizing (**2**). In addition to those mentioned above, the EI spectra of the total metabolites had weak peaks of ions with *m/z* 527 ($C_{29}H_{37}NO_8$) and 510 ($(M - OH)^+$)($C_{29}H_{36}NO_7$). The absence of the peaks of these ions in the spectrum of the total urine of control animals permits us to assign them to products of the metabolism of (**1**), the peak with *m/z* 527 being that of the molecular ion. This conclusion was confirmed by the LSIMS spectrum of the total metabolites isolated from the urine of rats that had received (**1**) in doses of 5 and 10 mg/kg: this contained the protonated ion $(M + H)^+$, *m/z* 528, which, on the addition of NaCl to the liquid matrix, shifted to *m/z* 550 $(M + Na)^+$. The composition and features of the fragmentation of this metabolite show that demethylation of the methoxyl at C-1 had taken place and so had an enlargement of the (**1**) molecule by CH_2O_2.

In view of the fact that the main metabolite is (**2**), a comparative investigation of the biological activities of (**1**) and (**2**) was of interest. The pharmacological properties of heteratisine [5] have been investigated in acute and chronic experiments on various species of laboratory animals using a large set of tests permitting the evaluation of its toxicity, specific antiarrhythmic activity, and influence on various organs and systems. We have compared the pharmacological effects of (**2**) and (**1**). As can be seen from the results given in Table 1, the main metabolite (**2**) is from 20 to 35.3 times less toxic than (**1**).

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TABLE 1. Comparative Biological Activities of Benzoylheteratisine and Heteratisine

Test	Effective doses, mg/kg		Ratio of the indices
	1	2	
Acute toxicity (LD_{50} in mice:			
intravenously	5.1	180	35.3
intraperitoneally	21.5	430	20.0
Antiarrhythmic activity:			
aconitine arrhythmia in rats	0.035	12.5	375.1
prevention (i/p) of fatal cardiac fibrillation caused by aconitine in mice	0.25	64	256
Arrhythmia caused by an electric discharge of the chambers of the heart in cats	0.1–0.2	15–20	150–200
Ventricular tachyarrhythmia in dogs after occlusion of the coronary artery	0.3	30	100
Arrhythmia caused by $BaCl_2$ in rats	0.025	–	
Cardiac fibrillation caused by $CaCl_2$ in rats	–	5	
Anesthesia of the rabbit cornea (concentration of the solution, %)	0.05	2	(no effect)

In experiments on various forms of arrhythmia, (**2**) possessed a pronounced antiarrhythmic action but its activity was from 150 to 357 times less than that of (**1**). In contrast to the latter, (**2**) exhibited a high antifibrillatory activity in arrhythmia caused by $CaCl_2$ and was ineffective in ventricular flutter induced by $BaCl_2$. Moreover, a solution of (**2**) with a concentration of 0.1-2% possessed no anesthetic action, on injection into a vein (10-20 mg/kg) caused a fall in the systemic arterial pressure, exhibited a ganglion-blocking action, decreased the frequency and increased the strength of cardiac contractions, and lengthened the Q–T interval.

Thus, benzoylheteratisine undergoes active metabolism in the organism.

Its main metabolite is heteratisine, which possesses a less pronounced antiarrhythmic activity. The physiological effects and therapeutic action observed on the administration of benzoylheteratisine must be considered as the summation of the action of the alkaloid benzoylheteratisine itself and that of its metabolites.

EXPERIMENTAL

In the experiments we used male rats weighing 180-220 g placed in special urine-collecting compartments.

The benzoylheteratisine was administered by a single intraperitoneal injection in a dose of 5 or 10 mg/kg, a group of 10 rats receiving each dose. A control group was given distilled water. The total metabolites were studied on a MKh 1310 mass spectrometer with double focusing having a SVP5 system for direct injection, at an accelerating potential of 5 kV.

The following regimes were used:

1) electron impact: EI source, temperature of the ionization chamber 150-170°C, temperature of the evaporator bulb 80-120°C, ionizing potential 70 V, collector current 60 μA;

2) secondary-ion mass spectrometry (LSIMS): LSIMS ion source, ionization by a beam of accelerated Cs^+ ions with an energy of 7 keV. The samples were dispersed in glycerol and deposited on the steel support of the direct sample injection system.

We investigated five samples on the MKh 1310: heteratisine, benzoylheteratisine, two sets of total metabolites after the administration of (**1**) in doses of 5 and 10 mg/kg, and the total extractive substances from the urine of the control animals.

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