STUDY OF STRUCTURE OF CHEMICAL COMPOUNDS, ANALYTICAL METHODS, AND PRODUCTION CONTROL

MASS-SPECTROMETRIC STUDY OF DIBASOLE METABOLITES

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The knowledge of the qualitative and quantitative composition of metabolites of medicinal compounds is necessary for studying their action and inactivation mechanism, directed search for new effective drugs and the solution of many practical problems of toxicology and forensic medicine [1-4]. In the present work, we studied the biotransformation products of dibasole (I) formed in blood, by mass spectrometry and chromatograph-mass spectrometry, by the action of electron bombardment (EB) under conditions of deutero-exchange (DE) and field desorption (FD). These methods made it possible to determine the number and quantitative ratio between metabolites of (I), to establish their chemical structure, using the dissociative ionization patterns of these compounds, and from a high-resolution mass-spectrum to obtain information on the total metabolites of I.

EXPERIMENTAL

The total metabolites of (I) were determined by the following procedure. An aqueous solution of the preparation containing 5 mg of the compound was added to 10 ml of blood. The mixture was left to stand for 24 h in a thermostat at 36.6°C. The pH was then adjusted to 2.5 by 10% sulfuric acid; the mixture was left to stand for 2 h, and then centrifuged for 30 min at 3000 rpm. The supernatant liquid was placed in a glass column (41 x 2.5 cm) filled with Sephadex G-25 gel. The column was eluted with 0.024 N sulfuric acid. The first 200 ml were discarded, and the following 550 ml were collected. The solution was extracted with benzene (3 x 50 ml) at pH 2.5-3.0 and 10.0, and with chloroform at pH 2.5-3.0 and 6.5-7.0. The organic solvents were combined after the separation of phases, and distilled. The dry residue was studied by mass spectrometry and chromatograph-mass spectrometry methods. As control, we studied the residue obtained from blood containing a conservant without the addition of the preparation, and also the dry residue from a physiological solution of (I) after thermostating. In the control experiments, compounds identified as metabolites of (I) were not detected.

The EB mass spectra and the EB mass spectra under DE conditions of vapors of total metabolites of (I) with vapors of D-O-methanol were obtained directly in the ionization chamber of the apparatus on the LKB-9000 apparatus (Sweden). The temperature of the measurement was 30°C, and the ionization voltage 70 V. The chromato-mass spectra of the metabolites were measured on the "Finnigan 3200F" mass spectrometer (U.S.A.). Ionization voltage 70 V, programmed temperature range 180-290°C (increase in temperature at rate of 6°C per min), temperature of injector 280°C, 25 m x 0.3 mm capillary column, SE-phase, gas carrier — helium, and feeding rate 1.3 ml/min.

To study the metastable transitions in the dissociative ionization of the metabolites of (I), their chromato-mass spectra were also obtained on the LKB-9000 mass spectrometer (Sweden). Ionization voltage 70 V, accelerating voltage 3.5 kV, cathode emission current 60 μA, programmed temperature range 100-200°C (increase in temperature at rate of 4°C per min), 1.1 m x 4 mm column, SE-30 phase (3%), gas carrier — helium, and feeding rate 30 ml/min.

The FD spectra of total metabolites of (I) were measured on the "Varian MATCH5" mass spectrometer (U.S.A.) at the emitter current of 5 mA, accelerating voltage of 3 kV (6 kV on emitter and 3 kV on cathode).

The high-resolution mass spectrum of the metabolites was obtained on the JMS-01-SG-2 apparatus (Japan) with an automatic system of information processing.

To determine the structure of the metabolites of (I) by chromato-mass spectra, we measured first the EB mass spectrum, EB mass spectrum under DE conditions and the FD mass spectrum of the pure preparation (I) under identical conditions and on the same instruments, and studied its dissociative ionization.

**RESULTS AND DISCUSSION**

The analysis of the EB mass spectra and EB mass spectra under DE conditions of (I) (Fig. 1, A-I, B-I) showed that the formation of the principal ions by the dissociative ionization of this compound can be represented by Scheme 1. A shift in molecular ion peak by 1 amu under DE conditions is caused by the presence of a labile hydrogen atom in the molecule of (I) at the nitrogen atom. The main direction of the dissociation of M+ ions of (I) is the elimination of H radicals. Analysis of the relative intensities of ions M+ (m/e 209), (M - H)+ (m/e 208), (M - H)++ (m/e 207) in the mass spectrum of a partially deuterated sample (Fig. 1, B-I) indicates a preferential splitting of an H atom from the methylene group of the benzyl substituent (see Scheme 1, path A). Fragment (M - H)+ with m/e 207 undergoes a rearrangement because of expansion of the imidazole ring into a pyrazine ring with the formation of a stable quinoxaline ring containing a nitrogen atom in the quaternary state. This supposition is confirmed by further splitting of a hydrogen atom and a HCN molecule from the (M - H)+ fragment with the formation of ions with m/e 206 and 180, respectively.

In the FD mass-spectrum of I (Fig. 1, C-I), only two ions are observed: the molecular ion with m/e 208, and a double-charged ion M++ with m/e 104, with a low peak intensity.