TRANSFORMATIONS OF ALKALOIDS OF THE QUINAZOLIN-4-ONE GROUP IN THE ANIMAL ORGANISM

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UDC 577.12 +543.51

The investigation of the mechanism of the action of biologically active substances requires a knowledge of the routes of transformations of these substances in the organism. Identification of the metabolites of widely used drugs has become a leading subject of special journals in recent years, and a number of reviews has been devoted to it [1-4]. One of the main methods used for recognizing metabolites is mass spectrometry in some type of combination with GLC, which is due mainly to their high sensitivity and selectivity.

The present paper describes the preliminary chromato-mass-spectrometric analysis of the metabolites isolated from rat urine after the administration of alkaloids of the 2,3-cycloalkylquinolizin-4-one group, which possess cholinotropic activity [5, 6]. We consider that experiments with a number of compounds of similar nature will lead to the elucidation of a definite regularity in metabolic transformations.

The biotransformation of quinazolin-4-one derivatives as drugs has been studied previously in relation to methaqualone [7, 8] and mecloqualone [9]. The results show that the main products of the transformations are hydroxy derivatives at one position or another of the initial molecules.

Our experiments were performed according to the following plan. The total metabolites were extracted from the material selected after a predetermined time from the introduction of the substance into the animal organism. Part of this total was separated by GLC to determine its quantitative composition. Another part was analyzed on the chromato-mass spectrometer in order to determine its qualitative composition. In addition, we studied the fragmentation of the initial substances and their derivatives including the spectra of possible metabolites.

The present paper gives the results relating to two quinazolin-4-one derivatives – deoxyvasicinone (DOV) (I) and vasicinone (II) [10, 11].

The mass spectrum of DOV is characterized by a doublet of strong peaks of the M^+ and $(M-1)^+$ ions (Fig. 1a). It is obvious that the $(M-1)^+$ fragment is formed by the detachment of a hydrogen atom from the C_9 position.

Examination by GLC of the total material isolated from the urine collected in the first six and the following 12 h after the administration of the DOV showed the presence of only the initial substance, but its amount in the first case was approximately 25 times higher than in the second.

The mass spectrum of the combined material (Fig. 1b) showed, in addition to the doublet of peaks with m/e 186 and 185 corresponding to (I), the peaks of ions with m/e 202, 200, 198, 196, 173, 146, and 119. When the energy of the ionizing electrons was reduced (12 eV) in the spectrum of the combined material (Fig. 1c), the relative intensity of the fragments with m/e 185, 173, 146, and 119 had decreased while the heights of the peaks of the ions with m/e 202, 200, 198, 196, and 186 remained at the same level. This permits the assumption that the latter are the peaks of molecular ions. A molecular weight of 202 may correspond to a hydroxy derivative of DOV. To increase the volatility of the possible metabolite, part of the total material was silylated. As a result, an additional maximum appeared on the chromatogram the retention time of which coincided with that of the TMS ether of vasicinone (II). The mass spectrum of this material had the peak of an ion with m/e 274 (M^+) and of the peak of the ion $(M-CH_3)^+$ that is characteristic for TMS ethers.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 250-254, March-April, 1977. Original article submitted December 30, 1976.

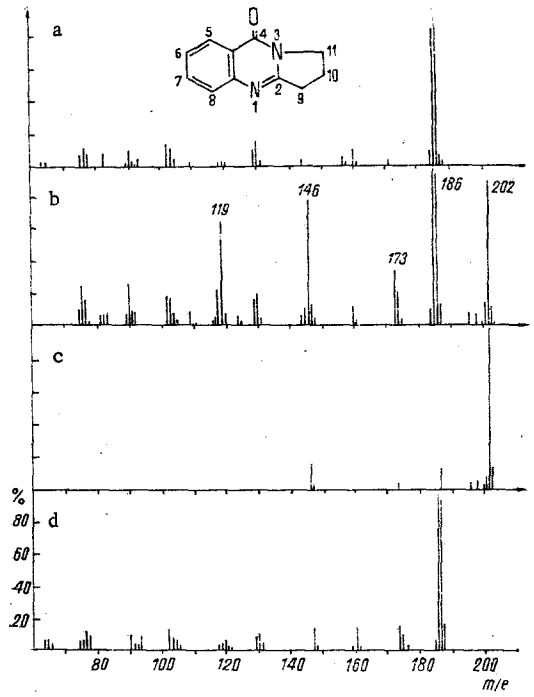


Fig. 1. Mass spectra of DOV (I) (a), of the combined metabolites of (I) at 40 eV (b) of the combined metabolites of (I) at 12 eV (c), and of DOV obtained on the chromato-mass spectrometer (d).

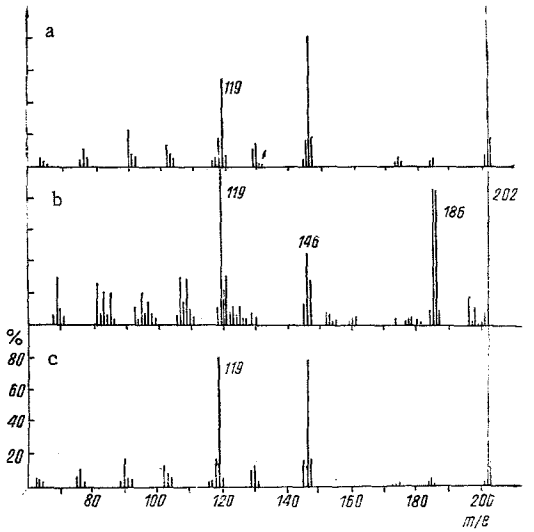


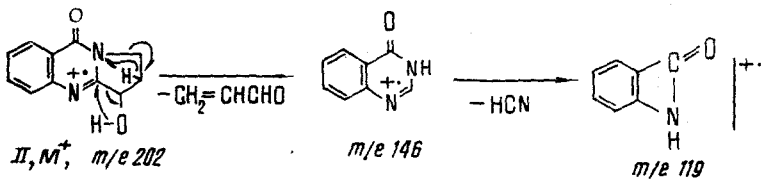
Fig. 2. Mass spectra of vasicinone (II) (a), of the total metabolites of (II) at 40 eV (b) and of vasicinone obtained on the chromato-mass spectrometer (c).

The relative amounts of (I) and (II) in the combined metabolites (i.e., obtained during the 18 h after the administration of the DOV) were, according to GLC, 7 and 93%, respectively.

The chromato-mass spectrometry of the nonsilylated total material also showed the presence of two quinazolinone bases, which were identified as (I) (Fig. 1d) and (II).

The predominant conversion of (I) to (II) in vivo permitted the assumption that the vasicinone shows a smaller tendency to undergo biotransformation. In actual fact, in the total obtained from the material collected during the 18 h after the administration of (II) we found chromatographically about 90% of vasicinone and 10% of DOV. A check on the chromato-mass spectrometer confirmed the presence of both compounds.

The main direction of fragmentation of the molecular ion of vasicinone (Fig. 2, a, c), unlike that of DOV, comprises the initial cleavage of the C_2-C_9 bond leading to the successive elimination of an acrolein molecule and HCN:



As in the case of DOV, the mass spectrum of the combined metabolites of (II) includes low-intensity peaks with m/e 200, 198, and 196. The absence of additional peaks on the chromatogram and in the mass spectra of components with the corresponding molecular weights on chromato-mass spectrometry does not permit the structure of these metabolities or their amounts in the mixture to be deduced at the present time. It is most likely that the compounds with molecular weights of 200 and 198 are products of the dehydrogenation of vasicinone or of the dehydration of its hydroxy derivatives.

EXPERIMENTAL

The experiments were performed on white rats (150-180 g). The alkaloids in the form of 0.5% aqueous solutions of the hydrochlorides were administered intraperitoneally at the rate of 50 mg/kg. Simultaneously, 4% on the body weight, of distilled water was administered per os to increase the secretion of the urine. Urine collected separately during the first 6 and the subsequent 12 h after the administration of the drug was treated with a 1 N solution of Na_2CO_3 to pH 10 and was extracted three times with chloroform. The chloroform layer was separated off and evaporation with the aid of a water pump.

A "Tsvet-4" chromatograph was used with a flame-ionizing detector and with a 2 m × 4 mm column filled with 5% of SE-30 on Chromaton N-AW (0.20-0.25 mm). The temperature of the column thermostat was 200°C, and the carrier gas was He (45-60 ml/min). The TMS ether of vasicinone was obtained by the action of chlorotrimethylsilane and hexamethyldisilazane in pyridine on (II) [12].

An LKB 2091 chromato-mass spectrometer (Institute of Petrochemical Synthesis of the Academy of Sciences of the USSR, V. G. Zaikin) was used with a 1.5 m × 3 mm packed column containing the stationary phase SE-30 on Chromosorb W. The temperature was programmed from 175°C at the rate of 5 degrees/min. The carrier gas was He (15 ml/min). The energy of the ionizing electrons was 70 eV, and the emission current 25 μA. The temperature of the molecular separator and of the ion source was 250°C.

A MKh 1303 mass spectrometer with a system for the direct introduction of the sample was used with a temperature of the inlet tube of 120-130°C at electron energies of 40 and 12 eV with an emission current of 50 μA.

SUMMARY

It has been found by chromato-mass spectrometry that the main product of the transformation of deoxyvasicinone excreted with rat urine in the unbound form is vasicinone. Vasicinone used as a drug is excreted predominantly in the unchanged form.

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MELANINS FORMED BY A CULTURE OF Aureobasidium (Pullularia) pullulans ARNAUD (DE BARY), 1910

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UDC 547.97:576.8.098

The investigation of the structure of melanins is difficult for the following reasons. In the first place, the chemical composition of the substances is complex and heterogeneous. The schemes for the synthesis of the chromophores known from the literature [1] give an idea of the formation of the monomers, but the process of the building up of the polymer and the question of its structure have so far remained unsolved; furthermore, a considerable number of materials are not covered by the existing classification [2]. In the second place, the structural and functional relationships between the components of the biopolymer – its protein moiety and the chromophore – are unknown. In the third place, the use of spectroscopic methods, which give extremely valuable information on structure at the molecular level, encounters fundamental difficulties in the case of multicomponent polymers. In spite of the voluminous literature devoted to the spectroscopic investigation of the melanins [2, 3], there are very few results giving information required for understanding their structure.

The problem of interpretation is the result of the absence of a well-defined structure of the electronic and IR absorption spectra. The lack of contrast of the melanin spectrum is due to the superposition of broad absorption bands characteristic for the fragments of the polymer molecules. The pattern is considerably complicated by intra- and intermolecular interactions. At the same time, the progress achieved recently in spectroscopic methods will, if only partially, enable the difficulties to be overcome and, in combination with other physicochemical methods, will permit the successful use of spectroscopy for the investigation of the structural changes of melanins.

The present paper gives the results of an investigation by physicochemical and spectral methods of the change in the composition and structure of melanins isolated from a culture of Aureobasidium (Pullularia) pullulans, strain 8. We set ourselves the following tasks: to determine the type of chromophore, to follow the change in the quantitative composition of the biopolymer during the growth of the culture, and to determine to what extent the structure and state of the melanin molecules change as the microorganism ages.

The absorption contours in the electron spectrum of alkaline solutions of the melanins before and after hydrolysis (Figs. 1 and 2) are not smooth lines as is stated in the literature [2]. They show inflections due to the fact that the spectrum consists of a number of broad overlapping absorption bands. The positions of the maxima of these bands were determined with the aid of special separating techniques [4]. In this way it was possible to isolate two absorption bands (λ_{max} 280 and 350 nm) in the spectrum of an alkaline solution of brown melanin, and four absorption bands (λ_{max} 280, 350, 490, 620 nm) in the spectrum of black melanin.

The maxima at 280 and 350 nm are common to all the samples investigated. They show the presence of a dicarbonyl fragment in the chromophore molecule [5, 6].

The infrared absorption spectra of the melanins (Fig. 3) contain features relating to the chromophore and to the protein moiety of the polymer, which complicates their assignment. The spectra of the melanins after

Institute of Pharmaceutical Chemistry, Leningrad. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 254-261, March-April, 1977. Original article submitted April 1, 1976.