MOLECULAR BIOLOGICAL PROBLEMS OF THE CREATION OF DRUGS AND STUDY OF THE MECHANISM OF THEIR ACTION

METABOLISM OF TETRINDOLE

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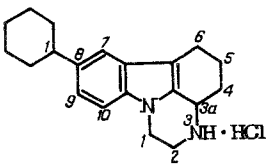
Tetrindole (2,3,3a,4,5,6-hexahydro-8-cyclohexyl-1H-pyrazino[3,2,1-j,k]-carbazole hydrochloride) is a novel domestic drug, belonging to the antidepressant group. The drug was synthesized and studied at the All-Union Chemicopharmaceutical Scientific-Research Institute.



The molecular weight of the base is equal to 294.

In its chemical structure, physicochemical properties, and pharmacological action tetrindole is close to the tetracyclic antidepressant pirazidol used in medical practice [2].

As a result of the development of a method of analysis of tetrindole in biomedia by HPLC, permitting quantitative estimation of the content of the unchanged preparation, it was established that in 72 h 3-12% of the introduced dose is excreted (about 1% with the urine, the remainder with the feces). These results served as the basis for suggesting that there is a high degree of metabolic processes occurring with the drug in the organism.

The aim of this work was to develop a procedure for the separation and isolation of tetrindole metabolites excreted from the rat organism with the urine, using HPLC, and to establish their structure by methods of NMR and mass spectrometry.

EXPERIMENTAL

Animals. The experiment was conducted on noninbred male rats, body weight 200-220 g. The animals were obtained from the nursery of the Academy of Medical Sciences of the Russian Federation. The rats were kept under stationary conditions with a natural daylight cycle and on a standard diet (combined feed and water). The animals were deprived of food for 18-20 h before the beginning of the experiment. After administration of the drug, the rats were transferred to special metabolic cages. The rats of the control group were kept under the same conditions.

Dose and Method of Administration. The drug was administered orally in starch gel in a dose of 300 mg of the drug per kg of body weight. Urine specimens were collected during the intervals 0-6, 6-24, and 24-48 h after administration of the drug.

Preparation of samples for Chromatographic and Spectrometric Analysis. The urine specimens (2 ml) were filtered off, alkalinized with a saturated $NaHCO_3$ solution to pH 8, and lyophilized to dryness. Tetrindole metabolites were extracted with 6 ml of chloroform, then with methanol. The extracts were evaporated; the dry residue was dissolved in 1.2 ml of methanol, filtered, and chromatographed. Fractions corresponding the the peaks were collected in polyethylene test tubes; the solutions were evaporated under vacuum, and the residues were analyzed using mass and NMR spectrometers.

Equipment. Liquid chromatograph from Filson with autosampler, sample volume introducible (loop) 50 µl, UV detector, wavelength 280 nm; Lichrosorb RP8 column, 10 µM 250·4.6 mm. Composition of eluent: 50% methanol and 50% water. Flow rate 1 ml/min. Volume of sample introducible 50 µl. Mass spectrometer MAT-122 from Gilson (Switzerland), direct introduction of

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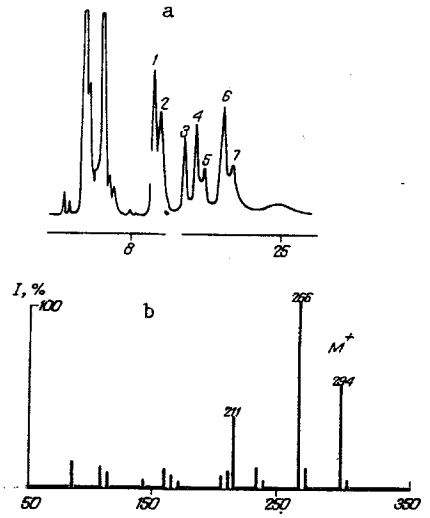


Fig. 1

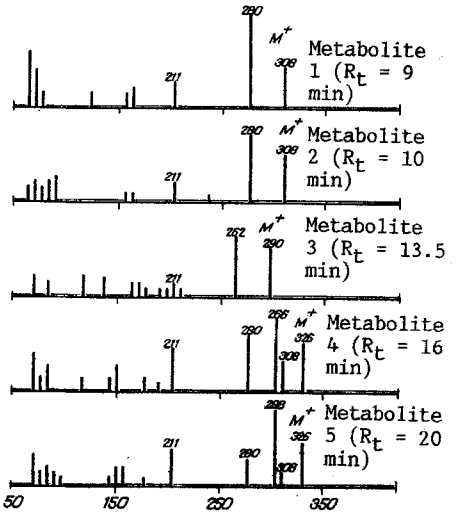


Fig. 2

Fig. 1. Chromatogram of a chloroform extract of urine of the experimental group of rats that received tetrindole (a) and electron impact mass spectrum of tetrindole (b). Along x-axis: time, min. In the control extract of urine from rats that did not receive the drug, the region on the chromatogram after 8 min contained no peaks. 1) R_t = 9 min; 2) R_t = 10 min; 3) R_t = 13.5 min; 4) R_t = 16 min; 5) R_t = 20 min; 6) R_t = 18.5 min; 7) R_t = 22.5 min. R_t = 9 (peak 1); 10 (2); 13.5 (3); 16 (4); 20 (5); 18.5 (6); 22.5 (7).

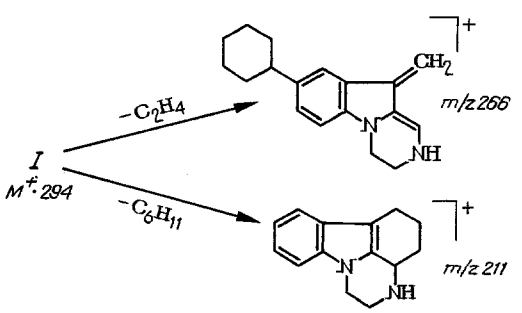
Fig. 2. Electron impact mass spectra of metabolites 1, 2, 3, 4, and 5.

sample into ion source, accelerating voltage 70 eV. NMR spectrometer from Varian (Switzerland), working frequency on channels 200 MHz.

RESULTS AND DISCUSSION

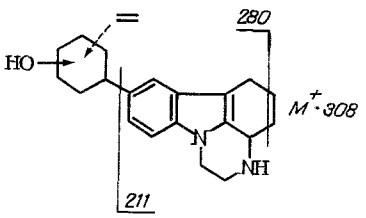
Up to seven peaks with R_t 9, 10, 13.5, 16, 18.5, 20 and 22.5 min, belonging to tetrindole metabolites, are observed on the chromatogram of a chloroform extract of the urine of the experimental rats (Fig. 1a). The drug itself has R_t > 25 min in this chromatographic system and is highly blurred. The methanol extract contained the same metabolites, only in smaller amounts. Only the ratio of the metabolites varied in different time intervals.

In order to identify the metabolites we made a preliminary study of the mass and NMR spectra of tetrindole. Breakdown of the drug molecule under the action of electron impact occurs chiefly along two pathways, giving a peak of maximum intensity with m/z 266 and a peak with m/z 211 in the mass spectrum. This characteristic breakdown was utilized in establishing the pathways of biotransformation of tetrindole (Fig. 1b).

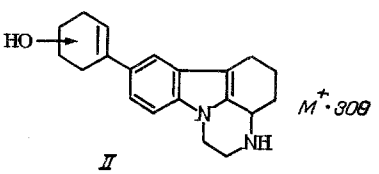


Signals of the aromatic protons at 7.58 ppm (H-7) and 7.48-7.50 ppm (H-9 and H-10); triplets from H-1 and H-2 at 4.15 and 4.38 ppm, respectively; signals at 3.12, 3.02, 2.32 ppm, belonging to H-4, H-6, and H-5, respectively; and broadened signals of the cyclohexyl residue at 1.3-1.9 and 2.62 ppm (H-1') are observed in the ^{3}H-NMR spectrum of tetrindole.

The mass spectra of the metabolites are presented in Fig. 2. As can be seen from the spectra, metabolite 1 with molecular weight 308 corresponds to the chromatographic peak 1 (R_t 9 min). There were no changes in the parity of the molecular weight in comparison with the original tetrindole molecule. The molecular weight was increased by 14 amu. A maximum-intensity peak$[M = 28]^+$, as well as a peak m/z 211, are observed in the spectrum, which is evidence that the right portion of the molecule (the tetrahydropyrazinocarbazole system) remained unchanged, and the conversions were associated with the cyclohexyl substituent. It is most natural to assume that these changes consist of the appearance of a double bond in the hydroxyl group in the cyclohexyl ring ($\Delta m = 14$).



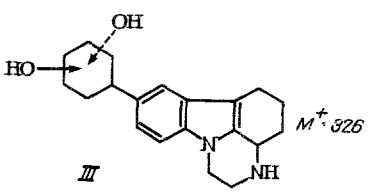
In the mass spectrum of metabolite 2 (R_t = 10 min), a peak of a molecular ion with m/z 308 is also observed. The values of the major characteristic ions of metabolites 1 and 2 also coincide. Evidently metabolite 2 is an isomer of 1 and differs from the latter in the position of the hydroxyl group of double bond. In a comparison of the ^{1}H NMR spectrum of tetrindole with the spectrum of the summary fraction 1 and 2, in the latter, together with signals in the aliphatic region (1.0-4.5 ppm), a number of signals are observed in the neighborhood of 7.1-7.7 ppm, along with broadened signals at 6.1 ppm. And yet it is known that the chemical shifts of olefinic protons in substituted cyclohexene lie within the interval 5.4-5.9 ppm in the absence of conjugation between the double bond and the phenyl substituent. At the same time, the signals of the protons in the presence of conjugation should be expected in the neighborhood of 6.0-7.3 ppm. These data agree with the earlier hypothesis of the presence of a double bond in the cyclohexyl ring; they suggest that this bond is conjugated with the indole ring:



II

Metabolite 3 (R_t = 13.5) has a molecular weight of 290. This is 4 amu less than the weight of the original tetrindole molecule. The mass spectrum of 3 retains the basic character of fragmentation typical of tetrindole, i.e., the presence of a maximum-intensity peak $[M = 28]^+$ m/z 262 and m/z 211. This suggests a structure of the metabolite with two double bonds in the cyclohexyl substituent. It can be assumed that the formation of metabolite 3 is associated with the appearance of two double bonds in the cyclohexyl substituent while the tetrahydropyrazolocarbazole ring system is preserved.

Metabolites 4 (R_t = 16 min) and 5 (R_t = 20 min) have the same molecular weight, 326. Their mass spectrometric breakdown is analogous to that observed for the preceding metabolites (see Fig. 2). The data obtained suggest that in this case the biotransformation affected only the cyclohexyl ring, and metabolites 4 and 5 are isomers hydroxylated twice on the ring.



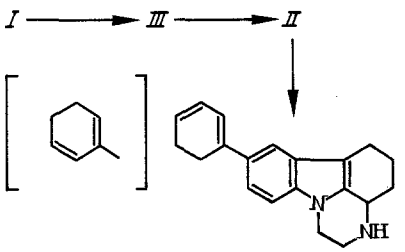
III

We should mention that recording the mass spectra of a mixture of metabolites (direct introduction of an unchromatographed sample into the ion source, temperature fractionation) made it possible to detect the molecular weights 290, 308, and 326, corresponding to those observed for the individual fractions of metabolites.

The ^1H NMR spectra of metabolites 3, 4, and 5 could not be recorded on account of the difficulty of isolating them in sufficient amounts. However, in the ^1H NMR spectra of a summary fraction of all the metabolites, a supplementary increase in the number of signals occurs in the neighborhood of 7-7.5 ppm, which cannot be attributed to the indole protons of the metabolites and which are evidently due to the unsaturated products formed on account of dehydration of metabolites hydroxylated on the cyclohexyl group. The metabolites with R_t = 18.5 and 22.5 min could not be identified on account of their low content.

Thus, the metabolism of tetrindole in the rat organism proceeds along the pathway of hydroxylation of the cyclohexyl substituent, followed by dehydration and the formation of a longer system of conjugated double bonds.*

The scheme of metabolism of the drug tetrindole can be represented as follows:



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*The processes of hydroxylation and double-bond formation in cyclohexyl derivatives during biotransformation were described in [1, 3].

INVESTIGATION OF INTERFERON INDUCERS AS GENOCORRECTORS IN VITRO AND IN VIVO

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Data have now been accumulated on the important role of gene and chromosomal mutations in the origin of malignant neoplasms [3, 14, 15]. In view of this, one of the approaches to the prophylaxis of oncologic diseases is a reduction of the amount of mutagenic compounds in the human environment. The use of rapid tests detecting mutagenic and potentially carcinogenic properties of chemical compounds takes on special significance here [11]. Another approach involves the discovery of substances possessing the ability to lower the mutagenic activity of genotoxic agents — antimutagens.

It is known that certain drugs give side effects, including genotoxic side effects [6, 17, 22]. One of the methods of neutralizing them may be the identification of antimutagens effective for the given drug and their use in clinical practice to compensate for undesirable genetic consequences. Many of the antimutagens are natural compounds, in particular, interferon, for which the presence both of antimutagenic and of anticarcinogenic properties has

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