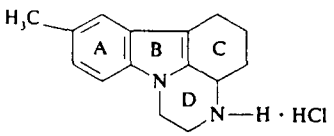
# STUDY OF PYRAZIDOL METABOLISM

**O. S. Anisimova,[1] A. V. Lopatin,[1] V. I. Pol'shakov,[1] V. V. Chistyakov,[1] I. A. Ermachenkov,[1] I. V. Golovanova,[1] P. Yu. Ivanov,[1] A. P. Pleshkova,[1] and Yu. N. Sheinker[1]**

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 30, No. 5, pp. 15 – 17, May, 1996.

*Original article submitted September 19, 1995.*

Pyrazidol, 8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole hydrochloride, is an original Russian drug belonging to the group of antidepressants [1].




In this work, we studied the metabolism of pyrazidol *in vivo* by experiments on rats. The structure of metabolites was established by the methods of electron impact mass spectrometry (EIMS), secondary ion mass spectrometry (SIMS), and $^1$H NMR spectroscopy.

## EXPERIMENTAL PART

**Experiments on animals.** The drug metabolism was studied on healthy white mongrel rats weighing 140 – 160 g (6 – 8 rats in each experiment). One day before the experiment, the rats were left in hunger and received only water. The administration of the drug was performed perorally as a tween suspension in an amount of 100 mg/kg. Urine was taken for analysis 6 and 24 h after the drug administration.

**Isolation of metabolites from the biological material.** Isolation of metabolites from the biological material was carried out according to the procedure described previously [2]. The urine was evaporated on the rotary evaporator at 37°C to obtain a thick mass, which was followed by multiply repeated extractions with chloroform and methanol. Thin-layer chromatography (TLC) showed that virtually the same metabolites were present in the methanol and chloroform extracts, although in somewhat lower amounts in the former case. For this reason, we used only the chloroform extracts for the study of metabolites.

**Thin-layer chromatography.** A benzene – ethyl alcohol – concentrated ammonia (30 : 90 : 1) system was used for the thin-layer chromatography of pyrazidol and its metabolites. The plates were developed using the Dragendorf reagent and the patterns were visualized by exposure to the UV light.

Isolation of the substance from the plate was performed by the descending elution method [3] using methanol as a mobile phase.

**Spectroscopy.** The electron-impact mass spectra of pyrazidol and its metabolites were obtained on a Varian MAT-112 spectrometer (Germany) with direct introduction of the sample into the ion source. The EIMS measurements were performed at an ionization energy of 70 eV and an ionization chamber temperature of 180°C. The secondary ion mass spectra were obtained on a Hitachi M-80A spectrometer (Japan) under the following conditions: ionization by $Xe^+$ ions; acceleration voltage of primary ions, 8 keV; current density of the primary ion beam, $10^{-7}$ A/cm$^2$. The samples were analyzed in a glycerin matrix on a gold target.

The $^1$H NMR spectra were recorded on a XL-200 spectrometer (Varian, USA) with a working frequency of 200 MHz, using deuteromethanol as the solvent and TMS as the internal standard for determining the chemical shifts.

**Synthesis.**

**8-Carboxy-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole (III)** was obtained in the form of methanesulfonate from 8-bromo-3-benzyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole (V) by lithiation and treatment with diethyl carbonate, with the following hydrolysis of the ester group and removal of the protecting benzene group by catalytic hydrogenation.

**3-Benzyl-8-ethoxycarbonyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole (VI).** To a solution of 7.62 g (0.02 mole) of V [4] in 70 ml of THF heated to 35°C was added 0.4 g of lithium. After the onset of the reaction, the mixture was cooled to 0°C for 10 min and allowed to stand at this temperature for 1 h. The resulting solution of organolithium compound was added at – 10°C to a solution of 11.8 g (0.1 mole) of diethyl carbonate in 30 ml of THF. After 30 min, the cold solution was acidified with 1.2 ml of acetic acid and THF was distilled off in vacuum. The residue was dissolved in benzene, washed with water, dried, and chromatographed on a short column with L 40/100 silica gel using benzene as the eluent. After distilling off the solvent, the

[1] Center for Drug Chemistry, All-Russia Research Institute of Pharmaceutical Chemistry, Moscow, Russia.



0091-150X/96/3005-0298$15.00 

product is recrystallized from acetone. Yield of compound VI, 3.1 g (41.4%), m.p. 154 – 156°C, mass spectrum: $M^+$ 374. $C_{24}H_{26}N_2O_2$.

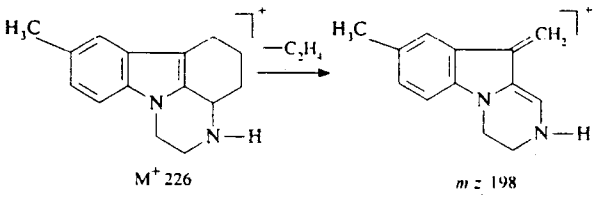
**3-Benzyl-8-carboxy-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole methanesulfonate hydrate (VII).** A mixture of 3.1 g (0.00083 mole) of VI, 1.4 g (0.025 mole) of KOH, 20 ml of ethanol, and 10 ml of water was boiled for 3 h. The resulting solution was evaporated in vacuum and the residue was diluted with 70 ml of water. Then activated charcoal was added, the mixture was filtered, and the filtrate was acidified with acetic acid. The precipitate was separated by filtration, washed with water and acetone, and dried. The resulting betaine (2.2 g) and 5 ml of 70% isopropyl alcohol were heated to boiling, 0.75 g (0.008 mole) of methanesulfonic acid were added to the resulting suspension, and the mixture was allowed to stand overnight. On the next day, the crystals were filtered off, washed with 6 ml of isopropyl alcohol, and dried. Yield of compound VII, 2.69 g (70%); m.p. 170 – 173°C (with decomposition); mass spectrum: $M^+$ 346 (betaine)[2]. $C_{23}H_{28}N_3O_6S$.

**8-Carboxy-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole methanesulfonate hydrate (VIII).** A mixture of 4.6 g (0.01 mole) of VII, 0.2 g of 20% palladium hydroxide on carbon [5], 50 ml of methanol, and 20 ml of water was hydrogenated at 15 atm and 60°C. Upon the completion of hydrogenation, the suspension was diluted with 30 ml of water, heated to boiling, and filtered to remove the catalyst. The filtrate was evaporated to dryness. The residue was doubly recrystallized from 50% ethanol. Yield of compound VIII, 2.3 g (65%); m.p. 300 – 303°C (with decomposition); mass spectrum: $M^+$ 256 (betaine). $C_{16}H_{20}N_2O_5S$.

## RESULTS AND DISCUSSION

To establish the structure of metabolites, we have done a preliminary study of the mass spectra of pyrazidol (I) and its dehydrogenated derivative II, a possible metabolite. We have also studied the behavior of pyrazidol under conditions of acid and base hydrolysis.

The EIMS of the drug (Table 1) exhibits three intense peaks, including the molecular ion peaks of the pyrazidol base $M^+$ ($m/z = 226$), $[M–H]^+$ (225), and $[M–C_2H_4]^+$ (198). The latter ion represents a product of the retro-diene decomposition of the molecular ion [6] and has the maximum peak intensity in the spectrum. Relative intensities of the other peaks do not exceed 10%.




$M^+$ 226 $m/z$ 198

The mass spectrum of dehydrogenated product II, shows only two intense peaks, belonging to the molecular ion ($m/z = 224$) and the $[M–H]^+$ species; intensity of the $[M–C_2H_4]^+$ peak does not exceed 9% (Table 1).

The study of the stability of pyrazidol in acid (pH 1) and base (pH 11) solutions by the TLC and mass spectrometry methods established that the drug was stable in acid solutions and exhibited dehydrogenation in the base solutions with the formation of compound II.

The SIMS of the urine extract samples showed, in addition to the signals observed in the control, intense peaks of the protonated molecular ions $MH^+$ with the mass numbers 257 and 255, and a low-intensity $MH^+$ peak of pyrazidol. The EIMS of the test urine extract contained intense peaks with $m/z = 256$ and 254. These results suggest that pyrazidol exhibits intensive metabolism in the animal organism with the formation of two main metabolites with molecular masses of 256 and 254.

A comparison of the TLC chromatograms of urine chloroform extracts of the control and test animals showed the presence of three additional spots in the patterns of test extracts as compared to the control. The chromatographic mobility of a small spot with $R_f$ 0.5 corresponds to the unchanged drug. Two poorly separated intense spots have $R_f$ 0.17 and 0.15; one of these has a violet fluorescence in the UV light, and the other one has a light-yellow fluorescence.

The structure of metabolites after the preparative isolation was established by means of the above-mentioned spectral methods. As expected, the mass spectrum of the compound isolated from zone I with $R_f$ 0.5 completely agreed with the spectrum of pyrazidol base. In a number of experiments, small peaks of the molecular ion ($m/z = 224$) and an ion with $m/z = 223$ are observed together with the peaks of pyrazidol in samples taken from the same band. These peaks are characteristic of the spectrum of dehydrogenated pyrazidol II. As the possibility of dehydropyrazidol formation in the course of metabolites isolation could not be ruled out, a special experiment was carried out to determine the stability of pyrazidol under the TLC conditions. Pyrazidol was added to the control urine, held in a thermostat at 40°C for 2 h, and extracted with chloroform. Chromatography of this preparation exhibited a single spot on the TLC plate, which was visualized in the UV light with a violet fluorescence typical of pyrazidol. Two-dimensional chromatography of pure pyrazidol showed that, immediately after separation on the plate, the compound was visualized in the UV light as a single violet spot. After 2 – 3 h the spot acquired a yellow tint and eventually was visualized in the UV light with a yellowish

[2] Under the electron impact conditions, methanesulfonates VII and VIII give the mass spectra of the corresponding betaines. This allows us to use EIMS spectra of compound VIII for the identification of metabolite III.

**TABLE 1.** Characteristic Peaks in EIMS Mass Spectra of Compounds I – III: Mass Numbers and Relative Intensities

| Compound | $m/z$ ($I_{rel}$) |
|---|---|
| I | 226(55), 225(24), 211(3), 199(16), 198(100), 182(6), 181(4), 115(4), 112(6), 99(14), 96(10), 79(9) |
| II | 224(100), 223(67), 196(9), 181(8), 180(7), 168(5) |
| III | 256(37), 255(36), 228(100), 211(5), 183(5), 180(6), 167(7) |

# ISM

hakov,[1] V. V. Chistyakov,[1] I. A. Ermachenkov,[1]
hkova,[1] and Yu. N. Sheinker[1]

hurnal, Vol. 30, No. 5, pp. 15 – 17, May, 1996.

ıber 19, 1995.

oped using the Dragendorf reagent and the patterns were visualized by exposure to the UV light.

Isolation of the substance from the plate was performed by the descending elution method [3] using methanol as a mobile phase.

**Spectroscopy.**

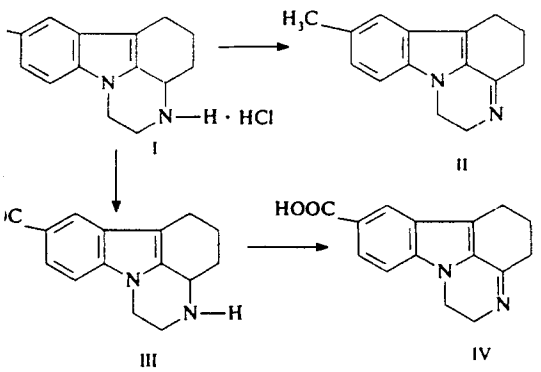
The electron-impact mass spectra of pyrazidol and its metabolites were obtained on a Varian MAT-112 spectrometer (Germany) with direct introduction of the sample into the ion source. The EIMS measurements were performed at an ionization energy of 70 eV and an ionization chamber temperature of 180°C. The secondary ion mass spectra were obtained on a Hitachi M-80A spectrometer (Japan) under the following conditions: ionization by $Xe^+$ ions; acceleration voltage of primary ions, 8 keV; current density of the primary ion beam, $10^{-7}$ A/cm$^2$. The samples were analyzed in a glycerin matrix on a gold target.

The $^1$H NMR spectra were recorded on a XL-200 spectrometer (Varian, USA) with a working frequency of 200 MHz, using deuteromethanol as the solvent and TMS as the internal standard for determining the chemical shifts.

**Synthesis.**

**8-Carboxy-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole (III)** was obtained in the form of methanesulfonate from 8-bromo-3-benzyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole (V) by lithiation and treatment with diethyl carbonate, with the following hydrolysis of the ester group and removal of the protecting benzene group by catalytic hydrogenation.

**3-Benzyl-8-ethoxycarbonyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole (VI).** To a solution of 7.62 g (0.02 mole) of V [4] in 70 ml of THF heated to 35°C was added 0.4 g of lithium. After the onset of the reaction, the mixture was cooled to 0°C for 10 min and allowed to stand at this temperature for 1 h. The resulting solution of organolithium compound was added at – 10°C to a solution of 11.8 g (0.1 mole) of diethyl carbonate in 30 ml of THF. After 30 min, the cold solution was acidified with 1.2 ml of acetic acid and THF was distilled off in vacuum. The residue was dissolved in benzene, washed with water, dried, and chromatographed on a short column with L 40/100 silica gel using benzene as the eluent. After distilling off the solvent, the products isolated from the band with $R_f$ 0.13 con-
ıetabolite IV mixed with metabolite III[4]. According
MS spectrum, metabolite IV has a molecular mass of
ich is 2 units lower as compared to the mass of com-
II. The SIMS spectrum contained the corresponding
$[M–H]^+$ (255). The $^1$H NMR spectrum of metabolite
'ed signals from the protons at positions 7, 9, and 10
o lower fields (by 0.1 – 0.2 ppm) relative to the same
n the spectrtum of metabolite III. This shift may be
dditional conjugation with the C=N bond of metabo-
vhile the carboxy substituent is retained in position 8.
ır shift of the $^1$H NMR signals was observed in going
razidol to dehydropyrazidol. Thus, a combination of
of mass spectrometry and $^1$H NMR spectroscopy al-
to identify metabolite IV as 8-carboxy-2,4,5,6-tetra-
H-pyrazino[3,2,1-jk]carbazole.
general scheme of pyrazidol metabolism may be pre-
s follows:




npounds III and IV are the main products of pyrazidol
sformation, which are excreted with the urine. Dehy-
ızidol is observed in the urine in minor amounts, and it
ible that dehydropyrazidol is an artifact product
in the course of metabolite isolation from the urine.

## RENCES

). Mashkovskii and N. I. Andreeva, *Farmakol. Toksikol.*, 1, 5 – 10 (1975).
. Anisimova, L. V. Frolova, V. V. Chistyakov, et al., *Khim.-n. Zh.*, **29**(2), 5 – 8 (1995).
'. Chistyakov, O. S. Anisimova, and Yu. N. Sheinker, *Analal Methods of Control in Development and Production of hetic Drugs. A Collection of Scientific Works* [in Russian], KhFI, Moscow (1985), pp. 13 – 19.
i. Glushkov, V. I. Shvedov, L. N. Yakhontov, et al., *Khim.-m. Zh.*, **24**(8), 23 – 26 (1990).
M. Pearlman, *Tetrahedron Lett.*, No 17, 1663 – 1664 (1967).
. Vul'fson, V. G. Zaikin, and A. I. Mikaya, *Mass Spectrome-of Organic Compounds* [in Russian], Khimiya, Moscow 36).
. Ionin, B. A. Ershov, and A. I. Kol'tsov, *NMR Spectroscopy Organic Chemistry* [in Russian], Khimiya, Leningrad (1983).

iiled to isolate pure metabolite IV because of its poor separation from ɔolite III.



0091-150X/96/3005-0298$15.00 © 1997 Plenum Publishing Corporation