INVESTIGATION OF THE STRUCTURE OF CHEMICAL COMPOUNDS; METHODS OF ANALYSIS AND QUALITY CONTROL

IDENTIFICATION OF PHENCYCLIDINE METABOLITES BY THE METHOD OF CHROMATO-MASS SPECTROMETRY

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Phencyclidine — 1-(1'-phenylcyclohexyl)-piperidine (I) — was previously used in medicine as an analgesic and anesthetic. However, because of its strong side effects (hallucinogenic, euphoric, and tranquilizing) [1-3], the use of this preparation is now limited to veterinary medicine.

The quantitative determination of I in the fluids of the organism [4], as well as its qualitative identification by the methods of thin-layer chromatography [5, 6] and mass spectroscopy [7, 8], has been reported. Earlier, metabolites of I, which are mono- and dihydroxyl derivatives, were reported in [4]. In this work we report on identical metabolites isolated after incubation of I with a microsomal preparation from rabbit liver.

EXPERIMENTAL

Preparation of Samples for Chromato-Mass Spectroscopy. Aqueous solutions of I were incubated with microsomal fractions of rabbit liver and the recently described [9] solutions of cofactors, then extracted with ether at pH 7.0-7.4. The combined ether extracts were concentrated and analyzed by chromato-mass spectroscopy, as described below.

Procedure of Chromato-Mass Spectroscopic Measurements. The analyses were performed on a Finnigan 4000 mass spectrometer (United States), connected through a glass jet separator to a Finnigan 9610 gas chromatograph (United States). The chromatographic separation was performed on a stainless steel column (3660×0.32 cm), stationary phase 3% SE-30, on Chromosorb W 80/100 mesh. Conditions of chromatographic separation: column temperature 200°C, temperature of injector 200°C, helium flow 20 ml/min, temperature of separator and delivery line 280 and 230°C, respectively. Conditions of mass spectrometry: electron energy 70 eV, source temperature 270°C, emission current 0.35 mA, scanning time 3 sec on the spectrum in the mass range 50-500 atomic units. Automatic repeated scanning with recording of the data on the magnetic disk of the Finnigan Incos system (United States) was used [10]. Methane, a mixture of which with helium served as the carrier gas, was used as the gas reagent for obtaining the mass spectra; methane was delivered under a pressure of 0.4 mm Hg. For all the spectra an increase in sensitivity was achieved by automatic deduction of the background. Perfluorokerosene was used as the calibrating substance for precise determination of the mass.

RESULTS AND DISCUSSION

A chromatogram based on the total ionic current of the ether extracts shows that they contain at least three compounds in amounts sufficient for identification. Figure 1 presents a chromatogram based on the total ionic current for chemical ionization (CI) by methane. In the mass spectra of electron impact (EI) and CI of the first compound (Fig. 2), the peaks of the molecular ion m/e 243 (see Fig. 2A) and the protonated molecular ion m/2 243 (see Fig. 2B) are observed, respectively. According to the composition of the molecular ions and the most intense ion fragments, this compound represents nonmetabolized I. The elementary composition of the ions is cited in Table 1. In a calculation of the elementary composition we used the following values of the atomic mass units for the elements: C 12.000, H 1.0078, N 14.0031, and O 15.9949; the maximum achievable discrepancy between the calculated and measured mass was considered as $30 \cdot 10^{-3}$ mass unit. A comparison of the mass spectra cited in Fig. 2 with the published spectra of I ,4% confirms the identity of this component of the ether extract with the nonmetabolized initial compound.

The monohydroxyl derivative of I, 1-(1'-phenylcyclohexyl)-4-hydroxypiperidine (II) was also identified by the method of chromato-mass spectroscopy. Figure 3 presents the mass spectra

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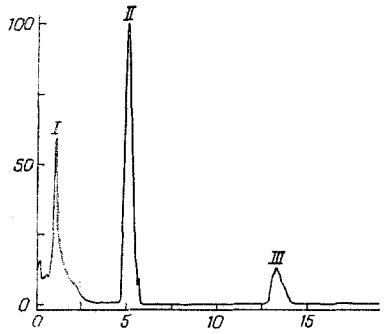

Fig. 1

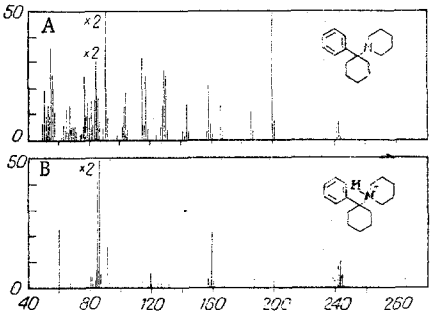

Fig. 2

Fig. 1. Chromatogram based on the total ionic current of a typical sample of a mixture of metabolites (in CI by methane). x axis: time of emergence of peak (in min); y axis: relative intensity (in %).

Fig. 2. Mass spectra of I in ionization by EI (A) and in CI (B). Here and in Figs. 3 and 4: x axes, m/e; y axes, relative intensity (in %).

TABLE 1. Elementary Composition Calculated for Certain Intense Ions in the Mass Spectra of Preparation I

Mass, atomic mass units	Intensity, %	Error, 10^{-3} amu	Number of equivalents of double bonds	Formula
243, 1889	6,66	9,8	6,0	$C_{17}H_{25}N$
200, 1429	38,77	1,0	6,0	$C_{14}H_{18}N$
186, 1230	8,88	5,3	6,0	$C_{13}H_{16}N$
166, 1392	10,19	20,4	2,5	$C_{11}H_{20}N$
158, 1026	28,41	7,0	6,0	$C_{12}H_{14}$
104, 0467	21,04	3,3	5,5	C_7H_6N
91, 0517	95,45	3,1	4,5	C_7H_7
84, 0663	58,85	15,0	1,5	$C_5H_{10}N$

Note. Here and in Table 2, the intensity of the peak with maximum intensity is taken as 100%.

TABLE 2. Elementary Composition Calculated for Certain Intense Ions in the Mass Spectra of Metabolite II

Mass, atomic mass units	Intensity, %	Error, 10^{-3} amu	Number of equivalents of double bond	Formula
258,9917	4,24	6,3	6,0	$C_{17}H_{25}NO$
216,1227	18,97	16,2	6,0	$C_{14}H_{18}NO$
200,1417	17,48	2,2	6,0	$C_{14}H_{18}N$
182,1281	5,77	25,5	2,5	$C_{11}H_{20}NO$
174,1166	6,39	11,7	5,5	$C_{12}H_{16}N$
158,0992	31,05	10,4	6,1	$C_{12}H_{14}$
143,1419	18,90	—10,9	1,0	$C_8H_{17}NO$
129,1436	55,89	7,6	0,0	$C_8H_{19}N$
115,0923	55,96	7,4	1,0	$C_6H_{13}NO$
104,0466	21,40	3,4	5,5	C_7H_6N
91,0505	100,00	3,3	4,5	C_7H_7
84,1026	30,03	—21,3	1,5	$C_5H_{10}N$

of EI and CI of the metabolite II, in which the peak of the molecular ion m/e 259 (EI, CI) and the peak of the fragment ion m/e 242 $M - 17^+$ (CI) are observed.

Table 2 presents the elementary composition of certain ions in the mass spectra of the metabolite II. According to the calculated elementary composition of the ions, the metabolite II contains a hydroxyl group. The presence of a hydroxyl group is confirmed by the spectrum obtained in CI by methane, where a quasimolecular ion m/e 260 $M + 1^+$ and a fragment ion m/e 242 $M - 17^+$ are observed. The position of the hydroxyl group in the molecule was established by Lin et al. [4], and with the exception of negligible differences associated with the experimental conditions, the mass spectra of CI and EI of the metabolite II are identical with the spectra published for 1-(1'-phenylcyclohexyl)-4-hydroxypiperidine.

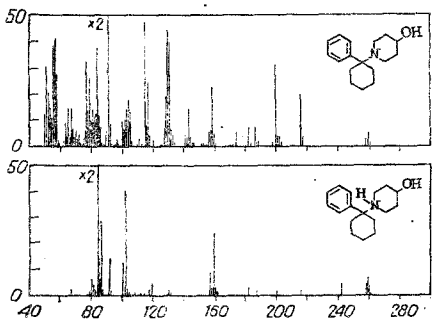

Fig. 3. Mass Spectra of II in ionization by EI (A) and in CI (B).

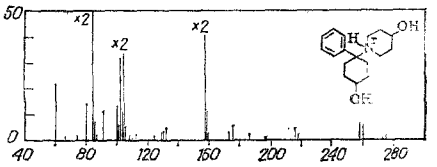

Fig. 4. Mass spectrum of III in CI by methane.

The mass spectrum of the metabolite III, obtained in CI by methane (Fig. 4), contains the molecular ion m/e 275 and the ion fragment $M - 17^+$; consequently, the metabolite III is a dihydroxyl derivative — 1-(1'-phenyl-4'-hydroxycyclohexyl)-4-hydroxypiperidine (III). Thanks to the use of CI, the phenylcyclohexyl portion of the molecule, together with the intense ion $M - 17^+$, is quite pronounced in the spectra, which is characteristic of the mass spectra of higher alcohols, taken under these conditions [11]. A comparison of the mass spectra of the CI of mono- and dioxyl metabolites provides evidence of the similarity of the ion fragments formed, with the exception of m/e 275 and 258. On this basis, it can be concluded that in the dihydroxyl metabolite III, one of the hydroxyl groups is at the carbon in the γ-position to the nitrogen of the piperidine ring, just as in the monohydroxyl metabolite. The previously published mass spectrum of dihydroxyphencyclidine, obtained under conditions of CI by methane, is similar to the spectrum depicted in Fig. 4, which is evidence of correspondence of the structure of the metabolite III to the formula cited above.

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