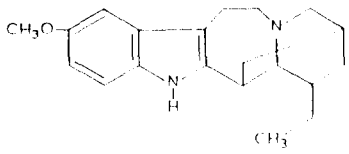
CHROM. 6050 Cartoni & Gianni 550

Gas chromatographic determination of ibogaine in biological fluids

Pharmacological studies of the alkaloid ibogaine showed that it has a stimulating action on the central nervous system; in small doses, it produces a light euphorizing effect, like morphine, and increases muscular and cerebral activity; at high doses, together with the central action, it produces apprehension, fear, a sense of inebriation and often hallucinations[1,2].

For these reasons, we have studied methods for the determination of this alkaloid in biological fluids.

Ibogaine is extracted from *Thabernanthe iboga* and is characterized by the following structure[3,4], in which an indolic group is present:



The compound has a m.p. of 153° at 0.01 mm Hg and pK_a 8.1 in 80% of methyl cellosolve. The absorption maxima in methanol are 226 and 298 nm. Log ε_{226} = 4.39 and log ε_{298} = 3.93.

The compound is soluble in ethanol, methanol, chloroform and acetone, and is insoluble in water. Ibogaine hydrochloride is soluble in water, methanol and ethanol, is scarcely soluble in acetone and chloroform and is insoluble in ether.

Ibogaine is decomposed by the action of heat and light. LLOYD *et al.*[5] examined the gas chromatography of ibogaine on a 6 ft. column, 4 mm I.D., packed with 2% SE-30 on Chromosorb W at 204°. BIEMAN AND FIEDMANN-SEIFELLER[6] determined the mass spectra of all the alkaloids extracted from *Thabernanthe iboga*, and discussed the fragmentation. DE SIO[7] reported the gas chromatographic separation of ibogaine from urine. Some drugs, such as Iperton, containing the total extract of *Thabernanthe iboga* are commercially available.

The determination of ibogaine in biological liquids, *e.g.*, urine, was carried out in this work by using gas and thin-layer chromatography.

Experimental

The standard solution (1 mg/ml) of ibogaine was prepared from the free base (Fluka) dissolved in ether and stored in a refrigerator in the dark. The extraction of ibogaine from an aqueous solution of the hydrochloride was investigated using ether, methylene chloride and chloroform as solvents at different pH values. With any of these solvents, the extraction was quantitative in the pH range 12–14. Below pH 12, the amount of drug extracted decreased. Ether was preferred among the solvents because of its greater volatility and smaller tendency to give emulsions with urine. The extraction from urine at a basic pH was preceded by three extractions with ether at an acidic pH (pH ca. 1) to remove from the sample other acidic or neutral impurities that would have interfered in the subsequent determinations.

For the *in vivo* extraction, the amounts of urine used were 2–10 ml in tests on rabbits and 10–100 ml in tests on humans. Thin-layer chromatography was

carried out with 20 × 20 cm plates pre-coated with cellulose (Merck), 0.1 μm in thickness.

The solvent was *n*-butanol–formic acid–water (20:1:2). The plates were sprayed with bromocresol green indicator buffered at pH 5.6. The ether extract of ibogaine (1–10 μg) was run for about 4 h and a spot with R_F = 0.6 was obtained.

For gas chromatography, two instruments were used; a Carlo Erba Fractovap, Model G.I., with a flame ionization detector, and a Hewlett-Packard, Model 5750 G, with a thermionic detector (AFID) using rubidium bromide, which is very specific and sensitive for nitrogen compounds.

Glass columns were used, 1.80 m long and 0.3 m I.D. packed (*a*) with 1% SE-30 on silanized Chromosorb W (80–100 mesh) or (*b*) with 0.1% SE-30 on silanized glass beads (Corning glass beads, GLC-110, 80–100 mesh). The column temperature was 180–250°, either isothermal or temperature-programmed as specified below. The injector temperature was 180°. The carrier gas was pure nitrogen.

The *in vivo* experiments were carried out as follows:

(*a*) Six albino rabbits received oral doses of 5, 10, 15 and 20 mg/kg of ibogaine hydrochloride dissolved in water. Urines were collected with a catheter at 0 (blank), 1, 2, 3, 4, 5 and 12 h after the administration.

(*b*) A single dose of six capsules of Iperton corresponding to a total of 240 mg of natural extract of *Thabernanthe iboga* was administered to a human subject of 70 kg weight. Two others received orally 5 mg of ibogaine hydrochloride. Urines were collected at the same time intervals as for (*a*).

Results and discussion

The determination of ibogaine either *in vivo* or *in vitro* is strongly influenced by its instability towards light and temperature. The samples should be stored

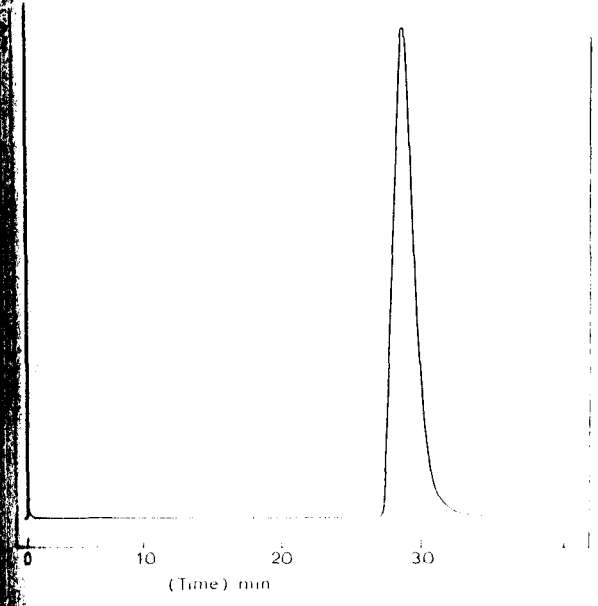


Fig. 1. Gas chromatogram of ibogaine. Column: 1% SE-30 on Chromosorb W, temperature-programmed from 180° to 250° (2°/min). AFID detector.

in a refrigerator at 0° and examined within 1–2 days. In acidic solution (pH = 1), ibogaine remains completely in the aqueous phase, and so it is possible to carry out, before the alkaline extraction, an extraction from the acidic solution to remove other possibly interfering substances.

Thin-layer chromatography of urine extracts showed well defined spots with a minimum detectable amount of about 1 μg. In *the vivo* experiments, two spots were

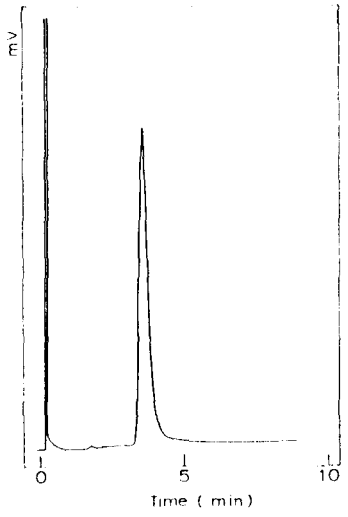


Fig. 2. Gas chromatogram of ibogaine. Column: 0.1% SE-30 on silanized glass beads, isothermal temperature 220°.

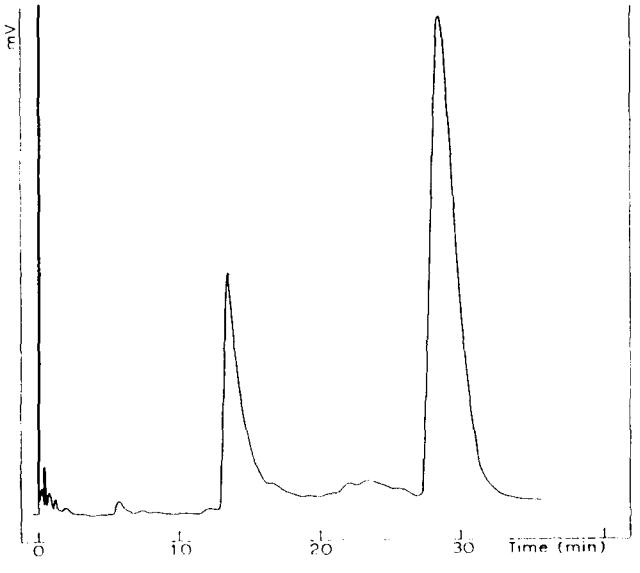


Fig. 3. Gas chromatogram of ether extract of human urine 3 h after administration of 5 mg of the drug. Operating conditions as in Fig. 1.

obtained, one with an R_F value of 0.80, *i.e.*, the same as for pure ibogaine, and the other with an R_F value of 0.80.

For the gas chromatographic determination, the column preparation and conditioning are very critical. The 1 % SE-30 column on silanized Chromosorb W is conditioned for 12 h at 300° and is silanized again every 10–15 h by injecting 10–15 μl of Sylil-8 (Pierce Chemical Co.). Only after this treatment is it possible to obtain symmetrical peaks with a linear response from 1 to 0.05 μg of ibogaine (Fig. 1). The operating temperature is in the range 180–225°, and at higher temperatures decomposition of the product occurs, as shown from the appearance on the gas chromatogram of another tailing peak after the standard one.

With the glass bead column, it is possible to work at a lower temperature, in a shorter time a more symmetrical and sharper peak is obtained and a minimum amount of 0.005 μg of ibogaine can be determined (Fig. 2).

The thermionic detector with rubidium bromide is very useful for the specific detection of ibogaine in extracts from urine and from the *Thabernanthe iboga* contained in the Iperton.

Excretion of the drug. In the urine extracts, it is possible to detect by either thin-layer or gas chromatography a fraction of non-metabolized ibogaine. In the rabbit, this fraction was found, after administration of 10 mg/kg, by extraction of 5 ml of urine (Fig. 4). The concentration reached a maximum 4–5 h after the administration and then decreased rapidly and disappeared after 6 h.

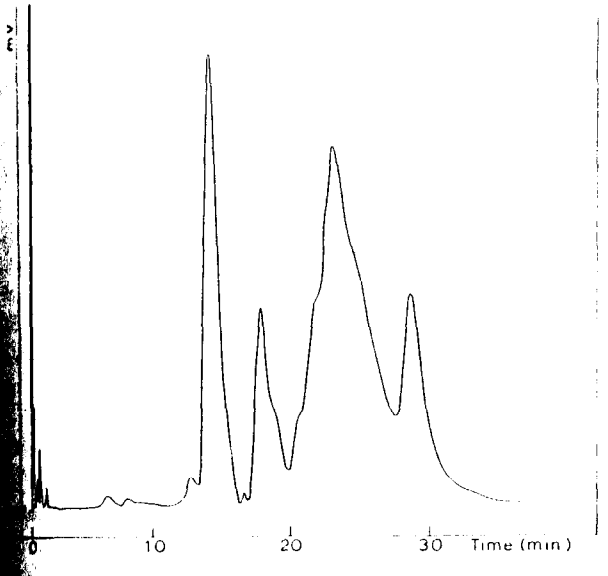


Fig. 4. Gas chromatogram of ether extract of rabbit urine 1 h after administration of 15 mg/kg of the drug. Operating conditions as in Fig. 1.

In humans it was possible, by extracting 50–100 ml of urine, to detect the drug in the urines collected within the first 3 h for an administration of 5 mg of ibogaine (Fig. 3). After the fourth hour, the drug could no longer be detected at this

dosage, by either thin-layer or gas chromatography. Ibogaine could not be detected at all in a subject who had taken a therapeutic dose of Iperton (six capsules).

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