TECHNICAL SECTION

Methods for the Detection and Determination of Ibogaine in Biological Materials *

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Ibogaine is a psychotropic alkaloid belonging to the indole group, originally obtained from the West African shrub Tabernanthe iboga (fam. Apocynaceae) (1). It was first isolated by Dybowski and Landrin in 1901 (2). The formula suggested by Taylor et al. (3) for this compound has been confirmed by X-ray crystallography (4) (Fig. 1).

IBOGAINE

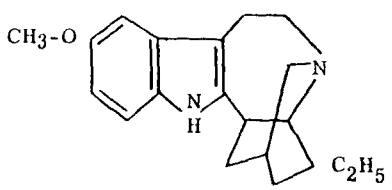

Fig. 1—Structure of ibogaine.

The extensive use of psychotropic drugs in modern therapy has created problems in the field of law enforcement. Traffic offenses are believed to occur with increased frequency especially when such drugs are taken with alcohol. Ibogaine has been reported to be used by addicts (5).

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This paper deals with two methods for the detection of ibogaine in biological materials which can be used in toxicology laboratories for forensic purposes.

Materials

Ibogaine stock solution: (1 mg/ml) Dissolve 100 mg of ibogaine hydrochloride (Aldrich Chemical Co., Inc., Milwaukee, Wis.) in 100 ml of distilled water. Further dilutions of 20, 40, 60, 80, 100, and 120 μg/ml were made from this stock solution.

Animals: Thirty-six white male Holtzman rats, each weighing about 200 grams, and divided in six equal groups.

Collection of specimens: Twenty-five ml of urine was collected from each group of rats using metabolism cages. The rats were then decapitated and blood was collected in test tubes containing 1–2 drops of heparin solution. Stomach, liver, kidneys and brain were removed, individually weighed, and homogenized using distilled water so that 5 ml of the homogenate contained one gm of wet tissue.

Reagents:

a. Hydrochloric acid (0.5 N).
b. Ammonium hydroxide, conc.
c. Potassium permanganate, aqueous solution, 0.5% w/v.
d. Potassium iodoplatinate reagent. Dissolve 0.25 g of chlorplatinic acid and 5 g of potassium iodide in water and dilute to 100 ml.
e. Petroleum ether, b.p. 30–60° C.

Preparation of thin-layer chromatographic plates: Glass plates, 10 × 20 cm, were coated to a thickness of 250 microns with a slurry of silica gel G (E. Merck AG) and distilled water in a ratio of 1:2 w/v. The plates were air dried for about one half hour and activated at 90° C for 90 minutes.

Methods

Extraction and spectrophotometric analysis: A known concentration of ibogaine hydrochloride solution was added to 5 ml of either tissue homogenate, blood or urine, mixed well, and then made alkaline with 5–6 drops of concentrated ammonium hydroxide. The mixture was extracted twice with 20 ml portions of petroleum ether using a 50 ml round bottom glass-stoppered centrifuge tube. The tube was centrifuged at 1500 rpm for five minutes and the solvent layer transferred to a separatory funnel. The volume of the solvent recovered was recorded.

The solvent was extracted with 10 ml of 0.5 N HCl. The UV absorbance of the aqueous phase was determined using a reference solution of 0.5 N HCl saturated with petroleum ether.

Ibogaine exhibited a characteristic absorbance with a maximum at 278 nm and a minimum at 249 nm (Fig. 2). A standard curve was pre-

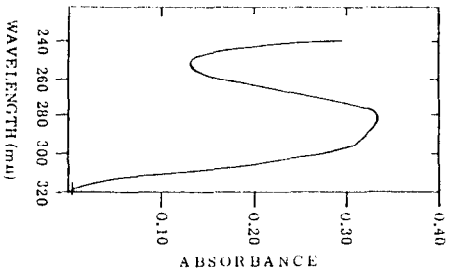

Fig. 2—UV spectrophotometric curve of ibogaine.

pared by plotting absorbance vs concentration of 20, 40, 60, 80, 100 and 120 μg/ml solutions of ibogaine hydrochloride. The absorption coefficient ($A^{1\,cm}_{1\%}$) of ibogaine hydrochloride in 0.5 N HCl was found to be 240.

Thin-layer chromatography: Following photometry the acid extract was evaporated to dryness on a steam bath. The residue was dissolved in 0.2 ml methanol and spotted on a thin-layer plate using 10 μl disposable pipets.

Two-solvent systems were found to be satisfactory in separating ibogaine from other drugs. System I: methanol-chloroform 1:9 (v/v); System II: ethanol-benzene 1:4 (v/v).

Ibogaine was located on the thin-layer plate by a distinct greyish violet spot given by iodoplatinate and/or a yellow spot given by potassium permanganate at appropriate R_f locations (Table I). The detection limit for ibogaine hydrochloride was found to be approximately one μg.

TABLE I

	Ibogaine	Amphetamine	Heroin	Novocaine	Quinine	Marihuana extract*	Morphine HCl	Demerol	Cocaine
Color of Spot in Potassium iodoplatinate Reagent	Greyish Violet	Greyish Violet	Dark Blue	Greyish Violet	Greyish Violet	Greyish Violet	Greyish Violet	Greyish Violet	Greyish Violet
Color of Spot in Potassium permanganate 0.5% aqueous solution	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
R_f in System • I Methanol : Chloroform 1 : 9	0.45	0.00	0.55	0.30	0.20	1.00	0.15	0.70	0.95
R_f in System •II Ethanol : Benzene 1 : 4	0.57	0.00	0.27	0.27	0.27	1.00 0.60	0.05	0.35	0.70

* Marihuana extract gave two spots with Potassium permanganate 0.5% aqueous solution only.

TLC separation and visualization of ibogaine and other drugs.

Discussion

The pH of the biological material and the solvent for extraction were investigated. Extraction of ibogaine was found to be optimum when the tissue homogenate, urine or blood was adjusted to pH 8 or above, and partition into petroleum ether was highly favorable, with little trouble from emulsion formation.

Ibogaine was then extracted from 5 ml volumes of water containing 20, 40, 60, 80, 100, and 120 μg ml concentrations of the drug using a single extraction with 20 ml petroleum ether. The recovery of ibogaine was approximately 97% up to 60 μg ml (Fig. 3). Above 60 μg ml extraction was increasingly incomplete and two 20 ml portions of the solvent were used to obtain a 97% recovery (Fig. 4).

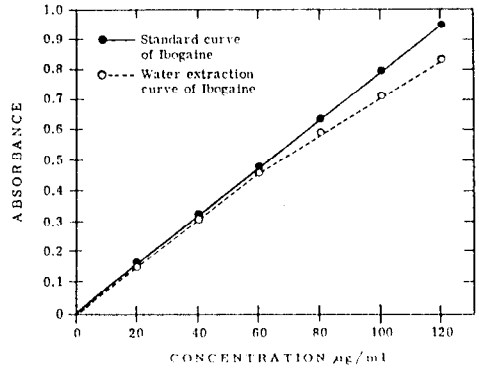

Fig. 3—Extraction of ibogaine from 5 ml volumes of water by a single extraction with 20 ml petroleum ether.

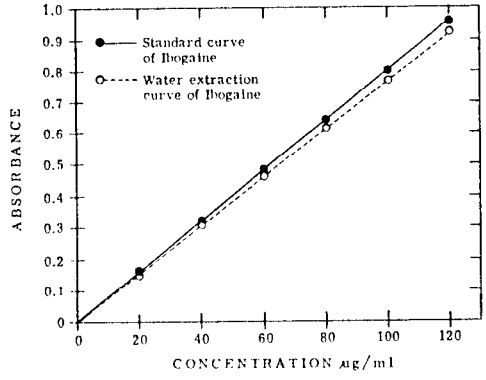

Fig. 4—Extraction of ibogaine from 5 ml volumes of water using 2 × 20 ml of petroleum ether.

The same series of concentrations of ibogaine hydrochloride solution were added to either 5 ml tissue homogenate, urine or blood, extracted in the same manner, and the percent recovery calculated. It was found that about 85% of ibogaine can be recovered from any tissue homogenate, urine or blood with two extractions (Fig. 5). Ibogaine was no longer detectable in a third extract of the material.

In the search for an appropriate TLC development several solvent systems were tried and the two described above were

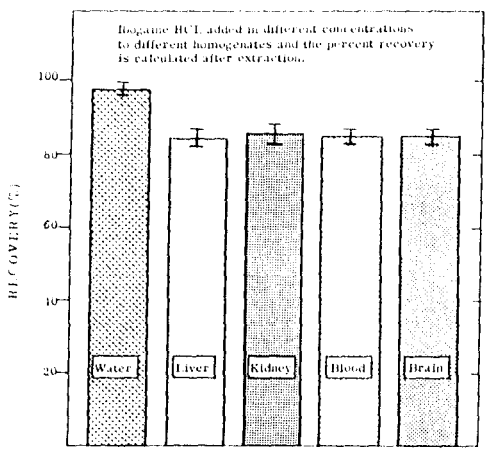

Fig. 5—Percent recovery of ibogaine from different tissue samples.

found most satisfactory. Table I gives the R_f values for several drugs which were also chromatographed with ibogaine using these solvent systems. Ibogaine has an R_f of 0.45 in system I and 0.57 in system II, whereas, the other drugs tested have different R_f values.

The spectrophotometric method described was employed for the recovery of ibogaine in tissue distribution studies in the rat. Free ibogaine could be detected in these biological materials up to eight hours following intraperitoneal injection of 50 mg/kg ibogaine HCl. At eight hours, the liver was found to contain 0.1% and blood 0.18% of the administered dose. A total of 12.6% of the administered dose was recovered in the whole rat at this time. It was also found that about 4% of the injected drug was excreted unchanged in urine.

Urine, from pretreated rats, was collected 24 hours post-injection and extracted with petroleum ether in the same man-

ner as described earlier. The solvent was evaporated and the residue dissolved in 0.2 ml methanol and chromatographed using TLC system II. Two major spots, giving the same color with either visualizing reagent, were detected. One spot with an R_f value of 0.57, corresponding to free ibogaine, and the other with an R_f value of 0.35 which was believed to be its metabolite. Studies on tissue distribution and metabolism of ibogaine will be discussed in detail in another paper.

Summary

UV and TLC methods for the detection and determination of ibogaine in biological materials have been described. Five ml of tissue homogenate, urine or blood are adjusted to pH 8 and extracted twice with 20 ml of petroleum ether. The solvent is then extracted with 0.5 N HCl. The UV absorbance is determined. Ibogaine exhibits a characteristic absorption curve with a maximum at 278 nm; $A\ {}^{1\%}_{1\ cm} = 240$.

Using TLC, two solvent systems, methanol and chloroform (1:9) and ethanol and benzene (1:4) give R_f values of 0.45 and 0.57 respectively for ibogaine hydrochloride. Ibogaine may be located by either potassium iodoplatinate reagent, which gives a greyish violet spot, or with 0.5% aqueous solution of potassium permanganate which gives a yellow spot.

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