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The Identification of Ibogaine in Biological Material

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A reliable method is described for the extraction of unchanged ibogaine from biological materials such as blood, urine, and tissue homogenates of liver, kidney, and brain by a simple extraction for basic drugs. Precise identification of the alkaloid may be achieved through the use of thin-layer chromatography, ultraviolet and infrared spectrophotometry.

Introduction

Ibogaine, an indole alkaloid extracted from the root of a West African plant, *Tabernanthe iboga*, was placed under the Federal Drug Abuse Control Act in November of 1967 (Federal Register, Nov. 3, 1967), presumably because of a purported hallucinogenic effect and the limited occurrence of the drug on the illicit market on the East Coast of the United States.

The alkaloid was first described by Dybowski and Landrin (1901), who reported that the natives of the Congo region considered the use of the material equivalent to alcohol. Pouchet and Chevalier (1905) reported that ibogaine given to dogs produced excitation and hallucinations. The findings of the latter authors were supported by Schneider and Sigg (1957), who observed fear and escape responses suggestive of hallucinations in dogs and cats having received 2–10mg/kg of ibogaine.

While the pharmacology of ibogaine and related alkaloids in animals has been treated in depth (Raymond-Hamet and Rothlin, 1939; Rothlin and Raymond-Hamet, 1938; Turner et al., 1955; Raymond-Hamet and Vincent, 1960; Gershon and Lang, 1962), a definitive work reporting the behavioral effects of the pure compound in man was not available until the Fall of 1967. At precisely the time that ibogaine and its salts were placed under strict Federal control.

Schmid (1967) reported that in tests with a series of human volunteers, hallucinogenic effects were not observed with doses ranging widely from 10mg/kg to 1·2g/kg of body weight. The effect was generally that of intoxication and mild sedation. Schmid considered it probable that the plants utilized by the natives contain other psychotropic substances, or that the ritualistic situation in which the drug is taken may exert a suggestive effect. The first hypothesis is at variance with the findings of Dybowski and Landrin (1901), who found the extracted alkaloid to be as active psychologically as the whole root. Consideration must be given, however, to the separation techniques available to chemists at the turn of the century, and the possibility that the ibogaine alkaloid extracted by Dybowski and Landrin was in fact a mixture of ibogaine and related *Voacanga* alkaloids, one or more of which might possibly be psychotropic, cannot be totally discounted. With respect to the ritualistic use of the raw

plant material by primitive societies of West Africa, Schultes (1967) indicates that there is no direct ethnopharmacologic evidence of any extensive use of the material as a vision-producing narcotic.

However erroneous, the notion that ibogaine is hallucinogenic in action has been widely publicized and circulated within the illicit drug market (*San Francisco Chronicle*, April 1, 1969). The placing of the material under strict regulation as a hallucinogenic drug by the Federal Government has done nothing to dispel this notion in the mind of the general public.

The chemistry of ibogaine and related alkaloids has been well elucidated, and reviews of the chemistry are available (Lebeau and Janot, 1956; Tatevosian, 1959; Saxton, 1960). Sullivan et al. (1967) reported a thin-layer chromatographic separation of ibogaine, heroin, and quinine, with color reactions of ibogaine and common alkaloidal reagents. Experience in the authors' laboratories indicates that reaction of ibogaine with common alkaloidal reagents is not particularly definitive, and of little diagnostic value in the identification of the alkaloid in an unknown mixture.

The extraction and identification methods presented here are appropriate for rapid toxicological investigations and make use of simple materials and chemicals.

Methods

For the thin-layer chromatographic identification of ibogaine, microscope slides or 5 × 20cm glass plates are used. A Silica Gel G layer 0·25mm thick is spread over the glass surface, the plates dried at room temperature (27°C) for half an hour after coating, and activated at 90°C for 90 minutes. All plates are equilibrated for five minutes in the solvent chamber and are chromatographed for a distance of 10cm from the origin. All experiments were conducted at an ambient temperature of 27°C.

Ibogaine HCl (Aldrich Chemical Company, Milwaukee, Wisconsin) solution 5mg/ml is treated with 2–3 drops ammonium hydroxide to liberate the base, which is then extracted with petroleum ether. The solvent layer is separated, allowed to dry, and the residue dissolved in 10ml of methanol for spotting on the thin-layer plate. To relate the mobility of the ibogaine alkaloid to other materials, standard solutions were prepared of heroin, amphetamine, quinine, and procaine in methanol to be run on the same TLC plate.

The use of the following seven alkaloidal reagents (US Treasury, 1960) was attempted for the purpose of the visualization of the spot on the developed thin-layer plate:

1. Zinc chlor-iodide Reagent
2. Wagner's Reagent
3. Ammonical Silver Nitrate Reagent
4. Potassium Ferrocyanide Reagent
5. p-Dimethylaminobenzaldehyde, 0·5% in acidified ethanol
6. Potassium Permanganate 0·5% aqueous Solution
7. Iodoplatinate Reagent

Of these reagents, only Potassium Permanganate solution and the Iodoplatinate Reagent developed color spots on the plates.

Eleven solvent systems were evaluated for the thin-layer chromatographic development:

System 1. Dioxane, Benzene, Ammonium hydroxide, Ethanol (40:50:5:5)
System 2. Chloroform, Dioxane, Ethyl acetate, Ammonium hydroxide (25:60:10:5)
System 3. Ethanol, Chloroform, Dioxane,–Petroleum ether, Benzene, Ammonium hydroxide, Ethyl acetate (5:10:50:15:10:5:5)
System 4. Ethyl acetate, Benzene, Ammonium hydroxide (60:35:5)
System 5. Ethyl acetate, Butyl ether, Ammonium hydroxide (60:35:5)

System 6. Ethyl acetate, Acetonitrile, Ammonium hydroxide (50:30:15:5)
System 7. Acetonitrile, Chloroform, Ethyl acetate, Ammonium hydroxide (40:30:25:5)
System 8. Acetonitrile, Benzene, Ethyl acetate, Ammonium hydroxide (40:30:25:5)
System 9. Methanol, Chloroform (10:90)
System 10. Ethanol, Benzene (20:80)
System 11. Ethyl acetate, Benzene, Butyl ether (20:60:20)

Two systems, numbers 9 and 10, were found to be particularly well suited for the separation of ibogaine and the other materials chromatographed, giving well-defined spots with little or no tailing.

TABLE 1

PROPERTIES OF TLC SYSTEMS No. 9 AND No. 10 AS DESCRIBED IN TEXT

	Ibogaine	*Amphetamine*	*Heroin*	*Procaine*	*Quinine*
Color of spot in Iodplatinate reagent	Greyish violet	Greyish violet	Blue	Greyish violet	Greyish violet
Color in Potassium Permanganate reagent	Yellow	Yellow	Yellow	Yellow	Yellow
Rf. in system no. 9	0·45±0·02	0·0	0·55±0·05	0·30±0·04	0·23±0·03
Rf. in system no. 10	0·57±0·09	0·0	0·27±0·04	0·27±0·04	0·27±0·04

Ibogaine HCl was given to Holtzman white male rats via intraperitoneal injections in doses of 50mg/kg of body weight. Tremor and ataxia appeared 3-4 minutes after injection and the animals became photophobic. These signs lasted for a period of 4–5 hours. Samples of 24-hour urine, blood from heart puncture or decapitation, and samples of liver, kidneys, and brain were taken one hour following administration of the drug. It would seem possible that the extreme avoidance reactions of the animals noted by other workers and attributed to hallucinations may be at least in part due to the photophobia exhibited by the animals.

5ml of blood or urine are made basic with 2–3 drops of ammonium hydroxide to bring the pH to 10·5–11. The material is then extracted twice with two 20ml portions of petroleum ether (30–60°C range). The petroleum ether extracts are pooled together. Half the volume is separated, dried under a stream of hot air, and the residue dissolved in methanol. This alcoholic extract is then spotted on the thin-layer plate using microliter pipettes. The remaining aliquot is shaken with 6ml of 0·5N hydrochloric acid and the acid layer taken for UV spectrophotometric analysis. Ibogaine gives a typical curve with an absorption maximum at 278nm and a minimum at 248nm. A bathochromic shift is noted when the solution is made basic to pH 10, as seen in Figure 1, and is accompanied by a slight hyperchromic effect upon the molar absorptivity.

Ibogaine gives a very characteristic infrared absorption spectrum, shown in Figure 2. The spectra of the hydrochloride salt and the free base are illustrated, the base having been run as a film by attenuated total reflectance infrared spectrophotometry on a KRS-5 25-pass crystal cut at 45°, and the salt run as a transmission spectrum in a KBr pellet.

Discussion

As the literature reveals no available information on the fate of ibogaine in the body after an ingested or injected dose, it was of interest to the authors to determine a reliable method for toxicological investigations of ibogaine in biological materials. It was established that free ibogaine may be obtained in biological samples of blood, urine, and tissue homogenates of liver, kidney, and brain by a simple extraction for basic drugs. Identification by thin-layer chromatography was found to be quite suitable, detecting as low as one microgram of ibogaine. UV and IR spectrophotometry, together with the thin-layer

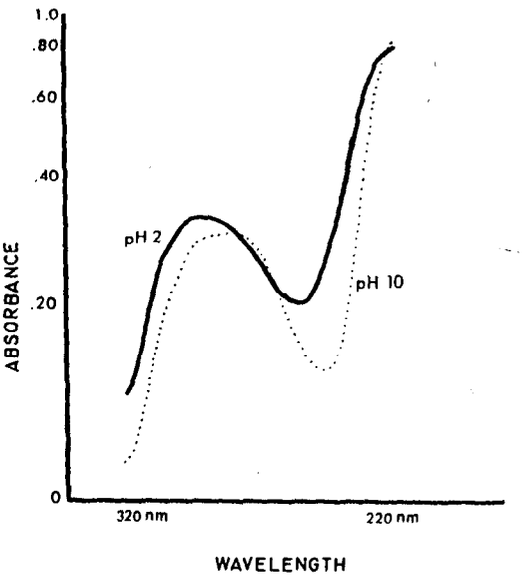

Fig. 1. Ultraviolet absorption spectra of Ibogaine.

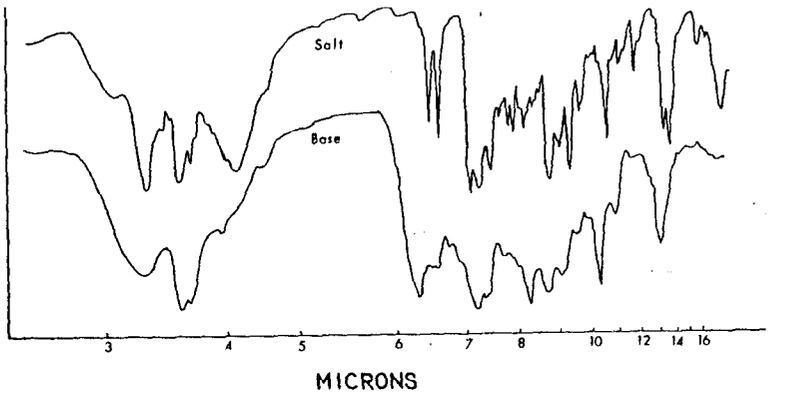

Fig. 2. Attenuated total reflectance infrared spectra of the salt and free base of Ibogaine.

chromatography described, is considered by the authors to constitute a positive criterion for the detection and identification of this drug.

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