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The identification and determination of lysergic acid diethylamide in narcotic seizures

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Methods are described by which lysergic acid diethylamide (LSD) can be identified and determined in the presence of heroin, other narcotics and controlled drugs. Thin layer chromatography and an Ehrlich's-reagent spray is applied to the LSD-containing material. For confirmation, thin layer chromatography after ultra-violet irradiation and acid hydrolysis should be carried out where ergot alkaloids are present. Spectrophotofluorometric analysis of LSD can be used in the presence of heroin, other narcotics and controlled drugs with a standard error of $\pm 2\%$. If ergot alkaloids are present, thin layer separation, elution and subsequent fluorometric analysis are recommended.

LYSERGIC acid diethylamide (LSD) has pharmacological properties (Rothlin, 1957) which have made it the subject of legislation. In Canada, under the Food and Drugs Act (1963), the sale of LSD is prohibited except to authorised persons for experimental research purposes. Rumours recently reached the Food and Drug Directorate that LSD was being used clandestinely and it became necessary to have methods for the detection and determination of LSD in narcotic seizures. Since LSD is a potent drug (normal dose 50–100 μg) it was necessary to develop methods on a microscale. Methods available for the identification of LSD and related drugs include colour tests (Pharmacopeia of the United States of America, 1955), paper chromatography (Foster, Macdonald & Jones, 1949; Stoll & Rüegger, 1954; Macek, 1954; Pöhm, 1958; Rochelmeyer, Stahl & Patani, 1958; Reio, 1960; Alexander, 1960, 1962; Heacock & Mahon, 1961; Taber & others, 1963a,b) and thin-layer chromatography (TLC) (Stahl, 1959; Hofmann, 1961; Stahl & Kaldewey, 1961; Waldi, Schnackerz & Munter, 1961; Gröger & Erge, 1963; Taber & others, 1963a,b).

We describe the development of a rapid, sensitive micro-identification procedure, and the adaptation of a spectrophotofluorometric method (Axelrod, Brady, Witkop & Ewarts, 1956, 1957) for the assay of LSD in presence of narcotics, such as heroin and controlled drugs, i.e., barbiturates and amphetamines.

Experimental

THIN LAYER CHROMATOGRAPHY

This was on Silica Gel G-layers (about 250μ) produced with Desaga-equipment according to Stahl (1959) on 20 × 20 cm glass plates.

System A: Chloroform: methanol (9:1) on plates prepared with Silica Gel G (30 g) and 0·1 N sodium hydroxide (60 ml). Time for a 10 cm-chromatogram is about 20 min. This solvent is applied to basic compounds and their salts before and after irradiation.

System B: Chloroform: methanol: ammonium hydroxide (28%) (40:40:20) on plates prepared with Silica Gel G (30 g) and water (60 ml)

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(Fahmy, Niederwieser, Pataki & Brenner, 1961). Time for a 10 cm-chromatogram is about 40 min. This solvent is used to examine the acid hydrolysis products of seizure material containing LSD and ergot alkaloids.

System C: Morpholine : isopropylether : chloroform (10 : 80 : 10) on Silica Gel G Eosin Y (30 g + 30 mg + 30 ml water)-plates. The time for a 10 cm-chromatogram is about 35 min. This solvent is for barbiturates.

All Rf values reported were measured on sets of two plates chromatographed simultaneously in the same jar.

CHROMOGENIC REAGENTS

1. *p*-Dimethylaminobenzaldehyde (DMBA) (0·5 g) in hydrochloric acid (37%) (5 ml) and ethanol (95 ml) (Hellberg, 1957). LSD and ergot-alkaloids give blue-violet spots. The highest intensity is reached about 10 min after spraying. This reagent is applied to material chromatographed in system A.

2. Ninhydrin (0·2%) in n-butanol (95 ml) and 2 N acetic acid (5 ml) (Farmilo & Genest, 1961). After spraying, the plates were heated for 5 min at 110°. This reagent is applied to acid hydrolysis products chromatographed in system B.

3. Potassium iodoplatinate (Farmilo & Genest, 1961). This reagent is applied to spots containing heroin and controlled drugs.

HYDROLYSIS

25 μl of the test solution is mixed with an equal amount of hydrochloric acid (37%) and sealed into two melting-point capillaries. The capillaries are then placed in a boiling water-bath for 2 hr, after which they are broken, drawn out and their contents applied directly to the thin layer plate to be chromatographed in system B.

IRRADIATION

The test material is spotted in the thin layer plate, irradiated with ultraviolet light from an Aristogrid lamp (3,660 Å; distance 1·7 cm) for 2 hr and chromatographed in system A.

SPECTROPHOTOFLUOROMETRY

A Bowman-Aminco spectrophotofluorometer, 1p28 phototube; multiplier position 0·01 and 0·03; slit arrangement No. 3 is used. The seizure material (10 mg, containing 10–20 μg LSD) is dissolved in water (10 ml) and diluted with 0·004 N hydrochloric acid (1 : 10). The fluorescent maximum is measured at 435 mμ after activation at 335 mμ and compared with values obtained for the standard curve (0·01–0·4 μg LSD* ml). All quantitative experiments were in darkened rooms.

* LSD-25 Sandoz used throughout.

ELUTION OF SPOTS

Seizure material is dissolved in water and an aliquot to contain 1–10 μg LSD per spot is chromatographed in system A. The spots are detected by ultra-violet light, encircled and transferred by suction into a small (5 ml) light-protected wash bottle containing 2 ml methanol. The eluate is shaken (5 min) and diluted with 0·004 N hydrochloric acid so that it contains 0·01–0·4 μg LSD/ml. Spectrofluorometric analysis against a blank eluate is as described above.

Results

Separation of LSD from heroin, other narcotics, and controlled drugs can be achieved by chromatography in system A. LSD (0·05 μg) can be identified by the light blue fluorescence in ultra-violet light and by the DMBA-spray. Because of the close relation of drugs of the ergot group to LSD they were also included in this study (Table 1); a good distribution of the group over the Rf range is given by system A.

TABLE 1. Rf VALUES OF LYSERGIC ACID DERIVATIVES, NARCOTICS AND CONTROLLED DRUGS

Compound	Rf values	Compound	Rf values
LSD	0·60	Dihydroergocristine	0·57
Ergonovine maleate	0·18	Ergotoxine	0·45–0·69–0·81
Ergometrinine	0·43	Heroin	0·48
Ergotamine tartrate	0·52	Morphine	0·10
Ergotaminine	0·72	Codeine	0·30
Ergocristine	0·71	Thebaine	0·53
Ergocristinine	0·77	Narcotine	0·77
Lysergic acid	0·05	Papaverine	0·77
Dihydroergotamine	0·35	Amphetamine	0·16
		Methamphetamine	0·17

TABLE 2. Rf VALUES OF LYSERGIC ACID DERIVATIVES AFTER IRRADIATION WITH ULTRA-VIOLET LIGHT

Compound	Rf values	
	DMBA-spray	Ultra-violet fluorescence
LSD	0·61	0·80 GB*
	0·38	0·73 Y
		0·61 B
		0·47 YB
Ergotamine tartrate	0·54	0·54 B
	0·51	0·35 Y
	0·31	
Ergocristine	0·72	0·72 B
	0·45	0·43 Y
Dihydroergocristine	0·57	0·66 YG
		0·35 YG
Ergotaminine	0·72	0·72 Gy
	0·52	0·52 Or
		0·20 Gy
		0·17 Ol
Heroin	0·48	0·48 B
		0·13 B

* G, green; Y, yellow; B, blue; Gy, grey; Or, orange; Ol, olive.

Since the positive DMBA reaction is given by all and a light blue fluorescence by most lysergic acid derivatives of pharmaceutical importance, further methods were developed to distinguish LSD from the more important ergot alkaloids. Table 2 shows chromatography results of LSD and related lysergic acid derivatives after irradiation with ultra-violet light. Compounds which travel close to LSD before irradiation develop additional spots after treatment with ultra-violet light and the DMBA spray. LSD, ergocristine and ergotaminine give one, dihydrocristine none and ergotamine three DMBA-positive spots. The fluorescent spots do not always coincide with the DMBA spots.

Both methods for the analysis of LSD in presence of other lysergic acid derivatives are based on Rf measurements after DMBA-treatment. Use was made of the fact that after acid hydrolysis of ergot alkaloids the lysergic acid moiety is decomposed completely, whereas the peptide part at C(8) is hydrolysed to yield amino-acids (Stoll & Hofmann, 1950). If a strongly alkaline solvent is chosen for the chromatography of the hydrolysis products, the amino-acids, after a ninhydrin spray, give reddish spots in amongst the ergot alkaloids, whereas LSD gives no reaction (Table 3).

TABLE 3. Rf VALUES OF AMINO-ACIDS AND ACID HYDROLYSIS PRODUCTS OF LYSERGIC ACID DERIVATIVES

Compound	Rf values
LSD	No spot
Dihydroergotamine	0·49–0·78
Ergotamine	0·48–0·78
Ergotaminine	0·49–0·78
Dihydroergocristine	0·49–0·79
Ergocristine	0·49–0·77
Ergotoxine	0·49–0·60–0·68 (faint) –0·78
Proline	0·49
Valine	0·61
Leucine	0·69
Phenylalanine	0·79

TABLE 4. Rf VALUES OF BARBITURATES, HEROIN AND LSD

Compound	Rf value
Pentobarbitone	0·40
Amylobarbitone	0·44
Quinalbarbitone	0·49
Phenobarbitone	0·23
Butabarbital	0·44
Barbitone	0·39
Hexobarbitone	0·46
LSD	0·34
Heroin	0·35*

* Elongated

The presence of barbiturates is anticipated in LSD-containing seizure material. If preliminary colour and crystal tests indicate barbiturates, chromatography in system C is suggested. In this system (for Rf values see Table 4) on eosin-impregnated plates (Eberhardt, Freundt & Langbein, 1962) the barbiturates show as dark spots on a light yellowish fluorescent

background, whereas LSD gives the usual blue fluorescence. Quinine, occasionally found in heroin seizures (Fulton, 1953), also gives a light blue fluorescence with slightly different maxima and is easily separable from LSD in system A (Rf quinine, 0·31).

For quantitative analysis we prepared seizure-like material by adding known amounts of LSD to heroin seizures and in one instance to a cube of sugar. The material was dissolved in water and, after appropriate dilution, a determination of LSD was made by spectrophotofluorometry (Table 5). Other substances were tested for interference in the fluorometric procedure. As well as heroin, barbitone, phenobarbitone, pentobarbitone, morphine, codeine, amphetamine and methamphetamine in a 1000-fold excess were measured in presence of LSD and found not to interfere. Most ergot alkaloids show a similar fluorescence curve to LSD.

TABLE 5. RECOVERY OF LSD ADDED TO SEIZURE MATERIAL

Seizure No.	LSD added μg	LSD found μg
Heroin 6	20	19·9
Sugar cube	20	19·8
Heroin 6	10	10·1
Heroin 122	10	10·1
Heroin 481 B	10	10·1

In presence of lysergic acid derivatives, LSD had to be chromatographed in system A, eluted and measured as described on page 252. Methanol gave an average recovery of 91% from 1–10 μg spots of LSD and is superior to methylene chloride : methanol (9 : 1) reported by Gröger & Erge (1963). These recoveries are better than those obtained in trials with a partition method (Axelrod & others, 1956, 1957).

Discussion

The method described is useful forensically because it is simple and rapid. For the qualitative identification of LSD in the presence of narcotics, other controlled drugs and pharmaceutically important ergot alkaloids, thin layer chromatography is preferred to pharmacopoeial colour tests or paper chromatography because the general colour tests for ergot alkaloids require 1 mg of substance while paper chromatography often needs pretreatment of the paper, and is more time consuming. Of the thin layer systems previously reported, that of Hofmann (1961) was closest to the one needed. Of the spray reagents, the DMBA spray was more specific and sensitive than potassium iodoplatinate (Farmilo & Genest, 1961), ninhydrin (Berg, 1952), vanillin (Nigam, Saharabudhe & Levi, 1963) *p*-toluene sulphonic acid (Leemann & Weller, 1960), and concentrated sulphuric acid (Stoll & Schlientz, 1955).

To obtain more values for identification, chromatography in a series of solvents, as described for narcotics by Genest & Farmilo (1961), or 2-dimensional chromatography, as reported by Heacock (personal communication) for lysergic acid derivatives, could be used. Thin layer

chromatography after ultra-violet irradiation in the same solvent is preferable to either procedure, because additional basic compounds characteristic for each lysergic acid derivative are produced. Through the work of Stoll & Schlientz (1955) and Hellberg (1957) it is known that lumi-ergot alkaloids are produced if acid aqueous solutions of ergot alkaloids are exposed to ultra-violet light. Water is added to the C(9–10) double bond thus creating another asymmetric C-atom at C(10). The lumi-alkaloids should exist, therefore, in four series designated as lumi-lysergic and lumi-isolysergic I and II. Assuming that no epimerisation takes place at C(8)—there should be two spots if lumi I- and lumi II-compounds are formed under our experimental conditions. This assumption is strengthened by the observation that no additional DMBA spots were found with dihydro-ergocristine. Two additional spots were found in ergotamine, but only one additional spot in LSD, ergocristine and ergotaminine. Either, one of the possible lumi-compounds is not formed, or, if they are both formed, the second one cannot be separated from the original alkaloid by our system. Stoll & Schlientz (1955) report that in aqueous solution preferably lumi-LSD I and to a much smaller degree lumi-LSD II are produced. Hellberg's data indicate that the "β-lumi"*-compound produced by irradiation could not be separated from the original alkaloid in the case of ergotaminine and ergocristinine. From ultra-violet observation of the thin layer plates chromatographed after irradiation, it is evident that under these experimental conditions transformation products other than lumi compounds I and II are formed. Schlientz, Brunner, Hofmann, Berle & Stürmer (1961) have reported aci-ergot alkaloid formation which was obtained under other experimental conditions. The finding that lumi-alkaloids give a characteristic reaction with concentrated sulphuric acid (Stoll & Schlientz, 1955) could not be confirmed, when applied to thin layer plates, since LSD and the natural ergot alkaloids gave spots of the same colour before irradiation. The fact remains that after ultra-violet treatment, characteristic DMBA- and ultra-violet patterns are produced which are helpful for micro-identification purposes. Microhydrolysis was used to confirm the identity of LSD in presence of related lysergic acid derivatives. Foster & others (1949) described the acidic hydrolysis of ergot alkaloids on a mg scale, and subsequent analysis of the hydrolysate on paper chromatograms. Diethylamine, the hydrolysis product of LSD, does not show up in system B as do the amino-acids produced from most ergot alkaloids. In the instance of ergonovine, which is easily separable from LSD in system A, a spot with an Rf between valine and leucine was found. This is probably 2-amino-1-propanol having the same Rf in system B as 1-amino-2-propanol.

Ultra-violet spectrometric assay of LSD in presence of heroin is possible but is not sensitive enough (log $\epsilon = 3{\cdot}99$ at 313 mμ) and is subject to interferences. The van Urk-reaction (Vining & Taber, 1959; Taber & others, 1962, 1963), is not as sensitive as spectrophotofluorometry (Axelrod & others, 1956, 1957; Vining & Taber, 1959; Taber & others,

* Stoll and Hellberg use different nomenclature.

1963a,b). Udenfriend, Duggan, Vasta & Brodie (1957) report 0·002 μg/ml to be the "practical" sensitivity for LSD as defined by Duggan, Bowman, Brodie & Udenfriend (1957). The range from 0·01 to 0·4 μg/ml was preferred because less background fluctuation of the fluorometer was encountered than at more sensitive photomultiplier positions. A straight-line relationship between concentrations and fluorometer-readings was confirmed for this range (Sprince, Rowley & Jameson, 1957). The method for LSD analysis in heroin seizure material is simple because there is no interference by heroin itself or by excipients normally found in such material. Although fluorometric methods for morphine, codeine (Brandt, Erlich, Rogosinky & Cheronis, 1961; Brandt, Olsen & Cheronis, 1963) and barbiturates (Duggan & others, 1957) have been reported in different media and at different activation and fluorescent wavelengths, these compounds, and the amphetamines, do not interfere in the direct LSD analysis.

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