Clin. chim. Acta 45, 375–386 (1973).

CCA 5599

ANALYTICAL STUDY OF SOME IMPORTANT HALLUCINOGENS BY A COMBINED FLUORIMETRIC AND PHOSPHORIMETRIC METHOD

J. J. AARON*, L. B. SANDERS AND J. D. WINEFORDNER**
Department of Chemistry, University of Florida, Gainesville, Fla. 32601 (U.S.A.)
(Received December 27, 1972)

SUMMARY

Room-temperature fluorescence and low-temperature phosphorescence excitation and emission spectra, phosphorescence lifetimes and/or fluorimetric and/or phosphorimetric analytical curves and limits of detection have been determined in methanol–water solution for nine hallucinogenic drugs, including lysergic acid diethylamide (LSD), lysergic acid, 2,5-dimethoxy-4-methylamphetamine (STP; DOM), mescaline and indole-type hallucinogens. Vibrational structure of phosphorescence bands, phosphorescence lifetimes and effect of pH on the phosphorescence spectra at 77°K are shown to be of value for the identification of these hallucinogens. Fluorimetric and phosphorimetric analytical curves are linear over large concentration ranges (10^2–10^4) concentration units. Detection limits are very low, between 1 and 23 ppb, according to the fluorescence and/or phosphorescence spectrum yield of the particular compound. The combined fluorimetric and phosphorimetric method is demonstrated to improve largely the sensitivity of previous analytical techniques used for the determination of hallucinogens.

INTRODUCTION

The identification and the quantitative determination of hallucinogenic drugs now represent an important analytical problem. However, although several analytical procedures have been recently described for these drugs[1], most of these techniques are not sufficiently simple, sensitive, and selective for the needs of pharmaceutical and forensic applications[1,2]. Methods currently available for the characterization and/or determination of hallucinogens are based upon gas–liquid chromatography[1,3], thin-layer chromatography (TLC)[1,3,4], mass spectrometry[1], infrared and ultraviolet absorption spectrophotometry[1,3,5,6] and spectrofluorimetry[1,4,6–11]. In terms of sensitivity and simplicity, fluorimetry seems to be a very promising technique because of the strong fluorescence of many hallucinogens[9,10]. For example, some in-

* On leave from: Laboratoire de Chimie Organique Physique, Paris, France.
** Author to whom reprint requests should be sent.

dole-type hallucinogens (psilocybin, *N,N*-dimethyltryptamine, *N,N*-diethyltryptamine), lysergic acid diethylamide (LSD) and 2,5-dimethoxy-4-methylamphetamine (STP; DOM) have been determined quantitatively by fluorimetry, with practical detection limits ranging between 0.01 and 1 μg/ml[1,9,10].

Combination of fluorimetric and TLC techniques have permitted the measurement of 0.1–2 μg of LSD[1,9,11] and the use of the photoproducts of LSD, obtained under ultraviolet irradiation, has also been proposed for its determination[8,11]. Fluorescence properties of several cannabis constituents and their photoproducts or their fluorescent-labeled derivatives have been used for their detection and quantitative determination at the nanogram level[6,12]. In spite of their high sensitivity, fluorimetric techniques suffer, to some extent, lack of selectivity, especially in the case or indole-type hallucinogens, for which excitation and emission maxima are broad and often occur at nearly the same spectral position[9,10]. In this regard, phosphorescence properties could be valuable for the identification of hallucinogenic compounds, because of

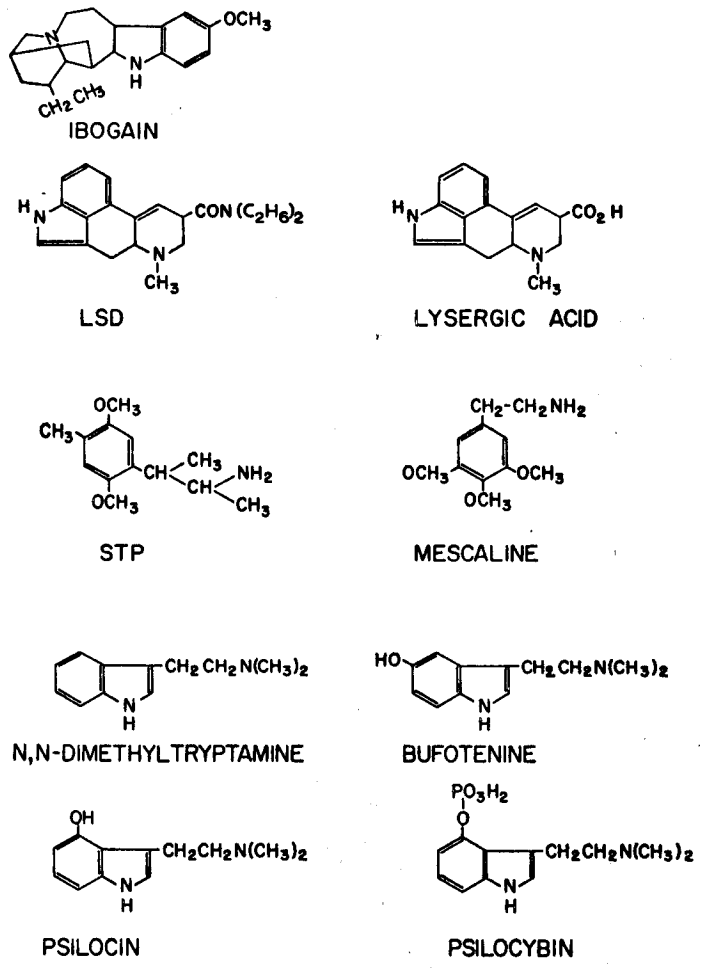

Fig. 1. Structure of hallucinogens.

the presence in most of these molecules of an indole-type structure, known to exhibit a phosphorescence spectrum sensitive to small variations of structural features[13]. Also, indole derivatives are very highly phosphorescent compounds[13]. Nevertheless, no attempt appears to have been made until now to perform phosphorescence analysis of hallucinogens as a general study.

The purpose of the present paper is to describe a combined fluorimetric and phosphorometric method for the characterization and quantitative determination of several important hallucinogenic drugs (structures in Fig. 1). We wish to show that the fluorescence properties of hallucinogens are of interest for their quantitative analysis at the nanogram level and their phosphorescence properties are useful for the identification of an hallucinogenic drug present in an unknown sample. For some hallucinogens, phosphorimetry can also be considered as a more sensitive quantitative method of analysis than fluorimetry. Recent instrumental improvements obtained in this laboratory for the application of phosphorimetry to aqueous, snowed matrices (refs. 14–17) have prompted us to use a predominantly aqueous solvent (methanol–water, 10:90, v/v) for both fluorimetric and phosphorimetric measurements.

EXPERIMENTAL

Apparatus

Phosphorescence and fluorescence excitation and emission spectra were recorded on an Aminco–Bowman spectrophotofluorimeter (SPF) equipped, for the phosphorescence spectra, with an Aminco–Keirs phosphoroscope attachment, and, in all cases, with an Aminco Ratio Photometer (American Instrument Co., Silver Spring, Md., U.S.A.). An Aminco xenon lamp power supply (Mode 422–818) was used to power the 150-W Hanovia xenon arc lamp. The RCA-1828 multiplier phototube (American Instrument Co.) was powered by the Aminco Ratio Photometer. While phosphorescence and fluorescence spectra were obtained on the Ratio Photometer in the ratio mode, phosphorescence and fluorescence intensities were measured in the normal mode with a low-noise nanoammeter previously described[18].

For the fluorescence measurements at room temperature (298°K), a regular 1 cm × 1 cm quartz cell was used as the sample cell and contained 1 ml of solution.

Phosphorescence measurements were performed at 77°K, with a rotating capillary tube, approximately 0.9 mm internal diameter and 5 mm outer diameter, made from T21 Suprasil quartz capillary tubing (Amersil Inc., Hillside, N.J., U.S.A.). This sample cell was used in the same conditions previously described[14] and contained about 20 μl of solution.

Phosphorescence lifetimes greater than 1 sec were measured by recording the nanoammeter output as a function of time with the Aminco model 1620-827 X–Y recorder in the time base mode, after complete termination of the exciting radiation by a guillotine-type shutter. Lifetimes of less than 1 sec were measured with the pulsed source, time resolved phosphorimeter recently described[19,20].

UV absorption spectra were measured at room temperature with a Beckman DB-G grating spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif., U.S.A.).

Chemicals

LSD, 2,5-dimethoxy-4-methylamphetamine (STP; DOM), psilocin and psilocybin were obtained from NIMH, Center for Studies of Narcotics and Drug Abuse, Rockville, Md., U.S.A. D-Lysergic acid (Grade II), mescaline hydrochloride, bufotenine monoxalate, ibogaine hydrochloride and *N,N*-dimethyltryptamine were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. All these drugs were used without further purification, and their UV absorption spectra were in good agreement with available literature data[3]. In the case of D-lysergic acid and mescaline, however, trace impurities were detected, respectively, by their phosphorescence and fluorescence spectra. These impurities were, however, in too small concentration ($\simeq$ 0.5%) to be of consequence in our study.

Solvents used were methanol (Matheson, Coleman and Bell, Manufacturing Chemists, Norwood, Ohio, U.S.A., "spectroquality" grade), and deionized water.

Sodium iodide and sodium bromide were used in the heavy atom effect studies (Fisher Scientific Co., Fair Lawn, U.S.A., "certified reagent" grade).

Procedure

Stock solutions of hallucinogenic drugs were prepared in attenuated daylight at concentrations between $3 \cdot 10^{-4}$ and $3 \cdot 10^{-3}$ M (according to the solubility of the compound) in methanol–water (10:90, v/v) mixtures. For some drugs, basic ($\simeq 2 \cdot 10^{-1}$ M NaOH, pH $\simeq$ 11) and acidic ($\simeq 6 \cdot 10^{-2}$ M HCl, pH $\simeq$ 2) methanol–water solutions were also prepared. Solutions of LSD in methanol–water (10:90, v/v) with 0.75 M sodium bromide or sodium iodide were also prepared for the phosphorescence studies. For the analytical curves, more dilute solutions (concentrations down to $3 \cdot 10^{-9}$ M) were obtained by successive dilutions with the appropriate solvent.

The slit arrangement used was 3,2,2,0.2 (in nm, corresponding to 18 and 1 nm spectral half-band passes) for fluorescence spectra; 3,2,2,3 (corresponding to 17 and 11 nm spectral half-band passes) for fluorescence intensity measurements; 3,3,3,0.2 (corresponding to 17 and 1 nm spectral half-band passes) for phosphorescence spectra and 3,3,3,3 (corresponding to 17 and 17 nm spectral half-band passes) for phosphorescence intensity measurements.

RESULTS AND DISCUSSION

Fluorescence spectral properties (see Table I)

Fluorescence excition and emission spectra of hallucinogens were obtained at 25° in methanol–water (10:90, v/v) (Table I). All fluorescence excitation spectra were corrected for the response and the intensity fluctuations of the xenon arc lamp with the ratio photometer. Maxima wavelengths values of the fluorescence excitation spectra are very close to the UV absorption maxima values for the same conditions of temperature and solvent (Table I), which confirms that we have observed the native fluorescence emission of the hallucinogenic drugs. Wavelength values for the main excitation and emission peaks agree also with previous literature fluorescence data obtained in different solvent conditions[10]. All the hallucinogens show similar emission fluorescence spectra with a single, broad peak. This prominent peak occurs in the 420–430-nm region for LSD and lysergic acid and in the 320–360-nm region for the indole-type hallucinogens, STP, and mescaline. Although it is therefore possible

TABLE I

FLUORESCENCE AND ABSORPTION SPECTRAL PROPERTIES OF HALLUCINOGENS IN METHANOL–WATER (10:90, v/v) AT 298° K
Concentrations approximately 10^{-5} to 10^{-4} M according to the compounds.

Hallucinogen	*Peak wavelength*, (nm)*		
	*Absorption spectra***	*Fluorescence excitation spectra**,†*	*Fluorescence emission spectra†*
LSD-25	(*242*), 312	246, *312*	430
D-Lysergic acid	(*240*), 312	248, *311*	420
Mescaline hydrochloride	(*225*), 270	(250), *270*	368
STP; DOM	(*220*), 290	221, *284*	330
N,N-Dimethyltryptamine	(*216*), 282, 288	229, *278*	359
Bufotenine monoxalate	(*222*), 277, (295)	223, *280*, (288)	342
Psilocin	*220*, 265, 283, 295	221, *276*	320
Psilocybin	*219*, 268	*222*, *270*	341
Ibogaine hydrochloride	*220*, 279, (290)	235, *290*	355

* Peak wavelength error = ± 3 nm.
** Wavelength of the main peak (or inflection) is in italics; wavelength of an inflection is given in parentheses.
† All fluorescence excitation and emission spectra obtained with the ratio photometer.

to distinguish LSD or lysergic acid from the other hallucinogens by their fluorescence spectra, a more precise identification of the individual drugs is not feasible by fluorescence, because of the broadness of the emission fluorescence peak, the absence of vibrational structure in it, and the similarity of the excitation spectra of the indole-type hallucinogens.

Phosphorescence spectral properties (see Tables II and III)

Phosphorescence excitation and emission spectra of hallucinogens were determined with the ratio photometer in methanol–water, (10:90, v/v), at 77°K. As expected,

TABLE II

PHOSPHORESCENCE PROPERTIES OF HALLUCINOGENS IN METHANOL–WATER (10:90, v/v) AT 77° K
Concentrations approximately 10^{-5} to 10^{-4} M according to the compounds. Phosphorescence excitation and emission spectra obtained with the ratio photometer.

Hallucinogen	*Peak wavelength* (nm)*		
	*Excitation spectra***	*Emission spectra***	*Lifetimes*** (sec)*
LSD-25	(250), *311*	*512*, 540	$0.004^{a,b}$
D-Lysergic acid	238, *310*	*518*, 552	$0.1_6{}^{a}$
Mescaline hydrochloride	250, *274*	*450*	1.3_5
STP; DOM	220, *289*	(390), *411*, 425, (435)	3.9_5
N,N-Dimethyltryptamine	230, *286*	419, *434*, 455	6.9
Bufotenine monoxalate	230, (293), *301*	(424), *447*	1.9^{c}
Psilocin	239, *292*	423, *443*, (465), (498)	4.0
Psilocybin	229, *280*	410, *430*, 450, (483)	5.5
Ibogaine hydrochloride	238, *292*	(415), *430*, (450)	8.6_5

* Peak wavelength error = ± 3 nm.
** Wavelength of the main peak is in italics. Wavelength of inflection is given in parentheses.
*** Precision of the lifetime values ± 5% except otherwise noticed.
[a] Precision of lifetime values of LSD and lysergic acid = ± 20%.
[b] Previous literature value: t = 0.0177 sec in ethanol glass at 77 °K (ref. 21).
[c] In NaOH 0.2 M methanol–water (10:90, v/v) solution.

TABLE III

SINGLET–TRIPLET SPLITTING OF HALLUCINOGENS

Hallucinogen	*Absorption maximum* (cm^{-1})	*Phosphorescence maximum* (cm^{-1})	S_1–T_1 *Energy difference* (cm^{-1})
LSD-25	32050	19500	12550
D-Lysergic acid	32050	19300	12750
Mescaline hydrochloride	37000	22200	14800
STP; DOM	34500	24300	10200
N,N-Dimethyltryptamine	35400	23050	12350
Bufotenine	36100	22380	13720
Psilocin	37700	22580	15120
Psilocybin	37300	23250	14050
Ibogaine hydrochloride	35800	23250	12550

wavelength values of the phosphorescence and fluorescence excitation maxima are very similar (Table II). Phosphorescence emission spectra are well resolved and show a characteristic vibrational structure for each particular compound (Figs. 2–8). With the exception of LSD (ref. 21), the triplet state of none of these hallucinogenic drugs has been investigated. Therefore, it is of interest to study their phosphorescence properties in more detail. All the indole-type hallucinogens show phosphorescence lifetimes longer than 1 sec, which indicates that the triplet state is of π,π^* type, in agreement with a similar assignment for the triplet state of indole and its carboxylic

TABLE IV

FLUORESCENCE AND PHOSPHORESCENCE ANALYTICAL CHARACTERISTICS OF HALLUCINOGENIC DRUGS

In neutral methanol–water (10:90, v/v) solution, except otherwise noticed. Fluorescence and phosphorescence measurements, respectively, at 298° K and 77° K.

Hallucinogen	*Fluorescence*			*Phosphorescence*		
	Concentration range (M) of near linearity[a]	*Limit of detection*[b] *(ng/ml)*	*Minimal detectable amount*[c] *(ng)*	*Concentration range (M) of near linearity*[a]	*Limit of detection*[b] *(ng/ml)*	*Minimal detectable amount*[c] *(ng)*
LSD-25[d]	10^3	6.5	6.5	10^4	8	0.16
D-Lysergic acid	10^4	1.3	1.3	—	—	—
STP; DOM	10^3	10	10	10^3	10	0.2
N,N-Dimethyltryptamine	10^3	15	15	10^3	15	0.3
Bufotenine monoxalate[e]	—	—	—	10^4	1	0.02
Psilocin	10^3	100	100	$2 \cdot 10^3$	6	0.1
Psilocybin	10^3	23	23	10^3	14	0.3
Ibogaine hydrochloride	10^3	7	7	10^3	10	0.2

[a] Near linearity means region over which analytical curve deviates by less than 1% from linearity.
[b] Limit of detection is defined as the concentration (in ng/ml or ppb) giving a fluorescence or phosphorescence signal (located on the linear part of the analytical curve) two times greater than the background noise. The background signal was subtracted from the observed signal value.
[c] Absolute limiting quantity of compound detected by the method—calculated from the limit of detection with a volume of sample of 1 ml for the fluorescence measurements and of 20 μl for the phosphorescence measurements.
[d] Phosphorescence analytical curve obtained in NaI, 0.75 M methanol–water (10:90, v/v) solution.
[e] Phosphorescence analytical curve obtained in NaOH, 0.2 M methanol–water (10:90, v/v) solution.

acid derivati
from the diffe
greater than
phosphorescei
and lysergic a
is not in agree
Further work
lysergic acid.

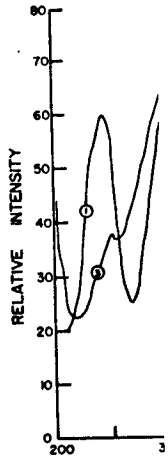

Fig. 2. Fluores
2, fluorescence
citation and em

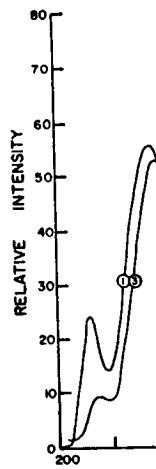

Fig. 3. Fluore
v/v). 1 and 2,
phorescence e

acid derivatives[13,22]. That the excited triplet–singlet energy differences calculated from the difference between absorption and phosphorescence maxima frequency* are greater than 10000 cm^{-1} for all the hallucinogens (Table III) is also in favor of a π,π^* phosphorescence[23]. However, the very short phosphorescence lifetime values for LSD and lysergic acid would rather indicate a n,π^* triplet state (Table II) although this is not in agreement with the very strong fluorescence of these particular compounds. Further work is necessary to investigate the nature of the triplet state of LSD and lysergic acid.

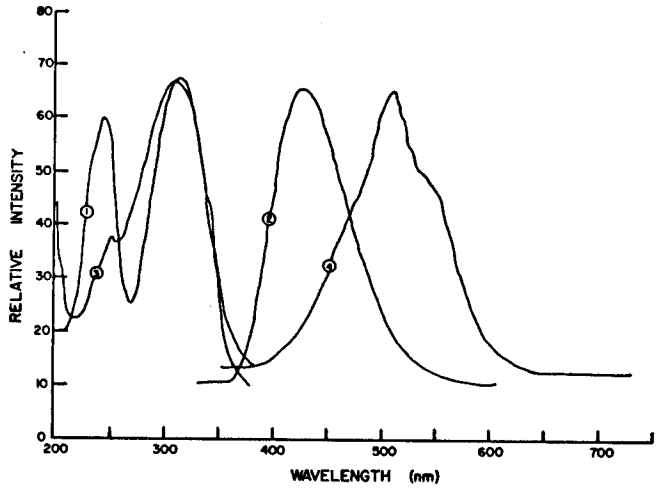

Fig. 2. Fluorescence and phosphorescence spectra of LSD in methanol–water (10:90, v/v). 1 and 2, fluorescence excitation and emission spectra at 298° ($2.5 \cdot 10^{-5}$M). 3 and 4, phosphorescence excitation and emission spectra at 77° K ($2.5 \cdot 10^{-4}$ M).

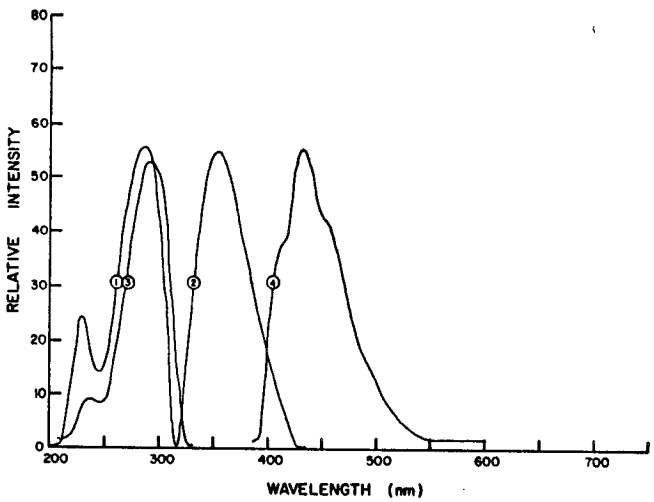

Fig. 3. Fluorescence and phosphorescence spectra of lysergic acid in methanol–water (10:90, v/v). 1 and 2, fluorescence excitation and emission spectra at 298° K (10^{-5} M). 3 and 4, phosphorescence excitation and emission spectra at 77° K ($5 \cdot 10^{-4}$ M).

Qualitative analysis (see Table IV)

Because of their specificity, phosphorescence spectra and phosphorescence lifetimes can be used in a complementary mode to identify the hallucinogen content of an unknown drug sample, after separation of the different constituents, for instance, by TLC. For example, LSD and lysergic acid exhibit a phosphorescence maximum at a much longer wavelength (512 nm, and 518 nm respectively, see Figs. 2 and 3) than all the other hallucinogens of the present study. Lysergic acid and LSD are easily distinguishable by the different vibrational structure and wavelength values of their phosphorescence bands. Psilocybin and ibogaine, which show very similar

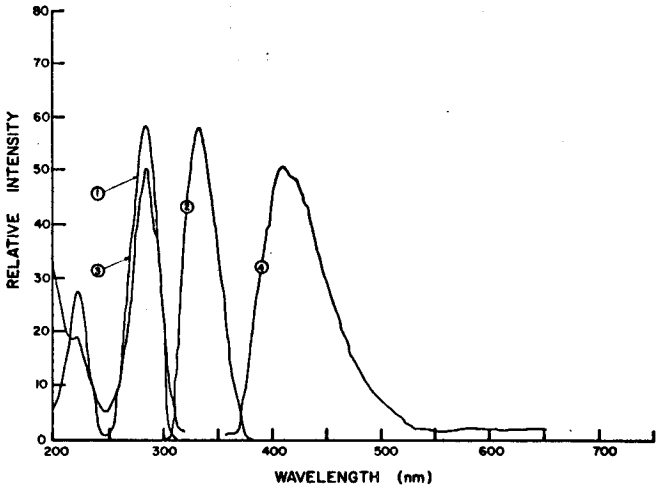

Fig. 4. Fluorescence and phosphorescence spectra of STP ($5 \cdot 10^{-5}$ M) in methanol–water (10:90 v/v). 1 and 2, fluorescence excitation and emission spectra at 298° K (10^{-5} M). 3 and 4, phosphorescence excitation and emission spectra at 77° K.

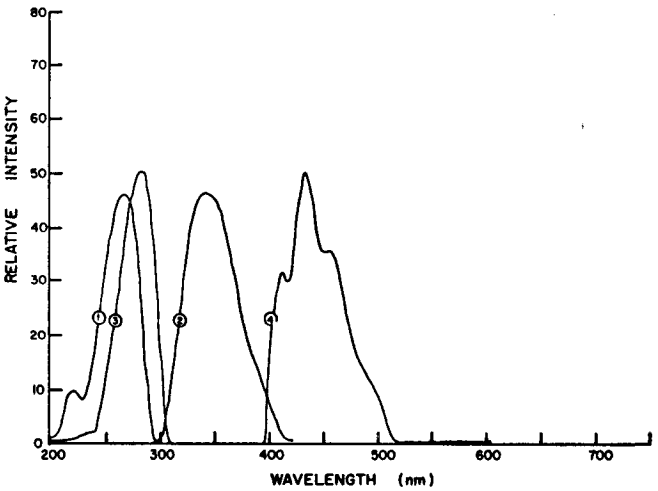

Fig. 5. Fluorescence and phosphorescence spectra of psilocybin ($5 \cdot 10^{-5}$ M) in methanol–water (10:90, v/v). 1 and 2, fluorescence excitation and emission spectra at 298° K. 3 and 4, phosphorescence excitation and emission spectra at 77° K.

vibrational structure and position of their phosphorescence bands (see Figs. 5 and 6) can be alternatively characterized by their different phosphorescence lifetime values, respectively 5.5 and 8.6 sec. Preliminary pH studies indicate that effect of pH on the phosphorescence spectra is also of value for the identification of an hallucinogen. In strongly alkaline solution, phosphorescence bands of psilocin and bufotenine (with phenolic groups) are red-shifted by about 400–600 cm^{-1}, while a non-phenolic indole hallucinogen like dimethyltryptamine does not exhibit any significant shift of its phosphorescence spectra (see Figs. 7 and 8).

Quantitative fluorimetric and phosphorimetric studies (see Table V)

All hallucinogens except mescaline have fluorescence and/or phosphorescence

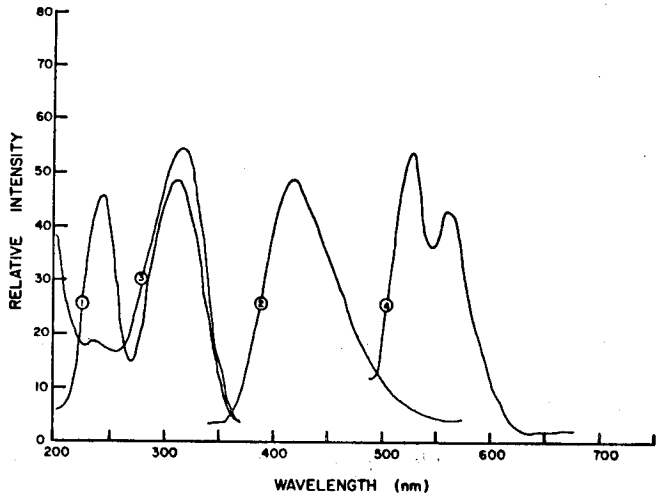

Fig. 6. Fluorescence and phosphorescence spectra of ibogaine in methanol–water (10:90, v/v). 1 and 2, fluorescence excitation and emission spectra at 298° K ($3 \cdot 10^{-5}$ M). 3 and 4, phosphorescence excitation and emission spectra at 77° K ($3 \cdot 10^{-4}$ M).

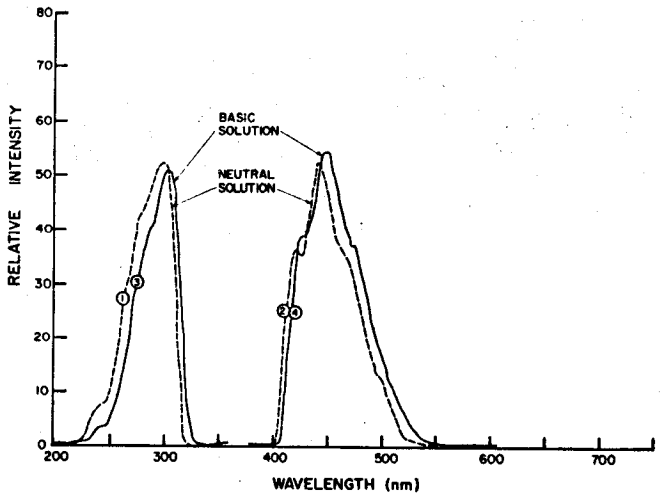

Fig. 7. Effect of pH upon phosphorescence excitation (1 and 3) and emission (2 and 4) spectra of psilocin ($5 \cdot 10^{-4}$ M) in methanol–water (10:90, v/v) at 77° K.

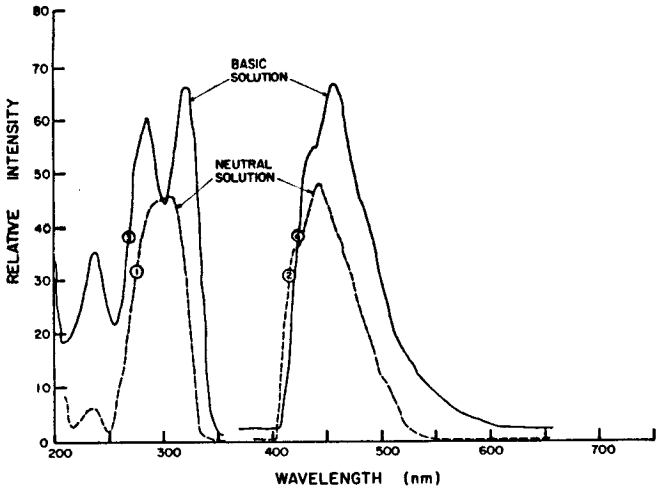

Fig. 8. Effect of pH upon phosphorescence excitation (1 and 3) and emission (2 and 4) spectra of bufotenine in methanol–water (10:90, v/v) at 77° K. Bufotenine concentration is $3 \cdot 10^{-4}$ M in neutral solution and $3 \cdot 10^{-5}$ M in basic solution (0.2 M NaOH).

quantum yields sufficiently high to be analytically useful. However, ratios of the fluorescence to phosphorescence quantum yields vary considerably according to the structure of the hallucinogen. Indeed, while LSD and lysergic acid are very strongly fluorescent but weakly phosphorescent, indole-type hallucinogens are generally more phosphorescent than fluorescent. Fluorescence and phosphorescence of mescaline are too weak to be useful in quantitative determination. Fluorescence and phosphorescence intensities of STP are at an equal high level. A preliminary study of the effect of pH upon the magnitude of phosphorescence or fluorescence signals of hallucinogenic drugs shows that a neutral medium is satisfactory for all the drugs, except in the case of bufotenine for which phosphorescence signals were about 2.5 times greater in basic than in neutral solution. Therefore, analytical curves were obtained in basic solution (0.2 M NaOH) for this last compound. In the case of LSD, phosphorescence signal is increased upon addition of sodium bromide and sodium iodide, 2.5 and 4 times respectively. This increase is typical of an external heavy-atom effect, characterized by the enhancement of the triplet population by radiationless intersystem-crossing and of the triplet-to-singlet transition probability[24]. Consequently, the phosphorescence analytical curve of LSD was determined in sodium iodide solution, which permitted a lower phosphorescence limit of detection of this weakly phosphorescent compound.

The fluorimetric and phosphorimetric limits of detection of hallucinogens are reported in Table V. All fluorescence and phosphorescence analytical curves have been determined with a 5% relative standard deviation. It is striking to notice the low detection limits of both fluorimetric and phosphorimetric methods, *i.e.*, limits of detection of most of the hallucinogens are in the range 1–20 ppb. In the case of LSD and lysergic acid, fluorimetry results in lower detection limits than phosphorimetry in spite of the enhancement of the phosphorescence of LSD in sodium iodide solutions. In the case of the indole-type hallucinogens, phosphorimetry results in lower detection limits for some molecules than by fluorimetry. However, much lower absolute quan-

TABLE V

COMPARISON OF DETECTION LIMITS OF FLUORIMETRY AND PHOSPHORIMETRY WITH OTHER ANALYTICAL METHODS

Hallucinogen	*Method of determination*	*Limit of detection (ng/ml)*	*Minimal detectable amount (ng)*	*Reference*
LSD-25	Color tests	1–50	1000	3
	Fluorimetry			1, 9, 10
	In situ fluorimetry on TCL		100	11
	Fluorimetry	6.5	6.5	This work
	Phosphorimetry	8	0.16	This work
D-Lysergic acid	Crystal test		250	3
	Fluorimetry	1.3	1.3	This work
STP; DOM	Color tests		100–250	3
	Gas chromatography		50	25
	Fluorimetry	500		10
	Fluorimetry	10	10	This work
	Phosphorimetry	10	0.2	This work
N,N-Dimethyltryptamine	Color tests		100–1000	3
	Crystal tests		500	3
	Fluorimetry	500		10
	Fluorimetry	15	15	This work
	Phosphorimetry	15	0.3	This work
Bufotenine monoxalate	Color tests		100–5000	3
	Crystal tests		250	3
	Phosphorimetry	1	0.02	This work
Psilocin	Color tests		100–250	3
	Crystal tests		1000	3
	Fluorimetry	100	100	This work
	Phosphorimetry	6	0.1	This work
Psilocybin	Color tests		500	3
	Fluorimetry	1000		10
	Fluorimetry	23	23	This work
	Phosphorimetry	14	0.3	This work
Ibogaine hydrochloride	TLC			26
	Fluorimetry	7	7	This work
	Phosphorimetry	10	0.2	This work

tities of hallucinogens can be detected by phosphorimetry than by fluorimetry, due to the small volume of sample (20 μl) needed in the phosphorimetric procedure. In this regard, quantities as low as $3 \cdot 10^{-10}$–$2 \cdot 10^{-11}$ g are detectable by the latter technique.

In Table V we have compared the limits of detection of hallucinogenic drugs by our combined fluorimetric and phosphorimetric methods with other analytical techniques. It is evident that fluorimetry and phosphorimetry compete very favorably with microcrystal, color tests, or chromatographic methods. Minimal detectable quantities are generally one to three orders of magnitude lower by fluorimetry–phosphorimetry than by the other methods. Therefore, we suggest that because of its simplicity, versatility and sensitivity, a combined fluorimetric and phosphorimetric method be used for the determination of hallucinogens, phosphorimetry being used as tool of identification of an unknown hallucinogenic drug, and fluorimetry or phosphorimetry alternatively used for quantitative determinations according to the fluorescence or phosphorescence yield of the particular drug. More work is now in progress

for the assay of mixtures of hallucinogens in real pharmaceutical samples by the present combined method.

ACKNOWLEDGEMENTS

Research was carried out as a part of a study on the phosphorimetric analysis of drugs in blood and urine, supported by U.S. Public Health Service Grant No. GM-11373-09.

We thank Karen Harbaugh for the determination of short phosphorescence lifetimes.

REFERENCES

1 A. S. PERLING, *J. Chromatog. Sci.*, 10 (1972) 268, and references.
2 B. S. FINKLE, *Anal. Chem.*, 44 9, (1972) 18A.
3 E. B. C. CLARK AND J. BERLE, *Isolation and Identification of Drugs*, The Pharmaceutical Press, London, 1969.
4 C. J. G. A. BOSS AND J. M. G. J. FRIJNS, *Pharm. Weekblad*, 107 (1972) 111.
5 H. J. VAN DER POL, *Pharm. Weekblad*, 106 (1971) 575.
6 A. BOWD, P. BYROM, J. B. HUDSON AND J. H. TURNBULL, *Talanta*, 18 (1971) 697.
7 P. J. KASVINSKY, *U.S. Nat. Tech. Inform. Serv.*, A. D. Rep. No. 737671 (1972).
8 D. G. UPSHALL AND D. G. WAILLING, *Clin. Chim. Acta*, 36 (1972) 67.
9 S. UDENFRIEND, *Fluorescence Assay in Biology and Medicine*, Academic Press, New York, 1969, Chapters 5 and 17.
10 A. GILLESPIE, *Anal. Letters*, 2 (1969) 609.
11 T. NIWAGUCHI AND T. INOVE, *J. Chromatog.*, 59 (1971) 127.
12 J. S. FORREST, D. E. GREEN, S. D. ROSE, G. C. SKINNER AND D. M. TORRES, *Res. Commun. Chem. Pathol. Pharmacol.*, 2 (1971) 787.
13 M. ZANDER, *Phosphorimetry*, Academic Press, New York, 1968, Chapters 2 and 4.
14 R. J. LUKASIEWICZ, P. ROZYNES, L. B. SANDERS AND J. D. WINEFORDNER, *Anal. Chem.*, 44 (1972) 237.
15 R. J. LUKASIEWICZ, J. J. MOUSA AND J. D. WINEFORDNER, *Anal. Chem.*, 44 (1972) 963.
16 J. J. AARON AND J. D. WINEFORDNER, *Anal. Chem.*, 44 (1972) 2122.
17 J. J. AARON AND J. D. WINEFORDNER, *Anal. Chem.*, 44 (1972) 2127.
18 T. C. O'HAVER AND J. D. WINEFORDNER, *J. Chem. Educ.*, 46 (1969) 241.
19 R. P. FISHER AND J. D. WINEFORDNER, *Anal. Chem.*, 44 (1972) 948.
20 C. M. O'DONNELL, K. F. HARBAUGH, R. P. FISHER AND J. D. WINEFORDNER, *Anal. Chem.*, in press.
21 A. BOWD, J. B. HUDSON AND J. H. TURNBULL, *J. Chem. Soc., B*, (1971) 1509.
22 P. S. SONG AND W. E. KURTIN, *J. Am. Chem. Soc.*, 91 (1969) 4892.
23 R. S. BECKER, *Theory and Interpretation of Fluorescence and Phosphorescence*, Wiley, New York, N.Y., 1969, Chapter 12.
24 S. P. MCGLYNN, T. AZUMI AND M. KINOSHITA, *Molecular Spectroscopy of the Triplet State*, Prentice-Hall, I. Englewood Cliffs, N.J., 1969, Chapter 8.
25 H. M. FALIS AND PISANO, *Anal. Biochem.*, 3 (1962) 337.
26 H. DHAMIR, N. JAIN, D. CRIM AND R. FORNEY, *J. Forensic Sci.*, 16 (1971) 103.