J.forens.Sci.Soc. 7, 46-50 (1967). LSD 1710/DES 443/MET 366/GY 1087/DHE 911/PSI 227

The Identification of some Proscribed Psychedelic Drugs

E. G. C. CLARKE

Department of Physiology, Royal Veterinary College, London, N.W. 1, England

Under the Drugs (Prevention of Misuse) Act, 1964, Modification Order, 1966, certain hallucinogenic drugs were brought under control, their unauthorised possession being prohibited. The substances in question are (i) lysergic acid amide and its N-alkyl derivatives; (ii) hydroxy-N, N-dimethyl tryptamines and their esters and ethers; and (iii) mescaline. The first group includes lysergamide itself, lysergide (LSD 25), its N, N-diethyl derivative and methysergide (the 1-(hydroxymethyl) propylamide of 1-methyl-lysergic acid).

The second group includes psilocin (4-hydroxydimethyltryptamine) and psilocybin, its phosphate ester; both these compounds are found in the Mexican sacred mushroom (*Psilocybe mexicana*) and various other fungi. Bufotonin (5-hydroxydimethyltryptamine) also occurs naturally, being found, *inter alia*, in both the toad (*Bufo spp.*) and the toadstool (*Amanita muscaria*). 6- and 7-hydroxydimethyltryptamine are synthetic compounds. 5-Methoxydimethyltryptamine is found in the Australian grass, *Phalaris tuberosa*, and gives rise to acute staggers in sheep (Gallagher *et al.*, 1964).

Mescaline is the only compound listed in the third category. One should, however, consider trimethoxyamphetamine, an extremely dangerous synthetic drug. Initially this compound produces hallucinations similar to those caused by mescaline. These are followed by anger, hostility and megalomaniac euphoria, which may escalate into a state of homicidal frenzy (Shulgin *et al.*, 1961). Strangely enough this compound is not at present controlled either as an hallucinogen or as an amphetamine-type drug.

The identification of very small quantities of any of these compounds is, therefore, a matter of considerable forensic interest. It is not proposed to consider here the extraction of these substances from tissues or body fluids, merely their identification if met with in the search for a 'general unknown'.

There are three classes of substance in which the Law is particularly interested: the narcotic drugs which are under international control; the amphetamine-type drugs which were brought under control by the Drugs (Prevention of Misuse) Act, 1964, and the psychedelic drugs now under discussion. Any system of screening should be capable of providing identification of drugs in any of these classes. There are, of course, no chemical tests for a psychedelic drug as such. Its hallucinogenic action can only be demonstrated by direct human experiment. But it should be noted that the order controls only the substances mentioned above, and does not apply to other drugs such as phencyclidine and hyoscine which may also cause hallucinations.

Materials and methods

Paper chromatography is carried out by the method of Curry and Powell (1954), slightly modified. Sheets of No. 1 Whatman paper, 14″ x 6″, are dipped in a 5 per cent. solution of sodium dihydrogen citrate, roughly blotted, and dried at room temperature. They may be stored indefinitely. The solvent is made by dissolving 2.4 g. of citric acid in a mixture of 65 ml of water and 435 ml of n-butanol. It may be used over a period of several weeks provided that water is added from time to time to keep the specific gravity at 0.843-0.844. The ascending method is used, four sheets at a time being run in a tank 8″ x 11″ x 15½″ deep. The system is not equilibrated, but experimental conditions must be strictly adhered to in order to get reasonably reproducible results (Clarke and Hawkins, 1963). After five hours the sheets are removed, dried,

and examined under ultraviolet light (254 mμ), absorbent and fluorescent spots being noted. They are then treated with the location reagents described below.

Thin Layer Chromatography 1: The method described by Sunshine (1963) is used. Glass plates, 20 cm x 20 cm, are coated with silica gel G to give a layer 250 μ thick, and dried at 110° for 1 hour. Glass developing tanks, 21 cm x 21 cm x 10 cm, are employed, the ends being covered with filter paper to assist saturation. 100 ml of methanol and 1.5 ml of 0.880 ammonium hydroxide solution are placed in each tank, which is allowed to stand for an hour before use in order to equilibrate. The liquid should be changed after two runs and the tank re-equilibrated.

Thin Layer Chromatography 2 : This follows the method described by Genest and Farmilo (1964). The plates are coated to a thickness of 250 μ with a slurry made by mixing 30 g. of silica gel G with 60 ml of 0.1 N sodium hydroxide solution and drying, as above. The general technique is the same but the solvent is a mixture of chloroform and methanol (9:1).

The test material for both paper and thin layer chromatography and for crystal tests should be dissolved in 2N acetic acid to give a one per cent. solution. 2.5 μl should be spotted on the paper and 1.0 μl on the thin layer plates.

Location Reagents: Iodoplatinate solution. Add 10 ml of 5 per cent. platinum chloride solution to 240 ml of 2 per cent. potassium iodide solution, and dilute with an equal volume of water.

Bromocresol green (BCG) 0.5 per cent. in alcohol. After spraying the paper should be exposed to ammonia vapour.

Diazo reagent (a) Dissolve 1 knife point p-nitraniline in 25 ml 2N HCl. Cool in refrigerator, and add 1 knife point sodium nitrite just before use. (b) Equal volumes of ethanol and 40 per cent. sodium hydroxide solution. Spray with (b) five minutes after spraying with (a).

Potassium permanganate, 1 per cent. in water.

Ninhydrin, 0.5 per cent. in acetone. Heat papers at 100° for five minutes after spraying.

para-Dimethylaminobenzaldehyde (pDB), 1 g. of p-dimethylaminobenzaldehyde is dissolved in 100 ml of alcohol and 10 ml of concentrated hydrochloric acid added.

Crystal Tests are carried out by the method of Clarke and Williams (1955).

Reagents for crystal tests: Styphnic acid, 5 per cent. in water *Gold Bromide,* 5 g. of gold chloride and 5 g. of sodium bromide in 100 ml of water. *Picric acid,* 5 per cent. in water.

Results

Table 1 shows the results obtained for the lysergic acid hallucinogens compared with those for some common ergot alkaloids. As this system does not separate ergocornine, ergocristine, and ergocryptine, a single entry has been shown as 'ergotoxin'; similarly for 'dihydroergotoxin'. The first column give the Rf values for the citrate buffered paper chromatograms. All these compounds give very elongated spots, so the figure is difficult to estimate exactly. The second column gives the fluorescence under ultraviolet light (254 mμ). It should be noted that the yellow-green fluorescence shown by the dihydro derivatives may easily be missed. The third and fourth columns show the reaction with iodoplatinate and bromocresol green respectively. The reactions shown as positive are usually very weak, and those indicated as doubtful are unlikely to be seen with the amounts of material employed in these experiments. The fifth column shows the result with the p-dimethylaminobenzaldehyde spray. The blue colour, which has a purple tinge, appears slowly and does not reach its maximum intensity for half an hour. With the permanganate spray (column six) reduction is almost instantaneous. Columns 7 and 8 give the Rf values with the two systems of thin layer chromatography described above; p-dimethylaminobenzaldehyde is the best location reagent. It will be noted that the

TABLE 1

	1	2	3	4	5	6	7	8
	P·C· Rf	U.V.	PtI_4	BCG	pDB	$KMnO_4$	T.L.C.1 Rf	T.L.C.2 Rf
"Ergotoxin"	0·82	Blue	—	—	Blue	++	0·73	0·78
"Dihydroergotoxin"	0·80	Green	—	—	Blue	++	0·71	0·68
Ergometrine	0·25	Blue	+	?	Blue	++	0·67	0·23
Methylergometrine	0·40	Blue	—	—	Blue	++	0·70	0·30
Lysergamide	0·11	Blue	+	+	Blue	++	0·60	0·18
Lysergide	0·47	Blue	+	?	Blue	++	0·66	0·63
Lysergic Acid	0·18	Blue	—	?	Blue	++	0·58	0·01
Methysergide	0·45	Blue	—	?	Blue	++	0·66	0·49
Ergosine	0·65	Blue	+	—	Blue	++	0·72	0·58
Ergotamine	0·65	Blue	—	—	Blue	++	0·68	0·58
Dihydroergotamine	0·63	Green	—	—	Blue	++	0·63	0·56

++—Strong Reaction +—Weak Reaction ?—Doubtful Reaction

TABLE 2

	1	2	3	4	5	6	7	8
	P.C. Rf	U.V.	PtI_4	BCG	pDB	Diazo	Ninhydrin	T·L·C· 1 Rf
Tryptamine	0·35	Blue	+ (w)	+	Purple	Orange	Grey	0·27
5-Methoxytryptamine	0·25	White	+ (w)	+	Purple	Orange	Grey	0·25
Serotonin	0·12	Pale Yellow	?	?	Purple	Orange—▶ Purple	Grey	0·25
Psilocybin	0·05	Dark Blue	—	—	Grey	—	—	0·04
Psilocin	0·31	Absorbs	++	?	Grey	Grey—▶ Purple	Orange	0·34
Bufotenin	0·16	Pale Blue	++	?	Purple	Orange—▶ Purple	Orange	0·32
6-Hydroxy DMT	0·13	Blue	++	—	Blue	Crimson—▶ Blue-Green	Orange	0·32
7-Hydroxy DMT	0·18	Absorbs	++	—	Green	Red-Brown—▶ Dark Green	Orange	0·33
5-Methoxy DMT	0·26	White	++	?	Purple	Orange	Orange	0·32
Dimethyltryptamine	0·38	Blue	++	?	Purple	Orange	Orange	0·34
N-Methyltryptamine	0·40	Blue	++	?	Purple	Orange	Orange	0·16

++—Strong Reaction +—Weak Reaction ?—Doubtful Reaction

first system, although most satisfactory as a general screening method, is of little value for distinguishing between these compounds, but that the second provides excellent differentiation. Compounds which run close together on the paper, e.g., lysergide, methysergide and methylergometrine (0.47, 0.45 and 0.40), are well separated by this method (0.63, 0.49 and 0.30).

Table 2 shows the results obtained for the dimethyltryptamines compared with those for some other tryptamine derivatives, the first five columns corresponding to those in Table 1. The fluorescence with serotonin and psilocybin is not very bright, and can easily be missed. With iodoplatinate, the spot given by tryptamine and by its 5-methoxy derivative usually appears white, while serotonin gives a white spot if sufficient material is present. The reactions with bromocresol green are weak and unsatisfactory in all cases. In column 6, where two colours are given, the first is that seen after spraying with diazotised p-nitraniline, the second after spraying with alkali. The ninhydrin spray (column 7) serves to distinguish the primary amines from the secondary and tertiary. The thin layer Rf values (column 8) are of little value for differentiating between these compounds, but would help in their identification should this method have been used as a part of a general screening procedure.

TABLE 3

	1 P.C. Rf	2 PtI_4	3 BCG	4 $KMnO_4$	5 Ninhydrin	6 Diazo	7 T.L.C.[1] Rf
Homoveratrylamine	0.22	+ (w)	+	+	Dull Purple	Purple	0.22
Mescaline	0.25	++	+	+	,,	,,	0.23
p-Methoxyphenethylamine	0.32	+	+	+	,,	,,	0.31
Trimethoxyamphetamine	0.41	+	+	+	,,	,,	0.35
Methoxamine	0.43	—	+	+	,,	,,	0.55
p-Methoxyamphetamine	0.50	—	+	?	,,	,,	0.40
Methoxyphenamine	0.58	+ (w)	+	?	—	—	0.24

++—Strong Reaction. +—Weak Reaction. ?—Doubtful Reaction

Table 3 shows the results for mescaline, trimethoxyamphetamine and some other methoxy derivatives of phenethylamine and phenylisopropylamine. All these compounds absorb weakly in ultraviolet light, and all react readily with bromocresol green. All reduce permanganate, although in the case of p-methoxyamphetamine and methoxyphenamine the reaction is very slow and may be missed. All give a purple colour with ninhydrin and with diazotised p-nitraniline, but here again the reaction with methoxyphenamine is very weak. The best means of distinguishing mescaline and trimethoxyamphetamine from the other members of the group is their reaction with concentrated nitric acid, with which these two compounds give a dull purple colour, the others giving either a faint yellow or no colour at all. This test may be carried out conveniently direct on a paper chromatogram by holding it over nitrous fumes generated from copper and nitric acid. Further confirmation may be made by means of crystal tests. Mescaline gives needles with styphnic acid while homoveratrylamine gives an oil. Trimethoxyamphetamine is only likely to be confused with paramethoxyphenethylamine or methoxamine from its Rf value. Here thin layer chromatography provides differentiation as methoxamine runs considerably faster and para-methoxyphenethylamine rather more slowly, but none of these compounds is particularly easy to locate on a thin layer plate; permanganate is the best reagent for this purpose. Once again crystal tests provide excellent differentiation as trimethoxyamphetamine gives bunches of serrated needles with gold bromide solution, whereas para-methoxyphenethylamine and methoxamine give no crystals at all. Trimethoxyamphetamine also gives typical crystals with picric acid.

Discussion

From the foregoing it will be seen that it is perfectly practicable to make a reasonably certain identification of any of the proscribed psychedelic drugs by means of the very simplest techniques. Once such provisional recognition has been made, final identification can really only be achieved by comparison of the reactions of the unknown with those of an authentic sample. Such comparison can be made either by means of the simple techniques outlined above, e.g., running the unknown and the control together and comparing Rf values and the results of the various colour reactions; in this case, of course, the shape of the spot and the exact shade it gives with the different reagents can be taken into account. Alternatively, recourse may be made to any of the instrumental techniques that are nowadays available in most laboratories. The chief difficulty will be the provision of the authentic sample. Many of these compounds are exceedingly hard to obtain as in most cases the manufacturers have stopped distribution, even to official laboratories.

I wish to acknowledge most gratefully gifts of drugs from Professor C. H. Gallagher, Professor R. T. Williams, Dr. E. R. Clark, Dr. J. J. Gordon, Dr. P. Hey, Dr. J. B. Jepson, The Pharmaceutical Society of Great Britain, Messrs. British Drug Houses Ltd., Burroughs Wellcome & Co., May & Baker Ltd., Sandoz Products Ltd., and Upjohn Ltd. I am also greatly indebted to Miss Sandra Gavin for technical assistance.

References

CLARKE, E. G. C., and HAWKINS, A. E., 1963, *J. Pharm. Pharmacol.*, **15**, 390.
CLARKE, E. G. C., and WILLIAMS, M., 1955, *J. Pharm. Pharmacol.*, **7**, 255.
CURRY, A. S., and POWELL, H., 1954, *Nature* (*Lond.*), **173**, 1143.
GALLAGHER, C. H., KOCH, J. H., MOOR, R. M., and STEEL, J. D., 1964, *Nature* (*Lond.*), **204**, 542.
GENEST, K., and FARMILO, C. G., 1964, *J. Pharm. Pharmacol.*, **16**, 250.
SHULGIN, A. J., BUNNELL, S., and SARGENT, T., 1961, *Nature* (*Lond.*), **189**, 1011.
SUNSHINE, I., 1963, *Am. J. Clin. Path.*, **40**, 576.