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The chromatographic identification of psychotropic drugs

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The thin-layer chromatography of three classes of psychotropic drugs—phenethylamines, tryptamines and erganes—has been investigated. Published methods are reviewed and Rf data, normalized by a graphical technique, are reported for extensions and modifications of some of these systems. Optimum forensic sorting procedures are recommended.

Recent papers from workers concerned with forensic identification of drugs seized in suspicious circumstances have reported the chromatographic mobility of a variety of psychotropic materials. For forensic purposes it is important to distinguish between potent psychotropic drugs for which restrictions are primarily concerned with supply, and other substances of closely related structure for which their actual or potential abuse has required their unauthorized possession to be made an offence. Chromatography on thin layers on glass plates or commercially coated polyester sheets provides rapid sorting methods for laboratories with limited time, sample and equipment.

It is convenient to summarize the published evidence, and to discuss our own contributions, in relation to three broad classes of bases—phenethylamines, tryptamines and erganes.

EXPERIMENTAL

Support

I.	Silica gel.
II.	Alkaline silica: I (30 g) with 0·1 N sodium hydroxide (60 ml).
III.	Alkaline silica: I (30 g) with N sodium hydroxide (60 ml).
IV.	Acidic silica: I (30 g) with 0·1 N potassium bisulphate (60 ml).
V.	Silica coating on polyester sheet (Eastman "Chromagram" 6061).
VI.	V, incorporating fluorescent indicator (Eastman "Chromagram" 6060).
VII.	V, to which 2 μl 0·1 N sodium hydroxide was added at each origin point.
I–IV.	Coating thickness 0·25 mm; plates 20 × 20 cm; coat applied with "Quickfit" and Shandon spreaders. Plates were dried at least $\frac{1}{2}$ h at room temperature, activated for 1 h at 110° and cooled in a desiccator cabinet.
V–VII.	Commercially prepared layers 0·10 mm thickness. Sheets from opened packets were stored in desiccator cabinet without activation.

Solvents (all of "Analytical Reagent" grade)

A. Methanol. B. Methanol–ammonia (sp.gr. 0·88) (100:1·5). C. Chloroform–methanol (9:1). D. Acetone. E. Cyclohexane–benzene–diethylamine (15:3:2).

About 100 ml of solvent was exposed in small (4 litre) paper-lined tanks and allowed to reach equilibrium before use. For solvent B, a tightly lidded chamber was necessary

to maintain an approximately constant ammonia concentration; but, providing internal normalization of Rf values (see below) was employed, the solvent could be used without renewal for several runs within a period of a few days.

Visualization

The following systems were employed: "a" 254 nm illumination; "b" 350 nm illumination; "c" sprayed with 1% iodine–methanol; "d" sprayed with mixture 10 ml platinic chloride (5%), 5 ml HCl (sp.gr. 1·18) and 240 ml potassium iodide (2%); "e" sprayed with 0·5 g dimethylaminobenzaldehyde dissolved in 5 ml HCl (sp.gr. 1·18) and 95 ml ethanol (99%); the plates heated for 5 to 10 min at 105°; "f" sprayed with sulphuric acid (sp.gr. 1·84).

The colour code employed in Table 2 refers consecutively to the initial colour, to the colour formed within 1 min, and to the time in minutes during which the second colour fades: g = grey, m = mauve or purple, n = brown, o = orange, p = pink or pinkish brown, y = yellow, yy = dark yellow.

Normalization

Although distortion of solvent fronts was prevented by lining the tanks with paper and allowing the solvent to come to equilibrium in the vapour phase (as recommended by French & Wehrli, 1965) there was still sufficient variation between runs to warrant including a reference substance on each plate. Sunshine (1963) used a single marker substance for each class of drug he examined (barbiturates, carbamates, phenothiazines) but he computed relative (Rx) values, equating the marker Rf to unity (i.e., some Rx values were greater than one). Many other workers include a standard substance of previously established Rf value when they wish to cite relative mobility in specified conditions. In a recent symposium on the standardization of TLC procedures, Stahl (1968) again referred to the use of a standard blend of defined substances in fixed concentrations in the same solvent. At the same symposium Gasparič (1968) recommended an internal standard comprising compounds selected evenly to cover the whole Rf range. We had independently decided to select a blend of related substances showing a range of Rf values; the apparent novelty of our technique lies in the graphical treatment, whereby a best-fit line takes account of all the marker points. By this technique, the spread of Rf values for a given substance and system is markedly reduced. The three mixtures routinely employed for phenethylamines, tryptamines and erganes are listed under Method. An example of the graphical normalization for one particular plate is shown in Fig. 1.

Method

Mean values for three or more reference spots were determined using optimum conditions previously established in at least 10 runs. For subsequent runs, 2 to 5 μg reference substances were applied accumulatively at each of two points trisecting the start-line, and the unknown substances symmetrically distributed either side of them. The plate was then developed and the uncorrected Rf values measured. Using the established mean values for the reference substances as abscissa (x-axis), the two observed Rf values for each of the standard spots were plotted from the ordinate (y-axis) and a smooth curve drawn through the family of intersections. Finally, entering all uncorrected Rf values from the y-axis, the "normalized" value (Rf*) was read from the x-axis.

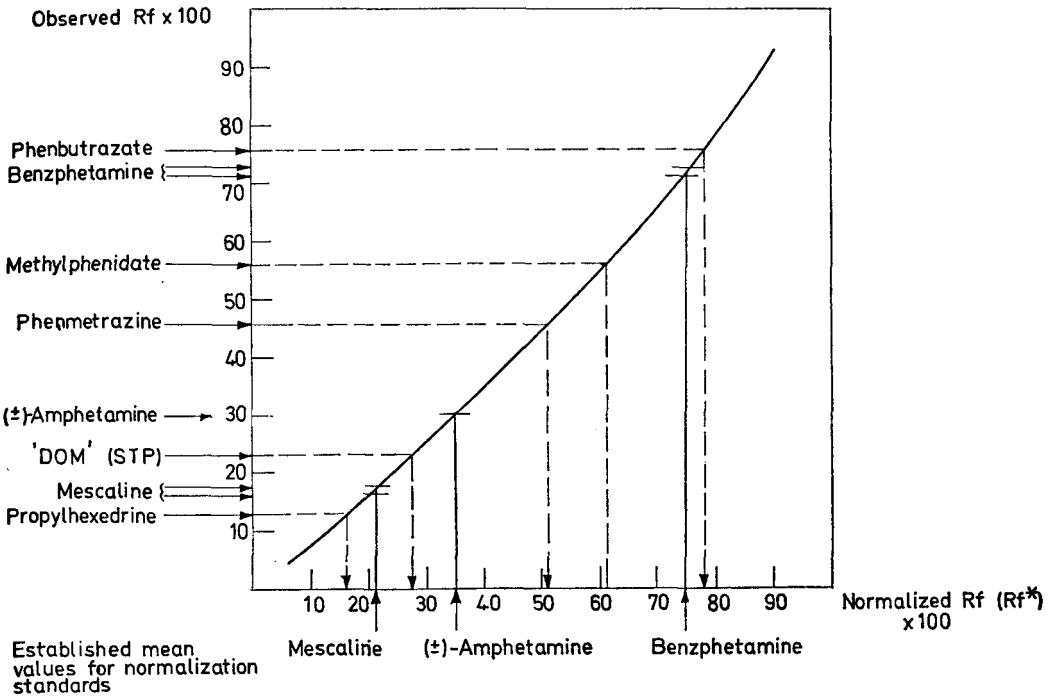

FIG. 1. Illustration of a normalization procedure for a typical plate in the phenethylamine series.

Standard substances selected for this normalization procedure were: (i) for phenethylamines—a blend of mescaline, amphetamine and benzphetamine; (ii) for tryptamines—*N*-methyl-, *NN*-dimethyl- and *NN*-dibutyltryptamine; (iii) for erganes—ergometrine (3 spots) and ergotamine (8β- and 8α-isomers).

PHENETHYLAMINES

Several workers have described the chromatographic resolution of psychotropic drugs in physiological fluids. Although Sunshine (1963) had analysed with a variety of thin-layer systems extracts from blood, urine and human stomach contents containing an extensive series of barbiturates, carbamates, phenothiazines and some narcotics and medicinal alkaloids, he did not examine psychotomimetics and psychotonic amines. Acetone–methanol chromatography on silica plates has been used for the separation of amphetamine, methylamphetamine and ephedrine from the urine of athletes (Beckett, Tucker & Moffat, 1967) and from horse urine (Karawya, El Keiy & others, 1968); for quantitative work the latter group removed zones and estimated the amines colorimetrically. Haywood & Moss (1968)—who also are especially concerned with racehorse "doping"—resolved alkaloids, stimulants and other psychotrophes in extracts from equine urine. They applied the Curry & Powell (1954) citrate buffer technique to thin layers of cellulose powder as well as using their published paper partition method.

Working in the main with the pure drug form, Clarke (1967a) applied convenient paper partition (Curry & Powell, 1954) and thin layer (Sunshine, 1963) chromatographic screening procedures to a number of amphetamines substituted in the aromatic ring. He later extended (Clarke, 1967b) these two techniques to twenty substances

recognized by the Home Office as being subsumed by the generic definition in the 1964 Drugs (Prevention of Misuse) Act. Between these two paper and TLC systems the twenty amines exhibited only moderately different mobilities: about half of each were concentrated within 10% of the respective paper and plate, although only five substances were common to this overlap. In his comprehensive scheme (Clarke & Berle, 1969) for the identification of basic drugs, Clarke recommends a variety of visualization procedures which in practice help to distinguish most amines that show comparable mobility and further assistance may be obtained from classical crystal tests or modern instrumental techniques. Nevertheless, for forensic purposes two distinct thin-layer separations are desirable.

Grant (1968) has recently reported a new solvent mixture, acetonitrile–benzene–ethyl acetate–ammonia for the TLC of extracts of stimulants: he achieved an adequate separation of methylamphetamine and ephedrine (Rf 0·34 and 0·26), but on the other hand phenylpropanolamine—which is well separated with methanol—fell between these two bases. In another recent paper, Genest & Hughes (1968) describe the TLC separation of 2,5-dimethoxy-4-methylamphetamine† from amphetamine, methylamphetamine and the pyschotomimetics mescaline, bufotenine and *NN*-dimethyltryptamine. They employed silica and alumina plates and three different solvent systems.

With chloroform–methanol (1 : 1) on alumina "DOM" is well resolved from the other five substances but in butanone–dimethylformamide–ammonia on silica the discrimination from amphetamine is only 0·04 Rf units, and in chloroform–methanol–acetic acid on silica methylamphetamine and "DOM" coincide.

Genest & Farmilo (1964) devised a system (IIC; see Discussion) to separate erganes from psychotonic amines and narcotics. Attempts in this laboratory to apply this system to the psychotomimetic ingredient isolated from a single tablet described as "STP" were not convincing: Maunder (1967) reported the TLC behaviour of the extracted base to be essentially similar to methoxamine, mescaline and (±)-amphetamine. Provision by the (then) U.S. Bureau of Drug Abuse Control of a reference sample of "DOM"† permitted a detailed examination of the behaviour of 2,5-dimethoxy-4-methylamphetamine in a variety of thin-layer systems. Phillips & Mesley (1969) have already reported the observed overlapping of this substance in relation to a number of α-methylphenethylamine (amphetamine) derivatives in three systems. We now report the investigation of nine systems and describe the application of two of these to thirty-four psychotropic amines of related structure; some are psychotonic (stimulant), others are sympathomimetics and a few psychotomimetic (hallucinogenic).

Results

Nine systems have been investigated; Table 1 shows the mean Rf values obtained in preliminary studies with eight of these systems, using eleven phenethylamine bases or their salts. In each system, the optimum loading was about 2 to 5 μg.

Table 2, most of which has appeared in the Government Chemist's Report for 1968, enumerates the Rf* values established with systems IIA and IB for thirty-four phenethylamine bases prepared as 0·1–0·2% solutions of (usually) a convenient salt in undiluted, or 50% aqueous, methanol or ethanol. Each Rf* value cited represents the mean of at least three runs and has been normalized by our graphical method.

† The Dow Co. experimental product "DOM" and the active ingredient of the illicit pyschotomimetic drug "STP" (Maunder, 1967; Phillips & Mesley, 1969).

Table 1. *Preliminary trials with phenethylamine derivatives*

Chromatographic system:	IIA	IIC	IIE	IIIA	IIIC	IVA	IVD	VIB
Phenylephrine hydrochloride	23	—	08 (43)	—	—	36	10, 13	—
Adrenaline sulphate	14, 70	—	09	—	—	07, 22	21	30, 61
Mescaline sulphate	00, 17, 46	15	—	—	17, 63, 79	—	—	00, 27
Methoxyphenamine hydrochloride	17	11	—	70	66	—	—	30
Ephedrine	22	—	—	—	—	09, 20	04, 31, 35	—
Ephedrine hydrochloride	07	06	—	—	45	—	—	31
Pseudoephedrine	—	—	—	—	—	08	—	—
N-Methylephedrine	—	10	—	—	—	—	—	—
(+)-Methylamphetamine	24	—	—	—	—	08, 35	06	—
(±)-Methylamphetamine	28	13	36	—	—	10, 35	08	—
(±)-Methylamphetamine hydrochloride	—	—	—	—	31	—	—	33
Phentermine	—	15	—	—	—	—	—	—
"STP" extract	21	16, 27[b]	—	65	48, 73	12, 65	—	—
"DOM" hydrochloride	21	17	—	—	—	—	—	39
(+)-Amphetamine	36	17	—	55	—	11	22	—
(±)-Amphetamine	35	19	38	—	—	08	19	—
(±)-Amphetamine hydrochloride	31	—	—	—	—	—	—	41
(±)-Amphetamine sulphate	31	—	—	50	69	—	—	—
Methoxamine hydrochloride	—	07, 40[b]	—	69	50, 74, 82	—	—	41
Benzphetamine hydrochloride	73	80	75	—	—	64	(11), 27	81

Observed mean Rf values (× 100) normalized to (±)-amphetamine marker only.
Visualization in all systems by method "c" except additional spots (b) revealed by method "b" (see p. 794).
For key to support, solvent and visualization code, refer to Experimental section.

Table 2. *Rf* values* × 100 *for phenethylamine derivatives*

		Control status	System IB	System IIA	Visualization c
1	Oxethazaine	S4B	05; (15)	02; (14)	g–p30
2	Propylhexedrine hydrochloride	PX	16	09; (11)	g–n5
3	Mephentermine sulphate	DPM	18	17	g–n5
4	Adrenaline sulphate	PX	21	21	y–yy30
5	Mescaline sulphate	DPM	21	13	g–n5
6	Methoxyphenamine hydrochloride	PX	21	16	g–p30
7	Ephedrine hydrochloride	PX	25	19	g–o30
8	Pseudoephedrine hydrochloride	PX	27	19	g–o30
9	Methylamphetamine hydrochloride	DPM	27	19	g–p5
10	2,5-Dimethoxy-4-methylamphetamine hydrochloride	S4B	28	19	g–p5
11	3-Methoxy-4,5-methylenedioxyamphetamine hydrochloride	S4B	29	23	g–p5
12	*N*-Methylephedrine hydrochloride	PX	30	28	g–y30
13	Phentermine hydrochloride	DPM	32	29	g–y5
14	3,4-Methylenedioxyamphetamine	S4B	33	24	g–p5
15	Methoxamine hydrochloride	(none)	34	28	g–y30
16	Dexamphetamine sulphate	DPM	33	28	g–p5
17	Levamphetamine sulphate	DPM	34	28	g–p5
18	(±)-Amphetamine hydrochloride	DPM	35	29	g–p5
19	Isoprenaline sulphate	PX	35	34; (48)	y–yy30
20	Chlorphentermine hydrochloride	DPM	38	(13); 34	g–y5
21	Phenylpropanolamine hydrochloride	PX	40	33	g–y5
22	Prolintane hydrochloride	DPM	43	42	g–o30
23	Fenfluramine hydrochloride	S4B	46	38	g–n5
24	Pipradrol hydrochloride	DPM	47	45	g–p30
25	Phenmetrazine theoclate	DPM	49	47	g–p5
26	Fencamfamin hydrochloride	DPM	(38); 51	(35); 50	g–p5
27	Fenethylline hydrochloride	DPM	59	53	g–o30
28	Methylphenidate hydrochloride	DPM	61	57	g–p5
29	Tranylcypromine hydrochloride	DPM	62	64	y–yy30
30	Doxapram hydrochloride	DPM	70	69	g–p30
31	Diethylpropion hydrochloride	DPM	73	73	g–y30
32	Benzphetamine hydrochloride	DPM	75	73	g–o30
33	Phenbutrazate hydrochloride	DPM	77	(66); 77	g–o30
34	Famprofazone	DPM	80	80	g–o30

For details of thin-layer chromatographic system see Experimental section.
Values for minor spots in parentheses.
DPM = Drugs (Prevention of Misuse) Act 1964.
S4B = Schedule 4B of Poisons (No. 2) Rules 1968.
PX = Part I, Poisons List (No. 2) 1968 but full or qualified exemption from S4B.

The visualization procedure "c"† easily reveals 1 μg of the amines cited in Table 2; the lower detection limit has not been fully investigated but is less than 0·5 μg for mescaline, (±)-amphetamine and benzphetamine.

Discussion

Preliminary investigations

With both methanol and acetone on the acid plate, IV, (bisulphate impregnated: Fike, 1966), amine bases not unexpectedly showed limited mobility. Moreover, a considerable proportion of salts or amines remained at the start when run in neutral conditions. More surprisingly, limited mobility was also found to apply to the basic plate, using chloroform–methanol (9:1) (IIC) (Genest & Farmilo, 1964). Better mobility was found with the solvent mixture E (Fike, 1966) but this did not appear to separate amphetamine from methylamphetamine. Experiments with methanol and chloroform–methanol (9:1) on a more strongly basic plate, III, showed a greatly enhanced mobility but poor separation of the bases examined. Methanol as solvent on the less basic plate, II, appeared to us to be the most useful system, IIA, to compare with the Sunshine (1963) system, IB, for which Clarke's results (1967b) with some similar bases were encouraging.

The frequent use in this and other laboratories (e.g., Schweda, 1967) of silica precoated polyester sheets, which have the advantage of permitting reduced sample loading (Maunder, 1969) as well as minimal preparation time, flexibility, durability and permanent record, prompted an investigation of an application to the chromatography of psychotropic drugs. However, with a number of amines, the advantage of the appreciably higher Rf values with ammoniacal methanol on Eastman "Chromagram" 6061 sheets was offset by poorer visualization: even on sheets incorporating fluorescent indicator ("Chromagram" 6060) there was little or no response to ultraviolet illumination and the iodine spray gave imperfectly resolved spots on a murky background.

Optimum systems

The differential resolution that is possible by use of the systems IB and IIA is apparent from Table 2. Especial interest attaches to the separation in system IIA of mescaline (compound 5) and methoxyphenamine (6) from adrenaline (4); of the hallucinogenic pair MMDA (11) from DOM‡ (10); of MDA (14) from dexamphetamine (16); of isoprenaline (19) from (±)-amphetamine (18); of the stimulant pair methylphenidate (28) from tranylcypromine (29); and the anoretic fenfluramine (23) from the stimulant pipradrol (24)—all of which show groups of overlapping mobilities in system IB. The converse is true for separation on IB of certain conjunctions not resolved on IIA, e.g., mephentermine (3) from methoxyphenamine (6); the sympathomimetic drugs *N*-methylephedrine (12) from methoxamine (15) and isoprenaline (19) from phenylpropanolamine (21); and MDA (14) from MMDA (11)—this last pair being hallucinogens bearing mescaline-like aromatic substitution in an amphetamine structure. Preliminary identification based upon these relatively slight differences in mobility is assisted by distinct nuances in the colour reaction with methanolic iodine spray on system IB: the observed sequences have been simplified in the code itemized in Table 2. It is of interest that the colour development can be arrested

† For visualization code, see Experimental section (p. 794).
‡ See footnote, p. 796.

by superimposing a second plate on the top of the silica gel layer. From the colour sequence it may be possible to suspect one component of a pair not resolved on either system. In particular it should be noted that methylamphetamine (9) may thereby be distinguished from ephedrine (7) and pseudo-ephedrine (8), dexamphetamine (16) and levamphetamine (17) from methoxamine (15), and pipradrol (24) from phenmetrazine (25).

The discrepancies between Rf* values we have observed in system IB for nine amines and those reported by Clarke (1967a) have been investigated. The principal cause appears to lie with the dissolution solvent chosen: while all our substances have been dissolved in aqueous or anhydrous alcohols, Clarke preferred to use 2 N acetic acid. Table 3 shows the spots resolved from both solvents and compared with Clarke's results; we have used both methanolic iodine visualization (c) (which we prefer), and the iodoplatinate reagent (d) used by Clarke. In our experience spots obtained from the acetic solutions exhibited considerable tailing, complicating the assignment of meaningful Rf values, as well as generally exhibiting higher mobility. Moreover, the iodoplatinate seemed a less sensitive reagent for these particular substances and did not always visualize the same spots as did the iodine spray. The van Urk reagent (visualization "e") is also less sensitive than "c".

Table 3. *Effect of dissolution solvent and visualization on mobility on system IB. Rf values × 100 for nine phenethylamines*

Solution in:	ethanol (0·1%)		2 N acetic acid (1%)		Clarke†
Visualization:	c	d	c	d	d
Mephentermine	18	(15)	(40); 50^t	40^t; 62	24
Amphetamine	35	—	44^t; 60	45^t; 63	48
Prolintane	43	31	50^t; (68^t)	48^t; (57)	48
"DOM"	28	—	63^t	53^t; 65^t	51
Chlorphentermine ..	38	—	(58); 66	50^t; (60)	54
Phentermine	32	—	58	(45^t); 59; (64)	56
Fencamfamin	(38); 51	(35)	70^t	59	60
Pipradrol	47	(32)	79	59	61
Benzphetamine	75	76	—	—	73

† Reference: Clarke (1967a), except "DOM" in Clarke & Berle (1969), p. 537.
t = tailing; relatively weak spots are enclosed in parentheses.

TRYPTAMINES

Martin & Alexander (1968) reported a variety of spectrometric and chromatographic procedures for suspected hallucinogens and related drugs. They described the separation of bicarbonate–chloroform extracts of *NN*-dimethyl- and diethyltryptamine (DMT, DET) on silica plates with aqueous ammoniacal ethanol (3 : 1 : 4), visualizing with formalin in aqueous ethanol and hydrochloric acid. Clarke (1967a) used a paper and a thin-layer system to examine eleven tryptamines, including both naturally occurring and specially synthesized hydroxylated derivatives of DMT which are controlled by the Drugs (Prevention of Misuse) Act, and—for comparison—the natural brain substance serotonin (5-hydroxytryptamine) which is presumed to be antagonized in psychotomimetic reactions. He reported a moderately good separation of these substances by the Curry & Powell (1954) paper partition method, but found bunching of equivalent mobilities using ammoniacal methanol on silica thin layer (Sunshine, 1963).

We have investigated five TLC systems with twenty tryptamine derivatives, most of which have been shown to exhibit psychotomimetic activity.

Results

The Rf* values enumerated in Table 4 were obtained on five systems with tryptamine and a variety of derivatives of the corresponding primary, secondary and tertiary amines. Data for systems IB and VIB are the mean of at least three runs; they have all been normalized to standard values for DMT and its monomethyl and di-n-butyl analogues. The data for IIC and IIA refer to less than three runs for some compounds. The optimum loading was 2 to 5 μg of salts from 0·2% ethanol solutions; 1 μg was easily detectable using visualization "c", and also with "a" on "Chromagram" sheets, but higher loadings were necessary for "a" on plates.

Discussion

The system IIC, which proved so successful in the ergane series (see the third part of this paper), provided very limited mobility for seventeen of the twenty tryptamines examined. When visualized in 254 nm ultraviolet illumination the basic plate seemed to minimize the already limited fluorescence of the tryptamines but spray "c" gave satisfactory visualization. Following our experience with phenethylamines (first part of this paper), we tried the more polar solvent methanol on the basic plate (i.e., system IIA) and found that generally mobility was increased but bufotenine (5-hydroxy-DMT) was no longer separated from 7-methyltryptamine. Employment of the Sunshine (1963) system, IB, further increased the mobilities (maximum Rf* 0·66 for *N*-dibutyltryptamine) but was without significant alteration of the sequence of bases. Clarke (1967a) used this system for his tryptamine series; for those five substances common to our investigation the Rf values are in substantial agreement. Fig. 2 illustrates the relative mobility in our three thin layer plate systems and with the polyester sheet equivalent (VIB) of IB. For the plates it is evident that IB is the system of first choice but that in some instances discrimination is possible from a second chromatogram using the system IIC.

Distinction between tertiary, and primary and secondary, bases is also possible by visualization "c"; the tertiary bases gave an orange colour which faded through yellow to a permanent but very faint yellow, whereas the primary and secondary bases gave an initial pale yellow which darkened and then slowly faded to a permanent fawn colour. This behaviour should be contrasted with the grey changing to orange or yellow and fading within 30 min, that we have reported for phenethylamine derivatives. With the ultraviolet 254 nm visualization the hydroxylated tryptamines (5-HT and bufotenine) gave a pinkish fluorescence contrasting with the dark purple of the other tryptamines.

On polyester sheets the tryptamines exhibited enhanced mobility (see Table 4) and all the main spots could be resolved by visualization "c" on "Chromagram" 6061 sheets. On sheets "6060" these tryptamines fluoresced (254 nm) strongly, appearing as blue or purple spots clearly distinguished from the orange background. The Rf values on the two types of sheet were essentially the same. Considering the speed and simplicity of the operation, this medium with ammoniacal methanol solvent and 254 nm visualization (i.e., system VIB a) is recommended for the forensic identification of tryptamine drugs. Mobility is compared with the three plate systems in Fig. 2.

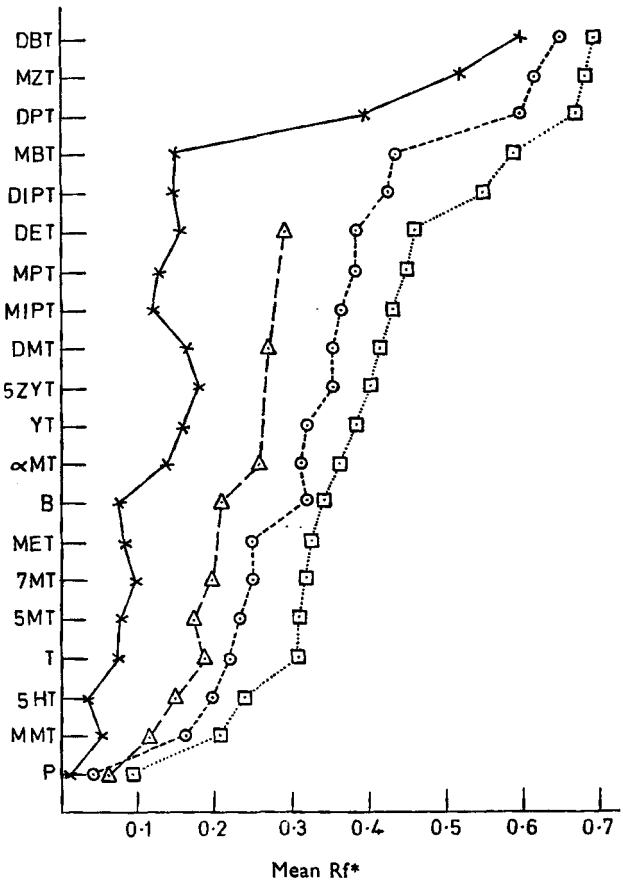

FIG. 2. Relative mobility of tryptamine derivatives in four TLC systems: ×, II C; △, II A; ⊙, IB and ⊡, VIB. For system code refer to experimental section. Full names of the compounds are given in Table 4.

The series of tryptamine derivatives is sufficiently extensive to permit deduction of a limited correlation between structure and mobility. Two homologous series may be distinguished. For the tertiary amines mobility increases smoothly through dimethyl, diethyl and di-isopropyl tryptamines and then somewhat faster through the di-n-propyl and di-n-butyl derivatives. The pyrrolidino analogue is slower than its uncyclized equivalent, diethyltryptamine. With the secondary (i.e., monoalkyl) tryptamines there is a regular, and (except for the propyl isomers) well resolved, series: methyl, ethyl, isopropyl, n-propyl, n-butyl, benzyl.

Ring substitution has less effect. Introduction of a 5-hydroxyl group marginally reduces the mobility of tryptamine and DMT; Clarke's (1967a) data also show this slight difference. It may be attributable to a reduction in the basicity of the indole nitrogen and/or participation of a semiquinone mesomer. On the other hand, substitution of a methyl group at positions 5 or 7 slightly increases the mobility. In the single instance of side chain substitution, an α-methyl appears to have somewhat more effect: thus, α-methyltryptamine (the indolyl analogue of amphetamine) runs ahead of its 5- and 7-isomers. The variation of mobility within the two homologous series and with ring or chain substitution is illustrated in Fig. 3.

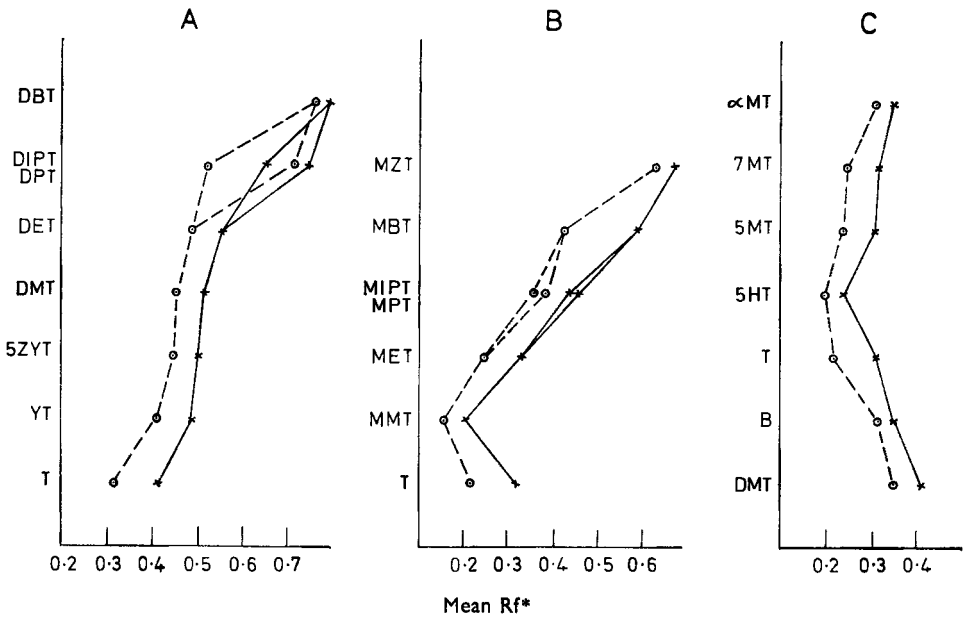

FIG. 3. Correlation of structure with mobility for tryptamine derivatives. A, tertiary amine series; B, secondary amine series; C, ring and side-chain substituents; ⊙ refers to system IB and × to system VIB. Full names of the bases are given in Table 4.

Table 4. *Rf* values* × 100 *for tryptamine derivatives in four TLC systems*

	Code	Psychotomimicry	IB	VB/VIB	IIA	IIC
Primary amines						
Tryptamine	T	− (i)	22	31	19	07
Serotonin (5-hydroxytryptamine)	5HT	− (i)	20	24	15	03
5-Methyltryptamine	5MT	− ? (iv)	23	31	18	08
7-Methyltryptamine	7MT	− ? (iv)	25	32	20	10
α-Methyltryptamine	αMT	+ (i)	31	36	26	14
Secondary amines						
N-Methyltryptamine	MMT	− ? (iv)	16	21	12	05
N-Ethyltryptamine	MET	+ (ii)	25	33	—	08
N-Isopropyltryptamine	MIPT	+ (ii)	36	43	—	12
N-Propyltryptamine	MPT	+ (ii)	38	45	—	13
N-Butyltryptamine	MBT	+ (ii)	43	59	—	16
N-Benzyltryptamine	MZT	+ (ii)	62	67	—	52
Tertiary amines						
Psilocybin	P	+ (i)	04	09	—	01
Bufotenine (5-hydroxy-DMT)	B	+ (i)	32	35	21	07
3-(2-Pyrrolidinoethyl)indole	YT	+ (iii)	32	38	—	16
5-Benzyloxy-3-(2-pyrrolidinoethyl)-indole	5ZYT	+ (iii)	35	40	—	18
NN-Dimethyltryptamine	DMT	+ (i)	35	41	27	16
NN-Diethyltryptamine	DET	+ (i)	38	46	29	16
NN-Diisopropyltryptamine	DIPT	+ (ii)	42	56	—	15
NN-Dipropyltryptamine	DPT	+ (i)	60	65	—	39
NN-Dibutyltryptamine	DBT	+ (i)	66	69	—	60

References: (i) Downing, 1964 (Review); (ii) Brimblecome & others, 1964; (iii) Hunt & Brimblecombe, 1967; (iv) R. W. Brimblecombe (unpublished work).
For system codes refer to Experimental section.

The results cited in Table 4 refer to authentic samples obtained either direct from the manufacturer or by the courtesy of other official laboratories. Examination by these systems of seizure samples of DMT (not at the moment a prohibited drug in the U.K.) and psilocybin have normally indicated a homogeneous product, implying commercial origin; but at least one DMT sample of U.S. origin contained secondary spots (Rf 0·02, 0·07) suggesting a probable illicit synthesis. Solutions of DMT and MMT have not produced additional spots on storage, but after 9 months in the dark at laboratory temperature psilocybin and bufotenine solutions each developed a relatively immobile (Rf 0·03 and 0·01 respectively) secondary spot.

ERGANES

According to Lerner (1967), Sandoz Pharmaceuticals recommended the solvent mixture dichloromethane–methanol (93:7) for the thin-layer chromatography of lysergide (LSD) (Rf value on silica 0·6), but the U.S. Food & Drug Administration (FDA) preferred chloroform-acetone (1:4) (Rf 0·4–0·5). Martin & Alexander (1967) subsequently reported that the FDA use acetone mobile phase for the separation of LSD, lysergamide (LAA) and lysergic acid seized from clandestine laboratories. Genest & Farmilo (1964) devized a different system to separate LSD and LAA from a number of ergot alkaloids and their various 8α-isomers, as well as from amphetamine, methylamphetamine and six narcotics. They used a basic plate (silica impregnated with 0·1 N sodium hydroxide) developed with chloroform–methanol (9:1); spots—not always the same ones—were visualized with dimethylaminobenzaldehyde spray and in ultraviolet light. Satisfactory resolution of a larger number of erganes was described by Clarke (1967a), who employed both the Genest & Farmilo (1964) thin-layer system and a modification of the Curry & Powell (1954) ascending buffered paper partition method. Clarke included methysergide, a potential hallucinogen arguably subsumed (Phillips, 1967) by the Drugs (Prevention of Misuse) Act, and methylergometrine—a synthetic homologue of the natural oxytocic ergometrine.

Our preliminary results with the Genest & Farmilo (1964) system have been reported (Government Chemist, 1968) and revealed satisfactory agreement with other published Rf values (Genest & Farmilo, 1964; French & Wehrli, 1965; Clarke, 1967a). We now publish fuller details of studies undertaken with authentic and seizure samples, comment on visualization techniques and secondary spots, and describe a convenient adaptation of the Genest & Farmilo (1964) conditions to commercially available silica impregnated polyester ("Chromagram") sheets as a rapid screening procedure for suspected erganes.

Results

Table 5 contrasts mean Rf* values for ten erganes and some of their 8α-isomers in three systems: methanol and ammoniacal methanol on neutral plates (systems IA and IB) and chloroform–methanol (9:1) on an alkaline plate (system IIC). For IB and IIC each value is the mean (±0·02) of at least nine runs (except 8β- and 8α-lysergamide, 8α-ergometrine and dihydroergotamine) normalized to values standardized for the three spots observed with commercial 8β-ergometrine (averaged from seventeen runs) and the two spots for 8β- and 8α-ergotamine (mean of fourteen runs). The erganes were applied as 2–10 μg of the salt from 0·1% solutions in aqueous methanol (1:1) or aqueous ethanol (7:3). In 254 nm illumination each main spot

Table 5. *Rf* values* × 100 *for erganes; subsidiary (impurity) spots in parentheses*

Chromatographic system:	Prepared plates			Precoated polyester sheets				
Visualization:	IA a,c	IB a,b,c	IIC a,b,c,e,f	VIA a	VIC a	VIB a	VB a	VIIC a,c
Lysergic acid	—	78 (15, 62)	02 (33†, 66†)	—	—	65	64	02 (22†, 42†, 72†)
8β-Lysergamide	60 (75)	—	24 (32, 55‡)	—	—	—	—	24 (30, 37, 51‡)
8β-Ergometrine maleate	63 (74)	74 (58)	25 (16, 31, 48‡)	76	36	69	70	25 (33, 40‡, 49)
Methylergometrine bimaleate	—	—	30 (37†, 42†, 55†)	—	—	—	—	31 (20†, 40†, 46†)
8α-Lysergamide	—	—	55 (08, 19, 24‡, 30†, 40)	—	—	—	—	53 (25‡, 41, 66)
8α-Lysergide‡§	27	62	42	—	44	—	—	34
8α-Ergometrine	—	—	48 (09, 20, 25‡, 35, 55)	—	—	—	—	39 (11, 20, 31, 67)
Methysergide bimaleate	—	75 (58)	47 (13†, 24, 31†, 35†, 54†, 62†, 67†)	—	—	74	73	51 (19†, 29†, 40†, 63†, 70†)
8β-Ergotamine tartrate	65 (73‡)	—	58 (30†, 42†, 48†, 61†, 66†, 76‡)	84	68	—	—	58 (37, 67, 76‡)
Dihydroergotamine	57 (65)	72	57 (17†, 25†, 35‡)	—	—	—	—	50 (19†, 27†, 34‡)
8β-Lysergide tartrate	61 (74)	71	63 (33†, 42‡, 71, 76†)	79	75	70	71	60 (34‡, 73)
1-Acetyl-lysergide	58	—	72 (45†, 49, 61, 77†, 85)	—	—	—	—	74 (58, 76, 82)
"Ergotoxine" ethanosulphate (8β-ergocristine)	73	—	76 (24†, 80†)	—	81	—	—	70
8α-Ergotamine tartrate‡§	73	—	76	—	—	—	—	76

† Unidentified spots observed after solution had been allowed to stand in the dark for several weeks.
‡ Spot weak or absent in freshly prepared solution and attributed to 8-epimer.
§ Values for these compounds are inferred from results for the 8-epimer.

was visualized by its bright blue fluorescence except dihydroergotamine, which had almost no fluorescence, and 1-acetyl-lysergide (ALD) which had bright green fluorescence; secondary spots from freshly prepared solutions, or those appearing in solutions that had been allowed to stand in the dark for several weeks, usually exhibited different fluorescent colours, but spots corresponding to 8-epimers gave the characteristic lysergic blue colour. On systems IA and IB loadings of 2–5 μg of the specified erganes gave, with spray "c", slowly fading yellow spots and significant tailing. In system IIC each main spot appeared purple to visualizations "e" and "f" and a slowly darkening yellow with "c", but few subsidiary spots could be visualized with these sprays.

Rf* values obtained with precoated polyester sheets are also summarized in Table 5. To simulate the basic plate of system II, 2 μl 0·1 N sodium hydroxide was pipetted on to each origin spot and the sheets dried before applying 5–10 μg of seven erganes.

Discussion

Ammoniacal methanol had been shown by Sunshine (1963) to be eminently suitable for the resolution of phenothiazines and alkaloids. However, our preliminary investigation indicated that neither this solvent nor methanol alone adequately separated a number of erganes, including lysergic acid itself, although all ran well ahead of tryptamines and most phenethylamines. Moreover, assignment of Rf values was obscured by considerable tailing. The principal application would therefore be an initial separation where these latter classes of psychotropic drugs were suspected of being mixed with, say, lysergide. It would appear that the system IIC is the one of choice for resolution of erganes on thin-layer plates.

Results with precoated polyester sheets confirmed our experience with silica gel on glass plates, namely that methanol (solvent A) and ammoniacal methanol (solvent B) gave poor resolution of substances that appeared as blue primary, but

suppressed secondary, spots with 254 nm illumination, and as blotchy badly resolved yellow areas with visualization "c". The sequence obtained on alkali-treated sheets (VII) with solvent C was similar to that with the plate system IIC but the observed Rf values were somewhat lower, with an appreciable proportion of the sample immobile. Although VII is not strictly comparable with the uniformly impregnated silica plates II, when adjusted to the previously well established mean values for ergometrine–ergotamine in system IIC the other principal spots showed good correlation but there was less satisfactory agreement for the subsidiary ones (see Table 5). The "Chromagram" 6061 sheets (without fluorescence indicator) are preferred because very few of the subsidiary spots could be detected by 254 nm visualization on the "6060" (with F.I.) sheets. The van Urk spray (visualization "e") gave, on warming, purple brown principal spots—except methysergide and 1-acetyl-lysergide, for which the faint grey brown colour may tentatively be attributed to substitution at the indole nitrogen modifying aromatic conjugation with the dimethylaminobenzaldehyde adduct.

Taking advantage of the sensitivity of system IIC for subsidiary spots, a special investigation of the homogeneity of lysergide samples was undertaken. From the results (see Table 6) it is clear that it may be possible, for a new seizure, to distinguish between recently diverted licitly manufactured material, and lysergide that has either been crudely synthesized or has been stored under adverse conditions. Subdivision of the latter category would depend upon detailed investigation of the likely by-products of various synthetic routes and breakdown mechanisms; this study has not yet been undertaken.

Table 6. *Multiplicity of spots observed in lysergide from various sources. Rf* values* $\times$ 100 *in system IIC; colour given by visualization "a"* (see p. 794)

Origin of sample	Main spot	8α-isomer	Subsidiary spots estimated less than 1% in fresh solutions		
Sandoz manufacture	63; blue	40†; faint blue (5%)	70†; faint green	76; orange	—
1966 Official synthesis	63; blue	40†; faint (1%)	71; green	76†; orange	30†; v. faint
1966 Illicit synthesis	64; blue	42; faint blue (5%)	72; green·	76†; orange	28†; v. faint
1968 Stained blotting paper	63; blue	45; blue (20%)	71; faint	75; v. faint	—
1967 Seized capsules	62; blue	42; blue (30%)	69; v. faint	73; v. faint	—
1969 Seized powder	64; blue	41; blue (50%)	71; v. faint	75; v. faint	—

† Spots observed only after solution had been standing for several weeks in the dark. Percentages refer to estimated proportion of 8α-lysergide.

In addition it may be possible tentatively to identify observed secondary spots in some other erganes. Thus, the impurities in ALD with Rf* 0·61 and 0·45 are probably 8β- and 8α-lysergide; and for pharmacopoeial purposes, conformers of lysergamide and ergometrine may be detected in ergometrine and methylergometrine.

The possibility that use of the basic plate (II) for chromatography of erganes might facilitate unintentional isomerization to 8α-conformers was considered.

Table 7. *Effect of basic plate on 8β-lysergide conformation. Uncorrected Rf values* × 100 *in methanol*

		Neutral plate (I)					Basic plate (II)				
Fluorescence colour:		orange	blue†	orange	blue	orange	orange	blue†	orange	blue	orange
Official synthesis	fresh solution	—	57	—	—	—	—	67	—	—	—
	old solution	70	57	40	—	27	77	66	59	—	47
Illicit synthesis	fresh solution	—	57	42	29	—	—	68	—	54	—
	old solution	71	58-(tail)-		29	—	77	68	—	52	—

† Major spot corresponding to 8β-lysergide.

Experiments with two samples of lysergide, one substantially free of the 8α form and one containing about 5%, are summarized in Table 7. Applications of 5 μl of methanolic solutions to neutral (I) and basic (II) plates were developed with methanol and the spots visualized in 254 nm illumination. Under conditions in which secondary spots arising in old solutions can be readily detected, there was no significant difference in the distribution of spots between the neutral and basic plates.

CONCLUSION

For reliable identification it is essential to run simultaneously a reference substance of well established mobility. Graphical normalization from multiple spot markers enables consistent correlation with previous data. When only a small amount of salt is available, a technique avoiding extraction of the free base is an advantage. For the resolution of *phenethylamine* drugs we recommend two separate chromatographic examinations of alcoholic solutions of the free bases or their salts, using ammoniacal methanol on silica plates and methanol on 0·1 N sodium hydroxide impregnated silica, visualizing spots in both systems with methanolic iodine spray. A blend of mescaline, amphetamine and benzphetamine is a convenient reference mixture; if mescaline is not available methoxyphenamine is an appropriate substitute.

For the preliminary sorting of tryptamines and erganes, silica coated polyester sheets (such as Eastman "Chromagram") provide a convenient, rapid and durable medium. The preferred solvent for *tryptamines* is ammoniacal methanol and for *erganes* we recommend chloroform–methanol (9 : 1) development after dosing the applied spots with 2 μl of 0·1 N sodium hydroxide solution.

It is desirable to repeat the chromatography (for the erganes preferably on a glass plate) concurrently with a known sample of the provisionally identified drug. Ultimate confirmation by chemical and spectrometric procedures will, of course, depend upon the particular substance suspected.

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