*Journal of Chromatography*, 86 (1973) 269–273


CHROM. 6903

**Note**

# Identification of N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine and bufotenin by cellulose TLC

P. BAUMANN* and N. NARASIMHACHARI

*Thudichum Psychiatric Research Laboratory, Galesburg State Research Hospital, Galesburg, Ill. 61401 (U.S.A.)*

(Received May 15th, 1973)

The psychogenic N,N-dimethylated tryptamines, N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) and bufotenin, are of considerable biological interest, especially since a role for them as possible abnormal metabolites in schizophrenia has been hypothesized. Moreover, the presence of one or more of the dimethylated tryptamines in urine samples of schizophrenic patients[1–4] and autistic children[5] has been demonstrated. Several thin-layer (TLC) and gas–liquid chromatographic methods for the separation and identification of these tryptamine derivatives have been reported in the literature. The method using *o*-phthalaldehyde (OPT) as a spray reagent is the most sensitive one for the qualitative identification and the quantitative determination of both 5-hydroxy- and 5-methoxytryptamine derivatives on silica gel G[6].

However, the spray reagents generally used for DMT, *p*-dimethylaminobenzaldehyde and *p*-dimethylaminocinnamaldehyde (DACA), are not as sensitive as OPT on silica gel G.

Baumann *et al.*[7] reported the detection of serotonin (5-HT) and its quantitation by densitometry using cellulose plates for TLC. 5-HT gave a characteristic blue color with DACA spray, the level of detection being 5 ng. Bufotenin also gave a similar blue color with DACA[8].

We have now examined the applicability of cellulose TLC to studies concerned with the determination of the three dimethylated tryptamines, DMT, 5-MeODMT and bufotenin, with a view to providing an additional parameter for their identification.

## EXPERIMENTAL

### *Materials*

*Standards.* Standard solutions of DMT, 5-MeODMT and bufotenin bioxalate were prepared to contain 1 $\mu$g/$\mu$l by dissolving DMT and 5-MeODMT in ethyl acetate and bufotenin in water. From the stock solutions serial dilutions containing from 5 to 100 ng/$\mu$l were prepared.

* Present address: Centre de Recherche Psychopathologique, Clinique Psychiatrique Universitaire, Hôpital De Cery, 1008, Prilly-Lausanne, Switzerland.

*Cellulose TLC plates.* 18 g of Merck Avicel cellulose (microcrystalline) were mixed for 30 sec with 100 ml of water in an osterizer. A layer of the mixture, 0.25 mm in thickness, was spread on five plates with a Desaga applicator.

*DACA spray reagent.* 200 mg of DACA were dissolved in 100 ml of ethanol, and 4 ml of this solution was mixed with 1 ml of concentrated HCl for use as a spray reagent.

*Solvent systems.* (A) *n*-Butanol–5 *N* acetic acid (100:35); (B) butanone-2–*n*-butanol–2.5 *N* acetic acid (70:20:20); (C) isopropanol–water–ammonia (17:3:1); (D) chloroform–methanol–ammonia (12:7:1).

*Methods*

*Thin-layer chromatography.* One-dimensional TLC was used to determine the sensitivity of the spray reagent. 1 $\mu$l of the serial dilutions of the standard samples was spotted on one cellulose and two silica gel G plates. Solvent A was used for developing the cellulose plate and solvent D for the silica gel G plates. The cellulose plate and one silica gel G plate were sprayed with DACA and the other silica gel G plate was sprayed with OPT. Two-dimensional TLC on cellulose was run by spotting mixtures of DMT, 5-MeODMT and bufotenin at two levels, 10 and 100 ng, and using solvent mixtures A and B. To study the effect of interfering materials in urine, the standards (5 $\mu$g each) were added to a 24-h urine sample and the tertiary amine fraction from the urine extract was used for TLC. The minimum detectable limit in urine was determined by adding 1, 2, 3, and 5 $\mu$g of the standards to the urine.

*Column chromatography.* To obtain cleaner chromatograms on TLC, it was found necessary to clean up the urinary extract by an additional step of ion-exchange column chromatography. Columns, 5 mm in diameter, were filled with Dowex 50 to a height of 5 cm and washed first with 10 ml of 2 *N* HCl, then with 20 ml of water to pH 4, and finally with ethanol. 5 $\mu$g of each of the three standards were used in the preliminary experiments. The column was eluted with ethanolic ammonia (30 ml of 35% $NH_4OH$ in 220 ml of 60% ethanol) and 1-ml fractions were collected. TLC monitoring of the eluate showed that DMT, 5-MeODMT and bufotenin appear in fractions 3 through 6. For the urinary fraction, the tertiary amine fraction from the urine extract was dissolved in 1 ml of ethanol and loaded in the column which had been previously washed with 12 ml of 60% ethanol. Elution was done with ethanolic ammonia. The loading, washing and elution were done at the rate of a drop every 7 sec. After the amine fraction (5–6 ml) had been concentrated under vacuum, the concentrate was extracted into ethyl acetate at pH 10 (a few drops of ammonia), evaporated to dryness and redissolved in 100 $\mu$l of ethyl acetate for use in TLC.

## RESULTS

The sensitivity data for the three compounds on cellulose and silica gel G TLC and for the OPT and DACA spray reagents are given in Table I. The level of sensitivity for DACA on silica gel G was considerably lower than on cellulose. With DACA, bufotenin and 5-MeODMT gave a blue color and DMT a violet color. The three compounds were well separated on two-dimensional TLC (Fig. 1) with solvent systems A and B. The $R_F$ values are given in Table II.

TABLE I

SENSITIVITY DATA WITH DIFFERENT SPRAY REAGENTS AND ADSORBENTS

| *N,N-Dimethylated tryptamine* | *DACA–cellulose (ng)* | *DACA–silica gel G (ng)* | *OPT–silica gel G (ng)* |
|---|---|---|---|
| DMT | 10 | 200 | ND* |
| 5-MeODMT | 5 | 200 | 5 |
| Bufotenin | 5 | 200 | 5 |

* ND = not detectable.

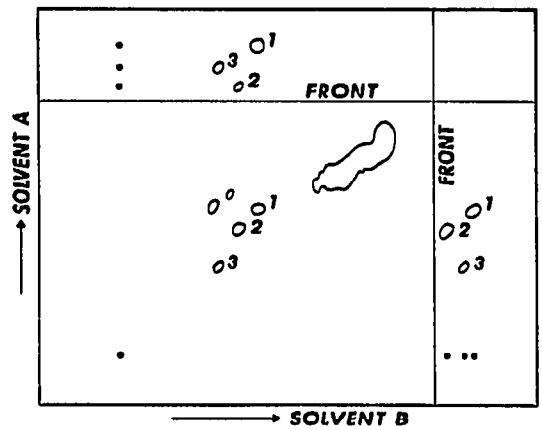


Fig. 1. TLC of a urine sample with added standards (2 μg each) after ion-exchange column chromatography (see text). 1 = DMT; 2 = 5-MeODMT; 3 = bufotenin.

TABLE II

$R_F$ VALUES OF N,N-DIMETHYLATED TRYPTAMINES

A = *n*-butanol–5 *N* acetic acid (100:35); B = butanone-2–*n*-butanol–2.5 *N* acetic acid (70:20:20); C = isopropanol–water–ammonia (17:3:1).

| *Compound* | *$R_F$ Values* | | |
|---|---|---|---|
| | *A* | *B* | *C* |
| DMT | 0.58 | 0.63 | 0.98 |
| 5-MeODMT | 0.52 | 0.56 | 0.96 |
| Bufotenin | 0.35 | 0.46 | 0.93 |

In the recovery experiments with standards added to the urine, it was possible to detect all three compounds at levels as low as 2 μg/24-h urine sample when a 10% aliquot of the final tertiary amine fraction was spotted on TLC. Bufotenin and 5-MeODMT could also be identified on silica gel G with OPT spray. When only the tertiary amine fraction was spotted on cellulose TLC, the chromatograms showed

only a few (3 or 4) spots when DACA was used as the spray reagent. The region where bufotenin and 5-MeODMT appeared was relatively free of any interfering material, whereas a larger spot appearing in the region of DMT interfered with its identification. However, when this tertiary amine fraction of urinary extraction was further purified by ion exchange chromatography, the combined extraction and column procedure gave a clear chromatogram with sharp, well-defined spots as shown by the recovery experiments.

In our opinion cellulose TLC provides another highly sensitive method for the identification of DMT, 5-MeODMT and bufotenin, a method which is capable of quantitation by densitometry. The use of the tertiary amine fraction from the urine extracts, which reduces interference by other basic substances giving a positive reaction to DACA spray, and the further cleanup of the tertiary amine fraction by ion-exchange column chromatography improve the method previously described by Baumann *et al.*[7] by increasing both the sensitivity to, and the identifiability of, DMT, bufotenin and 5-MeODMT.

The presence of bufotenin in the urine of some schizophrenics was reported on the basis of two-dimensional silica gel G TLC and OPT spray[4]. When the same samples were run on cellulose TLC with DACA spray, the identity of bufotenin was confirmed and comparable quantitative data were obtained. The identity of bufotenin from the same samples was confirmed by gas chromatography–mass spectrometry (GC–MS) of the TMS derivatives[9]. Thus for the routine screening of urine samples for bufotenin, both cellulose and silica gel G TLC, with DACA and OPT respectively as spray reagents, provide confirmatory evidence and comparable quantitative data.

Samples positive for bufotenin on silica gel G sprayed with OPT showed neither DMT nor bufotenin when the plates were sprayed with DACA. When these samples were reexamined on two-dimensional cellulose TLC, some from schizophrenic urines showed DMT. The semiquantitative data obtained by comparison of the color of the samples with that of the standards showed levels of DMT of about 1 to 2 $\mu$g in 24-h urine collections. The identity of DMT in urine samples from two drug-free chronic schizophrenics which were positive on cellulose TLC was confirmed by GC-MS analysis of both the free base and the TMS derivative (unpublished data). Thus cellulose TLC of the tertiary amine fraction is the method of choice for the identification and semiquantitation of DMT in the routine screening of urine samples, for it provides preliminary qualitative identification so that the samples can then be run on a GC–MS system.

ACKNOWLEDGEMENT

One of us (P.B) thanks the Académie Suisse des Sciences Médicales for a grant which made this visit possible.

REFERENCES

1 N. Narasimhachari, J. Avalos, M. Fujimori and H. E. Himwich, *Biol. Psychiatr.*, 5 (1972) 311.
2 N. Narasimhachari, B. Heller, J. Spaide, L. Haskovec, M. Fujimori, K. Tabushi and H. E. Himwich, *Life Sci.*, 9, Part I, (1970) 1021.
3 N. Narasimhachari, B. Heller, J. Spaide, L, Haskovec, M. Fujimori, K. Tabushi and H. E. Himwich, *Biol. Psychiatr.*, 3 (1971) 9.

4 N. Narasimhachari and H. E. Himwich, *J. Psychiatr. Res.*, 9 (1972) 113.
5 H. E. Himwich, R. L. Jenkins, M. Fujimori, N. Narasimhachari and M. Ebersole, *J. Autism Child. Schizo.*, 2 (1972) 114.
6 N. Narasimhachari and J. Plaut, *J. Chromatogr.*, 57 (1971) 433.
7 P. Baumann, B. Scherer, W. Krämer and N. Matussek, *J. Chromatogr.*, 59 (1971) 463.
8 P. Baumann, *Ph. D. Thesis*, in preparation.
9 N. Narasimhachari and H. E. Himwich, *Life Sci.*, 12, Part II, (1973) 475.