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REVERSED-PHASE HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF SOME TRYPTAMINE DERIVATIVES

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SUMMARY

A quantitative reversed-phase high-pressure liquid chromatographic method is described for tryptamine, bufotenine, N-methyltryptamine and N,N-dimethyltryptamine. All compounds were eluted with base-line separation within 15 min. The method is used to determine the tryptamine level in a leaf extract of *Acacia podalyriaefolia* A. Cunn. Satisfactory recoveries of tryptamine from plant material, and N,N-dimethyltryptamine from urine were obtained.

INTRODUCTION

Tryptophan-derived indole alkylamines occur widely in the plant family Leguminosae, especially in the genera *Acacia*, *Desmodium*, *Lespedeza*, *Mimosa*, *Piptadenia* and *Prosopsis*[1,2]. The N,N-dimethylated compounds, N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine and 5-hydroxy-N,N-dimethyltryptamine (bufotenine) are of particular interest, since they are considered to be hallucinogenic[3]. Tryptamine derivatives occur as normal constituents of blood and urine[4], and in the brain[5], and tryptamine is a putrefactive product in decaying human tissue[6]. N,N-dimethyltryptamine has been implicated as an abnormal metabolite in body fluids of schizophrenic patients[7,8].

Gas–liquid chromatography (GLC) has been widely used for the analysis of tryptamines, though not without difficulty. When the compounds are chromatographed without prior derivatization the primary amines tail severely on common stationary phases[9], and inadequate resolution is obtained between tryptamine, N-methyltryptamine and N,N-dimethyltryptamine[10]. The reactions of tryptamine with pentafluoropropionic anhydride[11] and 2-bromopropane-sodium hydride[12] lead to several products, although single, symmetrical peaks are reported by the formation of acetate[13], enamine (Schiff's base)[14] and isothiocyanate[3] derivatives. When the secondary amine N-methyltryptamine is silylated, silyl ethers of both the indole and alkyl –NH groups are formed[3]. More recent work, however, has demonstrated the applicability of GLC to the analysis of low levels of endogenous indole alkylamines using derivatives detected by electron capture[15,16].

A convenient method for the assay of selected tryptamines, avoiding the need for derivatization, is described in this communication. A primary, secondary and two tertiary indole alkylamines are rapidly resolved by isocratic elution using a reversed-phase high-pressure liquid chromatographic (HPLC) system. The method is used to quantitate the tryptamine level in an extract of *Acacia podalyriaefolia* leaves, and is currently being used to screen other species of the Leguminosae for the occurrence of tryptamine bases.

EXPERIMENTAL

HPLC separations were carried out using a Waters Assoc. (Milford, Mass., U.S.A.) Model 6000 liquid chromatograph, equipped with a Beckman 25 variable-wavelength UV spectrophotometer and recorder. Separations were conducted on a Waters Assoc. 30 × 4 mm I.D. μBondapak C_{18} column. Thin-layer chromatography (TLC) was performed on glass-backed pre-coated silica gel GF_{254} plates (20 × 20 cm, 0.25 mm thickness) (Merck, Darmstadt, G.F.R.). The low resolution mass spectrum was obtained by means of the Hitachi Perkin-Elmer Model RMU-6D spectrophotometer.

Chemicals

Tryptamine hydrochloride, bufotenine monooxalate hydrate and N-methyltryptamine were obtained from Sigma, St. Louis, Mo., U.S.A. Bufotenine was generously donated by Dr. J.-E. Lindgren, Swedish Medical Research Council, Karolinska Institutet, Stockholm, Sweden, and N,N-dimethyltryptamine by Dr. S. Khalil, College of Pharmacy, North Dakota State University, Fargo, N.D., U.S.A. All compounds were checked for purity by TLC. Solvents used for HPLC were of reagent grade and redistilled in glass.

Plant material

An 80% ethanolic extract of *Acacia podalyriaefolia* A. Cunn., collected in Australia, was supplied by the Developmental Therapeutics Program (Natural Products Branch) of the National Cancer Institute, formerly the Cancer Chemotherapy National Service Center, Bethesda, Md., U.S.A.

High-pressure liquid chromatography

Standard solutions of tryptamine hydrochloride, bufotenine monooxalate hydrate, N-methyltryptamine and N,N-dimethyltryptamine were dissolved in methanol–0.1 *M* ammonium carbonate (1:1), since this resulted in better resolution than when the eluting solvent was used to dissolve the compounds. These solutions were mixed and subjected to HPLC under the following operating conditions: eluting solvent, 1,4-dioxane–0.1 *M* ammonium carbonate (4:5); flow-rate, 1 ml/min at ambient temperature; wavelength of UV detector, 280 nm; 0.5 absorbance units full scale (a.u.f.s.) and recorder chart speed, 0.2 in./min.

Beer's law curves were obtained from triplicate 20 μl injections of four concentrations for each compound. The weights (in 20 μl) of the compounds applied to the column were: tryptamine (base equivalent) 0.99, 1.98, 2.97, 3.98 μg, bufotenine (base equivalent) 2.12, 4.24, 6.36, 8.48 μg, N-methyltryptamine, 2.43, 4.87, 7.29, 9.73

μg, N,N-dimethyltryptamine, 8.11, 16.21, 24.33, 32.43 μg. For tryptamine and bufotenine, peak heights were measured, while the peak areas of N-methyltryptamine and N,N-dimethyltryptamine were determined by measuring the product of height by width at half peak height.

The limit of detection for each compound was established by diluting a solution containing a known amount of the indole alkylamines, until the signal-to-noise ratio at the most sensitive UV setting (0.1 a.u.f.s.) was about 3:1.

Extraction of plant material

A dried 80% ethanolic extract of *Acacia podalyriaefolia* was subjected to a modification of a previously published extraction scheme[17]. About 2 g of accurately weighed plant material was thoroughly moistened with 28% NH_4OH solution and dried on a steambath. On cooling, the sample was placed in a 100-ml boiling flask fitted with a condenser, 20 ml of chloroform were added, and the mixture refluxed on a steam bath for 30 min. The refluxed mixture was cooled to room temperature and filtered. The residue was returned to the flask, an additional 15 ml of chloroform added, and the 30-min reflux period repeated. After cooling and filtering, the filtrates were combined, and partitioned with 10 ml of a 1% w/v HCl solution. Alkaloids were shown to be absent from the chloroform extract by TLC, and it was discarded. The acidic aqueous layer was adjusted to pH 8.5 with 28% NH_4OH, and the free base extracted into 3×10 ml chloroform. Chloroform was removed under reduced pressure, and the residue taken up in 6.0 ml methanol–0.1 *M* ammonium carbonate (1:1). Triplicate 20-μl injections were applied to the chromatographic column.

Although tryptamine has been isolated previously from *Acacia podalyriaefolia* leaves[18], the identity of the base obtained in this extraction procedure was checked by mass-spectral analysis after purification by preparative TLC in methanol–28% ammonia (131:2).

Recovery experiments

Tryptamine HCl (1.60 mg) was added to *Acacia podalyriaefolia* residue, from which all previous traces of tryptamine had been extracted, and the residue was re-extracted as described previously. The final residue was diluted to 8.0 ml with methanol–0.1 *M* ammonium carbonate (1:1), and the overall percentage recovery of tryptamine was obtained by analysis of peak heights from triplicate 20-μl injections of this solution.

The recovery of N,N-dimethyltryptamine in urine was investigated by dissolving 2.60 mg of the compound in 1.5 ml urine, and diluting with an equal volume of acetonitrile. Three 20-μl portions of the supernatant were directly injected onto the HPLC column, and the percentage recovery calculated from the mean peak area.

RESULTS AND DISCUSSION

The retention times of tryptamine, bufotenine, N-methyltryptamine and N,N-dimethyltryptamine are shown in Table I; Fig. 1 is a chromatogram showing base-line separation between the compounds within a short overall elution time. Attempts to remove the solvent impurity peak shown in Fig. 1 by double redistillation of solvents, and by using superior grades of solvents, were unsuccessful. However, this peak is

TABLE I

HPLC CHARACTERISTICS OF TRYPTAMINES

Compound	*Retention time (min)*	*Slope****	*Y-Axis intercept****	*Correlation coefficient****	*Lowest concentration detected (μg/ml)* §
Tryptamine*	5.1	5.14	+0.50	0.997	2.0
Bufotenine*	6.9	2.74	+0.56	0.996	3.8
N-Methyltryptamine**	8.7	0.85	−0.37	0.995	4.9
N,N-Dimethyltryptamine**	13.2	0.63	−0.85	0.994	11.9

* Peak height determination.
** Peak area determination.
*** UV detector 0.5 a.u.f.s. (within weight ranges in text).
§ UV detector 0.1 a.u.f.s.

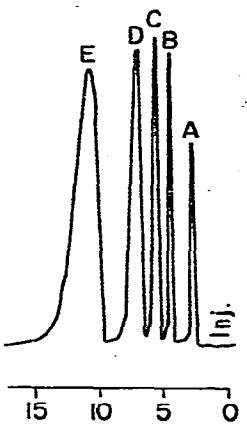

Fig. 1. Liquid chromatogram of standard tryptamines. For operating conditions, see text. A = Solvent impurity; B = tryptamine; C = bufotenine; D = N-methyltryptamine; E = N,N-dimethyltryptamine.

well removed from those of the four tryptamines assayed. Table I lists the slopes, *y*-axis (peak height/area) intercepts, and correlation coefficients of linear regression lines calculated for each compound from the points used to determine Beer's law standard curves. The detector response was linear at 0.5 a.u.f.s. for all the indole alkylamines, within the weight ranges listed in the experimental section of this paper.

Tryptamine was confirmed as a constituent of *Acacia podalyriaefolia* leaves, by comparison of its mass spectrum with published data[19], and was calculated to comprise 0.06% (w/w) of the plant extract by this HPLC assay. Fig. 2 shows a typical chromatogram of tryptamine from this plant source.

Of the three UV absorption bands that occur in the indole chromophore[20], the band at 280 nm, with a relatively low absorption intensity, was used to increase the selectivity of this HPLC assay for tryptamines in the presence of other plant constituents. The sensitivity of this method (detection limits for each compound are shown in Table I) does not approach those of other methods designed to estimate the very low levels of endogenous indole alkylamines[15,16].

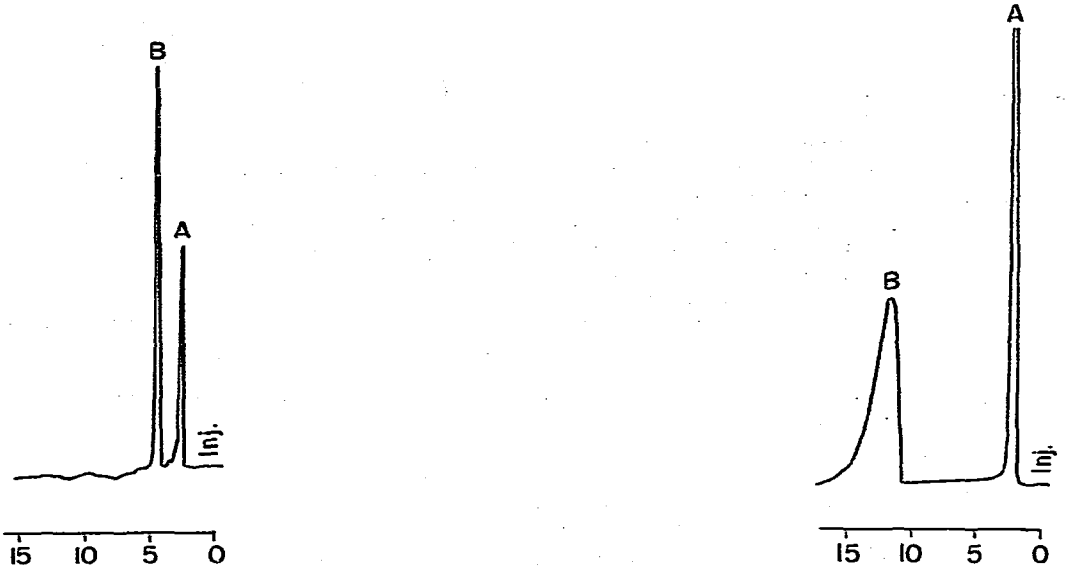

Fig. 2. Liquid chromatogram of *Acacia podalyriaefolia* leaf extract. A = Solvent impurity; B = tryptamine.

Fig. 3. Liquid chromatogram of N,N-dimethyltryptamine in urine. A = Urine impurity; B = N,N-dimethyltryptamine.

The recovery of tryptamine, introduced as a weighed amount of the hydrochloride salt, from the extraction and HPLC procedures was 95.3%. Accordingly, this method is suitable for the further investigation of tryptamines occurring as possible hallucinogenic constituents of plants, and could be also used to determine the normal levels of these bases in foodstuffs. It is unlikely that bufotenine would be quantitatively extracted by the method described in this study, due to the formation of a polar phenolate anion at basic pH[15]. However, if plant material containing bufotenine is purified by methods other than an acid-base shake out, such as by ion-exchange, this assay would be applicable to quantitate bufotenine levels.

N,N-Dimethyltryptamine was almost quantitatively recovered from urine (100.4%) by direct injection of urine diluted with acetonitrile. A typical chromatogram showing the resolution from urine impurities is shown in Fig. 3. Although the concentration of N,N-dimethyltryptamine used in this recovery experiment is far higher than endogenous levels in urine[4], the method might be of use in estimating this amine in urine after preliminary extraction and concentration procedures. It would be of interest to investigate the separation of N,N-dimethyltryptamine from its metabolites[21] using this HPLC system, although this is outside the scope of the present work.

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REFERENCES

1 T. E. Smith, *Phytochemistry*, 16 (1977) 171.
2 J. E. Saxton, in R. H. F. Manske (Editor), *The Alkaloids —Chemistry and Physiology*, Vol. 10, Academic Press, New York, 1968, p. 491.
3 N. Narasimhachari, J. Spaide and B. Heller, *J. Chromatogr. Sci.*, 9 (1971) 502.
4 F. Franzen and H. Gross, *Nature (London)*, 206 (1965) 195.
5 J. A. Saavedra and J. Axelrod, *Science*, 175 (1972) 1365.
6 J. S. Oliver, H. Smith and D. J. Williams, *Forensic Sci.*, 9 (1977) 1052.
7 R. J. Wyatt, J. C. Gillin, J. Kaplan, R. Stillman, L. Mandel, H. S. Ahn, W. J. A. VandenHeuvel and R. W. Walker, in E. Costa and P. Greengard (Editors), *Advances in Biochemical Pharmacology* Vol. 11, Raven Press, New York, 1974, p. 299.
8 B. Angrist, S. Gershon, G. Santhananthan, R. W. Walker, B. Lopez-Ramos, L. R. Mandel and W. J. A. VandenHeuvel, *Psychopharmacol.*, 47 (1976) 29.
9 H. M. Fales and J. J. Pisano, *Anal. Biochem.*, 3 (1962) 337.
10 S. Agurell, B. Holmstedt, J.-E. Lindgren and R. E. Schultes, *Acta Chem. Scand.*, 23 (1969) 903.
11 E. Gelpi, E. Peralta and J. Segura, *J. Chromatogr. Sci.*, 12 (1974) 701.
12 B. Blessington and N. I. Y. Fiagbe, *J. Chromatogr.*, 78 (1973) 343.
13 C. J. W. Brooks and E. C. Horning, *Anal. Chem.*, 36 (1964) 1540.
14 E. C. Horning, M. G. Horning, W. J. A. VandenHeuvel, K. L. Knox, B. Holmstedt and C. J. W. Brooks, *Anal. Chem.*, 36 (1964) 1546.
15 S. T. Christian, F. Benington, R. D. Morin and L. Corbett, *Biochem. Med.*, 14 (1975) 191.
16 F. Cattabeni, S. H. Koslow and E. Costa, *Science*, 178 (1972) 166.
17 N. R. Farnsworth and K. L. Euler, *Lloydia*, 25 (1962) 186.
18 J. J. Williaman and B. J. Schubert, *Alkaloid Bearing Plants and Their Contained Alkaloids*, Technical Bulletin, No. 1234, A.R.S. U.S.D.A. Washington, D.C., 1961, p. 101.
19 M. W. Couch and C. M. Williams, *Anal. Biochem.*, 50 (1972) 612.
20 A. I. Scott, in D. H. R. Barton and W. Doering (Editors), *International Series of Monographs on Organic Chemistry*, Vol. 7, Pergamon Press, Oxford, 1964, p. 172.
21 S. Szara, *Experientia*, 12 (1956) 441.