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Note

Identification and separation of indolealkylamines by gas–liquid chromatographic analysis of their heptafluorobutyryl derivatives

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In connection with our studies on the presence of trace levels of N- and/or O-methylated derivatives of tryptamine and 5-hydroxytryptamine in cerebrospinal fluid (CSF) of schizophrenic patients, we wish to report here the separation and identification of these putative abnormal metabolites by gas–liquid chromatography (GLC). Earlier, one of us reported[1] the presence of tryptamine in steer, dog and human brain at the nanogram level by means of GLC of the perfluoroacetyl derivative of this compound, as well as by fluorescence spectroscopy and thin-layer chromatography. These studies suggested that as a prerequisite to studying CSF a sensitive assay for these indolealkylamines would be required.

A specific gas chromatographic method, using flame ionization detection and mass spectrometric identification of this group of biogenetic amines, has been reported by Vessman *et al.*[2]. In this procedure, indolealkylamines were converted to heptafluorobutyryl (HFB) compounds by acyl transfer using N-heptafluorobutyrylimidazole as a derivatizing reagent. Each amine gives a single, well defined derivative. By first modifying somewhat Vessman's procedure for preparing and extracting these derivatives, then injecting them singly or as a mixture, it was possible for us to separate and identify each component present in purified toluene as they emerged from an OV-17 column, by means of a ^{63}Ni electron capture detector. Each of the HFB derivatives possesses a distinct, reproducible emergence time. Furthermore, excellent linearity is demonstrated between picograms of amine introduced into the column and the product of attenuation with peak area of the resulting signal.

EXPERIMENTAL

Materials

N-Methyltryptamine and N,N-dimethyltryptamine were synthesized in these laboratories by the method of Speeter and Anthony[3] and purified by distillation *in vacuo* and recrystallization from ethyl acetate–hexane (1:1). 5-Methoxy-N,N-dimethyltryptamine had been synthesized previously by us[4]. All other amines were obtained from Regis (Chicago, Ill., U.S.A.) and purified from ethyl acetate–hexane. N-Heptafluorobutyrylimidazole was obtained from Pierce (Rockford, Ill., U.S.A.) and toluene from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.).

Gas chromatography

A Hewlett-Packard Model 5700-A gas chromatograph with a Hewlett-Packard Model 18713A ^{63}Ni linear electron-capture detector and equipped with a Hewlett-Packard Model 7123A strip-chart recorder was used. Glass columns (1.8 m × 2 mm I.D.) were packed with 5% OV-17 on 80–100 mesh Gas-Chrom Q (Hewlett-Packard). The injector block was maintained at 250°, the column at 175° and the electron capture detector at 300°. A flow-rate of 50 ml/min was maintained for the carrier gas (argon–methane, 95:5).

Preparation and isolation of pure HFB derivatives

A mixture of 0.45 mmole of amine and 4.5 mmole of N-heptafluorobutyrylimidazole was heated at 85° for 1.25 h in a 3-ml Reacti-vial (Pierce) with the PTFE-lined screwcap closed. The reaction mixture was dissolved in toluene and transferred to a 60-ml separatory funnel with additional toluene (total volume, about 30 ml). This solution was cooled and washed with 5 ml of ice-cold 5% aqueous potassium carbonate and then with three 5-ml portions of water; after filtration through dry filter paper, the toluene solution was evaporated to dryness under reduced pressure with a rotary evaporator. The residue of the pure derivative was analyzed for C, H and N to verify the composition. Properties of pure derivatives isolated are shown in Table I. Solutions of these pure derivatives, made up to a concentration of 200 pg/μl in toluene, served as primary standards in the GLC analysis of these amines as their HFB derivatives.

Microscale runs

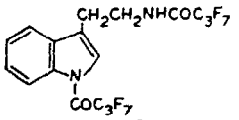
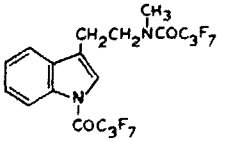
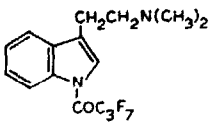
A mixture of 2 mg of the amine and 0.2 ml of N-heptafluorobutyrylimidazole was heated at 85° for 1 h in a 3-ml Reacti-vial. To this mixture was added 1 ml of water and 2 ml of toluene. After thorough mixing by means of a Vortex-Genie mixer (Scientific Instruments), the separated upper layer was withdrawn with a Pasteur pipet. The extraction with 2-ml portions of toluene was repeated five times. The pooled toluene extracts diluted to 25 ml with toluene were washed three times with 2-ml portions of water, and the solution was then filtered through dry paper into a 100-ml volumetric flask and diluted to volume with toluene. A 1:100 dilution of this stock solution gave a final solution which contained 200 pg/μl of the amine as its HFB derivative.

RESULTS AND DISCUSSION

As is shown in Table I, three of the HFB derivatives of the indolealkylamines studied here were isolated as pure compounds. Earlier work[2] showed that the N-heptafluorobutyrylimidazole reagent replaces all active protons in these amines. The derivative prepared from N,N-dimethyltryptamine was obtained in 72% yield. This lower yield might be attributed to the relative ease of hydrolysis of a compound having a single HFB moiety at the 1-position on the indole ring. All three of these isolated derivatives gave satisfactory C, H and N values on elemental analysis. The bis-HFB derivative obtained from tryptamine was found to be stable indefinitely in the absence of air and moisture, and a solution of this compound in purified toluene

TABLE I

PROPERTIES OF HFB DERIVATIVES OF TRYPTAMINE, N-METHYLTRYPTAMINE AND N,N-DIMETHYLTRYPTAMINE

Structure	*Mol. wt.*	*M.p.*	*Yield (%)*	*Analysis*					
				C		*H*		*N*	
				Calcd.	*Found*	*Calcd.*	*Found*	*Calcd.*	*Found*
	552	103–104°	95	39.1	39.4	1.81	2.04	5.07	5.24
	566	Oil	96	40.3	40.6	2.12	2.32	4.95	5.21
	384	Oil	72	50.0	49.7	3.91	4.04	7.29	7.17

served as a standard in these studies. It was not found necessary to prepare pure derivatives of the other amines shown in Table II.

Response, as measured by peak area times attenuation in the gas–liquid chromatogram of the HFB derivatives of indolealkylamines, was found to be linear in ranges varying from 4 to 400 pg of amine. Linear regression coefficients were calculated for a number of amines, and these data are summarized in Table III.

Recovery of tryptamine from CSF

In order to examine the applicability of this method to recovery of tryptamine from CSF, a sample of CSF obtained from the clinical pathology laboratory of the UAB Medical Center was spiked at the nanogram level with pure tryptamine. A 5-ml

TABLE II

GLC SEPARATION AND ANALYSIS OF INDOLEALKYLAMINES

No.	*Amine (as HFB derivative)*	*Emergence time (min)*	*Peak-area × attenuation* (cm^2)*	*Lower limit of detection** (pg)*
4	Tryptamine	14.3	744	1
5	N-Methyltryptamine	17.8	437	2
2	N,N-Dimethyltryptamine	7.8	123	10
6	O-Methylbufotenine	23.2	330	5
1	Bufotenine	6.5	60	20
7	5-Methoxytryptamine	37.8	863	1
3	5-Hydroxytryptamine	14.5	735	1

* Data in this column correspond to 400 pg of amine.

** The lower limit of detection is taken at an attenuation 1 (maximum sensitivity).

TABLE III

SUMMARY OF LINEAR REGRESSION COEFFICIENTS* RELATING Q, THE QUANTITY OF AMINE** CHROMATOGRAPHED, TO A, THE RECORDED SIGNAL (ATTENUATION × PEAK AREA), IN THE EQUATION $Q = mA + b$

Amine	*m*	*b*	*S.E. of estimate*	*Range Q (pg)*
Tryptamine	0.8668	+0.8337	0.9047	10–100
N-Methyltryptamine	0.4339	+0.7900	1.695	40–400
N-Methyltryptamine	0.4381	+0.1630	0.9863	4– 40
N,N-Dimethyltryptamine	0.3373	−0.0165	0.2947	5– 70
O-Methylbufotenine	0.0875	−0.4713	0.5586	10–200

* Calculated from experimental data using a Model 9821A Hewlett-Packard calculator with linear regression program.

** Data shown as quantity of amine which was chromatographed as the HFB derivative.

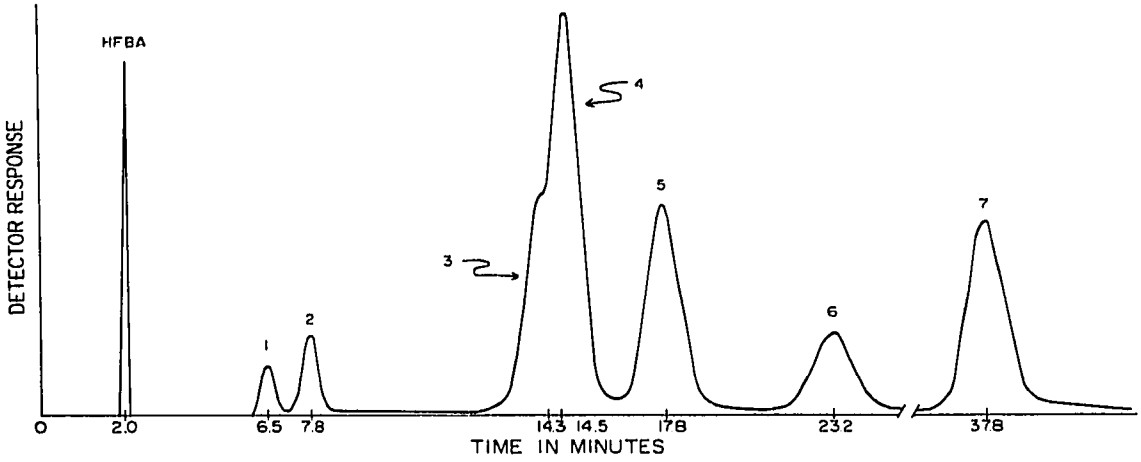


Fig. 1. Gas chromatogram of 400 pg of each amine shown in Table II as HFB derivative. HFBA = Heptafluorobutyric acid. Attenuation = 32.

sample of CSF containing the added tryptamine was deproteinized ($HClO_4$), adjusted to pH 11 (NaOH), extracted twice with equal volumes of methylene chloride, the extract dried (Na_2SO_4), evaporated to dryness, and the residue heated with 0.1 ml of N-heptafluorobutyrylimidazole at 85° for 1 h. The reaction mixture was dissolved in toluene (2 ml) and treated with water (1 ml). The separated toluene layer was dried (Na_2SO_4) and a 2.0-μl aliquot was injected into the gas chromatograph. A peak occurred at the emergence time corresponding to tryptamine-HFB (see Fig. 1), and calculation of peak area times attenuation to estimate the amount of tryptamine present showed excellent recovery of the tryptamine originally added to the CSF.

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