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GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC ANALYSIS OF AMINE METABOLITES IN URINE

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SUMMARY

An analytical method for the separation and identification of amine metabolites in human urine has been developed using a gas chromatograph—mass spectrometer—computer system. The sample preparation consists of organic solvent extraction, adsorption on a cation-exchange resin, evaporation, and acylation.

N-3-Hydroxypropyl-1, 4-diaminobutane was identified for the first time in urine. Methylguanidine, guanidine, putrescine, cadaverine, *p*-tyramine, 3-methoxytyramine, spermidine, and spermine were detected in normal urine with good gas chromatographic separation.

INTRODUCTION

The levels of aliphatic and phenolic amines in human urine have been determined by paper chromatography (PC), high-performance liquid chromatography (HPLC), and gas chromatography—mass spectrometry (GC—MS).

Kakimoto and Armstrong [1] analyzed the free and conjugated phenolic amines by two-dimensional PC and identified *p*-hydroxybenzylamine, *p*-sympathol, and vanillylamine, among other substances. Higa et al. [2] presented a new extraction method for urinary and plasma catecholamines using

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boric acid gel, and quantitated the level using mass fragmentography. Nelson et al. [3] analyzed 3-O-methylated catecholamine in human urine using ion-pair extraction and gas chromatography with electron-capture detection; they quantitated the amount of normetadrenaline and 3-methoxytyramine. Karoum et al. [4] investigated phenylethylamine and *m*- and *p*-tyramine in plasma, cerebrospinal fluid, urine, and brain using mass fragmentography. Scaro et al. [5] quantitated urinary free tyramine by HPLC with fluorescence detection, and reported that urines from patients with pheochromocytoma, neuroblastoma and Parkinson's disease have elevated tyramine levels.

Polyamines in urine have been analyzed by many investigatiors using HPLC [6], GC—MS [7, 8], and paper electrophoresis [9, 10] ever since Russell et al. [11] reported elevated polyamine concentrations in the urine of cancer patients.

Recently, GC—MS has been used to screen a number of metabolites in urine and plasma, especially organic acids and steroids, for the purposes of identifying abnormal substances [12—17].

This study reports an amine profile analysis of human urine using gas chromatography—mass spectrometry.

MATERIALS AND METHODS

Chemicals

Pentafluoropropionic anhydride was purchased from Gasukuro Kogyo Co. (Tokyo, Japan). Putrescine—2HCl, cadaverine—2HCl, spermidine—3HCl, spermine—4HCl, methylguanidine—HCl, guanidine—HCl, *p*-tyramine—HCl, and 3-methoxytyramine—HCl were the products of Sigma (St. Louis, MO, U.S.A.).

N-3-Hydroxypropyl-1,4-diaminobutane (HPDB) was synthesized according to the procedure of Tabor et al. [18].

Urine samples

Twenty-four-hour urine samples were collected in containers containing 50 ml of 3 *N* hydrochloric acid and stored at —20° C until analyzed.

Sample preparation

Fifty milliliters of urine were alkalinized to pH 13 by the addition of 3 *N* sodium hydroxide, extracted twice with 100 ml of *n*-butanol, and reextracted twice with 100 ml of 1 *N* hydrochloric acid. The extract was concentrated in vacuo to 3 ml, neutralized and applied to a Dowex 50W-X8 column (10 × 0.8 cm). After washing with 5 ml of distilled water, 40 ml of phosphate buffer (pH8), and 50 ml of 1 *N* hydrochloric acid, the amine was eluted with 100 ml of 6 *N* hydrochloric acid. The eluate was evaporated to dryness with a rotary evaporator. For acylation, 200 μl of anhydrous ethyl acetate and 200 μl of pentafluoropropionic anhydride were added to the dry residue, and the amines were acylated at 90° C for 20 min. After cooling the sample was dried with a stream of nitrogen at room temperature and redissolved in 200 μl of anhydrous ethyl acetate. Two microliters of this solution were subjected to GC—MS.

Gas chromatography—mass spectrometry

The instrument used for combined GC—MS consisted of a gas chromatograph, JGC-20K, a double focusing mass spectrometer, JMS D-300, and a data processing system, JMA 2000 (JEOL, Tokyo, Japan). The gas chromatograph was equipped with a 3 % OV-1 glass column (2 m × 2 mm I.D.). The carrier gas was helium at a flow-rate of 30 ml/min. Electron-impact ionization mass spectra were recorded under the following conditions: ionizing energy 70 eV, ionizing current 300 μA, separator temperature 230° C, ion-source temperature 200° C, and accelerating voltage 3 kV. Chemical-ionization mass spectra were recorded using methane as a reactant gas, and with an ionizing energy of 200 eV. The other conditions were the same as for electron-impact ionization.

RESULTS

Identification of HPDB in urine

Fig. 1 shows the reconstructed ion chromatogram (*m/z* 50—800) of the pentafluoropropionyl (PFP) derivatives of amines in urine. The electron-impact ionization mass spectrum of peak 43 is presented in Fig. 2 (top spectrum). The chemical-ionization mass spectrum of the peak indicates a molecular ion at *m/z* 584. High-resolution data of the compound are summarized in Table I. The original composition of the compound is considered to be $C_7H_{18}N_2O_1$. Fragment ions of 176, 190, 204, 216, and 218 are characteristic of butane diamino moiety. The fragment ion at *m/z* 393 (M—191) seems to be formed by the loss of $C_2H_4OCOC_2F_5$ from the molecular ion. The results suggest that the compound has the structure $NH_2(CH_2)_4NH(CH_2)_3OH$ or $NH_2(CH_2)_4NHCH_2CH(CH_3)OH$. The compound and authentic HPDB showed identical retention times and identical mass spectra (Fig. 2).

Amines detected in urine

Peaks 11, 12, 23, 28, 31, 41, 51, and 70 were identified as methylguanidine,

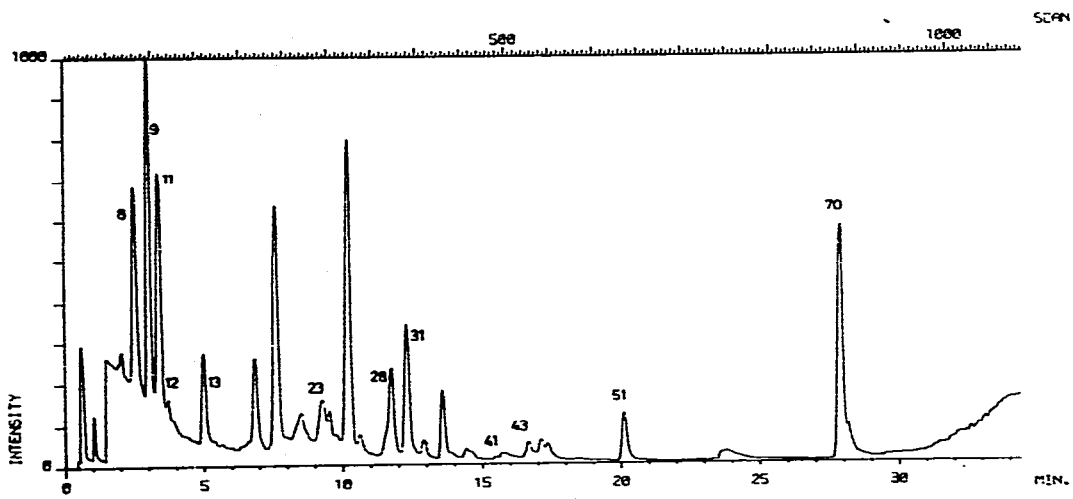

Fig. 1. Reconstructed ion chromatogram of the PFP derivatives of urine from a normal subject.

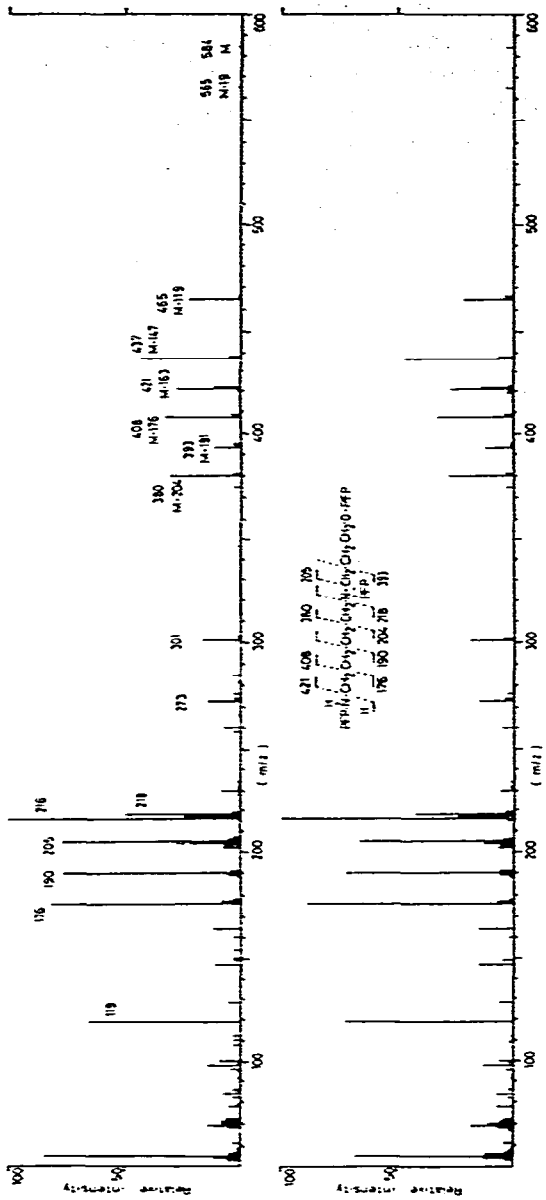

Fig. 2. Electron-impact ionization mass spectrum of the unknown peak 43 in Fig. 1 (top spectrum) and of N-3-hydroxypropyl-1,4-diaminobutane (bottom spectrum).

TABLE I

HIGH-RESOLUTION MASS SPECTRAL DATA

Observed *m/z*	Error (milli mass)	Unsaturation	Probable composition	Fragmentation
584.0790	—0.2	3.0	$C_{10}H_{15}N_2O_4(C_2F_5)_3$	M
465.0846	—2.5	3.5	$C_{10}H_{15}N_2O_4(C_2F_5)_2$	$M—C_2F_5$
437.0919	—0.3	2.5	$C_9H_{15}N_2O_3(C_2F_5)_2$	$M—COC_2F_5$
421.0783	4.5	3.0	$C_9H_{13}N_1O_3(C_2F_5)_2$	$M—NH_2COC_2F_5$
408.0658	0.0	2.5	$C_8H_{12}N_1O_3(C_2F_5)_2$	$M—CH_2NHCOC_2F_5$
380.0317	—2.7	2.5	$C_6H_8N_1O_3(C_2F_5)_2$	$M—(CH_2)_3NHCOC_2F_5$

guanidine, putrescine, cadaverine, *p*-tyramine, 3-methoxytyramine, spermidine, and spermine, respectively, by comparison with the mass spectra obtained in our laboratory from PFP derivatives of the authentic compounds (Table II). These amines were detected in healthy urine with good GC separation.

TABLE II

IDENTIFIED COMPOUNDS IN HEALTHY URINE

Peak No.	Compound	Mass spectrum of PFP derivative*
11	Methylguanidine	246 (100%, M^+ —119), 203 (32), 119 (45), 100 (7), 83 (95), 69 (38)
12	Guanidine	232 (88%, M^+ —119), 189 (18), 147 (9), 144 (8), 119 (57), 100 (17), 69 (100)
23	Putrescine	380 (6%, M^+), 261 (8), 218 (16), 217 (49), 216 (22), 176 (100)
28	Cadaverine	394 (11%, M^+), 275 (12), 247 (8), 232 (9), 230 (16), 218 (30), 190 (14), 176 (100)
31	*p*-Tyramine	266 (100%, M^+—163), 253 (25), 225 (11), 176 (15), 119 (24)
41	3-Methoxytyramine	459 (11%, M^+), 296 (100), 283 (26), 255 (5), 176 (7), 149 (53), 119 (18)
43	N-3-Hydroxypropyl-1,4-diaminobutane	584 (1%, M^+), 465 (22), 437 (43), 421 (27), 408 (32), 393 (11), 380 (30), 301 (16), 273 (14), 218 (49), 217 (24), 216 (100), 205 (76), 204 (22), 190 (76), 176 (81)
51	Spermidine	464 (24%, M^+—119), 436 (38), 273 (30), 218 (35), 216 (57), 204 (100), 202 (30), 190 (30), 176 (92), 119 (30)
70	Spermine	667 (27%, M^+ —119), 639 (30), 476 (20), 204 (100), 202 (19), 190 (22), 176 (41)

*Ionizing energy was 70 eV, and ionizing current 300 μA.

DISCUSSION

HPDB was identified for the first time in urine. However, the biosynthetic pathway of HPDB is obscure. One hypothesis is that HPDB is formed by reduction of N-4-aminobutyl-3-aminopropionaldehyde, which is a metabolite of

spermidine [18]. Tabor et al. [18], who investigated the oxidation of spermidine and spermine using purified bovine plasma amine oxidase, found that spermine was oxidized at both primary amino groups, giving dialdehyde, and spermidine was oxidized at the primary amino group of the aminopropyl moiety, giving N-4-aminobutyl-3-aminopropionaldehyde. On the other hand, N-4-aminobutyl-3-aminopropionic acid, putreanine, has been found in mammalian brain and other tissues [19]. The compound is considered to be the further oxidation product of N-4-aminobutyl-3-aminopropionaldehyde. There are no definite data that HPDB is a metabolite of spermidine as opposed to some other metabolite (Fig. 3).

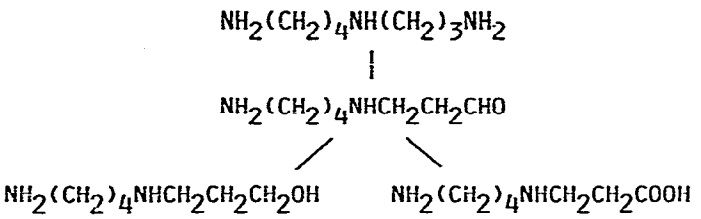

Fig. 3. Metabolic pathway of spermidine.

Our profiling amine analysis could detect phenolic amines such as *p*-tyramine and 3-methoxytyramine. A major component of peak 28 was cadaverine. A minor component of the peak had the fragment ion at *m/z* 253 and *m/z* 266. Moreover, a molecular ion at *m/z* 429 was obtained by chemical-ionization mass spectra using methane as a reactant gas. It is reasonable to suspect that the compound is *m*-tyramine according to retention times and mass spectra.

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