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GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC ANALYSIS OF ORGANIC ACIDS IN RENAL TISSUE BIOPSY

IDENTIFICATION OF 4-HYDROXYBUTYRIC ACID AND 4-HYDROXY-2-BUTENOIC ACID

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SUMMARY

The organic acids in renal tissue biopsy (0.5—1 mg) obtained from chronic glomerulonephritic patients were analyzed by capillary column gas chromatography—mass spectrometry. Some twenty compounds were identified in the renal tissue. The organic acid profile of renal tissue showed a marked difference from those of urine and serum. In particular, 4-hydroxybutyric acid and 4-hydroxy-2-butenoic acid, which are usually undetectable in urine and serum, were detected for the first time in renal tissue in considerably large amounts.

INTRODUCTION

So far the biopsy samples of renal tissue have been studied only by

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morphological methods such as light microscopy, fluorescence microscopy and electron microscopy. Recently Goodman et al. [1] described a method of analyzing the organic acids in various tissue specimens by gas chromatography—mass spectrometry (GC—MS). To determine the metabolism in renal tissue and, if possible, to diagnose renal disease from the viewpoint of metabolic profile, we have attempted to analyze the organic acids in renal tissue specimens by glass capillary column GC—MS. Organic acid profiles in renal tissue showed differences from those in urine or serum. 4-Hydroxybutyric acid and 4-hydroxy-2-butenoic acid have been detected in renal tissue for the first time.

EXPERIMENTAL

Materials

4-Hydroxybutyric acid was obtained from Tokyo Kasei Co. (Tokyo, Japan). 4-Hydroxy-2-butenoic acid was synthesized according to the method of McClure [2]. The synthesized compound was confirmed to be 4-hydroxy-2-butenoic acid by use of nuclear magnetic resonance, infrared spectroscopy and MS.

N,O-Bis(trimethylsilyl)trifluoroacetamide was purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.). All other reagents were commercially available products of analytical grade.

Sample preparation and GC—MS

Biopsied renal tissue samples were obtained from five patients with chronic glomerulonephritis. An amount of 0.5—1 mg of the tissue was homogenized with 0.2 ml of distilled water in a ground-glass homogenizer. After the addition of 0.8 ml of distilled water and sodium chloride, the homogenized solution was adjusted to pH 1.0 with 1 *N* hydrochloric acid, and the organic acids were extracted with 3 ml of ethyl acetate three times. After dehydration over anhydrous sodium sulfate, the extract was evaporated to dryness with a nitrogen stream. The extract was reacted with 1 mg of methoxylamine hydrochloride at 60°C for 1 h. After evaporation, the extract was trimethylsilylated with 20 μl of N,O-bis(trimethylsilyl)trifluoroacetamide at 90°C for 1 h. Three microlitres of the sample were subjected to GC—MS. The GC—MS instrument and the experimental conditions were the same as those reported previously [3].

RESULTS

Fig. 1 shows a gas chromatogram of organic acids in a renal tissue biopsy obtained from a patient with steroid-resistant nephrotic syndrome. The pathological finding was focal glomerulosclerosis. The peaks were identified by comparing their mass spectra with those of the trimethylsilylated authentic compounds or the mass spectra in the literature. The GC profile of renal tissue biopsy showed differences from those of urine and serum. In particular, the compound of peak 19 was detected in renal tissue in a comparatively large amount. In urine, however, the compound was barely detected or not detected at all. The electron-impact (EI) mass spectrum of peak 19 is shown in Fig. 2

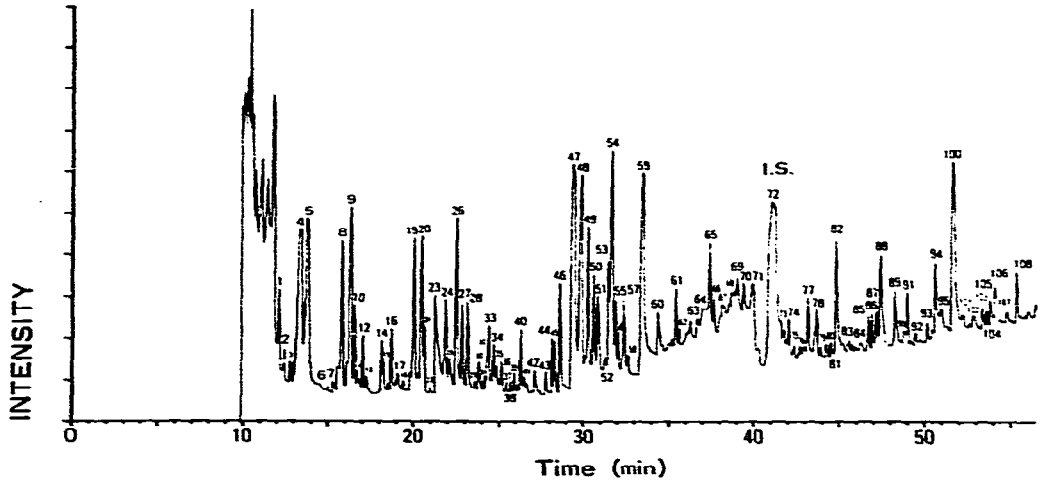

Fig. 1. Gas chromatogram of organic acids in renal tissue biopsy. The peaks were identified as follows: 3 = pyruvic acid; 4 = lactic acid; 5 = 2-hydroxyisobutyric acid (minor component), glycolic acid; 8 = 2-hydroxybutyric acid; 9 = 3-hydroxypropionic acid; 12 = 3-hydroxybutyric acid; 19 = 4-hydroxybutyric acid; 20 = diethylene glycol (artifact?); 25 = phosphoric acid; 26 = glycerol; 27 = 4-hydroxy-2-butenoic acid; 28 = succinic acid; 30 = methylsuccinic acid; 33 = 2-methylglyceric acid; 34 = glyceric acid; 36 = nonanoic acid; 37 = 4-deoxyerythronic acid; 42 = glutaric acid; 45 = 3-deoxytetronic acid; 47 = 2-deoxytetronic acid; 59 = 2,3-dideoxypentonic acid; 71 = isosaccharinolactone.

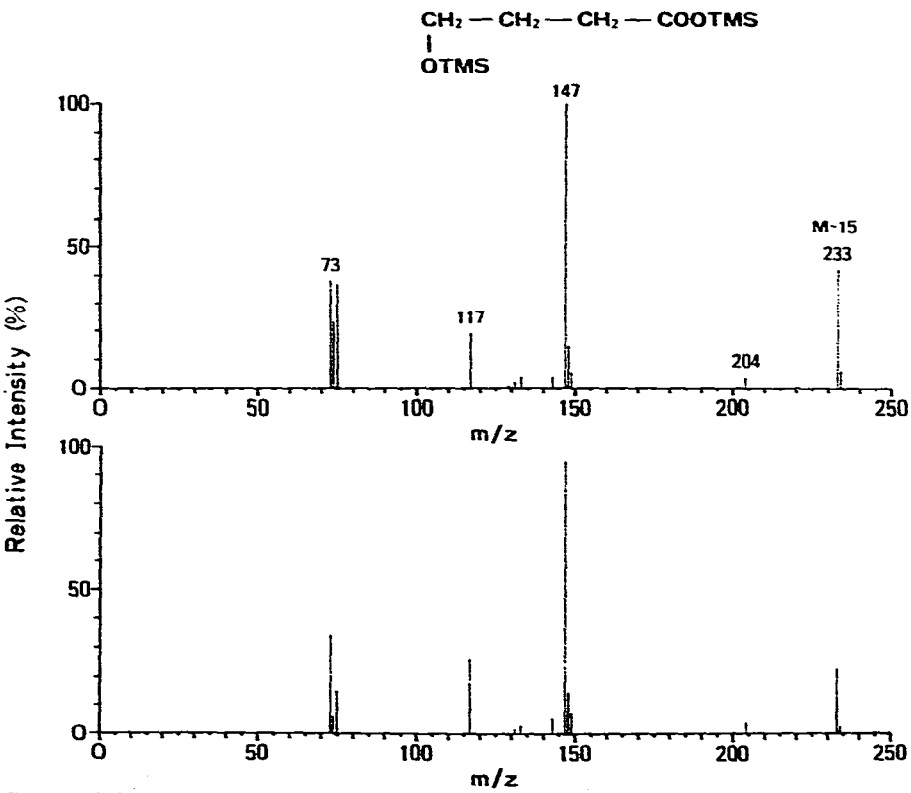

Fig. 2. EI-mass spectra of peak 19 in Fig. 1 (lower spectrum) and of trimethylsilylated 4-hydroxybutyric acid (upper spectrum).

(lower spectrum). The molecular ion of peak 19 was found to be *m/z* 248 by recording the chemical-ionization (CI) mass spectrum. High-resolution data of the *m/z* 233 ion indicated that the original molecular formula of the compound was $C_4H_8O_3$ and that the molecular structure was that of hydroxybutyric acid. The fact that the retention time of the compound on the gas chromatogram was delayed from 2-hydroxybutyric acid and 3-hydroxybutyric acid suggested the structure of 4-hydroxybutyric acid. The EI mass spectrum of trimethylsilylated 4-hydroxybutyric acid is shown in Fig. 2 (upper spectrum). Peak 19 was identified as trimethylsilylated 4-hydroxybutyric acid, since peak 19 and trimethylsilylated 4-hydroxybutyric acid showed identical retention times and identical mass spectra.

The compound of peak 27 in renal tissue was not detected in urine or serum at all. The EI mass spectrum of peak 27 is shown in Fig. 3 (lower spectrum). The CI mass spectrum of the peak indicated the molecular ion of *m/z* 246. High-resolution mass spectrometry of the *m/z* 246 ion showed an exact mass of 246.1089, an error of −1.6 millimass, an unsaturation of 2 and a probable composition of $C_{10}H_{22}O_3Si_2$. These data revealed the original composition of $C_4H_6O_3$ and the structure of hydroxybutenoic acid. The delayed retention time of peak 27 compared with 4-hydroxybutyric acid suggests the hydroxyl group at the C_4 position. The absence of an *m/z* 157 ion, $(M—OTMS)^+$, suggests unsaturation at C_2 not at C_3. 4-Hydroxy-2-butenoic acid was synthesized; the

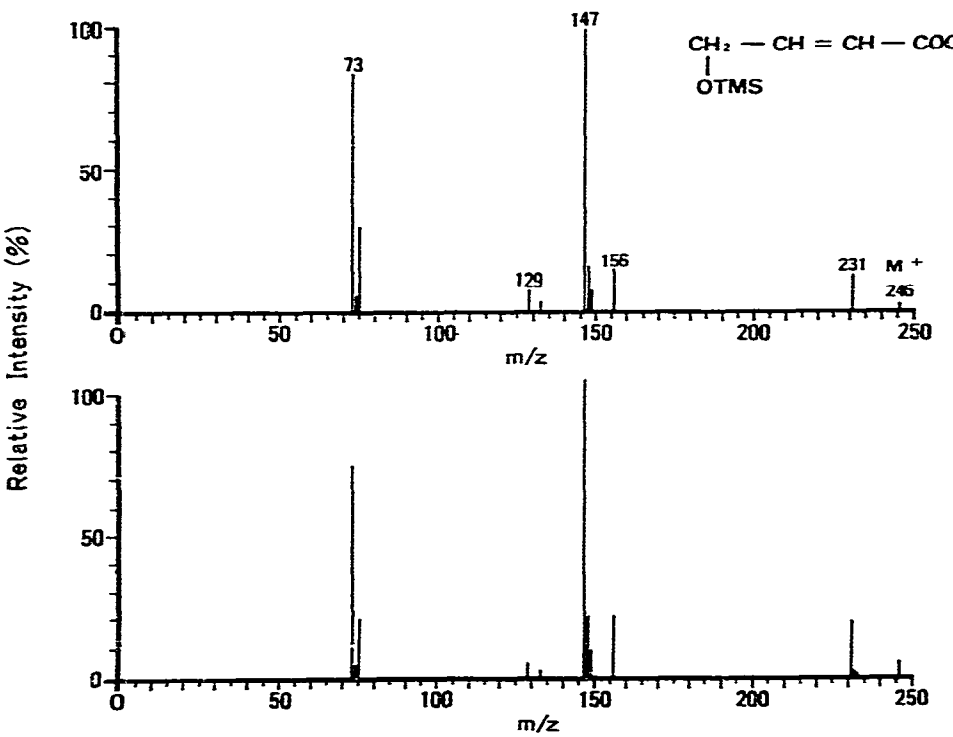

Fig. 3. EI mass spectra of peak 27 in Fig. 1 (lower spectrum) and of trimethylsilylated 4-hydroxy-2-butenoic acid (upper spectrum).

EI mass spectrum of the TMS derivative is shown in Fig. 3 (upper spectrum). Peak 27 was identified as trimethylsilylated 4-hydroxy-2-butenoic acid since peak 27 and trimethylsilylated 4-hydroxy-2-butenoic acid showed identical retention times and identical mass spectra.

DISCUSSION

By our profiling analysis of the renal tissue specimen, 4-hydroxybutyric acid and 4-hydroxy-2-butenoic acid were detected in renal tissue. So far 4-hydroxybutyric acid has been detected only in the human brain [4]. Recently, a case of inborn error of metabolism who excreted a large amount of 4-hydroxybutyric acid in the urine has been reported [5]. 4-Hydroxybutyric acid shows a pharmacological effect of central nervous system depression [6], and was synthesized as an intravenous anesthetic. The formation of 4-hydroxybutyric acid from 4-aminobutyric acid has been demonstrated in vitro [7] and in vivo [8], but this pathway seems to be a minor one for the degradation of 4-aminobutyric acid. The major portion of 4-aminobutyric acid is metabolized in brain and liver to succinic acid semialdehyde, which is further metabolized to succinic acid. 4-Aminobutyric acid is formed from glutamine via glutamic acid. The metabolic pathway of glutamine and glutamic acid is shown in Fig. 4. In the kidney, ammonia is produced from glutamine and glutamic acid, and excreted into the urine. The physiological significance of 4-hydroxybutyric acid in the kidney is at present unclear, but the formation of 4-hydroxybutyric acid in the kidney seems to be related to the ammonia production.

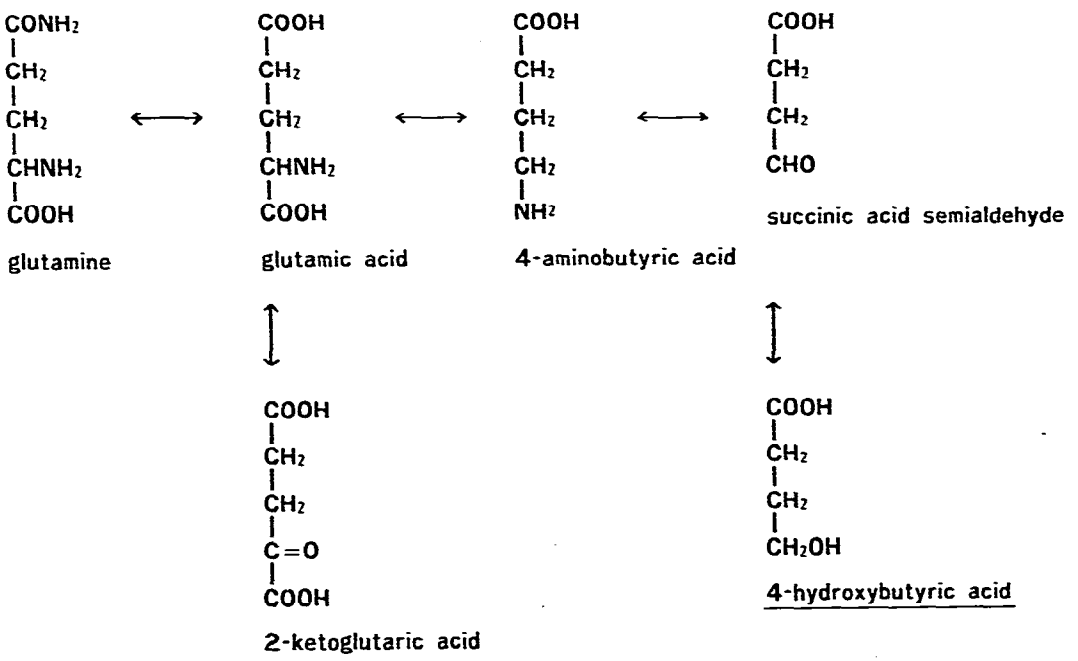

Fig. 4. Metabolic pathway of glutamine and glutamic acid.

4-Hydroxy-2-butenoic acid has not been reported to be present in human physiological fluids nor in tissues thus far. 4-Hydroxy-2-butenoic acid seems to be a metabolite of 4-hydroxybutyric acid. Walkenstein et al. [9] suggested the β-oxidation metabolic pathway of 4-hydroxybutyric acid (Fig. 5). The proposed pathway is considered to be a minor one, because the conversion of 4-hydroxybutyric acid via succinic semialdehyde to succinic acid seemed to be a more important pathway [10]. The detection of 4-hydroxy-2-butenoic acid as well as 4-hydroxybutyric acid in renal tissue, however, supports the β-oxidation pathway of 4-hydroxybutyric acid.

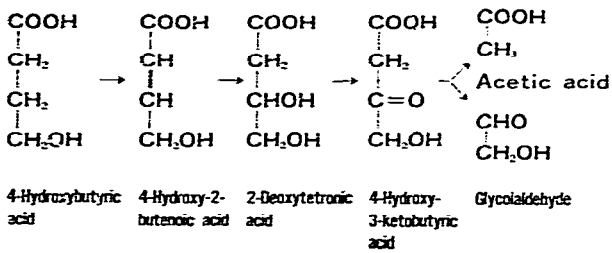

Fig. 5. Metabolic pathway of 4-hydroxybutyric acid.

The profiling analysis of organic acids in renal tissue biopsy shows the metabolic state mainly in the renal tubules, since most organic acids are known to be actively excreted into the urine by the renal tubular cells, and ammonia production is also performed in the renal tubular cells. The method may be useful for the investigation of renal disease which primarily affects the renal tubules, such as renal tubular acidosis and Fanconi's syndrome.

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