Gas Chromatography and Its Combination with Mass Spectrometry of Urinary Sugar Alcohols

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A gas chromatographic method and GC-MS is described for the analysis of urinary sugar alcohols as their trifluoroacetyl derivatives. Nine urinary sugar alcohols were identified: erythritol, threitol, fucitol, ribitol, arabinitol, xylitol, mannotol, glucitol and galactitol. The occurrence of fucitol in urine is noteworthy.

Concerned with galactosemia,2) pentosuria,3) cataract4) and the metabolism of sugars,5) the analysis of urinary sugar alcohols is important. Urinary sugar alcohols have been measured by many different methods, among these, gas chromatographic methods have distinct advantage in terms of speed and sensitivity. Wells, et al.6) described a method for the analysis of sugars and sugar alcohols in urine by gas chromatography of the corresponding trimethylsilyl (TMS) ethers, where separation of isomers of sugar alcohols was unsatisfactory. Matsui, et al.7) established the gas chromatography of sugar alcohols as their trifluoroacetyl (TFA) derivatives, where all of them, except D-glucitol and L-iditol, were separated from each other. For the application of this method, however, coexistence of sugars interferes with gas chromatography. We investigated removal of concomitant sugars and combination of gas chromatography with mass spectrometry (GC-MS) to establish an analytical method of sugar alcohols in urine.

Experimental

Materials—Ethyl acetate (GR; Kanto Chemical Co., Ltd.), trifluoroacetic anhydride (TFAA) (GR), phenylhydrazine (EP), phenylhydrazine-p-sulfonic acid (EP; Tokyo Kasei Kogyo Co., Ltd.), sodium boro-hydrate (GR; E. Merck AG) were used directly. Erythritol, D-arabinitol, D-xylitol were commercial samples. Fucitol and ribitol were prepared from the corresponding aldoses by NaBH₄ reduction.8) L-Threitol was prepared from L-threono lactone by treatment with NaBH₄.9) QAE-Sephadex (50—100 mesh) was exhaustively washed with water and the fines were removed by decantation after the treatment with acid and alkali, the resin was converted into the borate form by passing a saturated solution of K₂B₄O₇·5H₂O, washed with dist. water and stored moist.

Gas Chromatography—Gas chromatography was performed on a Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector. The glass tube (1.8 m x 4 mm i.d.) was packed with 2% XF-1105 on a support of Gas-Chrom P (80—100 mesh).

References:
1) Location: a) Hongo, Bunkyo-ku, Tokyo; b) Kamiyoga, Setagaya-ku, Tokyo.
Mass Spectrometry and GC-MS—Hitachi RMU-6 and RMU-7 mass spectrometers were used singly or being combined with Hitachi K-53 gas chromatograph.

A stainless steel tube (2 m x 3 mm i.d.) or a glass tube (1 m x 4 mm i.d.) packed with 2% XF-1105 on a support of Gas-Chrom P (80—100 mesh) was used.

Mass spectra were measured under the following conditions; ionizing voltage: 40 eV, accelerator voltage: 1200 V, Tl: 140°, Texp: 170°, Tsep: 200°.

Sugar alcohols-TFA derivatives were prepared as described below, and the reaction solutions were concentrated to remove ethyl acetate and excess TFAA and applied to a mass spectrometer.

In the case of GC-MS, sugar alcohol-TFA derivatives were dissolved again in 50 µl of ethyl acetate and 0.5 to 2 µl of the solution was injected to GC-MS.

Mass numbers were measured by the use of perfluorokerosene as an internal standard.

Gas Chromatographic Separation and Determination of Sugar Alcohols in Urine

The neutral fraction of urine includes sugar alcohols, aldoses and ketoses. We have successfully removed sugars by a reaction with a mixture of phenylhydrazine and phenyl-hydrazine-β-sulfonic acid. The reactions and excess reagents were removed by ion exchange resins. Then sugar alcohols were selectively separated from the other neutral materials by a QAE-Sephadex column (borate form).

Procedure

Two ml of normal urine was percolated through columns of Amberlite CG-120 (H⁺) (0.8 x 12 cm) and Amberlite CG-4B (Aco⁻) (0.8 x 12 cm) at room temperature. The effluent was evaporated to dryness under reduced pressure at below 50°. To the residue, 1 ml of dist. water, 60 mg of phenylhydrazine-β-sulfonic acid and 0.025 ml of phenylhydrazine were added. The solution was heated at 100° for 2 hr and centrifuged, then the supernatant was percolated through columns of Amberlite CG-4B (Aco⁻) (0.8 x 12 cm) and CG-120 (H⁺) (0.8 x 12 cm) at room temperature. The effluent was evaporated to dryness under reduced pressure. The residue was dissolved in 2 ml of water and percolated through a QAE-Sephadex column (borate form) (0.8 x 3 cm) and the column was washed with 10 ml of water. The sugar alcohols were eluted with 3 ml of a mixture of conc. HCl and CH₃OH (1:24) and the effluent was evaporated to dryness. To the residue, 1 ml of CH₃OH was added and evaporated to dryness to remove boric acid as methyl borate. After the repetition of this treatment two or three times, the residue was dried in vacuo. The sample was treated with 0.1 ml of ethyl acetate and 0.1 ml of trifluoroacetic anhydride (TFAA) for 15 min at room temperature. One to three µl of the reaction mixture was injected to a gas chromatograph.

Quantities of sugar alcohols were determined by the peak height ratio method using galactitol as an internal standard.

Recoveries of added sugar alcohols to urine were almost quantitative (Table I).

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<th>Added amount (µg)</th>
<th>Recovery (%)</th>
<th>Added amount (µg)</th>
<th>Recovery (%)</th>
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<td>50</td>
<td>103.6</td>
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<td>97.5</td>
<td>100</td>
<td>103.2</td>
</tr>
<tr>
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<td>99.3</td>
<td>200</td>
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<tr>
<td>500</td>
<td>97.2</td>
<td>300</td>
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Under the conditions described above we found nine discrete peaks corresponding to sugar alcohols on gas chromatograms of sugar alcohols from urines of apparently healthy individuals. A typical gas chromatogram is shown in Fig. 1.
The quantities of the various sugar alcohols, excreted in urine collected in 2 hours after overnight fasting of apparently healthy individuals, are shown in Table II.

<table>
<thead>
<tr>
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</table>

Ery.: erythritol, Thr.: threitol, Fuc.: fucitol, Rib.: ribitol, Ara.: arabinitol, Xyl.: xyitol, Man.: mannitol, Glu.: glucitol, Gal.: galactitol

**GC-MS for Identification of Urinary Sugar Alcohols**

The coincidence of the retention time, however, is not sufficient evidence for identification. Therefore we attempted to identify these nine peaks in Fig. 1. by GC-MS method. At first we measured mass spectra of authentic sugar alcohols-TFA derivatives by means of direct and indirect (heated) inlet systems and by GC-MS method. There was little difference among the spectra measured by these methods. The mass spectra of TFA derivatives of the isomeric sugar alcohols were quite similar and the fragmentation pattern was common to all the homologs of sugar alcohols as shown in Chart 1. Molecular peaks were not detected in any mass spectra but [M-19]+ peaks were detected in all mass spectra. Recently O.S. Chizhov, et al.\(^{10}\) reported that molecular peaks instead of [M-19]+ peaks had been detected in all spectra of sugar alcohol-TFA derivatives, however even by using the same reaction condition they described, we did not detect molecular peaks.

Secondly we measured mass spectra of sugar alcohol-TFA derivatives in urine using GC-MS method. These spectra completely coincided with the corresponding authentic spectra (Fig. 2,3,4,5), demonstrating that the nine peaks consisted of pure TFA derivatives.

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of sugar alcohols, erythritol,\textsuperscript{11} threitol,\textsuperscript{12} fucitol, ribitol,\textsuperscript{13} arabinitol,\textsuperscript{14} xylitol,\textsuperscript{15} mannitol,\textsuperscript{6} glucitol (sorbitol),\textsuperscript{16} galactitol (dulcitol).\textsuperscript{2,18}

**Discussion**

The occurrence of fucitol in urine is noteworthy as this deoxy sugar alcohol has not been detected in nature, while \(\alpha\)-fucose has a fairly widespread distribution. \(\alpha\)-Fucose occurs in seaweeds\textsuperscript{16} and a few gums, and found as a constituent of blood group substances from both animal and human sources,\textsuperscript{17} human milk oligosaccharides,\textsuperscript{18} urinary glycoprotein\textsuperscript{19} and other glycoproteins. \(\alpha\)-Fucose derived from these origins might be reduced in vivo by the action of aldose reductase which was proved to exist in the seminal vesicles and placenta of sheep and rat liver,\textsuperscript{20} and rat lens.\textsuperscript{4}

The mass spectra of isomeric sugar alcohol-TFA derivatives are very similar, though they are barely different in relative intensities of some peaks. Therefore it will be almost impossible to identify sugar alcohol-TFA derivatives by means of mere mass spectrometry, and the satisfactory resolution of isomeric sugar alcohol-TFA derivatives by gas chromatography is quite essential for their identification and separatory determination.

As the peaks obtained by our gas chromatographic method fully consist of TFA derivatives of urinary sugar alcohols, the method should be sufficiently reliable.

**Acknowledgement**

The authors wish to thank Mr. K. Kamei for his technical assistance.

\textsuperscript{12} N. Spencer, *J. Chromatog.*, 30, 566 (1967).