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Note

Identification of 6-deoxyallitol and 6-deoxygulitol in human urine

Electron-impact mass spectra of eight isomers of 6-deoxyhexitol

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Polyols form a large group of compounds in body fluids, and their study has become important since polyol levels in body fluids change in various diseases. In diabetes mellitus the urinary excretion of mannitol and myoinositol is increased [1], and the concentration of 1-deoxyglucose in plasma [2] and cerebrospinal fluid [3] is low. Increased production of sorbitol in the tissues is considered to be pathogenic in the complications of diabetes mellitus such as cataracts [4] and neuropathy [5]. In uraemia the serum and urinary levels of myoinositol are increased [1, 6], and the accumulation of myoinositol is considered to be a cause of uraemic polyneuropathy [7, 8]. We previously reported [6] that serum and urinary levels of chiroinositol and scylloinositol are increased and the serum level of 1-deoxyglucose is decreased in uraemia and that seven new deoxyalditols including 6-deoxymannitol and 6-deoxy-

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galactitol had been identified. In the present study 6-deoxyallitol and 6-deoxygulitol have been found as normal components of human urine for the first time.

MATERIALS AND METHODS

Chemicals

L-Rhamnose and L-fucose were the products of Tokyo Kasei (Tokyo, Japan). 6-Deoxyglucose was obtained from Sigma (St. Louis, MO, U.S.A.). D-Glucose, D-mannose and ribitol were the products of Yoneyama (Osaka, Japan).

6-Deoxymannitol was synthesized by the reduction of L-rhamnose with sodium borohydride, 6-deoxysorbitol by the reduction of 6-deoxyglucose with sodium borohydride, and 6-deoxygalactitol by the reduction of L-fucose with sodium borohydride. 6-Deoxyallitol was synthesized by the sodium borohydride reduction of 6-deoxy-D-allose [9], which was prepared by chemical conversion of D-glucose. 6-Deoxygulitol was synthesized by the sodium borohydride reduction of 6-deoxy-L-gulose [9], which was prepared by chemical conversion of D-mannose. 6-Deoxyiditol was synthesized by the sodium borohydride reduction of 6-deoxy-L-idose, which was obtained by the acid hydrolysis of methyl β-L-idopyranoside prepared from D-glucose according to the method of Ikeda et al. [10]. 6-Deoxyaltritol was synthesized by the sodium borohydride reduction of 6-deoxy-D-altrose, which was obtained by the acid hydrolysis followed by deacylation of methyl 2,3-di-O-acetyl-4-O-benzoyl-6-deoxy-α-D-altropyranoside prepared from D-glucose according to the method of Chiba and Tejima [11]. 6-Deoxytalitol was synthesized by the sodium borohydride reduction of 6-deoxy-L-talose [9], which was prepared by chemical conversion of D-glucose.

Samples

24-h Urine samples were obtained from five healthy adults and ten patients with chronic renal failure. Four of ten uraemic patients were on 5-h haemodialysis three times a week.

The urine samples were kept at −20° C prior to analysis.

Sample preparation

A volume of urine equivalent to 1 mg of creatinine was applied to a Dowex 50W-X8 column (H^+, 5 cm × 0.8 cm I.D.) after the addition of 50 μg of ribitol as internal standard. Polyols were eluted with 30 ml of distilled water. The eluate was applied to an Amberlite IRA 400 column ($HCOO^-$, 5 cm × 0.8 cm I.D.). Polyols were eluted with 30 ml of distilled water. After lyophilization, the polyols were redissolved with 9 ml of hot methanol, then transferred to a sample vial and dried with a nitrogen stream. The polyols were trimethylsilylated with 90 μl of N,O-bis(trimethylsilyl)trifluoroacetamide and 10 μl of trimethylchlorosilane at 60° C for 20 min. Of the sample 2 μl were subjected to gas chromatography—mass spectrometry.

Instrumentation

A Hewlett-Packard 5710A gas chromatograph was directly coupled to the

source of a JMS-D300 mass spectrometer (JEOL, Tokyo, Japan). The gas chromatograph was equipped with a 30 m × 0.25 mm I.D. OV-101 open tubular glass capillary column and a splitless injector. The column temperature was programmed from 120°C to 260°C at 3°C/min. Electron-impact ionization (EI) mass spectra were recorded at an ionizing energy of 70 eV, an ionization current of 300 μA and an accelerating voltage of 3 kV.

RESULTS AND DISCUSSION

Fig. 1 shows the gas chromatogram of polyols in the urine of a patient with chronic renal failure (upper chromatogram). The identification of the peaks

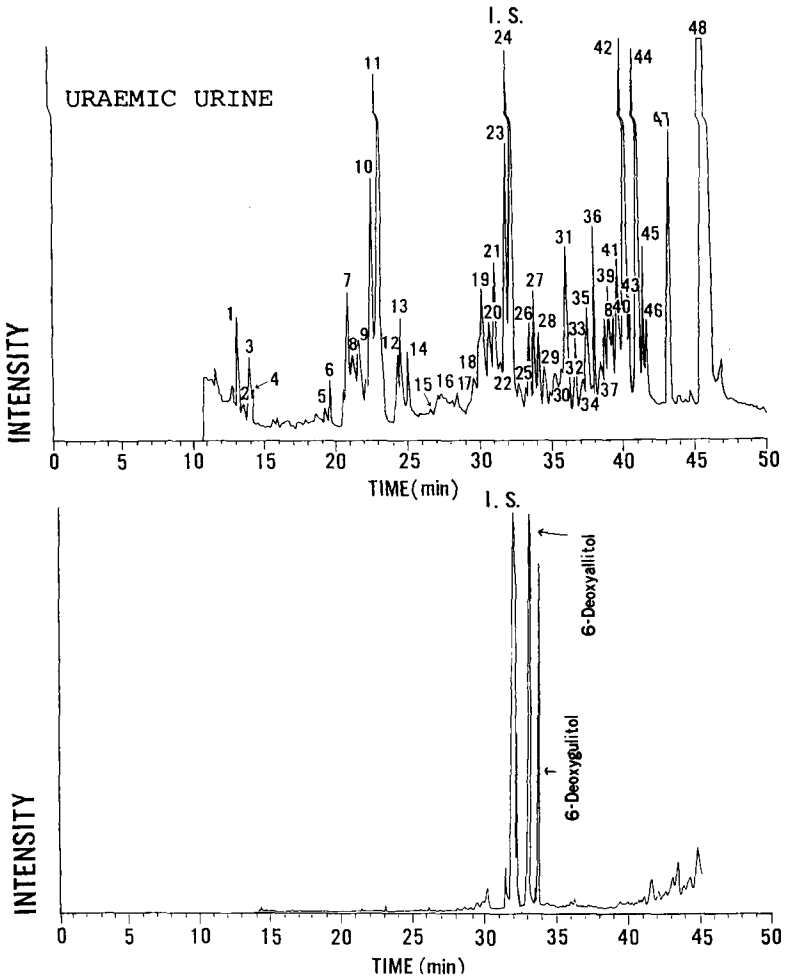

Fig. 1. Gas chromatograms of polyols in urine of a patient with chronic renal failure (upper chromatogram), and of synthesized deoxyhexitols (lower chromatogram). Peak identifications: 1 = glycerol, 3 = 4-deoxythreitol, 4 = 4-deoxyerythritol, 10 = threitol, 11 = erythritol, 12 = 5-deoxyxylitol, 13 = 5-deoxyarabitol, 15 = 2-deoxyribitol, 19 = xylulose, 22 = xylitol, 23 = arabitol, 24 = ribitol (internal standard), 26 = 6-deoxyallitol, 27 = 6-deoxymannitol, 28 = 6-deoxygulitol, 29 = 6-deoxygalactitol, 30 = fructose, 31 = 1-deoxyglucose, 36 = α-glucose, 37 = β-galactose, 39 = neoinositol, 42 = mannitol, 43 = sorbitol, 44 = chiro-inositol, 45 = β-glucose, 46 = epi- or *cis*-inositol, 47 = scylloinositol, 48 = myoinositol.

was based on the fact that the EI mass spectra and the relative retention times of the peaks were the same as those of the trimethylsilylated authentic compounds or that the EI mass spectra of the peaks were the same as those reported in the literature.

The EI mass spectrum of peak 26 is shown in Fig. 2 (lower spectrum). The molecular ion was found to be 526 by chemical ionization. The base peak at *m/z* 117 and the intense peak at *m/z* 219 in addition to the peaks at *m/z* 103, *m/z* 205, and *m/z* 307 suggested the structure of 6-deoxyhexitol. Eight isomers of 6-deoxyhexitol were synthesized, and the relative retention times and the EI mass spectra of the trimethylsilylated isomers were measured, and listed in Table I. Peak 26 was identified as 6-deoxyallitol, since only the trimethylsilyl (TMS) derivative of 6-deoxyallitol showed the same retention time (Fig. 1) and the same EI mass spectrum (Fig. 2) as those of peak 26.

The EI mass spectrum of peak 28 is shown in Fig. 3 (lower spectrum). The molecular ion of peak 28 was 526. Peak 28 and peak 26 showed similar mass spectra, suggesting an isomeric relationship. Although the TMS derivatives of 6-deoxygulitol, 6-deoxyiditol and 6-deoxyaltritol showed almost the same retention times as peak 28, only the TMS derivative of 6-deoxygulitol showed an EI mass spectrum identical with that of peak 28 (Fig. 3). The TMS derivative of 6-deoxyiditol was excluded because the relative intensity of *m/z* 217

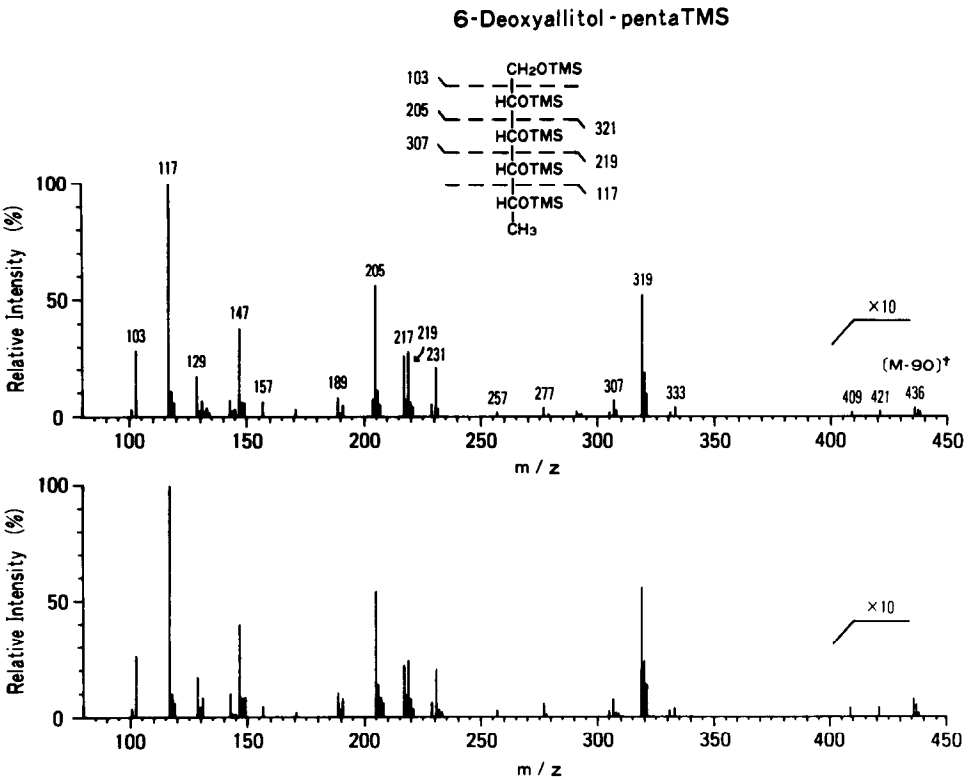

Fig. 2. EI mass spectra of TMS derivative of 6-deoxyallitol (upper spectrum) and of peak 26 (lower spectrum) in the gas chromatogram of Fig. 1.

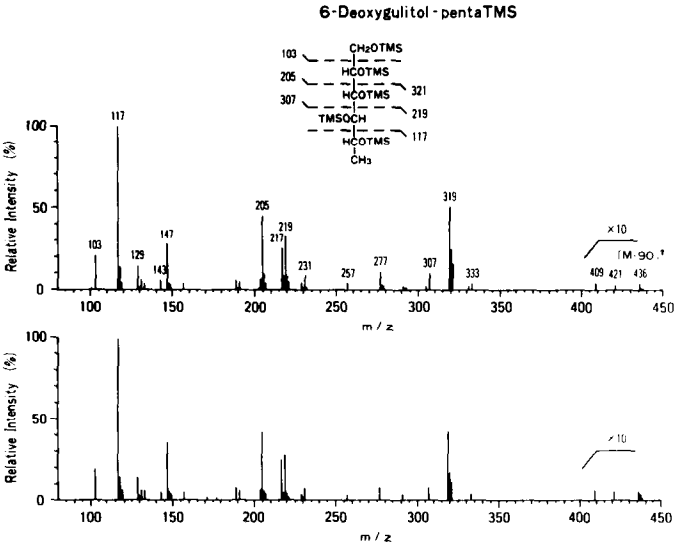

Fig. 3. EI mass spectra of TMS derivative of 6-deoxygulitol (upper spectrum) and of peak 28 (lower spectrum) in the gas chromatogram of Fig. 1.

TABLE I

RELATIVE RETENTION TIMES AND EI MASS SPECTRA OF EIGHT TRIMETHYLSILYLATED ISOMERS OF 6-DEOXYHEXITOL

m/z	Relative abundance (%)							
	6-Deoxy-allitol	6-Deoxy-mannitol	6-Deoxy-sorbitol	6-Deoxy-gulitol	6-Deoxy-iditol	6-Deoxy-altritol	6-Deoxy-galactitol	6-Deoxy-talitol
103	29	26	12	21	19	26	34	26
117	100	100	84	100	100	100	100	100
129	17	17	12	14	9	11	18	16
131	7	11	6	6	7	8	15	8
133	4	10	6	4	7	7	12	6
143	7	11	9	6	6	8	11	10
147	38	56	42	28	36	39	63	36
157	6	6	6	4	2	1	4	5
189	8	7	5	6	3	4	7	6
191	5	6	4	5	4	6	8	8
204	7	10	10	7	7	9	14	7
205	56	56	51	45	40	47	35	54
217	26	30	38	25	26	31	48	32
219	28	29	26	33	22	38	35	37
229	5	5	6	4	2	2	4	2
231	21	10	22	9	9	23	14	10
257	2	5	6	4	2	2	4	2
277	4	6	6	11	6	6	5	3
291	2	4	6	2	2	2	4	1
293	1	2	0.5	1	1	1	1	1
305	2	3	4	2	2	2	4	2
307	7	10	16	10	11	20	18	12
319	52	63	100	51	31	42	31	52
331	2	2	3	2	4	2	1	2
333	4	3	5	4	2	4	3	3
409	0.2	0.3	6	0.4	0.6	0.9	0.8	0.2
421	0.2	0.2	0.5	0.3	0.1	0.8	0.3	0.3
436	0.4	0.7	1	0.4	0.1	0.7	0.6	0.7
RRT*	1.04	1.05	1.05	1.06	1.06	1.06	1.07	1.08

*Relative retention time. Ribitol was used as standard (RRT = 1.0). The retention time of ribitol was 32.2 min on a 30 m × 0.25 mm I.D. OV-101 glass capillary column. The column temperature was programmed from 120°C to 260°C at 3°C/min.

(26%) was higher than that of *m/z* 219 (22%). The TMS derivative of 6-deoxyaltritol was excluded because the relative intensity of *m/z* 231 was as high as 23%. Peak 28 was then identified as 6-deoxygulitol.

Peak 27 and peak 29 were identified as 6-deoxymannitol and 6-deoxygalactitol, respectively, as reported in the literature [6].

6-Deoxyallitol and 6-deoxygulitol were newly detected in normal urine as well as in uraemic urine. The metabolic origin and the physiological significance of these new deoxyhexitols are obscure at present, and should be studied.

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