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### 2-ACETAMIDOGLUCAL, A NEW METABOLITE ISOLATED FROM THE URINE OF A PATIENT WITH SIALURIA

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#### Summary

A new metabolite, namely 2-acetamidoglucal, has been found in the urine of a patient with sialuria in addition to the metabolites *N*-acetylneuraminic acid, *N*-acetylmannosamine, *N*-acetylglucosamine and 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid reported earlier. The structure has been identified by mass spectrometry and 360 MHz proton nuclear magnetic resonance spectroscopy and verified by synthesis. All accumulated compounds fit into the metabolic pathway for the biosynthesis of CMP-*N*-acetylneuraminic acid. Sialuria is discussed in terms of a failure of regulation of UDP-*N*-acetylglucosamine 2-epimerase.

Previously, a mentally retarded boy with sialuria has been described. This patient excretes abnormal amounts of *N*-acetylneuraminic acid (NeuAc) [1,2], *N*-acetylmannosamine (ManNAc) [3], *N*-acetylglucosamine (GlcNAc) [3], and 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid ( $\Delta^{2,3}$ -NeuAc) [4] as is illustrated in Table I. In this paper the isolation and characterization of 2-acetamidoglucal, as an additional urinary compound, is reported. This new metabolite might give a clue to the origin of the inborn error of metabolism of the patient.

For the isolation of 2-acetamidoglucal from urine, 10 l urine were desalted in 1-l portions via passage through Dowex 50X8, H<sup>+</sup>-form, 20–50 mesh (3 × 30 cm) and Dowex 1X4, HCOO<sup>-</sup>-form, 200–400 mesh (3 × 30 cm), respectively. The neutral effluent was pooled, concentrated under reduced

TABLE I

AMOUNTS OF EXCRETED MONOSACCHARIDES IN THE URINE OF THE SIALURIA PATIENT, COMPARED WITH NORMAL VALUES

Monosaccharide	Sialuria (mg/l)	Normal (mg/l)
NeuAc	11 000—36 000	0—20
ManNAc	800—1 200	—*
GlcNAc	200—300	1—2
$\Delta^{2,3}$ -NeuAc	100—300	<1
2-Acetamidoglucal	30—40	—*

\*Not detected.

pressure to 2 l and then fractionated on a charcoal-Celite column (6 × 40 cm) [5]. After washing with 4 l of water, carbohydrates (essentially oligosaccharides) were desorbed from charcoal-Celite by stepwise elution with 5-l portions of 1.5, 3.5, 5, 7.5, 10 and 20% (v/v) ethanol in water, respectively. The solvents were evaporated and the residues of the six fractions were analysed by paper chromatography on Whatman No. 3 using the solvent system 1-butanol/acetic acid/water (4:1:5, v/v). Anilin oxalate [6], orcinol/trichloroacetic acid [7] and *p*-dimethylaminobenzaldehyde/trichloroacetic acid [8] were used as spray reagents. 2-Acetamidoglucal was isolated from the 10% ethanol fraction by preparative paper chromatography; the yield was 375 mg from 10 l of urine.

2-Acetamidoglucal (2-acetamido-1,2-dideoxy-D-*arabino*-hex-1-enopyranose) was prepared from 2-acetamido-2-deoxy-D-glucose via 2-methyl-(3',4',6'-tri-*O*-acetyl-1',2'-dideoxy- $\alpha$ -D-glucopyrano)[2',1':4,5]-2-oxazoline as an intermediate [9,10].

Gas-liquid chromatography was carried out on a Varian Aerograph 2740-30-01, equipped with dual flame ionization detector and glass columns (2.00 m × 4.0 mm) packed with 3.8% of SE-30 on Chromosorb W HP, 80—100 mesh. The oven temperature was programmed from 140 to 220°C at 2°C/min; the nitrogen flow rate was 40 ml/min. Trimethylsilylation of samples was performed with hexamethyldisilazane/trimethylchlorosilane/pyridine (2:1:10, v/v) or with [ $^2\text{H}_{18}$ ]hexamethyldisilazane/[ $^2\text{H}_9$ ]trimethylchlorosilane/pyridine (2:1:10, v/v), respectively [4].

Mass spectra at 75 eV were recorded on a JEOL JGC-1100/JMS-07 combination (column material, SE-30; oven temperature, programmed from 140 to 220°C at 2°C/min; ion source temperature, 250°C; accelerating voltage, 3 kV; ionizing current, 300  $\mu\text{A}$ ). High-resolution mass measurements were performed with a dynamic resolving power of 10 000 and a scan speed of 16 s per mass decade by using an AEI MS-902 mass spectrometer (ion source temperature, 100°C; accelerating voltage, 8 kV; ionizing current, 500  $\mu\text{A}$ ) connected on-line with a Ferranti Argus 500 computer.

360 MHz Proton NMR spectra were recorded with a Bruker HX-360 spectrometer, operating in the Fourier Transform mode at a probe temperature of 25°C. Before analysis, samples were exchanged three times in  $^2\text{H}_2\text{O}$  with intermediate lyophilization. Chemical shifts ( $\delta$ ) are given relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate in  $^2\text{H}_2\text{O}$  as solvent (indirectly to acetone:  $\delta = 2.225$  ppm). Spectrum simulations were run on a Varian CFT-

20 spectrometer using the modified spin simulation program SIMEQ CFT (de Bie, M.J.A., personal communication).

In Fig. 1 a paper chromatogram is given of the six urinary sugar fractions, desorbed from charcoal-Celite with a discontinuous gradient of ethanol/water. The fraction eluted with 10% ethanol contained an unknown component ( $R_F = 0.37$ ), which stained yellow-brown with anilin oxalate as well as with orcinol/trichloroacetic acid. Upon spraying with *p*-dimethylaminobenzaldehyde/trichloroacetic acid, the spot coloured intense violet at room temperature. For structural analysis the substance was isolated by preparative paper chromatography.

The pertrimethylsilylated compound showed a gas chromatographic retention time of about 27 min (oven temperature program: 140  $\rightarrow$  220°C, 2°C/min). The low- and high-resolution mass spectra of the pertrimethylsilyl derivative in combination with the low-resolution spectrum of the pertri(tri-

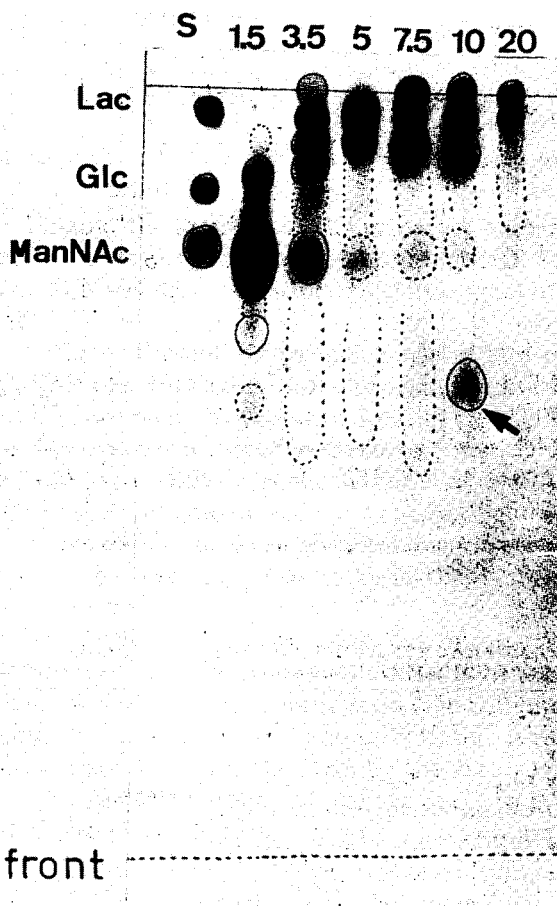


Fig. 1. Paper chromatography on Whatman No. 3 of six urinary sugar fractions from the sialuria patient, eluted from charcoal-Celite by a discontinuous gradient of ethanol/water (1.5, 3.5, 5, 7.5, 10 and 20%, v/v). Solvent system: 1-butanol/acetic acid/water (4:1:5, v/v). Time of migration: 16 h. The arrow indicates the position of 2-acetamidoglucose; the big spot in the 1.5% ethanol fraction corresponds with ManNAc + GlcNAc. S, standards; Lac, lactose; Glc, glucose; ManNAc, *N*-acetylmannosamine.

deuteromethyl)silyl analogue indicated the substance to be a 2-acetamido-1,2-dideoxy-hex-1-enopyranose (2-acetamidoglycal). The assignments of the most characteristic mass values in the spectrum of the trimethylsilylated 2-acetamidoglycal (Fig. 2) are presented in Table II.

The 360 MHz proton NMR spectrum of the non-derivatized compound recorded in  $^2\text{H}_2\text{O}$  is given in Fig. 3. The various resonances were assigned by selective homonuclear decoupling experiments. The assignments were checked by computer simulation of the spectrum. The resonance at 6.69 ppm represents the olefinic proton H-1, whereas H-3—H-6' resonate in the non-anomeric proton area of the spectrum.

The protons of the *N*-acetyl group are found at 2.07 ppm (singlet). The values of the coupling constants  $J_{4,5}$  (9.1 Hz; antiperiplanar orientation of the protons at C-4 and C-5),  $J_{3,4}$  (6.5 Hz) and  $J_{1,3}$  (0.6 Hz; allylic long-range coupling) indicate the *gluco*-configuration for the 2-acetamidoglycal

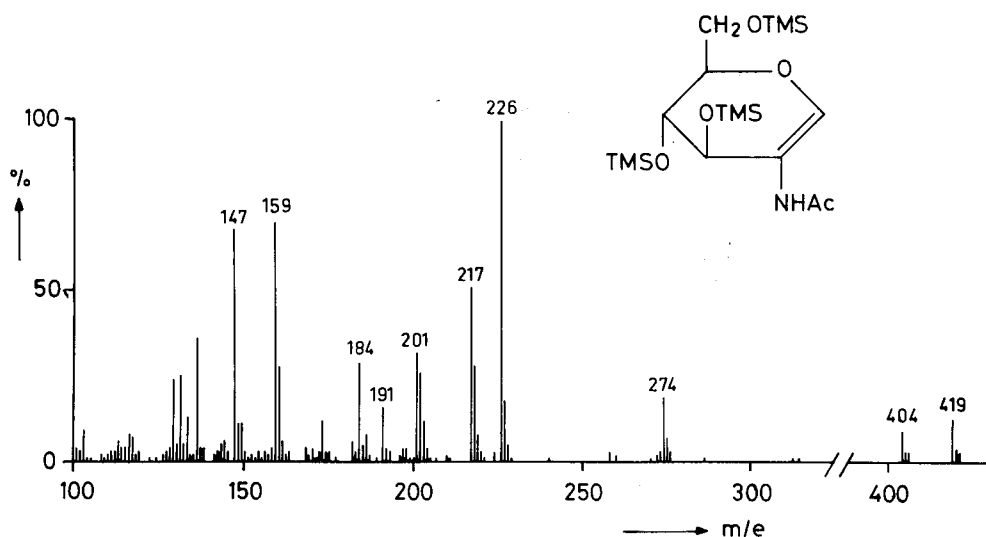


Fig. 2. 75 eV mass spectrum of trimethylsilylated 2-acetamidoglycal; only values  $m/e > 100$  and intensities  $\geq 1\%$  are given. TMS,  $\text{Si}(\text{CH}_3)_3$ ; Ac,  $\text{COCH}_3$ .

TABLE II

INTERPRETATION OF SOME IMPORTANT FRAGMENT IONS PRESENT IN THE MASS SPECTRUM OF TRIMETHYLSILYLATED 2-ACETAMIDOGLYCAL

$m/e$	General formula	Fragment
419	$\text{C}_{17}\text{H}_{37}\text{NO}_5\text{Si}_3$	$\text{M}^{++}$
404	$\text{C}_{16}\text{H}_{34}\text{NO}_5\text{Si}_3$	$\text{M}^{++}$ minus $\text{CH}_3$
274*	$\text{C}_{11}\text{H}_{24}\text{NO}_3\text{Si}_2$	$(\text{CH}_3)_3\text{SiO}=\text{CH}-\text{C}(\text{NHCOCH}_3)=\text{CH}-\text{OSi}(\text{CH}_3)_3$
226	$\text{C}_{10}\text{H}_{16}\text{NO}_3\text{Si}$	$\text{M}^{++}$ minus $\text{CH}_2\text{OSi}(\text{CH}_3)_3$ minus $\text{HOSi}(\text{CH}_3)_3$
217	$\text{C}_9\text{H}_{21}\text{O}_2\text{Si}_2$	$[\text{CH}-\text{CHOSi}(\text{CH}_3)_3-\text{CHOSi}(\text{CH}_3)_3]^+$
201	$\text{C}_8\text{H}_{15}\text{NO}_3\text{Si}$	$\text{M}^{++}$ minus $(\text{CH}_3)_3\text{SiOCH}_2-\text{CH}=\text{CHOSi}(\text{CH}_3)_3$
191	$\text{C}_7\text{H}_{19}\text{O}_2\text{Si}_2$	$(\text{CH}_3)_3\text{SiO}-\text{CH}=\text{OSi}(\text{CH}_3)_3$
184	$\text{C}_8\text{H}_{14}\text{NO}_2\text{Si}$	$226^+ \text{ minus } \text{CH}_2\text{CO}$
159	$\text{C}_6\text{H}_{13}\text{NO}_2\text{Si}$	$201^+ \text{ minus } \text{CH}_2\text{CO}$
147	$\text{C}_5\text{H}_{15}\text{OSi}_2$	$(\text{CH}_3)_2\text{Si}^+-\text{OSi}(\text{CH}_3)_3$

\* $\text{OSi}(\text{CH}_3)_3$  rearrangement to C-1.

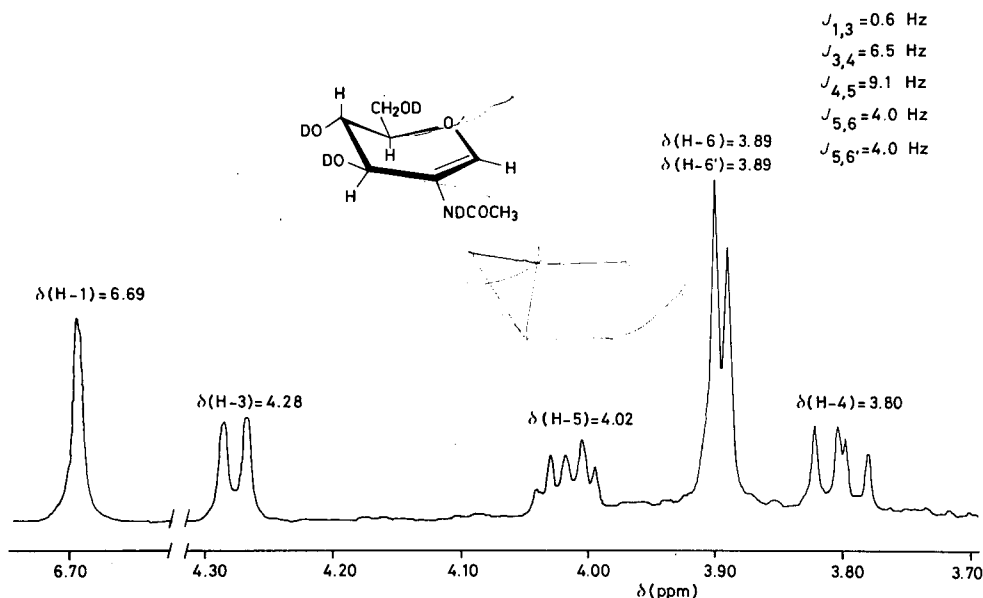


Fig. 3. Part of the 360 MHz proton NMR spectrum of 2-acetamidoglucal in  $^2\text{H}_2\text{O}$ .

Finally, the identity of the urinary 2-acetamidoglucal was verified by comparison with synthetic 2-acetamidoglucal. The mass spectrometric as well as the  $^1\text{H}$  NMR data of both compounds were in full agreement with each other.

Urine of the sialuria patient contains 30–40 mg of 2-acetamidoglucal per l. This unsaturated monosaccharide has not been detected before in nature. It has been proposed by Sommar and Ellis [11] that 2-acetamidoglucal should be an intermediate in the epimerization reaction of UDP-GlcNAc to ManNAc, the metabolic precursor of CMP-NeuAc. This reaction is catalysed by UDP-GlcNAc 2-epimerase. 2-Acetamidoglucal, formed after elimination of UDP from UDP-GlcNAc should be converted irreversibly into ManNAc. However, the presence of this intermediate could not be demonstrated by these authors.

The finding of 2-acetamidoglucal in the urine of the patient makes it reasonably to include this new metabolite in the metabolic pathway for the biosynthesis of sialoglycoconjugates (see Scheme I). All monosaccharides detected so far in abnormal amounts in the urine, form part of this scheme. GlcNAc can arise from ManNAc by the action of acylglucosamine 2-epimerase [12]. The relative amount of urinary GlcNAc is in line with the suggestion of McGuire [13], that this conversion is only important when a large amount of ManNAc is available, which is not directly converted into NeuAc. Concerning the formation of  $\Delta^{2,3}$ -NeuAc, it has been shown recently that CMP-NeuAc can in vitro degrade to this unsaturated sialic acid [14]. It is worthwhile to consider the possibility that  $\Delta^{2,3}$ -NeuAc could be an intermediate between CMP-NeuAc and NeuAc in the CMP-NeuAc hydrolase catalysed reaction.

In relation to the accumulated material, it is interesting to speculate on the biochemical origin of the inborn error of metabolism. Apparently, the



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