Isolation and characterization of 1-*O*-α-2-acetamido-2-deoxy-D-galactopyranosyl-*myo*-inositol from pregnancy urine

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A new neutral glycoside of *myo*-inositol was isolated from the pregnancy urine of a single donor. Its structure was investigated by ^{1}H-NMR spectroscopy and mass spectroscopy. It was identified as 1-*O*-α-2-acetamido-2-deoxy-D-galactopyranosyl-*myo*-inositol. No such structure or sequence has previously been reported in either *myo*-inositol or glucose glycosides.

myo-*Inositol* *Pregnancy* *Urine* *Blood group*

1. INTRODUCTION

Human urine is known to contain a number of oligosaccharides whose excretion greatly increases during pregnancy and lactation [1–3]. Most of these saccharides have a glucose residue at their reducing end; they are referred to here as glucose glycosides. Another type of glycoside has also been characterized in human urine: *myo*-inositol glycosides. So far, only 5 glycosides of this type have been isolated – one from the urine of normal subjects [4,5] and 4 from pregnancy urine [6,7] – and a sixth was identified in human milk [8]. All these *myo*-inositol glycosides are analogous to glucose glycosides by substitution of a *myo*-inositol residue for the terminal glucose.

Two of these *myo*-inositol glycosides are α-L-fucopyranosyl-*myo*-inositol [4,5] and 6-*O*-β-D-galactopyranosyl-*myo*-inositol (6-β-galactinol) [8]. However, screening of human urinary *myo*-inositol glycosides by gas chromatography/electron impact mass spectrometry (GC/EIMS) showed that more than two glycosides containing *myo*-inositol were sometimes present [9].

This work reports the isolation and characterization of one of these compounds as 1-*O*-α-2-acetamido-2-deoxy-D-galactopyranosyl-*myo*-inositol, a sequence which has not been reported previously.

2. MATERIALS AND METHODS

Third-term pregnancy urine was collected from a single donor of A blood group (secretor). Urine was kept frozen at −18°C until use.

Schleicher and Schüll 2043b was used for descending paper chromatography in the following solvent systems: (a) ethyl acetate/pyridine/water (10:4:3, v/v); (b) 1-propanol/ethyl acetate/water (6:1:3, v/v); (c) ethyl acetate/pyridine/acetic acid/water (5:5:1:3, v/v); (d) ethyl acetate/pyridine/water (12:5:4, v/v). All papers were stained with silver dip reagent [10]. Monosaccharides and *myo*-inositol were analyzed by GC [11] under the conditions reported in [7].

^{1}H-NMR spectroscopy (250.13 MHz) was performed on a Bruker SY-250 spectrometer

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operating in the pulsed Fourier transform mode at a probe temperature of 298 K. Chemical shifts were calculated in relation to sodium 4,4′-dimethyl-4-silapentane-1-sulfonate by indirect reference to acetone in ^2H_2O (δ = 2.225 ppm) with an accuracy of 0.15 Hz.

The isolated *myo*-inositol glycoside was permethylated as in [12] and analyzed on a Ribermag 10–10 quadripolar mass spectrometer equipped with a solid probe inlet, and using EIMS and direct chemical ionization (DCI) techniques. Ammonia was used as reactant gas (p_{NH_3} = 0.1 bar). For data acquisition and processing, the mass spectrometer was linked to a PDP-11 system. Operating conditions were as follows: filament current, 20 mA; ionization energy, 90 eV; ion source temperature, 120°C. The *myo*-inositol glycoside was desorbed at 7 mA/s.

Urine (1.5 l) was processed as in [7] using a column of Sephadex G-15 fine (Pharmacia). The deionized fraction which contained *myo*-inositol glycosides was fractionated on a column (2.5 × 16 cm) of DEAE–Sephadex A-25 (Pharmacia) equilibrated in 0.05 M pyridine acetate buffer (pH 6.1); the column was eluted first with this buffer and then successively with 5 other pyridine acetate buffers: 0.05 M (pH 5.3), 0.1 M (pH 5.3), 0.1 M (pH 4.8), 0.1 M (pH 4.3) and 0.5 M (pH 4.3). The fraction eluted with the 0.1 M (pH 4.8) buffer contained high levels of *myo*-inositol (0.1 residue for 1 glucose). It was submitted to preparative paper chromatography in solvent system (a) for 72 h.

Table 1

Chemical shifts and coupling constants observed for the isolated glycoside, and for the *N*-acetyl-D-galactosamine and 1α-galactinol, recorded on the same spectrometer

Residue	Proton	Chemical shifts (ppm) and coupling constants (Hz) in		
		Glycoside	Free *N*-acetyl-D-galactosamine	1α-Galactinol
N-Acetyl-D-galactosamine	H-1	5.46 ($J_{1\cdot2}$ = 3.5)	5.23 ($J_{1\cdot2}$ = 3.7)	
	H-2	4.16 ($J_{2\cdot3}$ = 9.0)	4.13 ($J_{2\ 3}$ = 11.0)	
	H-3	3.88 ($J_{3\cdot4}$ = 2.5)	3.91 ($J_{3\cdot4}$ = 3.2)	
	H-4	3.99	3.99	
	H-5	3.95 ($J_{5\cdot6}$ = 6.0)	4.10	
	H-6	n.a.	3.74	
	CH_3CO	2.05	2.04	
1α-Galactinol	H-1			5.14 ($J_{1\cdot2}$ = 3.7)
	H-2			3.86 ($J_{2\cdot3}$ = 10.1)
	H-3			3.96 ($J_{3\ 4}$ = 3.2)
	H-4			4.01 ($J_{4\cdot5}$ = 0.9)
	H-5			4.19 ($J_{5\cdot6}$ = 6.2)
	H-6			3.73
myo-Inositol	H-1	3.64 ($J_{1\cdot2}$ = 2.5)[a]		3.63 ($J_{1\cdot2}$ = 2.6)[a]
	H-2	4.24		4.28
	H-3	3.51 ($J_{3\cdot4}$ = 10.0)[a]		3.52 ($J_{3\cdot4}$ = 9.9)[a]
	H-4	n.a.		3.67
	H-5	3.41 ($J_{5\cdot6}$ = 8.2)[a]		3.32 ($J_{5\cdot6}$ = 9.2)[a]
	H-6	n.a.		3.76

[a] In the *myo*-inositol residue, $J_{1\ 2} = J_{2\cdot3}$, $J_{1\cdot6} = J_{3\cdot4}$ and $J_{4\ 5} = J_{5\ 6}$

n.a., not assigned (signals for these protons could not be assigned precisely since they appeared as multiplet between 3.70 and 3.80 ppm)

The fraction with the lowest mobility appeared as a pure *myo*-inositol glycoside after filtration on a 1.3 × 50 cm column of Biogel P-2 (200–400 mesh) (Bio-Rad) in 0.05 M pyridine acetate buffer (pH 5.3).

3. RESULTS AND DISCUSSION

The product was homogeneous in paper chromatography in the 4 solvent systems (a–d); the respective $B_{lactose}$ values were: (a) 0.1, (b) 0.45, (c) 0.24 and (d) 0.1. Monosaccharide analysis indicated the following molecular composition: *N*-acetyl-D-galactosamine to *myo*-inositol in molar ratios of 1.0:1.0; glucose accounting for less than 7% of the dry weight.

The ^{1}H-NMR spectrum of the *myo*-inositol glycoside was compared to that of *N*-acetyl-D-galactosamine and of 1-*O*-α-D-galactopyranosyl-*myo*-inositol (1α-galactinol) (table 1). The value observed for the coupling constant of the H-1 of *N*-acetyl-D-galactosamine ($J_{1\cdot2}$ = 3.5 Hz) was very similar to those observed for an α-type linkage ([13] and Davoust, D. et al., in preparation). This suggests for our glycoside that the *N*-acetyl-D-galactosamine is also on the α-configuration. The chemical shifts observed for the assigned protons of the *myo*-inositol residue were very similar in both the new glycoside and 1α-galactinol, particularly for the H-1 proton (table 1), thus suggesting the same type of linkage in the two compounds.

The permethylated glycoside (M_r = 509) was analyzed successively by EIMS and ammonia DCI: (i) Using EIMS, only 3 strong fragments derived from the *myo*-inositol were observed at *m/z* 233,

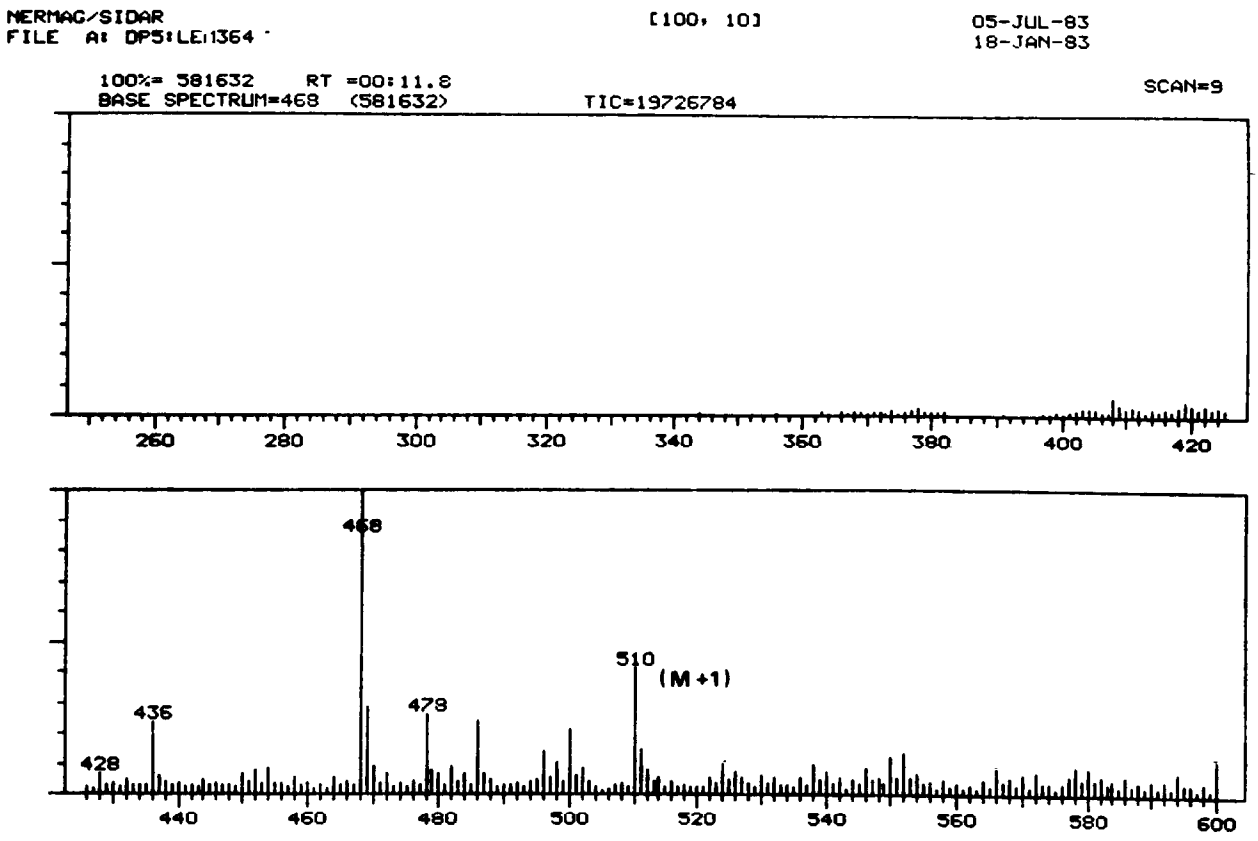

Fig.1. Ammonia DCI mass spectrum of the permethylated 1-*O*-α-2-acetamido-2-deoxy-D-galactopyranosyl-*myo*-inositol (M_r = 509). Fragments at *m/z* 478, 468 and 436 were obtained by loss of methanol, ketene and both methanol and ketene, respectively.

201 and 293 (J_1 fragment). Other fragments originating from the A-series were very weak (not shown); a similar phenomenon has been observed with different *myo*-inositol glycosides [5,7,9] although it is not fully understood.

(ii) Using ammonia DCI one would expect the 100% ion peak to be at m/z (M + 18) or m/z (M + 1) for a compound containing a nitrogen atom. The mass spectrum of the permethylated glycoside (fig.1) indeed exhibited the (M + 1) ion at m/z 510, but also some other fragments originating from the (M + 1) ion by the loss of methanol (m/z 478), or of ketene (m/z 468) or both (m/z 436). The presence of these ions was likely a consequence of pyrolytic effects during the process of desorption of the glycoside as reported in [14].

These results lead to the following structure assignment: 1-*O*-α-2-acetamido-2-deoxy-D-galactopyranosyl-*myo*-inositol.

The characterization of this new glycoside supports our work [9] demonstrating that more than two neutral *myo*-inositol glycosides of monosaccharides could be present in the urine of various donors.

The structure of the new compound is of great interest since no such structure has previously been reported in *myo*-inositol glycosides, nor has any glucoside (*N*-acetyl-D-galactosamine-glucose or -galactose) been characterized, even though these glycosides have been extensively studied [15,16], which excludes that this new compound is synthesized by the action of blood group A α-*N*-acetyl-D-galactosaminyltransferase on a *myo*-inositol acceptor. The possibility that this new glycoside originates from transglycosylation processes as reported for galactosyl inositol [17] is more likely.

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