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Carbohydrate Research 304 (1997) 77–79

CARBOHYDRATE RESEARCH

Preliminary communication

Chemical characterization of a new 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acid released by mild acid hydrolysis of the *Legionella pneumophila* serogroup 1 lipopolysaccharide

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Received 2 May 1997; accepted 17 July 1997

Abstract

A derivative of a new 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acid was released from the lipopolysaccharide of *Legionella pneumophila* serogroup 1 (strain Philadelphia 1) by mild acid hydrolysis, and identified, using NMR spectroscopy and GLC–MS, as 5,7-diacetamido-8-*O*-acetyl-3,5,7,9-tetradeoxy-L-*glycero*-D-*talo*-nonulosonic acid or its enantiomer. © 1997 Elsevier Science Ltd.

Keywords: Lipopolysaccharide; 5,7-Diamino-3,5,7,9-tetradeoxy-L-*glycero*-D-*talo*-nonulosonic acid; *Legionella pneumophila*

Legionella pneumophila is a facultative intracellular human pathogen which causes legionellosis, a severe respiratory disease in susceptible individuals [1]. We have found that the O-specific polysaccharide chain of *L. pneumophila* serogroup 1 (strain Philadelphia 1, ATCC 33152) lipopolysaccharide (LPS) is an α-2,4-linked homopolymer of 5-acetamidino-7-acetamido-8-*O*-acetyl-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-nonulosonic acid [2–4]. Now we report on the presence in this LPS of a derivative of another higher monosaccharide of this class.

LPS was isolated as described [2], degraded with 0.1 M sodium acetate buffer (pH 4.4, 100 °C, 6 h), and a low-molecular-mass fraction was isolated by GPC of the carbohydrate portion on Sephadex G-50 [3]. ^{1}H and ^{13}C NMR spectroscopic studies showed that this fraction was a mixture of almost equal amounts of a 5,7-diacetamido-8-*O*-acetyl-3,5,7,9-tetradeoxynonulosonic acid (**1**, $\alpha:\beta \sim 1:3$) and an α-mannopyranosyl-(1 → 8)-3-deoxy-D-*manno*-octulosonic acid disaccharide [α-Man*p*-(1 → 8)-Kdo] with the Kdo residue present in multiple forms (elucida-

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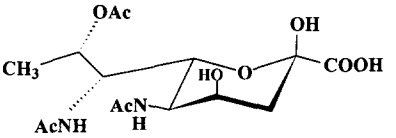
PII S0008-6215(97)00211-5

Table 1
600-MHz ^{1}H NMR data (δ, ppm; J, Hz) [a]

Compound	Proton						
	H-3eq H-3ax	H-4	H-5	H-6	H-7	H-8	H-9
Monosaccharide **1**							
1α	2.54 1.93	4.05	3.81	4.44	4.13	5.00	1.21
	$J_{3eq,4} \approx J_{3ax,4}$ 3.3 $J_{3eq,3ax}$ 14.6		$J_{4,5}$ 3.5	$J_{5,6}$ 10.8	$J_{6,7}$ 2.1	$J_{7,8}$ 9.2	$J_{8,9}$ 6.4
1β	2.10 [b] 2.13 [b]	4.11	3.87	4.45	4.20	4.97	1.22
	$J_{3eq,4} \approx J_{3ax,4}$ 3.3 $J_{3eq,3ax}$ 13.8		$J_{4,5}$ 3.1	$J_{5,6}$ 10.8	$J_{6,7}$ 2.1	$J_{7,8}$ 7.7	$J_{8,9}$ 6.5

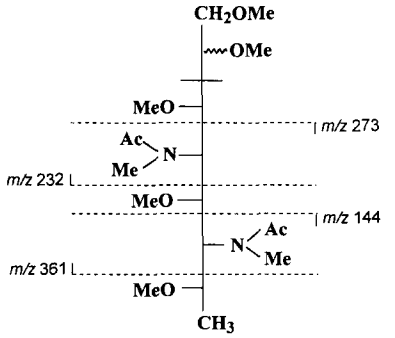
[a] The spectrum was run in D_2O at 305 K. Chemical shifts for OAc and NAc are δ 2.10 and 1.98, 2.00, respectively.
[b] Assignment could be interchanged.

tion of the structure of this disaccharide will be reported elsewhere). Similar degradation of *O*-deacylated LPS (anh N_2H_4, 37 °C, 2 h) resulted mainly in the same disaccharide with a much smaller amount (8:1) of *O*-deacetylated **1**. *O*-Deacetylation of **1** (aq 12% NH_4OH, 50 °C, 2 h) caused its conversion, most likely to a bicyclic derivative (^{1}H NMR data). Compound **1** was studied without further purification.



1β

Borohydride reduction of **1** followed by esterification (0.5 M HCl/MeOH, 20 °C), carboxyl reduction ($NaBH_4$, 20 °C) and methylation [5] resulted in a mixture of two stereoisomers (~ 1:1) of a 5,7-diacetamido-3,5,7,9-tetradeoxynonitol derivative (**2**) which was analyzed by GLC–MS. In accordance with the expected molecular mass of 420 amu, CI MS of **2** showed a peak for $[M + H]^+$ at m/z 421. The EI mass spectrum of **2** contained intense peaks of characteristic primary fragment ions at m/z 144 (C-7–C-9), 273 (C-5–C-9), 232 (C-1–C-5), and 361 (C-1–C-7), thus confirming the positions and *N*-acetylation of the amino groups at C-5 and C-7.



2

Table 2
90-MHz ^{13}C NMR data (δ, ppm) [a]

Compound	Carbon								
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
Monosaccharide **1**									
1α	[b]	[b]	39.7	67.5	49.8	69.5	52.2	70.4	17.1
1β	[b]	96.5	37.9	67.4	49.6	66.4	52.3	71.5	17.1

[a] The spectrum was run in D_2O at 300 K. Chemical shifts for OAc and NAc are δ 21.9 and 22.8, 23.0, respectively.
[b] Not found.

The ^1H and ^{13}C NMR spectra of **1** were assigned using two-dimensional COSY and $^1H,^{13}C$ HMQC experiments (Tables 1 and 2). Comparison of the $^3J_{H,H}$ coupling constant values for **1** (Table 1) and derivatives of 5,7-diamino-3,5,7,9-tetradeoxy-L-*glycero*-D-*galacto*-nonulosonic acid (legionaminic acid) [2,6–8] showed their similarity except for the $J_{3ax,4}$ and $J_{4,5}$ values, which are large in the latter monosaccharide (10–12 Hz) and small in **1** (3.1–3.5 Hz) indicating the equatorial orientation of H-4. A large $J_{5,6}$ value (10.8 Hz) demonstrated the *trans*-diaxial orientation of H-5 and H-6 and, thus, the *ribo* configuration of the fragment C-4–C-6. A small $J_{6,7}$ value (2.1 Hz) is typical of the *threo* configuration of the C-6–C-7 fragment, as in legionaminic acid [2,6,8], while a large value of ~ 10 Hz would be expected in the case of the *erythro* configuration characteristic for the L-*glycero*-L-*manno* isomer (pseudaminic acid) [9,10].

Comparison of the ^{13}C chemical shifts for **1** (Table 2) and the corresponding derivatives of legionaminic acid showed much similarity for the C-7–C-9 chemical shifts [2], but the signals for C-4–C-6 were shifted significantly upfield (by 2, 4, and 5 ppm, respectively) [6]. These shifts were in agreement with the change of the orientation of HO-4 from equatorial in legionaminic acid to axial in **1**. Close values of the C-9 chemical shifts in both monosaccharides (δ 17.3 [2] and 17.1 in the 8-*O*-acetyl derivatives, δ 19.4–20.1 [2,6] and 20.4 after *O*-deacetylation, respectively) were indicative of the *threo* configuration of the C-7–C-8 fragment (chemical shifts δ 16.2–17.9 were observed for non-*O*-acetylated derivatives of pseudaminic acid with the *erythro* configuration of this fragment [10]).

Comparison of the ^1H and ^{13}C NMR spectra of **1** and *O*-deacetylated **1** revealed displacements of the signals for C-9 from δ 17.1 to 20.4 and for H-8 from δ 4.97 to 4.23 (in the β-series) and, thus, the location of the *O*-acetyl group at position 8 was indicated.

Therefore, most likely **1** is 5,7-diacetamido-8-*O*-acetyl-3,5,7,9-tetradeoxy-L-*glycero*-D-*talo*-nonulosonic acid which differs from the known L-*glycero*-D-*galacto* isomer (legionaminic acid) [2,3,6–8] by the configuration at C-4 only. However, the enantiomeric structure is not strictly excluded, and synthesis of the authentic sample is necessary to assign unambiguously the absolute configuration in **1**. After the legionaminic acid and the pseudaminic acid (L-*glycero*-L-*manno*) [9–11], the new sugar is the third representative of aldulosonic acids of this class found in Nature. Although the exact location of monosaccharide **1** in LPS remains unknown, it can be hypothesized that the O-chain in *L. pneumophila* serogroup 1 is built up according a similar mechanism as the ABC-transporter-dependent assembly of homopolymeric O-polysaccharides [12]. In this case the isolegionaminic acid is expected to occupy the non-reducing end of the growing O-specific polysaccharide chain. According to our preliminary data, a derivative of the new isomer is also a component of the *L. pneumophila* serogroup 2 LPS.

Acknowledgements

We thank Mrs. R. Engel and Ms. K. Karpinski for help with HPLC and Mr. H.-P. Cordes for help with NMR spectroscopy. This work was supported by grant 436 RUS 113/314/0 (R,S) of Deutsche Forschungsgemeinschaft (DFG).

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