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Identification of a Homopolymer of 5-Acetamidino-7-acetamido-3,5,7,9-tetradeoxy-D-*glycero*-D-*talo*-nonulosonic Acid in the Lipopolysaccharides of *Legionella pneumophila* Non-1 Serogroups

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Abstract—O-Specific polysaccharides (OPS) were isolated by mild acid hydrolysis of the lipopolysaccharides (LPS) of strains of *Legionella pneumophila* serogroups 2-14, as well as strains Lansing 3 and 16453-92 from newly proposed serogroups. The OPS were studied by ^{1}H- and ^{13}C-NMR spectroscopy, GLC/mass spectrometry, and chemical modifications (mild alkaline O-deacetylation and conversion of the N-acetimidoyl group into the N-acetyl group). All OPS were found to be a homopolymer of a 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxynonulosonic acid, which in some strains is 8-O-acetylated. In most strains studied, the monosaccharide has the D-*glycero*-D-*talo* configuration and is thus the C4 epimer of legionaminic acid (4-epilegionaminic acid), which has been previously identified as the monomer in the OPS of *L. pneumophila* serogroup 1. Poly(4-epilegionaminic acid) occurs as a minor polysaccharide in serogroups 5 (strain Dallas 1) and 13 and is absent in serogroups 1 and 7. The chemical basis for serological differentiation of *L. pneumophila* strains is discussed.

Key words: lipopolysaccharide, O-specific polysaccharide, structure, 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxy-D-*glycero*-D-*talo*-nonulosonic acid, legionaminic acid, *Legionella pneumophila*

The facultative intracellular human pathogen *Legionella pneumophila* is the causative factor of legionellosis, a severe respiratory disease in susceptible individuals [1]. Based on the immunospecificity of the surface lipopolysaccharide (LPS, endotoxin, O-antigen) determined with polyclonal antibodies, strains of *L. pneumophila* may be classified into 14 numbered serogroups and one unnumbered serogroup Lansing 3 [2-4]. Using MAbs, isolates of *L. pneumophila* could be divided into 64 subgroups, termed LPS phenons [5].

The molecular basis for the diversity of *L. pneumophila* LPS antigens remained so far unknown. Aiming at creation of the chemical basis for the classification of *L. pneumophila* strains and determination of the specificity of LPS-associated epitopes, we have been elucidating the LPS structures in various serogroups. Recently, we have found that the LPS of *L. pneumophila* serogroup 1 (strain Philadelphia 1) has a unique O-specific polysaccharide (OPS) chain of an α2→4-linked homopolymer of a 5-N-acetimidoyl-7-N-acetyl-8-O-acetyl derivative of a 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acid, termed legionaminic acid [6, 7]. Originally, the D-*glycero*-L-*galacto* configuration was ascribed to legionaminic acid [6, 7], but later revised first to the L-*glycero*-D-*galacto* configuration [8] and finally to the D-*glycero*-D-*galacto* configuration [9]. A derivative of an isomer of legionaminic acid was released by mild acid hydrolysis from the same serogroup 1 LPS and identified as 5,7-diacetamido-8-O-acetyl-3,5,7,9-tetradeoxy-D-*glycero*-D-*talo*-nonulosonic acid (a derivative of 4-epilegionaminic acid) [9, 10]. Core of *L. pneumophila* LPS is a nonasaccharide lacking heptose and phosphate and containing a number of 6-deoxy sugars and O- and N-acetyl groups in the outer region [11-13]. The hydrophobicity of the carbohy-

Abbreviations: LPS) lipopolysaccharide; OPS) O-specific polysaccharide.

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drate portion of the LPS was suggested to promote spreading of *L. pneumophila* via aerosols and adherence to alveolar macrophages at the early stage of pulmonary infection; thus, it can be considered as a virulence factor of *L. pneumophila* [7].

In this work we report on the structure of another OPS of *L. pneumophila* that is characteristic of the most non-1 serogroup strains and found to be a homopolymer of 4-epilegionaminic acid that bears the same N-acyl substituents as legionaminic acid in serogroup 1 OPS.

MATERIALS AND METHODS

Cultivation of bacteria, isolation and degradation of LPS. *L. pneumophila* type strains were from the American Type Culture Collection (ATCC) or the National Collection of Type Cultures (NCTC) (Table 1). Strains were grown on buffered charcoal yeast agar at 37°C. Bacterial cells were digested with RNAse, DNAse, and proteinase K in 10 mM Tris-HCl buffer, pH 7.5, containing 4 mM $CaCl_2$ at 37°C, and lyophilized. The LPS were isolated in yields of 5-27% (Table 1) by a modified phenol–chloroform–petroleum ether procedure [14] using diethyl ether instead of water as solvent for LPS precipitation [6, 15].

The LPS (50-80 mg of each) were degraded with 0.1 M NaOAc-HOAc buffer, pH 4.4, at 100°C for 6 h. The carbohydrate portion was fractionated by gel-permeation chromatography on a column (60 × 2.5 cm) of Sephadex G-50 (S) in 0.05 M pyridinium acetate buffer, pH 4.5, monitored with a Knauer differential refractometer (Germany). High-molecular-mass OPS were isolated in yields of 13-43% of the LPS mass (Table 1).

Sugar analysis and chemical modifications. Methanolysis of the OPS (0.5 mg) was performed with 0.5 M hydrogen chloride in methanol (80°C, 8 h) and followed by acetylation with acetic anhydride in pyridine (1 :

Table 1. Yields of LPS and OPS, type of the monomer, and the degree of O-acetylation of OPS from different *L. pneumophila* serogroups

Serogroup	Strain	Reference strain	Yield, %		OPS type	
			LPS[a]	OPS[b]	monomer	degree of 8-O-acetylation, %
1	Philadelphia 1	ATCC 33152	7.3	50	Leg[c]	>90
2	Togus 1	ATCC 33154	20	43	4eLeg	80–90
3	Bloomington 1	ATCC 33155	16	40	4eLeg	<10
4	Los-Angeles 1	ATCC 33156	18	38	4eLeg	80–90
4	Portland 1	4	20	40	4eLeg	10–20
5	Dallas 1E	ATCC 33216	15	29	n.i.	>90
5	Cambridge 1	NCTC 11191	24	46	4eLeg	<10
6	Chicago 2	ATCC 33215	23	29	4eLeg	80–90
7	Chicago 8	ATCC 33823	8.2	22	n.i.	>50
8	Concord 3	ATCC 35096	25	19	4eLeg	80–90
9	IN-23-G1C2	ATCC 35289	5.4	13	4eLeg	~50
10	Leiden 1	ATCC 43283	5.7	16	4eLeg	80–90
11	797-PA-H	ATCC 43130	27	14	4eLeg	~70
12	570-CO-H	ATCC 43290	12	29	4eLeg	80–90
13	82-A-3105	ATCC 43736	9.2	20	n.i.	>90
14	1169-MN-H	ATCC 43703	10	20	4eLeg	80–90
(15)	Lansing 3	ATCC 35251	9.7	25	4eLeg	~40
(16)	16453-92[d]		20	33	4eLeg	80–90

Note: Leg and 4eLeg stand for 5-N-acetimidoyl-7-N-acetyl-legionaminic and -4-epilegionaminic acid, respectively; n.i.) monomer is not identified.

[a] Calculated to the dried cell mass.

[b] Calculated to the LPS mass.

[c] Structure of the OPS has been determined earlier [6-9].

[d] Strain from a proposed new serogroup [5].

1, 85°C, 30 min). GLC-MS analysis was carried out on a Hewlett-Packard 5989A instrument (USA) equipped with an HP-5 column using a temperature gradient 150°C (3 min) → 320°C at 5°/min.

2-Butanolysis of the OPS (0.5 mg) was performed with 0.5 M hydrogen chloride in (S)-2-butanol (70°C, 45 min) and followed by acetylation as described above. The acetylated (S)-2-butyl glycosides were identified by GLC-MS using for comparison the authentic samples derived from synthetic 5,7-diacetamido-3,5,7,9-tetradeoxynonulosonic acids with the D-*glycero*-D-*galacto*, L-*glycero*-D-*galacto*, D-*glycero*-D-*talo*, and L-*glycero*-D-*talo* configuration [9].

O-Deacetylation of the OPS (20 mg) was performed with aqueous 12% ammonia for 16 h at room temperature, and then the solution was concentrated in vacuum at 20°C and lyophilized. For conversion of the N-acetimidoyl group into the N-acetyl group, the O-deacetylated OPS (15 mg) was heated with aqueous 12% ammonia solution for 16 h at 80°C and treated as above.

NMR spectroscopy. Samples were lyophilized twice from solution in ^2H_2O and dissolved in 99.96% ^2H_2O. One-dimensional ^{1}H-NMR spectra and two-dimensional COSY and H-detected ^{1}H,^{13}C HMQC spectra were recorded on a Bruker DRX-500 spectrometer (Germany) at 50°C using sodium 3-trimethylsilylpropanoate-d_2 (δ_H 0.00) as internal standard. One-dimensional ^{13}C-NMR spectra were run on the same instrument or on a Bruker AMX-360 spectrometer at 50°C using acetone (δ_C 31.45) as internal standard.

RESULTS

OPS were prepared from *L. pneumophila* LPS serogroups 2-14 and strains Lansing 3 and 16453-92 from new proposed serogroups by degradation at pH 4.4 followed by fractionation on Sephadex G-50 (Table 1). Sugar analysis of the high-molecular-mass OPS from *L. pneumophila* serogroup 2 (strain Togus 1) after acidic methanolysis revealed rhamnose, mannose, 2-amino-2,6-dideoxyglucose, 2-amino-2-deoxyglucose, and 3-deoxy-*manno*-octulosonic acid, i.e., the same components that were found in the core oligosaccharide of *L. pneumophila* serogroup 1 LPS [11]. Therefore, the isolated OPS was attached to a core oligosaccharide.

No sugar was detected that might be considered as an OPS component. A similar result was obtained in studies of a homopolymer of legionaminic acid from *L. pneumophila* serogroup 1 LPS and was accounted for by the stability of the glycosidic linkage of the 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acid towards acid methanolysis and/or by destruction of the released monosaccharide under acidic conditions [6]. However, derivatives of a higher sugar were detected after acidic 2-butanolysis under milder conditions. They were indistinguishable by GLC-MS analysis data from the products derived from the authentic synthetic sample of 5,7-diacetamido-3,5,7,9-tetradeoxy-D-*glycero*-D-*talo*-nonulosonic acid, but differed from those of the corresponding L-*glycero*-D-*talo*, D-*glycero*-D-*galacto*, and L-*glycero*-D-*galacto* isomers.

The NMR spectra of the OPS of *L. pneumophila* serogroup 2 resembled those of serogroup 1 OPS [6]. The ^{13}C-NMR spectrum (Fig. 1b) showed characteristic signals for a 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acid (C2, C3, and C9 at δ 101.9, 40.5, and 17.4 ppm, respectively; C5 and C7 at δ 52.7 and 52.8 ppm) and for three different acyl substituents: N-acetyl, O-acetyl, and N-acetimidoyl groups at δ 23.5, 22.3, 20.5 (all CH_3), 174.8, 175.1 (both CO), and 167.0 ppm (C=N). Accordingly, the ^{1}H-NMR spectrum of the OPS contained signals for the deoxy groups of the nonulosonic acid at δ 2.81, 1.77, and 1.23 ppm (H3eq, H3ax, and H9, respectively), as well as N-acetyl and O-acetyl groups at δ 2.10-2.11 ppm and an N-acetimidoyl group at δ 2.23 ppm.

The ^{1}H- and ^{13}C-NMR spectra of the serogroup 2 OPS were assigned using two-dimensional COSY and ^{1}H,^{13}C HMQC experiments (Tables 2 and 3). The chemical shift data further confirmed the structure of the 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acid in the OPS. In particular, the characteristic proton-coupling pattern in the COSY spectrum demonstrated a 3-deoxy group of

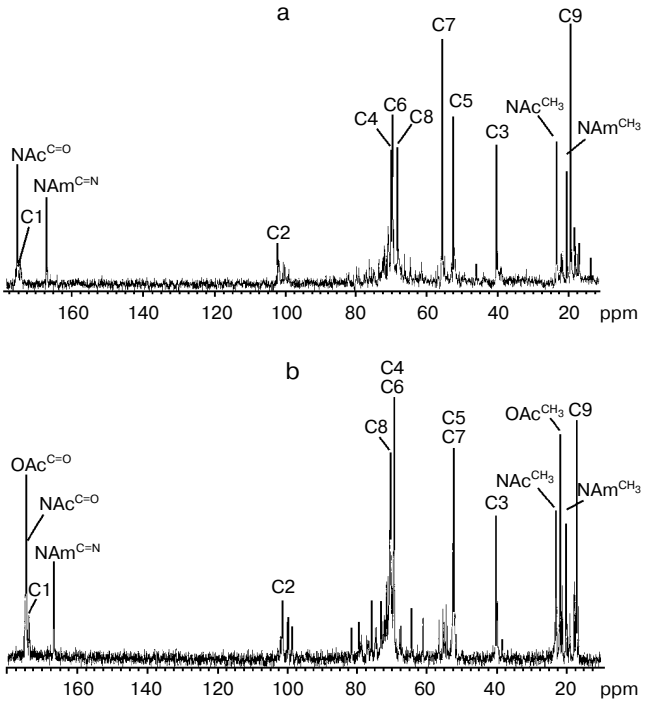


Fig. 1. 125-MHz ^{13}C-NMR spectra of the O-specific polysaccharides of *L. pneumophila* serogroup 2 strain Togus 1 (a) and serogroup 5 strain Cambridge 1 (b).

Table 2. ^{1}H-NMR data (δ in ppm, ^{3}J in Hz)

Compound	H3eq ($J_{3eq,3ax}$)	H3ax ($J_{3ax,4}$)	H4 ($J_{3eq,4}$)	H5 ($J_{4,5}$)	H6 ($J_{5,6}$)	H7 ($J_{6,7}$)	H8 ($J_{7,8}$)	H9 ($J_{8,9}$)
Polysaccharides of *L. pneumophila*								
Serogroups								
2 (Togus 1)	2.81	1.77	4.14	3.75	4.73	4.18	5.06	1.23
1 (Philadelphia 1) [6]	2.44	1.60	4.29	3.42 (9.5)	4.25 (9.5)	4.28 (<2)	4.90 (8.5)	1.27 (6.5)
5 (Cambridge 1)	2.90 (12)	1.88 (<3)	4.32 (<3)	3.75 (<3)	4.63 (9)	3.89 (<3)	3.97 (8)	1.18 (6)
O-Deacetylated polysaccharides								
2 (Togus 1)	2.90 (12)	1.87 (<3)	4.29 (<3)	3.75 (<3)	4.64 (9)	3.87 (<3)	3.96 (8)	1.18 (6)
1 (Philadelphia 1) [6]	2.63 (11)	1.63 (11)	4.05	3.50 (9.5)	3.98 (9.5)	3.91	3.91	1.20
Monosaccharides [9]								
*1*α	2.73 (12.9)	1.71 (11.9)	3.82 (4.7)	3.68	3.93 (10.3)	3.84	3.94	1.16
*1*β	2.31 (13.1)	1.87 (11.7)	3.98 (4.8)	3.72 (10.3)	4.31 (10.5)	3.91 (1.9)	3.85 (8.9)	1.16 (6.2)
*2*α	2.69 (14.4)	1.95 (3.5)	4.10 (3.0)	3.86 (2.9)	4.55 (10.8)	3.88 (2.3)	4.00 (8.6)	1.20 (6.4)
*2*β	2.19 (14.9)	2.14 (3.4)	4.13 (3.0)	3.90 (2.9)	4.63 (10.8)	3.92	3.92	1.18 (5.5)

Note: In the polysaccharides, chemical shifts for the N-acetyl, O-acetyl, and N-acetimidoyl groups are δ 2.01-2.11, 2.11-2.18, and 2.19-2.32 ppm, respectively.

a 2-keto sugar. The presence of amino groups at positions 5 and 7 followed from the position of the signals for C5 and C7 in the resonance region of carbons bearing nitrogen at δ 52.7 and 52.8 ppm, respectively.

Comparison of these data with the published data for polylegionaminic acid from *L. pneumophila* serogroup 1 LPS [6] revealed characteristic differences in ^{1}H- and ^{13}C-NMR chemical shifts of the two homopolysaccharides (Tables 2 and 3). The most significant differences were observed for atoms in the pyranose ring (e.g., compare the C4-C6 chemical shifts of δ 69.8, 52.7, and 69.8 ppm in serogroup 2 OPS with δ 71.4, 55.1, and 72.4 ppm in serogroup 1 OPS, respectively), whereas chemical shifts for atoms in the side chain were similar (Table 3). Similar differences and similarities were observed between the synthetic compounds 5,7-di-N-acetyllegionaminic acid (*1*) and 5,7-di-N-acetyl-4-epilegionaminic acid (*2*) having the D-*glycero*-D-*galacto* and D-*glycero*-D-*talo* configuration, respectively [9] (Fig. 2). Taking into account the sensitivity of the NMR chemical shifts to the configuration of both sugar ring and side chain of 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acids [9], it was concluded that the new homopolymer differs from polylegionaminic acid in the configuration at C4 only and, thus, is poly(4-epilegionaminic acid).

Treatment of the OPS with aqueous ammonia at ambient temperature resulted in O-deacetylation, as followed from the disappearance of the signals for the O-acetyl group from the ^{1}H- and ^{13}C-NMR spectra. The NMR spectra of the modified OPS that were assigned as described above were practically identical to the spectra of the non-modified OPS from *L. pneumophila* serogroup 5

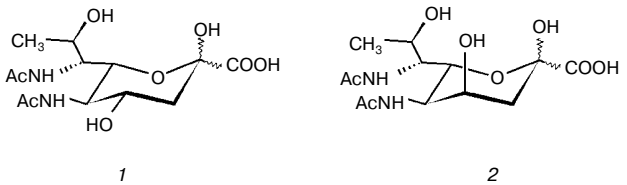
Table 3. ^{13}C-NMR data (δ in ppm)

Compound	C2	C3	C4	C5	C6	C7	C8	C9
Polysaccharides of *L. pneumophila*								
Serogroups								
2 (Togus 1)	101.9	40.5	69.8	52.7	69.8	52.8	70.7	17.4
1 (Philadelphia 1) [6]	101.6	40.6	71.4	55.1	72.4	52.6	70.8	17.3
5 (Cambridge 1)	102.3	40.4	70.2	52.6	69.9	55.9	68.3	19.5
O-Deacetylated polysaccharides								
2 (Togus 1)	102.0	40.4	70.3	52.6	69.9	55.8	68.4	19.5
1 (Philadelphia 1) [6]	101.8	39.2	71.6	54.2	72.4	55.2	67.7	19.4
Monosaccharides [9]								
*1*α		41.4	69.2	53.4	73.2	54.4	68.0	20.4
*1*β	96.6	40.3	68.4	53.9	70.9	54.4	67.5	20.4
*2*α		40.2	66.9	49.7	70.3	55.0	68.2	20.4
*2*β	96.3	37.6	67.1	49.7	66.5	54.7	67.2	20.4

Note: In the polysaccharides, chemical shifts for C1 of the nonulosonic acids and CO of N- and O-acetyl groups are δ 174-176 ppm, C=N of N-acetimidoyl group δ 167.0-168.0 ppm, Me of N-acetyl, O-acetyl, and N-acetimidoyl groups δ 23.0-23.6, 22.0-22.4, and 19.9-20.7 ppm, respectively.

strain Cambridge 1 (Tables 2 and 3; Fig. 1a). A better resolved ^{1}H-NMR spectrum of the O-deacetylated OPS enabled determination of $^3J_{H,H}$ coupling constant values, which confirmed the relative configuration of 4-epilegionaminic acid. Thus, relatively small $J_{3ax,4}$ and $J_{4,5}$ values of <3 Hz and a large $J_{5,6}$ value of 10 Hz demonstrated the equatorial orientation of H4 and axial orientation of H5 and H6; hence, the C4-C6 fragment has the ribo configuration. A small $J_{6,7}$ value (<3 Hz) was indicative of the *threo* configuration of the C6-C7 fragment [6, 9, 10, 16, 17]. Such $J_{6,7}$ value was observed for all derivatives of legionaminic acid and its C4 and C8 epimers studied earlier and could be predicted by molecular mechanics calculations [17]. The *erythro* configuration of the C7-C8 fragment was confirmed by the C6 and C8 chemical shifts of δ 69.9 and 68.4 ppm that were similar to those in the synthetic D-*glycero*-α-D-*talo* isomer (δ 70.3 and 68.2 ppm) and differed from those in the L-*glycero*-α-D-*talo* isomer (δ 72.6 and 69.7 ppm, respectively) [9].

Upon O-deacetylation, the signal for H8 in the ^{1}H-NMR spectrum shifted upfield from δ 5.06 to 3.96 ppm since the O-acetyl group no longer deshielded this proton. In the ^{13}C-NMR spectrum, an upfield displacement was observed for the C8 signal (from δ 70.7 to 68.4 ppm) and downfield displacements were observed for the signals of C7 and C9 (from δ 52.8 and 17.4 to 55.8 and 19.5 ppm, respectively). These changes corresponded to O-deacetylation at position 8 [18] and were similar to the chemical shift differences for C7, C8, and C9 of the 8-O-acetylated and non-O-acetylated forms of legionaminic acid [6] (Table 3) and 4-epilegionaminic acid [9, 10]. 8-O-Acetylation confirmed the (2→4)-linkage between 4-epilegionaminic acid residues in the OPS since O4 is the only possible glycosylation site.



1 *2*

Fig. 2. Structures of di-N-acetyllegionaminic acid (*1*) and di-N-acetyl-4-epilegionaminic acid (*2*).

The α configuration of 4-epilegionaminic acid in the OPS followed from a large difference of >1 ppm between the H3eq and H3ax chemical shifts (compare the corresponding differences of 0.71 and 0.05 ppm in the α- and β-anomers of the synthetic monosaccharide *2*, respectively; Table 2). In α-linked legionaminic acid this difference was 0.84 ppm in the initial OPS and 1.00 ppm in the O-deacetylated OPS [6]. The C6 chemical shift of δ 69.9 ppm confirmed the α configuration of 4-epilegionaminic acid (compare the C6 chemical shifts of δ 70.3 and 66.5 ppm for α- and β-anomers of the compound *2*, Table 3).

Comparison of the ^{1}H- and ^{13}C-NMR spectra of the initial and O-deacetylated OPS showed the presence in the former of a minor series of signals from non-O-acetylated 4-epilegionaminic acid. The degree of 8-O-acetylation in the initial OPS was estimated from the spectra as 80-90%. In addition, the spectra of the initial OPS contained minor signals for core constituents [11] and some other signals that were not assigned. They may belong to nonulosonic acid residues that differed from the major OPS monomer, e.g., those having the opposite anomeric configuration, a different general configuration, or different N-acyl substituents. Alternatively, they may originate from 4-epilegionaminic acid residues that occupy terminal positions in the polysaccharide chain.

Further treatment of the O-deacetylated OPS with aqueous ammonia at elevated temperature (37°C) resulted in conversion of the N-acetimidoyl group into the N-acetyl group and the corresponding changes in the ^{13}C-NMR spectrum. The signals for the N-acetimidoyl group disappeared from the spectrum, whereas signals for an additional N-acetyl group appeared instead. From the sugar carbon signals, the most significant shift (from δ 52.6 to 49.3 ppm) was observed for the C5 signal (compare published data δ 54.2 and 51.3 ppm in 5-N-acetimidoyl- and 5-N-acetyllegionaminic acid, respectively [6]). The position of the C7 signal at δ 55.8 ppm did not change, and, hence, the N-acetimidoyl group was located at position 5 and the N-acetyl group at position 7. It should be mentioned that, to some extent, hydrolysis of the 5-N-acetimidoyl group proceeded even when the aqueous ammonia treatment was performed at room temperature, thus showing that this group in 4-epilegionaminic acid is less stable than in legionaminic acid (in the latter the full conversion required heating with 0.1 M sodium hydroxide at 100°C for 5 h [6]).

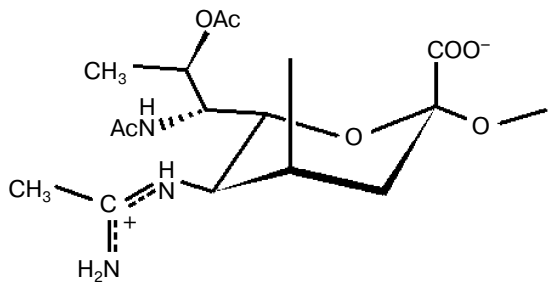


Fig. 3. Structure of the monosaccharide unit of the O-specific polysaccharide of *L. pneumophila* serogroup 2 strain Togus 1.

Therefore, the data obtained showed that the OPS of *L. pneumophila* serogroup 2 is an α2→4-linked homopolymer of 5-acetamidino-7-acetamido-8-O-acetyl-3,5,7,9-tetradeoxy-D-*glycero*-D-*talo*-nonulosonic acid or poly(4-epilegionaminic acid) (Fig. 3).

Similar ^{1}H- and ^{13}C-NMR spectroscopic studies showed that the OPS of most other *L. pneumophila* serogroups are the same homopolymer of 4-epilegionaminic acid with the same N-acyl substituents as in serogroup 2. The OPS of serogroups 4 (strain Los-Angeles 1), 6, 8, 10-12, 14, and strain 16453-92 are also 8-O-acetylated to a similar degree (>80%, Table 1). The OPS from serogroups 4 (strain Portland 1), 9, 11, and strain Lansing 3 are characterized by a lower degree of 8-O-acetylation (10 to 70%), whereas the OPS of serogroups 3 and 5 (strain Cambridge 1) were practically devoid of the 8-O-acetyl groups (Tables 2 and 3; Fig. 1a). The NMR spectra showed also that, as in serogroup 2 OPS, in all OPS studied, together with the major α-4-epilegionaminic acid, there are present minor unidentified components, most likely, other derivatives of the same monosaccharide or its isomer.

Although the OPS of serogroups 5 (strain Dallas 1), 7, and 13 are homopolymers of the same type as the OPS of the other serogroups, their ^{1}H- and ^{13}C-NMR spectra differed from those of both serogroup 1 and other serogroups mentioned above. The spectra showed that poly(4-epilegionaminic acid) occurs as a minor OPS in serogroups 5 (strain Dallas 1) and 13 and is completely absent in serogroup 7. The main OPS of serogroups 5 and 13 is the same and consists of an isomer of 5-acetamidino-7-acetamido-8-O-acetyl-3,5,7,9-tetradeoxynonulosonic acid that is not yet identified. The OPS of serogroup 7 is built up of yet another isomer of the monosaccharide of the same class that also remains to be identified.

The chemical data obtained in the present work and earlier [6, 7] showed that the *L. pneumophila* LPS is structurally rather conservative. In all serogroups, the LPS has a homopolysaccharide O-specific chain of a 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxynonulosonic acid that may be 8-O-acetylated. In serogroup 1, the monomer is legionaminic acid having the D-*glycero*-D-*galacto* configuration [6, 7], whereas the D-*glycero*-D-*talo* isomer is present in the most other serogroups (this work).

L. pneumophila serogroups 1 and 7 are unique with respect to the OPS structure since polylegionaminic acid is present in none of non-1 serogroups. This is consistent with the serological isolation of serogroups 1 and 7, which

was demonstrated using monoclonal antibodies [5]. Indeed, the LPS of serogroups 1 and 7 have no cross-reactive epitopes, whereas the LPS of all other serogroups share common epitopes. Most likely, the shared epitopes are associated with poly(4-epilegionaminic acid), which is a minor OPS of serogroups 5 (strain Dallas 1) and 13 and the major OPS in all other serogroups (except serogroups 1 and 7).

Strains from different serogroups may have the same OPS and, *vice versa*, strains of the same serogroup, like Dallas 1 and Cambridge 1 from serogroup 5, may produce different OPS. The exact chemical basis for serological differentiation of strains of cross-reactive (i.e., non-1, non-7) serogroups remains unknown. Their serological specificity may be defined by minor LPS constituents, like a minor nonulosonic acid derivative(s) different from the major OPS component. In this context it should be noted that *L. pneumophila* serogroup 1 strains, all having the OPS of polylegionaminic acid, could be clearly differentiated using monoclonal antibodies into 15 subgroups [5]. At the same time only one epitope on the serogroup 1 OPS could be chemically defined, namely, the epitope of monoclonal antibodies MAb 2 and 3/1, which is associated with the 8-O-acetyl group of legionaminic acid [19].

The role of the O-acetyl groups in *L. pneumophila* OPS remains unclear. A complex O-acetylation pattern has been recently revealed in serogroup 1 LPS, and the occurrence of at least two O-acetyl transferase-encoding genes suggested [13]. One of the genes, *lag-1*, may or may not be present in serogroup 1 strains and does not occur in non-1 serogroup strains [20]. The presence of *lag-1* correlates with binding MAb 2 and MAb 3/1 to *L. pneumophila* LPS but does not influence serogroup-specific epitope(s). Similarly, in non-1 serogroups the O-acetylation does not seem to define the serogroup specificity. Thus, two strains of the same serogroup 4, Los-Angeles 1 and Portland 1, belonged to high and low O-acetylated OPS chemotypes, respectively. Some non-1 serogroups are characterized by an intermediate chemotype, which may be indicative of a complex O-acetylation mechanism also in these serogroups.

Further studies of *L. pneumophila* LPS of various serogroups are necessary in order to determine the structures of yet unidentified OPS in serogroups 5, 7, and 13 and to reveal epitopes that are responsible for serological diversity of the OPS consisting of the same major homopolymer of 4-epilegionaminic acid.

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