

Note

A pseudoaminic acid-containing O-specific polysaccharide from a marine bacterium *Cellulophaga fucicola*

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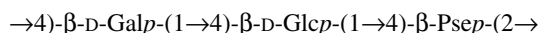
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Abstract—The O-polysaccharide was isolated from the lipopolysaccharide of *Cellulophaga fucicola* and studied by chemical analyses along with ¹H and ¹³C NMR spectroscopy. The following new structure of the O-polysaccharide of *C. fucicola* containing 5,7-diacetamido-3,5,7,9-tetra-deoxy-L-glycero-L-manno-non-2-ulonic acid residue (pseudoaminic acid, Psep) was elucidated as the following:



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The genus *Cellulophaga* belongs to the family Flavobacteriaceae of the phylum Bacteroidetes. It was created by Johansen et al.¹ to accommodate the heterotrophic aerobic Gram-negative yellow/orange pigmented gliding and agarolytic bacteria of marine origin. Currently this genus comprises five species: *Cellulophaga algicola*, *Cellulophaga baltica*, *Cellulophaga fucicola*, *Cellulophaga lytica*, and *Cellulophaga pacifica*.² Data on the lipopolysaccharide structure of *Cellulophaga* were reported only for *C. baltica*.³

The O-specific polysaccharide (OPS) of *C. fucicola* was obtained by mild acid degradation of the lipopolysaccharide (LPS) isolated from dried bacterial cells by the phenol–water procedure. Degradation of the LPS with sodium acetate buffer (pH 4.5) resulted in a high-molecular weight polysaccharide (OPS-I) and a low-molecular-

weight polymer (OPS-II). Sugar analysis by GLC of the alditol acetates derived after full acid hydrolysis of the OPS-I revealed Gal and Glc in the ratios ~1:1. GLC analysis of the acetylated (*S*)-2-octyl glycosides demonstrated the D configuration of both monosaccharides.

The ¹³C NMR spectrum of the OPS-I showed, inter alia, signals for three anomeric carbon atoms at δ 102.6, 102.8 (quaternary carbon; data from the DEPT experiment) and 104.2, two nitrogen-bearing carbon atoms of amino sugar(s) at δ 47.2 and 54.7, two CH₂OH groups at δ 61.3 and 62.2, one C–CH₂–C group at δ 36.4, two *N*-acetyl groups at δ 23.2 and 23.3 (both CH₃), one CH₃–C group at δ 17.6, and three CO groups at δ 173.2, 174.8, and 176.0. The ¹³C NMR spectrum of the OPS-II (Fig. 1) contained also a set of minor signals belonging to terminal residues (see below).

Four signals were observed in a low-field region of the ¹H NMR spectrum of the OPS-I at δ 4.34–4.64, which included only two signals for anomeric protons (δ 4.45 and 4.64, both doublets, *J*_{1,2} 8 Hz); the other

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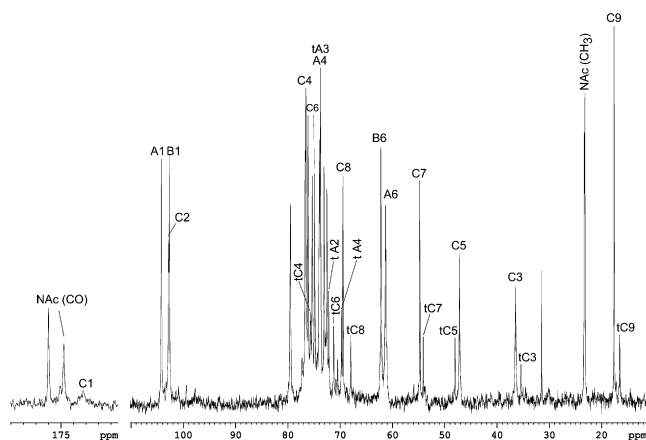


Figure 1. ^{13}C NMR spectrum of the low-molecular-weight fraction of the OPS-II of *C. fucicola*. Arabic numerals refer to the carbons in the sugar residues denoted as described in Table 1.

two signals at δ 4.34 and 4.52 were shown to belong to nonanomeric protons (see below). A high-field region of the spectrum contained two signals for *N*-acetyl groups at δ 1.94 and 2.00, one doublet (J 6 Hz) for a CH_3 group, and two one-proton signals at δ 2.67 (double doublets, J 5 and 13 Hz) and δ 1.77 (triplet, J 13 Hz).

The ^1H NMR spectrum of the OPS-I was assigned using 2D ^1H , ^1H COSY, TOCSY, and ROESY experiments (Table 1). The analysis of the correlations in the 2D spectra showed the presence in the repeating unit of spin systems for β -Galp (**A**), β -GlcP (**B**) and a 5,7-diacylamido-3,5,7,9-tetra-deoxynon-2-ulosonic acid (**C**). The last sugar residue was identified as 5,7-diacetyl-

amido-3,5,7,9-tetra-deoxy- β -L-glycero-L-manno-non-2-ulosonic acid (β -pseudaminic acid, β -Pse) on the basis of the following data:⁴

- (i) The ^{13}C NMR spectrum contained signals characteristic for C-1 (δ 173.2), C-2 (δ 102.8), C-3 (δ 36.4), C-5, C-7 (δ 47.2, 54.7), and C-9 (δ 17.6) of the 5,7-diamino-3,5,7,9-tetra-deoxynon-2-ulosonic acids.
- (ii) The positions of H-5 and H-7 (δ 4.34 and 4.02, correspondingly) in the ^1H NMR spectrum were typical of protons at *N*-acetylated carbons. The presence of only two *N*-acetyl groups in the repeating unit showed that the carbons C-5 and C-7 were *N*-acetylated.
- (iii) A large coupling constant ($J_{\text{H-3ax,H-4}}$ 13 Hz) indicated an axial position for H-4, small coupling constants $J_{\text{H-4,H-5}}$ and $J_{\text{H-5,H-6}}$ (4 and 2 Hz, correspondingly) displayed an equatorial position for H-5, and coupling constants $J_{\text{H-6,H-7}}$ (11 Hz) and $J_{\text{H-7,H-8}}$ (3 Hz) were characteristic for L-glycero-L-manno isomer of the 5,7-diamino-3,5,7,9-tetra-deoxynon-2-ulosonic acids.
- (iv) A large difference in the chemical shifts of H-3e and H-3a (0.9 ppm) was typical of the ulosonic acids with the axial position of the COOH group, that is the β -anomeric configuration of the pseudaminic acid residue.

A 2D ^1H , ^{13}C HSQC experiment was applied for the assignment of the ^{13}C NMR spectrum of the OPS-I (Table 1). Significant downfield displacement of the signals for C-4 of all sugar residues (**A**, **B**, and **C**), as

Table 1. ^1H and ^{13}C NMR data of the OPS-I and OPS-II of the OPS of *C. fucicola* (δ , ppm)^a

Sugar residue		1	2	3	4	5	6 (6a,6b)	7	8	9
<i>OPS-I</i>										
→4)- β -D-Galp-(1→ A	^1H	4.45	3.57	3.66	4.52	3.71	3.80; 3.98			
	^{13}C	104.2	72.1	73.1	73.7	76.7	61.3			
→4)- β -D-GlcP-(1→ B	^1H	4.64	3.23	3.65	3.62	3.61	3.66; 3.70			
	^{13}C	102.6	73.9	75.3	79.5	76.1	62.2			
→4)- β -Psep-(2→ C	^1H			1.77 (ax) 2.67 (eq)	4.06	4.34	3.73	4.02	4.15	1.16
	^{13}C	173.2	102.8	36.4	76.5	47.2	74.8	54.7	69.4	17.6
β -D-Galp-(1→ tA (from OPS-II)	^1H	n.d	3.53	3.66	3.93	n.d	n.d			
	^{13}C	n.d	72.2	73.7	69.8	n.d	n.d			
→4)- α -Psep tC (from OPS-II)	^1H			1.91 (ax) 2.10 (eq)	4.34	4.40	4.03	4.16	4.12	1.10
	^{13}C	n.d	n.d	35.9	75.7	48.0	71.2	54.1	68.0	16.5

^a The chemical shifts for the *N*-acetyl groups are δ_{H} 1.94 and 2.00; δ_{C} 23.2, 23.3 (both Me), and 174.8, 176.0 (2 CO).

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