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Structure of the O-polysaccharide of *Photorhabdus temperata* subsp. *temperata* XlNach^T containing a novel branched monosaccharide, 3,6-dideoxy-4-*C*-[(*S*)-1,2-dihydroxyethyl]-D-*xylo*-hexose

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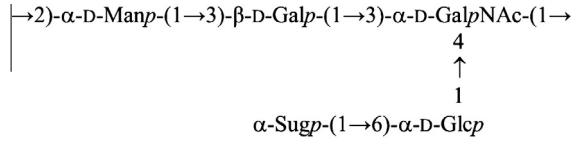
ABSTRACT

O-Polysaccharide was isolated from the lipopolysaccharide of an entomopathogenic bacterium *Photorhabdus temperata* subsp. *temperata* XlNach^T. Sugar analysis after full acid hydrolysis of the polysaccharide revealed D-glucose, D-mannose, D-galactose, D-GalNAc, and a branched monosaccharide, 3,6-dideoxy-4-*C*-[(*S*)-1′,2′-dihydroxyethyl]-D-*xylo*-hexose (Sug), which was isolated as a 1,2′-anhydro furanose derivative. The following structure of the polysaccharide was established by 1D and 2D ^{1}H and ^{13}C NMR spectroscopy:



1. Introduction

The genus *Photorhabdus* from the family Enterobacteriaceae includes three species of entomopathogenic bioluminescent bacteria *Photorhabdus temperata*, *Photorhabdus luminescens*, and *Photorhabdus asymbiotica*. They all have a mutualistic relationship with entomophagous nematodes from the family Heterorhabditis.[1] These bacteria synthesize an S-form lipopolysaccharide, which consists of lipid A, core oligosaccharide, and O-specific polysaccharide (O-antigen) and is important for both pathogenicity and symbiosis.[2] Recently, aiming at creation of the chemical basis for classification of *Photorhabdus* species, structures of the O-polysaccharides of representatives of *P. asymbiotica* subsp. *asymbiotica* and subsp. *australis*[3] and *P. luminescens* subsp. *laumondii*[4] have been elucidated. In this work, we established the O-polysaccharide structure of *P. temperata* subsp. *temperata* XlNach^T.

2. Results and discussion

The O-polysaccharide was obtained by mild acid hydrolysis of the lipopolysaccharide (1 % HOAc, 100 °C) isolated from bacterial cells by phenol–water extraction. Full acid hydrolysis of the O-polysaccharide with 3 M CF_3CO_2H (120 °C) released almost equal amounts of glucose, mannose, and galactose as well as 2-amino-2-deoxygalactose (GalN), which were identified by GLC of the acetylated alditols. Their D configuration was established by GLC of the acetylated (*S*)-2-octyl glycosides. After hydrolysis under milder conditions (1 M CF_3CO_2H, 100 °C), a branched deoxy sugar derivative **1** also was isolated by reversed-phase HPLC and studied by NMR spectroscopy.

The ^{1}H and ^{13}C NMR spectra and the 2D heteronuclear ^{1}H,^{13}C HSQC spectrum of **1** revealed 9 proton and 8 carbon signals, including those for one quaternary carbon at δ 90.3, one anomeric center at δ_H/δ_C 5.17/105.0, and one group each of CH_3–C, C–CH_2–C, and OCH_2–C (Table 1). The signals were assigned using 2D COSY, TOCSY, ^{1}H,^{13}C HSQC, and HMBC experiments, and three separate

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spin-systems were identified for C-1–C-3, C-5–C-6, and C-1′–C-2′ fragments (Table 1). Altogether these data suggested that **1** is a derivative of a branched octose with branching at C-4 (Sug).

Structure of **1** shown in Chart 1 was established by HMBC and NOESY data. Correlations of H-1 with both C-4 and C-2′ in the HMBC spectrum demonstrated a bicyclic structure with a sugar furanose ring and a 1,2′-anhydro dioxane cycle. It was confirmed by H-3a,b/C-1′, H-2b′/C-1, and H-2b′/C-4 correlations in the HMBC spectrum as well as an H-3a/H-5 correlation in the NOESY spectrum (Chart 1). The configuration of **1** was inferred by correlations of H-2 and H-3a with H-2a′ in the NOESY spectrum (Chart 1) and a relatively large coupling constant $J_{1',2a'}$ 9.7 Hz indicating the axial orientation of H-1′. The last finding was the key for determination of the side chain configuration in Sug. The structure of **1** was confirmed by the molecular mass 213.0736 Da for the $[M+Na]^+$ ion determined by high-resolution ESI MS (calculated molecular mass 213.0733 Da for $C_8H_{14}O_5Na$).

To further confirm the structure and to determine the absolute configuration of Sug the O-polysaccharide was subjected to periodate oxidation followed by borohydride reduction, which converted Sug to a 3,6-dideoxyhexose. Acid hydrolysis of the degraded polysaccharide with 1 M CF_3CO_2H (100 °C) afforded paratose (3,6-dideoxy-D-*ribo*-hexose), which was isolated by reversed-phase HPLC and identified by GLC of the acetylated (*S*)-2-octyl glycoside. Hence, Sug had the D configuration and, combined with structural data of **1**, was 3,6-dideoxy-4-*C*-[(*S*)-1,2-dihydroxyethyl]-D-*xylo*-hexose. To the best of our knowledge, this monosaccharide has not been hitherto found in nature. Two related branched octoses, 3,6-dideoxy-4-*C*-[(*S*)- and (*R*)-1-hydroxyethyl]-D-*xylo*-hexoses (yersinioses A and B, respectively), have been reported as components of a number of bacterial polysaccharides (see Bacterial Carbohydrate Structure Database at http://csdb.glycoscience.ru/bacterial). They differ from Sug in deoxygenation of the terminal carbon (C-2′) of the side chain.

The ^{13}C NMR spectrum of the O-polysaccharide (Fig. 1) showed signals for five anomeric carbons at δ 96.2–106.9, one CH_3–C group (C-6 of Sug) at δ 13.6, one C–CH_2–C group (C-3 of Sug) at δ 32.3, five OCH_2–C groups at δ 62.0–62.8 and 65.6, one nitrogen-bearing carbon (C-2 of GalN) at δ 50.8, other sugar carbons at δ 66.0–81.0, and one *N*-acetyl group at δ 23.5 (CH_3) and 175.9 (CO). The ^1H NMR spectrum of the polysaccharide (Fig. 2) contained signals for five anomeric protons at δ 4.49–5.29, one CH_3–C group (H-6 of Sug) at δ 1.14, one C–CH_2–C group (H-3 of Sug) at δ 1.69 (H-3eq) and 2.03 (H-3ax), other sugar protons at δ 3.46–4.46, and one *N*-acetyl group at δ 2.01. Therefore, the repeating unit of the O-polysaccharide consists of five sugar residues including one residue each of D-Glc, D-Man, D-Gal, D-GalNAc, and Sug.

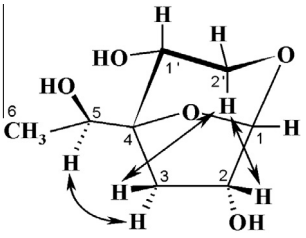

Chart 1. Structure of bicyclic branched monosaccharide **1**. Arrows indicate H,H correlations observed in the NOESY spectrum of **1**.

The ^1H and ^{13}C NMR spectra of the O-polysaccharide were assigned using 2D $^1H,^1H$ COSY, TOCSY, ROESY, $^1H,^{13}C$ HSQC, and HMBC experiments. Spin systems for α-Glc*p*, α-Man*p*, β-Gal*p*, and α-Gal*p*NAc were identified by tracing connectivities starting from H-1 combined with coupling constants and ^1H and ^{13}C NMR chemical shifts data (Table 1). The C-1–C-3 fragment of Sug was recognized too. A relatively large coupling constant $J_{2,3ax}$ ~10 Hz and a relatively small value $J_{1,2}$ ~2 Hz indicated the axial orientation of H-2 and equatorial orientation of H-1; hence, Sug is α-linked. Other signals of Sug, including that for the quaternary carbon (C-4), were assigned by cross-peaks for C-4 (with H-3eq, H-6, and H-1′) and C-5 (with H-1, H-3eq, H-6, and H-1′) in the $^1H,^{13}C$ HMBC spectrum (Fig. 3). The H-1/C-5 correlation at δ 4.82/67.9 demonstrated the 1,5-pyranose form of Sug, and, hence, the C-1′–C-2′ fragment represented the side chain of the branched monosaccharide.

Downfield displacements of the signals for C-2 of Man, C-3 of Gal, C-3 and C-4 of GalNAc to δ 78.1–81.0, as well as C-6 of Glc to δ 65.6, as compared with their positions in the spectra of the corresponding non-substituted monosaccharides,[5] revealed the glycosylation pattern in the repeating unit. Therefore, the O-polysaccharide was branched with a 3,4-disubstituted GalNAc residue at the branching point and Sug as the terminal monosaccharide of a side chain.

In the ROESY spectrum, there were interresidue cross-peaks between the following anomeric protons and protons at the linkage carbons: Man H-1,Gal H-3; Gal H-1,GalNAc H-3; GalNAc H-1,Man H-2; Sug H-1,Glc H-6a; and Glc H-1,GalNAc H-4. These data were in agreement with the positions of substitution of the sugar residues inferred from the ^{13}C NMR chemical shift data and defined their sequence in the repeating unit. The glycosylation pattern and monosaccharide sequence were confirmed by correlations between anomeric protons and linkage carbons revealed by the $^1H,^{13}C$ HMBC spectrum (data not shown).

Table 1
^1H and ^{13}C NMR chemical shifts (δ, ppm) and $^3J_{H,H}$ coupling constants (Hz)

Sugar residue	H-1 *C-1*	H-2 *C-2*	H-3 (eq, ax) *C-3*	H-4 *C-4*	H-5 *C-5*	H-6 (a,b) *C-6*	H-1′ *C-1′*	H-2′ (a,b) *C-2′*
O-polysaccharide								
→3,4)-α-D-Gal*p*NAc-(1→	5.15	4.46	4.11	4.35	4.20	3.81, 3.87		2.01
	101.5	*50.8*	*78.3*	*78.4*	*73.7*	*62.0*	*175.9*	*23.5*
→3)-β-D-Gal*p*-(1→	4.49	3.46	3.69	4.11	3.60	3.74, 3.82		
	106.9	*70.4*	*78.1*	*66.0*	*76.1*	*62.6*		
→2)-α-D-Man*p*-(1→	5.29	4.01	4.06	3.83	3.91	3.80, 3.88		
	96.2	*81.0*	*71.5*	*68.1*	*73.9*	*62.0*		
→6)-α-D-Glc*p*-(1→	5.00	3.54	3.82	3.67	4.32	3.86, 4.07		
	100.7	*73.2*	*74.5*	*70.5*	*72.0*	*65.6*		
α-Sug*p*-(1→	4.82	3.97	1.69, 2.03		4.21	1.14	3.60	3.61, 3.85
	98.2	*66.0*	*32.3*	*75.7*	*67.9*	*13.6*	*75.6*	*62.8*
Monosaccharide ***1***								
	5.17	4.55	1.78, 2.45		4.02	1.33	4.03	3.36, 3.95
	105.0	*74.9*	*35.2*	*90.3*	*69.9*	*18.3*	64.9	65.4
	$J_{1,2}$	$J_{2,3a}$	$J_{2,3b}$	$J_{3a,3b}$	$J_{5,6}$	$J_{1',2a'}$	$J_{1',2b'}$	$J_{2a',2b'}$
	~0	2.2	7.4	14.2	6.8	9.7	6.2	11.3