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Carbohydrate Research 340 (2005) 1419–1423
Carbohydrate RESEARCH

Note

The O-polysaccharide from the lipopolysaccharide of *Providencia stuartii* O44 contains L-quinovose, a 6-deoxy sugar rarely occurring in bacterial polysaccharides

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Received 28 January 2005; received in revised form 22 February 2005; accepted 22 February 2005
Available online 18 March 2005

Abstract—The O-polysaccharide (O-antigen) of *Providencia stuartii* O44:H4 (strain 3768/51) was obtained by mild acid degradation of the lipopolysaccharide and studied by sugar and methylation analyses along with ^{1}H and ^{13}C NMR spectroscopy, including 2D $^{1}H,^{1}H$ COSY, TOCSY, ROESY, and H-detected $^{1}H,^{13}C$ HSQC, and HMQC-TOCSY experiments. The O-polysaccharide was found to have a branched hexasaccharide repeating unit of the following structure:

β-D-Glc*p*A
1
↓
4
[3)-α-L-Fuc*p*-(1→3)-α-D-Glc*p*-(1→4)-α-L-Qui*p*-(1→3)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*NAc-(1→]$_n$

Keywords: *Providencia stuartii*; O-antigen; Lipopolysaccharide; Polysaccharide structure

Gram-negative bacteria of the genus *Providencia* are divided into five species, including *Providencia alcalifaciens*, *Providencia rustigianii*, *Providencia stuartii*, *Providencia heimbachae*, and *Providencia rettgerii*.[1] They are facultative pathogens that under favorable conditions cause enteric diseases, as well as wound and urinary-tract infections. Particularly, *Providencia stuartii* has been recognized as a pathogen with an increasing involvement in urinary tract infections primarily in nursing home patients with long-term urinary catheters in place. These infections are frequently persistent, difficult to treat, and may even result in fatal bacteremia. The serological classification scheme of three *Providencia* species, *P. alcalifaciens*, *P. rustigianii*, and *P. stuartii*, used in serotyping of clinical isolates, is based on the lipopolysaccharide (LPS, O-antigen, endotoxin) and flagella (H-antigens) and includes 63 serogroups.[2] Immunochemical studies of *Providencia* O-antigens aim at the creation of the molecular basis for the serological classification and cross-reactivity of *Providencia* strains and related bacteria, including *Proteus*. Recently, structures of the O-polysaccharides of the LPS of *P. stuartii* serogroups O4, O18, O33, O47, and O49 have been elucidated.[3–7] Now we report on the structure of the O-polysaccharide of *P. stuartii* O44.

A high-molecular-mass polysaccharide, eluted immediately after the void volume on GPC on Sephadex G-50, was isolated by mild acid degradation of the

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doi:10.1016/j.carres.2005.02.020

lipopolysaccharide of *P. stuartii* O44. Sugar analysis obtained by GLC of the acetylated alditols revealed fucose (Fuc), quinovose (Qui), glucose (Glc), 2-aminodeoxyglucose (GlcN), and 2-aminodeoxygalactose (GalN) in the ratios ~0.4:0.6:1:0.7:0.6. In addition, glucuronic acid (GlcA) was identified by anion-exchange chromatography using a sugar analyzer. An enzymatic assay with D-glucose oxidase showed that Glc has the D configuration. The L configuration of the 6-deoxy sugars and the D configuration of the amino sugars and GlcA were determined by GLC of the acetylated (+)-2-octyl glycosides.

GLC–MS of the partially methylated alditol acetates derived from the methylated polysaccharide revealed a 4-substituted 6-deoxyhexose, a 3,4-disubstituted 6-deoxyhexose, a 3-substituted hexose, a 3-substituted hexosamine, and a 4-substituted hexosamine. In addition to these monosaccharides, similar analysis after carboxyl reduction of the methylated polysaccharide showed a 6-substituted hexose, which was evidently derived from a terminal nonreducing GlcA residue. The pyranose form of the 4-substituted monosaccharides was shown by NMR spectroscopic data (see below).

The ^{13}C NMR spectrum of the polysaccharide (Fig. 1) demonstrated a regular structure. It contained anomeric signals for six sugar residues, three of which were separated and resonated at δ 99.6, 100.0, and 105.1 and three others overlapped at δ 100.5. There were also signals for two nitrogen-bearing carbons at δ 50.9 and 54.7 (C-2 of *N*-acetylhexosamines), three unsubstituted $HOCH_2$ groups of hexoses and hexosamines at δ 61.1 (2 C) and 62.0 (data of a DEPT-135 experiment), two methyl groups (C-6 of 6-deoxy sugars) at δ 16.2 and 17.8, one carboxyl group of GlcA at δ 175.3, 22 sugar-ring oxygen-bearing carbons in the region δ 68-84, some of the signals being overlapped, and two *N*-acetyl groups at δ 23.4, 23.6 (both Me), 175.7 and 175.9 (both CO). Accordingly, the ^{1}H NMR spectrum (data not shown) contained signals for six anomeric protons at δ 4.55, 4.86, 5.01, 5.03, 5.24, and 5.27, and two *N*-acetyl groups at δ 2.04 and 2.07. As judged by the absence of signals within δ 84–88 from the ^{13}C NMR spectrum, all sugar residues are in the pyranose form.[8]

The ^{1}H and ^{13}C NMR spectra of the polysaccharide were assigned using ^{1}H,^{1}H COSY, TOCSY, ROESY (Fig. 2), ^{1}H,^{13}C HSQC (Fig. 3), and ^{1}H,^{13}C HMQC-TOCSY experiments. The TOCSY spectrum showed correlation from H-6 to all protons of Qui, from H-1 to H-5 of Fuc, and from H-1 to H-4 for the other sugar residues. The COSY spectrum showed most of correlations between the neighboring protons within each spin system. The spin system of GlcA was identified by the absence of any H-6 signal, and the spin systems of GlcN and GalN were distinguished using the characteristic chemical shifts of C-2 as a nitrogen-bearing carbon, which were determined based on the ^{1}H,^{13}C HSQC data (Fig. 3). Using these data, protons and carbons of all six sugar residues were fully assigned (Table 1).

The small $J_{1,2}$ coupling constant values (<3 Hz, signals not resolved) showed that all residues except GlcA are α-linked, whereas GlcA showing $J_{1,2}$ 7.2 Hz is β-linked. Significant downfield displacements in the ^{13}C NMR spectrum of the signals for α-Fuc*p* C-3 (from δ 70.4 to 75.2), α-Fuc*p* C-4 (from δ 73.0 to 81.5), α-Glc*p*

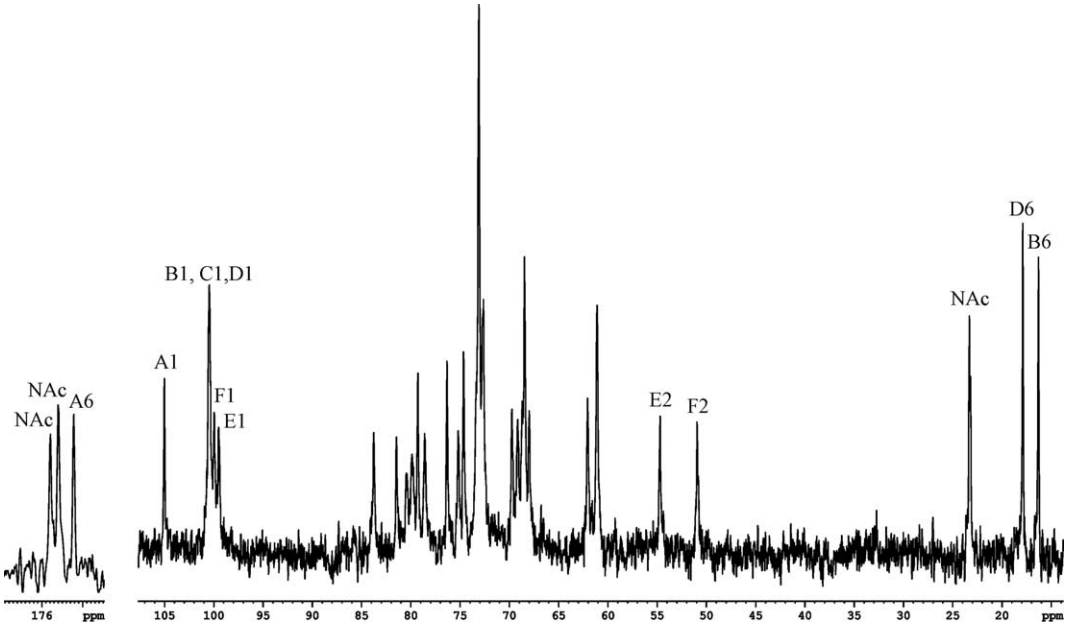

Figure 1. ^{13}C NMR spectrum of the O-polysaccharide from *P. stuartii* O44. Arabic numerals refer to atoms of sugar residues denoted by letters as shown in Table 1.