Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 1116–1121
Carbohydrate RESEARCH

Note

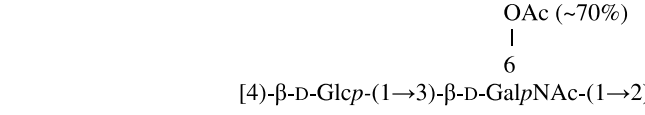
New structure for the O-polysaccharide of *Providencia alcalifaciens* O27 and revised structure for the O-polysaccharide of *Providencia stuartii* O43

Olga G. Ovchinnikova,[a,*] Ivan S. Bushmarinov,[a] Nina A. Kocharova,[a] Filip V. Toukach,[a] Marianna Wykrota,[b] Alexander S. Shashkov,[a] Yuriy A. Knirel[a] and Antoni Rozalski[b]

[a]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation*
[b]*Department of Immunobiology of Bacteria, Institute of Microbiology and Immunology, University of Lodz, PL 90-237 Lodz, Poland*

Received 13 January 2007; received in revised form 9 February 2007; accepted 19 February 2007
Available online 28 February 2007

Abstract—The O-polysaccharide was obtained by mild acid degradation of the lipopolysaccharide from *Providencia alcalifaciens* O27 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, H-detected $^{1}H,^{13}C$ HSQC, and HMBC experiments. It was found that the polysaccharide is built up of linear partially O-acetylated tetrasaccharide repeating units and has the following structure:



where Qui4NFo stands for 4-formamido-4,6-dideoxyglucose (4-formamido-4-deoxyquinovose).

The O-polysaccharide structure of *Providencia stuartii* O43 established earlier was revised with respect to the configuration of the constituent 4-amino-4,6-dideoxyhexose (from Rha4N to Qui4N).

Keywords: *Providencia alcalifaciens*; *Providencia stuartii*; O-Antigen; Lipopolysaccharide; Polysaccharide structure

Gram-negative bacteria of the genus *Providencia* are divided into six species, including *P. alcalifaciens*, *P. rustigianii*, *P. stuartii*, *P. heimbachae*, *P. rettgerii*, and *P. vermicola*.[1,2] They are facultative pathogens that under favorable conditions cause enteric diseases, as well as wound and urinary-tract infections.[2] 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.[3] 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*. At present, 25 *Providencia* O-polysaccharide structures have been established (Refs. 4–14 and references cited in Refs. 4 and 7). In this paper, we report on a new structure of the O-polysaccharide of *P. alcalifaciens* O27 and a revised structure of the O-polysaccharide of *P. stuartii* O43 studied by us earlier.[8]

Mild acid degradation of the LPS of *P. alcalifaciens* O27 gave a high-molecular-mass polysaccharide eluted from Sephadex G-50 immediately after the void volume.

* Corresponding author. Tel.: +7 495 1376148; fax: +7 495 1355328; e-mail: olga.ovchinnikova@gmail.com

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.02.028

Sugar analysis using GLC–MS of the alditol acetates derived after hydrolysis with CF_3CO_2H showed the presence of glucose (Glc), 2-amino-2-deoxygalactose (GalN), and 4-amino-4,6-dideoxyglucose (Qui4N) in ratios ~1:0.6:0.8. GLC–MS of the acetylated methyl glycosides demonstrated the presence of glucuronic acid (GlcA). The D configuration of all monosaccharides was determined by GLC of the acetylated (*S*)-2-octyl glycosides and confirmed by NMR spectroscopy (see below).

Linkage analysis by GLC–MS of the partially methylated alditol acetates derived from the methylated polysaccharide revealed 4-substituted Glc, 3-substituted GalN, and 2-substituted Qui4N. In addition to these monosaccharides, similar analysis after carboxyl-reduction of the methylated polysaccharide showed the presence of 4,6-disubstituted Glc, which was evidently derived from 4-substituted GlcA.

The ^{13}C NMR spectrum of the polysaccharide showed structural heterogeneity, which, most likely, was caused by nonstoichiometric O-acetylation. The ^{13}C JMOD NMR spectrum of the O-deacetylated polysaccharide (OPS) (Fig. 1) showed a regular structure. It contained signals for four sugar residues, including those for four anomeric carbons at δ 99.0, 101.5, 104.8, and 105.7, 14 sugar-ring carbons in the region δ 67–83, one C–CH_3 group at δ 18.0 (minor *E* isomer) and 18.1 (major *Z* isomer), two C–CH_2OH groups at δ 61.9 and 62.6, one C–CO_2H group at δ 176.5, and two nitrogen-bearing carbons at δ 52.8 and 57.0. The spectrum also contained signals for one *N*-acetyl group at δ 176.7 (CO) and δ 23.8 (CH_3) as well as one *N*-formyl group at δ 166.1 and 169.0 (major *Z* and minor *E* isomer, respectively). There was only one signal within the region δ 82–88, at δ 82.5 later assigned to C-2 of Qui4N; hence, all sugar residues in the repeating unit are pyranosidic.[15] The ^1H spectrum of the OPS contained signals for four anomeric protons at δ 4.52, 4.67, 5.32, and 5.69, one C–CH_3 group at δ 1.13 (major) and 1.18 (minor), one *N*-acetyl group at δ 2.01, one *N*-formyl group at 8.18 (major *Z* isomer) and 7.99 (minor *E* isomer) (compare published data[10]), and other protons in the region δ 3.33–4.18.

The ^1H and ^{13}C NMR spectra of the OPS were assigned using $^1H,^1H$ COSY, TOCSY, ROESY, $^1H,^{13}C$ HSQC (Fig. 2), $^1H,^{13}C$ HSQC–TOCSY, and $^1H,^{13}C$ HMBC (Fig. 3) experiments (Table 1). The spin system of Qui4N was distinguished by correlations of each of H-1 and H-6 with all other protons of this residue in the TOCSY spectrum, and the H-2 to H-5 signals were assigned using the COSY spectrum. The ^{13}C NMR signals were assigned by H/C correlations in the HSQC spectrum, H-6/C-4 and H-6/C-5 correlations in the HMBC spectrum, and H-6/C-2 and H-6/C-3 correlations in the HSQC–TOCSY spectrum. A difficulty in the assignment of the C-2 and C-3 signals owing to an overlap of the H-2 and H-3 signals at δ 3.71 was overcome by taking into account the methylation analysis data: the lower field resonance at δ 82.5 was assigned to C-2 of the 2-substituted Qui*p*4N and, accordingly, the other signal at δ 70.6 to C-3. A cross-peak at δ 8.18/57.0 between the *N*-formyl group and C-4 of Qui4N in the HMBC spectrum demonstrated formylation at N-4. A small $J_{1,2}$ coupling constant <3 Hz and chemical shifts of H-1 (δ 5.69) and C-5 (δ 68.3, compare published data[16]) indicated that Qui*p*4NFo is α-linked.

The assignment of the ^1H NMR signals for another monosaccharide was performed using the COSY spectrum, which demonstrated correlations between all neighboring protons from H-1 to H-5, and were confirmed by the TOCSY experiment. A triplet form of the H-3 signal at δ 3.96 ($J_{2,3} \approx J_{3,4} \sim 9$ Hz) revealed the *gluco*-configuration of this residue. A doublet splitting of the H-5 signal and a H-5/C-6 cross-peak at δ 4.07/176.5 in the HMBC spectrum indicated that this residue is GlcA. A small $J_{1,2}$ coupling constant <3 Hz and chemical shift of the H-1 (δ 5.32) showed its α configuration.

The ^{13}C NMR signals of Glc*p* were distinguished by the HSQC–TOCSY experiment showing correlations

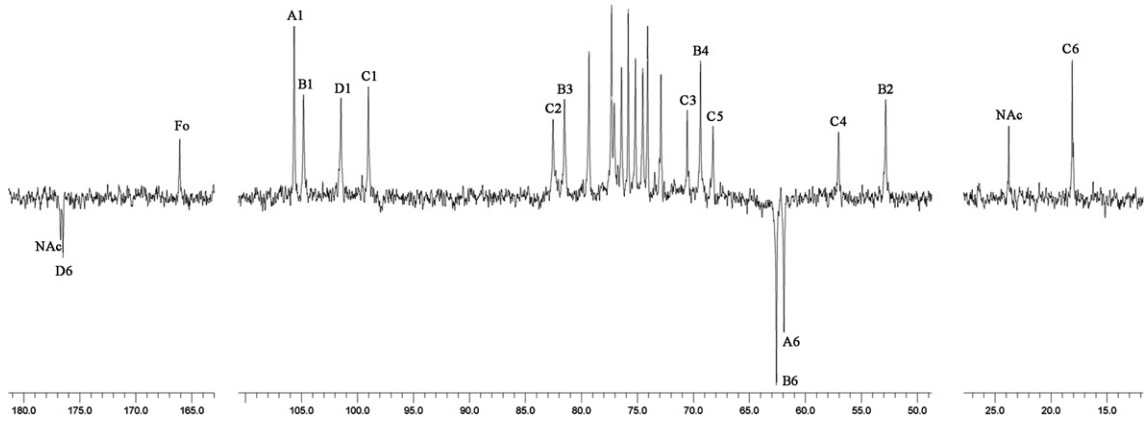


Figure 1. JMOD spectrum of the O-deacetylated polysaccharide from *P. alcalifaciens* O27. Arabic numerals refer to carbons in sugar residues denoted by capital letters as shown in Table 1.