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Note

Structure and genetics of the O-antigen of *Escherichia coli* O169 related to the O-antigen of *Shigella boydii* type 6Andrei V. Perepelov^{a,*}, Alexander S. Shashkov^a, Xi Guo^b, Andrei V. Filatov^a, Andrej Weintraub^c, Göran Widmalm^d, Yuriy A. Knirel^a^a N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation^b TEDA School of Biological Sciences and Biotechnology, Nankai University, TEDA, 300457 Tianjin, China^c Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska Institute, Karolinska University Hospital, S-141 86 Stockholm, Sweden^d Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

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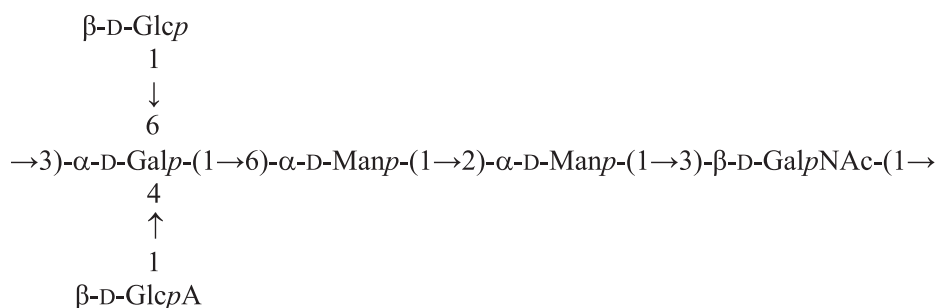
O-Antigen

Bacterial polysaccharide structure

O-Antigen gene cluster

ABSTRACT

The O-polysaccharide (O-antigen) of *Escherichia coli* O169 was studied by sugar analysis along with 1D and 2D ¹H and ¹³C NMR spectroscopy. The following structure of the branched hexasaccharide repeating unit was established:



The O-polysaccharide of *E. coli* O169 differs from that of *Shigella boydii* type 6 only in the presence of a side-chain glucose residue. A comparison of the O-antigen biosynthesis gene clusters between the *galF* to *gnd* genes in the genomes of the two bacteria revealed their close relationship. The glycosyltransferase gene responsible for the formation of the $\beta\text{-D-Glcp}\text{-(1}\rightarrow 6\text{)-}\alpha\text{-D-Galp}$ linkage in the O-antigen was identified in the gene cluster.

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Escherichia coli is a clonal species, including both commensals and pathogens. The bacteria are normally identified by a combination of their O- and H- (and sometimes K-) antigens. Variations in types of sugars present, their arrangement and linkages make the O-antigen or O-polysaccharide (OPS) the most variable constituent on the cell surface and provide the basis for serotyping of the bacteria.¹ By now about 180 O-serotypes of *E. coli* have been

recognized. Genes for O-antigen synthesis are normally located on the chromosome as an O-antigen gene cluster, and genetic variations in the cluster are the major basis for the diversity of the O-antigen forms. OPS structures for most serogroups have been elucidated (see *E. coli* O-antigen database at <http://www.casper.org.su.se/ECODAB/>)² but several still remain to be established.

Serogroup O169 belongs to enterotoxigenic *E. coli* (ETEC) group, which has been recognized as a common cause of foodborne outbreaks.³ *E. coli* O169 has notably become one of the most prevalent ETEC pathogens associated with foodborne outbreaks in many countries including the Republic of Korea,⁴ Japan,⁵ and the USA.⁶ In

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The ^1H and ^{13}C NMR spectra of the OPS were assigned using 2D COSY, TOCSY, ^1H , ^{13}C HSQC, HMBC, and HSQC-TOCSY experiments (Table 1). Based on intra-residue ^1H , ^1H and ^1H , ^{13}C correlations and coupling constant values estimated from the 2D NMR spectra, six spin systems, including two systems each for *gluco*- (Glc and GlcA, denoted as units **A** and **B**), *galacto*- (Gal and GalNAc, units **C** and **F**), and *manno*-configured sugars (two Man residues, units **D** and **E**)

The OPS was subjected to a Smith degradation and the products were fractionated by GPC. The main oligosaccharide product was studied by 1D and 2D NMR spectroscopy, including the full assignment of the ^1H and ^{13}C NMR signals (Fig. 1, Table 1). As a result, the following structure was elucidated:

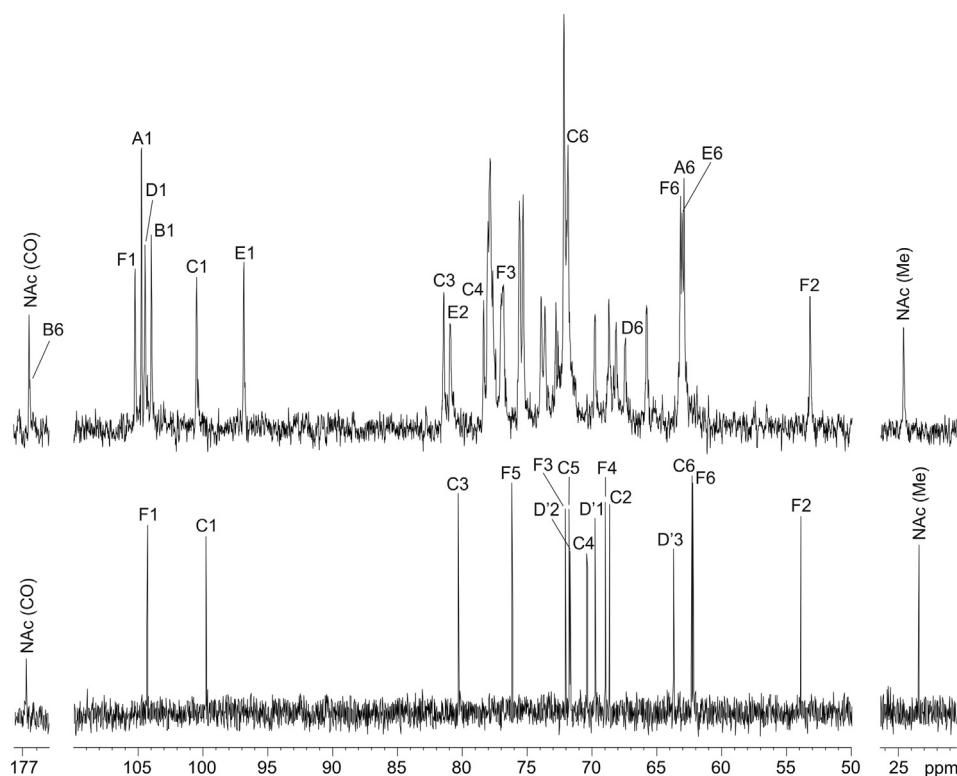
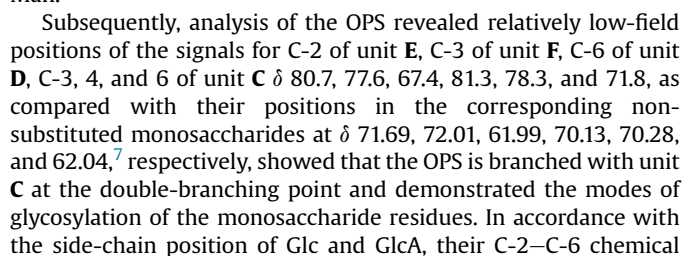


Fig. 1. ^{13}C NMR spectra of the OPS (top) and oligosaccharide derived by Smith degradation of the OPS (bottom) from *E. coli* O169. Arabic numerals refer to carbons in sugar residues denoted by letters as shown in Table 1 and Chart 1.

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