ELSEVIER

Carbohydrate Research 291 (1996) 209–216

CARBOHYDRATE RESEARCH

Note

Structure determination of the O-antigen polysaccharide from the enteroinvasive *Escherichia coli* (EIEC) O143 by component analysis and NMR spectroscopy

Clas Landersjö [a], Andrej Weintraub [b], Göran Widmalm [a,*]

[a] *Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*

[b] *Karolinska Institute, Department of Immunology, Microbiology, Pathology and Infectious Diseases, Division of Clinical Bacteriology, Huddinge University Hospital, S-141 86 Huddinge, Sweden*

Received 9 May 1996; accepted 7 June 1996

Keywords: *Escherichia coli*; *Shigella boydii*; Lipopolysaccharide; O-Antigen; 2-Amino-1,3-propanediol

Certain *Escherichia coli* serogroups may cause an illness that resembles bacillary dysentery. These strains have been classified as enteroinvasive *E. coli* (EIEC) and cause diarrhoea. The *E. coli* O143 belongs to this group [1]. Strong cross-reactivity between *Shigella boydii* type 8 and *E. coli* O143 has been reported [2]. In order to investigate the possible basis of this cross-reactivity and the structure of the O-antigenic polysaccharide from the pathogenic *E. coli* O143, we have performed structural studies which we report here.

The lipopolysaccharide from *E. coli* O143 was delipidated under mild acidic conditions and purified by gel permeation chromatography to give a polysaccharide. Component analysis of the polysaccharide showed 2-amino-1,3-propanediol, D-glucosamine, D-galactosamine, D-glucuronic acid, and D-galacturonic acid. Sugar components considered to derive from the core of the LPS were also found. The ^{1}H and ^{13}C NMR spectra of the polysaccharide showed the presence of *O*-acetyl groups, inter alia, at δ_C 20.9 and 21.5. The native O-antigen polysaccharide was subsequently *O*-deacetylated by dilute NaOH (aq). The ^{1}H NMR spectrum of the *O*-deacetylated polysaccharide showed the presence of four signals in the anomeric region (Fig. 1). The presence of four sugars

* Corresponding author.

PII S0008-6215(96)00168-1

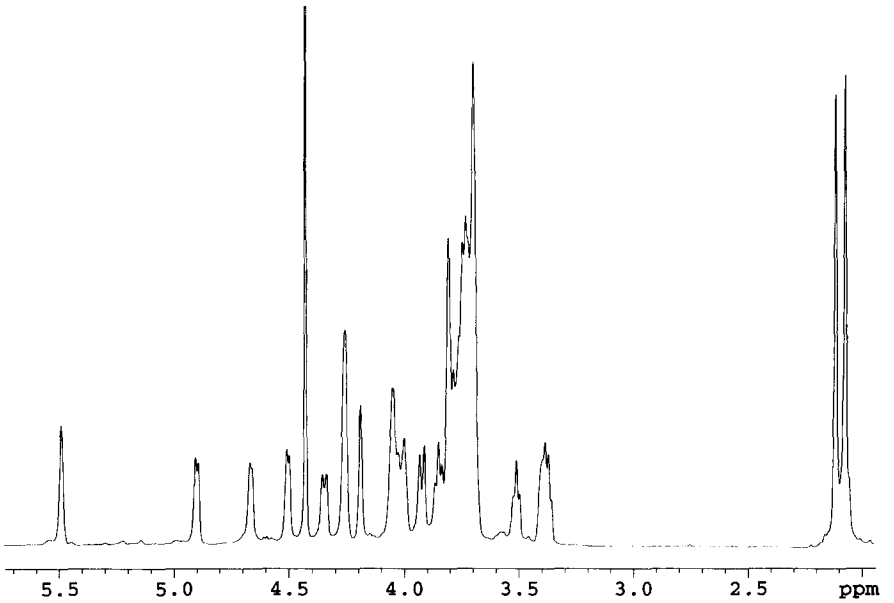


Fig. 1. The ^{1}H NMR spectrum at 600 MHz of the *O*-deacetylated *E. coli* O143 O-antigen polysaccharide.

in the repeating unit was corroborated by the ^{13}C NMR spectrum where signals were observed at δ_C 97.6, 101.8, 103.4, and 103.6. The amino sugars are *N*-acetylated as signals were observed at δ_H 2.07 (3 H), 2.11 (3 H), δ_C 23.1, 23.4, 174.6, and 175.4. The ^{13}C NMR spectrum showed in total 31 signals, in agreement with equimolar amounts of the five components described above. Values of $^{1}J_{C,H}$ and $^{3}J_{H,H}$ for the anomeric protons and ^{13}C NMR chemical shifts show that the sugar residues in the polysaccharide are pyranoid. The ^{13}C NMR spectrum also showed signals from an amide-linked carbonyl group (170.9 ppm), a carboxylic acid group (175.6 ppm), and three nitrogen-carrying carbons (48.9, 53.2, and 55.5 ppm), and four signals deriving from hydroxymethyl groups (61.3, 61.4, 61.6, and 61.8 ppm).

The ^{1}H and ^{13}C NMR spectra of the polysaccharide were completely assigned using one-dimensional (1D) ^{1}H,^{1}H TOCSY experiments and 2D ^{1}H,^{1}H COSY, relayed ^{1}H,^{1}H COSY, double-relayed ^{1}H,^{1}H COSY, ^{1}H,^{1}H NOESY, ^{1}H,^{13}C gHSQC, and ^{1}H,^{13}C HMBC experiments. The assignments of NMR signals to the components of the polysaccharide are based on $^{3}J_{H,H}$ couplings, ^{1}H and ^{13}C chemical shifts, and chemical shift displacements due to glycosylation, and are given in Table 1, where the sugar residues are labelled from **A**–**D** with respect to the decreasing chemical shift of their anomeric protons. From $J_{H\text{-}1,H\text{-}2}$ couplings residue **A** is α-linked and residues **B**–**D** are β-linked. Residues **A** and **B** could be assigned to amino sugars because of the chemical shifts of their C-2 signals. From $^{3}J_{H,H}$ couplings and chemical shifts **A** is the D-Gal*p*NAc residue and **B** is the D-Glc*p*NAc residue. Both are 3-substituted since large downfield ^{13}C chemical shift displacements ($\Delta\delta_C \sim 10$ ppm) due to glycosylation are observed. In a similar manner **C** could be assigned to a β-linked 2-substituted Gal*p*A

Table 1
Chemical shift (δ, ppm) of the signals in the ^{1}H and ^{13}C NMR spectra [a] of the *O*-deacetylated *E. coli* O143 O-antigen polysaccharide

Sugar residue	$^{1}H/^{13}C$										
	1	2	3	4	5	6	NAc	CO	1′	2′	3′
→ 3)-α-D-Gal*p*NAc-(1 →	5.49 (3.3)	4.35	4.04	4.26	4.00	3.73, 3.76	2.11				
A	97.6 [180]	48.9	78.6	69.4	71.0	61.6	23.4	174.6			
→ 3)-β-D-Glc*p*NAc-(1 →	4.90 (8.5)	3.85	3.75	3.51	3.40	3.73,3.92	2.07				
B	101.8 [166]	55.5	83.5	69.6	76.2	61.8	23.1	175.4			
→ 2)-β-D-Gal*p*A6NHCH$(CH_2OH)_2$-(1 →	4.66 (7.3)	3.77	3.79	4.19	4.25				~ 3.7	4.05	~ 3.7
C	103.4 [165]	77.4	74.6	70.1	75.3	170.9			61.3	53.2	61.4
→ 4)-β-D-Glc*p*A-(1 →	4.50 (7.9)	3.38	3.71	3.81	3.81						
D	103.6 [163]	74.0	77.2	76.1	77.4	175.6					

[a] $J_{H\text{-}1,H\text{-}2}$ values are given in Hz in parentheses and $J_{H\text{-}1,C\text{-}1}$ values in Hz in square brackets.

Table 2
Intra-residue and inter-glycosidic correlations from the anomeric atoms observed in the HMBC spectrum of the *O*-deacetylated *E. coli* O143 O-antigen polysaccharide

Sugar residue	Anomeric atom		Connectivity to		Residue, atom
	δ_H	δ_C	δ_C	δ_H	
→3)-α-D-Gal*p*NAc-(1→	5.49		78.6		**A**, C-3
A			76.1		**D**, C-4
			71.0		**A**, C-5
		97.6		3.81	**D**, H-4
→3)-β-D-Glc*p*NAc-(1→	4.90		77.4		**C**, C-2
B			76.2		**B**, C-5
		101.8		3.85	**B**, H-2
				3.77	**C**, H-2
→2)-β-D-Gal*p*A6NHCH$(CH_2OH)_2$-(1→	4.66		78.6		**A**, C-3
C		103.4		4.25	**C**, H-5
				4.04	**A**, H-3
				3.77	**C**, H-2
→4)-β-D-Glc*p*A-(1→	4.50		83.5		**B**, C-3
D			77.4		**D**, C-5
			74.0		**D**, C-2
		103.6		3.38	**D**, H-2
				3.75	**B**, H-3

residue and **D** to a β-linked 4-substituted D-Glc*p*A residue. The 2-amino-1,3-propanediol moiety is amide-linked to C-6 of the galacturonic acid residue **C**, as is evident from correlations in the HMBC spectrum from H-2′, δ_H 4.05, to C-6, δ_C 170.9. Heteronuclear correlations within the 2-amino-1,3-propanediol moiety were also observed from C-2′, δ_C 53.2, to hydroxymethyl protons, $\delta_H \sim 3.7$, and from C-1′ and C-3′, δ_C 61.3 and 61.4, to H-3′ and H-1′ hydroxymethyl protons, respectively. It can be noted that the signals for H-4 and H-5, δ_H 3.81, in residue **D** overlap extensively.

The sequence of sugar residues in the polysaccharide was determined by $^1H,^1H$ NOESY and $^1H,^{13}C$ HMBC experiments. In the spectra from the ^1H-detected HMBC experiment one can observe both intra- and inter-residue correlations from the anomeric protons as well as the anomeric carbons (Table 2). The 3-substituted 2-acetamido-2-deoxygalactopyranose residue, **A**, shows a three-bond correlation from the anomeric proton over the glycosidic linkage to C-4 of the 4-substituted glucuronic acid residue, **D**. This fact and the long-range correlation from C-1 in **A** to H-4 in **D** demonstrate structural element **1**:

→3)-α-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→

A **D**

1

Table 3
Intra-residue and inter-glycosidic NOEs from the anomeric protons of the *O*-deacetylated *E. coli* O143 O-antigen polysaccharide

Sugar residue	Anomeric proton	NOE to proton	Residue, atom
	δ_H	δ_H	
→ 3)-α-D-Gal*p*NAc-(1 →	5.49	4.35	**A**, H-2
A		3.81	**D**, H-4
→ 3)-β-D-Glc*p*NAc-(1 →	4.90	~ 3.76	**C**, H-2/**B**, H-3
B		3.40	**B**, H-5
→ 2)-β-D-Gal*p*A6NHCH(CH$_2$OH)$_2$-(1 →	4.66	4.25	**C**, H-5
C		4.04	**A**, H-3
		3.79	**C**, H-3
→ 4)-β-D-Glc*p*A-(1 →	4.50	3.81–3.71	**D**, H-5/**B**, H-3/**D**, H-3
D			

One inter-residue NOE from the NOESY experiment (Table 3), between H-1 in residue **A** and H-4 in residue **D**, confirms structure **1** above.

The 4-substituted glucuronic acid residue, **D**, is linked to the 3-substituted 2-acetamido-2-deoxyglucopyranose residue, **B**, as is evident from three-bond correlations from H-1 and C-1 in **D** to C-3 and H-3 in **B**, respectively, as well as an inter-residue NOE between H-1 in **D** and H-3 in **B**, giving partial structure **2**:

→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→

D **B**

2

Inter-residue correlations from the HMBC spectrum between H-1 in the 3-substituted 2-acetamido-2-deoxyglucopyranose residue, **B**, and C-2 in the 2-substituted galacturonic acid residue, **C**, and between C-1 and H-2 of the same residues define, together with an observed NOE between H-1 in **B** and H-2 in **C**, structure element **3**:

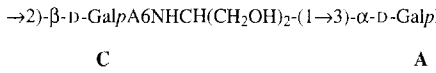
→3)-β-D-Glc*p*NAc-(1→2)-β-D-Gal*p*A6NHCH(CH$_2$OH)$_2$-(1→

B **C**

3

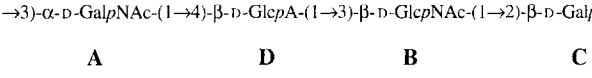
The 2-substituted galacturonic acid residue, **C**, shows inter-residue three-bond correlations from its H-1 and C-1 to C-3 and H-3, respectively, in **A**, that is, the 3-substituted

2-acetamido-2-deoxygalactopyranose residue. An NOE between H-1 in **C** and H-3 in **A** was also observed. Structure element **4** is thus defined as follows:



4

Thus, it is concluded that the structure of the *O*-deacetylated O-antigen polysaccharide from *E. coli* O143 is composed of tetrasaccharide repeating units having structure **5**:



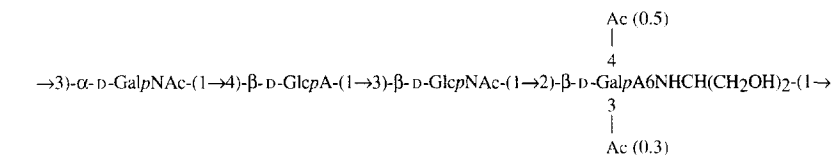
5

2-Amino-1,3-propanediol amide-linked to a D-galacturonic acid residue also occurs in the O-antigen polysaccharide from *S. boydii* serotype 8. By comparison of the above structure **5** with that of the O-antigen polysaccharide of *S. boydii* serotype 8 [3] it is concluded that the O-antigen polysaccharides from *E. coli* O143 and *S. boydii* serotype 8 have the same polysaccharide backbone. The *S. boydii* serotype 8 O-antigen polysaccharide was also shown to carry *O*-acetyl groups on positions 3 and 4 of the galacturonic acid residue.

The ^{13}C NMR spectrum of the native O143 polysaccharide showed the presence of *O*-acetyl groups, with signals at δ_C 20.9 and 21.5 as well as a signal at δ_C 67.5. Chemical shift displacements upon *O*-acetyl substitution of sugars have been investigated [4]. These were used for the determination of the *O*-acetyl substitution pattern of the native O143 polysaccharide. The signal at δ_C 67.5 is assigned to C-4 of the galacturonic acid residue upon *O*-acetyl substitution of position 3 of the same residue. In agreement with this a signal was observed in the ^1H NMR spectrum at δ_H 4.99 (0.3 H) assigned to H-3 as well as a signal at δ_H 4.36 (not integrated) assigned to H-4. Thus, the galacturonic acid residue carries 0.3 mole *O*-acetyl group per repeating unit at position 3; *O*-acetyl substitution also occurs at position 4 of the same residue, since signals occur, inter alia, at δ_H 5.52 (0.5 H) assigned to H-4 and at δ_H 4.40 (0.5 H) assigned to H-5. A signal occurs at δ_H 4.19 (0.2 H) for the non-substituted form and is assigned to H-4 of the galacturonic acid residue (cf. Table 1) which corroborates the above assignments. Thus, the galacturonic acid residue carries 0.5 mole *O*-acetyl group per repeating unit at position 4. The ^1H NMR signals in the region of acetyl groups are in agreement with the above data and occur at δ_H 2.28 (0.9 H) for 3-OAc, 2.15 (1.5 H) for 4-OAc, 2.11 (3.0 H) for NAc of galactosamine, 2.07 (2.1 H) for NAc of glucosamine being non-substituted or having a 4-OAc group at the galacturonic acid residue, and 2.00 (0.9 H) for NAc of glucosamine having a 3-OAc group at the galacturonic acid residue.

The signals at δ_C 21.5 and 20.9 could consequently be assigned to 3-OAc and 4-OAc, respectively.

The structure of the O-antigen polysaccharide from *E. coli* O143 is composed of tetrasaccharide repeating units containing 0.8 mole *O*-acetyl group per repeating unit, located on the galacturonic acid residue. The complete structure of the repeating unit of the O-antigen polysaccharide is given by structure **6**:



6

The strong cross-reactivity between *S. boydii* type 8 and *E. coli* O143 can consequently be explained be their identical O-antigen polysaccharides.

1. Experimental

General methods.—Concentrations were performed under diminished pressure at < 40 °C or under a stream of N_2 or air. For GLC a Hewlett–Packard 5890A instrument fitted with a flame-ionisation detector was used with HP-5 and DB225 capillary columns. GLC–MS was performed on a Hewlett–Packard 5890-5970 instrument equipped with an HP-5-MS capillary column.

Component analysis.—Hydrolysis of polysaccharide material was performed with 2 M CF_3CO_2H at 120 °C for 2 h. The sugars were then converted into alditol acetates and analysed by GLC and GLC–MS (EI). Determination of the absolute configurations of the sugars was performed essentially as described by Leontein et al. [5] by GLC of their glycosides, using optically active 2-butanol [6].

NMR spectroscopy.—NMR spectra of solutions in D_2O were recorded at 70 °C for the native polysaccharide and at 62 °C for the *O*-deacetylated polysaccharide on JEOL GSX-270, Varian Unity+ 400, and Varian Unity+ 600 spectrometers. Chemical shifts are reported in ppm relative to sodium 4,4-dimethyl-4-sila(2,2,3,3-$^2H_4$)pentanoate (δ_H 0.00) and acetone (δ_C 31.00) as internal references. Chemical shifts were obtained from 1D spectra when possible. Assignments were obtained from 1D proton–proton TOCSY experiments [7] with mixing times of 30, 70, 100, and 140 ms, using a selective E-BURP-2 pulse [8] of duration 115 ms, 2D proton–proton-correlated spectroscopy (COSY), relayed COSY, double-relayed COSY, and gradient-enhanced ^{13}C-decoupled inverse ^1H-detected $^1H,^{13}C$ heteronuclear single quantum coherence (HSQC) experiments [9]. Relayed COSY spectra were performed using a delay time of 30 or 60 ms. For sequential information, two-dimensional nuclear Overhauser effect (NOESY) exper-

iments, using mixing times of 150 and 350 ms, and a heteronuclear multiple-bond connectivity (HMBC) [10] experiment with a delay time of 50 ms were performed.

Bacterial strain.—E. coli O143:K$^-$:H$^-$ (CCUG 11443) was obtained from the Culture Collection, University of Göteborg, Sweden.

Isolation and purification of the O-polysaccharide.—E. coli O143 bacteria were grown in TY medium (30 L) in a fermentor (Belach, Sweden) at 37 °C and constant pH (7.1). Bacteria were killed by the addition of formaldehyde (1% final concentration) and harvested by centrifugation. Lipopolysaccharide (LPS) was extracted by the hot phenol–water method [11]. The LPS was treated with aq 1% AcOH at 100 °C for 1 h. Liberated lipid A was centrifuged off, and the supernatant solution was neutralised and subsequently lyophilised. The product was further purified by column chromatography on a column (2.6 × 90 cm) of Bio-Gel P-2. *O*-Deacetylation of the polysaccharide was performed using 0.1 M NaOH (aq) for 16 h at room temperature.

Acknowledgements

This work was supported by grants from the Swedish Natural Science Research Council and the Swedish Agency for Research Cooperation with Developing Countries (SAREC).

References

[1] R.J. Gross and B. Rowe, *J. Hyg.*, 95 (1985) 531–550.
[2] F. Ørskov and I. Ørskov, *Methods Microbiol.*, 14 (1984) 43–112.
[3] V.L. L'vov, N.V. Tokhtamysheva, A.S. Shashkov, B.A. Dmitriev, and N.K. Kochetkov, *Bioorg. Khim.*, 9 (1983) 60–73.
[4] P.-E. Jansson, L. Kenne, and E. Schweda, *J. Chem. Soc., Perkin Trans. 1*, (1987) 377–383.
[5] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res*, 62 (1978) 359–362.
[6] G.J. Gerwig, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 77 (1979) 1–7.
[7] H. Kessler, H. Oschkinat, and C. Griesinger, *J. Magn. Reson.*, 70 (1986) 106–133.
[8] H. Geen and R. Freeman, *J. Magn. Reson.*, 93 (1991) 93–141.
[9] W. Willker, D. Leibfritz, R. Kerssebaum, and W. Bermel, *Magn. Reson. Chem.*, 31 (1993) 287–292.
[10] M.F. Summers and A. Bax, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.
[11] O. Westphal, O. Lüderlitz, and F. Bister, *Z. Naturforsch.*, 7 (1952) 148–155.