Carbohydrate Research, 176 (1988) 231–236
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STRUCTURAL STUDIES OF THE *Escherichia coli* O-149 O-ANTIGEN POLYSACCHARIDE

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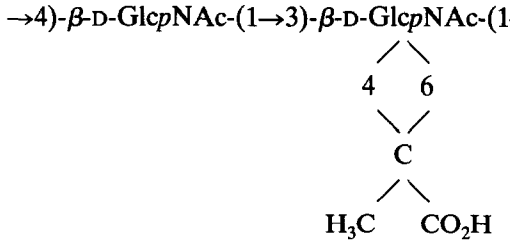
(Received October 10th, 1987; accepted for publication, November 12th, 1987)

ABSTRACT

The structure of the O-antigen polysaccharide from *Escherichia coli* O-149 has been investigated; methylation analysis, partial hydrolysis with acid, and n.m.r. spectroscopy were the principal methods used. It is concluded that the polysaccharide is composed of trisaccharide repeating-units having the following structure.



The absolute configuration at the acetalic carbon atom of the pyruvic acid residue is *S*.

INTRODUCTION

Neonatal piglet diarrhoea is often caused by *Escherichia coli* O-149[1], and strains belonging to this O-serotype also colonise the intestine of man[2]. These strains often elaborate the K-88 fimbriae. We now report structural studies of the *E. coli* O-149 O-polysaccharide, isolated from a strain obtained from piglets with diarrhoea.

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0008-6215/88/$ 03.50

RESULTS AND DISCUSSION

The lipopolysaccharide (LPS) was isolated from *E. coli* O-149, strain 853-76 and 512-N, as previously described[3]. Both LPS were investigated and gave similar results. The polysaccharide (PS) is generally prepared from the LPS by treatment with aqueous acetic acid at 100°, followed by conventional work-up. When this procedure was applied, a product was obtained which gave complicated ^{1}H- and ^{13}C-n.m.r. spectra, containing, *inter alia,* signals at δ_H 1.50 and δ_C 25.4. The intensities of these signals, relative to those given by *N*-acetyl groups and the methyl groups of 6-deoxyhexose residues, decreased with increasing time of hydrolysis. The signals are typical for pyruvic acid residues of 4,6-*O*-(carboxyethylidene)glycopyranosides in which the methyl group occupies an equatorial position[4]. The presence of pyruvic acid in the hydrolysate of the LPS was also demonstrated by the procedure devised by Nimmich[5].

A PS that was free from pyruvic acid residues was prepared by extending the hydrolysis time of the LPS. A sample of partially delipidated LPS was also prepared by treatment of the LPS with aqueous sodium hydroxide. This material gave several weak extra resonances in the anomeric region of the n.m.r. spectra and prolonged treatment increased their intensity. Sugar analysis of the PS gave L-rhamnose, glucose, galactose, a heptose, and 2-amino-2-deoxy-D-glucose in the relative proportions 37:9:4:trace:50. N.m.r. evidence, discussed below, demonstrates that the amino sugars were *N*-acetylated. In this analysis, hydrolysis with acid was preceded by solvolysis with liquid hydrogen fluoride, in order to make sure of complete hydrolysis of the 2-acetamido-2-deoxy-D-glucosidic linkages.

The ^{1}H-n.m.r. spectrum of the completely depyruvylated PS contained, *inter alia,* signals for anomeric protons at δ 4.85 (b, 1 H), 4.72 ($J_{1,2}$ ~7 Hz, 1 H), and 4.62 ($J_{1,2}$ ~7 Hz, 1 H), for *N*-acetyl groups at δ 2.02 (s, 6 H), and for H-6 of L-rhamnose residues at δ 1.30 ($J_{1,2}$ 5.0 Hz, 3 H). The corresponding signals for the partially delipidated LPS occurred at δ 4.82 (2 H), 4.75 (1 H), 2.02 (6 H), and 1.30 (9 H); a signal for the methyl group of the pyruvic acid residue occurred at δ 1.50 (s, 3 H). The agreement between the two spectra is good, except for the large signal at δ 1.30 in the spectrum of the partially delipidated LPS. The reason for this discrepancy is not understood. The ^{13}C-n.m.r. spectrum of the depyruvylated PS contained, *inter alia,* signals for anomeric carbons at δ 103.2, 101.8, and 101.2, for carbons linked to nitrogen at δ 56.8 and 55.6, for methyl of *N*-acetyl groups at δ 23.3 and 23.2, and for C-6 of L-rhamnose residues at δ 17.5. The corresponding signals in the partially delipidated LPS occurred at δ 103.2, 102.2, 101.3, 56.8, 55.6, 23.3, 23.2, and 17.5, together with signals given by the pyruvic acid residue at δ 101.3 and 25.4.

Methylation analysis of the samples without and with pyruvic acid gave the sugars listed in Table I, columns A and B. The results, in conjunction with the n.m.r. results discussed above, demonstrate that the PS is composed of trisaccharide repeating-units containing two residues of 2-acetamido-2-deoxy-β-D-

TABLE I

METHYLATION ANALYSIS OF THE DEPYRUVYLATED PS (A) AND THE PARTIALLY DELIPIDATED LPS (B) FROM *E. coli* O-149

Methylated sugar[a]	T[b]	*Detector resp. (%)*	
		A	B
2,4-Rha	0.93	33	51
2,3,6-GlcNAc	2.38	39	26
2,4,6-GlcNAc	2.56	28	
2-GlcNAc	3.18		23

[a]2,4-Rha = 2,4-di-*O*-methyl-L-rhamnose, *etc.* [b]Retention time of the corresponding alditol acetate under conditions described in the Experimental.

glucopyranose, linked through O-3 and O-4, respectively, and one residue of L-rhamnopyranose linked through O-3. Pyruvic acid is linked as an acetal, with the *S* configuration[4], to the 4- and 6-positions of the 3-linked 2-acetamido-2-deoxy-D-glucopyranosyl residue. The anomeric nature of the L-rhamnopyranosyl residue was still undetermined. The chemical shift of H-5 in this residue in the depyruvylated PS was found to be δ 3.39 by using a COSY-spectrum. The corresponding values for α- and β-L-rhamnopyranose are δ 3.86 and 3.39, respectively. The H-5 resonances in α-L-rhamnopyranosyl residues may appear considerably upfield when H-5 is close to a proton in the opposing ring[6,7]. Assuming that the L-rhamnopyranosyl residue is α-linked, no such interactions are indicated, independent of whether it is linked to O-3 or to O-4 of a 2-acetamido-2-deoxy-D-glucopyranosyl residue. Thus, it is concluded that the L-rhamnopyranosyl residue in the PS has the β configuration.

In order to distinguish between the two possible trisaccharide repeating-units, **1** and **2**, of the depyruvylated PS, the PS was subjected to partial hydrolysis, and the products were reduced with sodium borodeuteride, methylated, and analysed

$\rightarrow$3)-β-D-Glc*p*NAc-(1$\rightarrow$4)-β-D-Glc*p*NAc-(1$\rightarrow$3)-β-L-Rha*p*-(1$\rightarrow$

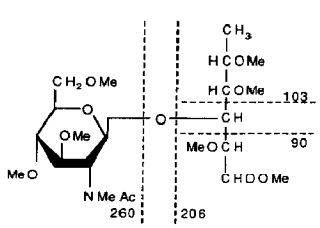
1

$\rightarrow$4)-β-D-Glc*p*NAc-(1$\rightarrow$3)-β-D-Glc*p*NAc-(1$\rightarrow$3)-β-L-Rha*p*-(1$\rightarrow$

2

by g.l.c.–m.s. The disaccharide-alditols **3**, **4**, and **5** were obtained, and identified from their mass spectra (the origins of some pertinent fragments are indicated in the formulas).

From the combined results, it is concluded that the *E. coli* O-149 O-polysaccharide is composed of trisaccharide repeating-units having the structure **6**.



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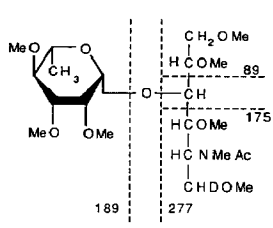

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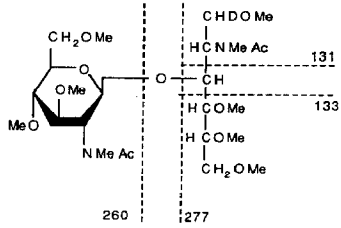

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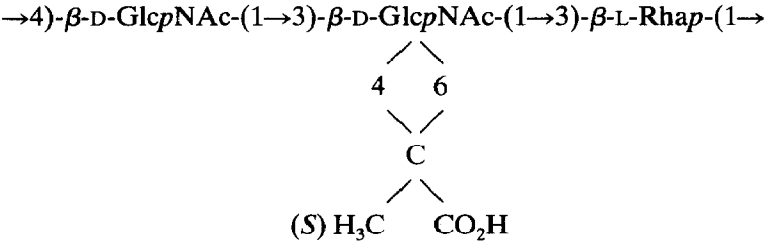
Pyruvic acetals are common in capsular polysaccharides from Gram-positive and Gram-negative bacteria but have only been found once before in a lipopolysaccharide[8], namely, that elaborated by *Shigella dysenterieae* type 9.



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EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at <40° (bath) or at room temperature by flushing with air. For g.l.c., a Hewlett-Packard 5830A instrument fitted with a flame-ionisation detector was used. Separations of alditol acetates and of partially methylated alditol acetates were performed on an SE 54 fused-silica capillary column, using a temperature programme 210 (3 min) →250° at 2°/min and 150 (2 min) →220° at 2°/min, respectively. G.l.c.–m.s. was performed on a Hewlett-Packard 5970 instrument. Hydrolyses were performed by solvolysis with anhydrous hydrogen fluoride for 3 h at room temperature followed by hydrolysis with 2M trifluoroacetic acid as earlier described[9,10].

Absolute configurations were determined by the method of Gerwig *et al.*[11]. Methylation analyses were performed as previously described[12]. Methylated products were recovered by reversed-phase chromatography on Sep-Pak C_{18} cartridges[13]. The sample was diluted with an equal part of water and applied to the cartridge, which was washed with water and acetonitrile–water (15:85), and the methylated product was eluted with acetonitrile.

N.m.r. spectra of solutions in deuterium oxide were recorded at 70° (^{13}C) and

85° (^{1}H) with a JEOL GX-400 instrument. Chemical shifts are reported in p.p.m. relative to internal 1,4-dioxane (δ 67.4) for ^{13}C and internal sodium trimethylsilylpropanoate-d_4 (δ 0.00) for ^{1}H. COSY experiments were performed according to JEOL standard pulse sequences.

Preparations of polysaccharide samples. — The LPS was isolated and purified as earlier described[3]. The PS was isolated from the LPS by treatment with 0.1M aqueous acetic acid for 1 h at 100°, followed by conventional work-up[3]. The depyruvylated PS was obtained by prolonging the hydrolysis time to 6 h.

The partially delipidated LPS was prepared by treating the LPS with 0.1M aqueous sodium hydroxide at room temperature for 16 h, dialysis, concentration, and chromatography on a Bio-Gel P-10 column irrigated with mM aqueous formic acid. The eluate was monitored by using a differential refractometer and the modified LPS was eluted in the void volume.

Partial hydrolysis. — The depyruvylated PS (15 mg) was treated with 0.5M trifluoroacetic acid at 100° for 2 h. After concentration and freeze-drying of the hydrolysate, the product was fractionated on a Bio-Gel P-4 column irrigated with mM aqueous formic acid. A fraction (3.7 mg), eluted in the disaccharide region, was reduced with sodium borodeuteride, methylated, and analysed by g.l.c.–m.s., using a methyl silicone column operated at 200° (5 min) →300° at 8°/min. Three products, **3** (45%), **4** (34%), and **5** (21%), were obtained with the retention times, relative to fully methylated lactitol, of 1.24, 1.25, and 1.70, respectively. The mass spectra showed, *inter alia*, the following peaks (relative intensities in parenthesis) for **3**, *m/z* 90 (23%), 101 (100), 103 (26), 206 (9), 260 (21), 266 (23), and 276 (39); **4**, *m/z* 88 (100%), 89 (64), 101 (54), 175 (21), 189 (22), and 277 (63); **5**, *m/z* 89 (60%), 131 (23), 133 (7), 260 (93), and 277 (100).

ACKNOWLEDGMENTS

This work was suppored by grants from the Swedish Medical Research Council (O3X-02533 and 16X-07927) and the National Swedish Board for Technical Development, and by a maintenance grant from the International Seminar in Chemistry, University of Uppsala (to A.A.).

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