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Carbohydrate Research 319 (1999) 124–132

CARBOHYDRATE RESEARCH

Chemical structure of a polysaccharide from *Campylobacter jejuni* 176.83 (serotype O:41) containing only furanose sugars☆

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Received 26 January 1999; accepted 24 May 1999

Abstract

A neutral polysaccharide was obtained by hot phenol–water extraction of biomass from *Campylobacter jejuni* 176.83 and subsequently separated from acid-liberated core oligosaccharide of lipopolysaccharide by sequential GPC on Bio-Gel P6 and TSK-40 columns. All sugar components of the trisaccharide repeating unit of the polysaccharide were found to be of the furanose ring form. The major trisaccharide contained β-L-arabinose, 6-deoxy-β-D-*altro*-heptose (β-D-6d-altHep) and 6-deoxy-β-L-altrose (β-L-6d-Alt), whereas in the minor trisaccharide the β-L-6d-Alt is replaced by its C-5 epimer α-D-Fuc. On the basis of ^{1}H and ^{13}C NMR spectroscopic studies, including 2D ROESY, HMQC and HMQC-TOCSY experiments, the following structures of the repeating units were established:

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Keywords: *Campylobacter jejuni*; O:41 Polysaccharide, structure; 6-Deoxy-D-*altro*-heptose; 6-Deoxy-L-altrose; D-Fucose; L-Arabinose; NMR spectroscopy

1. Introduction

Campylobacter jejuni is recognised as one of the most frequent causes of enteritis in humans [1]. Furthermore, infection with *C. jejuni* has been identified as the single most important predisposing factor for the development of the neurological disorder Guillain–Barré syndrome (GBS) [2]. However, some reports suggest that only specific serotypes are associated with GBS [3–5]. Structural studies of lipopolysaccharide (LPS) extracted from *C. jejuni* have shown that the terminal regions of the LPS core oligosaccharide (OS) of specific serotypes mimic the structures of human gangliosides [3,4,6,7], particularly strains associated with GBS development [8–10].

☆ Preliminary data were presented at the 19th International Carbohydrate Symposium, August 1998, San Diego, CA, USA.

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PII: S0008-6215(99)00129-9

Research has focused on the view that molecular mimicry in *C. jejuni* LPS may be a factor in the pathogenesis of GBS by inducing autoreactive antibodies to human gangliosides [3,4]. In a previous serological investigation of four *C. jejuni* strains belonging to serotype O:41, three of which were recovered from patients who developed GBS and one from a patient who developed enteritis alone, the presence of ganglioside-like epitopes was demonstrated in LPS of all the strains [11]. These results prompted a structural investigation of the LPS from the enteritis-associated strain, *C. jejuni* 176.83. Silver staining of electrophoresis gels and immunoblotting experiments with homologous antisera showed that this strain produced an LPS of low relative molecular mass characteristic of core OS linked to lipid A [11]. No ladder-like banding patterns indicative of extended O antigen chains were seen. Initial chemical studies revealed the presence of LPS of low relative molecular mass and an associated extracellular polysaccharide in phenol–water extracts of *C. jejuni* 176.83. The results of a structural investigation of this polysaccharide, which is associated with LPS extracts, but not covalently linked to LPS, are reported in this paper.

2. Results and discussion

The ^{13}C NMR spectrum of the polysaccharide (Fig. 1) contained two series of signals with integral intensity of about 3:1. Both the major and minor series corresponded to a repeating unit of three sugar residues. The signals at 18.8 (minor) and 19.6 (major) ppm indicated the presence of 6-deoxy sugars. The signals of C–CH$_2$–C groups at 35.4 (minor) and 34.2 (major) ppm revealed the presence of another deoxy sugar in the repeating unit. The ^{1}H NMR spectrum of the polysaccharide (Fig. 2) was well resolved and confirmed the presence of deoxy sugars (signals for CH$_3$–C at 1.22 and 1.27 ppm, and for C–CH$_2$–C at 1.70 and 1.81 ppm. The ^{1}H and ^{13}C spectra were assigned using 2D homonuclear ^{1}H, ^{1}H COSY, TOCSY, and H-detected ^{1}H, ^{13}C HMQC and HMQC-TOCSY [12] (Fig. 3) experiments. Analysis of the spectra showed that the polysaccharide was composed of two types of trisaccharide repeating units (Tables 1 and 2). The predominant repeating unit (about 75%) contained three furanose residues, as determined from the chemical shifts of C-4 for each sugar residue, which were typical of the furanoid form of the sugar ring [13]. The gated decoupled spectrum showed $^1J_{C-H}$ coupling constants for anomeric carbon atoms in the range 175–177 Hz; such values are typical for sugars in the furanoid ring form [14]. One of the sugar residues was a pentofuranose, the second a 6-deoxyhexofuranose, and the third a 6-deoxyheptofuranose. The minor repeating unit greatly resembled the predominant one; where differences occurred in the ^{13}C chemical shifts of the carbons these were small (<1.4 ppm, Table 1).

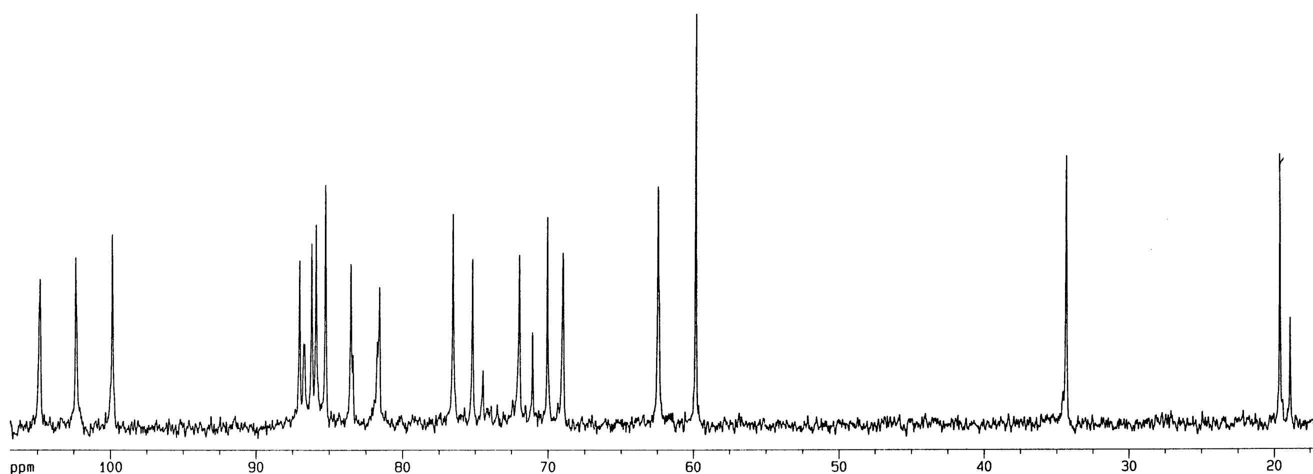

Fig. 1. ^{13}C NMR spectrum of the polysaccharide.

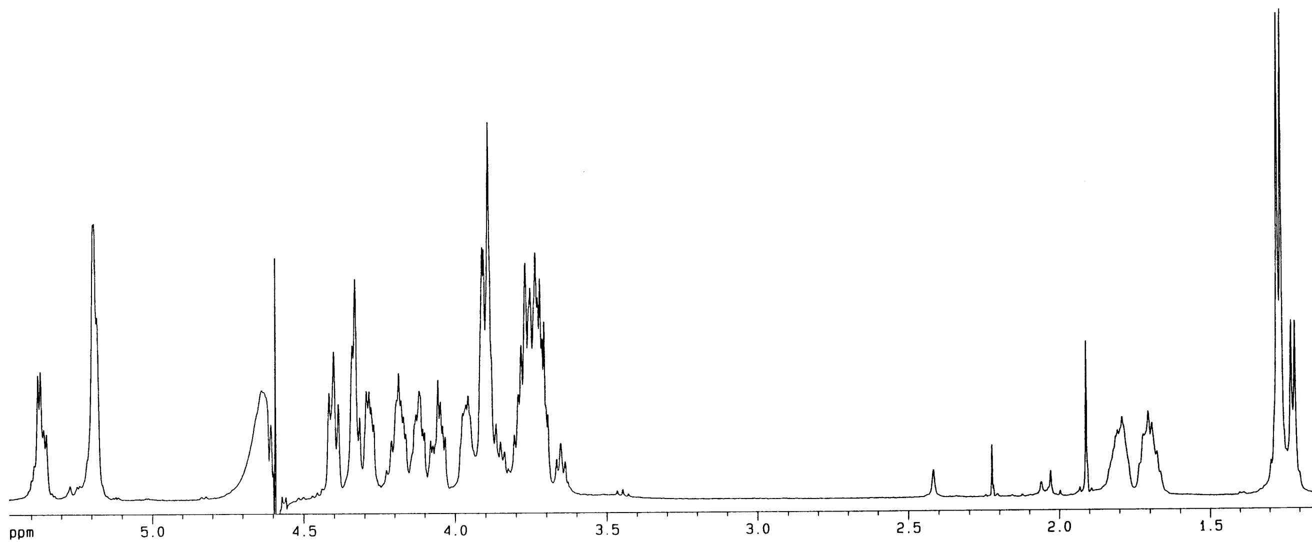

Fig. 2. ^{1}H NMR spectrum of the polysaccharide.

Fig. 3. 2D HMQC-TOCSY spectrum of the polysaccharide.

Although chemical shift and $^3J_{H,H}$ coupling constant values (Table 2) were available for constituent sugars, the identification of each sugar proved problematic. This was due to the flexibility of the furanoid rings and the dependence of their conformation on the position of the linkage; moreover, some of the coupling constants were similar to those in the corresponding C-5-epimeric sugars in the furanoid form [15]. In contrast, a definite set of coupling constants is characteristic for most sugars with pyranoid ring size [16]. Therefore, in order to determine the identities of the constituent sugars, the products of full acid hydrolysis of the polysaccharide, a mixture of mutarotating sugars, were investigated using both 1D ^{1}H and ^{13}C NMR spectra as well as 2D COSY, TOCSY and H-detected ^{1}H, ^{13}C HMQC experiments. Analysis of the spectra revealed that the pentose in the repeating unit of the polysaccharide was arabinose. All proton signals of this sugar with the coupling constants typical of β- and α-arabinopyranoses were identified in the ^{1}H NMR spectrum of the mixture. Comparison of the ^{1}H and ^{13}C NMR spectra of the mixture with those of L-arabinose (Table 3) confirmed the presence of β- and α-arabinopyranoses.

A similar approach allowed identification of the hexoses in the repeating unit of the polysaccharide as fucose and 6-deoxyaltrose. The predominant series of signals of these sugars with coupling constants typical of the β-pyranosidic form were found in the ^{1}H NMR spectrum of the mixture of monosaccharides. The ^{1}H and ^{13}C NMR spectra of the mixture were also compared with those of L-fucose (Table 4) and 6-deoxy-L-altrose

Table 1
^{13}C NMR chemical shifts for the polysaccharide (δ in ppm)

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6	C-7
Repeating unit containing β-L-6d-Alt*f*							
→2)-β-L-Ara*f*-(1→	104.8	87.0	76.55	83.5	62.45		
→2)-β-D-6d-altHep*f*-(1→	102.35	81.6	72.0	85.25	69.0	34.35	59.85
→2)-β-L-6d-Alt*f*-(1→	99.85	85.9	75.2	86.2	70.05	19.6	
Repeating unit containing α-D-Fuc*f*							
→2)-β-L-Ara*f*-(1→	104.8	86.75	76.55	83.5	62.45		
→2)-β-D-6d-altHep*f*-(1→	102.35	81.7	72.0	85.25	69.0	34.35	59.85
→2)-α-D-Fuc*f*-(1→	99.85	85.9	74.5	86.6	71.05	18.8	

Table 2
^{1}H NMR data for the polysaccharide (δ in ppm, ^3J in Hz)

Sugar residue	H-1	H-2	H-3	H-4					
Repeating unit containing β-L-6d-Alt*f*									
					H-5a	H-5b			
→2)-β-L-Ara*f*-(1→	5.195	4.34	4.06	4.12	3.90	3.74			
	$J_{1,2}$4.6	$J_{2,3}$4.1	$J_{3,4}$7.8	$J_{4,5a}$2.9	$J_{4,5b}$6.0				
					H-5	H-6a	H-6b	H-7a	H-7b
→2)-β-D-6d-altHep*f*-(1→	5.38	4.30	4.405	3.90	3.96	1.81	1.70	3.78	3.73
	$J_{1,2}$4.4	$J_{2,3}$8.1	$J_{3,4}$7.2	$J_{4,5}$6.7					
					H-5	H-6			
→2)-β-L-6d-Alt*f*-(1→	5.185	4.19	4.34	3.71	3.89	1.27			
	$J_{1,2}$3.8	$J_{2,3}$7.9	$J_{3,4}$7.0	$J_{4,5}$6.7	$J_{5,6}$6.3				
Repeating unit containing α-D-Fuc*f*									
					H-5a	H-5b			
→2)-β-L-Ara*f*-(1→	5.195	4.42	4.10	4.07	3.90	3.74			
					H-5	H-6a	H-6b	H-7a	H-7b
→2)-β-D-6d-altHep*f*-(1→	5.38	4.28	4.405	3.90	3.96	1.81	1.70	3.78	3.73
					H-5	H-6			
→2)-α-D-Fuc*f*-(1→	5.185	4.19	4.21	3.65	3.85	1.22			
	$J_{1,2}$4.2	$J_{2,3}$7.8	$J_{3,4}$6.6	$J_{4,5}$7.0	$J_{5,6}$6.4				

Table 3
^{13}C and ^{1}H NMR data for mutarotating solution of L-arabinose in D_2O at 303 K [a]

Monosaccharide		C-1	C-2	C-3	C-4	C-5	H-1	H-2	H-3	H-4	H-5	H-5′
L-Ara*p*	α	97.6	72.7	73.3	69.45	67.2	4.505	3.50	3.655	3.93	3.89	3.67
							$J_{1,2}$ 7.8	$J_{2,3}$ 9.7	$J_{3,4}$ 3.5	$J_{4,5}$ 2.1	$J_{5,5'}$ 13.0	$J_{4,5'} < 1$
	β	93.4	69.3	69.5	69.5	63.3	5.23	3.815	3.865	4.00	4.01	3.64
							$J_{1,2}$ 3.5	$J_{2,3}$ 9.8	$J_{3,4}$ 3.2	$J_{4,5} < 1$	$J_{5,5'}$ 12.6	$J_{4,5'}$ 2.1
L-Ara*f*	α	101.9	82.3	76.5	83.8	62.0	5.235	4.03	3.975	4.11	3.79	3.68
							$J_{1,2}$ 2.1	$J_{2,3}$ 4.3	$J_{3,4}$ 6.3	$J_{4,5}$ 5.7	$J_{5,5'}$ 12.4	$J_{4,5'}$ 3.4
	β	96.0	77.05	75.0	82.2	62.0	5.29	4.085	4.04	3.84	3.78	3.65
							$J_{1,2}$ 4.5	$J_{2,3}$ 7.2	$J_{3,4}$ 6.9	$J_{4,5}$ 3.1	$J_{5,5'}$ 12.4	

[a] The ratio of α*f*:β*f*:α*p*:β*p* 1:1:25:10.

Table 4
^{13}C and ^{1}H NMR data for mutarotating solution of L-fucose in D_2O at 303 K [a]

Monosaccharide		C-1	C-2	C-3	C-4	C-5	C-6	H-1	H-2	H-3	H-4	H-5	H-6
L-Fuc*p*	α	93.4	69.3	70.5	73.05	67.5	16.7	5.19	3.76	3.85	3.80	4.20	1.20
								$J_{1,2}$ 4.2	$J_{2,3}$ 10.4	$J_{3,4}$ 11.5	$J_{4,5}$ 1.0	$J_{5,6}$ 6.6	
	β	97.4	72.9	74.1	72.6	72.0	16.7	4.54	3.44	3.63	3.74	3.79	1.24
								$J_{1,2}$ 7.1	$J_{2,3}$ 10.5	$J_{3,4}$ 10.2	$J_{4,5}$ 4.3	$J_{5,6}$ 6.4	
L-Fuc*f*	α	96.1	77.85	76.1	85.95	70.05	19.05	5.27	4.07	4.03	3.57	3.62	1.21
								$J_{1,2}$ 4.2	$J_{2,3}$ 7.4	$J_{3,4}$ 6.3	$J_{4,5}$ 6.3	$J_{5,6}$ 6.5	
	β	102.05	82.75	77.6	87.5	68.4	19.4	5.22	4.01	3.97	3.87	3.90	1.25
								$J_{1,2}$ 2.9	$J_{2,3}$ 4.5	$J_{3,4}$ 5.8	$J_{4,5}$ 4.2	$J_{5,6}$ 6.6	
								$J_{1,3}$ 0.6					

[a] The ratio of α*f*:β*f*:α*p*:β*p* 1.0:1.5:13:31.

Table 5
^{13}C and ^{1}H NMR data for mutarotating solution of 6-deoxy-L-altrose in D_2O at 303 K [a]

Monosaccharide		C-1	C-2	C-3	C-4	C-5	C-6	H-1	H-2	H-3	H-4	H-5	H-6
L-6d-Alt*p*	α	94.3	72.1	71.15	71.6	69.6	17.2	4.915	3.755	3.935	3.71	4.155	1.29
								$J_{1,2}$ 4.4	$J_{2,3}$ 6.5	$J_{3,4}$ 3.3	$J_{4,5}$ 6.3	$J_{5,6}$ 6.8	
	β	92.85	72.3	71.8	70.65	70.95	18.4	5.08	3.81	4.025	3.56	3.84	1.265
								$J_{1,2}$ 1.1	$J_{2,3}$ 3.9	$J_{3,4}$ 3.1	$J_{4,5}$ 9.7	$J_{5,6}$ 6.3	
								$J_{1,3}$ 0.5	$J_{2,4}$ 0.5				
L-6d-Alt*f*	α	102.2	82.8	76.7	87.6	70.0	18.6	5.235	4.01	4.10	3.97	3.91	1.22
								$J_{1,2}$ 3.0	$J_{2,3}$ 4.0	$J_{3,4}$ 5.1		$J_{5,6}$ 6.3	
								$J_{1,3}$ 0.7					
	β	96.2	77.9	75.5	85.7	70.8	18.5	5.275	4.065	4.175	3.635	3.93	1.215
								$J_{1,2}$ 4.5	$J_{2,3}$ 7.3	$J_{3,4}$ 5.8	$J_{4,5}$ 7.7	$J_{5,6}$ 6.6	

[a] The ratio of α*f*:β*f*:α*p*:β*p* 1.2:1.0:2.6:3.0.

(Table 5), and the presence of β- and α-fucopyranose and 6-deoxy-β- and α-altropyranoses was confirmed. The signals of 6-deoxy-β- and α-altropyranose were significantly more intense than those of the corresponding signals of β- and α-fucopyranose. In addition, some signals of 6-deoxy-β- and α-altrofuranose were found in the ^{1}H and ^{13}C NMR spectra of the mixture.

Two series of signals belonging to a 6-deoxy-β- and α-heptopyranose were found in the TOCSY spectrum of the monosaccharide mixture. The chemical shifts of H-1 and H-4 of the 6-deoxyheptopyranose were close to those of the corresponding protons of 6-deoxy-β- and α-altropyranose. The $^{3}J_{H,H}$ coupling constant values for the ring protons were also in agreement with those characteristic of altropyranose. Comparison of the ^{1}H and ^{13}C NMR spectra of the mixture with those of 6-deoxy-D-*altro*-heptose (Table 6) confirmed the presence of 6-deoxy-*altro*-heptose in the repeating unit of the polysaccharide.

High-performance anion exchange chromatography (HPAEC) of the hydrolysed and reduced polysaccharide revealed the presence of three peaks corresponding to fucitol (8.4 min), arabinitol and 6-deoxyaltritol (12.0 min), and a 6-deoxyheptitol (17.7 min).

The monosaccharide composition was confirmed and the absolute configurations were determined by chemical methods. The polysaccharide was subjected to alcoholysis with (−)-2-octanol and acetylated glycosides derived were studied by GLC. As a result, the presence of L-arabinose, 6-deoxy-L-altrose, D-fucose, and 6-deoxy-D-*altro*-heptose was demonstrated. Methylation analysis indicated the presence of 2-linked arabinose, 2-linked 6-deoxyheptose, 2-linked 6-deoxyaltrose and minor amounts of 2-linked fucose. These data also confirmed that all sugar residues are in the furanose form. The inability to detect sugar constituents such as L-*glycero*-D-*manno*-heptose and D-hexosamines, which are normally found in the core OS of *C. jejuni* LPS, indicated that this polysaccharide, although isolated by the phenol–water procedure that is used for LPS extraction, was not the O-specific polysaccharide chain of high molecular mass LPS. This is consistent with the previously observed inability to detect LPS ladder-like banding patterns indicative of O antigen chains in electrophoresis and immunoblotting experiments with *C. jejuni* 176.83 [11].

Chemical shifts of C-1 (Table 1) pointed to the β configuration of L-arabinofuranose, 6-deoxy-L-altrofuranose, and 6-deoxy-D-*altro*-heptofuranose in the predominant repeating unit of the polysaccharide [13]. In the minor repeating unit (about 25%), the 6-deoxy-β-L-altrofuranose residue is replaced by an α-D-fucofuranose residue. A low-field chemical shift of C-2 for each of the residues confirmed the substitution at position 2 and, as a consequence, indicated a linear repeating unit. The sequence of the residues was established using a 2D ROESY experiment on the polysaccharide (Fig. 4). Separation of the correlation peaks in the ROESY spectrum into intra- and inter-residue cross-peaks and the interpretation of the latter were difficult due to overlapping of the H-1 signals of the pentose and heptose residues. However, the presence of the correlation peaks H-1(6d-altHep)/H-2(6d-Alt) in the major series and H-1(6d-altHep)/H-2(Fuc) in the minor series defined the following sequences of the sugar residues:

→2)-β-L-Ara*f*-(1→2)-β-D-6d-altHep*f*-(1→2)-β-L-6d-Alt*f*-(1→ (major repeating unit);

→2)-β-L-Ara*f*-(1→2)-β-D-6d-altHep*f*-(1→2)-α-D-Fuc*f*-(1→ (minor repeating unit).

The HMBC spectrum confirmed the sequences shown above. It contained inter alia trans-gly-

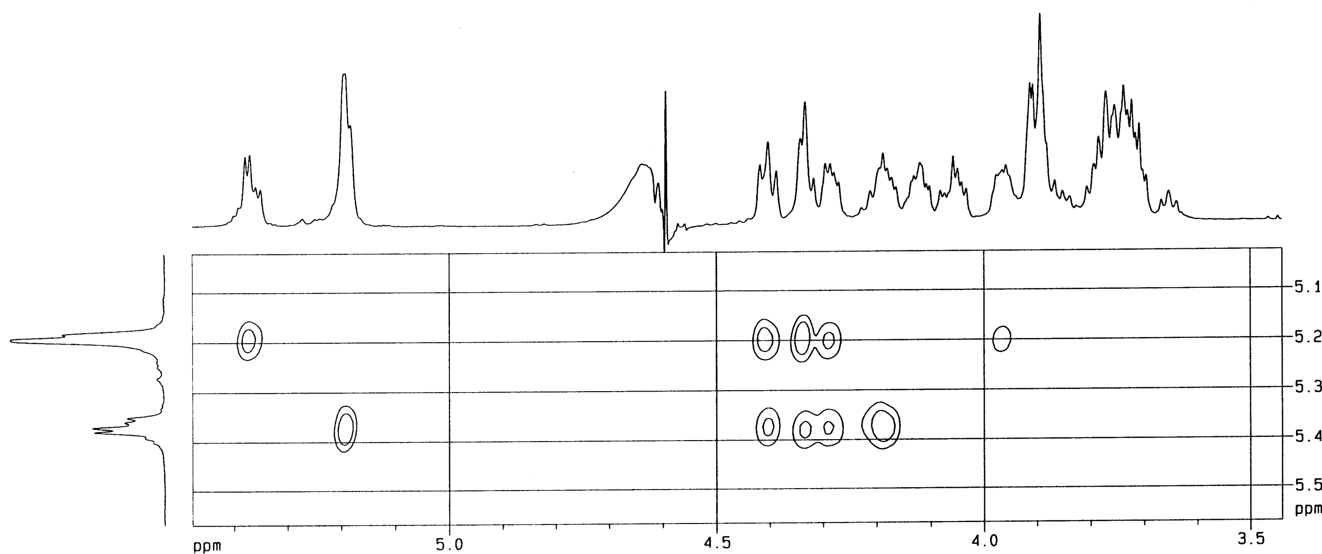

Fig. 4. 2D ROESY spectrum of the polysaccharide.

Table 6
^{13}C and ^{1}H NMR data for mutarotating solution of 6-deoxy-D-*altro*-heptose in D_2O at 303 K [a]

Monosaccharide		C-1	C-2	C-3	C-4	C-5	C-6	C-7	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	H-7,7′
D-6d-altHep*p*	α	94.4	72.1	71.25	70.6	71.4	33.35	59.35	4.875	3.73	3.905	3.79	4.11	1.90	1.865	~3.75
									$J_{1,2}$ 5.0	$J_{2,3}$ 7.2	$J_{3,4}$ 3.5	$J_{4,5}$ 5.5	$J_{5,6}$ 4.5	$J_{6,6'}$ 14.1	$J_{6',7}$ 5.0	$J_{6,7'}$ 8.1
													$J_{5,6'}$ 7.9	$J_{6,7}$ 8.1	$J_{6',7'}$ 5.0	$J_{7,7'}$ 12.7
	β	93.3	71.95	71.7	69.0	71.85	35.25	59.3	5.08	3.815	4.045	3.655	3.82	2.025	1.715	~3.75
									$J_{1,2}$ <2	$J_{2,3}$ 4.0	$J_{3,4}$ 3.3	$J_{4,5}$ 9.7	$J_{5,6}$ 2.9	$J_{6,6'}$ 14.5	$J_{6',7}$ 5.6	$J_{6,7'}$ 7.6
													$J_{5,6'}$ 9.7	$J_{6,7}$ 7.6	$J_{6',7'}$ 5.6	$J_{7,7'}$ 12.7
D-6d-altHep*f*	α	102.4	83.0	76.9	87.05	70.0	35.6	59.5	5.24	4.02	4.13	4.005	3.94	1.85	1.70	~3.75
									$J_{1,2}$ 2.6	$J_{2,3}$ 3.5	$J_{3,4}$ 5.0	$J_{4,5}$ 5.0				
	β	96.5	78.15	75.8	85.15	70.8	35.65	59.55	5.28	4.07	4.21	3.73	3.88	1.875	1.68	~3.75
									$J_{1,2}$ 4.6	$J_{2,3}$ 7.1	$J_{3,4}$ 6.7	$J_{4,5}$ 7.0				

[a] The ratio of α*f*:β*f*:α*p*:β*p* 1.4:1.0:3.2:4.3.

cosidic correlation peaks C-1(Ara)/H-2(6d-altHep), C-1(6d-altHep)/H-2(6d-Alt) and C-1(6d-Alt)/H-2(Ara) for the major repeating unit, and C-1(Ara)/H-2(6d-altHep), C-1(6d-altHep)/H-2(Fuc) and C-1(Fuc)/H-2(Ara) for the minor repeating unit. Whether the sample contained two polysaccharides, each containing one of the above repeating units, or consisted of one polysaccharide where β-L-6d-Alt*f* is sometimes replaced by α-D-Fuc*f*, cannot be ascertained.

The *C. jejuni* 176.83, serotype O:41, is similar to several other *Campylobacter* species in producing heptose sugars of unusual configuration. Other examples include D-*glycero*-D-*altro*-heptose (and three derivatives from deoxygenation at C-6 and/or O-methylation at C-3) in *C. jejuni* O:23 and O:36 [17], 6-deoxy-D-*talo*-heptose in *Campylobacter coli* serotype O:30 [18], and 6-deoxy-L-*galacto*-heptose and 6-deoxy-L-*gulo*-heptose in strains of *Campylobacter lari* [19,20]. Pathways are documented for the biosynthesis and interconversions of hexose derivatives involving glycosyl esters of nucleotides, but little is known of similar transformations of heptoses [21,22]. In contrast to *C. jejuni* O:23 and O:36, where 6-deoxy-D-*altro*-heptose occurs as a pyranose sugar ring [17], it occurs in *C. jejuni* O:41 in the furanosyl form. Moreover, an α-linked, rather than a β-linked, 2-substituted 6-deoxy-D-*altro*-heptofuranose occurs in capsular polysaccharides from *Eubacterium saburreum* [23].

The C-5-epimeric sugars 6-deoxy-β-L-altrofuranose and α-D-fucofuranose replace each other in the major and minor repeating units, respectively, of the polysaccharide. The ability of *C. jejuni* 176.83 to produce units of differing composition can possibly reflect antigenic variation by the bacterium to overcome the host immune response. Likewise, this may also explain the production of polysaccharide repeating units of differing heptopyranose composition in *C. jejuni* O:23 and O:36 [17]. No evidence was obtained to indicate that the extracellular polysaccharide of *C. jejuni* 176.83 was the O antigen chain of high molecular mass LPS. This is similar to findings with *C. jejuni* serotype O:3, *C. coli* O:30 and *C. lari* strains, where polysaccharides associated with

LPS extracts from the phenol–water procedure have been described [17–20]. Therefore, they may be more similar to capsular polysaccharides that carry lipid termini such as di-O-acyl glycerol units [24], which were not detected, but which are worthy of further investigation.

Specific bacterial polysaccharides containing only furanose sugars are rare; one of a few known examples is the O-specific polysaccharide of *Pectinatus cerevisiiphilus* [29].

3. Experimental

Bacterial strain, growth conditions, and isolation of the polysaccharide.—*C. jejuni* 176.83 was isolated from a 9-year-old male suffering from enteritis and diarrhoea in Cape Town, South Africa, using an established protocol [25]. The strain was serotyped as *C. jejuni* O:41 using the Penner scheme for thermostable somatic O antigens [26]. Large quantities of the bacterium were grown on blood agar under microaerobic conditions and harvested as described previously [27]. The LPS and associated polysaccharide produced by the bacterium were extracted by hot phenol–water treatment [28] into the water phase, and subsequently this phase was enzymatically treated with RNase A, DNase II and proteinase K to ensure purity from contaminating nucleic acids and proteins [27]. The extract was dispersed in water, degraded with 1% HOAc at 100 °C for 1 h, lipid A was precipitated by centrifugation, and the supernatant solution was subjected to GPC on a Bio-Gel P6 column (100 × 1 cm) with water as eluent as described previously [21]. Chromatography yielded a peak containing both core oligosaccharide of LPS and polysaccharide, which were subsequently separated by GPC on a TSK-40 column.

NMR spectroscopy.—Samples were deuterium-exchanged by freeze-drying three times from ^2H_2O and then examined in solutions of 99.97% ^2H_2O. Spectra of the polysaccharides were recorded at 36 °C on a Bruker DRX-500 spectrometer using internal acetone (δ_H 2.225, δ_C 31.45) as reference. Data were acquired and performed using XWINNMR 1.1 version software. Mixing times of 120 and 300 ms were used in TOCSY and ROESY experiments, respectively. A mixing time of 100 ms and recycle delay of 0.5 s were used in a HMQC-TOCSY [29] experiment. NMR spectra of the monosaccharides were recorded on a Jeol 400 MHz spectrometer equipped with a DEC AXP 300 computer as described by Hanniffy et al. [30].

HPAEC.—The hydrolysed and reduced polysaccharide was analysed by HPAEC at superhigh pH using pulsed amperometric detection [31]. The chromatography was performed on a Dionex DX-500 chromatography system equipped with an MA1 column. An isocratic eluent of 600 mM NaOH and a flow rate of 0.55 mL/min at ambient temperature were used. An ED-40 electrochemical detector, in the integrated amperometry mode, facilitated the detection of the eluted product peaks. Data were collected and processed using Peak Net software on a Gateway 2000 PC.

Other analytical methods.—The absolute configurations of the monosaccharides were determined by a modified method for GLC analysis of chiral glycosides [32,33] using synthetic 6-deoxy-D-*altro*-heptose [34] and 6-deoxy-L-altrose from the O-specific polysaccharide of *P. cerevisiiphilus* [29] as authentic samples. Permethylated polysaccharide was prepared by the procedure of Ciucanu and Kerek [35] and partially methylated alditol acetates were identified using published data [17,36]. Analysis of the methylated products was by GLC on a Hewlett–Packard model 5890 chromatograph using capillary columns with the following programs: DB-23 (15 m × 0.25 mm) at 190 °C isothermally and DB-17 (30 m × 0.35 mm) at 190 °C isothermally and by GLC–MS using the same chromatograph equipped with a mass-selective detector (model 5971A).

Acknowledgements

The authors thank Professor G.O. Aspinall (York University, Toronto) for the gift of 6-deoxy-*galacto*-heptose, Professor A. Zamojski (Polish Academy of Sciences, Warsaw) for the gift of 6-deoxy-D-*altro*-heptose and Dr

A.J. Lastovica (University of Cape Town, South Africa) for providing the *C. jejuni* strain. This study was financially supported by grants from the Wellcome Trust, BioResearch Ireland (to A.V.S. for the visit of A.S.S.) and the Irish Health Research Board (to A.P.M.). A.V.S. and O.M.H. gratefully acknowledge the award of an International Collaboration Grant from Enterprise Ireland.

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