ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 330 (2001) 223–229

CARBOHYDRATE RESEARCH

Chemical structure of the core oligosaccharide of aerotolerant *Campylobacter jejuni* O:2 lipopolysaccharide☆

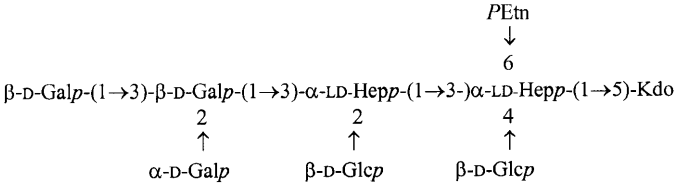
Orla M. Hanniffy,[a] Alexander S. Shashkov,[b] Anthony P. Moran,[c] Sof'ya N. Senchenkova,[b] Angela V. Savage [a,*]

[a] *Department of Chemistry, National University of Ireland, Galway, Ireland*
[b] *N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow 117913, Russia*
[c] *Department of Microbiology, National University of Ireland, Galway, Ireland*

Received 19 April 2000; received in revised form 16 October 2000; accepted 23 October 2000

Abstract

The structure of the core oligosaccharide of aerotolerant *Campylobacter jejuni* O:2 lipopolysaccharide was determined and found to contain 3-deoxy-D-*manno*-octulosonic acid (Kdo), L-*glycero*-D-*manno*-heptose (LD-Hep), D-galactose, D-glucose, and phosphorylethanolamine (*P*Etn). Based on ^{1}H, ^{13}C and ^{31}P NMR spectroscopic studies including 2D COSY, TOCSY, ROESY and heteronuclear $^{1}H-^{31}P$ and HMQC experiments it was established that the oligosaccharide has the following structure:



Keywords: *Campylobacter jejuni*; Aerotolerant O:2 core oligosaccharide, structure; NMR spectroscopy

1. Introduction

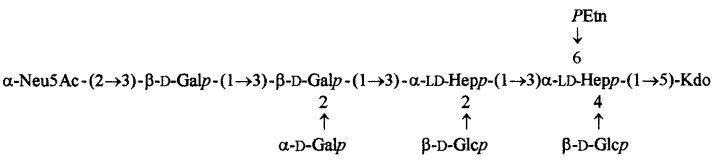
Campylobacter jejuni is recognised as a leading cause of human enteritis,[1] and is an enteric commensal in other warm-blooded animals, including poultry, which may act as a source of the bacterium for humans.[2,3] Also, infection with *C. jejuni* has been determined to be the single most important predisposing factor for the development of the neurological disorder Guillain–Barré syndrome (GBS).[4] Recent research has proposed that molecular mimicry in *C. jejuni* lipopolysaccharide (LPS) may be a factor in the pathogenesis of GBS by inducing autoreactive antibodies to human gangliosides.[5,6] It has been shown previously that the terminal regions of the core oligosaccharides (OS) from the LPS of specific serotypes mimic the structures of human gangliosides,[5–8] in particular those strains associated with GBS development.[9–11]

☆ Preliminary data were presented at the 10th European Carbohydrate Symposium, July 1999, Galway, Ireland.

* Corresponding author. Tel.: +353-91-750447; fax: +353-91-525700.
E-mail address: angela.savage@nuigalway.ie (A.V. Savage).

0008-6215/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0008-6215(00)00281-0

Although microaerobic conditions aid the survival and growth of the bacterium in the host digestive tract, these organisms are sensitive to atmospheric levels of oxygen when removed from such an environment.[12] Nevertheless, studies have implicated raw water supplies as a major potential source of transmission and infection, both to humans and to broiler flocks.[13] Therefore, it has been suggested that survival in such well-oxygenated conditions, which are unfavourable to the bacterium, may lead to the induction of cellular modifications such as aerotolerant forms,[14,15] to aid their survival and transmission. Comparative investigations of microaerophilic and aerotolerant forms of *C. jejuni* have shown differences in antibiotic sensitivities, enzyme levels and outer membrane proteins between these differing forms of the same strain.[16] Despite LPS being the predominant surface antigen of *C. jejuni*,[16] these molecules have not been characterised in aerotolerant forms of *C. jejuni*. Structural studies on the core region of low-M_r LPS of microaerophilic *C. jejuni* serotype O:2 have been undertaken.[8] These studies showed the presence of sugars commonly found as constituents of the core OS:3-deoxy-D-manno-octulosonic acid (Kdo), L-*glycero*-D-*manno*-heptose (LD-Hep), D-galactose, D-glucose, *N*-acetylneuraminic acid (Neu5Ac) and phosphorylethanolamine (*P*Etn). The following structure was determined:[8]



The detailed structural examination of the core OS of low-M_r LPS from aerotolerant *C. jejuni* serotype O:2 is described in this paper.

2. Results and discussion

The ^{13}C NMR spectrum of the core fraction **I** was typical for an oligosaccharide (Table 1) and also contained some minor peaks. This spectrum also contained an intense signal at 41.5 ppm, which is typical for the C-2 of a phosphoethanolamine group (*P*Etn).[17] The ^{31}P spectrum confirmed the presence of phosphorus in the core fraction **I**. This spectrum contained two signals at 2.2 ppm (major) and 2.05 ppm (minor). A very intense signal from H-2,2′ of *P*Etn was seen at 3.30 ppm.

The ^{13}C and ^{1}H NMR spectra of the dephosphorylated core fraction **II** (Figs. 1 and

Table 1
^{1}H and ^{13}C chemical shifts of the phosphorylated OS of core fraction **I** (ppm)

Residue		H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6	H-6′	H-7 C-7	H-7′
→3)-α-LD-Hep-(1→		5.11[a]	4.19	4.26	4.33	3.89	4.62[b]		3.90	3.84
4	(A)	102.3[c]	71.2	74.6	72.4	72.4	74.9		62.7	
↑										
*P*Etn		H-1,1′ 4.17 63.4	H-2,2′ 3.30 41.5							
→3)-α-LD-Hep-(1→		5.42	4.40	4.29	4.06	3.72	4.10		3.75	3.67
2	(B)	100.2	74.6	76.1	66.3	72.7	69.7		64.2	
↑										
→3)-β-D-Gal-(1→		4.98	3.99	3.99	4.24	3.78	3.78	3.78		
2	(C)	100.1	73.7	81.6	70.3	76.1	62.15			
↑										
β-D-Gal-(1→	(D)	4.64	3.59	3.64	3.93	3.69	3.77	3.77		
		105.2	72.5	74.6	70.1	76.5	62.4[d]			
β-D-Glc-(1→	(E)	4.88	3.33	3.59	3.28	3.43	3.97	3.64		
		103.0[e]	75.2	77.0	72.1	77.9	62.7[f]			
β-D-Glc-(1→	(F)	4.47	3.38	3.48	3.51	3.37	3.96	3.82		
		103.7	73.9	76.9	70.5	77.6	62.1			
α-D-Gal-(1→	(G)	5.63	3.82	3.93	3.97	4.46	3.76			
		98.8	70.0	70.8	70.8	72.0	62.1[d]			

Minor signals: [a], 5.06 and 5.04 ppm; [b], 4.63; [c], 101.8 and 101.7; [e], 102.8; [f], 62.9. [d], alternative asignments

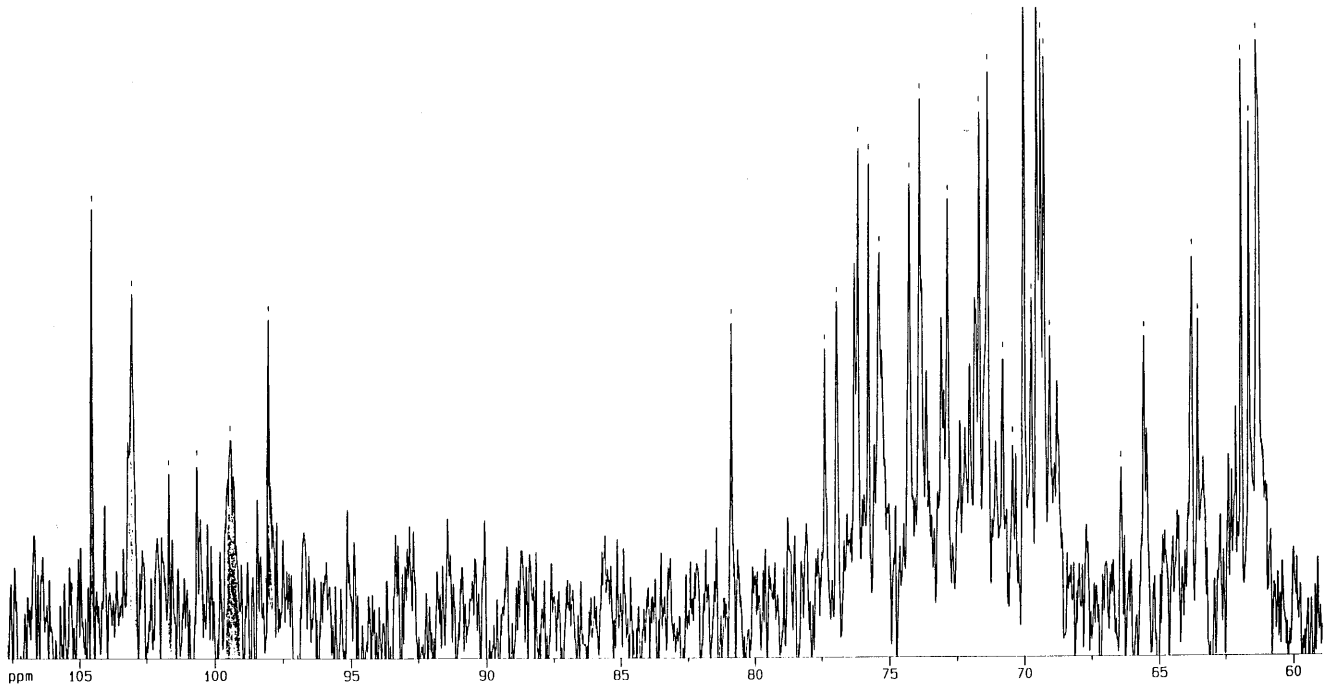


Fig. 1. ^{13}C NMR spectrum of the dephosphorylated core oligosaccharide.

2) proved to be more regular. The anomeric regions contained signals with the most intense peaks from the predominant component of the mixture. The signals of the predominant oligosaccharide in the ^{13}C and ^{1}H NMR spectra of fraction **II** were assigned using homonuclear 2D COSY, TOCSY, ROESY and heteronuclear 2D HMQC and HMBC spectra. The H-detected $^{1}H-^{13}C$ HMQC spectrum showed seven intense correlation peaks in the resonance region characteristic for anomeric carbons and protons of aldoses. The analysis of the 1D ^{13}C APT spectrum[18] revealed in the anomeric region one additional signal of a ketose at 97.1 ppm. Thus, the main component of fraction **II** was an octasaccharide. Analysis of the COSY and TOCSY spectra and intra-residue peaks in the ROESY spectrum revealed the sugar composition of the oligosaccharide. It contained Kdo, two residues of α-L-*glycero*-D-*manno*-heptopyranose (residues **A** and **B**, see Table 2), two residues of β-galactopyranose (**C** and **D**), one residue of α-galactopyranose (**G**), and two residues of β-glucopyranose (**E** and **F**). The HMQC spectrum allowed for the definite assignment of the ^{13}C NMR spectrum (Table 2). The positions of substitution in the residues were found after analysis of the glycosylation effects in the ^{13}C NMR spectrum. The comparison of sub-spectra of the residues **D**, **E**, **F** and **G** with the spectra of the corresponding pyranoses or their methyl glycosides[19] showed that the residues were terminal ones in the OS under consideration. A downfield shift was found for C-2 and C-3 of the residue **C** in comparison with that of the corresponding carbon atoms of Me-β-Gal*p*. Thus, the residue **C** was substituted at C-2 and C-3. The comparison of the sub-spectra of the residues **A** and **B** with spectra of α-LD-Hep*p*[20] displayed substitution of **A** at C-3 and C-4 and of **B** at C-2 and C-3. The downfield shift of C-5 of Kdo was in agreement with the substitution of the residue at C-5.[19]

The analysis of the inter-residue peaks in the ROESY spectrum revealed the sequence of the residues. The observation of a very intense trans-glycosydic correlation peak H-1(**A**)/H-5(Kdo) corroborated the sequence **A**-(1→5)-Kdo. The anomeric proton of the second α-LD-Hep*p* (residue B) proved to be in spatial contact with H-1 of **F** and, simultaneously, with H-2 and H-3 of **A**. The first contact is typical for 1→2-linkages, and the presence of correlation peak H-1(**F**)–H-2(**B**) confirmed

the 1→2-linkage between **F** and **B**. Thus, the correlation peaks H-1(**B**)–H-2(**A**) and H-1(**B**)–H-3(**A**) proved the existence of a 1→3-linkage between **B** and **A**. The observation of the inter-residue peaks H-1(**C**)–H-2(**B**) and H-1(**C**)–H-3(**B**) demonstrates with certainty the presence of a 1→3-linkage between these residues (taking into account the occupation of position 2 of residue **B** by residue **F**). The substitution of residue **A** at H-4 by the residue **E** was established by the presence of the correlation peaks H-1(**E**)–H-4(**A**) and H-1(**E**)–H-

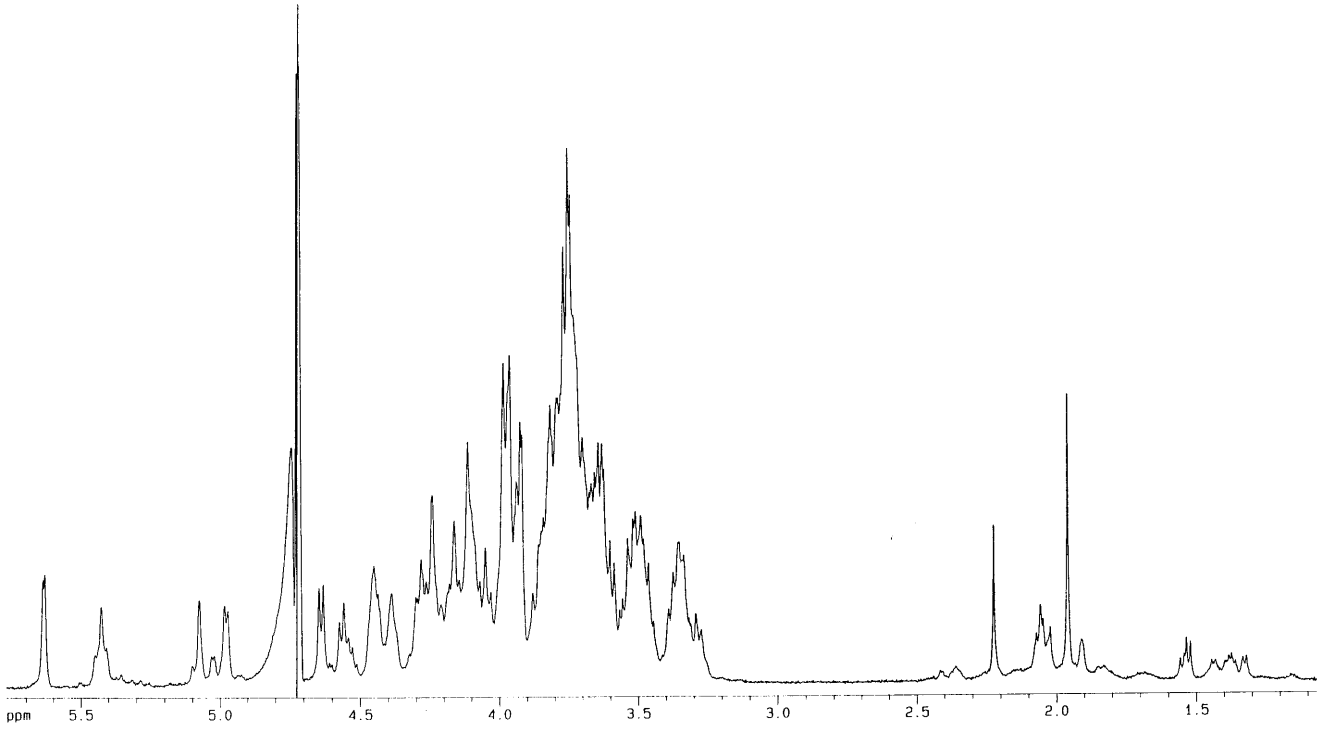


Fig. 2. ^{1}H NMR spectrum of the dephosphorylated core oligosaccharide.

Table 2
^{1}H and ^{13}C chemical shifts of the dephosphorylated OS of core fraction **II** (ppm)

Residue		H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6	H-6'	H-7 C-7	H-7'	H-8,8' C-8
→5)-Kdo[a]				2.02	3.73	4.10	3.865		3.70		3.73
			97.8	36.4	67.15	76.05	72.5		70.5		64.3
→3)-α-LD-Hep-(1→		5.08[b]	4.16	4.22	4.27	4.14	4.10		3.79	3.66	
4	(A)	102.4	71.5	74.2	74.6	72.7	69.4		64.1		
↑											
→3)-α-LD-Hep-(1→		5.42[c]	4.385	4.26	4.04	3.70	4.09		3.80	3.65	
2	(B)	100.1	74.6	76.1	66.3	72.5	69.8		64.5		
↑											
→3)-β-D-Gal-(1→		4.98	3.97	3.98	4.23	3.77	3.78	3.73			
2	(C)	100.1	73.6	81.6	70.3	76.1	62.4				
↑											
β-D-Gal-(1→	(D)	4.63	3.48	3.62	3.92	3.68	3.74	3.74			
		105.2	72.4	74.6	70.1	76.5	62.4[d]				
β-D-Glc-(1→	(E)	4.47	3.33	3.53	3.28	3.48	3.97	3.63			
		103.7	75.0	77.0	72.1	77.9	62.7				
β-D-Glc-(1→	(F)	4.44	3.37	3.45	3.49	3.335	3.94	3.82			
		103.8	73.7	76.9	70.1	77.7	62.1				
α-D-Gal-(1→	(G)	5.63	3.80	3.92	3.95	4.45	3.80	3.74			
		98.7	69.7	70.75	70.75	72.1	62.1[d]				

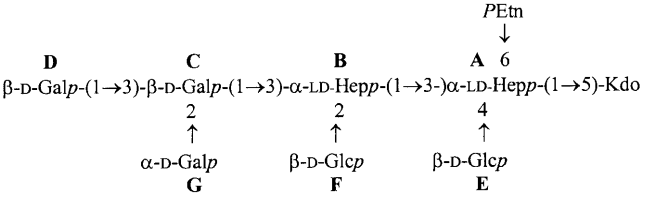
[a] Predominant form
[b] Minor signals at 5.01 and 5.02 ppm. [c] Minor signal at 5.40 ppm.
[d] alternative assignments.

6(**B**). The signals of H-2 and H-3 of the 2,3-substituted residue **C** were strongly overlapped (3.97 and 3.98 ppm, respectively, Table 2). Both H-1(**D**) and H-1(**G**) displayed correlation peaks with one of the protons in this region of the spectrum. However, for the anomeric proton of residue **D** an additionally long-range inter-residue correlation peak H-1(**D**)–H-4(**C**) was observed. The appearance of this peak proved the existence of a $1\rightarrow3$ linkage between **D** and **C** and, as a consequence, the presence of a $1\rightarrow2$ linkage between **G** and **C**. The last conclusion was confirmed by the presence of an intense H-1(**D**)–C-3(**C**) correlation peak in the HMBC spectrum. In addition, the HMBC spectrum contained H-1(**F**)–C-2(**B**), H-1(**B**)–C-3(**A**), and H-1(**A**)–C-5(Kdo) correlation peaks, which confirmed the sequence established by the analysis of the inter-residue peaks in the ROESY spectrum.

Similar NMR investigations were completed for the core fraction **I**. The comparison of the ^{1}H and ^{13}C (Tables 1 and 2) chemical shifts for the corresponding residues in two fractions showed that *P*Etn is located at position 6 of residue **A**. The presence of *P*Etn led to the downfield shift of both H-6(**A**) and C-6(**A**) and provides for the less significant changes in the chemical shifts of neighbouring atoms. The location of *P*Etn at position 6 of **A** was confirmed by the analysis of the 2D $^{1}H-^{31}P$ spectrum. This spectrum showed correlation peaks at 4.62(H-6**A**), 4.17(H-1 *P*Etn) and 3.30(H-2 *P*Etn) ppm for the major signal ^{31}P at 2.2 ppm and 4.63(H-6A), 4.17(H-1 *P*Etn) and 3.30(H-2 *P*Etn) ppm for the minor signal ^{31}P at 2.05 ppm. Thus, the small differences in the positions of the major and minor signals in the ^{31}P spectra probably arise due to the different forms of Kdo at the 'reducing' end of the residue α-LD-Hep*p* (**A**). By the same reasoning, the presence of the minor signals for C-1 and H-1 of α-LD-Hep*p* (**A**) and C-1, C-6 (**E**) or for H-1 **A** and **B** (Table 1) may be explained.

The same monosaccharide composition of LPS as that of liberated core OS was confirmed, and the absolute configurations were determined by chemical methods. The LPS and OS preparations were subjected to alcoholysis with (−)-2-octanol and the acetylated glycosides derived were studied by GLC. As a result, the presence of D-Gal, D-Glc, D-LD-Hep, and Kdo residues were demonstrated. Upon methanolysis and peracetylation, the peracetylated methyl ketoside methyl ester derivative of Neu5Ac was not detected in aerotolerant *C. jejuni* O:2 LPS, but was detected in control microaerophilic *C. jejuni* O:2 LPS, where its presence has been described previously.[8] Methylation analysis indicated the occurrence of 2,3-linked Gal, 2,3-linked LD-Hep, 3,4-linked LD-Hep, possibly 3,4,6-linked LD-Hep, 5-linked Kdo, and terminal Gal and Glc residues in aerotolerant *C. jejuni* O:2 LPS and core OS. These data also confirmed that all residues were in the pyranose form.

The following structure of the core OS from aerotolerant *C. jejuni* O:2 has been proposed:



The ^{1}H and ^{13}C NMR spectra of the core fraction **I** contained minor signals which may be attributable to amino-sugars and of 6-deoxy-sugars. However, such sugars were not evident in chemical analyses.

The structure of the core OS of aerotolerant *C. jejuni* serotype O:2 is similar to that of the microaerophilic O:2 strain;[8] the only difference is the absence of Neu5Ac. This residue may be labile under the acidic conditions used to liberate core OS from *C. jejuni* LPS, and hence can be lost from liberated OS.[7,8] Therefore, the absence of Neu5Ac from intact aerotolerant *C. jejuni* serotype O:2 LPS, as well as liberated core OS was confirmed by chemical methods in this study. This study also allows the opportunity of revising some previously published[8] NMR assignments.

Aerotolerant forms of normally microaerophilic *C. jejuni* and other microaerophilic bacteria have been described previously,[14,15,21] but little is known of the biological relevance of this phenomenon. The suggestion that these forms could aid the survival of *C. jejuni* in well-oxygenated environments, and hence aid

transmission,[14] has not been supported in studies in which such strains are subjected to environmental stresses.[15] Therefore, it is unlikely that adaptation to growth in air confers these micro-organisms with an advantageous survival strategy outside of the intestine. It is more likely that the rapid adaptation of *C. jejuni* to grow in higher levels of oxygen reflects an emergency response of the bacterium to these unfavourable conditions.[15] This is supported by observations of increased susceptibility to antibiotics and to several environmental stresses by aerotolerant compared with microaerophilic forms.[15] Consistent with changes in the LPS of an aerotolerant form of *C. jejuni* in this study, subtle changes in the outer membrane proteins of such forms has been observed previously,[15] and suggest that adaptation to aerobic growth requires de novo protein synthesis.

Despite the implication of molecular mimicry of sialylated gangliosides by LPS of certain strains of *C. jejuni*, including those of serotype O:2, in the induction of autoreactive antibodies contributing to the pathogenesis of GBS,[5,6,16] the advantage for the bacterium of producing such structures remains unclear.[16] Thus, future physiological studies on the reason(s) for the loss of sialylation of LPS by the aerotolerant form of *C. jejuni* O:2 may give better insights into the biological relevance of the sialylation phenomenon.

3. Experimental

Bacterial strain, growth and isolation of the polysaccharide.—Aerotolerant *C. jejuni* serotype O:2 was grown under aerobic conditions on blood agar,[15] biomass harvested, and LPS extracted as described previously.[22] The LPS and associated polysaccharide produced by the bacterium were extracted by a hot phenol–water treatment[23] into the water phase, and subsequently this phase was enzymatically treated with RNase A, DNAse II and proteinase to ensure purity from contaminating nucleic acids and proteins.[24] The extract was dispersed in water and 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.[25]

NMR spectroscopy.—Samples were deuterium-exchanged by freeze-drying three times from D_2O and then examined in solutions of 99.97% D_2O. Spectra of the polysaccharides were recorded, using internal acetone (δ_H 2.225, δ_C 31.45) as a reference, at 30 °C on a Bruker DRX-500 spectrometer, where data were acquired and performed using XWINNMR 1.1 version software and on a JEOL 400 MHz spectrometer equipped with a DEC AXP 300 computer.

All 2D NMR experiments[26] were performed using standard Bruker software. For the phosphorylated oligosaccharide the mixing time for ROESY was 300 ms and for TOCSY the duration of the MLEV 17 spin-lock was 200 ms. For the dephosphorylated oligosaccharide the mixing time for the ROESY was 200 ms. For the TOCSY the mixing time was 120 ms and the duration of the MLEV 17 spin-lock was 200 ms. For HMQC/TOCSY the mixing time was 150 ms and the duration of the MLEV 17 spin-lock was 200 ms.

Other analytical methods.—LPS was hydrolysed with 2 M trifluoroacetic acid (120 °C, 2 h), the hydrolysate evaporated, and monosaccharides identified by GLC of the derived alditol acetates[27] as described previously.[8] The absolute configurations of the monosaccharides were determined by a method for GLC analysis of chiral glycosides essentially as described.[28] For analysis of the occurrence of Neu5Ac in intact LPS, Neu5Ac was detected and characterised as its peracetylated methyl ketoside methyl ester derivative after acidic methanolysis (1 M HCl, 86 °C, 1 h) of LPS and peracetylation under the conditions described previously.[29] Permethylated OS was prepared by the procedure Ciucanu and Kerek[30] and partially methylated alditol acetates were identified using published data.[8,31] 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 J. McGovern for technical assistance during growth of *C. jejuni*. This study was financially supported by grants from the Wellcome Trust (to A.V.S.), 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.

References

1. Penner, J. L. *Clin. Microbiol Rev.* **1988**, *1*, 157–172.
2. Skirrow, M. B. *J. Com. Pathol.* **1993**, *111*, 113–149.
3. Wallis, M. R. *Br. J. Med. Sci.* **1994**, *51*, 57–64.
4. Mishu, B.; Blaser, M. J. *Clin. Infect. Dis.* **1993**, *17*, 104–108.
5. Moran, A. P.; Prendergast, M. M.; Appelmelk, B. J. *FEMS Immunol. Med. Microbiol.* **1996**, *16*, 105–115.
6. Moran, A. P.; Appelmelk, B. J.; Aspinall, G. O. *J. Endotoxin Res.* **1996**, *3*, 521–531.
7. Aspinall, G. O.; McDonald, A. G.; Raju, T. S.; Pang, H.; Moran, A. P.; Penner, J. L. *Eur. J. Biochem.* **1993**, *213*, 1017–1027.
8. Aspinall, G. O.; McDonald, A. G.; Raju, T. S.; Pang, H.; Kurjancyk, L. A.; Penner, J. L.; Moran, A. P. *Eur. J. Biochem.* **1993**, *213*, 1029–1037.
9. Aspinall, G. O.; McDonald, A. G.; Pang, H.; Kurjancyk, L. A.; Penner, J. L. *Biochemistry* **1994**, *33*, 241–249.
10. Moran, A. P.; O'Malley, D. T. *J. Endotoxin Res.* **1995**, *2*, 233–235.
11. Nam Shin, J. E.; Ackloo, S.; Mainkar, A. S.; Monteiro, M. A.; Pang, H.; Penner, J. L.; Aspinall, G. O. *Carbohydr. Res.* **1998**, *305*, 223–232.
12. Moran, A. P.; Upton, M. E. *J. Appl. Bacteriol.* **1987**, *62*, 43–51.
13. Pearson, S. R.; Greenwood, M.; Helaing, T. D. *Appl. Environ. Microbiol.* **1993**, *59*, 987–996.
14. Jones, D. M.; Sutcliffe, C. M.; Rios, R.; Fox, A. J.; Curry, A. *J. Med. Microbiol.* **1993**, *38*, 145–150.
15. McCole, J. C.; Moran, A. P. In *Campylobacter, Helicobacter and Related Organisms*; Lastovica, A. J.; Newell, D. G.; Lastovica, E. E., Eds.; Institute of Child Health: Cape Town, South Africa, 1998; pp. 184–188.
16. Moran, A. P.; Penner, J. L. *J. Appl. Microbiol.* **1999**, *86*, 361–377.
17. Edebrink, P.; Jansson, P.-E.; Bogwald, J.; Hoffman, J. *Carbohydr. Res.* **1996**, *287*, 225–245.
18. Patt, S. L.; Shoolery, J. N. *J. Magn. Reson.* **1982**, *46*, 535–539.
19. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
20. Muller-Loennies, S.; Holst, O.; Brade, H. *Eur. J. Biochem.* **1994**, *224*, 751–760.
21. Tompkins, D. S.; Dave, J.; Mapstone, N. P. *Eur. J. Clin. Microbiol. Infect. Dis.* **1994**, *13*, 409–412.
22. Moran, A. P.; Zähringer, U.; Seydel, U.; Scholz, D.; Stütz, P.; Rietschel, E. T. *Eur. J. Biochem.* **1991**, *198*, 459–469.
23. Westphal, O.; Jann, K. *Methods Carbohydr. Chem.* **1965**, *5*, 83–92.
24. Moran, A. P.; Rietschel, E. T.; Kosunen, T. U.; Zähringer, U. *J. Bacteriol.* **1991**, *173*, 618–626.
25. Aspinall, G. O.; Lynch, C. M.; Pang, H.; Shaver, R. T.; Moran, A. P. *Eur. J. Biochem.* **1995**, *231*, 570–578.
26. Sawardeker, J. S.; Sloneker, J. H.; Jeanes, A. *Anal. Chem.* **1965**, *37*, 1602–1603.
27. Hanniffy, O. M.; Shashkov, A. S.; Senchenkova, S. N.; Tomshich, S. V.; Komandrova, N. A.; Romanenko, L. A.; Knirel, Y. A.; Savage, A. V. *Carbohydr. Res.* **1999**, *321*, 132–138.
28. Leontein, K.; Lindberg, B.; Lönngren, J. *Carbohydr. Res.* **1978**, *62*, 359–362.
29. Moran, A. P.; Rietschel, E. T.; Kosunen, T. U.; Zähringer, U. *J. Bacteriol.* **1991**, *173*, 618–626.
30. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.
31. Jansson, P.-E.; Kenne, L.; Liedgren, H.; Lindberg, B.; Lönngren, *Chem. Commun. Univ. Stockholm* **1976**, *8*, 1–75.