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STRUCTURAL STUDIES OF THE O-ANTIGENIC POLYSACCHARIDE OF *Escherichia coli* O86, WHICH POSSESSES BLOOD-GROUP B ACTIVITY

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ABSTRACT

The O-specific side-chains of the lipopolysaccharide from *Escherichia coli* O86:K2:H2 have been investigated using n.m.r. spectroscopy, methylation analysis, and specific degradations, and shown to be composed of the pentasaccharide repeating-unit

→4)-α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→
3
↑
1
α-D-Gal*p*

which represents the biological repeating-unit. The blood-group B activity was confirmed by an enzyme-linked immunosorbent assay.

INTRODUCTION

Carbohydrate structures, exposed on the surface of bacterial cells, are important virulence factors and antigens for many pathogenic micro-organisms. Several micro-organisms possess human blood-group activity and, in Gram-negative bacteria, this specificity is often associated with the bacterial O-antigen[1]. Springer *et al.*[1,2] have shown that *E. coli* O86 has a high blood-group B activity. On the basis of chemical, enzymic, and serological investigations and by analogy with the structure of the blood-group B specific oligosaccharide, Springer *et al.*[2,3] and Kochibe *et al.*[4] proposed partial structures for the terminus of the lipopolysaccharide from *E. coli* O86 containing the blood-group B trisaccharide **1**.

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→
3
↑
1
α-D-Gal*p*

1

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It is evident that there is a similarity in the terminal structure of the lipopolysaccharide of *E. coli* O86 and the human blood-group B structure. We now report structural studies of the O-specific side-chains of the *E. coli* O86 lipopolysaccharide.

RESULTS AND DISCUSSION

The lipopolysaccharide (LPS) from *E. coli* O86:K2:H2 was delipidated to give the polysaccharide (PS). Sugar analysis of the PS and determination of the absolute configuration[5,6] revealed L-fucose, D-galactose, and 2-amino-2-deoxy-D-galactose in the relative proportions 1:2:2 as the main components.

Methylation analysis (Table I, column A) revealed terminal and 2,3-linked D-Gal*p*, 3-linked D-Gal*p*NAc, 4-linked L-Fuc*p*, and a small amount of terminal L-Fuc*p*.

The ^{1}H-n.m.r. spectrum of the PS (Table IIA and Fig. 1) contained, *inter alia*, five signals in the region for anomeric protons at δ ($J_{1,2}$) 5.22 (2 H, not resolved), 5.04 (1 H, 3.1 Hz), 4.68 (1 H, 7.0 Hz), and 4.56 (1 H, 8.4 Hz), a signal for the NAc groups at δ 2.05 (6 H), and a signal for H-6 of the 6-deoxyhexosyl residue at δ 1.21 (3 H, $J_{5,6}$ 6.2 Hz). The ^{13}C-n.m.r. spectrum of the PS (Table IIIA and Fig. 2) contained five signals for anomeric carbons at δ ($J_{C\text{-}1,H\text{-}1}$) 103.6 (164 Hz), 103.2 (164 Hz), 99.7 (173 Hz), 94.3 (170 Hz), and 94.1 (173 Hz). The last two signals appear at unusually high field for α sugars. Similar high-field signals have been observed, all associated with a typical steric arrangement of substituents around the glycosidic linkage[7–9]. In the present PS, where all the sugars have the

TABLE I

METHYLATION ANALYSES OF NATIVE O86 AND DEGRADATION PRODUCTS[a]

Sugar[b]	*T*[c]	*Mole %*					
		A	*B*	*C*	*D*	*E*	*F*
2,3,4-Fuc	0.81	3					
2,3-Fuc	0.95	20		25			
2,3,4,6-Gal	1.05	25	47	10		42	32
3,4,6-Gal	1.21			25			
2,4,6-Gal	1.22						30
4,6-Gal	1.34	19		4			
1,2,4,5,6-GalNAc	1.37				44	32	21
2,3,4,6-GalNAc	1.65				56		
2,4,6-GalNAc	1.80	33	53	35		26	27

[a]Key: A, native O86; B, Smith-degradation product; C, product after lead tetra-acetate degradation; D–F, oligosaccharides obtained by anhydrous hydrogen fluoride solvolysis and subsequent reduction with sodium borodeuteride; D, disaccharide **4**; E, trisaccharide **5**; F, tetrasaccharide **6**. [b]2,3,4-Fuc = 2,3,4-tri-*O*-methyl-L-fucose *etc*. [c]Retention time of the derived alditol acetate, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an Ultra 2 column (see Experimental).

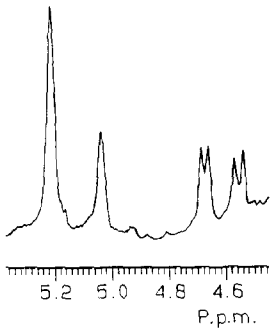


Fig. 1. ^{1}H-N.m.r. spectrum of native polysaccharide (anomeric region).

galacto configuration, only the structural element α-D-Gal*p*-(1→3)-D-Gal*p*, or a corresponding structure in which one or both of the sugars are changed to D-Gal*p*NAc, will fulfil the steric requirements. Bock *et al.*[10] have shown that the glycosylation shift for the resonances of anomeric carbons can be correlated with the distance between the protons on the anomeric and aglycon carbon atoms, respectively. In the region where signals for carbon linked to nitrogen appear, signals were found at δ 51.9 and 49.6, indicating the presence of one β and one α D-Gal*p*NAc. The ^{13}C-n.m.r. spectrum also contained signals for two NAc groups at

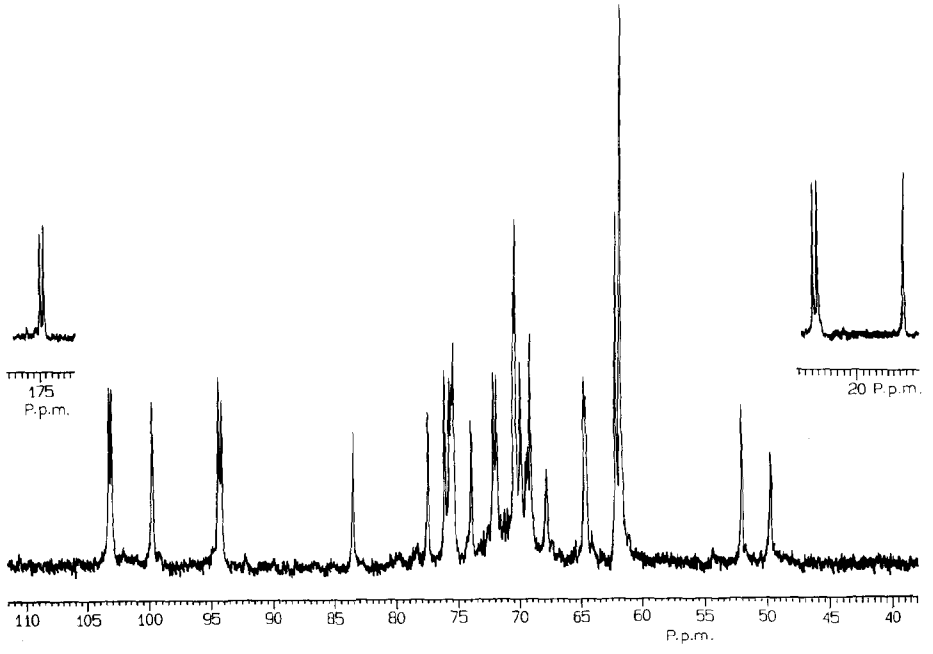


Fig. 2. ^{13}C-N.m.r. spectrum of native polysaccharide.

TABLE II

^{1}H-N.M.R. DATA OF NATIVE O86 AND DEGRADATION PRODUCTS[a,b]

A Native O86

Atom	α-D-*Galp*-(*1→3*)-β-D-*Galp*-(*1→3*)-α-D-*GalpNAc*-(*1→3*)-β-D-*GalpNAc*-(*1→4*)-α-L-*Fucp*-(*1→* 2 ↑				
H-1	5.22 (n.r.)	4.68 (7.0)	5.04 (3.1)	4.56 (8.4)	5.22 (n.r.)
H-2	3.89	3.89	4.23	4.14	3.78
H-3	3.89	3.95	3.94	3.78	3.54
H-4	3.98	4.27	4.21	4.06	3.78
H-5	4.20	3.65	3.86	3.66	4.33
H-6	3.73	3.78	3.76	3.78	1.21

NAc, 2.05 and 2.05.

B Product after lead tetra-acetate oxidation

Atom	→2)-β-D-*Galp*-(*1→3*)-α-D-*GalpNAc*-(*1→3*)-β-D-*GalpNAc*-(*1→4*)-α-L-*Fucp*-(*1→*			
H-1	4.64 (7.5)	5.07 (3.5)	4.58 (8.2)	5.22 (3.7)
H-2	3.65	4.24	4.14	3.81
H-3	3.84	3.96	3.80	3.64
H-4	3.93	4.20	4.07	3.83
H-5	3.67	3.85	3.67	4.29
H-6	3.84	3.79	3.78	1.19

NAc, 2.05 and 2.03.

C Smith-degradation product

Atom	β-D-*Galp*-(*1→3*)-α-D-*GalpNAc*-(*1→3*)-β-D-*GalpNAc*-(*1→2*)-*4-deoxy*-L-*threitol*			
H-1	4.46 (7.5)	5.07 (3.8)	4.63 (8.6)	3.73
H-2	3.52	4.38	4.08	3.63
H-3	3.62	3.86	3.80	3.91
H-4	3.92	4.22	4.09	1.16
H-5	3.63	3.86	3.64	
H-6	3.76	3.76	3.80	

NAc, 2.04 and 2.01.

[a]See Experimental. [b]Chemical shifts (p.p.m.), $J_{1,2}$ values (Hz) in brackets.

δ 23.4 and 23.0 and one signal for C-6 of the 6-deoxyhexosyl residue at δ 16.2. Thus, it is concluded that the polysaccharide is composed of pentasaccharide repeating-units containing two β- and three α-pyranosyl residues.

Smith degradation[11] of the PS followed by gel chromatography yielded a product that was eluted in the trisaccharide region. Methylation analysis (Table I, column B) showed that this product contained one terminal D-Gal*p* group and two

TABLE III

^{13}C-N.M.R. DATA OF NATIVE O86 AND DEGRADATION PRODUCTS[a,b]

A Native O86

Atom	α-D-*Galp*-(1→3)-β-D-*Galp*-(1→3)-α-D-*GalpNAc*-(1→3)-β-D-*GalpNAc*-(1→4)-α-L-*Fucp*-(1→ (2 ↑)				
C-1	94.3 (170)	103.2 (164)	94.1 (173)	103.6 (164)	99.7 (173)
C-2	69.0	73.9	49.6	51.9	69.3
C-3	70.4	75.5	77.4	75.6	70.3
C-4	70.3	64.7	69.8	64.5	83.4
C-5	72.0	75.3	71.8	76.0	67.8
C-6	62.2	61.8	61.7	61.8	16.2

NCO*C*H$_3$, 23.4 and 23.0; N*C*OCH$_3$, 175.0 and 174.7.

B Product after lead tetra-acetate oxidation

Atom	→2)-β-D-*Galp*-(1→3)-α-D-*GalpNAc*-(1→3)-β-D-*GalpNAc*-(1→4)-α-L-*Fucp*-(1→			
C-1	103.0	94.1	103.0	100.1
C-2	77.3	49.7	51.9	69.7
C-3	74.4	75.2	75.8	70.0
C-4	69.9	69.6	64.5	83.3
C-5	75.8[c]	71.8	76.0[c]	67.8
C-6	61.8	61.8	61.8	16.3

NCO*C*H$_3$, 23.3 and 23.0; N*C*OCH$_3$, 174.9 and 174.7.

C Smith-degradation product

Atom	β-D-*Galp*-(1→3)-α-D-*GalpNAc*-(1→3)-β-D-*GalpNAc*-(1→2)-4-*deoxy*-L-*threitol*			
C-1	105.5 (162)	94.7 (172)	102.6 (163)	62.5
C-2	71.7	49.1	52.0	85.8
C-3	73.5	78.0	75.8	67.9
C-4	69.5	69.4	64.7	18.5
C-5	75.8	71.9	75.8	
C-6	61.8	61.8	61.8	

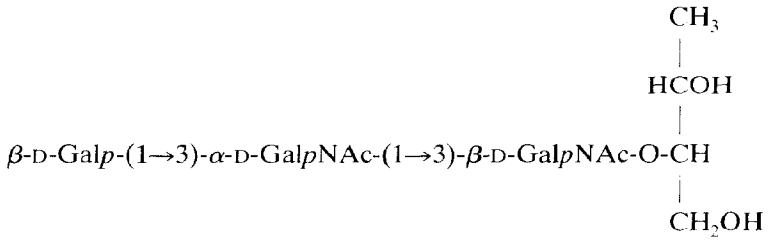
NCO*C*H$_3$, 23.2 and 23.0; N*C*OCH$_3$, 175.4 and 175.2.

[a]See Experimental. [b]Chemical shifts (p.p.m.), $J_{C\text{-}1,H\text{-}1}$ values (Hz) in brackets. [c]These assignments may be reversed.

3-linked D-Gal*p*NAc residues. The ^{1}H-n.m.r. spectrum (Table IIC) indicated, *inter alia*, the presence of one α-hexopyranosyl residue [δ 5.07 ($J_{1,2}$ 3.8 Hz)] and two β-hexopyranosyl residues [δ 4.63 ($J_{1,2}$ 8.6 Hz) and 4.46 ($J_{1,2}$ 7.5 Hz)]. The ^{1}H-n.m.r. spectrum also contained signals for NAc groups at δ 2.04 (3 H) and 2.01 (3 H), and a signal at δ 1.16 (3 H, 6.4 Hz), which could be assigned to H-4 of the 2-substituted

4-deoxy-L-threitol derived from the 4-substituted L-Fuc*p* residue. The ^{13}C-n.m.r. spectrum (Table IIIC) contained, *inter alia*, signals for anomeric carbons at δ 105.5 ($J_{C\text{-}1,H\text{-}1}$ 162 Hz), 102.6 (163 Hz), and 94.7 (172 Hz). The ^{1}H- and ^{13}C-n.m.r. spectra could be fully assigned using 2D-techniques.

The results of the methylation analysis indicated two alternative structures as one D-Gal*p*NAc residue is α and the other is β. The signal at δ 94.7, however, could be assigned to an α-D-Gal*p*NAc residue in the structural element α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc, as discussed above. The 2D-n.O.e. spectrum (NOESY) also gave connectivities consistent with these results. The oligosaccharide glycoside consequently has structure **2**.



2

Among the sugar residues in the PS, only the 4-linked L-Fuc*p* residue and the terminal D-Gal*p* group should be oxidised with periodate. The initial periodate-oxidation step in the Smith degradation was carried out under conditions that were expected to cleave all the vicinal diol groups in the polysaccharide. The terminal D-Gal*p* group, which contains *cis*-hydroxyl groups, should be more readily oxidised by periodate than the 4-substituted L-Fuc*p* residue which contains *trans*-hydroxyl groups. However, modified Smith-degradations, using 1.1–1.5 mol of periodate per repeating unit in order to selectively oxidise the former group, gave inconclusive results.

Perlin *et al.*[12,13] showed that internal glycosyl residues of many oligosaccharides, particularly those that are 4-linked and contain a 2,3-*trans*-diol, are resistant to cleavage by lead tetra-acetate in aqueous acetic acid. The PS was treated in sequence with lead tetra-acetate in aqueous acetic acid, sodium borohydride, and acid under mild conditions. Gel chromatography then yielded one main product eluted in the void volume. Methylation analysis (Table I, column C) showed that the product contained one 4-linked L-Fuc*p*, two 3-linked D-Gal*p*NAc residues, and a 2-linked D-Gal*p* residue. In addition, small amounts of 2,3-linked D-Gal*p* and terminal D-Gal*p* were detected, indicating that the oxidation was incomplete. However, it can be concluded that the D-Gal*p* unit removed on oxidation had been 3-linked to the branched D-Gal*p* residue. This result, in conjunction with the ^{1}H (Table IIB) and ^{13}C-n.m.r. (Table IIIB) data, demonstrate that the degradation product contains structure **3**.

→4)-α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→

3

Treatment[14,15] of the PS with anhydrous hydrogen fluoride for 20 min at −50° followed by the addition of 25 vol. of cold anhydrous ether[15,16], reduction with sodium borodeuteride, gel chromatography, and h.p.l.c. gave a di-, a tri-, and a

TABLE IV

^{1}H-N.M.R. DATA FOR OLIGOSACCHARIDES OBTAINED BY HYDROGEN FLUORIDE SOLVOLYSIS AND SUBSEQUENT REDUCTION WITH SODIUM BORODEUTERIDE[a,b]

Atom	α-D-*Gal*p*NAc-(1→3)*-D-*GalNAc-ol*						
H-1	5.10 (4.0)	3.76, 3.68					
H-2	4.23	4.34					
H-3	3.89	3.87					
H-4	4.04	3.70					
H-5	4.02	3.75					
H-6	3.77	3.65					
NAc, 2.05 and 2.04.							
Atom	β-D-*Gal*p*-(1→3)*-α-D-*Gal*p*NAc-(1→3)*-D-*GalNAc-ol*						
H-1	4.48 (7.3)	5.11 (3.5)	3.76, 3.68				
H-2	3.52	4.40	4.35				
H-3	3.61	4.01	3.89				
H-4	3.91	4.26	3.73				
H-5	3.64	4.05	3.77				
H-6	3.77	3.77	3.65				
NAc, 2.04 and 2.03.							
Atom	α-D-*Gal*p*-(1→3)*-β-D-*Gal*p*-(1→3)*-α-D-*Gal*p*NAc-(1→3)*-D-*GalNAc-ol*						
H-1	5.13 (3.1)	4.54 (7.2)	5.11 (3.1)	3.76, 3.68			
H-2	3.86	3.66	4.41	4.35			
H-3	3.94	3.71	4.02	3.89			
H-4	4.01	4.13	4.30	3.72			
H-5	4.18	3.66	4.06	3.75			
H-6	3.72	3.77	3.76	3.66			
NAc, 2.04 and 2.02.							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*NAc*
D-GalNAc-ol	3.73, 3.64	4.21	3.85	3.41	3.91	3.64	2.03

[a]See Experimental. [b]Chemical shifts (p.p.m.), $J_{1,2}$ values (Hz) in brackets.

TABLE V

^{13}C-N.M.R. DATA FOR OLIGOSACCHARIDES OBTAINED BY HYDROGEN FLUORIDE SOLVOLYSIS AND SUBSEQUENT REDUCTION WITH SODIUM BORODEUTERIDE[a,b]

Atom	*α*-D-*GalpNAc-(1→3)*-D-*GalNAc-ol*	
C-1	98.8	—
C-2	50.8	52.9
C-3	68.5	77.6
C-4	69.4	71.1[c]
C-5	72.6	71.2[c]
C-6	61.9	63.9

$NCOCH_3$, 23.0 and 22.9; $NCOCH_3$, 175.4 and 175.1.

Atom	*β*-D-*Galp-(1→3)*-*α*-D-*GalpNAc-(1→3)*-D-*GalNAc-ol*		
C-1	105.2	98.9	—
C-2	71.7	49.4	52.8
C-3	73.6	77.5[c]	77.6[c]
C-4	69.6	69.3	71.1
C-5	75.9	72.2	71.1
C-6	61.8	61.9	63.8

$NCOCH_3$, 23.0 and 22.9; $NCOCH_3$, 175.3 and 175.0

Atom	*α*-D-*Galp-(1→3)*-*β*-D-*Galp-(1→3)*-*α*-D-*GalpNAc-(1→3)*-D-*GalNAc-ol*			
C-1	96.6	105.2	99.0	—
C-2	69.2	70.3	49.4	52.9
C-3	70.2	78.7	78.0[c]	77.6[c]
C-4	70.2	66.1	69.3	71.1
C-5	71.8	75.6	72.2	71.1
C-6	62.0	61.8	62.0	63.9

$NCOCH_3$, 23.0 and 23.0; $NCOCH_3$, 175.3 and 175.3.

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*$NCOCH_3$*	*$NCOCH_3$*
D-GalNAc-ol	62.6	52.5	69.9	70.8	71.0	64.2	22.8	175.6

[a]See Experimental. [b]Chemical shifts (p.p.m.). [c]These assignments may be reversed.

tetra-saccharide. Methylation analysis of these saccharides (Table I, columns D–F) showed that each contained 3-linked 2-acetamido-2-deoxy-D-galactitol-*1-d* but no L-fucose derivative.

Methylation analysis of the disaccharide-alditol revealed a terminal D-Gal*p*NAc group and 3-linked 2-acetamido-2-deoxy-D-galactitol-*1-d*. In the ^{1}H- and ^{13}C-n.m.r. spectra of the disaccharide-alditol (Tables IV and V), the signals for the anomeric proton and carbon appeared at δ 5.10 ($J_{1,2}$ 4.0 Hz) and 98.8, respectively, indicating the structure **4**.

α-D-Gal*p*NAc-(1→3)-D-GalNAc-ol

4

Methylation analysis showed that the trisaccharide-alditol contained a terminal D-Gal*p* group, a 3-linked D-Gal*p*NAc residue, and 3-linked 2-acetamido-2-deoxy-D-galactitol-*1-d*. The ^{1}H- and ^{13}C-n.m.r. spectra of the trisaccharide-alditol (Tables IV and V) contained, *inter alia*, the signals for the anomeric protons and carbons at δ 5.11 ($J_{1,2}$ 3.5 Hz), 4.48 ($J_{1,2}$ 7.3 Hz), and 105.2 and 98.9, respectively, indicating the structure **5**.

β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-D-GalNAc-ol

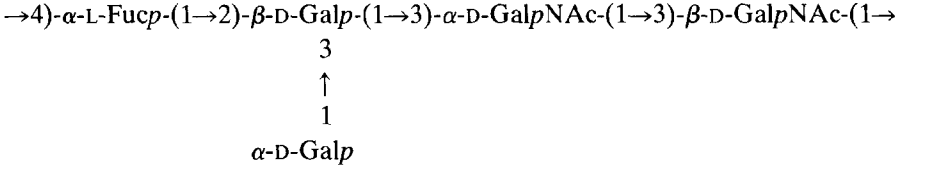
5

Methylation analysis of the tetrasaccharide-alditol revealed terminal D-Gal*p*, 3-linked D-Gal*p*, 3-linked D-Gal*p*NAc, and 3-linked 2-acetamido-2-deoxy-D-galactitol-*1-d*. In the ^{1}H- and ^{13}C-n.m.r. spectra of the tetrasaccharide-alditol (Tables IV and V), the signals for the anomeric protons and carbons appeared at δ 5.13 ($J_{1,2}$ 3.1 Hz), 5.11 ($J_{1,2}$ 3.1 Hz), and 4.54 ($J_{1,2}$ 7.2 Hz), and 105.2, 99.0, and 96.6, respectively, indicating the structure **6**.

α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-D-Gal*p*NAc-ol

6

Thus, it is concluded that the O-antigenic polysaccharide of *E. coli* O86 is composed of pentasaccharide repeating-units with the structure **7**.



7

The n.m.r. spectra of the compounds discussed could be assigned using 2D-techniques (Tables II–V). In the ^{13}C-n.m.r. spectrum of the native PS, two signals corresponding to ring carbons appeared at unusually high field (δ 64.7 and 64.5). These signals were assigned to C-4 in the β-D-Gal*p* and β-D-Gal*p*NAc residues, each 3-substituted by an α-D sugar with the *galacto* configuration[17]. In unsubstituted β-D-Gal*p* and β-D-Gal*p*NAc, the resonance of C-4 appears at δ ~69. A signal at δ

~64 was also observed in the ^{13}C-n.m.r. spectra of **2**, **3**, and **6**, but not in those of **4** and **5**. This is in agreement with the proposed assignment.

In the spectrum of the native PS, the signal at δ 83.4 was assigned to C-4 of the 4-linked α-L-Fuc*p* residue. A corresponding signal at δ 83.3 was observed in the spectrum of **3**. The ^{13}C-n.m.r. spectrum of the Smith-degradation product contained, *inter alia*, a signal at δ 85.8 which was assigned to C-2 of the 4-deoxy-L-threitol. No other signals were found in this region. In the disaccharide β-D-Glc*p*-(1→4)-α-L-Fuc*p*-O-Me, the resonance of C-4 of the α-L-Fuc*p* residue appeared at δ 81.9, compared to δ 72.8 in unsubstituted α-L-Fuc*p*[18].

In order to verify the blood-group B activity of the *E. coli* O86:K2:H2 isolate, the bacteria were agglutinated with a blood-group B specific human antiserum. The antigenicity of the purified LPS was tested in an enzyme-linked immunosorbent assay (ELISA) against the same blood-group B antisera used above. The antiserum had an ELISA end-point titer against *E. coli* O86 LPS of ~1000, whereas the binding to the negative control LPS (*E. coli* R3 rough LPS and *Salmonella typhimurium* smooth LPS) was ~10 fold lower, *i.e.*, at background level. Thus, the results confirm Springer's finding that *E. coli* O86 possesses blood-group B activity.

In the methylation analyses of native PS, a small amount of terminal Fuc*p* was detected which could be obtained only from the non-reducing end of the lipopolysaccharide. However, no signals from this residue were observed in the ^{1}H- or ^{13}C-n.m.r. spectra, probably due to overlapping signals. The PS also contains terminal α-D-Gal*p* groups. Terminal β-D-Gal*p*, present in **2**, gives a signal at δ 105.5, and a weak signal in this position was also observed in the spectrum of the product obtained on lead tetra-acetate oxidation. This β-D-Gal*p* group must have been derived from the terminal repeating-unit, in which both the α-L-Fuc*p* and α-D-Gal*p* groups have been oxidised. However, no signal in this region was observed in the spectrum of the native PS. These results therefore confirm that structure **7** represents the biological repeating-unit.

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 5890A instrument, fitted with a flame-ionisation detector, was used. Separations were performed on an Ultra 2 (cross-linked 5% phenyl methyl silicone) fused-silica capillary column, using the temperature programme 150° for 1 min, 150°→250° at 5°/min, and 250° for 20 min. G.l.c.–m.s. was performed on a Hewlett–Packard 5790–5970 instrument, using the same phase. All identifications of mass spectra were unambiguous and will not be discussed. Hydrolysis of underivatised material was performed with 4M hydrochloric acid for 2 h at 100°, and hydrolysis of methylated material was performed with 2M trifluoroacetic acid for 1 h at 120°. Methylation analyses were performed as previously described[19,20]. The

absolute configurations of fucose, galactose, and 2-acetamido-2-deoxygalactose were determined by g.l.c. of their glycosides with a chiral alcohol[5,6]. A differential refractometer was used for monitoring the effluents from gel chromatography columns. The treatment with anhydrous hydrogen fluoride was performed in a specially designed apparatus made of Teflon and Kel-F (Penninsula Laboratories Inc.). The hydrogen fluoride was dried by distillation over cobalt trifluoride. The h.p.l.c. equipment comprised a Waters Model 6000A solvent delivery system and Model U 6K injector. Separations were performed on a Waters Carbohydrate Analysis Column (3.9 × 300 mm stainless-steel column) with acetonitrile–water as eluent.

N.m.r. spectroscopy. — Spectra were recorded at 70° for solutions in D_2O, using a JEOL GX-270 instrument. Chemical shifts are reported in p.p.m. relative to internal 1,4-dioxane (δ 67.4) for ^{13}C and internal acetone (δ 2.21) for ^{1}H resonances. For assignment of ^{1}H signals, proton–proton correlation techniques were used, *e.g.*, COSY, relayed COSY, double relayed COSY, and TQF-COSY. Sequence information was obtained by NOESY experiments with mixing times of 200 and 400 ms. ^{13}C-N.m.r. spectra were assigned mainly using 2D-^{13}C–^{1}H heteronuclear correlation spectroscopy. The amounts of the oligosaccharides isolated after hydrogen fluoride solvolysis of the PS was insufficient for heteronuclear correlation spectroscopy. When assignments were not straightforward, selective decoupling experiments were used to distinguish between signals in the ^{13}C-n.m.r. spectrum.

Bacterial strain. — *E. coli* O86:K2:H2 (85:85 F) was isolated from children at a neonatal ward at Danderyd Hospital (Stockholm), and verified by Drs. F. and I. Örskov at Statens Seruminstitut (Copenhagen)[21].

Isolation and purification of the O-polysaccharide. — *E. coli* O86:K2:H2 bacteria were grown in brain heart infusion broth batch cultures (10 L). 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[22]. The LPS was treated with acetic acid (2%, pH 3.1) for 2 h at 100°, liberated lipid A was removed by centrifugation, and the supernatant solution was neutralised, dialysed, and lyophilised. The product was further purified by chromatography on a column (2.6 × 90 cm) of Bio-Gel P-10.

Enzyme-linked immunosorbent assay (ELISA)[23]. — Briefly, LPS was coated to flat-bottomed microtiter plates (A/S Nunc, Roskilde, Denmark) in coating buffer (0.05M sodium carbonate, pH 9.6) at an optimal coating dose (0.1 mL, 10 μg/mL) overnight at 20°. Residual binding sites were blocked by the addition of 1% bovine serum albumin (0.1 mL) in coating buffer. A blood-group B specific human antiserum (Anti B BCA, AB0 blood-group serum, BCA, West Chester, U.S.A.) was used. ELISA end-point titers were determined as described[23], employing an alkaline phosphatase-conjugated swine anti-human IgM (μ-chain specific) preparation (Orion diagnostica, Helsinki, Finland) in conjunction with *p*-nitrophenyl phosphate (1 mg/mL; disodium salt in M diethanolamine buffer, pH 9.6, containing mM

magnesium chloride). Absorbance was read with a Titertek Multiscan MCC spectrophotometer at 405 nm after 100 min.

Smith degradation[11]. — PS (35 mg) was dissolved in 0.1M sodium acetate buffer (10 mL, pH 3.9), and sodium metaperiodate (27 mg) was added. The solution was kept in the dark for 96 h at 4°. The excess of periodate was reduced with ethylene glycol (0.1 mL) and the product was isolated by chromatography on a column (2.6 × 90 cm) of Bio-Gel P-2. A solution of the product in water (10 mL) was treated with sodium borohydride (500 mg) for 16 h at room temperature, excess of sodium borohydride was decomposed with acetic acid, and the product was recovered by gel chromatography (as described above). The product (20 mg) was treated with 0.5M trifluoroacetic acid (5 mL) for 48 h at room temperature, the hydrolysate was concentrated to dryness, and the residue was dissolved in water and fractionated on a column of Bio-Gel P-2 (as described above). One major product, **2** (8 mg), was isolated (Table I, column B; Tables IIC and IIIC).

Degradation with lead tetra-acetate[12,13]. — The PS (31 mg) was dissolved in water (1.3 mL), acetic acid (6.2 mL) was added, and the mixture was treated with lead tetra-acetate (33 mg). After 21 h, oxalic acid (oxalic acid in acetic acid, 100 mg/mL, 0.2 mL) was added, the mixture was centrifuged, the supernatant solution was concentrated to dryness, and a solution of the residue in water was lyophilised. A solution of the product in water (10 mL) was treated with sodium borohydride (500 mg) for 15 h at room temperature, excess of reagent was decomposed with acetic acid, and the product was isolated by chromatography on a column (2.6 × 90 cm) of Bio-Gel P-2. The product (20 mg) was treated with 0.5M trifluoroacetic acid (5 mL) for 43 h at room temperature, the solution was freeze-dried, and the residue was fractionated on a column of Bio-Gel P-2 (as above). One major product, **3** (10 mg), was eluted in the void volume (Table I, column C; Tables IIB and IIIB).

Partial degradation with anhydrous hydrogen fluoride[14–16]. — The PS (50 mg) was dissolved in anhydrous hydrogen fluoride (2 mL) at −78°. The temperature was allowed to rise to −50° and the mixture was stirred thereat for 20 min. Cool anhydrous ether (50 mL) was added, and the mixture was concentrated and co-distilled with cool ether (4 × 50 mL). A solution of the product in 0.05M trifluoroacetic acid (25 mL) was kept for 30 min at room temperature, then freeze-dried. The product was dissolved in water and treated with sodium borodeuteride (100 mg) for 16 h at room temperature, the excess of reagent was decomposed with acetic acid, and the product was fractionated on a column (2.6 × 90 cm) of Bio-Gel P-2 and further purified by h.p.l.c. Three products were obtained: **4** (2 mg), **5** (2 mg), and **6** (3 mg) (Table I columns D–F, Tables IV and V).

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