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STRUCTURAL STUDIES OF THE O-SPECIFIC SIDE CHAIN OF THE LIPOPOLYSACCHARIDE FROM *Escherichia coli* O:7

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ABSTRACT

The structure of the O-specific side-chain of the lipopolysaccharide from *Escherichia coli* O:7 has been investigated, using n.m.r. spectroscopy, methylation analysis, partial hydrolysis, and Smith degradation as the principal methods. It is concluded that the polysaccharide is constructed of repeating pentasaccharide units having the structure

$$\rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow 3)\text{-}\beta\text{-D-Qui}p4\text{NAc-}(1\rightarrow 2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow,$$
$$\begin{array}{c} 3 \\ \uparrow \\ 1 \\ \alpha\text{-L-Rha}p \end{array}$$

where D-Qui*p*NAc stands for 4-acetamido-4,6-dideoxy-D-glucopyranose. The ^{13}C-n.m.r. spectrum of the polysaccharide has been interpreted completely.

INTRODUCTION

In studies of the lipopolysaccharides of Enteric bacteria belonging to different genera and causing related diseases, we have established the identity of the chemical structures and serological properties for the O-specific polysaccharide from *E. coli* O:124 and *Shigella dysenteriae* type 3[1], and *E. coli* O:58 and *Sh. dysenteriae* type 5[2], respectively. We have now investigated a pair of lipopolysaccharides from *E. coli* O:7 and *Sh. boydii* type 12, since both micro-organisms cross-react with appropriate antisera[3]. Since the cross-reactivity was incomplete, the O-specific polysaccharides of these two bacteria were expected to possess different but related structures. We now report on the structure of the O-specific side-chain of the lipopolysaccharide from *E. coli* O:7. The structural analysis of the *Sh. boydii* type 12 O-specific polysaccharide and the results of a comparative immunochemi-

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cal study of the lipopolysaccharides and polysaccharides will be reported elsewhere.

RESULTS AND DISCUSSION

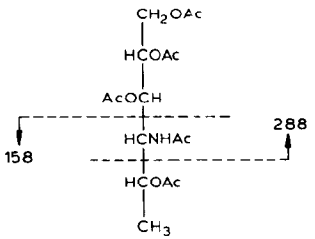
As found previously[4], the lipopolysaccharide from *E. coli* O:7 is composed of rhamnose, mannose, galactose, glucose, 2-acetamido-2-deoxyglucose, and a 4-amino-4,6-dideoxyhexose, the latter being tentatively identified as the D-*gluco* isomer.

Monosaccharide composition and n.m.r. spectra. — The lipopolysaccharide was isolated from the dried bacterial cells by extraction with hot aqueous phenol and purified by ultracentrifugation[5]. The preparation was degraded with aqueous 1% acetic acid (2 h, 100°) to give, after ultracentrifugation followed by chromatography on Sephadex G-50, a polysaccharide (PS), $[\alpha]_D$ +40° (water), which was neutral in paper electrophoresis.

Hydrolysis (4M HCl, 100°, 16 h) of PS gave (amino acid analyser) approximately equimolar amounts of 2-amino-2-deoxyglucose (17%) and an amino sugar having T_{GlcN} 1.27, which was expected to be a 4-amino-4,6-dideoxyhexose. Analysis of a hydrolysate (2M HCl, 100°, 3 h) of PS, using a Technicon sugar analyser, revealed rhamnose, mannose, and galactose (17, 16, and 20%, respectively), together with a small proportion of glucose which seemed to originate from the core moiety.

The components in the hydrolysate were deaminated[6] and then reduced with borohydride and acetylated. G.l.c. of the resulting alditol acetates revealed acetylated 2,5-anhydromannitol, rhamnitol, mannitol, and galactitol in approximately equimolar proportions, together with some 6-deoxyglucitol (indistinguishable from the authentic compound by g.l.c.–m.s.). The presence of 6-deoxyglucose after the deamination stage can be rationalised if 4-amino-4,6-dideoxyglucose was present in the original hydrolysate, since Williams and his co-workers[7] have shown that methyl α-D-glucopyranoside is the major product of deamination of methyl 4-amino-4-deoxy-α-D-glucopyranoside.

In order to identify all of the monosaccharide constituents, PS was treated with anhydrous hydrogen fluoride[8]. Depolymerisation was complete and no



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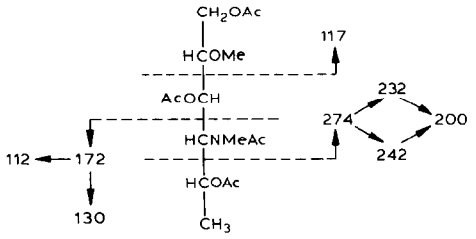

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oligosaccharides were detected by chromatography on Sephadex G-15. A portion of the resulting monosaccharide mixture was analysed as the alditol acetates by g.l.c.–m.s., which revealed acetylated rhamnitol, 4-acetamido-4,6-dideoxyhexitol (**1**), mannitol, galactitol, and 2-acetamido-2-deoxyglucitol (listed in the order of elution from an OV-1 column, 180–240°). The peaks at *m/z* 288, 246, 228, 186, 126, and 84 in the mass spectrum of **1** (see formula) corresponded to the primary C-1/C-4 fragment ($CH_2OAcCHOAcCHOAcCH{=}NHAc$) and to the secondary fragments derived therefrom by successive elimination of acetic acid and ketene, thus indicating that the acetamido group in **1** is located at position 4. Another series of peaks at *m/z* 158, 116 (158 − CH_2CO), and 98 (158 − AcOH) corresponded to the primary C-4/C-6 fragment ($CH_3CHOAc{=}NHAc$), proving that 1 was generated from a 4-acetamido-4,6-dideoxyhexose.

The polysaccharide solvolysate was subjected to preparative p.c., to give L-rhamnose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and a 4-acetamido-4,6-dideoxyhexose, the latter being slightly contaminated with L-rhamnose. The assignment of absolute configurations was based on the optical rotation data. The crude 4-acetamido-4,6-dideoxyhexose was acetylated and purified by column chromatography on silica gel. The ^{1}H-n.m.r. spectrum (250 MHz) of the resulting triacetate contained two series of signals which reflected an $\alpha\beta$-ratio of 1:1. The well-resolved signals for the ring hydrogens of the α and β anomers exhibited large coupling constants (Table I) consistent with the *gluco* configuration. The $[\alpha]_D$ value (+17°) of 4-amino-4,6-dideoxyglucose hydrochloride obtained by hydrolysis of the triacetate (2M HCl, 100°, 4 h) was similar to the literature value (+20.1°) for the D-isomer[9]. Thus, it is established that 4-amino-4,6-dideoxy-D-glucose is a constituent of the O-specific side-chain of the lipopolysaccharide from *E. coli* O:7.

The ^{1}H-n.m.r. spectrum of PS exhibited, *inter alia,* two strong doublets at δ 1.21 ($J_{5,6}$ ~6 Hz) and 1.29 ($J_{5,6}$ ~6 Hz), assigned to the CMe groups of the L-rhamnosyl group and the 4-acetamido-4,6-dideoxy-D-glucosyl residue, as well as two singlets at 1.97 and 2.03 assigned to the NAc groups of 2-acetamido-2-deoxy-D-

TABLE I

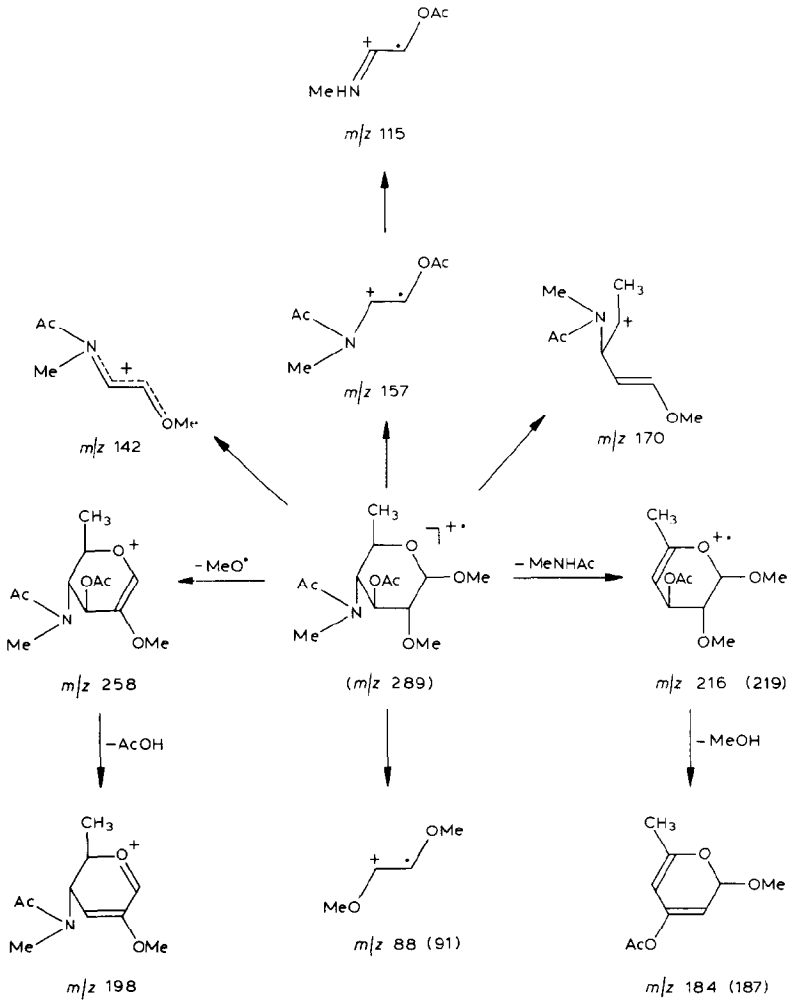
^{1}H-N.M.R. DATA ($CDCl_3$, 250 MHz) FOR 4-ACETAMIDO-1,2,3-TRI-*O*-ACETYL-4,6-DIDEOXY-α,β-D-GLUCOPYRANOSE[a]

	α Anomer		*β Anomer*	
H-1	6.27 d	($J_{1,2}$ 3.6 Hz)	5.63 d	($J_{1,2}$ 8.2 Hz)
H-2	5.05 dd	($J_{2,3}$ 10.1 Hz)	5.09 dd	($J_{2,3}$ 9.5 Hz)
H-3	5.22 t	($J_{3,4}$ 10.1 Hz)	5.03 t	($J_{3,4}$ 9.7 Hz)
H-4	4.73 ddd	($J_{4,5}$ 10.0 Hz)	4.72 ddd	($J_{4,5}$ 10.0 Hz)
H-5	4.54 dq	($J_{5,6}$ 6.0 Hz)	4.22 dq	($J_{5,6}$ 6.1 Hz)
Me-5	1.21 d	($J_{5,6}$ 6.0 Hz)	1.26 d	($J_{5,6}$ 6.1 Hz)
NH	5.50 d	($J_{NH,4}$ 9.6 Hz)	5.64 d	($J_{NH,4}$ 9.5 Hz)
AcN	1.93 s		1.93 s	

[a] δ scale. The OAc groups gave six singlets in the range δ 1.98–2.12. The signals were assigned by means of homonuclear double-resonance.

glucosyl and 4-acetamido-4,6-dideoxy-D-glucosyl residues. Two broad signals in the anomeric region at δ 4.50 (2 H) and 5.08 (3 H) indicated the repeating unit of the polysaccharide to be a pentasaccharide containing three α- and two β-linkages.

The ^{13}C-n.m.r. spectrum of PS corroborated the foregoing conclusions. The signal of double intensity at 17.8 p.p.m. was assigned to the CMe groups of L-rhamnose and 4-acetamido-4,6-dideoxy-D-glucose, and the pairs of signals at 23.3/23.6 and 174.9/175.4 p.p.m. were asigned to two NAc groups of 2-acetamido-2-deoxy-D-glucose and 4-acetamido-4,6-dideoxy-D-glucose. Two resonances for carbons linked to nitrogen were present at 53.5 and 57.6 p.p.m., corroborating the existence of two *N*-acetylhexosamines. The signals at 61.35, 61.5, and 61.65 p.p.m. were attributed to C-6 of D-mannose, D-galactose, and 2-acetamido-2-deoxy-D-



Scheme 1

glucose, and indicated that these positions were unsubstituted. Five signals were present in the anomeric region at 97.3, 98.85, 100.6, 103.9, and 104.7 p.p.m. The total number of signals in the spectrum of PS was 34, confirming a pentasaccharide repeating-unit.

Thus, the O-specific polysaccharide from *E. coli* O:7 is composed of a pentasaccharide repeating-unit containing L-rhamnose, D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and 4-acetamido-4,6-dideoxy-D-glucose.

Methylation analysis. — Methylation[10] of PS, followed by hydrolysis and conversion of the products into their alditol acetates[11], yielded approximately equimolar proportions of 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylgalactitol, and 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methylmannitol, which were identified by g.l.c.–m.s.[11] and comparison with the authentic compounds. 3-*O*-Acetyl-4,6-dideoxy-2-*O*-methyl-4-*N*-methylacetamidoglucitol (**2**, see formula for fragmentation pattern) and 3-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-*N*-methylacetamidoglucitol were also detected in comparable amounts. The detector responses of the aminoalditol derivatives, which are lower than those of the neutral sugars[12], were not quantified, and hence a precise ratio was not determined. Methanolysis of methylated PS followed by acetylation and g.l.c. of the derived, acetylated methyl glycosides revealed methyl 3-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-*N*-methylacetamidoglucopyranoside[13] and methyl 3-*O*-acetyl-4,6-dideoxy-2-*O*-methyl-4-*N*-methylacetamidoglucopyranoside. The e.i. fragmentation pattern of the latter compound is shown in Scheme 1. The peak at *m/z* 88, which shifted to a higher *m/z* value by three mass units when CD_3OD was used for the methanolysis, therefore corresponds to the primary C-1/C-2 fragment ($CH_3OCHCHOCH_3$), thus demonstrating the MeO group to be located at position 2. The appearance of the peak at *m/z* 157, corresponding to the primary C-3/C-4 fragment, proved that the AcO group occupies position 3.

These results demonstrated the repeating unit of PS to be a branched pentasaccharide. The presence of the 4,6-di-*O*-methyl-D-mannosyl residue is attributable to a branch point, and that of the 2,3,4-tri-*O*-methyl-L-rhamnosyl group to the terminal sugar of a side chain. The results also indicated that all of the sugars were pyranoid. Unequivocal evidence for the presence of a galactopyranosyl residue was obtained from the ^{13}C-n.m.r. data for PS (see below).

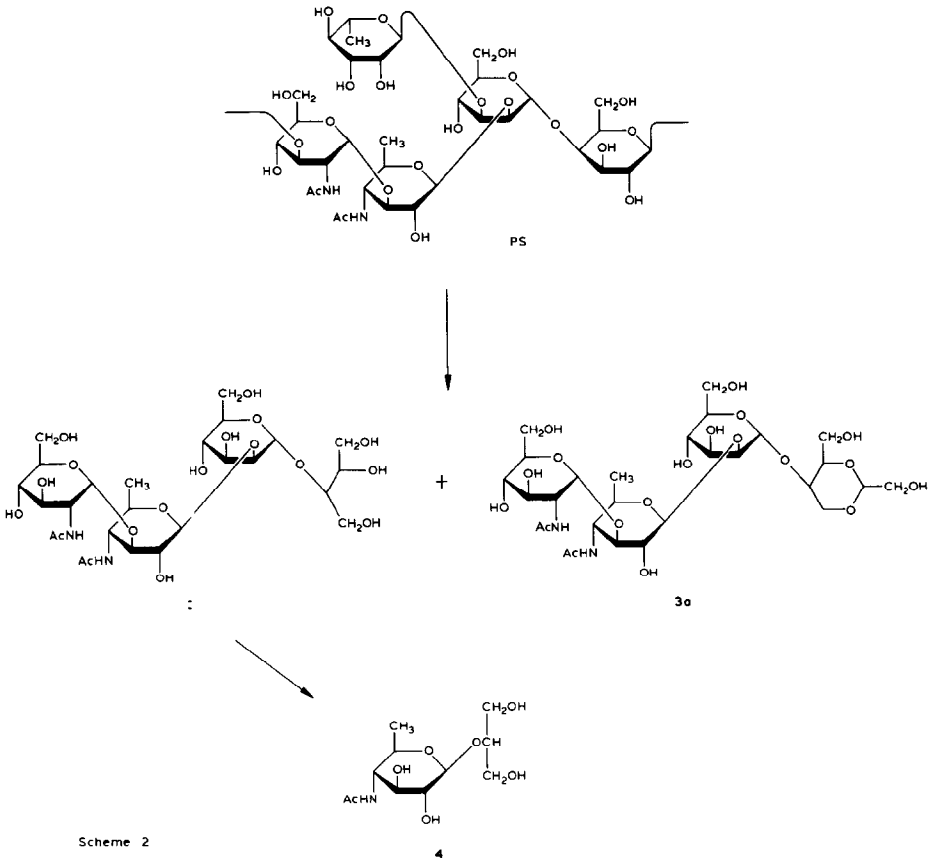
Methylated PS was also reduced with lithium aluminium hydride[12] and then hydrolysed. Analysis of the hydrolysate revealed that the neutral methylated sugars were unaltered, but that there were no hexosamine derivatives. These data strongly indicated that PS contains the sequence 2-acetamido-2-deoxy-D-glucose→4-acetamido-4,6-dideoxy-D-glucose.

Partial hydrolysis. Hydrolysis of PS with 0.3M hydrochloric acid (100°, 40 min) followed by chromatography on Sephadex G-50 gave a modified polymer (PS_M) containing 5% of the L-rhamnose content of PS. Methylation analysis of PS_M revealed a considerable loss of 2,3,4-tri-*O*-methyl-L-rhamnose and the appearance of 3,4,6-tri-*O*-methyl-D-mannose instead of 4,6-di-*O*-methyl-D-mannose.

Thus, the side chains in PS contained single L-rhamnosyl groups attached to position 3 of 2,3-disubstituted D-mannosyl residues.

The linear polysaccharide PS_M had a positive $[\alpha]_D$ value (+50.5°), which was higher than that (+40°) of PS, indicating indirectly that the L-rhamnosyl groups in PS are α.

Smith degradation. — Treatment of PS, in sequence, with periodate, borohydride, and mild acid (Smith degradation[14]) gave oligosaccharides **3** and **3a**, which were isolated by chromatography on Sephadex G-15 followed by preparative p.c. Oligosaccharide **3** (lower R_F) was the main product and treatment with anhydrous hydrogen fluoride followed by sugar analysis (g.l.c. of alditol acetates) revealed it to contain D-mannose, 4-acetamido-4,6-dideoxy-D-glucose, 2-acetamido-2-deoxy-D-glucose, and threitol, in equimolar proportions. The ^{13}C-n.m.r. spectrum of **3** (see Table II) contained 26 signals, which accorded with its monosaccharide composition, and included three for anomeric carbons, four (61–64 p.p.m.) for hydroxymethyl groups of D-mannose, 2-acetamido-2-deoxy-D-glucose, and threitol, one for C-6 of the 4-acetamido-4,6-dideoxy-D-glucosyl residue, and two pairs for the AcN groups of the amino sugar residues. Moreover, the downfield location (54.95



Scheme 2

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS (p.p.m.) IN D_2O[a]

		C-1	C-2	C-3	C-4	C-5	C-6
PS	→3)-α-D-Glc*p*NAc-(1→	98.85	53.5	81.9	69.4	72.5	61.65[b]
	→3)-β-D-Qui*p*4NAc-(1→	103.9	74.3	79.35	57.3	72.2[c]	17.8
	→2)-α-D-Man*p*-(1→	100.6	74.3	75.15	65.9	73.3[c]	61.5[b]
	3 ↑						
	→4)-β-D-Gal*p*-(1→	104.7	72.0	73.3	78.0	76.15	61.35[b]
	α-L-Rha*p*-(1→	97.3	71.15	71.65	73.3	69.9	17.8
PS_M	→3)-α-D-Glc*p*NAc-(1→	98.85	53.6	81.7	69.45	72.5	61.7[d]
	→3)-β-D-Qui*p*4NAc-(1→	103.0	74.3	79.2	57.4	72.5	17.8
	→2)-α-D-Man*p*-(1→	100.6	78.7	70.9	68.1	73.3	61.7[d]
	→4)-β-D-Gal*p*-(1→	104.7	72.0	73.3	78.4	76.3	61.4[d]
3	α-D-Glc*p*NAc-(1→	98.8	54.95	72.4	70.85	72.85	61.8[f]
	→3)-β-D-Qui*p*4NAc-(1→	103.2	74.4	79.2	57.7	72.2[e]	17.8
	→2)-α-D-Man*p*-(1→	100.7	79.2	70.85	68.25	73.4[e]	62.5[f]
	→2)-L-Thr-ol	61.25	81.2	72.4	63.7		
4	β-D-Qui*p*4NAc-(1→	103.7	74.8	74.8	57.95	72.45	18.05
	→2)-Glycerol	62.4	80.7	62.4			
→4)-β-D-Gal*p*-(1→[g]		103.9	71.8	73.8	78.3	76.3	61.2
Me β-D-Qui[h]		104.3	74.5	76.7	76.2	73.0	17.8
Me α-L-Rha[h]		101.9	71.0	71.3	73.1	69.4	17.7
Me α-D-Glc*p*NAc[i]		98.6	54.25	71.9	70.4	72.2	61.4
Me 3-*O*-Me-α-D-Glc*p*NAc[i]		98.7	52.4	81.1	69.6	72.2	61.2
2-*O*-Me-α-D-Man*p*[j]		91.8	81.6	71.0	68.3	73.3	62.1

[a]δ scale. CH_3OH (δ 50.15) was used as the internal standard in the calculation of chemical shifts. [b–f]Groups of several peaks, the assignments for which may be interchangeable. [g]Ref. 24. [h]Ref. 19. [i]Ref. 22. [j]Ref. 23.

p.p.m.) of the C-2 resonance for the 2-acetamido-2-deoxy-D-glucosyl residue of **3**, as compared with that (53.5 p.p.m.) for PS, strongly indicated that a 2-acetamido-2-deoxy-D-glucosyl group was the non-reducing terminus in **3** (see below). This inference was confirmed when Smith degradation of **3** followed by borohydride reduction gave glycoside **4** (see Table II for the chemical shifts) composed of 4-acetamido-4,6-dideoxy-D-glucose and glycerol. It also follows that the D-mannosyl residue was linked to threitol as shown in Scheme 2.

The structure of the oligosaccharide **3a** merits comment. The yield reached 25% and did not decrease on repeated mild hydrolysis with acid. Comparison of the ^{13}C-n.m.r. spectra of **3** and **3** + **3a** demonstrated that **3** and **3a** differed only in the aglycon moiety. The ^{13}C signal at 100.7 for **3** and assigned to C-1 of the D-mannosyl residue (see below) was observed at 101.0 p.p.m. for **3a**, reflecting the sensitivity of the C-1 resonance to changes in the aglycon. Comparison of the ^{13}C-n.m.r. data also revealed that the aglycon in **3a** exhibited six resonances, two of which (62.1 and 63.5 p.p.m.) were assigned to two hydroxymethyl groups, one (101.8 p.p.m.) was assigned to an acetal carbon, and three (71.35, 73.35, and 80.3 p.p.m.) were in the region for ring non-anomeric carbons. Thus, **3a** is a 2-hydro-

xyethylidene derivative of **3**, the threitol residue of which is involved in cyclic acetal formation with glycolaldehyde, the C-1/C-2 fragment produced from the 4-*O*-substituted galactopyranosyl residue on Smith degradation. Such cyclic acetals retaining C-1/C-2 moieties of the degraded pyranose ring were observed on Smith degradation of (1→4)-glycans[14,15], and this reaction can result in the formation of a five-[15] or six-membered[14] cyclic derivative. The ^{13}C resonances of the acetal carbons in analogous *m*-dioxane and *m*-dioxolane systems occur at 99–102 and 110–112 p.p.m., respectively[16]. Thus, the ^{13}C resonance (101.8 p.p.m.) corresponding to the acetal carbon of the glycolaldehyde in **3a** is consistent with the six-membered *m*-dioxane structure of the cyclic acetal.

Linkage configuration. — Assignment of the anomeric configurations in PS was based on ^{13}C-n.m.r. spectroscopy (gated-decoupling technique). The $^1J_{C,H}$ values for the anomeric signals at 104.7 and 103.9 p.p.m. were 160 Hz, and those for the signals at 100.6, 98.85, and 97.3 p.p.m. were ~170 Hz. Therefore, the two downfield signals originate from C-1 of β-linked units and the three upfield signals from C-1 of α-linked residues[17]. Comparison of the ^{13}C-n.m.r. data for PS, PS_M, oligosaccharide **3**, and glycoside **4** allowed assignment of all of the signals for the anomeric carbons. The signal at 103.9 p.p.m. for PS, which was also given by **3** and **4**, originates from C-1 of the 4-acetamido-4,6-dideoxy-β-D-glucose residue. Further, the signals at 100.7 and 98.9 p.p.m. for **3** were assigned to C-1 of α-D-mannose and 2-acetamido-2-deoxy-α-D-glucose, respectively. Comparison of ^{13}C-data for PS and PS_M revealed a strong decrease in the intensity of the signal at 97.3 p.p.m., thus confirming its assignment to the α-L-rhamnopyranosyl group in the side chain; hence, the fifth signal (104.7 p.p.m.) for PS originated from the D-galactosyl residue. In the gated-decoupling spectrum, this signal gave a doublet with $^1J_{C,H}$ 160 Hz, confirming that it originated from a β-pyranose residue (*cf.* 175 and 172.3 Hz, respectively, for methyl α- and β-D-galactofuranosides).

^{13}C-N.m.r. spectrum of PS. — The complete interpretation given in Table II was accomplished on the basis of the data for PS_M, oligosaccharide **3**, glycoside **4**, and model glycosides. The signals for **4** were identified by using the data for methyl 6-deoxy-β-D-glucopyranoside[19], taking into consideration the magnitudes of the α- and β-effects for substitution of HO-4 by an acetamido group[20,21].

The signals of the terminal 2-acetamido-2-deoxy-α-D-glucopyranosyl group and 2-substituted α-D-mannopyranosyl residue in the spectrum of **3** were assigned by comparison with data for methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[22] and 2-*O*-methyl-α-D-mannopyranose[23], respectively. The ^{13}C data for methyl 2-acetamido-2-deoxy-3-*O*-methyl-α-D-glucopyranoside[22] and methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside[24] confirmed the assignments of the signals of the 3-subsituted 2-acetamido-2-deoxy-α-D-glucopyranosyl and the 4-substituted β-D-galactopyranosyl residues. The signals of the terminal α-L-rhamnopyranosyl group were identified by comparison with the data for PS and PS_M, and were in reasonable agreement with those for methyl α-L-rhamnopyranoside[19]. Similarly, comparison of the ^{13}C data for PS and PS_M al-

lowed the signals belonging to the 2,3-disubstituted α-D-mannopyranosyl residue to be assigned. Thus, the interpretation of the ^{13}C-n.m.r. data accords with the chemical evidence for the structure of PS.

EXPERIMENTAL

General methods. — P.c. was performed on Whatman No. 1 paper with 1-butanol–pyridine–water (6:4:3; solvent *A*), and electrophoresis (27 V/cm) on the same paper with 0.025M pyridine–acetic acid (pH 4.5) or 0.05M triethylammonium hydrogencarbonate. Reducing sugars were detected with alkaline silver nitrate (pretreatment with KIO_4 for non-reducing sugars) and amino sugars with ninhydrin. Sugar analysis was performed with a Technicon SC-2 Analyser, using 0.5M sodium tetraborate (pH 8.9). Amino sugars were analysed with an Amino Acid Analyser BC-200 (BIO-CAL), using a column (27 × 0.9 cm) of Chromex UA-8 resin and elution with 0.35M sodium citrate buffer (pH 5.28) at 65°. G.l.c. was performed with a Pye Unicam Model 104 instrument, using glass columns (150 × 0.4 cm) containing *A*, ECNSS-M on Gas Chrom Q (100–200 mesh); and *B*, OV-1. G.l.c.–m.s. was performed with a Varian-MAT GNOM 111 instrument and the above liquid phases. Gel chromatography was effected on Sephadex G-50 (column, 80 × 4 cm) and G-15 (column, 100 × 1.5 cm) with a pyridine–acetate buffer (pH 4.5). Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter. N.m.r. spectra (^{1}H, ^{13}C) were recorded with a Bruker–Physik WP 250 spectrometer. Evaporations were effected under diminished pressure below 40°.

Materials. — The lipopolysaccharide, isolated[4] from *E. coli* O:7, was treated (1-g portions) with aqueous 1% acetic acid (110 mL, 2 h, 100°). The precipitate of lipid A was removed by centrifugation, and the supernatant solution was freeze-dried. The residue was fractionated on Sephadex G-50, to give a polysaccharide (PS, 540 mg), $[\alpha]_D$ +40° (*c* 1, water), and an oligosaccharide fraction (190 mg).

Investigation of PS. — (*a*) *Monosaccharide composition.* PS (2 mg) was hydrolysed with 2M HCl (2 mL, 100°, 3 h) for analysis of the neutral monosaccharides, and 4M HCl (100°, 16 h) for identification of the amino sugars. The hydrolysates were concentrated, and the residues were dried *in vacuo* over KOH. Identification of neutral and amino sugars and their simultaneous quantification after deamination[6] were carried out as described previously[21,25].

PS (110 mg) was treated with anhydrous hydrogen fluoride (7 mL, distilled over CoF_3) in a Teflon vessel for 3 h at 20–25°. Hydrogen fluoride was then evaporated *in vacuo*, the residue was treated with aqueous 10% acetic acid (75 mL), and the solution was concentrated. A solution of a portion (1 mg) of the residue in water (0.5 mL) was treated with sodium borohydride (15 mg) overnight at 4°. After routine work-up, the resulting alditols were treated with acetic anhydride (0.5 mL) and pyridine (0.5 mL) for 0.5 h at 100°, and the products were analysed by g.l.c.–m.s. (column *B*).

The remainder of the solvolysate was subjected to preparative p.c. (solvent

A), to give L-rhamnose (5.2 mg), $[\alpha]_D$ +8° (*c* 0.5, water) (lit.[26] $[\alpha]_D$ +9.1°); 2-acetamido-2-deoxy-D-glucose (8.3 mg), $[\alpha]_D$ +45° (*c* 0.8, water) (lit.[26] $[\alpha]_D$ +41.3°); D-mannose (11.2 mg), $[\alpha]_D$ +11.5° (*c* 0.9, water) (lit.[26] $[\alpha]_D$ +14.2°); D-galactose (14.2 mg), $[\alpha]_D$ +79° (*c* 1, water) (lit.[26] $[\alpha]_D$ +81.1°); and a mixture (15.3 mg) of 4-acetamido-4,6-dideoxy-D-glucose and L-rhamnose. The mixture was acetylated conventionally and the products were eluted from a column (12 × 0.8 cm) of silica gel (100–200 mesh) with benzene–acetone (7:3), to give 4-acetamido-1,2,3-tri-*O*-acetyl-4,6-dideoxy-α,β-D-glucopyranose (13 mg). The material was hydrolysed with 2M HCl (100°, 2 h), to give 4-amino-4,6-dideoxy-D-glucose hydrochloride (8.1 mg), $[\alpha]_D$ +17° (*c* 0.85, water), lit.[9] $[\alpha]_D$ +20.1° (*c* 0.76, water).

(*b*) *Partial hydrolysis.* PS (50 mg) was hydrolysed with 0.3M HCl (5 mL) for 40 min at 100°, and the hydrolysate was freeze-dried. Fractionation of the residue on a column of Sephadex G-50 gave PS_M (28 mg), $[\alpha]_D$ +50.5° (*c* 1, water). A portion (1 mg) of PS_M was subjected to sugar analysis (after treatment with hydrogen fluoride[8], as described above) and another (2 mg) to methylation analysis.

(*c*) *Smith degradation.* A solution of PS (117 mg) in 0.1M sodium metaperiodate (30 mL) was kept for 60 h at 20° in the dark. Sodium borohydride (350 mg) was added with cooling, and the mixture was stirred for 4 h. The excess of borohydride was decomposed with 50% acetic acid, and the product (89 mg), isolated by gel filtration on a column of Sephadex G-50, was treated with 0.5M HCl (50 mL) at room temperature for 48 h. The solution was freeze-dried, and the residue behaved as a trisaccharide when isolated by gel filtration on a column of Sephadex G-15. Preparative p.c. (solvent *A*) then gave oligosaccharides **3** (R_{Rha} 0.29, 27.5 mg) and **3a** (R_{Rha} 0.35, 6.9 mg). A portion (1 mg) of **3** was subjected to sugar analysis as above for PS and PS_M.

Oligosaccharide **3** was Smith-degraded essentially as described above. After borohydride reduction, the product was treated with KU-2 (H^+) resin, and boric acid was removed conventionally. A sample (1.5 mg) of the residue was hydrolysed with 2M HCl (1 mL, 100°, 3 h). The hydrolysate contained (amino acid and sugar analysis) 4-amino-4,6-dideoxy-D-glucose, but no 2-amino-2-deoxy-D-glucose or D-mannose. The remaining material was treated with 0.5M HCl for 18 h at room temperature. The hydrolysate was neutralised with 2M NaOH and freeze-dried. The product was reduced with sodium borohydride and, after the usual work-up, purified by preparative p.c. (solvent *A*), to give the glycoside **4** (5.1 mg), R_{Rha} 0.95.

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