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Structural analysis of the heptose/hexose region of the lipopolysaccharide from *Escherichia coli* K-12 strain W3100*

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ABSTRACT

The disaccharide L-*glycero*-D-*manno*-heptosyl-D-glucose was isolated from the lipopolysaccharide (LPS) of *Escherichia coli* K-12 strain W3100 after partial hydrolysis with acid, and the structure was determined by methylation analysis, n.m.r. spectroscopy, and comparison with a synthetic standard. In addition, the oligosaccharides L,D-Hep–D-Glc–D-Glc and L,D-Hep–D-Glc–D-Glc-D-Glc were isolated, and their structures were established by g.l.c.–m.s. and methylation analysis. The results indicated that L-*glycero*-D-*manno*-heptose, a characteristic constituent of the inner core region, may also occur in the outer core region which, in *E. coli*, is generally composed of hexoses. A revised structure of the carbohydrate backbone of the hexose/heptose region of the LPS is given.

INTRODUCTION

Many lipopolysaccharides (LPS), particularly those of the Enterobacteriaceae, consist of three defined regions, namely, the O-side-chain, the core, and the lipid A region[1]. Whereas the biological function of the O-side-chain, *e.g.*, as a serotype-specific antigen or as a receptor for bacteriophages, is well documented, that of the core oligosaccharide is not fully understood. Since many details of the structure of the core region are not known, the core types Ra, R1, R2, R3, R4, and K-12, which occur in many enterobacterial species, are being investigated. The results[2–5] indicate that these core types have a common general architecture. The lipid A-proximal inner core is composed of L-*glycero*-D-*manno*-heptose and 3-deoxy-D-*manno*-octulosonic acid (Kdo), whereas the outer core contains glucose, galactose, and 2-acetamido-2-deoxy-D-glucose. We have reported on patterns of substitution that differ from this general structure, namely, the occurrence of hexoses in the inner core region, the substitution of Kdo with galactose[6] and rhamnose[7,8], and the presence of a 2-amino-2-deoxy-D-glucose residue in the heptose region[9].

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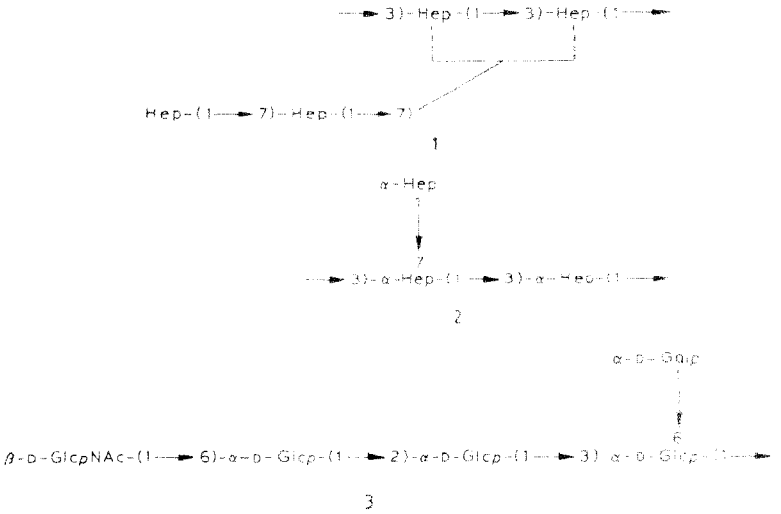
The structure of the core region of the LPS from *E. coli* K-12 has been investigated by several groups. Prehm *et al.*[2], who worked on strain W3100, proposed the branched heptose tetrasaccharide **1** to be present in the inner core region, whereas Jansson *et al.*[3] and Wollin[4], who investigated strain W3110, suggested only three heptose residues, arranged as shown in **2**, and proposed structure **3** for the outer core. These conflicting results prompted a reinvestigation of the structure, and an L-*glycero*-D-*manno*-heptosyl-glucose disaccharide was detected which showed unequivocally one heptose residue to be linked to the hexose region. This structure was not detected in earlier investigations, irrespective of the bacterial strain used. We now report a revised structure of the K-12 core region.



1 2 3

EXPERIMENTAL

General. — *Escherichia coli* K-12 strains W3100 (obtained from Dr. G. Schmidt, Forschungsinstitut Borstel) and W3110 (ATCC 27325, obtained from the American Type Culture Collection) were used. L-*glycero*-D-*manno*-Heptopyranose was a gift from Professor Dr. H. Paulsen (University of Hamburg).

The cultivation of bacteria and the isolation of the LPS were performed as described[8]. The yields (based on the dry weight of the bacteria) were 3.5‰ (strain W3100) and 1.8‰ (strain W3110). Analytical and general methods for reduction, acetylation, high-voltage paper electrophoresis (p.e.), gel-filtration on Bio-Gel P2 (Bio-Rad), and the conditions for g.l.c.–m.s. were as described[9,10]. The temperature programmes in g.l.c. were 150° for 5 min, then 5°/min →300° for **7**–**9**, 180° for 5 min, then 5°/min →300° for **10**, and 150° for 3 min, then 3°/min →250° at 0.07 MPa for methylation analysis.

The ^{1}H- and ^{13}C-n.m.r. spectroscopy on solutions of **5** in C_5D_5N (internal Me_4Si) and OS-HF in D_2O (internal CH_3CN, 1.70 p.p.m.) was performed as described[9].

Methylation involved a modified[10] Hakomori[11] procedure. Purification of the methylated samples was performed as described by Waeghe *et al.*[12]

Preparation of O-*deacylated and dephosphorylated LPS (LPS-HF).* — The LPS was dried over P_2O_5 and treated with hydrazine (25 mg/mL, 37°, 30 min). Acetone was added to the suspension (acetone–hydrazine, 15:1 by vol., under cooling), and the sediment (*O*-deacylated LPS, 80%) was washed several times with acetone, then dried. Aqueous HF (48%) was added (15 mg/mL), and the mixture was stirred for 48 h at 4°, then neutralised with 4M NaOH, dialysed, and lyophilised to give LPS-HF (43.5%).

Smith degradation. — A solution of LPS-HF (70 mg) in 0.04M sodium periodate (50 mL) was stored for 120 h at 4° in the dark. Ethylene glycol (445 μL) was added, the mixture was stirred at room temperature for 1 h, then neutralised, reduced ($NaBH_4$), and dialysed, and the product was hydrolysed with aqueous 1% acetic acid (90 min, 100°). The hydrolysate was centrifuged (~2500*g*), and the product of Smith degradation, present in the supernatant solution, was isolated using Bio-Gel P2.

Preparation of the dephosphorylated core-oligosaccharide (OS-HF). — LPS-HF (45 mg) was hydrolysed with aqueous 1% acetic acid (90 min, 100°), the hydrolysate was centrifuged (100 000*g*), then lyophilised, and the residue (27 mg, 60%) was fractionated on Bio-Gel P2 to give OS-HF (20 mg, 44%), part of which was purified by preparative p.e. for quantitative and methylation analyses.

*Isolation and purification of 1,2,3,4-tetra-*O*-acetyl-6-*O*-(2,3,4,6,7-penta-*O*-acetyl-*L*-glycero-α-*D*-manno-heptopyranosyl)-*D*-glucopyranose* (**5**). — LPS (180 mg) was hydrolysed (70 min, 100°) in M HCl and the hydrolysate was centrifuged (~2500*g*). The sediment was hydrolysed as described for LPS, the combined supernatant solutions were concentrated, and the residue was fractionated on Bio-Gel P2. The fractions obtained were acetylated (16 h, room temperature, acetic anhydride–pyridine–4-dimethylaminopyridine) and the products were analysed by t.l.c. on Silica Gel 60 (Merck), using toluene–acetone (5:1). The fraction that contained acetylated disaccharide was purified further on a column (18 × 1 cm) of Silica Gel 60, using toluene–acetone (5:1). The fraction that contained acetylated disaccharide was subjected to h.p.l.c., using two connected columns (250 × 8 mm) of Nucleosil 50 (5 μm; Bischoff, F.R.G.) and chloroform–ethyl acetate (32:1) at 2.5 mL/min and 10.3 MPa on a two-pump gradient system (Gilson 302), to give purified **5** (2.25 mg, 1.25% of the LPS).

Isolation of the methylated Hep–Glc–Glc-ol (**7**) *and Hep–Glc–Glc–Glc-ol* (**8**). — OS-HF (40 mg) was hydrolysed (6 h, 100°) in 0.1M trifluoroacetic acid. The products in the hydrolysate were fractionated by liquid chromatography, using a Büchi system [681 pump and a borosilicate 3.3 column (920 × 26 mm)] and TSK-40 (Merck) in deionised water. The flow rate was ~2 mL/min at 0.3 MPa. The second of three fractions contained di-, tri-, and tetra-saccharides, which were reduced (NaB^2H_4), methylated, and then fractionated on a SEP-PAK C_{18} cartridge[12]. The fractions eluted with acetonitrile–water, 1:5 (*a*) and 1:2 (*b*), were further fractionated by h.p.l.c. [LiChrosorb RP-18 (5 μm) column (250 × 4.6 mm), Bischoff, F.R.G.], using linear gradients of aqueous 10% acetonitrile→acetonitrile during 75 min, then acetonitrile for 5 min for (*a*); and aqueous 20% acetonitrile→acetonitrile during 75 min, then acetonitrile for 5 min for (*b*). Fractions were monitored by t.l.c. on Silica Gel 60 (Merck) with detection by charring with sulfuric acid. The appropriate fractions were subjected to g.l.c. H.p.l.c.

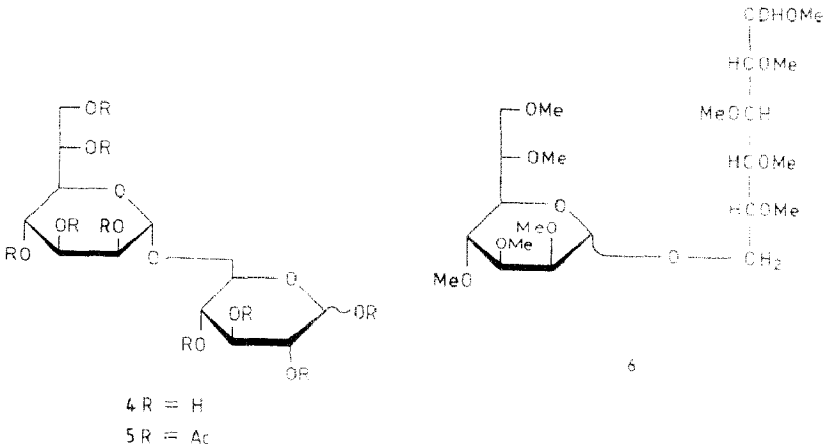
of fraction (*a*) gave pure methylated Hep–Glc–Glc-ol, which was eluted at 36 min, and of (*b*) gave pure methylated Hep–Glc–Glc–Glc-ol, which was eluted also at 36 min.

Methylation analyses. — OS-HF was methylated[10,11], and the product was hydrolysed (4 h, 100°) with 8M trifluoroacetic acid, followed by reduction (NaB^2H_4) and acetylation (16 h, 37°, acetic anhydride–pyridine–4-dimethylaminopyridine). In methylation analyses of saponified and reduced (NaB^2H_4) **5**, methylated Hep–Glc–Glc-ol (**7**), and Hep–Glc–Glc–Glc-ol (**8**), hydrolyses were done successively in aqueous 90% formic acid (3 h, 100°) and 4M trifluoroacetic acid (4 h, 100°).

The reduced ($NaBH_4$) product from the Smith degradation was methylated, carboxyl-reduced (NaB^2H_4), and remethylated with CD_3I, and the product was subjected to g.l.c.–m.s. After successive hydrolyses in aqueous 90% formic acid (3 h, 100°) and 4M trifluoroacetic acid (4 h, 100°), reduction (NaB^2H_4), and acetylation, the products were analysed by g.l.c.–m.s.

RESULTS

Identification of 1,2,3,4-tetra-O-acetyl-6-O-(2,3,4,6,7-penta-O-acetyl-L-glycero-α-D-manno-heptopyranosyl)-D-glucopyranose (**5**). — After partial hydrolysis (M HCl) of the LPS from strain W3100 and fractionation of the hydrolysate on Bio-Gel P2, **4** was isolated and acetylated to give **5**. The e.i.-mass spectral data for **5** were identical to those described for the synthetic counterpart[13].



After *O*-deacetylation (methanolic 0.25M sodium methoxide) of **5**, the product **4** was reduced (NaB^2H_4) and then methylated to give **6** [mol wt. 515, *m/z* 516 $(M + 1)^+$ and 533 $(M + 18)^+$], which showed the same e.i.-mass spectrum as that for the synthetic derivative[13]. Methylation analysis of **6** gave 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methyl-(1-^{2}H)glucitol and 1,5-di-*O*-acetyl-2,3,4,6,7-penta-*O*-methyl-(1-^{2}H)heptitol in the molar ratio ~ 1:1.

^{1}H-N.m.r. spectroscopy of **5** (Table I) revealed signals of an L,D-heptopyranoside, the H-1′ resonance of which was shifted 1.7 p.p.m. to higher field compared to that of

TABLE I

^{1}H-N.m.r. data (360 MHz, C_6D_6, internal Me_4Si) of **5**α and **5**β (isolated from the LPS of *E. coli* K-12), L,D-heptopyranose hexa-acetate, and α- and β-D-glucopyranose penta-acetate

Assignment	**5**				L,D-*Hep*		D-*Glc*			
	δ (p.p.m.)		J *(Hz)*		*δ (p.p.m.)*	J *(Hz)*	*δ (p.p.m.)*		J *(Hz)*	
	α	β	α	β			α	β	α	β
H-1	6.64	5.73	3.9	7.4			6.59	5.82	3.6	8.3
H-2	5.31	5.39	10.4[a]				5.23	5.39	10.4[a]	
H-3	5.86	5.39	10.5[a]				5.80	5.39	9.4[a]	
H-4	5.24	5.14	10.4	9.2			5.32	5.28	9.8[a]	
H-5	4.15	3.22	5.7	6.2			4.09	3.25	4.4	4.4
H-6a	3.59	3.50	11.2	10.8			4.29	4.01	12.3	12.6
H-6b	3.38	3.26	3.5	3.4			4.04	3.96	2.2	2.1
H-1′	4.52	4.63	1.5	1.7	6.22	1.8				
H-2′	5.59	5.62	3.2	3.1	5.51	3.5				
H-3′	5.64	5.70	9.7	9.6	5.57	10.5				
H-4′	5.79	5.79	10.3	10.3	5.75	10.2				
H-5′	4.43	4.29	2.4	2.2	4.14	2.3				
H-6′	5.72	5.64	4.4	4.7	5.56	4.8				
H-7′a	4.63	4.50	12.1	11.9	4.41	11.9				
H-7′b	4.40	4.40	8.2	8.2	4.25	7.6				

[a] Non-resolved high-order multiplet.

TABLE II

^{13}C-N.m.r. data[a] (in p.p.m., 90.6 MHz, C_6D_6, internal Me_4Si) of the anomers of **5** isolated from the LPS of *E. coli* K-12, L,D-heptopyranose hexa-acetate, and α- and β-D-glucopyranose penta-acetate

Assignment	**5**[c]		L,D-*Hep*[b]	D-*Glc*[c]	
	α	β		α	β
C-1	89.48	92.19		89.2	91.8
C-2	69.81	71.09		69.2	70.5
C-3	70.47	73.41		69.9	72.8
C-4	69.93[d]	69.65[d]		68.0	68.1
C-5	70.81	73.15		69.9	72.8
C-6	67.55	67.04		61.6	61.7
C-1′	98.06	98.27	91.19		
C-2′	70.04	69.27	68.94		
C-3′	69.93[d]	69.65[d]	67.18		
C-4′	65.02	65.05	64.69		
C-5′	69.93[d]	69.70	71.47		
C-6′	67.86	67.53	69.61		
C-7′	63.21	63.10	62.77		

[a] Other signals: $COCH_3$ (13 s) 168–171 p.p.m., $COCH_3$ (8 s) 20–21 p.p.m. [b] Assignment by ^{1}H–^{13}C-COSY-n.m.r. [c] Data taken from ref. 24. [d] Non-resolved signal.

authentic acetylated L-α-D-heptopyranose, proving its involvement in a glycosidic linkage. A GATED-^{13}C-n.m.r. experiment showed[14] the heptopyranose residue to be α ($J_{C-1,H-1'}$ 171 Hz). Furthermore, shifts of the resonances for H-6a (0.7 p.p.m. for the α and 0.5 p.p.m. for the β anomer) and H-6b (0.6 p.p.m. for the α and 0.7 p.p.m. for the β anomer) of the glucose residue to higher field compared to those of α- and β-D-glucopyranose penta-acetate identified a (1→6) linkage between heptose and glucose.

The ^{13}C-n.m.r. spectrum (Table II) confirmed these results, in showing shifts to higher field for C-6 (~6 p.p.m.) and C-1′ (~7 p.p.m.). The n.m.r. data for **5** were identical with those[13] for the synthetic compound. Thus, **4** is 6-*O*-L-*glycero*-D-*manno*-heptopyranosyl-D-glucopyranose.

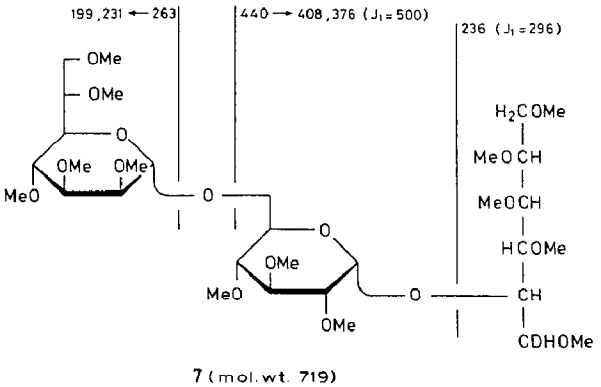
Isolation and identification of methylated 2-O-{6-O-L-glycero-D-manno-*heptopyranosyl-glucopyranosyl}glucitol (**7**) *and* 3-O-{2-O-{6-O-L-glycero-D-manno-*heptopyranosyl-glucopyranosyl}glucopyranosyl}glucitol (**8**). — After partial hydrolysis of OS-HF (0.1M trifluoroacetic acid), fractionation on TSK-40, and methylation of the appropriate fractions, **7** and **8** were obtained by h.p.l.c. on LiChrosorb RP-18, but in amounts too small for n.m.r. studies. The *T* values are listed in Table III, and the e.i.-mass spectra and fragmentation patterns are shown in Figs. 1 and 2. For **7**, the typical fragments for a terminal heptose and a glucitol residue were observed at *m/z* 263 and 236, respectively, as were the daughter ions after the loss of methanol. The J_1 fragment[15] (*m/z* 296) of the glucitol residue was also detected. Another J_1 fragment at *m/z* 500 originated from the ion at *m/z* 440 (cleavage between the terminal heptose and C-6 of glucose) which, by loss of methanol, gave the secondary ions at *m/z* 408 and 376. For **8**, the fragments at *m/z* 263, 236, 231, 199, and 296 (J_1) were present. The mol. wts. of 719 for **7** and 923 for **8** were determined in c.i. (ammonia)-m.s. [*m/z* 737 $(M + 18)^+$ for **7**, *m/z* 941 $(M + 18)^+$ for **8**].

TABLE III

Retention times (*T*) of derivatives in g.l.c.

Compound	T
6	3.18[a]
7	3.87[a]
8	4.99[a]
9	4.45[a]
10	1.64[b]

[a] Relative to that of α-D-glucose penta-acetate. [b] Relative to that of maltitol nona-acetate.



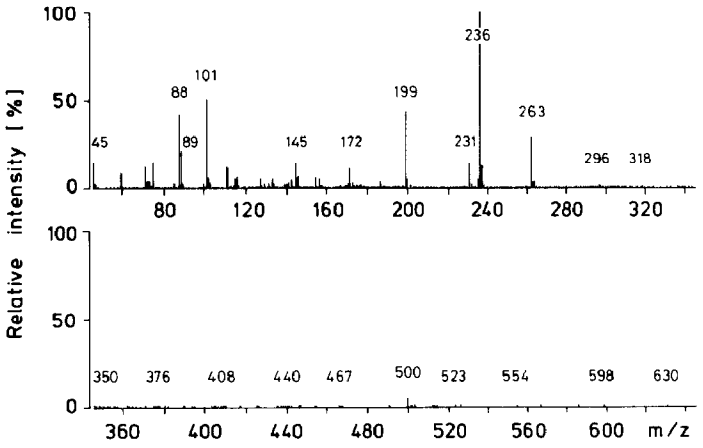


Fig. 1. E.i.-mass spectrum and fragmentation pattern of **7**.

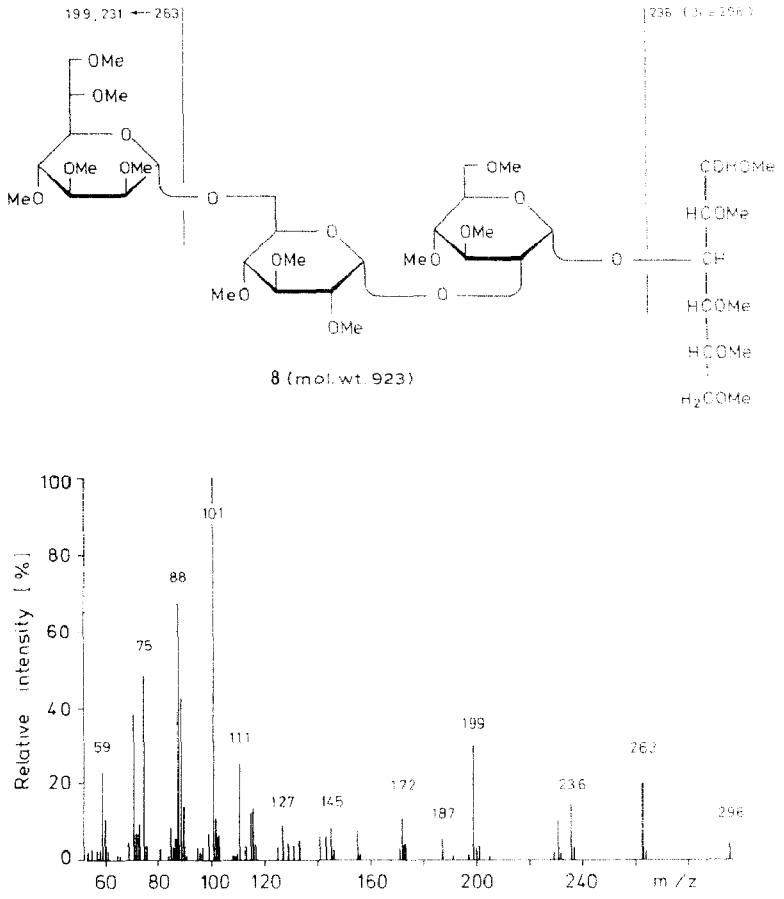
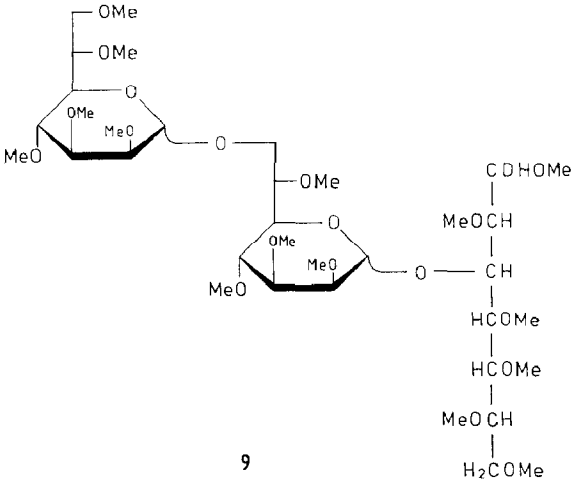


Fig. 2. E.i.-mass spectrum and fragmentation pattern of **8**.

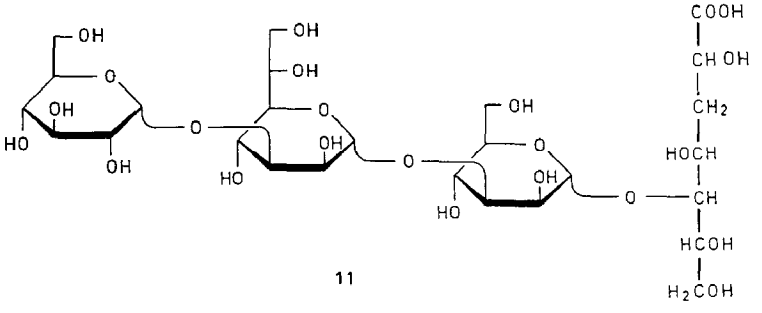
Methylation analyses of **7** yielded 2-*O*-acetyl-1,3,4,5,6-penta-*O*-methylglucitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-(1-^{2}H)glucitol, and 1,5-di-*O*-acetyl-2,3,4,6,7-penta-*O*-methyl-(1-^{2}H)heptitol, and of **8** gave 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylglucitol, 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-(1-^{2}H)glucitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-(1-^{2}H)glucitol, and 1,5-di-*O*-acetyl-2,3,4,6,7-penta-*O*-methyl-(1-^{2}H)heptitol, and proved the structures shown in Figs. 1 and 2.

Furthermore, during h.p.l.c. for the purification of **8**, a fraction was obtained that consisted of a mixture of **7** and methylated 3-*O*-(7-*O*-L-*glycero*-D-*manno*-heptopyranosyl-L-*glycero*-D-*manno*-heptopyranosyl)-(1-^{2}H)heptitol (**9**). The latter compound had the same *T* value and e.i.-mass spectrum as reported[16] for the synthetic compound [mol. wt. 807, *m/z* 825 $(M + 18)^+$].



9

Smith degradation of LPS-HF. — The reduced ($NaBH_4$) product from the Smith degradation was methylated, carboxyl-reduced (NaB^2H_4), remethylated with CD_3I, and fractionated on SEP-PAK C_{18}. G.l.c. of the fraction that was eluted with acetonitrile–water (2:1) revealed a peak which e.i.-m.s. indicated to be a methylated glucose–heptose–mannose–3-deoxyheptitol [**10**, mol. wt. 941, *m/z* 959 $(M + 18)^+$]. The e.i.-mass spectrum and fragmentation pattern of **10** are shown in Fig. 3. The sequence of constituents in **10** was indicated by ions at *m/z* 219 (terminal glucose, secondary ions at *m/z* 187 and 155 by loss of methanol) and 467 (secondary ion at *m/z* 403, loss of two methanol residues), the latter of which showed that the glucose was linked to a heptose. The ion at *m/z* 254 identified the 3-deoxyheptitol residue, and that at *m/z* 426 (a secondary ion which originated from *m/z* 458 by loss of methanol) proved that the mannose was linked to the 3-deoxyheptitol. Methylation analysis of **10** gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(1-^{2}H)glucitol, 1,3,5-tri-*O*-acetyl-2,4,6,7-tetra-*O*-methyl-(1-^{2}H)heptitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-(1-^{2}H)mannitol, and 5-*O*-acetyl-3-deoxy-2,4,6,7-tetra-*O*-methyl-1-*O*-trideuteriomethyl-(1,1-$^2H_2$)heptitol. Therefore, **11** was proved to be the product of Smith-degraded LPS-HF.



11

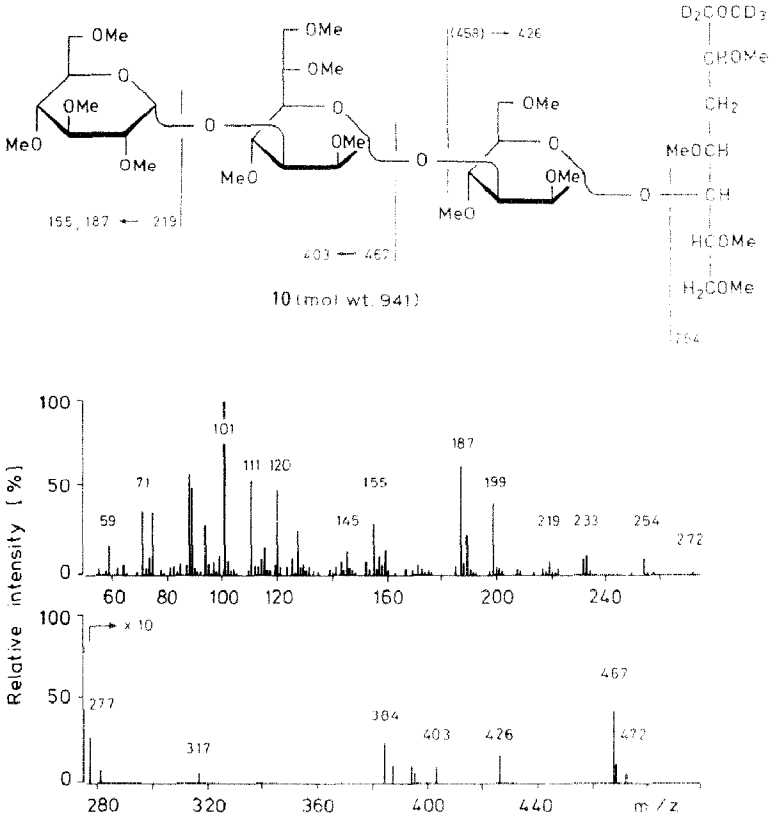


Fig. 3. E.i.-mass spectrum and fragmentation pattern of **10**.

Analysis of OS-HF. – The quantitative analytical data for OS-HF are shown in Table IV; 4 mol. equiv. of L,D-heptose were present and only small proportions of D-GlcN were identified.

Methylation analysis of OS-HF (Table V) confirmed the presence of ~4 mol. equiv. of L,D-heptose. Smaller proportions of terminal Glc and terminal GlcN were also identified. The proportion of the latter was similar to that of 1,5,7-tri-*O*-acetyl-2,3,4,6-penta-*O*-methyl-(1-^{2}H)heptitol. Furthermore, the lower proportion of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-(1-^{2}H)glucitol (relative ratio, 0.65) indicated a partial substitution of glucose I in the main chain (see **12**), which was confirmed by the amounts of terminal Glc (relative ratio, 0.46).

In a GATED-^{13}C-n.m.r. experiment, all of the $J_{C,H}$ values were found to be in the range 170–172 Hz, which indicated[14] the sugar moieties to be α.

The above results indicate the carbohydrate backbone of the core from *E. coli* K-12 W3100 LPS to be as shown in **12**.

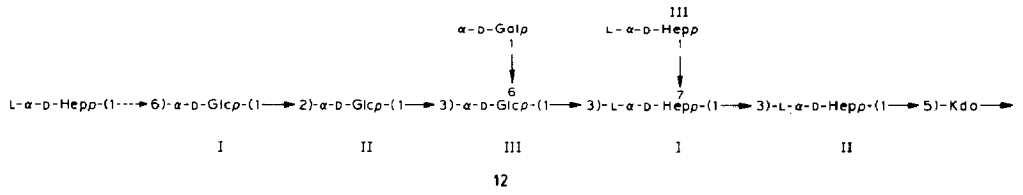


TABLE IV

Quantitative analysis of OS-HF from the LPS of *E. coli* K-12

Compound	*nMol/mg*	*Rel. ratio*[a]
D-Glc	1326	2.8
D-Gal	425	1.0
L,D-Hep	1807	3.9
D-GlcN	139	0.3
Kdo	n.q.[b]	

[a] Relative to D-Gal. [b] Not quantified, due to the difficulty[17] of releasing Kdo from 5-substituted derivatives and the production of artefacts under acid conditions[18].

TABLE V

Methylation analysis data for OS-HF isolated from the LPS of *E. coli* K-12

Compound	*Ratio*[a]
2,3,4,6-Glc[b]	0.46
2,3,4,6-Gal	0.88
3,4,6-Glc	0.79
2,3,4,-Glc	0.65
2,4-Glc	1.00
2,3,4,6,7-Hep	1.31
2,4,6,7-Hep	1.05
2,3,4,6-Hep	0.24
2,4,6-Hep	1.06
2,3,4,6-GlcNAc	0.15

[a] Relative to 2,4-Glc. [b] 2,3,4,6-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(1-^{2}H)glucitol, *etc.*

DISCUSSION

Earlier publications[2-4] on the structure of the LPS from *E. coli* K-12 reported various proportions (3–4 mol) of L,D-Hep. Since these investigations were carried out on strains W3100 and W3110, the LPS from each strain were used for a comparative analysis. Preliminary experiments revealed 4 mol of L,D-Hep per mol of D-Gal in each LPS. In addition, a heptose–hexose disaccharide and a heptose–(hexose)$_2$ trisaccharide were detected, which indicated that one heptose residue was linked to the hexose region

(outer core). Since this finding was not reported[2-4] earlier for any strain, the carbohydrate chain of the core oligosaccharide from K-12 LPS was investigated, using strain W3100, and the structure shown in **12** was identified.

The quantitative and methylation analyses of purified OS-HF revealed 4 mol of L,D-Hep. The isolation of 6-*O*-L-*glycero*-D-*manno*-heptopyranosyl-D-glucose, the structure of which was determined unequivocally by comparison of the n.m.r. and g.l.c.–m.s. data with those of a synthetic standard, indicated one of the heptose residues to be linked to the hexose region. The isolation of methylated 2-*O*-(6-*O*-L-*glycero*-D-*manno*-heptopyranosyl-glucopyranosyl)glucitol (**7**) and 3-*O*-[2-*O*-(6-*O*-L-*glycero*-D-*manno*-heptopyranosyl-glucopyranosyl)glucopyranosyl]glucitol (**8**), the structures of which were established by g.l.c.–m.s., indicated the heptose residue to be 6-linked to the Glc I residue in the outer core. Methylation analysis of OS-HF confirmed the above linkages, and showed that the Gal residue was 6-linked to the Glc III residue. Furthermore, all hexoses and heptoses were proved to be pyranoid, since each of the partially methylated alditol acetates carried a 4-*O*-methyl group and acetyl groups at positions 1 and 5. The GATED-^{13}C-n.m.r. experiment showed that each sugar in the core oligosaccharide was α. It was not possible to confirm the reported[2-5] attachment of GlcNAc at position 6 of the Glc I residue in the outer core. Although small proportions of GlcNAc were detected in OS-HF by quantitative and methylation analyses, no GlcNAc-containing oligosaccharide(s) could be identified. The small proportions of 1,5,7-tri-*O*-acetyl-2,3,4,6-tetra-*O*-methylheptitol, identified in the methylation analysis of OS-HF, did not necessarily show the linkage point of the GlcNAc, since small amounts of any of the terminal sugars could be located there. Work is in progress to identify the position of the GlcNAc residue.

The 5-substitution of the Kdo residue in the main chain of the core oligosaccharide was proved for the first time by the isolation and g.l.c.–m.s. analysis of the product (**10**) from Smith degradation. The Kdo was identified by conversion into a 3-deoxyheptitol, which showed that it was not substituted at positions 7 and 8. The e.i.-mass spectrum and methylation analysis of **10** showed Man to be 5-linked to the 3-deoxyheptitol. The Man, which was the product of oxidation of an L,D-Hep with HO-6,7 unsubstituted, was found to be 3-substituted by another L,D-Hep residue that was not destroyed during the Smith degradation. Therefore, in addition to 3-substitution by Glc, the latter residue was also substituted either at position 6 or 7. These data, together with those of the methylation analysis and the identification of 3-*O*-(7-*O*-L-*glycero*-D-*manno*-heptopyranosyl-L-*glycero*-D-*manno*-heptopyranosyl)-(1-^{2}H)heptitol (**9**), confirmed the structure of the heptose region as shown in **12**. Furthermore, the analyses of **10** proved the outer core to be linked via glucose to position 3 of the Hep I residue in the main chain. Compounds **8** and **10** are related structures in which the terminal Glc residue in **10** is the glucitol residue in **8**.

Heptopyranose residues linked to the outer core region (or to the O-antigen) have been identified[19-23] in the LPS from other bacteria, but to our knowledge, when the configuration has been determined, it was usually D-*glycero*-D-*manno*. In some of the core types of the LPS from *Proteus mirabilis*, a terminal D,D-heptose may be replaced by another L,D-heptose[19].

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