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Note

Structural studies of the core region of the lipopolysaccharide from *Salmonella minnesota* strain R7 (rough mutant chemotype Rd_1)

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The inner core region of enterobacterial lipopolysaccharides (LPS) is highly conserved in bacterial evolution. It is composed of 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptopyranose. Whereas the Kdo region of different LPS has been investigated intensively[1], little is known about the heptose part of the inner core. In enterobacterial LPS, the first heptose in linked to position 5 of the Kdo residue in the main chain[2-4]. From the core-deficient rough mutant strain R4 of *Salmonella minnesota*, the disaccharide 3-deoxy-5-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-D-*manno*-octulopyranosonic acid has been isolated and characterised by ^{13}C-n.m.r. spectroscopy[5] and mass spectrometry[4,6]. We now report on the methylation analysis of the core region of another rough mutant strain (*S. minnesota* strain R7, Rd_1 chemotype), and on the isolation and characterisation of the trisaccharide 3-deoxy-5-*O*-(3-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-D-*manno*-2-octulopyranosonic acid (**1**).

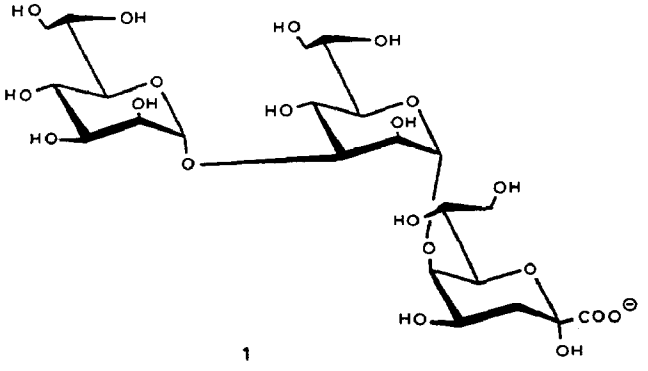

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TABLE I

Sugar composition of the LPS from *Salmonella minnesota* strain R7

Compound	*nMol/mg*	*Ratio*[a]
D-GlcN	712	2.0
L,D-Hep	755	2.1
Kdo	783	2.2

[a] Relative to D-GlcN = 2.0.

The LPS from *S. minnesota* R7 comprised, as sugar constituents, D-GlcN, Kdo, and L-*glycero*-D-*manno*-heptose in the molar ratios 2.0:2.2:2.1 (Table I).

Methylation analyses[4] of the LPS revealed g.l.c.–m.s. peaks originating from a terminal and a 4-substituted pyranosidic Kdo[7] in the molar ratio ~1:0.2, together with a terminal and a 3-substituted L-*glycero*-D-*manno*-heptopyranose residue in the molar ratio ~1:1. In addition, a peak corresponding to a 4,5-disubstituted Kdo was observed, which, due to the unknown response factor of this compound, was not quantified; however, it was smaller than those of the heptose derivatives.

The core constituents of the LPS were released by hydrolysis under conditions that cleave exclusively the ketosidic bonds of Kdo. When the combined diffusates of dialysis were analysed by high-voltage paper electrophoresis (p.e.), Kdo, 3-deoxy-D-*manno*-2-octulopyranosonate 7-(2-aminoethyl phosphate)[8], and a product with M_{Kdo} 0.49 were detected. The last component was isolated and purified by gel-permeation chromatography and preparative p.e., and shown to have structure **1**.

Chemical analysis of **1** identified L-*glycero*-D-*manno*-heptose and Kdo in the molar ratio ~2:1. After reduction (NaB^2H_4) and methylation of **1**, methylated methyl 3-deoxy-5-*O*-(3-*O*-L-*glycero*-D-*manno*-heptopyranosyl-L-*glycero*-D-*manno*-heptopyranosyl)-D-*glycero*-D-*talo*/*galacto*-octonate was identified in g.l.c.–m.s. by its e.i.-mass spectrum[9] [mol. wt. 835, $(M + 18)^+$ 853 (57.7% of base peak), as determined by c.i.-(ammonia)-m.s.]. Methylation analysis after carboxyl reduction[4] (hydrolysis in 4M trifluoroacetic acid, 100°, 4 h) followed by g.l.c.–m.s. revealed 1,5-di-*O*-acetyl-2,3,4,6,7-penta-*O*-methyl-[1-^{2}H]heptitol, 1,3,5-tri-*O*-acetyl-2,4,6,7-tetra-*O*-methyl-[1-^{2}H]heptitol, and 1,5-di-*O*-acetyl-3-deoxy-2,4,6,7,8-penta-*O*-methyl-[2-^{2}H]octitol in the molar ratios ~1:1:1. Thus, **1** was 3-deoxy-5-*O*-(3-*O*-L-*glycero*-D-*manno*-heptopyranosyl-L-*glycero*-D-*manno*-heptopyranosyl)-D-*manno*-octulopyranosonic acid.

The chemical shifts obtained in ^{13}C-n.m.r. spectroscopy (Table II) were assigned by comparison with those published[5,10] for 3-deoxy-5-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-D-*manno*-octulopyranosonic acid and 3-*O*-α-L-*glycero*-D-*manno*-heptopyranosyl-L-*glycero*-D-*manno*-heptopyranose, and with those of authentic Kdo. The 5-substitution of Kdo was indicated by a downfield shift of ~9 p.p.m. for the C-5 signal, compared to Kdo, and the 3-substitution of the first heptose residue by a downfield shift of ~6.3 p.p.m. for C-3, compared to the C-3 signals of the terminal heptose residues of

TABLE II

^{13}C-N.m.r. data (90.6 MHz, D_2O, CH_3CN = 1.70 p.p.m) of **1**, compared with Kdo, LαD-Hepp1→5Kdo[a], and LαD-Hepp1→3L,D-Hepp[b]

Assignment[c]	**1**	*Kdo*	LαD-*Hepp1→5Kdo*	LαD-*Hepp1→3*L,D-*Hepp*
C-1	177.10	177.59	177.0	
C-2	97.18	97.23	97.3	
C-3	34.74	34.45	35.1	
C-4	66.64[e]	67.02	66.7	
C-5	76.31	67.41	76.5	
C-6	71.91	71.95	72.1	
C-7	70.04	70.03	70.0	
C-8	63.84[g]	63.81	64.0	
C-1′	102.27[f]		102.5	94.95
C-2′	70.91[d]		71.2	71.36
C-3′	77.63		71.3	78.61
C-4′	66.92[e]		67.1	66.92
C-5′	72.86		72.9	72.65
C-6′	69.68		69.8	69.70
C-7′	64.14[g]		64.1	63.80
C-1″	102.65[f]			103.18
C-2″	70.91[d]			70.89
C-3″	71.40			71.36
C-4″	66.85[e]			66.94
C-5″	72.51			72.06
C-6″	69.39			69.54
C-7″	63.95[g]			63.54

[a] Data taken from ref. 5. [b] Data taken from ref. 10. [c] Empirical assignment by comparison with Kdo, LαD-Hepp1→5Kdo, and LαD-Hepp1→3L,D-Hepp. [d] Non-resolved signal. [e–g] Assignment interchangeable.

the two disaccharides. A GATED-^{13}C-n.m.r. experiment (Table III) proved all linkages to be α ($J_{C\text{-}1',H\text{-}1'}$ 171 Hz, $J_{C\text{-}1'',H\text{-}1''}$ 172 Hz). Furthermore, carbon atoms with axial hydroxyl groups could be identified by their higher $^1J_{C,H}$ coupling constants[5], *e.g.*, C-5 of Kdo ($J_{C\text{-}5,H\text{-}5}$ 151 Hz) and C-2 of both heptose residues ($J_{C\text{-}2',H\text{-}2'}$ 150 Hz, $J_{C\text{-}2''H\text{-}2''}$ 150 Hz). All the other coupling constants were in the range 143–146 Hz.

Taken together, our results identified the structure of the isolated trisaccharide as **1**. Furthermore, the results of the quantitative and methylation analyses accord with the proposed[9] structure of the core region of LPS from *S. minnesota* R7 as shown in **2**. The n.m.r. data for **1** and for 3-deoxy-5-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-D-*manno*-octulopyranosonic acid will be useful for the interpretation of more complex n.m.r. spectra of higher oligosaccharides that are isolated from less defective mutants of *S. minnesota* and other bacteria.

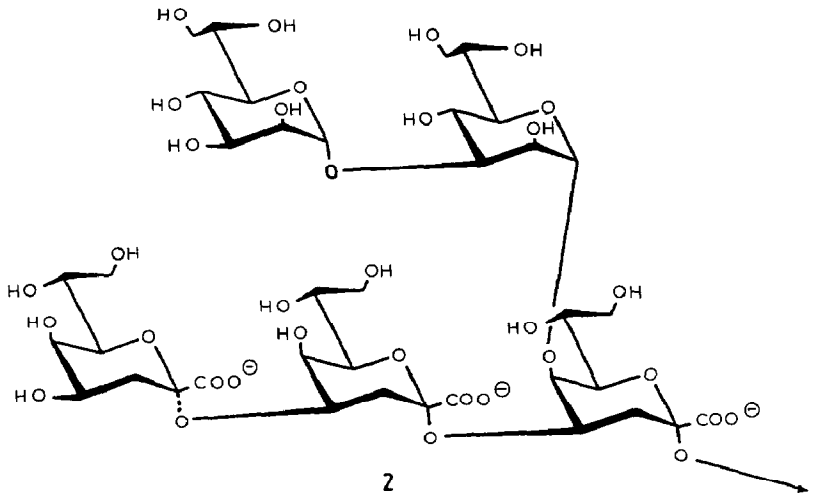

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TABLE III

$^{1}J_{C,H}$ coupling constants (Hz) of **1**, compared with those of LαD-Hepp1→5Kdo[a]

C-Atom	**1**	LαD-*Hepp1→5Kdo*
1	—	—
2	—	—
3	127	132
4	143	143
5	151	148
6	143	145
7	146	145
8	143	145
1′	171	170
2′	150	149
3′	144	146
4′	143	146
5′	143	145
6′	143	140
7′	144	144
1″	172	
2″	150	
3″	143	
4″	144	
5″	143	
6″	143	
7″	143	

[a] Data taken from ref. 5.

EXPERIMENTAL

General methods for quantitative and methylation analyses, and the conditions for n.m.r. spectroscopy were as described[4,11]. Temperature programme for g.l.c. in methylation analysis: 140° for 3 min, 3°/min→250°. G.l.c.–m.s. of methylated methyl 3-deoxy-5-*O*-(3-*O*-L-*glycero*-D-*manno*-heptopyranosyl-L-*glycero*-D-*manno*-heptopyranosyl)-D-*glycero*-D-*talo*/*galacato*-octonate was as described[9].

Bacteria and bacterial LPS. — *Salmonella minnesota* strain R7 (chemotype Rd_1) was grown in a fermenter (14 L), killed with phenol (0.5%), centrifuged, and washed with ethanol, acetone (twice), and ether. The LPS was extracted[12] from the dry bacteria in a yield of 3.3%.

*Isolation and purification of 3-deoxy-5-*O*-(3-*O*-*L*-glycero-α-*D*-manno-heptopyranosyl-*L*-glycero-α-*D*-manno-heptopyranosyl)-*D*-manno-octulosonic acid* (**1**). — The LPS (1 g) was hydrolysed (1 h, 100°) in 100mM sodium acetate buffer (pH 4.4, 100 mL), and the hydrolysate was dialysed against water (3 × 500 mL) at 4°. The combined diffusates were concentrated and desalted on a column (100 × 1.5 cm) of Bio-Gel P2 (Bio-Rad) in water. Three fractions were obtained and analysed by p.e. Compound **1** (M_{Kdo} 0.49) was isolated from fraction 1 by preparative p.e. and lyophilisation (yield, 30 mg, 3% of LPS).

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