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Note

Isolation and identification of 3-deoxy-5-*O*-α-L-rhamnopyranosyl-D-*manno*-2-octulopyranosonate from the inner core region of the lipopolysaccharide of *Escherichia coli* K-12*

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Six different core types have been identified[1] in lipopolysaccharides (LPS) of *Escherichia coli* on the basis of chemical, serological, and genetic investigations, namely Ra, R1–R4, and K-12. Structural investigations have been performed on different parts[2–11] of the LPS from *E. coli* K-12. The Kdo–lipid A linkage involves HO-6′ of the lipid A backbone[10], and 3-deoxy-D-*manno*-2-octulopyranosonate 7-(2-aminoethyl phosphate) (PE-Kdo) has been identified[11]. Furthermore, a rhamnosyl-Kdo disaccharide was detected in several strains[8,9], but its structure was not determined unequivocally[8]. The rhamnose is the immunodominant sugar[9]. We now report that the structure of this disaccharide is **1**.

After hydrolysis of LPS from *E. coli* K-12 strain W3100 (complete core) in acetate buffer (0.1M, pH 4.4) followed by dialysis and high-voltage paper electrophoresis (p.e.), one major spot (**1**, M_{Kdo} 0.63) was detected by staining with silver nitrate and thiobarbituric acid.

In addition to **1**, Kdo (M_{Kdo} 1.0)[12], PE-Kdo[11] (M_{Kdo} 0.73), and Kdo-phosphate (M_{Kdo} 2.33, traces) were identified. After purification of the last-named product by preparative p.e. and treatment with alkaline phosphatase, Kdo was detected as the reduced and methylated derivative[12] by g.l.c. Due to the small amounts available, the position of the phosphate residue was not determined. However, the Kdo-phosphate was not[11] a hydrolysis product of PE-Kdo.

Analysis[11] of **1**, isolated by ion-exchange and gel-permeation chromatography, followed by preparative p.e., revealed Kdo and Rha in the molar ratio ~1:1. The Rha was shown[13] to be L. For g.l.c.–m.s., **1** was reduced with NaB^2H_4 and methylated to give **2** [Fig. 1, *T* 2.04, mol. wt. 513, *m/z* 514 $(M + 1)^+$ and 531 $(M + 18)^+$], which, after carboxyl-reduction (NaB^2H_4), yielded **3**. Methylation of **3** yielded **4** [*T* 1.95/1.99, mol. wt. 501, *m/z* 502 $(M + 1)^+$ and 519 $(M + 18)^+$], methylation analysis (hydrolysis in 2M

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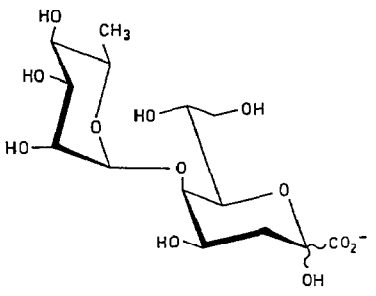

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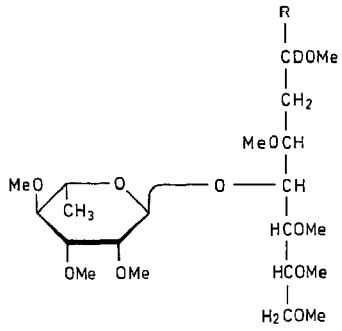
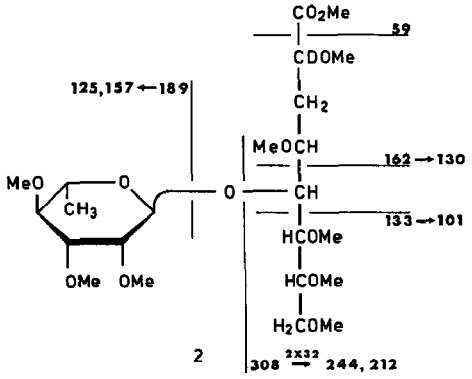


2 R = CO_2Me

3 R = D_2COH

4 R = D_2COMe



M = 513

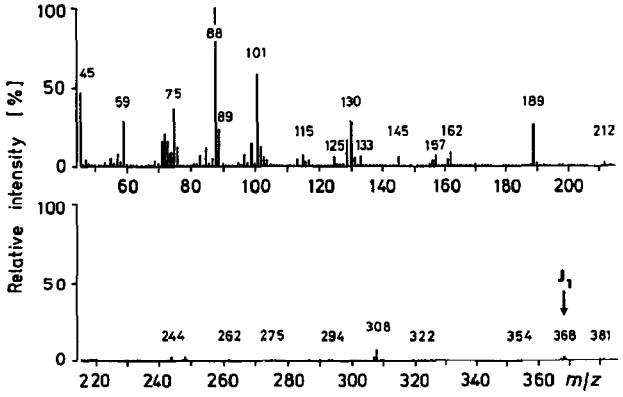


Fig. 1. E.i.-mass spectrum and fragmentation pattern of **2**.

trifluoroacetic acid at 120° for 2 h followed by reduction and acetylation) of which yielded equimolar amounts of 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methyl-L-mannitol and 5-*O*-acetyl-3-deoxy-1,2,4,6,7,8-hexa-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-(1,1,2-$^2H_3$) octitol[12] (**5**). Methylation analysis of **3** yielded 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methyl-L-mannitol and 1,5-di-*O*-acetyl-3-deoxy-2,4,6,7,8-penta-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-(1,1,2-$^2H_3$)octitol[12] (**6**). These data indicated that an L-Rha*p* was 5-linked to Kdo.

TABLE I

^{13}C-N.m.r. data for **1**, α-L-rhamnose, and the ammonium salt of Kdo (in p.p.m.)

Carbon atom	**1**	*Kdo*	*α-L-Rha*
1	177.19	177.59	
2	97.34	97.23	
3	34.75	34.45	
4	68.32	67.02	
5	73.89	67.41	
6	71.19	71.95	
7	70.23	70.03	
8	63.72	63.81	
1′	102.16		94.80
2′	71.49		71.62
3′	70.95		70.76
4′	72.78		73.00
5′	69.98		69.10
6′	17.66		17.61

Table I shows the ^{13}C-n.m.r. data for **1**. The C-1 resonance at 102.16 p.p.m. did not allow the anomeric configuration of the L-Rha*p* unit to be determined (*cf.* 101.9 and 102.0 p.p.m., respectively, for C-1 of methyl α- and β-rhamnopyranoside[14]). The anomeric configuration was determined[15] as α from the $J_{C\text{-}1,H\text{-}1}$ value of 174.08 Hz. The resonances of C-1/3 and C-7/8 of the Kdo moiety of **1** were similar to those of Kdo. The signal for C-5 at 73.89 p.p.m. and the shifts of the resonances for C-4 and C-6 indicated 5-substitution of the Kdo unit. Unexpectedly, the resonance (68.32 p.p.m.) for C-4 of the Kdo unit was shifted downfield compared to that (67.02 p.p.m.) of Kdo. Thus, the structure of **1** is established as 3-deoxy-5-*O*-α-L-rhamnopyranosyl-D-*manno*-2-octulosonic acid.

3-Deoxy-8-*O*-[3-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-D-*manno*-octulosonate has been identified[16] as a component of the LPS from *Acinetobacter calcoaceticus*. L-*glycero*-D-*manno*-Heptopyranose is 5-linked to Kdo in many LPS, and 5-linked D-mannose[17] and 7-linked D-galactose[18] have been described also. The substitution of Kdo with L-rhamnose has been reported[19,20] in cell walls of certain plants (where **1** was also identified) and [21] in the capsular polysaccharide of *E. coli* O4:K12:H$^-$.

EXPERIMENTAL

General methods were as described[18]. The absolute configuration of the rhamnose released (4M trifluoroacetic acid, 4 h, 100°) from **1** was determined according to König *et al.*[13]. All *T* values were relative to that of α-D-glucopyranose penta-acetate.

Bacteria and bacterial lipopolysaccharides. — *E. coli* K-12 strain W3100[22] was grown in a fermenter (14 L), and the cells were killed with phenol and centrifuged. The sedimented bacteria were washed successively with ethanol, acetone (twice), and ether, then dried. The LPS was isolated (3.5%) from dry bacteria by the phenol–chloroform–light petroleum method[23].

*Isolation and purification of 3-deoxy-5-*O*-α-*L*-rhamnopyranosyl-*D*-manno-2-octulosonic acid* (**1**). — The procedure was essentially that described[18] for 3-deoxy-7-*O*-α-D-galactopyranosyl-D-*manno*-2-octulosonic acid. Briefly, LPS was hydrolysed (1 h, 100°) in sodium acetate buffer (pH 4.4, 0.1M), then dialysed against water (3 × 100 mL), and the combined diffusates were desalted [IR-120 (H^+) resin], neutralised with pyridine, concentrated, and fractionated on a column of polyethyleneimine cellulose. The fraction eluted with pyridinium acetate (pH 5.5, 50mM) was eluted from a column of Bio-Gel P-2 with pyridinium acetate (pH 5.2, 50mM). The fraction that contained **1** was purified by preparative p.e. (yield 1%), $[\alpha]_D$ +4.25° (*c* 0.5, water).

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