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Note

Structure of the O-specific side-chains of the *Escherichia coli* O 75 lipopolysaccharide: a revision

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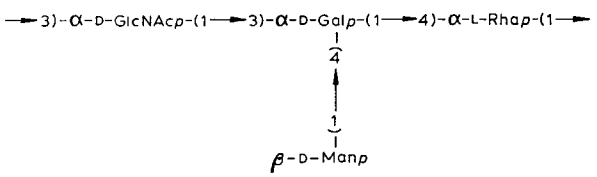
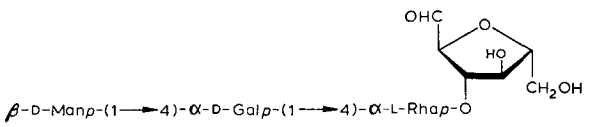
We recently proposed[1] a structure (**1**) for the O-specific side-chains of the *Escherichia coli* O 75 lipopolysaccharide (LPS), based upon methylation analysis, Smith degradation, and optical rotations of original and degraded materials.



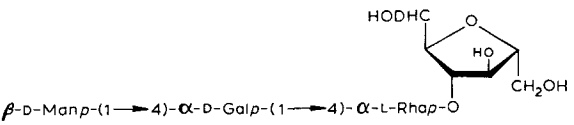
1

A 100-MHz ^{1}H-n.m.r. spectrum of the polysaccharide (PS), $[\alpha]_{578}$ +43°, obtained from the LPS on mild hydrolysis with acid, showed, *inter alia*, signals at δ 1.33 (3 H, $J_{5,6}$ 6 Hz, CH_3 of L-rhamnose residues), and 2.07 (s, 3 H, CH_3 of *N*-acetyl groups), and, in the region for anomeric protons, at δ 4.82 (1 H, $J_{1,2}$ 8 Hz), 4.91 (1 H, $J_{1,2}$ small), 4.97 (1 H, $J_{1,2}$ small), and 5.10 (1 H, $J_{1,2}$ small). This confirmed the presence of a tetrasaccharide repeating-unit with one L-rhamnosyl and one 2-acetamido-2-deoxy-D-glucosyl residue. The signal at δ 4.82, $J_{1,2}$ 8 Hz, because of the high value for its coupling constant, must derive from a sugar residue having a *trans*-diaxial arrangement of H-1 and H-2. Consequently, either the D-galactopyranosyl or the 2-acetamido-2-deoxy-D-glucopyranosyl residue should be β-linked, in disagreement with structure **1**. This signal showed a considerable shift, to δ 4.61, $J_{1,2}$ 8 Hz, on *N*-deacetylation, indicating that it derived from the amino sugar. The results, therefore, demonstrated that structure **1** had to be revised.

The *N*-deacetylated[2] LPS, on acid hydrolysis, yielded D-mannose and L-rhamnose (1:1) as monomers. As the 2-amino-2-deoxy-D-glucopyranosidic linkage



2



3

is resistant to acid hydrolysis, the result confirms that the 2-acetamido-2-deoxy-D-glucose in the original polysaccharide is linked to D-galactose.

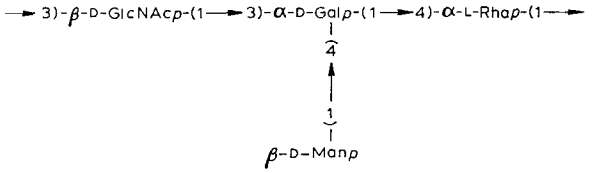
Deamination of the *N*-deacetylated LPS yielded the tetrasaccharide **2**, part of which was reduced, with sodium borodeuteride, to give the alditol **3**, $[\alpha]_{578}$ +32° (the complete structures are given, although they were not proved at this stage of the investigation.) The alditol, on sugar analysis, yielded 2,5-anhydro-D-mannitol-*1-d*, L-rhamnose, D-mannose, and D-galactose in the proportions 28:26:21:25. Methylation analysis of **3** yielded 2,5-anhydro-1,4,6-tri-*O*-methyl-D-mannitol-*1-d*, 2,3-di-*O*-methyl-L-rhamnose, 2,3,6-tri-*O*-methyl-D-galactose, and 2,3,4,6-tetra-*O*-methyl-D-mannose (23:22:25:30). The identification of the 2,5-anhydro-D-mannitol derivative, from the mass spectrum of its acetate, has been discussed[3]. The n.m.r. spectrum of **3** showed, *inter alia*, signals at δ 1.25 (3 H, $J_{5,6}$ 6 Hz), 4.71 (1 H, $J_{1,2}$ small), 4.75 (1 H, $J_{1,2}$ small), and 4.98 (1 H, $J_{1,2}$ small). The absence of a signal with a high coupling constant in the anomeric region demonstrates that the D-galactopyranosyl residue is α-linked and, consequently, that the 2-acetamido-2-deoxy-D-glucopyranosyl residue in the original LPS is β-linked.

Part of the tetrasaccharide **2** was treated with base, when the trisaccharide substituent in the β-position to the aldehyde group was eliminated. The trisaccharide was reduced to its alditol (**4**), $[\alpha]_{578}$ +73°. The n.m.r. spectrum of **4** showed, *inter alia*, signals at δ 1.30 (3 H, $J_{5,6}$ 6 Hz), 4.84 (1 H, $J_{1,2}$ 1 Hz), and 5.28 (1 H, $J_{1,2}$ 3 Hz). An acid hydrolysate of **4** contained equimolecular proportions of L-rhamnitol, D-mannose, and D-galactose. Methylation analysis of **4** yielded 1,2,3,5-tetra-*O*-methyl-L-rhamnitol, 2,3,4,6-tetra-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl-D-galactose. As the D-galactopyranosyl residue in **4** is α-linked, the D-mannopyranosyl group must be β-linked, in order to account for the observed optical rotation. This assignment is also in agreement with the observation[1] that the LPS did not precipitate with concanavalin A. The structure **4** of the trisaccharide is thereby established, and the sequence of sugar residues in **3** should, consequently, be as depicted. From the optical rotation of **3**, and the known configurations of the D-galactopyranosyl and D-mannopyranosyl units, it can further be concluded that the L-rhamnopyranosyl residue is α-linked.

β-D-Manp-(1→4)-α-D-Galp-(1→4)-α-L-Rhamnitol

4

All of the structural features in **1**, except the anomeric nature of the 2-acetamido-2-deoxy-D-glucopyranosyl residue, have thus been confirmed, and the correct structure (**5**) of the tetrasaccharide repeating-unit of *E. coli* O 75 O-antigen has been established.



5

EXPERIMENTAL

General methods. — These were the same as in an investigation[4] of the *E. coli* O 69 LPS. Preparation of PS from LPS was also performed as previously described[1].

For sugar analysis, the product (1 mg or less) was hydrolysed with 0.25M sulfuric acid at 100° overnight, and the resulting sugars were analysed, by g.l.c., as the alditol acetates[5]. Methylation analysis was performed as previously described[6].

N-*Deacetylation.* — A solution of LPS (500 mg), sodium hydroxide (2 g), and thiophenol (2 g) in water (5 ml) and methyl sulfoxide (25 ml) was kept at 100° for 15 h, neutralised with acetic acid, filtered, dialysed, and freeze-dried. The product (200 mg) was devoid of *N*-acetyl groups, as evident from its n.m.r. spectrum, which showed, *inter alia*, signals at δ 1.36 (3H, $J_{5,6}$ 6 Hz), 4.61 (1 H, $J_{1,2}$ 8 Hz), 5.02 (1 H, $J_{1,2}$ small), 5.06 (1 H, $J_{1,2}$ small), and 5.13 (1 H, $J_{1,2}$ small).

Deamination. — *N*-Deacetylated LPS (30 mg) was dissolved in water (0.6 ml); 33% aqueous acetic acid (1 ml) and 5% aqueous sodium nitrite (1 ml) were added, and the solution was kept at 25° for 40 min, passed through a column of Dowex 50 (H^+) resin, and freeze-dried. The product was dissolved in water (1 ml), sodium borodeuteride (20 mg) was added, the solution was kept at room temperature for 2 h, filtered through a column of Dowex 50 (H^+) resin, and concentrated, and boric acid was removed by codistillation with methanol (3 × 3 ml). Fractionation, first on a column (80 × 0.8 cm) of Sephadex G-25 and then on a column (80 × 0.8 cm) of Sephadex G-15, yielded oligosaccharide **3** (4.5 mg), $[\alpha]_{578}^{25}$ +32° (*c* 0.4, water), eluted in the tri- to tetra-saccharide region.

Another sample of *N*-deacetylated LPS (53 mg) was deaminated as described above, and the freeze-dried product was dissolved in 0.05M sodium hydroxide (1 ml). The solution was kept at 37° for 30 min, neutralised with M hydrochloric acid, and reduced with sodium borodeuteride as described above. Fractionation of the product on a column (80 × 0.8 cm) of Sephadex G-15 yielded oligosaccharide **4** (15 mg), $[\alpha]_{578}^{25}$ +73° (*c* 0.5, water), eluted in the trisaccharide region.

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