

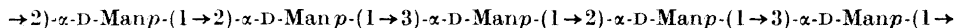
Structural Studies on the *Klebsiella* O Group 3 Lipopolysaccharide

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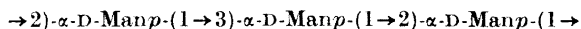
The structure of the O-specific side chains in a *Klebsiella* O group 3 lipopolysaccharide has been investigated, using methylation analysis and Smith degradation as the principal methods. The results are consistent with the assumption that the side chains are composed of repeating units containing five α -D-mannopyranose residues, two which are (1 \rightarrow 3)-linked and adjacent and the other three (1 \rightarrow 2)-linked.

The O-specific side chains in the lipopolysaccharide (LPS) from two of the twelve serologically defined O groups of *Klebsiella*, O group 3 and O group 5, are composed of D-mannose residues.¹ Those from O group 5 contain in addition a low percentage of 3-O-methyl-D-mannose. The O-antigen from *E. coli* O8 also contains D-mannose and its 3-O-methyl ether,² and is serologically identical with the *Klebsiella* O5 antigen.³ According to our recent studies,⁴ the O-specific side chains in the *Klebsiella* O5 LPS should be composed of pentasaccharide repeating units of structure I, and be terminated by 3-O-methyl-D-mannose residues.



I

In a recent investigation of the *E. coli* O8 LPS a trisaccharide repeating unit (II) was proposed.⁵



II

We now report structural studies on the *Klebsiella* O3 LPS.

RESULTS AND DISCUSSION

The LPS was isolated from *Klebsiella* O3:K58 as previously described.¹ A hydrolysate of the LPS contained mannose and trace amounts of galactose, glucose, and a heptose. The three latter sugars, which are found in all *Klebsiella* LPS,¹ are probably components of the core region, whereas the mannose represents the sole component of the O-specific side chains. D-Arabinose was used as internal standard in the analysis and the percentage of mannose was found to be 43 %. Mannose was isolated from a hydrolysate and the D-configuration was assigned from its optical rotation. No significant absorption around 1735 cm⁻¹ in the IR was observed, demonstrating the absence of O-acyl groups. This was confirmed by methylation analysis of the acetalated polysaccharide, according to the method devised by de Belder and Norrman.⁶ The optical rotation of the LPS, $[\alpha]_{589} + 38^\circ$, indicates that the D-mannose residues are α -linked, assuming that the rotation is essentially due to these residues. Sugar analysis of acetylated LPS that had been treated with chromium trioxide in acetic acid, using *myo*-inositol hexaacetate as internal standard, also showed that no D-mannose residues had been oxidized. These results indicate that the D-mannose residues are α -linked.⁷

Fully methylated polysaccharide, prepared by the Hakomori procedure,⁸ was hydrolysed and the sugars in the hydrolysate analysed, as alditol acetates, by GLC-MS.⁹ The results (Table 1) suggest that the O-specific side chains are composed of linear pentasaccharide residues, with two 3-O-substituted and

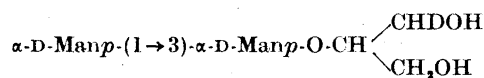
Table 1. Methyl ethers obtained in the methylation analysis of the *Klebsiella* O group 3 LPS.

| Sugars ^a | T ^b | Mol % ^c |
|------------------------------|----------------|--------------------|
| 2,3,4,6-G } 2,3,4,6-Man } | 1.00 | 2 |
| 3,4,6-Man | 1.95 | 56 |
| 2,4,6-Man | 2.09 | 38 |
| Others ^d | — | 4 |

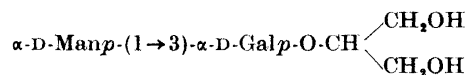
^a 2,3,4,6-G = 2,3,4,6-tetra-O-methyl-D-glucose, etc. ^b Retention times of the corresponding alditol acetates on an ECNSS-M column at 170° relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol. ^c Average values from three determinations. ^d Some of these are probably non-sugar components.

three 2-O-substituted α -D-mannopyranose residues, as was also observed for the *Klebsiella* 05 LPS. The terminal D-mannose residue will give 2,3,4,6-tetra-O-methyl-D-mannose, and from the percentage of this sugar, which is not well separated from the corresponding D-glucose derivative, the average number of pentasaccharide repeating units in the O-specific side chains should be 9 or higher.

In this repeating unit the two 3-*O*-substituted α -D-mannopyranose residues could either be adjacent or separated. In order to distinguish between these alternatives, the LPS was subjected to a Smith degradation.^{4,10} The products after periodate oxidation, reduction with sodium borohydride and hydrolysis under mild conditions were reduced with sodium borodeuteride. Sugar analysis, using D-arabinose as internal standard, of the material obtained after periodate oxidation and borohydride reduction revealed a mannose content similar to the original, (1 \rightarrow 3)-linked D-mannose residues. In a methylation analysis of this product the only methyl ether obtained was 2,4,6-tri-*O*-methyl-D-mannose. These results demonstrate that the oxidation had gone to completion and that no significant amounts of material had been lost. Part of the product obtained after hydrolysis and borodeuteride reduction was subjected to methylation analysis. The only sugars obtained in significant quantities were 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,4,6-tri-*O*-methyl-D-mannose, in the proportion 0.7:1.0. Another part of the product was trimethylsilylated and analysed by GLC-MS.^{4,11} No trimethylsilyl (TMS) derivatives of mannitol, glycerol mannoside, or mannitol mannoside were observed. The main component, $T_{\text{melibiitol}} = 1.04$ (retention time relative to the TMS derivative of melibiitol) was identified as the TMS derivative of mannosyl glycerol (III), as it gave the same MS, except for the shifts caused by the deuterium labelling, as that of the mannosylgalactosyl glycerol (IV) ($T_{\text{melibiitol}} = 1.4$) isolated from a red alga.¹² The yield of III in the Smith degradation was estimated to $\sim 35\%$ of the theoretical value, assuming that the response factor on GLC of its TMS derivative is the same as that of the melibiitol derivative. The estimate of the yield is also corrected for the finding that only about 85% of the oxidized-reduced residues had been hydrolysed, as demonstrated by the methylation analysis of the degraded material.



III



IV

From the evidence presented above it is proposed that the O-specific side chains of the *Klebsiella* O3:K58 LPS are composed of pentasaccharide repeating units with the structure V. This is similar to the repeating unit of the *Klebsiella* O group 5 LPS (I) but the 3-*O*-substituted D-mannose residues are adjacent in the former and separated in the latter. This difference, and the absence of 3-*O*-methyl-D-mannose in the *Klebsiella* O group 3 LPS, could account for observed serological differences between the two species.



V

EXPERIMENTAL

General methods were the same as in the investigation of *Klebsiella* O group 5 LPS⁴ except for the GLC analyses of TMS derivatives which were performed on a glass column (180 × 0.15 cm) containing 3 % XE-60 on Gas Chrom Q (100/120 mesh). Separations were performed at 175° or 120° to 180° (3°/min) for quantitative estimations.

The LPS was isolated from strain *Klebsiella* O3:K58 (636/52) as previously described.¹ The LPS showed $[\alpha]_{589}^{20} + 38^\circ$ (c 0.1, water). In the IR spectrum (KBr) no absorption around 1735 cm⁻¹ was observed. The content of phosphorus in the material was < 0.1 %.

Sugar and methylation analyses were performed as previously described.^{4,8,9,13,14} Mannose was isolated from a hydrolysate of LPS by preparative paper chromatography. The sample showed $[\alpha]_{578}^{20} + 16^\circ$ (c 0.1, water).

Methylation analysis of acetalated LPS. Preparation of the acetalated LPS by reaction with methyl vinyl ether and methylation analysis of the product was performed as previously described.^{8,15} Only mannitol hexaacetate was obtained in the analysis.

Oxidation with chromium trioxide. LPS (20 mg) was dissolved in formamide (20 ml), acetic anhydride (5 ml) and pyridine (5 ml) were added and the solution was kept at room temperature overnight. The reaction mixture was added to the top of a Sephadex LH-20 column (25 × 3 cm) which was irrigated with chloroform:acetone (2:1). The eluate was monitored polarimetrically and the acetylated polysaccharide (28 mg) was eluted as a single peak with the void volume. The acetylated polysaccharide showed no absorption in the hydroxyl region in the IR. *myo*-Inositol hexaacetate (7 mg) was added to the polysaccharide material and this was divided into two equal portions. One portion was dissolved in acetic acid (0.4 ml), chromium trioxide (40 mg) was added and the mixture was treated in an ultrasonic bath at 50° for 1 h. The other portion was treated likewise but the chromium trioxide was omitted. The samples were recovered by partition between chloroform and water and subjected to sugar analysis as described earlier.^{4,13,14} The proportion between mannitol hexaacetate and *myo*-inositol hexaacetate was the same in the two samples.

Smith degradation of the LPS. A mixture of LPS (30 mg) in 0.1 M acetate buffer of pH 3.9 (20 ml) and 0.2 M sodium metaperiodate (5 ml) was kept in the dark at 4° for 120 h. Excess periodate was destroyed with ethylene glycol (1 ml), the solution was dialysed overnight and concentrated to 50 ml, sodium borohydride (300 mg) was added and the solution was kept at room temperature for 9 h. Excess borohydride was decomposed with 50 % acetic acid and the solution dialysed overnight. Part of the recovered material (1/15) was used for sugar analysis with D-arabinose as internal standard. Another part (1/3) was lyophilized and subjected to methylation analysis. After addition of 0.5 mg of D-arabinose (internal standard) the main part was treated with 0.25 M sulphuric acid at room temperature for 60 h, neutralized with Dowex 3 (free base) and reduced with sodium borodeuteride (50 mg) during 3 h at room temperature. The solution was treated with acetic acid and boric acid was removed by repeated distillations with methanol. The material was divided into two equal parts which were lyophilized and dried in a vacuum over phosphorus pentoxide. One part was subjected to methylation analysis. The other part was dissolved in dry pyridine (2 ml) and treated with a mixture of trimethylchlorosilane (0.2 ml) and hexamethyldisilazane (0.4 ml). After 20 min at 40° the mixture was concentrated to dryness, dissolved in carbon tetrachloride, filtered and used for GLC-MS. The ratio of the responses for the TMS-derivatives of D-arabinitol and melibiitol, determined in a separate experiment, was 3.4:1. Using the same factor the main peak ($T_{\text{melibiitol}} = 1.04$) accounted for 0.4 mg. The mass spectrum of this component showed, *inter alia*, the following signals (relative intensities in brackets): 73(100), 103(37), 104(20), 129(23), 147(23), 204(30), 217(29), 361(33), 451(5) and 467(3).

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