Structural Studies on the Lipopolysaccharide from *Klebsiella* K73:O10

I. Methylation Analysis, Identification and Location of 3-*O*-Methyl-L-rhamnose

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Nimmich and Korten [1] recently determined the sugar composition of lipopolysaccharides (LPS) from 19 *Klebsiella* strains, belonging to the O-groups 1—12. Several of these LPS contained D-ribose, a sugar which is seldom found in polysaccharides. In the present paper, structural studies on the O-specific side chains in one of these LPS, from *Klebsiella* K73:O10, are reported.

The LPS, which was isolated as described previously,[1] yielded on acid hydrolysis D-glucose, D-ribose, L-rhamnose and another sugar in the relative proportions 5:36:57:1, as determined by GLC [2] of their alditol acetates. In addition to these sugars, Nimmich and Korten [1] demonstrated the presence of 3-deoxy-octulosonic acid (KDO), *N*-acetyl-D-glucosamine and heptose residues in the LPS.

The minor component in the hydrolysate had a higher mobility than L-rhamnose on paper chromatography and was identified by the MS of its alditol acetate as a 6-deoxy-3-*O*-methyl hexose. On demethylation, with boron trichloride,[3] followed by reduction and acetylation, a product was obtained, which was indistinguishable, on GLC, from L-rhamnitol acetate. Because of the small amount available, no reliable value for the optical rotation of the sugar could be obtained. As L-rhamnose is one of the main components of the LPS it seems, however, reasonable to assume that the minor sugar is 3-*O*-methyl-L-rhamnose. This sugar, acofriose, has previously been found in cardiac glycosides [4] and glycolipids from mycobacteria.[5]

The LPS was methylated by the Hakomori procedure.[6] By using trideuteriomethyl iodide, examination of the methylated sugars obtained on hydrolysis of the methylated product should permit the original and the introduced *O*-methyl groups to be distinguished. The sugars, as their alditol acetates, were investigated by GLC—MS.[7] ECNSS—M columns, generally used for this analysis, gave incomplete separation of the components. A good separation was, however, obtained on an OS-138 (polyphenyl ether) SCOT-column (Fig. 1). The results of the analysis are summarised in Table 1, column A.

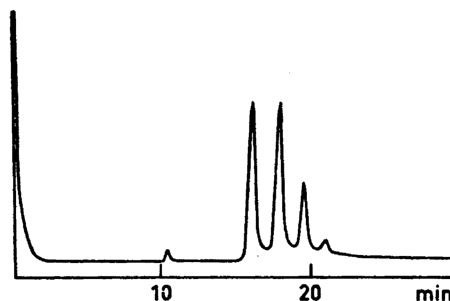


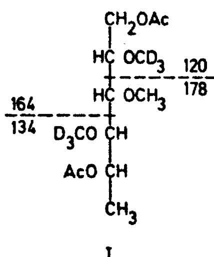
Fig. 1. GLC separation, on the OS-138 SCOT-column at 180°, of the alditol acetates of the methylated sugars, obtained from the hydrolysate of the fully methylated polysaccharide.

Table 1. Methyl ethers obtained in the methylation analysis of the polysaccharide (A) and the partially hydrolysed polysaccharide (B).

Sugars	T^a	Mol %	
		A	B
2,3,4-Tri-*O*-Me-L-Rha	0.49	1.6	5.4
2,5-Di-*O*-Me-D-Rib	0.77	36.1	36.2
2,3-Di-*O*-Me-L-Rha	0.86	39.8	34.5
2,4-Di-*O*-Me-L-Rha	0.94	19.4	22.6
2,3,4,6-Tetra-*O*-Me-D-G	1.00	2.1	1.3

[a] Retention times of the corresponding alditol acetates on the OS-138 SCOT-column relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

The component in the first peak was identified from its MS as 1,5-di-*O*-acetyl-2,4-di-*O*-trideuteriomethyl-3-*O*-methyl-L-rhamnitol (I), all the expected primary and



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secondary fragments being observed. As the percentage of this component corresponds to that of 3-*O*-methyl-L-rhamnose in the sugar analysis, it is inferred that essentially all the 3-*O*-methyl-L-rhamnose residues, but no L-rhamnose residues, occupy terminal positions in the LPS.

The other components were readily identified by comparing their MS with those of the corresponding, non-deuterated analogues.

It is evident, from the results of the methylation analysis, that all D-ribose residues are furanosidic, and that the terminal 3-*O*-methyl-L-rhamnose residues and the 3-linked L-rhamnose residues are pyranosidic. It seems reasonable to assume that the 2,3-di-*O*-methyl-L-rhamnose is also derived from pyranosidic L-rhamnose residues.

The LPS was treated with acid under mild conditions, in order to hydrolyse a considerable proportion of the D-ribofuranosidic linkages. The oligo- and polymeric material was recovered by dialysis and subjected to methylation analysis (Table 1, column B). The increase in 2,3,4-tri-*O*-methyl-L-rhamnose and the corresponding decrease in 2,3-di-*O*-methyl-L-rhamnose indicates that the D-ribofuranose residues are linked to the 4-position of L-rhamnose residues, and lends support to the assumption that all the L-rhamnose residues are pyranosidic.

From the low optical rotation of the LPS, $[\alpha]_{578}^{20}$ $-60°$ (c 0.25, H_2O), it is inferred that the L-rhamnopyranose residues are α-linked, and the D-ribofuranose residues β-linked.

The O-specific side chains of LPS are generally composed of oligosaccharide repeating units. In the present LPS, D-ribose, L-rhamnose and 3-*O*-methyl-L-rhamnose are clearly derived from the O-specific side chains whereas the other sugars originate from the basal core (*cf.* Ref. 1). As the ratio of L-rhamnose to D-ribose residues is approximate 3:2, the simplest repeating unit should contain five sugar residues. The tentative structure II accounts for the results discussed above. In the terminal repeating unit, the 3-linked L-rhamnose residue in II should be replaced by a 3-*O*-methyl-L-rhamnose residue. From the percentages of the methylated sugars (Table 1, column A),

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the average number of repeating units in the O-specific side chains is approximately 12. Fragmentation analysis of the LPS is in progress.

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Experimental. The methods used are the same as described previously.[8] In the sugar analysis, the hydrolysis was performed with 0.05 M sulphuric acid at 100° for 14 h. For the graded acid hydrolysis, the polysaccharide (3 mg) was dissolved in water (3 ml), 0.05 M sulphuric acid (3 ml) was added, and the solution kept at 100° for 10 min. The hydrolysate was, after neutralisation with $BaCO_3$, dialysed overnight and evaporated to dryness, and the residue was subjected to methylation analysis as described before.[8]

1. Nimmich, W. and Korten, G. *Pathol. Microbiol.* **36** (1970) 179.
2. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. *Anal. Chem.* **12** (1965) 1602.
3. Bonner, T. G. and Bourne, E. J. *Methods Carbohydrate Chem.* **2** (1963) 206.
4. Thudium, F., Schindler, O. and Reichstein, T. *Helv. Chim. Acta* **42** (1959) 2.
5. MacLennan, A. P. *Biochem. J.* **82** (1962) 394.
6. Hakomori, S. *J. Biochem. (Tokyo)* **55** (1964) 205.
7. Björndal, H., Hellerqvist, C. G., Lindberg, B. and Svensson, S. *Angew. Chem.* **82** (1970) 643.
8. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A. A. *Carbohyd. Res.* **8** (1968) 43.

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