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Structural Studies on the O-Specific Side-Chains of the Cell-Wall Lipopolysaccharide from *Pasteurella pseudotuberculosis* Group II A

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The structure of the O-specific side-chains of the cell-wall lipopolysaccharide from *Pasteurella pseudotuberculosis* group II A has been investigated, using methylation analysis and partial hydrolysis studies. One of the sugar components of this lipopolysaccharide is a 6-deoxyheptose, possibly with the D-*manno*-configuration. As a result of these studies a tentative structure for the repeating unit of the O-specific side-chains is proposed.

Pasteurella pseudotuberculosis group II has recently been divided into two subgroups, II A and II B, containing the antigens 5,6 and 5,7, respectively.[1] The structure of the O-specific side-chains of the group II B lipopolysaccharide (LPS), which carries the O-antigens, has recently been investigated [2] and a structure for the repeating unit of the O-specific side-chains proposed. In the present communication, similar studies on the group II A LPS are reported.

RESULTS

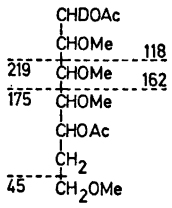
The LPS was isolated from formaldehyde killed cells of *P. pseudotuberculosis* group II A by extraction with phenol-water.[3] A lipid-free polysaccharide (PS) was prepared from the LPS by treatment with aqueous acetic acid at 100°, partition between aqueous ethanol and hexane and gel filtration on Sephadex G-25.

A hydrolysate of the PS contained abequose, mannose, galactose, glucose, an unknown sugar, and heptose in the relative percentages 16 : 3 : 21 : 9 : 22 : 30. Two heptoses, the alditol acetates of which had the same retention times on GLC as those derived from D-*glycero*-D-*manno*-heptose (8 %) and L-*glycero*-D-*manno*-heptose (22 %) were present. Abequose was identified by Davies.[4]

In the present investigation, the sugars were identified by GLC-MS. It seems reasonable to assume that the hexoses have the D-configuration.

The unknown sugar yielded an alditol acetate which, from its MS, was a 2-deoxy-heptitol acetate. It had the same retention time as the acetate of 2-(6-)deoxy-D-*manno*-heptitol (T = 1.25, relative to D-glucitol hexaacetate on an ECNSS-M column). 2-Deoxy-D-*galacto*-heptitol acetate showed T = 1.4, and no other reference substances were available. MS of an alditol acetate, obtained after reduction with borodeuteride, proved that the parent sugar was a 6-deoxy-heptose and not a 2-deoxy-heptose. The same evidence was obtained on MS of some partially methylated alditol acetates of this sugar, as will be discussed below. The amount of PS available did not allow the isolation of this sugar in sufficient amount to determine its physical properties. On paper chromatography (ethyl acetate – acetic acid – water, 3 : 1 : 1), it moved slightly faster than D-mannose. It gave a yellowish brown colour with anisidine hydrochloride, more yellowish than that given by the aldohexoses. To the best of our knowledge, 6-deoxy-heptoses have not been found in Nature before, nor have they been synthesized.

Methylation of the PS by the Hakomori procedure,[5] followed by acid hydrolysis, reduction with borodeuteride and acetylation, produced a mixture



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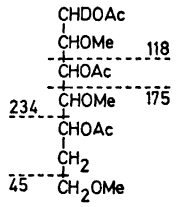


II

of alditol acetates which was analysed by GLC-MS.[6] The results are given in Table 1, column A. The identification of most of the components requires no comments. Two alditol acetates (I and II) from their MS, especially the primary fragments formed on fission of the alditol chain, were identified as deriving from a 6-deoxy-2,3,4,7-tetra-*O*-methyl-heptose and a 6-deoxy-2,4,7-tri-*O*-methyl-heptose, respectively.

Part of the PS was hydrolysed under mild conditions, when essentially all the abequosidic linkages should be cleaved, and the carbohydrate material was recovered and subjected to methylation analysis (Table 1, column B).

For another part of the PS, the change in optical rotation with time on acid hydrolysis was followed (Fig. 1). The initial fast decrease in optical rotation should be due to hydrolysis of abequosidic linkages, which consequently have the α-configuration. After about 15 min substancially all abequosidic linkages are hydrolysed, as indicated by the experiment described above. The subsequent decrease should be due to hydrolysis of the second most readily

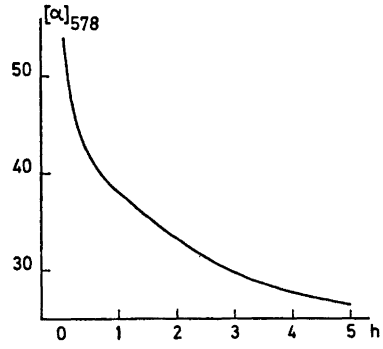


Fig. 1. Optical rotation *versus* time on acid hydrolysis of the *P. pseudotuberculosis* II A PS.

Table 1. Methyl ethers obtained from the hydrolysates of the fully methylated PS (A) and the partially hydrolysed, fully methylated PS (B).

Sugar[a]	T[b]	Mol %[c]	
		A	B
2,4-Abe	0.32	12.0	4.9
2,3,4,6-Man	1.00	5.5	2.0
2,3,4,7-6d-Hep	1.27	5.6	22.1
2,3,4,6,7-Hep	1.68	6.8	2.7
2,3,6-Gal	2.42	5.9	8.2
2,4,7-6d-Hep	2.90	16.5	0
2,6-Gal	3.65	17.6	26.8
2,3,6,7-Hep	5.6	4.1	1.8
2,3,4,6-Hep	5.9	2.1	0.9
2,4,6-Hep	12	1.1	4.5

[a] 2,4-Abe = 2,4-di-*O*-methyl-abequose, 2,3,4,7-6d-Hep = 6-deoxy-2,3,4,7-tetra-*O*-methyl-heptose, *etc.*

[b] Retention time of the corresponding alditol acetate on the ECNSS-M column, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

[c] As considerable amounts of the volatile 2,4-di-*O*-methyl-abequose and derivatives were lost during the analysis, the molar percentages are given relative to that of total 6-deoxy-heptose derivatives, which are assumed to represent all the 6-deoxy-heptose in the sugar analysis.

hydrolysed linkages. If it is assumed that these are the 6-deoxy-heptosidic linkages and further that the 6-deoxy-heptose has the D-*manno*-configuration (see below) it should consequently be α-linked.

DISCUSSION

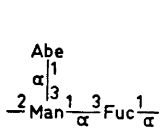
The O-specific side-chains of LPS are generally composed of oligosaccharide repeating units. The simplest unit, for the *P. pseudotuberculosis* group II A LPS, should contain one residue each of abequose, D-galactose and 6-deoxy-heptose. The heptose almost certainly derives from the core. The origin of the

other sugars is less certain, they may derive from contaminating carbohydrate material.

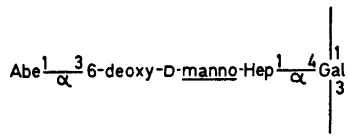
From the methylation analysis it is evident that all sugars are pyranosidic. Abequose is terminal. As all the 6-deoxy-2,4,7-tri-*O*-methyl-heptose was replaced by 6-deoxy-2,3,4,7-tetra-*O*-methyl-heptose after the mild acid hydrolysis, abequose should be linked to the 6-deoxy-heptose in the 3-position. The percentage of 6-deoxy-2,3,4,7-tetra-*O*-methyl-heptose in the methylation analysis of the orginal PS indicates that part of the abequosidic linkages was hydrolysed already during the preparation of PS from the LPS.

The 6-deoxy-heptose should be linked to D-galactose in the 3- or 4-position. As the 2,3,6-tri-*O*-methyl-D-galactose most probably derives from the terminal repeating unit, the 6-deoxy-heptose residue is consequently linked to the 4 position and the chain of D-galactose residues is linked through (1→3)-linkages.

The repeating unit of the group II B LPS (III) contains a 3-*O*-α-abequosyl-α-D-mannose residue.[2] As groups II A and II B have a common antigen, 5, they should contain similar structural features in the O-specific side-chains of their LPS. It therefore seems reasonable to assure that the 6-deoxy-heptose has the D-*manno*-configuration and that IV is the structure of the repeating unit. There is no evidence concerning the anomeric nature of the D-galactose



III



IV

residue. From the proportion between 2,4,6-tri-*O*-methyl-D-galactose and 2,6-di-*O*-methyl-D-galactose in the methylation analysis of the original PS, there are, at average, four repeating units in a side-chain. The same heptose ethers were found in studies of the group II A and group II B PS, indicating that they derive from the core part of the LPS. The D-glucose found in the sugar analysis is not accounted for in the methylation analysis, and the structural significance of the D-mannose and its tetra-*O*-methyl derivative is uncertain. The proposed structure for the repeating unit should therefore be regarded as tentatively only.

The structural studies demonstrate considerable differences between the II A and II B LPS. Davies [7] reported the presence of glucose, galactose, mannose, and abequose residues in the LPS from *P. pseudotuberculosis* group II. The presence of galactose and absence of fucose indicates that he studied a group II A LPS, but mistook the 6-deoxy-heptose for mannose.

EXPERIMENTAL

Isolation of LPS and PS. P. pseudotuberculosis strain 7 of serotype II A, obtained from Dr. W. Knapp, was employed as a source of LPS. The same methods as for group II B were used,[2] but a sugar analysis of the polysaccharide fraction still showed the

presence of non-sugar components; thus it was further fractionated on a Sephadex G-25 column. The PS fraction was eluted as a sharp peak, detected by its optical rotation. The two 2-deoxy-heptoses were kindly supported from Dr. M. B. Perry, Ottawa.

Added in proof. 6-Deoxy-D-*manno*-heptose has now been synthesised (to be published) and proved to be indistinguishable from the natural 6-deoxyheptose. The two sugars showed the same optical rotation, $[\alpha]_D + 30° \pm 5$ (water), and the same mobility on paper chromatography and paper electrophoresis. Derivatives of the two sugars were also indistinguishable by GLC.

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