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STRUCTURE OF THE POLYSACCHARIDE ANTIGEN OF *Eubacterium saburreum*, STRAIN L44*

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ABSTRACT

Structural studies of the polysaccharide antigen produced by the anaerobic, oral filamentous micro-organism *Eubacterium saburreum*, strain L44, are reported. It is concluded that the polysaccharide is linear and composed of β-(1→6)-linked D-*glycero*-D-*galacto*-heptopyranose residues. About 65% of these residues carry an *O*-acetyl group in the 7-position.

INTRODUCTION

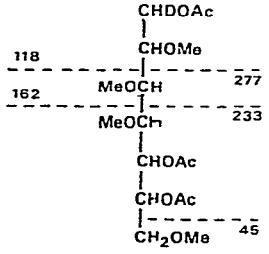
Although heptoses are components of several bacterial polysaccharides[1], there is only one example of a homoglycan composed of heptose residues. This is the polysaccharide antigen, PS L44, produced by an anaerobic, oral filamentous micro-organism, *Eubacterium saburreum*[2]. This antigen, in addition to a heptose component, tentatively identified as D-*glycero*-D-*galacto*-heptose, also contains a high percentage of *O*-acetyl groups. Its physical properties, location in the cell wall[3], and serological properties[4] have been investigated. We now report structural studies of this polysaccharide.

RESULTS AND DISCUSSION

The heptose, isolated from a hydrolysate of PS L44, and the derived alditol acetate were chromatographically indistinguishable from D-*glycero*-D-*galacto*-heptose and its alditol acetate, respectively. The heptose, which showed $[\alpha]_{578}$ +60°, gave a crystalline diethyl dithioacetal which was indistinguishable (m.p., i.r.) from the corresponding derivative[5] prepared from an authentic sample of D-*glycero*-D-*galacto*-heptose. The previous, tentative identification of the sugar as D-*glycero*-D-*galacto*-heptose is thus confirmed.

*Dedicated to Dr. Horace S. Isbell, in honour of his 75th birthday.

The polysaccharide was methylated by the Hakomori procedure[6], and hydrolysed, and the sugars in the hydrolysate were reduced with borodeuteride, acetylated, and analysed by g.l.c.–m.s.[7]. One main component was obtained which, according to its mass spectrum, was derived from 2,3,4,7-tetra-*O*-methyl-D-*glycero*-D-*galacto*-heptose. The primary fragments formed are indicated in formula **1**. The expected secondary fragments were also observed. Of these, that having *m/e* 113, with the probable structure **2**, is typical[8] for the primary fragment *m/e* 233 having both methoxyl groups in terminal positions, as indicated in **1**.



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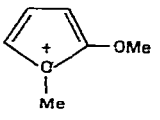

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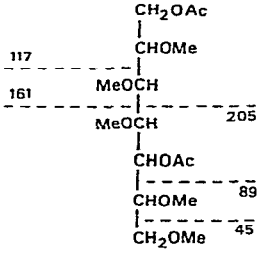
The polysaccharide is thus composed either of (1→6)-linked heptopyranose residues or, less probably, of (1→5)-linked heptoseptanose residues. The latter alternative was eliminated by subjecting the polysaccharide to partial hydrolysis with acid, borodeuteride reduction, methylation, hydrolysis, borohydride reduction, acetylation, and analysis of the products by g.l.c.–m.s. In addition to the 2,3,4,7-tetra-*O*-methyl derivative discussed above, 1,5-di-*O*-acetyl-2,3,4,6,7-penta-*O*-methylheptitol (**3**) and 6-*O*-acetyl-1,2,3,4,5,7-hexa-*O*-methylheptitol (**4**), deuterated at C-1, were observed.



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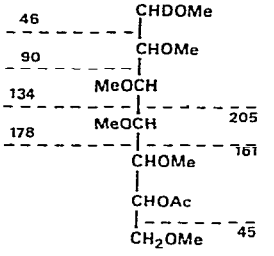

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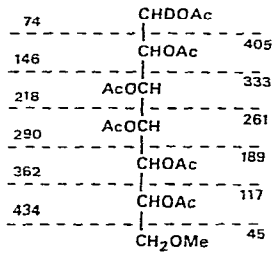
Compound **3** is derived from heptopyranose residues, and **4** from heptose residues substituted in the 6-position, thus precluding the heptoseptanose alternative. Some pertinent fragments, demonstrating that C-6 is methoxylated in **3** and acetoxylated in **4**, respectively, are indicated in the formulae.

The methylation analysis of PS L44 was repeated, using a dextran of known structure[9] as internal standard. Assuming the same response factors for the different, partially methylated alditol acetates, the 1,5,6-tri-*O*-acetyl-2,3,4,7-tetra-*O*-methyl-D-*glycero*-D-*galacto*-heptitol accounted for approximately 76% of the polysaccharide

material. This agrees well with previous analyses[2] of PS L44. In g.l.c. of some methylation analyses, peaks other than those given by the heptose derivatives were observed. The components in these peaks gave only weak mass spectra, which could not be interpreted. These components most probably do not derive from the polysaccharide but from a contamination.

No penta-*O*-methylheptose was observed in the methylation analysis of PS L44, indicating that the polysaccharide has a high molecular weight. Gel filtration and ultracentrifugation experiments[2] indicated a high molecular weight and a considerable polydispersity. The polysaccharide has a negative rotation, $[\alpha]_{578}$ −35°, indicating that all sugar residues are β-D-linked. Methyl β-D-*glycero*-D-*galacto*-heptopyranoside[10] shows $[\alpha]_D$ −5°.

O-Acetyl groups were located by the method devised by de Belder and Norrman[11]. The polysaccharide was treated with methyl vinyl ether and a catalytic amount of toluene-*p*-sulphonic acid in methyl sulphoxide, the acetalated material was methylated and hydrolysed, and the sugars in the hydrolysate were reduced with borodeuteride, acetylated, and analysed by g.l.c.–m.s. The alditol acetates of the heptose and of 7-*O*-methylheptose were obtained in the approximate proportion 35:65, demonstrating that 65% of the heptose residues are acetylated in the 7-position. The mass spectrum of the 7-*O*-methylheptitol derivative (**5**) was typical for a mono-*O*-methylalditol acetate with the methoxyl group in the primary position. A reasonably strong fragment with *m/e* 45 was observed, and the other ions were weak, compared to those given by similar derivatives with methoxyl groups in secondary positions[7]. The percentage of *O*-acetyl groups estimated from this analysis agrees well with that previously determined[2].



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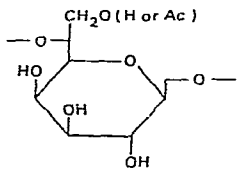

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Thus, the polysaccharide antigen from *Eubacterium saburreum* is shown to be composed of β-(1→6)-linked D-*glycero*-D-*galacto*-heptopyranose residues, and approximately 65% of these carry an *O*-acetyl group in the 7-position (**6**).

EXPERIMENTAL

General methods. — Concentrations were performed under diminished pressure at 40°. Melting points are corrected. For g.l.c., a Perkin–Elmer 990 instrument with

flame-ionisation detectors was used, and for g.l.c.–m.s. a Perkin–Elmer 270 instrument. For quantitative evaluations of the g.l.c., a Hewlett Packard 3370 B integrator was used. Optical rotations were recorded with a Perkin–Elmer 141 photo-electric polarimeter.

Identification of the heptose. — The polysaccharide, isolated as previously described[2], and further purified by passage through a column of hydroxylapatite (Bio Gel HTP, Bio Gel Laboratories, Richmond, Calif.) equilibrated with mM phosphate buffer (pH 6.8) showed $[\alpha]_{578}$ −35° (*c* 0.1, water). Part of the polysaccharide (10 mg) was hydrolysed, and the major component in the hydrolysate was isolated by preparative p.c. on Whatman No. 1 filter paper, using acetone–water (19:1) as irrigant. It had $[\alpha]_{578}$ +60° and was indistinguishable from D-*glycero*-D-*galacto*-heptose on p.c. (same solvent), and the derived alditol acetate had the same retention time as the hepta-acetate of D-*glycero*-D-*galacto*-heptitol on an ECNSS-M column and an OV 225 column at 190°. It was transformed into the diethyl dithioacetal[5], m.p. 197–199°. A sample prepared from authentic D-*glycero*-D-*galacto*-heptose had m.p. 198–200°; lit.[5] m.p. 189–190°. The i.r. spectra of the two preparations were superimposable.

Methylation analyses. — The polysaccharide (5 mg), in a 5-ml serum bottle sealed with a rubber cap, was dissolved in dry methyl sulphoxide (1 ml). The bottle was flushed with nitrogen, and 2M methylsulphinyl-sodium in methyl sulphoxide (1 ml) was added using a syringe. The gelatinous solution was agitated in an ultrasonic bath (40 kc/s) for 30 min and kept at room temperature for 8 h. Methyl iodide (1 ml) was added dropwise with external cooling, and the resulting, turbid solution was agitated for 30 min in the ultrasonic bath. The clear solution was diluted with water (10 ml), dialysed against distilled water, and evaporated to dryness. A solution of the methylated polysaccharide in 90% formic acid (3 ml) was kept for 2 h at 100°, concentrated to dryness, and then hydrolysed in 0.25M sulphuric acid (3 ml) at 100° for 12 h. The hydrolysate was neutralised with barium carbonate, and the sugars were reduced with sodium borodeuteride, acetylated, and analysed by g.l.c.–m.s.

G.l.c. of this product showed one main component with a mobility (*T* value) relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol of 4.92 on an ECNSS-M column (3% on Gas Chrom Q 100–120 mesh) at 170°, and 4.69 on an OV-225 column (3% on Gas Chrom Q 100–120 mesh) at 170°. It gave the following ions on m.s. (relative intensities are given in brackets): 43(100), 45(25), 71(5), 75(7), 87(11), 99(13), 101(7), 102(30), 113(18), 117(5), 118(38), 129(8), 131(6), 143(16), 156(6), 162(9), 173(2), 203(4), 233(13), 277(6).

The methylation analysis was repeated, using 5.9 mg of PS L44 and 2.9 mg of dextran from *Leuconostoc mesenteroides* NRRL B512[9] as an internal standard. The alditol acetates derived from 2,3,4-tri-*O*-methyl-D-glucose (obtained from 89% of the D-glucose residues in the dextran) and 2,3,4,7-tetra-*O*-methyl-D-*glycero*-D-*galacto*-heptose were present in the proportion ≃ 1:2. The heptose residues therefore account for 76% of the PS L44 material.

Another part of PS L44 (4 mg) was hydrolysed in 0.5M sulphuric acid (1 ml) for 4 h at 100°, the sugars in the hydrolysate were reduced with sodium borodeuteride,

and the product was subjected to methylation analysis, essentially as described above. The methylated product was, however, isolated by partition between chloroform and water, and not by dialysis. The alditol acetates were separated on an OV-225 column at 170°, and identified by m.s. as the acetates of 1,2,3,4,5,7-hexa-*O*-methylheptitol (45%, *T* 0.86), 2,3,4,6,7-penta-*O*-methylheptitol-1-*d* (30%, *T* 2.22), and 2,3,4,7-tetra-*O*-methylheptitol (25%, *T* 4.11).

Location of the O-*acetyl groups.* — The polysaccharide (20 mg) and toluene-*p*-sulphonic acid (5 mg) were dissolved in methyl sulphoxide (2 ml) in a stoppered flask. Methyl vinyl ether (1 ml) was added and the solution was kept at 14–15° for 3 h. The clear, yellow reaction mixture was placed on a column (25 × 3 cm) of Sephadex LH 20, which was then eluted with anhydrous acetone. The separation was monitored by optical rotation, and the acetalated polysaccharide was eluted with the void volume free of reagent. The acetalated polysaccharide was methylated as described above, hydrolysed, reduced with sodium borodeuteride, acetylated, and analysed by g.l.c.–m.s.

G.l.c. (OV-225 column) of this mixture showed the presence of two main components, one (35%, *T* 2.42, relative to D-glucitol hexa-acetate) derived from unchanged heptose, and the other (65%, *T* 1.34) was identified as 1,2,3,4,5,6-hexa-*O*-acetyl-7-*O*-methylheptitol-*l-d*. It gave the following ions on m.s.: 43(100), 45(7), 85(2), 87(8), 99(3), 103(2), 110(2), 111(2), 115(9), 116(3), 128(4), 129(11), 140(3), 157(5), 159(3), 170(7), 171(4), 183(2), 188(2), 197(6), 201(1), 213(2), 218(1), 231(1), 257(1), 260(1), 261(1), 290(1), 303(1), 333(2), 362(1), 405(1), 434(1).

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