Carbohydrate Research, 58 (1977) 439–442

STRUCTURAL STUDIES OF THE POLYSACCHARIDE ANTIGEN OF *Eubacterium saburreum*, STRAIN L 452

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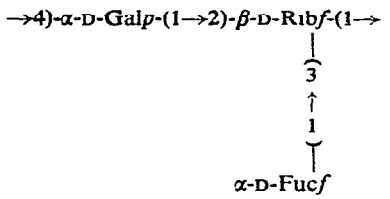
(Received December 2nd, 1976, accepted for publication, December 22nd, 1976)

ABSTRACT

The structure of the polysaccharide antigen produced by *Eubacterium saburreum*, strain L 452, has been investigated Methylation analysis, graded hydrolysis with acid, and n m r spectroscopy were the principal methods used The polysaccharide is composed of trisaccharide repeating-units having the following structure



The assignment of the β configuration to the D-ribofuranosyl residue is tentative

INTRIDUCTION

Investigations of the cell-wall polysaccharide antigens produced by *Eubacterium saburreum*, strains L 44[1] and L 49[2], have shown them to have unusual structures The former is composed of D-*glycero*-D-*galacto*-heptose and *O*-acetyl groups, whereas the latter contains, in addition to these components, a new sugar which has been identified as 6-deoxy-D-*altro*-heptose and is present as furanosidic end-groups We now report structural studies of the antigen produced by another strain, L 452, of the same organism[3]

RESULTS AND DISCUSSION

The antigen had $[\alpha]_{578}$ +170° and, on acid hydrolysis, yielded D-ribose, D-fucose, and D-galactose in approximately equimolecular proportions The sugars

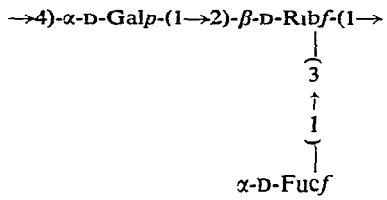
were characterised by g l c –m s of their alditol acetates and by their optical rotations The identity of D-fucose was confirmed by the formation of its methylphenylhydrazone, which was indistinguishable from an authentic sample (m p , i r) Bacterial polysaccharides are often composed of oligosaccharide repeating-units, and on this basis, these results indicate the antigen to contain a trisaccharide repeating-structure This was supported by the ^{1}H-n m r spectrum which showed, *inter alia*, a signal (3 H) for the methyl groups of the D-fucose residues at δ 1 20 ($J_{5,6}$ 6 Hz) and signals for anomeric protons at 5 10–5 20 (overlapping signals, 2 H) and 5 40 ($J_{1,2}$ low, 1 H) It was also evident from the n m r spectrum that the polysaccharide did not contain *O*-acetyl groups, and this conclusion was further confirmed by the i r spectrum where there was no absorption in the carbonyl region

Methylation analysis of the polysaccharide, with analysis of the partially methylated sugars as their alditol acetates[4], yielded 2,3,5-tri-*O*-methyl-D-fucose, 5-*O*-methyl-D-ribose, and 2,3,6-tri-*O*-methyl-D-galactose in the proportions 32 37 31 The results demonstrate that the D-fucose is furanosidic and terminal, and that the D-ribose is also furanosidic and linked through O-2 and O-3 The D-galactose is most probably pyranosidic and linked through O-4 Mild, acid hydrolysis of the polysaccharide (0 05M sulfuric acid at 80° for 3 h), with fractionation of the product on a column of Sephadex G-15, gave three main fractions The first fraction contained polymeric material, $[\alpha]_{578}$ +152°, on acid hydrolysis, it gave D-ribose and D-galactose, but only small proportions of D-fucose Methylation analysis gave approximately equal amounts of 3,5-di-*O*-methyl-D-ribose and 2,3,6-tri-*O*-methyl-D-galactose The terminal D-fucofuranosyl groups are therefore linked to O-3 of the D-ribofuranosyl residues The ^{1}H-n m r spectrum showed, *inter alia*, signals for the anomeric protons at δ 5 22 ($J_{1,2}$ 4 Hz, 1 H) and 5 40 ($J_{1,2}$ low, 1 H)

The second fraction, eluted in the disaccharide region, was shown by acid hydrolysis to be composed of equimolecular amounts of D-galactose and D-ribose It contained one main component which, after further purification by p c , had $[\alpha]_{578}$ +142°, on reduction with borodeuteride and methylation analysis, it gave 2,3,4,6-tetra-*O*-methyl-D-galactose and 1,3,4,5-tetra-*O*-methyl-D-ribitol-*1-d* It was thus identified as 2-*O*-α-D-galactopyranosyl-D-ribose The methylated disaccharide alditol was also investigated by g l c –m s The fragmentation pattern was in agreement with the postulated structure, using principles outlined for related substances[5] The results consequently demonstrate that the D-galactosyl residue is pyranosidic and α-linked, The last fraction from the Sephadex column contained almost pure D-fucose

During the graded, acid hydrolysis of the polysaccharide described above, an initial decrease in optical rotation was observed, indicating that the D-fucofuranosidic residues cleaved during this treatment were α-linked It has already been demonstrated that the D-galactopyranose residues are α-linked, but it is difficult to determine, either from the n m r evidence or from the optical rotations, whether the D-ribofuranosyl groups are α- or β-linked However, as β-D-ribofuranose residues are commonly observed in Nature, whereas the occurrence of the corresponding α-linked

units has not been conclusively established, it is tentatively assumed that the assignment of a β-linkage to the ribosyl units in the *E saburreum* polysaccharide is correct Consequently, the structure of the repeating unit of the polysaccharide is established as



The occurrence of D-fucofuranosyl groups in this structure is an unusual feature D-Fucose is a rare sugar in Nature[6], and fucofuranosyl residues, of undetermined absolute configuration, have only been observed once, in a polysaccharide from a diatom[7]

During this work, it was observed that 2,3,5-tri-*O*-methyl-D-fucitol obtained in methylation analyses, was only partially acetylated under the conditions normally applied The secondary hydroxyl group showed low reactivity, and the related 2,3,5,6-tetra-*O*-methyl-D-galactitol behaved similarly Complete acetylation could be achieved by prolonging the reaction time

EXPERIMENTAL

General methods — These were the same as previously described[1] N m r spectra for solutions in D_2O at 85° were recorded with a Varian XL-100 instrument operated in the PFT mode

Identification of components — The polysaccharide, isolated as previously described[3], showed $[\alpha]_{578}^{22}$ +170° (*c* 0 1, water) The value is corrected to apply to the carbohydrate content of the polysaccharide material, determined by sugar analysis in the presence of an internal standard Acid hydrolysis of the polysaccharide, with 0 25M sulfuric acid at 100° for 16 h, gave three components in the proportions 37 30 33 They were identified as fucose, ribose, and galactose by g l c –m s of their alditol acetates The free sugars were fractionated on two columns of micro-Bondapak Carbohydrate (30 × 5 cm), with acetonitrile–water (9 1) as eluant, and showed $[\alpha]_{578}^{22}$ +83°, −24°, and +80°, respectively The D-fucose was further characterised as its methylphenylhydrazone, m p 172–173°, and this derivative was indistinguishable from an authentic sample (m p , i r)

Methylation analyses — These were performed as previously described[1] The identifications of components were unambiguous and will not be discussed The following retention times of the derived alditol acetates, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, on g l c (SP-1000 column at 220°) were observed 2,3,5-tri-*O*-methyl-D-fucose, $T = 0\ 59$, 3,5-di-*O*-methyl-D-ribose, $T = 0\ 81$, 5-*O*-methyl-D-ribose, $T = 1\ 29$, and 2,3,6-tri-*O*-methyl-D-galactose, $T = 1\ 86$

During the methylation analysis, the acetylation of 2,3,5-tri-*O*-methylfucitol was incomplete using routine conditions [acetic anhydride–pyridine (1 1), 100°, 15 min] In addition to the expected 1,4-diacetate, a comparable amount of the 1-acetate ($T = 0$ 50) was obtained, and identified by its mass spectrum, which showed, *inter alia*, the following fragments (relative intensities in brackets) *m/e* 43(50), 59(100), 71(15), 87(22), 89(9), 101(27), 117(37), 131(11), 145(2), and 161(2)

The same behaviour was observed for 2,3,5,6-tetra-*O*-methyl-D-galactitol, which gave the analogous 1-acetate ($T = 1$ 04) Mass spectrum. *m/e* 43(95), 45(62), 59(56), 71(37), 87(47), 89(62), 99(20), 101(100), 117(62), 131(31), 143(8), and 161(11)

This incomplete acetylation has not been observed for other sugars, but could be overcome here by extending the reaction time to 1 h

Partial hydrolysis with acid — The polysaccharide (2 7 mg), in 0 05M sulfuric acid (1 ml), was kept at 80°, and the change in optical rotation was followed in a 10-cm tube; the $[\alpha]_{578}$ value dropped from +0 466° to +0 330° in 4 h The hydrolysis was repeated with 8 mg of polysaccharide, the solution was neutralised with barium carbonate and concentrated, and the products were fractionated on a column (1 5 × 90 cm) of Sephadex G-15 The elution was monitored by differential refractometry, and three main fractions were obtained polymeric (3 mg), D-galactosyl-D-ribose (0 5 mg), and D-fucose

The disaccharide was reduced with sodium borodeuteride, and subjected to methylation analysis on g l c , using an OV-1 column at 150°

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council (B76-03X-2522-08), Harald Jeanssons Stiftelse, Stiftelsen Sigurd och Elsa Goljes Minne, Knut och Alice Wallenbergs Stiftelse, the Norwegian Research Council for Science and the Humanities, and by NIH Research Grant No OE 03821-01 from the United States Public Health Service

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