Carbohydrate Research, 66 (1978) 67–70

STRUCTURAL STUDIES OF THE POLYSACCHARIDE ANTIGEN OF *Eubacterium saburreum*, STRAIN L 32*

JAMES HOFFMAN, BENGT LINDBERG,

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

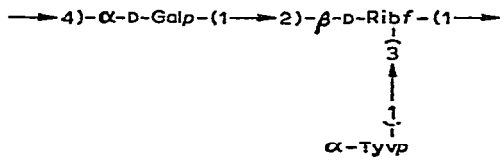
TOR HOFSTAD, AND NILS SKAUG

Department of Microbiology, The Gade Institute, Schools of Dentistry and Medicine, University of Bergen, Bergen (Norway)

(Received January 3rd, 1978; accepted for publication, January 10th, 1978)

ABSTRACT

The structure of the polysaccharide antigen produced by *Eubacterium saburreum*, strain L 32, has been investigated. The principal methods used were methylation analysis, graded hydrolysis with acid, and n.m.r. spectroscopy. The polysaccharide, which contains the unusual sugar 3,6-dideoxy-D-*arabino*-hexose (tyvelose, Tyv), is composed of trisaccharide repeating-units having the following structure:



INTRODUCTION

The cell-wall polysaccharide antigens produced by *Eubacterium saburreum*, strains L 44[1], L 49[2], and L 452[3], contain several unusual sugar components, namely, D-*glycero*-D-*galacto*-heptopyranosyl, 6-deoxy-D-*altro*-heptofuranosyl, D-ribofuranosyl, and D-fucofuranosyl residues. We now report structural studies of the polysaccharide antigen from *E. saburreum*, strain L 32.

RESULTS AND DISCUSSION

The antigen had $[\alpha]_D$ +102°, and yielded D-ribose, D-galactose, and a third sugar in approximately equimolecular proportions on acid hydrolysis. From the mass spectrum of its alditol acetate, the last sugar is a 3,6-dideoxyhexose. The optical

*Dedicated to Dr. Elizabeth Percival.

rotation ($[\alpha]_D$ +23°), the mobility in p.c., and the retention time of the alditol acetate in g.l.c. indicated it to be 3,6-dideoxy-D-*arabino*-hexose (tyvelose). This conclusion was further confirmed by preparing the alditol, which had m.p. 113–114° and $[\alpha]_{578}$ −38° in good agreement with published values[4]. 3,6-Dideoxy sugars are not uncommon as components of lipopolysaccharides from Gram-negative bacteria[5], but this is the first report of a 3,6-dideoxyhexose in a polysaccharide from a Gram-positive bacterium. The D configurations of the three sugar components were demonstrated by treating them with (+)-2-octanol and an acidic catalyst followed by acetylation, and comparing the gas chromatograms of the resulting products with those prepared from authentic materials[6].

Methylation analysis of the polysaccharide gave 2,4-di-*O*-methyltyvelose, 5-*O*-methyl-D-ribose, and 2,3,6-tri-*O*-methyl-D-galactose, which were identified by g.l.c.–m.s. of their alditol acetates. The stoichiometry was not good, probably because of the volatility of the two former sugars. The results demonstrate that tyvelose occurs as pyranosidic end-groups, D-ribose as branching residues, linked through O-2 and O-3, and D-galactose as chain residues linked through O-4. The presence of D-galactose as furanosidic residues linked through O-5 was excluded on the basis of the stability of the galactosidic linkages towards acid hydrolysis. Investigation of a D-galactosylribitol obtained on graded hydrolysis followed by borohydride reduction (see below) confirmed this conclusion.

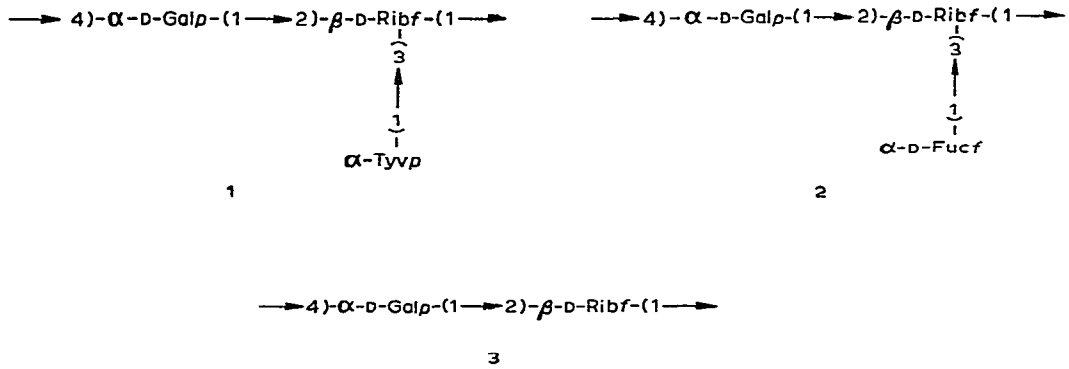
The ^{1}H-n.m.r. spectrum of the polysaccharide showed, *inter alia*, signals for H-6 and H-3 of the tyvelosyl group at δ 1.24 (3 H, $J_{5,6}$ 4 Hz) and 1.8 (multiplet, 2 H), respectively, and for three anomeric protons at δ 4.76 (1 H, $J_{1,2}$ low), 5.20 (1 H, $J_{1,2}$ 4 Hz), and 5.40 (1 H, $J_{1,2}$ low). The signal at the lowest field was assigned to H-1 of the ribofuranosyl residue. The signal at δ 4.76 was weak in samples that had been subjected to graded hydrolysis with acid and contained a low percentage of tyvelose, and was consequently assigned to H-1 of that sugar. The remaining signal, at δ 5.20, was assigned to the D-galactopyranosyl residue which, according to the shift and coupling constant of this signal, should be α-linked.

The ^{13}C-n.m.r. spectrum showed, *inter alia*, signals at δ 17.8 and 34.4, assigned to C-6 and C-3 of the tyvelopyranosyl group, respectively, and three signals for anomeric carbon atoms, at δ 99.0, 99.3, and 108.5. The signals for C-1 in methyl α- and β-D-ribofuranoside appear at δ 103.1 and 108.0, respectively, and the signal at δ 108.5 was therefore assigned to the D-ribofuranosyl residue, which should be β-linked[7].

The results given above indicate that the polysaccharide is composed of trisaccharide repeating-units. Graded hydrolysis of the polysaccharide with acid gave several products that were separated by gel-permeation chromatography. Methylation analysis of the material of high molecular weight gave only low percentages of 2,4-di-*O*-methyltyvelose and 5-*O*-methyl-D-ribose, demonstrating that the tyvelopyranosyl groups had been preferentially cleaved. The preponderant D-ribose derivative was the 3,5-dimethyl ether, demonstrating that tyvelose is linked to O-3 of D-ribofuranose in the original polysaccharide. A D-galactosyl-D-ribose, $[\alpha]_{578}$ +129°, was also isolated

from the hydrolysate. The product obtained on reduction of this disaccharide with sodium borodeuteride, followed by methylation, gave a mass spectrum which was indistinguishable from that[3] given by 2-*O*-α-D-galactopyranosyl-D-ribitol-*1-d*. During the graded hydrolysis, an initial decrease in optical rotation was observed, indicating that the tyvelopyranosyl linkages cleaved during this treatment were α-linked.

From the results given above, structure **1** is proposed for the repeating unit of the polysaccharide of *E. saburreum*, strain L 32. In the related structures for the repeating units of the antigen[3] (**2**) of *E. saburreum*, strain L 452, and the *Klebsiella* O-group 4 O-antigen[8] (**3**), the assignment of β configuration to the D-ribofuranosyl residue was only tentative. As the signal for the anomeric proton of the ribofuranosyl residue in each of the three polysaccharides appears at δ 5.40 ($J_{1,2}$ low), the assignment of β configuration to this residue in the two latter polysaccharides has now received strong support.



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 and glass columns [180 cm × 2 mm (i.d.)] containing 3% of OV-225 on 100/120 mesh Gas-Chrom Q was used. G.l.c.–m.s. was performed with a Varian MAT 311-SS instrument at an ionisation potential of 70 eV. N.m.r. spectra were recorded in the PFT mode with a JEOL FX-100 instrument, in D_2O at 85° for ^1H-spectra, and in D_2O at room temperature for ^{13}C-spectra. Optical rotations were determined with a Perkin–Elmer 241 instrument. Gel filtrations were performed on columns of Biogel-P 2. Paper chromatography was performed on Whatman No. 1 paper with 1-butanol–pyridine–water (6:4:3) as irrigant.

Identification of components. — The polysaccharide, isolated as previously described[9], was hydrolysed in 0.25M sulfuric acid at 100° for 3 h. The sugars were identified as tyvelose, ribose, and galactose by g.l.c.–m.s. of their alditol acetates, and were obtained in equimolecular proportions. The sugars were separated by p.c., and

proved to have the D configuration by comparing the gas chromatograms of their acetylated (+)-2-octyl glycosides with those prepared from authentic samples[6]; for this experiment, a column of SP-1000 was used. The tyvelose was aiso reduced with sodium borohydride to give the alditol, which was crystallized from acetone and had m.p. 113–114°, $[\alpha]_{578}$ −38°.

Methylation analysis. — The polysaccharide was treated by the procedure previously described[1]. The proportions of 2,4-di-*O*-methyltyvelose, 5-*O*-methylribose, and 2,3,6-tri-*O*-methyl-D-galactose were 2:3:5.

Graded, acid hydrolysis. — The polysaccharide (20 mg) was dissolved in 0.25M sulfuric acid, the solution kept at 80° for 3.5 h, the acid neutralized with barium carbonate, and the solution concentrated. The product was fractionated on a column (1.6 × 100 cm) of Biogel-P 2 by using water as irrigant. On methylation analysis, the first fraction eluted from the column yielded 2,4-di-*O*-methyltyvelose, 3,5-di-*O*-methyl-D-ribose, 5-*O*-methyl-D-ribose, and 2,3,6-tri-*O*-methyl-D-galactose in the proportions 1:2:1:6. After some higher oligosaccharides, a disaccharide was eluted, and purified by rechromatography on the same column. It had $[\alpha]_{578}$ +129°, and gave equimolecular parts of D-galactose and D-ribose on acid hydrolysis. Part of the disaccharide was reduced with sodium borodeuteride, methylated, and investigated by g.l.c.–m.s.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council (B77-03X-02522-09A), Harald Jeanssons Stiftelse, Stiftelsen Sigurd och Elsa Goljes Minne, Knut och Alice Wallenbergs Stiftelse, and the Norwegian Research Council for Science and the Humanities, and by NIH Research Grant No. DE 03821-01 from the United States Public Health Service.

REFERENCES

1 J. HOFFMAN, B. LINDBERG, S. SVENSSON, AND T. HOFSTAD, *Carbohydr. Res.*, 35 (1974) 49–53.
2 J. HOFFMAN, B. LINDBERG, J. LÖNNGREN, AND T. HOFSTAD, *Carbohydr. Res.*, 47 (1976) 261–267.
3 J. HOFFMAN, B. LINDBERG, T. HOFSTAD, AND H. LYGRE, *Carbohydr. Res.*, 58 (1977) 439–442.
4 O. WESTPHAL AND O. LÜDERITZ, *Angew. Chem.*, 72 (1960) 881–891.
5 O. LÜDERITZ, O. WESTPHAL, A. M. STAUB, AND H. NIKAIDO, in G. WEINBAUM, S. KADIS, AND S. J. AJL (Eds.), *Microbial Toxins*, Vol. IV, Academic Press, New York, 1971, pp. 145–233.
6 K. LEONTEIN, B. LINDBERG, AND J. LÖNNGREN, *Carbohydr. Res.*, 62 (1978) 359–362.
7 R. G. S. RITCIE, N. CYR, B. KORSCH, H. J. KOCH, AND A. S. PERLIN, *Can. J. Chem.*, 53 (1975) 1424–1433.
8 H. BJÖRNDAL, B. LINDBERG, J. LÖNNGREN, K. NILSSON, AND W. NIMMICH, *Acta Chem. Scand.*, 26 (1972) 1269–1271.
9 T. HOFSTAD AND N. SKAUG, *J. Gen. Microbiol.*, in press.