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Note

Structural studies of the O-antigen polysaccharide of *Vibrio fluvialis* AA-18239*

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Vibrio fluvialis, a species of micro-organism that causes diarrhoea and other symptoms similar to, although not as severe as, those caused by *V. cholerae* O-1 (Asian cholera) has been divided into 18 O-antigenic types[1]. A marine vibro, *V. fluvialis* 181–86 Kobe, which does not belong to any of these types, possesses[2] an O-antigenic factor in common with *V. cholerae* O-1. Its lipopolysaccharide (LPS) also contains 4-amino-4,6-dideoxy-D-mannose ("perosamine"), which is the main, possibly the only, sugar component of the *V. cholerae* O-antigen[3]. The structure of the O-antigen polysaccharide from another strain, *V. fluvialis* OKA-82-708, has been determined[4], and these seem to be the only chemical studies of LPS from *V. fluvialis*. We now report studies of the O-antigen polysaccharide of *V. fluvialis* AA-18239, a clinical strain isolated at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Dhaka.

The LPS was isolated from killed, freeze-dried cells of *V. fluvialis* AA-18239 by extraction with phenol–water[5] and purified by ultracentrifugation. The polysaccharide (PS) was prepared from the LPS by hydrolysis with aqueous 1% acetic acid at 100°, and was eluted from Bio-Gel P-10 shortly after the void volume.

The PS had $[\alpha]_D$ +107° (*c* 0.3, water) and, on hydrolysis with acid, yielded D-ribose, D-glucose, 2-amino-2-deoxy-D-galactose, and two heptoses in the molar proportions 3.0:1:6.2:0.5:0.3. The alditol acetates of the heptoses had the same retention times in g.l.c. as those of L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*-heptose, respectively. The absolute configurations of the sugars were determined as devised by Gerwig *et al.*[6].

Methylation analysis of the PS gave 3,5-di-*O*-methyl-D-ribose, 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2-deoxy-3,6-di-*O*-methyl-2-*N*-methylacetamido-D-galactose in the molar proportions 3.5:2.0:1:10.5.

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The ^{13}C-n.m.r. spectrum of the PS (Fig. 1) contained 13 strong and several weak signals, the former most probably from the O-specific side-chains and the latter from the core oligosaccharide. The simple spectrum indicated the O-specific side-chains to be composed of disaccharide repeating-units which, according to the results of methylation analysis, should be a 4-linked 2-acetamido-2-deoxy-D-galactopyranosyl and a 2-linked D-ribofuranosyl residue. This conclusion agrees well with the spectrum, and the signals for anomeric carbons at δ 96.61 and 107.69 further demonstrate[7] that the 2-acetamido-2-deoxy-D-galactopyranosyl residue is α and the D-ribofuranosyl residue β. The stoichiometries of the results of methylation and sugar analyses are poor, probably because a considerable part of the D-ribose and its dimethyl ether was destroyed during the hydrolyses or lost during the work-up. Nevertheless, the results demonstrate that the O-antigen polysaccharide is composed of disaccharide repeating-unit **1**.

$$\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1\rightarrow 2)\text{-}\beta\text{-D-Rib}f\text{-}(1\rightarrow$$

1

The ^{1}H-n.m.r. spectrum of PS was also in agreement with this structure and contained, *inter alia*, signals for two anomeric protons at δ 5.10 (J 3.4 Hz) and 5.23 (not resolved, $v_{1/2}$ 2.9 Hz), and for an *N*-acetyl group at δ 2.02.

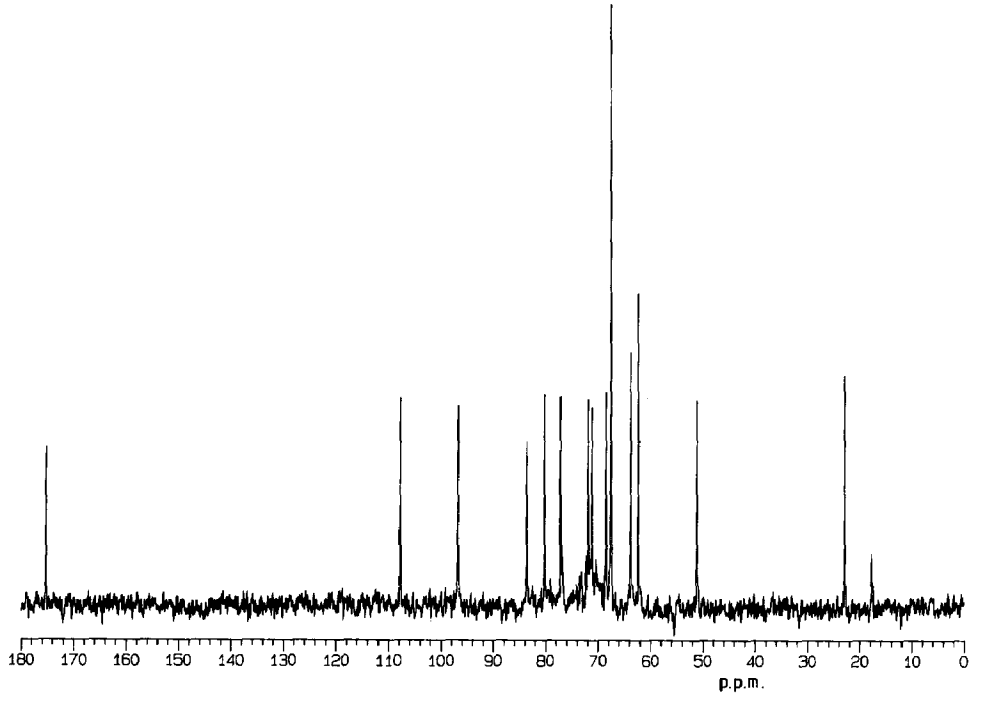

Fig. 1. ^{13}C-N.m.r. spectrum (internal 1,4-dioxane, δ 67.40) of the *Vibrio fluvialis* AA-18239 O-antigen polysaccharide.

Treatment of PS with acid under mild conditions, followed by reduction with sodium borohydride and chromatography on a reversed phase C_{18} column, gave two oligosaccharide-alditols as the main products. The ^{1}H-n.m.r. spectrum of one of these contained a single signal at δ 5.15 (J 3.8 Hz) in the region for anomeric protons. Hydrolysis with acid yielded ribitol and 2-acetamido-2-deoxy-D-galactose, as required from **2**. The other product gave signals for three anomeric protons, at δ 5.13 (J 3.6 Hz), 5.17 (J 3.6 Hz), and 5.29 (not resolved, $\nu_{1/2}$ 2.9 Hz), and gave ribitol, ribose, and 2-acetamido-2-deoxy-D-galactose on acid hydrolysis, as required from **3**. The main ions on f.a.b.-m.s. of **3** in the positive mode had m/z 691.4 for $[M + H]^+$ and 713.5 for $[M + Na]^+$. The characterization of **2** and **3** thus lends further support to the structure **1** postulated for the repeating unit of the O-antigen polysaccharide.

The same O-antigen as in *Vibrio fluvialis* AA-18239 has been found[9] in *Pseudomonas cepacia*, serogroup 3, and in some types of *Serratia marcescens* and *Pseudomonas aeruginosa* (see ref. 9.).

α-D-Gal*p*NAc-(1→2)-D-Ribitol

2

α-D-Gal*p*NAc-(1→2)-β-D-Rib*f*-(1→4)-α-D-Gal*p*NAc-(1→2)-D-Ribitol

3

EXPERIMENTAL

General methods. — These were the same as used in the investigation of the *Klebsiella* K21b extracellular polysaccharide[8]. F.a.b.-m.s. was performed on a JEOL SX 102 instrument, with a matrix of glycerol–thioglycerol (1:1). H.p.l.c. was carried out on a Waters μ Bondapak C_{18} column, using a Shimadzu LC 6A system and a UV detector at 195 nm.

Isolation of the O-antigen polysaccharide. — A culture of *V. fluvialis* AA-18239 was grown in Roux flasks on nutrient agar at 37° for 18 h. The cells were harvested by gentle shaking with aqueous 0.85% sodium chloride, collected by centrifugation, and killed by suspension in chloroform. The killed bacteria were suspended in water and centrifuged, and this operation was repeated twice, after which the cells were freeze-dried.

The LPS (450 mg) was extracted from the freeze-dried cells (100 g) by the phenol–water method[5], and purified by ultracentrifugation and freeze-drying.

The LPS (400 mg) in aqueous 1% acetic acid was kept at 100° for 90 min, the precipitate was removed by centrifugation, and the supernatant solution was freeze-dried. The product (160 mg) was eluted from a column (90 × 3 cm) of Bio-Gel P-10 with water. The PS (70 mg) was eluted shortly after the void volume.

Hydrolysis with acid under mild conditions. — A solution of the PS (3 mg) in 0.2M aqueous trifluoroacetic acid (1 mL) was kept at 80° for 2 h, then concentrated to dryness, and the product was reduced by borohydride. After conventional work-up, the product was fractionated, first on a Bio-Gel P-2 column and then by h.p.l.c. using acetonitrile–water (1:99).

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