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Note

Structural studies of the O-antigen polysaccharide of *Escherichia coli* 09a

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The O-antigens associated with strains of *E. coli* that form thick capsules are commonly 08 and 09 (ref. 1). The O-antigen carried by the serotype K26 (09a:K26:H$^-$) is stated to be 09a, suggesting a substance almost identical to, yet slightly different serologically, from the 09 antigen. The structure of the O-polysaccharide of *E. coli* 09 was reported by Prehm *et al.*[2] to be a mannan based on the pentasaccharide repeating unit **1**.

$$\underset{\text{e}}{\rightarrow 3)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow}\underset{\text{d}}{3)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow}\underset{\text{c}}{2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow}\underset{\text{b}}{2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow}\underset{\text{a}}{2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow}$$

1

The slight serological difference that exists between the 09 and 09a antigens has prompted us to investigate the 09a polysaccharide to establish a chemical basis for the difference. The structural elucidation of the polysaccharide is the subject of this note.

The O-antigen polysaccharide (PS) was isolated as described in the Experimental section, after removal of the capsular polysaccharide by an established procedure[3]. The PS, which had $[\alpha]_D^{23}$ +81.6°, gave only D-mannose on hydrolysis. Methylation analysis (Fig. 1A) of PS afforded roughly equimolar proportions of 3,4,6- and 2,4,6-tri-*O*-methylmannose (1.00:0.93). The methylation analysis (Fig. 1B) of 09 PS (isolated from 09:K9:H12 bacteria in the same manner as for 09a) showed these two sugars in the ratio 1.45:1.00, consistent with a theoretical ratio of 3:2 for the pentasaccharide repeating unit.

The high resolution ^{1}H- and ^{13}C-n.m.r. spectra of 09a PS and 09 PS are shown in Figs. 2 and 3 (A and B); no n.m.r. data were reported by Prehm *et al.*[2] for 09

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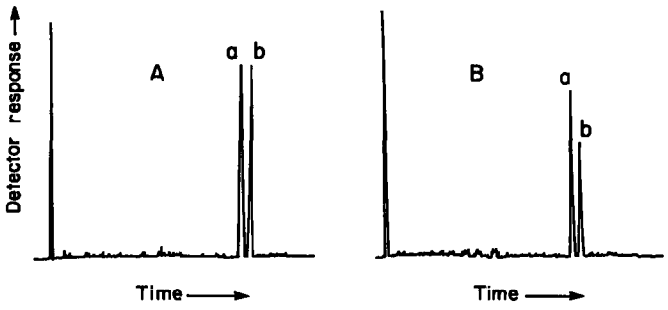

Fig. 1. Gas chromatogram of partially methylated alditol acetates from the polysaccharides of *E. coli* 09a (A) and 09 (B). a = 1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methylmannitol, b = 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol.

PS. The anomeric linkages in 09 PS have been conclusively proved to be α-linkages by both α-mannosidase digestion and optical rotation[2] (09 PS has $[\alpha]_D$ +89° (ref. 2), $[\alpha]_D$ of methyl α-D-mannopyranoside is +79.2°). Thus it can be taken that the anomeric signals in the n.m.r. spectra of 09 PS are all α-signals. The proton and carbon spectra of 09 and 09a PS are almost identical, the only visible difference being in the ^{1}H-spectra where the resonance at δ 5.27, due to a 2-linked mannose unit, integrates for two protons in the 09 PS but for only one proton in the 09a PS. Thus the ^{1}H-n.m.r. spectra are consistent with a pentasaccharide repeating unit for 09 PS and a tetrasaccharide repeating unit for 09a PS.

Ogawa and Yamamoto[4] have synthesized unambiguously a pentasaccharide corresponding to the 09 PS repeating unit **1** and have assigned the proton anomeric resonances and all the carbon resonances of this oligosaccharide. The assignments rested on the results of model studies of (1→2)- and (1→3)-α-linked mannans and oligomannoses, as detailed by Ogawa and Yamamoto[5], Ogawa and Sasajima[6], and Gorin[7]. It is on the basis of these studies that we have assigned the resonances in

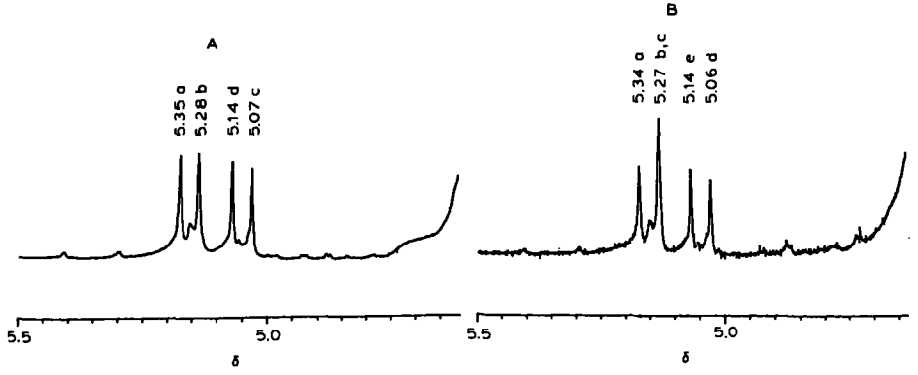

Fig. 2. 500 MHz ^{1}H-N.m.r. spectra of *E. coli* 09a PS (A), and 09 PS (B), recorded in D_2O at 95°. Values are expressed in p.p.m. downfield from DSS. The letters a, b, c, etc. above the peaks refer to the respective mannosyl residues in the repeating units of the PS's, as shown in structures **1** and **2**.

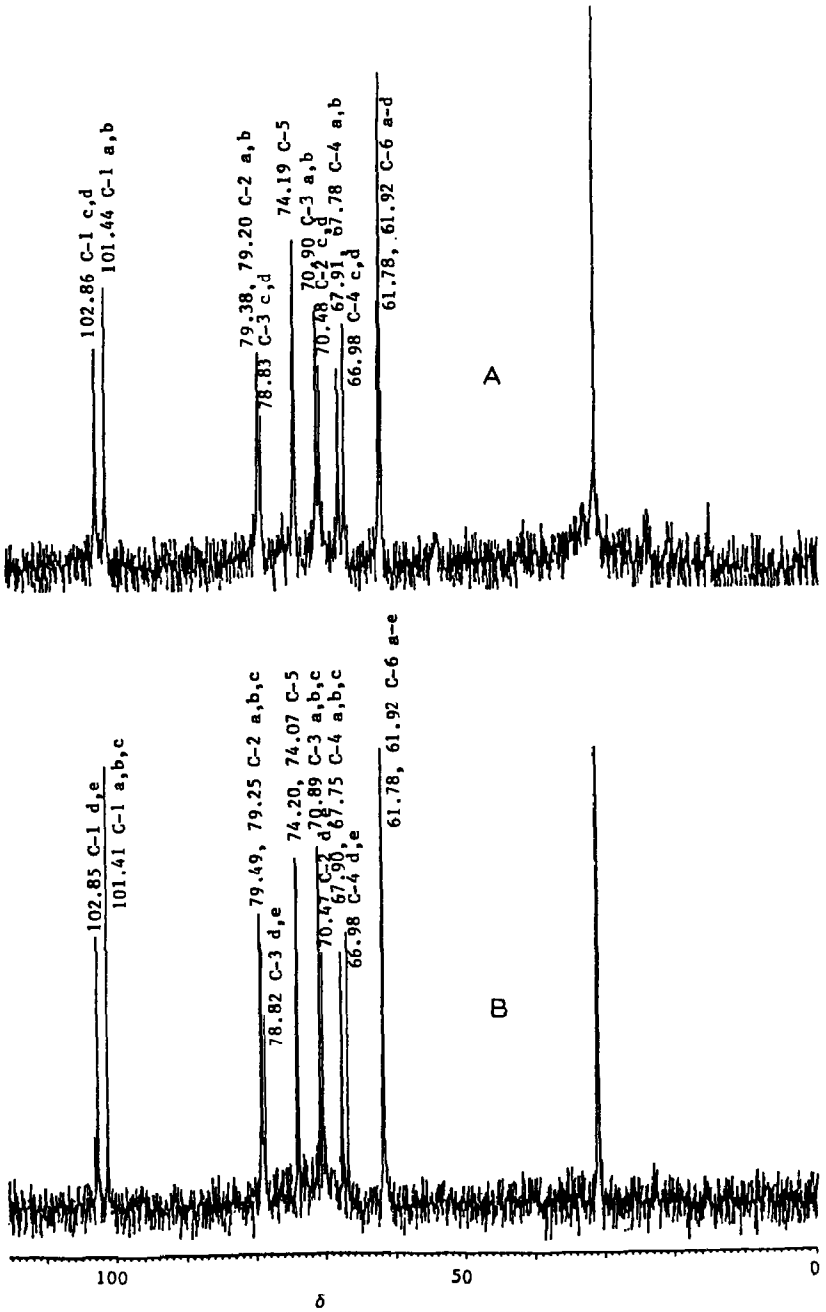

Fig. 3. 125 MHz ^{13}C-N.m.r. spectra of *E. coli* 09a PS (A), and 09 PS (B), recorded in D_2O at 30°. Chemical shifts are expressed in p.p.m. downfield from DSS. The letters a, b, c, have the same meaning as in Fig. 2.

the spectra of the 09 and 09a polysaccharides. The ^{1}H-n.m.r. spectrum of 09a PS shows that the H-1 protons are present in four distinct chemical environments, which can exist only if the (1→2)- and (1→3)-linked sugars occur in blocks in the repeating unit of the PS. An alternating structure of (1→2) and (1→3) linkages would show anomeric protons in only two different environments. The structure of the 09a polysaccharide from *E. coli* 09a:K26:H$^-$ must therefore consist of the following repeating unit **2**.

d c b a
→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→
2

EXPERIMENTAL

Polarimetry was performed in a 1 cm cell at 23° using a Perkin–Elmer model 141 instrument. Evaporations were carried out under reduced pressure at temperatures not exceeding 40°. ^{1}H- And ^{13}C-n.m.r. spectra were recorded on a Bruker WM-500 Ft spectrometer at 95° and 30°, respectively. Samples run in D_2O were first hydrogen-exchanged by freeze-drying from 99.7% D_2O. In all cases, acetone (δ 2.23 for ^{1}H and 31.07 p.p.m. for ^{13}C, measured against aqueous sodium 4,4-dimethyl-4-silapentane-1-sulfonate, DSS) was used as the internal standard. Gas-liquid chromatography was performed on a Hewlett–Packard HP 5890 chromatograph fitted with a flame ionization detector and employing a capillary column (30 m × 0.25 mm) of OV-225 (Durabond®) having a film thickness of 0.25 μm. Mass spectrometry was carried out on a Micromass VG 16F instrument at the mass spectrometry unit of the University of Cape Town.

Cultures of *E. coli* 09:K26:H$^-$ and 09:K9:H12 bacteria were obtained from Dr. I. Ørskov, Copenhagen, and were propagated on solid media (Mueller–Hinton agar). The capsular polysaccharides were isolated from the bacteria by CTAB precipitation as described previously[3], and the lipopolysaccharides were obtained from the supernatant CTAB solution after removal of the capsular material. Dialysis and freeze-drying gave the lipopolysaccharides, and the 09 and 09a polysaccharides (80 mg and 60 mg respectively) were separated from the lipid moieties by treatment with 1% acetic acid (1.5 h, 100°), followed by centrifugation and freeze-drying. Gel chromatography of the polymers on Sepharose 4B, with M aqueous NaCl as eluent, yielded purified polysaccharide in each case. A portion of 09a PS (1 mg) was hydrolyzed with 2M trifluoroacetic acid (TFA) at 100° for 16 h and the residue was converted to the peracetylated aldononitriles[8]. G.l.c. analysis showed only mannonitrile pentaacetate. Another portion (5 mg) was methylated by the Hakomori method[9] as modified by Sandford and Conrad[10], hydrolyzed (2M TFA, 100°, 16 h), reduced ($NaBH_4$, 1 h), acetylated (1:1 pyridine–acetic anhydride, 1 mL, 100°), and analyzed by g.l.c. A further portion of PS (8 mg) was hydrolyzed (2M TFA, 100°, 16 h), the acid was removed by evaporation, and the residue was

dissolved in water and freeze-dried. A specific optical rotation of +12.1° was obtained, indicating that the mannose units in the PS have the D-configuration (authentic D-mannose has $[\alpha]_D$ +14.2°).

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