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Note

Structure of a neutral polymer isolated from the lipopolysaccharide of the reference strain (C.D.C. 4523-60) for *Serratia marcescens* serogroup O15

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In continuation of our studies of the surface polysaccharides produced by reference strains of *Serratia marcescens*, we have isolated the lipopolysaccharide of the O15 strain C.D.C. 4523-60 (yield, 18% of the cell walls). The neutral monosaccharide composition (expressed as percentages of the total peak area on g.l.c. of the alditol acetates) was: rhamnose, 37.6; mannose, 22.4; glucose, 21.3; galactose, 1.6; D-*glycero*-D-*manno*-heptose, 3.8; L-*glycero*-D-*manno*-heptose, 13.2%. Other components were 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and galacturonic acid (no attempt was made to detect the 3-deoxy-2-octulosonic acid also expected to be present). On polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate, the lipopolysaccharide gave a ladder pattern typical of S-form products.

During treatment of the lipopolysaccharide with aqueous 1% acetic acid at 100°, the hydrolysate darkened considerably as found with almost all lipopolysaccharides from *S. marcescens* (*e.g.*, refs. 1 and 2). After freeze-drying, the water-soluble products were eluted from Sephadex G-50 to provide two polymeric fractions (F1a and F1b) in addition to an oligosaccharide fraction (F2) and minor, monomeric products. Fraction F1a (yield, 13% of the lipopolysaccharide) gave a sharp peak at the void volume, contained only glucose, mannose, and galacturonic acid, and was adsorbed by DEAE-Sepharose (requiring 0.2 or 0.3M NaCl for elution). This polymer will be the subject of a separate report.

Fraction F1b gave a very broad elution profile, extending almost from the void volume to the oligosaccharide (F2) fraction, and was mainly (83%) recovered from DEAE-Sepharose by elution with water. The apparent yield of F1b (14% of the lipopolysaccharide) was almost certainly an underestimate, as the isolated polymer would not dissolve completely in water after freeze-drying. Thus, material was probably lost when the initial hydrolysate was clarified by centrifugation before Sephadex chromatography. Fraction F1b consisted mainly of L-rhamnose (33.3%, corrected) and 2-amino-2-deoxy-D-galactose (25.0%, uncorrected), accompanied

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by small amounts of glucose (5.2%) and heptoses. Signals in the n.m.r. spectra at δ 2.15 (^{1}H), 177.68 and 25.46 (^{13}C), all referenced to external DSS, showed that the amino sugar was present as its *N*-acetyl derivative. Although the poor water-solubility of the polymer made it difficult to get good n.m.r. spectra, the presence of a disaccharide repeating-unit was clear from major anomeric signals at δ 4.97 (unresolved) and 4.88 ($J_{1,2}$ ~8 Hz) in the ^{1}H-n.m.r. spectrum, for example. Minor signals in the n.m.r. spectra pointed to some form of heterogeneity in fraction F1b.

The results of methylation analysis confirmed the heterogeneity of F1b: products derived from 3-substituted 2-acetamido-2-deoxygalactopyranose, 4-substituted rhamnopyranose, and 3-substituted rhamnopyranose residues were obtained. The ratio of the peak areas for the two methylated rhamnitol acetates in g.l.c. was 5.7:1.0. After *N*-deacetylation and deamination of F1b, followed by reduction ($NaBD_4$) of the products, most of the material was eluted from Sephadex G-15 in about the position of a disaccharide. Acid hydrolysis of the product (F1bDA) gave only rhamnose and 2,5-anhydrotalitol (ratio of the peak areas for the alditol acetates in g.l.c., 1.0:0.7). After methylation of F1bDA, a single g.l.c. peak was detected in the region expected for a permethylated disaccharide-alditol. A peak in the mass spectrum at *m/z* 189 showed that rhamnose was the non-reducing terminal residue in the methylated product, and conversion into methylated alditol acetates gave the products from unsubstituted rhamnopyranose and 3-substituted 2,5-anhydrotalitol residues (relative peak area, 1.0:0.8). The ^{1}H-n.m.r. spectrum of F1bDA contained a single anomeric signal at δ 4.91 ($J_{1,2}$ 1.6 Hz), and the ^{13}C-n.m.r. spectrum one at δ 101.13 ($^1J_{CH}$ 167 Hz). These data allow structure **1** to be proposed for fraction F1bDA, and indeed the ^{13}C-n.m.r. spectrum was identical with that of the product with this structure previously isolated[2] from the lipopolysaccharide of *S. marcescens* serogroup O9.

α-L-Rha*p*-(1→3)-2,5-anhydro-D-talitol

1

As the β configuration can be assigned to the 2-acetamido-2-deoxygalactose residues in F1b on the basis of the ^{1}H-n.m.r. spectrum for the polymer, structure **2** can be assigned to the disaccharide repeating-unit. The significance of the minor proportion of 3-substituted rhamnose residues in F1b is unclear. However, it is intriguing to note that the putative O-specific polymer of *S. marcescens* O9 (which can give cross-reactions with serogroup O15) is built mainly from disaccharide repeating-units of structure **3**, but also contains a minor proportion of 4-substituted rhamnose residues[2], *i.e.*, the converse of the situation in the neutral polymer from *S. marcescens* O15.

→4)-α-L-Rha*p*-(1→3)-β-D-Gal*p*NAc-(1→

2

→3)-α-L-Rha*p*-(1→3)-β-D-Gal*p*NAc-(1→

3

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of lipopolysaccharide. — *S. marcescens* O15 (strain C.D.C. 4523-60) was grown for 16 h at 30° in Nutrient Broth No. 2 (Oxoid) as a 20-L batch culture aerated at 20 L.min^{-1}. The lipopolysaccharide (0.52 g) was extracted from cell walls (2.77 g) prepared by mechanical disintegration of the cells (70 g wet weight), as in previous studies[1,2]. After mild hydrolysis (aqueous 1% acetic acid, 2.25 h, 100°) of the lipopolysaccharide, the water-soluble products were fractionated by chromatography on Sephadex G-50 and DEAE-Sepharose CL-6B (refs. 1 and 2).

General methods. Methods for the release, identification, and determination of monosaccharides (including chromatographic and electrophoretic methods) were mainly those described previously[2]. 2-Amino-2-deoxygalactose was determined by g.l.c. of the aminodeoxyalditol acetate, and was assigned to the D-series by g.l.c. of the oct-2-yl glycoside acetates[3]. N.m.r. spectra were obtained either with a Bruker WH-400 spectrometer (for product F1bDA) or a JEOL GX-270 spectrometer (for F1b itself). In the former case, the ^{1}H-n.m.r. spectrum for the sample in D_2O was recorded at 60° with TSP as the external reference, while the ^{13}C-n.m.r. spectra (with complete proton-decoupling or with gated decoupling) were recorded at 50° with Me_4Si as the external standard. In the second case (F1b), both spectra were recorded with DSS as the external reference at either 65° (^{1}H) or 22° (^{13}C).

Methylation analysis. — Methylated alditol acetates from F1b and F1bDA were prepared, and were identified by g.l.c. and m.s. as before[1,2]. On g.l.c. using a capillary column (25 m) of BP1 at 220°, permethylated F1bDA was eluted after 2.95 min (*cf.* ~3.5 min for permethylated maltitol).

N-*Deacetylation and deamination.* — Fraction F1b was *N*-deacetylated by treatment with NaOH in aqueous Me_2SO (ref. 4) for 22 h at 100° under nitrogen. The work-up, deamination, reduction ($NaBD_4$), and fractionation of the products on Sephadex G-15 were carried out as previously[2].

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