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Note

Structure of a neutral polymer isolated from the lipopolysaccharide of *Serratia marcescens* O1 (strain C.D.C. 866-57)

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We have recently shown[1,2] that both neutral and acidic polymers can be isolated from cell-wall lipopolysaccharides of the Gram-negative bacterium *Serratia marcescens* after mild acid hydrolysis. There are indications[2] that the acidic polymers may be (micro)capsular components and that, in the case of the closely related serogroups O14 and O6, at least, they are responsible for O specificity as presently defined. It was therefore of interest to examine the lipopolysaccharides of other strains of *S. marcescens* representing different O serogroups. Here we describe the fractionation of lipopolysaccharide of the reference strain (C.D.C. 866-57) for serogroup O1, and the structure of the neutral polymer thereby obtained.

Like other lipopolysaccharides from *S. marcescens*[1–3], the product from the O1 strain gave a dark-brown suspension on hydrolysis with aqueous 1% acetic acid at 100°. From the water-soluble products, a polymeric fraction (yield, 50% of the lipopolysaccharide) was obtained by chromatography on Sephadex G-50. The heterogeneity indicated by the elution profile for this fraction was confirmed by stepwise elution from a column of DEAE-Sepharose. About 40% of the material was eluted with water or 0.1M NaCl, and had only rhamnose and 2-amino-2-deoxyglucose as major monosaccharide components. Glucose was a trace component of the aqueous eluate and a minor one of the 0.1M NaCl eluate (ratio of peak areas on g.l.c. of the alditol acetates from rhamnose and glucose, ~4:1). The latter eluate also contained small but significant amounts of L-*glycero*-D-*manno*-heptose and D-*glycero*-D-*manno*-heptose: glucose and the heptoses are likely to be components of a core oligosaccharide[1,3]. The material eluted with higher concentrations of NaCl (0.2M and 0.3M) was composed of D-glucose, D-mannose, 2-amino-2-deoxy-D-glucose, and galacturonic acid (molar ratios ~2:1:1:1). Further studies of this acidic, *O*-acetylated polymer will be reported separately.

The i.r. spectrum of the neutral polymer from the aqueous eluate showed the absence of *O*-acyl substituents and indicated that the 2-amino-2-deoxyglucose was

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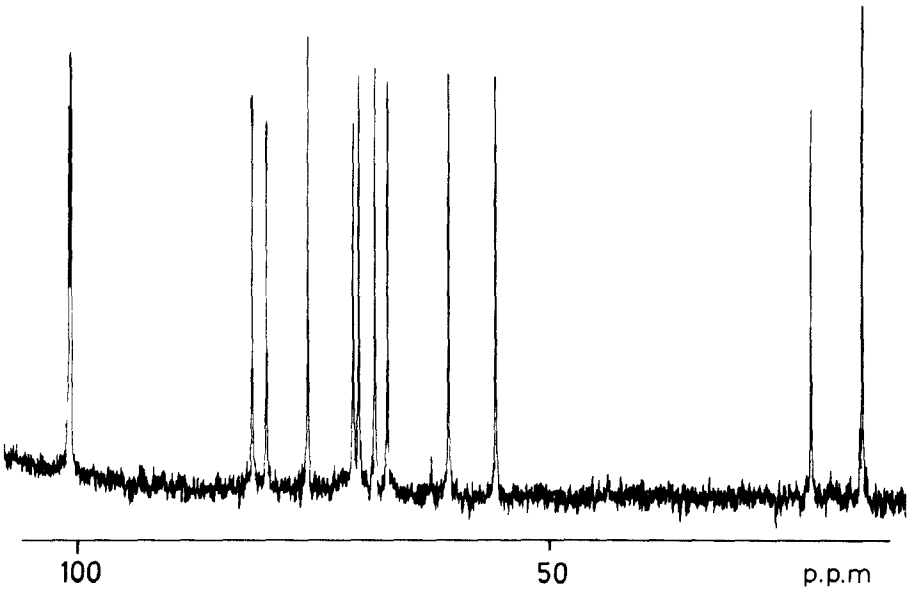

Fig. 1. ^{13}C-N.m.r. spectrum of the neutral polymer from the lipopolysaccharide of *S. marcescens* C.D.C. 866-57. The spectrum for the sample in D_2O was obtained at 100.61 MHz and 50° with complete proton-decoupling. In addition to the signals shown, the spectrum contained a signal for a carbonyl carbon at δ 174.48 with reference to external tetramethylsilane.

present as its *N*-acetyl derivative (bands at 1650 and 1560 cm^{-1}). This was confirmed by a methyl singlet at δ 2.06 in the ^{1}H-n.m.r. spectrum and signals at δ 174.48 and 22.46 in the ^{13}C-n.m.r. spectrum (Fig. 1). Both spectra also indicated that the polymer had a disaccharide repeating-unit. The ^{1}H-n.m.r. spectrum contained one-proton anomeric signals at δ 4.87 (broad singlet) and 4.81 ($J_{1,2}$ 8.8 Hz) and a methyl doublet ($J_{5,6}$ ~6 Hz) at δ 1.28. The ^{13}C-n.m.r. spectrum contained anomeric signals at δ 101.17 and 101.00, too close for the *J* values to be determined by gated decoupling of the signals. Nevertheless, the ^{1}H-n.m.r. spectrum showed that at least the 2-acetamido-2-deoxyglucosyl residue was β-linked, and pyranoid structures could be inferred for both sugars.

The presence in the ^{13}C-n.m.r. spectrum of a signal at δ 60.98 showed that the 2-acetamido-2-deoxyglucosyl residue was not substituted at position 6. Survival of the sugar during periodate oxidation confirmed that it must be substituted at position 3 or 4, while destruction of the rhamnose indicated substitution at position 2 or 4. Only two significant products were obtained on methylation analysis of the polymer. The identification of these products as 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol-*1-d* and 1,3,5-tri-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-2-*N*-methylacetamidoglucitol-*1-d* showed the polymer to be constructed from alternating residues of 4-substituted rhamnose and 3-substituted 2-acetamido-2-deoxyglucose. From an interpretation of the ^{13}C-n.m.r. spectrum (Table I)[4–8], it could be inferred that the rhamnosyl residue was α-linked, making **1** the structure of the repeating-unit. If the

TABLE I

ASSIGNMENT OF SIGNALS[a] IN THE ^{13}C-N M.R SPECTRUM OF THE NEUTRAL POLYMER FROM THE LIPOPOLYSACCHARIDE OF *S. marcescens* C.D.C. 866-57

Carbon atom	*3-β-GlcpNAc-1*	*4-α-Rhap-1*
C-1	101.00[b]	101.17[b]
C-2	55.91	71.08
C-3	81.81	70.49
C-4	68.77	80.28
C-5	75.95	67.44
C-6	60.98	17.06
-NHC(O)CH_3	174.48	
-NHC(O)CH_3	22.46	

[a]Chemical shifts are given in p.p.m. downfield from external tetramethylsilane. [b]Pair of signals for which the assignments may be interchanged.

rhamnose had been β-linked, the signals for C-3 and C-5 would have been at $\delta \sim 74$ and ~72, respectively[9–12].

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

1

Neither the polymer characterised above nor the acidic polymer briefly described has yet been reported for other strains of *S. marcescens*, so both remain candidates as the O1 antigen. The presence of core-specific heptoses only in the neutral material indicates that this is derived from the lipopolysaccharide. The fractionation of this material on DEAE-Sepharose may be a consequence of heterogeneity in chain length (shorter chains being retarded by gel permeation and by weak adsorption through ionic residues in the terminal core oligosaccharide), or of the presence in extracts of free hapten lacking the core oligosaccharide.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of lipopolysaccharide. — Cells of *S. marcescens* C.D.C. 866-57 were grown at 30° for 16 h in Nutrient Broth No. 2 (Oxoid) as 20-L batch cultures aerated at 20 $L.min^{-1}$. Lipopolysaccharide was extracted by the hot, aqueous phenol method from defatted cell-walls[1]. After mild acid hydrolysis[1], the water-soluble products were fractionated[2] first on Sephadex G-50 and then on DEAE-Sepharose CL6B. After elution from the anion-exchange column with water or aqueous NaCl (0.1–0.3M), fractions containing polymeric material were dialysed and freeze-dried.

Chromatographic and electrophoretic methods. — The following solvents were used for p.c.: *A*, ethyl acetate–pyridine–water (13:5:4); *B*, ethyl acetate–pyridine–acetic acid–water (5:5:1:3). The pyridine–acetic acid buffer systems (pH

5.3 or 2.7) used for paper electrophoresis, and the detection reagents used on chromatograms and electrophoretograms, have been described[1]. Glass columns packed with stationary phases of Silar 10c (I) or OS-138 (II) were used for g.l.c. of (methylated) alditol acetates. G.l.c.–m.s. was carried out with a Finnigan 1020 mass spectrometer and a fused-silica capillary column of SE-54. Autoanalysis of amino sugars was done with a Locarte instrument.

Determination of monosaccharide composition[1,2]. — For the release of neutral sugars, samples were hydrolysed with 2M HCl at 105° for 2 h. After neutralisation and deionisation of the hydrolysates, sugars were identified by p.c. (solvent *A*) and by g.l.c. of the alditol acetates (column I). The D configuration of glucose and mannose was established by using enzymic assays[2], and the L configuration of rhamnose by g.l.c. of the acetylated oct-2-yl glycosides[13]. For the release of amino sugars, samples were hydrolysed with 6.1M HCl at 105° for 4 h under nitrogen. 2-Amino-2-deoxyglucose in hydrolysates was identified by p.c. (solvent *B*), paper electrophoresis at pH 5.3, and autoanalysis. Its D configuration was apparent from the $[\alpha]_D$ value of the hydrochloride [+73.3° (*c* 0.6, water; based on a colorimetric analysis[14])]. For the release of hexuronic acids, samples were hydrolysed with 0.5M H_2SO_4 at 105° for 4 h, the hydrolysates were neutralised ($BaCO_3$), and uronic acids were identified by p.c. (solvent *B*) and by paper electrophoresis at pH 2.7.

Degradative methods. — Methylation analysis was carried out by standard procedures[1–3], and the methylated alditol acetates were identified by g.l.c. (columns I and II) and g.l.c.–m.s. The oxidation of samples with sodium periodate was also carried out and monitored as described previously[1–3].

N.m.r. spectroscopy. — N.m.r. spectra (^1H and ^{13}C) were obtained with a Bruker WH-400 spectrometer for solutions in D_2O. The ^1H spectrum was recorded at 80° with sodium 4,4-dimethyl-4-silapentane-1-sulphonate as the internal standard. The proton-decoupled ^{13}C spectrum was recorded at 50° with tetramethylsilane as the external standard.

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