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Note

A common structure for neutral polymers isolated from the lipopolysaccharides of reference strains for *Serratia marcescens* serogroups O17 and O19

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Serogroups O17 and O19 of *Serratia marcescens* were first defined by Le Minor and Pigache[1]. Relationships involving both these groups and others (including O1, O9, and O15) were apparent in this and subsequent studies[2–4], and in one study[4] it was shown that absorption of O17 antiserum with O19 cells removed both O17 and O19 reactivity (in contrast to earlier reports[1–3]). We have previously determined the structures of the repeating units of neutral polymers isolated from the reference strains for serogroups O1 (ref. 5), O9 (ref. 6), and O15 (ref. 7), and of an acidic polymer from the O15 strain[8]. It was therefore of interest to extend the structural studies to the polysaccharides produced by the O17 and O19 reference strains[1].

Lipopolysaccharides were extracted from the bacterial cell walls by the aqueous phenol method with yields of 23% (O17) and 40% (O19). Both products contained the same neutral sugars—rhamnose, glucose, L-*glycero*-D-*manno*-heptose, and D-*glycero*-D-*manno*-heptose—and also 2-amino-2-deoxyglucose. The ratios of neutral sugars (expressed as percentages of the total peak area in g.l.c. of the alditol acetates) were 36:35:22:7 for O17 and 70:23:7:trace for O19. The impression, given by the relatively high content of aldoheptose for the O17 lipopolysaccharide, that this product was richer in molecular species lacking a polymeric side-chain was reinforced by fractionation (Sephadex G-50) of the water-soluble products obtained on mild acid hydrolysis of the lipopolysaccharides. The elution profile for O17 showed a relatively prominent peak for oligomeric material (partly degraded core), and the yield of polymeric material (25% of the lipopolysaccharide) was substantially less than for O19 (41%). When the polymeric products were fractionated further on DEAE-Sepharose CL-6B, only materials eluted with water or 0.1M NaCl were recovered, indicating the presence of "neutral" but not acidic polymers. The products eluted with water contained mainly 2-amino-2-deoxy-D-glucose and L-rhamnose (O17, 33.0%; O19, 29.5%), together with traces of glucose and aldoheptoses. The products eluted with 0.1M NaCl were very similar, but con-

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tained rather more of the minor components, consistent with higher contents of core oligosaccharide (*i.e.*, shorter side-chains). N.m.r. spectroscopy confirmed that the aqueous eluates, which were used for further study, were the more homogeneous.

The n.m.r. spectra (^1H and ^{13}C) showed that both the O17 and O19 products consisted mainly of polymers with a disaccharide repeating-unit. Furthermore, the n.m.r. spectra were superimposable, indicating structural identity: the numerical data reported here are those obtained for the O19 product. The ^1H-n.m.r. spectrum contained major anomeric signals at δ 4.87 (1 H, unresolved) and 4.71 (0.8 H, $J_{1,2}$ 8.5 Hz), methyl signals at δ 2.05 (s) and 1.24 ($J_{5,6}$ 6.2 Hz), and a minor anomeric signal at δ 4.80 (0.2 H, $J_{1,2}$ 8.5 Hz). The ^{13}C-n.m.r. spectrum (see Fig. 1 for the O17 polymer) contained 14 major signals, including anomeric signals at δ 102.88 ($^1J_{CH}$ 162 Hz) and 101.94 ($^1J_{CH}$ 170 Hz), and signals for a 2-acetamido group at δ 175.30, 56.43, and 23.04, an unsubstituted hydroxymethyl carbon at δ 61.32, and C-6 of rhamnose at δ 17.28. As expected from other data, the spectrum also contained several minor signals. From the n.m.r. results, it can be inferred that the O17 and O19 polymers are constructed from α-L-rhamnopyranosyl and 2-acetamido-2-deoxy-β-D-glucopyranosyl residues, and that position 6 in the latter sugar is unsubstituted. Methylation analysis of both polymers showed that the amino sugar was actually 3-substituted, but that the rhamnose residues were variously substituted in position 3 (~80%) or position 4 (~20%). Thus, the major structural element in both the O17 and O19 polymers is the disaccharide residue of structure **1**. It is not clear whether similar units containing 4-substituted α-L-rhamnopyranosyl residues are present as integral components of the polymer, or whether they constitute the repeating units of a separate glycan.

$$\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow$$

1

In order to confirm the structural conclusions, the O17 polymer was subjected to a reaction sequence of *N*-deacetylation, deamination, and reduction ($NaBD_4$). The major product (DO17) was isolated by h.p.l.c. and had the retention time expected for a disaccharide derivative. Its ^1H-n.m.r. spectrum contained one anomeric signal (1 H) at δ 4.91 ($J_{1,2}$ 1.7 Hz) and a methyl signal at δ 1.34 ($J_{5,6}$ 6.3 Hz). Its ^{13}C-n.m.r. spectrum contained the expected 11 strong signals (including an anomeric signal at δ 100.53 and a signal at δ 17.39 for C-6 of rhamnose) and a weak triplet at δ 62.00 (corresponding to C-1 of the 2,5-anhydromannitol-*1-d* residue). Permethylated DO17 showed only one peak in g.l.c., and primary fragments in the mass spectrum (listed in the Experimental) are consistent with the identification of DO17 as a rhamnosyl derivative of 2,5-anhydromannitol-*1-d*. Methylation analysis of DO17 confirmed this partial structure, and showed that the 2,5-anhydromannitol-*1-d* was 3-substituted. The ^{13}C-n.m.r. data for both the original polymer (Table I) and the deamination product DO17 (Table II) are in accord with the structures proposed.

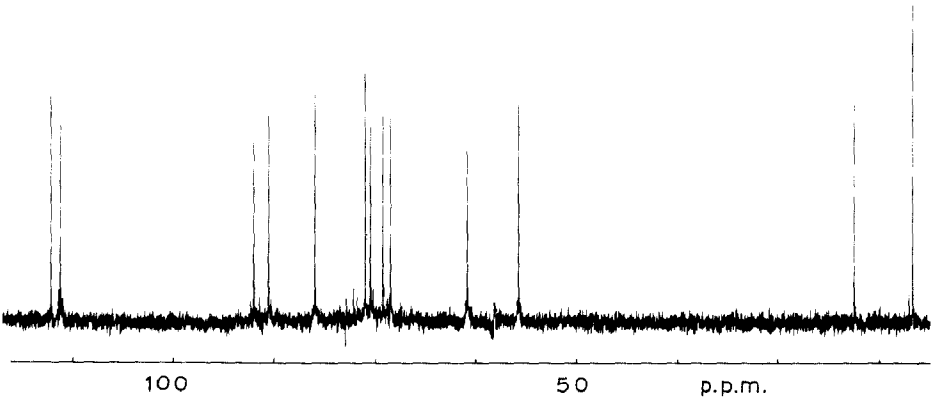

Fig. 1. ^{13}C-N.m.r. spectrum of the O17 glycan. The spectrum for the sample in D_2O was obtained at 100.62 MHz and 50°, with complete proton-decoupling and tetramethylsilane as the external reference. In addition to the signals shown, the spectrum contained a carbonyl resonance at δ 174.60.

The establishment of structure **1** as the major unit in both the O17 and O19 polymers, and even the presence in both of a minor proportion of 4-substituted α-L-rhamnopyranosyl residues, seems to account for the close serological relationship between the parent organisms[1–4]. However, it is not apparent why monospecific antisera can be prepared by absorption with the cross-reacting bacteria[1–3]. Presumably, there are other heat-stable antigens produced in addition to those isolated and characterized in this study. Nevertheless, there is now a clear chemical basis for the existence[4] of a well-defined complex of cross-reacting serogroups (O1, O17, and O19). Thus, the reference strain for serogroup O1 produces a neutral polymer[5] with the disaccharide repeating-unit of structure **2**, corresponding to the minor structural unit (or separate polymer) for O17 and O19.

TABLE I

^{13}C-N.M.R. DATA FOR THE O19 POLYMER

Carbon atom	*Chemical shift (p.p.m.)*[a]	
	→3)-α-L-Rhap-(1→	*→3)-β-D-GlcpNAc-(1→*
C-1	101.94	102.88
C-2	71.16	56.43
C-3	81.20	82.59
C-4	71.52	68.93
C-5	69.86	76.57
C-6	17.28	61.32
-NHC(O)CH_3		175.30
-NHC(O)CH_3		23.04

[a]The spectrum for the sample in D_2O was recorded at 100.62 MHz and 27° with proton decoupling and 1,4-dioxane as the internal reference (δ 67.40). The assignments were made with the aid of literature data[9,10], but may be interchanged for closely spaced signals.

TABLE II

^{13}C-N.M.R. DATA FOR THE DEAMINATION PRODUCT DO17

Carbon atom	*Chemical shift (p.p.m.)*[a]	
	α-L-*Rha*p-(*1*→	→*3)-2,5-Anhydro*-D-*mannitol*-1-d
C-1	100.53	62.00
C-2	71.09	82.51
C-3	70.98	84.00
C-4	72.86	76.42
C-5	69.87	83.94
C-6	17.39	61.73

[a]The spectrum for the sample in D_2O was recorded at 100.62 MHz and 26° with proton decoupling and 1,4-dioxane as the internal reference (δ 67.40). Assignments were made with the aid of literature data[9] but may be interchanged in the case of some closely spaced signals.

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

2

In addition, there are also structural similarities between the O17/O19 polymer and the neutral polymers from the O9 reference strain (repeating unit of structure **3**)[6] and the O15 reference strain (repeating unit of structure **4**)[7]. Curiously, in both of the last two polymers, there is heterogeneity of the same type as that reported here for the O17/O19 polymer. For O9, there is a minor proportion of 4-substituted rhamnosyl residues, and for O15 a minor proportion of 3-substituted rhamnosyl residues.

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

3

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

4

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of lipopolysaccharides. — Cells of the O17 and O19 reference strains for *S. marcescens* were grown and processed as in related studies[5–8]. The yields of wet cells, freeze-dried cell walls, and lipopolysaccharides, respectively, were as follows: O17, 196 g, 5.47 g, and 1.25 g; O19, 154 g, 2.44 g, 0.97 g. After mild acid hydrolysis of the lipopolysaccharides (aq. 1% acetic acid, 2.25 h, 100°), the water-soluble products were fractionated first on Sephadex G-50 and then on DEAE-Sepharose CL-6B. Of the O17 polymeric material, 49% was eluted from the ion-exchange column with water and the remainder with 0.1M NaCl; the corresponding values for O19 were 61% and 31%, respectively.

General methods. — N.m.r. spectra (^1H and ^{13}C) were recorded with a Bruker WH-400 (all data cited) or a JEOL JNM-GX270 spectrometer for samples dissolved in D_2O. ^1H-N.m.r. spectra were recorded at 60° (400 MHz) or 70° (270 MHz) with sodium 3-trimethylsilylpropanoate-d_4 (400 MHz) or sodium 4,4-dimethyl-4-silapentane-1-sulphonate (270 MHz) as the external reference. ^{13}C-N.m.r. spectra were recorded at ~27° with 1,4-dioxane as the internal reference, or at 50° with tetramethylsilane as the external reference, and with complete or gated decoupling. Chromatographic and electrophoretic methods used to identify and determine monosaccharides were those described in related studies[11]. Methylation analyses also followed standard procedures[7].

Preparation and characterization of a deamination product (DO17). — A sample of the O17 polymer was *N*-deacetylated for 22 h at 95°, deaminated, and reduced ($NaBD_4$): the major product (DO17) was isolated by h.p.l.c. as described[12]. The permethylated derivative was examined by g.l.c., using a fused-silica capillary column (25 m) of BP1 at 200°, by conversion into methylated alditol acetates, and by direct e.i.-m.s. Diagnostic fragment ions included the following (relative intensities in brackets and assignments[13] in square brackets): *m/z* 88 (100), 101 (27), 157 (3) [aA_2], 158 (9) [bA_2], 189 (3) [aA_1], 190 (7) [bA_1], and 250 (13) [abJ_1].

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