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Structure of the O21 antigen from *Serratia marcescens*

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ABSTRACT

Lipopolysaccharide was isolated from both phases of an aqueous-phenol extraction of defatted cell walls from the reference strain for *Serratia marcescens* serogroup O21. The product from the aqueous phase was of the R type, lacking a polymeric side-chain. The polymeric fraction of the lipopolysaccharide from the phenolic phase (the O21 antigen) had a disaccharide repeating-unit with the following structure: →4)-α-D-Glc*p*-(1→4)-β-D-Man*p*NAc-(1→.

INTRODUCTION

Serogroup O21 of *Serratia marcescens* was defined by Le Minor and Sauvageot-Pigache[1], and was shown by these and other workers[2,3] to share antigenic factors with serogroups O2 and O3. During the course of systematic studies of polysaccharides present in lipopolysaccharide extracts from reference strains for the different O serogroups of the organism, we have characterised acidic glycans from the O2 (ref. 4) and O3 (ref. 5) strains. We now report the isolation of lipopolysaccharides from the O21 reference strain (IP-1521) and the structure of the O-antigenic side chain of the lipopolysaccharide.

RESULTS AND DISCUSSION

When defatted cell walls of strain IP-1521 were extracted by the aqueous-phenol method, ~4% of the original cell-wall material was recovered from the aqueous phase. No polysaccharide fraction was obtained when the extract was subjected to mild acid hydrolysis and the water-soluble products were separated by chromatography on Sephadex G-50. Examination of the extract by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) confirmed that it consisted of R-type lipopolysaccharide. However, SDS-PAGE of whole cells treated with proteinase K (protease type XI)[6] gave the "ladder" pattern typical of S-type lipopolysaccharides. Consequently, the phenolic layer from the cell-wall extract was checked for the presence of lipopolysaccharide. The material recovered (5.8% of the whole cell wall) gave a "ladder" pattern on SDS-PAGE and released a polymeric product (yield, 40%) as well as an oligosaccharide fraction (yield, 11%) on mild acid hydrolysis. Monosaccharide analysis of the phenol-soluble lipopolysaccharide gave the following results (expressed as the relative peak areas in g.l.c. of the alditol acetates): glucose (50), 2-amino-2-

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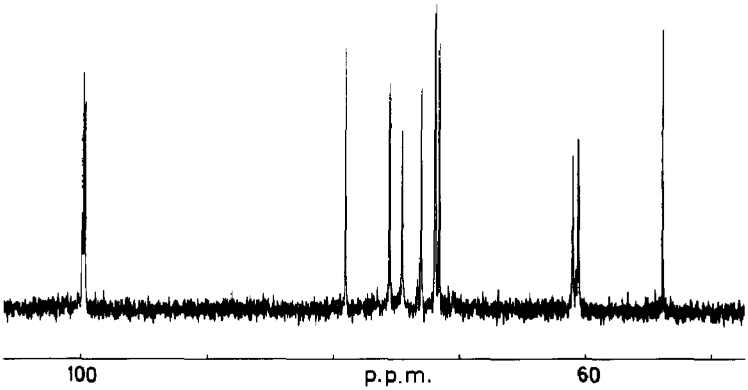


Fig. 1. ^{13}C-N.m.r. spectrum of a solution of the polymer in D_2O, recorded at 27° and 100.62 MHz with complete proton-decoupling and tetramethylsilane as the external reference. In addition to the signals shown, the spectrum contained others at δ 175.97 and 22.58 from an acetamido group.

deoxyglucose (6), 2-amino-2-deoxymannose (25), D-*glycero*-D-*manno*-heptose (4), and L-*glycero*-D-*manno*-heptose (15). Chromatography on DEAE-Sepharose CL-6B of the polymeric product from the lipopolysaccharide gave only "neutral" material, eluted with water (21%) or 0.1M NaCl (79%). Both fractions had the same monosaccharide composition and gave the same n.m.r. spectra, so only the more abundant fraction was studied in detail.

The neutral O21 polymer had only glucose and 2-amino-2-deoxymannose as significant monosaccharide components, both identified as the D isomers by g.l.c. of the but-2-yl glycoside acetates. Methylation analysis showed that both monosaccharides were present as 4-substituted pyranoside residues (the ring size being indicated by the n.m.r. spectra). The ^{1}H-n.m.r. spectrum contained a methyl singlet at δ 2.07 (attributable to an *N*-acetyl group), and anomeric signals (each 1 H) at δ 5.31 ($J_{1,2}$ ~4 Hz) and 4.87 (unresolved). A further signal at δ 4.54 ($J_{2,3}$ ~5 Hz) was assigned to H-2 of ManNAc. The ^{13}C-n.m.r. spectrum of the polymer contained 14 discrete signals (Fig. 1), including two in the anomeric region at δ 99.87 ($^1J_{CH}$ 172 Hz) and 99.71 ($^1J_{CH}$ ~ 165 Hz). The n.m.r. data for the polymer point to a disaccharide repeating-unit of structure **1**. Provisional assignments of the signals in the ^{13}C-n.m.r. spectrum (Table I) were made with the aid of literature data[7-9].

→4)-α-D-Glc*p*-(1→4)-β-D-Man*p*NAc-(1→

1

In order to confirm the structure of the repeating unit, the polymer was subjected to a Smith degradation. A product (SD) eluted in the region expected for a disaccharide-alditol was isolated by h.p.l.c. G.l.c. analysis of permethylated SD showed that two compounds were present (relative peak areas 1.00:0.72). G.l.c.–e.i.-m.s. indicated that the major component (SD1) was derived from the classical Smith-degradation product (**2**). The minor component (SD2) was apparently a by-product from transacetalation

TABLE I

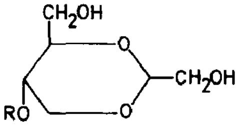
^{13}C-N.m.r. data for the O21 polymer

Carbon atom	*Chemical shift (p.p.m.)*[a]	
	4)-α-D-Glcp-(1	*4)-β-D-ManpNAc-(1*
C-1	99.87	99.71
C-2	71.97	53.84
C-3	73.07	71.91
C-4	79.06	74.56
C-5	71.63	75.55
C-6	61.00	60.56
-NH*C*(O)CH$_3$		175.97
-NHC(O)*C*H$_3$		22.58

[a] The spectrum for a solution of the polymer in D$_2$O was recorded at 27° and 100.62 MHz with tetramethylsilane as the external reference. Assignments for some signals with closely similar chemical shifts may be interchanged.

during the hydrolytic step of the Smith degradation[10]. The ^{1}H-n.m.r. spectrum of the mixture SD contained two anomeric signals for ManNAc at δ 4.87 (1 H, $J_{1,2}$ 1.7 Hz) and 4.94 (0.7 H, $J_{1,2}$ 1.7 Hz), and the corresponding methyl signals at δ 2.07 (3 H) and 2.06 (2.1 H). The spectrum also included a triplet at δ 5.00 (0.7 H, J_{HH} 3.4 Hz) attributable to the acetal proton (H-2) in the minor product SD2, expected[10] to be derived from the substituted 1,3-dioxane (**3**) or the related 1,3-dioxolane. This inference was supported by the ^{13}C-n.m.r. spectrum (Fig. 2) for the mixture. The signals attributable to C-1, C-2, and C-3 of ManNAc clearly consist of major and minor components, and there are corresponding sets of signals for the erythritol and 1,3-dioxane (or 1,3-dioxolane) residues. Assignments of the signals, assuming structure **3**, are given in Table II.

β-D-Man*p*NAc-(1→2)-Erythritol
2



3 R = β-Man*p*NAc

This study has shown that the lipopolysaccharides from *S. marcescens* strain IP-1521 are unusually hydrophobic. The product (R-type) lacking a polymeric side chain was isolated from both the aqueous and the phenolic phases of the extraction mixture, while the S-type lipopolysaccharide was found exclusively in the phenolic phase. Many examples of such lipopolysaccharides are now known, but the explanation for the phase preference of the O21 product is unclear: similar lipopolysaccharides from *S. marcescens*, in which the repeating unit of the O-specific polymer is constructed from a 2-acetamido-2-deoxyhexose and a 6-deoxyhexose, partition preferentially into the aqueous phase[11].

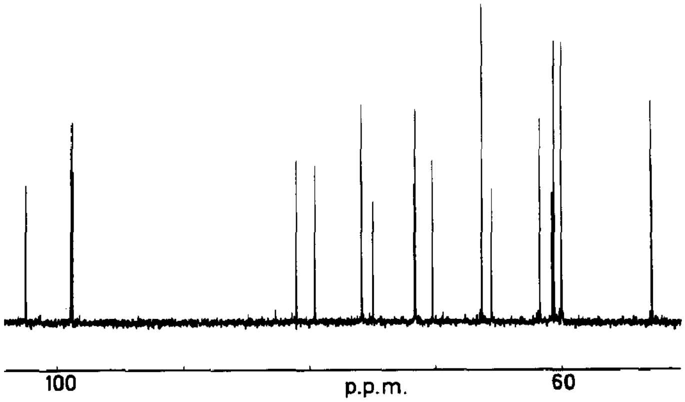


Fig. 2. ^{13}C-N.m.r. spectrum of a solution of the product (SD) obtained on Smith degradation of the polymer, recorded at 27° and 100.62 MHz with complete proton-decoupling and acetone (δ_{Me} 29.79) as the internal reference. In addition to the signals shown, the spectrum contained others at δ 175.03, 174.91, and 21.58 from acetamido groups.

The polymer with the repeating unit **1** is clearly the O21 antigen of *S. marcescens*. For this serogroup, there is none of the ambiguity caused by the presence of both neutral and acidic polymers in the lipopolysaccharide extracts[12]. It is also clear that the O21 polymer described must be involved in the serological cross-reactions[1-3] with groups O2 and O3, and that it must be present in some strains initially typed[13] as O14. The spurious assignment of the latter strains (and similarly of others which actually possess the neutral O6 or O8 antigens[13]) is caused by the presence of a masking, "microcapsular"

TABLE II

^{13}C-N.m.r. data for the Smith-degradation products SD1 (**2**) and SD2 (**3**)

Carbon atom	*Chemical shift (p.p.m.)*[a]			
	SD1		*SD2*	
	ManpNAc	*Erythritol*	*ManpNAc*	*1,3-Dioxane*
C-1	100.11	61.82	100.02	–
C-2	54.15	82.24	54.10	103.74
C-3	72.80	71.46	72.89	–
C-4	67.54	62.96	67.54	76.17
C-5	77.09	–	77.09	80.76
C-6	61.24	–	61.24	66.77
-NH*C*(O)CH$_3$	176.24	–	176.12	–
-NHC(O)*C*H$_3$	22.78	–	22.78	–
2-CH$_2$OH	–	–	–	61.98[b]
4-CH$_2$OH	–	–	–	61.82[b]

[a] The spectrum for a solution of SD in D$_2$O was recorded at 27° and 100.62 MHz with acetone as the internal reference (δ_{Me} set at 29.79 p.p.m., but the chemical shifts tabulated were adjusted to δ_{Me} 31.00). [b] Assignments may be interchanged.

acidic glucomannan[14]. The present results also demand a re-examination of polysaccharide antigens in the O2 and O3 reference strains. The lipopolysaccharides previously isolated[4,5] from the aqueous phase for these organisms did not give a "ladder" pattern on SDS-PAGE and yielded only acidic polymers unrelated to the O21 antigen, whereas a "ladder" pattern was given by whole cells treated with proteinase K (ref. 3). Thus, it is likely that these strains also produce a phenol-soluble lipopolysaccharide related to the O21 antigen.

EXPERIMENTAL

Growth of the bacteria, and isolation and frationation of the lipopolysaccharide. — *S. marcescens* strain IP-1521 was grown for 16 h at 30° in Nutrient Broth No. 2 (Oxoid) as a 20-L batch culture aerated at 20 $L.min^{-1}$, to give 166 g of wet cells. Isolated walls (6.93 g) were prepared by mechanical distintegration of the cells. Extraction of the defatted walls with aqueous phenol, as in previous studies[4,5,11], provided two fractions of lipopolysaccharide, derived from the aqueous phase (270 mg) and the phenolic phase (400 mg). Mild acid hydrolysis of the lipopolysaccharides, followed by chromatography of the water-soluble products on Sephadex G-50 and DEAE-Sepharose CL-6B, involved standard procedures[4,5].

General methods. — Isolated lipopolysaccharides and also proteinase K-treated whole cells[6] were examined by SDS-PAGE[5]. The enzyme used was protease type XI (Sigma). Chromatographic and electrophoretic methods used to identify monosaccharides and to determine their absolute configurations were as in previous studies[11]. Methylation analysis of the O21 polymer, monitored by g.l.c and e.i.-m.s. of the methylated alditol acetates, also involved standard procedures[15]. N.m.r. spectra (^{1}H and ^{13}C) were recorded with a Bruker WH-400 (all ^{13}C data cited) or JEOL JNM-GX270 (^{1}H data for the polymer) spectrometer for samples dissolved in D_2O. ^{13}C-N.m.r. spectra were recorded at 27° with tetramethylsilane as the external reference (for the polymer) or acetone as the internal reference (for the Smith-degradation products). ^{1}H-N.m.r. spectra were recorded at 70° with sodium 4,4-dimethyl-4-silapentane-1-sulphonate as the external reference (for the polymer) or at 60° with acetone as the internal reference (δ 2.22, for the Smith-degradation products).

Smith degradation. — A sample of the O21 polymer was subjected to periodate oxidation, followed by reduction ($NaBH_4$), and mild acid hydrolysis[4,5]. The major product (SD) was isolated by h.p.l.c.[5], and its n.m.r. spectra were recorded. After permethylation, two components (SD1 and SD2) were detected by g.l.c. (relative retention times 1.00:1.94 on a fused-silica capillary column of BP1 at 220°). E.i.-m.s. showed that both components had permethylated ManNAc at the non-reducing terminus, giving ions with m/z 260 [aA_1] and 228 [aA_2]. The presence of a methylated erythritol residue in SD1 was indicated by peaks at m/z 207 [abJ_1] and 147 [bA_1], while corresponding peaks at m/z 235 and 175 for SD2 were consistent with the presence of a 1,3-dioxane residue as shown in structure **3**.

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