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Note

Structural studies of neutral polymers isolated from the lipopolysaccharides of *Serratia marcescens* O6 (strain C.D.C. 862-57) and O12 (C.D.C. 6320-58)

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We have recently shown[1] that different strains of *Serratia marcescens* belonging to the same O serogroup (O14) produce different neutral polysaccharides. Although apparently derived from the cell-wall lipopolysaccharides, these polymers cannot (alone) be responsible for O specificity, and circumstantial evidence has been obtained that this specificity is conferred by (micro)capsular acidic glucomannans[2]. In a continuation of this study, we have begun to analyse the surface carbohydrates from strains of *S. marcescens* representing serogroups known to have cross-reactions[3,4] with serogroup O14. Here we describe the structures of the neutral polymers obtained from the reference strains for serogroup O6 (C.D.C. 862-57) and O12 (C.D.C. 6320-58).

The lipopolysaccharides were extracted from isolated cell-walls and degraded by mild acid hydrolysis (aqueous 1% acetic acid, 100°, 2.25 h). The O6 lipopolysaccharide was exceptional in our experience[1,2,5], as the hydrolysate did not turn dark brown during this process. The water-soluble products were fractionated by chromatography, first on Sephadex G-50 and then on DEAE-Sepharose CL-6B. In the case of the O6 lipopolysaccharide, the material eluted from the ion-exchange column with water (fraction F1) represented only 3% of the polymeric products, whereas that eluted with 0.1M NaCl (F2) accounted for 55%. The acidic material eluted with higher concentrations of NaCl was the glucomannan described elsewhere[2]. The corresponding F1 and F2 fractions from the O12 lipopolysaccharide represented 18 and 20% of the polymeric products, respectively: later fractions of eluate contained an acidic galactoglucomannan which has yet to be fully characterised.

The monosaccharide compositions of the O12 F1 and F2 fractions were very similar: ribose and 2-amino-2-deoxygalactose were the only major components, but small or trace amounts of rhamnose, glucose, 2-amino-2-deoxyglucose, and heptoses were also detected. Quantitative data obtained for F1 (2-amino-2-deoxygalactose, 29.2%; ribose, 21.5%) were close to those for another polymer obtained[1]

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from an O14 strain (C.D.C. 4444-60). 2-Amino-2-deoxygalactose was shown to be present as the *N*-acetyl derivative by the i.r. spectrum (bands at 1640 and 1550 cm^{-1}), the ^{1}H-n.m.r. spectrum (a methyl singlet at δ 2.03), and the ^{13}C-n.m.r. spectrum (signals at δ 174.67 and 22.11). Both n.m.r. spectra showed that the polymer has a disaccharide repeating-unit. The ^{1}H spectrum contained one-proton anomeric signals at δ 5.25 (unresolved) and 5.12 ($J_{1,2}$ 3.5 Hz), while the ^{13}C spectrum contained 13 discrete signals, including anomeric signals at δ 106.98 and 95.81. In fact, the ^{13}C-n.m.r. spectrum could be superimposed on that recorded for the polymer from strain C.D.C. 4444-60. Confirmation that the two polymers had identical structures was provided by periodate oxidation (both major components resisted oxidation) and methylation analysis (g.l.c.–m.s. evidence for residues of 2-substituted ribofuranose and 4-substituted 2-acetamido-2-deoxygalactopyranose). Thus, the repeating unit in the neutral polymer from strains C.D.C. 4444-60 (O14) and 6320-58 (O12) has the structure **1**. At least some of the minor components of both polymers are likely to arise from a terminal core oligosaccharide of the parent lipopolysaccharide.

$\rightarrow$4)-α-D-Gal*p*NAc-(1$\rightarrow$2)-β-D-Rib*f*-(1$\rightarrow$

1

The small amount (4 mg) of the O6 F1 fraction available precluded structural studies, but the 2-amino-2-deoxygalactose content (18.2%) and the presence of ribose as the major neutral sugar suggested that the fraction included a polymer similar or identical to that described above. Glucose, rhamnose, and 2-amino-2-deoxyglucose were minor or trace components of the fraction. By contrast, the O6 F2 fraction consisted mainly of D-glucose (35.3%) and L-rhamnose (33.3%), along with smaller amounts of heptoses (mainly L-*glycero*-D-*manno*-heptose). The presence of an *O*-acetyl group was apparent from the i.r. spectrum (bands at 1720 and 1250 cm^{-1}), the ^{1}H-n.m.r. spectrum (a signal at δ 2.16), and the ^{13}C-n.m.r. spectrum (signals at δ 173.67 and 20.45). From the ^{1}H spectrum, the acetyl–rhamnose ratio was judged to be about 1:2. Both n.m.r. spectra were considerably simplified by deacetylation of the polymer, for which a disaccharide repeating-unit was indicated by signals in the ^{1}H spectrum at δ 5.09 ($J_{1,2}$ ~4 Hz) and 4.86 (broad singlet), and in the ^{13}C spectrum at δ 100.71 (J_{CH} 164 Hz) and 95.62 (J_{CH} 170 Hz). For both the native and the deacetylated polymer, the rate and the extent of periodate consumption were similar, only glucose was destroyed during the process, and erythritol was formed after borohydride reduction and acid hydrolysis of the oxidised polymer. Methylation analysis showed that the polymer was constructed from 3-substituted rhamnopyranosyl and 4-substituted glucopyranosyl residues. From these results, structure **2** can be assigned to the repeating unit. This structure has previously been assigned[5] to the repeating unit of a polymeric fraction from the lipopolysaccharide of *S. marcescens* N.C.T.C. 1377. As expected, the ^{13}C-n.m.r. spectra of the two deacetylated polymers were essentially identical. Although the native polymers differed in the extent of acetylation (N.C.T.C. 1377, 80–90%; C.D.C. 862-57, ~50%), the location of the acetyl group (O-2 of rhamnose) was apparently the same. In the case of the O6 polymer, evidence for this location was provided by the spectral changes which accompanied deacetylation. For the native polymer, two comparable signals in the ^{13}C-n.m.r. spectrum at δ 68.64 and 67.49 could be assigned to C-2 of rhamnose residues with and without an acetyl substituent, respectively. On deacetylation, the downfield member of this pair was lost along with the upfield member of the pair of signals (δ 100.71 and 99.34) assigned to C-1 of rhamnose. Similar changes have been reported for the polymer from strain N.C.T.C. 1377.

→4)-α-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→

2

The isolation of the same acetylated glucorhamnan from the lipopolysaccharides of the O6 reference strain and strain N.C.T.C. 1377 (as well as[5] strain A.T.C.C. 264) prompted us to submit strain N.C.T.C. 1377 for O serotyping. The result — O14, cross-reacting with O1, O6, and O7 — complicates still further the picture of surface carbohydrates produced by strains of *S. marcescens*. Our studies of lipopolysaccharides from four strains of serogroup O14 have led to the characterisation of three neutral polymers. The polymer of ribose and 2-acetamido-2-deoxygalactose with the repeating-unit **1** has been isolated[1] from two strains of serovar O14:H12 as well as the C.D.C. strain 6320-58 (O12:H9), and may be a minor component in the C.D.C. strain 862-57 (O6:H3). The acetylated glucorhamnan has been isolated from one O14 strain (N.C.T.C. 1377) and the C.D.C. strain 862-57 (O6:H3). The third neutral polymer of glucose, galactose, and 2-acetamido-2-deoxyglucose[1] has been found only in one strain of serovar O14:H9 (C.D.C. 1783-57). Although the acidic glucomannans described elsewhere[2] may provide the antigenic factor (6_2) common to O6 and O14 strains[4], the acetylated glucorhamnan provides a further possibility for serological cross-reactions between some strains in this complex. Similarly, the polymer of ribose and 2-acetamido-2-deoxygalactose links some O14 strains (and possibly the O6 reference strain) with the O12 reference strain, and could be the antigen known to be shared[4] by the C.D.C. strains 4444-60 (O14) and 6320-58 (O12). Serological studies using undegraded polymers are obviously necessary to settle these matters.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of lipopolysaccharide. — Cells of *S. marcescens* strains C.D.C. 862-57 and 6320-58 were grown at 30° for 16 h in Nutrient Broth No. 2 (Oxoid) as 20-L batch cultures aerated at 20 L.min^{-1}. Lipopolysaccharides were extracted from defatted cell-walls, and the polymeric, water-soluble products obtained on mild acid hydrolysis were fractionated as described previously[2].

Determination of monosaccharide compositions. — The conditions of acid hydrolysis used for the release of neutral and amino sugars were those described in related studies[1,2]. Neutral sugars were identified by p.c. with ethyl acetate–pyridine–water (13:5:4) as the solvent, and alkaline $AgNO_3$ and aniline hydrogenoxalate as the detection reagents. Quantitative data were obtained by g.l.c. of the alditol acetates on Silar 10c. D-Glucose was determined by using D-glucose oxidase (EC 1.1.3.4, Boehringer), and L-rhamnose was identified by g.l.c. of the acetylated oct-2-yl glycosides[6]. Amino sugars were identified by p.c. with ethyl acetate–pyridine–acetic acid–water (5:5:1:3) and by paper electrophoresis at pH 5.3, with ninhydrin and alkaline $AgNO_3$ as the detection reagents. Autoanalysis (Locarte) provided confirmation of identities and quantitative data.

Degradative methods. — The oxidation of polymers with sodium periodate, selective *O*-deacetylation, and methylation analyses were carried out as described previously[1,2]. Methylated alditol acetates were identified by g.l.c. on Silar 10c, and by g.l.c.–m.s. using a Finnigan 1020B mass spectrometer fitted with a fused-silica capillary column (30 m) of SE-54.

Spectroscopic methods. — I.r. spectra were recorded with a Unicam SP-200 spectrophotometer and samples dispersed in KCl. N.m.r. spectra (^1H and ^{13}C) were recorded for solutions in D_2O with a Bruker WH-400 spectrometer. ^{13}C Spectra (with complete proton-decoupling or with gated decoupling) were recorded at ~50° with tetramethylsilane as the external standard. ^1H Spectra were recorded at ~80° with sodium 4,4-dimethyl-4-silapentane-1-sulphonate as the internal standard.

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