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STRUCTURAL STUDIES OF AN ACIDIC GALACTOGLUCOMANNAN FROM THE O15 REFERENCE STRAIN (C.D.C. 4523-60) OF *Serratia marcescens*

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ABSTRACT

Both neutral and acidic polymers have been isolated from the lipopolysaccharide extract of the reference strain (C.D.C. 4523-60) for *Serratia marcescens* serogroup O15. By means of n.m.r. spectroscopy, methylation analysis, and studies of degradation products, the acidic polysaccharide was shown to have a branched pentasaccharide repeating-unit with the following structure.

$$\begin{array}{c} \alpha\text{-D-Man}p \\ 1 \\ \downarrow \\ 4 \\ \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Gal}p\text{A-}(1\rightarrow 3)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow \end{array}$$

INTRODUCTION

About half of the reference strains for serogroups O1 to O15 of *Serratia marcescens* each produce a neutral and an acidic polysaccharide, both of which are present in lipopolysaccharide extracts from isolated cell walls. Studies of the distribution of these polymers[1–6] and the results of serological tests[7,8] indicate that the neutral polymers are integral components of the lipopolysaccharides and that the acidic polymers are microcapsular components which complicate the typing of strains by their heat-stable antigens. In a previous study[9], we characterized the neutral polymer of the O15 reference strain with the repeating unit **1** but containing a minor proportion of 3-substituted α-L-rhamnopyranosyl residues. We now report the structure of the repeating unit of the acidic polymer from the same strain.

$$\begin{array}{c} \rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{NAc-}(1\rightarrow \\ \mathbf{1} \end{array}$$

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RESULTS AND DISCUSSION

The monosaccharide composition of the polymer was determined as mannose (55.1%), glucose (16.4%), and galacturonic acid (15.0%). Both hexoses were identified as the D isomers by g.l.c. of the acetylated oct-2-yl glycosides, and the galacturonic acid was assigned the same configuration by enzymic assay of D-galactose in the carboxyl-reduced Smith-degradation product SD1 (see below). Methylation analysis of the native polymer (Table I, column A) showed the presence of pyranosyl residues of unsubstituted mannose (1), 3-substituted glucose (1), and 3-substituted mannose (2). Similar analysis of the carboxyl-reduced polymer (Table I, column B) indicated the additional presence of a 2,4-disubstituted galactopyranosyluronic acid residue.

A pentasaccharide repeating-unit for the acidic polymer was confirmed by the n.m.r. spectra. The ^{1}H-n.m.r. spectrum contained five anomeric signals (each 1 H) at δ 5.52 ($J_{1,2}$ 3.6 Hz), 5.10 (unresolved), 4.94 ($J_{1,2}$ 1.5 Hz), 4.92 (unresolved), and 4.68 ($J_{1,2}$ 8.0 Hz), and showed the absence of *O*-acetyl substitution. The ^{13}C-n.m.r. spectrum (Fig. 1) contained 29 signals (one of double intensity), including anomeric signals at δ 104.08 ($^1J_{CH}$ 162 Hz), 102.43 ($^1J_{CH}$ 172 Hz), 101.61 ($^1J_{CH}$ 170 Hz), 100.59 ($^1J_{CH}$ 158 Hz), and 100.46 ($^1J_{CH}$ 177 Hz), as well as a carbonyl signal at δ 174.44 and four signals with δ ~60, corresponding to unsubstituted hydroxymethyl groups. Although the $^1J_{CH}$ values for the anomeric signals at δ 100.59 and 100.46 are somewhat atypical[10], it can be inferred that one mannosyl residue and either the glucopyranosyl or the galactopyranosyluronic acid residue are β-linked, and that the remaining residues are α-linked.

Smith degradation of the native polysaccharide gave a product (SD1) which remained polymeric, but was not very water-soluble, and had a hexose composition of mannose (33.5%) and glucose (16.4%). After carboxyl reduction, the hexose composition (molar ratios) was mannose (2.00), glucose (1.02), and galactose (0.81), showing that most of the galacturonic acid residues had been reduced. Methylation analysis of SD1 (Table I, column C) confirmed the loss of the terminal

TABLE I

METHYLATION ANALYSES OF THE O15 ACIDIC POLYMER AND SOME DEGRADATION PRODUCTS[a]

Methylation product[b]	*Relative peak area (g.l.c.)*				
	A	*B*	*C*	*D*	*E*
2,3,4,6-Man	1.00	0.82		1.01	1.00
2,4,6-Glc	1.03	1.04	1.24	1.00	0.64
2,4,6-Man	2.00	2.00	2.00	0.95	
3-Gal		0.71			

[a]Key: A, native polymer; B, native polymer with reduction of the uronic acid methyl ester; C, SD1; D, SD2; E, SD3. [b]2,3,4,6-Man = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol, *etc.*

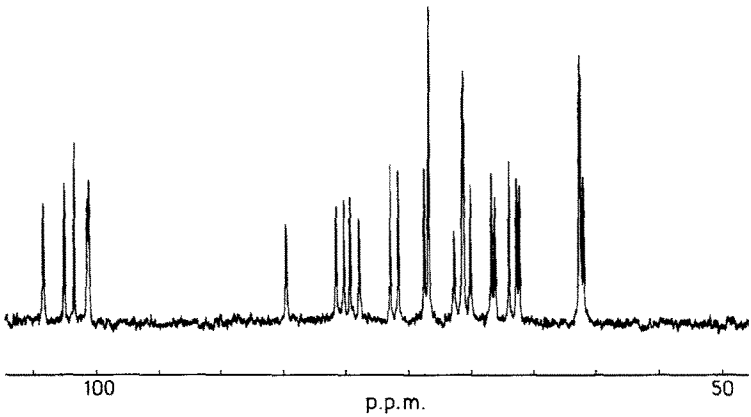

Fig. 1. ^{13}C-N.m.r. spectrum of the O15 acidic polymer. The spectrum for the sample in D_2O was obtained at 100.61 MHz and 50° with complete proton-decoupling and Me_4Si as the external reference. In addition to the signals shown, the spectrum contained a signal for a carbonyl carbon at δ 174.44.

mannopyranosyl group, which correlated with disappearance from the ^{13}C-n.m.r. spectrum of the signal at δ 101.61, from which it could be concluded that the terminal residue in the original polymer was α-linked. Because of heterogeneity of SD1 (assumed to be caused by the presence of the uronic acid residue), detailed interpretation of its ^{13}C-n.m.r. spectrum was not possible, but it was notable that heterogeneity particularly affected (split) the signal at δ ~104 attributed to a β-linked residue in the proximity of the uronic acid. An attempt was made to identify the proximal residue by lithium–ethylenediamine degradation[11] of SD1. Because of poor solubility in ethylenediamine, degradation was incomplete, and products were eluted from Sephadex G-15 from the void volume to the trisaccharide region. The material giving a peak in the latter region gave three spots in t.l.c., the major product having $R_{Maltose}$ 1.0 and the minor products $R_{Maltose}$ 1.2 and 0.6, respectively. Analyses of the major product (isolated by preparative t.l.c.) showed the presence of glucitol and a terminal hexose (mannose), consistent with glucosyl substitution of the galactopyranosyluronic acid residue in SD1. Insufficient material was available for further characterization of the degradation products.

$$\begin{array}{c} \alpha\text{-D-Man}p \\ 1 \\ \downarrow \\ 4 \\ \rightarrow 3)\text{-D-Glc}p\text{-}(1\rightarrow 2)\text{-D-Gal}p\text{A-}(1\rightarrow 3)\text{-D-Man}p\text{-}(1\rightarrow \\ \mathbf{2} \end{array}$$

Methylation analysis of carboxyl-reduced SD1 gave an extra product (derived from a 2-substituted hexopyranosyl residue) compared with those from the unreduced material (Table I, column C). Taken with the results described above, this

finding permits the partial structure **2** to be assigned to the branch-point region of the acidic polymer. To complete the structure, carboxyl-reduced SD1 was subjected to two further Smith degradations. The first product (SD2) had a mannose–glucose ratio of 2.00:1.12. All of the glucose and ~50% of the mannose was destroyed on oxidation of peracetylated SD2 with CrO_3, indicating that only one of the two mannose residues had the α configuration and, at the same time, that the galacturonic acid residue (not the glucose) in the original polymer was also α-linked. The results of methylation analysis of SD2 (Table I, column D) confirmed that mannose was the hexose substituted by the uronic acid, as shown in **2**. Further Smith degradation of SD2 gave SD3, which had a mannose–glucose ratio of 1.00:0.97. Both hexoses were destroyed by CrO_3 oxidation of peracetylated SD3 and therefore had the β configuration. G.l.c. of the permethylated oligosaccharide-alditol gave one major peak, for which m.s. showed fragment ions at *m/z* 219 and 187 (aA series), 103 ($aldA_1$), 307 ($baldA_1$), 367 ($abaldJ_1$), and 359 (abA_3), consistent with a hexosyl-hexosyl-glycerol structure for SD3. The results of methylation analysis (Table I, column E), although giving an unexpectedly low proportion of the product from 3-substituted glucose, confirmed mannose as the non-reducing-terminal residue, and permitted structure **3** to be assigned to SD3. Accordingly, structures **4, 5,** and **6** can be assigned to SD2, the repeating unit of SD1, and the repeating unit of the native polymer, respectively.

β-D-Man*p*-(1→3)-β-D-Glc*p*-(1→2)-Glycerol

3

α-D-Man*p*-(1→3)-β-D-Man*p*-(1→3)-β-D-Glc*p*-(1→2)-Glycerol

4

→2)-α-D-Gal*p*A-(1→3)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→3)-β-D-Glc*p*-(1→

5

α-D-Man*p*

1

↓

4

→2)-α-D-Gal*p*A-(1→3)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→3)-β-D-Glc*p*-(1→

6

On the basis of the work carried out, it is not possible to state whether the O15 group antigen of *S. marcescens* is the acidic polymer with repeating unit **6** or the neutral polymer with repeating unit **1**. However, by analogy with the results for some other serogroups[2,5,6], the acidic polymer is likely to be a microcapsular antigen partially masking the lipopolysaccharide with the neutral polymer as the O-specific side-chain. Serological cross-reactions between *S. marcescens* O15 and

O9 (the reference strain for which lacks an acidic polymer[12]) are probably explained by structural similarities between the neutral polymers. The O9 polymer has the disaccharide repeating-unit **7**, but also contains a minor proportion of 4-substituted rhamnose residues. The acidic O15 polymer has not been found in other serogroup reference strains of *S. marcescens*, although some of its structural features (*e.g.*, a relatively large repeating-unit, and the presence of branching and of 3-substituted hexose residues) apply to other acidic polymers that have been characterized, *e.g.*, the glucomannans from O6 and O14 strains[2,6], an O4 polymer[13], and the O13 polymer[5].

→3)-α-L-Rha*p*-(1→3)-β-D-Gal*p*NAc-(1→
7

EXPERIMENTAL

Isolation of the acidic polymer. — The polymer was obtained from the reference strain (C.D.C. 4523-60) for *S. marcescens* O15 as described previously[9], and constituted 13% of the lipopolysaccharide extracted from isolated cell walls.

General methods. — The methods used for the determination of monosaccharide composition have been described[5,9]. Monosaccharides were assigned to stereochemical series by g.l.c. of the acetylated oct-2-yl glycosides[14] (for glucose and mannose) or by using D-galactose oxidase (EC 1.1.3.9), after carboxyl reduction[15] of the corresponding uronic acid. N.m.r. spectra were recorded with a Bruker WH-400 or a JEOL GX-270 spectrometer. The ^{1}H-n.m.r. spectrum of the acidic polymer was obtained for a solution of the sample in D_2O, and was recorded at 60° with TSP as the external reference. ^{13}C-N.m.r. spectra (with complete proton-decoupling or with gated decoupling) were recorded at 50° and either 100 MHz or 68 MHz with Me_4Si as the external reference. G.l.c.–m.s. was carried out by using a Finnigan 1020B instrument.

Degradative methods. — Poly- and oligo-saccharides were methylated and converted into partially methylated sugars by standard procedures[9]. The products were identified by g.l.c.–m.s. of the methylated alditol acetates or by g.l.c. of the methylated aldose acetates (to differentiate the products from terminal glucose and mannose residues[16]). The reduction of carboxylic ester groups in the permethylated acidic polymer was carried out with $LiBH_4$ in tetrahydrofuran[5]. Carboxyl reduction of uronic acid residues in the Smith-degradation product SD1 was carried out by a carbodi-imide–$NaBH_4$ method[15]. Successive oxidations of the native polysaccharide and of its Smith-degradation products followed standard procedures[12]. In the case of carboxyl-reduced SD1, the initial product from Smith degradation was treated with $NaBH_4$ in order to convert the terminal glyceraldehyde residue into a glycerol residue. Products of low molecular weight obtained by lithium–ethylenediamine degradation[11] of SD1 were isolated by chromatography on Sephadex G-15, followed by t.l.c. with propan-2-ol–acetone–M lactic acid (2:2:1) and deionisation

of the eluates. The oxidation of peracetylated oligosaccharides to determine anomeric configurations[17] was carried out with CrO_3 for 1 h at 50°.

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