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Structure of a mannan isolated from the lipopolysaccharide of the reference strain (S3255) for a new serogroup of *Serratia marcescens*

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ABSTRACT

Both a neutral and an acidic polymer have been isolated from a lipopolysaccharide extract of *Serratia marcescens* strain S3255. The neutral polymer is a linear mannan with the repeating unit shown. The same repeating unit has been described for the O-specific polymers from *Escherichia coli* O8 and *Klebsiella* O5.

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

INTRODUCTION

In a recent study of the O serology of clinical isolates of *Serratia marcescens*, two new antigens (S1254 and S3255) were described[1]. We have shown[2] that the former antigen is a glucorhamnan with a disaccharide repeating-unit which also occurs[3] in a neutral polymer isolated from the lipopolysaccharide of the reference strain for serogroup O4. In the O4 polymer, most repeating units are acetylated at position 2 of the rhamnose residue. In continuing our structural studies of the surface polysaccharides of *S. marcescens*, we now report the structure of a neutral polymer isolated from the lipopolysaccharide of the S3255 reference strain.

RESULTS AND DISCUSSION

Lipopolysaccharide was extracted by the aqueous phenol method from defatted cell walls of strain S3255, the only pigmented reference strain for the O serogroups of *S. marcescens*. The product (yield, 23% of the originial walls) had galactose, glucose, mannose, 2-amino-2-deoxyglucose, and aldoheptoses as major monosaccharide components. Mild acid hydrolysis (aqueous 1% acetic acid, 2.25 h, 100°), followed by chromatography (Sephadex G-50) of the water-soluble products, gave a polymeric fraction (yield, 69%), as expected from the "ladder" pattern given by cells treated with proteinase K on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE)[1]. Further chromatography of the mixture on DEAE-Sepharose CL-6B gave a neutral polymer eluted partly with water (yield, 8%) and partly with 0.1M

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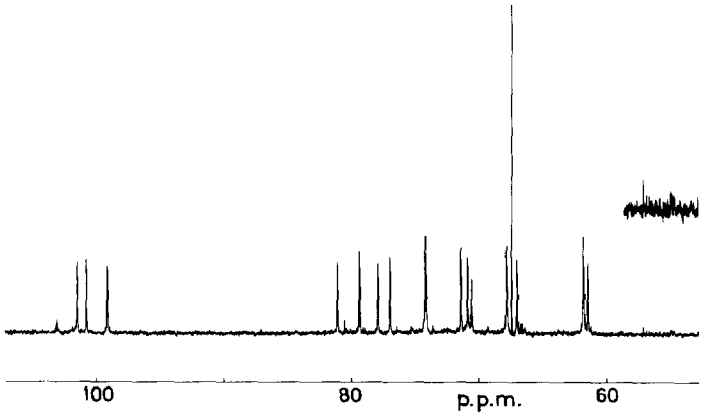

Fig. 1. ^{13}C-N.m.r. spectrum of the S3255 mannan. The spectrum for the sample in D_2O was obtained at 100.62 MHz and 27°, with complete proton-decoupling and 1,4-dioxane (δ 67.40) as the internal reference. The inset is an expansion showing the minor signal at δ 57.16 for the carbon atom of a methoxyl group.

NaCl (yield, 22%), and also an acidic polymer eluted with 0.3M NaCl (yield, 70%). The results of structural studies of the acidic polymer will be reported elsewhere.

Both fractions of the neutral polymer had the same monosaccharide composition (essentially mannose, with traces of glucose, heptoses, and an acetamido sugar) and gave the same n.m.r. spectra. Only the major fraction was studied further. Methylation analysis of the polymer showed the presence of 2-substituted and 3-substituted manno-pyranosyl residues (relative peak areas in g.l.c. of the methylated alditol acetates, 1.7:1.0). The ^{1}H-n.m.r. spectrum contained three major, unresolved anomeric signals (each 1 H) at δ 5.34, 5.16, and 4.79. The chemical shifts and the relatively large line width for the first two signals point to their derivation from α-pyranosyl residues. The presence in the polymer of a trisaccharide repeating-unit, with two residues in the α configuration and the other β, was confirmed by the ^{13}C-n.m.r. spectrum (Fig. 1), which contained 15 signals (three corresponding to 2 C each) including anomeric signals at δ 101.50 ($^1J_{CH}$ 174 Hz), 100.80 ($^1J_{CH}$ 172 Hz), and 99.14 ($^1J_{CH} \sim 164$ Hz).

The structure of the repeating unit was established by further n.m.r. studies. Most of the signals in the ^{1}H-n.m.r. spectrum were assigned with the aid of connectivities in 2D spectra (COSY and relayed COSY). The data are given in Table I, in which the residues designated a, b, and c are listed in order of decreasing chemical shift for H-1. The signals for H-1a to H-5a were readily traced, but those for H-6 and H-6′ could not be identified because of the complexity of the spectrum in the range δ 3.7–3.8. For residue b, signals could be traced only as far as H-4b, but H-5b was subsequently identified from a CH-correlation spectrum. In the case of residue c, connectivities were clear from H-1c to H-4c, and H-5c was readily identified by its characteristic high-field signal[4] for the β-pyranosyl residue, thus enabling the signals for H-6c and H-6′c to be located.

TABLE I

Chemical shifts (p.p.m.) of signals in the ^{1}H- and the ^{13}C-n.m.r. spectra for the S3255 mannan[a]

Atom		*Residue* *a*	*b*	*c*
1	H	5.34	5.16	4.79
	C	101.50	100.80	99.14
2	H	4.12	4.28	4.17
	C	79.36	77.91	71.38
3	H	4.01	3.87	3.73
	C	70.87	70.55	81.09
4	H	3.70	3.71	~3.73
	C	67.81	67.81	66.96
5	H	3.77	3.77	3.42
	C	74.18	74.18	76.96
6	H	nd[b]	nd	3.93
	C	61.80	61.80	61.46
6′	H	nd	nd	~3.76

[a] Assignments for C-6 may be interchanged. [b] Not determined.

Assignment of signals in the ^{13}C-n.m.r. spectrum (Table I) was carried out with the aid of the CH-correlation spectrum and relevant literature data[4-6]. From the downfield location of the signals for C-2a, C-2b, and C-3c, it is clear that residues a and b are both 2-substituted, and that the β-pyranosyl residue c is 3-substituted. Structure **1** can therefore be assigned to the repeating unit. In order to confirm this sequence, a series of 1D n.O.e. difference spectra was obtained. The results (Table II) are consistent with the proposed sequence →2a→3c→2b→.

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TABLE II

Observed n.O.e. contacts and assignments for the S3255 mannan

Proton irradiated	*Observed n.O.e. effect*[a] *Intra-residue*	*Inter-residue*
H-1a	H-2a (m)	H-3c (s), H-1b (m), H-1c (w), H-2c (w)
H-1b	H-2b (m)	H-2a (s), H-1c (s), H-3c (m), H-1a (w), H-2c (w)
H-1c	H-3c (s), H-5c (m), H-2c (w)	H-2b (m), H-1b (m), H-1a (w), H-2a (w)

[a] Magnitudes are indicated by s (strong), m (moderate), and w (weak).

Although the side chains of bacterial lipopolysaccharides often have complex structures and contain unusual sugars, simple homoglycans are produced by various bacteria. Examples of mannans are the O8, O9, and O9a antigens of *Escherichia coli*[7-9], a polymer from *Pseudomonas diminuta*[10], and one from a *Synechococcus* strain[11]. In fact, the repeating unit of the mannan from *E. coli* O8 (which is the same as that from *Klebsiella* O5)[7] also has the structure **1**, and the ^{13}C-n.m.r. spectrum obtained for the S3255 polymer (Fig. 1) was identical to that described for the *E. coli* O8 mannan[7]. Furthermore, minor signals at δ 103.1 and 57.1 in the latter spectrum were derived from C-1 and the methoxyl C of a 3-*O*-methyl-α-D-mannopyranosyl group at the non-reducing terminus of the mannan. Closer inspection of Figure 1 reveals the same minor signals in the spectrum for the S3255 mannan, and the ^{1}H-n.m.r. spectrum for the polymer contained a minor signal at δ 3.47 (s, 0.2 H) attributable to methoxyl H, indicating an average chain-length of ~15 repeating units. G.l.c.–m.s. of the alditol acetates derived from the polymer also revealed a minor component, the m.s. fragmentation pattern of which was consistent with its derivation from a 3- or 4-*O*-methylhexose. Thus, it seems likely that the mannan from *S. marcescens* S3255, like those from *E. coli* O8 and *Klebsiella* O5, incorporates a 3-*O*-methyl-α-D-mannopyranose residue, and that the attachment of this unit at the non-reducing end terminates biosynthesis of the polymer[7]. Further studies[12] have confirmed the close serological relationship between *S. marcescens* strain S3255, *E. coli* O8, and *Klebsiella* O5, which is clearly based on the common structure for their mannans. The antigen was also found[1] in three other strains of *S. marcescens* (also pigmented) in a survey of 104 clinical isolates. The need to absorb S3255 antisera with the O5 antigen of *S. marcescens*[1] to obtain monospecificity may suggest a common antigenic factor in the acidic polymers produced by the two reference strains, as the neutral polymer present in the O5 lipopolysaccharide is a partially acetylated galactoglucan[13] unrelated to the S3255 mannan.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of the lipopolysaccharide. — *S. marcescens* strain S3255 was grown and the cells were processed as in previous studies[2,3,13]. From a 20-L batch culture, the following yields were obtained: wet cells, 80 g; freeze-dried cell walls, 1.6 g; lipopolysaccharide, 372 mg. The water-soluble products from mild acid hydrolysis of the lipopolysaccharide were fractionated successively on Sephadex G-50 and DEAE-Sepharose CL-6B, to give a mannan and an acidic polymer.

Structural methods. — Monosaccharide compositions were determined as described[2], and the D configuration was assigned to mannose after g.l.c. of the but-2-yl glycoside acetates[14]. Methylation analysis followed standard procedures[3]. N.m.r. spectra (^{1}H and ^{13}C) were recorded with a Bruker WH-400 spectrometer. The ^{13}C-n.m.r. spectra for a solution of the mannan in D_2O (with or without gated decoupling) were recorded at 27° with 1,4-dioxane (δ 67.40) as the internal reference. The 1D ^{1}H-n.m.r. spectrum was recorded at 50° with sodium 3-trimethylsilylpropanoate-d_4 as the external

reference. The 2D spectra (COSY, relayed COSY, and CH-shift correlation) were also recorded at 50° with standard pulse sequences, as were n.O.e. difference spectra.

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