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STRUCTURE OF THE PUTATIVE O23 ANTIGEN OF *Serratia marcescens*

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ABSTRACT

A neutral glycan containing L-rhamnose and 2-acetamido-2-deoxy-D-galactose is one of two polymers present in the lipopolysaccharide extract from the reference strain for *Serratia marcescens* serogroup O23. The glycan, which has the disaccharide repeating-unit shown, shares structural features with polymers from several other O serogroups.

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

INTRODUCTION

Serogroup O23 of *Serratia marcescens* was first described[1] in 1983 by Le Minor *et al.* Although an antigenic relationship with some strains of serogroups O12, O13, and O14 has been demonstrated[1–3], antisera specific to O23 can be prepared by absorption with cells containing the common antigen. During systematic studies of the surface polysaccharides of *S. marcescens*, we have obtained circumstantial evidence[4–6] that the antigen common to some O12, O13, and O14 strains has the disaccharide repeating-unit **1**. It was therefore of interest to characterize the polymer(s) present in the O23 reference strain.

→2)-β-D-Rib*f*-(1→4)-α-D-Gal*p*NAc-(1→

1

RESULTS AND DISCUSSION

Water-soluble material extracted by the aqueous-phenol method from isolated cell walls of *S. marcescens* O23 was obtained in 31% yield. Monosaccharide components detected after total acid hydrolysis were glucose, galactose, mannose, rhamnose, aldoheptoses, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose. After mild acid hydrolysis (aq. 1% acetic acid, 2.25 h, 100°), 38% of the material remained water-soluble and polymeric (Sephadex G-50). Four fractions were obtained by chromatography on DEAE-Sepharose CL-6B: fraction 1 (42%), eluted

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with water; fraction 2 (13%), eluted with 0.1M NaCl; fraction 3 (30%), eluted with 0.3M NaCl; fraction 4 (15%), eluted with M NaCl. This paper is concerned only with the "neutral" material present in fractions 1 and 2, both of which consisted mainly of L-rhamnose and 2-amino-2-deoxy-D-galactose. The n.m.r. spectra (^{1}H and ^{13}C) of the fractions were very similar (although minor signals were more prominent for fraction 2) and indicative of a polymer with a disaccharide repeating-unit. Structural studies were confined to the cleaner fraction (1).

The ^{1}H-n.m.r. spectrum contained two major anomeric signals (each 1 H) at δ 5.16 (unresolved) and 4.80 ($J_{1,2}$ 8.0 Hz) and methyl signals at δ 2.08 (s) and 1.36 ($J_{5,6}$ 6.2 Hz): minor signals in the spectrum indicated only a low level of contamination or heterogeneity (the material contained traces of glucose and aldoheptoses, probably derived from a terminal core oligosaccharide). The ^{13}C-n.m.r. spectrum (Fig. 1) contained 14 major signals (and several minor signals), including anomeric signals at δ 101.93 ($^1J_{CH}$ 162 Hz) and 101.44 ($^1J_{CH}$ 173 Hz), signals for a 2-acetamido group at δ 175.00, 52.85, and 22.40, and for C-6 of rhamnose at δ 17.18. From these spectral data, it could be inferred that the disaccharide repeating-unit was constructed from α-rhamnopyranosyl and 2-acetamido-2-deoxy-β-galactopyranosyl residues. The results of methylation analysis showed that both residues were 4-substituted, and therefore that the repeating unit had structure **2**. The ^{13}C-n.m.r. data for the polymer (Table I) were consistent with this structure.

$\rightarrow$4)-α-L-Rha*p*-(1$\rightarrow$4)-β-D-Gal*p*NAc-(1$\rightarrow$

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A Smith degradation was carried out on the polymer in order to consolidate the structural conclusions. By means of h.p.l.c., a single major product (over 80%

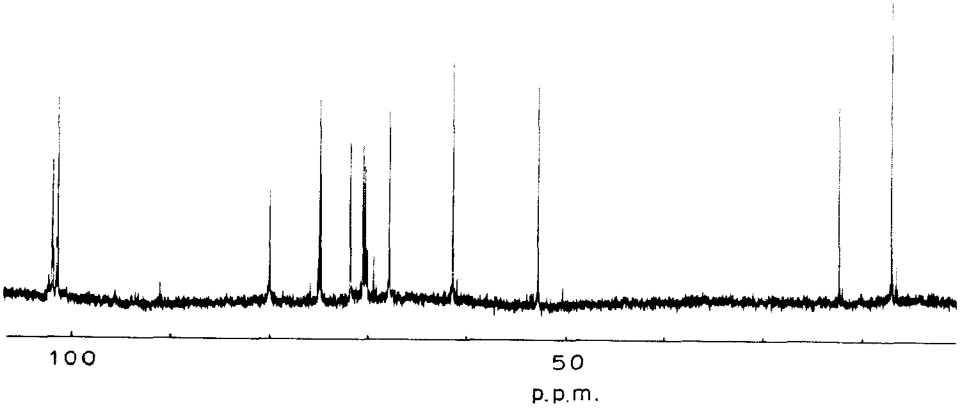

Fig. 1. ^{13}C-N.m.r. spectrum of a solution of the O23 glycan in D_2O obtained at 100.62 MHz and 27°, with complete proton-decoupling and tetramethylsilane as the external reference. In addition to the signals shown, the spectrum contained a carbonyl resonance at δ 175.00.

TABLE I

^{13}C-N.M.R. DATA FOR THE O23 NEUTRAL GLYCAN

Carbon atom	*Chemical shift (p.p.m.)*[a]	
	→4)-α-L-Rhap-(1→	*→4)-β-D-GalpNAc-(1→*
C-1	101.44	101.93
C-2	70.56	52.85
C-3	70.34	71.95
C-4	80.04	74.85
C-5	67.95	74.96
C-6	17.18	61.43
-NHC(O)CH$_3$		175.00
-NHC(O)CH$_3$		22.40

[a] The spectrum for a solution of the sample in D$_2$O was recorded at 27° and 100.62 MHz with tetramethylsilane as the external reference. Tentative assignments (some for signals with closely similar chemical shifts may be interchanged) were made with the aid of literature data[7], and making allowance for an upfield displacement of ~0.8 p.p.m. (compared with values based on the use of 1,4-dioxane as internal reference with δ 67.40).

of the total peak area), with the elution characteristic of a disaccharide-alditol, was detected. Its ^{1}H-n.m.r. spectrum contained one anomeric signal (1 H) at δ 4.62 ($J_{1,2}$ 8.3 Hz), and methyl signals at δ 2.05 (s) and 1.17 (d, J 6.6 Hz). These data are in accord with those reported previously[8] for compound **3**, the expected product of the degradation. Also, the ^{13}C-n.m.r. spectrum of the product contained the expected 12 signals, including an anomeric signal at δ 101.66, signals for the 2-acetamido group at δ 175.01, 52.84, and 22.25, and a methyl signal at δ 16.74. Finally, the permethylated disaccharide-alditol gave a single peak in g.l.c., and e.i.-m.s. of the derivative gave fragment ions (listed in the Experimental) also in accord with structure **3**.

β-D-Gal*p*NAc-(1→3)-1-Deoxy-D-erythritol

3

The O23 glycan described here is the third example of a polymer produced by *S. marcescens* in which the repeating unit is a disaccharide constructed from α-L-rhamnopyranosyl and 2-acetamido-2-deoxy-β-D-galactopyranosyl residues. However, one or both positions of glycosylation are different in the polymers from the O9 (ref. 8) and O15 (ref. 9) strains, compared with O23, and no serological relationship has been described between serogroup O23 and the others. The results of this study do not account for the cross-reactions between O23 and strains belonging to serogroups O12, O13, and O14 (refs. 1–3). Certainly, the polymer with repeating unit **1**, which seems to be the common antigen in some O12, O13, and O14 strains, was not detected in the O23 reference strain, and, if present, could only be a trace component. Also, it seems unlikely that the residue of 2-acetamido-2-deoxy-D-galactopyranose present in both repeating units **1** and **2** accounts for the cross-reaction, because of the different anomeric configuration. Equally, preliminary analyses of the acidic O23 glycan have not revealed any structural features in common with the polymer having repeating unit **1**. Thus, at present, we have no explanation for the serological observations in terms of surface polysaccharides.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of the lipopolysaccharide. — The O23 reference strain of *S. marcescens* was grown and processed as in related studies[6,8,9]. From a 20-L batch culture, 151 g of wet cells were obtained and used to prepare cell walls (3.19 g) and lipopolysaccharide (0.98 g). The water-soluble products from mild acid hydrolysis of the lipopolysaccharide were fractionated by chromatography on Sephadex G-50 and on DEAE-Sepharose CL-6B.

Structural methods. — Monosaccharides were released and identified as described[8,9]: absolute configurations were determined by g.l.c. of the but-2-yl glycoside[10] acetates. Methylation analysis, monitored by g.l.c. and e.i.-m.s. of the methylated alditol acetates, involved standard procedures[11]. N.m.r. spectra (^{1}H and ^{13}C) were mainly recorded (all data cited) with a Bruker WH-400 spectrometer for solutions in D_2O. ^{1}H-n.m.r. spectra were recorded at ~60° with sodium 3-trimethylsilylpropanoate-d_4 as the external reference. ^{13}C-N.m.r. spectra (with complete proton-decoupling or gated decoupling) were recorded at 27° with tetramethylsilane as the external reference or with 1,4-dioxane as internal reference.

A Smith degradation was carried out[8] on a sample (20 mg) of the O23 glycan. The major product was isolated by h.p.l.c.[12] and, after permethylation, was examined by g.l.c. and by e.i.-m.s. Significant fragment ions (relative intensities in brackets and some assignments[13] in square brackets) included the following: *m/z* 71(100), 87(80), 98(32), 117(2) [bA$_1$], 129(67), 142(30), 177(12) [abJ$_1$], 187 (33), 219(32), 228(6) [aA$_2$], 260(4) [aA$_1$], and 320(2).

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