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Note

Structure of a glucorhamnan from the lipopolysaccharide of *Serratia marcescens* strain S1254

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The current scheme for typing strains of *Serratia marcescens* by their heat-stable antigens is undergoing major reconstruction as a result of chemical studies allied with serological reappraisal[1,2]. Evidence has accumulated that many of the existing 24 O serogroups are actually defined by acidic, microcapsular polymers, which can camouflage structural variations in lipopolysaccharide side-chains (the conventional O antigens). For example, an acidic glucomannan[3] apparently accounts for the prevalence[4] of "O14" strains, the lipopolysaccharides of which differ in their neutral polymeric fractions (at least 4 polymers are represented, each of which also occurs in lipopolysaccharides from strains of other O serogroups)[5].

In a recent survey of clinical isolates of *S. marcescens*[2], 2 new O antigens were identified, one of which (S1254) was present in 13.5% of the strains tested and, apparently, was related to the O4 antigen. During our studies of the surface polysaccharides of *S. marcescens*, we have isolated both a partially acetylated glucorhamnan[6] and an acidic galactomannan[7] from the O4 reference strain, and it was therefore of interest to characterise the S1254 antigen.

Lipopolysaccharide was isolated (yield, 36%) from cell walls of strain S1254 by the aqueous phenol method. 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, 43%). Most of the fraction (61%) was eluted with water from a column of DEAE-Sepharose CL-6B, and the remainder with 0.1M NaCl; there was no evidence for the presence of an acidic polymer. Both subfractions had the same sugar composition and gave identical n.m.r. spectra, so further studies were confined to the material eluted with water.

The major components of the polymer were L-rhamnose and D-glucose (ratio of peak areas in g.l.c. of the alditol acetates, 1.00:1.15); small proportions of aldoheptoses were also present. The n.m.r. spectra of the polymer confirmed that the repeating unit was a disaccharide of pyranosyl residues, and showed the absence of *O*-acetyl groups. In the ^{1}H-n.m.r. spectrum, there were anomeric signals (each 1 H) at δ 5.07 ($J_{1,2}$ 3.8 Hz) and 4.95 ($J_{1,2}$ 1.6 Hz), pointing to α-pyranosyl residues, and a methyl signal at δ 1.29 ($J_{5,6}$ 6.2

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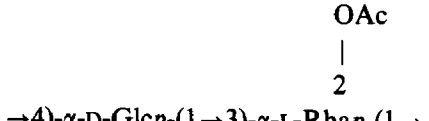
Hz) from Rha H-6. A complete assignment of the spectrum (Table I) was possible with the aid of a COSY experiment. The ^{13}C-n.m.r. spectrum of the polymer contained 11 discrete signals (one corresponding to 2 C), including anomeric signals at δ 101.23 and 96.31. All signals could be assigned (Table I) from the results of a heteronuclear

TABLE I

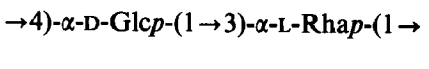
Chemical shifts (p.p.m.) of signals in the ^{1}H- and the ^{13}C-n.m.r. spectra for the S1254 polymer

Atom		*→4)-α-D-Glcp-(1→*	*→3)-α-L-Rhap-(1→*
1	H	5.07	4.95
	C	96.31	101.23
2	H	3.60	4.17
	C	72.46	68.02
3	H	3.88	3.82
	C	72.46	76.58
4	H	3.61	3.59
	C	78.04	71.05
5	H	4.03	4.04
	C	71.61	70.03
6	H	3.81	1.29
	C	60.77	17.47
6′	H	3.73	

shift-correlation experiment. From the large chemical shifts for Glc C-4 and Rha C-3, it was inferred that these were the positions of *O*-glycosylation. In fact, the ^{13}C-n.m.r. spectrum could be superimposed on that obtained[6] for the neutral polymer from the O4 reference strain after *O*-deacetylation, allowing for a displacement of ~0.8 p.p.m. attributable to the use of different operating parameters, and the assignments made (Table I) are in accord with those made[6] provisionally for the deacetylated O4 polymer. As the native O4 polymer has the repeating unit **1**, it is clear that the repeating unit in the S1254 polymer has the structure **2**.



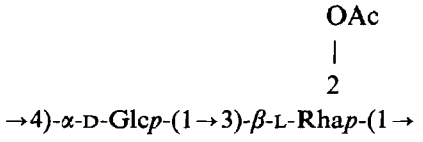
1



2

Further evidence for structure **2** was provided by n.m.r. spectroscopy. The chemical shift for Rha H-5 (Table I) is consistent with the α configuration[8,9], as is the chemical shift for C-5 (ref. 10), even though the data for the anomeric signals are not conclusive. Confirmation of the positions of the glycosidic linkages was provided by 1D n.O.e. difference spectra. Irradiation at δ 4.95 (Rha H-1) showed an intra-residue n.O.e. at Rha H-2 (6%) and an inter-residue n.O.e. at Glc H-4 (13%). Similarly, irradiation at δ 5.07 (Glc H-1) gave effects at Glc H-2 (15%), Rha H-3 (9%), and Rha H-2 (6%).

The serological relationship between the S1254 and O4 antigens is obviously accounted for by a common glucorhamnan structure. In the O4 antigen, as isolated, acetylation at O-2 of rhamnose is ~90% complete, whereas no *O*-acetyl group was detected for S1254. A related glucorhamnan having an acetyl group at position 2 of the β-rhamnopyranosyl residues (structure **3**) has been isolated from lipopolysaccharides of *S. marcescens* strains assigned to serogroup O6 (ref. 11), O7 (ref. 6), and O14 (refs. 6 and 12).



3

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of the lipopolysaccharide. — *S. marcescens* strain S1254 was grown and the cells were processed as in previous studies[6]. From a 20-L batch culture, the following yields were obtained: wet cells, 54 g; dry cell walls, 1.9 g; lipopolysaccharide, 684 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 the neutral glucorhamnan.

Structural methods. — The identification of monosaccharides and determination of their absolute configuration were carried out as described[6,13]. N.m.r. spectra (^1H and ^{13}C) were recorded with a Bruker WH-400 (all data cited) or JEOL JNM-GX270 spectrometer. The ^{13}C-n.m.r. spectrum for a solution of glucorhamnan in D_2O was recorded at 27° with complete proton-decoupling and 1,4-dioxane (δ 67.40) as the internal reference. The 1D ^1H-n.m.r. spectrum was recorded at 60° with acetone (δ 2.22) as the internal standard. 2D-N.m.r. spectra (homo- and hetero-nuclear) were obtained by using standard pulse sequences and solutions at 50°. N.O.e. difference spectra were also recorded at 50°.

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