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STRUCTURE OF THE O-SPECIFIC GALACTAN FROM THE LIPOPOLYSACCHARIDE OF THE REFERENCE STRAIN FOR *Serratia marcescens* SEROGROUP O24

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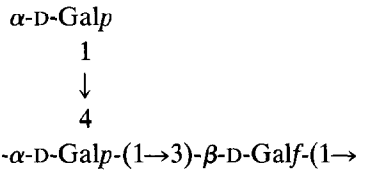
ABSTRACT

The putative O-specific polysaccharide for *Serratia marcescens* serogroup O24 is a galactan with a branched, trisaccharide repeating-unit of the structure shown. The structure of the backbone is identical to that of the linear galactans isolated from the reference strains for *S. marcescens* serogroups O16 and O20, presumably accounting for the serological cross-reactions observed.



INTRODUCTION

A new serogroup, O24, of *Serratia marcescens* was described by Traub[1] in 1985, and a serological relationship with groups O16 and O20 has been reported[1–3]. As part of a comprehensive study of the heat-stable antigens produced by *S. marcescens*, we have recently characterized glycans isolated from lipopolysaccharide extracts of the O16 and O20 reference strains[4]. Both strains produced a galactan with the disaccharide repeating-unit **1**. In the case of the O16 strain, this galactan was apparently accompanied by a polymer of 2-substituted β-D-ribofuranosyl residues[4]. We have therefore extended our studies to the O24 reference strain, and now report the structure of the galactan present.

1

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RESULTS AND DISCUSSION

The lipopolysaccharide extracted from isolated cell walls in 23% yield had galactose as the major sugar component, and glucose, heptoses, and 2-amino-2-deoxyglucose as lesser components. After mild acid hydrolysis, 38% of the lipopolysaccharide was recovered as water-soluble, polymeric products. Chromatography on DEAE-Sepharose CL-6B gave two fractions indistinguishable by their monosaccharide composition and n.m.r. spectra, eluted with water (72% of recovered products) or 0.1M NaCl (28%). No acidic polymer was detected.

The polymer isolated was essentially a galactan, with only traces of the other sugars detected in the parent lipopolysaccharide. Galactose was identified as the D isomer by g.l.c. of the but-2-yl glycoside acetates. Methylation analysis of the galactan showed three major components derived from unsubstituted galactopyranosyl, 3-substituted galactofuranosyl, and 3,4-disubstituted galactopyranosyl (or 3,5-disubstituted galactofuranosyl) residues (relative peak areas in g.l.c. of the methylated alditol acetates, 0.95:1.00:1.03). Two minor products derived from 3-substituted and 4-substituted galactopyranosyl residues (relative peak areas, ~0.2) were also detected. The ^{1}H-n.m.r. spectrum of the galactan contained 3 anomeric signals (each 1 H) at δ 5.22 ($J_{1,2}$ 2.5 Hz), 5.08 ($J_{1,2}$ ~4 Hz), and 5.04 ($J_{1,2}$ 3.5 Hz). A trisaccharide repeating-unit was also indicated by the ^{13}C-n.m.r. spectrum (Fig. 1), which contained 18 signals, including anomeric signals at δ 109.80, 100.44, and 100.19, and signals for free hydroxymethyl carbons at δ 63.06, 60.60, and 60.48. The data confirm the presence in the polymer of a single (β) furanosyl and two (α) pyranosyl residues.

The location of the α-D-galactopyranosyl substituent in the branched repeating-unit was determined from studies of the products obtained on partial hydrolysis (aq. 90% formic acid, 1 h, 70°) of the methylated galactan[4]. After reduction ($NaBD_4$) and trideuteriomethylation of the products, g.l.c. revealed one major component (PH1) and four minor components (PH2–5). The retention times indicated that PH1 and PH2 were derived from disaccharides, PH3 and PH4 from trisaccharides, and PH5 from a tetrasaccharide. The mass spectrum of PH1 con-

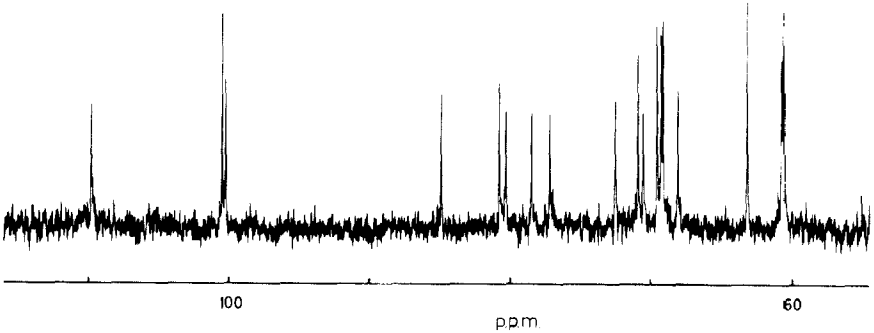
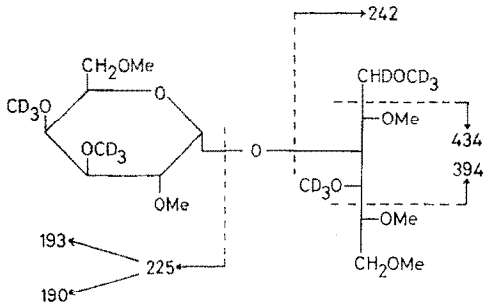


Fig. 1. ^{13}C-N.m.r. spectrum of the O24 galactan. The spectrum for the sample in D_2O was obtained at 100.62 MHz and 50°, with complete proton-decoupling and tetramethylsilane as the external reference.



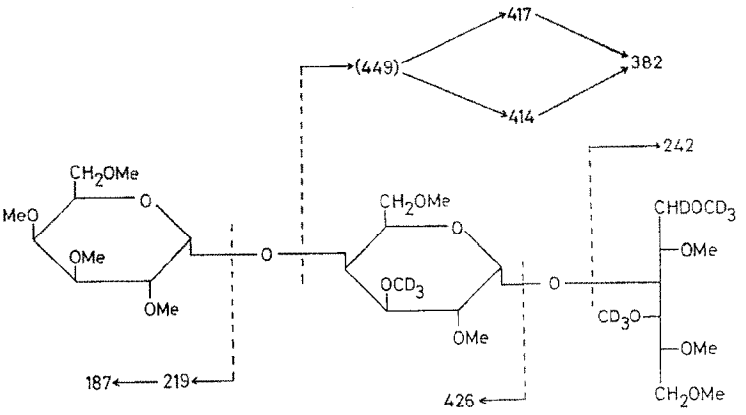
Scheme 1. Fragmentation in e.i.-m.s. of the *O*-alkylated disaccharide-alditol (PH1).

firmed that it was a permethylated galactosylgalactitol and showed that both component residues carried two deuteriomethyl groups [fragment ions at *m/z* 225 (aA_1) and 242 (bA_1)]. The structure inferred for PH1 (Scheme 1) is supported by other fragment ions indicated and by a J_1 fragment at *m/z* 305, and is consistent with the expectation that cleavage of the furanosidic linkage and removal of the lateral substituent should be preferred hydrolytic steps, producing the derivative of disaccharide **2**. The mass spectrum of the minor disaccharide derivative PH2, which included the aA series of fragments at *m/z* 219, 187, and 155 as well as the bA_1 fragment of *m/z* 245, showed that the galactosyl residue did not carry a deuteriomethyl group and that the galactitol residue carried three such groups. Thus, the parent disaccharide of partial structure **3** arose through cleavage of both main-chain linkages involving the branch-point galactose residue.

α-D-Gal*p*-(1→3)-β-D-Gal*f* α-D-Gal*p*-(1→3/4)-α-D-Gal*p*

2 **3**

Because the derivatives PH1 and PH2 were more highly resolved by g.l.c.



Scheme 2. Fragmentation in e.i.-m.s. of the *O*-alkylated trisaccharide-alditol (PH3).

than might be expected purely from differences in the number and location of trideuteriomethyl groups, it could be inferred that the glycosidic bond in **3** involved the 4 position. Confirmation was provided by a study of the trisaccharide derivative PH3, isolated by preparative h.p.l.c. Characteristic fragments in the mass spectrum of the derivative (Scheme 2) indicated the structure shown. Of particular significance was a large peak at *m/z* 305 (bcJ_1) as for PH1, indicating that the internal galactose residue carried a trideuteriomethyl group in position 3 and was glycosidically substituted in position 4 (ref. 5). This inference was substantiated by conversion of PH3 into methylated alditol acetates: the products obtained were the derivatives of 2,3,4,6-tetra-*O*-methylgalactopyranosyl, 4-substituted 2,6-di-*O*-methyl-3-*O*-trideuteriomethylgalactopyranosyl, and 3-substituted 2,5,6-tri-*O*-methyl-1,4-di-*O*-trideuteriomethylgalactitol residues. Thus, the trisaccharide parent of PH3 has the structure **4**, and the repeating unit of the original galactan has the structure **5**. Mass-spectral data for PH4 were consistent with its derivation from the backbone trisaccharide of structure **6**, with retention of a furanosidic linkage, but this was not rigorously proved. No useful information was obtained for product PH5.

α-D-Gal*p*-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*f*

4

α-D-Gal*p*
1
↓
4
→3)-α-D-Gal*p*-(1→3)-β-D-Gal*f*-(1→

5

β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→3)-β-D-Gal*f*

6

These studies have shown that the repeating disaccharide which forms the backbone of the O24 galactan has the same structure as that (**1**) which is the repeating unit of the linear O16 and O20 galactans[4]. The same linear galactan also occurs as the side chain of lipopolysaccharides from *Pasteurella haemolytica* serotypes T4 (ref. 6) and T10 (ref. 7). The relative lability of the galactofuranosyl linkage may account for the detection of a minor proportion of the product from 4-substituted galactopyranosyl residues during methylation analysis of the O24 galactan (some hydrolysis occurred during treatment of the lipopolysaccharide with 1% acetic acid at 100°). The small amount of product from 3-substituted galacto-

TABLE I

^{13}C-N.M.R. DATA FOR THE O24 GALACTAN

Carbon atom	*Chemical shift (p.p.m.)*[a]		
			↓ 4
	α-D-*Gal*p-(1→	→3)-β-D-*Gal*f-(1→	→3)-α-D-*Gal*p-(1→
C-1	100.44	109.80	100.19
C-2	69.05	80.20	67.98
C-3	69.45	84.83	77.06
C-4	69.19	80.68[b]	78.37
C-5	70.87	70.50	72.46
C-6	60.48	63.06	60.60

[a]The spectrum was recorded at 50° with tetramethylsilane as the external reference. The provisional assignments were made with the aid of literature data for linear galactans[4,7] and a terminal α-D-galactopyranosyl residue[8], together with data on glycosylation effects[9], but assignments for some signals with closely similar chemical shifts may be interchanged. [b]The chemical shift assigned is unexpectedly different from that (~82.3 p.p.m., adjusted for the different reference used) observed[4,7] for the corresponding carbon atom in the linear galactans.

pyranosyl residues could be due to the absence of the lateral substituent from some repeating units, although the n.m.r. spectra (*e.g.*, Fig. 1) did not point to any significant heterogeneity in the galactan. A provisional assignment (Table I) of the signals in the ^{13}C-n.m.r. spectrum could be made by using data[4,7] for the linear galactan as a starting point.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of the lipopolysaccharide. — The reference strain for *S. marcescens* serogroup O24 was grown for 16 h at 30° in Nutrient Broth No. 2 (Oxoid). From a 20-L batch culture, aerated at 20 L.min^{-1}, 158 g of wet cells were obtained and used to prepare[4] cell walls (6.12 g) and lipopolysaccharide (1.41 g). After mild hydrolysis of the lipopolysaccharide (aq. 1% acetic acid, 2.25 h, 100°), the water-soluble polymeric products were obtained by chromatography on Sephadex G-50 and fractionated[4] on DEAE-Sepharose CL-6B.

General methods. — Methods used for the identification and assignment of configuration to monosaccharide components were those described previously[4]. N.m.r. spectra (^{13}C and ^{1}H) of samples in D_2O were recorded with a Bruker WH-400 or JEOL JNM-GX270 spectrometer. ^{1}H-N.m.r. spectra were recorded at 70° with sodium 3-trimethylsilylpropanoate-d_4 as the external reference, and ^{13}C-n.m.r. spectra were recorded at 50° with tetramethylsilane as the external reference. Methylation analyses were carried out by standard procedures[4], and were monitored by g.l.c. and by e.i.-m.s.

Preparation of methylated oligosaccharide-alditols[4]. — A sample (~3 mg) of methylated O24 galactan was hydrolysed, and the products were reduced ($NaBD_4$), then trideuteriomethylated, and examined by g.l.c. on a fused-silica capillary column (25 m) of BP1 (isothermal at 220° for 4 min, then programmed to 290° at 15° min^{-1}). The only major product (PH1) was eluted in 3 min 23 s; four minor or trace products were also eluted (PH2, 3 min 32 s; PH3, 9 min 49 s; PH4, 9 min 37 s; PH5, 20 min 40 s). Mass spectra for PH1 and PH2 were obtained by g.l.c.–m.s.; the other products were isolated by h.p.l.c. (ref. 4) and mass spectra were obtained by direct insertion on the probe. Significant fragments ions (relative intensities in brackets and some assignments[5] in square brackets, ALD signifying fission within the alditol residue) for PH1 and three of the minor products (PH2 to PH4) are listed below. PH1: *m/z* 88(35), 91(75), 104(97), 111(41), 114(47), 190(100) [aA_1 – CD_3OH], 193(10) [aA_1 – CH_3OH], 225(36) [aA_1], 242(83) [bA_1], 305(30) [abJ_1], 394(4) [ALD], and 434(trace) [ALD]. PH2: *m/z* 155(23) [aA_3], 187(100) [aA_2], 219(43) [aA_1], and 245(75) [bA_1] (incomplete g.l.c. resolution from PH1 prevented the acquisition of other specific data). PH3: *m/z* 88(100), 91(48), 101(55), 104(50), 111(56), 155(44) [aA_3], 175(19), 187(83) [aA_2], 219(36) [aA_1], 242(83) [cA_1], 305(49) [bcJ_1], 382(35) [bcA_3], 414(4) [bcA_1 – CD_3OH], 417(2) [bcA_1 – CH_3OH], 426(1) [baA_1], and 509(12) [$abcJ_1$]. PH4: *m/z* 104(100), 111(12), 155(55) [aA_3], 187(21) [aA_1 – CD_3OH], 190(25) [aA_1 – CH_3OH], 222(45) [aA_1], 242(37) [cA_1], 382(5) [bcA_3], 414(1) [bcA_1 – CD_3OH], 417(1) [bcA_1 – CH_3OH], 429(16) [baA_1], 449(2) [bcA_1], 512(1) [$abcJ_1$], and 598(1) [ALD].

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