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Note

Structure of a neutral polymer isolated from the lipopolysaccharide of *Serratia marcescens* O5 (C.D.C. 867-57)

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As part of a systematic study of the surface polysaccharides elaborated by strains of *Serratia marcescens* representing different O serogroups, we have examined products from the O5 reference strain. As with most other lipopolysaccharides of this organism, mild, acid hydrolysis of the product extracted from defatted cell walls gave a dark-brown suspension. Fractionation of the water-soluble material on Sephadex G-50 gave a polymeric fraction representing 39% of the parent lipopolysaccharide. Further chromatography on DEAE-Sepharose CL-6B, with stepwise elution by water and aqueous NaCl, provided a neutral polysaccharide and an acidic one in equal yield. Some of the neutral polymer was eluted by water but most (76%) by 0.1M NaCl. The acidic polymer, which has yet to be characterised, was mainly (82%) eluted by 0.2M NaCl, and the balance by 0.3M NaCl.

The monosaccharide compositions of both fractions of neutral polymer were very similar — essentially glucose and galactose, with traces of heptoses and rhamnose — and the n.m.r. spectra (^1H and ^{13}C) were indistinguishable. Structural studies were confined to the more abundant fraction. Quantitative analysis of the polymer gave the composition as D-glucose (37.2%) and D-galactose (46.1%). The presence of an *O*-acetyl substituent was apparent from signals in the n.m.r. spectra at δ 2.14 (^1H) and at δ 173.34 and 20.44 (^{13}C). From the complexity of the spectra (*e.g.*, Fig. 1) and integration of the ^1H-n.m.r. spectrum, it was inferred that *O*-acetylation was not stoichiometric and amounted to about two groups per three (disaccharide) repeating-units. Both n.m.r. spectra were greatly simplified by *O*-deacetylation. The ^{13}C-n.m.r. spectrum (Fig. 2) contained 12 discrete, major signals, including two in the anomeric region at δ 108.13 and 102.22, indicative of β-furanosyl and β-pyranosyl residues, respectively. These assignments were supported by anomeric signals of equal integrated intensity at δ 5.17 (unresolved) and 4.65 ($J_{1,2}$ 7.9 Hz) in the ^1H-n.m.r. spectrum.

Only two significant products were obtained on methylation analysis of the

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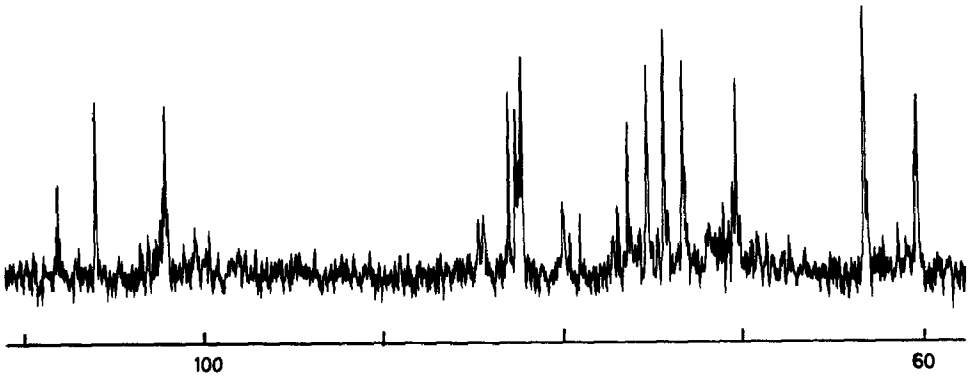

Fig. 1. ^{13}C-N.m.r. spectrum of the native polymer. The spectrum for a solution of the sample in D_2O was obtained at 100.61 MHz and 50°, with complete proton-decoupling and external tetramethylsilane as the reference. Additional signals at δ 173.34 and 20.44 are attributable to partial *O*-acetylation.

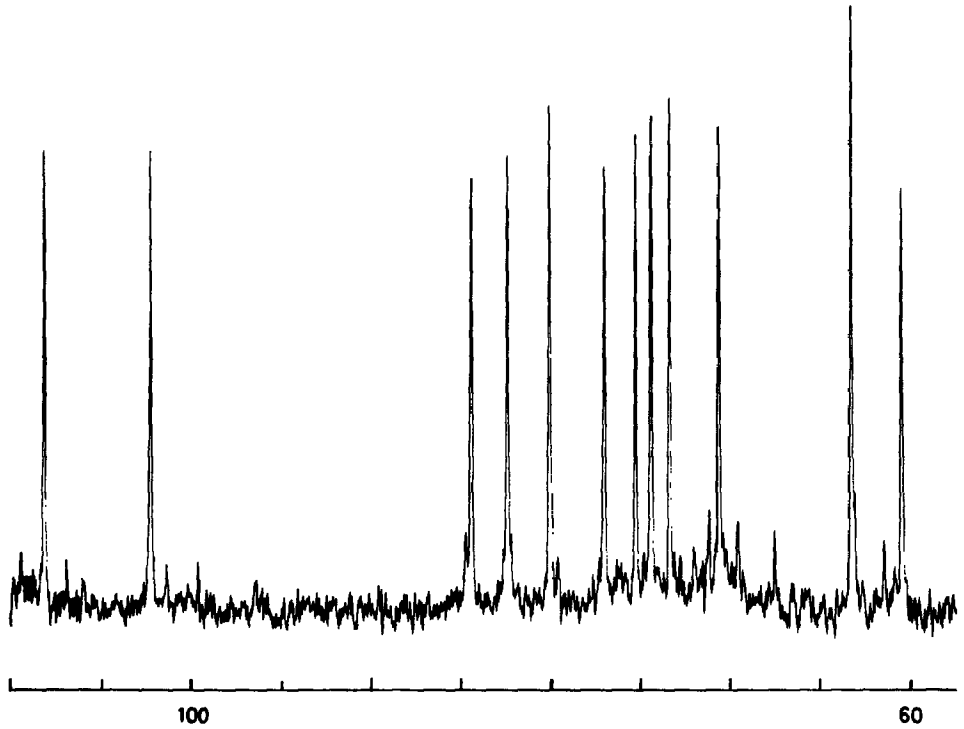

Fig. 2. ^{13}C-N.m.r. spectrum of the *O*-deacetylated polymer. The spectrum was obtained as for the native polymer (Fig. 1).

polymer. One of them had the g.l.c. retention time and mass spectrum of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol-*1-d*. The second product had the mass spectrum of a 1,3,4-tri-*O*-acetyl-2,5,6-tri-*O*-methylhexitol-*1-d*, and therefore was derived from

a 3-substituted galactofuranosyl residue. The evidence for a disaccharide repeating-unit of structure **1** was supported by the results obtained on periodate oxidation followed by borohydride reduction of the *O*-deacetylated polymer. Analysis of the product showed that both hexoses had been destroyed, and acid hydrolysis gave arabinose and erythritol in the molar ratio 1.00:0.96.

$$\rightarrow 3)\text{-}\beta\text{-D-Gal}f\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow$$
1

A comparison of the ^{13}C-n.m.r. spectra of the native and the *O*-deacetylated polymer (Figs. 1 and 2) allowed the *O*-acetyl group in the former to be located at position 2 of the galactosyl residue. The signals assigned to C-1 and C-3 of the galactose were shifted upfield by 2.1 and ~1.5 p.p.m., respectively, in the *O*-acetylated polymer, while the signal assigned to C-2 was shifted downfield by ~2.5 p.p.m. (other signals were only slightly affected). A full assignment of the signals for the *O*-deacetylated polymer is given in Table I. Structure **2** applies to about two-thirds of the repeating units in the native polysaccharide.

$$\begin{array}{l} \qquad\quad \text{OAc} \\ \qquad\quad | \\ \qquad\quad 2 \\ \rightarrow 3)\text{-}\beta\text{-D-Gal}f\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow \\ \qquad\quad 2 \end{array}$$

Whereas several neutral polymers produced by *S. marcescens* occur in strains representing more than one O serogroup[3–5], the polymer described here has not been isolated previously. Galactofuranosyl residues are fairly uncommon in bacterial polysaccharides, but examples are to be found among the O-specific polysaccharides produced by *Shigella* and *Klebsiella* species, the capsular polysaccharides of *Klebsiella* and *Streptococcus*, and in the *Salmonella* T1 polysaccharide *inter alia*[6]. The simultaneous presence of neutral and acidic polymers in lipopolysacchar-

TABLE I

ASSIGNMENTS OF SIGNALS[a] IN THE ^{13}C-N.M.R. SPECTRUM OF THE *O*-DEACETYLATED NEUTRAL POLYMER

Carbon atom	3)-β-D-*Galf*-(*1*	4)-β-D-*Glc*p-(1
C-1	108.13	102.22
C-2	79.99	73.25
C-3	84.36	74.30
C-4	82.35	76.91
C-5	70.50	75.16
C-6	63.14	60.37

[a] The spectrum was recorded at 50° with external tetramethylsilane as reference. Compilations[1,2] of reference data were used in making the assignments.

ide extracts is common for strains of *S. marcescens*. In some such cases, there is circumstantial evidence that (*a*) the neutral polymer is an integral part of the lipopolysaccharide, and (*b*) the acidic polymer is microcapsular, but is the antigen which defines the O serogroup[5,7]. It has not yet been determined whether this applies to the O5 reference strain studied here.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of the lipopolysaccharide. — *S. marcescens* strain C.D.C. 867-57 (O5:H1) was grown in Nutrient Broth No. 2 (Oxoid, 20 L) for 16 h at 30° with aeration at 20 L.min $^{-1}$, giving 92 g of wet cells. Cell walls (2.33 g) were obtained by mechanical disintegration of the cells[4,7] and, after the extraction of lipids, were used for the isolation[4,7] of lipopolysaccharide (315 mg). After mild, acid hydrolysis of the lipopolysaccharide (1% acetic acid, 2.25 h, 100°), the water-soluble polymeric products were obtained by chromatography on Sephadex G-50 (yield, 39% of the parent lipopolysaccharide) and fractionated by chromatography on DEAE-Sepharose CL-6B (ref. 7).

General methods. — Chromatographic, electrophoretic, and other physicochemical methods used were essentially those described previously[4,5]. N.m.r. spectra were obtained with a Bruker WH-400 spectrometer for samples dissolved in D_2O. ^{1}H-N.m.r. spectra were recorded at 80° with TSP-d_4 as an external reference; ^{13}C-n.m.r. spectra with complete proton decoupling were recorded at 50° with tetramethylsilane as an external reference. G.l.c. separations were done with a Mega chromatograph (Carlo Erba) fitted with fused-silica capillary columns of BP1 (for alditol acetates) or BP10 (for methylated alditol acetates), supplied by Scientific Glass Engineering (U.K.) Ltd. G.l.c.–m.s. was carried out with a Finnigan 1020B instrument.

Other methods. — The configurations of hexoses were established by g.l.c. of the diastereoisomeric oct-2-yl glycoside acetates[8], using the BP1 column. Methylation analysis and periodate oxidation were carried out as in a previous study[4].

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