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Note

Structure of the polysaccharide S-84 elaborated by *Pseudomonas* ATCC 31562: depolymerisation using fuming hydrochloric acid

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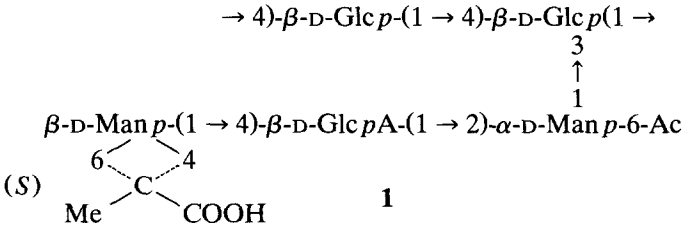
The extracellular polysaccharide (S-84) from *Pseudomonas* ATCC 31562 (ref. 1) has valuable rheological properties and can be used as a thickening, suspending, and stabilising agent in aqueous systems. A hydrolysate of S-84 contained glucose, mannose, and glucuronic acid, and the polysaccharide also contained O-acetyl groups and pyruvic acid residues. These component sugars are present in xanthan, the extracellular polysaccharide from *Xanthomonas campestris*[2,3], which has properties similar to those of S-84. Xanthan has the structure **1** and it seemed possible that S-84 has a related structure.



1

A hydrolysate of S-84 contained glucose and mannose in the ratio 9:11, and that of a carboxyl-reduced sample showed the same sugars in the ratio 61:39. Each sugar was demonstrated to be D by the method of Gerwig et al.[4]. This analysis also demonstrated that the acid was glucuronic acid. The ^{1}H-NMR spectrum of native S-84 was poor, but the signals at δ 2.14 (~ 3 H) and 1.47 (~ 3 H) indicated the presence of *O*-acetyl and pyruvic acid groups. Methylation analysis together with NMR data (see below) of S-84 (Table I, A) revealed 2-linked Man, 4-linked Glc, 3,4-linked Glc, 4,6-linked Man, and some terminal Man residues. Methylation

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TABLE I

Methylation analyses of S-84 and of different modified products

Sugar	Detector response					
	A[a]	B	C	D	E	F
2,3,4,6-Man[b]	4	7	35			5
2*,3,4,6-Man[c]					32	
3,4,6-Man	19	17	15	23		7
2,3,6-Glc	27	22	33	33	35	26
2,6-Glc	29	22	13	44	33	18
2,3-Man	21	14	4			25
3,4-Man						18
2,3-Glc		19				

[a] Key: A, native polysaccharide; B, carboxyl-reduced polysaccharide; C, partially hydrolysed, depyruvated polysaccharide; D, uronic acid-degraded polysaccharide; E, uronic acid-degraded and trideuteriomethylated polysaccharide; F, methyl trifluoromethanesulfonate-methylated polysaccharide. [b] 2,3,4,6-Man = 2,3,4,6-tetra-*O*-methyl-D-mannose, etc. [c] * indicates a trideuteriomethyl group.

analysis of carboxyl-reduced S-84 (Table I, B) revealed that a 4-linked GlcA residue was also present. Methylation analysis of the partially hydrolysed polysaccharide (Table I, C) further showed that the pyruvic acid was 4,6-linked to the terminal D-Man residue. A difference between S-84 and xanthan is that, in the former, almost all of the terminal Man residues are pyruvated but only 50% in the latter. However, different conditions of culture can result in different proportions of pyruvic acid in the polysaccharide.

A uronic acid degradation, i.e., treatment of the methylated polysaccharide with base followed by trideuteriomethylation, demonstrated the presence of a terminal trisaccharide element, D-Man*p*-4,6-Pyr-(1 → 4)-D-Glc*p*A-(1 → 2)-D-Man (Table I, D and E), identical to that in the side chain in xanthan.

A comparison of the ^{13}C-NMR spectra of S-84 and **1** should establish possible identity, except for the lower percentage of pyruvic acid groups in the xanthan. However, solutions of a pyruvic acid-rich *X. campestris* polysaccharide and S-84 were very viscous and, even after extensive high-energy ultrasonication, only limited improvement of spectra could be obtained.

Treatment of the *O*-deacetylated polysaccharides with fuming hydrochloric acid at room temperature[5] for 0.5 h, followed by workup and gel filtration, gave modified polysaccharides, the solutions of which had reduced viscosities, and acceptable ^{13}C-NMR spectra were obtained (Fig. 1). Furthermore, the pyruvic acid acetals were not cleaved substantially, as indicated by the strong ^{13}C signal at 25.6 ppm (the acetals have the *S*-configuration[6]). The ^{13}C-NMR spectra contained only weak signals in the region 92–99 ppm, indicating that few glycosidic linkages had been cleaved. The spectra of the two modified polysaccharides were similar and, except for the signal at δ 25.6, showed only small differences in the intensities of some signals. Thus, it can be concluded that the backbone of S-84 is identical to that of xanthan (**1**).

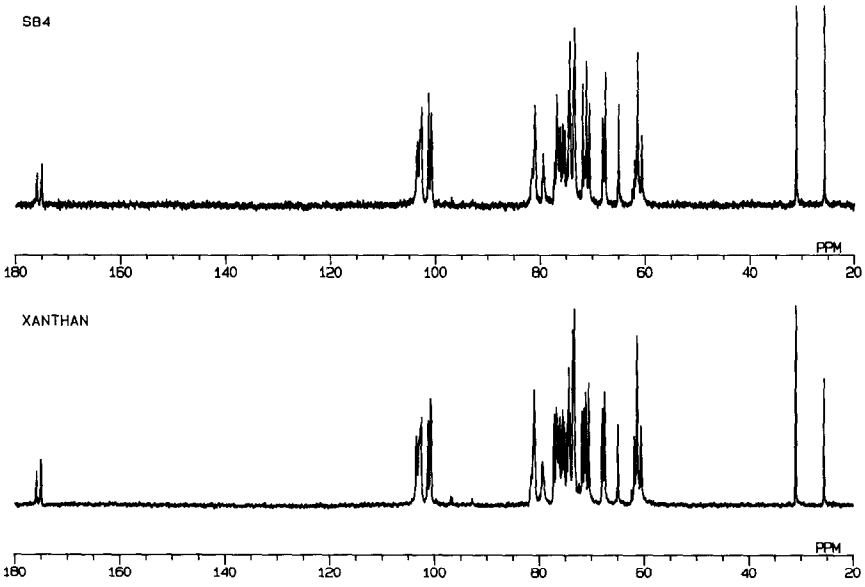

Fig. 1. ^{13}C-NMR spectra of *O*-deacetylated and depolymerised S-84 and xanthan polysaccharides.

Methylation analysis of the native S-84 polysaccharide, using methyl trifluoromethanesulfonate and a sterically hindered base[7], demonstrated (Table I, F) the *O*-acetyl groups to be located at position 6 of the 2-linked Man residues, as also found in xanthan.

The use of fuming hydrochloric acid for limited non-specific depolymerisation of the S-84 polysaccharide and xanthan may be useful for other polysaccharides, provided that the rates of hydrolysis of different glycosidic linkages do not differ significantly.

EXPERIMENTAL

General methods.—Sugar and methylation analyses were performed as described[8]. NMR spectra of solutions in D_2O were recorded at 70° (^{13}C) or 85° (^{1}H) with a JEOL GSX 270 spectrometer. Chemical shifts are referenced to internal trimethylsilylpropanoate-d_4 (^{1}H) or internal acetone (^{13}C, δ 31.00). The absolute configuration of sugars was determined by the method of Gerwig et al.[4]. GLC was performed using an HP-5 fused-silica column. Peaks were identified using GLC–MS and the assignments were unambiguous. *O*-Acetyl groups were located by the method of Prehm[7]. The uronic degradation was performed as described[9].

Depolymerisation of S-84.—The polysaccharide (100 mg) was *O*-deacetylated using 0.1 M NaOH for 16 h at room temperature. The solution was neutralised, then freeze-dried, and fuming HCl (5 mL) was added to the dry residue. The mixture was stirred at room temperature for 30 min, then cooled with ice,

neutralised with 2 M NaOH, applied to a column (3 × 80 cm) of Bio-Gel P-4, and eluted with water, and the material in the void volume was recovered.

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