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Note

Comparative studies of polysaccharides elaborated by *Rhizobium*, *Alcaligenes*, and *Agrobacterium*

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The extracellular heteropolysaccharides elaborated by *Rhizobium meliloti*[1,2] and *Alcaligenes faecalis* var. *myxogenes*[3–5] are both composed of D-glucose, D-galactose, and pyruvic acid residues in the molar proportions 7:1:1. They are partially esterified, the former with acetic acid, the latter with succinic acid. The *Rhizobium meliloti* polysaccharide (*R.m.*-PS) is composed of octasaccharide repeating-units (**1**), with *O*-acetyl groups linked to position 6 of some D-glucopyranosyl residues[2]. Structural studies of the *Alcaligenes faecalis* var. *myxogenes* polysaccharide (*Al.f.*-PS)[6,7] revealed that it contains the same types of linkages as the *R.m.*-PS. Succinoglycans elaborated by nine strains of different species of *Agrobacterium* also seem to be closely related to the *Al.f.*-PS[8] and are hydrolysed into octasaccharides by the same, highly specific enzyme (succinoglycan depolymerase) from a *Flavobacterium* sp.[4,9]. These octasaccharides are hydrolysed into tetrasaccharides by another, specific, intracellular β-D-glucanase from the same *Flavobacterium* sp.[5]. In agreement with these results, methylation analyses of the extracellular polysaccharides from *R.m.* and *Agrobacterium tumefaciens* gave similar results[10]. It therefore seemed possible that all of these polysaccharides had identical structures, disregarding the different modes of acylation. We now report comparative studies of the polysaccharides elaborated by *R.m.*, *Al.f.*, and *Agrobacterium radiobacter* (*Ag.r.*).

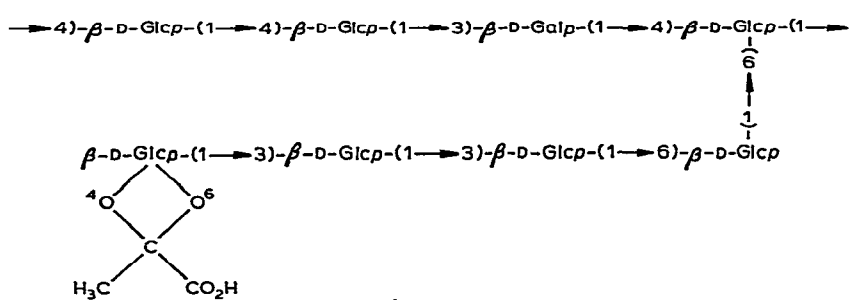

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TABLE I

METHYLATION ANALYSIS OF THE *Al.f.*, *Ag.r.* AND *R.m.* POLYSACCHARIDES

A *(OV-225)*					B *(SP-1000)*					C *(OS-138)*				
Methylated sugar[a]	T[b]	Al.f.	Ag.r.	R.m.	*Methylated sugar*[a]	T[b]	Al.f.	Ag.r.	R.m.	*Methylated sugar*[a]	T[b]	Al.f.	Ag.r.	R.m.
2,4,6-Glc	1.82	26	30	29	2,4,6-Glc	1.72	23	22	26	2,4,6-Glc	1.63	25	28	27
2,4,6-Gal	2.03	11	12	11	2,3,6-Glc	1.94	33	34	32	2,3,6-Glc	1.75	24	25	21
2,3,4-Glc	2.22	41	33	33	2,4,6-Gal	1.94				2,3,4-Glc	1.88	26	23	22
2,3,6-Glc	2.32				2,3,4-Glc	2.00	17	17	18	2,4,6-Gal	1.88			
2,3-Glc	4.5	21	23	26	2,3-Glc	3.5	28	27	23	2,3-Glc	3.0	29	23	29

[a]2,4,6-Glc = 2,4,6-tri-*O*-methyl-D-glucose, *etc.* [b]Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

In the ^{1}H-n.m.r. spectra of the polysaccharides, a signal at δ 1.48 (s) was assigned to the methyl protons of the pyruvic acid residues. A multiplet at δ 2.59 was present in the spectra of the *Al.f.*-PS and the *Ag.r.*-PS and confirmed the presence of succinyl groups in these PS. From the intensities of the signals, the number of succinyl groups per pyruvic acid residue was estimated to be 1.0 (*Al.f.*) and 0.45 (*Ag.r.*), respectively, as already shown[8]. Some batches of the *R.m.*-PS contained only low percentages of *O*-acyl groups, according to their ^{1}H-n.m.r. spectra. In one batch, signals both for *O*-acetyl groups (δ 2.15, s) and *O*-succinyl groups (δ 2.59, m) were observed. In this batch, the number of acyl groups per pyruvic acid residue was 1.05, and the ratio between *O*-acetyl groups and *O*-succinyl groups was 1 : 1.4. The presence of *O*-succinyl groups in the *R.m.*-PS was overlooked in previous investigations[1,2].

The three polysaccharides were subjected to methylation analysis, and the methylated sugars analysed, as the alditol acetates, by g.l.c.–m.s.[11]. G.l.c. was performed on glass-capillary columns of SP-1000 and on packed columns of OV-225 and OS-138. On each column, a pair of components was not, or only partially, separated. This was a different pair on each column, but the combined results (Table I) demonstrate that, within the experimental limits, each polysaccharide gives the analysis expected for a polymer having the structure **1**.

In order to get well-resolved ^{13}C-n.m.r. spectra, the polysaccharides were deacylated and carboxyl-reduced[12] and the acetal groups selectively cleaved by acid hydrolysis under mild conditions. The ^{13}C-n.m.r. spectra of the modified polysaccharides were superposable.

The three polysaccharides were treated with succinoglycan depolymerase, followed by the intracellular, specific β-D-glucanase from *Flavobacterium* M 64. Paper chromatograms of the products were indistinguishable and showed two spots, R_{Glc} 0.08 and 0.24, each of which (unpublished results) is due to a tetrasaccharide.

The foregoing results strongly indicate that the three polysaccharides studied are identical, except for the mode of acylation. *Rhizobium* and *Agrobacterium* belong to the same family, *Rhizobiaceae*. However, the family to which *Alcaligenes* belongs seems to be uncertain[13]. The strain producing the polysaccharide now investigated is a variety of *A. faecalis*. It is generally recognized that differentiation between non-pathogenic *Agrobacterium* and *Alcaligenes* species is difficult. At any rate, the finding that the same, rather complicated polysaccharide is elaborated by bacteria from these three groups is remarkable.

EXPERIMENTAL

Exocellular, acidic polysaccharides from *Rhizobium meliloti* U27, *Alcaligenes faecalis* var. *myxogenes* 10C3, and *Agrobacterium radiobacter* IFO 12665 were used.

Methylations of the polysaccharides were performed by the Hakomori[14] procedure with sodium methylsulfinylmethanide–methyl iodide in methyl sulfoxide. Transformations into alditol acetates were effected as described earlier[11]. G.l.c. separations were performed on packed columns (OV-225, 3% on Gas Chrom Q;

or OS-138, 3% on Gas Chrom Q) or on a glass-capillary column, wall-coated with SP-1000. For g.l.c.–m.s., a Varian 311 instrument was used. Polysaccharides were deacylated with 0.1M sodium hydroxide at 25° overnight. The carboxyl-reduction was performed by Conrad's method[12]. Depyruvylations were effected on carboxyl-reduced material by treating the polysaccharides with 0.05M HCl at 100° for 1 h.

For n.m.r. spectra, a JEOL FX-100 instrument was used. All samples were dissolved in D_2O. For ^{1}H-n.m.r., the chemical shifts are given in p.p.m. relative to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate; for ^{13}C-n.m.r., the chemical shifts are given in p.p.m. relative to external tetramethylsilane. Spectra were run at 85° and proton-decoupled.

Succinoglycan depolymerase and intracellular, specific β-D-glucanase were prepared from a culture of *Flavobacterium* M 64 capable of growing in a medium containing succinoglycan, as described previously[10,15]. The products obtained on hydrolysis of the three polysaccharides by successive treatment with succinoglycan depolymerase and the intracellular β-D-glucanase were investigated by paper chromatography, as previously described[8].

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