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Note

Neutral oligosaccharides from *Rhizobium trifolii* Bart A*

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The ability to fix nitrogen is possessed by *Rhizobium* bacteria which live symbiotically in nodules on the roots of legume plants. Since the extracellular polysaccharides of the bacteria participate in the specific host-symbiont selection[1], structural variations might be expected in the polysaccharides produced by different strains of the bacteria. Such conclusions cannot be drawn at present, since only sparse information is available, some obtained by methylation analysis of the polysaccharides[2–5] and some by identification of the products of partial hydrolysis with acid[6,7]. Several glucuronic acid-containing oligosaccharides[6] and pyruvic acid-containing saccharides[7] have been characterised. The identification of some neutral oligosaccharides is now reported.

EXPERIMENTAL

The *Rhizobium* strain was *Rh. trifolii* Bart A. The cultivation technique and the preparation of the polysaccharide were as reported earlier[8]. The partial hydrolysis of the polysaccharide with acid, the fractionation of the products, and the general methods were the same as formerly reported[7]. The components in the neutral fraction were separated by preparative paper chromatography using the descending method with 1-butanol–pyridine–water (5:3:2).

RESULTS AND DISCUSSION

The oligosaccharides **1–9** were obtained from the polysaccharide (PS) by partial hydrolysis with acid, and their paper-chromatographic (p.c.) mobilities and yields are given in Table I. The oligosaccharides were totally hydrolysed with acid to identify the hexose constituents. Each oligosaccharide was subjected in sequence to reduction, hydrolysis, and acetylation, and the products were examined by g.l.c.–m.s.;

*Fragmentation Analysis of Extracellular Acid Polysaccharides from Seven *Rhizobium* Strains, Part III. For Part II, see ref. 7.

TABLE I

YIELDS AND R_{GLC} VALUES FOR **1–9**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Mg/g of PS	5	9	1	5	4	2	3	5	2
R_{GLC}[a]	0.61	0.55	0.44	0.28	0.18	0.15	0.09	0.04	0.02

[a]1-Butanol–pyridine–water (5:3:2).

TABLE II

G.L.C. DATA FOR ACETATES OF METHYLATED ALDITOLS

Methylated sugar[a]	T[b]	*Mole* %								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
2,3,6-Glc	1.50					36	36	25	50	38
2,4,6-Glc	1.36				35			25		21
2,3,4,6-Glc	1.00		56	54		34	35		27	
2,3,4,6-Gal	1.09	55			34			26		22
1,2,4,5,6-Glc	0.54	45								
1,2,3,5,6-Glc	0.60		44		31	30		24		
1,2,3,4,5-Glc	0.61			46			29		23	19

[a]2,3,6-Glc = 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol, *etc.* [b]Retention time of the methylated alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an OV-225 column programmed at 2°/min from 160→200°.

D-glucose was always the reducing residue. Conventional methylation analysis (methylation, hydrolysis, reduction, acetylation, and g.l.c.–m.s.) of **1–9** revealed the amounts and linkages of the hexoses (Table II). Compounds **1**, **4**, **7**, and **9**, which contained D-galactose and D-glucose, were hydrolysed by β-D-galactosidase. Partially degraded **4**, **7**, and **9**, and **2**, **3**, **5**, **6**, and **8** contained only D-glucose, and were completely hydrolysed by β-D-glucosidase. These results revealed that all of the glycosidic linkages have the β-D configuration and that a D-galactosyl group is the terminal non-reducing unit of **1**, **4**, **7**, and **9**. Although it has not been proved that D-galactose in **7** and **9** is (1→3)-linked to D-glucose, it is most likely from the results for **1** and **4** that the postulated structures are correct. (1→3)-Linked D-glucosyl residues have not been found in the polysaccharide from *Rh. trifolii* Bart A or in the oligosaccharides derived therefrom. The following structures are proposed for **1–9**.

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

1 **2**

β-D-Glc*p*-(1→6)-D-Glc*p* β-D-Gal*p*-(1→3)-β-D-Glc*p*-(1→4)-D-Glc*p*

3 **4**

β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-D-Glc*p*

5

β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-D-Glc*p*

6

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

7

β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-D-Glc*p*

8

β-D-Gal*p*-(1→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-D-Glc*p*

9

The identification of **3**, **6**, **8**, and **9** confirms the results[9] obtained by methylation analysis of the polysaccharide, which revealed that the side chains probably are (1→6)-linked to (1→4)-linked glucose residues of the backbone. The same proposals have been made following methylation analysis of polysaccharides from other *Rhizobium* strains[2-5]. The identification of these components suggests that some of the side chains are released, together with a D-glucose residue of the backbone, during the hydrolysis. Some breakage of the backbone was also observed in the study of the acidic fraction, as it contained cellobiouronic acid[7].

Compounds **1** and **4** have the same structures as the oligosaccharides previously identified as acidic fragments having pyruvic acid linked as an acetal to positions 4 and 6 and 3 and 4 of the D-galactosyl group.

From the results of enzymic degradation of the PS, it has been suggested[10] that the side chains might consist of more than three, consecutive, (1→4)-linked D-glucosyl residues. This possibility is unlikely, as no trace of cellotetraose or oligosaccharides containing a cellotetraose residue could be detected in the partial, acid hydrolysate.

As a result of these studies, it is proposed that the sugar residues of the side chains form a tetrasaccharide represented by **7**, which is in agreement with the results obtained by sequential degradation of the PS produced by another *Rh. trifolii* strain[3].

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