

Studies on the Polysaccharides of Lichens. III.¹ The Structure of Alkali-Soluble Polysaccharides in *Umbilicaria pustulata* (L.) Hoffm. and *Umbilicaria spodochoa* (Ach.) Hoffm.

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Kjølberg, O. and Kvernheim, A. L., 1989. Studies on the Polysaccharides of Lichens. III. The Structure of Alkali-Soluble Polysaccharides in *Umbilicaria pustulata* (L.) Hoffm. and *Umbilicaria spodochoa* (Ach.) Hoffm. – Acta Chem. Scand. 43: 280–285.

D-Galacto-D-mannans have been isolated from two species of lichen, *Umbilicaria pustulata* and *Umbilicaria spodochoa*, by extraction with alkali. The polysaccharide composition of the various fractions is dependent on the sequence and vigour of the extraction procedures. One fraction from each lichen was purified and investigated in detail. The glycans in these fractions were found to have closely related structures, comprising a main chain of (1→6)-linked α -D-mannopyranosyl units with side chains attached to approximately every other mannose unit at positions O-2, O-4 or both. The side chains were short stubs consisting mainly of 2- and 3-linked α -D-mannopyranosyl and 3-linked α -D-glucopyranosyl units. All galactose existed as β -D-galactofuranosyl units, as non-reducing end groups attached to the main chain at O-4, or as sequences of 5- and 3-linked β -D-galactofuranosyl units in the side chains. The major fractions of the polysaccharides were found to have a molecular weight of about 50 000 (M_w).

Water-soluble polysaccharides from the lichens *Umbilicaria pustulata*, (*UP*) and *Umbilicaria spodochoa* (*US*) have been described in a previous paper.¹ While many water-soluble polysaccharides from different lichens have been described, mainly of the homoglycan type, only a few papers report the structures of alkali-soluble polysaccharides from lichens. In one of these, Gorin and Iacomini² described the structure of an alkali-soluble polysaccharide from *Actinogyra muehlenbergii*, which is closely related to *UP* and *US*. According to Dahl and Krog³ the family *Actinogyra* Schol. is synonymous with *Umbilicaria* Hoffm. This polysaccharide, which was extracted from the lichen with 2% KOH, precipitated with Fehling's solution and purified by the freeze-thaw procedure, was characterised as a galactomannan containing Man, Gal and Glc in a 62:30:8 ratio. It consisted of a (1→6)-linked α -D-mannopyranosyl main chain having side chains of α -D-Man_p, α -D-Glc_p, β -D-Gal_p and β -D-Gal_i units, either monosubstituted in the O-2, O-4 positions, or disubstituted in O-2,4 positions in the main chain. Also alkali-soluble polysaccharides isolated from other lichens have been described^{2,4,6} as galactomannans with (1→6)-linked α -D-mannopyranosyl units in the main chain, and with varying types of side chains. We now describe alkali-soluble polysaccharides from the lichens *U. pustulata* and *U. spodochoa* which are mainly of the same type as those described for *A. muehlenbergii*.

Results and discussion

After the water-soluble polysaccharides had been extracted, the alkali-soluble polysaccharides were obtained from two extractions with 2% sodium hydroxide, to give the fractions *UP-6*, *UP-7* and *US-6*, *US-7*, respectively. Subsequently, the lichens were extracted twice with 10% sodium hydroxide to give the fractions *UP-9*, *UP-11* and *US-9*, *US-11*. Additional fractions *UP-8*, *UP-10*, *US-8* and *US-10* were obtained by isolation of precipitates formed during dialysis of the 10% NaOH extracts.

Analytical data of the fractions are recorded in Table 1. All of the analytical methods were modified to enable the use of Me₂SO as solvent. None of the fractions contained hexamine⁷ or phenols.⁸ As can be seen from Table 1, all the fractions from the same lichen had varying compositions and consequently could not be mixed. Only the largest fractions *UP-6* and *US-6*, were used for further investigations. These two fractions were partly soluble in water, and the soluble parts gave nearly colourless fractions, *UP-6S* and *US-6S*, of very low protein, sulfate and uronic acid content compared with the parent fractions *UP-6* and *US-6*. The specific rotations for *UP-6S* and *US-6S* were found to be $[\alpha]_D -81$ and -42° ($c = 0.16$, water), respectively. Size-exclusion chromatography (SEC) of *UP-6* and *US-6* gave very similar distribution curves, both with two main peaks having molecular weights as recorded in Table 2. The low molecular-weight peaks, *UP-62* and *US-62*, were found to contain more than 75% of the parent

Table 1. Monosaccharide composition, protein, sulfate and uronic acid content, and total carbohydrate content using the phenol/sulfuric acid assay (method 1) and GLC of alditol acetates using internal standard (method 2), and specific rotation of fractions.

Sample	Monosaccharide composition ^a						Protein	Sulfate	Uronic acid	Carbohydrate		Specific rotation ^b
	Rha	Fuc	Xyl	Man	Gal	Glc				Method 1	Method 2	
UP-6			5.4	39.8	20.0	30.3	22.7	11.1	3.6	42.7	69.5	–
UP-7	4.2	2.9	1.0	31.5	23.4	35.0	22.1	14.6	1.5	49.1	66.5	–
UP-8				7.6	5.0	84.2	4.5	4.7	1.8	57.4	51.1	–30.1
UP-9	2.8	2.8	2.0	20.5	25.5	45.0	1.7	2.9	1.1	62.5	67.9	–27.4
UP-10				8.5	5.0	81.1	7.1	7.2	3.9	55.1	62.2	–22.5
UP-11	1.5			10.5	8.5	78.0	4.0	2.4	1.0	58.2	61.6	–
US-6	5.6	3.6		32.4	19.1	32.3	22.8	15.0	3.7	46.3	45.1	–
US-7	2.1	2.6		49.0	19.1	19.0	21.0	14.5	5.8	43.7	59.6	–
US-8				3.8	2.9	89.4	3.7	5.7	2.7	60.2	61.7	–32.0
US-9	3.3		1.4	14.9	13.3	64.3	4.9	5.1	2.8	58.4	88.0	–45.1
US-10				2.4	2.0	95.2	6.0	5.8	0.2	40.0	47.8	–35.4
US-11				8.1	6.3	80.3	6.1	6.1	3.9	56.6	63.9	–24.3

^aMonosaccharide composition is expressed as a percentage of the total carbohydrate. Protein, sulfate, uronic acid and carbohydrate are given as percentages of the total amount in the fraction. ^bSome values are omitted because of high absorbance. For the values listed: $c = 0.16$, 10% NaOH.

fractions. The high molecular-weight fractions, UP-61 and US-61, gave polymodal distributions indicative of heterogeneous material.

Ion-exchange chromatography of UP-62 and US-62 on DEAE-cellulose, using 0.1 M phosphate buffer as the eluant gave colourless fractions, UP-621 and US-621 which had the same low content of protein, sulfate and uronic acid as UP-6S and US-6S. Subsequent elution with 1.0 M phosphate buffer, 4.5 M sodium chloride and 0.5 M sodium hydroxide, respectively, gave polysaccharide fractions which were not investigated further except for monosaccharide composition as shown in Table 3. Attempts at further purification of the fractions UP-621 and US-621 by precipitation with Fehling's solution and by freezing and

thawing did not alter the monosaccharide composition (Table 3).

Methylation analysis was carried out on UP-6S, UP-621, US-6S and US-621, and the fragments were analysed as partially methylated alditol acetates and also as anhydroalditols formed by reductive cleavage of the methylated polysaccharides.⁹ The results, (Table 4), show that the two UP-fractions and the two US-fractions were similar (the data for UP-61 and US-61 are also shown for comparison). Discrepancies may be due to some impurities present in UP-6S and US-6S. The large proportion of tetra-*O*-methylated sugars indicates a highly branched structure.

In order to elucidate further the complex structures, mild acid hydrolysis of UP-6S and US-6S with 0.05 M trifluoroacetic acid (conditions likely to hydrolyse galactofuranosidic linkages selectively²) was carried out. Methylation data for the fractions retained in dialysis tubing, UP-6SI and US-6SI, are found in Table 5. These results show a branched mannan (or glucomannan) similar to the unhydrolysed fractions UP-6S and US-6S, but without galactose. The increase in 2,3,4-tri-*O*-methylmannitol and the corresponding decrease in 2,3-di-*O*-methylmannitol indicate that galactofuranose units represent a branch point at O-4 in mannose. This was confirmed by selective, mild hydrolysis of the methylated polysaccharides, which were subsequently ethylated.¹⁰ The ethyl groups were found by GLC-MS analysis to be located at O-4 in almost every 2,4-dibranched mannose residue and also to a smaller extent at the O-3 and O-6 positions of other mannose residues.

The presence of 2,5,6- and 2,3,6-tri-*O*-methyl galactitols (Table 4) indicate that some of the side chains are extended by linkages to the O-3 and O-5 positions of galactofuranosyl units. Comparison with the methylated products present in the dialysates, (UP-6SO and US-6SO, after selective,

Table 2. Size-exclusion chromatography measurements on UP-6 and US-6 on Sepharose 6B CL and Sephacryl S-300 superfine using pullulan and dextran as standards. UP-6 peak 1 is denoted UP-61, UP-6 peak 2 is denoted UP-62.

Sample	Sepharose 6B CL		Sephacryl S-300 s	
	10 ³ M _w	M _w /M _n	10 ³ M _w	M _w /M _n
Pullulan standard				
UP-61	665	1.13	143	1.24
UP-62	43.2	2.58	33.7	2.12
US-61	695	1.14	184	1.11
US-62	37.2	1.64	27.8	1.43
Dextran standard				
UP-61	1650	1.19	153	1.27
UP-62	69.9	3.81	33.2	2.34
US-61	1740	1.21	198	1.12
US-62	55.8	2.09	26.6	1.50

Table 3. Monosaccharide composition and protein, sulfate and uronic acid content of purified UP-6 and US-6 fractions.

Sample	Monosaccharide composition ^a							Protein ^b	Sulfate ^b	Uronic acid ^b
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc			
UP-6S				1.9	52.0	38.5	7.5	7.3	2.9	1.4
UP-61	3.6	1.4	2.6	4.0	13.9	21.9	52.7	14.4	4.7	0.8
UP-621			1.4		61.0	28.0	9.6	3.3	4.0	0.1
UP-622			3.5		54.6	31.3	10.6			
UP-623				4.8	51.9	26.5	16.7			
UP-624				15.3	21.8	22.7	40.3			
US-6S				2.8	46.5	40.6	10.1	9.2	6.1	1.4
US-61	2.0		3.4	3.7	16.8	26.9	47.2	20.8	5.0	0.5
US-621			1.7	0.8	65.7	25.6	6.2	2.6	3.0	0.1
US-622			2.0	1.4	51.6	36.2	8.8			
US-623			5.1	9.4	40.3	26.8	18.4			
US-624			5.7	26.6	30.4	13.9	20.7			

^aMonosaccharide composition is expressed as a percentage of the total amount of carbohydrate. ^bProtein, sulfate and uronic acid were not monitored in the 622, 623 and 624 fractions.

Table 4. Methylation data on fractions. Pyranose/furanose identification of residues from methyl positions, reductive cleavage and partial hydrolyses. Amounts in mol %. The ratio of non-reducing end-groups to branch-points are shown on the bottom line.

Methylating species	UP-61	UP-621	UP-6S	US-61	US-621	US-6S
2,3,4-Xyl _p	7			5		
2,3,5-Ara				1		
2,3-Xyl	5			2		
2,4-Fuc _p	2					
3,4-Rha _p				3		
4-Rha _p	5			4		1
2,3,4,6-Man _p	10	30	22	7	36	28
2,3,4,6-Glc _p	14		3	8		3
2,3,5,6-Gal _f			5		6	5
2,3,4-Man _p		13	10		8	7
2,3,4-Glc _p	2		2	2	2	2
2,3,5-Gal _f	1	2	1	1		1
2,3,6-Man _p			2			1
2,3,6-Glc _p		5		1		
2,3,6-Gal _f	3	7	5	2	2	2
2,4,6-Man _p ^a		6	6		20	9
2,4,6-Glc _p	18	2	6	32		5
2,5,6-Gal _f	18	4	4	8		4
3,4,6-Man _p ^a	6	4		16		4
2,3-Man _p		9	7		6	5
2,3-Glc _p		1				
2,3-Gal			2			1
2,4-Man _p			2			1
2,4-Glc _p	6		2	4		
2,5-Gal _f	3			2		
3,4-Man _p		11	12	1	13	11
4,6-Man _p		2	2	1		
3-Man _p ^a		6	2		6	7
4-Man _p ^a			6			3
End/branch	2.1	0.9	0.7	1.8	1.4	0.9

^aQuantitative measurements of 2,4,6-Man_p/3,4,6-Man_p and 3-Man_p/4-Man_p based on MS.

mild hydrolysis of UP-6S and US-6S) confirms that the side chains contain sequences of (1→3)- and (1→5)-linked galactofuranosyl units. The presence of 2,3,4- and 2,4,6-tri-O-methylmannitol in the dialysate indicates that mannose

units are linked in the O-3 and O-6 positions in the side chains. They are terminated mainly by mannopyranosyl units, and to a small extent by glucopyranosyl units.

The ¹³C NMR spectra of the anomeric region of the

Table 5. Partially hydrolysed and methylated samples. UP-6SI and US-6SI were retained in the dialysis tubing after hydrolysis. UP-6SO and US-6SO were dialysed, M_w cut-off 3500.

Methylating species	UP-6SI	UP-6SO	US-6SI	US-6SO
2,3,4,6-Man _p	21	38	22	48
2,3,4,6-Glc _p	2	14	1	16
2,3,4,6-Gal _p		20		11
2,3,5,6-Gal _r		5		3
2,3,4-Man _p	31	6	24	7
2,3,6-Gal _r		3		4
2,4,6-Man _p	3	8	9	7
2,4,6-Glc _p	5		3	
2,5,6-Gal _r		5		5
3,4,6-Man _p	9		5	
2,3-Man _p	2		2	
2,4-Man _p	2		3	
2,4-Glc _p	2		1	
3,4-Man _p	18		22	
3 or 4-Man _p	4		8	
End/branch ^a	0.7		0.5	

^aThe ratio of non-reducing end groups to branchpoints.

fractions US-621, UP-6S and US-6S were very similar except for a downfield displacement of the low-field C-1 signal in the sparingly soluble US-621 sample, Fig. 1. Comparison of these spectra with that of *A. muhlenbergii*² shows that the polysaccharides in this investigation are more complex. The signals at δ 109.2 and in the proximity of δ 108.3 may all be ascribed to C-1 of β -D-galactofuranosyl units. The δ 109.2 signal ($J_{C-1,H-1}$ ca. 178 Hz) could be a single side-chain unit (1 \rightarrow 4)-linked to an α -D-mannopyranosyl residue,² or (1 \rightarrow 6)-linked to an α -D-mannopyranosyl unit.¹¹ The δ 108.3 signal probably originates from a

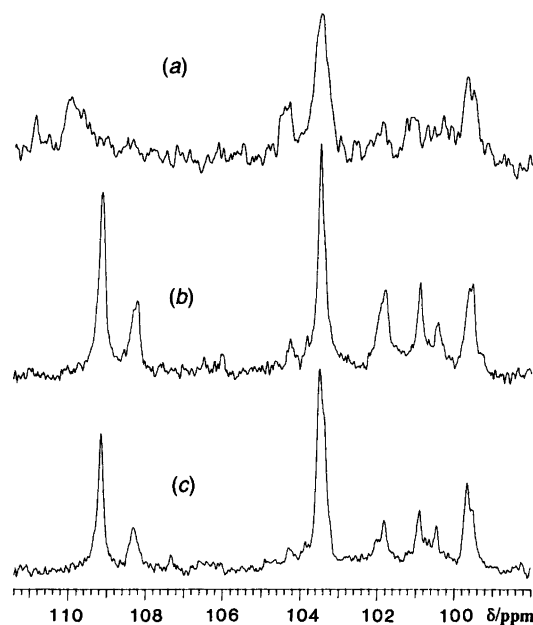


Fig. 1. ¹³C NMR spectra of the anomeric region of (a) UP-621, (b) UP-6S and (c) US-6S.

C-1 in a (1 \rightarrow 5)-linked β -D-galactofuranosyl chain.¹¹ The complexity of this region, however, indicates that these chains are short, and may be terminated by residues other than β -D-galactofuranosyl units. The δ 103.5 signal ($J_{C-1,H-1}$ ca. 172 Hz) can be assigned to α -D-mannopyranosyl non-reducing end-groups (1 \rightarrow 2)- or (1 \rightarrow 3)-linked to α -D-mannopyranosyl residues.^{2,12} The fine structure of this signal indicates that a C-1 signal from 3-*O*-substituted α -D-mannopyranosyl groups may also be present.¹² The δ 101.8 signal is very complex. Signals found in this region may be due to α -D-mannopyranosyl non-reducing end-groups, (1 \rightarrow 4)-linked to α -D-mannopyranosyl units,¹³ or 3,6-disubstituted α -D-mannopyranosyl units.¹⁴ The δ 100.9 signal is attributed to the unsubstituted (1 \rightarrow 6)-linked main chain of α -D-mannopyranosyl residues.² The signals in the δ 99.5–99.7 region are assigned to 2,6-di-*O*-substituted α -D-mannopyranosyl units² and 4,6-di-*O*-substituted α -D-mannopyranosyl residues.¹⁵ Residues of 2-mono-*O*-substituted α -D-mannopyranosyl groups linked to (1 \rightarrow 6)-linked α -D-mannopyranosyl chains may also be found in this region.¹³

Even though it is not possible to provide a complete interpretation of the ¹³C NMR spectra, there is good correlation with the methylation analysis. There is, however, an apparent discrepancy in the amounts of β -D-galactofuranosyl units present when the two methods are compared. The reason for this may be that some tetra-*O*-methylated galactofuranose is lost during work-up.

It may be concluded that the polysaccharides isolated from the two *Umbilicaria* species have similar structures. Only small differences in the branching patterns have been detected. The UP-621 fraction has a higher proportion of unsubstituted (1 \rightarrow 6)-linked α -D-mannopyranosyl groups, while US-621 has a higher proportion of (1 \rightarrow 3) substituted α -D-mannopyranosyl groups (in the side chains). The proportion of β -D-galactofuranosyl units substituted in the O-5 positions is higher in UP-621 than in US-621. The two polysaccharides are also similar to the alkali-soluble galactomannans isolated from other lichen species. They differ, however, in the branching patterns and in their solubility parameters. The specific rotations of UP-6S and US-6S (see previously) are very low compared with values found by others.²

Experimental

General methods. GLC was performed with a Varian 3400 instrument and with the following columns: 1, fused silica capillary SP-2340 (30 m, 0.25 mm id); 2, fused silica capillary SP-2380 (30 m, 0.35 mm id). Program 1: 190 °C, 4 min; 190–240 °C, 4 °C min⁻¹; 240 °C, 10 min; Program 2: 80 °C, 0.5 min; 80–160 °C, 20 °C min⁻¹; 160 °C, 1 min; 160–240 °C, 2 °C min⁻¹; 240 °C, 5 min. Program 3: 80 °C, 0.75 min; 80–120 °C, 20 °C min⁻¹; 120 °C, 0.5 min; 120–240 °C, 2 °C min⁻¹; 240 °C, 5 min. Injector and detector (FID) were held at 250 °C. Splitless injection for 0.75 min was used.

GLC/MS was carried out using the same temperature programming and column as described for GLC. MS system 1: Carlo Erba 4200 gas chromatograph coupled with a Micromass 7070F MS and a VG 2200 data system. MS system 2: HP 18947A GC/MS Capillary interfacer, and a HP 5992A GC/MS system.

Absorbance was measured with: spectrophotometer 1, Hitachi UV-VIS 101 and 1 cm glass cuvettes. Spectrophotometer 2, Perkin Elmer 295 with 9 mm id glass tubes.

Specific rotations were measured on a Perkin Elmer 241 polarimeter at 589 nm using a 1 dm, 5 ml cell ($c \approx 0.16$, 2% NaOH, 10% NaOH or H₂O).

¹³C NMR spectra were recorded on a Varian XL-300 (7.2 T) instrument. Samples were dissolved in D₂O using (CD₃)₂SO as an internal reference at 70 °C. The number of transients were 60–400 000 depending on sample amount (10–80 mg).

Isolation of polysaccharides. The residues (100 g) after the first hot-water extraction,¹ were extracted three times more with boiling water (1000 ml) for 3 h. The residues were then suspended in 2% NaOH (1000 ml) for 3 h in a sonification bath (temperature 20–40 °C) under an N₂ atmosphere. The suspensions were filtrated through muslin and centrifuged, and precipitation was achieved by the addition of ethanol (60% v/v). After being cooled to 5 °C the mixtures were centrifuged. The precipitates were dissolved in 2% NaOH (600 ml) at room temperature and under an N₂ atmosphere and reprecipitated with ethanol as above. After centrifugation the precipitates were dissolved in 2% NaOH (400 ml) and the solutions were adjusted to pH 5 with acetic acid, and dialysed against tap water for 5 days before being freeze-dried, to give UP-6 (3.85 g) and US-6 (4.40 g). A further extraction with 2% NaOH gave UP-7 (2.17 g) and US-7 (2.19 g). The procedure was repeated twice with 10% NaOH. During dialysis precipitation occurred. After being centrifuged the solutions were precipitated with ethanol, dissolved in water and freeze-dried to give UP-9 (0.83 g), UP-11 (0.87 g), US-9 (1.71 g) and US-11 (0.76 g). The material precipitated during dialysis was washed twice with ethanol and twice with diethyl ether before being dried in a vacuum desiccator. This gave UP-8 (5.54 g), UP-10 (1.14 g), US-8 (4.64 g) and US-10 (0.54 g).

O-Sulfate was determined by the method of Chandrasekaran *et al.*⁷ Samples, dissolved in Me₂SO (0.9 ml, 1.6 mg ml⁻¹), were mixed with an equal volume of HCl (0.5 M) and hydrolysed for 4 h at 100 °C.⁷ Of the hydrolysed material, 0.5 ml was withdrawn, and diluted to 1.5 ml with water, for analysis. The standard curve for sulfate determination was made with potassium sulphate (2.01 μmol ml⁻¹). Of this 0.0–1.0 μl were withdrawn, Me₂SO (0.25 ml) was added and the solutions were diluted to 1.5 ml prior to being analysed. The absorbance was measured with spectrophotometer 1 at 360 nm.

Protein was analysed according to Lowry *et al.*¹⁶ Samples dissolved in Me₂SO (0.2 ml, 1.6 mg ml⁻¹) were diluted to 1.0 ml with water. Standard curves were made with bovine serum albumin (106 μg ml⁻¹) in water. From this, 0.0–0.8 ml in steps of 0.2 ml were withdrawn, Me₂SO (0.2 ml) was added, and the samples were diluted to 1.0 ml prior to being analysed. The absorbance was assayed using spectrophotometer 1 at 750 nm.

Uronic acid was determined by the method of Blumenkrantz *et al.*¹⁷ Samples dissolved in Me₂SO (50 μl, 1.6 mg ml⁻¹) were diluted to 200 μl with Me₂SO. A standard curve was made with D-galacturonic acid monohydrate (0.522 μmol ml⁻¹) in Me₂SO. From this solution, 0–200 μl in steps of 40 μl were withdrawn and the samples were diluted to 200 μl with Me₂SO prior to being analysed. The absorbance was measured with spectrophotometer 2 at 520 nm.

Monosaccharides were assayed by the method of Blakeney *et al.*¹⁸ The samples, dissolved in Me₂SO, (0.5 ml, 1.6 mg ml⁻¹), were mixed with *myo*-inositol (25 μl, 9.37 mg ml⁻¹), before being hydrolysed. Monosaccharides were analysed as alditol acetates using GLC with column 1, program 1 (basis for total carbohydrate method 2 in Table 1).

Total carbohydrate was determined by the method of Dubois *et al.*¹⁹ To the samples dissolved in Me₂SO (40 μl, 1.6 mg ml⁻¹), was added 60 μl Me₂SO. Phenol (100 μl, 4% in water) and conc. H₂SO₄ (2 ml) were added and the sample was shaken after each addition. The tubes were heated on a boiling water bath for 5 min. and cooled to room temperature before the absorbances were measured at 487 nm using spectrophotometer 2 (basis for total carbohydrate method 1 in Table 1).

Purification of polysaccharides. UP-6 and US-6 (3 g) were stirred with 100 ml boiling water for 2 h. The centrifuged solutions were precipitated with ethanol (60% v/v final concentration) and the precipitates were redissolved in water. Further precipitation was obtained with acetone. After the precipitates had been dissolved in water, dialysed and freeze-dried, the fractions UP-6S (673 mg) and US-6S (680 mg) were obtained. The freeze-dried products were only partly soluble in water and only the soluble fractions were used in the subsequent analyses.

SEC analyses were carried out on a Sepharose 6B CL (60 cm, 2.5 cm id) column, void volume $V_0 = 119$ ml and total volume $V_T = 320$ ml, and a Sephacryl S-300 superfine (84 cm, 2.6 cm id) column with $V_0 = 170$ ml and $V_T = 413$ ml. Dextran (Pharmacia) and pullulan (Showa Denko) were used as standards. Phosphate buffer (0.5 M), containing 0.02% sodium azide, was used as the eluant. A Waters 6000A solvent delivery system with a 5 ml sample injection loop was used. A Waters R-400 refractometer was used as the detector, and the fractions were collected with a Pharmacia Frac-100 fraction collector in the peak-detection

mode. Samples (50 mg) were dissolved in NaOH (2%), dialysed against running tap water and adjusted to 0.05 M in phosphate before injection.

Ion-exchange chromatography. A column of DEAE-cellulose (Fluka), packed after being allowed to swell in 0.5 M NaOH at room temperature for 20 h and then washed with water until neutral, was used. After the column had been equilibrated with 0.01 M phosphate buffer, samples (0.2–0.3 g), dissolved in NaOH (2%), dialysed and adjusted to 0.01 M in phosphate, were applied. Elution was carried out with 0.01 M (UP-621, 143 mg, and US-621, 173 mg) and 1.0 M (UP-622, 39 mg, and US-622, 46 mg) phosphate buffers in succession followed by 4.5 M NaCl (UP-623, 10 mg, and US-623, 51 mg) and 0.5 M NaOH (UP-624, 22 mg, and US-624, 33 mg) with gravity flow at about 1 ml min⁻¹. Amounts isolated after a typical elution are given together with names of the fractions. The carbohydrate content of the fractions was monitored using the phenol/sulfuric acid assay.

Methylations were carried out as previously reported.²⁰ The partly methylated alditol acetates were analysed on GLC using column 1 or 2, program 2, and by GLC–MS using the GLC with column 1, program 2 and MS 1 or 2. Partly methylated alditol acetate standards were used for identification of the different peaks.

Reductive cleavage of the methylated polysaccharides was undertaken according to Rolf *et al.*,⁹ using the Et₃SiH, Me₃SiO₃SCF₃ reagent. The samples were analysed on GLC using column 1, program 3, and by GLC–MS with the GLC running with column 1, program 3 and MS 2.

Partial hydrolysis. UP-6S (23.0 mg) and US-6S (24.1 mg) were dissolved in 0.05 M TFA (10 ml) and hydrolysed for 3 h at 100°C. After being cooled to room temperature the solutions were dialysed, using Spectrapor dialysis tubing with an M_w cut-off at 3 500 daltons. The dialysis was run for two 24 h periods with distilled water and subsequently with running water for 24 h. The retained fractions and the combined distilled-water fractions were evaporated to dryness and dissolved in distilled water (5 ml) before being freeze-dried. Retained fractions: UP-6SI (11.0 mg) and US-6SI (14.7 mg). Dialysed fractions: UP-6SO (7.8 mg) and US-6SO (9.2 mg).

Partial hydrolysis of methylated samples was carried out according to Valent *et al.*¹⁰ to determine the positions of acid-labile residues. The ethylated alditol acetates found when this procedure was used on UP-621 were: 3-O-ethyl-2,4,6-tri-O-methylhexitol (0.9%); 3-O-ethyl-4,6-di-O-methylhexitol (1.9%); 6-O-ethyl-3,4-di-O-methylhexitol (1.2%) and 4-O-ethyl-3-O-methylhexitol (4.2%).

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Received October 19, 1988.