Carbohydrate Polymers 17 (1992) 137–144

Separation, isolation and characterization of acidic polysaccharides from the inner bark of *Ulmus glabra* Huds.

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(Received 5 October 1990; revised version received 7 February 1991; accepted 12 February 1991)

Pectin type polysaccharides were extracted from the inner bark of *Ulmus glabra* Huds., using water at 50°C. After redissolving the product by boiling, fractionation into 9 polymers was achieved by a combination of two different ion exchange columns. The three most abundant polymers were isolated and characterized. The polysaccharides appeared to have structural differences. The two fractions A_{FPLC} and B_{FPLC} contain galacturonic acid, galactose and rhamnose, while C_{FPLC} in addition contains 3-*O*-methylgalactose, glucuronic acid and acetyl groups. The backbone of all three polysaccharides consists of 4-linked α-*D*-galactopyranosyl-uronic acid interspersed with 2-linked α-*L*-rhamnopyranosyl residues. The side chains on A_{FPLC} and B_{FPLC} consist of galactose units attached to C-3 of the rhamnose, while the side chains of C_{FPLC} are mainly linked through C-4 of rhamnose. These include 4-linked 3-*O*-methylgalactose. After weak acid hydrolysis, 5 acidic oligosaccharides were isolated and characterized.

NOTATION

Galp	Galactopyranose
GalpA	Galactopyranosyluronic acid
Rhap	Rhamnopyranose

INTRODUCTION

The common elm, *Ulmus glabra* Huds., was in former days a tree of great value in Norway (Reichborn-Kjennerud, 1922; Nordhagen, 1954; Høeg, 1974). The general interest was concentrated on the inner bark, being rich in mucilage-containing sacs. The mucilagineous properties of this bark were the reason for the interest in the tree, and powdered inner bark was added to the dough when the harvest was poor. The gelling and thickening properties made better bread. The mucilage was also used as a demulcent when the digestive tract was inflamed and the inner bark was believed to be of nutritional value. The United States Pharmacopea (Grieve, 1976) has registered Slippery Elm (*U. fulva* Michaux) as an official drug. The structure of the mucilage from *U. fulva* has been studied by Beveridge *et al* (1971) and Hirst *et al.* (1951).

Little is known of the structure of the mucilage from the inner bark of *U. glabra.* Øyseth (1954) reported the total monomer composition of the crude extract, but no fractionation was then performed.

Preliminary studies indicated that a partly purified extract from the bark of *U. glabra* have antiinflammatory effect. The authors have published the fractionation of the polysaccharide material into 9 different polymers, and the isolation of three of these by a combination of DEAE-Sepharose CL-6B chromatography and the Mono P column in the FPLC system (Barsett & Smestad Paulsen, 1985). The present paper reports on the total carbohydrate composition and partial structural elucidation of the isolated polysaccharides, and separation and characterization of 5 oligosaccharides isolated from the crude extract of the inner bark of *Ulmus glabra* Huds.

METHODS

General methods

The water used was always distilled. Solutions were concentrated under reduced pressure at 40°C in a

Carbohydrate Polymers 0144-8617/91/$03.50
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Büchi Rotavapor®. All dialyses were performed using tubing with a nominal molecular-weight cutoff of 12 000 or 3500, and all filtrations were performed through Whatman GF/A glassfibre filter. Centrifugations were performed by a Sorvall® RC2-B automatic superspeed refrigerated centrifuge with 4000 rpm at 10 °C for 20 min, or by a MSE table centrifuge with 1800 rpm for 5 min. Absorbance was measured using a Cecil 292 digital ultraviolet spectrophotometer. Specific rotation was determined by means of a Perkin-Elmer 141 polarimeter. ^{1}H-NMR spectra were recorded with a Bruker 400 MHz FT-NMR spectrometer for solutions in D_2O at 99 °C.

Preparation of the elm bark extract for chromatography

The inner bark of common elm (*Ulmus glabra* Huds.) (200 g) was extracted with ethanol–water (3 : 1) at 80 °C for 2 h to remove low molecular weight material and coloured matter. The extraction was repeated 4 times. The carbohydrate polymer was then extracted at 50 °C with water (16 l), dialysed and lyophilized. The crude extract thus obtained (14 g) was further purified after redissolution at 100 °C for 4 h, centrifuged at 1800 g and filtrated through Whatman GF/A glassfibre filter to obtain a purified crude extract.

Ion exchange chromatography of the purified crude extract

The purified crude extract (2 g) was applied onto a DEAE-Sepharose CL-6B column (60 × 5 cm) converted into chloride form. The column was coupled to a P-1 peristaltic pump (Pharmacia), and fractions of 8·8 ml were collected using an Ultrorac 7000 fraction collector (LKB). The column was eluted at 1 ml/min, first with water (500 ml), then by gradient elution using 0–1 M sodium chloride for elution of the acidic polymers (Fig. 1). The eluent was monitored at 206 nm in a 2158 Uvicord SD detector, (LKB) coupled to a Cole-Parmer (K8387-321) recorder. The fractions were tested for carbohydrate content colorimetrically (487 nm) by the phenol-sulphuric acid method (Dubois *et al.*, 1956). Fractions 77–91 were pooled, dialysed, freeze dried and dessignated Fr.I (0·2 g).

Further separation of Fr.I on a Mono P column in the fast protein liquid chromatography (FPLC) system

The anion-exchange column, Mono P, was fitted in the FPLC system (Pharmacia) consisting of two P-500 pumps, a gradient programmer GP-250, a V-7 valve, a UV-1 monitor at 214 nm, a FRAC-100 fraction collector and a two-channel recorder with event marker, REC 482. For analytical purposes 2 mg Fr.I in 500 µl were injected, whereas for preparative purposes 10 mg Fr.I in 5 ml were injected onto the column via a superloop.

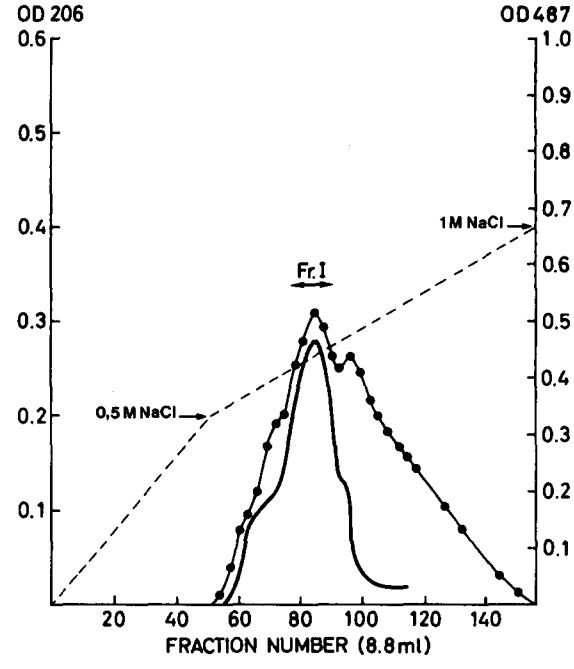

Fig. 1. Chromatography of the mucilage from common elm (*Ulmus glabra*) on a DEAE-Sepharose CL-6B column. ——, Carbohydrate profile; ●——●, optical density at 206 nm (OD 206) (corrected for the absorption due to the salt gradient); - - -, salt gradient (0–1 M sodium chloride).

The column was eluted at 1 ml/min, using 0–1 M sodium chloride in 15 mM phosphate-sodium chloride buffer (pH 7). The elution programme is given in Fig. 2. Fractions of 1 ml were collected and tested for carbohydrate content as above. Nine acidic polymers were separated, and the three most abundant were isolated, rechromatographed and dessignated A_{FPLC}, B_{FPLC} and C_{FPLC} (Barsett & Smestad Paulsen, 1985).

Molecular weight estimation by high-performance liquid chromatography (HPLC)

The HPLC system consisted of a LKB 2150 HPLC pump, a Reodyne 7125 injector fitted with a 100 µl loop, DuPont high-performance size-exclusion chromatography (HPSEC) columns SE60, SE100, SE500, SE1000 coupled in series (Wilmington), Optilab 5902 interference refractometer (Tecator AB) and a Cole-Parmer K8387-32 recorder. The column temperature was maintained at 30 °C by a Thermomix 1441 (Braun) thermostat in a water-bath. Dextrans T70, T250 and T500 (Pharmacia) were used as standards. The eluent was 0·05 M acetate buffer (pH 4·7), with a flow rate of 0·25 ml/min.

Weak acid hydrolysis and isolation of oligosaccharides

The crude extract was suspended in 0·5 M sulphuric acid (500 ml) and heated at 100 °C for 3 h. After neutralization with barium carbonate, Dowex-50 (H^+)

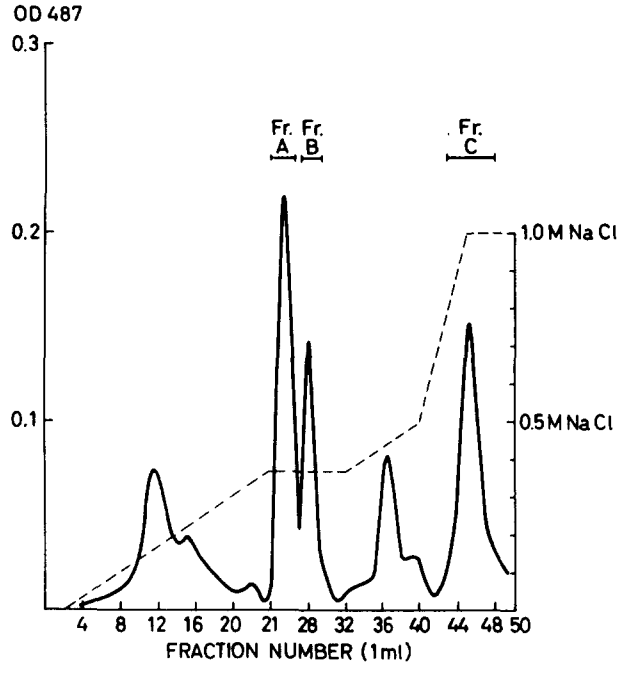

Fig. 2. Elution profile (——) (carbohydrate) of Fr.I from Fig. 1 applied on a Mono P column in the FPLC system (- - -, 0–1 M sodium chloride elution programme).

was added to the filtrate to pH 4. The solution was filtrated and concentrated, and the oligosaccharides obtained were separated by high voltage paper electrophoresis (HVE) and preparative paper chromatography. High voltage electrophoresis was performed on Whatman No. 3 mm filter paper at 60 V/cm for 2 h in 10% pyridin/0·5% acetic acid, pH 6·42. Preparative paper chromatography was performed on Whatman No. 3 mm filter paper in the solvent system (v/v) ethyl acetate, acetic acid, formic acid and water (18 : 3 : 1 : 4) for 52 h. All filter papers were washed with solvent prior to application. The mono- and oligosaccharides were located on chromatograms and electropherograms with the following reagents:

(a) aniline oxalate, saturated aqueous solution (Hough *et al.*, 1950);
(b) periodate/benzidine (Gordon *et al.*, 1956). The located zones with oligosaccharides were eluted with water.

Quantitative determination of the carbohydrate composition

The samples (100–200 μg) were subjected to methanolysis with 1 M or 4 M hydrochloric acid in anhydrous methanol for 24 h at 80 °C (Chambers & Clamp, 1971). The reagent was removed with nitrogen and the methylglycosides dried *in vacuo* prior to conversion into the corresponding trimethylsilylethers. Mannitol was included as an internal standard. The samples (*O*-silylated methylglycosides) were subjected to gas chromatography analysis on a Carlo Erba 4200 Fractovap chromatograph with a 430 LT programmer. The chromatograph was equipped with a flame-ionization detector, a Grob split-splitless injector, used in the split mode, and a 3390A Hewlett-Packard reporting integrator. The column was a DB-5 fused-silica capillary column (15 m × 0·32 mm i.d.), film thickness 0·25 μm (Durabond™). Helium was used as the carrier gas at a flow rate of 5·0 ml/min. The *O*-silylated methylglycosides were analysed using the following conditions: injector temperature, 250 °C; detector temperature, 260 °C; column temperature, 140 °C when injected, followed by an increase of 1 °C/min to 160 °C, then 6 °C/min to 250 °C and then kept at 300 °C for 5 min.

Periodate oxidation

The sample (1–2 mg) was subjected to oxidation with 0·01 M $NaIO_4$ in 0·1 M NaOAc buffer pH 4 (10 ml) at 4 °C in the dark. The consumption of periodate was followed spectrophotometrically at 223 nm (Aspinall & Ferrier 1957), and the reaction terminated after 14 h by addition of ethylene glycol. Free aldehyde groups were reduced by $NaBH_4$. The solution was desalted, freeze dried and subjected to methanolysis and analysis of monosaccharides by GC as described above.

Determination of methoxyl groups

The samples (4 mg in 1 ml distilled water) were subjected to saponification with 1 ml 1 M NaOH and 1 mg $NaBH_4$ for 2 h at 100 °C. The methanol content was determined by a spectrophotometric method (Wood & Siddiqui, 1971) and headspace gas analysis by gas chromatography (Drozd & Novak, 1979; Hinshaw, 1990). Headspace gas analysis was performed on a Carlo Erba 6000 Vega series 2 gas chromatograph with control module ICU 600. The chromatograph was equipped with a flame-ionization detector and a Hewlett-Packard HP-3396A Recording Integrator. The column was a glass column (2 m × 3 mm i.d.) packed with GP 60/80 Carbopack B/5% Carbowax® 20 M. Nitrogen was used as the carrier gas at a flow rate of 25 ml/min. The column temperature was 90 °C; injector temperature, 120 °C and detector temperature, 150 °C. Ethanol was used as an internal standard. The vials were heated for 30 min at 60 °C just before 1 ml gas from the upper gas-layer of the samples was injected directly on the column. The gas was injected by a gas- and liquid-tight syringe with a PTFE sealing male Luer to accept Luer Lock needles (Scientific Glass Engineering Pty Ltd.).

Reduction of uronic acids in polymers and oligomers

Prior to reduction, the polysaccharides and oligosaccharides were de-esterified in 0·05 M NaOH for 2 h

at 2°C, then neutralized to pH 4–5 with 0·5 M HCl (Saulnier *et al.*, 1988). 1-Cyclohexyl-3-(2-morpholino-ethyl)-carbodi-imide metho-*p*-toluenesulphonate (Taylor & Conrad, 1972) (10 mol of CMC per carboxylic acid equivalent) was added, and the pH was kept at 4·75 with 0·01 M HCl for 2 h. $NaBH_4$ solution (250 mol of $NaBH_4$ per carboxylic acid equivalent) was then added during 30 min, maintaining the pH at 7 with 1 M HCl (1-octanol as anti-foaming agent). After 17 h the $NaBH_4$ solution (125 mol $NaBH_4$ per carboxylic acid equivalent) was added during 30 min, maintaining the pH at 7 with M HCl. The mixture containing the reduced polysaccharide was then extensively dialysed, concentrated and feeze dried. Oligosaccharide-samples were concentrated to 0·5 ml, centrifuged and the supernatant dried *in vacuo*.

Methylation analysis

The polysaccharides and oligosaccharides were methylated by the method described by Harris *et al.* (1984), modified by Kvernheim (1987), using the lithium salt of methylsulphinyl carbanion (Blakeney & Stone 1985). Both CH_3I and CD_3I were used as methylating agents. The partially methylated alditol acetates were analysed by GC/MS. Electron impact ionization mass spectra were obtained using Hewlett-Packard Mass Selective Detector 5970 with a Hewlett-Packard 5890 GC, a Disk Drive 9130 and a Think Jet recorder. The gas chromatograph (GC/MS) was fitted with a split-splitless injector, used in the split mode and a Hewlett-Packard methyl silicone capillary column (12 m × 0·20 mm i.d.) with film thickness 0·33 μm. The column was inserted directly into the ion source of the mass spectrometer. The injector temperature was 250°C, the detector temperature was 300°C and the column temperature was 80°C when injected and kept for 1 min, then 30°C/min to 160°C, followed by 0·5°C/min to 200°C and then kept at 300°C for 5 min. The carrier gas was helium and the flow rate was 0·9 ml/min.

Ethylation analysis

The samples were ethylated by the same procedure as described for methylation, except for using ethyliodid as the ethylating agent. The ethylated derivatives were analysed in the GC/MS system described above.

RESULTS AND DISCUSSION

The inner bark of *U. glabra* contains 7% water-soluble carbohydrate of polymeric nature. The extract was extremely viscous, and after freeze drying the polymeric substance was difficult to dissolve. The total carbohydrate content in the crude extract was 70% and the protein content was determined to 12·5% (Lowry *et al.*, 1951). The carbohydrate composition is given in Table 1. Of the galacturonic acid present, 64% appeared to be esterified as determined by the method of Hestrin (1949). Acetyl (Tomoda *et al.*, 1974) and pyruvate (Boehringer Mannheim, 1977) was detected only in negligible amounts. Before structural studies on the polymer were carried out, various fractionation methods were tested after dissolution of the polymer at room temperature. The solution became too viscous and clogged the various columns tried (e.g. DEAE-Sephadex A-25 and Sepharose 4B). A clear solution was first achieved after heating for 4 h at 100°C, followed by centrifugation and filtration and then a considerable reduction in viscosity was observed. This treatment might result in cleavage of a few glycosidic linkages, thus giving fragments with a somewhat lower molecular weight than that of the native polymer. The partly purified crude extract was applied onto a DEAE-Sepharose 6B CL column. The acidic polymers were eluted from the column by a salt gradient elution. As shown in Fig. 1, the carbohydrate material started to be eluted first at 0·5 M sodium chloride, and total exclusion was achieved at 0·8 M sodium chloride. The elution profile clearly shows that several polymeric fractions of an acidic nature are present in the purified crude extract from the inner bark. The fraction marked Fr.I on Fig. 1, was pooled, dialysed and freeze dried. The total carbohydrate composition is given in Table 1. After dissolution, Fr.I was applied to a Mono P™ column fitted in the FPLC system (Barsett & Smestad Paulsen, 1985). This matrix, primarily developed for chromatofocusing of proteins (Søderberg *et al.*, 1983),

Table 1. Relative carbohydrate composition, before and after periodate oxidation, of polysaccharide fractions isolated from inner bark of *Ulmus glabra*

Monosaccharides	Cruce extract[a]		Fr.I		A_{FPLC}		B_{FPLC}		C_{FPLC}	
	Native	Peri.ox.	Native	Peri.ox.	Native	Peri.ox.	Native	Peri.ox.	Native	Peri.ox.
Rha	16	12	20	14	25	19	34	22	16	12
3-*O*-Me-Gal	14	14	7	7	—	—	—	—	11	11
Gal	33	2	56	5	46	trace	32	trace	22	trace
Gal A	22	7	17	5	26	trace	28	trace	38	13
Glc A	12	—	trace	—	—	—	trace	—	14	—

[a] Arabinose is present in trace amounts.

Table 2. Specific rotation, molecular weight and total carbohydrate content of polysaccharide fractions from inner bark of *Ulmus glabra*, isolated on the Mono P column

Fraction	[α]D	$\overline{MW}$	Total carbohydrate content
A_{FPLC}	+50·0	475 000	99%
B_{FPLC}	+34·2	710 000	98%
C_{FPLC}	+8·1	1 120 000	81%

separated the fraction into at least 9 different acidic polymers (Fig. 2). The three most abundant fractions were isolated and rechromatographed in order to obtain pure polymers. The fractions were dessignated A_{FPLC}, B_{FPLC} and C_{FPLC}. $[\alpha]_D$, $\overline{MW}$ and total carbohydrate contents are given in Table 2. The molecular weights ($\overline{MW}$) of the FPLC-isolated fractions A, B and C are given with reference to standard dextrans with a different molecular structure from the polymers isolated. As the shapes of these polymers differ from those of the dextrans, there is a great deal of uncertainty as to the real molecular weight of the fractions. The degree of esterification was determined spectrophotometrically and by headspace gas analysis by gas chromatography. Due to the great amount needed for this determination, an average of the degree of esterification of FPLC-fractions A and B was determined. The values for Fr.I with the two methods were 28% and 23% respectively, for FPLC-fractions A and B the average values were 93% and 50% respectively, and for FPLC-fraction C, 22% and 24% respectively. The spectrophotometrical method showed a higher average degree of esterification of A + B than the headspace gas analysis. Both Fr.I and the FPLC fractions were subjected to periodate oxidation. Results of the carbohydrate composition before and after this treatment are given in Table 1. The results show that only a part of the rhamnose present is oxidised in all fractions in contrast to the results obtained for galactose and galacturonic acid. The FPLC-fractions A and B are quite similar with respect to carbohydrate composition. Only fraction C_{FPLC} contains glucuronic acid and 3-*O*-methyl-galactose. This fraction also contains the highest amount of uronic acids and the lowest degree of esterification. It seems to be a clear connection between the amount of uronic acid with free COOH-groups and the elution profile of Mono P™ column (Fig. 2). A_{FPLC} and B_{FPLC} both contain galacturonic acid and rhamnose in the ratio 1 : 1, while C_{FPLC} contain the same sugars in the ratio 2 : 1. All fractions were methylated both before and after reduction of the carboxyl-groups (Table 3). There are some structural differences, but all fractions contain galacturonic acid which is mainly (1 → 4) linked. The fractions A_{FPLC} and B_{FPLC} seem quite similar, but A_{FPLC} contains (1 → 3) galactose, (1 → 6) galactose and (1 → 4) galactose, all being absent in B_{FPLC}. Both fractions contain a great amount of terminal galactose. The rhamnose in these fractions is linked mainly both (1 → 2) and (1 → 3). The FPLC-

Table 3. Methylation/ethylation analysis of various native and carboxyl-reduced polysaccharide fractions from the inner bark of elm

Glycosyl residue	Position of *O*-methyl/ *O*-ethyl groups	Methylated products (mol. %)						Ethylated products (mol. %)[a]	
		with CH_3I					with CD_3I[a]		
		Rel. R_t[b]	Fr.I	A_{FPLC}	B_{FPLC}	C_{FPLC}	C_{FPLC}	Rel. R_t[b]	C_{FPLC}
Rha	2,3,4	0·69	0·4						
	3,4	0·88	8·2	2·6					
	4	1·13	4·2	25·3	36·8	1·9			
	3	1·15	9·6	1·2	3·7	17·0	35·6	1·25	35·5
Gal	2,3,4,6	1·06	13·2	35·1	31·2	12·4	21·9	1·50	29·8
	2,3,6	1·33	39·8	0·3		15·6	10·7	1·77	14·6
	2,4,6	1·38	2·6	2·9					
	2,3,4	1·59	0·7	8·8					
	2,6	1·63				6·2	11·6		
	2,3	1·93	0·5	1·4	3·5				
	2,4	2·09	6·6						
3-*O*-Me-Gal	2,4,6	1·06						1·42	1·6
	2,6	1·33					20·2	1·68	18·5
GalA[c]	2,3,4,6	1·06	0·3						
	2,3,6	1·33	13·7	22·4	24·7	33·2			
GlcA[c]	2,3,4,6	1·00	tr.		tr.	5·5			
	2,3,6	1·35				8·2			

[a] Ethylation and methylation with CD_3I were performed on the native C_{FPLC}-fraction only.
[b] Relative to 1,5-di-*O*-acetyl 2,3,4,6 tetra-*O*-methyl-glucitol.
[c] Determined as the increase of methylated glycosyl residues after carboxyl-reduction.

fraction B seems to have a greater amount of branched galactose linked both (1 → 4) and (1 → 6). The fraction C_{FPLC} differs from these two fractions by containing 3-*O*-methyl-galactose and glucuronic acid. It also differs in the way the rhamnose is branched. In this fraction rhamnose is mainly linked both (1 → 2) and (1 → 4). Due to the content of 3-*O*-methyl-galactose this fraction was also methylated with CD_3I and ethylated. The results showed that the FPLC-fraction C contains (1 → 4) linked 3-*O*-methyl-galactose and trace amounts of terminal 3-*O*-methyl-galactose. Glucuronic acid appeared to be terminal and (1 → 4) linked. ^{1}H-NMR spectra of A_{FPLC}, B_{FPLC} and C_{FPLC} were obtained. The spectra of FPLC-fractions A and B both showed resonance at 5·31 ppm indicating *α-L*-rhamnose, resonance at 5·08 ppm indicating *α-D*-galacturonic acid and resonance at 4·64 ppm indicating *β-D*-galactose (Dutton *et al.*, 1980; Jaworska & Zamojski, 1984). The NMR-spectrum verifies a more complex structure for FPLC-fraction C, resonance at 2·19 ppm indicates *O*-acetyl which leads to changes in the chemical shift. The anomeric region seems more complex, but the spectrum indicates the same anomeric configurations as for the FPLC-fractions A and B. Although the total extract contained a negligible amount of acetyl, the NMR spectrum indicates acetyl groups linked either to galactose or galacturonic acid in the C_{FPLC} fraction.

In order to obtain further details of the polysaccharides present in the elm bark, the crude extract was subjected to weak acid hydrolysis. The hydrolysate obtained was separated on HVE into one neutral fraction and one broad acidic fraction. The neutral fraction consisted of galactose and 3-*O*-methyl-galactose as monomers. The acidic fraction contained all the sugars present in the polymer. Various methods, including modifications of HVE and various types of column chromatography, were tested for separation of the fraction with no success. PC was the only method giving fractions which apparently consisted of one compound. Five different oligosaccharides, PC.A-E, were isolated. The PC.E fraction had the lowest retardation of these fractions. The carbohydrate compositions before and after periodate oxidation, are given in Table 4. The fractions were methylated both before and after reduction of the carboxyl-groups, and the results are given in Table 5. On the basis of these results the suggested structure of the isolated oligosaccharides are given in Table 6. The methylation results showed that all monomers were in a pyranose form, and on the basis of the ^{1}H-NMR spectra of the polymers A_{FPLC}, B_{FPLC} and C_{FPLC} it is likely that also the isolated oligosaccharides contain *α-D*-galacturonic acid, *α-L*-rhamnose and *β-D*-galactose.

On the basis of the total results obtained, it seems likely that the backbone of the acidic polymers isolated from inner bark of *U. glabra* consist of a rhamnogalacturonan with side chains consisting of galactose,

Table 4. The relative carbohydrate composition, before and after periodate oxidation, of various oligosaccharide fractions isolated by preparative paper chromatography

Monosaccharide	PC.A		PC.B		PC.C		PC.D		PC.E	
	Native	Peri.ox.	Native	Peri.ox.	Native	Peri.ox.	Native	Peri.ox.	Native	Peri.ox.
Rha	1	0·5	2	1	1	1	1	—	1	—
3-*O*-Me-Gal			1	1	2	2				
Gal	1	—	1	—						
GalA	2	—	3	—	3	—	2	—	1	—

Table 5. Methylation analysis of native and carboxyl-reduced oligosaccharide fractions isolated by preparative paper chromatography

Glycosyl residue	Position of *O*-methyl groups	Methylated products (mol. %)					
		Rel. R_t[a]	PC.A	PC.B	PC.C	PC.D	PC.E
Rha	3,4	0·88	16·7	14·4		38·0	57·4
	4	1·13	13·8				
	3	1·15		17·3	20·7		
Gal	2,3,4,6	1·06	10·6	16·1	14·8		
	2,3,6	1·33	13·7	12·8	19·2		
GalA[b]	2,3,4,6	1·06	9·7	12·3	13·7	29·2	42·6
	2,3,6	1·33	35·5	27·1	31·6	32·8	

[a]Relative to 1,5 di-*O*-acetyl 2,3,4,6-tetra-*O*-methyl glucitol.
[b]Determined as the increase of methylated glycosyl residues after carboxyl-reduction.

Table 6. Proposed structure of the isolated oligosaccharides

Fraction	Oligosaccharides
PC.A	Gal 1 ↓ 4 Gal 1 ↓ 3 GalA(1 → 4)GalA(1 → 2)Rha(1 → 4)GalA(1 → 4)GalA(1 → 2)Rha-
PC.B	Gal 1 ↓ 4 3-*O*-Me-Gal 1 ↓ 4 GalA(1 → 4)GalA(1 → 2)Rha(1 → 4)GalA(1 → 2)Rha-
PC.C	3-*O*-Me-Gal 1 ↓ 4 3-*O*-Me-Gal 1 ↓ 4 GalA(1 → 4)GalA(1 → 4)GalA(1 → 2)Rha-
PC.D	GalA(1 → 4)GalA(1 → 2)Rha-
PC.E	GalA(1 → 2)Rha-

3-*O*-methyl-galactose and/or glucuronic acid. It seems also likely that the backbone consists of disaccharide repeating unit → 4)-α-*D*-Gal*p*A-(1 → 2)-α-*L*-Rha*p*-(1 → and trisaccharide repeating unit → 4)-α-*D*-Gal*p*A-(1 → 4)-α-*D*-Gal*p*A-(1 → 2)-α-*L*-Rha*p*-(1 →, and that there are sequences with even more galacturonic acid units per rhamnose unit. The side chains consisting of 3-*O*-methyl-galactose are attached to the C-4 position of the backbone rhamnose residue, while side chains consisting of galactose usually are attached to the C-3 position of other backbone rhamnose residues.

The structure of these isolated polysaccharides have much in common with the suggested structure of the mucilage isolated from the bark of the slippery elm tree (*Ulmus fulva*) (Beveridge *et al.*, 1971). Three oligosaccharides isolated from *U. fulva* showed the same structure as proposed for the oligosaccharides isolated from *U. glabra*.

ACKNOWLEDGEMENTS

The authors are indebted to T. Aslaksen, T. Haugli and G. Skodbo for their skillful technical assistance, to H. Grasdalen (Trondheim-NTH) for valuable discussions of NMR-spectra and to F. Tønnesen for recording all the GC/MS data.

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