Carbohydrate Research, 185 (1989) 199–210
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STUDIES OF THE POLYSACCHARIDES OF *Populus alba L.*: ISOLATION AND CHARACTERISATION OF XYLOGLUCANS

ŠTEFAN KARÁCSONYI* AND VLADIMÍR KOVÁČIK
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava (Czechoslovakia)
(Received March 21st, 1988; accepted for publication, July 19th, 1988)

ABSTRACT

Extraction with alkali of the polysaccharides from the tissue culture of primary cell walls of poplar *(Populus alba L.)* gave the major fraction of hemicellulose B, from which two homogeneous xyloglucans were isolated by fractionation using ion-exchange and gel-permeation chromatography followed by precipitation with Fehling's solution. These xyloglucans had highly branched structures which differed in the degree of side-chain substitution, but the basic structure was similar to those of xyloglucans isolated from other dicotyledons.

INTRODUCTION

Various aspects of physiology and metabolism in the development and growth of higher plants have been investigated by using *in vitro* cell and tissue cultures. Suspension cultures of *Acer pseudoplatanus* have been used in investigations of the structure of a cell-wall glycoprotein[1], cell-wall and extracellular polysaccharides[2], and the molecular structure of cell walls[3].

We have described[4,5] the composition and ultrastructure of the primary cell wall of *Populus alba L.* together with changes in composition during tissue growth and now report on two neutral polysaccharides isolated from the plant.

RESULTS AND DISCUSSION

Cell walls of high purity were isolated[4] from the green tissue culture of *Populus alba L.* harvested at the stationary phase of growth (*i.e.*, on the 20th day after inoculation). Fractional extraction of the lipid-free cell walls afforded the pectic, hemicellulose, and α-cellulose components (Table I).

Xyloglucan, the main structural component of hemicelluloses of the primary cell wall of dicotyledons[6], was found in proportions of 40% and 23%, respectively, in the polysaccharide fractions *C* and *D* obtained by extraction with alkali (Table

*Author for correspondence.

0008-6215/89/$ 03.50

TABLE I

EXTRACTION OF POLYSACCHARIDES FROM *Populus alba L.* CELL-WALL MATERIAL (CWM)

Fraction	*Extractant*	*Yield*[a] *(%)*	$[\alpha]_D$ *(degrees)*	*Protein (%)*	*Uronic acid (%)*	*XG*[b]	*Molar ratios of monosaccharides*						
							Gal	*Glc*	*Man*	*Ara*	*Xyl*	*Rha*	*Fuc*
CWM				12.1	19.1	10.1	5.4	28.3	1.5	10.0	8.9	1.2	1.5
A	Water	4.0	−36	5.1	12.5	—	2.0	tr	—	10.0	0.6	0.7	0.3
B[c]	Aq. 0.5% ammonium oxalate	15.3	+178	2.3	78.5	—	3.4	0.4	0.1	10.0	0.9	1.7	0.6
C	Aq. 15% potassium hydroxide	19.4	+21	8.9	7.1	40.0	7.1	13.3	2.3	10.0	15.0	1.3	2.8
D	Aq. 17.5% sodium hydroxide + 4% boric acid	10.1	+27	—	20.4	23.0	7.7	16.8	3.7	10.0	11.2	1.2	0.7
E	Residue	34.7	—	—	8.9	—	7.4	114.3	1.7	10.0	13.6	1.7	1.7

[a]Based on lipid-free CWM. [b]Xyloglucan determined by the modified iodine–sulphate method[28]. [c]Degree of esterification, 17.6%. This fraction contained also 2-*O*-methyl-deoxyhexose (1.8 mol %) and 2-*O*-methylxylose (2.0 mol %).

TABLE II

FRACTIONATION OF POLYSACCHARIDE *C* (TABLE I) ON DEAE-SEPHADEX A-50

Fraction	*Eluant*	*Yield*[a]	$[\alpha]_D$[b] *(degrees)*	*Molar ratios of monosaccharides*							*Uronic acid*
				Gal	*Glc*	*Man*	*Ara*	*Xyl*	*Rha*	*Fuc*	
1	2.0mM Potassium acetate	20.0	+42	4.9	18.8	2.3	0.3	10.0	—	2.5	—
2	0.01M	8.7	+58	6.0	23.3	0.4	2.6	10.0	0.2	2.3	—
3	0.1M	18.3	+27	3.5	12.3	0.3	0.9	10.0	—	1.8	+
4	0.5M	16.8	−1.9	21.3	2.9	0.4	35.7	10.0	4.9	1.8	+++
5	0.3M Sodium hydroxide	4.3	+9	0.8	1.3	0.2	3.6	10.0	—	0.3	++

[a]Percentage of weight of material applied to column. [b]Water.

I, column 6). The content (~10%) of xyloglucan in the primary cell wall of poplar is equal to, or only a little higher than, that in the cambial tissue of *Populus tremuloides* and *Tilia americana*[7], but half of that in the cell wall of the suspension culture of *Acer pseudoplatanus*[2]. However, the proportions in plant cell walls are dependent on the methods of determination and isolation as well as on the stage of development of the plant tissue[8].

Fractionation of the polysaccharide mixture *C*, which represents the main portion of the non-cellulose polysaccharides, on a column of DEAE-Sephadex A-50 (AcO^-) (see Table II) yielded xyloglucans mainly in fractions *1* and *3*. Subsequent purification of fraction *1*, $[\alpha]_D$ +42° (water), by repeated precipitation with Fehling's solution[9] gave polysaccharide XG-1.

Chromatography on Sephadex G-75 of fraction *3*, $[\alpha]_D$ +27° (water), gave two fractions. The main, xyloglucan-containing fraction was purified by precipitation with Fehling's solution to give xyloglucan XG-2. The chemical compositions of XG-1 and XG-2 were different, but remained constant on further purification and were comparable with that of xyloglucans isolated from various other plants[6]. XG-1 and XG-2 were homogeneous in electrophoresis, sedimentation analysis, and gel filtration. The $\overline{M}_w/\overline{M}_n$ ratios 1.14 and 1.08, respectively, indicate low polydispersity. Physico-chemical constants and the compositions of these polysaccharides are presented in Table III.

The molecular weights of XG-1 and XG-2 (37.2×10^3 and 28.7×10^3, respectively) are higher than those ($7.6–11.5 \times 10^3$) of xyloglucans of *Acer pseudoplatanus* or present in seeds of various plants, but lower than those of xyloglucans of cambial tissues of *Populus tremuloides*[7] (62×10^3), *Phaseolus coccineus*[10] (11×10^4), and *Simmondsia chinensis*[11] (17.4×10^4). Different molecular weights have been ob-

TABLE III

DATA ON XYLOGLUCANS XG-1 AND XG-2

Polysaccharide	*XG-1*	*XG-2*
$[\alpha]_D$ (water)	+50°	+45°
Electrophoretic mobility	5.39×10^{-5} cm^2.V^{-1}.s^{-1}	5.15×10^{-5} cm^2.V^{-1}.s^{-1}
Diffusion coefficient, D_{20}	7.12×10^{-7} cm^2.s^{-1}	7.19×10^{-7} cm^2.s^{-1}
Sedimentation constant, S_{20}	4.14×10^{-13}	3.22×10^{-13}
Partial specific volume	0.6191	0.6202
$\overline{M}_w$	37300	28800
$\overline{M}_n$	32700	26600
$\overline{M}_w/\overline{M}_n$	1.14	1.08
Carbohydrate composition (mol %)		
Fuc	4.9	6.2
Gal	17.0	11.8
Xyl	32.8	34.6
Glc	38.7	46.0
Ara	tr	1.4
Man	6.6	tr

served for xyloglucans isolated from the same plant tissue in different phases of development[12,13].

The partially methylated saccharides, obtained by hydrolysis of the methylated xyloglucans, were identified as the corresponding alditol acetates by g.l.c.[14] and g.l.c.–m.s.[15]. The results are listed in Table IV.

Partial acid hydrolysis of XG-1 and XG-2 with acid gave a mixture of sugars and di-, tri-, and tetra-saccharides *1–5* which were resolved on Sephadex G-15 and by p.c., and identified by methylation analysis and mass spectrometry of the methylated compounds as D-Xyl*p*-(1→6)-D-Glc*p* (*1*), D-Glc*p*-(1→4)-D-Glc*p* (*2*), D-Gal*p*-(1→2)-D-Xyl*p* (*3*), D-Xyl*p*-(1→6)-D-Glc*p*-(1→4)-D-Glc*p* (*4*), and a mixture (*5*) of two tetrasaccharides (*5a* and *5b*), the structures of which were identified on the basis of methylation analysis and interpretation[16] of the mass spectra of methylated *5*.

From the structures of *1–5* and the results of methylation analysis, it follows that the average repeating-units of XG-1 and XG-2 contain 19 and 15 sugar residues, respectively, and the structures are highly branched with the side chains α-D-Xyl*p*-(1→ (*6*), β-D-Gal*p*-[1→2(4)]-α-D-Xyl*p*-(1→ (*7*), and α-L-Fuc*p*-(1→2)-β-D-Gal*p*-[1→4(2)]-α-D-Xyl*p*-(1→ (*8*), variously attached to O-6 of (1→4)-linked D-glucopyranosyl residues.

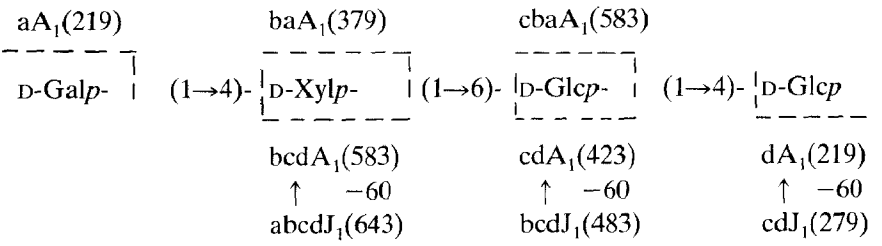
Although the precise location of the terminal fucosyl groups was not determined, methylation analysis of partially degraded XG-3 (Table IV, column 3) indicated their probable position, consistent with the structure of xyloglucans of primary cell walls of dicotyledons[6].

TABLE IV

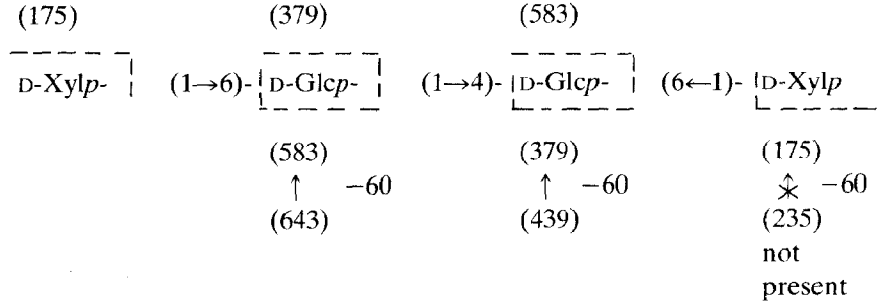
METHYLATED SUGAR FROM THE HYDROLYSATE OF THE METHYLATED XYLOGLUCANS

Sugar (as alditol acetates)	T[b]		*Mole %*			*Deduced linkage*
	B	C	*XG-1*	*XG-2*	*XG-3*[c]	
2,3,4,6-Me_4-Gal[a]	1.21	1.12	7.6	5.0	7.1	D-Gal*p*-(1→
3,4,6-Me_3-Gal	1.86	1.92	9.6	6.9	4.4	→2)-D-Gal*p*-(1→
2,3,6-Me_3-Glc	1.96	1.87	15.1	13.8	14.4	→4)-D-Glc*p*-(1→
2,3-Me_2Glc	2.80	3.54	26.3	32.8	36.0	→4,6)-D-Glc*p*-(1→
2,3,6-Me_3-Man	1.78	2.03	3.0	—	—	→4)-D-Man*p*-(1→
2,3-Me_2Man	2.57	2.85	3.3	—	—	→4,6)-D-Man*p*-(1→
2,3,4-Me_3-Xyl	0.76	0.64	14.2	20.7	21.3	D-Xyl*p*-(1→
2,3-Me_2-Xyl	1.48	1.64	5.1	3.8	4.4	→4)-D-Xyl*p*-(1→
3,4-Me_2-Xyl	1.48	1.64	10.6	9.4	9.0	→2)-D-Xyl*p*-(1→
2,3,5-Me_3-Ara	0.57	0.53	—	1.3	—	L-Ara*f*-(1→
2,3,4-Me_3Fuc	0.69	0.55	5.2	6.3	3.4	L-Fuc*p*-(1→

[a]2,3,4,6-Me_4-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, *etc.* [b]Retention time of the corresponding alditol acetate, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, on columns *B* and *C* (see Experimental). [c]Degraded XG-2.



5a



5b

The essentially similar structural features of xyloglucans were confirmed by the ^{13}C-n.m.r. spectra, which differed only in the intensities of the signals (Fig. 1). The signals in the region for anomeric carbons at 104.4, 103.6, 100.6, and 100.1 p.p.m. may be assigned to β-D-Gal*p*, β-D-Glc*p*, α-D-Xyl*p*, and α-L-Fuc*p* units, in accordance with the anomeric configuration of the sugar units in these polymers[6].

Although XG-1 and XG-2 have similar gross structural features, there are differences in the fine structures, mainly in the sugar composition, polydispersity, and the ratios of (1→4)- and (1→4,6)-linked Glc*p* units (1:1.8 in XG-1 and 1:2.4 in XG-2). The heterogeneity of XG-1 is emphasised by the presence of (1→4)- and (1→4,6)-linked Man*p* units, the structural significance of which is not clear. They may be an integral part of the xyloglucan, but the presence of a contaminant galactoglucomannan cannot be excluded[17].

The identification of the structural unit *5a* in XG-1 and XG-2 indicates a heterogeneity of branching, hitherto unknown, which is inherent in the xyloglucans formed in cultures of poplar tissue. There are other differences in the fine structures of xyloglucans isolated from different plants. Thus, *O*-acetyl groups are present in the xyloglucans isolated from the extracellular polysaccharides in suspension cultures of sycamore[18]. Multiple forms of xyloglucans, as found in the primary cell wall of poplar, have been found in suspension cultures of cells of *Rosa glauca*[19] and in parenchymatous tissues of apple[20]. It has been assumed that the xyloglucans in seeds and primary cell walls of plants are reserve materials in seed germination[21] or active components of molecular structure of cell walls, and modification of the structure makes possible the growth and development of cells[22]. Oligosaccharides

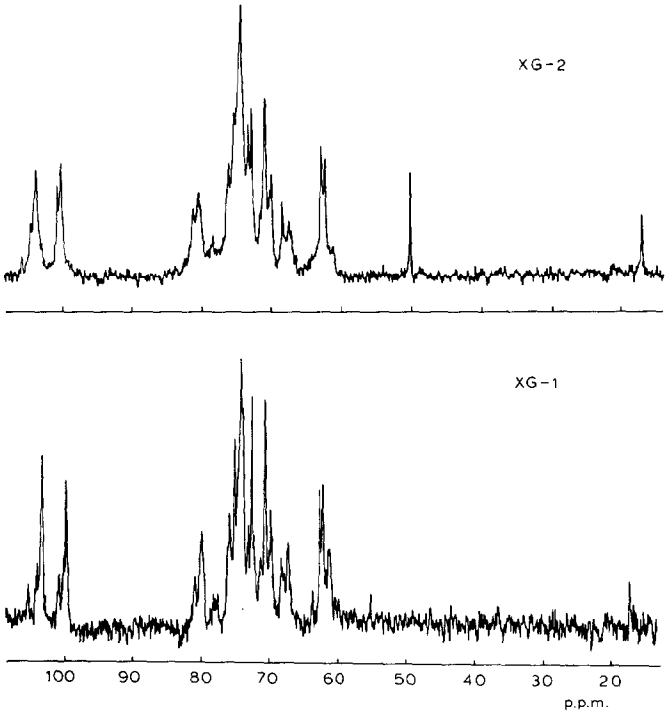


Fig. 1. ^{13}C-N.m.r. spectra (75.46 MHz) of 3% solutions of XG-1 and XG-2 in D_2O.

derived from xyloglucans can inhibit auxin-induced pea epicotyl growth[23]. It is possible that the xyloglucans found in the cell walls of poplar and other plants have specific functions in plant development.

EXPERIMENTAL

General. — All evaporations were conducted under diminished pressure at <40°. Optical rotations (1-mL cell) were measured at 20 ±1° with a Perkin–Elmer Model 141 polarimeter. Free-boundary electrophoresis of solutions of polysaccharides (10 mg/mL) was performed in 0.05M sodium tetraborate buffer (pH 9.2) with a Zeiss 35 apparatus at 180 V and 6 mA for 30 min. G.l.c. was performed on a Hewlett–Packard Model 5711 A instrument with *A*, a column (305 × 0.3 cm) of 1% of XE-60 on Gas Chrom Z (80–100 mesh) at 130→150° at 1°/min, with a N_2 flow of 36 mL/min; *B*, a column (200 × 0.3 cm) of 3% of SP-2340 on Chromosorb WAW-DMCS (80–100 mesh) for 4 min at 180° then →210° at 2°/min, with a N_2 flow of 30 mL/min; *C*, a column (200 × 0.5 cm) of 3% of OV-225 on Chromosorb WAW-DMCS (80–100 mesh) for 16 min at 180° then →210° at 2°/min, with a N_2 flow of 20 mL/min; *D*, a column (400 × 0.4 cm) of 10% of Carbowax 400 on Chromosorb WAW (80–100 mesh) at 45°, with a N_2 flow of 17 mL/min. Column *A* was used for quantitative analysis of sugar trifluoroacetates[24].

G.l.c.–m.s. was carried out with a JGC-20 K gas chromatograph fitted with column *B* or *C* and with helium (inlet pressure, 101.3 kPa) as the carrier gas. Mass spectra were obtained at 23 eV and an emission current of 300 μA, using a JMS-D 100 (JEOL) spectrometer. The inlet temperature was 220° and that of the ionising chamber was 200°.

Descending p.c. was performed on Whatman Nos 1 and 3MM paper, using *E*, ethyl acetate–pyridine–water (8:2:1); and *F*, ethyl acetate–acetic acid–water (18:7:8); and detection with aniline hydrogen phthalate. Uronic acids were determined by the modified carbazole method[25], carbohydrates by the phenol–sulphuric acid method[26], acetyl content by g.l.c. (β-D-glucose penta-acetate as the standard) (column *D*), protein by the Lowry method[27] with bovine serum albumin as the standard, and xyloglucan by the iodine–sodium sulphate method[28].

Proton-decoupled ^{13}C-n.m.r. spectra (75.46 MHz) were recorded in the pulsed F.t. mode with a Bruker AM-300 spectrometer. The spectral width was 17 kHz, pulse width 12 μs, and the number of data points 16k. Methanol was used as the internal standard (50.15 p.p.m.).

Sedimentation analysis (at various concentrations) was performed with an MOM 110 ultracentrifuge. Photographs were taken at intervals of 6 min (50,000 r.p.m.). Diffusion coefficients were calculated by the maximum ordinate method. The partial specific volumes of the polysaccharides were determined from the sum of the specific volumes of the monomers[29,30]. Number-average molecular weights were determined using a Knauer Membrane Osmometer with a two-layered membrane.

Analysis of the component sugars. — Samples (~3 mg) of CWM and polysaccharide *E* (~3 mg) were each suspended in aqueous 72% sulfuric acid (0.2 mL) for 1 h at 30°. Each mixture was then diluted with water (5.6 mL), heated for 6 h at 100°, cooled, neutralised ($BaCO_3$), filtered, treated with Dowex 50W(H^+) resin, and concentrated.

The polysaccharide obtained after fractionation was heated with aqueous 90% formic acid (0.4 mL) for 7 h at 100°. After evaporation of the acid, sugars were determined as alditol trifluoroacetates by g.l.c.

Oligosaccharides (~1 mg) were hydrolysed with aqueous 90% formic acid (0.2 mL) for 2 h at 100°.

Plant material. — The green callus tissue, isolated from the cambial region of the stem of *Populus alba L.* var. pyramidalis, had been maintained in culture since March 1979, and the callus explants had been subcultured in a basal medium according to Diaz-Colon *et al.*[31]. The explants were cultured in 0.8% agar at 20 ±1° in the dark or diffuse light. The tissue cultures were harvested at the stationary phase of growth and the cell walls, obtained as described previously[5], contained (%) lipids (4.7), uronic acid (19.2), protein (12.1), lignin (Klason; 4.8), OAc (1.5), Gal (6.4), Glc (33.1), Man (1.8), Ara (9.7), Xyl (8.7), Rha (1.3), and Fuc (1.5).

Fractionation of the cell-wall material (CWM). — The CWM was exhaustively extracted with chloroform–methanol (2:1), then air-dried. The lipid-free material,

suspended in 0.05M phosphate buffer (pH 6.9, 100 mL) containing sodium azide (5mM) as preservative, was incubated with alpha-amylase [pancreas (Sigma), 100 μg/mL] for 48 h at 37° in the presence of toluene. The CWM (18 g) was depectinated by extraction (g/100 mL) twice with water [5 h, 95° (bath)], and then twice with aqueous 0.5% ammonium oxalate (1 h, 90°). The insoluble material (14.0 g) was treated with acidified sodium chlorite solution [acetic acid (0.3 mL) and sodium chlorite (0.9 g) in water (100 mL)] for 2 h at 35°, and the resulting material (11.2 g, 64% of the CWM) was extracted (4 h, 25° under N_2) successively with aqueous 15% potassium hydroxide and then aqueous 17.5% sodium hydroxide containing 4% of boric acid (400 mL each). Each extract was adjusted to pH 6 with acetic acid, dialysed, and freeze-dried, and the residue was washed with water and acetone, then dried *in vacuo* to give the α-cellulose. The yields and gross compositions of these fractions are detailed in Table I.

Xyloglucan. — (*a*) *Isolation.* A solution of fraction *C* (Table I, 1850 mg) in 2mM potassium acetate (80 mL) was applied to a column (40 × 4 cm) of DEAE-Sephadex A-50 (AcO^- form) and eluted stepwise at 45 mL/h with potassium acetate (2mM, 0.05, 0.1, and 0.5M; 500 mL each) and 0.3M potassium hydroxide (500 mL). Each eluate was dialysed and freeze-dried (see Table II). The xyloglucan-rich (at 640 nm) fractions *1* and *3* were used for further study.

Fraction *1* (545 mg) was fractionated further by precipitation from aqueous solutions (50 mL) with Fehling's solution[9] (30 mL), dispersal of the resulting precipitate in water (100 mL), addition of 0.2M hydrochloric acid, and precipitation with ethanol, to give XG-1 (260 mg). Repetition of this procedure gave no further change in sugar composition. Data on XG-1 are given in Table III. A solution of fraction *3* (490 mg) in water (2 mL) was applied to a column (2.5 × 100 cm) of Sephadex G-75 (40–120 μm) which was irrigated with water. Assay of the eluate for total carbohydrates revealed two polysaccharides. The xyloglucan-rich fraction was collected, dialysed, and freeze-dried, and the crude xyloglucan (300 mg) was treated with Fehling's reagent, as described above, to give XG-2 (250 mg) (see Table III). Sedimentation analyses and electrophoretic mobilities confirmed the homogeneity of XG-1 and XG-2 (Table III).

(*b*) *Partial hydrolysis.* — Each polysaccharide was treated for 2 h at 100°, first with aqueous 10% formic acid (20 mL) and then with aqueous 20% formic acid. The hydrolysate was concentrated after each treatment and the part to be hydrolysed was precipitated with ethanol. The low-molecular-weight fragments (450 mg) were eluted from a column (2.5 × 100 cm) of Sephadex G-15 with water to give glucose, xylose, galactose, fucose, small amounts of mannose and arabinose, and a mixture (110 mg) of di-, tri-, and tetra-saccharides. P.c. of the last mixture on Whatman No 3MM paper yielded components with R_{Gal} 0.36 (solvent *E*), 0.84, 0.73, 0.61, and 0.51 (solvent *F*), each (~3 mg) of which was methylated with methyl iodide (2 mL) and sodium hydride (15 mg) in *N,N*-dimethylformamide (2 mL). The products were identified by m.s.

Compound *1* (23.6 mg), R_{Gal} 0.84 (solvent *F*), $[\alpha]_D$ +64° (*c* 1.9, water), gave

Xyl and Glc (molar ratio 1:1) on hydrolysis. The mass spectrum of methylated *1* contained characteristic peaks of ions abJ_1 (*m/z* 279), bA_1 (219), and aA_1 (175). The calculated mol. wt. of 410 and the intense ions abD_1 at *m/z* 309, respectively, proved the (1→6)-linked pentose–hexose structure[32].

Compound *2* (15 mg), R_{Gal} 0.36 (solvent *E*), $[\alpha]_D$ +34.5° (*c* 0.9, water), gave Glc on hydrolysis. The characteristic[16] fragment ions abJ_1 (*m/z* 279), aA_1 and bA_1 (219), and the ratio of peak intensities at *m/z* 161/159 in the mass spectrum of methylated *2* identified *2* as a (1→4)-linked hexose disaccharide.

Compound *3* (7 mg), R_{Gal} 0.73 (solvent *F*), $[\alpha]_D$ +8.2° (*c* 0.4, water), gave Xyl and Gal (molar ratio 0.9:1) on hydrolysis. The mass spectrum of methylated alditol (NaB^2H_4) of *3* contained peaks of ions bA_1 (192) and aA_1 (219) and an ion at *m/z* 133 which identified[33] *3* as a (1→2)-linked hexose–pentose disaccharide.

Compound *4* (12.8 mg), R_{Gal} 0.61 (solvent *F*), $[\alpha]_D$ +45° (*c* 1, water), gave Xyl and Glc (molar ratio 1:2) on hydrolysis. The mass spectrum of methylated *4* contained characteristic peaks of ions $abcJ_1$ (*m/z* 483), bcA_1 (423), bcJ_1 (279), cA_1 (219), and aA_1 (175), which proved[16] a pentose–hexose–hexose structure. The mass spectrum of the methylated trisaccharide-alditol (NaB^2H_4) of *4* contained intense peaks of fragments H_1 (*m/z* 88), bcA_1 (440), and bcJ_1 (236), which characterise[33] the (1→6) linkage between units *a* and *b*. The methylated trisaccharide-alditol was hydrolysed with 0.1M hydrochloric acid and the products were converted into alditol acetates. G.l.c.–m.s. then identified 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methyl-D-glucitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol, and 1-*O*-acetyl-2,3,4-tri-*O*-methyl-D-xylitol. Thus, *4* is Xyl*p*-(1→6)-Glc*p*-(1→4)-Glc*p*.

Compound *5* (9.6 mg), R_{Gal} 0.51 (solvent *F*), $[\alpha]_D$ +44° (*c* 0.87, water), gave Gal, Xyl, and Glc on hydrolysis. The mass spectrum of methylated *5* contained intense ions of the A_1 series (*m/z* 583, 423, 379, 219, and 175) suited for calculation of the mol. wt. Thus, the mol. wts. of 818 and 774 proved the presence of two hexose- and pentose-containing tetrasaccharides. Methylated *5* was hydrolysed and the products were converted into alditol acetate derivatives. G.l.c.–m.s.[15] then identified 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-D-xylitol, 1,5,4-tri-*O*-acetyl-2,3-di-*O*-methyl-D-xylitol, 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol, and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol. These findings, together with the fact that the mass spectrum of methylated *5* contained all the peaks of ions J, except that of *m/z* 235, provide evidence[16] that *5* consisted of at least two tetrasaccharides.

(*c*) *Mild acid hydrolysis*. XG-2 (15.4 mg) was treated with 10mM oxalic acid (4 mL) for 90 min at 100°. The solution was dialysed and freeze-dried to give XG-3 (14.4 mg), hydrolysis of which yielded Fuc, Gal, Xyl, and Glc in the molar proportions 3.8:10.7:35.9:49.6.

(*d*) *Methylation analysis*. Each polysaccharide (~5 mg) was methylated once by the Hakomori method[34] and twice by the Purdie method[35], to give products which had negligible i.r. absorption for hydroxyl. Each methylated polysaccharide

(~6 mg) was treated with aqueous 90% formic acid (1.5 mL) for 1 h at 100°, the hydrolysate was concentrated to dryness, and the residue was hydrolysed with 2M hydrochloric acid (1 mL) for 6 h at 100°. The sugars were converted into their alditol (NaB^2H_4) acetates and identified by g.l.c. and g.l.c.–m.s.[15] (columns *B* and *C*). The results are given in Table IV. The identification of 2,3- and 3,4-di-*O*-methylxylitol derivatives was confirmed by the presence of fragments *m/z* 117, 118, 161, 162, 189, and 190. Their molar proportions were calculated from peak intensities of the corresponding ions.

ACKNOWLEDGMENT

We thank Mr. G. Košický for performing the electrophoretic measurements and the molecular weight determinations.

REFERENCES

1 M. F. Heath and D. H. Northcote, *Biochem. J.*, 125 (1971) 953–961.
2 K. W. Talmadge, K. Keegstra, W. D. Bauer, and P. Albersheim, *Plant Physiol.*, 51 (1973) 158–173; W. D. Bauer, K. W. Talmadge, K. Keegstra, and P. Albersheim, *ibid.*, 51 (1973) 174–187; G. O. Aspinall, J. A. Molloy, and J. W. T. Craig, *Can. J. Biochem.*, 47 (1969) 1063–1070.
3 K. Keegstra, K. W. Talmadge, W. D. Bauer, and P. Albersheim, *Plant Physiol.*, 51 (1973) 188–196.
4 M. Kubačková, Š. Karácsonyi, L. Bilisics, and L. Zákutná, *Drev. Vysk.*, 27 (1982) 1–20; *Chem. Abstr.*, 98 (1983) 50376w.
5 M. Kubačková, Š. Karácsonyi, L. Bilisics, D. Lišková, and F. Janeček, *Drev. Vysk.*, 26 (1981) 1–17; *Chem. Abstr.*, 97 (1982) 36190d.
6 A. G. Darvill, M. McNeil, P. Albersheim, and D. P. Delmer, in N. E. Tolbert (Ed.), *The Biochemistry of Plants*, Vol. 1, Academic Press, New York, 1980, pp. 92–162.
7 B. W. Simson and T. E. Timell, *Cellul. Chem. Technol.*, 12 (1978) 39–50.
8 Y. Takeuchi and A. Komamine, *Physiol. Plant.*, 42 (1978) 21–28.
9 J. K. N. Jones and R. J. Stoodley, *Methods Carbohydr. Chem.*, 5 (1965) 36–38.
10 M. A. O'Neill and R. R. Selvendran, *Carbohydr. Res.*, 111 (1983) 239–255.
11 T. Watanabe, K. Takahashi, and K. Matsuda, *Agric. Biol. Chem.*, 44 (1980) 791–799.
12 Y. Kato and K. Matsuda, *Agric. Biol. Chem.*, 45 (1981) 1–8.
13 T. Hayashi, Y. Kato, and K. Matsuda, *Plant Cell Physiol.*, 21 (1980) 1405–1418.
14 J. Lönngren and A. Pilotti, *Acta Chem. Scand.*, 25 (1971) 1144–1145.
15 P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–75.
16 V. Kováčik and P. Kováč, *Carbohydr. Res.*, 103 (1982) 251–257.
17 Y. Akiyama, S. Eda, M. Mori, and K. Kato, *Phytochemistry*, 22 (1983) 1177–1180.
18 W. S. York, J. E. Oates, H. van Halbeek, A. G. Darvill, P. Albersheim, P. R. Tiller, and A. Dell, *Carbohydr. Res.*, 173 (1988) 113–132.
19 J. P. Joseleau and G. Chambat, *Plant Physiol.*, 74 (1984) 694–700.
20 P. Ruperez, R. R. Selvendran, and B. J. H. Stevens, *Carbohydr. Res.*, 142 (1985) 107–113.
21 S. E. B. Gould, D. A. Rees, and N. J. Wight, *Biochem. J.*, 124 (1971) 47–53.
22 J. M. Labavitch and P. M. Ray, *Plant Physiol.*, 54 (1974) 499–502.
23 W. S. York, A. G. Darvill, and P. Albersheim, *Plant Physiol.*, 75 (1984) 295–297.
24 J. Shapira, *Nature (London)*, 222 (1969) 792–793.
25 T. Bitter and H. M. Muir, *Anal. Biochem.*, 4 (1962) 330–334.
26 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.

27 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265–275.
28 P. KOOIMAN, *Recl. Trav. Chim. Pays-Bas*, 79 (1960) 675–678.
29 R. A. GIBBONS, in A. GOTTSCHALK (Ed.), *Glycoproteins—Their Composition, Structure and Function*, Elsevier, Amsterdam, 1966, pp. 61–95.
30 A. K. ALLEN, N. N. DESAI, A. NEUBERGER, AND J. M. CREETH, *Biochem. J.*, 171 (1978) 665–674.
31 J. D. DIAZ-COLON, R. W. BOWERY, F. S. DAWIS, AND J. R. BAUR, *Physiol. Plant.*, 27 (1972) 60–64.
32 O. S. CHIZHOV, L. A. POLJAKOVÁ, AND N. K. KOCHETKOV, *Dokl. Akad. Nauk SSSR*, 158 (1964) 291–294.
33 J. KÄRKKÄINEN, *Carbohydr. Res.*, 17 (1971) 1–10.
34 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
35 E. L. HIRST AND E. PERCIVAL, *Methods Carbohydr. Chem.*, 5 (1965) 287–296.