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Water-Soluble Polysaccharides of Poplar (*Populus alba* L.) Callus Tissue Cell Wall

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Two water-soluble arabinose-rich polysaccharides have been isolated from the primary wall of poplar (*Populus alba* L.) callus culture cells. Their structures have been investigated using chemical methods and NMR spectroscopy. The general, structural features of the polysaccharides are discussed.

Primary cell walls of plants contain considerable amounts of arabinose-rich polysaccharides as hemicellulosic components or constituents of pectic polymers [1, 2]. The occurrence of these polymers in the cell walls of actively growing cells may assume a certain nonspecified physiological function [3, 4]. In the framework of the research of cell wall polysaccharides of poplar (*Populus alba* L.) callus tissue a water-soluble, arabinose-rich fraction of pectic polymers was isolated [5]. Fractionation and structural characterization of this material is the subject of this report.

EXPERIMENTAL

Material and Methods

Cell walls were isolated from poplar (*Populus alba* L. var. *pyramidalis*) callus culture cells as described [5].

All evaporations were carried out below 40 °C. Spectral data were recorded with a Perkin—Elmer

G 9836 (for IR) and a Specord UV VIS spectrometer. Optical rotations (1 cm³ cell) were measured with a Perkin—Elmer Model 141 polarimeter at (20 ± 1) °C and λ = 585 nm, in water. Free-boundary electrophoresis of polysaccharide solutions (ρ = 10 mg cm⁻³) was performed in 50 mM sodium tetraborate buffer of pH 9.2 with a Zeiss 35 apparatus for 30 min at 150 V and 8 mA. Analytical GLC separations were performed on a Hewlett—Packard Model 5711 A instrument. A stainless steel column (2.0 m × 3 mm) coated with A, 3 % of SP-2340 on Chromosorb WAW-DMCS (150—170 μm) was used with N₂ carrier-gas flow rate 30 cm³ min⁻¹. The temperature program was 120 °C → 250 °C at 4 °C min⁻¹ for the separation of fully acetylated alditols.

GLC-MS analysis was performed on a Hewlett—Packard 5985 A instrument equipped with B, OV-17 capillary glass-column (25 m × 0.21 mm) and the temperature program was 180 °C → 260 °C at 3 °C min⁻¹ with initial hold of 1 min. Paper chromatography was performed on Whatman No. 1 and No. 3 MM papers, using C ethyl acetate—acetic acid—for-

mic acid—water ($\varphi_r = 18 : 3 : 1 : 4$) and detection with anilinium hydrogen phthalate. The mobilities R_{GalA} are expressed relative to that of D-galacturonic acid. Zone-electrophoresis was performed in high-voltage (5 kV) system on Whatman GF 81 glass microfibre paper (34 cm \times 12 cm) in a pyridine—acetate buffer (pH 6.3, containing 10 vol. % pyridine, 0.3 vol. % acetic acid, and 10 mM-EDTA) with application of 40 V cm^{-1} for 50 min. 1-Naphthol—sulfuric acid reagent was used for detection [6]. Total carbohydrate, uronic acid, protein, and acetyl content was determined as described [5].

The ^1H (300 MHz) and ^{13}C (75.13 MHz) NMR spectra were obtained with a Bruker AM-300 FT-spectrometer for solutions (3 mass % in D_2O). Chemical shifts were measured relative to internal HOD ($\delta = 4.8$) and methanol ($\delta = 50.15$), respectively.

The polysaccharides (≈ 5 mg) were methylated once by the Hakomori [7] and twice by the Purdie [8] method to give products showing no IR absorption for hydroxyl. The fully methylated polysaccharides were treated first with aqueous 90 vol. % formic acid (1 cm^3) for 1 h at 100 $^\circ\text{C}$ and then with 2 M hydrochloric acid for 6 h at 100 $^\circ\text{C}$, the hydrolyzates were reduced with NaBD_4 (4 h) and the alditols were acetylated with acetic anhydride—pyridine ($\varphi_r = 1 : 1$) for 1 h at 100 $^\circ\text{C}$. Uronic acid in the methylated sample was reduced with LiAlD_4 (30 mg) in anhydrous tetrahydrofuran (2 cm^3) and the eventual alditol acetates were analyzed by GLC-MS [9]. The results are given in Tables 2 and 3.

The number average molecular mass (\bar{M}_N) was determined by osmotic pressure measurements in water at 35 $^\circ\text{C}$ with a Knauer vapour-pressure osmometer using Dextran T-10 (Pharmacia Fine Chemicals, \bar{M}_N 5700 as standard) and Knauer membrane osmometer with a two-layered membrane (Amicon). The \bar{M}_N values for NA arabinan and the acidic polysaccharide AP were 11900 g mol^{-1} (average DP = 90) and 29200 g mol^{-1} , respectively.

The NA polysaccharide (5.4 mg) was oxidized with sodium periodate (7 cm^3 , $c = 15$ mmol dm^{-3}) at 5 $^\circ\text{C}$ in the dark. Periodate consumption was monitored spectrophotometrically [10]. The consumption of oxidant was complete corresponding to 0.70 mol per sugar residues. After completion of the oxidation, sodium borohydride was added and the product was hydrolyzed with 0.25 M trifluoroacetic acid. After deionization arabinose, glycerol, and traces of erythritol could be detected by GLC (column A) as their acetate derivatives.

Isolation and Fractionation of Polysaccharides

The cell wall material (CWM) was preextracted with chloroform—methanol ($\varphi_r = 2 : 1$), then air-dried. The

extractive-free material, suspended in 50 mM phosphate buffer of pH 6.9 (100 cm^3) containing 5 mM sodium azide as preservative, was incubated with α -amylase (Sigma) ($\rho = 100$ $\mu\text{g cm}^{-3}$) for 48 h at 37 $^\circ\text{C}$. The CWM (24.4 g) was extracted (2.4 dm^3) with water twice at continuous stirring for 2 h at 95 $^\circ\text{C}$. The combined extracts were dialyzed, concentrated, and freeze-dried to give polysaccharide mixture A (980 mg, w_r (per dry mass of CWM) = 4 %) of $[\alpha]_D$ ($\rho = 10.0$ g dm^{-3}) = -32.2° containing 12.5 mass % uronic acid, 5.1 mass % protein, and 2.4 mass % acetyl. Hydrolysis of this material provided arabinose, galactose, xylose, rhamnose, and fucose in the mole ratio $x_r = 10 : 2 : 0.6 : 0.7 : 0.3$ together with traces of glucose (column A).

Paper electrophoresis of polysaccharide mixture A suggested the presence of a small portion of a neutral polymer (hydrolysis of which gave mainly arabinose) and a major portion of an acidic polysaccharide. A solution of polysaccharide A (640 mg) in 2 mM potassium acetate (2 cm^3) was applied to a column (2.5 cm \times 30 cm) of QAE-Sephadex A-50 (AcO^-) and eluted with 2 mM potassium acetate (pH 6.37, 300 cm^3) initially, and then with a linear gradient 2 mM \rightarrow 0.6 M potassium acetate (500 cm^3). Appropriate fractions (7 cm^3) were combined, dialyzed and freeze-dried to yield fraction 1 (136 mg, not retained on the column) and 2 (406 mg), which was eluted with 0.3 M \rightarrow 0.5 M potassium acetate (Fig. 1). Data on these fractions are given in Table 1.

The polysaccharide fraction 1 (130 mg) was suspended in aqueous ethanol (75 vol. %, 10 cm^3) and stirred overnight at ambient temperature. The residue was separated by centrifugation and the ethanol-soluble portion (72 mg), polysaccharide NA, which had $[\alpha]_D$ ($\rho = 10.0$ g dm^{-3}) = -141° gave on hydrolysis arabinose and galactose ($x_r = 11.2 : 1.0$) and traces of xylose. Rechromatography of fraction 2 (130 mg) on a QAE-Sephadex A-50 (AcO^-) column using 0.5 M potassium acetate as eluant gave polysaccharide AP the composition of which remained unchanged (Table 1). The IR spectrum of the polysaccharide had absorption bands at $\tilde{\nu} = 1732$ cm^{-1} , 1618 cm^{-1} , and 1250 cm^{-1} characteristic of ester groups and carboxylate anions. NMR spectra (^1H and ^{13}C) of the AP polysaccharide exhibited signals at $\delta = 2.15$ and 21.53, respectively, due to the presence of OCOCH_3 groups. The homogeneity of NA and AP polysaccharides was confirmed by electrophoretic mobilities (12.5×10^{-5} $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$ and 3.2×10^{-5} $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$, respectively) and gel permeation chromatography.

Acidic Polysaccharide AP — Partial Hydrolysis. The polysaccharide AP (350 mg) was treated twice with 0.4 M trifluoroacetic acid (10 cm^3) for 2 h at 100 $^\circ\text{C}$. The hydrolyzate was concentrated after each treat-

ment and the part to be hydrolyzed further was precipitated with ethanol. The combined hydrolyzates were concentrated to dryness and the residue was fractionated on a column of Dowex 1 × 8 (AcO⁻) resin by elution with water and 3 M acetic acid. The material that eluted with 3 M acetic acid was concentrated to dryness and the residue (41 mg) was chromatographed on a column (1.3 cm × 200 cm) containing Bio-Gel P-4 with water and the disaccharide fraction was further purified by paper chromatography (solvent C) to give two major components *I* ($R_{\text{GalA}} = 0.17$) and *II* ($R_{\text{GalA}} = 0.76$). The compounds *I* and *II* were reduced (NaBD₄) and deionized with Dowex 50W (H⁺) resin. The disaccharide alditols were methylated [7], then purified using Sep-Pak C₁₈ cartridge (Waters Assoc.) and analyzed by MS.

Compound *I*. The ¹H NMR spectrum of the alditol derivative of *I* showed an anomeric signal at $\delta = 5.13$ ($J_{1,2} = 3.4$ Hz) that was assigned to α -linked galacturonic acid residue. MS of the methylated alditol gave the characteristic peaks of aA₁, aA₂, bA₁, and abJ₁ at m/z 233, 201, 250, and 310 which characterized [11] the hexA-hexA-cl-1-d structure. The methylated and carboxyl-reduced alditol was hydrolyzed (90 vol. % formic acid, 2 h at 100 °C) and the products were converted into alditol acetates. GLC-MS [9] then identified 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol and 4,6-di-*O*-acetyl-1,2,3,5-tetra-*O*-methylgalactitol. Thus *I* is α -D-GalpA-(1 → 4)-D-GalpA.

Compound *II*. The ¹H NMR spectrum of the alditol derivative of *II* suggested that galacturonic acid residues had the α -anomeric configuration ($\delta = 5.13$, $J_{1,2} = 3.4$ Hz). The mass spectrum of the methylated alditol of *II* gave the ions aA₁, aA₂, abJ₁, and bA₁ at $m/z = 233, 201, 266, \text{ and } 206$ which characterized the hexA-deoxyhexitol-1-d structure. GLC-MS of the alditol acetates obtained after hydrolysis, reduction, and acetylation of the methylated and

carboxyl-reduced disaccharide alditol, identified 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol and 2-*O*-acetyl-1,3,4,5-tetra-*O*-methylrhamnitol, respectively. Thus, *II* is α -D-GalpA-(1 → 2)-L-Rhap.

Enzyme Treatment of Polysaccharide AP. The polysaccharide AP (10 mg) was deesterified (10 mM sodium hydroxide, pH 12.3, 3 h) and treated with endo- α -1,4-polygalacturonase [12] in 0.1 M sodium acetate buffer of pH 4.2 (2.5 cm³) for 40 h at 25 °C. After neutralization the enzyme was inactivated for 10 min at 100 °C and the polysaccharide was subjected to gel filtration on a column (1.3 cm × 95 cm) of Sephadex G-75 (40–120 μm). The column was eluted with water and fractions of 4 cm³ were collected and analyzed by the phenol–sulfuric acid assay [13].

RESULTS AND DISCUSSION

The hot-water extract of lipid-free and α -amylase treated wall contained polymeric material (w_r per dry mass of the wall) = 4 %) which consisted of D-galacturonic acid (12.5 mass %, degree of esterification 20.4 mass %), arabinose and galactose (mole ratio $x_r = 10 : 2$), and low proportions of xylose, rhamnose, fucose, and glucose. High-voltage paper electrophoresis indicated the polymers to be composed of a low proportion of neutral polysaccharides containing arabinose as the main constituent and of acidic components having different electrophoretic mobilities.

Fractionation of the polysaccharide mixture on a column of QAE-Sephadex A-50 gave two main polysaccharide fractions 1 and 2 (Fig. 1 and Table 1). Subsequent purification of 1 by precipitation with aqueous 75 vol. % ethanol gave a neutral polymer NA composed of arabinose and galactose ($x_r = 11.2 : 1.0$) which was essentially arabinan. Rechrom-

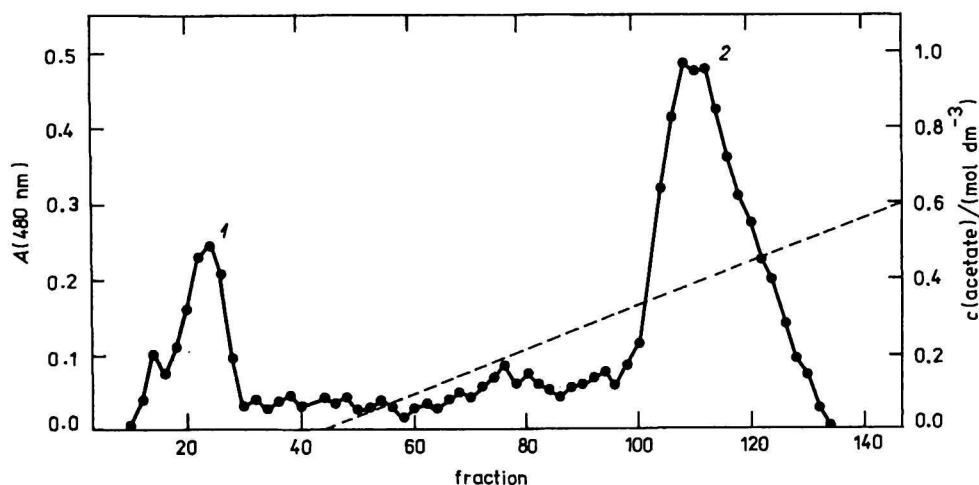


Fig. 1. Chromatography of the water-soluble polysaccharides from poplar cell wall on QAE-Sephadex A-50. — Carbohydrate, --- solvent gradient.

Table 1. Fractionation of Water-Soluble Polysaccharides from *Populus alba* L. on QAE-Sephadex A-50

Fraction	Eluant	Yield ^a %	[α] _D ^o	Mole ratios of monosaccharides						Uronic acid mass %
				Gal	Glc	Ara	Xyl	Rha	Fuc	
1	2.0 mM Potassium acetate	21.2	- 109.0	3.0		8.2	1.0	0.2		
2 ^b	0.5 M Potassium acetate	63.7	- 11.7	3.1	0.2	20.4	1.0	1.8	0.7	15.2

a) Content of the material applied to the column; b) degree of esterification 20.6 %.

atography of the fraction 2 on QAE-Sephadex A-50 with 0.5 M acetate yielded an acidic polysaccharide AP which had negative optical rotation [α]_D = - 11.7° and high content of arabinose (\approx 75 mass % of total carbohydrate) in addition to small amounts of D-galacturonic acid, rhamnose, galactose, and fucose (Table 1). The number average molecular mass (\bar{M}_N) determined osmotically was 29200 g mol⁻¹. Both the polymers NA and AP were homogeneous in electrophoresis and gel permeation chromatography.

The molecular mass of NA arabinan ($\bar{M}_N = 11900$ g mol⁻¹, corresponding to an average DP = 90) was equal to that of arabinans in the bark of *Salix alba* L. [14] and roots of *Althea officinalis* L. [15] but slightly lower than that in the bark of *Rosa glauca* [16].

Glycosyl-linkage composition analysis [9] reflected the highly branched character of the NA arabinan, which consisted of 84 arabinose residues including 24 terminal and 34 internal mainly (1 → 5)-linked ones and to a lesser extent of (1 → 3)- and (1 → 2)-linked arabinose residues. There were also 26 arabinose residues (1 → 2,5)-, (1 → 3,5)-, and (1 → 2,3,5)-linked, constituting branch points of the arabinan framework. Some of the terminal arabinose residues were present in the pyranoid form (Table 2). The periodate consumption of 0.70 mol per sugar residue substantiated the proposed structure of the polymer. In addition to the above linkages, NA

arabinan contained small amounts of (1 → 4)- and (1 → 4,6)-linked galactose residues, the structural significance of which is not clear. They might be an integral part of the arabinan, but the presence of a contaminant β -galactan cannot be excluded [2, 17]. The configuration of the linkages, established by ¹³C NMR measurements (δ), was shown to be α for arabinofuranose ($\delta = 108.6$ and 108.2) and β for galactopyranose ($\delta = 105.5$) residues [18, 19]. These configurations agree with the highly negative optical rotation [α]_D = - 141° of NA arabinan.

The acidic polysaccharide AP was the main component of water-soluble poplar polysaccharides. The glycosyl residue and linkage compositions as well as spectral data of the polysaccharide resembled those of pectic polymers partially substituted by O-acetyl groups [17]. The deesterified AP was degraded slightly by endopolygalacturonase [12] (Fig. 2) suggesting a highly branched "ramified" regions containing rhamnogalacturonan core (rhamnose and galacturonic acid, $x_r = 0.46 : 1$) and side chains that contained mainly arabinose residues.

Hydrolysis of the AP polysaccharide with acid gave acidic oligosaccharides that were isolated by ion-exchange and paper chromatography and characterized by ¹H NMR spectroscopy, mass spectrometry, and linkage-composition analysis as α -D-GalpA-(1 → 4)-D-GalpA and α -D-GalpA-(1 → 2)-L-Rhap, the common structural components of pectic polymers [17].

Table 2. Glycosyl-Linkage Composition of *Populus alba* L. Arabinans

Glycosyl residue	Position of O-methyl groups	$t_{R,r}$ ^a	Mole ratio		Linkage	Number of sugar residues in NA
			NA	AP		
Ara	2,3,5	0.64	1.00	1.00	T(f) ^b	24
	2,3,4	0.77	0.08	tr	T(p)	
	3,5	0.94	0.06		2	
	2,5	1.03	0.22		3	34
	2,3	1.14	1.27	1.10	5	
	2	1.46	0.95	0.96	3,5	23
	3	1.77	tr ^c	0.15	2,5	
	none	1.64	0.12	0.06	2,3,5	3
Gal	2,3,6	1.50	0.22		4	4 or 5
	2,3	1.88	0.10		4,6	

a) G.l.c. retention time relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylglucitol (column B); b) T = a nonreducing terminal residue; c) tr = traces.

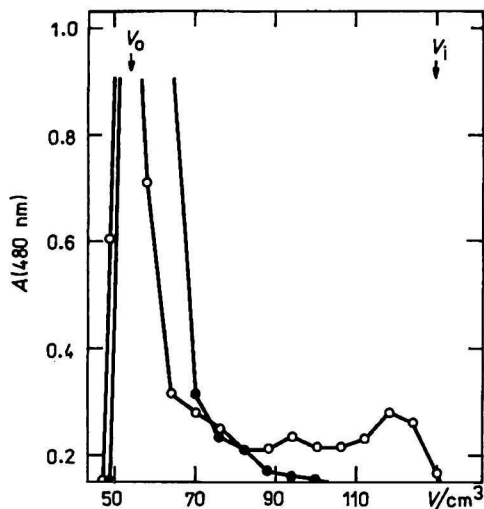


Fig. 2. Elution profiles from Sephadex G-75 for polysaccharide AP before (●) and after (○) digestion with endo-1,4-polygalacturonase; V_0 — void volume, V_i — included volume.

Linkage compositions of the methylated AP polysaccharide were determined both before and after carboxyl reduction by GLC-MS [9]. The results (Table 3) showed that AP contained terminal, 5-, 3,5-, 2,5-, and 2,3,5-linked arabinose, terminal, 3-,

4-, 6-, 3,6-, and 4,6-linked galactose, 2- and 2,4-linked rhamnose, 4- and 3,4-linked glucose, and terminal xylose residues. AP polysaccharide after reduction (Table 3) also contained 4-, 2,4-, and 3,4-linked 6,6'-dideuterated galactose residues originating from galacturonic acid residues. The configurations of the anomeric linkages were the same as those found in pectic polymers from plant cell walls [2, 17]. In addition, weak signals at $\delta = 21.53$ and 2.15 in ^{13}C and ^1H NMR spectra, respectively, corroborated the presence of acetyl groups.

From these results it may be concluded that (1→4)-linked α -D-galacturonic acid and (1→2)-linked α -L-rhamnose residues in the mole ratio about 2 : 1 formed the rhamnogalacturonan core of AP polysaccharide. The various side chains were 4-linked to approximately 80 % of the rhamnose (4 of every 5) and 3- or 2-linked to 23 % of the galacturonic acid (approximately 1 of every 5) residues. Recently, some pectic polymers [20–22] containing branched galacturonic acid residues have been obtained from plants, indicating that galacturonic acid residues might be points of attachment of side chains in rhamnogalacturonans.

The arabinan side chains consisted of (1 → 5)-linked arabinose residues substituted mainly at O-3

Table 3. Glycosyl-Linkage Composition of Acidic Polysaccharide AP

Glycosyl residue	Position of O-methyl	$t_{R,r}^a$	Linkage	x/mole % ^b	
				A ^c	B ^c
Ara	2,3,5	0.63	T ^d	22.0	18.6
	2,3	0.92	5	24.3	20.5
	2	1.16	3,5	21.1	17.8
	3	1.18	2,5	3.3	2.7
	none	1.27	2,3,5	1.4	1.7
Xyl	2,3,4	0.70	T	1.3	tr ^e
	2,3	0.93	4	tr	
Gal	2,3,4,6	1.05	T	3.4	3.3
	2,3,6	1.29	4	2.9	1.8
	3,4,6	1.33	2	tr	
	2,4,6	1.36	3	5.8	5.1
	2,3,4	1.43	6	1.0	1.9 ^f
	2,6	1.52	3,4	tr	
	2,3	1.65	4,6	0.6	
	2,4	1.76	3,6	2.5	3.0
	2	1.86	3,4,6	tr	
	3	1.91	2,4,6	tr	
Rha	3,4	0.86	2	1.4	1.5
	3	1.15	2,4	5.9	5.4
Glc	2,3,6	1.30	4	0.4	tr
	2,6	1.54	3,4	1.3	1.5
Fuc	2,3,4	0.73	T	1.4	1.0
GalA ^g	2,3		4		10.6 ^h
	2		3,4		2.3
	3		2,4		1.0

a) G.l.c. retention time relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylglucitol (column B). b) Derivatives of the composition lower than 0.4 mole % were omitted and the data were normalized to 100 mole %. c) A — original AP, B — methylated carboxyl-reduced AP; d) T — a nonreducing terminal residue; e) tr — the content lower than 0.4 mole % detected; f) small amounts of 6,6-dideutero derivatives were included; g) analyzed as 6,6-dideutero glycosyl residues; h) including amounts (≈ 0.6 mole %) of 4,6-linked galactosyl derivative.

and to a lesser extent at O-2 and were branched to a slightly higher degree than NA arabinan (35.8 % vs. 28.9 %) (Table 2). The basic structure of both neutral and acidic arabinans, however, was similar to that of L-arabinans originating from other plants [2, 17]. Regarding the conditions of isolation and the presence of weak furanosidic linkages in the polysaccharide structure, the neutral NA arabinan might originate from a complex heteropolysaccharide. Thus, its occurrence in the poplar cell wall in a form of native homopolymer remains to be established.

The galactose containing chains consisted of highly branched $\beta(1 \rightarrow 3,6)$ -linked galactooligosaccharides together with lesser amounts of $\beta(1 \rightarrow 4,6)$ -linked oligomers. The average length of the side chains of both structures was about 4 units by comparing the ratio of terminal nonreducing galactose residues to internal galactose residues in the side chains. This value was in close agreement with that evidenced for pectic polymers isolated from flax [23] but lower than that in the cell walls of the suspension cultured *Acer pseudoplatanus* [24] and *Allium cepa* [25] cells (DP 6 and 7–8, respectively). Selective cleavage of the galacturonic acid residues of the backbone of AP polysaccharide with lithium in ethylenediamine [26, 27] provided additional evidence [28] that low-molecular mass galactooligomers (DP = 2–7) and high-molecular mass arabinooligomers (DP \approx 20) constituted the acidic polysaccharide complex.

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