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Structural Studies of Arabinogalactan and Pectin from *Silene vulgaris* (M.) G. Callus

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Abstract—Arabinogalactan and pectin (named silenan) were isolated from *Silene vulgaris* (M.) G. callus. Fractionation by ion-exchange chromatography on DEAE-cellulose and digestion with pectinase demonstrated that silenan from *S. vulgaris* callus (80% of D-galacturonic acid) and silenan from the aerial part of the campion *S. vulgaris* are similar: both pectins contain a high quantity of homogalacturonan segments. The NMR spectral data and mass spectrometry of the purified polysaccharide and its fragment obtained by Smith degradation confirmed that the core of the arabinogalactan consisted of the different segments of β-1,3-D-galactopyranan. Some of the β-galactopyranose residues of the backbone are branched at O-6. The side chains of the arabinogalactan were shown to contain residues of terminal and 3-O-substituted β-galactopyranose, terminal α-arabinofuranose and α-rhamnopyranose, and 2-O-substituted α-rhamnopyranose. The α-rhamnopyranose residues in the sugar chain appeared to be 2-O-glycosylated by the β-1,4-D-galactopyranosyl uronic acid residues.

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Key words: arabinogalactans, pectin, *Silene vulgaris*, NMR spectroscopy of polysaccharides, methylation analysis, GLC-MS of methylated sugars, cell culture

Arabinogalactans are widespread as plant constituents. They occur in cell walls as individual components [1, 2] and as constituents of other polysaccharides as follows: gums, pectic substances, and hemicelluloses [2, 3]. Arabinogalactans as well galactans and arabinans are known to be present as side chains of pectic polysaccharides [2, 4]. They appeared to be the parent intermediates in biosynthesis of the ramified region of pectins: they influence pectin structure attachment in the process of biosynthesis to the backbone of the macromolecule—galacturonan.

Polysaccharides produced by plant cell cultures [4] are often used as an available model for studying structure and biosynthesis of polysaccharides. Polysaccharides isolated from cell walls of *in vitro* cultivated cells are suggested to be the same ones as in the intact plant [5, 6]. Callus cultures are highly productive by biomass and polysaccharides synthesized and, in contrast to intact plants, present a homogenous system consisting of cells with primary cell walls mainly [7].

Callus polysaccharides are known to possess immunomodulatory activity [2] similar to the intact plant polysaccharides. Callus cultures may be a suitable model system for elucidation of dependence of physiological activity from structural patterns of plant polysaccharide macromolecules. These cultures may be used for producing physiologically active polysaccharides bearing certain structural features and properties.

The optimal conditions for *Silene vulgaris* (Moench) Garcke callus growth and production of polysaccharides were worked out earlier [8], and arabinogalactan and pectin were isolated from the callus of this plant [9].

Abbreviations: AG-C) crude arabinogalactan from *S. vulgaris* callus; AG-C-1, AG-C-2) fractions obtained by gel permeation chromatography of crude arabinogalactan AG-C; AG (AG-C-2-100)) purified arabinogalactan from *S. vulgaris* callus; AG-S) fragment obtained after Smith degradation of arabinogalactan AG; SV) silenan, pectin from *S. vulgaris*; SV-P) silenan SV fragment resistant toward pectinase enzymatic treatment; SVC) silenan, pectin from *S. vulgaris* callus; SVC-P) silenan SVC fragment resistant toward pectinase enzymatic treatment.

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Qualitative and quantitative changes in polysaccharides during the growth cycle of the callus were elucidated as well an influence of phytohormones and carbohydrates on cell growth and production of polysaccharides [10, 11].

The present paper reveals an elucidation of the structural features of arabinogalactan AG produced by the *S. vulgaris* callus and a comparative study of pectic polysaccharides from the callus (SVC) and those from the intact plant (SV).

MATERIALS AND METHODS

General analytic procedures. The contents of glycuronic acids were estimated using interaction with 3,5-dimethyl phenol in the presence of concentrated H_2SO_4 [12] (a calibration curve was plotted for D-galacturonic acid), the protein contents were determined in the accord with Lowry's procedure [13] (a calibration curve was plotted for BSA); the contents of methoxyl groups were calculated using the method described in [14] (a calibration curve for methanol was used). All the spectrophotometric measurements were carried out on an Ultrospec 3000 instrument (England). Specific optical rotations were determined using a Polartronic MHZ polarimeter (Germany).

Qualitative and quantitative determinations of the neutral sugars as the corresponding alditol acetates were achieved using gas-liquid chromatography (GLC) on a Hewlett-Packard 4890A chromatograph (USA) with flame ionization detector and integrator HP 3395A using a RTX-1 capillary column (0.25 mm × 30 m; Restek, USA), the carrier gas was argon, and the following program was used: from 175 (1 min) up to 250°C (2 min) with a rate of 3°C/min. Percents of sugars from the total mixture were calculated from peak areas using coefficients of detector response [1].

Combined GLC and mass spectrometry (GLC-MS) of the partially methylated alditol acetates was run as follows: GLC on a Varian 3300 instrument (England) on a DB-5 capillary column (0.25 mm × 30 m; J2W Scientific), the carrier gas being helium; 5°C/min gradient from 150 to 280°C. MS: a Finnigan MAT ITD-700 ion trap (England), mass range from m/z 44 to 500. Energy of ionizing electrons was ≈70 eV. The temperature of the interface was 220°C, scanning frequency was 1 scan/sec, acquisition delay was 250 sec.

NMR spectra were recorded with a Bruker DRX-500 spectrometer (Germany) using 3-5% oligo- and polysaccharide solutions in D_2O at 303°K (the internal standard was acetone, δ_H 2.225 ppm, δ_C 31.45 ppm). The signal assignments in NMR spectra were made on the basis of two-dimensional (2D) spectral data (COSY, TOCSY, ROESY, and ^1H-,^{13}C HSQC with proton detection) as described earlier [15-18]. 2D spectra were performed using the standard Bruker procedures. The ROESY spectra were run using the mixing time 200 msec. A 60 msec duration of the MLEV 17 spin-lock for TOCSY experiments was used. The points of substitution and sequence of the sugar residues were estimated using 2D experiments in estimation of Overhauser effects (ROESY).

Gel-permeation and ion-exchange chromatography were run on chromatographic equipment (Pharmacia, Sweden). The sugar elution was checked by a reaction of the eluate aliquot with phenol in the presence of concentrated H_2SO_4 [19].

All aqueous solutions were concentrated in vacuum at 40-45°C using a rotor evaporator and centrifuged at 7000-8000*g* for 10-20 min. The samples were lyophilized.

Extraction of polysaccharide fractions from callus of *S. vulgaris*. Callus culture of *S. vulgaris* obtained as described earlier [8] was used as the parent material. A frozen biomass of the callus (2.1 kg) was thermostatted on a water bath at 30°C. The solution obtained was separated by filtration. The residual material was extracted twofold with distilled water at 70°C for 3 h. All the solutions obtained were combined and concentrated, the residual raw material was removed by centrifugation, and the polysaccharide was precipitated with four volumes of 96% ethanol. The precipitate was dissolved in water, dialyzed against distilled water, and lyophilized to furnish the parent crude arabinogalactan AG-C with a yield 6.4% of the air-dried raw material.

Silenan, the pectic polysaccharide, was extracted from the *S. vulgaris* callus with aqueous ammonium oxalate as described earlier [20]. Previously, the residual raw material was treated sequentially with 0.5% aqueous formaldehyde and with aqueous hydrochloride at pH 4.0 and 50°C for 3 h. The residual material was treated with 0.5% aqueous ammonium oxalate. The combined extract was concentrated, centrifuged, and precipitated with four volumes of 96% ethanol. The precipitate was redissolved in distilled water, dialyzed, and lyophilized. As a result, silenan SVC was obtained with a yield 11.9% of the air-dried parent material.

Fractionation of the parent arabinogalactan AG-C. The crude arabinogalactan AG-C (60 mg) was dissolved in 0.01 M NaCl (2 ml) and the solution was subjected to chromatography on a Sephacryl S-500 column (2 × 85 cm, void volume V_0 = 75 ml, eluent 0.01 M NaCl, elution rate 0.4 ml/min). Fractions corresponding to the separate peaks on the elution curve were combined, concentrated, dialyzed, and lyophilized to afford two polysaccharide fractions as follows: the main one as AG-C-2, yield 37 mg, K_{av} 0.5, and the minor fraction of AG-C-1, yield 5.2 mg, K_{av} 0. The procedure was repeated several times, and as a result fraction AG-C-2 was obtained in sufficient amounts for further investigations.

The fraction AG-C-2 (268 mg) was dissolved in distilled water (50 ml) and separated successively using various ultrafiltration membranes as follows: polysulfone, 100 and 50 kD (Millipore, USA). The fractions were concen-

trated and lyophilized to furnish the purified arabinogalactan AG-C-2-100, yield 184 mg, molecular mass within 100-300 kD, and fraction AG-C-2-50, yield 15 mg, molecular mass within 50-100 kD. AG-C-2-100 was termed AG and used for further structural investigation.

Specific viscosity of the solutions of arabinogalactan and silenan was determined as described earlier [21]. The samples of polysaccharides were dissolved in 0.01 M NaCl, the solutions were filtered, and the specific viscosity ([η], dl/g) was measured at 25°C using the following viscometers: VPG-2 (time of solvent flow is 76 sec) for AG and VPG-4 (time of solvent flow is 90 sec) for silenan SVC.

Molecular masses of arabinogalactan and its fragments AG-C-2-50 and AG-S were estimated as described earlier [22]. The polysaccharide sample (3 mg) was dissolved in 0.15 M NaCl (1 ml) prepared using bidistilled water, and the solution was filtered and subjected to analysis using the following chromatographic system: a SD-200 pump (Dynamax, USA), a Shodex Asahipak GS-620 HQ column (7.6 mm × 30 cm; Shimadzu, Japan) with a Shodex GS-26 7B pre-column (7.6 mm × 5 cm; Shimadzu), CTO-10AS thermostat (Shimadzu), and RID G136A refractometer (Shimadzu). Elution was carried out with 0.15 M NaCl at 40°C with an effluent rate of 0.5 ml/min. Dextran sulfates with molecular masses in ranges 36-50, 400-600, and 1400 kD (Sigma, USA) were employed for column calibration.

Ion-exchange chromatography of silenan SVC on DEAE-cellulose. A sample of silenan (20 mg) was dissolved in 0.01 M NaCl (1 ml) and the solution was chromatographed on DEAE-cellulose (OH^- form, 25 × 3 cm). The column was eluted consecutively with 0.01, 0.1, 0.2, 0.3, and 0.4 M NaCl with a rate of 0.4 ml/min. The fractions corresponding to separate peaks on the elution curve were combined, concentrated, dialyzed, and lyophilized to afford the following polysaccharide fractions: SVC-D-1 (eluted with 0.2 M NaCl, yield 10 mg) and SVC-D-2 (eluted with 0.3 M NaCl, yield 6.3 mg).

Complete acid hydrolysis. Trifluoroacetic acid (TFA; 2 M, 0.5-1.0 ml) containing *myo*-inositol (0.5-1.0 mg/ml) was added to the investigated sample (2-5 mg), and the mixture was thermostatted for 4-6 h at 100°C. Excess TFA was removed by repeated evaporation with methanol. Sugars obtained were identified using descending paper chromatography [18] and GLC as the corresponding alditol acetates [1].

Smith degradation of arabinogalactan AG. AG (91 mg) was subjected to Smith degradation as described earlier [23] to furnish a crude polysaccharide fraction. The fraction was purified using ultrafiltration membranes (polysulfone, 100, 50 kD; Millipore) to afford AG-S as fragment AG, molecular mass within 50-100 kD, yield 49 mg.

Digestion of silenan SVC. SVC (200 mg) was dissolved in water (40 ml) and subjected to saponification as described previously [24]. Endo-polygalacturonase (8 mg, activity 500 U/mg, EC 3.2.1.15; Fluka, Germany) was added to the solution obtained and the mixture was incubated at 37°C under a control of increasing amounts of reducing sugars using the procedure of Nelson and Somogyi [25]. Pectinase was deactivated by boiling at 100°C, and a precipitate obtained was removed by centrifugation. The solution was concentrated and polysaccharides were precipitated with four volumes of 96% ethanol. The precipitate was separated by centrifugation, dissolved in distilled water, and lyophilized. The polysaccharide mixture was dissolved in distilled water and separated on Bio-Gel P-4 (Bio-Rad, USA). Fractions corresponding to the main peak on the elution curve (K_{av} 0.02) were collected, concentrated, and lyophilized to give SVC-P as a fragment of silenan, yield 14.8 mg.

Methylation analysis of arabinogalactan and its fragments. Methylation of arabinogalactan AG and its fragment AG-S was carried out in accord with Hakomori as described previously [1]. The dry sample of permethylated AG obtained was dissolved in tetrahydrofuran (1 ml) and $LiAlH_4$ (5 mg) was added [22] to the mixture followed by treatment as describer earlier [18]. The methylated sugars were converted into the corresponding partially methylated alditol acetates and identified using GLC and GLC-MS.

RESULTS AND DISCUSSION

Isolation of arabinogalactan. The crude arabinogalactan AG-C (Table 1) was isolated from the frozen callus of *S. vulgaris*. The main constituents of the AG-C sugar chain were shown using complete acid hydrolysis to be residues of D-galactose, L-arabinose, and D-galacturonic acid (Table 1). The configuration of the sugar residues was determined using NMR spectral data. In addition, residues of glucose and xylose were present indicating the possible occurrence of stored glucan and/or xyloglucan as representatives of hemicelluloses of the plant cell walls. In addition, AG-C was found to contain protein, which appeared to form a complex with arabinogalactan [3].

Fractionation of AG-C. The parent arabinogalactan AG-C isolated from the callus of *S. vulgaris* proved to be a mixture of bioglycans with various sugar compositions and different contents of protein (Table 1) accordingly to the data of successive fractionation using molecular-sieve chromatography on Sephacryl S-500 followed by ultrafiltration on membranes with pore sizes 100 and 50 kD. The main polysaccharide fraction obtained was found to represent the purified arabinogalactan AG with a molecular masses more than 100 kD, $[\alpha]_D^{20} = -21°$ (*c* 0.4; water). Using HPLC, the purified AG was shown to possess high homogeneity as shown by show the single narrow peak on chromatograms. As can be seen from Table 1, the purified

Table 1. Yields and characterization of arabinogalactan and its fragments

Fraction	Yield, %	Content, %*****							
		GalA	Neutral monosaccharides						Protein
			Gal	Ara	Glc	Xyl	Rha	Man	
AG-C	6.5*	9.1	41.1	8.9	6.8	4.0	4.6	2.1	20.0
AG-C-1	9.0**	5.9	27.4	3.2	4.0	3.5	0.9	1.2	15.0
AG-C-2	61.6**	7.2	51.1	11.0	6.5	4.5	4.7	1.7	6.2
AG (AG-C-2-100)	70.0***	10.6	50.8	9.3	1.5	2.5	3.4	1.1	5.5
AG-C-2-50	6.0***	11.5	25.8	5.2	14.0	10.2	2.7	1.1	10.0
AG-S	53.8****	7.0	84.0	—	—	—	1.0	—	—

* From air-dried plant raw material.
** From fraction AG-C.
*** From fraction AG-C-2.
**** From arabinogalactan AG.
***** Contents of galacturonic acid, neutral sugars, and protein were determined as wt. %.

Table 2. Chemical shifts of the atom resonances in the ^{13}C- and ^{1}H-NMR spectra of arabinogalactan AG

Residue	Chemical shifts (δ, ppm) $^{13}C/^{1}H$ (acetone 31.45/2.225 ppm)					
	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6, 6'
→3)-β-Gal*p*-(1→	103.4/4.58	72.2/3.54	83.2/3.78	69.7/4.12	76.3/3.67	62.5/3.77
	104.3/4.52	72.2/3.54	83.2/3.91	n.d.	76.3/3.69	
	104.7/4.46	72.2/3.56	83.2/3.91	69.8/4.24	76.3/3.69	
	104.8/4.43	72.2/3.54	81.7/3.70	69.8/4.09	76.3/3.69	
→3,6)-β-Gal*p*-(1→	105.2/4.69	71.6/3.78	83.3/3.85	70.0/4.20	75.0/3.91	70.7/3.91;4.03
α-Araf-(1→	110.4/5.24	82.8/4.21	78.2/3.94	85.7/4.13	62.4/3.71;3.82	
→4)-β-Gal*p*A-(1→	103.4/4.56	72.5/3.63	74.3/3.64	78.3/4.66	76.5/4.55	175.6/–
→2)-α-Rha*p*-(1→	99.3/5.14	82.4/4.12	70.9/3.76	73.6/3.43	70.8/4.02	17.5/1.25

Note: n.d., not determined.

AG and the parent crude arabinogalactan AG-C appeared to have a close related correlation of the galactose and arabinose residues. The galacturonic acid contents are corresponding to the usual values of those in arabinogalactans [3].

Fractions AG-C-1 and AG-C-2-50 accompanying arabinogalactan AG and differing in molecular weights appeared to be carbohydrate–protein complexes which contained galactose residues as the main sugar constituents (Table 1). In addition to the residues of galactose, AG-C-2-50 differed in a considerable quantity of glucose and xylose residues occurring in a crude arabinogalactan AG-C also.

The purified arabinogalactan AG was used for further structural studies.

NMR spectroscopy of arabinogalactan AG. The ^{1}H- and ^{13}C-NMR spectra of AG were interpreted (Table 2) using joint analysis of two-dimension correlation spectra TOCSY, COSY, ROESY, HMQC/TOCSY, and HSQC. Analysis of the COSY and TOCSY spectra revealed residues with configuration of β-galactopyranose, α-rhamnopyranose, and α-arabinofuranose as constituents of AG. The correlation peaks H1/H5 in the ROESY spectrum (Table 2) were found for the residues possessing a pyranose configuration.

Some intense signals (103.4, 104.3, 104.7, 104.8, and 110.4 ppm) of the anomeric carbon atoms of the residues of 3-O-β-D-galactopyranose and terminal arabinofuranose, respectively [17], are observed in the resonance region of anomeric atoms of the ^{13}C-NMR spectrum of

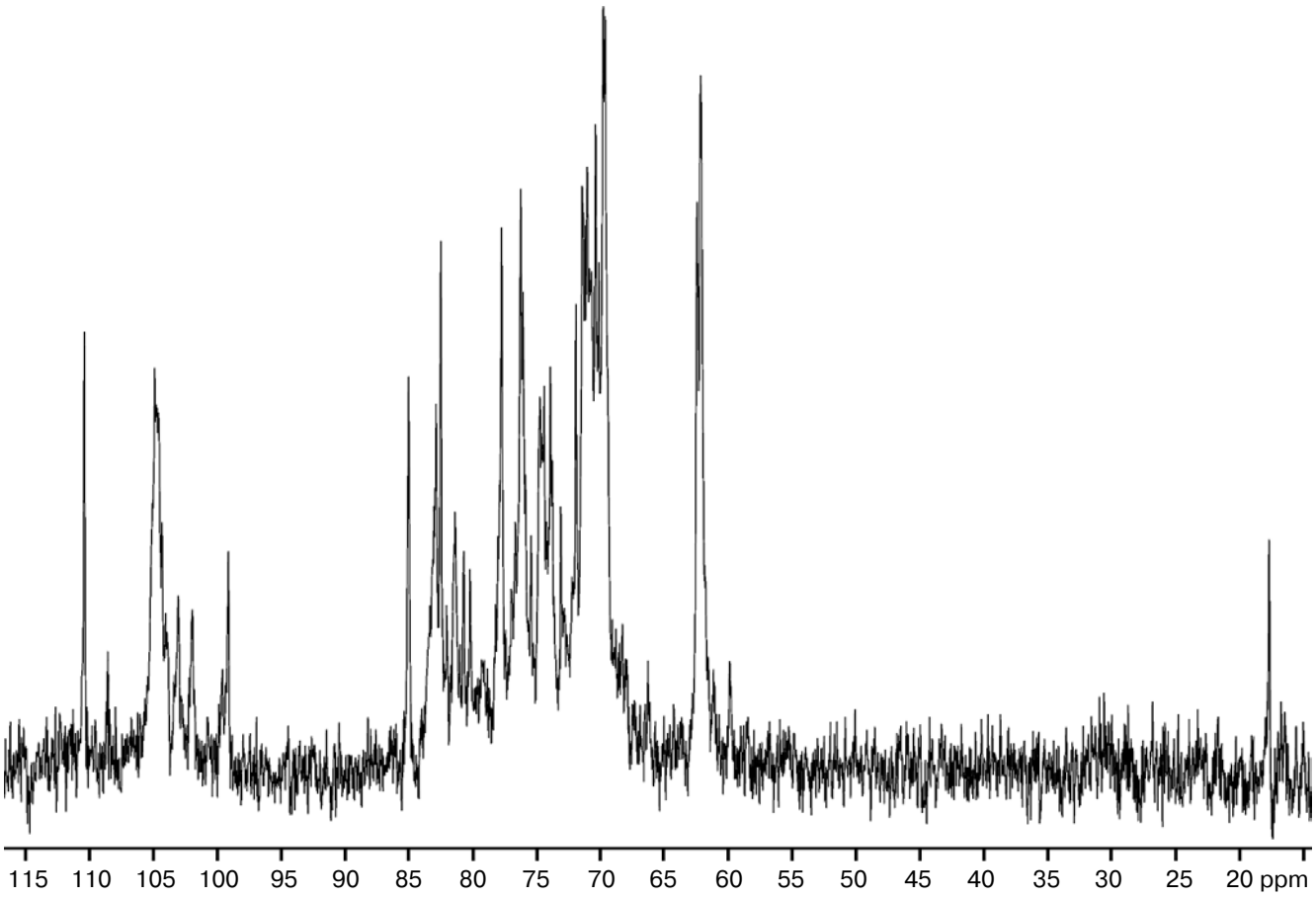


Fig. 1. The ^{13}C-NMR spectrum of arabinogalactan AG.

arabinogalactan (Fig. 1). In the same region of the spectra, signals (99.3, 103.4, and 108.8 ppm) of the residues of α-rhamnopyranose, β-galactopyranosyl uronic acid, and 5-O-α-arabinofuranose are present. The occurrence of small amounts of methyl groups in arabinogalactan as elements of the non-established sugar residues was confirmed by signals of C/H-atoms of the CH_3O-group at 59.4/3.49 ppm in the HSQC spectrum.

An intense *trans*-glycosyl correlation signal between a proton of the anomeric carbon atom and H3 of the galactopyranose residues substituted at C3 (4.58/3.78 ppm) is observed in the ROESY spectrum suggesting the presence of a sugar chain consisting of residues of β-1,3-linked D-galactopyranose in the macromolecule of arabinogalactan.

The *trans*-glycosyl correlation signal between the proton of the anomeric carbon atom of the galactopyranose residues substituted at C3 position and H6,6′ of the galactopyranose residue substituted at 3- and 6-positions (4.46/3.91, 4.03 ppm) is observed in the ROESY spectrum indicating the occurrence of substitutions at 6-position of the β-1,3-linked D-galactopyranose residues.

The intense correlation signal between proton at the anomeric carbon atom of the terminal arabinofuranose residues and H3 of the galactopyranose residues substituted at 3-position (5.24/3.70 ppm) is observed in the ROESY spectrum demonstrating that some arabinofuranose residues are present at the non-reducing points of β-1,3-linked D-galactopyranose chains.

In addition to signal at 99.3 ppm in the resonance region of the anomeric carbon atoms in the ^{13}C-NMR spectrum, signal in high field at 17.5 ppm caused by methyl group (Fig. 1) is obvious indicating the presence of the rhamnopyranose residues in the sugar chain of arabinogalactan. The ^{1}H/^{13}C HSQC spectrum confirms α-configuration of the rhamnopyranose residues (the chemical shift of C5/H5 at 70.8/4.02 ppm) and indicates their substitution at 2-position (C2/H2 82.4/4.12 ppm). An assignment of signals of the D-galactopyranosyl uronic acid residues in the heteronuclear ^{1}H/^{13}C HSQC spectrum (Table 2) was achieved using the data of two-dimensional spectra as described earlier [15, 16].

The *trans*-glycosyl correlation signal between the proton of the anomeric carbon atom of the α-rhamnopyranose residues substituted at 2-position and H3 of the β-galactopyranose residues substituted at 3-position (5.14/3.78 ppm) as well as between H1 of the β-galactopyranosyl uronic acid residues and H2 of the α-rham-

Table 3. Chemical shifts of the atom resonances in the ^{13}C- and ^{1}H-NMR spectra of the arabinogalactan fragment AG-S

Residue	Chemical shifts (δ, ppm) $^{13}C/^{1}H$ (acetone 31.45/2.225 ppm)					
	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
→3)-β-Gal*p*-(1→	104.9/4.45; 104.9/4.44	72.2/3.54; 72.2/3.54	83.4/3.82 83.4/3.82	70.1/3.97; 70.7/4.13	76.5/3.68; 76.2/3.78	62.4/3.78; 62.4/3.78
→3,6)-β-Gal*p*-(1→	105.2/4.70	71.7/3.79	83.4/3.87	69.9/4.22	75.1/3.92	70.7/3.92; 4.04
→4)-β-Gal*p*A-(1→	103.3/4.60	72.3/3.57	74.3/3.66	78.6/4.67	76.7/4.58	175.8/–

nopyranose residues substituted at 2-position (4.56/4.12 ppm) is observed in the ROESY spectrum. Such fragments of the sugar chain as →2)-α-Rha*p*-(1→3)-β-Gal*p*-(1→ and →4)-β-Gal*p*A-(1→2)-α-Rha*p*-(1→ appeared to occur in the arabinogalactan macromolecule.

Smith degradation of arabinogalactan. Smith degradation of arabinogalactan AG was carried out and the mixture obtained was separated using ultrafiltration membranes to furnish arabinogalactan AG-S with molecular mass 50-100 kD (Table 1).

As can be seen from Table 1, a complete degradation of the arabinofuranose residues is observed as a result of Smith degradation with a simultaneous increasing content of the galactose residues. This change in sugar composition of parent arabinogalactan confirmed the absence of 1,3-linked arabinofuranose residues and the presence of the linear chains consisting of β-1,3-D-galactopyranose residues which appeared to represent the backbone of the macromolecule. Some galactose residues appeared to be branching points and to bear side chains of arabinogalactan.

NMR spectroscopy of AG-S fragments. The data of NMR spectroscopy of AG-S confirmed the above suggestions indicating that the β-1,3-D-galactopyranose residues appeared to be the main constituents of the AG-S sugar chain (Table 3). These data confirmed and added information concerning the structure of arabinogalactan provided by analysis of NMR spectra of the parent polysaccharide.

In the anomeric region of the ^{13}C-NMR spectrum of AG-S (Fig. 2), four main signals are observed for C1 atoms of the following residues: α-rhamnopyranose (99.4 ppm), 4-O-substituted β-galactopyranosyl uronic acid (103.3 ppm), and 3-O-substituted (104.9 ppm) and

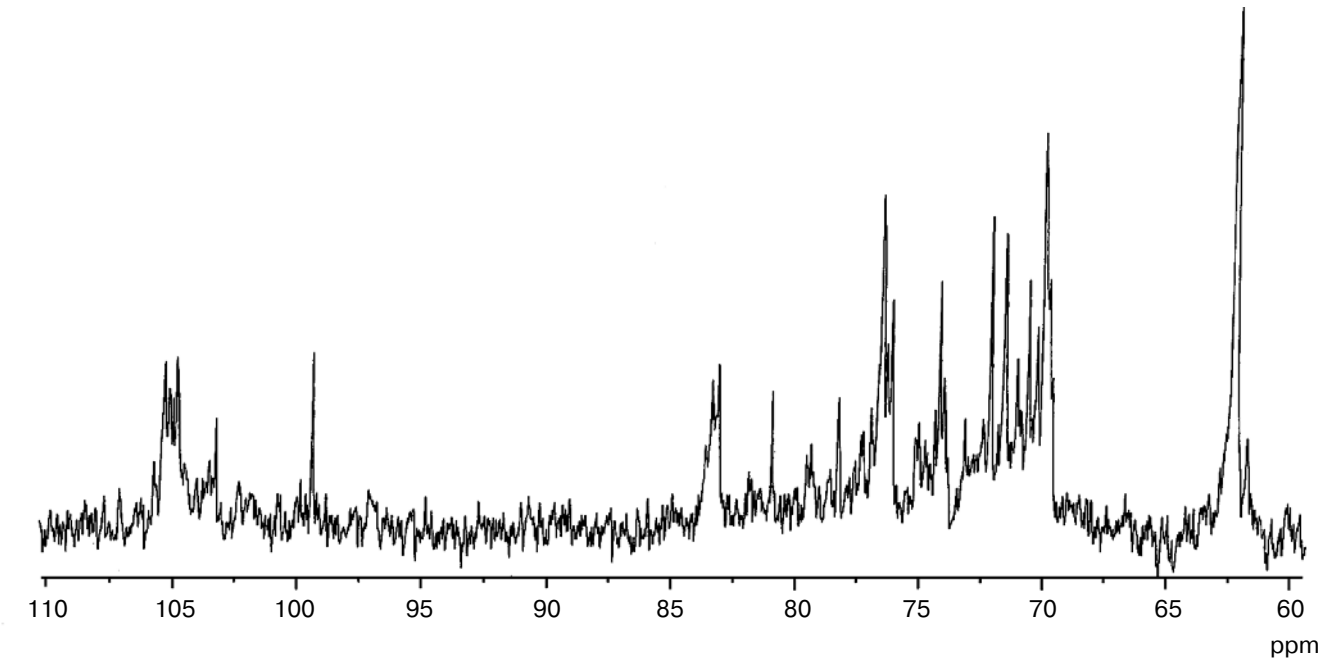


Fig. 2. Part of the ^{13}C-NMR spectrum of the fragment AG-S.

Table 4. Methylation analysis of arabinogalactan and its fragment

Methylated sugars*	Fragments	AG	AG-S
2,3,4-O-Me_3-Rha*p*	Rha*p*-(1→	+	—
2,3,5-OMe_3-Ara*f*	Ara*f*-(1→	++	—
2,3,4,6-Me_4-Gal*p*	Gal*p*-(1→	++	+
2,4,6-Me_3-Gal*p*	→3)-Gal*p*-(1→	++++	+++
2,3,6-Me_3-Gal*p*	→4)-Gal*p*-(1→	+	—
2,3,4-Me_3-Gal*p*	→6)-Gal*p*-(1→	+	—
2,6-Me_2-Gal*p*	→3,4)-Gal*p*-(1→	+	+
2,4-Me_2-Gal*p*	→3,6)-Gal*p*-(1→	+++	++
3,4-Me_2-Gal*p*	→4,6)-Gal*p*-(1→	+	—

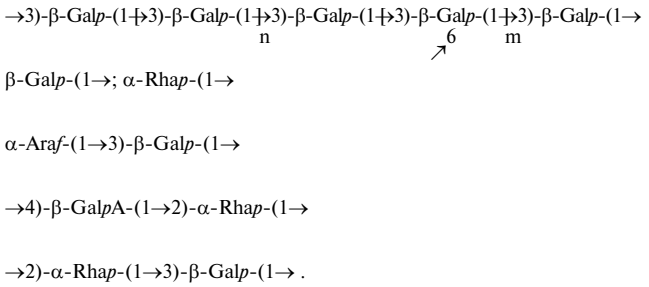
* Identified as the corresponding alditol acetates.

3,6-di-O-substituted (105.2 ppm) β-galactopyranose. The chemical shift at 62.4 ppm is assigned to signal of the $HOCH_2$-group of the galactopyranose residues. Signals with various intensities are present in the anomeric proton resonance region of the ^{1}H-NMR spectrum (Table 3).

In addition to signals of the residues of 3-O-, 3,6-di-O-substituted β-galactopyranose, and 4-O-substituted β-galactopyranosyl uronic acid (Table 3), signals of the α-rhamnopyranose residues (C1/H1 99.4/5.16, C6/H6 17.6/1.25 ppm) were detected in the ^{1}H-,^{13}C HSQC spectrum. The correlation peaks of H1/H5 (Table 3) were found in the ROESY spectrum for the residues of pyranose configuration.

Methylation analysis of arabinogalactan. Methylation studies involving permethylation in accord with Hakomori [1] followed by reduction with $LiAlH_4$ in tetrahydrofuran and acid hydrolysis [22] were used for elucidation of linkages between sugar residues and sizes of sugar circles in arabinogalactan and its fragments. Methylated sugars identified as the corresponding alditol acetates using GLC-MS (Table 4) indicated that the main constituents of the arabinogalactan sugar chain appeared to be 1,3-linked galactopyranose residues. Sugar chains of the macromolecule are highly branched, the branching points are represented by 3,6-di-O-substituted residues of galactopyranose. Therefore, the arabinogalactan appeared to belong to the group of arabino-3,6-galactans [2]. 2,3,5-tri-O-methyl-arabinofuranose and 2,3,4-tri-O-methyl-rhamnopyranose were found among products of arabinogalactan methylation. These data suggest that some arabinose and rhamnose residues are present in arabinogalactan as terminal residues.

Thus, on the basis of all the data of NMR spectroscopy and methylation analysis of AG the presence of the following structural features in the sugar chain of arabinogalactan AG can be suggested:



The presence of 4-O-substituted residues of D-galactopyranosyl uronic acid confirms our suggestion that acidic arabinogalactan isolated from callus culture of *S. vulgaris* is an intermediate product of biosynthesis of callus pectin [9].

Silenan SVC, pectin of the callus of *S. vulgaris*, and its comparison with silenan SV of the intact plant. The procedure described previously [24] was used for isolation pectin from the callus of *S. vulgaris*. As a result, silenan SVC, $[\alpha]_D^{20}$ +220.0° (*c* 0.1; H_2O) was obtained with higher yield than silenan SV obtained from the intact plant [24]. The sugar compositions of both samples of silenan were found to be closely related (Table 5), having high contents of D-galacturonic acid residues (80 and 81%) with low amount of methoxyl groups (2.0 and 1.5%).

As a result of fractionation of silenan SVC on DEAE-cellulose (OH^- form) with 0.01-0.3 M NaCl, silenan SVC was shown to represent galacturonoglycan of insignificant heterogeneity (Table 5) similarly to silenan SV [24]. Both polysaccharides were found to contain a mixture of acidic polysaccharides differing in contents of galacturonic acid residues only and, therefore, in length of sugar chains consisting of these residues.

Like silenan of the intact plant, silenan SVC proved subject to substantial cleavage during digestion with pectinase (endo-α-1,4-D-polygalacturonase). Using PC, considerable quantities of released galacturonic acid and oligogalacturonides were detected in the enzymic lysate. The fragment SVC-P, $[\alpha]_D^{20}$ +53.3° (*c* 0.1; H_2O), resistant to further enzymic treatment and purified using gel filtration on Bio-Gel P-4, was shown to represent 7.4% of the parent silenan only, thus confirming that α-1,4-D-galacturonan appeared to represent the backbone of silenan SVC as in the case of silenan SV. The fragment SVC-P (2.1% methoxyl groups) was found to contain substantial amounts of neutral sugar residues as follows: galactose, arabinose, and rhamnose (Table 5). The data of structural analysis of fragment SV-P [23] suggests that SVC-P and silenan SVC similarly to silenan SV and fragment SV-P contained ramified regions represented by rhamnogalacturonan I (RG I).

Thus, silenan of the callus of *S. vulgaris* proved to be closely related to silenan of the intact plant in relation to structural pattern of the sugar chain; in particular, both pectins are characterized by high contents of the linear α-1,4-D-galacturonan regions.

Table 5. Yields and comparative characteristic of silenans SV and SVC and their fragments

Fraction	Yield, %	Content, %****							
		GalA	Neutral monosaccharides						Protein
			Gal	Ara	Glc	Xyl	Rha	Man	
SV*	2.2**	80.0	2.2	3.4	0.7	0.3	1.7	tr.	11.0
SV-D-1*	7***	43.2	6.4	5.4	2.1	2.2	2.0	1.6	tr.
SV-D-2*	10***	70.4	4.4	4.1	1.1	0.8	2.1	0.7	tr.
SV-D-3*	19***	85.0	2.1	2.3	1.1	2.0	1.5	0.9	tr.
SV-P*	6***	39.2	12.3	9.3	3.6	1.2	5.3	2.3	tr.
SVC	11.9**	81.4	3.6	2.2	0.5	tr.	1.6	0.5	9.0
SVC-D-1	50.0***	66.0	3.3	1.9	3.1	3.4	1.8	1.4	tr.
SVC-D-2	31.7***	79.5	1.4	1.0	tr.	tr.	1.1	tr.	tr.
SVC-P	7.4***	44.0	15.9	9.8	1.3	1.2	7.7	1.4	tr.

Note: tr., trace.
* Data from [24].
** From air-dried raw material.
*** From the parent silenans SV and SVC, respectively.
**** Contents of D-galacturonic acid, neutral sugars, and protein were determined in wt. %.

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