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Structure of Silenan, a Pectic Polysaccharide from Campion *Silene vulgaris* (Moench) Garcke

O. A. Bushneva[1], R. G. Ovodova[1], A. S. Shashkov[2], A. O. Chizhov[2], and Yu. S. Ovodov[1]*

[1]Institute of Physiology, Komi Science Center, The Ural Branch of the Russian Academy of Sciences, Pervomaiskaya ul. 50, Syktyvkar 167982, Russia; fax: (8212) 24-1001; E-mail: ovoys@physiol.komisc.ru or bushneva@physiol.komisc.ru
[2]Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky pr. 47, Moscow 119991, Russia; fax: (7-095) 135-5328; E-mail: shash@ioc.ac.ru or chizhov@ioc.ac.ru

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Abstract—A pectic polysaccharide named silenan, $[\alpha]_D^{20}$ +148.6° (*c* 0.1; H_2O), was isolated earlier from the aerial part of campion, *Silene vulgaris* (Moench) Garcke. Silenan has been shown to contain homogalacturonan segments as "smooth regions" and rhamnogalacturonan fragments as "hairy regions". The present study reveals a generalization of structural features of silenan. Silenan was subjected to enzymic digestion with pectinase, to Smith degradation, and to lithium-degradation to determine the conforming poly- and oligosaccharide fragments of "hairy regions" of silenan. The NMR-spectral data and mass-spectrometry confirmed that the core of the ramified region of silenan consisted of residues of α-rhamnopyranose 2-O-glycosylated with the residues of α-1,4-D-galactopyranosyl uronic acid. The part of the α-rhamnopyranose residues of the backbone are branched at O-4. On the basis of the data, the hairy regions of silenan proved to contain mainly linear chains of β-1,3-, β-1,4-, and β-1,6-galactopyranan and α-1,5-arabinofuranan. The side chains of the ramified region were shown to have branching points represented 2,3-, 3,6-, 4,6-di-O-substituted β-galactopyranose residues.

Key words: campion, *Silene vulgaris*, *Oberna behen*, pectin, silenan, structure, NMR spectroscopy, GLC-MS

Glycanogalacturonan pectic polysaccharides are widespread in aquatic and terrestrial plants [1, 2]. Summarizing data concerning the structure of pectins has resulted in a common structural model demonstrating that pectins consist of macromolecular segments of the linear region such as α-D-galacturonan and rhamnogalacturonan and the ramified regions such heteroglycanogalacturonans as rhamnogalacturonan I, rhamnogalacturonan II, apiogalacturonan, and xylogalacturonan [3, 4]. A peculiarity of pectins from various plants is their fine structural features such as sugar residue kinds, linkages between them, and their attachment to the backbone (core) [5].

The fine structure is suggested to be of primary importance in the ability of polysaccharides to influence the immunity of mammals [6, 7]. Elucidation of not only the main pattern but also of the peculiarity of fine structural features of the polysaccharides is necessary for molecular understanding of the correlation between physiological activity and chemical properties of pectic substances. The search for new physiologically active pectins and studies of their structures to elucidate the influence of structural features on activities are important for this purpose.

Earlier [8], we isolated a pectic polysaccharide named silenan from the aerial part of campion, *Silene vulgaris* (Moench) Garcke (*Oberna behen* (L.) Ikonn) (Caryophyllaceae family), which is a perennial medicinal plant. On screening polysaccharides from plants of the European North of Russia, silenan was shown to stimulate phagocytosis [7, 9]. Silenan enhances oxygen metabolism of peritoneal macrophages on peroral introduction, influencing functional activity of the cell receptors [9]. The stimulatory action of silenan on blood neutrophils and peritoneal macrophages *in vitro* is manifested in increasing secretory function without changes in cell adhesive properties [7].

The sugar chain of silenan was found earlier to contain residues of D-galacturonic acid, arabinose, galactose, and rhamnose as the main constituents [8]. Similarly to the known pectic polysaccharides [3, 4], silenan has a block composition of sugar chain and consists of blocks of the linear galacturonan and rhamnogalacturonan and the ramified regions of rhamnogalacturonan I [10].

The present work is devoted to further elucidation of the fine structural features of silenan using enzyme diges-

* To whom correspondence should be addressed.

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tion and lithium cleavage followed by structural analysis of the fragments obtained using NMR spectroscopy and methylation analysis.

MATERIALS AND METHODS

Isolation of silenan SV. Silenan SV was isolated from the fresh aerial part of the plant as described earlier [8]. Plant material was harvested during the flowering period of campion in the Botanical Garden of Syktyvkar State University (Komi Republic, Russia).

General analytical methods. Glycuronic acid contents were determined using interaction of polysaccharide fractions with 3,5-dimethylphenol in the presence of concentrated sulfuric acid [11] using a standard curve for D-galacturonic acid. The total protein contents were estimated according to the Lowry method [12] using a standard curve for BSA. All the spectrophotometric measurements were run on an Ultrospec 3000 instrument (England). The specific optical rotations were determined using a Polatronic MHZ polarimeter (Germany).

Gel permeation chromatography was carried out on a Bio Gel P-4 column (1.4 × 55 cm, void volume 27 ml, total volume 80 ml); distilled water was used as the eluent with flow rate of 0.3 ml/min. The sugar elution was monitored by the reaction of eluate aliquots with phenol in the presence of concentrated H_2SO_4 [13].

NMR spectra were recorded with a Bruker DRX-500 spectrometer (Germany) using 3-5% oligo- and polysaccharide solutions in D_2O at 313°K; the internal standard was acetone, δ_H 2.225 ppm, δ_c 31.45 ppm. Two-dimensional spectra were performed using the standard Bruker procedures.

Qualitative and quantitative analysis of the neutral monosaccharides as the corresponding alditol acetates were carried out using GLC. GLC was performed on a Hewlett-Packard 4890A instrument (USA) using a flame-ionization detector and a HP 3395A integrator on a RTX-1 capillary column (0.25 mm × 30 m, Restek) with argon as carrier gas using the following temperature program: isothermal at 175°C for 1 min, then 3°C/min gradient up and isothermal at 250°C for 2 min. The percentage of sugars in the sample was calculated from the peak areas using response factors [14].

Methylation was carried out in accord with the Hakomori procedure as described earlier [14]. The methylated sample was dried and dissolved in tetrahydrofuran (1 ml) and $LiBH_4$ (5 mg) was added. The mixture was heated at 70°C for 1 h, neutralized with 10% acetic acid (200 μl) in methanol, dialyzed, and lyophilized [15]. The sample obtained was repeatedly methylated in accord with Hakomori [14]. The material obtained was hydrolyzed with 2 M TFA (0.5-1.0 ml) at 100°C for 5 h. Acid was evaporated with methanol, and the methylated sugars obtained were transformed into alditol acetates [15] and determined using GLC and GLC-MS.

GLC-MS of the partially methylated alditol acetates was performed on a Carlo Erba 4200 instrument with an Ultra-1 capillary column (0.2 mm × 30 m, Hewlett-Packard); the carrier gas was helium; 5°C/min gradient from 150 to 280°C. MS: a Finnigan MAT ITD-700 ion trap, 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. The percentage of the methylated sugars was estimated as ratios of the peak areas (total ion current).

All aqueous solutions were concentrated in vacuum at 40-45°C followed by centrifugation at 7000-8000*g* for 10-20 min. The sugars obtained were subjected to lyophilization.

Enzymic digestion of silenan SV after preliminary saponification. Silenan (500 mg) was dissolved in water (75 ml), and 2 M NaOH (25 ml) was added. The solution was maintained for 2 h at 20°C. Excess alkali was neutralized to pH 4.5 with acetic acid. The precipitate obtained was separated by centrifugation. Pectinase (20 mg, Ferak, Germany; protein content 6.6 mg, activity 56.7 U/mg) was added to the supernatant, and the solution was incubated at 37°C. Hydrolysis was monitored by measuring the quantity of reducing sugars according to the procedure of Nelson and Somogyi [16]. Pectinase was deactivated by heating at 100°C, and the precipitate obtained was removed by centrifugation. The solution was concentrated and the sample precipitated with 4 volumes of 96% ethanol. The precipitate was separated by centrifugation, dissolved in 1 ml distilled water, and purified by chromatography on Bio Gel P-4 resulting in fraction SVP-1 (yield 38 mg, K_{av} 0).

Enzymic digestion of SVP after preliminary saponification. Fraction SVP was obtained as described earlier [8] on a digestion of silenan omitting preliminary saponification. Fraction SVP (66 mg) was dissolved in water (6 ml), 2 M NaOH (2 ml) was added, and the solution was kept for 2 h at 20°C. Excess alkali was neutralized with acetic acid to pH 4.5. Pectinase (6 mg) was added, the solution was treated as above. The precipitate obtained on adding 96% ethanol was separated by chromatography on Bio Gel P-4 to furnish fraction SVPP (yield 25 mg, K_{av} 0.02).

Cleavage of fraction SVP with lithium. Fraction SVP (114 mg) dehydrated previously in a Fischer pistol *in vacuo* over P_2O_5 at 40°C was suspended in ethylene diamine (18 ml) for 2 h. Lithium (15 mg) was added to the solution. After appearance of blue color, the reaction mixture was kept for 1 h. Reaction was monitored colorimetrically [17]. The reaction was stopped by adding cold water (30 ml) followed by cooling the mixture in ice. All procedures were carried out with constant rigorous mixing of the reaction mixture. Ethylene diamine was

removed by evaporation *in vacuo* at 30°C, adding toluene (1 ml) several times. The precipitate obtained was dissolved in water on cooling in ice. The solution was passed through a column with Amberlite IR-120 (H^+-form; distilled water was used as eluent, 60 ml). Elution of sugar was monitored using the reaction of aliquots with phenol in the presence of concentrated sulfuric acid [13]. The fraction after lithium cleavage was obtained with yield 62 mg. The fraction obtained (20 mg × 3) was separated using chromatography on Bio Gel P-4 yielding two oligosaccharide fractions as follows: SVPL-1 (yield 6.2 mg, K_{av} 0) and SVPL-2 (yield 12.5 mg, K_{av} 0.5).

RESULTS AND DISCUSSION

Isolation of silenan SV. Silenan was isolated from the aerial part of campion as described earlier [8]. The data of qualitative and quantitative analysis of silenan SV sugar composition are listed in Table 1.

Digestion. Digestion of silenan with pectinase resulted in fraction SVP and a large quantity of galacturonic acid (more than 50% of the silenan was digested) confirming the presence of substantial regions of the linear α-1,4-D-galacturonan in its composition [10]. Preliminary saponification of SVP with 0.5 M NaOH to eliminate methoxyl groups (3.2%) followed by digestion with pectinase of demethoxylated SVP led to its further cleavage resulting in a fraction resistant to the action of this enzyme. Gel filtration of the fraction on Bio Gel P-4 gave rise to fragment SVPP (Table 1) purified from low molecular weight admixtures and devoid of methoxyl groups, $[\alpha]_D^{20}$ +49.2° (*c* 0.1, H_2O). Therefore, the methoxylated residues of D-galacturonic acid of silenan occurred mainly in the linear region of α-1,4-D-galacturonan.

Similar results were obtained on digestion with pectinase of the parent silenan (2.0% methoxyl groups) after preliminary saponification. As a result, fragment SVP-1 was isolated (Table 1), $[\alpha]_D^{20}$ +42.1° (*c* 0.1, H_2O). Fragments SVPP and SVP-1 are similar in relation to specific optical rotation, sugar compositions, and differed in substantially higher contents of neutral sugar residues, especially rhamnose (up to 16%), in comparison with fragment SVP.

Thus, analysis of fragments SVP, SVPP, and SVP-1 demonstrated that the regions of silenan sugar chain resistant to digestion with pectinase represented the ramified region RG-I, as noted earlier [10].

NMR spectroscopy of fragment SVPP. This suggestion is confirmed by the results of studies of fragments SVP [10] and SVPP using NMR spectroscopy. NMR spectra of fragments SVP and SVPP show certain similarities and differences.

A group of intensive signals including a signal at 99.7 ppm, which is assigned to C1 of the α-D-galactopyranosyl uronic acid residue bearing a substitution of C4-atom in the backbone, are observed in the ^{13}C-NMR spectrum (Table 2) of fragment SVPP in the anomeric region [18]. Signals of the similar intensity assigned to carboxyl group of the non-methoxylated galacturonic acid residue are observed in the region of 175.5-175.9 ppm. In addition, the ^{13}C- and ^{1}H-NMR spectra were interpreted using two-dimensional spectroscopy TOCSY, HSQC/TOCSY, HSQC, and ROESY.

Analysis of the COSY and TOCSY spectra of fraction SVPP revealed residues of α-rhamnopyranose in the com-

Table 1. Yields and sugar composition of silenan and its fragments: SVP, SVPP, SVP-1, SVPL-1, SVPL-2, and SVPS

Silenan fragments	Yield, %	Content, %*#						
		D-Gal*p*A	Neutral monosaccharides					
			Rha	Gal	Ara	Xyl	Man	Glc
SV	—	63	2.2	3.2	4.2	1.9	1.1	2.7
SVP	13*	44	4.3	9.3	7.8	1.7	2.9	3.5
SVPP	6*	32	15.1	25.6	11.9	0.9	3.2	1.3
SVP-1	7*	35	15.8	22.1	12.1	1.5	4.2	1.5
SVPL-1	10**	22	11.6	27.6	23.4	trace	trace	trace
SVPL-2	12**	29	24.0	33.0	35.0	trace	trace	trace
SVPS	2.3***	28	5.8	35.6	12.9	1.5	0.9	1.0

* From parent silenan SV.
** From lithium-degraded polysaccharide.
*** From SVP fragment.
*# Content of D-galacturonic acid and neutral sugars is expressed as wt. %.

Table 2. Chemical shifts for the resonances of C-atom signals in the ^{13}C-NMR spectrum of SVPP fragment

Residue	Chemical shifts (δ), ppm (acetone, δ_H 2.225 ppm, δ_C 31.45 ppm)					
	C1	C2	C3	C4	C5	C6
→4)-α-Gal*p*A-(1→	99.7	69.6	69.9	78.4	72.6	175.9-177.9
→2)-α-Rha*p*-(1→	98.8	77.9	71.4	73.2	70.2	17.8
α-Gal*p*A-(1→(2)	98.8	70.6	70.6	72.1	72.6	175.9
→2,4)-α-Rha*p*-(1→	99.7	77.5	70.6	81.6	68.0	17.9
→3)-β-Gal*p*-(1→	104.9	71.4	83.0	69.7	75.8	62.5
β-Gal*p*-(1→	104.7	72.0	74.0	69.9	76.3	62.2
Ara*f*-(1→	109.5	82.6	77.0	85.3	62.4	
Ara*f*-(1→	110.8	82.6	77.0	85.3	62.4	

position of oligosaccharides (Table 3). The presence of the corresponding intensive correlation peaks in the ^{1}H/^{13}C HSQC spectrum (Fig. 1) confirmed the occurrence of considerable numbers of 2-O-substituted α-rhamnopyranose residues in fragment SVPP. The correlation peak of the H2-atom of the rhamnopyranose residue with the anomeric atom of the D-galactopyranosyl uronic acid residue glycosylating the rhamnopyranose residues in the second position is observed in the ROESY spectrum (Fig. 2) at 5.03/3.93 ppm. The data indicate the occurrence of the linear rhamnogalacturonan regions in silenan.

The presence of 2,4-di-O-substituted α-rhamnopyranose residues was ascertained on comparison of the chemical shifts of corresponding signals in the HSQC spectra of fragments silenan (SVPP) and tanacetan, a pectic polysaccharide from tansy *Tanacetum vulgare* [19]. In particular, the correlation peak of C4/H4-atoms at 81.6/3.80 ppm confirming substitution of the 2-O-glycosylated α-rhamnopyranose residues into the fourth position (the centers of signals are shown in Tables 2 and 3) is observed in the HSQC spectra (Fig. 1) of both silenan (SVPP) and tanacetan fragments.

Signals of similar intensity assigned to the residues of the terminal (C1 104.7 ppm) and 3-O-substituted β-galactopyranose (C1 104.9 ppm) are observed in the anomeric area of the ^{13}C-NMR spectrum. The correlation peak of H1/H3-atoms of the terminal and 3-O-substituted β-galactopyranose residues at 4.42/3.86 ppm was detected in the ROESY spectrum (Fig. 2). In addition, the correlation peak of the H1/H3-atoms of the 3-O-substituted β-galactopyranose residues at 4.68/3.86 ppm is present in the ROESY spectrum. These data indicate the occurrence of a disaccharide fragment composed of β-1,3-linked galactopyranose residues. The correlation peaks of H1/H5-atoms of the 3-O-substituted and terminal β-galactopyranose residues at 4.68/3.75 and

Table 3. Chemical shifts for the resonances of the proton signals in the ^{1}H-NMR spectrum of SVPP fragment

Residue	Chemical shifts (δ), ppm (acetone, δ_H 2.225 ppm)					
	H1	H2	H3	H4	H5	H6,6'
→4)-α-Gal*p*A-(1→	5.25	3.83-3.87	3.97	4.40-4.44	4.64-4.69	n.d.
→2)-α-Rha*p*-(1→	5.32	3.93	3.78	3.42	4.02	1.25
α-Gal*p*A-(1→(2)	5.03	3.96	4.05	4.31	4.64	
→2,4)-α-Rha*p*-(1→	5.25	4.12	3.96	3.80	3.79	1.32
→3)-β-Gal*p*-(1→	4.68	3.78	3.86	4.23	3.75	3.78
β-Gal*p*-(1→	4.42	3.55	3.68	3.93	3.70	3.78
Ara*f*-(1→	5.42	4.19	3.92	4.08	3.80; 3.69	
Ara*f*-(1→	5.24	4.21	3.98	4.12	3.83; 3.71	

Note: n.d., not determined.

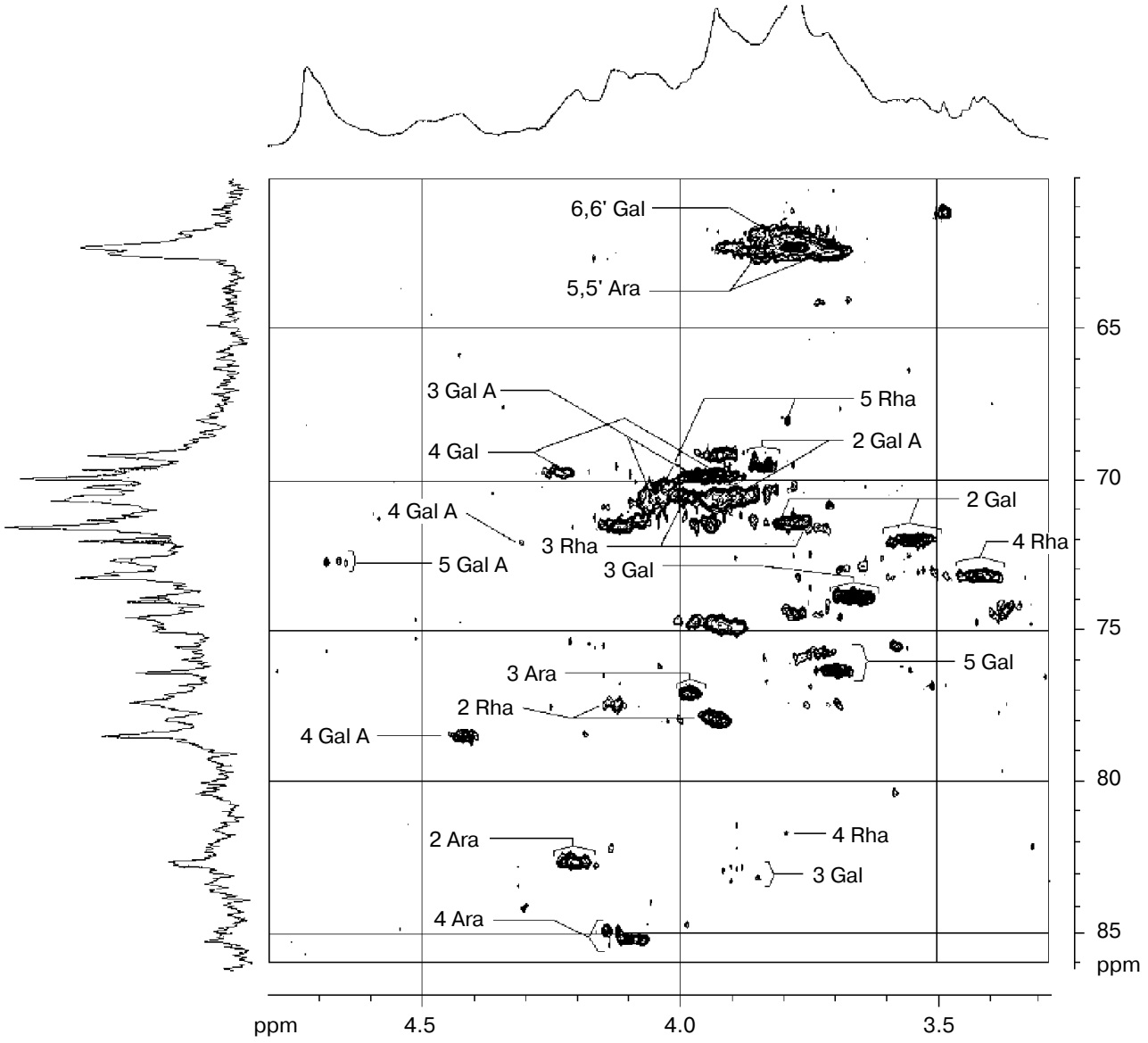


Fig. 1. $^{1}H/^{13}C$ HSQC spectrum of SVPP fragment.

4.42/3.70 ppm, respectively, are also present in the ROESY spectrum.

In the NMR spectra of fragment SVPP, in contrast to fragment SVP [10], signals of α-1,5-linked arabinofuranose residues are absent and only weak signals of β-1,4-linked galactopyranose residues are present. The blocks of sugar chains consisting of β-1,4-linked galactopyranose residues and α-1,5-linked arabinofuranose residues appeared to have lower molecular weight in a comparison with those composed by the residues of β-1,4-linked galactopyranose. In this connection, the low molecular weight fragments remained in the supernatant after repeated digestion with pectinase followed by precipitation of the enzymatic hydrolyzate with 96% ethanol.

Thus, digestion of silenan with pectinase demonstrated that it contained the regions of the linear α-1,4-D-galacturonan and rhamnogalacturonan. In addition, a ramified region is present in its composition. The side chains are α-1,5-linked arabinofuranan, and β-1,3- and β-1,4-linked galactopyranan appeared to be terminated by the α-arabinofuranose residues. The core of the ramified region consists of the residues of α-1,4-linked D-galactopyranosyl uronic acid and α-rhamnopyranose 2-O-glycosylated by the residues of α-1,4-D-galactopyranosyl uronic acid. In addition, the NMR spectral data

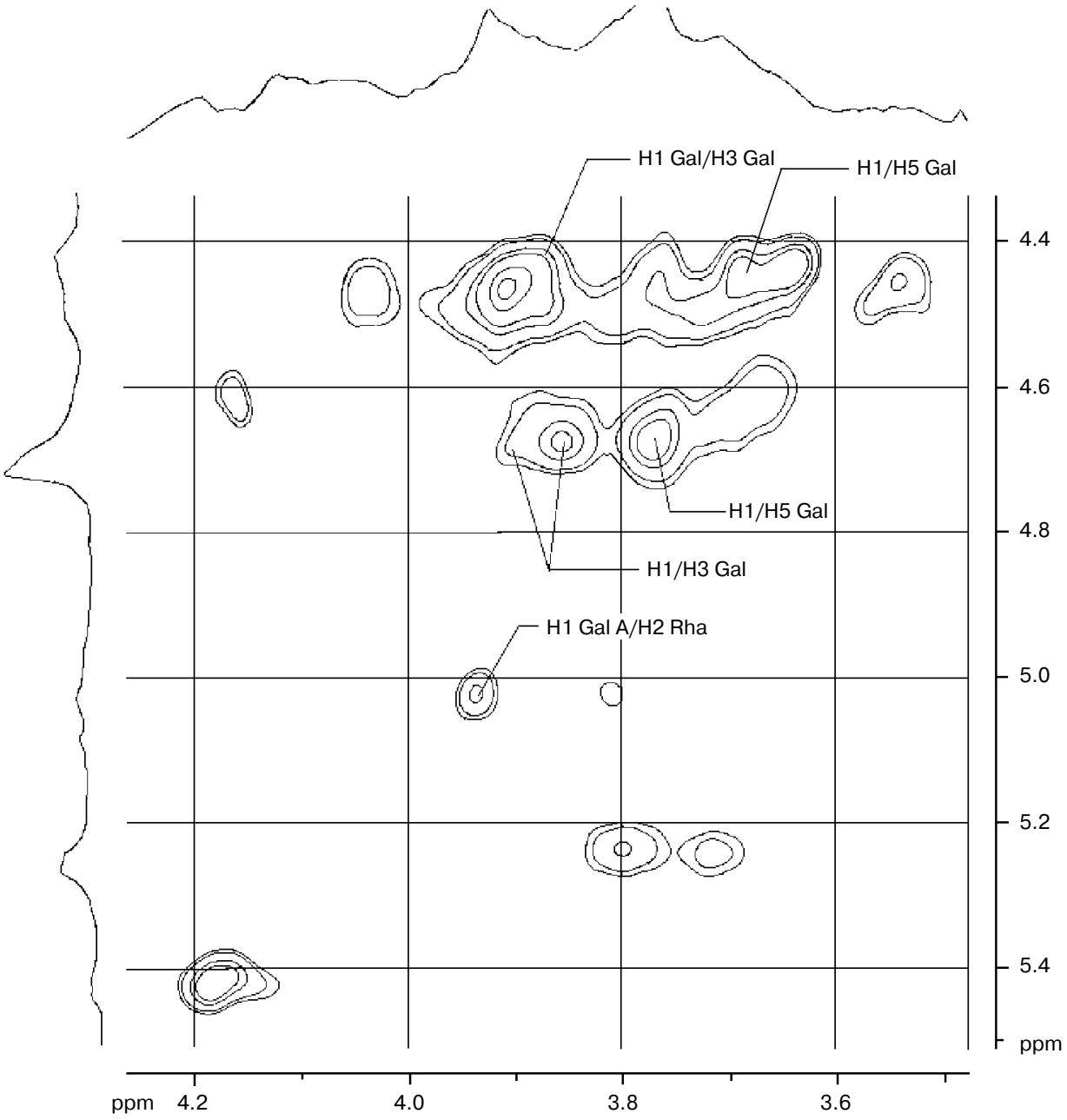


Fig. 2. ROESY spectrum of SVPP fragment.

indicated occurrence of some 2,4-di-O-substituted α-rhamnopyranose residues in the backbone of fragment SVPP. Namely, they appeared to be the branching points of the silenan core bearing the side chains of the macromolecule representing the ramified RG-I blocks.

Cleavage with lithium of fragment SVP. Selective decomposition of the galacturonic acid residues of the RG-I backbone with lithium in ethylene diamine allowed isolation of the neutral segments of the side chains and proof of their bond with core in the parent polysaccharide. Further structural analysis of the isolated oligosaccharides gives rise to additional data concerning the structure of the side chains of the investigated pectin [17]. Lithium cleavage was carried out using fragment SVP obtained from digestion of silenan.

Treatment of SVP with lithium in ethylene diamine [17] provided a mixture of two oligosaccharides with yield 37.8%, fractionated on Bio Gel P-4 into two oligosaccharide fraction: SVPL-1, yield 10%, $[\alpha]_D^{20}$ +39.0° (*c* 0.06, H_2O); and SVPL-2, yield 12%, $[\alpha]_D^{20}$ +37.0°

(*c* 0.06, H_2O). An appreciable decrease in the contents of the D-galacturonic acid residues (to 22-29%) is observed as a result of lithium cleavage of the sugar chain of silenan. The content of the neutral sugar residues, especially galactose, arabinose, and rhamnose, increased (Table 1).

Methylation analysis of SVPL-1 and SVPL-2. Methylation analysis was used for elucidation of linkages between sugar residues and confirmation of the ring forms of the monosaccharide residues in the fragments. Permethylation of SVPL-1 and SVPL-2 according to Hakomori [14] followed by a reduction of carboxyl groups of the permethylated fragments obtained with lithium borohydride in tetrahydrofuran [15] and a repeated permethylation of the material obtained followed by acidic hydrolysis yielded a mixture of the methylated sugars, which were reduced with sodium borohydride followed by acetylation to furnish a mixture of the methylated alditol acetates. Acetates of the methylated alditols (Table 4) were identified using GLC-MS.

The GLC-MS data confirmed information obtained earlier concerning the structure of the side chains of silenan and demonstrated that the residues of terminal and 5-O-substituted arabinofuranose, terminal, 4-O-, 3-O-, 6-O-, and 3,6-di-O-substituted galactopyranose are present in SVPL-1 and SVPL-2 fragments, and the branching points of the backbone appeared to be 2,4-di-O-substituted rhamnopyranose residues (Table 4).

In addition, 3,5-di-O-substituted arabinofuranose and 4,6-di-O-galactopyranose residues of the ramified region were detected as minor constituents, and 2,4- and 3,4-di-O-substituted residues of galactopyranose represented the branching points of the backbone or the ramified region of silenan.

Methylation analysis of SVPS. The polysaccharide fragment SVPS analyzed structurally earlier using NMR spectroscopy [10] was obtained by digestion of SVP.

SVPS was subjected to the further methylation studies followed by reduction of carboxyl groups as above. The data of methylation analysis of SVPS (Table 4) confirmed and supplemented conclusions concerning the structural features of sugar chains and the main substituents on the parent silenan. The residues of terminal, 3-O-, 6-O-, and 3,6-O-di-substituted galactopyranose and the residues of terminal arabinofuranose were detected as the main constituents of fragment SVPS in addition to the 4-O-substituted galactopyranose residues of the backbone. Residues of 3,5-di-O-substituted arabinofuranose and 4,6-di-O-substituted galactopyranose which appeared to be the branching points of the RG-I side chains are present in minor amounts. The minor residues of 2,4- and 3,4-di-O-substituted galactopyranose might represent the branching points of the side chains as well as the backbone of silenan.

Structure of silenan. Based on the data, some propositions can be made concerning the macromolecule of silenan. Like many known pectic polysaccharides [3-5], silenan (molecular mass, M_{SD}, 22 kD [20]) has a block sugar chain structure and consists of some blocks of linear and ramified regions.

Table 4. Methylation analysis of the SVPL-1, SVPL-2, and SVPS fragments of silenan (molar ratios of the methylated alditol acetates)

Methylated sugar*	Mode of linkage	SVPS	SVPL-1	SVPL-2
3-O-Me-Rha*p*	→2,4)-Rhap-(1→	—	1	1
2,3,5-Me_3-Ara*f*	Ara*f*-(1→	3	7	6
2,3-Me_2-Ara*f*	→5)-Ara*f*-(1→	—	6	2
2-Me-Ara*f*	→3,5)-Ara*f*-(1→	1	—	1
2,3,4,6-Me_4-Gal*p*	Gal*p*-(1→	3	5	4
2,3,6-Me_3-Gal*p*	→4)-Gal*p*-(1→	6	6	4
2,4,6-Me_3-Gal*p*	→3)-Gal*p*-(1→	6	9	4
2,3,4-Me_3-Gal*p*	→6)-Gal*p*-(1→	3	3	2
2,4-Me_2-Gal*p*	→3,6)-Gal*p*-(1→	4	6	4
2,3-Me_2-Gal*p*	→4,6)-Gal*p*-(1→	1	1	—
2,6-Me_2-Gal*p*	→3,4)-Gal*p*-(1→	1	2	1
3,6-Me_2-Gal*p*	→2,4)-Gal*p*-(1→	1	1	—

* Identified as the corresponding alditol acetates.

The blocks of the linear region consist of galacturonan and rhamnogalacturonan. The results of a partial acidic hydrolysis and digestion of silenan combined with the NMR spectral data indicated that α-1,4-D-galactopyranosyluronan occupied the main part of the silenan macromolecule core. Some residues of galacturonic acid in the linear region are methoxylated. The occurrence of the linear rhamnogalacturonan in silenan was shown using NMR spectroscopy of the fragment SVPP obtained on digestion. This segment consists of separate parts of galacturonan interlinked by rhamnopyranose residues involved in 1,2-linkage in the sugar chain.

Small blocks of the hairy region of silenan represent RG-I. The results of digestion and Smith degradation in combination with the NMR spectral data demonstrated that the backbone of the hairy region of silenan consists of the α-1,4-D-galactopyranosyl uronic acid and α-rhamnopyranose residues substituted by the residues of α-1,4-D-galactopyranosyl uronic acid in the second position.

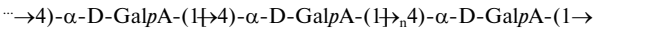
A covalent bond proved to be present between the core of the ramified region and the side sugar chains of silenan. Using methylation analysis and NMR spectroscopy, some α-rhamnopyranose residues were shown to be branching points bearing a substitution in the fourth position. Simultaneously, the occurrence of a certain amount of galacturonic acid residues in fragments obtained on Smith degradation suggests that they seemed to represent the branching points of the sugar chain of silenan and/or contain *O*-acetyl groups. These residues of galacturonic acid appeared to make up the segments of the linear galacturonan resistant to periodate oxidation due to steric factors.

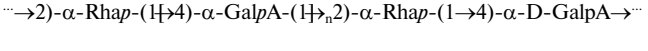
The side chains of the hairy regions of silenan represent blocks that appeared to consist of various kinds of arabinogalactan. The side chains of RG-I proved to contain blocks of α-1,5-arabinofuranan and β-1,3- and β-1,4-galactopyranan bearing the α-arabinofuranose residues at the terminal positions.

In accord with analysis of the NMR spectra of the fragment SVPS obtained as a result of Smith degradation, it can be suggested that the residues of α-arabinofuranose being constituents of the other side chains (possibly α-1,5-arabinofuranan) degraded on periodate oxidation of the silenan macromolecule are attached in the ramified region of silenan to the side chains of β-1,3-galactopyranan via the branching points presented by 2,3-di-O-substituted galactopyranose residues. The occurrence of 3,5-di-O-substituted arabinofuranose residues, 3,6- and 4,6-di-O-substituted galactopyranose residues in the permethylated fragments SVPS, SVPL-1, and SVPL-2 demonstrated also the presence of covalent bonds between various sugar chains of arabinofuranan and galactopyranan.

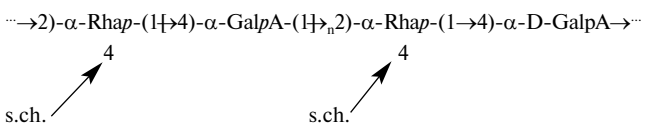
Based on the data, the following scheme of the structural features of silenan is suggested:

Segments of the linear region of silenan:

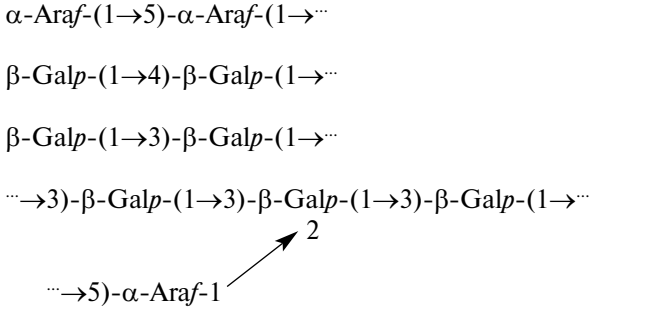




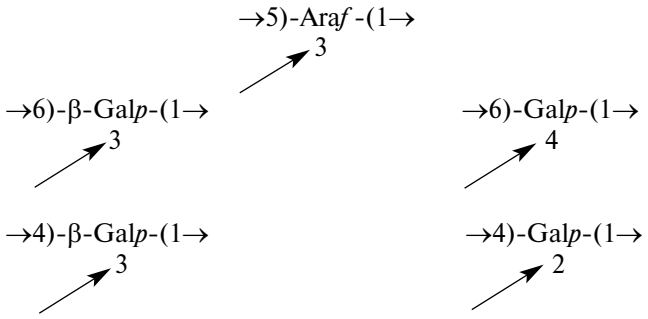
and the ramified region:



bearing the following side chains (s.ch.):



and possible additional branching points of the side chains:



and the backbone:

Thus, the principal structural pattern and some features of the fine structure of silenan were elucidated.

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