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Structural studies on hairy region of pectic polysaccharide from campion *Silene vulgaris* (*Oberna behen*)

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Abstract

Using enzymic digestion with pectinase, controlled Smith degradation and NMR-spectroscopy, some structural features of the hairy region of pectic polysaccharide termed silenan SV from the aerial part of campion *Silene vulgaris* (Moench) Garke (*Oberna behen* (L.) Ikonn) were elucidated.

Silenan was subjected to enzymic digestion with pectinase to furnish the polysaccharide fraction (SVP). The contained residues of D-galacturonic acid (43%), arabinose, galactose and rhamnose as main constituents. The backbone of the hairy region of silenan was found to consist of α-1,4-galactopyranosyl uronic acid and 2-*O*-glycosylated rhamnopyranose residues. The side chains contained linear regions of residues of α-1,5-linked arabinofuranose and β-1,3-, β-1,4-linked galactopyranose. Silenan SV and its fragment SVP were subjected to Smith degradation to give fractions SVS and SVPS. These contain the residues of terminal and 2-substituted α-arabinofuranose as well as residues of terminal, 3-, and 2,3-substituted β-galactopyranose. In addition, NMR-spectral data confirmed that the residues of α-rhamnopyranose 2-*O*-glycosylated with the residues of α-1,4-galactopyranosyl uronic acid of the backbone occurred in the core of SVPS and, therefore, in the backbone of silenan SV.

On the basis of data obtained, the hairy regions of silenan were suggested to contain mainly the linear chains of β-1,3-, β-1,4-galactopyranan and α-1,5-arabinofuranan. The chains of α-1,5-linked arabinofuranose, β-1,3- and β-1,4-linked galactopyranose were shown to be involved in the side chains of the hairy region having branching points at 2,3-substituted β-galactopyranose residues.

Keywords: Plant polysaccharides; *Silene vulgaris* (*Oberna behen*); Pectin; Silenan; Arabinogalactan; Rhamnogalacturonan; Enzymic digestion; Smith degradation; NMR-spectroscopy of polysaccharides

1. Introduction

Pectic polysaccharide named silenan SV (yield 2.0–2.5% dry material) were isolated from the aerial part of a campion *Silene vulgaris* (Moench) Garke (*Oberna behen* (L.) Ikonn which is a medicinal plant abundant through the European North of Russia (Ovodova, Bushneva, Shashkov, & Ovodov, 2000). Silenan SV has been found to possess an immunomodulatory activity (Popov, Popova, Ovodova, Bushneva, & Ovodov, 1999).

Silenan SV, $[\alpha]_D^{20} + 148.6°$ (c 0.1; H_2O) was rich in galacturonic acid (63%), and rhamnose, galactose, and arabinose were also present in reasonable proportions (Ovodova et al., 2000). Silenan was shown to contain the homogalacturonan segments as the backbone or 'smooth regions' and rhamnogalacturonan fragments as ramified or 'hairy regions' (Ovodova et al., 2000). The structural features of the hairy regions are unknown. The present paper elucidates some structural features of the hairy regions of silenan SV.

2. Experimental

2.1. Plant material and isolation of silenan SV

The aerial parts of *S. vulgaris* were collected at the bloom period of the plant in June near Syktyvkar (Komi Republik, Russia). Isolation followed by purification of silenan SV was performed as described earlier (Ovodova et al., 2000).

2.2. Enzymic digestion

A solution of silenan SV (500 mg) was dissolved in 50 ml of water, and the aqueous solution of pectinase (10 mg, 30%

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Table 1
Analytical data for silenan SV and its fragments after pectinase digestion and Smith degradation. n.d. —not determined

Sample	Gal*p*A[a]	OMe[a]	Rha[b]	Gal[b]	Ara[b]	Xyl[b]	Man[b]	Glc[b]	Protein[a]
SV	63	2.0	2.2	3.2	4.2	1.9	1.1	2.7	13
SVP	44	3.2	4.3	9.3	7.8	1.7	2.9	3.5	–
SVS-1	17	n.d.	4.9	31.1	16.7	0.6	2.0	2.3	–
SVS-2	24	n.d.	3.9	20.5	13.6	0.9	2.6	2.4	–
SVPS	28	n.d.	5.8	35.6	12.9	1.5	0.9	1.0	–

[a] Weight percentange.

[b] Expressed as mol%.

active protein, 57 IU, Ferbak, Berlin) was added. The mixture was incubated in the dialysis tube with simultaneous dialysis against distilled water at 37 °C for 1 h. The resulting polysaccharide fraction (60 mg) was subjected to a further purification using gel filtration on Sephacryl S-500 to afford a single peak with Kav = 0. Fractions corresponding to this peak were collected and lyophilized to furnish the homogeneous polysaccharide fraction SVP (yield 52 mg). The analytical data for the fraction SVP are given in Table 1.

2.3. Smith degradation of silenan SV and the polysaccharide SVP

A solution of silenan SV (1 g) in water (400 ml) was treated in dark with 0.02 M aqueous sodium metaperiodate (400 ml) for 7 days at +5 °C. The mixture obtained was then treated with ethylene glycol (10 ml) for 2 h under vigorous stirring, $NaBH_4$ (2.5 g) was added, and the mixture was kept overnight. The material obtained was acidified with acetic acid to pH 5.0, dialyzed, and lyophilized to yield the corresponding polyalcohol (0.590 g). The material (0.385 g) obtained was dissolved in 2% acetic acid (150 ml) and the solution was heated for 2 h at 100 °C. After neutralization and evaporation with methanol, the mixture was dissolved in a minimum volume of water and applied to a Sephadex G-25 column. Fractions corresponding to the single peak with Kav = 0 were combined and the solution obtained was lyophilized to give degraded silenan as the crude polysaccharide fraction (yield 16.0 mg). The material obtained (10.4 mg) was applied to a Sephacryl S-500 column. Elution with water furnished the purified polysaccharide fractions as follows: SVS-1 (Kav = 0, yield 2.4 mg) and SVS-2 (Kav = 0.3, yield 7.6 mg). The analytical data for the polysaccharide fractions SVS-1 and SVS-2 obtained are listed in Table 1.

Fraction SVP (0.406 g) in water (160 ml) was treated in the dark with 0.02 M aqueous sodium metaperiodate (160 ml) for 3 days at +5 °C. A treatment of the mixture obtained was carried out as mentioned earlier to afford the crude polysaccharide fraction, which was purified on Sephacryl S-500. The single peak eluted with distilled water with Kav = 0.8. Fractions corresponding to this peak were combined and lyophilized to give the purified polysaccharide SVPS (yield 9.5 mg). The analytical data for the polysaccharide fraction SVPS are given in Table 1.

2.4. General methods

Glycuronic acid contents were determined using interaction of polysaccharide fractions with 3,5-dimethylphenol in the presence of concentrated sulphuric acid (Usov, Bilan, & Klochkova, 1995) using the standard curve for galacturonic acid. The total protein contents were estimated according to the Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951) using the standard curve for bovine serum albumin. The contents of methoxyl groups were estimated as described earlier (Wood & Siddiqui, 1971). Spectrophotometric measurements were run on Ultrospec 3000 instrument (England).

Samples were exchanged by deuterium using twofold evaporation with D_2O. NMR spectra were recorded with a Bruker DRX-500 spectrometer (Germany) using 3–5% carbohydrate solution in D_2O at 55 and 70 °C, the internal standard was acetone, δ_H 2.225 ppm, δ_C 31.45 ppm. Two-dimensional spectra COSY, TOCSY, ROESY and HSQC were recorded using the standard Bruker procedures. The spectra ROESY were performed using the mixing time 200 ms. The 60 ms duration of the MLEV17 spin-lock for TOCSY experiments was used.

Optical rotations were measured on a Polatronic MHZ (Germany) polarimeter at 20 °C in water.

Complete acidic hydrolysis was performed using trifluoroacetic acid (2 M, 1 ml) containing *myo*-inositol (0.3 mg/ml) as the internal standard. Acid was added to the polysaccharide fractions (3–5 mg), and the mixture obtained was heated for 8 h at 100 °C. The acid was then removed by the repeated co-evaporation with methanol. Monosaccharides obtained were separated and quantified by paper chromatography. An identification and quantitation were achieved using gas–liquid chromatography.

Gas–liquid chromatography was performed on a Hewlett–Packard 4890A instrument on a RTX-1 capillary column (0.25 mm × 30 m) with argon as a carrier gas using a flame-ionization detector and a HP 3395A integrator. The neutral monosaccharides were qualitatively and quantitatively determined as the corresponding alditiol acetates (York, Darvill, McNeil, Stevenson, & Albersheim, 1985)

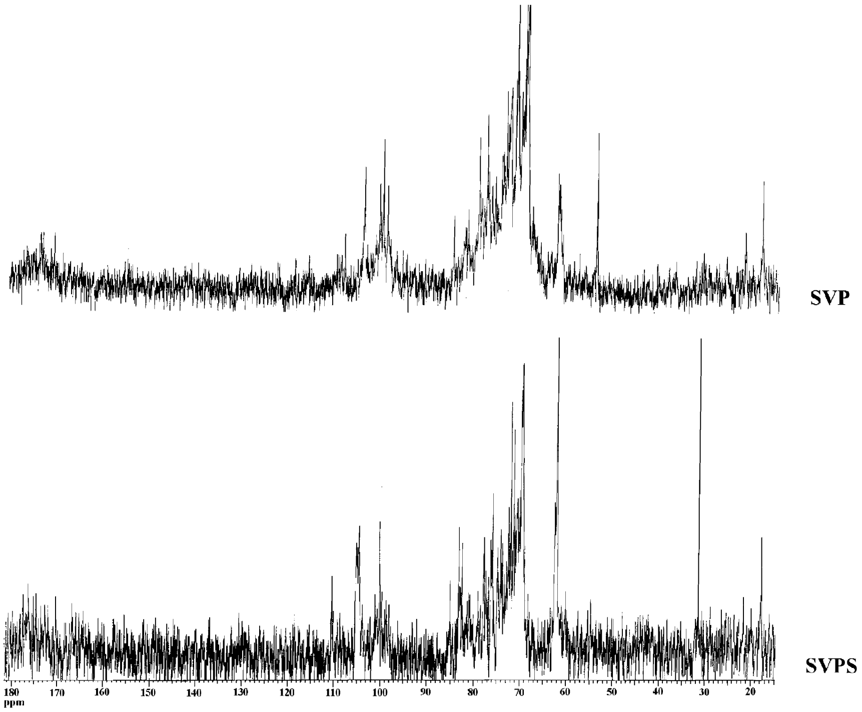

Fig. 1. ^{13}C NMR spectra of the SVP and SVPS fractions.

using the following temperature program: isothermal at 175 °C for 1 min, then 3 °C/min gradient up and isothermal 250 °C for 2 min.

The descending paper chromatography was carried out on Filtrak FN-12 and Filtrak FN-13 papers in a 6:4:3 (v/v/v) *n*-butanol–pyridine–water developing system. The sugars were detected by spraying with a solution of aniline hydrogen phthalate following heating at 105 °C.

Gel filtration was carried out on a Sephacryl S-500 column (1.4 × 48 cm, a void volume $V_0 = 32$ ml), using distilled water as the eluent. The flow rate of 0.3 ml/min, and 3 ml fractions were collected. When gel filtration was performed on a Sephadex G-25 column (2.5 × 75 cm, a void volume $V_0 = 108$ ml) using distilled water as the eluent with a flow rate of 0.5 ml/min, 8 ml fractions were collected. The elution of fractions was controlled by the positive reaction of the eluate for carbohydrates using the phenol–sulphuric acid procedure as described earlier (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

All aqueous solutions were concentrated in vacuo at 40–45 °C followed by centrifugation at 7500 × *g* for 10–20 min, and the supernatants were lyophilized.

3. Results and discussion

3.1. Degradation of silenan SV by pectinase

Silenan SV was isolated from the aerial parts of *Silene vulgaris* and purified as described earlier (Ovodova et al., 20000. Silenan SV was subjected to digestion with pectinase in order to achieve a partial fragmentation of the macromolecule. Like all pectic polysaccharides, silenan SV undergoes cleavage on pectinase digestion to give galacturonic acid only. The polysaccharide fraction SVP obtained was found to account for only 15% of the total material. Thus, α-1,4-galacturonan is suggested as the backbone of silenan. In addition to polysaccharide SVP, a mixture of free galacturonic acid, oligogalacturonides, and other oligosaccharides (more than 50% of the parent silenan SV) was obtained (Ovodova et al., 2000). The analytical data for SVP are given in Table 1. The sugar chains of the fragment, $[\alpha]_{\mathrm{D}}^{20} + 54.5°$ (*c* 0.1; H_2O), were shown to contain considerable amounts of the following neutral sugars: galactose, arabinose, and rhamnose. However, the residues of D-galacturonic acid (44%) are present also indicating an occurrence of acetyl groups and/or side chains attached to the backbone. Thus, a digestion of silenan SV with pectinase resulted in polysaccharide fragment SVP which was studied using NMR spectroscopy.

The ^{13}C NMR spectrum of the SVP fraction (Fig. 1) demonstrated the resonance from C1 of α-D-galactopyranosyluronic acid at 100.6 ppm that was most intensive in the high-field anomeric signal area. This should be assigned to the galacturonic acid residues of the α-1,4-linked backbone (Keenan, Belton, Matthew, & Howson, 1985). The resonance from C6 of unmethoxylated galacturonic acid residues is located at 174.9 ppm (Catoire, Goldberg, Pierron, Morvan, & Herve de Penhoat, 1998). The occurrence of more than one carboxyl signal (at 174.9 and 175.2 ppm, Fig. 1) might be due to the presence of unbranched and branched rhamnosyl residues in the main pectic chain. A

Table 2
Chemical shifts for the resonances of the glycosyl residues of SVP in the ^{13}C- and ^{1}H-NMR spectra

Residue	Chemical shifts, δ, ppm (internal standard acetone, δ_C 31.45/δ_H 2.25 ppm)					
	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6,6′
→ 4)-α-Gal*p*A-(1 →	100.6/5.17	69.6/3.78	70.1/3.98	80.1/4.47	n.d.	174.9
→ 2)-α-Rha*p*-(1 →	99.7/5.25	77.5/3.99	71.6/3.94	73.6/3.45	69.5/3.76	17.9/1.25
β-Gal*p*-(1 →	104.8/4.48	72.4/3.56	74.3/3.67	69.9/4.14	76.5/3.73	62.1/3.76
→ 3)-β-Gal*p*-(1 →	105.6/4.70	71.7/3.80	83.6/3.86	69.9/4.21	75.5/3.72	62.1/3.76
→ 4)-β-Gal*p*-(1 →	105.6/4.70	71.5/3.67	73.6/3.76	78.4/3.97	75.5/3.72	62.1/3.76
α-Ara*f*-(1 →	109.2/5.25, 111.0/5.45	82.9/4.22	78.2/3.95	85.4/4.15	62.5/3.83; 3.73′	
→ 5)-α-Ara*f*-(1 →	108.9/5.10	82.4/4.13	78.3/4.03	83.8/4.19	68.3/3.90; 3.80′	

similar phenomenon was previously described for the native pectin of tobacco (Westerlund, Aman, Andersson, & Andersson, 1991). The resonance from the other atoms of α-1,4-linked D-galactopyranosyl uronic acid residues were broadened in both the ^{1}H- and ^{13}C-NMR spectra probably due to an irregularity in the oligosaccharide chains. Alternatively, this part of the molecule may probably be too bulky and rigid and thus fail to show detectable signals (Pressey & Himmelsbach, 1984). COSY and TOCSY spectra show a weak and broad peak for H2–H4 (centres of the signals are shown in Table 2). The HSQC spectrum (Fig. 2) revealed the resonance of the corresponding carbon atoms,

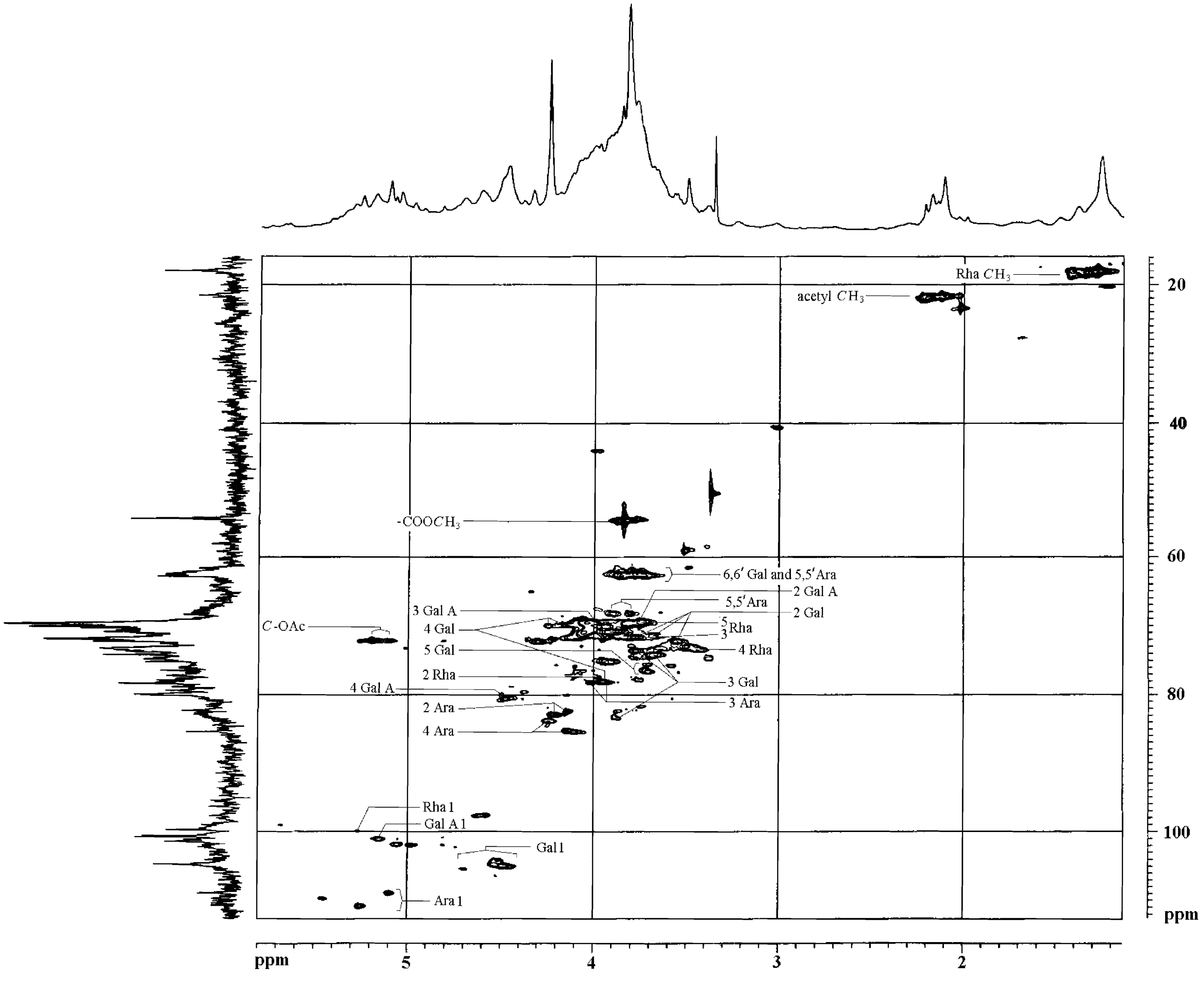

Fig. 2. ^{1}H/^{13}C HSQC spectrum of SVP fraction.

chemical shifts of which are similar to those described earlier (Keenan et al., 1985).

The resonances from methyl groups of the methoxylated D-galactopyranosyl uronic acid residues at 54.1 ppm should also be mentioned among other signals in the ^{13}C NMR spectrum indicating partial methoxylation of carboxyl groups of the galacturonic acid residues (Odonmažig, Badga, Ebringerová, & Alföldi, 1992). The resonance from C6 of methoxylated galacturonic acid at 172.0 ppm was also detected in the ^{13}C NMR spectrum (Catoire et al., 1998) (Fig. 1). The presence of methoxylated carboxyl groups in the residues of D-galacturonic acid was also confirmed by the following signals: C 54.1/H 3.85 ppm in ^{1}H/^{13}C HSQC spectra (Fig. 2).

An intensive resonance from the anomeric carbon atom of the rhamnopyranose residue at 99.7 ppm is observed (Fig. 1). The high-field resonance at 17.9 ppm from the rhamnopyranose methyl group is easily identified (Keenan et al., 1985; Pressey & Himmelsbach, 1984). The two-dimensional NMR spectra (TOCSY and ^{1}H/^{13}C HSQC) showed the signals of the anomeric and C2/H2-atoms of 2-*O*-glycosylated α-rhamnopyranose (Table 2). Analysis of the COSY and TOCSY spectra showed proton signals from the rhamnopyranosyl residues. The HSQC spectrum showed the α-configuration for the rhamnopyranosyl residues (chemical shift of C5 at 69.5 ppm) and substitution of the residues at position 2 (signal of C2 at 77.5 ppm) (Fig. 2). However, the correlation peak of H2 with an anomeric atom of some other residues in the ROESY spectrum was not observed.

The signals of the residues with a β-galactopyranosidic configuration are observed in the high-field region of the ^{1}H-NMR spectrum at 4.48 and 4.70 ppm (COSY and TOCSY spectra). The ROESY spectrum showed spatial interactions of the anomeric protons with H3-atom and revealed a signal from H5 at 3.72–3.73 ppm. The HSQC spectrum displayed correlation peaks C1 104.8/H1 4.48 and C1 105.6/H1 4.70 ppm (Fig. 2). Thus, the position of the anomeric carbon atoms is similar to that of the β-galactopyranose residue (Keenan et al., 1985; Pressey & Himmelsbach, 1984). Analysis of the TOCSY and HSQC spectra revealed the absence of substitution on residues with chemical shift of C1 at 104.8 ppm and a substitution on residues with chemical shift of C1 at 105.4 ppm at position 3 (C3 83.6 ppm) or 4 (C4 78.4 ppm).

Part of the signals in the low-field region of the ^{1}H-NMR spectrum (5.10, 5.25 and 5.45 ppm) was assigned to the anomeric proton of α-arabinofuranosidic residues (COSY and TOCSY). The HSQC spectrum revealed both non-substituted (C5 62.5 ppm) and 5-substituted (C5 68.3 ppm) residues. The presence of two signals in the ^{13}C- and ^{1}H-NMR spectra of the non-substituted residues (Table 2) may be explained by the different nature of the 'aglycones'. These data demonstrated that silenan contained blocks of sugar chains composed of residues of α-1,5-linked arabinofuranose as well as β-1,3- and β-1,4-linked galactopyranose. These blocks appeared to be terminated with α-arabinofuranose residues. The neutral fragments of arabinan and galactan are most likely the side chains of the ramified region of silenan attached to the backbone of rhamnogalacturonan, as previously noted for the other pectic polysaccharides (Pressey and Himmelsbach, 1984).

The resonance at 21.6 ppm (Fig. 1) showed the presence of some *O*-acetyl groups (Keenan et al., 1985). The correlation signal at C 21.6/H 2.10 ppm in the two-dimensional NMR-spectra confirmed this. In addition, the correlation peaks C 72.2/H 5.10 and H 5.18 ppm in the HSQC spectrum indicated *O*-acetyl groups in molecule of silenan SV (Fig. 2). A low-field shift of the resonance in ^{1}H-NMR spectrum is connected with the effect of acetyl groups. This influence is less than that observed in ^{13}C-NMR spectrum showed the signals of the corresponding C-atoms in the field of non-anomeric carbon atoms.

Thus, NMR spectral data for the fragment SVP demonstrated an occurrence in silenan SV of the ramified regions contained β-1,3- and β-1,4-linked galactopyranan and α-1,5-arabinofuranan side chains in the presence of 2-*O*-glycosylated α-rhamnopyranose residues allowed to suggest that these regions represented rhamnogalacturonan I. (O'Neill, Albersheim, & Darvill, 1990; Ovodov, 1998; Yamada, Hirano, & Kiyohara, 1991)

3.2. Smith degradation of silenan SV and its fragment SVP

To obtain additional information concerning structural pattern of silenan a comparative study of products of Smith degradation of silenan and its fragment SVP was carried out.

Smith degradation of silenan followed by separation and purification of the materials obtained using gel filtration on Sephadex G-25 and a Sephacryl S-500 was found to result in two homogeneous polysaccharide fractions as follows: SVS-1, $[\alpha]_{\mathrm{D}}^{20}$ + 21.1° (*c* 0.15; H_2O) as the minor component and SVS-2, $[\alpha]_{\mathrm{D}}^{20}$ + 35.6° (*c* 0.62; H_2O) as the main constituent of the polysaccharide mixture (yield 0.8% of the parent SV). Smith degradation of the fraction SVP afforded the fragment SVPS, yield 2.3% of the parent SVP, $[\alpha]_{\mathrm{D}}^{20}$ + 29.5° (*c* 0.37; H_2O).

The analytical data for the fractions SVS-1, SVS-2 and SVPS are given in Table 1. As may be seen from this table a substantial decrease of the D-galacturonic acid residues in both cases confirmed an occurrence of the linear α-1,4-homogalacturonan chains as the backbone of the macromolecule. However, some residues of galacturonic acid are refined in the degraded polysaccharides thus indicating that they represented branching points of the macromolecule or contained acetyl groups. In addition, the retained residues of arabinose and galactose demonstrated that side chains of the hairy regions are resistant to periodate oxidation. In addition, an occurrence of the rhamnose residues in the fragments SVS-1, SVS-2 and SVPS indicated that some of these residues appeared to be substituted with side chains.

These statements were confirmed by the NMR spectral

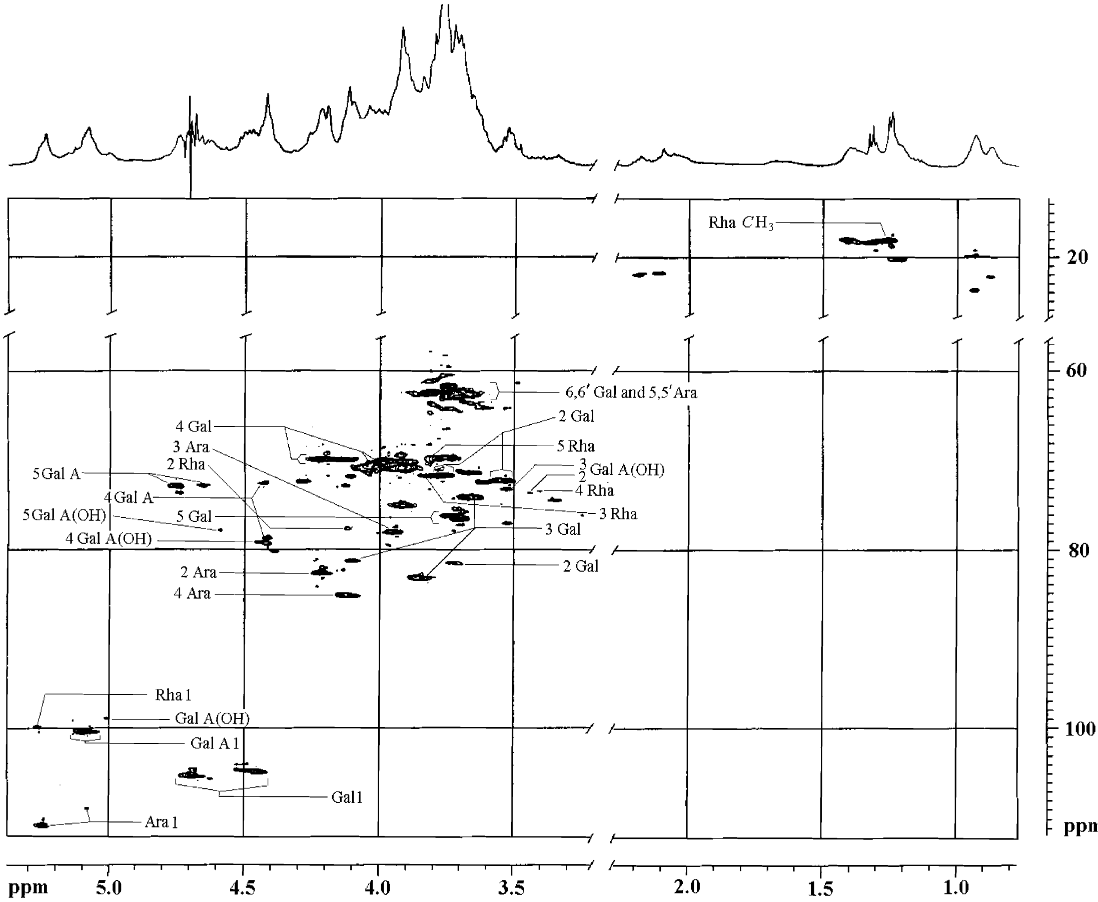

Fig. 3. $^1H/^{13}C$ HSQC spectrum of SVPS fraction.

data. The NMR-spectra of the fractions SVS-2 and SVPS were similar. In this connection, the NMR-spectral data for SVPS only are given below. The ^{13}C-NMR spectrum of the fraction SVPS is typical for the mixture of oligosaccharides in non-equimolar ratio due to the presence of signals with different intensity (Fig. 1). Five signals at 98.9, 100.4, 104.6, 105.3, and 110.4 ppm among other signals in the anomeric field of the spectrum were found to possess the most intensity. In addition, the resonances at 62.15 and 17.9 ppm in ^{13}C-NMR spectrum is associated with the signals of the $-CH_2OH$ group of the galactopyranose and CH_3-group of rhamnopyranose residues, respectively (Fig. 1), (Odonmažig et al., 1992).

The analysis of the COSY and TOCSY spectra of SVPS revealed the residues with α- and β-galactopyranosidic, α-rhamnopyranosidic and α-arabinofuranosidic configuration. Position of H5 signal for the residues with the galacto-configuration was found in the ROESY spectrum (correlation peaks H4/H5). The correlation peaks H1/H5 were also found in the spectrum for the residues with β-galactopyranosidic configuration. The HSQC spectrum (Fig. 3) indicated that the residues with α-galacto-configuration corresponded to the α-galactopyranosyl uronic acid residues substituted at position 4 (C4 in the range of 75.5–80.2 ppm). All the correlation peaks of these residues were significantly broadened or split into several peaks with insignificant differences in chemical shifts, probably, due to heterogeneity of the nearest environment of the residues. In addition, the HSQC spectrum (Fig. 3) revealed the residues of the terminal β-galactopyranose (C1 104.6–104.8 ppm), 3-substituted β-galactopyranose (C3 83.2 ppm), and 2,3-di-substituted β-galactopyranose (C3 81.4 ppm and C4 81.5 ppm), as well as 2-substituted α-rhamnopyranose (C2 77.7 ppm) and the terminal α-arabinofuranose (C1 110.4 ppm) residues. Minor correlation peak (C1/H1 108.8/5.08 ppm) probably belonged to 2-substituted α-arabinofuranose residues. More sensitive (in comparison with one-dimensional ^{13}C-NMR) H-detected HSQC spectrum revealed also signals of 4-substituted β-galactopyranosyl uronic acid residues (C4 79.5) with a reducing anomeric centre (C1 97.3 ppm).

Analysis of the ROESY spectra of SVPS confirmed the presence of the α-1,4-linked D-galactopyranosyl uronic acid residues. The correlation peak of H1/H4-atoms of the galacturonic acid residues at 5.08/4.43 ppm was observed. Some galacturonic acid residues are linked to 2-position of rhamnose residues as may be seen from the correlation peak of H1(Gal*p*A)/H2(Rha*p*) at 5.00/4.10 ppm. In addition, the correlation peak of H1(Rha*p*)/H4(Gal*p*A) at 5.25/4.43 ppm demonstrated that the residues of rhamnopyranose

Table 3

Chemical shifts for the resonances of the glycosyl residues of SVPS in the ^{13}C- and ^{1}H-NMR spectra. n.d.—not determined. The range of the chemical shifts are shown in the parenthesis

Residue	Chemical shifts, δ, ppm (internal standard acetone, δ_C 31.45/δ_H 2.25 ppm)					
	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6,6′
→ 4)-α-Gal*p*A-(1 →	100.4/5.08/(5.05–5.12)	69.8/3.78/(3.75–3.80)	(70.3–71.2)/(3.96–4.02)	(78.5–80.2)/(4.40–4.47)	(72.7–72.9)/(4.65–4.73)	≈ 175.5
α-Gal*p*A-(1 → 2)-α-Rha*p*-(1 → 4)-β-GalA(OH)						
α-Gal*p*A-(1 → (2)	98.9/5.00	70.4/3.93	70.6/4.08	72.4/4.43	72.7/4.65	175.5
→ 2)-α-Rha*p*-(1 → (4)	99.8/5.25	77.7/4.10	71.3/3.87	73.3/3.38	69.9/3.79	17.9/1.25
→ 4)-β-GalA(OH)	97.3/4.36	73.6/3.42	73.2/3.57	79.5/4.43	78.1/4.59	62.4
β-Gal*p*-(1 → (3)	104.6/4.48	72.2/3.54	74.0/3.66	70.5/4.03	76.5/3.69	62.1/3.76
→ 3)-β-Gal*p*-(1 →	105.3/4.69	71.7/3.79	83.2/3.83	69.7/(4.17–4.24)	76.1/3.72	62.1/3.76
β-Gal*p*-(1 → 3)-β-Gal*p*-(1 → ↗ 2 α-Ara*f*-1						
→3)-β-Gal*p*-(1→ ↗2	104.0/4.49	81.4/3.71	81.5/4.11	69.7/4.13	76.1/3.72	62.1/3.76
β-Gal*p*-(1 → (3)	104.8/4.44	72.2/3.54	74.0/3.66	70.0/4.01	76.1/3.69	62.1/3.76
α-Ara*f*-(1 → (2)	110.4/5.26	82.6/4.23	78.0/3.96	85.3/4.12	62.7/3.82; 3.69′	

are linked to 4-position of the β-galacturonic acid residues. Oligosaccharides contained the similar glycosidic bonds have been isolated earlier from the apple pectin (Colquhoun, Ruiter, Schols, & Voragen, 1990), from the yellow mustard *Sinapis alba* L. pectin (Cui, Eskin, Biliaderis, & Marat, 1996), and from the rhamnogalacturonans of the sycamore *Acer pseudoplatanus* (An, Zhang, O'Neill, Albersheim, & Darvill, 1994).

In addition, the correlation peak of H1(Gal*p*)/H3(Gal*p*) at 4.69/3.83 ppm in the ROESY spectrum of SVPS showed that some terminal residues of β-galactopyranose are attached to 3-position of the galactopyranose residues.

The terminal residues of α-arabinofuranose are attached to 2-position of the β-galactopyranose residues of β-1,3-galactopyranan chain according to the correlation peak of H1(Ara*f*)/H2(Gal*p*) at 5.26/3.71 ppm. In addition, the correlation peak H1(Gal*p*)/H3(Gal*p*) at 4.44/4.11 ppm indicated the presence of β-1,3-linkage between two galactopyranose residues in the sugar chain (at the branching points of the sugar chain).

The chemical shifts for the resonances of the glycosyl residues of SVPS in the NMR spectra are listed in Table 3.

On the basis of the data obtained, it may be suggested that the core of the ramified regions of silenan is composed of the residues of α-1,4-D-galactopyranosyl uronic acid and α-1,2-rhamnopyranose. In addition, some residues of rhamnose and galacturonic acid appeared to be the branching points of the backbone. The side chains of the hairy regions of silenan were found to represent the different blocks which consisted of the residues of arabinofuranose and galactopyranose. The linear chains of α-1,5-linked arabinofuranose, β-1,3- and β-1,4-linked galactopyranose were shown to be involved in the side chains of the hairy region having the branching points represented by 2,3-substituted β-galactopyranose (Table 3).

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