



Structure of arabinogalactan and pectin from the *Silybum marianum*

R.K. Rakhmanberdyeva^a, K.S. Zhaunbayeva^a, S.N. Senchenkova^b, A.S. Shashkov^b,
Kh.M. Bobakulov^{c,*}

^a Department of Chemistry of High Molecular Plant Substances, Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, 77, M. Ulugbek str., 100170, Tashkent, Uzbekistan

^b N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, B-334, Leninsky Pros., 47, Moscow, Russia

^c Department of Physical Methods of Research, Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, 77, M. Ulugbek str., 100170, Tashkent, Uzbekistan

ARTICLE INFO

Keywords:

Silybum marianum
Arabinogalactan
Pectin
NMR spectroscopy
GC-MS

ABSTRACT

From the leaves of *Silybum marianum* L. were isolated arabinogalactan with molecular weight 38 kDa and pectic substances. The monosaccharide composition of arabinogalactan was represented by β -galactose and α -arabinose in a ratio of 2.6:1.0 and β -galacturonic acid as a minor component. By chemical methods and GC, GC-MS, 1D and 2D NMR spectroscopy was established that the arabinogalactan consists of *D*-galactopyranose residues linked by β -1,6-glycosidic bonds as a main chain, and the side chain was represented by α -arabinose, β -galactose and 4-O-methylglucuronic acid. Pectic substance was found in small amounts. According to NMR data it contains also a branched rhamnogalacturonan.

1. Introduction

Silybum marianum (L.) Gaertn. (Milk thistle) belongs to the family Asteraceae and as a medicinal plant has long been used in folk medicine to treat liver diseases and normalize digestion, to stimulate lactation, inflammation of the upper respiratory tract and lungs. It is known from the literature that the fruits and seeds of *S. marianum* contain flavonoids, alkaloids, saponins, fatty oils, proteins, vitamins K, resins, mucus, as well as macro- and microelements. But should be noted not enough information about the structure and biological activity of polysaccharides. In medical practice on the basis of *S. marianum* fruits have been created hepatoprotective preparations, such as Carsil, Silibor, Legadone et al. The presence of a silymarin complex (a mixture of three flavonolignans) determines healing properties of *S. marianum* for the liver. There is a report on the study of milk thistle extract as an additional treatment for cancer. Possible indications during cancer treatment include cleaning, detoxification and prevention of hepatotoxicity during chemotherapy and radiation therapy [1].

Earlier, we studied the carbohydrate composition of the aerial part of *S. marianum*. The presence of water-soluble polysaccharides and pectin was found [2]. It is known that polysaccharides have a pronounced anti-inflammatory, antioxidant, wound healing effect and activate the functions of the immune system and therefore, on their basis can be created new preparations [3–6]. It follows that the study of

polysaccharides of *S. marianum* is relevant.

The purpose of this study was to establish the structure of arabinogalactan (AG) and pectin isolated from the *S. marianum* by chemical methods as well as GC, GC-MS and NMR spectroscopy.

2. Results and discussion

2.1. Isolation of polysaccharides

Continuing the study to establish the structure of polysaccharides by fractional precipitation with alcohol of a water-soluble polysaccharide (WSPS) from the *S. marianum* leaves, a crude fraction was obtained with a yield of 42.3% [2]. For purification from non-carbohydrate components the fraction was applied to column with a Sephadex G-50. The monosaccharide composition of the purified fraction is represented by galactose, glucose, arabinose, mannose and uronic acid. Therefore, the fraction consists of mixture of neutral and acidic polysaccharides. Next, the fraction was passed sequentially through a column with DEAE-cellulose and TSK-40. As a result, the neutral fraction (minor) and the main acidic fraction were obtained. In the hydrolyzate of the neutral fraction, glucose and mannose were identified, in approximately equal proportions. The main acidic fraction contained galactose and arabinose in a ratio of 2.6:1 and was designated by us AGS, as well as glucose, mannose and rhamnose as minor components. An attempt to

* Corresponding author.

E-mail addresses: rakhmanberdieva@mail.ru (R.K. Rakhmanberdyeva), shash@ioc.ac.ru (A.S. Shashkov), khayrulla@rambler.ru (K.M. Bobakulov).

<https://doi.org/10.1016/j.carres.2019.107797>

Received 15 May 2019; Received in revised form 11 August 2019; Accepted 3 September 2019

Available online 04 September 2019

0008-6215/© 2019 Published by Elsevier Ltd.

separate the two acidic polysaccharides on TSK-40 did not lead to success.

AGS was white amorphous powder, completely soluble in water. Molecular weight characteristics of polysaccharides were determined by high performance exclusion chromatography (Fig. S1) and were 38–40 kDa. In the IR spectrum of AGS were detected absorption bands in the 800, 860 and 981 cm^{-1} regions (Fig. S2) characteristic of arabinogalactans [7].

2.2. Smith degradation results

In the results of Smith degradation of raw AG was identified glycerin and as a free monosaccharide. Detection of glycerin indicates the branching of the polymer and the presence of 1,6 type of bond between the monosaccharide residues. The identification of free monosaccharide implies presence of the 1,3 type of bond in the AGS chain.

2.3. Methylation and GC-MS analysis

Methylation of raw AG was carried out according to the Hakomori method [8]. The completeness of methylation was monitored by IR spectroscopy by the absence of an absorption band of 3400–3200 cm^{-1} (OH group). Permethyl product was subjected to methanolysis, the product of methanolysis was acetylated and obtained trimethylsilyl derivatives, which were analyzed by GC-MS [8,9]. As a result, the main compounds were identified: 2,3,4-tri-O-Me- β -D-Galp (15.2%), 2,4,6-tri-O-Me- β -D-Galp- (15.4%). In addition, 2,3,4,6-tetra-O-Me- β -D-Galp (6.2%), 2,3,5-tri-O-Me- α -Araf (2.6%), 2,3-di-Me- β -D-Glcp (9.4%), 2,5-di-O-Me- α -D-Araf (6.2%) were detected and identified in the product (Table 1).

2.4. NMR analysis

The structure of AGS was studied using 1D and 2D NMR spectroscopy. The structure of arabinogalactan was studied on the basis of analysis of 1D (^1H , ^{13}C NMR) and 2D COSY, TOCSY, ROESY, HSQC and

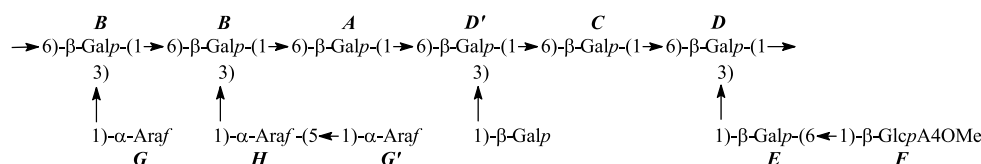
The ^1H NMR signals were assigned by 2D COSY, TOCSY and ROESY spectra. The spectra revealed the presence of β -galactopyranose (β -Galp), α -arabinofuranose (α -Araf), 4-OMe- β -glucuronic acid residues (β -GlcpA-4-OMe) as the main components of polysaccharides, as well as α -galacturonic acid residues (α -GalpA) and α -rhamnopyranose (α -Rhap) as minor constituents.

The type of substitution in the residues was determined by comparing the chemical shifts of the carbon atoms in the residues with those in the corresponding unsubstituted sugars. The HSQC spectrum (Fig. 1) revealed downfield shifts for carbon atoms C-6 (β -Galp) of residue **A** (Table 2.), C-3 and C-6 (β -Galp, residue **B**), C-5 (α -Araf) of residue **H**, C-4 (β -GlcpA4OMe) of residue **F**, C-4 (α -GalpA) of residues **I** and **I'**, C-4 (α -Rhap) of residue **J** and C-2,4 (α -Rhap) of residue **J'**. In addition were found terminal (unsubstituted) residues of α -Araf **G** and **G'**, as well as terminal residues β -Galp, judging by the presence of signals $\delta_{\text{C}}/\delta_{\text{H}}$ 63.2/3.76 (C-6 and H-6,6' unsubstituted galactopyranose) and 76.4/3.63 (C-5 and H-5). The remaining signals of unsubstituted galactopyranose overlap with the corresponding signals of residue **A**.

The linkage sequences of residues were deduced by analyzing the correlation peaks in the ROESY (Fig. 3) and HMBC spectra. The following inter-residual correlation peaks were observed in the ROESY spectrum: H-1(A)/H-6'(B), H-1(B)/H-6'(A), H-1(F)/H-6(E), H-1(D)/H-6(A), H-1(A')/H-4(J'), H-1(J, J')/H-4(I, I'), H-1(J)/H-1(I') and H-1(G, G')/H-3(B). Based on these data, it can be concluded that two acidic polymers are present in the polysaccharide, the typical structural fragments of which are listed in Table 2.

As well as the glycosidic linkages in the polysaccharides was confirmed by analyzing the correlation peaks in the HMBC spectrum, where the following inter-residual correlation peaks are observed $\delta_{\text{H}}/\delta_{\text{C}}$: 1G/H/3B, 1A/B/6B,D, 1B/6A, 1F/6E (Fig. S5). The structure of the acidic galactan is rather unusual, but not unique. For example, the β -GlcpA-4-OMe-(1 $\rightarrow$ 6)- β -Galp-(1 $\rightarrow$) fragment was found among oligosaccharides isolated from polysaccharides of the fig tree's cell wall (Sycamore) [10].

Based on the obtained chemical and spectral data, arabinogalactan and pectin substances have the following structures.

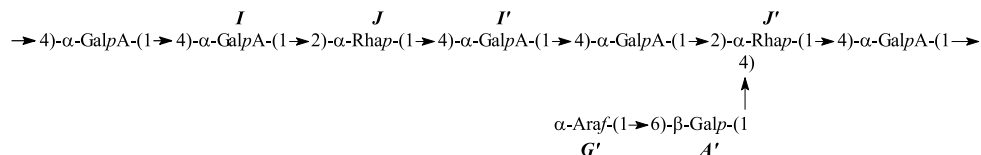


HMBC spectra.

The ^1H NMR spectrum (Fig. 1, upper projection and Fig. S3) contained the signals of anomeric atoms in the region of δ_{H} 4.42–5.24 ppm,

The main structural fragment of arabinogalactan.

According to NMR spectroscopy data, pectin substance is branched ramnagalacturonan:



as well as the characteristic signals at δ_{H} 3.52 (singlet, OMe), 1.25 and 1.31 (H-6, Rhap).

In the ^{13}C NMR spectrum (Fig. 1, the projections on the left) were visible the signals of anomeric atoms at δ_{C} 104.3–110.6 ppm, as well as the characteristic signals of OCH_3 at δ_{C} 61.4 and C- CH_3 at δ_{C} 17.8 and 18.0 and COOH at δ_{C} 173.8 and 174.2 (Fig. 2.).

The main structural fragment of pectin substance.

3. Conclusion

Thus, the data obtained revealed that the main chain of arabinogalactan is a branched galactan with β -1 $\rightarrow$ 6 glycosidic bonds where

Table 1
Methylation analysis data for AGS.

Retention time (min)	Methylation sugar	Relative amount (%)	Major mass fragments (<i>m/z</i>)
14.3	2,3,5-Me ₃ -Araf	2.6	29, 45, 87, 101, 114
21.1	2,3,4,6-Me ₄ -Galp	6.2	45, 75, 88, 101, 149
21.4	2,3,4-Me ₃ -Galp	15.2	45, 55, 88, 101, 73,75, 176
22.9	2,5-Me ₂ -Araf	6.2	59, 71, 87, 99, 118, 160, 173
29.2	2,3-Me ₂ -Glc	9.4	45, 73, 75, 88, 130, 161
30.5	2,4,6-Me ₃ -Galp-	15.4	45, 59, 71, 74, 87, 101, 102,

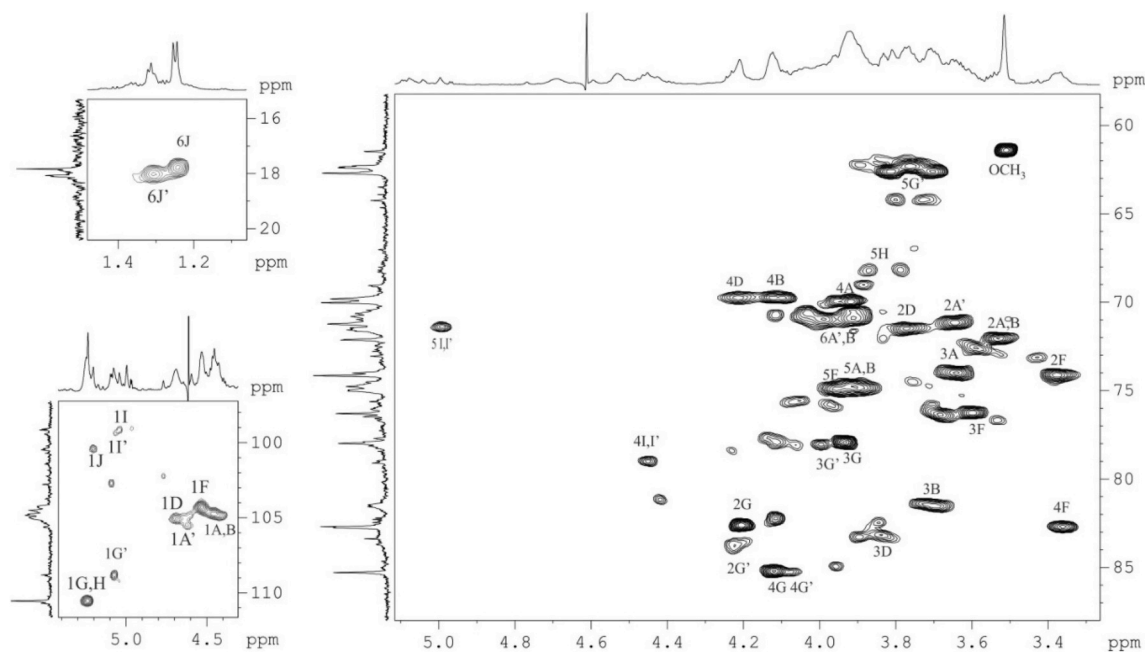


Fig. 1. Parts of HSQC spectrum of arabinogalactan and pectin from the *S. marianum*. Arabic numerals belong to the atoms in the residues denoted by Latin letters as in Table 2.

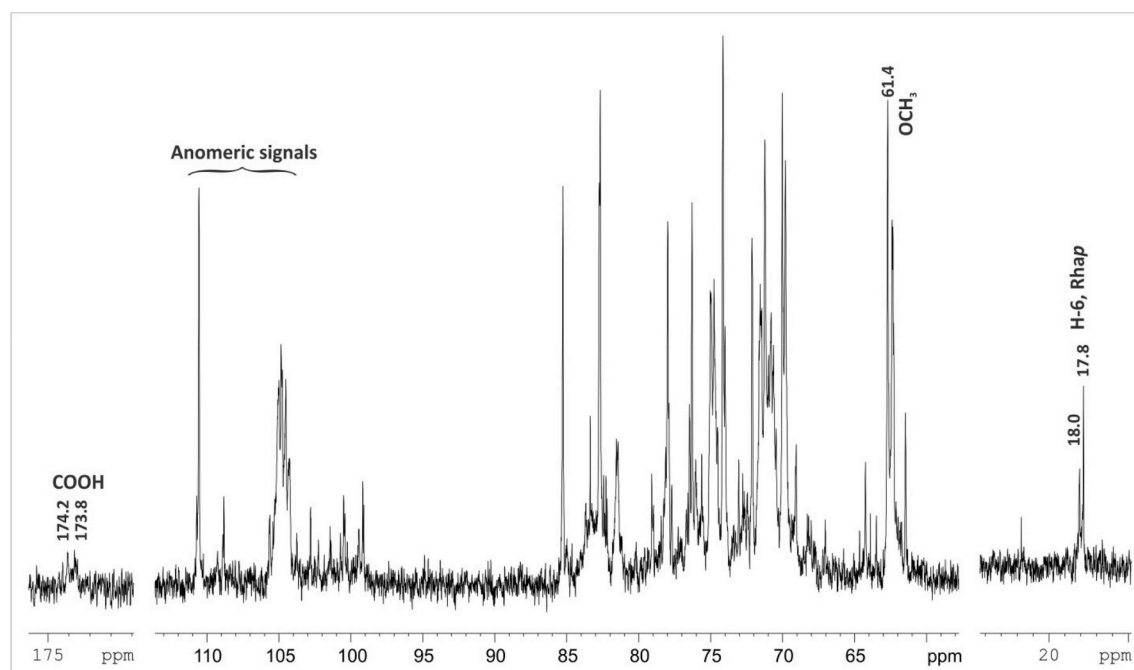


Fig. 2. ¹³C NMR spectrum of arabinogalactan and pectin from the *S. marianum*.

Table 2

Chemical shifts of ^1H and ^{13}C NMR of the main fragments of arabinogalactan and pectin from the *S. marianum*.

Sugar residue	C1 H1	C2 H2	C3 H3	C4 H4	C5 H5,5'	C6 H6, H6'
Arabinogalactan						
→6)-β-Galp-(1→A	104.7	72.1	74.0	70.8	74.8	70.9 ^a
	4.46	3.54	3.65	3.93	3.91	4.00, 3.92
→6)-β-Galp-(1→B	104.8	71.2	81.5	69.8	74.8	70.7 ^a
3)	4.42	3.65	3.71	4.12	3.91	4.04, 3.92
↑	110.6	82.7	77.9	85.3	62.7	
α-Araf-(1G	5.24	4.21	3.94	4.13	3.83, 3.71	
→5)-α-Araf-(1→H	110.6	82.7	77.9	83.3	68.2	
	5.24	4.21	3.94	4.12	3.88, 3.79	
α-Araf-(1→G'	108.8	83.8	78.1	85.3	62.7	
	5.08	4.21	4.00	4.08	3.83, 3.71	
→6)-β-Galp-(1→D	105.1	71.5	83.2	69.8	74.8	70.7 ^a
3)	4.69	3.78	3.87	4.22	3.91	4.04, 3.92
↑	104.8	71.2	74.0	69.8	74.8	70.7 ^a
1)-β-GalpE	4.42	3.65	3.65	4.12	3.91	4.04, 3.92
6)						
↑						
1)-β-GlcpA4OMeF	104.3	74.1	76.2	82.7 ^b	74.8	174.2
	4.53	3.38	3.60	3.37	3.97	
Pectin						
→4)-α-GalpA-(1→I	99.3	69.0	70.6	78.9	71.4	173.8
	5.06	3.89	4.12	4.46	5.00	
→4)-α-GalpA-(1→I'	99.1	69.0	70.6	78.9	71.4	173.8
	5.04 _{xw}	3.89	4.12	4.46	5.00	
→2)-α-Rhap-(1→J	100.4	82.3	69.8	73.2	69.6	17.8
	5.21	4.13	4.01	3.43	3.84	1.25
→2)-α-Rhap-(1→J'	100.4	82.3	69.8	81.3	69.0	18.0
4)	5.21	4.13	4.01	3.71	3.70	1.31
↑	105.6	71.2	74.0	70.8	74.8	70.9 ^a
→6)-β-Galp-(1A'	4.62	3.65	3.65	3.93	3.91	4.00, 3.92
α-Araf-(1→G'	108.8	83.8	78.1	85.3	62.7	
	5.08	4.21	4.00	4.08	3.83, 3.71	

^a The assignment in the column may be interchanged.

^b OMe at δ_{C} 61.4 and δ_{H} 3.52.

part of D-galactopyranose residues is substituted at position C-3 by single residues of L-arabinofuranose or its disaccharide, as well as by β-galactopyranose or by disaccharide β-GlcpA-4-OMe-(1→6)-β-Galp-(1→ (Fig. 2). The structure of the acidic galactan is rather unusual, but not unique. For example, the β-GlcpA-4-OMe-(1→6)-β-Galp-(1→) fragment was found among oligosaccharides isolated from polysaccharides of the fig tree's cell wall (*Sycamore*) [10].

4. Experimental

4.1. Paper chromatography analysis

The solutions were evaporated at $40 \pm 5^\circ\text{C}$. Paper chromatography was carried out on paper Filtrak-FN 13 and 18 in the solvent system: *n*-butanol-pyridine- H_2O (6:4:3, in descending mode). Substances were detected by spraying the following solvents: acidic aniline phthalate (5 min, 100°C), bromophenol blue.

4.2. Plant material

S. marianum is a two-year-old herbaceous plant reaching 1.5 m in height with variegated (spotted) large leaves and purplish red inflorescences (baskets with spikes along the edges) up to 4–5 cm in diameter. Milk thistle in Uzbekistan mainly grows Surkhandarya, Kashkadarya and Jizzakh regions. The productivity of the aerial parts in dry form is 74–89 kg/ha, and seeds 10–12 t/ha. The permissible annual rate of raw material procurement is 27.3 tons [3,11]. The plant materials were collected in Surkhandarya region in 2013 during the rest period.

4.3. IR spectra of samples

The IR spectra of the samples were taken on a Perkin-Elmer System 2000 FT-IR spectrometer in plates pressed with KBr. The number of scans was 100.

4.4. GC analysis of samples

GC analysis of the samples was carried out on a Shimadzu GC-2010 chromatograph with a flame ionization detector, on quartz capillary column Shimadzu Rxi-624Sil MS (30 m × 0.25 mm × 1.40 μm) in mobile phase (N_2) rate 1.5 ml/min and injector temperature was 260°C , the detector temperature was 280°C and the column temperature was 230°C . Samples were taken in the form of aldonitrile acetate.

4.5. GC-MS analysis of samples

GC-MS analysis of the obtained methyl products of arabinogalactan was performed on an Agilent 5975C inert MSD/7890A GC chromatography-mass spectrometer. Separation of the components of the mixture was carried out on an Agilent HP-INNOWax quartz capillary column (30m × 250 μm × 0.25 μm) in the temperature mode: 100°C (2 min) – $4^\circ\text{C}/\text{min}$ to 220°C (5 min) – $15^\circ\text{C}/\text{min}$ up to 250°C (15 min). The injection volume of sample was 1.0 μl, the flow rate of the mobile phase (He) was 1.1 ml/min. The injector temperature was 250°C . EI-MS spectra were obtained in the m/z range of 10–550 a.e.m. The compounds were identified by comparison of their mass-spectral data with those of the Wiley Registry of Mass Spectral Data (9th Ed.), NIST Mass Spectral Library (2011) (W9N11.L).

4.6. NMR spectroscopy

The NMR spectra were measured with Avance AV600 spectrometer (Bruker, Germany) with an operating frequency of 600 MHz for ^1H , using standard software of the company. Chemical shifts were counted from TSP as an internal standard (δ_{H} 0.0, δ_{C} 1.6).

4.7. Monosaccharide analysis

Full acid hydrolysis of WSPS. Samples were hydrolyzed with 1 N H_2SO_4 at 100°C , 8 h, and the hydrolyzate was neutralized as described [10]. The content of uronic acid was determined by the carbazole method [12].

4.8. Fractionation of WSPS

9 g of purified WSPS was dissolved in 500 ml of water and 250 ml of alcohol by dropwise was added to the solution, the precipitate was separated by centrifugation and dried. The yield of fraction 1 was 2.6 g. To the supernatant solution was added another 250 ml of alcohol and fraction 2 was obtained with a yield of 4.2 g (AGS). Fractions 3 were obtained by adding 250 ml of alcohol with a yield of 1.6 g. 250 ml of alcohol was added to the mother aqueous-alcohol solution and was received fraction with a yield of 0.2 g.

4.9. Purification of the fraction

1 g of fraction 2 was dissolved in 50 ml of water and applied to column (30 × 1 cm) with Sephadex G-50, the column was eluted with water. The eluate was evaporated and precipitated with ethanol. The yield of purified fraction from the low molecular weight compounds was 0.82 g. Then 0.82 g of the polysaccharide was dissolved in 10 ml of water and loaded onto a column with DEAE cellulose (OH form) (42 × 3 cm). The column was successively eluted with water, then 0.1 N and 0.5 N alkali, until the polysaccharides were negatively reacted by the phenol-sulfuric acid method [13]. The aqueous eluate was

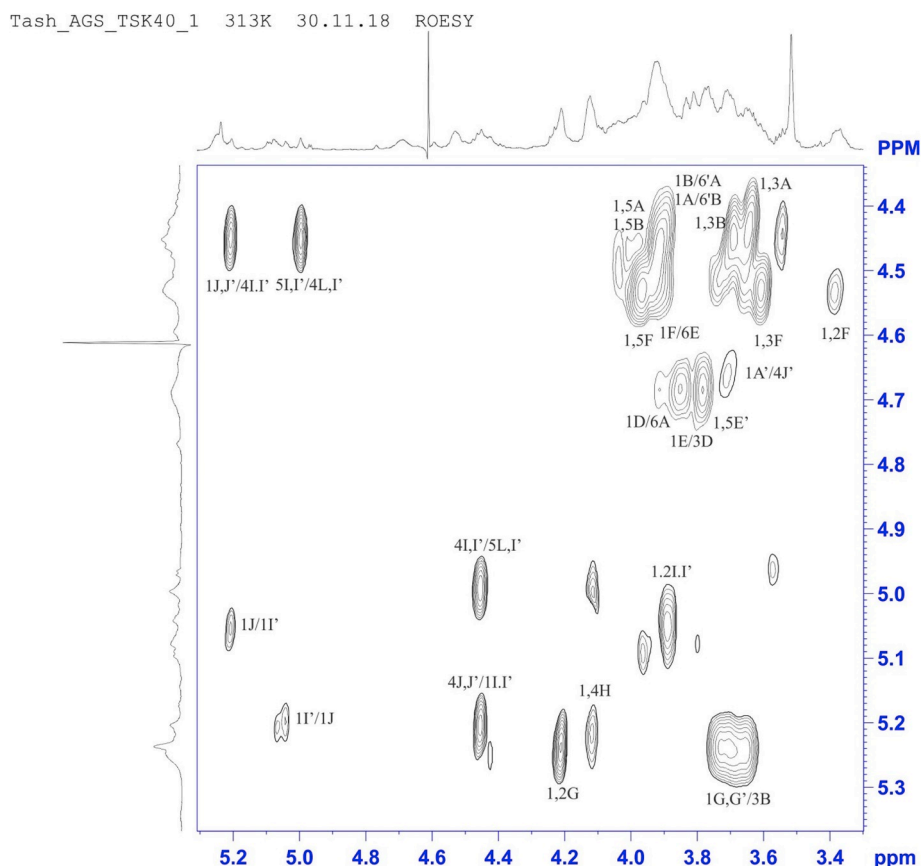


Fig. 3. Part of ROESY spectrum of arabinogalactan and pectin from the *S. marianum*.

evaporated to a certain volume, precipitated two times the volume of ethyl alcohol, then the precipitate was separated, washed with alcohol, and dried in vacuum over P_2O_5 . A neutral polysaccharide was obtained with a yield of 0.05 g. The alkaline extracts were neutralized with CH_3COOH , dialyzed, evaporated and precipitated with two volumes of alcohol. The precipitate was treated as described above. The yield of acid polysaccharide AGS was 0.609 g.

Gel chromatography on a column with Sephadex G-75. 30 mg AGS was dissolved in 1 ml of water and applied on a Sephadex G-75 column (52×1.8 cm). Collected aliquots of 3 ml, the yield of polysaccharides was controlled by the phenol-sulfuric acid method [13].

4.10. Determination of the molecular weight of polysaccharides

The molecular weight of polysaccharides was determined by high-performance exclusion chromatography on an Agilent 1260 Infinity liquid chromatograph using a PL Aquagel OH Mixed chromatographic column (USA) 300 mm long and 8 mm inner diameter. Concentrations of injected samples were 1–4 mg/ml, volume - 20 μ l. Aqueous 0.1 N sodium nitrate solution was used as eluent. The volumetric flow rate of eluent was 0.8 ml/min. The temperature of the systems of columns and detectors was maintained at 25 °C. A differential refractometer was used as a detector. The chromatographic column was calibrated using linear, highly dispersed polymer standards of pullulan (Showa Denko, Japan).

4.11. Smith degradation

0.03225 g of AGS dried to constant weight were dissolved in 24.9 ml of water, then was added 5.1 ml of 0.25 M $NaIO_4$ and kept at a temperature of +5 °C. Every four days, 1 ml aliquots were taken and titrated with 0.01 N thiosulfate sodium solutions. After 16 days, the

consumption of $NaIO_4$ was 2.7 mol, and in the future this amount did not change. The formic acid released during the reaction was titrated with 0.01 N $NaOH$ and it was calculated that its amount was 0.33 mol. To destroy the oxidizing agent, 4–5 drops of ethylene glycol were added, dialyzed, then 0.1 g of $NaBH_4$ was added, and left overnight. The solution was treated with cation exchanger KU-2 (H^+), filtered and the filtrate was evaporated with methanol. The dry residue was hydrolyzed in 3 ml of 0.5 N HCl at 85 °C for 4 h. In the product of the hydrolysis of PC (system: n-BuOH-Pyr- H_2O 6:4:3) and by GLC were found mainly glycerin.

4.12. Methylation of AGS

Arabinogalactan was methylated according to the Hakomori method [8]. 0.5 ml of 0.5 N HCl in methanol was added to the permethylate and heated at 65 °C for 16 h. The resulting methylglycoside was evaporated to dryness, and then the product was acetylated. To do this, 0.5 ml of absolute methanol, 10 μ l of absolute pyridine and 50 μ l of acetic anhydride were added to the sample and the mixture was kept at room temperature for 15 min. The resulting acetyl derivative was dried in a desiccator over $NaOH$.

The obtaining trimethylsilyl derivatives. 50 μ l of absolute pyridine, 10 μ l of HMDS and 5 μ l of TMSC were added to the sample and kept at room temperature for 30 min. The reaction mixture was centrifuged at 3000 rpm, for 10 min, the precipitate was separated, the supernatant solution was dried, dissolved in hexane and analyzed by GC-MS [14].

Disclosure statement

No potential conflict of interest was reported by the authors.

Acknowledgements

This work was financially supported by the Programs for Fundamental Scientific Researches of the Uzbekistan Academy of Sciences under Grants VA-FA-F6-007 and VA-FA-F6-010.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carres.2019.107797>.

References

- [1] K. Abascal, E. Yarnell, The Many Faces of *Silybum marianum* (Milk Thistle): Part 1 - Treating Cancer and Hyperlipidemia and Restoring Kidney Function Alternative and Complementary Therapies vol. 9, (2004), pp. 170–175 <https://doi.org/10.1089/107628003322256878>.
- [2] K.S. Zhauynbaeva, R.K. Rakhmanberdyeva, B.A. Abdurakhmanov, Polysaccharides from *Silybum marianum*, Chem. Nat. Compd. 53 (2017) 820–822 <https://doi.org/10.1007/s10600-017-2132-y>.
- [3] V.A. Kurkin, Saint-mary thistle: a source of medicinals, Pharm. Chem. J. 37 (2003) 189–202 <https://doi.org/10.1023/A:1024782728074>.
- [4] E.N. Medvedeva, V.A. Babkin, O.A. Makarenko, S.M. Nikolaev, V.B. Khobrakova, A.M. Shulunova, T.E. Fedorova, L.A. Es'kova, The obtaining a high arabinogalactan from larch and study of its immunomodulatory properties, Khimija rastitel'nogo syr'ja (Chemistry of plant raw material) (4) (2004) 17–23 (in Russian).
- [5] E.N. Medvedeva, V.A. Babkin, L.A. Ostroukhova, Arabinogalaktan of the larch - properties and prospects of use, Khimija rastitel'nogo syr'ja (Chemistry of plant raw material) (1) (2003) 27–37 (in Russian).
- [6] C.S. Nergard, T. Matsumoto, M. Inngjerdingen, K. Inngjerdingen, S. Hokputsa, S.E. Harding, T.E. Michaelsen, D. Diallo, H. Kiyohara, B.S. Paulsen, H. Yamada, Structural and immunological studies of a pectin and a pectic arabinogalactan from *Vernonia kotschyana* Sch. Bip. ex Walp. (Asteraceae), Carbohydr. Res. 340 (2005) 115–130 <https://doi.org/10.1016/j.carres.2004.10.023>.
- [7] R.K. Rakhmanberdyeva, Arabinogalactomannan from *Gleditsia macracantha* seeds, Chem. Nat. Compd. 45 (2009) 145–147 <https://doi.org/10.1007/s10600-009-9263-8>.
- [8] S. Hakomori, A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide, J. Biochem. 55 (1964) 205–208.
- [9] S. Willför, R. Sjöholm, C. Laine, B. Holmbom, Structural features of water-soluble arabinogalactans from Norway spruce and Scots pine heartwood, Wood Sci. Technol. 36 (2002) 101–110 <https://doi.org/10.1007/s00226-001-0137-x>.
- [10] F. Kodiralieva, A.S. Shahshkov, R.K. Rakhmanberdyeva, Structure of galactomannan from seeds of *Crotalaria alata*, Chem. Nat. Compd. 51 (2015) 405–408 <https://doi.org/10.1007/s10600-015-1303-y>.
- [11] B. Nigmatullaev, Dissertation, Institute of Botany of the Uzbek Academy of Sciences, 2019.
- [12] V.V. Arasimovich (Ed.), Biochemical Methods for Fruit Analysis, Shtiintsa, Kishinev, 1984, p. 12 (in Russian).
- [13] N.K. Kochetkov (Ed.), Methods of Carbohydrate Chemistry, Mir, Moscow, 1967, p. 259 (in Russian).
- [14] J. An, M.A. O'Neill, P. Albersheim, A.G. Darvill, Isolation and structural characterization of β -D-glucosyluronic acid and 4-O-methyl β -D-glucosyluronic acid-containing oligosaccharides from the cell-wall pectic polysaccharide, rhamnogalacturonan I, Carbohydr. Res. 252 (1994) 235 [https://doi.org/10.1016/0008-6215\(94\)90018-3](https://doi.org/10.1016/0008-6215(94)90018-3).