Carbohydrate Research 365 (2013) 20–25

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Isolation and structural characterization of a polysaccharide LRP4-A from *Lycium ruthenicum* Murr.

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ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 22 September 2012
Accepted 16 October 2012
Available online 24 October 2012

Keywords:
Lycium ruthenicum Murr.
Polysaccharide
Structure

ABSTRACT

A complex polysaccharide, termed LRP4-A, was isolated from the fruit of *Lycium ruthenicum* Murr. and its structure was characterized. The crude polysaccharide LRP was obtained from the fruit of *L. ruthenicum* Murr. using hot water extraction followed by ethanol precipitation. The water-soluble polysaccharide LRP4-A was purified from LRP by anion-exchange chromatography and gel filtration chromatography. Its molecular weight was 1.05×10^5 Da. Monosaccharide composition analysis revealed that LRP4-A mainly consisted of rhamnose, arabinose, glucose, and galactose in the molar ratio of 1:7.6:0.5:8.6, with a trace of xylose. Structure of the polysaccharide LRP4-A was characterized using a series of analytical techniques, including methylation analysis, partial acid hydrolysis, IR, NMR, and ESI-MS. LRP4-A was identified to be a highly branching polysaccharide with a backbone of β-(1→6)-linked galactose partially substituted at O-3 position. The branches were composed of (1→3)-linked-Gal, (1→3)-linked-Ara, (1→5)-linked-Ara, and (1→2,4)-linked-Rha. Arabinose, galactose, and glucose were located at the termini of the branches.

1. Introduction

Lycium ruthenicum Murr., which belongs to the genus *Lycium* of the family *Solanaceae*, is a kind of wild resource plant in the northwest of China. It has high photosynthetic efficiency and strong resistance to environmental pressure and lives mainly in Qinghai and Xinjiang Provinces of China.[1] Because of the characteristics of sweetness and juiciness, the fructification of *Lycium ruthenicum* Murr. has been eaten as a fruit or been used as a raw material to produce beverage for a long term. As all know, another species of the genus *Lycium*, *Lycium barbarum* L., has been extensively utilized as a tonic for thousands of years, which was believed to have a variety of biological activities, including immunoregulation, anti-aging, lowering blood-sugar and blood-fat levels, and anti-fatigue.[2,3] Compared with *Lycium barbarum* L., *Lycium ruthenicum* Murr. has same types but much higher content of nutritional ingredients.[4] Moreover, quite a few biologically active molecules from *Lycium ruthenicum* Murr. have also been proved to have a series of biological effects, such as cell-mediated immunity enhancement, anti-oxidation, anti-aging, anti-fatigue, and hypoglycemic activity.[5–8]

Previously, a lot of studies have been focused on polysaccharide of *Lycium barbarum* L., which was revealed to be one of the main active components.[9–12] Polysaccharide from *Lycium ruthenicum* Murr., however, has been seldom investigated. Previous research on *Lycium ruthenicum* Murr. polysaccharide was merely preliminary analysis of its extraction method and bioactivity. Li et al.[13] extracted the crude polysaccharide from *Lycium ruthenicum* Murr., and analyzed its physical and chemical properties. Wang et al.[14] optimized the extraction conditions for the crude polysaccharide of *Lycium ruthenicum* Murr. Many other studies[15–17] disclosed the good biological activities of the crude polysaccharide of *Lycium ruthenicum* Murr. in immunoregulation, anti-fatigue, and blood-sugar reduction.

The current study is focused on the structural analysis of the polysaccharide components of *Lycium ruthenicum* Murr. The crude polysaccharide was first extracted from *Lycium ruthenicum* Murr., and then the ion-exchange and gel-permeation chromatography was used to isolate and purify the crude polysaccharides and a homogeneous polysaccharide LRP4-A was obtained. Structure of the polysaccharide LRP4-A was characterized by a series of analytical techniques including methylation analysis, partial acid hydrolysis, GC, GC–MS, ESI-MS, and NMR. These analysis results could provide a foundation for both the further investigation on the structure–activity relationship of *Lycium* polysaccharide and the deep exploitation of *Lycium ruthenicum* Murr.

2. Experimental

2.1. Plant material, chemicals, and instruments

The fruit of *Lycium ruthenicum* Murr. was purchased from Jinpenyuan Wolfberry Com. Ltd, Qinghai province. Monosaccharide

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http://dx.doi.org/10.1016/j.carres.2012.10.013

standards (D-galactose, L-rhamnose, L-arabinose, D-glucose, D-xylose, and D-galacturonic acid) and dextrans (5000, 12,000, 25,000, 50,000, 80,000, and 150,000 Da) were purchased from Sigma (St. Louis, MO, USA). Sephadex G-100 was purchased from Pharmacia Co. (Uppsala, Sweden). DEAE-cellulose 52 was purchased from HengXin Chemical Reagent Co. (Shanghai, China). All other chemicals used were of analytical grade.

HPLC analysis was carried out on a waters 2695 HPLC system (2695 HPLC pump, 2414 refractive index detector). GC analysis was carried out using a Shimadzu GC-2010 instrument. GC–MS analysis was carried out using a Shimadzu GCMS-QP 2010 system. ESI-MS data were acquired on a Thermo Scientific LTQ XL ion trap mass spectrometer. NMR spectra were recorded using a Varian Inova 400 spectrometer.

2.2. Extraction of crude polysaccharides

The dry fruit of *Lycium ruthenicum* Murr. was pulverized and soaked in distilled water at 80 °C for 2 h. After filtration through a piece of 100-mesh gauze fabric, the obtained solid residue was reprocessed with distilled water at 80 °C for 2 h. The aqueous extracts obtained during the two extraction steps were pooled and concentrated under reduced pressure. A four-fold volume of EtOH was then added to the concentrated extracts, followed by an incubation step at 4 °C for 24 h. The mixture was subjected to centrifugation, and the resulting precipitate was collected and redissolved in distilled water. The obtained aqueous solution was deproteinated according to the Sevage method,[18] dialyzed against distilled water for 48 h, concentrated under reduced pressure and finally lyophilized. The crude polysaccharides termed LRP was obtained.

LRP was dissolved in distilled water at 40 °C and the solution was adjusted to pH 9 with aqueous ammonia. H_2O_2 (30%) was added dropwise until the color faded· After stirring for several hours, the color turned yellow. The solution was dialyzed against distilled water for 48 h, neutralized with 0.5 mol/L HCl and finally lyophilized.[19]

2.3. Fractionation of LRP

DEAE-cellulose (DE-52) anion-exchange chromatography was used to fractionate the decolored LRP. LRP was dissolved in water and loaded onto a DEAE-cellulose column (5 × 25 cm, HCO_3^- form), followed by elution with water and 0.05, 0.1, 0.25, and 0.5 M $NaHCO_3$ at a flow rate of 1 mL/min, successively. The eluted fractions were monitored spectrophotometrically at 490 nm using the phenol–sulfuric acid method. Five fractions designated as LRP1, LRP2, LRP3, LRP4, and LRP5 were obtained. LRP4 was further fractionated with gel-permeation chromatography on a Sephadex G-100 column using 0.1 mol/L NaCl solution as an eluent, and two fractions were obtained. The main fraction, designated as LRP4-A, was collected, dialyzed, and lyophilized. It was further purified using the Sephadex G-100 column chromatography. After dialysis and lyophilization, a white fluffy material termed polysaccharide LRP4-A was obtained.

2.4. Determination of homogeneity and molecular weight

The homogeneity and molecular weight of LRP4-A were evaluated and determined by high performance gel-permeation chromatography (HPGPC). The sample solution was subjected to a Waters 2695 High Performance Liquid Chromatography (HPLC) system equipped with a TSK-GEL G4000 SWXL column (7.8 × 300 mm) and a Waters 2414 Refractive Index Detector. The mobile phase was 0.1 mol/L phosphate buffer (pH 6.0) containing 0.1 mol/L Na_2SO_4 at a flow rate of 0.4 mL/min. The linear regression was calibrated with dextran standards of known molecular weights (5000, 12,000, 25,000, 50,000, 80,000, and 150,000 Da). V_t and V_0 were calibrated with glucose and dextran blue (2000,000), respectively. The molecular weight of LRP4-A was estimated by reference to the calibration curve made above.

2.5. Monosaccharide composition analysis

The polysaccharide sample was hydrolyzed in 2 mol/L TFA at 121 °C for 2 h. The acid was then completely removed by co-distillation with water. Neutral sugars and uronic acids were simultaneously detected by GC utilizing the method described previously.[20] GC was performed on a Shimadzu GC-2010 system equipped with a capillary column of rtx-50 (30.0 m × 0.25 mm × 0.25 μm). The temperature program was: 180 °C for 2 min, then to 210 °C at 6 °C/min, then to 215 °C at 0.3 °C/min, then to 240 °C at 6 °C/min for 30 min. Nitrogen was used as the carrier gas at 0.6 mL/min.

2.6. Determination of sugar and protein contents

The carbohydrate content of each sample was determined using the phenol–sulfuric acid method with glucose as the standard.[21] Protein content of each sample was carried out according to the procedure of Bradford with chicken egg white albumin as the standard.[22]

2.7. FT-IR analysis

The IR spectrum of LRP4-A was recorded in the range from 4000 to 400 cm^{-1} using KBr discs on an EQUINOX 55 Fourier transform infrared spectrophotometer (Bruker, Germany).

2.8. Partial acid hydrolysis

LRP4-A was hydrolyzed in 20 mmol/L H_2SO_4 at 80 °C for 12 h. The solution was dialyzed against distilled water for 72 h using a dialysis bag with a MWCO of 8 kDa. The non-dialyzable (inside the dialysis bag) sample was concentrated and lyophilized, giving the partially hydrolyzed polysaccharide LRP4-A-I. The dialyzable (outside the dialysis bag) fraction was concentrated and neutralized with barium carbonate. After centrifugation, the supernatant was freeze-dried and designated as LRP4-A-O. Sugar compositions of LRP4-A-I and LRP4-A-O were analyzed by GC as described above.

2.9. ESI-MS of the dialyzable oligosaccharides

The sample of LRP4-A-O was desalted using a Dowex 50 WX8-400 cation exchange column according to our previously described method.[23]

The desalted sample was detected by a Thermo Scientific LTQ XL ion trap mass spectrometer (USA) in positive ion mode (sheath gas N_2, flow rate 30.0 arb, auxiliary gas flow rate 5.0 arb, spray voltage 5 kV, heated capillary temperature 350 °C, capillary voltage 48 V, tube lens voltage 220 V). Samples were injected into the ion source by a 2 μL fixed quantity (Rheodyne) loop, and the mobile phase was methanol/water (1:1, v/v) solution at a flow rate of 200 μL/min.

2.10. Methylation analysis

Both the samples of LRP4-A and LRP4-A-I were methylated three times according to the method of Needs and Selvendran.[24] The methylated products were extracted using chloroform and examined by IR spectroscopy. The absence of the absorption peak corresponding to hydroxyl indicated the complete methylation. The per-methylated polysaccharide was hydrolyzed by treatment

with 90% formic acid at 100 °C for 3 h. Excess acid was removed by co-distillation with methanol for three times after the hydrolysis was completed. The products were hydrolyzed in 2 mol/L TFA at 121 °C for 2 h, followed by reduction with $NaBH_4$ and acetylation. The resulting partially methylated alditol acetates were analyzed by GC and GC–MS. GC–MS was performed using a Shimadzu GC–MS-QP2010 system equipped with a capillary column of rtx-5 ms (30.0 m × 0.25 mm × 0.25 μm), and the temperature program was: 140 °C for 2 min, then to 250 °C at 2 °C/min for 20 min. Helium was used as the carrier gas and maintained at 1.24 mL/min.

2.11. NMR spectroscopy

Before NMR analysis, the polysaccharide samples were exchanged three times with 99.9% D_2O, lyophilized and finally dissolved in 99.9% D_2O. ^1H and ^{13}C NMR spectra were recorded at 30 °C on a Varian Inova 400 spectrometer.

3. Results and discussion

3.1. Isolation and fractionation of LRP

The water-soluble crude polysaccharide, designed as LRP, was obtained from the fruit of *Lycium ruthenicum* Murr. by hot water extraction, ethanol precipitation, deproteinization, dialysis, and lyophilization. LRP was a black polysaccharide, the pigment of which could not be removed by column chromatography. To avoid the influence of the pigment on structure analysis, LRP was decolored with 30% H_2O_2, followed by dialysis and lyophilization, giving a pale yellow solid material (yield is 0.96% of the crude herb).

Decolored LRP was fractionated by anion-exchange chromatography. As shown in Figure 1, five sub-fractions, designed as LRP1, LRP2, LRP3, LRP4, and LRP5, respectively, were obtained.

The polysaccharide component LRP4 was further fractionated by gel-permeation chromatography on a Sephadex G-100 column, and two sub-fractions, LRP4-A and LRP4-B, were obtained (see Fig. 2). LRP4-A, the dominant sub-fraction, was further purified by gel-permeation chromatography on a Sephadex G-100 column, and a symmetrical sugar peak was obtained (LRP4-A) with a yield of 0.19% of the crude extracts.

3.2. Homogeneity and molecular weight

The homogeneity and molecular weight of LRP4-A were determined by high performance gel-permeation chromatography (HPGPC). LRP4-A was eluted with 0.1 mol/L phosphate buffer (pH 6.0) containing 0.1 mol/L Na_2SO_4, and a single symmetrical peak was obtained, indicating that the polymer was homogeneous. The calibration curve equation was: $lgMw = 7.925–0.145t_R$,

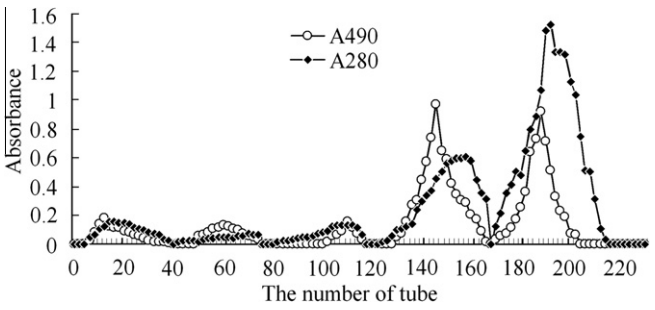

Figure 1. Chromatographic elution profile of LRP on DEAE-cellulose column (5 × 25 cm). LRP1–LRP5 were obtained by elution with H_2O, 0.05, 0.1, 0.25, and 0.5 mol/L $NaHCO_3$ solution, respectively. A490 was determined by sulfate–phenol method.

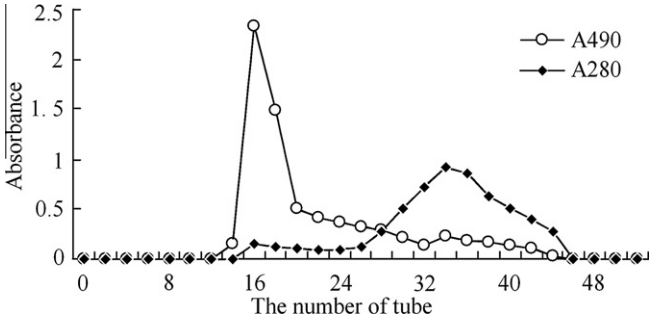

Figure 2. Chromatographic elution profile of LRP4 on a Sephadex G-100 column with 0.1 mol/L NaCl as the eluant.

$R^2 = 0.998$, in which Mw denoted the molecular weight of the standard dextran and t_R represented the retention time. Based on the calibration with standard dextrans, the average molecular weight (MW) of LRP4-A was estimated to be 1.05×10^5 Da.

3.3. Monosaccharide composition, character, and partial acid hydrolysis analysis of LRP4-A

Sugar composition of LRP4-A was determined by GC and the results were shown in Figure 3. LRP4-A mainly consisted of rhamnose, arabinose, glucose, and galactose in the molar ratio of 1:7.6:0.5:8.6, with a trace of xylose. No uronic acid was detected in LRP4-A, and arabinose and galactose accounted for 42.9% and 48.6% of the total sugar content, respectively. As determined by a colorimetric method, the total carbohydrate content was 95.7%, and the protein content was 1.4%. A UV scan in the region of 200–400 nm showed strong absorbance at about 200 nm but no distinct absorbance at 280 nm, indicating further that the LRP4-A sample contained a trace of protein. In the FT-IR spectrum, a typical major broad stretching peak in the region of 3600–3200 cm^{-1} was attributed to the O–H stretching and bending vibrations; the small band at around 2926 cm^{-1} was attributed to the C–H stretching and bending vibrations; the relatively strong absorption peak at 1642 cm^{-1} reflected the absorption of the C–O group that is part of glycosides;[25] the weak absorbance at 895 cm^{-1} suggested that pyranoses existed in the β-configuration. The IR spectrum showed no specific signature of uronic acid in consistence with the data of GC analysis. These results showed that LRP4-A was a neutral polysaccharide which was rich in arabinose and galactose.

Partial acid hydrolysis was carried out for the existence of abundant arabinose in LRP4-A, because furanose has a hydrolysis rate nearly two orders of magnitude higher than that of pyranose in dilute sulfuric acid.[26] LRP4-A was partially hydrolyzed with 20 mmol/L H_2SO_4 at 80 °C for 12 h and then dialyzed against distilled water. The sugar compositions of the non-dialyzable fraction LRP4-A-I and the dialyzable fraction LRP4-A-O were separately analyzed. The results were shown in Figure 4. The dialyzable fraction LRP4-A-O was composed of rhamnose, arabinose, glucose, and galactose in a molar ratio of 1:12.5:0.4:1.8 and contained a trace of xylose, indicating that they were present on the outer chains. The non-dialyzable fraction LRP4-A-I contained Gal and a trace of Glc, suggesting that Gal and a trace of Glc were present as the main chain or brink of the main chain. LRP4-A-I had no rhamnose, arabinose or xylose, suggesting that all rhamnose, arabinose, and xylose were present on the branches of the polysaccharide.

3.4. Methylation analysis

Methylation of LRP4-A and LRP4-A-I was performed three times to obtain per-methylated polysaccharides. After complete acid

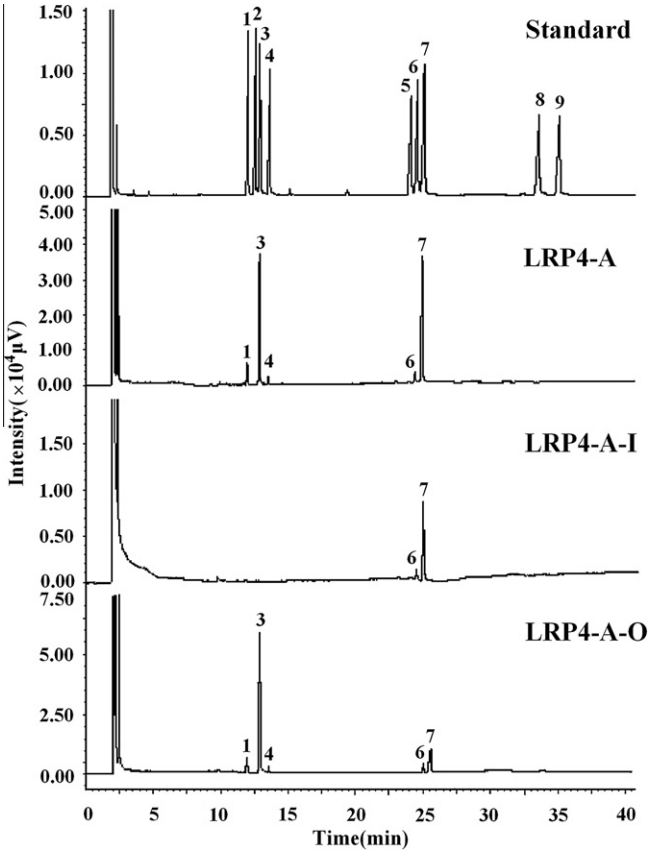

Figure 3. Gas chromatograms of the monosaccharides derived from LRP4-A, LRP4-A-I, and LRP4-A-O. (1) Rhamnose (Rha); (2) Fucose (Fuc); (3) Arabinose (Ara); (4) Xylose (Xyl); (5) Mannose (Man); (6) Glucose (Glc); (7) Galactose (Gal); (8) Glucuronic acid (GlcA); (9) Galacturonic acid (GalA).

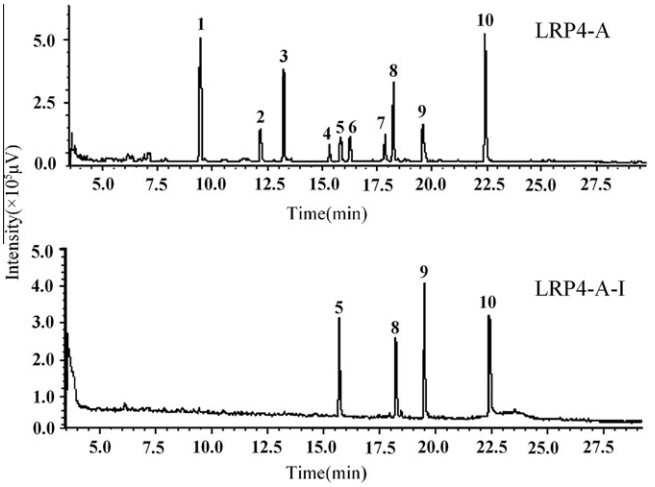

Figure 4. Gas chromatograms of partially methylated alditol acetates derived from LRP4-A and LRP4-A-I. Column: rtx-5 ms (30 m × 0.25 mm × 0.25 μm); temperature: 140 °C (2 min)–2 °C/min, 250 °C (20 min). (1) 2,3,5-Me_3-Ara; (2) 2,5-Me_2-Ara; (3) 2,3-Me_2-Ara; (4) 2,3,4,6-Me_4-Glc; (5) 2,3,4,6-Me_4-Gal; (6) 2,4-Me_2-Ara; (7) 3-Me-Rha; (8) 2,4,6-Me_3-Gal; (9) 2,3,4-Me_3-Gal; (10) 2,4-Me_2-Gal.

hydrolysis and alditol acetylation, the obtained partially methylated alditol acetates were analyzed by GC and GC–MS. The GC chromatograms of LRP4-A and LRP4-A-I were shown in Figure 4. The chromatogram of LRP4-A exhibited ten peaks, while that of LRP4-A-I showed only four peaks. Peaks of methylated sugars were identified by their retention times and mass spectra. The molar ratio of partially methylated alditol acetates was calculated based on both peak area and molar response factors, which were evaluated by the 'effective carbon response', originally according to the predicted ionization potential of organic constituents in an FID.[27] The results of methylation analysis of LRP4-A and LRP4-A-I were listed in Table 1.

As shown in Table 1, the non-reducing ends of LRP4-A contained arabinose, glucose, and galactose. Branches were present on galactosyl residues and on a trace of rhamnosyl residues. The types of arabinosyl residues included terminal Ara, (1→3)-linked-Ara_f, (1→3)-linked-Ara_p, and (1→5)-linked-Ara. The glucosyl residues were present at the non-reducing end. In addition, the galactosyl residues were composed of terminal-, (1→3)-linked, (1→6)-linked, and (1→3,6)-linked galactose. The branching unit was →3,6) Gal(1→, which accounted for about 40.9% of all hexoses. Obviously, the glycan branches highly. Termini of LRP4-A-I were composed of galactose. The branching unit of LRP4-A-I was (1→3,6)-linked Gal, which indicated that the backbone of LRP4-I was composed of either (1→3)-linked Gal or (1→6)-linked Gal. The amount of total terminal sugars was approximately equal to that of branched sugars, suggesting the completeness of methylation. Compared with LRP4-A, LRP4-A-I had no terminal arabinose, terminal glucose, (1→3)-linked Ara_f, (1→3)-linked Ara_p, (1→5)-linked Ara or (1→2,4)-linked Rha, indicating that they existed as branches. The

Table 1
Results of methylation analysis of LRP4-A and LRP4-A-I

Peak number[a]	Partially methylated sugar	Deduced linkage	Relative molar ratio	
			LRP4-A	LRP4-A-I
1	2,3,5-Me_3-Ara	Ara(1-	8	
2	2,5-Me_2-Ara	-3)Ara_f(1-	2	
3	2,3-Me_2-Ara	-5)Ara(1-	6	
4	2,3,4,6-Me_4-Glc	Glc(1-	1	
5	2,3,4,6-Me_4-Gal	Gal(1-	2	5
6	2,4-Me_2-Ara	-3)Ara_p(1-	2	
7	3-Me-Rha	-2,4)Rha(1-	2	
8	2,4,6-Me_3-Gal	-3)Gal(1-	7	4
9	2,3,4-Me_3-Gal	-6)Gal(1-	3	7
10	2,4-Me_2-Gal	-3,6)Gal(1-	9	5

[a] Number of the peaks are the same as those designated in Figure 4.

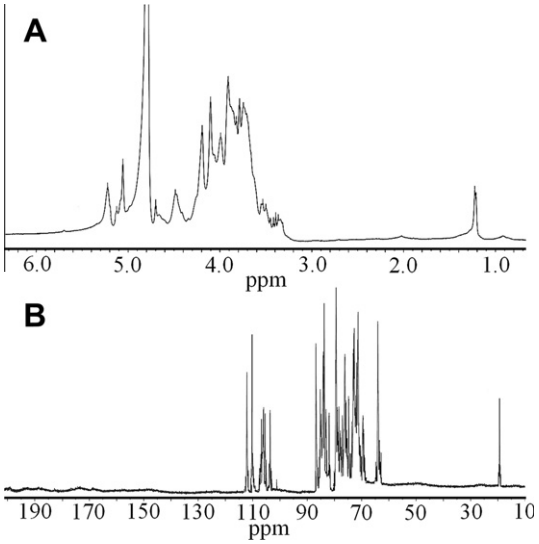

Figure 5. ^1H (A) and ^{13}C (B) NMR spectra of LRP4-A.

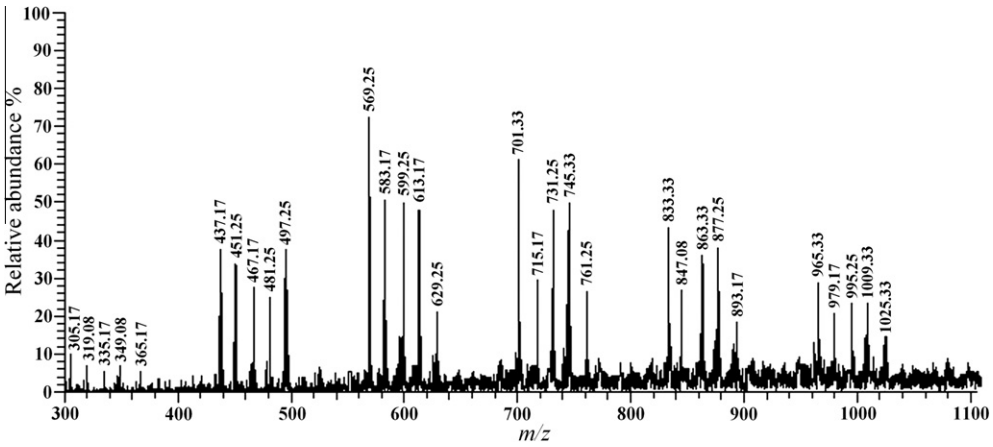

Figure 6. ESI-MS profile of LRP4-A-O.

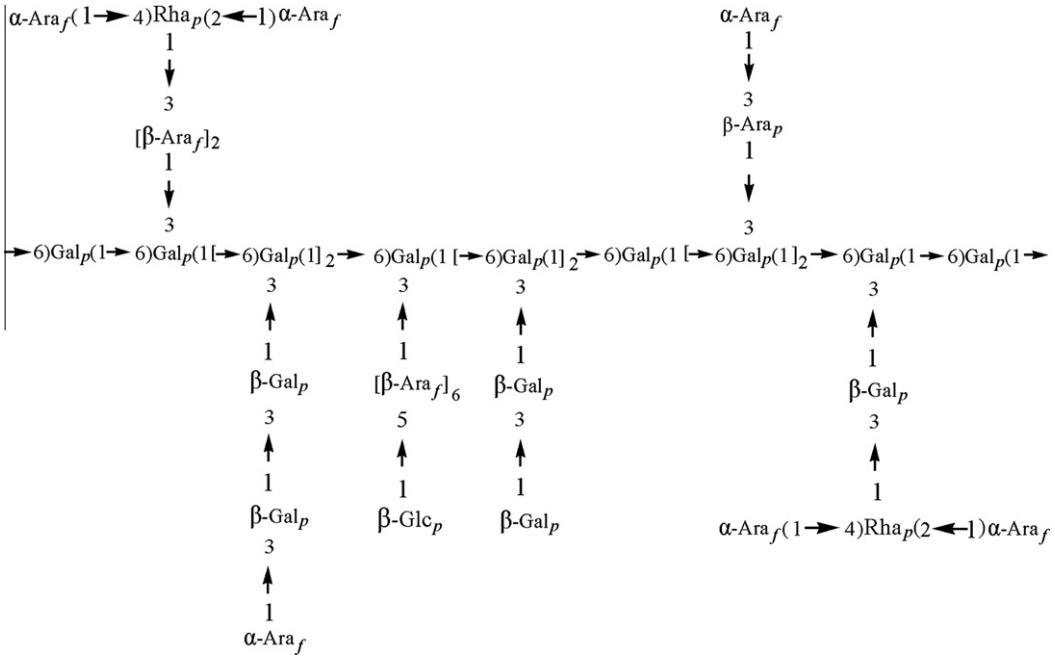

Figure 7. Scheme of the structural unit of LRP4-A.

increase of four →6)Gal(1→ in LRP4-A-I was concomitant with the decrease of four →3,6)Gal(1→ branching unit in LRP4-A, showing that the main chain was composed of →6)Gal(1→. In addition, the three new terminal Gal(1→ in LRP4-A-I was accompanied with the decrease of three →3)Gal(1→, implying that (1→3)-linked Gal was located at branches. It can be deduced from the results that the backbone of LRP4-A was composed of (1→6)-linked Gal.

3.5. NMR analysis

Signals in the ^{1}H and ^{13}C NMR spectra of LRP4-A and LRP4-A-I were assigned as completely as possible, based on the analysis of component and linkage as well as previously reported values.[28–30] In the ^{1}H NMR spectrum of LRP4-A (see Fig. 5A), signals for two anomeric protons occurred near 5.22 and 5.06 ppm, respectively. The signals at 1.24 ppm was assigned to α-Rha_p residues. The signals at ^{1}H 5.22 ppm of LRP4-A were not found in the spectrum of LRP4-A-I, indicating that Ara (1- was α-furanose. The signals of LRP4-A at ^{1}H 5.22 and 5.06 ppm disappeared in the spectrum of LRP4-A-I and the ratio of integral area between ^{1}H 5.22 and 5.06 ppm was 1.2:1. Therefore, (1→3)-linked Ara and (1→5)-linked Ara were of β-configuration.

The signals at 110.07–111.95 ppm, which could be attributed to non-reducing α-Ara_f units, were present in the ^{13}C NMR spectrum of LRP4-A (see Fig. 5B) but absent in that of LRP4-A-I. Signals at 105.29–105.83 ppm were assigned to β-Gal*p*, corresponding to the characteristic absorption at 895 cm^{-1} in IR spectrum. The δ 103.44 ppm resonance signal was assigned to the anomeric carbon of β-Ara_f.

3.6. ESI-MS analysis of LRP4-A-O

The MS profiling of LRP4-A-O was shown in Figure 6. Molecular ions at *m*/*z* 305.17, 437.17, 569.25, 701.33, 833.33, and 965.33, corresponding to $[M+Na]^+$ adducts of pentose polymers with degrees of polymerization ranging from two to seven, respectively, suggested that the side chains of LRP4-A contain different degrees of polymerization of arabinose, consisting with the results analyzed by GC. Ions at *m*/*z* 319.08, 451.25, 583.17, 715.17, 847.08, and 979.17, corresponding, respectively, to $[M+Na]^+$ adducts of dHex-

$Pent_{(1-6)}$, manifested that the side chains of LRP4-A contain rhamnose and different degrees of polymerization of arabinose ranging from one to six. Ions at *m*/*z* 335.17, 467.17, 599.25, 731.25, 863.33, and 995.25, corresponding to $[M+Na]^+$ of $HexPent_{(1-6)}$, respectively, demonstrated the side chains of LRP4-A contain one hexose (galactose or glucose) and different degrees of polymerization of arabinose ranging from one to six. Ions at *m*/*z* 349.08, 481.25, 613.17, 745.33, 877.25, 1009.33, 365.17, 497.25, 629.25, 761.25, 893.17, and 1025.33, were ascribed to $[M+Na]^+$ adducts of HexdHex, $Pent_1HexdHex$, $Pent_2HexdHex$, $Pent_3HexdHex$, $Pent_4HexdHex$, $Pent_5HexdHex$, Hex_2, $Pent_1Hex_2$, $Pent_2Hex_2$, $Pent_3Hex_2$, $Pent_4Hex_2$, $Pent_5Hex_2$, respectively (Pent: arabinose; dHex: rhamnose; Hex: galactose or glucose).

Therefore, based on the obtained data, the structure of the repeating unit of LRP4-A was assigned to that shown in Figure 7. However, this is only one of the possible structures, and the branches can be arranged in other reasonable order.

The polysaccharides isolated from the fruit of *Lycium Barbarum* L. and *Lycium ruthenicum* Murr. mainly belong to a type of arabinogalactans, the linkages of main chains of which comprise backbone of β-(1→6)-linked galactose, β-(1→3)-linked galactose or β-(1→4)-linked galactose.[12,31–33] The structure of the main chain of LRP4-A was shown to be correspondent with that of a polysaccharide LbGP extracted from *Lycium Barbarum* L.,[31] as they shared a main chain structure consisting of β-(1→6)-linked galactose. However, LRP4-A also had some structural features: (1) an obviously high total carbohydrate content, 95.7%, which was much higher than that of LbGP (70%); (2) much more and more complex branching structures than those of LbGP; (3) more diverse monosaccharide types (Gal, Ara, Glc, and Rha) than those of LbGP (Gal and Ara).

4. Conclusion

In this study, a complex polysaccharide LRP4-A was isolated and purified from the *Lycium ruthenicum* Murr., and then its structure was characterized. LRP4-A, a heteropolysaccharide with a molecular weight of 1.05×10^5 Da, was mainly composed of rhamnose, arabinose, xylose, glucose, and galactose, among which arabinose and galactose accounted for 42.9% and 48.6% of the total carbohydrate content, respectively. LRP4-A was identified to be a highly branching polysaccharide with a backbone of β-(1→6)-linked-Galactose. The backbone was partially substituted at O-3 of galactose residues for (1→3)-linked Gal and Gal(1→. The branching side chain was comprised of Ara (1→, (1→3)-linked Ara, (1→5)-linked Ara, Glc(1→, and (1→2,4)-linked Rha. This kind of structure is commonly found in plant arabinogalactan, which comprises generally arabino-3,6-galactan (type II). The research on the biological activities of LRP4-A is proceeding steadily.

Acknowledgment

This study is carried out with financial support from the National Natural Science Foundation of China (No. 31071506, 31170773).

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