Carbohydrate Polymers 84 (2011) 402–406

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Isolation and structural characterization of a novel galactomannan from *Eremurus anisopterus* (Ker. et Kir) Regel roots

Changfeng Hu, Qingjun Kong, Deyu Yang, Yuanjiang Pan*

Department of Chemistry, Zhejiang University, Hangzhou 310027, People's Republic of China

ARTICLE INFO

Article history:
Received 26 August 2010
Received in revised form
17 November 2010
Accepted 28 November 2010
Available online 3 December 2010

Keywords:
Eremurus anisopterus
Polysaccharide
Galactomannan
Gel filtration

ABSTRACT

A novel galactomannan was obtained from the roots of *Eremurus anisopterus* (Ker. et Kir) Regel, an ephemeroid plant in the Gurbantunggut Desert in Xinjiang. The crude polysaccharide was extracted following the usual separation procedure, including water dissolution, centrifugation and precipitation with ethanol. The crude polysaccharide was then applied to anion-exchange chromatography and gel filtration to obtain a homogeneous polysaccharide E_1. The average molecular weight of E_1 was estimated to be 6 kDa according to the calibration curve. On the basis of composition analysis, methylation analysis, periodate oxidation analysis, Smith degradation, infrared spectra (IR) and nuclear magnetic resonance (NMR) experiments, E_1 is a novel linear galactomannan with a main chain composed of (1 → 6)-linked α-D-Gal*p* and (1 → 2)-α-D-Man*p* at a relative molar ratio of 1:3.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Roots of medical plants are important resources of interesting bioactive polysaccharide, many of which have been reported to possess various biological functions. For instance, a glucan isolated from the roots of *Rubus crataegifolius* Bge. exhibits strong immunological activity (Ni et al., 2009). An arabinogalactan extracted from the roots of *Angelica acutiloba* Kitagawa was found to possess anti-complementary activity (Kiyohara & Yamada, 1989); a heteropolysaccharide consisting of Ara, Glc, Gal obtained from the root of *Ophiopogon japonicus* displays hypoglycemic activity (Chen et al., 2011); and an arabinoglucogalactan isolated from *Panax notoginseng* root has antioxidant activity (Wu & Wang, 2008). Moreover, some polysaccharides isolated from plant roots show special structure (Dong, Ding, Yang, & Fang, 1999; Matsumoto, Honsono-Nishiyama, Guo, Ikejima, & Yamada, 2005; Sun & Liu, 2008).

The genus *Eremurus* (Liliaceae), which includes about 50 species, mainly distributes in the mountains of central and western Asia. Four species are known to occur in China (Hou, 1982). *Eremurus anisopterus* (Ker. et Kir) Regel, one of the four species, is an ephemeroid plant in the Gurbantunggut Desert in Xinjiang, China. Its roots can be used as Chinese traditional medicine for the treatment of rheumatism and physical weakness (Liu, Jiang, Dan, Wang, & Zhao, 2002; Yunnan Materia Medica Co., 1993). Several kinds of anthraquinones, naphthalenes and other compounds have been isolated from the genus *Eremurus* (Li, Shi, Zhang, & Zhang, 2000; Zhang, Zhang, Tao, & Li, 2000). In addition, many polysaccharides are present in the genus *Eremurus* as chemical constituents of the species. However, there is no report on purification and structural elucidation of the polysaccharides from the roots of the genus *Eremurus*. Therefore, further studies on this subject of the genus *Eremurus* are desirable.

In this work, we present a detailed study of the purification and structural characterization of a water-soluble polysaccharide E_1 from *E. anisopterus* roots based on ion-exchange chromatography, gel filtration, acid hydrolysis, methylation analysis, periodate oxidation, Smith degradation, NMR experiments, and IR.

2. Experimental

2.1. General procedures

The IR spectra (KBr pellet, 4000–400 cm^{-1}) were determined using a Beckman Acculab 10 instrument. Ultraviolet (UV) absorptions were recorded on a JASCO V-530 UV-Via spectrophotometer. NMR spectra were obtained at 25 °C, using a Brucker 500 MHz instrument. For NMR spectroscopy, E_1 (90 mg) was dissolved in 1 mL of 99.99% D_2O and chemical shifts of samples are expressed in ppm (δ) relative to the signal for Me_4Si. GC–MS was carried out on a Trace DSQ GC-MS instrument (Thermo Fisher Scientific) using an HP-5 column (30 m × 0.25 mm i.d., Agilent) and a temperature program (150–300 °C, 2 min initial hold, 15 °C/min ramp rate, and hold until the end of run). The ionization potential was 70 eV, and the temperature of the ion source was 200 °C.

* Corresponding author. Tel.: +86 571 87951264; fax: +86 571 87951264.
E-mail address: cheyjpan@zju.edu.cn (Y. Pan).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.11.054

2.2. Plant material

Roots of *E. anisopterus* were collected in August 2008 in Shihezi, Xinjiang Province, China and identified by Dr. Yongxiang Cheng (Shihezi University, Shihezi, China).

2.3. Extraction and isolation

After defatted with petroleum ether and EtOAc, the powdered roots (2 kg) were extracted with deionized water at the room temperature. The aqueous was concentrated to 200 mL under vacuum. To precipitate the polysaccharide, four volumes of 95% ethanol were gradually added to the solution. After 12 h, the deposit was collected by centrifugation, and washed with acetone and 95% ethanol three times. Protein was removed using Sevage reagent (*n*-butanol:chloroform at the ratio of 1:5, v/v) according to the Sevage procedure (Sevag, Lackman, & Smolens, 1938). The solution was concentrated and subjected to a column of weakly basic anion-exchange styrene macroreticular resin (D 315) as decolorizer, yielding 6.35 g decolorized fraction.

The decolorized fraction (400 mg) was dissolved in deionized water (2.0 mL) and centrifuged. The supernatant was then applied to a column of fast-flow DEAE-Sepharose (Pharmacia) (2.6 cm × 40 cm) eluted successively with deionized water, 0.1 M, 0.3 M, and 0.5 M NaCl (300 mL each). The content of polysaccharide was detected by phenol–H_2SO_4 spectrophotometric method (Dubois, Gilles, Hamilton, & Rebers, 1956). According to the elution profile, main part obtained from the deionized water elute was dialyzed against deionized water with a tubular membrane (MWCO: 3500 Da, Huamei Bioengineering Company, Shanghai, China) for 2 d to remove small molecules. The residue within membrane was then concentrated, lyophilized, and further chromatographed on a Sephadex G-200 (Pharmacia) column (2.6 cm × 40 cm) using 0.05 M NaCl as eluent. Appropriate fractions were combined, dialyzed and lyophilized to yield E_1 (92 mg).

2.4. Determination of purity and molecular weight

The purity of E_1 was determined by high-performance gel permeation chromatography (HPGPC) carried out on a Waters 515 instruments (Milford, MA) fitted with a Waters 2410 refractive index detector and a TSK G4000-SWXL (Tosoh Biosep, made in Japan) size exclusion analytical column (7.8 mm × 300 mm). The mobile phase was deionized water and the sample concentration was 5 mg/mL. T-series Dextran standards (Sigma), which have definite molecular masses ranging from 10 to 500 kDa, were used to calibrate the HPGPC system. During the experiment process, the column was kept at 40 °C. All data obtained by the system were collected and analyzed using the Waters Millenium 32 software package.

2.5. Component analysis

Polysaccharide E_1 was hydrolyzed with 2 M trifluoroacetic acid (TFA) at 100 °C for 6 h, followed by evaporation to dryness. The product was divided into two portions. One portion was used for paper partition chromatograph (PC). The PC was performed on Whatman 3 MM filter paper, and the solvents used were as follows: A, 3:3:1 EtOAc–HOAc–H_2O; B, 6:4:3 *n*-butanol–pyridine–H_2O. Sugars were detected by aniline–diphenylamine and *p*-anisidine (Bailey & Bourne, 1960). Authentic standards including D-glucose, D-mannose, D-galactose, D-arbinose, D-xylose, L-rhamnose, and D-glucuronic acid were used as reference. The other portion was reduced with $NaBH_4$ at room temperature for 12 h and acetylated with Ac_2O-pyridine (1 mL each) at 100 °C for 10 h (Wolfrom & Thrompson, 1963). Finally, the resulting alditol acetates were analyzed by GC–MS and identified by their typical retention times and electron impact profiles.

2.6. Methylation

Per-*O*-methylation of E_1 was performed in dried DMSO–CH_3I using NaOH as a catalyst (Ciucanu & Kerek, 1984). The entire process was repeated three times. The product showed no –OH absorption bands in the IR spectrum. After complete acid hydrolysis was carried out with 2 M TFA in a boiling water bath for 8 h, the methylated E_1 was reduced with $NaBH_4$, converted into partially methylated alditol acetates, and analyzed by GC–MS.

2.7. Periodate oxidation and smith degradation

E_1 (19.6 mg) was oxidized with 0.04 M $NaIO_4$ (25 mL) in the dark at 4 °C until stabilization of periodate consumed (measured at 233 nm) (Goldstein, Hay, Lewis, & Smith, 1965; Hay, Lewis, & Smith, 1965). The excess periodate was destroyed by adding ethylene glycol, and the solution was dialyzed against deionized water for 2 d. After it was reduced with $NaBH_4$ (50 mg) for 12 h at 25 °C, the residue was subjected to complete hydrolysis with 2 M TFA. The acid was removed by co-distillation with methanol under vacuum. Finally, the products were acetylated and analyzed by GC–MS.

3. Results and discussion

The powdered roots were extracted with deionized water at the room temperature. After it was concentrated, the aqueous solution was defatted with petroleum ether and EtOAc, respectively. The resulting fraction was decolorized using a weakly basic anion-exchange styrene macroreticular resin. Then the decolorized part was sequentially separated through fast-flow DEAE-Sepharose and Sephadex G-200 chromatography to yield a purified polysaccharide E_1. The HPGPC profile (Fig. 1) showed a single and symmetrically sharp peak, indicating that E_1 was a homogeneous polysaccharide. According to the calibration curve, the average molecular weight of E_1 was about 6 kDa. E_1 appeared as a white powder and had no absorption at 280 or 260 nm in the UV spectrum, suggesting the absence of protein and nucleic acid.

Component analysis was conducted with 2 M TFA for 12 h at 100 °C. PC showed that galactose and mannose were present in E_1. In addition, the result of GC–MS indicated only galactose and mannose were produced as the neutral sugars at a molar ratio of 1:3. According to the literature, the absolute configuration of the sugars was determined by GLC of their acetylated glycosides, using (+)-2-butanol (Gerwig, Kamarling, & Vliegenthart, 1978). Both of them had D-configuration.

The IR spectrum of E_1 showed strong bands at 3405, 2930, 1635, 1030, 932, 869, 815, and 595 cm^{-1}. The bands at 869 and 815 cm^{-1} were characteristics of D-mannose present in the polysaccharide. The broad band at 1635 cm^{-1} was due to bound water. The band at 2930 cm^{-1} was ascribed to C–H stretching vibration, whereas the strong band at 3405 cm^{-1} was contributed to the hydroxyl stretching vibration of the polysaccharide (Zhang, 1997).

The oxidation of E_1 was completed in 4 d with 0.04 M sodium periodate at 4 °C. The periodate consumption of polysaccharide was 1.31 mol/mol sugar residue. The oxidized E_1 was reduced with $NaBH_4$ to yield corresponding polyalcohol. After Smith degradation of the polyalcohol, the glycol and glycerol were found in GC–MS analysis through conversion to the alditol acetates. The absence of monoses and erythritol suggested that no (1 → 3)-and (1 → 4)-linked glycosyl bonds were presented in E_1. The amount of periodate consumed indicated that E_1 was a polysaccharide with (1 → 2)- and (1 → 6)-linked backbone.

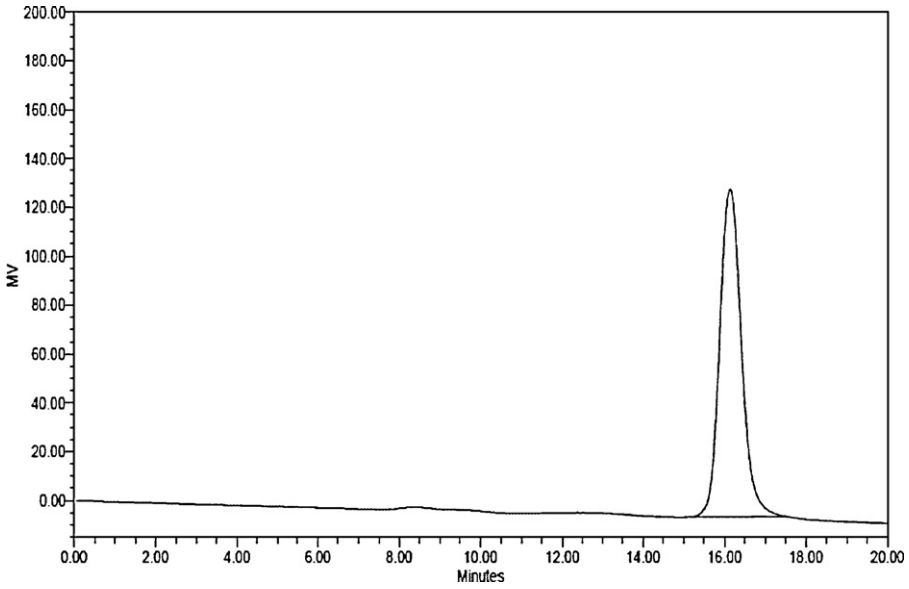

Fig. 1. The HPGPC profile of E_1 isolated from *E. anisopterus* roots.

Table 1
GC–MS results of partially *O*-methylalditol acetates of methylation analysis of E_1 isolated from *E. anisopterus* roots.

Alditol acetate	Retention time[a]	Approximate molar ratio[b]	Fragments (diagnostic ions, *m*/*z*)	Proposed structure
2,3,4-Tri-*O*-methylgalactitol	1.02	1.00	87,99,101,117,129,161,189,233	→ 6)-Gal*p*-(1 →
3,4,6-Tri-*O*-methylmanitol	1.00	3.18	87,101,129,130,145,161,189, 190	→ 2)-Man*p*-(1 →
2,3,4,6-Tetra-*O*-methylglucitol	0.95	Trace	87,88,101,117,129,145,161,205	Glc-(1 →

[a] Relative to 3,4,6-tri-*O*-methylmanitol.
[b] Relative to 2,3,4-tri-*O*-methylgalactitol.

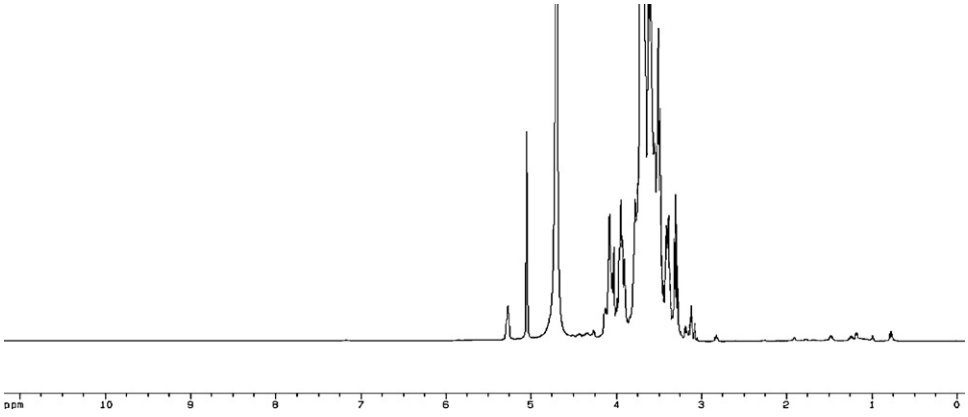

Fig. 2. 500 MHz ^{1}H NMR spectrum of E_1 isolated from *E. anisopterus* roots in D_2O at 25 °C.

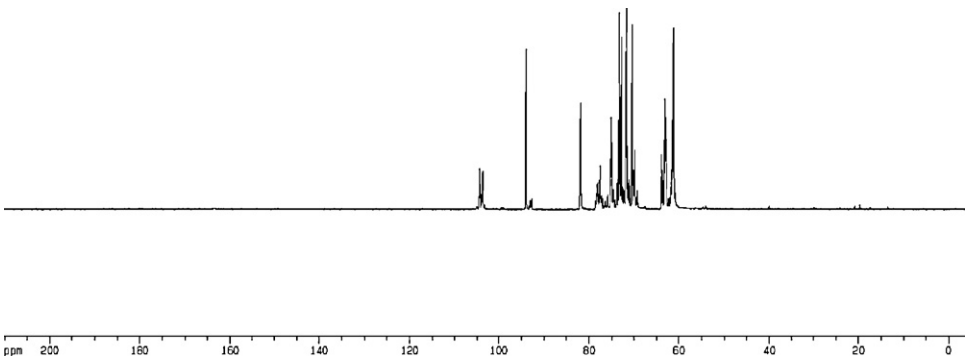

Fig. 3. 125 MHz ^{13}C NMR spectrum of E_1 isolated from *E. anisopterus* roots in D_2O at 25 °C.

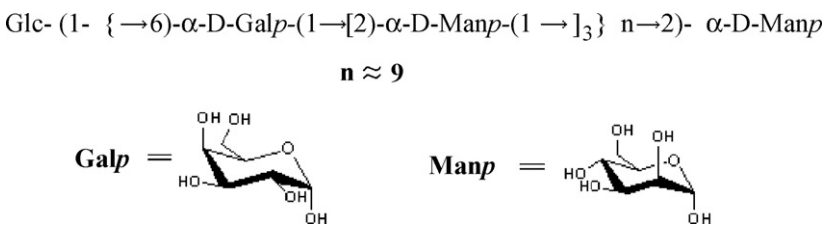

Fig. 4. The proposed structure of E_1.

Permethylated E_1 was obtained by repeating the procedure of a modified Hakomori method three times. After the product was hydrolyzed, reduced and acetylated, GC–MS showed two main peaks corresponding to 2,3,4-tri-*O*-methyl-Gal and 3,4,6-tri-*O*-methyl-Man derivatives at an approximate molar ratio of 1:3.18 (Table 1), which coincided with the result of periodate oxidation and Smith degradation. In the 500 MHz ^{1}H NMR spectrum of E_1, the resonances of the anomeric protons were separated at δ 5.26 and 5.05 with the ratio of peak areas at approximate 1:3 (Fig. 2), which were assigned as (1 → 6)-α-D-Gal*p* (residue A) and (1 → 2)-α-D-Man*p* (residue B), respectively. The other signals occurred in the region of δ 4.2–3.2, which showed overlapping peaks, were assigned to the protons of carbons C-2 to C-5 (or C-6) of the glycosidic ring. In the broadband decoupled ^{13}C NMR spectrum (Fig. 3) of the galactomannan, the signal at δ 104.4 and 103.8 (a complex resonance) was from the anomeric carbons of residue A and B, respectively (Ahrazem, Prieto, Leal, Jiménez-Barbero, & Bernabé, 2002; Zhang, 1997). The signals at 94.0 and 93.8 were ascribed to the reducing Man residue which has α/β configurations (anomerization effect) (Davis, Hoffmann, Russell, & Debet, 1995). In conclusion, E_1 is a linear galactomannan with a main chain composed of (1 → 6)-α-D-Gal*p* and (1 → 2)-α-D-Man*p* in 1:3.

Galactomannans are the seed energy reserve polysaccharides of many plants (Chaubey & Kapoor, 2001; Joshi & Kapoor, 2003) and the minor components of the fungal cell wall (Ahrazem et al., 2002; Giménez-Abián, Bernabé, Leal, Jiménez-Barbero, & Prietoa, 2007). They have an enormous structural variability between species. The units of the backbone can be unsubstituted, monosubstituted or disubstituted at different positions (Baron, Iacomini, Fanta, & Gorin, 1991; Carbonero, Tischer, Cosentino, Gorin, & Iacomini, 2003; Cunha, Vieira, Arriaga, de Paula, & Feitosa, 2009; Ishrud, Zahid, Zhou, & Pan, 2001). However, linear galactomannans are considered to be rare polymers. So special attention is now being given to the bioactivities of this polysaccharide, and the results will be reported elsewhere in the future.

4. Conclusion

A water soluble polysaccharide E_1 was isolated from hot-water extraction of the roots of *E. anisopterus* and purified by anion-exchange chromatography and gel-filtration chromatography. On the basis of chemical analysis and NMR studies, E_1 is a novel galactomannan, the average molecular weight of which was estimated to be about 6 kDa, with a main chain composed of (1 → 6)-α-D-Gal*p* and (1 → 2)-α-D-Man*p* in 1:3 (Fig. 4). So E_1 is a rare linear galactomannan and may exhibit some special bioactivities.

Acknowledgment

This work was supported by the National Science Foundation of the People's Republic of China [grant number 20975092].

References

Ahrazem, O., Prieto, A., Leal, J. A., Jiménez-Barbero, J., & Bernabé, M. (2002). Fungal cell-wall galactomannans isolated from *Geotrichum* spp. and their teleomorphs, *Dipodascus* and *Galactomyces*. *Carbohydrate Research*, *337*, 2347–2351.

Bailey, R. W., & Bourne, E. J. (1960). Colour reactions given by sugars and diphenylamine-aniline spray reagents on paper chromatograms. *Journal of Chromatography A*, *4*, 206–213.

Baron, M., Iacomini, M., Fanta, E. S., & Gorin, P. A. J. (1991). Galactomannan, lichenan and isolichenan from the polysaccharide rich lichen Newropogon aurantiacoater. *Phytochemistry*, *30*, 3125–3126.

Carbonero, E. R., Tischer, C. A., Cosentino, C., Gorin, P. A. J., & Iacomini, M. (2003). Structural characterization of galactomannan from the cyanolichen *Leptogium azureum*. *Carbohydrate Polymers*, *53*, 469–473.

Chaubey, M., & Kapoor, V. P. (2001). Structure of a galactomannan from the seeds of *Cassia angustifolia* Vahl. *Carbohydrate Research*, *332*, 439–444.

Chen, X. M., Jin, J., Tang, J., Wang, Z. F., Wang, J. J., Jin, L. Q., et al. (2011). Extraction, purification, characterization and hypoglycemic activity of a polysaccharide isolated from the root of *Ophiopogon japonicus*. *Carbohydrate Polymers*, *83*, 749–754.

Ciucanu, I., & Kerek, F. (1984). Simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Cunha, P. L. R., Vieira, Í. G. P., Arriaga, Â. M. C., de Paula, R. C. M., & Feitosa, J. P. A. (2009). Isolation and characterization of galactomannan from *Dimorphandra gardneriana* Tul. seeds as a potential guar gum substitute. *Food Hydrocolloids*, *23*, 880–885.

Davis, A. L., Hoffmann, R. A., Russell, A. L., & Debet, M. (1995). ^{1}H- and ^{13}C-NMR characterization of the digalactosylmannopentaose liberated from legume seed galactomannan by β-mannanase action. *Carbohydrate Research*, *271*, 43–54.

Dong, Q., Ding, S. W., Yang, X., & Fang, J. N. (1999). Structural features of a heteroxylan from *Sophora subprostrata* roots. *Phytochemistry*, *50*, 81–84.

Dubois, M., Gilles, K. A., Hamilton, J. K., & Rebers, P. A. (1956). Colorimetric method for determination of sugars related substances. *Analytical Chemistry*, *28*, 350–356.

Gerwig, G. J., Kamarling, J. P., & Vliegenthart, J. F. G. (1978). Determination of the D and L configuration of neutral monosaccharides by high-resolution capillary g.l.c. *Carbohydrate Research*, *62*, 349–357.

Giménez-Abián, M. I., Bernabé, M., Leal, J. A., Jiménez-Barbero, J., & Prietoa, A. (2007). Structure of a galactomannan isolated from the cell wall of the fungus *Lineolata rhizophorae*. *Carbohydrate Research*, *342*, 2599–2603.

Goldstein, I. J., Hay, G. W., Lewis, B. A., & Smith, F. (1965). Controlled degradation of polysaccharides by periodate oxidation, reduction and hydrolysis. *Methods in Carbohydrate Chemistry*, *5*, 361–370.

Hay, G. W., Lewis, B. A., & Smith, F. (1965). Periodate oxidation of polysaccharides: general procedures. *Methods in Carbohydrate Chemistry*, *5*, 357–361.

Hou, F. C. (1982). In J. E. Xia (Ed.), *A dictionary of the families and genera of Chinese seed plants*. Beijing: Science Press (Revised by Wu, T.L., Ko, W.O.C., & Chen, T.C.).

Ishrud, O., Zahid, M., Zhou, H., & Pan, Y. J. (2001). A water-soluble galactomannan from the seeds of *Phoenix dactylifera* L. *Carbohydrate Research*, *335*, 297–301.

Joshi, H., & Kapoor, V. P. (2003). *Cassia grandis* Linn. f. seed galactomannan: structural and crystallographical studies. *Carbohydrate Research*, *338*, 1907–1912.

Kiyohara, H., & Yamada, H. (1989). Structure characterization of an anti-complementary arabinogalactan from the roots of *Angelica acutiloba* Kitagawa. *Carbohydrate Research*, *193*, 193–200.

Li, C., Shi, J. G., Zhang, Y. P., & Zhang, C. Z. (2000). Constituents of *Eremurus chinensis*. *Journal of Natural Products*, *63*, 653–656.

Liu, J. R., Jiang, F. S., Dan, J. M., Wang, H. Y., & Zhao, W. B. (2002). Ultrasonic extraction and determination of polysaccharides of *Eremurus anisopterus*. *Chinese Traditional and Herbal Drugs*, 322–323.

Matsumoto, T., Honsono-Nishiyama, K., Guo, Y. J., Ikejima, T., & Yamada, H. (2005). A possible signal transduction pathway for cyclin D2 expression by a pectic polysaccharide from the roots of *Bupleurum falcatum* L. in murine B cell. *International Immunopharmacology*, *5*, 1373–1386.

Ni, W. H., Zhang, X., Bi, H. T., Iteku, J., Ji, L., Sun, C. X., et al. (2009). Preparation of a glucan from the roots of *Rubus crataegifolius* Bge. and its immunological activity. *Carbohydrate Research*, *344*, 2512–2518.

Sevag, M. G., Lackman, D. B., & Smolens, J. (1938). The isolation of components of streptococcal nucleoproteins in serologically active form. *Journal of Biological Chemistry*, *124*, 425–436.

Sun, Y. X., & Liu, J. C. (2008). Structural characterization of a water-soluble polysaccharide from the Roots of *Codonopsis pilosula* and its immunity activity. *International Journal of Biological Marcromolecules*, *43*, 279–282.

Wolfrom, M. L., & Thrompson, A. (1963). Reduction products-reduction with sodium borohydrate. In R. L. Whistler, & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (pp. 65–68). New York: Academic Press.

Wu, Y. L., & Wang, D. N. (2008). Structural characterization and DPPH radical scavenging activity of an arabinoglucogalactan from *Panax notoginseng* root. *Journal of Natural Products*, *71*, 241–245.

Yunnan Materia Medica Co. (1993). *Names of Yunnan traditional materia medica resources*. Beijing: Science Press., p. 633.

Zhang, W. J. (1997). *Biochemical technology of glycoconjugate research*. China: Zhejiang University Press., pp. 196, 245–247.

Zhang, Y. P., Zhang, C. Z., Tao, B. Q., & Li, C. (2000). Chemical constituents from *Eremurus chinensis* Fedtsch. *China Journal of Chinese Materia Medica*, *25*, 355–356.