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Structural analysis and anti-tumor activity comparison of polysaccharides from *Astragalus*

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ABSTRACT

Two polysaccharides: APS-I and APS-II, were obtained after a successive purification consisting of (1) fractional precipitation using ethyl alcohol; (2) filtration through DEAE-Sephadex A-25 and Sephadex G-100 eluted by water. The molecular weights of APS-I and APS-II were 4.77×10^6 and 8.68×10^3 Da, respectively, and they were mainly composed of glucose, and a small amount of arabinose and xylose, with their molar ratios of 0.54:1:18.14 and 0.23:1:29.39, respectively for APS-I and APS-II. FTIR, methylation, periodate oxidation, Smith degradation, ^{1}H NMR and ^{13}C NMR were used in their structure analysis. The results indicated that structurally, APS-I and APS-II were similar, with their main chains mainly composed of major α-(1 → 3) glucose and a few 1 → 4, 1 → 6 glucoses, while the side chain contained arabinoses and xyloses. The tumor inhibition ratios of APS-I and APS-II were 55.47% and 47.72%, respectively.

1. Introduction

Radix *Astragalus* is the dry root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge. It belongs to Leguminous plant which has been used as a health-promoting herb in China for more than 2000 years. Many pharmacological functions of different components from *Astragalus* have been recognized in "Chinese Pharmacopoeia". Particularly for its polysaccharide fractions which show a variety of biological activities, such as anti-tumor, immune modulation and hypoglycemic effects (Lin & Chiang, 2008; Liu, Wu, Mao, Wu, & Ouyang, 2009; Shao et al., 2004; State Pharmacopoeia Committee, 2010).

Polysaccharides from the functional fractions of *Astragalus* have received much attention in the research community. Previously, one kind of polysaccharides (APS) has been isolated from *Astragalus*, which is an α-(1 → 4)-D-glucan, containing one single α-D-glucose at the C-6 position of every nine residue, with a molecular weight of approximately 3.6×10^4 Da (Li, Chen, Wang, & Tian, 2009). The animal experiments indicated that APS could have a glomerulonephritis therapeutic potential (Li & Zhang, 2009). Others reported that APS was an α-(1 → 4)-D-glucan with α-(1 → 6)-linked branches attached to the O-6 of branch points and biological tests showed that APS had significant immune modulating activity, thus can be used to treat the gastric cancer (Li et al., 2009).

Recently, we isolated several kinds of polysaccharides from *A. mongholicus* and studied their effects on cancer cells. Interestingly, two novel polysaccharides, named APS-I and APS-II, have a backbone composed of (1,3)-β-D-glucopyranosyl (Glcp) residues. It has been shown that the bioactivities of polysaccharides are most closely related to their chemical composition, configuration and molecular weight, as well as their physical properties. In this paper, we report the extraction and purification of two novel polysaccharides from *A. mongholicus* based on the DEAE-A25 and Sephadex G-100 column chromatography, and their structure were identified by a series of chemical and instrumental analyses. In addition, the correlations between the structural characteristics and anti-tumor activities *in vitro* were also assayed.

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Table 1
The GC result of standard monosaccharides.

Name	Rha	Ara	Xyl	Man	Glu	Gal	Internal standard
Retention time (min)	6.682	7.307	7.902	17.359	18.682	20.066	28.898

2. Materials and methods

2.1. Materials

The dry roots of *A. mongholicus* were commercially obtained from Datong, Shanxi, China, and were crushed to coarse powder.

D-Glucose, D-galactose, D-xylose, L-arabinose, D-mannose, T-series dextran were purchased from Solarbio Corp. (Beijing, China). DEAE-Sephadex A-25 and Sephadex G-100 were purchased from Sigma (St, Louis, MO, USA). All other chemicals were of analytical reagent grade, and were used as received.

2.2. Extraction and purification of polysaccharides

The pre-degreased powdered roots (50 g) of *A. mongholicus* were extracted three times by using CaO solution (pH 9–10, 300 mL) for 90 min at 100 °C (Li, 2000), the filtrate was combined and concentrated to 200 mL using a rotary evaporator at 60 °C. The protein was removed by following the Sevag method (Alam & Gupta, 1986). The mixture was precipitated by using 3 volumes of ethanol overnight after removal of the Sevag reagent. The crude polysaccharide (8.95 g) was obtained by centrifugation at 2000 r/min for 20 min and washed using ethanol and acetone alternately 5 times.

The polysaccharide was re-dissolved and fractioned by using 30% and 70% ethanol solutions, successively. Subsequently, the fractions were separately by applying a DEAE-Sephadex A-25 column (30 cm × 3 cm) that was eluted with distilled water. The yielded fractions were concentrated and combined according to the phenol–sulfuric acid method. The solutions were concentrated in a rotary evaporator and applied to Sephadex G 100 (30 cm × 3 cm) and equilibrated with distilled water. Each fraction had only one main peak, and it was collected and freeze-dried.

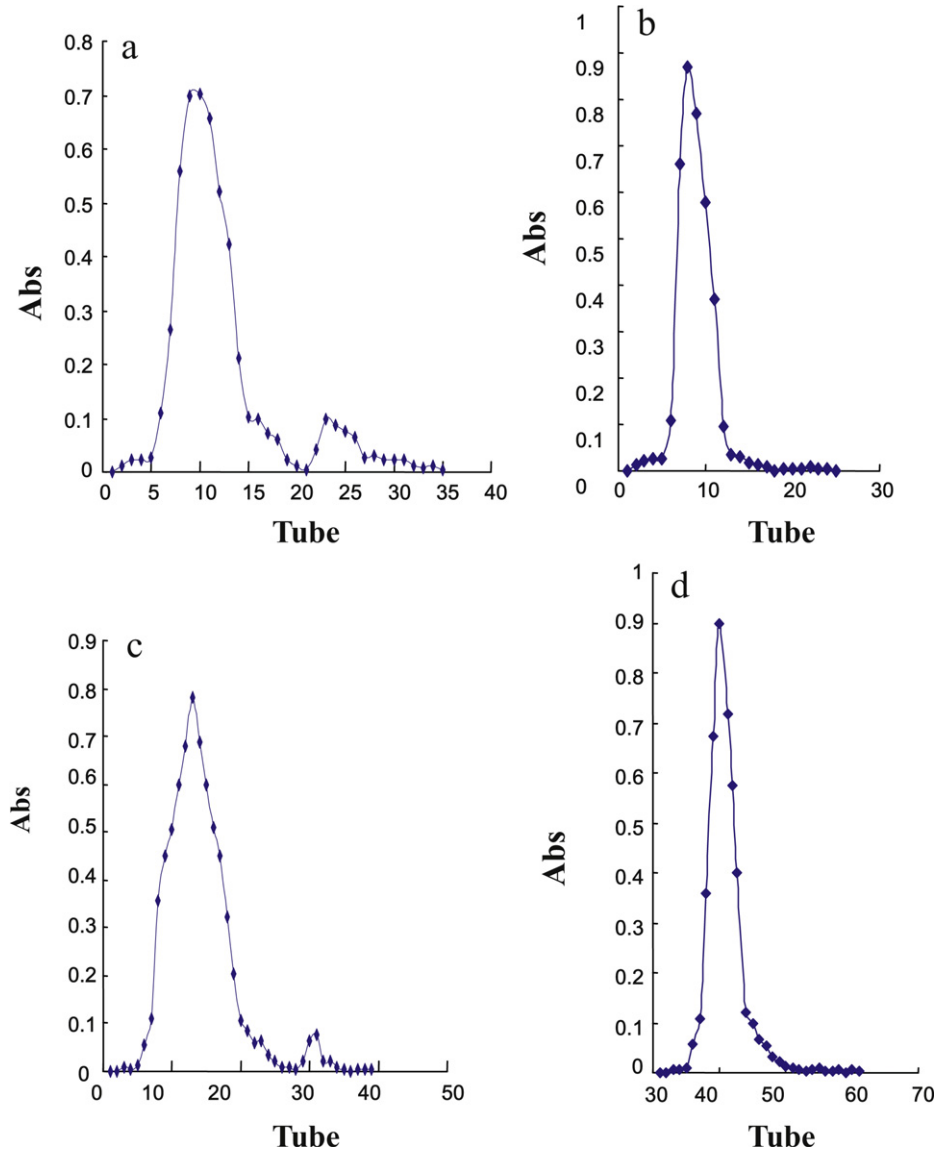

Fig. 1. Elution profiles of APS-I on DEAE-Sephadex A-25 (a), APS-I on Sephadex G-100 (b), APS-II on DEAE-Sephadex A-25 (c), and APS-II on Sephadex G-100 (d).

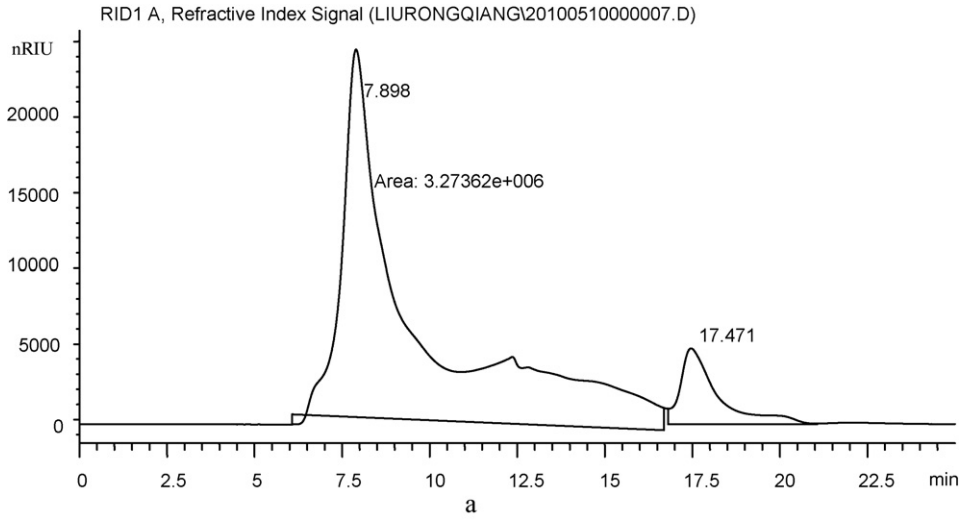

Fig. 2. HPGPC profiles of APS-I (a) and APS-II (b). The results supported the conclusion that APS-I and APS-II were homogeneous polysaccharides. The weighted-average molecular weight of APS-I and APS-II were 5.02×10^6 and 8.5×10^3 Da, respectively, based on the column calibration.

Table 2
Monosaccharides composition and molar ratio of APS-I.

Sugar	Retention time (min)	Molar ratio
Ara	7.319	0.54
Xyl	7.895	1
Glc	18.996	18.14

Table 3
Monosaccharides composition and molar ratio of APS-II.

Sugar	Retention time (min)	Molar ratio
Ara	7.295	0.23
Xyl	7.889	1
Glc	19.019	29.39

Table 4
Results of the methylation analysis of APS-I.

Methylation positions	Linkages	Major mass fragments (*m*/*z*)
2,3-Me_2-Ara	1,4-Linked Ara	44,88,101,131
2,3-Me_2-Xyl	1,4-Linked Xyl	43,73,100,103,142
2,3,4-Me_3-Glc	1,6-Linked Glc	58,71,87,101,117,129,143,161,189
2,3,6-Me_3-Glc	1,4-Linked Glc	45,75,103,115,143,173
2,4,6-Me_3-Glc	1,3-Linked Glc	75,88,103,139,159,189,201,231,233,259
2,3,4,6-Me_4-Glc	Terminal Glc	45,71,87,101,117,129,145,161,205

Two main polysaccharides APS-I and APS-II were obtained as white powders.

2.3. Determination of molecular weight by high performance gel-permeation chromatography (HPGPC)

The molecular weights and homogeneity of APS-I and APS-II were determined by using a HPLC (Agilent-1200) equipped with a TSK-gel G4000PWxl column (7.8 mm × 300 mm, column temperature 30 °C) and Refractive Index Detector (RID, detecting temperature 40 °C). A sample solution (20 μL) was injected and run with purified water at 0.6 mL/min as mobile phase. The standard curve was established using T-series Dextran as the standards (T-10, T-40, T-70, T-500 and T-2000) (Chang, Qu, Lin, Duan, & Weng; Fei, Yua, & Yin, 2010).

Table 5
Results of the methylation analysis of APS-II.

Methylation positions	Linkages	Major mass fragments (*m*/*z*)
2,3-Me_2-Ara	1,4-Linked Ara	43,45,75,88,97,110,157
2,3-Me_2-Xyl	1,4-Linked Xyl	42,44,71,88,101,103,129
2,3,4-Me_3-Glc	1,6-Linked Glc	43,45,58,88,101,117,129,143,161,189
2,3,6-Me_3-Glc	1,4-Linked Glc	58,75,103,115,143,173
2,4,6-Me_3-Glc	1,3-Linked Glc	75,101,116,127,139,159,189,231,233,259
2,3,4,6-Me_4-Glc	Terminal Glc	45,71,87,101,117,129,145,161,205

2.4. IR analysis

IR spectra were collected on the polysaccharides samples. 1 mg sample was mixed with 150 mg of dry KBr, and pressed into a disk for the analysis. The IR spectra were recorded in the range of 400–4000 cm^{-1} on a Fourier transformed IR spectrophotometer (VECTOR-22).

2.5. Monosaccharide and methylation analysis

The polysaccharide sample (5 mg) was dissolved in 4 mL of TFA (2 M) and hydrolyzed to monosaccharides at 110 °C for 3 h in a sealed tube. Subsequently, thin layer chromatography (TLC) was performed using silica gel plate (G254), and the elute was a mixture of *n*-butyl alcohol–methyl alcohol–chloroform–acetic acid–H_2O (12.5/4.5/5/1.5/1.5; v/v/v/v/v) while aminobenzene–diphenylamine was the visualizing aid, under the conditions of 110 °C for 10 min. GC was used to determine the mono sugar concentration (He, 2008), using a capillary column (OV-225, China) and a FID detector, while nitrogen was used as the carrier gas (40 mL/min). Other conditions were: the injector temperature of 250 °C, and the detector temperature of 280 °C.

The methylation analysis was performed based on the Hakomori method (Hakomori, 1964). The sample was treated with 90% formic acid (4 mL) for 6 h at 110 °C, then the residue was hydrolyzed using 2 M TFA (4 mL) for 3 h at 110 °C. After removal of formic

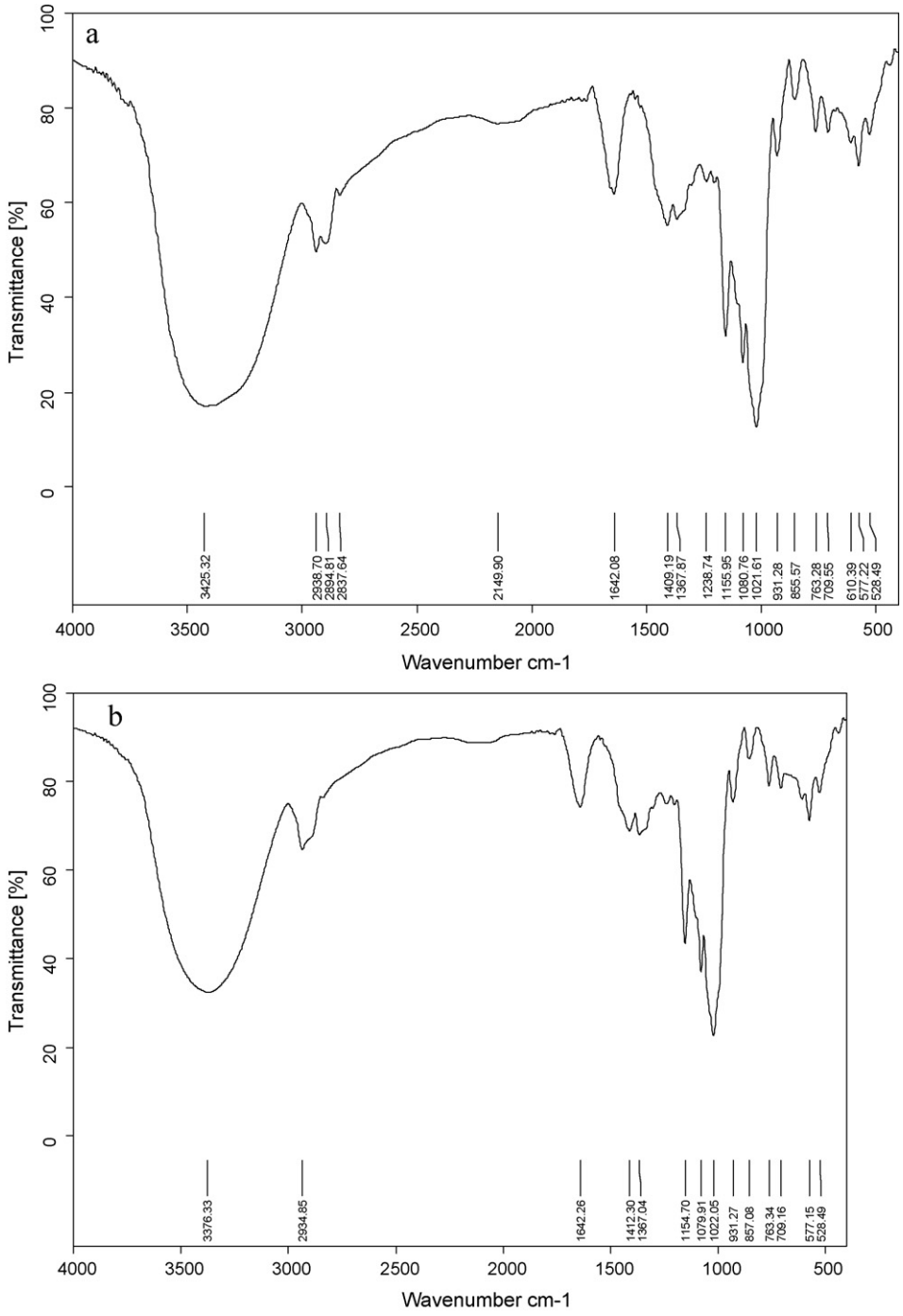

Fig. 3. FTIR spectra of APS-I (a) and APS-II (b).

acid, the hydrolysate was concentrated to dryness. The methylated monosaccharides were converted into their corresponding alditol acetates by reduction with $NaBH_4$ at room temperature for 3 h. The reduced polysaccharide was acetylated with acetic anhydride, and dissolved in chloroform and ready for GC–MS analysis.

2.6. Periodate oxidation and Smith degradation analysis

The periodate oxidation and Smith degradation analysis were performed based on the literature methods (Chaplin & Kennedy, 1994; Dixon & Lipkin, 1954; Fang, 1988).

2.7. NMR spectroscopy

The sample was dried using P_2O_5 in vacuum for several days, and then exchanged with deuterium by lyophilizing with D_2O four times. The ^1H and ^{13}C NMR spectra were recorded at 27 °C on a Bruker Advance DPX-500 spectrometer.

2.8. Animal experiments

Kuming mice (6–8 weeks old, 18–22 g, half male half female) were obtained from Shanchuanhong Experimental Animals Cor-

poration of Tianjin, China (Licence Number: SCXK (Tianjin, China) 2009-0001) and were kept at 25 ± 1 °C in a humidity of 60 ± 10% with 12 h light/dark cycle. There were six groups of mice including one control group, one positive control group and 4 experimental groups (APS-I at 50 mg/kg and 100 mg/kg, APS-II at 50 mg/kg and 100 mg/kg), each group had 10 mice and were implanted with H22 cells (2×10^6 cells) by subcutaneous injection at the right gluteal region. After 7 days when the cells were grew, the control group was administrated by saline through intragastric way. For the positive control group, cyclophosphamide (30 mg/kg) was administrated through intragastric way. And for the experimental groups, polysaccharides samples were administrated by the same way. After 10 days of intragastric administration, the mice were sacrificed and the tumor and visceral organ weights were measured and made into sections (Lin & Chiang, 2008; Ren, 2010).

2.9. Statistical analysis

All data were expressed as means ± SD of three parallel measurements. Analysis of variance (ANOVA) was used to evaluate the significant difference among various treatments with the criterion of $P < 0.05$.

3. Results and discussion

3.1. Structural analysis

The yield of crude polysaccharide fraction was 12.96% of the coarse powder of *A. mongholicus*. The crude polysaccharides were separated and sequentially purified through DEAE-A25 and Sephadex G-100, each showing a main product (Fig. 1), as detected by the phenol–sulfuric acid assay.

The total carbohydrate content was determined based on the phenol–sulfuric acid method as D-glucose equivalents (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), and the total sugar contents of APS-I and APS-II were 94.59% and 97.57%, respectively. The protein content was determined by the Coomassie brilliant blue method as BSA equivalents (Bradford, 1976). UV–vis absorption spectra were recorded on a spectrum-2102UV spectrophotometer, and no absorption was observed, indicating that APS-I and APS-II did not contain protein and nucleic acid. The specific rotation, determined at 25 °C with an automatic polarimeter (Model WZZ-2B, China) were recorded $[\alpha]_D^{25} = +185^\circ$ (C, 0.001 g/mL, H_2O) and $[\alpha]_D^{25} = +170^\circ$ (C, 0.001 g/mL, H_2O) for APS-I and APS-II, respectively.

The result of HPGPC analysis (Fig. 2) indicated that APS-I and APS-II were homogeneous polysaccharides. The estimated weight-average molecular weights of APS-I and APS-II were 5.02×10^6 Da and 8.5×10^3 Da, respectively, which were obtained based on the calibration of using dextran.

The IR spectra of APS-I and APS-II (Fig. 3) showed two strong bands at 3425and 3376 cm^{-1}, attributing to the hydroxyl stretching vibration of the polysaccharide; the bands at 2939 and 2935 cm^{-1} were due to the C–H stretching vibration; the band at 1642 cm^{-1} was due to the presence of bound water. The three bands at 1021–1155 cm^{-1} indicated the pyran configurations of polysaccharides. The bands at 855 and 931 cm^{-1} were characteristic of (1 → 3)-α-glucan (Barker, Bourne, Stacey, & Whiffen, 1954). The IR spectra did not yield significant differences between the two APS.

TLC analysis indicated that monosaccharides of APS-I and APS-II mainly contained glucose, while arabinose and xylose were also present in small amounts. The GC analysis showed that the monosaccharides of APS-I and APS-II were arabinose, xylose and glucose with molar ratios of 2.32:4.29:93.39 and 0.62:2.74:95.97, respectively (Tables 1–3), suggesting that glucose is the main sugar in APS-I and APS-II.

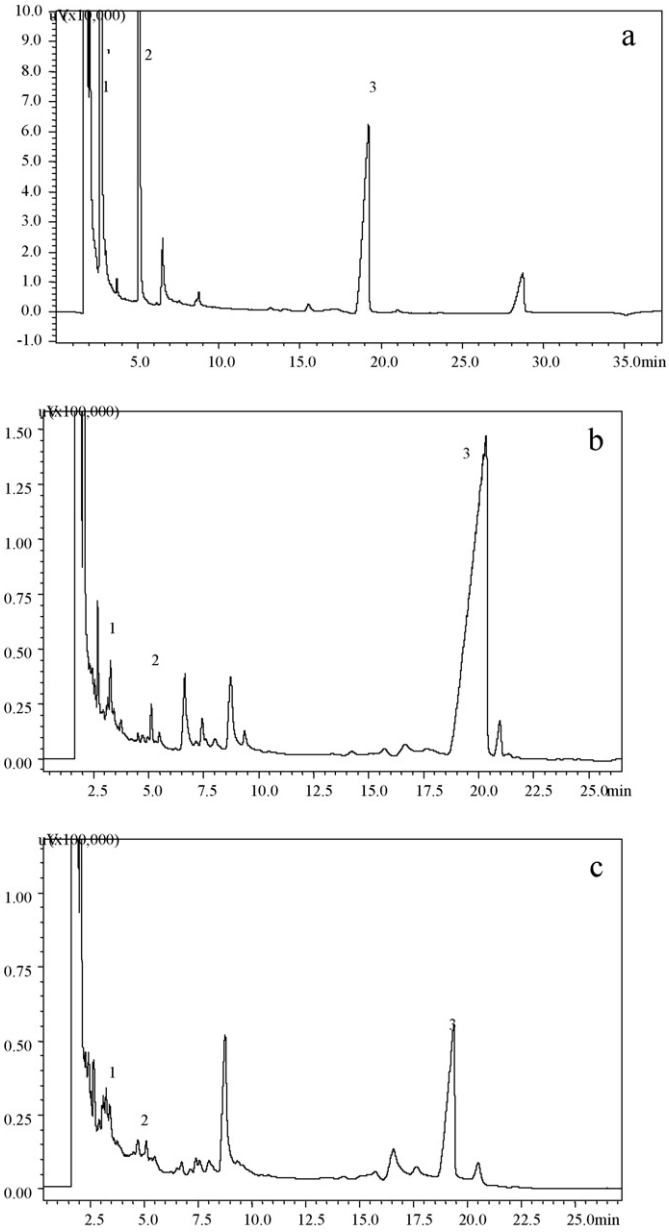

Fig. 4. GC profiles of standard monosaccharides (a), Smith degradation products of APS-I (b), and Smith degradation products of APS-II (c). Peak identity: 1-glycerol, 2-erythritol, and 3-glucose.

APS-I and APS-II were methylated using the Hakomori method (Hakomori, 1964), followed by hydrolysis and conversion into alditol acetates (Tables 4 and 5). The results indicated that APS-I and APS-II had the similar structure; the linear chain was mainly composed of major α-(1 → 3) glucose, while the side chain was made of (1 → 4), (1 → 6) glucose, arabinose and xylose.

The results of periodate oxidation showed that the consumption of $NaIO_4$ was 0.011 mmol and of the amount of generated formic acid was 0.0016 mmol for APS-I, while for APS-II, 0.012 mmol of $NaIO_4$ was consumed and 0.0018 mmol of formic acid was generated. The GC analysis of the Smith degradation of the periodate-oxidized APS-I and APS-II showed that they contain glycerol, erythritol and glucose (Fig. 4). The molar ratio of glycerol,

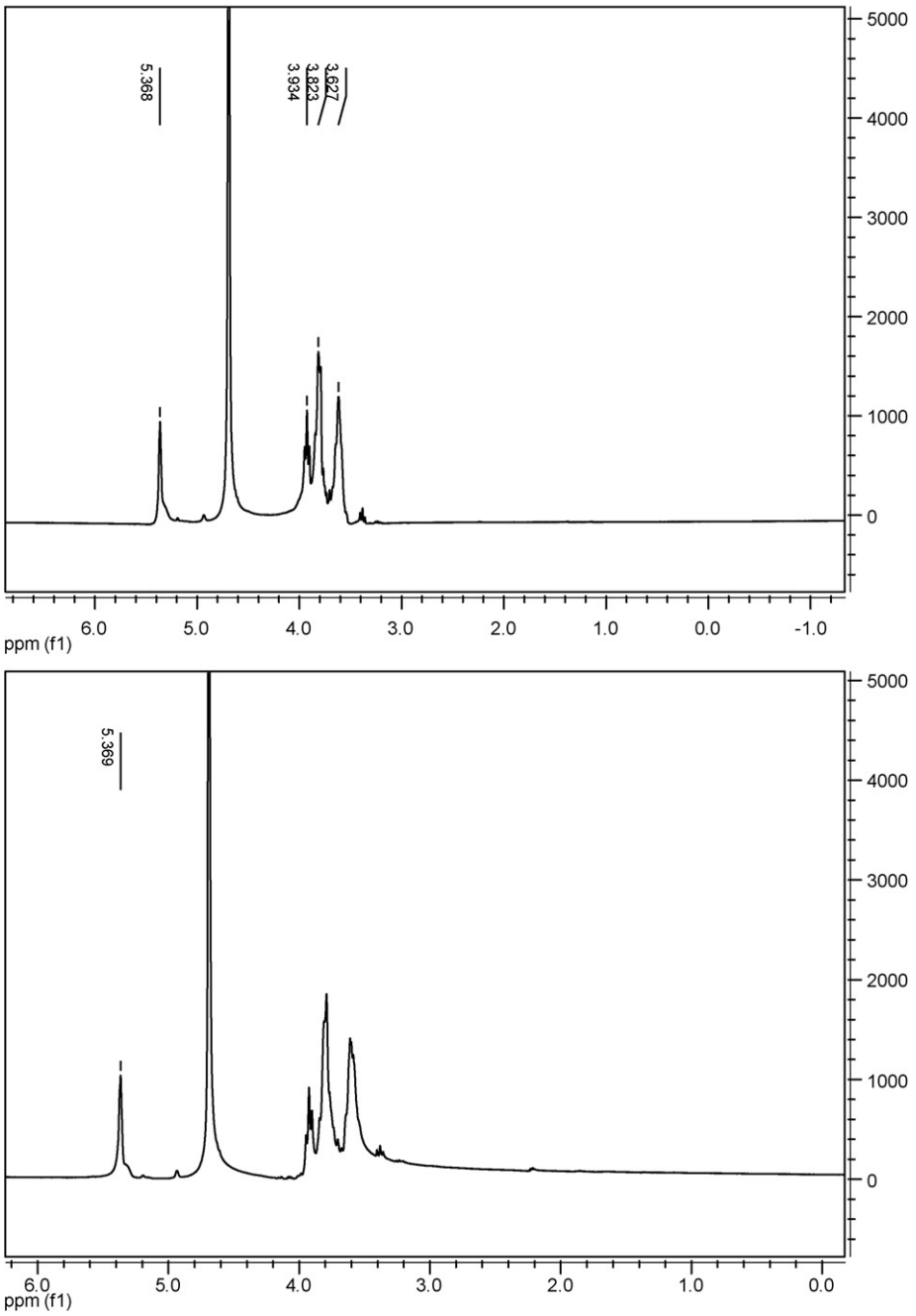

Fig. 5. ^{1}H NMR spectra of APS-I (a) and APS-II (b).

erythritol and glucose was in the ratio of 4.36:2.41:87.21 for APS-I, and 5.6:4.64:81.15 for APS-II. Based on the above results, it can be concluded that APS-I and APS-II were mainly made up of major α-(1 → 3) glucose (Goldstein, 1965).

The ^{1}H and ^{13}C NMR spectra of APS-I and APS-II were shown in Figs. 5 and 6. The results supported the conclusion that that the main polysaccharides for both APS-I and APS-II were composed of glucoses. The resonances in 99.70–99.65 ppm could be attributed to the anomeric carbon atoms of Glcp. The characteristics of the carbon signals suggested the α-anomeric configuration of the Glcp moieties (Uzochukwu, Balogh, Loefler, & Ngoddy, 2002), which is in agreement with the results of IR and methylation. Therefore, it was concluded that APS-I and APS-II had the same structure of (1 → 3)-linked glucan and the degree of polymerization is the only difference between APS-I and APS-II.

3.2. Anti-tumor activities of APS-I and APS-II

The animal experiment results in Table 6 showed that the positive group had the minimum tumor weight, and the difference between APS-I and APS-II groups would be negligible. However, it was very different when compared with the control group, and the ratios of anti-tumor were 49.71%, 55.47%, 45.46% and 47.72%, respectively. Thus the activity of anti-tumor of APS-I was higher than that of APS-II. These results suggested that the anti-tumor activities of polysaccharides from *Astragalus*, may be related to their

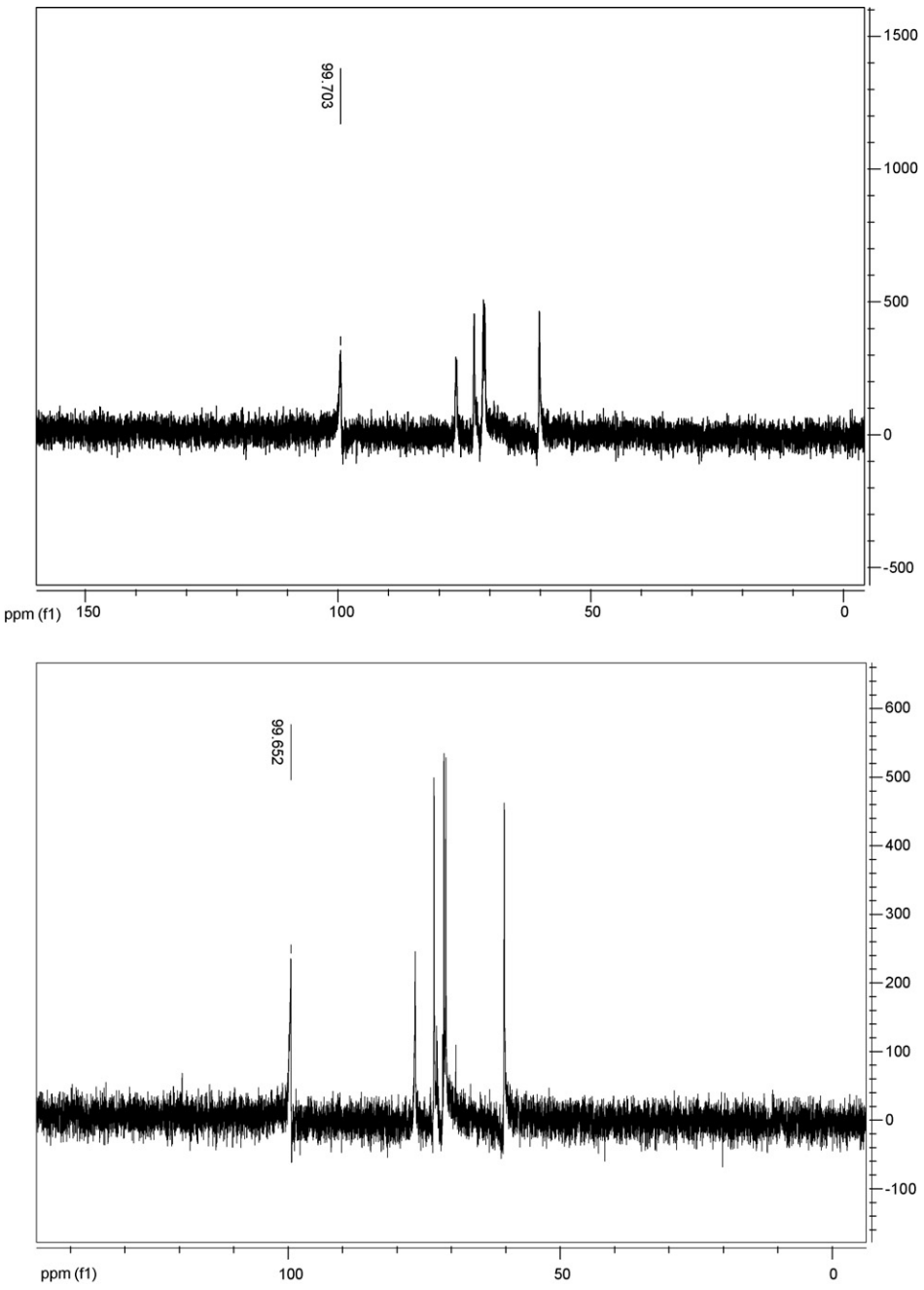

Fig. 6. ^{13}C NMR spectra of APS-I (a) and APS-II (b).

Table 6
The result of anti-tumor-H22 effect by APS-I and APS-II.

Group	Liver weight (g)	Tumor weight (g)	Ratio of anti-tumor (%)	Spleen index (mg/g)	Thymus index (mg/g)
Control	2.17 ± 0.32	2.27 ± 1.02	–	83.81 ± 16.38	28.07 ± 9.33
Positive control	1.83 ± 0.22[a]	0.98 ± 0.54[a]	56.77	34.71 ± 11.04[a]	4.63 ± 0.89[a]
APS-I 100 mg/kg	2.28 ± 0.27[a]	1.14 ± 0.79[a]	49.71	72.57 ± 11.23	24.65 ± 5.50
APS-I 50 mg/kg	2.10 ± 0.29[a]	1.01 ± 0.66[a]	55.47	81.27 ± 19.61	25.38 ± 7.13
APS-II 100 mg/kg	2.11 ± 0.21[a]	1.23 ± 0.53[b]	45.46	77.16 ± 11.47	21.68 ± 6.69
APS-II 50 mg/kg	2.40 ± 0.23[a]	1.19 ± 0.69[b]	47.72	79.51 ± 23.38	23.98 ± 5.05

[a,b]Means significantly different compared to control group ($P < 0.05$).

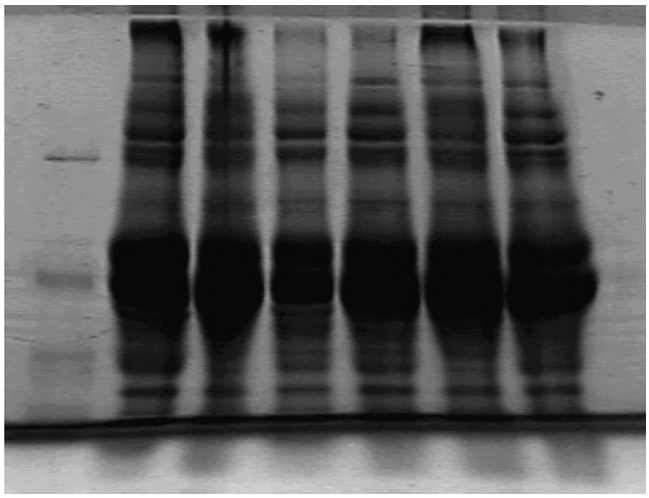

Fig. 7. Serum protein analysis by SDS-PAGE. (a) Marker, (b) control group, (c) APS-I (100 mg/kg), (d) APS-I (50 mg/kg), (e) APS-II (100 mg/kg), (f) APS-II (50 mg/kg), and (g) positive control group.

structures. Table 6 also shows that the APS-I and APS-II treatment could protect the organs of animal like liver, spleen and thymus in comparison with the cyclophosphamide (positive control group) treatment (Fig. 7).

Acknowledgments

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References

Alam, N., & Gupta, P. C. (1986). Structure of a water-soluble polysaccharide from the seeds of *Cassia angustifolia*. *Planta Medica*, *52*, 308–310.

Barker, S. A., Bourne, E. J., Stacey, M., & Whiffen, D. H. (1954). Infrared spectra of carbohydrates. Part I. Some derivatives of D-glucopyranose. *Journal of the Chemical Society*, *17*, 1–176.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, *72*, 248–254.

Chang, L. N., Qu, S. X., Lin, S. Z., Duan, K., & Weng, J. (2011). Preparation and biological properties of HA containing *Astragalus* polysaccharides. *Journal of Inorganic Materials*, *26*, 22–28.

Chaplin, M. F., & Kennedy, J. F. (Eds.). (1994). *Carbohydrate analysis: A practica approach*. (2nd ed., pp. 76–82). New York: Oxford University Press.

Dixon, J. S., & Lipkin, D. (1954). Spectrophotometric determination of vicinal glycols. *Analytical Chemistry*, *26*, 1092–1093.

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

Fang, J. N. (1988). Chemical structure of a glucan from *Astragalus mongholicus*. *Acta Chimica Sinica*, *46*, 1101–1104.

Fei, Y., Yua, M., & Yin, Y. N. (2010). Structure characterization and antioxidant activity of a novel polysaccharide isolated from *Ginkgo biloba*. *International Journal of Biological Macromolecules*, *46*, 436–439.

Goldstein, I. J. (1965). *Methods in Carbohydrate Chemistry*, *5*, 361–370.

Hakomori, S. I. (1964). A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry*, *55*, 205–208.

He, J. Z. (2008). The analysis study on polysaccharide structure and monosaccharide constituents from the *Lycium barbarum*. *Food and Fermentation Industries*, *34*, 48–50.

Li, H. M. (2000). A technological study on enhancing the extraction rate of *Astragalus* polysaccharides. *Journal of Northwest University (Natural Science Edition)*, *30*, 509–510.

Li, R., Chen, W. C., Wang, W. P., & Tian, W. Y. (2009). Extraction, characterization of *Astragalus* polysaccharides and its immune modulating activities in rats with gastric cancer. *Carbohydrate Polymers*, *78*, 738–742.

Li, S. G., & Zhang, Y. Q. (2009). Characterization and renal protective effect of a polysaccharide from *Astragalus membranaceus*. *Carbohydrate Polymers*, *78*, 343–348.

Lin, Y. W., & Chiang, B. H. (2008). Anti-tumor activity of the fermentation broth of *Cordyceps militaris* cultured in the medium of Radix Astragali. *Process Biochemistry*, *43*, 244–250.

Liu, M., Wu, K., Mao, X., Wu, Y., & Ouyang, J. (2009). *Astragalus* polysaccharide improves insulin sensitivity in KKAy mice: Regulation of PKB/GLUT4 signaling in skeletal muscle. *Journal of Ethnopharmacology*, *127*, 32–37.

Ren, M. P. (2010). Antitumor activity of Astragalus polysaccharides. *Chinese Journal of New Drugs*, *19*, 221–224.

Shao, B. M., Xu, W., Dai, H., Tu, P., Li, Z., & Gao, X. M. (2004). A study on the immune receptors for polysaccharide from the roots of *Astragalus membranaceus*, a Chinese medicinal herb. *Biochemical and Biophysical Research Communications*, *320*, 1103–1111.

State Pharmacopoeia Committee (Ed.). (2010). *Chinese Pharmacopoeia*. Beijing: Chinese Medical Department of Science and Technology Publishing House.

Uzochukwu, S., Balogh, E., Loefler, R. T., & Ngoddy, P. O. (2002). Structural analysis by ^{13}C nuclear magnetic resonance spectroscopy of glucan extracted from natural palm wine. *Food Chemistry*, *76*, 287–291.