# Structural Characterization and DPPH Radical Scavenging Activity of an Arabinoglucogalactan from *Panax notoginseng* Root

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The structure of an antiradical arabinoglucogalactan (**1**) from *Panax notoginseng* roots was determined. This polymeric carbohydrate was obtained through successive phosphate buffer (pH 7.0) extraction after cold-water pretreatment and purification by ion-exchange and gel-filtration chromatography. Monosaccharide analysis, permethylation analysis, $NaIO_4$ and $CrO_3$ oxidations, Smith degradation, graded acid hydrolysis, and IR and NMR experiments indicated that **1** possesses a backbone of (1→3)-linked *β*-D-galactofuranosyl residues, with branches of α-L-Ara*f*-(1→4)-*β*-D-Glc*p*-(1→ residues at O-6. Additionally, **1** exhibited high scavenging activity against DPPH free radicals with a 50% scavenging concentration ($SC_{50}$) of 11.72 ± 0.91 *μ*g/mL, suggesting that this arabinoglucogalactan is a potential antiradical.

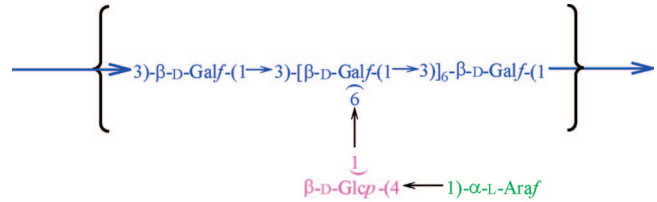
*Panax notoginseng* (Burk.) F. H. Chen, also named "Sanqi" or "Tianqi" in mainland China, is a highly valued and important Chinese medicinal herb belonging to the Araliaceae family. This plant is cultivated in the Yunnan and Guangxi Provinces of mainland China, and its root is well-known for its efficacy in promoting blood clotting, relieving swelling, and alleviating pain.[1–3] It has been used for the treatment of hemoptysis, hemostasis, and hematoma in China for more than four centuries.[1,3] The chemical constituents of *P. notoginseng* root, which include saponins, amino acids, and flavonoids, have been reported.[4] Recent phytochemical and pharmacologic studies revealed that the major bioactive substances are saponins (ginsenosides, notoginsenosides, and gypenosides).[4] Although there have been some preliminary studies of the structures and biologic activities of the high-molecular-weight (HMW) fractions (mainly polysaccharides), data on the water-soluble/insoluble polysaccharides are scarce and only the general structural features have been determined.[5–10]

Radicals, such as superoxide anion and hydroxyl, are generated in living organisms as byproducts through many metabolic pathways and readily react with and oxidize biomolecules, including carbohydrates, proteins, lipids, and DNA. There is increasing evidence that the accumulation of free radicals in biologic systems causes oxidative damage to tissues, which affects cellular integrity and function. Free radicals are associated with the pathogenesis of various diseases, such as arthritis, cancer, inflammation, and heart diseases, as well as aging-related health deterioration in humans.[11–13] Thus, the development of beneficial antioxidants that can directly terminate free radical-mediated oxidative reactions is important for protecting humans from disease.

In the present study, we investigated the structural characteristics of an arabinoglucogalactan purified from *P. notoginseng* root. We also evaluated its free radical scavenging activity by determining its ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) in vitro.

## Results and Discussion

Compound **1**, extracted from the roots of *P. notoginseng* with hot phosphate buffer (85 °C) after cold-water pretreatment, had a specific rotation $[\alpha]^{22}_D$ +11.4 (*c* 0.5, $H_2O$). Sephacryl S-300 gel-filtration chromatography followed by phenol-sulfuric acid assay




**1**

and high-performance gel permeation chromatography (HPGPC) using a refractive-index detector (Figure 1, PD = $M_w/M_n$ = 1.07) produced a single symmetrical peak, indicating that **1** was homogeneous. Its weight-average molecular mass was approximately 6.7 × $10^4$ Da. A lack of ultraviolet absorption at 280 nm and a negative response to the Lowry method[14] confirmed that **1** contained no proteins. The *m*-hydroxybiphenyl method and IR spectrum indicated that it did not contain uronic acids. Sugar analysis of **1** revealed the presence of arabinose/glucose/galactose in relative proportions of 1:1:8. Determination of the absolute configurations of the sugar residues as acetylated (+)-2-butyl glycosides by gas–liquid chromatography (GLC) indicated that the sugar residues were L-arabinose, D-glucose, and D-galactose.

Compound **1** had characteristic absorption bands at 3389, 2953, 2928, 1641 (br), 1421, 1175−1040, 937, 890, and 816 $cm^{-1}$. The broad band at 1641 $cm^{-1}$ was associated with absorbed water.[15] The bands between 1175 and 1040 $cm^{-1}$ were dominated by the glycosidic linkage *ν*(C−O−C) and *ν* (C−O−H) contributions. An intense band at 1421 $cm^{-1}$ corresponded to a C−O stretch and C−H or OH bending. The weak band at 890 $cm^{-1}$ was ascribed to *β*-pyranoid-type glycosidic linkages.[15] Two bands at 937 and 816 $cm^{-1}$, respectively, corresponded to a symmetrical stretch and C−H variable-angle vibrations of furan-type glycosidic rings.

The position of the glycosidic linkage in **1** was determined by permethylation analysis. Compound **1** was methylated three times until there were no hydroxy absorption bands in the 3600–3000 $cm^{-1}$ region in their IR spectra. Following hydrolysis, reduction, and acetylation, 2,5,6-tri-*O*-methyl-D-Gal*f*, 2,5-di-*O*-methyl-D-Gal*f*, 2,3,5-tri-*O*-methyl-L-Ara*f*, and 2,4,6-tri-*O*-methyl-D-Glc*p* derivatives were present in relative proportions of 7.06:1.09:1.00:0.98 (Table 1). Thus, we hypothesized that **1** should have a (1→3)-linked D-Gal*f* backbone with some branching sites at O-6 and terminal L-Ara*f* residues contained exclusively in the branching-chains as nonreducing ends. However, it was not known whether (1→4)-linked D-Glc*p* residues were inserted directly into the backbone or into the branches. To test our hypothesis and to detect the branches,

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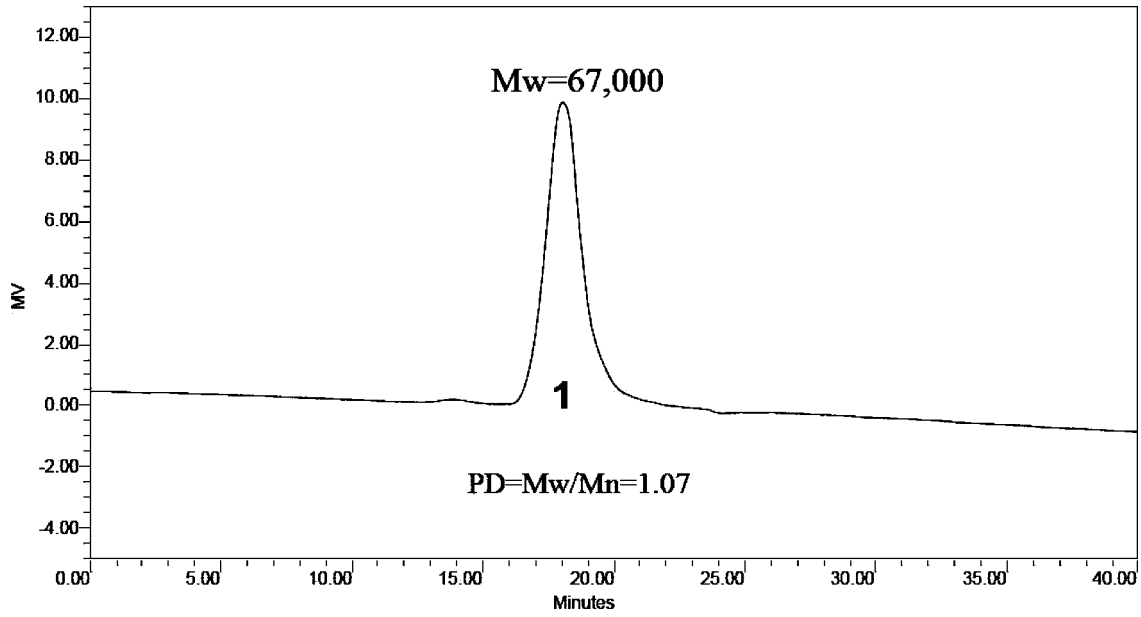


**Figure 1.** Profile of **1** in HPGPC. Mw, weight-average molecular mass; Mn, number-average molecular mass; PD, polydispersity.

**Table 1.** Partial *O*-Methylalditol Acetates Obtained via Methylation Analysis of **1** Derived from *P. notoginseng* Roots

| alditol acetate | approximate molar ratio[a] | fragments (diagnostic ions, *m*/*z*) | linkage indicated |
|---|---|---|---|
| 2,3,5-tri-*O*-methylarabitol | 1.00 | 87,101,102,118,129,161 | 1-Ara*f* |
| 2,5,6-tri-*O*-methylgalactitol | 7.06 | 87,89,102,118,161,173,203,233 | 1,3-Gal*f* |
| 2,5-di-*O*-methylgalactitol | 1.09 | 87,102,118,131,173,190,203,233 | 1,3,6-Gal*f* |
| 2,3,6-tri-*O*-methyglucitol | 0.98 | 87,99,101,113,117,129,161,233 | 1,4-Glc*p* |

[a] Relative to 2,3,5-tri-*O*-methylarabitol.

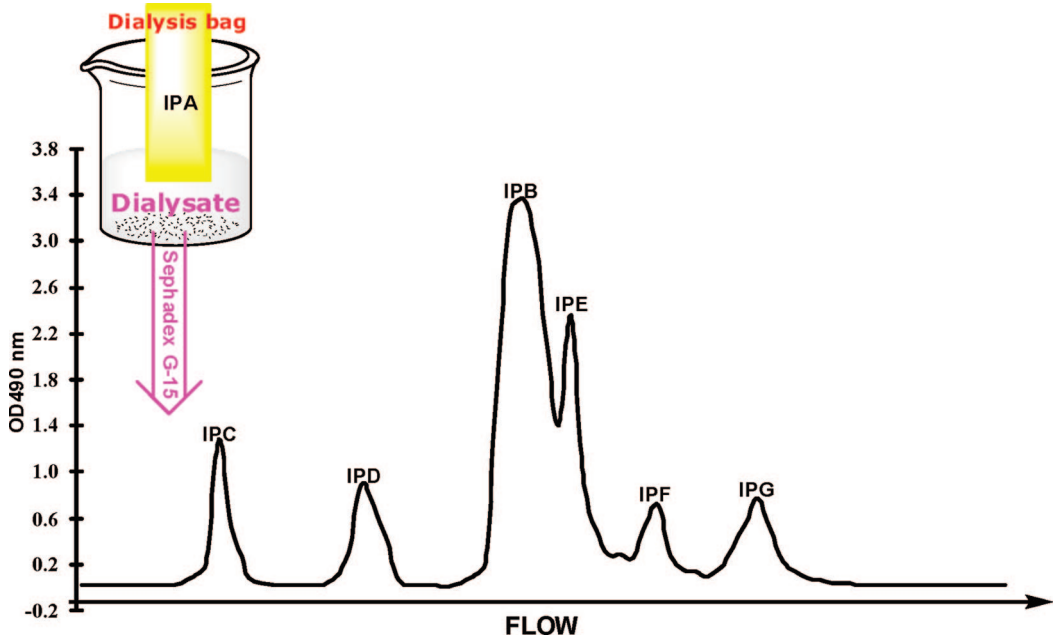


**Figure 2.** Profile of the isolation of **IPA**, **IPB**, **IPC**, **IPD**, **IPE**, **IPF**, and **IPG** by dialysis and Sephadex G-15 column chromatography, eluted with $H_2O$, 0.02, 0.02–0.1 M NaCl (100 mL/each).

we performed graded acid hydrolysis, dialysis, and column chromatography experiments, resulting in seven fragments (**IPA**−**IPG**) (Figure 2). The seven fragments were separately permethylated using the Ciucanu & Kerek method,[16] and each product was isolated by $CHCl_3$ extraction. The extract was washed with distilled $H_2O$, dried with anhydrous $Na_2SO_4$, and evaporated. The final product was dried over $P_2O_5$ in vacuo. These partially methylated products were further hydrolyzed, converted into alditol acetates, and analyzed by GLC/MS. The linkage analysis results for fragments **IPA**−**IPG** are shown in Table 2. The structure of **IPC** (Figure 3) supported our hypothesis, indicating that 1,4-linked *β*-D-Glc*p* residues are branches tied into C-6 of the (1→3)-Gal*f* backbone, and the other fragments further showed that **1** had a main chain composed of 1,3-linked D-galactofuranosyl residues and side chains at C-6 of the L-Ara*f*-(1→4)-D-Glc*p*-(1→ groups.

Compound **1** was completely oxidized with 40 mM $NaIO_4$ for 72 h at 4 °C in the dark. A total of 0.907 mol of $NaIO_4$ was consumed and almost no formic acid was produced per mole of

**Table 2.** Linkage Analysis of the Products (**IPA**−**IPG**) Produced by Graded Acid Hydrolysis (molar %)

| alditol acetate | linkage | IPA | IPB | IPC | IPD | IPE | IPF | IPG |
|---|---|---|---|---|---|---|---|---|
| 2,3,5-$Me_3$-Ara | 1-Ara*f* | 8.7 | 15.5 | 31.3 | | 15.8 | 14.1 | 16.3 |
| 2,3,5,6-$Me_4$-Gal | 1-Gal*f* | | 17.8 | | 21.1 | 19.2 | 15.3 | 8.9 |
| 2,3,5-$Me_3$-Gal | 1,6-Gal*f* | | | 34.9 | | | | |
| 2,5-$Me_2$-Gal | 1,3,6-Gal*f* | 9.6 | 17.6 | | | 18.5 | 12.8 | 17.1 |
| 2,5,6-$Me_3$-Gal | 1,3-Gal*f* | 71.2 | 34.2 | | 78.9 | 22.0 | 41.9 | 43.3 |
| 2,3,6-$Me_3$-Glc | 1,4-Glc*p* | 10.5 | 14.9 | 33.8 | | 24.5 | 15.9 | 14.4 |

glycosyl residues, based on the average molar mass (159) of the component residues. Composition analysis of the polyalcohol resulting from metaperiodate oxidation gave dehydroxymethenylgalactose and galactose, which respectively arose from 1,3- and 1,3,6-linked Gal*f* residues, and erythritol attributed to 1,4-linked Glc*p* residues as well as glycerol from terminal Ara*f* residues. These results were in approximate agreement with the expected results based on linkage analysis of **1** ($NaIO_4$ consumption: 0.892; formic acid production: 0). The anomeric configurations of sugar residues were determined by oxidation of acetylated **1** with chromium trioxide. Both Gal*f* and Glc*p* residues were almost completely destroyed and a large amount (>95%) of Ara*f* residues survived, suggesting that L-Ara*f* was α and both D-Glc*p* and D-Gal*f* were $\beta$.

The broadband-decoupled $^{13}C$ NMR spectrum (Figure 4, **X**) showed **1** to be a complex polysaccharide because four signals were observed in the anomeric region. The sugar residues were designated **A**−**D** according to decreasing $^{13}C$ NMR chemical shifts of the resonances of their respective anomeric carbons. Three downfield signals at $\delta$ 108.2, 107.8, and 107.1, indicative of furanosyl rings, were attributed to C-1 of **A**, **B**, and **C**, respectively. The remaining signal at $\delta$ 105.1 was assigned to C-1 of **D**. All of the resonances were resolved[17–22] and recorded in Table 3. For residue **A**, there was a large downfield chemical shift displacement of $\Delta\delta_C$ of 7.8 for C-3. Thus, **A** was 3-substituted, and the residue was consequently →3)-$\beta$-D-Gal*f*-(1→. Residue **B** gave two large downfield chemical shift displacements of $\Delta\delta_C$'s of 7.7 and 4.7 for C-3 and C-6, respectively. Thus, **B** is 3,6-disubstituted and the residue is consequently →3,6)-$\beta$-D-Gal*f*-(1→. The signal at $\delta$ 78.8 in residue **D**, showing a large downfield chemical shift displacement of $\Delta\delta_C$ of 8.1 for C-4, indicated that **D** was 4-substituted and the residue was →4)-$\beta$-D-Glc*p*-(1→. Additionally, residue **C** was nonreducing Ara*f* (1→. The $^{13}C$ DEPT 135 spectrum of **1** (Figure 4, **Y**) showed that the signals at $\delta$ 61.5, 61.7, and 62.6 were separately assigned to C-6 of C-6-unsubstituted Glc*p* and Gal*f* residues and C-5 of nonreducing Ara*f* residues. The relatively upfield signal at $\delta$ 67.7, however, resulted from C-6 of C-6-substituted Gal*f* residues.

The 500 MHz $^1H$ NMR spectrum of **1** showed the resonances of four anomeric protons to be well separated at $\delta$ 5.28, 5.25, 5.19, and 4.76, which were exclusively assigned to H-1 of **C**, **B**, **A**, and **D**, respectively, according to the HMQC experiment. The chemical shifts from $\delta$ 3.5 to 4.1, showing overlapping peaks, were assigned to the protons of carbons C-2 to C-5 (or C-6) of the glycosidic rings. The HMBC spectrum (Figure 5) gave the correlations between proton and carbon in **1**, viz., C-1 in **A** to H-3 in **A** or **B**, and H-6 in **B** to C-1 in **D**. Thus, it was further corroborated that **1** had a backbone consisting of 1,3-linked $\beta$-D-Gal*f* residues with a side chain of 1,4-linked Glc*p* residues at O-6. Thus, it was deduced that the nonreducing end units of L-Ara*f* were linked to position 4 of **D**.

From the results of chemical analysis and IR and NMR spectra, **1** was suggested to be an arabinoglucogalactan. The free radical

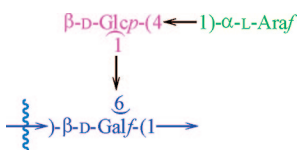


**Figure 3.** Proposed structure of **IPC**.

scavenging activity of **1** and of the positive control (ascorbic acid) were assayed using DPPH, as this molecule containing a stable free radical is widely used to evaluate the radical scavenging ability of antioxidants. Both **1** and ascorbic acid significantly quenched DPPH radicals in a concentration-dependent manner (Figure 6). The $SC_{50}$ of **1** was 11.72 ± 0.91 $\mu$g/mL, almost twice that of ascorbic acid, with an $SC_{50}$ value of 5.24 ± 0.46 $\mu$g/mL, indicating that the scavenging activity of **1** was slightly weaker than that of ascorbic acid. As a high-molecular-weight biomacromolecule, however, **1** is a strong scavenger of DPPH radicals and represents as a possible new natural antioxidant source. Further studies will focus on the relationship between the structure and activity of this arabinoglucogalactan.

## Experimental Section

**General Experimental Procedures.** All evaporations were performed at <50 °C under reduced pressure. Optical rotation was determined with a Perkin-Elmer 141 (Waltham, MA) polarimeter. IR spectra were recorded with a Beckman Acculab 10 instrument (Beckman Instruments, Fullerton, CA) with KBr pellets. NMR spectra were recorded on a Bruker 500 instrument (Billerica, MA). For $^1H$ NMR spectroscopy at 70 °C, the sample (9.6 mg) was repeatedly dissolved in $D_2O$ (5 × 5 mL), and the solution was lyophilized. The final lyophilized sample was dissolved in 1 mL of 99.99% $D_2O$. For $^{13}C$ NMR spectroscopy at 50 °C, the sample (48 mg/mL) was dissolved in $D_2O$. Data processing was performed using vendor-supplied software. Chemical shifts are reported in parts per million using external tetramethylsilane (TMS, $\delta_H$ 0.00) or internal acetone in $D_2O$ ($\delta_C$ 31.45) as references. The NMR DEPT experiment was performed using a polarization-transfer pulse of 135°. The HMQC experiment was performed at a $^1H$ frequency of 500.13 MHz (125.77 MHz for $^{13}C$) with a spectral width of 1247 Hz for $t_2$ and 10 000 Hz for $t_1$, using the standard pulse sequence. The time domain data sets were multiplied with a phase-shifted sine curve, and after Fourier transformation and zero-filling, data sets of 4k × 4k points were obtained. The HMBC spectra were recorded on the Bruker Avance DPX-500 spectrometer using different mixing times: 50, 100, and 200 ms. The centrifugation conditions were 8000 rpm for 45 min at 4 °C. Uronic acid was estimated using the improved *m*-hydroxybiphenyl method.[23]

**Materials and Chemicals.** The dried roots of *P. notoginseng* were purchased from a Chinese Medicine Corporation in Yanji City and authenticated by Dr. Hongxiang Sun (College of Animal Science, Zhejiang University, People's Republic of China). A sample was deposited at Ankang Medicine Co, Ltd., Yanji, Jilin Province, People's Republic of China, as voucher no. AK0137. The roots were cut into small pieces in a commercial slicer and used in the subsequent studies. DPPH was purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO).

**Isolation and Purification.** Dried crushed roots (250 g), previously defatted with light petroleum and 95% EtOH under reflux conditions and pretreated with cold water at room temperature overnight, were successively extracted with phosphate buffer (pH7.0) at 85 °C for 5 h. The buffer extracts were further treated with TFA to remove protein and extensively dialyzed (molecular weight cutoff 3500–5000 Da) against distilled $H_2O$ for 48 h. The retentate was centrifuged, precipitated with EtOH, and vacuum-dried at 37.5 °C to afford the crude polysaccharide (**PNCP**, yield: 2.5%, based on the original dried material). A portion (5 g) of **PNCP** was fractionated on a DEAE-cellulose ($Cl^-$ form) column (60 × 10 cm), eluted with distilled $H_2O$ and stepwise by 0.1, 0.3, 0.5, and 1.0 M NaCl to give seven subfractions. The water-eluted fraction (907 mg, yield: 18.1%, based on the **PNCP**) was further separated on a DEAE-cellulose ($AcO^-$ form) column (50 × 5 cm) eluted with distilled $H_2O$ and 0.05, 0.1, 0.2, and

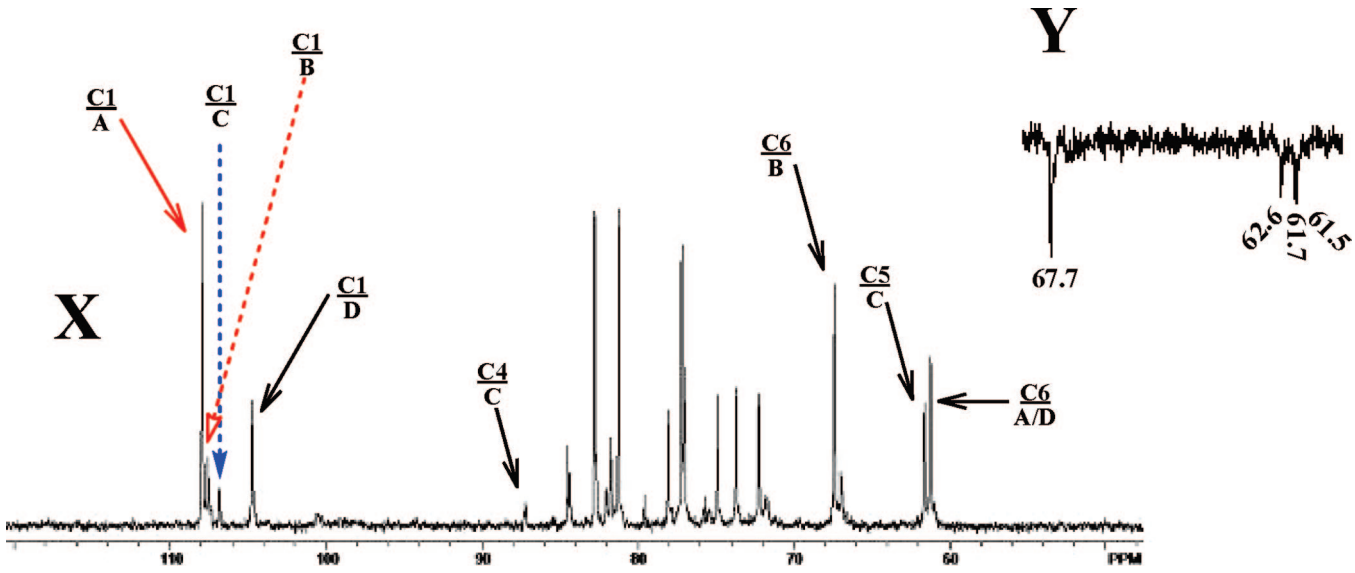


**Figure 4.** (**X**) $^{13}$C NMR spectrum of **1** obtained from *P. notoginseng* roots in $D_2O$; (**Y**) $^{13}$C DEPT 135 NMR spectrum of **1** in $D_2O$. Numerical values are in $\delta$ (ppm); **A**, residue A; **B**, residue B; **C**, residue C.

**Table 3.** Chemical Shifts (ppm) of the Signals in the $^{13}$C NMR Spectrum of **1** from *P. notoginseng* Roots[a]

| | $^{13}$C/chemical shifts | | | | | |
|---|---|---|---|---|---|---|
| sugar residues | 1 | 2 | 3 | 4 | 5 | 6 |
| →3)-$\beta$-D-Gal*f*-(1→ | 108.2 | 81.5 | 84.8 | 82.4 | 72.5 | 61.7 |
| **A** | (6.2) | (−0.4) | (7.8) | (−0.8) | (0.8) | (−1.3) |
| →3,6)-$\beta$-D-Gal*f*-(1→ | 107.8 | 79.8 | 84.7 | 82.1 | 72.4 | 67.7 |
| **B** | (5.8) | (−2.1) | (7.7) | (−1.1) | (0.7) | (4.7) |
| α-L-Ara*f*-(1→ | 107.1 | 83.1 | 77.3 | 86.5 | 62.6 | - |
| **C** | (6.9) | (0.9) | (−0.2) | (1.7) | (0.3) | |
| →4)-$\beta$-D-Glc*p*-(1→ | 105.1 | 74.0 | 75.3 | 78.8 | 75.9 | 61.5 |
| **D** | (8.3) | (−0.9) | (−1.2) | (8.1) | (−0.7) | (0.0) |

[a] Chemical shift displacements ($\Delta\delta$) are reported in parentheses and compared to those of the corresponding monosaccharide.

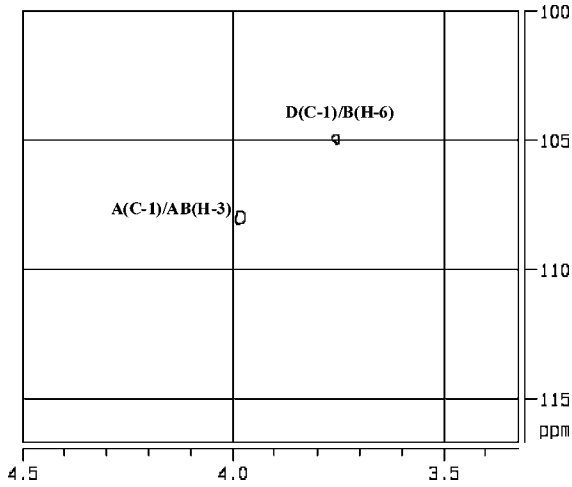


**Figure 5.** Part of the $^{1}$H,$^{13}$C-HMBC spectrum of **1**.

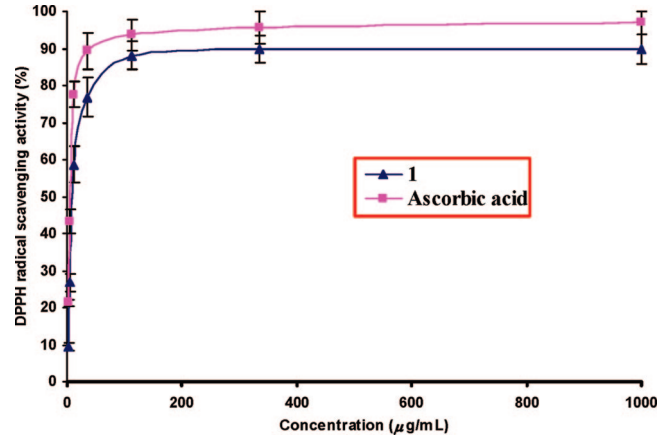


**Figure 6.** Scavenging effects of **1** and ascorbic acid on DPPH radicals. Each sample was assayed in triplicate for each concentration. Experimental results are mean ± SD of three parallel measurements. The $SC_{50}$ ($\mu$g/mL) values were calculated from the dose–response curves.

1.0 M NaOAc to give four fractions. The water-eluted fraction was further purified on a Sephadex G-100 column (60 × 2.6 cm) to afford **1** (yield: 3.3%, based on the **PNCP**).

**Homogeneity and Molecular Mass Determination. (a) Gel Filtration.** A solution of **1** (5 mg) in 0.02 M acetate buffer (1 mL) was applied to a Sephacryl S-300 column (1.6 × 45 cm) and eluted with a gradient of 0.02–0.10 M acetate buffer.

**(b) High-Performance Gel-Permeation Chromatography.** Molecular mass determination was conducted by HPGPC on a tandem column composed of Ultrahydrogel 250 and 2000 columns, eluted with distilled $H_2O$ at a flow rate of 0.5 mL/min. The column was kept at 35.0 ± 0.1 °C and precalibrated using standard Dextrans (T-580, T-200, T-110, T-70, T-40, and T-10, Pharmacia). The HPGPC system was incorporated in a Waters 515 instrument (Milford, MA) as well as the Waters Millenium 32 software package, and the eluent was monitored with a 2410 refractive-index detector and a 2487 dual $\lambda$ absorbance detector. The sample was prepared as 0.4% (w/v) solutions, and 20 $\mu$L of solution was analyzed in each run.

**Monosaccharide Analysis.** Hydrolysis was performed with 2.0 M TFA at 110 °C for 5.5 h, followed by evaporation to dryness with distilled $H_2O$ as codistillation, successive reduction with $NaBD_4$, and acetylation with $Ac_2O$−pyridine (1:1, v/v, 2 mL) at 50 °C for 6 h. The

absolute configuration of sugar residues present in **1** were determined essentially as described by Leontein et al.[24] by GLC of their acetylated glycosides, using (+)-2-butanol.[25] Alditol acetates and acetylated 2-butyl glycosides were separated on an HP-5 fused Si column using a temperature program of 180 °C (1 min) and 3 °C $min^{-1}$ to 210 °C, analyzed by GLC/MS, and identified by their typical retention times and electron impact profiles. Hydrogen was used as the carrier gas. The column was fitted to a Hewlett-Packard model 5713 gas chromatograph equipped with flame-ionization detector.

**Permethylation.** The vacuum-desiccated **1** was permethylated using the Ciucanu & Kerek procedure[16] with anhydrous DMSO, NaOH, and MeI. To obtain significant results, three consecutive treatments were performed. Complete hydrolysis of the methylated **1** was then conducted with 90% aqueous formic acid (3 mL, 2 h, reflux) followed by 2.0 M TFA (2.5 mL, 4 h, reflux) to afford monomeric units with different degrees of permethylation depending on their positions in the chain. The permethylated sugars were derived into the alditol acetates by reducing with $NaBD_4$ and acetylating with $Ac_2O$−pyridine, and identified by GLC using a temperature program of 180 °C (1 min) and 1.5 °C $min^{-1}$ to 210 °C. GLC/MS analysis was performed on a Thermo Quest Trace GC2000/Trace MS equipped with a DB-225 capillary column (30 m × 0.25 mm). A temperature program of 100 °C (2 min) and 10 °C $min^{-1}$ to 250 °C was used with helium as a carrier gas (1 mL/min, flow rate). All partially methylated alditol acetates were positively identified using authentic standards.

**Periodate Oxidation and Smith Degradation.** A solution of **1** (22 mg) was mixed with 0.04 M $NaIO_4$ (20 mL) and kept at 4 °C in the dark.[26] The oxidation was quenched by the addition of ethylene glycol (1.0 mL) after 72 h, and the solution was concentrated, reduced, neutralized, dialyzed, and lyophilized to give a degraded product (**Id**, 12.7 mg). A portion (1.5 mg) of **Id** was further hydrolyzed with 1.5 M TFA at 100 °C for 5 h, converted into the alditol acetate, and analyzed by GLC.

**$CrO_3$ Oxidation.** A mixture of **1** (8 mg) and *myo*-inositol (3 mg) in formamide (3.0 mL) was acetylated with $Ac_2O$ (2 mL) and pyridine (2 mL) for 24 h at room temperature. The mixture was dissolved in $CHCl_3$ (15 mL), and a portion (0.5 mL) of this solution was used as a control. The remaining solution was made according to the published procedure.[27] Sugar analysis was performed before and after oxidation.

**Graded Acid Hydrolysis.** Compound **1** (65 mg) was dissolved in 20 mL of 50% aqueous formic acid and then hydrolyzed for 3 h at 100 °C. After cooling, the solution was concentrated and dialyzed against distilled $H_2O$, and the retentate was lyophilized to give one polymer (**IPA**, 11.4 mg). The dialysate was fractionated on a Sephadex G-15 column eluted with $H_2O$ and 0.02, 0.02–0.1 M NaCl to afford a main fraction (**IPB**) and a series of oligosaccharides (**IPC**, **IPD**, **IPE**, **IPF**, and **IPG**).

**Radical Scavenging Activity Using the DPPH Method.**[28] To determine the antioxidant activity of **1**, the stable DPPH radical was used. The test was performed in 96-well microplates. Fifty microliters of a 0.025% DPPH solution in MeOH was added to a range of solutions of different concentrations (seven serial 3-fold dilutions to give a final range of 1000 to 1.5 $\mu$g/mL) of **1** to be tested in distilled $H_2O$ (220 $\mu$L). Absorbance at 518 nm was determined 30 min after the addition of the **1** solution, and the percentage of activity was calculated. Ascorbic acid was used as a positive control.

**Statistical Analysis.** The antioxidant activity of each sample was expressed as the $SC_{50}$ value, which is the concentration in $\mu g \cdot mL^{-1}$ of **1** that scavenged 50% of the DPPH radicals. All of the results are expressed as mean ± SD of three different trials.

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