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Chemical components and molecular mass of six polysaccharides isolated from the sclerotium of *Poria cocos*

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Abstract—Six polysaccharides were extracted sequentially from the fresh sclerotium of *Poria cocos* cultivated in China using 0.9% NaCl (PCS1), hot water (PCS2), 0.5 M NaOH (PCS3-I and PCS3-II), and 88% formic acid (PCS4-I and PCS4-II). Their chemical and physical characteristics were determined using infrared spectroscopy (IR), gas chromatography (GC), GC–MS methylation analysis, ^{13}C NMR spectroscopy, elementary analysis (EA), protein analysis, size exclusion chromatography combined with laser light scattering (SEC-LLS), light scattering (LS), and viscometry. The results indicated that the polysaccharides PCS1, PCS2, and PCS3-I were heteropolysaccharides containing D-glucose, D-galactose, D-mannose, D-fucose, and D-xylose; the predominant monosaccharide was D-glucose except for PCS1 where it was D-galactose. PCS3-II, the main component of the sclerotium of *P. cocos*, was a linear (1→3)-β-D-glucan of high purity. PCS4-I consisted of (1→3)-β-D-glucan with some β-(1→6) linked branches. PCS4-II was mainly composed of (1→3)-β-D-glucan containing some glucose branches. The M_w values of the six polysaccharides PCS1, PCS2, PCS3-I, PCS4-I in 0.2 M NaCl aqueous solution, PCS3-II, and PCS4-II in dimethyl sulfoxide (Me_2SO) were determined to be 11.6×10^4, 20.8×10^4, 17.1×10^4, 9.1×10^4, 12.3×10^4, and 21.1×10^4, respectively. The six polysaccharides in aqueous solution or Me_2SO exist as flexible chains.

Keywords: *Poria cocos* sclerotium; Polysaccharide; Molecular mass; ^{13}C NMR; Chemical component; Light scattering

1. Introduction

Poria cocos, a fungus that grows on the roots of pine trees, is one of the most important traditional medicines in China and Japan, and has pharmacological importance in diuretic,[1] antibacterial, antitumor,[2–4] mitogenic, complement activating,[5] and immune stimulating activities.[6] The highest proportion of (1→3)-β-D-glucan extracted from *P. cocos* sclerotium is called pachyman. Chihara et al.[3] have reported the presence of β-(1→6)-glucosyl branches on the backbone of (1→3)-β-D-glucan. When the β-(1→6)-glucoside linkages are severed by sodium metaperiodate oxidation, the linear (1→3)-β-D-glucan through obtained has antitumor activites.[3] It is worth noting that chemical components, molecular weight, water solubility, conformation, and chemical modification of polysaccharides have significant effects on their antitumor and immunomodulatory activities.[7–10] However, the chemical components and molecular mass of various polysaccharides from *P. cocos* sclerotium and mycelia have still not been fully studied. In particular, there are some discrepancies in the literature data, where the reported values of molecular mass range from 4.1×10^4 to 5×10^6.[3,11,12] In our laboratory, a linear (1→3)-β-D-glucan (PCS3) isolated from *P. cocos* sclerotium has been shown to form aggregates in aqueous solution, leading to large apparent molecular mass, and in dimethyl sulfoxide (Me_2SO) or cadoxen to be dissolved as single chains with M_w about 9×10^4.[13–15] The higher values of M_w reported in the literature are

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believed to have resulted from extensive aggregation, which can be avoided in good solvents such as concentrated cadoxen or Me_2SO.

In previous work we reported on the effect of culture media on the chemical and physical characteristics of polysaccharides isolated from *P. cocos* mycelia[16] and on the antitumor activities of these polysaccharides.[17] Here, we attempt to study systematically the chemical components and molecular mass of the various polysaccharides extracted from fresh sclerotium of *P. cocos*. Infrared spectroscopy (IR), gas chromatography (GC), GC–MS methylation analysis, ^{13}C NMR spectroscopy, elementary analysis (EA), laser light scattering (LLS), size-exclusion chromatography (SEC) combined with LLS, and viscometry were used to obtain information dealing with composition and conformation in detail. This is the first integrated report on the molecular mass of the various polysaccharides extracted from fresh sclerotium of *P. cocos*.

2. Experimental

2.1. Isolation and purification of polysaccharides[16–18]

Fresh sclerotium of *P. cocos* cultivated in Luotian (Hubei, China) was peeled, and the white body of the sclerotium was dried and powdered. The powder was defatted by Soxhlet extraction with EtOAc for 6 h and acetone for 6 h. The resulting residue was immersed in aq 0.9% NaCl and stirred by a mechanical stirrer overnight to yield a syrup before being centrifuged to give the supernatant PCS1. Further extraction was made with hot water, 0.5 M NaOH, and 88% HCO_2H and sample codes are outlined in Scheme 1. Each concentrated supernatant was decolorized with 30% H_2O_2 and deproteinated by the Sevag method 10 times and then dialyzed (regenerated cellulose tubing; M_w cut-off 8000, USA) against tap water for five days and distilled water for four days. Each polysaccharide examined by UV spectroscopy (UV-160, Shimadzu, Japan) showed a main peak at 200 nm for polysaccharide, no absorption peaks at 280 nm for protein and 600 nm for pigment, except for PCS3-I. The polysaccharides were finally lyophilized (Christ Alpha 1-2, Germany) to obtain a white powder for PCS1, PCS3-II, and PCS4-I, a light yellow powder for PCS2 and PCS3-I, and a light brown powder for PCS4-II.

2.2. Analysis of chemical composition

Infrared spectra of the polysaccharides were recorded with a Nicolet 170SX FT-IR (Spectrum One, Perkin Elmer Co., USA) spectrometer in the range 4000–400 cm^{-1} using the KBr disk method. The elemental compositions of the products were determined by an elemental analyzer (CHN-O-RAPID Heraeus Co., Germany). Protein content in the polysaccharides was measured using a Kjeletc 1030 self-analyzer (Switzerland) according to the semi-micro Kjeldahl principle.

Gas chromatography (GC) of the alditol acetates derivatives of the polysaccharides was performed with an HP-6890 gas chromatography (Hewlett Packard, USA) using an Alltech DB-225 capillary column (15 m × 0.25 mm) programmed from 180 to 220 °C at 4 °C/min and held at 220 °C for 30 min. The injection sample volume was 2 μL, the carrier gas was high-purity helium, and detection was by flame ionization. For the methylation analysis, the polysaccharides PCS1 and PCS2 were permethylated twice using CH_3I and solid NaOH in dimethyl sulfoxide (Me_2SO) as a sequential method as described.[19,20] The methylated polysaccharide was subsequently hydrolyzed with 2 M trifluoroacetic acid (2 h, 120 °C) and reduced with $NaBH_4$. After neutralization and removal of boric acid by coevaporation with MeOH, the mixture of partially methylated alditols was acetylated with Ac_2O (3 h, 120 °C). The resulting products were analyzed using a mass spectrometer (MS, QP5050A, Shimadzu, Japan) fitted with a capillary column (30 m × 0.75 mm i.d., Restek OV225) in the Chinese University of Hong Kong.

High resolution ^{13}C NMR spectra were recorded on a Jeol Lambda 400 MHz spectrometer equipped with a DEC AXP 300 computer workstation (Jeol Co., Herts., England) at 60 °C. All the samples, except PCS3-I, were deuterium exchanged by freeze-drying three times from D_2O and then examined in solutions of 99.96% D_2O, using internal acetone as reference (δ_H 2.225, δ_C 31.45) for PCS1 and PCS2 or 99.97% Me_2SO-d_6 for PCS3-II, PCS4-I, and PCS4-II.

2.3. Viscometry

Intrinsic viscosities [η] of the polysaccharide solutions were measured at 25 ± 0.1 °C using an Ubbelohde capillary viscometer. The 0.2 M NaCl aqueous solution or Me_2SO were used as sample solvents, respectively. The kinetic energy correction was always negligible. Huggins and Kraemer equations were used to estimate the [η] value by extrapolation to concentration (c) to be zero as follows:

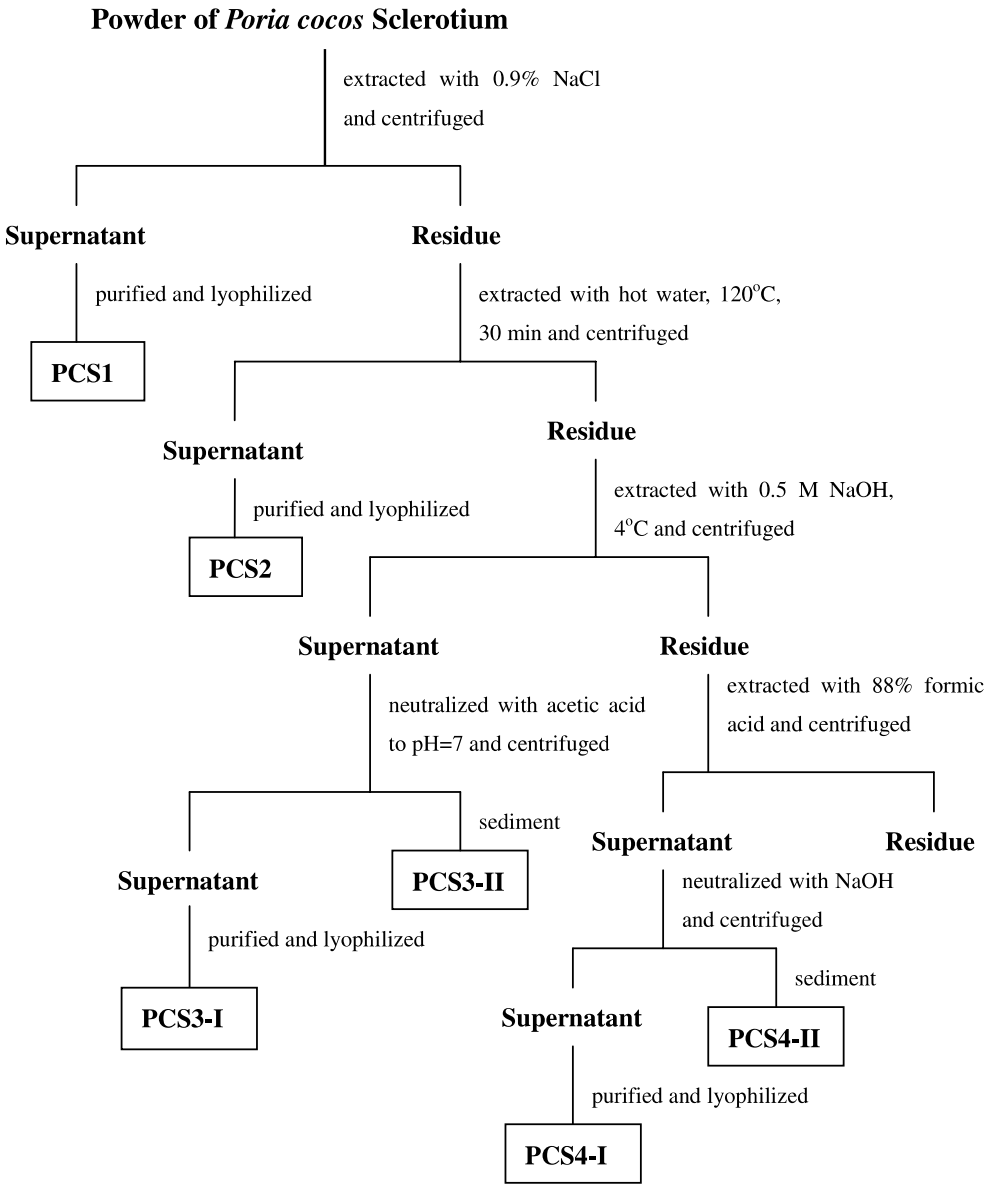
$$\eta_{sp}/c = [\eta] + k'[\eta^2]c \quad (1)$$

$$(\ln \eta_r)/c = [\eta] + k''[\eta]^2 c \quad (2)$$

where k' and k'' are constants for a given polymer at a given temperature in a given solvent; η_{sp}/c, the reduced specific viscosity; $(\ln \eta_r)/c$, inherent viscosity.

2.4. Laser light scattering

The light-scattering intensities of polysaccharides solution were determined with a multi-angle laser light scattering instrument equipped with a He–Ne laser



Scheme 1. Extraction of the polysaccharides from sclerotium of *Poria cocos*.

($\lambda = 633$ nm; Dawn® DSP, Wyatt Technology Co., USA) in the angles of 43°, 49°, 56°, 63°, 71°, 81°, 90°, 99°, 109°, 118°, 127°, 136°, and 152° at 25 °C. The polysaccharide solutions of desired concentrations were prepared, and optical clarification of the solution was achieved by filtration through a 0.2 μm pore size filter (Whatman, England) into the scattering cell (SV mode). The refractive index increments (dn/dc) were determined using an Optilab refractometer (Dawn-DSP, Wyatt Technology Co., USA) at 633 nm and 25 °C. The dn/dc values of samples in aqueous 0.2 M NaCl and Me_2SO solutions were determined to be 0.140 and 0.060 mL g^{-1}, respectively. Astra software (Version 4.70.07) was utilized for data acquisition and analysis.

2.5. SEC-LLS measurements

Size exclusion chromatography combined with laser light scattering (SEC-LLS) measurements were carried out on a Dawn® DSP multi-angle laser photometer already mentioned, combined with a P100 pump (Thermo Separation Products, San Jose, USA) equipped with TSK-GEL G5000 and G3000 PWXL column (7.8 × 300 mm) in series for aqueous solution, or a G4000 H_6 column (7.5 × 300 mm) for Me_2SO at 25 °C. A differential refractive index detector (RI-150) was simultaneously connected. The eluent was aqueous 0.2 M NaCl or Me_2SO with a flow rate of 1.0 mL/min. All solutions having a polysaccharide concentration of 1.0×10^{-3} to 2.0×10^{-3} g/mL were filtered first with a

sand filter followed by a 0.20 μm filter (Whatman, England), then kept in sealed glass bottles before injection onto the SEC column. Astra software (Version 4.70.07) was utilized for the data acquisition and analysis.

3. Results and discussion

3.1. Chemical composition of PCS

The IR spectra of the samples PCS1–PCS4-II are shown in Figure 1. All samples exhibited the characteristic IR absorption of polysaccharide at 1250 and 1650 cm^{-1}. The IR absorption peak at 800 cm^{-1} for PCS1 was the characteristic absorption of mannose. The polysaccharides PCS2, PCS3-I, PCS3-II, PCS4-I, and PCS4-II showed IR absorption at 890 cm^{-1}, which is characteristic of β-D-glucan. The sample PCS4-II exhibited a specific absorption peak at 1720 cm^{-1}, suggesting the presence of uronic acid.

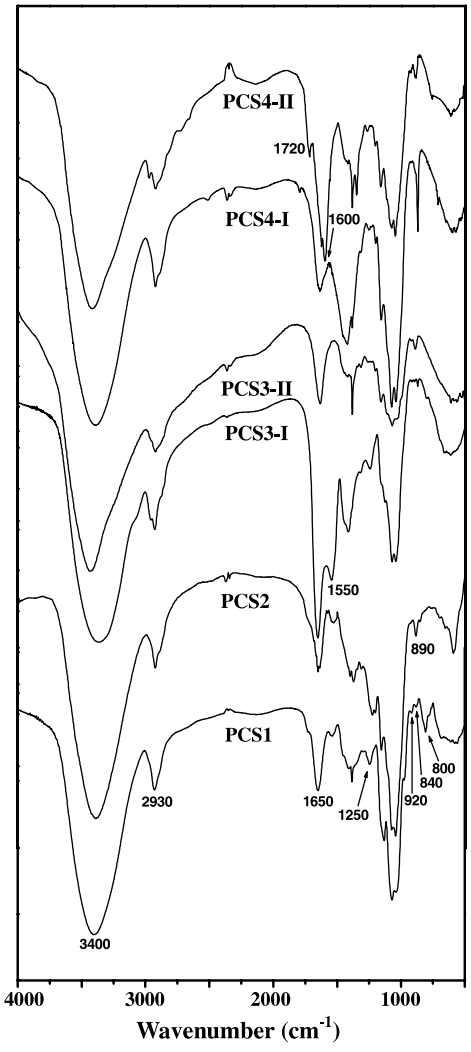


Figure 1. FT-IR spectra of the polysaccharide fractions from sclerotium of *Poria cocos*.

GC traces of the polysaccharides hydrolyzates, in comparison with standard saccharides, showed the monosaccharide components and are summarized in Table 1 together with protein content and yields of the polysaccharides. The results revealed that D-glucose was the predominant monosaccharide of PCS2–PCS4-II, and its content increased with the progress of isolation on the whole. The polysaccharides PCS1, PCS2, and PCS3-I were heteropolysaccharides containing D-glucose, D-mannose, D-galactose, D-fucose, and D-xylose. The polysaccharides PCS3-II, PCS4-I, and PCS4-II mainly consisted of D-glucose. D-mannose was present in a high amount in both PCS1 and PCS3-I while D-galactose was present in the highest amount in PCS1. The results of methylation analysis by GC–MS further confirmed that the samples PCS1 and PCS2 were heteropolysaccharides with various linkages. PCS1 mainly contained →3)-D-Glc-(1→, →6)-D-Glc-(1→, →6)-D-Gal-(1→, →4,6)-D-Gal-(1→, →2,6)-D-Man-(1→ and → 3,6)-D-Man-(1→. PCS2 mainly contained →3)-D-Glc-(1→, D-Glc(1→ (terminal), →6)-D-Glc-(1→, →2)-D-Gal-(1→ and →3,6)-D-Man-(1→. The components and structure of the heteropolysaccharides PCS1, PCS2, and PCS3-I were very complex, and will be further investigated in detail.

The protein content of each polysaccharide calculated from *N* elemental analysis was similar to the results of protein analysis. The average values of the protein content are summarized in Table 1. The protein content in PCS3-I was much higher than in other samples and can be attributed to the probability that the protein was bound to the polysaccharides, as the Sevag procedure was repeated over 10 times to remove free protein. From Table 1, it was found that the yield of PCS3-II (84.2%) was much higher than the other polysaccharides, so it was the main component of *P. cocos* sclerotium.

The ^{13}C NMR spectra of five polysaccharides PCS1, PCS2, PCS3-II, PCS4-I, and PCS4-II are shown in Figure 2, and the assigned chemical shifts of the spectra of the samples and those previously reported for (1 → 3)-β-D-glucan[21,22] are in Table 2. The strong signals around 103.0, 86.2, 76.3, 72.7, 68.3, and 60.8 ppm for the polysaccharides PCS2 to PCS4-II were assigned as C-1, C-3, C-5, C-2, C-4, and C-6 of (1 → 3)-β-D-glucan.[21–23] The ^{13}C NMR spectrum of PCS3-II showed one anomeric peak at 102.9 ppm indicating that there was a polysaccharide of one residue present. The ^{1}H spectrum also showed one signal in the anomeric region further confirming the deduction of a one-sugar polysaccharide. The ^{13}C–^{1}H HMQC spectrum correlated proton and carbon signals, and lead to the conclusion from chemical shift values and approximate coupling constant data that the repeating unit was (1 → 3)-β-D-glucan. Thus,

Table 1. Monosaccharide composition, protein content and yield of the polysaccharides from *Poria cocos* sclerotium

Sample	Monosaccharide content (wt.%)							Protein (wt.%)	Yield (wt.%)
	Rha	Fuc	Xyl	Man	Gal	Ara	Glc		
PCS1	—	9.2	—	25.7	47.9	—	17.1	6.2	0.34
PCS2	+	1.5	+	8.8	6.5	+	82.4	12.4	0.11
PCS3-I	—	9.0	4.0	39.3	10.4	—	37.2	40.1	0.08
PCS3-II	+	—	—	+	+	—	98.4	—	84.2
PCS4-I	+	1.2	+	2.9	+	+	93.1	5.5	0.03
PCS4-II	+	—	—	+	—	—	97.2	—	0.51

—: not detected.
+: trace amount.
Rha: rhamnose; Fuc: fucose; Xyl: xylose; Man: mannose; Gal: galactose; Ara: arabinose; Glc: glucose.

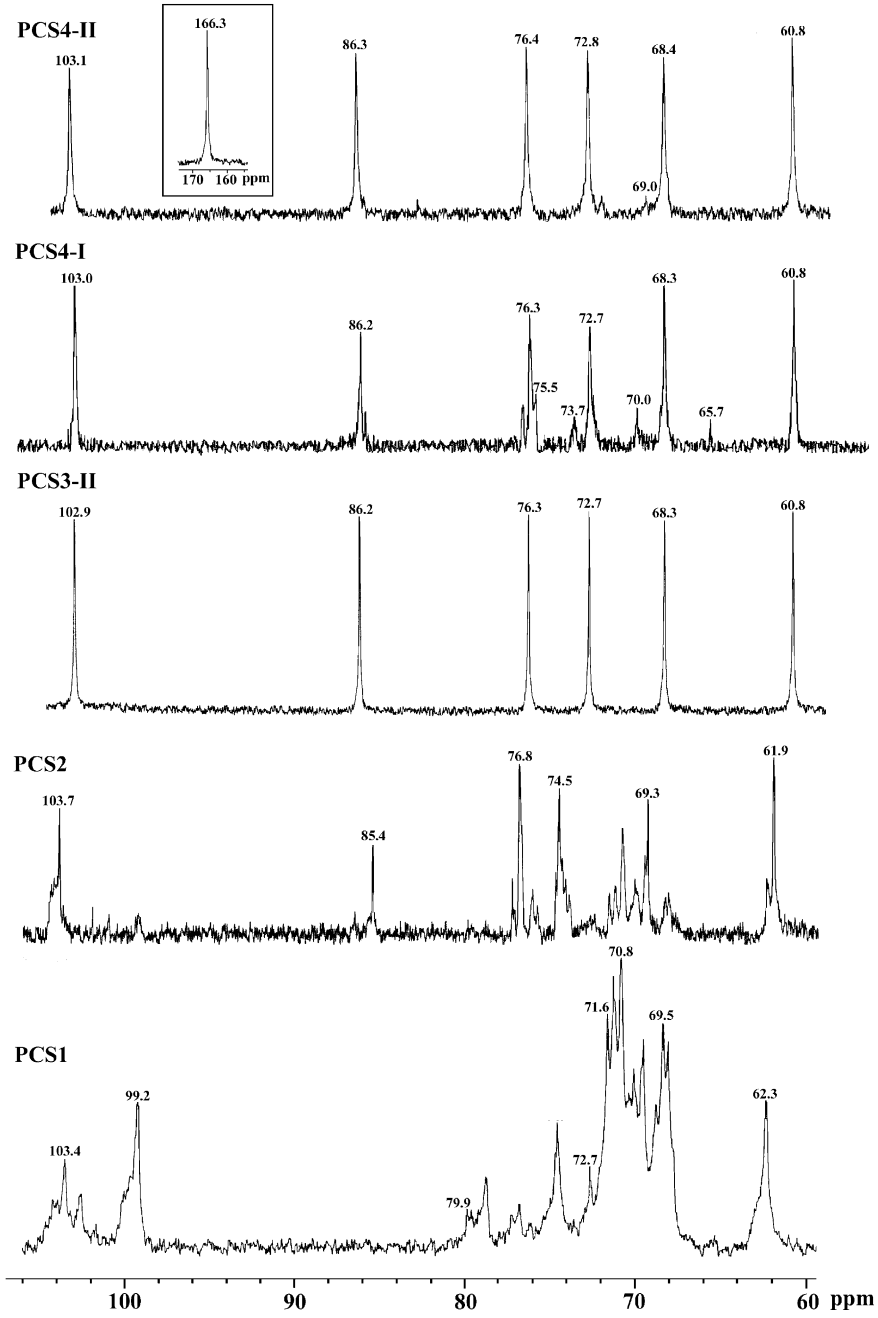


Figure 2. ^{13}C NMR spectra of fractions PCS1 and PCS2 in D_2O, PCS3-II, PCS4-I, and PCS4-II in Me_2SO-d_6.

Table 2. ^{13}C NMR chemical shifts for the polysaccharides (δ, ppm)

Sample	C-1	C-2	C-3	C-4	C-5	C-6	C-6′
PCS2	103.7	74.5	85.4	69.3	76.8	61.9	
PCS3-II	102.9	72.7	86.2	68.3	76.3	60.8	
PCS4-I	103.0	72.7	86.2	68.3	76.3	60.8	70.0
PCS4-II	103.1	72.8	86.3	68.4	76.4	60.8	69.0
Branched (1→3)-β-D-glucan[21]	103.0	72.8	86.2	68.4	76.3	60.9	70.0
Linear (1→3)-β-D-glucan[22]	102.9	72.6	86.1	68.2	76.1	60.8	

PCS3-II was characteristic of a linear (1→3)-β-D-glucan. The chemical shifts for PCS4-I showed that the signal for C-6 had moved from 60.8 to 70.0 ppm (C-6′), indicating the existence of a β-(1→6) branch. Additional small signals for C-3′ at 73.7, C-4′ at 65.7, and C-5′ at 75.5 ppm confirmed the presence of branches. The signal in the spectrum of PCS4-II at 69.0 ppm revealed that there were branches on C-6. Other chemical shifts at 83.6 and 72.0 ppm can be attributed to C-2′ and C-4′ for (1→2) linked branching.[24,25] However, there was a signal at 166.3 ppm for PCS4-II, suggesting the existence of some acid. It was attributed to an impurity rather than glucuronic acid, as the $-CO_2H$ signal for uronic acid in Me_2SO-d_6 at 60 °C would be expected to appear between ~172–173 ppm, approximately. Therefore, PCS4-II contained mainly (1→3)-β-D-glucan with some β-(1→6) and (1→2) branching.

The strong signals in the spectrum of PCS1 at 99.2, 70.8, 69.5, 71.6, and 62.3 ppm were assigned to C-1, C-3, C-2, C-5, and C-6 of α-D-galactose bound to mannose, while those at 103.4, 79.9, 72.7, and 62.3 ppm were assigned to C-1, C-4, C-3, and C-6 of mannose,[26,27] indicating that PCS1 mainly contained galactose and mannose. Although signals for glucose and fucose were not seen, these data are in good agreement with the GC, GC–MS of methylated products and IR results indicating that PCS1 is a heteropolysaccharide. The predominant component of polysaccharides isolated from the sclerotium of *P. cocos*, PCS3-II, was (1→3)-β-D-glucan.

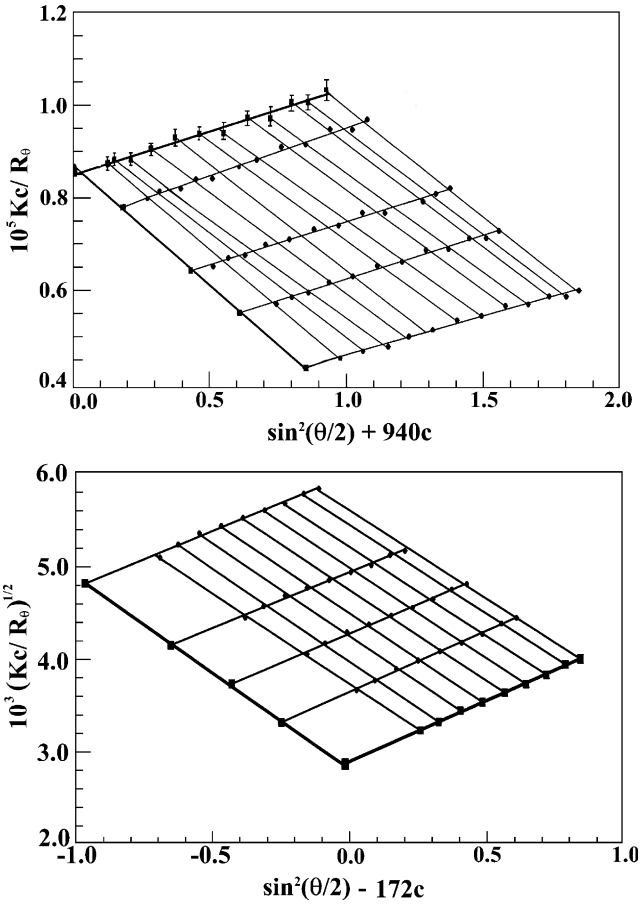


Figure 3. Zimm plots for PCS1 in 0.2 M NaCl aq solution (top) and PCS3-II in Me_2SO (bottom).

3.2. Molecular mass and intrinsic viscosity

Figure 3 shows the Zimm plots for PCS1 in 0.2 M NaCl aqueous solution and for PCS3-II in Me_2SO at 25 °C. Here K is the light scattering constant, R_θ is the reduced Rayleigh ratio at angle θ, and c is polysaccharide concentration. From LLS, SEC-LLS and viscometry measurements, the weight-average molecular mass M_w root mean square radius of gyration $\langle s^2\rangle^{1/2}$ and intrinsic viscosity [η] of the polysaccharides in 0.2 M NaCl aqueous solution or Me_2SO are summarized in Table 3. The M_w values measured by LLS were similar to those by SEC-LLS, which proved the agreement of two methods. In general, the values of [η] and $\langle s^2\rangle^{1/2}$ relate to chain stiffness of the polymer, and the relatively higher values of [η] and $\langle s^2\rangle^{1/2}$ reflect a relatively expanded chain of the polymer. From the data listed in Table 3, the extent of the chain expansion for the polysaccharides from PCS1 to PCS4-II increased with the isolation progress on the whole. Compared with a flexible polymer, the polysaccharides PCS1, PCS2, and PCS3-I exist as random coils in aqueous solution, while PCS3-II and PCS4-II exist as relatively expanded flexible chains in Me_2SO. As shown in Figure 3, the A_2 value of PCS1 and PCS3-II were positive (2.37×10^{-3} and 1.03×10^{-3} mol mL g^{-2}), suggesting that 0.2 M NaCl aqueous solution and Me_2SO were good solvents for PCS1 and PCS3-II, respectively, at 25 °C. Interestingly, the M_w values of β-glucan from *P. cocos* were significantly lower

Table 3. Experimental results from viscosity, LLS and SEC-LLS for the polysaccharides from *Poria cocos* sclerotium at 25 °C

Sample	Solvent	$[\eta]$ ($cm^3\,g^{-1}$)	$\langle s^2\rangle^{1/2}$ (nm)	LLS	SEC-LLS	
				$M_w \times 10^{-4}$ ($g\,mol^{-1}$)	$M_w \times 10^{-4}$ ($g\,mol^{-1}$)	Polydispersity index
PCS1	0.2 M NaCl	5.96	31.5[a]	11.6	11.2	1.6
PCS2	0.2 M NaCl	8.50	39.1[b]	20.8	23.9	1.6
PCS3-I	0.2 M NaCl	17.6	35.9[b]	17.1	15.8	1.8
PCS3-II	Me_2SO	76.3	56.4[a]	12.3	14.0	1.7
PCS4-I	0.2 M NaCl	93.2	50.2[b]	—	9.1	1.6
PCS4-II	Me_2SO	96.3	79.8[a]	21.1	19.1	2.0

—: not detected.
[a]Data obtained from LLS.
[b]Data obtained from SEC-LLS.

than those of polysaccharides from other basidiomycetes. For example, the M_w values of (1→3)-β-D-glucan from *Lentinus edodes*[28,29] ranged from 8.8×10^5 to 1.2×10^6 while those from *Auricularia auricula judae* were 1.17×10^6 and 1.44×10^6 for (1→3)-β-D-glucans A and C.[30]

4. Conclusions

P. cocos sclerotium contained more than six polysaccharide fractions. The predominant monosaccharide was D-glucose for the polysaccharides PCS2 to PCS4-II, whose content increased with the progress of isolation on the whole. The polysaccharides PCS1, PCS2, and PCS3-I were heteropolysaccharides containing D-glucose, D-galactose, D-mannose, D-fucose and a trace of D-xylose. The polysaccharide PCS3-I was a protein-bound heteropolysaccharide while PCS3-II, the main component of the *P. cocos* sclerotium, was a linear (1→3)-β-D-glucan of high purity. PCS4-I consisted of (1→3)-β-D-glucan with a few β-(1→6) linked branches, while PCS4-II was mainly composed of (1→3)-β-D-glucan with a few β-(1→2) linked and β-(1→6) linked branches. The M_w values of the six polysaccharides were determined to be 11.6, 20.8, 17.1, 12.3, 9.1, and 21.1×10^4 for the samples PCS1–PCS4-II, respectively. The polysaccharides PCS1, PCS2, and PCS3-I exist as random coils in 0.2 M NaCl aqueous solution, while PCS3-II and PCS4-II exist as relatively expanded flexible chains in Me_2SO.

Acknowledgements

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References

1. Narui, T.; Takakashi, K.; Kobayashi, M.; Shibata, S. *Carbohydr. Res.* **1980**, *87*, 161–163.
2. Ding, Q.; Zhang, L.; Zeng, F. *Chinese J. Polym. Sci.* **1998**, *308*, 339–343.
3. Chihara, G.; Hamuro, J.; Maeda, Y.; Arai, Y.; Fukuoka, F. *Nature* **1970**, *225*, 943–944.
4. Kanayama, H.; Adachi, N.; Togami, M. *Chem. Pharm. Bull.* **1983**, *31*, 1115–1118.
5. Yamada, H.; Kiyohara, H.; Takemoto, N.; Zhao, J. F.; Kawamura, H.; Komatsu, Y.; Cyong, J. C.; Aburada, M.; Hosoya, E. *Planta Med.* **1992**, *58*, 166–170.
6. Wang, S. X.; Wen, Y. Y.; Hu, C. X. *Phytother Res.* **1995**, *9*, 448–451.
7. Mizuna, M.; Morimoto, M.; Minato, K.; Tsuchida, H. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 434–437.
8. Adachi, Y.; Ohno, N.; Ohsawa, M.; Yadomae, T. *Chem. Pharm. Bull.* **1990**, *38*, 477–481.
9. Young, S. H.; Jacobs, R. R. *Carbohydr. Res.* **1998**, *310*, 91–99.
10. Kiho, T.; Yoshida, I.; Nagai, K.; Ukai, S. *Carbohydr. Res.* **1989**, *189*, 273–279.
11. Hoffman, G. C.; Simson, B. W.; Timell, T. E. *Carbohydr. Res.* **1971**, *20*, 185–188.
12. Kanayama, H.; Adachi, N.; Fukai, Y.; Takeuchi, I.; Togami, M. *Kakugaku Zasshi* **1986**, *106*(3), 206–211.
13. Zhang, L.; Ding, Q.; Zhang, P.; Zhu, R.; Zhou, Y. *Carbohydr. Res.* **1997**, *303*, 193–197.
14. Ding, Q.; Jiang, S.; Zhang, L.; Wu, C. *Carbohydr. Res.* **1998**, *308*, 339–343.
15. Zhang, L.; Ding, Q.; Meng, D.; Ren, L.; Yang, G.; Liu, Y. *J. Chromatogr. A* **1999**, *839*, 49–55.
16. Jin, Y.; Zhang, L.; Chen, L.; Chen, Y.; Cheung, P. C. K.; Chen, L. G. *Carbohydr. Res.* **2003**, *338*, 1507–1515.
17. Jin, Y.; Zhang, L.; Zhang, M.; Chen, L.; Cheung, P. C. K.; Oi, V. E. C.; Lin, Y. *Carbohydr. Res.* **2003**, *338*, 1517–1521.
18. Zhang, L.; Ding, Q.; Zhang, P.; Feng, H. *Chem. J. Chinese Univ.* **1997**, *6*, 990–993.
19. Needs, P. W.; Selvendran, R. R. *Carbohydr. Res.* **1993**, *245*, 1–10.
20. Needs, P. W.; Selvendran, R. R. *Phytochem. Anal.* **1993**, *4*, 210–216.

21. Misaki, A.; Kakuta, M.; Sasaki, T.; Tanaka, M.; Miyaji, H. *Carbohydr. Res.* **1981**, *92*, 115–129.
22. Saito, H.; Tabeta, R.; Sasaki, T.; Yoshioka, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2093–2101.
23. Kogan, G.; Alfoldi, J.; Masler, L. *Biopolymers* **1988**, *27*, 1055–1063.
24. Deng, C.; Yang, X.; Gu, X.; Wang, Y.; Zhou, J.; Xu, H. *Carbohydr. Res.* **2000**, *328*, 629–633.
25. Bao, X.; Liu, C.; Fang, J.; Li, X. *Carbohydr. Res.* **2001**, *332*, 67–74.
26. Gorin, P. A. J. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 13–104.
27. Chaubey, M.; Kapoor, V. P. *Carbohydr. Res.* **2001**, *332*, 439–444.
28. Zhang, L.; Li, X.; Zhou, Q.; Zhang, X.; Chen, R. *Polym. J.* **2002**, *34*, 443–449.
29. Zhang, L.; Zhang, X.; Zhou, Q.; Zhang, P.; Zhang, M.; Li, X. *Polym. J.* **2001**, *33*, 317–321.
30. Zhang, L.; Yang, L.; Ding, Q.; Chen, X. *Carbohydr. Res.* **1995**, *270*, 1–10.