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Carbohydrate Polymers 67 (2007) 343–346
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Structural studies of *Paecilomyces tenuipes* Samson polysaccharide-part-2

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Received 2 February 2006; received in revised form 1 June 2006; accepted 1 June 2006
Available online 21 July 2006

Abstract

A water-soluble polysaccharide-part-2 separated from *Paecilomyces tenuipes* Samson was determined to be homogeneous by gel permeation chromatography (GPC). HPLC showed a monosaccharide containing D-glucose, D-galactose, and D-mannose at a ratio of about 2:1:1. The number-average molecular weight was estimated to be 1.02×10^4. The specific rotation was determined to be $[\alpha]_D^{20} = +54°$. The GC–MS analysis of hydrolysates obtained from acid-catalyzed hydrolysis of both *Paecilomyces tenuipes* Samson-part-2 and its methylated product showed that it has a β-(1 → 6)-D-glucose main chain with β-(1 → 6)-D-mannose and β-(2 → 6)-D-galactose side chains. The results obtained from IR, ^{1}H NMR, and ^{13}C NMR analyses confirmed the proposed structure.

Keywords: Polysaccharide; GPC; HPLC; GC–MS

1. Introduction

Paecilomyces tenuipes Samson (PTPS) is a fungus belonging to the cordycepic genus. A polysaccharide from *Paecilomyces tenuipes* Samson has been found to have bioactivities, such as promoting the activities of LDH, ACPase, and Argase, similar to the source of a valuable traditional high tonic of the same genus, *Paecilomyces sinensis* Berksacc (Gong, Zhu, & Wang, 1990; Xu & Zhou, 2000). Studies of the structure of the polysaccharide from *Paecilomyces sinensis* Berksacc showed that it has a β-(1 → 2) mannose main chain with β-(1 → 2, 6) mannose and β-(1 → 3, 5, 6) galactose as side chains (Chen, Shiao, Lee, & Wang, 1997; Chu, 1996). We previously analyzed the Asian lacquer polysaccharide by means of NMR measurement (Lu et al., 1999; Lu & Yoshida, 2003) and reported its specific biological activities (Lu et al., 2000). In addition, the structure of the polysaccharide purified from *Paecilomyces tenuipes* Samson-part-1 was analyzed using NMR and GC–MS measurements in our previous study (Lu, Sun, Wang, Tian, & Yoshida, 2001), where we found that the polysaccharide of *Paecilomyces tenuipes* Samson-part-1 was composed of glucose with only an α-(1 → 6) linkage.

In this study, we report the structural characteristics of the polysaccharide-part-2 separated from *Paecilomyces tenuipes* Samson using GC–MS analysis of hydrolysates obtained from acid-catalyzed hydrolysis of PTPS-part-2 and its methylated product, and by IR and NMR spectroscopic techniques.

2. Experimental

2.1. Materials

Paecilomyces tenuipes Samson was kindly supplied by the Wenzhou Medical College (Wenzhou, Zhejiang, China). The crude material was purified through a column

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doi:10.1016/j.carbpol.2006.06.003

(2.5 cm i.d × 120 cm) of Sephadex G-100 (Amersham Pharmacia Biotech, Uppsala, Sweden) using deionized water as the eluent. The polysaccharide fraction was pooled, dialyzed, and then freeze-dried.

2.2. Measurements

The molecular weight of the polysaccharide was estimated by aqueous phase GPC using the following columns: TOSOH TSK-gel, G2500PW$_{XL}$, G3000PW$_{XL}$, and G4000PW$_{XL}$, ϕ7.6 mm × 300 mm × 3, eluted with 66.7 mM L^{-1} of phosphate buffer (pH 6.86), using pullulan as the standard.

The infrared spectra were taken using a Shimadzu FT-IR 8300 spectrometer by the KBr pellet method. Specific rotations were measured using a JASCO DIP-140 digital polarimeter in water.

PTPS-part-2 (50 mg) was freeze-dried several times from 99.96% D_2O before preparing the sample for NMR measurement. All NMR spectra were recorded at 40 °C in D_2O by a JEOL α-500 spectrometer using the phase-sensitive mode and a field gradient probe. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as the internal standard at δ 0 ppm for the ^{1}H and δ 0.015 ppm for the ^{13}C spectra.

2.3. Sugar analysis

Samples were hydrolyzed in 2 N trifluoroacetic acid at 100 °C for 8 h. The quantities of sugars in the resulting hydrolysates were first estimated by HPLC analysis on a TOSOH TSK-gel amide-80 column using acetonitrile–water (80:20 v/v) as eluent with flow rate of 1 ml min^{-1} at 80 °C. The sample was completely hydrolyzed when the disaccharide and/or oligosaccharide peaks disappeared in the chromatogram. Because galactose and glucose cannot be separated by this column, the hydrolysate was further analyzed with a DX500 high performance anion exchange chromatograph (HPAEC) system equipped with a pulsed amperometric detector (Dionex, Sapporo, Japan). The monosaccharides were isolated on a CarboPac PA-1 column using 20 mM NaOH as eluent with a flow rate of 1 ml min^{-1} at room temperature.

2.4. Methylation analysis

The methylation of PTPS-part-2 was analyzed using methylsulfinylcarbanion in DMSO according to the method of Hakomori (Hakomori, 1964). The methylated polysaccharide was hydrolyzed in 90% formic acid at 100 °C for 12 h. After evaporation, the partially methylated sugars were converted to alditol acetates (Englyst & Cummings, 1984), and the partially methylated alditol acetates were analyzed using Shimadzu GC-17A and QP-5000 apparatuses equipped with a fused silica capillary column (J and W DB-1, 0.25 mm × 30 m) and using He as the carrier gas with flow of 50 ml min^{-1}.

3. Results and discussion

The crude polysaccharides produced by *Paecilomyces tenuipes* Samson owed two peaks on GPC and were separated on a Sephadex G-100 column, as shown in Fig. 1. The high molecular weight fraction, PTPS-part-1, was characterized in our previous study (Lu et al., 2001). The low molecular weight fraction, PTPS-part-2, had a number-average molecular weight of 1.02×10^4, and was analyzed in this study.

The specific rotation of PTPS-part-2 was $[\alpha]_D^{20} = +54°$ (c 1 mg ml^{-1}, H_2O). The strong absorption in the range of 1200–1000 cm^{-1} that appeared in the IR spectrum suggested that the monosaccharide in PTPS-part-2 has a pyran structure (Fig. 2). The absorption band at 889 cm^{-1} indicated that the glucosidic bond in PTPS-part-2 was a β-linkage (Du, Kong, & Li, 1994). This is in agreement with the specific rotation data.

In the analysis of the hydrolysate from native PTPS-part-2 by HPLC on a Tosoh TSK-gel Amide-80 column, the two peaks in the chromatogram were assigned to D-Man, D-Glc, or D-Gal (Fig. 3B). D-Glc and D-Gal overlapped in this column. In order to determine whether it was D-Glc or D-Gal, the hydrolysate was subjected to HPAEC-PAD analysis. To prepare a standard chromatogram of the monosaccharide (Fig. 4A), the ratio of Glc is 4-fold of the other monosaccharide because D-Glc is difficult to detect. In the chromatogram of the hydrolysate from PTPS-part-2 (Fig. 4B), the ratio of D-Glc, D-Gal, and D-Man was calculated to be about 2:1:1 according to the monosaccharide standards.

The sugar linkages in methylated PTPS-part-2 were elucidated by acid-catalyzed hydrolysis, and then the resulting products were converted to the corresponding alditols, which were characterized by GC–MS spectrometry (Fig. 5). The main peak, with a retention time of 8.39 min, was elucidated to be 2, 3, 4-trimethyl-1, 6-diacethyl-glucopyranan according to its mass spectrum. The other peaks were determined to be 2, 3, 4, 6-tetramethyl-1, 5-diacethyl-glucopyranan, 1, 3, 4, 6-tetramethyl-2, 5-diacethyl-galactopyranan, 2, 3, 4-trimethyl-1, 5,

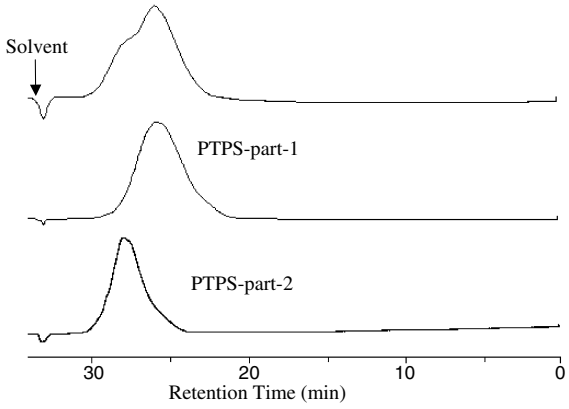

Fig. 1. GPC profiles of PTPS, column: TOSOH TSK-gel, G2500PW$_{XL}$, G3000PW$_{XL}$, G4000PW$_{XL}$, ϕ7.5 × 300 mm × 3, eluted with 66.7 mM L^{-1} of phosphate buffer (pH 6.86), using pullulan as standard.

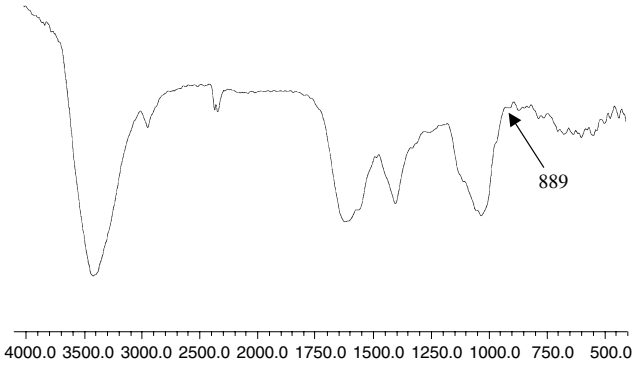

Fig. 2. IR spectrum of PTPS-part-2.

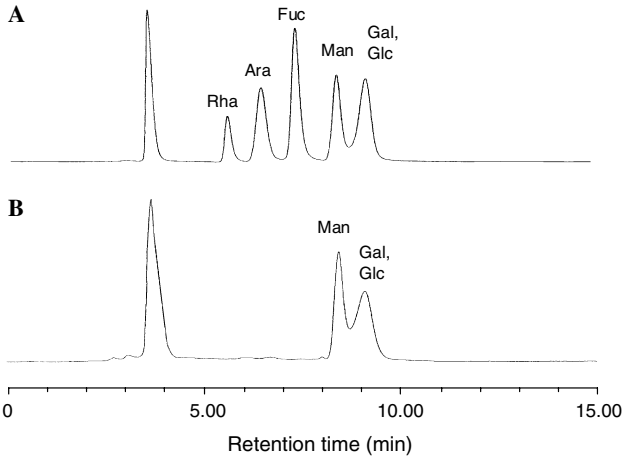

Fig. 3. Amide-80 column chromatograms of (A) monosaccharide standard and (B) monosaccharide residues of hydrolysis product of PTPS-part-2.

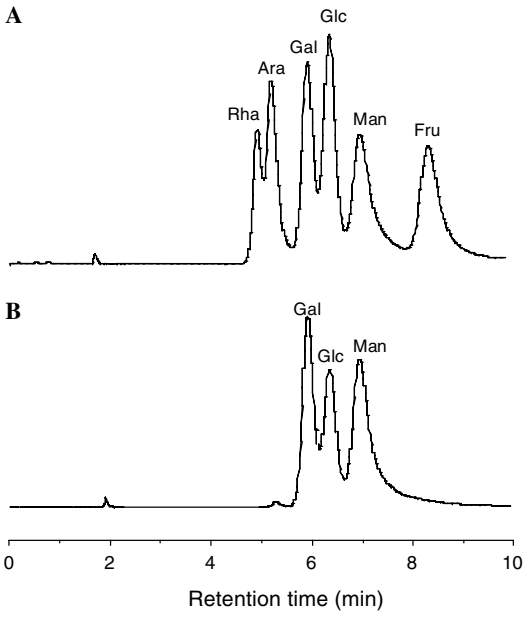

Fig. 4. HPAEC-PAD chromatograms of (A) monosaccharide standard and (B) monosaccharide residues of hydrolysis product of PTPS-part-2.

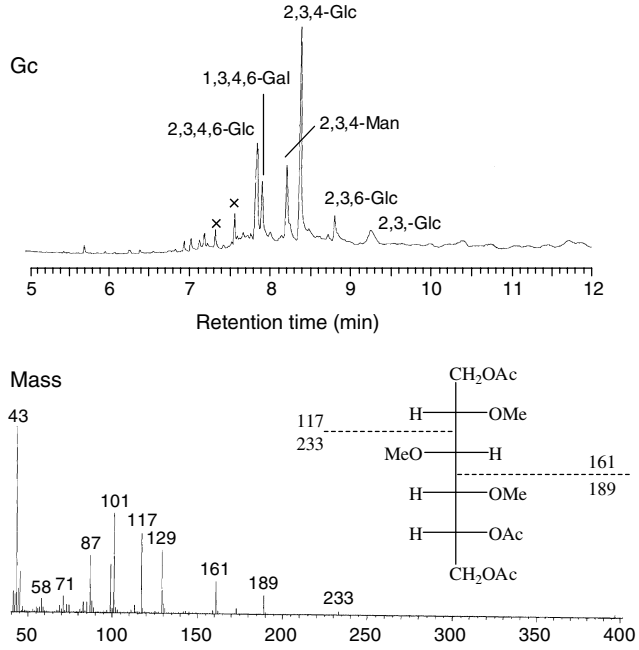

Fig. 5. GC and mass of main peak (retention time 8.39 min) spectra of PTPS-part-2.

6-triacethyl-mannopyranan, 2, 3, 6-trimethyl-1, 4, 5-triacethyl-glucopyranan, and 2, 3-dimethyl-1, 4, 5, 6-tetraacethyl-glucopyranan according to their mass spectra. Most possible configurations of the glycosidic bonds are summarized in Table 1. The results showed that PTPS-part-2 has a β-(1 → 6)-linkage glucopyranan main chain with β-(2→)-galactopyranan and β-(1→)-mannopyranan as side chains in the native polysaccharide.

In the anomeric region (90–110 ppm) of the ^{13}C NMR spectrum of PTPS-part-2, the signals at δ 100.2, 101.5, and 108.7 were assigned to β-(1 → 6)-Glc, β-(2→)-Gal, and β-(1→)-Man, respectively (Fig. 6). The ^{1}H NMR spectrum (not shown) confirmed the proposed three monosaccharide-repeating unit because it exhibited three strong

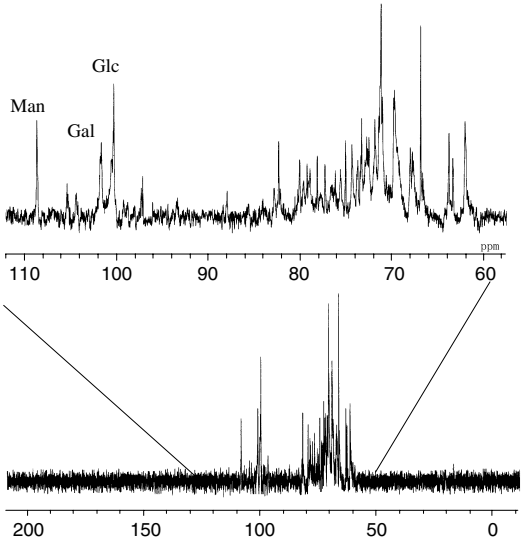

Fig. 6. ^{13}C NMR spectra of PTPS-part-2 in D_2O at 40 °C.

Table 1
Results of the analysis of GC–MS

Retention time (min)	Fragments	Configuration
7.82	2, 3, 4, 6-Tetramethyl-1, 5-diacetyl-glucotitol	Glc(1-
7.90	1, 3, 4, 6-Tetramethyl-2, 5-diacetyl-gala1titol	Gal(2-
8.19	2, 3, 4-Trimethyl-1, 5, 6-triacetyl-mannitol	Man(1-
8.39	2, 3, 4-Trimethyl-1, 5, 6-triacetyl-glucotitol	Glc(1-, -6)Glc
8.79	2, 3, 6-Triamethyl-1, 4, 5-triacetyl-glucotitol	Glc(1-, -4)Glc
9.22	2, 3-Diamethyl-1, 4, 5, 6-triacetyl-glucotitol	Glc(1–4), Glc(1–6)

anomeric signals of δ 4.91, 5.15, and 5.22 in the anomeric region (δ 5.5–4.5). The $^3J_{1,2}$ coupling constants were calculated to be 4.9, 7.3, and 5.9 Hz, respectively, suggesting that there is a β-pyranosyl residue.

As discussed above, it was evident that the main structure of polysaccharide-part-2 produced by *Paecilomyces tenuipes* Samson has a β-(1 → 6)-linkage glucopyranan main chain with β-(2→)-galactopyranan, and β-(1→)-mannopyranan as the side chains with the ratio of D-Glc, D-Gal, and D-Man about 2:1:1. It has been reported that PTPS has biological activities similar to *Paecilomyces sinensis* Berksacc (Jin, Lu, & Yuan, 2002a; Jin, Lu, Yuan, & Li, 2002b). Investigation on the biological activity of PTPS-part-2 is now in progress.

Acknowledgement

The authors are deeply grateful to Professor Lu Jianxin (Wenzhou Medical College, Wenzhou, China) for kindly supplying the *Paecilomyces tenuipes* Samson.

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