Carbohydrate Polymers 92 (2013) 2239–2244

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Structural elucidation of a novel heteropolysaccharide from the fruiting bodies of *Pleurotus eryngii*

An-qiang Zhang, Yang Zhang, Jun-hong Yang, Pei-long Sun*

College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310014, China

ARTICLE INFO

Article history:
Received 10 September 2012
Received in revised form
23 November 2012
Accepted 24 November 2012
Available online 1 December 2012

Keywords:
Pleurotus eryngii
Heteropolysaccharide
Structural elucidation
NMR analysis

ABSTRACT

A novel water-soluble heteropolysaccharide (PEPS1), with a molecular weight of 1.88×10^4 Da as determined by high performance liquid chromatography (HPLC), specifically size exclusion chromatography, was isolated from the fruiting bodies of *Pleurotus eryngii* by hot water extraction and further purification by DEAE Sepharose Fast Flow and Sephacryl S-300 and S-100 High-Resolution chromatography. By use of compositional analysis, methylation analysis, together with ^{1}H, ^{13}C NMR and 2D NMR spectroscopy including COSY, TOCSY, HMQC, HMBC and NOESY experiments, the PEPS1 was identified as a heteropolysaccharide that consists of a α-D-(1 → 6)-Galactopyranan backbone with a β-D-Mannosyl unit on *O*-2 of the 2,6-di-*O*-substituted-D-Galactosyl units, α-(1 → 6)-3-*O*-Me-D-Galactopyranan backbone with a terminal α-3-*O*-Me-D-Galactosyl unit and it also contained a minor 2,3,6-α-D-Galatopyranan units and 4-β-D-Mannopyranan residues.

1. Introduction

Mushrooms are well known for their rich diversity of bioactive components which contribute to exceptional advantage on both nutritive and pharmaceutical value (Ng, 1998). Nowadays, they have attracted growing attention of the whole world as a functional food and a source for development of new drugs. The extracts from specific mushrooms, such as polysaccharide, had been proved its profound health promoting benefits (Manzi, Aguzzi, & Pizzoferrato, 2001).

Pleurotus is an important genus which contains many edible and medicinal species. Among them, several species have been commercially cultivated due to their high mineral and protein contents (Yildiz, Yildiz, Gezer, & Temiz, 2002). People's growing interests in *Pleurotus* are partially because of its β-glucan, which demonstrates great immune modulation, antioxidant, anti-inflammatory (Bobek & Galbavy, 2001; Smiderle et al., 2008). *Pleurotus eryngii*, a delicious edible mushroom belongs to *Pleurotus* (*P.*) family, is also named as "king oyster mushroom" due to its positive health effects and it is commonly cultivated in Europe, Middle East, North America and some parts of Asia (Royse, 1995).

Among the very important metabolites derived from mushrooms, polysaccharides have attracted much attention owing to their demonstrated health benefits. Several polysaccharides have been isolated from *Pleurotus* mushrooms. From *P. ostreatus*, a most studied β-D-glucan which is composed of (1 → 3)-linked β-D-glucopyranosyl and named pleuran was extracted (Karacsonyi & Kuniak, 1994). Other polysaccharides with different linkage patterns which demonstrate health benefits were also found in *Pleurotus*, including heteropolysaccharides, mannogalactans, xylomannans and so on (Gutiérrez, Prieto, & Martínez, 1996).

β-Glucans are the most studied and characterized fungal polysaccharides. Besides, the 3-*O*-methyl galactoses are also common in mushrooms which have been isolated from the fruiting bodies of *Phellinus igniarius* (Yang et al., 2007) and *Lampteromyces japonicus* (Fukuda & Hamada, 1978).

Recently, the biotechnological potential of *P. eryngii* has been exploited to be a good source of molecules such as polysaccharides that can function as immunomodulatory, antitumor, hypolipidemic and antioxidant properties (Jeong, Jeong, Gu, Islam, & Song, 2010; Kalyoncu, Oskay, Saglam, Erdogan, & Tamer, 2010; Mizutani, Inatomi, Inazu, & Kawahara, 2010). Since structure and functions are intimately related, for polysaccharides, the elucidation of molecular structure is essential issue for better investigating its biological behavior and exploring its structure–activity relationship (Srivastava & Kulshreshth, 1989).

The aim of this work was mainly focus on a new structure of heteropolysaccharide from the fruiting bodies of *P. eryngii*. The fractionation, isolation, and purification of polysaccharides are described together with the elucidation of the structural features.

2. Experimental

2.1. Materials

Fruiting bodies were purchased from Qingyuan (Zhejiang Province, China) and identified by Prof. Weiming Cai, Zhejiang

* Corresponding author. Tel.: +86 571 883 20779; fax: +86 571 883 20345.
E-mail addresses: sun_pl@126.com, sun_pl@zjut.edu.cn (P.-l. Sun).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.11.069

Academy of Agricultural Science. DEAE Sepharose Fast Flow and Sephacryl S-300 and S-100 High-Resolution were purchased from GE Healthcare. Dextrans, trifluoroacetic acid (TFA) and monosaccharide standards (D-Gal, L-Fuc, L-Rha, D-Man, D-Xyl, D-Glc) were from Sigma. HPLC was carried out on a Waters 2695 HPLC system (2695 HPLC Pump, 2414 Refractive Index Detector). GC–MS was carried out using a ThermoFinnigan TRACE MS, and NMR spectra were determined with a Varian INOVA 500.

2.2. Isolation and purification

The dried fruiting bodies of *P. eryngii* were exhaustively reflux extracted with 95% EtOH for 12 h to remove lipids. After filtration, the residue was air dried and extracted with boiling distilled water thrice (2 h for each). The combined supernatant was concentrated into one-tenth of the original volume, and 95% EtOH was added slowly with stirring to the final alcohol concentration reached 30%. The precipitate was removed by centrifugation (4000 rpm, 10 min, 4 °C), and 95% EtOH was continually added slowly to final concentration of 60%. The precipitate obtained by centrifugation (4000 rpm, 10 min, 4 °C) was dissolved in distilled water and lyophilized, and defined as PEPS60. A portion of PEPS60 was dissolved in water and insoluble residue was removed by centrifugation (10,000 rpm, 10 min, 4 °C). The supernatant was applied to a DEAE Sepharose Fast Flow column (XK 26 mm × 100 cm), and eluted with water. The main fraction was collected based on the phenol–sulfuric acid assay (Zhang, 1999), and then further purified using High-Resolution Sephacryl S-300 and S-100 (XK 26 mm × 100 cm) gel-permeation chromatography. The main fraction was collected, concentrated and freeze dried to get a white purified *P. eryngii* polysaccharide (PEPS1).

2.3. Homogeneity and molecular weight

The homogeneity and molecular weight of PEPS1 were determined by high performance liquid chromatography (HPLC) on a Waters 2695 HPLC system equipped with a TSK-gel PW_{XL} G4000 column, eluted with distilled water at a flow rate of 1.0 mL/min and detected by a refractive index detector (RID). 10 μL of sample solution (1.0 mg/mL) was injected in each run. The column was kept at 30.0 ± 0.1 °C. The standard curve of molecular weight was obtained with the T-series Dextrans standards (1000, 5000, 12,000, 80,000, 150,000, 270,000 and 670,000 Da). The molecular weight of PEPS1 was estimated by reference to the calibration curve made above.

2.4. Monosaccharide composition analysis and methylation analysis

PEPS1 (2 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 4 mL) at 110 °C for 2 h. The excess TFA was removed by vacuum evaporation with MeOH. The hydrolyzed mixture were reduced with $NaBH_4$ (20 mg) and acetylated with acetic anhydride (Albersheim, Nevins, English, & Karr, 1967). The acetylated derivatives of PEPS1 were analyzed by gas chromatography (GC) using an Agilent 7890N instrument equipped with an HP-5 capillary column (30 m × 0.32 mm × 0.25 μm) a flame-ionization detector (FID). The temperature program consists of 120–240 °C at 10 °C/min and then held at 240 °C for 6.5 min. The heater temperatures of the injector and detector were both at 250 °C. Nitrogen was used as carrier gas.

PEPS1 (2 mg) was methylated twice by the method (Anumula & Taylor, 1992). The reaction mixture was extracted with $CHCl_3$, and the solvent was then removed by evaporation. Complete methylation was confirmed by the disappearance of the OH band (3200–3700 cm^{-1}) in the IR spectrum. The permethylated polysaccharide was hydrolyzed by treatment with HCO_2H (88%, 3 mL) at 100 °C for 3 h, evaporated to dryness and further hydrolyzed with 2 M TFA (4 mL) at 100 °C for 6 h. The partially methylated sugar in the hydrolysate were reacted with $NaBH_4$ and acetylated with AC_2O, and the resulting mixture of methylated alditol acetates were analyzed by GC–MS.

2.5. NMR analysis

PEPS1 (30 mg) was dried in a vacuum over P_2O_5 for 72 h, and then exchanged with deuterium by lyophilizing with D_2O (0.5 mL) for three times. ^1H NMR (25 °C, 60 °C) and ^{13}C NMR (60 °C) spectra were determined in 5-mm tubes using a Varian INOVA 500 NMR spectrometer. ^1H Chemical shifts were referenced to residual HDO at δ 4.78 (25 °C) as the internal standard. ^{13}C chemical shifts were determined in relation to DSS (δ 0.00) calibrated externally. ^1H–^1H correlated spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple quantum correlation spectroscopy (HMQC) were used to assign signals. Two-dimensional heteronuclear multiple-bond correlation spectroscopy (HMBC) and two-dimensional overhauser effect spectroscopy (NOESY) were used to assign inter-residue linkages and sequences.

3. Results and discussion

The novel water-soluble polysaccharide PEPS1 was obtained by DEAE Sepharose Fast Flow column, High Resolution Sephacryl S-300 and S-100 gel-permeation chromatography. PEPS1 was tested as a single symmetric peak on the HPLC, indicating it was a homogenous polysaccharide (Fig. 1). And the molecular weight of PEPS1 was estimated to be 1.88×10^4 Da in reference to the calibration curve of Dextran standards. Lack of absorption at 280 nm indicated that PEPS1 contained no protein.

The monosaccharide composition analysis of PEPS1 determined by sugar analysis indicated that it consists of D-mannose and D-galactose. In addition, another peak was observed eluting before D-mannose that did not match any of our monosaccharide standards. The 3-*O*-Me-D-galactose was confirmed by comparison of retention times and mass spectra of partially *O*-methylated derivatives. The mass spectrum of 3-*O*-methylgalactose is dominated by

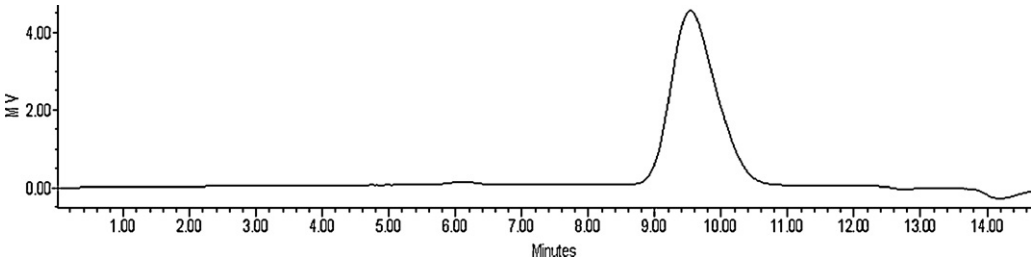

Fig. 1. Curve of PEPS1 polysaccharide isolated from the fruiting bodies of *P. eryngii* with HPLC.

Table 1
The methylation analysis of PEPS1 isolated from the fruiting bodies of *P. eryngii*.

Methylated sugars	Substituted sugar residue	Molar ratios	Major mass fragments (*m*/*z*)
2,3,4,6-Me_4-Gal*p*	Non-reducing end 3-*O*-Me-D-Gal*p* unit	0.06	43,71,89,101,117,129,145,161,203
2,3,4,6-Me_4-Man*p*	Non-reducing end Man*p* unit	0.87	43,71,87,101,117,129, 161,203,233
2,3,6-Me_3-Man*p*	4-Substituted Man*p* unit	0.12	43,71,87,101,117,129,145,161,205
2,3,4-Me_3-Gal*p*	6-Substituted 3-*O*-Me-D-Gal*p* unit	1.09	43,71,87,99,101,117,129,161,173,189,233
3,4-Me_2-Gal*p*	2,6-di-Substituted Gal*p* unit	1.00	43,71,87,99,129,159,173,189,233
4-Me-Gal*p*	2,3,6-tri-Substituted Gal*p* unit	0.07	43,71,87,129,159,189,201,231,261

the cleavage of bonds between *O*-methylated carbons and adjacent *O*-acetylated carbons. For 3-*O*-methylgalactose alditol acetate, this breakage produced *m*/*z* 261 and 189 as the primary fragments, and *m*/*z* 87, 99, 129, 159 and 201 as the other main fragments (Stepan, Bleckmann, Geyer, Geyer, & Staudacher, 2010; Watt, O'Neill, Percy, & Brasch, 2002). 3-*O*-methylgalactose was further confirmed by GC–MS of the alditol acetates.

Methylation analysis (Table 1) of the PEPS1 detected 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4,6-tetra-*O*-methylmannose, 2,3,6-tri-*O*-methylmannose, 2,3,4-tri-*O*-methylgalactose, 3,4-di-*O*-methylgalactose, 4-*O*-methylgalactose in a ratio of 0.06:0.87:0.12:1.09:1.00:0.07, respectively.

The ^{1}H NMR spectrum (Fig. 2) of PEPS1 showed that contained anomeric proton signals at δ 4.77–5.12, one *O*-Me group signal at δ 3.44. Other sugar protons were in the region of δ 3.32–4.33. The ^{13}C and DEPT-135 NMR Spectrum (Fig. 3) of polysaccharide mainly contained signal for anomeric carbons at δ 100.71–104.26. Two *O*-Me group signals at δ 58.78 and δ 59.16,

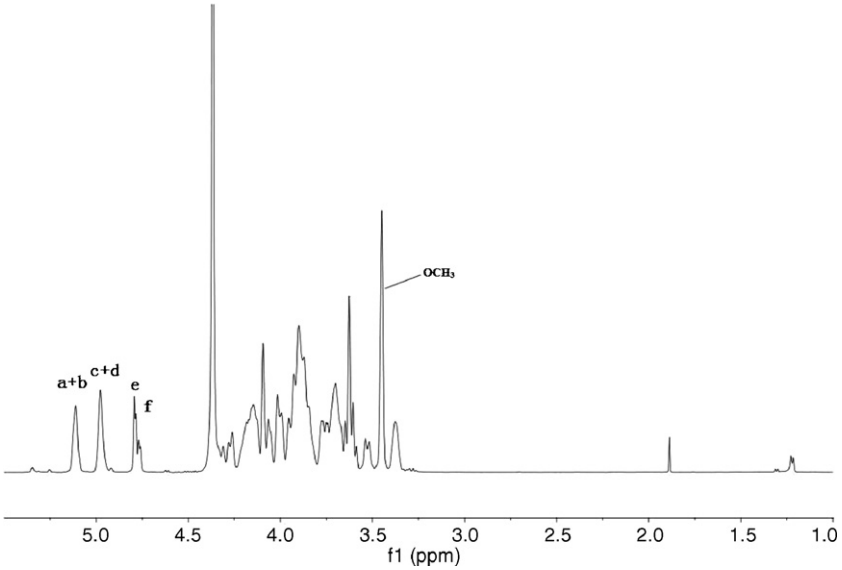

Fig. 2. 500 MHz ^{1}H NMR spectrum of PEPS1 polysaccharide isolated from the fruiting bodies of *P. eryngii* in D_2O at 60 °C. The anomeric protons are labeled a–f.

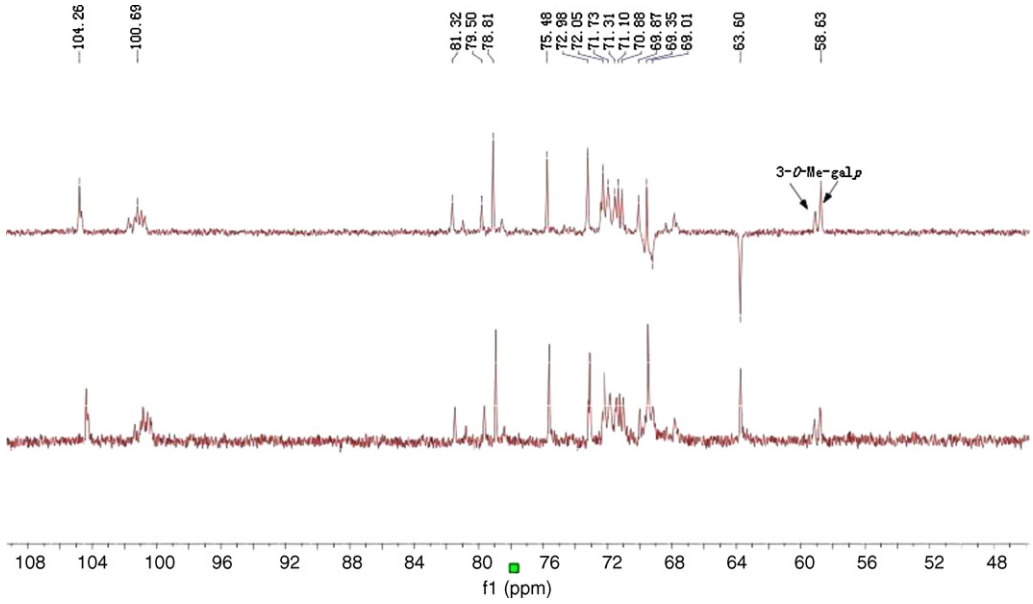

Fig. 3. 500 MHz ^{13}C and DEPT-135 NMR spectra of PEPS1 polysaccharide isolated from the fruiting bodies of *P. eryngii*.

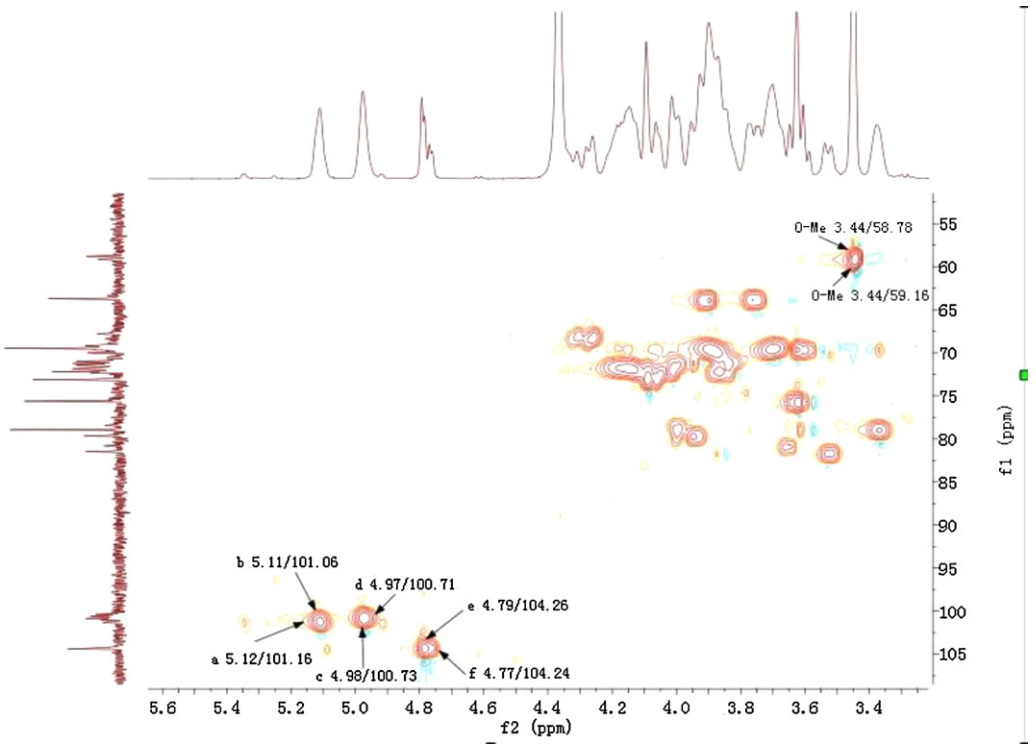

Fig. 4. 500-MHz ^{1}H–^{13}C HMQC spectrum of PEPS1 polysaccharide isolated from the fruiting bodies of *P. eryngii*.

and sugar ring carbons linked to oxygen in the region of δ 63.78–81.63.

Residue **a** →2,6)-α-D-Gal*p*-(1→. The ^{1}H resonances for H-1 to H-4 of residue **a** were assigned from the cross-peaks in the ^{1}H–^{1}H COSY and TOCSY spectra. H-5, H-6a and H-6b were assigned from the cross-peaks of TOCSY spectrum and validated by HMQC (Fig. 4) and NOESY spectra. ^{13}C NMR resonances were assigned from the HMQC spectrum (Table 2). H-4 displays strong NOEs to both H-3 and H-5, which indicated that residue **a** is a Gal-type residue. H-1 appears as a singlet ($J_{H\text{-}1,H\text{-}2} < 3$ Hz) in the ^{1}H NMR spectrum and the H-1/H-2 intra-residue correlation in the NOESY spectrum indicate the residue **a** is an α-configuration. The downfield shifts of the C-2 and C-6 carbon signals with respect to the standard values for glycopyrannoses indicated that residue **a** was identified as →2,6)-α-D-Gal*p*-(1→.Residue **b** →2,3, 6)-α-D-Gal*p*-(1→. The ^{1}H NMR resonances for H-1, -2, -3 were assigned from the cross-peaks in the ^{1}H–^{1}H COSY spectrum. H-4 and H-5 were assigned from the cross-peaks of ^{1}H–^{1}H COSY and TOCSY spectra, validated by the strong cross-peaks H-3/H-4 and H-4/H-5 in the NOESY spectrum. The H-6a and H-6b resonance were assigned from the TOCSY spectrum. The H-5 and H-4 and H-6b were obtained from the TOCSY spectrum. ^{13}C resonances were assigned from the HMQC spectrum (Table 2). H-4 displays strong NOEs to both H-3 and H-5, which indicated that residue **b** is a Gal-type residue. H-1 appears as a singlet ($J_{H\text{-}1,H\text{-}2} < 3$ Hz) in the ^{1}H NMR spectrum, and the H-1/H-2 intra-residue correlation in the NOESY spectrum and the cross-peaks of H-1 and C-3, C-5 in the HMBC spectrum indicate the residue **b** is an α-configuration. The downfield shifts of the C-2, C-3 and C-6 carbon signals with respect to the standard values for glycopyrannoses indicated that residue **b** was identified as →2,3,6)-α-D-Gal*p*-(1→. Residue **c** →6)-α-3-*O*-Me-D-Gal*p*-(1→. The ^{1}H resonances for H-1 to H-4 of residue **c** were assigned from the cross-peaks in the ^{1}H–^{1}H COSY and TOCSY spectra. The H-5 resonance was assigned from the NOESY spectrum. The cross-peaks of H-2 and H-4, H-5,

Table 2
Chemical shifts data for PEPS1 isolated from the fruiting bodies of *P. eryngii*.

Residue		Proton or carbon[a]							
		1	2	3	4	5	6a	6b	*O*-Me
→2,6)-α-D-Gal*p* (**a**)	H	5.12	**3.96**	3.66	4.28	4.00	**3.71**	3.90	
	C	101.16	**79.65**	75.74	68.36	71.48	**69.67**		
→2,3,6)-α-D-Gal*p* (**b**)	H	5.11	**3.99**	**3.66**	4.33	4.00	3.71	3.90	
	C	101.06	**78.31**	**80.85**	68.35	71.22	**69.67**		
→6)-α-3-*O*-Me-D-Gal*p* (**c**)	H	4.98	3.85	**3.53**	4.26	3.87	**3.70**	4.01	3.44
	C	100.73	70.86	**81.63**	68.12	72.27	**69.14**		58.78
α-3-*O*-Me-D-Gal*p* (**d**)	H	4.97	3.88	**3.52**	4.31	3.87	4.00	4.08	3.44
	C	100.71	70.09	**81.56**	68.08	72.19	63.83		59.16
β-D-Man*p* (**e**)	H	4.79	4.09	3.63	3.59	3.37	3.77	3.91	
	C	104.26	72.99	75.70	69.56	78.91	63.78		
→ 4)-β-D-Man*p* (**f**)		4.77	4.07	3.83	**3.63**	3.37	3.77		
		104.24	73.05	71.48	**78.93**	78.91	63.78	3.91	

[a] Bold numbers repsent glycosylation sites.

Table 3
Interglycosidic correlations from NOESY spectrum of PEPS1 isolated from the fruiting bodies of *P. eryngii*.

Residue	Proton	Intra-correlation[a]
→2,6)-α-D-Gal*p* (a)	H-1	3.96 (**a**; H-2), 3.66 (**a**; H-3), 4.28 (**a**; H-4), **3.71** (**a**; **H-6a**)
→2,3,6)-α-D-Gal*p* (b)	H-4	3.66 (a; H-3), 4.00 (**a**; H-5)
	H-1	3.99 (**b**; H-2), **3.66** (**b**; **H-3**), 4.33 (**b**; H-4), 4.00 (**b**; H-5)
→6)-α-3-*O*-Me-D-Gal*p* (c)	H-4	3.66 (**b**; H-3), 4.00 (**b**; H-5)
	H-1	3.85 (**c**; H-2), 3.53 (**c**; H-3), 4.26 (**c**; H-4), 3.87 (**c**; H-5), **3.70** (**c**; **H-6a**)
α-3-*O*-Me-D-Gal*p* (d)	H-4	3.53 (**c**; H-3), 3.87 (**c**; H-5)
	H-1	3.88 (**d**; H-2), 3.52 (**d**; H-3), 4.31 (**d**; H-4), **3.70** (**c**; **H-6a**)
β-D-Man*p* (e)	H-4	3.52 (**d**; H-3), 3.87 (**d**; H-5)
	H-1	4.09 (**d**; H-2), **3.96** (**a**; **H-2**)
→ 4)-β-D-Man*p* (f)	H-1	4.07 (f; H-2), **3.63** (**f**; **H-4**)

[a] Inter-residue NOEs are showed in bold font.

H-6a and H-6b of TOCSY spectrum showed H-5, H-6 are located on residue **c**. The carbon chemical shifts from the C-1 to C-6 were assigned from the HMQC spectrum (Table 2). The chemical shifts of *O*-CH_3 (δ 3.44/58.78) were assigned form ^{1}H NMR, ^{13}C NMR and HMQC spectra. ^{1}H resonances for *O*-CH_3 correlated with C-3 (δ 3.44/81.63) of residue **c** in the HMBC spectrum showed that *O*-CH_3 is located on residue **c**. The H-4/H-5 coupling constant was small in the ^{1}H–^{1}H COSY spectrum (Staaf, Urbina, Weintraub, & Widmalm, 1999) and H-4 and H-3, H-5 displays strong NOE signals in the NOESY spectrum, as expected for a Gal-type residue. H-1 appears as a singlet ($J_{H\text{-}1,H\text{-}2}$ <3 Hz) in the ^{1}H NMR spectrum, and the H-1/H-2 intra-residue correlation in the NOESY spectrum indicate the residue **c** is an α-configuration. The downfield shift of C-6 carbon signal with respect to the standard values for glycopyrannoses indicated that residue **c** was identified as →6)-α-3-*O*-Me-D-Gal*p*-(1→ Residue **d** α-3-*O*-Me-D-Gal*p*-(1→. The ^{1}H resonances for H-1 to H-6 of residue **c** were assigned from the cross-peaks in the ^{1}H–^{1}H COSY, TOCSY and NOESY spectra. The carbon chemical shifts from the C-1 to C-6 were assigned from the HMQC spectrum (Table 2). The chemical shifts of *O*-CH_3 (δ 3.44/59.16) were assigned form ^{1}H NMR, ^{13}C NMR and HMQC spectra. ^{1}H resonances for *O*-CH_3 correlated with C-3 (δ 3.44/81.56) of residue **d** in the HMBC spectrum showed that *O*-CH_3 is located on residue **d**. The H-4 and H-3, H-5 displays strong NOE signals in the NOESY spectrum, as expected for a Gal-type residue. H-1 appears as a singlet ($J_{H\text{-}1,H\text{-}2}$ < 3 Hz) in the ^{1}H NMR spectrum, and the H-1/H-2 intra-residue correlation in the NOESY spectrum indicate the residue **d** is an α-configuration. The combination of these data identified residue **d** as α-3-*O*-Me-D-Gal*p*-(1→ Residue **e** β-D-Man*p* (1→. The ^{1}H resonances for H-1 to H-6 residue **e** were assigned from the cross-peaks in the ^{1}H–^{1}H COSY and TOCSY spectra. The carbon chemical shifts from the C-1 to C-6 were assigned from the HMQC spectrum (Table 2). The manno-configuration for residue **e** was supported from a coupling constant value of $J_{H\text{-}1,H\text{-}2}$ = 4.5 Hz and a large coupling constant value of $J_{H\text{-}4,H\text{-}5}$ = 9.5 Hz. A small $J_{H\text{-}1,H\text{-}2}$ values for D-mannosyl residue did not give information about the anomeric configuration (Paramonov et al., 2001). The β configuration of Man was inferred by the H-5 and C-5 chemical shifts at δ_H 3.37 and δ_C 78.91 (compare published data δ_H 3.82 and δ_C 73.34 for α-mannopyranose, δ_H 3.38 and δ_C 77.00 for β-mannopyranose, Jansson, Kenne, & Widmalm, 1989). The combination of these data identified residue **e** as β-D-Man*p*.

Residue **f** → 4)-β-D-Man*p*-(1→. The ^{1}H resonances for H-1 to H-6 residue **f** were assigned from the cross-peaks in the ^{1}H–^{1}H COSY and TOCSY spectra. The carbon chemical shifts were assigned from the HMQC spectrum (Table 2). The manno-configuration from residue **f** was supported from a coupling constant value of $J_{H\text{-}1,H\text{-}2}$ = 4.5 Hz and a large coupling constant value of $J_{H\text{-}4,H\text{-}5}$ = 9.5 Hz. A small $J_{H\text{-}1,H\text{-}2}$ values for D-mannosyl residue did not give information about the anomeric configuration (Paramonov et al., 2001). The β configuration of Man was inferred by the H-5 and C-5 chemical shifts at δ_H 3.37 and δ_C 78.91. The downfield shift of C-4 carbon signal with respect to the standard values for glycopyrannoses indicated that residue **f** was identified as →4)-β-D-Man*p*-(1→.

The sequence of glycosyl residues was determined from NOESY studies followed by confirmation with HMBC experiments. Inter-residue NOEs correlations (Table 3) were observed between H-1 of residue **a** and H-6 of residue **a**, between H-1 of residue **b** and H-3 of residue **b**, between H-1 of residue **c** and H-6a of residue **c**, between H-1 of residue **d** and H-6a of residue **c**, between H-1 of residue **e** and H-2 of residue **a**, between H-1 of residue **f** and H-4 of residue **f**. HMBC experiments (Table 4) showed clear correlations between H-1 of residue **a** and C-6 of residue **a**, between H-1 of residue **b** and C-3 of residue **b**, between H-1 of residue **c** and C-6 of residue **c**, between H-1 of residue **d** and C-6 of residue **c**, between H-1 of residue e and C-2 of residue **a**, between H-1 of residue **f** and C-2 of residue **b.**

Based on the data presented above, it demonstrated that PEPS1 consisted of a α-D-(1 → 6)-galactopyranan backbone with a β-D-Mannosyl unit on *O*-2 of the 2,6-di-*O*-substituted-D-galactosyl

Table 4
Two-and three-bond ^{1}H–^{13}C correlations for the PEPS1 isolated from the fruiting bodies of *P. eryngii*.

Residue	Proton	Proton correlation[a]
→2,6)-α-D-Gal*p* (a)	H-1	79.65 (**a**; C-2), 71.49 (**a**; C-5), **69.67 (a**; **C-6)**
→2,3,6)-α-D-Gal*p* (b)	H-1	80.78 (**b**; C-3), 71.17 (**b**; C-5)
→6)-α-3-*O*-Me-D-Gal*p* (c)	H-1	70.86 (**c**; C-2), 81.63 (**c**; C-3), **69.14 (c**; **C-6)**
	H (*O*-Me)	**81.63 (c**; **C-3)**
α-3-*O*-Me-D-Gal*p* (d)	H-1	81.56 (**d**; C-3), 72.19 (**d**; C-5), **69.14 (c**; **C-6)**
	H (*O*-Me)	**81.56 (d**; **C-3)**
β-D-Man*p* (e)	H-1	72.95 (**d**; C-2), **79.65 (a**; **C-2)**
→ 4)-β-D-Man*p* (f)	H-1	73.05 (**f**; C-2), **78.31 (b**; **C-2)**

[a] Inter-residue correlations are shown in bold font.

units, α-(1 → 6)-3-*O*-Me-D-galactopyranan backbone with a terminal α-D-3-*O*-Me-D-galactosyl unit and it also contained a minor 2,3,6-α-D-Galatopyranan units and 4-β-D-Mannopyranan residues.

4. Conclusion

The polysaccharide fractions of *P. eryngii* have been widely investigated and reported, but mainly focused on their immunostimulating and antitumor activities while the structural elucidation has not been previously reported. In the present study, a novel water-soluble heteropolysaccharide was isolated from the fruiting bodies of *P. eryngii* with hot water and then further purified by DEAE Sepharose Fast Flow and Sephacryl S-300 and S-100 High-Resolution chromatogram, which has a molecular weight of 1.88×10^4 Da. Compositional analysis, methylation analysis, together with NMR spectroscopy established that PEPS1 consists of a α-D-(1 → 6)-galactopyranan backbone with a β-D-Mannosyl unit on *O*-2 of the 2,6-di-*O*-substituted-D-galactosyl units, α-(1 → 6)-3-*O*-Me-D-galactopyranan backbone with a terminal α-D-3-*O*-Me-D-galactosyl unit and it also contained a minor 2,3,6-α-D-Galatopyranan unit and 4-β-D-Mannopyranan residues, PEPS1 is therefore a novel fungal polysaccharide.

Acknowledgment

The study was supported by Zhejiang major science and technology projects and special priority themes (No. 2009C13029).

References

Albersheim, P., Nevins, D. J., English, P. D., & Karr, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharide by gasliquid chromatography. *Carbohydrate Research*, *5*, 340–345.

Anumula, K. R., & Taylor, P. B. (1992). A comprehensive procedure for preparation of partially methylated alditol acetates from glycoprotein carbohydrates. *Analytical Biochemistry*, *203*, 101–108.

Bobek, P., & Galbavy, S. (2001). Effect of pleuran (β-glucan from Pleurotus ostreatus) on the antioxidant status of the organism and on dimethylhydrazine-induced precancerous lesions in rat colon. *British Journal of Biomedical Sciences*, *58*, 164–168.

Fukuda, K., & Hamada, A. (1978). 3-O-methyl galactose found in polysaccharides of *Lampteromyces japonicus*. *Biochimica et Biophysica Acta*, *544*, 445–447.

Gutiérrez, A., Prieto, A., & Martínez, A. T. (1996). Structural characterization of extracellular polysaccharides produced by fungi from the genus *Pleurotus*. *Carbohydrate Research*, *281*, 143–154.

Jansson, P. E., Kenne, L., & Widmalm, G. (1989). Computer-assisted structural analysis of polysaccharides with an extended version of CASPER using ^{1}H-and ^{13}C-n.m.r. *data. Carbohydrate Research*, *188*, 169–191.

Jeong, Y. T., Jeong, S. C., Gu, Y. A., Islam, R., & Song, C. H. (2010). Antitumor and immunomodulating activities of endo-biopolymers obtained from a submerged culture of *Pleurotus eryngii*. *Food Science and Biotechnology*, *19*(2), 399–404.

Kalyoncu, F., Oskay, M., Saglam, H., Erdogan, T. F., & Tamer, A. U. (2010). Antimicrobial and antioxidant activities of Mycelia of 10 wild mushroom species. *Journal of Medicinal Food*, *13*(2), 415–419.

Karacsonyi, S., & Kuniak, L. (1994). Polysaccharides of *Pleurotus ostreatus*: Isolation and structure of pleuran an alkali-insoluble β-D-glucan. *Carbohydrate Polymers*, *24*, 107–111.

Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, *73*, 321–325.

Mizutani, T., Inatomi, S., Inazu, A., & Kawahara, E. (2010). Hypolipidemic effect of *Pleurotus eryngii* extract in fat-loaded mice. *Journal of Nutritional Science and Vitaminology*, *56*(1), 48–53.

Ng, T. B. (1998). A review of research on the protein-bound polysaccharide (polysaccharopeptide PSP) from the mushroom *Coriolus versicolor* (Basidiomycetes: Polyporaceae). *General Pharmacology*, *30*, 1–4.

Paramonov, N., Bailey, D., Rangarajan, M., Hashim, A., Kelly, G., Curtis, M. A., et al. (2001). Structure analysis of the polysaccharide from the lipopolysaccharide of *Porphyromonas gingivalis* strain W50. *European Journal of Biochemistry*, *268*, 4698–4707.

Royse, D. J. (1995). Specialty mushrooms. *Mushroom News*, *43*, 4–21.

Smiderle, F. R., Olsen, L. M., Carbonero, E. R., Baggio, C. H., Freitas, C. S., Marcon, R., et al. (2008). Anti-inflammatory and analgesic properties in a rodent model of a (1 → 3),(1 → 6)-linked β-glucan isolated from *Pleurotus pulmonarius*. *European Journal of Pharmacology*, *597*, 86–91.

Srivastava, R., & Kulshreshth, D. K. (1989). Bioactive polysaccharides from plants. *Phytochemistry*, *28*, 2877–2883.

Staaf, M., Urbina, F., Weintraub, A., & Widmalml, G. (1999). Structural elucidation of the O-antigenic polysaccharides from the enteroaggregative *Escherichia coli* strain 62D1. *European Journal of Biochemistry*, *262*, 56–62.

Stepan, H., Bleckmann, C., Geyer, H., Geyer, R., & Staudacher, E. (2010). Determination of 3-*O*- and 4-*O*-methylated monosaccharide constituents in snail glycans. *Carbohydrate Research*, *345*(10), 1504–1507.

Watt, D. K., O'Neill, S. A., Percy, A. E., & Brasch, D. J. (2002). Isolation and characterisation of a partially methylated galacto-glucurono-xylo-glycan a unique polysaccharide from the red seaweed *Apophloea lyallii*. *Carbohydrate Polymers*, *50*, 283–294.

Yang, Y., Zhang, J. S., Liu, Y. F., Tang, Q. J., Zhao, Z. G., & Xia, W. S. (2007). Structural elucidation of a 3-O-methyl-D-galactose-containing neutral polysaccharide from the fruiting bodies of *Phellinus igniarius*. *Carbohydrate Research*, *342*, 1063–1070.

Yildiz, S., Yildiz, U. C., Gezer, E. D., & Temiz, A. (2002). Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom. *Process Biochemistry*, *38*, 301–306.

Zhang, W. J. (1999). *Biochemical techniques in complex carbohydrates* (2nd ed.). Hangzhou: Zhejiang University Press.