

PREPARATION AND STRUCTURE OF FIVE DERIVATIVES OF β -(1 $\rightarrow$ 3)-D-GLUCAN ISOLATED FROM *PORIA COCOS* SCLEROTIUM*

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Abstract A new solvent of cellulose (1.5 mol/L NaOH/0.5 mol/L urea aqueous solution) was used as one of the homogeneous reaction media of polysaccharides for methylation, hydroxyethylation and hydroxypropylation. A water insoluble β -(1 $\rightarrow$ 3)-D-glucan, sample PCS3-II, isolated from fresh sclerotium of *Poria cocos* was sulfated in dimethyl sulfoxide (Me₂SO), carboxymethylated in NaOH, isopropanol solution, as well as methylated, hydroxyethylated and hydroxypropylated in the new solvent system, respectively, to obtain five water-soluble derivatives coded as S-PCS3-II, C-PCS3-II, M-PCS3-II, HE-PCS3-II and HP-PCS3-II. Their chemical structure and distribution of substitution were characterized by infrared spectroscopy (IR), elementary analysis (EA), ¹H-NMR, ¹³C-NMR, 2D-COSY, 2D-TOCSY and 2D-¹H-detected ¹H ¹³C HMQC spectra. The results reveal that the relative reactivity of hydroxyl groups of the β -(1 $\rightarrow$ 3)-D-glucan is in the order C-6 > C-4 > C-2 on the whole. The substitution of the samples S-PCS3-II, C-PCS3-II and M-PCS3-II occurred mainly at C-6 position and secondly at C-4 and C-2 positions, and that of HE-PCS3-II occurred at C-6 and C-4 positions and of HP-PCS3-II almost completely occurred at C-6 position. The degrees of substitution (DS) obtained from ¹³C-NMR range from 0.23 to 1.27. The water solubility of the derivatives is in the order S-PCS3-II > C-PCS3-II > M-PCS3-II > HE-PCS3-II > HP-PCS3-II. This work provides a novel and nonpolluting process for the methylation, hydroxyethylation and hydroxypropylation of β -(1 $\rightarrow$ 3)-D-glucan.

Keywords Chemical structure, Sulfation, Carboxymethylation, Methylation, Hydroxyethylation, Hydroxypropylation, β -D-glucan, *Poria cocos* sclerotium, NMR

INTRODUCTION

Recently, more attention has been directed towards the modified polysaccharides, such as sulfated and carboxylmethylated derivatives, which can obviously improve antitumor and antiviral actions^[1–5]. The induction of suitable ionic groups with appropriate degrees of substitution can not only enhance the water solubility of the polysaccharides, but also change the polymer chain to adopt a certain conformation in aqueous solutions^[6]. Some glucan derivatives such as dextran sulfate, *Lentinan* sulfate and curdlan sulfate exhibit high anti-HIV activities or low anti-coagulant activities^[7, 8]. A carboxymethylation derivative of a water insoluble α -(1 $\rightarrow$ 3)-D-glucan from *Ganoderma lucidum* has shown better water solubility and potent antitumor activity against Ehrlich ascites carcinoma (EAC) in mice^[9]. Moreover, hydroxyethylation, hydroxypropylation and methylation of the polysaccharides also can improve the water-solubility and bioactivity^[10, 11]. The effects of groups, positions and degrees of substitution on the antitumor activity and bioactivity of polysaccharides have been reported^[10, 12]. Thus, structure analysis of modified polysaccharides, including the degree of substitution (DS), the substitution position and non-selective or selective substitution, may provide a basic understanding of the correlation of

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position and non-selective or selective substitution, may provide a basic understanding of the correlation of structure to bioactivities. NMR is an effective tool in structure research on polysaccharide and polysaccharide derivatives^[13–17]. It has been reported that two-dimensional NMR spectroscopy (2D-NMR) experiments could provide complete assignment of high-resolution 1D spectra of polysaccharides^[18,19], and the technique has also been extensively used as a tool for carbohydrate analysis^[20–22].

Poria cocos has been long used in China and Japan as a traditional medicine^[23–26]. The main component of sclerotium of *Poria cocos* is a water-insoluble β -(1 $\rightarrow$ 3)-*D*-glucan, which hardly exhibits antitumor activity^[27–29]. In our previous work^[30], six polysaccharides have been isolated from sclerotium of *Poria cocos*, and the main component is a water insoluble polysaccharide (PCS3-II) extracted with a 0.5 mol/L NaOH aqueous solution. It has been demonstrated to be a linear β -(1 $\rightarrow$ 3)-*D*-glucan of high purity. In this work, we attempted to synthesize five water-soluble derivatives from PCS3-II in order to improve its water solubility and antitumor activity. The synthesis of methylated, hydroxyethylated and hydroxypropylated derivatives was performed in 1.5 mol/L NaOH/0.5 mol/L urea aqueous solution, a new solvent of cellulose in our laboratory^[31]. It is believed that this is a novel and nonpolluting process for the chemical modification of polysaccharides. The structure of the modified polysaccharides was determined by infrared spectroscopy (IR), elemental analysis (EA), ¹H-NMR, ¹³C-NMR, 2D-COSY, 2D-TOCSY and 2D-¹H-detected ¹H ¹³C HMQC NMR experiments. This is the first integrated report on the chemical modifications of β -*D*-glucan isolated from *Poria cocos* sclerotium.

EXPERIMENTAL

Materials

A linear β -(1 $\rightarrow$ 3)-*D*-glucan PCS3-II was extracted with 0.5 mol/L NaOH aqueous solutions from fresh sclerotium of *Poria cocos* cultivated in Luotian (Hubei, China), and used as native sample in this work. All the chemical reagents used were analytical grade, and were obtained from commercial resources in China. Dimethyl sulfoxide (Me₂SO) and pyridine were dehydrated with molecular sieves before use. The other reagents were used without further purification.

Preparation of Sulfated Derivative

600 mg PCS3-II dissolved in 50 mL Me₂SO was kept at room temperature overnight with stirring, and then 9 mL pyridine was slowly added and stirred for 30 min. Then 3.7 mL chlorosulfonic acid (for every two moles of pyridine, one mole of chlorosulfonic acid was added) was added dropwise to the solution in an ice bath with stirring. The resulting solution was heated to 80°C and the reaction was continued for 2 h. After the solution was cooled to room temperature, 5% NaOH solution was added to adjust the pH to 7. The solution was dialyzed in slightly alkaline water (pH = 9) to remove pyridine. Finally, the sulfated polysaccharide was subjected to dialysis against distilled water and lyophilized using lyophilizer (CHRIST Alpha1-2, Germany) to obtain the sample coded as S-PC3-II.

Preparation of Carboxymethylated Derivative

600 mg PCS3-II was suspended in a mixture of 10 mL 20% NaOH and 25 mL isopropanol in an ice bath with stirring for 3 h. Then, a mixture of 5.25 g chloroacetic acid, 10 mL 20% NaOH and 25 mL isopropanol was slowly added with stirring. The reaction was continued at room temperature for 3 h and then at 60°C for 1.5 h. After the solution was cooled to room temperature, 0.5 mol/L HCl was added to adjust the pH to 7. The carboxymethylated derivative was purified according to the above method and lyophilized to obtain the sample coded as C-PC3-II.

Preparation of Methylated, Hydroxyethylated and Hydroxypropylated Derivatives

600 mg PCS3-II was dissolved in 50 mL 1.5 mol/L NaOH/0.5 mol/L urea aqueous solution in an ice bath with stirring for 3 h. Then the corresponding derivatization reactant (3.5 g dimethyl sulfate, 8.0 mL 2-chloroethanol or 6.0 mL 1,2-epoxypropane) was slowly added to the solution with stirring. The solution was stirred at room temperature for 3 h and then at 60°C for 2 h to obtain, respectively, methylated, hydroxyethylated and

hydroxypropylated derivatives, and coded as M-PCS3-II, HE-PCS3-II and HP-PCS3-II. These samples were purified and lyophilized according to the above method and process.

Characterization

Infrared spectra of the samples were recorded with a Nicolet 170 SX FT-IR (Spectrum One, Perkin Elmer Co., USA) spectrometer in the range $4000\text{--}400\text{ cm}^{-1}$ using the KBr-disk method. The elemental compositions C, H, O and S of the products were determined by using an elemental analyzer (CHN-O-RAPID Heraeus Co., Germany).

All the samples were deuterium exchanged with D_2O three times, then freeze-dried. High-resolution ^{13}C -NMR spectra of the samples S-PCS3-II, HP-PCS3-II and HE-PCS3-II were recorded on a JEOL Lambda 400 MHz spectrometer equipped with a DEC AXP 300 computer workstation at 60°C , using internal acetone as reference ($\delta_{\text{H}} = 2.225$, $\delta_{\text{C}} = 31.45$). The polymer concentration was adjusted to 5 wt% in all experiments. D_2O was used as solvent for S-PCS3-II, and dimethyl sulfoxide- d_6 ($\text{Me}_2\text{SO}-d_6$) for HE-PCS3-II and HP-PCS3-II. The parameters used for 2D-NMR experiments were as follows: COSY (256×512 data matrix; zero-filled to 512 data points in t_1 ; 16 scans per t_1 value; spectral width 1908.0 Hz; recycle delay 0.7317 s; unshifted sine-bell filtering in t_1 and t_2); In the TOCSY experiment the duration of the spin-lock was 80 ms; HMQC (256×512 data matrix; zero-filled to 512 data points in t_1 ; 64 scans per t_1 value; spectral width in t_1 1749.2 Hz and in t_2 9643.2 Hz; delay for coupling constants 145 Hz; recycle delay 1.0 s; shifted sine-squared filtering in t_1 and t_2); HMBC (512×1024 data matrix; zero-filled to 1024 data points in t_1 ; 56 scans per t_1 value; spectral width in t_1 1873.0 Hz and in t_2 16977.9 Hz; delay for coupling constants 145 Hz; recycle delay 1.5 s; shifted sine-squared filtering in t_1 and t_2). The 1D and 2D-NMR experiments mentioned above was performed in the laboratory of Dr. Savage (Department of Chemistry, National University of Ireland, Galway). High-resolution ^{13}C -NMR spectra of the samples C-PCS3-II and M-PCS3-II were recorded on a 600 MHz INOVA-600 NMR spectrometer (Varian Inc., USA) equipped with a ULTRA60 SUN computer workstation at 25°C , using internal acetone as reference ($\delta_{\text{C}} = 31.45$). The polymer concentration was adjusted to 5 wt%, and D_2O was used as solvent for the samples.

RESULTS AND DISCUSSION

The degrees of substitution (DS) of the derivatives were calculated from element content ($W\%$), total atomic weight of the element calculated from the sugar unit (M_{A}), total atomic weight of the element calculated from the substitution group (W_{A}), and molecular weight of a substitution group (M_0) by the following formula:

$$DS = (162 \times W\% - M_{\text{A}}) / (W_{\text{A}} - M_0 \times W\%)$$

The yield and DS of five derivatives calculated from EA and ^{13}C -NMR are summarized in Table 1. All the derivatives are white powder and the yield of each derivative is high. Values of DS obtained from EA and ^{13}C -NMR range from 0.33 to 1.34 and from 0.23 to 1.27, respectively, indicating a relatively low DS of M-PCS3-II, HE-PCS3-II and HP-PCS3-II obtained from PCS3-II in 1.5 mol/L NaOH/0.5 mol/L urea aqueous solution. We attempted to synthesize the methylated, hydroxyethylated and hydroxypropylated products of PCS3-II in organic media such as dimethyl sulfoxide (Me_2SO), but the resulting samples are water insoluble and their DS values are too low. So 1.5 mol/L NaOH/0.5 mol/L urea aqueous solution as a novel and nonpolluting solvent was used as the homogeneous reaction media of water-insoluble polysaccharides for their derivation.

Table 1. The yield, solubility and degree of substitution (DS) of native sample PCS3-II and its derivatives

Sample	Yield (g)	Solubility ^a		DS from EA results	DS from ^{13}C -NMR			
		Water	Me_2SO		C-6	C-4	C-2	Total DS
PCS3-II		-	+					
S-PCS3-II	0.924	++	-	1.21	0.44	0.24	0.34	1.02
C-PCS3-II	0.967	++	-	1.34	0.71	0.35	0.21	1.27
M-PCS3-II	0.587	++	+	0.82	0.33	0.21	0.15	0.69
HE-PCS3-II	0.645	+	+	0.60	0.25	0.24	*	0.49
HP-PCS3-II	0.622	+	+	0.33	0.23	*	*	0.23

^a Determined by naked eye;

++ Good solubility; +: soluble; -: insoluble; * No substitution

The solubility of the derivatives in water and Me_2SO is also listed in Table 1. The results were determined by the naked eye. It is noted that all the derivatives exhibit better water solubility than native PCS3-II. This is because the introduction of suitable ionic groups greatly enhances the water solubility of polysaccharides. Furthermore, S-PCS3-II and C-PCS3-II show better water solubility than HE-PCS3-II and HP-PCS3-II. This indicates that with the increase of the carbon number of hydrophobic alkyl groups of the derivatives, the substitution reactions and the DS decrease, leading to the decrease of water solubility. In view of the results, the water solubility of the derivatives is in the order S-PCS3-II > C-PCS3-II > M-PCS3-II > HE-PCS3-II > HP-PCS3-II, which is similar to the order of their DS.

Figure 1 shows the FT-IR spectra of the samples PCS3-II and its derivatives. All samples exhibit the characteristic IR absorption of polysaccharides at 1250 and 1650 cm^{-1} ^[32]. Compared to PCS3-II, the characteristic peak of β -glucan at 890 cm^{-1} for S-PCS3-II is weakened, and two new absorption bands at 815 and 1210 cm^{-1} for the sulfated derivative appear due to the presence of the groups $\text{C}-\text{S}-\text{O}$ and $\text{S}=\text{O}$ ^[5, 10], respectively, indicating that sulfated derivative has been synthesized successfully. In the IR spectrum of C-PCS3-II, the characteristic peak of β -glucan at 890 cm^{-1} is also weakened. Moreover, two new absorption bands at 1600 and 1340 cm^{-1} appear due to the existence of the $-\text{COO}$ group and the $-\text{CH}_2-$ group of the carboxymethylated derivative^[9]. The peaks at 1380 and 1450 cm^{-1} in the IR spectrum of M-PCS3-II indicate the existence of $-\text{CH}_3$ groups. Compared with PCS3-II, the relatively low intensity of the $-\text{OH}$ vibration band at 3400 cm^{-1} for M-PCS3-II and the relatively high intensity of the $-\text{CH}_3$ or the $-\text{CH}_2-$ vibration bands at 2920 cm^{-1} and the $\text{C}-\text{O}-\text{C}$ vibration band at 1070 cm^{-1} suggest that methylation of the sample PCS3-II has occurred. Similar results such as the relatively high intensity of a $\text{C}-\text{O}-\text{C}$ band at 1070 cm^{-1} in the IR spectra of HE-PCS3-II and HP-PCS3-II indicate the existence of $\text{C}-\text{O}-\text{C}$ groups^[10].

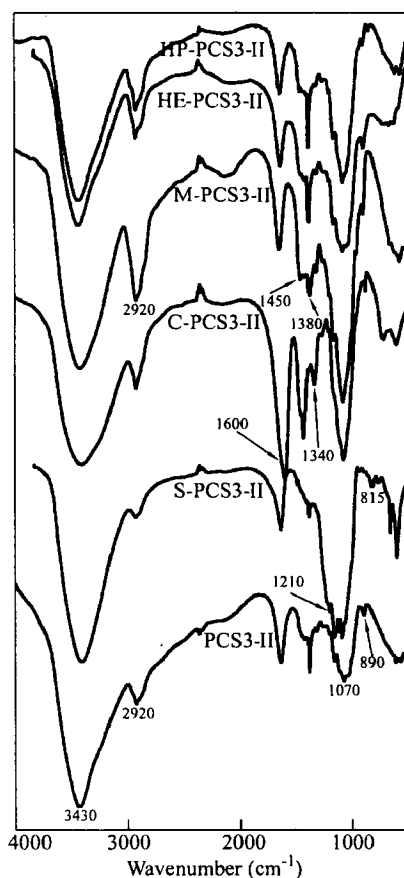
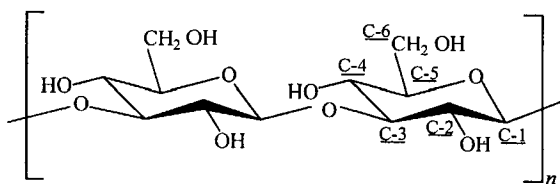


Fig. 1 FT-IR spectra of PCS3-II and its derivatives

Scheme 1 Chemical structure of linear β -(1 $\rightarrow$ 3)-*D*-glucanTable 2. ^{13}C -NMR chemical shifts for native sample PCS3-II and its derivatives in solution

Sample	δ											
	C1	C1'	C2	C2s	C3	C3'	C4	C4s	C5	C5'	C6	C6s
PCS3-II ^a	102.9		72.7		86.2		68.3		76.3		60.8	
C-PCS3-II	103.7		74.4	82.3	85.3	84.9	69.4	74.9	76.8	75.5	61.9	71.0
M-PCS3-II	103.8		74.4	80.2	85.5	83.8	69.5	75.7	76.5	74.9	61.9	72.3
HE-PCS3-II	102.8		72.7		86.1	83.4	68.3	76.4	76.2	74.5	60.7	70.0
HP-PCS3-II	102.8		72.6		86.1		68.2		76.2	74.5	60.7	70.2
S-PCS3-II	103.6	102.2	74.3	80.1	85.4		69.3	75.0	76.8	74.6	61.9	68.3

^a Data obtained from previous work^[30]

Figures 2 and 3 show the ^{13}C -NMR spectra of PCS3-II and the five derivatives in solution. The comparisons of ^{13}C -NMR chemical shifts for the samples in solution are summarized in Table 2. The ^{13}C -NMR spectrum of PCS3-II shows only six strong chemical shifts at $\delta = 102.9$, 86.2, 76.3, 72.7, 68.3 and 60.8 assigned as C-1, C-3, C-5, C-2, C-4 and C-6, respectively, indicating a linear β -(1 $\rightarrow$ 3)-*D*-glucan with high purity^[30]. The structure of linear β -(1 $\rightarrow$ 3)-*D*-glucan is shown in Scheme 1. The main signals in the ^{13}C -NMR spectrum of C-PCS3-II are assigned to β -(1 $\rightarrow$ 3)-*D*-glucan at $\delta = 103.7$ (C-1), $\delta = 85.3$ (C-3), $\delta = 76.8$ (C-5), 74.4 (C-2), $\delta = 69.4$ (C-4) and $\delta = 61.9$ (C-6). The peak of C-6 shifts from $\delta = 61.9$ to 71.0 (C-6s) and C-5 from $\delta = 76.8$ to 75.5 (C-5'), indicating that the carboxymethylation of PCS3-II has occurred at C-6 position, and the DS is relatively high. The peak of C-4 partially shifts from $\delta = 69.4$ to 74.9, and C-2 partially shifts from $\delta = 74.4$ to 82.3, indicating the substitution has also occurred at C-4 and C-2 position. A series of peaks at $\delta = 82.4$ –85.8 are due to the combination substitution effect of C-2 and C-4 to C-3, and C-4 and C-6 to C-3^[33]. The new chemical shifts at $\delta = 179.1$ can be attributed to the C=O carbon of carboxymethyl group. These results indicate that the ease of carboxymethylation is in the order C-6 > C-4 > C-2.

For the ^{13}C -NMR spectrum of M-PCS3-II, there are some peaks at $\delta = 59.8$ –61.9, which are assigned to the O–CH₃ group. The peak area (about 33%) of C-6s at $\delta = 72.3$ indicates that the methylation mainly has occurred at C-6 position, which can be further proved by the C-5 shift from $\delta = 76.5$ to 74.9. The new peaks at $\delta = 80.2$ for C-2s and $\delta = 75.7$ for C-4s prove the substitution at C-4 and C-2 positions. Many peaks in the ^{13}C -NMR spectrum of M-PCS3-II can be attributed to the effect of the multi-substitution at C-2, C-4 and C-6. The relative reactivity of hydroxyl group of the β -glucan in 1.5 mol/L NaOH/0.5 mol/L urea aqueous solutions is in the order C-6 > C-4 > C-2.

Figure 4 shows the 2D-COSY and 2D- ^1H -detected ^1H ^{13}C HMQC spectra of HE-PCS3-II. The ^{13}C -NMR spectrum of HE-PCS3-II in Fig. 3 contains 20 signals. In addition to the main signals of β -(1 $\rightarrow$ 3)-*D*-glucan, a DEPT experiment shows the signals at $\delta = 73.3$, 72.4, 70.0, 60.5 and 60.1 can be assigned to methylene groups. The 2D- ^1H -detected ^1H ^{13}C HMQC spectra show the correlation of methylene protons at $\delta = 3.70/3.45$ to carbon at $\delta = 60.7$, and protons at $\delta = 3.73/3.48$ to carbon at $\delta = 70.0$, which are assigned to the C-6 and C-6s of β -(1 $\rightarrow$ 3)-*D*-glucan. The protons at $\delta = 3.73/3.48$ to carbon at $\delta = 60.1$ are assigned to substitution ethoxyl group. The similar signals, assigned to ethoxyl group, can be found at $\delta = 72.4$ and 73.3 for the carbon bearing two hydrogens at $\delta = 3.73/3.48$ and $\delta = 3.88/3.50$, indicating successful hydroxyethylation of PCS3-II. The peaks of C-6 have shifted from $\delta = 60.7$ to 70.0 (C-6s) and of C-5 from $\delta = 76.2$ to 74.5 (C-5'), suggesting that hydroxyethylation of PCS3-II happens at C-6 position. The shift of peak at C-4 from $\delta = 68.3$ to 76.4 indicate

that the substitution also has occurred at C-4 position. The C-2 position is not substituted because of the absence of substitution effect on the C-1 peak^[34]. Therefore, the possible structure of the substitution group is sugar $-\text{O}-\text{CH}_2-\text{CH}_2-\text{OH}$ or sugar $-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_2-\text{OH}$. In view of the relatively small areas of the substitution peaks of C-6s and C-4s, the DS of HE-PCS3-II is not high.

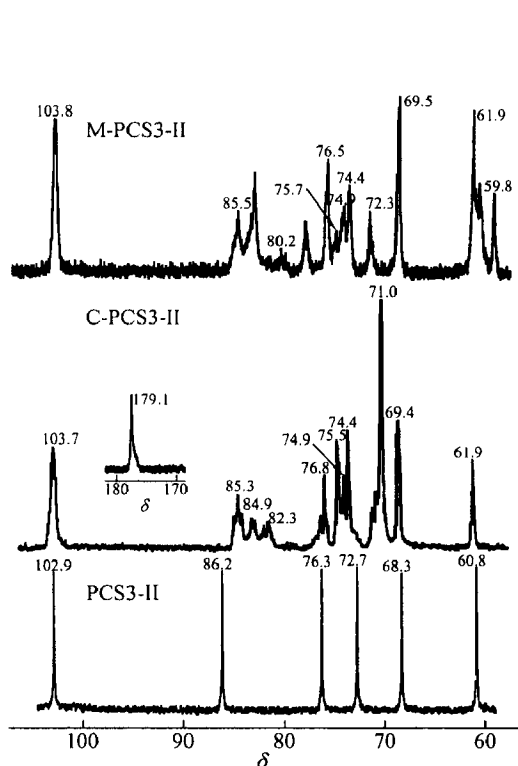


Fig. 2 ^{13}C -NMR spectra of PCS3-II in $\text{Me}_2\text{SO}-d_6$ at 60°C , C-PCS3-II in D_2O at 25°C and M-PCS3-II in D_2O at 25°C

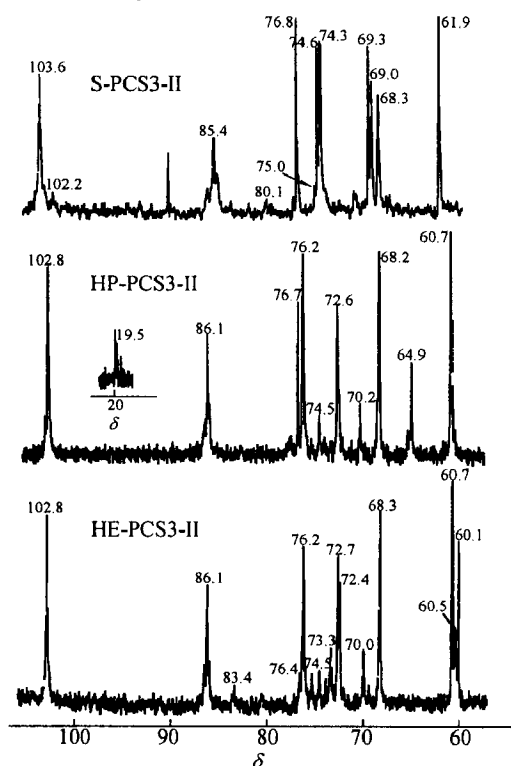


Fig. 3 ^{13}C -NMR spectra of HE-PCS3-II in $\text{Me}_2\text{SO}-d_6$ at 60°C , HP-PCS3-II in $\text{Me}_2\text{SO}-d_6$ at 60°C and S-PCS3-II in D_2O at 40°C

Figure 5 shows the 2D- ^1H -detected ^1H ^{13}C HMQC spectra of HP-PCS3-II. The ^{13}C -NMR spectrum of HP-PCS3-II in Fig. 3 contains 14 signals. Except for the main signals of β -(1 $\rightarrow$ 3)-*D*-glucan, the chemical shifts at $\delta = 70.2$ and 74.5 are assigned as C-6s and C-5', respectively, indicating substitution at C-6 position. The results from 2D- ^1H -detected ^1H ^{13}C HMQC experiments show the correlation of methylene protons at $\delta = 3.26$ to carbon at $\delta = 76.7$, protons at $\delta = 1.05$ to carbon at $\delta = 19.5$ and protons at $\delta = 3.73$ to carbon at $\delta = 64.9$, and all the signals are assigned to the substitution group of hydroxypropyl. Obviously, C-2 and C-4 positions are not substituted because of the absence of substitution effect C-1' and C-4s peaks. Moreover, the DS of C-6 is not high (23%) because of the small peak area of C-6s at $\delta = 70.2$ and $-\text{CH}_2-$ at $\delta = 19.5$. Therefore, the substitution of HP-PC3-II has entirely occurred at C-6 position.

Figure 6 shows the 2D-COSY and 2D- ^1H -detected ^1H ^{13}C HMQC spectra of S-PCS3-II. 44% of $-\text{OH}$ groups at C-6 position has been substituted, according to the peak areas of C-6 at $\delta = 61.9$ and C-6s at $\delta = 68.3$. The COSY spectrum shows the correlation of H-6' (substituted C-6) at $\delta = 4.53$ and 4.23 to H-5' (substitution effect) at $\delta = 3.72$. The shifts at $\delta = 4.80, 3.58, 3.75, 3.52, 3.53, 3.92$ and 3.72 are assigned as H-1, H-3, H-5, H-2, H-4 and H-6, respectively. The results from HMQC experiments show the correlation of H-5' at $\delta = 3.72$ to C-5' at $\delta = 74.6$. The chemical shift at $\delta = 69.0$ is assigned to C-4' due to the substitution effect of C-6s. The substitution of S-PC3-II has also occurred on C-4 and C-2 positions, because the chemical shift of C-4 moves from $\delta = 69.3$ to 75.0 , C-1 from $\delta = 103.6$ to 102.2 and C-2 from $\delta = 74.3$ to 80.1 . There is little difference in the distribution of substitution of S-PC3-II from other derivatives, indicating that the relative reactivity of sulfation

for the β -glucan is in the order C-6 > C-4 and C-2.

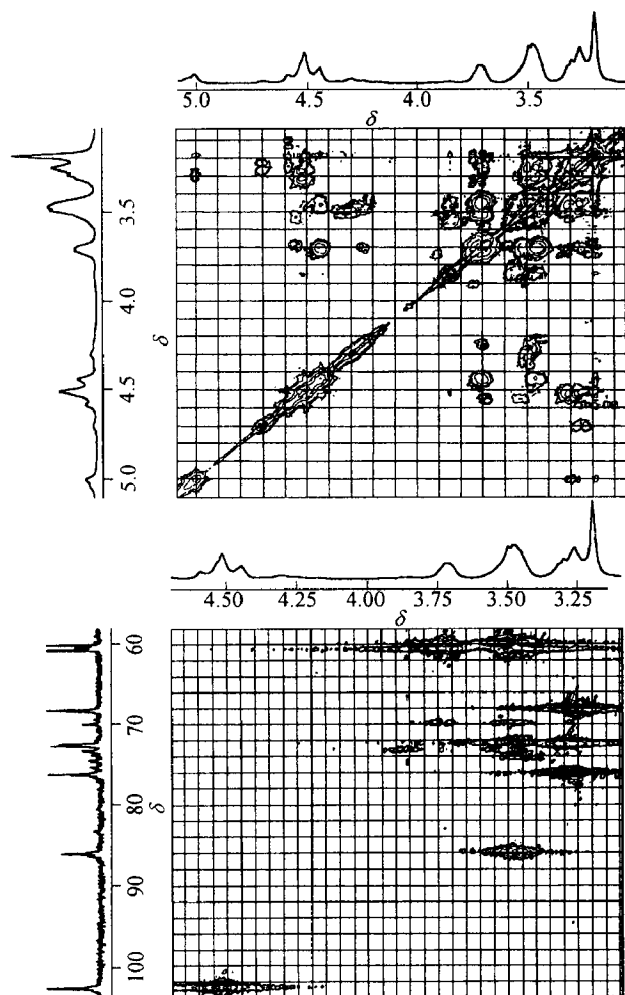


Fig. 4 2D-COSY spectra (top) and part of a ^1H -detected ^1H ^{13}C HMQC spectrum (bottom) of HE-PCS3-II at 60°C. The corresponding parts of the ^{13}C and ^1H -NMR spectra are displayed along the vertical and horizontal axes, respectively.

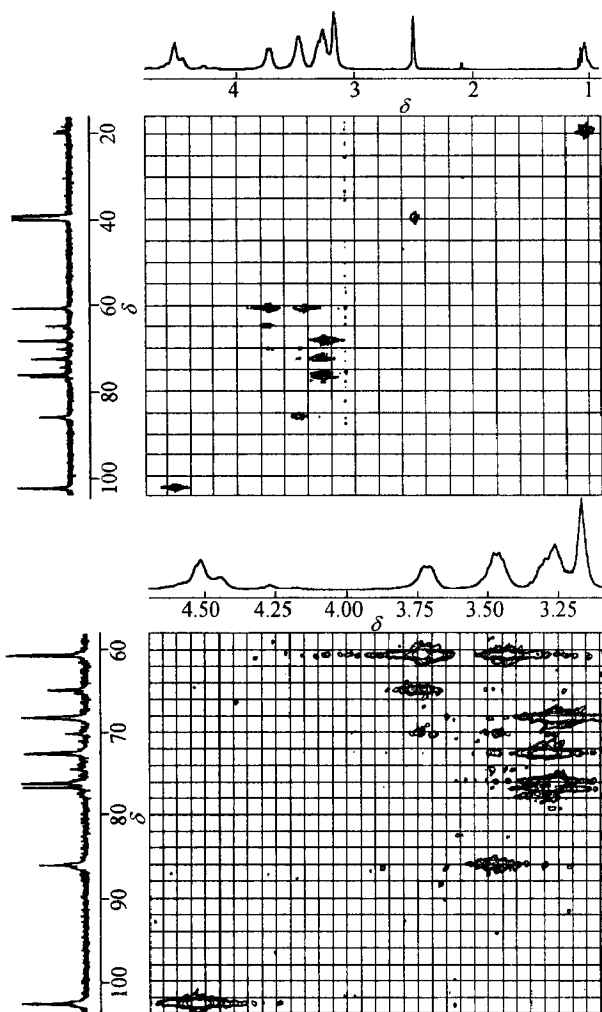


Fig. 5 Part of a ^1H -detected ^1H ^{13}C HMQC spectrum of HP-PCS3-II at 60°C.

The corresponding parts of the ^{13}C and ^1H -NMR spectra are displayed along the vertical and horizontal axes, respectively.

CONCLUSIONS

Two derivatives were satisfactorily synthesized from water insoluble β -(1 $\rightarrow$ 3)-*D*-glucan isolated from fresh sclerotium of *Poria cocos* by sulfation in dimethyl sulfoxide (Me_2SO) at 80°C for 2 h, and carboxymethylation in NaOH, isopropanol solution at room temperature for 3 h and then at 60°C for 1.5 h. Three derivatives were successfully synthesized from the β -*D*-glucan in 1.5 mol/L NaOH/0.5 mol/L urea aqueous solvent system as the homogeneous reaction media by using methylation, hydroxyethylation and hydroxypropylation at room temperature for 3 h and then at 60°C for 2 h. The derivatives all exhibit better water solubility than the native sample PCS3-II. The water solubility of the derivatives is in the order S-PCS3-II > C-PCS3-II > M-PCS3-II > HE-PCS3-II > HP-PCS3-II. Moreover, the relative reactivity of hydroxyl groups of β -(1 $\rightarrow$ 3)-*D*-glucan is in the order C-6 > C-4 > C-2 on the whole. The substitution of S-PCS3-II, C-PCS3-II and M-PCS3-II mainly occurred at C-6 position and secondly at C-4 and C-2 positions. The substitution of HE-PCS3-II occurred mainly at C-6 and secondly at C-4 position, and that of HP-PCS3-II was almost fully at C-6 position. All of them are non-

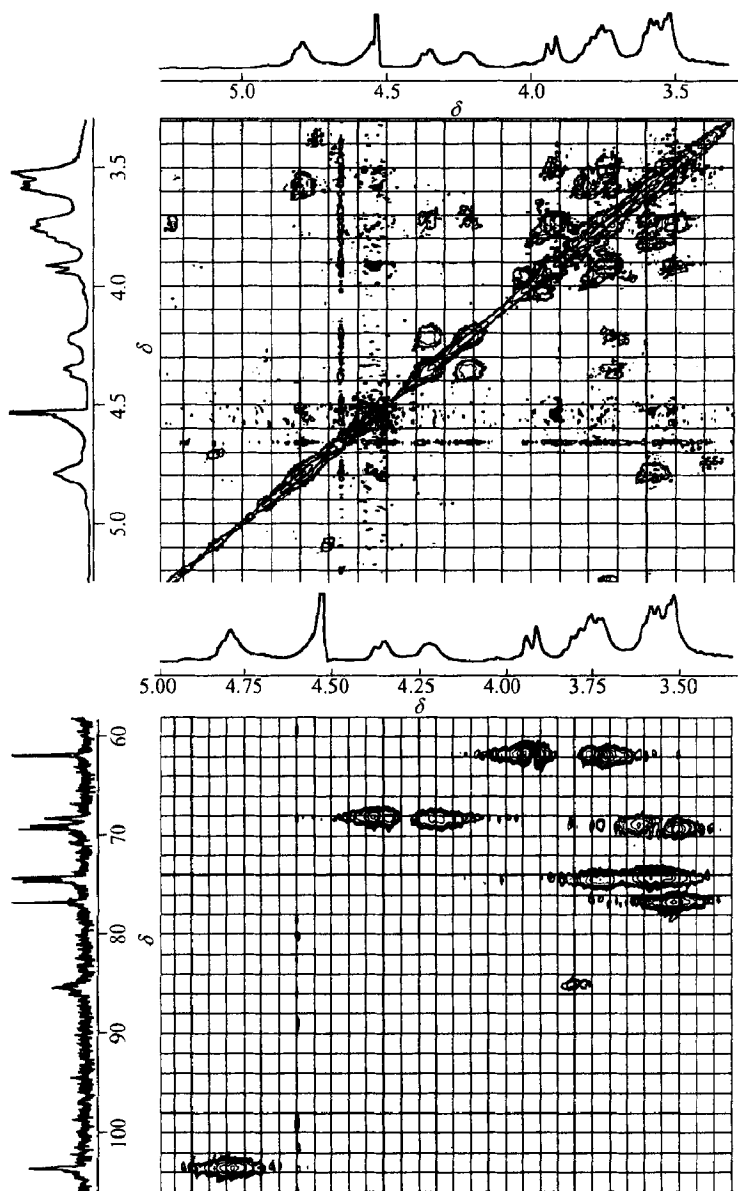


Fig. 6 2D-COSY spectrum (top) and part of a ^1H -detected ^1H ^{13}C HMQC spectrum (bottom) of S-PCS3-II at 50°C

The corresponding parts of the ^{13}C and ^1H -NMR spectra are displayed along the vertical and horizontal axes, respectively.

selective substitutions. The DS and ease of substitution of the five derivatives are in the order C-PCS3-II > S-PCS3-II > M-PCS3-II > HE-PCS3-II > HP-PCS3-II. It implies that the substitution reactivity and DS of the β -(1 $\rightarrow$ 3)-*D*-glucan decrease with an increase of the carbon number in hydrophobic alkyl groups of the derivatives, which leads to the decrease of water solubility.

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