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Preparation and antiherpetic activities of chemically modified polysaccharides from *Polygonatum cyrtonema* Hua

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ABSTRACT

The chemically modified polysaccharides, including sulfated, phosphorylated, carboxymethylated, acetylated and sulfonylated derivatives, were prepared from a neutral polysaccharide (PD) extracted from *Polygonatum cyrtonema* Hua. These compounds were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Antiherpetic activities of natural and modified *P. cyrtonema* polysaccharide against herpes simplex virus (HSV) induced by cyclophosphamide (CP) were then evaluated on vero cells using cytopathic effect (CPE) inhibition assay. The phorphorylated derivative (P-PD) and sulfated derivative (S-PD), exhibited significant inhibitory activity against HSV in comparison with the native ones, especially, P-PD presented better antiviral potency. In addition, the sulfonylated derivative (Ts-PD) was found to be as effective as PD, and the acetylated derivative (Ac-PD) to be slightly less antiviral effective, whereas carboxymethylated derivative (C-PD) was shown to be almost inactive. The results indicated that the types of function groups appeared to be very important for the antiherpetic activity of polysaccharides.

1. Introduction

Polysaccharides are known to present a broad range of biological activities comprising antiviral and antitumor action, variable effects on the immune system and anticoagulant activity (Bohn & BeMiller, 1995; Hardena, Falshaw, Carnachan, Kerna, & Pricharda, 2009; Kennedy, 1989; Kennedy & White, 1983). In particular, the antiviral effect of polysaccharides against the herpes simplex virus (HSV) has been reported (Arad, Ginsberg, & Huleihel, 2006; Damonte, Matulewicz, & Cerezo, 2004; Karmakar, Pujol, Damonte, Tuhin Ghosh, & Ray, 2010; Luescher-Mattli, 2003; Pujol, Carlucci, Matulewicz, & Damonte, 2007) and some of these polysaccharides are currently undergoing either preclinical or clinical evaluation (Kleymann, 2005; McReynolds & Garvey-Hague, 2007). Unlike the widely used acyclovir (ACV)-related nucleoside analogues, which all targeted against viral DNA synthesis, polysaccharides interfere with the attachment of virus to host cell surface by binding to viral envelope glycoprotein (Damonte et al., 2004; Duarte et al., 2001; Neyts et al., 1992; Witvrouw & De Clercq, 1997). In contrast with ACV, which may result in some undesirable complications (Richman et al., 1987) and also induce the emergence of drug-resistant viruses (Coen, 1991; Larder, Darby, & Richman, 1989) after prolonged treatment in immunocompromised patients, polysaccharides have the relatively low mammalian toxicity and the novel antiviral mechanism. Thus, the development of the potential of polysaccharides as anti-HSV agents will be of considerable interest.

Polygonatum cyrtonema Hua, a species of the Liliaceae family, is a widely used traditional Chinese medicine for the treatment of cough, dizziness and lung problems. The neutral polysaccharide (PD) isolated from *P. cyrtonema* Hua has been reported to show immunomodulatory, anti-aging and antiviral activities (Gu, Meng, & Pu, 2003; Shi, Meng, & Li, 1999), and has been implicated to be the major bioactive component of the herb. The structure of PD is a branched fructan core with (2→6)-linked β-D-fructofuranosyl (Fruf) residues every three (2→1)-linked β-D-Fruf residues and an average degree of polymerization (DP) of 28 (Liu, Liu, Meng, Yan, & He, 2004). Compared to other antiherpetic polysaccharides, such as sulfated polysaccharides, PD possesses a low molecular weight and no ionic groups. A structure activity relationship (SAR) of polysaccharide has been proposed, which suggests that the antiviral activity of polysaccharide is strongly dependent on the presence of suitable ionic groups with appropriate degree of substitution (DS) (Bohn & BeMiller, 1995; Kulicke, Lettau, & Thielking, 1997). Moreover, some reports revealed that the polysaccharide linked to anionic features of the molecules showed more potent antiherpetic activity than the neutral ones (Baba, Snoeck, Pauwels, & Clercq, 1988; Talarico et al., 2004). To our knowledge, neither the introduction of ionic groups to the PD, nor the antiviral activity of their chemically modified derivatives has been reported. Evidently, modifying the structure of PD is favorable and necessary to provide an opportunity to obtain new pharmacological agents with possible therapeutic uses. Herein we prepared a series of chemically

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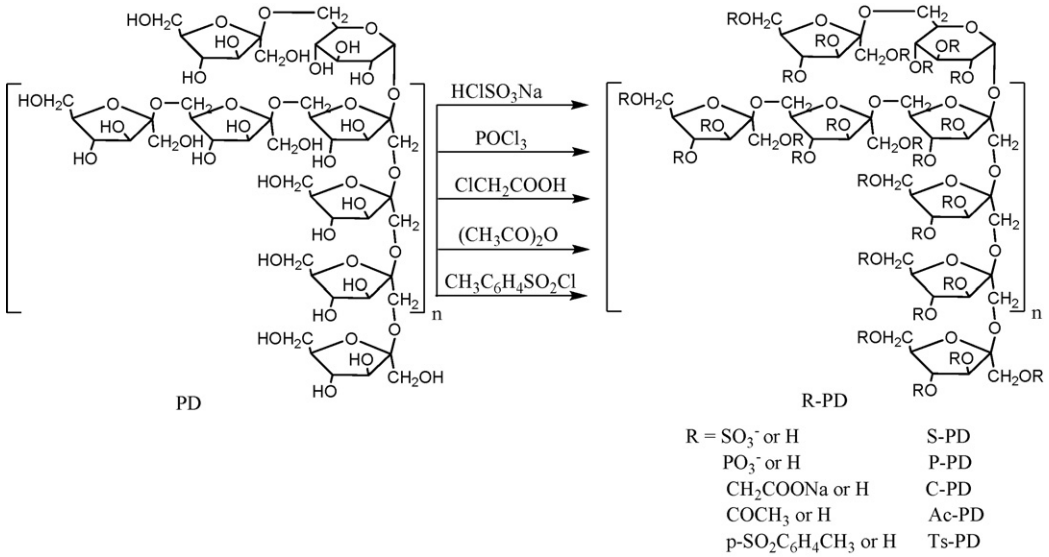

Chart 1. Synthesis of chemically modified polysaccharide from *Polygonatum cyrtonema* Hua.

modified polysaccharides, including sulfated, phosphorylated, carboxymethylated, acetylated and sulfonylated derivatives (Chart 1). The comparison of their in vitro antiherpetic activities was also reported for the first time.

2. Experimental

2.1. Materials

The rhizome of *P. cyrtonema* Hua was collected from Yibin city, Sichuan Province, China, in autumn and authenticated by Professor Zuocheng Zhao at Chengdu Institute of Biology, Chinese Academy of Sciences. PD was isolated from 85% ethanol soluble extracts of the rhizome of *P. cyrtonema* Hua according to the method previously reported (Liu et al., 2004), using multistep anion exchange and size exclusion chromatography. Bio Gel P2, P4 were purchased from Bio-rad Laboratories, USA. RPMI-1640, trypsin, 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT), and fetal bovine serum (FBS) were obtained from Gibco, USA. All other chemicals were of analytical grade unless otherwise claimed.

2.2. General methods

The FT-IR spectra (KBr pellets) of the PD and its modified derivatives were recorded on a PerkinElmer Spectrum 100 spectrometer. Optical rotations of 0.2% aqueous solution of the PD and its derivatives were measured at 20 °C, using a 10 cm cell and sodium D line (589.3 nm) with a Rudolph Autopol III automatic polarimeter. ^{1}H and ^{13}C $\{^{1}H\}$ NMR spectra were recorded on an Advance Bruker 600 MHz spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) in D_2O at 298 K. ^{31}P $\{^{1}H\}$ NMR spectra were run on an Advance Bruker 300 MHz spectrometer (121 MHz for ^{31}P) in D_2O at 298 K. Chemical shifts (in ppm) of ^{1}H and ^{13}C $\{^{1}H\}$ NMR spectra were recorded relative to tetramethylsilane (Me_4Si), while that of ^{31}P $\{^{1}H\}$ spectra were recoded relative to 85% H_3PO_4 (external). The sulfate content was determined using turbidimetric method (Dodgson & Price, 1962). The ascorbic acid method was applied to determine the total phosphate content (Lowry, Roberts, Leiner, Wu, & Farr, 1954). The carboxymethyl content was determined by neutralization titration (Regina, Heatley, & Budd, 1998). The degree of acetylation was determined using the method of Gröndahl (Gröndahl, Teleman, & Gatenholm, 2003) and the 4-toluene sulfonyl content was determined according to ^{1}H NMR.

2.3. Preparation of the different derivatives of P. cyrtonema polysaccharide

2.3.1. Sulfation of P. cyrtonema polysaccharide

This was prepared according to a modified method (Tao, Zhang, & Zhang, 2009). Chlorosulfonic acid (2 mL) was added dropwise to pyridine (6 mL) under ice-water bath with stirring. Anhydrous DMF (6 mL) was then added to the sulfating agent, followed by adding lyophilized and grinded polysaccharide (0.5 g). The reaction mixture was stirred at 0 °C for 6 h, and then neutralized with a saturated aqueous $NaHCO_3$ solution. The product was precipitated with anhydrous ethanol. The resulting precipitate was dissolved in water and then desalted with a Bio Gel P4 column (1.6 cm × 100 cm) using distilled water as the eluent. The main fraction (monitored by anthrone assay) was collected, concentrated and dried under vacuum to yield the sulfated polysaccharide as a pale yellow solid.

2.3.2. Phosphorylation of P. cyrtonema polysaccharide

This was prepared according to a modified method (Yuan et al., 2005). Under a nitrogen atmosphere, phosphorus oxychloride (200 μL) was added dropwise to a stirred solution of triethyl phosphate (400 μL) and pyridine (1.6 mL) at 0 °C. The mixture was allowed to stir at the same temperature for 30 min, and then a DMSO solution (5 mL) containing polysaccharide (0.5 g) was added. The reaction mixture was stirred for 6 h and neutralized with aqueous NaOH. The product was precipitated with anhydrous ethanol. The desalting procedure was similar to that described for the preparation of sulfated polysaccharide to provide the phosphorylated polysaccharide as a pale brown solid.

2.3.3. Carboxymethylation of P. cyrtonema polysaccharide

This was prepared according to a modified method (Tao et al., 2009). Polysaccharide (0.5 g) was dissolved in distilled water (2 mL) and then aqueous NaOH (1 N, 6.5 mL) was added. The solution was allowed to stir for 30 min at room temperature before the addition of chloroacetic acid (0.48 g). The reaction mixture was heated to 75 °C and stirred for 4 h. After cooling to room temperature, the solution was neutralized with hydrochloric acid and concentrated under reduced pressure. The desalting procedure was similar to

that described for the preparation of sulfated polysaccharide to provide the carboxymethylated polysaccharide as a white solid.

2.3.4. Acetylation of P. cyrtonema polysaccharide

This was prepared according to a modified method (Zhang et al., 2010). Acetic anhydride (0.75 mL) was added dropwise to a stirred solution of polysaccharide (0.5 g) and pyridine (2 mL) in DMSO (2 mL) at 0 °C. The mixture was allowed to stir at the same temperature for 30 min. The reaction was quenched with distilled water and was precipitated with anhydrous ethanol. The desalting procedure was similar to that described for the preparation of sulfated polysaccharide to provide the acetylated polysaccharide as a reddish brown solid.

2.3.5. Sulfonylation of P. cyrtonema polysaccharide

To a stirred solution of polysaccharide (0.5 g) in DMSO (2 mL) was added pyridine (2 mL) and 4-toluene sulfonyl chloride (1.4 g) under ice-water bath. The mixture was stirred for 6 h at room temperature and was quenched with distilled water. Anhydrous ethanol was then added to precipitate. The desalting procedure was similar to that described for the preparation of sulfated polysaccharide to provide the sulfonylated polysaccharide as a dark red solid.

2.4. Cells and virus

African green monkey kidney-derived cells (vero cells) provided by West China University of Medical Sciences and herpes simplex virus (HSV-2) strain 333 obtained from Wuhan Institute of Virology, Chinese Academy of Sciences, were used in the antiviral bioassay throughout. Vero cells were propagated in sterile RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin mixture. The maintenance medium contains 2% FBS instead. The cell line was incubated in a humidified 5% CO_2 atmosphere at 37 °C. Virus stocks were titrated using cytopathic effect (CPE) assay (Mahmoud et al., 2002) in vero cells and 50% tissue culture infectious dose ($TCID_{50}$) was measured before use.

2.5. Cytotoxicity assay

Vero cells were seeded onto 24-well microplates at a concentration of 10^5 cells per well and incubated for 24 h at 37 °C with 5% CO_2. The growth medium was then replaced by maintenance medium, in which the tested compounds with serial dilutions were added. Cells were cultured for 72 h under conditions described above. Subsequently, the medium was removed and MTT (final concentration 0.5 mg/mL) was added. After incubating for 4 h in the dark, MTT was removed, followed by the addition of DMSO. The microplates were shaken for 5 min and the optical densities were measured on a Bio-rad Microplate Reader at 570 nm (630 nm as the reference wavelength). Percent cytotoxicity was calculated according to the formula: $1 - (A_{sample}/A_{blank})) \times 100\%$. Each sample was measured in triplicate.

2.6. Antiviral assay

Vero cells were seeded and incubated to confluent monolayer in 24-well plates under conditions described above. Medium was discarded and maintenance medium containing tested compounds with various concentrations were added. Vero cells were then infected with virus (100 $TCID_{50}$) and incubated for 72 h. The cytopathic effect was examined under inverted light miroscopy daily and the results scored. Antiviral activities were further measured by adding MTT after the removal of medium. The procedure was similar to that described in cytotoxic assay. The inhibition rate was calculated as $[OD_{sample} - OD_{virus\ control}]/[OD_{cell\ control} - OD_{virus\ control}] \times 100\%$. The 50% inhibitory concentration was obtained by Four-Parameter Logistic Model. ACV was used as positive control. Each sample was measured in triplicate. The 50% cell-inhibitory concentration (IC_{50}) was calculated by Reed–Muench method (Zhang, Dong, & She, 1998).

Table 1
The isolated yield, DS and the optical rotations of *P. cyrtonema* polysaccharide (PD) and its sulfated (S-PD), phosphorylated (P-PD), carboxymethylated (C-PD), acetylated (Ac-PD) and sulfonylated (Ts-PD) derivatives.

Sample	R	Isolated yield (g)[a]	DS (%)	α_D^{20} (°)
S-PD	SO_3Na or H	0.53	0.82	−8.0
P-PD	PO_3Na or H	0.46	0.65	−23.0
C-PD	CH_2COONa or H	0.51	0.73	−32.5
Ac-PD	$COCH_3$ or H	0.45	0.74	−25.0
Ts-PD	p-$SO_2C_6H_4CH_3$ or H	0.54	0.22	−7.5
PD	H	–	–	−36.7

[a] Yields are given per 0.5 g of PD.

3. Results and discussion

3.1. Preparation and characterization of PD derivatives

Starting from a neutral polysaccharide (PD) extracted from *P. cyrtonema* Hua, five chemically modified derivatives of PD were readily synthesized as a mixture with different substituted positions purified by a Bio Gel P4 column according to the method described in the literature (Tao et al., 2009; Vogl, Paper, & Franz, 2000; Xu et al., 2009). Their yields, degrees of substitution and optical rotations were given in Table 1. Relatively to PD, the weights of sulfated (S-PD), carboxymethylated (C-PD), and sulfonylated derivatives (Ts-PD) slightly increased after modification, whereas a remarkable decrease for phosphorylated (P-PD) and acetylated derivatives (Ac-PD) were observed. As shown in Table 1, the sulfation reaction underwent smoothly in 0.82 DS. Other substitutions such as carboxymethylation, acetylation and phosphorylation also proceeded rapidly, but with lower efficiency, affording DS in the ranges of 0.74, 0.73 and 0.65, respectively. Low degrees of substitution were obtained for sulfonylation in 0.22 DS under such condition.

The FT-IR spectrums of the crude polysaccharide (PD) and its derivatives (R-PD) were shown in Fig. 1. The spectra of PD and R-PD were very similar and typical signals of polysaccharide at 1635, 1455, 1296, 1270, 1131, 1024, 931 and 817 cm^{-1} were clear for all the samples, indicating these polysaccharides should have the similar structures. The strong absorption band at about 3400 cm^{-1} in the spectra of PD and R-PD was assigned to OH stretching vibrations and the peak at about 2920 cm^{-1} was assigned to C–H stretching of the CH_2 groups. The bands attributed to primary alcoholic-CH_2OH stretching mode and C–O–C stretching vibrations appeared at about 1075 cm^{-1}. By comparison with the FT-IR spectra of PD, two characteristic absorption bands appeared in the FT-IR spectra of the sulfated derivative (S-PD), one at near 1239 cm^{-1} describing an asymmetrical S=O stretching vibration and the other at near 815 cm^{-1} representing a symmetrical C–O–S vibration associated with a C–O–SO_3 group, indicating S-PD was successfully sulfated (Mähner, Lechner, & Nordmeier, 2001). For phosphorylation, two new bands at 1262 and 929 cm^{-1} were ascribed to the P=O stretching vibration and P–O stretching vibration, respectively. This assignment was consistent with the similar bands commonly observed in other related phosphorylation of polysaccharide systems (Yuan et al., 2005). In the carboxymethylated derivative, two new strong absorption bands appeared at 1601 and 1320 cm^{-1}, as a result of the existence of the COO^- and CH_2 groups, respec-

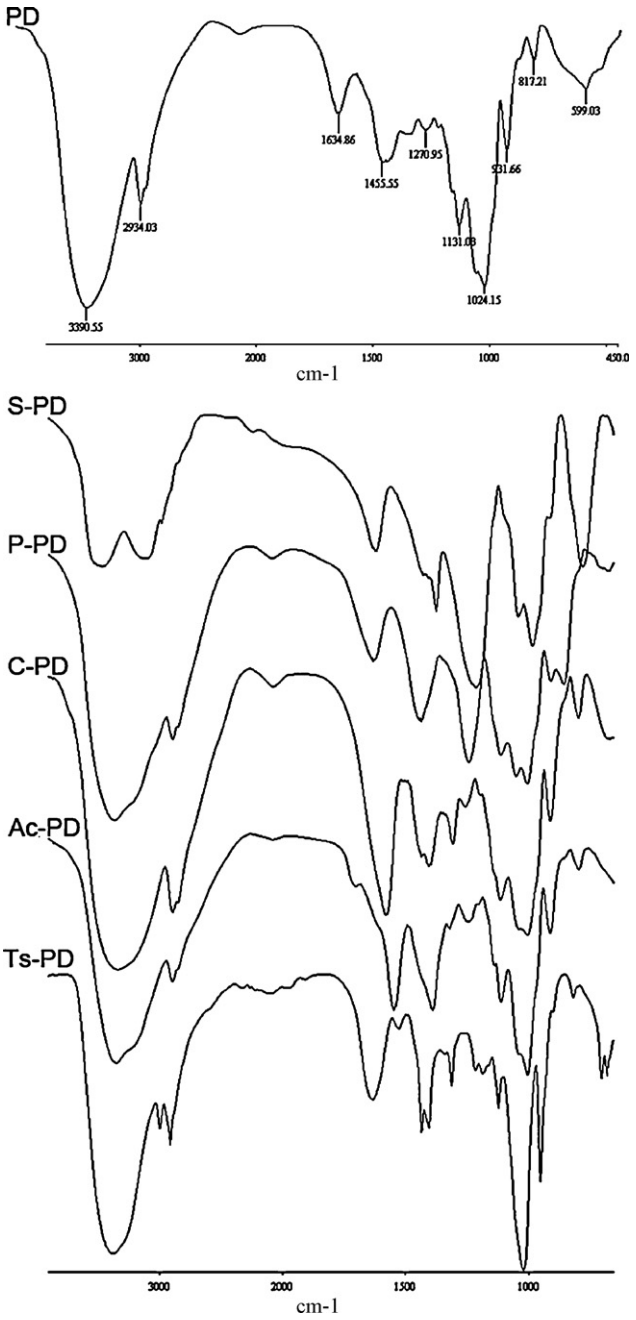

Fig. 1. FT-IR spectra of *P. cyrtonema* polysaccharide (PD), and its sulfated (S-PD), phosphorylated (P-PD), carboxymethylated (C-PD), acetylated (Ac-PD), sulfonylated (Ts-PD) derivatives.

tively. The IR spectra of the acetylated derivative (Ac-PD) exhibited the spectral characteristics of esterified products by displaying essential bands of ester compounds at 1726 cm^{-1} (C=O stretching vibration) and 1373 cm^{-1} (–C–O–C– st.). For the sulfonylated derivative, the peaks at 1724 cm^{-1} were assigned to the characteristic absorbance of C=O stretching vibration and the signals at 1561, and 1447 cm^{-1} were attributed to the C=C of toluene group, indicating that Ts-PD were obtained.

The chemical shifts and ^{13}C NMR spectra of the PD and its chemically modified derivatives R-PD are listed in Table 2 and Fig. 2, respectively. From the NMR spectra of PD and its chemically modified derivatives R-PD, the chemical shifts of the protons and carbons of the preponderant residue were fully assigned, through a comparison with literature data (Lopez, Mancilla-Margalli, &

Table 2
^{1}H and ^{13}C NMR spectroscopic data of *P. cyrtonema* polysaccharide (PD) and its sulfated (S-PD), phosphorylated (P-PD), carboxymethylated (C-PD), acetylated (Ac-PD) and sulfonylated (Ts-PD) derivatives.

Sample	Chemical shifts (ppm)					
	H-1/C-1β-Fru_f	C-2β-Fru_f	H-3/C-3β-Fru_f	H-4/C-4β-Fru_f	H-5/C-5β-Fru_f	H-6/C-6β-Fru_f
S-PD	3.67–3.88/60.1, 60.6, 60.8	103.1, 103.7, 103.9	4.13–4.16/76.9, 77.2	4.04–4.07/74.5, 74.7, 75.2	3.79–3.82/80.3, 81.1	3.62–3.63/62.3, 63.0
P-PD	3.68–3.87/60.1, 60.5, 60.9	103.3, 103.7, 103.9, 104.0	4.12–4.18/76.6, 76.8	4.05–4.08/74.5, 74.8, 75.3	3.78–3.81/80.3, 81.1	3.63–3.64/62.3, 62.6, 63.2
C-PD	3.69–3.87/60.1, 60.5, 60.9	103.2, 103.7, 103.9, 103.4	4.12–4.17/76.6, 76.8	4.04/74.5, 74.8, 75.3	3.74–3.79/80.2, 81.1	3.62–3.63/62.3, 62.6, 63.2
Ac-PD	3.68–3.70, 3.89/60.2, 60.6, 61.0	103.2, 103.7, 103.8, 104.0	4.13–4.19/76.6, 76.8	4.05–4.06/74.5, 74.8, 75.2	3.76–3.81/80.3, 81.1	3.63–3.65/62.3, 62.8, 63.1
Ts-PD	3.69–3.87/60.5	103.3, 103.7	4.12–4.17/76.8	4.05–4.09/74.7	3.77–3.81/81.1	3.64/62.2
PD	3.65–3.91/59.4, 60.0, 60.5	103.2, 103.7, 103.8, 104.0	4.25–4.19/76.6, 76.8, 77.1	4.07/74.4, 74.7, 75.3	3.80–3.88/80.3, 81.1	3.70–3.71/61.4, 62.3, 62.6, 63.3

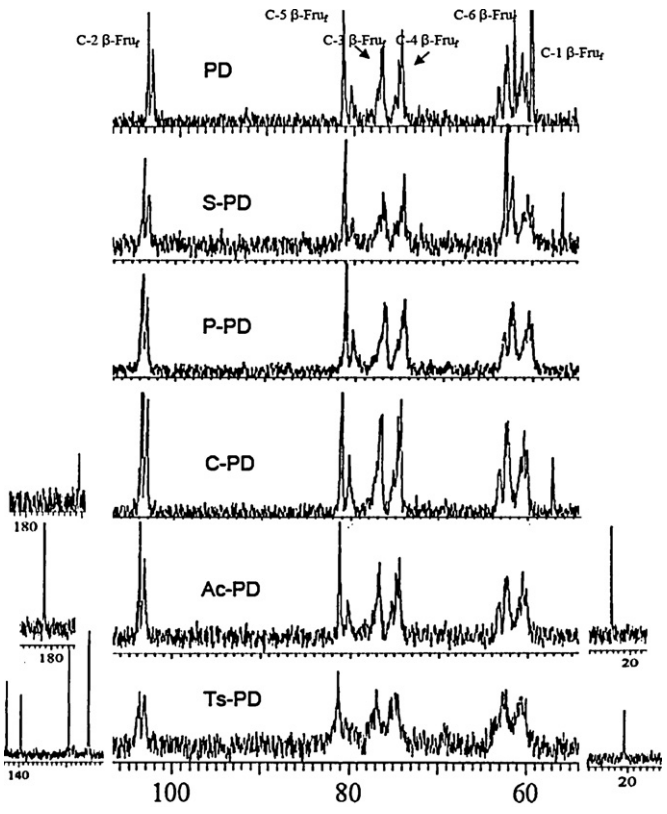

Fig. 2. ^{13}C NMR spectra of *P. cyrtonema* polysaccharide (PD), and its sulfated (S-PD), phosphorylated (P-PD), carboxymethylated (C-PD), acetylated (Ac-PD), sulfonylated (Ts-PD) derivatives.

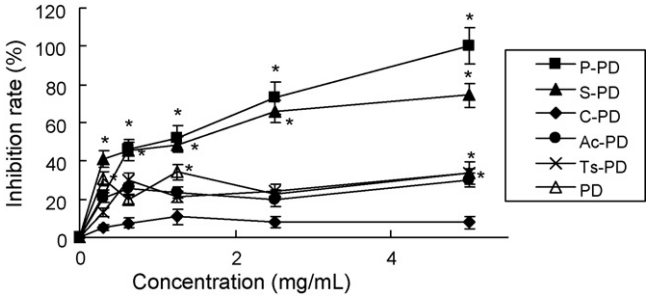

Fig. 3. Inhibitory effects of *P. cyrtonema* polysaccharide (PD), and its sulfated (S-PD), phosphorylated (P-PD), carboxymethylated (C-PD), acetylated (Ac-PD), sulfonylated (Ts-PD) derivatives. Values are means ± S.D. of three determinations. Statistically significant differences at P values of <0.05 (*).

Mendoza-Diaz, 2003; Ravenscroft et al., 2009), to those of a β-D-fructofuranosyl residue with a β-anomeric configuration. By comparison with β-D-frutan, the absorptions at around 3.65–3.91 (H-1), 4.25–4.19 (H-3), 4.06–4.07 (H-4), 3.80–3.88 (H-5), 3.70–3.71 (H-6) ppm in ^1H NMR and 60.0 (C-1), 103.7 (C-2), 76.8 (C-3), 74.7 (C-4), 81.5 (C-5), 62.3 (C-6) ppm in ^{13}C NMR should be attribute to the signals of the backbone carbon chain. After chemical modification, the signals of the backbone chain of PD mentioned above still existed, indicating that the main structure of PD in its chemically modified derivatives was reserved. When the ^{13}C NMR spectrum of the native unmodified PD were compared, the spectral absorption at 59.4 ppm, the chemical shift of C-6 for PD, disappeared in the ^{13}C NMR spectrum of S-PD. In addition, a new absorption at 63.0 ppm appeared in the spectra of S-PD, indicating that C-6 of PD had substituted by sulfo group, so the C-6 signal was shifted downfield. Similarly, the peak of 61.44 ppm for C-6 of the PD disappeared in the ^{13}C NMR spectrum of P-PD was observed, indicating that the C-6 of different β-D-Fruf residues was substituted by a phosphor group. More noticeable evidence could be observed in the ^{31}P NMR spectra with intense signals at −7.64 and −21.0 ppm, which suggested the polysaccharide was successfully substituted by phosphorus group ($-PO_3$). For carboxymethylation, although no obvious peak in ^1H NMR spectra was observed due to the overlapping of signals at 3.62–4.17 ppm, a significant peak of the carbonyl group at 170.4 ppm was observed in ^{13}C NMR spectra. When comparing the ^1H and ^{13}C NMR spectra of PD and Ac-PD, the main spectral features of Ac-PD were the presence of new signals at 1.86 and 2.67 ppm in ^1H NMR, and 23.2 and 38.8 ppm absorption in ^{13}C NMR spectrum, respectively. These signals can be assigned to $-CH_3$ of acetyl, while the characteristic signal at 181.3 ppm in ^{13}C NMR can be assigned to the –C=O group. For the spectral analysis of the sulfonylated product, new peaks at about 7.64, 7.32 and 2.34 ppm in ^1H NMR spectrum and absorptions at 142.5, 139.5, 129.5, 125.4 and 20.5 ppm in ^{13}C NMR spectrum, respectively, were assigned to the toluene group. Those also could be seen as an evidence of the success of the sulfonyl substitution.

3.2. Cytotoxic effects of PD and its derivatives on cell viability

Vero cell viability was evaluated by MTT assay after 3 days of incubation in the presence of serial dilutions of PD and its chemically modified derivatives R-PD. The results indicated that all the derivatives had no obvious cytotoxicity on vero cells even at a maximal concentration of 10 mg/mL. MTT assay was in agreement with the observations of cell morphology under light microscopy. Thus, we can conclude that the CC_{50} of these compounds was more than 10 mg/mL.

3.3. Anti-HSV-2 effects of PD and its derivatives

Introduction of different function groups can affect the polarity, conformation or charge density of native polysaccharide, which may lead to the change of antiviral activity. This in turn provides valuable information on structure–activity relationship. To clarify the main factor influencing bioactivity, we examined the antiherpetic activities against HSV-2 (333 strain) of *P. cyrtonema* polysaccharide and its sulfated (S-PD), phosphorylated (P-PD), carboxymethylated (C-PD), acetylated (Ac-PD) and sulfonylated (Ts-PD) derivatives on vero cells using cytopathic effect (CPE) inhibition assay. Fig. 3 shows that only P-PD and S-PD gave significant inhibition (≥50% reduction of infectivity) at all concentrations. The IC_{50} of P-PD and S-PD for HSV-2 (333 strain) were calculated to be 1.22 mg/mL and 2.20 mg/mL, respectively. Accordingly, P-PD and S-PD displayed anti-HSV-2 properties superior to that of native polysaccharide in vitro assay. Fig. 4 shows the in vitro inhibition ratios of PD and its derivatives at a concentration of 2.5 mg/mL for comparison. As shown in Fig. 4, *P. cyrtonema* polysaccharides failed to show obvious inhibitory effect at these concentrations with inhibition rate lower than 35%, in accordance with a previous report (Gu et al., 2003). To our surprise, P-PD and S-PD exhibited high antiviral activity against HSV-2 in a concentration-dependant way. The phorphorylated PD was found more effective than the sulfated derivative, with the inhibition rate reaching 100% at the maximum concentration. In contrast, carbomethylated, acetylated and sulfonylated PD derivatives showed no improvement of the antiviral activity against HSV-2. For acetylated and sulfonylated PD, no significant change of activity was observed, while the carboxymethylated PD showed slightly decreased inhibitory effect comparing to the native polysaccharide. These results revealed that not only the charge density, but also the chemical properties of the function groups on derivatized PD affected its antiherpetic activities.

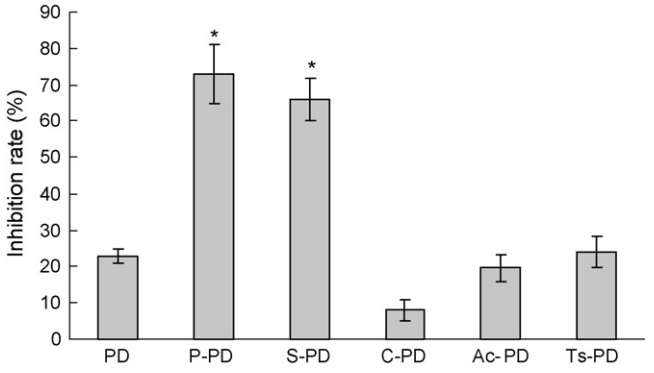

Fig. 4. Inhibitory effects of all the compounds on HSV-2 infectivity at the concentration of 2.5 mg/mL. Values are means ± S.D. of three determinations. Statistically significant differences at P values of <0.05 (*).

4. Conclusion

The chemical modification of polysaccharides from *P. cyrtonema* Hua was carried out in the present paper. The successful synthesis of the chemically modified polysaccharide was verified either by IR analysis or by NMR spectroscopy. We found that the phosphorylated and sulfated derivatives of the *P. cyrtonema* polysaccharides exhibited higher inhibitory activity against HSV-2 than that of the native material. Compared to S-PD, P-PD seemed to be a better inhibitor. Given the interesting chemical characteristics of the sulfated and phosphorylated polysaccharides derived from *P. cyrtonema* Hua and the significant in vitro antiherpetic properties reported here, these macromolecules could be promising candidates for further pharmacological and clinical studies. Meanwhile, the study on its antiviral mechanism is underway in our research group.

Acknowledgements

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