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Structural Characterization of α-D-Glucan Obtained from *Angelica sinensis* Roots

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α-D-Glucan (ASP-1) was isolated from the hot water extract of *Angelica sinensis* roots by ethanol precipitation, anion-exchange and gel-permeation chromatography. Smith degradation, NMR spectroscopy, composition and methylation analyses confirmed the ASP-1 structure comprises a (1→6) linked α-D-glucopyranosyl backbone, coupled with two branches per unit bonded through α(1→3) linkages, where each branch contains Glc-(1→3)-Glc-(1- and Glc-(1→4)-Glc-(1- chains at either end.

Key Words: *Angelicae sinensis*, Umbelliferae, Polysaccharide, Glucan.

INTRODUCTION

Angelica sinensis (Oliv.) Diels (Umbelliferae) has been widely used in Chinese traditional medicine. It is reported to exhibit anti-thrombotic, anti-tumour and spasm-relieving activities, while also promoting immunologic function and demonstrating a marked resistance to radiation[1]. Previous analyses have shown that the main components of *Radix Angelicae sinensis* are volatile oils, organic acids and low molecular weight carbohydrates. It is widely believed that the various pharmacological activities of *Radix Angelicae sinensis* are related to the polysaccharide components[2,3]. Previous reports have described the isolation of several polysaccharides from *Radix Angelicae sinensis*; however, the biological activities of these components have yet to be investigated[4–6]. To further the study of the biological activities of these components, and thus the corresponding pharmacological action of *Radix Angelicae sinensis*, a high molecular weight polysaccharide (ASP-1) from the hot water extract of *Radix Angelicae sinensis* roots has been isolated. Preliminary investigations have indicated that ASP-1 inhibits several tumour cell lines and improves immunity.

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RESULTS AND DISCUSSION

An elemental analysis of α-D-glucan (ASP-1) confirmed that the polysaccharide contained no nitrogen, while an optical rotation study revealed that ASP-1 had a specific rotation $[\alpha]_D^{15}$ of +128°, implying the presence of mainly-linked sugars. The average molecular weight of ASP-1 was estimated to be 5.37×10^5, as determined from a calibration curve of the log (molecular weights of standard dextrans) *vs.* elution volume. Thin layer chromatography (TLC) of ASP-1 following hydrolysis revealed only D-glucose in the hydrolysate. Gas chromatography (GC) studies of ASP-1, its hydrolysate, the corresponding O-acetal-alditol cyanide and several isolated monosaccharides, all confirmed D-glucan to be the major component in ASP-1. This was further corroborated by the appearance of three bands (1019, 1106 and 1155 cm^{-1}) in the IR spectrum, characteristic of the modes in glucopyranose, respectively. Moreover, the appearance of a peak at 846 cm^{-1}, strongly suggests the presence of an α-configuration linkage between glucopyranoses.

ASP-1 was methylated according to methods described in literature[7]. The permethylated polysaccharide was then subjected to acid hydrolysis, reduction and acetylation The partially methylated alditol acetate was analyzed using GC and GC-MS, and the results shown in Table-1. Compounds (i) 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl glucitol, (ii) 1,3-di-O-acetyl-2,4,6-tri-O-methyl glucitol, (iii) 1,4-di-O-acetyl-2,3,6-tri-O-methyl glucitol, (iv) 1,6-di-O-acetyl-2,3,4-tri-O-methyl glucitol, (v) 1,3,6-tri-O-acetyl-2,4-di-O-methyl glucitol were identified in the molar ratio of 2 : 1 : 1 : 12 : 2. GC-MS analysis showed that the main chain in these species is composed of glucose α(1→6) linkages, connected *via* two branches per unit linked through α(1→3) linkages, on which there are Glc-(1→3)-Glc-(1- and Glc-(1→4)-Glc-(1- chains at either end of each branch.

TABLE-1
ANALYSIS DATA OF PARTIALLY METHYLATED ALDITOL ACETATES OBTAINED FROM α-D-GLUCAN (ASP-1)

Methylated alditol acetate derivates	Main fragment		Molar ration	Linkage
	ASP-1	Reference [9]		
2,3,4,6-Me_4-Glc	43, 45, 71, 87, 101, 117, 129, 145, 161	43, 45, 71, 87, 101, 117, 129, 145, 161, 205	2	Glc→1
2,4,6-Me_3-Glc	43, 45, 71, 87, 101, 117, 129	43, 45, 71, 87, 101, 117, 129, 161	1	Glc→1,3
2,3,6-Me_3-Glc	43, 45, 87, 99, 101, 117	43, 45, 87, 99, 101, 113, 117, 223	1	Glc-(1,4→)
2,3,4-Me_3-Glc	43, 87, 89, 101, 117, 129, 161, 189	43, 87, 89, 101, 117, 129, 161, 189	12	Glc-(1,6→)
2,4-Me_2-Glc	43, 87, 117, 129, 189	43, 87, 117, 129, 189	2	Glc-(1,3,6→)

ASP-1 was degraded by perioxidation[8] and the resulting product was converted to the corresponding alditol acetate and analyzed by GC. Here, the observation of a large concentration of glycerol confirmed the presence of a high percentage of 1,6-linked glucosol residues in the alditol acetate. Interestingly, the main degradation product of the corresponding 1,4-linked glucose analog was determined to be erythitol. The appearance of glucose is believed to be due to the presence of oxidation-resistant glucose residues such as 1,3,6-linked glucopyranose, an observation that is further corroborated by the results obtained from the methylation experiments.

The ^{1}H-NMR and ^{13}C-NMR results for ASP-1 in D_2O revealed spectra similar to those of dextran, which is known to contain α-glucose moieties coupled through an α(1→6) linkage. Moreover, the appearance of additional peaks in the ^{1}H and ^{13}C-NMR spectra of ASP-1 at δ 4.87 and δ 99.9 ppm, respectively, are attributed to the corresponding anomeric proton and carbon signals. The observed chemical shifts (δ 4.87 and δ 99.9 ppm) provide strong indications that the linkage between the glucose units adopts an α-configuration.

EXPERIMENTAL

General experimental procedures: CD data were recorded on a JASCO-20C automatic recording spectropolarimeter. IR and GC spectra were obtained on IR-170X and GC-Shimadzu-9A spectrometers, respectively. ^{1}H and ^{13}C-NMR analyses were performed on a Bruker AM-400 (400 MHz) spectrometer using a TMS internal standard. GC-MS was performed on a QP-1000A instrument using the corresponding TMS-derivatized samples (TMS-O-methylglycosides).

Plant material: The roots of *Angelicae sinensis* were collected from Minxian County, Gansu Province, China and a voucher specimen was deposited in the Shanghai Institute of Material Medica.

Extraction and isolation: The powder (1 kg) was first steam distilled to remove the volatile oil components. The residue was then extracted with water, filtered and condensed, and the whole process repeated twice. The filtrate was then precipitated with three volumes of ethanol and centrifuged. The resulting precipitate was then dissolved in water and deproteinated repeatedly (10 times) using *n*-BuOH : $CHCl_3$ (4 : 1). The water solution was dialyzed for 24 h and finally condensed. Three volumes of ethanol was then added to the condensate and the solution was then stirred overnight. The resulting solution was filtered and the filtrate washed with ethanol (500 mL), propanone (500 mL) and then finally dried *in vacuo* to give the crude polysaccharide (ASP) in good yield (20.5 g, 2.05%).

ASP (5.0 g) was dissolved in water (40 mL) and applied to a cellulose DE-52 column (2.9 × 44 cm), which was first eluted with H_2O (400 mL) and then by a gradient elution with (1000 mL) of 0.1–2.0 M NaCl solution. The process was monitored using the anthrone-sulfuric acid method and the subsequent fractions (83–102) were collected in tubes (6 mL). The fractions were then purified on a

Sephadex G-100 (2.6 × 90 cm) column using a 0.1 M NaCl solution. The first fraction of the NaCl eluate was collected and dialyzed for 24 h. The corresponding dialysate was then precipitated with three volumes of ethanol and finally centrifuged at 3000 g. The resulting precipitate was washed first with ethanol (200 mL) and then with propanone (200 mL) and finally dried to give a white powder (ASP-1) in good yield (1.5 g, 0.15%).

Homogeneity and molecular weight: The homogeneity of ASP-1 was determined by cellulose-acetate membrane electrophoresis [membrane: 2 × 10 cm; buffer solution: borax-boric acid, pH 9.0; current: 0.5 mA/cm; time: 45 min; stain: alcian blue ethyl alcohol solution], which revealed a single blue band. The molcular weight of ASP-1 was determined by G-200 chromatography, using a 0.1 M NaCl mobile phase and a flow rate of 6 mL/h, and calibrated against curves derived from 6 dextran standards with molcular weight of 1×10^4, 4×10^4, 7×10^4, 1.1×10^5, 5×10^5 and 2×10^6, respectively.

Composition analysis: ASP-1 (10 mg) dissolved in 2 M TFA (4 mL) was hydrolyzed at 110°C for 2 h. The TFA was removed by repeated evaporation with MeOH and the remaining hydrolysate was analyzed using TLC on PEI-cellulose plates (E. Merck), developed with 5 : 5 : 1 : 3 EtOAc-pyridine-HOAc-water. The plate was visualized by spraying with *o*-phthalic acid and heating at 100°C for 5 min. The hydrolysate was then reduced using $NaBH_4$ (25 mg) at room temperature for 3 h. The reduced hydrosylate was neutralized with AcOH and evaporated to dryness and then finally acetylated with Ac_2O (100°C, 1 h). The resulting alditol acetate was analyzed using GC.

Methylation analysis: ASP-1 (5 mg) was methylated according to the modified method described in literature[7] and repeated three times to ensure completeness. The reaction was confirmed by the disappearance of the hydroxyl absorption in the corresponding IR spectrum. The permethylated polysaccharide was first hydrolyzed in 90% formic acid (100°C, 3 h) and then further hydrolyzed in 2 M TFA (100°C, 4 h). The partially methylated sugars were reduced and acetylated according to the method described above.

Periodate oxidation: ASP-1 (10 mg) was dissolved in 0.015 M $NaIO_4$ (20 mL) and maintained in the dark at 4°C. The oxidation of ASP-1 was followed by monitoring the UV absorption band at 224 nm each day. After oxidation was complete, ethylene glycol (0.2 mL) was added to the solution with stirring for 0.5 h and the formic acid produced was titrated with 0.01 N NaOH. The reaction mixture was dialyzed against distilled water and the non-dialysate was reduced using $NaBH_4$ (50 mg, 12 h). The pH was adjusted to 5.0 and the non-dialysate solution was dialyzed again. The resulting solution was lyophilized and then hydrolyzed with 1 M TFA at 100°C for 6 h, and finally analyzed by TLC.

NMR spectroscopy: ASP-1 (30 mg) was dissolved in D_2O (0.5 mL), freeze-dried and then redissolved in D_2O (0.5 mL) and ^{13}C and ^1H NMR spectra were recorded at room temperature on a Bruker AM400 spectrometer (400 MHz). All chemical shifts were recorded relative to Me_4Si.

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