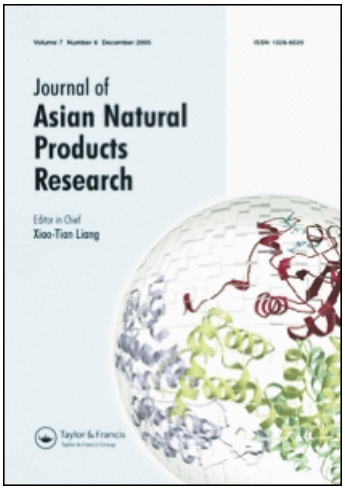

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Structural characterization of polysaccharides from the roots of *Urtica fissa*

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Three polysaccharides were isolated from the roots of *Urtica fissa* by extraction, ultrafiltration, anion-exchange, and gel-filtration chromatography. The structures were characterized using acetylation, methylation, and spectral methods (GCMS, NMR). All three polysaccharides are mainly composed of D-arabinofuranosyl, D-galactopyranosyl, D-glucopyranosyl residues with different structural characteristics. Polysaccharide A of MW 5.2×10^3 contained a linear chain of 1-linked β-D-glucopyranosyl, 1,6-linked β-D-glucopyranosyl, 1,6-linked α-galactopyranosyl, and 1,5-linked β-arabinofuranosyl moieties. Polysaccharide B of MW 7.7×10^4 possessed a chain consisting of 1,5-linked α-D-arabinofuranosyl, 1,3-linked β-D-mannopyranosyl, 1,6-linked β-D-glucopyranosyl, and 1,6-linked α-D-galactopyranosyl residues, but 4-*O* of α-D-galactopyranosyl residues were branched by terminal β-D-glucopyranosyl residues. Polysaccharide C of MW 5.3×10^4 composed of a chain of 1,5-linked α-D-arabinofuranosyl, 1,4-linked β-D-galactopyranosyl, 1,5-linked β-D-xylopyranosyl, 1,4-linked β-D-mannopyranosyl, 1-linked β-D-glucopyranosyl residues, and the terminal β-D-glucopyranosyl residues are attached to 3-*O* positions of 1,6-linked α-D-glucopyranosyl residues.

Keywords: *Urtica fissa*; Urticaceae family; polysaccharide; isolation; structural characterization

1. Introduction

The genus *Urtica* (Urticaceae family) has been used as a herbal medicine worldwide. Various water extracts from the roots of *Urtica dioica* (stinging nettle) are widely used for the treatment of benign prostatic hyperplasia [1–4]. The genus *Urtica* has also been used as a traditional medicine in China for the treatment of eczema, rheumatism, and inflammation. Wang reported that there were 11 species and subspecies of the genus *Urtica* in Sichuan Province, China, out of which eight nettles were commonly used for the treatment of rheumatism [5,6]. Many compounds were reported to exist in water or aqueous alcohol extracts of *Urtica* such as fatty acids, sterols, flavonoids, proteins, polysaccharides, and lectins [7–13]. However, the main active compounds are not yet clear, and some of the pharmacologic components are considered to be polysaccharides or glycoproteins. The polysaccharide fraction of the 20% methanol extract of the roots of *U. dioica* showed an inhibitory effect on the growth of human prostatic epithelium LNCaP cells [14]. The crude polysaccharide from *Urtica fissa* was shown to have significant anti-inflammatory activities and immunological enhancement

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in a rat footpad swelling and pinna swelling mode [15]. Our research showed that the crude polysaccharide fractions extracted using water could inhibit castrated rat's prostate hyperplasia induced by testosterone propionate [16]. However, the structures of polysaccharides present in *U. fissa* have not yet been characterized. Therefore, the purpose of this work was to isolate, purify, and characterize the polysaccharides present in the *Urtica* species.

2. Results and discussion

The hot-water extract of the roots of *U. fissa* was ultrafiltered to remove small molecular compounds and obtain the crude polysaccharide fraction. The fraction was repeatedly subjected to remove protein by Sevage method and separated on a DEAE-Sephadex A-25 column. Two major fractions were obtained with water elution (42%) and 0.2 M NaCl elution (36.8%). The water fraction was further chromatographed on a Sepharose 6B column with water elution to obtain a homogeneous polysaccharide A (49.8%). The fraction eluted with 0.2 M NaCl was further separated to yield two fractions B (35.6%) and C (45.8%).

The homogeneity and molecular weight of the polysaccharides A–C were determined using a Sephacryl S-200-HR column with water elution. Each of the polysaccharides A–C was eluted as a single symmetrical peak (Figure 1). The molecular weights of the three polysaccharides were determined from the calibration curve established with reference to standard dextrans, which were 5.2×10^3, 7.7×10^4, and 5.3×10^4, respectively. The polysaccharides A–C were regarded as neutral polysaccharides as no acidic sugar was found. According to their GC of alditol acetates, all three polysaccharides mainly consisted of D-arabinose, D-glucose, and D-galactose.

The types of linkage in the three polysaccharides were determined

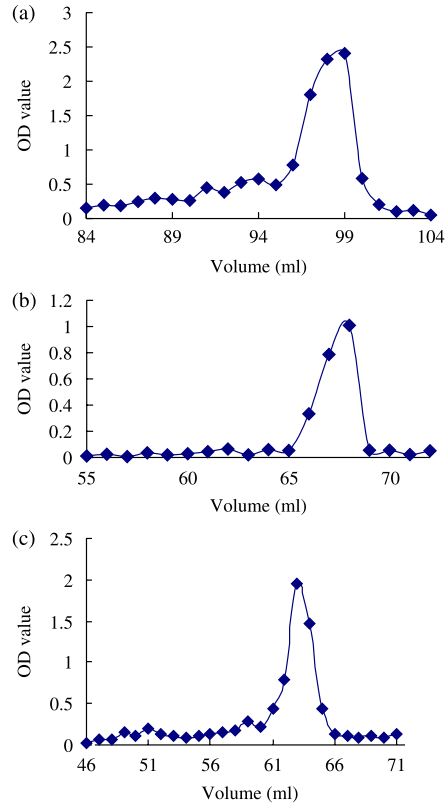

Figure 1. Size-exclusion chromatography of (a) A, (b) B, and (c) C on a Sephacryl S-200-HR column, eluted with water.

by methylation and GC-MS analysis. Anomeric carbon configurations were determined as possible by ^{13}C NMR spectroscopy based on the component analysis, methylation analysis, and literature data [17–19]. GC-MS of methylated derivatives of polysaccharide A resulted in four peaks (Table 1): 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-arabinitol (*m/z*: 58, 71, 87, 101, 117, 129, 161, 189), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol (*m/z*: 43, 59, 71, 87, 101, 117, 129, 145 161, 173, 205), 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-galactitol (*m/z*: 43, 58, 71, 87, 99, 101, 117, 131, 147, 161, 191, 233), and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-glucitol (*m/z*: 43, 58, 75, 87, 99, 101, 117, 131, 147, 161, 191, 203, 233) in a molar ratio of

Table 1. The GC-MS data of polysaccharide A after methylation.

Composition	Relative retention time (min)	Molar ratio	Fragment peaks of MS	Connection
1,4,5-Tri-*O*-acetyl-2,3-di-*O*-Me-Ara*f*	1.10	1	58, 71, 87, 101, 117, 129, 161, 189	5 1 → Ara—
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-Me-Glu*p*	1.15	2.4	43, 59, 71, 87, 101, 117, 129, 145, 161, 173, 205	1 Glu—
1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-Me-Gal*p*	1.17	2.8	43, 58, 71, 87, 99, 101, 117, 131, 147, 161, 191, 233	6 1 → Gal—
1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-Me-Glu*p*	1.22	7.6	43, 58, 75, 87, 99, 101, 117, 131, 147, 161, 191, 203, 233	6 1 → Glu—

Note: Relative retention time was determined using 2,3,4,6-tetra-*O*-methyl-*O*-glucopyranose as a standard.

1:2.4:2.8:7.6. These results indicate non-reducing-end D-glucopyranosyl, 1,6-linked D-glucopyranosyl, 1,6-linked galactopyranosyl, and 1,5-linked arabinofuranosyl moieties present in polysaccharide A. Four anomeric carbon peaks at δ 105.8, 102.8, 100.9, and 95.5 in the ^{13}C NMR spectrum of polysaccharide A (Table 2) were assigned to C-1 of β-arabinofuranosyl, β-glucopyranosyl, α-galactopyranosyl, and α-glucopyranosyl residues, respectively. Polysaccharide A was proposed to be a linear backbone mainly consisting of 1,6-linked β-D-glucopyranosyl residues with insertion of 1,6-linked α-D-galactopyranosyl and 1,5-linked β-D-arabinofuranosyl residues.

Methylation analysis of polysaccharide B gave mainly the peaks of 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-arabinitol (*m/z*: 43, 87, 101, 117, 129, 161, 189), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol (*m/z*: 43, 59, 71, 87, 101, 117, 129, 145 161, 173, 205), 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-mannitol (*m/z*: 43, 75, 88, 101, 117, 129, 161, 189, 217, 233), 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-glucitol (*m/z*: 43, 59, 74, 87, 101, 117, 129, 149, 161, 189, 221, 233), and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-galactitol (*m/z*: 43, 58, 75, 87, 101, 113, 131, 145, 161, 178, 191, 205, 233, 261) in an approximate molar ratio of 3.0:2.4:1:7.2:3.4 (Table 3), which revealed the presence of 1,5-linked D-arabinofuranosyl, non-reducing-end D-glucopyranosyl, 1,3-linked D-mannopyranosyl, 1,6-linked D-glucopyranosyl, and 1,4,6-linked D-galactopyranosyl moieties in polysaccharide B. The ^{13}C NMR spectrum (Table 2) confirmed the anomeric carbon configurations of α-D-arabinofuranosyl, β-D-glucopyranosyl, α-D-galactopyranosyl, β-D-mannopyranosyl, and β-D-glucopyranosyl residues based on the anomeric carbon signals at δ 106.4, 102.7, 99.7, 98.8, and 95.5, respectively. According to the analysis above, polysaccharide B may have a chain consisting of 1,5-linked α-D-arabinofuranosyl, 1,6-linked α-D-galactopyranosyl, 1,3-linked β-D-mannopyranosyl, and 1,6-linked β-D-glucopyranosyl residues, but 4-*O* of α-D-galactopyranosyl residues were branched by terminal β-D-glucopyranosyl residues.

Methylation analysis of polysaccharide C (Table 4) showed the presence of 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-xylinitol (*m/z*: 43, 87, 101, 117, 129, 161, 189), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-

Table 2. The ^{13}C NMR data of polysaccharides A–C.

Polysaccharides	Composition	δ (ppm)					
		C-1	C-2	C-3	C-4	C-5	C-6
A	β-Ara(1 → 5)	105.8	87.6	76.3	83.8	67.2	
	β-Glu(1 →)	95.5	74.3	75.8	69.1	74.8	68.0
	α-Gal(1 → 6)	100.9	69.2	70.9	70.0	72.2	63.5
	β-Glu(1 → 6)	102.8	73.1	75.7	69.9	74.9	69.1
B	α-Ara(1 → 5)	106.4	73.2	74.9	70.6	69.2	
	β-Glu(1 →)	95.5	74.3	75.7	69.2	74.8	69.1
	β-Man(1 → 3)	98.8	69.2	80.4	65.5	77.6	63.6
	β-Glu(1 → 6)	102.7	73.0	75.5	69.0	74.8	63.8
	α-Gal(1,4 → 6)	99.7	69.2	71.5	71.3	72.5	63.6
C	β-Xyl(1 → 5)	102.7	73.8	74.6	77.8	63.8	
	α-Ara(1 → 5)	106.4	87.5	76.3	83.8	67.1	
	β-Man(1 → 4)	101.2	71.4	73.8	67.2	75.5	67.1
	β-Glu(1 →)	95.5	74.3	75.7	69.1	74.9	69.1
	β-Gal(1 → 4)	105.4	72.7	73.8	69.2	76.3	63.6
	α-Glu(1,3 → 6)	99.7	72.2	73.8	78.6	70.9	60.0

glucitol (*m/z*: 43, 71, 87, 101, 117, 129, 145, 161, 191, 205), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-galactitol (*m/z*: 43, 76, 87, 99, 101, 117, 131, 161, 173, 205, 233, 261), 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-glucitol (*m/z*: 43, 59, 74, 87, 101, 117, 129, 149, 161, 189, 221, 233), 1,4,5-di-*O*-acetyl-2,3,5-tri-*O*-Me-arafuranosyl (*m/z*: 43, 58, 74, 87, 101, 117, 129, 161), and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-Me-mannitol in a molar ratio of 1.4:2.8:3.7:7.3:3.5:1, and indicated the presence of 1,5-linked xylopyranosyl, non-reducing-end D-glucopyranosyl, 1,4-linked galactopyranosyl, 1,3,6-linked glucopyranosyl residues, 1,5-linked arabinofuranosyl, and

Table 3. The GCMS data of polysaccharide B after methylation.

Composition	Relative retention time (min)	Molar ratio	Fragment peaks of MS	Connection
1,4,5-Tri-*O*-acetyl-2,3-di-*O*-Me-Ara*f*	1.02	3.0	43, 87, 101, 117, 129, 161, 189	5 1 → Ara—
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-Me-Glu*p*	1.09	2.4	43, 59, 71, 87, 101, 117, 129, 145, 161, 173, 205	1 Glu—
1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-Me-Man*p*	1.10	1	43, 75, 88, 101, 117, 129, 161, 189, 217, 233	3 1 → Man—
1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-Me-Glu*p*	1.18	7.2	43, 59, 74, 87, 101, 117, 129, 149, 161, 189, 221, 233	6 1 → Glu—
1,4,5,6-Tetra-*O*-acetyl-2,3-di-*O*-Me-Gal*p*	1.22	3.4	43, 58, 75, 87, 101, 113, 131, 145, 161, 178, 191, 205, 233, 261	6 4 1 ↓ →Glu—

Note: Relative retention time was determined using 2,3,4,6-tetra-*O*-methyl-*O*-glucopyranose as a standard.

Table 4. The GCMS data of polysaccharide C after methylation.

Composition	Relative retention time (min)	Molar ratio	Fragment peaks of MS	Connection
1,4,5-Tri-*O*-acetyl-2,3-di-*O*-Me-Xyl*f*	0.84	1.4	43, 87, 101, 117, 129, 161, 189	5 1 → Xyl—
1,4,5-Di-*O*-acetyl-2,3,5-tri-*O*-Me-Ara*f*	0.84	3.5	43, 58, 74, 87, 101, 117, 129, 161	5 1 → Ara—
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-Me-Man*p*	1.09	1	43, 45, 58, 71, 87, 101, 113, 117, 129, 143, 161, 173, 203, 223, 233	4 1 → Man—
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-Me-Glu*p*	1.16	2.8	43, 71, 87, 101, 117, 129, 145, 161, 191, 205	1 Glu—
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-Me-Gal*p*	1.22	3.7	43, 76, 87, 99, 101, 117, 131, 161, 173, 205, 233, 261	4 1 → Gal—
1,3,5,6-Tetra-*O*-acetyl-2,4-di-*O*-Me-Glu*p*	1.36	7.3	43, 59, 74, 87, 101, 117, 129, 149, 161, 189, 221, 233	6 3 1 ↓ →Glu—

Note: Relative retention time was determined using 2,3,4,6-tetra-*O*-methyl-*O*-glucopyranose as a standard.

1,4-linked mannopyranosyl. The ^{13}C NMR spectrum (Table 2) showed six peaks in the region of anomeric carbon resonances at δ 106.4, 105.4, 102.7, 101.2, 99.7, and 95.5 assigned to be C-1 of α-D-arabinofuranosyl, β-D-galacopyranosyl, β-D-xylopyranosyl, β-D-mannopyranosyl, α-D-glucopyranosyl, and β-D-glucopyranosyl residues, respectively. These results revealed that polysaccharide C may possess a backbone consisting of 1,5-linked α-D-arabinofuranosyl, 1,4-linked β-D-galactopyranosyl, 1,5-linked β-D-xylopyranosyl, 1,4-linked β-D-mannopyranosyl, 1-linked β-D-glucopyranosyl residues, and the terminal β-D-glucopyranosyl residues are attached to 3-*O* positions of 1,6-linked α-D-glucopyranosyl residues.

3. Experimental

3.1 General experimental procedures

Total sugars were determined by the phenol–sulfuric acid analysis at 490 nm using a UV-2100 spectrophotometer (Unico, Shanghai, China). GC analysis was performed using an HP5890 instrument equipped with a DB-5 fused-silica capillary column (60 m × 0.32 mm × 0.25 μm; Agilent, CA, USA). GC-MS was analyzed by HP5890 and VG-TRIOL MICROMASS (Agilent). NMR was analyzed in D_2O (30 mg/ml), with TMS as the internal standard, using JNM-ECA400 NMR (JEOL, Tokyo, Japan) at normal temperature.

3.2 Plant material

The root of *U. fissa* was collected in July, 2005, in Anshun mountains of Guizhou Province, China. It was identified by Prof. Cun-Sheng Liu in the Beijing University of Chinese Traditional Medicine. A voucher specimen (No. 200507) is deposited in our laboratory.

3.3 Plant extraction

The roots of *U. fissa* (200 g) were pre-extracted with 95% ethanol (2000 ml) to remove pigments. The depigmented plant residue was extracted with boiling water (1600 ml) for 3 h twice. The aqueous

extract was centrifuged to remove water-insoluble materials and ultrafiltrated to remove small molecular substances using an ultrafiltration system including a pump and a hollow fiber microporous membrane cartridge (5 cm i.d. × 30 cm) with a cut-off molecular weight of 5000. The concentrated large molecule fraction (100 ml) was lyophilized to yield the crude polysaccharide extract (9.5 g).

3.4 Purification of the polysaccharides

The protein in the crude polysaccharide extract was removed by Sevage method (chloroform–*n*-butanol, 4:1, v/v) for seven times. Deprotein crude polysaccharide (800 mg) was purified by chromatography on a DEAE-Sephadex A-25 (2.5 cm i.d. × 50 cm; Amersham Biosciences, GE Healthcare Life Science, Fairfield, CT, USA) column using water elution, followed by elution on the stepwise gradient of NaCl (0.2–1.0 M). The fractions were monitored spectrophotometrically using the phenol–sulfuric acid analysis.

The main fraction (336 mg) eluted with water was further separated by a Sepharose 6B (2 cm i.d. × 70 cm; Amersham Biosciences, GE Healthcare Life Science) column with water elution to yield a homogeneous polysaccharide A (167 mg). The other main fraction (294 mg) eluted with 0.2 M NaCl from a DEAE-Sephadex A-25 column was further separated by Sepharose 6B and obtained two homogeneous polysaccharides B (105 mg) and C (135 mg).

3.5 Homogeneity and molecular weight determination

Polysaccharides A–C (5 mg each) were loaded on a Sephacryl S-200-HR (1 × 100 cm; Amersham Biosciences, GE Healthcare Life Science) column and eluted with water. Fractions (1 ml) were collected consecutively and monitored with the phenol–sulfuric acid analysis. The column was calibrated with standard molecular weight dextrans (D7100, D10000, D41100, D84400, D133800 from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China).

3.6 Compositional analysis

The polysaccharides were hydrolyzed with 2 M trifluoroacetic acid at 110°C for 5 h, and the monosaccharides were analyzed by GC of their alditol acetates (Blakeney, Harris, Henry, and Bruce, 1983) with a temperature gradient of 150–310°C at an ascending rate of 5°C/min.

3.7 Methylation analysis

Methylation of polysaccharides (5 mg) was carried out according to Needs and Selvendran method [20]. The fully methylated polysaccharides were hydrolyzed, acetylated, and analyzed by GC-MS. The partially methylated alditol acetates were identified by their fragment ions in EI-MS.

4. Conclusion

As far as we know, there has been no report on the structural characterization of the polysaccharides from the roots of *U. fissa*. We isolated three neutral polysaccharides A–C and some structural features were characterized. All three polysaccharides are mainly composed of D-arabinofuranosyl, D-galactopyranosyl, and D-glucopyranosyl residues. Polysaccharide A consists of a linear chain of 1,6-linked β-D-glucopyranosyl, 1,6-linked α-D-galactopyranosyl, and 1,5-linked β-D-arabinofuranosyl residues. Polysaccharide B possesses a chain consisting of 1,5-linked β-D-arabinofuranosyl, 1,6-linked α-D-galactopyranosyl, and 1,6-linked β-D-glucopyranosyl residues, but terminal α-D-glucopyranosyl residues were attached to 4-*O* of α-D-galactopyranosyl residues. Polysaccharide C may contain a chain consisting of 1,5-linked β-D-arabinofuranosyl, 1,4-linked α-D-galactopyranosyl,

and 1,6-linked β-D-glucopyranosyl residues, but 3-*O*-positions of 1,6-linked β-D-glucopyranosyl residues were substituted by terminal α-D-glucopyranosyl residues.

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