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Structural features of a heteroxylan from *Sophora subprostrata* roots

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Abstract

An acidic heteroxylan, SSb2, was isolated from the alkaline extract of *Sophora subprostrata* by DEAE-Sephadex A-25 and Sephacryl S-300 chromatography, with an M_r of 4.4×10^5 and a specific optical rotation of -49.2° (*c* 0.5, H_2O). Its structural features were elucidated by methylation analysis, partial acid hydrolysis, periodate oxidation and ^{13}C NMR spectroscopy. The data obtained indicated that SSb2 has a backbone consisting of 1,4-linked xylopyranosyl residues with branches at O-2 or O-3, composed of L-Ara$_f$(1 → 5)L-Ara$_f$(1 → , 4-*O*-Me-D-GlcA(1→ or D-GlcA(1 → . SSb-2 exhibited an immunostimulating activity in vivo in pharmacological experiments.

Keywords: *Sophora subprostrata*; Leguminosae; Heteroxylan; Polysaccharides

1. Introduction

Sophora subprostrata, a widely used Chinese medicinal plant, is distributed mainly in southern China, whose roots are used as an antipyretic, antidote or analgesic; it has been reported to have antitumour activity (Chen & Jiang, 1994). From its 1 M sodium hydroxide extract, we isolated three polysaccharide fractions, among which, SSb1, an xyloglucan with an immunosupressive effect, has been reported elsewhere (Dong & Fang, 1998). In this communication, we describe the structure of SSb2, an acidic heteroxylan.

2. Results and discussion

The dried roots were refluxed with ethanol to remove lipids and then extracted with boiling water three times. The residue was extracted with 1 M sodium hydroxide at 4°C. After neutralization, dialysis and precipitation with ethanol, a crude preparation (SSb) was obtained as a brownish powder. The crude polysaccharide was fractionated by anion-exchange chromatography (DEAE-Sephadex A-25, Cl^- form) with water and NaCl gradient elution (0–2 M). The eluate was monitored polarimetrically and SSb1 recovered from the aqueous eluate and SSb2 and SSb3 from the saline eluate (cf. Fig. 1). SSb2 was rechromatographed on a Sephacryl S-300 column and shown to be homogeneous by high performance gel permeation chromatography. Its M_r was estimated to be 4.4×10^5 based on comparison with dextran samples of known M_rs. The specific rotation $[\alpha]_D^{20}$ was -49.2° (*c* 0.5, H_2O). The polysaccharide was free of nitrogen, based on elementary analysis.

After complete hydrolysis with 2 M TFA, SSb2 was shown by TLC to contain neutral monosaccharides and uronic acid. The polysaccharide was reduced with $NaBH_4$ three times as described by Conrad and Taylor (SSb2R) (Taylor & Conrad, 1972). The sugar compositions of native and reduced SSb2 were analyzed by GC after the hydrolysates were converted into their alditol acetates (Table 1). The polysaccharide was composed of L-arabinose, D-xylose, 4-*O*-Me-D-glucuronic acid and D-glucuronic acid in the molar ratios of ca. 0.25:1.0:0.11:0.03.

Glycosyl linkages were determined by methylation analysis of SSb2R. The partially methylated alditol acetates were identified by GC–mass spectrometry

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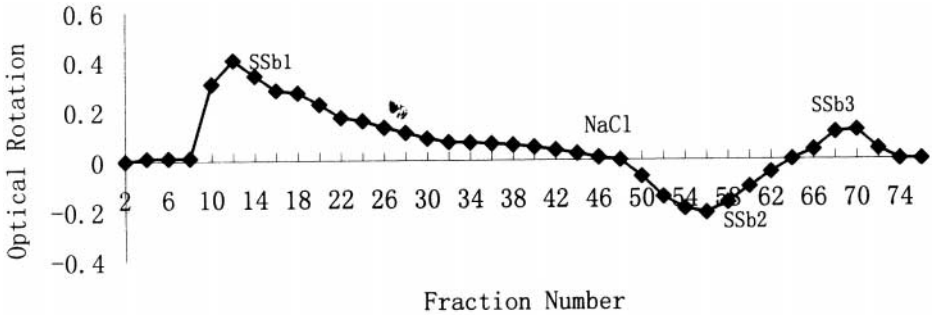

Fig. 1. Separation of SSb on DEAE-Sephadex A-25.

Table 1. Composition of hydrolysates of SSb2[a] before and after reduction

Residues	L-Ara	D-Xyl	4-*O*-Me-D-Glc	D-Glc
SSB-2	13.1	63.0	–	–
SSB-2R	18.5	70.0	8.0	2.4

[a]SSb2 is a fraction isolated from crude polysaccharide (cf. Fig. 1).

(Table 2). The results showed that the polysaccharide contains 1- and 1,5-linked L-arabinofuranose, 1,4- 1,3,4- and 1,2,4-linked D-xylopyranose and non-reducing terminal 4-*O*-Me-D-glucuronic acid or D-glucuronic acid, indicating a backbone composed of $1 \rightarrow 4$ linked D-xylosyl residues, with branches at *O*-2 or *O*-3 of some xylosyl residues. Nonreducing terminals include L-Ara_f, 4-*O*-Me-D-GlcA and D-GlcA.

SSb2 consumed 0.75 sodium periodate and produced 0.03 formic acid for each pentosyl residue, indicating that some of the uronic acid was not methylated at position 4, in agreement with composition analysis. The oxidized SSb2 was reduced and then completely hydrolyzed with 1 M TFA; the hydrolysates included glycerol, 3-*O*-Me-erythronic acid and D-xylose, as detected by TLC. D-Xylose resulted from the branch point residues and 3-*O*-Me-erythronic acid from terminal 4-*O*-Me-D-GlcA. Glyceric acid, expected from terminal D-GlcA, was not detected.

The anomeric configuration of each residue was elucidated by ^{13}C NMR which involved 21 signals. The signal at 178.9 ppm can undoubtedly be assigned to C-6 of uronic acid residues. The anomeric region includes four signals, among which 109.7 ppm arises from α-L-Ara_f, the strong signal at 103.8 ppm from internal β-D-Xyl_p and 103.5 ppm from β-D-xyl_p at branch points. Thus, 99.7 ppm can only be assigned to 4-*O*-Me-α-D-GlcA and so the anomeric configurations are all determined. The other four strong signals at 78.6, 75.9, 74.9 and 65.2 ppm can be assigned to C-4, C-3, C-2 and C-5 of xylopyranosyl residues, respectively. The three weaker signals at 86.2, 84.6 and 79.0 ppm probably arise from C-4, C-2 and C-3 of arabinofuranosyl residues. In the secondary carbon (CH_2) region, 67.4 ppm can be assigned to C-5 of 1,5-linked Ara_f and 63.3 ppm from C-5 of non-reducing L-Ara_f. The signal at 62.0 ppm is from methyl carbon of 4-*O*-Me-D-GlcA.

After hydrolysis with 0.1 M TFA at 80°C for 2 h, products soluble and insoluble in ethanol were separated giving PHA (supernatant) and PHB (precipitate), respectively. The former was further separated on

Table 2. GC–MS data for methylation analysis of SSb2 and PHB[a]

Components	Molar ratios		Mass fragments (*m*/*z*)	Linkages
	SSb-2	PHB		
2,3,5-Me_3-Ara	8.2	–	45, 101, 117, 129, 161, 205	Ara_f(1→
2,3,4-Me_3-Xyl	–	5.4	101, 117, 129, 161	Xyl(1→
2,3-Me_2-Ara_f	8.0	–	87, 101, 117, 161, 189	→5)Ara_f(1→
2,3-Me_2-Xyl	53.6	62.9	101, 117, 129, 161, 189	→4)Xyl(1→
2,3,4,6-Me_4-Glc	11.0	–	45, 117, 129, 161, 205	Glc(1→
2-Me-Xyl	6.4	–	117, 129, 161, 201, 261	→3,4)Xyl(1→
3-Me-Xyl	12.8	15.9	87, 129, 189	→2,4)Xyl(1→
2,3,4-Me_3-Glc	–	15.7	101, 117, 129, 161, 189, 233	GlcA(1→

[a]PHB is a polysaccharide fraction from partial hydrolysis of SSb2 (see Section 3).

Sephadex G-15 giving mono-, di- and trisaccharides. The first was only L-arabinose. Hydrolysis of the second two by 2 M TFA indicated that the sole di- and trisaccharides were xylobiose and xylotriose. PHB was purified by gel filtration and then hydrolyzed with 2 M TFA. The hydrolysate was analyzed by TLC, showing the presence of xylose and 4-*O*-Me-glucuronic acid, suggesting that uronic acids are attached directly to backbone. The positions to which uronic acids are linked were O-2, as indicated by methylation analysis on PHB (Table 2). This was supported by the almost equal molar ratios of 2,3,4,6-Me_4-Glc and 3-Me-Xyl after methylation analysis. Thus, the arabinosyl residues were probably linked in branches as L-Ara_f(1 → 5)L-Ara_f(1→ at O-3 of xylosyl residues.

From the above results, we concluded that SSb2 is an acidic heteroxylan containing a (1 → 4) β-D-xylan backbone with branches composed of L-Ara_f(1 → 5)L-Ara_f(1→ linked to O-3 and 4-*O*-Me-D-GlcA(1→ or D-GlcA(1→ linked to O-2. The backbone was the same as that of the acidic heteroxylans reported earlier, such as those from *Acanthopanax senticosus* (Fang & Proksch, 1985), beeswing wheat bran (Asensio, 1987), *Quercus suber* (Dupont & Selvendran, 1987) and wheat endosperm (Hoffmann, 1992). The difference is mainly in the degree of branching and the fine structure of branches, for example, the branch of Ara_f(1 → 5)L-Ara_f(1 → , which was less reported in other heteroxylans. SSb2 is freely soluble in water and cannot be precipitated from aqueous solution by adjusting the pH to 4.5. This may be accounted for by the branching structure which substantially modifies the molecular conformation in solution. SSb2 was extracted along with xyloglucan under alkaline condition, possibly indicating the xylans are to some extent bonded to xyloglucans in the cell wall, as proposed before (Asensio, 1987). In vivo pharmacological experiments on mice (unpublished data) showed that SSb2 was active in increasing the weight of the spleen and in stimulating the proliferation of B lymphocytes, indicating immunostimulating activity.

3. Experimental

3.1. General

Evaporations were carried out under red. pres. below 45°C. TLCs were performed on Pei-cellulose (Merck) with EtOAc–pyridine–HOAc–H_2O (5:5:1:3) and detected by spraying with aniline-*o*-phthalic acid reagent. GC was performed on a capillary column DP-1701, (0.255 mm × 30 m, temp.: 200°C) with a FID. HPGPC was performed with a RI detector and a Sugar KS-805 column (Showa Denko K.K.).

3.2. Plant material

Roots of *S. subprostrata* Chun et T. Chen as dried crude drug were collected from the Zhejiang province of China.

3.3. Isolation and purification

Dried roots (5 kg) were first refluxed with EtOH for 16 h and then extracted ×3 with boiling H_2O for 8 h. After filtration, the residue was extracted with 1 M NaOH at 4°C for 8 h, the alkaline extract neutralized with 2 M HCl and dialyzed against running H_2O. The nondialysate was concd to small vol. and ppted with 3 vol. of EtOH. The ppt. was obtained by centrifugation and washed sequentially with EtOH and Et_2O and gave a brownish powder SSb (78 g). An aliquot (10 g) in H_2O (20 ml) was fractionated on DEAE-Sephadex A-25 (2.6 × 90 cm). The column was first eluted with H_2O, followed by gradient elution with 0–2 M NaCl. SSb2 (0.8 g) was obtained in the NaCl eluate. On HPGPC, it gave a symmetrical peak, indicating homogeneity.

3.4. Homogeneity and M_r

These were determined by HPGPC on a Sugars KS-805 column. Calibration was made with dextrans T-2000, T-110, T-70, T-40 and T-20. 0.002 M NaOH was used as the mobile phase and the flow rate was maintained at 0.4 ml min^{-1}. All samples were prepd as 0.1% (w/v) solns; 20 μl of soln was injected in each run.

3.5. Compositional analysis

SSb2 (5 mg) was hydrolyzed with 2 M TFA (4 ml) at 110°C for 1.5 h in a sealed tube. After removal of TFA, the hydrolyzate was analyzed by TLC. One part of the hydrolyzate was dissolved in H_2O (2 ml) and reduced with $NaBH_4$ (25 mg) at room temp. for 2 h. The resulting alditols were acetylated with Ac_2O (2 ml) at 100°C for 1 h. The alditol acetates were analyzed by GC. Another portion of SSb2 (20 mg) was reduced ×3 according to Taylor and Conrad (1972) to give a carboxyl-reduced polysaccharide SSb2R (15 mg). This material was hydrolyzed and analyzed as described for SSb2.

3.6. Methylation analysis

SSb2 (6 mg) was methylated ×3 according to the modified NaOH–DMSO procedure (Needs, 1993) and the completeness of methylation confirmed by IR from the absence of OH absorption at 3500 cm^{-1}. Partially

methylated alditol acetates were prepd and analyzed by GC–MS.

3.7. $NaIO_4$ oxidation

SSb2 (20 mg) was oxidized with 0.01 M sodium periodate for 4 days. Consumption of $NaIO_4$ was measured by spectrometrically and HCO_2H production by titration with 0.01 M NaOH. The resulting polyhydroxyl derivative was reduced and obtained by dialysis, then subject to hydrolysis with 1 M TFA at 100°C for 8 h. After evapn, the residue was analysed by TLC.

3.8. Partial hydrolysis

SSb2 (50 mg) was dissolved in 0.1 M TFA (8 ml) and hydrolyzed at 80°C for 2 h. After evapn to remove TFA, the hydrolyzate was redissolved in water (3 ml) and ppted with EtOH (14 ml) and centrifuged. The supernatant was concd and separated by Sephadex G-15 chromatography (1.6×90 cm). Frs were detected by the H_2SO_4–PhOH method and analyzed by TLC before and after complete hydrolysis with 2 M TFA. The ppt. was chromatographed on Sephadex G-200 (2.6×90 cm) and the main polysaccharide fr. collected, dialyzed and freezed-dried (PHB). PHB (2 mg) was hydrolyzed with 2 M TFA for 1.5 h at 110° and analyzed by TLC. PHB (4 mg) was permethylated and reduced with $LiAlH_4$ (Teixeira, Iocomini, McCune, & Gorin, 1994), then analyzed using the usual procedure for methylation analysis.

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