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POLYSACCHARIDES OF SAPONIN-BEARING PLANTS. XV. STRUCTURE OF GLUCOARABINOGALACTAN FROM *Acantophyllum Borszczowii* ROOTS

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The structure of the branched polysaccharide glucoarabinogalactan was investigated by periodate oxidation, methylation, and ^{13}C NMR spectroscopy. The main chain consists of α-1→6-bonded galactopyranoses. 1→2-Bonded D-glucopyranoses and 1→3-bonded D-galactopyranoses are located on the unreducing ends. There is a short side chain with β-1→3-bonded L-arabinopyranoses.

Key words: *Acantophyllum Borszczowii*, glucoarabinogalactan, structure, oligosaccharide, galactobiose, galactotriose, galactotetrose, galactopentose.

We found during an investigation of polysaccharides of *Acantophyllum Borszczowii* [1] that the main polysaccharide from the plant roots is a water-soluble polysaccharide (WSPS) from which a neutral polydisperse polysaccharide (NPS) was obtained by separation over DEAE-cellulose. Fractional precipitation with alcohol from an aqueous solution of the NPS isolated three homogeneous fractions I, II, and III in yields of 8, 66, and 11%, respectively.

Fraction II was quantitatively the main one. Paper chromatography (PC) and GC detected glucose, galactose, and arabinose in the ratio 2:5.3:1, respectively, in the acid hydrolysate. Therefore, fraction II contains a glucoarabinogalactan as a white amorphous powder that is freely soluble in water. Its IR spectrum exhibits absorption bands at 3375, 2937, 1638, 1419, 1350, 1075, 920, 860, and 799 cm^{-1}.

Periodate oxidation of the glucoarabinogalactan consumes 1.56 mole of periodate per mole of anhydrous unit and releases 0.58 mole of formic acid. The products of Smith degradation [2] include glycerine, galactose, and arabinose according to PC and GC. This is consistent with the presence of 1→2, 1→3, or 1→6 bonds between the monosaccharides.

Hakomori methylation [3] produced the fully methylated product. Its IR spectrum lacked absorption bands for hydroxyls. After formolysis and hydrolysis of the permethylate, PC and GC of the cleavage products detected 2,3,4,6-tetra-O-Me-Gal*p*, 2,3,4,6-tetra-O-Me-Glc*p*, 3,4-di-O-Me-Gal*p*, 2,4-di-O-Me-Gal*p*, 2,3,4-tri-O-Me-Gal*p*, 2,3,4-tri-O-Me-Ara*p*, and 2,4-di-O-Me-Ara*p* in a 2:6:3:6:5:2:1 ratio according to comparison with authentic samples.

The observation of 2,3,4-tri-O-Me-Gal*p* indicates that the main chain contains a polysaccharide of (1→6)-bonded D-galactoses. The presence of 3,4-di-O-Me-Gal*p* and 2,4-di-O-Me-Gal*p* indicates that the main chain is branched at C-2 and C-3. The observation of 2,3,4-tri-O-Me-Ara*p* and 2,4-di-O-Me-Ara*p* indicates that a short chain forms the side chain. The appearance of 2,3,4,6-tetra-O-Me-Gal*p* and 2,3,4,6-tetra-O-Me-Glc*p* is consistent with D-galactose and D-glucose at the unreducing ends of the polysaccharide.

The products from oxidation of the polysaccharide by chromic anhydride contain free glucose and galactose, which confirms the presence of α-glycoside bonds. Only the arabinoses, which have β-glycoside bonds between them, are oxidized.

The glucoarabinogalactan was partially acid-hydrolyzed for a complete structure determination. The hydrolysis products included arabinose, glucose, galactose, galactobiose (A), galactotriose (B), galactotetrose (C), and galactopentose (D).

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TABLE 1. Properties of Oligosaccharides A-D of Glucoarabinogalactan

Oligosaccharide	$[\alpha]_D^{25}$, deg (*c* 1.0; water)	Product		CP
		reduction (hydrolysate)	methylation (hydrolysate)	
		galactose	(**2**)	
OC-A	+142	1	1	2
OC-B	+160	2	2	3
OC-C	+175	3	3	4
OC-D	+180	4	4	5

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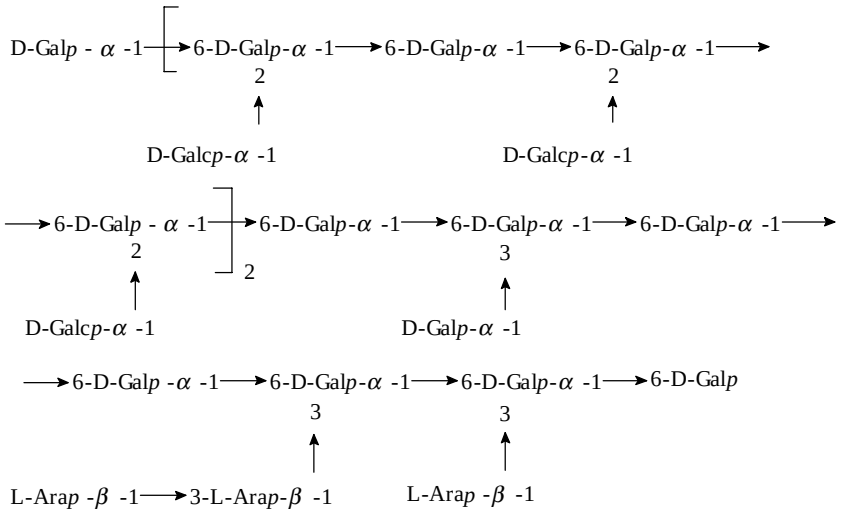
α-(1→6) Bonds; the product of reduction dulcite and methylation (**1**) are equal to 1.

TABLE 2. ^{13}C NMR Chemical Shifts of *A. Borszczowii* Glucoarabinogalactan

Compound	^{13}C chemical shifts, ppm from TMS					
	C-1	C-2	C-3	C-4	C-5	C-6
→6-Gal*p*-α-1 →	100.15	69.49	70.49	70.49	72.25	62.28
D-Glc*p*-α-1 →	99.60	72.25	74.34	70.5	73.25	61.85
L-Ara*p*-β-1 →	101.7	69.62	69.95	70.1	63.38	-
→3-Ara*p*-β-1 →	93.01	69.41	74.34	70.1	63.38	-



The total oligosaccharides were separated preparatively by PC to afford four pure oligosaccharides (OS). Total acid hydrolysis of these gave only D-galactopyranose (PC and GC). Periodate oxidation and Smith degradation (A-D) formed only glycerine. This is consistent with α-(1→2) or α-(1→6) bonds.

Hakomori methylation of OS A-D produced fully methylated products. After work up and total acid hydrolysis, TLC and GC showed that permethylates of OS A-D consisted of 2,3,4-6-tetra-O-Me-Gal*p* (**1**) and 2,3,4-tri-O-Me-Gal*p* (**2**) in ratios 1:1, 1:2, 1:3, and 1:4, respectively (Table 1).

The glucoarabinogalactan and its OS were studied by ^{13}C NMR spectroscopy to confirm the chemical data.

The spectrum of the glucoarabinogalactan contains strong signals at 100.15 and 99.60 ppm. This indicates that Gal*p* and Glc*p* in the α-configuration substituted with C-6 galactopyranoses resonate at 68.09 ppm.

Resonances at 102.48, 103.5, and 104.47 ppm are typical of C-1 of galactopyranose at branching points. Substituted C-2 and C-3 of galactopyranose appear at 82.02 and 85.31 ppm. Unreducing C-1 of β-Ara*p* resonates at 93.01 ppm. C-3 of bound Ara*p* appears at 74.34 ppm (Table 2) [4-6].

Thus, we propose a possible structure for glucoarabinogalactan from *A. Borszczowii* based on chemical and spectral data.

EXPERIMENTAL

GC was performed on a Chrom-5 instrument with a flame-ionization detector and stainless-steel column (200 × 0.01 mm), 5% Silicone XE-60 on Chromaton NAW (0.200-0.255 mm), 210°C, and He carrier gas (60 mL/min) for acetates of aldononitriles. Acetates of aldononitriles and polyols were prepared as before [7].

TLC was carried out on Silufol UV-254 plates and over LS-5/40 mm silica gel using benzene:acetone (2:1) with anilinium acid phthalate developer.

PC was carried out on FN-3, 11, and 12 paper using 1-butanol:pyridine:water (6:4:3) and anilinium acid phthalate as developer.

IR spectra were recorded on a Perkin—Elmer Model 2000 IR-Fourier spectrometer in KBr disks (5 mg compound per 200 mg KBr).

Polysaccharides and oligosaccharides were reduced by an excess of $NaBH_4$ using the literature method [8].

Total acid hydrolysis was carried out as usual [1].

^{13}C NMR spectra of oligo- and polysaccharides were recorded on a WM-250 Bruker instrument at working frequency 62.5 MHz for C nuclei using the literature conditions [8].

Specific rotations were measured on a Zeiss polarimeter in a 1-dm tube of 10 mL volume and a 0.5-dm tube of 1-mL volume at 20-23°C.

Separation of WSPS over DEAE-cellulose. DEAE-cellulose (200 g, beaded polymer) was treated successively with HCl (0.5 N, 1 L), water until neutral, NaOH (0.5 N, 1 L), water, $(NH_4)_2CO_3$ (1 M, 1 L), and water until neutral. DEAE-cellulose was packed into a column and washed with water (1 L). Then, a solution of polysaccharide in water (15.7 g/200 mL) was passed over the column and eluted with water (1 L). The elution of polysaccharides was monitored using phenol—sulfuric acid.

Aqueous eluates were evaporated to 200 mL and precipitated with alcohol (1 L). The resulting precipitate was separated, washed with alcohol and acetone, and dried in vacuum over P_2O_5. Yield of NPS, 11.3 g. PC and GC detected only galactose in its hydrolysate. Polysaccharides eluted by $(NH_4)_2CO_3$ (1 M) were concentrated and precipitated by alcohol (1:3). The resulting precipitate was separated, washed with alcohol, and dried in vacuum over P_2O_5. Yield of acidic polysaccharide (APS), 1.02 g. PC of its hydrolysate detected galacturonic acid, galactose, glucose, mannose, and arabinose.

Fractional Precipitation of NPS with Alcohol. NPS (2 g) was dissolved in H_2O (100 mL) and treated dropwise with alcohol (50 mL) with vigorous stirring. The resulting precipitate (fraction I) was separated by centrifugation, washed with alcohol (96°), and dried in vacuum over P_2O_5. Yield, 0.16 g. The mother liquor was treated with another portion of alcohol (50 mL). The precipitate (GAG-B, fraction II) was separated and dried. Yield 1.12 g. The mother liquor was treated again with alcohol. The precipitate (fraction III) was separated by centrifugation and dried over P_2O_5. Yield 0.22 g. The aqueous-alcohol mother liquor was evaporated and precipitated with alcohol (1:3). The precipitate was separated and dried (solid). Yield 0.16 g.

Gel Filtration of Fractions I, II, and III. Fractions I, II, and III (0.01 g each) were dissolved in distilled water (1 mL) and placed on a Sephadex G-50 column (2 × 40 cm) (each fraction was chromatographed separately). The column was calibrated using dextrans of known masses 20,000, 6,000, and 2,000 and rafinose of molecular weight 504. The molecular weights of the fractions were 6,000, 4,000, and 2,300, respectively.

Periodate Oxidation and Smith Degradation. Glucoarabinogalactan (0.05 g) was dissolved in water (25 mL), treated with sodium periodate solution (5 mL, 0.25 M), and left at 5°C. Each day aliquots (1 mL) were collected and titrated with sodium thiosulfate solution (0.01 N). The oxidation was stopped on the 14th day. The periodate consumption was 1.2 mole. The yield of formic acid was 0.8 mole. The periodate was destroyed with ethyleneglycol. The products were diluted with water, treated with $NaBH_4$ (0.1 g), and left overnight. The solutions were treated with cation-exchanger KU-2 (H^+). The filtrate was

evaporated with methanol. The dried precipitate was hydrolyzed with H_2SO_4 (4 mL, 1 N) for 10 h at 100°C. PC and GC of the hydrolysis products detected galactose, arabinose, and glycerine.

Methylation of Glucoarabinogalactan. Fractions (0.1 g) underwent Hakomori methylation twice. The product was extracted with $CHCl_3$ and evaporated to dryness. Yield 0.085 g. The completeness of methylation was checked by TLC.

Formolysis and Hydrolysis of the Permethylate. The permethylate was boiled on a water bath with formic acid (5 mL, 85%) for one hour, cooled, and evaporated. The solid was treated with H_2SO_4 (5 mL, 1 N) and hydrolyzed for 10 h at 100°C. The hydrolysate was worked up as usual. TLC and GC of the products detected 2,3,4,6-tetra-O-Me-Gal*p*, 2,3,4,6-tetra-O-Me-Glc*p*, 3,4-di-O-Me-Gal*p*, 2,4-di-O-Me-Gal*p*, 2,3,4-tri-O-Me-Gal*p*, 2,3,4-tri-O-Me-Ara*p*, and 2,4-di-O-Me-Ara*p* in the ratio 2:6:3:6:5:2:1.

Acetylation of Glucoarabinogalactan. Glucoarabinogalactan (0.1 g) was dissolved in formamide (5 mL), treated with anhydrous pyridine (7 mL), and dropwise with acetic anhydride (7 mL), and stirred for 6 d. The mixture was cooled with icewater (0.5 L). The solid was separated by centrifugation, washed with icewater, and dried in vacuum over P_2O_5. Yield of peracetate, 0.139 g.

Oxidation of Glucoarabinogalactan Peracetate with Chromic Anhydride. Chromic anhydride (0.2 g) was placed in a flask, dissolved in glacial acetic acid (5 mL), treated with glucoarabinogalactan peracetate (0.1 g), and heated for 5 h at 50°C. The mixture was diluted with water, extracted with $CHCl_3$, dried over anhydrous Na_2SO_4, and evaporated to dryness. The solid was dissolved in H_2SO_4 (2 mL, 1 N) and hydrolyzed for 10 h on a boiling water bath. The hydrolysate was treated as above. PC and GC detected free glucose and galactose. This is consistent with the presence of α-glycoside bonds between the monosaccharides.

Partial Hydrolysis of Glucoarabinogalactan. Fraction II (2.0 g) was dissolved in H_2SO_4 (10 mL, 0.25 N), hydrolyzed at 100°C for 5 min, neutralized with $BaCO_3$ and then cation-exchanger KU-2 (H^+), and evaporated to a syrup. PC detected arabinose, glucose, galactose, and OS A-D.

The hydrolysate was separated by PC. Bands corresponding to OS were cut out, extracted with water, and evaporated to dryness to afford four pure OS.

Hydrolysis of Oligosaccharides. OS A-D (0.1 g each) were treated with H_2SO_4 (0.25 N) at 100°C for 5 h. The hydrolysates were worked up as usual. PC of hydrolysates detected galactose.

Periodate Oxidation of Oligosaccharides. OS A-D (0.01 g each) were oxidized with sodium periodate (10 mL, 0.05 M, 15°C, 5 d). Then, they were treated with ethyleneglycol (two drops) and reduced with $NaBH_4$ (0.03 g) for 3 h. The mixtures were treated with cation-exchanger KU-2 (H^+) and evaporated to dryness. The dry solids were hydrolyzed with H_2SO_4 (0.25 N) at 100°C for 4 h. The hydrolysates were neutralized, deionized, evaporated, and investigated by PC and GC. Only glycerine was found.

Methylation of Oligosaccharides. OS A-D (0.01 g each) were methylated by the Hakomori method. The completeness of the process was checked by TLC. The hydrolysates of all permethylates contained 2,3,4,6-tetra-O-Me-D-galactose and 2,3,4-tri-O-Me-D-galactose.

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