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POLYSACCHARIDES OF SAPONIN-BEARING PLANTS. XIV. STRUCTURAL STUDY OF GLUCOARABINOGALACTAN FROM *Acanthophyllum pungens* ROOTS

A. O. Arifkhodzhaev,[1] A. D. Kurbanova,[1] D. A. Rakhimov,[1] and A. S. Shashkov[2]

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The structure of the polysaccharide glucoarabinogalactan, which is a branched polysaccharide, was established by chemical and spectral methods. Its main chain consists of α-1→6-bound galactopyranose units. Galactopyranose atoms C-2 and C-3 act as branching points. The side chains contain galactopyranose, glucopyranose, and chains of β-(1→3)-bound arabinopyranose units. The linear structures of three galactooligosaccharides were identified and proved by partial acid hydrolysis.

Key words: *Acanthophyllum pungens*, polysaccharide, glucoarabinogalactan, oligosaccharides, galactobiose, galactotriose, galactotetraose, structure.

We investigated polysaccharides of *Acanthophyllum pungens* [1]. The principal polysaccharide of the plant roots is water soluble. Separation over DEAE-cellulose afforded neutral polysaccharide (NPS) in 50% yield. The NPS, according to gel chromatography over Sephadex, is nonuniform. The NPS hydrolysate contains mainly glucose, arabinose, and galactose. The NPS was fractionally precipitated by ethanol to produce homogeneous fractions. Three fractions were obtained in yields of 17 (I), 76 (II), and 6.5% (III).

Fraction II contained the bulk of the material and was homogeneous according to gel chromatography over Sephadex G-50. Paper chromatography (PC) and GC of its hydrolysate detected D-glucose, L-arabinose, and D-galactose in the ratio 1.3:1.0:5.3. Therefore, this fraction contains a glucoarabinogalactan.

The glucoarabinogalactan is an amorphous white powder that is very soluble in water, forms a nonviscous solution with $[\alpha]_D^{25}$ +168° (*c* 1.0, H_2O), does not give a positive reaction with iodine for starch, and lacks nitrogen. The molecular weight determined by gel chromatography over Sephadex G-50 using a calibration curve and known dextran standards is 3700. The IR spectrum contains absorption bands at 860, 920, 980, 1079, 1152, 1350, 1420, 1655, 2935, 3359, and 3470 cm^{-1} and lacks signals corresponding to methyl, acetyl, and sulfate groups.

Periodate oxidation consumes 1.4 mole of $NaIO_4$ per mole of anhydrous polysaccharide unit and forms 0.52 mole of HCOOH. PC and GC of the decomposition products by the Smith method [2] detected glycerin, galactose, and arabinose, which is consistent with the presence of 1→2, 1→3, and 1→6 linkages between monosaccharide units, where the arabinose and galactose units have 1→3-glycoside bonds.

Methylation of the glucoarabinogalactan according to Hakomori [3] produced the methylated product, the IR spectrum of which lacked absorption bands of hydroxyls. The cleavage products after formolysis and hydrolysis of the permethylate included 2,3,4,6-*tetra-O*-Me-Gal*p*; 2,3,4,6-*tetra-O*-Me-Glc*p*; 2,3,4-*tri-O*-Me-Gal*p*; 3,4-*di-O*-Me-Gal*p*; 2,4-*di-O*-Me-Gal*p*; 2,3,4-*tri-O*-Me-Ara*p*, and 2,4-*di-O*-Me-Ara*p* in the ratio 3:4:6:4:3:1:2 according to PC and GC with authentic samples. The presence of 2,3,4-*tri-O*-Me-Gal*p* indicates that the main polysaccharide chain has α-1→6-bound Gal*p* units. The detection of 2,3,4,6-*tetra-O*-Me-Gal*p* and 2,3,4,6-*tetra-O*-Me-Glc*p* indicates that the D-galactose and D-glucose units are located on the nonreducible ends.

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1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, cnc@icps.org.uz; 2) N. D. Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Leninskii pr., 47. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 111-114, March-April, 2003. Original article submitted March 31, 2003.

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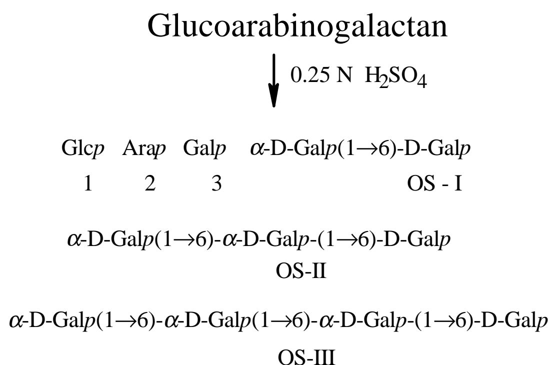
TABLE 1. ^{13}C NMR Chemical Shifts of *A. pungens* Glucoarabinogalactan

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→	100.14	69.47	70.5	70.4	72.15	62.32
D-Glc*p*-α-1→	99.58	72.28	74.37	70.5	73.26	61.84
L-Ara*p*-β-1→	101.58	69.64	69.91	70.16	63.33	
-3-Ara*p*-β-1→	93.10	69.4	74.37	70.16	63.33	



Scheme 1. Partial acid hydrolysis of *A. pungens* glucoarabinogalactan.

The presence of 3,4-*di-O*-Me-Gal*p* and 2,4-*di-O*-Me-Gal*p* proves that the main glucoarabinogalactan chain consists of D-Gal*p* units with 1→6 bonds and that the galactopyranose is branched at C-2 and C-3. The presence of 2,3,4-*tri-O*-Me-Ara*p* and 2,4-*di-O*-Me-Ara*p* indicates that the short chain is located in the side chain where the L-Ara*p* units are bound to each other by 1→3 bonds.

The high positive specific rotation of the glucoarabinogalactan and absorption bands at 860 and 920 cm^{-1} in the IR spectrum are consistent with the presence of an α-glycoside bond between the monosaccharide units. This hypothesis is confirmed by oxidation of the acetylated polysaccharide by chromic anhydride. The oxidation products include free glucose and galactose units. Only the arabinopyranose units, which have β-glycoside bonds between them, are oxidized. Therefore, free arabinose is not observed in this instance [4].

The glucoarabinogalactan was studied by ^{13}C NMR spectroscopy to confirm the chemical data. The chemical shifts of the C atoms are listed in Table 1.

Chemical shifts at 100.14 and 99.58 ppm indicate that the galactopyranose and glucopyranose units have the α-configuration. Substituted C-6 atoms of galactopyranose resonate at 67.13-68.79 ppm. The resonance regions at 102.45 and 104.45 ppm are typical of the galactopyranose C-1 atom located at the branching sites. Substituted C-2 and C-3 atoms of galactopyranose appear at 81.9 and 85.2 ppm. The C-1 atom of nonreducible β-Ara*p* resonates at 101.58; anomeric C atoms β-1→3-bonded to Ara*p*, at 93.10. Those C-3-bonded to Ara*p* appear at 74.37 ppm [5-7].

The structure of the polysaccharide was completely established using partial acid hydrolysis of the glucoarabinogalactan. The products contained glucose (**1**), arabinose (**2**), galactose (**3**), and three oligosaccharides according to PC (Scheme 1).

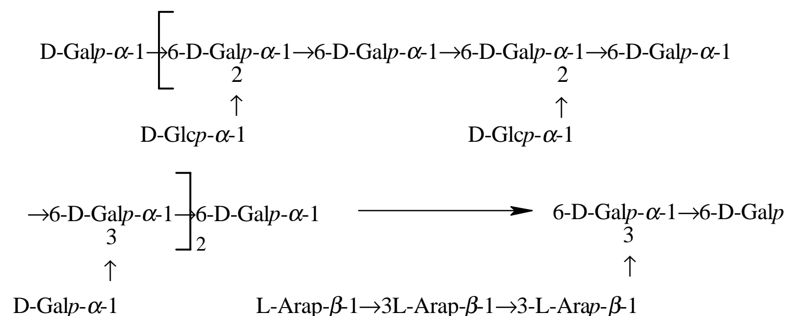
Preparative separation of the total oligosaccharides produced chromatographically pure compounds (OS-I, -II, and -III), the monosaccharide composition of which contains only D-galactopyranose (PC and GC). The structures of OS-I—III were studied by chemical and spectral methods.

Periodate oxidation and Smith decomposition formed only glycerin. Therefore, the monosaccharides in the oligosaccharides are bonded to each other by either an α-(1→2) or α-(1→6) bond. Methylation of OS-I—III according to Hakimori produced the methylated products (completion of the reaction monitored by IR spectroscopy). After the appropriate treatment and total acid hydrolysis, TLC and GC determined that the permethylates of OS-I, OS-II, and OS-III consist of 2,3,4,6-*tetra-O*-Me-D-Gal*p* (**4**) and 2,3,4-*tri-O*-Me-D-Gal*p* (**5**) in the ratios 1:1, 1:2, and 1:3, respectively.

Dulcite and galactose were detected in the products of oligosaccharide reduction by $NaBH_4$ after the appropriate treatment. The methylation and reduction data make it possible to determine the polymerization degree of OS-I—OS-III.

The results obtained by studying the oligosaccharide structures by ^{13}C NMR confirm the chemical data. The spectra of the oligosaccharides differ little from each other. The spectrum has strong signals at 99.6 (C-1), 69.8 (C-2), 70.6 (C-3), 70.9 (C-4), 72.3 (C-5), and 62.5 (C-6) ppm for the C atoms of the galactopyranose units.

We propose a possible chemical structure for the glucoarabinogalactan of *A. pungens* roots based on the chemical and spectral data presented above and the study of the structures of the oligosaccharides produced by partial acid hydrolysis.



EXPERIMENTAL

TLC was performed on Silufol UV-254 plates and on LS-5/40 mm silica gel. PC used Filtrak FN-3,11,12 paper and the solvent systems and developers described before [8].

GC was carried out on a Chrom 5 chromatograph with a flame-ionization detector and stainless-steel column (200×0.01 mm) with 5% silicone XE-60 on Chromaton NAW -0.200-0.255 at 210°C with He carrier gas flowing at 60 mL/min for aldonitrile acetates. The aldonitrile acetates were prepared as before [9]. The specific rotations of the compounds were measured on a Zeiss polarimeter using a tube 1-dm in length with a volume of 1 mL.

Products were reduced by an excess of $NaBH_4$ as before [10].

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

Total acid hydrolysis was performed using the literature conditions [1].

^{13}C NMR spectra of glucoarabinogalactan were taken on a Bruker WM-250 instrument at working frequency 62.5 MHz and the literature conditions [10].

Separation of Water-soluble Polysaccharide (WSPS) over DEAE-cellulose. The separation was performed over a DEAE-cellulose column as before [1]. An aqueous solution of WSPS (5.0 g) was separated into NPS and acidic PS (APS).

Elution with water produced NPS (2.5 g); with CH_3COONa (1 M), APS (0.75 g).

Fractionation of NPS by Alcohol. NPS (2 g) were dissolved in water (10 mL), stirred vigorously, and treated dropwise with alcohol (50 mL). The precipitate (fraction I) was separated by centrifugation, washed with alcohol (96°) and acetone, and dried in vacuo over P_2O_5. Yield 0.34 g. The supernatant liquid was treated with more alcohol (50 mL). The precipitate (fraction II) was separated and dried. Yield 1.52 g. The aqueous-alcoholic mother liquor was evaporated and treated with acetone (50 mL). The precipitate (fraction III) was separated and dried. Yield 0.13 g.

$[\alpha]_D^{25}$ +175, +168, +150 (*c* 1.0, H_2O) for fractions I, II, and III, respectively.

Periodate Oxidation and Smith Decomposition. The principal fraction II (0.05 g) was dissolved in water (25 mL), treated with sodium periodate solution (5 mL, 0.25 M), and left at +5°C. Aliquots (1 mL) were taken each day and titrated with sodium thiosulfate solution (0.01 N). After seven days, 1.4 moles of $NaIO_4$ were consumed. The amount did not change after this. The formic acid released during the reaction was titrated with NaOH solution (0.01 N). The calculated amount was 0.52 moles. The excess of periodate was destroyed by ethyleneglycol. The product was 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 CH_3OH. The dry solids were hydrolyzed in H_2SO_4 (3 mL, 1 N) for 6 h at 100°C. The hydrolysis products of fraction II contained glycerin in addition to galactose and arabinose.

Methylation of Glucoarabinogalactan. Glucoarabinogalactan (0.1 g) was methylated twice according to Hakimori [3] to afford the fully methylated product (the IR spectrum lacks absorption bands for OH) in 0.095 g yield; O–CH_3, 41.7%.

Formolysis and Hydrolysis of the Permethylate. The glucoarabinogalactan permethylate (0.05 g) was boiled on a water bath with formic acid (5 mL, 85%) for 1 h, cooled, and evaporated. The solid was treated with H_2SO_4 (5 mL, 0.5 N) and hydrolyzed for 10 h at 100°C. The hydrolysates were treated as usual. TLC and GC detected 2,3,4,6-*tetra-O*-Me-Gal*p*, 2,3,4,6-*tetra-O*-Me-Glc*p*, 2,3,4-*tri-O*-Me-Gal*p*, 3,4-*di-O*-Me-Gal*p*, 2,4-*di-O*-Me-Gal*p*, 2,3,4-*tri-O*-Me-Ara*p*, and 2,4-*di-O*-Me-Ara*p* in the ratio 3:4:6:4:3:1:2, respectively.

Acetylation of Glucoarabinogalactan. The compound (0.1 g) was dissolved in formamide (5 mL), treated with anhydrous pyridine (7 mL), dropwise with acetic anhydride (7 mL), and stirred for five days. The mixture was precipitated by icewater (0.5 L) and dried in vacuo over P_2O_5 to afford the peracetate in 0.130 g yield.

Oxidation of the Glucoarabinogalactan Peracetate by Chromic Anhydride. Chromic anhydride (0.2 g) was placed into a flask, dissolved in glacial acetic acid (5 mL), treated with the peracetate of fraction II (0.1 g), heated for 4 h at 50°C, diluted with water, and extracted with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The solid was dissolved in H_2SO_4 (1 mL, 1 N) and hydrolyzed for 10 h on a boiling-water bath. The hydrolysate was treated as usual. PC and GC of the hydrolysate detected free glucose and galactose in a 1:4 ratio.

Partial Acid Hydrolysis of Glucoarabinogalactan. Glucoarabinogalactan (1 g) was dissolved in H_2SO_4 (10 mL, 0.25 N), heated at 100°C for 10 min, neutralized by $BaCO_3$, deionized by cation-exchanger KU-2 (H^+), and evaporated to a syrup. PC detected glucose, arabinose, galactose, and three oligosaccharides.

The glucoarabinogalactan hydrolysate was separated by PC. Bands corresponding to pure oligosaccharides were cut out, extracted with water, and evaporated to dryness to afford three chromatographically pure oligosaccharides I-III in yields of 0.18, 0.15, and 0.07 g, respectively. Specific rotations $[\alpha]_D^{25}$ (*c* 1.0): 141.6 (I), 160.0 (II), 172.4 (III).

Oligosaccharide Hydrolysis. Compounds I, II, and III (0.01 g each) were hydrolyzed by H_2SO_4 (0.25 N) at 100°C for 4 h. Only galactose was observed in the hydrolysis products after the appropriate treatment, i.e., the oligosaccharides consist of D-galactopyranose units.

Periodate Oxidation and Smith Decomposition of the Oligosaccharides. Oligosaccharides were oxidized by sodium periodate solution (0.05 M) under the conditions described above. The reaction consumed 2 (OS-I), 2 (OS-II), and 1.8 (OS-III) moles of periodate. The amount of formic acid produced was 1 (OS-I), 1 (OS-II), and 0.9 (OS-III) moles.

PC and GC detected only glycerin in the hydrolysate of the oxidation products.

Methylation of Oligosaccharides. Oligosaccharides (0.01 g each) were methylated according to Hakimori, as described above to produce the fully methylated products. After the appropriate treatment and total acid hydrolysis, TLC and GC found that permethylates of I, II, and III consist of 2,3,4,6-*tetra-O*-Me-D-Gal*p* and 2,3,4-*tri-O*-Me-D-Gal*p* in the ratios 1:1, 1:2, and 1:3, respectively.

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