Chemistry of Natural Compounds, Vol. 48, No. 5, November, 2012 [Russian original No. 5, September–October, 2012]

ARABINOGALACTAN FROM THE AERIAL PART OF *Cardaria repens*

R. K. Rakhmanberdyeva

UDC 547.917

A polysaccharide with $[\alpha]_D$ –40.1° and MW 56,000 that consisted of L-arabinose, D-galactose, and galacturonic acid was obtained for the first time from the aerial part of Cardaria repens. *It was found that the polysaccharide was a branched arabinogalactan where the main chain was 1,6-β-D-galactan and several galactose units were substituted by 1,3-α-Araf and 1,2-α-D-GalUA units.*

Keywords: arabinogalactan, arabinose, galactose, methylation, periodate oxidation, partial hydrolysis.

The aerial part of *Cardaria repens* (Cruciferae) is known to contain 8.4% water-soluble polysaccharide (WSPS) [1, 2]. Fractionation by alcohol of the WSPS produced arabinogalactan (AG) with $[\alpha]_D$ –40.1° and MW 56,000 with an L-arabinose:D-galactose ratio of 1:2.2 and 1.1% galacturonic acid.

Smith degradation established that 2.7 moles of oxidant were consumed per mole of AG anhydrounit and that 0.33 moles of formic acid were released. The presence of glycerin in the polyalcohol hydrolysis products indicated that the monosaccharide units in the AG were joined through either 1,3- or 1,6-glycoside bonds. The identification of free arabinose was consistent with a branched polysaccharide structure.

Methylation of AG was performed by the Hakomori method [3]. The permethylate was subjected to formolysis and hydrolysis. Then, hydrolysis products were separated over a column of silica gel (L 150/250). The methylated product was washed by $CHCl_3$:MeOH (9:1) and analyzed by TLC. The presence of 2,3,4-tri-*O*-Me-D-Gal*p* in the AG hydrolyzate proved that the galactopyranose units in the polymer chain were 1,6-bonded. The presence of 2,3,4,6-tetra-*O*-Me-D-Gal*p* indicated that the AG chain had a galactopyranose unit at the non-reducing terminus. The AG macromolecule was reduced by $NaBH_4$ in order to identify galacturonic acid in it. The resulting reduced product was methylated as before [3] and by diazomethane. The identification in the hydrolyzate of 3,4,6-tri-*O*-Me-D-Gal*p* permethylate suggested that galacturonic acid in the AG was bonded to the main chain through an α-1,2-bond. The detection of trace quantities of di-*O*-Me-D-hexose and 2,5-di-*O*-Me-pentose confirmed again data from periodate oxidation regarding possible branching in the AG chain. Demethylation of the last produced galactose and arabinose.

The high negative specific rotation of AG $[\alpha]_D$ –40.1° and its methyl derivative $[\alpha]_D$ –78.3° provided evidence that the galactose units in the AG had the β-configuration; the arabinose units, the α-configuration of the glycoside bond. This hypothesis was confirmed by total oxidation of acetylated AG using chromic anhydride [4]. Paper chromatography (PC) of the hydrolyzate of the last detected only free arabinose. Therefore, the arabinose was bonded to the main chain through an α-glycoside bond. This was also consistent with resonances in the ^{13}C NMR spectrum of the AG with chemical shifts 104.4 (C-1), 72.01 (C-2), 73.82 (C-3), 68.7 (C-4), and 76.5 (C-5) ppm. These were characteristic of 1,6-bonded galactopyranose units. A resonance at 82.1 ppm was probably related to a substituted galactopyranose unit on C-3. The spectrum had three anomeric resonances at 101.07, 104.4, and 108.2 ppm. The first two resonances belonged to the C-1 atoms of galactopyranose and galacturonic acid. The last resonance was characteristic of L-arabinofuranose C-1. The resonance at 104.4 ppm belonged to C-1 of Gal*p* that was situated at a branching point; at 101.07 ppm, to galacturonic acid C-1. The resonance at 53.7 ppm was indicative of the presence in the polysaccharide of OCH_3 groups as uronic acid methyl esters. Chemical shifts assigned to arabinofuranose units were located at 68.7 and 108.2 ppm.

Partial cleavage of the AG macromolecule produced mainly three oligosaccharides (O-1–O-3) and an insignificant amount of disaccharide with R_f 0.8 that gave only arabinose upon hydrolysis.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences, Republic of Uzbekistan, Tashkent, fax: (99871) 120 64 75, e-mail: rakhmanberdieva@mail.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 5, September–October, 2012, pp. 654–656. Original article submitted March 12, 2012.

 0009-3130/12/4805-0728

The composition and structure of the oligosaccharides were established from total acid hydrolysis before and after reduction by $NaBH_4$, methylation, and ^{13}C NMR spectroscopy. The degree of polymerization of the oligosaccharides was determined by GC using the ratio of the monosaccharides.

Oligosaccharide O-1, $[\alpha]_D$ +17.8°. PC of the total acid hydrolysis products of O-1 identified galactose and arabinose. The hydrolysis products after $NaBH_4$ reduction contained galactose and arabite in a 1:1 ratio. Hakomori methylation [3] produced the permethylate, which formed after hydrolysis 2,3,5-tri-*O*-Me-L-arabinose and 2,4,6-tri-*O*-Me-D-galactose in a 1:1 ratio. The detection of 2,4,6-tri-*O*-Me-D-galactose meant that the oligosaccharide main chain contained 1→3 bonds.

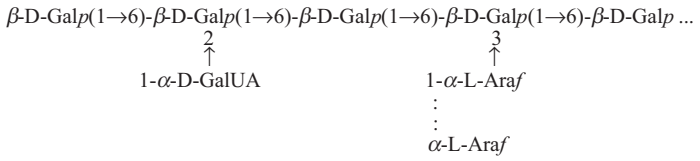
Smith degradation produced erythrite and glycerin in a 1:1 ratio. Based on the results, it was concluded that oligosaccharide O-1 was a disaccharide, i.e., α-L-Ara*f*-(1→3)-β-D-Gal*p*.

Oligosaccharide O-2, $[\alpha]_D$ +42.3°. Only galactose was identified after total acid hydrolysis of oligosaccharide O-2. Its reduction products were dulcite and galactose in a 1:2 ratio. The hydrolysis products of O-2 permethylate included mainly 2,3,4-tri-*O*-Me-D-galactose, which indicated the presence of 1→6-bonds in the monosaccharide units of the oligosaccharide chain. Periodate oxidation produced mainly glycerin. The combined results showed that oligosaccharide O-2 was a trisaccharide, i.e., β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*.

Oligosaccharide O-3, $[\alpha]_D$ +34.2°. Judging from the results of total acid hydrolysis of oligosaccharide O-3, it consisted only of galactose units. Reduction of it by $NaBH_4$ and hydrolysis formed dulcite and galactose in a 1:3 ratio. The hydrolyzate of the permethylate contained mainly 2,3,4-tri-*O*-Me-D-galactose, which showed the presence of 1→6-bonds between the monosaccharide units in the oligosaccharide chain. The periodate oxidation product was glycerin. According to the results, oligosaccharide O-3 was a tetrasaccharide, i.e., β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*.

Thus, a branched AG where the main chain was 1,6-β-D-galactan in which several galactose units were substituted by 1,3-α-L-Ara*f* and 1,2-α-D-GalUA and the arabinose units were bonded to the main polysaccharide chain as a disaccharide was isolated for the first time from the aerial part of *C. repens*.

Based on the chemical and spectral data, the AG had the most probable structure **1**.



1

EXPERIMENTAL

TLC was carried out on Silufol UV-254 plates with KSK silica gel (LS-5/40 mm) using the solvent systems (by volume) $CHCl_3$:MeOH (9:1, 1) and MEK:NH_4OH (1%) (30:1, 2). PC used Filtrak FN 1,3,7,11,12 paper and solvent system BuOH-1:Py:H_2O (6:4:3, 3).

Compounds were detected by spraying with conc. H_2SO_4 (1), anilinium acid phthalate (2), and KIO_4:$KMnO_4$:benzidine (3). GC was performed in a Chrom-5 chromatograph with a flame-ionization detector under the following conditions: a) stainless-steel column (200 × 0.3 cm), 5% Silicone XE-60 on Chromaton NAW (0.200–0.250 mm), 210°C, N_2 carrier gas, and gas flow rate 60 mL/min for aldononitrile acetates; b) 3% polyneopentylglycol adipate on Chromaton NAW-DMC (0.125 × 0.160 mm), 210°C, N_2 carrier gas, and gas flow rate 60 mL/min for polyol acetates.

Specific rotation of polysaccharides and their methyl derivatives were measured on a Zeiss polarimeter in a 1-dm tube of volume 10 mL and a 0.5-dm tube of volume 1 mL at 20–23°C.

Ultracentrifugation was carried out on a MOM-3170 instrument at 50,000 rpm and 20°C at 30° angle at 10-min intervals for centrifugation time 1 h 45 min.

^{13}C NMR spectra were recorded on a Unity Plus-400 spectrometer at operating frequency for C nuclei of 15.08 Hz with full suppression of protons. Solutions (3%) in D_2O with MeOH internal standard were prepared. Chemical shifts are given relative to TMS (50.18 ppm).

Fractional Precipitation of *C. repens* WSPS by EtOH. WSPS (10 g) were dissolved in H_2O (250 mL) to give a slightly cloudy solution that was centrifuged. The precipitate was separated, washed with EtOH, and dried in vacuo over P_2O_5. Yield of water-insoluble polysaccharide (WISPS), 0.23 g.

The centrifugate (250 mL) was stirred constantly and treated with EtOH (125 mL). The resulting precipitate was separated, washed with EtOH, dehydrated by acetone, and dried in vacuo over P_2O_5 to afford fraction 1 (0.43 g). Then, the supernatant solution was treated with another portion of EtOH (125 mL). The resulting precipitate was worked up analogously. Yield of fraction 2, 1.32 g. Fractions 3 (AG) and 4 were obtained by adding EtOH (250 mL) to the supernatant solutions. The yields of fractions 3 and 4 were 2.0 and 0.73 g, respectively. The aqueous EtOH solution was evaporated to a syrup and precipitated by EtOH. Yield of fraction 5, 3.68 g.

Gel-filtration of AG. AG (0.01 g) was dissolved in NaCl solution (1 mL, 0.3%), placed on a column of Sephadex G-100 (2 × 55 cm), and eluted by the same solution. Fractions (3 mL) were collected and analyzed by the literature method [5]. The column was calibrated by passing dextrans of molecular weights 110,000 (V_1 = 21.8 mL), 80,000 (V_2 = 24.6 mL), and 40,000 (V_3 = 32.8 mL). The molecular weight (V_4 = 28.6 mL) of 56,000 for *C. repens* AG was calculated from a calibration curve.

Periodate Oxidation and Smith Degradation. AG (0.0322 g) was dissolved in H_2O (24.9 mL), treated with $NaIO_4$ (5.1 mL, 0.25 M), left for 24 h at 20°C, and then stored at 5°C. Aliquots (1 mL) were taken every day and titrated with sodium thiosulfate solution (0.01 N). The consumption of $NaIO_4$ after 26 d was 2.7 moles. Its value did not change further. The formic acid that was released during the reaction was titrated with NaOH solution (0.01 N). Its amount was calculated as 0.33 moles for AG. Oxidant in the reaction mixture was destroyed by adding ethyleneglycol (0.1 mL). The solution was dialyzed, treated with $NaBH_4$ (0.1 g), and left overnight. The solution was treated with KU-2 cation-exchanger (H^+) and filtered. The filtrate was evaporated with MeOH. The dry solid was hydrolyzed in HCl (3 mL, 0.5 N) at 85°C for 4 h. PC of the hydrolysis products (system 3, detectors 2 and 3) and GC (conditions a and b) detected mainly glycerin and arabinose.

Methylation of AG. AG (0.1 g) was methylated by the literature method [3]. The completeness of the methylation was checked by TLC (system 1, detector 1) and by IR spectra (OH absorption band at 3200–3600 cm^{-1} missing). The yield of permethylate was 0.08 g, $[\alpha]_D$ –24.4° (*c* 0.5%, acetone), OCH_3 40.8%.

AG permethylate (0.05 g) was refluxed in formic acid (1 mL, 85%) for 1.5 h, cooled, and evaporated. The solid was dissolved in H_2SO_4 solution (2 mL, 0.5 N) and hydrolyzed for 6 h at 100°C. The hydrolyzate was worked up as usual. Methyl derivatives were separated over a column (L = 50 cm, d = 2 cm) of silica gel (L 100/250) with elution by $CHCl_3$:MeOH (9:1). Aliquots (5 mL) were collected and checked by TLC using system 2. Pure 2,3,4,6-tetra-*O*-Me-D-Gal; 2,3,4-tri-*O*-Me-D-Gal; 2,3,5-tri-*O*-Me-L-Ara; and two compounds (di-*O*-Me-derivatives) with R_f values 1.0, 0.9, 0.55, 0.05, and 0.03, respectively, were obtained.

Demethylation of Di-*O*-Me-derivatives of AG. The di-*O*-Me-derivatives (0.005 and 0.003 g) were dissolved separately in HBr solution (2 mL, 45%, in a 2-mL ampul) and hydrolyzed for 1.5 h. The hydrolyzates were evaporated to dryness and distilled with MeOH. PC (system 3, detector 2) identified arabinose and galactose.

Acetylation of AG. AG (0.1 g) was acetylated and oxidized by CrO_3 as before [4]. PC (system 3, detector 3) detected arabinose in the final products.

Partial Acid Hydrolysis of AG. AG (0.2 g) was dissolved in CF_3COOH solution (20 mL, 0.5 M) and hydrolyzed for 2.5 h at 100°C. The hydrolyzate was neutralized with anion-exchanger, condensed, and studied by PC (system 3, detector 2). Arabinose, galactose, and three oligosaccharides with $R_{f\text{Gal}}$ 0.54, 0.32, and 0.1 were detected.

Total oligosaccharides were loaded onto a column of DEAE-cellulose-Sephadex A-25 (formate form) and eluted sequentially by HCOOH solutions (0.1 M, 0.4, 0.6, and 0.8). The yields of oligosaccharides were monitored using phenol:H_2SO_4 [5]. The resulting oligosaccharides were rechromatographed to afford pure oligosaccharides in yields of 0.026 g (O-1), 0.053 g (O-2), and 0.0126 g (O-3). PC (system 3, detector 2) identified galactosylarabinose (O-1), galactotriose (O-2), and galactotetraose (O-3) with $[\alpha]_D$ +17.8° (*c* 0.1%, H_2O) for O-1, lit. [6] $[\alpha]_D$ +7° (*c* 0.2%, H_2O); $[\alpha]_D$ +42.3° (*c* 0.25%, H_2O) for O-2, lit. [7] $[\alpha]_D$ +48° (*c* 0.1%, H_2O); and $[\alpha]_D$ +34.2° (*c* 0.23%, H_2O) for O-3, lit. [6] $[\alpha]_D$ +28.2° (*c* 0.25%, H_2O).

Hydrolysis of *C. repens* Oligosaccharides. Oligosaccharides O-1, O-2, and O-3 (0.005 g each) were hydrolyzed by HCl solution (0.5 N) at 100°C for 4 h and worked up appropriately. PC (system 3, detector 2) detected in the hydrolyzate of O-1 galactose and arabinose; of O-2 and O-3, only galactose.

Periodate Oxidation of Oligosaccharides. Oligosaccharides O-1, O-2, and O-3 (0.03 g each) were oxidized by sodium periodate. PC (system 3, detectors 2 and 3) of the hydrolyzate of the reduced product and GC (condition b) detected glycerin and erythrite in ratios of 1:1, 1:2, and 1:3, respectively.

Methylation of Oligosaccharides. Oligosaccharides O-1, O-2, and O-3 (0.01 g each) were methylated according to Hakomori [3].

REFERENCES

1. R. K. Rakhmanberdyeva and A. M. Nigmatullaev, *Rastit. Resur.*, **1**, 78 (2003).
2. R. K. Rakhmanberdyeva, E. L. Kristallovich, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 202 (1995).
3. S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
4. J. Hoffman and S. Svensson, *Acta Chem. Scand.*, **2** (26), 661 (1972).
5. M. Dubois, K. A. Gilles, J. K. Hamilton, P. Q. Rebers, and F. Smith, *Anal. Chem.*, **3** (28), 350 (1956).
6. N. Shimizu, M. Tomoda, M. Satoh, R. Gonda, and N. Ohara, *Chem. Pharm. Bull.*, **8** (39), 2082 (1991).
7. R. Gonda, M. Tomoda, N. Shimizu, and M. Kanari, *Planta Med.*, **56**, 73 (1990).