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AN ARABINOGALACTAN FROM THE SEEDS OF *Pseudium guava*

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ABSTRACT

Hemicelluloses of seeds of *Pseudium guava* containing D-galactose (59.6), D-arabinose (35.9), and a uronic acid (4.5%) were analyzed by methylation analysis and Smith-degradation analysis, and the following structural elements were deduced; chain residues of (1→4)-linked D-galactose, (1→5)-linked D-arabinose, and terminal D-arabinose residues. The following structure was assigned to the polysaccharide.

→5)-D-Ara*f*-(1→4)-D-Gal*f*-(1→5)-D-Ara*f*-(1→

INTRODUCTION

Arabinogalactans are widely distributed in plant hemicelluloses as major constituents of the cell walls[1–5]. Water has seldom been used for the isolation of hemicelluloses from hard woods, but Adams[6] extracted the hemicellulose from American beech with hot water, and obtained a xylan in a yield of 13.4%; the yield is quite high, considering the limited swelling power of water. Several attempts have been made to employ liquid ammonia for the isolation of hemicelluloses from woods. The use of this solvent was introduced by Yan and Purves[7], and the method was subsequently applied in isolating xylans from sugar maple[8] and trembling aspen[9]. Hemicelluloses can be partly extracted from holocelluloses by dilute alkali[10–12]; 4–10% sodium or potassium hydroxide solution is usually used as the extractant, and up to 24% potassium hydroxide solution[12] when exhaustive extraction is desired. Extraction of hemicelluloses by dilute alkali under nitrogen causes little change, but prolonged treatment of hemicelluloses with alkali leads to diminution in their molecular weight[13].

EXPERIMENTAL

Isolation of the hemicellulose. — The powdered seeds (1 kg) were defatted by successively soaking in acetone, ether, and methanol for two weeks each. The defatted seeds (850 g) were then soaked in 3% sodium hydroxide solution (3 L) under a nitrogen atmosphere. The mixture was heated on a boiling-water bath for 3 h, the suspension was filtered through muslin, and the filtrate was centrifuged. In an ice

bath, the supernatant liquor was made neutral with 50% acetic acid solution, kept for 1 h, and centrifuged. The clear centrifugate was poured into acetone (6 vol.), with stirring. The resulting, amorphous substance was collected by centrifugation at 7000 r.p.m., washed with alcohol, and then dialyzed for 3 days against tap water. Acetone (6 vol.) was added to the concentrated dialyzate. The precipitate was collected, dissolved in water, reprecipitated with acetone, dissolved in water, and the solution dialyzed against distilled water for 3 days, and then freeze-dried.

Purification and characterization of the isolated hemicellulose. — The homogeneity of the hemicellulose was demonstrated by gel filtration on Sepharose 4B. Before use, the gel was washed thoroughly with 3% NaCl until free from azide. The swollen gel was then packed into a column (80 × 1.5 cm) at 4°, and a 0.2% (w/v) solution (2 mL) of the hemicellulose was applied to the column, and eluted with 0.3% NaCl. A 2-mL portion of each of the 3-mL fractions collected was used for reaction with 4 mL of 0.2% anthrone in conc. H_2SO_4. The absorption was measured at[14] 625 nm. Blue Dextran 2000 (average molecular weight, 2×10^6) was eluted from the column, either prior to, or after, the hemicellulose studied, by application of 2 mL of 0.2% (w/v) Blue Dextran 2000 and measurement of the absorbance (at 264 nm) of each of the 3-mL eluates collected.

Characterization and quantitative determination of component sugars of the hemicellulose. — The hemicellulose was hydrolyzed with 0.5M sulfuric acid for 12 h at 100°. The hydrolyzate was made neutral with $BaCO_3$, the suspension filtered, and the filtrate evaporated to a residue containing monosaccharides; these were reduced with sodium borohydride, and the resulting alditols converted into their acetates. These were characterized, and quantitatively determined relative to each other, by g.l.c.[15]. G.l.c. was performed with a Varian 1700 chromatograph fitted with columns (1–2 m) of 5% of NPGS, 5% of SE, or 3% of ECNSS-3M on Chromosorb W (80–100 mesh).

Methylation of the hemicellulose. — The hemicellulose (50 mg) was dried over phosphorus pentaoxide for 3 h at 60°, and then transferred to a test tube fitted with a serum cap and a magnetic stirring-bar, and containing dimethyl sulfoxide (12 mL). The vial was purged with nitrogen, and kept in a sonic bath for 1 h at room temperature. The mixture was injected with the Hakomori reagent[16] (3 mL), and sonicated for 30 min, to yield a clear solution. [The Hakomori reagent was prepared by adding Me_2SO (10 mL) to 1:1 sodium hydride–oil suspension (1.0 g), and sonicating at 80° until gas evolution ceased.] Methyl iodide (3 mL) was cautiously injected (with venting) into the vial, cooled in ice–water, and the mixture was sonicated for 30 min, to yield a clear solution which was poured directly into a dialysis bag. After dialysis overnight against running tap-water, the solution was lyophilized to dryness. This procedure was then repeated twice, with the following modification: Me_2SO (3 mL), Hakomori reagent (1 mL), and methyl iodide (1 mL). The product showed negligible absorption in the hydroxyl-group region (~3500 cm^{-1}) of the infrared spectrum.

Hydrolysis of the methylated hemicellulose. — The methylated hemicellulose (10 mg) was first treated with 90% formic acid (6 mL) and magnetically stirred for

2 h on a boiling-water bath. The resulting solution was evaporated in a rotary evaporator, and water (5 mL) was added to, and evaporated from, the residue. To this residue was added 0.5M sulfuric acid–acetic acid[17] (1 mL), and the solution was magnetically stirred for 14 h at 70°. Water (0.1 mL × 4) was added at 1-h intervals, with continued stirring, and heating at 70°. The hydrolyzate was made neutral with saturated, aqueous barium hydroxide, and centrifuged. The methylated sugars in the hydrolyzate were reduced with sodium borohydride (40 mg) for at least 4 h. The mixture was then made neutral with acetic acid, and de-ionized with Amberlite IR-120 (H^+) resin. After evaporation *in vacuo*, boric acid was removed by addition and evaporation of methanol (2 × 5 mL), and the residue was converted into alditol acetates by treatment with 1:1 acetic anhydride–pyridine (4 mL) for 20 min at 100°. The mixture was evaporated to dryness *in vacuo*, and the residue was dissolved in chloroform, and analyzed by g.l.c.

Periodate oxidation. — The hemicellulose (100 mg) was oxidized in 15mM sodium metaperiodate (100 mL), with stirring, in the dark. Samples (10 mL) were taken at different times for spectrophotometric determination (223 nm) of periodate consumption, and for potentiometric determination of the formic acid produced[18].

Smith degradation[18]. — The hemicellulose (197 mg) was dissolved in 0.1M sodium acetate buffer, pH 3.9 (200 mL), and 0.2M sodium metaperiodate (50 mL) was added. After 120 h in the dark at 4°, the excess of periodate was reduced with ethylene glycol (1 mL), the mixture was dialyzed, the dialyzate concentrated to 50 mL, and sodium borohydride (2 g) was added to the concentrate. After stirring overnight at room temperature, the excess of borohydride was decomposed by acidifying with 50% acetic acid. The solution was dialyzed, and evaporated to dryness. The residue was treated with 0.5M trifluoroacetic acid (25 mL) for 48 h at 23° and the solution was evaporated to dryness. The residue was separated on Whatman No. 1 paper with pyridine–ethyl acetate–water (2:5:7, upper phase), and the chromatograms were sprayed with benzidine–sodium metaperiodate.

RESULTS AND DISCUSSION

The hemicellulose was isolated from the seeds by using 3% NaOH under a nitrogen atmosphere (to avoid any tautomerization of the hemicellulose). The hemicellulose was precipitated with acetone, and its homogeneity was demonstrated by gel filtration on Sepharose 4B; yield 72.59 g/kg of seeds, and the product had $[\alpha]_D^{23}$ −175° (*c* 0.1, 3% NaOH); Found: ash 0.03; N, 0.03%. D-Galactose, D-arabinose, and D-galacturonic acid[19] were identified in the acid hydrolyzate of the hemicellulose. The approximate relative proportions of the sugar components in the hemicellulose were 13:8:1, respectively.

The hemicellulose was methylated according to Hakomori[16]. The fully methylated hemicellulose (indicated by infrared analysis) was hydrolyzed, and the resulting methylated sugars were converted into their alditol acetates. G.l.c. analysis of the *O*-methylalditol acetates gave derivatives of 2,3,6-tri-*O*-methyl-D-galactose (61),

2,3-di-*O*-methyl-D-arabinose (38), and 2,3,5-tri-*O*-methyl-D-arabinose (1 mol %).

The absence of mono-*O*-methylarabinose or di-*O*-methylgalactose indicated that the polymer was an unbranched chain, and not a branched form. The presence of 2,3,6-tri-*O*-methyl-D-galactose indicated (1→4)-linked D-galactose. The presence of 2,3-di-*O*-methyl-D-arabinose indicated (1→4)-linked D-arabinopyranose or (1→5)-linked D-arabinofuranose. The presence of 2,3,5-tri-*O*-methyl-D-arabinose indicated D-arabinofuranosyl residues, and that the arabinose residue is the nonreducing end-group.

The hemicellulose was oxidized with periodate; after 100 h, 0.85 mol of periodate was consumed, and 0.02 mol of formic acid released, per sugar residue. The periodate-oxidized hemicellulose was reduced with sodium borohydride, and the resulting polyalcohol was hydrolyzed with sulfuric acid. Galactopyranose residues (1→2)-linked will consume one mol of periodate to produce two mol of glycerol per sugar unit; galactopyranose residues (1→3)-linked are periodate-resistant hexose residues. Galactopyranose residues (1→4)-linked will consume one mol of periodate, to produce one mol of erythritol quantitatively. Galactopyranose (1→6)-linked will consume two mol of periodate, to produce one mol of formic acid and one mol of glycerol per galactose residue. Arabinofuranose (1→2)- and (1→3)-linked are periodate-resistant residues. Arabinofuranose (1→5)-linked will consume one mol of periodate to produce one mol of glycerol quantitatively.

Chromatography of the hydrolyzate showed that nearly all of the sugar residues were oxidized by periodate to give glycerol (35–40) and erythritol (58–62 mol %). Isolation of erythritol (quantitatively) equal to the proportion of galactose confirmed the (1→4)-linked D-galactopyranose residues. Isolation of glycerol in the same proportion as arabinose confirmed the (1→5)-linked D-arabinofuranose residues.

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