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AN ACIDIC XYLAN FROM THE CAPSULAR POLYSACCHARIDE-COMPLEX OF *Ocimum gratissimum* SEEDS

YERNOOL V. ANJANEYALU, MUJEEBUR-RAHMAN KHAN,

Department of Post-graduate Studies and Research in Chemistry, University of Mysore, Manasagangothri, Mysore-570 006 (India)

AND RUDRAPATNAM N. THARANATHAN

Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore-570 013 (India)

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ABSTRACT

An acidic xylan composed of D-xylose (48%), L-arabinose (16%), D-galactose (16%), and D-galacturonic acid (~20%) has been isolated from the capsular, mucilaginous polysaccharide-complex of the seeds of *Ocimum gratissimum*. Graded, acid hydrolysis afforded *O*-β-D-galactopyranosyl-(1→3)-L-arabinose, an aldobiouronic acid, and an aldotriouronic acid composed of D-xylose and D-galacturonic acid. Methylation analysis, together with the isolation of a xylan core from the acid-degraded polysaccharide, indicated that the polysaccharide contains a (1→4)-linked xylan backbone with 60% of the xylosyl residues substituted either on O-2 or O-3 with *O*-β-D-galactopyranosyl-(1→3)-L-arabinofuranosyl, L-arabinofuranosyl, or D-galactopyranosyluronic acid side-chains. These structural features for the polysaccharide accord with periodate-oxidation studies.

INTRODUCTION

In continuing our studies of the capsular, mucilaginous polysaccharide-complexes from the seeds of *Ocimum* species[1–6] with a view to comparing the main structural features of the major acidic polysaccharides of different *Ocimum* species, the structural features of an acidic xylan from *O. gratissimum* are now reported.

RESULTS AND DISCUSSION

The polysaccharide-complex of the seeds of *O. gratissimum*, isolated in a yield of 15%, was composed of glucose, galactose, mannose, xylose, and arabinose, in the molar ratios ~2:2:0.1:1:1, and galacturonic acid (~4.8%). The constituent sugars were isolated from a hydrolysate by cellulose-column chromatography, and the $[\alpha]_D$ values indicated that the arabinose was L and the

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other sugars were D. Fractionation of the polysaccharide-complex with 3mM hydrochloric acid[1] gave an acid-soluble (20%) and an acid-insoluble fraction (75%). The acid-soluble fraction was composed of xylose, arabinose, and galactose, in the molar ratios ~3:1:1, and galacturonic acid (~20%). The acid-insoluble fraction was composed of arabinose, galactose, glucose, and mannose in the molar ratios ~1:0.5:1.5:1.

DEAE-cellulose chromatography (CO_3^{2-} form)[7] of the acid-soluble fraction gave a major acidic polysaccharide (80%) and a minor fraction (3%). The major acidic polysaccharide, on re-chromatography on DEAE-cellulose (PO_4^{3-} form), emerged as a single peak. On ultracentrifugation, the polysaccharide gave a single, symmetrical peak, and microzone electrophoresis[8] indicated a single band. The polysaccharide, $[\alpha]_D$ +40° (*c* 0.2, water), had M_n 321,000, as determined by gel filtration[9].

The purified polysaccharide was composed of xylose (48%), arabinose (16%), galactose (16%), and galacturonic acid (20%). The carboxyl-reduced polysaccharide[10] was methylated (Hakomori[11]) and then hydrolysed, and the products were converted into their alditol acetates and subjected to g.l.c. and g.l.c.–m.s.[12]. In this way, derivatives of 2,3,5-tri-*O*-methylarabinose, 2,5-di-*O*-methylarabinose, 2,3,4,6-tetra-*O*-methylgalactose, 2,3-di-*O*-methylxylose, and 2-*O*- and 3-*O*-methylxylose were identified in the molar ratios 1:4:8:6.9. The derivatives of 2-*O*- and 3-*O*-methylxylose were not separated by g.l.c., but were identified by their characteristic mass-spectral fragments. The formation of 2,3,4,6-tetra-*O*-methylgalactose (8 mol) indicated that galactose and galacturonic acid were present as non-reducing, pyranosyl end-groups. The formation of 2,3,5-tri-*O*-methylarabinose (1 mol) and 2,5-di-*O*-methylarabinose (4 mol) indicated that arabinose was present both as non-reducing, terminal furanosidic residues, and non-terminal furanosidic residues substituted at O-3. The formation of 2,3-di-*O*-methylxylose (6 mol) and 2-*O*- and 3-*O*-methylxylose (9 mol) indicated that the xylose residues were present as (1→4)- and/or (1→5)-linked non-terminal units, the most probable linkage being the former in view of the stability of the xylosyl residues to acid hydrolysis observed subsequently. Some of these xylosyl residues were further substituted either at O-2 or O-3.

Mild, acid hydrolysis of the acidic polysaccharide afforded an acid-degraded polysaccharide that was homogeneous by microzone electrophoresis and composed mainly of xylose and galacturonic acid. This result, together with the methylation data, indicated that xylosyl residues probably formed the backbone of the polysaccharide, since galacturonic acid constituted the non-reducing end-groups. Further, the formation of the acid-degraded polysaccharide indicated that the xylosyl residues of the backbone were probably pyranoid and (1→4)-linked.

Graded, acid hydrolysis of the polysaccharide gave three oligosaccharides (**1**–**3**), with R_{Gal} values of 0.75, 0.43, and 0.26, which were isolated in yields 5, 7, and 4%, respectively.

Oligosaccharide **1**, $[\alpha]_D$ +55° (*c* 0.5, water), was composed of arabinose and

galactose in the ratio 1:1, with arabinose as the reducing end, since borohydride reduction of **1** followed by methylation analysis gave 2,3,4,6-tetra-*O*-methylgalactose and 1,2,4,5-tetra-*O*-methylarabinitol. Further, incubation of **1** with β-D-galactosidase liberated D-galactose. Hence, **1** was *O*-β-D-galactopyranosyl-(1→3)-L-arabinose. The reported $[\alpha]_D$ values for 1 are +60° (*c* 1.8) when isolated from *Albizzia zygia*[13], and +97→+67° (2 h, *c* 0.45) when isolated from *Anogiessus schimperi* gum[14].

Oligosaccharides **2** and **3** had $[\alpha]_D$ +114 °(*c* 0.7) and +75° (*c* 0.4), respectively, and were composed of xylose and galacturonic acid in the ratios 1:1 and 2:1, respectively. Xylose was the reducing terminus in **2** and **3**, which were aldobiouronic and aldotriouronic acids, respectively. They were not further characterised.

On periodate oxidation, the polysaccharide consumed 0.81 mol of periodate and liberated 0.28 mol of formic acid per sugar residue. Smith degradation of the polysaccharide followed by ethanol precipitation gave a residue which, on acid hydrolysis, afforded xylose (p.c.). The supernatant solution contained arabinose as the only intact sugar. Thus, periodate oxidation had cleaved all the galactosyl and galactosyluronic acid groups, which constitute the non-reducing end-groups. The liberation of arabinose could be due to its being present in the furanoid form, and the periodate-resistant xylose could reflect the presence of side chains attached to O-2 or O-3 of some of the xylosyl residues in the parent polysaccharide.

The results of methylation analysis, mild hydrolysis with acid, oligosaccharide formation, and periodate oxidation indicate the acidic polysaccharide to be a (1→4)-linked xylan with 60% of the xylosyl residues substituted either on O-2 or O-3 with *O*-β-D-galactopyranosyl-(1→3)-L-arabinofuranosyl, L-arabinofuranosyl, or D-galactopyranosyluronic acid side-chains.

Similar structural features have been inferred for the major acidic polysaccharide of *O. canum* and *O. basilicum*, but the *O. gratissimum* polysaccharide contains simpler side-chains.

EXPERIMENTAL

Materials. — Seeds of *O. gratissimum* were obtained locally during December–January.

General methods.— These have been previously reported[1–6]. G.l.c. of alditol acetates was performed at 190° with a Varian Aerograph Series 1400 fitted with a flame-ionisation detector and a stainless-steel column (8 ft ×0.125 in.) containing 3% of OV-225 on Chromosorb-W (H.P., 80–100 mesh). Alditol acetates of partially methylated sugars were analysed by using a Packard Model 428 gas chromatograph fitted with a flame-ionisation detector and a glass column (2 m × 2 mm) containing 3% of OV-225 on Gas Chrom Q (80–100 mesh). Nitrogen was the carrier gas. G.l.c.–m.s. was performed with a Varian 3700 gas chromatograph (OV-225 column) coupled to a Varian MAT 445 mass spectrometer and a Varian

Spectrospin MAT-200 data-processing system.

Electrophoresis of the polysaccharide fractions, dyed with Procion Brilliant Red 2 BS, in 0.05M borate buffer (pH 9.3) was carried out at an applied voltage of 180 V with a Beckman Microzone Electrophoretic cell (Model R101) and Millipore Phoroslide[8].

Isolation of the acidic polysaccharide. — The seeds of *O. gratissimum* (100 g) were soaked overnight in water (1.5 L). The mixture was then stirred vigorously for 3 h, and the viscous, aqueous extract was isolated by brief centrifugation. Addition of ethanol (2 vol.) to the extract precipitated the polysaccharide-complex as a thick gel, which was collected by centrifugation, washed repeatedly with ethanol and then ether, and dried (yield, 15 g).

Hydrolysis[3] of the polysaccharide-complex (1 g) gave (p.c.) glucose, galactose, mannose, arabinose, xylose, galacturonic acid, and small proportions of oligosaccharides, probably aldobiouronic acids. The mixture was subjected to chromatography on a column (45 × 12 cm) of cellulose with 1-butanol–ethanol–water (10:1:2), to give 6 fractions. Each fraction was further purified by preparative p.c., and $[\alpha]_D$ values were determined.

To an aqueous dispersion of the polysaccharide-complex (400 mg) in water (200 mL) was added M hydrochloric acid, dropwise with stirring, to 3mM. The precipitate was collected by centrifugation, washed, and dried (yield, 305 mg). Ethanol (3 vol.) was added to the supernatant solution, and the precipitate was collected and dried (yield, 80 mg). Acid hydrolysis[3] of the fractions gave (p.c.) xylose, arabinose, and galactose (3:1:1), together with galacturonic acid (20%) in the latter fraction (acid-soluble); the former fraction contained only arabinose, galactose, glucose, and mannose (1:0.5:1.5:1).

A solution of the acid-soluble polysaccharide (500 mg) in the minimum amount of water was layered on a column (90 × 2.5 cm) of DEAE-cellulose (CO_3^{2-} form) and eluted with water and then 0.1→0.5M ammonium carbonate. Fractions (~12 mL) were assayed for carbohydrate by the phenol–sulfuric acid method[15]. Appropriate fractions were combined, dialysed against water, and lyophilised. In this way, major (421 mg) and minor polysaccharide fractions (16 mg) were obtained. Acid hydrolysis[3] of the major fraction gave (p.c.) xylose, arabinose, galactose, and galacturonic acid. This acidic polysaccharide was further investigated.

Investigation of the polysaccharide. — *(a) Homogeneity.* A 1% solution of the acidic polysaccharide in 0.1M sodium chloride was subjected to ultracentrifugation. Sedimentation occurred as a single, symmetrical peak. Microzone cell-electrophoresis of the dyed polysaccharide[8] also gave a single band.

(b) Molecular size. A column (80 × 1.5 cm) of Bio-Gel P-200 was used with 0.1M sodium chloride as eluant[9]. The column was calibrated with dextrans of known molecular weight (Pharmacia).

(c) Sugar composition. The polysaccharide (5 mg) was hydrolysed[3] and the products were converted into alditol acetates. G.l.c. revealed derivatives of xylose,

arabinose, and galactose (3:1:1).

(d) Carboxyl-reduction. A solution of the polysaccharide (15 mg) in water (~3 mL) was reduced three times by the method of Taylor and Conrad[10]. The carboxyl-reduced polysaccharide (14 mg) contained only traces of uronic acid.

A solution of the carboxyl-reduced polysaccharide in dry dimethyl sulfoxide (1 mL) was methylated by the Hakomori procedure[11]. The reaction mixture was poured into water (~5 mL) and dialysed. The dialysate was extracted with chloroform, and the extract was concentrated to dryness. The methylated polysaccharide was hydrolysed, and the products were converted into alditol acetates and subjected to g.l.c. and g.l.c.–m.s.[12].

Isolation and characterisation of the oligosaccharides and the degraded polysaccharide. — The acidic polysaccharide (100 mg) was hydrolysed with 0.05M sulfuric acid (20 mL) at 90° for 40 min. Addition of acetone (60 mL) to the hydrolysate precipitated the degraded polysaccharide (60 mg), which was collected by centrifugation. The supernatant solution was neutralised and deionised. P.c. then revealed traces of arabinose and galactose, together with oligosaccharide **1**.

Hydrolysis of the degraded polysaccharide with 0.25M acid for 30 min, as described above, gave a further degraded polysaccharide (10 mg) and a hydrolysate containing oligosaccharides **2** and **3**. Finally, hydrolysis of the degraded polysaccharide with 0.5M acid for 1 h released traces of xylose and oligosaccharides **2** and **3**, but no degraded polysaccharide could be isolated by the addition of acetone. The three oligosaccharides **1**–**3** (5, 7, and 4 mg, respectively), isolated from the combined hydrolysate, had R_{Gal} values of 0.75, 0.43, and 0.27 (1-propanol–ethanol–water, 7:1:2), and $[\alpha]_D$ values of +55°, +115°, and +75°, respectively. Acid hydrolysis of the oligosaccharides gave (p.c.) arabinose and galactose (1:1) from **1**, and xylose and galacturonic acid from **2** (1:1) and **3** (2:1).

Each oligosaccharide (5 mg) was reduced with sodium borohydride (20 mg). Acid hydrolysis then gave (p.c.) galactose and arabinitol from **1**, galacturonic acid and xylitol from **2**, and galacturonic acid, xylose, and xylitol from **3**.

Oligosaccharide **1** (5 mg) was reduced with borohydride, methylated (Hakomori), hydrolysed, reduced with borohydride, and acetylated. G.l.c. then revealed the alditol acetates of 2,3,4,6-tetra-*O*-methylgalactose and 1,2,4,5-tetra-*O*-methylarabinose.

A solution of **1** (2 mg) in 0.05M phosphate buffer (pH 7.2, 1 mL) was incubated for 12 h at 37° with β-D-galactosidase (10 μL, 11 units/mg of protein). Ethanol (3.3 mL) was added to the reaction mixture, which was then centrifuged, deionised, and concentrated. P.c. revealed galactose.

The acidic polysaccharide (37 mg) was treated with 0.25M sulfuric acid (2 mL) at 90° for 2 h followed by the addition of ethanol (2 vol.). The precipitated, degraded polysaccharide (~10 mg) was collected by centrifugation. The supernatant solution contained (p.c.) arabinose and galactose. The degraded polysaccharide gave a single band in microzone electrophoresis and, on acid hydrolysis, gave (p.c.) mainly xylose and galacturonic acid.

Periodate-oxidation studies. — *(a)* A mixture of the polysaccharide (50 mg) and 0.05M sodium metaperiodate (50 mL) was kept in the dark at 5°. Periodate consumption[16], which was followed iodimetrically and became constant after 8 h, was 0.81 mol per sugar residue, and 0.28 mol of formic acid was liberated[16].

(b) The polysaccharide (10 mg) was treated with 0.05M sodium metaperiodate (10 mL) for 72 h at 5°, excess of periodate was reduced with ethylene glycol, and the reaction mixture was dialysed. The dialysate was concentrated, and reduced with borohydride[17]. The reduced product was treated[17] with M sulfuric acid at room temperature for 48 h. Ethanol (2 vol.) was added to the mixture, followed by centrifugation, neutralisation, deionisation, and concentration. The resulting syrup contained (p.c.) only arabinose. Acid hydrolysis of the residue gave only xylose.

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