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AN ACIDIC POLYSACCHARIDE FROM THE SEEDS OF *OCIMUM ADSCENDENS*

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Abstract—The mucilaginous polysaccharide-complex found in the seeds *O adscendens* contains an acidic polysaccharide which has been isolated It is composed of D-galactose (~ 20%), D-galacturonic acid (~ 35%) and L-rhamnose (~ 39%) Methylation analysis using GC and GC/MS, Smith degradation, isolation of an acidic oligosaccharide from partial hydrolysates, and enzymic hydrolysis using β-D-galactosidase indicated the polysaccharide backbone to be → 4)-GalpA-(1 → 2)-L-rhap-(1 → Nearly two-thirds of the rhamnopyranosyl units are O-4 substituted by D-galactopyranosyl non-reducing end groups

INTRODUCTION

Hitherto we have reported the main structural features of the major acidic xylans present in the capsular mucilaginous polysaccharide-complexes of the seeds of *Ocimum* species, viz *O canum* [1–3], *O basilicum* [4] and *O gratissimum* [5], in order to compare the main structural features of the acidic polysaccharides of different *Ocimum* species In continuation of such studies, we undertook the investigation of the major acidic polysaccharide present in the capsular polysaccharide-complex of the seeds of *Ocimum adscendens* Unlike in the other *Ocimum* species, *O adscendens* does not have an acidic xylan but a different type of polysaccharide whose main structural features are reported in this paper

RESULTS AND DISCUSSION

A mucilaginous capsular polysaccharide-complex found in the seeds of *O adscendens* was isolated in 18% yield by cold water extraction of the seeds, followed by ethanol precipitation Acid hydrolysis [1], of the polysaccharide-complex, separation of the constituent sugars by PC and cellulose column chromatography [6], and measurement of their specific rotations revealed the presence of L-arabinose, L-rhamnose, D-glucose and D-galactose in the molar ratio 3 2 2 2 5 The polysaccharide-complex also contained D-galacturonic acid (~ 5 6%) D-Xylose residues could not be detected

In order to isolate the major acidic polysaccharide fraction, the crude polysaccharide-complex was subjected to different fractionation techniques, viz preferential extraction [7], precipitation with quaternary ammonium salts [8], ion-exchange chromatography [9] and molecular sieve chromatography Thus extraction of the crude polysaccharide-complex with aqueous EDTA (2%) gave an 'EDTA-soluble' fraction, yield 10%, composed of arabinose, rhamnose, galactose and galacturonic acid in the ratio 1 2 2 5 2, and an 'EDTA-insoluble' fraction, yield 85%, composed of arabinose, rhamnose, glucose and galactose in the ratio 2 0 5 1 5 1 with a trace of galacturonic acid

The 'EDTA-soluble' fraction with cetavlon (6 8%), gave 'cetavlon-precipitable' and 'cetavlon-non-precipitable' fractions in yields 66 and 20% respectively Acid hydrolysis [1] of the fractions and identification of the sugars indicated arabinose, rhamnose, galactose and galacturonic acid in the ratio 0 5 1 5 1 1 in the precipitable fraction while the non-precipitable fraction had only the neutral sugars in the ratio 1 1 5 2 with trace amounts of galacturonic acid

The 'cetavlon-precipitable' fraction on DEAE-cellulose chromatography (PO_4^{3-} form) emerged as a single peak, indicating its possible homogeneity However, on DEAE-sephadex A-50 (Cl^- form), two acidic polysaccharide fractions, OAP_1 yield 20%, and OAP_2 yield 62%, were isolated The former, OAP_1, contained galacturonic acid (~ 10%) and neutral sugars arabinose, rhamnose and galactose (23 4 4 1), and the latter, OAP_2 contained galacturonic acid (~ 35%) and rhamnose and galactose (~ 1 5 1)

The major acidic polysaccharide OAP_2 on micro-zone cell electrophoresis [10] moved as a single spot and on gel filtration chromatography [11], emerged as a single peak indicating its possible homogeneity The polysaccharide had $\bar{M}_n$ 50 120 and $[\alpha]_D$ +20° (*c* 0 2, water)

The acidic polysaccharide was subjected to carboxyl-reduction according to Conrad and Taylor's method [12] The carboxyl-reduced polysaccharide contained only traces of unreduced uronic acid A single Hakomori methylation [13] gave a permethylated product This, on complete hydrolysis and derivatization into alditol acetates followed by GC/MS [14], indicated the presence of 3,4-di-*O*-methylrhamnose, 3-*O*-methylrhamnose, 2,3,6-trio-methylgalactose and 2,3,4,6-tetra-*O*-methylgalactose in the approximate mole ratio 1 2 2 3 2 respectively The identification of 2,3,4,6-tetra-*O*-methylgalactose (~ 2 mole proportion) as the only fully methylated sugar

derivative indicated that these residues constitute the only non-reducing end-groups in the carboxyl-reduced polysaccharide chain However, the native polysaccharide OAP_2 could contain either galacturonic acid or galactose or both as non-reducing end groups The formation of 3,4-di-*O*-methyl and 3-*O*-methylrhamnoses indicated that the rhamnose units are (1 → 2) glycosidically linked The near equivalence of 2,3,6-tri-*O*-methylgalactose with the di-*O*-methyl- and mono-*O*-methylrhamnoses indicate that these are linked to one another, galactose linked by (1 → 4) glycosidic bonds The mono-*O*-methylrhamnose derivative and the tetra-*O*-methylgalactose derivative formed in equal proportion point to the presence of non-reducing galactosyl residues on O-2 of rhamnose

Partial acid hydrolysis of the native polysaccharide OAP_2 with TFA [15] and isolation of the products gave a pure oligosaccharide in 10% yield (R_{gal} 0 27, solvent b), $[\alpha]_D + 2°$ (*c* 0 2, water), an immovable residue in 26% yield, and significant amounts of galactose and rhamnose The residue was composed of galacturonic acid and rhamnose Complete hydrolysis of the oligosaccharide revealed galacturonic acid and rhamnose almost in equal proportion with trace amounts of an oligosaccharide spot These results indicate the presence of →4)-D-galpA-(1 → 2)-L-rhap-(1 →, or →2)-L-rhap-(1 → 4)-D-galpA-(1 →, or both sequences in the native polysaccharide It appeared that the native polysaccharide contained D-galactose as the non-reducing sidechains This was indicated by enzymic hydrolysis of OAP_2 with β-D-galactosidase by which only D-galactopyranosyl units were released In the native polysaccharide, the backbone is essentially →4)-D-galpA-(1 → 2)-L-rhap-(1 →

Smith degradation [16] of the polysaccharide OAP_2 gave only rhamnose as the intact sugar Apparently the units of rhamnose carry the galactopyranosyl branch points on O-4 of these units and hence survive periodate attack Thus methylation analysis, partial hydrolysis leading to the acidic oligosaccharide, enzymic hydrolysis and Smith degradation support the partial structure proposed for the polysaccharide Similar galactopyranosyl uronic acid-(1 → 2)-rhamnose units have been reported in the backbone of polysaccharides from *Opuntia-Ficus-Indica* [17] and mangle gum [18]

EXPERIMENTAL

General methods Unless stated otherwise all evaporations were carried out under red pres below 45° and all hydrolyses at 100° Acid hydrolysis of the polysaccharide was done by solubilization in H_2SO_4, (72%) at 0° After 1 hr at room temp, the acid strength was adjusted to 8% and refluxed at 100° for 12–16 hr The hydrolysate was neutralized with solid $BaCO_3$, filtered, and the filtrate was passed through a column of Amberlite IR-120 (H^+) The effluent was concd and examined by PC

Descending PC was performed on Whatman No 1 and 3 MM papers with the solvent systems (a) *n*-BuOH–EtOH–H_2O (10 1 2), (b) *n*-PrOH–EtOH–H_2O (7 1 2), (c) *n*-BuOH–C_6H_6–C_5H_5N–H_2O (5 1 3 3, upper), (d) *n*-BuOH–HOAc–H_2O (4 1 5, upper) Sugars were detected with aniline phthalate

GC was performed (FID) using a glass column (2 m × 2 mm) packed with 3% OV-225 on Gas Chrom Q, 80–100 mesh, at 180° for partially methylated alditol acetates, with N_2 as the carrier gas

GC/MS was carried out on a Varian 3700 gas chromatograph coupled to a Varian MAT 445 mass spectrometer and a Varian Spectrospin MAT 200 data processing system

Isolation of the polysaccharide From the locally available *O adscendens* plants, seeds were collected during December–January The polysaccharide-complex (18 g) was isolated from the seeds (100 g) as already described [5]

The crude polysaccharide-complex (10 g) was suspended in aq EDTA (2%, 1 l), stirred mechanically for 2 hr and centrifuged After removing the centrifugate, the residue was repeatedly extracted with EDTA soln The combined extracts on dialysis, concn and lyophilization gave 'EDTA-soluble' polysaccharide, yield 1 g The 'EDTA-insoluble' fraction was washed and dried, yield 8 5 g.

To the 'EDTA-soluble' polysaccharide (1 g) in H_2O (100 ml) a few drops of octanol, and aq cetavlon (6 8%) was added dropwise with stirring till the precipitation was complete [7] The ppt was collected and decomposed This gave 'cetavlon-precipitable-polysaccharide' fraction OA, yield 100 mg, and the 'cetavlon-non-precipitable' fraction was recovered [7], yield 200 mg

Purification of 'cetavlon-precipitable' polysaccharide The polysaccharide fraction OA (500 mg), dissolved in a minimum amount of H_2O was layered on a DEAE-cellulose column (PO_4^{3-} form) 32 5 × 25 cm, and was eluted sequentially with H_2O, NaPi buffer (0 1–0 5 M, pH 8), and finally with 0 5 M NaOH A fraction OAP was isolated, yield 425 mg

The polysaccharide fraction OAP (325 mg), dissolved in minimum quantity of NaPi buffer, was fractionated on DEAE-sephadex A-50 (Cl^- form), 32 5 × 2 5 cm, and was eluted successively with NaPi buffer (0 05 M and 0 1 M, pH 8) Two fractions OAP_1, yield 40 mg, and OAP_2, yield 250 mg, were isolated

Electrophoresis Micro-zone cell electrophoresis of the dyed polysaccharide fraction OAP_2 [10] was performed in borate buffer (0 05 M, pH 9 3) containing NaCl (0 05 M)

Sugar composition The crude polysaccharide and its fractions were hydrolysed [1] The neutral sugars were examined by PC in solvents (a) and (b) and the acidic sugars in (d) Optical rotations were determined in H_2O after quantitative separation of the sugars by cellulose column chromatography using solvent (a)

Partial hydrolysis The polysaccharide OAP_2 (50 mg) was hydrolysed [15] with 2 M TFA (~ 16 ml) for 2 hr and examination by PC of the hydrolysate in solvent (b) revealed galactose, rhamnose, one major oligosaccharide and an immovable residue at the origin The latter was extracted with H_2O and precipitated with EtOH, yield 13 mg The oligosaccharide was separated and purified by repeated PC, yield 5 mg Both were hydrolysed with 2 M TFA for 10–12 hr

Carboxyl reduction The polysaccharide OAP_2 (15 mg) in H_2O (~ 3 ml) was reduced ×3 by the method of ref [12] The carboxyl-reduced polysaccharide was isolated, yield 13 mg

Methylation analysis The carboxyl-reduced polysaccharide (5 mg) was methylated according to ref [13] The permethylated product was hydrolysed with HCOOH for 2 hr, and after evaporation of excess of HCOOH, with 0 25 M H_2SO_4 for 10 hr The product after $NaBH_4$ reduction and acetylation with Ac_2O–pyridine was analysed by GC and GC/MS

Smith degradation [16] The polysaccharide (10 mg) was oxidized with 0 05 M $NaIO_4$ (10 ml) for 72 hr at 5° Excess of IO_4^- was destroyed with ethylene glycol (0 5 ml), dialysed and reduced with $NaBH_4$ After 12 hr, the soln was dialysed and concd The residue was treated with 1 M H_2SO_4 at room temp for 48 hr Two vols of EtOH were added and the soln was neutralized, deionized, concd and examined by PC in solvent (b)

Enzymic hydrolysis The polysaccharide OAP_2 (2 mg) in NaPi buffer (1 ml, 0 05 M, pH 7 2) was incubated for 12 hr at 37° with β-D-galactosidase (10 μl) (Sigma) EtOH (3 3 ml) was added and centrifuged The supernatant was deionized on Dowex-50 (H^+), concentrated and examined by PC

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