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STRUCTURAL STUDIES OF AN ACIDIC POLYSACCHARIDE FROM *Ocimum basilicum* SEEDS

YERNOOL V. ANJANEYALU AND D. CHANNE GOWDA

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

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ABSTRACT

An acidic polysaccharide isolated from the seeds of *Ocimum basilicum* by DEAE-cellulose fractionation was ~92% pure, having an associated glucan impurity (~8%). The polysaccharide is composed of D-xylose, L-arabinose, L-rhamnose, and D-galacturonic acid in the molar ratios 15:9:7:12, together with traces of galactose and glucose. Methylation analysis indicated that the polysaccharide contained a (1→4)-linked xylan backbone carrying branch-points at C-2 and C-3 of the xylosyl residues, and revealed the structural features of the side chains. Periodate-oxidation and Smith-degradation studies support the results of methylation analysis.

INTRODUCTION

In continuation of our studies[1,2] of the mucilaginous polysaccharides from the seeds of *Ocimum basilicum*, DEAE-cellulose fractionation[3] of the acid-soluble portion of the polysaccharides gave one major, acidic polysaccharide along with three minor fractions. The main structural features of this acidic polysaccharide are now reported.

RESULTS AND DISCUSSION

An acidic polysaccharide was isolated in 46% yield by DEAE-cellulose fractionation[3] of the acid-soluble portion of the mucilaginous polysaccharides from the seeds of *Ocimum basilicum*, and was further purified by DEAE-cellulose chromatography. Ultracentrifugal analysis of the polysaccharide indicated a symmetrical peak (sedimentation coefficient, 5.9S), while free-boundary electrophoresis showed a major, symmetrical peak (~92% of the total; electrophoretic mobility -1.5×10^{-4} $cm^2.sec^{-1}.volt^{-1}$) together with a slow-moving, minor peak (~8%). This minor peak could be due to an associated glucan that is precipitated (~6%) under the highly alkaline conditions of Haworth methylation. The major polysaccharide contained residues of xylose, arabinose, rhamnose, and galacturonic acid in the molar proportions 15:9:7:12, together with traces of galactose and glucose, as determined by g.l.c. analysis of their alditol acetates. P.c. examination of the acid hydrolysate in-

TABLE I

ANALYSIS OF THE ALDITOL ACETATES DERIVED FROM THE METHYLATED POLYSACCHARIDE (A), AND THE METHYLATED AND CARBOXYL-REDUCED POLYSACCHARIDE (B)

Alditol acetates of	T[a]	*Molar proportions from*	
		A	*B*
2,3,4-Tri-*O*-methylxylose	0.46	1.0	1.0
2,3,4-Tri-*O*-methylrhamnose	0.51	4.74	4.8
2,4-Di-*O*-methylrhamnose	0.89	1.0	0.92
2,3-Di-*O*-methylarabinose	1.05	4.64	4.84
2,3-Di-*O*-methylxylose	1.16	4.14	5.7
5-*O*-Methylarabinose	1.62	4.22	5.5
2-*O*-Methylxylose + 3-*O*-Methylxylose	1.96	13.97	16.4
2,3,4-Tri-*O*-methylgalactose	2.71	—	5.3

[a]Retention times relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on an OV-225 S.C.O.T. column at 170°.

dicated that, in addition to these sugars, there was an aldobiouronic acid that was characterised as 3-*O*-(D-galactopyranosyluronic acid)-D-xylose.

When the polysaccharide was methylated by the Haworth procedure[4], a small proportion of the material was precipitated from the reaction mixture. This was recovered in ~6% yield and shown to be a glucan that probably represents the slow-moving component observed during free-boundary electrophoresis. The major polysaccharide recovered from the aqueous solution gave a fully methylated product on Kuhn methylation[5] (4 treatments) followed by Purdie methylation[6] (4 treatments). Acid hydrolysis of the methylated polysaccharide and analysis of the products, as their alditol acetates by g.l.c. and g.l.c.–m.s.[7,8], gave the results shown in Table I. On carboxyl reduction[9] and acid hydrolysis, the methylated polysaccharide gave the partially methylated sugars shown in Table I.

From these results, it is evident that the major portion of the xylose in the polysaccharide is converted into the 2-*O*- and 3-*O*-methyl derivatives, which indicates the possible presence of a (1→4)-linked xylan backbone in which most of the xylosyl residues carry branch points either at C-2 or at C-3. The relative proportions of these branch points could not be determined, as 2-*O*-methylxylose and 3-*O*-methylxylose were not separated by g.l.c.; however, their presence was inferred from their characteristic mass-spectral fragments. Furthermore, their mass spectra indicated a higher proportion of 3-*O*-methylxylose, suggesting a preponderance of branch points on C-2 of the xylosyl residues. Formation of 2,3,4-tri-*O*-methylrhamnose and 2,3-di-*O*-methylarabinose, 2,3,4-tri-*O*-methylxylose and 2,4-di-*O*-methylrhamnose, and 2,3-di-*O*-methylxylose and 5-*O*-methylarabinose in equal proportions indicated the presence of 4- or 5-*O*-rhamnosylarabinose, 3-*O*-xylosylrhamnose, and 2- or 3-*O*-xylosylarabinose chains attached to the xylan backbone either at C-2 or C-3. Galacturo-

nic acid, obtained as 2,3,4-tri-*O*-methylgalactose from the methylated and carboxyl-reduced polysaccharide, indicated its presence exclusively as non-reducing end-groups linked to the xylan backbone either directly or through some xylose residues as evidenced by the formation of 3-*O*-(D-galactopyranosyluronic acid)-D-xylose. In the case of the methylated and carboxyl-reduced polysaccharide, there is an increase in the proportions of 2,3-di-*O*-methylxylose, 5-*O*-methylarabinose, and 2-*O*- and 3-*O*-methylxylose compared to the methylated acidic polysaccharide. This can be explained only if galacturonic acid residues are directly attached to these sugar residues, which, on carboxyl-reduction, become more susceptible to acid hydrolysis, thereby completely releasing the above sugar derivatives. However, in the methylated acidic polysaccharide, due to the formation of aldobiouronic acids, the proportions of these methylated sugars are comparatively low. On the basis of these results, the following structural features are tentatively inferred for the acidic polysaccharide:

$$\ldots \rightarrow 4)\text{-Xyl}p\text{-}(1\rightarrow 4)\text{-Xyl}p\text{-}(1\rightarrow 4)\text{-Xyl}p\text{-}(1\rightarrow 4)\text{-Xyl}p\text{-}(1\rightarrow \ldots,$$
$$\qquad\qquad\uparrow \qquad\qquad\uparrow \qquad\qquad\uparrow \qquad\qquad\uparrow$$

where → indicates that the following side-chains are attached to the xylan backbone: Gal*p*A-(1→, Rha*p*-(1→ 4 or 5)-Ara-(1→, Xyl*p*-(1→3)-Rha*p*-(1→, and Gal*p*A-(1→4)-Xyl*p*-(1→2 or 3)-Araf-(1→.

$$\begin{array}{c} \text{2 or 3} \\ \uparrow \\ \text{1} \\ \text{Gal}p\text{A} \end{array}$$

The presence of a xylan backbone in the polysaccharide is supported by periodate-oxidation[10] and Smith-degradation[11] studies.

On periodate oxidation, the polysaccharide consumed 0.86 mol of periodate per mol of hexosyl residue, and a major portion of the xylose, together with traces of arabinose and rhamnose, remained intact. The cleavage of all sugar residues by periodate, except for a major portion of the xylose and traces of arabinose and rhamnose, can be explained on the basis of the above structural features.

Smith degradation of the polysaccharide gave a xylan core composed mainly of xylose, periodate-degraded acidic components derived from uronic acid residues, and traces of arabinose and rhamnose. On periodate oxidation, the xylan core consumed 0.55 mol of periodate per mol of pentosyl residue, and ~50% of the xylosyl residues resisted attack by periodate. This resistance could be due to the presence of periodate-degraded, acidic components attached to these residues. Hence, at least part of the galacturonic acid residues should be directly attached to the xylan backbone.

EXPERIMENTAL

Materials and methods. — Descending paper chromatography (p.c.) was per-

formed on Whatman No. 1 and 3MM filter papers with the solvent systems (v/v): *A*, 1-butanol–ethanol–water (10:1:2); *B*, 1-butanol–benzene–pyridine–water (5:1:3:3, upper layer); *C*, 1-butanol–acetic acid–water (4:1:5, upper layer); *D*, ethyl acetate–pyridine–acetic acid–water (5:5:1:3); and *E*, ethyl acetate–pyridine–water (8:2:1). Detection was effected with *p*-anisidine hydrochloride[12] and alkaline silver nitrate[13]. Whatman DE1 DEAE-cellulose (coarse, fibrous powder (floc)] was used for fractionation of polysaccharides, and the elution pattern was followed by using the phenol–sulphuric acid reagent.

Unless otherwise stated, all acid hydrolyses were performed with 0.5M sulphuric acid on a boiling water-bath. The hydrolysates were neutralised with barium carbonate, and the clear filtrates were deionised with Amberlite IR-120(H^+) and IRA-400 (CO_3^{2-}) resins, and concentrated under reduced pressure below 45°. The residues were examined by p.c., and, after conversion into the alditol acetates, were also analysed by g.l.c. on 3% ECNSS-M. The column of Amberlite IRA-400 resin was eluted with 2M formic acid, the eluate was evaporated under reduced pressure to dryness, and a portion of the residue was examined by p.c. Another portion of the residue was analysed by g.l.c. on 3% of ECNSS-M after conversion into the alditol acetates[14]. G.l.c. of alditol acetates was performed on a Willy Giede GCHF 18.3 gas chromatograph fitted with a flame-ionisation detector and a stainless-steel column (3 m × 4 mm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) with nitrogen as the carrier gas. G.l.c. of the alditol acetates of partially methylated sugars was performed on a Perkin–Elmer 990 gas chromatograph with an OV-225 S.C.O.T. column (15 m × 0.5 mm) and nitrogen as the carrier gas. G.l.c.–m.s. was performed with a Varian MAT 311-SS instrument.

Isolation of the acidic polysaccharide. — The acid-soluble fraction (300 mg) of *Ocimum basilicum* seed-polysaccharides dissolved in water (50 ml) was fractionated on a column (22 × 4.5 cm) of DEAE-cellulose by elution successively with water, and 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5M sodium hydroxide (1–1.5 litres of each). An acidic polysaccharide (140 mg) was recovered from the 0.05M sodium hydroxide eluate, which was neutralised with 50% acetic acid and then concentrated before the polysaccharide was precipitated by the addition of ethanol (4 vol.). This product was twice rechromatographed on DEAE-cellulose by elution with 0.05M sodium hydroxide. During gel filtration on a column (70 × 2.5 cm) of Sephadex G-200 with 0.1M sodium chloride, the polysaccharide was found to be excluded from the column. Ultracentrifugal analysis of a 0.5% solution of the polysaccharide in 0.1M sodium acetate–acetic acid buffer (pH 4.8) revealed a symmetrical peak (sedimentation coefficient 5.9S). Free-boundary electrophoresis of a 1% solution in 0.05M sodium tetraborate (pH 9.26) indicated a major, symmetrical peak (electrophoretic mobility -1.5×10^4 $cm^2.sec^{-1}.volt^{-1}$) together with a minor peak having a lower mobility. The polysaccharide had a uronic acid content of 27.5% as determined by the carbazole method[15].

Acid hydrolysis of the acidic polysaccharide. — The major acidic polysaccharide (20 mg) was hydrolysed with 0.5M sulphuric acid at 100° for 16 h. P.c. (solvents *A* and *B*) of the resulting neutral sugars and g.l.c. of their alditol acetates indicated the

presence of xylose, arabinose, rhamnose, and traces of galactose and glucose. P.c. (solvents *C* and *D*) of the acidic sugars indicated mainly galacturonic acid and a small proportion of an aldobiouronic acid (R_{GalA} 0.54, solvent *C*). The acidic sugars were converted into their methyl ester methyl glycosides with 2% methanolic hydrogen chloride, and then reduced with sodium borohydride and hydrolysed with acid. The resulting sugars were converted into their alditol acetates and analysed by g.l.c. which indicated the presence of mainly galactose and xylose, a small proportion of arabinose, and a trace of rhamnose. The overall composition of the polysaccharide by g.l.c. analysis was found to be xylose, arabinose, rhamnose, and galacturonic acid in the molar ratios 15:9:7:12, together with traces of galactose and glucose.

Characterisation of the aldobiouronic acid. — An aldobiouronic acid (4 mg) was isolated from the acidic portion of the polysaccharide hydrolysate by preparative p.c. on Whatman 3MM filter paper with solvent *C*. Acid hydrolysis of the aldobiouronic acid gave xylose and galacturonic acid in equal amounts. Reduction with sodium borohydride followed by acid hydrolysis gave galacturonic acid, but no xylose. The aldobiouronic acid was boiled under reflux with dry, methanolic hydrogen chloride (2%) for 5 h, and the resulting methyl ester methyl glycoside, on periodate oxidation followed by acid hydrolysis, gave xylose as the only intact sugar. Hence, the aldobiouronic acid was 3-*O*-(D-galactopyranosyluronic acid)-D-xylose.

Methylation analysis. — The acidic polysaccharide (500 mg) was methylated by the Haworth method[4] for 6 h. The material which precipitated after warming the reaction mixture to 65–70° was recovered by centrifugation, washed with water, and dried over phosphorus pentaoxide; yield, 29 mg. This material was treated with 48% hydrobromic acid followed by acid hydrolysis, and p.c. then indicated the presence of glucose, together with a small proportion of xylose and traces of arabinose and rhamnose. The major polysaccharide was recovered from the reaction mixture, after dialysis, by evaporation; yield, 465 mg. This polysaccharide (400 mg) was suspended in dimethyl sulphoxide (20 ml) and *N*,*N*-dimethylformamide (10 ml) and methylated twice by the Kuhn method[5] with barium oxide, barium hydroxide, and dimethyl sulphate, and twice with barium oxide and methyl iodide. The methylated product was extracted each time with chloroform. The final product was obtained as a white powder (365 mg). Four treatments of this material by the Purdie method[6] gave a fully methylated product (320 mg) that showed no i.r. absorption for hydroxyl.

The methylated polysaccharide (20 mg) was treated with cold 72% sulphuric acid for 1 h at room temperature and then hydrolysed for 12 h at 100° after dilution to 8% acid concentration. The resulting, partially methylated, neutral sugars were converted into their alditol acetates and analysed by g.l.c. and g.l.c.–m.s. The results are given in Table I.

The methylated polysaccharide (10 mg) dissolved in dry tetrahydrofuran (2 ml) was twice reduced with lithium aluminium hydride[9] (10 mg). The carboxyl-reduced polysaccharide was then hydrolysed, and the products were analysed by g.l.c. and g.l.c.–m.s. as described above. The results are shown in Table I.

Periodate oxidation and Smith degradation of the acidic polysaccharide. — The

acidic polysaccharide (100 mg) was oxidised with 45mM sodium metaperiodate (100 ml) at 5° in the dark. The periodate consumption[10] became constant (0.86 mol per mol of hexosyl residue) after 24 h. The periodate-oxidised material was treated with ethylene glycol, reduced with sodium borohydride, and hydrolysed with 0.5M sulphuric acid for 8 h at 100°. The resulting sugars were examined by p.c., and also analysed by g.l.c., after borohydride reduction and acetylation, which indicated the presence of mainly xylose, periodate-degraded components, and traces of arabinose and rhamnose.

The periodate-oxidised and borohydride-reduced material (20 mg) was treated with M sulphuric acid for 48 h at room temperature. The solution was neutralised with barium carbonate, deionised with Amberlite IR-120(H^+) resin, and evaporated to dryness, and the residue was extracted with methanol. The clear, methanolic extract was concentrated, and p.c. examination then indicated the presence of mainly periodate-degraded, neutral components together with traces of arabinose and rhamnose (but no xylose). After methanol extraction, the residue was obtained as a white powder (4.5 mg). A portion of the residue (2 mg) was hydrolysed, and p.c. examination of the products indicated the presence of xylose and periodate-degraded, acidic components. Another portion of the residue (1.5 mg), on oxidation with 0.09M sodium metaperiodate (1 ml) for 120 h, consumed 0.55 mol of periodate per mol of pentosyl residue, as determined spectrophotometrically[10]. The periodate-oxidised material, on treatment with ethylene glycol followed by sodium borohydride, dialysis, and acid hydrolysis, gave (p.c.) xylose and periodate-degraded components.

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REFERENCES

1 R. N. THARANATHAN AND Y. V. ANJANEYALU, *Indian J. Chem.*, 12 (1974) 1164–1165.
2 R. N. THARANATHAN AND Y. V. ANJANEYALU, *Aust. J. Chem.*, 24 (1975) 1345–1350.
3 D. CHANNE GOWDA, Ph. D. Thesis, University of Mysore, 1977.
4 W. N. HAWORTH, *J. Chem. Soc.*, 107 (1915) 8–16.
5 R. KUHN AND H. TRISCHMANN, *Chem. Ber.*, 96 (1963) 284–287.
6 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 85 (1904) 1049–1070.
7 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610–619.
8 P. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun., Univ. Stockholm*, 8 (1976) 1–75.
9 G. O. ASPINALL AND A. K. BHATTACHARJEE, *J. Chem. Soc.*, (1970) 361–365.
10 G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem.*, 5 (1965) 357–361.
11 M. ABDEL-AKHER, J. K. HAMILTON, R. MONTGOMERY, AND F. SMITH, *J. Am. Chem. Soc.*, 74 (1952) 4970–4971.
12 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702–1706.
13 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature (London)*, 166 (1950) 444–445.
14 J. H. SLONEKER, *Methods Carbohydr. Chem.*, 6 (1972) 20–24.
15 Z. DISCHE, *Methods Carbohydr. Chem.*, 1 (1962) 497–501.