Carbohydrate Research, 126 (1984) 297–302
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SOME STRUCTURAL ASPECTS OF THE MUCILAGE ISOLATED FROM THE LEAVES OF *Litsea polyantha*

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(Received September 23rd, 1983; accepted for publication, October 14th, 1983)

ABSTRACT

The purified polysaccharide from the mucilage of the leaves of *Litsea polyantha* was found to contain D-xylose and L-arabinose in the molar ratio of 1:4. A homogeneous, degraded polysaccharide consisting of L-arabinose and D-xylose in the molar ratio of 2:3 was obtained on mild hydrolysis of the native polysaccharide. Permethylation studies were conducted on both the native and degraded polysaccharides, and the results were in good agreement with those obtained from periodate oxidation followed by Smith degradation. Graded-hydrolysis studies established the presence of some of the sugar groupings in the polysaccharide. All of these data indicated that the polysaccharide is a heavily branched arabinoxylan.

INTRODUCTION

The plant *Litsea polyantha* (Lauraceae) grows abundantly in India, and its bark and roots are used for their medicinal action[1]. The seed fat is used in an ointment for rheumatism, and the leaves have a cinnamon-like odor when bruised. The water extract of the leaves is used by village people as a purgative and laxative. Interestingly, the water extract of the mucilage is highly viscous: the present chemical investigation was undertaken because of both the high viscosity and the medicinal value of this mucilage.

RESULTS AND DISCUSSION

Cold-water extraction of the leaves of *Litsea polyantha*, followed by repeated precipitation with ethanol, yielded a polysaccharide material. On hydrolysis, this gave L-arabinose and D-xylose as the constituent sugars, together with traces of D-glucose and D-galactose. Purification of the polysaccharide *via* the Cetavlon complex[2] removed the hexose-containing impurities. On gel filtration through a column of Sephadex G-100, the material was eluted as a single peak; it had $[\alpha]_{589.5}^{26}$ −106°, contained D-xylose and L-arabinose in 1:4 molar ratio, and was paper-electrophoretically homogeneous. A degraded polysaccharide was prepared by heating

0008-6215/84/$ 03.00

a solution of the polysaccharide in 15mM trifluoroacetic acid for 45 min at 100°. The degraded polysaccharide had $[\alpha]_{589.5}^{26}$ −86°, was electrophoretically homogeneous, and contained D-xylose and L-arabinose in the molar ratio of 3:2.

To ascertain the structural features, the native polysaccharide and its degradation product were fully methylated by the Hakomori method[3], followed by the Purdie method[4]. The results of the methylation analysis are shown in Table I, columns A and B.

In the hydrolyzate of the methylated, native product, L-arabinose was found to be present as its 2,3,5-tri-, 2,5-di-, and 2-mono-*O*-methyl derivatives, which indicated that all of the arabinose residues are in the furanose form, and that the di- and mono-*O*-methyl derivatives are engaged in (1→3) and (1→3,5) linkages. Of these methyl derivatives of arabinose, a major portion (~72%) is present as non-reducing end-units, while the rest is either singly or doubly branched. The isolation of unsubstituted D-xylose in the hydrolyzate of the per-*O*-methyl derivative indicated that all of the xylose units are highly branched. In the case of the methylated, degraded polysaccharide, the arabinose was present as the 2,3,5-tri-*O*-methyl derivative, and the xylose, as the 2,3-di- and 2-mono-*O*-methyl derivatives.

On periodate oxidation, the native and the degraded polysaccharide respectively consumed 0.60 and 0.66 mol of the oxidant per mol of pentosyl residue in 12 and 7 h, respectively, and there was almost negligible liberation of formic acid as estimated by the method of Hirst *et al.*[5]. The theoretical values for periodate consumption and formic acid liberation, calculated on the basis of the results of the methylation studies, are 0.57 and 0.60 mol, with no formic acid liberated. Thus, the periodate-oxidation results are in close agreement with those of the methylation studies.

TABLE I

METHYLATION STUDIES OF: A, NATIVE POLYSACCHARIDE; B, DEGRADED POLYSACCHARIDE; C, 1ST SMITH-DEGRADED PRODUCT; D, 2ND SMITH-DEGRADED PRODUCT; AND E, OLIGOSACCHARIDES I, II, AND III FROM GRADED HYDROLYSIS

Sugars[a]	*T*[b]		*Approximate mol %*							*Structural unit deduced*
	1	2	*A*	*B*	*C*	*D*	*E*			
							I	*II*	*III*	
2,3,5-Ara	0.49	0.44	56	38	46	—	48	28	—	Ara*f*-(1→
2,3,4-Xyl	0.67	0.55	—	—	—	—	—	—	32	Xyl*p*-(1→
2,4-Xyl	1.34	1.06	—	—	—	—	51	34	—	→3)-Xyl*p*-(1→
2,3-Xyl	1.56	1.21	—	18	—	96	—	36	65	→4)-Xyl*p*-(1→
2,5-Ara	1.12	0.88	7	—	—	—	—	—	—	→3)-Ara*f*-(1→
2-Ara		1.95	14	—	—	—	—	—	—	→3,5)-Ara*f*-(1→
2-Xyl	2.97	2.21	—	41	51	—	—	—	—	→3,4)-Xyl*p*-(1→
Xyl			20	2	—	—	—	—	—	→2,3,4)-Xyl*p*-(1→

[a]2,3,5-Ara = 2,3,5-tri-*O*-methyl-L-arabinose, etc. [b]Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

On hydrolysis, the product from periodate oxidation of the native polysaccharide yielded equimolar proportions of L-arabinose and D-xylose, which proved that ~72% of the arabinose had been oxidized. After subjection to Smith degradation, the polysaccharide was methylated. The result, given in Table I, column C, showed that all of the xylosyl residues are resistant to periodate oxidation, and that the rest of the arabinosyl units are nonreducing end-groups after the first periodate oxidation.

The material obtained after a second Smith degradation had a xylan chain that, on methylation and hydrolysis, gave 2,3-di-*O*-methylxylose as the sole methylated sugar derivative.

On graded hydrolysis, the polysaccharide gave three oligomers, which were isolated as homogeneous fractions. They were characterized by methylation studies. The results are shown in Table I, column E. Oligosaccharide I was found to be a disaccharide having an arabinosyl group at the nonreducing end, as the methylation analysis gave 2,3,5-tri-*O*-methylarabinose and 2,4-di-*O*-methylxylose in equimolar proportions. On methylation analysis, oligosaccharide II gave 2,3,5-tri-*O*-methylarabinose, and 2,4- and 2,3-di-*O*-methylxylose in almost equimolar proportions. The third oligomer gave 2,3,4-tri- and 2,3-di-*O*-methylxylose in the molar ratio of 1:2.

From all of these results, it is possible to outline the structural features of the polysaccharide from the mucilage of the leaves of *Litsea polyantha*. The macromolecule contains a xylan backbone of xylosyl residues glycosidically linked with each other by (1→4) linkages; these residues are heavily branched by arabinosyl residues attached by (1→2), (1→3) linkages, and some of the arabinosyl residues are further branched by arabinose, and they are joined through O-1, O-3, and O-5. The specific rotation values show that most of the linkages are β.

EXPERIMENTAL

General methods. — All evaporations were performed under diminshed pressure at a bath temperature below 40°. A Perkin–Elmer Model 241 MC polarimeter and 1-dm tubes were used for measuring optical rotations, and i.r. spectra were recorded with a Perkin–Elmer 177 spectrophotometer. G.l.c. was performed with a Hewlett–Packard Model 5730A gas chromatograph fitted with an f.i.d. and a t.c.d. detector, with use of two different glass columns (1.83 m × 6 mm) containing (*1*) 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 190° (for alditol acetates of sugars) and at 170° (for partially methylated alditol acetates), and (*2*) 3% of OV-225 at 155°. For quantitative evaluation of the g.l.c., a Hewlett–Packard 3390A integrator was used. The methyl derivatives of some of the sugars were identified by mixed injection with an authentic sample. Paper chromatography was performed on Whatman No. 1 (for qualitative work) and on Whatman No. 3 (for quantitative) sheets, with 6:4:3 1-butanol–pyridine–water as the developer, and indication with aniline oxalate. Gel-filtration chromatography was conducted with Sephadex LH-

20, and G-100. Elutions were monitored both with a differential refractometer, and by the phenol–sulfuric acid method (for unmethylated compounds), and polarimetrically (for methylated compounds). A Shandon, high-voltage, electrophoresis apparatus, model L-24, was used for testing the homogeneity of the materials.

Isolation and purification of the polysaccharide. — The dried and powdered leaves (25 g) were exhaustively extracted successively in a Soxhlet apparatus with alcohol, 1:2 benzene–methanol, and dry methanol, and dried. The dry material was allowed to swell in water overnight, diluted to a 1% solution, and stirred vigorously for 24 h at room temperature. The insoluble material was removed by squeezing the suspension through a Nylon cloth, and the cloudy solution was centrifuged at 25,000–30,000 r.p.m. for clarification. The wine-red, viscous solution was passed through a bed of Dowex 50 (H^+) resin, and then dialyzed. The dialyzed material was concentrated to a small volume, and the polysaccharide was precipitated by adding ethanol. The precipitate was dried, mixed with water, and the mixture stirred for 10 h, and kept overnight at 5°. A gel-like substance that settled was removed at the centrifuge, and to the pink, viscous, supernatant liquor was added, in the cold, ethanol (2 vol., acidified to pH 4.5 with acetic acid); the pinkish precipitate was collected by centrifuging, washed several times with ethanol, and dried. The process of dissolution in water and precipitation with alcohol was repeated five times. Finally, the precipitate was dissolved in water, and the solution was lyophilized; yield, 1.8 g. On hydrolysis, arabinose, xylose, and traces of glucose and galactose were identified by p.c. and g.l.c.

The polysaccharide was further purified by complexing with a quaternary ammonium salt[2]. The material (1.5 g) was dissolved in water (200 mL) with stirring, and a small amount of insoluble material was removed by centrifugation. A portion of cetyltrimethylammonium hydroxide (CTA-OH), in the form of a 10% (w/v) aqueous solution, was added to the polysaccharide solution, which became cloudy and then aggregated on addition of a small amount of 0.5M sodium hydroxide solution. The sticky precipitate thus obtained was removed by centrifugation, dissolved in the minimal volume of 6% acetic acid, and the solution poured into ethanol. An almost white powder was obtained after centrifuging and drying.

The purified polysaccharide (40 mg) was dissolved in the minimal volume of water, and the solution was applied to a column (80 × 2 cm) of Sephadex G-100, and eluted with water. The major portion of the material, eluted as a single fraction, moved as a single component when subjected to high-voltage electrophoresis; it had $[\alpha]_{589.5}^{26}$ −106° (in water).

Preparation of degraded polysaccharide. — A solution of the polysaccharide (500 mg) in 15mM trifluoroacetic acid solution (100 mL) was heated on a boiling-water bath for 45 min. Arabinose was detected first, and, after 45 min, xylose appeared. The hydrolyzate was dialyzed, concentrated to a small volume, and poured, with stirring, into ethanol (3 vol., containing 1% of lithium chloride). The precipitate was separated by centrifugation, and triturated thrice with ethanol and

once with dry ether; yield, 175 mg. The degraded polysaccharide had $[\alpha]_{589.5}^{26}$ −86° (in water), and was found to be electrophoretically homogeneous.

Sugar analysis. — The native and the degraded polysaccharide (3.9 mg and 2.1 mg, respectively) were each mixed with *myo*-inositol (250 μg for each 1 mL of solution, as an internal standard), and hydrolyzed with 2M trifluoroacetic acid (TFA) for 3 h at 110° in a sealed tube. The excess of acid was removed by codistillation with water. The hydrolyzates were converted into their alditol acetates[6], and these were analyzed by g.l.c. using column *1*. The native and the degraded polysaccharide both contained arabinose and xylose, in the molar ratios of 4:1 and 2:3, respectively.

Methylation analysis. — Thoroughly dried native, and degraded, polysaccharide (15 mg, and 6.5 mg, respectively) were methylated once by the Hakomori method[3] and twice by the Purdie[4] method. After the usual treatment, the final products were purified by passing them through a column of Sephadex LH-20. The i.r. spectrum showed no absorption in the hydroxyl-group region. The fully methylated polysaccharides were hydrolyzed with 90% formic acid, followed by 0.25M sulfuric acid[7]. The sugars were analyzed by g.l.c. (columns *1* and *2*) as their alditol acetates. The results are shown in Table I, columns A and B.

Periodate oxidation. — The native and the degraded polysaccharide were each treated with 0.04M sodium metaperiodate in the dark at 4°. Consumption of the oxidant became constant (monitored spectrophotometrically[8]) for the native polysaccharide in 12 h, and for the degraded, in 7 h, at a value corresponding respectively to 0.60 mol and 0.66 mol of the oxidant per pentosyl residue. The product of oxidation of the native polysaccharide (50 mg) was used for the following experiments.

Part (~5 mg) of the oxidation product was hydrolyzed with 0.5M sulfuric acid for 5 h at 100°. G.l.c. analysis then showed the presence of arabinose and xylose in almost equimolar proportions.

Smith degradation. — Part (~50 mg) of the oxidation product was reduced with sodium borohydride[9]. The reaction mixture was acidified with acetic acid to pH 4, dialyzed to remove salts, and lyophilized. The reduced, periodate-oxidized product was then treated with 0.05M sulfuric acid (5 mL) for 48 h at room temperature. After the usual treatment, a part (~5 mg) was fully methylated; the analysis result is shown in Table I, column C.

Second periodate oxidation. — The solution containing partially hydrolyzed, reduced, periodate-oxidized, native polysaccharide (~20 mg) was further oxidized with sodium metaperiodate solution. After removal of iodate and periodate ions, the material was reduced with sodium borohydride, and the excess of $NaBH_4$ removed; the product was treated with 0.05M sulfuric acid (2 mL) for 24 h at room temperature. The hydrolyzate was made neutral, and, after the usual treatment, was fully methylated. The result of the methylation analysis is shown in Table I, column D.

Graded hydrolysis of the native polysaccharide. — Aided by the results of a

pilot experiment, the polysaccharide (1.25 g) was heated with 0.02M oxalic acid for 10 h at 100°. The solution was made neutral with 0.02M calcium hydroxide solution, and the suspension centrifuged. The supernatant liquor was de-ionized with Dowex 50 (H^+) and Dowex-1 X-4 (OH^-) resins. Paper-chromatographic examination of the hydrolyzate indicated three oligosaccharides, namely I, II, and III (designated according to their mobility), together with arabinose and xylose. Each oligomer was resolved on Whatman No. 3 filter paper, and was then found to be homogeneous by paper chromatography. On hydrolysis, oligosaccharide I, $[\alpha]_{589.5}^{26}$ −35.5°, gave arabinose and xylose in almost equimolar proportions. Methylation analysis (see Table I, column E) showed the presence of 2,3,5-tri-*O*-methylarabinose and 2,4-di-*O*-methylxylose in the molar ratio of 1:1. This indicated that arabinose was the nonreducing end-group, glycosylically (1→3)-linked to xylose. Oligosaccharide II, $[\alpha]_{589.5}^{26}$ −14.9°, contained arabinose and xylose in 1:2 molar ratio. On hydrolysis, the permethylated derivative yielded 2,3,5-tri-*O*-methylarabinose, and 2,3- and 2,4-di-*O*-methylxylose in almost equimolar ratios. Oligosaccharide III, $[\alpha]_{589.5}^{26}$ −44.5°, contained xylose only. The permethylated derivative yielded 2,3,4-tri- and 2,3-di-*O*-methylxylose in 1:2 molar ratio.

ACKNOWLEDGMENTS

The authors thank Dr. N. Roy of the Department of Macromolecules, Indian Association for the Cultivation of Science, Jadavpur, Calcutta 700032 (India) for supplying some methylated sugar derivatives, and also Mr. A. K. Das and Mr. S. Choudhuri for their technical assistance.

REFERENCES

1 R. N. CHOPRA, I. C CHOPRA, AND B. S. VARMA, *Supplement to Glossary of Indian Medicinal Plants*, Publication and Information Directorate, Council of Scientific and Industrial Research, Hillside Road, New Delhi-12, 1969, p. 57; K. BISWAS AND E. GHOSH, *Bharatiya Bonoausudi* (Bengali), Vol. 4, Calcutta University, 1973, p. 1016.
2 G. A. ADAMS, *Methods Carbohydr. Chem.*, 5 (1965) 75–78.
3 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
4 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 85 (1904) 1049–1070.
5 T. G. HALSALL, E L. HIRST, AND J. K. N. JONES, *J. Chem. Soc.*, (1947) 1427–1432.
6 P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH, AND A. KARR, *Carbohydr. Res.*, 5 (1967) 340–345.
7 B. LINDBERG, J. LONNGREN, J. L. THOMPSON, AND W. NIMMICH, *Carbohydr. Res.*, 25 (1972) 49–57.
8 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind. (London)*, (1957) 1216.
9 M. ABDEL-AKHER, J. K. HAMILTON, R. MONTOGOMERY, AND F. SMITH, *J. Am. Chem. Soc.*, 74 (1952) 4970–4971.