Carbohydrate Research, 87 (1980) 241–248
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

STRUCTURE OF A NEW ARABINOXYLAN FROM THE BARK OF *Cinnamomum iners*

J. PAPE GOWDA, D. CHANNE GOWDA, AND YERNOOL V. ANJANEYALU

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

(Received April 8th, 1980; accepted for publication, May 14th, 1980)

ABSTRACT

Two polysaccharide fractions, water-soluble (24%) composed of D-xylose and L-arabinose, and alkali-soluble (15%) composed of L-arabinose, D-xylose, and D-glucose, have been isolated from the delignified bark of *Cinnamomum zeylanicum*. The water-soluble fraction gave an arabinoxylan (60%) composed of L-arabinose and D-xylose in the molar ratio 1.45:1.00. Methylation analysis, together with the isolation of the oligosaccharides 3-*O*-α-D-xylopyranosyl-L-arabinose, 4-*O*-β-D-xylopyranosyl-D-xylose, *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, and *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose after partial hydrolysis with acid, suggested that the polysaccharide contained a backbone of (1→4)-linked β-D-xylosyl residues each of which was substituted both at O-2 and O-3 with L-arabinofuranosyl and 3-*O*-α-D-xylopyranosyl-L-arabinofuranosyl groups. Very mild hydrolysis with acid gave a degraded polysaccharide, containing 10% of L-arabinose and 90% of D-xylose, which was essentially a (1→4)-linked β-D-xylan carrying a limited number of branches at O-2 and O-3. These results together with those from periodate-oxidation and Smith-degradation studies support the proposed structure.

INTRODUCTION

The powdered, dry bark of *Cinnamomum iners*[1], which is dark brown in colour and has a characteristic odour due to cinnamaldehyde, is used extensively in making incense sticks, because of its good binding properties and also to provide bulk. It is a rich source of a hitherto unstudied mucilage which is responsible for its binding properties. The mucilage consists of water-soluble and alkali-soluble polysaccharides, and we now report on the chemical nature of the former.

RESULTS AND DISCUSSION

Delignification[2] of the dry-bark powder gave a white, insoluble residue and an aqueous extract. The polysaccharide in the aqueous extract was precipitated with

0008-6215/80/0000-0000/$ 02.25,

ethanol and isolated (24%) as a colourless powder (*A*) which contained 55% of L-arabinose, 40% of D-xylose, and 5% of D-glucose; nitrogen, *O*-acetyl groups, and uronic acids were absent. Extraction of the insoluble residue with aqueous sodium hydroxide followed by precipitation with ethanol gave a nitrogen-free, alkali-soluble polysaccharide (*B*, 15%) as a colourless powder which contained 23% of L-arabinose, 20% of D-xylose, 51% of D-glucose, and traces of uronic acid.

Polysaccharide *A* was almost completely soluble in aqueous acetic acid; the residue (P-1) (10%) had the same sugar composition as polysaccharide *B*. Addition of ethanol (2 vol.) to the acetic acid solution precipitated 60% of the polysaccharide (P-2), and neutralisation of the remaining solution with sodium hydroxide gave polysaccharide P-3 (25%). Fractions P-2 and P-3 contained L-arabinose and D-xylose in the ratio 1.45:1, as determined by g.l.c. of the derived alditol acetates[3]. Polysaccharide P-2 was completely and sharply precipitated from aqueous solution by Fehling's solution or aqueous barium hydroxide; it appeared to be a single species and is designated as an arabinoxylan.

Partial hydrolysis of the arabinoxylan gave four oligosaccharide fractions (**1**–**4**). Results of borohydride reduction and periodate oxidation indicated fraction **1** to be 3-*O*-α-D-xylopyranosyl-L-arabinose. However, its $[\alpha]_D$ value (+101°) was low compared to the reported[4] value (+172–183°), and g.l.c. of the products obtained after methylation and hydrolysis revealed 2,3,4-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose, and 2,5-di-*O*-methylarabinose in the molar ratios 1:0.43:0.57. Hence, fraction **1** was a mixture of 3-*O*-α-D-xylopyranosyl-L-arabinose (58%) and 4-*O*-β-D-xylopyranosyl-D-xylose (42%), which had similar R_{Glc} values in the solvent employed for chromatography.

Fractions **2**–**4** were characterised as 4-*O*-β-D-xylopyranosyl-D-xylose, *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, and *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose. On graded hydrolysis of the arabinoxylan, L-arabinose and 3-*O*-α-D-xylopyranosyl-L-arabinose were released under very mild conditions, suggesting the presence of L-arabinofuranosyl residues.

Hakomori methylation[5] of the arabinoxylan, followed by acid hydrolysis and g.l.c. and g.l.c.–m.s. of the products as the partially methylated alditol acetates[6], gave the results shown in Table I. The presence of a high proportion of xylose in the hydrolysate of the methylated polysaccharide, and also the formation of 4-*O*-β-D-xylopyranosyl-D-xylose and the corresponding xylotriose and xylotetraose on partial hydrolysis, suggested that the arabinoxylan contained a backbone of (1→4)-linked β-D-xylosyl residues. The formation of 2,3,4-tri-*O*-methyl-D-xylose and 2,5-di-*O*-methyl-L-arabinose, together with the formation of 3-*O*-α-D-xylopyranosyl-L-arabinose on partial hydrolysis of the polysaccharide, indicated that the remainder of the D-xylose residues were present as non-reducing end-groups and attached to the backbone through (1→3)-linked L-arabinofuranosyl residues at O-2 and/or O-3. The formation of 2,3,5-tri-*O*-methyl-L-arabinose in high yield suggested that the major

TABLE I

G.L.C. AND G.L.C.–M.S. OF ALDITOL ACETATES DERIVED FROM PERMETHYLATED ARABINOXYLAN

Alditol acetates of	*Molar proportions*	*Characteristic fragments* (m/z)	*Mode of linkage*
2,3,5-Tri-*O*-methyl-L-arabinose	8.2	45,101,117,129,161	L-Ara*f*-(1→
2,3,4-Tri-*O*-methyl-D-xylose	4.1	101,117,161	D-Xyl*p*-(1→
2,5-Di-*O*-methyl-L-arabinose	4.8	45,117,173,189,233	→3)-L-Ara*f*-(1→
D-Xylose	5.9	103,115,145,187,217	→2,3,4)-D-Xyl*p*-(1→

portion of the L-arabinose residues were present as non-reducing end-groups directly linked to the D-xylan backbone at O-2 and/or O-3.

Thus, the average structural features of the arabinoxylan can be tentatively represented as shown in Fig. 1; the sequence of the different branches along the polysaccharide chain is not known.

The above structural assignment was supported by the results of periodate-oxidation[7] and Smith-degradation[8] studies. Thus, in accord with expectation, the arabinoxylan consumed 0.65 mol of periodate and liberated 0.14 mol of formic acid per pentosyl residue. Reduction of the periodate-oxidised material with borohydride followed by acid hydrolysis and g.l.c. of the products as the alditol acetates gave glycerol, L-arabinose, and D-xylose in the molar ratios 1.20:1.16:1.00. These results also accord with the structure in Fig. 1, as did those of partial hydrolysis with acid.

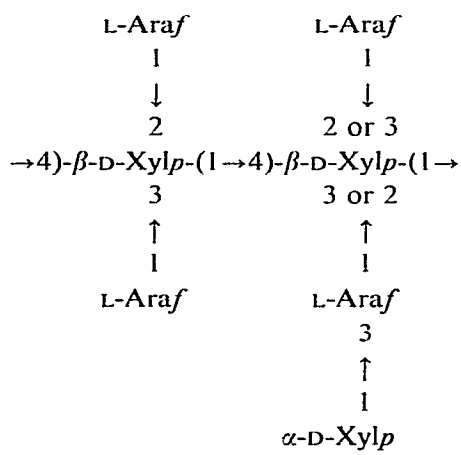

Fig. 1. Average repeating-unit of the arabinoxylan from *C. iners*.

Hydrolysis of the arabinoxylan under very mild conditions gave degraded polysaccharide, $[\alpha]_D$ −61.5°, containing 10% of L-arabinose and 90% of D-xylose. Methylation analysis of the degraded polysaccharide, coupled with g.l.c. and g.l.c.–m.s. of the resulting, partially methylated alditol acetates, gave the results shown in Table II. The formation of 2,3-di-*O*-methyl-D-xylose in high proportion and 3-*O*-

TABLE II

G.L.C. AND G.L.C.–M.S. OF ALDITOL ACETATES DERIVED FROM THE PERMETHYLATED, PARTIALLY DEGRADED ARABINOXYLAN

Alditol acetates of	*Molar proportions*	*Characteristic fragments* (m/z)	*Mode of linkage*
2,3,5-Tri-*O*-methyl-L-arabinose	0.95	45,101,117,129,161	L-Ara*f*-(1→
2,3,4-Tri-*O*-methyl-D-xylose	1.90	101,117,161	D-Xyl*p*-(1→
2,5-Di-*O*-methyl-L-arabinose	2.00	45,117,173,233	→3)-L-Ara*f*-(1→
2,3-Di-*O*-methyl-D-xylose	12.50	101,117,129,161 189,233	→4)-D-Xyl*p*-(1→
2-*O*-Methyl- and 3-*O*-methyl-D-xylose	2.90	117,129,189,261	→3,4)-D-Xyl*p*-(1→ and →2,4)-D-Xyl*p*-(1→

and/or 2-*O*-methyl-D-xylose in relatively small proportion indicated that the degraded polysaccharide was essentially a (1→4)-linked D-xylan having a few branches at O-2 and O-3. The formation of a very small amount of 2,3,5-tri-*O*-methyl-L-arabinose, together with that of 2,3,4-tri-*O*-methyl-D-xylose and 2,5-di-*O*-methyl-L-arabinose in the molar ratio 1:1, suggested that the branch points consisted of L-arabinofuranosyl and 3-*O*-D-xylopyranosyl-L-arabinofuranosyl groups. The high, negative $[\alpha]_D$ value of the degraded polysaccharide indicated a preponderance of β-D linkages.

The degraded polysaccharide consumed 1.2 mol of periodate per pentosyl residue, and small proportions of L-arabinose and D-xylose survived. The formation of such a degraded polysaccharide further supports the structure proposed in Fig. 1.

Highly branched L-arabino-D-xylans containing (1→4)-linked β-D-xylan backbones with branch points usually at O-3, and to some extent at O-2, as well as both at O-2 and O-3, have been reported from various sources[9]. However, there has been no report of an L-arabino-D-xylan having a (1→4)-linked β-D-xylan backbone in which all of the D-xylosyl residues carry branches both at O-2 and O-3, as in the arabinoxylan from *C. iners*. A highly branched polysaccharide containing a D-xylan core has been isolated from the corm sacs of *Watsonia pyrimidata*[10], but the branches have D-galactopyranosyl end-groups.

EXPERIMENTAL

General. — The finely powdered bark of *C. iners* was obtained from the local market. Decending paper chromatography (p.c.) was performed on Whatman No. 1 and 3MM papers with *A*, 1-butanol–benzene–pyridine–water (5:1:3:3, upper layer); *B*, 1-butanol–acetic acid–water (4:1:5, upper layer), *C*, ethyl acetate–pyridine–water (8:2:1); *D*, 1-butanol–pyridine–water (6:4:3); and detection with *p*-anisidine hydrochloride[11] and alkaline silver nitrate[12].

Polysaccharide fractions were hydrolysed with 0.125 or 0.25M sulphuric acid at ~100°, and the hydrolysates were neutralised ($BaCO_3$), filtered, deionised with

Amberlite IR-120(H^+) and IRA-400(CO_3^{2-}) resins, and concentrated under diminished pressure below 45°. The residues were examined by p.c., and the neutral sugars were also analysed by g.l.c. as their alditol acetates[3]. The Amberlite IRA-400 (CO_3^{2-}) resin was eluted with 2M formic acid, the eluate was concentrated to dryness, and the residue was examined by p.c. for acidic sugars. The constituent sugars were isolated by preparative p.c., and their absolute configurations were assigned on the basis of $[\alpha]_D$ values and by conversion into suitable derivatives (di-*O*-benzylidene-xylose[13], and arabinose and glucose *p*-nitroanilides[14]).

Oligosaccharides were converted into their methyl glycosides by treatment with dry 2% methanolic hydrogen chloride overnight at room temperature. Each methyl glycoside was methylated (Hakomori) and then hydrolysed with 0.25M sulphuric acid; the products were converted into their alditol acetates[3] and analysed by g.l.c. using a CIC (India) AC.1-F.i. gas chromatograph fitted with a flame-ionisation detector and a stainless-steel column (2 m × 3 mm) containing 5% of OV-225 on Gas Chrom Q (100–120 mesh) with nitrogen as the carrier gas. Retention times were determined relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. G.l.c.–m.s. was performed with a Pye 204 gas chromatograph attached to a V.G. Micromass 16E mass spectrometer.

Isolation of the polysaccharide. — Finely powdered, dark-coloured, bark powder (10 g) was stirred with water (500 ml) and delignified with sodium chlorite in the presence of acetic acid[2]. The insoluble residue was collected and dried. Ethanol (2 vol.) was added to the filtrate, to give a white precipitate. After storage overnight, the supernatant solution was decanted, and the polysaccharide (2.4 g) was collected by centrifugation and dried.

The insoluble residue was extracted thrice with 5% sodium hydroxide. To the combined extracts (500 ml) was added ethanol (2 vol.). The white precipitate (1.5 g) was collected, washed thoroughly with ethanol, and dried.

The water-soluble and alkali-soluble polysaccharides were hydrolysed with 0.25M sulphuric acid for 8 h at 100°. The sugars in the hydrolysates were isolated by preparative p.c. (solvent *A*) and their absolute configurations were determined. The water-soluble polysaccharide contained 55% of L-arabinose, 40% of D-xylose, and 5% of D-glucose; nitrogen, *O*-acetyl groups, and uronic acids were absent. The alkali-soluble polysaccharide was nitrogen-free and contained 51% of D-glucose, 26% of D-xylose, 23% of L-arabinose, and traces of a uronic acid.

Fractionation of the water-soluble polysaccharide. — A solution of the water-soluble polysaccharide (1 g) in 5% acetic acid (1 litre) was centrifuged, and the insoluble residue (P-1, 100 mg) was collected and dried. Ethanol (2 vol.) was added to the clear centrifugate with continuous stirring, and the precipitate (P-2, 600 mg) was collected and dried. Further addition of ethanol (4 vol.) did not yield more precipitate. Neutralisation of the remaining aqueous portion with 20% aqueous sodium hydroxide gave a precipitate, which was collected, dissolved in water (50 ml), and dialysed against running tap-water for 24 h and then against distilled water for

24 h. From the resulting highly viscous solution, the polysaccharide (P-3, 250 mg) was recovered either by lyophilisation or by the addition of excess of ethanol.

Acid hydrolysis and p.c. of the products revealed that P-1 and the alkali-soluble fraction were similar in sugar composition. P-2 and P-3 contained L-arabinose and D-xylose in the molar ratio 1.5:1.0, as determined by g.l.c. of the derived alditol acetates[3]. P-2 was completely and sharply precipitated from its aqueous solution with Fehling's solution or aqueous barium hydroxide, and its sugar composition remained unaltered after repeated precipitation. Thus, P-2 appeared to be a single species and was designated as arabinoxylan.

Investigation of the arabinoxylan P-2. — (*a*) *Methylation analysis.* A single Hakomori methylation[5] of P-2 (10 mg) gave a fully methylated product. The methylated product was extracted with chloroform, and the extract was dried (Na_2SO_4) and concentrated. The residue was hydrolysed with 90% formic acid (1 ml) at 100° for 2 h in a sealed tube and then, after evaporation of the formic acid, with 0.25M sulphuric acid for 6 h at 100°. The resulting, partially methylated sugars were converted into their alditol acetates, which were extracted with chloroform, and analysed by g.l.c. and g.l.c.–m.s. The results are given in Table I.

(*b*) *Partial hydrolysis.* P-2 (0.5 g) was hydrolysed with 0.125M sulphuric acid (50 ml) at 100° for 1 h, to give a maximum yield of small oligosaccharides. The hydrolysate was neutralised, deionised, and concentrated. P.c. (solvent *A*) of the residue revealed oligosaccharide fractions **1**–**4** (R_{Glc} 0.83, 0.71, 0.34, and 0.17, respectively) in addition to L-arabinose and D-xylose. The oligosaccharides were isolated by preparative p.c. on Whatman 3MM paper and their homogeneity was ascertained by p.c.

Fraction **1** had $[\alpha]_D$ +101° (*c* 0.25 water). Acid hydrolysis gave (p.c.) D-xylose and L-arabinose. Reduction with sodium borohydride followed by acid hydrolysis gave D-xylose. Periodate oxidation of the methyl glycoside followed by acid hydrolysis gave (p.c.) L-arabinose as the only intact sugar. Methylation of the methyl glycoside, followed by acid hydrolysis and g.l.c. of the resulting, partially methylated sugars as their alditol acetates, gave 2,3,4-tri-*O*-methyl-D-xylose (1 mol), 2,5-di-*O*-methyl-L-arabinose (0.57 mol), and 2,3-di-*O*-methyl-D-xylose (0.43 mol). Hence, fraction **1** was a mixture of 3-*O*-α-D-xylopyranosyl-L-arabinose and 4-*O*-β-D-xylopyranosyl-D-xylose.

Fraction **2** had m.p. 186–189° (from aqueous methanol), $[\alpha]_D$ −24° (*c* 0.25, water), and was 4-*O*-β-D-xylopyranosyl-D-xylose; lit.[4] m.p. 186–189°, $[\alpha]_D$ −20–−30°. Acid hydrolysis gave (p.c.) only D-xylose. Periodate oxidation of the methyl glycoside followed by borohydride reduction and acid hydrolysis gave (p.c.) glycerol, but no D-xylose. Methylation analysis of the methyl glycoside gave 2,3,4-tri-*O*-methyl-D-xylose and 2,3-di-*O*-methyl-D-xylose in equal proportions.

Fraction **3** was *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose. Acid hydrolysis gave only D-xylose. Periodate oxidation of the methyl glycoside followed by borohydride reduction and acid hydrolysis gave (p.c.) glycerol, but no

intact D-xylose. Methylation analysis gave 2,3,4-tri-*O*-methyl-D-xylose (1 mol) and 2,3-di-*O*-methyl-D-xylose (2 mol).

Fraction **4** was *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, m.p. 215–218° (from water–methanol–1-butanol), $[\alpha]_D$ −61.5° (*c* 0.2, water); lit.[4] m.p. 219–220°, $[\alpha]_D$ −60°. Acid hydrolysis gave only D-xylose. Periodate oxidation of the methyl glycoside followed by borohydride reduction and acid hydrolysis gave (p.c.) glycerol, but no intact D-xylose. Methylation analysis gave 2,3,4-tri-*O*-methyl-D-xylose (1 mol) and 2,3-di-*O*-methyl-D-xylose (3 mol).

(*c*) *Periodate oxidation.* The polysaccharide (100 mg) was treated with 45mM sodium metaperiodate (100 ml) at room temperature in the dark. Periodate consumption and the liberation of formic acid were monitored by titration against standard sodium thiosulphate and sodium hydroxide, respectively. The periodate consumption (0.65 mol) became constant after 10 h, and 0.14 mol of formic acid per mol of pentosyl residue was liberated.

The oxidation mixture was treated with ethylene glycol, dialysed, reduced with sodium borohydride, and hydrolysed with 0.5M sulphuric acid for 5 h at 100°. The resulting sugars were examined by p.c. and also by g.l.c. after borohydride reduction and acetylation, which indicated the formation of glycerol, L-arabinose, and D-xylose in the molar ratios 1.20:1.14:1.00.

Partially degraded arabinoxylan. — The arabinoxylan (200 mg) was hydrolysed with 0.125M sulphuric acid (20 ml) for 80 min at 80°. The hydrolysate was quickly neutralised ($BaCO_3$), deionised, and concentrated under diminished pressure. The degraded polysaccharide (30 mg), which was precipitated by the addition of excess of ethanol, had $[\alpha]_D$ −61.5° (*c* 0.2, water).

Acid hydrolysis of the degraded polysaccharide and g.l.c. of the resulting sugars as their alditol acetates indicated the presence of 90% of D-xylose and 10% of L-arabinose.

The degraded polysaccharide (10 mg) was methylated[5] (Hakomori), and then hydrolysed with 90% formic acid (1 ml) for 2 h at 100° in a sealed tube and, after evaporation of the formic acid, with 0.25M sulphuric acid for 6 h at 100°. The resulting, partially methylated 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 II.

The degraded polysaccharide (10 mg), when treated with 45mM sodium metaperiodate (10 ml) at room temperature in the dark, consumed 1.2 mol of oxidant per pentosyl residue. The oxidation mixture was treated with ethylene glycol, dialysed, reduced with sodium borohydride, and hydrolysed. P.c. of the hydrolysate revealed glycerol together with small proportions of D-xylose and L-arabinose.

ACKNOWLEDGMENTS

We thank Professor T. R. Ramaiah (Department of Biochemistry, University of Mysore) for providing g.l.c. facilities, and Professor G. O. Aspinall (Department

of Chemistry, York University, Ontario, Canada) for the g.l.c.–m.s. analysis. One of us (J.P.G.) thanks the U.G.C. for an F.I.P. fellowship, and the Department of Collegiate Education (Government of Karnataka) for study leave.

REFERENCES

1 *The Wealth of India–Raw Materials*, C.S.I.R. Publication, New Delhi, India, Vol. II, 1950, pp. 179–181.
2 R. L. WHISTLER, *Methods Carbohydr. Chem.*, 5 (1965) 171–175.
3 J. H. SLONEKER, *Methods Carbohydr. Chem.*, 6 (1972) 20–24.
4 R. W. BAILEY, *Oligosaccharides*, Pergamon, Oxford, 1965, pp. 43–44 and 79.
5 S.-I. HAKOMORI, *J. Biochem (Tokyo)*, 55 (1964) 205–209.
6 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610–619.
7 G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods. Carbohydr. Chem.*, 5 (1965) 357–365.
8 M. ABDEL-AKHER, J. K. HAMILTON, R. MONTGOMERY, AND F. SMITH, *J. Am. Chem. Soc.*, 74 (1952) 4970–4971.
9 G. O. ASPINALL, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 353–379.
10 D. H. SHAW AND A. M. STEPHEN, *Carbohydr. Res.*, 1 (1966) 400–413.
11 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702–1706.
12 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature (London)*, 166 (1950) 444–445.
13 R. L. WHISTLER AND J. N. BEMILLER, *Methods Carbohydr. Chem.*, 1 (1962) 88–90.
14 R. L. WHISTLER AND J. N. BEMILLER, *Methods Carbohydr. Chem.*, 1 (1962) 71–73.