Carbohydrate Research, 131 (1984) 149–155
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STRUCTURAL STUDIES ON A POLYSACCHARIDE FRACTION FROM THE FRUITS OF *Cordia dichotoma* FORST

NANDA G. BASU, PRADYOT K. GHOSAL, AND SWAPNADIP THAKUR
Department of Chemistry, Natural Products Laboratory, University of Burdwan, Burdwan 713 104 (India)

(Received February 2nd, 1984; accepted for publication, February 23rd, 1984)

ABSTRACT

The neutral polysaccharide isolated from the fruits of *Cordia dichotoma* was separated into two fractions, I and II, by gel filtration using Sephadex G-100. The major fraction, II, was found to contain D-glucose and L-arabinose in the molar ratio of 21:4. The results of complete hydrolysis with acid, permethylation studies, periodate oxidation, and Smith degradation suggested it to be an arabinoglucan, and the backbone of the polysaccharide to be composed of (1→6)-linked D-glucopyranosyl and (1→2)-linked L-arabinofuranosyl residues. The average degree of polymerization, determined through end-group analysis, was found to be ~144.

INTRODUCTION

The medicinally important[1], deciduous plant *Cordia dichotoma* (Boraginaceae) is available all over India. Its ripe fruit produces a sticky, jelly-like mass. Constituents of the polysaccharide present in the fruits of *C. myxa* have been reported[2], but no attempt has yet been made to elucidate the structure of the neutral polysaccharide isolated from the fruits of *C. dichotoma*. We now report the structural elucidation of the major polysaccharide fraction, II (separated by gel filtration of the polysaccharide), on the basis of the results of complete hydrolysis with acid, methylation analysis, periodate oxidation, and Smith degradation. Also, the degree of polymerization (d.p.) of the polysaccharide was determined by end-group analysis.

RESULTS AND DISCUSSION

The polysaccharide isolated from the ripe fruit by extraction with water was purified by repeated precipitation from the aqueous solution with 9:1 (v/v) ethanol–acetone. The ionic impurities were removed by passing a dilute, aqueous solution of the polysaccharide through columns of Amberlite IR-120 (H^+) and Amberlite IRA-400 ($HCOO^-$) resins. The eluate was concentrated, and the concentrate lyophilized. The polysaccharide was then subjected to gel filtration through

0008-6215/84/$ 03.00

TABLE I

CONTENTS OF MONOSACCHARIDES IN THE POLYSACCHARIDE

Monosaccharide	*Percent*	*Fragments obtained in g.l.c.–m.s. analysis* (m/z)
D-Glucose	86.7	43(100%), 73(5), 85(8), 97(7), 103(15), 115(28), 128(15), 139(14), 145(21), 157(14), 170(11), 187(13), 217(11), 259(3), 289(6), 361(1)
L-Arabinose	13.2	43(100%), 73(4), 85(10), 103(13), 115(28), 127(13), 145(17), 157(6), 158(5), 187(11), 200(4), 217(9)

Sephadex G-100 in 0.1M sodium chloride–0.1M sodium hydroxide buffer, the eluate being monitored spectrophotometrically by using the phenol–sulfuric acid reagent[3]. It was observed that two fractions (I and II) were present, their relative distribution being 23 and 77%, respectively.

Fraction II was further gel-filtered through G-200, using the same buffer, and there was no further fractionation. This fraction was also passed through a DEAE-cellulose column. using phosphate buffer, and the homogeneity of the polysaccharide thus obtained was tested by high-voltage electrophoresis in sodium tetraborate buffer, which gave a single band, either on treatment with periodic acid–fuchsin sulfite reagent[4] or using benzidine periodate[5] as the spray reagent. The present communication deals with the structural investigation of this purified fraction II, $[\alpha]_D^{22}$ +69° (*c* 0.27 in 0.1M NaOH).

The optimum conditions for complete hydrolysis were ascertained from the results of a number of pilot experiments, hydrolysis with M H_2SO_4 for 20 h at 100° being finally chosen. The monosaccharides were D-glucose and L-arabinose. The identity of the monosaccharides was determined by paper chromatography using solvent systems *A*, *B*, and *C*, and was confirmed by isolation of the sugars by preparative, paper chromatography, followed by measurement of the specific rotation of each. Further confirmation regarding their identity was achieved through g.l.c. and g.l.c.–m.s.[6] analysis (see Table I). The ratio of the component sugars was estimated by g.l.c. as their alditol acetates, using D-mannose as the internal standard.

Permethylation (twice) by the Hakomori method[7] gave completely methylated products showing no infrared absorption for hydroxyl group. The methylated material was completely hydrolyzed, the sugars converted into the methylated alditol acetates, and these characterized by g.l.c.[8] using column *1*. The methylated sugars were further characterized by g.l.c.–m.s. analysis[6,8,9], using an SE-30 capillary column.

The results of the methylation analysis (see Table II) could be assessed by considering the characterized products from the methylated polysaccharide. The occurrence of 3,5-di-*O*-methylarabinose (4 mol) could be explained by considering

TABLE II

RESULTS OF METHYLATION ANALYSIS OF THE POLYSACCHARIDE

Methylated sugars	*Retention time*[a]	*Molar proportion*	*Linkage pattern*	*Fragments obtained in g.l.c.–m.s. analysis*[b] (m/z)
3,5-Di-*O*-methylarabinofuranose	0.79	4	→2)-Ara*f*-(1→	43 (84%), 45(34), 75(25), 87(100), 99(61), 129(79), 130(35), 131(20), 189(28)
2,3,4,6-Tetra-*O*-methylglucopyranose	**1.00**	1	Glc*p*-(1→	43(100%), 45(30), 71(7), 85(18), 87(19), 101(53), 117(71), 129(67), 145(49), 161(64), 205(21)
2,4,6-Tri-*O*-methylglucopyranose	1.82	3	→3)-Glc*p*-(1→	43(100%), 45(15), 58(15), 71(16), 87(63), 99(17), 101(71), 103(16), 117(71), 129(16), 161(25)
2,3,4-Tri-*O*-methylglucopyranose	2.23	15	→6)-Glc*p*-(1→	43(100%), 87(32), 99(39), 101(60), 117(62), 129(49), 161(21), 189(12)
2,4-Di-*O*-methylglucopyranose	4.21	2	→3,6)-Glc*p*-(1→	43(100%), 44(24), 85(20), 87(29), 117(91), 129(62), 189(29)

[a]Retention times were calculated by taking 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol as 1.00, and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol as 2.32, using a column of 3% of OV-225 at 170°. [b]G.l.c.–m.s. analyses were performed by using an SE-30 capillary column (12 m).

(1→2)-linked L-arabinofuranosyl residues. The presence of 2,3,4,6-tetra-*O*-methylglucose (1 mol) indicated that one D-glucopyranosyl unit is present as a non-reducing group. The unbranched, interior parts constituting the branch chain were composed of (1→3)-linked D-glucosyl (3 mol) and (1→6)-linked D-glucosyl (15 mol) residues, as is evident from the formation of the respective products, *viz.*, 2,4,6-tri- and 2,3,4-tri-*O*-methyl-D-glucose. The presence of 2,4-di-*O*-methyl-D-glucose (2 mol) could be explained by considering (1→6)-linked D-glucosyl residues carrying branches at O-3. The alternative possibility, namely, (1→3)-linked D-glucose having branching at O-6 was discarded on the basis of the results of a second Smith degradation, where it was found that no D-glucose residue survived after the second Smith degradation.

The results of periodate-oxidation studies supported the proposed mode of linkages in the polysaccharide. Periodate kinetic-studies[10,11] showed that the consumption of periodate became constant within 22 h. The periodate uptake was 1.26 mol per mol of hexosyl residue. The value of periodate uptake observed was in close agreement with the theoretical amount required for the mode of linkages pro-

posed for them. The polysaccharide was subjected to periodate oxidation followed by borohydride reduction, and complete hydrolysis of part of the periodate-oxidized, borohydride-reduced material revealed the presence of D-glucose (68%) and L-arabinose (32%). When the rest was subjected to a second Smith degradation, only L-arabinose survived.

An end-group analysis[12] showed a value of ~144 for the average degree of polymerization ($\overline{d.p.}$).

The results of the methylation analysis and the Smith degradation studies could be readily explained by considering an arabinoglucan backbone involving (1→6)-linked D-glucopyranosyl (2 mol) and (1→2)-linked L-arabinofuranosyl (4 mol) residues for the average repeating-unit of the polysaccharide.

To the best of our knowledge, this is possibly the first report of the occurrence of an arabinoglucan from a natural source.

EXPERIMENTAL

General methods. — Specific rotations were measured with a Perkin–Elmer Model 241 MC spectrophotometer at 22°. Infrared spectra were recorded with an Acculab-10 Beckman instrument for KBr pellets. The homogeneity of the polysaccharide was tested by high-voltage electrophoresis at 4° with a Labor Model No. OE 201 instrument, using borate buffer (pH 9.2) on Whatman No. 1 filter paper. Kinetic study of periodate consumption at room temperature (32°) was conducted with a Beckman Model 26 spectrophotometer at 225 nm, and all other spectrophotometric estimations of sugars (by the phenol–sulfuric acid method) in the visible region were performed with this instrument.

Descending paper-chromatography was performed on Whatman No. 1 paper for detection, and Whatman No. 3 MM paper for preparative purposes. The solvent systems (v/v) used for paper chromatography were (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 9:2:2 ethyl acetate–acetic acid–water, and (*C*) 4:1:5 1-butanol–acetic acid–water (upper layer). Spray reagents were (*a*) alkaline silver nitrate solution in aqueous acetone and (*b*) a saturated, aqueous solution of aniline hydrogenoxalate.

Gel-filtration chromatography was conducted on a column (60 × 2.5 cm) of Sephadex G-100 and a column (40 × 2.5 cm) of G-200 with 0.1M sodium chloride–0.1M sodium hydroxide buffer (pH ~10.9). Chromatography on a column (50 × 1.5 cm) of DEAE-cellulose was performed by using 25mM phosphate buffer (pH 11.0). Aqueous solutions of sugars were concentrated in a rotary evaporator at bath temperatures below 50°. Concentrated sugar solutions were lyophilized.

Gas–liquid chromatography (g.l.c.) of the monosaccharides, as their alditol acetates and partly methylated alditol acetates, was conducted in glass columns containing (*1*) 3% of OV-225 on Gas-chrom Q (100–200 mesh) and (*2*) 3% of ECNSS-M on Gas-chrom Q (100–200 mesh) with nitrogen as the carrier gas, in Hewlett–Packard Model 5713A and Packard Model 419 instruments fitted with

flame-ionization detectors. Alditol acetates were characterized by matching of g.l.c. peaks against those of standard alditol acetates produced from mixtures of D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, D-fucose, and L-rhamnose. The partially methylated alditol acetates were identified by standard retention-times relative to those of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol. G.l.c.–m.s. analyses of the alditol acetates and methylated alditol acetates were performed in an SE-30 glass-capillary column (12 m) in a Hewlett–Packard 5895 instrument.

Source of the polysaccharide. — The ripe fruits of *Cordia dichotoma* were collected from the University campus during the month of August, 1981. The polysaccharide present in the mesocarp was used in the investigation.

Isolation and purification of polysaccharide. — The ripe fruits (500 g) were decoated, and the mesocarp was squeezed with water (2 L). The reddish-brown, viscous mixture was centrifuged for 30 min at 8,000 r.p.m. The polysaccharide was precipitated from the centrifugate with 9:1 (v/v) ethanol–acetone (10 L). The precipitation process was repeated thrice, ultimately giving a brown, spongy mass (10 g). This material was dissolved in water (1 L), and the solution was passed through a column (80 × 1 cm) of Amberlite IR-120 (H^+) resin and a column (60 × 1 cm) of Amberlite IRA-400 (HCO_2^-) resin. The eluate (1.5 L) was concentrated, and then lyophilized (9.2 g).

Fractionation of polysaccharide. — The polysaccharide (250 mg) was fractionated in a column (60 × 2.5 cm) of Sephadex G-100. The column was equilibrated with 0.1M sodium chloride–0.1M sodium hydroxide buffer, and eluted with the same buffer, the eluate being monitored by the spectrophotometric method[3]. Two fractions, namely, I (yield 55 mg), $[\alpha]_D^{22}$ +75° (*c* 0.24, 0.1M NaOH), and II (yield 185 mg), $[\alpha]_D^{22}$ +69° (*c* 0.27, 0.1M NaOH), were obtained. Gel filtration of fraction II was further conducted on a column (40 × 2.5 cm) of Sephadex G-200, using the same buffer solution. This fraction was rechromatographed on a column (50 × 1.5 cm) of DEAE-cellulose, using 25mM phosphate buffer (yield 180 mg).

Test of homogeneity by electrophoresis of fraction II. — Electrophoresis of the polysaccharide was performed in 0.01M sodium tetraborate buffer (pH 9.2) on a strip of Whatman No. 1 filter-paper, with an applied voltage of 20 V/cm at 4°. It migrated as a single band, as seen when sprayed with benzidine–periodate reagent[5] or treated with periodic acid–fuchsin sulfite reagent[4].

Estimation of monosaccharides in the polysaccharide. — On the basis of the results of pilot experiments, the polysaccharide (25 mg) was hydrolyzed with M H_2SO_4 (20 mL) for 20 h at 100°, using D-mannose as the internal standard. The excess of acid was neutralized with $BaCO_3$, and the solution decationized with Amberlite IR-120 (H^+) resin. The monosaccharides were detected by paper chromatography using solvent systems *A*, *B*, and *C*, and quantitatively assayed as their alditol acetates by g.l.c. in columns *1* and *2*. The monosaccharides were characterized as being glucose (86.7%) and arabinose (13.2%), and this was confirmed by g.l.c.–m.s. analysis using an SE-30 capillary column. After isolation of these

monosaccharides by preparative paper-chromatography, using solvent *C*, their specific rotations were measured; these showed them to be D-glucose and L-arabinose.

Methylation analysis. — The dry polysaccharide (25 mg) was mixed with dry dimethyl sulfoxide (25 mL) in an air-tight vial, and the mixture was stirred. The solution was then treated with 2M methylsulfinyl sodium (25 mL) under an atmosphere of dry nitrogen. After being stirred overnight, the solution was treated with methyl iodide (25 mL) by dropwise addition, and kept stirred for a further 4 h at 4°. The mixture was dialyzed and concentrated, and the product was extracted with chloroform. This treatment was repeated. The infrared spectrum of the permethylated product showed no absorption band in the region of 3600–3300 cm^{-1}; yield 14 mg.

The methylation product was treated with 90% formic acid (10 mL) for 5 h at 100°, and then the formic acid was removed, as the water azeotrope, in a rotary evaporator. The material was now completely hydrolyzed with M sulfuric acid (10 mL) for 10 h at 100°. After the usual treatment, the partially methylated monosaccharides were converted into their alditol acetates, and these were characterized by g.l.c. using column *1* (see Table II) and by g.l.c.–m.s. analysis.

Periodate-oxidation and Smith-degradation studies on the polysaccharide. — The polysaccharide was dissolved in 0.1M sodium hydroxide solution, and the pH was adjusted to 7 by adding hydrochloric acid. This solution was treated with 0.04M sodium metaperiodate in the dark at 32°, the rate of consumption of the oxidant being monitored spectrophotometrically[9,10]. The periodate consumption became constant at 22 h, and was found to correspond to 1.26 mol of periodate per mol of hexosyl residue.

Smith degradation of the polysaccharide was conducted as follows. The polysaccharide (50 mg) was dissolved in 0.1M NaOH (20 mL), the base neutralized with hydrochloric acid, and the volume made up to 50 mL with water. To this solution was added 0.08M sodium metaperiodate solution (50 mL), and the mixture was kept for 27 h at 32° in the dark. The solution was then treated with ethylene glycol (10 mL), kept overnight, dialyzed, and concentrated, and the contents were reduced with sodium borohydride (850 mg) at room temperature. The excess of borohydride was decomposed with acetic acid, the solution decationized with Amberlite IR-120 (H^+) resin, the boric acid removed by addition of methanol and evaporation, and an aqueous solution of the residue lyophilized (18 mg).

Part (6 mg) of the borohydride-reduced, periodate-oxidized material was hydrolyzed with M sulfuric acid (6 mL) for 8 h at 100°. The neutral hydrolyzate obtained after the usual treatment was examined by p.c. (solvent *C*) and as alditol acetates by g.l.c. using column *2*. Besides lower polyhydric alcohols and aldehydes, glucose and arabinose were detected, their relative proportions being 68 and 32% respectively. The other part (12 mg) was hydrolyzed with M sulfuric acid (12 mL) for 10 h at room temperature, the acid neutralized with $BaCO_3$, and the solution decationized, and dialyzed. The dialyzate was concentrated to 10 mL, and the con-

tents were subjected to a second Smith degradation. After the usual processing, the fragment of the polysaccharide that resisted periodate oxidation was isolated by precipitation with ethanol–acetone (yield 1.5 mg).

This periodate-immune fragment was hydrolyzed with 0.5M sulfuric acid for 6 h, and the monosaccharide was characterized as arabinose by p.c. (solvent *C*) and by g.l.c. in column *2*.

End-group analysis. — The polysaccharide (30 mg) was dissolved in 0.05M NaOH solution (10 mL). Sodium borohydride (250 mg) was added to the solution, and the mixture was kept for 48 h at room temperature. The excess of borohydride was decomposed with dilute acetic acid, and the pH was adjusted to 5.5; then, 0.5M sodium metaperiodate solution (5 mL) was added, and the volume of the mixture was brought to 25 mL. The oxidation was allowed to proceed in the dark, and, periodically, aliquots (2 mL) of the mixture were transferred to test tubes, and the excess of periodate immediately precipitated by addition of a saturated solution of lead acetate (3 mL). A length of dialysis tubing containing distilled water (5 mL) was introduced into all of the test tubes, and the concentration of formaldehyde in the tubing was determined by the chromotropic acid procedure[12]. A similar set of experiments was performed with D-mannitol.

ACKNOWLEDGMENTS

The authors express their heartfelt thanks to Professor N. H. Fischer, Louisiana State University, U.S.A., for the g.l.c.–m.s. analyses, and to Professor C. V. N. Rao and Dr. S. Basu of IACS, Calcutta, for some instrumental facilities. Financial assistance from C.S.I.R. (New Delhi) to one of us (N.G.B.) is gratefully acknowledged.

REFERENCES

1 R. N. CHOPRA, S. L. NAYAR, AND I. C. CHOPRA, *Glossary of Indian Medicinal Plants*, C.S.I.R., New Delhi, 1956, p. 77.
2 I. M. SAYED AND A. QURENHI, *Pak. J. Sci. Ind. Res.*, 19 (1976) 64–65.
3 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
4 E. KOIW AND A. GRONWALL, *Scand. J. Clin. Lab. Invest.*, 4 (1952) 244–246.
5 J. A. CIFONELLI AND F. SMITH, *Anal. Chem.*, 26 (1954) 1132.
6 P.-E. JANSSON, L. KENNE, B. LINDBERG, H. LIEDGREN, AND J. LONNGREN, *Chem. Commun., Univ. Stockholm*, 8 (1976).
7 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
8 H. BJORNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem., Int. Ed. Engl.*, 9 (1970) 610–619.
9 H. BJORNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohydr. Res.*, 5 (1967) 433–441.
10 J. S. DIXON AND D. LIPKIN, *Anal. Chem.*, 26 (1954) 1092–1093.
11 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind. (London)*, (1957) 1216.
12 G. W. HAY, B. A. LEWIS, F. SMITH, AND A. M. UNRAU, *Methods Carbohydr. Chem.*, 5 (1965) 251–253.