Carbohydrate Research, 159 (1987) 336–340
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

A neutral seed-gum from *Cassia renigera*

REKHA GUPTA*, NEETA KHARE, VANDANA SINGH, AND PURNA CHANDRA GUPTA
Chemical Laboratory, Department of Chemistry, University of Allahabad, Allahabad-211002, (India)

(Received August 12th, 1985; accepted for publication in revised form, August 20th, 1986)

Species of Cassia[1] (N.O. Leguminoseae) are described as being highly medicinal, and rich sources of polysaccharide. With a view to studying the nature of the seed gum of *Cassia renigera*, a chemical examination was undertaken. It was found to contain a galactomannan that possessed a novel structural feature; the presence of a galactobiose moiety not reported by Seth *et al.*[2]. Earlier studies had shown its presence in seeds of *Cassia corymbosa*[3].

RESULTS AND DISCUSSION

The polysaccharide was conveniently extracted from the extractive-free seeds (light petroleum, 90% ethanol) with 1% aqueous acetic acid, and isolated by repeated precipitation from the extract and then its aqueous solution with ethanol. It was purified by repeated fractionation with ethanol, deproteinization with chloroform, and complexation with Fehling solution. Xylose, present as the free sugar, was removed by the Fehling solution. The homogeneity was verified by zone electrophoresis, and *via* acetylation and deacetylation. The white, amorphous polysaccharide had $[\alpha]_D^{25}$ +40.2° (water), ash content 0.32%, and a negligible percentage of methoxyl, acetyl, and uronic acid contents. Acid catalyzed hydrolysis gave galactose and mannose in the molar ratio of 3:5.

To determine the mode of union of the building units in the galactomannan, it was exhaustively methylated, first by the Hakomori and then by the Purdie method. Hydrolysis of the fully methylated polysaccharide, $[\alpha]_D^{28}$ +23° (chloroform), gave four methylated sugars, as follows. (*1*) 2,3,4-Tri-*O*-methyl-D-galactose (2 mol); this must represent the nonterminal, unbranched D-galactosyl end-groups in the side chain. (*2*) 2,3,4,6-Tetra-*O*-methyl-D-galactose (4 mol); this must have arisen from the terminal D-galactosyl end-groups in the side chain. (*3*) 2,3-Di-*O*-methyl-D-mannose (4 mol); this constitutes the branch points in the main chain of the molecule. (*4*) 2,3,6-Tri-*O*-methyl-D-mannose (6 mol); this indicates

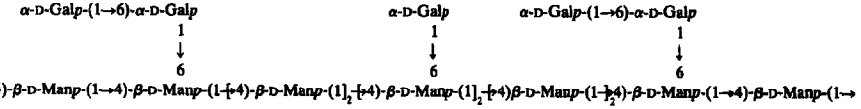
*To whom correspondence should be addressed.

0008-6215/87/$ 03.50

the presence of (1→4)-linked D-mannose units forming the main chain of the molecule. The percentage of terminal groups calculated from methylation studies was 36.4%.

The anomeric nature of the glycosidic bonds and the sequential arrangement of the sugar units were determined by characterization (see Experimental section) of the following oligosaccharides: trisaccharides identified as α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-D-Man*p*, α-D-Gal*p*-(1→6)-β-D-Man-*(1*→4)-D-Man*p*, and β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p*; disaccharides identified as α-D-Gal*p*-(1→6)-D-Gal*p*, α-D-Gal*p*-(1→6)-D-Man*p*, and β-D-Man*p*-(1→4)-D-Man*p*, along with the component sugars D-galactose and D-mannose.

The accumulation of evidence suggested the most suitable pattern of structure as follows, although the sequential arrangement of the side chains has not yet been ascertained.



EXPERIMENTAL

All evaporations were conducted under diminished pressure, and all specific rotations are equilibrium values. Amberlite IR-120 (H^+) ion-exchange resin was used for decationizing the hydrolyzates. The emulsin used was extracted from almonds.

Paper chromatography was performed at room temperature by the descending technique, using the following solvent systems (v/v). (S_1) 5:1:4 1-butanol–ethanol–water[4]; (S_2) 11:6:3 1-butanol–2-propanol–water[5]; (S_3) 10:4:3 ethyl acetate–pyridine–water[6]; and (S_4) 2:1:2 ethyl acetate–pyridine–water[7]. The spray reagent used for detecting the sugar spots was aniline hydrogenphthalate.

Isolation of the polysaccharide. — Crushed seeds were successively extracted with light petroleum, 90% ethanol, and 1% aqueous acetic acid. The acid extract was slowly added, with stirring, to ethanol (4 vol), and the crude polysaccharide was collected. Dissolation and reprecipitation were repeated, in order to minimize the impurities.

Purification of the polysaccharide. — *Deproteinization*[8]. The aqueous solution of the crude polysaccharide was shaken well with chloroform, and then centrifuged, whereupon the denatured protein formed, at the water–chloroform interface, a gel that was removed. This treatment was repeated several times, to remove almost all of the protein.

Complexation[9]. The protein-free, mucilaginous solution was slowly poured into an excess of Fehling solution, and the copper complex was precipitated. The complex was separated, thoroughly washed with water, and then decomposed with M hydrochloric acid. The polysaccharide was regenerated by slowly adding the acid

extract to ethanol (5 vol.), with stirring. The pure product was reprecipitated from its solution in 1% aqueous acetic acid by ethanol, to yield a nonreducing, white, amorphous material having ash content 0.32% and $[\alpha]_D^{25}$ +40.2° (water); its i.r. spectrum[10] showed bands at 3400 (OH, prominent), 2850, 2300, 890, and 810 cm^{-1}.

Homogeneity of the polysaccharide. — Fractional precipitation[11]. The polysaccharide (1.5 g) was fractionally precipitated from aqueous solution (300 mL) by addition of ethanol (400 and 800 mL). The fractions (*a* and *b*) were collected by centrifugation, washed with ether, and dried. Hydrolysis of fractions *a* and *b* gave D-galactose and D-mannose in 3:5 molar ratio, and both fractions retained the original specific rotation.

Acetylation and deacetylation[12]. The polysaccharide (1.0 g) was acetylated, giving a peracetate having $[\alpha]_D^{28}$ +19° (chloroform). Deacetylation regenerated the polysaccharide, $[\alpha]_D^{25}$ +40° (water).

Zone electrophoresis[13]. A 0.3% solution (50.2 mL) of polysaccharide in water was subjected to zone electrophoresis on Whatman No. 1 MM paper in 0.1M sodium tetraborate (pH 9.3) for 6.5 h at 320 V and 320 mA. A plot of the absorbance against segment number showed only a single, sharp peak.

Structural investigation of the polysaccharide. — Complete hydrolysis. The polysaccharide (1.0 g) was hydrolyzed with M sulfuric acid at 100° for 42 h P.c. (solvent S_2) revealed D-galactose and D-mannose (Co-p.c., m.p., $[\alpha]_D$ values, and preparation of three crystalline derivatives).

The polysaccharide (300 mg), together with D-ribose (30 mg) as a reference sugar, was hydrolyzed, and the hydrolyzate fractionated (solvent S_2) on Whatman No. 3 MM paper. After separation on a preparative scale, quantification[14] of the D-galactose and D-mannose by periodate oxidation indicated a molar ratio of 3:5.

Methylation analysis. The polysaccharide (8 g) was first methylated by the Hakomori method[15]. The product was twice methylated by the Purdie method[16], to yield fully methylated derivative, $[\alpha]_D^{28}$ +23° (chloroform). The i.r. spectrum of this product had no absorption at 3700–3200 cm^{-1}.

The methylated galactomannan (3.2 g) was hydrolyzed with 90% formic acid for 6 h at 100°. The hydrolyzate was fractionated on Whatman No. 3 MM paper (solvent S_1) using 2,3,4,6-tetra-*O*-methyl-D-glucose (TMG) as the reference sugar, to give the following compounds.

(1) *2,3,4-Tri*-O-*methyl*-D-*galactose*, m.p. 82°, $[\alpha]_D^{25}$ +148→+153° (water); lit.[17] m.p. 85°, $[\alpha]_D$ +154°; R_{TMG} 0.64; Found: OMe, 42.1; Calc.: OMe, 41.8%; 2,3,4-tri-*O*-methyl-*N*-phenylgalactosylamine, m.p. 164°, $[\alpha]_D^{25}$ +42° (methanol), lit.[18] the same.

(2) *2,3,4,6-Tetra-O-methyl*-D-*galactose*, m.p. 72–73° (light petroleum), $[\alpha]_D^{32}$ +120.3° (water); lit.[19] m.p. 74°, $[\alpha]_D$ +121°; R_{TMG} 0.89; Found: OMe, 51.32; Calc.: OMe 52.54%; 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m.p. 192–193°, $[\alpha]_D^{32}$ +42.2° (acetone), lit.[18] the same.

(3) *2,3-Di*-O-*methyl*-D-*mannose*, $[\alpha]_D^{32}$ −16.8° (water); lit.[20] $[\alpha]_D$ −15.8°; R_{TMG} 0.53; syrup, Found: OMe, 40.2; Calc.: OMe, 29.81%; 1,4,6-tri-*O*-*p*-nitrobenzoate, m.p. 191–192°, $[\alpha]_D^{32}$ +63.8° (chloroform), lit.[18] the same.

(4) *2,3,6-Tri-O-methyl-D-mannose*, syrup, $[\alpha]_D^{25}$ −11° (water); lit.[21] $[\alpha]_D$ −10°; R_{TMG} 0.81; syrup, Found: OMe, 40.2; Calc.: OMe, 41.8%; 2,3,6-tri-*O*-methyl-D-mannono-1,4-lactone, m.p. 80°; lit.[22] the same; 2,3,6-tri-*O*-methyl-D-mannonic acid phenylhydrazide, m.p. 128–129°, $[\alpha]_D^{32}$ −18.9° (water); lit.[22] the same.

The methylated polysaccharide (1.0 g) together with added D-glucose as a reference was hydrolyzed with 0.75M sulfuric acid for 18 h at 100°. The resulting sugars were separated by p.c. (solvent S_1), and quantified by the alkaline hypoiodite[23] method. The molar ratios of fractions 1 to 4 were 2:4:4:6.

Partial hydrolysis with acid. The polysaccharide (8.0 g) was hydrolyzed with 0.05M sulfuric acid for 12 h at 100°. The hydrolyzate was fractionated by p.c. on a preparative scale (solvents S_3 and S_4), and elution of fractions with distilled water gave D-galatose and D-mannose, along with the following oligosaccharides.

(1) *Galactosylgalactose: α-D-Galp-(1→6)-D-Galp*, m.p. 128°, $[\alpha]_D^{25}$ +150° (water); lit.[23] m.p. 130°, $[\alpha]_D$ +154°. The octaacetate had m.p. 223°, $[\alpha]_D^{25}$ +179.3° (chloroform); phenylosazone, m.p. 191; lit.[24] the same. Acid hydrolysis gave [p.c. (solventS_1)] D-galactose only. The high, positive optical rotation and the resistance to emulsin hydrolysis indicated α linkages. During 48 h, the disaccharide consumed 4.96 mol of oxidant, and liberated 2.98 mol of formic acid. Methylation and subsequent hydrolysis gave [p.c. (solvent S_1)] 2,3,4-tri- and 2,3,4,6-tetra-*O*-methyl-D-galactose.

(2) *Galactosyl-mannose: α-D-Galp-(1→6)-D-Manp*, m.p. 199°, $[\alpha]_D^{32}$ +120° (water); lit.[25] m.p. 201–202°, $[\alpha]_D$ +123→124°; phenylosazone, m.p. 173° lit.[25] the same. Acid hydrolysis gave [p.c. (solvent S_2)] galactose and mannose. Periodate oxidation (48 h) liberated 3.02 mol of formic acid, and consumed 5.02 mol of oxidant. Resistance to emulsin, and the high, positive optical rotation, support α linkages. Methylation followed by hydrolysis gave, by p.c. (solvent S_1), 2,3,4,6-tetra-*O*-methylgalactose and 2,3,4-tri-*O*-methylmannose.

(3) *Mannosylmannose: β-D-Manp-(1→4)-D-Manp*, m.p. 200°, $[\alpha]_D^{25}$ −9° (water); lit.[25] m.p. 203–204°, $[\alpha]_D$ +7→8.5°; phenylosazone, m.p. 204°. Acid and emulsin hydrolyses gave [p.c. (solvent S_2)] mannose only. Successful emulsin hydrolysis and the negative optical rotation support β-linkages. Periodate oxidation liberated 2.02 mol of formic acid and consumed 4.01 mol of sodium metaperiodate. Methylation followed by hydrolysis gave [p.c. (solvent S_1)] 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-mannose.

(4) *Galactosylepimelibiose: α-D-Galp-(1→6)-α-D-Galp-(1→6)-D-Manp*, m.p. 122°, $[\alpha]_D^{25}$ +124° (water), lit.[24,25] m.p. 124°, $[\alpha]_D$ +131° (water). Controlled acid hydrolysis gave [p.c. (solvent S_2)] galactosylgalactose, galatosylmannose, galactose, and mannose. Periodate oxidation liberated 3.98 mol of formic acid and consumed 7.01 mol of sodium metaperiodate. Methylation and subsequent hydrolysis gave [p.c. (solvent S_1)] 2,3,4,6-tetra- and 2,3,4-tri-*O*-methylgalactose, and 2,3,4-tri-*O*-methylmannose.

(5) *Galactosylmannobiose: α-D-Galp-(1→6)-β-D-Manp-(1→4)-D-Manp*, m.p. 225–227°, $[\alpha]_D^{25}$ +93° (water); lit.[26] m.p. 228–229°, $[\alpha]_D$ +98.4°. Controlled acid

hydrolysis gave [p.c. (solvent S_2)] epimelibiose, mannobiose, galactose, and mannose. Emulsin hydrolysis gave (p.c.) epiamelibiose and mannose. Periodate oxidation liberated 2.98 mol of formic acid and consumed 6.04 mol of sodium metaperiodate. Methylation and subsequent hydrolysis gave, by p.c. (solvent S_1), 2,3,4,6-tetra-*O*-methylgalactose, and 2,3,4- and 2,3,6-tri-*O*-methylmannose.

(6) *Mannotriose; β*-D-*Man*p-*(1→4)-β*-D-*Man*p-*(1→4)*-D-*Man*p, m.p. 164–166°, $[\alpha]_D^{25}$ −18° (water); lit.[27,28] m.p. 169.5°, $[\alpha]_D$ −15→−26°. Controlled acid hydrolysis gave [p.c. (solvent S_2)] mannobiose and mannose. Successful emulsin hydrolysis and the high, negative optical rotation suggests that the inter-sugar linkage is *β*. Periodate oxidation liberated 2.02 mol of formic acid, and consumed 5.03 mol of oxidant. Methylation analysis revealed [p.c. (solvent S_1)] 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylmannose.

ACKNOWLEDGMENT

The authors thank the Council of Scientific and Industrial Research, New Delhi, for granting them Fellowships.

REFERENCES

1 L. H. BAILEY, *The Standard Cyclopedia of Horticulture*, Vol. 1, Macmillan, New York, 1961, p. 680.
2 R. P. SETH, S. MUKHERJEE, AND S. D. VERMA, *Carbohydr. Res.*, 125 (1984) 336–339.
3 K. TEWARI, N. KHARE, V. SINGH, AND P. C. GUPTA, *Carbohydr. Res.*, 135 (1984) 141–146.
4 E. L. HIRST AND J. K. N. JONES, *Discuss. Faraday Soc.*, 7 (1949) 268–271.
5 S. A. I. RIZVI, P. C. GUPTA, AND R. K. KAUL, *Planta Med.*, 19 (1971) 222–233.
6 G. O. ASPINALL, R. BEGBIE, AND J. E. MCKAY, *J. Chem. Soc.*, (1962) 214–219.
7 H. MEIER, *Acta Chem. Scand.*, 14 (1960) 749–752.
8 A. M. STAUB, *Methods Carbohydr. Chem.*, 5 (1965) 5–6.
9 H. C. SRIVASTAVA AND P. P. SINGH, *Carbohydr. Res.*, 4 (1967) 326–331.
10 S. A. BARKER, E. J. BOURNE, AND D. H. WHIFFEN, *Methods Biochem. Anal.*, 3 (1956) 213–243.
11 R. L. WHISTLER AND G. E. LAUTERBACH, *Arch. Biochem. Biophys.*, 77 (1958) 289–292.
12 A. S. CEREZO, *J. Org. Chem.*, 30 (1965) 924–927.
13 A. B. FOSTER, *Adv. Carbohydr. Chem.*, 12 (1957) 81–115, see pp. 86–90.
14 E. L. HIRST AND J. K. N. JONES, *J. Chem. Soc.*, (1949) 1659–1662.
15 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–207.
16 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021–1026.
17 M. ONUKI, *Nippon Nogei Kagaku Zasshi*, 9 (1933) 90–111; *Chem. Abstr.*, 27 (1933) 2138.
18 F. SMITH AND R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, Reinhold, New York, 1959, pp. 514–553.
19 W. CHARLTON, W. N. HAWORTH, AND W. J. HICKINBOTTOM, *J. Chem. Soc.*, (1927) 1527–1536.
20 G. J. ROBERTSON, *J. Chem. Soc.*, (1934) 330–336.
21 E. L. HIRST AND J. K. N. JONES, *J. Chem. Soc.*, (1948) 1278–1282.
22 P. ANDREWS, L. HOUGH, AND J. K. N. JONES, *J. Am. Chem. Soc.*, 74 (1952) 4029–4032.
23 E. L. HIRST, L. HOUGH, AND J. K. N. JONES, *J. Chem. Soc.*, (1949) 928–932.
24 A. DE GRANDCHAMP-CHAUDUN, J. E. COURTOIS, AND P. LE DIZET, *Bull. Soc. Chim. Biol.*, 42 (1960) 227–230.
25 R. W. BAILEY, *Oligosaccharides*, Vol. 4, Pergamon, Oxford, 1965, pp. 46–54.
26 R. W. BAILEY, *Oligosaccharides*, Vol. 4, Pergamon, Oxford, 1965, pp. 91–104.
27 G. O. ASPINALL, R. B. RASHBROOK, AND G. KESSLER, *J. Chem. Soc.*, (1958) 215–221.
28 A. TYMINSKI AND T. E. TIMELL, *J. Am. Chem. Soc.*, 823 (1960) 2823–2827.