SULPHATED POLYSACCHARIDES OF THE *Grateloupiaceae* FAMILY PART VI[1]. A POLYSACCHARIDE FROM *Pachymenia carnosa*

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ABSTRACT

Pachymenia carnosa, a red seaweed of the *Grateloupiaceae*, yielded a sulphated polysaccharide which, on acid hydrolysis, gave D-galactose, 2-*O*-methyl-D-galactose, 4-*O*-methylgalactose, and 6-*O*-methyl-D-galactose. Partial hydrolysis of the polysaccharide resulted in the isolation and characterisation of 4-*O*-β-D-galactopyranosyl-D-galactose, 4-*O*-β-D-galactopyranosyl-2-*O*-methyl-D-galactose, 3-*O*-(2-*O*-methyl-D-galactopyranosyl)-D-galactose, 4-*O*-(6-*O*-methyl-β-D-galactopyranosyl)-D-galactose, 2-*O*-methyl-4-*O*-(6-*O*-methyl-β-D-galactopyranosyl)-D-galactose, and a 6-*O*-methyl-(2-*O*-methyl-D-galactopyranosyl)-D-galactose. At present, it is not possible to postulate a structure for the polymer.

INTRODUCTION

Pachymenia carnosa is a red seaweed of the *Grateloupiaceae* family. The weed used in the present investigation was collected near Cape Town, South Africa. The results obtained so far indicate that this polysaccharide contains many of the structural features found in the polysaccharides of other members of the *Grateloupiaceae* family[2-5].

RESULTS AND DISCUSSION

Extraction of *Pachymenia carnosa* with hot water, followed by centrifugation and precipitation of the mucilage into ethanol, afforded a sulphated polysaccharide, purification of which was effected by dissolution in water followed by centrifugation and subsequent precipitation into ethanol. The infrared spectrum of the polymer showed the ester sulphate peak[6] at 1240 cm^{-1}, but there was no resolution into discrete peaks of the broad peak centred at 815 cm^{-1}; thus, no indication of the type of ester sulphate present was given.

The products of total hydrolysis of the polysaccharide were separated on a charcoal–Celite column, using water and aqueous ethanol, to give D-galactose, 2-*O*-methyl-D-galactose, 6-*O*-methyl-D-galactose, all in crystalline form, and a trace of

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4-*O*-methylgalactose, which was characterized as 4-*O*-methyl-*N*-phenylgalactosylamine. The low optical rotation of the 4-*O*-methylgalactose indicated that it was a mixture of D and L forms. The molar ratios of the components (galactose, 2-*O*-methylgalactose, 6-*O*-methylgalactose, 4-*O*-methylgalactose, xylose, $NaSO_3^-$) was 6.15:1.0:0.52:0.21:0.03:6.59. The monosaccharides were determined by quantitative g.l.c. of their alditol acetates[7], and sulphate was determined by a spectrophotometric method[8,9].

Treatment of the polysaccharide with alkali and sodium borohydride resulted in the formation of a small amount (1%) of 3,6-anhydrogalactose, indicating that any (1→2)- and/or (1→4)-linked units present in the polymer are not predominantly sulphated on positions 3 or 6.

Partial hydrolysis of the polysaccharide with acid, followed by fractionation of the neutral products on a charcoal–Celite column, yielded, in addition to the monosaccharides above, several disaccharides and a trace of an, as yet, unidentified monosaccharide. Only two disaccharides were obtained in crystalline form, namely 4-*O*-*β*-D-galactopyranosyl-D-galactose and 4-*O*-*β*-D-galactopyranosyl-2-*O*-methyl-D-galactose. Other disaccharides (all chromatographically homogeneous, but non-crystalline) characterised were

3-*O*-(2-*O*-methyl-D-galactopyranosyl)-D-galactose,

4-*O*-(6-*O*-methyl-*β*-D-galactopyranosyl)-D-galactose,

2-*O*-methyl-4-*O*-(6-*O*-methyl-*β*-D-galactopyranosyl)-D-galactose,

and a 6-*O*-methyl-(2-*O*-methyl-D-galactopyranosyl)-D-galactose. In each case, the component units of the disaccharide were obtained by hydrolysis followed by paper chromatography. The relative positions of the two component sugar units in the molecule were obtained by reduction of the disaccharide with sodium borohydride and characterization of the remaining reducing sugar by means of paper chromatography after hydrolysis. The linkage of the disaccharides was obtained by methylation studies. In all cases, 2,3,4,6-tetra-*O*-methylgalactose and either 2,3,6- or 2,4,6-tri-*O*-methylgalactose were obtained. No disaccharide yielded both tri-*O*-methyl sugars.

The evidence presented suggests that the polymer contains mainly *β*-D-(1→4) links with a smaller number of (1→3) links. Because[10] of the known ease of acid hydrolysis of (1→3)-linked, as compared with (1→4)-linked, galactose residues, it is possible that the number of (1→3)-linked galactose residues is greater than indicated by this study. A preliminary study indicated that desulphation is relatively easily effected, whereas total methylation of the native polymer is difficult if not impossible. Studies of the desulphated polysaccharide will be reported later.

EXPERIMENTAL

Concentration of solutions was carried out at 40° under reduced pressure, and specific rotations were measured in water unless otherwise stated. Paper chromatography was carried out on Whatman No. 1 filter paper, using the following solvent

systems: (*a*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), (*b*) butyl alcohol–pyridine–water (9:2:2), (*c*) butyl alcohol–ethyl alcohol–water (40:11:9), and (*d*) ethyl acetate–pyridine–water (8:2:1). Sprays (*i*), (*ii*), and (*iii*) were, respectively, 2% *p*-anisidine hydrochloride in butyl alcohol containing 5% of water[11]; 5% triphenyltetrazolium chloride in methanol mixed with equal parts of a mixture of 2 ml of 2.5M aqueous sodium hydroxide in 3 ml of methanol, just before spraying; 20% sulphuric acid in ethanol. R_{Gal} values refer to rates of movement of sugars on chromatograms relative to galactose. Thin-layer chromatography (t.l.c.) was carried out on glass plates coated with Silica Gel G containing calcium sulphate as binder, using methyl ethyl ketone–water (85:7). R_{TMG} values of methylated sugars refer to the rates of travel relative to that of 2,3,4,6-tetra-*O*-methyl-D-galactose. Sulphate was determined by the 4′-chlorobiphenyl-4-ylamine method[8,9]. Infrared spectra were recorded on a Beckman IR-8 spectrophotometer, using KBr discs. Gas–liquid chromatography (g.l.c.) of methylated sugars was carried out on a Beckman GC 4 chromatograph equipped with dual flame-ionization detectors, using nitrogen as carrier gas. Separations were effected on a column containing 15% by weight of poly(butane-1,4-diol succinate) on acid-washed Celite (80–100 mesh) at 175°. Retention times (T) are relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Isolation and purification of polysaccharide. — Steam was passed into wet *Pachymenia carnosa* (2 kg) in water with constant stirring, the pH being kept at 5–6 by adding glacial acetic acid when necessary. After the weed had disintegrated, steam was passed through the mixture for a further 0.5 h. The resulting slurry was fed into a basket centrifuge and the centrifugate precipitated with ethanol (5 vol.). The product was washed with ethanol, and finally with ether to give 203 g (10% yield on a wet-weight basis) of crude, off-white, fibrous polymer.

Further purification of the polysaccharide for analysis was effected by repeated (4 times) dissolution in water, centrifugation of the solution, and precipitation with ethanol (5 vol.). This yielded a white, fibrous polymer having (on material dried *in vacuo* over P_2O_5 at 60° for 24 h) $[\alpha]_D^{20}$ +73° (*c* 0.52), ν_{max} 1240 and 815 cm^{-1} [Found: OMe, 2.2; N, 0.3; 3,6-anhydrogalactose[12], 1.4; SO_4^{2-}, 30.3; $NaSO_3^-$, 32.5%; equiv. wt. (from SO_4^{2-} determination), 317].

Chromatographic examination of a neutralized acid-hydrolysate (solvents *a*, *b*, *c*, and *d*) revealed the presence of galactose (major; yellow–brown); 2-*O*-methylgalactose (orange–pink), R_{Gal} 2.72 (solvent *d*); 4-*O*-methylgalactose (minor; yellow), R_{Gal} 1.77 (solvent *d*); 6-*O*-methylgalactose (brown), R_{Gal} 2.39 (solvent *d*); and xylose (trace, red) (spray *i*). The molar ratios of the component sugars were determined by g.l.c. of their alditol acetates[7] at 175°, using a column filling of 20% Apiezon M on Chromosorb W (80–100 mesh; acid-washed and treated with chlorodimethylsilane).

Hydrolysis of the polysaccharide and separation of the component sugars. — Polysaccharide (8.6 g) and sulphuric acid (0.375M; 100 ml) were heated for 16 h on a boiling-water bath. After neutralization ($BaCO_3$), centrifugation, and evaporation to dryness, the residue was extracted with aqueous methanol. Evaporation of the extract gave a syrup (3.19 g), which, in the minimal amount of water, was applied to

a charcoal–Celite column (1:1 w/w; 4.5 × 35 cm). Initially, the column was eluted with water, followed by 0.5, 2, and 5% aqueous ethanol. Fractions (*ca.* 15 ml) were collected and analyzed by paper chromatography with solvents (*a*) and (*b*).

Fraction I. The syrup (1.73 g), which was shown to contain galactose and a trace of xylose, was crystallised and recrystallized from aqueous ethanol to give D-galactose, m.p. and mixed m.p. 161–162°, $[\alpha]_D^{23}$ +120 (3 min) → +72° (*c* 0.50). The sugar (50 mg) in nitric acid–water (1:1; 1 ml) was heated on a water bath at 80° for 2 h. The galactavic acid which crystallized on cooling had, after recrystallization from water, m.p. and mixed m.p. 212–213°.

Fraction II. The syrup (0.175 g) contained (paper chromatography) galactose and 2-*O*-methylgalactose (major sugar).

Fraction III. The syrup (0.410 g) contained 2-*O*-methylgalactose (major) and 4-*O*-methylgalactose (paper chromatography). The 2-methyl ether crystallized from methanol and had m.p. and mixed m.p. 148–149°, $[\alpha]_D^{20}$ +54 (3 min) → +84° (*c* 0.5). The mother liquor was concentrated and chromatographed on several sheets of paper. The areas corresponding to the 4-methyl ether were extracted with methanol, and the extract was filtered and evaporated to a syrup (10 mg) which had $[\alpha]_D^{22}$ −9° (*c* 0.77) on a sample dried at 60°/0.1 mmHg. The sugar (8 mg), freshly distilled aniline (16 mg), and a drop of acetic acid in ethanol (0.5 ml) were refluxed for 4 h. The "anilide", after recrystallization from ethanol, had m.p. 166–168°; lit. m.p. 167–168° for 4-*O*-methyl-*N*-phenyl-L-galactosylamine[13] and 168° for 4-*O*-methyl-*N*-phenyl-D-galactosylamine[14].

Fraction IV. The syrup (0.225 g) was (paper chromatography) a mixture of 2-*O*-methylgalactose (major) and 6-*O*-methylgalactose.

Fraction V. The syrup (0.350 g) contained (paper chromatography) 6-*O*-methylgalactose and a trace of 2-*O*-methylgalactose. The 6-*O*-methylgalactose crystallized as plates from ethanol–ethyl acetate and had m.p. 119–121° and mixed m.p. 116–117°, $[\alpha]_D^{19}$ +138 (2 min) → +80° (*c* 0.65).

Treatment of the polysaccharide with alkali. — To the polysaccharide (1.0 g) in water (75 ml) was added sodium borohydride (0.2 g), and the solution was set aside at room temperature for 48 h, with occasional shaking. Sodium hydroxide (7 g) and sodium borohydride (0.8 g) were then added, and the mixture was heated to, and maintained at, 80° (±2°). After 4 h, a second portion of sodium borohydride (0.8 g) was added. After a further 3 h, the solution was cooled, made slightly acid with hydrochloric acid, dialysed, and concentrated. The polymer was isolated by freeze-drying to give a white foam (877 mg) having $[\alpha]_D^{19}$ +76° (*c* 1.0) (Found: SO_4^{2-}, 30.6; 3,6-anhydrogalactose[12], 2.35%). Paper chromatography of an acid hydrolysate showed the presence of only the sugars found in the native polymer, *viz.* galactose, 2-*O*-methylgalactose, 4-*O*-methylgalactose, 6-*O*-methylgalactose, and xylose (trace). The chromatogram was slightly streaked due to the presence of 3,6-anhydrogalactose.

Partial hydrolysis of the polysaccharide. — In order to determine the optimal conditions for the formation of oligosaccharides, the polysaccharide (0.75 g) in sulphuric acid (0.5M; 15 ml) was heated on a boiling-water bath. Aliquots (1 ml)

were withdrawn at regular intervals, neutralized ($BaCO_3$), centrifuged, concentrated, and subjected to paper chromatography (solvents *a* and *d*) for 16 h. The optimal time of hydrolysis for the production of the maximal amount of oligosaccharides was 2.5 h.

The polysaccharide (20 g) was hydrolysed (0.5M sulphuric acid, 400 ml) for 2.5 h under the above conditions, and the neutralized ($BaCO_3$) hydrolysate was centrifuged, and evaporated to dryness. The product (12.9 g), dissolved in the minimal quantity of water, was deionized by passage first through a column (20 × 4 cm) of Amberlite IR-120(H^+) resin and then through a column (21 × 5 cm) of Amberlite IRA-400 resin in the acetate form. Evaporation of the resulting solution yielded a white foam (7.1 g) which was dissolved in the minimal quantity of water, applied to a charcoal–Celite column (1:1 w/w; 65 × 5.4 cm), and eluted with water and aqueous ethanol (2–30%) by the gradient technique. Fractions (*ca.* 30 ml) were collected and sorted into the following 11 major fractions with the aid of paper chromatography.

Fraction I. The partly crystalline, white foam (2.20 g), eluted with water (0.7 l), was shown to be predominantly galactose by paper chromatography. It also contained traces of xylose and a sugar of R_{Gal} 2.3 (solvent *d*).

Fraction II. The syrup (553 mg), eluted with water (3.6 l), consisted of galactose 2-*O*-methylgalactose, and 4-*O*-methylgalactose (paper chromatography).

Fraction III. The syrup (458 mg), eluted with water (9.0 l), consisted of galactose (trace), 4-*O*-methylgalactose, 2-*O*-methylgalactose (major component), and a sugar (yellow, spray *i*) having R_{Gal} 2.70 (solvent *a*), 4.35 (solvent *d*). In order to isolate the fast-moving sugar, the syrup (235 mg) was fractionated on Whatman No. 1 paper (solvent *d*; 24 h). Extraction of the relevant portion of the papers with water–methanol (1:1) yielded a syrup (48 mg), $[\alpha]_D^{21}$ *ca.* 0° (*c* 0.96). After demethylation[11] of the sugar (2 mg) with 48% hydrobromic acid (0.2 ml) on a boiling-water bath for 5 min, chromatography (solvents *a* and *d*) of the neutralized (Ag_2CO_3) solution revealed the presence of galactose and a sugar (yellow; spray *i*) having R_{Gal} 1.57 (solvent *a*), 3.04 (solvent *d*). The sugar was unchanged by acid hydrolysis (M HCl, 16 h at 100°). Work is continuing on this sugar.

Fraction IV. The syrup (363 mg) was eluted with 2% aqueous ethanol (7.2 l) and shown (paper chromatography) to consist predominantly of a sugar chromatographically indistinguishable from 6-*O*-methylgalactose. It also contained traces of sugars having $R_{Gal} < 1$ (solvent *a*).

Fraction V. The syrup (464 mg) was eluted with 5% aqueous ethanol (4.5 l). Paper chromatography revealed the presence of a single sugar having R_{Gal} 0.33 (solvent *a*), 0.27 (solvent *d*). It crystallized readily from aqueous methanol; m.p. 204–205° (decomp.), $[\alpha]_D^{20}$ +77 (4 min) → +67° (*c* 0.52). Partial hydrolysis, followed by paper chromatography, revealed the presence of galactose and the original material, whereas complete hydrolysis gave galactose only. A portion[15] (5 mg) was dissolved in redistilled *N*,*N*-dimethylformamide (0.5 ml), and the solution cooled to 0°. Redistilled methyl iodide (0.5 ml) and dry, freshly prepared silver oxide (0.5 g) were added, and the mixture was stirred vigorously in the dark with ice-cooling for 3 h, and then at room temperature for 24 h. The partly methylated oligosaccharide was extracted

with chloroform. After a single treatment with Purdie's reagents[16], t.l.c. of the product revealed (spray *iii*) the presence of two discrete spots, indicating that methylation was complete. Acid hydrolysis (0.5M sulphuric acid, 4 h) of the methyl glycosides revealed (t.l.c. and paper chromatography) products having the mobilities of 2,3,4,6-tetra-*O*-methylgalactose (R_{TMG} 1.00) and 2,3,6-tri-*O*-methylgalactose (R_{TMG} 0.87). A sample of the methylated oligosaccharide was refluxed with 3% methanolic hydrogen chloride for 6 h, and the derived methyl glycosides were examined by g.l.c. Peaks corresponding to 2,3,4,6-tetra-*O*-methylgalactose (*T* 1.80) and 2,3,6-tri-*O*-methylgalactose (*T* 3.17, 3.89, 4.20, 4.49), in the molar ratio 1.0:1.0, were observed. The infrared spectrum of this oligosaccharide was identical with that of authentic 4-*O*-β-D-galactopyranosyl-D-galactose.

Fraction VI. The syrup (136 mg) was eluted with 5% aqueous ethanol (4.5 l), and paper chromatography (solvent *a*) showed the presence of sugars having R_{Gal} 0.34 and 0.62 and traces having R_{Gal} 0.54, 0.96, and 1.09. Fractionation on Whatman No.1 paper (solvent *a*, 72 h), followed by extraction of the appropriate portions of the papers with 50% aqueous methanol, afforded a chromatographically homogeneous syrup, $[\alpha]_D^{21}$ +32° (*c* 0.50), R_{Gal} 0.62 (solvent *a*), 0.36 (solvent *d*); it gave a red spot with spray (*ii*), indicating a free hydroxyl group on C-2 of the reducing residue. Partial hydrolysis of the sugar gave (paper chromatography) galactose and 2-*O*-methylgalactose in addition to the original material, whereas total hydrolysis gave galactose and 2-*O*-methylgalactose only.

Sodium borohydride (6 mg) was added to the sugar (4 mg) dissolved in water (2 ml), and the mixture was allowed to stand overnight. The solution was then passed through a column of Amberlite IR-120(H^+) resin and evaporated, and boric acid was removed by repeated distillation of methanol from the residue. Hydrolysis of the non-reducing syrup gave [paper chromatography (solvent *d*; spray *i*)] 2-*O*-methylgalactose as the only reducing sugar. Methylation of the sugar (5 mg), as described above, gave a fully methylated oligosaccharide (t.l.c., spray *iii*), and methanolysis of this product with refluxing, 3% methanolic hydrogen chloride for 6 h gave (g.l.c.) 2,3,4,6-tetra-*O*-methylgalactose (*T* 1.83) and 2,4,6-tri-*O*-methylgalactose (*T* 3.91, 4.19). Paper chromatography of a hydrolysate of the methylated oligosaccharide revealed 2,4,6-tri-*O*-methylgalactose and 2,3,4,6-tetra-*O*-methylgalactose. No trace of a sugar having the mobility of 2,3,6-tri-*O*-methylgalactose was observed. The above results indicate that the oligosaccharide is 3-*O*-(2-*O*-methyl-D-galactopyranosyl)-D-galactose.

Fraction VII. The syrup (134 mg), eluted with 7.5% aqueous ethanol (1.8 l), was a mixture of five oligosaccharides. Fractionation on Whatman No. 1 filter paper (solvent *a*, 60 h), followed by double extraction with methanol–water (1:1), yielded a chromatographically homogeneous sample of the major sugar (46 mg) R_{Gal} 0.96 (solvent *a*), 0.76 (solvent *d*). After crystallization from methanol, this had m.p. 213–214°, $[\alpha]_D^{18}$ +86 (3 min) → +62° (*c* 0.49). Partial hydrolysis gave (paper chromatography) galactose and 2-*O*-methylgalactose, in addition to the original sugar, and complete hydrolysis revealed the presence of approximately

equal amounts of galactose and 2-*O*-methylgalactose. After reduction of the oligosaccharide with borohydride, paper chromatography of an acid hydrolysate revealed the presence of galactose only (spray *i*). The sugar (5 mg) was methylated and methanolysed, and the derived methyl glycosides were examined by g.l.c. Peaks corresponding to 2,3,4,6-tetra-*O*-methylgalactose (*T* 1.80) and 2,3,6-tri-*O*-methylgalactose (*T* 3.19, 3.98, 4.21, 4.48) in the molar ratio 1.0:1.10 were observed. The oligosaccharide is therefore 4-*O*-*β*-D-galactopyranosyl-2-*O*-methyl-D-galactose; lit.[4] m.p. 213–214°, $[\alpha]_D^{16}$ +87.6 (4 min)→ +70.1°.

Fraction VIII. The syrup (472 mg), eluted with 10% aqueous ethanol (10.5 l), consisted (paper chromatography, solvent *d*) predominantly of 4-*O*-*β*-D-galactopyranosyl-2-*O*-methyl-D-galactose and 4-*O*-(6-*O*-methyl-*β*-D-galactopyranosyl)-D-galactose (see fraction IX). Traces of oligosaccharides having R_{Gal} 0.13, 0.27, and 0.43 (solvent *a*) were also observed.

Fraction IX. The syrup (205 mg) was eluted with 10% aqueous ethanol (4.3 l). Paper chromatography (solvent *a*) revealed the presence of saccharides having R_{Gal} 0.96 (major component), 0.16, 0.21, 0.31, 0.46, 0.57, and 1.05. Fractionation on Whatman No. 1 filter paper (solvent *a*, 48 h) afforded the main component (51 mg), R_{Gal} 0.68 (solvent *d*), $[\alpha]_D^{18}$ +35° (*c* 0.52). Partial hydrolysis of a sample yielded galactose, 6-*O*-methylgalactose and a small proportion of the original sugar, whereas only galactose and 6-*O*-methylgalactose (in approximately equal amounts) were obtained on total hydrolysis. On examination by paper chromatography of the acid hydrolysate of a reduced sample, only 6-*O*-methylgalactose was observed with spray (*i*). The sugar was methylated by using the modified Kuhn procedure[15], followed by a treatment with Purdie's reagents[16], and the derived methyl glycosides were methanolysed (3% methanolic hydrogen chloride) and examined by g.l.c. Peaks corresponding to 2,3,4,6-tetra-*O*-methylgalactose (*T* 1.83) and 2,3,6-tri-*O*-methylgalactose (*T* 3.21, 3.79, 4.18, 4.49) were observed in the molar ratio of 1.0:1.03. The above results indicate that the oligosaccharide was 4-*O*-(6-*O*-methyl-*β*-D-galactopyranosyl)-D-galactose; the *β*-D configuration is inferred from the $[\alpha]_D$ value.

Fraction X. The syrup (243 mg) was eluted with 15% aqueous ethanol (7.5 l) and contained several slow-moving oligosaccharides (paper chromatography). This fraction was not further investigated.

Fraction XI. The syrup (253 mg), eluted with 20% aqueous ethanol (3.0 l), contained traces of many oligosaccharides having $R_{Gal} < 1$, as well as two fast-moving oligosaccharides. It was separated on Whatman No. 1 paper (solvent *a*, 24 h), and the relevant portions of the papers were extracted with aqueous methanol to yield the following two sub-fractions.

Fraction XIA. The chromatographically homogeneous syrup (91 mg), R_{Gal} 1.84 (solvent *a*), R_{Gal} 1.98 (solvent *d*), $[\alpha]_D^{21}$ +54° (*c* 0.59), yielded 2-*O*-methylgalactose and 6-*O*-methylgalactose on partial hydrolysis with acid. After reduction with borohydride, paper chromatography of the acid hydrolysate of the non-reducing syrup revealed (spray *i*) the presence of 6-*O*-methylgalactose. A portion of the oligosaccharide (5 mg) was methylated and methanolysed, and the derived methyl glyco-

sides were examined by g.l.c. Only peaks corresponding to 2,3,4,6-tetra-*O*-methylgalactose (*T* 1.89) and 2,3,6-tri-*O*-methylgalactose (*T* 3.27, 4.01, 4.25, 4.54) in the molar ratio 1.0:1.13 were observed. The above evidence suggests that the structure of the oligosaccharide is 2-*O*-methyl-4-*O*-(6-*O*-methyl-β-D-galactopyranosyl)-D-galactose. The β-D configuration is assigned from the $[\alpha]_D$ value.

Fraction XIB. The syrup (4 mg) had R_{Gal} 2.28 (solvent *a*), 2.87 (solvent *d*), $[\alpha]_D^{20}$ +7° (*c* 0.88). Paper-chromatographic examination of a partial, acid hydrolysate of the sugar revealed the presence of 2-*O*-methylgalactose and 6-*O*-methylgalactose only. After reduction of the saccharide with borohydride, paper chromatography of an acid hydrolysate of the non-reducing syrup revealed 2-*O*-methylgalactose only (spray *i*). The above evidence suggests that the oligosaccharide is a 6-*O*-methyl-(2-*O*-methyl-D-galactopyranosyl)-D-galactose.

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