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CHEMICAL INVESTIGATIONS ON THE GUM EXUDATE FROM SAJNA (*Moringa oleifera*)

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ABSTRACT

The purified, whole-gum exudate from the drum-stick plant (*Moringa oleifera*) was found to contain L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, and D-xylose in the molar ratios of ~ 14.5 : 11.3 : 3 : 2 : 1 : 1. A homogeneous, degraded-gum polysaccharide consisting of D-galactose, D-glucuronic acid, and D-mannose in the molar ratios of ~ 11.7 : 3.9 : 1, was obtained on mild hydrolysis of the whole gum with acid. Permethylation studies were conducted on the whole gum, the degraded gum, and their carboxyl-reduced products, and the results were in good agreement with those obtained from periodate oxidation followed by Smith degradation. Also, isolation and characterization of the oligosaccharides obtained from the mother liquor during preparation of the degraded gum, and by graded hydrolysis of the degraded gum, were achieved. On the basis of the results obtained from these studies, a tentative structure was assigned to the average repeating-unit of the gum.

INTRODUCTION

The gum from the drum-stick plant (*Moringa oleifera*), commonly known as Sajna gum, is well known in India due to its medicinal values[1]. Earlier investigators[2] reported considerable work on this gum, but no detailed attempt to elucidate the structure of the polysaccharide seems to have been made so far. Consequently, a detailed investigation has now been undertaken.

RESULTS AND DISCUSSION

The water-soluble portion of the gum yielded a polysaccharide material that was repeatedly precipitated with ethanol. After gel filtration through a column of Sephadex G-100, the major portion of the material, obtained as a single component, had $[\alpha]^{26}_{589.5}$ −45.6°, and was found to be electrophoretically homogeneous. The

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TABLE I

METHYL ETHERS OF SUGARS OBTAINED FROM THE HYDROLYZATE OF (*A*) METHYLATED, DEGRADED GUM; (*B*) METHYLATED, CARBOXYL-REDUCED, DEGRADED GUM; (*C*) METHYLATED, WHOLE GUM; AND (*D*) METHYLATED, CARBOXYL-REDUCED, WHOLE GUM

Sugars[a]	T[b]		*Approximate mol %*				*Mode of linkage*
	a	*b*	A	B	C	D	
2,3,4,6-Glc	1.00	1.00	—	20	—	6	Glc*p*[c]-(1→
2,3,4,6-Gal	1.24	1.19	8	6	—	—	Gal*p*-(1→
2,3,6-Man	2.20	2.03	8	6	3	3	→4)Man*p*-(1→
2,3,4-Xyl	0.67	0.55	—	—	3	3	Xyl*p*-(1→
2,3,5-Ara	0.48	0.41	—	—	20	19	Ara*f*-(1→
2,3,4-Rha	0.44	0.35	—	—	7	6	Rha*p*-(1→
2,3,6-Glc	2.48	2.30	—	—	—	3	→4)Glc*p*[c]-(1→
2,3,4-Gal	3.38	2.90	48	41	7	6	→6)Gal*p*-(1→
2,3-Gal	5.66	4.72	31	26	29	28	→4,6)Gal*p*-(1→
2,5-Ara	1.12	0.88	—	—	12	13	→3)Ara*f*-(1→
2,3-Ara	1.33	1.10	—	—	7	6	→5)Ara*f*-(1→
2-Ara		1.95	—	—	7	6	→3,5)Ara*f*-(1→

[a]2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose, 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 as unity, on (a) a 3% of ECNSS-M column at 170°, and (b) a 3% of OV-225 column at 155°. [c]Glucose from glucuronic acid by reduction.

polysaccharide contained D-galactose, D-mannose, L-arabinose, L-rhamnose, D-xylose, and D-glucuronic acid in the molar ratios of 11.3:1:14.5:2:1:3, as estimated by g.l.c. of the carboxyl-reduced product. A degraded gum was prepared by heating a 1% solution of the gum in 15mM trifluoroacetic acid for 6.5 h on a boiling-water bath, cooling, dialyzing, and isolating by precipitation with ethanol. The precipitate had $[\alpha]_{589.5}^{26}$ +16.6°, and was electrophoretically homogeneous. It contained D-galactose, D-mannose, and D-glucuronic acid in the molar ratios of 11.73:1:3.9, as estimated from the carboxyl-reduced product.

The dialyzate obtained during the preparation of the degraded gum was found to contain arabinose, rhamnose, xylose, and two oligosaccharides. The oligosaccharides were separated, and isolated in the homogeneous state, by preparative paper chromatography. Oligosaccharide I was a disaccharide having only arabinose units; it was identified as 3-*O*-β-L-arabinofuranosyl-L-arabinose from its specific rotation, and from methylation studies. Oligosaccharide II was a disaccharide having rhamnose and arabinose as constituent units, the latter being found to be at the reducing end. From the specific rotation, and the results of methylation studies, it was characterized as 5-*O*-β-L-rhamnopyranosyl-L-arabinose.

The whole gum, its carboxyl-reduced product, and the degraded gum and its carboxyl-reduced product were methylated by the Hakomori method[3] followed by Purdie methylation[4], to yield a fully methylated derivative. After conversion into

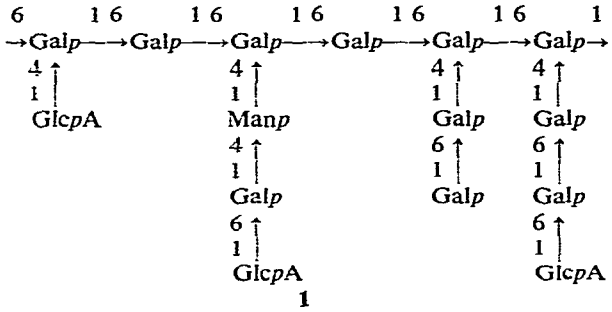
their alditol acetates, the methylated sugars obtained on hydrolysis thereof were analyzed by g.l.c., using columns (*1*) and (*2*); the results are given in Table I.

The data thus far obtained gave some insight into the structural details of the polysaccharides respectively present in the degraded and the whole gum. In the hydrolyzate of methylated whole gum and its carboxyl-reduced product, L-arabinose was found to be present as its 2,3,5-tri-, 2,5-di-, 2,3-di-, and 2-mono-*O*-methyl derivatives, which indicated that all of the arabinose residues are in the furanose form, and the di- and mono-*O*-methyl derivatives are engaged in (1→3), (1→5) and (1→3,5) linkages. L-Rhamnosyl and D-xylosyl groups are in the pyranoid form at the nonreducing end, as they are present as their 2,3,4-tri-*O*-methyl derivatives. The presence of a small proportion of 2,3,6-tri-*O*-methylglucose (in the methylated, carboxyl-reduced, whole gum) indicated that a portion of the glucuronic acid is substituted at O-4. A trace of 2,3,6-tri-*O*-methyl-D-mannose, substituted at O-4, was found in the hydrolyzate of the methylated and the carboxyl-reduced, methylated, degraded gum. Characterization of a large proportion of 2,3-di-*O*-methylgalactose (for both the degraded and the whole gum) indicated that the polysaccharide is highly branched. The rest of the galactose residues are (1→6)-linked, as 2,3,4-tri-*O*-methylgalactose was obtained (for both the degraded and the whole gum). Traces of 2,3,4,6-tetra-*O*-methylgalactose and 2,3,4,6-tetra-*O*-methylglucose were obtained from the carboxyl-reduced, methylated product, as the corresponding units occupy the nonreducing end after the removal of all of the labile sugars during preparation of the degraded gum.

Periodate oxidation showed that the degraded and the whole gum consumed 1.70 and 1.08 mol of the oxidant in 8 and 12 h, liberating 0.62 and 0.23 mol of formic acid, respectively, per mol of hexosyl residue. The theoretical values for periodate consumption are 1.66 and 1.06 mol, and for formic acid liberation, 0.66 and 0.22 mol, calculated on the basis of the results of the methylation studies. Thus, the periodate-oxidation results are in good agreement with those of the methylation studies.

On hydrolysis, the periodate-oxidized, degraded gum yielded only lower alcohols, whereas the whole gum gave arabinose and lower alcohols. On the basis of the methylation studies, the proportion of this periodate-resistant arabinose was estimated to be 14.5%. These results are in close agreement. On Smith degradation, the hydrolyzate was found to contain an oligosaccharide, besides other polyhydric alcohols. The oligosaccharide was separated on thick filter-papers, and was isolated in homogeneous state. On methylation and hydrolysis, it gave 2,3,5-tri- and 2,3-di-*O*-methylarabinose in equimolar proportions, which indicated it to be an arabinose disaccharide. This result showed that, at some places in the whole gum, the arabinose is present as Ara-(1→3)-[Ara-(1→3)-Ara-(1→5)]-Ara residues.

On graded hydrolysis, the degraded gum gave one acidic and two neutral oligomer fraction in the homogeneous state and a mixture. On rechromatography, the mixture yielded two homogeneous, acidic oligosaccharides. All five oligosaccharides were separated, and isolated, by preparative paper-chromatography. The neutral oligosaccharides were confirmed as being 4-*O*-β-D-galactopyranosyl-D-mannose and



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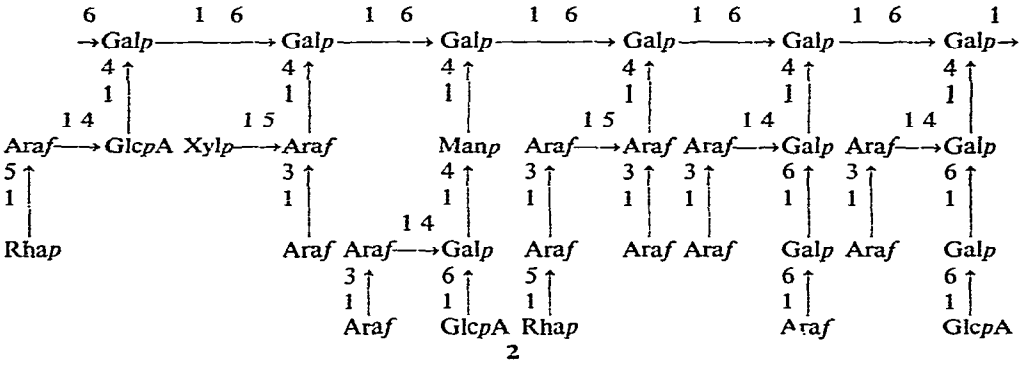

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6-*O*-β-D-galactopyranosyl-D-galactose, and the acidic oligomers were 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, 4-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactopyranosyl-D-galactose. They were characterized by their specific rotation, and by methylation and periodate-oxidation studies.

On considering all of the foregoing results (obtained by permethylation, Smith-degradation, and graded-hydrolysis studies), the structure of the average repeating unit of the degraded gum polysaccharide was assigned to be as depicted in **1**. This structure represents the general linkage of the different monosaccharide units, and accounts for the different fragmentation-products obtained. Structure **2** was assigned to the average repeating-unit of the whole gum, on the basis of the structure of the degraded gum coupled with that of the oligosaccharide obtained in the mother liquor during the preparation of the degraded gum, combined with the other analytical data already mentioned. The structure assigned well explains the linkages of the different monosaccharide units, and also those of the various oligosaccharides identified, but it does not necessarily depict the correct sequence of branches, because the data thus far acquired are insufficient for this purpose.

EXPERIMENTAL

General methods. — Evaporations were conducted in a rotary evaporator below

40° (bath temperature). All specific rotations were determined with a Perkin–Elmer Model 241 MC spectropolarimeter at 26 ±1° and 589.6 nm. Paper-chromatographic separations were performed by the descending technique on Whatman No. 1 and No. 3 MM paper. The solvent systems employed were *A*, 6:4:3 1-butanol–pyridine–water; *B*, 5:5:1:3 ethyl acetate–pyridine–acetic acid–water, and *C*, 1-butanol–acetic acid–water, 4:1:5 (upper layer). Aniline oxalate and alkaline silver nitrate were used for detecting the spots. Gel-filtration chromatography was conducted with Sephadex LH-20 and G-100. Elutions were monitored by the phenol–sulfuric acid method (for unmethylated compounds), and polarimetrically (for methylated compounds). For g.l.c., a Hewlett–Packard Model 5730A gas chromatograph fitted with an f.i.d. and a t.c.d. detector and a glass column (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° were used. A Shandon high-voltage electrophoresis apparatus, model L-24, was used for determining the homogeneity of the materials with (*a*) borate buffer, pH 9.5, and (*b*) phosphate buffer, pH 6.5, and the spots were developed with benzidine periodate. I.r. spectra were recorded with a Perkin–Elmer 177 spectrophotometer.

Isolation of purified gum. — The crude gum (100 g) was powdered, washed several times with alcohol, exhaustively extracted with 1:2 benzene–methanol in a Soxhlet apparatus, and then with dry methanol, and dried. The dry material was allowed to swell in water overnight. The viscous solution was diluted with sufficient water to make it a 1% solution, and this was stirred 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 for clarification. From the aqueous solution, the polysaccharide was precipitated by adding ethanol. The precipitate was dried, mixed with water, and the mixture stirred for 6 h, and kept overnight at 4°. A gel-like substance that settled was removed at the centrifuge, and to the pink, viscous, supernatant liquor was added, in the cold, ethanol (3 vol., acidified with acetic acid to pH 4.5); 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 six times, until an almost white precipitate was obtained. Finally, the precipitate was dissolved in water, and the solution was lyophilized; yield 18.5 g; $[\alpha]_{589.5}^{26}$ −46.15° (*c* 1, water).

The polysaccharide (100 mg) was dissolved in the minimum volume of ammonium hydrogencarbonate buffer (pH 8.0), and the solution was applied to a column (80 × 2 cm) of Sephadex G-100, and eluted with the same buffer. Each fraction was monitored by the phenol–sulfuric acid method. The major portion of the material, eluted as a single fraction, moved as a single component when subjected to high-voltage electrophoresis; it had $[\alpha]_{589.5}^{26}$ −45.6° (*c* 0.04, water).

The polysaccharide (5 mg) was hydrolyzed with 2M trifluoroacetic acid (TFA) for 16 h at 100°, and the excess of acid was removed by codistillation with water.

Paper chromatography using solvent systems *A* and *B* showed the presence of galactose, rhamnose, mannose, xylose, arabinose, and glucuronic acid.

Preparation of degraded gum. — A solution of whole gum (3 g) in 15mM trifluoroacetic acid solution (300 mL) was heated on a boiling-water bath for 6.5 h in order to ensure maximal release of the labile sugars (other than galactose). The hydrolyzate was dialyzed against distilled water, concentrated to a small volume, and the concentrate poured, with stirring, into ethanol (3 vol., acidified with acetic acid to pH 4.5). The resulting, flocculent precipitate was successively triturated with absolute ethanol (three times) and acetone, and dried *in vacuo*; yield 2.20 g; $[\alpha]_{589.5}^{26}$ +16.6° (*c* 1.02, water). It was found to be electrophoretically homogeneous. Hydrolysis of the degraded gum gave galactose, mannose, and glucuronic acid. Chromatographic examination of the dialyzate showed the presence of a few oligosaccharides besides the monomers.

Carboxyl reduction with CMC[5]. — The uronic acid in both the whole gum and the degraded gum was reduced with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC). To a solution of the polysaccharide (whole gum, 52 mg in 50 mL of water; degraded gum, 38 mg in 35 mL of water) was added CMC (1.3 g, 900 mg, respectively) with continuous stirring. The pH of the solutions was kept at 4.75 by dropwise addition of 0.01M hydrochloric acid, and the solution was kept for 2 h. Aqueous sodium borohydride (2M, 30 mL, 18 mL, respectively) was added dropwise during 1 h, the pH being kept at ~7 by concurrent addition of 4M HCl. After being stirred for 1 h, the solutions were dialyzed against distilled water for two days, and then lyophilized. A quantitative yield was obtained after two repetitions of the process.

Estimation of sugars. — The uronic acid content of the whole gum and the degraded gum was estimated spectrophotometrically by the carbazole–sulfuric acid method[6], and the content was found to be 10% for the whole gum, and 24.5% for the degraded gum. The carboxyl-reduced, whole gum (8.5 mg) and the degraded gum (5.1 mg) were separately mixed with *myo*-inositol (3.13 mg, 1.23 mg, respectively), used as the internal standard, and hydrolyzed with 2M trifluoroacetic acid as already described. The alditol acetates[7] were analyzed by g.l.c., using column *I*. For the whole gum, the contents found were galactose (34.00) mannose (3.0), arabinose (43.50), rhamnose (6.15), xylose (2.93), and glucose (9.37%), whereas, for the degraded gum, galactose (70.4), mannose (6.0), and glucose (23.2%) were found. The presence of glucose in the reduction products indicated that the uronic acid was glucuronic acid.

Methylation analysis. — The whole gum, the carboxyl-reduced whole gum, the degraded gum, and the carboxyl-reduced degraded gum (6.1, 4.5, 5.7, and 4.8 mg, respectively, dried over P_2O_5) were dispersed in dry dimethyl sulfoxide (6, 5, 6, and 5 mL, respectively) in separate vials. The vials were flushed with nitrogen, and 2M methylsulfinyl sodium in dimethyl sulfoxide was added (6, 5, 6, and 5 mL, respectively) by using a syringe. The gelatinous solutions were agitated in an ultrasonic bath for 30 min, and then kept overnight at room temperature. Methyl iodide

(4.5, 3.0, 4.0, and 2.5 mL, respectively) was added dropwise, with external cooling, and the turbid solutions resulting were agitated for 30 min in an ultrasonic bath. The products were then dialyzed, lyophilized, and remethylated by the Purdie method. No absorption for OH bands was observed in the i.r. spectrum; the product from whole gum, yield 6.2 mg, had $[\alpha]_{589.5}^{26}$ −27.2° (*c* 0.23, $CHCl_3$); that from carboxyl-reduced whole gum, yield 4.4 mg, had $[\alpha]_{589.5}^{26}$ −24.1° (*c* 0.52, $CHCl_3$); that from degraded gum, yield 5.9 mg, had $[\alpha]_{589.5}^{26}$ +12.1° (*c* 0.16, $CHCl_3$); and that from carboxyl-reduced degraded gum, yield 4.75 mg, had $[\alpha]_{589.5}^{26}$ +11.5° (*c* 0.35, $CHCl_3$).

All of the methylation products were hydrolyzed separately with 2M trifluoroacetic acid for 18 h at 100°, and the excess of acid was removed by codistillation with water. The partially methylated sugars were converted into their alditol acetates, and these were analyzed by g.l.c., using columns *1* and *2*. The results are shown in Table I.

Periodate oxidation. — The degraded gum and the whole gum were treated with 0.04M sodium metaperiodate in the dark at 4°. Consumption of the oxidant was monitored spectrophotometrically[8], and the formic acid liberated was estimated[9] in each case. For the degraded gum, the periodate consumption and the liberation of formic acid became constant in 8 h, corresponding to 1.70 and 0.62 mol, respectively, and, for the whole gum, became constant in 12 h, corresponding to 1.08 and 0.23 mol, respectively, per mol of hexosyl residue.

The periodate-oxidized, degraded gum (70 mg) and whole gum (350 mg) were reduced with sodium borohydride[10]. Part (~10 mg each) of the oxidized product was hydrolyzed with 0.5M sulfuric acid for 14 h at 100°. After neutralization of the acid with $BaCO_3$, and subsequent treatment, the alditol acetates were analyzed by g.l.c., and (other than polyhydric alcohols) only arabinose was detected in the oxidized-reduced product from the whole gum.

Another portion of periodate-oxidized, reduced whole gum (175 mg) was dissolved in M sulfuric acid (20 mL), and the solution was kept for 2 days at room temperature. The acid was neutralized with $BaCO_3$, the suspension filtered, and the filtrate concentrated to a small volume. Chromatographic examination (solvent *A*) showed the presence not only of arabinose but also of a spot above that of authentic L-arabinose. The spot was separated on Whatman No. 3 MM filter paper. Methylation analysis showed the presence of 2,3,5-tri- and 2,3-di-*O*-methylarabinose in equimolar proportions, which indicated that the saccharide was a disaccharide.

Graded hydrolysis of the degraded gum. — The degraded gum (1.10 g) was heated with 40% formic acid on a boiling-water bath for 4.5 h, the optimal conditions being ascertained by pilot experiments. The acid was removed by codistillation, and the hydrolyzate was passed through a column of Dowex-1 X-4 (HCO_3^-) resin, on which the acidic oligosaccharides were absorbed. The column was eluted exhaustively with distilled water. The neutral eluate and washings were combined, and concentrated to a small volume. The Dowex column was next eluted with 30% formic acid, and then with water. The eluates were combined, and the acid was removed *in vacuo*.

Both the acidic and the neutral fractions were resolved on Whatman No. 3 MM filter paper with solvent *B*, and the separate sugars were isolated by eluting the corresponding strips with water. One acid and two neutral fractions were isolated in homogeneous state, in addition to a mixture of acid fractions. On rechromatography with solvent *C*, the mixture gave rise to two homogeneous fractions.

Characterization of 4-O-β-D-*galactopyranosyl*-D-*mannose.* — The syrup had $[\alpha]_{589.5}^{26}$ +34° (*c* 0.1, water); mol. wt. 336 (determined by the alkaline hypoiodite method[11]) (Calc. for $C_{12}H_{22}O_{11}$, 342). On hydrolysis, it gave galactose and mannose in equimolar proportions, as estimated by g.l.c. after converting the sugars into the alditol acetates. Sodium borohydride reduction, and subsequent hydrolysis, gave galactose as the reducing sugar. Methylation analysis showed the presence of 2,3,4,6-tetra-*O*-methylgalactose and 2,3,6-tri-*O*-methylmannose, indicating that the galactose was glycosidically (1→4)-linked to the mannose, the galactosyl group being the non-reducing end.

Characterization of 6-O-β-D-*galactopyranosyl*-D-*galactose.* — This syrup had $[\alpha]_{589.5}^{26}$ +29.5° (*c* 0.2, water); mol. wt. 347 (Calc. for $C_{12}H_{22}O_{11}$, 342). On hydrolysis, it gave galactose only. One mol of the methyl glycosides of the disaccharide consumed 4.05 mol of periodate. On complete methylation of a portion of the disaccharide, hydrolysis gave 2,3,4,6-tetra- and 2,3,4-tri-*O*-methylgalactose, indicating that the two galactose units were glycosidically (1→6)-linked.

Characterization of 6-O-(β-D-*glucopyranosyluronic acid*)-D-*galactose.* — This compound had $[\alpha]_{589.5}^{26}$ −4° (*c* 0.5, water); mol. wt. 350 (Calc. for $C_{12}H_{20}O_{12}$, 356). On hydrolysis, paper-chromatographic examination showed the presence of galactose and glucuronic acid. Reduction with sodium borohydride, followed by hydrolysis, gave glucuronic acid only. The aldobiouronic acid was fully methylated by the Hakomori method. A portion of the methylation product was reduced with lithium aluminum hydride, and then remethylated by the Purdie method. On hydrolysis, the fully methylated product gave 2,3,4-tri-*O*-methylgalactose, whereas the reduced compound gave 2,3,4,6-tetra-*O*-methylglucose and 2,3,4-tri-*O*-methylgalactose in the molar ratio of 1:1, therefore the glucuronic acid is glycosidically (1→6)-linked to galactose.

Characterization of 4-O-(β-D-*glucopyranosyluronic acid*)-D-*galactose.* — The yield of this fraction was very small. It had $[\alpha]_{589.5}^{26}$ +16° (*c* 0.2, water). On hydrolysis, it gave galactose and glucuronic acid. The oligosaccharide was fully methylated, and the product hydrolyzed. A portion of the methylated product was reduced with $LiAlH_4$, and the product remethylated. On g.l.c. analysis, the fully methylated product gave 2,3,6-tri-*O*-methylgalactose, whereas the reduced compound gave 2,3,4,6-tetra-*O*-methylglucose and 2,3,6-tri-*O*-methylgalactose in equimolar proportions. In this disaccharide, glucuronic acid is (1→4)-linked to galactose.

Characterization of 6-O-(β-D-*glucopyranosyluronic acid*)-D-*galactopyranosyl*-D-*galactose.* — This syrup had $[\alpha]_{589.5}^{26}$ −6° (*c* 0.1, water); mol. wt. 510 (Calc. for $C_{18}H_{30}O_{17}$, 518). The hydrolyzate of the oligosaccharide and of its sodium borohydride-reduced product showed spots for galactose and glucuronic acid on paper

chromatograms. The oligosaccharide was fully methylated. A portion of the methylated product was reduced with $LiAlH_4$, and remethylated. On hydrolysis, the fully methylated product gave 2,3,4-tri-*O*-methylgalactose, whereas its reduced product gave 2,3,4,6-tetra-*O*-methylglucose and 2,3,4-tri-*O*-methylgalactose in the molar ratio of 1:2, as estimated by g.l.c. On periodate oxidation, the methyl ester methyl glycoside consumed 5.80 mol of periodate per mol.

Examination of the supernatant liquor obtained during preparation of the degraded gum. — The procedure for preparation of the degraded gum has already been described. The hydrolyzate was dialyzed, and the dialyzate concentrated to a small volume which, on paper-chromatographic examination, was found to contain arabinose, xylose, rhamnose, and two oligosaccharides (R_{Gal} 0.33 and 0.23 in solvent *C*). The mixture was resolved on Whatman No. 3 MM paper, and the zones corresponding to each sugar were excised, and eluted with water. Each fraction was found to be homogeneous, and was characterized as follows.

Characterization of 3-O-β-L-arabinofuranosyl-L-arabinose. — The syrup had $[\alpha]_{589.5}^{26}$ +86° (*c* 0.1, water); mol. wt. 276 (Calc. for $C_{10}H_{18}O_9$, 282). On hydrolysis, it gave arabinose only. Methylation analysis showed the presence of 2,3,5-tri- and 2,5-di-*O*-methylarabinose in equimolar proportions. Periodate oxidation of the methyl glycoside showed that, in 5 h, one mol of the oxidant was consumed per mol of the disaccharide, and no formic acid was liberated.

Characterization of 5-O-β-L-rhamnopyranosyl-L-arabinose. — The syrup had $[\alpha]_{589.5}^{26}$ −15°; mol. wt. 285 (Calc. for $C_{11}H_{20}O_9$, 296). It contained equimolar proportions of rhamnose and arabinose, as estimated by g.l.c. On reduction with sodium borohydride, followed by hydrolysis, and subsequent treatments, it gave a peak for rhamnose only. This showed that arabinose was at the reducing end of the disaccharide. Methylation analysis showed the presence of 2,3,4-tri-*O*-methylrhamnose and 2,3-di-*O*-methylarabinose.

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