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THE STRUCTURE OF *Lannea coromandelica* GUM*

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ABSTRACT

Lannea coromandelica trees exude a water-soluble gum polysaccharide containing galactose (70%), arabinose (11%), rhamnose (2%), and uronic acids (17%). Three aldobiouronic acids are present (chromatographic analysis), namely 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and 6-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-galactose. Linkage analysis of degraded gum *A*, obtained by controlled, acid hydrolysis, gave (chromatographic analysis) 3-*O*-β-L-arabinofuranosyl-L-arabinose, 3-*O*-β-L-arabinopyranosyl-L-arabinose, 3-*O*-α-D-galactopyranosyl-L-arabinose, 3-*O*-β-D-galactopyranosyl-D-galactose, and 6-*O*-β-D-galactopyranosyl-D-galactose. Degraded gum *A* was examined by methylation analysis, and was subjected to a Smith-degradation, giving degraded gum *B*, which was studied by linkage and methylation analysis. The *O*-methyl derivative of the whole gum was prepared by the Haworth and Purdie procedures and examined, after methanolysis, by g.l.c.: 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,5- and 2,3,4-tri- and 2,5-di-*O*-methyl-L-arabinose; 2,3,4,6-tetra-, 2,3,6-, 2,4,6-, and 2,3,4-tri-, and 2,6- and 2,4-di-*O*-methyl-D-galactose; 2,3,4-tri-*O*-methyl-D-glucuronic acid and 2,3,4-tri-*O*-methyl-D-galacturonic acid were identified. The whole gum was subjected to three successive Smith-degradations, giving Polysaccharides I–III which were examined by linkage and methylation analysis. The structural evidence obtained indicates that the gum molecules are very highly branched, based on a galactan framework consisting of short chains of β-(1→3)-linked D-galactose residues, branched and interspersed with β-(1→6) linkages. To positions 3 and 6 of this framework are attached either single D-galactose end-groups or short side-chains of D-galactose or of L-arabinose residues, and three aldobiouronic acids. The structure therefore appears to be very similar to that established recently for *Lannea humilis* gum.

INTRODUCTION

The polyphenols associated with *Lannea coromandelica* trees have been investigated[1], and there have been two major, but unconvincing, attempts to establish the structure of *L. coromandelica* gum. Ramachandran and Joshi[2] suggested a struc-

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ture based on a backbone of β-(1→4)-linked galactose residues, some of which were substituted at the 3-position by arabinose or galactose; unfortunately, these authors reported *L. coromandelica* gum to be a *neutral* polysaccharide and, since it contains 17% of uronic acids, there appears to be little alternative to discounting their work.

Bhattacharyya and Rao[3] proposed a repeating structure for the degraded gum based on a main chain of (1→6)-linked galactose residues to some of which were attached galactose or galacturonic acid at the 3-position, or two galactose residues at positions 3 and 4. This structure was extended by Bhattacharyya and Mukherjee[4] to incorporate arabinose side-chains in a structure for the whole gum, and this model has been used[5–7] in attempted interpretations of physico-chemical measurements. Unfortunately, the analytical data on which this structure was based[3] differ in several important respects from values established recently[8], in comparison with which Bhattacharyya and Rao's values for the arabinose content appear to be high. Furthermore, Bhattacharyya and Rao[3] did not detect rhamnose or nitrogen, a significant methoxyl content was ignored, galacturonic acid was the only uronic acid identified (three acids are present), and a negative specific rotation (−44°) was recorded, as opposed to the positive values observed by other authors (+45°, ref. 2; +27°, ref. 8; +29°, ref. 9).

In consequence, it appeared that the structure of *Lannea coromandelica* gum should be re-investigated.

EXPERIMENTAL AND RESULTS

Origin and purification of gum specimen. — Gum from *L. coromandelica* (Houtt.) Merrill was obtained in October 1967 from Mr. L. Wijesinghe, Research Laboratory of the Conservator of Forests, Kew Road, Colombo, Ceylon. The crude gum was readily soluble in cold water; the method of purification and the analytical data for this gum have been given[8].

Analytical methods. — The standard analytical methods have been described[10], except that g.l.c. (Pye Argon Chromatograph) of mixtures of *O*-methyl sugars was carried out at argon flow-rates of ~100 ml.min^{-1} on columns (120 × 0.5 cm) of (*i*) 15% by weight of poly(ethylene glycol adipate) on Phase-sep Universal B at 175°, and (*ii*) 15% by weight of poly(butane-1,4-diol succinate) on Phase-sep Universal B at 185°.

Homogeneity of the gum. — The gum migrated as a single band on thin-layer electrophoresis[11] on "Phoroslide" strips at pH 8.9 (0.1M ammonium carbonate buffer), pH 4.7 (0.1M sodium acetate buffer), and pH 9.2 (50mM sodium borate buffer). A solution (0.5%) of the gum in 0.5M sodium chloride, when examined by ultracentrifugation at 44,770 r.p.m., showed a single, broad peak.

The neutral and acidic components. — The gum (2.36 g) was hydrolysed in 0.5M sulphuric acid (200 ml) for 6 h at 100°. The solution was cooled, neutralised with barium carbonate, deionised with Amberlite IR-120(H^+) resin, concentrated, and fractionated on a column (30 × 1.5 cm) of Duolite A-4 resin in the formate form.

Elution with water and then with 5% formic acid gave the neutral and acidic fractions, respectively. After concentration to a syrup, the neutral fraction was chromatographed in solvents (*a*), (*b*), and (*c*); the presence of components having mobilities identical with those of authentic D-galactose, L-arabinose, and L-rhamnose was indicated.

The acidic fraction was concentrated, and the residual formic acid was removed by repeated additions of water followed by concentration to a syrup. Paper chromatography in solvents (*c*) and (*d*) showed the presence of two acidic components, having R_{Gal} 0.21 and 0.61, respectively, in solvent (*c*), and R_{Gal} 0.70 and 0.92, respectively, in solvent (*d*). The faster-moving component was the more abundant. The components were fractionated on Whatman 3MM papers in solvent (*c*).

Fraction **1** had $[\alpha]_D$ +47° (*c* 0.86). The syrup (8 mg) was hydrolysed (M sulphuric acid for 7 h at 100°); paper chromatography of the hydrolysate in solvents (*a*), (*c*), (*h*), and (*i*) indicated the presence of a galactose, galacturonic and glucuronic acids, and a glucurono-6,3-lactone. This suggested that fraction **1** was a mixture of aldobiouronic acids, and paper chromatography in solvents (*c*) and (*d*) showed that this fraction behaved identically with acidic fraction **1** of *L. humilis* gum[10]. Accordingly, the conditions devised[10] for separating the aldobiouronic acids in *L. humilis* gum were applied. Paper chromatography of fraction **1** on 3MM paper in solvent (*a*) for 150 h gave separation of two components which were eluted separately and concentrated. Fraction **1**(a) had R_{Gal} 0.14 [solvent (*a*)], 0.21 [solvent (*c*)], and 0.70 [solvent (*d*)], and was identical chromatographically in these solvents with the specimen of 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose characterized completely in the study of *L. humilis* gum[10]. Fraction **1**(**b**) had R_{Gal} 0.19 [solvent (*a*)], 0.21 [solvent (*c*)], and 0.73 [solvent (*d*)], and was identical chromatographically in these solvents with the specimen of 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose characterized completely in the study of *L. humilis* gum[10].

Fraction **2** had $[\alpha]_D$ +9° (*c* 0.84) and was identical chromatographically in solvent (*c*) (R_{Gal} 0.61) and solvent (*d*) (R_{Gal} 0.92) with the specimen of 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose characterized completely in the study of *L. humilis* gum. It should be pointed out that the R_{Gal} values for fractions **1**(**a**), **1**(**b**), and **2** are for aged-solvent systems; for example, the R_{Gal} value for fraction **2** was 0.70 in freshly prepared solvent (*c*), and for fraction **1**(**a**) R_{Gal} was 0.52 in freshly prepared solvent (*d*).

Partial, acid hydrolysis of L. coromandelica *gum.* — *L. coromandelica* gum (25 mg) was hydrolysed with 0.25M sulphuric acid (25 ml) for 1 h at 100°. The solution was neutralised with barium carbonate, filtered, deionised with Amberlite IR-120(H^+) resin, and concentrated. Paper chromatography in solvents (*a*), (*b*), and (*c*) indicated the presence of a galactose, a smaller amount of an arabinose, a trace of rhamnose, and three neutral disaccharides chromatographically identical with authentic 6-*O*-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.30 in solvent (*a*); 0.38 in solvent (*b*)], 3-*O*-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.49 in solvent (*a*); 0.55 in solvent (*b*)] (major components, in approximately equal amounts), and 3-*O*-α-D-galactopyranosyl-L-arabinose [R_{Gal} 0.67 in solvent (*a*); 0.73 in solvent (*b*), and 0.55 in solvent (*c*)]

TABLE I

DATA FOR *Lannea coromandelica* GUM AND ITS DEGRADATION PRODUCTS

Polysaccharide	*Yield* (%)	$[\alpha]_D$ (*degrees*)	$\overline{M}_n$ *x* 10^3	*Constituent sugars* (%)				*Periodate reduced* (*mmoles.g*$^{-1}$)	*Formic acid released* (*mmoles.g*$^{-1}$)
				Gal	*Ara*	*Rha*	*Uronic acid*		
L. coromandelica gum	78	+27	[a]	70	11	2	17	9.26	3.54(72 h)
Degraded-gum *A*	69	+20	3.27	84	1		15	12.7	5.33(96 h)
Degraded-gum *B*	27	+19	1.84	100				—	—
Polysaccharide I	31	+13	2.04	96	4			4.43	0.95(48 h)
Polysaccharide II	37	+12	4.08	99	1			2.37	0.78(48 h)
Polysaccharide III	47	−6	3.18	99.5	0.5			n.d.	n.d.

[a] $\overline{M}_W$ 2.57×10^5. [b] n.d., not determined.

(minor component, pink spot with aniline oxalate), together with some higher oligosaccharides.

Preparation of degraded gum A. — *L. coromandelica* gum (1.97 g) was dissolved in 5mM sulphuric acid (100 ml) and kept at 100° for 100 h. Aliquots *1*, *2*, and *3* (1.5 ml) were withdrawn after 6, 30, and 100 h, respectively, neutralised with barium carbonate, filtered, deionised with Amberlite IR-120(H^+) resin, and concentrated. The syrups were examined by paper chromatography in solvents (*a*), (*b*), (*c*), and (*f*). Aliquot *1* contained an appreciable amount of arabinose, and traces of galactose, rhamnose, and arabinose disaccharides. Aliquot *2* contained galactose, much arabinose, a trace of rhamnose, and three disaccharides chromatographically identical with 3-*O*-α-D-galactopyranosyl-L-arabinose [R_{Gal} 0.67 in solvent (*a*), 0.73 in solvent (*b*), and 0.55 in solvent (*c*)], 3-*O*-β-L-arabinofuranosyl-L-arabinose [R_{Gal} 1.20 in solvent (*c*), 1.40 in solvent (*f*)], 3-*O*-β-L-arabinopyranosyl-L-arabinose [R_{Gal} 0.82 in solvent (*a*), 0.91 in solvent (*b*), and 0.76 in solvent (*c*)], and traces of higher oligosaccharides. Aliquot *3* contained galactose, much arabinose, a trace of rhamnose, small amounts of 3-*O*-α-D-galactopyranosyl-L-arabinose and 3-*O*-β-L-arabinopyranosyl-L-arabinose, and barely detectable traces of 6-*O*-β-D-galactopyranosyl-D-galactose and 3-*O*-β-D-galactopyranosyl-D-galactose. Higher oligosaccharides were present.

After 100 h, the bulk of the brown solution was cooled, filtered, dialysed exhaustively against running tap-water, and freeze-dried to give degraded gum *A* as a pale-brown product (1.31 g, 69% when corrected for the aliquots withdrawn).

Examination of degraded gum A. — The analytical data obtained are shown in Table I. After hydrolysis of the degraded gum (20 mg) with 0.5M sulphuric acid for 7 h at 100°, paper chromatography of the resulting syrup in solvents (*c*) and (*d*) indicated the presence of a galactose, an arabinose, and the aldobiouronic acids already identified in the whole gum. Degraded gum *A* (210 mg) was methylated by the Haworth and Purdie procedures to give a product (128 mg) (Found: OMe, 40.7%). After methanolysis of a portion of this product, g.l.c. of the products gave the results shown in Table II.

Preparation of degraded gum B *by Smith-degradation of degraded gum* A. — Degraded gum *A* (0.86 g) was dissolved in water (25 ml), and 0.75M sodium metaperiodate (25 ml) was added. After 96 h in darkness at room temperature (when the amounts of periodate reduced and formic acid produced were as shown in Table I), the reaction was stopped by the addition of ethylene glycol (3 ml). The solution was dialysed against running tap-water for 72 h, sodium borohydride (0.47 g) was added, and, after 30 h at room temperature, the solution was dialysed for a further 72 h. The solution was made 0.5M with respect to sulphuric acid, kept for 48 h at room temperature, and then dialysed for 48 h. Degraded gum *B* was isolated as the freeze-dried product (0.24 g, 27%).

Examination of degraded gum B. — After hydrolysis of degraded gum *B* (30 mg) with 0.25M sulphuric acid for 1 h at 100°, paper chromatography of the resulting syrup in solvents (*a*) and (*b*) indicated the presence of galactose and disaccharides chromatographically identical with authentic 3-*O*-β-D-galactopyranosyl-D-galactose

TABLE II

EXAMINATION OF METHANOLYSIS PRODUCTS FROM METHYLATED DEGRADED-GUMS *A* AND *B*

O-Methyl sugar identified[a]	*Relative molar proportions*	
	A	B
2,3,5-Tri-*O*-methyl-L-arabinose	tr[c]	
2,3,4-Tri-*O*-methyl-L-arabinose	tr	
2,5-Di-*O*-methyl-L-arabinose	tr	
2,3,4,6-Tetra-*O*-methyl-D-galactose	7	1
2,3,6-Tri-*O*-methyl-D-galactose	0.1	0.1
2,4,6-Tri-*O*-methyl-D-galactose	4	9
2,3,4-Tri-*O*-methyl-D-galactose	1	0.1
2,6-Di-*O*-methyl-D-galactose	0.1	1
2,4-Di-*O*-methyl-D-galactose	5	tr
2,3,4-Tri-*O*-methyl-D-glucuronic acid[b]	2	
2,3,4-Tri-*O*-methyl-D-galacturonic acid[b]	1	

[a]See Table III for retention times and R_{Glc} values. [b]As methyl ester methyl glycoside. [c]tr, trace.

(major component), 6-*O*-β-D-galactopyranosyl-D-galactose (trace), and higher oligosaccharides. Degraded gum *B* (110 mg) was methylated by the Haworth and Purdie procedures to give a product (72 mg) having OMe, 40.5%. After methanolysis, g.l.c. gave the results shown in Table II.

Methylation of L. coromandelica *gum.* — The purified gum (249 mg) was methylated by the Haworth and Purdie procedures to give a product (186 mg), $[\alpha]_D$ −4° (*c* 0.83, chloroform) (Found: OMe, 40.7%). After methanolysis, the methyl

TABLE III

EXAMINATION OF METHANOLYSIS PRODUCTS FROM METHYLATED *L. coromandelica* GUM

Relative retention time (T) *of methyl glycosides*[a]		R_{Glc} *after hydrolysis Solvents*		*O-Methyl sugar identified*
Column (i)	*Column* (ii)	(e)	(f)	
0.51	0.51	1.01	1.03	2,3,4-Tri-*O*-methyl-L-rhamnose
0.58, 0.70	0.61, 0.76	0.97	1.01	2,3,5-Tri-*O*-methyl-L-arabinose
1.02	1.03	0.78	0.75	2,3,4-Tri-*O*-methyl-L-arabinose
1.83, 3.14	1.75, (2.69)	0.80	0.80	2,5-Di-*O*-methyl-L-arabinose
1.58	1.60	0.87	0.78	2,3,4,6-Tetra-*O*-methyl-D-galactose
(2.86)	(2.69)	0.74	0.49	2,3,6-Tri-*O*-methyl-D-galactose
3.59, 3.80	3.48, 3.74	0.72	0.40	2,4,6-Tri-*O*-methyl-D-galactose
(6.22)	(5.54)	0.72	0.34	2,3,4-Tri-*O*-methyl-D-galactose
9.06	7.92	0.55	0.19	2,6-Di-*O*-methyl-D-galactose
14.37, 15.58	12.45, 13.84	0.53	0.12	2,4-Di-*O*-methyl-D-galactose
2.27, (2.86)	2.18, (2.69)			2,3,4-Tri-*O*-methyl-D-glucuronic acid[b]
(6.22)	(5.54)			2,3,4-Tri-*O*-methyl-D-galacturonic acid[b]

[a]Figures in parenthesis indicate *T* values of components which were not completely resolved. [b]As methyl ester methyl glycoside.

glycosides shown in Table III were identified by g.l.c. The relative amounts of the *O*-methyl sugars could not be estimated satisfactorily because of incomplete resolution of several of the components, but 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, and 2,4-di-*O*-methyl-D-galactose were present in ratios of ~7:2:1:7. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*), indicated the presence of 2-*O*-methyl-D-galactose in addition to the *O*-methyl sugars already identified by g.l.c. of their methyl glycosides.

Preparation, partial hydrolysis with acid, and methylation of Polysaccharide I. — *L. coromandelica* gum (27.0 g) was dissolved in water (750 ml), 0.5M sodium metaperiodate (750 ml) was added, and the solution kept in darkness at room temperature. The oxidation was followed by measuring the release of formic acid with time. After 72 h, the amounts of periodate reduced and formic acid released were as shown in Table I. The reaction was stopped by the addition of ethylene glycol (16 ml), and the solution was dialysed against running tap-water for 48 h. Sodium borohydride (8.1 g) was added, and the mixture was kept at room temperature for 36 h, and then dialysed for 48 h. The solution was made 0.5M with respect to sulphuric acid, and the polyalcohol was hydrolysed for 48 h at room temperature. After dialysis against running tap-water for 72 h, the Smith-degradation product (Polysaccharide I) was isolated by freeze-drying (see Table I).

Polysaccharide I (22 mg) was hydrolysed with M sulphuric acid for 7 h at 100°. Paper chromatography of the hydrolysate in solvents (*a*), (*h*), and (*i*) indicated the presence of a galactose and an arabinose only. The gum (21 mg) was hydrolysed with 0.25M sulphuric acid for 1 h at 100°, and paper chromatography of the resulting syrup in solvents (*a*) and (*b*) indicated the presence of a galactose, an arabinose, two neutral disaccharides having the mobilities of 6-*O*-β-D-galactopyranosyl-D-galactose [minor component: R_{Gal} 0.31 in solvent (*a*) and 0.39 in solvent (*b*)] and 3-*O*-β-D-galactopyranosyl-D-galactose [major component: R_{Gal} 0.48 in solvent (*a*) and 0.56 in solvent (*b*)] and higher, neutral oligosaccharides.

Polysaccharide I (218 mg) was methylated by the Haworth and Purdie procedures (see Table IV). Methylation of a portion of the product, followed by g.l.c. examination of the mixtures of methyl glycosides, gave the results shown in Table IV. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*), indicated the presence of 2-*O*-methylgalactose.

Preparation of the sequential, Smith-degradation products: Polysaccharides II and III. — The following weights of the sequence of Smith-degradation products were periodate-oxidised, borohydride-reduced, and hydrolysed, and the corresponding degraded polysaccharides were recovered, all (except for the periodate-oxidation conditions) as already described for Polysaccharide I. Polysaccharide I (7.62 g) gave Polysaccharide II (2.81 g); II (2.05 g) gave III (0.97 g). The percentage yields, specific rotations, number-average molecular weights, sugar compositions, and periodate-oxidation data for Polysaccharides II and III are shown in Table I.

Partial, acid hydrolysis of Polysaccharides II and III. — After hydrolysis (0.25M sulphuric acid, 1 h, 100°) of II (26 mg) and III (28 mg), paper chromatography

TABLE IV

METHYLATION DATA AND RELATIVE PROPORTIONS OF *O*-METHYL SUGARS PRESENT IN METHYLATED POLYSACCHARIDES I–III

	Polysaccharides		
	I	*II*	*III*
Methylation data			
Weight of polysaccharide used (mg)	218	229	252
Weight of product (mg)	99	145	148
$[\alpha]_D$ of product (degrees)	−3	−5	−12
OMe of product (%)	41.4	40.9	40.4
O-Methyl sugars identified[a]			
2,3,5-Tri-*O*-methyl-L-arabinose	0.5	tr	tr
2,3,4-Tri-*O*-methyl-L-arabinose	0.1	—	—
2,5-Di-*O*-methyl-L-arabinose	tr	—	—
2,3,4,6-Tetra-*O*-methyl-D-galactose	1	1	1
2,3,6-Tri-*O*-methyl-D-galactose	0.1	0.1	0.1
2,4,6-Tri-*O*-methyl-D-galactose	5	4	5
2,3,4-Tri-*O*-methyl-D-galactose	0.1	tr	tr
2,6-Di-*O*-methyl-D-galactose	0.8	0.5	0.5
2,4-Di-*O*-methyl-D-galactose	0.1	tr	tr

[a]See Table III for retention times and R_{Glc} data.

[solvents (*a*) and (*b*)] indicated the presence in each of a galactose, very small amounts of an arabinose, disaccharides having the mobilities of 3-*O*-β-D-galactopyranosyl-D-galactose (major component) and 6-*O*-β-D-galactopyranosyl-D-galactose (minor component), and higher neutral oligosaccharides.

Methylation of Polysaccharides II and III. — Polysaccharides II and III were methylated by the Haworth and Purdie procedures; data for the products are shown in Table IV. Methanolysis of a portion of each *O*-methyl derivative, followed by g.l.c. examination of the mixture of methyl glycosides, gave the results shown in Table IV. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*), indicated the presence of 2-*O*-methylgalactose, in addition to those *O*-methyl sugars already characterized by g.l.c. of their methyl glycosides (Table IV).

Molecular weight ($\bar{M}_n$) *of degraded polysaccharides by end-group analysis.* — The polysaccharide (30–50 mg) was dissolved in 0.1% aqueous *p*-hydroxybenzaldehyde (10 ml), which prevents recombination of formaldehyde with the oxopolysaccharide[12]. To the solution was added 1 ml of aqueous sodium metaperiodate, such that a slight excess of periodate was present. At suitable time-intervals, samples (1 ml) were transferred to centrifuge tubes, and treated with 0.5M sodium sulphite (1 ml) to destroy excess periodate and with ethanol (4 ml) to precipitate the oxopolysaccharide. The tubes were stored for 48 h at 2° and then centrifuged. Portions (1 ml) of the supernatant were treated with 9 ml of chromotropic acid reagent [2 g of the sodium salt of chromotropic acid dissolved in a solution of AnalaR sulphuric acid (566 ml) and water (320 ml)] and kept for 30 min at 100°. After cooling, thiourea solution

(2 ml, 4.6%, w/v) was added, and the absorbance was measured on a Unicam SP1300 spectrometer using filter 4. A calibration curve for formaldehyde was prepared by oxidising known weights of AnalaR D-glucose. Assuming the production of 1 mole of formaldehyde per average polymer unit, a value for $\overline{M}_n$ may be calculated for the polysaccharide. The results are recorded in Table I.

DISCUSSION

The gum from *Lannea coromandelica* contains galactose, arabinose, rhamnose, galacturonic acid, glucuronic acid, and 4-*O*-methylglucuronic acid. In earlier studies, it was reported[13] to contain 6-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-galactose, but, in contrast, the only aldobiouronic acid identified by other Indian workers[14] was 3-*O*-(D-galactopyranosyluronic acid)-D-galactose. Careful chromatographic analyses have now established that *L. coromandelica* gum contains the same three aldobiouronic acids as *L. humilis* gum[10], *viz.* 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, with the distinction that the last-named is the major aldobiouronic acid in *L. coromandelica* gum. This is substantiated by the methoxyl contents[8] of these two species (*L. humilis*, 0.4%; *L. coromandelica*, 1.6%).

Controlled, acid hydrolysis of *L. coromandelica* gum led to chromatographic evidence for the presence of 3-*O*-β-L-arabinofuranosyl-L-arabinose, 3-*O*-α-D-galactopyranosyl-L-arabinose, and 3-*O*-β-L-arabinopyranosyl-L-arabinose. The presence of a very large proportion of 2,3,4,6-tetra-*O*-methyl-D-galactose in the methanolysate of methylated, degraded-gum *A* confirms the report[3] that *Lannea* gums contain characteristically large amounts of non-reducing D-galactopyranose end-groups. The unusually high proportion of 2,4-di-*O*-methyl-D-galactose also suggests that degraded gum *A* is very highly branched, and the presence of only trace amounts of 2,5-di-*O*-methyl-L-arabinose indicates that there is little arabinose involved in the formation of side-chains involving more than one arabinose residue.

Controlled hydrolysis of degraded-gum *A* led to evidence for the presence of 3-*O*-β-D-galactopyranosyl-D-galactose and 6-*O*-β-D-galactopyranosyl-D-galactose. The evidence indicates that L-Ara*f*-(1→ , L-Ara*p*-(1→ , and D-Gal*p*-(1→ act as non-reducing end-groups, together with the three aldobiouronic acids. From the data presented in Tables I and II, it is seen that rhamnose residues also act as non-reducing end-groups; that there is only a small proportion of 1,3-disubstituted L-arabinofuranose residues in the side-chains; that there are 3-*O*-, 4-*O*-, and 3,6-di-*O*-substituted D-galactose residues; that there are many terminal, non-reducing D-galactopyranosyl residues; and that the arabinose-containing side-chains are attached to positions 3 or 6 of certain D-galactose residues. Possible structural fragments include: →4)-D-Gal*p*-(1→ ; →6)-D-Gal*p*-(1→ ; β-L-Ara*f*-(1→3)-L-Ara*f*-(1→ ; β-L-Ara*p*-(1→3)-L-Ara*f*-(1→ ; α-D-Gal*p*-(1→3)-L-Ara*f*-(1→ ; β-D-Gal*p*-(1→3)-D-Gal*p*-(1→ ; β-D-Gal*p*-(1→6)-D-Gal*p*-(1→ ; and →3-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→ .

The sequence of Smith-degradations (see Tables I and IV) indicates that very few of the arabinose-containing side-chains contain more than 3 residues. In this structural feature, as indeed in all such respects, *L. coromandelica* gum is very similar indeed to *L. humilis* gum, for which a possible structural fragment of the branched galactan framework has been published[10]. The low yield (31%) of polysaccharide I and the high reduction of periodate (9.26 mmole.g^{-1}) by the whole gum are only marginally less than the values recorded[10] for *L. humilis* gum. Partial, acid hydrolysis of the Smith-degraded Polysaccharides I–III each gave the β-(1→3)-linked D-galactose disaccharide as the major component, with steadily decreasing proportions of the β-(1→6)-linked-D-galactose disaccharide present. The core of the gum is therefore a D-galactan based mainly on β-(1→3)-linked residues, but it must contain periodate-vulnerable β-(1→6)-units, and long sequences of β-(1→3)-linked residues are unlikely to occur. The periodate-vulnerable β-(1→6)-linkages may possibly arise at each stage of the Smith-degradation sequence by the progressive removal of arabinose-containing side-chains or D-galactose residues from position 3 of 6-*O*-substituted D-galactose residues. The random distribution of a significant number of such β-(1→6)-linked D-galactose residues provides a reasonable explanation for the drastic fragmentation of *L. coromandelica* gum by the first Smith-degradation.

By end-group assay, the value of $\overline{M}_n$ for Polysaccharide I was found to be lower than that for Polysaccharides II and III. These experiments were repeated, and the values obtained initially were confirmed. Studies will be undertaken to try to find an explanation for this apparent anomaly.

It is clear that, in almost every respect, the structural features of *L. coromandelica* gum are closely similar to those of *L. humilis* gum[10]. The major differences involve the higher uronic acid content of *L. coromandelica* gum and the presence in it of 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose as the major aldobiouronic acid. Direct comparisons of the appropriate chromatograms for *L. humilis* and *L. coromandelica* gums showed that (*a*) methylated degraded gum *A* from *L. coromandelica* contained relatively smaller proportions of 2,3,5-tri-, 2,3,6-tri- and 2,6-di-*O*-methyl-L-arabinose; (*b*) methylated polysaccharide I from *L. coromandelica* gum contained a smaller amount of 2,6-di-*O*-methyl-D-galactose and a proportionately larger amount of 2,4-di-*O*-methyl-D-galactose; (*c*) methylated polysaccharide II from *L. coromandelica* gum contained relatively smaller amounts of 2,3,5-tri-*O*-methyl-L-arabinose, 2,4,6-tri-*O*-methyl-D-galactose, and 2,6-di-*O*-methyl-galactose; (*d*) methylated polysaccharide III of *L. coromandelica* gum contained relatively smaller amounts of 2,3,5-tri-*O*-methyl-L-arabinose (although for both gums this is reported as "trace"), and 2,3,6-tri-, 2,4,6-tri-, and 2,6-di-*O*-methyl-D-galactose. At each stage of the structural analysis, the polysaccharide and degraded polysaccharides from *L. coromandelica* gum appear to have been more easily methylated than the corresponding polysaccharides from *L. humilis* gum, so that slightly higher methoxyl-contents were achieved. Consequently, the relative proportions of the 2,6-di- and 2,3,6-tri-*O*-methylgalactose fragments (the presence of which is frequently ascribed to undermethylation or demethylation in structural analyses) appear to be

smaller in *L. coromandelica* gum than in *L. humilis* gum, and the significance of these slight differences between the two gums should not be interpreted too rigorously.

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