THE STRUCTURE OF *Lannea humilis* GUM*

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ABSTRACT

Lannea humilis trees exude a water-soluble gum polysaccharide containing galactose (75%), arabinose (11%), rhamnose (2%), and uronic acids (12%). 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-β-D-glucopyranosyluronic acid)-D-galactose. Linkage analysis of degraded gums *A* and *B*, 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 gums *A* and *B* were examined by methylation analysis, and the former was subjected to a Smith-degradation, giving degraded gum *C*, which was studied by linkage and methylation analysis. The *O*-methyl derivative of the whole gum was prepared (*a*) by the Haworth and Purdie procedures, and (*b*) by the sodium hydride–methyl iodide–methyl sulphoxide technique. Both products were 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-, 2,6- and 2,4-di-, and 2-*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 four successive Smith-degradations giving Polysaccharides I–IV, which were examined by linkage and methylation analysis. Polysaccharide IV is a branched galactan; the arabinose-containing side-chains in *L. humilis* gum therefore do not contain more than four residues, and only a few of that length occur. The evidence obtained indicates that the gum molecules are very highly branched. The galactan framework consists 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. A possible structural fragment that shows these features is proposed.

INTRODUCTION

A recent paper[1] reported the results obtained in an analytical study of the water-soluble gums from *Lannea schimperi*, *Lannea humilis*, and *Lannea coromande-*

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lica. Of these species, only the latter (under its synonyms[1] *Lannea grandis*, "gum jeol", etc.) has been studied structurally[2,3]. We now report the results of a structural study of the gum from *Lannea humilis* (Oliv.) Engler.

EXPERIMENTAL AND RESULTS

Origin and purification of gum sample. — Gum from *L. humilis* (Oliv.) Engler was collected in May 1969 at Layyuna Central Forest Reserve, Central Kordofan, by Mr. A. G. Seif-el-Din, M.Sc., Gum Research Officer to the Republic of the Sudan. The crude gum was soluble in cold water. The methods of extraction and purification, and the values obtained for the analytical parameters of two specimens of this gum have been given[1].

Analytical methods. — The standard analytical methods have been described[4,5], but paper chromatography was carried out on Whatman No. 1 paper by using the organic phase of the following solvent systems (v/v); (*a*) benzene–butyl alcohol–pyridine–water (1:5:3:3); (*b*) ethyl acetate–pyridine–water (10:4:3); (*c*) acetic acid–ethyl acetate–formic acid–water (3:18:1:4); (*d*) acetic acid–ethyl acetate–formic acid–water (8:18:3:9); (*e*) butyl alcohol–ethanol–water (4:1:5); (*f*) ammonia (sp. gr. 0.880)–butanone–water (1:200:17); (*g*) acetic acid–butanone–water (1:9:1, saturated with boric acid); (*h*) butyl alcohol–ethanol–0.1M hydrochloric acid (1:10:5); (*i*) butyl alcohol–ethanol–0.1N phosphoric acid (1:10:5). Before using the latter two solvents, the paper was dipped in 0.3M sodium dihydrogen phosphate and allowed to dry. G.l.c. (Pye Argon Chromatograph) of mixtures of *O*-methyl sugars was carried out at argon flow-rates of *ca.* 30 ml.min^{-1} on columns of (*i*) 10% by weight of poly(ethylene glycol adipate) on 45–60 mesh Gas-Chrom Z at 175°, and (*ii*) 10% by weight of poly(butane-1,4-diol succinate) on 80–100 mesh Gas-Chrom P at 160°. Retention times (*T*) are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Homogeneity of the gum. — The gum migrated as a single band on (*a*) zone electrophoresis on cellulose acetate film at both pH 8.9 (0.1M ammonium carbonate buffer) and pH 4.7 (0.1M sodium acetate buffer), and (*b*) thin-layer electrophoresis on "Phoroslide" strips at pH 8.9, 4.7, and 9.2 (50mM sodium borate buffer).

The gum was also chromatographed on a column (46 × 1.3 cm) of DEAE cellulose at pH 4.1 (20mM acetate buffer); gradient elution with sodium chloride (0→0.5M) in 20mM acetate buffer gave a single, symmetric peak. A solution (0.5%) of the gum in 0.5M sodium chloride was examined by ultracentrifugation at 44,770 r.p.m.; this showed a single, broad peak.

The neutral and acidic components. — The gum (10 g) was hydrolysed (0.5M sulphuric acid (500 ml) for 7.5 h at 100°. The solution was cooled, neutralised with barium carbonate, deionised with Amberlite IR-120(H^+) resin, concentrated, and fractionated on a column (34 × 3.8 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*), (*c*), (*h*), and (*i*); the presence of three 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 solvent (*c*) indicated the presence of a trace of a galactose and two acidic components, having R_{Gal} 0.21 and 0.61, which were fractionated on Whatman 3MM papers in solvent (*c*).

Fraction 1 (186 mg) had $[\alpha]_D$ +48° (*c* 0.93). The syrup (10 mg) was hydrolysed (M sulphuric acid for 7 h at 100°); paper chromatography of the hydrolysate in solvents (*a*), (*c*), (*h*), and (*i*) revealed 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. Chromatography in solvent (*c*) again revealed only 1 spot (R_{Gal} 0.21). Solvent (*d*) showed two incompletely resolved spots (R_{Gal} 0.70, 0.73), but double development in solvents (*c*) and (*d*) gave no improvement in resolution. Zone electrophoresis in 50mM borate buffer (pH 9.2) also gave only one spot. Paper chromatography in solvent (*a*) for 168 h eventually separated the two components (R_{Gal} 0.14, 0.19) sufficiently for the isolation of each in reasonably pure form. The remainder of fraction 1 was treated on 3MM papers in solvent (a) for 168 h, and the two components were eluted separately and concentrated.

Fraction 1a (33 mg) had $[\alpha]_D$ +107° (*c* 1.09), suggesting the presence of an α-D linkage. Paper chromatography in solvent (*c*) gave a single spot (R_{Gal} 0.21); solvent (*a*) gave a spot (R_{Gal} 0.14) with a trace of fraction 1b (R_{Gal} 0.19). Fraction 1a (7 mg) was hydrolysed (M sulphuric acid, 7 h, 100°), and paper chromatography of the hydrolysate in solvents (*a*), (*c*), and (*h*) revealed equal amounts of a galactose and a galacturonic acid with a trace of a glucurono-6,3-lactone. Sodium borohydride (25 mg in 5 ml of water) was added to fraction 1a (8 mg) and the mixture was left at room temperature for 18 h. Excess borohydride was destroyed by the addition of Amberlite IR-120(H^+) resin. Resin and solvent were removed, and borate was volatilised as methyl borate by repeated additions of methanol followed by concentration to dryness. The product was hydrolysed (M sulphuric acid, 7 h, 100°), and paper chromatography of the hydrolysate in solvents (*a*), (*c*), (*g*), and (*h*) indicated equal amounts of galactitol and a galacturonic acid, and a trace of a galactose. Fraction 1a (10 mg) was methylated by the Kuhn method. G.l.c. of the methanolysis products on column (*i*) indicated the presence of a 2,3,4-tri-*O*-methylgalacturonic acid (*T* 5.25) and a 2,3,6-tri-*O*-methylgalactose (*T* 2.30, 3.10, 3.58). The methanolysate was then reduced with sodium borohydride. After removal of methyl glycosides by mild, acid hydrolysis (0.5M sulphuric acid, 4 h, 100°), the products were examined by paper chromatography in solvents (*e*) and (*f*). This revealed a 2,3,4-tri-*O*-methylgalactose [R_{Glc} 0.74, solvent (*e*); 0.35, solvent (*f*)] and a 2,3,6-tri-*O*-methylgalactose [R_{Glc} 0.74, solvent (*e*); 0.50, solvent (*f*)]. These experiments and the $[\alpha]_D$ value led to the identification of fraction 1a as 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose.

Fraction 1b (48 mg) had $[\alpha]_D$ +8° (*c* 1.06), suggesting the presence of a β-D

linkage, and had the same chromatographic mobility as authentic 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose [R_{Gal} 0.19, 0.21, and 0.72 in solvents (*a*), (*c*), and (*d*), respectively]. Chromatography in solvent (*a*) also revealed a small amount of fraction 1a as impurity. Fraction 1b (10 mg) was hydrolysed (M sulphuric acid, 7 h, 100°), and paper chromatography of the hydrolysate in solvents (*a*), (*c*), and (*h*) revealed a galactose, together with a similar amount of a glucuronic acid and glucurono-6,3-lactone, and a trace of a galacturonic acid. Fraction 1b (10 mg) was reduced with sodium borohydride as described for fraction 1a, and the product was hydrolysed (M sulphuric acid, 7 h, 100°). Paper chromatography of the hydrolysate in solvents (*a*), (*c*), (*g*), and (*h*) indicated the presence of galactitol and a similar amount of a glucuronic acid and glucurono-6,3-lactone. Fraction 1b (15 mg) was methylated by the Kuhn method. G.l.c. of the methanolysis products on column (*i*) indicated the presence of a 2,3,4-tri-*O*-methylglucuronic acid (*T* 2.14, 2.70), a 2,3,4-tri-*O*-methylgalactose (*T* 5.55), and a 2,3,5-tri-*O*-methylgalactose (*T* 3.69, 4.32). The methanolysate was then reduced with sodium borohydride. After removal of methyl glycosides by mild, acid hydrolysis (see above), the products were examined chromatographically in solvents (*e*) and (*f*). This revealed a 2,3,4-tri-*O*-methylgalactose [red spot: R_{Glc} 0.72, solvent (*e*); 0.36, solvent (*f*)] and a red-black spot [R_{Glc} 0.84, solvent (*e*); 0.72, solvent (*f*)], corresponding to a 2,3,4-tri-*O*-methylglucose and 2,3,5-tri-*O*-methylgalactose. Double development in solvent (*f*) separated the 2,3,4-tri-*O*-methylglucose (red spot) from the 2,3,5-tri-*O*-methylgalactose (black spot). These experiments and the $[\alpha]_D$ value led to the identification of fraction 1b as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose.

Fraction 2 (151 mg) had $[\alpha]_D$ +10° (*c* 0.75), suggesting the presence of a β-D linkage, and had the same chromatographic mobility as 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose in solvents (*c*) (R_{Gal} 0.61) and (*d*) (R_{Gal} 0.92). These chromatograms also revealed a trace of a galactose as impurity. Fraction 2 (8 mg) was hydrolysed (M sulphuric acid, 7 h, 100°), and paper chromatography of the hydrolysate in solvents (*a*), (*c*), (*h*), and (*i*) revealed equal amounts of a 4-*O*-methylglucuronic acid and galactose. Reduction of fraction 2 (15 mg) with sodium borohydride, as described for fraction 1a, was followed by hydrolysis of the product (M sulphuric acid, 7 h, 100°). Paper chromatography of the hydrolysate in solvents (*a*), (*c*), (*g*), and (*h*) indicated equal amounts of galactitol and a 4-*O*-methylglucuronic acid, with a trace of a galactose. Fraction 2 (15 mg) was methylated by the Kuhn method. G.l.c. of the methanolysis products indicated the presence of the *O*-methyl sugars found in the methanolysate of fraction 1b. After reduction of the methanolysate followed by removal of methyl glycosides, paper chromatography in solvents (*e*) and (*f*) again showed the presence of the *O*-methyl sugars found in the methanolysate of fraction 1b. These experiments and the $[\alpha]_D$ value led to the identification of fraction 2 as 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

Preparation of degraded gum A. — To determine suitable conditions for the preparation of degraded gum *A*, *L. humilis* gum (1 g) was dissolved in 5mM sulphuric acid (40 ml) and kept at 100° for 120 h. Aliquots (1 ml) were withdrawn at intervals,

neutralised with barium carbonate, filtered, deionised with Amberlite IR-120(H^+) resin, and concentrated. Paper chromatography in solvent (*c*) showed that an arabinose appeared within 1 h and the quantity released increased with time. A galactose first appeared in trace amount after 4 h and then increased with time, but was released in much smaller quantity than the arabinose. Traces of a rhamnose and components having the same chromatographic mobilities as authentic 3-*O*-β-L-arabinofuranosyl-L-arabinose (R_{Gal} 1.20), 3-*O*-β-L-arabinopyranosyl-L-arabinose (R_{Gal} 0.76), and 3-*O*-α-D-galactopyranosyl-L-arabinose (R_{Gal} 0.55) were also found. These components reached a maximum in 36–48 h. Galactose disaccharides and higher oligosaccharides appeared in trace amounts after 36 h, but the amounts of these were still extremely small after 100 h.

The syrups in which arabinose-containing disaccharides were present were further chromatographed in solvents (*a*), (*b*), and (*f*); the disaccharides were chromatographically identical with authentic 3-*O*-α-D-galactopyranosyl-L-arabinose [pink spot with aniline oxalate: R_{Gal} 0.67, solvent (*a*); 0.73, solvent (*b*)], 3-*O*-β-L-arabinofuranosyl-L-arabinose [R_{Gal} 1.40, solvent (*f*)], and 3-*O*-β-L-arabinopyranosyl-L-arabinose [R_{Gal} 0.82, solvent (*a*); 0.91, solvent (*b*); 0.55, solvent (*f*)].

Degraded gum *A* was prepared by heating a solution of *L. humilis* gum (25 g) in 5mM sulphuric acid (1 litre) for 100 h at 100°. After cooling, the brown solution was filtered, dialysed exhaustively against running tap-water, and freeze-dried to give degraded gum *A* as a pale-brown product (18.9 g; 76%).

Examination of degraded gum A. — An attempt to record the optical rotation of degraded gum *A* was unsuccessful because of the brown colour of its solution; the other analytical data obtained are shown in Table I. After hydrolysis of the degraded gum (50 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 characterized in the whole gum. Degraded gum *A* (322 mg) was methylated by the Haworth and Purdie procedures to give a product (219 mg) (Found: OMe, 39.5%). After methanolysis of a portion of this product, g.l.c. of the products gave the results shown in Table II, except for 2-*O*-methylgalactose. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*), indicated the presence of a 2-*O*-methylgalactose in addition to those *O*-methyl sugars already characterized by g.l.c. of their methyl glycosides.

Preparation of degraded gum B. — Degraded gum *A* (12.7 g) was hydrolysed with 0.25M sulphuric acid (1 litre) for 1 h at 100°. After removal of an aliquot (8 ml), the remaining brown solution was dialysed against running tap-water, and degraded gum *B* was isolated by freeze-drying as a pale-brown product (9.9 g, 78%). The aliquot removed 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, and three neutral disaccharides chromatographically identical with authentic 6-*O*-β-D-galactopyranosyl-D-galactose [R_{Gal} 0.30 in solvent (*a*) and 0.38 in solvent (*b*)], 3-*O*-β-D-

galactopyranosyl-D-galactose [R_{Gal} 0.49 in solvent (*a*) and 0.55 in solvent (*b*)] (major components), 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*) (trace component, pink spot with aniline oxalate)], together with higher oligosaccharides.

Examination of degraded gum B. — The optical rotation of degraded gum *B* could not be recorded because of the brown colour of its solution; the other analytical data obtained are shown in Table I. After hydrolysis of the degraded gum (50 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 a galactose, a trace of an arabinose, disaccharides chromatographically identical with authentic 3-*O*-β-D-galactopyranosyl-D-galactose and (rather less) 6-*O*-β-D-galactopyranosyl-D-galactose, and higher oligosaccharides. After hydrolysis of the gum (50 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 the aldobiouronic acids already characterized in the whole gum. Degraded gum *B* (342 mg) was methylated by the Haworth and Purdie procedures to give a product (294 mg) having OMe, 39.9%. After methanolysis of a portion of this product, g.l.c. gave the results shown in Table II, except for 2-*O*-methylgalactose. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*), indicated the presence of a 2-*O*-methylgalactose in addition to those *O*-methyl sugars already characterized by g.l.c. of their methyl glycosides.

Preparation of degraded gum C *by Smith-degradation of degraded gum* A. — Degraded gum *A* (2.04 g) was dissolved in water (50 ml), and 0.75M sodium metaperiodate (50 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 (6 ml). The solution was dialysed against running tap-water for 72 h, sodium borohydride (1 g) was then 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 *C* was isolated as the freeze-dried product (0.514 g, 25%).

Examination of degraded gum C. — The analytical data are shown in Table I. After hydrolysis of degraded gum *C* (50 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, disaccharides chromatographically identical with authentic 3-*O*-β-D-galactopyranosyl-D-galactose (major component) and 6-*O*-β-D-galactopyranosyl-D-galactose (trace), and higher oligosaccharides. Degraded gum *C* (268 mg) was methylated by the Haworth and Purdie procedures to give a product (177 mg) having OMe, 39.1%. After methanolysis of a portion of this product, g.l.c. gave the results shown in Table II, except for 2-*O*-methylgalactose. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*), indicated the presence of a 2-*O*-methylgalactose in addition to those *O*-methyl sugars already characterized by g.l.c. of their methyl glycosides.

TABLE I

DATA FOR *Lannea humilis* GUM AND ITS DEGRADATION PRODUCTS

Polysaccharide	*Yield* (%)	$[\alpha]_D$ (*degrees*)	$\bar{M}_n \times 10^3$	*Constituent sugars* (%)				*Periodate reduced* (*mmoles.g*$^{-1}$)	*Formic acid released* (*mmoles.g*$^{-1}$)
				Gal	*Ara*	*Rha*	*Uronic acid*		
L. humilis gum	80	+43	[a]	75	11	2	12	10.0	4.13 (72 h)
Degraded-gum *A*	76	n.d.[b]	4.32	86	2		12	13.4	6.5 (96 h)
Degraded-gum *B*	78	n.d.	3.74	90	1		9	n.d.	n.d.
Degraded-gum *C*	25	+25	1.95	100				n.d.	n.d.
Polysaccharide I	28	+27	1.96	95	5			3.37	1.20 (48 h)
Polysaccharide II	53	+21	3.29	98	2			2.20	0.89 (48 h)
Polysaccharide III	34	+5	2.93	99	1			2.20	0.72 (48 h)
Polysaccharide IV	36	−19	2.20	100				n.d.	n.d.

[a] $\bar{M}_w = 2.57 \times 10^5$; [b] n.d. = not determined.

Methylation of L. humilis *gum.* — The purified gum (324 mg) was methylated by the Haworth and Purdie procedures to give a product (246 mg), $[\alpha]_D$ 0° (*c* 1.15, chloroform) (Found: OMe, 41.6%). The gum (372 mg) was also methylated[6] by the sodium hydride–methyl iodide–methyl sulphoxide procedure to give a product (319 mg), $[\alpha]_D$ −11° (*c* 0.95, chloroform) (Found: OMe, 40.5%). After methanolysis of a portion of each product, the methyl 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-*O*-methylgalactoses were present in ratios of approximately 8:5:1 in both methanolysates; the ratio of 2,3,4- to 2,3,5-tri-*O*-methylarabinoses was smaller in the product from the Haworth and Purdie methylation procedures. Large amounts of 2,4-di-*O*-methylgalactose were found. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*), indicated the presence in both products of 2-*O*-methylgalactose in addition to the *O*-methyl sugars already identified by g.l.c. of their methyl glycosides. Only a trace of 2-*O*-methylgalactose was found in the product from the sodium hydride methylation procedure; a much larger quantity was found in the product from the Haworth and Purdie procedures.

Preparation, partial hydrolysis with acid, and methylation of Polysaccharide I. — Preliminary, small-scale experiments established that an oxidation time of 72 h with 0.25M sodium metaperiodate was required for *L. humilis* gum.

L. humilis gum (44.4 g) was dissolved in water (1250 ml), 0.5M sodium metaperiodate (1250 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 (26.5 ml), and the solution was dialysed against running tap-water for 48 h. Sodium borohydride (13.3 g) was added, and the mixture was kept at room temperature for 30 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 (30 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 (20 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} 0.30 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 0.55 in solvent (*b*)], and higher, neutral oligosaccharides.

Polysaccharide I (259 and 294 mg, respectively) was methylated by the Haworth and Purdie procedures and by the sodium hydride–methyl iodide–methyl sulphoxide procedure[6] (see Table IV). Methanolysis of a portion of each product, followed by

g.l.c. examination of the mixtures of methyl glycosides, gave the results shown in Table IV, except for 2-*O*-methylgalactose. Hydrolysis of the methyl glycosides, followed by paper chromatography in solvents (*e*) and (*f*) indicated the presence of 2-*O*-methylgalactose (see Table IV).

Preparation of the sequential, Smith-degradation products, Polysaccharides II–IV. — 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 (11.5 g) gave Polysaccharide II (6.08 g); II (5.2 g) gave III (1.75 g); III (1.1 g) gave IV (0.40 g). The percentage yields, specific rotations, number-average molecular weights, sugar compositions, and periodate-oxidation data for Polysaccharides II–IV are given in Table I.

Partial, acid hydrolysis of Polysaccharides II–IV. — After hydrolysis (0.25M sulphuric acid, 1 h, 100°) of II (40 mg), III (42 mg), and IV (34 mg), paper chromatography [solvents (*a*) and (*b*)] indicated the presence in each of a galactose, small amounts of an arabinose (none in Polysaccharide IV), disaccharides having the mobilities of 3-*O*-β-D-galactopyranosyl-D-galactose (major component in each case) and 6-*O*-β-D-galactopyranosyl-D-galactose (minor component in each case, but present as a trace only in Polysaccharide IV), and higher neutral oligosaccharides.

Methylation of Polysaccharides II–IV. — Polysaccharides II, III, and IV 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, except for 2-*O*-methylgalactose. 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-sieve chromatography (m.s.c.) of the whole gum, degraded gums A *and* B, *and Polysaccharides I and II.* — Portions (50 mg) of *L. humilis* gum, degraded gums *A* and *B*, and polysaccharides I and II were treated[7] with Procion Red M-2B dye. *L. humilis* gum and degraded gums *A* and *B* were subjected[8] to m.s.c. on a column (40 × 1.5 cm) of Bio-Gel A-5M agarose; Polysaccharides I and II were treated on a column (35 × 1.5 cm) of Bio-Gel P10 polyacrylamide. For both columns, the eluent was M sodium chloride at a flow-rate of 0.5 ml.min^{-1}. Fig. 1 shows the elution profiles obtained.

DISCUSSION

The purified gum from *Lannea humilis*[1] has a weight-average molecular weight of 2.57×10^5. Zone electrophoresis, thin-layer electrophoresis, ion-exchange chromatography, and ultracentrifugation did not indicate any sharp discontinuity in the properties of the system of molecular species involved. *L. humilis* gum therefore behaves similarly to *Acacia* exudates[9,10], although molecular-sieve chromatography

suggests that it has a broader distribution[11] of molecular weight, skewed in the opposite sense to that found in *Acacia senegal* gum[9].

The gum from *L. humilis*, like the *Acacia* spp. studied to date, contains galactose, arabinose, and rhamnose (the proportion of which was less in the batch purified for this study than was found[1] in an earlier preparation). *Lannea humilis* gum, however, has a more complex uronic acid system involving galacturonic acid in addition to glucuronic acid and its 4-*O*-methyl ether, which both occur in almost all *Acacia* species[12,13]. *L. coromandelica* gum has been reported[14] to contain 6-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-galactose, but a galacturonic acid was the only uronic acid identified in a later study[15], in which the aldobiouronic acid was characterized as 3-*O*-(D-galactopyranosyluronic acid)-D-galactose.

Controlled, acid hydrolysis of *L. humilis* gum gives 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. These disaccharides are found frequently in *Acacia* gum exudates, but only the first named had been detected previously in a *Lannea* gum[16]. The presence of a large proportion of 2,3,4,6-tetra-*O*-methyl-D-galactose in the methanolysate of methylated, degraded gum *A* confirms the report[2] 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 branched side-chains.

Controlled hydrolysis of degraded gum *A* leads to evidence for the presence of 3-*O*-β-D-galactopyranosyl-D-galactose, and 6-*O*-β-D-galactopyranosyl-D-galactose. The values for $\bar{M}_n$ of degraded gums *A* and *B* do not differ greatly; molecular-sieve chromatography (Fig. 1) shows that the major difference between them is the absence in degraded gum *B* of traces of material of high molecular weight having an elution volume in the range 30–60 ml. The drastic difference between the molecular weight of *L. humilis* gum and degraded gum *A* is much greater than can be explained in terms of the removal of arabinose, rhamnose, and some of the uronic acids from peripheral positions in the whole gum. This effect is closely analogous to that already noted[10] for gums from *Acacia* species.

Degraded gum *C* was prepared by Smith-degradation of degraded gum *A* in order to obtain information about the distribution of β-(1→3) and β-(1→6) linkages in the D-galactan core of the gum. The data in Table II show that degraded gum *C* is composed predominantly of β-(1→3) linkages with a few β-(1→6) branches.

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 characterized. There are also the following possible structural fragments: →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-Galp-(1→; →3)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→.

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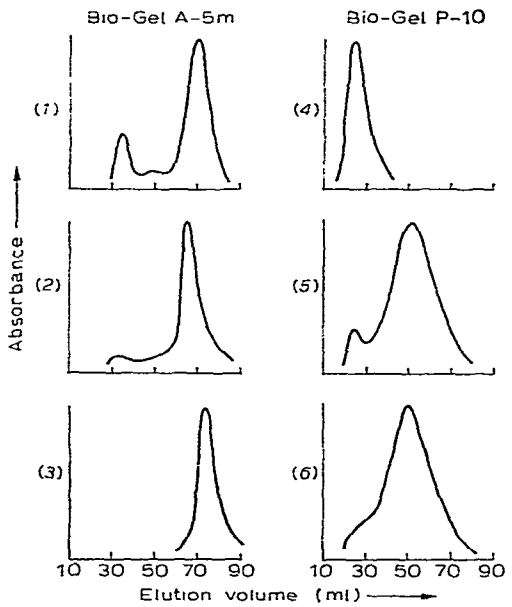

Fig. 1. Elution patterns from molecular-sieve chromatography of *Lannea humilis* gum and its degradation products. (*1*) *L. humilis* gum; (*2*) Degraded gum *A*; (*3*) Degraded gum *B*; (*4*) *L. humilis* gum; (*5*) Polysaccharide I; (*6*) Polysaccharide II.

There is evidence from the studies of the whole gum and its degradation products, summarised in Tables I and II, that rhamnose residues act as non-reducing,

TABLE II

EXAMINATION OF METHANOLYSIS AND HYDROLYSIS PRODUCTS FROM METHYLATED, DEGRADED GUMS *A*, *B*, AND *C*

O-*Methyl sugar identified*[a]	*Approximate, relative molar proportions*		
	A	B	C
2,3,5-Tri-*O*-methyl-L-arabinose	0.1	tr	
2,3,4-Tri-*O*-methyl-L-arabinose	tr	tr	
2,5-Di-*O*-methyl-L-arabinose	tr		
2,3,4,6-Tetra-*O*-methyl-D-galactose	7	6	1
2,3,6-Tri-*O*-methyl-D-galactose	1	0.5	0.1
2,4,6-Tri-*O*-methyl-D-galactose	4	4	9
2,3,4-Tri-*O*-methyl-D-galactose	0.5	0.2	0.1
2,6-Di-*O*-methyl-D-galactose	0.5	0.2	1
2,4-Di-*O*-methyl-D-galactose	5	4	tr
2-*O*-Methyl-D-galactose	1	1	1
2,3,4-Tri-*O*-methyl-D-glucuronic acid[b]	2	1.5	
2,3,4-Tri-*O*-methyl-D-galacturonic acid[b]	1	0.5	

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

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TABLE III

EXAMINATION OF METHANOLYSIS AND HYDROLYSIS PRODUCTS FROM METHYLATED *L. humilis* GUM

Relative retention time (T) *of methyl glycosides*[a]		R_{Glc} *after hydrolysis*		O-*Methyl sugar identified*
Column (i)	*Column (ii)*	*Solvents* (e)	(f)	
0.54	0.49	1.02	1.03	2,3,4-Tri-*O*-methyl-L-rhamnose
0.63, 0.77	0.58, 0.74	0.95	1.03	2,3,5-Tri-*O*-methyl-L-arabinose
1.07	1.25	0.79	0.75	2,3,4-Tri-*O*-methyl-L-arabinose
(1.42), (2.10)	(1.95), (2.35)	0.81	0.80	2,5-Di-*O*-methyl-L-arabinose
1.62	1.78	0.88	0.80	2,3,4,6-Tetra-*O*-methyl-D-galactose
(2.30), (3.10), (3.58)	(3.23), (4.00), (4.41)	0.74	0.48	2,3,6-Tri-*O*-methyl-D-galactose
3.33, 3.72	4.21, 4.55	0.74	0.40	2,4,6-Tri-*O*-methyl-D-galactose
(5.48)	(7.45)	0.74	0.35	2,3,4-Tri-*O*-methyl-D-galactose
7.49	11.36	0.53	0.20	2,6-Di-*O*-methyl-D-galactose
11.68, 13.22	17.80, 19.60	0.53	0.12	2,4-Di-*O*-methyl-D-galactose
		0.37	0.04	2-*O*-Methyl-D-galactose
(2.10), (2.65)	(2.35), (3.21)			2,3,4-Tri-*O*-methyl-D-glucuronic acid[b]
(5.48)	(7.45)			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.

terminal 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*-, 6-*O*-, and 3,6-di-*O*-substituted D-galactose residues; that there are many terminal, non-reducing, D-galactopyranose residues; and that the arabinose-containing side-chains are attached to positions 3 or 6 of certain D-galactose residues.

The sequence of Smith-degradations (see Tables I and IV) indicates that none of the arabinose-containing side-chains contain more than 4 residues. The low yield (28%) and high reduction of periodate by the whole gum indicate that it must contain periodate-vulnerable β-(1→6)-units, although the core of the gum is a D-galactan based mainly on β-(1→3)-linked residues. Partial, acid hydrolysis of each of the Smith-degraded polysaccharides I–IV gave the β-(1→3)-linked D-galactose disaccharide as the major component. The β-(1→6)-linked D-galactose disaccharide is always present, but in steadily decreasing proportion; there is only a trace in polysaccharide IV. Long sequences of β-(1→3)-linked D-galactose residues are therefore unlikely to occur, although the predominance of 2,4,6- over 2,3,4-tri-*O*-methyl-D-galactose in each of methylated Polysaccharides I–IV confirms that most of the D-galactose residues are β-(1→3)-linked. The continuing appearance of periodate-vulnerable β-(1→6) linkages indicates, however, that these must 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.

TABLE IV

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

	Polysaccharides				
	I[a]	*I*[b]	*II*	*III*	*IV*
Methylation data					
Weight of polysaccharide used (mg)	259	294	262	273	234
Weight of product (mg)	202	277	181	240	157
$[\alpha]_D$ of product (degrees)	−0.5	−36	−3.8	−13	−16
OMe of product (%)	40.4	41.9	40.0	40.0	39.0
O-Methyl sugars identified[c]					
2,3,5-Tri-*O*-methyl-L-arabinose	0.5	0.3	0.1	tr	0
2,3,4-Tri-*O*-methyl-L-arabinose	0.1	0.3	0	0	0
2,5-Di-*O*-methyl-L-arabinose	tr[d]	tr	0	0	0
2,3,4,6-Tetra-*O*-methyl-D-galactose	1	1	1	1	1
2,3,6-Tri-*O*-methyl-D-galactose	0.1	tr	0.1	0.2	0.1
2,4,6-Tri-*O*-methyl-D-galactose	5	5	5	7	6
2,3,4-Tri-*O*-methyl-D-galactose	0.1	0.1	tr	tr	tr
2,6-Di-*O*-methyl-D-galactose	1.5	tr	1.5	2	1.5
2,4-Di-*O*-methyl-D-galactose	tr	0.2	tr	tr	tr
2-*O*-Methyl-D-galactose	0.5	tr	0.3	0.5	0.3

[a]Methylated by the Haworth and Purdie procedures. [b]Methylated by the sodium hydride –Me_2SO procedure. [c]See Table III for retention times and R_{Glc} data. [d]tr = trace.

The ratio of 2,4,6-tri- to 2,3,4,6-tetra- and 2,4-di-*O*-methyl-D-galactose, as estimated from peak areas in g.l.c., does not vary significantly in Polysaccharides I–IV, in which a similar degree of branching is probably present. The small decreases in molecular weight of these successive Smith-degradation products suggests that degradations II, III, and IV merely removed peripheral residues and/or the few vulnerable β-(1→6) linkages exposed in the previous stage. The branched galactan core of *L. humilis* gum is produced essentially by the first Smith degradation, in which drastic fragmentation of the whole gum occurs with a correspondingly large reduction of periodate, presumably as a result of the random distribution of a significant number of periodate-vulnerable β-(1→6)-linked D-galactose residues.

From the structural evidence obtained, some suggestions for the structure of the gum from *L. humilis* can be made. It is a highly branched structure, which probably resembles *Acacia arabica* gum in being densely packed and globular in shape[9], a suggestion supported by the low viscosity given[1] by its solutions. The branched galactan framework of the whole gum appears to be built up from the very much smaller branched galactan cores, revealed in degraded gum *C* and the Smith-degrada-

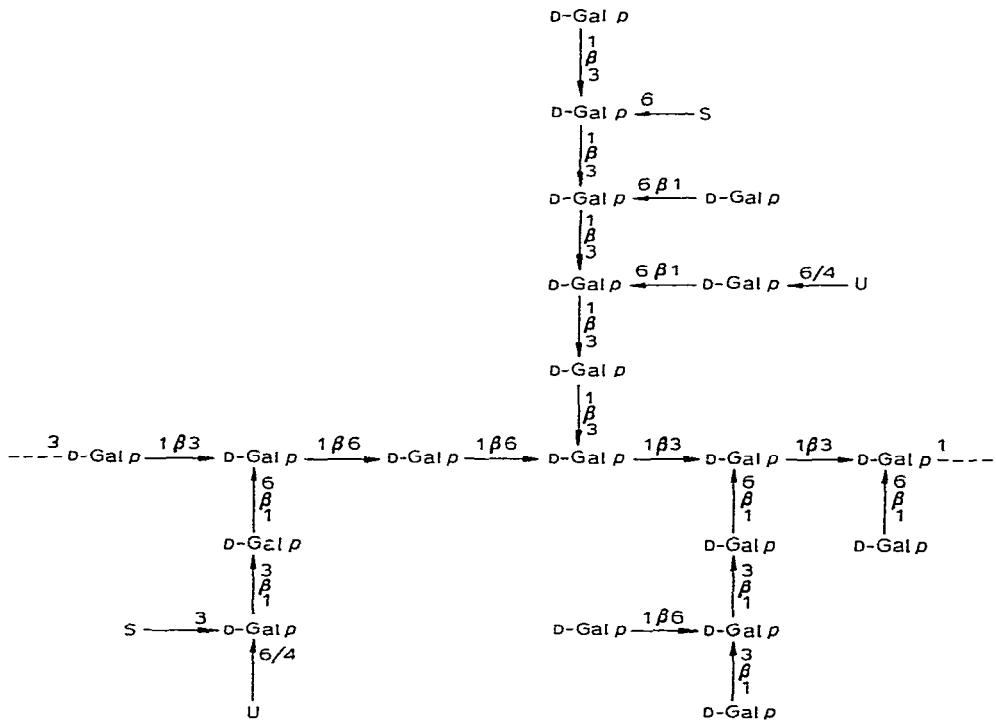

Fig. 2. A possible structural fragment of the branched galactan framework of *Lannea humilis* gum U represents uronic acid residues, which may be D-Gal*p*A>D-Glc*p*A≃4-*O*-Me-D-Glc*p*A. A few D-Glc*p*A residues may be substituted by L-Rha*p*. S represents D-Gal*p*-, L-Ara*p*-, and L-Ara*f*-containing side-chains. These side-chains may be up to 4 units long and contain ···^{3}L-Ara*f*^{1}··· residues. They may be terminated by L-Ara*f*^{1}···, L-Ara*p*^{1}···, or D-Gal*p*$\xrightarrow{1\alpha 3}$L-Ara*f*^{1}··· residues. 6/4 = 6 or 4.

tion Polysaccharides. These cores could be linked by periodate-vulnerable β-(1→6) linkages, with some of the D-galactose residues so involved being further substituted at position 3. Although β-(1→3) linkages are by far the most frequently involved in the galactan, there cannot be any tendency for long β-(1→3)-linked chains to form. The β-(1→3)-linked D-galactose residues must be very frequently substituted at position 6, by single D-galactose residues or by short D-galactose side-chains, to account for the unusually large number of terminal, non-reducing D-galactose residues. The arabinose-containing side-chains are also short, only a few being as much as 4 units long; some of them may be terminated by non-reducing D-galactopyranose, L-arabinopyranose, or L-arabinofuranose residues, and the evidence indicates that these side-chains are also based predominantly on β-(1→3) linkages. The three aldobiouronic acids characterized and the small proportion of rhamnose are present as terminal, non-reducing units; the mode of attachment of the rhamnose has not been established.

Fig. 2 shows a possible structural fragment that illustrates the essential features of this acidic polysaccharide. This fragment is not intended to represent any form of repeating unit; it is only one of many alternative, but broadly similar, structures which would satisfy all of the analytical evidence obtained.

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