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THE STRUCTURE OF *Chorisia speciosa* GUM

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ABSTRACT

The purified, gum exudate from *Chorisia speciosa* (Palo borracho) was studied. It contains, in moles per mole, L-arabinose, ~1; L-rhamnose, 2; D-mannose, 1; D-galactose, 8; and D-glucuronic acid, 3; and a trace of D-xylose. The results from methylation analysis and selective, alkaline degradation, combined with the characterization of the oligosaccharides resulting from partial hydrolysis with acid, made possible the assignment of a tentative "average structure".

INTRODUCTION

Chorisia speciosa St. Hil. is a large tree from the genus *Bombacaceae*, originally from tropical South America[1], having typical, bulging trunks allowing for water storage[2]. When the trunk suffers injury, a gum exudes, apparently to heal wounds. It was of interest to analyze the composition of this gum, due to the relationship of *Bombacaceae* to *Sterculiaceae*[2], as well as for its possible uses.

RESULTS AND DISCUSSION

Composition. — The gum was collected from a single tree of *Chorisia speciosa* St. Hil.[1], and after purification by precipitation with acidified ethanol, the polysaccharide had $[\alpha]_D$ +18°. Gel-permeation chromatography showed the gum to consist of two fractions, of molecular weight 1.05×10^5 (80%) and 40,000 (20%).

Hydrolysis of the gum with trifluoroacetic acid (TFA) for 4 h, and examination of the hydrolyzate by paper chromatography demonstrated the presence of rhamnose, arabinose, galactose, and several oligosaccharides. When the time of hydrolysis was extended to 48 h, mannose and glucuronic acid were also detected. Quantitative analysis of the gum showed rhamnose, arabinose, mannose, galactose, and glucuronic acid in the mole ratios of 1.8:0.9:1.0:7.8:2.8, with a trace of xylose.

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TABLE I

METHYLATION ANALYSIS OF *Chorisia speciosa* GUM, AND DERIVED PRODUCTS

Methylated sugars[a] *(as alditol acetates)*	*Relative retention times*		*Mole %*[b]				
	OV-225	*SP-1000*	*I*[c]	*II*	*III*	*IV*	*V*
	(180°, 4 min; 2°/min, 230°)	*220°*					
2,3,4-Rha	0 47	0.46	10.9	4.3	—	—	—
2,3,4-Ara	0.57	0 59	5.4	6.0	—	—	—
2,3- + 3,4-Rha	0 84	0.80 + 0.76	3.6	—	—	—	—
2,3-Ara	0 95	1.00	2.8	1.2	—	—	—
2,3,4,6-Gal	1 00	1 00	4.8	34.0	4.1	42.6	35.0
3,4,6-Man	1.32	1.44	3.4	—	40 9	—	—
2,4,6-Man	1.33	1.63	—	4.0	—	7.4	22.8
2,4,6-Gal	1.39	1.69	7.8	12 5	1 2	31 5	33.3
2,3,4-Glc (GlcA)	1.49	2 10	5 2	—	8.8	—	—
2,3,4-Gal	1.93	2.20	21.8	28.8	5.2	—	—
4,6-Man	1.93	2.27	5.9	—	2.0	18.5	8.9
2,3-Glc (GlcA)	2.08	2.98	13.6	—	37.8	—	—
2,4-Gal	2 20	3.56	10 0	9.2	—	—	—
2-Gal	2.44	4.00	2.3	—	—	—	—
4-Gal	2 57	4 24	2.3	—	—	—	—

[a]2,3,4,6-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, etc. [b]Values were corrected by use of the effective, carbon-response factors given by Albersheim *et al.*[13]. [c]I, original gum; II, product from β-elimination; III, methylation of **P1**; IV, product **S1** from Smith hydrolysis; and V, product **S2** from Smith hydrolysis.

Methylation analysis. — The gum was methylated by the methods of Hakomori[3] and Purdie[4], and analysis gave the results presented in Table I (column I), from which it may be seen that the principal component corresponds to 6-linked galactose, and that most of the glucuronic acid is 4-linked. The gum clearly has several types of terminal residue. Degradation of the permethylated gum with base[5,6] gave the results shown in Table I (column II). These indicate that much of the rhamnose is linked to O-4 of glucuronic acid; the increase in 2,3,4,6-tetra-*O*-methyl-D-galactose and the replacement of 4,6-di- by 2,4,6-tri-*O*-methyl-D-mannose are consistent with the results obtained on partial hydrolysis of the gum (see later). The small amount of the last-named compound found in the hydrolyzate is presumably due to incomplete degradation with base.

Partial hydrolysis. — Two different experiments were conducted with M TFA. In the first, hydrolysis for 1 h, and separation of the fragments having low molecular weight into neutral and acidic components by ion-exchange chromatography, gave three neutral oligosaccharides, **N1**, **N2**, and **N3**, together with two acidic ones, **A1** and **A2**. From the analytical results summarized in Table II, and obtained as explained

TABLE II

ANALYSIS OF NEUTRAL AND ACIDIC OLIGOSACCHARIDES FROM PARTIAL HYDROLYSIS OF THE GUM

Oligosaccharide[a]	R_{Glc}[b]	$[\alpha]_D$ *(degrees)*	*Sugar analysis*	*Methylation analysis*
A1	0.36	+13	glucuronic acid (1.0) galactose (1.0)	2,3,4-glucose (1.0) 2,3,4-galactose (0.5) 2,3,5-galactose (0.3)
A2	0.15	−30	glucuronic acid (1.0) galactose (2 0)	2,3,4-glucose (1.0) 2,3,4-galactose (1.5) 2,3,5-galactose (0 3)
A3	0.60	−36	glucuronic acid (1 0) mannose (1.0)	3,4,6-mannose (0 9) 2,3,4-glucose (1.0)
A4	0.14	−8	glucuronic acid (1.0) mannose (1.0)	3,4,6-mannose (1.9) 2,3,4-glucose (1.0) 2,3-glucose (0.9)
N1	0.50	+40	galactose	2,3,4,6-galactose (1 0) 2,4,6-galactose (0.9)
N2	0.41	+14	galactose	2,3,4,6-galactose (1.0) 2,3,4-galactose (0 5) 2,3,5-galactose (0 3)
N3	0.22	+22	galactose	2,3,4,6-galactose (1 0) 2,4,6-galactose (1 0) 2,3,4-galactose (0.8)

[a]For the source of the oligosaccharides, see the text. [b]Compared to that of Glc as unity.

in the Experimental section, it was concluded that the structures of these compounds are as follows.

β-Gal-(1→3)-Gal **N1** β-Gal-(1→6)-Gal **N2** β-Gal-(1→3)-β-Gal-(1→6)-Gal **N3**

β-GlcA-(1→6)-β-Gal-(1→6)-Gal **A2** β-GlcA-(1→6)-Gal **A1**

In the second experiment, the gum was hydrolyzed for 1.5 h, the acid was evaporated, the residue was dissolved in water, and the solution was dialyzed against distilled water. It should be noted that the dialyzate did not contain any mannose, nor was this sugar found in any of the oligosaccharides isolated in the previous experiment, suggesting that this component forms part of a resistant core. The non-dialyzable material (**P1**) had $[\alpha]_D$ +20°, and its ^{1}H-n.m.r. spectrum showed signals at δ 5.40 and 4.53 ($J_{1,2}$ 8 Hz) in the ratio of 1 : 1. Analysis of $\mathbf{P_1}$ showed that mannose, galactose, and glucuronic acid were present in the mole ratios of 3.9 : 1.0 : 4.1, and methylation analysis indicated (see Table I, column III) that 3,4,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-glucuronic acid were the main components; this suggested that **P1** is a polymer having alternating mannose and glucuronic acid residues.

When **P1** was further hydrolyzed, an additional aldobiouronic acid (**A3**) was isolated, together with a higher oligomer (**A4**) that was shown to be a dimer of **A3**.

β-GlcA-(1→2)-Man — **A3**

β-GlcA-(1→2)-α-Man-(1→4)-β-GlcA-(1→2)-Man — **A4**

Periodate oxidation. — On oxidation with 0.1M $NaIO_4$ solution, the gum consumed 9.8 mmol of $NaIO_4$ per g, consistent with the methylation results. Mild hydrolysis[7] of the polyol with acid, and precipitation with ethanol, yielded a product (**S1**) which, on methylation, gave the results shown in Table I, column IV. The presence of a large amount of 4,6-di-*O*-methyl-D-mannose was explained by the resistance to hydrolysis of the residue of oxidized glucuronic acid. Accordingly, **S1** was hydrolyzed with 0.1M TFA for 1 h on a steam bath, to yield a product (**S2**) which, on methylation (Table I, column V), showed an increase in the ratio of 2,4,6-tri- to 4,6-di-*O*-methyl-D-mannose. The formation of this compound, together with 2,4.6-tri- and 2,3,4,6-tetra-*O*-methyl-D-galactose, indicates that periodate-resistant galactose residues are attached directly to the backbone, at O-3 of the D-mannosyl residues.

CONCLUSIONS

From the sum of these experiments, certain conclusions may be drawn. The gum has a backbone, composed of glucosyluronic-mannose units, wherein ~65% of the mannosyl residues are branched at O-3. These branches consist of a framework of galactosyl residues that are mainly β-(1→6)-linked, and may also be substituted at O-3.

The main, (terminal) nonreducing group is L-rhamnosyl, most of which is linked to glucuronic acid at O-4.

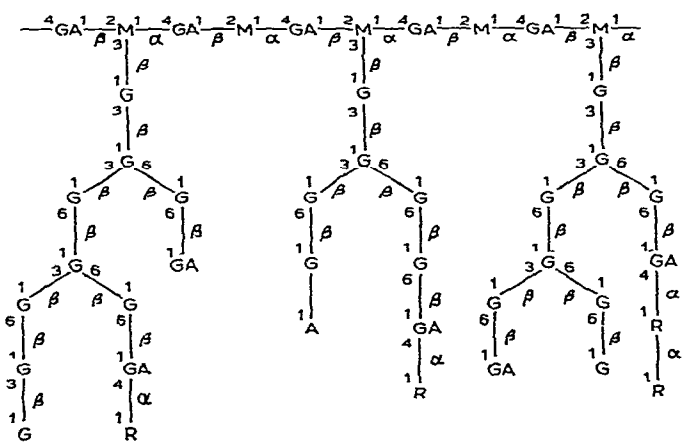

Fig. 1. A possible structure for *Chorisia speciosa* gum. [A = arabinose, G = galactose, GA = glucuronic acid, M = mannose, and R = rhamnose.]

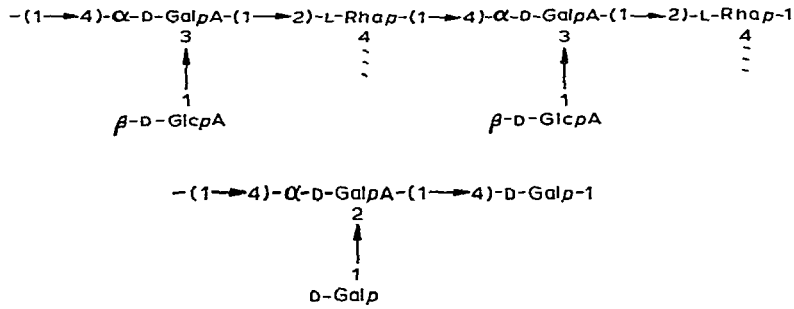

Fig. 2. The two partial structures constitute the major part of the polysaccharide from *Sterculia urens*[14].

Fig. 1 shows an average structure that satisfies the analytical results, but represents only one of the many possibilities that can be conceived. All of them have to contain a backbone of mannosyl and glucosyluronic acid residues, with side chains ending in Rha-(1→4)-GlcA-(1→6)-Gal, arabinose, or galactose.

There are clear differences from the related, *Sterculia* gums[8,9]. Those gums have galactosyluronic acid, rhamnosyl, and galactosyl residues in the central backbone[10], and, in them, glucuronic acid is only a minor, terminal group (see Fig. 2); on the other hand mannose, is absent.

EXPERIMENTAL

General methods. — Optical rotations (1-mL cell) were measured at 20 ± 3° in a Perkin–Elmer model 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer 457 spectrophotometer. Ion-exchange chromatography for separation of neutral and acidic oligosaccharides was performed in a column (2 × 28 cm) of BioRad AG1-X2 (formate) resin (200–400 mesh). The neutral fraction was eluted with water, and the acidic, with 10% formic acid. Paper chromatography (p.c.) was conducted by the descending method, using Whatman No. 1 paper and the following solvent systems (v/v): (*1*) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, (*2*) 8:2:1 ethyl acetate–pyridine–water, (*3*) 2:1:1 1-butanol–acetic acid–water, and (*4*) 4:1:5 1-butanol–ethanol–water (upper phase). Chromatograms were developed with silver nitrate, or with *p*-anisidine hydrochloride in 1-butanol, by heating the papers for 10 min at 110°. Preparative paper-chromatography was conducted by the descending method using Whatman No. 3 MM paper, and solvent *3*. Analytical (qualitative and quantitative) separations by gas–liquid chromatography (g.l.c.) were performed in a Hewlett–Packard 5700 instrument fitted with dual f.i.d., and an Infotronics CRS-100 electronic integrator was used to measure peak areas. Stainless-steel columns (1.8 m × 3 mm) were used with a carrier-gas flow-rate of 20 mL/min. The columns used were: (*A*) 3% of SP-2340 on Supelcoport (100–200 mesh), and (*B*) 5% of ECNSS-M, (*C*) 3% of OV-225, and (*D*) 5% of SP-1000, all on Gas Chrom Q (100–120 mesh). G.l.c.–m.s. was performed with a Micromass 12 instrument, fitted with a Watson–

Biemann separator. Spectra were recorded at 70 eV, with an ionization current of 100 μA and an ion-source temperature of 200°.

Sugar analysis. — Acidic polysaccharides or oligosaccharides were refluxed in methanolic hydrogen chloride (3%) overnight, the acid was neutralized with $PbCO_3$, the suspension filtered, and the filtrate evaporated *in vacuo*. The methyl esters of uronic acids were reduced with sodium borohydride in anhydrous methanol. The excess of $NaBH_4$ was decomposed with Amberlite IR-120 (H^+) resin, and the borate removed by several evaporations with methanol. Oligomers were hydrolyzed with 2M TFA for 3 h, and polymers for 8 h, on a steam bath. The acid was then removed by several evaporations with H_2O, and the sugars liberated by hydrolysis were reduced with $NaBH_4$ in water. Conversion of the alditols into their acetates was achieved with 1 : 1 acetic anhydride–pyridine on a steam bath for 1 h. The alditol acetates were separated and quantified by g.l.c. in column *A* (program: 4 min at 195°; then 2°/min to 260°; 32 min).

Methylation analysis. — Methylations were conducted according to the method of Hakomori[3]. The uronic esters in polymeric material were reduced with $LiAlH_4$ in anhydrous oxolane, and the oligosaccharides, with $Ca(BH_4)_2$ in the same solvent[11]. After hydrolysis with 2M TFA on a steam bath, the methylated sugars liberated were converted into the alditol acetates as already described. The partially methylated, alditol acetates obtained were separated and quantified by g.l.c.: column *B* (4 min at 160°; then 2°/min to 190°; 32 min), *C* (4 min at 180°; then 2°/min to 230°; 32 min), and *D* (isothermal, 220°). The identity of the alditol acetates was confirmed by g l.c.–m.s. Oligosaccharides **A1**, **A2**, and **N2** (having a reducing galactose residue) gave appreciable amounts of 2,3,5-tri-*O*-methylgalactofuranose

Collection of the sample. — The sample of *Chorisia speciosa* gum was obtained from a large tree planted in Montevideo, Uruguay (calle Francisco Simon 2359). Cuts were made into the bark in January 1979, and gum tears were collected by scraping after two weeks.

Purification of the gum. — The gum (5.0 g) was allowed to swell in water (500 mL) for 24 h. It was then heated for 8 h on a steam bath (pH kept at 7–8), centrifuged, and precipitated into ethanol acidified with HCl (pH 2.5; 2.0 L). No sugars were detected in the supernatant liquor. The precipitated gum was dissolved in water, and the solution freeze-dried; yield 3.4 g.

Determination of molecular weight — A sample analyzed by gel-permeation chromatography was found to consist of two fractions; one (80%) had $\overline{M.w.}$ 1.05×10^5 and the other (20%) $\overline{M.w.}$ 40,000. In the experiments reported next, the whole gum was used.

Composition of the gum. — A sample of the gum (30 mg) was heated with 2M trifluoroacetic acid on a steam bath for 4 h. After removal of the acid, examination by p.c. in solvent *1* showed the presence of rhamnose, arabinose, galactose, and several oligosaccharides. After hydrolysis for 48 h, rhamnose, arabinose, mannose, galactose, and glucuronic acid were detected in p.c. (solvent *1*). Conversion of the neutral sugars into alditol acetates, and g l.c. thereof, showed rhamnose, arabinose, mannose,

and galactose in the ratios of 1.9:0.9:1.0:6.7, and a trace of xylose. Total sugar analysis of the gum, as already described, showed rhamnose, arabinose, mannose, galactose, and glucose in the ratios of 1.8:0.9:1.0:7.8:2.8.

Configuration of the sugars. — Preparative g.l c. (column of Silar 10C on Gas Chrom Q, programmed from 210° at 4°/min to 250°), followed by measurement of the c.d. spectra, showed the rhamnitol pentaacetate to be of the L configuration, and both the mannitol hexaacetate and the glucitol (derived from glucuronic acid) hexaacetate to be of the D configuration. Arabinose was assigned the L configuration by measurement of the optical rotation of the free sugar, isolated from the gum hydrolyzate by paper chromatography. Galactose was assigned the D configuration on the basis of measurement of the optical rotations of the oligosaccharides that contained it.

Methylation of the gum. — The gum (150 mg) was methylated by the Hakomori procedure[3], followed by a Purdie treatment[4]. The product (125 mg) showed no hydroxyl absorption in the i.r. spectrum Methylation analysis of this material gave the percentages of sugars shown in Table I, column I.

Base-catalyzed, uronic acid degradation. — Part of the permethylated gum (50 mg) was carefully dried *in vacuo*, dissolved in 19·1 dimethyl sulfoxide–2,2-dimethoxypropane (15 mL) containing *p*-toluenesulfonic acid (1 mg), and stirred under N_2 for 2 h. Sodium methylsulfinylmethanide (2M) in dimethyl sulfoxide (15 mL) was added, and stirring was continued overnight. The mixture was frozen, methyl iodide (10 mL) was added, and the solution was stirred for 1.5 h at room temperature. The excess of methyl iodide was evaporated, and the solution was diluted with water (4 vol.) and extracted with chloroform. Hydrolysis of the product, and methylation analysis of the sugars released, gave the results shown in Table I, column II.

Partial hydrolysis. — *Experiment 1.* The gum (450 mg) was hydrolyzed with M trifluoroacetic acid for 1 h on a steam bath. The acid was removed by evaporation with water, and the products were separated into acidic (220 mg) and neutral (160 mg) compounds on a column of Bio-Rad AGl-X2. Two fractions, **A1** (40 mg), and **A2** (25 mg), were isolated by preparative p.c. of the acidic fraction; the results of their analyses are given in Table II.

The ^{1}H-n m r. spectrum of **A1** showed signals at δ 5.27 ($J_{1,2}$ 2 Hz, 0.4 H), 4.61 ($J_{1,2}$ 8 Hz, 0.6 H), and 4.52 ($J_{1,2}$ 8 Hz, 1 H); that of **A2** showed signals at δ 5.25 ($J_{1,2}$ 2 Hz, 0.4 H), 4.87 ($J_{1,2}$ 7 Hz, 0.6 H), 4.52 ($J_{1,2}$ 7 Hz, 1 H), and 4.42 ($J_{1,2}$ 8 Hz, 1 H). **A1** was identified as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and this confirmed by co-chromatography with an authentic sample. **A2** was identified as 6-*O*-[6-*O*-(β-D-glucopyranosyluronic acid)-(β-D-galactopyranosyl]-D-galactose.

Three neutral oligosaccharides, namely, **N1** (10 mg), **N2** (15 mg), and **N3** (5 mg), were isolated from the neutral fraction by preparative p.c. Analysis of these oligosaccharides as shown in Table II indicated the following structures: **N1**, 3-*O*-β-D-galactopyranosyl-D-galactose; **N2**, 6-*O*-β-D-galactopyranosyl-D-galactose; and **N3**, 6-*O*-(3-O-β-D-galactopyranosyl-β-D-galactopyranosyl)-D-galactose.

Experiment 2. A sample of the gum (250 mg) was treated with M TFA for 1.5 h

TABLE III

^{1}H-N.M R. ANALYSIS OF ACIDIC OLIGOSACCHARIDES FROM PARTIAL HYDROLYSIS OF *Chorisia speciosa* GUM

Oligosaccharide[a]	*δ*[b] *(p.p.m.)*	$J_{1,2}$ *(Hz)*	*Integral proton*	*Assignment*
GlcA$\frac{1\,6}{\beta}$Gal	5.27	2	0 4	$\overset{6}{-}$Gal$\underset{\alpha}{-}$OH
A1	4.61	8	0.6	$\overset{6}{-}$Gal$\underset{\beta}{-}$OH
	4.52	8	1.0	GlcA$\overline{\beta}$
GlcA$\frac{1\,6}{\beta}$Gal$\frac{1\,6}{\beta}$Gal	5.25	2	0.4	$\overset{6}{-}$Gal$\underset{\alpha}{-}$OH
	4.87	7	0 6	$\overset{6}{-}$Gal$\underset{\beta}{-}$OH
A2	4.52	7	1.0	GlcA$\overline{\beta}$
	4.42	8	1.0	$\overset{6}{-}$Gal$\overline{\beta}$
	5.30	s	0.8	$\overset{2}{-}$Man$\underset{\alpha}{-}$OH
GlcA$\frac{1\,2}{\beta}$Man	4 99	s	0.2	$\overset{2}{-}$Man$\underset{\beta}{-}$OH
A3	4.55	8	1.0	GlcA$\overline{\beta}$
	5.40	s	1.0	$\overset{2}{-}$Man$\underset{\alpha}{-}$
	5 29	s	0.8	$\overset{2}{-}$Man$\underset{\alpha}{-}$OH
GlcA$\frac{1\,2}{\beta}$Man$\frac{1\,4}{\alpha}$GlcA$\frac{1\,2}{\beta}$Man	4.98	s	0.2	$\overset{2}{-}$Man$\underset{\beta}{-}$OH
A4	4.53	8	1.0	GlcA$\overline{\beta}$
	4.49	8	1.0	$\overset{4}{-}$GlcA$\overline{\beta}$

[a]For the source of **A1**, **A2**, **A3**, and **A4**, see text. [b]Derived shift, relative to internal acetone at *δ* 2.23 downfield from sodium 4,4-dimethyl-4-silapentane-1-sulfonate.

on a steam bath. The acid was removed, the residue was dissolved in water (10 mL), and the solution was dialyzed for 72 h against distilled water (1.0 L). The non-dialyzable material (50 mg) had $[\alpha]_D$ +20° (*c* 1.7, H_2O), and its ^{1}H-n.m.r. spectrum in D_2O showed signals at δ 5.40 and 4.53 ($J_{1,2}$ 8 Hz) in the ratio of 1:1. Sugar analysis showed mannose, galactose, and glucose in the ratios of 3.9:1.0:4.1. Methylation analysis gave the results shown in Table I, column III. On further hydrolysis of this nondialyzable material (20 mg), with 2M TFA for 7 h on a steam bath, p.c. (solvent *1*) showed galactose, mannose, glucuronic acid, and aldobiouronic acid **A3** (R_{Glc} 0.6), the aldobiouronic acid **A1**, and higher oligomers. Compound **A3** (6 mg) was isolated by paper chromatography, and analyzed as shown in Table II. The ^{1}H-n.m.r. spectrum of **A3** showed signals at δ 5.30 (s, 0.8 H), 4.99 (s, 0.2 H), and 4.55 ($J_{1,2}$ 8 Hz, 1 H), and was thus identified as 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose.

Compound **A4** (5 mg) was also isolated from the hydrolyzate by paper chromatography, and analyzed as shown in Table III. The ^{1}H-n.m.r. spectrum of **A4** showed signals at δ 5.40 (s, 1 H), 5.29 (s, 0.8 H), 4.98 (s, 0.2 H), 4.53 ($J_{1,2}$ 8 Hz, 1 H), and 4.49 ($J_{1,2}$ 8 Hz, 1 H). **A4** was hydrolyzed with 2M TFA for 4 h, and paper chromatography of the hydrolyzate showed the aldobiouronic acid **A3**, mannose, and glucuronic acid. Compound **A4** was identified as a dimer of **A3**.

Periodate oxidation. — A solution of the gum (200 mg) in water (100 mL) was treated with 0.1M $NaIO_4$ (100 mL) for 96 h at 4° in the dark, the periodate consumption being monitored[12] by the Fleury–Lange method[7]. The final consumption of periodate was 9.8 mmol/g of the gum. Ethylene glycol (10 mL) was added, the polysaccharide was dialyzed overnight, and then reduced with $NaBH_4$ (1.0 g), and the solution made neutral with 50% acetic acid, dialyzed, and freeze-dried, to yield the polyalcohol (120 mg). This product (5 mg) was hydrolyzed with 2M TFA overnight on a steam bath, and the sugars present in the hydrolyzate (detected by paper chromatography in solvent *1*) were found to be galactose and mannose. Quantitation of the sugars (as their alditol acetates) by g.l.c. showed galactitol and mannitol hexaacetates in the ratio of 2.8:1.0. The polyalcohol (100 mg) was hydrolyzed[7] with 0.75M TFA during 20 h at room temperature, the acid removed by evaporation, and the residue dissolved in water (3 mL); precipitation with ethanol gave precipitate **S1** (25 mg). Methylation analysis of part (5 mg) of this material gave the results shown in Table I, column IV.

The rest of the precipitate (20 mg) was heated with 0.1M TFA for 1 h on a steam bath, the acid was evaporated, and the residue (**S2**) was methylated by the Hakomori procedure; sequential hydrolysis, reduction, and acetylation gave the results shown in Table I, column V.

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