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STRUCTURAL INVESTIGATIONS ON BAEL EXUDATE GUM

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ABSTRACT

Purified, exudate gum from the bael (*Aegle marmelos*) tree contains D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid in the molar ratios of ~9:3:1:3. Degraded gum was prepared by autohydrolysis in its aqueous solution at 100°. Methylation analyses were conducted on the degraded gum, the whole gum, and the carboxyl-reduced, whole gum, and the results were corroborated by those from periodate oxidation followed by Smith degradation. The anomeric configurations of the different sugar residues in the whole-gum polysaccharide were determined by chromium trioxide oxidation of the acetylated polysaccharide. Finally, the oligosaccharides obtained by autohydrolysis of the whole gum, and by graded hydrolysis of the degraded gum, were characterized. From these results, a tentative structure was assigned to the average repeating-unit in the polysaccharide.

INTRODUCTION

The structure of the gummy material engulfing the seeds of bael (*Aegle marmelos*) fruit has been reported[1–3]. When injuries are made on its trunk, the tree also exudes gum, to heal up the wounds. It was therefore of interest to study the structure of this exudate gum, to ascertain whether all of the structures are of similar type; this is important, as an insufficient number of exudate gums has been studied in sufficient detail to permit assessment of their potential in the chemical taxonomy of plants[4]. Only mature bael trees have well developed, gum cavities in their fruits, and these trees exude plenty of gum from injured trunks.

RESULTS AND DISCUSSION

The exudate gum from bael trees was isolated by repeated precipitation of an aqueous solution of the gum with ethanol. The polysaccharide finally precipitated was almost white, and had $[\alpha]_{589.6}^{23}$ −22°. On hydrolysis with M sulfuric acid, it yielded galactose (54.26), arabinose (6.1), rhamnose (18.83), and glucuronic acid (20.8%). The gum polysaccharide was purified by passing it through a column (95 × 1.5 cm) of Sephadex G-100; most of it (88%) emerged from the column as a single peak, and the

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TABLE I

SURVIVAL OF SUGARS[a] IN THE OXIDATION OF ACETYLATED, CARBOXYL-REDUCED, WHOLE GUM WITH CHROMIUM TRIOXIDE

Time (h)	myo-*Inositol*	*Galactose*	*Glucose*	*Rhamnose*	*Arabinose*
0	10	7.20	2.40	2.25	0.88
0.5	10	1.56	0.82	1.87	0.28
1.5	10	0.89	0.27	0.87	0.17

[a]The sugars were analyzed and estimated by g.l.c., using column *a* at 190°.

effluent was lyophilized. On hydrolysis with M sulfuric acid, the lyophilizate, $[\alpha]_{589.6}^{23}$ −23°, gave the same sugars in the same proportions. The homogeneity of the polysaccharide was further confirmed by (*a*) electrophoresis in borate buffer, when a single spot was obtained, and (*b*) ultracentrifugal analysis using Schlieren optics, when a single peak was obtained. The carboxyl groups in the gum polysaccharide were reduced with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate[5] and sodium borohydride. On hydrolysis, the product yielded galactose, rhamnose, arabinose, and glucose (confirming the presence of glucuronic acid in the polysaccharide). From the specific rotations of the sugars isolated, the configurations of these sugar residues were determined to be D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid.

In order to ascertain the anomeric configurations of the different sugar residues, the acetylated derivative of the carboxyl-reduced gum was subjected to oxidation with chromium trioxide[6,7] in acetic acid for different time-intervals at 50°, using *myo*-inositol as the internal standard. After deacetylation with sodium methoxide, the products were hydrolyzed, and the alditol acetates of the different sugars were estimated by g.l.c. The results are shown in Table I. From these results, it appears that galactose, glucose (formed from glucuronic acid), and arabinose were oxidized, and consequently decreased rapidly in their percentage, but that the rhamnose was oxidized comparatively slowly, thereby showing that only the rhamnose had the α configuration, the rest having the β configuration. However, the rate at which the L-arabinose was autohydrolyzed showed that it possibly had the α configuration, and as acetylated furanoses are nonspecifically oxidized by chromium trioxide[6], we cannot as yet specify its anomeric configuration.

Aided by the results of a pilot experiment, degraded gum was prepared by autohydrolysis of an aqueous solution of the whole gum for 25 h on a boiling-water bath, and the loosely bound sugar residues (mainly those of arabinose and rhamnose) were preferentially removed. The degraded gum was obtained by precipitating the product with four volumes of ethanol; it had $[\alpha]_{589.6}^{23}$ −9°. The carboxyl groups of the degraded gum were reduced as for the whole polysaccharide, and upon hydrolysis of the product, and g.l.c. analysis of the alditol acetates, the hydrolyzate showed galactose (75.4), glucose (16), rhamnose (4.2), and arabinose (4.1%).

In the mother liquor from the foregoing precipitation, a few mono- and oligo-

saccharides were detected by paper chromatography. The monosaccharides included arabinose, rhamnose, and a very minute proportion of galactose. The oligosaccharides were separated (and isolated) by preparative paper-chromatography into three pure fractions. Fraction 1, $[\alpha]_{589.6}^{23}$ −12.5°, contained only rhamnose, and, on methylation by the Kuhn method[8,8a] and subsequent hydrolysis of the product, 2,3,4-tri-*O*-methylrhamnose and 3,4-di-*O*-methylrhamnose were obtained in almost equal amounts, proving that fraction 1 was 2-*O*-rhamnopyranosylrhamnose. As the configuration of the rhamnose had been found to be L, and its anomeric configuration to be α, the disaccharide is 2-*O*-α-L-rhamnopyranosyl-L-rhamnose. Fraction 2, $[\alpha]_{589.6}^{23}$ −17°, contained arabinose and rhamnose in the ratio of 1:2. Reduction of the oligosaccharide with sodium borohydride, and subsequent hydrolysis, showed that the arabinose was at the reducing end of the oligosaccharide. The compound was methylated by the Kuhn method[8,8a], and the product hydrolyzed: 2,3,4-tri- and 3,4-di-*O*-methylrhamnose, and 2,3-di-*O*-methylarabinose were obtained in almost equal proportions. The oligosaccharide therefore appears to be a trisaccharide having the structure *O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→5)-L-arabinose. On hydrolysis, fraction 3, $[\alpha]_{589.6}^{23}$ −10.5°, obtained in small amounts, yielded rhamnose, galactose, and glucuronic acid. On reduction with sodium borohydride, and subsequent hydrolysis, the rhamnose was found to have been reduced, indicating that this residue had been at the reducing end.

The degraded gum, the whole gum, and the carboxyl-reduced, whole gum were first permethylated by the Hakomori method[9], but, as the products still showed some OH absorption bands in their i.r. spectra, they were further methylated by the Purdie method[10] until the products showed no OH absorption bands in their i.r. spectra. The permethylated, degraded gum, the whole gum, and the carboxyl-reduced, whole

TABLE II

METHYL ETHERS OF SUGARS FROM THE HYDROLYZATES OF METHYLATED, WHOLE GUM (A), METHYLATED, CARBOXYL-REDUCED, WHOLE GUM (B), AND METHYLATED, DEGRADED GUM (C)

Sugars[a]	*T*[b]		*Approximate mol-%*			*Mode of linkage*
	a	b	*A*	*B*	*C*	
2,3,4-Rha	0.47	0.35	6	4	4	Rha*p*-(1→
2,3,5-Ara	0.49	0.41	4	4	trace	Ara*f*-(1→
3,5-Ara	0.90	0.79	1	1	4	→2)-Ara*f*-(1→
3,4-Rha	0.91	0.86	18	17	trace	→2)-Rha*p*-(1→
2,3,4,6-Glc	**1.00**	**1.00**	—	18	—	Glc*p*A-(1→
2,3-Ara	1.09	1.06	2	1	—	→4)-Ara*f*-(1→
2,3,4,6-Gal	1.24	1.19	15	10	22	Gal*p*-(1→
2,4,6-Gal	2.25	2.03	18	15	31	→3)-Gal*p*-(1→
2,4-Gal	6.34	5.1	35	29	37	→3,6)-Gal*p*-(1→

[a]2,3,4-Rha = 2,3,4-tri-*O*-methyl-L-rhamnose, 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% ECNSS-M column at 155°, and (*b*) a 5% OV-225 column at 170°.

gum respectively had $[\alpha]_{589.6}^{23}$ −6°, −8.2°, and −5.7°; they were hydrolyzed, first with 85% formic acid for 2 h at 100°, and, after removal of the formic acid, with 0.5M sulfuric acid for 18 h at 100°. The acid was neutralized with barium carbonate, and the solutions were evaporated to dryness. The alditol acetates of the partially methylated sugars in each hydrolyzate were prepared, and analyzed by g.l.c. using columns (*b*) and (*c*). The results are shown in Table II.

From these results, some of the structure of the bael gum polysaccharide may be developed. The only glucose derivative that could be characterized was 2,3,4,6-tetra-*O*-methylglucose, and this was detected only in the carboxyl-reduced, whole gum, showing that glucuronic acid is present only as nonreducing end-groups in the polysaccharide. All of the other sugars, *viz.*, galactose, rhamnose, and arabinose, are present both in the interior, and in the outer parts of the molecule as nonreducing end-groups. Characterization of 2,3,5-tri- and 2,3- and 3,5-di-*O*-methylarabinose in the hydrolyzates of methylated whole gum and carboxyl-reduced, whole gum showed that the L-arabinose is in the furanose form, and that the residues present in the interior part of the whole gum have both (1→2)- and (1→5)-linkages. Interestingly, 2,3-di-*O*-methylarabinose was absent from the hydrolyzate of the methylated, degraded gum, showing that the degraded gum contains only (1→2)-linked arabinose. Similarly, L-rhamnose is in the pyranose form, and those residues present in the chain have (1→2)-linkages, as 2,3,4-tri- and 3,4-di-*O*-methylrhamnose were obtained. Traces of 2,3,5-tri-*O*-methylarabinose and 3,4-di-*O*-methylrhamnose were found in the hydrolyzate of the methylated, degraded gum. Characterization of large proportions of 2,4-di-*O*-methylgalactose (and of tetra-*O*-methylhexose and tri-*O*-methylpentose) in all three of the fractions indicated that the molecule is highly branched, and that the branch points originate only at O-1, O-3, and O-6 of D-galactosyl residues. The rest of the galactosyl residues in the chain are (1→3)-linked. A point still not properly explained is that the sum of the nonreducing ends [*viz.*, tetra-*O*-methyl-galactose and -glucose (carboxyl-reduced gum) and tri-*O*-methyl-arabinose and -rhamnose] exceeds the branch-points, *viz.*, the di-*O*-methylgalactose. The methylated, degraded gum gave only a trace of 2,3,5-tri-*O*-methylarabinose, showing that most of the terminal, arabinofuranosyl groups were removed during autohydrolysis of the whole gum. As a result, the proportion of 2,4,6-tri-*O*-methylgalactose is also higher in the degraded gum. The unmethylated, degraded gum contains a relatively smaller proportion of glucuronic acid, showing that some of these residues, also, were eliminated during preparation of the degraded gum.

Both the whole gum and the degraded gum were oxidized with sodium meta-periodate. The consumption of periodate, 0.80 and 0.70 mol, respectively, per mol of hexosyl residue, was as expected from such linkages, but Smith degradation[11] gave more-positive results. The Smith-degradation products of both the whole gum and the degraded gum showed the presence of galactose and glycerol, with a trace of arabinose. The Smith-degradation products of whole gum and degraded gum were both subjected to a second Smith degradation, and the products, which could be precipitated with ethanol, showed the presence of galactose, only. These results

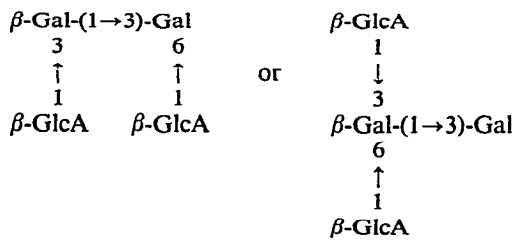
suggest that there is, possibly, a periodate-immune backbone-chain of galactopyranosyl residues to which various branches are attached (as branch points contain only 1,3,6-linked galactopyranosyl residues).

The degraded gum was subjected to graded hydrolysis with 42.5% formic acid for 4 h at 100°, the optimal conditions being found with the aid of pilot experiments. A paper chromatogram of the hydrolyzate showed spots for monosaccharides, spots in the region of oligosaccharides, and one at the origin. The oligosaccharides were resolved, and isolated, by preparative paper-chromatography. After repeated chromatography on paper, one neutral and four acidic oligosaccharides were obtained.

Fraction 1, $[\alpha]_{589.6}^{23}$ +11.5°, equivalent weight 348, on hydrolysis and paper-chromatographic examination, showed spots of galactose and glucuronic acid, whereas, on reduction ($NaBH_4$) and hydrolysis, it showed only glucuronic acid. On methylation by the Kuhn method[8], hydrolysis, and analysis of the product by g.l.c. using column (*c*), a peak for 2,4,6-tri-*O*-methylgalactose, only, was obtained. All these observations indicate that fraction 1 is 3-*O*-*β*-(D-glucopyranosyluronic acid)-D-galactose.

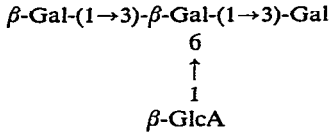
Fraction 2, $[\alpha]_{589.6}^{23}$ +6.6°, equivalent weight 506, showed spots of galactose and glucuronic acid on paper chromatograms of its hydrolyzate and on that of its reduction product, obtained with sodium borohydride. The oligosaccharide was methylated, and the product hydrolyzed, and the alditol acetate prepared from the hydrolyzate was analyzed by g.l.c. using column (*c*); a peak of 2,4,6-tri-*O*-methylgalactose, only, was obtained, showing glucuronic acid to be at the nonreducing end. Hence, fraction 2 is 3-*O*-*β*-(D-glucopyranosyluronic acid)-3-*O*-*β*-D-galactopyranosyl-D-galactose.

Fraction 3, $[\alpha]_{589.6}^{23}$ −9°, equivalent weight 335, on hydrolysis and paper-chromatographic examination, showed spots of galactose and glucuronic acid. On reduction ($NaBH_4$) and hydrolysis, it showed spots of galactose and glucuronic acid. On methylation, hydrolysis, and g.l.c. analysis of the product as before, it showed peaks of 2,4,6-tri-*O*-methylgalactose and 2,4-di-*O*-methylgalactose in equal amounts. As the oligomer contained two neutral sugar residues, and as it had an equivalent weight of 335, the compound is likely to be a tetramer containing two glucuronic acid residues. No further work was performed on it, but one of two possible structures (A or B) may be assigned to it.



A B

Fraction 4, $[\alpha]_{589.6}^{23}$ −6.3°, equivalent weight 670, on hydrolysis and paper-chromatographic analysis, showed spots of galactose and glucuronic acid. Reduction ($NaBH_4$) and hydrolysis gave material that showed the same spots. After methylation and hydrolysis, g.l.c. analysis of the alditol acetates of the product showed the presence of 2,3,4,6-tetra-, 2,4,6-tri- and 2,4-di-*O*-methylgalactose. From these data, structure C may be assigned to this oligomer.



C

Fraction 5, $[\alpha]_{589.6}^{23}$ +58°, was the only neutral oligosaccharide isolated in appreciable amount and, on hydrolysis, it yielded galactose only. Upon methylation and hydrolysis, and analysis of the alditol acetates of the hydrolyzate by g.l.c., peaks of 2,3,4,6-tetra and 2,4,6-tri-*O*-methylgalactose in almost equal amounts were found, indicating that the oligosaccharide is 3-*O*-β-D-galactopyranosyl-D-galactose.

From consideration of the results of analysis of the sugars of the whole gum, the degraded gum, and the carboxyl-reduced, whole gum, their methylation analyses, Smith degradation, and graded hydrolysis, the general structure shown in **1** may be assigned to the average repeating-unit of the degraded-gum polysaccharide. This structure merely represents the general nature of the linkages of the different monosaccharide units, but it well explains the different fragmentation products obtained, although it does not necessarily depict the true sequence of the branches present in the polysaccharide.

From the structure (**1**) of the degraded gum, coupled with (*a*) the structure of the oligosaccharides obtained during the preparation of the degraded gum and (*b*) the other analytical results mentioned, the general structure **2** may be assigned to the average repeating-unit of the whole gum. In this case, also, the structure well explains the linkages of different monosaccharide units, and also the various oligosaccharides characterized, but it does not necessarily depict the correct sequence of branches, because the data thus far acquired are insufficient for this purpose.

This structure (**2**) of the exudate gum of bael tree bears some similarities to that of the gum surrounding the seeds of bael fruit[1-3]. The sugar components*, the backbone chain, and the presence of all of the glucuronic acid in the nonreducing ends, are similar in both gums. There are, however, some differences in the linkages in the branches.

*The authors of references 1–3 greatly regret their wrong reporting of the presence of galacturonic acid in the gum of bael fruit. A reinvestigation thereof revealed that its sugar components are actually galactose, rhamnose, arabinose, and glucuronic acid; the neutral methyl sugars reported therein are in order.

→3)-β-Gal-(1→3)-Gal-β-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→
6 6 6 6
↑ ↑ ↑ ↑
1 1 1 1
→6)-β-Gal β-Gal Ara β-Gal β-Gal
3 3 2 3 3
↑ ↑ ↑ ↑ ↑
β-GlcA-(1→ 1 1 1 1 1
→6)-β-Gal β-Gal α-Rha β-GlcA β-GlcA-(1→6)-β-Gal
3 3
↑ ↑
1 1
β-GlcA β-Gal
1

→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→3)-β-Gal-(1→
6 6 6 6 6 6 6
↑ ↑ ↑ ↑ ↑ ↑ ↑
1 1 1 1 1 1 1
→6)-β-Gal α-Rha β-Gal Ara β-Gal Ara α-Rha β-Gal
3 2 3 2 (or 5) 3 2 3
↑ ↑ ↑ ↑ ↑ ↑ ↑
β-GlcA-(1→ 1 1 1 1 1 1 1
→6)-β-Gal α-Rha β-Gal α-Rha β-GlcA α-Rha β-GlcA-(1→6)-β-Gal
3 2 2 2 3
↑ ↑ ↑ ↑ ↑
1 1 1 1 1
β-GlcA β-Gal α-Rha β-Gal β-Gal
3 3 3
↑ ↑ ↑
1 1 1
β-GlcA β-GlcA β-GlcA
2

EXPERIMENTAL

General methods. — All specific rotations are equilibrium values, and were measured with a Perkin–Elmer Model 241 MC spectropolarimeter at 23 ±1° and 589.6 nm. The homogeneity of the polysaccharide was determined by using the Schlieren optics of a Beckman L-5 65 ultracentrifuge for a 1% solution of the polysaccharide in phosphate buffer, and also by high-voltage electrophoresis on Whatman No. 1 filter paper, using borate buffer (pH 9.4), with a Shandon High Voltage Electrophoresis apparatus Model L-24. Infrared spectra were recorded with a Beckman IR-20A instrument having cesium bromide optics, and ultraviolet and visible spectra were respectively recorded with a Carl Zeiss VSU 2-P and a Hilger spectrophotometer. Paper partition-chromatography was performed on Whatman No. 1 and 3MM papers (for preparative purposes), with the following solvent systems (v/v): *A*, 8:2:1 ethyl acetate–pyridine–water; *B*, 5:5:1:3 ethyl acetate–pyridine–acetic acid–water; *C*, 9:2:2 ethyl acetate–acetic acid–water; and *D*, the upper layer of 4:1:5 1-butanol–acetic acid–water. The sugars were detected with (*1*) alkaline silver nitrate, (*2*) aniline hydrogenoxalate, and (*3*) benzidine periodate. For gas–liquid chromatography, a Hewlett–Packard 5730A gas chromatograph with flame-ionization detector was used. Resolutions were performed on glass columns (1.83 m × 6 mm) containing *a*, 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 190° (for alditol acetates of sugars) and at 155° (for alditol acetates of partially methylated sugars); *b*, 5% of OV-225 on Sil Rub (80–100 mesh) at 170°; and *c*, 1% of OV-225 on Gas Chrom Q (80–100 mesh) at 170° (for alditol acetates of partially methylated sugars). Mole proportions of the alditol acetates were determined by cutting out their peak areas and weighing them. All evaporations were performed under diminished pressure at bath temperatures below 40°. Small volumes of aqueous solutions were lyophilized. The alditol acetates of sugars were prepared[10] as follows. To a solution of the sugar(s) (~5 mg) in water (10 mL) was added sodium borohydride (~30 mg), and the solution was kept for 5 h at room temperature, and then decationized with Dowex 50W X-8 (H^+) ion-exchange resin to pH 4, the suspension filtered, and the filtrate evaporated to dryness. Boric acid was removed by repeated evaporation with methanol (5-mL portions). The resulting alditol was dried, and then acetylated with acetic anhydride (2 mL) in pyridine (2 mL) by heating on a boiling-water bath for 1 h. The excess of the reagents was removed by codistillation with toluene, and then the product was dried. Chloroform solutions of the alditol acetates were injected into the g.l.c. apparatus.

Isolation of the exudate, bael-gum polysaccharide. — Crude, exudate gum (5.1 g) was obtained from a large bael tree seven days after injuries had been made on different parts of its trunk, and the gum was dispersed in distilled water. The particles of bark were removed by filtration through Nylon cloth, and the filtrate was centrifuged for 30 min at 14,000 r.p.m. To the cold, clear liquid was added cold ethanol (3 vol., acidified with acetic acid to pH 4.5), and a brownish precipitate separated out. This was centrifuged off, washed three times with ethanol, and dried. The poly-

saccharide was dissolved in water (300 mL), re-precipitated with ethanol (900 mL), and the precipitate collected by centrifugation. The process of dissolution in water and precipitation with ethanol was repeated five times, until a fairly white precipitate was obtained. It was dried over phosphorus pentaoxide; yield 3.75 g, $[\alpha]_D^{23}$ −22° (*c* 1, water).

Purification of the gum polysaccharide. — The polysaccharide (50 mg) was purified by passing an aqueous solution of it through a column (95 × 1.5 cm) of Sephadex G-100. The column was eluted with water, 5-mL fractions being collected. The carbohydrate content in each fraction was determined by the phenol–sulfuric acid method; the elution pattern showed a single peak. The recovery (44 mg) in this peak region was 88%; $[\alpha]_D^{23}$ −23° (*c* 1, water).

Hydrolysis of the polysaccharide. — The gum polysaccharide (10 mg) was hydrolyzed with M sulfuric acid on a boiling-water bath for 20 h. The solution was cooled, made neutral ($BaCO_3$), and the suspension centrifuged. Part of the centrifugate was analyzed on paper, using solvents *A* and *B*, and spots for galactose, rhamnose, arabinose, glucuronic acid, and an aldobiouronic acid (faint) were detected. The other part was converted into the alditol acetates, and analyzed by g.l.c. using column *a*. The chromatogram showed peaks for galactose, rhamnose, and arabinose.

*Preparation of carboxyl-reduced gum with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CMC)*[5]. — CMC (500 mg) was added to a stirred solution of the polysaccharide (20 mg) in water (20 mL), and the pH of the solution was kept at 4.75 by dropwise addition of 0.01M hydrochloric acid. After 2 h, 2M aqueous sodium borohydride (35 mL) was added dropwise during 45 min, and the pH was kept at ~7 by concurrent addition of 4M hydrochloric acid. After being stirred for 1 h, the solution was dialyzed against distilled water for 24 h, and then lyophilized. The whole process was repeated once.

Sugar analysis. — The carboxyl-reduced gum (8.12 mg) was mixed with *myo*-inositol (3.55 mg, used as the internal standard), and hydrolyzed with M sulfuric acid as already described. The alditol acetates were analyzed by g.l.c., using column *a*, and were found to contain galactose (54), rhamnose (18), glucose (20), and arabinose (6%). The presence of glucose in this hydrolyzate confirmed that the uronic acid was glucuronic acid (glucose was absent from the hydrolyzate of the whole gum). The uronic acid in the whole gum was also estimated; the carbazole–sulfuric acid method[12] was used, with D-glucuronic acid as the standard, and the content was found to be 21.2%.

Nature of the sugars. — A batch (160 mg) of carboxyl-reduced gum was prepared, and hydrolyzed as described earlier. The sugars were resolved, and isolated, by preparative paper-chromatography using solvent *A*. The specific rotations of these sugars were: galactose, +77° (lit.[13a] +79° for D-galactose); glucose, +54° (lit.[13b] +52.5° for D-glucose); rhamnose, +7.5° (lit.[13c] +9.18° for L-rhamnose); and arabinose, +98° (lit.[13d] +105° for L-arabinose).

Oxidation of the carboxyl-reduced, whole gum with chromium trioxide[6,7]. — A mixture of carboxyl-reduced, whole gum (3.8 mg) and *myo*-inositol (2.6 mg) was

dissolved in formamide (0.5 mL), acetic anhydride (1 mL) and pyridine (1.5 mL) were added, and the mixture was stirred overnight at room temperature. The mixture was evaporated under diminished pressure, and the residue partitioned between chloroform and water. The chloroform layer was washed with water, dried (anhydrous sodium sulfate), and evaporated to dryness. The residue was dissolved in glacial acetic acid (4 mL), and powdered chromium trioxide (400 mg) was added, with stirring, at 50°. Aliquots were removed at 0, 0.5, and 1.5 h, diluted with water immediately after removal, extracted with chloroform, and the extracts dried (anhydrous sodium sulfate), and evaporated to dryness. The products were deacetylated with sodium methoxide, decationized with Dowex 50W X-8 (H^+) ion-exchange resin, and hydrolyzed. After the usual treatment, the hydrolyzates were converted into the alditol acetates, and the mixtures analyzed by g.l.c. using column *a* (see Table I).

Preparation of the degraded gum. — Pilot experiments with dilute mineral acids, formic acid, and water (autohydrolysis) for different time-intervals indicated that autohydrolysis for 25 h at 100° is best suited for the preparation of degraded gum. A solution of whole gum (620 mg) in water (50 mL) was heated on a boiling-water bath for 25 h, cooled, and poured, with stirring, into cold ethanol (150 mL). The resulting precipitate was centrifuged off, washed several times with ethanol, and dried; yield 465.4 mg, $[\alpha]_{589.6}^{23}$ −9.3° (*c* 1.07, water). On chromatographic examination, the mother liquor from the precipitation showed spots for arabinose and rhamnose, and a few spots in the oligosaccharide region. The oligosaccharides were isolated by preparative paper-chromatography using solvent *C*. The degraded gum (15.7 mg) was converted into carboxyl-reduced, degraded gum as already described. The product was completely hydrolyzed with M sulfuric acid, and the alditol acetates of the hydrolyzate were analyzed by g.l.c. using column *a*. The degraded gum contained galactose (75), glucose (16), rhamnose (4.2), and arabinose (4.1%).

Methylation analysis. — The whole gum (5.9 mg), degraded gum (5 mg), and carboxyl-reduced, whole gum (3.3 mg) were mixed with dimethyl sulfoxide (6, 5, and 4 mL, respectively) in separate vials, and treated with 2M methylsulfinyl sodium (6, 5, and 4 mL, respectively) under nitrogen. The solutions were stirred overnight, methyl iodide (6, 5, and 4 mL, respectively) was added dropwise, with external cooling, to the vials, and the mixtures were stirred for 2 h. The products were then dialyzed, lyophilized, and remethylated by the Purdie method. The fully methylated samples thus obtained showed no OH bands in their i.r. spectra; that from whole gum (6.1 mg) had $[\alpha]_{589.6}^{23}$ −6.2° (*c* 0.16, chloroform), from degraded gum (5.9 mg), −8° (*c* 0.33, chloroform), and from carboxyl-reduced, whole gum (4.1 mg), −5.7° (*c* 0.52, chloroform).

These methylated samples were separately hydrolyzed, first with 85% formic acid for 2 h at 100°, and then, after removal of the formic acid by codistillation with water, with 0.5M sulfuric acid for 18 h at 100°. After neutralization of the acid with barium carbonate and the usual treatment, the partially methylated sugars were converted into their alditol acetates, which were analyzed by g.l.c. using columns *a* and *b* (see Table II).

Graded-hydrolysis studies. — A solution of the degraded gum (200 mg) in 42.5% formic acid (50 mL) was heated on a boiling-water bath for 5 h (the optimal conditions for release of the maximum amount of oligosaccharides being ascertained by pilot experiments). Formic acid was removed by codistillation with water under diminished pressure, and the product was passed through a column (20 × 2.5 cm) of Dowex-1 X-4 (OAc^-) ion-exchange resin in order to separate the acidic from the neutral oligosaccharides. The eluate and washings (with distilled water) were concentrated to 1 mL, to give the neutral oligomers. The column was then eluted with 30% acetic acid (100 mL), and the eluate was evaporated to dryness, giving the acidic oligosaccharides. Both the neutral and the acidic oligosaccharides were resolved on Whatman No. 3MM paper, using solvents *B* and *C*, and the separate sugars were isolated by eluting the corresponding strips with water.

These oligosaccharides and those obtained from the autohydrolysis products of whole gum were treated similarly. They were hydrolyzed with M sulfuric acid for 20 h at 100°, and, after neutralization and the usual treatment, the sugars were analyzed both by paper chromatography, and by g.l.c. (as their alditol acetates) using column *a*. Their reducing ends were reduced by treatment of the aqueous solutions with sodium borohydride (8–10 times) for 4–5 h at room temperature. The products were then hydrolyzed, and analyzed as before. The equivalent weights of the acidic oligomers were determined by direct titration of a known amount of the oligosaccharide in water with 0.02M sodium hydroxide solution, using phenolphthalein as the indicator. The oligosaccharides in *N*,*N*-dimethylformamide were methylated by the Kuhn method[8,8a], with Drierite as the internal desiccant, and methyl iodide and silver oxide as the methylating agents. The methylated oligosaccharides were hydrolyzed directly with M sulfuric acid on a boiling-water bath for 20 h; the hydrolyzates were converted into their alditol acetates, and analyzed by g.l.c., using columns *b* and *c*.

Periodate oxidation and Smith-degradation studies of the whole gum and the degraded gum. — The whole gum and the degraded gum were treated with 0.1M sodium metaperiodate in the dark at 5°. Consumption of the oxidant (monitored spectrophotometrically[14]) became constant in 24 h, corresponding to a consumption of 0.75 and 0.70 mol, respectively, per mol of hexosyl residue.

In separate experiments, the whole gum (50 mg) and the degraded gum (50 mg) were each treated with 0.1M sodium metaperiodate (50 mL) in the dark for 24 h at 5°. The excess of periodate was decomposed by adding ethylene glycol (2 mL) and keeping the mixture for 3 h. The solution was dialyzed, and the dialyzate was concentrated to ~10 mL, treated with sodium borohydride (300 mg), and kept overnight at room temperature. The mixture was acidified with acetic acid and dialyzed, and the dialyzate concentrated to 10 mL. Part of the solution (1 mL) was hydrolyzed with M sulfuric acid, and after the usual treatment, the alditol acetates were analyzed by g.l.c., using column *a*. The remaining solution (9 mL) was treated with 0.5M sulfuric acid for 8 h at room temperature. The acid was neutralized ($BaCo_3$), the barium salt removed by centrifugation, the supernatant solution dialyzed, and the

dialyzate concentrated to 10 mL. The solution was again Smith-degraded, as already described, and the final solution was added to 4 vol. of cold ethanol, giving a precipitate which was centrifuged off, washed once with ethanol, and dried. The solid (~4 mg in each case) was hydrolyzed, and, after the usual treatment, the alditol acetates were examined by g.l.c., using column *a*.

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