

STRUCTURAL INVESTIGATION ON DEGRADED *Spondias dulcis* GUM

SUMANTA BASU AND CHINTALACHARUVU V. N. RAO

Department of Macromolecules, Indian Association for the Cultivation of Science, Jadavpur, Calcutta-700032 (India)

(Received March 6th, 1981; accepted for publication, March 27th, 1981)

ABSTRACT

Autohydrolysis of an aqueous solution of purified, exudate gum from *Spondias dulcis* trees yielded a degraded gum containing D-galactose, L-arabinose, and D-galacturonic acid in the mole ratios of 3:3:1. Methylation studies were conducted on the degraded gum and its carboxyl-reduced derivative. Three neutral and three acidic oligosaccharides were obtained on graded hydrolysis of the degraded gum, and these were characterized. Based on the results, a tentative structure was proposed for the repeating unit in the polysaccharide. The results of periodate oxidation supported the structure assigned. The anomeric configurations of the sugar residues were determined by studies of oxidation with chromium trioxide.

INTRODUCTION

The plant *Spondias dulcis* (Anacardiaceae family) grows abundantly in India, and the exudate gum is known for its refrigerant, medicinal action. The structure of the gum has not been studied in detail, but some preliminary investigations were reported¹ recently. This communication contains the results of studies on the degraded gum.

RESULTS AND DISCUSSION

Based on the results of pilot experiments, the degraded gum was prepared by autohydrolysis of an aqueous solution of the whole gum for 50 h at 100°. The polysaccharide was precipitated with three volumes of ethanol, and purified by passing it through a column of Sephadex G-100. The material eluted as a single peak and was obtained by lyophilization of the eluate. In high-voltage electrophoresis in 0.01M borate buffer, and ultracentrifugal analysis using Schlieren optics, the degraded gum moved as a single substance, indicating its homogeneity. It had $[\alpha]_D^{26} -14^\circ$. On hydrolysis with 0.5M sulfuric acid, the purified, degraded gum gave arabinose (41.0%), galactose (37.7%), and galacturonic acid (13.0%). The carboxyl groups in the degraded gum were reduced by using the method of Taylor and Conrad²,

TABLE I

METHYLATION ANALYSIS OF THE DEGRADED GUM (A), AND CARBOXYL-REDUCED DEGRADED GUM (B)

Sugars	T ^a		Mole proportion		Mode of linkage
	i	ii	i	ii	
2,3,4-Tri- <i>O</i> -methyl-L-arabinose	0.73	0.54	2.0	1.8	Arap-(1→
2,3,5-Tri- <i>O</i> -methyl-L-arabinose	0.48	0.41	2.6	2.5	Araf-(1→
2,4-Di- <i>O</i> -methyl-L-arabinose	1.40	1.10	4.8	4.6	→3)-Arap-(1→
2,5-Di- <i>O</i> -methyl-L-arabinose	1.10	0.84	1.8	1.7	→3)-Araf-(1→
2,6-Di- <i>O</i> -methyl-D-galactose	3.65	3.14	3.7	4.6	→3,4)-Galp-(1→
2,4-Di- <i>O</i> -methyl-D-galactose	6.35	5.10	3.6	3.7	→3,6)-Galp-(1→
2,4,6-Tri- <i>O</i> -methyl-D-galactose	2.28	2.03	0.8	1.8	→3)-Galp-(1→
2,3,4,6-Tetra- <i>O</i> -methyl-D-galactose	1.25	1.19	—	3.6	GalpA-(1→

^aRetention times of the corresponding alditol acetates, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity, on a column of (i) 3% of ECNSS-M (170°), and (ii) 3% of OV-225 (170°).

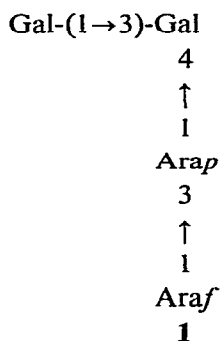
and the resulting product gave, on hydrolysis, galactose and arabinose in the ratio of 1.3:1, confirming the presence of galacturonic acid in the degraded gum.

The degraded gum and its carboxyl-reduced derivative were methylated by the Hakomori method³, followed by the Purdie method⁴, to yield fully methylated products. The permethylated polysaccharides were hydrolyzed with 90% formic acid, and then with 0.5M sulfuric acid. The alditol acetates of the resulting methylated sugars in each hydrolyzate were identified, and their relative mole proportions determined, by g.l.c. The results are summarized in Table I. From these results, the structural features of the degraded gum could be ascertained.

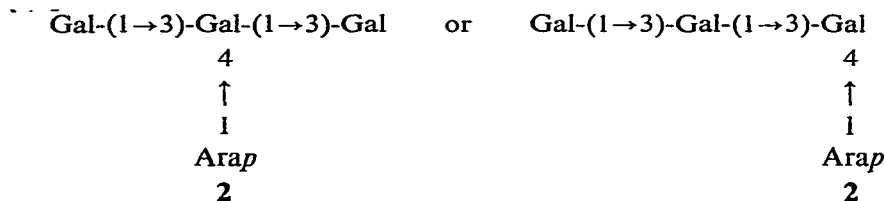
The molecule of the gum contains both L-arabino-pyranose and -furanose residues, and all of the galactose units are in the pyranose form. The non-reducing ends are occupied by L-arabino-furanosyl and -pyranosyl units, as indicated by the presence of 2,3,4- (2.0 mol) and 2,3,5-tri-*O*-methyl-L-arabinose (2.6 mol). No tetra-*O*-methyl derivative of galactose was detected in the hydrolyzate of the methylated, degraded gum, whereas the reduced product yielded 3.6 mol of 2,3,4,6-tetra-*O*-methyl-D-galactose, indicating that all of the galacturonic acid units occupy non-reducing ends. The doubly linked arabinose units, either in the pyranose (4.8 mol) or the furanose form (1.8 mol) are (1→3)-linked, and all of the doubly linked galactopyranose residues are also (1→3)-linked. Isolation and characterization of 2,6-di- (3.7 mol) and 2,4-di-*O*-methyl-D-galactose (3.6 mol) indicated that these D-galactopyranose units in the molecule are 1,3,4- and 1,3,6-linked. As the aldobiouronic acid¹ was characterized as GalA-(1→3)-Gal, and as a 3.6 mol proportion of the uronic acid residues was present in the polysaccharide, it was expected to generate equal amounts of (1→3)-linked galactopyranose residues in the hydrolyzate of the reduced, methylated material, but only 1.8 mol proportion of this methylated sugar was actually obtained, indicating that some of the galactose units to which uronic acid is linked are involved in branch points.

To ascertain the sequence of the different sugar residues, the polysaccharide was subjected to graded hydrolysis. Guided by the results of pilot experiments for maximal release of oligosaccharides, the degraded gum was hydrolyzed with 20% formic acid for 5 h at 100°. The mixture was separated into three neutral and three acid fractions by using ion-exchange columns, followed by p.c. Each fraction was found to be homogeneous in paper chromatography, and was characterized as follows.

Neutral oligosaccharides. — On hydrolysis, oligosaccharide **1**, 7.4 mg { R_{Gal} 0.4 (solvent C), $[\alpha]_D^{24} +68^\circ$ (c 0.4, water)} gave galactose and arabinose in the mole ratio of 1:0.8. On reduction ($NaBH_4$) of the oligosaccharide, and hydrolysis of the product, spots for galactose and arabinose were found by p.c. The material was fully methylated by the Hakomori method, and the product hydrolyzed. The methylated sugars were identified and estimated in the usual way by g.l.c. using columns (a) and (b); they were 2,3,5-tri-*O*-methyl- (0.8 mol) and 2,4-di-*O*-methyl-L-arabinose (0.86 mol), and 2,6-di-*O*-methyl- (0.92 mol) and 2,3,4,6-tetra-*O*-methyl-D-galactose (1.0 mol). This oligosaccharide was therefore assigned the following structure.

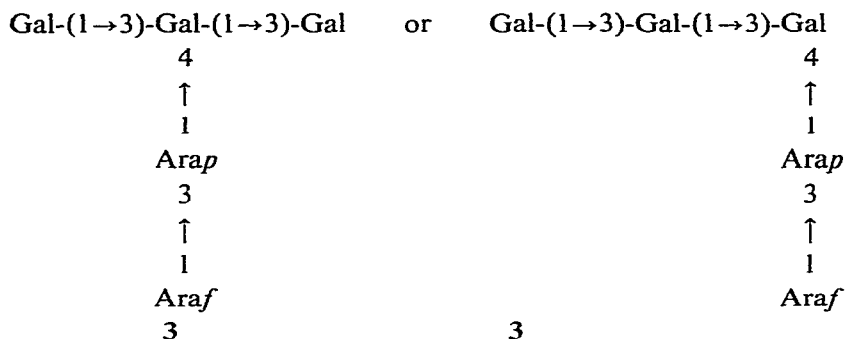


On hydrolysis, oligosaccharide **2**, 5.1 mg { R_{Gal} 0.3 (solvent C), $[\alpha]_D^{24} +28^\circ$ (c 0.5, water)} gave galactose and arabinose in the molar ratio of 2.9:1. On reduction with $NaBH_4$ of the oligosaccharide, and hydrolysis, the products gave spots corresponding to galactose and arabinose, showing that the galactose unit was at the reducing end. The material was fully methylated, the product hydrolyzed, and the alditol acetates of the resulting methylated sugars were identified by g.l.c. as being 2,3,4-tri-*O*-methyl-L-arabinose (0.87 mol), and 2,6-di-*O*-methyl- (0.95 mol), 2,4,6-tri-*O*-methyl- (0.92 mol), and 2,3,4,6-tetra-*O*-methyl-D-galactose (1.0 mol). Thus, the structure that may be assigned to this oligosaccharide is either



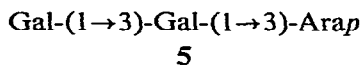
On hydrolysis, oligosaccharide **3**, 5 mg { R_{Gal} 0.14 (solvent C), $[\alpha]_D^{24} +43^\circ$

(*c* 0.5, water)} gave galactose and arabinose in the mole ratio of 3:1.8. On reduction (NaBH_4) and hydrolysis, this oligosaccharide showed spots of galactose and arabinose. On methylation, hydrolysis, and g.l.c. analysis, the oligosaccharide yielded 2,3,5-tri-*O*-methyl- (0.89 mol) and 2,4-di-*O*-methyl-L-arabinose (0.95 mol), and 2,6-di-*O*-methyl- (0.88 mol), 2,4,6-tri-*O*-methyl- (0.96 mol), and 2,3,4,6-tetra-*O*-methyl-D-galactose (1.0 mol). Based on these results, the structure assigned to this oligosaccharide is either



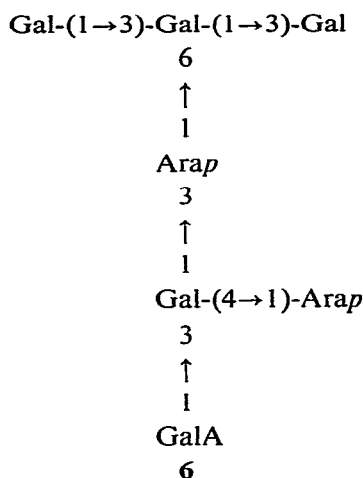
Acidic oligosaccharides. — On hydrolysis, oligosaccharide 4, 12 mg { R_{GalA} 0.7 (solvent C), $[\alpha]_{\text{D}}^{24} + 52^\circ$ (*c* 0.7, water); lit. $[\alpha]_{\text{D}} + 56.2^\circ$ } gave galactose and galacturonic acid, whereas, on reduction (NaBH_4) and hydrolysis, only galacturonic acid was detected. The galactose¹⁰ and galacturonic acid⁵ in it were estimated to be present in the mole ratio of 1:0.9. On methylation, hydrolysis, and analysis by g.l.c., 2,4,6-tri-*O*-methyl-D-galactose was obtained. Hence, the structure of the aldobiouronic acid (4) is 3-*O*-(β-D-galactopyranosyluronic acid)-D-galactose.

On hydrolysis, oligosaccharide 5, 8.3 mg { R_{GalA} 0.34 (solvent C), $[\alpha]_{\text{D}}^{24} + 56^\circ$ (*c* 0.5, water)} gave galactose, arabinose, and galacturonic acid, estimated to be in the mole ratios of 1.0:0.94:1.0. On reduction (NaBH_4) and hydrolysis, it gave galactose and galacturonic acid. The fully methylated oligosaccharide, on hydrolysis followed by g.l.c. examination, yielded 2,4-di-*O*-methyl-L-arabinose (0.89 mol) and 2,4,6-tri-*O*-methyl-D-galactose (0.75 mol). The methylated oligosaccharide was reduced with LiAlH_4 , and the product hydrolyzed. The hydrolyzate was found to contain 2,4-di-*O*-methyl-L-arabinose (0.88 mol), and 2,4,6-tri-*O*-methyl- (1.0 mol), and 2,3,4-tri-*O*-methyl-D-galactose (0.85 mol). All of these results indicated that the structure of the oligosaccharide is as follows.



Oligosaccharide 6, 28.7 mg { R_{GalA} 0.14 (solvent C), $[\alpha]_{\text{D}}^{24} + 22^\circ$ (*c* 1, water)} contained galactose, arabinose, and galacturonic acid in the mole ratios of 3.8:1.9:1. On methylation, hydrolysis, and g.l.c. examination, it gave 2,3,4-tri-*O*-methyl- (0.83 mol) and 2,4-di-*O*-methyl-L-arabinose (0.87 mol), and 2,4-di-*O*-methyl- (0.85 mol), 2,6-di-*O*-methyl- (0.75 mol), 2,4,6-tri-*O*-methyl- (0.93 mol), and 2,3,4,6-tetra-

O-methyl-D-galactose (1.00 mol). The methylated product was reduced with LiAlH_4 , and the product hydrolyzed. The hydrolyzate was found to contain 2,3,4-tri-*O*-methyl-D-galactose (0.83 mol), besides the aforementioned methylated sugars. On mild hydrolysis with acid, the oligosaccharide yielded arabinose and two oligosaccharides whose mobilities in p.c. were identical with those of oligosaccharides **2** and **4**. Based on these data, the structure assigned to this oligosaccharide is as follows.



The structures of oligosaccharides **1**, **2**, **3**, and **6** indicate that the main chain of the polysaccharide is composed of (1→3)-linked galactose residues to which arabinose units are glycosidically linked at either O-4 or O-6; no oligomer isolated had a galactose residue linked to the main chain. It is evident from the structures of oligosaccharides **1** and **3** that an arabinofuranosyl residue is linked to O-3 of an arabinopyranose unit.

The structures of oligosaccharides **5** and **6** show that the aldobiouronic acid is glycosidically linked to O-3 of an arabinopyranose unit in the molecule. The structure of the latter further indicates that the aldotriouronic acid is glycosidically linked to O-6 of a galactose residue in the main chain, and that O-4 of the galactose residues in some of the aldobiouronic acid units is joined to an arabinopyranose residue.

From these results and those of methylation studies, one of the possible, and simplest, structures that could be assigned to the repeating unit of the degraded gum molecule is as follows.

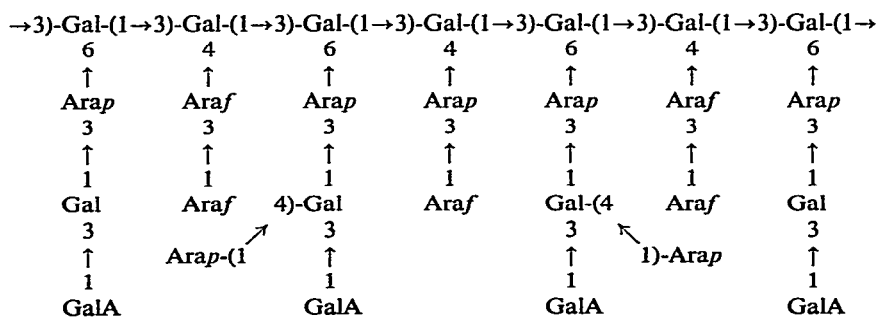


TABLE II

OXIDATION OF PERACETYLATED, DEGRADED GUM AND CARBOXYL-REDUCED, PERACETYLATED, DEGRADED GUM WITH CrO_3

<i>Material</i>	<i>Time of oxidation (h)</i>	<i>Galactose</i>	<i>Arabinose</i>	<i>myo-Inositol</i>
Peracetylated, degraded gum	0	5.6	8.4	10
	1.5	1.6	5.3	10
	2.5	0.8	3.3	10
Carboxyl-reduced, peracetylated, degraded gum	0	6.5	5	10
	1.5	3.6	3.2	10
	2.5	1.8	2.0	10

The degraded gum was subjected to periodate oxidation, the consumption of the oxidant being monitored spectrophotometrically⁷. During the reaction, 0.48 mol of oxidant was consumed per mol of hexosyl residue in 7 h. The theoretical value expected from the assigned structure is 0.55. The periodate-oxidized and then NaBH_4 -reduced material was found to contain 63.8% of galactose and 32.5% of arabinose; the calculated values for the proposed structure are 65 and 35%, respectively. When this product was treated with 0.5M sulfuric acid for 24 h at room temperature, followed by the usual treatments, and examination by p.c., one spot, corresponding to arabinose, was detected, indicating that arabinose residues had been released during mild treatment with acid. The Smith-degraded material, on submission to a second periodate oxidation followed by reduction with NaBH_4 , yielded a product containing galactose. Identification of such a product after the second periodate oxidation indicated that there is a sequence of (1→3)-linked galactose residues in the polysaccharide. Thus, the results from the periodate oxidation support the structure assigned to the repeating unit of the degraded gum.

Attempts were made to determine the anomeric configurations of the different sugar residues in the molecule by chromium trioxide oxidation⁸ of the peracetylated derivative of the degraded gum and that of the carboxyl-reduced product. The oxidation was conducted in acetic acid at 50°, and *myo*-inositol was used as the internal standard. Aliquots were removed at different time-intervals, deacetylated, the products hydrolyzed, and the sugars estimated by g.l.c. in the usual way. The results are shown in Table II. The rapid disappearance of galactose during the reaction indicates that most of the galactose units are β -linked. The value of the specific rotation of oligosaccharide 4, and the rate of disappearance of galactose from the carboxyl-reduced, degraded gum revealed that most of the galacturonic acid units are also β -linked. The results of the CrO_3 oxidation, and the ease with which the majority of the arabinose residues are removed during mild treatment with acid, show that the majority of the arabinose residues are α -linked.

EXPERIMENTAL

General methods. — Paper chromatography was performed 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, and (C) 4:1:5 (upper layer) 1-butanol–acetic acid–water. Whatman No. 1 filter papers were used for qualitative, paper chromatography, and large amounts of sugar mixtures were separated on Whatman No. 3MM sheets. The sugars were detected with (a) alkaline silver nitrate, and (b) benzidine periodate spray-reagents. All evaporations were conducted under diminished pressure at bath temperatures not exceeding 40°. Optical rotations were measured with a Perkin–Elmer model 241 MC spectropolarimeter. Ultraviolet spectra were recorded with Yanaco SP-1 and Scimadzu UV-201A spectrophotometers, and i.r. spectra with a Beckman IR-20A instrument. A Hewlett–Packard Model 5730 A gas chromatograph, fitted with a flame-ionization detector and glass columns (180 × 6 mm) packed with (i), 3% of ECNSS-M on Gas Chrom Q (100–200 mesh) and (ii) 3% of OV-225 on Gas Chrom Q (100–120 mesh), was used for g.l.c. Periodate oxidation was conducted in the dark at 4°, and the consumption of the oxidant was monitored spectrophotometrically⁷. A Shandon high-voltage electrophoresis instrument (Model L 24) and a Beckman Model L5-65 Ultracentrifuge fitted with a Schlieren optical system were used for testing the homogeneity of the materials. The quantitative results are reported as weight percentage of the material.

Isolation of the degraded gum. — In a pilot experiment, the whole gum (100 mg) in water (25 mL) was treated with Amberlite IR-120 (H⁺) resin, and the resulting solution (pH 4.7) was heated in a boiling-water bath. The progress of hydrolysis was monitored by paper-chromatographic examination of the hydrolyzate at regular intervals; arabinose was detected first, and, after 50 h, galactose appeared. Whole gum (1 g) in water (250 mL) was subjected to such autohydrolysis for 50 h, the solution was cooled, and to it was added ethanol containing 1% of lithium chloride. The degraded material was precipitated with ethanol (3 vol.), and the precipitate was separated by centrifugation, and triturated thrice with ethanol and once with acetone. This material was dissolved in water and reprecipitated in the same way; yield 760 mg.

Purification of the polysaccharide. — A solution of the degraded gum (60 mg) in 0.05M NH₄HCO₃ solution (3 mL) was applied to a column (100 × 2.2 cm) of Sephadex G-100. The column was eluted with the same solution (pH 8.0), and the eluate was collected in 5-mL fractions. Each fraction was tested for the presence of carbohydrates by the phenol–sulfuric acid method⁹. It was found that the desired material eluted as a single peak. The polysaccharide was recovered in 85% yield; $[\alpha]_D^{24} - 14^\circ$ (c 1, 0.1M NaOH).

The purified material was subjected to high-voltage electrophoresis in 0.01M borate buffer, pH 9.2, at 68 V/cm for 1.5 h, and the paper was developed with spray reagent (b). The material moved 3 cm towards the cathode as a single spot. A 1% solution of the material in 0.1M phosphate buffer containing 0.1M sodium chloride (pH 7.8) gave a single peak on ultracentrifugation.

The degraded gum (5 mg) was hydrolyzed with 0.5M sulfuric acid for 16 h at 100°; the acid was neutralized with BaCO₃, and the suspension centrifuged. In paper chromatography using solvents (A, B, and C), and spray reagent (a), spots corresponding to galactose, arabinose, and galacturonic acid were detected. Neutral sugars, as their alditol acetates, were estimated by g.l.c., using *myo*-inositol as the internal standard. Uronic acid was estimated by the carbazole-sulfuric acid method⁵, with D-galacturonic acid as the standard.

*Preparation of carboxyl-reduced, degraded gum*². — To a solution of the degraded gum (30 mg) in water (30 mL) was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (750 mg), and the pH was kept at ~4.75 during the reaction by adding 0.01M hydrochloric acid. After 2 h, 2M aqueous NaBH₄ (14 mL) was added dropwise during 45 min, and the pH was kept at 7 by simultaneous addition of 4M hydrochloric acid. After 1 h, the solution was dialyzed against distilled water and lyophilized. The procedure was repeated once, to ensure complete reduction. Carboxyl-reduced, degraded gum (2 mg) was hydrolyzed with 0.5M sulfuric acid for 16 h at 100°, and after the usual treatment, the sugars were estimated by g.l.c.

Methylation analysis of degraded gum and carboxyl-reduced, degraded gum. — Degraded gum (15 mg), and carboxyl-reduced, degraded gum (10 mg), in dimethyl sulfoxide (15 mL and 10 mL, respectively) were each methylated by the Hakomori procedure³, and the methylated products were remethylated twice by the Purdie method⁴. The i.r. spectrum of the materials did not contain any band in the region of 3600–3300 cm⁻¹.

The methylated products (8 mg) were hydrolyzed with 90% formic acid for 2 h at 100°, the acid was removed in *vacuo*, and the products were heated with 0.5M sulfuric acid for 10 h at 100°. The solutions were made neutral, and the methylated sugars were converted into their alditol acetates in the usual way, and identified, and estimated, by g.l.c. The results are given in Table I.

Graded hydrolysis of the degraded gum. — A solution of the degraded gum (200 mg) in 20% formic acid (50 mL) was heated on a boiling-water bath for 5 h. Formic acid was removed under diminished pressure, and the hydrolyzate was passed successively through columns of Dowex-50W X-8 (H⁺) and Dowex-1 X-4 (HCO₂⁻) ion-exchange resin. The eluate and washings containing neutral oligosaccharides were concentrated to a small volume. The column of anion-exchange resin was eluted with 10% formic acid (200 mL), and the eluate was evaporated to dryness, yielding the acidic oligosaccharides. The mixtures containing the neutral and acidic oligosaccharides were resolved on Whatman No. 3MM papers, using solvent C, and the separated sugars were isolated by eluting the corresponding strips with aqueous ethanol. The homogeneity of each oligosaccharide was checked by p.c.

The oligosaccharides were hydrolyzed with 0.5M sulfuric acid for 10 h at 100°, and, after the usual treatment, the sugars were identified, and their amounts determined, by p.c. and g.l.c. (as their alditol acetates, using column *i* at 190°). The reducing-end residues in them were identified by treatment with NaBH₄, followed by hydrolysis and the usual treatment; the sugars in the hydrolyzate were identified by

p.c., using aniline oxalate as the spray reagent. The oligosaccharides were methylated by the Hakomori method. The methylated, acidic oligosaccharides were reduced⁶ with LiAlH_4 , and the resulting products were hydrolyzed with 2M trifluoroacetic acid for 16 h at 95°. The methylated sugars obtained were converted into their alditol acetates, and these were identified, and estimated, by g.l.c.

Periodate oxidation of the degraded gum. — The degraded gum was treated with 0.1M sodium metaperiodate in the dark at 4°. Consumption of the oxidant became constant within 7 h, at a value corresponding to 0.48 mol of the oxidant per hexosyl residue. In a separate experiment, the degraded gum (50 mg) in water (50 mL) was oxidized with sodium metaperiodate solution (0.2M, 50 mL) for 7 h at 4°. The excess of periodate was decomposed with ethylene glycol, and the solution was dialyzed for three days against distilled water, and freeze-dried; yield, 35 mg.

The periodate-oxidized, degraded gum (30 mg) was reduced with NaBH_4 , and the resulting material (2 mg) was hydrolyzed with 0.5M sulfuric acid for 16 h at 100°. The neutral hydrolyzate obtained after the usual treatment was examined by p.c. (solvent *A*), and also by g.l.c. (as the alditol acetates). Besides lower polyhydric alcohols and aldehydes, galactose and arabinose were detected: galactose, 63.8%, and arabinose, 32.5%. A portion of the periodate-oxidized and reduced, degraded gum (15 mg) was kept with 0.5M sulfuric acid (15 mL) for 24 h at room temperature, and then made neutral, and centrifuged, and the supernatant liquor was concentrated to a small volume. P.c. examination of this solution (solvents *A* and *B*) revealed the presence of arabinose. The material (8 mg) was subjected to a second periodate oxidation at 4° in the dark. After the usual treatment, the solution was desalted by passage through a column (30 × 2.5 cm) of Sephadex G-50, and freeze-dried. On complete hydrolysis of this material, followed by the usual treatment and p.c. examination (solvents *A* and *B*), only one spot, corresponding to galactose, was detected.

Oxidation of the degraded gum and the carboxyl-reduced, degraded gum with chromium trioxide. — A mixture containing degraded gum (12.6 mg) and *myo*-inositol (16 mg) was dissolved in formamide (4 mL). To the solution were added acetic anhydride (3 mL) and pyridine (4 mL), and the mixture was stirred for 16 h at room temperature, dissolved in chloroform (25 mL), and the solution washed with water (3 × 20 mL), dried (anhydrous Na_2SO_4), and evaporated to dryness.

The product was reacylated, to ensure complete acetylation of the degraded gum. Powdered chromium trioxide (1.2 g) was added to a solution of the acetylated product in glacial acetic acid (12 mL), and the mixture was stirred at 50°. Aliquots were removed at 0, 1.5, and 2.5 h, and each was immediately diluted with water. The solution was extracted thrice with chloroform, and the extracts were combined, dried (Na_2SO_4), and evaporated to dryness. The product was deacetylated with 0.2M sodium methoxide for 3 h, decationized with Dowex-50W X-8 (H^+) ion-exchange resin, and the product hydrolyzed with M sulfuric acid for 8 h, and the alditol acetates were analyzed by g.l.c. The results are given in Table II.

A mixture of carboxyl-reduced, degraded gum (10 mg) and *myo*-inositol

(8 mg) was acetylated, and the product was oxidized with chromium trioxide as already described. The results of the g.l.c. analysis are summarized in Table II.

ACKNOWLEDGMENTS

The authors are indebted to Dr. S. Thakur, Burdwan University, for his generous supply of the gum. The authors also thank Dr. A. K. Mukherjee of this Department for his constant cooperation during the work.

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