Mesquite gum. I. The 4-*O*-methylglucuronogalactan core

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A re-examination of mesquite gum has shown that partial hydrolysis of the gum furnishes a mixture of acidic oligosaccharides including 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose (**1**), 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose (**2**), 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (**3**), *O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-D-galactose (**4**), and *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→4)-*O*-β-D-galactopyranosyl-(1→3)-D-galactose (**5**). A series of degradations of the gum affords successively, arabinose-free degraded gum A, carboxy-reduced degraded gum A, and degraded gums B and C. Degraded gum C contains a high proportion of 1→3′ linked β-D-galactopyranose residues. The structure of the 4-*O*-methylglucuronogalactan core of the gum is re-assessed in the light of these and other results.

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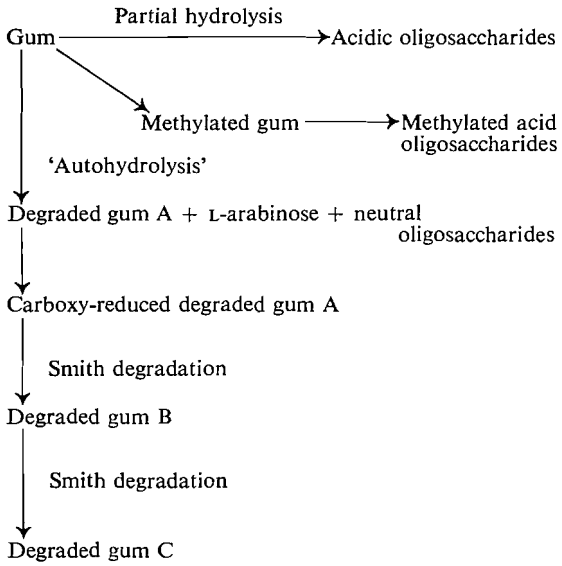
Earlier investigations by White (1–4) and by Smith and his collaborators (5–7) showed that mesquite gum, the exudate from *Prosopsis juliflora*, is a highly branched polysaccharide with residues of L-arabinose, D-galactose, and 4-*O*-methyl-D-glucuronic acid. Some of the main structural features of the gum were established by both workers but differences in the modes of linkage of D-glucuronic acid (or its 4-methyl ether) to D-galactose residues were reported. Cuneen and Smith (5) isolated on partial acid hydrolysis of the gum a mixture of monomethyl derivatives of the aldobiouronic acids, 4-*O*- and 6-*O*-(D-glucopyranosyluronic acid)-D-galactoses. The second aldobiouronic acid was later shown to be 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose (7). In contrast, White (3) isolated from the methylated gum a partially methylated aldobiouronic acid for which the structure, 3-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-2,4-di-*O*-methyl-D-galactose, was assigned. The present investigation was undertaken to re-examine the linkages in these oligosaccharides and to ascertain the distribution of the 1→3′ and 1→6′ linkages between D-galactopyranose residues in the interior chains of the gum. The degradations performed on mesquite gum are summarized in the flow sheet labelled Scheme 1.

The purified gum acid, which was obtained by precipitation from an acidified solution of the gum, was shown to be chemically homogeneous within acceptable limits by chromatography on diethylaminoethylcellulose (8). Partial acid



SCHEME 1

hydrolysis of the gum gave a series of acidic oligosaccharides which were separated by chromatography on diethylaminoethyl–Sephadex, followed by filter sheet chromatography, to give five acidic oligosaccharides (**1**–**5**) and three fractions containing mixtures of higher oligosaccharides. Oligosaccharide **1** was identified as the previously characterized 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose. The structure of oligosaccharide **2** was established as 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose by methylation analysis, and the configuration of the glycosidic linkage was

assigned on the basis of the observed specific rotation ($[\alpha]_D$ +94°) and by the presence of a doublet (τ 4.84; J = 3 Hz) in the n.m.r. spectrum of the disaccharide glycitol which was characteristic of an α-D-glycoside (9). Oligosaccharide **3**, which was isolated only in small amounts, was characterized as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose by methylation analysis and by conversion into a crystalline derivative.

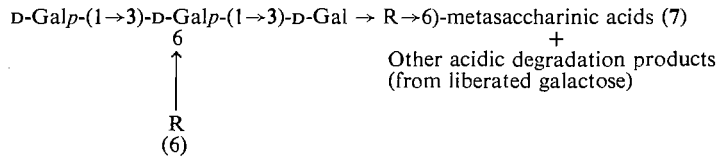
Oligosaccharides **4** and **5** were not isolated in sufficient quantity to permit the formation of crystalline derivatives. Their structures were established on the basis of their specific rotations, by paper chromatography of various transformation products, and by gas chromatography of fragments formed from the methylated trisaccharides and methylated modified derivatives. The structure, *O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-D-galactose, was assigned to oligosaccharide **4** on the basis of the following observations: (*i*) partial hydrolysis gave oligosaccharide **1**; (*ii*) partial hydrolysis of the carboxy-reduced trisaccharide glycoside afforded a disaccharide which was indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose and whose structure was confirmed by methylation analysis; (*iii*) g.l.c. showed that methanolysis of the methylated trisaccharide furnished methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid, and 2,3,4- and 2,3,5-tri-*O*-methylgalactose,[1] whilst methanolysis of the methylated trisaccharide glycitol gave methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid and 2,3,4-tri-*O*-methylgalactose, together with 1,2,3,4,5-penta-*O*-methylgalactitol. The following observations permitted the assignment of the structure, *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→4)-*O*-β-D-galactopyranosyl-(1→3)-D-galactose, for oligosaccharide **5**: (*i*) partial hydrolysis gave oligosaccharide **2**; (*ii*) methanolysis of the methylated trisaccharide gave methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid, and 2,3,6- and 2,4,6-tri-*O*-methylgalactose; (*iii*) the presence of a 3-*O*-substituted reducing galactose residue was established by conversion of the trisaccharide into methyl furanosides which were selectively oxidized with sodium periodate and reduced with sodium borohydride to give 3-*O*-substituted arabinofuranose units whose presence was detected by methylation analysis; (*iv*) the presence of the 3-*O*-substituted reducing galactose residue was further indicated by controlled oxidation of the trisaccharide with lead tetraacetate to give an alkali-stable 2-*O*-substituted lyxose residue.

4-Me-β-D-G*p*A-(1→6)-D-Gal (**1**)
4-Me-α-D-G*p*A-(1→4)-D-Gal (**2**)
β-D-G*p*A-(1→6)-D-Gal (**3**)
4-Me-β-D-G*p*A-(1→6)-β-D-Gal*p*-(1→6)-D-Gal (**4**)
4-Me-α-D-G*p*A-(1→4)-β-D-Gal*p*-(1→3)-D-Gal (**5**)

The three higher acidic oligosaccharide fractions were chromatographically homogeneous, but further examination of the fractions indicated that each contained a mixture of components. Although the separation of individual oligosaccharides was not feasible, some structural information was obtained by methylation analysis of the oligosaccharides, the oligosaccharide glycitols, and the products of alkaline degradation. For example, extended treatment of oligosaccharide fraction 7 with oxygen-free lime-water resulted in the disappearance of galactopyranose end groups, branch points, and 3-*O*-substituted galactose residues. This observation is consistent with the transformation of oligosaccharides (such as **6**), which contain 1→3′ linked galactose residues in chains interior to units of aldobiouronic acids (**1** or **3**), to *O*-glycosyl metasaccharinic acids (**7**) (see Scheme 2).

Since these experimental results failed to provide evidence for the formation of an oligosaccharide containing a 3-*O*-(D-glycopyranosyluronic acid)-D-galactose linkage, a sample of methylated mesquite gum was prepared for depolymerization under the conditions used earlier by White (3). An exhaustive series of fractionations was carried out on the products of methanolysis (see Experimental section), but no indication was obtained for the formation of the partially methylated aldobiouronic acid, 3-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-2,4-di-*O*-methyl-D-galactose, nor could a crystalline derivative of the type reported by White (3) be isolated. The presence of fully or partially etherified derivatives (**8**–**11**) of aldobiouronic acids **1** and **2** was clearly indicated. In so far as residues of 3-*O*-substituted 2,4-di-*O*-methyl-D-galactose were present in oligosaccharides formed on methanolysis of methylated mesquite gum they were constituents either of

[1]Where sites of substitution permit, methylation of reducing galactose and arabinose residues in *N*,*N*-dimethylformamide (10) gives rise to both pyranose and furanose derivatives.



R = 4-Me-β-D-GpA-(1→6)-D-Galp-(1- or β-D-GpA)-(1→6)-D-Galp-(1-

SCHEME 2

partially etherified neutral oligosaccharides, *e.g.* derivatives of 3-*O*-D-galactopyranosyl-D-galactose, or of higher acidic oligosaccharides such as oligosaccharide **5**. We conclude therefore that the linkages of D-glucuronic acid (or the 4-methyl ether) to D-galactose residues are of two types only, namely β-D-(1→6′) and α-D-(1→4′), and that those of the 1→3′ type reported by White (3) were based on valid conclusions from the incomplete data obtained by the techniques then available.

2,3,4-Me_3-α-D-GpA-(1→4)-2,3,6-Me_3-D-Gal (**8**)
2,3,4-Me_3-β-D-GpA-(1→6)-2,3,4-Me_3-D-Gal (**9**)
2,3,4-Me_3-β-D-GpA-(1→6)-2,4-Me_2-D-Gal (**10**)
2,3,4-Me_3-β-D-GpA-(1→6)-2-Me-D-Gal (**11**)

The mixtures of methyl glycosides of methylated sugars formed on methanolysis of methylated mesquite gum and of the reduced methylated gum were examined by gas chromatography (Table 1). The identities of the more fully substituted derivatives of arabinose and galactose were confirmed by g.l.c. of the corresponding methylated aldonolactones. The results confirm and extend earlier observations by White (1) and Cuneen and Smith (6) who identified 2,3,5-tri- and 3,5-di-*O*-methyl-L-arabinose, 2,4-di-*O*-methyl-D-galactose, and 2,3,4-tri-*O*-methyl-D-glucuronic acid as cleavage products from the methylated gum. In addition, 2,5-di-*O*-methylarabinose together with smaller amounts of 2,3,4-tri-*O*-methylrhamnose, 2,3-di-*O*-methylarabinose, 2,3,4,6-tetra-, 2,3,4-tri-, and 2,3,6-tri-*O*-methylgalactose, and 2,3-di-*O*-methylglucuronic acid have now been shown to be present. 2-*O*-Methyl-D-galactose was recognized as a further constituent of the gum by examination of hydrolysis products, but the structural significance of this sugar must be regarded as unproven since the axial 4-hydroxyl group of 3-*O*-substituted D-galactose residues is known to be difficult to methylate. No monomethyl ethers of arabinose could be detected amongst the cleavage products from the methylated gum and no evidence for branching through residues of this sugar has been obtained. The recognition of variously substituted arabinose residues in the gum prompted an examination of oligosaccharides formed on controlled hydrolysis of the gum and these experiments are reported in the following paper (11). The formation of acidic oligosaccharide **3** on partial hydrolysis of the gum, taken together with the detection of 2,3-di-*O*-methylglucuronic acid and 2,3,4-tri-*O*-methylrhamnose as minor products from the methylated gum indicates the presence of a small proportion of unmethylated D-glucuronic acid residues and suggests that these units may be 4-*O*-substituted by (presumably L-) rhamnose residues as in gum arabic (12) and *Araucaria bidwillii* gum (13).

Mesquite gum, like many other exudate gums (14, 15), may be degraded by controlled acid hydrolysis to give a degraded polysaccharide essentially devoid of arabinose residues. Autohydrolysis of gum acid afforded degraded gum A. A sample of degraded gum A was methylated and the cleavage products from the methylated degraded gum (Table 1) were examined by gas chromatography of the methyl glycosides formed on methanolysis. The highly branched nature of the interior portion of the gum was clearly shown

TABLE 1

Methanolysis products from methylated gum and methylated degraded gum A

Methyl glycosides	Methylated gum*	Methylated degraded gum A*
2,3,4-Me_3 Rhamnose	+	tr
2,3,5-Me_3 Arabinose	+ + +	tr
2,3-Me_2 Arabinose	+	n.d.
2,5-Me_2 Arabinose	+ +	tr
3,5-Me_2 Arabinose	+ + + +	tr
2,3,4-Me_3 Glucuronic acid	+ + +	+ + +
2,3-Me_2 Glucuronic acid	+	n.d.
2,3,4,6-Me_4 Galactose	+	+ +
2,3,4-Me_3 Galactose	+	+ + +
2,3,6-Me_3 Galactose	+	+
2,4,6-Me_3 Galactose	n.d.	+
2,4-Me_2 Galactose	+ + + +	+ + +

*tr = trace; n.d. = not detected.

with residues of glucuronic acid (or the 4-methyl ether) as end groups. Galactose residues, with the exception of those which were 4-*O*-substituted by 4-*O*-methylglucuronic acid residues as in the aldobiouronic acid (**2**), were mutually joined by 1→3′ and/or 1→6′ linkages. Comparison of the cleavage products from methylated mesquite and from the methylated degraded gum indicated that the dominant site of attachment of arabinose-containing side-chains was by 1→3′ linkages to 6-*O*-substituted galactose residues as in the partial structure (**12**).

. . .6)-β-D-Gal*p*-(1. . . **12**, where R = [L-Ara*f*]$_n$. . .)
3
↑
R

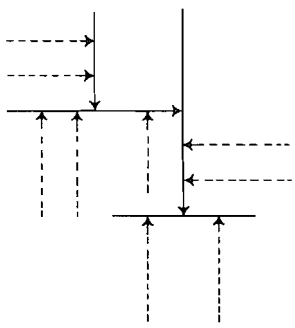
In order to obtain information on the distribution of 1→6′ and 1→3′ linkages between galactose residues in the interior chains of the gum, degraded gum A was converted into the carboxy-reduced derivative (16), which was degraded by Smith's procedure (17) of sequential periodate oxidation, borohydride reduction, and mild acid hydrolysis, to give degraded gum B. Degraded gum B similarly afforded degraded gum C. Degraded gums B and C were examined by (*a*) paper chromatography of oligosaccharides formed on partial acid hydrolysis, (*b*) analysis of reagent consumed during periodate oxidation, and (*c*) gas chromatography of the methyl glycosides of methylated sugars formed on methanolysis of the methylated derivatives (Table 2). The results clearly indicated that the sequence of reactions had resulted in removal of most of the outer chains and that degraded gum C approximated to a linear 1→3′ linked β-D-galactan. Degraded gum C, however, still contains one branch point per 10–12 residues. It is probable, therefore, that the interior chains of the galactan framework of mesquite gum which still remain in degraded gum C, like those in the interior chains of gum arabic (18) and other *Acacia* gums (19, 20), are of the ramified type (as in **13**) rather than consisting of a single 1→3′ linked chain with comb-like branches (as in **14**). Evidence from the specific rotations of the degraded polysaccharides and of oligosaccharides formed in small amount on autohydrolysis of the gum (11) suggests that all the galactose residues, whether in 1→3′ or 1→6′ linkages, have the β-D-configuration.

TABLE 2

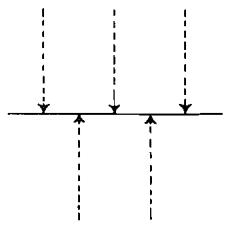
Relative proportions of methanolysis products from methylated degraded gums B and C

Methyl glycosides	Gum B	Gum C
2,3,4,6-Me_4 Galactose	1.5	1.5
2,3,4-Me_3 Galactose	1	0.5
2,4,6-Me_3 Galactose	4	15
2,4-Me_2 Galactose	1	1.8

Residues of 4-*O*-methyl-D-glucuronic acid (and presumably also of the small proportion of D-glucuronic acid) terminate outer chains in degraded gum A. Acidic oligosaccharides **1**, **4**, and probably **3**, presumably arise from the extremities of chains of 1→6′ linked β-D-galactopyranose residues. This type of linkage has been recognized in a number of other polysaccharides of the same general type (15). On the other hand, although some of these polysac-



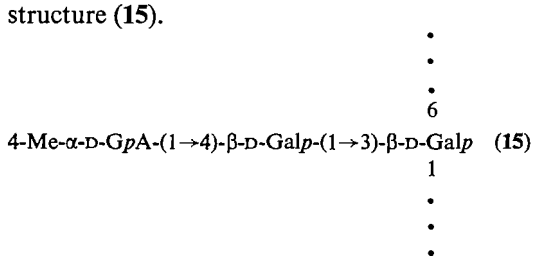
(13)



(14)

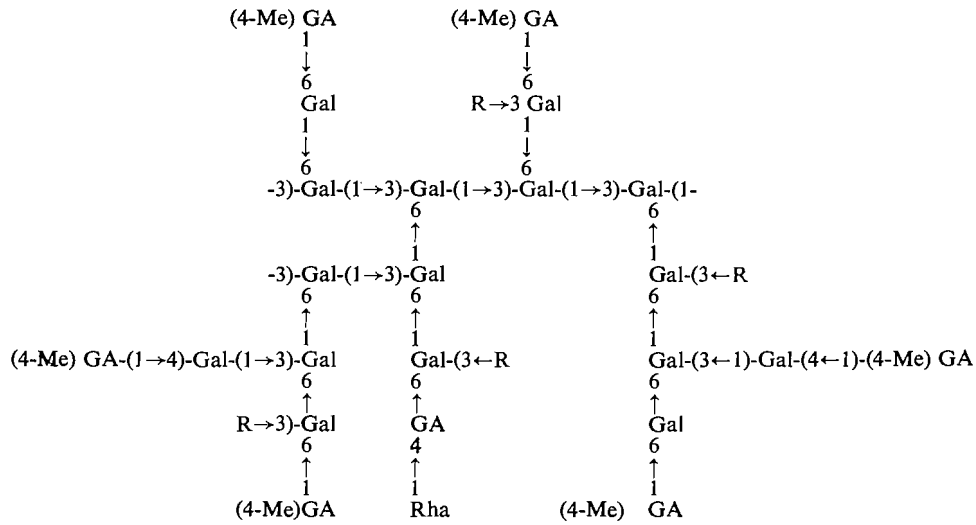
——— chains of 1→3′ linked β-D-galactopyranose units
- - - - - - - - - chains of 1→6′ linked β-D-galactopyranose units
→ 1→6′ linkages at branch points

charides also give rise on partial hydrolysis to the aldobiouronic acid (**2**), such units have not been placed within the overall molecular architecture. In mesquite gum, two observations, (*i*) the characterization of the trisaccharide (**5**) and (*ii*) the isolation of the fully etherified aldobiouronic acid (**8**) from the methylated gum, indicate that units of the aldobiouronic acid themselves carry no side-chains and that they are joined to the next D-galactose residues by 1→3′ linkages. Since virtually all the 3-*O*-substituted galactose residues in the gum are also 6-*O*-substituted, it is probable that units of the aldobiouronic acid (**2**) are attached as side-chains to the exterior chains of 1→6′ linked galactose residues as in the partial structure (**15**).



(**15**)

The present results permit the formulation of the following partial structure (**16**) for the 4-*O*-methylglucuronogalactan framework of mesquite gum in which the known structural features are accommodated. The following paper provides evidence for the sequences and types of linkage between L-arabinofuranose and other sugar residues in the acid-labile outer chains of the gum.



(**16**)

Experimental

General Methods

Paper chromatography was carried out in the following solvent systems (v/v): (*A*) ethyl acetate – pyridine – water (10:4:3); (*B*) ethyl acetate – acetic acid – formic acid – water (18:3:1:4); (*C*) ethyl acetate – acetic acid – formic acid – water (18:8:3:9); (*D*) ethyl acetate – pyridine – acetic acid – water (5:5:1:3); (*E*) 1-butanol – ethanol – water (4:1:5, upper layer).

Gas-liquid partition chromatography was carried out on columns of acid-washed Celite coated with: (*a*) 3% (w/w) of neopentylglycol adipate polyester at 150°; (*b*) 5% (w/w) of polyethyleneglycol adipate polyester at 175°; (*c*) 3% (w/w) of silicone gum XE-60 at 125°; and (*d*) 10% (w/w) of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)benzene] at 200°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside. Paper ionophoresis was performed in borate buffer at p*H* 10. Evaporations were carried out under reduced pressure at bath temperatures not exceeding 40°. Unless otherwise stated, optical rotations were observed for aqueous solutions at *ca.* 18°.

Small-scale methylations of oligosaccharides were performed by the method of Kuhn *et al.* (10). Uronic acid was determined by the carbazole method (21). Degrees of polymerization of oligosaccharides were determined by measurement of carbohydrate content by the phenol – sulfuric acid reagent (22) before and after reduction with sodium borohydride.

Isolation of Gum Acid

Mesquite gum was obtained as hard vitreous amber-yellow nodules, embedded with bark, twigs, and other foreign material. The nodules (50 g) were dispersed in water (150 ml), insoluble material was removed by filtration through muslin, the solution was acidified with dilute hydrochloric acid, and polysaccharide was precipitated by pouring the solution into ethanol (6 volumes). The polysaccharide was twice reprecipitated and redissolved in water to give a solution which was freeze-dried to afford gum acid (28 g), $[\alpha]_D$ +60° (*c*, 2.0), [Found:

uronic acid, 17%]. Hydrolysis of the gum gave galactose and arabinose as the main neutral sugar components together with a trace of rhamnose.

A portion of the gum acid (470 mg) was chromatographed on diethylaminoethylcellulose (phosphate form) (8) and the column was eluted with sodium dihydrogen phosphate buffer (p*H* 6) of increasing concentrations and finally with 0.5 *M* potassium chloride. The major peak eluted with 0.25 *M* buffer contained *ca.* 75% of the polysaccharide. The uronic acid content of the minor peaks was estimated by the carbazole method (21), and the results showed that all peaks, with the single exception of a neutral peak eluted with 0.005 *M* buffer and amounting to less than 5% of the whole, contained similar proportions of acid. The polysaccharide preparation was used without further purification in later experiments.

Partial Hydrolysis of Mesquite Gum

Mesquite gum (20 g) in 0.5 *N* sulfuric acid (2000 ml) was heated under reflux for 1.5 h. The cooled solution was neutralized with barium carbonate and filtered, and the filtrate was concentrated (to 200 ml). After treatment with Amberlite resin IR-120(H), the solution was further concentrated and adsorbed on a column (20 × 3 cm) of diethylaminoethyl–Sephadex A-25 (formate form). The column was eluted with water (1500 ml) to remove neutral sugars, and then 0.05 *M* formic acid to give fractions (total weight 3.1 g) containing acidic sugars. Acidic sugars were separated in sufficient quantity by filter-sheet chromatography in solvents *B* and *C* to give oligosaccharide fractions **1** (480 mg), **2** (205 mg), **3** (150 mg), **4** (210 mg), **5** (87 mg), **6** (150 mg), **7** (140 mg), and **8** (22 mg).

Examination of Oligosaccharide Fractions 1–8

Oligosaccharide **1**, $R_{galactose}$ 0.65 in solvent *B*, M_G 1.06, had $[\alpha]_D$ +20° (*c*, 1.0), and gave 4-*O*-methylglucuronic acid and galactose on hydrolysis. Methanolysis of the methylated derivative gave products which were shown by g.l.c. on column *a* to have the retention times of methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid, and 2,3,4- and 2,3,5-tri-*O*-methylgalactose. Methanolysis of the methylated glycitol similarly afforded 1,2,3,4,5-penta-*O*-methylgalactitol and methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid. Methylation of the disaccharide with methyl sulfate and sodium hydroxide, and methyl iodide and silver oxide furnished the methyl ester methyl glycoside hexamethyl ether of 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, m.p. 89–90° and mixed m.p. (with sample of m.p. 90–91°) 90°.

Oligosaccharide **2**, $R_{galactose}$ 0.75 in solvent *B*, M_G 0.69, had $[\alpha]_D$ + 94° (*c*, 0.5), gave 4-*O*-methylglucuronic acid and galactose on hydrolysis, and contained 48% of uronic acid. The g.l.c. on column *a* of the methanolysis products from the methylated derivative showed the presence of methyl glycosides of 2,3,4-tri-*O*-methyl glucuronic acid and 2,3,6-tri-*O*-methylgalactose. Methanolysis of the methylated glycitol similarly afforded 1,2,3,5,6-penta-*O*-methylgalactitol and methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid. The p.m.r. spectrum of the disaccharide glycitol in deuterium oxide showed a doublet (τ 4.84, *J* 3 Hz) attributable (9) to the anomeric proton of an α-D-glycoside. The disaccharide (150 mg) was converted into the methyl ester methyl glycosides by standing overnight in methanolic 1% hydrogen chloride (10 ml), and then heating under reflux for 0.75 h. The cooled solution was neutralized with silver carbonate, filtered, and concentrated to a syrup which was treated with sodium borohydride (200 mg) in water (10 ml) for 18 h. Excess of borohydride was destroyed with Amberlite resin IR-120(H), the reduced disaccharide was methylated with methyl sulfate and sodium hydroxide, and the methylated disaccharide (105 mg) was isolated by extraction with chloroform. The methylated disaccharide was hydrolyzed with *N* hydrochloric acid at 100° for 6 h, and the neutralized hydrolysate was concentrated to a syrup. The syrup was fractionated on a cellulose column (35 × 2 cm) with light petroleum (b.p. 100–120°) – 1-butanol (7:3), saturated with water as eluant, to give (*i*) 2,3,4,6-tetra-*O*-methyl-D-glucose (45 mg), m.p. 79–80° and mixed m.p. (with sample of m.p. 81–82°) 79–80°, and (*ii*) 2,3,6-tri-*O*-methyl-D-galactose (32 mg), which was characterized by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, m.p. 98–99° and mixed m.p. (with sample of m.p. 97–98°) 97–98°.

Oligosaccharide **3**, $R_{galactose}$ 0.26 in solvent *B*, M_G 1.14, gave glucuronic acid and galactose on hydrolysis, contained 46% of uronic acid, and was chromatographically indistinguishable from 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. The g.l.c. on columns *a* and *b* of the methanolysis products from the methylated derivative showed the presence of methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid, and 2,3,4- and 2,3,5-tri-*O*-methylgalactose. Methanolysis of the methylated glycitol likewise furnished 1,2,3,4,5-penta-*O*-methylgalactitol and methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid. The disaccharide was characterized by conversion into the methyl ester methyl glycoside hexamethyl ether, m.p. 86–87° and mixed m.p. (with sample of 90–91°) 86–88°.

Oligosaccharide **4**, $R_{galactose}$ 0.15 in solvent *B*, M_G 0.92, $[\alpha]_D$ +2° (*c*, 1.0), contained 29% of uronic acid and was shown to be a trisaccharide. Partial hydrolysis gave 4-*O*-methylglucuronic acid, galactose, and oligosaccharide **1**. Methanolysis of the methylated trisaccharide and of the methylated trisaccharide glycitol gave methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid and 2,3,4-tri-*O*-methylgalactose from both, methyl glycosides of 2,3,5-tri-*O*-methylgalactose from the former, and 1,2,3,4,5-penta-*O*-methylgalactitol from the latter [g.l.c. on columns *a* and *b* respectively]. The trisaccharide (50 mg) was converted into the methyl glycoside of the carboxy-reduced derivative as described for oligosaccharide **2**. The reduction product was heated in 0.5 *N* sulfuric acid at 100° for 0.5 h. The neutralized hydrolysate was separated by filter sheet chromatography in solvent *B* to give a disaccharide (6 mg), $[\alpha]_D$ +25° (*c*, 0.5), $R_{galactose}$ 0.31, which gave galactose only on hydrolysis and was chromatographically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose. Methanolysis of the methylated disaccharide afforded methyl glycosides of 2,3,4,6-tetra-, and 2,3,4- and 2,3,5-tri-*O*-methylgalactose [g.l.c. on column *b*].

Oligosaccharide **5**, $R_{galactose}$ 0.24 in solvent *B*, M_G 0.72, $[\alpha]_D$ +56° (*c*, 1.0), contained 31% of uronic acid and was shown to be a trisaccharide. Partial hydrolysis gave 4-*O*-methylglucuronic acid, galactose, and oligosaccharide **2**. Methanolysis of the methylated trisaccharide and of the reduced [$LiAlH_4$] methylated trisaccharide

TABLE 3

Methylation of oligosaccharide fractions 6–8 and derivatives

Cleavage products from methylated derivatives	Oligosaccharide fractions*§							
	6			7			8	
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*
2,3,4-Me_3 Glucuronic acid†	+	+	+	+	+	+	+	+
2,3,4,6-Me_4 Galactose†	+	+		+	+	tr	+	+
2,3,4-Me_3 Galactose†	+	+	+	+	+	+	+	+
2,3,5-Me_3 Galactose†‡	+		tr	+		tr	+	
2,4,6-Me_3 Galactose†	tr			+	+		+	+
2,5,6-Me_3 Galactose†‡				+		tr	+	
2,4-Me_2 Galactose†	n.d.						n.d.	+
1,2,3,4,5-Me_5 Galactitol		+			+			+
1,2,3,5,6-Me_5 Galactitol		+			+			+

*a, Methylation of oligosaccharide fraction; *b*, methylation of oligosaccharide glycitol; *c*, methylation of oligosaccharide fraction after treatment with lime-water.
†As methyl glycosides.
‡From methylation of reducing sugar unit.
§tr = trace; n.d. = not detected.

gave methyl glycosides of 2,3,6- and 2,4,6-tri-*O*-methylgalactose from both, 2,3,4-tri-*O*-methylglucuronic acid from the former, and 2,3,4-tri-*O*-methylglucose from the latter [g.l.c. on columns *a* and *b*, and *b* respectively].

Oligosaccharide **5** (20 mg) was kept in methanolic 4% hydrogen chloride (2 ml) at room temperature for 165 min [$[\alpha]_D$ $+56° \rightarrow +42°$ (constant)]. The solution was neutralized with silver carbonate, filtered, and concentrated to a syrup which consisted of one major product, $R_{galactose}$ 0.46 in solvent *B*, and a trace of starting material. A portion (5 mg) of the syrup was oxidized with periodic acid (3 mg) in water (2 ml) at 5° for 1 h. Periodic and iodic acids were precipitated by neutralization with barium hydroxide, the solution was centrifuged, and barium ions were removed from the supernatant liquid by treatment with Amberlite resin IR-120(H). The solution containing the oxidation product was reduced with potassium borohydride (15 mg) for 18 h. Excess of borohydride was destroyed with Amberlite resin IR-120(H), the solution was concentrated, and boric acid was removed by repeated distillations with methanol. Partial hydrolysis of the degraded trisaccharide gave 4-*O*-methylglucuronic acid, galactose, arabinose, and oligosaccharide **2**. Methanolysis of the methylated degraded trisaccharide gave methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid, 2,3,6-tri-*O*-methylgalactose, and 2,5-di-*O*-methylarabinose [g.l.c. on columns *a* and *b*].

Lead tetraacetate (10 mg) in acetic acid (1 ml) was added to oligosaccharide **5** (12 mg) in water (0.5 ml) and acetic acid (1 ml), and the solution was kept for 15 min. Hydrogen sulfide was passed through the solution to precipitate lead salts, and the filtered solution was concentrated to a syrup which contained one major component, $R_{galactose}$ 1.13 in solvent *B*, which gave a characteristic pink stain with aniline oxalate, and a trace of starting material. Hydrolysis of a portion of the syrup gave lyxose, galactose, and oligosaccharide **2**. The remainder of the oxidized syrup was kept in oxygen-free saturated lime-water (15 ml) for 10 days. The solution was neutralized with Amberlite resin IR-120(H), filtered, and concentrated to a syrup, which contained a single reducing sugar, $R_{galactose}$ 0.59 in solvent *B*, gave no stain with triphenyltetrazolium hydroxide thus indicating the presence of a 2-*O*-substituted reducing unit (23), and yielded lyxose, galactose, and oligosaccharide **2** on partial hydrolysis.

Oligosaccharide fractions 6, 7, and 8 were homogeneous by paper chromatography and had $R_{galactose}$ 0.06 and 0.30 in solvents *B* and *C*, 0.02 and 0.20, and 0.01 and 0.13 respectively. Ionophoresis later showed that fraction 6 was heterogeneous, M_G 0.7 and 0.9, but only one component was detected in fraction 8. Partial hydrolysis of fractions 6, 7, and 8 gave galactose and oligosaccharides **1**, **3**, and **4**. Samples of each oligosaccharide fraction, the corresponding glycitols, and the products from treatment of fractions 6 and 7 with oxygen-free saturated lime-water for 7 days, were methylated and the methanolysis products from the various methylated derivatives were examined by g.l.c. [see Table 3].

Methylated Mesquite Gum

Gum nodules (30 g) were dissolved in water (100 ml) by stirring overnight, insoluble material was removed by filtration, and methylation was carried out by the addition of methyl sulfate (200 ml) and aqueous 30% sodium hydroxide (400 ml) during 4 h. After six further treatments the partially methylated gum was isolated by extraction with chloroform, converted into the silver salt (19.7 g), and methylated further with methyl iodide and silver oxide to give methylated mesquite gum (15 g), $[\alpha]_D$ $+59°$ (*c*, 0.8 $CHCl_3$),[Found: OMe, 43.1%], whose i.r. spectrum showed negligible hydroxyl absorption. A sample of the methylated gum was heated in methanolic 4% hydrogen chloride at 100° for 18 h, and after neutralization with silver carbonate the methanolysate was examined by g.l.c. on columns *a*, *b*, and *d* [see Table 4]. A portion of the methanolysate was hydrolyzed in *N* sulfuric acid at 100° for 12 h, and the hydrolysate was oxidized with bromine water for 3 days. Examination of the resulting mixture of aldonolactones by g.l.c. on column *b* showed the presence of components with the retention times of 2,3,5-tri- (*T*, 1.74), 2,3- (*T*, 8.1), 2,5-

TABLE 4

Gas-liquid chromatography of methanolysate from methylated mesquite gum

Methyl glycosides of	Relative retention times (T) on*								
	Column *a*			Column *b*			Column *d*		
2,3,4-Me_3 Rhamnose					0.46			0.43	
2,3,5-Me_3 Arabinose	0.49		0.63	0.54		0.72	0.49		(0.63)
2,3-Me_2 Arabinose		1.19			1.49				
2,5-Me_2 Arabinose		1.49			1.79		0.71		1.08
3,5-Me_2 Arabinose	0.83		1.98	1.05		2.40	(0.63)		0.84
2,3,4,6-Me_4 Galactose		1.77						1.59	
2,3,4-Me_3 Galactose		6.54			7.02		2.55		2.82
2,3,6-Me_3 Galactose	2.74	3.54	4.07	(3.13)	3.95	4.52			
2,4-Me_2 Galactose	13.5		15.5				3.66		4.21
2,3,4-Me_3 Glucuronic acid	2.28		2.99	2.40		3.13	1.75		2.18
2,3-Me_2 Glucuronic acid				7.7	8.45	10.3			

*Figures in parentheses represent T values for methyl glycosides whose anomers were detected in separate non-overlapping peaks; no figures are quoted for T values where no individual glycoside of an individual sugar was detected with certainty on a particular column.

(T, 14.8), and 3,5-di-*O*-methylarabinonolactone (T, 5.41), and 2,3,4,6-tetra- (T, 6.80) and 2,3,6-tri-*O*-methylgalactonolactone (T, 13.6).

Lithium aluminum hydride (200 mg) in tetrahydrofuran (8 ml) was added slowly to methylated mesquite gum (150 mg) in tetrahydrofuran (8 ml). The mixture was stirred for 30 min, and then heated under reflux for 3 h. Excess of hydride was destroyed by the addition of ethyl acetate, water was added, and the mixture was acidified to pH 4 by the addition of dilute sulfuric acid. The mixture was extracted with chloroform, and the extract was concentrated and poured into light petroleum to give reduced methylated gum (50 mg), $[\alpha]_D$ +61° (*c*, 1.0 $CHCl_3$), [Found: OMe, 39.4%]. A sample of the reduced methylated gum was methanolyzed and the resulting methyl glycosides were examined by g.l.c. on columns *a* and *b*. Similar products to those formed from methylated mesquite gum were detected except that methyl glycosides of 2,3,4-tri-*O*-methylglucose replaced those of 2,3,4-tri-*O*-methylglucuronic acid [T, 2.29 and 3.36, and 2.39 and 3.58 on columns *a* and *b* respectively]. No glycosides of 2,3-di-*O*-methylglucuronic acid or its reduction product, 2,3-di-*O*-methylglucose, could be detected.

Partial Methanolysis of Methylated Mesquite Gum

In order to simulate the conditions used by White in the examination of cleavage products from methylated mesquite gum (3), methylated gum (13 g) in methanolic 4% hydrogen chloride (110 ml) was heated under reflux for 5 h. The cooled solution was neutralized with silver carbonate, filtered, and concentrated to a syrup. The syrup was extracted five times with light petroleum at 100°, and the petrol-insoluble residue was dissolved in chloroform and concentrated to a syrup (3.0 g).

A portion of the petrol-insoluble residue was treated with dilute sodium hydroxide at pH 12 for 2 h. Sodium ions were removed with Amberlite resin IR-120(H), and the resulting solution was adsorbed on diethylaminoethyl–Sephadex A-25 (formate form) and separated into an acidic fraction (340 mg) and a neutral fraction (460 mg). The neutral fraction was hydrolyzed with N hydrochloric acid (25 ml) at 100° for 15 h, and the solution was neutralized and concentrated to a syrup. A portion (65 mg) of the syrup was separated by filter sheet chromatography in solvent E to give two major components, 2-*O*-methyl-D-galactose (21 mg), m.p. 148–150° and mixed m.p. (with sample of m.p. 149–151°) 148–151°, and 2,4-di-*O*-methyl-D-galactose, m.p. 101–103°, together with small amounts of 2,3,4-tri-*O*-methylgalactose, and 2,5- and 3,5-di-*O*-methylarabinose, which were identified by g.l.c. of their methyl glycosides, and traces of arabinose and an unidentified sugar (possibly a mono-*O*-methylarabinose).

A further quantity (1.8 g) of the petrol-insoluble residue was fractionally distilled under reduced pressure (0.005 mm) to give fractions 1 (34 mg, b.p. 88–160°), 2 (393 mg, b.p. 160–180°), and 3 (300 mg, b.p. 180–210°). The g.l.c. of fraction 1 showed the presence of methyl glycosides of 2,5- and 3,5-di-*O*-methylarabinose. Treatment of the fraction with methanolic hydrogen chloride caused no further depolymerization. The g.l.c. of fraction 2 showed the presence of methyl glycosides of the sugars in fraction 1 together with 2,4-di-*O*-methylgalactose. Further treatment of fraction 2 with methanolic hydrogen chloride gave in addition small amounts of methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid and 2,3,4-tri-*O*-methylgalactose. The g.l.c. of fraction 3 showed the presence of methyl glycosides of 2,4-di-*O*-methylgalactose, and, after methanolysis, of 2,3,4-tri-*O*-methylglucuronic acid, and 2,3,4- and 2,3,6-tri-*O*-methylgalactose. A sample of fraction 3 was remethylated, and the product was methanolyzed and acetylated. The g.l.c. indicated the formation of derivatives of 2,3,4-tri-*O*-methylglucuronic acid, 2,3,4,6-tetra-, and 2,3,4-, 2,3,6-, and 2,4,6-tri-*O*-methylgalactose. Treatment of a portion of fraction 3 with methanolic ammonia afforded a syrup from which no crystalline derivative could be obtained.

Separation of Acidic Oligosaccharide Fractions

The remainder of fraction 3 was saponified with dilute sodium hydroxide at pH 12.5 and fractionated by chromatography on diethylaminoethyl-Sephadex A-25 (formate form) as described previously to give neutral fraction 3A (160 mg) and acidic fraction 3B (90 mg). The g.l.c. of fraction 3A showed the presence of methyl glycosides of 2,4-di-*O*-methylgalactose, and, after treat-

TABLE 5

Cleavage products from partially methylated acidic oligosaccharide fractions 3B(*i*)–3B(*iii*)

Fraction	Cleavage products*	Cleavage products after remethylation
3B(*i*)	2,3,4-Me_3 Glucuronic acid 2,3,4-Me_3 Galactose 2,3,6-Me_3 Galactose 2,4-Me_2 Galactose (tr)	2,3,4-Me_3 Glucuronic acid 2,3,4-Me_3 Galactose 2,3,6-Me_3 Galactose
3B(*ii*)	2,3,4-Me_3 Glucuronic acid 2,3,4-Me_3 Galactose (tr) 2,4-Me_2 Galactose	2,3,4-Me_3 Glucuronic acid 2,3,4-Me_3 Galactose
3B(*iii*)	2,3,4-Me_3 Glucuronic acid 2,4-Me_2 Galactose 2-Me Galactose†	2,3,4-Me_3 Glucuronic acid 2,3,4-Me_3 Galactose

*tr = trace.
†Detected by paper chromatography of hydrolysate.

ment with methanolic hydrogen chloride, of a small amount of 2,3,4-tri-*O*-methylgalactose. Methylation of fraction 3A, followed by methanolysis and acetylation of the methyl glycosides, afforded derivatives of 2,3,4,6-tetra-, and 2,3,4-, 2,3,6- (trace), and 2,4,6-tri-*O*-methylgalactose (g.l.c.), indicating the presence in fraction 3A of partially methylated neutral oligosaccharides containing galactose residues mutually joined by 1→3′ and 1→6′ linkages.

Methanolysis of fraction 3B afforded methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid, 2,3,6- and 2,3,4-tri-, and 2,4-di-*O*-methylgalactose [g.l.c. of methyl glycosides on column *a* and of acetylated methyl glycosides on column *c*]. A portion of fraction 3B was remethylated, and the product was methanolyzed and acetylated. The g.l.c. indicated the formation of derivatives of 2,3,4-tri-*O*-methylglucuronic acid and 2,3,4-tri-*O*-methylgalactose as major products, and in smaller amounts of 2,3,6- and 2,4,6-tri-*O*-methylgalactose.

In an attempt to separate partially methylated acidic oligosaccharides containing galactose residues unsubstituted at C-6, fraction 3B (75 mg) in pyridine (2 ml) was heated with triphenylmethyl chloride (80 mg) at 100° for 1 h. The t.l.c. indicated no formation of products of increased chromatographic mobility due to tritylation, but the unchanged components were separated by preparative t.l.c. to give fractions 3B(*i*), 3B(*ii*), and 3B(*iii*). Samples of these fractions were methanolyzed and the resulting methyl glycosides (column *b*) and/or the derived acetates (column *c*) were examined by g.l.c. Further samples of the fractions were remethylated and the methanolysis products were similarly examined. The results are summarized in Table 5.

Degraded Gum A

Gum acid (25.5 g) in water (1275 ml) was boiled under reflux for 24 h, and the cooled solution was poured into ethanol (6 l) containing 1% of hydrochloric acid. The precipitated degraded polysaccharide was twice reprecipitated, redissolved in water, and the solution was freeze-dried to give degraded gum A (7.0 g), $[\alpha]_D$ −8° (*c*, 1) [Found: uronic acid (carbazole method), 40%]. Hydrolysis of the degraded polysaccharide gave acidic sugars, galactose, and only a trace of arabinose.

Degraded gum A (5 g) was methylated, as described for mesquite gum, with methyl sulfate and 30% sodium hydroxide. The partially methylated polysaccharide was isolated as the silver salt (2.5 g) which was further methylated with methyl iodide and silver oxide to give methylated degraded gum A (1.2 g), $[\alpha]_D$ −5° (*c*, 1.0 $CHCl_3$), [Found: OMe, 43.6%]. A sample of the methylated polysaccharide was heated in methanolic 4% hydrogen chloride at 100° for 18 h, and after neutralization with silver carbonate the methanolysate was examined by g.l.c. on columns *a* and *b* [for retention times of methyl glycosides see Table 4]. The results are quoted in Table 1.

Carboxy-reduced Degraded Gum A

Degraded gum A (9 g) was kept in water (600 ml) containing ethylene oxide (100 ml) at room temperature for 3 days during which time the p*H* rose from 2 to 7.1. Excess of ethylene oxide was removed by evaporation, and the glycol ester (9.5 g) was removed by precipitation with ethanol, dissolved in water, and the solution was freeze-dried. The glycol ester (9.4 g) was treated with acetic anhydride and pyridine in formamide (24) to give glycol ester acetate (15.7 g). Lithium borohydride (16 g) in tetrahydrofuran (250 ml) was added to glycol ester acetate (15.5 g) in tetrahydrofuran (250 ml) with immediate formation of a precipitate. The reaction mixture was stirred at room temperature for 2 h, and then heated under reflux for 18 h with stirring. Excess of hydride was destroyed by the careful addition of water. The mixture was then acidified to p*H* 3 by the addition of dilute sulfuric acid, dialyzed, concentrated (to 200 ml), and freeze-dried to give carboxy-reduced degraded gum A (6.8 g), $[\alpha]_D$ +50° (*c*, 1.0). Hydrolysis of the polysaccharide gave galactose and 4-*O*-methylglucose, and small amounts of arabinose and glucose but no acidic sugars.

Degraded Gum B

Carboxy-reduced degraded gum A (6.8 g) was oxidized by sodium metaperiodate (17.12 g) in water (1000 ml) for 7 h [consumption of oxidant, measured spectrophoto-

metrically (25), was constant and corresponded to 0.87 mole of reagent per sugar residue]. The excess of periodate was destroyed by the addition of ethylene glycol, the solution was dialyzed to remove sodium iodate and concentrated, and potassium borohydride (5 g) was added in two portions during 3 days. The excess of borohydride was destroyed and sodium ions were removed by treatment of the solution with Amberlite resin IR-120(H), and the solution was concentrated several times with methanol to remove boric acid as methyl borate, and furnished polyalcohol as a syrup (3.6 g). The polyalcohol was hydrolyzed with *N* sulfuric acid at room temperature for 3 h, and the solution was neutralized with barium hydroxide and barium carbonate, filtered, and treated with Amberlite resin IR-120(H). The solution was then concentrated and poured into ethanol (6 volumes). The precipitated polysaccharide was washed with ethanol and dissolved in water, and the solution was freeze-dried to give degraded gum B (1.2 g), $[\alpha]_D$ +18° (*c*, 1.0). The supernatant solution from the above precipitation was concentrated to a syrup (2.0 g). Paper chromatography showed glycerol to be the main component and that no reducing sugars were present. Hydrolysis of the syrup gave small amounts of galactose and arabinose.

Hydrolysis of degraded gum B gave galactose and a trace of arabinose. Partial hydrolysis of the degraded gum in 0.5 *N* sulfuric acid at 100° for 1 h gave as major components oligosaccharides with the mobilities of 3-*O*-β-D-galactopyranosyl-D-galactose and the polymer-homologous trisaccharide, and as a minor component 6-*O*-D-galactopyranosyl-D-galactose. Degraded gum B (200 mg) was methylated with methyl iodide and sodium hydride in dimethylsulfoxide as described by Sandford and Conrad (26), and furnished methylated degraded gum B (83 mg), $[\alpha]_D$ −24° (*c*, 1.0 $CHCl_3$) [Found: OMe, 43.8%]. A sample of the methylated polysaccharide was methanolyzed and g.l.c. of the resulting methyl glycosides on columns *a* and *b* showed the presence of components listed in Table 2, the relative proportions being determined by measurement of peak areas on the chromatograms.

Degraded Gum C

Degraded gum B (800 mg) was oxidized with sodium metaperiodate [consumption of reagent, 0.78 mole per sugar residue], reduced with sodium borohydride, and hydrolyzed with *N* sulfuric acid at room temperature as described previously, to give degraded gum C (360 mg), $[\alpha]_D$ +19° (*c*, 1.0). Partial hydrolysis of the polysaccharide gave galactose as the sole monosaccharide, 3-*O*-galactopyranosylgalactose, and in small amount 6-*O*-galactopyranosylgalactose. Oxidation of the polysaccharide with sodium metaperiodate resulted in the consumption of 0.25 mole of reagent per sugar residue. Degraded gum C (80 mg) was methylated with methyl iodide and sodium hydride in dimethylsulfoxide to give methylated degraded gum C (30 mg), $[\alpha]_D$ −16° (*c*, 1.0 $CHCl_3$). A sample of the methylated polysaccharide was methanolyzed to give methyl glycosides of the sugars listed in Table 2 [g.l.c. on columns *a* and *b*].

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