

## THE STRUCTURE OF DEGRADED, MANGLE GUM

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### ABSTRACT

A degraded gum obtained from mangle gum by autohydrolysis consists of residues of L-arabinose, D-galactose, L-rhamnose, D-galacturonic acid, and 4-O-methyl-D-glucuronic acid in the molar proportions of 15 13 5 26 7 36 4 1. Electrophoretic data indicated that the degraded gum is homogeneous. Methylation studies showed that the polysaccharide has a highly branched structure. Hydrolysis of one mole of (a) the neutral part of the methylated polysaccharide yielded 2,3,5-tri-O-methyl-L-arabinose (4 moles), 2,3,4,6-tetra-O-methyl-D-galactose (1 mole), 3,4-di-O-methyl-L-rhamnose (1 mole), 2,5-di-O-methyl-L-arabinose (2 moles), 2,4,6-tri-O-methyl-D-galactose (1 mole), and 3-O-methyl-L-rhamnose (1 mole), and of (b) the acidic part of the methylated polysaccharide yielded seven sugars, namely, 2,3,4-tri-O-methyl-D-galacturonic acid (3 moles), 2,3-di-O-methyl-D-galacturonic acid (1 mole), 4-O-methyl-2-O-(2-O-methyl-D-galactopyranosyluronic acid)-L-rhamnose (4 moles), 3,4-di-O-methyl-2-O-(2-O-methyl-D-galactopyranosyluronic acid)-L-rhamnose (2 moles), 3-O-methyl-2-O-(2,3,4-tri-O-methyl-D-galactopyranosyluronic acid)-L-rhamnose (2 moles), 2,4,6-tri-O-methyl-3-O-(2,3,4-tri-O-methyl-D-galactopyranosyluronic acid)-D-galactose (2 moles), and 2,3,6-tri-O-methyl-4-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-D-galactose (2 moles). Periodate-oxidation data substantiated the results of methylation studies. Smith and Barry degradations were also performed. From all these results, a tentative structure for the degraded gum is given.

### INTRODUCTION

In a previous communication<sup>1</sup>, it was reported that, after being subjected to purification and fractionation, the mangle gum obtained from the South American plant *Rhizophora mangle* L. gave one fraction that was homogeneous. On graded hydrolysis, this yielded neutral and acidic oligosaccharides which were isolated and their structures established. For a degraded derivative of mangle gum that was obtained, a tentative structure has now been assigned to its repeating unit from the results of methylation, periodate oxidation, Smith degradation, and Barry degradation studies.

## RESULTS AND DISCUSSION

The homogeneous fraction of the mangle gum was subjected to autohydrolysis, and a degraded gum having  $[\alpha]_D^{30} + 30^\circ$  was isolated. Electrophoresis of this product on silica gel plates, with borate (pH 9.5) and phosphate (pH 8.0) buffers, showed that it was homogeneous. It was found to contain residues of galactose<sup>2</sup>, 13.5; arabinose<sup>3</sup>, 15.0; rhamnose<sup>4</sup>, 26.7; galacturonic acid<sup>5</sup>, 36.0; and glucuronic acid<sup>6</sup>, 4.1%.

The polysaccharide was methylated by Kuhn's method<sup>7</sup>, followed by Purdie's method<sup>8</sup>, to yield a fully methylated derivative which was subjected to methanolysis and then de-esterification. The acid fraction was adsorbed on a column of an anion-exchange resin, and the neutral sugar fraction in the eluate was hydrolyzed. It was found to contain six methylated sugars, which were separated into homogeneous fractions and identified, the mole proportion of each was also determined. The acid sugar portion was then displaced from the resin column and, after hydrolysis, was separated into its individual components. Each sugar was converted into its methyl ester methyl glycoside, which was reduced and the product hydrolyzed, two methylated monosaccharides and five methylated disaccharides were obtained. On further hydrolysis, the latter group yielded methylated sugars, these were separated into the individual components, which were then identified through the preparation of suitable, crystalline derivatives. A portion of the mixture of methylated, acid sugars was separated by quantitative, paper chromatography, and then amounts were determined by the dry-weight method. The results are given in Table I.

As the degraded gum contains 40% of uronic acid residues, it should theoretically be possible to obtain about 80% of the material as aldobiouronic acids, provided that one uronic acid unit is not linked glycosidically to another. Of the 38 sugar residues in the repeating unit of the degraded gum, 10 were found to be present in the neutral fraction, and 4 as monoglycuronic acid residues. The remaining 24 units are present as 12 aldobiouronic acid residues, of which 2-*O*-( $\alpha$ -D-galactopyranosyluronic acid)-L-rhamnose constitutes about 75%. From these results, it is obvious that the main chain of the polysaccharide is composed of aldobiouronic acid residues, namely, those of 2-*O*-( $\alpha$ -D-galactopyranosyluronic acid)-L-rhamnose. The O-3 atoms of all of the D-galacturonic acid residues, and O-3 of most of the L-rhamnose residues in the main chain, are glycosidically linked to other sugars. Of the units linked to acid sugar residues in the main chain, some are D-galacturonic acid residues which either form the nonreducing ends or are, in turn, joined through O-4 to other sugar units. The remaining aldobiouronic acid units are present as 4-*O*-(4-*O*-methyl- $\beta$ -D-glucopyranosyluronic acid)-D-galactose and 3-*O*-( $\beta$ -D-galactopyranosyluronic acid)-D-galactose. In these two aldobiouronic acids, the reducing-end sugars are not linked at any position other than O-1. For the degraded gum, one of the possible structures of the repeating unit that can accommodate all of these facts is shown in Fig. 1.

To verify the results of the methylation studies, the polysaccharide was subjected to periodate oxidation, the progress of the reaction being monitored by a spectrophotometric method<sup>9, 10</sup>. One molar proportion of hexose residue consumed 0.69 mole

TABLE I  
RESULTS OF METHYLATION ANALYSIS OF DEGRADED, MANGLE GUM

<i>Methylated sugars</i>	<i>Mole ratio</i>	<i>Mode of linkages</i>
<i>Neutral sugars</i>		
2,3,5-Tri- <i>O</i> -methyl-L-arabinose	4	L-Araf-(1→
2,3,4,6-Tetra <i>O</i> -methyl-D-galactose	1	D-Galp-(1→
3,4-Di- <i>O</i> -methyl-L-rhamnose	1	→2)-L-Rhap-(1→
2,5 Di- <i>O</i> -methyl-L-arabinose	2	→3)-L-Araf (1→
2,4,6-Tri- <i>O</i> -methyl-D galactose	1	→3)-L-Galp-(1→
3- <i>O</i> -Methyl-L-rhamnose	1	→2,4)-L-Rhap-(1→
<i>Acid sugars</i>		
2,3,4-Tri- <i>O</i> -methyl-D galactopyranuronic acid	2 8	D-GalpA-(1→
2,3 Di- <i>O</i> -methyl-D-galactopyranuronic acid	0 9	→4)-D-GalpA-(1→
4- <i>O</i> -Methyl-2- <i>O</i> (2- <i>O</i> -methyl-D-galactopyranosyluronic acid)-L-rhamnose	3 7	→4)-D-GalpA-(1→2)-L Rhap (1→ 3 3 ↑ ↑
3,4-Di- <i>O</i> -methyl-2- <i>O</i> (2- <i>O</i> methyl-D-galactopyranosyluronic acid) L-rhamnose	1 9	→4)-D-GalpA-(1→2)-L-Rhap (1→ 3 3 ↑
3- <i>O</i> -Methyl-2- <i>O</i> -(2,3,4-tri <i>O</i> -methyl-D-galactopyranosyluronic acid) L-rhamnose	1 8	D-GalpA-(1→2)-L-Rhap (1→ 4 ↑
2,4,6-Tri- <i>O</i> -methyl-3- <i>O</i> (2,3,4-tri- <i>O</i> -methyl-D galactopyranosyluronic acid) D-galactose	1 9	D-GalpA-(1→3)-Galp-(1→
2,3,6-Tri- <i>O</i> -methyl-4- <i>O</i> (2,3,4-tri- <i>O</i> -methyl-D glucopyranosyluronic acid)-D galactose	1 8	4- <i>O</i> -methyl D-GlcApA-(1→4)-D-Galp (1→



of the oxidant in 28 h, simultaneously liberating 1 mole of formic acid per 5.6 molar proportions of hexose residue. Assuming the correctness of the structure deduced from the results of methylation studies, the results for periodate uptake and formic acid liberation are 0.71 mole and 4.8 moles, respectively. The results are in reasonable agreement with the experimental values (within the limit of the experimental error).

Smith degradation<sup>13</sup> of the oxopolysaccharide yielded galactose, arabinose, rhamnose, and galacturonic acid, but no glucuronic acid. The proportions of the sugars resistant to periodate oxidation were estimated to be galactose, 8.5, arabinose, 12.9, rhamnose, 20.5, and galacturonic acid, 17.0%. The values calculated on the basis of the structure proposed were galactose, 10.5, arabinose, 5.3, rhamnose, 18.3, and galacturonic acid, 13.8%. The percentages of galactose, rhamnose, and galacturonic acid residues resistant to periodate are in reasonable agreement with the theoretical values, but, for arabinose, the expected value is less than that found experimentally. It is possible that some of the sugar residues at the nonreducing ends are linked at O-3 to other sugar units, but the methylation studies definitely indicate the presence of L-arabinofuranose residues as nonreducing ends. This discrepancy could not be resolved from the data thus far obtained.

On mild hydrolysis, the Smith-degraded product liberated galactose, rhamnose, and galacturonic acid (besides polyhydric alcohols, and other aldehydes). After treatment with acid, the resulting material was subjected to a second periodate oxidation, and it was found that some of the galactose, arabinose, rhamnose, and galacturonic acid residues resisted this second oxidation. This result is explicable if the structure contains a chain of (1→3)-linked arabinose residues, a chain of (1→3)-linked galactose residues, and rhamnose and galacturonic acid residues that are linked at O-3 to the (1→3)-linked side-chains.

Barry degradation<sup>14</sup> of the degraded gum yielded galacturonic acid, galactose, rhamnose, an aldatriuronic acid, and a reducing material that remained at the origin of a paper chromatogram. From the assigned structure (see Fig. 1) of the repeating unit of the degraded gum, galactose, rhamnose, and galacturonic acid should be present in the Barry-degraded product as monosaccharide residues. Besides an aldatriuronic acid containing rhamnose and galacturonic acid residues in the ratio of 2:1, another fragment, containing galactose, arabinose, rhamnose, and galacturonic acid residues, should also be present. Actually, the aldatriuronic acid obtained was found to contain rhamnose and galacturonic acid residues in the ratio of 2:1. The periodate-oxidation studies on this material suggest the structure depicted in Fig. 2. The material retained at the origin was electrophoretically homogeneous, and contained Gal, 22.0, Ara, 12.5, Rha, 32.3, and GalA, 30.4%. On the basis of the

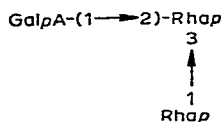


Fig. 2. Structure of fraction 1 isolated from the Barry-degraded product.

structure proposed, a large fragment of the polysaccharide, whose structure is given in Fig 3, should be obtained on Barry degradation of the degraded gum. This fragment should contain Gal, 23.0; Ara, 14.0, Rha, 30.0, and GalA, 30.0%. Thus, the results of periodate oxidation and Smith and Barry degradation gave further support to the structure assigned to the repeating unit of the degraded mangle gum.

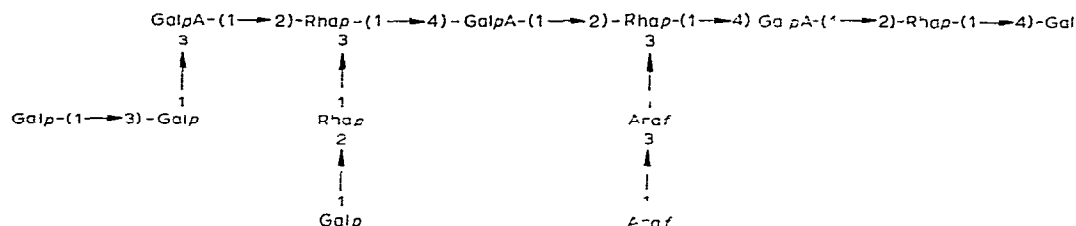


Fig 3 Structure of fraction 2 isolated from the Barry-degraded product

#### EXPERIMENTAL

**General** — All specific rotations are equilibrium values. Unless otherwise stated, all evaporations were conducted *in vacuo* at 30–40°. Whatman No. 1 MM filter paper was used for partition paper chromatography, and large quantities of sugar mixtures (up to 200 mg) were separated on Whatman No. 3 MM paper. The solvent mixtures (v/v) used for partition chromatography of sugars and their derivatives were (A) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, (B) upper layer of 4:1:5 1-butanol–acetic acid–water, (C) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water, (D) 8:2:1 ethyl acetate–pyridine–water, (E) upper layer of 4:1:5 1-butanol–ethanol–water, and (F) azeotrope of 2-butanone–water. The spray reagents used were (a) aniline oxalate, (b) aniline hydrogen phthalate, and (c) alkaline silver nitrate.

The kinetics of periodate oxidation were studied spectrophotometrically<sup>9,10</sup> and the amount of formic acid liberated was determined by titrating it with standard sodium hydroxide.<sup>11</sup> The mole fraction of each methylated sugar was determined by the alkaline hypoiodite method.

**Autohydrolysis of the purified gum, and isolation of the degraded gum** — A solution of the purified gum (1 g) in water (50 ml) was de-ionized with Amberlite IR-120 (H<sup>+</sup>) ion-exchange resin, and the resulting solution (pH 3–4) was diluted to 100 ml with water and heated on a boiling-water bath. At regular intervals, a small portion of the hydrolyzate was added to an excess of ethanol (to precipitate the hydrolyzed material) and the supernatant liquor was examined by paper chromatography. Arabinose was detected first, and then two slow-moving spots, after 9 h, galactose began to appear.

The gum (10 g) was, therefore, subjected to autohydrolysis for 9 h as just described, and the product was dialyzed against distilled water. The degraded gum was isolated by lyophilizing the dialyzate; yield 8.29 g, moisture, 3%,  $[\alpha]_D^{30} +30^\circ$  (c 0.1, water), OMe (found), 1.2%. The degraded gum was found to contain

galactose<sup>2</sup>, 13.5, arabinose<sup>3</sup>, 15.0, rhamnose<sup>4</sup>, 26.7, galacturonic acid<sup>5</sup>, 36.0, and glucuronic acid<sup>6</sup>, 4.1%

*Methylation of the degraded gum* — The degraded gum was methylated according to Kuhn's method<sup>7</sup>. Dried, degraded gum (1.4 g) was well dispersed in dimethyl sulfoxide (70 ml), and the suspension was diluted with *N,N*-dimethylformamide (35 ml). A mixture of barium oxide (11.3 g) and barium hydroxide (11.3 g) was added in small lots, followed by dimethyl sulfate (25 ml), while it was stirred at 0° under a nitrogen atmosphere. After 2 h, the mixture was made alkaline with liquid ammonia, and extracted with chloroform. The extract was concentrated, and the partially methylated polysaccharide was precipitated with acetone, yield, 1.2 g. This product (1 g) was subjected to Purdie methylation<sup>8</sup> six times, the product was then considered to be fully methylated, as it showed no OH band in its infrared spectrum, yield 900 mg,  $[\alpha]_D^{30} -34^\circ$  (c 0.5, chloroform), OMe, 42.0%.

*Methanolysis, and separation of the neutral and acidic components of the methylated, degraded gum* — A solution of the methylated, degraded gum (600 mg) in dry, methanolic hydrogen chloride (2.5%, 20 ml) was boiled for 16 h under reflux, the optical rotation of the solution had then become constant. The solvent was removed under diminished pressure, and the resulting syrup was dissolved in water. The solution was neutralized ( $\text{Ag}_2\text{CO}_3$ ), the precipitate was centrifuged off, and the solution was evaporated to a syrup, this was heated with 2% barium hydroxide solution for 4 h at 80°, and the base was then neutralized by passing in carbon dioxide gas. The solid was centrifuged off, and washed with warm water. The resulting clear solutions were combined, and passed through a column of Amberlite IR-120 ( $\text{H}^+$ ) ion-exchange resin, and then through a column of Dowex-1 X-4 ( $\text{HCO}_3^-$ ) resin to adsorb the acid sugars. The Dowex column was washed with water (2 litres), and the neutral solution and washings were combined and concentrated.

The neutral solution (10 ml), containing the methyl glycosides of the neutral sugars, was hydrolyzed with 0.5M sulfuric acid for 12 h on a boiling-water bath (until the optical rotation was constant). The solution was then cooled, made neutral with  $\text{BaCO}_3$ , the suspension centrifuged, and the supernatant liquor de-ionized and evaporated to a syrup (220 mg). On paper-chromatographic examination of the syrup (solvent *F*), spots corresponding to six methylated sugars were detected.

The mixture (200 mg) was separated into its components on thick filter-paper, and each of the individual methylated sugars was obtained in a homogeneous state. They were identified through their specific rotations, by other properties of the crystalline products, or by preparation of suitable derivatives (see Table II).

*Examination of the acidic components of the methylated, degraded gum* — The column of Dowex-1 X-4 resin which had adsorbed the acid sugars from the hydrolyzate was eluted with 0.2M sulfuric acid. The eluate was made neutral with  $\text{BaCO}_3$ , the suspension was filtered, and the solid was washed with water. The filtrate and washings were combined, passed through a column of Amberlite IR-120 ( $\text{H}^+$ ) resin, and the eluate evaporated to a syrup. The resulting methyl glycosides were hydrolyzed with 0.05M sulfuric acid for 6 h on a boiling-water bath, and the hydrolyzate was made

TABLE II  
ANALYSIS OF THE NEUTRAL FRACTION OF THE METHYLATED, DEGRADED GUM

Fraction no	Methylated sugars (yield in mg)	$[\alpha]_D^{30}$ (degrees)	Derivative or crystalline product		
			Name	$[\alpha]_D^{30}$ (degrees)	<i>M p.</i> (degrees)
1	2,3,5-Tri- <i>O</i> -methyl-L-arabinose (72)	-39 (lit <sup>15</sup> -44)	amide	+17 [lit +16 (ref 15), +20 (ref 16)]	134-136 [lit 138 (ref 16), 136-138 (ref 17)]
2	2,3,4,6-Tetra- <i>O</i> -methyl D-galactose (14)	+110 (lit <sup>18</sup> +104.5)	anilide	+41 (lit <sup>21</sup> +39)	185 [lit 187 (ref 19), 186-188 (ref 20)]
3	3,4-Di- <i>O</i> -methyl-L-rhamnose (32)	+13 (lit <sup>23</sup> +18.6)	crystalline product		95 (lit <sup>23</sup> 95-96)
4	2,5-Di- <i>O</i> -methyl-L-arabinose (32)	+80 (lit <sup>24</sup> +83)	amide	+41 (lit <sup>24</sup> +38)	129-130 (lit <sup>24</sup> 132)
5	2,4,6-Tri- <i>O</i> -methyl-D-galactose (14)	+87 (lit <sup>25</sup> +91.6)	anilide		175 [lit 178 (ref 26), 175 (ref. 27)]
6	3- <i>O</i> -Methyl-L rhamnose	+32 (lit <sup>28</sup> +35)	crystalline product		110 [lit 113 (ref 28), 114-115 (ref 29)]



neutral and filtered. The filtrate was passed through a column of Amberlite IR-120 ( $H^+$ ) resin, and the eluate was evaporated to a syrup (330 mg). On paper-chromatographic examination with solvent *E*, seven spots were detected.

The mixture of methylated, acid sugars (300 mg) was separated into its components by paper chromatography with solvent *E*. Each fraction was converted into its methyl ester methyl glycoside, and each of these was reduced with lithium aluminum hydride in dry ether. Hydrolysis followed by the usual treatments yielded a methylated, neutral sugar from each (see Table III).

*Periodate oxidation of the degraded gum* — The degraded gum (2 mg) was treated with 0.1M sodium metaperiodate in the dark at 0°. Consumption of the oxidant and liberation of formic acid became constant in 27 h, corresponding to 0.69 mole of oxidant per mole of hexose residue and 5.6 moles of hexose residue per mole of formic acid.

*Smith degradation* — The periodate-oxidized, degraded gum (175 mg) was reduced with sodium borohydride. Part (5 mg) of the resulting material was hydrolyzed with 0.5M sulfuric acid for 12 h at 100°. The hydrolyzate was made neutral with  $BaCO_3$ , treated in the usual way, and examined chromatographically. Besides spots corresponding to lower polyhydric alcohols, aldehydes, and acids, zones of galactose, arabinose, and galacturonic acid were detected. The proportion of each sugar resistant to periodate oxidation in the reduced, periodate-oxidized, degraded gum was estimated to be: galactose<sup>2</sup>, 8.5, arabinose<sup>3</sup>, 12.9, rhamnose<sup>4</sup>, 20.4, and galacturonic acid<sup>5</sup>, 18.2%.

A portion (175 mg) of the reduced, periodate-oxidized, degraded gum was kept with 0.5M sulfuric acid (40 ml) for 3 days at room temperature, and then the solution was made neutral, the suspension was filtered, and the filtrate was concentrated to a small volume. On paper-chromatographic examination, in addition to galacturonic acid, rhamnose, and galactose, one more spot was detected at the origin. This mixture (30 mg) was subjected to a second periodate oxidation for 2 days at 0° in the dark. Iodate and periodate ions were removed as the insoluble barium salts, and the resulting solution was concentrated to 5 ml. On complete hydrolysis of the twice-oxidized material, followed by the usual treatments and paper-chromatographic examination, spots corresponding to galactose, arabinose, rhamnose, and galacturonic acid were detected.

*Barry degradation* — A mixture of periodate-oxidized, degraded gum with 45% ethanol (50 ml), phenylhydrazine (1.5 ml), and acetic acid (1.5 ml) was heated for 1.5 h on a steam bath. The solution was concentrated to 20 ml, and then extracted with ether. Chromatographic examination of the ether layer did not show the presence of any sugar or its derivative. On chromatographic examination, the aqueous layer showed the presence of galacturonic acid, galactose, rhamnose, and one spot at the origin.

The aqueous layer was concentrated to 5 ml, and then heated for 8 h on a steam bath with benzoic acid (950 mg) and benzaldehyde (0.7 ml) in ethanol (10 ml). The solution was evaporated to dryness, the product was dissolved in water, and the

TABLE III

ANALYSIS OF METHYLATED, ACID SUGARS

Fraction no	Methylated sugars (yield in mg)	Neutral sugars obtained on reduction and hydrolysis	[ $\alpha$ ] <sub>D</sub> <sup>20</sup> (degrees)	Derivative or crystalline product		
				Name	M p (degrees)	[ $\alpha$ ] <sub>D</sub> <sup>20</sup> (degrees)
1	2,3,4-Tri- <i>O</i> -methyl-D-galactopyranuronic acid (42)	2,3,4-tri- <i>O</i> -methyl-D galactose	+ 114 (lit <sup>30</sup> 114)	amide	165 (lit. <sup>31</sup> 166)	+ 37 (lit. <sup>33</sup> + 43)
2	2,3 Di- <i>O</i> -methyl-D galactopyranuronic acid (15)	2,3-di- <i>O</i> -methyl-D galactose	+ 10.3 (lit <sup>34</sup> + 11.3)	amide	130 (lit <sup>34</sup> 130-131)	+ 118 (lit <sup>34</sup> + 119.4)
3	4- <i>O</i> -Methyl-2- <i>O</i> -(2- <i>O</i> -methyl-D galactopyranosyluronic acid)-L-rhamnose	4- <i>O</i> methyl-L-rhamnose	+ 130 (lit <sup>35</sup> 150)	crystalline product	80 (lit <sup>36</sup> 82)	—
4	3,4-Di- <i>O</i> -methyl-2- <i>O</i> -(2- <i>O</i> -methyl-D-galactopyranosyluronic acid)-L-rhamnose (35)	2- <i>O</i> -methyl-D-galactose	+ 78 (lit <sup>28</sup> + 80)	amide	163-164 (lit <sup>28</sup> 163)	—
5	3- <i>O</i> -Methyl-2- <i>O</i> -(2,3,4-tri- <i>O</i> -methyl-D-galactopyranosyluronic acid)-L-rhamnose (32)	2- <i>O</i> -methyl-D-galactose	same as in acid fraction 3	same as in acid fraction 3		
6	2,4,6-Tri- <i>O</i> -methyl-3- <i>O</i> -(2,3,4-tri- <i>O</i> -methyl-D-galactopyranosyluronic acid)-D-galactose (30)	3,4-di- <i>O</i> -methyl-L-rhamnose	same as in neutral fraction 6	same as in neutral fraction 6		
7	2,3,6-Tri- <i>O</i> -methyl-4- <i>O</i> -(2,3,4-tri- <i>O</i> -methyl-D-glucopyranosyluronic acid) D galactose (30)	2,3,4-tri- <i>O</i> -methyl-D galactose	+ 87 (lit <sup>25</sup> + 91.6)	amide	175 [lit 178 (ref 26), 174 (ref 27)]	+ 36 (lit. <sup>37</sup> + 38)
		2,3,6-tri- <i>O</i> -methyl-D-galactose	+ 78 (lit <sup>39</sup> + 79.4)	lactone	96-97 [lit. 97-98 (ref 40), 99 (ref 41), 101 (ref 42)]	—
		2,3,4-tri- <i>O</i> -methyl-D-glucose	+ 58 (lit <sup>29</sup> + 62)	amide	152 (lit. <sup>29</sup> 150)	— 100 (lit. <sup>38</sup> — 103)

solution was exhaustively extracted with chloroform and then with ether. The chloroform and ether extracts were combined, the solvents were evaporated off, and the resulting syrup showed no spots corresponding to any sugars.

The aqueous layer was concentrated, and examined by paper chromatography with solvents *A*, *B*, and *D*, it was found to contain galacturonic acid, galactose, and rhamnose, and two other spots were noted (one having  $R_{\text{Gal}} 0.16$  in solvent *A*, and the other at the origin). A portion (60 mg) of the mixture was separated on thick filterpapers (with solvent *A*) into its components, and the zones containing the slow-moving spot (fraction 1) and the stationary compound (fraction 2) were cut out, and eluted with water. The resulting solutions were evaporated to syrups, yield, fraction 1, 11.7 mg, and fraction 2, 18.7 mg. Fraction 1: this syrup had  $[\alpha]_{\text{D}}^{30} + 28^\circ$  (*c* 0.5, water) and mol wt 510, it was found to be homogeneous by electrophoresis. It contained residues of galacturonic acid (30%) and rhamnose (62%). A portion (6 mg) was converted into its methyl ester methyl glycoside, and this was subjected to periodate oxidation. The periodate uptake became constant in 2.5 h, and corresponded to 3.9 moles of the oxidant per mole of the methyl ester methyl glycoside. Fraction 2: the syrup, having  $[\alpha]_{\text{D}}^{30} + 120^\circ$  (*c* 0.1, water), moved as a single compound on electrophoresis. The material contained residues of galactose, 22.8, arabinose, 12.5, rhamnose, 32.3, and galacturonic acid, 30.5%.

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