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THE STRUCTURE OF DEGRADED BAEL (*Aegle marmelos*) GUM*

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ABSTRACT

Mild, acid hydrolysis of purified bael gum yielded a homogeneous, degraded product consisting of residues of L-arabinose, D-galactose, L-rhamnose, and D-galacturonic acid in the molar proportions of 1:19:1:2. The homogeneity of this degraded gum was proved from electrophoretic data. Hydrolysis of one mole of the fully methylated polysaccharide gave (*a*) from the neutral part, 2,3,4,6-tetra-*O*-methyl-D-galactose (12 moles), 3,4-di-*O*-methyl-L-rhamnose (2 moles), 2,5-di-*O*-methyl-L-arabinose (1 mole), 2,4,6-tri-*O*-methyl-D-galactose (8 moles), 2,3-di-*O*-methyl-L-arabinose (1 mole), 2,4-di-*O*-methyl-D-galactose (12 moles), and 2-*O*-methyl-D-galactose (2 moles), and (*b*) from the acidic part, 2,3,4-tri-*O*-methyl-D-galacturonic acid (1 mole), 2,4,6-tri-*O*-methyl-3-*O*-(2,3,4-tri-*O*-methyl-D-galactopyranosyluronic acid)-D-galactose (2.5 moles), and 2,4,6-tri-*O*-methyl-3-*O*-[2,4,6-tri-*O*-methyl-3-*O*-(2,3,4-tri-*O*-methyl-D-galactopyranosyluronic acid)-D-galactopyranosyl]-D-galactose (1 mole). The results of periodate oxidation were in agreement with those of methylation studies. Smith, and Barry, degradation studies on the degraded gum were also conducted. From the results of all these experiments, a tentative structure for the average repeating-unit of the degraded gum is proposed.

INTRODUCTION

In a previous communication[1], it was reported that, on mild, acid hydrolysis under controlled conditions, the purified gum from bael fruit (*Aegle marmelos*) yielded degraded gum ($[\alpha]_D$ +44°), galactose (79.5%), arabinose (3.5%), galacturonic acid (8.6%), and rhamnose (4.2%). When examined by paper electrophoresis, the degraded gum was found to be homogeneous. The oligosaccharides obtained by graded hydrolysis of this degraded gum were also characterized.

We now report the results of methylation, periodate oxidation, and Smith, and Barry, degradation studies on the degraded product.

*Structural Investigations on Bael (*Aegle marmelos*) Gum, Part II. For Part I, see ref. 1.

RESULTS AND DISCUSSION

The homogeneous, degraded gum was methylated by the Hakomori method[2], followed by Purdie methylation[3], to yield a fully methylated derivative which was subjected to methanolysis and then de-esterification. The acid fraction was adsorbed on a column of anion-exchange resin, and the neutral fraction was hydrolyzed. Seven methylated sugars were detected in the hydrolyzate, separated into homogeneous fractions, and identified; the mole proportion of each was also determined. The results are given in Table I. The acid sugar portion was displaced from the resin column and, on hydrolysis, gave a mixture of three components. These were separated, and each was converted into its methyl ester methyl glycoside, which was reduced and the product hydrolyzed; one methylated monosaccharide, one methylated disaccharide, and one methylated trisaccharide were obtained. On further hydrolysis, the last two yielded methylated sugars which were separated into individual components, and these identified through the preparation of suitable, crystalline derivatives. A portion of the mixture of methylated acid sugars was separated by quantitative, paper chromatography, and their amounts were determined by the dry-weight method. The results are given in Table I.

TABLE I

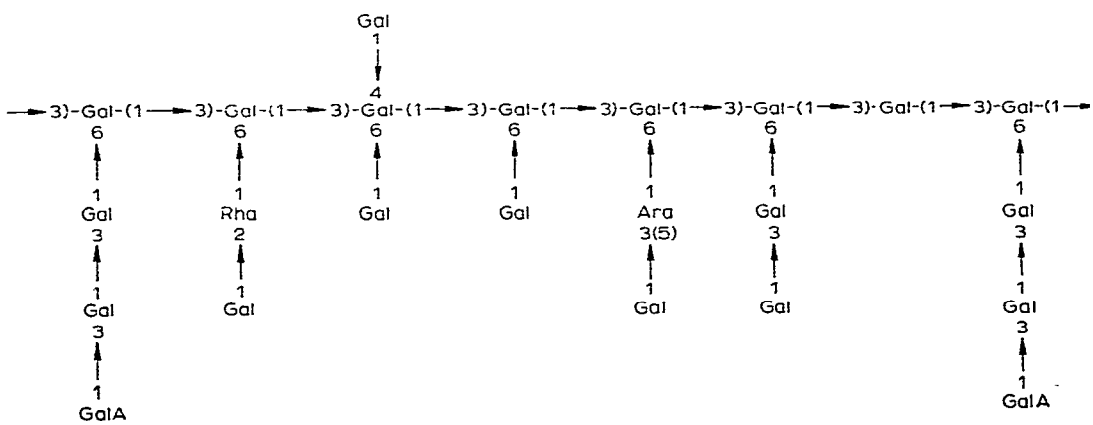
RESULTS OF METHYLATION ANALYSIS OF DEGRADED BAEL GUM

Methylated sugars	*Approximate mole ratio*	*Mode of linkage*
Neutral sugars		
2,3,4,6-Tetra-*O*-methyl-D-galactose	12	D-Gal*p*-(1→
3,4-Di-*O*-methyl-L-rhamnose	2	→2)-L-Rha*p*-(1→
2,5-Di-*O*-methyl-L-arabinose	1	→3)-L-Ara*f*-(1→
2,4,6-Tri-*O*-methyl-D-galactose	8	→3)-D-Gal*p*-(1→
2,3-Di-*O*-methyl-L-arabinose	1	→5)-L-Ara*f*-(1→
2,4-Di-*O*-methyl-D-galactose	12	→3,6)-D-Gal*p*-(1→
2-*O*-Methyl-D-galactose	2	→3,4,6)-D-Gal*p*-(1→
Acid sugars		
2,3,4-Tri-*O*-methyl-D-galactopyranosyluronic acid	1	D-Gal*p*A-(1→
2,4,6-Tri-*O*-methyl-3-*O*-(2,3,4-tri-*O*-methyl-D-galactopyranosyluronic acid)-D-galactose	2.5	D-Gal*p*A-(1→3)-D-Gal*p*-(1→
2,4,6-Tri-*O*-methyl-3-*O*-[2,4,6-tri-*O*-methyl-3-*O*-(2,3,4-tri-*O*-methyl-D-galactopyranosyluronic acid)-D-galactopyranosyl]-D-galactose	1	D-Gal*p*A-(1→3)-D-Gal*p*-(1→3)-D-Gal*p*-(1→

From these results, it is possible to assign a structure to the average repeating-unit of the degraded polysaccharide from bael gum. Isolation (and characterization) of 2,3,4,6-tetra-*O*-methyl-D-galactose from the neutral fraction of the hydrolyzate, and of 2,3,4-tri-*O*-methyl-D-galacturonic acid from the acid fraction, and the absence of any tri-*O*-methylpentose, indicates that D-galactopyranosyl and D-galacto-

pyranosyluronic acid groups constitute the nonreducing ends. The interior part of the molecule is made up of D-galactopyranosyl, L-arabinofuranosyl, and L-rhamnopyranosyl residues, of which D-galactopyranosyl residues constitute the major portion; this is evident from the fact that the tri-, di-, and mono-*O*-methyl-D-galactose residues constitute 22 molar proportions compared to only 2 of di-*O*-methyl-L-rhamnose and 2 of di-*O*-methyl-L-arabinose. Presence of 2,4-di-*O*-methyl-D-galactose indicates that the molecule is highly branched and that, at the branch points, D-galactopyranosyl residues are joined through O-1, O-3, and O-6; characterization of 2-*O*-methyl-D-galactose reveals that some of the D-galactosyl residues are linked through O-1, O-3, O-4, and O-6. Demonstration of the presence of only 2,4,6-tri-*O*-methyl-D-galactose in the tri-*O*-methyl fraction indicates that the backbone chain is composed of (1→3)-linked D-galactosyl residues, and this was confirmed by the results of Smith, and Barry, degradation studies. Isolation (and characterization) of 3,4-di-*O*-methyl-L-rhamnose, 2,5-di-*O*-methyl-L-arabinose, and 2,3-di-*O*-methyl-L-arabinose from the hydrolyzate indicates that the unbranched, interior parts of the molecule consist of (1→2)-linked L-rhamnosyl, and (1→3)- and (1→5)-linked L-arabinosyl, residues.

In the acid fraction, most of the D-galacturonic acid was obtained as aldobiouronic acids. As the degraded gum contained 8.6% of D-galactosyluronic acid residues, it should theoretically be possible to obtain about 17.2% of the material as aldobiouronic acids, provided that one uronic acid residue is not linked glycosidically to another. Of the 23 sugar residues in the average repeating-unit of the degraded gum, 19 were found to be present in the neutral fraction, and the four residues remaining are present as two aldobiouronic acid residues. For every singly branched unit in the molecule, there should be one nonreducing end-group, and, for every doubly branched residue, there should be two nonreducing end-groups. In one mole of methylated, degraded gum there were present 12 moles of 2,4-di-*O*-methyl-D-galactose and 2 moles of 2-*O*-methyl-D-galactose residues, requiring, altogether, 16 moles of nonreducing end-groups. In the hydrolyzate of the methylated product, 12 moles of 2,3,4,6-tetra-*O*-methyl-D-galactose and 4.5 moles of 2,3,4-tri-*O*-methyl-D-galacturonic acid equivalents were obtained as the nonreducing ends. The simplest structure which can accommodate all of these facts is as follows.



where Gal represents a D-galactopyranosyl residue or group; GalA, a D-galactopyranosyluronic acid group; Rha, an L-rhamnopyranosyl residue; and Ara, an L-arabinofuranosyl residue.

This structure describes the general nature of the linkages between the various monosaccharide units. Graded-hydrolysis studies gave additional evidence regarding the existence of such branches as those shown. This structure does not, however, depict the correct sequence of different branches present in the molecule. The isolation of 2,3-di- and 2,5-di-*O*-methyl-L-arabinose in equal proportions indicates that some of the L-arabinose-containing branches are (1→3)- and some are (1→5)-linked.

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[4,5]. The polysaccharide consumed 0.80 mole of periodate, liberating 0.40 mole of formic acid, per molar equivalent of hexose residue in ~10 h. From theoretical considerations, a polysaccharide with the structure shown would liberate 0.34 mole of formic acid and consume 0.76 mole of periodate per molar equivalent of hexose residue. The experimental results are, within the limits of experimental error, in reasonable agreement with the calculated values. All of the (nonreducing) end-groups, the L-rhamnose residues, and the L-arabinose residues joined through O-1 and O-5 linkages in the chain were oxidized by periodate. The D-galactose residues present in the interior of the molecule are joined through O-1 and O-3, or O-1, O-3, and O-6, or O-1, O-3, O-4, and O-6, and, as such, are immune to reaction with periodate; as these residues constitute the bulk of the molecule, a large part of the molecule (consisting mostly of D-galactose residues) would be expected to remain unoxidized on periodate oxidation of the degraded gum. This was found to be correct from the results of Smith-degradation studies[6]. The Smith-degraded product had a high molecular weight (2056) and contained 94% of galactose and ~5% of arabinose. When this substance was subjected to a second periodate oxidation and the oxidation product was reduced with sodium borohydride, a material of reasonably high molecular weight and consisting only of galactose residues was obtained. Isolation of such a product after the second periodate oxidation leads to the conclusion that only galactose residues are present in the backbone chain of the molecule, and justifies the placement of L-rhamnosyl and L-arabinosyl residues in the side chains. Part of the L-arabinosyl residues that survived the first periodate oxidation were those joined through O-1 and O-3, and these were vulnerable to the second periodate oxidation. Hence, it may be concluded that O-3 in each of these L-arabinose residues is glycosidically linked to a residue that was oxidized by the first periodate oxidation. Therefore, a D-galactose-containing material is obtained after two successive periodate oxidations of the degraded gum, as would be expected from a polysaccharide having the structure depicted.

The results of Barry degradation[7] also support these contentions. The periodate-oxidized material was subjected to Barry degradation, and, after the usual treatment of the product, the unoxidized portion was recovered. No monosaccharide was present,

but a polymeric substance was isolated from the origin of a paper chromatogram; this material contained 95% of galactose and ~5% of arabinose. These results are in good agreement with those expected on the basis of the structure shown.

The results of all these experiments therefore support the structure proposed for the average repeating-unit of the molecule. There may be some occasional linkages, vulnerable to periodate, lying between such units in the molecule of the whole polysaccharide.

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. Spray reagents used were: (*a*) aniline oxalate, (*b*) aniline hydrogen phthalate, and (*c*) alkaline silver nitrate.

The kinetics of periodate oxidation were studied spectrophotometrically[4,5] and the amount of formic acid liberated during the reaction was determined by titrating it with standard sodium hydroxide solution[8]. The mole fraction of each methylated sugar was determined by the alkaline hypoiodite method.

Isolation of the degraded gum. — The purified bael gum (8 g) was heated with 0.05M oxalic acid for 75 min on a boiling-water bath to obtain the degraded gum[1]. The degraded polysaccharide was electrophoretically homogeneous; moisture, 0.3%; ash (as Li), 2.6%; galacturonic acid[9], 3.6%; galactose[10], 79.5%; arabinose[11], 3.8%; and rhamnose[12], 4.2%.

Methylation of the degraded gum. — The degraded gum (1.5 g) was methylated by the Hakomori method[2]. The product was remethylated by the Purdie method[3], to yield a fully methylated derivative showing no OH absorption band in its infrared spectrum; yield 1.65 g, $[\alpha]_D^{30}$ −5° (*c* 1.4, chloroform); found: OMe, 41.0%.

Methanolysis, and separation of the neutral and acidic components of the methylated, degraded gum. — A solution of the methylated, degraded gum (1.5 g) in dry, methanolic hydrogen chloride (2.5%, 90 ml) was boiled under reflux for 16 h; 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 made neutral (Ag_2CO_3), the precipitate was centrifuged off, and the solution was evaporated to a syrup; this was heated with 2% barium hydroxide solution (90 ml) for 4 h at 80°, the solution was made neutral by passing in carbon dioxide gas, and the solid was centrifuged off. The residue was washed several times with warm water, and the washings were combined with the main solution. The

resulting, clear solution was then passed successively through columns of Amberlite IR-120 (H^+) and Dowex I X-4 (HCO_3^-) resin. The Dowex column was washed thoroughly with water (5 litres), and the neutral solution and washings were combined and concentrated.

The mixture containing the methyl glycosides of the neutral sugars was hydrolyzed with 0.5M sulfuric acid on a boiling-water bath until the optical rotation was constant (12 h). The solution was cooled, made neutral ($BaCO_3$), the suspension centrifuged, and the supernatant liquor de-ionized and then evaporated to a syrup (1.2 g). On paper-chromatographic examination (solvent *E*), 7 methylated sugars were detected.

The mixture was separated into its components on thick filter-papers, and each methylated sugar was obtained homogeneous. They were identified through their specific rotations, and by preparing crystalline products. The results are given in Table II.

Examination of the acidic components of the methylated, degraded gum. — The Dowex column (which had adsorbed the methylated, acidic sugars) was thoroughly washed with distilled water (4 litres), and the acidic fraction was displaced from the resin column with 0.5M sulfuric acid (100 ml). The eluate was made neutral with barium carbonate, the suspension filtered, and the filtrate de-ionized with Amberlite IR-120 (H^+), and evaporated to a syrup (240 mg), which was hydrolyzed by heating it with 0.25M sulfuric acid (25 ml) for 6 h on a boiling-water bath. The hydrolyzate was made neutral with barium carbonate, and the suspension filtered; the filtrate and washings were combined, passed through a column of Amberlite IR-120 (H^+) resin, and the eluate evaporated to a syrup. On paper-chromatographic examination (solvent *E*), the mixture gave three spots. It was separated on Whatman No. 3 MM filter paper into its components, and each of the three fractions was isolated in homogeneous state. The individual sugars were converted into their methyl ester methyl glycosides, and these were each reduced with lithium aluminium hydride in dry ether. Hydrolysis followed by the usual treatment yielded neutral sugars which were characterized. The results are given in Table III.

Periodate oxidation of the degraded gum. — The degraded gum was treated with 0.1M sodium metaperiodate in the dark at 0°. Consumption of the oxidant and liberation of formic acid became constant within 10 h, corresponding to consumption of 0.80 mole of the oxidant and liberation of 0.40 mole of formic acid per molar equivalent of hexose residue.

Smith degradation. — The periodate-oxidized, degraded gum (200 mg) was reduced with sodium borohydride. Part (5 mg) of the resulting material was hydrolyzed with 0.5M sulfuric acid for 18 h at 100°. The hydrolyzate was made neutral ($BaCO_3$) and, after the usual treatment, was examined chromatographically (solvent *B*). Besides spots corresponding to lower polyhydric alcohols and aldehydes, galactose and arabinose were detected. The proportion of each sugar resistant to periodate oxidation was estimated to be: galactose[10], 94%; and arabinose[11], 5%.

A portion of the reduced, periodate-oxidized, degraded gum (50 mg) was kept

TABLE II

ANALYSIS OF THE NEUTRAL FRACTION OF THE METHYLATED, DEGRADED GUM

Fraction No.	*Methylated sugars*	*Yield (mg)*	$[\alpha]_D^{30}$ *(degrees)*	*Derivative or crystalline product*		
				Name	$[\alpha]_D^{30}$ *(degrees)*	*M.p. (degrees)*
1	2,3,4,6-Tetra-*O*-methyl-D-galactose	330.6	+110 (lit.[13] +104.5)	anilide	+41 (lit.[14] +39)	186 (lit.[15] 187)
2	3,4-Di-*O*-methyl-L-rhamnose	49.3	+13 (lit.[16] +18.6)	crystalline		95 (lit.[16] 95–96)
3	2,5-Di-*O*-methyl-L-arabinose	25.3	+80 (lit.[17] +83)	amide	+41 (lit.[17] +38)	130 (lit.[17] 132)
4	2,4,6-Tri-*O*-methyl-D-galactose	225.6	+87 (lit.[18] +91.6)	anilide	+36 (lit.[19] +38)	175 (lit.[20] 178)
5	2,3-Di-*O*-methyl-L-arabinose	26.8	+102 (lit.[14] +101)	anilide		133 (lit.[21] 138)
6	2,4-Di-*O*-methyl-D-galactose	313.4	+78 (lit.[22] +85)	anilide		210 (lit.[23] 216)
7	2-*O*-Methyl-D-galactose	47.4	+80 (lit.[24] +83)	anilide		161 (lit.[24] 165)

TABLE III

ANALYSIS OF METHYLATED ACID SUGARS

Fraction No.	*Methylated sugars*	*Yield (mg)*	*Neutral sugars obtained on reduction and hydrolysis*	$[\alpha]_D^{30}$ *(degrees)*	*Derivative*		
					Name	*M.p. (degrees)*	$[\alpha]_D^{30}$ *(degrees)*
1	2,3,4-Tri-*O*-methyl-D-galacturonic acid	20.0	2,3,4-tri-*O*-methyl-D-galactose	+111 (lit.[25] +114)	anilide	160 (lit.[26] 166)	+37 (lit.[22] +43)
2	2,4,6-Tri-*O*-methyl-3-*O*-(2,3,4-tri-*O*-methyl-D-galactopyranosyluronic acid)-D-galactose	107.3	2,4,6-tri-*O*-methyl-D-galactose + 2,3,4-tri-*O*-methyl-D-galactose	+87 (lit.[18] +91.6) same as in acid fraction 1	anilide	175 (lit.[20] 178)	+36 (lit.[19] +38)
3	2,4,6-Tri-*O*-methyl-3-*O*-[2,4,6-tri-*O*-methyl-3-*O*-(2,3,4-tri-*O*-methyl-D-galactopyranosyl-uronic acid)-D-galactopyranosyl]-D-galactose	64.0	2,3,4-tri-*O*-methyl-D-galactose + 2,4,6-tri-*O*-methyl-D-galactose	same as in acid fraction 1 same as in acid fraction 2			

with 0.5M sulfuric acid (25 ml) for two days at room temperature and then made neutral with barium carbonate, the suspension filtered, and the filtrate concentrated to a small volume. Chromatographic examination of this solution (solvents *C* and *D*) revealed the presence of galactose only. The mixture (20 mg) was subjected to a second periodate-oxidation at 3° in the dark. Iodate and periodate ions were removed as the insoluble barium salts, and the resulting solution was concentrated to ~1 ml. On complete hydrolysis of this material, followed by the usual treatments and paper-chromatographic examination (solvents *C* and *D*), only a spot corresponding to galactose was detected.

Barry degradation. — A mixture of periodate-oxidized, degraded gum (150 mg), 9:11 ethanol–water (14 ml), phenylhydrazine (0.45 ml), and acetic acid (0.45 ml) was heated for 1.5 h on a steam bath, concentrated to 2 ml, and extracted with ether. Chromatographic examination of the ether layer did not show the presence of any sugar or its derivative, but the aqueous layer gave one spot at the origin.

The aqueous layer was concentrated to 10 ml, and then heated for 8 h on a steam bath with benzoic acid (33 mg), ethanol (3.3 ml), and benzaldehyde (0.33 ml). The solution was evaporated to dryness, the product was taken up in water, and the solution was exhaustively extracted, first with chloroform and then with ether. The extracts were combined, and the solvents were evaporated off. The resulting syrup showed no spots corresponding to any sugar.

The aqueous layer was concentrated, and examined by paper chromatography (solvents *B* and *D*); only one spot at the origin was detected. A portion (~125 mg) of the mixture was separated on thick filter-papers, and the portion at the origin of the papers was excised, and eluted with water. The aqueous solution was concentrated to a small volume, and freeze-dried, to give a solid product (90 mg) that contained galactose (95%) and arabinose (~5%).

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