# 336. *The Structure of* Acacia pycnantha *Gum.*

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Controlled acid-hydrolysis of the gum liberates L-arabinose and L-rhamnose with the formation of a degraded gum containing residues of D-galactose and D-glucuronic acid. 3-*O*-L-Arabofuranosyl-L-arabinose has been isolated during the early stages of the hydrolysis. Partial acid-hydrolysis of the fragment remaining after degradation of the periodate-oxidised degraded gum with phenylhydrazine affords 3-*O*-β-D-galactopyranosyl-D-galactose and only small amounts of a second disaccharide. Hydrolysis of the methylated degraded gum yields 2 : 3 : 4 : 6-tetra-, 2 : 4 : 6-tri-, 2 : 4- and 2 : 6-di-, and 2-*O*-methyl-D-galactose, (2 : 3 : 4-tri-*O*-methyl-6-D-galactopyranose 2 : 3 : 4-tri-*O*-methyl-β-D-glucopyranosid)uronic acid, and traces of other sugars. Hydrolysis of the methylated gum affords 2 : 3 : 5-tri- and 2 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4- and 2 : 4 : 6-tri-, 2 : 4- and 2 : 6-di-, and 2-*O*-methyl-D-galactose, 2 : 3 : 4-tri-*O*-methyl-L-rhamnose, (2 : 3 : 4-tri-*O*-methyl-6-D-galactopyranose 2 : 3 : 4-tri-*O*-methyl-β-D-glucopyranosid)uronic acid, and traces of other sugars. It is concluded that the gum is a highly branched polysaccharide containing a framework of D-galactopyranose residues with main chains linked 1 ⟶ 3 and with side-chains attached by 1 ⟶ 6 linkages; to this branched framework are attached side-chains of L-rhamnopyranose, L-arabofuranose, 3-*O*-L-arabofuranosyl-L-arabofuranose, and (6-D-galactopyranose β-D-glucopyranosid)uronic acid residues. The structure of the gum is compared with that of gum arabic.

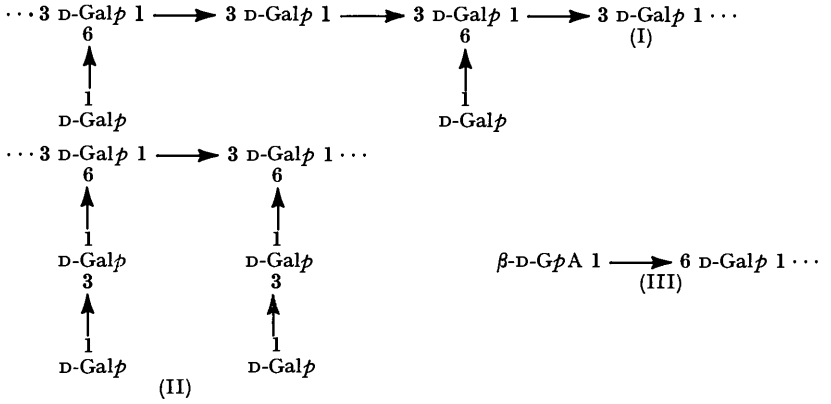
A previous investigation [1] of *Acacia pycnantha* gum showed that the gum is composed of residues of D-galactose (65%), L-arabinose (27%), L-rhamnose (1—2%), and D-glucuronic acid (5%). Although containing the same sugar units, the gum differs considerably from gum arabic [2,3] and the gums from other *Acacia* species [4] in the proportions of the constituent sugars. The gum is similar to these gums in giving rise to the aldobiouronic acid (6-D-galactose β-D-glucopyranosid)uronic acid, as a product of partial acid-hydrolysis. A further similarity with gum arabic is the formation of 3-*O*-β-D-galactopyranosyl-D-galactose as another product of partial acid hydrolysis. In this paper the results of further structural investigations on *A. pycnantha* gum are reported and in consequence a more detailed comparison of the structure of the gum with that of gum arabic is possible.

Hydrolysis of the gum under controlled conditions resulted in the release of arabinose and rhamnose, and only traces of galactose, with the formation of a stable degraded gum virtually free from arabinose residues. When the mild hydrolysis was arrested at an early stage an arabinose-containing disaccharide was present amongst the products. A quantity of this material was isolated by chromatography on cellulose; it had chromatographic mobility and optical rotation ($[\alpha]_D$ +89°) similar to those reported for 3-*O*-L-arabofuranosyl-L-arabinose isolated from sugar-beet araban.[5] Since the disaccharide was readily hydrolysed by acid it probably contained a furanosyl linkage, and its absence at later stages in the hydrolysis of the gum indicated that it was not an acid reversion product.[6] The presence of a 3-*O*-substituted arabinose residue was indicated by the formation of *ca.* 1 mol. of formaldehyde on periodate oxidation of the disaccharide and of 1·54 mols. of formaldehyde on similar oxidation of the derived glycitol.[7] Confirmation of the structure of the

[1] Hirst and Perlin, *J.*, 1954, 2622; Perlin, *Analyt. Chem.*, 1955, **27**, 396.
[2] Smith, *J.*, 1939, 744, 1724; 1940, 1035; Jackson and Smith, *J.*, 1940, 74, 79.
[3] Smith and Spriestersbach, Amer. Chem. Soc. Meeting, Minneapolis, Sept., 1955, Abs. Papers, 15D; Dillon, O'Ceallachain, and O'Colla, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 331; 1954, **57**, *B*, 31.
[4] Stephen, *J.*, 1951, 646; Charlson, Nunn, and Stephen, *J.*, 1955, 269, 1428; Hulyachar, Ingle, and Bhide, *J. Indian Chem. Soc.*, 1956, **33**, 861; Mukherjee and Shrivastava, *J. Amer. Chem. Soc.*, 1958, **80**, 2536.
[5] Andrews, Hough, and Powell, *Chem. and Ind.*, 1956, 658.
[6] Jones and Nicholson, *J.*, 1958, 27.
[7] Hough, Woods, and Perry, *Chem. and Ind.*, 1957, 1100.

disaccharide as 3-*O*-L-arabofuranosyl-L-arabinose was obtained by the isolation of 2 : 3 : 5-tri- and a mixture of 2 : 4- and 2 : 5-di-*O*-methyl-L-arabinose on hydrolysis of the methylated disaccharide.

The degraded gum was converted into the fully methylated derivative, hydrolysis of which afforded 2 : 3 : 4 : 6-tetra-, 2 : 4 : 6-tri-, and 2 : 4-di-*O*-methyl-D-galactose, and a methylated aldobiouronic acid, together with smaller amounts of 2 : 6-di- and 2-*O*-methyl-D-galactose. Very small amounts of 2 : 3 : 4-tri-*O*-methyl-D-galactose and 2 : 3-di-*O*-methyl-L-arabinose were probably also present in the hydrolysate. The methylated aldobiouronic acid was shown to be (2 : 3 : 4-tri-*O*-methyl-6-D-galactopyranose 2 : 3 : 4-tri-*O*-methyl-β-D-glucopyranosid)uronic acid since reduction of the derived methyl ester methyl glycoside with lithium aluminium hydride followed by hydrolysis yielded 2 : 3 : 4-tri-*O*-methyl-D-glucose and 2 : 3 : 4-tri-*O*-methyl-D-galactose. It follows from the isolation from the methylated degraded gum of 2 : 3 : 4 : 6-tetra-, 2 : 4 : 6-tri-, and 2 : 4-di-*O*-methyl-D-galactose in approximately equimolecular amounts that the degraded gum contains a highly branched stable framework of 1 : 3- and 1 : 6-linked D-galactopyranose residues, for which partial structures (I), (II), and other variants may be advanced. The isolation of the fully etherified aldobiouronic acid, (2 : 3 : 4-tri-*O*-methyl-6-D-galactopyranose 2 : 3 : 4-tri-*O*-methyl-β-D-glucopyranosid)uronic acid, indicates that aldobiouronic acid units (III) must be attached as side-chains to the galactan backbone, but there is no evidence yet for the positions of linkage of these units. It is not yet known if the minor products of hydrolysis of this methylated polysaccharide are of structural significance.




Evidence in favour of structure (I) for the major part of the degraded gum was obtained by application of Barry's method of degradation [8] to the polysaccharide. Periodate-oxidised degraded gum was treated with phenylhydrazine and partial acid hydrolysis of the residual polymer afforded 3-*O*-β-D-galactopyranosyl-D-galactose as the main disaccharide product with only traces of the 1 : 6-linked isomer being detected chromatographically. It follows that the main chain is composed essentially of 1 : 3-linked β-D-galactopyranose residues as required by structure (I) and that the 1 : 6-linkages in the degraded gum are those of D-galactose residues attached as side-chains. The isolation of a small amount of a 1 : 3-linked galactotriose and higher homologues provides further evidence in support of this structure. The 1 : 3- and 1 : 6-linked D-galactopyranose residues in *Acacia pycnantha* gum are, therefore, similarly arranged to those in gum arabic [3] and in larch ε-galactan.[9]

The characteristic linkages of the sugar residues in the undegraded gum were established by analysis of the complex mixture of methylated sugars obtained on hydrolysis of the methylated gum. The following sugars were characterised by the formation of crystalline

[8] Barry and Mitchell, *J.*, 1954, 4020.
[9] Aspinall, Hirst, and Ramstad, *J.*, 1958, 593.

derivatives: 2 : 3 : 5-tri- and 2 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-L-rhamnose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4- and 2 : 4 : 6-tri-, and 2 : 4-di-*O*-methyl-D-galactose, and the methylated aldobiouronic acid, (2 : 3 : 4-tri-*O*-methyl-6-D-galactopyranose 2 : 3 : 4-tri-*O*-methyl-β-D-glucopyranosid)uronic acid. In addition small amounts of 2 : 6-di- and 2-*O*-methyl-D-galactose were identified, and two unidentified dimethyl ethers of arabinose (probably the 3 : 5- and 2 : 3-isomers) were also present. These sugars were not present in sufficient quantity to be considered as major structural units and some, indeed, may be products of incomplete methylation.

The significance of these results may be assessed by comparing the nature and amounts of the various methylated sugars isolated from the methylated derivatives of the degraded and undegraded gums. Taking the galactan structure (I) with attached aldobiouronic acid units (III) as a working model for the degraded gum, we may consider first the nature of the acid-labile residues removed during the mild acid-hydrolysis, and, secondly, the points of attachment of these groups to the galactan framework. The isolation of the same fully etherified aldobiouronic acid from methylated degraded and undegraded gums indicates that no substituents are linked to the acidic disaccharide units (III).

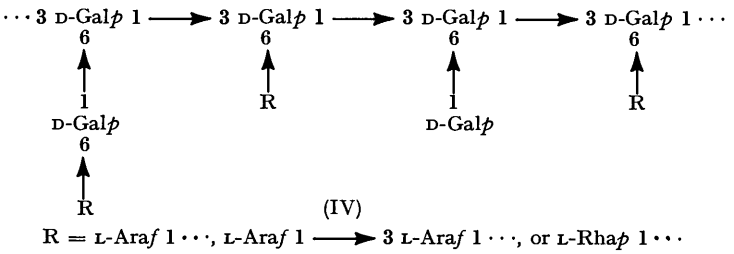
The only L-rhamnose derivative found on hydrolysis of the methylated gum was the 2 : 3 : 4-trimethyl ether, therefore the small proportions of these sugar residues in the gum occur solely as end groups in the pyranose form. Apart from traces of arabinose residues giving rise to 2 : 3-di-*O*-methylarabinose and which may be present in either furanose or pyranose form, L-arabinose residues in the gum occur solely in the furanose form, approximately two-thirds in terminal and one-third in non-terminal positions linked 1 ⟶ 3. Since 3-*O*-L-arabofuranosyl-L-arabinose has been identified as a partial acid hydrolysis product of the gum, the simplest, but not the only, assignment of arabinose residues is of equal proportions of single L-arabofuranose and of 3-*O*-L-arabofuranosyl-L-arabofuranose residues attached as side chains to the galactan framework. The points of attachment of the acid-labile residues to galactose residues in the gum follow from a comparison of the proportions of D-galactose methyl ethers formed on hydrolysis of the methylated gum and of the methylated degraded gum. In the absence of galactose and galactose-containing oligosaccharides amongst the low-molecular-weight products of mild acid hydrolysis of the gum it may be assumed that the galactan framework of the gum is substantially undisturbed during the formation of the degraded gum. The most striking difference is the large increase in the proportion of 2 : 4 : 6-tri-*O*-methyl-D-galactose from the methylated degraded gum and the corresponding decrease in the proportion of 2 : 4-di-*O*-methyl-D-galactose. It follows that a large proportion of the acid-labile groups are attached to position 6 of 1 ⟶ 3 linked D-galactopyranose residues in the main chains of the molecular structure. A second but significant difference is the increase in the proportion of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose which corresponds approximately with the decrease in the proportion of 2 : 3 : 4-tri-*O*-methyl-D-galactose. Further acid-labile groups are therefore linked to position 6 of D-galactopyranose residues present as side chains in the gum. It is not possible to ascribe particular points of attachment to the various types of acid-labile groups, but on the basis of present knowledge the main structural features of *Acacia pycnantha* gum may be summarised in structure (IV), with aldobiouronic acid units (III) attached in a manner as yet unknown.

Sufficient is now known of the mode and order of linkage of the constituent sugar residues of *Acacia pycnantha* gum for a broad comparison of its structure to be made with that of gum arabic. The structure (V) for gum arabic is based upon the classic work of Smith [2] and upon more recent investigations [3] in which the presence of the backbone of 1 : 3-linked galactose residues has been established.

The two gums contain highly branched backbones of galactose residues in which the main chain is linked 1 ⟶ 3 and side-chains are attached by 1 ⟶ 6 linkages, but whereas all the galactose residues in the main chain of gum arabic carry galactose-containing side-chains, such side-chains are attached only to every second galactose residue in the main

*Proportions of methyl ethers of* D-*galactose.*

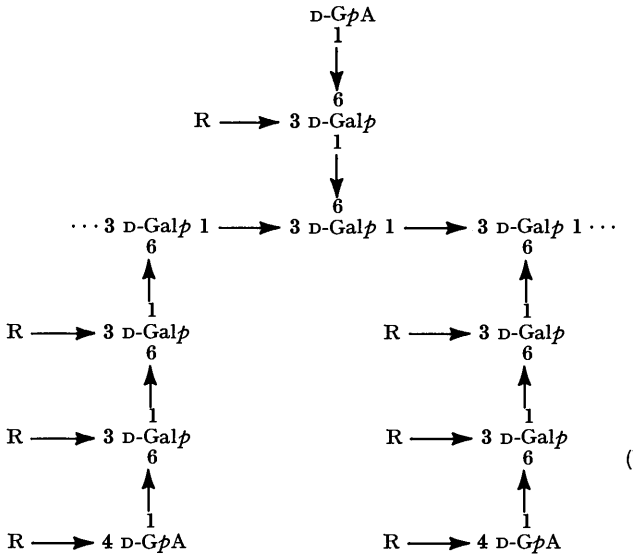
| Methyl ether | Hydrolysis of methylated gum | Hydrolysis of methylated degraded gum | Difference |
|---|---|---|---|
| 2 : 3 : 4 : 6-Tetra- ..... | 1·0 | 1·6 | +0·6 |
| 2 : 3 : 4-Tri- ..... | 0·65 | 0·25 | −0·4 |
| 2 : 4 : 6-Tri- ..... | 0·2 | 1·7 | +1·5 |
| 2 : 4-Di- ..... | 3·4 | 1·4 | −2·0 |




(IV)

R = L-Araf 1 ···, L-Araf 1 → 3 L-Araf 1 ···, or L-Rhap 1 ···

chain of *Acacia pycnantha* gum. Both gums contain the same aldobiouronic acid units in the side-chains of the molecule but the precise mode of attachment to the backbone in the latter case is not known. Similarities between the two gums are also shown by the L-arabofuranose and L-rhamnopyranose end groups which are removed by mild acid hydrolysis. The gums differ, however, in the nature of the more complex side-chains also removed under these conditions. In gum arabic these are 3-*O*-D-galactopyranosyl-L-arabofuranose and in some cases[10] 3-*O*-L-arabopyranosyl-L-arabofuranose groups, but in *A. pycnantha* gum they are 3-*O*-L-arabofuranosyl-L-arabofuranose groups. It may be noted, however, that in both cases the non-terminal L-arabofuranose residues carry substituents at position 3. The most marked differences between the two gums are shown in the positions of linkage to galactose or glucuronic acid residues of the various groups on the periphery of the gum




(V)

R = L-Araf 1 ···, L-Rhap 1 ···, D-Galp 1 → 3 L-Araf 1 ···, or in some cases[10] L-Arap 1 → 3 L-Araf 1 ···

structure. Although it is not known to which positions specific groups are attached, in gum arabic they are linked to $C_{(3)}$ of galactose residues in the outer chains and to $C_{(4)}$ of

[10] Andrews and Jones, *J.*, 1955, 583.

glucuronic acid residues, whereas in *Acacia pycnantha* gum they are linked to $C_{(6)}$ of galactose residues in both the main and outer chains.

In the absence of any strict proof of homogeneity caution must be exercised in the interpretation of the results of structural investigations of complex polysaccharides such as the plant gums. There is indeed evidence for a measure of heterogeneity in some samples of gum arabic [11,12] and of other *Acacia* gums [12] including *A. pycnantha* gum. Professor F. Smith (personal communication) informs us that glass-fibre paper ionophoresis in 2N-sodium hydroxide shows his sample of *Acacia pycnantha* gum to contain a major and a minor component. Glass-fibre paper ionophoresis of the gum sample used by us showed only one component, although another sample of the gum showed a similar degree of heterogeneity to that examined by Professor Smith. There is however no detailed information regarding the nature of the heterogeneity and it is not possible therefore to state whether different fractions from a single gum consist of similarly constituted polysaccharides differing only in some minor respect, or whether there are more fundamental differences of structure. Nevertheless, it is already clear from the comparisons made that the major components of the two gums have important structural features in common. It is indeed possible that just as many polysaccharides of the hemicellulose groups have been shown to be members of the same general family but differing considerably in fine structure,[13] so the vastly more complicated gums from related botanical species may be shown to contain similar basal units of molecular structure upon which are superimposed even wider variations in detailed structure.

## Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (D) butan-2-one, half saturated with water; (E) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer). Unless otherwise stated, chromatography of methylated sugars was carried out in solvent B, and $R_G$ values refer to the rate of movement relative to 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in that solvent. Demethylations of methylated sugars were performed by the method of Hough, Jones, and Wadman.[14] Paper ionophoresis was in borate buffer at pH 10. Optical rotations were observed at 18° ± 2°.

The gum was prepared from crude nodules as described previously, and the sample of methylated gum was that prepared by Hirst and Perlin.[1]

*Partial Acid Hydrolysis and Preparation of the Degraded Gum.*—The gum (1 g.) was heated with 0·01N-sulphuric acid (100 ml.) at 100°. The optical rotation of the solution was observed every hour, and every two hours samples (5 ml.) were withdrawn, neutralised with barium carbonate, filtered, shaken with Amberlite resin IR–120(H) to remove barium ions, and poured into ethanol (3 vol.) to precipitate degraded gum. The supernatant liquor and the hydrolysate from the degraded gum were examined chromatographically in solvent A. The results showed that the arabinose was almost completely removed after 10 hr. and the rhamnose after 14 hr. (solution had $[\alpha]_D$ +39°); only small quantities of galactose were released. In a more careful search for oligosaccharides released during the mild acid-hydrolysis, samples were passed through charcoal–Celite columns (1 : 1; 5 g.). Elution with water removed most of the monosaccharides and elution with 15% ethanol removed an unknown sugar of $R_{Gal}$ 1·4 in solvents C and D. This sugar was present in the hydrolysate after 1 hr., in decreased amount after 2 hr., and was no longer present after 4 hr. or at later stages in the hydrolysis.

The gum (29 g.) was heated at 100° with 0·01N-sulphuric acid (1·5 l.) for 14 hr. ($[\alpha]_D$ −8° ⟶ +38°). The cooled solution was neutralised with Amberlite resin IR–4B(OH), concentrated, and poured into ethanol (4 vol.) to give the degraded gum (15 g.), $[\alpha]_D$ +20° (*c.* 1·2 in $H_2O$) (Found: uronic anhydride, 6%).

*Partial Acid Hydrolysis and Isolation of* 3-O-L-*Arabofuranosyl*-L-*arabinose.*—Further examination of the products of mild acid hydrolysis of the gum showed that the unknown sugar of

[11] Heidelberger, Adams, and Dische, *J. Amer. Chem. Soc.*, 1956, **78**, 2853.
[12] Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.
[13] Hirst, *J.*, 1955, 2974.
[14] Hough, Jones, and Wadman, *J.*, 1950, 1705.

$R_{Gal}$ 1·4 was present in greatest amount after the gum had been heated with 0·01N-sulphuric acid at 85° for 4 hr. The gum (10 g.) was heated at 85° with 0·01N-sulphuric acid (300 ml.) for 4 hr., and the cooled solution was neutralised with Amberlite resin IR–4B(OH), concentrated, and poured into ethanol (3 vol.). The precipitated degraded polysaccharide was removed and the supernatant liquor was concentrated to a syrupy mixture of sugars (2·2 g.), chromatography of which showed arabinose and small quantities of rhamnose, galactose, and the sugar of $R_{Gal}$ 1·4. The syrup was fractionated on cellulose (2·8 × 80 cm.) by use of solvent D to give three fractions. Fraction 1 (1·7 g.) contained arabinose and traces of rhamnose and the disaccharide ($R_{Gal}$ 1·4). Fraction 2 (105 mg.) contained the disaccharide with traces (*ca.* 1%) of galactose and arabinose. Fraction 3 (70 mg.) contained the disaccharide, galactose, and unidentified oligosaccharides.

The disaccharide (fraction 2) had $[\alpha]_D$ +89° (*c* 1·0 in $H_2O$) and gave only arabinose on hydrolysis. Periodate oxidation [15] of a sample (10 mg.) gave 0·87 mol. of formaldehyde, identified as the dimedone compound, m. p. 189—190°. A second sample (10 mg.) in water (2 ml.) was reduced with potassium borohydride (20 mg.) for 16 hr., and oxidation with periodate afforded formaldehyde corresponding to 1·54 mol. per mol. of glycitol.

Methyl sulphate (2 ml.) and 40% aqueous sodium hydroxide (3 ml.) were added dropwise to the sugar (80 mg.) in water (8 ml.) at 0°. Further additions of methyl sulphate (2 × 12 ml. + 6 ml.) and 40% sodium hydroxide (2 × 22 ml. + 9 ml.) were made at room temperature and the reaction was completed by heating the solution on the boiling-water bath for 1 hr. The cooled mixture was extracted with chloroform for 16 hr. to give methylated disaccharide (80 mg.). Hydrolysis of a sample (3 mg.) gave 2 : 3 : 5-tri- and 2 : 5- and 2 : 4-di-*O*-methylarabinose, with only traces of products of incomplete methylation. Hydrolysis of the methylated disaccharide (77 mg.) with N-sulphuric acid at 100° for 4 hr., followed by neutralisation with barium carbonate and deionisation, gave a syrup (68 mg.) which was separated on filter sheets with solvent B, to give three fractions. Fraction *a* (20 mg.), $R_G$ 0·98, was identified as 2 : 3 : 5-tri-*O*-methyl-L-arabinose by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 132—133°. Fraction *b* (10 mg.), $R_G$ 0·84, was characterised as 2 : 5-di-*O*-methyl-L-arabinose by conversion into aldonamide, m. p. and mixed m. p. 123—124°. Fraction *c* (14 mg.), $R_G$ 0·65, was identified as 2 : 4-di-*O*-methyl-L-arabinose by conversion into the aniline derivative, m. p. and mixed m. p. 131°.

*Preparation and Hydrolysis of Methylated Degraded Gum.*—The degraded gum (10 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide, to give methylated degraded gum (3·1 g.), $[\alpha]_D$ −35° (*c* 1·0 in $CHCl_3$) (Found: OMe, 45·0%). The methylated degraded gum (2·9 g.) was suspended in N-hydrochloric acid (300 ml.) at 40° for 8 days. The resulting solution was heated at 100° for 16 hr. (constant rotation), cooled, neutralised with silver carbonate, and, after removal of silver ions as silver sulphide, treated with barium carbonate and concentrated to a syrup (2·4 g.). The syrupy mixture of sugars was separated on cellulose (3·5 × 90 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later 1 : 1) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give ten fractions, and a further fraction was obtained by elution of the cellulose with water.

*Fraction* 1. The syrup (40 mg.), $[\alpha]_D$ +44° (*c* 0·4 in $H_2O$), contained a mixture of 2 : 3 : 5-tri-*O*-methylarabinose ($R_G$ 0·98), 2 : 3 : 4-tri-*O*-methylrhamnose ($R_G$ (1·01), and 2 : 3 : 4 : 6-tetra-*O*-methylgalactose ($R_G$ 0·89). Demethylation gave rhamnose, arabinose, and galactose.

*Fraction* 2. The chromatographically pure sugar (525 mg.), $R_G$ 0·89, crystallised from ether–light petroleum and had m. p. and mixed m. p. 68° (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose) and $[\alpha]_D$ +140° ⟶ +117° (*c* 1·0 in $H_2O$) (Found: OMe, 52·2. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The aniline derivative had m. p. and mixed m. p. 194°.

*Fraction* 3. The syrup (56 mg.), $[\alpha]_D$ +90° (*c* 1·1 in $H_2O$), contained tetra-*O*-methylgalactose ($R_G$ 0·89) and a small quantity of 2 : 5-di-*O*-methylarabinose. Demethylation gave galactose and arabinose. The major component was identified by conversion into 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. 191°.

*Fraction* 4. Chromatography of the partly crystalline material (583 mg.), $[\alpha]_D$ +128° ⟶ +94° (*c* 0·61 in $H_2O$), showed 2 : 4 : 6- and/or 2 : 3 : 4-tri-*O*-methylgalactose. Recrystallisation from acetone–ether–light petroleum afforded 2 : 4 : 6-tri-*O*-methyl-D-galactose, m. p. and mixed m. p. 101° and $[\alpha]_D$ +120° ⟶ +90° (*c* 0·71 in $H_2O$) (Found: OMe, 41·8%. Calc. for

[15] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.

$C_9H_{18}O_6$: OMe, 41·9%) (aniline derivative, m. p. and mixed m. p. 167—168°). A sample (10 mg.) of the fraction in water (10 ml.) was reduced with potassium borohydride (20 mg.) for 16 hr., excess of hydride was destroyed by addition of acetic acid, *p*-hydroxybenzaldehyde (2—3 mg.) was added, and the solution was made up to a standard volume. Equal volumes of sodium metaperiodate–sodium hydrogen carbonate solution were added to aliquot parts of the reduced sugar solution, and the formaldehyde released was estimated colorimetrically by the method of O'Dea and Gibbons.[16] The formaldehyde formed corresponded to the presence of 8% of the 2 : 3 : 4-trimethyl ether in the fraction. Attempts to characterise the second sugar by fractional crystallisation of the aniline derivatives failed.

*Fraction* 5. Chromatrography of the syrup (134 mg.), $[\alpha]_D$ +103° (*c* 1·2 in $H_2O$) showed 2 : 4 : 6- and/or 2 : 3 : 4-tri-*O*-methylgalactose and 2 : 3-di-*O*-methylarabinose. Demethylation gave galactose and arabinose. Treatment of the syrup with aniline furnished the characteristic needles of 2 : 4 : 6-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 165—166°. Concentration of the mother-liquor yielded a mixture, m. p. 146°, of needles and plates (characteristic of 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine), but it was not possible to separate the two components. Periodate oxidation of the derived mixture of glycitols gave 0·45 mol. of formaldehyde. If the presence of 10% of di-*O*-methylarabinose in the fraction is assumed, the fraction contained 2 : 3 : 4- (35%) and 2 : 4 : 6-tri-*O*-methyl-D-galactose (55%).

*Fraction* 6. The sugar (56 mg.), $R_G$ 0·54, after recrystallisation from chloroform–light petroleum had m. p. and mixed m. p. (with 2 : 6-di-*O*-methyl-D-galactose monohydrate) 98—99°, and $[\alpha]_D$ +52° ⟶ +82° (*c* 0·55 in $H_2O$) (Found: OMe, 27·0. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·2%). The aniline derivative had m. p. and mixed m. p. 119°

*Fraction* 7. Chromatography of the fraction (223 mg.), $[\alpha]_D$ +105° ⟶ +86° (*c* 0·88 in $H_2O$), showed 2 : 4- and 2 : 6-di-*O*-methyl-D-galactose, $R_G$ 0·47 and 0·54, in the approximate proportion of 5 : 1. Demethylation gave galactose. Recrystallisation from acetone containing 1% of water gave 2 : 4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 101° (aniline derivative, m. p. and mixed m. p. 216—217°). Chromatography of the products of periodate oxidation [17] of the mother-liquor showed unchanged 2 : 4-di-*O*-methylgalactose and methoxymalondialdehyde (from the 2 : 6-dimethyl ether), but no products characteristic of the 2 : 3-dimethyl ether.

*Fraction* 8. The sugar (212 mg.), $R_G$ 0·47, after recrystallisation from acetone containing 1% of water had m. p. and mixed m. p. (with 2 : 4-di-*O*-methyl-D-galactose monohydrate) 102° and $[\alpha]_D$ +123° ⟶ +85° (*c* 0·68 in $H_2O$) (aniline derivative, m. p. and mixed m. p. 218°).

*Fraction* 9. The syrup (47 mg.), $[\alpha]_D$ +83° (*c* 0·47 in $H_2O$), contained 2 : 4-di-*O*-methylgalactose ($R_G$ 0·47) and a small amount of 2-*O*-methylgalactose ($R_G$ 0·29). Demethylation gave galactose.

*Fraction* 10. The sugar (49 mg.), $R_G$ 0·29 and $[\alpha]_D$ +63° ⟶ +82° (*c* 0·49 in $H_2O$), after recrystallisation from glacial acetic acid had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 148—149°.

*Fraction* 11. Removal of barium ions by treatment with Amberlite resin IR–120(H) followed by concentration gave a syrup (108 mg.), which was converted into the methyl ester methyl glycoside by refluxing it with methanolic 3% hydrogen chloride (40 ml.) for 6 hr. Lithium aluminium hydride in tetrahydrofuran was added slowly to a boiling solution of the ester glycoside in tetrahydrofuran, and the mixture was refluxed for 2 hr. Excess of hydride was destroyed by water, the mixture was taken to dryness, and the residue was exhaustively extracted with chloroform and acetone to give a syrup (85 mg.). Hydrolysis of the syrup with N-hydrochloric acid at 100° for 4 hr. gave a mixture of sugars (69 mg.) which was separated on cellulose (50 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol, saturated with water. to give fractions *a* (23 mg.) and *b* (28 mg.). Fraction *a* was characterised as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 134°. Fraction *b*, $[\alpha]_D$ +116° (*c* 0·28 in $H_2O$), was characterised as 2 : 3 : 4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 169°.

*Degradation of Periodate-oxidised Degraded Gum with Phenylhydrazine.*—Degraded gum (3·5 g.) was oxidised with sodium metaperiodate (8·5 g.) in water (100 ml.) for 48 hr. in the dark (complete oxidation). The solution was treated with lead acetate to remove iodate and periodate, and then with dilute sulphuric acid to precipitate excess of lead. The resulting solution

[16] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.
[17] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

was heated with phenylhydrazine (17 ml.) and glacial acetic acid (10 ml.) at 100° for 5 hr. The cooled solution was repeatedly extracted with ether, made 0·2N with respect to sulphuric acid and heated at 100° for 40 min. to cleave phenylosazone residues. The cooled solution was neutralised with Amberlite resin IR–4B(OH), extracted with ether, concentrated to 50 ml., and poured into ethanol (150 ml.) to precipitate the polysaccharide residue (1·0 g.).

Small-scale experiments showed that hydrolysis of the degraded polysaccharide with 0·5N-sulphuric acid at 100° for 56 min. gave a solution with 50% of the reducing power obtained on prolonged hydrolysis. Chromatography of the hydrolysate after this period showed galactose and 3-*O*-galactopyranosylgalactose. The degraded polysaccharide (0·9 g.) was heated with 0·5N-sulphuric acid (75 ml.) at 100° for 1 hr. The cooled solution was neutralised with Amberlite resin IR–4B(OH), concentrated, and poured into ethanol (3 vol.). The precipitated degraded polysaccharide was rehydrolysed under the same conditions and combination of the soluble hydrolysates afforded a syrup (350 mg.). The syrup (350 mg.) was dissolved in water and poured on charcoal–Celite (1 : 1; 100 g.). Elution with water afforded galactose (212 mg.). Elution with water containing 5% of ethanol gave a syrup (31 mg.) containing 3-*O*-galactopyranosylgalactose ($R_{Gal}$ 0·60 in solvent A) and a trace of 6-*O*-galactopyranosylgalactose ($R_{Gal}$ 0·40). Recrystallisation from ethanol–water gave 3-*O*-D-galactopyranosyl-D-galactose, identified by m. p. and mixed m. p. 145—147° and by *X*-ray powder photography. Elution with water containing 10% of ethanol gave a syrup (7 mg.) containing a sugar with $R_{Gal}$ 0·33 in solvent A and higher oligosaccharides. Partial acid-hydrolysis gave galactose and 3-*O*-galactopyranosylgalactose.

*Hydrolysis of Methylated Gum and Separation of Methylated Sugars.*—The methylated gum (6·0 g.) was refluxed with methanolic 4% hydrogen chloride (300 ml.) for 18 hr. (constant rotation). Methanol was removed under reduced pressure and the product was heated with 0·5N-hydrochloric acid (300 ml.) on the boiling-water bath for 14 hr. (constant rotation). The cooled solution was neutralised with silver carbonate, then filtered, silver was removed with hydrogen sulphide, and the filtrate was concentrated. Sugars were extracted from the residue with methanol, and the resulting syrup was dissolved in water, neutralised with barium carbonate, filtered, and concentrated to a syrup (5·1 g.). The syrupy mixture of sugars was separated on cellulose (90 × 3·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give eleven fractions, and a further fraction was obtained by elution of the cellulose with water.

*Fraction* 1. Chromatography of the syrup (640 mg.) showed 2 : 3 : 4-tri-*O*-methylrhamnose ($R_G$ 1·01) and 2 : 3 : 5-tri-*O*-methylarabinose ($R_G$ 0·98). The optical rotation $[\alpha]_D$ −23° (*c* 0·64 in $H_2O$) of the syrup corresponded to that of a mixture of 2 : 3 : 4-tri-*O*-methyl-L-rhamnose ($[\alpha]_D$ +24°) and 2 : 3 : 5-tri-*O*-methyl-L-arabinose ($[\alpha]_D$ −37°) in the proportions of 23 : 77. Demethylation with hydriodic acid gave rhamnose and arabinose. A portion (360 mg.) of the syrup was fractionated on cellulose (50 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol, saturated with water, to give four fractions. Fraction 1*a* (47 mg.), $[\alpha]_D$ +24° (*c* 0·4 in $H_2O$), was characterised as 2 : 3 : 4-tri-*O*-methyl-L-rhamnose (aniline derivative, m. p. and mixed m. p. 111°). Fraction 1*b* (232 mg.), $[\alpha]_D$ −28° (*c* 1·0 in $H_2O$), contained a mixture of 2 : 3 : 4-tri-*O*-methyl-L-rhamnose and 2 : 3 : 5-tri-*O*-methyl-L-arabinose. Fraction 1*c* (47 mg.), $[\alpha]_D$ −37° (*c* 0·47 in $H_2O$), was chromatographically pure 2 : 3 : 5-tri-*O*-methyl-L-arabinose and afforded 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 135—136°. Fraction 1*d* (7 mg.), $[\alpha]_D$ +100° (*c* 0·14 in $H_2O$), was 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose ($R_G$ 0·89) which had not been detected previously in the fraction.

*Fraction* 2. Chromatography of the syrup (717 mg.) showed two components, $R_G$ 0·98 and 0·89, and the optical rotation {$[\alpha]_D$ +92° (*c* 0·71 in $H_2O$)} corresponded to that of a mixture of 2 : 3 : 5-tri-*O*-methyl-L-arabinose ($[\alpha]_D$ −37°) and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose ($[\alpha]_D$ +117°) in the proportion of 16 : 84. The major component was identified by conversion into 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 191°.

*Fraction* 3. Chromatography of the syrup (42 mg.), $[\alpha]_D$ +62° (*c* 0·41 in $H_2O$), showed 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (identified as the aniline derivative, m. p. and mixed m. p. 189°) and small amounts of 2 : 3 : 5-tri- and 2 : 5-di-*O*-methylarabinose.

*Fraction* 4. Chromatography of the syrup (276 mg.), $[\alpha]_D$ +4·5° (*c* 1·1 in $H_2O$), showed di-*O*-methylarabinose ($R_G$ 0·84) and a small amount of tetra-*O*-methylgalactose ($R_G$ 0·89). Demethylation gave arabinose and a small amount of galactose. Separation of a portion (160 mg.) on cellulose using light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water,

gave two fractions. Fraction 4*a* (18 mg.) was 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (aniline derivative m. p. and mixed m. p. 190°). Chromatography of fraction 4*b* (122 mg.) showed one sugar ($R_G$ 0·84), but ionophoresis showed 2 : 5-di-*O*-methylarabinose together with a small amount of 3 : 5-di-*O*-methyl-arabinose. Demethylation gave only arabinose. The major component was identified by conversion into 2 : 5-di-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 125—126°.

*Fraction* 5. Chromatography of the syrup (237 mg.), $[\alpha]_D$ +113° (*c* 1·0 in $H_2O$), showed a tri-*O*-methylgalactose ($R_G$ 0·73) (Found: OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Demethylation gave galactose. Treatment of the syrup with ethanolic aniline furnished the aniline derivative of 2 : 3 : 4-tri-*O*-methyl-D-galactose, m. p. and mixed m. p. 168°. Reduction with potassium borohydride, followed by periodate oxidation, gave 0·91 mol. of formaldehyde per mol. of sugar.

*Fraction* 6. Chromatography of the syrup (351 mg.), $[\alpha]_D$ +108° (*c* 1·1 in $H_2O$), showed tri-*O*-methylgalactose ($R_G$ 0·73) and *ca.* 10% of 2 : 3-di-*O*-methylarabinose ($R_G$ 0·67). Demethylation gave galactose and a small amount of arabinose. Reaction with ethanolic aniline afforded the aniline derivative of 2 : 3 : 4-tri-*O*-methyl-D-galactose, m. p. and mixed m. p. 167°, and from the mother-liquors, after repeated fractional crystallisations, the aniline derivative of 2 : 4 : 6-tri-*O*-methyl-D-galactose, m. p. and mixed m. p. 178—180°. Periodate oxidation of the derived mixture of glycitols gave 0·64 mol. of formaldehyde per mol. of sugar. If 10% of 2 : 3-di-*O*-methylarabinose is assumed to be present in the fraction, the fraction contained 2 : 3 : 4- (54%) and 2 : 4 : 6-tri-*O*-methyl-D-galactose (36%).

*Fraction* 7. Chromatography of the syrup (53 mg.), $[\alpha]_D$ +109° (*c* 0·53 in $H_2O$), showed a mixture of tri-*O*-methylgalactose ($R_G$ 0·73) and 2 : 6-di-*O*-methylgalactose ($R_G$ 0·54).

*Fraction* 8. The crystalline material (1·885 g.) had $[\alpha]_D$ +112° ⟶ +85° (*c* 0·91 in $H_2O$), and recrystallisation from acetone containing 1% of water furnished 2 : 4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 103° and $[\alpha]_D$ +122° ⟶ +85° (*c* 1·0 in $H_2O$) (Found: OMe, 27·1. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·2%) (aniline derivative, m. p. and mixed m. p. 217—218°). Chromatography and ionophoresis of the mother-liquor from the above recrystallisation showed a mixture of 2 : 4- and 2 : 6-di-*O*-methylgalactose. The syrup (228 mg.) was separated on cellulose (50 × 2 cm.) with solvent D as eluant to give three fractions. Chromatography of fraction 8(i) (39 mg.) showed 2 : 6-di-*O*-methylgalactose ($R_G$ 0·54) and a trace of (?) 2-*O*-methylarabinose ($R_G$ 0·38). Demethylation gave galactose and a trace of arabinose. Chromatography of the periodate-oxidation products showed methoxymalondialdehyde (from 2 : 6-di-*O*-methylhexoses and 2-*O*-methylpentoses). The main component was characterised as 2 : 6-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 99—100° and $[\alpha]_D$ +52° ⟶ +84° (*c* 0·36 in $H_2O$) (Found: OMe, 27·0. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·2%) (aniline derivative, m. p. and mixed m. p. 121—122°). Chromatography of fraction 8 (ii) (27 mg.) and its periodate-oxidation products [17] showed 2 : 4- and 2 : 6-di-*O*-methylgalactose together with a trace of the 2 : 3-dimethyl ether. Fraction 8(iii) (151 mg.), $[\alpha]_D$ +115° ⟶ +85° (*c* 1·0 in $H_2O$), after recrystallisation afforded 2 : 4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 102° (aniline derivative, m. p. and mixed m. p. 216—218°).

*Fraction* 9. Chromatography of the syrup (108 mg.) and its periodate-oxidation products [17] showed a complex mixture of sugars, including 2 : 3-, 2 : 4-, and 2 : 6-di-*O*-methylgalactose, 2-*O*-methylarabinose, and 2-*O*-methylgalactose, which was not examined further.

*Fraction* 10. The chromatographically pure sugar (224 mg.), $R_G$ 0·29 and $[\alpha]_D$ +61° ⟶ +85° (*c* 1·0 in $H_2O$), crystallised from glacial acetic acid and had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 146°.

*Fraction* 11. Chromatography of the syrup (42 mg.) showed galactose, arabinose, and 2-*O*-methylgalactose in approximately equal amounts.

*Fraction* 12. Removal of barium ions by treatment with Amberlite resin IR–120(H) followed by concentration gave a syrup (165 mg.), which was converted into the methyl ester methyl glycoside by refluxing methanolic 3% hydrogen chloride (50 ml.) during 6 hr. (constant rotation). The cooled solution was neutralised with silver carbonate, filtered and concentrated, and the product was heated with 0·15N-barium hydroxide (10 ml.) at 60° for 5 hr. After removal of barium ions with Amberlite resin IR–120(H), the acid glycoside was absorbed on Amberlite resin IR–4B(OH). Elution of the resin with N-sodium hydroxide and removal of sodium ions gave a syrup (102 mg.), which was re-esterified by refluxing it with methanolc hydrogen chloride. Lithium aluminium hydride (240 mg.) in tetrahydrofuran (10 ml.) was

added slowly to a solution of the ester glycoside in tetrahydrofuran (30 ml.), and the mixture was refluxed for 2 hr. Excess of hydride was destroyed by water, the mixture was taken to dryness, and the residue was extracted exhaustively with chloroform and acetone to give a syrup (91 mg.). Hydrolysis of the syrup with N-hydrochloric acid at 100° for 4 hr. gave a mixture of sugars (74 mg.) which was separated on cellulose (50 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol, saturated with water, to give fractions (i) (26 mg.) and (ii) (30 mg.). Fraction (i) was characterised as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 133—135°. Fraction (ii), $[\alpha]_D$ +115° (*c* 0·3 in $H_2O$), was characterised as 2 : 3 : 4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 169°.

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