47. *Gum Ghatti (Indian Gum). Part II.* The Hydrolysis Products obtained from the Methylated Degraded Gum and the Methylated Gum.*

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Hydrolysis of methylated degraded gum ghatti yields 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, 2 : 3-, and 2 : 4-di-*O*-methyl-D-galactose, 3 : 4 : 6-tri-*O*-methyl-D-mannose, and 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, together with traces of other sugars. Hydrolysis of methylated gum ghatti affords 2 : 3 : 5-tri-, 2 : 3-, 2 : 4-, 2 : 5-, and 3 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, 2 : 4-di-, and 2-mono-*O*-methyl-D-galactose, 4-*O*-methyl-D-mannose, 2 : 3 : 4-tri-*O*-methyl-L-rhamnose, 2 : 3 : 4-tri-, and 2 : 3-di-*O*-methyl-D-glucuronic acid, together with traces of other sugars. Partial structures for the gum are discussed in the light of these and previous results.

It was shown in Part I that gum ghatti (Indian gum) from *Anogeissus latifolia*, Wall, is composed of the following sugar residues, L-arabinose (5 parts), D-galactose (3 parts), D-mannose (1 part), and D-glucuronic acid (1 part), together with the small amounts of xylose and a 6-deoxyhexose (probably rhamnose). Most of the arabinose residues are located in the outer parts of the molecular structure since autohydrolysis of the gum acid leads to the removal of these residues and the formation of a degraded gum, in which only a small proportion of arabinose residues are attached to the more resistant backbone of galactose, mannose, and glucuronic acid residues. Further hydrolysis of the gum under more drastic conditions gives D-galactose and a mixture of two aldobiouronic acids, characterised as 6-*O*-β-D-glucuronosyl-D-galactose and 2-*O*-β-D-glucuronosyl-D-mannose. An examination of the methylated sugars formed on hydrolysis of the methylated gum and the methylated degraded gum is now reported, and partial structures for the gum are discussed.

A sample of degraded gum ghatti (substantially arabinose-free), prepared by autohydrolysis of the gum acid, was converted into its fully methylated derivative. The methylated polysaccharide was hydrolysed, and the hydrolysate (containing acidic components as barium salts) was separated into an ether-soluble fraction and an ether-insoluble residue. The neutral methylated sugars present in the ether-soluble fraction were fractionated chromatographically on cellulose,[1] giving 2 : 3 : 4 : 6-tetra-*O*-methyl-, 2 : 3 : 4-tri-*O*-methyl-, 2 : 3- and 2 : 4-di-*O*-methyl-D-galactose, all of which were characterised as crystalline derivatives. Although no methyl ethers of D-mannose could be isolated in pure form and identified as crystalline derivatives, the presence of 3 : 4 : 6-tri-*O*-methylmannose was established by chromatography, paper ionophoresis, the detection of 2 : 3 : 5-tri-*O*-methylarabinose when a fraction containing the sugar was oxidised with periodate,[2] and the observation that a fraction containing the sugar in

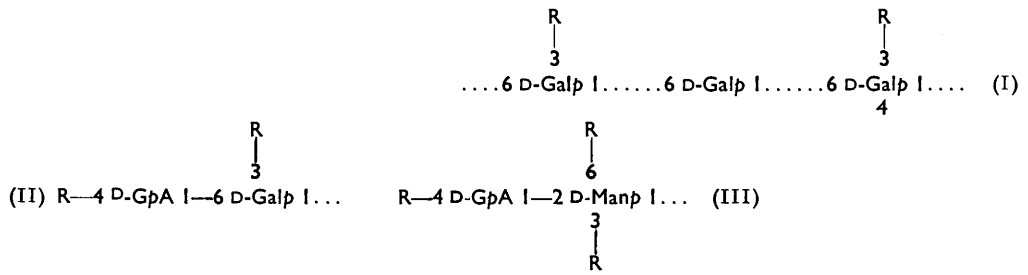
* The paper by Aspinall, Hirst, and Wickstrøm (*J.*, 1955, 1160) is to be regarded as Part I.

[1] Hough, Jones, and Wadman, *J.*, 1949, 2511.

[2] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

admixture with tetra- and 2 : 3 : 4-tri-*O*-methylgalactose was converted into the corresponding mixture of aldonamides, which gave a positive Weerman test. This sugar was the most important mannose derivative present, although traces of a di-*O*-methyl- (probably 3 : 4-) and 4-*O*-methyl-mannose were also observed. In addition, traces of 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methyl-xylose and -rhamnose were detected chromatographically, but in insufficient quantity to be of structural significance. The ether-insoluble residue afforded further quantities of 2 : 3 : 4-tri- and di-*O*-methylgalactose, which were separated from the barium salts on filter sheets. The acidic fraction, consisting mainly of methylated aldobiouronic acids, was submitted to further acid hydrolysis under vigorous conditions, and again the acidic were separated from the neutral sugars. The neutral fraction contained 2 : 3 : 4-tri-*O*-methyl-D-galactose, identified as the aniline derivative, and 3 : 4 : 6-tri-*O*-methylmannose and 2 : 3-di-*O*-methylgalactose were identified chromatographically. The main component of the acidic fraction was identified chromatographically as 2 : 3 : 4-tri-*O*-methylglucuronic acid, and, after reduction of the methyl ester methyl glycoside and hydrolysis, as 2 : 3 : 4-tri-*O*-methylglucose. The minor component of the acidic fraction was converted into the methyl ester methyl glycoside, reduced with lithium aluminium hydride, and hydrolysed to give 2 : 3-di-*O*-methylglucose together with small amounts of tri-*O*-methylglucose and tri- and di-*O*-methylgalactose. Some of the glucuronic acid residues in the degraded gum, therefore, were present in non-terminal positions linked through $C_{(1)}$ and $C_{(4)}$.

It is clear from these results that the gum contains a backbone of 1 : 6-linked D-galactopyranose units (I; R = H). Although both 2 : 3- and 2 : 4-di-*O*-methyl-D-galactose were characterised, evidence from ionophoresis and from chromatographic examination of the periodate oxidation products of the di-*O*-methylgalactose fractions showed the 2 : 3-dimethyl ether to be the main component of the mixture. (When mixtures of these two sugars are present, the 2 : 4-dimethyl ether is more readily characterised as the relatively insoluble aniline derivative.) It follows that the main branching point in the degraded gum is through position 4 of galactose, although some small proportion of branching may also occur through position 3. Since the main products of hydrolysis of the methylated aldobiouronic acids were 2 : 3 : 4-tri-*O*-methylglucuronic acid, 2 : 3 : 4-tri-*O*-methylgalactose, and 3 : 4 : 6-tri-*O*-methylmannose, the aldobiouronic acid groups are



present as terminal groups (II and III; R = H). These groups are, therefore, attached as side-chains probably through position 4 of galactose residues in the backbone. At present, there is no indication whether these units are attached directly to the backbone or whether 1 : 6-linked galactose residues are interposed. The structural significance of the non-reducing D-galactopyranose end groups in the degraded gum is not yet clear. Although these end groups may terminate another type of side-chain, it is also possible that they are present at the non-reducing end of the backbone and arise from scission of the main chain during the autohydrolysis.

Fully methylated gum ghatti was hydrolysed, and the hydrolysate (containing acidic

components as barium salts) was separated into an ether-soluble fraction (A) and an ether-insoluble residue (B). The residue (B) was then separated by chromatography on cellulose to give neutral sugars (C), a pure sample of 2-*O*-methyl-D-galactose, and an acidic fraction. The combined neutral sugars (A) + (C) were chromatographed on cellulose, giving pure samples of most of the major components and mixtures containing the minor components, which were refractionated. The following methylated sugars were characterised as crystalline derivatives: 2 : 3 : 5-tri-, 2 : 3-, 2: 4-, 2 : 5-, and 3 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, 2 : 4-di-, and 2-mono-*O*-methyl-D-galactose, 4-*O*-methyl-D-mannose, and 2 : 3 : 4-tri-*O*-methyl-L-rhamnose. Traces of some other sugars were detected chromatographically, but these were present in insufficient amount to be of structural significance. The acidic fraction, which was contaminated by a small amount of 2-*O*-methylgalactose, was converted into the corresponding mixture of methyl ester methyl glycosides, which was reduced with lithium aluminium hydride and hydrolysed to give a mixture of neutral sugars. The methylated sugars were fractionated on cellulose, and the following sugars were identified as crystalline derivatives: 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-glucose, 2 : 4-di- and 2-mono-*O*-methyl-D-galactose, and 4-*O*-methyl-D-mannose. The isolation of 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-glucose indicates the presence in the methylated gum of residues of 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-glucuronic acid. 2 : 4-Di-*O*-methyl-D-galactose and 4-*O*-methyl-D-mannose are the main neutral fragments arising from the aldobiouronic acid groupings. It is probable that the 2-*O*-methyl-D-galactose arose entirely from the contaminating neutral sugar and not from hydrolysis of a partially methylated aldobiouronic acid. In addition, a trace of 2 : 3 : 4-tri-*O*-methylgalactose was detected chromatographically

In view of the several stages involved in the separation and identification of the many methylated sugars formed on hydrolysis of the methylated gum, it is not possible to give more than an approximate estimate of the proportions of some of the constituent sugars arising from the neutral part of the gum. Since even more operations were involved in the identification of the acidic residues and of the neutral sugar residues attached thereto, and since the hydrolyses of acidic polysaccharides are usually accompanied by some decomposition, it is again only possible to estimate their relative proportions approximately. The significance of these results, taken together with previous results, may be assessed most conveniently by considering, in turn, the L-arabinose residues removed during the autohydrolysis, the backbone of 1 : 6-linked D-galactopyranose residues, and the aldobiouronic acid units. The approximate composition of the gum determined in Part I and expressed as parts per equivalent weight of gum acid provides a useful working model.

The quantity of 2 : 3 : 5-tri-*O*-methyl-L-arabinose isolated from the methylated gum accounts for approximately four of the five parts of L-arabinose present per equivalent of gum. Although relatively small amounts of 2 : 3 : 4-tri-*O*-methyl-L-rhamnose, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, and 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid were also present as units of the methylated gum, terminal L-arabofuranose residues account for most of the non-reducing end groups in the gum. The fifth part of L-arabinose is accounted for by approximately equal amounts of 2 : 3-, 2 : 4-, 2 : 5-, and 3 : 5-di-*O*-methyl-L-arabinose. Three of these four sugars represent units not susceptible to attack by periodate. Since it was shown in Part I that about 20% of the arabinose residues in the gum are not attacked by periodate, it follows that most, if not all, of these sugars are of structural significance and do not arise from incomplete methylation of the gum or from demethylation during hydrolysis. These non-terminal arabinose residues must also occur in the outer parts of the molecule since they are removed as free arabinose during the autohydrolysis of the gum acid. It is clear, therefore, that the majority of L-arabinose residues in the gum occur in the furanose form as single-unit side-chains attached to the more resistant part of the structure. In a few cases, however, non-terminal L-arabinose residues must be interposed between the end groups and the other sugar residues. The small proportion of L-arabopyranose residues in the gum, as shown by the isolation of 2 : 4-di-*O*-methyl-L-arabinose,

is of particular interest as until recently [3] L-arabinose had been found in combination only in the furanose form.

The D-galactose residues present in the gum occur in three main types of combination as shown by the isolation of 2 : 3 : 4-tri- (<1 part), 2 : 4-di- (>1 part), and 2-mono- (>1 part) *O*-methyl-D-galactose from the methylated gum. Since the corresponding residues in the methylated degraded gum afford 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-galactose, it follows that the preferred mode of attachment of arabinose is to position 3 of galactose, although it is possible that some arabinose residues may also be linked to position 4. It is probable, however, that most of the 2-*O*-methyl-D-galactose represents a double branching point, to arabinose through position 3, and to aldobiuronic acid through position 4. These results are in reasonable agreement with the results of periodate oxidation of the gum (Part I) where it was shown that about a third of the galactose residues in the gum were attacked by periodate. There is no evidence at present as to the rôle of the very small proportion of D-galactopyranose end groups.

The sugar residues present in the aldobiouronic acid groupings in the methylated gum are those of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, 2 : 3-di-*O*-methyl-D-glucuronic acid (main acid component), 2 : 4-di-*O*-methyl-D-galactose, and 4-*O*-methyl-D-mannose, whereas the corresponding residues in the methylated degraded gum are those of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid (main acid component), 2 : 3-di-*O*-methyl-D-glucuronic acid, 2 : 3 : 4-tri-*O*-methyl-D-galactose, and 3 : 4 : 6-tri-*O*-methyl-D-mannose. It follows that arabinose residues are attached to these sugars through position 3 of galactose, positions 3 and 6 of mannose, and through position 4 of some glucuronic acid residues. Apart from chromatographic and ionophoretic evidence for traces of 3 : 4 : 6-tri-*O*-methylmannose, the only derivative of D-mannose found in the hydrolysate from the methylated gum was the 4-methyl ether, indicating one main mode of linkage of mannose residues in the gum. The evidence adduced in Part I indicated, on the one hand, the presence of one part of mannose and one part of glucuronic acid per equivalent of gum, and, on the other hand, the linking of glucuronic acid to both galactose and mannose, and suggested that some mannose residues in the gum may be linked to neutral sugar residues only. Experiments to provide further evidence on this point are in progress.

In Part I it was shown that hydrolysis of gum ghatti affords small amounts of xylose and a 6-deoxyhexose (probably rhamnose) in addition to the main constituent sugars. The isolation of 2 : 3 : 4-tri-*O*-methyl-L-rhamnose from the methylated gum confirms the presence of L-rhamnose residues in the gum. No other methyl ethers of rhamnose were detected. Since this sugar has persisted throughout the various operations it seems probable that it is an integral part of the gum structure and does not arise from a contaminating polysaccharide. On the other hand, only traces of xylose derivatives (as the 2 : 3 : 4-trimethyl ether) could be detected on hydrolysis of the methylated gum. It is unlikely, therefore, that xylose is a constituent of the gum itself.

Our present knowledge of the detailed molecular structure of gum ghatti may be summarised in terms of the partial structures (I, II, and III), with the substituent groups R representing mainly single L-arabofuranose residues, but in a few cases more complex arabinose-containing side-chains terminated again by L-arabofuranose residues. Experiments to determine the mode of attachment of the aldobiouronic acid side-chains (II and III) to the backbone of galactose residues (I) will be reported later. It is already clear that gum ghatti resembles several other plant gums, notably damson, cherry, and egg-plum gums,[4-6] in containing a high proportion of L-arabofuranose residues in the outer parts of the molecular structure. It differs, however, from these gums in containing galactose residues mutually linked mainly through $C_{(1)}$ and $C_{(6)}$, and not through $C_{(3)}$ also. Gum

[3] For references see Aspinall and Schwarz, *Ann. Reports*, 1955, **52**, 267.
[4] Hirst and Jones, *J.*, 1938, 1174; 1939, 1482; 1946, 506.
[5] Jones, *J.*, 1939, 558; 1947, 1055; 1949, 3141.
[6] Hirst and Jones, *J.*, 1947, 1064; 1948, 120; 1949, 1757.

ghatti differs also in this respect from gum arabic, which is now known to contain a backbone of 1 : 3-linked D-galactopyranose units to which are attached side-chains of 1 : 6-linked galactose units.[7]

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper using the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer); (B) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (C) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (D) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (E) benzene–ethanol–water (169 : 47 : 15, upper layer); (F) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (G) butan-2-one, half saturated with water containing 1% of ammonia. Unless otherwise stated, chromatography of methylated sugars was carried out in solvent D and R_G values refer to rate of movement relative to 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in that solvent. Demethylations of methylated sugars were carried out by the procedure of Hough, Jones, and Wadman.[8] Paper ionophoresis was carried out in borate buffer at pH 10 at a potential of 500 v. Aniline derivatives of methylated sugars were prepared by refluxing the sugar in ethanolic aniline for 30 min.; further heating resulting in darkening of the solution. Optical rotations were observed at 18° ± 2°.

Samples of the gum acid and the degraded gum acid were prepared as described in Part I. During the preparation of the degraded gum acid the autohydrolysis of the gum acid caused the release of arabinose and only traces of xylose, rhamnose, and galactose. The various samples of degraded gum acid had slightly different optical rotations, $[\alpha]_D$ (as barium salt) varying from +2° to +9°. These samples were combined for subsequent experiments since the chromatographic patterns, after hydrolysis with N-sulphuric acid for 6 hr. at 100°, were similar in each case showing galactose in quantity, two aldobiouronic acids, small amounts of arabinose and mannose, and a trace of xylose.

Preparation and Hydrolysis of Methylated Degraded Gum.—The degraded gum (15 g.) was methylated extensively with methyl sulphate and sodium hydroxide following the procedure of Brown, Hirst, and Jones,[9] The product isolated as methylated degraded gum acid (8·2 g.; OMe, 36·9%) was further methylated with methyl iodide and silver oxide, giving methylated degraded gum (6·7 g.). Fractional precipitation of the methylated polysaccharide from chloroform by light petroleum gave fraction (*a*) (3·7 g.; OMe, 44·4%), which had $[\alpha]_D$ −28° (*c* 1·0 in $CHCl_3$), and fraction (*b*) (3·0 g.; OMe, 45·1%), which had $[\alpha]_D$ −11·5° (*c* 1·0 in $CHCl_3$). Chromatographic examination of the hydrolysates of the two fractions in solvent D showed similar complex mixtures of sugars.

Methylated degraded gum (fraction *a*; 3·2 g.) was suspended in N-hydrochloric acid (500 ml.) at 35° for 7 days, and the resulting solution was heated at 100° for 12·5 hr. (constant rotation), cooled, neutralised with silver carbonate, and filtered, and the filtrate was treated with hydrogen sulphide to remove silver ions, filtered, and concentrated. The resulting syrup was dissolved in water, and the solution was neutralised with barium carbonate, filtered, and concentrated to a dark syrup (3·0 g.). The dry syrup was repeatedly extracted with dry ether, to give an ether-soluble fraction (1·92 g.) and an ether-insoluble residue.

Examination of the Ether-soluble Fraction.—The syrup was separated on cellulose (60 × 3·4 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 eight fractions, and a further fraction was obtained by elution of the cellulose with water.

Fraction 1. The syrup (23 mg.) contained a mixture of sugars (R_G 0·97—0·93) and a trace of tri-*O*-methylgalactose (R_G 0·72). Chromatographic examination in solvent E showed 2 : 3 : 4-tri-*O*-methylxylose and two sugars having similar mobilities and staining properties with aniline oxalate to 2 : 3 : 4-tri-*O*-methylrhamnose and 2 : 3 : 5-tri-*O*-methylarabinose. Demethylation gave galactose and traces of xylose and arabinose. The remainder of the syrup was hydrolysed with N-hydrochloric acid at 100 for 6 hr., and chromatography showed much di-*O*-methylgalactose in the hydrolysate. The origin of the latter sugar is obscure.

Fraction 2. The syrup (97 mg.) had $[\alpha]_D$ +100° (*c* 0·34) and R_G 0·89. The sugar was

[7] Dillon, O'Ceallachain, and O'Colla, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 331; 1954, **57**, *B*, 31; Smith and Spriestersbach, Amer. Chem. Soc. Meeting, Minneapolis, Sept., 1955, Abs. Papers, 15D.
[8] Hough, Jones, and Wadman, *J.*, 1950, 1705.
[9] Brown, Hirst, and Jones, 1949, 1761.

identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 189—190°.

Fraction 3. Chromatography of the syrup (92 mg.) showed three components corresponding to 2 : 3 : 4 : 6-tetra-*O*-methylgalactose, 3 : 4 : 6-tri-*O*-methylmannose, and 2 : 3 : 4-tri-*O*-methylgalactose, R_G 0·89, 0·82, and 0·72. Demethylation gave galactose and mannose. Paper ionophoresis showed a component having the same mobility as 3 : 4 : 6-tri-*O*-methyl-D-mannose. Chromatographic examination of the products of periodate oxidation [2] showed 2 : 3 : 5-tri-*O*-methylarabinose (R_G 0·95) in addition to unchanged starting material. The syrup (50 mg.) was converted into the corresponding mixture of aldonamides, which, after treatment with sodium hypochlorite and addition of semicarbazide, afforded hydrazodicarbonamide, m. p. 268° and mixed m. p. (with sample of m. p. 263—264°) 264°.

Fraction 4. The chromatographically pure syrup (0·904 g.) had $[\alpha]_D$ +118° (*c* 0·42) and R_G 0·72 (Found: OMe, 41·7. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). Demethylation gave only galactose. The sugar was identified as 2 : 3 : 4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. 165—167° and mixed m. p. (with sample of m. p. 159—161°) 159—162°. The sugar subsequently crystallised from acetone–ether as the monohydrate, m. p. 71° and mixed m. p. (with sample m. p. 73—76°) 72—73°.

Fraction 5. The syrup (112 mg.) contained two components, R_G 0·72 (2 : 3 : 4-tri-*O*-methylgalactose) and 0·60. The second component showed similar chromatographic and ionophoretic behaviour to 3 : 4-di-*O*-methyl-D-mannose. The mixture gave galactose and mannose on demethylation. Attempts to separate the two components by chromatography on Amberlite resin IRA-400 (borate form) were unsuccessful.

Fraction 6. The syrup (203 mg.) contained two components, having R_G 0·49 and 0·75 respectively, the coloration of the former with aniline oxalate suggesting 2 : 3-di-*O*-methylgalactose. Demethylation gave only galactose. Separation of the syrup (190 mg.) on filter sheets using solvent D gave fractions 6*a* (70 mg.) and 6*b* (54 mg.). Paper ionophoresis of fraction 6*a* showed 2 : 3-di-*O*-methylgalactose and a trace of the 2 : 4-dimethyl ether. Chromatographic examination of the products of periodate oxidation [2] confirmed the presence of 2 : 3-di-*O*-methylgalactose. An authentic sample of 2 : 3-di-*O*-methyl-D-galactose when oxidised with periodate showed three oxidation products with R_F 0·66 (grey), 0·78 (brown), and 0·87 (grey) respectively, whereas 2 : 4-di-*O*-methyl-D-galactose showed only unchanged sugar (R_F 0·37). The sugar had $[\alpha]_D$ +69° ⟶ +80° (*c* 0·37) and the identity of the main component was proved by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. 125—128° and mixed m. p. 124—127°. Fraction 6*b* was hydrolysed with N-hydrochloric acid for 6 hr. at 100° and chromatographic examination showed only 2 : 3 : 4-tri-*O*-methylgalactose; it is probable that this fraction contains a polymer of 2 : 3 : 4-tri-*O*-methyl-D-galactose arising from incomplete hydrolysis of the methylated polysaccharide.

Fraction 7. The syrup (91 mg.) contained at least two components (having R_G 0·48 and 0·75 respectively). Separation of the mixture (88 mg.) on filter sheets using solvent D gave fractions 7*a* (53 mg.) and 7*b* (17 mg.). Paper ionophoresis and chromatographic examination of the products of periodate oxidation [2] indicated the presence in fraction 7*a* of 2 : 3- and 2 : 4-di-*O*-methylgalactose. The presence in the mixture of 2 : 4-di-*O*-methyl-D-galactose was shown by conversion into the aniline derivative, m. p. and mixed m. p. 202—204°. Fraction 7*b* contained the same substance (R_G 0·75) as fraction 6*b* and small amounts of di-*O*-methylgalactose. On hydrolysis the main component gave 2 : 3 : 4-tri-*O*-methylgalactose.

Fraction 8. The syrup (31 mg.) contained a di-*O*-methylgalactose (R_G 0·45) and small amounts of 4-*O*-methylmannose and an unidentified sugar (R_G 0·65).

Fraction 9. The syrup (48 mg.) contained a complex mixture of acidic and neutral sugars and was not examined further.

Examination of the Ether-insoluble Fraction.—The ether-insoluble residue was treated with Amberlite resin IR-120(H) to remove barium ions, and concentration gave a syrup (0·714 g.) (equiv. wt., 597). Chromatography showed neutral sugars in addition to acidic substances. The syrup (0·63 g.) was dissolved in water and neutralised with barium carbonate, and the resulting mixture was separated on filter sheets using solvent D, to give barium salts (A) (365 mg.), and fractions B (i) (190 mg.) and B (ii) (92 mg.), both contaminated with acidic substances. Chromatography showed fraction B (i) to contain mainly 2 : 3 : 4-tri-*O*-methylgalactose (R_G 0·72), and fraction B (ii) to contain di-*O*-methylgalactose (ionophoresis and chromatography of the periodate oxidation products [2] indicating the 2 : 3-isomer to be the

main component). A sample (25 mg.) of barium salts (A) was treated with Amberlite resin IR-120(H) to give a mixture of acids (equiv. wt., 370, indicating mainly aldobiouronic acid) which on hydrolysis yielded acidic and neutral sugars.

Barium salts (A) (330 mg.) were converted into the corresponding acids (307 mg.) which were hydrolysed with N-hydrochloric acid at 100° for 7 hr. After neutralisation with silver carbonate, separation on filter sheets in solvent D afforded neutral sugars [fractions C (i)—C (iv)] and silver salts (D). The silver salts (D) were converted into acids (143 mg.) which were separated on filter sheets by solvent C into fractions D (i) (48 mg.) and (D) (ii) (36 mg.).

Fraction C (i) (9 mg.) contained 3 : 4 : 6-tri-*O*-methylmannose (R_G 0·82). Fraction C (ii) (35 mg.) contained 2 : 3 : 4-tri-*O*-methyl-D-galactose (R_G 0·72), identified as the aniline derivative, m. p. 159—162° and mixed m. p. 156—159°. Fraction C (iii) (26 mg.) contained 2 : 3 : 4-tri-*O*-methylgalactose (R_G 0·72) and a trace of a second component (R_G 0·60). Fraction C (iv) (28 mg.) contained di-*O*-methylgalactose (R_G 0·49), shown by ionophoresis and chromatography of the periodate oxidation products [2] to be mainly the 2 : 3-dimethyl ether. Fraction (i) was chromatographically pure 2 : 3 : 4-tri-*O*-methylglucuronic acid. Attempts to characterise the sugar by conversion into the crystalline amide of methyl 2 : 3 : 4-tri-*O*-methyl-α-D-glucuronoside failed. The syrupy product was treated with methanolic hydrogen chloride, and the resulting ester was reduced with lithium aluminium hydride and hydrolysed. Chromatography showed only 2 : 3 : 4-tri-*O*-methylglucose (R_G 0·85). Fraction D (ii), which contained at least three components, was converted into the methyl ester methyl glycosides, reduced with lithium aluminium hydride, and hydrolysed. Chromatography showed 2 : 3-di-*O*-methylglucose, small amounts of tri- and di-*O*-methylgalactose, and a trace of 2 : 3 : 4-tri-*O*-methylglucose.

Preparation and Hydrolysis of Methylated Gum.—The gum acid (25 g.) was methylated extensively with methyl sulphate and sodium hydroxide by the procedure of Brown, Hirst, and Jones.[9] The product isolated as the methylated gum acid (18 g.; OMe, 35·0%; ash, 5·8%) was further methylated with methyl iodide and silver oxide (three treatments) to give methylated gum (7·3 g.), $[\alpha]_D$ −72° (*c* 1·0 in $CHCl_3$) (Found: OMe, 42·8%).

The methylated gum (7·3 g.) was refluxed with methanolic 2% hydrogen chloride (500 ml.) for 12 hr. (constant rotation). Methanol was removed under reduced pressure and the product was heated with 0·55N-hydrochloric acid (600 ml.) on the boiling-water bath for 12 hr. (constant rotation). The cooled solution was neutralised with silver carbonate, then filtered, and hydrogen sulphide was passed through the filtrate to precipitate silver ions, 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 (7·3 g.).

The mixture of methylated sugars (7·3 g.) was repeatedly extracted with dry ether to give ether-soluble sugars (A) (4·76 g.) and an ether-insoluble residue (B) (2·10 g.). The ether-insoluble sugars (B) were separated into neutral and acid fractions by chromatography on cellulose (50 × 2·5 cm.) with butan-1-ol, 80% saturated with water, as eluant, four fractions being isolated. Fraction (C) (0·80 g.) contained a mixture of neutral sugars. Fraction (D) (0·224 g.) contained chromatographically pure 2-*O*-methyl-D-galactose, m. p. 157—158° (from acetone–water), $[\alpha]_D$ +55° (5 min.) ⟶ +89° (120 min., const.) (*c* 1·91 in H_2O). Fraction (E) (0·14 g.) contained a mixture of 2-*O*-methylgalactose and acidic sugars. Fraction (F) (0·398 g.) contained acidic components and a trace of 2-*O*-methylgalactose.

Examination of Neutral Methylated Sugars.—The ether-soluble sugars (A) and fraction (C) were combined and separated on cellulose (76 × 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 eighteen fractions. A further fraction (19) (64 mg.) was obtained by elution of the cellulose with water.

Fraction 1. The syrup (119 mg.) had $[\alpha]_D$ +15° (*c* 0·39), and chromatography showed a main component having R_G 1·03 (cf. 2 : 3 : 4-tri-*O*-methyl-L-rhamnose) and traces of other sugars. Chromatography in solvent E showed a second component travelling faster. Hydrolysis of a sample with N-sulphuric acid, followed by neutralisation with barium carbonate and chromatography showed tri-*O*-methylrhamnose, 2 : 3 : 5-tri-*O*-methylarabinose, and the barium salt of an acid (at the starting line of the paper). Treatment of a second sample with cold barium hydroxide, followed by neutralisation with carbon dioxide and chromatography, gave a similar result. The remaining syrup (*ca.* 80 mg.) was treated with cold 5% barium hydroxide solution for 30 min., and the solution was neutralised with carbon dioxide, filtered

and concentrated. The product was separated on a filter sheet with solvent D, to give fractions 1*a* and 1*b*. Fraction 1*a* contained 2 : 3 : 4-tri-*O*-methylrhamnose (R_G 1·03) and a trace of 2 : 3 : 5-tri-*O*-methylarabinose (R_G 0·97). The main component was identified by conversion into 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-L-rhamnosylamine, m. p. and mixed m. p. 98—100°. Fraction 1*b* (barium salt) was deionised with Amberlite resin IR-120(H), and chromatography in solvent C showed 2 : 3 : 4-tri-*O*-methylglucuronic acid. Conversion of the acid into the methyl ester methyl glycoside with dry methanolic hydrogen chloride, followed by reduction with lithium aluminium hydride in methylal, hydrolysis with N-hydrochloric acid, and chromatography in solvent D, showed only 2 : 3 : 4-tri-*O*-methylglucose. It is concluded that tri-*O*-methylglucuronic acid was present in fraction 1 as an ester.

Fraction 2. Chromatography of the syrup (150 mg.) showed 2 : 3 : 5-tri-*O*-methylarabinose and a trace of 2 : 3 : 4-tri-*O*-methylrhamnose. The optical rotation, $[\alpha]_D$ −41° (*c* 0·59), indicated almost pure 2 : 3 : 5-tri-*O*-methyl-L-arabinose (cf. 2 : 3 : 5-tri-*O*-methyl-L-arabinose,[10] $[\alpha]_D$ −39·5°).

Fraction 3. The syrup (1·835 g.), which had $[\alpha]_D$ −42·5° (*c* 0·68), was almost pure 2 : 3 : 5-tri-*O*-methyl-L-arabinose with a trace of 2 : 3 : 4 : 6-tetra-*O*-methylgalactose. Demethylation gave arabinose and a trace of galactose. The identity of the main component was confirmed by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. 132—133° and mixed m. p. (with sample of m. p. 129—130°) 129°.

Fraction 4. Chromatography of the syrup (34 mg.), which had $[\alpha]_D$ +66° (*c* 0·48), showed a single component, R_G 0·97. Re-examination in solvent E showed 2 : 3 : 4-tri-*O*-methylxylose, 2 : 3 : 5-tri-*O*-methylarabinose, and an unidentified sugar. Demethylation gave xylose, arabinose, and galactose.

Fraction 5. Chromatography of the syrup (34 mg.) which had $[\alpha]_D$ +30° (*c* 0·59) in solvent E, showed 2 : 3 : 4 : 6-tetra-*O*-methylgalactose and 2 : 3 : 5-tri- and di-*O*-methylarabofuranose. The presence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose was shown by conversion into the aniline derivative, m. p. and mixed m. p. 179—180°.

Fraction 6. The syrup (17 mg.) had $[\alpha]_D$ −35° (*c* 0·29) and chromatography showed a main component with R_G 0·83, giving a brown stain (and yellow fluorescence in ultraviolet light) with aniline oxalate (cf. 3 : 5-di-*O*-methyl-L-arabinose, R_G 0·83). Demethylation gave arabinose and traces of galactose and mannose. Paper ionophoresis showed that 3 : 4 : 6-tri-*O*-methylmannose (R_G 0·82) and 2 : 5- (R_G 0·85) and 3 : 5-di-*O*-methylarabinose (R_G 0·83) could be readily distinguished in mixtures; fraction 6 showed mainly 3 : 5-di-*O*-methylarabinose with small amounts of the other two sugars.

Fraction 7. The syrup (230 mg.), which had $[\alpha]_D$ −24° (*c* 0·42), was shown by chromatography and ionophoresis to contain approximately equal amounts of 2 : 5- and 3 : 5-di-*O*-methylarabinose. Attempts to separate the two components by gradient elution from charcoal containing borate buffer (pH 10) with butan-2-one [11] were unsuccessful. The major portion (160 mg.) was fractionated by elution from Amberlite resin IRA-400 (borate form) with 0·5M-boric acid.[12] Although much sugar was irreversibly absorbed on the resin, two ionophoretically pure fractions 7*a* (40 mg.) and 7*b* (10 mg.) were obtained. Fraction 7*a* was identified as 2 : 5-di-*O*-methyl-L-arabinose by conversion into 2 : 5-di-*O*-methyl-L-arabonamide, m. p. 122° and mixed m. p. 123—124°. Fraction 7*b* was identified as 3 : 5-di-*O*-methyl-L-arabinose by conversion into 3 : 5-di-*O*-methyl-L-arabonolactone, m. p. 65° and mixed m. p. (with sample m. p. 69—71°) 67—69°.

Fraction 8. Chromatography and ionophoresis showed the syrup (80 mg.) to contain 2 : 5- and 3 : 5-di-*O*-methylarabinose, 3 : 4 : 6-tri-*O*-methylmannose, and a trace of 2 : 3 : 4-tri-*O*-methylgalactose. Attempts to separate the components by chromatography on charcoal containing borate buffer [11] failed.

Fraction 9. The syrup (174 mg.), which had $[\alpha]_D$ +72° ⟶ +119° (*c* 0·42), contained two components, R_G 0·65 and 0·70 respectively, present in approximately equal quantities and indistinguishable from 2 : 3-di-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylgalactose. Demethylation gave arabinose and galactose. The syrup was converted into the corresponding mixture of aldonamides, from which 2 : 3-di-*O*-methyl-L-arabonamide readily crystallised, m. p. 154° and mixed m. p. 153—154°.

[10] Baker and Haworth, *J.*, 1925, 365.
[11] Bouveng and Lindberg, *Acta Chem. Scand.*, 1956, **10**, 1283.
[12] Lock and Richards, *J.*, 1955, 3025.

Fraction 10. The chromatographically pure syrup (299 mg.) had $[\alpha]_D$ +109° (*c* 0·51) and R_G 0·70. Recrystallisation from acetone–ether afforded 2 : 3 : 4-tri-*O*-methyl-D-galactose hydrate, m. p. 66—67°. The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine had m. p. and mixed m. p. 161—162°.

Fraction 11. Chromatography of the syrup (59 mg.), which had $[\alpha]_D$ +88° (*c* 0·33), showed 2 : 3 : 4-tri-*O*-methylgalactose (R_G 0·70) and a trace of an unknown sugar (R_G 0·52). The main component was identified by conversion into 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 164—165°.

Fraction 12. Chromatography of the syrup (155 mg.) showed a main component, R_G 0·60, and a small amount of 2 : 3 : 4-tri-*O*-methylgalactose. Demethylation gave arabinose and a small amount of galactose. The optical rotation, $[\alpha]_D$ +118° (*c* 0·38), indicated that the major component was a di-*O*-methyl-L-arabopyranose. Chromatographic examination in solvent F differentiated the sugar from 2 : 3- and 3 : 4-di-*O*-methylarabinose, and ionophoresis showed 3 : 4-di-*O*-methylarabinose to be absent. The syrup (60 mg.), when heated with ethanolic aniline, afforded an aniline derivative, m. p. 129—130° and mixed m. p. (with sample, 139—140°) 125—126°, whose *X*-ray powder photograph was identical with that of 2 : 4-di-*O*-methyl-*N*-phenyl-L-arabinosylamine.

Fraction 13. Chromatography of the syrup (280 mg.), which had $[\alpha]_D$ +55° (*c* 0·29), showed a major component (R_G 0·49), a small amount of tri-*O*-methylgalactose, and a trace of a sugar (R_G 0·32) suspected of being 2-*O*-methylarabinose. Demethylation gave galactose and a trace of arabinose. Paper ionophoresis showed three components, a small component travelling at the same rate as 2 : 3-di-*O*-methylgalactose, an unidentified component in traces [(?) 2-*O*-methylarabinose], and the main component stationary (cf. 2 : 4-di-*O*-methylgalactose). Chromatography of the products of periodate oxidation showed 2 : 4-di-*O*-methylgalactose (R_F 0·37, unchanged), and small amounts of oxidation products with R_F 0·66 (grey), 0·78 (brown), and 0·15 (bright yellow). The first two oxidation products are formed from 2 : 3-di-*O*-methylgalactose, and the third from 2-*O*-methylaldoses (probably from 2-*O*-methylarabinose). The major component was identified as 2 : 4-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 206—208°.

Fraction 14. The chromatographically pure sugar (393 mg.) crystallised from acetone–water and had m. p. and mixed m. p. (with 2 : 4-di-*O*-methyl-D-galactose monohydrate) 97—99° and $[\alpha]_D$ +133° ⟶ +89° (equil.) (*c* 0·54) (Found: OMe, 27·8. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·4%). The derived 2 : 4-di-*O*-methyl-*N*-phenyl-D-galactosylamine had m. p. and mixed m. p. 213—214°.

Fraction 15. Chromatography of the syrup (109 mg.) showed 2 : 4-di-*O*-methylgalactose, a sugar with R_G 0·36, and a trace of (?) 2-*O*-methylarabinose. Demethylation gave galactose, mannose, and a trace of arabinose. Ionophoresis showed di-*O*-methylgalactose (stationary), and a second sugar moving faster than 2- and 3-*O*-methylmannose. Separation of the syrup (100 mg.) on cellulose with solvent G gave fractions 15*a* and 15*b*. Fraction 15*a* contained 2 : 4-di-*O*-methylgalactose and (?) 2-*O*-methylarabinose (R_G 0·32) and gave galactose and arabinose on demethylation. Chromatography of the products of periodate oxidation [2] showed unchanged di-*O*-methylgalactose and a component, R_F 0·15 (bright yellow), formed from 2-*O*-methylaldoses. Fraction 15*b* was identified as 4-*O*-methyl-D-mannose by conversion into 4-*O*-methyl-D-mannonolactone, m. p. and mixed m. p. 159—160°.

Fraction 16. Chromatography of the syrup (47 mg.) showed 4-*O*-methylmannose (R_G 0·36) and a second sugar in smaller amount (R_G 0·32). The optical rotation, $[\alpha]_D$ +43° ⟶ +37° (*c* 0·33) (cf. 4-*O*-methyl-D-mannose, $[\alpha]_D$ +32° ⟶ +22°), and methoxyl content (Found: OMe, 17·3. Calc. for $C_7H_{14}O_6$: OMe, 16·0. Calc. for $C_6H_{12}O_5$: OMe, 18·9%) were consistent with those of a mixture of 4-*O*-methyl-D-mannose and a mono-*O*-methyl-L-arabinose. Furthermore, chromatography showed periodate oxidation products with R_F 0·60 (brown) and 0·15 (yellow) identical with those from 4-*O*-methyl-D-mannose and 2-*O*-methylaldoses. Attempts to characterise the sugar by conversion into 4-*O*-methyl-D-glucosazone failed, although an impure fraction, m. p. 135—136°, was shown by circular paper chromatography to contain the desired compound together with a second component.

Fraction 17. Chromatography showed the syrup (84 mg.) to contain 2 : 4-di-*O*-methylgalactose, 4-*O*-methylmannose, and 2-*O*-methylgalactose.

Fraction 18. The chromatographically pure sugar (431 mg.; R_G 0·25) crystallised from glacial acetic acid and had m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 146—147°

and $[\alpha]_D$ +64° ⟶ +91° (equil.) (*c* 0·37). After recrystallisation from acetone–water the sugar had m. p. 154°.

Examination of Acidic Components.—Acidic fractions (F) and (19) (as barium salts), containing a trace of 2-*O*-methylgalactose, were combined and dissolved in water, barium ions were removed by passage through Amberlite resin IR-120(H), and the solution was concentrated to a syrup (376 mg.). The mixture of acids was refluxed with methanolic 1·3% hydrogen chloride (50 ml.) for 6 hr. The product, after neutralisation with silver carbonate and concentration, was dissolved in formaldehyde dimethyl acetal (40 ml.), lithium aluminium hydride (0·2 g.) was added, and the solution was refluxed for 2 hr. Excess of hydride was destroyed by water, the acetal layer was separated, the aqueous layer was taken to dryness, and the residue was extracted with chloroform and acetone. The combined organic extracts were concentrated to a syrup (330 mg.) which was hydrolysed with N-hydrochloric acid (30 ml.) for 6 hr. at 100° to give, after neutralisation and concentration, a syrupy mixture of sugars (230 mg.). Separation of the methylated sugars on cellulose (50 × 2·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (1 : 1), saturated with water, as eluant, gave nine fractions. Fraction *a* (21 mg., R_G 0·85) was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 134—135°. Fraction *b* (19 mg.; R_G 0·72 and 0·58) contained 2 : 3 : 4-tri-*O*-methylgalactose and 2 : 3-di-*O*-methylglucose (major component). Fraction *c* (47 mg.; R_G 0·58) was identified as 2 : 3-di-*O*-methyl-D-glucose by chromatography of the sugar and its periodate oxidation products [R_F 0·73 (bright yellow) and 0·63 (brown), ionophoresis, and by conversion into 2 : 3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. 173—174° and mixed m. p. (with sample, m. p. 168—169°) 169—171°. Fraction *d* (11 mg.; R_G 0·58 and 0·54) contained 2 : 3-di-*O*-methylglucose and a trace of an unidentified sugar. Fraction *e* (9 mg.; R_G 0·49) was identified as 2 : 4-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 212—214°. Fraction *f* (10 mg.; R_G 0·49 and 0·36) contained a mixture of 2 : 4-di-*O*-methylgalactose and 4-*O*-methylmannose. Fraction *g* (20 mg.; R_G 0·36) was identified as 4-*O*-methyl-D-mannose by chromatography of the sugar and its periodate oxidation product [R_F 0·60 (brown)], ionophoresis, and by conversion into 4-*O*-methyl-D-mannonolactone, m. p. and mixed m. p. (with sample of m. p. 161—162°) 150—151°. Fraction *h* (13 mg.; R_G 0·32) contained at least two components giving periodate oxidation products having R_F 0·60 (brown) and 0·15 (bright yellow). Fraction *j* (8 mg.; R_G 0·25) was identified as 2-*O*-methyl-D-galactose by chromatography of the sugar and its periodate oxidation product R_F 0·23 (bright yellow), and as the crystalline sugar, m. p. and mixed m. p. 139—140°.

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