228. *The Constitution of Mesquite Gum. Part II. Methylated Mesquite Gum.*

By J. I. Cunneen and F. Smith.

Methylation of mesquite gum or its acetate with methyl sulphate and sodium hydroxide affords the corresponding methylated mesquitic acid. By means of silver oxide and methyl iodide the latter is converted into the methyl ester. Methanolysis of the methyl ester of methylated mesquitic acid by methyl-alcoholic hydrogen chloride gives rise to 2 : 3 : 5-trimethyl methyl-L-arabofuranoside (I) (3 mols.); 3 : 5-*dimethyl methyl-L-arabofuranoside* (V) (6—7 mols.); 2 : 4-dimethyl methyl-D-galactopyranoside (IX) (4 mols.); a dimethyl methylhexoside (probably a galactoside) (1 mol.) and the methyl ester of 2 : 3 : 4-trimethyl methyl-D-glucuronoside (XI) (2 mols.). With the exception of the unknown dimethyl methylhexoside, these constituents of the methyl gum have been identified by their conversion into characteristic crystalline derivatives. The structural significance of the formation of the various methyl-glycosides is discussed.

In Part I (Cunneen and Smith, preceding paper) there was described the isolation and characterisation of two aldobiuronic acids, 6- and 4-glucuronosidogalactose, which form part of the complex molecular structure of mesquite gum. This paper is concerned with the determination of the mode of linking between these two aldobionic acids and the sugar components, galactose and arabinose, which constitute the rest of the complex structure.

The various methyl derivatives of galactose, arabinose, and glucuronic acid obtained by cleavage of methylated mesquite gum have been separated and identified, and an estimate made of the proportion of each of the substances present in the repeating unit of the complex molecule. Although there has been some hesitation in the past in drawing conclusions from the isolation of partly methylated glycosides formed by hydrolysis of methylated polysaccharides, it is now recognised that these partly methylated sugars are of structural significance (cf. Freudenberg, *Ber.*, 1940, **73**, 609), and, because of this recognition, it has been possible to make some progress in the determination of the constitution of highly complex polysaccharides such as damson gum (Hirst and Jones, *J.*, 1946, 506) and gum arabic (Smith, *J.*, 1940, 1035). The same significance is to be attached to the isolation of dimethyl glucose from the cleavage of methylated glycogen and methylated starch.

As the fractional precipitation of gums is often somewhat difficult, an investigation as to the homogeneity of mesquite gum was made on the methyl derivative and not on the gum itself. The methylated gum was obtained by the action of methyl sulphate and sodium hydroxide on either the gum or its acetyl derivative. The methylated mesquitic acid so prepared was esterified, and completely methylated by treatment with methyl iodide and silver oxide. Fractional precipitation of the methyl ester of methylated mesquitic acid from ethereal solution with light petroleum showed that the main part of the material was essentially homogeneous; it had an equivalent weight of 1550, $[\alpha]_D$ + 55° in chloroform, and a methoxyl content of 40%.

When boiled with 2% methyl-alcoholic hydrogen chloride, the methyl ester of methylated mesquitic acid undergoes simultaneous hydrolysis and glycoside formation with formation of a mixture of methylated sugars, methyl esters of methylated uronic, and methylated aldobionic acids. Treatment of the cleavage fragments with dilute barium hydroxide solution converted the esters of the acids into their barium salts. Extraction of a dry mixture of the barium salts and the methylated glycosides with ether readily removed the latter and left the barium salts as a pale yellow powder.

The mixture of methylated glycosides was separated by fractional distillation; this was simple compared with the separation of the methylglycosides from methylated arabic acid (Smith, *J.*, 1940, 1035), mainly owing to the larger difference in the boiling points of the constituents and to the fact that there was no tendency to form constant-boiling mixtures. The methylglycosides were identified as 2 : 3 : 5-trimethyl methyl-L-arabofuranoside, 3 : 5-*dimethyl methyl*-L-*arabofuranoside*, and 2 : 4-dimethyl methyl-D-galactopyranoside.

2 : 3 : 5-Trimethyl methylarabofuranoside (I) was characterised by the following facts. It was hydrolysed by dilute sulphuric acid to the trimethyl arabinose (II) which on oxidation with bromine in aqueous solution afforded the characteristic crystalline lactone (III) (Baker and Haworth, *J.*, 1925, 365). When allowed to react with methyl-alcoholic ammonia, the lactone (III) gave the characteristic crystalline amide (IV) of 2 : 3 : 5-trimethyl L-arabonic acid (Pryde, Humphreys, and Waters, *J.*, 1931, 1298; Haworth, Hirst, and Oliver, *J.*, 1934, 1922; Hirst and Jones, *J.*, 1939, 496; Smith, *J.*, 1940, 1035).

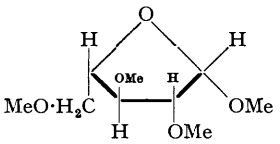
The structure assigned to the *dimethyl methylpentoside* (V) was ascertained in the following manner. Methylation of (V) with silver oxide and methyl iodide gave a trimethyl methylpentoside, identified as (I) by the reactions already outlined. This evidence demonstrated that the dimethyl methylpentoside was a derivative of methyl-L-arabofuranoside and that the two methyl groups must occupy the positions 2 : 3, 2 : 5, or 3 : 5.

To decide between these possibilities, the *dimethyl arabinose* (VI) obtained by hydrolysis of the arabinoside (V) was oxidised with bromine to the corresponding *dimethyl arabonolactone* (VII). This lactone was crystalline (m. p. 75°), and its slow rate of mutarotation in aqueous solution ($[\alpha]_D$ — 84° changing to — 69° in 28 days) indicated that it belonged to the γ-series of arabonolactones. The properties of the lactone (VII), and of the crystalline *amide* derived from it, distinguished it from the known 2 : 5-dimethyl L-arabonolactone (Smith, *J.*, 1939, 751) and also from the 2 : 3-isomer (Hirst and Jones, *J.*, 1938, 504; Smith, *J.*, 1939, 753).

It was thus inferred that the two methyl groups must be at C_3 and C_5, and this was confirmed by the fact that the amide (VIII) showed a positive test for α-hydroxy-amides (Weerman, *Rec. Trav. chim.*, 1917, **36**, 16).

Although at the time the lactone (VII) had not been described it has since been characterised

by White (*J. Amer. Chem. Soc.*, 1946, **68**, 272) who demonstrated the presence of a free hydroxyl group at C_2 by the fact that the dimethyl arabinose gives a phenylosazone without loss of methoxyl.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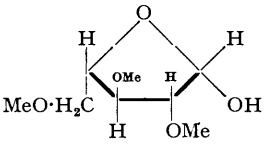
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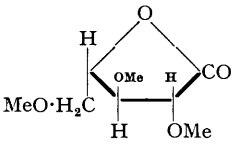
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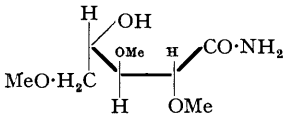
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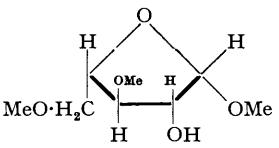
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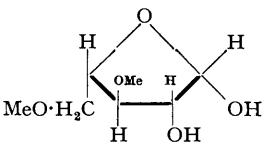
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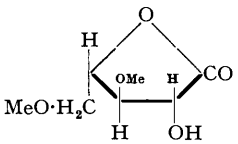
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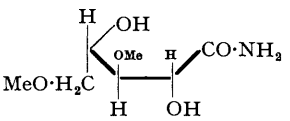
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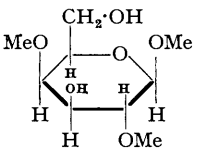
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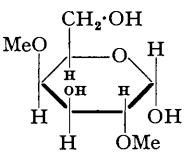
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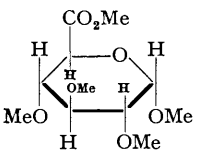
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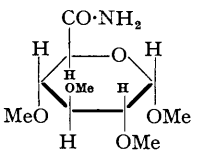
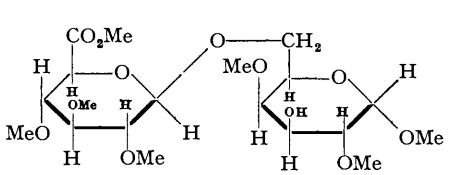

(XII.)

The third component of the mixture of methylated glycosides, the crystalline dimethyl methylhexoside (IX), was identified as 2 : 4-dimethyl β-methylgalactopyranoside by comparison with an authentic specimen previously obtained from arabic acid (Smith, *J.*, 1939, 1724). This constitution was confirmed by the observation that on hydrolysis with N-sulphuric acid (IX) gave 2 : 4-dimethyl galactose (X) as the crystalline monohydrate. Furthermore, treatment of (X) with ethyl-alcoholic aniline yielded the characteristic crystalline anilide (McCreath and Smith, *J.*, 1939, 387; Smith, *J.*, 1939, 1736).

The acidic components of the cleavage fragments isolated as barium salts were treated with acid methyl alcohol at room temperature, a procedure which esterifies, without cleaving the glycosidic bonds (Jackson and Smith, *J.*, 1940, 74). Fractional distillation of the mixture of ester glycosides thus formed gave the methyl ester of trimethyl methylglucuronoside (XI) and the methyl ester of a pentamethyl aldobiuronoside (XIII). A residue (F) remained.

The structure of the methyl ester of the trimethyl methylglucuronoside (XI) was proved by the observation that its treatment with methyl-alcoholic ammonia yielded the characteristic amide of trimethyl α-methylglucopyruronoside (XII), identified by comparison with an authentic specimen (Smith, *J.*, 1939, 1732).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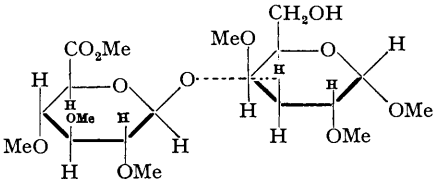

(XIV.)

The facts available strongly suggest that the methyl ester of the *pentamethyl methyl-aldobiuronoside* (XIII) is composed of a molecule of 2 : 3 : 4-trimethyl glucuronic acid joined

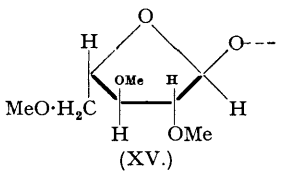
through its reducing group C_1 to position 6 of a molecule of 2 : 4-dimethyl methylgalactoside. On boiling the aldobionic ester (XIII) with 8% methyl-alcoholic hydrogen chloride, simultaneous hydrolysis and glycoside formation take place with production of almost equimolecular amounts of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) and crystalline 2 : 4 dimethyl methylgalactopyranoside (IX). These were readily separable by fractional distillation and were identified in the manner already described.

The two fragments (XI) and (IX) of the methylated aldobiuronic acid would result from a biuronic acid composed of a 2 : 3 : 4-trimethyl methylglucuronic acid joined through its reducing group either to position 3 or to position 6 of the 2 : 4-dimethyl methylgalactopyranoside. The two possibilities are shown by the formulations (XIII) and (XIV) respectively both of which would give on cleavage the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) and crystalline 2 : 4-dimethyl methylgalactopyranoside (IX) as required by the experimental facts. Previous investigations have established the fact that two aldobiuronic acids are produced by hydrolysis of mesquite gum, namely, 6- and 4-glucuronosido-galactose (Cunneen and Smith, *loc. cit.*). Only the former will allow of the formation of a 2 : 4-dimethyl galactose residue, and for this reason it is believed that in the methylated aldobionic acid obtained from methylated mesquitic acid there is present a 1 : 6 link and that it is therefore to be represented by (XIII) and not by (XIV).

The existence of an aldobiuronic acid in mesquite gum having a 1 : 3-glycosidic linkage cannot, however, be entirely dismissed on the evidence produced above because it may be that such an aldobionic acid would be more easily hydrolysed than those containing the 1 : 6 and the 1 : 4 linkages.

The incompletely hydrolysed acid fragments (residue F, see above) gave upon further hydrolysis with acid methyl alcohol more 2 : 3 : 4-trimethyl methylglucuronoside and 2 : 4-dimethyl methylgalactoside, but there still remained an acid-resistant residue approximating analytically to a methylated aldobionic acid. This required treatment with methyl-alcoholic hydrogen chloride in a sealed tube at 130° before it underwent cleavage to give a trimethyl methylglucuronoside and a dimethyl methylhexoside. The identity of the latter was not established, but it is believed that these two fragments are derived by hydrolysis of a methylated aldobiuronic acid which corresponds to the 4-glucuronosidogalactose previously isolated from mesquite gum (Cunneen and Smith, *loc. cit.*).

As a result of these constitutional studies it is at once apparent that although the structure of methylated mesquite gum, as a whole, is complicated, it is nevertheless characterised by some simple features. Thus the characterisation of the 2 : 3 : 5-trimethyl methyl-L-arabinoside (I) and the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) prove that these units must constitute " end " residues in the complex and consequently they are joined to other units in the methylated polysaccharide gum by glycosidic bonds shown by broken lines in formula (XV) and (XVI).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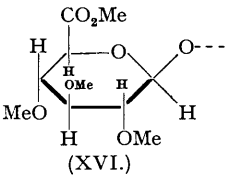
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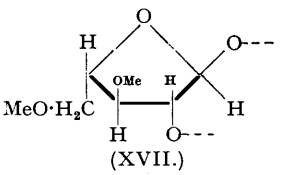
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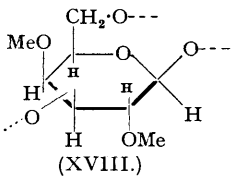
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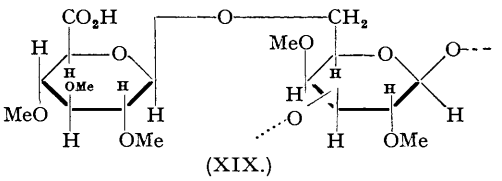
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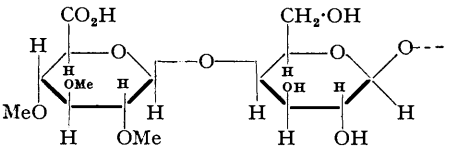

(XX.)

The dimethyl methyl-L-arabinoside with its substituent methyl groups in positions 3 and 5 can arise only from L-arabofuranose residues which are joined by glycosidic bonds to other units in the complex as in (XVII).

By the same reasoning it is clear that the 2 : 4-dimethyl methylgalactoside is produced from galactose units involved in glycosidic union with other units through positions 3 and 6 as in (XVIII) (see White, *loc. cit.*).

Isolation and characterisation of 6 β-D-glucuronosido-D-galactose by direct hydrolysis of mesquite gum establishes the fact that some of the end glucuronic acid units are joined to position 6 of a galactose residue (Cunneen and Smith, *loc. cit.*). This is now confirmed by the isolation of the methyl ester of a pentamethyl 6-glucuronosidomethylgalactoside from the methylated mesquitic acid. The experimental proof that this pentamethyl derivative is composed of a unit of 2 : 3 : 4-trimethyl glucuronic acid joined by a 1 : 6-linkage to a unit of 2 : 4-dimethyl galactose enables the further deduction to be made that the galactose residue in the aldobionic acid must be joined to other residues of the complex through positions 1 and 3 as in (XIX).

Since the existence of an aldobiuronic acid having a 1 : 4-linkage has been established by the isolation of 4-glucuronosidogalactose from the hydrolytic fragments of mesquite gum (Cunneen and Smith, *loc. cit.*) it is also apparent that some glucuronic acid units are joined to other galactose units through position 4 as in (XX). The manner in which the 4-glucuronosido-galactose fragment is linked to the rest of the mesquitic acid molecule has not yet been determined, but if it proves to be correct that the dimethyl hexose isolated as described above from methylated mesquitic acid is in fact derived from the 4-glucuronosidogalactose, then it is obvious that this aldobionic acid is attached to the rest of the complex by two linkages. One link involves the reducing group (C_1) of the galactose unit, the other engages one of the positions 2, 3, or 6. The elucidation of the structure of this dimethyl galactose unit will be the subject of further investigation.

Plant gums are composed of several kinds of sugar units, some of which may be present as pyranose and others as furanose forms. Most plant gums contain the oxidised form of a hexose such as glucuronic or galacturonic acid, and some contain the reduced form of a hexose such as rhamnose (gum arabic) or fucose (gum tragacanth), but, so far, such reduced forms of the sugars as hexitols or pentitols have not been detected. Another characteristic of plant gums is that the units are joined by several types of glycosidic linkages. The gum now being discussed is a typical plant gum inasmuch as the component monosaccharide residues consisting of galactose, arabinose, and glucuronic acid are mutually joined by no less than four types of linkage, namely the 1 : 2-pentose and the 1 : 3-, 1 : 4-, and 1 : 6-hexose types. The presence of the 1 : 2-linkage is deduced from the isolation of 3 : 5-dimethyl arabinose, the 1 : 3-linkage from the isolation of 2 : 4-dimethyl galactose, the 1 : 4-linkage from the characterisation of 4-glucuronosidogalactose, and the fourth type, the 1 : 6-linkage, follows from the isolation of 6-glucuronosidogalactose and 2 : 4-dimethyl galactose.

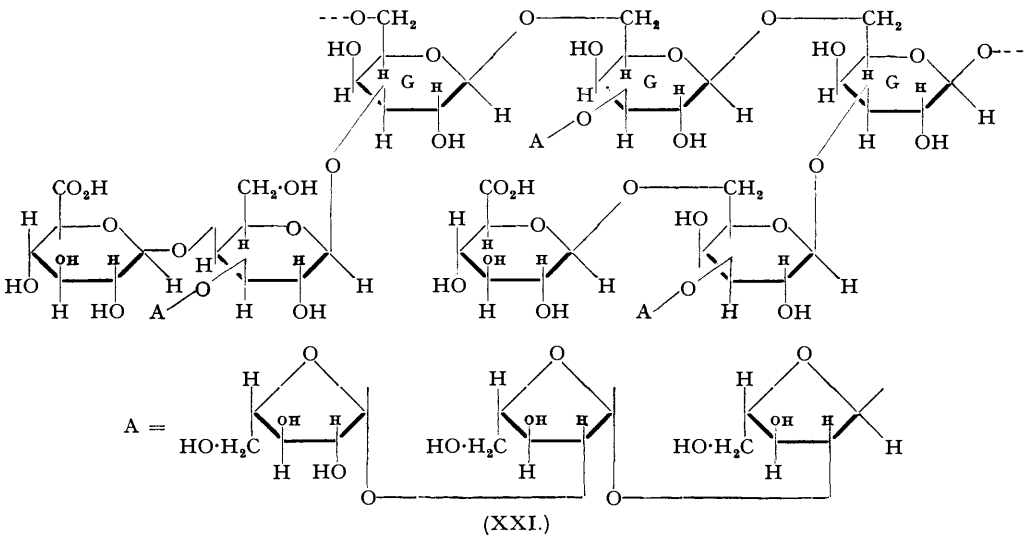
The identification of the products of hydrolysis of the methylated polysaccharides is not an easy problem, and some difficulty attends the determination of the amounts of each of these fragments. No unique solution of the structure of mesquite gum can, therefore, be advanced, but it is of interest to consider, in the light of the available evidence, the possible types of complex structure that may represent its constitution.

Calculations based upon the weights of the various constituents of the mixture of the cleavage fragments obtained from the methylated mesquite gum indicate that the approximate relative proportions of the fragments are as follows : 2 : 3 : 5-trimethyl methylarabinoside (3 mols.); 3 : 5-dimethyl methylarabinoside (6—7 mols.); 2 : 4-dimethyl methylgalactoside (4 mols.); 2 : 3 : 4-trimethyl methylglucuronoside (2 mols.); an unknown dimethyl methylhexoside (? galactoside) (1 mol.). Glycosidic union of these 17 fragments will give a repeating unit having an equivalent weight of 1250 for the mesquitic acid (found, 1350) and 1570 for the methylated substance (found, 1550).

The isolation of 2 : 3 : 5-trimethyl and 3 : 5-dimethyl arabinose shows that the pentose units are present in the complex as furanose units, while the identification of the galactose units as a 2 : 4-dimethyl derivative and the glucuronic acid as the 2 : 3 : 4-trimethyl derivative, and the very probable formation of an unidentified dimethyl galactose from the aldobionic acid which possesses the 1 : 4-linkage, strongly suggest that the galactose and glucuronic acid units all have the pyranose structure. It is believed that there exists in mesquitic acid a nucleus, relatively stable to acid hydrolysis, which is composed of pyranose units of glucuronic acid and galactose and to which is attached the arabofuranose residue (cf. arabic acid). Such a

representation derives support from the fact that hydrolysis of the gum with 0·1N-sulphuric acid gives arabinose and a degraded mesquitic acid which is composed of glucuronic acid and galactose residues only. The methylated gum is degraded in an analogous fashion by boiling 0·5% methyl-alcoholic hydrogen chloride. A mixture of 3 : 5-dimethyl and 2 : 3 : 5-trimethyl methylarabofuranoside is produced, while the stable nucleus of methylated degraded mesquitic acid, composed solely of galactose and glucuronic acid units, remains unaffected. The hydrolysis of this relatively stable methylated nucleus demands a much more drastic treatment than that required for the cleavage of the arabofuranoside links, an observation which supports the view that the residues of galactose and glucuronic acid, of which this nucleus is composed, are of the pyranose type.

The presence of branched chains is clearly proved not only by the isolation of 2 : 4-dimethyl galactose but by the isolation of three molecular proportions of 2 : 3 : 5-trimethyl arabinose and two of 2 : 3 : 4-trimethyl glucuronic acid. The arabinose units which afford the 2 : 3 : 5-trimethyl derivative and the glucuronic acid units from which the corresponding 2 : 3 : 4-trimethyl derivative arises, constitute five end groups in the structure of a repeating unit composed of approximately seventeen sugar residues. This high proportion of end groups can arise only from a multiple branched-chain structure.



(XXI.)

Several structures may be assigned to mesquitic acid on the basis of the above evidence. One possible formulation of the repeating unit is shown by (XXI); this consists of three galactose units G joined by 1 : 6-linkages to form a main chain, and to position 3 of any two of the three galactose units of this main chain are attached the two branching side chains consisting of two aldobiuronic acids the presence of which in mesquitic acid has been shown both in this work and in that recorded in Part I. Thus five galactose units and two glucuronic acid residues constitute the stable nucleus of degraded mesquitic acid.

To this stable nucleus are attached three other side chains designated by (A) in (XXI). These three groups are composed entirely of the arabofuranose units which are split off by mild acid hydrolysis from the relatively stable nucleus of mesquitic acid. Of the methylated arabofuranose units that are formed from the methyl ester of methylated mesquitic acid by methanolysis, three are fully methylated and must constitute terminal units. The remaining arabinose units appear as the 3 : 5-dimethyl derivatives and must therefore be distributed as non-terminal units of three chains in which the arabofuranose units are mutually joined by 1 : 2-glycosidic linkages. There are several arrangements of the pentose constituents which would satisfy the above requirements. The simplest is that given in (XXI). Here the three acid labile side chains (A) are represented as being each composed of three arabofuranose units. One of these side chains (A) is joined to that galactose unit of the main chain which is not involved in the branching of the nucleus of degraded mesquitic acid. The other two side chains (A) are joined as shown to those galactose units which form part of the aldobiuronic acid residues.

The structure (XXI) used for purposes of discussion shows three galactopyranose units in

the main chain united by 1 : 6-linkages, and to position 3 of each of these units a side chain is attached. These side chains, three in number, are composed of two residues of aldobionic acid and one (A) of arabofuranose units. It will be apparent, however, that it is also possible for the three galactose units to be joined by 1 : 3-linkages while the side chains are attached to position 6 of each of the three galactose units in the main chain. Such a modification of the union of the 3 galactose units in the main chain would still be in conformity with the established fact that these galactose units give rise to 2 : 4-dimethyl galactose.

Furthermore, the possibility of the three galactose units of the main chain being mutually joined by 1 : 6- and 1 : 3-linkages cannot be excluded. In such a case, the side chain (A) and the two aldobionic acid side chains would be attached to position 3 of the galactose units already linked in the main chain through position 6, and to position 6 of the galactose units which have position 3 involved in the main chain linkage (White, *J. Amer. Chem. Soc.*, 1947, **69**, 622). Substantial evidence as to the precise mode of linkage of the galactose constituents of the main chain will best be obtained by the methylation of degraded mesquitic acid [*i.e.*, of mesquitic acid from which the labile arabofuranose side chains (A) have been removed by acid hydrolysis] followed by a study of the partly methylated products of hydrolysis. A preliminary study of this methylated degraded mesquitic acid was made in 1939, but owing to the war the completion of the examination had to be postponed; this will form the subject of a later communication.

In mesquite gum, as in gum arabic, damson gum, and cherry gum, the complex structures are composed of several kinds of sugars joined by different types of glycosidic bonds. Both hexoses and pentoses are found as members of polysaccharide gums, and it is of particular interest that hexoses and their derivatives—for example, rhamnose, fucose, glucuronic acid, and galacturonic acid—occur only in pyranose form (Hirst, *J.*, 1942, 77). Of the pentoses, arabinose seems to exist only in the furanose form as in the gums and arabans, while xylose occurs for example in xylan (Haworth, Hirst, and Oliver, *J.*, 1934, 1917), gum tragacanth (Luckett and Smith, *J.*, 1945, 739), damson gum (Hirst, *loc. cit.*), and the mucilage of *Plantago lanceolata* (Mullan and Percival, *J.*, 1940, 1501) in the pyranose form. It is also curious that L-arabinose and D-glucuronic acid often form constituents of the same molecular complex, as in the gum under review, gum arabic (Smith, *J.*, 1940, 1035), and cherry and damson gums (Hirst, *loc. cit.*), while D-galacturonic acid and D-xylose have been identified as constituents of gum tragacanth (Luckett and Smith, *loc. cit.*). Seldom does D-galacturonic acid occur with L-arabinose, and, although D-glucuronic acid and D-xylose form members of the same complex as in damson gum (Hirst and Jones, *J.*, 1938, 1174), the proportion of xylose is small, and furthermore there is present also a much larger proportion of L-arabinose. Glucose still remains to be identified as a constituent of plant gums. The significance of these facts is not yet clear, but they seem to indicate that in the plant the formation of a pentose from a hexose, if it occurs, does not involve the intermediate formation and decarboxylation of a uronic acid. For example, D-galacturonic acid could not be intermediate in the formation of L-arabofuranose units from D-galactopyranose unless a change from a pyranose to a furanose ring structure took place analogous to that which occurs with remarkable ease in the acid-catalysed conversion of 3 : 6-anhydro-α-methylglucopyranoside into 3 : 6-anhydro-α-methylglucofuranoside (Haworth, Owen. and Smith *J.*, 1941, 88). Nevertheless, the fact remains that oxidation or reduction of terminal groups of hexoses does occur in Nature as is shown by the inter-relationship of D-glucose, D-glucuronic acid, and D-xylose; of D-galactose, D-galacturonic acid, and L-arabinose; of D-galactose and L-fucose; and of D-mannose and L-rhamnose.

Experimental.

Methylation of Mesquitic Acid.—Mesquitic acid (20 g.) which had equiv. wt. of 1300 (by titration with sodium hydroxide) and 1290 (from uronic anhydride content of 13·7%) was methylated by dissolving it in sodium hydroxide (200 c.c. of a 30% solution) and gradually adding methyl sulphate (250 c.c.) and sodium hydroxide (500 c.c. of a 30% solution) during 4 hours at room temperature with vigorous stirring, which was continued for a further 8 hours after the addition of all the methyl sulphate. The resulting solution was cooled in an ice-bath, almost neutralised with dilute sulphuric acid, and filtered to remove sodium sulphate, the filtrate concentrated to a suitable volume (100—150 c.c.) under reduced pressure, and the methylation repeated using methyl sulphate (250 c.c.) and sodium hydroxide (700 c.c. of a 30% solution). During the methylation, acetone was added as required to keep the methylated material in solution. After the fifth methylation the sodium salt of the methylated polysaccharide, which separated readily upon completion of the methylation by heating for 15—20 minutes on the boiling water-bath, was dissolved in aqueous acetone (60 c.c. water, 40 c.c. acetone) and treated with methyl sulphate (250 c.c.) and sodium hydroxide (750 c.c. of a 30% solution) at 35°, the reagents being added gradually during 2½ hours. The methylation was completed by heating the solution for 15 minutes on the boiling water-bath, whereby the excess of acetone was expelled and the sodium salt of methylated mesquitic acid separated on the surface of the mixture as yellow nodules. These were removed by filtration while

the solution was still hot, since the sodium salt of methylated mesquitic acid was less soluble in hot than in cold water. After two more methylations at 35° a portion of the sodium salt of methylated mesquitic acid was suspended in water, acidified with dilute sulphuric acid, and extracted with chloroform. After being washed with sodium sulphate solution (20%) and dried ($MgSO_4$), the chloroform solution was concentrated under reduced pressure to give a glassy solid, which after dissolution in acetone, was poured into excess of light petroleum. This precipitated the methylated polysaccharide as a white amorphous powder which after being dried in a vacuum at 60° had $[\alpha]_D^{20°}$ + 60° in chloroform (*c*, 1·3) (Found : OMe, 39·1%; equiv., 1540). The remainder of the sodium salt of methylated mesquitic acid was subjected to 3 further methylations, and the product (8 g.) was purified as above (Found : OMe, 39·0%). Three treatments of mesquite gum with methyl sulphate and sodium hydroxide are probably enough to give complete methylation.

The Acetate of Mesquitic Acid.—Freshly prepared mesquitic acid (100 g.) (Cunneen and Smith, *loc. cit.*) was dissolved in pyridine (1 l.), and acetic anhydride (1 l.) gradually added during 2 hours. After 2 more hours, the solution was poured slowly with stirring into water (10 l.), the precipitated mesquitic acetate was washed 3 times with water, filtered off, and washed once with ethyl alcohol and finally with ether. After being dried in a vacuum at 45° it was obtained as a fine white amorphous powder (114 g.).

Methylation of the Acetate of Mesquitic Acid.—Mesquitic acetate was methylated in portions of 20 g. by dissolving each portion in aqueous acetone (200 c.c. acetone, 100 c.c. water) and gradually adding sodium hydroxide (700 c.c. of a 30% solution) and methyl sulphate (250 c.c.) during 1½ hours at 45°. The methylation was completed by heating the mixture at 90° for 20 minutes to remove all acetone. The sodium salt of the methylated mesquitic acid separated as yellow nodules which were removed by filtration and subjected to a further 5 methylations. After this treatment the sodium salt of the partially methylated gum was suspended in water (500 c.c.) and acidified with dilute sulphuric acid, and the solution extracted 3 times with chloroform. Evaporation of the chloroform solution, after it had been washed with a concentrated solution (*ca.* 20%) of sodium sulphate and dried (Na_2SO_4), yielded the methylated polysaccharide (17 g.) which had $[\alpha]_D^{18°}$ + 62° in chloroform (*c*, 1·0) (Found : OMe, 39·0%; equiv., 1625).

Methylation was completed by boiling a solution of the polysaccharide in methyl iodide (30 c.c.) under reflux for 6 hours, during which time silver oxide (15 g.) was gradually added. Following removal of the excess of methyl iodide by distillation the methylated product was extracted with acetone; evaporation of the acetone solution gave the methylated gum as a glassy solid (16 g.) which was subjected to three further methylations with Purdie's reagents (silver oxide and methyl iodide).

Fractionation of Methylated Mesquitic Gum.—To a solution of the methylated polysaccharide (32 g.) in ether (250 c.c.), light petroleum (b. p. 40—60°) was added slowly with stirring to give a precipitate (fraction 1). The mother liquor was decanted and from it two more fractions (2 and 3) were successively precipitated with light petroleum. Fraction 4 was obtained as a white amorphous powder by pouring the solution, left after the precipitation of the first 3 fractions, into excess of light petroleum. Each fraction was then redissolved in ether (50 c.c.) and reprecipitated by pouring the solution into excess of light petroleum. The properties of these fractions after being dried in a vacuum are given in the Table. Fractions 2 and 3 showed almost identical properties and were accordingly subjected to hydrolytic examination.

Fraction.	Wt (g.).	OMe (%).	$[\alpha]_D^{20°}$ ($CHCl_3$).	Ash (%).	Equiv.	η in *m*-cresol (*c*, 0·46).
1	4	40·1	44°	0·4	1320	0·323
2	10	40·0	55	nil	1525	0·152
3	9	40·25	56	nil	1542	0·135
4	2·5	40·2	64	nil	1385	0·235

Hydrolysis of the Methyl Ester of Methylated Mesquitic Acid. Experiment I.—In this preliminary experiment the methylated polysaccharide (9·4 g.) was boiled for 6 hours with methyl alcohol (350 c.c.) containing 2% of dry hydrogen chloride. The solution was neutralised with silver carbonate and filtered, the residue being repeatedly washed with hot methyl alcohol. The combined filtrate and washings were evaporated under reduced pressure, and a syrup (9·2 g.) remained which was dissolved in water (100 c.c.) and heated for 2 hours at 60° with barium hydroxide (1·5 g.). Excess of alkali was neutralised with carbon dioxide, the mixture heated for 15 minutes at 85° to decompose any barium hydrogen carbonate and filtered, and the precipitates washed with hot water. The filtrate was evaporated to a small volume (50 c.c.) and extracted 10 times with chloroform (300 c.c.); and the dried chloroform solution was filtered and concentrated to give a mobile mixture of methylated glycosides (*A*) (5·25 g.). The aqueous solution left after the chloroform extraction furnished on evaporation a syrup consisting of the barium salts of the methylated uronic and aldobionic acids contaminated with some methylated glycosides. The latter were removed by exhaustive extraction with ether; evaporation of this ethereal extract gave syrupy glycosides (*B*) (0·9 g.) which partially crystallised. The residual barium salt (*C*) amounted to 4·2 g.

Examination of the Methylated Glycosides (A).—Distillation of the methylated glycosides gave :

Fraction.	Wt. (g.).	B.p. (bath temp.).	Pressure (mm.).	$n_D^{19°}$.	OMe (%).
(I)	1·6	137—140°	0·05	1·4365	59·0
(II)	3·0	150—180	0·02	1·4465	48·8
(III)	0·39	180—200	0·02	1·4565	45·3

Identification of 2 : 3 : 5-*Trimethyl Methylarabinoside* (I).—When a solution of fraction (I) in 0·2N-sulphuric acid (50 c.c.) was heated on the boiling water-bath for 7 hours, the rotation changed from $[\alpha]_D^{18°}$ — 58° (initial value) to — 43° (constant value). The acid was neutralised with barium

carbonate, and the solution filtered and evaporated to dryness. Extraction of the residue with ether gave a syrup (0·96 g.) consisting of 2 : 3 : 5-trimethyl arabinose (II) which had $[\alpha]_D^{20°}$ — 37° in water (*c*, 0·65), n_D^{17} 1·4555 (Found : OMe, 44·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

Oxidation with bromine. The syrup was dissolved in water (10 c.c.) and treated with bromine (1·5 c.c.) for 48 hours at room temperature. The bromine was removed by aeration, and the non-reducing solution neutralised with silver carbonate and filtered before and after passing in hydrogen sulphide. The clear solution was evaporated to dryness and extracted with dry ether; concentration of the extracts gave 2 : 3 : 5-trimethyl γ-arabonolactone (III), b. p. 120° (bath temp.)/0·024 mm., $n_D^{19°}$ 1·4465 (0·7 g.), which crystallised on nucleation and had $[\alpha]_D^{17°}$ — 47° (initial value) in water (*c*, 1·0) changing to $[\alpha]_D^{17°}$ — 36° (constant value) in 20 days (Found : OMe, 46·6. Calc. for $C_8H_{14}O_5$: OMe, 48·9%).

2 : 3 : 5-*Trimethyl* L-*Arabonamide* (IV).—Treatment of the 2 : 3 : 5-trimethyl γ-arabonolactone with methyl-alcoholic ammonia at 0° for 2 days followed by removal of the solvent under reduced pressure afforded the crystalline amide of 2 : 3 : 5-trimethyl L-arabonic acid, m. p. and mixed m. p. 138°, $[\alpha]_D^{18°}$ + 18° in water (*c*, 1·3) after crystallisation from acetone–ether (Found : OMe, 44·6. Calc. for $C_8H_{17}O_5N$: OMe, 44·9%).

Identification of 3 : 5-*Dimethyl Methyl*-L-*Arabinoside* (V).—A portion of fraction (II) (0·6 g.) after being methylated twice with Purdie's reagents in the usual way gave a syrupy 2 : 3 : 5-trimethyl methylarabinoside (0·45 g.) (Found : OMe, 59·0. Calc. for $C_9H_{18}O_5$: OMe, 60·25%), recognised by its conversion into the crystalline 2 : 3 : 5-trimethyl γ-arabonolactone which the corresponding 2 : 3 : 5-trimethyl arabinose furnished on oxidation with bromine. Treatment of this lactone with methanolic ammonia yielded 2 : 3 : 5-trimethyl L-arabonamide, m. p. and mixed m. p. 138°, $[\alpha]_D^{15°}$ + 15° in water (*c*, 1·4) (Found : OMe, 44·7. Calc. for $C_8H_{17}O_5N$: OMe, 44·9%).

3 : 5-*Dimethyl Arabinose* (VI).—When a solution of fraction (II) (1·0 g.) in 0·1N-sulphuric acid (50 c.c.) was heated on the boiling water-bath it showed $[\alpha]_D^{19°}$ — 87° (initial value), — 56° (after 2 hours), — 50° (3 hours), — 45° (4 hours), — 45° (8 hours) (constant value). The concentration of acid was increased to 1N and the solution heated for 4 hours, but no further change in the rotation was observed. The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure. Extraction of the residue with alcohol followed by filtration and removal of solvent yielded 3 : 5-dimethyl arabinose (VI) (Found : OMe, 35·7. Calc. for $C_7H_{14}O_5$: OMe, 34·8%).

3 : 5-*Dimethyl γ-Arabonolactone* (VII).—After treatment of a solution of the dimethyl sugar (0·6 g.) in water (2 c.c.) with bromine (0·6 c.c.) for 48 hours at room temperature, the solution, after removal of the excess of bromine, no longer reduced Fehling's solution. The lactone, isolated in the usual way, gave on distillation a colourless liquid, b. p. 135° (bath temp.)/0·025 mm. (0·35 g.), $n_D^{20°}$ 1·4580, which crystallised spontaneously. The crystals of 3 : 5-dimethyl γ-arabonolactone, after recrystallisation from ether, had m. p. 75°, $[\alpha]_D^{17°}$ — 84·5° (initial value in water, *c*, 1·5), — 84° (after 3 hours), — 79° (10 hours), — 73° (20 hours), — 71° (25 hours), — 69° (28 hours), — 69° (50 hours) (Found : C, 47·35; H, 7·2; OMe, 35·3. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·9; OMe, 35·2%).

3 : 5-*Dimethyl* L-*Arabonamide* (VIII).—Treatment of the lactone (0·25 g.) with dry methyl-alcoholic ammonia for 24 hours at 0° gave the amide, which after removal of solvent and crystallisation from acetone had m. p. 145°, $[\alpha]_D^{18°}$ + 10° in water (*c*, 2·6) (Found : OMe, 32·1; N, 7·1. Calc. for $C_7H_{15}O_5N$: OMe, 32·1; N, 7·25%).

A portion of the amide (9 mg.) was dissolved in water (0·2 c.c.) and a solution of 1·5N-sodium hypochlorite (0·2 c.c.) added. The mixture was kept at 0° for ½ hour and the excess of hypochlorite destroyed by the addition of 2 drops of sodium thiosulphate solution. Addition of sodium acetate (0·5 g.) to the solution, followed by semicarbazide hydrochloride, gave in 2—3 minutes a white precipitate of hydrazodicarbonamide, m. p. and mixed m. p. 256° (decomp.); yield 70%.

Fraction (III).—This fraction which had $[\alpha]_D^{18°}$ — 35° in water (*c*, 1·2) appeared to be a mixture of 3 : 5-dimethyl methylarabinoside and 2 : 4-dimethyl methylgalactoside and was not further examined (Found : OMe, 45·3%).

Examination of the Methylated Glycosides (B). *Identification of* 2 : 4-*Dimethyl β-Methylgalactoside* (IX).—The partly crystalline glycosides (*B*) furnished on distillation :

Fraction.	Wt. (g.).	B.p. (bath temp.) at 0·02 mm.	$n_D^{16·5°}$.	OMe (%).
(IV)	0·12	138—150°	1·4575	44·4
(V)	0·71	160—165	1·4670	40·0

Examination of Fraction (IV).—This fraction had $[\alpha]_D^{18°}$ — 28·4° in water (*c*, 1·27), and like fraction (III) appeared to be a mixture of 3 : 5-dimethyl methylarabinoside and 2 : 4-dimethyl methylgalactoside.

Trituration of the crystalline fraction (V) with acetone and ether gave 2 : 4-dimethyl β-methylgalactoside (IX) which, after 3 crystallisations from acetone–ether, had m. p. and mixed m. p. 167°; $[\alpha]_D^{13°}$ + 2·0° in water (*c*, 1·0) (Found : C, 48·3; H, 7·7; OMe, 41·25. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2; OMe, 41·9%).

When a solution of a portion (0·36 g.) of the crystalline galactoside in N-sulphuric acid (10 c.c.) was boiled it showed the following rotations : $[\alpha]_D^{16°}$ + 2·0° (initial value); + 31° (after 1 hour); + 91° (5 hours); + 88° (10 hours) (constant value). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure to give the free sugar (0·3 g.) which was purified by extraction with ethyl alcohol followed by removal of the solvent.

2 : 4-*Dimethyl Galactose Anilide.*—A portion (0·15 g.) of the dimethyl sugar was boiled with alcohol (5 c.c.) containing aniline (0·07 g.) for 3 hours; the alcohol was distilled off and the anilide crystallised on cooling. The crystals of 2 : 4-dimethyl galactose anilide had m. p. 214° after recrystallisation from ethyl alcohol. It gave no depression when in admixture with an authentic specimen.

Identification of the Methyl Ester of 2 : 3 : 4-*Trimethyl Methylglucuronoside* (XI).—The ether-insoluble barium salts (*C*) (4·2 g.) were dissolved in methyl alcohol (200 c.c.) containing 1% of hydrogen chloride and left for 2 days at room temperature. The solution was neutralised with silver carbonate and filtered.

Evaporation of the filtrate gave a syrup (2·5 g.) (Found : OMe, 47·0%) consisting of the methyl esters of the methylated uronic and aldobiuronic acids and probably some dimethyl methylgalactoside which upon fractional distillation gave :

Fraction.	Wt. (g.).	B. p. (bath temp.).	$n_D^{22°}$.	OMe (%).	Equiv.
(VI)	0·5	160—165°/0·01 mm.	1·4507—1·4595	49·6	353
(VII)	1·3	225—270/0·01 mm.	1·4705—1·4735	47·6	467

There remained a residue of 0·7 g.

Fraction (VI).—This fraction (OMe, 49·6%; equiv., 353) was probably a mixture of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (OMe, 58·6%; equiv., 264) and 2 : 4-dimethyl methylgalactoside (OMe, 41·9%). The latter was not sufficiently soluble in ether to be completely removed by the ether extraction procedure which was applied above for the separation of the mixture of barium salts and methylated glycosides.

The presence of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside in fraction (VI) was proved by boiling the latter (0·3 g.) for 6 hours with 3% methyl-alcoholic hydrogen chloride (20 c.c.). The cooled solution was neutralised with silver oxide, filtered, and evaporated to dryness. Purification of the syrupy residue by extraction with ether followed by distillation in a vacuum gave a colourless liquid containing the methyl ester of 2 : 3 : 4-trimethyl α-methylglucuronoside (Smith, *J.*, 1939, 1724). Treatment of this distillate with methyl-alcoholic ammonia for 2 days at 0° followed by removal of the excess of solvent gave a crystalline residue from which the amide (XII) of 2 : 3 : 4-trimethyl α-methylglucuronoside was separated by crystallisation from ethyl alcohol–light petroleum; m. p. 184° alone or in admixture with an authentic specimen.

Fraction (VII).—This fraction, which had $n_D^{18°}$ 1·4735, $[\alpha]_D^{17°}$ + 47° in water (*c*, 0·5), appeared to be the methyl ester of hexamethyl aldobiuronic acid (Found : OMe, 47·6; equiv., 467. Calc. for $C_{19}H_{34}O_{12}$: OMe, 47·8%; equiv., 454).

Hydrolysis of the Methyl Ester of Methylated Mesquitic Acid. Experiment II.—This experiment was carried out in order to secure enough of the methylated aldobiuronic acid [see fraction (VII) above] for purposes of identification and with the object of estimating the relative quantities of the different hydrolytic cleavage fragments.

The methylated mesquitic gum (20 g.) was hydrolysed by boiling it for 6 hours with 2% methyl-alcoholic hydrogen chloride (320 c.c.); the specific rotation then changed from + 57° to − 18°. The mixture was cooled, neutralised with silver carbonate, and filtered. After the residue had been washed several times with hot methyl alcohol, the combined filtrate and washings were evaporated to dryness under reduced pressure. The syrupy residue (20 g.) was heated for 2 hours at 60° with barium hydroxide (2·5 g.) in water (200 c.c.). The excess of barium hydroxide was neutralised with carbon dioxide and the solution was heated on the boiling water-bath for 15 minutes to decompose barium hydrogen carbonate. The solution was filtered and evaporated to dryness under reduced pressure. Six extractions of this pale yellow syrup with ether gave a mixture of ether-soluble glycosides (*D*) (11·7 g.), and there remained the ether-insoluble barium salt (*E*).

Fractional Distillation of the Glycosides (D).—Slow distillation of the mixture through a narrow 4-inch vacuum jacketed column yielded : *fraction* (a), 2 : 3 : 5-trimethyl methyl-L-arabinoside (3·1 g.), b. p. 115—125° (bath temp.)/0·17 mm., $n_D^{19°}$ 1·4380, $[\alpha]_D^{17°}$ − 56° in water (*c*, 1·2) (Found : OMe, 59·2%); *fraction* (b), 3 : 5-dimethyl methyl-L-arabinoside (6·3 g.), b. p. 130—135° (bath temp.)/0·1 mm., n_D^{19} 1·4475, $[\alpha]_D^{20°}$ − 66° in water (*c*, 1·3) (Found : OMe, 48·5%); *fraction* (c), 2 : 4-dimethyl β-methylgalactoside (1·8 g.), b. p. 160—180° (bath temp.)/0·033 mm., $[\alpha]_D^{16°}$ + 2° in water (*c*, 0·8), m. p. and mixed m. p. 168° (Found : OMe, 41·7%). The residue (0·52 g.) in the distilling flask crystallised on cooling, and recrystallisation showed that it was a mixture of the α and the β form of 2 : 4-dimethyl methylgalactoside.

Examination of the Barium Salts (E).—The ether-insoluble barium salts (*E*) were converted into the corresponding methyl esters by treatment with methyl alcohol containing 2% of dry hydrogen chloride for 2 days at room temperature. After neutralisation with silver carbonate the filtered solution gave, on removal of solvent, a syrupy product (5·0 g.) which upon fractional distillation yielded : *fraction* (d) (0·9 g.), b. p. 155—190° (bath temp.)/0·02 mm., $n_D^{19°}$ 1·4565; *fraction* (e) (2·1 g.), b. p. 230—265° (bath temp.)/0·02 mm., $n_D^{19°}$ 1·4770 (Found : OMe, 46·5; equiv., 474. Calc. for $C_{19}H_{34}O_{12}$: OMe, 47·8%; equiv., 454).

The *residue* (F) (2·9 g.) which remained in the flask was examined later.

Identification of the Methyl Ester of 2 : 3 : 4-*Trimethyl Methylglucuronoside* (XI).—Redistillation of fraction (*d*) gave the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (0·7 g.), b. p. 115—120° (bath temp.)/0·03 mm., $n_D^{19°}$ 1·4495 (Found : OMe, 56·3; equiv., 284. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264). A crystalline residue of almost pure 2 : 4-dimethyl β-methylgalactoside (0·2 g.) remained.

Treatment of the ester with methyl-alcoholic ammonia at 0° gave the amide of 2 : 3 : 4-trimethyl α-methylglucuronic acid which after crystallisation from ethyl alcohol–ether–light petroleum had m. p. and mixed m. p. 185°, $[\alpha]_D^{18°}$ + 138° in water (*c*, 0·53) (Found : C, 48·5; H, 7·7; N, 5·6; OMe, 49·4. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·7; N, 5·6; OMe, 49·8%).

Identification of the Methyl Ester [*Pentamethyl Methylaldobiuronoside* (XIII)] *of the Hexamethyl Aldobiuronic Acid.*—A solution of *fraction* (e) (2·0 g.) in dry methyl alcohol (100 c.c.) containing 8% of dry hydrogen chloride was boiled for 22 hours; the specific rotation had then changed from $[\alpha]_D^{17°}$ + 80° to $[\alpha]_D^{17°}$ + 69°. The solution was neutralised with silver carbonate and filtered, and the residue washed well with methyl alcohol. Evaporation of the filtrate gave a syrup (2·0 g.) which on fractional distillation yielded : *fraction* (f) (0·59 g.), b. p. 112—120° (bath temp.)/0·025 mm., $n_D^{18°}$ 1·4520 (Found : OMe, 58·1; equiv., 275. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264); *fraction* (g) (0·61 g.), b. p. 145—165° (bath temp.)/0·025 mm., $n_D^{18°}$ 1·4720 (Found : OMe, 42·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%) (this fraction crystallised spontaneously); *fraction* (h) (0·45 g.), b. p. 165—170° (bath temp.)/0·025 mm., $n_D^{18°}$ 1·4810. A *residue* (G) (0·35 g.) remained in the distilling flask.

Examination of Fraction (f).—This fraction was identified as the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI), since treatment with methyl-alcoholic ammonia furnished, in good yield, the crystalline amide (XII) of 2 : 3 : 4-trimethyl α-methylglucuronoside, m. p. 185°, $[\alpha]_D^{18°}$ + 133° in water (*c*, 0·6) (Found : C, 48·5; H, 7·6; N, 5·6; OMe, 48·7. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·75; N, 5·6; OMe, 49·8%).

Examination of Fraction (g).—After recrystallisation from acetone–ether the crystalline fraction gave 2 : 4-dimethyl β-methylgalactoside (IX), $[\alpha]_D^{16°}$ + 4° in water (*c*, 0·8), m. p. and mixed m. p. 166°. Hydrolysis of a small portion of fraction (*g*) by heating it on a boiling water-bath with N-sulphuric acid for 12 hours followed by neutralisation with barium carbonate gave 2 : 4-dimethyl galactose (X) which crystallised readily as the monohydrate on the addition of a little water; m. p. and mixed m. p. 102° (after recrystallisation from ethyl alcohol–acetone–ether). Treatment of anhydrous 2 : 4-dimethyl galactose with boiling ethyl-alcoholic aniline gave crystalline 2 : 4-dimethyl galactose anilide which separated from the mixture, m. p. 214°, mixed m. p. 216°.

Examination of Fraction (h).—This fraction like the distillation residue (*G*) appeared to be the methyl ester of a hexamethyl aldobionic acid (Found : OMe, 46·4; equiv., 460. Calc. for $C_{18}H_{34}O_{12}$: OMe, 47·8%; equiv., 454). This methyl ester of the methylated aldobionic acid is relatively stable, since further treatment of fraction (*h*) with boiling methyl alcohol containing 8% of dry hydrogen chloride for 25 hours effected no further hydrolysis.

Further Hydrolysis of the Residue (F).—A solution of this syrupy residue (*F*) (2·9 g.) in methyl alcohol (30 c.c.) containing 8% of dry hydrogen chloride was boiled for 12 hours. The solution was neutralised with silver carbonate, filtered, and evaporated to dryness under reduced pressure. The syrupy residue was purified by extraction with a mixture of acetone (1 part) and ether (5 parts). Distillation of the syrup gave : *fraction* (i) (0·2 g.), b. p. 132—145° (bath temp.)/0·04 mm., $n_D^{21°}$ 1·4505 (Found : equiv., 270); *fraction* (j) (1·3 g.), b. p. 155—165° (bath temp.)/0·05 mm. (Found : OMe, 41·3%). A *residue* (H) (1·4 g.) remained in the distillation flask and had $n_D^{21°}$ 1·4820 (Found : OMe, 46·2%; equiv., 485).

Examination of Fraction (i).—This was the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) and was characterised by its conversion into the crystalline amide, m. p. and mixed m. p. 185°, in the manner already described.

Examination of Fraction (j).—This crystalline fraction was 2 : 4-dimethyl β-methylgalactoside (IX) and had m. p. and mixed m. p. 167° (after recrystallisation from acetone–ether).

Examination of the Residue (H).—This was the hexamethyl aldobionic acid characterised by its resistance towards hydrolysis.

Hydrolysis of the Residues (H) *and* (G) *and Fraction* (h) *Consisting of the Resistant Hexamethyl Aldobionic Acid.*—The residue (*H*) was combined with the residue (*G*) and fraction (*h*) since all these fractions had proved resistant towards hydrolysis by 8% methyl-alcoholic hydrogen chloride.

A portion (0·52 g.) of this mixture (2·2 g.) was dissolved in methyl alcohol (20 c.c.) containing 2% of dry hydrogen chloride and heated in a Carius tube at 130° for 2 days. The solution was neutralised with silver carbonate and filtered, and the filtrate evaporated to yield a syrup (0·5 g.) which on fractional distillation gave : *fraction* (k) (the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside) (0·22 g.), b. p. 110—135° (bath temp.)/0·02 mm., $n_D^{20°}$ 1·4515 (Found : OMe, 57·5; equiv., 281. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264); *fraction* (l) a dimethyl methyl(?)galactoside (0·25 g.), b. p. 160—210° (bath temp.)/0·02 mm., $n_D^{20°}$ 1·4745 (Found : OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). This fraction (*l*) failed to crystallise. It is believed that it consists of a dimethyl methylgalactoside and probably arises from the methylated aldobiuronic acid (?) (XX), stable to 8% methyl-alcoholic hydrogen chloride, which possesses the 1 : 4-glycosidic linkage. For this reason the dimethyl methylgalactoside is probably a 2 : 3, 2 : 6, or a 3 : 6 derivative. It will be examined further.

The results of this second hydrolysis of the methyl ester of methylated mesquitic acid (20 g.) were as follows :

Compound.	Wt. (g.).	*M*.	Wt. × 100, mols.	Molar prop. (approx.).
2 : 3 : 5-Trimethyl methylarabinoside	3·1	206	1·51	3
3 : 5-Dimethyl methylarabinoside	6·3	192	3·28	6—7
2 : 4-Dimethyl methylgalactoside	4·43	222	2·0	4
Methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside	2·46	264	0·93	2
Unknown dimethyl methylgalactoside	1·1	222	0·495	1

Although a considerable loss of material has occurred this was not unexpected in view of the extensive handling of the products, and it is believed that the above relative proportions of the cleavage fragments are approximately correct.

Hydrolysis of the Methyl Ester of Methylated Mesquitic Acid. Experiment III.—A solution of the methyl ester of methylated mesquitic acid (12 g.) in 0·5% methyl-alcoholic hydrogen chloride (300 c.c.) was boiled under reflux, during which the following changes occurred : $[\alpha]_D$ + 53° (initial value); + 49° (after 0·5 hours); + 44° (1 hour); + 36° (2 hours); + 22° (4 hours); + 9° (7 hours); + 7° (9 hours); + 1·5° (13 hours). The hydrolysis was arrested at this point and the solution was neutralised with silver carbonate, filtered, and concentrated to a syrup (12 g.). This was dissolved in water (100 c.c.) containing barium hydroxide (1·6 g.) and the solution heated for 2 hours at 60—70°. The excess of barium hydroxide was neutralised with carbon dioxide and the solution filtered and evaporated to dryness under reduced pressure. The dry residue consisting of the barium salts of the methylated acids and the methylated glycosides was extracted 6 times with ether under reflux. Removal of the solvent from the ethereal solution gave a mixture of the methylated glycosides (3·8 g.). There remained an ether-insoluble residue of barium salts (4·2 g.).

Examination of the Ether-insoluble Barium Salts.—The barium salts (4·2 g.) were esterified by treatment with 1% methyl-alcoholic hydrogen chloride (150 c.c.) for 2 days at room temperature. The solution was then neutralised with silver carbonate, filtered, and concentrated to give the methyl ester

as a glassy residue (2·86 g.) [Found : equiv., 760. Calc. for the methyl ester of a degraded mesquitic acid (in the form of a methyl glycoside) composed of 5 units of dimethyl galactose and 2 units of 2 : 3 : 4-trimethyl glucuronic acid : equiv., 723].

Further Hydrolysis of the Methyl Ester of Methylated Degraded Mesquitic Acid.—When a solution of the methyl ester (2·86 g.) in 1% methyl-alcoholic hydrogen (100 c.c.) was boiled, the following changes were observed : $[\alpha]_D$ + 35° (initial value); + 33·5° (after 1 hour) (constant for a further 3 hours). Since no hydrolysis seemed to have taken place the concentration of the hydrogen chloride in the methyl-alcoholic solution was increased to 2% by the addition of the requisite amount of 10% methyl-alcoholic hydrogen chloride. The mixture was boiled again and the following changes were observed : $[\alpha]_D$ + 33·5° (initial value); + 42·5° (after 2 hours); + 45° (4 hours) (constant for 1 hour).

The solution was then neutralised with silver carbonate, filtered, and concentrated to give a syrupy mixture consisting of methyl esters and methylated glycosides (2·7 g.) which was treated with barium hydroxide (1·0 g.) in water (50 c.c.) for 2 hours at 60°. The excess of barium hydroxide was neutralised with carbon dioxide and the solution was filtered and evaporated to dryness under reduced pressure. The mixture of barium salts and methylated glycosides thus produced was exhaustively extracted with dry ether under reflux. Concentration of the ethereal solution gave a syrup (0·95 g.) which on being kept afforded crystalline 2 : 4-dimethyl β-methylgalactoside (IX), m. p. and mixed m. p. 167° (Found : OMe, 41·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The ether-insoluble barium salts were dissolved in 1% methyl-alcoholic hydrogen chloride (50 c.c.) and left for several days at room temperature in order to effect esterification without hydrolysis. The solution was neutralised with silver carbonate, filtered, and concentrated to a syrup. This was extracted with dry ether under reflux, and removal of the solvent gave the methyl esters as a syrup (1·24 g.)

From this hydrolsysis of methylated mesquitic gum it will be seen that 2% methyl-alcoholic hydrogen chloride is necessary to hydrolyse the methyl ester of the methylated degraded mesquitic acid. In addition 0·5% and 1% methyl-alcoholic hydrogen chloride appear to effect cleavage of the arabinose residues from the polysaccharide molecule, leaving the methylated degraded mesquitic acid. The latter will be studied further.

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