THE CHEMISTRY OF PLANT GUMS AND MUCILAGES: A STUDY OF THE GUMS PRODUCED BY TREES OF THE GENUS KHAYA.

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Contents

Introduction

Most polysaccharide material is structural (e.g. cellulose) or serves as food reserve (e.g. starch and glycogen), but the plant gums do not belong in either of these categories. Jones and Smith (1) define them as "substances of plant origin which are obtained as an exudation from the fruit, trunks or branches of trees, spontaneously or after mechanical injury of the plant by invasion of the bark or by removal of a branch or after invasion by bacteria or fungi". This exudate is a mixture of polysaccharide, inorganic and organic material, plus mechanical impurities.

The polysaccharide portion contains uronic acid and so resembles mucilages, hemicellulose, bacterial polysaccharides and pectic materials. In chemical constitution the plant gums are similar to mucilages and bacterial polysaccharides; hexose, pentose, deoxyhexose and uronic acid units are joined in a complex pattern by primary valences. Their structure so closely resembles the mucilages that in reviews (1-6) both are considered under a single classification, but they differ in their environment and mode of origin. Hirst and Jones give the following description and method of differentiation. "The gums and mucilages are hydrophilic colloids of high molecular weight. Some are completely soluble in water, forming viscous

solutions of gels, others swell and absorb considerable quantities of solvent without dissolving. They are not soluble in the common organic solvents. Gums are usually characterised as plant exudates, often from infected tissues, whilst mucilages are obtained by the extraction, with water, of various plant organs". In the following discussion the structures considered have been limited to plant exudates containing uronic acids.

The relative proportion of polysaccharide in the crude gum varies greatly according to the genus which produces it. Gum myrrh (Commiphora myrrha)(7) contains a large amount of resin, while many other gums are exuded as almost pure polysaccharide, but gum tragacanth (Astragalus) is a mixture of a water insoluble polysaccharide, an acidic water-soluble polysaccharide and a neutral galacto-araban (8).

Different sugars compose the acidic polysaccharides isolated from different genuses (Table 1), while within a genus the gums of various species appear to have the same component sugars but in varying proportions. Five Acacia species have been found to contain different amounts of the same monosaccharides (9,10). Slight variations in the quantities of the component sugars have been found in the exudates from the bark and fruit of the Victoria plum tree (11).

Gum is produced in greater quantity after hot dry weather and it has been suggested that the formation of it indicates a tree in poor health. For commercial production the trees are deliberately punctured. Two different mechanisms have been proposed for the formation of gums. They may be formed by the tree after puncturing, to seal the wound and prevent infection, or they may be the result of bacterial infection.

Gums are commercially important and are used as binding agents and inert dispersion media in textile, cosmetic and food industries, and also in pharmaceutical products.

Interest in the plant gums derives from their structural similarity to the bacterial polysaccharides. Partly hydrolysed gums give a precipitin reaction with anti-pneumococcus sera (12).

The biochemistry of the production of the monosaccharides of plant gums and their conversion to polymers is a fundamental problem.

Purification and Properties

Polysaccharides can be removed from their native environment by neutral or alkaline aqueous extraction. Crude gum extracts are often contaminated with mechanical impurities, low molecular weight organic material, waxes, inorganic salts, proteins, enzymes

and lignin. After removal of the mechanical impurities at a centrifuge or by filtration, the organic material, waxes and inorganic salts are removed by precipitation of the polymers from an aqueous solution, by the addition of a water miscible organic solvent. Alternatively, the solid can be extracted with aqueous organic solvent solutions. Low molecular weight contaminants can also be removed by dialysis or electrodialysis. Lignin is removed by treatment with chlorite solutions (13).

The separation of polymers is more difficult and no general method has been devised. Fractional precipitation can be used, either by adding organic solvents to an aqueous solution of the polysaccharide, or adding solvents to a solution of a derivative (e.g. methyl ether, acetate)(15). Fractional solution can also be used. Occasionally an unusual solubility effect affords a means of separation. Methylated gum tragacanth has been separated by hot water extraction into a neutral araban and an acidic polysaccharide, as the former is insoluble in hot water, while the sodium salt of the acid is soluble (8). The ability of some polysaccharides to form insoluble complexes has been used in their purification - yeast mannan can be precipitated as the copper complex (17). The separation of fractions by precipitation or solution does not always mean that different molecular types are present but may only be

a separation of chemically similar molecules of different size, as a polysaccharide sample is an aggregate of various molecular weight fractions. Purity with respect to inorganic or protein contaminants can easily be determined by elementary analysis, but no general test for deciding whether a polysaccharide is homogeneous has been devised. In examining fractions it is necessary to make chemical tests, as molecules which are chemically identical but differ in molecular weight can have different physical properties.

Some polysaccharides have been examined using electrophoretic techniques - polyuronic acids, heparin, mucoitin sulphate, chondroitin sulphate, hyaluronic acid (18,19), plant gums (11), various acidic polysaccharides of gastric mucosa (20,21) and carrageenin (22,44). The homogeneity of polysaccharides in molar alkali has been examined (23) and neutral polysaccharides have been separated in a borate buffer solution (24). All these results were obtained using a Tiselius electrophoretic apparatus. Paper electrophoresis has been used to separate hyaluronic acid and chondroitin sulphate (25) and to examine the mucopolysaccharides bound to thyroglobulin (26). The use of a continuous electrophoresis apparatus, to separate iodine complexes of partially hydrolysed amylose fractions has been described (27), in which a solution of the mixture was allowed

to travel downwards, through a bed of glass beads, in an electric field directed across the flow. Several systems for continuous electrophoresis on paper have been described (28).

Linear dextrins have been separated into molecular weight fractions by electrokinetic ultrafiltration, using collodion membranes of graded porosity (29).

Electrophoretic behaviour and ultracentrifuge measurements give the most information about the homogeneity of a polymer and if a substance cannot be separated into fractions by either of these methods, it is considered to be a single molecular species.

There are few examples of chromatographic procedures being applied to the fractionation of polysaccharides. Starch has been separated into amylose and amylopectin on alumina (37) and paper (38). Hyaluronic acid and chondroitin sulphuric acid have been separated on a column of silica-coated Celite, using as the solvent water saturated with n-butanol, plus 3% laurylamine and eluting with increasing concentrations of sodium chloride in water almost saturated with n-butanol (53). This method was adapted from a description of counter-current fractionation of heparin, using a "carrier" amine (92). The separation of some polysaccharides or partially hydrolysed polysaccharides

may be possible by reversed phase partition chromatography (39). Proteins have been partitioned between two liquid phases produced when salts are added to a mixture of water and a water-miscible organic solvent. The phase in which the protein is most soluble is held stationary by adsorption on kieselguhr and the phase in which it is least soluble becomes the moving phase.

An ash content determination indicates any inorganic impurity in the final product and the presence of protein can be found by an elementary nitrogen analysis. The optical rotation gives information about the type of glycosidic linkages of the component sugars. Infra-red spectra have been found to yield information about structural features (36). The methoxyl content of acidic polysaccharides indicates methyl ester or methyl ether. Uronic acid can be estimated from the carbon dioxide liberated on heating with strong hydrochloric acid (30,31), from the equivalent weight and by colorimetric estimation of derivatives (32). Some gums contain acetyl groups, which can be estimated as acetic acid (33) produced on acidification after heating with alkali.

Methods have been described for the identification and estimation of individual sugars, formed after hydrolysis, as insoluble derivatives and by colorimetric techniques (34,35), but these have largely

been superseded by chromatographic procedures.

Gum tragacanth is the only exudate examined in which the polysaccharide portion has been found to be heterogeneous, but as only a few attempts have been made to fractionate plant gums it is possible that others are mixtures.

Molecular Size and Shape

The molecular weight of polysaccharides can be determined by chemical or physical means. Some chemical methods depend on the reaction of the hemiacetal group of the monosaccharide, at the end of the molecule, with oxidising agents (alkaline hypoiodite, alkaline ferricyanide) and colour complexing reagents (alkaline copper solutions)(40). Radioactive cyanide has also been used in end group estimation (41). These methods cannot be applied with any accuracy to high molecular weight polysaccharides and are more suitable for the determination of the molecular weights of oligosaccharides. The possibility that some of the end groups are substituted or that the molecule is cyclic must be considered. An estimate of the molecular weight of linear polymers can be made from the amount of fully methylated sugar isolated after methylation and hydrolysis (43). In branched chain polymers, methylation results can indicate the degree of branching.

Information about the size and shape of

polymeric molecules can be obtained by physical measurement (45), but as polysaccharides do not exist as a single molecular weight species, the value obtained depends on the method used. The measurement of colligative properties (osmotic pressure, isothermal distillation) gives a number average molecular weight. Viscosity, light scattering power, sedimentation velocity and diffusion, and sedimentation equilibrium give a weight average molecular weight. Sedimentation data give information about the shape of the molecule. Each method has an optimum region for measurement. Isothermal distillation can be used for the region 5,000 to 10,000, osmotic pressure 10,000 to 20,000 and light scattering and ultracentrifuge methods above 20,000.

Few physical measurements have been made on gums and their high molecular weight and highly branched structure make them unsuitable for estimation of molecular weight by chemical methods. Gum arabic has been investigated using osmotic pressure measurements (46) and sedimentation and diffusion data (47). Values of 191,000 to 326,000 have been found. Gum tragacanth has been found to have a molecular weight of 840,000 (48) and Karaya gum 9,500,000 (49), using sedimentation and diffusion methods. All the molecules were described as being highly branched and possessing an elongated

shape in solution. Highly branched structures have been indicated from methylation.

Isolation and Identification of Monosaccharides

The breakdown of polysaccharides into their components occurs on heating with dilute acid or by the action of enzymes. The glycosidic link of furanosides is more readily broken by acids than that of pyranosides. Glycosides of uronic acids are very resistant to hydrolysis and as uronic acids are readily degraded in solutions of low pH, considerable destruction accompanies their hydrolysis. Some destruction of neutral sugars also occurs in acid solutions at high temperatures, so the time of heating and acidic concentration should be kept as low as possible (52). Both acidic solutions and crude enzyme preparations can cause "reversion" - the resynthesis of oligosaccharides from hydrolysis products (50) - and to avoid this in acidic hydrolysis, it is desirable to keep the concentration of monosaccharides as low as possible. Degradation of polysaccharides also takes place under alkaline conditions (51).

If sulphuric acid is used for hydrolysis, this can be removed as barium sulphate by the addition of barium carbonate, but care must be taken to avoid heating aldose solutions in the presence of barium carbonate, as epimerisation occurs. If hydrochloric

acid or methanolic hydrogen chloride is used, this can be removed with silver carbonate. Hydrolysis with formic acid should be followed by treatment with dilute mineral acid, to hydrolyse any formyl esters. Strongly basic resins in the hydroxyl form should not be used for removing anions, as they degrade sugars to smaller chain length acids and cause epimerisation (54).

Early methods of identification of the sugars, formed on hydrolysis, depended on the selective precipitation of each sugar by the addition of a specific reagent (35). These methods have the disadvantage that they are seldom quantitative and unless a sugar is present in appreciable quantity, its derivatives may not easily be crystallised from the solution.

The introduction of multi-stage separation processes has made the isolation and estimation of the component sugars more quantitative and less tedious. Chromatography has been used extensively. Adsorption chromatography, although a useful method for many organic substances, is generally unsuitable for sugars and their derivatives, although some separations have been effected (55). Compounds of this type have been successfully separated using partition chromatography (60,61,62). In 1941, Martin, Gordon and Synge (56) separated amino acids on a column of silica gel treated with water, followed by irrigation with organic solvents.

In 1944, Consden, Gordon and Martin (57) used a strip of filter paper as the carrier and introduced "paper chromatography". This method was later applied to monosaccharides, oligosaccharides and methylated sugars (58,69).

Different solvent systems are used for different types of sugars. The most general is n-butanol-ethanol-water and all neutral monosaccharides can be examined in this solvent. For highly methylated derivatives a solution of benzene-ethanol-water (63) is more satisfactory, while for unmethylated sugars and oligosaccharides solvents containing pyridine are used. A method has been described in which the paper is first treated with borate buffer giving a greater separation of certain sugars (64), according to their ability to form complexes with this reagent. Uronic acids and O-methyl-uronic acids travel slowly and streak in neutral or basic solvents, due to ionisation and it is necessary to use a solvent system containing an acid to suppress this ionisation. A mixture of butanol-water and formic or acetic acid is satisfactory (58). If the paper is first treated with alginic acid solution, then neutral solvent systems can be used for irrigation (66).

Each substance has a characteristic rate of movement at a certain temperature, for a particular type

of paper and solvent system. This can be described as the R_F value, i.e. the distance travelled by the substance, relative to the distance travelled by the solvent front. A better constant is the R_G value (59), i.e. the distance travelled by the substance, relative to the distance travelled by 2:3:4:6-tetra-O-methyl-glucose, in the same time. Other reference sugars can be used.

A general method of detection of the position of polyhydric substances on the paper is to spray with ammoniacal silver nitrate (58,69,70). This reagent is not specific, but is valuable because almost every compound on a paper chromatogram either produces a stain or inhibits background colour. Other sprays that indicate polyhydroxy compounds are potassium permanganate solution, iodine vapour, potassium meta-periodate followed by fuchsine which reacts with the aldehydes formed, or potassium iodide which reacts with the formic acid (71). Lead tetra-acetate in benzene and a mixture of permanganate and metaperiodate (72) give brown spots. More specific reagents can be used. Aldoses, on spraying with amine salts and subsequent heating, appear as coloured spots. A saturated aqueous solution of aniline oxalate (73) is an example of this type of spray reagent. Different combinations of amine and acid give different colours, according to

the class of sugar (74). Many of the spots fluoresce in ultra-violet light thus increasing the sensitivity of the reagent. Ketoses can be detected with urea hydrochloride solution (75). Acids can be shown by using a spray composed of an indicator (76) and lactones and esters have been indicated by spraying with alkaline hydroxylamine, followed by ferric chloride solution (77).

A useful application of paper chromatography is the micro-estimation of the component sugars of a mixture. The amounts can be estimated by spraying the paper with a reagent that produces a colour and comparing the intensity of the spot with that produced by known weights of the same substance. An example of this method is the addition of silver nitrate to the flowing solvent, followed by exposure of the dried paper to ammonia fumes and heating. The intensity of the spot is measured in a densitometer (78).

More accurate determinations involve detection of the position of the sugar by spraying a guide strip and then elution of the material, followed by estimation. Titration of the formic acid produced by the action of metaperiodate (59) and the Somogyi copper reagent (80) have been used for the estimation of sugars.

The separation on paper strips is useful for indicating the sugars present in a mixture, but as

final identification depends on the isolation of the components and their characterisation, the minute quantities that can be separated limit its use. Using thick sheets of paper, it is possible to separate up to 100 mg. of a mixture on a single strip. For preparative purposes cellulose powder packed in columns is used. In 1944 (81), several highly methylated sugars were separated on silica gel columns, using chloroform and water as eluant. In 1949, Wadman, Hough, and Jones (82) described a general method of separation of sugars and their derivatives, using a column of powdered cellulose. n-Butanol-water or n-butanol-ethanol-water solvent systems have been used to separate monosaccharides. Automatic fraction cutting devices are used for collecting and at regular intervals the contents of a tube examined on a paper chromatogram.

Another method of separation of neutral sugars depends on the adsorption of negatively charged borate complexes on a strongly basic ion exchange resin and preferential elution with dilute sodium borate solution (85).

Column chromatography of uronic acids has not been described. As hydrolysis to a uronic acid is so difficult and accompanied by extensive degradation, they are more readily identified by comparing

the sugars, produced on hydrolysis of the polysaccharide, with those formed after reduction with potassium borohydride of the methyl glycoside, methyl ester of the acidic fraction (83), obtained on partial hydrolysis. Care must be taken when using this reagent, as it slowly attacks glycosidic linkages (84).

Galacturonic and glucuronic acids have been separated by adsorption on the formate form of a strongly basic resin and elution with formic acid, but mannuronic and glucuronic acids were not separated (65).

The ionophoretic behaviour of the borate complexes of sugars can be used for compounds that show similar chromatographic behaviour and several descriptions of this method have been published (86-89). The carrier is a strip of filter paper and the borate complexes migrate towards the anode. Amino sugars and uronic acids can be separated from neutral sugars, using non-borate buffer solutions. The term M_G value has been introduced and this is constant for a particular sugar. It describes the distance of migration of the substance, relative to the distance of migration of glucose.

The paper strip method has the disadvantage that only small quantities can be separated. Several authors have described methods that can be used for larger quantities. In these, the mixture is allowed

to fall under gravity down a strip of the carrier (e.g. sand, glass or paper) and a potential is applied across the direction of flow. The various components are deflected from a vertical path according to their electrophoretic mobility. The merits of various systems have been discussed (28) and an apparatus has been described that uses thick paper as the carrier and is claimed to obviate difficulties associated with former designs.

A large number of plant gums have been hydrolysed and the component sugars characterised. The results obtained are recorded in table 1. Several other gums have been examined using paper chromatographic procedures to indicate the components. Cashew gum (Anacardium occidentale) contains galactose and arabinose (109), jeol gum (Lannea grandis) galacturonic acid, galactose and arabinose (110). Chile plum gum contains partially methylated monosaccharides (7) and golden apple gum (Spondias cytheria) galactose, arabinose, xylose, glucuronic acid (mainly, if not entirely as a mono-O-methyl ether) and traces of rhamnose and fucose (91). Ketha gum (Feronia elephantum)(79) contains L-arabinose, D-xylose, D-galactose, L-rhamnose (trace) and uronic acid, silky oak gum (Grevilla robusta)(111) L-arabinose, D-galactose and D-glucuronic acid, and Anogeissus schimperi gum (121) arabinose, galactose

Table 1

Plant gum	Uronic acid	Hexose	Pentose	Methyl pentose	Other sugars	Ref.
Arabic(Kordofan)	D-Glu[+]	D-Ga	L-Ar	L-Rh		93
Acacia mollissima	D-Glu	D-Ga	L-Ar	L-Rh		94
Acacia pycantha	D-Glu	D-Ga	L-Ar	L-Rh		9
Acacia cyanophylla	D-Glu	D-Ga	L-Ar	L-Rh		10
Acacia karroo	D-Glu	D-Ga	L-Ar	L-Rh		156
Egg plum	D-Glu	D-Ga	L-Ar,D-Xy			98
Almond	D-Glu	D-Ga	L-Ar,D-Xy			99
Peach(*Prunus persica*)	D-Glu	D-Ga	L-Ar,D-Xy	L-Rh		100
Cherry	D-Glu	D-Ga, D-Ma	L-Ar,D-Xy			67
Damson plum	D-Glu	D-Ga, D-Ma	L-Ar,D-Xy			96
Ghatti(*Anogeissus latifolia*)	D-Glu	D-Ga, D-Ma	L-Ar,D-Xy	L-Rh		97
Phormium tenax	D-Glu		D-Xy			101
Mesquite(*Prosopis juliflora*)	4-Me-D-Glu	D-Ga	L-Ar			102
Myrrh(*Commiphora myrrha*)	4-Me-D-Glu	D-Ga	L-Ar			7
Lemon	4-Me-D-Glu	D-Ga	L-Ar			150
Grapefruit	4-Me-D-Glu	D-Ga	L-Ar			103
Sapote	Me-D-Glu		L-Ar,D-Xy			104
Sterculia setigera	D-Gal	D-Ga		L-Rh	D-Ta	105
Cochlospermum gossypium	D-Gal	D-Ga		L-Rh	D-Ta	106
Cholla(*Opuntia fulgida*)	D-Gal	D-Ga	L-Ar,D-Xy	L-Rh		107
Tragacanth (*Astralagus*)	D-Gal	D-Ga	D-Xy	L-Fu		8
Neem(*Melia azadirachta*)	D-Glu	D-Ga	L-Ar	L-Fu		108

[+]D-Glu:- D-glucuronic acid; 4-Me-Glu:- 4-O-methyl-D-glucuronic; D-Gal:- D-galacturonic; D-Ga:- D-galactose; L-Ar:- L-arabinose; D-Ma:- D-mannose; D-Xy:- D-xylose; L-Rh:- L-rhamnose; L-Fu:- L-fucose; D-Ta:- D-tagatose.

and glucuronic acid.

All the plant gums contain uronic acid in varying quantity: D-glucuronic acid or its 4-O-methyl ether occurs in most gums, although several have been found to contain D-galacturonic acid: D-galactose and L-arabinose are present in most gums while D-xylose and L-rhamnose are often found; D-mannose, L-fucose and D-tagatose have been found in several cases. The absence of D-glucose is common to all the gums.

Mode of Linking Monosaccharides

After the detection and estimation of the monosaccharides, it is necessary to find the mode of linking of the individual sugars. This is not random and one particular sugar has a regular manner of linking. In a polysaccharide composed of one or two sugars, periodate oxidation can give information about the linkages and the degree of branching (90). In structures as complex as the plant gums the interpretation of results is difficult.

Enzyme action can be used to determine the manner of linking and whether the hemi-acetal bond has an α or β configuration. This method has been useful in structural work on starch and glycogen, but only a few enzyme systems capable of degrading gums have been investigated (112), although this method would appear to be promising for the determination of fine

structure.

Methylation, followed by acidic hydrolysis and identification of the methylated sugars, is the usual method of finding the manner of linking. Methylation is accomplished by repeated treatment with dimethyl sulphate and alkali (131), reaction of the thallous derivative with methyl iodide (114) or addition of methyl iodide to the polysaccharide in a solution of sodamide in liquid ammonia (115). Another method is to suspend the finely divided polysaccharide (from freeze-drying) in an ethereal solution of diazomethane (116). As the methoxyl content increases, the material becomes soluble in organic solvents and it is possible to use methyl iodide and silver oxide (117). Several of these methods expose the polysaccharide to conditions that degrade certain linkages (118) and it is always possible that the methylated product is partly degraded. Incomplete methylation and demethylation during hydrolysis limit the use of the methylation procedure for determining fine structure, but the main linkage types are readily detected.

Characterisation of the methylated sugars, produced on hydrolysis, often does not define the original linkages. A 2:3:6-tri-O-methyl-hexose can come from a 1:4 linked pyranoside or a 1:5 linked furanoside and further evidence (enzymic hydrolysis, periodate oxidation

or rate of acid hydrolysis) must be considered. The linkage is always a hemi-acetal type, involving the oxygen on the first carbon of one sugar with another sugar. The determination of which anomeric configuration is present becomes more difficult as the number of sugars and degree of branching increases. In gums, the probable configuration can be predicted from the change in rotation during acidic hydrolysis and from the ease of hydrolysis.

Methylated polysaccharides cannot be hydrolysed by heating in aqueous acid, as they are precipitated from hot solutions. It is necessary to reduce the chain length first, by heating in methanolic hydrogen chloride (119), formic acid (120) or keeping at room temperature in strong mineral acid, and then heating with dilute aqueous acid.

Formerly, the isolation of the various methylated sugars, produced by hydrolysis, was effected by fractional distillation under reduced pressure of the methyl glycosides, formed by methanolysis of the methylated polysaccharide (43). Now the methyl sugars are separated by chromatographic methods. For chromatography on paper, n-butanol-ethanol-water and benzene-ethanol-water are useful solvent systems for neutral sugars and amine-acid sprays suitable for indicating the components. By using different combinations of

amine and acid and noting the colours produced, it is often possible to gain useful information about the nature of a particular product (74).

The quantitative estimation of methylated sugars by paper chromatography is more difficult than for the parent sugars. Most methods depend on colorimetric comparison with standard samples (74). Recently a method has been described in which the reaction with sodium borohydride has been used for estimation (125). Usually, the quantitative estimates have been obtained by separation of larger quantities on cellulose columns. The solvent system used is n-butanol-light petroleum-water and the proportion of light petroleum is decreased as the more highly methylated sugars are eluted (82). Fractionation of tri-O-methyl-glucoses on charcoal-celite columns has been described (126).

Ionophoresis has been used to separate certain methylated sugars that cannot be separated by cellulose chromatography - 3:4-di-O-methyl-L-rhamnose travels at a much faster rate in borate buffer than 2:3 or 2:4-di-O-methyl-L-rhamnose (88), as in the first compound, complexing occurs between borate ion and the oxygen atoms at C_1 and C_2, while the other compounds contain no contiguous hydroxyl groups. 2:4 and 3:4-Di-O-methyl-D-glucoses have also been separated (89).

Methylated uronic acids occur in the hydrolysates

of plant gums. Their slow rate of travel in neutral solvents can be used to separate them from neutral methylated sugars. If the acid can form a lactone, this will move like a neutral sugar in neutral solvents, so it is necessary to run acids, capable of forming lactones, as their salts, in order to separate them from neutral sugars when using neutral solvent systems. The acids can be separated on paper, using as the solvent n-butanol-water and acetic or formic acid.

The large scale chromatography of O-methyl-uronic acids has been found to be unsatisfactory (129). They are readily characterised by conversion to the methyl ester, methyl glycosides after separation from the neutral sugars and then reduction to the methyl glycosides with lithium aluminium hydride, followed by hydrolysis of the glycosides to a mixture of neutral methylated aldoses (130).

The following O-methyl sugars have been isolated from methylated plant gums. (The figures in brackets after the name of a sugar refer to the positions of the methyl groups).

Gum arabic (131) gave D-glucuronic acid (2:3:4, 2:3), D-galactose (2:3:4:6, 2:4), L-arabinose (2:3:5, 2:5), L-rhamnose (2:3:4).

Cherry gum (132) gave D-glucuronic acid (2:3:4, 2:3), D-galactose (2:4:6, 2:4), L-arabinose (2:3:5, 2:5).

Damson plum gum (133) gave D-glucuronic acid (2:3:4, 2:3), D-galactose (2:4:6, 2:4, 2), L-arabinose (2:3:5, 2:3).

Egg plum gum (134) gave D-glucuronic acid (2:3), D-galactose (2:4:6, 2:4), D-xylose (2:3:4), L-arabinose (2:3:5, 2:5, 2).

Mesquite gum (135) gave D-glucuronic acid (2:3:4), D-galactose (2:4), L-arabinose (2:3:5, 3:5).

Sapote gum (136) gave D-glucuronic acid (3:4), D-xylose (2:3:4, 3), L-arabinose (2:3:4).

Sterculia setigera gum (137) gave D-galacturonic acid (2), D-galactose (2:3:4:6, 2:3:6, 2:6), L-rhamnose 3:4, 2 and/or 3).

Cholla gum (107) gave D-galacturonic acid (2), D-galactose (2:4), D-xylose (2:3:4), L-arabinose (2:3:5, 2:3).

Tragacanth gum (8) gave D-galacturonic acid (2:3, mono), D-xylose (2:3:4, 3:4), L-fucose (2:3:4).

Cochlospermum gossypium gum (106) gave methylated uronic acids, D-galactose (2:3:4:6, 2:3:6, 2:6), L-rhamnose (2:3:4, 3:4, 3).

Acacia pycantha gum (9) has been methylated and the products of hydrolysis examined on a paper chromatogram. The R_G values obtained, indicated the presence of 2:3:5-tri-O-methyl-arabinose, 2:3:4:6-tetra-O-methyl-galactose, an unknown sugar,

2:3:4-tri-O-methyl-galactose, a di-O-methyl-galactose, an unknown sugar and a methylated uronic acid. Periodate oxidation of Phormium tenax gum (101) indicated 1,2' or 1,4' glycosidic linkages.

Isolation of Partial Hydrolysis Products

Further information about structure can be gained from the isolation of oligosaccharides produced by mild acidic hydrolysis or enzyme action. Disaccharides and partly degraded polymers have been examined, but much information about structure could be obtained from fractions of intermediate size.

The isolation of oligosaccharides from acidic or enzymic hydrolysis can give misleading information, as the oligosaccharide isolated may not be an essential structural unit, but may have been synthesized from other fragments, formed in an earlier stage of hydrolysis (42,139). A study of the amount of oligosaccharide produced from likely fragments in the presence of the enzyme or acid solution, compared with the amount formed in the breakdown of the polysaccharide, indicates whether it is a product of re-synthesis or a genuine fragment.

The isolation of disaccharides composed of a uronic acid linked through C_1 to a neutral sugar is facilitated by the resistance of this linkage to acidic hydrolysis.

6-O-D-Glucuronopyranosyl-D-galactose has been

isolated from arabic (123), Acacia mollissima (94), Acacia pycantha (9), Acacia cyanophylla (10), Acacia karroo (156), egg-plum (142), almond (99), peach (100) and ghatti (97) gums.

6-O-(4-O-Methyl-D-glucuronosyl)-D-galactose has been isolated from mesquite gum (161).

4-O-D-Glucuronosyl-D-galactose has been isolated from Acacia karroo (156) and neem (108) gums.

4-O-(4-O-Methyl-D-glucuronosyl)-D-galactose has been isolated from gum myrrh (160).

4-O-(4-O-Methyl-D-glucuronosyl)-L-arabinose has been isolated from lemon gum (144).

2-O-D-Glucuronosyl-D-mannose has been isolated from cherry (67), damson plum (96) and ghatti (97) gums.

2-O-D-Galacturonosyl-L-rhamnose and 4-O-D-galacturonosyl-D-galactose have been isolated from Sterculia setigera (105) and Cochlospermum gossypium (106) gums.

An aldobiouronic acid in which the uronic acid is linked to the 4 position of D-galactose has been isolated from lemon (103) and grapefruit (103) gums. Mesquite gum (140,143,157,161) has been reported to contain three aldobiouronic acids in which D-galactose is joined through the 3, 4 or 6 position to 4-O-methyl-D-glucuronic acid. In all cases the high yields obtained, relative to the amount of uronic acid, in these

gums excluded the possibility that they were reversion products. The aldobiouronic acid is isolated as the barium salt, by precipitation with alcohol, from a neutralised partial hydrolysate and the structure established by methylation and identification of the hydrolysis products. The introduction of lithium aluminium hydride as a reducing agent has simplified identification, as the final hydrolysis is carried out on a neutral disaccharide, which splits readily and both products of hydrolysis are neutral sugars, the derivatives of which are more extensively described than those of O-methyluronic acids (145).

Recently, the isolation of two acidic trisaccharides from an hydrolysate of okra mucilage (146), using cellulose partition chromatography and n-butanol-acetic acid-water as the eluant, has been described and an aldotriouronic acid has been separated from an hydrolysate of corn cob hemicellulose by a similar method (16). The oligosaccharides, produced by enzymic action from hyaluronic acid, have been separated on a column of the formate form of a polystyrene quaternary ammonium salt of 200-400 mesh (147), by elution with continuously increasing concentrations of aqueous formic acid and in a similar manner mono, di, tri, and tetra-galacturonic acids from enzymic hydrolysis of pectic acid (148,149). Continuous electrophoresis would

appear to be another possible method of purification. Charcoal-Celite chromatography has been found unsuitable for preparative purposes (149).

Using very dilute acidic solutions or autohydrolysis, neutral oligosaccharides have been isolated from some gums.

3-O-β-L-Arabinopyranosyl-L-arabinose has been isolated from Acacia karroo (156), peach, cherry, and golden apple gums (91,150,151).

(4 or) 5-O-β-D-Xylopyranosyl-L-arabinose has been isolated from peach and cholla gums (151).

3-O-D-Galactopyranosyl-D-galactose has been isolated from Acacia pycantha and arabic gums (9,113).

3-O-D-Galactopyranosyl-L-arabinose has been isolated from arabic (93) and Acacia cyanophylla (10) gums.

3-O-α-D-Xylopyranosyl-L-arabinose has been isolated from golden apple gum (91).

Cellulose partition chromatography separates disaccharides and as small quantities are produced, thick sheets of paper have often been used. The rate of movement of disaccharides on paper chromatograms is slow and for qualitative purposes they can be more quickly separated as their N-benzyl glycosylamine derivatives, formed in situ on the paper (153). Paper ionophoresis in borate buffer also provides a rapid

means of separating oligosaccharides (154). Charcoal-Celite (1:1) columns can be used for preparative purposes. The oligosaccharides are eluted with increasing concentrations of ethanol or other organic solvents in water (155).

The disaccharides are usually characterised by methylation, hydrolysis and identification of the fragments. Recently, measurement of the amount of formic acid produced on oxidation with lead tetra-acetate has been used and this has the advantage that it can be carried out on small quantities of substance (152).

Some polysaccharides, but not gums, have been subjected to partial acetolysis with an acetic anhydride, sulphuric acid mixture, then dissolved in benzene and the products separated by chromatography on Silene EF-Celite, using benzene-ethanol for developing (14).

If an aqueous solution of an acidic polysaccharide is heated, slow hydrolysis occurs and if some of the linkages are labile (e.g. pentofuranose), these are preferentially removed leaving a degraded residue. If the labile links are on the periphery of the molecule, the residue will still be of high molecular weight. Methylation and hydrolysis of this gives information about the inner part of the molecular structure. Autohydrolysis of gum arabic yielded arabic acid (122,95),

which on methylation and hydrolysis gave D-galactose (2:3:4:6, 2:3:4, 2:4) and D-glucuronic acid (2:3:4).

Degraded mesquite gum (143) gave D-galactose (2:3:4, 2:4:6) and O-methyl-D-glucuronic acid.

Degraded egg plum gum (142) gave D-galactose (2:3:4:6, 2:4:6, 2:3:4, 2:4) and D-glucuronic acid (2:3).

Degraded damson plum gum (138) gave D-galactose (2:3:4:6, 2:4:6, 2:3:4, 4:6), D-xylose (2:3:4) and D-glucuronic acid (2:3, 2:3:4).

Degraded cherry gum (68) gave D-galactose (2:3:4:6, 2:4:6, 2:4, 2:6), D-xylose (2:3:4, 2:4) and D-glucuronic acid (2:3:4, 2:3).

Another method of partial degradation is by periodate oxidation. The oxidised polysaccharide can then be reduced with sodium borohydride or Raney nickel, followed by hydrolysis and analysis of the various products (124). Alternatively, the oxidised polysaccharide can be degraded by heating with phenylhydrazine acetate (127) or hydroxylamine acetate (128). This degraded polymer can again be treated with periodate followed by phenylhydrazine acetate and the process repeated until only one sugar is found on hydrolysis. In this manner the less highly substituted outer sugars are progressively removed. Using this method it has been possible to modify (141) a previously postulated structure for arabic acid.

Discussion

The gum exudate of Khaya grandifolia, a west African tree, has been examined by McIlroy (121). He found that the crude gum softened and swelled in cold water and dissolved in hot water with difficulty, to give a clear, neutral solution. The gum was prepared as a white powder by dissolution in 4% alkali, acidification of the solution and precipitation by ethanol. Re-precipitation did not reduce the ash content below 10.5%. An aqueous solution of the gum was non-reducing towards Fehling's solution, gave a negative test for protein with Millon's reagent, and was dextro-rotatory ($[\alpha]_D^{20}$ +104°). On acidic hydrolysis the rotation decreased, indicating α-linkages and the production of galactose and rhamnose was shown by paper chromatography. The barium salt of the acidic material was obtained after neutralisation and on further hydrolysis this liberated more rhamnose and galactose. On heating with basic lead acetate a brick-red precipitate formed. This test is claimed to be specific for galacturonic acid (158).

In the present investigation, the gum, which was supplied by Professor R. J. McIlroy, was purified by dissolving in acid, precipitating twice from water by the addition of ethanol (3 parts of ethanol to 1 part of water), once by acetone (4 parts of acetone to 1 part

of water) and then by ethanol (3 parts of ethanol to 2 parts of water). The addition of more ethanol in this last precipitation produced only a small amount of substance. This gave a product, free from ash, with a rotation of $+122^{\circ}$ which was used for the determination of properties and the rate of hydrolysis, and it has been referred to as the purified gum. For the large scale hydrolysis, methylation and subsequent experiments, the gum was prepared by dissolving in acid, removing mechanical impurities, precipitating by the addition of ethanol (3 parts of ethanol to 2 parts of water) and washing the precipitate until free from chloride ions. This product has been referred to as the re-precipitated gum.

The electrophoretic behaviour, in a borate buffer of pH 10, of the material obtained from Professor R. J. McIlroy, indicated a single polymeric component. The measurements were carried out in an Antweiler semi-micro apparatus.

The purified product was readily soluble in water, in contrast to the crude gum. Apparently the treatment with alkali removed ester groups. Acetyl groups have been found in some other gums, e.g. Sterculia gums (105). A low, but significant, methoxyl content (1.0%) was obtained after a portion of the purified gum had been precipitated from water by acetic

acid and freeze-dried from aqueous solution, indicating the presence of methyl ether groups. The equivalent weight was determined by direct titration with N/10 sodium hydroxide solution, using phenolphthalein as the indicator. This gave a value of 344 which indicates 51% uronic anhydride in the gum. The uronic anhydride content, estimated from the carbon dioxide evolved on heating with 19% hydrochloric acid, was 47.2% - no explanation is apparent for the discrepancy in the results obtained by these two methods.

The hydrolysis of the purified gum with N-sulphuric acid at 100° was followed polarimetrically, and the iodine consumption of the hydrolysate in alkaline solution estimated. The neutral sugars produced were examined on a paper chromatogram. The rotation changed from +122 to +93 in 5.0 hours and the iodine number (millilitres of N/10 iodine solution consumed by the hydrolysate of one gram of the polysaccharide) from 1.9 to 46. Only a slight change occurred on heating another hour.

Galactose and arabinose appeared after heating for half-an-hour and rhamnose after 3 hours. After 15 hours, the iodine number was 75 and after 20 hours the rotation changed to +50. On heating an aqueous solution of the gum at 100° (autohydrolysis) the iodine number changed to 11.5 in 8 hours.

As one gram of hexose reacts with 111 ml. of N/10 iodine solution, hydrolysis of the polysaccharide cannot have been complete after 6 hours heating, although the reaction slowed down. After 15 hours, the hydrolysis was still not complete. The slow rate of hydrolysis is explained by the high uronic acid content of the gum, as the glycosidic linkage of uronic acids is resistant to acidic hydrolysis. The high proportion of uronic acid made the total estimation of the component sugars, formed on hydrolysis, impossible. After heating for 18 hours at 100° with 2N-sulphuric acid and estimating the component sugars by the formic acid produced on the addition of meta-periodate, the total recovery was only 80% - the percentages based on the original polysaccharide were anhydro-galactose 18%, anhydro-rhamnose 15%, anhydro-arabinose <1% and the uronic acid, estimated by the carbon dioxide evolved on heating with 19% hydrochloric acid, 47%.

A large scale hydrolysis of the re-precipitated gum with N-sulphuric acid at 100° and separation of the products by partition chromatography on a column of powdered cellulose, using as the eluant n-butanol-ethanol-water, gave four fractions:-

L-rhamnose	3.7%
L-arabinose	0.2%
D-galactose	18.0%

an acidic fraction	52.0%
Total	74%

The three neutral sugars were obtained crystalline and were characterised by their melting points, mixed melting points with an authentic sample and their optical rotations. The acidic fraction was obtained as an amorphous solid by washing the column with water and removing the barium ions with Amberlite IR-120 resin. An examination on a paper chromatogram, using a neutral solvent for irrigation, revealed a faint trace of galactose in this fraction.

On heating a portion of the acidic fraction with 2N-sulphuric acid for 18 hours, galactose and rhamnose were produced.

The acidic fraction was converted to the methyl ester, methyl glycoside and reduced in aqueous solution with potassium borohydride. The glycosides were hydrolysed and the mixture of neutral sugars separated into five fractions by partition chromatography on a column of powdered cellulose. (The amounts of sugar isolated are expressed as a percentage of the weight of original polysaccharide).

L-rhamnose	10.1%
4-O-methyl-D-glucose	2.0%
D-glucose	0.1%
D-galactose	26.6%

L-Rhamnose and D-galactose were obtained crystalline. 4-O-Methyl-D-glucose was characterised as the osazone and the other fraction was assumed to be D-glucose on the basis of its R_G value and destruction by glucose-oxidase (notatin). The D-glucose was probably formed by demethylation of 4-O-methyl-D-glucose.

As 4-O-methyl-D-glucose was not found in the hydrolysis before reduction, even after using vigorous conditions for hydrolysis, then presumably 4-O-methyl-D-glucuronic acid is present in the gum. The total quantity of D-galactose isolated indicates that D-galacturonic acid is also a constituent. The amount of D-galactose and L-rhamnose found was 58.4% of the original polysaccharide. As the gum contains 47 - 51% of uronic acid, some of the D-galactose must have been produced by the reduction of D-galacturonic acid.

The presence of two different uronic acid residues in a single gum is unique, although the two uronic acids have been previously found in different gums. 4-O-Methyl-D-glucuronic acid occurs in mesquite (161), myrrh (7), lemon (150), grapefruit (150) and probably sapote (104) gums, and D-galacturonic acid in four other cases; gum tragacanth (8), cholla gum (107), *Sterculia setigera* gum (105) and *Cochlospermum gossypium* gum (106). The gum of *Khaya grandifolia* resembles

the gums of Sterculia setigera and Cochlospermum gossypium in its high uronic acid content and in having L-rhamnose and D-galactose as the neutral sugar components. It differs in having 4-O-methyl-D-glucuronic acid and a trace of L-arabinose.

The re-precipitated gum was methylated by six treatments with dimethyl sulphate and alkali, and converted to the ester by methyl iodide and silver oxide in methanol. The ester was heated with methyl iodide and silver oxide. After five treatments, the material soluble in chloroform-light petroleum (1:4) (50%) was isolated and had a methoxyl content of 40.2%.

The methylated gum was hydrolysed by keeping in 2N-sulphuric acid at room temperature for 10 days and then heating at 100° for 20 hours. Five fractions were obtained by partition chromatography on a column of powdered cellulose, using n-butanol-light petroleum-water as the eluant.

2:3:4:6-Tetra-O-methyl-D-galactose was characterised as the aniline derivative.

2:3:6-Tri-O-methyl-D-galactose was characterised as the 2:3:6-tri-O-methyl-D-galactonolactone, after oxidation with bromine water.

3-O-Methyl-L-rhamnose was obtained crystalline. Oxidation with bromine water produced a syrupy lactone and its optical rotation agreed with the previous value

for 3-O-methyl-L-rhamnonolactone. The methyl glycoside was not attacked by meta-periodate. Methyl-2 and 4-O-methyl-L-rhamnosides consume one mole. of meta-periodate.

The fourth fraction was obtained in <1% yield and was a mixture, as indicated by paper chromatography, of 3-O-methyl-L-rhamnose and a fraction with an R_G value of .45. The methoxyl content indicated a mixture of 2 parts of a di-O-methyl-hexose and 1 part of 3-O-methyl-L-rhamnose.

By washing the column with water, the barium salt of an acidic fraction was eluted. After removal of the barium ions by Amberlite IR-120 resin, this was separated into two fractions by partition chromatography on a column of powdered cellulose, using as the eluant n-butanol-acetic acid-water. As the separation was not efficient, it was necessary to re-cycle the mixed fraction twice. The first fraction had an R_G value of .81, with a trace of substance of R_G value of .95, when examined in an acidic solvent. On heating with acid it was unchanged. The second fraction was a mixture of substances with R_G values in an acidic solvent of less than .81, and on hydrolysis further quantities of 2:3:6-tri-O-methyl-D-galactose and 3-O-methyl-L-rhamnose were produced. There were also traces of substances with R_G values of .45, .30 and .25.

Washing with water eluted an acidic fraction which was combined with the rest of the uronic acid, converted to the methyl glycoside, methyl ester and reduced with lithium aluminium hydride in tetrahydrofuran. Hydrolysis and separation of the mixture of neutral sugars, by partition chromatography on a column of powdered cellulose, gave four fractions.

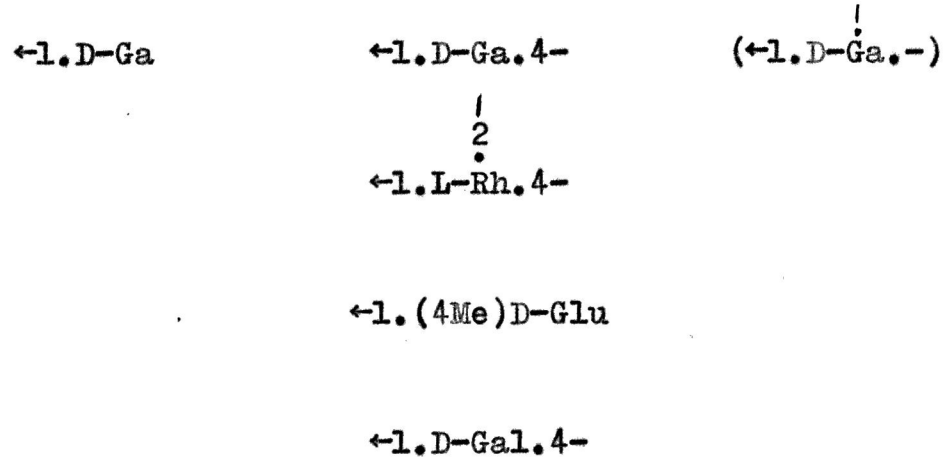
2:3:4-Tri-O-methyl-D-glucose was characterised as the aniline derivative and by methylation to 2:3:4:6-tetra-O-methyl-D-glucose. It was presumably formed by the reduction of 2:3:4-tri-O-methyl-D-glucuronic acid.

2:3:6-Tri-O-methyl-D-galactose was characterised as the lactone. This cannot have been formed by the reduction of a uronic acid, as the 6 position is substituted. The isolation of this compound in earlier stages of the hydrolysis indicates that it arose from incomplete hydrolysis of the acidic portion.

3-O-Methyl-L-rhamnose was isolated as the crystalline sugar.

2:3-Di-O-methyl-D-galactose was characterised as the aniline derivative. This, presumably, was formed by the reduction of 2:3-di-O-methyl-D-galacturonic acid, as the yield, although low compared with the expected amount, was higher than the quantity of neutral sugar of similar R_G value already isolated.

Assuming that the sugars were present in the pyranose form in the original polysaccharide, the isolation of these sugars indicates that the following structural units[+] are present in the gum.



The slow rate of autohydrolysis and hydrolysis makes the assumption of pyranose rings reasonable. If the small amount of L-arabinose is an integral part of the molecule, this may be present as the furanose sugar, but all the arabinose was lost during the methylation.

[+]D-Ga, D-galactose

L-Rh, L-rhamnose

(4Me)D-Glu, 4-O-methyl-D-glucuronic acid

D-Gal, D-galacturonic acid

The numbers refer to the positions where one monosaccharide is joined to another.

The small quantity of substances of R_G values lower than .45 isolated, made it improbable that these were structurally significant as they could have arisen from undermethylation or demethylation. The total yield of monosaccharides was 37%. This low recovery was due to the decomposition of the uronic acids and probably some of the neutral sugars during the prolonged heating with strong acid, which was necessary for hydrolysis. As the difficulty of hydrolysis is associated with the uronic acid linkages, the methylated gum was reduced with lithium aluminium hydride in tetrahydrofuran. It was possible to hydrolyse the neutral polysaccharide produced, using less vigorous conditions. On hydrolysis and separation of the components on sheets of thick paper (Whatman 3 MM), five fractions were obtained and the total yield of monosaccharides was 80% (average of two results). As two of the components could not be separated, the reduced gum was methylated and on hydrolysis, six fractions were obtained. The recovery was 76%.

Reduced gum	%
2:3:4:6-tetra-O-methyl-D-galactose 2:3:4-tri-O-methyl-D-glucose	22
tri-O-methyl-D-galactose	23
3-O-methyl-L-rhamnose	24
di-O-methyl-D-galactose	29
R_G .30	2

Methylated, reduced gum	%
2:3:4:6-tetra-O-methyl-D-glucose	8
2:3:4:6-tetra-O-methyl-D-galactose	19
tri-O-methyl-D-galactose	43
3-O-methyl-L-rhamnose	24
di-O-methyl-D-galactose	5
R_G .30	1

The total of tetra-methyl sugars in the methylated product is higher than the total of tetra-O-methyl-galactose and tri-O-methyl-glucose in the reduced gum, and the amount of tri-O-methyl-galactose in the methylated material is not enough to account for the amount of di-O-methyl-galactose which has been methylated to tri-O-methyl-galactose. These results suggest the presence of 2:3:4-tri-O-methyl-D-galactose in the tri-O-methyl-galactose fraction of the reduced material, presumably formed by the reduction of 2:3:4-tri-O-methyl-D-galacturonic acid. Assuming this, it is possible to balance the two sets of analyses and the following approximate percentages have been estimated for the original polysaccharide.

	%		%
←1.D-Ga,	12	←1.D-Ga.4-,	17
←1.D-Ga.-, (3)	5	←1.L-Rh.4-, (2)	26

	%		%
←1.(4Me)D-Glu,	8	←1.D-Gal,	6
		←1.D-Gal.4-,	25

These figures can only be approximate, as the total uronic acid (39%) is lower than the value for the original gum (47%).

The methylation results show that Khaya grandifolia gum resembles in some aspects Sterculia setigera and Cochlospermum gossypium gums. Galactose is linked through the 1 and 4 positions in contrast to most of the gums that have been examined, where the linking is 1-3, 1-6, or 1-3-6, e.g. Acacia (131), mesquite (135), cherry (132), damson plum (133), egg-plum (134) and cholla (107) gums. L-Rhamnose is the main branching point and not on the periphery of the molecule as in Acacia, cherry, cholla and damson plum gums. The linking of D-galacturonic acid through the 1 and 4 positions is similar to pectic acid (159).

The acidic portion, formed after partial hydrolysis of the original polysaccharide, was found to be a mixture of oligosaccharides. Examination on a paper chromatogram, in an acidic solvent system, indicated a number of substances which varied in their rate of movement, relative to galactose, from zero to .85. One gram of the mixture reacted with 36 ml. of N/10 iodine in alkaline solution.

Six fractions were obtained by adsorption on the acetate form of Amberlite IRA-400 resin and eluting with increasing concentrations of aqueous acetic acid. The resin was prepared by grinding the commercial product and packed as a slurry in dilute acetic acid and washed with water. The concentration of the acetic acid was increased stepwise. The increases were small (0.1%) at low concentrations but larger (2.5%) at higher concentrations.

A uronic acid, a mixture of two aldobiouronic acids, three acidic trisaccharides and a mixture of higher oligosaccharides were obtained.

The uronic acid was characterised as D-galacturonic acid by oxidation to mucic acid. The yield, based on the original polysaccharide, was 1.3%. The aldobiouronic acid fraction was obtained in 10% yield. Only one spot was obtained after paper chromatography and spraying with aniline oxalate, but this was brown at the head and red-brown at the tail. Two substances with slightly different M_G values were indicated by paper electrophoresis in a borate buffer. One gram of the mixture consumed 44 ml. of N/10 iodine. This value is low for a disaccharide but rhamnose does not react completely. On acidic hydrolysis, rhamnose, galactose, galacturonic acid and 4-O-methyl-glucuronic acid were liberated. After oxidation of the disaccharides

with bromine water, the rhamnose and galactose were destroyed but the two acids were not.

The mixture was methylated with dimethyl sulphate and alkali (four treatments) and esterified with silver oxide and methyl iodide. After reduction with lithium aluminium hydride in tetrahydrofuran, the neutral disaccharides produced were completely methylated by silver oxide and methyl iodide. Hydrolysis with N-sulphuric acid and separation of the components by partition chromatography on a column of powdered cellulose, using n-butanol-light petroleum-water as the eluant, gave three fractions. The second fraction was separated into two parts by partition chromatography on sheets of thick paper, using benzene-ethanol-water for irrigation.

2:3:4:6-Tetra-O-methyl-D-glucose was isolated as the crystalline sugar.

2:3:4:6-Tetra-O-methyl-D-galactose was characterised as the aniline derivative.

3:4-Di-O-methyl-L-rhamnose was characterised as 3:4-di-O-methyl-L-rhamnonolactone, after oxidation with bromine water.

2:3:6-Tri-O-methyl-D-galactose was characterised as 2:3:6-tri-O-methyl-D-galactonolactone, after oxidation with bromine water.

These results indicate a mixture of two

aldobiouronic acids, either,

←1.D-Ga.4←1.(4Me)D-Glu

and

←1.L-Rh.2←1.D-Gal

or the other combination with D-galacturonic acid joined to D-galactose and 4-O-methyl-D-glucuronic acid linked to L-rhamnose. Three pieces of evidence suggest that the formulation, as shown, is correct.

The two aldobiouronic acids have the same rate of movement on a paper chromatogram. A disaccharide composed of L-rhamnose and 4-O-methyl-D-glucuronic acid would move at a faster rate than one composed of D-galactose and D-galacturonic acid.

The rate of movement in a borate buffer on paper electrophoresis is similar. As the compounds are arranged they have the same charges in borate buffer but in the other arrangement the charges would be different.

2-O-D-Galacturonosyl-L-rhamnose and 4-O-(4-O-methyl-D-glucuronosyl)-D-galactose occur in other plant gums and mucilages.

From the results obtained by hydrolysis of the methylated gum, it is possible to describe the mode of linking of these aldobiouronic acids in the

gum. All the tetra-O-methyl-D-galactose was removed in the first stage of hydrolysis, so the following units must be present:-

←1.L-Rh.2 ←1.D-Gal.4-

and

←1.D-Ga.4 ←1.(4Me)D-Glu

While the presence of two different uronic acid residues in a single gum is unusual, the isolation of two aldobiouronic acids is not. Whenever two disaccharides have been found, they have had the same constituent uronic acid, e.g. mesquite (140,143, 157,161), ghatti (97), Cochlospermum gossypium (106), Sterculia setigera (105) and Acacia karroo (156) gums have given mixtures of aldobiouronic acids.

2-O-D-Galacturonosyl-L-rhamnose has been isolated from the partial hydrolysis of Sterculia setigera and Cochlospermum gossypium gums and 4-O-(4-O-methyl-D-glucuronopyranosyl)-D-galactose from gum myrrh (160).

Three substances were isolated which were probably trisaccharides, as indicated by their rate of movement on a paper chromatogram. The first was isolated in 2.0% yield and contained some impurities. On hydrolysis, rhamnose and galactose were liberated and after reduction of the methyl ester, methyl

glycoside with potassium borohydride, followed by hydrolysis, galactose and rhamnose were indicated by paper chromatography. By oxidation of the trisaccharide with bromine, most of the rhamnose was destroyed. This suggests a trisaccharide composed of rhamnose, galactose and galacturonic acid in which the rhamnose has the 1 position unsubstituted:-

$$\leftarrow.\mathrm{Rh}\begin{cases}\mathrm{Gal}\\ \mathrm{Ga}\end{cases}$$

The second, isolated in 0.8% yield, gave a single spot on a paper chromatogram and on paper electrophoresis. Rhamnose and galactose were produced on hydrolysis and after bromine oxidation the galactose disappeared. On reduction of the methyl ester, methyl glycoside and hydrolysis, galactose and rhamnose were produced, suggesting a structure of:-

$$\leftarrow.\mathrm{Ga}\begin{cases}\mathrm{Gal}\\ \mathrm{Rh}\end{cases}$$

The third was isolated in 7.0% yield and was impure. On hydrolysis, galactose and rhamnose were found and on bromine oxidation the galactose was destroyed. Reduction and hydrolysis produced galactose and rhamnose. The equivalent weight was 490, indicating one uronic acid linked to two neutral monosaccharides with a structure of:-

←.Ga $\begin{pmatrix}\text{Gal}\\ \text{Rh}\end{pmatrix}$

The isolation of these three products indicates that at least three of the sugars are in a single polymer.

The final fraction was a mixture of substances, all of which moved more slowly than the probable trisaccharides. Hydrolysis produced galactose and rhamnose and the same sugars were found after reduction and hydrolysis. The equivalent weight of 300 indicated more than 50% uronic acid in the mixture, which suggests the presence of oligosaccharides in which uronic acids are linked together.

The recovery from the column was 60%.

In view of the investigations on Acacia species, which have indicated that the different species have the same component sugars but in differing proportions, another Khaya species, senegalensis, was subjected to preliminary examination.

Khaya senegalensis gum swelled and dissolved slowly in water but was readily soluble after treatment with alkali. One particular nodule of the gum had an acetyl content of 2.7% and a methoxyl content of 2.2%. The gum was purified by dissolution in alkali and precipitation from acidic solution by ethanol. After four precipitations, the rotation was constant

at a slightly higher value than the rotation of Khaya grandifolia gum. An attempt at fractionation between water and ethanol was unsuccessful. After precipitation from acetic acid and freeze-drying, the methoxyl content was 1.2%. The equivalent weight was 412, indicating 42% uronic anhydride in the gum.

On hydrolysis of the purified gum with N-sulphuric acid at 100° for 6 hours, L-rhamnose (4.0%), L-arabinose (2.1%), D-galactose (16.1%) and an acidic fraction (37.8%) were produced. The acidic fraction had an equivalent weight of 348 and on further hydrolysis galactose and rhamnose were liberated. It was converted to the methyl glycoside, methyl ester and reduced with potassium borohydride. Examination on a paper chromatogram indicated three substances and a trace of another. These corresponded in R_G values to rhamnose, 4-O-methyl-glucose, glucose (trace) and galactose. A paper chromatogram of the acidic fraction, which was irrigated with an acidic solvent and sprayed with aniline oxalate, gave a pattern of spots similar to the acidic fraction obtained by partial hydrolysis of Khaya grandifolia gum.

From these preliminary results it appears that the same monosaccharides are present in both species of Khaya genus. The proportions of uronic acid and L-arabinose differ and there are probably

differences in the other sugars. As in the Acacia genus, different species have the same component sugars but in different quantity.

EXPERIMENTAL

The following solvent systems were used for paper chromatography: A, n-butanol-pyridine-water-benzene, (5:3:3:1); B, n-butanol-ethanol-water, (4:1:5); C, n-butanol-acetic acid-water (4:1:5); D, n-butanol-acetic acid-water (8:2:5); E, benzene-ethanol-water, (169:15:47). All these are two phase systems and the upper phase was used for irrigation of the paper. Unless otherwise stated, Whatman no.1 filter paper was the carrier and spraying with a saturated aqueous solution of aniline oxalate was used for development. The term R_G refers to the rate of movement relative to 2:3:4:6-tetra-O-methyl-D-glucose in solvent B, except when another solvent system is specified. Paper electrophoresis was carried out in borate buffer of pH 10.

Purification of Khaya grandifolia Gum.- The gum was obtained as a light grey powder which had been re-precipitated by ethanol, after solution in 4% aqueous sodium hydroxide (121). The crude gum (4.0g.) was dissolved in water (150 ml.) and after addition of concentrated hydrochloric acid (5 ml.) the mechanical impurities were removed at the centrifuge. The gum was precipitated by pouring the solution into ethanol (500 ml.). The precipitate was dissolved in water (100 ml.), re-precipitated in ethanol (300 ml.),

dissolved in water (100 ml.) again, and acetone (400 ml.) added. The white powder was washed with ethanol (3 times), kept overnight with anhydrous ethanol, washed with dry ether and dried (2.8g). After dissolution in water (200 ml.), ethanol was added in 50 ml. portions to effect a fractionation. The bulk of the material (2.3g.) precipitated when 300 ml. of ethanol had been added. This precipitate was removed at the centrifuge and dried (100°/12mm.), to give a white amorphous powder, free from chloride ions, $[\alpha]_D^{18}$ +122° (c, 1.88 in H_2O). This value did not change on re-precipitation. Hydrolysis of a sample for 6 hours at 100° with N-sulphuric acid and examination of the products on a paper chromatogram, indicated the presence of galactose, rhamnose, acidic material and a trace of arabinose. This sample was used for the determination of properties and the hydrolysis of the gum. On addition of ethanol (600 ml.) to the mother liquors, only a trace of material was precipitated (.03g.). For the partial hydrolysis, methylation and subsequent experiments, the polysaccharide was dissolved in water, hydrochloric acid added and then precipitated by the addition of ethanol (3 parts of ethanol to 2 parts of water). The precipitate was washed with ethanol until free from chloride ions and then dried, $[\alpha]_D^{19}$ +120° (c, 1.80 in H_2O).

Properties of K. grandifolia Gum.- A 1.7% solution of the crude polysaccharide, in borate buffer of pH 10, gave a single symmetrical peak when examined in an Antweiler micro-electrophoresis apparatus. The purified polysaccharide was free from ash and had an equivalent weight of 344, found by titration with N/10 sodium hydroxide solution, using phenolphthalein as the indicator . (Found: OMe, 1.0%). The uronic anhydride content, estimated as carbon dioxide formed on heating with 19% hydrochloric acid (30), was 47.2%. Hydrolysis with N-sulphuric acid in a boiling water bath for 6 hours and estimation of the liberated aldoses by the method of Hirst and Jones (59), indicated the presence of anhydro-galactose 16.1%, anhydro-rhamnose 8.2%, and anhydro-arabinose <1% in the gum. After hydrolysis with 2N-sulphuric acid for 18 hours, the values were anhydro-galactose 18.0%, anhydro-rhamnose 14.7%, and anhydro-arabinose <1%. Oxidation of the gum with nitric acid and estimation of the mucic acid (m.p. 216°), indicated 58% galacturonic and galactose anhydride. Paper chromatograms of the neutral sugars, produced on hydrolysis, were sprayed with urea hydrochloride, ammoniacal silver nitrate and a mixture of periodate and permanganate to detect any other substances but none were indicated.

Hydrolysis of K. grandifolia Gum.- The purified

gum was heated in a boiling water bath with N-sulphuric acid and the rate of hydrolysis followed polarimetrically and by the iodine consumed on reaction with hypoiodite. The neutral sugars produced were examined on a paper chromatogram. Originally the solution had $[\alpha]_D$ +122° and one gram of polysaccharide consumed 1.9 ml. of N/10 iodine solution. After 0.5 hours 16.5 ml. of iodine was consumed; 1 hour, 22 ml.; 1.5 hours, $[\alpha]_D$ +113°, 26 ml.; 2.0 hours, $[\alpha]_D$ +110°, 30 ml.; 3.1 hours, $[\alpha]_D$ +107°, 37 ml.; 4.1 hours, $[\alpha]_D$ +97°, 42 ml.; 5.0 hours, $[\alpha]_D$ +93°, 46 ml.; 6.0 hours, $[\alpha]_D$ +92°, 47 ml. Galactose and arabinose appeared after heating for 0.5 hours and rhamnose after 3 hours. Some decomposition started after 6 hours heating and the iodine values became erratic and the solution too dark to measure the optical rotation. The heating was continued for 15 hours when the iodine consumption was 75 ml. After 20 hours, the rotation of a solution which had been decolourised with charcoal was +50°.

On heating the gum in aqueous solution, after 8 hours one gram of the hydrolysate consumed 11.5 ml. of N/10 iodine solution.

Partial Hydrolysis of K. grandifolia Gum.- The re-precipitated gum (10.0g.) was dissolved in N-sulphuric acid and heated in a boiling water-bath

for 6 hours, the solution neutralised with barium hydroxide and the excess alkali quickly destroyed with carbon dioxide. The precipitated barium salts were removed at the centrifuge and washed three times with water. The water was removed by distillation under diminished pressure and the residue separated into four fractions by chromatography on a column of powdered cellulose, using n-butanol saturated with water, plus 10% ethanol as the eluant.

Fraction A.- 0.370g., $[\alpha]_D^{17}$ +9.8° (c, 4.44 in H_2O), R_G .30. This fraction crystallised on removal of the solvent and was recrystallised from ethanol to give L-rhamnose hydrate, m.p. 95° (not depressed on admixture with an authentic specimen).

Fraction B.- .023g., $[\alpha]_D^{17}$ +104° (c, 1.13 in H_2O), R_G .12. This fraction crystallised on removal of the solvent and was recrystallised from methanol to give L-arabinose, m.p. 155° (not depressed on admixture with an authentic sample).

Fraction C.- 1.80g., $[\alpha]_D^{16}$ +81° (c, 1.61 in H_2O), R_G .07. This fraction crystallised on removal of the solvent and was recrystallised from methanol to give D-galactose, m.p. 164° (not depressed on admixture with an authentic sample).

Fraction D was obtained by washing the column with water and was composed of a mixture of barium

salts of acidic material. (Found: Ba content 17.4%). The barium was removed by passage through a column of Amberlite IR-120 resin (acid form) and the solvent removed by freeze-drying to leave a yellow powder (5.2g.); one gram consumed 36 ml. of N/10 iodine. Hydrolysis with 2N-sulphuric acid at 100° for 18 hours, followed by examination of the hydrolysate on a paper chromatogram (solvent A), indicated the presence of rhamnose and galactose.

Identification of the Constituent Sugars of the Acidic Fraction Produced on Partial Hydrolysis of K. grandifolia Gum.- The freeze-dried product (4.5g.) was kept overnight with 4% hydrogen chloride in methanol and then boiled for 7 hours. The solution was cooled, neutralised with silver carbonate and filtered. After distillation of the methanol, the residue was dissolved in water (100 ml.) and added slowly to a solution of potassium borohydride (2.3g.) in water (100 ml.). After 2 hours, the excess borohydride was destroyed by the addition of dilute acetic acid and ions removed by passage through columns of ion exchange resins (Amberlite IR-120 and IR-4B). After removal of the water, the residue was hydrolysed by heating with N-sulphuric acid at 100° for 18 hours. The sulphuric acid was neutralised with barium carbonate and the syrup, left after distillation of the

water, was separated into four components by partition chromatography on cellulose powder, using as eluant n-butanol saturated with water, changing to n-butanol saturated with water plus 10% ethanol. Fractions A and B were only partially separated. The mixed fraction was triturated with wet butanol, when most of fraction A crystallised. The residue from the mother liquors was then separated by chromatography on a smaller column.

Fraction A.- 0.878g., $[\alpha]_D^{18}$ +10° (c, 2.35 in H_2O), R_G .30. This fraction crystallised on removal of the solvent and was recrystallised from wet butanol to give L-rhamnose hydrate m.p. 95° (not depressed on admixture with an authentic sample).

Fraction B.- 0.173g., $[\alpha]_D^{20}$ +62° (c, .74 in H_2O), R_G .27. (Found: OMe, 15.5). (Calc. for a mono-O-methyl-hexose 15.9%). A portion (.030g.) was heated in a boiling water bath for 30 minutes with phenylhydrazine (.08 ml.), acetic acid (.08 ml.) and water (0.5 ml.). The precipitated osazone (.022g.) was recrystallised from ethanol, m.p. 154°, $[\alpha]_D$ -31° → -14° (equilibrium value) (c, 1.11 in H_2O).

Fraction C.- .011g., R_G .09 (solvent A). A portion was incubated with glucose-oxidase and the solution examined on a paper chromatogram (solvent A). On development with aniline oxalate, no spot at R_G .09

was found, indicating that the original substance was D-glucose.

Fraction D.- 2.30g., $[\alpha]_D^{18}$ +81° (c, 2.34 in H_2O), R_G .07. This fraction crystallised on removal of the solvent and was recrystallised from methanol to give D-galactose, m.p. 163° (not depressed on admixture with an authentic sample).

Methylation of K. grandifolia Gum.- An atmosphere of nitrogen was maintained inside the reaction vessel, during all methylations using aqueous sodium hydroxide.

The re-precipitated gum (20g.) was dissolved in 30% aqueous sodium hydroxide solution (600 ml.) and dimethyl sulphate (270 ml.) added, with vigorous stirring, over a period of 8 hours. During the addition of the dimethyl sulphate, the temperature of the reaction was kept below 20°. After 24 hours, more sodium hydroxide solution (30%, 600 ml.) was added, followed by the gradual addition of dimethyl sulphate (270 ml.) over a period of 5 hours. The next day, sodium hydroxide (180g.) in water (200 ml.) was added, followed by dimethyl sulphate (270 ml.) over a period of 5 hours. The reaction mixture was acidified and dialysed against running tap water for 24 hours. The volume of the solution was reduced to 200 ml. by distillation of the water under diminished pressure.

After three more methylations, the solution was dialysed against running tap water for 24 hours. The volume of the solution was reduced to 200 ml.; this was acidified with dilute sulphuric acid and dialysed for 48 hours against distilled water. The water was removed by freeze-drying to give a yellow powder which was dissolved in water and silver carbonate added. (Found; OMe, 24.2; ash, 7.5%). The solution was filtered, the water removed by freeze-drying and the residue boiled with methanol (100 ml.), methyl iodide (80 ml.) and silver oxide (5g.) for two hours, during which time two further additions of silver oxide were made. The silver oxide was removed by filtration, the solvent distilled and the residue methylated five times with methyl iodide and silver oxide. The time of heating in each methylation was 24 hours. After each treatment, the material soluble in petroleum ether (80 parts) and chloroform (20 parts by volume) was isolated. Any material soluble in petroleum ether was discarded. This yielded 10.1g., $[\alpha]_D^{16}$ +53° (c, 1.32 in $CHCl_3$). (Found: OMe, 40.2%).

Hydrolysis of Methylated K. grandifolia Gum and Identification of the Neutral Sugars.- The methylated gum (8.0g.) was kept at room temperature for 10 days with 2N-sulphuric acid (125 ml.). The solution was heated at 100° for 20 hours and neutralised

with barium hydroxide, followed by carbon dioxide. The precipitated barium salts were removed at the centrifuge, washed with water, and the solvent removed by distillation under diminished pressure. The residue was separated into five fractions by partition chromatography on a column of cellulose powder, using as eluants light petroleum (60 parts)-n-butanol (40 parts), saturated with water, changing to light petroleum (50 parts)-n-butanol (50 parts), saturated with water and finally water.

Fraction A.- 0.90g., $[\alpha]_D^{15}$ +111° (c, 0.96 in H_2O), R_G .88. (Found: OMe, 52.0. Calc. for a tetra-O-methyl-hexose 52.5%). A portion (0.100g.) was heated under reflux with aniline (0.1 ml.) in ethanol (2.0 ml.) for 3 hours. On cooling, needles (.098g.) appeared having m.p. 190° (not depressed on admixture with the aniline derivative of 2:3:4:6-tetra-O-methyl-D-galactose) and $[\alpha]_D^{16}$ -63° → +34° (equilibrium value) (c, 0.83 in $(CH_3)_2CO$).

Fraction B.- 0.84g., $[\alpha]_D^{15}$ +86° (c, 1.07 in H_2O), R_G .71. (Found: OMe, 42.4. Calc. for a tri-O-methyl-hexose 41.9%). A portion (0.168g.) was oxidised with bromine water at 60° for 6 hours, the solution aerated to remove excess bromine and neutralised with silver carbonate. The solution was filtered, hydrogen sulphide passed and the solution filtered

again. The water was distilled under diminished pressure and the residue extracted with ether. Distillation of the ether left a syrup, which crystallised (0.129g.) on cooling. This was examined on a paper chromatogram (solvent B). The paper was dried and kept in a covered dish with ethereal diazo-methane, sprayed with an alkaline methanolic solution of hydroxylamine followed by ferric chloride. Only one spot was observed and this was chromatographically identical with 2:3:6-tri-O-methyl-D-galactonolactone. Using bromo-cresol green as the spray, a faint streak from the origin was observed. This might indicate the presence of 2:4:6-tri-O-methyl-D-galactonic acid, but all attempts to prepare an aniline derivative of any 2:4:6-tri-O-methyl-D-galactose in the original syrup were unsuccessful. The crude lactone was purified by distillation under diminished pressure (0.08 mm./bath temperature 100-110°) and recrystallised from benzene-light petroleum to give needles, m.p. 98° (not depressed on admixture with 2:3:6-tri-O-methyl-D-galactono-lactone) $[\alpha]_D^{17}$ -40° → -29° (equilibrium value) (c, 0.55, in H_2O).

Fraction C.- 0.28g., $[\alpha]_D^{16}$ +37° (c, 0.95 in H_2O), $[\alpha]_D^{15}$ +11° (c, 1.93 in C_2H_5OH), R_G .55. (Found: OMe, 18.5. Calc. for a mono-O-methyl-deoxy-hexose 17.4%). This fraction crystallised on removal of the solvent, and had m.p. 115°. A portion (.068g.) was

kept with bromine water for 20 hours and then heated for 6 hours at 60°. The bromine was removed by aeration, the solution neutralised with silver carbonate, filtered, the silver ions precipitated by passing hydrogen sulphide and the solution filtered again. The water was distilled under diminished pressure and the residue extracted with acetone. On distillation of the acetone, a syrup (.032g.) remained, $[\alpha]_D$ -20° → -18° (equilibrium value) (c, 1.6 in H_2O). The glycoside, prepared by boiling for 7 hours with 3% hydrogen chloride in methanol, was not oxidised by potassium meta-periodate solution.

Fraction D.- .023g., R_G .55 and .45. This fraction was combined with a later fraction.

The ions of methylated uronic acids then appeared.

Fraction E.- (water wash). The solvent was distilled, the residue dissolved in water and the solution passed through a column of Amberlite IR-120 (acid form) resin to remove cations. The water was distilled and the residue separated into two portions by partition chromatography on a column of powdered cellulose, using as the eluant n-butanol half saturated with water plus 5% acetic acid. Three cycles were necessary.

Fraction 1.- 0.70g., R_G .81 and a trace of .95, was unchanged on heating at 100° with 2N-sulphuric acid

for 24 hours.

Fraction 2.- 3.1g., R_G values less than .81. This was heated with 2N-sulphuric acid at 100° for 24 hours. The solution was neutralised with barium carbonate, the insoluble barium salts removed at a centrifuge and the solvent removed by distillation under diminished pressure. The residual syrup was separated into six fractions by partition chromatography on a column of powdered cellulose, using light petroleum-butanol, butanol-water and water as eluants.

Fraction B(i).- .045g., R_G .71.

Fraction C(i).- 0.110g., R_G .55.

Fraction D(i).- .033g., R_G .55 + .45. This was combined with fraction D, total .056g., $[\alpha]_D^{18}$ +57° (c, 0.63 in H_2O). (Found: OMe, 25.2%). The methoxyl content indicates a mixture of di-O-methyl-hexose (.04g.) and mono-O-methyl-rhamnose (.02g.).

Fraction F.- .020g., R_G .30.

Fraction G.- .020g., R_G .25.

Fractions D, F, and G were isolated in such small quantity that they cannot be considered to be structurally significant, as they could easily have arisen from undermethylation or demethylation.

Fraction 3.- (water wash). The barium ions were removed by passing an aqueous solution through a column of ion exchange resin (Amberlite IR-120) to

yield 1.2g. Examination on a paper chromatogram (solvent C) indicated one main component R_G .45 with traces of other compounds. On heating a portion with 2N-sulphuric acid at 100° for 16 hours and examination of the product on a paper chromatogram, two neutral components of R_G values .71 and .55 were indicated.

Reduction of Acidic Fraction from the Hydrolysate of Methylated K. grandifolia Gum.- The two acidic fractions from hydrolysis of the methylated gum (1 and 3) were combined (2.1g.) and boiled with 2% hydrogen chloride in methanol for 10 hours. The acid was neutralised with silver carbonate and the solvent distilled to leave a yellow syrup, which was dissolved in dry tetrahydrofuran (10 ml.) and the solution added slowly to lithium aluminium hydride (1.0g.) in dry tetrahydrofuran (10 ml.). (The tetrahydrofuran was purified by distillation from lithium aluminium hydride, after it had been kept at least one week over sodium wire). After 4 hours, the excess lithium aluminium hydride was destroyed with water and the solution acidified with dilute sulphuric acid. The sulphate and aluminium ions were removed as precipitates, by the addition of barium hydroxide and the barium and lithium ions removed as the insoluble carbonates. The water was distilled under diminished pressure to leave a syrup (1.1g.), which was hydrolysed by heating with N-sulphuric

acid at 100° for 16 hours. The sulphate ions were removed as barium sulphate and the water distilled. The residue was separated into four fractions, using as the eluants n-butanol, light petroleum, water and n-butanol half-saturated with water.

Fraction A.- 0.111g., $[\alpha]_D^{16}$ +73° (c, 0.63 in H_2O), R_G .85. (Found: OMe, 40.3. Calc. for a tri-O-methyl-hexose 41.9%). A portion (.010g.) was boiled for 6 hours with aniline (.02 ml.) and ethanol (0.4 ml.). The solvent was removed in a desiccator leaving crystals, which were recrystallised from benzene-light petroleum (.011g.), m.p. 146°. A further portion (.030g.) was boiled with 4% hydrogen chloride in methanol for 6 hours. The acid was removed with silver carbonate and the methanol distilled. The residue was methylated with silver oxide and methyl iodide. The glycoside was hydrolysed with N-sulphuric acid and the acid neutralised with barium carbonate. On distillation of the water, crystalline 2:3:4:6-tetra-O-methyl-D-glucose (.019g.) was obtained, m.p. 82°, $[\alpha]_D^{14}$ +81° (equilibrium value) (c, 0.32 in H_2O).

Fraction B.- .068g., $[\alpha]_D^{16}$ +85° (c, 0.99 in H_2O), R_G .71. (Found: OMe, 40.5. Calc. for a tri-O-methyl-hexose 41.9%). A portion (.042g.) was oxidised with bromine water for 6 hours at 60°. The bromine was removed by aeration and the hydrobromic acid as

silver bromide. Hydrogen sulphide was passed through the solution, which was filtered. The water was removed by distillation under diminished pressure and the residue extracted with ether. On distillation of the ether, the lactone crystallised and was purified by distillation under diminished pressure (bath temperature 80°- 90°/.05 mm.) and recrystallised from benzene-light petroleum to give crystals, m.p. 97°, (not depressed on admixture with 2:3:6-tri-O-methyl-D-galactonolactone).

Fraction C.- 0.154g., $[\alpha]_D^{15}$ +14° (c, 0.57 in C_2H_5OH), R_G .55. (Found: OMe, 17.0. Calc. for a mono-O-methyl-deoxyhexose 17.4%). The m.p. was 115° (not depressed on admixture with 3-O-methyl-L-rhamnose).

Fraction D.- 0.250g., $[\alpha]_D^{15}$ +103° (c, 0.84 in H_2O), R_G .46. (Found: OMe, 30.2. Calc. for a di-O-methyl-hexose 29.8%). A portion (.020g.) was boiled with aniline (.02 ml.) and ethanol (0.4 ml.) for 8 hours. The ethanol and aniline were removed in a desiccator and the residue dissolved in benzene and precipitated as an amorphous solid, by the addition of light petroleum. After crystallisation from acetone (.013g.), the m.p. was 154° (not depressed on admixture with the aniline derivative of 2:3-di-O-methyl-D-galactose), $[\alpha]_D^{19}$ -46.5° → +12° (equilibrium value, 120 hours), (c, 1.0 in C_2H_5OH).

Reduction of Methylated K. grandifolia Gum.- The methylated gum (0.50g.) was dissolved in dry tetra-hydrofuran (10 ml.) and added slowly to lithium aluminium hydride (0.25g.) in tetra-hydrofuran (10 ml.). After 2 hours, the solution was boiled for 2 hours. On standing for 16 hours at room temperature, the excess lithium aluminium hydride was destroyed with ethyl acetate, the solution acidified with dilute sulphuric acid and extracted with chloroform. The chloroform extract was washed with sodium bicarbonate solution and dried over anhydrous calcium chloride. Distillation of the chloroform left a yellow amorphous solid (0.39g.), $[\alpha]_D^{18}$ +46° (c, 1.44 in $CHCl_3$). (Found: OMe, 31.6%).

Hydrolysis of Reduced Methylated K. grandifolia Gum.- The reduced, methylated gum was heated in a boiling water bath with formic acid for 4 hours. The formic acid was distilled under diminished pressure, the residue dissolved in water and the water distilled. Water was added again and distilled. The residue was heated at 100° with N-hydrochloric acid for 16 hours. The hydrochloric acid was removed with silver carbonate, the solution filtered and hydrogen sulphide passed. The solution was filtered again and the water distilled. The residue was separated into five components by chromatography on Whatman 3 MM paper (two sheets 17 cm.

wide). The paper had been exhaustively extracted with hot methanol in a soxhlet apparatus. The positions of the sugars were found by spraying a guide strip, prepared from a separate hydrolysate and the sugars eluted by extraction with hot methanol in a soxhlet apparatus. The extract was filtered under gravity through a no.3 sintered glass funnel, the methanol distilled and the residue weighed. Two hydrolyses were carried out.

	Wt.(mg.)
Original polysaccharide	87.3, 91.0
R_G .88 + .85	16.6, 18.6
" .71	17.6, 18.6
" .55	17.9, 20.0
" .46	22.8, 22.3
" .30	2.9, 3.0
Blank	1.7, 1.8
Total (corrected for blanks)	69.3, 73.5

Methylation of Reduced Methylated K. grandifolia Gum.- The reduced, methylated gum (0.30g.) was heated with silver oxide and methyl iodide (4 x 24 hours) and the product isolated (0.26g.), $[\alpha]_D^{17}$ +34° (c, 1.12 in $CHCl_3$). (Found: OMe, 40.8%).

Hydrolysis of Methylated Reduced Methyl K. grandifolia Gum.- The methylated, reduced, methyl gum was heated in a boiling water bath with formic acid and

then with N-hydrochloric acid. The sugars were isolated and separated on two strips of Whatman 3 MM filter paper and the fractions isolated in the same manner as described for the methylated, reduced gum.

	Wt. (mg.)
Original polysaccharide	86.9
R_G 1.00	6.7
" .88	14.3
" .71	30.0
" .55	17.0
" .46	5.2
" .30	2.4
Blank	1.6
Total (corrected for blanks)	66.0

Separation of Acidic Components Obtained by Partial Hydrolysis of K. grandifolia Gum.- Examination of the acidic fraction on a paper chromatogram (solvent D), revealed a number of substances:- ($R_{Galactose}$ values .80 (intense), .40, .19, .09 with other faint spots and streaking). The $R_{Galactose}$ values were not reproducable, but the pattern of spots, produced on spraying with aniline oxalate solution, was always the same. The mixture was fractionated by chromatography on the acetate form of Amberlite IRA-400 resin, using aqueous acetic acid as the eluant. The resin was prepared by grinding in a mortar and taking the

material that passed through a 100 mesh seive. After stirring in water, the fraction that settled between 10 and 30 minutes was used. The freeze-dried acidic fraction (4.0g.) was adsorbed on a column 30 cm. x 3.4 cm. and eluted with increasing concentrations of dilute acetic acid (from 0.1 to 1.0% by stages of 0.1%, to 2.0 by 0.2%, to 5 by 0.5%, and to 15 by 2.5%). The column was then washed with 30% formic acid solution; (R_{Ga} refers to the rate of movement, relative to galactose, in solvent D).

Fraction 1.- Eluted with 0.1% acetic acid, 0.105g., R_{Ga} 1.00, (.81), .67, .52, (.35), zero (solvent D). Similar R_{Ga} values were obtained using a basic solvent system (solvent A) for irrigation.

Fraction 2.- 0.2 - 0.7% acetic solution, 0.172g., R_{Ga} .40 with streaking from .1 - .5, M_G .65 with a diffuse spot at .81. On hydrolysis with 2N-sulphuric acid at 100° for 18 hours, galactose and rhamnose were produced (paper chromatography). After oxidation with bromine water and hydrolysis, most of the rhamnose was removed. A portion was boiled with hydrogen chloride in methanol, reduced with potassium borohydride and hydrolysed. Galactose and rhamnose were formed (paper chromatography).

Fraction 3.- 0.8 - 1.0% acetic acid, 0.731g., $[\alpha]_D^{16}$ +130° (c, 1.83 in H_2O), R_{Ga} .80 (brown at the head and reddish-brown at the tail), M_G .82 and .69.

One gram consumed 44 ml. of N/10 iodine solution on oxidation. Hydrolysis (2N-sulphuric acid at 100° for 18 hours) indicated the presence of rhamnose, galactose, galacturonic acid and mono-O-methyl-uronic acid (paper chromatography). After oxidation with bromine water, the neutral sugars were destroyed, but both uronic acids remained.

A portion (0.600g.) was dissolved in water (5.0 ml.) and dimethyl sulphate (3.0 ml.) added, and 40% sodium hydroxide solution (6.0 ml.) added during 6 hours. After 12 hours, dimethyl sulphate (6.0 ml.) was added, followed by 40% sodium hydroxide (15 ml.) during 6 hours. After another 12 hours, dimethyl sulphate (7 ml.) was added and sodium hydroxide (15 ml.) over 6 hours. This was repeated using dimethyl sulphate (10 ml.) and sodium hydroxide (20 ml.). The solution was neutralised with sulphuric acid and extracted with chloroform. The chloroform was distilled to leave a pale yellow syrup (0.684g.), which was heated with methyl iodide and silver oxide for 24 hours, filtered and the methyl iodide distilled. The syrup was dissolved in dry tetrahydrofuran and added slowly to lithium aluminium hydride (0.30g.) in tetrahydrofuran. After 3 hours the excess lithium aluminium hydride was destroyed with water, the solution acidified with dilute sulphuric acid and extracted with chloroform.

The chloroform was washed with sodium bicarbonate solution and water, and the chloroform distilled. After methylation with silver oxide and methyl iodide (3 x 24 hours), the methylated product (0.410g.) was isolated as a yellow syrup. (Found: OMe, 52.1%). A methylated disaccharide composed of a methyl-pentose and a hexose requires 51.1% and a methylated disaccharide composed of two hexoses 54.6%. After hydrolysis with N-sulphuric acid (10 ml.) at 100° for 16 hours, the acid was neutralised with barium carbonate and the mixture of sugars, obtained after distillation of the water under diminished pressure, separated into three fractions by partition chromatography on a column of powdered cellulose, using n-butanol, (30 parts), light petroleum, (70 parts), saturated with water as the eluant.

Faction A.- .093g., $[\alpha]_D^{17}$ +82° (c, 1.12 in H_2O), R_G 1.00. (Found: OMe, 52.8. Calc. for a tetra-O-methyl-hexose 52.5%). This fraction crystallised on distillation of the solvent to give 2:3:4:6-tetra-O-methyl-D-glucose, having m.p. 84°.

Fraction B.- 0.200g., R_G .88 and .84. This was separated into two components by chromatography on Whatman 3 MM paper (solvent E).

Fraction B(a).- .082g., $[\alpha]_D^{18}$ +97° (c, 1.32 in H_2O), R_G .85 (solvent E). (Found: OMe, 48.6. Calc. for a tetra-O-methyl-hexose 52.5%). A portion (.040g.)

was heated with aniline (.04 ml.) in ethanol (0.5 ml.) for 3 hours. On cooling, crystals separated (.039g.). Recrystallisation from ethanol gave needles, m.p. 194° (not depressed on admixture with the aniline derivative of 2:3:4:6-tetra-O-methyl-D-galactose), $[\alpha]_D$ -60° → +18° (equilibrium value) (c, 0.80 in $(CH_3)_2CO$).

Fraction B(b).- .076g., $[\alpha]_D^{17}$ +20° (c, 1.42 in H_2O), R_G .54 (solvent E), M_G .4. (Found: OMe, 32.2. Calc. for a di-O-methyl-deoxyhexose 32.3%). A portion (.021g.) was kept with bromine water for 24 hours and then heated at 60° for 6 hours. The bromine was removed by aeration and the solution neutralised with silver carbonate. The solution was filtered, hydrogen sulphide passed and the solution filtered again. The water was distilled under diminished pressure and the residue extracted with acetone. The acetone was distilled to leave a syrup, which crystallised on cooling. This was purified by distillation under diminished pressure (0.2 mm./bath temperature 90°- 100°) to give needles (.005g.), m.p. 80°, $[\alpha]_D$ -120 ±10(equilibrium value) (c, 0.20 in H_2O).

Fraction C.- .073g., $[\alpha]_D^{18}$ +88° (c, 0.50 in H_2O), R_G .71. (Found: OMe, 41.0. Calc. for a tri-O-methyl-hexose 41.9%). A portion (.053g.) was oxidised with bromine water for 6 hours at 60°. The excess bromine was removed by aeration and acid removed

with silver carbonate. The water was distilled under diminished pressure and the residue extracted with ether. On distillation of the ether, the residue (.037g.) crystallised. Recrystallisation from benzene-light petroleum gave needles, m.p. 96° (not depressed on admixture with 2:3:6-tri-O-methyl-D- galactonolactone), $[\alpha]_D$ -42° → -25° (equilibrium value) (c, 0.57 in H_2O).

Fraction 4.- 1.0 - 2.0% acetic acid, .060g., R_{Ga} .38, M_G 1.06. On hydrolysis with 2N-sulphuric acid at 100° for 18 hours, galactose and rhamnose were found (paper chromatography). After oxidation with bromine water, no galactose was found. On heating with hydrogen chloride in methanol, followed by reduction with potassium borohydride and hydrolysis, galactose and rhamnose were formed (paper chromatography).

Fraction 5.- 2.5 - 3.0% acetic acid, 0.102g., R_{Ga} .85, M_G 1.07 (with a faint spot travelling behind this value), $[\alpha]_D^{18}$ +30° (c, 1.42 in H_2O). On heating with 2N-sulphuric acid for 18 hours at 100° and examination of the products on a paper chromatogram, a trace of galactose was indicated but the main product appeared unchanged. A portion (.028g.) was oxidised at room temperature with bromine water. Crystals of mucic acid (.006g.) formed, m.p. 220° (dec.).

Fraction 6.- 4.0 - 12.5% acetic acid, 0.528g.,

R_{Ga} .36 (with faint streak to the starting line), M_G .79 (with diffuse spot travelling faster). Hydrolysis with 2N-sulphuric acid for 18 hours at 100°, produced galactose and rhamnose (paper chromatography). The galactose was almost completely removed by oxidation with bromine water. Reduction of the glycoside, ester with potassium borohydride followed by hydrolysis, indicated galactose and rhamnose. The E.W. was 490 (by titration with N/10 sodium hydroxide with phenolphthalein as indicator).

Fraction 7.- 12.5 - 15.0% acetic acid, 0.650g., R_{Ga} .19, .09 and zero (with streaking from zero to .35), M_G .93 (elongated spot), E.W. 300 (by titration with N/10 sodium hydroxide with phenolphthalein as indicator). On hydrolysis with 2N-sulphuric acid for 18 hours at 100° and examination on a paper chromatogram, the presence of galactose and rhamnose was indicated. Reduction of the methyl glycoside ester with potassium borohydride, followed by hydrolysis and examination of the products on a paper chromatogram, indicated the presence of galactose and rhamnose.

Elution with 30% formic acid gave a further .085g. of material.

Purification of Khaya senegalensis Gum.- The gum was obtained as hard nodules, of varying size and colour, mixed with a small amount of bark. The nodules

varied from colourless to dark red-brown. Hydrolysis of a nodule with N-sulphuric acid at 100° for 6 hours, followed by examination of the product on a paper chromatogram, indicated the presence of galactose, arabinose, rhamnose and acidic material. One particular nodule had OMe, 2.2% and acetyl, 2.7%. The crude gum was powdered and dissolved, by stirring in 4% sodium hydroxide solution (200 ml.). The mechanical impurities were removed at the centrifuge, the solution cooled, acidified with hydrochloric acid and poured into ethanol (600 ml.), to which had been added concentrated hydrochloric acid (5 ml.). The precipitate was removed at the centrifuge, dissolved in water (100 ml.) and precipitated by addition to ethanol (300 ml.). After two more precipitations the product was washed with ethanol, then ether and dried (4 hours, 60°/12 mm.) to give a white powder (1.8g.). An attempted fractionation between water and ethanol was unsuccessful.

Properties of K. senegalensis Gum.- The purified polysaccharide contained 2.0% sulphated ash, $[\alpha]_D^{16}$ +124° (c, 0.971 in H_2O). A portion was dissolved in N/10 sodium hydroxide solution and after 12 hours, precipitated by pouring the solution into acetic acid. The precipitate was dissolved in water and the water removed at 0°/0.5 mm. The residue had OMe, 1.2%. The

E.W. (found by titration with N/10 sodium hydroxide using phenolphthalein as indicator) was 412.

Partial Hydrolysis of K. senegalensis Gum.- The purified gum (2.30g.) was dissolved in N-sulphuric acid and heated in a boiling water bath for 6 hours; the solution neutralised with barium hydroxide and carbon dioxide. The precipitated barium sulphate was removed at the centrifuge and washed several times with water. The water was removed by distillation under diminished pressure and the residue separated into four fractions by partition chromatography on a column of powdered cellulose, using as eluant n-butanol saturated with water, plus 5% ethanol.

Fraction A.- .091g., $[\alpha]_D^{18}$ +10° (c, 1.02 in H_2O), R_G .30. This fraction crystallised on removal of the solvent and was recrystallised from wet n-butanol to give L-rhamnose hydrate, m.p. 92° (not depressed on admixture with an authentic sample).

Fraction B.- .048g., $[\alpha]_D^{17}$ +113° (c, 0.79 in H_2O), R_G .12. This fraction crystallised on removal of the solvent and was recrystallised from ethanol to give L-arabinose, m.p. 156° (not depressed on admixture with an authentic sample).

Fraction C.- 0.370g., $[\alpha]_D^{18}$ +80° (c, 2.14 in H_2O), R_G .07. This fraction crystallised on removal of the solvent and was recrystallised from methanol to

give D-galactose, m.p. 164° (not depressed on admixture with an authentic sample).

Fraction D was obtained by washing the column with water. The barium was removed by passing a solution through a column of ion exchange resin (Amberlite IR-120, acid form) and the water removed at 0°/0.5 mm. to give a yellow powder (0.87g.). Examination on a paper chromatogram (solvent D), indicated a complex mixture of substances with the same $R_{Galactose}$ values as the mixture of acidic substances, obtained on partial hydrolysis of Khaya grandifolia gum:- $R_{Galactose}$ .80 (intense), .40, .19, .09 with other faint spots and streaking. The E.W. was 348 (obtained by titration with N/10 sodium hydroxide solution, using phenolphthalein as the indicator). Hydrolysis with 2N-sulphuric acid at 100° for 18 hours and examination of the hydrolysate on a paper chromatogram (solvent A), indicated the presence of rhamnose and galactose.

Reduction of the Methyl Ester Glycosides of the Partial Hydrolysate of K. senegalensis Gum.- The freeze-dried acidic fraction (0.70g.) was kept overnight with 5% hydrogen chloride in methanol and then boiled for 6 hours. The solution was cooled, neutralised with silver carbonate and filtered. After distillation of the methanol, the residue was dissolved in water (10 ml.) and added slowly to a solution of

potassium borohydride (0.35g.) in water (10 ml.). After 2 hours, the excess borohydride was destroyed by the addition of dilute acetic acid and the ions removed by passing the solution through two columns of ion exchange resins (Amberlite IR-4B, hydroxyl form, and IR-120, acid form). The water was removed by distillation and the glycosides hydrolysed by heating with N-sulphuric acid at 100° for 18 hours. The acid was removed with barium carbonate and the water removed by distillation under diminished pressure, to leave a yellow syrup (0.51g.). Examination on a paper chromatogram (solvent A) and spraying with aniline oxalate, revealed three substances, R_G values .07, .25, .30 and a trace of material R_G .09.

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