# 206. *Plant Gums of the Genus* Khaya. *The Structure of* Khaya grandifolia *Gum.*

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*Khaya grandifolia* gum has been shown to be composed of residues of D-galactose, L-rhamnose, D-galacturonic acid, and 4-*O*-methyl-D-glucuronic acid, with traces of L-arabinose. Hydrolysis of the methylated gum indicated the presence therein of residues of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2 : 3 : 6-tri-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid and 2 : 3-di-*O*-methyl-D-galacturonic acid. On partial hydrolysis the gum gave two aldobiouronic acids, 2-*O*-D-galacturonosyl-L-rhamnose and 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose, an aldotriouronic acid, *O*-D-galacturonosyl-(1→2)-*O*-L-rhamnose-(1→4)-D-galactose, and other acidic oligosaccharides.

*Khaya senegalensis* gum has been shown to be composed of the same sugar residues, but in different proportions.

The gum exudate of *Khaya grandifolia*, the West African mahogany tree, has been examined by McIlroy,[1] who obtained evidence for the presence of residues of galactose, rhamnose, and galacturonic acid. The gum, therefore, appeared to be similar to the gums of *Sterculia setigera* [2] and *Cochlospermum gossypium* ("Karaya" gum) [3] rather than to the glucuronic acid-containing gums of the *Acacia* [4-6] and *Prunus* [7-9] genera. A quantity of the gum was kindly placed at our disposal by Dr. R. J. McIlroy and in the present study the main structural features of the gum have been investigated.

The gum we examined had been purified by dissolution in 4% sodium hydroxide solution, and was further purified by precipitation from acid solution with acetone and then by re-precipitation from aqueous solution with ethanol. The purified gum, in contrast with the crude material, was readily soluble in water. It is possible that the treatment with alkali had removed ester groupings as several plant gums [2,3] are known to occur as partly acetylated polysaccharides. The gum had a uronic anhydride content [10] of 47% (calculated for a substance of equivalent weight 344, 51%) and a low, but significant, methoxyl content (1%).

The gum was extremely resistant to hydrolysis, and even after hydrolysis with 2N-sulphuric acid for 18 hr. at 100° the yield of neutral sugars was low, quantitative estimation [11] indicating the presence of galactose (18%), rhamnose (15%), and arabinose (1%) (results expressed as percentages of anhydro-sugar originally present in the gum). After hydrolysis of the gum with N-sulphuric acid at 100° for 6 hr. L-rhamnose (3·7%), L-arabinose (0·2%), and D-galactose (18·0%) were isolated as crystalline compounds. The incompletely hydrolysed acidic residue (52% of the original weight of gum) was converted into the methyl ester methyl glycoside, reduced with potassium borohydride, and rehydrolysed, the following sugars being then identified : L-rhamnose (10·1%), 4-*O*-methyl-D-glucose (2·0%), D-glucose (trace), and D-galactose (26·6%). It is probable that the trace of glucose arose from the demethylation of 4-*O*-methyl-D-glucose during the hydrolysis. As 4-*O*-methyl-D-glucose was not found in the hydrolysate of the gum before treatment of the gum with potassium borohydride, it must have been formed by the reduction of

[1] McIlroy, *J.*, 1952, 1918.
[2] Hirst, Hough, and Jones, *J.*, 1949, 3145; Hough and Jones, *J.*, 1950, 1199.
[3] Hirst and Dunstan, *J.*, 1953, 2332.
[4] Challinor, Haworth, and Hirst, *J.*, 1931, 258; Smith, *J.*, 1939, 744, 1724; Jackson and Smith, *J.*, 1940, 74, 79.
[5] Stephen, *J.*, 1951, 646; Hirst and Perlin, *J.*, 1945, 2622; Charlson, Nunn, and Stephen, *J.*, 1955, 269.
[6] Charlson, Nunn, and Stephen, *J.*, 1955, 1428.
[7] Hirst and Jones, *J.*, 1938, 1174; 1939, 1482; 1946, 506.
[8] Jones, *J.*, 1939, 558; 1947, 1055; 1949, 3141.
[9] Hirst and Jones, *J.*, 1947, 1064; 1948, 120; Brown, Hirst, and Jones, *J.*, 1949, 1757.
[10] McCready, Swenson, and Maclay, *Ind. Eng. Chem. Anal.*, 1946, **18**, 290.
[11] Hirst and Jones, *J.*, 1949, 1659.

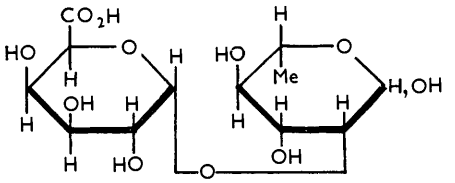
4-*O*-methyl-D-glucuronic acid residues. The combined yield of L-rhamnose and D-galactose (58·4%) was greater than could be derived from neutral sugar residues in a gum containing 47% of uronic anhydride, therefore some of the D-galactose could only have arisen from the reduction of D-galacturonic acid residues in the original gum. Further evidence for the presence of galacturonic residues was obtained on nitric acid oxidation of the gum; the yield of mucic acid indicated the presence in the gum of 58% of galactose and/or galacturonic acid residues.

The methylated gum was prepared in the usual way and like the parent gum was extremely resistant to hydrolysis. The hydrolysate was fractionated chromatographically on cellulose,[12] giving 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 2 : 3 : 6-tri-*O*-methyl-D-galactose, and 3-*O*-methyl-L-rhamnose (all identified as crystalline derivatives), a trace of an unidentified di-*O*-methylgalactose (probably of no structural significance), and a mixture of acidic substances. After attempts to separate the acidic components had failed, the acids were converted into the methyl ester methyl glycosides, which were reduced with lithium aluminium hydride. The reduced glycosides were hydrolysed and the resulting neutral sugars were partitioned on cellulose. Further quantities of 2 : 3 : 6-tri-*O*-methyl-D-galactose and 3-*O*-methyl-L-rhamnose were isolated, and in addition 2 : 3 : 4-tri-*O*-methyl-D-glucose and 2 : 3-di-*O*-methyl-D-galactose were characterised by the formation of crystalline derivatives. As the last two sugars were not present amongst the neutral sugars isolated on direct hydrolysis of the methylated gum it is clear that they were formed by the reduction of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic and 2 : 3-di-*O*-methyl-D-galacturonic acids respectively. No methyl ethers of L-arabinose were detected.

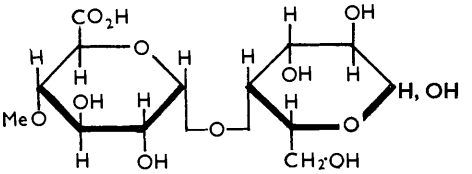
The mixture of acidic substances obtained on partial hydrolysis of the gum was fractionated by stepwise elution from a column of an anion-exchange resin [13] with increasing concentrations of acetic acid, to give a hexuronic acid, a mixture of aldobiouronic acids, an aldotriouronic acid, and other incompletely identified acidic oligosaccharides. The hexuronic acid, isolated in 1·3% yield from the gum, was identified as galacturonic acid by bromine oxidation to mucic acid. The aldobiouronic acid fraction, isolated in 10% yield, was shown by paper ionophoresis [14] to contain two components with slightly different $M_G$ values, although the two substances travelled at the same rate on a paper chromatogram. Hydrolysis of the mixture of acids gave rhamnose, galactose, galacturonic acid, and 4-*O*-methylglucuronic acid, but, after oxidation of the disaccharides with bromine water, rhamnose and galactose could no longer be detected on hydrolysis. The aldobiouronic acids were converted into a mixture of fully methylated disaccharides by reduction of the acidic residues in the methylated aldobiouronic acids with lithium aluminium hydride followed by further methylation of the reduction products. Hydrolysis of the methylated disaccharides then yielded the following sugars, which were identified as crystalline derivatives: 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 6-tri-*O*-methyl-D-galactose. Although these results indicate the mode of linkage of the sugar residues in the aldobiouronic acids, they do not provide evidence for the combination of sugars. Two observations, however, point to 2-*O*-D-galacturonosyl-L-rhamnose (I) and 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose (II) as structures of the aldobiouronic acids. First, the two aldobiouronic acids travel on the chromatogram at the same rate, whereas it would be expected from the known rates of movement of the constituent sugars that aldobiouronic acids, in which the sugars were linked in the alternative manner, would travel at different rates. Secondly, only a small difference in the ionophoretic mobilities of the aldobiouronic acids (I) and (II) would be expected, as each disaccharide contains one pair of hydroxyl groups [the 3 : 4-hydroxyl groups of the galacturonosyl residue in (I) and the 1 : 2-hydroxyl groups of the galactose residue in (II)] capable of complex formation with boric acid and the carboxyl group of the acidic residue. On the other hand, the alternative pair of aldobiouronic acids, 4-*O*-D-galacturonosyl-D-galactose and 2-*O*-(4-*O*-methyl-D-glucuronosyl)-L-rhamnose, would be

[12] Hough, Jones, and Wadman, *J.*, 1949, 2511.
[13] Weissman, Meyer, Sampson, and Linker, *J. Biol. Chem.*, 1954, **208**, 417; Derungs and Deuel, *Helv. Chim. Acta*, 1954, **37**, 657.
[14] Consden and Stanier, *Nature*, 1952, **169**, 783.

expected to show greatly different ionophoretic mobilities [15] as the former compound would contain two pairs of hydroxyl groups capable of complex formation with boric acid, whereas the latter would contain none. These two aldobiouronic acids have been previously isolated from the hydrolysis of plant gums, namely, 2-*O*-D-galacturonosyl-L-rhamnose from *Sterculia setigera* [2] and *Cochlospermum gossypium* [3] gums, and 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose from gum myrrh.[16]



(I)



(II)

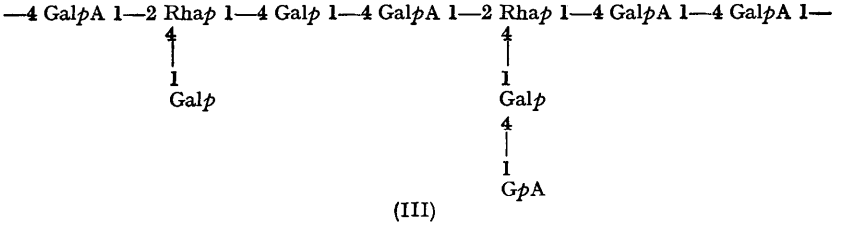
An aldotriouronic acid (equivalent weight 490) was isolated in 7·0% yield, and gave on hydrolysis galactose and rhamnose. After oxidation of the trisaccharide with bromine water only rhamnose was detected on hydrolysis, and after reduction of the acidic residue with potassium borohydride and hydrolysis the products were galactose and rhamnose. The acidic trisaccharide, therefore, contained residues of galactose, rhamnose, and galacturonic acid, the galactose residue forming the reducing group. The aldotriouronic acid was methylated in the same way as the mixture of aldobiouronic acids and was converted into a fully methylated neutral trisaccharide, hydrolysis of which gave sugars travelling on the paper chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 6-tri-*O*-methyl-D-galactose. The tri-*O*-methyl-D-galactose must have been derived from the reducing galactose residue linked through position 4, therefore the tetra-*O*-methyl-D-galactose could only have been formed from a galacturonic acid residue at the non-reducing end of the molecule. It follows from this evidence that the acidic trisaccharide was *O*-D-galacturonosyl-(1→2)-*O*-L-rhamnosyl-(1→4)-D-galactose. Other acidic oligosaccharides were obtained in low yield, but although these were all shown to be composed of galactose, rhamnose, and galacturonic acid residues, insufficient quantities were isolated for detailed structural studies. The high proportion of acid residues in one of these fractions (equivalent weight 300) suggested that the gum may contain adjacent galacturonic residues.

The main structural features of *K. grandifolia* gum are now clear from this investigation. From the methylation studies it is evident that end-groups of D-galactose and 4-*O*-methyl-D-glucuronic acid occur in the gum whilst L-rhamnose residues provide the only branching points in the molecule and 1 : 4-linked D-galacturonic acid residues occur in the main chains. The evidence for the presence of the aldobiouronic acid 2-*O*-D-galacturonosyl-L-rhamnose (I) in the products of partial hydrolysis shows that the L-rhamnose residues are linked through positions 1 and 2 in the main chain and that the side chains must be linked through position 4. As glycosiduronic acid linkages are particularly resistant to acid hydrolysis, the evidence that on mild acid hydrolysis of the gum only galactose, together with small amounts of arabinose, was formed and no significant amounts of neutral disaccharides were detected, suggests that the D-galactose end-groups are linked directly to the L-rhamnose residues. On the other hand, the identification of the aldobiouronic acid 4-*O*-(4-*O*-methyl-D-glucuronosyl)-D-galactose (II) on partial acid hydrolysis of the gum shows that the 4-*O*-methyl-D-glucuronic acid end-groups are linked to the main chain through 1 : 4-linked D-galactose residues in two-residue side-chains. The isolation of the aldotriouronic acid, *O*-D-galacturonosyl-(1→2)-*O*-L-rhamnosyl(1→4)-D-galactose, shows that some 1 : 4-linked D-galactose residues are also present in the main chain. In addition, the main chain

[15] Foster, *J.*, 1953, 982; Foster and Stacey, *J.*, 1955, 1778.
[16] Jones and Nunn, *J.*, 1955, 3001.

probably contains adjacent D-galacturonic acid residues, as indicated by the oligosaccharides of high uronic acid content isolated on partial acid hydrolysis of the gum; indeed, the high uronic acid content of the gum itself can only be explained on this basis.

Throughout this investigation the hydrolyses of both the original gum and its methyl ether were accompanied by considerable decomposition and the yields of sugars isolated were low. An attempt to obtain a quantitative estimate of the ratio of methylated sugar residues present in the methylated gum was made by hydrolysing samples of the methylated polysaccharide after reduction of the acidic residues with lithium aluminium hydride, but only an 80% recovery of sugars on hydrolysis was obtained. Consequently only an approximate estimate of the constituent sugars present in the gum can be made. Ultracentrifugal and electrophoretic examinations of the gum (in collaboration with Dr. C. T. Greenwood) indicated the presence of only one molecular species, and all the structural features must therefore be accommodated within a single molecule. Although no unique structure can be put forward for the gum, the repeating unit (III) shown includes all the known types of linkage present in the gum, and the ratio of constituent sugar residues, namely, D-galactose (3 parts), L-rhamnose (2 parts), D-galacturonic acid (4 parts) and 4-*O*-methyl-D-glucuronic acid (1 part), is consistent with the quantitative measurements made, when allowance is made for the decomposition of sugars during hydrolysis. The ultracentrifugal studies indicated that the gum was of high molecular weight although no accurate value is yet available, and the end-groups of D-galactose and 4-*O*-methyl-D-glucuronic acid must therefore occur in the side-chains rather than as the non-reducing terminal residues of the backbone of the molecule.




(III)

(Gal*p*A = D-galactopyranuronic acid, Gal*p* = D-galactopyranose, Rha*p* = L-rhamnopyranose, and G*p*A = 4-*O*-methyl-D-glucopyranuronic acid.)

These results show that *Khaya grandifolia* gum contains several interesting structural features. The occurrence of two different uronic acid residues within a single molecule is a unique feature of the plant gums so far examined. On previous occasions when two aldobiouronic acids have been isolated from the partial hydrolysis of a plant gum, these have contained the same uronic acid residue linked either to different sugars or to the same sugar through different positions. The gum resembles in some respects those from *Sterculia setigera* [2] and *Cochlospermum gossypium*,[3] which also contain D-galactose, L-rhamnose, and D-galacturonic acid residues, although the present gum contains in addition 4-*O*-methyl-D-glucuronic acid residues. As is also the case for these gums the D-galactose residues in *K. grandifolia* gum are linked through positions 1 and 4, in contrast with damson,[7] cherry,[8] and egg-plum [9] gums, and the gums of the *Acacia* group [5, 6] where the D-galactose residues are linked through positions 1 and 3, and 1 and 6. *K. grandifolia* gum, however, differs from *S. setigera* and *C. gossypium* gums in that L-rhamnose occurs solely as branching points in the central part of the molecular structure and is not also found linked through positions 1 and 2 only. The gum differs also from *S. setigera* gum in that the D-galacturonic acid residues are linked only through positions 1 and 4 in the main chain and do not form the branching points of the gum molecule.

Recent investigations of the gums of the *Acacia* genus [5, 6] have shown that different gums contain the same constituent sugars and in each case the aldobiouronic acid, 6-*O*-D-glucuronosyl-D-galctose, has been isolated from the products of partial hydrolysis, but the proportions in which the sugar residues are present differ markedly. It was of interest therefore to examine another gum of the *Khaya* genus. *Khaya senegalensis* gum was

obtained in the natural state as a partially acetylated polysaccharide, and was purified by dissolution in aqueous sodium hydroxide and isolated as a white amorphous powder after three reprecipitations from aqueous solution with ethanol. The purified gum had an equivalent weight of 412 and a methoxyl content of 1·2%. After partial hydrolysis, L-rhamnose (4·0%), L-arabinose (2·1%), and D-galactose (16·1%) were isolated as crystalline compounds. The incompletely hydrolysed acidic residue (37·8%), which had an equivalent weight of 348, liberated further quantities of rhamnose and galactose on extended hydrolysis. Chromatographic examination of the acidic residue indicated a mixture of acidic oligosaccharides similar to that obtained on partial hydrolysis of *K. grandifolia* gum. After conversion into the methyl ester methyl glycoside, reduction with potassium borohydride, and hydrolysis of the reduction product, chromatographic examination of the hydrolysate showed rhamnose, 4-*O*-methylglucose, glucose (trace), and galactose to be present. The methoxyl content of the gum was not high enough for all the uronic acid to be present as 4-*O*-methylglucuronic acid, so it is probable that galacturonic acid is also a constituent sugar of the gum. *K. senegalensis* gum, therefore, contains the same sugar residues as *K. grandifolia* gum, but the proportions of uronic acid and L-arabinose are different. The evidence at present available indicates that in the *Khaya* genus the position in regard to the composition of the various gums is similar to that found for *Acacia* species.

## Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper, with the upper layers of the following solvent systems (v/v): (*A*) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (*B*) butan-1-ol–ethanol–water (4 : 1 : 5); (*C*) butan-1-ol–acetic acid–water (4 : 1 : 5); (*D*) butal-1-ol–acetic acid–water (8 : 2 : 5); (*E*) benzene–ethanol–water (169 : 47 : 15). Paper ionophoresis [14] was carried out in borate buffer at pH 10.

*Purification and Properties of* Khaya grandifolia *Gum.*—The gum was received from Dr. R. J. McIlroy as a light grey powder which had been reprecipitated with ethanol after dissolution in 4% aqueous sodium hydroxide.[1] The gum acid was precipitated from solution in dilute hydrochloric acid with ethanol and reprecipitated from aqueous solution with acetone, to give a white amorphous powder, $[\alpha]_D^{18}$ +122° (unchanged on further reprecipitation) (*c*, 1·88 in $H_2O$) [Found: equiv., 344 (by titration); uronic anhydride, 47·2% (by decarboxylation); ash, nil; OMe, 1·0%].

Chromatographic examination of the hydrolysate of the gum after it had been heated with N-sulphuric acid at 100° showed that galactose and arabinose (trace) were released after 0·5 hr. and that rhamnose appeared after 3 hr. The hydrolysis of the gum under milder conditions was also followed (autohydrolysis at 100° for 40 hr., hydrolysis with 0·01N-sulphuric acid at 100° for 40 hr., and hydrolysis with 0·1N-sulphuric acid at 100° for 14 hr.), but in no case were significant amounts of neutral disaccharides detected in addition to galactose and arabinose.

Quantitative estimation, by the method of Hirst and Jones,[11] of the sugars produced on hydrolysis of the gum with N-sulphuric acid at 100° for 6 hr. indicated the presence of the following sugars (calc. as percentage of anhydro-sugar in the original gum): galactose, 16·1; rhamnose, 8·2; arabinose, <1%. After hydrolysis of the gum with 2N-sulphuric acid at 100° for 18 hr. the same sugars were present in the following quantities: galactose, 18·0; rhamnose, 14·7; arabinose, <1%. Oxidation of the gum with nitric acid and estimation of the mucic acid (m. p. and mixed m. p. 216°) formed indicated the presence in the gum of 58% of galactose and/or galacturonic acid residues.

*Partial Hydrolysis of the Gum.*—The purified gum (10 g.) was heated with N-sulphuric acid (250 c.c.) at 100° for 6 hr., the hydrolysate was neutralised with barium hydroxide, the excess of alkali rapidly destroyed with carbon dioxide, and the filtrate was taken to dryness. The residue was fractionated on cellulose,[12] with butan-1-ol saturated with water plus 10% of ethanol, as the eluant, giving four fractions.

Fraction 1 (0·370 g.) had $[\alpha]_D^{17}$ +9·8° (equil.) (*c*, 4·44 in $H_2O$) and after recrystallisation from ethanol had m. p. and mixed m. p. (with L-rhamnose hydrate) 95°. Fraction 2 (0·023 g.) had $[\alpha]_D^{17}$ +104° (equil.) and after recrystallisation from methanol had m. p. and mixed m. p. (with L-arabinose) 155°. Fraction 3 (1·80 g.) had $[\alpha]_D^{16}$ +81° (equil.) and after recrystallisation from methanol had m. p. and mixed m. p. (with D-galactose) 164°. Fraction 4, obtained by elution

of the cellulose with water, consisted of the barium salts of acidic material (Found : Ba, 17·4%). Barium ions were removed by passage through a column of Amberlite resin IR-120(H) and the solution was freeze-dried to give the acidic fraction (A) (5·2 g.) which was examined later.

*Hydrolysis of the Acidic Fraction (A) after Reduction with Potassium Borohydride.*—The acidic fraction (A) (4·5 g.) was set aside overnight in methanolic 4% hydrogen chloride and then refluxed for 7 hr. After neutralisation with silver carbonate, the dry residue was dissolved in water (100 c.c.) and the solution was added slowly to a solution of potassium borohydride (2·3 g.) in water (100 c.c.). After 2 hr., the excess of borohydride was destroyed by the addition of dilute acetic acid, and the solution was de-ionised by passage through columns of Amberlite resins IR-120 and IR-4B. After being taken to dryness the residue was hydrolysed by N-sulphuric acid at 100° for 18 hr. Chromatographic examination of the hydrolysate showed rhamnose, 4-*O*-methylglucose, glucose (trace), and galactose, and partition on cellulose, with, as eluant, butan-1-ol saturated with water, gave pure fractions of glucose and galactose, and a mixture of rhamnose and 4-*O*-methylglucose. Most of the rhamnose crystallised on trituration of the mixed fraction with moist butan-1-ol and complete separation of the two components was achieved by further partition on cellulose. Examination of the four fractions confirmed the identity of the sugars. Fraction 1 (0·878 g.) had $[\alpha]_D^{18}$ +10° (equil.) (*c*, 2·35 in $H_2O$) and after recrystallisation from moist butan-1-ol had m. p. and mixed m. p. (with L-rhamnose hydrate) 95°. Fraction 2 (0·173 g.) had $[\alpha]_D^{20}$ +62° (Found : OMe, 15·5. Calc. for $C_7H_{14}O_6$ : OMe, 15·9%), and was identified as 4-*O*-methyl-D-glucose by conversion into the osazone, m. p. 154°, $[\alpha]_D^{19}$ −31°→−14° (equil.) (*c*, 1·11 in $H_2O$). Fraction 3 (11 mg.) travelled on the chromatogram at the same rate as D-glucose and was destroyed on incubation with glucose-oxidase. Fraction 4 (2·30 g.) had $[\alpha]_D^{18}$ +81° (equil.) (*c*, 2·34 in $H_2O$) and after recrystallisation from methanol had m. p. and mixed m. p. (with D-galactose) 163°.

*Separation of Acidic Components obtained on Partial Hydrolysis of the Gum.*—The acidic fraction (A) (4·0 g.) was absorbed on a column of Amberlite resin IRA-400 (acetate form) (30 × 3·4 cm.; 100 mesh) and the column was eluted with increasing concentrations of dilute acetic acid (0·1—1·0 by stages of 0·1%, 1·0—2·0 by 0·2%, 2·0—5·0 by 0·5%, and 5·0—15·0 by 2·5%). Six main fractions were obtained together with small quantities of complex mixtures which were not examined further.

*Fraction* 1. The syrup (0·172 g., eluted with 0·2—0·7% acetic acid) contained a main component having $R_{Gal}$ 0·40 (rate of movement relative to galactose in solvent *D*) and $M_G$ 0·65 (rate of movement on the ionophoretogram relative to glucose). Hydrolysis with 2N-sulphuric acid at 100° for 18 hr. gave galactose and rhamnose, but after oxidation with bromine water hydrolysis gave galactose and only a trace of rhamnose. Hydrolysis of the syrup after reduction of the methyl ester methyl glycoside with potassium borohydride gave galactose and rhamnose.

*Fraction* 2. The syrup (0·731 g., eluted with 0·8—1·0% acetic acid) had $[\alpha]_D^{16}$ +130° (*c*, 1·83 in $H_2O$). Chromatographic examination suggested the presence of two components, $R_{Gal}$ 0·80, which were incompletely resolved, but complete resolution was achieved on the ionophoretogram ($M_G$ 0·82 and 0·69). Hydrolysis with 2N-sulphuric acid gave rhamnose, galactose, galacturonic acid, and 4-*O*-methylglucuronic acid, but after oxidation with bromine water hydrolysis gave the acidic components only.

A portion of the syrup (0·60 g.) was methylated four times with methyl sulphate and sodium hydroxide, and the product (after extraction of the acidified reaction mixture with chloroform) (0·684 g.) was further methylated with methyl iodide and silver oxide. After removal of the solvent, the syrup was dissolved in dry tetrahydrofuran and added slowly to lithium aluminium hydride (0·30 g.) in tetrahydrofuran. After 3 hr. the excess of lithium aluminium hydride was destroyed by the addition of water, and the solution was acidified and extracted with chloroform. The extract was re-methylated three times with methyl iodide and silver oxide, and the mixture of methylated disaccharides was isolated as a yellow syrup (0·410 g.) (Found : OMe, 52·1. A methylated disaccharide composed of a hexose and a deoxyhexose residue requires OMe, 51·1%, and a methylated disaccharide composed of two hexose residues requires OMe, 54·6%). The methylated sugars were hydrolysed with N-sulphuric acid (10 c.c.) at 100° for 16 hr. and the hydrolysate was separated on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, as eluant, to give three fractions. Fraction *a* (93 mg.) crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 84°, $[\alpha]_D^{17}$ +82° (equil.) (*c*, 1·12 in $H_2O$) (Found : OMe, 52·8. Calc. for $C_{10}H_{20}O_6$ : OMe, 52·5%). Fraction *b* (200 mg.) contained two sugars having $R_G$ 0·88 and 0·84 in solvent *B*, which were separated on filter sheets with solvent *E* to give fractions *b*(i) (82 mg.) and *b*(ii) (76 mg.). Fraction *b*(i) had $[\alpha]_D^{18}$ +97° (equil.) (*c*, 1·32 in $H_2O$) and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose

by conversion into the aniline derivative, m. p. and mixed m. p. 194°, $[\alpha]_D^{18}$ −60°→+18° (equil.) (*c*, 0·8 in $Me_2CO$). Fraction *b*(ii) had $[\alpha]_D^{17}$ +20° (equil.) (*c*, 1·42 in $H_2O$) (Found : OMe, 32·2. Calc. for $C_8H_{16}O_5$ : OMe, 32·3%) and was identified as 3 : 4-di-*O*-methyl-L-rhamnose by conversion into 3 : 4-di-*O*-methyl-L-rhamnono-1 : 5-lactone, m. p. 80°. Fraction *c* (73 mg.) had $[\alpha]_D^{18}$ +88 (equil.) (*c*, 0·5 in $H_2O$) (Found : OMe, 41·0. Calc. for $C_9H_{18}O_6$ : OMe, 41·9%) and was identified as 2 : 3 : 6-tri-*O*-methyl-D-galactose by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactono-1 : 4-lactone, m. p. and mixed m. p. 96°, $[\alpha]_D^{20}$ −42°→−25° (equil.) (*c*, 0·57 in $H_2O$).

*Fraction* 3. The syrup (60 mg., eluted with 1·0—2·0% acetic acid) had $R_{Gal}$ 0·38 and $M_G$ 1·06. Hydrolysis with 2N-sulphuric acid gave rhamnose and galactose, but after oxidation with bromine water hydrolysis gave only rhamnose. Hydrolysis of the syrup after reduction of the methyl ester methyl glycoside with potassium borohydride gave galactose and rhamnose.

*Fraction* 4. The syrup (0·102 g., eluted with 2·5—3·0% acetic acid) had $[\alpha]_D^{18}$ +30° (*c*, 1·42 in $H_2O$) and contained a main component, $R_{Gal}$ 0·85 and $M_G$ 1·07, which was identified as D-galacturonic acid by conversion into mucic acid, m. p. and mixed m. p. 220° (decomp.).

*Fraction* 5. The syrup (0·528 g., eluted with 4·0—12·5% acetic acid) had $R_{Gal}$ 0·36 and $M_G$ 0·79 (Found : equiv., 490). Hydrolysis with 2N-sulphuric acid gave rhamnose and galactose, but after oxidation with bromine water hydrolysis gave only rhamnose. Hydrolysis of the syrup after reduction of the methyl ester methyl glycoside with potassium borohydride gave galactose and rhamnose. A portion of the syrup (0·346 g.) was methylated in the same way as fraction 2 and after reduction of the acidic residues was converted into the fully methylated trisaccharide (0·071 g.). Chromatographic examination of the hydrolysate after it had been heated with N-hydrochloric acid at 100° for 4 hr. showed the presence of sugars travelling at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, 3 : 4-di-*O*-methyl-L-rhamnose, and 2 : 3 : 6-tri-*O*-methyl-D-galactose in solvents *B* and *E*.

*Fraction* 6. The syrup (0·65 g., eluted with 12·5—15·0% acetic acid) contained a mixture of substances of $R_{Gal}$ 0·0—0·19 with a main component having $M_G$ 0·93 (Found : equiv., 300). Hydrolysis with 2N-sulphuric acid gave galactose and rhamnose, and the same sugars only were formed on hydrolysis after reduction of the methyl ester methyl glycoside with potassium borohydride.

*Methylation of the Gum.*—The purified gum (20 g.) was methylated six times with methyl sulphate and sodium hydroxide and, after careful acidification of the reaction mixture and dialysis to remove inorganic ions, the partially methylated gum was converted into the silver salt by treatment of an aqueous solution with silver carbonate and the silver salt was isolated by freeze-drying. The silver salt was suspended in a boiling mixture of methyl iodide (80 c.c.) and methanol (100 c.c.), and silver oxide (15 g.) was added during 2 hr. After removal of insoluble silver salts five further methylations were carried out with methyl iodide and silver oxide, the methylated polysaccharide (10·1 g.), soluble in chloroform–light petroleum (b. p. 60—80°) (1 : 4), was isolated having $[\alpha]_D^{16}$ +53° (*c*, 1·32 in $CHCl_3$) (Found : OMe, 40·2%).

*Hydrolysis of the Methylated Gum and Identification of the Neutral Sugars.*—The methylated gum (8·0 g.) was dissolved in 2N-sulphuric acid (125 c.c.) and kept at room temperature for 10 days. The solution was then heated at 100° for 20 hr., cooled, and carefully neutralised with barium hydroxide. After carbon dioxide had been bubbled through the mixture to remove excess of hydroxide as barium carbonate, the precipitated barium salts were removed and the solution was taken to dryness. The resulting mixture of sugars was separated on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol (6 : 4), saturated with water, as eluant, to give four fractions containing neutral sugars, and elution with water gave a mixture of acidic substances (as barium salts) (E) which was examined later.

*Fraction* A. The syrup (0·90 g.) had $[\alpha]_D^{15}$ +111° (*c*, 0·96 in $H_2O$) and $R_G$ 0·88 in solvent *B* (Found : OMe, 52·0. Calc. for $C_{10}H_{20}O_6$ : OMe, 52·5%), and was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 190°, $[\alpha]_D^{16}$ −63°→+34° (equil.) (*c*, 0·83 in $Me_2CO$).

*Fraction* B. The syrup (0·84 g.) had $[\alpha]_D^{15}$ +86° (*c*, 1·07 in $H_2O$) and $R_G$ 0·71 (Found : OMe, 42·4. Calc. for $C_9H_{18}O_6$ : OMe, 41·9%), and was identified as 2 : 3 : 6-tri-*O*-methyl-D-galactose by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactono-1 : 4-lactone, m. p. and mixed m. p. 98°, $[\alpha]_D^{17}$ −40°→−29° (equil.) (*c*, 0·55 in $H_2O$). As the 2 : 3 : 6- and the 2 : 4 : 6-trimethyl ether of D-galactose travel at similar rates on the chromatogram evidence for the presence of the 2 : 4 : 6-isomer in this fraction was sought but none was found : all attempts to prepare the aniline derivative failed and chromatographic examination of the tri-*O*-methylgalactonolactone indicated the presence of only the one component.

*Fraction* C. The sugar (0·28 g.) had $[\alpha]_D^{16}$ +37° (equil.) (*c*, 0·95 in $H_2O$), $[\alpha]_D^{15}$ +11° (equil.)

(*c*, 1·93 in EtOH), and $R_G$ 0·55 (Found : OMe, 18·5. Calc. for $C_7H_{14}O_5$ : OMe, 17·4%), and crystallised [3] on removal of solvent, m. p. 115°. The methyl glycoside of the sugar was unattacked by periodate and the derived 3-*O*-methyl-L-rhamnono-1 : 4-lactone had $[\alpha]_D^{16}$ −20° → −18° (equil.) (*c*, 1·6 in $H_2O$).

*Fraction* D. The syrup (23 mg.) contained two sugars with $R_G$ 0·55 and 0·45 and was examined after combination with a later fraction.

*Examination of the Acidic Fraction.*—The mixture of barium salts (E) was converted into the corresponding mixture of acids by removal of barium ions with Amberlite resin IR-120(H). The mixture was separated into two fractions by partition on cellulose, with, as eluant, butan-1-ol 50% saturated with water to which glacial acetic acid (5%, v/v) was added. Fraction 1 (0·70 g.) contained a main component having $R_G$ 0·81 in solvent *C* together with a trace of a substance with $R_G$ 0·95, and was unchanged by hot 2N-sulphuric acid. Fraction 2 (3·1 g.) contained substances with $R_G$ less than 0·81 and was further hydrolysed by 2N-sulphuric acid at 100° for 24 hr. and the hydrolysate was neutralised with barium carbonate. The neutral sugars were separated from the barium salts of the acidic components by partition on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol, saturated with water, butan-1-ol saturated with water, and water as eluants. Fraction B(i) (45 mg.) had $R_G$ 0·71 (solvent *B*) and fraction C(i) (110 mg.) had $R_G$ 0·55. Fraction D(i) (33 mg.) contained sugars of $R_G$ 0·55 and 0·45 and was combined with fraction D (total 56 mg.), $[\alpha]_D^{15}$ +57° (*c*, 0·63 in $H_2O$) [Found : OMe, 25·2. A mixture of di-*O*-methyl-hexose and mono-*O*-methylrhamnose (2 : 1) requires OMe, 25·7%]. Fraction F (20 mg.) travelled on the chromatogram at the same rate as rhamnose ($R_G$ 0·30) and fraction G (20 mg.) had $R_G$ 0·25 but was not examined further. Fraction 3, obtained as barium salts by elution of the column with water, was converted into a mixture of acids by de-ionisation with Amberlite resin IR-120(H), and when taken to dryness gave a syrup (1·2 g.) in which one main component ($R_G$ 0·45 in solvent *C*) was present, together with traces of other compounds.

*Reduction of the Acidic Fractions with Lithium Aluminium Hydride.*—The two acidic fractions 1 and 2 were combined (2·1 g.) and refluxed with methanolic 2% hydrogen chloride for 10 hr. After neutralisation with silver carbonate, the solution was taken to dryness, the resulting syrup was dissolved in dry tetrahydrofuran (10 c.c.), and the solution was added slowly to lithium aluminium hydride (1·0 g.) in tetrahydrofuran (10 c.c.). After 4 hr., the excess of hydride was destroyed by water, the solution was acidified with dilute sulphuric acid, the sulphate and aluminium ions were removed as precipitates on addition of barium hydroxide, and the barium and lithium ions were removed as the insoluble carbonates. The resulting solution was taken to dryness, to yield a syrup (1·1 g.), which was hydrolysed by N-sulphuric acid at 100° for 16 hr., and the hydrolysate was separated on cellulose, eluants being light petroleum (b. p. 100—120°)–butan-1-ol, saturated with water, and butan-1-ol 50% saturated with water, to give four fractions.

*Fraction a.* The syrup (0·111 g.) had $[\alpha]_D^{16}$ +73° (*c*, 0·63 in $H_2O$) and $R_G$ 0·85 in solvent *B* (Found : OMe, 40·3. Calc. for $C_9H_{18}O_6$ : OMe, 41·9%). Methylation of the sugar and hydrolysis of the resulting methyl glycoside yielded 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. 82° and $[\alpha]_D^{14}$ +81° (equil.) (*c*, 0·32 in $H_2O$), and the sugar was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the aniline derivative, m. p. 146°.

*Fraction b.* The sugar (0·068 g.), $[\alpha]_D^{16}$ +85° (*c*, 0·99 in $H_2O$) and $R_G$ 0·71 (Found : OMe, 40·5. Calc. for $C_9H_{18}O_6$ : OMe, 41·9%), was identified as 2 : 3 : 6-tri-*O*-methyl-D-galactose by conversion into 2 : 3 : 6-tri-*O*-methyl-D-galactono-1 : 4-lactone, m. p. and mixed m. p. 97°.

*Fraction c.* The sugar (0·154 g.), $[\alpha]_D^{15}$ +14° (*c*, 0·57 in EtOH) and $R_G$ 0·55, crystallised and had m. p. and mixed m. p. (with 3-*O*-methyl-L-rhamnose) 115° (Found : OMe, 17·0. Calc. for $C_7H_{14}O_5$ : OMe, 17·4%).

*Fraction d.* The syrup (0·25 g.) had $[\alpha]_D^{18}$ +103° (*c*, 0·84 in $H_2O$) and $R_G$ 0·46 in solvent *B*, and was identified as 2 : 3-di-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 154°, $[\alpha]_D^{19}$ −46·5° → +12° (equil.) (*c*, 1·0 in EtOH).[17]

*Reduction of the Methylated Gum.*—The methylated gum (0·50 g.) was dissolved in dry tetrahydrofuran (10 c.c.) and added slowly to lithium aluminium hydride (0·25 g.) in tetrahydrofuran (10 c.c.). After 2 hr. at room temperature, the solution was refluxed for 2 hr. and set aside overnight. Excess of hydride was destroyed by ethyl acetate, and the solution was acidified with dilute sulphuric acid and extracted with chloroform. The chloroform extract yielded the reduced methylated gum (A) (0·39 g.), $[\alpha]_D^{18}$ +46° (*c*, 1·44 in $CHCl_3$) (Found : OMe, 31·6%).

The reduced methylated gum A (0·30 g.) was further methylated by four treatments with

[17] Bell and Greville, *J.*, 1955, 1136.

methyl iodide and silver oxide, to give reduced methylated gum (B) (0·26 g.), $[\alpha]_D^{17}$ +34° (*c*, 1·12 in $CHCl_3$) (Found : OMe, 40·8%).

*Hydrolysis of the Reduced Methylated Gums.*—The reduced methylated gums (*ca.* 100 mg.) were hydrolysed by formic acid at 100° for 4 hr. and after removal of formic acid with N-hydrochloric acid at 100° for 16 hr. After neutralisation with silver carbonate the hydrolysates were separated on extractive-free Whatman 3MM paper with solvent *B*. The appropriate sections of the papers were extracted with hot methanol, and the extracts were filtered and taken to dryness. The annexed weights of sugars are corrected for blanks.

| Sugar | $R_G$ in solvent *B* | Wt. of sugar from gum A (89·1 mg.) | Wt. of sugar from gum B (86·9 mg.) |
|---|---|---|---|
| Tetra-*O*-methylglucose | 1·00 | — | 5·1 |
| Tetra-*O*-methylgalactose | 0·88 | 15·9 | 12·7 |
| Tri-*O*-methylglucose | 0·85 | 15·9 | — |
| Tri-*O*-methylgalactose | 0·71 | 16·4 | 28·4 |
| Mono-*O*-methylrhamnose | 0·55 | 17·2 | 15·4 |
| Di-*O*-methylgalactose | 0·46 | 20·8 | 3·6 |
| Rhamnose | 0·30 | 1·2 | 0·8 |

*Purification and Properties of* Khaya senegalensis *Gum.*—The gum was obtained as colourless to dark brown nodules admixed with small particles of bark. One particular nodule had OMe, 2·2, and OAc, 2·7%, and chromatographic examination of the hydrolysate showed galactose, arabinose, rhamnose, and a complex mixture of acidic substances. The gum was purified by dissolving it in 4% sodium hydroxide solution, removing the mechanical impurities at the centrifuge, acidifying the resulting solution with hydrochloric acid, and precipitating the polysaccharide with ethanol. After three reprecipitations from aqueous solution with ethanol, the gum was isolated as a white powder, $[\alpha]_D^{16}$ +124° (*c*, 0·97 in $H_2O$) [Found : equiv., 412 (by titration); sulphated ash, 2·2; OMe, 1·2%].

*Partial Hydrolysis of* K. senegalensis *Gum.*—The purified gum (2·30 g.) was heated with N-sulphuric acid at 100° for 6 hr., the hydrolysate was neutralised with barium hydroxide, the excess of alkali was rapidly destroyed with carbon dioxide, and the filtrate was taken to dryness. The residue was fractionated on cellulose, eluant being butan-1-ol saturated with water plus 5% ethanol, to give four fractions. Fraction 1 (0·091 g.) had $[\alpha]_D^{18}$ +10° (equil.) (*c*, 1·02 in $H_2O$) and after recrystallisation from moist butan-1-ol had m. p. and mixed m. p. (with L-rhamnose hydrate) 92°. Fraction 2 (0·048 g.) had $[\alpha]_D^{17}$ +113° (equil.) (*c*, 0·79 in $H_2O$) and after recrystallisation from ethanol had m. p. and mixed m. p. (with L-arabinose) 156°. Fraction 3 (0·370 g.) had $[\alpha]_D^{18}$ +80° (equil.) (*c*, 2·14 in $H_2O$) and after recrystallisation from methanol had m. p. and mixed m. p. (with D-galactose) 164°. Fraction 4 was obtained by elution of the cellulose with water and consisted of the barium salts of acidic substances. The barium ions were removed with Amberlite resin IR-120, and the acidic fraction was freeze-dried to give a yellow powder (0·87 g.). Chromatographic examination in solvent *D* indicated a complex mixture of acids, $R_{Gal}$ 0·80 (intense), 0·40, 0·19, and 0·09, similar to those formed on partial hydrolysis of *K. grandifolia* gum. The fraction had equiv. 348 (by titration) and hydrolysis with 2N-sulphuric acid gave rhamnose and galactose. A portion of the fraction was converted into the methyl ester methyl glycoside by heating it with methanolic hydrogen chloride, then reduced with potassium borohydride, and the product was hydrolysed and shown by chromatography to contain rhamnose, 4-*O*-methylglucose, galactose, and a trace of glucose.

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